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Noninvasive transcranial focal stimulation via tripolar concentric ring electrodes lessens behavioral seizure activity of recurrent pentylenetetrazole administrations in rats

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Abstract

Epilepsy affects approximately one percent of the world population. Antiepileptic drugs are ineffective in approximately 30% of patients and have side effects. We have been developing a noninvasive transcranial focal electrical stimulation with our novel tripolar concentric ring electrodes as an alternative/complementary therapy for seizure control. In this study we demonstrate the effect of focal stimulation on behavioral seizure activity induced by two successive pentylenetetrazole administrations in rats. Seizure onset latency, time of the first behavioral change, duration of seizure, and maximal seizure severity score were studied and compared for focal stimulation treated (n = 9) and control groups (n = 10). First, we demonstrate that no significant difference was found in behavioral activity for focal stimulation treated and control groups after the first pentylenetetrazole administration. Next, comparing first and second pentylenetetrazole administrations, we demonstrate there was a significant change in behavioral activity (time of the first behavioral change) in both groups that was not related to focal stimulation. Finally, we demonstrate focal stimulation provoking a significant change in seizure onset latency, duration of seizure, and maximal seizure severity score. We believe that these results, combined with our previous reports, suggest that transcranial focal stimulation may have an anticonvulsant effect.

Index Terms

Epilepsy; pentylenetetrazole; noninvasive transcranial focal electrical stimulation; tripolar concentric ring electrode; seizure

I. Introduction

Epilepsy is a neurological disorder that affects approximately one percent of the world population with up to three-fourths of all people with epilepsy living in developing countries [1]. Over 50 million people worldwide are affected by epilepsy. Anti-epileptic drugs are ineffective in up to 30% of persons with epilepsy and can cause side effects [2]. Surgery is another option available, but carries risks and many persons are not candidates [3].

Electrical brain stimulation has shown promise in reducing seizure frequency. Implantable techniques such as deep brain stimulation (DBS) [4–8], responsive neurostimulation (RNS) [9, 10], vagus nerve stimulation (VNS) [11–15], and trigeminal nerve stimulation (TNS) [16, 17] have been widely studied. Noninvasive forms of brain stimulation for epilepsy are gaining acceptance. There is a growing body of research on different forms of noninvasive

electrical stimulation including transcranial magnetic stimulation (TMS) [18–21] and transcranial direct current stimulation (tDCS) [22]. 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 [23].

Concentric ring electrodes (CREs) have unique capabilities. They perform the second spatial derivative, the Laplacian, on the scalp potentials. Previously we have shown that tEEG, Laplacian electroencephalography (EEG) with the tripolar concentric ring electrode (TCRE) configuration is superior to conventional EEG with disc electrodes since the tEEG has significantly better spatial selectivity, signal-to-noise ratio, localization, approximation of the analytical Laplacian, and mutual information [24–26]. Also, stimulation via CREs is unique. Unlike electrical stimulation via conventional disc electrodes that is usually applied across the head, transcranial electrical stimulation via CREs has a much more uniform current density [27] and focuses the stimulation directly below the electrodes. Therefore, we call this form of stimulation transcranial focal 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 CREs on rat skin were quantitatively analyzed in [28] 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 [29]. 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.

Most importantly, promising results using TFS to attenuate acute seizures in a pilocarpine-induced status epilepticus (SE) model have been previously achieved by our group [30] where TFS via TCREs attenuated electrographic seizure activity toward baseline and halted the progression of behavioral seizures. Moreover, interruption of the seizure activity appeared to be a long-lasting effect and the TFS treatment significantly enhanced the survival of rats after SE. More experiments showed that TFS, after severe penicillin-induced [31] myoclonic jerks, significantly decreased their number and duration.

For further validation TFS was used in a third animal model, the pentylenetetrazole (PTZ) model, widely used for testing both seizure susceptibility and screening of new antiepileptic drugs [32]. As a first step, the potential of TFS to reduce pathological synchronization of PTZ-induced electrographic activity was studied [33]. Cross-channel coherence was used to measure synchrony changes at particular frequency bands in electrographic activity recorded from TCREs on the rat scalp. Cross-channel coherence was performed on tEEG segments recorded (a) during the pre-seizure 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.

Next, the potential of TFS to reduce power of PTZ-induced electrographic seizure activity was studied [34]. 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. For the TFS-treated group, after TFS, the tEEG 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. The application of general likelihood ratio test showed that TFS significantly reduced the power of electrographic seizure activity in the TFS-treated group compared to controls in more than 86% of the cases.

As the next fundamental step we study the effect of TFS on PTZ-induced behavioral seizure activity. Our recent preliminary results showed that TFS caused a significant reduction in duration of myoclonic activity [35]. In this study we expand our analysis of the effect of TFS on behavioral seizure activity and summarize our findings. The analysis is expanded in two ways. First, four different metrics were used including time of the first behavioral change, seizure onset latency, seizure duration, and maximal seizure severity score allowing better description of the effect of TFS. Second, in our preliminary study two independent groups of animals were used. The PTZ was administered to animals in both groups only once with only the TFS-treated group receiving TFS but not the control group. This approach does not account for variability (resistance to PTZ, etc) among the animals of the two groups potentially obscuring the effect of TFS. The only way to reduce the effect of this factor is to average the results for larger numbers of animals in each group as was attempted in our preliminary study ($n = 14$ and $n = 21$ for TFS-treated and control groups respectively) [35]. In this study we use a different experimental design administering PTZ to the animals in both groups twice and giving TFS to the animals in the TFS-treated group after the second PTZ administration only. This approach allows us to compare the results from the first PTZ administration in the TFS-treated and control groups confirming that there is no significant difference between controls and TFS-treated groups. After that we use the results for the first seizure as a baseline to study the difference between the first and the second PTZ-induced seizures in each group separately. Finally, we compare the rates of change caused by recurrent PTZ administrations in control and TFS-treated groups to evaluate the effect of TFS.

II. Methods

A. Animals

Naïve male Sprague-Dawley rats ($n = 19$, 220–320 g body weight) were used. Rats were maintained under controlled conditions (12:12 h light/dark cycle, 25°C) with food and water ad libitum. The experimental protocol was approved by the University of Rhode Island IACUC. All experiments were performed in the early afternoon.

B. Drug

Pentylenetetrazol (PTZ) was dissolved in saline solution (0.9%) and administered intraperitoneally in a constant volume of 1.0 ml/kg. A dose of 45 mg/kg was used in this study.

C. Habituation

In order to habituate the animals to handling related to injections, rats received five successive daily injections with saline solution (0.9%) before each PTZ administration.

D. PTZ administration and scoring behavioral activity

The day of the PTZ administration, animals were allowed to habituate to the experiment room for approximately 10 min. After the video recording was started, the rat's behavior was observed for five minutes as a basal level after which the animals received the PTZ injection (45 mg/kg, ip.). Monitoring and video recording continued for another 25 minutes or until the animal returned to its regular activity. Behavioral analysis was conducted in real time with video recordings serving as a backup. Both PTZ administrations were conducted and scored according to the same protocol with the second administration performed one week after the first one.

To score seizure-related behavioral activity we used the following stages of the revised Racine's scale for PTZ-induced seizures in rats [36]: R = 0, no seizure activity; R = 1, sudden behavioral arrest and/or motionless staring; R = 2, myoclonic jerk (sudden and fast neck jerk); R = 3, clonic seizure in a sitting position; R = 4, clonic seizures while lying on the belly; R = 5, tonic-clonic seizure while lying on the belly; R = 6, tonic-clonic seizures while lying on the side and/or wild jumping.

Derived from these scores the following four metrics were established: (1) *Time of the first behavioral change* was defined as the time in seconds between PTZ administration and observation of the first behavioral manifestation: sudden behavioral arrest and/or motionless staring (R = 1) for at least 10 s or the first myoclonic jerk (R = 2). (2) *Seizure onset latency* was defined as the time in seconds between PTZ administration and the moment the seizure reached stage R = 3 or higher (clonic or tonic-clonic seizure). (3) *Seizure duration* was defined as the cumulative time in seconds the animal spent having a seizure corresponding to stages R = 3 or higher (clonic or tonic-clonic seizure). (4) *Maximal seizure severity score* was defined as the highest R value for the animal.

E. Electrode attachment

One day prior to the second administration of PTZ, the electrodes were attached to the rats scalp. Rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (12 mg/kg, ip). The rats scalp and the top of the neck were shaved and prepared with NuPrep abrasive gel (D. O. Weaver & Co., Aurora, CO, USA). Two custom-designed TCREs [24] with diameter equal to 1.0 cm were placed, one on the rat scalp and one on the top of the neck, using conductive paste (1 mm Ten20, Grass Technologies, RI, USA) and fixed with dental acrylic (Pearson Lab Supply, Sylmar, CA, USA). The electrodes were made of gold-plated copper and each ring was 0.9 mm wide (Fig. 1, panel A). The rat was returned to its cage and allowed food and water ad libitum for approximately 24 h until the experimental procedure of second PTZ administration began.

As shown in Fig. 1, panel B one TCRE that was used to stimulate primarily the cerebral cortex, was centered on the top of the head (s). The front edge of the electrode was placed near the site that should be the bregma since we were not able to see it. An isolated ground electrode was attached on the top of the neck behind the ears (g) and used for impedance measurement only. To serve as ground all three recording surfaces of the TCRE (g) were shorted together (Fig. 1, panel B). These particular electrode locations were chosen due to size constraints and brain anatomy of adult rats. To allow reliable impedance measurements for the recording surfaces of TCRE (s) to the isolated ground (g) the distance between two

TCREs should be kept small. Since fitting and fixing two 1.0 cm TCREs on the rat's head may be problematic the top of the neck provides a viable alternative for (g).

F. TFS administration

First, the skin-to-electrode impedance was measured. If the outer ring and central disc skin-to-electrode impedance for the 1.0 cm dia. electrode (s) to the isolated ground electrode (g) of Fig. 1 were less than 10 KΩ, then the rat was administered TFS (n = 9). If this impedance was greater than 10 KΩ the rat was assigned to the control group (n = 10) not receiving TFS. Lower impedances for electrode (s) for the TFS-treated group ensured that the TFS would penetrate into the rat and not be dissipated over the high impedance. The skin-to-electrode impedance was rechecked at the end of the experiment.

After seizures were induced with PTZ the TFS-treated group received TFS (300 Hz, 50 mA, 200 μs, biphasic square pulses for 2 minutes) after the first behavioral change was observed. TFS was administered between the outer ring and the central disc of electrode (s). The TFS pulses were generated by a custom-built stimulator that was controlled with a BS2P-24 microcontroller (Parallax Inc., CA, USA).

Slight inconsistencies in TFS parameters occurred due to a problem with equipment resulting in 2 out of 9 TFS-treated rats receiving stimulation with up to an order of magnitude higher current (500 mA) for a few seconds. The higher current melted the conductive paste lowering its conductivity returning the current to the target range for the remainder of the stimulation duration.

G. Statistical analysis

All the statistical analysis was performed using Minitab (Minitab Inc., State College, PA, USA). Two types of statistical tests were used for two separate tasks addressed in this study.

For the first task, evaluating the significant difference of the four behavioral activity metrics between control and TFS-treated groups for a seizure induced by the first PTZ administration, we used unpaired or "independent samples" tests: parametric two-sample Student's t-test (alternative hypothesis of sample means being not equal) and non-parametric Mann-Whitney test (alternative hypothesis of sample medians being not equal) [37]. Tests for independent samples were used in this case because the two samples to be compared represented two different groups of animals. An exception had to be made for the maximal seizure severity score because the maximal seizure severity score for the first PTZ administration in all the control group were equal to three. This caused an error in the Minitab implementation of Mann-Whitney test. The Kruskal-Wallis test, an extension of Mann-Whitney, was used instead in this case.

The second task was to evaluate if there was significant difference in four behavioral activity metrics between first and second PTZ-induced seizures in control and TFS-treated groups. We used paired or "dependent samples" tests: parametric paired t-test (alternative hypothesis of mean difference between two samples being not equal to 0) and non-parametric Wilcoxon signed-rank test (alternative hypothesis of median difference between two samples being not equal to 0) [37]. Tests for dependent samples were used in this case since the two samples compared were repeated measures on the same animals, i.e. they represented a single group of animals that has been tested twice.

The Ryan-Joiner (similar to Shapiro-Wilk) normality test was used for all the samples compared. For the first task, a parametric test was used only when both samples compared were normally distributed. Otherwise, a non-parametric test was used. For the second task, a parametric test was used only when the corresponding differences between pairs of samples

compared were normally distributed for both control and TFS-treated groups. Otherwise a non-parametric test was used in both groups for consistency. The same exception as in the case of Mann-Whitney test had to be made for maximal seizure severity score since Ryan-Joiner test uses the correlation of sample order statistics (the sample values arranged in ascending order) with those of a normal distribution so samples whose values are all equal or contain few different values may produce erroneous results. The Anderson-Darling test that uses the sum of the weighted squared vertical distances between the normal cumulative distribution function and the sample cumulative frequency distribution is less sensitive to sample ordering and was used for maximal seizure severity score instead [38].

Some of the results obtained in this study are illustrated in Fig. 2–4 in the form of box plots (also called box-and-whisker plots) that are widely used to assess and compare sample distributions [39]. All these box plots follow a standard form [39]. The center line represents the sample median. The top and bottom of the box represent the third (75th percentile) and the first (25th percentile) quartiles respectively. The upper and lower whisker limits are equal to the third quartile plus 1.5 times the interquartile range and the first quartile minus 1.5 times the interquartile range respectively where the interquartile range is equal to the difference between the third and the first quartiles. Finally, data points beyond the whiskers are marked as outliers using open circles.

III. Results

After five days of habituation to handling PTZ was administered to the rats and behavioral activity was assessed. One week later including five more days of habituation right before the procedure the rats were anesthetized and the electrodes were attached. On the following day the PTZ and the TFS, in the TFS-treated group, were administered. We found no significant difference in behavioral activity between the two groups after the first PTZ administration. After the second PTZ administration there were some significant differences between the first and the second PTZ-induced seizures in both groups which are described below.

A. First PTZ administration (controls vs. TFS-treated)

Due to non-normally distributed data the non-parametric Mann-Whitney or Kruskal-Wallis tests were used to compare the results between the control and the TFS-treated groups.

Time of the first behavioral change—There was no statistically significant difference ($p = 0.97$) in the time to the first behavioral change with a median of 88 s for the control group (n = 10) and 94 s for the TFS-treated group (n = 9).

Seizure onset latency—There was no statistically significant difference ($p = 0.188$) in the seizure onset latency with a median of 1673 s for the control group (n = 10) and 132 s for the TFS-treated group (n = 8, one of the animals never developed a seizure even thought it presented a behavioral arrest resulting in undefined seizure onset latency and seizure duration and maximal seizure severity score both equal to 0).

Seizure duration—There was no statistically significant difference ($p = 0.44$) in seizure duration with a median of 38 s for the control group (n = 10) and 46 s for the TFS-treated group (n = 9).

Maximal seizure severity score—There was no statistically significant difference in maximal seizure severity score ($p = 0.68$) with a median equal to 3 for both control (n = 10) and TFS-treated (n = 9) groups.

B. Second PTZ administration (controls vs. controls, TFS-treated vs. TFS-treated)

For the normally distributed data, time of the first behavioral change, the parametric paired t-test was used to compare behavioral activity between the first and the second PTZ-induced seizure in the control and TFS-treated groups. The remaining data was non-normally distributed and the non-parametric Wilcoxon signed-rank test was used.

Time of the first behavioral change—The paired t-test showed a statistically significant difference in the time of first behavioral change for both the control (n = 10, means of 96 s and 45 s for first and second PTZ administrations respectively, $p = 0.016$) and TFS-treated (n = 9, means of 106 s and 41 s, $p = 0.012$) groups. The corresponding box plots are presented in Fig. 2.

Seizure onset latency—There was a statistically significant difference in the seizure onset latency change for the control group (n = 10, medians of 1673 s and 85.5 s, $p = 0.019$) and none for the TFS-treated group (n = 7, medians of 132 s and 89 s, $p = 0.933$). For the TFS-treated group two animals never developed a seizure: one during the first PTZ administration and the other during the second PTZ administration resulting in undefined seizure onset latency and were excluded from paired testing. The corresponding box plots for unpaired data without any exclusions are presented in Fig. 3.

Seizure duration—There was a statistically significant difference in the seizure duration for the control group (n = 8, medians of 38.5 s and 255 s, $p = 0.03$) and none for the TFS-treated group (n = 7, medians of 42 s and 100 s, $p = 0.052$). For each of control and TFS-treated groups two animals expired during the second PTZ-induced seizure resulting in undefined seizure durations and were excluded from the paired testing. The corresponding box plots for unpaired data without any exclusions are presented in Fig. 4.

Maximal seizure severity score—There was a statistically significant difference in maximal seizure severity score for the control group (n = 10, medians of 3 and 6, $p = 0.036$) and none for the TFS-treated group (n = 9, both medians equal to 3, $p = 0.173$).

IV. Discussion

A. Effect of TFS on behavioral seizure activity

The first step of this study was to establish that there was no significant difference between results obtained for control and TFS-treated groups for the first PTZ-induced seizure. Once we established there were no significant differences in behavioral activity from the first PTZ treatment (Results, section 3.A) the second step was to study the difference between the first and the second PTZ-induced seizures in each group separately. The same behavioral seizure activity metrics (seizure onset latency, time of the first behavioral change, duration of seizure and maximal seizure severity score) were used in both cases. While there was no significant difference between the behavioral seizure activity for the first PTZ-induced seizure, time of the first behavioral change, was significantly different between the first and second PTZ-induced seizures for both groups. It should be noted that TFS would not have any effect on time of the first behavioral change since the TFS was not turned on until the first behavioral change was observed. Therefore, until the time of the first behavioral change both groups are treated the same and are not statistically different. This significant difference is reflected in the results (Results, section 3.B) outlining the main finding of this study: while the general trend was the same in control and TFS-treated groups (mean/median decrease in time of the first behavioral change and seizure onset latency and increase in seizure duration and maximal seizure severity score) the difference was statistically significant for all four metrics of behavioral activity in the control group but only for time of

first behavioral change, that could not have been affected by TFS in the TFS-treated group. The fact that there was no statistically significant change in three behavioral seizure activity metrics that could have been affected by TFS clearly suggests that TFS may have an anticonvulsant effect. If this difference between two groups was due to some factor other than TFS all four behavioral seizure activity metrics affected or not affected by TFS would have been likely to exhibit similar behavior.

B. Potential limitations of current study

Discussion of two potential limitations of the current study is presented below.

First, it can be seen from the results presented in Results (section 3.A) for seizure onset latency and Fig. 3 that the animals in the control group had longer seizure onset delays overall than animals in the TFS treated group for the first PTZ-induced seizure (medians of 1673 s and 132 s respectively). Even though there was no statistically significant difference ($p = 0.188$) between the two groups we found that there were some rats with short and long seizure onset delays. It may be plausible to subdivide them further in future studies. In this study the only criteria for such division was based on skin-to-electrode impedance of the electrode (s). At the same time longer seizure onset delays in the control group suggest that animals in that group could have initially been less susceptible to PTZ than animals in the TFS-treated group. In this case the fact that median seizure onset latency of the second PTZ-induced seizure was shorter for the control group than for the TFS-treated group (Fig. 3, Results section 3.B) further suggests the anticonvulsant effect of TFS. Finally, even if the initial difference in median seizure onset latencies between the control and TFS-treated groups could have influenced the results presented in this study it would not have affected seizure duration and maximal seizure severity score.

Second, compared to seizure onset latency and maximal seizure severity score (Results, section 3.B), the results of seizure duration analysis seem less conclusive with smaller difference in p values corresponding to tests for control and TFS-treated groups respectively. This may be partially attributed to not using all the available data for paired testing. Two rats expired in each group during the second PTZ-induced seizure forcing us to exclude seizure duration data from four rats for the first PTZ administrations. The corresponding box plots for the complete data, with no exclusions related to paired testing, presented in Fig. 4 clearly suggests there is significant difference in results between the two PTZ administrations for the control group and no such difference for TFS-treated group. Unpaired Mann-Whitney test was applied to these unpaired data to further confirm the statistically significant difference in the seizure duration for the control group (n = 10 and n = 8 for the first and second PTZ administrations respectively, medians of 38 s and 255 s, $p = 0.002$) and no such difference for the TFS treated group (n = 9 and n = 7 respectively, medians of 46 s and 100 s, $p = 0.169$).

C. Recurrent PTZ administrations and selection of validation approach

The effect of recurrent administrations of PTZ producing a gradual increase in the seizure intensity is well established and used for the development of PTZ-induced kindling in rats [40–43]. Recurrent intraperitoneal administration of doses equal to 30mg/kg [40], 35 mg/kg [41] and 40 mg/kg [42, 43], comparable to the dose used in the current study (45 mg/kg), were shown to produce progressive sensitization to the convulsive effect of PTZ. Due to this sensitization it would be difficult to reliably evaluate the effect of TFS using the same animals first as a part of the TFS-treated group and then as a control or vice versa. Even though such an approach allows for a within-subject comparison it also convolutes the effect of TFS with the increased sensitivity to PTZ. This sensitization along with the possible obscuring/masking of PTZ tolerance when using a single PTZ administration on two

separate groups of animals (Introduction) further justifies the validation approach used in this study. We used two PTZ administrations on two groups of animals with comparisons evaluating the effect of TFS being drawn between the first and the second PTZ administrations in control and TFS-treated groups separately to overcome these limitations.

D. Other noninvasive/minimally invasive electromagnetic brain stimulation techniques

It was found that noninvasive electrical stimulation via ear bars captured penicillin-induced seizures in rats [44]. They applied electrical stimulation to cause seizures in hopes of controlling seizures. The electrical stimulation also caused strong tonic activity in contrast to what we observe with TFS. Cathodal tDCS, applied on the skull of rats, was found to significantly alter the threshold localized seizure activity (TLS) induced with a transcranial cortical ramp-stimulation model of focal epilepsy [45]. They found significant differences in the TLS, due to 30 minutes of tDCS applied on the cranium prior to ramp-stimulation, which 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 in [35] and in this study with TFS significantly attenuating behavioral seizure activity and preventing its return. Cathodal tDCS has also been applied on the skull after inducing pilocarpine-induced status epilepticus in immature rats [46]. In [46] they found reductions in cell loss, cognitive impairment, and frequency of convulsions. However, they do not report how they stopped the status epilepticus, whether they used an anticonvulsant or let it continue. The tDCS was applied for two consecutive weeks starting two days after the termination of status epilepticus [46]. In contrast we applied TFS for one minute on the scalp five minutes after the onset of pilocarpine-induced status epilepticus, and reapplied five minutes later if there did not appear to be a favorable response [30]. The TFS stopped or reduced electrographic and behavioral activity and was also long lasting [30]. Finally, tDCS has also been applied to PTZ-induced seizures in rats. In [47] anodal and cathodal tDCS initiated immediately after PTZ injection and continued for 120 minutes at 2 mA significantly reduced seizure-induced expression of c-Fos, a marker of excessive neuronal activation, in hippocampus and neocortex of seizing young rats. The PTZ-induced seizure model has also been used for assessment of reduction of behavioral seizure activity using TMS in rats [48, 49]. In [48] a significant increase in latency to PTZ-induced myoclonic and tonic-clonic behavioral activity due to 0.5 Hz repeated TMS (rTMS) has been reported. In [49] 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, in our studies we administered TFS after the first MJ [30, 31, 33–35] rather than immediately after administering PTZ [47], before administering PTZ [48], or after the first electrographic signs of seizures [49] and found a significant reduction in the total duration of behavioral PTZ-induced MJs due to TFS [35] as well as significant reduction of behavioral seizure activity due to recurrent PTZ administrations shown in the current study. In summary there are no direct comparisons for TFS seizure control. However, we show results that are in line with other reports.

E. Summary and future work

Taken together with previous effects observed by our group with models of status epilepticus induced by pilocarpine [30] and penicillin [31] as well as the reduction of pathological synchronization [33] and power [34] of PTZ-induced electrographic seizure activity and the PTZ-induced behavioral activity presented in this study indicate that the seizure control achieved with noninvasive TFS is applicable to diverse acute seizure models. These results also suggest that TFS may have the potential to be a viable noninvasive therapy for intractable epilepsy. Directions of future work include testing different stimulation parameters for optimal efficacy of the TFS, determining the specific

mechanisms of action of TFS via TCREs, and investigating how the results in rats may translate to human epilepsy.

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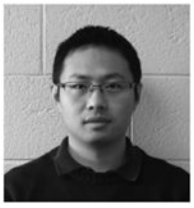

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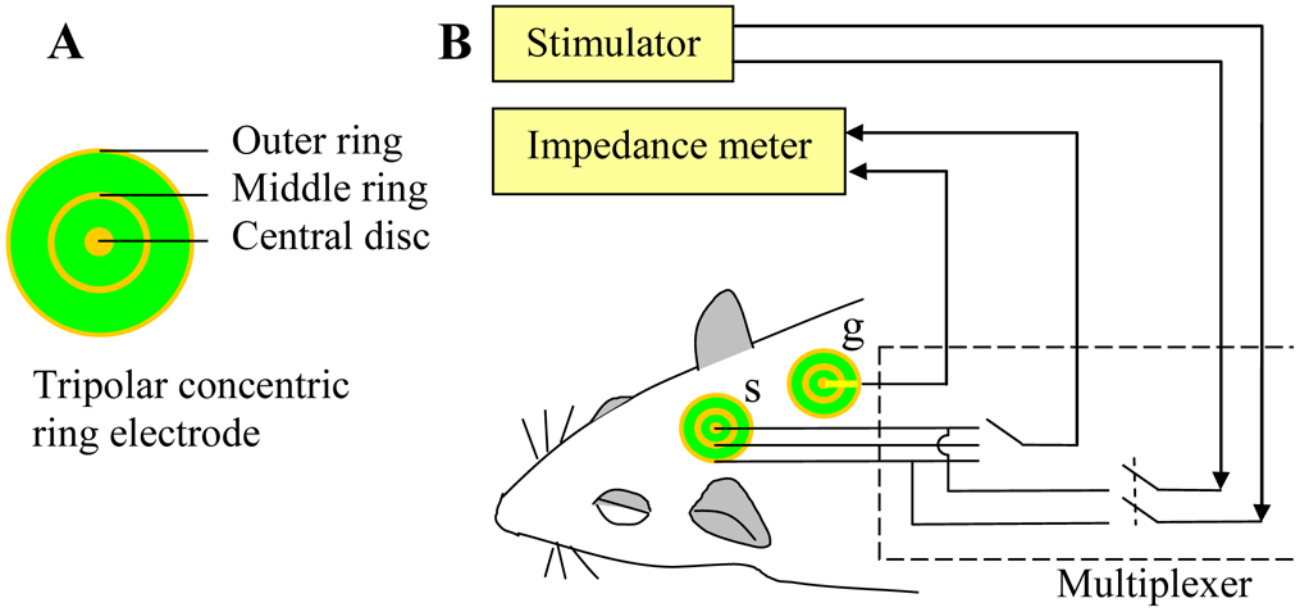

Fig. 1.
Schematic representations of the tripolar concentric ring electrode (A) and the experimental setup (B). The TFS was applied between the outer ring and the central disc of electrode (s). Electrode (g) was the ground.

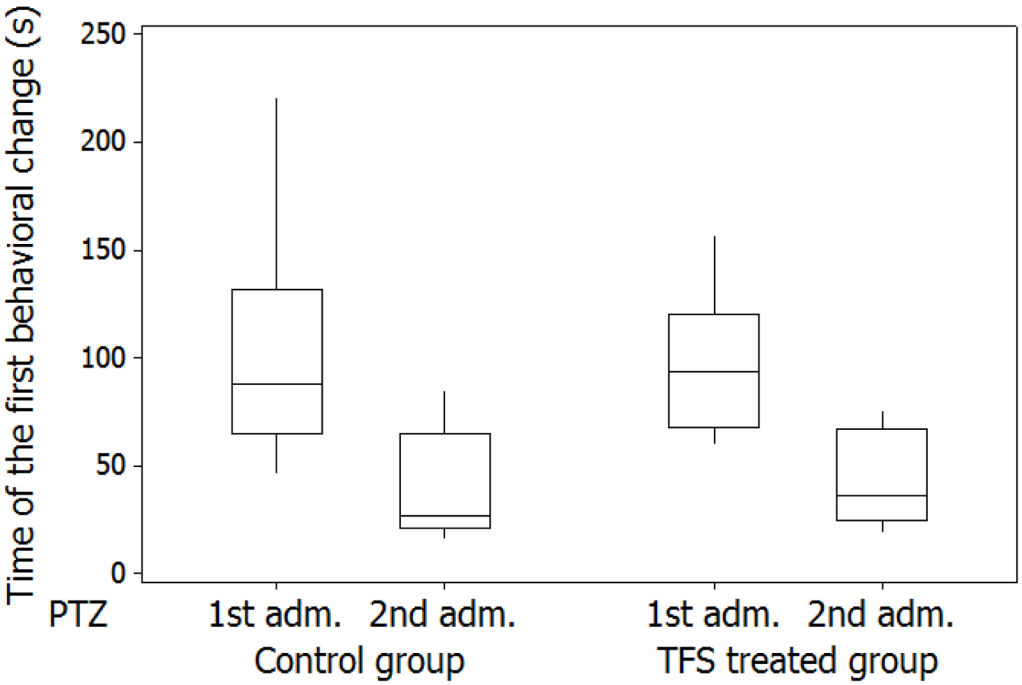

Fig. 2.

Time of the first behavior change caused by first and second PTZ administrations in TFS-treated (n = 9 for both administrations) and control groups (n = 10 for both administrations). There was a statistically significant difference in the time of first behavioral change for both TFS-treated (means of 106 s and 41 s, p = 0.012) and control (means of 96 s and 45 s for first and second PTZ administrations respectively, p = 0.016) groups. Details on box plot form can be found in Section 2.7.

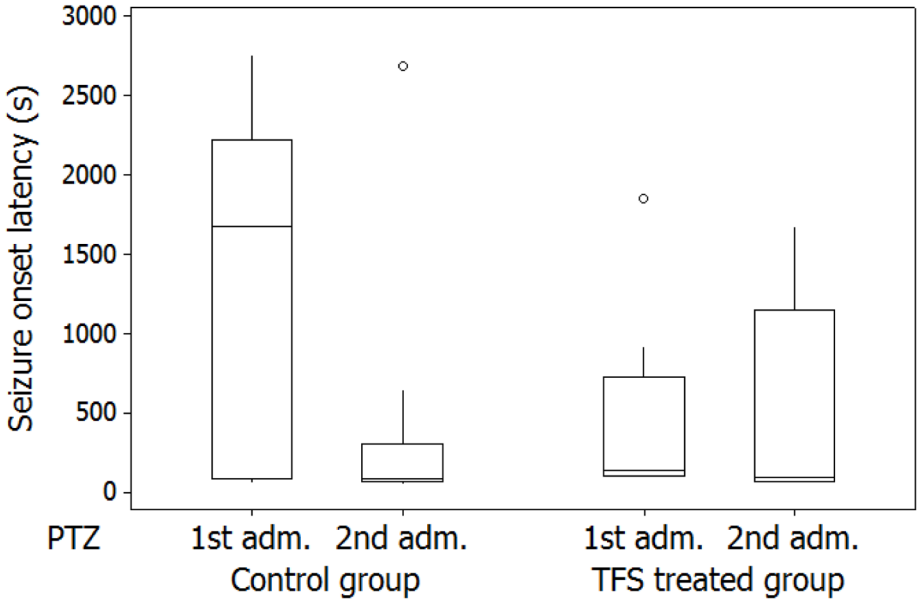

Fig. 3.

Seizure onset latency caused by first and second PTZ administrations in TFS-treated (n = 8 for both administrations, two animals never developed a seizure: one during the first PTZ administration and another during the second administration resulting in undefined seizure onset latency) and control groups (n = 10 for both administrations). Details on box plot form can be found in Section 2.7.

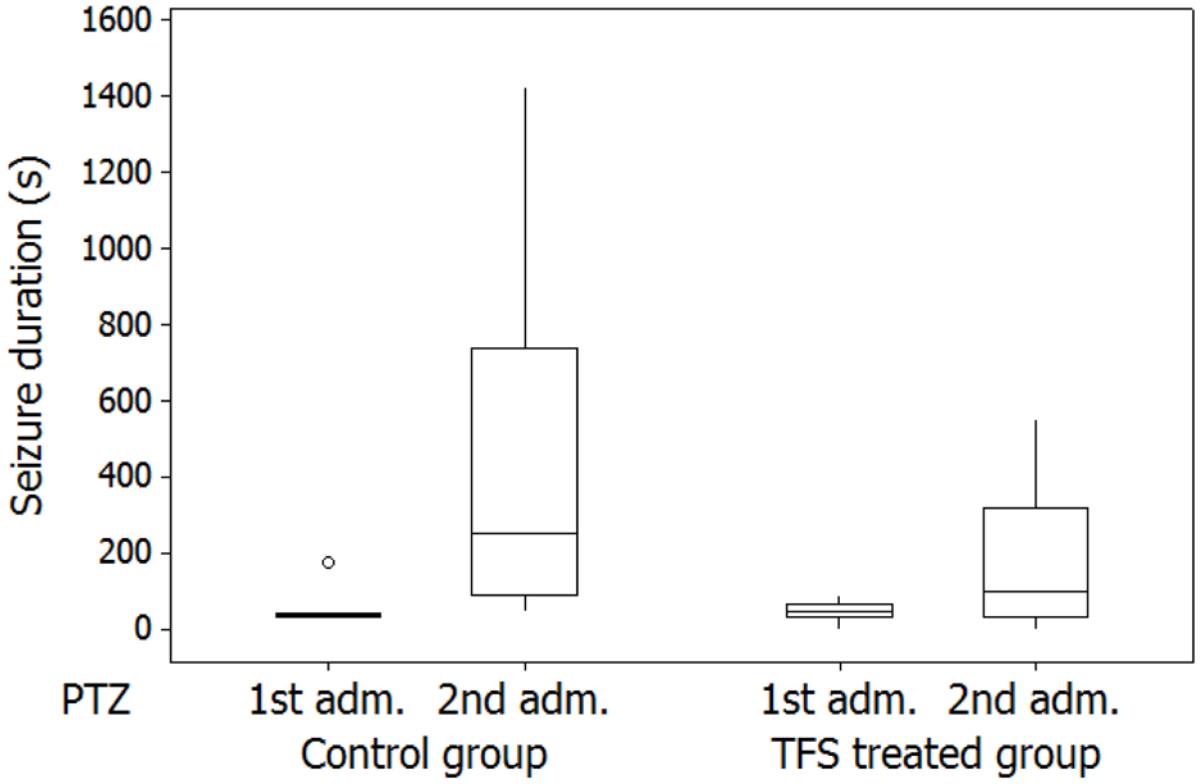

Fig. 4.

Seizure duration caused by first and second PTZ administrations in TFS-treated (n = 9 and n = 7 for first and second administrations, respectively) and control groups (n = 10 and n = 8 for first and second administrations, respectively). In both groups, two animals expired during the seizure caused by the second PTZ administration resulting in undefined seizure durations. Details on box plot form can be found in Section 2.7.