

Feasibility of Recording High Frequency Oscillations with Tripolar Concentric Ring Electrodes during Pentylentetrazole-induced Seizures in Rats

Oleksandr Makeyev, *Member, IEEE*, Xiang Liu, *Student Member, IEEE*, Liling Wang, Zhenghan Zhu, Aristides Taveras, Derek Troiano, Andrei V. Medvedev, *Member, IEEE*, and Walter G. Besio, *Senior Member, IEEE*

Abstract— As epilepsy remains a refractory condition in about 30% of patients with complex partial seizures, electrical stimulation of the brain has recently shown potential for additive seizure control therapy. Previously, we applied noninvasive transcranial focal stimulation via novel tripolar concentric ring electrodes (TCREs) on the scalp of rats after inducing seizures with pentylentetrazole (PTZ). We developed a close-loop system to detect seizures and automatically trigger the stimulation and evaluated its effect on the electrographic activity recorded by TCREs in rats. In our previous work the detectors of seizure onset were based on seizure-induced changes in signal power in the frequency range up to 100 Hz, while in this preliminary study we assess the feasibility of recording high frequency oscillations (HFOs) in the range up to 300 Hz noninvasively with scalp TCREs during PTZ-induced seizures. Grand average power spectral density estimate and generalized likelihood ratio tests were used to compare power of electrographic activity at different stages of seizure development in a group of rats ($n = 8$). The results suggest that TCREs have the ability to record HFOs from the scalp as well as that scalp-recorded HFOs can potentially be used as features for seizure onset detection.

I. INTRODUCTION

Epilepsy is a neurological disorder that affects approximately one percent of the world population [1] and in about one third of patients with complex partial seizures antiepileptic drug therapy is ineffective [2]. Recently, electrical stimulation of the brain has shown promise in reducing seizure frequency. Different forms of noninvasive electrical stimulation including transcranial magnetic stimulation [3], [4] and transcranial direct current stimulation [5] have received increasing attention compared to implantable techniques. Yet, as previously concluded in a review of various brain stimulation techniques, the best

structures to stimulate and the most effective stimuli to use are still unknown [6].

Concentric ring electrodes (CREs) have unique capabilities. They perform the second spatial derivative, the Laplacian, on the surface potentials. Previously, we have shown that tEEG, Laplacian electroencephalography (EEG) with the tripolar CRE (TCRE) configuration, is superior to conventional EEG with disc electrodes since tEEG has significantly better spatial selectivity, signal-to-noise ratio, localization, approximation of the analytical Laplacian, and lower mutual information [7]-[9].

Unlike electrical stimulation via conventional disc electrodes applied across the head, transcranial electrical stimulation via the CRE has a much more uniform current density [10] and focuses the stimulation directly below the electrodes. In our previous works, we have achieved promising results using transcranial focal stimulation (TFS) via TCRE to attenuate acute seizures in common animal models induced by pilocarpine [11], penicillin [12], and pentylentetrazole (PTZ) [13]-[15]. For a pilocarpine-induced status epilepticus, TFS attenuated electrographic seizure activity and halted the progression of behavioral seizures significantly enhancing the survival of rats [11]. TFS also significantly decreased severe penicillin induced myoclonic jerks (MJs) in number and duration [12]. Finally, for the PTZ-induced model, a significant increase in synchrony within the beta-gamma frequency bands during seizures was demonstrated as well as the ability of TFS to significantly reduce this synchrony [13]. We also found that TFS caused reductions of both power of electrographic seizure activity [14] and duration of behavioral myoclonic activity [15]. For all these models, TFS was triggered manually immediately after the first MJ was observed. In a recent study, we proposed a cumulative sum (CUSUM) seizure detecting algorithm to trigger automatic application of TFS [16]. The CUSUM algorithm was evaluated on pre-recorded data and detected the electrographic seizure activity in all experiments well in advance of the behavioral seizure activity.

As the next fundamental step, we demonstrated feasibility of an automatic seizure control system through single and multiple TFS administrations in rats with PTZ-induced seizures [17], [18]. TFS was automatically triggered by a real-time electrographic seizure activity detector based on a disjunctive combination of CUSUM and generalized likelihood ratio test (GLRT). The detection methodology

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O. Makeyev, X. Liu, L. Wang, Z. Zhu, A. Taveras, D. Troiano, and W. G. Besio are with the Department of Electrical, Computer, and Biomedical Engineering, University of Rhode Island, Kingston, RI 02881, USA (phone: 401-874-9208; fax: 401-782-6422; e-mail: omakeyev@ele.uri.edu, liu@ele.uri.edu, lwang@ele.uri.edu, zhu.zhenghan1987@gmail.com, ataveras@my.uri.edu, derek_troiano@my.uri.edu, besio@ele.uri.edu).

A. V. Medvedev is with the Department of Neurology, Georgetown University, Washington, DC, USA (email: am236@georgetown.edu).

proposed in [16]-[18] was based on detecting the changes in signal power since in our previous works we found a significant increase in tEEG power corresponding to seizure onset [14], [16]. Frequency bands up to 30 Hz [14] and 100 Hz [16] were previously studied.

There is growing evidence that increased high frequency oscillations (HFOs) within several frequency bands >30 Hz may be indicative of either early seizure development [19]-[21] and/or tissue epileptogenicity [22]-[24]. Increased HFOs within the gamma band 30-80 Hz have been described in the *in vivo* kainic acid models by Medvedev et al [19] and suggested to form a pre-seizure state [20] as well as facilitate seizure propagation within the amygdalo-hippocampal network [21]. Also, HFOs in the ranges 80-250 Hz (ripples) and 250-500 Hz (fast ripples) have been considered as a marker of epileptogenic tissue [22]-[24]. While several animal models have been used to study HFOs including kainic acid [20], pilocarpine [25], and tetanus toxin [26] models, to the best of our knowledge, no results on HFOs in PTZ-induced seizures are currently reported. Moreover, conventional disc electrodes are very limited in their ability to record HFOs (along with other types of epileptiform activity) from the scalp and this is a major reason why intracranial recordings are currently used for better seizure localization during presurgical evaluations. The ability to record and detect HFOs from the scalp could be extremely useful for the proper diagnosis of epilepsy and more accurate localization of the epileptic focus.

In this preliminary study we assess feasibility of recording HFOs noninvasively using TCRES during PTZ-induced seizures in rats and evaluate their potential as features for seizure onset detection.

II. METHODS

Approximately 24 h before the induction of seizures adult male Sprague-Dawley rats were anesthetized, their scalps were shaved, and prepared with abrasive gel. Three custom-designed TCRES [7] were placed on the scalp with conductive paste and adhered with dental acrylic cement. One recording TCRES (1.0 cm dia.) was centered on the top of the head. Two other recording TCRES (6 mm dia.) were placed bilaterally behind the eyes, but in front of the ears. A shorted 1.0 cm TCRES served as an isolated ground electrode attached on the top of the neck behind the ears. A schematic of the experimental setup is shown in Fig. 1.

On the following afternoon skin-to-electrode impedance was measured. After that tEEG and video recording were started. For tEEG derivation the EEG signals were preamplified (gain 100 and 0.3 Hz high pass filter) with a custom built preamplifier, amplified (gain 1000 and band pass of 1-300 Hz with the 60 Hz notch filter active), and digitized (16 bits, 1500 S/s). Next, two differential EEG signals from recording surfaces of TCRES were algebraically summed with the appropriate weights to give Laplacian derivation of the signal as reported previously by Besio et al [7]. Due to the acquisition software constraint on the maximum size of the recording file we could not record tEEG continuously at the sampling rate of 1500 samples per second for longer than 10 min. Therefore, three separate 10 min

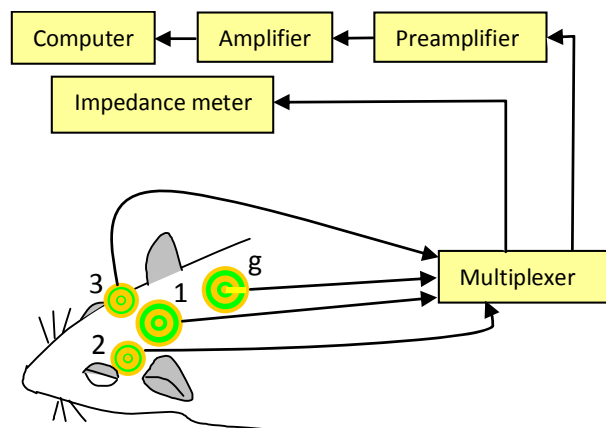


Fig. 1. Schematic representation of the experimental setup. Electrodes (1), (2), and (3) were used for recording. Shorted electrode (g) was the ground. A personal computer was used to control the system and store the data.

tEEG recording were made for each rat while the video was recorded continuously. After the first recording of 10 min baseline tEEG, the PTZ was administered (45 mg/kg, i.p.) and the second recording was started. The third recording was started 10 min after the end of the second one. For each rat data recorded from one TCRES was selected for further analysis based on the signal-to-noise ratio, skin-to-electrode impedance, and visual inspection for presence of artifacts. Prior to any further processing described below additional ± 1 Hz notch filters (4th order zero-phase Butterworth) were applied at 60 Hz and its harmonics up to and including 300 Hz.

Grand average power spectral density (PSD) estimate was calculated to compare different stages of seizure development. Three one minute long segments were processed for each rat. A "Baseline" segment was selected from each first recording during a period when the rats were relatively still for at least 1 min resulting in artifact free baseline tEEG. An "Ictal" segment was selected from the second recording starting 1 min before the first MJ. For two rats whom the first MJ's occurred in less than one minute after the PTZ injection (at 57 and 52 s respectively), the first minute after the injection was used as the Ictal segment. A "Postictal" segment was selected for each rat as the last minute of the third recording representing the 30th minute after the PTZ injection. Ictal segments were selected as described because they contained electrographic activity immediately preceding the first MJ. Since myoclonic jerks are the first clear behavioral seizure manifestation in this model, Ictal segments were not affected by strong seizure-induced movement artifacts during more advanced stages of behavioral manifestations (forelimb clonus, rearing and falling, etc) [27]. Postictal segments were selected in such a way to contain electrographic activity after the advanced stages of seizure are over but the rats have not quite yet resumed the normal activity with roaming and eating causing movement artifacts.

We used Welch's method to calculate PSD estimates since it reduces noise in the estimated power spectra compared to the standard periodogram approach [28]. First, for each rat the tEEG segments were de-meant and digitally

filtered 1–300 Hz. Next, Welch’s method was used to calculate the PSD estimates for each segment with the following parameters: the Hamming window size and the number of points for Fast Fourier Transform equal to 1024 with 50% window overlap. Finally, for Baseline, Ictal and Postictal segments PSD estimates were averaged over all animals to produce grand average estimates. For two animals that expired during the seizure and did not have Postictal segments, only Baseline and Ictal segments were used.

The GLRT was used to compare the power of electrographic activity between Baseline, Ictal, Postictal segments in each rat to assess the significance of differences [29]. As for the PSD estimates, for two animals that expired during the seizure only the Baseline and Ictal segments were compared. Different frequency bands were analyzed including: 1-30 Hz (delta, theta, alpha, and beta activities), 30-80 Hz (gamma activity), 80-250 Hz (ripples), and 250-300 Hz (a part of the fast ripple band) as well as the complete spectra (1-300 Hz). For each band, segments were digitally band pass filtered and de-meaned. Due to the zero mean signal value for each segment (as a result of de-meaning), the total spectral power of the segments was equal to their sample variance and, assuming segments to be white Gaussian noise with unknown variance, the GLRT test hypotheses were defined as follows: under the null hypothesis, the variances of two segments being compared were equal i.e., no difference in power in the current frequency band. The three alternative hypotheses were: the variance of the Ictal segment is higher than the variances of both the Baseline and the Postictal segments as well as the variance for the Postictal segment is higher than the variance for the Baseline segment. In the test statistic variance was replaced by its maximum likelihood estimate. The results were averaged for all the rats.

III. RESULTS

The grand average PSD estimate for the group of animals ($n = 8$) is presented in Fig. 2. It can be seen that administration of PTZ caused an increase in tEEG power (comparison of Baseline and Ictal traces) which is expected since PTZ induces high frequency electrographic spiking activity. When seizure activity ceased or was weaker, the PSD was reduced towards the pre-seizure Baseline (comparison of Ictal and Postictal traces). In both cases the difference appears to be distributed evenly across the spectrum including the HFO bands of gamma activity (30-80 Hz), ripples (80-250 Hz) and fast ripples (250-300 Hz).

The GLRT results for the significance of difference in power between three categories of segments are presented in Table 1. GLRT was applied to pairs of segment sample variances for each rat and the percentage of animals in the group ($n = 8$) showing a significant ($p = 0.01$) difference in power was calculated. It can be seen from Table 1 that the Ictal power was significantly higher than the Baseline power for 100% of the animals in all the frequency bands. At the same time the results obtained for the rest of the comparisons (Baseline vs. Postictal and Postictal vs. Ictal) are not as conclusive, especially so for the HFO ripples and fast ripples with as low as 66.67% of animals showing a significant

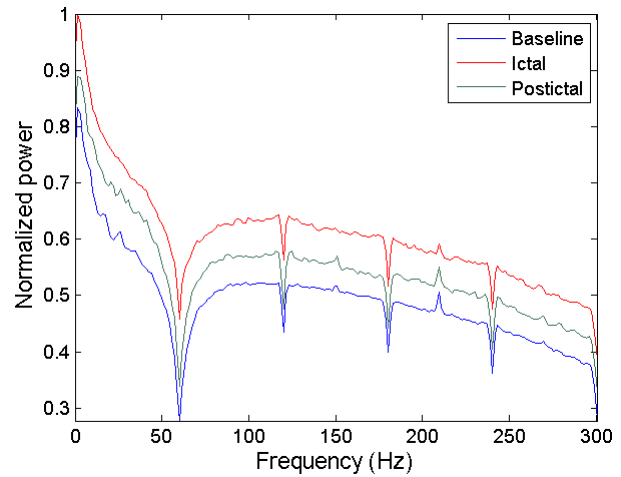


Fig. 2. Grand average power spectral density estimates.

difference. Still, these results suggest that in the majority of the animals the power of the Ictal segment is significantly ($p = 0.01$) higher than the power of the Postictal segment which, in its turn, is significantly ($p = 0.01$) higher than the power of the Baseline segment.

IV. DISCUSSION

The results obtained in this study show a significant increase in tEEG power at the early stage of seizures (before the first MJ) which is consistent with our previous findings for frequency ranges 1-100 Hz [14], [16]. This increase is shown using the grand average PSD estimate (Fig. 2) and confirmed with GLRT (Table 1) for three categories of segments over the group of eight rats.

There are two important advantages of our approach for the selection of Baseline, Ictal, and Postictal segments. First, it was kept consistent for all the animals allowing one to average the PSD and GLRT results for the group. Second, the selection criteria for all three segments were defined to minimize the presence of movement artifacts. It is especially important for Ictal and Postictal segments that were selected before and after strong seizure-induced movement artifacts respectively.

Even though the obtained results are preliminary and further investigation is needed for confirmation, they indicate the feasibility of noninvasively recording HFOs with TCRES. As can be seen from Table 1, the power of the Ictal segments was significantly higher ($p = 0.01$) than the power of the

TABLE I
GENERALIZED LIKELIHOOD RATIO TEST RESULTS FOR SIGNIFICANCE OF DIFFERENCE IN POWER BETWEEN THREE CATEGORIES OF SEGMENTS.

Frequency band (Hz)	Animals with significant ($p = 0.01$) difference in power (%)		
	Baseline vs. Ictal	Baseline vs. Postictal	Postictal vs. Ictal
1-30	100	83.34	83.34
30-80	100	83.34	83.34
80-250	100	66.67	83.34
250-300	100	66.67	66.67
1-300	100	83.34	83.34

Baseline segments for all the animals in all frequency bands including the HFO frequency range (30-300 Hz) which may result in a useful feature for seizure onset detection in future studies. Moreover, in majority of the animals the power of Postictal segments in the same frequency range was higher than the power of Baseline segments. Taking into account that the Postictal segments represent the 30th minute after the injection of PTZ and that the only remaining behavioral seizure activity at this time point and for the current dose of PTZ is behavioral arrest intermittent with periods of normal activity, this may suggest the potential of using the increased tEEG power (in particular, in the HFO bands >30 Hz) as an electrographic biomarker for duration of seizure activity.

It should be pointed out that to the best of our knowledge these findings demonstrating the feasibility of recording HFOs with TCRES during PTZ-induced seizures are novel regarding both the detection of HFOs in the PTZ-induced seizure model and recording of HFOs with noninvasive electrodes from scalp. Our plans for future work include the development of automatic detection and classification approaches for HFOs recorded by TCRES and the assessment of their use for seizure onset detection.

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