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# Electric Fields in Hippocampus Due to Transcranial Focal Electrical Stimulation via Concentric Ring Electrodes

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*Abstract*— **As epilepsy affects approximately one percent of the world population, electrical stimulation of brain has recently shown potential as an additive seizure control therapy. In this study we applied focal transcranial electrical stimulation (TFS) on the surface of the skull of rats via concentric ring electrodes. We recorded electric potentials with a bipolar electrode consisting of two stainless steel wires implanted into the left ventral hippocampus. TFS current was gradually increased by 20% starting at 103 μA allowing us to assess the relationship between TFS current and both potentials recorded from the bipolar electrode and the resulting electric field. Generally, increases in TFS current resulted in increases in the electric field. This allows us to estimate what extra-cranial TFS current would be sufficient to cause the activation of neurons in the hippocampus.**

## I. Introduction

EPILEPSY is a neurological disorder that affects approximately one percent of the world population with up to three-fourths of all persons with epilepsy in developing countries [1]. Over 50 million people worldwide are affected by epilepsy. Anti-epileptic drugs used to treat epilepsy are ineffective in 25~30% of cases and can cause side effects. Surgery is another option available, but carries risks.

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) [2]-[5] and transcranial direct current stimulation (tDCS) [6]. 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 [7].

Previously we have shown that noninvasive transcranial focal electrical stimulation (TFS) via tripolar concentric ring electrodes (TCRE) has been effective in controlling seizures. When TFS was triggered manually after severe penicillin-induced myoclonic jerks there was a significant reduction in the number and length of myoclonic jerks [8]-[9]. We also found that there was a significant reduction of the effects of pilocarpine-induced status epilepticus with the effects lasting at least several hours [10]. Finally, we recently showed that TFS significantly reduced pentylenetetrazole (PTZ)-induced hypersynchrony in electrographic activity at the beta and gamma frequencies [11].

When electrical stimulation is administered via concentric ring electrodes, TFS, unlike conventional electrical stimulation where stimulation is usually applied across the head, has a much more uniform current density and focuses the stimulation directly below the stimulating electrode. We found in a multiconductivity agarose head phantom that TFS was attenuated dramatically off center from the TCRE but was strongest just below the disc [12]. According to Kowalski *et al.* based on estimates of the current density thresholds for stimulation of the motor cortex using magnetic stimulation they found a rheobase value of approximately 2.5 A/m$^2$ for activation [13]. Assuming the conductivity of the brain is 0.25 S/m this represents an electric field of 10 mV/mm. There is also evidence that rectangular pulses, which we apply with TFS, require less energy to activate neurons than sine waves [14]. The electric field magnitude required to modify the activity of a neuron is also much smaller than the magnitude needed to provoke neuronal firing from rest [15].

For this study we wanted to measure potentials deep in the brain, *in vivo,* generated by TFS. Normally we apply TFS from the scalp surface however due to the implantation of recording electrodes in the brain we were not able to apply TFS from the scalp but rather from the skull. To anchor the implanted electrode a cement base is laid which is an insulator preventing conduction of the TFS. Therefore, we applied TFS on the skull via concentric ring electrodes.

## II. Methods

### A. Animals

Male Wistar rats (250 – 300 g body weight), individually housed and maintained under environmentally controlled conditions (12 h normal light/dark cycles, 22-25ºC, and 38% relative humidity) with food and water *ad libitum*, were used in the present study. The protocol was conducted according to the Mexican Official Norm ("Norma Oficial Mexicana"

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### *B. Surgery*

Rats (n = 2) were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.m.). Then, bipolar electrodes, consisting of two twisted strands of stainless steel wire, insulated except at the cross-section of their tips, were stereotactically implanted into the left and right ventral hippocampus using the following coordinates in mm from bregma and skull surface: anteroposterior -5.3; lateral 5.2; depth 7.5 [16]. Stainless steel screws were threaded into the cranium over the frontal cortex to fix the electrode assembly. The implated bipolar electrodes were attached to male connector pins, which were inserted into an Amphenol connector strip [17]. A 6.0 mm diameter TCRE [18] was centered on the top of the skull with the front ring behind the bregma. The bipolar electrode was just outside the outer ring of the TCRE with the bare conductors inside the hippocampus. Fig. 1 shows the electrode configuration and placement. The electrodes assembly were then fixed to the skull with dental acrylic. Animals were allowed to recover with water and food provided *ad libitum* for one week before any further manipulation.

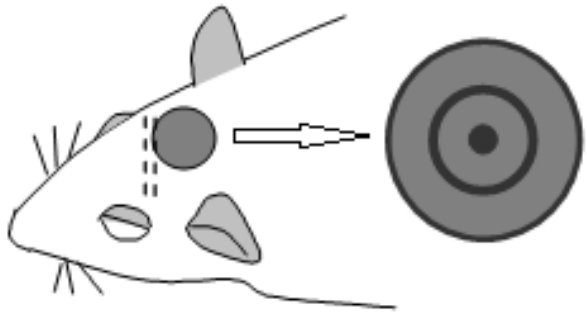

Fig. 1. The location of the implanted bipolar electrode in the hippocampus (two dashed lines) and the stimulating tripolar concentric ring electrode with its configuration.

### *C. Focal Stimulation*

After surgery recovery the animals were fasted. On the second day of fasting TFS was applied with a custom built biphasic stimulator using a Parallax Basic Stamp 2P24 to control frequency, phase, and time duration of the TFS output signals. The magnitude of the stimulation was adjusted manually. The TFS was delivered at 300 Hz with 200 µS biphasic pulses starting at 103 µA and gradually increased by 20% at a time. The TFS application was discontinued when motor behaviors (head nodding or backing up) occurred during short (15 s) stimulation trains with 2 min pauses.

### *D. Data Acquisition*

The potentials from the implanted bipolar electrodes were amplified with a P511 High Performance AC Amplifier (Grass Technologies, West Warwick, RI). Variable amplifier gain was determined using a 500 µV calibration pulse and calculated to be equal to 9660 for rat #1 and 9827 for rat #2. The signals were band-pass filtered (1-100 Hz) with line filter active, reviewed with Polyview (Grass Technologies), and digitized (250 samples per second, 16 bit) and stored to the hard drive.

### *E. Data Processing*

The data were exported using Polyview in text file format. Custom Matlab (Mathworks, Natick, MA) programs were written to perform further processing. The average potential voltage was calculated for each 15 s TFS train. Fifth order Butterworth low-pass filter was used to detect the starting and ending point of each train accurately. A cutoff frequency of 0.1 Hz was determined empirically.

## III. Results

Both TCREs were functional after nine-days of implantation on the surface of the skull (extra-cranial). The rats did not have any problems with surface infections. The TFS was definitely penetrating into the brain as witnessed by behavioral activity at the higher TFS intensities.

Fig. 2 shows the raw potentials recorded from the bipolar electrodes for two rats (panels *A*, *B*) and the corresponding filtered signals (panels *C*, *D*). The two lower panels of Fig. 2 clearly show when the stimulation was on even at the lowest stimulation current of 103 µA. As you observe left-to-right in Fig. 2 the TFS current of the potentials recorded from the bipolar electrode increased over time.

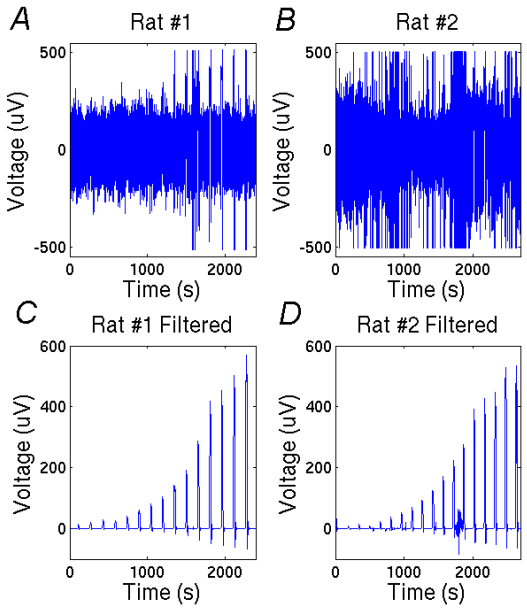


Fig. 2. Raw potentials recorded from bipolar electrodes for two rats (panels *A*, *B*) due to varying intensities of focal electrical stimulation (FS) from a tripolar concentric ring electrode placed on the skull surface (extra-cranial). Panels *C* and *D* show corresponding filtered potentials recorded from the bipolar electrodes revealing the envelope due to the TFS.

Fig. 3 shows the relationship between the TFS current and the average potential voltages for each TFS train recorded from the bipolar electrodes. Generally, as the TFS current increased the potentials recorded from the bipolar electrodes also increased. However, the last stimulation current for rat #2 did not cause an increase in the potential recorded from the bipolar electrode, it actually decreased.

Table 1 tabulates the average potential voltages and the resulting electric fields from various TFS intensities applied. Electric fields were calculated based on the distance of 1 mm between the wires of the bipolar electrode.

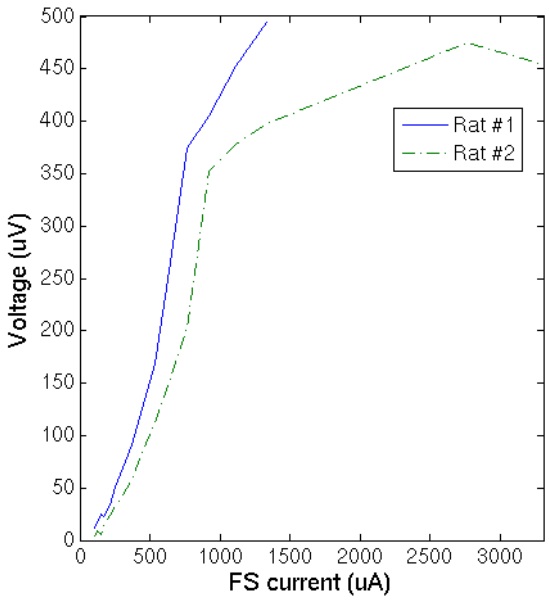


Fig. 3. The relationship between the transcranial focal electrical stimulation (TFS) intensity and average potential voltages recorded from the bipolar electrode for two rats. As the TFS current increased the potentials recorded from the bipolar electrodes also increased except for the highest current level for rat #2.

## IV. Discussion

Observing the raw recordings from the implanted bipolar electrodes (Fig. 2, *A* and *B*), both recordings appear to be noisy. Therefore, it is not apparent whether TFS is reaching deep into the rat brain. After further signal processing, the corresponding filtered signals (Fig. 2. *C* and *D*) demonstrate that even the weakest TFS (103 µA) is penetrating into the hippocampus. The distance from the center disc of the TCRE to the implanted bipolar electrode is approximately 9.01 mm. Radially, the bipolar electrode was located just outside the outer ring of the TCRE.

Even at the highest stimulation currents, the electric field resulting from the potentials recorded by the bipolar electrode was below 0.5 mV/mm. Higher TFS intensities would have been needed to achieve electric fields that would be effective at activating neurons in the hippocampus where the bipolar electrode was implanted. This may be due to the fact that electrical stimulation using TCREs is highly localized, as opposed to conventional disc electrodes. The

TABLE I
THE POTENTIALS RECORDED FROM THE IMPLANTED BIPOLAR ELECTRODES AND THE RESULTANT ELECTRIC FIELDS.

| FS current (µA) | Voltage (µV) | | Electric field (mV/mm) | |
|---|---|---|---|---|
| | Rat #1 | Rat #2 | Rat #1 | Rat #2 |
| 103 | 11 | 5 | 0.011 | 0.005 |
| 124 | 18 | 9 | 0.018 | 0.009 |
| 149 | 26 | 5 | 0.026 | 0.005 |
| 170 | 22 | 14 | 0.022 | 0.014 |
| 215 | 34 | 23 | 0.034 | 0.023 |
| 250 | 51 | 33 | 0.051 | 0.033 |
| 310 | 69 | 44 | 0.069 | 0.044 |
| 372 | 92 | 58 | 0.092 | 0.058 |
| 446 | 125 | 84 | 0.125 | 0.084 |
| 535 | 168 | 112 | 0.168 | 0.112 |
| 642 | 265 | 155 | 0.265 | 0.155 |
| 770 | 374 | 203 | 0.374 | 0.203 |
| 924 | 405 | 352 | 0.405 | 0.352 |
| 1110 | 453 | 378 | 0.453 | 0.378 |
| 1330 | 495 | 398 | 0.495 | 0.398 |
| 2760 | | 475 | | 0.475 |
| 3310 | | 454 | | 0.454 |

TCRE focuses the stimulation directly below the electrode central disc while attenuating it sharply radially outside of the TCRE. Therefore, current TFS intensities may have been high enough to generate electric fields needed to reach the minimum threshold for neuronal activation directly below the TCRE.

In the future, it would be beneficial to record potentials directly below the center of the TCRE central disc. There are two possible approaches to achieve this. However, both of them are challenging. The first approach is to put a hole in the middle of the disc and run the bipolar electrode through it. In this configuration, the TFS could induce more artifact in the bipolar electrode. Also, the connector for the bipolar electrode is directly above it so that would have to be accounted for. The second approach is to access the hippocampus from below and between the ear and the eye. A significant amount of work would be needed to try different coordinates and then perform histology to verify positioning of the electrode in the hippocampus.

It can be noticed from Fig. 3 that the potentials recorded from the bipolar electrode did not always increase with each increment of the TFS current. This may be due to the manual adjustment of the custom stimulator used for these experiments. The stimulator was designed for use in the mA ranges and adjustments in the µA ranges were not as accurate. Even though the results obtained for the two rats seem reasonably consistent, more experiments would be needed for conclusive proof as well as their validation with results from numerical simulations.

Typically we apply TFS on the scalp rather than on the skull. The thickness of the rat scalp is approximately 1.0 mm. The scalp is also much more conductive than the skull,

0.44 and 0.018 S/m, respectively. From the scalp the TFS would be attenuated further. It remains to be seen how much weaker the TFS would be. It may be possible to estimate what electric field could be expected from TFS through the scalp via modeling.

There may have been tissue damage to the skull caused by TFS even though to the naked eye there was none. Tissue analysis was not performed to determine this conclusively. In our work we prefer the noninvasive TFS usage. Placement of TCRE on the skull in this study was only due to experimental necessity. The main goal of this study was to determine if weak TFS could reach deep into the brain.

## V. Conclusion

In conclusion it can be seen that from the skull surface, minimally invasive TFS can generate electric fields deep into the rat brain in the hippocampus. Even though the achieved electric field could not be considered large enough to activate neurons at the bipolar recording site it suggests the potential of the approach and allows us to estimate what extra-cranial TFS current would be sufficient to cause neuron activation.

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