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Focal suppression of epileptiform activity in the hippocampus by a high-frequency magnetic field

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ABSTRACT

Electric current has been used for epilepsy treatment by targeting specific neural circuitries. Despite its success, direct contact between the electrode and tissue could cause side effects including pain, inflammation, and adverse biological reactions. Magnetic stimulation overcomes these limitations by offering advantages over biocompatibility and operational feasibility. However, the underlying neurological mechanisms of its action are largely unknown. In this work, a magnetic generating system was assembled that included a miniature coil. The coil was positioned above the CA3 area of mouse hippocampal slices. Epileptiform activity (EFA) was induced with low Mg^{2+}/high K^{+} perfusion or with 100 μM 4-aminopyridine (4-AP). The miniature coil generated a sizable electric field that suppressed the local EFA in the hippocampus in the low-Mg^{2+}/high-K^{+} model. The inhibition effect was dependent on the frequency and duration of the magnetic stimulus, with high frequency being more effective in suppressing EFA. EFA suppression by the magnetic field was also observed in the 4-AP model, in a frequency and duration - dependent manner. The study provides a platform for further investigation of cellular and molecular mechanisms underlying epilepsy treatment with time varying magnetic fields.

Key Words: magnetic coil; induced electric field; hippocampus; epilepsy; electrophysiology

Introduction

Epilepsy is one of the most prevalent neurological disorders affecting one out of every twenty-six people, with one-third of the patients remaining pharmacologically intractable over their seizures (Kwan and Brodie, 2000, Laxer et al., 2014). Therefore, resection and disconnection surgeries that are irreversible have to be considered, despite their association with neurological deficits such as memory loss, aphasia, and motor and visual impairments (Josephson et al., 2013).

Deep brain stimulation (DBS) has been used to suppress seizure activity in clinical settings by applying electrical stimulation to various parts of the brain, including centromedian (Velasco et al., 2000) and anterior nuclei (Fisher et al., 2010) of the thalamus, cerebellum (Cooper, 1973, Cooper et al., 1973), locus coeruleus (Faber and Vladyka, 1983), and hippocampus (Velasco et al., 2007, Smart et al., 2013, Thomas and Jobst, 2015). This reversible and adjustable alternative to resection has been proved to be successful in alleviating seizures in humans and has been used widely in clinical practices. Although effective, there are several limitations associated with DBS. First, maintaining response consistency over time with implanted electrodes has proved to be challenging. Second, inflammatory and immune reactions of the tissue occur in response to direct contact with the stimulation electrode, with restricted formation of glial scars surrounding the electrode (Polikov et al., 2005, Orlowski et al., 2017). The formation of glial scarring will alter or block the stimulation induced electric fields (Grill et al., 2009, Sillay et al., 2013). Due to the reduced effectiveness, biocompatibility of the implanted electrodes has become the primary concern in device design (Polikov et al., 2005).

An alternative method to generate electric current inside the brain is via electromagnetic induction of magnetic coils. Emerging evidence advocate the use of magnetic coil as an alternative treatment for epilepsy (Ye and Kaszuba, 2019). Recently, miniature-sized magnetic coils have been used to activate selected neuronal subpopulations while avoiding others (Bonmassar et al., 2012, Lee and Fried, 2014). In comparison to implanted electrodes, miniature coils offer advantages in preventing direct contact between the electrode and neural tissue, therefore eliminating numerous problems that may arise at the brain-electrode interface (Polikov et al., 2005, Cogan, 2008, Koivuniemi et al., 2011). These coils may be implanted for focal stimulation by being coated with insulated biocompatible materials. Such practices can increase the target tissue stimulation focality and avoid the adverse effects associated with implants (Saxena et al., 2013, Canales et al., 2015, Lee et al., 2016). However, it is unknown if the miniature coil could be effective in suppressing epileptic activity.

Here, we designed a system that used a miniature coil to generate a sufficiently strong electric field. We recorded the *in vitro* epileptiform activity from the prepared hippocampal slices while applying the miniature coil stimulation to the CA3 area. We found that local EFA can be reliably inhibited by our miniature coils, especially at the higher frequency range of magnetic stimulation.

Experimental Procedures

Animals

All experimental protocols in this study were approved by the animal care committee at Loyola University Chicago in accordance with the policies established by Institutional Animal Care and Use Committee (IACUC). Care was taken to avoid unnecessary pain and suffering of the animals during the study. A total of 30 C57BL/6J mice of either gender (2-3 month of age, Charles River Laboratories, Wilmington, MA) were used in this study. Mice were kept in the Loyola University Chicago Animal Facility under continuous care by facility technicians.

System/coil design

We purchased commercial multilayer surface mount inductors (100nH, MLG1005SR10JTD25, TDK U.S.A. Corporation, Uniondale, NY). Each lead of the coil was soldered with a copper wire (magnetic wire 32-AWG, GC electronics, IL, L3-616). The coil was then covered with silicon glue (DAP All-Purpose 100% Silicone Adhesive Sealant, DAP Products Inc., Baltimore, MD 21224) and allowed to cure in room temperature overnight before use. Silicon glue was chosen because of its very low electric conductivity, high dielectric strength, and excellent biocompatibility after *in vivo* implantation into animals (Ye et al., 2006b, a) .

The coil was positioned on the tip of a glass pipette (TW150F-4, WPS), with the two copper wires running through the inside of the pipette. The pipette was 4 inches long, and the inner diameter was 1.12mm, which secured the two wires inside the pipette. The pipette was then mounted on and secured to a micromanipulation for positioning the coil above the brain slices. The ends of the two wires were attached to the output of a 1000 W power amplifier with a bandwidth of 70 kHz (Pyramid PB 717X 2 channel, Pyramid Car Audio, Brooklyn, NY, 11204). The amplifier was powered by a Triple Channel DC Power Supply (2231A-30-3, Keithley). Voltage pulses (-10 V/10 V) were generated by an arbitrary function generator (AFG1022, Tektronix) and were delivered to the power amplifier (Fig. 1A). Since preliminary experiment with low frequency (less than 5 Hz) did not produce any suppression effects of epileptiform activity, frequency ranging from 20 Hz to 400 Hz were tested. Pulses with width of 1 ms and duration of 10 s were generated.

Longer durations of 20 s and 30 s were also tested whenever needed. The voltage across the coil during pulse train stimulation was measured for the computation of the induced electric field. Electric conductivity of the coil was confirmed by measuring its DC resistance (1.37 Ω). Potential leakage of the current from the coil was checked before and after each experiment. If presented, this current generated extremely large level of noise.

Brain slice preparation

Mice were anaesthetized with isoflurane and decapitated, and the brain was quickly removed and placed in ice-cold (2–5°C) sucrose-based artificial cerebrospinal fluid (ACSF) for ~ 3-5min. Sucrose-based ACSF contained (in mM): 210 sucrose, 26 $NaHCO_3$, 2.5 KCl, 1 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose, and was continuously bubbled with 95% O_2 – 5% CO_2. This high Mg^{2+} - low Na^{2+} - containing ACSF was used only during the tissue preparation to minimize neurotoxicity, mediated by Na^+ and neurotransmitter release (Jahromi et al., 2000). The cerebellum was removed and the brain was bisected along the midsagittal line. The superior cortex was removed and the dorsal cortex was cut parallel to the longitudinal axis. The brain tissue was then glued to an aluminum block with Cyanoacrylate glue with its ventral side up. The block was secured in a vibratome (Series 1000, Technical Products International, St. Louis, MO) so that the caudal end of the brain faced the blade. Slices (400 μm) were cut, collected, and incubated in room temperature ACSF for at least 1h before being transferred to a recording chamber. This normal ACSF contained (in mM): 123 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.8 $CaCl_2$, 0.9 $MgCl_2$, 1.25 NaH_2PO_4, and 10 glucose, and was continuously bubbled with 95% O_2–5% CO_2.

***In vitro* electrophysiology**

After 1 hour incubation, slices were moved to a recording chamber for extracellular field potential recording. Since cell viability has to be guaranteed through the time course of long-time recording, we have improved viability of the slice to more than 6 hours by implementing an *in vitro* slice recording membrane chamber (Hill and Greenfield, 2011) into our recording system. Slices were perfused with normal ACSF at a flow rate of 4-6 ml/min at room temperature (21 ^{0}C). To precipitate EFA, the tissue was perfused with low Mg^{2+}/high K^+ ACSF containing the following (in mM): 123 NaCl, 5 KCl, 1.5 CaCl2, 0.25 MgSO4, 25 NaHCO3, 1.2 NaH2PO4, and 15 glucose. For the 4-AP model of epilepsy, 100 μM 4-aminopyridine was added into the normal ACSF during perfusion.

The mini coil was positioned above the CA3 stratum radiatum, with the winding of the coil parallel to the dendritic-somatic axis of the glutamatergic pyramidal neurons (Fig. 1B). No temperature change was observed in the recording chamber during our experiment. A recording

borosilicate glass electrode, filled with 150 mM NaCl, was placed in the CA3 stratum pyramidale to record EFA. The resistance of the electrode was around 10 MΩ. In some experiments, the recording electrode was positioned in the CA1 area. Responses were filtered, amplified, and recorded with a PClamp 10 amplifier (Axon Instruments, Foster city, CA) and acquisition of data was performed using Clampex 10 (Axon Instruments) at a sampling rate of 25 kHz.

Pharmacology

The blocker of α-amino-3-hydroxy-5-methyl-4-isoxalone/kainite (AMPA/KA) glutamate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), and the blocker of N-methyl-D-aspartate (NMDA) glutamate receptors, D-(-)-2-amino-5-phosphonopentanoic acid (D-APV, 10 μM), were used to block glutamate receptors. All drugs were purchased from Sigma (Sigma, St. Louis, MO).

Data analysis and statistics

To improve the signal-to-noise ratio during magnetic stimulation, the raw recording was filtered by a band pass filter (2.5 Hz – 35 Hz) to eliminate the high frequency noise picked up by the field recording electrode. After filtering, spontaneous spikes were counted with a threshold detecting program in Clampfit (Fig. 1C). To quantify the effects of magnetic stimulation, frequency (number of events/second) of the EFA was calculated (Chiang et al., 2014, Ledri et al., 2014). The spontaneous burst frequencies were compared in a 10 s period immediate before (Before group), during (During group), and after (After group) magnetic stimulation. Data was normalized to the mean frequency of the baseline data in each trial. Data from multiple stimulus trials and multiple slices was collected for each stimulation condition and pooled together for the analysis. Throughout the text, mean ± standard error (SE) was reported. All data was verified for normal distribution and homogeneity of variance by KS normality and Levene test, respectively. Statistical significance was determined with one way repeated measures ANOVA followed by multiple comparisons with Bonferroni test for data with equal variance and normal distribution, and Garnes-Howell test for the data with no equal variance and/or no normal distribution using the Systat software (v. 13.1, Systat Software, Inc. San Jose, CA, USA). Effects were considered statistically significant at $p < 0.05$. We acquired multiple slices from individual animals without within subject averaging in the final statistics (nested design). Ethically, this is a good method to reduce the number of animals in each experiment, however, this kind of data analysis, although commonly used in neuroscience, may reduce the statistical power (Aarts et al., 2014).

Chemical dissolving of the inductor encapsulation

To examine the detailed physical structure of the magnetic coil, the coil was placed into cell culture plate (24 well) and was treated with 1 ml, 40% liquid hydrofluoric acid at room temperature for 48 hours. The acid was removed with a pipette and the coil was then gently washed three-times with deionized water. Thereafter, 10 N HCL was added into the well for 1 hour and the acid was removed. The coil was again gently washed three times with deionized water. The coil was allowed to dry in room temperature (Fig. 2).

Calculation of the induced electric field by the magnetic coil

Magnetic field generated by a magnetic coil with radius *r* was calculated by Faraday's law of induction, which made use of the magnetic flux Φ_B through a hypothetical surface Σ whose boundary was a wire loop (Fig. 3). It stated that the EMF was given by the rate of change of the magnetic flux:

$$\varepsilon = -\frac{d\phi_B}{dt} \quad (1)$$

where ε was the electromotive force (EMF) and Φ_B was the magnetic flux. Direction of the EMF followed left handed rule. It could also be written in an integral form by the Kelvin–Stokes theorem. $\oint \vec{E} \cdot d\vec{l} = -\iint \frac{\partial \vec{B}}{\partial t} \cdot d\vec{A}$ (2)

Where *B* was the magnetic field inside the coil. *E* was the induced electric field. *dl* was an infinitesimal vector element or the path element. *dA* was an infinitesimal vector element of the area considered. If we were to consider the electric field inside the magnetic coil, at a point whose distance was *r* from the coil center, by integrate

$$2\pi rE = -\pi r^2 \frac{\partial B}{\partial t} \quad (3)$$

we obtained the intensity of the induced electric field

$$E = -\frac{r}{2}\frac{\partial B}{\partial t} \quad (r < R) \quad (4)$$

Where R was the radius of the coil.

Outside the coil,

$$2\pi rE = -\pi R^2 \frac{\partial B}{\partial t} \quad (5)$$

The induced electric field was

$$E = -\frac{R^2}{2r}\frac{\partial B}{\partial t} \quad (r > R) \quad (6)$$

Based on the “Ohm’s law” for an inductor, we had

$$v = L\frac{dI}{dt} \quad (7)$$

Where *v* was the instantaneous voltage across the inductor, *L* the inductance in Henrys, and *dI/dt* the instantaneous current changes in A/s. For an inductor (coil) with a flowing current (*I*) inside, the magnetic field was calculated by

$$B = \mu_0 \frac{NI}{l} \quad (8)$$

Where *N* was the loop number and *l* the length of the coil.

By combining (7) and (8), we had

$$\frac{\partial B}{\partial t} = \frac{v\mu_0 N}{Ll} \quad (9)$$

From (4) and (9), we had

$$E = -\frac{r}{2}\frac{v\mu_0 N}{Ll} \quad (r < R) \quad (10)$$

From (6) and (9), we had

$$E = -\frac{R^2}{2r}\frac{v\mu_0 N}{Ll} \quad (r > R) \quad (11)$$

Distribution of the induced electric field generated by the miniature coil was plotted (Fig.4).

Results

Mini coil induced sizable electric field in the hippocampal slices

Under time-varying magnetic stimulation, an electric field is induced by electromagnetic induction through the coil current. Previous study suggests that the intensity of an electric field plays a significant role in controlling neuronal activation and suppressing epileptiform activity in rat hippocampal slices (Gluckman et al., 1996). A 10 V/m threshold for neuronal activation was reported by Chan and Nicholson (Chan and Nicholson, 1986). Fields at 10–20 V/m have been shown to modulate neurone-firing patterns of Purkinje and stellate cells in the isolated turtle cerebellum *in vitro* (Chan and Nicholson, 1986) or the guinea-pig hippocampus (Jefferys, 1981). Electric field suppression of epileptiform activity in rat hippocampal slices has been demonstrated for fields as low as 5–10 V/m (Gluckman et al., 1996).

We calculated the intensity of the electric field induced by the coil and found that the small size of the coil ensured a relatively large electric field to be generated locally around the coil. The output voltage across the coil was measured. We obtained coil parameters (TDK Corporation, MLG 1005 SR10JJD25) from the manufacture's data sheet. Length of the coil: *l* = 1 mm; Inductance: L=100nH; $\mu_0 = 4\pi\, X\, 10^{-7} H/m$; Structure of the inductor was exposed by chemically dissolving the outer layer. It contains 20 rectangular loops (1 mm length X 0.5 mm width) (Fig. 2).

The intensity of the field inside the coil increased linearly with the distance to the center of the coil (Equ.10). Outside the coil, the field intensity decayed as a function of the distance between the targeted tissue and the center of the coil (Equ.11, Fig. 4A). When estimating the field intensity on the epileptic hippocampal tissue, two factors were considered. First, the coated material has an average thickness of about 200 μm. Second, the slicing process likely damaged cells on the top and bottom 50 μm of the brain slices. As a result, these cells were likely not involved in generating epileptiform activity. Therefore, electric field that was 500-800 μm from the center of the coil was controlled the neural activity, which was within a range of 40-70 V/m (Fig. 4B), strong enough to modulate cellular excitability and suppress epileptiform activity.

The miniature coil suppressed EFA in a low-Mg^{2+}/high-K^{+} model and demonstrated frequency and duration dependencies

To elicit EFA, ACSF with low-Mg^{2+}/high-K^{+} concentration was used to continuously perfuse the brain slices. This manipulation induced stable bursting, epileptiform activity for up to several hours, as seen in other studies (Lian et al., 2003, Isaev et al., 2005, Kang et al., 2010).

These EFAs can be recorded in several anatomic locations such as CA3, CA2, and CA1 areas. The EFA could be completely eliminated by the ionotropic glutamate receptor antagonists CNQX (10 μM) and AP5 (50 μM), indicating that they were glutamate-mediated events (data not shown).

We focused on the CA3 area of the hippocampus, a critical region for the initiation of EFA (Dzhala and Staley, 2003, Derchansky et al., 2006, Zhang et al., 2012). Previous studies using direct electric stimulation have indicated that EFA suppression was most effective when electric current flows through the dendritic-somatic axis of the glutamatergic pyramidal neurons (Jefferys, 1981, Gluckman et al., 1996). We therefore positioned the coil in an orientation so that the magnetically-induced electric field can meet this requirement for the CA3 pyramidal neurons (Fig. 1B).

Previous work with electric (Lian et al., 2003) or optical (Chiang et al., 2014) stimulation suggested that the efficacy of *in vitro* EFA suppression was dependent on the stimulus frequency. Therefore, we systematically tested the pulse frequency on EFA inhibition by the miniature magnetic coil using 20 Hz, 50 Hz, 100 Hz, 200 Hz, and 400 Hz (pulse width was 1 ms and duration was 10 s), and received the following results.

20 Hz stimulus (Fig. 5A) caused slight decrease of EFA spiking frequency (to 87.5% ± 9.7% of the base line). However, when EFA frequencies before, during, and after stimulus were compared, one way repeated measures ANOVA revealed that 20 Hz stimulation had no significant effect ($F_{(2,30)}$=1.6, p=0.219). 50 Hz stimulus (Fig. 5B) significantly altered the EFA frequency during stimulation ($F_{(2,15)}$=5.7, p<0.05). The During group showed significantly lower frequency than the Before group (p<0.05), whereas Before and After groups were not significantly different from each other (p>0.05), suggesting a full recovery of network activity post-stimulus. 100 Hz stimulus (Fig. 5C) provided further alteration of the EFA ($F_{(2,12)}$=8.3, p<0.01). Both Before and After groups were different from the During group (p<0.05), whereas Before and After groups were not significantly different from each other (p>0.05), suggesting a full recovery of network activity post-stimulus. 200 Hz stimulus (Fig. 5D) had a significant effect in altering EFA activity during stimulation ($F_{(2,24)}$=38.2, p<0.001). Both the Before and After groups were different from the During group (p<0.001), whereas Before and After groups were not significantly different from each other (p>0.05). However, the EFA frequency in the After group was slightly lower after a 10 s post-stimulus period.

Complete blockage of EFA in CA3 was easily achieved with 400 Hz pulses (1 ms, 10 s duration) (Fig. 5E). One-way repeated measures ANOVA on the three groups (Before, During and After) revealed that 400 Hz magnetic field stimulation had a significant effect ($F_{(2,12)}$ = 242.7, p < 0.001). Each two of these three groups were significantly different (p<0.001). 400 Hz magnetic

stimulus temporally enhanced the EFA during magnetic stimulation. EFA was completely suppressed by the 10 s stimulation, for more than 1 minute (82.1 ± 9.6s; n=5) post-stimulus.

In summary, there was a dynamic EFA change associated with the increase in the stimulus frequency during magnetic stimulation. The magnetic field partially suppressed EFA during stimulation when the field frequency was lower than 200 Hz. Higher frequency magnetic field (>200 Hz) temporally intensified the EFA during stimulation but alleviated or completely suppressed the EFA post-stimulus. To illustrate this pattern change, we analyzed all the data with frequency as a single factor. One-way repeated measures ANOVA revealed that field frequency was a significant factor in altering the EFA during stimulation ($F_{(4,31)}$=43.7, p<0.001) and in suppressing post-stimulus EFA ($F_{(4,31)}$=17.0, p<0.001) (Figs. 5F and 5G).

To investigate the threshold of stimulus duration that could ensure complete EFA suppression, we systematically applied 400 Hz magnetic stimulus while varying the stimulus duration between 1 to 10 s to the brain slice (Fig. 6). The minimal duration for causing complete suppression of post-stimulus EFA was 4s. When stimulation duration was > 4 s, there was a linear relationship between the duration of complete EFA suppression and the duration of stimulation (R^2=0.988, p<0.001).

Prolonged magnetic stimulation can produce extra suppressive effects on the EFA. We tested this possibility for 200 Hz stimulus, a frequency that was incapable of producing a complete EFA suppression, if stimulus duration was short (10 s, Fig. 5D). Indeed, when we doubled or tripled the stimulus duration (to 20 s or 30 s), 200 Hz stimulus completely suppressed EFA post-stimulus (n=3, Fig. 7). With the prolonged stimulation, 200 Hz stimulus was able to eliminate EFA for about 30 second post-stimulus.

Suppression of EFA in CA3 was a localized phenomenon

Intensity of the induced electric field decreased dramatically in the area farther away from the coil (Fig. 4). Therefore, we hypothesized that the inhibitory effect of the coil was limited to the local area (CA3). We recorded EFA from the CA1 area when the CA3 area was stimulated (Fig. 8) with 400 Hz (1 ms width and 10 s duration) pulses and failed to observe EFA suppression in CA1 ($F_{(2,21)}$=0.389. p=0.682). Further increase of stimulation duration in CA3 to 30 seconds was not effective in EFA suppression in CA1 (data not shown). We concluded that the miniature coil only suppressed local EFA.

The miniature coil suppressed EFA in the 4-AP model and also demonstrated frequency and duration dependencies

EFA suppression in CA3 by the miniature coil was also observed in the 4-AP model of epilepsy. Hippocampal slices were perfused with ACSF that contained 100 μM 4-aminopyridine (4-AP), which generated EFA featured as spikes of constant frequency (2-4 spikes/10 seconds) (Chiang et al., 2014, Ledri et al., 2014). The 10 s, 20 Hz magnetic field induced a significant EFA suppression ($F_{(2,15)}$=26.9, $p<0.01$) during stimulation (Fig. 9A). The 50 Hz (n=5) or 200 Hz (n=4) stimulation completely suppressed the EFA in CA3. Stimulation with longer duration caused prolonged EFA suppression. Figs. 9B and 9C illustrate two examples in which 50 Hz or 200 Hz stimulation were delivered to the hippocampal slices. Complete suppression was observed in both short (10 s) and long (30 s) stimulation episodes. Unlike what occurred in the low-Mg^{2+}/high-K^{+} model (in which post-stimulus EFA was inhibited), EFA in the 4-AP model resumed to its normal frequency once the magnetic stimulation was terminated. Figs 9D and 9E summarize the EFA changes under different stimulation frequency. Overall, field frequency was a significant factor for EFA suppression during stimulation ($F_{(2,12)}$=32.4, $p<0.001$).

Discussion

Despite the great potential for magnetic treatment of epilepsy, laboratory research that focuses on the mechanisms of magnetic field stimulation at the molecular, cellular, and network levels are of great value in facilitating this translation. With a mini-coil that delivered magnetic fields, we observed partial or complete focal EFA inhibition in two different *in vitro* models of epilepsy in the hippocampus. The work composes an important step towards in-depth cellular/molecular studies on the mechanisms of magnetic control over abnormal excitability in epilepsy. The miniature coil has a dimension of 1mm × 0.5 mm × 0.5 mm. This small dimension is comparable to the cylinder shaped electrode (1.5 mm in height and 1.27 mm in diameter) in the Medtronic 3387/3389 quadripolar DBS electrode (Medtronic Inc, Minneapolis, MN). Covered by biocompatible material, the miniature coil could potentially be implanted into the epileptic foci as a valuable alternative to the existing electrode-based method used in the treatment of epilepsy.

Both high frequency and low frequency have been used for seizure suppression with electric stimulation

A large range of frequency spectrum has been explored in electric stimulation for seizure suppression, including high frequency stimulation (HFS) and low frequency stimulation (LFS). Deep brain stimulation with high frequency stimulus has targeted gray matter structures. Although the optimal stimulation parameters are undetermined (Saillet et al., 2009), clinical and experimental studies suggest that HFS produces a palliative effect on seizures. For example, 60 Hz stimulation decreased the primary and secondary generalized tonic-clonic seizures in the

thalamic centromedian nucleus in patients with medically intractable epilepsy (Velasco et al., 2000). In patients with non-lesional temporal lobe epilepsy, HFS (130 Hz)stimulation caused a reduction of the interictal discharges and absence of seizures (Boex et al., 2007). Low frequency electrical stimulation (LFS) is also a potential therapeutic method for epilepsy treatment. LFS was applied to reduce seizures in patients with epileptic disorders but refractory to conventional antiepileptic treatment, including temporal lobe epilepsy (Theodore and Fisher, 2004, Yamamoto et al., 2006). It has also been reported that LFS on a fiber track (fornix) reduced epileptiform discharges and seizures in patients with intractable mesial temporal lobe epilepsy (Koubeissi et al., 2013).

Animal models of epilepsy were used to test the parameters of stimulations and reveal neurological mechanisms of stimulation. In pentylenetetrazole (PTZ)-induced seizures, 100 Hz electric pulses were anti-epileptogenic in stimulating thalamic reticular nucleus (Pantoja-Jimenez et al., 2014) and anterior thalamic nuclear complex (AN) (Mirski et al., 1997). In the kindling model of rats, 50 Hz stimulation in thalamic reticular nucleus caused suppression of limbic motor seizures (Nanobashvili et al., 2003). Animal research also showed positive anti-epileptic effects of LFS. In hippocampus - kindling rats, LFS in the hippocampus showed improved long-term spatial learning and memory (Esmaeilpour et al., 2017). In amygdala-kindled rats, LFS combined with sub-threshold dosages of anticonvulsant drug phenobarbital significantly reduced seizures (Asgari et al., 2014). Targeting on the fiber-track, such as corpus callosum, LFS suppressed seizures in a rat model of focal cortical seizures (Couturier and Durand, 2018). It has further been reported that LFS on the hippocampal commissure reduced seizures in a rat model of chronic temporal lobe epilepsy (Rashid et al., 2012).

Brain slices were used to further investigate the cellular/ionic mechanism of electric stimulation on seizure suppression. For HFS, 50 Hz pulses suppressed EFA in several *in vitro* models of epilepsy (low Ca^{2+}, picrotoxin, and high K^{+}) (Lian et al., 2003). Sinusoidal high frequency (20-50 Hz) electric fields across rat hippocampal slices were found to suppress zero-Ca^{2+}, low-Ca^{2+}, picrotoxin, and high-K^{+} epileptiform activity throughout the duration of the stimulus and for up to several minutes following the stimulus. EFA suppression was also found at even higher frequencies (< 500 Hz) (Bikson et al., 2001). For LFS, 0.1 or 1 Hz LFS in the lateral nucleus of the amygdala abolished the ictal-like epileptiform discharges induced by 4-AP in the perirhinal cortex in the rate brain slices preparations. These effects were mediated by $GABA_B$ receptors (Kano et al., 2015). LFS on the commissural fibre tract reduces bilateral hippocampal epileptic activity, due to the stimulation-induced long-lasting hyperpolarizing, which is mediated through $GABA_B$ receptor activity (Toprani and Durand, 2013). The 1Hz LFS on the Schaffer collaterals

prevented high-K^+ - induced neuronal hyper-excitability in the hippocampal neurons (Ghasemi et al., 2018).

Implementation of clinically unattainable high frequency magnetic stimulation

In contrast to the successful seizure suppression with both high frequency and low frequency electric stimulation, epilepsy treatment with magnetic stimulation are mainly using low frequency stimulation.

Starting from the 1990s, magnetic fields have been applied in epilepsy treatment (Anninos et al., 1991, Anninos et al., 1999) and have shown some anticonvulsant effects in epileptic patients (Anninos et al., 1999) (Sandyk and Anninos, 1992). Case reports demonstrated reduction of seizure frequency and/or epileptic discharges after repetitive transcranial magnetic stimulation (rTMS) (Menkes and Gruenthal, 2000) (Brasil-Neto et al., 2004) (Kinoshita et al., 2005) (Liu et al., 2013) (Fregni et al., 2006) (Sun et al., 2012). The rTMS studies use relative low frequency (<1 Hz) (Rossi et al., 2009) (Muller et al., 2014). For example, 0.5 Hz slow-frequency rTMS led to a 70% reduction in the frequency of seizures in a patient with medically refractory partial seizures (Menkes and Gruenthal, 2000). The 0.3 Hz rTMS decreased mean daily number of seizures in patients with intractable epilepsy by 22.8% (Brasil-Neto et al., 2004). These low frequency rTMS protocols also showed their anti-epileptic effects in mice work, with picrotoxin-induced (Kistsen et al., 2016) or bicuculline-induced convulsions (Sung et al., 2003).

Apparently, the frequency band of interests in those clinical applications was much lower than our *in vitro* implementation. This was partially due to the technical difficulties encountered in delivering larger currents to the coil at higher frequencies in rTMS practice (much higher impedance, much higher energy-storage requirement, and much severe cooling issues) and the concern that high frequency stimulation had the tendency of inducing seizures (Rossi et al., 2009). However, anti-epileptic effects of high frequency magnetic stimuli started to appear in some animal studies. Short duration, high frequency (20 Hz) rTMS train has long-term anticonvulsant effects (Ebert and Ziemann, 1999). Animal work also shows that at high frequency, rTMS could decrease epileptic spike frequency acutely (Gersner et al., 2016), which was supported by this study. Further studies are needed to explore the possibility of adapting such high frequency spectrum into future rTMS treatment of epilepsy.

Possible mechanisms of action of high frequency magnetic field on EFA suppression

In this study, we reported that magnetic stimulation at high frequencies can generate reliable focal EFA suppression. In the low-Mg^{2+}/high-K^+ model, EFA was reduced during 20-100 Hz stimulation. For frequency at 200 Hz, the field reduced EFA post-stimulus. Higher frequency

(400 Hz) caused complete suppression of EFA for up to 90 seconds, nine times of the stimulation duration. Longer duration of coil stimulation would enhance the suppressive effects of the magnetic field at both 200 Hz and 400 Hz. For the 4-AP model, magnetic stimulation at 50 Hz or higher was sufficient for complete EFA suppression, even for stimulus with long durations (up to 30 second). The inhibiting effects were local to the stimulation site, due to a quick decay of coil-induced electric field around the coil.

Similarity between the electrode-produced HFS in EFA suppression, and our coil-produced results at high frequency range, leads to the speculation that the neurological mechanisms behind the two stimuli share certain similarity. There are several possible biophysics and neurological mechanisms for EFA suppression by the time-varying magnetic field and the induced secondary electric field. First, the magnetic stimulation, via its induced electric field, could have caused excessive K^+ release, generating the associated depolarization block of epileptic neurons (Bikson et al., 2001, Lian et al., 2003).

Second, magnetic field could alter synaptic transmission via its induced electric field, including long- and short-term modulations. Short-term depression of synaptic transmission was observed during high frequency electric stimulation in the globus pallidus in rats (Rav-Acha et al., 2005) and in primates (Erez et al., 2009). Depression of synaptic transmission by HFS could be due to the fact that HFS-induced release of inhibitory GABA molecules is more prominent than the excitatory neurotransmitter (Feuerstein et al., 2011). Alternatively, it could be due to axonal and/or synaptic failure, which suppress the synaptic transfer of firing rate oscillations, synchrony, and rate-coded information during high frequency stimulation (Rosenbaum et al., 2014). Magnetic field could also modulates long term potential (LTP) induction in the hippocampal area of rats (Komaki et al., 2014, Dong et al., 2018), probably due to a NMDAR-dependent mechanism (Tokay et al., 2009).

Third, neurons affect each other via extracellular electric fields and ephaptic interactions. As extracellular fields and ephaptic interactions respectively play significant roles in recruitment/synchronization of neuronal activity (Mann-Metzer and Yarom, 2000) and nonsynaptic epileptogenesis (Haas and Jefferys, 1984) (Richardson and O'Reilly, 1995) (Bawin et al., 1986) (Jefferys, 1981) (Konnerth et al., 1986) (Snow and Dudek, 1984). It is possible that magnetically-induced electric fields will interrupt this “coupling” and desynchronize the neuronal firing of an epileptic circuit (Ye and Steiger, 2015). This possibility could be tested in the future by using *in vitro* models that are elicited by zero Ca^{2+} (Bikson et al., 1999), which presumably lack

synaptic transmission capabilities but with field coupling still available. Methods established in this brain slice work could lead to in-depth cellular /molecular studies on these mechanisms.

Potential long-term effects of magnetic stimulation

We observed acute inhibition of epileptic form activity by the magnetic stimulation in this *in vitro* study using brain slices. We, however, cannot exclude the possibility that miniature coil stimulation could cause long-term effects in epileptic suppression, as those observed in the clinical cases during DBS treatment of epilepsy (Velasco et al., 2007). Electric stimulation could introduce modulation of neurotrophic factors at the site of stimulation (Ho et al., 2014). It could also introduce genetic modulation locally and in the regions receiving projects from the area undergoing stimulation (Ewing et al., 2013). Similarly, evidence of long-term effects of magnetic stimulation is abundant, including its enhancing effect on adult neural stem and progenitor cell proliferation (Cullen and Young, 2016). Further *in vivo* work, using chronical implantation of miniature coil (covered with biocompatible materials) into the animals, will ultimately reveal this possibility.

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Figure legends

Figure 1. A. Magnetic field generator, which included a signal generator, a power amplifier and a coil held by a glass tube. B. Orientation of the magnetic coil and the hippocampal slice. The coil was positioned in the CA3 area, with the winding of the coil parallel to the dendritic-somatic axis of the CA3 pyramidal cells. Recording electrode was inserted into the CA3 stratum pyramidale. C. Filtering method for the detection of the epileptiform activity in the low Mg^{2+}/high K^{+} model. 1. Epileptiform activity recorded from the CA3 area, which was stimulated for 10 s by a 200 Hz magnetic field. 2. Filtered data by a band pass filter showed an improved signal-noise ratio. 3. Spike detection based on the threshold method.

Figure 2. Size and structure of the magnetic coil. A. Image of a miniature coil used in the study relative to a pencil point. B. The outer cover of the coil was chemically dissolved to reveal the

structure of the solenoid. The coil consists of 20 loops of wire (approximately 0.1 mm in width) that is 1 mm X 0.5 mm rectangular in shape.

Figure 3. Magnetic flux (*B*) generated by the current (*I*) inside a modeled coil with a radius *R*. The induced electric field was *E*.

Figure 4. Intensity of the induced electric field inside and outside of the modeled mini-coil. A. Color map plot for the field intensity and its distribution around the coil (represented by the black ring). B. Relationship between field intensity and the distance from the coil center. Area of the locations of the brain slice was illustrated.

Figure 5. Suppression of epileptiform activity (EFA) by the high-frequency magnetic field in the hippocampus CA3 area in the low Mg^{2+}/high-K^{+} model. A. 20 Hz. B. 50 Hz. C. 100 Hz. D. 200 Hz. E. 400 Hz magnetic stimulation (1ms pulse width, 10 second duration). Red bars represented the time of magnetic stimulation. Blue bars represented the complete suppression of the EFA. F. Frequency-dependency of EFA suppression during magnetic stimulation. G. Frequency-dependency of EFA suppression post-stimulation. Asterisks represented the statistical significance when compared with baseline frequency. *p<0.05; ** p<0.01; *** p<0.001.

Figure 6. Threshold of stimulation duration that ensured complete EFA suppression at 400 Hz. A. Magnetic stimulus with a duration 1s to 10 s was applied, with 1 s as increment. B. Expanded traces for 1s, 7s, and 10 s stimulation. Blue bars represented the complete suppression of the EFA. C. Relationship between the stimulus duration and the duration of the complete EFA inhibition. A minimal of 4 s was needed for 400 Hz stimulation to cause complete suppression post-stimulus. Linear regression showed duration of complete suppression (s) = -78.3 + 18.95 X stimulation duration (s).

Figure 7. Prolonged magnetic stimulation produced extra suppression effects on EFA. A prolonged magnetic stimulation (30 s) at 200 Hz led to complete inhibition post-stimulus, while shorter stimulation (10 s) at the same frequency only provided partial inhibition post-stimulus. Red bars represented the time of magnetic stimulation. Blue bars represented the complete suppression of the EFA.

Figure 8. EFA suppression by the magnetic coil was a local phenomenon and was not observed in the CA1 area. A. Illustration of coil position in CA3, and electrode position in CA1 pyramidal. B.

Raw data for 400Hz, 1 ms, and 10 s stimulation. Red bars represented the time of magnetic stimulation. C. Statistic summary of the changes in the EFA frequency before, during, and after magnetic stimulation.

Figure 9. Suppression of EFA by a high-frequency magnetic field in the hippocampus in the 4-AP model (100 μM). A. 20 Hz magnetic field partially suppressed EFA. B. 50 Hz magnetic field completely suppressed EFA during stimulation. C. 200 Hz magnetic field completely suppressed EFA. Longer (30s) stimulation caused prolonged suppression. Red bars represented the time of magnetic stimulation. D and E. Summary of frequency-dependency of the magnetic suppression on 4-AP induced EFA during stimulation and post-stimulation. Asterisks represent the statistical significance when compared with baseline frequency. *$p<0.05$; ** $p<0.01$; *** $p<0.001$.

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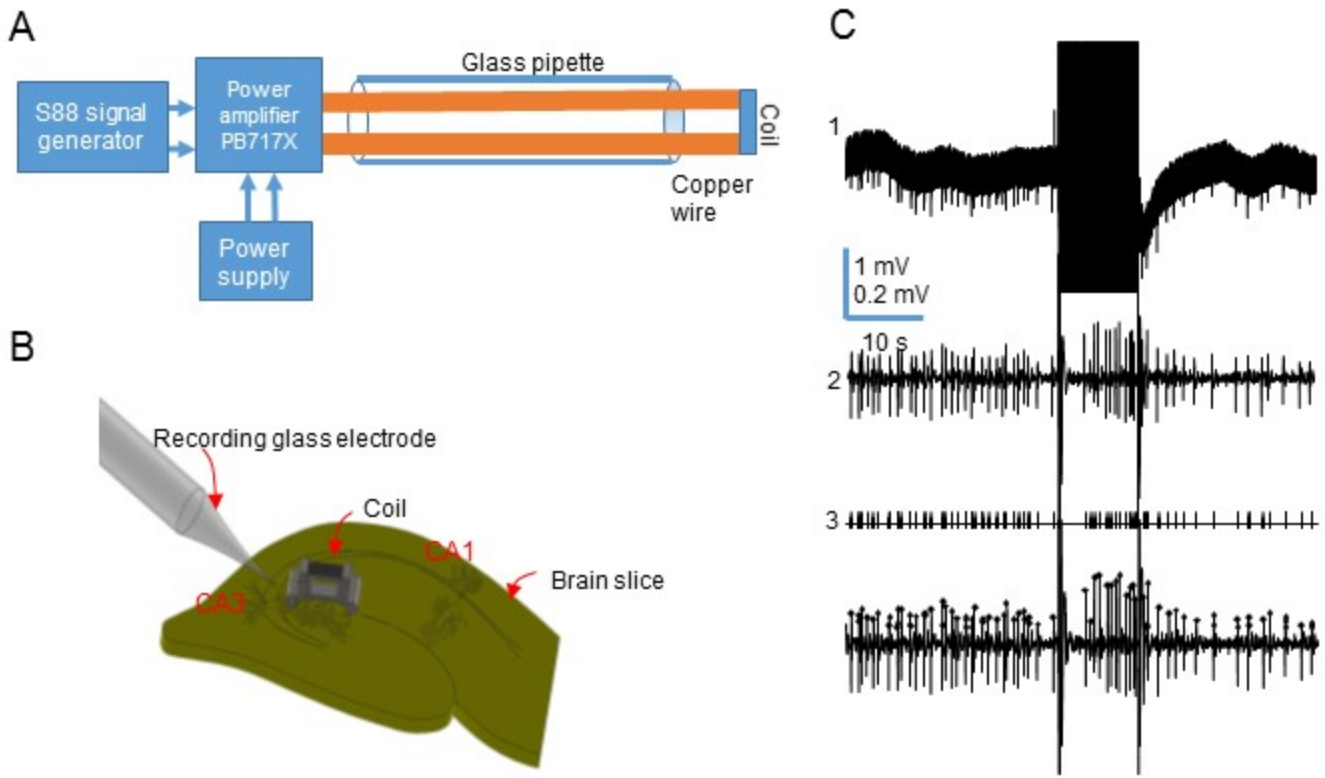
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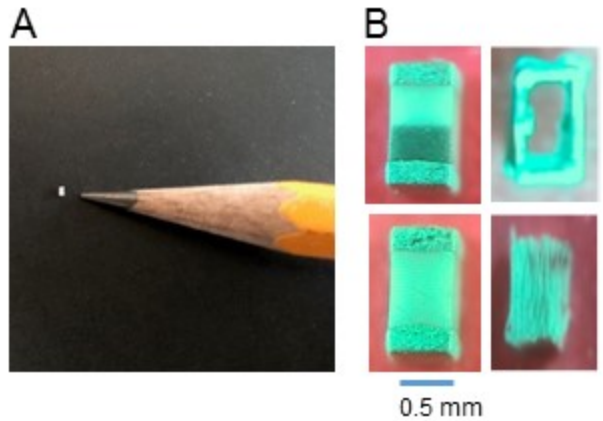
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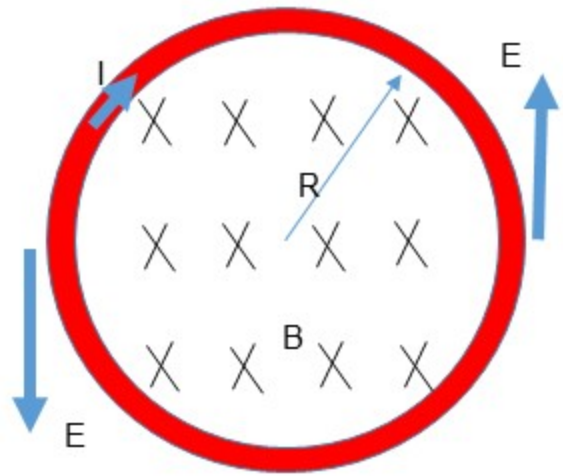
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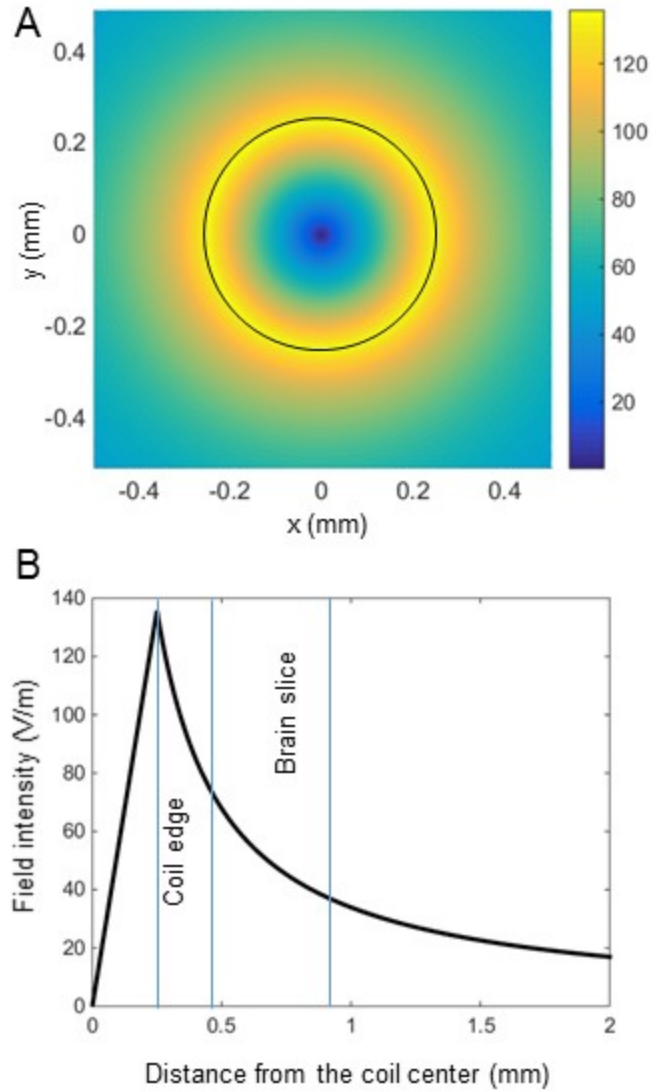
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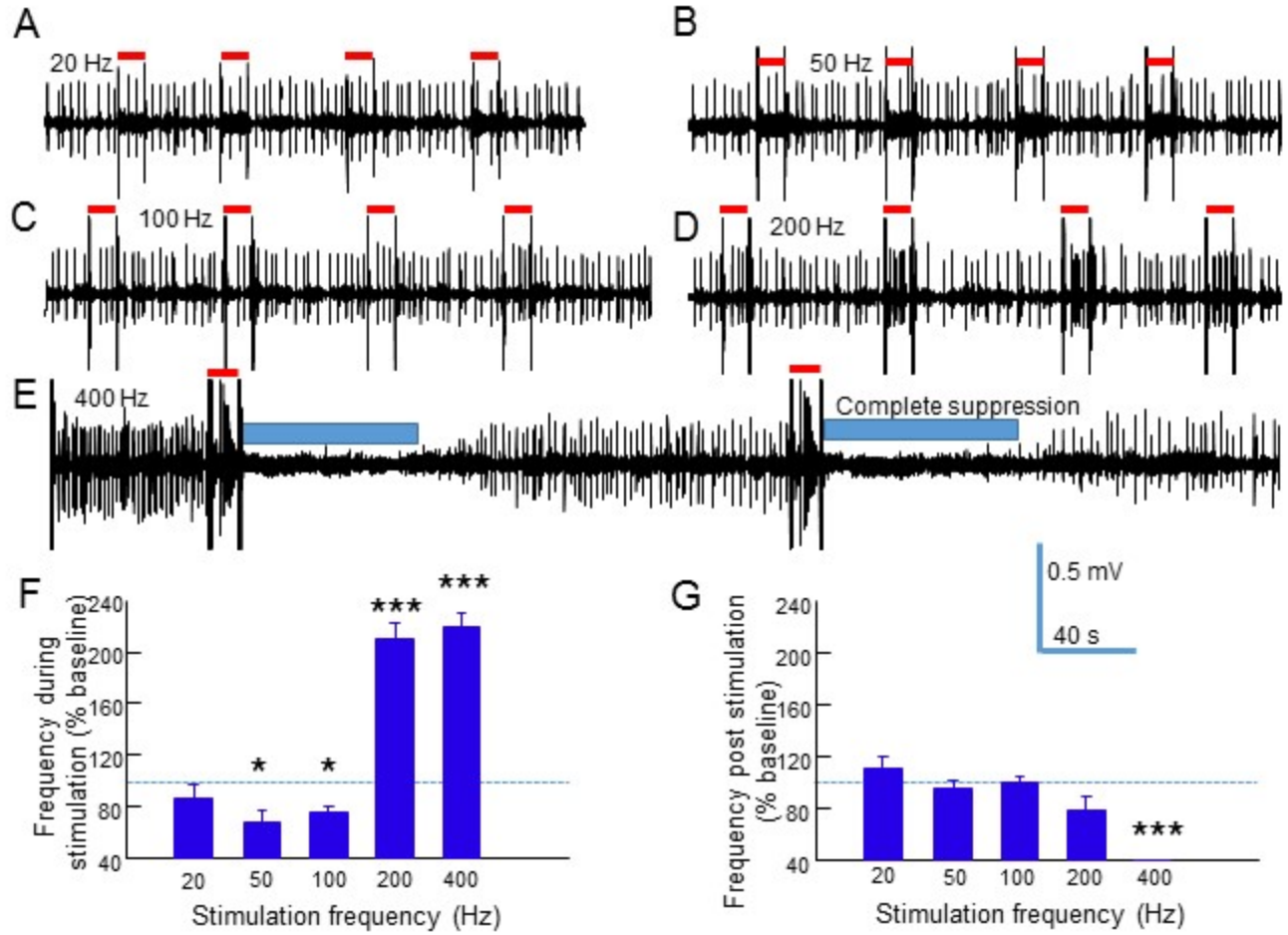
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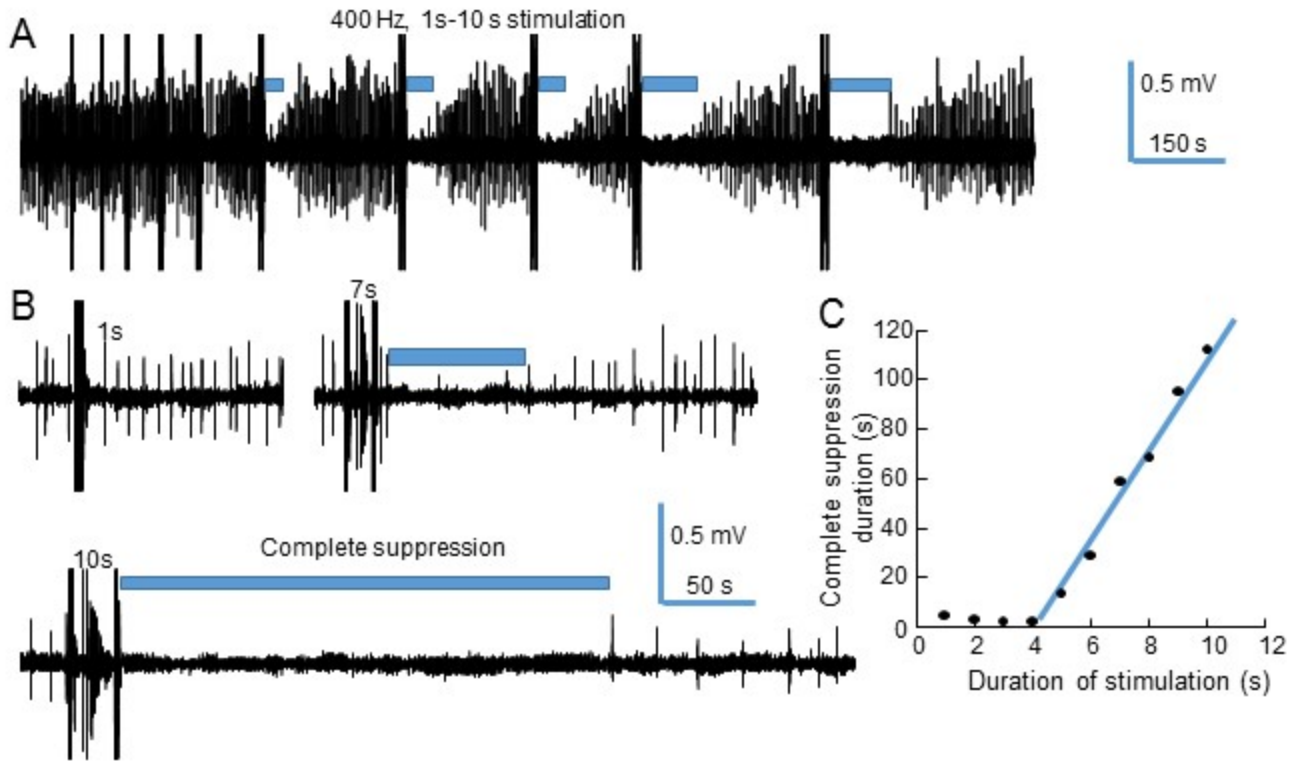
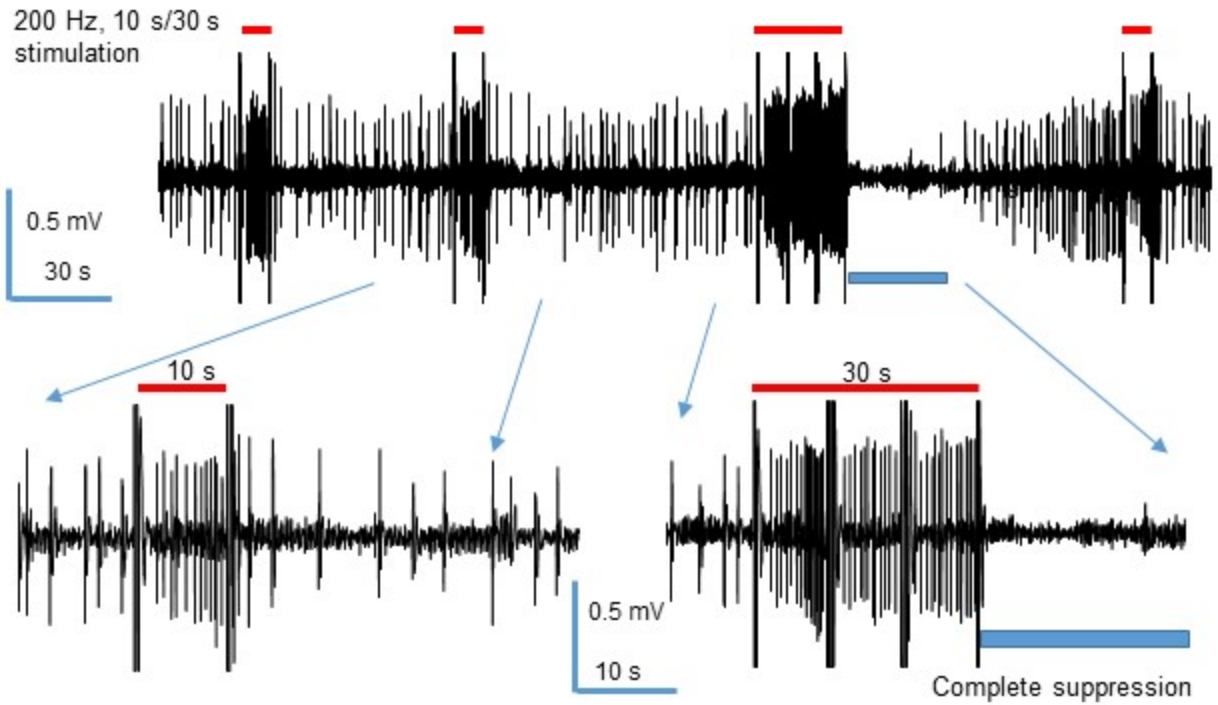
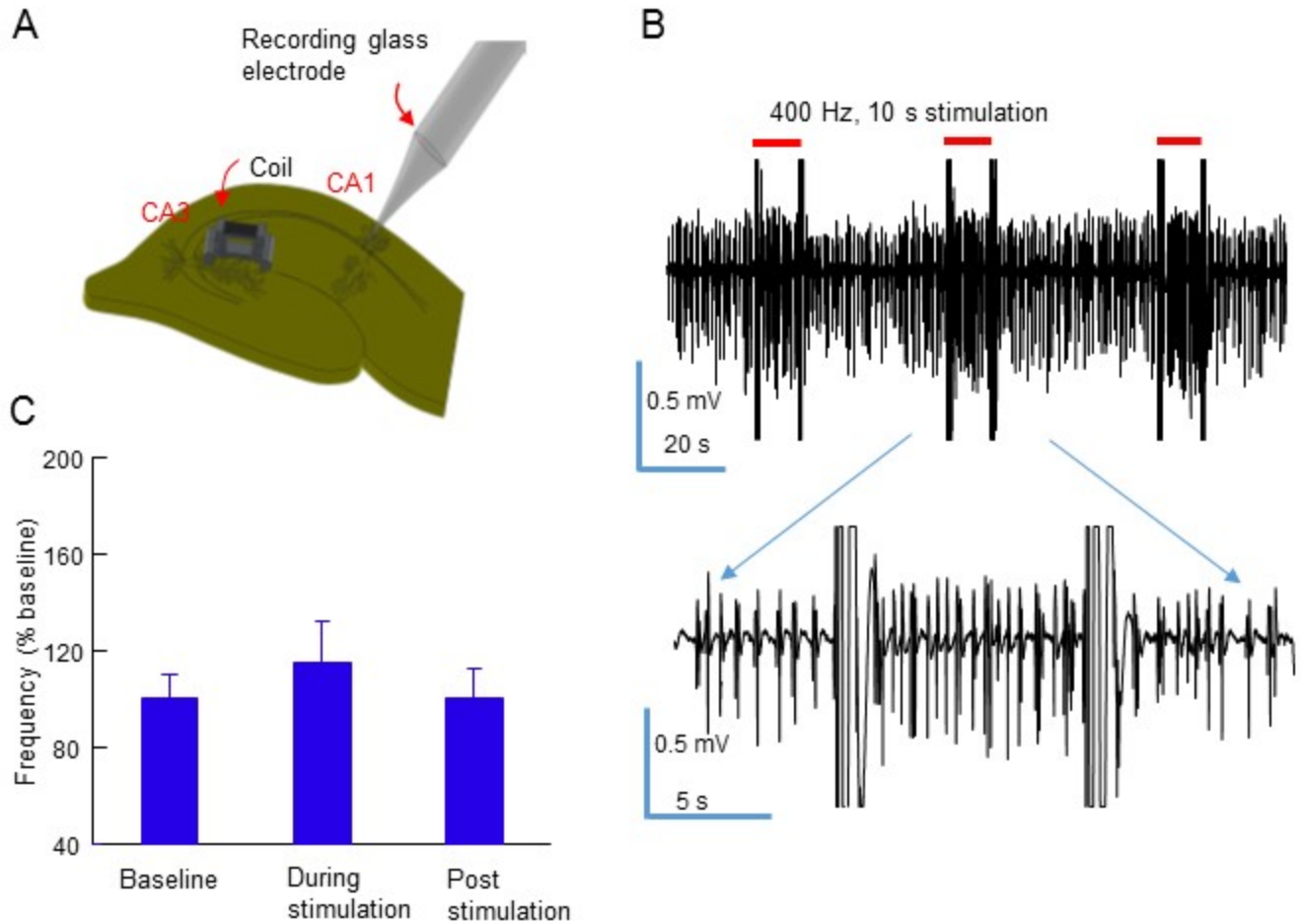
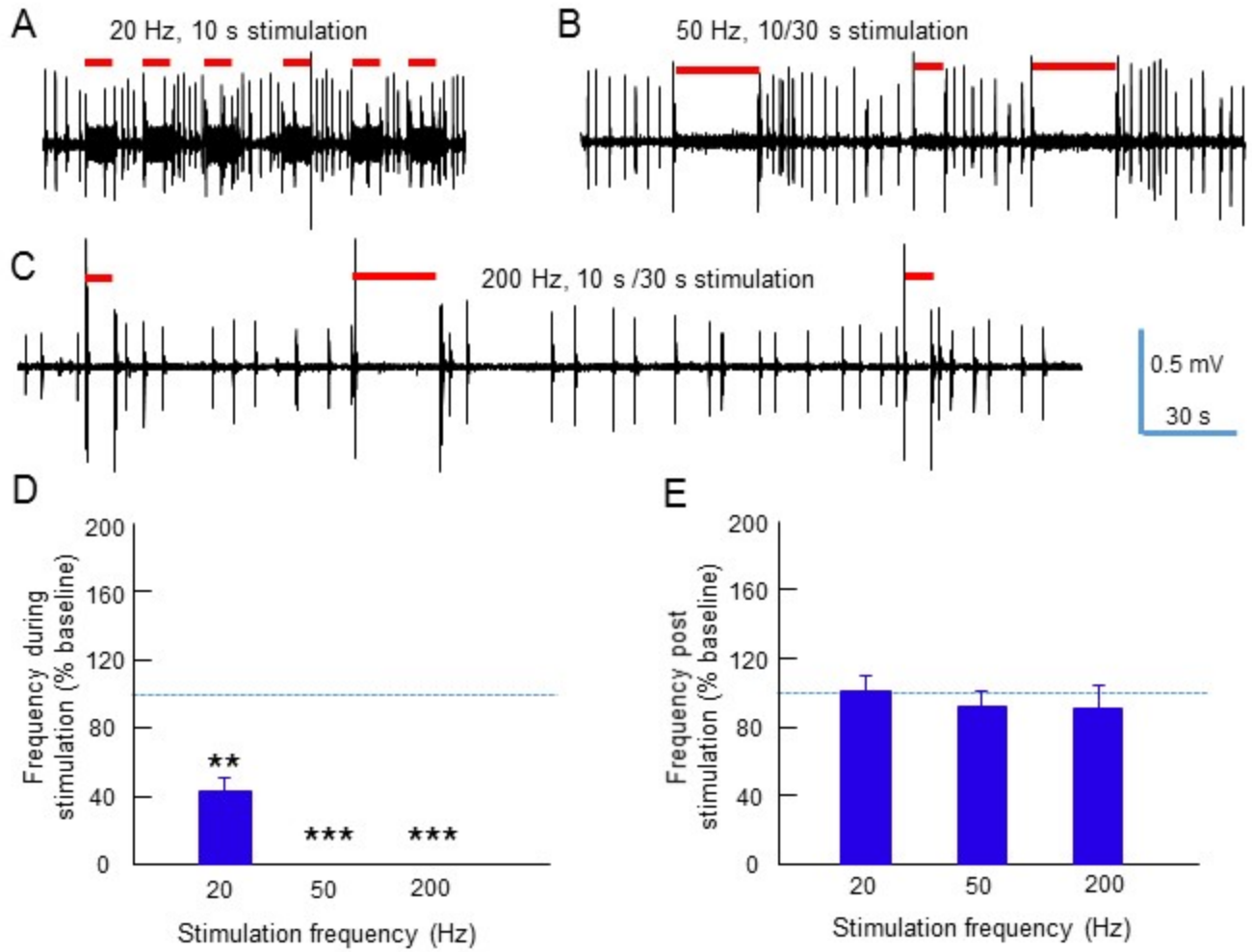
200
400
Stimulation frequency (Hz)
G
Frequency post stimulation (% baseline)
240
200
160
120
80
40
***
20
50
100
200
400
Stimulation frequency (Hz)


A
400 Hz, 1s-10 s stimulation
0.5 mV
150 s
B
1s
7s
10s
Complete suppression
0.5 mV
50 s
C
Complete suppression duration (s)
Duration of stimulation (s)
0
20
40
60
80
100
120
0
2
4
6
8
10
12


200 Hz, 10 s/30 s
stimulation
0.5 mV
30 s
10 s
30 s
0.5 mV
10 s
Complete suppression


A
Recording glass electrode
Coil
CA1
CA2
B
400 Hz, 10 s stimulation
0.5 mV
20 s
0.5 mV
5 s
C
Frequency (% baseline)
200
160
120
80
40
Baseline
During stimulation
Post stimulation


A
20 Hz, 10 s stimulation
B
50 Hz, 10/30 s stimulation
C
200 Hz, 10 s /30 s stimulation
0.5 mV
30 s
D
Frequency during stimulation (% baseline)
200
160
120
80
40
0
**
***
***
20
50
200
Stimulation frequency (Hz)
E
Frequency post stimulation (% baseline)
200
160
120
80
40
0
20
50
200
Stimulation frequency (Hz)