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Transcranial Focal Stimulation via Concentric Ring Electrodes Reduced Power of Pentylenetetrazole-induced Seizure Activity in Rat Electroencephalogram

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***Abstract*— As epilepsy affects approximately one percent of the world population, electrical stimulation of the brain has recently shown potential for additive seizure control therapy. In this study we applied noninvasive transcranial focal stimulation (TFS) via concentric ring electrodes on the scalp of rats after inducing seizures with pentylenetetrazole (PTZ) to assess the effect of TFS on the electrographic activity. Grand average power spectral densities were calculated to compare different stages of seizure development. They showed a significant difference between the TFS treated group and the control group. In case of the TFS treated group, after TFS, the power spectral density was reduced further towards a pre-seizure "baseline" than it was for the control group. The difference is the most drastic in delta, theta and alpha frequency bands. Application of general likelihood ratio test showed that TFS significantly ($p<0.001$) reduced the power of electrographic seizure activity in the TFS treated group compared to controls in more than 86% of the cases. These results suggest that TFS may have an anticonvulsant effect.**

I. Introduction

EPILEPSY is a neurological disorder that affects approximately one percent of the world population [1]. During recent years, electrical stimulation of the brain has shown promise in reducing seizure frequency. Noninvasive forms of stimulation receive increasing attention compared to implantable techniques with a growing body of research on different forms of noninvasive electrical stimulation including transcranial magnetic stimulation [2], [3] and transcranial direct current stimulation [4]. Yet, as previously concluded by Theodore and Fisher in a review of various brain stimulation techniques, the best structures to stimulate and the most effective stimuli to use are still unknown [5].

Concentric ring electrodes have unique capabilities. They perform the second spatial derivative, the Laplacian, on the surface potentials.

Previously we have shown that Laplacian electroencephalography (EEG) with the tripolar concentric ring electrode configuration is superior to conventional EEG with disc electrodes because the former has significantly better spatial selectivity, signal-to-noise ratio, localization, approximation of the analytical Laplacian, and mutual information [6]-[8].

When electrical stimulation is administered via the concentric ring electrodes, unlike conventional electrical stimulation where stimulation is usually applied across the head, TFS via concentric ring electrodes has a much more uniform current density and focuses the stimulation directly below the electrodes. In a pilot study, we have achieved promising results using TFS to attenuate acute seizures in a pilocarpine-induced status epilepticus (SE) model [9]. Namely, it was shown that TFS via tripolar concentric ring electrodes attenuated electrographic seizure activity and halted the progression of behavioral seizures. Moreover, interruption of seizure activity appeared to be long-lasting and TFS treatment significantly extended life and enhanced the survival of rats after SE.

To further validate the effect of TFS the authors extended the prior work by testing the effect of TFS via concentric ring electrodes in a second animal model - a pentylenetetrazole (PTZ) induced rat seizure model, which is one of the most commonly used models for testing anticonvulsant effects [10]. As a first step, potential of TFS to reduce pathological synchronization of brain potential was studied [11]. Cross-channel coherence was used to measure synchrony changes at particular frequency bands in electrographic activity recorded from three tripolar concentric ring electrodes on the rat scalp. It was applied to EEG segments recorded during the (a) preictal stage, (b) after administration of PTZ, and (c) immediately after application of TFS. A significant increase in synchrony within the beta-gamma frequency bands during seizures was demonstrated as well as the potential of TFS to significantly reduce this synchrony.

As the next fundamental step, in this study, we continue using TFS via concentric ring electrodes on the scalp of rats after inducing seizure with PTZ to assess the effect of TFS on the electrographic activity.

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II. Methods

Our animal protocol was approved by the IACUC. Approximately 24 h before the induction of seizures, an adult male 220~320 g Sprague-Dawley rat was given a combination of 80 mg/kg of ketamine and 12 mg/kg xylazine (i.p.) for anesthesia. The rat scalp was shaved and prepared with NuPrep abrasive gel (D. O. Weaver & Co., Aurora, CO, USA.). Three custom-designed tripolar concentric ring electrodes [6] were applied to the rat scalp using conductive paste (0.5 mm Ten20, Grass Technologies, RI, USA.) and adhered with Teets dental acrylic (Pearson Lab Supply, Sylmar, CA, USA.). A schematic of the experimental setup is shown in Fig. 1.

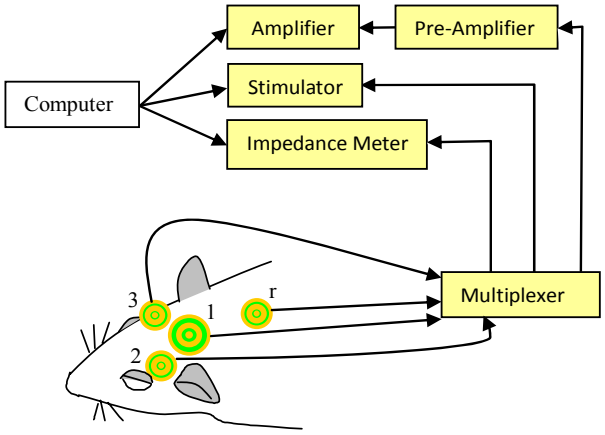

Fig. 1. Schematic representation of the experimental setup. The TFS was applied between the outer ring and the middle disc of electrode (1). Electrodes (1), (2), and (3) were used for recording. Electrode (r) was the reference. A personal computer was used to control the system and store the data.

One tripolar concentric ring electrode (1) (diameter = 1.0 cm with the width of each ring being 0.9 mm), used to record from and stimulate primarily the cerebral cortex, was centered on the top of the head. The front edge of the electrode was placed as close to the bregma as possible. Two other recording electrodes (2, 3) (diameter = 6 mm, ring width 0.4 mm) were placed bilaterally behind the eyes, but in front of the ears closer to the subcortical structures such as the hippocampus, thalamus, and midbrain than electrode (1) (A 2.0 mm, L 9.0 mm relative to the central electrode). A reference electrode (r) was attached on the top of the neck behind the ears. The electrodes were made of gold-plated copper. Rats were returned to their cages and allowed food and water ad libitum for approximately 24 hours until the experimental procedure began.

On the following afternoon the rats were placed in a transparent plastic cage and the electrodes via a commutator and cables (Plastics One, Roanoke, VA,) were connected to a manual multiplexer. The multiplexer was first set to connect the electrodes to a Prep-Check Plus EIM-107 (General Devices, Ridgefield, N.J.) to measure skin-to-electrode impedance. If the impedance for the outer ring and middle disc of the 1.0 cm dia. electrode (1) on Fig. 1 was less than 10 KΩ the rat was given TFS (n = 6). If the outer ring or center disc skin-to-electrode impedance for electrode (1) to the reference electrode (r) was greater than 10 KΩ, but less than 25 KΩ, the rat did not receive TFS and was put into the control group (n = 5). Lower impedances for TFS treated group ensured effectiveness of TFS. The EEG and the video recordings were then started. After five minutes of baseline EEG recording the PTZ was administered (55 mg/kg, i.p.). To determine when to administer the TFS, the rat behavioral activity was closely monitored and scored for seizure-related phenomena according to a scheme adapted from Mirski *et al.* [12]. The TFS (300 Hz, 50 mA, 200 μs, biphasic square pulses for 2 minutes) was administered immediately after the first myoclonic jerk was observed, which corresponded to a seizure activity level of R = 3.

The EEG signals were preamplified (gain 100 and 0.3 Hz high pass filter) with a custom built preamplifier and then amplified using a Grass Model NRS2 Neurological Research System with Model 15A54 AC amplifiers (Grass Technologies, West Warwick, RI, USA) with a gain of 1000 and band pass of 1.0–100 Hz with the 60 Hz notch filter active, and digitized (16 bits, 256 S/s). Two differential signals from each electrode were combined with an algorithm to give Laplacian derivation of the signal as reported previously by Besio [6]. For each rat data recorded from one electrode was selected for further analysis based on the signal-to-noise ratio, skin-to-electrode impedance and visual inspection for presence of artifacts. All the signal processing was performed using Matlab (Mathworks, Natick, MA, USA).

Grand average PSD estimates were calculated to compare different stages of seizure development. Three thirty-second long segments were processed for each rat. These segments were selected in the same way for control and TFS treated groups. In both groups the "Baseline" segment was selected during the time period when the rats were relatively still for at least 30 seconds resulting in artifact free baseline EEG. The "Pre-TFS" and "Post-TFS" segments were selected starting 30 s before and 2.5 min after the first R = 3 myoclonic jerk, respectively. Shifts of up to 10 s were allowed to obtain the most artifact-free segments possible. Pre-TFS segments were selected in such a way to contain the electrographic activity that preceded the first R = 3 myoclonic jerk that was detected as the cue to turn the TFS on for the TFS treated group. Post-TFS segments were selected in such a way to let the amplifiers recover from the application of 2 min long TFS for the TFS treated group and to start as soon after the amplifiers recover as possible before the rats started roaming and eating causing movement artifacts. For the control group the Post-TFS segment was emulating the time after the application of TFS would have been stopped for a TFS treated rat. We used Welch's method to calculate PSD estimates since it allows reducing noise in the estimated power spectra compared to standard periodogram approach [13]. First, for each rat the Laplacian segment's means were subtracted and filtered 1.0–30 Hz. Next, Welch's method was used to calculate the PSD estimates for each segment with the following parameters: the window size and the number of points for Fast Fourier Transform equal to 512, 50% window overlap, Hamming

window function. Finally, for Baseline, Pre-TFS and Post-TFS segments PSD estimates were averaged together to produce grand average estimates for all control and all TFS treated rats respectively.

General likelihood ratio test (GLRT) was used to compare the average power of electrographic seizure activity in TFS treated and control groups [14]. Four minute long segments of data were processed for each rat. These segments were selected in the same way for both control and TFS treated groups: the beginning of the segment was 3 minutes after the first R = 3 myoclonic jerk. In this way we account for the duration of TFS and recovery of the amplifiers in case of TFS treated group. Since the number of samples in segments being compared has to be equal for GLRT, duration of all the segments was selected to be equal to the minimal duration from the beginning of the segment to the end of the recording for all the rats. Each extracted segment was band pass filtered, 1.0–30 Hz, and demeaned. Due to the mean subtraction the power of the segments was equal to their sample variance and assuming segments to be white Gaussian noise with unknown variance the test hypotheses were defined in the following way: under the null hypothesis variances of two segments corresponding to control and TFS treated rats respectively were equal meaning that TFS was not effective. The alternative being the variance for the segment corresponding to the TFS treated rat is less than the variance for the control rat. In the test statistic variance was replaced by its maximum likelihood estimate.

III. Results

Grand average PSD estimates for control and TFS treated groups are presented in Fig. 2 and Fig. 3 respectively. It can be seen from the figures that while administration of PTZ caused increases in power of EEG for both groups (comparison of Baseline and Pre-TFS traces) which is expected since PTZ induces high-frequency electrographic spiking activity, there is a significant difference between the TFS treated group and the control group. For the TFS treated group, after TFS, the power spectral density was reduced further towards a pre-seizure "baseline" than it was for the control group. The difference can be seen mostly in

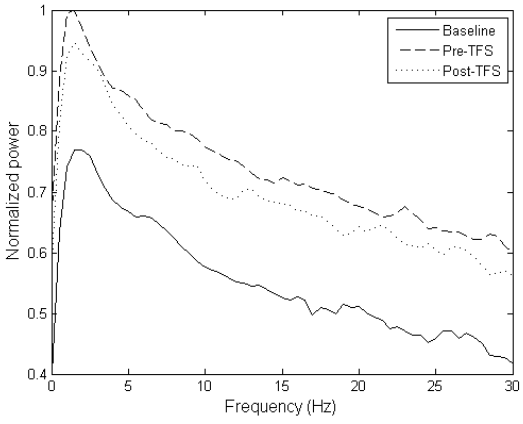

Fig. 2. Grand average PSD estimates for the control group.

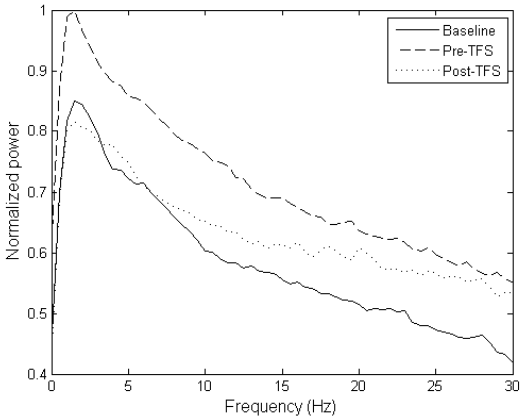

Fig. 3. Grand average PSD estimates for the TFS treated group.

delta (1 - 4.5 Hz), theta (5 – 8.5 Hz), and alpha (9 – 13.5 Hz) frequency bands (comparison of Pre-TFS and Post-TFS traces).

The signal power calculated on per second basis for segments corresponding to control and TFS treated groups are presented in Fig. 4 and Fig. 5 respectively. GLRT was applied to pairs of segment sample variances corresponding to control and TFS treated groups and the results were averaged for all the pairs. The results suggest that TFS significantly ($p<0.001$) reduced the power of electrographic seizure activity in the TFS treated group compared to controls in 86.67% of the cases. It can be seen from the six traces in Fig. 5 from the TFS treated rats that (*F*) has significantly higher signal power compared to the rest of the group. Examination of the video recording for this rat revealed that TFS was started too early, approximately 30 s before the actual first R = 3 myoclonic jerk. Since it is clear not to what extent premature initiation of TFS may have influenced its anticonvulsant effect we didn't exclude rat *F* from the dataset. However, recalculating the GLRT without this rat raises the percentage of cases where TFS has significantly ($p<0.001$) reduced the power of electrographic seizure activity in the TFS treated group compared to controls to 96%.

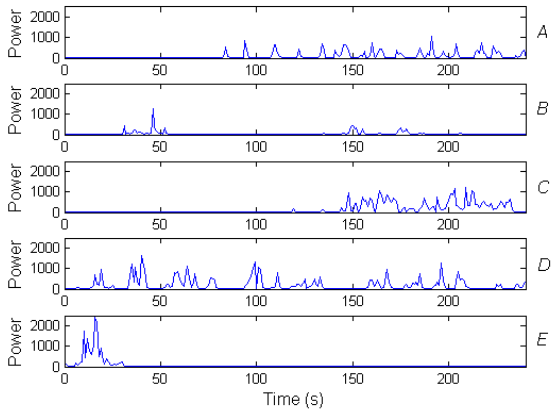

Fig. 4. Signal power calculated on per second basis for data segments corresponding to control group (n = 5; letters *A*-*E* denote individual rats).

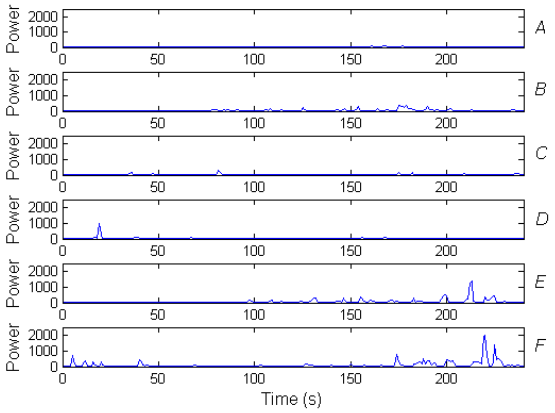

Fig. 5. Signal power calculated on per second basis for data segments corresponding to TFS treated group (n = 6; letters *A-F* denote individual rats).

IV. Discussion

In the previous study [9], we used a pilocarpine-induced SE model which represents an extreme form of seizures. Because of the severity and prolonged nature of the seizures, this model is not the best candidate for the parametric testing of anticonvulsant effect of electrical stimulation which is a key direction of our work. Thus, for the current study, we chose the PTZ seizure model, one of the most commonly used models for testing anticonvulsant effects and a rapid and efficient measure of both seizure susceptibility and screening of new drugs [10].

Our present results show that after the application of TFS, the power of the electrographic activity of the TFS treated rats reduced further towards the "baseline" than for the control group suggesting that TFS may have an anticonvulsant effect (Post-TFS traces in Fig. 2 and Fig. 3). An important advantage of our approach to selection of Pre-TFS and Post-TFS segments was that keeping it consistent for both the control and TFS treated groups allows one to draw a direct comparison between corresponding traces. However, this approach has significant disadvantages. First, starting the Post-TFS segment as soon as possible to minimize movement artifacts may not have allowed enough time for the amplifiers to recover after application of TFS for the TFS treated rats causing high frequency noise. We believe that this noise is attributing to higher power in the beta (14 – 31.5 Hz) frequency band of Post-TFS trace in Fig. 3. Second, as can be seen in Fig. 4 and Fig. 5, electrographic activity caused by PTZ-induced seizures is highly variable with periods of intense spiking activity interchanging with periods of absence with very low activity intensities. Strict guidelines for selection of Post-TFS segment make them vulnerable to being selected for the absence periods making them less representative of the induced seizure activity. The only way to reduce the effect of this factor is to use grand average PSD estimates as was done in this study. Still, for the control rats, which were not treated with TFS, we expect the seizure electrographic activity to continue until PTZ diminishes so that it no longer interferes with brain activity. We believe that selection of Post-TFS falling completely or partially on the periods of absence for rats in control group may have attributed to the resulting reduced power of the Post-TFS trace compared to the Pre-TFS trace in Fig. 2. For conclusive proof that TFS significantly reduced the power of electrographic seizure activity in the TFS treated group compared to controls GLRT was used on significantly larger (4 min) segments of data.

As the next step behavioral manifestations of seizures should be considered and compared for TFS treated and control groups including the number of myoclonic jerks, duration of seizure and seizure activity level. Another direction of future work is determining the specific mechanisms of action of TFS via concentric ring electrodes.

References

[1] J. W. Sander, "The epidemiology of epilepsy revisited," *Curr. Opin. Neurol.*, vol. 16, no. 2, pp. 165-170, April 2003.

[2] C. A. Tassinari, M. Cincotta, G. Zaccara, and R. Michelucci, "Transcranial magnetic stimulation and epilepsy," *Clinical Neurophysiology*, vol. 114, pp. 777–798, May 2003.

[3] W. H. Theodore, K. Hunter, R. Chen, F. Vega–Bermudez, B. Boroojerdi, P. Reeves–Tyer, K. Werhahn, K. R. Kelley, and L. Cohen, "Transcranial magnetic stimulation for the treatment of seizures: A controlled study," *Neurology*, vol. 59, no. 4, pp. 560-562, August 2002.

[4] F. Fregni, S. Thome-Souza, M. A. Nitsche, S. D. Freedman, K. D. Valente, and A. Pascual-Leone, "A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy," *Epilepsia*, vol. 47, no. 2, pp. 335–342, February 2006.

[5] W. Theodore and R. Fisher, "Brain stimulation for epilepsy," *The Lancet*, vol. 3, no. 2, pp. 111-118, February 2004.

[6] W. Besio, K. Koka, R. Aakula, and W. Dai, "Tri-polar concentric electrode development for high resolution EEG Laplacian electroencephalography using tri-polar concentric ring electrodes," *IEEE Trans. BME.*, vol. 53, no. 5, pp. 926-933, May 2006.

[7] W. Besio, R. Aakula, K. Koka, and W. Dai, W, "Development of a tri-polar concentric ring electrode for acquiring accurate Laplacian body surface potentials," *Annals of Biomedical Engineering*, vol. 34, no. 3, pp. 426-435, March 2006.

[8] K. Koka and W. Besio, "Improvement of spatial selectivity and decrease of mutual information of tri-polar concentric ring electrodes," *J of Neuroscience Methods*, vol. 165, pp. 216–222, September 2007.

[9] W. Besio, K. Koka, and A. Cole, "Effects of noninvasive transcutaneous electrical stimulation via concentric ring electrodes on pilocarpine-induced status epilepticus in rats," *Epilepsia*, vol. 48, no. 12, pp. 2273–2279, December 2007.

[10] M. Sarkisian, "Overview of the current animal models for human seizure and epileptic disorders," *Epilepsy & Behavior*, vol. 2, pp. 201-216, June 2001.

[11] W. Besio, X. Liu, L. Wang, A. Medvedev, and K. Koka, "Transcutaneous electrical stimulation via concentric ring electrodes reduced pentylenetetrazole-induced synchrony in beta and gamma bands in rats," *International Journal of Neural Systems*, vol. 21, no. 2, pp. 139-149, April 2011.

[12] M. Mirski, L. Rossell, J. Terry, R. Fisher, "Anticonvulsant effect of anterior thalamic high frequency electrical stimulation in the rat," *Epilepsy Research*, vol. 28, pp. 89-100, September 1997.

[13] P. D. Welch, "The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms," *IEEE Transactions on Audio Electroacoustics*, vol. AU-15, pp. 70–73, June 1967.

[14] S. Kay, *Fundamentals of Statistical Signal Processing, Volume 2: Detection Theory*. Upper Saddle River: Prentice Hall, 1998, ch. 6.