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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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Abstract

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 towards 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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The authors confirm that they have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Keywords

Transcranial focal electrical stimulation; TFS; tripolar concentric ring electrode; TCRE; EEG; PTZ; seizure; epilepsy

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, Besio et al. 2006a, Besio et al. 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 in (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^4s^{-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 in (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. achieved promising results using TFS to attenuate acute seizures in a pilocarpine-induced status epilepticus (SE) model (Besio et al. 2007). 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 minutes 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 processed separately for the EA and BA parts of this study to avoid the possibility of inconsistency influencing the obtained results.

Animal preparation

Our animal protocol was approved by the University of Rhode Island IACUC. Approximately 24 h before the induction of seizures, an adult male 220~320g Sprague-Dawley rat was given either 80 mg/kg of ketamine and 12 mg/kg xylazine i.p. (EA) or 120 mg/kg of ketamine i.p. (BA) for brief anesthesia. The rat scalp was shaved and prepared with NuPrep abrasive gel (D. O. Weaver & Co., Aurora, CO, USA). Three custom-designed TCREs (Besio et al., 2006a) 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).

As shown in Fig. 1 panel A one TCRE (diameter = 1.0 cm), was centered on the top of the head (1). The front edge of the electrode was placed near the site that should be the bregma since we were not able to see it. The TFS (300 Hz, 50 mA, 200 μs, biphasic square pulses for 2 minutes) was administered once between the outer ring and the central disc of electrode (1). Two other TCRE recording electrodes (diameter = 0.6 cm) were placed bilaterally behind the eyes, but in front of the ears (A 2.0 mm, L 9.0 mm relative to the central electrode) on both sides of the head (2,3). An isolated ground electrode was attached on the top of the neck behind the ears (g). These particular electrode locations were chosen due to size constraints and brain anatomy of adult rats. The electrodes were made of gold-plated copper. The rat was returned to its cage and allowed food and water ad libitum for approximately 24 h until the experimental procedure began. All experiments were performed in the afternoon. Flow charts of the experimental procedures for the EA and BA parts of this study are presented in panels A and B of Fig. 2 respectively.

Electrographic activity

Recording system—The Laplacian 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).

tEEG—Two differential signals from each electrode (Fig. 1, panel B) were combined algorithmically for a tEEG derivation (Fig. 1, panel C) of the signal as reported previously in (Besio et al., 2006a). Briefly, the algorithm is two-dimensional and weights the middle ring and central disc difference sixteen times greater than the outer ring and central disc difference. For each rat the 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).

Power spectral density analysis—Grand average power spectral density (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 tEEG. The “pre-TFS” and “post-TFS” segments were selected starting 30 s before and 2.5 min after the first R = 3 MJ, respectively. 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. Shifts of up to 10 s were allowed to obtain the most artifact-free segments possible. For the TFS treated group, Pre-TFS segments were selected in such a way to contain the electrographic activity that preceded the first R = 3 MJ that was detected as the cue to turn the TFS on. Post-TFS segments were selected to account for a small delay due to manually changing the electrode from recording to stimulation and back, to let the amplifiers recover from the application of 2 min long TFS, and to start as soon after the amplifiers recover as possible before the rats started roaming and eating causing movement artifacts.

First, for each rat the tEEG segments were demeaned and filtered 1.0–30 Hz. Next, Welch’s method (Barbé et al. 2010) was used to calculate the PSD estimates for each segment

(window size and the number of points for Fast Fourier Transform equal to 256, 50% window overlap, Hamming window). Finally, PSD estimates were averaged to produce grand average estimates for all control and all TFS treated rats respectively.

Statistical analysis—The generalized likelihood ratio test (GLRT, Kay 1998) was used to compare the average power of electrographic seizure activity between the TFS treated and control groups. Since the exact values of power to be compared are generally unknown a composite hypothesis test is needed to accommodate unknown parameters. Although there is no optimality associated with the GLRT it is widely used in practice due to its ease of implementation and less restrictive assumptions (Kay 1998).

For each rat 900 s (15 min) long segments of data were processed. The segments for both control and TFS treated groups began 3 minutes after the first R = 3 MJ to account for the duration of TFS and recovery of the amplifiers (TFS treated group). Since the segments compared must be equal length for GLRT, the length of all segments was set equal to the minimal length of the recording for all the rats. Each extracted segment was de-meaned. By de-meaning the power of the segments was equal to their sample variance (Shiavi 2007). Assuming the segments were 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. The GLRT was applied to segment pairs of control and TFS treated groups verifying the difference in variance and the results were then averaged for all the pairs.

Behavioral activity

Behavioral activity metrics—After the administration of PTZ the following stages were used to score seizure-related behavioral activity (Mirski et al. 1997): R = 0, no seizure activity; R = 1, oral-facial movements only; R = 2, head nodding; R = 3, MJ; R = 4, forelimb clonus; R = 5, rearing; R = 6, severe clonic activity with rearing and falling. The latency for behavioral seizure activity was defined as the time to the first MJ, R = 3. The maximal R value and the total number of MJs were counted (first to last) for each animal as well as the duration of myoclonic activity (the duration was the time elapsed between the first and the last MJs). *Statistical analysis:* Given the non-normality of distribution (Ryan-Joiner test) of at least one of the two samples for all four behavioral seizure activity metrics the non-parametric unpaired Mann–Whitney U test was used to compare the results obtained for TFS and control groups.

RESULTS

Electrographic activity

A total of 10 TFS treated rats and 10 controls were used to assess the effect of TFS on electrographic seizure activity. Electrographic seizure activity preceded behavioral activity and appeared as clear short high-frequency bursts and sometimes continued after the behavioral activity had ceased. Figure 3 panel A shows 30 s segments of tEEG for a typical control rat. The top (blue) trace shows the “baseline” tEEG prior to the administration of PTZ, the middle (red) trace shows the PTZ-induced seizure activity just before the first R = 3 MJ and prior to the application of TFS, and the bottom (green) trace showing the tEEG 2.5 minutes after the first MJ emulating the segment just after the application of TFS would have been stopped for a TFS treated rat. Figure 3 panel B shows similar segments for a typical TFS treated rat. For the TFS treated rat, the bottom (green) trace is from 2.5 min after the first MJ immediately after the application of TFS.

As illustrated in panels A and B, the “baseline” (blue) traces are quiet while the administration of PTZ caused an increase in seizure activity in the tEEG of both rats (middle, red, traces). After the application of TFS, the seizure activity of the TFS treated rat diminished (bottom, green, trace in panel B). In contrast, the seizure activity of the control rat continued to persist (bottom, green, trace in panel A).

The corresponding PSD estimates for the traces shown in panels A and B are shown in panels C and D, respectively. The “baseline” (blue traces in both panels C and D) has the least power across the spectrum. After the administration of PTZ, the tEEG power of both rats increased (red traces in panels C and D). After the application of TFS, the power for the TFS treated rat is reduced toward the baseline (green trace in panel D). In contrast, the power of the tEEG higher frequencies for the control rat continued to increase (green trace in panel C).

Grand average PSD estimates for control and TFS treated groups are presented in Fig. 4. As in the case of individual rats, it can be seen from the figures that while administration of PTZ caused increases in power of the tEEG for both groups which is expected since PTZ induces high-frequency electrographic spiking activity, there is a difference between the TFS treated group and the control group. For the TFS treated group (Fig. 4, panel B), after TFS, the PSD was reduced further towards a pre-seizure “baseline” than it was for the control group (Fig. 4, panel A) in delta (1 - 4.5 Hz), theta (5 – 8.5 Hz), and alpha (9 – 13.5 Hz) frequency bands (comparison of red and green traces).

The signal power calculated on per second basis for segments corresponding to control and TFS treated groups are presented in panels A and B of Fig. 5 respectively. The GLRT results show that TFS significantly ($p = 0.001$) reduced the power of electrographic seizure activity in the TFS treated group compared to controls in 87% of the segment pairs.

Figure 6 provides an example of superimposition of the electrographic and the behavioral activities for two representative animals: one TFS-treated (panels A-B) and one control (panels C-E). Panels A and C are spectrograms of the tEEG signals with panel A representing signals from a rat who received TFS at the onset of PTZ-induced seizure (black block shows when the TFS was on). Panels B and D show the tEEG waveforms corresponding to the spectrograms in panels A and C respectively. The top row of panels A and C (turquoise background) shows when the PTZ was given (dark blue mark) as well as any behavioral seizure activity corresponding to stages $R \geq 3$ (red marks). It should be noted that the animals presented in Fig. 6 were originally used for the EA part of this study. As such they received a higher dose of PTZ than the rats from the BA part of the study (55 vs. 45 mg/kg respectively). Because of this, the animals for Fig. 6 experienced stronger seizures with faster progression from MJs to higher R stages like tonic-clonic seizures and, therefore, smaller numbers of MJs compared to results obtained in the BA study. For an accurate representation of behavioral activity in Fig. 6 all seizure activity corresponding to stages equal or higher than MJ ($R = 3$) was selected for inclusion in the figure.

As can be seen from Fig. 6 the TFS-treated rat had significantly shorter electrographic and behavioral seizure activities unlike the control rat who was having a tonic-clonic seizure for almost 800 seconds. The spectrogram for the control rat (panel C) shows that there was more power in the higher frequencies during tonic-clonic seizure than there was during the baseline or after the seizure stopped while the rat was roaming, chewing, and grooming. This is a clear sign that tEEG greatly attenuates severe movement/muscle artifacts. Panel E is an expanded portion of the tEEG in panel D during the tonic-clonic stage showing clear seizure activity and the lack of muscular and movement artifacts.

Behavioral activity

A total of 18 TFS treated and 20 control rats were used to assess the effect of TFS on behavioral seizure activity. Out of 20 controls, 14 rats were used twice, first as part of the TFS treated group and then as a control 1-2 days after the TFS treatment. This was done to minimize the effect of variability (resistance to PTZ, etc) among the rats of both groups and to allow the within-subject comparison.

Number of MJs—Figure 7 shows a comparison of the number of MJs between the control group (n = 20) and the TFS treated group (n = 18). The number of MJs was measured from the first MJ until the last. There was a statistically significant difference in the number of MJs with a median of 22 for the control group and 4.5 for the TFS treated group ($p = 0.002$).

Duration—Figure 8 shows a comparison of the durations of MJ activity in minutes between the control group (n = 20) and the TFS treated group (n = 18). The duration of MJs was time elapsed from the first MJ until the last. There was a statistically significant difference in duration with a median of 17.56 min for the control group and 8.63 min for the TFS treated group ($p = 0.031$).

Seizure onset latency and the maximal behavioral activity score—There was no statistically significant difference in seizure onset latency with a median of 108.5 s for the control group and 112 s for the TFS treated group ($p = 0.63$). Neither was there a statistically significant difference in the maximal behavioral activity score with a median of R = 5 for the control group and R = 4.5 for the TFS treated group ($p = 0.78$)

DISCUSSION

Anticonvulsant effect

Our present results show that TFS significantly reduced PTZ-induced seizures. After the application of TFS, the electrographic activity of TFS treated rats was reduced towards the “baseline”, suggesting that TFS may have an anticonvulsant effect. We expected the power to increase during the PTZ-induced seizures and be greater than the “baseline” activity because PTZ induces high-frequency electrographic spiking activity. This increased electrographic activity and the resultant increased power can be observed in both typical rats (red traces in Fig. 3 panels A/B and C/D, respectively) and group grand averages (red traces in Fig. 4 panels A/B). After applying TFS, the power of the electrographic activity of the TFS treated rats was reduced further towards the pre-seizure baseline levels compared to controls in delta, theta and alpha frequency bands. This reduction is evident in both typical rats (green traces in Fig. 3 panels A/B and C/D, respectively) and the group grand averages (green traces in Fig. 4 panels A/B). 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. Evidence of PTZ-induced seizure activity still persisting in the control rat can be clearly seen from the green traces in Fig. 3 panels A and C. Unsurprisingly, the power of the seizure electrographic activity for the control rat (green trace in Fig. 3 panel C) continued to rise due to the effect of the PTZ still enhancing the seizure.

TFS also led to a reduction in the number of MJs. This provides further evidence that TFS has an anticonvulsant effect. Figure 7 shows that the median number of MJs was 22 for the control group and 4.5 for the TFS treated group. The dramatic reduction in the number of MJs is not surprising because there was also a significant reduction in the duration of the myoclonic activity after TFS was applied, as illustrated in Fig. 8. At the same time there was no significant difference in seizure onset latency and the maximal behavioral activity score

between control and TFS treated groups. Figure 6 further illustrates the potential of TFS to reduce both electrographic and behavioral seizure activity showing their superimposition for typical TFS-treated and control rats.

Model selection

The PTZ seizure model is one of the most commonly used models for testing anticonvulsant effects (De Sarro 1999, Fisher 1989, Sarkisian 2001). PTZ, a $GABA_A$ antagonist, is well documented for eliciting traits from absence-like seizures to generalized tonic-clonic seizures (Fisher 1989). Depending upon the dose of PTZ given, it is possible to have any of several endpoints: arrest and staring, MJs, limbic seizures (facial and forelimb clonus with rearing), clonic convulsive seizures, and tonic extensor seizures and is used for seizure susceptibility and screening of new drugs (Sarkisian 2001). Small doses of PTZ given repeatedly provoke chemical kindling (De Sarro 1999).

Data segment selection

An important advantage of our approach to selection of 30 s segments preceding and following the application of TFS was consistency for both the control and TFS treated groups. Time-synchronized data segmentation allowed us to directly compare between corresponding traces. However, this data segmentation approach has significant disadvantages. First, starting the segment 2.5 min after the first MJ, in order to minimize movement artifacts, may not have allowed enough time for the amplifiers in some channels to recover after the 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 (green trace in Fig. 4 panel B). Second, as can be seen in Fig. 5 electrographic activity caused by PTZ-induced seizures is highly variable with periods of intense spiking activity interchanging with quiet periods with very low activity intensities. Strict guidelines for data segment selection causes vulnerability to selecting data segments partially or fully during quiet periods making them less representative of the induced seizure activity. The only way to reduce the effect of this nuisance factor is to use grand average PSD estimates as was done in this study. 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 (15 min long) segments of data.

Other noninvasive/minimally invasive brain stimulation techniques

The effect of TFS on seizures was first reported by Besio (Besio et al. 2004b). This original research was conducted with a intracisternal penicillin model in rats. Our current findings that TFS reduced the electrographic activity, duration, and number of MJs of PTZ-induced seizures in rats are consistent with the results previously achieved with pilocarpine-induced SE in rats (Besio et al. 2007).

Patwardhan et al. (2005) found that noninvasive electrical stimulation via ear bars captured penicillin-induced seizures in rats. Patwardhan et al. generated seizures to control seizures. The stimulation, via ear bars, also caused strong tonic activity.

Cathodal tDCS, applied on the skull of rats, was found by Liebetanz et al. (2006) to significantly alter the threshold for localized seizure activity (TLS) induced with a transcranial cortical ramp-stimulation. They found significant differences in the TLS, due to 30 minutes of tDCS applied on the skull prior to ramp-stimulation that lasted up to 90 minutes after stopping the tDCS. We have not tested TFS on the transcranial cortical ramp-stimulation model of focal epilepsy however we have found similar long lasting effects on PTZ-induced seizures. Fig. 8 illustrates how TFS decreased the duration of PTZ-induced myoclonic seizure activity which on average lasted 18 min in the control group. If TFS had

no lasting effect the mean duration would be the same for both groups. However, it appears that the short two-minute TFS lasts at least 18 min, nine times longer than it was applied. Similar to Liebetanz, Kamida et al. (2011) also used cathodal tDCS applied on the skull. They applied tDCS after administering pilocarpine-induced status epilepticus (SE) in immature rats. They found reductions in cell loss, cognitive impairment, and frequency of convulsions as a result of the stimulation. Kamida et al. applied tDCS for two consecutive weeks starting two days after the termination of SE. In contrast Besio et al. (2007) applied TFS for one or two minutes, on the scalp, 5 minutes after the onset of pilocarpine-induced SE which stopped or reduced electrographic and behavioral activity and was also long lasting.

Seizure reduction has also been reported using TMS on seizing rats. Akamatsu et al. (2001) found a significant increase in latency to PTZ-induced myoclonic and tonic-clonic behavioral activity due to 0.5 Hz repeated TMS (rTMS). Rotenberg et al. (2008) found that rTMS at frequencies of 0.5 and 0.75 Hz, manually triggered by the onset of EEG seizure activity, reduced the duration of individual PTZ-induced seizures. In contrast we administered TFS after the first MJ, when the seizure is quite severe, rather than before administering PTZ or after the first electrographic signs of seizures and found a significant reduction in the total duration of behavioral PTZ-induced MJs due to TFS.

Limitations of the methods

For the placement of the stimulation electrode (1) we are certain there are variations in where the electrodes are placed from rat-to-rat. There is no definite way, noninvasively, to determine exactly where the electrode is placed relative to brain structures. It is also unknown whether the TFS anticonvulsant effect is from stimulation of peripheral nerves in the skin, meninges, brain, or something else. We are not able to implant electrodes into the brain to conclusively determine when the electrographic seizure activity has ceased since that would compromise the scalp where the TCREs are attached and skull altering TFS pathways. Therefore, we use the end of MJ activity as endpoint for seizure duration.

Our findings suggest that TFS may have an anticonvulsant effect. In the present study TFS was administered after the first MJ was observed. In the future, it would also be of interest to analyze whether applying TFS before administering PTZ or at the first onset of PTZ-induced electrographic activity blocks the behavioral seizure activity and/or alters the progression of the electrographic activity.

Finally, we intended this paper to report the results on electrographic and behavioral aspects independently rather than to integrate and compare them as the direct comparison is not feasible in this case due to the use of two different groups of animals with different data collection protocols for two objectives of this study.

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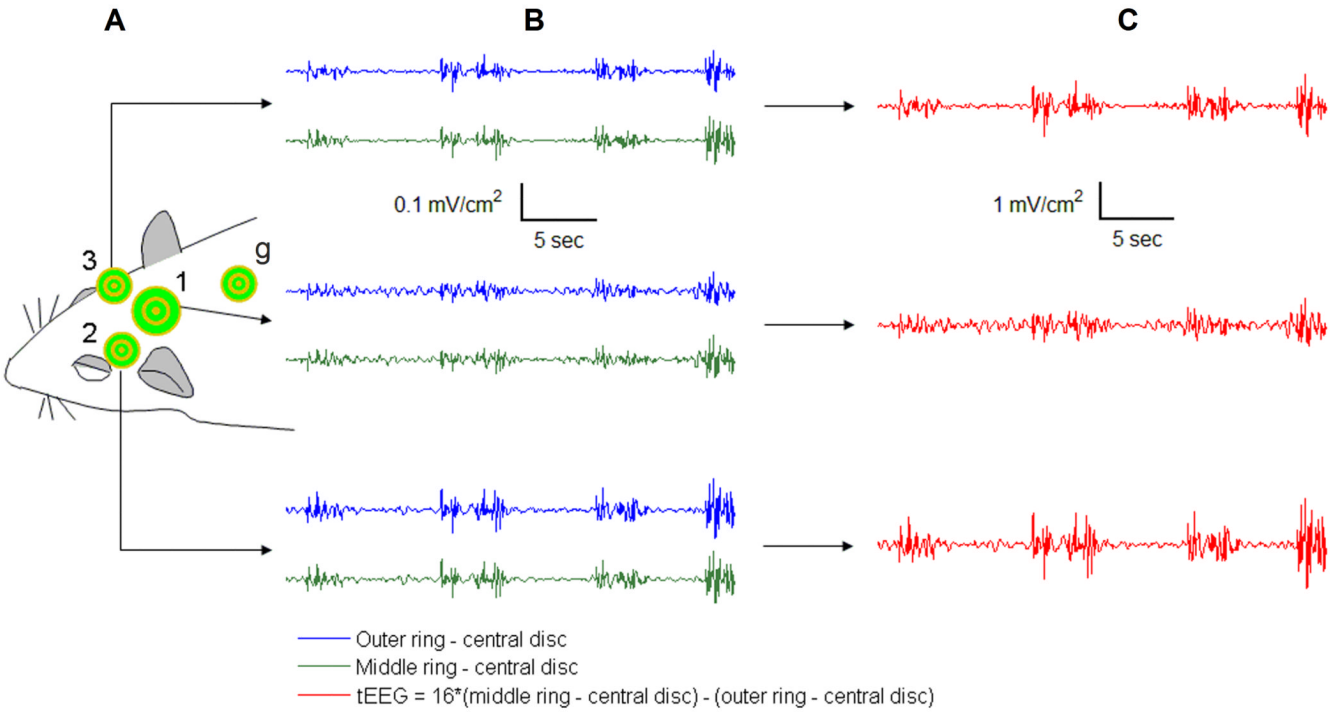

Figure 1.

Schematic representation of the electrode placement and Laplacian derivation of the acquired signal. The Laplacian algorithm is two-dimensional and weights the middle ring and central disc signal difference sixteen times greater than the outer ring and central disc signal difference. Panel A – TFS was applied between the outer ring and the central disc of electrode (1). Electrodes (1), (2), and (3) were used for recording. Electrode (g) was the isolated ground electrode. Panel B – two signal differences from each electrode: top (blue) outer ring and central disc signal difference and bottom (green) middle ring and central disc signal difference. Panel C – the resulting Lapacian tEEG outputs after combining the signals of panel B with the Laplacian algorithm (Besio et al. 2006a).

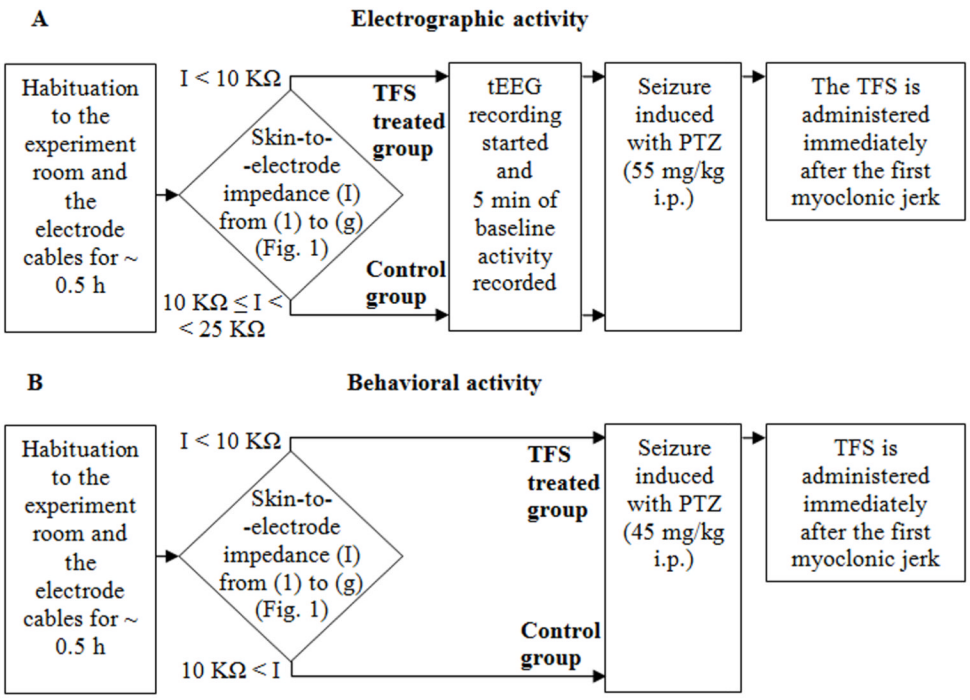

Figure 2.
Flow charts of the experimental procedures for the electrographic (A) and behavioral (B) activity parts of this study.

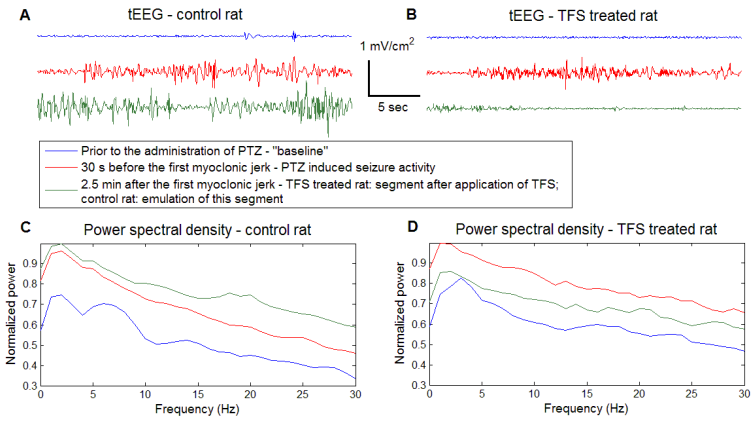

Figure 3.

Panel A – Control rat. Top (blue) trace: “baseline” tEEG prior to the administration of PTZ. Middle (red) trace: PTZ-induced seizure activity recorded 30 s before the first MJ and immediately prior to the application of TFS. Bottom (green) trace: tEEG recorded 2.5 min after the first MJ emulating the segment just after the application of TFS would have been stopped for a TFS treated rat. Panel B – TFS treated rat. Top (blue) trace: “baseline” tEEG prior to the administration of PTZ. Middle (red) trace: PTZ-induced seizure activity 30 s before the first MJ. Bottom (green) trace: tEEG recorded 2.5 min after the first MJ immediately after the application of TFS.

Panels C and D show PSD estimates for tEEG segments from panels A and D, respectively.

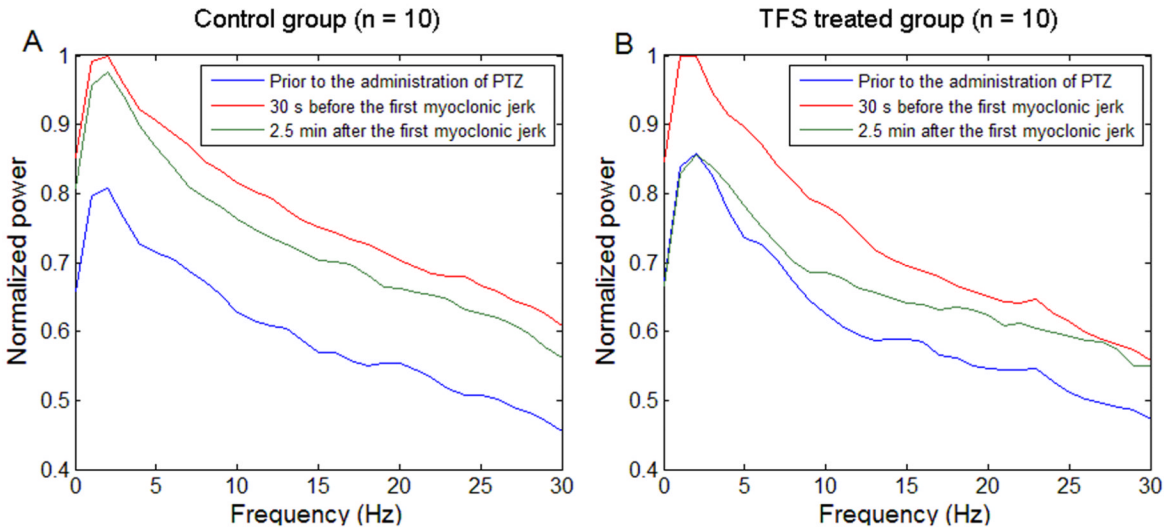

Figure 4.
Grand average PSD estimates for the control (A) and TFS treated (B) groups (n = 10 for each group).

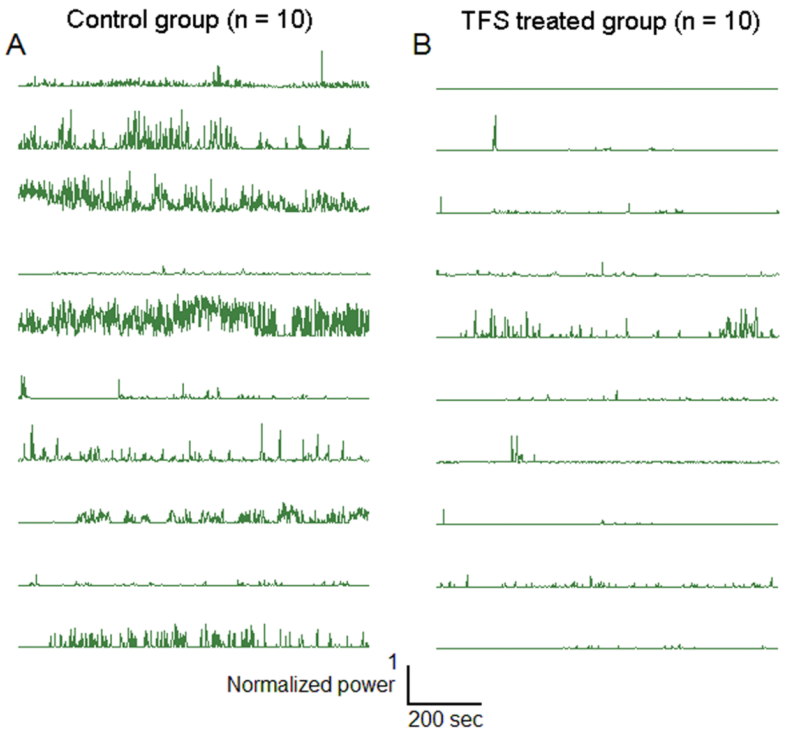

Figure 5.
Signal power calculated on per second basis for data segments corresponding to control (A) and TFS treated (B) groups (n = 10 for each group; traces correspond to individual rats).

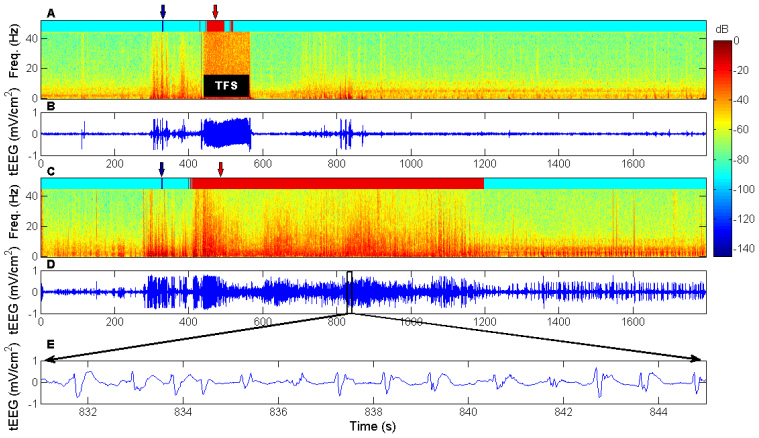

Figure 6.

Example of superimposition of the electrographic and the behavioral activities for two representative animals: one TFS-treated (panels A-B) and one control (panels C-E). Panels A and C – spectrograms of the tEEG signals with panel A representing signals from a rat who received TFS at the onset of PTZ-induced seizure (black block shows when the TFS was on). The top row of panels A and C (turquoise background) shows when the PTZ was given (dark blue mark and an arrow) as well as any behavioral seizure activity corresponding to stages R ≥ 3 (red marks and an arrow). Panels B and D – tEEG wavefroms corresponding to the spectrograms in panels A and C respectively. Panel E – an expanded portion of the tEEG in panel D during the tonic-clonic stage showing clear seizure activity and the lack of muscular and movement artifacts.

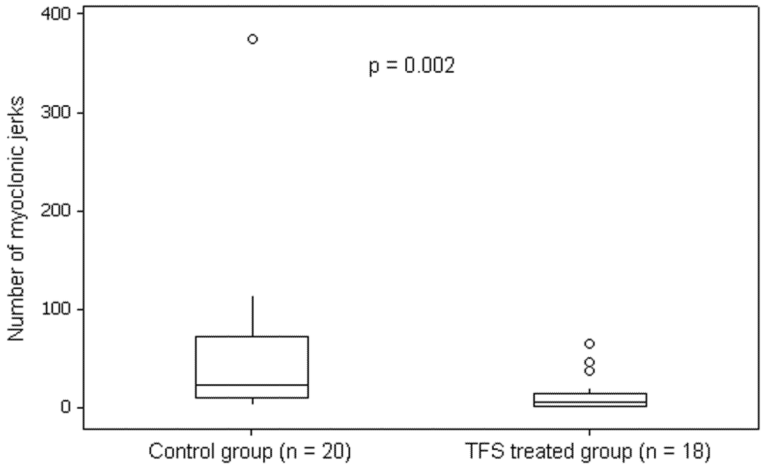

Figure 7.

After the application of TFS, the number of MJs in the TFS treated group (n = 18; sample minimum: 0, maximum: 65, mean: 12.28, first quartile: 1.25, third quartile: 10.75) reduced to a median of 4.5. This is significantly less (p = 0.002, Mann–Whitney U test) than the median of 22 for the control group (n = 20; sample minimum: 2, maximum: 374, mean: 53.95, first quartile: 9.75, third quartile: 69.75). Outliers, defined by Minitab, (version 16) are marked with open circles.

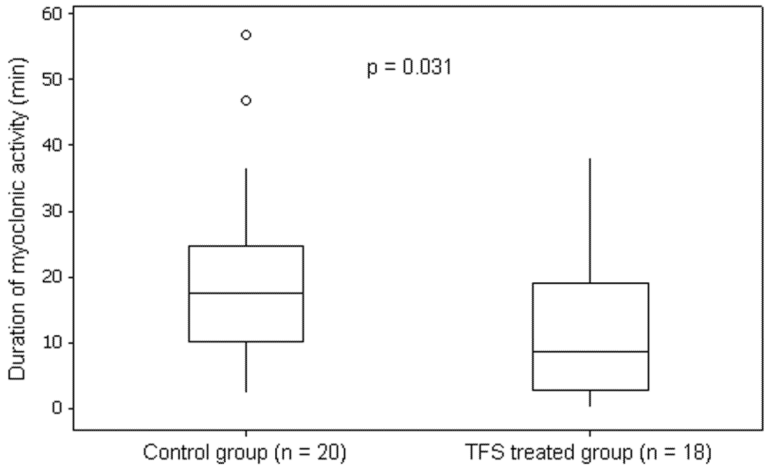

Figure 8.

After the application of TFS, the duration of myoclonic activity of PTZ-induced seizures in the TFS treated group (n = 18; sample minimum: 0, maximum: 38, mean: 10.95, first quartile: 3.08, third quartile: 17.67) reduced to a median of 8.63 min. This is significantly less (p = 0.031, Mann–Whitney U test) than the median of 17.56 min for the control group (n = 20; sample minimum: 2.17, maximum: 56.83, mean: 20.28, first quartile: 10.56, third quartile: 23.56). Outliers, defined by Minitab, (version 16) are marked with open circles.