# Comparative Analysis of Seizure Control Efficacy of 5Hz and 20Hz Responsive Deep Brain Stimulation in Rodent Models of Epilepsy

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***Abstract*— We assess the effects of low-frequency (5Hz) responsive stimulation (LFRS) and high-frequency (20Hz) responsive stimulation (HFRS) of the rat hippocampus on the spontaneous seizure suppression in two rodent models of epilepsy. Acute seizures in 12 rats were induced by intra-hippocampal injection of 4-amynopyridine (4-AP) and chronic seizures in six rats were induced by intraperitoneal injection of kainic acid. Two bipolar electrodes were implanted into the CA1 regions of both hippocampi. The electrodes were connected to a custom-built responsive neurostimulator that detects the intracerebral electroencephalographic (icEEE) seizure onset and triggers a responsive electrical stimulation. The rats were randomly divided into two groups: non-stimulation and stimulation group. The non-stimulation group did not receive stimulation, whereas the stimulation group received LFRS and HFRS. The baseline average seizure rate in the non-stimulation group was ~6.5 seizures per 30-minute in the acute model and ~5 seizures per day in the chronic model. The seizure rate in the stimulation group was reduced by 80.8% during the LFRS, while the HFRS reduced seizure frequency only by 26.9% and in the chronic model, 91.6% during the LFRS, while the HFRS reduced seizure frequency only by 15%. The seizure formation was effectively aborted using the LFRS by means of the neural inhibition mechanism, which is similar to that of anti-epileptic drugs. In this responsive stimulation technique, the inhibition lasted only for several seconds, as needed for the seizure suppression, unlike the continuous inhibition (neural activity suppression) in the case of anti-epileptic drugs.**

## I. Introduction

Temporal lobe epilepsy is common and often refractory to antiepileptic drugs. Only 11-25% of patients with temporal lobe epilepsy successfully control their seizures using the pharmacological treatments [1], whereas the rest of patients have either systemic and central nervous system side effects or drug-resistant epilepsy. An alternative treatment option for these patients is surgical intervention [2]. However, many patients have the epileptogenic zone overlying eloquent areas (language, primary motor or visual areas) that cannot be resected without permanent sequelae, while others have multifocal epilepsy [2]. Another relatively new alternative treatment option is neurostimulation which has several advantages for the treatment of refractory epilepsy due to specific targeting of the treatment and adjustment as required [3]. This treatment option avoids many adverse effects that are typically associated with the antiepileptic drugs and also facilitates the reversal procedure by removing the implant, unlikely the surgical option.

Several neurostimulators have been introduced over the last decade for the treatment of refractory epilepsy. Among all, Vagus Nerve Simulator (VNS, Cyberonics, Inc.) and Responsive Neurostimulator (RNS, Neuropace Inc.) have been approved by FDA for the treatment of refractory epilepsy. The VNS system performs open-loop stimulation (OLS) [4] and the RNS system performs closed-loop stimulation (CLS) [5]. A CLS system is advantageous over an OLS system for several reasons [3], [5]. The OLS approach has been applied to various deep brain structures (e.g., subthalamic nucleus, anterior nucleus of the thalamus, cerebellum, caudate nucleus, and hippocampus) [3], and the CLS technique has targeted the epileptogenic zone or adjacent regions [5]. The hippocampus is a common epileptogenic zone in temporal lobe epilepsy [6].

High-frequency and low-frequency deep brain stimulation has shown antiepileptic effect in patients and animal models [3], [5], [7], [8]. The high (130 Hz) and relatively high (20 Hz) frequency stimulation has resulted in shorter seizure latency and propagation, both achieving similar results [9]. Also, seizure shortening and suppression were observed when using low-frequency (0.5 Hz - 5 Hz) stimulation [7], [8]; and the 5 Hz stimulation was more effective in the CLS [8]. Low-frequency stimulation may be advantageous over high-frequency one due to the smaller current requirement and subsequently lower risk of tissue damage.

In this study, we have investigated the seizure suppression effects of 5 Hz and 20 Hz responsive deep brain stimulation in a rat model of chronic seizures. We used a 22 mm × 30 mm responsive neurostimulator on freely moving animals. Intraperitoneal injection of kainic acid was used for inducing spontaneous seizures in rats that mimic human temporal lobe epilepsy. The treatment efficacy using the two stimulation paradigms in the hippocampus was demonstrated comparing the stimulation effects in non-treatment and treatment groups. Following the experiments, the histology results were analyzed to determine the hippocampus damage due to the stimulation. Furthermore, a possible mechanism is discussed to hypothesize the effect of seizure suppression and progression due to the 5Hz and 20Hz responsive neurostimulation.

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## II. METHODS AND MATERIALS

### A. Animals

24 male Wistar rats (275– 400 g) were used in these experiments. Among them, only 18 rats were qualified for this study and the rest were sacrificed or died due to the complications. All the experimental procedures were conducted at the Hospital for Sick Children (Toronto, Canada) and performed according to the protocols approved by the Animal Care and Ethics Committee.

### B. Seizure Models

Two rodent models of human temporal lobe epilepsy were used in this study. These models are described below.

*1*) *Acute model*: 4-amynopyridine (4-AP) was injected intra-hippocampally into 14 rats to induce seizures for a few hours. Following the injection of 6-8μL 4-AP (300 - 500 nmol), 12 of the 14 rats had spontaneous recurrent electrographic seizures for at least two hours, which were used in this study.

*2*) *Chronic model*: Kainic acid (KA) was injected intraperitoneally into ten rats to induce chronic seizures. One to two months after the injection (13 mg/Kg dissolved in saline), recurrent spontaneous seizures developed in six rats, which were used in this study.

### C. Electrode Implantation

All seizure-induced rats were anesthetized with isoflurane and oxygen, and placed in a stereotaxic frame (Stoelting Co., Germany). Two burr holes were drilled in the skull overlying the right and left temporal lobes. One bipolar electrode (chronic model) or one bipolar electrode with canula (acute model) was chronically implanted bilaterally into the right CA1 regions of the hippocampi using a stereotaxic micro-manipulator and similarly another bipolar electrode was implanted into the left CA1, for a total of four recording and four stimulation channels.

### D. Responsive Neurostimulator

The responsive neurostimulator is a custom-built 22 mm × 30 mm PCB carrying two main components: a neuro-interface integrated circuit (chip) and a field-programmable gate array (FPGA) [10]. This neurostimulator interfaces the implanted bipolar electrodes with neural amplifies, filters neural signals, processes the signals in real time, detects a seizure onset, and triggers a programmable electrical stimulation pattern upon a seizure onset detection.

*(i) Amplifier and Stimulator*: A microchip was custom-designed to provide 256 recording and 64 stimulation channels [11]. The chip was wire-bonded onto the PCB and was protected by epoxy [10]. The amplifier in each recording channel has a programmable mid-band gain from 54 dB to 72 dB, programmable bandwidth of 1 Hz to 5 kHz with 7.99 $\mu V_{rms}$ input-referred noise. The stimulation channel has a programmable current from 20 μA to 250 μA.

*(ii) Seizure Detector*: A small, low-power FPGA was as well soldered to the neurostimulator PCB for controlling the neuro-interface chip and performing signal processing. The icEEG recordings were processed in the FPGA and a computer in real time to trigger responsive neurostimulation for suppressing seizures. This seizure detection algorithm was previously introduced in [10]. It has been demonstrated

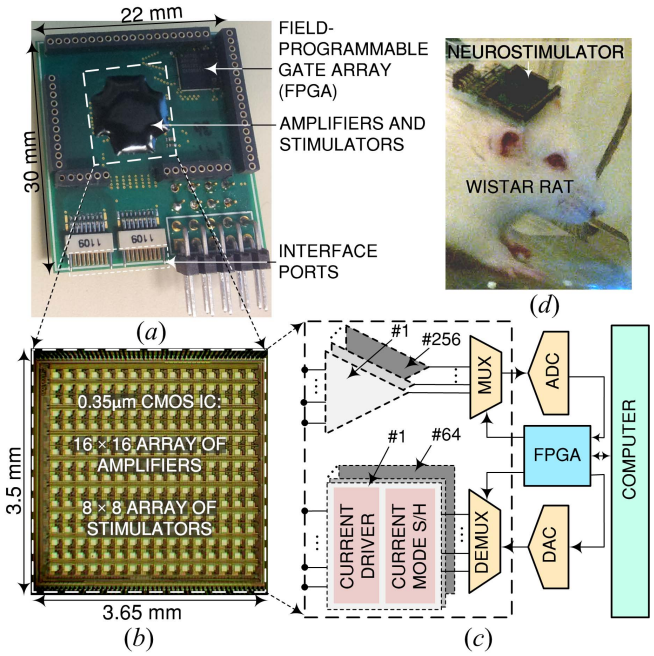



Fig. 1. (a) A custom-made responsive neurostimulator, (b) a neurochip with 265-channel amplifers and 64-channel stimulators, (c) system-level block diagram, and (d) free moving animals with the responsive neurostimulator.

that the change in phase synchrony between two bipolar recordings from both hippocampi is a good precursor of a seizure onset.

### E. Video-EEG Monitoring

Following electrode implantation, the rats were placed in electrically screened Plexiglas chambers. The implanted electrodes were connected to the responsive neurostimulator for icEEG recording and hippocampus stimulation. The icEEG recordings were acquired at 10 kHz using the neurostimulator and the behavior of the animals was also video-recorded simultaneously with the icEEG recording. The two seizure models had different experimental periods.

*1) Acute model*: two hours.

*2*) *Chronic model:* 24 hours a day, 7 days a week for three weeks.

### F. Electrical Stimulation Parameters

The stimulation consisted of bipolar monophasic current pulses (pulse width 100 μs) delivered to the hippocampus for 5 sec. The current amplitude was set to 150 μA due to the safety considerations in order not to damage the tissue. The safety of the current stimulation was estimated using the Shannon model [12], $Q = \sqrt{A \times 10^k}$, where Q is the charge per phase in μC, $A$ is the electrode surface area in $cm^2$, and $k$ is a constant of 1.5. Following this model, and considering the electrode pad area of ~12000 $\mu m^2$, the maximum deliverable charge per phase for the electrodes should be 0.062 μC/phase in order to avoid tissue damage [8], [10]. The chosen stimulation current in these experiments was two times lower than the maximum deliverable charge per phase. The two stimulation frequencies were used to investigate the seizure suppression rate in the epileptic rats:

*(i) Low-frequency responsive stimulation (LFRS)*: 5Hz

*(ii) High-frequency responsive stimulation (HFRS)*: 20Hz

*G. Responsive Stimulation Method*

All implanted rats with seizures were divided into two groups: (1) non-stimulation and (2) stimulation groups. In the non-stimulation group (four in the acute and three in the chronic models), seizures were monitored and labeled by the responsive neurostimulator while stimulator turned OFF; and later, quantified the seizure frequency per half an hour (acute condition) and per day (chronic condition). The stimulation group was treated differently in the following two models.

(i) *Acute model*: The stimulator was turned ON for triggering a stimulation upon a seizure precursor detection. Four rats were stimulated with the LFRS and other four were stimulated with the HFRS.

(ii) *Chronic model*: Seizures of the stimulation group (n = 3) were monitored, labeled and quantified during the first week, and afterwards the stimulator was turned ON to trigger a stimulation upon a seizure precursor detection. At first, the neurostimulator was set to the LFRS for a week and during the following week, the frequency of stimulation was set to the HFRS.

## III. Results

A total of 410 seizures was recorded behaviorally and electrographically from the 14 rats used in the acute (n = 8) and chronic (n = 6) experiments, and all the events were cross-validated using video-icEEG recordings. Fig. 2 shows a representative electrographic seizure (a), and both successful (b) and failed (c) responsive stimulation for the seizure suppression.

*A. No-stimulation group*:

The non-stimulation group members suffered from 13.1 seizures per hour in the acute model and 5.9 seizures per day in the chronic model. A total of 104 seizures (acute) and 308 seizures (chronic) were recorded during the experiment. The neurostimulator monitored the icEEG recordings in real time, and detected 86 acute seizures correctly (with 18 seizures missed) with 0.47 false alarms per hour and detected 282 chronic seizures correctly (with only 26 seizures missed) with 0.67 false alarms per day. The average number of seizures in the non-stimulation group were quantified in 30 minute periods in the acute model (6.5 seizures per 30-minute in Fig. 3(a)) and in one day periods in the chronic model (5.7 seizures per day in Fig. 3(b)).

*B. Stimulation group*:

*1*) *Acute model*: Eight rats received the responsive stimulation as soon as their seizures started.

*i*) *HFRS*: Four rats, that received the 20Hz HFRS right after the detection of the seizure precursor, had a reduced 25.1% seizure frequency (4.8 seizures per 30 minutes).

*ii*) *LFRS*: The other four rats received the 5Hz LFRS right after the seizure precursor detection, and had a reduced 80.8% seizure frequency (1.25 seizures per 30 minutes in Fig. 5(a)) compared to the non-stimulation group.

*2*) *Chronic model*: Three rats in the stimulation group had a similar baseline seizure frequency average as the non-stimulation group, at 4.28 seizures per day in the first week (without feedback stimulation). The rats received the responsive stimulation in the second and third weeks.

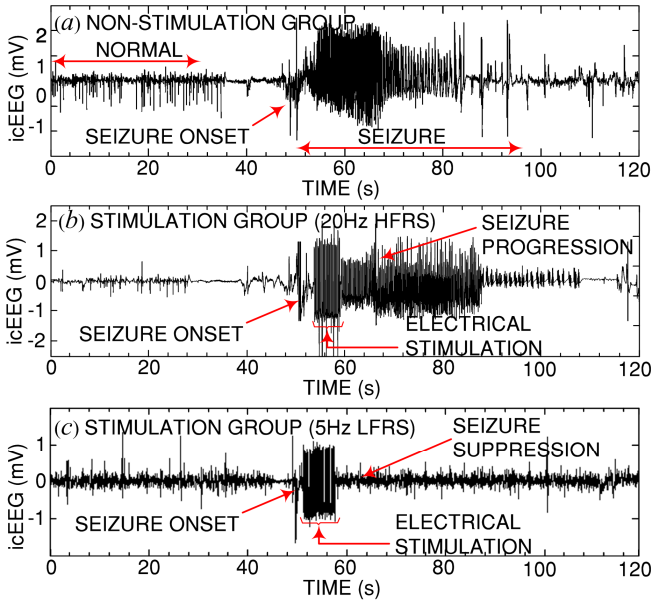


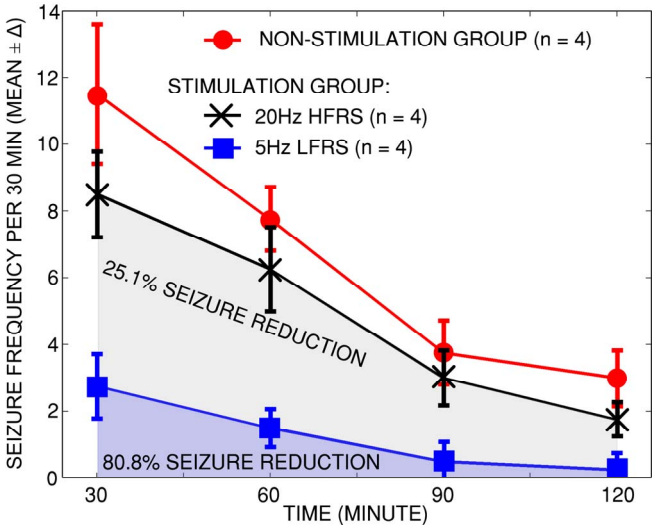

Fig. 2. Effects of 5Hz LFRS and 20Hz HFRS on seizure frequency: (a) an electrographic seizure in the non-treatment group; (b) automatic seizure onset detection, self-triggered 20Hz electrical stimulation for 5s period, but subsequent seizure progression; (c) automatic seizure onset detection, self-triggered 5Hz electrical stimulation for 5s period, and subsequent seizure suppression.

*i*) *LFRS*: In the second week, the rats received a 5 sec burst of 5 Hz upon seizure onset detection. Fig. 2(b) illustrates a seizure onset detection, responsive 5 Hz electrical stimulation and seizure abortion. The seizure frequency in the treatment group dropped down to 1 seizures per day on average (76.6% and 82.4% seizure rate reductions compared to the no-stimulation phase in the stimulation group and the non-stimulation group, respectively), as shown in Fig. 3(b).

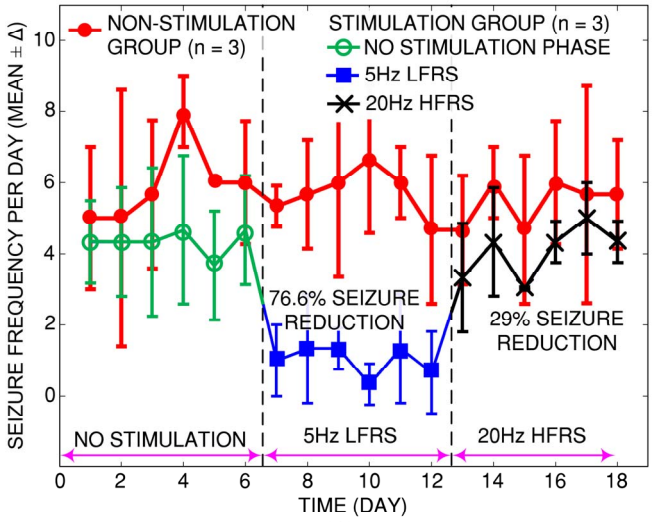
*ii*) *HFRS*: In the third week, the neurostimulator was reconfigured to trigger a 5 sec burst of 20 Hz stimulation upon seizure detection. Fig. 2(c) depicts a failed seizure abortion attempt using the 20 Hz stimulation. The seizure reduction in the third week during 20 Hz stimulation was only by 29%, as shown in Fig. 3(b).

## IV. Discussion

These two stimulation results were further analyzed to explore the possible mechanisms for the seizure suppression during LFRS (5 Hz) and the lack of effectiveness at 20 Hz (HFRS). The icEEG recordings power levels during the normal, seizure onset, seizure, and after 5Hz LFRS and 20Hz HFRS were quantified in different frequency bands (delta, theta, alpha, beta and gamma). Fig. 4 illustrates the power spectrum analysis of the icEEG recordings, and depicts a power divergence in the theta band at the seizure onset (local oscillation) compared to the normal state and a further increase of power levels in the different frequency bands during the seizure. The LFRS at the seizure onset reduced the power divergence seen in the absence of stimuli perhaps because the frequency of pulses used (5 Hz) corresponds to the theta band (well-known for being anti-epileptic [13]). However, the HFRS often yielded more of the initial power divergence at the seizure onset gradually progressing to a seizure episode. Therefore, the LFRS may




(*a*)




(*b*)

Fig. 3. Seizure frequencies in the non-stimulation and stimulation groups: (a) acute and (b) chronic models. The seizure frequency during the 5Hz LFRS period reduced significantly compared to the 20Hz HFRS period and non-stimulation group.

inhibit the focal neural excitation (similar to the anti-epileptic drugs effect [14],[15]) at seizure onset and disrupt the seizure progression, while the HFRS may support and further promote the local oscillations at the seizure onset.

## V. Conclusion

In this paper, we have demonstrated the effects of 5Hz LFRS and 20Hz HFRS on the suppression of spontaneous seizures in two rat models of epilepsy. The seizure frequency was reduced dramatically during the 5Hz LFRS period in both models, compared to the non-treatment group. However, the seizure frequency was reduced little (only 25% in acute model and 29% in chronic model reduction versus the baseline seizure frequency) during the 20Hz HFRS period. The presented results suggest that the neural inhibition at the seizure onset using the 5Hz LFRS could stop the transition to seizure and the neural excitation using the 20Hz HFRS at the seizure onset may have little effect or could even boost the transition to seizure. Thus the 5Hz LFRS of the hippocampus could be an effective technique

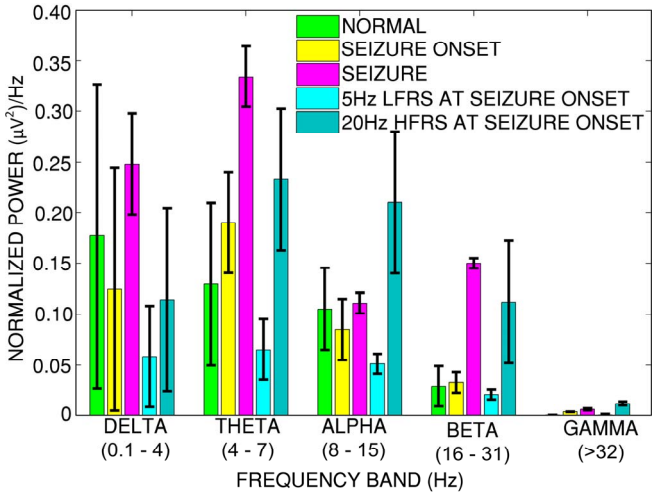



Fig. 4. Mean power spectrum (± standard deviation) analysis during normal, seizure onset, seizure, after 5Hz LFRS and 20Hz HFRS. The power levels in the lower frequency bands diverged from the normal to seizure onset periods, but the power levels augmented in different frequency bands during seizure. From a seizure onset period, a 5Hz LFRS diminished the power in the lower frequency bands and suppressed the seizure, and 20Hz HFRS enhanced the power levels in the theta, alpha and beta frequency bands compared to the seizure onset and developed a seizure.

for developing a new therapeutic implantable device for human patients with temporal lobe epilepsy.

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