TMS Disruption of Time Encoding in Human Primary Visual Cortex

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This report details my summer research project for the REU Theoretical and Computational Neuroscience program as part of Dr. Michael Beauchamp's lab. This summer, I studied time encoding in the primary visual cortex using Transcranial Magnetic Stimulation. TMS is a technique that perturbs brain activation by running a current through a coil and causing a large change in magnetic field, thus creating a momentary electric field within the conductive medium of the cortex that briefly affects the electrical signaling of the brain. Single pulse TMS is a safe, non-invasive, effective way of linking change in behavioral responses to an area of stimulation. In the case of this experiment, TMS is used to imply causation between V1 and time perception. The goals of this project are to give evidence of time encoding in human primary visual cortex, to use subsequent data to form ideas about how disruption of visual pathways affects timing judgments, and to use this information to posit about the neural mechanism for timing in this area.

Introduction

The primary visual cortex is one of the best-studied areas in systems neuroscience research. Most of the research examines visual encoding and retinotopic organization, as well as the role of vision in areas such as perception, attention, and multisensory integration. However, a recent study has given evidence that primary visual cortex (V1) has the ability to encode secondary information, such as timing (Shuler, Science 2006). Shuler, et al used implanted electrode recordings to show that primary visual neurons in rats have three separate mechanisms for encoding timing information. The rats learned to time a consistent interval between a visual stimulus, in one eye, and a reward. The authors then found right and left eye preferential neurons whose activations at presentation of visual stimulus correlated to when a reward occurred, or was supposed to occur. The correlated timings of activation, in addition to the observation that the same neurons when untrained did not perform this way, led researchers to conclude the brief interval triggered by the visual stimulus was learned and programmed within the neurons that encoded the visual information as well.

However, Shuler and Bear did not show how these neurons affect timing ability. In order to fill in this gap, we endeavored to do two things: to determine if there is a corresponding mechanism in human primary visual neurons, and to test its effect on timing. Our overall method was to train subjects to estimate an interval of time, and then to disrupt V1 during the interval in order see how accuracy and precision of the response times were affected. To do this, we first found a location on each subject's V1 that produced a visual disruption when TMS was delivered. Then we used a visual discrimination task to behaviorally confirm the area of the visual field that was being affected by the TMS. We then trained each subject to estimate a two second interval following the presentation of a visual stimulus in this area. This learned ability was then tested by delivering TMS to the V1 location during the interval. Our hypothesis was that, if these trained neurons are disrupted using TMS, then ability to time will be affected. If so, it can be concluded that not only do human V1 neurons have similar timing mechanisms, but also that they can be used to time.

Methods

In order to complete our main experiment of testing timing ability with and without TMS, several preliminary experiments were needed for both logistical information and behavioral evidence of V1 disruption.

Locating Consistent Area of Visual Disruption

First, a location on the subject's visual cortex was found that consistently produced a phosphene in the lower left area of the visual field. Phosphenes represent disruptions in the visual field due to signal interference created by the TMS. TMS pulses were delivered by a MagStim Rapid TMS (Magstim, Wales, UK). MRI data of the subject is uploaded to the TMS interface software, Brainsight (Rogue Research, Montreal, Quebec), so that the subject's own brain was the map used to locate the visual cortex. The Brainsight program is a neuronavigation tool that allows the experimenter to view the subject's brain in reference to the TMS coil on a computer screen, so that positioning of the coil is spacially accurate. Since the lower left visual field was the target of the disruption, the right, slightly dorsal areas of V1 were targeted with the TMS. Certain areas of the cortex are more susceptible to inducing current (Thielscher et al, 2009), so the location of stimulation was found through localized trial and error for each subject. The TMS coil was positioned over an area in the right dorsal primary visual cortex and triggered, until the subject reported a visual disruption. Once the location was identified, a marker was set and recorded on the brain image so that it could be returned to for all subsequent steps. This location was targeted in subsequent experiments in order perturb the subject's visual pathway during the interval estimation task.

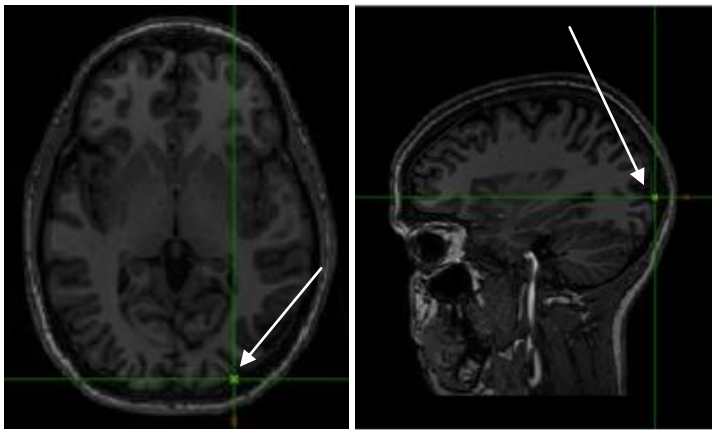

Figure 1: Axial (left) and sagittal (right) views of one subject's brain. The yellow spot indicated by the arrows is the area stimulated by the TMS in order to produce the phosphene.

Behavioral Confirmation

The next step confirmed behaviorally that the phosphene and stimulus overlapped. Using the software Presentation (v. 13.1 Neurobehavioral Systems, Inc., Albany, CA), a "U" shaped stimulus was presented in the lower left part of the subject's visual field, in any of four directions, up, down, left or right (see figure 2). The program then delivered a TMS pulse immediately after presentation of the stimulus. The subject then determined which direction the stimulus was facing, and responded using the arrow

Figure 2: Example of stimulus on computer screen

keys. If the phosphene and stimulus both occupied overlapping visual space, then the subject's ability to determine the orientation of the stimulus decreased in comparison to a control. Forty trials per TMS delay were run until the optimal timing was identified. There were also two controls; one round where no TMS pulse was delivered, and another where a TMS pulse was given to a non-visual, non-interfering area of the brain like the frontal lobe.

Since the subjects' cortexes varied in both connectivity and area of TMS stimulation, the optimal delay of the TMS pulse was variable between subjects. Literature tells us that there is generally strong disruption with an 80-100 ms timing delay (Amassian et al, 1989), but subsequent studies have shown that there are potentially two intervals of optimal timing delay, an earlier disruption just as visual information is being encoded, and a later one for visual perception (Corthout et al, 1999; Juan et al, 2003). The optimal delay for each subject was found by running the experiment with varying delays and choosing the one with the lowest percent correct in the task. The percent correct for a four alternative forced choice (4AFC) task with complete interference should be around 25%.

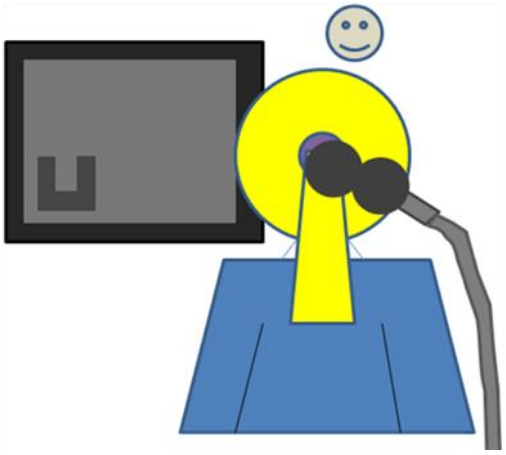

Figure 3: Example set up of behavioral experiment. TMS figure of eight coil is over area of stimulation; smiley face is the point of fixation so that the stimulus (U) is in the lower left visual field.

Time Interval Training

After confirming the location of disruption in the visual field produced by the TMS, the subject was then trained to know the duration of a two second interval. A visual stimulus was presented in the confirmed visual field location, and the subject was then told to respond when they believed two seconds had passed. The program then provided calibrated feedback for all responses farther than +- 100 ms away from 2000 ms. A calibrated feedback bar showed up in red if the response was over 100 ms too early or too late; the length of the bar was directly proportional to how far off the response was (see figure 4). On correct trials (those where the subject guessed within the acceptable range) the same bar was shown in green, depicting the precision of the response, on half of the trials. Feedback only occurred on half the correct trials so that the subject did not learn the response, just the stimulus and the time interval. Since we wanted to test the encoding of time in visual neurons, recording time by counting beats, numbers, or any other typical method was discouraged. There were forty trials of this interval learning; stimulus shown, interval guessed, feedback given. This part of the methods was used to train V1 neurons to encode time through repetition, as Shuler and Bear reported no corresponding activation in untrained neurons.

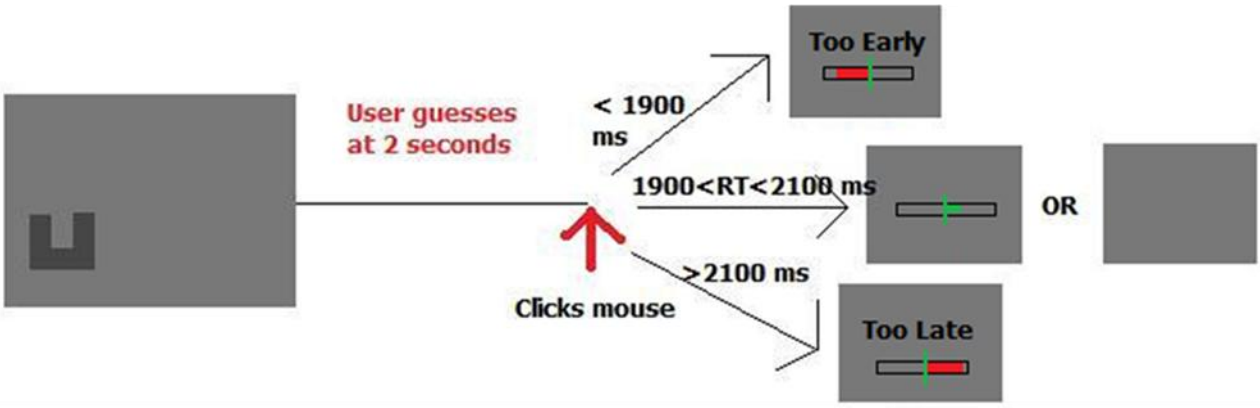

Figure 4: Training procedure. Calibrated responses for inaccurate trials, and positive or no response for others

Testing TMS Effect on Timing Ability

The last step tested the effect of disruption in the primary visual cortex on the ability to time this two second interval. Presentation ran the following trial forty times: the same stimulus was presented, the subject responded when they believe two seconds has passed, and no feedback was given. On half of these trials, the program delivered a TMS pulse, at a random point between 200 and 1800 ms, to V1.

The purpose was to compare these two types of trials. After the training procedure, the V1 neurons have been trained for time encoding. The no-TMS trials were run so that subjects were able to use this timing information to help them estimate the interval. V1 TMS disrupted this information during the interval, so it could not be used. The response times for each of these kinds of trials were compared to see if disrupting V1 activation hindered estimation.

The control for the experiment involved the same paradigm, but instead of delivering a TMS pulse to the visual cortex, a TMS pulse is delivered to a non-involved area of the brain with minimal disruption of possible confounds like motor cortex stimulation. This was done to control for non-specific side-effects of TMS.

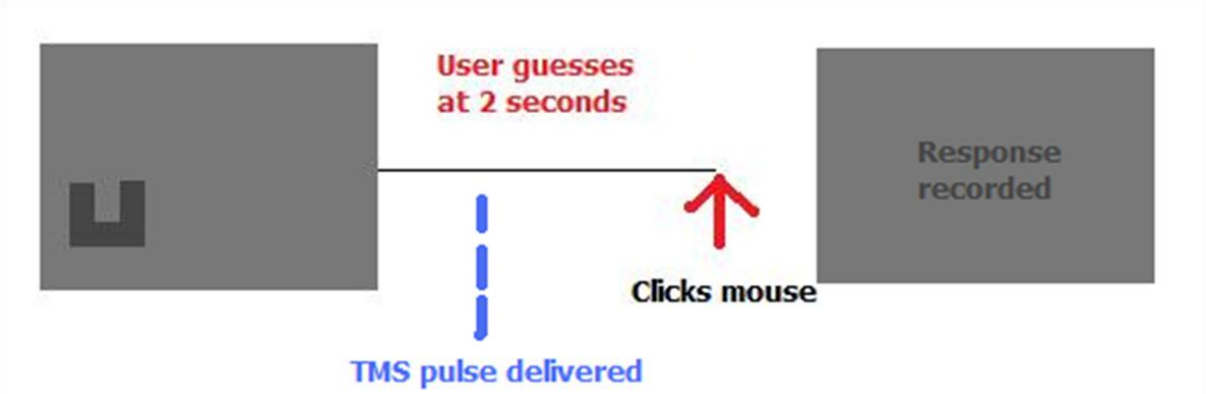

Figure 5: Testing procedure. TMS pulse (indicated by blue line) occurs at a random point between 200 ms and 1800 ms after presentation of stimulus

Results

Behavioral Results

With all eleven subjects there were varying optimal timing delays ranging from 45 ms to 70 ms. These numbers are consistent with the literature values, especially considering the slight timing delays caused by the program sending a signal to the TMS and also the brief time the TMS spends charging and pulsing. With these considerations, the actual timing delays are probably a bit longer than what the program recorded. All eleven subjects showed behavioral deficits ranging from 15% to 54% below a control baseline, and the average percent correct over all subjects at each subject's optimal delay was 53%. An example graph of accuracy in the task vs. TMS delay is given in figure 6. There was also a qualitative trend between strength and consistency of the phosphene effect and behavioral deficit at optimal TMS delay. For example, the subject who experienced an immediate, consistent and broad phosphene also recorded the greatest drop in accuracy for the behavioral task.

Another trend was observed upon examination of the behavioral data. In error trials, there were a disproportionate amount of 180 degree flip errors as opposed to either 90 degree rotation errors (i.e. when an 'up' stimulus was presented, subject chose 'down' not 'right' or 'left'). Since there are 3 possible errors a subject can make, on error trials, the probability of a 180 degree error is 33%. In our results, we observed that, on average, this error was made 67% of the time. Another interesting correlated trend was that this proportion was even higher at non-optimal TMS delays. A subject at non-optimal TMS delays might make the 180 degree error ~90% of the time, as opposed to 60% of the time at optimal TMS delay. We concluded from this that with a more congruent delay, the 90 degree options were *more* difficult to automatically dismiss in the decision process, but still dismissed much earlier than the last two options (correct and 180 degree flip). We believe that the baseline around 50% in other subjects was due to this ability to dismiss the 90 degree options quickly. Even at optimal rates, subjects were not as susceptible to the TMS disrupting all orientation information (hence their 180 flip rates being closer to twice the theoretical rate even at optimal TMS delay). Since 90 degree errors, even at an optimal TMS delay, were fairly easily dismissed, the task for three of four subjects still resembled a two choice forced task rather than a four choice one, and baselines approached 50% instead of 25%. The subject with the greatest behavioral deficit (29% accuracy at optimal delay) had a corresponding 180 degree error rate of 37%, meaning that the accuracy fell to chance as the 180 flip error rate also fell to chance. All subjects represented corresponding trends of accuracy with 180 flip error rates; as a TMS delay became more optimal, the ability to dismiss 90 degree errors also decreased *at a similar rate* as accuracy in the task (see figures 6 and 7).

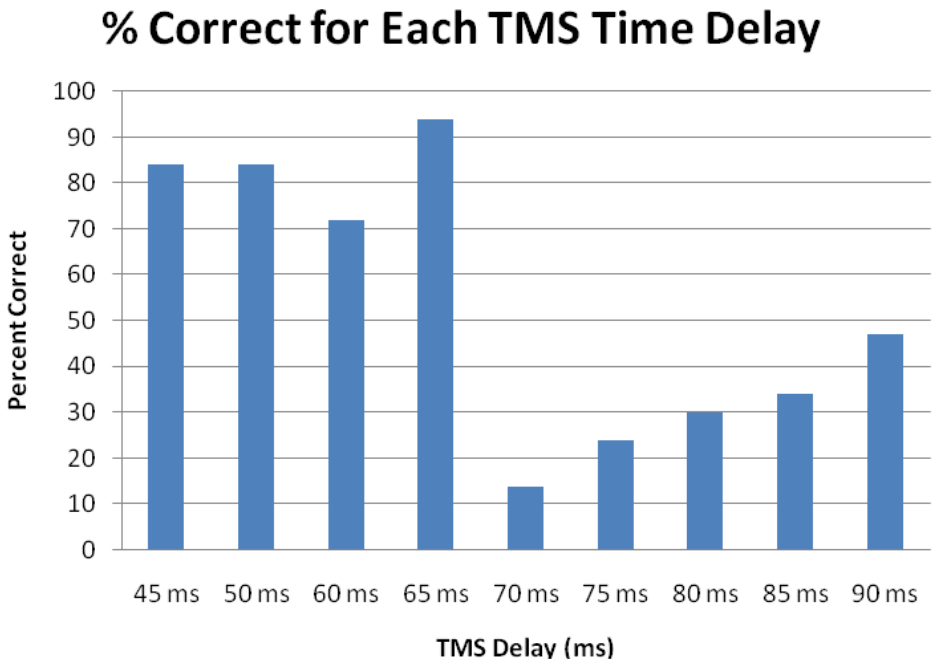

Figure 6: Accuracy in behavioral task over an array of TMS delays, for one subject.

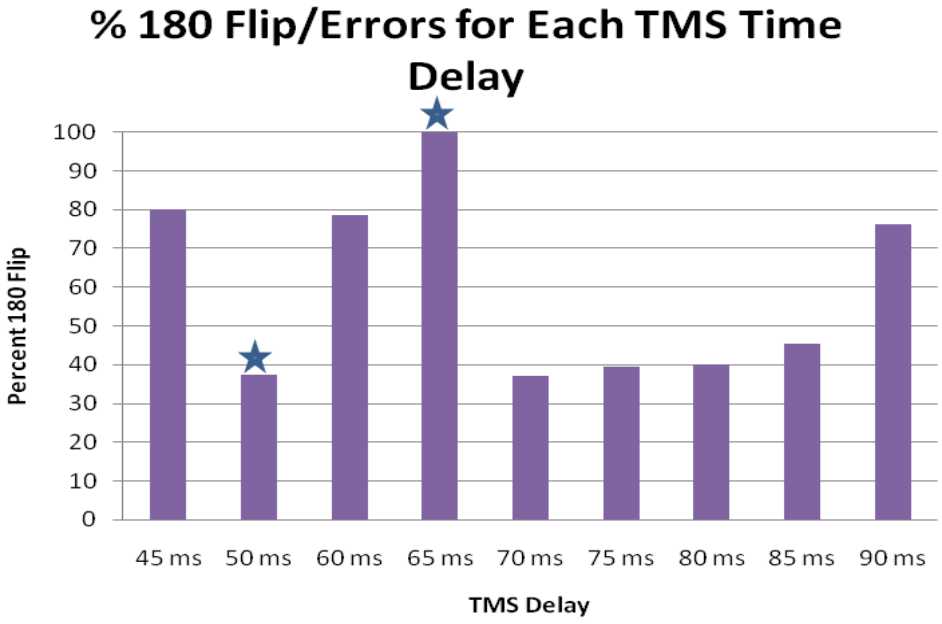

Figure 7: 180 degree error rate across an array of TMS delays. The stars indicate trials where an insufficient amount of errors were made to determine significance.

Results of TMS Effect on Timing Ability

Each trial in the testing program presented the following sequence: stimulus, wait, subject response, screen with the words "response recorded" but no other feedback. On half of these trials, a random TMS was delivered between 200 ms and 1800 ms after the end of the stimulus.

The TMS and No-TMS trials were randomly interleaved. Out of four rounds of forty trials for each subject, three were experimental (V1 TMS) and one was a control (frontal lobe TMS). Upon analysis, the trials were separated into the separate groups and compared.

Fig 8 shows data for one subject. There was a distribution of response times (RTs). Most RTs were clustered around the desired duration of 2 seconds, with a mean RT of 2311 ms. There was a roughly Gaussian distribution of RTs, with a standard deviation of 302.

Figure 9 shows data for the same subject with TMS. The distribution has a mean of 2416, and a standard deviation of 540. There was less of a Gaussian distribution and more of an even distribution for this data series, as well as a larger range.

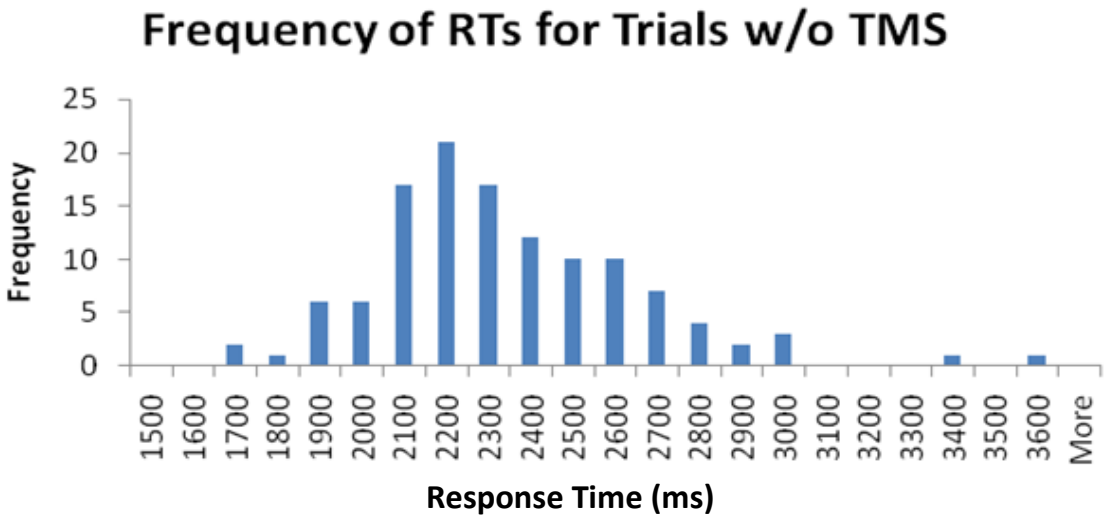

Figure 8: Histogram of trials without TMS for one subject.

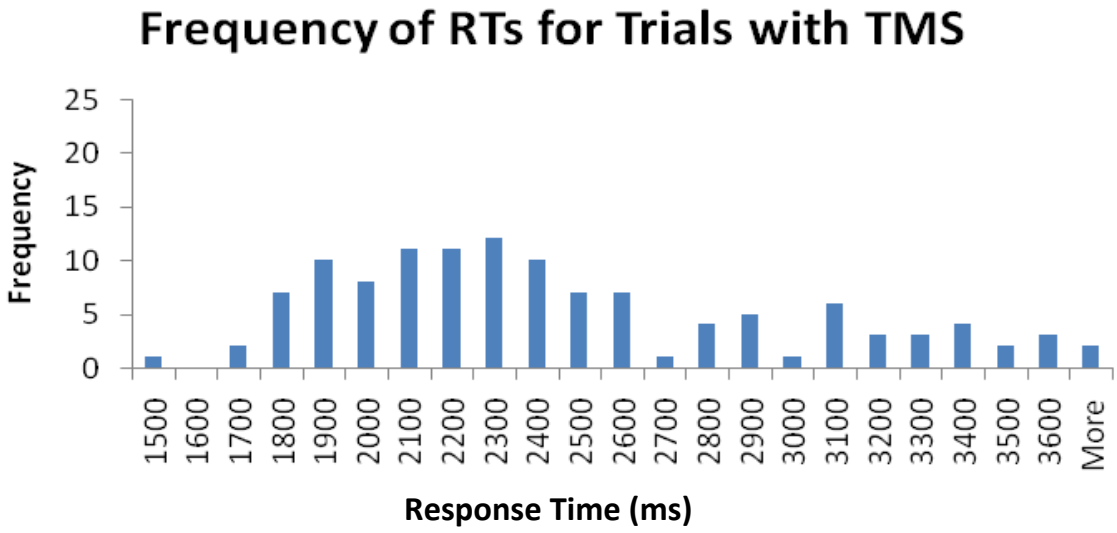

Figure 9: Histogram of TMS trials for the same subject.

We performed analysis across nine subjects, after screening those subjects that did not fall below 70% accuracy in the orientation task. In each subject, the standard deviation for TMS

trials was larger than for no TMS trials. Averaged across subjects, the standard deviation for no TMS trials was 381, and 475 for trials where V1 TMS was delivered, p = .00924. There was no difference in the mean RT; the average mean response time was 2211 for no TMS trials and 2263 for V1 TMS trials, p = .147.

In order to remove the possibility that non-specific confounds of the TMS were the source of the change in standard deviation, we performed a control experiment where the same 40 trial paradigm was used, but instead of delivering a TMS pulse to the visual cortex, the frontal lobe was targeted. This allowed for no-TMS trials and V1 TMS trials to each be compared to the control TMS trials. The statistics for the no-TMS trials were similar to the control and those for the V1 TMS trials differed significantly, further supporting that change in timing ability is a result from disruption of V1 neurons. If there were confounds because of properties of the physical TMS pulse and not because of directed neuronal disruption, then the standard deviation of these control TMS trials would have been closer to that of the V1 TMS trials.

We found that the average standard deviation of control TMS trials was 348, slightly greater than the standard deviation of no-TMS trials at 381, p = .104. Since this was not statistically significant, we said that the control TMS trials were virtually the same as the no-TMS trials. The significant change in timing ability from V1 TMS trials to no-TMS trials was not based on confounds of the TMS like anticipation or muscle movement.

Figure 10 shows the average standard deviation for each type of TMS trial, across all subjects.

Figure 11 shows the average mean response time for each type of TMS trial, across all subjects.

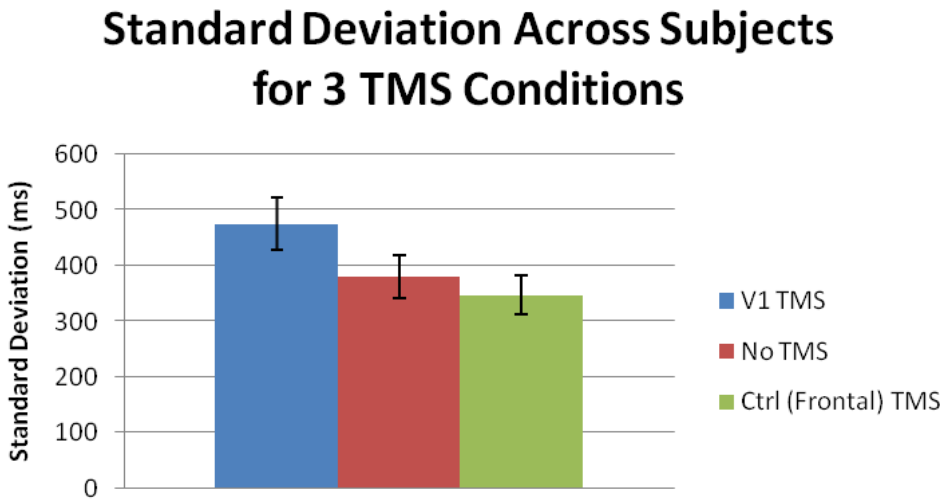

Figure 10: Comparison of average standard deviation of response times (across all subjects) for the three different TMS locations.

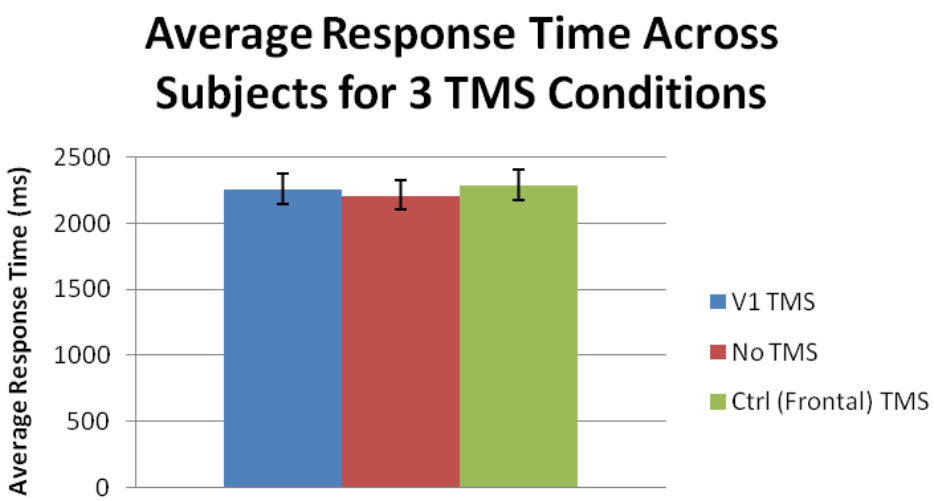

Figure 11: Comparison of average response time across all subjects, for three different TMS locations.

Discussion

Our results showed a significant difference across subjects between the standard deviation of response times when TMS was delivered to V1, and the standard deviation of response times when no TMS was given. In comparison to a frontal lobe TMS control, V1 TMS resulted in a significantly larger standard deviation. Also, the standard deviation for the control TMS response times was not significantly different from the standard deviation of the no-TMS response times. From this, we concluded that disrupting V1 does affect one's ability to time. We can further posit that because there is a significant effect from TMS pulses in V1 and not in a control site, the reason for this change is because V1 neurons are encoding timing information that is altered upon delivery of a TMS pulse. Furthermore, our results agree with those found in Shuler and Bear, that V1 neurons can be trained to encode time.

There are also a few minor changes to the paradigm that could be added for increased effectiveness. The experiment could be run in the dark, so that the only visual information being encoded would be from the stimulus. Also, the firing rate of the V1 neurons would be higher because of the drastic change of visual information as the bright stimulus flashes in the dark room. This would possibly cause TMS to be more effective, and amplify the results of our paradigm.

The Shuler and Bear finding was such a novel discovery about the brain that there are innumerable questions that could be posed in response to it. With this additional information, that humans have and are able to use timing information in our primary visual cortex, we can further direct our questions to include the following: how large of a role does timing play in the visual cortex? Do more complex visual neurons encode similar information about timing? Could there be other sensory modalities that store secondary information about time as well? If so, can timing information from these areas be used to make judgments? How strongly do we rely on timing information from these areas in comparison to each other? Continuing to strengthen the evidence for time encoding in primary visual cortex will undoubtedly spur a line of further research in related areas.

References

Amassian,V. E. *et al.* Suppression of visual perception by magnetic coil stimulation of human occipital cortex. *Electroencephalogr. Clin. Neurophysiol.* **74,** 458–462 (1989)

Corthout, E., Uttl, B., Ziemann, U., Cowey, A. & Hallett, M. Two periods of processing in the (circum)striate visual cortex as revealed by transcranial magnetic stimulation. *Neuropsychologia* **37,** 137–145 (1999)

Juan CH, & Walsh V. Feedback to V1: a reverse hierarchy in vision. *Exp Brain Res*. **150**, 259-263 (2003)

Shuler, M. G., and M. F. Bear. Reward timing in the primary visual cortex. *Science.* **311**, 1606-1609 (2006)

Thielscher, A., Reichenbach, A., Ugurbil, K., Uludag, K., The Cortical Site of Visual Suppression by Transcranial Magnetic Stimulation. *Cerebral Cortex.* Epublished ahead of print. (2009)