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Effects of transcranial focal electrical stimulation via tripolar concentric ring electrodes on pentylenetetrazole-induced seizures in rats

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Summary
Purpose: To study the effects of noninvasive transcranial focal electrical stimulation (TFS) via tripolar concentric ring electrodes (TCRE) on the electrographic and behavioral activity from pentylenetetrazole (PTZ)-induced seizures in rats.
Methods: The TCREs were attached to the rat scalp. PTZ was administered and, after the first myoclonic jerk was observed, TFS was applied to the TFS treated group. The electroencephalogram (EEG) and behavioral activity were recorded and studied.
Results: In the case of the TFS treated group, after TFS, there was a significant ($p = 0.001$) decrease in power compared to the control group in delta, theta, and alpha frequency bands. The number of myoclonic jerks was significantly different ($p = 0.002$) with median of 22 and 4.5 for the control group and the TFS treated groups, respectively. The duration of myoclonic activity was also significantly different ($p = 0.031$) with median of 17.56 min for the control group versus 8.63 min for the TFS treated group. At the same time there was no significant difference in seizure onset latency and maximal behavioral seizure activity score between control and TFS treated groups.
Conclusions: TFS via TCREs interrupted PTZ-induced seizures and electrographic activity was reduced toward the ''baseline.'' The significantly reduced electrographic power, number of myoclonic jerks, and duration of myoclonic activity of PTZ-induced seizures suggests that TFS may have an anticonvulsant effect.

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Introduction

During recent years, electrical stimulation of the brain has shown promise in reducing seizure frequency. Implantable techniques such as the deep brain stimulation (DBS) (Chabardes et al., 2002; Vonck et al., 2002; Kerrigan et al., 2004; Usui et al., 2005; Velasco et al., 2005), the responsive neurostimulator (RNS) (Kossoff et al., 2004; Goodman et al., 2005), and the vagus nerve stimulation (VNS) (Ben-Menachem et al., 1994; The VNS Study Group, 1995; Handforth et al., 1998; George et al., 2000; Patwardhan et al., 2000), trigeminal nerve stimulation (Fanselow et al., 2000; DeGiorgio et al., 2003) have been widely studied.

Noninvasive forms of brain stimulation for epilepsy are also receiving increasing attention. There is a growing body of research on different forms of noninvasive electrical stimulation including transcranial magnetic stimulation (TMS) (Wassermann, 1998; Hallett, 2002; Theodore et al., 2002; Tassinari et al., 2003) and transcranial direct current stimulation (tDCS) (Fregni et al., 2006). Yet, as previously concluded by Theodore and Fisher (2004) in a review of various brain stimulation techniques, the best structures to stimulate and the most effective stimuli to use are still unknown.

Concentric ring electrodes have unique capabilities. They perform the second spatial derivative, i.e. the Laplacian, on the surface potentials. Previously we have shown that Laplacian electroencephalography (EEG) with the tripolar concentric ring electrode (TCRE) configuration (Fig. 1, panel A) has significantly better spatial selectivity, signal-to-noise ratio, localization, approximation of the analytical Laplacian, and mutual information than conventional EEG with disc electrodes (Besio et al., 2004a, 2006a, 2006b; Koka & Besio, 2007). These findings suggest that EEG with TCREs (tEEG) may be superior at detecting seizures or other neurological disorders than conventional EEG with disc electrodes.

Unlike electrical stimulation between two conventional disc electrodes applied across the head, electrical stimulation via concentric ring electrodes has a much more uniform current density (Wiley and Webster, 1982) and focuses the stimulation directly below the electrodes. Therefore, we call this form of stimulation transcranial focal electrical stimulation (TFS).

An important advantage of TFS is that it does not cause motor contractions as is common with electroconvulsive therapy, another form of transcranial electrical stimulation. The rats do not show signs of pain or aversion when TFS is applied via TCRE and continue to roam freely. The effects of TFS via concentric ring electrodes (CREs) on rat skin were quantitatively analyzed (Besio et al., 2010) through calculation of the temperature profile under the CRE and the corresponding energy density with electrical-thermal coupled field analysis using a three-dimensional multi-layer model. Infrared thermography was also used to measure skin temperature during electrical stimulation to verify the computer simulations. Histological analysis was performed to study cell morphology and characterize any resulting tissue damage. It was concluded that as long as the specified energy density applied through the CRE was kept below 0.92 ($A^2/cm^4\,s^{-1}$), the maximum temperature will remain within the safe limits and also within the limits of the melting point of conductive paste and provide a safe current density distribution. Effects of TFS via TCRE on rat cortical integrity were studied (Mucio-Ramirez et al., 2011). Histomorphological analysis was used to assess cortical areas below the TFS site for neuronal damage. Control and TFS treated animals were anaesthetized and transcardially perfused. The brains were removed, post-fixed, and cut into coronal sections. Slices were mounted on gelatinized slides, Nissl stained for brightfield analysis, and photographed with a microscope equipped with a digital camera. Images where digitized to grayscale and the integrated optical density was measured with densitometry software. No significant difference in integrated optical density values was found for control and TFS-treated rat brains and morphological analysis did not show any pyknotic neurons, cell loss or gliosis that might confirm any neuronal damage.

Previously Besio et al. (2007) achieved promising results using TFS to attenuate acute seizures in a pilocarpine-induced status epilepticus (SE) model. Pilocarpine is a cholinergic muscarinic agonist. After the application of TFS via concentric ring electrodes, the electrographic activity visually resembled the ''baseline'' activity and the behavioral seizure activity was reduced (Besio et al., 2007). The seizure activity still had not returned for two or more hours after the stimulation (Besio et al., 2007).

To further validate TFS Besio et al. 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. PTZ is a $GABA_A$ receptor antagonist allowing us to evaluate TFS on another mechanism of seizure induction. As a first step, TFS was shown to reduce pathological synchronization of PTZ-induced electrographic activity within the beta—gamma frequency bands (Besio et al., 2011). In this study we expand our analysis of the effect of TFS on both electrographic and behavioral activity and summarize our findings.

Methods

We now report on the use of longer electrographic activity time windows (15 min long) analyzed to confirm if TFS has long-lasting effects. For behavioral activity, four different metrics were used including the latency of the seizure onset, the number of myoclonic jerks (MJs), duration of myoclonic activity and the maximal behavioral seizure activity score to better assess the effect(s) of TFS.

Data collection

Data collection for this study was performed separately for two parts of the study corresponding to assessment of the effect of TFS on (1) electrographic activity (EA) and (2) behavioral activity (BA) respectively. The two parts of the study were conducted in two different laboratories and data collection protocols have differed slightly in such aspects as type and amount of anesthesia given and amount of PTZ given. These differences are specified in the corresponding sections of the manuscript. Because of this inconsistency in the data collection process the corresponding data were