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Effects of Responsive and Scheduled Stimulation in the Subiculum on Seizures and Excitability in the Kainate Induced Temporal Lobe Epilepsy Model

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Abstract

Objective: The subiculum, one major output structure in the hippocampus, is considered to participate in seizure generation and modulation in temporal lobe epilepsy (TLE) since stimulation of the subiculum was found to suppress seizures in a seizure model. The current study aimed to investigate whether acute scheduled or responsive stimulation of the subiculum can suppress spontaneous seizures and affect local excitability of the subiculum in a chronic TLE model.

Methods: Wistar rats were administrated intraperitoneally with kainic acid (KA) repeatedly to induce status epilepticus (SE). 4 months later the rats were implanted with stimulation electrodes in the subiculum. After one-week baseline recording, they received either responsive or scheduled high frequency stimulation (HFS, 125 Hz) for two days and then were swapped to the other type of stimulation for two days with one-week interval. All the rats also received pulse stimuli (100 μs, interval of 5-7 s) at different intensities (20, 50, 100 mA) before and after stimulation while evoked responses were elicited.

Results: Acute HFS of the subiculum-both scheduled and responsive stimulation-suppressed spontaneous focal seizures in rats. The excitability of the subiculum, measured by evoked responses, did not show obvious changes before and after HFS.

Conclusion: The preliminary outcomes revealed the anticonvulsant effects of subicular stimulation on spontaneous seizures, suggesting that the subiculum is a promising target candidate for deep brain stimulation to control seizures in TLE. The absence of changes in subicular excitability indicates that HFS affects the hippocampal network rather than the excitability of the subiculum per se.

Keywords: High frequency stimulation; Responsive; Scheduled; Subiculum; TLE; Rats

Introduction

Temporal lobe epilepsy (TLE) is the most common type of refractory epilepsy in adults. More than 30% of epilepsy patients cannot be well controlled by antiepileptic drugs [1]. Deep brain stimulation (DBS) is considered as a viable treatment option for patients who are not suitable for resective surgery.

Neuropathological and neurophysiological evidence [2-4] suggest that the hippocampus plays an important role in seizure generation in TLE. DBS, especially, high frequency stimulation (HFS), has been delivered to the hippocampus in order to control seizures in different animal models [5-8] as well as in patients with refractory epilepsy [9-14]. The common target region for DBS in these studies is the CA3 area. Recently, another sub-region within the hippocampus-the subiculum-has received increasing attention [15-17].

The subiculum, situated between the CA1 and entorhinal cortex (Ecx), is the major output structure in the hippocampal formation. It is also considered to participate in the generation and maintenance of epileptic activity [18]. *In vitro* study showed that the subiculum is hyperexcitable in human with TLE. Importantly, spontaneous rhythmic activities were found in hippocampal slices in human [15,17], reminiscent of interictal spikes observed in epilepsy patients. All together, these outcomes raise the possibility of the subiculum as a potential target for DBS.

Indeed, more evidence from *in vivo* studies confirmed the anticonvulsant effects of subicular stimulation. Zhong et al. [19] applied low frequency stimulation (LFS, 1 Hz) to the subiculum in a kindling model of TLE. These authors found that LFS of the subiculum slowed the progression of kindling acquisition, reduced average seizure stage in fully kindled rats, and inhibited occurrence of spontaneous generalized seizures in the pilocarpine treated rats. Meanwhile, our group [20] applied responsive HFS (125 Hz) to the subiculum when seizures were visually detected on EEG in a seizure model of TLE. The results showed acute anticonvulsant effects of subicular stimulation: less focal seizures and longer inter-seizure interval. Further, with help of an automatic seizure detection program, both responsive and scheduled stimulation were delivered to the subiculum in a similar seizure model [21]. The anticonvulsant effects were found on both types of stimulation, dependent on the severity of seizures (reaching SE or not) and type of seizures (immediate and lasting effects on focal seizures and latent effects on generalized seizures). Based on these positive outcomes, it would be interesting to further investigate whether subicular stimulation is effective in a chronic TLE model.

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Chemical induction of status epilepticus (SE) has been one of the most common approaches for animal models of TLE. A single large bolus of kainic acid (KA) was first used [22-24] to induce SE. However, a relatively high mortality rate was observed [25,26] and sometimes only a small number of animals developed spontaneous seizures [27]. Hellier et al. [28,29] developed a new protocol to induce SE by titration of low-dose KA, resulting in higher survival rate and more animals that develop spontaneous seizures. We adapted their protocol with some modifications to further lower the mortality rate (see methods).

The aim in the present paper was to investigate the effects of subicular stimulation-both responsive and scheduled way-in a chronic TLE model. In addition, local excitability of the subiculum, measured by the amplitude of evoked potentials (EPs) that were induced by a series of single pulses, was also investigated before and after responsive and scheduled stimulation.

Materials and Methods

Animals

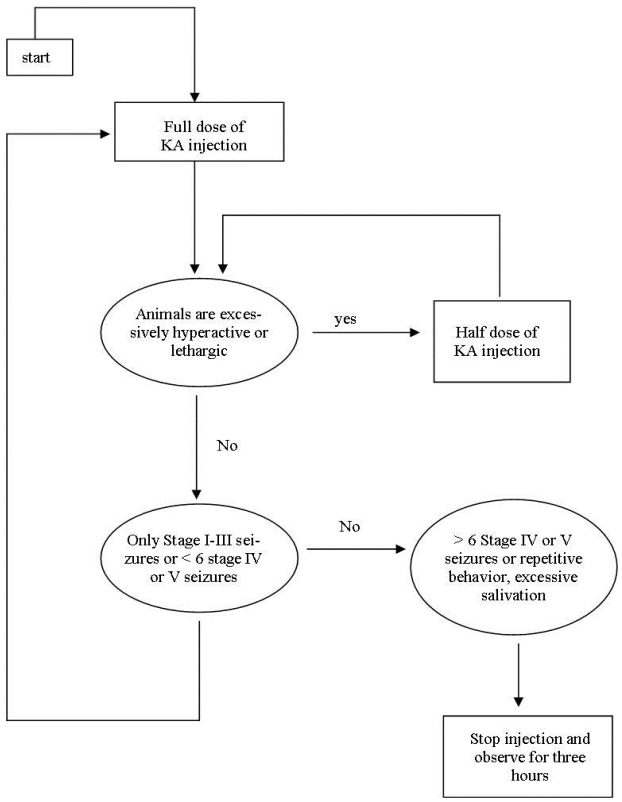
Male Wistar rats (n=14) weighing 120-150 g, were used (bred at the Biological Psychology Department, Donders Center for Cognition, Radboud University Nijmegen). The rats were housed under controlled temperature (20 °C, relative humidity 50~70%) and 12-hour light-dark conditions (with light at 8 am off and 8 pm on), with *ad libitum* access to food and water. The local medical-ethical committee of the Radboud University Nijmegen (RU-DEC) approved all procedures on animal experimentation in the study. Efforts were taken to alleviate discomfort and number of animals as much as possible.

KA administration protocol

The KA administration protocol was based on a previous protocol [28] with some modifications to reduce suffering of animals and lower the mortality rate of animals (see flowchart). The rats were injected with multiple KA (Ascent Scientific Ltd, U.K.) intraperitoneally while the behaviour of the rats was continuously monitored by two researchers. In our protocol, the rats received a full dose KA (5 mg/kg) or half dose (2.5 mg/kg) per hour depending on their seizure severity until they reached 3-hour SE (flowchart). In total, the rats received 7 ± 2 injections and 2.1 ± 0.6 mg of KA on average. Diazepam (5 mg/kg, i.p.) was given to the rats if they showed severe behaviors such as wild jumping. Subcutaneous administration of saline (1 ml) was given to all the animals after SE induction and was continued for two or three days if they appeared lethargic.

Surgery

The rats were anesthetized with isoflurane inhalation and fixed in a stereotaxic frame. At the start of surgery atropine sulfate (0.1 ml, i.m.) was given to reduce saliva secretion and the analgesic Rimadyl (4 mg/kg, i.v.) was administered. Temperature was monitored and maintained at 37°C with a heating pad throughout surgery. A tripolar electrode set (MS333/2a, Plastics One, Roanoke, VA, USA) was placed 5.6 mm posterior, 4.8 mm lateral to bregma at the right hemisphere at 3.2 mm depth in the subiculum [30], serving as stimulation electrodes and recording electrode. Another tripolar electrode containing three stainless wires was placed on the left hemisphere, with the frontal wire targeting the motor cortex and the other two wires located in the cerebellum serving as reference and ground electrode respectively. The tripolar electrodes and several screws were attached to the skull with dental acrylic cement. After surgery, the animals were housed individually and were given two weeks to recover.



Flowchart: This kainate acid (KA) administration protocol was based on the protocol by Hellier and Dudek (2005). In their protocol, the endpoint for multiple injections is three-hour (status epilepticus) SE, that is, at least one convulsive seizure per hour for three consecutive hours. Animals need to receive full dose or half dose of KA injection every hour depending on the intensity of the seizures. Animals continue to receive KA until they reach 3-hour SE in the end. In our study, we modified the protocol to reduce the discomfort that animals suffered from KA administration, based on the protocol used by Wyckhuys et al, 2010. A main difference is the endpoint of the modified protocol: when animals display more than 6 stage IV or V seizures, repetitive behaviors, or excessive salivation, KA injections were terminated and animal behaviors were then observed for the next three hours instead of giving another injection. This modification is based on the observation that when animals display such severities of seizure behaviors, they always show at least one convulsive seizure per hour for three consecutive hours without receiving any further KA injection. By doing so, animals receive less injections and thus less suffering and lower mortality rate.

Video and local field potentials (LFP) Monitoring

One LFP recording was recorded from the ipsilateral hippocampus and the other was recorded from the contralateral motor cortex. All these LFP recordings were made against the cerebellar reference electrode. LFP signals, fed into a multi-channel differential amplifier via a swivel contact that enables the animals to move freely, were amplified (5000x), band-pass (1-100Hz) and notch filtered (50 Hz). The output was sampled at 256 Hz and digitized with a WINDAQ/Pro data acquisition system in combination with a DI410-interface (DATAQ Instruments 2.49, Akron, OH, USA). Video was captured with a camera placed in the recording chamber and recorded with the aid of the Observer® (Noldus Information Technology BV, Wageningen, The Netherlands).

Video and LFP monitoring were performed under controlled conditions (20°C, relative humidity 50~70%). The recording took place in a noise-isolated experimental chamber. Two days before LFP

recording, the animals were placed in a Plexiglas recording cage (30×25 cm, high 35 cm) so as to habituate to the recording system. Later, the animals were recorded with LFP monitoring for 24-hour baseline recording for 7 days to measure the rate of spontaneous seizures.

HFS

HFS was delivered at 125 Hz, bipolar, biphasic, square wave with a width of 100 μs. The stimulation intensity was determined for each rat at the end of baseline recording. Starting with 100 μA, the intensity was step-wisely increased by 100 μA until motor effects (twitching, head nodding, rearing etc.) or LFP abnormalities were observed. Then the intensity was reduced by 100 μA and was kept at that level for the rest of the experiment. HFS parameters and the protocol to determine stimulation intensity were similar to what was previously used [11-14]. The rats were randomly divided into two groups-responsive and scheduled stimulation groups. In the responsive stimulation group, the digitized signal was also connected to a previously developed and validated automatic seizure detection program [21,31]. When the program detected a seizure, it triggered the stimulator to deliver stimulation to the animals until the seizure was over. The rats in the scheduled stimulation group received continuous stimulation whose duration was based on the total duration of spontaneous seizures during the baseline. Both stimulation groups received stimulation for two days. The stimulation conditions were swapped one week later, so a cross-over design was used.

Stimuli to measure excitability

To measure local excitability, all the rats also received electrical stimuli (pulse width: 100 μs) with an interval of 5-7s at different intensities (20, 50, 100 mA). Pulses were given at four occasions: before and after HFS (Figure 1). All the animals received a total of 50 stimuli for each intensity at each occasion.

Histology

At the end of the experiment, the animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and then a DC current (25 μA, 15 sec) was delivered through the electrodes to create a lesion around the electrode tips. Afterwards, the animals were perfused transcardially with 2% potassium ferrocyanide in a solution of 4% formaldehyde in 0.04 M phosphate buffer (PH=7.3). The brains were removed and post-fixed in the same solution overnight at 4 °C. After post-fixation, the brains were placed into a 30% sucrose solution and remained there until they sank 3 or 4 days later. Then coronal sections (60 μm) were cut by a microtome (HM 440E, Waldorf, Germany) and the slices containing the track of the electrodes were stained with cresyl violet. In the end, these slices were examined under a light microscope to verify the positions of the electrodes using the same atlas of the rat brain [30]. This study included only the rats whose histological examination confirmed the location of the stimulation electrode at the subiculum region.

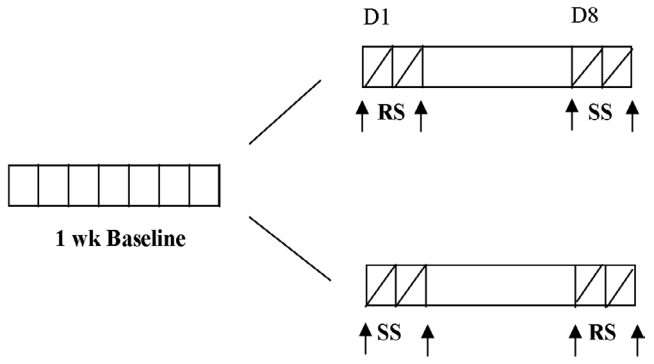


Figure 1: Indicates the stimulation protocol in the study. All the rats first received one-week baseline recording to measure the rate of spontaneous seizures. Then they were randomly classified into two groups to receive either responsive stimulation (RS) or scheduled stimulation (SS) in the subiculum for two days. One week later, they received the other type of stimulation for another two days. All the rats also received single pulse stimuli (with interval of 5-7 s) before and after RS or SS, as shown by the arrows, in order to measure excitability of the subiculum.

Data analysis

The recorded LFP were reviewed with the WINDAQ/Pro browser. Statistical analysis was done in SPSS 15.0. The definition of the start point of seizures is when the amplitude of the epileptiformic activity is twice the amplitude of the baseline LFP. Racine scale [32] was used to classify the severity of behavioral seizures: Stage I (immobility, facial automatism), II (head nodding, wet dog shakes), III (unilateral myoclonus), IV (bilateral myoclonus or tonic-myoclonic behavior, rearing without falling) and V (bilateral myoclonus or tonic-myoclonic behavior, rearing and falling). Focal seizures were defined both by severity of seizures (behavior, Stage I or II) and by epileptic activity on the LFP of the hippocampal channels only. Generalized seizures were defined both by severity of seizure behavior (Stage III, IV, or V) and by synchronous epileptic activity on the LFP of the hippocampus and motor cortex. Seizure characteristics such as seizure number and duration were calculated.

The rate of spontaneous seizures, measured by averaging seizures during the baseline period, serves as the control. One-way ANOVA was used to compare average seizure rate and duration of the two stimulation groups to the baseline period. Given the small number for each group, effective size square (Eta^2) was considered to co-evaluate the outcomes of the statistical tests. If $p<0.1$ and $Eta^2>0.2$, the outcome was still considered as significant. Post-hoc tests with Helmert contrast were chosen to reveal the difference between the baseline period and stimulation period as well as the difference between the two stimulation groups. Considering the sample size and probability distribution of the data, Kruskal Wallis non-parametric ANOVA was also performed. The outcomes of Kruskal Wallis test was reported in details only when there were inconsistent results between this test and one-way ANOVA.

Brain vision analyzer (Brain Products GmbH, Gilching, Germany) was used for averaging evoked potentials (EPs) (n=30) offline per rat per intensity, and before and after scheduled (PreSS and PostSS) and responsive (PreRS and Post RS) stimulation. The main components of the EPs were identified and characterized by their latency. Repeated measure ANOVA was conducted to compare the amplitude of EPs with different order (RS-SS, SS-RS) as between-subjects factor, and different stimulation type (RS, SS), day (before and after stimulation) and intensity as within-subjects factors. In addition, non-parametric Mann-Whitney and Wilcoxon tests were also performed.

Results

In total, 14 rats received KA administrations to develop 3-hour SE on the first day. Four months later, four rats were excluded as they did not show sufficient convulsive seizures (at least one convulsive seizure/hour) during 6-hour behavior monitor prior to surgery. One rat died after implantation surgery and one died during stimulation recording. Two rats lost electrodes during stimulation recording. Thus, six rats were finally included in the study for data analysis. The scheduled

stimulation group received a controlled amount of stimulation (4.1 ± 1.8 minute) during the 48-hour recording session based on the total duration of spontaneous seizures during the baseline period. The responsive stimulation group received stimulation (1.5 ± 0.7 minute) depending on the total duration of all seizures during the 48-hour recording session.

Effects of HFS on spontaneous seizures

The mean focal seizure number during the baseline period 5.2 ± 0.7 seizures per day (mean ± S.E.M, range from 0 – 15 seizures). The mean seizure duration during the baseline period was 8.2 ± 1.3 s (range from 3 – 24 s). There were very few generalized seizures (0.3 ± 0.7 seizures per day) with duration of 59.2 ± 7.0 s. The inter seizure interval was variable, from less than 1 hour to 1 day.

During the two-day stimulation period, the responsive stimulation group had 1.6 ± 0.4 focal seizures per day, and the scheduled stimulation group had 1.7 ± 0.3 focal seizures per day. One-way ANOVA showed a group effect ($F_{(2, 15)}$ =10.78, p<0.01, eta^2=0.6) and post-hoc tests suggested that both stimulation groups had reduced focal seizure number (p<0.01) compared to the baseline period (Figure 2A). Likewise, focal seizures lasted 4.4 ± 1.8 s in the responsive stimulation group and 2.8 ± 1.2 s in the scheduled stimulation group. One-way ANOVA showed that there was a group effect ($F_{(2, 15)}$ =3.44, p=0.06, eta^2=0.3) and post-hoc t test showed that both stimulation groups had shorter duration of focal seizures (p<0.05) compared to the baseline period (Figure 2B). Meanwhile, the Kruskal-Wallis non-parametric ANOVA also showed that both stimulation groups had less and shorter focal seizures, consistent with the outcomes of the parametric ANOVA.

Considering that there were very few generalized seizures in general and in particular after stimulation in both groups (in fact four rats did not show any generalized seizures after stimulation), generalized seizures were excluded from statistical analyses.

Evoked potentials (EPs)

Evoked potentials induced by electrical pulse stimuli were mainly visible on LFP recorded at the subiculum site near the stimulation electrodes. The first negative response at 2 ms was considered as a stimulation artifact, which can also affect the following positive response at 4 ms. Thus, these two responses were not identified as main components. In total, three main components of EPs were identified: N1 (latency at 5 ms), N2 (latency at 13 ms), P1 (latency at 23 ms), as illustrated in Figure 3.

Repeated measures ANOVA showed the following outcomes

Amplitude of N1: only a main effect on intensity (Figure 4A) was found: the stimulation at higher intensity induced larger evoked potentials ($F_{(2, 8)}$ =4.95, p<0.05) than lower intensity stimulation.

Amplitude of N2: a main effect on intensity ($F_{(2, 8)}$ =4.52, p<0.05) (Figure 4A) as well as an interaction between stimulation group, intensity and day effect ($F_{(2, 8)}$ =4.18, p=0.06, eta^2=0.51). Post-hoc t test showed that evoked potentials were marginally higher ($t_{(5)}$ =2.45, p=0.06) after scheduled stimulation compared to before stimulation but only at 100 mA. This was confirmed by the outcomes of the non-parametric Wilcoxon test showing higher evoked potentials after scheduled stimulation at 100 mA. Different stimulation groups showed different tendency in EPs changes (post-pre) after stimulation at 100 mA: the rats seemed to have higher EPs after receiving scheduled stimulation, whereas no such changes were observed in the rats after responsive stimulation ($t_{(5)}$=4.35, p=0.01), as shown in Figure 4B. Similarly, non-parametric Mann-Whitney test also confirmed the difference between the two stimulation groups.

Amplitude of P1: neither main effects on order, intensity, group, or day, nor interactions effects were observed.

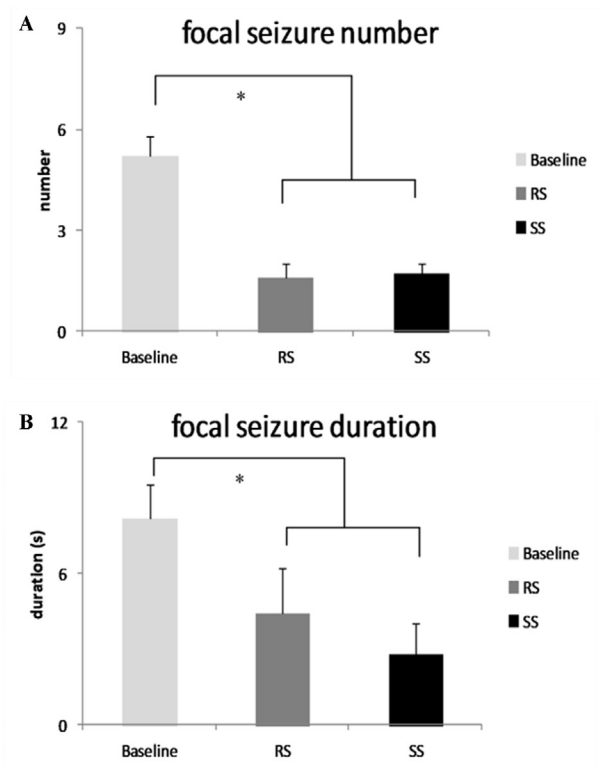


Figure 2: Mean and sem of the focal seizure number and duration during baseline, and after responsive and scheduled information. Both stimulation groups had less (A) and shorter (B) focal seizures compared to the baseline period. RS: responsive stimulation. SS: scheduled stimulation. *: p<0.05; **: p≤0.01.

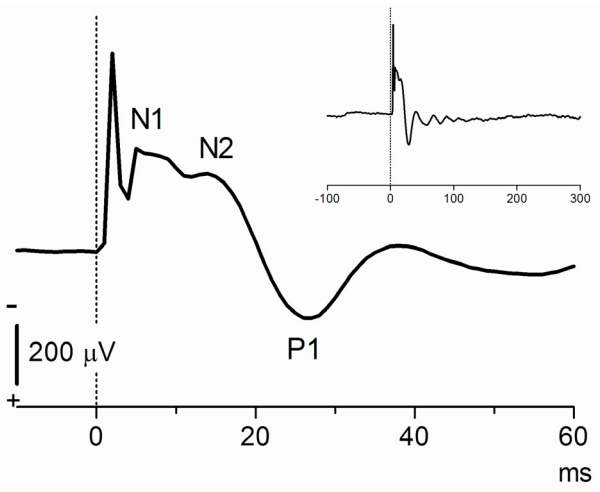


Figure 3: Example of average evoked potential (EPs) induced by electrical stimulation of the subiculum. The upper right panel represents a grand average of all EPs averaged at three intensities (20, 50, and 100 mA). The main panel is the magnification of EPs (-10: 60 ms) with the three main components at different latencies: N1 (at 5 ms), N2 (at 13 ms), P1 (at 23 ms).

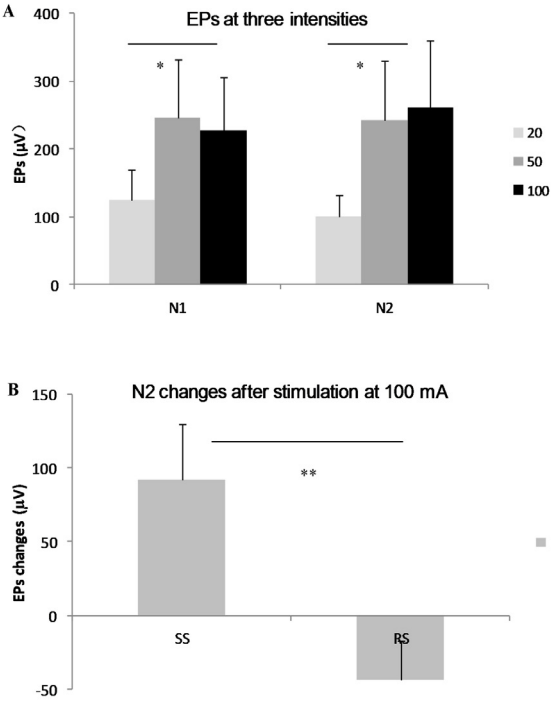


Figure 4: Mean and sem of the amplitude of the evoked potentials (EPs) at three intensities. A: significant intensity effects were found for N1 and N2 component when EPs were elicited by stimuli at three intensities (20, 50, 100 mA). B: Mean and sem of change in amplitude of N2 after stimulation (Post-Pre) at 100 mA in the two stimulation groups. N2 was increased after scheduled stimulation (SS), whereas it was reduced after responsive stimulation (RS). *: $p<0.05$; **: $p\leq0.01$.

Discussion

The effects of responsive and scheduled stimulation of the subiculum in the SE model of TLE induced by repeated KA administrations were compared. The preliminary results (from both parametric and non-parametric statistical analyses) showed that acute HFS of the subiculum-both scheduled and responsive stimulation-suppressed spontaneous focal seizures in rats. Meanwhile, local excitability, measured by evoked potentials, did not show obvious changes before and after HFS.

Effects of HFS on spontaneous seizures

The outcomes of our study confirmed the anticonvulsant effects of subicular stimulation, consistent with the limited evidence from animal studies. Zhong et al. [19] applied continuous LFS to the subiculum for 15 minutes daily for 35 days after induction of SE in the pilocarpine induced epilepsy model. They found that LFS of the subiculum can prevent occurrence of spontaneous seizures. This is the only study that proved the anti-epileptogenesis effects of subicular stimulation in the chronic epilepsy of TLE. Meanwhile, our previous work investigated whether HFS of the subiculum with different fashion-responsive and scheduled stimulation-would result in beneficial effects in a seizure model of TLE [21]. The results showed that both types of stimulation can inhibit seizures in rats that did not develop SE after acute administration of KA. Such anticonvulsant effects were different for focal and generalized seizures: immediate and persistent effects on focal seizures, whereas for generalized seizures only delayed effects (two weeks later) were found. The current study, despite preliminary (considering the small number of animals), further confirmed the effects of subicular stimulation in a chronic TLE model, indicating that the subiculum is a potential target for DBS to control seizures.

Despite the beneficial effects of stimulation, some differences existed between the outcomes of these two studies. For instance, in our previous study scheduled stimulation seemed more effective than responsive stimulation in seizure suppression, whereas no such differences were found between the two stimulation groups in the current study. This discrepancy might be explained by the fact that the amount of stimulation was not controlled in the previous study (16 hours and 5 min for the SS and RS group respectively), in comparison to the controlled amount of stimulation for the two stimulation groups in the current study. Another important fact that needs to take into account is that different models were used in these two studies. Many intense focal and generalized seizures were induced in the acute seizure model in the previous study while only a few slowly emerging spontaneous seizures were noticed in the current epilepsy model. This may account for different effects of stimulation between these two studies. In all, it is not surprising that the current study showed that even a small amount of either scheduled or responsive stimulation (around 2 minutes) can suppress spontaneous focal seizures.

Besides evidence from animal models, Bondallaz et al. [33] recently delivered HFS (130 Hz) in the hippocampus to investigate the relationship of stimulation and distance between stimulation contact and focus in eight patients with refractory epilepsy. The outcomes showed that when the stimulation electrode was closer to the subiculum, the effects of stimulation were higher. The Bondallaz et al. [33] study is the first clinical study supporting the participation of the subiculum in seizure generation and propagation.

One highlight in our outcomes is that responsive HFS in the subiculum is beneficial for seizure suppression besides the classic scheduled stimulation. Zhong et al. [19] investigated LFS of subiculum at different delivery time points such as at double ADD (after discharge duration) delay, 0.5 hr delay, 2h delay on kindling acquisition and found that LFS at double ADD delay can slow the progression of kindling. They proposed that such a wide time window for the subiculum indicates that the subiculum is suitable for responsive stimulation. The outcomes of our work, in favor of their assumption, proved that responsive stimulation of the subiculum for only 2 minutes is effective in suppression focal seizures.

Excitability of the subiculum and hippocampal network

Our data suggests that local excitability in the subiculum in general was not altered by both types of stimulations. Such absence of change in the local excitability of the subiculum was in contrast with HFS induced decrease of spontaneous seizures.

The subiculum is one major output station in the hippocampus, receiving primary inputs from the CA1 area and sending projections directly or indirectly via the pre- and para-subiculum [34] to the entorhinal cortex (EC). It is possible that the excitability of the subiculum per se was not altered by stimulation. Instead, HFS of the subiculum might alter the excitability of the entire hippocampus network, resulting in suppression of spontaneous seizures. Stypulkowski et al. [35] have investigated the hippocampal excitability during remote thalamic stimulation (5 Hz) and direct hippocampal stimulation in rats. They found that the amplitude of the hippocampal evoked potentials were reduced when local field potentials (LFPs) were suppressed, indicating a reduction of hippocampal excitability by both remote stimulation and direct stimulation. More experiments need to be conducted in the future to investigate whether and how much the excitability of different

subareas of the hippocampus such as the CA3 and CA1 area are altered during and after different stimulation protocols.

Epilepsy model

The rate of spontaneous seizures in the present study (5-10 seizures per day) is relatively low compared to previous studies (20-25 seizures per day) at the same period after SE. One reason could be different strain of rats. In our study, Wistar rats were used compared to Sprague-Dawley (SD) rats in other studies. It is possible that genetic factors contribute to different levels of spontaneous seizures. A previous study [36] reported differential sensitivity of strain to KA induced seizures in adult rats: SD rats seemed more insensitive to KA compared to other strains such as Wistar-Furth and Fisher 344. Later, the same group [37] found that such strain difference was not observed in juvenile rats. If so, considering that juvenile rats were used in our study, rat strain might not affect expression of spontaneous seizures as expected. A more plausible explanation could be induction of less intense SE at the early phase in our study. For mortality concern, an adjusted protocol was adopted in our study to induce SE. In total, the rats received 7 ± 2 injections and 2.1±0.6 mg of KA to reach 3-hour SE on the first day. This is less intense compared to data from others [38] (3.0 ± 1.3 mg). Therefore, it is more likely that induction of less intense SE led to lower level of spontaneous seizures in our study.

Conclusion

In summary, different types of acute stimulation in the subiculum were delivered in a chronic epilepsy model. The preliminary outcomes showed that both responsive and scheduled HFS of the subiculum can suppress spontaneous seizures, indicating that the subiculum is a promising target candidate for DBS to control seizures. In contrast, focal excitability – measured by evoked potentials in the subiculum – did not alter after either type of HFS. Taken together, these outcomes suggest the beneficial effects of HFS of the subiculum on the hippocampal network rather than the excitability of the subiculum per se.

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