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Low Intensity Repetitive Transcranial Magnetic Stimulation Modulates Spontaneous Spiking Activities in Rat Cortex

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***Abstract*—Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique for neuromodulation. Even at low intensities, rTMS can alter the structure and function of neural circuits; yet the underlying mechanism remains unclear. Here we report a new experimental paradigm for studying the effect of low intensity rTMS (LI-rTMS) on single neuron spiking activities in the sensorimotor cortex of anesthetized rats. We designed, built, and tested a miniaturized TMS coil for use on small animals such as rats. The induced electric field in different 3D locations was measured along different directions using a dipole probe. A maximum electric field strength of 2.3 V/m was achieved. LI-rTMS (10 Hz, 3 min) was delivered to the rat primary motor and somatosensory cortices. Single-unit activities were recorded before and after LI-rTMS. Results showed that LI-rTMS increased the spontaneous firing rates of primary motor and somatosensory cortical neurons. Diverse modulatory patterns were observed in different neurons. These results indicated the feasibility of using miniaturized coil in rodents as an experimental platform for evaluating the effect of LI-rTMS on the brain and developing therapeutic strategies for treating neurological disorders.**

I. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique for treating neurological and neuropsychiatric conditions. Even at low intensities, rTMS has been shown to have therapeutic effect in patients with depression [1][2]. In addition, recent work suggests that low intensity rTMS (LI-rTMS) alters the structure and function of neural circuits *in vivo* [3][4]. Moreover, LI-rTMS has been shown to lower action potential threshold and increase evoked spike firing rate *in vitro* [5]. Although LI-rTMS has been widely used in clinical and experimental practice, the underlying mechanism of its effect remains unclear. Rodent models of LI-rTMS are needed for studying the neurobiological mechanisms with simultaneous electrophysiological recordings and pharmacological manipulations, which are difficult or impossible in human patients. However, a major limitation of performing TMS in rodents is the lack of small size coils. Most commercially available coils are designed for human brain stimulation. Their sizes are much bigger than the rodent brain, which results a loss of stimulation focality. Several recent studies have developed small coils for use on rodents and demonstrated the effect of LI-rTMS on modulation of motor evoked potential amplitude and skilled motor learning [6][7]. However, to the best of our knowledge, no previous study has evaluated immediate effects of LI-rTMS on spontaneous neuronal spiking activities, which are the most important neural signals underlying sensory, motor, and cognitive processes.

To address this problem, we designed, built, and tested a miniaturized coil for rodent LI-rTMS studies. The induced electric field was measured and validated in saline with a dipole probe (Fig. 1). We further delivered high frequency LI-rTMS to the primary motor and somatosensory cortices of anaesthetized rats and recorded spontaneous spiking activities of single neurons before and after stimulation using microelectrodes (Fig. 2). This experimental paradigm makes it possible to evaluate the effect of LI-rTMS on single neuron activities in small animals and provides a useful platform for studying the underlying mechanisms of TMS and developing therapeutic strategies for treating neurological disorders.

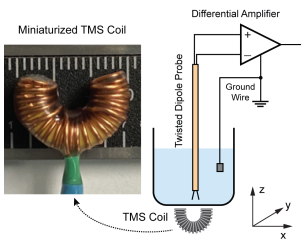

Figure 1. Measure electric field of the C-shaped miniaturized TMS coil with a dipole probe in saline.

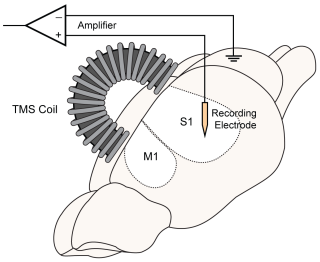

Figure 2. Recording cortical activities before and after TMS in a rat brain.

II. Method

A. Miniaturized TMS coil

A miniaturized TMS coil (Fig. 1) was designed and built from a commercially available 47 μH toroidal inductor. Insulated copper wires were wound around the C-shaped half ring and evenly distributed over the circumference. Electrical current flows in opposite directions at both ends of the coil to increase the strength of the induced electric field at the center, which is similar to a traditional figure-of-eight TMS coil. The

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magnetic core is made of micron-sized iron powders with high saturation flux density which further focuses the induced electric field.

The stimulation waveforms and patterns were generated with a customized MATLAB script and delivered from a personal computer. Gaussian pulses with maximum amplitude of ~-1.4 V and STD of ~45 μs were used as the input. An audio interface card and a voltage to current converter were implemented in the electrical circuit to convert the stimulation waveform into the current pulse in the coil. Metal blocks were placed around the circuit for heat dissipation during stimulation.

B. Electric field measurements

A dipole probe was made with a pair of insulated wires, which are twisted to minimize the electromagnetic interference [8]. The wires were exposed at the ends with a 3 mm separation [9]. Electric field were measured in a 300 mL beaker filled with 0.9% sodium chloride solution. The coil was placed under the beaker and positioned at the center. The dipole probe was placed at various 3D positions in the beaker during stimulation (Fig. 1). It was connected to a DAM50 differential amplifier (World Precision Instruments, Sarasota FL, USA) with a gain of 1000. Signals were recorded with a Digidata 1322A acquisition system and pClamp 9 software (Molecular Devices, Sunnyvale CA, USA). Since the electric field between two close points is approximately linear [8], the induced electric field in different directions is calculated as

$$E_x = -\frac{\Delta V}{\Delta x},\ E_y = -\frac{\Delta V}{\Delta y},\ E_z = -\frac{\Delta V}{\Delta z} \quad (1)$$

where ΔV denotes the first peak amplitudes of the recorded waveforms; E_x, E_y, and E_z are the electric fields measured along x, y, and z direction, respectively (Fig. 1); Δx, Δy, and Δz are the distance between the exposed ends of the dipole probe, which are aligned along measuring directions during measurements.

C. Electrophysiological recording

Male Sprague-Dawley rats (n = 3, 300-350 g, 3-4 months) were used for LI-rTMS and electrophysiological recordings. Animals were anaesthetized with a ketamine (75 mg/kg) and xylazine (10 mg/kg) cocktail after a brief induction with 4% isoflurane and 95% oxygen at 1 L/min. Additional doses of ketamine (30 mg/kg) were administered to maintain a constant level of anesthesia, which was assessed by breathing rate and the toe-pinch reflex. Animals were mounted on a stereotaxic frame by ear bars and a nose cone. Craniotomy was performed above the right hemisphere to expose the sensorimotor cortex. Dura matter was carefully resected to expose the cortex. A Tungsten microelectrode (0.1 MΩ at 1 kHz) was implanted in the sensorimotor cortex using the micromanipulators (Fig. 2). A ground wire was placed in the hindbrain. Spontaneous spiking activities (1 min) were recorded before and immediately after LI-rTMS (3 min, 10 Hz). Data were digitized and collected with a Digidata 1322A acquisition system and the pClamp 9 software (Molecular Devices, Sunnyvale CA, USA) with gain of 1000 and a filter of 300 Hz to 10 kHz at a 100 MHz sampling rate. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee from the University of the Southern California.

D. Spike sorting and statistical analysis

Spontaneous spikes before and after LI-rTMS were sorted using a Plexon offline sorter. Firing rate histograms and mean firing rates of neurons were calculated with NeuroExplorer. A paired *t*-test was performed to compare the firing rates before and after stimulation. A significance level of $\alpha = 0.05$ was used to determine whether the mean firing rates were significantly altered after TMS.

III. Results

A. Coil input signal and coil current

A Gaussian pulse with peak value of 1 and STD of ~45 μs was generated in Matlab as the control signal (Fig. 3A). The actual input signal generated by the audio interface card was measured to have a peak amplitude of ~-1.4 V at maximum audio volume (Fig. 3B). The TMS coil current was generated as a result of capacitive discharge and showed peak amplitude of ~160 A across a 0.025 Ω resistor (Fig. 3C). The resulting skewed Gaussian waveform was due to the nonlinear voltage-to-current converter in the circuit. This coil current further induced a biphasic electric field waveform that can be measured with a dipole probe in saline (Fig. 3D).

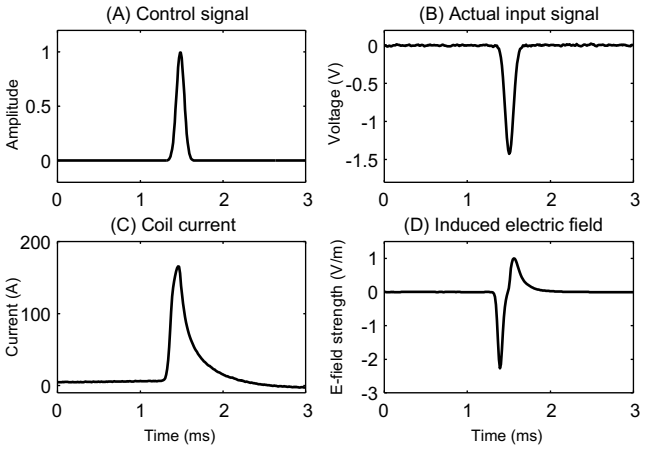


Figure 3. Signal waveforms of the TMS circuit. A: control signal; B: actual input signal from the audio interface card; C: current in the TMS coil; D: induced biphasic electric field waveform measured in saline.

B. Induced electric field

In a cylindrical container filled with saline, the induced electric field was measured in the transverse (x-y) plane with a distance of 4 mm from the coil. X-direction was defined as the line connecting the two ends of the coil; y-direction was the line perpendicular to the x-direction (Fig. 1). The center point of the coil was the origin in the plane where those two lines intersected. The electric field was sampled at 2 mm along x-direction and 1 mm along y-direction. Figure 4A and B illustrated the 3D plots of the x and y component of induced electric field in the plane. For the x component of the electric field, maximum points were detected at the four corners of the coil. Those peaks had different polarities at the adjacent corners but same polarities at the opposite corners. For the y component of the electric field, the maximum point (~2.3 V/m) existed at the center of the coil where the x component had a minimum strength. This result was observed because the current flowed in the opposite direction at both ends of the

coil, which reinforced the y component of induced electric field but canceled the x component at the center. The overall electric field with both x and y components is shown in a quiver plot (Fig. 4C). The arrow indicates the direction of the electric field vector, and the length represents the field strength. The quiver plot demonstrated a ring-shaped induced electric field in the transverse plane at z = 4 mm. The dipole probe was also moved vertically along z-direction to measure the electric field at various depths. There was a rapid decay of the electric field within 10 mm (Fig. 4D).

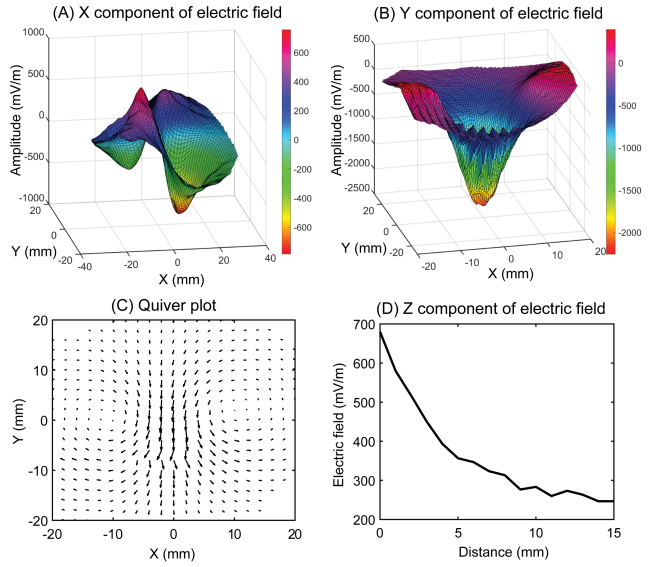


Figure 4. Induced electric field measured in saline. A: x-component of the electric field in the transverse plane; B: y-component of the electric field in the transverse plane; C: quiver plot of the electric field in the transverse plane; D: z-component of the electric field at different depths.

C. LI-rTMS modulates spontaneous firing in neurons

We performed 4 trials of LI-rTMS experiments in 3 animals (Table 1). The microelectrode was implanted in either the somatosensory cortex (S1) or the primary motor cortex (M1). Trial 2 and trial 3 were conducted on the same animal. In each trial, one minute of spontaneous activities was recorded as the baseline (Fig. 5A). The TMS coil was then placed over the craniotomy window with a medial-lateral orientation so that the induced current flowed from the medial to the lateral part of the brain (Fig. 2). The coil was tilted at 15 degrees to ensure its center aligned with the electrode position. The distance from the coil to the cortex surface was approximately 4 mm. Three minutes of 10 Hz LI-rTMS was delivered to the sensorimotor cortex, followed by another one minute of spontaneous activity recordings to measure the effect of TMS (Fig. 5B). The neuronal spikes were sorted offline. We analyzed the changes in firing patterns and rates using the firing histograms of each neuron. Results showed a consistent increase of firing in neurons (Fig. 6). The mean firing rates were significantly higher after LI-rTMS than the baseline values (Fig. 7; paired *t*-test: $t = 2.6824$, $df = 11$, $p = 0.0107$). However, diverse levels of modulation to the spontaneous firing were observed in different neurons. For example, neuron 1b's firing rate increased by 140% after TMS, while neuron 1a's firing rate showed a 30% decrease. Among the 12 trials, 11 trials showed increase of firing rates. Linear regression analyses to the relation between firing rates before and after LI-rTMS indicated that S1 neurons exhibited a more prominent multiplicative increase while M1 neurons showed a moderate additive increase (Fig. 7).

TABLE I. BRIAN COORDINATES FOR EACH TRIAL

# of trials	Target	ML[a] (mm)	PA[b] (mm)	Depth (mm)
Trial 1	S1	2.4	-1.19	-1.5
Trial 2	M1	3	1.15	-1.7
Trial 3	S1	2.8	-2.5	-1.9
Trial 4	M1	3	1.25	-1.8

a. Medial-lateral axis; b. Posterior-anterior axis

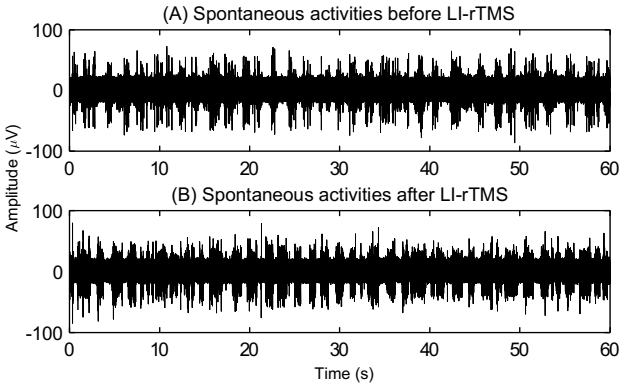
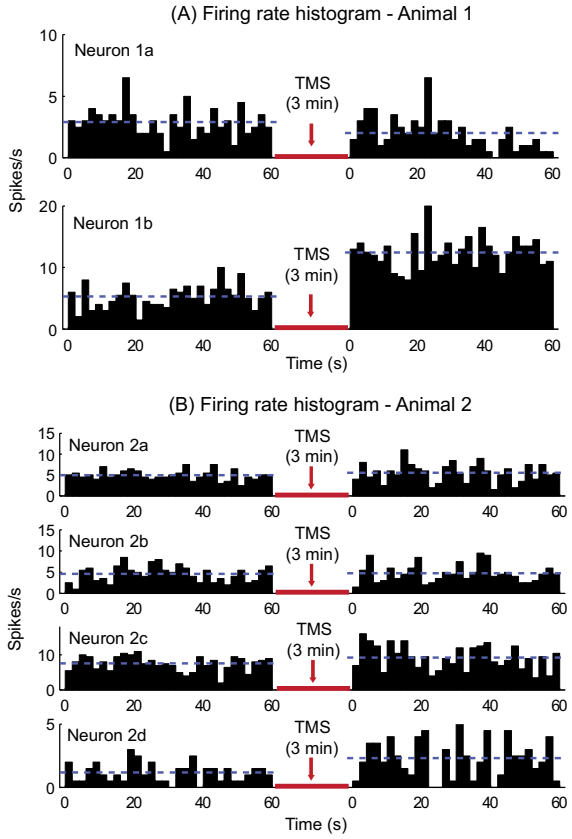


Figure 5. Example of 1-minute spontaneous activity recordings before (top) and after (bottom) the 3-min LI-rTMS (trial 1).



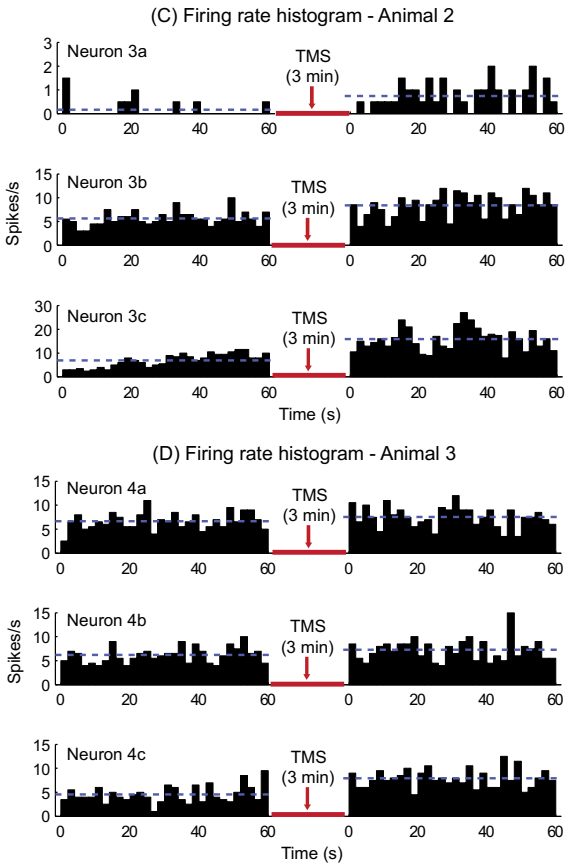


Figure 6. Firing rate histograms (bin size: 2 s) of neurons recorded before and after the 3-min LI-rTMS. The dashed lines denote the mean firing rates.

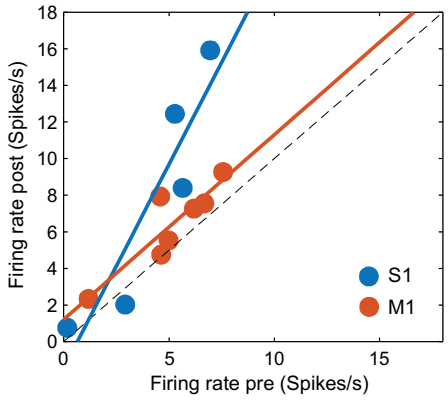


Figure 7. Comparison of mean firing rates of neurons before and after the 3-min LI-rTMS. Linear fits (blue and red solid lines) are superimposed on the scatter plots. The dashed line denotes the 45-degree line.

IV. Discussion

In this study, we designed, built, and evaluated a miniaturized TMS coil in rats. This TMS coil was able to induce an electric field higher than 2 V/m within a 4 by 4 mm window at a depth of ~4 mm, that can be used as a focal stimulation above the rat sensorimotor cortex. To investigate whether this low intensity stimulation could produce neuromodulatory effects, we delivered 10 Hz LI-rTMS in anaesthetized rats and assessed firing rate changes in the primary motor cortex and the somatosensory cortex. Previous studies have indicated that low intensity electric field can affect the evoked neural responses and behaviors [6][7][10]. Our results are consistent with these previous findings and provide new data on the effect of LI-rTMS on spontaneous neural activities.

V. Conclusion

We demonstrate, for the first time, that 10 Hz LI-rTMS delivered with a miniaturized coil can consistently increase the firing rate of spontaneous spiking activities in both the primary motor cortex and the somatosensory cortex in rats. This work shows the feasibility of using rodent models and miniaturized coils as a platform for investigating the neural effects and underlying neurobiological mechanisms of TMS and developing therapeutic strategies for treating neurological disorders.

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