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Measuring Epileptogenicity in Kainic Acid Injected Rats

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***Abstract*—The present work aims at improving the validation of therapeutic approaches to treat temporal lobe epilepsy. Today's antiepileptic drugs perform only poorly for this form of epilepsy, and electrical stimulation might be an alternative for these patients. Finding the optimal stimulation parameters, however, is difficult as the underlying mechanisms, both of the disease and the stimulation procedure, and even the evaluation of the therapeutic effect are still poorly understood. Here we used a method to obtain a quantitative measure - the relative phase clustering index (rPCI) - of the epileptogenicity of a brain region in a simple and straightforward way.**

I. Introduction

TEMPORAL lobe epilepsy (TLE) is the most common form of focal epilepsy in adults [1]. Often, patients fail to respond to antiepileptic drugs. Resecting an epileptogenic hippocampus is the last possible therapeutic option for many patients, but bears, among others, the risk of memory and visual deficits [2, 3]. Another promising approach is electrical brain stimulation [4-7], but optimal target areas and stimulation parameters still need to be determined.

We consider the hippocampus a particularly qualified area for stimulation as the activity is limited to this structure longer than at other foci [8, 9]. Still, the choice of stimulation parameters needs to be addressed. As the underlying mechanisms for the efficacy of the stimulation remain unknown, the optimal parameters need to be found with an empirical approach [10, 11]. The most important feature seems to be the stimulus frequency [12, 13].

To systematically study and compare the effects of different stimulation patterns and different electrode designs, quantitative measures of therapeutic success are of key importance. So far, these measures depend strongly on the experimental model used. An important model for epilepsy research is the kainate model. In this animal model, kainic acid (KA) is injected into the hippocampus to provoke neural loss and cell dispersion resembling the hippocampal sclerosis observed in about 90% of TLE patients [14] and leading to spontaneous seizures [15-17]. A problem with the kainate model, however, is that spontaneous seizures especially in the first 3 months the seizure frequency is very low and unstable (around 3 per month [18]), impeding statistical analyses and necessitating high numbers of experimental animals as well as long-term recordings to gain the numbers of seizures necessary for validation of the tested stimulation parameters, especially as the yield of animals developing seizures at all in this model is quite low.

Therefore, we are looking for a new method for evaluating the effect of therapeutic electrical brain stimulation in the epilepsy context. This study uses an experimental measure to quantify the epileptogenicity of the hippocampus that shall be used later for evaluation. For this, the relative phase clustering index (rPCI), a measure of phase demodulation introduced by Kalitzin and colleagues [19, 20] as a possibility to predict seizures in an electrical brain-stimulation paradigm, is adapted to the rat kainate model. Our aim is to use this measurement to detect possible changes in neuronal excitability from local field potential (LFP) signals, and to correlate these changes to the occurrence of electrographic and behavioral seizures.

II. Materials and Methods

A. Animals

Female Wistar rats were used for this experiment. Animals were kept under a 12-hour light (day) and 12 hour (night)

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cycle. Temperature (21°C) and humidity were maintained at a constant level. Food and water were delivered *ad libitum*.

B. Electrodes

The stimulating/recording electrode consisted of 125 µm thick Teflon-coated stainless steel wires, which were twisted to a tetrode. The active electrode surfaces at the tips were approximately aligned (Fig. 1), with a distance of 0.5 mm between outer and inner contacts, and 1.0 mm between the two inner contacts. In addition, a single Teflon-coated tungsten wire (60 µm diameter) was used as a reference electrode, whereas the ground electrode was realized by a miniature screw placed in the skull.

Prior to implantation, all electrodes were characterized by an impedance analyzer with electro-chemical interface (Solartron 1260 and 1287, Farnborough Hampshire, UK) to ensure similar electrical characteristics.

C. Surgical Procedure

For KA injection and implantation of the electrodes, rats were anesthetized with intraperitoneal injection of ketamin (100 mg/kg), xylazine (3 mg/kg), atropine (0.1 mg/kg) and post-operative analgesia with buprenorphin (0.05 mg/kg). The head was shaved and the animal fixed in a stereotaxic frame. Following antisepsis, the skull surface was exposed by a median longitudinal skin incision. Burr holes in the skull were made under the magnified view of a surgical microscope.

KA was injected into the hippocampus at the stereotactic coordinates AP -5.5 L -4.8 V -5.0 with regard to bregma by means of a Hamilton syringe, applying 0.4µg/0.1 µl continuously over one minute. The injection needle was removed five minutes afterwards, to ensure the dispersion of KA at the intended site.

The stimulating/recording electrode was placed at the injection site in 6.5 mm depth, such that the epileptic focus generated by KA should lie between the two inner contacts of the electrode.

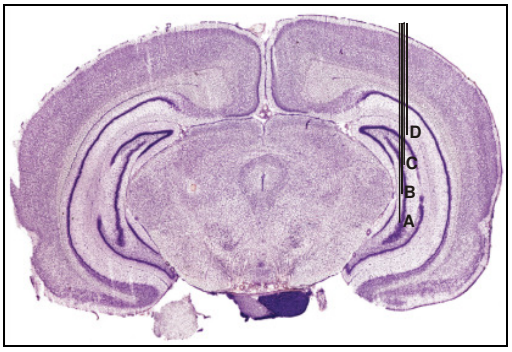

Figure 1: Placement of the stimulating/recording electrode (adapted from Paxinos and Watson [21]). KA injection site was located between the two inner contacts (B and C), which were used for stimulation. Data from the two outer contacts (A and D) were used for analysis.

The reference and ground electrodes were placed 1 mm posterior to the lambdoid suture. To fixate the implant, screws were placed around the electrodes and the connector was fixed to the skull by dental cement.

D. Data Recording and Stimulation

After implantation, animals were allowed one week of recovery before recordings were started. LFP signals were amplified and filtered (1Hz - 5 kHz) with a MPA81 pre-amplifier and PGA32 signal amplifier (both: Multi Channel Systems, Reutlingen, Germany), A-D converted at a sampling rate of 10 kHz with a CED power 1401 and recorded with Spike2 (both: Cambridge Electronic Design, Cambridge, England). During recordings, animals were able to move freely inside their cage. Data was recorded continuously for 46 hours starting at the 7th day after implantation, and then again 6 hours at the 10th day. Simultaneous video monitoring was performed with a normal camcorder at 25 fps and synchronized to the recorded signals by Spike2 (version 5.08). For stimulation, the STG2008 (Multi Channel Systems, Reutlingen, Germany) was used at the two inner contacts (B and C) of the tetrode. Stimulation consisted of a 30 s-train of bipolar rectangular pulses with amplitude of 5 µA and a pulse width of 260 µs, at a frequency of 20 Hz. These values were found to be optimal in an earlier related study (unpublished) on rats using the kindling model for epilepsy. During the recording period, stimulation was repeated every hour.

E. Data Analysis

Stimulation was applied to the two inner contacts (denoted B and C, Fig. 1) each hour, while LFP signals were recorded from the two outer contacts (denoted A and D). Epileptiform activities (e.g. spikes and ripples) were identified by visual inspection of the recordings. Due to its different forms, the amount and severity of epileptic activity was difficult to quantify. Therefore, only differences between channels and changes over recording time were evaluated.

In addition, we computed the phase clustering index (PCI) and relative PCI (rPCI), as described in [19, 20], from the LFP signals recorded at contacts A and D. The PCI measures the amount of time-locking of different frequency components of the stimulus-evoked LFP signals. Its values range from 0 (randomly distributed phases) to 1 (aligned phases). The rPCI is defined as the difference between the highest PCI at any of the harmonic frequencies and PCI at the stimulation frequency. It was suggested that high values of rPCI indicate increased tissue excitability [20]. Data processing was done using Matlab R2007b (The MathWorks, Inc., Natick, MA, United States).

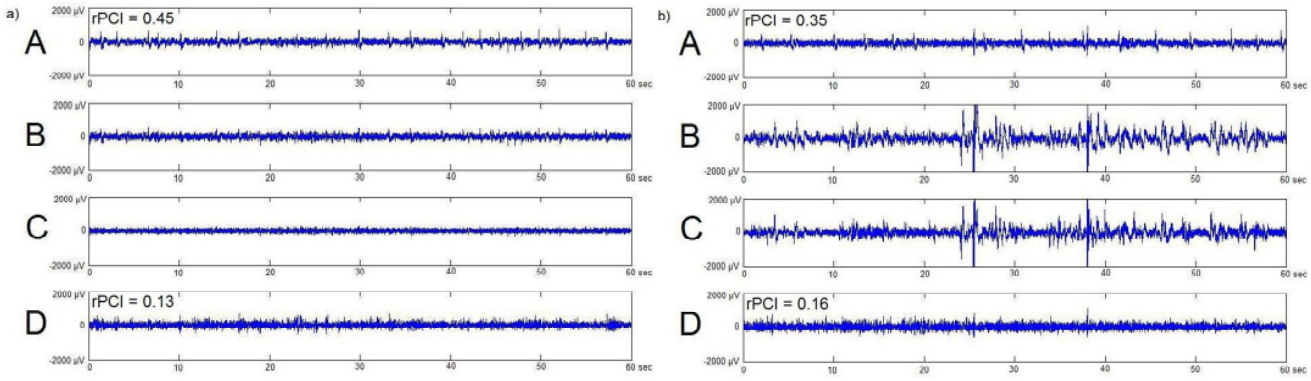

Figure 2: Examples of spiking activity recorded on channel A, outside (a) and during (b) an electrographic seizure. In b), seizure activity is observed on channels B and C. In both periods, no epileptic patterns are observed on channel D. The rPCI values in the graphs were computed 5 to 10 minutes after the recordings.

III. Results

Recordings were performed 7-10 days after KA injection. At this stage, the process of epileptogenesis has just started. It takes three to four months to establish a stable 'epileptic state'. As spontaneous epileptic seizures are sparse in the kainate model, it is not surprising that no behavioral seizures were observed in our experiment. However, the LFP signals showed typical seizure patterns especially at the epileptic focus, i.e. on electrode channels B and C.

In particular, on the first two recording days (7th and 8th day after surgery), channel A repeatedly exhibited intervals with spiking activity, sometimes leading to or going along with epileptic activity at channels B and C (the contacts closest to the injection site), but not on channel D (Fig. 2a). These intervals lasted typically about 2 minutes and contained 20-40 spikes. Some intervals lasted much longer, e.g. one recorded seizure with spiking activity on channel A and seizure patterns on channel B and C continued for more than 15 minutes and contained 250-300 spikes (Fig. 2b). In these three channels (A-C), no special trend concerning spike densities or prolonged intervals of electrographic seizures was observed over the course of the recording. Strikingly, the LFP on channel D did not show any such epileptiform activity during these recording days. Even during the seizure activity on channels B and C, hardly any changes were observed in the signal of this channel. Only short intervals (1-3 seconds) with fast ripples occurred on channel D, in most cases concomitant with ripples on channel A.

Interestingly, when comparing these observations to the rPCI values computed for these two days, it was striking that rPCI was consistently higher on channel A than on channel D. Indeed, on channel A all values were between 0.30 and 0.69 (mean 0.43 ± 0.12), whereas values on channel D ranged from 0.10 to 0.24 (mean 0.16 ± 0.03).

Furthermore, on the 10th day after surgery, the seizure activity originating from channels B and C spread to channel D as well (Fig. 3). Although still no spike intervals, like those seen on channel A in Fig. 2a, occurred on this channel, the rPCI values rose to values now ranging from 0.33 to 0.52 (mean 0.44 ± 0.07). At the same time, rPCI values for channel A were also higher (ranging from 0.34 to 0.72 (mean 0.58 ± 0.13)), although no obvious changes could be seen within the signals of this channel compared to the first two days. We never observed epileptiform activities on channel D without associated activity on channel A.

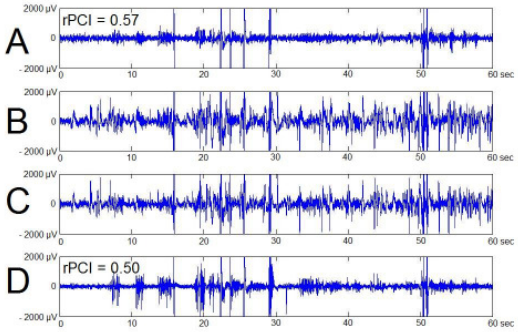

Figure 3: Example of seizure activity originating at channels B and C and spreading to channels A and D. The rPCI values included were obtained 20 minutes after this recording.

IV. Discussion

While the quantitative evaluation of the amount and severity of epileptic activity on individual channels remains difficult, the results obtained thus far suggest that the rPCI might provide a useful quantitative measure for epileptogenicity. By comparing the occurrence of epileptiform activity on the recorded channels with the rPCI values computed for each hour, we observed a clear trend, that rPCI values were higher for channels with more epileptiform activity. The problem remains, though, to quantify this activity itself, with different forms of such activity, moreover of different magnitude impeding a simple quantitative measure. Therefore, only this general trend can be reported at this point, whereas no explanation can be given for the variance between the individual rPCI values themselves. Whether these differences are merely stochastic or possibly reflect some further, thus far unobserved changes in the neural network cannot be answered now. For this, it would be necessary to compare single rPCI values with some overall measure of epileptiform activity in the recorded data shortly before and after the test stimulation.

The rPCI measurement was already used by Kalitzin and colleagues to help them find the seizure onset site in epileptic patients, and may lead to the ability to predict the occurrence of seizures [20]. As shown here, the method can be adapted to the kainate model of the rat. It results in higher rPCI values for channels with more epileptiform activity. The possibility to predict the occurrence of seizures remains to be analyzed.

Further experiments, over longer time intervals with more recording sites, need to be performed in order to validate the possibilities of this measurement. However, the results obtained thus far are promising that we may gain a method that would help the validation of therapeutic approaches in epilepsy treatment.

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