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Corpus callosum low frequency stimulation suppresses seizures in an acute rat model of focal cortical seizures

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Summary

Objective: Low frequency fiber-tract stimulation has been shown to be effective in treating mesial temporal lobe epilepsies through activation of the hippocampal commissure in rodents and human patients. The corpus callosum (CC) is a major pathway connecting the two hemispheres of the brain however few experiments have documented corpus callosum stimulation. The objective is to determine the efficacy of corpus callosum stimulation at low frequencies to suppress cortical seizures.

Methods: 4-aminopyridine (4-AP) was injected in the primary motor cortex of 24 rats under anesthesia. Recording electrodes were placed in the contralateral motor cortex and hippocampus. Three pairs of stimulating electrodes were inserted into the corpus callosum along its longitudinal axis. Local field potentials were recorded one hour before, during, and after stimulation to determine the effect of stimulation on seizure duration. Stimulation was delivered from each pair of electrodes independently in separate experiments. Furthermore, electrical stimulation was injected from the region of the corpus callosum with the highest degree of innervation of the seizure focus in order to compare the efficacy of different stimulation frequencies (1–30Hz) on seizure suppression.

Results: Corpus callosum stimulation was effective at suppressing seizures at 10Hz by 76% ($p < 0.05$, $n = 5$) and 20Hz by 95% ($p < 0.0001$, $n = 14$). Stimulation at frequencies of 1 and 30Hz did not have a significant effect on reducing the total time spent seizing ($p > 0.9999$, $n = 5$). Furthermore, stimulation was only effective at suppressing seizures when the pair of electrodes was placed within the section of corpus callosum containing fibers innervating the seizure focus. Secondly generalized seizures in the hippocampus were eliminated when seizures in the cortical focus were suppressed.

Significance: Low frequency fiber tract stimulation of the corpus callosum suppresses both cortical and cortically induced hippocampal seizures in an acute model of focal cortical seizures. The stimulation paradigm is selective as it is only effective when targeted to specific regions of the corpus callosum that project maximally to cortical regions generating the seizure activity. Selective

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placement of stimulation electrodes along the corpus callosum could be used as a patient-specific treatment for cortical epilepsies.

Keywords

4-aminopyridine; primary motor cortex; hippocampus; deep brain stimulation; neocortex

Introduction

Epilepsy is one of the most pervasive neurological disorders affecting approximately 1% of the world's population¹. Each year it is estimated that there are between 16 and 51 new patients diagnosed with an epilepsy disorder per one hundred thousand people². Unfortunately, around 20% of these patients do not respond to anti-epileptic medication². Of those patients that are refractory to pharmacological treatment about half have focal or multifocal cortical epilepsy³. Focal cortical epilepsies are known to be particularly resistant to medication^{3–5}. The difficulty in treating cortical epilepsies is thought to be due to the wide variability in localization and pathology among individual patients⁶. The most common alternative to medication is surgical resection however most patients are not eligible for resection due to an inability to identify a well-defined epileptogenic region that is not co-localized with any eloquent cortex. Furthermore, of the patients that are eligible for surgical resection only about half gain freedom from seizures. The success rate of resection decreases precipitously over time to only about 30% of patients experiencing no seizures after 5 years^{3; 4; 6–11}.

More recently several deep brain stimulation (DBS) technologies have been developed around the unmet need for seizure suppression in patients for which medication and surgery are not viable options. Electrical stimulation has the advantage of being less invasive, adjustable, and reversible compared to surgical resection. Clinical studies have shown that seizure suppression can be achieved with different stimulation parameters and grey matter locations. In one such study of bilateral stimulation of the anterior nuclei of the thalamus, a 56% median seizure frequency reduction was reported after 2 years post implantation¹². Another implementation of deep brain stimulation for epilepsy uses a feedback system to stimulate in response to detected seizure activity. This responsive cortical neuro-stimulation reduced seizure frequency by about 37% after 2 years¹³. In a follow-up pivotal trial of the responsive neurostimulation device (RNS) a seizure suppression rate of 53% after 2 years was reported¹⁴. The limited success of this responsive closed loop paradigm in seizure suppression is likely the result of a persistent difficulty in adequately localizing seizure foci. Seizure foci are rarely well defined or involve multiple epileptogenic regions that evolve over time^{15; 16}. Even in situations where the seizure focus is perfectly circumscribed within a fixed region the spread of highly synchronized neuronal activity often occurs too quickly to be terminated with stimulation at the site of origination¹⁷.

Stimulation of white matter tracts is an alternative method for suppressing seizures by lowering excitability in a large portion of the brain simultaneously^{18–21}. Low frequency stimulation (LFS) which we define to be between 1 to 20 Hz was applied to the hippocampal commissure and yielded a greater than 90% reduction in seizure frequency in *in-vitro*

experiments, chronic animal studies, and in one clinical trial for patients with mesial temporal lobe epilepsy seizure odds ($n=7$) were reduced by 92% in the 2 days that followed stimulation^{18–21}. For cortical seizures, a similar approach would be to target the corpus callosum. Surprisingly, despite the long history of research and surgical interventions involving the corpus callosum in epilepsy there is only one study on the effect of electrical stimulation of this large fiber tract²². Although this study reported no effect on seizure activity in a feline penicillin generalized epilepsy model, the experiment looked only at recordings before and following stimulation due to an inability to resolve activity masked by stimulation artifacts. Moreover, the topical penicillin generalized model of epilepsy is primarily dependent on inputs from the thalamus which have a very different topographic distribution compared to colossal inputs to the cortex²².

Although the functional characteristics of callosal fibers has been the subject of active debate, the corpus callosum is thought to consist predominantly of excitatory axons that synapse on both pyramidal cells and inhibitory neurons in the corresponding cortical hemispheres^{23; 24}. Regardless of the exact nature of the transcallosal fibers we speculate that a low frequency stimulation protocol should suppress seizures similar to the effect observed in the hippocampus with commissural stimulation. Based on the positive results from other fiber tract stimulation paradigms, a more clearly defined focal cortical model of epilepsy along with recordings taken during stimulation will lead to a better understanding of the potential suppressive effects of corpus callosum stimulation. Here we test the hypothesis that targeted low frequency stimulation of specific tracts of the corpus callosum will lead to seizure inhibition of foci located in the cortical regions innervated by those fibers. We describe the use of direct corpus callosum stimulation for focal cortical epilepsy in an acute rodent model of neocortical seizures.

Materials and Methods

Animals

All animal procedures were conducted in accordance with the guidelines, reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University. Twenty nine adult male Sprague–Dawley rats (150–300g) (Charles River, Wilmington, MA, USA) were used in this study. Isoflurane anesthesia was administered at a concentration of 1–3% and vitals were monitored during the entire experiment. Animals were secured via a stereotactic apparatus. Subsequently, a small incision was made along the rostro-caudal axis to expose the skull. All connective tissue and vasculature was removed from the skull by scrubbing with hydrogen peroxide and electric cautery. A total of 10 burr holes were drilled into the skull. Stainless steel monopolar electrodes were placed in the primary motor cortex, CA3 region of the hippocampus, and in the corpus callosum at six separate locations along the rostro-caudal axis (diameter 200 μ m, PlasticsOne, Part No. E363/3). All coordinates were determined via rat brain atlas; motor cortex (AP: –1.00mm, Lateral: –2.40mm, Depth: –1.60mm), hippocampus (AP: –3.14mm, Lateral: –3.00mm, Depth: –3.75mm), corpus callosum (AP: [+0.20mm, –1.88mm, –4.16mm], Lateral: +/- 1.00mm, Depth: [–3.00mm, –3.10mm, –2.60mm])²⁵. A stainless steel screw electrode was embedded into the skull near the most posterior bone sutures and

used as a reference. All electrodes were secured to the skull using a dental cement (TEETS Denture Material, Co-Oral-lite Dental Mfg. Co., Diamond Springs, CA, USA). A 5 μ L microsyringe (Hamilton, Reno, NV, USA) was secured to the stereotaxic frame and inserted into the contralateral motor cortex (AP: +1.00mm, Lateral: -2.40mm, Depth: -1.60mm). The location of the electrodes and injection site as well as the corresponding coronal slices of the rodent brain are shown in figure 1A.

Focal Cortical Model

In order to test the hypothesis that selective corpus callosum stimulation can reduce seizures, we developed a stable model of focal cortical seizures using repeated local injections of 4-AP. Injections of a cocktail of 30mM 4-aminopyridine (4-AP), 1.2mM CaCl₂, and 0.6mM MgSO₄ were injected at a rate of 1 μ L bolus per hour under a slightly reduced Isoflurane anesthesia concentration (<2%). Injections were delivered via a 5 μ L syringe 1 μ L bolus given at the start of every hour during the experiment. The injections were administered to the contralateral cortex in order to allow seizures to spread through the corpus callosum to the unaffected hemisphere where we had placed recording electrodes in the corresponding motor cortex and hippocampus. The first seizure usually occurred within 20 minutes to 1 hour following the initial injection. In order to ensure the seizures did not disappear over time we recorded 3 hours of baseline activity prior to every experiment. We found that the seizure rate actually increased over time. Moreover, we found that it did not take very long for activity to generalize from the motor cortex to the hippocampus as can be seen in figure 2B. The total duration of time spent seizing in each of the three hours of baseline was almost identical between the cortex and hippocampus. To ensure the reproducibility of this model the corresponding author will respond to any requests for clarification on the details of this protocol.

Data Acquisition and Seizure Identification

EEG recordings were sampled at 100kHz (PowerLab DAQ, AD Instruments Inc, Colorado Springs, CO USA) and amplified by 100. Recordings were taken continuously over the course of the experiment in order to monitor seizures in the cortex and secondarily in the hippocampus. Seizures were identified according to a metric established by Nissinen et al where in the EEG segment must be double the amplitude of the baseline amplitude, the majority of the frequency content must be at least 5Hz, and this activity must continue for longer than 5 seconds²⁶. Seizure onset was identified as the time at which the first spike from the first segment of EEG activity satisfying these criteria occurred. The duration of each of these seizures was recorded for purposes of comparison across experimental periods. Periods of time during stimulation were cleaned of stimulation artifacts by applying template subtraction and a median filter. The artifact removal did not affect seizure identification as can be seen in supplemental figure 2.

Electrical Stimulation

Each pair of stimulation electrodes in the corpus callosum was utilized independently of the rest. The appropriate stimulation amplitude was determined by finding 50% of the maximum amplitude of the evoked potential in the cortex using the pair of electrodes closest to the recording electrodes. Based on the evoked potentials in the cortex the amplitude was fixed at

4mA for all experiments. Stimulation was delivered through a digital stimulator with a current isolator (DS8000 Digital Stimulator, World Precision Instruments Inc, Sarasota, FL USA) driven by a separate function generator (FG-8002, Goldstar) in the form of a 2mA biphasic (4mA pk-pk) current pulse with a 100 μ s pulse width (each phase was 100 μ s giving a total biphasic pulsewidth of 200 μ s). The current pulses were delivered continuously for one hour at a frequency of 1, 10, 20, or 30Hz depending on the experiment. The different frequencies were applied only to the pair of electrodes closest to the recording electrodes. We only used 20Hz stimulation in the other 2 pairs of electrodes. The configuration of the electrodes used to evaluate the frequency range of stimulation efficacy is shown in the figure 3A insert.

Experimental Design and Statistical Methods

In each experiment we recorded activity for 3 hours prior to the initiation of any stimulation protocol in order to validate our acute model. In 14 animals we applied 20Hz stimulation to the electrode pair in the corpus callosum positioned closest to the recording electrode in the cortex. Following one hour of this stimulation a period of 1 hour of post stimulation was recorded to monitor for any after-effect. In sets of 5 animals we also tested stimulation at 1, 10, or 30Hz from the same location in order to compare efficacy. We also tested the efficacy of stimulation from the other two pairs of electrodes in the corpus callosum farther from the motor cortex. The experiments conducted using these other two pairs of electrodes were carried out using an additional set of 5 animals. Finally, we tested the effect of moving the seizure focus, recording, and stimulating electrodes on the efficacy of stimulation. The entire cortical/callosal assembly placement was shifted to a more posterior aspect of the corpus callosal axis. The experiments conducted using this alternate seizure focus location required the use of 5 additional animals. The experimental design of this study is shown in figure 3A. To compare the efficacy of stimulation at different frequencies and in different locations we used a Friedman's test with Dunn's multiple comparisons post-hoc test with a significance level of 0.05. A non-parametric ANOVA was utilized due to the non-normal distribution of these data as determined by a D'Agostino-Pearson omnibus normality test ($p < 0.05$). Total time spent seizing during baseline, stimulation, and post stimulation were compared for statistical significance.

Results

Seizure activity during baseline

The temporal distribution of seizures generated by this focal model of cortical epileptiform activity was first examined. Two typical examples of seizures recorded are shown in figure 2A. The seizures generated in the cortex have patterns similar to those observed with hippocampal applications of 4-AP^{20; 21; 27}. The seizures typically begin with a single high amplitude spike and then develop into high frequency firing followed by lower frequency high amplitude spikes. The spectrogram in figure 2A demonstrates the clear difference in power spectral density between seizure activity and the underlying baseline. Seizure activity similar to that observed in the cortex was also generated in the hippocampus often with an onset delay of several seconds. To determine the stability of seizure activity, the total time spent seizing was measured three hours prior to stimulation to establish a baseline. Figure

2B shows that the seizure activity generated by the 4-AP focal model did not decrease over time but increased. The total time spent seizing significantly increased following a three hour baseline period compared to the initial seizure activity during the first hour ($p < 0.0001$, $n = 24$). Furthermore, by the end of hour three seizures had almost completely generalized to the hippocampus with the majority of cortical seizures triggering hippocampal seizures. During the first hour of the baseline period all seizures were less than 200s long with a significantly higher proportion of the seizure duration generated in the cortex as shown in figure 2C. In the second hour of baseline recording seizure durations in the hippocampus and cortex were similar with some seizures lasting as long as 500 seconds (figure 2D). By the third hour of baseline the hippocampus and cortex had become nearly synchronized with individual seizures of the same duration. Moreover, some seizures in both the cortex and hippocampus were observed to last for as long as 10 mins.

The 4-AP model of acute focal cortical seizures produces a stable baseline seizure frequency and duration. This model is also shown to exhibit generalization of seizures in the cortex to the hippocampus. The seizure focus is known to be extensively innervated by corpus callosum fibers and should therefore serve as a suitable model to evaluate the effect of low frequency stimulation for cortical seizure suppression.

20Hz Stimulation Suppresses Cortical Seizures

To determine the effect of corpus callosum stimulation on the seizure activity described above, two stimulation electrodes were positioned along the anterior-posterior axis of the corpus callosum to activate specifically those fibers innervating the location of the focus in the cortex. Single pulse stimulation was applied to generate evoked potentials to confirm that the focus was innervated by the fibers activated. In each experiment three hours of baseline activity was recorded followed by a recording period of one hour during which stimulation was applied (figure 3A). Figure 3B shows distinct seizures along with a large number of interictal spikes during 1 Hz stimulation. Similarly seizures and interictal spiking were prevalent during 30 Hz stimulation. In figure 3C only sporadic interictal spiking is observed during 20Hz stimulation.

Figure 3D shows the effect of stimulation at 1, 10, 20, and 30Hz. 10 and 20 Hz generated a 76% and 95% reduction in seizures respectively ($p < 0.05$, $n = 5$; $p < 0.0001$, $n = 14$). This effect was most pronounced at 20Hz with complete seizure suppression in the majority of experiments. There was no statistically significant effect of either 1 or 30Hz at suppressing seizures.

20Hz Stimulation also suppresses hippocampal seizures induced by a cortical focus

Recordings in the hippocampus show that the cortical seizures generalized to the hippocampus (see figure 4A). When 20 Hz stimulation was applied to the corpus callosum (figure 4B) activity in both the cortex and the hippocampus was limited to occasional interictal spiking. The total time spent seizing in the hippocampus was at a minimum during stimulation at 10 and 20 Hz as was the case in the cortex. It is important to distinguish propagation of individual spikes originating in the cortex from the seizure wavefront itself. In figure 4C one can see that the seizure spikes are fairly well synchronized between the

hippocampus and cortex with roughly equal proportions emanating from either structure. The seizure wavefront however, which we define as the onset of the seizure, always occurs first in the cortex indicating that there is not an independent seizure focus in the hippocampus can be seen in figure 4D. According to our criteria that seizures must consist of greater than 5Hz spectral power for longer than 5 seconds figure 2A shows the delay between the cortex and hippocampus in terms of both the spectral power and amplitude ($>2\times$ baseline amplitude). Figure 4E shows that 10 and 20Hz are the only frequencies effective at suppressing seizures with 20Hz providing the greatest suppressive effect in the hippocampus ($p < 0.05$, $n=5$; $p=0.0003$, $n=14$).

Stimulation Efficacy is Spatially Selective

Corpus callosum fibers are topographically organized and only a subset of those fibers can be activated by a single bipolar electrode pair. We determined the feasibility of selectively activating a cortical region of interest (seizure focus) with corpus callosum stimulation, thereby minimizing the amount of cortical tissue activated and limiting the potential side effects. To determine the spatial selectivity of corpus callosum stimulation, bipolar electrodes were placed at locations along the anterior/posterior axis at +0.2mm, -1.88mm, and -4.16mm (figure 5A, C, and E). The stimulation frequency was fixed at 20Hz based upon our previous findings. In figure 5A, C, and E the evoked potentials from all three stimulation locations are shown. Seizure suppression generated by stimulation electrodes from each location is also shown in figure 5B, D, and F showing that suppression is greatest for electrode positions generating the largest evoked potential. This result indicates that the stimulation paradigm is selective as even the most proximal stimulation site to the optimal target (2mm posterior to the optimal portion of the corpus callosum for this particular focus) has no statistically significant effect on seizure suppression (figure 5D). The stimulation location 4 mm posterior to the optimal portion of the corpus callosum tract also showed no effect on seizure suppression (figure 5E). To further validate the ability to target specific tracts of the corpus callosum based upon the location of the seizure focus, the seizure focus/recording electrode and stimulating electrodes were moved 2 mm posterior from their original location as shown in figure 6A. Similar to the results shown in figure 5, seizures were suppressed by 95% during stimulation in the more posterior location ($p=0.03$, $n=5$) as shown in figure 6B. Seizures were also inhibited identically in the hippocampus as well ($p=0.03$, $n=5$).

Discussion

Cortical epilepsies are among the most difficult to effectively manage due to the extent of functional and organizational diversity inherent to the cortex. Consequently, patients often present with a wide variety of symptom pathologies³⁻⁶. Depending on where the seizure focus is located in the cortex patients may or may not be candidates for surgical resection. However, such a procedure can cause many functional deficits and often fails to permanently decrease seizure frequency⁶⁻¹¹. To study the effect of an electrical stimulation paradigm on modulating seizures an animal model with a well-defined seizure focus must be utilized. Preferably such a model would produce stable seizures over a relatively long period of time. Furthermore, these seizures should be very distinct from other forms of activity produced by

hyperexcitability (e.g. interictal spiking, spike ripples, waves) with detectable start and end times. However, to date no such model for acute focal cortical seizures exists in the literature. Previous studies have used a single 4-AP injection in the neocortex to produce seizures over the course of 2–3 hours however a slight decrease in seizure frequency and duration over the course of the experiment was observed^{28–32}. Therefore, in order to determine the efficacy of corpus callosum stimulation for focal cortical seizures we developed a modified 4-AP acute model of focal seizures. This modified 4-AP model is unique because 1 μ L injections of 30mM 4-AP are administered repeatedly once per hour over the entire duration of the experiment and because the ACSF media was adjusted to further increase tissue excitability by lowering magnesium and calcium concentrations. The important feature of this model is that it produces sustained spontaneous seizures that become more prevalent with time and can be characterized as discrete events (figure 2A). This model is stable over time periods (>5 hours under anesthesia) necessary to conduct acute experiments with a percent time spent in seizure of between 10–15%.

Unlike the tetanus toxin model, 4-AP elicits seizures with a distinct morphology and duration both *in-vitro* and *in-vivo*^{20; 21; 27}. The reproducibility of seizures with clear initiation and termination times across experimental setups make 4-AP an attractive chemical model of seizures. Previous studies have shown that low frequency fiber tract stimulation, specifically of the hippocampal commissure can suppress seizures consistently by greater than 90%^{18–21}. In this study we have shown that direct stimulation of the corpus callosum can inhibit cortical seizures by >90%. This suppression rate is similar to previous results obtained with electrical stimulation of the hippocampal commissure, 100% suppression *in-vitro*²¹, 90% *in-vivo* chronically¹⁹, and 87% in patients¹⁸. Corpus callosum stimulation produced an identical suppressive effect on seizures that had spread to the hippocampus from a single stimulation site. This can be explained by the fact that the seizures generated by the 4-AP focus in the cortex generalized to the hippocampus and were therefore produced by the cortical focus. There was no indication of additional foci developing in the hippocampus because individual hippocampal spikes during seizures were synchronized to cortical spikes during seizures (figure 4C). Furthermore, the seizure wavefront always originated in the cortex with seizures arriving in the hippocampus anywhere from a few milliseconds to seconds after initiating in the cortex (figure 4D). Based on these findings it seems likely that corpus callosum stimulation acts directly upon the seizure focus within the region of the cortex directly innervated by the fiber tract being stimulated. There was a clear difference in the cortex versus the hippocampus in terms of the optimal frequency to suppress seizures. Unlike studies involving LFS in the hippocampal commissure where the effective window of the suppressive effect was found to be between 1 and 10 Hz^{21; 27}, cortical tissue responds optimally to 10–20Hz. Interestingly, several studies investigating the mechanisms of vagal nerve stimulation (VNS) seizure reduction found that stimulation between 10–20Hz suppressed seizures better than any other frequencies^{33–35}. This finding is the basis for the current frequencies utilized in vagal nerve stimulation therapy. The vagus nerve has a number of projections to the cortex that could inhibit cortical tissue by activating simultaneously large areas of the cortex similar to corpus callosum stimulation. Several studies utilizing transcranial magnetic stimulation (TMS) to assess cortical excitability found a significant reduction in cortical activity during vagal nerve

stimulation^{33; 36}. However, unlike VNS, CC low frequency stimulation involves a much more direct activation of cortical structures. Consequently, it is more likely that the underlying mechanism for seizure suppression differs between VNS and CC low frequency stimulation even if the frequency window of therapeutic efficacy is very similar.

Low frequency stimulation has been used to suppress seizures through electrical stimulation^{18–21; 27; 37–46}, repetitive transcranial magnetic stimulation (rTMS)^{47–50}, optically using optogenetics⁵¹, and even with auditory stimulation^{52; 53}. Furthermore LFS has been applied to a number of different brain regions including the hippocampus^{18–21; 27; 38; 51}, cortex^{41; 45}, amygdala³⁹, anterior nucleus of the thalamus^{42; 46}, Nucleus caudatus³⁷, and to a wide variety of patient specific epileptic foci via rTMS⁵⁰. The majority of these studies activate grey matter to achieve seizure suppression. Unlike grey matter stimulation, low frequency stimulation of white matter tracts^{21; 27} has the advantage of activating much larger regions of the brain and therefore can prevent seizures by lowering the excitability over a large area of neural tissue.

The mechanisms of seizure suppression by low frequency stimulation have been studied in the hippocampus through a series of *in-vitro* and *in-vivo* experiments^{27; 51}. In an *in-vitro* slice preparation of bilateral hippocampi connected by an intact ventral hippocampal commissure (VHC), low frequency stimulation was applied to the VHC fiber tract to activate both hippocampi in a 4-AP model of epilepsy. Results suggested that LFS produced a long-lasting-hyperpolarization mediated by the GABA_B inhibitory post-synaptic potential (IPSP) and slow afterhyperpolarization (sAHP)²⁷. In an *in-vivo* study optogenetic stimulation was applied selectively to activate GABA interneurons in the CA3 region of the hippocampus where a focal 4-AP injection was administered to generate a seizure focus. The activation of GABA interneurons entrained CA3 pyramidal cells through a GABA_A mediated mechanism⁵¹. This cell-specific optical LFS significantly reduced seizure frequency similar to what had been reported with electrical low frequency stimulation of the hippocampus.

Corpus callosum stimulation could in principle activate most of the cortex if all of the fibers were to be activated simultaneously. However, a single pair of electrodes positioned within the CC in a direction parallel to the fibers to maximize selectivity could only activate a subset of the CC fibers. The suppressive effect of cortical stimulation was highly specific to the location of the electrodes within the corpus callosum tract. Such a spatially specific effect was not reported in studies of low-frequency fiber tract stimulation for the hippocampus likely due to the fact that the ventral hippocampal commissure innervates most of the hippocampus bilaterally from a small tract. The suppressive effect of low frequency stimulation of the corpus callosum is particularly sensitive to the colocalization of the seizure focus with specific regions of the fiber tract that are activated. This sensitivity to fiber tract selection within the corpus callosum may place some limitations on the translation of this stimulation paradigm into chronic models of epilepsy where axonal remodeling, non-synaptic seizure spread, and the existence of diffuse cortical foci with hard to define axonal projections may diminish the effect of such a targeted form of white matter stimulation.

There is however a clear advantage to the unexpected spatial limitations of the effect of corpus callosum stimulation. For a clinical implementation of this paradigm, the stimulation

electrode could be positioned within the corpus callosum in a location specific to each individual patient's seizure focus location. Unlike current DBS systems that target specific grey matter foci, stimulating specific tracts of the corpus callosum could allow a greater degree of flexibility in determining the appropriate regions of the cortex to stimulate. With a low frequency requirement (10–20Hz), highly selective electrode placement, and a high seizure suppression rate this new stimulation paradigm could provide a significant improvement over currently available brain stimulation paradigms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- Stable spontaneous seizures are induced in a novel acute animal model of focal cortical seizures with local 4-AP injections.
- Stimulation of the corpus callosum suppresses seizures originating in the motor cortex between 10–20Hz with an efficacy of 76% and 95% respectively
- Both cortical and cortically-induced seizures that spread to the hippocampus were inhibited by the same amount
- Corpus callosum stimulation is selective and only inhibits cortical tissue that is specifically innervated by distinct fiber tracts within the corpus callosum

Figure 1: Electrode and MicroSyringe Placement

(A) Horizontal plane view of electrode and microneedle placement. Monopolar stainless steel electrodes placed in the left primary motor cortex (M1), CA3 region of the left hippocampus (Hipp), bilaterally in the medial aspect of the corpus callosum (CC) within an optimal location (anterior), proximal location (slightly posterior to optimal location), and distal location (most posterior to optimal location). A stainless steel screw electrode was placed near the back of the brain (Ref). A microneedle with syringe was inserted contralateral to the cortical recording site (M1 Inj). (B) Sagittal view of the positioning described in (A) with an emphasis on the anatomy inherent to each coronal slice containing either an electrode or microneedle. (*Rat Brain 3D image retraced from*⁵⁴⁻⁵⁶)

Figure 2: Characterization of Acute Focal Cortical Model of Epilepsy

(A) Simultaneous recordings of seizures in the cortex and hippocampus during the baseline.

In the expanded view a seizure beginning in the cortex (red trace) and propagating to the

hippocampus (blue trace). Adjacent to the expanded view of both seizures are the

corresponding spectrograms over the same period of time. (B) Percent of each hour of

baseline where seizures were present in either the motor cortex or the hippocampus (*

$p < 0.0001$, ** $p = 0.0001$, *** $p < 0.0001$, **** $p < 0.0001$, $n = 24$). (C-E) Histograms of seizure

duration over the course of the three hours of baseline recording.

Figure 3: Corpus Callosum Stimulation Frequency Range of Cortical Inhibitory Effect
(A) Electrode placement for each experiment and experimental design for each stimulation procedure where 3 hours of baseline activity was recorded followed by 1 hour of recording during stimulation and 1 hour of recording after stimulation. **(B)** Sample recording from the cortex before stimulation and during 1Hz stimulation **(C)** Sample recording of baseline and during 20Hz stimulation **(D)** Percent time spent seizing in the cortex (* $p < 0.05$, $n = 5$; ** $p < 0.0001$, *** $p < 0.05$, $n = 14$).

Figure 4: Corpus Callosum Stimulation Frequency Effect on Hippocampal Seizures
(A) Simultaneous recording from the cortex (top trace) and hippocampus (bottom trace) during a seizure originating in the cortex that can be seen propagating to the hippocampus
(B) Sample recording of the cortex and hippocampus during 20Hz stimulation (stimulation artifact and evoked potential removed) indicates that both cortical and hippocampal seizures are suppressed **(C)** Histogram of cross-correlation results generated from hippocampal and cortical seizure segments ($n=7$). The delay in individual seizure spikes between the cortex and hippocampus are shown with negative latencies indicating that the cortical spikes are leading. **(D)** Histogram of differences in seizure onset times between the cortex and hippocampus with negative latency time corresponding to a seizure originating in the cortex ($n=7$). **(E)** Percent of each hour spent seizing in the hippocampus (* $p < 0.05$, $n = 5$; ** $p = 0.0003$, *** $p = 0.0014$, $n = 14$).

Figure 5: Spatial Extent of Corpus Callosum Stimulation

Effect of 20 Hz stimulation from 3 different locations along the longitudinal axis of the corpus callosum. (**A**, **C**, and **E**) Evoked potentials from the motor cortex (top traces) and the hippocampus (bottom traces) moving from the most anterior position for stimulation to the most posterior position. (**B**, **D**, and **F**) Percent of each hour spent in seizure in either the cortex or hippocampus (**B**) (* $p < 0.0001$, ** $p = 0.0003$, *** $p < 0.05$, **** $p = 0.0014$, $n = 14$).

Figure 6: Effect of Corpus Callosum Stimulation on a Different Epileptic Focus

Focus generated in the posterior region of the primary motor cortex (M1). **(A)** Electrode and microsyringe placement for the experiment (Left) and an evoked potential from the M1 (top trace) with no evoked activity in the hippocampus (bottom trace) during stimulation. **(B)** Percent time spent in seizure during the hour before stimulation, during stimulation, and the hour after ($*p<0.05$, $**p<0.05$, $n=5$). **(C)** Example of activity recorded from the M1 (top trace) and hippocampus (bottom trace) during the baseline period. **(D)** Example activity recorded from same locations during 20Hz corpus callosum stimulation after artifacts and evoked potentials were removed.