Chronic Transcranial Focal Stimulation from Tripolar Concentric Ring Electrodes does not Disrupt Memory Formation in Rats

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***Abstract*— Non-invasive electrical brain stimulation has shown potential utility as a treatment for seizures in epilepsy patients. Transcranial focal stimulation (TFS) via tripolar concentric ring electrodes (TCREs) has been effective in reducing seizure severity in acute rodent models, but it has yet to be determined whether or not it will serve as a viable long-term treatment strategy. Prior experiments indicate that a single dose of TFS via TCRE does not impact short- or long-term memory formation. The present study investigated if five daily doses of TFS via a TCRE on the scalp affected the memory. The spontaneous object recognition (SOR) test was used to evaluate the memory. Sham and TFS-treated groups were evaluated and both showed comparable levels of preference for novel objects, indicating successful memory formation. More work on repeated dosage strategies is important for establishing the safety and efficacy of TFS as a putative treatment.**

I. Introduction

Electrical brain stimulation has been demonstrated to be a useful methodology for treatment of epilepsy [1]. Tripolar concentric-ring electrodes (TCREs) have unique capabilities that provide advantages over conventional disc electrodes for neurological applications, including more uniform current density [2] and locally focused stimulation directly below the electrodes. Prior work has shown promise in using transcranial electrical stimulation with TCREs to reduce seizure activity in pilocarpine- [3], penicillin- [4] and pentylenetetrazole-induced [5-7] animal models. Stimulation via TCREs, referred to as transcranial focal stimulation (TFS), has shown effectiveness in reducing seizure activity [7-9].

Numerous studies have been conducted to establish the efficacy and safety of TFS [8-11]. Tissue from both the scalp [10] and cortex [11] of stimulated rats was studied with no apparent damage to the tissue occurring as a result of TFS application.

Various stimulation-based approaches to treating epilepsy have been evaluated for their safety and efficacy. Deep Brain Stimulation (DBS) of the hippocampus has been used to safely reduce or abolish seizures in epilepsy patients [12,13]. Transcranial Direct Current Stimulation (tDCS) has also been used to treat epilepsy patients safely, and there is a growing body of evidence that tDCS may be capable of improving both long- and short-term memory [14-16]. To assess whether TFS would impact memory or cognition, the spontaneous object recognition (SOR) test was applied to naïve rats being treated with single doses of TFS or sham-stimulation [17,18]. No significant difference between stimulated and sham groups was detected for post-stimulation memory test intervals varying between 10 sec and 48 hours.

It is unclear whether or not multiple doses of TFS will be necessary for success as a treatment strategy for epilepsy. Therefore, the present experiment was conducted to further investigate whether and how the application of TFS may affect memory using a multiple-dose paradigm. Following handling and conditioning, rats were given a single 2-minute TFS dose at 24-hour intervals for five consecutive days. At the end of the final stimulation, the timed intervals of the memory test began and the performance of the rats in sham and stimulated groups was assessed. Our preliminary findings suggest that chronic application of TFS has no adverse effect on memory formation as assessed by the SOR test.

II. Methods

A. Subjects

Male Sprague-Dawley rats (n=11) weighing 300-400g were housed in groups of 2-3 subjects. Rats were kept at a 12:12h light/dark cycle at 25°C and were provided with access to food and water ad libitum. All experiments were conducted between 1000hr and 1400hr. The experimental protocol was approved by the University of Rhode Island IACUC.

B. Spontaneous Object-Recognition Apparatus

The SOR test was performed in an opaque blue acrylic open-field chamber (60cm x 60cm x 60cm) with 15cm black squares (Clever System Inc.) painted onto the bottom surface. The open-field chamber was placed on a table in a dark room lit only by a 60-W light bulb placed 1m above the bottom of the chamber. A fume hood was used to generate constant white-noise at a volume of 72 dB. A video camera mounted above the chamber was used to record the locomotion and behavioral tests.

Familiar objects used in the test were identical glass beakers of approximately the same size as the novel objects.

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The novel objects were plastic and varied in color and shape; they were no smaller than the size of the rat and no larger than 2.5 times the size of the rat [21]. Objects were secured in the same location each trial by Velcro strips adhered to the floor of the apparatus [26]. The same sequence of objects was provided to all rats during memory tests.

C. Habituation and TFS Stimulation

Prior to TFS treatment, rats were habituated with each animal held gently by the experimenter(s) for five minutes daily for five consecutive days. On the sixth day of the experiment, the rats were exposed either to sham- or TFS-stimulation and were subsequently given the same treatment for four more days at 24hr intervals. Following the final treatment, the rats were placed into the empty open-field chamber facing the wall where objects would later be placed to explore and familiarize with the chamber for five minutes. No objects were in the chamber during this phase. The chamber was cleaned with 60% ethanol between individual rats' trials.

D. SOR Testing

SOR testing consisted of four stages: re-habituation, familiarization, delay, and test. Rats were videotaped during this process for behavioral assessment at a later time. Between each step, the box and objects were cleaned with 60% ethanol to prevent scent contamination between trials. During rehabituation, each rat explored the empty open-field chamber for 1 min. Rats were then returned to their home cage for one minute while the two familiar objects were placed into the chamber. During familiarization, rats were placed in the open-field chamber to explore the identical familiar objects for three minutes. During the delay phase, the rats were returned to their home cage for 1min, 1hr, 24hr time intervals and the familiar object was paired with a novel object following familiarization and stimulation. The rats were then placed into the chamber at the following test intervals: 1min, 1hr, 24hr.

During the test phase, rats were returned to the open-field chamber and allowed to explore the two objects for three minutes at the conclusion of each delay interval. For scoring purposes exploration was defined as the rats placing the snout within 2cm of the object while investigating the object. Other kinds of contact with the object were not scored. Cognitive function was evaluated using the recognition index (RI). The RI was calculated by dividing the time spent investigating the novel object (t_{novel}) by the total time spent exploring novel and familiar objects (t_{novel} /{t_{novel}+$t_{familiar}$}) [27]. An RI value exceeding 0.5 indicates a preference for novel object exploration.

E. Sham/TFS-Stimulation via TCREs

On the day following the fifth handling, rats' scalps were shaved. While one researcher held the rats, the other applied conductive paste (Ten-20 electrode paste, Grass Technologies, West Warwick, RI) to the scalp and placed the TCRE. Stimulation condition (TFS vs sham-TFS) was assigned to rats randomly; only TFS-treated animals were exposed to nonzero current from the TCREs while the sham TFS group received 0mA. The TFS (300 Hz, 200 μs charge-balanced biphasic pulses at 50 mA) was applied for five consecutive days at 24-hour intervals beginning the first day after handling. The TFS methods and parameters used for this study were chosen from prior experiments where seizure attenuation in penicillin-, pilocarpine-, and pentylenetetrazole-induced acute seizures was observed [2-4]. The TCRE was placed near the center of the top of the head.

F. Activity Test

Locomotor activity was assessed during familiarization and three memory test intervals by counting the number of times rats moved from one square to another with all four paws [25]. The number of crossings was counted in three minute time bins.

G. Experimental Groups

To evaluate the effects of TFS on performance in the SOR test, two groups were used: sham (n=5) and TFS (n=6). Both groups underwent handling, attachment to the stimulation apparatus, familiarization, and the SOR test. After familiarization, the TCRE was placed on the rats' head for the final TFS or sham TFS-treatment. In the case of sham rats, the power remained off; TFS rats received their fifth stimulation at this time. The delay intervals were selected to represent two short-term memory time points (1min, 1hr) as well as one long-term memory time point (24hr) [17, 19-24].

H. Statistical Analysis

The results are presented as the mean +/- the standard error of the mean (SEM). A two-way repeated analysis of variance (ANOVA) and subsequent Holm-Sidak test was performed to assess differences between responses to novel objects and delay intervals or group (TFS, sham-TFS) during the SOR test. A p value of less than 0.05 was considered significant. GraphPad Prism (version 6.04, GraphPad Software Inc., La Jolla, California, USA) was used for all statistical analyses.

III. Results

During familiarization, the number of seconds that rats spent exploring each of the identical objects was recorded to evaluate whether the location of the objects on the left or right side of the test chamber would have any bearing on exploration time. Fig. 1 shows the mean time spent exploring the right and left familiar objects for both the sham and TFS-treated groups of rats. There was no statistically significant preference between object locations ($p = 0.5538$), nor was there any difference in total exploration time between groups ($p = 0.7598$).

Fig. 2 shows the RIs determined for each of the three delay intervals tested grouped by stimulation condition. For

each of the delay intervals, there is no significant difference between the RI values for sham and TFS-treated groups ($p = 0.2305$). Between the one minute and one hour intervals, there was a significant difference in RI values ($p = 0.0112$) for the sham group.

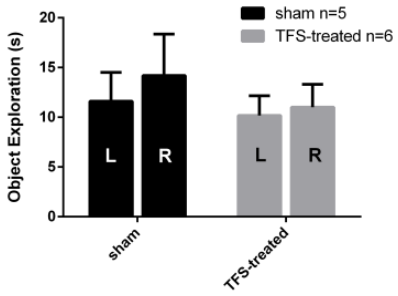

Figure 1. Mean time spent exploring identical objects during the familiarization phase. Left and right object exploration times correspond to the left and right columns respectively; each group spent similar amounts of time exploring both objects. Data are presented as mean +/- SEM (n=5, 6)

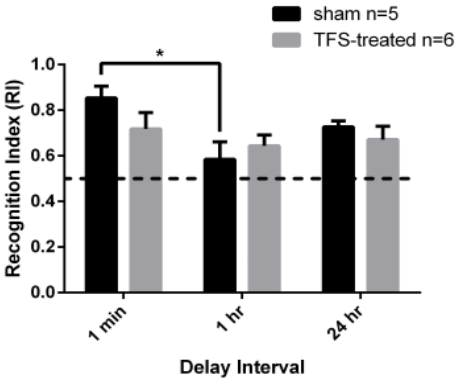

Figure 2. Effects of TFS on memory performance of rats tested in the spontaneous object recognition test. Animals were stimulated four consecutive days. On the fifth day, after TFS, the testing began. Each animal was then tested at 1min, 1hr, 24hr delay intervals. Data are presented as mean +/- SEM (n=5-6)

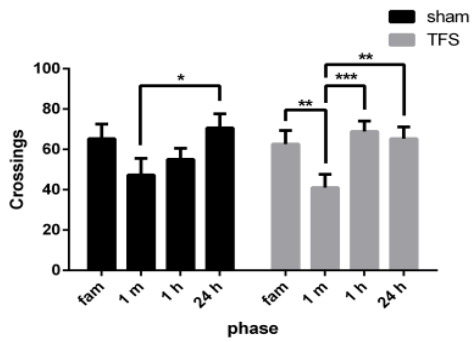

Figure 3. Number of test chamber grid crossings counted in each phase of the experiment. Both groups of rats showed a similar mean number of crossings during familiarization and at each memory test interval. Data are presented as mean +/- SEM (n=5-6)

Locomotor activity was measured during familiarization and each delay interval as the number of complete crossings between 15cm squares marked on the floor of the test chamber (Fig. 3). For the sham group there was only a significant difference in the number of crossings between 1min and 24 hr ($p < 0.05$). Within the TFS-treated group, there were significantly fewer crossings during the 1min delay interval test compared to the familiarization, 1hr and 24hr tests ($p < 0.01$).

IV. Discussion

Prior work in our group with the SOR memory test paradigm has established that single doses of TFS from TCRE's do not disrupt recognition memory in naïve rats exposed to novel and familiar objects [18]. Our results in the present study are consistent with those of the single-dose TFS study: rats stimulated with TFS via TCRE's for five consecutive days at 24 hour intervals exhibited comparable performance to the sham control group in recognizing novel objects. The RI values calculated for the sham group at the one minute and one hour time points are significantly different [18]. Moreover, the mean RI for the sham group at one hour is close to 0.5 indicating nearly equal time spent exploring each object. A more granular review of the data revealed that one of the rats in the sham group performed very poorly on the test (RI = 0.333), a result which could have an exaggerated influence on the mean in a data set of n=5. We would not expect that the sham group experienced any stress or stimuli that the TFS-treated group did not, so it is unlikely that this difference is indicative of adverse experimental conditions or an effect on memory that is not related to TFS treatment itself.

The locomotor activity test is intended to provide a metric of the level of anxiety that the rats may be experiencing during the test [25]. Although TFS-treated rats had activity levels comparable to those of the sham group at all stages of the experiment, the difference between the number of crossings at the one minute time point and the number at the one hour and 24 hour time points bears consideration. It seems reasonable to conclude that there may be an impact on the activity level as a result of a higher rate of environments being presented to the rats in proximity to the first memory test. Four prior TFS treatments were followed by a prolonged return to the home cage whereas the final treatment was followed by a minute-long return and subsequent presentation of the first novel object. Given the absence of stimulation just before the 1hr and 24hr memory tests and increased familiarity to the test chamber following the 1min memory test, it seems reasonable to conclude that the rats could have reduced anxiety during the second and third memory tests.

Although we followed the methodology of the single-dosage TFS experiment as closely as possible, some changes were necessary and should be considered in the interpretation of the results. In the present experiment, the rats' were shaved and given either two-minutes of TFS or two minutes contact to an inactive TCRE for four days prior

to the SOR testing. The fifth time they were given TFS or sham TFS was after the familiarization phase; in the single-dosage experiments, only this final stimulation session was present. Thus, it may be possible that the animals were not in the same sense naïve, although any putative interference with normal cognitive function was not apparent in the data collected.

Another important consideration for this study is the limited number of subjects and testing intervals. Based on the single-dosage experiments, we would expect that the more important of these variables to increase would be the number of subjects, as a comparison of the RI values of different intervals in each study shows fairly consistent results between both the short- and long-term memory delay intervals [18]. For this reason, the present study only included one delay length that would assess long-term memory. Two delay intervals on the scale of short-term memory were chosen to allow for a possible distinction between memory on the scale of minutes and hours. It does not represent a comprehensive evaluation of putative cognitive impacts caused by repeated TFS application but rather an informative first step. Further investigation of chronic stimulation paradigms for TFS from TCRE's will be necessary to establish safe and effective constraints for developing treatment strategies that do not interfere with the cognitive capacity of treated individuals.

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