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High frequency stimulation can suppress globally seizures induced by 4-AP in the rat hippocampus: An acute *in vivo* study

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Abstract

Background—High frequency stimulation (HFS) on the hippocampus can locally suppress epileptiform activity *in-vitro* and decrease seizure frequency *in vivo*. *In-vitro* HFS on the ventral commissural tract, a novel target, was shown to block the axonal conduction and suppress activity in the CA1 and CA3 neuron.

Objective—To study the spatial extent of seizure suppression by HFS applied on the tract and focus site in an *in vivo* experiment.

Methods—Five adult Sprague–Dawley rats were used for the study. Six electrodes were placed on the septal, middle, and temporal hippocampus bilaterally to simultaneously record seizure activity in the entire hippocampus. Seizure activity was induced by injecting 4-aminopyridine (4-AP) into the right middle part of the hippocampus. Following induction, HFS (100 Hz) was applied to the tract and the focus site at 100, 300 and 500 μ A.

Results—The induced seizure activity was dominated by two patterns, high frequency spiking and pseudo-periodic spikes. Either tract or focus site stimulation could generate suppression of only the pseudo-periodic spikes. The suppression rates were dependent on stimulation amplitude ($P < 0.005$, chi square test). However, HFS also caused conversion of the seizure pattern. The conversion rates increased with higher stimulation amplitudes and were higher with focus site stimulation ($P < 0.01$, Fisher's exact test).

Conclusions—The results of this study have two practical implications [1], both tract and focus site stimulation can produce global suppression of hippocampus and [2] the choice of stimulation parameters is critical in order to produce suppression, but not conversion, of seizure pattern.

Keywords

High frequency electrical stimulation; Hippocampus; Seizure suppression

Introduction

Epilepsy is one of the most common neurological disorders. It is caused by the abnormal discharge of neurons of the brain. The most effective treatments are anti-epileptic drug therapy and surgical resection of the epilepsy focus. However, approximately one third of the patients remain that cannot benefit from either treatment and as a result suffer from recurrent seizures [1]. Therefore, researchers have explored alternative therapies such as electrical stimulation [2–5].

Brain stimulation can be applied either within or outside the seizure focus. Non-focal stimulation assumes that stimulation applied to a critical location can suppress seizures in a wide brain region. Several attempts have concentrated on the thalamus [6,7] and other neuron-rich structures such as cerebellum [8], sub-thalamic nucleus [9], and substantia nigra [10]. Focal stimulation assumes that the electrical stimuli applied directly into the focus can suppress seizures originating at that location [11,12]. The effects of stimulation with various frequencies have also been investigated. The frequency range can be roughly divided into low frequencies (<10 Hz) and high frequencies (greater than 50 Hz) [2]. Low frequency stimulation has been shown to generate a reduction of seizure frequency *in-vitro* [13] and to suppress the expression of amygdala kindled seizures *in vivo* [11]. High frequency stimulation (HFS) used in deep brain stimulation (DBS) has been shown to reduce seizure frequency in clinical trials. From *in-silico* and *in-vitro* studies, it is commonly accepted that suppression of neuronal activity with HFS is generated by depolarization block [14–17] likely due to the sodium channel inactivation [16]. Other lines of evidence have suggested that HFS could suppress and block axonal conduction accompanied with an increase of extracellular potassium [18,19]. HFS applied *in-vitro* showed that HFS caused local suppression and the extent of suppression within hundreds of micrometers around the electrode was dependent on the current amplitude [15]. Another study showed that HFS decreased the excitatory postsynaptic potential of the soma thereby impairing the ability of the cells to generate action potentials in response to depolarizing current [17]. Furthermore, two *in vivo* studies using the kindling model of epilepsy showed that 130 Hz stimulation reduced tissue excitability and modified epileptogenesis of the tissue [12,20].

Most experimental designs apply stimulation directly to the neural tissue where the focus is likely to be located. Another stimulation strategy is to focus on axonal tracts since a single tract can activate a large number of regional neurons in the brain. Low frequency stimulation applied to the ventral hippocampal commissural tract has been shown to reduce seizure frequency in mice with a sodium channel mutation and in a chronic model of epilepsy in rats [21,22]. *In-vitro* analysis has shown that HFS of the same tract could block axonal conduction and suppress the activity in the CA3 and CA1 neurons connected to these tracts [18,19]. Tract activation could affect a major portion of the spatial extent of the hippocampus bilaterally thereby preventing seizure generation and propagation simultaneously. This concept was shown to be effective for low frequency [21,22] but not for high frequency stimulation. Moreover, suppression of seizure activity by tract stimulation has not been directly compared to focal stimulation in a controlled rat experiment. Since HFS for seizure suppression is currently applied in clinical trials, an analysis of tract versus focal stimulation could provide new information on the mechanism of seizure suppression.

The main purpose of this study was to investigate the effect of HFS on seizure activity in the hippocampus *in vivo* and to compare the results of applying HFS to fiber tracts versus lesion site. Focal epileptiform activity was induced by 4-AP in an acute *in vivo* rat preparation and activity was monitored throughout the hippocampus bilaterally. The spatial extent of the

seizures and the effects of HFS in the fiber tract and the injection focus (lesion site) were both investigated and compared.

Methods

Experimental set-up and induction of seizure

Five adults Sprague–Dawley rats (260–350 g) were used for the study. The animals were first anesthetized by urethane (1.5 g/kg, i.p.) and then placed on the stereotaxic frame. The experimental protocol was reviewed and approved by the Institution Animal Care and Use Committee at CWRU. Neural activity from the hippocampus was obtained from six 280 μ m stainless wire electrodes (E363/1, Plastics One Inc.) placed into the septal, middle and temporal parts of the hippocampus bilaterally (Fig. 1). The coordinates of the electrodes were obtained from an atlas of the rat's brain [23]. All electrodes were placed on the CA1 of the hippocampus and their coordinates were (AP - 2.5, ML $\pm$ 2.0, DV 3.0), (AP - 4.0, ML $\pm$ 3.0, DV 2.8), and (AP - 5.5, ML $\pm$ 4.0, DV 3.0) respectively. Two stimulation electrodes were placed on the ventral commissural tract (AP - 1.5, ML -0.5, DV 4.5) and the focus site, where 4-AP was injected (AP - 4.0, ML 3.0, DV 3.5), respectively (Fig. 1). Two screw electrodes were fixed on the parietal and frontal as the reference and ground electrodes. The location of the recording electrodes were adjusted by delivering two pulse currents (2 mA, 40 Hz, 100 μ s pulse width) to the ventral commissural tracts and inducing orthodromic evoked potential in the CA1 of the corresponding six areas [24]. The rat was allowed to recover 30 min and neural activity is acquired for about 10 min as baseline recordings. The signals were amplified 100 times by two 4-channel amplifiers (Model 1700, A-M System Inc.), with the hardware pass band in 1~5 kHz. Signals were acquired by the ML795 PowerLab/16SP data acquisition system (AD Instruments Inc.) and stored in the computer for the offline analysis. The sampling rate was 20 kHz.

Seizures were induced by injecting 4-AP [25–27]. A concentration of 40 mM with volume of 0.5 μ L was chosen because it induced intermittent seizures instead of status epilepticus and the period of interictal activity was shorter than that induced by 20 mM concentration of 4-AP. Following the injection and onset of seizures, the syringe was removed and another stimulation electrode for stimulating the injection site was inserted into the same location. Seizures started within 30 min in all experiments. Neural signals were acquired before and following the injection. Stimulation was applied only when seizures had propagated throughout the whole hippocampus.

Stimulation parameters

The ability of electrical stimulation to suppress seizure activity induced by 4-AP was tested by stimulating the two sites described above (tract and focus site). Based on previous *in-vitro* studies [15,18,19], stimulation parameters were chosen as follows: frequency 100 Hz, amplitude 100, 300 and 500 μ A, with a pulse width of 100 μ s, and duration of 0.5 s. The range of amplitudes was selected based on the preliminary observation that 100 μ A was close to the lower threshold of amplitudes needed for suppression and that amplitudes exceeding 500 μ A modified the epileptiform activity.

Removal of stimulation artifacts

A signal processing algorithm was developed to remove the stimulation artifacts. A template of the stimulation artifact was first generated and the raw data were subtracted from the template at each artifact period. It was assumed that the stimulation artifact was deterministic and the signal was random relative to the stimulation artifacts. Therefore, the shape of stimulation artifact could be obtained from phase-locked ensemble average and the artifacts were removed by subtracting the template from the raw data. To construct the

template, the artifacts were collected from the raw data, aligned by the time of the peak, and ensemble averaged. The baseline shift in the template was removed by shifting the first data point of the template to zero. The template was aligned to all the peaks of raw data and subtracted.

The artifact removal algorithm was first tested with an experiment performed in a saline bath (Fig. 2A). Recording electrodes were positioned in the center of the 9 cm Petri dish. A 10 Hz sinusoid signal (alpha band) and 10 MHz band limited white noise were generated independently by two waveform generators and applied to the saline bath through the stimulating electrodes placed on the circumference of the Petri dish. Stimulation pulses (100 Hz) were applied from another pair of electrodes 4.5 cm away to generate artifacts and vertical to the line linking the recording electrodes. Therefore, all signals could be recorded through the recording electrodes.

Statistical analysis

Since the results show that HFS generates an all-or-none complete suppression, the outcome of HFS could be treated as categorical data with binomial distribution. To obtain the relationship among suppression rate, stimulation strength, and sites of stimulation, Chi square independency test was applied for dependency test and Fisher's exact test ($P = 0.05$) was applied for statistical comparison.

Results

Validation of removal of stimulation artifact

The artifact removal algorithm was first validated and the results are shown in Fig. 2. The artifact was 1 mV and the signal to noise ratio was 0.1 (Fig. 2B). In the time domain, the stimulation artifact was clearly removed (Fig. 2C). Spectral analysis of the signals showed that the power of stimulation artifacts (100 Hz and its harmonic frequencies) was reduced. The mean peak power and its standard deviation was reduced from -37.24 ± 0.18 dB to -63.11 ± 4.39 dB at 100 Hz and from -34.74 ± 0.13 dB to -63.58 ± 4.01 dB at 200 Hz for 30 trials ($P < 0.005$ by paired t test). The power of other frequency bands and even at 10 Hz was not affected.

Two alternating seizure patterns in 4-AP induced seizures

4-AP was injected unilaterally into the right CA3 of the middle region of the hippocampus. However, seizure activity could be observed and was often synchronized in the whole hippocampus. In four out of the five animals, seizures first occurred at the injection site, spread to the whole hippocampus and then became synchronized. In the one other animal, the seizures appeared almost simultaneously in all six regions. The progression and the propagation of the seizure activity are shown in Fig. 3A–D. Spikes appeared initially in the right side of the hippocampus (Fig. 3A). Eleven minutes following the 4-AP injection (Fig. 3B), the spikes appeared in the right middle hippocampus and spread to the entire hippocampus (Fig. 3C). Finally, seizure events appeared at the same time and were synchronized in the six regions (Fig. 3D). Synchronization was quantified in one of five rats by estimating the latency of spikes. Ten spikes from each area were randomly chosen and the peak times were used for comparison. When the right middle site (focus) was used as the baseline, the latency in the left septal, middle, temporal, right septal and temporal were 1.5 ± 8.5 , 2.9 ± 2.3 , 3.8 ± 3.1 , 6.9 ± 4.6 , and 0.8 ± 3.1 ms, respectively.

Two main patterns of waveforms of 4-AP induced seizures were observed: high frequency spiking and low frequency pseudo-periodic spikes (PPSs). An example of high frequency spiking is shown in Fig. 4B. The frequency content of this pattern was in the range of 10–30

Hz as determined by spectral analysis. The other pattern was a sequence of PPSs shown in Fig. 4C. The frequency of spike repetition was less than 10 Hz. A seizure episode always started with high frequency spiking and was followed by PPSs (Fig. 4A). The PPSs would sometimes reverse back to the high frequency spiking, or would attenuate in amplitude and decrease in frequency until the seizure stopped. The high frequency spiking was always followed by the PPSs but sometimes the spikes could appear independently. In the experiments on the 5 animals, the number of high frequency spikes and PPSs were 21 ± 5.4 and 24 ± 6.6 times, respectively, during a mean recording time of 68.8 ± 29.5 min.

HFS could suppress seizure activity

HFS (100 Hz) was applied to the commissural tract to determine the effect of tract stimulation on the seizure activity. The stimulation was applied to both patterns of seizure activity in the preliminary experiments. The results show that HFS was unable to affect significantly the high frequency spiking activity, but could suppress the PPSs (Fig. 4D,E). The stimulation artifacts of Fig. 4D,E were eliminated by the removal algorithm. Fig. 4E shows a successful case of complete suppression of PPSs (500 μ A) during the stimulation and for a period of time following the stimulation and then spikes gradually reappeared. Hence, to quantify the outcome of stimulation, the suppression rate (p) was defined as the ratio of the number of seizures blocked to the total number (N) of stimulation in the experiment involving the 5 rats and was represented as percentage (mean $\pm$ standard deviation %). The mean (μ) and standard deviation (sd) defined in (Equation (1)) and (Equation (2)) were based on the following binominal distribution:

$$\mu = 1 * p + 0 * (1 - p) = p \times 100\% \quad (1)$$

$$sd = \sqrt{\frac{p(1-p)}{N}} \times 100\% \quad (2)$$

Experiments were designed to generate information about the amount of suppression as well as the spatial extent of the suppression of the PPSs by tract or focus site stimulation. The responses were recorded in the bilateral septal (LS, RS), middle (LM, RM), and temporal (LT, RT) hippocampus. Fig. 5A,B shows two typical examples of spatial effect of the stimulation. In Fig. 5A, HFS with 500 μ A was applied on the tract and suppression occurred in all six regions of the hippocampus. This effect is defined as global region suppression. But sometimes, suppression did not occur in some of the six regions. In Fig. 5B, HFS with 500 μ A was also applied to the tract but suppression did not occur in the right middle and temporal (RM and RT) regions. This phenomenon is defined as partial region suppression. Therefore, stimulation generated various suppression rates in the six regions. The total numbers of trials of HFS were different due to the variable numbers of available PPS periods. The total number of trials of tract stimulation with 100, 300 and 500 μ A was 16, 24 and 29, respectively. The total number of trials of focus site stimulation with 100, 300 and 500 μ A was 14, 26 and 25, respectively. The suppression rates in the six regions were calculated by Equation (2) as shown in Fig. 5C.

When stimulation was applied to the commissural tract, the suppression rates at the six regions depended strongly on the stimulation amplitude ($P < 0.005$, $\chi^2 = 20.1$, chi square test). The suppression rates with stimulation amplitude of 500 μ A was higher than those of 300 and 100 μ A ($P < 0.01$, Fisher's exact test), but there was no significant difference between moderate and lower stimulation amplitudes (300 and 100 μ A). With stimulation amplitude of 500 μ A, the suppression rates reached 100% in all three left regions. However,

on the right side, the rates were only 65.5, 51.7, and 69.0% for septal, middle and temporal regions, respectively (Fig. 5C).

Stimulation applied to the focus site also showed an amplitude-dependent suppression ($P < 0.005$, $\chi^2 = 28.8$, chi square test). Focus site stimulation generated a higher suppression rate than the tract stimulation especially in the left side where suppression rate reached 100% at 300 and 500 μA (Fig. 5C). In the right septal region, the stimulation amplitude of 300 μA had a higher suppression rate than that of 100 μA ($P < 0.05$, Fisher's exact test). In the right temporal region, the suppression rates of 300 and 500 μA were higher than that of 100 μA ($P < 0.05$, Fisher's exact test).

The suppression rates generated by the two stimulation sites are compared in Fig. 5C. It is easier to suppress seizures by focus site stimulation than by tract stimulation. Focus site stimulation with 100 μA had higher suppression rates than tract stimulation only in the left regions of the hippocampus ($P < 0.05$, Fisher's exact test). By increasing the stimulation amplitude to 300 μA , focus site stimulation produced higher suppression rates in all six regions ($P < 0.01$, Fisher's exact test).

Taken together, these results indicate that both tract and focus site stimulation by HFS could suppress seizure activity in both sides of the brain in an amplitude-dependant manner and focus site stimulation was somewhat more effective than tract stimulation.

Spatial extent of the suppression by tract or focus comparison

To quantify the spatial effects of the HFS on either the tract or the focus site, the cumulative suppression rate of total trials in six regions were pooled and plotted in Fig. 6. Originally, the suppression rate should be identical in six regions since global suppression occurred and caused suppression in six regions. However, the different suppression rates in six regions shown in Fig. 6 were due to the partial region suppression which stimulation made suppression only in some of six regions. Therefore, the minimal suppression rate among six regions could indicate the global suppression rate. Global suppression was observed with both tract and focus site stimulation except for tract stimulation with 100 μA . Tract stimulation with 500 μA could generate 52% global suppression. Similarly, tract stimulation with 300 μA could generate 21% global suppression but there was no global suppression when stimulation amplitude was 100 μA . Focus site stimulation with 500, 300, 100 μA could generate 60, 69, and 50% suppression respectively.

Fig. 6 also shows the effects of partial region suppression. The partial region suppression caused the suppression rates in the six regions to be different. The suppression occurred only in some regions so that these regions would have higher suppression rates. Therefore, the partial region suppression rate could be treated as the difference between the global suppression rate and the maximal suppression rate among six regions. Tract stimulation with 500, 300, and 100 μA generated 48, 38, and 44% partial region suppression in all stimulation trials, respectively. Focus site stimulation with 500 and 300 μA generated 40 and 31% partial region suppression. Partial region suppression was most likely to occur in the left side, suggesting that the suppression was not localized to the stimulation site but instead involved the contralateral hippocampus.

Conversion of seizure pattern by HFS

In addition to suppression, HFS could also convert the PPSs into high frequency spiking during and following stimulation. Fig. 7A shows an example of such conversion of the seizure pattern due to HFS. PPSs were suppressed for a period of time during and following the stimulation but the high frequency spiking appeared instead of PPSs after stimulation.

In order to quantify this phenomenon, the rate of conversion was estimated and defined as the ratio of the number of stimulation inducing pattern conversion to the total number of stimulation trials. Fig. 7B summarizes the rates of seizure pattern conversion by the tract and focus site stimulation in the six regions of the hippocampus. When the stimulation was applied to the tract, the pattern conversion appeared only with the amplitude set at 500 μ A. In general, focus site stimulation generated more pattern conversions than tract stimulation ($P < 0.01$, Fisher's exact test). The rates of conversion increased with an increase of the stimulation amplitude with the focus site stimulation. In the left brain regions, the stimulation amplitude of 500 μ A induced significantly more conversions than that of 300 and 100 μ A ($P < 0.05$, Fisher's exact test). In the right brain regions, stimulation with 100 μ A induced lower conversion rate than that of 500 μ A, but only the temporal part reached the significance ($P < 0.05$, Fisher's exact test).

Discussion

The experiments for this study were carried out using 4-AP as a model of epilepsy. 4-aminopyridine (4-AP) is mainly a potassium (K^+) channel blocker, although it can also affect calcium (Ca^{2+}) current [28]. Studies [29,30] have shown that the gene family encodes many highly homologous K^+ channel forming proteins with subtle differences in properties. Potassium current could be broadly subdivided into slow inactivating delayed rectifiers (D-type) and rapidly inactivating A-type current [31]. In the CA1 and CA3 pyramidal cells of the hippocampus, the main potassium currents include slow delayed rectifier contributed by Kv1.1, 1.2, 1.3, and 1.4 D-type subunits, fast delayed rectifier attributable to Kv 3 subunits, and A-type current mediated by Kv4 channels [32–37]. 4-AP can block delayed rectifiers with a low concentration of 40 μ M and block A-type currents with a high concentration of 1.5 mM [38]. Other evidence suggests that 4-AP can enhance the release of GABA from presynaptic terminal and increase NMDA current in rat hippocampal slices [39]. Therefore, 4-AP with a concentration of 50 μ M has been used to generate spontaneous epileptiform bursts characteristic by interictal and ictal-like discharges *in-vitro* [40,41]. Moreover, 4-AP with high concentration (20–100 mM) has been used in many studies to generate epilepsy (status epilepticus) *in vivo* [25–27] and in several studies of the effect of stimulation for seizure control [42,43].

Previous *in-vitro* studies have shown that biphasic pulse trains ($300 \pm 100 \mu$ A, 120 μ s, 140 Hz) applied directly to the hippocampus were effective in blocking seizure activity [15] and monophasic pulse trains ($405 \pm 101 \mu$ A, 120 μ s, 150 Hz) applied to the commissural tract could completely block axon conduction [19]. These results clearly indicate that HFS could suppress neural activity when applied to neurons or fiber tracts. The present *in vivo* study further shows that electrical stimulation applied to either the focus site or the commissural tract could suppress seizure activity in the whole hippocampus bilaterally. However, the suppression effect was dependant of the pattern of seizure activity.

In this study, we found that 4-AP treatment induced two patterns of seizures. A previous *in-vitro* study reported that 4-AP treatment produced two types of field potentials in the hippocampus [40]. One was a short duration interictal-like epileptiform field potentials occurring at a frequency of 0.3–1.3 Hz and the other was a longer-lasting interictal spikes at a lower frequency of 0.036 ± 0.013 Hz. However, these findings were interictal-like and had very low frequency (0.036–1.3 Hz). This is to our knowledge the first report describing these two types of epileptiform activity *in-vitro*. The kindling model induced by electrical stimulation of the amygdala was reported to produce a type of seizures characterized by high-amplitude and frequency discharges [44]. The kainate model could induce both non-convulsive and convulsive seizures but the electrographic patterns were similar [45]. The seizure pattern produced by kainate model was similar to the PPSs described in the present

study. However, the frequency range was not clearly defined in the study of kainite model. The lithium-pilocarpine model induced four patterns of seizures during status epilepticus [46], and two of patterns were morphologically similar to those induced by 4-AP, although the frequency ranges were somewhat different. This comparison suggests that the seizure patterns are different for different seizure models.

HFS suppressed the PPSs but could not suppress the high frequency spiking. Conversely, HFS at both the tract and the focus site could cause conversion of seizure pattern to high frequency spiking after or even during stimulation. There is no report in the related literature on this phenomenon, though after-discharge induced by HFS has been documented. In clinical trials, HFS (100–500 Hz) applied to the epileptogenic zone in four patients and to the anterior thalamus in four patients reported that HFS triggered after-discharge in 0.2% of the 1491 instances of closed-loop stimulation [47]. In the kainate animal model, electrical stimulation (5–130 Hz) applied to hippocampus bilaterally also caused after-discharge and high frequency stimulation was more prone to trigger the after-discharge than low frequency stimulation [48]. The authors suggested that the after-discharge was caused by the kindling effect. It is not clear whether the seizure pattern conversion and the after-discharge were identical or whether their mechanisms were similar. Regardless of its mechanisms, seizure pattern conversion would prolong the duration of seizures because high frequency spiking could not be suppressed by HFS, but needed to be converted back to PPS first. Therefore, in addition to the potential neuronal damage caused by HFS, seizure pattern conversion or after-discharge would be another important consideration for implementing closed-loop stimulation.

Both tract and focus site stimulation produced global suppression of seizures in the hippocampus. One possible explanation for the global suppression by tract stimulation is the connections of the tract to the entire hippocampus [21]. A previous study showed that continuous open-loop stimulation (3 Hz) on the ventral commissural tract could reduce the seizure frequency and duration in a mutation model of epilepsy [21]. This study further shows that short duration HFS applied to the same tract could immediately suppress 4-AP induced seizure globally in the hippocampus. However, tract stimulation was not able to generate global suppression in all cases and sometimes generated only partial region suppression. In the case of partial region suppression, the lower suppression rate always occurred in the right side (the side of injection) of the hippocampus (Fig. 6). Therefore it is possible that 4-AP injection induced focal damage and prevented seizure suppression or that the higher excitability in the injected region made seizures more difficult to be suppressed. In most cases, the seizure patterns were similar in all six regions and HFS produced global suppression (Fig. 5A). However, for some periods of time the seizure pattern in the right side, especially in the RM region, was different from those in other regions (Fig. 5B). If HFS was applied at these periods, seizures may not be suppressed in the right side. Focus site stimulation was able to generate partial region suppression with more suppression in the left side of the hippocampus. Wyckhuys et al. [49] showed that unilateral HFS on the hippocampus induced relative hypoperfusion in the hippocampus bilaterally and when the stimulation amplitude was higher (similar to those that we used in this study) the hypoperfusion was more pronounced on the contralateral side. The fact that unilateral HFS had a strong effect on bilateral hippocampal regions in [49] is compatible with the results reported in the present study.

Stimulation of the focus site could also produce global suppression. Although the effect of HFS *in-vitro* was shown to be localized [15], the axonal block could affect the propagation of seizure and induce a more global effect. In *in vivo* animal studies of closed-loop focal stimulation, the recording electrode was usually close to the stimulation electrode so the extent of the stimulation effect could not be determined [48]. In clinical trials, closed-loop

focal stimulation electrodes are placed close to the focus for seizure detection or use an array needle electrode integrated with stimulating and sensing electrodes [47,50]. Therefore, the spatial extent of the suppression is also difficult to assess. In the present study, both focal and tract stimulation could suppress seizures in the whole hippocampus bilaterally. The mechanism of global suppression by focal stimulation is not clear but the most likely explanation for this effect is activation of the longitudinal fibers tracts in the hippocampus. CA3 neurons project to the contralateral CA3 neurons through commissural fibers transversely and to ipsilateral CA3 neurons through associate fibers longitudinally [51]. Furthermore, CA1 neurons are activated by CA3 neurons through Schaffer collaterals. It has been previously shown that local stimulation could activate the longitudinal fibers tracts [52] and thereby activation the CA3 neurons first ipsilaterally and contralaterally through the commissural tract. Although the longitudinal fibers could provide a pathway to explain the propagation of seizure activities from a single site, the actual mechanism of the global suppression by HFS stimulation remains unknown and further studies are needed to elucidate this issue.

Conclusions

The results of this study have two practical implications, both tract and focus site stimulation can produce global suppression in the hippocampus and the choice of stimulation parameters is critical in order to produce suppression but not conversion of seizure pattern. These results could prove to be important in order to determine the choice of the location of DBS electrodes for seizure suppression with high frequency stimulation.

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Figure 1.

Locations of the recording and stimulation electrodes. Six stainless electrodes were inserted into CA1 of the left septal (LS), right septal (RS), left middle (LM), right middle (RM), left temporal (LT) and right temporal (RT) regions of the hippocampus, respectively. Stimulation electrodes were inserted into the ventral commissural tract (VCT) and the CA3 of the RM hippocampus. Two screw electrodes were fixed on the midline parietal and frontal areas of the skull as the reference and the ground. AP:-1.5 was 1.5 mm back from the Bregma.

Figure 2. Validation of the artifact removal algorithm: (A) Set-up of the verification experiment. Signals before and after removing the stimulation artifact (B, C), and their corresponding spectra (D). (E) Difference in power between the signals before and after removing stimulation artifacts.

Figure 3.

Gradual propagation and spreading of the seizure activities to the six regions at 7 min, 11 min, 12 min, and 13 min after 4-AP injection (A–D). Eleven minutes after injection, spikes appear in the right middle (RM) regions of the hippocampus, spread to the whole hippocampus and finally become synchronized. LS: left septal; RS: right septal; LM: left middle; LT: left temporal; RT: right temporal regions of the hippocampus.

Figure 4.

Two different types of activities. (A) Segment of typical seizure activity, consisting of the high frequency-low amplitude spiking (B) and the pseudo-periodic spikes (C). High frequency stimulation could not significantly affect high frequency spiking (D) but completely suppressed pseudo-periodic spikes (E). The stimulation was applied during a solid line and the artifacts were removed. The signals were all recorded from the left septal region in the hippocampus.

Figure 5.

Typical spatial response to the tract and focus site stimulation. (A) An example of global suppression by HFS. Tract stimulation with 500 μA was applied between two dash lines and suppression occurred in six regions. (B) An example of partial region suppression by HFS. Tract stimulation with 500 μA was applied between two dash lines. The suppression occurred except for the right middle and temporal (RM and RT) regions. (C) Suppression rates of HFS in the six regions where are the left septal (LS), right septal (RS), left middle (LM), right middle (RM), left temporal (LT) and right temporal (RT) regions of the hippocampus. The number of trials of tract stimulation with 100, 300 and 500 μA was 16, 24 and 29, respectively. The number of trails of focus site stimulation with 100, 300 and 500 μA was 14, 26 and 25, respectively. **: $P < 0.01$ and *: $P < 0.05$, Fisher's exact test.

Figure 6.

Cumulative suppression rates versus locations of the left septal (LS), right septal (RS), left middle (LM), right middle (RM), left temporal (LT) and right temporal (RT) of the hippocampus when stimulation is applied with three stimulation amplitudes (100, 300 and 500 μ A) at the tract or the focus site (i.e., the injection site of 4-AP at the right middle CA3). The minimal suppression rate among six regions was the global suppression rate.

Figure 7.

Conversion of type of activity with stimulation. (A) Tract stimulation with 500 mA suppressed pseudo-periodic spikes but induced pattern conversion to high frequency spiking after stimulation. (B) The rates of seizure pattern conversions in the six regions where are the left septal (LS), right septal (RS), left middle (LM), right middle (RM), left temporal (LT) and right temporal (RT) regions of the hippocampus. **: $P < 0.01$ and *: $P < 0.05$, Fisher's exact test.