Evaluation of low frequency stimulation in fully kindled rats

Joacir G. Cordeiro¹,²,³, Alberto Capurro¹, Karina K. Cordeiro¹,³, Ad Aertsen¹ and Andreas Schulze-Bonhage¹,²

¹ Bernstein Center for Computational Neuroscience Freiburg, Albert-Ludwigs-University, Hansastrasse 9A, 79104 Freiburg, Germany.
² Epilepsy Center, University Hospital, Breisacher Str. 64, 79106 Freiburg, Germany
³ Federal University of Paraná-UFPR, R: General Carneiro 180, Curitiba, Brazil

Abstract

To evaluate the potential of stimulation techniques to suppress epileptic activity, human studies are limited regarding the analysis of stimulation parameters. Animal models can serve for a systematic study of the efficacy of different stimulation paradigms. Earlier studies demonstrated that in animal models of kindling, short term low frequency stimulation (LFS) applied during the kindling process is able to interfere with epileptogenesis. We here studied the effects of LFS after consolidation of the epileptogenic process. Wistar rats underwent hippocampal kindling (60Hz, 1.6s, 500µA, once daily stimulation) until reaching the fully kindled state. Stimuli to evoke seizures were then adjusted to individual afterdischarge (AD) thresholds. Animals were then submitted to infrathreshold short term LFS protocol delivered through 4 electrodes on the ipsilateral hippocampus using bipolar stimulation (alternating between each pair of electrodes) with square waves at 1Hz, 50-150µA, 15 minutes per day for 7 days. The AD latency was significantly longer after the LFS application and a statistically non-significant increase tendency was also observed on the AD threshold and AD duration. The AD amplitude and the behavioural seizure duration were not significantly influenced. These data suggest that short term infrathreshold LFS is not able to suppress continued ictogenesis in fully kindled rats.

Key Words: hippocampal kindling, low frequency stimulation, brain stimulation

1 Introduction

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in adults. One of its major causes is the hippocampal sclerosis. Seizures are resistant to currently available antiepileptic drugs in the majority of cases, therefore the mainstay of the mesiotemporal epilepsy treatment presently consist of the resection of the epileptogenic area¹. The use of electric brain stimulation represent an alternative approach, but so far its efficacy is lower than of resective procedures²,³,⁴. One of the possible reasons for this may be related to the lack of adequate stimulation parameters⁵. Animal models can serve for a systematic study of the effect of different stimulation paradigms. The aim of this study was to evaluate the effect of low frequency stimulation (LFS) applied on the epileptic focus of fully kindled rats.

2 Method

2.1 Animal population

Experiments were carried out on five adult Wistar rats of both sexes with weights between 250 and 350 grams at the time of the electrodes implantation. The animals were obtained through the Freiburg Clinic University and their utilization was approved by its ethical committee and experimental statute for animal models. The rats were housed during all the experiment in individual transparent plastic cages under a 12-hour light (day) and 12-hour darkness cycle (night). Food and water were delivered ad libitum.

2.2 Electrodes placement

The electrophysiological recordings and behavioral seizures used in this study were obtained through electrical kindling of the dorsal hippocampus of rats as animal model of temporal lobe epilepsy. Kindling was performed with bipolar stimulation through platinum electrodes (125µm of diameter) placed in the right dorsal hippocampus at stereotaxic coordinates AP=-3.6, L=-2.4, V=-2.5 and AP=-3.6, L=-2.4, V=-3.5. In addition two tungsten electrodes (50 µm of diameter) were implanted on the ipsilateral hippocampus 1 mm anterior and 1 mm posterior to the kindling site (coordinates AP=-2.6, L=-1.5, V=-3.2 and AP=-4.6, L=-3.5, V=-3.4); a common reference tungsten electrode was placed 1mm posterior to the lambdoid suture (cerebellar surface); an additional occipital screw served as ground electrode (Fig 1). Electrode positions were controlled in post-mortem using Nissl stains of brain slices after intracardiac perfusion (Fig 2). The surgical procedures were done under general anesthesia with intraperitoneal atropin(0,5mg/ml) 0,1mg/kg, ketamin10%.
100mg/kg, xylazine 2% 3mg/kg, and post-surgical analgesy with buprenorphin (0.05mg/kg). All steps of animal care were done according to German regulations (Az.: 35-9185.81/1/923, Reg.-Nr. G-06/27).

Fig. 1 Schematic representation of the electrodes implanted in the right dorsal hippocampus. Kindling stimuli were delivered through H2-H3 electrodes. The four electrodes were used to deliver the LFS protocol. (Reproduced and adapted from Paxinos & Watson, 2007)

Fig. 2 Representative coronal slice (50µm, Nissl stain) displaying the position of the bipolar electrode in the right dorsal hippocampus (arrow).

2.3 Hippocampal Kindling

The electric stimulation used to induce the hippocampal kindling was bipolar delivered through the H2-H3 electrodes pair (Fig 1). It consisted of 200 pulses of biphasic square waves at 60 Hz (0.4 ms width) and amplitude of ± 500µA (suprathreshold stimulation). The stimulation train was applied daily in each rat in series of 5 consecutive days, separated by intervals of 2 days without stimulation. The process was continued until the rats reached behavioral seizures of degree 5 on the Racine scale for 10 consecutive sessions (i.e. fully kindled stage). In each kindling session the EEGs were 500 times amplified (signal amplifier PGA32 from Multi Channel Systems, Reutlingen, Germany), broad-band filtered (from 1 Hz to 5 KHz) and recorded at 10.4 kHz sampling rate (analogic-digital converter CED Power 1401 from Cambridge Electronic Design, Cambridge, England), with simultaneous 25 frames/second video-monitoring (model Sony HDV1080i from Sony Corporation, Tokyo, Japan). The signal was synchronized to the video-monitoring through the Spike2 software (from Cambridge Electronic Design, Cambridge, England), which was also used to analyze the obtained data.

2.4 Low frequency stimulation protocol

Stimuli to evoke seizures were then adjusted to individual afterdischarge thresholds (ADT) which were determined in steps of 50µA. After the ADT determination, the animals were submitted to the short term LFS protocol delivered through the four hippocampal electrodes H1, H2, H3 and H4 (Fig 1). The protocol consisted of bipolar stimulation (alternated between each pair of electrodes) with square waves at 1Hz, 50-150mA of amplitude (individually adjusted) and lasted 15 minutes per day during one week. On the last protocol day after LFs, and seizure induction was resumed after determination of ADT. The AD features (pre and post LFS treatment) were compared regarding to AD threshold, duration, latency (time between the stimulus and AD start) and amplitude; similarly, the behavioural seizures duration was determined on videos. For the statistical analysis the U-test was applied.

3 Results

LFS was well tolerated without seizure induction; animal behaviour remained unaltered during stimulation sessions.

Before LFS treatment, mean AD threshold was 221µA (SD ±182); mean AD duration was 87s (SD ±11); mean AD latency was 1,3s (SD ±0,9); mean AD amplitude was 2,6mV(SD ±1,6) and mean behavioural seizure duration was 41,5s (SD ±11).

After LFS treatment, mean AD threshold was 304µA (SD ±262); mean AD duration was 117s (SD ±73); mean AD latency was 0,79s (SD ±0,3); mean AD amplitude was 3,5mV(SD ±1,7) and mean behavioural seizure duration was 38,3s (SD ±8,48).

An increase in mean post LFS AD threshold was observed, but this trend was not statistically significant. Post LFS AD duration and AD latency were not statistically significant when compared to pre LFS. The mean AD amplitude and the behavioural seizure duration were also not significantly influenced by LFS.
4 Discussion

Electric brain stimulation increasingly plays a role in the armamentarium of epilepsy treatment. The current strategies may be divided into non-selective stimulation (i.e. vagus nerve stimulation) and the stimulation of the epileptic focus and its vicinities\textsuperscript{2,4,7,8}. In cases where no single and precise focus may be attributed as responsible for the seizures, the non-selective techniques are usually preferred. When a precise focus can be determined, focal stimulation may be applied aiming to alter the local neural network dynamics. Recently, two studies by Boon et al. and Velasco et al. reported on significant reductions in seizure frequency by long term intrahippocampal high frequency stimulation\textsuperscript{5,8}. Targeting higher levels of efficacy, other stimulation strategies may offer chances.

Experimental data suggest the potential benefit of low frequency stimulation paradigms; thus Mohammad-Zadeh et al. reported an anti-kindling effect of infrathreshold LFS at 1Hz\textsuperscript{9}. Weiss et al. reported quenching of epileptic activity using suprathreshold LFS at 1Hz during the amygdalar kindling and in the fully kindling state in rats\textsuperscript{10}. LFS quenching applied during the kindling process was able to inhibit seizure development and to increase the AD threshold. In addition it was reported a decrease seizure duration and increase AD threshold when the quenching was applied to fully amygdala kindled rats. Similar to these finding, LFS stimulation in our study resulted in a tendency to increase AD threshold tendency to increase, but changes were not significant regarding either AD threshold or seizure duration.

The low efficacy of LFS stimulation applied here may after reaching the fully kindled state shows the high degree of epileptogenicity. It may be related to the low stimulus intensity applied, as the degree of depression of excitability may be related to stimulus intensities.

5 Conclusion

Short term infrathreshold LFS can be applied safely in fully kindled rats and does not modify their behavior. Our findings suggest that the efficacy of infrathreshold LFS may not be sufficient for a consistent ictal focus depression once kindling has been fully established. Further studies are necessary to determine the possible benefit of longer term LFS applied at different intensities on fully kindled rats.

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6 Literature