The Effects of Transcranial Magnetic Stimulation (TMS) on the Neural Activity of Awake Non-Human Primates

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Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biomedical Engineering in the Graduate School of Duke University 2015
Abstract

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Abstract

Transcranial magnetic stimulation, or TMS, is a non-invasive stimulation method which induces an electric field in the brain. For the past two decades it has been used extensively in clinical and research settings for basic research and treatment studies of neurological disorders such as depression. Despite its widespread use and established safety, the mechanism of effect for the stimulation is still poorly understood. The goal of this project is to study the effect of single pulse TMS on single neurons in awake rhesus macaques. By using the modified electronics developed by Mueller et al. (2014), we were able to minimize the duration of the stimulus artifact in the recordings down to a few milliseconds, allowing us to capture and characterize the neural activity in the frontal eye field (FEF) and primary motor cortex (M1) immediately following a TMS pulse. We found that the intracranial electric field induced by TMS has a variety of effects on individual neurons but a distinct pattern of effect on the population activity: a short-latency excitation (< 20ms latency) followed by a longer-lasting inhibition (for ~100 ms). These effects were absent in Sham TMS treatments. Our single pulse TMS protocol caused no long term effects on neural activity, but repetitive TMS (rTMS) protocols of 1 and 5 Hz changed spontaneous firing rates. The outcome of this work is to demonstrate empirically how TMS affects neurons in the primate brain.
To my parents, my brother, and John, who have always supported me through my endeavors and taught me the importance of taking a step back in order to see the full solution. Thank you for everything.
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List of Abbreviations and Symbols

Symbols

- ∇ Curl operator
- E Electric Field
- B Magnetic Field
- $E_{th}$ Estimated neural activation threshold

Abbreviations

- TMS Transcranial Magnetic Stimulation
- spTMS Single pulse transcranial magnetic stimulation
- rTMS Repetitive transcranial magnetic stimulation
- ECT Electroconvulsive therapy
- TRD Treatment-resistant depression
- FEF Frontal Eye Field
- M1 Primary Motor Cortex
- V1 Primary Visual Cortex
- fMRI Functional magnetic resonance imaging
- MRI Magnetic resonance imaging
- DTI Diffusion tensor imaging
- MEP Motor evoked potential
- ISI Inter-spike Interval
Acknowledgements

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Introduction

Transcranial magnetic stimulation (TMS) was first successfully demonstrated in 1985 by Barker et al, when they discovered that by placing a high intensity, rapid changing magnetic field above the human motor cortex, they were able to illicit twitch motor responses similar to those produced by electrical stimulation (Barker et al., 1985; Merton et al., 1982). The explanation and theory behind magnetic stimulation is based on Maxwell’s equation for electromagnetic induction where a time-varying magnetic field will induce an electric field in nearby conductive material, such as the brain (1.1).

\[ \nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \]  
(1.1)

\[ \oint_{\Sigma} \mathbf{E} \cdot d\ell = -\frac{d}{dt} \int_{\Sigma} \mathbf{B} \cdot dS \]  
(1.2)

It was later suggested that since repetitive pulse magnetic stimulation, or rTMS, produces long-term effects on the brain, it could be used as an alternative to electroconvulsive therapy (ECT) for treatment-resistant depression (TRD) (Höflich et al., 1993; Kolbinger et al., 1995). ECT is a highly effective treatment with a sustained response observed in about 79% of patients and a remission rate around 75% (Husain et al., 2004). Still, many patients are wary of the treatment method because of its negative public connotation and potential side effects.
that include nausea, muscle aches, headaches, prolonged seizures, skin burns, pulmonary complications, and memory loss (Park, 2011). By comparison, TMS is a non-invasive, painless stimulation technique with minimal reported side effects.

In 2008, the FDA approved the first TMS machine to be used clinically to treat depression, though there were restrictions on intended patient groups. In order for a patient to be eligible for TMS treatment, the patient could fail no more than one previous antidepressant treatment and could not have any history of epilepsy (Lisanby et al., 2009).

Even with these restrictions to optimize safety and efficacy, the success of TMS in treating TRD remains modest at best. A pivotal study found that while TMS did produce remission of depression, the efficacy was limited, with only 14% remission of the stimulation patient population, and unfortunately the efficacy has not seen much improvement since then (Deng et al., 2015). One glaring problem is that we still don’t know what TMS does to neurons. This lack of data leads to trial-and-error design of TMS protocols, rather than rational design.

Several studies have shown correlations between rTMS pulse frequency and neural excitation, though with varying observed responses and neural resolution. In anesthetized cat studies, investigators were able to record neural activity at a single cell level and determine cell type responses and brain wave frequency changes (Moliadze et al., 2003; Allen et al., 2007; Hallett, 2007). In human studies, investigators were able to use a twitch response (motor threshold) and fMRI to relate behavioral changes to stimulation effect with limited spatial and temporal resolution (Hallett, 2007; Luber et al., 2008; Najib et al., 2010; Luber and Lisanby, 2014). We focused on studying the effects of single pulse TMS (spTMS) in rhesus macaques because of their homologous brain anatomy. We used single neuron recordings for high spatial resolution and custom-designed electronics to suppress stimulation artifacts that blocked investigation of immediate post-TMS effects in previous work. In rhesus macaques, our laboratory is able to record from single neurons in a variety of brain areas while the animal sits, alert but still. It is also possible to extend the work to conditions where the same animals are engaged in
My overall hypothesis was that a single pulse of TMS causes changes in the neural activity within the first hundred milliseconds following stimulation, and possibly even within the first couple of milliseconds. It is only through determining this neural response that we can begin to have a greater understanding of how TMS influences the brain, and will be able to design more effective clinical treatments. My thesis project focused on three aims to characterize the neural response. Aim 1 was to determine the types of neural responses to spTMS in single neurons. The responses were analyzed to look for patterns in stimulation effects compared with a Sham spTMS condition, when everything was the same except that no electric field was induced at the site of neural recordings. Aim 2 was to quantify the dose response of the neural population to the various intensity levels of the TMS machine. If the neuronal responses we observed were truly due to the induced electric field in the brain, we would expect to see them change as intensities increase. Currently most TMS studies base their intensity level on the motor threshold, which is the intensity necessary to evoke an involuntary twitch in the thumb or forefinger 50% of the time when stimulated. Another test of whether the neural responses are truly due to induced electric fields would be if they emerge near the motor thresholds of the tested animals. Aim 3 was to characterize changes in baseline (spontaneous) activity and TMS pulse-evoked activity across repeated applications of TMS. We checked whether our spTMS protocol, at very low frequency (.067 Hz), had long term effects, and evaluated neural changes to the clinically relevant frequencies of 1, 5, and 10 Hz. The results of these three Aims suggest that TMS evokes a specific, dose-dependent pattern of short-latency excitation followed by inhibition after individual pulses, and changes in long-term spontaneous firing rates after higher frequency trains of pulses.
Experimental Design

Within the experimental design there were numerous challenges and modifications that we undertook in order to effectively record neural activity while stimulating with TMS. These modifications were divided into two groups, the changes made to a traditional electrophysiology set up and the changes made to the TMS equipment. For more details of the modifications made to the experimental set up, see (Mueller et al., 2014).

2.1 Electrophysiology

Three rhesus macaques (monkey H, K, and M) were used in this study. Monkeys H and K had previously been implanted with FEF chambers over their left hemisphere and a headpost slightly posterior of the brow. The chambers were standard cilux chambers from Crist. The rest of the implant was formed with dental acrylic and affixed to the head with ceramic bone screws. Monkey M was surgically implanted the same way, but with a M1 chamber over the left hemisphere. This implant was designed specifically so that the custom TMS coil could be placed around the M1 chamber in a medial-lateral alignment along the central sulcus without directly touching the chamber, headpost or acrylic implant. The alignment of the headpost and chamber also meant that there was little variability in coil positioning day to day (Figure 2.8). Additionally, the dental acrylic used in
Figure 2.1: A recreation of the alignment between the TMS coil and the M1 chamber on Monkey M. The TMS coil, recording chamber, and headpost are labeled as TMS coil, RC, and H respectively in the figure. Noticed that in order to allow for the correct stimulation alignment, the TMS coil had to fit around both the headpost and the recording chamber.

This implant was applied more thinly than traditional electrophysiology implants. This permitted the TMS coil to rest closer to the surface of the brain without direct contact with the implant, which could cause mechanical vibrations during stimulation. We recently installed an identical recording implant in a second M1 monkey and will begin recording from it in April 2015 to replicate the M1 findings reported in this thesis.

Single neuron recordings were taken in two different brain areas in order to determine if the response to the stimulation was dependent and varying according to the brain region. The frontal eye field (FEF) was selected because of the lab’s experience with the visual-oculomotor system and the neural activity properties and stimulation-related characteristics of this brain region (Shin and Sommer, 2012; Tehovnik and Sommer, 1997; Sommer and Wurtz, 1998, 2008). M1, i.e. primary motor cortex, is a major target in TMS studies because stimulation of it activates limb muscles, allowing the motor evoked potential (MEP) and the motor
Table 2.1: Motor thresholds in our monkeys. All from Monkey M except where noted. The threshold values recorded prior to the implant surgery required anesthesia with Ketamine because the head could not be restrained. The first column lists whether there was an implant. The second column lists the determined intensity for causing muscle twitches 50% of the time. The final column lists any additional information or comments about the threshold value.

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<th>Implant Present</th>
<th>Threshold Intensity %</th>
<th>Additional Comments</th>
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<tr>
<td>No</td>
<td>70</td>
<td>Anesthesia was light: Ketamine=0.7 mL, Aknosine=0.3mL</td>
</tr>
<tr>
<td>No</td>
<td>34</td>
<td>Anesthetized: Middle Finger Twitch</td>
</tr>
<tr>
<td>No</td>
<td>31</td>
<td>Anesthetized</td>
</tr>
<tr>
<td>Yes</td>
<td>59</td>
<td>Anesthetized: Monkey K</td>
</tr>
<tr>
<td>Yes</td>
<td>63</td>
<td>Awake ipsilateral</td>
</tr>
<tr>
<td>Yes</td>
<td>65</td>
<td>Awake</td>
</tr>
<tr>
<td>Yes</td>
<td>53</td>
<td>Awake</td>
</tr>
<tr>
<td>Yes</td>
<td>65</td>
<td>Awake</td>
</tr>
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threshold response to be determined non-invasively. Since many TMS research and clinical studies use stimulation intensities based on the motor threshold, we also collected the motor threshold of monkey M prior to any cranial implant surgeries and several times throughout the recording period (2013 through 2015, see table 2.1). Motor thresholds were variable and could be quite low when the the TMS coil was positioned against the intact scalp, before implant surgery. After the implant was in place, however, motor thresholds were more consistent and averaged 61% (of max possible TMS output).

2.1.1 Amplification Circuit Design

Also of critical importance was the modification of the preamplifier, or headstage, and the main rack amplifier used for recording the neuronal voltage traces. As mentioned in Chapter 1, the magnetic field produced by the TMS coil induces an electric field in any nearby conducting material. This would include the headstage which traditionally sits on the primate chair a few inches above the recording chamber to limit noise. The proximity of the TMS coil to the headstage typically produces a large pulse artifact on the scale of volts at the input leads. Since
the voltages recorded from the brain are typically on the scale of several hundred micro-volts, the pulse artifact will swamp the neuronal signals and cause over saturation of the opamps at each stage of gain that can last up to 200ms. This voltage artifact has typically restricted other groups to studying the mid to late latency effects of TMS (Moliadze et al., 2003; Hallett, 2007). Other groups have removed the ringing of the pulse artifact offline using cosine fitting and template matching (Fisher et al., 2012). While this method can be effective it can limit the investigator’s knowledge about the neural response to the stimulation during the recording.

In order to evaluate neural response online during a recording session we had to remove the part of the TMS pulse that caused over saturation of the opamps. There were two headstage designs that we used throughout the recording period, which are shown in Figure 2.2. Circuit A was modified to include anti-parallel diodes at each stage of gain, which would clip any input voltage above a certain threshold to prevent over saturation, as can be seen in Figure 2.3(a). This method was fairly effective at limiting the ringing following the TMS pulse to the point where individual cells could be determined within 10ms following stimulation. The limitation on this method was that spikes riding along the pulse or oscillation waves could be clipped and distorted. In practice, this meant that if neuronal activity occurred earlier than 10ms on the trace, it could usually not be used. Circuit B was designed to remove the majority of the TMS artifact from the voltage prior to most stages of gain, in order to limit the amount of observed distortion. This circuit worked by having a small inductive 'sense' loop of wire arranged so that it followed the path of the electrode. The electric field artifact produced in the electrode would then be replicated in the loop. The loop signal was inverted with its gain adjusted so it matched the electrode’s artifact. The electrode and the inverted loop signals were then added together with a passive resistance adder, removing the majority of the TMS pulse artifact. This method allowed for neural activity to be recorded within 1 ms following the stimulation pulse (Figure 2.3(b) and additional examples in Appendix A).
Figure 2.2: The modified headstage designed in this project to allow for immediate online recordings of the neural activity following a TMS pulse. A) The circuit eliminates the possibility of over saturating the opamps by clipping any signal above a certain threshold prior to any gain stage. This limits the ringing artifact down to around 10ms consistently, however, there is distortion of the TMS pulse waveform and any spikes that were potentially riding the oscillation wave. B) This circuit actively records the TMS pulse artifacts and then removes it from the recorded voltage trace with a passive resistance adder. This enabled us to record spiking activity within 1 ms of the TMS pulse. Figure modified from (Mueller et al., 2014).

2.2 Coil Modifications

2.2.1 Custom D-Shaped Coil

Our interest in recording from the focal center of stimulation meant that a figure-8 shaped coil, as is typically used by clinicians and human brain researchers, would be better suited than a circular TMS coil, which was used in other primate TMS studies (Tischler et al., 2011). Figure-8 coils produce the strongest electric field in the brain in a single region, under the intersection of the two wings. Figure 2.4 shows a model representation of the electric field produced in a macaque brain.
Figure 2.3: Minimal artifacts (the burst of noise starting at time 0, when spTMS was applied) recorded *in vivo* with the circuits of Figure 2.2. (A) Superimposed, repeated recordings aligned to the start of spTMS using Circuit A. (B) Improved artifact reduction from using Circuit B.

![Artifact Reduction](image)

Figure 2.4: A model of the normalized electric field produced by the figure-8 coil. The electric field is modeled in an uniform sphere roughly the same size as a rhesus macaque's brain. Courtesy of Angel Peterchev's laboratory; modified from (Mueller et al., 2014).

by a figure-8 coil. Notice the highest intensity electric is directly below the focal center of the TMS coil.

A figure-8 coil configuration was problematic, however, since we wanted to record neurons located in the induced electric field (red region of Figure 2.4). To access the brain for recording, we require a craniotomy onto which a sterile chamber is attached, but this recording chamber would block the figure-8 coil from having close contact with the head, meaning there would be little or no electric field produced in the brain. Several iterations of coils were developed in collaboration with Warren Grill's laboratory (see Appendix B for details of design history and Appendix C for details of final coil construction). The main innovation was
Figure 2.5: A) The cross-sectional area of the electric field produced by the custom coil. B) Oblique top view of the coil’s electric field. C) The relationship between intensity of the electric field and the depth into the brain. The dotted line represents the estimated electric field threshold necessary to stimulate neuronal elements. Note that all plots here are based off of modeling with an uniform sphere approximately the same size as a primate skull.

A slot between the two wings of the figure-8. That is, the wings were separated so that they could fit around the recording chamber. Increasing the distance between the wings, however, reduced the amount of overlap between the wings’ magnetic fields, meaning a weaker induced electric field between them, at the site of neural recordings. In order to compensate for the separation, the wings were modified so that they were slightly angled towards the focal center in the chamber. This resulted in an improved interaction of the magnetic fields over more depth into the brain, and therefore an improved strength and focal volume of stimulation in the brain. Figure 2.5 shows various view angles of computer simulations of the electric field produced by the final coil design (courtesy Peterchev lab). Furthermore, the figure also plots the normalized electric field vs surface depth for the coil, demonstrating that when the coil is outputting maximum intensity, it should produce an electric field above threshold for activating neurons for the first centimeter and a half below the surface of the dura. Hence, this TMS coil’s novel design allows for plenty of depth access to the brain for recording in cerebral cortex.

Figure 2.6 is a side by side comparison of our modified TMS coil and a conventional Magstim figure-8 coil, according to their shape (top), electric field (center), and electric field fall off (bottom). As can be seen, the modified TMS coil has larger area of stimulation of the surface of the head than the traditional coil, how-
Figure 2.6: The comparison between the traditional figure-8 coil and the modified coil. Data in the plots are modeled or measured electric field strengths, $E$, relative to the estimated neural activation threshold, $E_{th}$.

However, because of the angle of the wings on the coil, there is a greater interaction between the magnetic fields created along the edge of the coil. This interaction meant that the coil’s induced electric field could penetrate to the same depth as the figure-8 coil without the high level scalp stimulation associated in a traditional figure-8 coil.

2.2.2 Stim vs. Sham

In addition to modifying the coil to optimize recording at the site of stimulation, we also modified the power line connections to our TMS coil so it was possible to record Stim and Sham protocols without disrupting the experimental setup at the monkey’s head. We separated the power lines of each winding of our TMS
Figure 2.7: Top row) The direction of the current is labeled on each wing of the TMS coil, with the Stim configuration currents going in the same direction past the recording chamber (opposite directions around the loops), while the Sham currents move in opposite directions past the recording chamber (both clockwise around the loops). Bottom row) The resulting electric fields produced in the brain. Notice how Stim has a high intensity electric field between the two wings but the Sham produces almost zero electric field in the area between the two wings.

coil, so that we had control of the direction of current flow. This meant that we could control the constructive and deconstructive properties of the interacting magnetic fields at the intersection of the two wings. Figure 2.7 illustrates the constructive properties when the currents flow in opposing directions, creating our Stim condition, and deconstructive properties observed when the currents flow in the same direction, creating our Sham condition.

A crucial feature of this design is that since the same amount of current will go through the windings during Sham, the same ”clicking” sound will be produced as in the Stim condition, and edge of the wings will still create a small magnetic field which will induce a similar scalp sensation. It is important to note, however, that because the winding of the coils were angled slightly towards the center, there is a
FIGURE 2.8: Contour plots of the electric fields produced by the custom coil.  
A) A top view of the electric field for the Stim configuration.  
B) A top view of the electric field for the Sham configuration.

stronger magnetic field around than edge of the Sham configuration than would be for a non-angled figure-8 coil. This additional field interaction means that there is likely indirect annular stimulation at the sight of recording. This is still an acceptable control since the interest of the project is on the immediate effects of TMS on neurons, that is, the effects of the central, focused electric field at the recording site. Figure 2.8 shows the expected magnetic fields in the head for both Stim and Sham configuration.
Effects of Single Pulse TMS on Neural Activity

From the summer of 2012 through 2015 my colleagues and I recorded from the frontal eye field (FEF), the primary visual cortex (V1), and the primary motor cortex (M1). During this time we studied the effect of TMS on neural activity using a variety of intensities and Stim/Sham configurations. Each application of a TMS pulse constituted one "trial". A collection of trials at a single intensity and constant Stim or Sham configuration was termed a "block" and saved in a discrete data file. Examples of raw data, as observed in the lab, are shown in Appendix A. For each recorded neuron, we used a fast, coarse range of intensities (10, 50, and 90%) if we were unsure about the stability of the neural isolation, or a more time-consuming fine range of intensities (10% to 90% in 10% increments, in mixed up order) if we were confident that the isolation would endure for long enough (Figure 3.1). Given the small sample size collected from V1 we did not include its data in our analysis or figures.

3.1 Single Cell Examples

The first main result was that single neuron responses to spTMS are remarkably heterogeneous. TMS-evoked response patterns included transient excitation, transient inhibition, periods of complete inhibition of activity, and other, more complex patterns. Figure 3.2 shows a few different examples of the single neuron
Figure 3.1: Diagram of the two protocols used during recording sessions. Protocol 1 (Left): Blocks where recorded for a well isolated unit where each block consistent of 15 TMS pulses 15 seconds apart with baseline collected for a minute before and after each block. The protocol would always alternate blocks between Stim and Sham, however, the starting configuration was pseudo-randomly selected. Likewise the order of the blocks for 90% and 10% intensity could vary. Protocol 2 (Right): The order of intensities and Stim/Sham configuration varied across recording sessions, with the only designation being to record all nine intensities for both Stim and Sham with a similar ordering pattern. When a new neuron was isolated, its activity was recorded for at least a minute prior to starting the stimulation protocol. Each block consisted of 20 pulses separated by 15 seconds with an uniform jitter of up to 1 second.

responses we observed. The three examples are all from the Stim configuration, at 50% or higher intensity, selected from different recording sessions. Example neuron 1 showed an immediate high activity response to TMS between 10 and 20 ms following the pulse. The response was so consistent, and so short latency, that it resembles a vertical line just to the right of time 0. After this quick burst of activity the cell returned to baseline activity. Example neuron 2 also demonstrated some initial bursting activity. This activity was over a longer time period and also appeared to be centered at a later time, around 35 ms. Much more surprising
Figure 3.2: Some examples of the variability of neural response observed during the recording session. The raster plots show the activity 500 ms before to 500 ms after the TMS pulse (at time 0; stimulus artifact duration shown in gray). Below each raster plot is the superimposed action potential waveforms for each action potential tick mark represented above it. The before and after data are shown in different colors (red and blue, respectively) in order to demonstrate that they are from the same neuron.

About this example was that starting at 50 ms the cell was completely inhibited for almost 100 ms. The cell activity started again with a much higher firing rate than before the pulse. Example neuron 3 was especially complex, in that it showed an initial inhibition of cell activity, followed by a short burst of activity, followed by another period of inhibition before the cell returned to normal firing.

These example neurons were typical, and illustrated two main characteristics of all the individual neuron responses. First, spTMS could evoke excitation, inhibition, or both for several hundred ms. This suggests that the < 1 ms induced electric field does not only evoke single spikes, but triggers a prolonged circuit response. Second, although there was heterogeneity of responses between neurons, there was precise consistency in responses, trial-by-trial, within individual neurons. This indicates that our methods of recording and stimulation were highly reliable, and that the neuronal responses were "stationary", affected specifically and transiently by the TMS pulses rather than gradually by unknown factors.

Given the reliability of the individual neuron responses to spTMS, we felt justified in pooling the neurons to answer the larger question of what TMS does to the aggregate, population activity in a brain area. The rest of our analyses focus on such population results.
3.2 Population Examples

First, let’s look at a summary of all the neurons I recorded from in FEF and M1. Figure 3.3 shows the average recorded activity for each block of data collected from FEF and M1 in chronological order, with the oldest files in the top rows and the newest files towards the bottom. Within the plots, the TMS pulse is marked with a black vertical line across the trials at time 0 and the excitatory activity, or activity greater than baseline, and inhibitory activity are shown with shades of red and blue respectively. For each block, or row, the individual spiking times in each trial were convolved with a normalized 5 ms width Gaussian kernel in order to create spike density functions (MacPherson and Aldridge, 1979). These trial-by-trial spike density functions were then baseline corrected using the average activity prior to the TMS pulse and averaged across the number of TMS pulses in the block (Equation (3.2)).

\[
\text{Spike Density Function} = \text{Spike Train} \ast \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}} \quad (3.1)
\]

\[
SDF_{\text{average}} = \frac{1}{\# \text{TMS pulses}} \sum_{n=1}^{\# \text{TMS pulses}} SDF_n \quad (3.2)
\]

Finally, for comparison across neurons which could have different ranges of absolute firing rates, we performed most analyses on normalized firing rates to emphasize the temporal patterns (see legend of Figure 3.3). Overall, then, each row in Figure 3.3 represents the average response pattern of a neuron to a particular intensity and Stim/Sham configuration.

In Figure 3.3, there are several clear trends to observe following the stimulation pulse. First, and most fundamentally, spTMS has an effect on most, but not all, neurons. In the majority of blocks, there is a clear overall change in activity following the TMS pulse when compared to the baseline activity prior. Additionally, in the majority of the blocks, there is a sharp burst of activity within the first 100ms following the TMS pulse. In the next section we will sort out how much of this effect is attributable to the induced electric field, in Stim trials, as opposed to spurious factors such as noise or vibration, which also occur in Sham trials. In a
Figure 3.3: Heatplots of the neural activity 500 ms before and after TMS for all collected M1 and FEF data. Each row is the spike density function for a different block, where the function is the average activity across TMS pulses. Left) The raw activity heatplot. The baseline firing rate prior to TMS was determined for each block and then subtracted from that row. The colorscale, in units of spikes/s was determined for the whole population. Center) The normalized heatplot. Each row was normalized by finding the maximum absolute value of the row and then dividing every point by the value. The colorscale is unitless and runs from -1 (inhibition, below baseline activity) to 1 (excitation, toward max activity). Right) A legend defining which blocks were recorded from M1 and FEF.
large number of block, there also appears to be a period of inhibition immediately following the excitatory peak. This inhibition roughly lasts from 50 to 200 ms. In sum, looking at the entire dataset, we can see that there is a stimulation effect on the neurons, but it is not present in all neurons or all blocks of testing. Next we will sort the data by intensity and Stim/Sham conditions to determine what experimental factors explain these differences in responses to spTMS.

3.3 Stim vs Sham

As mentioned in Chapter 2, our Sham condition was designed so that it would produce comparable auditory clicks and scalp sensations as the Stim condition, but without an induced electric field at the center of the recording chamber. This Sham design is therefore a suitable control to demonstrate the immediate and direct effects of TMS on single units, although it should be noted that many other types of Sham TMS are available and it would be interesting to test them in future studies (Deng and Peterchev, 2011; Deng et al., 2013). Figure 3.4 shows the average activity plots from Figure 3.3 graphed separately for Stim and Sham conditions. In general, within the Sham heatplot the burst of activity noted above occurs around 50 ms following the TMS pulse, while the onset time appears to be shorter in the Stim plot (this is easiest to see in the M1 data, and by zooming in with a pdf reader). Furthermore, the width of the peak activity for Sham appears to be much broader than for Stim. While there is some observed inhibition gaps in the Sham protocol, they are not as distinct as the inhibition periods observed in the Stim blocks.

In order to determine if these timing patterns of post-TMS activity were significantly different between Stim and Sham, we used the \texttt{findpeaks} in MATLAB on each block in order to find the first peak time immediately following the TMS pulse that passed a threshold value of 0.2 for the normalized heatplot (It is important to note that since the amplitudes were all relative to the absolute max value for each block the maximum amplitude range is [-1 1]). This threshold limited the search to only find peaks with bursting activity greater than baseline; we
verified by eye for each block that the method excluded spurious post-TMS peaks similar to those in the pre-TMS period. The peak times were binned every 6 ms for averaging across blocks. The frequencies of occurrence of post-TMS bursts were then graphed as a function of post-TMS time as shown in Figure 3.5. The area-normalized graph at the bottom illustrates that the Stim blocks typically have a burst of activity following the TMS pulse about 12 ms while the Sham blocks have high activity around 18 ms. Importantly, note that we have not even separated the data by intensity yet. While the Stim and Sham data in Figure 3.5 may seem surprisingly similar, half of the Stim data were collected at less than 50% intensity, below motor threshold (recall, that average was 61%), and thus could be effectively Sham-like.

3.4 Dose Response

As noted above, low intensity Stim is much like a Sham condition, so about half the Stim data are "diluted" by blocks in which we don’t expect an effect (the
Figure 3.5: Histograms of the peak activity times following the TMS pulse. The histograms are binned in 6 ms intervals and any cell where no peaks in activity were found were not included in the figure. Top) Raw histogram shown in block count per bin. Bottom) Normalized histogram where each bin has been divided by the total number of blocks with a peak time.

< 50% intensity conditions). Furthermore, with higher intensity Sham, such as 80% or 90%, we would expect a greater magnetic field interaction towards the focal center of the chamber which could create small, but significant focal stimulation. Therefore, next we separated Stim and Sham data by intensity levels. Figure 3.6 shows the nine intensity heatplots for the Stim configuration and Figure 3.7 shows the same heatplots for the Sham configuration. In addition to the heatplots, we also graphed the average spike density function for the blocks at each intensity, shown below each of the main graphs.

As observed in the full population plot (Figure 3.4), there is a peak of excitation within the first 100 ms following the TMS pulse for all intensities of the Stim configuration (Figure 3.6). The amplitude and consistency of this activity appears to increase proportionally with the intensity of the stimulation, with 10% intensity showing a weak trend of activity and 40% showing the clear onset pattern. At 50% intensity and above the onset time of the bursting activity is shifted earlier to occur almost immediately after the TMS pulse. Following this very short-latency
burst activity is a period of inhibition lasting between 30 and 100 ms seen most clearly for 60% and above. The length of this inhibition period tends to increase with stimulation intensity.

The Sham plots in Figure 3.7 also exhibit burst activity following the TMS pulse, but in general it is similar across intensities, and specifically it does not shift to a pattern of very short-latency excitation followed by inhibition at higher intensities (Figure 3.7), as we found in the Stim data (cf. Figure 3.6). In other
Figure 3.7: Heatplots for Sham separated by intensity with the average spike density function plotted for each intensity.

In words, there is no obvious dose response effect in the Sham data. Moreover, the low-intensity Stim data (≤50% intensity graphs in Figure 3.6) looks similar to all the Sham data. These observations suggest that the Sham responses are due to non-electric field effects such as noise and vibration. An influence of the electric field at the recording site, which should increase as intensity increases, is present in the Stim configuration only.
Smoothed Average Peak Times

To compare the data from Figures 3.6 and 3.7 directly, we smoothed the spike density functions at each intensity and superimposed them in Figure 3.8 for Stim (above) and Sham (below). There is a clear shift in bursting activity onset for the Stim configuration (marked with red arrow, Figure 3.8 top) that is not observed in the Sham configuration. Additionally, following higher intensity stimulation there is an inhibition period that lasts until about 100 ms (marked with blue arrow, Figure 3.8 top), which again was not observed in Sham.

To quantify these observations, we then found the time of the peak in the spike density functions at each intensity, which are plotted in Figure 3.9. The figure shows a clear decrease in peak onset time for the Stim configuration that starts around 50% and begins to clearly diverge from the Sham condition around 60%. This same drop is also noticeable in Figure 3.8, top graph, where 60% (yellow line) is shifted to the left of 50% (green line). This same shift was not observed in the Sham data.

For a clean, overall summary of our main result, we grouped the intensities
according to low (10-30%), medium (40-60%), and high (70-90%) intensities (Figure 3.10). Note that the high intensity category is solidly in the supra-motor threshold range for our preparation (which averaged 61%; Table 2.1). This final summary illustration shows explicitly that for higher intensity stimulation, electric field-induced bursting activity (i.e. in the Stim configuration) occurs within milliseconds after the TMS pulse and that this burst activity is followed by an inhibition period until 100 ms after the TMS pulse. In sum, we find that the TMS-evoked induction of an electric field in the cerebral cortex causes a reliable excitation-then-inhibition pattern of population neural activity that emerges at and above the motor threshold of the subject.
Figure 3.10: The average spike density function from the heatplots of Figures 3.6 and 3.7. The functions have been grouped into low (10-30%), medium (40-60%), and high intensities (70-90%) and then smoothed with a moving average over every 25 points. The waveforms are shaded according to stimulation intensity.
Effects of Repetitive TMS on Neural Activity

The focus of this thesis so far has been to characterize the immediate neural activity response to spTMS, rather than rTMS which is used extensively in clinical settings. A single session of rTMS has been observed to cause activity changes in the brain that can last as long as several hours (Allen et al., 2007; Hallett, 2007). When the treatment is applied daily over the course of several weeks, investigators and clinicians have observed more permanent changes to brain function and activity through fMRI’s and EEGs (Chang et al., 2010; Shindo et al., 2006; Fregni and Pascual-Leone, 2007; Eschweiler et al., 2000; Berman et al., 2000; Speer et al., 2000; Lisanby et al., 2009). The mechanism of change for rTMS is not understood; however, the absences of such long-term effects in well designed spTMS experiments suggests that it is a compounded effect of the pulses in the train. From inspection of single neuron data across many trials (Figure 3.3) it appeared that our spTMS protocol caused no long lasting effects, but we wanted to confirm this quantitatively This chapter will demonstrate through single cell and population examples that no significant prolonged neural activity changes were observed during the Stim configuration spTMS recordings. The same analysis will then be applied to experiments that used varying frequencies of rTMS.
Figure 4.1: (A) Activity of an example M1 neuron during the spTMS protocol. (B) The waveform of the same cell before (red) and after (teal) the TMS pulse and the ISI of the cell demonstrating well isolated single-unit activity. (C) The average "pulse response" (average activity in a 200 ms epoch following TMS minus baseline activity) after each TMS pulse during a recording block for this neuron. The line represents the linear regression. (D) Scatter plot of the linear regression slopes for the population, for both baseline changes (top) and pulse responses (bottom). The example cell is highlighted in the pulse response graph. The red points represent cells in which the baseline or pulse response of a neuron changed significantly during the spTMS pulse train (p=0.05, Spearmans Correlation).

4.0.1 Single Cell Example

Panel B of Figure 4.1 shows the waveform and ISI of an example neuron, and the raster of the neural activity during the spTMS train (0.067Hz) is shown in panel A. To quantify changes of average firing rate during a pulse train, we plotted two measures of neural activity associated with each pulse in consecutive order. The first measure was "baseline", or the spontaneous activity in the 200 ms period just before each pulse. The second measure was "pulse response", or the average activity for 200 ms after each pulse minus the baseline activity before each pulse. We then performed a linear regression and Spearman’s correlation test to determine if there was a significant change in baseline firing rates over the block’s pulse train (Figure 4.1 panel C). This neuron exhibited no significant changes in baseline (not shown) or pulse response (marked lime green in bottom graph of panel D),
although in the population (Figure 4.1 panel D), some neurons did change their baseline activity or pulse response as indicated with red dots.

4.0.2 Population

The example data illustrated in Figure 4.1 were collected at 50% intensity. The same analyses were performed, as well, on all neurons collected at 10, 70, and 90% intensity, as shown in Figure 4.2. The top graph for each intensity shows the total change in baseline firing rate during a TMS pulse train, represented as the linear regression slope as in Figure 4.1, with the red points again representing cells with significant change. The majority of neurons for all intensities show no significant baseline change for the pulse train. Likewise, there were few cells that experienced significant changes to pulse responses across the train. Overall, none of the distributions of linear regression slopes deviated significantly from zero (Wilcoxon signed rank tests, all p>.05) (Grigsby et al., 2014). This finding agrees with the qualitative assessment in Chapter 3 that responses to individual TMS pulses were highly consistent from trial to trial (Figure 3.2). These results also confirm the conclusions of previous studies that spTMS does not produce long term effects on the brain, and that single pulses applied at sufficiently slow frequencies can be treated as independent events.

4.1 rTMS Intra-train Effects

Since long term effects are observed in rTMS protocols, we wanted to determine whether such changes are baseline dependent, pulse response dependent, or some combination of the two. For a subset of our M1 neurons we recorded low (1Hz), medium (5Hz), and high (10Hz) rTMS data at either 50, 70, or 90% intensity. These rTMS blocks were all collected following the collection of a full 18 block spTMS protocol and typically included at least three separate pulse trains of rTMS. The same analysis described in Section 4.0.1 was performed on a population of neurons stimulated with 70% rTMS, with the results shown in Figures 4.3 and 4.4. The population size for 10Hz was too small to determine statistical changes,
Figure 4.2: Scatter plots for intensities 10, 50, 70, and 90% of the linear regression slopes for the population, for both baseline changes (top) and pulse response (bottom). The red points represent cells in which the firing changed significantly during the block of spTMS trials (p=0.05, Spearman’s Correlation).

However, the data are still shown in the figures.
Figure 4.3: (A) Histograms of the population baseline regression slopes for varying rTMS frequencies. 70% intensity. (B) The scatter plots of the baseline regression slopes. In the population, baselines showed significant suppression during 1 and 5Hz rTMS trains (Wilcoxon signed rank tests vs. zero, $p < 0.05$).
Figure 4.4: A) Histograms of the population pulse response regression slopes for varying rTMS frequencies. (B) Scatter plots. In the population, pulse responses were unchanged during the train for all rTMS frequencies.
At the various frequencies of rTMS, there were clear changes in neural activity over time. During 1 and 5 Hz rTMS trains, average baseline firing rate activity dropped significantly, suggesting a suppression of overall activity (Figure 4.3). This shift in activity can be seen in the left side histograms, where the majority of the firing rate slopes are less than zero and in the right side scatter plots where all significant values (marked yellow) are below zero.

There was no significant change in pulse response for either 1 Hz or 5 Hz. This makes sense since there is limited time during the pulse train for changes in cellular activity to occur; our rTMS protocols were significantly shorter than most clinical rTMS protocols, which generally include hundreds of pulses per train. It would be interesting to extend the length of our rTMS protocol to determine if the pulse response changes become more significant, and to see if more significant pulse responses lead to more or less significant baseline changes.

In addition to calculating the significance of baseline change across a series of rTMS pulse trains, we also plotted the baseline after the entire rTMS train finished, compared with the baseline just before that train started (Figure 4.5). The only significant change was seen for 1 Hz rTMS. After 1 Hz rTMS trains, baseline firing rates increased. This result may appear to be surprising given the fact that often 1 Hz rTMS has been observed to cause suppression in behavior and markers of neural activity such as MEPs and fMRI signals. Previous findings from our work, which are excluded from this report for brevity, may explain this discrepancy (Grigsby et al., 2013). In general, we have found that putative inhibitory cells typically have a more immediate bursting response to TMS than putative excitatory cells. Therefore we speculate that the main effects of 1 Hz rTMS are mediated by increased activity of the inhibitory cells which suppress the stimulated brain area’s long-range projection neurons, all of which are excitatory in cerebral cortex.
Figure 4.5: Scatter plot of the firing rates after the last pulse of an rTMS train vs before the first pulse of the rTMS train (n=56 neurons). At 1Hz an increase in activity was observed. The 5Hz data suggested a suppression of activity, though more data must be collected.
5

Conclusion

5.1 Overview

The overall goal of this thesis research was to determine what TMS does to neural activity. By using custom made electronics optimized to record neural activity in high magnetic fields, we were able to record single cell activity immediately following a TMS pulse. I found that, from neuron to neuron, single pulse TMS causes a variety of effects, but in the population, the overall change is a dose-dependent pattern of excitation-inhibition that emerges around motor threshold (> 60% intensity). The pattern consists of a short-latency, brief excitation, followed by a broader inhibition, and concludes 100 ms after the TMS pulse. Somewhat unexpectedly, a consistent Sham effect was also observed in that there was a brief excitation period 50ms following the TMS pulse.

We also found that, in the population, sequential spTMS at the very low rates we used caused no consistent changes in baseline activity or pulse responses from trial to trial. This means that each pulse of spTMS can be assumed to be an independent event and that, for our spTMS protocol, pulse order is insignificant. Using rTMS, however, we did find long-term effects during pulse trains. These effects included significant mean firing rate changes during and after pulse trains, as well as noticeable pulse response variability changes. While the pulse response changes were not significant within the population, there were enough cells with
a significant change to warrant further study. Specifically, it would be of interest
to determine if the significant pulse response changes are dependent on cell type.
Furthermore, we observed that 1Hz rTMS causes excitation in some neurons fol-
lowing the pulse train, which is unexpected in light of both the literature and
the long term effects on baseline activity that we found. It seems likely that this
initial excitation recruits inhibitory neurons preferentially, which then suppress
larger networks of neurons in the area.

5.2 Comparison to Previous Primate TMS studies

As briefly referenced in Chapter 2, there have been other primate groups working
to understand the mechanism of stimulation for TMS at a neuronal level. These
groups have experienced the same technical challenges we had and approached
them in different ways. These approaches include amplification circuit design,
artifact removal protocols, and coil modifications. Additionally, there have been
variations on the stimulation protocol and targeted brain area.

One of the earliest studies of TMS in primates was in 1994 by Baker et al.
In this experiment they recorded from the pyramidal tract in conscious monkeys,
which were identified by a collision test (Baker et al., 1994). While the group was
studying a different response than this thesis, the group observed a similar dosage
response to the one discussed in Chapter 3. Specifically, an increase in neural
response around 50% intensity and a potential increase in inhibition at higher
intensities.

Baker’s group would later go on to study reticular formation responses to
TMS stimulation of M1 (Fisher et al., 2012). In order to determine the neural
activity immediately following a TMS pulse, the group used exponential fitting
to remove the TMS artifact. One limitation of using a fitting or average method
to remove artifact, is that it obtains the best results offline. This might limit
the experimenters knowledge of the cellular activity during the recording, such
as whether or not a neuron experiences excitation or inhibition immediately after
the pulse. Within their neural data results we do observe single cell responses
very similar to our own. There are cells that had an inhibitory response to TMS stimulation and there are some that experience an initial burst of activity followed by 100 ms period of inhibition after the TMS pulse. Baker also observed that the neural response was dependent on the stimulation intensity.

Finally, Tischler et al approached the artifact issue by using a mini-coil (Tischler et al., 2011). They designed a coil that would fit inside the recording chamber. This significantly smaller design would create a very focal stimulation area in the recording chamber without requiring the same power or magnetic field interactions of larger commercial TMS coils. Since the coil would require less power and would have a much smaller magnetic field, the charging pulse artifact following stimulation was dramatically smaller. This meant that they were also able to observe cellular activity within a few milliseconds (<2.5ms) of the TMS pulse. Our results also agree with Tischler’s where there was a distinction between sub and supra motor threshold responses. During supra motor threshold stimulation, they observed a short-latency excitation peak, followed by a 100 ms period of inhibition, just like Fisher and the results presented in this thesis.

These results suggest to us that there is consistent neuronal TMS response that can be observed across subjects, scientific groups, and even decades. Further work will need to be done in order determine the direct and indirect stimulation within the collected neural data.

5.3 Limitations

There are several limitations to this study that must be considered. First and foremost is that all M1 data came from the same subject. We still need to test whether the M1 results are reproducible in a second monkey, and we will do that imminently. Secondly, while our Sham was effective in eliminating the electric field from the recording site for most intensities, at higher intensities such as 80% and 90% Sham conditions seemed to cause a slight but noticeable shortening of neural response latencies. This effect will need to be studied further with modeling and in vivo testing so that it can be characterized to potentially inform an improved
Sham design. Finally, we are still in the midst of studying rTMS. We will need to collect more rTMS data to fill in frequency gaps such as 10Hz. Additionally, to match the protocols used most often in clinical applications, we will likely need to record rTMS data with longer pulse trains.

5.4 Future Directions

Since the neural activation found with Sham spTMS was very much unexpected, it is important to determine if it resulted from annular electrical stimulation, scalp stimulation, auditory response, or something else. By using an alternative Sham, such as a bone vibrator, we can determine whether the effect was electrical stimulation or mechanical. It would also be beneficial to create more thorough computational models of TMS in the monkey brain. These models would be based on DTI and MRI recordings of the monkey subjects and would allow us to more accurately estimate the electric field for varying depths and intensities. Dr. Peterchev’s lab is currently working on creating this improved head model design. Lastly, we would want to test different spatial orientations of the TMS coil to see whether the apparent heterogeneity of individual neurons might be attributable, in part, to the neurons’ relative orientations with respect to the induced electric field.

5.5 Take Home Message

Within this project we have demonstrated that TMS does create neuromodulation in the brain that can be consistently characterized. These results are among the first to provide a fundamental understanding of the relationship between TMS parameters and neural activity. Ultimately, this line of research should lead to a more rational approach to designing TMS protocols for specific clinical needs.
Appendix A

In Vivo Recording Traces

Here are examples of raw voltage traces from the data collected. Figures A.1 and A.2 show how soon after the TMS pulse we begin to see and record neural activity. An example of a single pulse recording block is shown in Figure A.3.

Figure A.1: Overlay of TMS pulses showing identifiable action potentials just before and after stimulation.
Figure A.2: Overlay of TMS pulses showing identifiable short latency action potentials (< 5ms) just before after stimulation.

Figure A.3: An example of the raw voltage trace collected during one block of a single pulse recording. (See Chapter 3 for the definition of a recording block.) At the beginning of a recording block the baseline activity was recorded for 60 seconds. There were then 15 TMS pulses separated by 15 seconds. In order to prevent an anticipatory response in the primates, there was an additional uniformly distributed jitter of up to one second between each pulse. After the 15 pulses, the neural activity was recorded for another 60 seconds. The TMS pulses are shown in red.
I worked closely with members of the Grill laboratory to design the novel TMS coil used in my work. Dr. Warren Grill and his then-Master’s student, Jerel Mueller, constructed the coil, and I helped to test it at the bench (using saline flasks and measurement equipment) and in vivo (during preliminary neural recordings). The common theme throughout the coil design was to permit access to the middle of the induced electric field in the brain, which required the innovative inclusion of a slot between the two “wings” of the figure-8 or butterfly coil model. The first attempt was a ”double racetrack coil” (schematic in Figure B.1; induced electric field modeling in Figures B.2 and B.3). The second design was a ”double triangle coil” (schematic in Figure B.4; induced electric field modeling in Figures B.5 and B.6). This design was then angled 30 degrees towards the recording chamber to increase magnetic field interactions. (schematic in Figure B.7; induced electric field modeling in Figures B.8). The final design changed the wing shape from a triangle to D-shape in order to minimize stress on the coil’s wire windings (schematic in Figure B.9; depth threshold comparison B.10).
**Figure B.1:** The finite element model for the double racetrack coil v1.0.

**Figure B.2:** The electric field for the double racetrack coil. The model is of the electric field in an uniform sphere with a 3.5 cm radius. *Left*) The frontal view of the electric field. The strongest points of the electric field are immediately under the edges of the racetrack coils. The electric field under the recording chamber is weaker than the field under the coil edges, however, it appears fairly constant and well distributed for about a third of the depth into the brain. *Right*) The top view of the electric field produced on the surface of the sphere. The majority of the field between the coils has a large magnitude.
Figure B.3: The electric field depth threshold for the double racetrack coil v1.0. The blue line shows the expected strength of the field at the center of the recording chamber at various depths from the surface. The red line marks the minimum electric field strength required to illicit a neural response, $E_{th} = 0.92 V/cm$ (based on the average human motor threshold, adjusted for waveform). The interception between the two lines is the maximum depth where the stimulation will cause a direct effect. The maximum depth estimated for the double racetrack coil was about 0.8 cm. $V_c = 1650 V$ (100% max output of Magstim Rapid) and $L = 9.9 \mu H$. 
Figure B.4: The finite element model for the double triangle coil v1.5. There are 11 winding layers of wire for each coil. The triangle shape was to strengthen the electric field in the recording chamber and increasing the magnetic field strength.
Figure B.5: The electric field for the double triangle coil. The model is of the electric field in an uniform sphere with a 3.5 cm radius. Left) The frontal view of the electric field. The strongest points of the electric field are again immediately under the edges of the coils. The electric field under the recording chamber, however, is much stronger than the field observed with the racetrack coil model. Right) The top view of the electric field produced on the surface of the sphere. The majority of the field between the coils has a large magnitude that is very similar to the field observed with the racetrack coil.
Figure B.6: The electric field depth threshold for the double triangle coil v1.5. The blue line shows the expected strength of the field at the center of the recording chamber at various depths from the surface. The red line marks the minimum electric field strength required to illicit a neural response, $E_{th} = 0.87\text{V/cm}$ (adjusted for waveform). The maximum depth estimated for the double triangle coil was about 1 cm. $V_c = 1650\text{V}$ (100% max output of Magstim Rapid) and $L = 11\mu\text{H}$. 
Tilt wings down 30 degrees

Figure B.7: The finite element model for the double triangle coil v2.5. In this version, the wings were tilted down by 30 degrees. This tilt allowed for greater interaction of the magnetic fields produced in each wing, especially at the center of the recording chamber. This allowed for a stronger electric field in the recording chamber and the deeper penetration of the stimulation into the cortex. Ultimately, the flaw with this design was that the sharp corners of the triangle and the high current in the windings could lead to wire breakage in the wings.
Figure B.8: The electric field depth threshold for the double triangle coil v2.5. The blue line shows the expected strength of the field at the center of the recording chamber at various depths from the surface. The red line marks the minimum electric field strength required to illicit a neural response, $E_{th} = 0.87 V/cm$ (adjusted for waveform). The field strength increased significantly after the triangle wings were angled towards the head. The electric field at the head surface is almost double the surface field strength of the double triangle coil v1.5. The maximum depth estimated for the angled double triangle coil was a little over 1.5 cm. $V_c = 1650 V$ (100% max output of Magstim Rapid) and $L = 11 \mu H$. 
Figure B.9: The angled D-shape coil. This coil was designed to create a strong electric field similar to the one observed with the angled triangle coil while decreasing the amount of stress on the wire windings experienced with the double triangle coil designs. As observed in Figure 2.5 the 30° angle of the windings created a strong electric field at the center of the recording chamber.
Figure B.10: The depth thresholds for various coil designs and recording chamber diameters. In addition to modeling the threshold depth for various coil designs, we also considered the possibility of modifying the recording chambers, specifically the recording chamber’s diameter. Decreasing the separation between the wings leads to an increase of the electric field. By optimizing both the wings’ separation and design, the threshold depth could go as far as 2 cm into the brain. The modifications of the recording chambers were ultimately not completed in order to not limit our results and to allow for easier implementation both within our lab and in other primate labs.
Appendix C

TMS Coil Casting Implementation

In addition to assisting in the design and testing of novel TMS coils, I also worked with members of the Grill laboratory to create a robust mold for building consistent quality D-shape TMS coils. Figure C.1 shows the number of wire windings per coil. Figures C.2 through C.4 show the dimension of the coil wings and their internal spools. Figures C.5 and C.6 show previous casting mold shapes. Finally, Figure C.7 show the machine shop measurements necessary for the stable arm connector.
Figure C.1: The loop dimensions for the final coil design (ie angled D-shaped coil). There are nine wire winding layers and an induction value of $L = 12.7 \mu H$. 
Figure C.2: The dimensions of the spools used to make the angled D-shaped coil.

Figure C.3: Top view of the coil casting dimensions.
Figure C.4: The cross sectional front view of the coil casting dimension.

Figure C.5: The initial purposed casting mold design. In this design the weakest points on the coil (ie where in the angle occurs) were supported. This design was not supported enough of the structure so inconsistent proxy casting was observed.
Figure C.6: Final Casting Mold Design. This design completely surrounding the coil and maintained secure contacts once locked together. The design enable additional proxy to be added to the cast after the mold was locked together in order to fill any air bubbles that may have formed. This mold was significantly more consistent between coil builds than the previous molds.
Figure C.7: The dimensions of the arm connector for the TMS coil. The TMS coil was secured to the chair using a small mechanical arm that would screw into a shock on the chair. This connection allowed for consistent coil placement and also limited the mechanical noise within the recorded signal.


