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Signal in the Noise? The Effect of Non-Invasive Brain Stimulation on Contrast Perception

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Chapter 1

Non-Invasive Brain Stimulation:

Past and Present

What is Non-Invasive Brain Stimulation?

Non-invasive brain stimulation (NIBS) is a set of techniques used to modulate brain activity. NIBS is becoming a popular technique in both clinical and basic research, due to its ability to modulate behavior. This is directly evident in the rehabilitation of patients with visual or motor deficits following stroke impairment, the treatment of depression in treatment resistant patients, and even Parkinson's disease, among other clinical applications (Khedr et al., 2005, Chang et al., 2005; Chang et al., 2010; Fitzgerald et al, 2009; Shimamoto et al., 2001). However, there is still a lack of understanding of the mechanisms through which these forms of stimulation affect the underlying cortex regarding effects observed on behavioral output. In light of the number of NIBS methods and protocols available, we will first address the main aspects of these neurostimulation techniques, and, specifically, how each is hypothesized to interact with cortical functions and behavior. Further to this, we will explore some key factors that come into play when determining the appropriate mechanistic and design aspects necessary to develop a well-rounded study. These aspects include parameter choices in the magnetic and electrical stimulation, as well as the importance of calibrating stimulation intensity to individual thresholds. Present research indicates these protocol choices are non-trivial, likely affecting the main source of contradiction in results in the past and current literature (Miniussi et al., 2013). Additionally, we propose that the neural activation state prior to stimulation plays a significant role in how transcranial magnetic stimulation (TMS) and (transcranial electric stimulation) TES parameters affect subsequent neural activity (Perini et al., 2012; Silvanto et al., 2008a). We present a brief review of studies manipulating the cortical activation state prior to brain stimulation, and discuss the implications in study design. We then present research which critically examines the interaction of stimulation and cortical activation state using several types of brain stimulation on healthy subjects. This paves the way for future research to explore the

possible application of these methods in the clinical population, as well as the division of clinical sub-populations when taking into account individual variability and the tailoring of the intervention to each patient. However, while the wealth of research produced by initial studies using NIBS has been crucial for scientific advancement in cognitive neuroscience. However, recent research in the development of TMS, and other NIBS techniques, have demonstrated a more complex picture of neural and behavioral impact following stimulation than initially thought. For example, in some studies NIBS has been shown to not only impact the targeted brain region, but also the areas functionally connected to the targeted region (Battelli et al., 2017). We are becoming more capable of critically assessing these non-target specific impacts on behavior by combining stimulation with other neuroimaging techniques, however, basic understanding of the techniques on behavior remains ill-defined.

These points define the scope of the thesis, our aims being to principally understand how these mechanisms of non-invasive stimulation contribute to consequent behavioral output. Namely, we investigate how two different NIBS techniques (TMS and tRNS) are crucially affected by external input factors such as stimulus properties (specifically visual contrast intensity, in the case of the present studies) which recruits a linearly increasing amount of neuronal activity with signal strength, as well as the internal input properties of each stimulation technique. With this aim, we examine the properties of these techniques and resultant behavioral outputs in regards to the theory of stochastic resonance, a recently proposed explanation as a potential mechanism by which these NIBS techniques may moderate the variety of behavioral outcomes shown in previous work.

TMS: Principles of TMS Methodology

In the 1980's TMS was primarily used for creating transient focal disruptions to cortical functions (Pascual-Leone et al., 2000). These focal disruptions are generated by a high intensity magnetic pulse which is converted to an electrical pulse within the brain through electromagnetic induction described by of Faraday's law (Rossi et al., 2009). The TMS pulse's effect on the underlying cortical tissue is to trigger depolarization of cell membranes resulting in transynaptic depolarization or hyperpolarization (Minussi et al. 2013). Using this mechanism, researchers were able to temporarily inhibit cortical function at a high enough intensity output,

and causally align a specific brain region to the subsequently disrupted behavior (Pascual-Leone et al., 2000). Researchers hoped the short-lived inhibition protocols would shed light on the numerous deficits caused in a wide variety of patient populations who had damaged in one region or another of the brain. Because it passes a magnetic pulse into the brain, it is generally more tolerable for both healthy and patient populations than direct electrical stimulation used in the past (Hallett, 2007). This method gave cognitive neuroscientists at that time a way to "simulate" brain lesions in healthy subjects by momentarily taking the stimulated area "offline" (Pascual-Leone et al., 2000). Generally, these changes are measured through behavioral and reaction time data, and show a direct causal influence of the transient pulse directly below the coil, (Robertson et al., 2003). Previously, with the use of techniques like functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG), behavior could only be correlated to various types of brain related activity elicited by psychophysical tasks (Crosson et al., 2010). Each of these neuroimaging methods also have their own disadvantages, be it the inverse problem (MEG), poor spatial resolution (EEG), or low temporal resolution (fMRI), impacting the interpretation of the correlation with behavioral affect. However, using TMS, the researcher implements a transient magnetic impulse into the system directly, creating a direct link between stimulation area and behavior. The potential to make these direct causal links has been monumental in developing the field of cognitive neuroscience and consequently our understanding of the role of individual areas and functional networks. TMS was further deemed in the field of neuropsychology as a tool that had not only excellent spatial and temporal resolution, but a tool that also had a "cognitive resolution" (Walsh and Rushworth, 1999).

Exciting new developments have been made in both clinical and healthy populations, and the application of TMS has become over the years more thoroughly established, and diverse, (single pulse, double pulse, triple pulse, repetitive transcranial stimulation (rTMS) at high frequencies or low frequencies, and theta burst stimulation; Fried et al. 2017; Thut et al., 2003; Pitcher et al., 2007; Houdayer et al., 2008; Striemer et al., 2011; Huang et al. 2011). TMS in all its modalities has been found to elicit very different effects under different conditions, or even similar conditions with pulses applied at different time intervals relative to stimulus onset (Paulus et al., 1999; Pitcher et al., 2007; Camprodon et al., 2010; Silvanto and Walsh, 2005; Cowey, 2005; Grosbras and Paus, 2003). Whereas studies with single pulse TMS (spTMS)

applied shortly before stimulus onset showed facilitatory effects, other studies applying spTMS concurrent with stimulus onset exhibited the classical “virtual lesion” effects (Grosbras and Paus, 2003; Topper et al., 1998; Silvanto and Muggleton 2008a).

Recent literature exploring the effects of TMS indicate that the neuronal activation state of the brain prior to stimulation may play a role in the paradoxical and diverse effects found within otherwise seemingly comparable studies. For instance, aspects such as initial state of the brain at the start of the intervention or task, as well as individual neurological traits and physiology play a key role in modulating effects derived from stimulation (Silvanto et al., 2008, Hsu et al., 2016). In a study by Silvanto and coworkers, they showed that when a population of neurons is temporary inhibited with low frequency TMS, and then additional TMS is applied to the cortex, a paradoxical effect occurs: stimulation that normally causes a decrement in performance becomes facilitatory in nature. The pre-stimulation of 1hZ rTMS results in the suppression of cortical activity under the stimulated area. The authors then applied single pulse TMS and showed in the control condition (when the pre-stimulation with rTMS did not occur) that subjects were similarly impaired in task performance. By putting subjects’ cortical states into one of suppression prior to applying a suppressive single pulse stimulation, the authors exhibited a reversal of effects, the interaction of an inhibitory single pulse on a system already under cortical suppression facilitated performance. This indicates that the physiological responses can vary extensively not only in patients, but also in normal subjects whose physiology and baseline level of cortex excitability extend upon a wide range, and can play a key role in determining how their brain responds to stimulation. Similarly, in a TMS study conducted by Brighina et al. (2002), the same pulse at the same intensity, applied to different populations (normal versus those who suffer from migraines) elicited a paradoxical effect. The same 1Hz rTMS pulse applied to a healthy subject exerted an inhibitory effect whereas it caused a facilitatory effect when applied to migraine sufferers. Even within this neurological population there is a variety of subtypes and this likely results in changes in excitability and hence different response to NIBS. Yet another study showed that individuals suffering from migraine with aura showed a further reduced threshold for TMS-induced phosphenes (Badaway et al., 2012), similarly to what has also been found for moving phosphenes (Battelli et al., 2002). In an animal model study on anethesized cats, Pasley, Allen, and Freeman (2009) showed that response variability of the population of neurons stimulated in the cats’ visual cortex was partially modulated by the pre-existing state of the network. Using a partial correlation method,

they compared post-TMS responses with pre-TMS cortical activity to visual stimuli for bursting (more responsive to visual stimuli), and non-bursting (less responsive) cell types. When bursting cells were in a spontaneously high active state, spontaneous discharge was more likely to occur after TMS than when the bursting cells were in a spontaneously lower activity state. This result suggests that TMS is not an all or nothing method with a unilateral effect, the state of the system at the time of stimulation and the inherent noise in the non-linear system is crucially important to elicit effects. Importantly, this points to a graded effect of TMS on a non-linear system that is dependent not only on ongoing cortical activation as in this study with cats, but crucially, point to evidence that different levels of pulse intensity could potentially modify outcomes substantially. It is consequently possible that an overly strong pulse could in effect overpower the system even if the "more active" bursting cells were in a low activity state, forcing them over threshold, too weak of a pulse might not be able to push the bursting cells to threshold, while an intermediary (defined "optimal" in stochastic resonance literature) pulse could provide just enough of a push to depolarize enough of bursting cells over the threshold for detection even the low-active state. Overall, this indicates that it is necessary to move from studies using fixed parameters for all subjects to a more individualized approach with the goal to *precisely* adapt stimulation parameters in a subject specific manner.

As noted, the effects we see with TMS might not be as straightforward as previously believed (Siebner et al., 2009, Pasley et al., 2009). Furthermore, studies such as Garcia et al. (2011) and Ilmoniemi et al., (1997) show that when used in conjunction with other methodologies such as EEG and fMRI, we can gain significant insight into the spread of TMS across the cortex. This allowed critical insight to its potentially network-wide effects across time, but we are still trying to understand how these neurophysiological effects correlate with external factors even in simpler and more focal paradigms. The significance of this is directly relevant to TMS literature and it will be discussed in the present studies. This is important to determine because the resultant behavioral effects that spTMS has had on behavioral data, have also led to a variety of sometimes contradictory results. Current studies seem to suggest that TMS may be less focal or linear than previously imagined to due evidence of its potential to spread over the cortex under certain conditions (Garcia et al. 2011, Ilmoniemi et al., 1997). The results researchers therefore consider localized to one region maybe be an effect of a whole network change. With evidence exemplifying this potential exposure to network-wide spread, controlling for this confound allows us to discretely study the area of interest, but implications can be limited

due factors relating to functional connectivity. Specifically, to dispel these confounding effects, the implementation of an active control condition within the stimulation paradigm itself is extremely compelling and worth examining, allowing the researcher to examine the effects of function of a discrete brain region, permitting more concrete conclusions to be drawn from the resultant data.

Nevertheless, without precise knowledge regarding the interaction of these techniques with cortical function, we inevitably limit our ability to make potentially groundbreaking discoveries, both theoretical and clinical. This is especially important considering the newer devices such as tRNS and tDCS about which much less is known than TMS.

Transcranial Electrical Stimulation: tRNS

Transcranial random noise stimulation (tRNS) was successfully implemented as a neuromodulatory technique by Terney et al. (2008), and has recently gained consideration as a promising method which promotes cortical plasticity and enhanced learning across a variety of modalities (Fertonani et al. 2011; Snowball et al. 2013; Cappaletti et al., 2013; Pasqualotto et al, 2015; Pasqualotto 2016; Tyler et al. 2018). In tRNS, weak electrical currents are delivered to the cortex through electrodes placed on the scalp. The electrical currents are non-polarity specific, and the alternating currents produced by the electrodes oscillate randomly across frequency ranges selected by the researcher. According to the research question, either low- (lf-tRNS = .1 to 100Hz) or high-frequency stimulation (hf-tRNS = 101-640hz) is selected (Cohen Kadosh, 2013). hf-tRNS, in particular, has been shown as the range of frequencies that most often improve cognitive performance, and is believed to exert excitation on the cortex equally through both electrodes (Pirulli et al., 2016). Whereas TMS transynaptically depolarizes pyramidal neurons below the coil directly causing action potentials, the mechanism of HF-tRNS acts to excite the pyramidal tract neurons, but does not result in direct depolarization of these neurons (Miniussi et al. 2013). Instead, the current passed through the scalp via the electrodes potentially causes depolarization to be more likely which in turn alters the current state of neuronal excitability (Reed and Kadosh, 2018). This effect is more often produced in the high frequency spectrum, and it is likely caused by repeated opening of the sodium channels, resulting in a maintained yet fluctuating state of excitability (Terney et al. 2008). This helpful addition of electrical activity reacts with ongoing neuronal processes and network fluctuations,

which in turn enhance the sensitivity of neurons that are subsequently in a primed state to react to supplementary input. Therefore, when visual stimuli are presented during a task, it is plausible that the visual signal from peri-threshold (weak) stimuli could be facilitated in this enhanced network state, and push these neurons close to threshold in this sensitive state over the threshold for depolarization (Miniussi et al., 2013). This tendency to encourage a state of randomly oscillating excitation at a variety of frequencies and not allowing the network state to settle into or adapt to an equilibrium is a primary reason to suspect that tRNS may be intrinsically stochastic, lending helpful noise to a system that aids but does not directly result in depolarization (and therefore signal detection) without relevant additional external input to the excited neurons (van der Groen and Wenderoth, 2016; van der Groen et al., 2018).

The effect of HF-tRNS has been shown to outlast the time of stimulation (Herpich et al., 2018), and therefore lends itself well to situations in which it is used as an adjunct for rehabilitation or training paradigms following cortical damage or perceptual learning (Wessel et al., 2015; Herpich et al. 2019). However, in studies such as the current one, this potentially useful augmentation to therapeutic/learning settings should be controlled for when studying transient effects of tRNS. Bilateral montages with tRNS is a particularly useful method for enhancing homologous areas concurrently. With the long-lasting beneficial impacts on behavior, tRNS could also be a useful technique for future clinical paradigms. However, the behavioral enhancements in clinical and non-clinical paradigms have often proven to be highly task specific (Contemori et al., 2019). In order to translate the results from basic research techniques, and developing clinical methods in the future to generalize these benefits of effects on cognitive tasks in healthy participants, this should be a priority, moving towards translation to the clinical domain (Campana and Casco, 2003). Nevertheless, tRNS is proving to be a powerful tool. Inukai et al., 2016, showed that of the three methods (tDCS, tACS, and tRNS), tRNS showed the most statistically significant improvements in increasing cortical excitability measured by motor evoked potentials (MEPs) compared to both pre-stimulation levels and when compared to sham, at all time points. The changes produced by this mechanism still remain unclear, but, for instance, success in perceptual learning paradigms has shown evidence it might function as a mean to increase long-term potentiation by depolarizing cortical neurons underneath the stimulation site, a function critical for learning, thereby inducing long lasting changes in plasticity (Fertonani et al., 2011; Miniussi et al., 2013; Cappalletti et al.,

2013). This provides proof of concept that this technique could be a compelling rehabilitative instrument that can augment already pre-existing rehabilitation exercises for stroke patient populations, as well as for enhancing rate of learning in cognitive tasks in healthy subjects.

Despite its promising effects, it remains unclear how the behavioral impact of tRNS changes when parameters are manipulated in the healthy population. For example, optimal amplitude delivery is yet to be determined. Van der Groen et al., (2016) varied the amplitude of tRNS stimulation from 0-1.5 milliamperes (mA) and found stochastic resonance like effects of an inverted-U shape peaking around 1 mA. Stochastic resonance theory suggests that stimulation techniques such as TMS and tRNS adds noise to the already oscillating brain activity, bringing the neurons closer to firing threshold (van der Groen and Wenderoth, 2016; van der Groen et al., 2018). More details on stochastic resonance will follow in a later section of the introduction. Furthermore, low intensity tRNS at .400mA has also been shown to have an inhibition like effect and induce a behavioral decrement (Moliadze et al., 2012). As in foundational studies of TMS, there is a tendency for new tRNS experiments to base stimulation protocols on the parameters selected by previous experiments that have shown significant results; however, this emerging research indicates a more in-depth examination of how each parameter influences behavioral and neural effect. In turn, targeted stimulation protocols can then be refined in their parameter selections for future testing in the clinical domain, to attain a specifically desired behavioral response based on the results of this foundational research in healthy participants.

Mechanistic Factors Affecting Stimulation Outcome

Transcranial magnetic stimulation

When using NIBS techniques, there are a multitude of factors which influence the type of behavioral output, and not all of them may be visible or mechanistic in property. However, the mechanistic aspects of NIBS techniques are a primary measure that we researchers can take to ensure that we create a well-controlled study which easily affords itself to replication. The literature exemplifies a variety of factors such stimulation device, including coil or electrode type, that could account for the high variability of published data (Deng et al., 2013, Klooster et

al. 2016). The coils which produce the magnetic stimulation, in particular, can either be more focal (figure of 8), or diffuse (round coil), and both are useful for different paradigms (Ruohonen and Ilmoniemi, 2002; Deng et al. 2013). Here we focus on the method used in the experiments in the following chapters, the more focal figure of 8 coil, which can reach a depth of around 2-3 centimeters (Deng et al. 2013). In recent decades, a multitude of factors have come to light which we now know directly affect the stimulation produced by TMS such as geometry and direction of the induced magnetic field (modulated by the precise directionality in all axes in which the TMS coil is placed on the scalp), pulse waveform (Hallett, 2000, Kaminski et al 2011), aspects regarding subjects' different cortical anatomy (Siebner et al 2009, Wagner et al 2007, Janssen et al. 2014), and the level of stimulation intensity needed to reliably produce measurable effects such as phosphenes or motor evoked potentials (Stewart 2001, Sandrini et al. 2011, Boroojerdi et al., 2002). Another methodological issue put forth by Paus et al. (1997) and Ilmoniemi et al. (1997), is that even while we believe that these effects are focal, the pulse transmits effects that may spread, moreover, to anatomically connected areas in ways that without a concordant set-up of EEG to evaluate such a possibility, presents further potential for confound, even in carefully controlled studies. This is especially pertinent in the occipital lobe given its relatively close distance to its homologue, the main stimulation site we use when exploring paradigms of this study, both in our study involving TMS and our studies employing other forms of electrical stimulation as previously described, and will not be discounted as a potential confound in our TMS experiment.

The parameters used in both TMS and tRNS stimulation paradigms cannot be considered in a vacuum. The mechanistic properties of the stimulation type being used, the behavioral paradigm itself, and the subject specific considerations must be taken into account. Each of these are considered to play a crucial role in the cumulative behavioral result, but little is known about what constitutes the optimal set of parameters for different paradigms. Importantly, specific aspects of the stimulation paradigm such as stimulation intensity and the brain state at the time of stimulation may interact (Tseng et al., 2012, Schwarzkopf et al., 2011, Perini et al., 2012, Abrahamyan et al., 2011, Abrahamyan et al., 2015, Silvanto and Cattaneo, 2017, van der Groen and Wenderoth, 2016). This has important theoretical and clinical implications, and the aspects under which these conditions interact will be the main topic of my thesis work. Particularly, we will focus on one theory under which these conditions interact, that of stochastic resonance, now gaining momentum in the field.

The interactions of the mechanistic properties of stimulation such as intensity, and the neural recruitment of signal based on low-level features such as intrinsic stimulus properties varying the signal-to-noise ratio in a non-linear system such as the brain is critical. When different studies using what appears to be a similar procedure shown both facilitatory as well as inhibitory effects, as in the case of TMS, this creates a controversy within the field that is hard to mitigate. The lack of replicability between studies hinders drawing solid conclusions about the efficacy to modulate function in the hypothesized manner across research in the same area. Future work should be focused on systematically evaluating each parameter in NIBS and determining any interaction effects between parameters. For example, a study exploring the interaction between stimulation intensity and task specific stimulus properties could uncover the reasons behind seemingly contradictory evidence. Furthermore, comprehension of these interactions can afford careful experimental design with clearer hypothesized outcomes. This also brings us to the importance of physiological assessment of online (stimulation applied during task/intervention) and offline (stimulation applied prior to task/intervention) paradigm network effects to directly assess changes in cortical activity immediately and over time. This will necessarily lead to more concrete conclusions about how the stimulation not only directly affects the cortex at the moment of stimulation, but further, how long the duration of these effects last, an important issue in translating theory to clinical practice (Edwards et al., 2019).

Transcranial electric stimulation

TES methods have many similarities that influence the way that their weak electrical currents (direct as in tDCS; randomly oscillating alternating currents in tRNS) are delivered to the cortex. One of these factors is the montage of the electrodes on the scalp. Electrode placement is generally selected by using the 10-20 system, and the electrode(s) are placed on cortical areas of specific interest, determined to be of importance in the task being investigated and the direct cortical area or networks being modulated by these particular nodes (Klem et al., 1999). However, a very pertinent concern is bridging of the electrodes due to oversaturation of the electrode sponges. Bridging of electrodes occurs when the saline solution, gel, or paste, from two separate electrodes comes into contact with each other. Effectively, it creates increased conductance between the two electrodes themselves, promoting cross-talk, and

therefore, an undetermined, but certainly smaller amount of signal is input to the cortex than predetermined by the researcher under the paradigm. This is therefore a critical point to consider in experimental design; while the researcher believes the current is being applied to the cortex, it is in fact being distributed across the scalp in an unspecific or unpredictable manner that does not correspond directly with the electrode placement implemented in the specific paradigm, (Vanneste et al., 2013; Woods et al., 2016). To address this issue, we applied electrodes with 10-20 electrode paste, given that this is especially relevant for areas that are close together, such as the occipital cortex, and bridging using saline solution is more likely. Another, principle factor influencing the effect of tRNS on the system is the voltage that is applied, expressed in milliamperes (mA), (Caumo et al., 2012). Currently, many studies stay within the ranges of 0.5-2mA, but the range of effects due to different stimulation intensities within this methodology varies, and it is difficult to make comparisons between a variety of studies using similar but slightly different parameters or placements (Caumo et al., 2012; Nitsche and Paulus, 2000). The intensity of stimulation directed into the scalp is not only affected by the stimulation intensity chosen by the researcher, but also, critically, the size of the electrodes that are used. Large electrodes typically used in stimulation (the 5x7 electrodes) are generally limited in their focality compared to smaller 5x5 or ring electrodes, (Paulus, 2011). The amount of current that is delivered to the cortical area is directly impacted by the electrode size because the total amount of electricity being delivered is being uniformly spread across the electrodes, thus, the larger the electrodes, the more diffuse and less focal the stimulation. Further factors relating to subject variability which reduce or modulate the efficacy of stimulation are known to be the positioning of the electrodes, the thickness of the skin, skull, and, as previously mentioned, whether a monocephalic or bicephalic montage is used. The choice of montage is highly task specific, given the wide variety of evidence that one application is more desirable than another even in similar task recruitment paradigms (Parkin et al., 2018). Concerning the visual cortex, which we are most interested in, both have been tested, but biphasic montages seem, at this point, most interesting in their ability to modulate the affected area in the intended direction and will be the main focus of our tES paradigm.

Stimulation Intensity and the Importance of Thresholding for Individual Differences

Both TMS (magnetic) and tES (electrical) applications of non-invasive brain stimulation have a common goal: how do we set participants in as equal of a state as possible? Importantly, stimulation intensity in single pulse TMS studies is one of the TMS parameters that was believed to behave in a linear manner, with higher intensities producing greater effects on cortical activity (Siebner et al 2009). However, current research directly implicates the importance of the interaction between task difficulty and stimulation parameters, and calls into question the previous assumption that TMS affects all types of neurons indiscriminately in a target region, and that this is independent of the brain state at the time of stimulation, (Silvanto et al 2008b). Even in rTMS studies, uncertainty about the interactions of high and low frequency stimulation, which were believed to be relatively consistent in their effects (high frequency faciliatory effects, low frequency inhibitory effects), have been shown to be far more complicated, and due to an interaction of factors than originally thought (Houdayer et al., 2008). Though we investigate the properties of spTMS, these differential results found also in the rTMS literature illustrates yet again that the properties of the techniques that we have relied on in the past to “guide” our current models of methodology can no longer be taken for granted, and that the problem extends across the range of parameters for different NIBS techniques. It illustrates the irrefutable necessity to explore how TMS in its most straightforward implementation (transient single pulse) specifically affects neural activation and consequent behavioral outcomes, as the current "one-size-fits-all" application of brain stimulation techniques across groups of individuals necessarily limits any scientific interpretation of the resultant behavioral outputs (Kaminski et al 2011). This is the goal of our study, to elucidate the behavioral effects of spTMS and tRNS in a well-controlled within-subjects design that can illustrate effects that occur at different parameters within the same individual, and those that are common across the group when individual factors are taken into account. Both TMS and tES lack clarification in how they directly impact behavior in the healthy subject, and this needs to be reconciled if researchers are to proceed with certainty about what they are directly modifying.

In a combined fMRI-TMS paradigm implemented by Nahas et al. (2001), the intensity of stimulation relative to subject specific motor thresholds was shown to modulate the amount of activation measured by changes in blood oxygen level dependency (BOLD) signal in a distinctly

linear pattern. This experiment showed that lower intensities produced minimal changes in activation whereas stronger intensities resulted in significantly larger contralateral activation (Nahas et al 2001). However, the TMS intensity required to produce behavioral results varies from person to person. This is crucial to consider when designing a stimulation experiment, as the effects will necessarily vary with the amount of stimulation applied. A variety of studies chose to use a fixed amount of max stimulator output for all participants, and did not take into account individual differences. However, TMS studies have demonstrated that to elicit visual phosphenes (a brief flash of light that can be perceived when TMS is delivered over the visual cortex) very different stimulation intensities (and hence different phosphene thresholds) are necessary to report a phosphene, indicating high inter-subject variability. In a subset of subjects with potentially drastic individual differences, this may create widely variable differences in behavioral outcome on opposite ends of the spectrum. This creates a potential "washing out" of effects that may have been otherwise significant had these individual aspects been taken into account, and therefore, potentially relevant effects go undetected. Furthermore, it has been shown that motor threshold, measured through motor evoked potentials (MEPs – the motor response of the muscles when TMS is delivered over the motor cortex), and phosphene thresholds, even within an individual, do not correlate (Stewart et al. 2001). If these within subject's measures are an unreliable substitute for one another, these differences may relate not only to stimulation parameters that vary between these cortical regions (motor and vision) but should also be considered determining appropriate TMS intensity to other regions of the cortex. This gives further credence that it is unjustifiable to implement paradigms using these techniques at a "blanket" level of stimulation across all subjects, as individual (and therefore group) differences even within the same modality will also likely be variable (Stewart, 2001). A variety of studies investigating TMS with ongoing brain oscillations found discordant results between the sets of studies, that showed a significant negative correlation between alpha power MEP amplitude, while others found the inverse, (Maki et al., 2010; Berger et al, 2014; Sauseng et al., 2009; Zarkowski et al., 2006). Consequently, we deem it paramount to consider the individual cortical state of the subject as non-trivial factor, given each individual's level of cortical excitability is unique, and if not taken into account, this could create contradictory results leading to research that may mask true effects impacting ongoing development in both research and clinical applications. We are not alone in this concern, and in the past several years, researchers in multiple groups have highlighted both the heterogeneity between studies and

prevalence of contradictory findings. This has caused an upsurgance in recent attempts to incorporate aspects regarding subject-specific differences into the design of recent experiments. We consider this an important step forward in the field, towards discerning true effects due to stimulation and paradigm based parameters that sufficiently accounts for subject specific parameters.

In light of this, recent studies using transcranial electrical stimulation in its various potential applications have investigated the importance of stimulation intensity as a crucial factor with strong evidence for significant modulation differences when intensity is modulated, with results that are not immediately intuitive or predicted by past models. This study is different in that it considers a wider range of stimulation intensities and their effects on individual subject cortical excitability in a within-subjects design that can disentangle more readily differences across many parameters, and account for individual differences. In this study by Moliadze et al. (2012), the authors show that there is a clear shift from the activation of inhibitory networks to excitation when increasing the stimulation intensity of high-frequency tRNS and tACS. They explored a range of 5 intensities (0.2, 0.4, 0.6, 0.8, and 1.0 mA), for each subject, conducted on different days, in order to explore the relationship between intensity and cortical excitability measured by MEPs before and after each stimulation session. They found that the intensity of the stimulation was significant at varying levels for both tRNS and tACS, such that at 0.4mA, the effect was inhibitory rather than excitatory, while at 1mA an excitatory effect was recorded, with the middle levels of stimulation creating no significant change compared to sham condition. Similarly, a tDCS study by Batsikadze et al. (2013) showed significant intensity dependent effects on motor cortical excitability as measured by the pre- and post- MEP measurements. They found that stimulation modulated the corticospinal excitability differentially dependent on stimulation intensity for both anodal and cathodal tDCS, such that at stimulation of cathodal 2mA significantly increased MEP amplitudes, whereas at 1mA cathodal tDCS reduced corticospinal excitability. Hence, across multiple methods of brain stimulation the amount of stimulation intensity creates differential effects, which do not follow the previous assumption of linear relationships between stimulation and effect. Understanding the individual properties of the neurotypical or atypical patient, adjusting for cortical excitability, and using appropriate mechanistic properties of NIBS techniques will allow us to better interpret our results and the outcomes of our hypotheses.

Stochastic Resonance in tRNS and TMS

One of the most interesting hypotheses on how methods of brain stimulation work is that it acts as a "noise inducer" producing a stochastic resonance effect by enhancing the strength of the signal (Schwarzkopf et al., 2011; Miniussi et al., 2013; Van der Groen et al., 2016; 2018). Here we will briefly overview studies which explore NIBS methods in regards to stochastic resonance theory. We will attempt to compare different studies that might help elucidate how individual factors interplay. We will mainly focus on works that are relevant to studies on vision and visual discrimination, hence the effects of brain stimulation on the visual cortex and its behavioral effects will be analyzed more in detail.

Stochastic resonance cannot occur in a linear, or optimized system, where the signal is already optimized and noise can only act to degrade the signal, (McDonnell and Abbott, 2009). Instead, in non-linear systems such as the brain which are constantly fluctuating, external noise input can be deemed potentially beneficial if it leads to signal smoothing, by boosting below threshold neuronal firing above the detection threshold. This is the basic tenant of stochastic resonance theory, that optimal levels of non-relevant external noise added to a system can be paradoxically helpful. One of the principles aims of our research is to determine if NIBS methods can act as a "helpful" method of noise induction in the system. Another question that remains contested due to variance in the present literature is how is the network signal affected by this noise after "optimal threshold" has been reached; at what point does the noise become "unhelpful", and what are the characteristics of behavioral performance before it reaches an intensity at which it becomes detrimental? There are various responses to this, with the most commonly espoused model being that of the inverted "U" shape model, that indicates only a brief optimal period where noise enhances the behavioral response, followed quickly by behavioral decrements as intensity increases (Abrahamyan et al., 2011; 2015; Schwarzkopf et al. 2011). Another theory by Wenning and Obermeyer (2003) purports mechanisms of stochastic resonance that are activity driven and adaptive. They propose that their models of single neurons undergoing external noise show that at subthreshold levels, noise indeed enhances the signal strength and optimize neuronal output, but that once this threshold is met, overabundant noise is effectively "tuned out" of the system able to give more importance to the true internal neuronal signal and allowing a more constant average firing rate of the neuron in question. This is different from the more commonly adopted "U" shape model in that this model's

predictions are that it would take an extremely disruptive amount of noise to disrupt an already strong signal, whereas the U shape would predict that any amount of noise after peri-threshold noise should elicit a decrement in behavior. This activity driven adaptive theory of stochastic resonance was supported by results of a later study on an anesthetized cat model by Funke et al. (2007).

The single unit recording study by Funke et al. (2007) tested the model of noise induction by Wenning and Obermeyer (2003). In 17 anesthetized cats, they implemented image jitter in cat area 17 on a visual stimulus with frequencies in a plausible range to imitate microtremors or micro-saccades that occur in a natural state when the animals are visually exploring their world. This is a practical example of inherent noise that is also present in everyday activities during visual search of our environment, and which the visual system might use to enhance our perception of the real world by using this noise in a helpful manner. Their results exhibited increased performance with small levels of jitter to stimuli with an internally weak cortical response, whereas stimuli with an already strong cortical response showed little to no decrement. The most helpful conditions seemed to be with a moderate and weak signals paired with moderate tremor, in support of stochastic resonance and the helpful effect of external noise on the system, and simultaneously, only a small decrease in visual response to strong signals with a strong tremor. However, they did not show support for the added noise creating a tonic increase in background activity that proportionally results in a decrease of the signal-to-noise ratio as proposed by the U shaped model. Instead, their results indicate that there was a broadening of the response peaks, such that the mean response relative to the stimulus signal was still governing the overall cortical response, and that the background noise, while not helpful, was not inherently detrimental at the intensities it was employed in their study. The authors attribute this as support for the activity driven dependent theory of stochastic resonance of the visual system response in the cat cortex.

Proponents of the model of stochastic resonance that follow a U shaped model are typically more abundant, and is what is more often touted in the behavioral literature in human participants where single cell studies are not possible. One such study by Schwarzkopf et al. 2011 examines the behavioral reactions to noise added by TMS in the visual system. This is a well-designed study that first acknowledged the importance of examining the input on a gradation; they operationalized noise in their study at different levels of intensity, allowing a closer examination of the properties and potential interactions of external and internal signal on

behavior. Schwarzkopf and coworkers (2011) used a classic motion coherence detection task (Newsome and Pare, 1988) and asked subjects to report the direction of motion of a subgroup of coherently moving dots (signal) among randomly moving dots (noise) while they received online TMS. They found that low intensity TMS can facilitate the detection of a weak motion signal, while high intensity TMS impairs motion detection when the motion signal is strong. To explain their results, the authors proposed that the effects of both TMS intensity and stimulus noise interact such that only TMS at low intensity would result in a stochastic resonance effect, artificially enhancing the signal visibility and detection. Specifically, because the brain is a nonlinear system, it relies on signal detection processing, and the addition of non-discriminant noise (via brain stimulation) to a pre-existing signal should impact the signal in differential ways. This theory posits that under optimal conditions the signal to noise ratio is increased, allowing for enhanced detection of a previously subthreshold signal receiving this additional "push" towards the detection threshold. In other words, when an "optimal" low-level amount of external random noise is added to the signal already present in the system, stochastic resonance theory predicts that is that it decrease the detection threshold by increasing the signal to noise ration; conversely, if too much noise is added to the system, the resultant signal-to-noise ratio will effectively decrease, increasing the threshold that is needed to reach detection (Perini et al. 2012, Walsh and Cowey, 2000). A handful of other studies have attempted to understand the different parameters of non-invasive brain stimulation affecting behavioral outcome under the theory of stochastic resonance, with mixed results (Schwarzkopf et al., 2011; Perini et al., 2012; van der Groen and Wenderoth et al., 2016; Abrahamyan et al., 2015).

Schwarzkopf et al. was the first group to operationalize the amount of external noise added to the system by implementing a variety of stimulation intensities (60%, 80%, and 100% of phosphene threshold). They simultaneously varied the initial amount of intrinsic stimulus noise to create low and high levels of neuronal activation, based on the amount of motion coherence signal present in each trial. Their results indicated that there is, in fact, an interaction between the stimulation intensity and stimulus signal, (amount of coherence), such that participants' performance improved only when the lowest stimulation intensity was applied during low coherence (low signal) trials. Further, as predicted under their model, adding too much external noise to the system will "drown out" the signal; for high coherence stimuli, participants' accuracy was impaired by medium and high TMS.

At first glance this evidence seems to support the stochastic resonance theory. However, the motion coherence paradigm as implemented in their study presents a difficult caveat to untangle, as mentioned by the 2015 study by Abrahamyan et al. Specifically, in their stimulus the overall number of dots was held constant, and in the low coherence condition, they presented a weaker left or right motion signal by increasing the motion noise, that instead was reduced in the high coherence stimulus condition (Abrahamyan et al., 2015). Hence, it becomes difficult to discern whether the effect of TMS was to push the (physiological) subthreshold signal present in the neural population above the level for conscious detection, or simply to decrease the noise present in the signal.

A subsequent study by Perini et al. (2012) attempted to address these issues by using a different experimental paradigm using adaptive and non-adaptive state conditions. They based their study on the hypothesis of stochastic resonance as one of four potential explanations for the contradictory nature of results acquired up to that point in the literature. This group chose stimulation intensity of 120% phosphene threshold to operationalize "external noise" in the system, but used a wider gradient of pre-existing "stimulus" noise by using visual stimuli (Gabors) across a wider range of contrast levels than previous studies. By using gradients of a static low-level visual stimulus, this study eliminated the potential motion confound (Schwarzkopf et al. 2011). The experiment tested whether or not the differential predictions of these state-dependent hypotheses could wholly or partially account for observed task performance, determined by shifts of the contrast response functions (CRF) compared to performance in discrimination when no TMS or adaptation was applied. They found that while in the adaptive state, performance increased for the middle contrast levels in the TMS compared to the non-TMS condition selectively shifting the contrast response function at the middle level only, conversely, in the non-adaptive state, the opposite effect occurred. None of their proposed models, and relevant to the current paper, that of stochastic resonance could fully account for these results. The question therefore remains as to whether the implementation of noise is facilitatory or inhibitory. Notably, one major point not accounted for by this group is that while they tailored TMS intensity to participants' individual phosphene levels, Perini and colleagues used only suprathreshold stimulation (120% phosphene threshold), and thus may have missed effects present in the Schwarzkopf and colleagues 2011 study. Hence, we integrate in our study the need for further investigation using more graded levels of TMS

intensity over simultaneously graded levels of stimulus intensity, warranted by the lack of incorporation of both in either of these past studies.

Another interesting more recent modality of brain stimulation that might act as a “noise inducer” is transcranial random noise stimulation (tRNS, Terney et al. 2008), a type of direct current stimulation procedure that has shown great potential to enhance cognitive functions when applied during behavioral testing (Fertonani et al., 2011).

Recent studies have demonstrated that tRNS potentially operates under the mechanism of stochastic resonance by inducing noise into the system that is both dependent on the intensity of the external noise added, as well as the relative strength of the already present neuronal signal activated by the stimuli itself (van der Groen et al., 2016; van der Groen et al., 2018; Schwarzkopf et al. 2011). A perhaps more straightforward method of “random noise” in comparison to TMS (generally considered more focal because of its direct effect on depolarization of neurons underneath the coil), the effects which we see here may help shed insight into the effects of other mechanisms of brain stimulation, and help us determine if they work under the stochastic principles when the optimal parameters are applied.

In their study, van der Groen and coworkers (2016) investigated tRNS intensities applied at 0, 0.5, 0.75, 1, and 1.5 mA over frequencies randomly oscillating between 100–640 Hz zero-mean Gaussian white noise to the visual cortex while participants performed a two-alternative forced choice task. During each trial, Gabors (stimuli used to measure contrast sensitivity, Perini et al., 2012) were presented in one of 8 locations at either the first or second presentation interval and participants were required to indicate in which interval the Gabor appeared. While the authors did not take into account individual levels of sensitivity or overall cortical excitability prior to stimulation as in the previously discussed TMS paradigms (Perini et al., 2012), they did manipulate both the overall stimulation intensity level, as well as the stimulus intensity level. The stimulus intensity level was calibrated for each individual by their contrast detection threshold assessed at 75% detection accuracy during a staircase procedure, and the task was performed at either 60% of this threshold, or 80% of this threshold, depending on which group to which they were randomly assigned.

Since not all participants participated in both the low threshold and high threshold variation of the experiment, it makes it difficult to compare between the tasks used in the previously described TMS experiments, where subjects underwent all conditions, shortcomings that we will try to address in the present research. These caveats aside, the authors did find

significant evidence for the theory that tRNS is both stimulation and stimulus intensity dependent, as predicted under the theory proposed by Schwarzkopf et al. (2011). Specifically, they found that participants' performance at low intensity stimulation (we cannot say subthreshold here, as they were not thresholded for this parameter), significantly increased in a u-shaped function, characteristic of the SR phenomenon. In essence, as stimulus intensity increased, subjects who were subthreshold for performance at 60% correct performed better at increasing stimulus intensities than those in the 80% correct condition, with performance peaking at the 1mA level.

Their study seemingly supported the results of the TMS study found by Schwarzkopf et al. (2011) in their motion coherence paradigm, such that the subthreshold performer group indicated significant performance increase at the subthreshold stimulus intensity level, only for "mid-levels" of stimulation intensity. Due to the recency of this technique, however, it is still unclear what is "low" stimulation and where on the gradient the stimulation becomes "high" stimulation. This is a substantial caveat, given that currently, we do not have a method to individualize stimulation intensity to the subject. Because of this, we cannot say for sure if the results of this study correspond to the findings that their defined values of "low" noise, such as those operationalized through consideration of individual phosphene level calibrations in the TMS studies, correspond with these parameters of tRNS as a low, medium, or high intensity level, that modulates behavior in a subject dependent manner. As in the study of TMS, this discrepancy makes discerning results problematic, as for some subjects the author's definition of "low stimulation" could be, for a specific set of subjects, considered medium or high-level stimulation intensity, while for others it could be considered a low-level stimulation intensity. We are currently unable to untangle this possible confound. A paradigm including both thresholding for individual levels of cortical excitability in the visual cortex with phosphene threshold is an appropriate method given that our study involves visual perception. As a direct measure of cortical excitability in the area of cortex being studied, it could potentially eliminate or reduce performance variability of subjects based individual brain state. We will implement this factor into our design by measuring phosphene threshold before the actual experiment is performed, and split subjects into comparable groups with similar levels of cortical excitability in post-task analyses. If such a subdivision is deemed relevant, it could be considered an integral measure to determine optimal levels of stimulation for individual participants in future studies. This would also allow us to further classify the effects of stimulation in a manner similar to that of TMS that

allow us to personalize the amount of stimulation received as a measure of a percentage of their cortical excitability, for future studies involving tRNS. This is of critical importance given that dose-dependent application, if this mechanism works under the principle of stochastic resonance as predicted, is not only non-trivial, but an absolutely crucial factor such that we deliver a current intensity that drives activity towards performance benefit, rather than decrement. This is important not only in the study of healthy patients, but has implications as both a potential mechanism to study the field of consciousness (bringing a non-conscious stimulus up to a level for conscious detection) and as a clinical tool mitigating improvement regarding functional impairment following infarct. Particularly regarding the latter, understanding the functions of this mechanism are crucial, especially now that it is currently being considered as a potential supplementary therapy for stroke patients undergoing motor and visual rehabilitation (Arnao et al., 2019; Herpich et al. 2019).

The aims of this thesis are three-fold:

Aim 1) Investigate the effects of both TMS and tRNS upon carefully controlled visual psychophysical parameters. Specifically, we investigate applications of carefully controlled parameters in both TMS and tRNS studies using an orientation discrimination paradigm. We aim to elucidate whether these effects can be attributed to stochastic resonance.

Aim 2) In terms of TMS, we have designed a model that we believe can shed light on the disparate effects of other studies, by combining techniques used across multiple studies into one paradigm. We consider this an important step forward towards determining the impact of two notably crucial factors in the field, stimulation intensity, and the amount of neural signal generated by the stimuli properties in its own right (in our experiment, contrast intensity). We also carefully control for individual cortical excitability by using intensities tailored to each subject's level of cortical excitability.

Aim 3) we used tRNS as a way to control state-dependent effects in vision. The aim of our main experiment in the occipital cortex is to combine a wide range of both parameters regarding tRNS stimulation intensity and stimulus intensity identical to the experimental paradigm used in our TMS study. The goal is to better characterize the transient effects of this technique, and to determine if under bilateral stimulation conditions, it acts in the manner predicted by stochastic resonance theory. Comparatively to TMS, which directly depolarizes neurons underneath the cortical area of stimulation, we propose that tRNS seems like an even more optimal method of NIBS that could act as a noise inducer in the brain.

Chapter 2

Transcranial Magnetic Stimulation

TMS in a stochastic system: The Effect of TMS Intensity on Perception

Abstract

Transcranial magnetic stimulation (TMS) is a popular tool to study cognition. However, the manner in which TMS actually affects neuronal populations remains unclear. One theory postulates that TMS works similarly to an input-gain model, while opposing views posit that it acts as a noise inducer under the principle of stochastic resonance. In this work, we sought to investigate whether different levels of visual contrast are differentially affected by varying levels of TMS intensity. Single pulse TMS was delivered to V1 while participants performed a two-alternative forced choice orientation discrimination (OD) task of one of two Gabor patches presented on either side of fixation. Gabors were presented at five contrast levels and four TMS intensities, based on individualized phosphene thresholds. Participants' performance in the OD improved with increasing stimulus contrast, irrespective of TMS intensity, and both visual fields were affected by TMS, with increasing TMS intensity yielding decreasing performance at different contrast levels. Additionally, we found an interaction between the effects of TMS intensity and stimulus contrast, in which TMS intensity increased performance predominantly at the middle contrast levels, but only at TMS intensity levels that were below phosphene threshold. However, these effects were themselves dichotomous: TMS 60% produced an interaction between TMS Intensity and contrast, improving performance at the one subthreshold contrast level, whereas TMS 80% yielded and overall main effect of increasing performance compared to sham, also producing a trend towards relative increase also at the suprathreshold contrast level. Reaction times were overall faster in the field contralateral to TMS potentially indicating an overall performance enhancement regardless of stimulation intensity. However, performance results suggest that the enhancement depends on a combination of stimulation intensity and visual stimulus intensity, relative to individualized

phosphene intensities, supporting leading theories of TMS as a noise inducer under the stochastic resonance model.

Introduction

The relationship between transcranial magnetic stimulation (TMS) and its influence on behavioral effects might not be as straightforward as previously thought. The long-held assumption was that TMS affects behavior acting by suppressing cortical activity, inhibiting the most active neurons independent of their state, resulting in a "virtual lesion" (Amassian et al., 1989; Pascual-Leone et al., 2000). Typically, stimulation intensity for a TMS study is set as a percentage of the individual motor threshold (MT), a motor response (a visible twitch) of a hand muscle that is detected when TMS is delivered over the motor cortex. Motor evoked potentials can also be recorded by placing electrodes over the hand muscles, thus the amplitude of the response can be measured, yielding a more precise estimate of the MT. As demonstrated in a combined functional magnetic resonance imaging-TMS (fMRI-TMS) paradigm implemented by Nahas et al. (2001), the intensity of stimulation calculated relative to subject specific motor thresholds has been shown to modulate the amount of activation measured by changes in blood oxygen level (BOLD) signal in a distinctly linear pattern in non-motor (prefrontal) areas. Specifically, they showed that lower intensities produced minimal changes in activation within the stimulated areas and no changes in distal areas, whereas stronger intensities resulted in significantly larger contralateral activation, thus a simultaneous response in both the stimulated and unstimulated hemisphere. This was generally considered to be the case across different cortical areas, with the underlying assumption that a subject with a low motor threshold needed lower stimulation intensities to elicit a response also in other cortical areas. Stimulation intensity was therefore believed to behave in a linear manner, with higher intensities producing greater effects on cortical activity (Siebner et al., 2009). Similarly, in a study investigating contrast intensity and its recruitment of neuronal signal, the investigators found significant evidence that neuronal recruitment created by increasing contrast intensity behaves in a comparable fashion, recruiting a significantly higher neural response as contrast increases (Boynton et al., 1999). While evidence for the linear increase of contrast intensity was shown to be highly significant in this study which examined the magnitude of neuronal responses to contrast stimuli of

increasing contrast with fMRI, evidence for the supposedly linear effects of stimulation found in Siebner et al.'s (2009) work are subject to highly more heterogenous results across different experimental paradigms.

Results from different studies using the exact same procedure are sometimes controversial in that they have shown both facilitatory as well as inhibitory effects (Moliadze et al., 2003; Allen et al., 2007). This lack of replicability, and in fact counterintuitive findings, hinders drawing solid conclusions about the efficacy of TMS to modulate function in the hypothesized manner across different research paradigms in the same cortical area. In vision, for example, recent research has suggested TMS might differentially affect distinct populations coding for opposing visual features (e.g. in a motion coherence task, where a subset of target stimuli move in one direction among distracters moving in random directions), in various paradigms exploring the use of adaptation and TMS (Van Wezel and Britten, 2002; Engel and Furmanski, 2001; Silvanto et al. 2007; Silvanto and Muggleton 2008b; and Cattaneo and Silvanto 2008). This indicates that behavioral results may not solely be a product of one or a few simple parameters, but, instead, rely on a combination of factors related to stimulation, stimulus properties, and the baseline state of the cortical network and the ongoing neural processes at the time of stimulation. These paradigms, in contrast to the virtual lesion theory, often support the idea that TMS results in the perceptual facilitation of the least active neurons, such that only those neurons in an adapted state are subsequently facilitated by an online TMS pulse, instead of inhibited, as it occurs in non-adaptive paradigms (Silvanto & Muggleton, 2008a). Conversely, other studies proposed models whereby TMS acts as a mechanism of noise induction, based on the hypothesis that TMS can differentially affect perception of stimuli either depending on stimulation intensity, independent of stimulation intensity or a combination of both (Ruzzoli et al., 2010). This is also called the stochastic resonance (SR) effect (Schwarzkopf et al., 2011, Simonotto et al., 1997; Abrahamyan et al., 2011; Abrahamyan et al. 2015), widely used in physiology to describe the effect of noise on neurons (McDonnell and Abbott, 2009). To date, it is still unclear which of these models best encompasses the full variation of effects produced by TMS when used in adaptive and non-adaptive states (Perini et al. 2012).

The model proposing TMS as a mechanism of noise induction has gained traction in the past several years (Schwarzkopf et al. 2011; Abrahamyan et al., 2011, Perini et al., 2012,

Abrahamyan et al., 2015, van der Groen and Wenderoth, 2016.). The stochastic resonance theory postulates that when systems reliant on non-linear signal detection processing, such as the brain, experience the addition of white noise to a pre-existing signal generated by ongoing neural processes related to the original stimulus, the signal-to-noise ratio (SNR) is increased. This additional noise affects the system by amplifying original (subthreshold) activity, pushing it just above the threshold for detection. This is only posited to be the case, however, if an "optimal" amount of noise is added to the system. In a situation where too much noise is added to the system with pre-existing activity related to low stimulus signal, the opposite effect might occur, or alternatively, in the case that the signal is already very strong, no effects may occur (see Fig. 1). The most commonly proposed model however, states that too much noise decreases the SNR, which in turn decreases detectability and discriminability of a stimulus, causing suprathreshold stimuli to either receive little benefit from added noise, or to subsequently cause a decrease in discrimination ability (Perini et al 2012, Walsh & Cowey 2000). Instead, when the intensity of the stimulus is already strong (suprathreshold), low-level noise may have relatively little to no effect. The question remains, how do we quantify the optimal amount of noise to add a system with TMS? Is it possible to determine the amount of noise that will produce the most benefit and increase performance, and to clarify if different levels of noise produce differential performance benefits and decrements?

A recent psychophysical work studied the state-dependency effect of TMS on orientation discrimination of gratings that varied in contrast (Perini et al 2012) while subjects were either in an adapted (contrast adaptation to flickering gratings preceding the target stimuli) or non-adapted state. They investigated varying theoretical accounts of TMS effects based on previous models (Amassian et al., 1989; Naka & Rushton, 1966; Ruzzoli et al. 2010; Schwarzkopf et al., 2011; Silvanto & Muggleton 2008b; Simonotto et al., 1997). Interestingly, their results demonstrated that none of the proposed models satisfactorily accounted for the full range of behavioral findings exhibited in both adaptive and non-adaptive states (Perini et al 2012, Schwarzkopf et al. 2011). Without adaptation, TMS caused behavioral responses predicted by a model of TMS as a suppressive mechanism, where stimulation significantly decreased contrast sensitivity, resulting in impaired performance across contrast levels. However, this model was not compatible with facilitatory effects of TMS when subjects were tested with adaptation. A recent model proposed by Schwarzkopf et al. (2011) studied intensity dependent

effects on visual perception of moving stimuli presented at different levels of motion coherence held at a constant rate of contrast. Subjects were stimulated with triple pulse TMS at 60%, 80%, and 100% of subject specific phosphene thresholds, as well as a no-TMS condition. Their results showed that compared to the sham TMS, low-TMS intensity significantly increased performance in the low-coherence condition, but showed detrimental effects with application of medium or high intensity TMS. Regarding high-coherence stimuli (strong signal-to-noise-ratio), subjects experienced significant detection impairment at both medium and high TMS intensity levels, but low TMS intensity was not strong enough to modulate noise in a decremental fashion, compared to baseline. This significant interaction seemingly supports effects predicted by a model of TMS as a modulator of noise-induction in a neural system based on stochastic resonance properties, however, there are several caveats to this experiment that limit its conclusions. For example, the behavioral improvements found by Schwarzkopf et al. (2011) may be due to TMS reducing the noise rather than improving the weak signal, since the level of dots in the motion stimuli were held constant, and the “higher noise” condition also had higher coherence, (Abrahamyan et al. 2015). Additionally, they used a triple pulse design, unlike other studies using single pulse, and may therefore not be comparable to other studies due to numerous effects such as pulse timing and onset, both key factors in manipulating behavioral responses, as noted in a study by Abrahamyan et al., (2015).

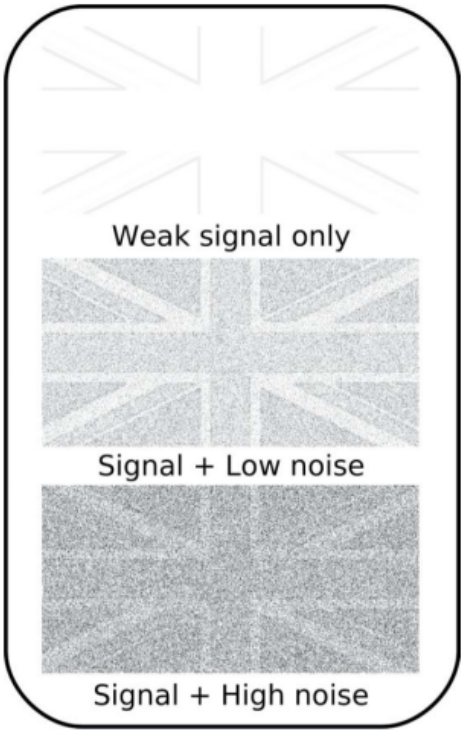


Figure 1. Example of predicted effects of the stochastic resonance phenomenon, adapted from Schwarzkopf et al. (2011). As a low level noise is added to the system, details that were previously subthreshold are pushed above the level for detection: In this graph, flag 2, where more details are exposed. Consequently, they predicted a decrease in detail (experimentally, performance), when too much additional noise is added to the system.

The current study aims to elucidate whether TMS intensity modulates stimulus visual perception in an independent manner, or if TMS intensity and stimulus contrast interact when other visual properties (orientation of the stimuli) are held constant, at different levels of contrast. While we expect an increase of performance with increasing contrast intensity (Boynton et al., 1999), a potential evidence in favor of stochastic resonance would be an interaction between the two independent variables, most notably an increase of performance accuracy at subthreshold stimulus intensity levels. This will be assessed behaviorally through discrimination performance and through any effects evident in the shifting of the contrast response function. We hypothesize three ways in which this could occur: 1) The application of different TMS stimulation intensities modulates performance of orientation discrimination similarly across all contrast levels, and differential effects are due to intensity, not contrast; 2) The application of different TMS stimulation intensities modulates performance of orientation discrimination differentially according to contrast level, independent of stimulation intensity: and 3) The application of different TMS stimulation intensities interacts differentially with stimuli of

varying contrast levels.

Materials and Methods

Participants

Fourteen participants (7 males, 7 females, mean age: 24.125 years [SD: ± 2.1 years]) with normal or corrected-to-normal vision, including one author (DP), participated in the study. Participants had no history of neurological or psychiatric illness and were not taking any medications for the duration of the experiment. Participants were selected based on their ability to detect the presence of phosphenes induced by occipital TMS. Each participant was screened for TMS contraindications prior to participation. Participants completed a form detailing any short-term side-effects after each session and were screened with the Mini-mental state examination (MMSE) (Folstein et al 1975). All participants gave informed consent to participate in the study, which was approved by the ethics committee of the University of Trento.

Experimental setup

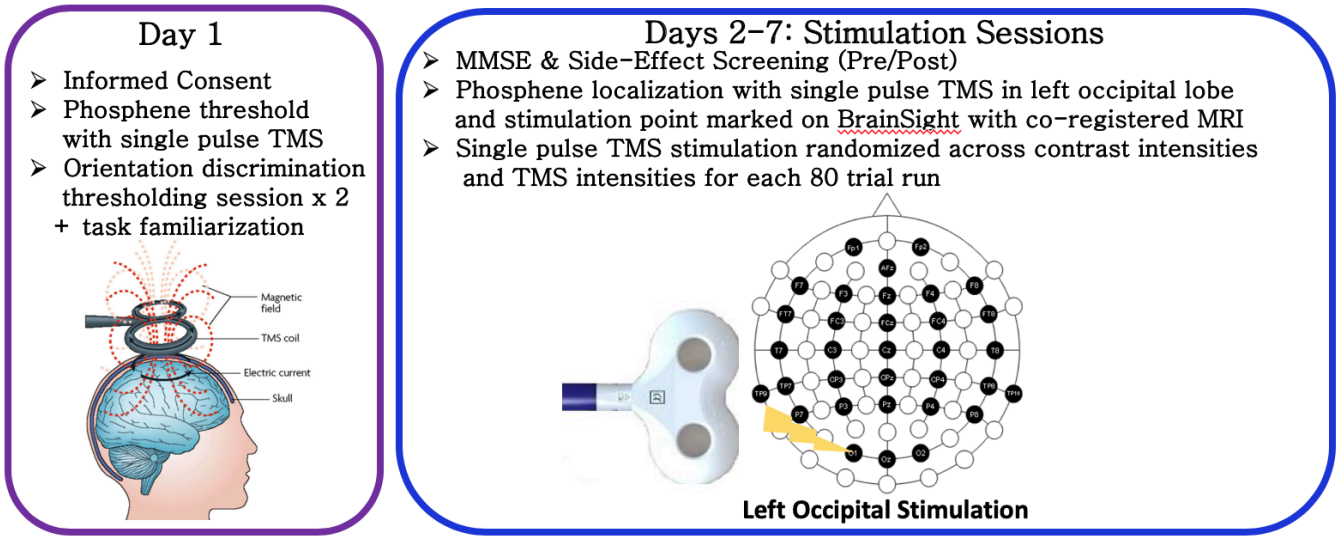


Figure 2. Breakdown of the experimental protocol by day. On day 1 participants were explained the task and the set-up of the experiment. All were screened for contraindications of stimulation. Subjects were thresholded for the orientation discrimination task threshold values 2 times, in order to account for task practice. If results differed significantly, participants were thresholded a third time. Participants were thresholded for individual phosphene levels with single pulse TMS (standard figure of 8, 70mm coil). Phosphene localization was performed each day at the start of stimulation. During each experimental session 2-7 ,participants received TMS stimulation at one of four intensities according to individual phosphene threshold simultaneous to stimulus onset, randomized across

trials. They performed the orientation discrimination task, randomized across contrast intensities 1.56, 3.125, 6.25, 12.5, 25 and TMS intensities to prevent order confounds.

Task Setup

The experiment was run in a dimly lit room using a chin-rest to ensure participants' heads would remain as still as possible at a distance of 57 cm from the monitor throughout the duration of each run. Stimuli were presented on a 22 inch (~55.8 cm) Samsung 2233RZ LCD monitor with the screen set to a resolution of 1680 x 1050 at a refresh rate of 120 Hz. The experiment was presented via Matlab R2010B (The Mathworks, Massachusetts) and ASF: A Simple Framework, an add-on to the Psychophysics toolbox on a Dell desktop computer running Windows 7.

TMS Setup

Single pulse TMS was delivered to the left visual cortex (V1) using a 70 mm figure-8-coil connected to a Magstim Rapid2 stimulator (Magstim Co., UK). MATLAB communicated with the MagStim (via a serial port connection) for automated pulse delivery. Previously acquired magnetic resonance image scans were used in co-registering the head position of each subject in space in order to stimulate the exact brain region across different sessions. Scans of each participant were high-resolution, T1-weighted (magnetization-prepared rapid gradient echo sequence (MPRAGE) with 176 slices, in-plane resolution 256 × 224, 1 mm isotropic voxels, using Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) with acceleration factor of 2, time to repeat (TR) = 2700 ms, time to echo (TE) = 4.18 ms, time to inversion (TI) = 1020 ms, flip angle = 7°) with a MedSpec 4-T head scanner (Bruker BioSpin, Ettlingen, Germany) equipped with an 8-channel array head coil (USA Instruments, Aurora, Ohio, USA).

Psychophysics session

Participants completed 2 runs of a thresholding procedure based on García-Pérez (2000) in order to determine the minimum tilt away from vertical discriminated with 80% accuracy at a 12.5% level of contrast. The first of these sessions was used to familiarize the participant with the task, while the second session value was used to obtain the orientation thresholds used in the main experiment. If the thresholding session resulted in values that were very different from one another, a third session was completed.

Phosphene session

Prior to each of the five TMS sessions, area left V1 corresponding to the right visual field was determined by moving the coil over the left occipital area until a phosphene was reported at least 3 out of 5 times similar to the method used to measure MEPs thresholds in the motor cortex (Borsook et al., 2012). Position O1 was located on each subject using 10-20 EEG system standard measurement positions, marked on a swim cap. The coil was positioned at this start point with the handle pointed leftward away from the scalp, 10% up from the inion (point OZ) and 5% to the left of OZ based on individual scalp measurements, and moved discretely across the scalp in an average of 4cm by 4cm grid area. Average stimulation intensity to induce a phosphene for each participant was determined during this session by incrementally increasing TMS intensity from a starting value of 50% and increasing by 1% of the maximum stimulator output until subjects reported visual phosphenes and the coil position was held constant over each of the following sessions. Phosphene location was sampled at the start of each session and marked on a reconstructed 3D MRI scan with the Brainsight neuronavigation system (Rogue Research Inc., Montreal Canada), to allow for online motion correction ensuring stimulation of the phosphene induced area. Average stimulation location is reported in Figure 3. The TMS intensity levels thus used in the experiment corresponded to 0%, 60%, 80%, and 100% of the individual thresholds obtained during this initial session for each subject. Average maximum stimulation intensity across participants was 67.07% maximum stimulation output (SD: ± 5.94%, range = 55-76%).

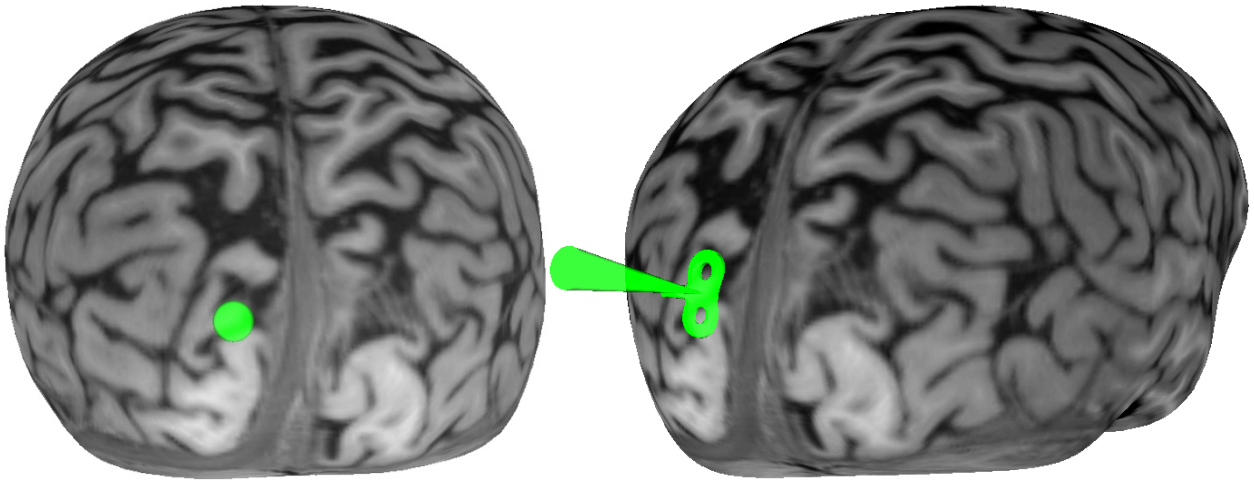


Figure 3. Average location of stimulation point on a representative subject. Average Talairach coordinates yielded an approximate stimulation point on the cortex at X= -13.55 (SD=4.65), Y= -98.81(SD=3.90), Z= 3.03 (SD=(9.27), (MNI Coordinates: X=-12.98, Y=-104.8.22, Z=8.22 (see **Fig. 1**) which corresponds to LVF V1.

Experimental Session

Participants took part in a 2 alternative forced choice (2AFC) orientation discrimination task. Subjects were told to maintain fixation for the duration of each trial on a centralized fixation dot. Each trial began with a fixation dot, followed by the presentation of two tilted peripheral Gabors (diameter = 4.98 degrees of visual angle [DVA]; see **Fig. 4**), the centers of which were presented simultaneously 5.01 DVA to the left and right of (and 2.51 DVA below) a central fixation dot (diameter=0.5 DVA) for a period of 30ms. TMS pulses were delivered simultaneously with the onset of Gabor presentation with an average temporal spacing of ~6 seconds in between pulses. These Gabors were presented at 5 different contrast levels tilted left or right away from the vertical under 4 different TMS intensities, resulting in a factorial structure of 2 (left / right) × 5 (1.56% / 3.12% / 6.25% / 12.5% / 25%) × 2 (oriented left / oriented right) × 4 (0%, 60%, 80%, 100%). After the Gabors disappeared, a horizontal line appeared to the left or right of the fixation dot, indicating on which of the two previously presented Gabors a subject should make an orientation judgment. The fixation dot turning green signaled to the participant they should respond. Response collection lasted for a total of 3 seconds (see Figure 3). Participants were instructed to make the orientation judgment with the index fingers of the left and right hand using a mouse (left finger for leftward orientation, right finger for rightward orientation). If a response was not made within the 3 seconds time window, the response was marked as incorrect and the experiment proceeded to the next trial.

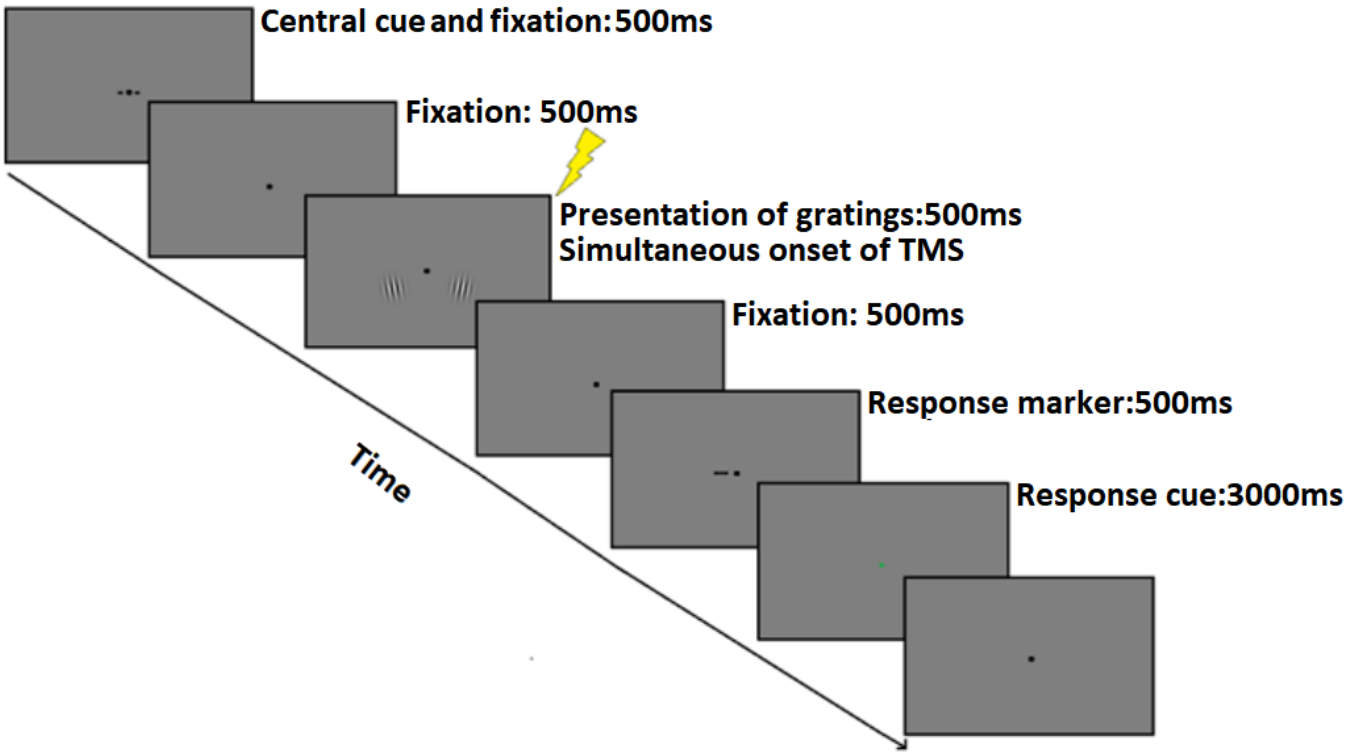


Figure 4. Example trial of the orientation discrimination task. After perceiving two tilted Gabor patches, participants were cued toward which stimulus they should respond. After a short delay, the fixation dot turned green, signaling participants to make the response. Participant responses terminated the trial, but if no response was made, the trial was marked incorrect and moved onto the next trial. All contrast intensities were presented in randomized order with randomized levels of TMS intensity, resulting in a total of trials per condition. Each experimental session separated by a minimum of 24 hours. The experiment took place in a dimly lit room, and participants maintained fixation for the duration of the session, while their head was placed in a head-rest to prevent movement.

Data analysis

All behavioral and statistical analyses were performed using MATLAB and SPSS. Mauchly's test of Sphericity was performed, and when violated, Greenhouse-Geisser corrections of degrees of freedom and resultant p-values were reported accordingly. All values are corrected for Bonferroni multiple comparisons.

Behavioral performance

Percent correct of our binomial data were calculated for each intensity by comparing correct responses trials to compared to incorrect response trials. These data were normalized using an arcsine transformation, a method shown to normalize data for comparison to that of a Gaussian distribution when data comes from designs based on a finite number of Bernoulli trials, since this type of data only approach the Gaussian distribution with large N, allowing for

stabilization and appropriate approximation for a general linear model (GLM) Gaussian test by permitting separation of the variance from the means (Bromiley and Thacker, 2002). The data were then submitted to a 3-way repeated measures ANOVA was computed with factors visual field x TMS intensity x contrast level to determine if performance differed between the left and right visual fields. Data for each visual field were subsequently submitted to a 2-way repeated measures ANOVA computed with factors tRNS intensity x contrast level to determine if performance differed according to stimulation intensity, contrast intensity, or an interaction of the two independent factors, due to differential visual field predictions regarding TMS effects due to stimulation site of the left occipital lobe. Post-hoc 2x5 ANOVAS comparing RVF and LVF performance at each TMS intensity across contrast levels to determine the drivers of the main effects and interactions. When appropriate, post-hoc paired t-test were also performed.

Field specific 2-way 4x5 repeated-measures analysis of variance (ANOVA) were computed with factors TMS intensity x Contrast level to determine differences within each visual field, due to differential visual field predictions regarding TMS effects due to stimulation site of the left occipital lobe. Post-hoc TMS (2)x Contrast (5 levels) ANOVAS were computed to compare performance at each TMS level to Sham condition separately for the RVF and LVF to determine any differences in sham performance. When appropriate, based on the results of 2x5 ANOVAs, paired t-tests were computed between specific levels of interest to sham condition. This functions to limit the correction due to paired t-tests based on specific predictions of interaction at subthreshold and suprathreshold contrast levels, and because we were not interested in comparisons between different TMS intensities, only differences between TMS intensities compared to sham.

Reaction Time Performance

Repeated Measures ANOVAs

A 3-way repeated measures ANOVA was computed with factors side x TMS intensity x contrast level to determine if participants' reaction times significantly differed between the left and right visual fields. Post-hoc 2x5 ANOVAs at each TMS intensity comparing RVF and LVF performance were computed across contrast levels, to determine if participants were responding differently to either one of the visual fields.

RVF and LVF field specific 2-way RM ANOVAs were then computed with factors TMS

intensity (4) x Contrast level (5). 2x5 ANOVAS were computed to compare performance at each TMS level to Sham condition separately for the RVF and LVF across contrasts. A priori paired t-tests were computed for Contrast 6.25 in the RVF and Contrast level 25 in the LVF to determine if timing effects could explain significant results found at each of these contrast levels in the behavioral data.

Results

Behavioral Analysis

Right Visual Field vs Left Visual Field

The 2x4x5 RM ANOVA of Visual Field by TMS Intensity by Contrast Intensity was calculated to determine if the effect was selective by visual field. The ANOVA showed a significant main effect due to Visual Field ($F_{(1,13)}$=7.5295, p=.017), Contrast intensity ($F_{(1.876,24.394)}$=194.353, p< .000), and TMS Intensity ($F_{(3,39)}$=4.636, p=.007). Furthermore, it showed significant interactions for TMS x Contrast Intensity ($F_{(12,156)}$ =2.222, p=.013), and VF x TMS x Contrast intensity ($F_{(5.861,76.195)}$=2.354, p=.040). All other interactions were non-significant. Overall, pairwise comparisons between Visual Field showed that participants responded better to stimuli in the RVF (M=98.2, SE=3.0) by a mean performance of 9.0 percentage points over the LVF (M=89.2, SE=2.8), (pairwise comparison SE= 3.3, p=.017).

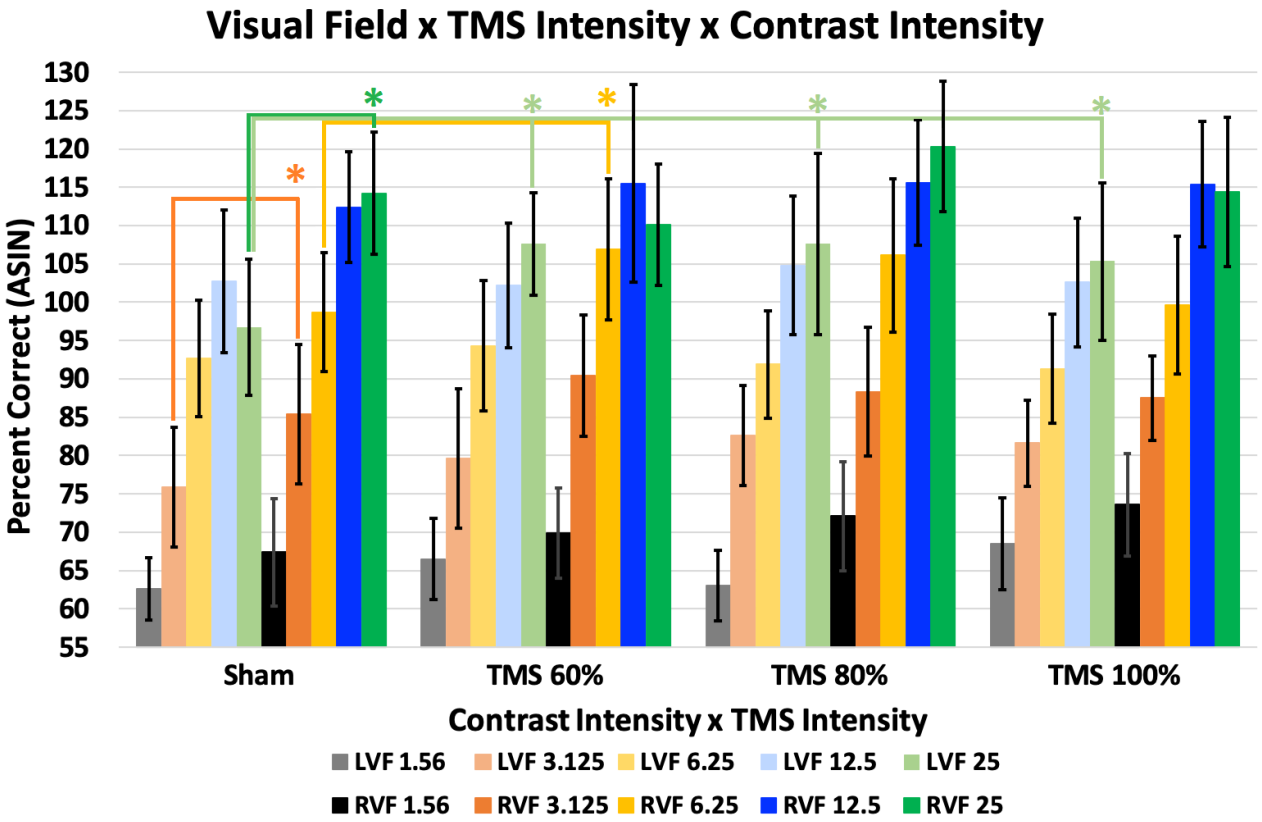


Figure 5. Right visual field and left visual field performance data (arcsine transformed). Participants were significantly better in the field contralateral to stimulation, and specifically at certain contrast levels at sham for contrasts 3.125 and Contrast 25 in which RVF showed significantly higher accuracy values compared to the RVF.. Additionally, performance differed within respective LVF and RVF fields independently, interacting with contrast intensity and TMS intensity. In the LVF, all TMS intensities resulted in significantly better accuracy performance compared to LVF sham condition. In the RVF, performance was only significantly better for Contrast 6.25 compared to sham after Bonferroni corrections for multiple comparisons, the likely driver of the main effect seen in the RVF, and in line with the theory of SR.

2x5 ANOVAs for each TMS intensity across all contrast intensities were computed to determine the driver of the Visual Field x TMS x Contrast interaction. The VF by Contrast intensity ANOVAS at each separate TMS intensity (Sham, 60%, 80%, and 100%), all showed significant main effects for Contrast intensity, (Sham: $F_{(4,52)}$=131.150, p<.000; TMS 60%: $F_{(4,52)}$=105.755,p<.000); TMS 80%: $F_{(2.203,28.636)}$=98.763, p<.000; TMS 100%: $F_{(4,52)}$=81.179, p<.000).

In the Sham condition between RVF and LVF, there was a main effect of Visual Field ($F_{(1,13)}$=10.374, p= .007), which showed that when stimuli were presented in the RVF (M=95.6, SE=3.2) participants were an average of 9.5 percentage points (SE= 3.0) more accurate at responding in the RVF than when stimuli were presented in the LVF (M=86.1, SE=3.0).

Interestingly, differences between RVF and LVF during Sham stimulation resulted in a significant interaction of VF x Contrast Intensity ($F_{(4,52)}$=3.437, p=.001), a likely contributor to the interaction in the main 2x4x5 ANOVA. Post-hoc paired t-tests computed between each contrast level at Sham stimulation between the RVF and LVF showed that only two of the five paired t-tests at each contrast between the RVF and LVF remained significant after Bonferroni corrections for multiple comparisons. At contrast 3.125, participants were better at discriminating stimuli in the RVF (M=67.41, SD=12.22) than in the LVF (M=62.55, SD=7.01), with an average mean difference of 4.86 points, ($t_{(13)}$=3.821, p=.002). Interestingly, however is the performance difference specifically at Contrast 25 between the RVF (M=114.18,SD=13.8) and the LVF (M=96.70,SD=15.48) with an average difference of 17.48 points at Contrast level 25 ($t_{(13)}$=3.742, p= .002), see Table 1.

For TMS 60%, 80%, and 100%, all main effects of TMS intensity showed that performance in the RVF was better than performance in the LVF (TMS 60%: $F_{(1,13)}$=5.894, p=.030; TMS 80%: $F_{(1,13)}$=6.976, p=.020; TMS 100%: $F_{(1.13)}$ =4.922, p=.045).

Pair	Contrast	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	1.56	RVF LVF	$t_{(13)}$ =1.823, p=.091	NO
2	**3.125**	RVF LVF	$t_{(13)}$=3.821, p=.002	**YES**
3	6.25	RVF LVF	$t_{(13)}$=1.414, p=.181	NO
4	12.5	RVF LVF	$t_{(13)}$=2.258, p=.042	NO
5	**25**	RVF LVF	$t_{(13)}$ =8.955, p<.000	**YES**

Table 1. Paired T-Test comparisons of performance comparing the RVF and LVF at Sham. Compared to the LVF, participants performed more accurately in RVF. Between RVF and LVF paired t-test comparisons between specific contrast intensities showed that participants performed significantly better in the RVF at contrast 3.125 and contrast 25, with other comparisons either showing insignificant results, or not surviving Bonferroni corrections for multiple comparisons. These results could not explain the significant results seen at contrast 3.125 in the

performance data for the LVF or RVF compared at different TMS intensities to sham, but were likely meaningful for contrast 25, as LVF performance at this contrast was markedly decreased for all TMS intensities in the LVF, a likely driver of the significant results seen at this contrast level in the LVF for all intensities compared to sham.

Right Visual Field

The 4x5 TMS Intensity by Contrast RM ANOVA of the RVF indicated increasing contrast causes systematic performance improvement in the RVF ($F_{(2.062,26.800)}$=113.409, p<.000), and was significantly modulated by TMS pulse intensity ($F_{(3,39)}$=3.525, p=.024). Crucially, participants' performance appeared affected by differing TMS intensities only at specific contrast levels as evidenced by the significant TMS Intensity x Contrast Intensity interaction, ($F_{(12,156)}$=1.893, p=.039) (see **Fig. 6**).

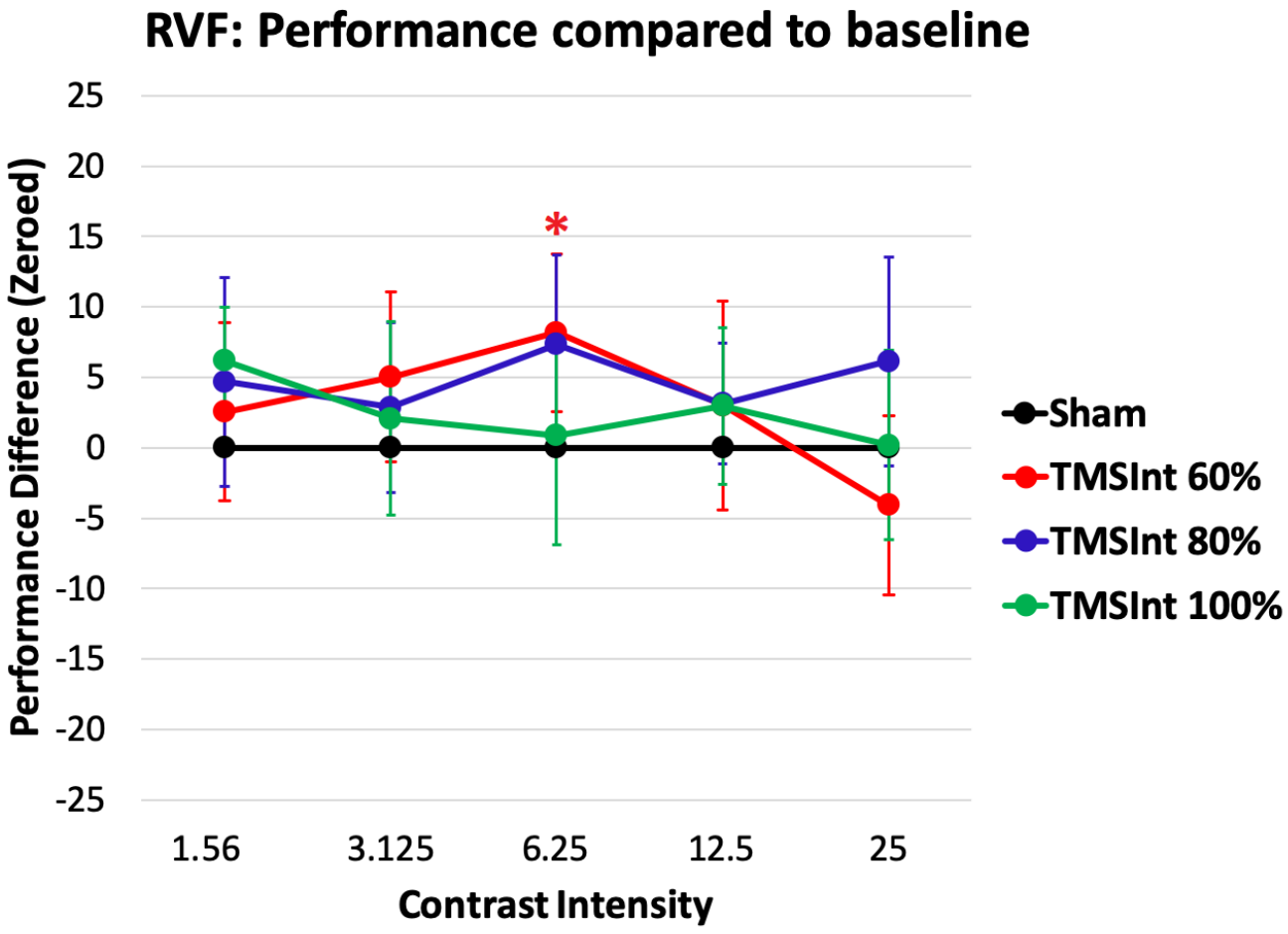


Figure 6. Performance Results for the Right Visual Field. Accuracy performance at TMS 60% was significantly better only at Contrast 6.25 compared to sham after Bonferroni corrections for multiple comparisons, the likely

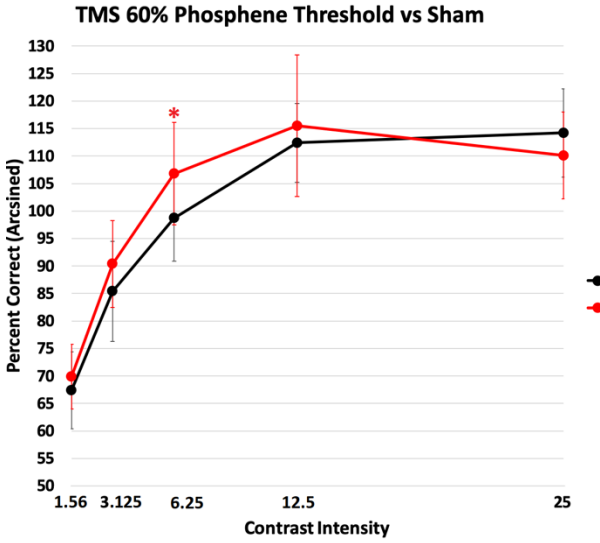
driver of the main effect seen in the RVF, and in line with the theory of SR.

Follow-up analysis were investigated by performing 2x5 ANOVAS at each TMS intensity compared to sham (60%, 80%, 100%) to determine the driver of the interaction. For all TMS intensities there was a significant main effect of Contrast Intensity (TMS 60%: $(F_{(4,52)}=101.727$, $p<.000)$; TMS 80%: $(F_{(4,52)}=100.596$, $p<.000)$; TMS 100%: $(F_{(1.979, 25.726)}=110.975$, $p<.000)$ This was expected, as an integral feature of the experimental design.

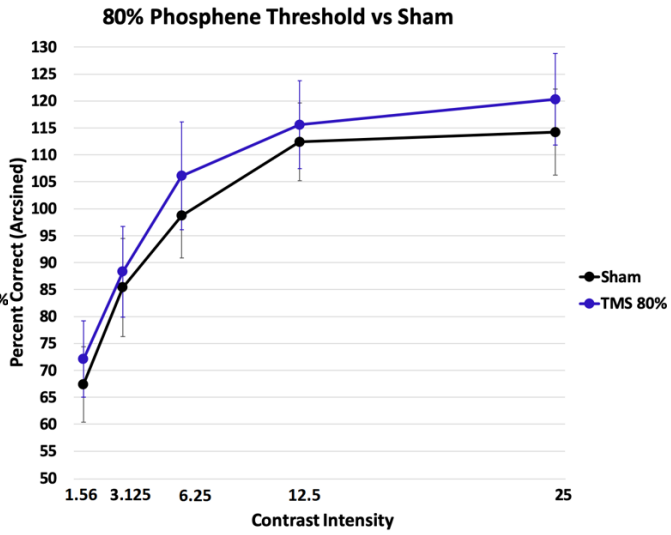
The 2x5 Sham vs TMS 60% showed no main effect of TMS but a significant interaction of TMS Intensity x Contrast Intensity, $(F_{(4,52)}=2.867$, $p=.032)$. A follow up paired t-test indicated a performance enhancement when sham (M=98.71, SD=13.615), was compared to TMS 60% (M=106.89, SD=15.856), at the subthreshold contrast level (6.25 Contrast) directly below threshold, $t_{(13)}=3.150$, $p=.008)$. A paired t-test at suprathreshold Contrast 25 showed no statistically significant effects, though with a trend towards performance decrement at this level started to appear, likely the driver of the interaction in this ANOVA and the main RVF 4x5 ANOVA, evident in **Fig. 7a**.

The 2X5 RM ANOVA of Sham vs TMS 80%, however, showed only a significant main effect of TMS Intensity, $(F_{(1,13)}=10.424, p=.007)$ with no significant interaction **(Fig.7b),** in which paired comparisons of the main effects showed that 80% TMS (M=100.5, SE=3.0) improved performance more than Sham performance (M=95.6, SD=3.3), by an average difference of 4.8 points (SE=1.5), and surviving Bonferroni corrections. Follow-up paired t-tests were conducted between Sham and TMS 80% at contrasts 6.25 and 25. At contrast 6.25, improvement with TMS 80%TMS (M=106.06, SD=17.22) was evident compared to sham (M=98.71, SD=13.615; $t_{(13)}=2.502$, $p=.026)$, similar to the results seen at this contrast level when TMS 60% was compared to sham. Performance compared to sham at Contrast 25 (M=120.32, SD=14.7) seemed to increase at the suprathreshold level, but results from the paired t-test were also not significant compared to sham performance (M=114.18, SD=13.795), $t_{(13)}=1.790$, $p=.097$, despite an indication of a trend towards performance enhancement. While for the uncorrected values, performance at 80% TMS for at contrast 6.25 was significantly better than sham,

comparable to the effect at TMS 60%, due to the stringent Bonferroni corrections for multiple comparisons, the performance improvement was deemed insignificant (See **Table 2**).



7a. Significant Interaction effect at TMS 60%



7b. Significant main effect of TMS 80%

Figure 7. RVF Main Effects and Interactions by TMS Intensity. Figure 6a shows the main interaction effect such that only at contrast 6.25 does a subthreshold TMS intensity (60%) induce a significant performance improvement compared to sham, creating an interaction effect, whereas Figure 6b shows a main effect of TMS at 80% phosphene threshold. While it survives correction as a main effect, the most performance increase is seen at contrast 6.25, as in the 60% subthreshold phosphene stimulation condition, however, this is only true before stringent corrections for multiple comparisons with the Bonferroni correction. Additionally, in TMS 80% main effect graph, we do not see the trend towards decreasing performance at increasing contrast, a fundamental factor proposed by the SR model.

Pair	Contrast	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	**6.125**	60% Sham	**$t_{(1,13)}$=3.150, p= .008**	**YES**
2	25	60% Sham	$t_{(1,13)}$= -1.382, p=.190	NO
3	6.125	80% Sham	$t_{(1,13)}$= 2.502, p=.026	NO
4	25	80% Sham	$t_{(1,13)}$= 1.790, p=.097	NO

Table 2. RVF Paired T-Test comparisons of performance accuracy across TMS intensity and contrast intensity. Results show that only the subthreshold contrast level 6.125 at subthreshold TMS intensity level 60% was significantly better than performance compared to sham levels. While the same subthreshold contrast level (6.125)

showed considerably better performance results compared to sham, after Bonferroni corrections for multiple comparisons, this result was no longer significant. While performance appeared to decrease at the suprathreshold contrast (25) compared to sham performance, this result was also statistically insignificant. There was however, a trend towards significant for performance improvement at suprathreshold contrast with TMS 80%, likely the reason that this TMS intensity level showed a main effect of performance enhancement even when compared for pairwise comparisons between other TMS intensities.

Left Visual Field

The 4x5 TMS Intensity by Contrast Intensity RM ANOVA of the LVF indicates increasing contrast caused systematic performance improvement in the LVF as contrast increased ($F_{(2.23,29.03)}$=101.011, p<.000), a significant main effect of TMS pulse intensity ($F_{(3,39)}$=2.949, p<.045), and a significant interaction between TMS Intensity and Contrast intensity ($F_{(12,156)}$= 2.715, p=.002), likely the result of differences at the last contrast level for all intensities compared to the zero TMS intensity level, see **Fig. 8**.

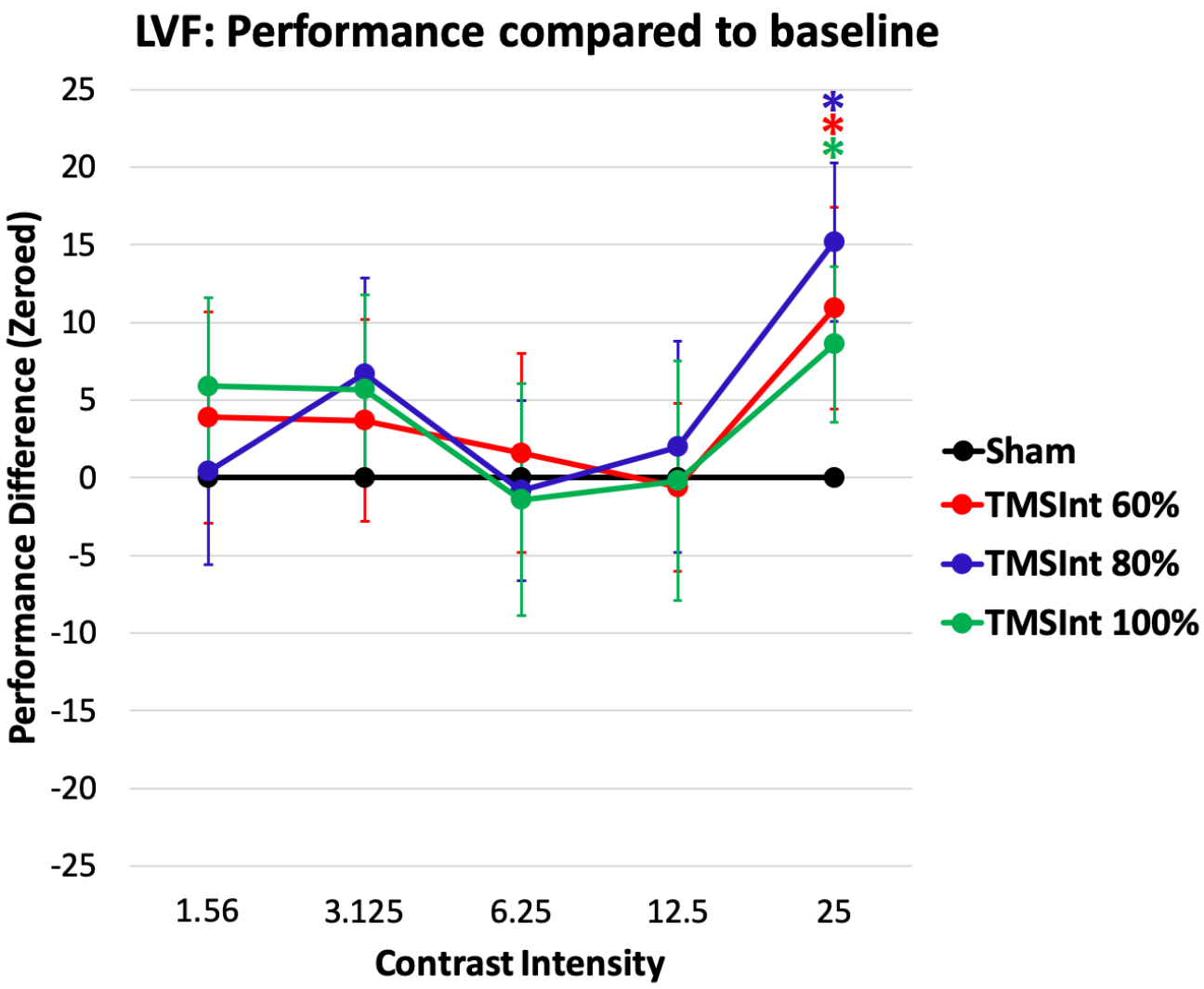


Figure 8. LVF Performance results subtracted by sham performance, indicating overall accuracy improvement compared to sham at each contrast intensity by TMS intensity level. Results show that compared to sham, at contrast 25, the suprathreshold contrast, participants were significantly more accurate at all three levels of stimulation. Performance for all other comparisons was not significant after Bonferroni corrections for multiple comparisons.

Post-hoc 2x5 ANOVAS were conducted at each TMS Intensity level across all contrast levels vs sham separately to determine the driver of the TMS main effect and interaction. Across all analyses in the 2x5 ANOVAS, as expected, we found a main effect of Contrast intensity, with performance improving as Contrast level increased (TMS 60%: ($F_{(4,52)}$=85.841, p< .000); TMS 80%: ($F_{(2.039, 26.503)}$=83.785, p<.000); TMS 100%: $F_{(4,52)}$=68.33, p<.000). The only 2x5 RM TMS x Contrast Intensity ANOVA to show a significant main effect of TMS when compared to Sham was TMS 80%, which indicated TMS 80% (M=90.8, SE=3.0) improved discrimination

compared to Sham performance (M=90.8, S=3.0), by a difference of 4.7 points (SE, 1.4), ($F_{(1,13)}$=10.863, p=.006), but this was shown to be driven by performance increase at only 2 levels of contrast, 3.125 ($t_{(13)}$=2.362, p=.034) and 25 ($t_{(13)}$=6.374, p<.000).

All 2x5 ANOVAS comparing Sham TMS to each stimulation intensity individually indicated significant interactions between TMS and Contrast Intensity, (TMS 60%: ($F_{(4,52)}$=3.805, p=.024); TMS 80%:($F_{(4,52)}$=6.033, p<.000); TMS 100%: $F_{(4,52)}$=2.762, p=.037). This is believed to mainly be driven by performance at the last contrast level (25) so paired t tests were submitted comparing each TMS intensity performance at this level to Sham performance, including other pairs of interest.

At Contrast 25, all TMS intensities produced performance benefits that were better than Sham performance, surviving Bonferroni corrections for multiple comparisons. Paired t-tests indicated a significant difference between Sham (M=96.70, SD=15.481) and TMS 60% (M=107.63, SD=11.669), $t_{(13)}$=3.620, p=.003), TMS 80% (M=111.90, SD=20.46; $t_{(13)}$=6.374, p< .000), and TMS 100% (M=105.33, SD=17.90; $t_{(13)}$=3.775, p= .002), in which performance at all three TMS Intensities was better than Sham. Other paired comparisons of interest were identified as contrast 3.125 for 80% TMS and contrasts 1.56 and 3.125 at TMS 100%, compared to Sham. However, none of these paired t-tests survived after stringent Bonferroni correction for multiple comparisons (See **Table 3**).

Pair	Contrast	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	**25**	60% Sham	**$t_{(1,13)}$=3.620, p=.003**	**YES**
2	**25**	80% Sham	**$t_{(1,13)}$=6.374, p<.000**	**YES**
3	**25**	100% Sham	**$t_{(1,13)}$=3.775, p=.002**	**YES**
4	1.56	100% Sham	$t_{(1,13)}$=2.262, p=.041	NO
5	3.125	80% Sham	$t_{(1,13)}$=2.362, p=.034	NO

Table 3. LVF Paired T-Test comparisons at specific contrast intensities and TMS intensities of interest. The main results seen by the paired t-tests were that at every single level of TMS intensity, performance at contrast 25 was significantly better than performance in the sham condition. While there were several interesting results indicating that perhaps TMS 80% was able to successfully push subthreshold contrast stimuli across the level for detection and improve performance accuracy, this did not survive the stringent Bonferroni corrections for multiple comparisons. This was similarly the case with TMS at 100% of phosphene threshold for the lowest contrast intensity.

Reaction Time Analysis Results

Comparison of Left Visual Field to Right Visual Field

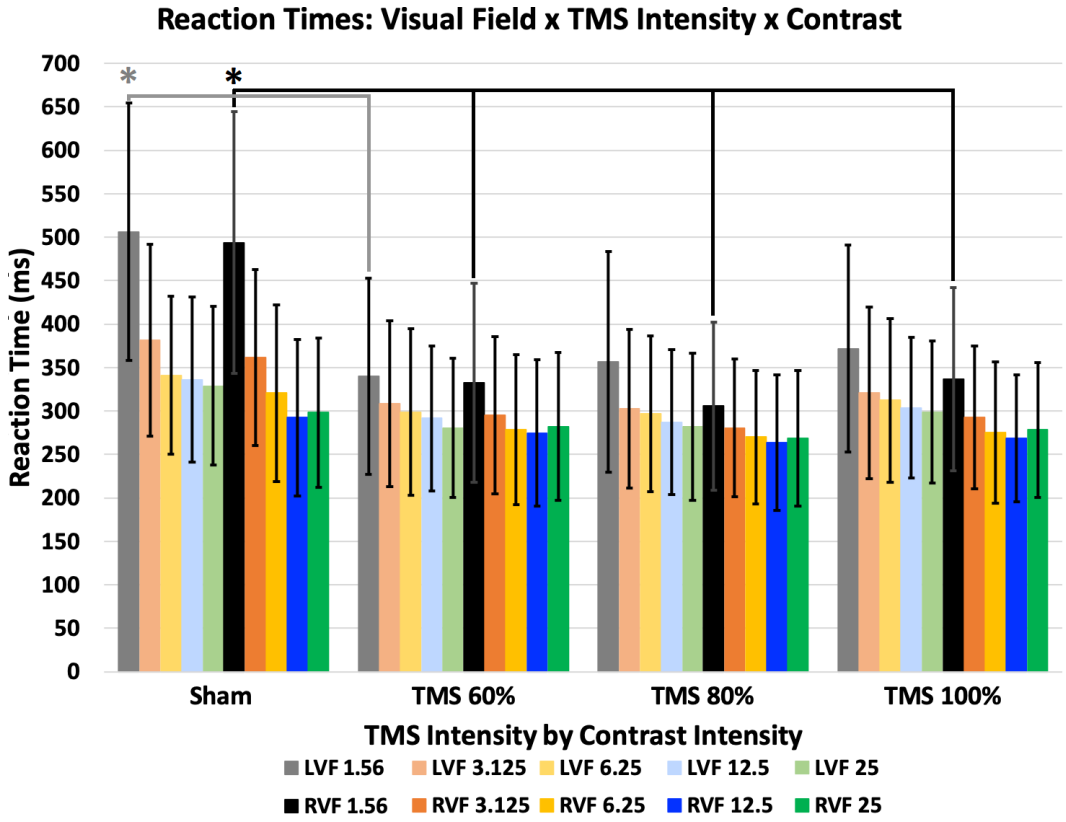


Figure 9. Reaction time results for the RVF and LVF. RT performance differed significantly in the LVF only when comparing Sham to TMS 60%, such that participants responded significantly slower when comparing Sham at contrast 1.56 compared to TMS 60%. In the RVF, all participants responded faster at all TMS intensities compared to Sham.

The Visual Field by TMS Intensity by Contrast intensity (2x4x5) 3-way RM ANOVA was computed to determine if there were significant differences between the LVF and RVF reaction times (Figure 8). The ANOVA showed significant Main effects of Visual Field ($F_{(1,13)}$=5.280, p=.039), TMS Intensity ($F_{(1.222,15.885)}$=17.396, p=.005), and Contrast ($F_{(1.120, 14.564)}$=17.396, p=.001). Furthermore, it indicated a significant TMS Intensity by Contrast interaction ($F_{(2.221,28.875)}$=8.542, p=.001). Pairwise comparisons between Visual Field indicated reaction time performance in the RVF (M=303.395, SE=39.22) was overall faster than participant reaction times in the LVF (M=327.230, SE=43.127), with the RVF showing overall faster performance times with a mean difference of 23.835ms (SE=10.372). Pairwise comparisons for the main effect of TMS intensity showed that RT at sham condition (M=365.94, SE=47.02) was significantly slower than both TMS conditions of 60% (M=298.16, SE=42.09) and TMS 80% (M=291.33, SE=39.73), by a mean difference of 67.78ms and 74.61ms, respectively.

When a VF(2)xTMS(3)xContrast(5) ANOVA was computed, omitting sham condition, it

was evident that the results of the Interaction in the 3-way ANOVA were largely due to the changes in response times in the sham condition compared to the conditions in which TMS stimulation was actually applied. This ANOVA showed only main effects of Visual Field, ($F_{(1,13)}$=5.025, p=.043), and Contrast ($F_{(1.143,14.865)}$=8.372, p=.009), in which RT were significantly faster in the RVF than the LVF, as in the VFxTMSxContrast ANOVA. Looking at the graph between the RVF and LVF RT performances, it is clear that the largest difference occurs at the lowest contrast level (1.56). Thus, post-hoc paired t-tests were performed to evaluate this, comparing Sham stimulation to each TMS intensity for the RVF and the LVF at this level, (See **Table 4**). All paired T-tests were initially significant. However, after stringent Bonferroni corrections for multiple comparisons, results indicated that for all RVF Stimulation levels compared to Sham at Contrast 1.56, participants responded more slowly for sham than at any TMS stimulation level. In the LVF, after correction, only TMS 60% showed response times that were significantly faster than sham condition.

Pair	Visual Field	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	**RVF**	Sham 60%	**$t_{(1,13)}$=3.287, p= .006**	**YES**
2	**RVF**	Sham 80%	**$t_{(1,13)}$=4.001, p= .002**	**YES**
3	**RVF**	Sham 100%	**$t_{(1,13)}$=3.140, p= .008**	**YES**
4	**LVF**	Sham 60%	**$t_{(1,13)}$=3.756, p= .002**	**YES**
5	LVF	Sham 80%	$t_{(1,13)}$=3.058, p= .009	NO
6	LVF	Sham 100%	$t_{(1,13)}$=2.763, p= .016	NO

Table 4. Paired t-test results comparing RT data between the LVF and RVF. While uncorrected results showed that RT performance differed significantly in the LVF for all TMS intensities compared to Sham, only the comparison between Sham RT to TMS 60% RT, remained significant after Bonferroni corrections for multiple comparisons, such that participants responded significantly slower when comparing Sham at contrast 1.56 compared to TMS 60% In the LVF. In the RVF, all participants responded faster at all TMS intensities compared to Sham, surviving

Bonferroni corrections for multiple comparisons.

Visual Field(2)x Contrast(5) ANOVAs were computed at each stimulation level compared to Sham, and there was a significant main effect of Contrast intensity for each pair (Sham: ($F_{(1.169, 15.191)}$=19.059, p<.000); TMS 60%: ($F_{(1.562,20.302)}$=5.944, p=.014; TMS 80%: ($F_{(1.468, 19.083)}$=5.644, p=.018); TMS 100%: ($F_{(1.260,16.379)}$= 8.878, p=.006). When comparing the RVF and the LVF, a main effect of Visual Field indicated significant differences were found only when RVF and LVF were compared at TMS 100%, ($F_{(1,13)}$=6.203, p=.027). Pairwise comparisons showed that participants were significantly slower in responding to visual stimuli in the LVF (M=321.47, SE=43.08) than stimuli to the RVF (M=290.18, SE=38.15), with a mean difference of 31.289ms (SE=12.56).

Right Visual Field

The RVF 4x5 Analysis conducted in the right visual field closely mimicked the pattern of results above with main effects of TMS Intensity ($F_{(1.496,19.442)}$=8.122, p=.005), Contrast Intensity ($F_{(1.453,18.892)}$=17.252, p<.000), and TMS Intensity by Contrast interaction ($F_{(2.442, 31.748)}$=8.115, p=.001). Main effects pairwise comparisons between TMS Sham (M=353.19ms, SE=46.99ms), and all other TMS stimulation intensities showed that only one intensity was significantly faster than Sham stimulation. TMS 80% (M =277.68, SE=56.96ms), showed that RT performance at Sham stimulation was an average of 75.52ms slower than RT Performance at TMS 80%, (SE=21.28ms, p=.021).

2x5 ANOVAs were computed between Sham and each TMS intensity level to determine the driver of the interaction, with follow up paired t-tests in the case these differences were significant. All main effects of Sham versus TMS intensity were significant (TMS 60%: $F_{(1,13)}$ =8.849, p=.011; TMS 80%: $F_{(1,13)}$=12.590, p=.004; TMS 100%: $F_{(1,13)}$=7.240, p=.019). Similarly there were main effects at all levels of TMS compared to Sham for Contrast Intensity (TMS 60%: $F_{(1.478,19.211)}$=20.481, p<.000; TMS 80%: $F_{(1.53,19.894)}$=16.346, p<.000; TMS

100%: $F_{(1.519,19.749)}$=19.054, p<.000). For all TMS intensities compared to Sham there was a significant TMS intensity by contrast interaction (TMS 60%: $F_{(1.588,20.650)}$=8.621, p=.003; TMS 80%: $F_{(1.483,19.273)}$=12.677, p=.001; TMS 100%: $F_{(1.417,18.422)}$=9.793, p=.003). Due to the fact that all intensities had main effects and interactions, a full set of paired t-tests were performed for each TMS intensity compared to Sham at each Contrast. Only one comparison TMS 80% (M=305.627ms, SD=167.780ms) versus Sham at Contrast 1.56 (M=493.684ms, SD=260.869) survived Bonferroni corrections for multiple comparisons ($t_{(13)}$=4.001, p=.002). Before Bonferroni corrections, all three TMS intensities were significantly faster than Sham for the first 3 contrast levels, but not at the threshold or suprathreshold levels, with the exception of TMS 80% at Contrast 25 (M=268.709, SD=134.777) compared to Sham at 25 (M=298.080, SD=148.990), likely the driver of the interaction due to insignificance different only at threshold level (See Table 5). However, after stringent corrections for multiple comparisons, only RT performance at contrast 1.56 during TMS 80% was statistically significant, a finding that did not correspond to the RVF accuracy performance data, indicating speed-accuracy trade-off was not the main driver of the performance value effects.

Pair	Contrast	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	1.56	60% Sham	$t_{(1,13)}$=3.287, p= .006	NO
2	3.125	60% Sham	$t_{(1,13)}$=2.609, p=.022	NO
3	6.25	60% Sham	$t_{(1,13)}$=2.577, p=.023	NO
4	12.5	60% Sham	$t_{(1,13)}$=1.055, p=.311	NO
5	25	60% Sham	$t_{(1,13)}$=1.326, p=.208	NO
6	**1.56**	**80% Sham**	**$t_{(1,13)}$=4.001, p=.002**	**YES**
7	3.125	80% Sham	$t_{(1,13)}$=3.059, p=.009	NO
8	6.25	80% Sham	$t_{(1,13)}$=2.780, p=.016	NO
9	12.5	80% Sham	$t_{(1,13)}$=1.654, p=.122	NO
10	25	80% Sham	$t_{(1,13)}$=2.710, p=.018	NO

Table 5. RVF Paired t-tests of RT across TMS and contrast intensities. Results show that participants were overall faster in both the 60% and 80% conditions at making a decision compared to sham, except for the threshold contrast (12.5), before Bonferroni correction for multiple comparisons. This indicates that results of the performance data cannot be attributed to a speed-accuracy trade off, in that participants were both faster and more accurate at these TMS intensities, and did not slow down their response times in the effort to produce more accurate results. After stringent corrections for multiple comparisons, only 80% TMS showed significantly faster RT than sham, but this level was not more accurate in the accuracy performance related data.

Left Visual Field

The ANOVA of reaction time data showed that reaction times were significantly modulated by TMS intensity ($F_{(1.183,15.374)}$=9.368, p=.006), Contrast intensity ($F_{(1.098,14.269)}$=9.368, p=.002), and resulted in a significant interaction between TMS Intensity and Contrast intensity ($F_{(2.749,35.732)}$=5.270, p<.000). Pairwise comparisons of the main effect

of TMS intensity indicated significantly slower reaction times at Sham compared to both the 60% Stimulation (Mean difference=74.89ms, SE=20.09ms; p =.015) and Stimulation at 80% (Mean difference= 73.703ms, SE=22.97ms; p=.041), but not when compared to RTs at TMS 100%.

2x5 ANOVAs were computed between Sham and each TMS intensity level to determine the driver of the interaction, with follow up paired t-tests in the case these differences were significant. All main effects of Sham versus TMS intensity were significant (TMS 60%: $F_{(1,13)}$=13.899, p=.003; TMS 80%:$F_{(1,13)}$=10.298, p=.007; TMS 100%: $F_{(1,13)}$=6.020, p=.029). Similarly there were main effects at all levels of TMS compared to Sham for Contrast Intensity (TMS 60%: $F_{(1.204,15.654)}$=14.897, p<.001; TMS 80%: $F_{(1.211,15.737)}$=17.454, p<.000; TMS 100%: $F_{(1.240,16.117)}$=16.586, p<.000). For all TMS intensities compared to Sham there was a significant TMS intensity by contrast interaction (TMS 60%: $F_{(1.981,24.580)}$=8.429, p=.002; TMS 80%: $F_{(1.464,19.037)}$=5.804, p=.017; TMS 100%: $F_{(1.603,20.839)}$=6.156, p=.011)

Due to the fact that all intensities had main effects and interactions, a full set of paired t-tests were performed for each TMS intensity compared to Sham at each Contrast. Only one comparison TMS 60% (M=339.93ms, SD=195.34ms) versus Sham at Contrast 1.56 (M=506.10ms, SD=256.87ms) survived Bonferroni corrections for multiple comparisons ($t_{(13)}$=-3.756, p=.002). Without Bonferroni corrections, all three TMS intensities were significantly faster compared to Sham at all contrast levels, except for the last 3 contrast levels at TMS 100%, likely the driver of the interaction due to insignificance different only at threshold level (See **Table 5**).

Importantly, in light of behavioral results listed above, we looked with specific interest at the set of paired t-tests at Contrast 25 across TMS intensities in the LVF to see if differences in RT performance could shed light on the unexpected significant differences in performance behavior (percent correct) that we see at this suprathreshold contrast between the TMS and sham conditions. However, follow up paired t-tests computed between the performances at Contrast 25 showed RTs were faster only at TMS 60% (M=280.64, SD= 139.14) and 80% (M=282.06, SD=146.55) compared to Sham (M=328.94, SD=158.51), ($t_{(13)}$=-3.185, p= .007;

$t_{(13)}$=2.167, p=.049), whereas TMS 100% was not (see **Table 6**).

Pair	Contrast	TMS Intensity	Significance	Significant after Bonferroni Correction?
1	**1.56**	60% Sham	$t_{(1,13)}$=-3.756, p=.002	**YES**
2	*25*	*60% Sham*	*$t_{(1,13)}$=-3.185, p=.007*	*NO*
3	*25*	*80% Sham*	*$t_{(1,13)}$=-2.167,p=.049*	*NO*
4	*25*	*100% Sham*	*$t_{(1,13)}$=-1.840,p=.089*	*NO*

Table 6. Paired T-Test comparisons of interest in the LVF. Paired T-tests at Contrast 25 for the LVF were of considerable interest to us, considering the results of unexpected behavioral performance increases at each of the TMS intensity levels compared to sham at this contrast. However, as we see here, while it is apparent that participants responded faster in at least 2 of the TMS conditions 60% and 80%) compared to sham reaction times, none of these survive Bonferroni corrections for multiple comparisons. A significant comparison of interest is, however, that at the lowest level contrast, for contrast 1.56, 60% TMS RT performance is faster than sham RT Performance

Discussion

Contralateral effects to stimulation

The present study aimed at understanding the direct causal effect of TMS intensity and its interaction with visual stimulus contrast, to determine whether its effects upon the visual cortex are linear depending on the intensity of stimulation (e.g. the stronger the intensity the higher the effect) or whether the interaction of contrast and intensity vary non-linearly, potentially indicating a stochastic-like effect of stimulation upon the cortex.

We used TMS over the left early visual cortical area V1 and found an effect on orientation discrimination that was selective for the condition in which TMS was delivered at 80% of phosphene threshold. Specifically, there was an overall increase in performance at all contrast levels in the right visual field (contralateral to stimulation) at 80% TMS intensity (**Fig. 7b**). Moreover, when TMS was delivered at 60% of phosphene threshold, we found an improvement in the right visual field that was selective for the medium contrast level (**Fig. 7a**).

Our data show that a simple model of stochastic resonance cannot fully account for all the conditions we tested. In particular, analysis of the RVF effects show not only a main effect of stimulation intensity for the subthreshold level of 80% stimulation of phosphene threshold, but importantly, a significant interaction effect, mainly due to increased performance benefit at the first subthreshold contrast level at 60% of phosphene threshold. The follow-up analyses showed that the likely drivers of the interaction clearly indicate divergent characteristics of performance at 60% and 80% stimulation intensities vs sham performance, but in different ways. The results are in one case, dependent on the stimulation intensity, and in the other case (60%) dependent on both stimulus contrast and stimulation intensity. While in both conditions most performance benefits are at a subthreshold contrast level, in the case of TMS 60%, behavioral performance starts to go back to baseline at suprathreshold levels. The question is whether what we see at 60% is really different than what we are seeing at 80%. In other words, do these stimulation intensities denote the same effect, or can they truly show divergent characteristics when applied to higher contrasts, and higher TMS intensities? It could be argued that in fact they both support stochastic resonance, but in different ways. Both seem to operate stochastically as we would expect in pushing up performance at the contrast hovering just below threshold for detection where some noise might be beneficial, but neither explicitly follow the inverse "U" that is so typical of the presence of stochastic resonance phenomenon in other perceptual studies, and in nature (Perini et al, 2012; Schwarzkopf et al., 2011; Abrahamyan et al., 2015). Concurrently, we also see an overall effect of better performance in the RVF compared to the LVF, a result coherent with a modulation of behavior in the field contralateral to stimulation, but also potentially relevant to signifying a right visual field advantage for simple visual stimuli (Railo et al. 2011), The interaction of subthreshold contrast intensity 6.25 with a subthreshold stimulation level 60% does seem to at least partially support the theory of stochastic resonance for single pulse TMS effects. Similarly, we find that it is not implausible that the effects seen in studies of adaptation are discordant with this hypothesis. The main results of the RVF do show at least partial support for stochastic resonance in general and could partially be explained by the same mechanisms studied in a previous paper with similar results (Perini et al. 2012) whereby TMS facilitated performance for stimuli below threshold. We did not find, however, a suppressive effect of TMS at supra-contrast thresholds like in the Perini et al. study. For instance, stimulation intensity was held constant at 120% (of phosphenes threshold) in the experiment conducted by Perini et al. (2012), whereas in the experiment

conducted by Schwarzkopf et al. (2011) TMS intensity was varied, a variable that was also manipulated in subsequent studies (Abrahamyan et al., 2011; 2015). Altogether, previous data align with the idea that TMS might act by adding noise into the system, and the effects are at least dependent both on stimulation parameters and stimulus intensities.

Ipsilateral effects to stimulation

We found improvement of performance at each TMS Intensity level in the field (left) ipsilateral to stimulation, however, it is evident in the graphs (**Fig. 8**) that sham condition at this contrast level performed in an unexpected manner. While increasing contrast intensities are generally considered to produce a significant boost in performance (Boynton et al., 1999) we show that these effects are not consistent with the performance results we see here. Our data show a decrease from threshold contrast 12.5 to suprathreshold contrast 25, a condition that should overall be much easier for the participants to detect, in line with visual signaling of neurons being sufficiently higher at this level (25) than at 12.5, evidenced by a neuroimaging study that directly shows this increased neuronal recruitment. Therefore, in a sham condition with no external intervention exerted upon the signal to noise ratio, the signal at 25 is expected to produce a performance at least as equal, if not higher than a contrast twice as low in luminance intensity (Boynton et al., 1999). Therefore, we posit that this effect at contrast 25 is likely due to this unexpected violation of evidence of linear increase in performance as contrast increases, as no external force was applied to mitigate the neural response to the stimuli, since the performance decrement occurred during the sham condition. This violation does not follow the expected linearity related to neuronal recruitment raising in magnitude of response with higher contrast, therefore presenting a potential explanation for the unexpected performance drop at this level during sham condition, rather than a true effect of TMS stimulation during non-sham conditions mitigating the behavioral response, in which the neuronal signal was twice as strong at sham in the thresholded contrast. Such effect might indicate a potential attentional bias favoring the right over the left hemifield at higher contrast indicated by a right visual field advantage for low-level visual stimuli and perceived contrast, (Railo et al., 2011). Furthermore, this unpredicted drop in behavioral performance at Sham stimulation at the highest contrast level in the ipsilateral field cannot be accounted for by differences in reaction times. Even though participants responded overall more slowly in the sham condition, and in the LVF compared to the RVF, they were less accurate at Sham at this suprathreshold contrast, and

several participants (5 of 14) verbally reported a “pull” of attention towards the target in the RVF during at least one of their stimulation sessions. Therefore, the differences in performance, which were significant at all 3 TMS levels at the highest contrast level cannot be accounted for by differences in the reaction time. This indicates that improvement for all TMS intensities at this contrast level was not modulated by reaction time, and resultant effects were not due to a speed-accuracy trade-off, but instead, by effects of the variables of the experimental paradigm itself, potentially, as indicated a right visual field advantage when competing stimuli strength is high enough (Railo et al., 2011). However, it is also important to note that there may have been a cumulative effect over time of TMS intensity in the ipsilateral field, despite trials being separated about 6-7 seconds apart, and the fact that sham stimulation was interwoven into the design. This could be one explanation for the results seen, but due to a lack of other studies reporting their results with single pulse TMS in the field ipsilateral to stimulation, this has yet to be determined and is, at the moment, speculative.

When TMS was delivered at 80% of phosphene threshold, a significant effect was present at different contrasts in the LVF, with a performance improvement at two contrast levels, a subthreshold contrast (3.125) and a suprathreshold contrast (25), though performance benefit at the highest contrast may be due to the unexpected drop in Sham performance. The main point of interest in this result is the increase at subthreshold contrast 3.125 at 80% PT, a result similar to that found in the RVF at contrast 6.125, though both subthreshold TMS intensities 60% and 80% phosphene threshold showed significant performance enhancement over sham in the RVF. This could indicate potential evidence for ipsilateral effects of single pulse TMS not yet reported in other studies, and therefore, which cannot be accounted for by current models. This effect was unexpected given that it occurred in the field ipsilateral to stimulation, however, it could potentially be that there was perhaps some cross-over from the ipsilateral field of stimulation to the contralateral site, as the networks are functionally connected. The study by Garcia et al., (2011) used EEG in conjunction with TMS to show that while TMS effects are predominantly local in the occipital cortex and in V1, and that this effect is manipulated linearly by TMS intensity, network sites that V1 is connected to, i.e. parietal lobe and thalamic areas further on in the spread of the pulse across connected networks, also showed significant differences in modulation at higher TMS intensities. Since the left and right occipital lobes are connected by transcallosal fibers, some effects of the pulse in the ipsilateral stimulation site

may potentially have crossed over to the contralateral stimulation site, causing weak but perceptible influences on behavior. This may be why the pulse had to be stronger (80% in LVF compared to 60% and 80% in RVF) to create a performance enhancement. Importantly, we cannot rule out that some immediate effects affected performance in the field ipsilateral to stimulation (in our case, the LVF). Studies using concurrent PET and TMS, as well as EEG and TMS have both shown that there is evidence for ipsilateral TMS effects crossing over via transcallosal fibers to the contralateral hemisphere in the occipital lobe, (Paus et al., 1997; Ilmoniemi et al., 1997). Furthermore, in a study by Fuggetta et al., (2005) the authors showed a similar finding while delivering stimulation in the motor cortex. They suggested that some levels of intensity manipulated the cortical areas of M1 just beneath the cortex in one manner, but manipulated at higher TMS intensities effects across the network differentially potentially due to a diffuse spreading that targets different structures. These two studies then look not just at the noise injected in the peripheral system close to the area being stimulated, but also how it interacts with areas that are functionally connected with it (Garcia et al. 2011), and how the depth might be affecting the perturbance created by the single pulse. For instance, the study by Fuggetta et al. (2005) hypothesized that low intensity TMS elicited direct and indirect excitation of pyramidal neurons through transynaptical volleys, such that, at high intensities, stimulation acted more similarly to electrical stimulation by directly activating the axonal pathways. In line with previous studies that showed an ipsilateral effect of TMS (Paus et al., 1997) and evidence by Garcia et al., (2011), that supports the spread of the signal across the network, the results from Fuggetta et al. (2005) indicate that while the low intensity results of TMS may themselves be confined to stimulating the cortex directly underneath the cortical area (resulting, as in our experiment, an expected effect in the RVF with left occipital stimulation), pulses at higher intensities could potentially "bypass the source of cortical oscillatory activity", thereby activating deeper cortical structures, or passing over through the transcallosal connections to the other hemisphere. This could then suggest a reason for why not only our study, but others, find significant findings in both hemispheres. Specifically, in the set-up of our experimental paradigm, stimulation close to midline may make this even more likely, though to produce effects similar to the effects we see with left hemisphere stimulation at lower intensities, the intensity (being higher) would likely have a markedly reduced effect. Thus, when we send the single pulse TMS of high enough intensities, its effects could potentially exert effects in the LVF through transcallosal connections, and when the stimulation is high enough to pass via

transcallosal connections, but not so strong as to directly inhibit the underlying cortical area producing focal suppression (as seen in Perini et al., 2012; Amassian et al., 1989), some effects of TMS single pulse stimulation could potentially produce bilateral effects.

Conclusions

Under the scope of the current experiment, it is evident that TMS might indeed act as a mechanism of stochastic resonance, especially with stimuli just below detectability threshold. Our finding supports that low-level stimulation, regarded under the theory of stochastic resonance as a potentially "optimal" injection of low-level noise, at subthreshold contrast intensities can be beneficial under very specific conditions.

Our results therefore show that no model can account for the full range of effects, and that perhaps searching for a one-size-fits-all model to fit all results for this technique may not be the appropriate approach. While stochastic resonance certainly seems to play a role at certain TMS intensities dependent on modification of task difficulty (in this case, the contrast intensity), it is also clear that performance benefit can occur in increasing fashion as long as stimulation remains at subthreshold phosphene threshold levels, whereas once the phosphene threshold is reached or superseded, we begin to notice either no improvement, or a performance decrement (Perini et al., 2012). These results seem to be most in line with the activity driven adaptive account of stochastic resonance espoused by Wenning and Obermeyer (2003), as we do not see a significantly marked decrease in performance indicative of the inverse "U". This denotes a possibility that the system makes use of helpful amounts of external noise only when it is in an optimal intensity range for the system, a principle tenant of stochastic resonance theory. The results of our experiment point to the necessity to tailor the type of TMS, as well as the stimulus properties in conjunction to the desired TMS effect.

Our findings conclusively contradict previous work which led to the belief that TMS intensity behaved in a relatively linear manner (Siebner et al., 2009), or as a sole method for focal suppression, (Naka and Rushton, 1966; Amassian et al., 1989; Pascual-Leone et al., 2000), and provides further proof of confidence that TMS acts as a method of helpful noise induction under the concept of stochastic resonance exhibited by recent evidence in the field in line with our results (Wenning and Obermeyer, 2003; Schwarzkopf et al., 2011; Abrahamyan et al., 2011; Abrahamyan et al., 2015).

Chapter 3

Transcranial Random Noise Stimulation

Stochastic Noise in a Stochastic System: Does tRNS aid sub-threshold stimuli perception?

Abstract

High frequency transcranial random noise stimulation (hf-tRNS) is a relatively new form of brain stimulation, whose influence on transient behavioral effects are not well studied, though often shown to be facilitatory in nature. This is especially well marked in studies of perceptual learning, but these paradigms often extend multiple days, and the immediate behavioral impact of hf-TRNS it is still unclear. Stochastic resonance has been explored as an explanation of the tRNS effects, however the findings are inconsistent across studies (Moss et al, 2004; van der Groen and Wenderoth, 2016; van der Groen et al., 2018; Pavan et al., 2019). These results are highly variable with regards to methodology, stimulation parameters, task, and electrode montage. To account for this, we combined multiple features of past paradigms regarding stimulation intensity and an orientation discrimination paradigm to determine if there was evidence that tRNS acts by adding helpful noise into a stochastic system (the brain) in a carefully designed within-subjects paradigm, with bilateral occipital stimulation. We used a low-level visual orientation discrimination task, thresholded for difficulty at contrast level (12.5), and increased task difficulty by decreasing contrast at three sub-threshold contrast levels (1.56, 3.125, 6.25), and facilitated discrimination by including a suprathreshold contrast level (25). Participants were stimulated over 4 different days at 4 different tRNS intensity levels, (Sham, .250mA, .500mA, .750mA) in a within-subjects design. The main experiment, where we apply tRNS to the occipital cortex, tests predictions made by other groups in the field, that by adding noise directly into the system, we observe behavioral results that indicate evidence of stochastic resonance. The second (parietal) experiment acts as a control experiment to ensure that effects due to stimulation were local and not reproducible at locations distal to the area of occipital application. For the parietal experiment, we use exactly the same parameters as the

occipital experiment, but with stimulation in the parietal areas (P3/P4). In the main (occipital) experiment, measures of cortical excitability were obtained using single pulse TMS (spTMS) to induce phosphenes. Presentation of each contrast intensity in the orientation discrimination task was randomized, and order of stimulation intensity was randomized across subjects to prevent order effects. We hypothesized that, in experiment 1, if tRNS acts under the principle of stochastic resonance, we would see performance benefits at subthreshold contrast intensities, and performance decrement at the suprathreshold contrast intensity. Our results showed an interaction effect of tRNS intensity and contrast intensity in the orientation discrimination task, but the results were in the opposite direction predicted by models of stochastic resonance. We found that at .750mA, participants' accuracy performance was significantly reduced compared to sham. This performance decrement at a subthreshold level of contrast at .750mA shows that introducing this amount of noise into the system is detrimental, especially for subthreshold contrast levels.

Introduction

TRNS was introduced as a new non-invasive brain stimulation technique which can induce cortical excitability changes that can last up to 90 minutes following only 10 minutes of stimulation (Terney et al., 2008). As tRNS is used more widely, some inconsistencies have been discovered, akin to those which limited understanding of the properties of TMS (Pascual-Leone et al, 2000; Paulus et al., 1999; Pitcher et al., 2007; Camprodon et al., 2010; Silvanto and Walsh, 2005). Works by Abrahamyan et al. (2011, 2015), van der Groen and Wenderoth (2016), van der Groen et al., (2018), and more recently, Pavan et al., (2019) are working towards amending these discrepancies, but differences in paradigms limit the conclusions that we can currently make. One of the main aims of this study is to implement a more systematic approach that incorporates crucial elements of these designs. While more rigorous, we propose that a within-subjects design is paramount towards bringing out subject specific differences. We believe that by implementing this factor, along with measures of cortical excitability will allows us to make comparisons about how the technique influences behavior in a way that accounts for individual variability. This of course, requires more time than a between subjects design, and is, in itself liable to confounds such as learning, which we will attempt to control by re-setting task difficulty to the same limit each day, so in the case that subjects do learn, they

will not reach ceiling effects. Even with this potential confound, we believe the within subjects design is a much stronger method with which to assess this technique across a variety of stimulation parameters and task difficulty (stimulus parameters), and how they affect the subjects behavioral output. Clarification from this type of design could be highly beneficial to identifying subject specific factors as well as understanding the overall effect at the group level.

tRNS has been predominantly used in the motor cortex, likely following the successful results of Ternery et al., (2008). This technique may act differently across cortical regions and networks, evidenced by the discrepant findings in the occipital cortex (van der Groen and Wenderoth, 2016; van der Groen et al., 2018; Pavan et al., 2019). Furthermore, within the same cortical regions, discrepancies indicate tRNS could even affect cortical excitation differently based on the experimental paradigm (van der Groen and Wenderoth, 2016; van der Groen et al., 2018). For example V1 and V5 areas were both stimulated under a unilateral montage in two separate studies, yet the findings were that in one study, the unilateral montage in V1 produced beneficial effects on performance, while it did not create beneficial effects on performance in the study on a motion coherence task when v5 was stimulated (van der Groen and Wenderoth, 2016; van der Groen et al., 2018). These discrepancies support the necessity of further studies scrutinizing a larger variety of stimulation ranges in specific areas, with the idea that these findings might not translate from one paradigm to another.

In the field right now, the primary view is that tRNS operates as an optimal noise inducer, adding helpful noise into the system, under the theory of stochastic resonance. The critical point in the literature right now is that we do not know what that “optimal” level is, if, indeed, tRNS does work by adding helpful noise into the system and plays a role in stochastic resonance, which has yet to be decisively determined. While it is true that research has indicated a potential interaction effect between subthreshold stimuli and tRNS intensity as low as .250mA (van der Groen et al., 2018), others have also shown a benefit well above these stimulation intensity levels, such as 1mA, 1.5mA, or higher (van der Groen et al., 2016; Pavan et al., 2019).

With these objectives in mind, we present several aims of Experiment 1 (Occipital tRNS) and Experiment 2 (Parietal tRNS, control study). In both experiments, we will examine the effects of tRNS applied bilaterally to the cortex. In Experiment 1 (the main experiment), participants received hf-tRNS directly to the occipital cortex through bilateral stimulation. In control Experiment 2, participants received hf-tRNS to the parietal cortex. Participants in both experiments underwent the same stimulation paradigm. Stimulation intensities varied across a

range of .250mA - .750mA and a Sham stimulation condition. Participants took part in a 2 alternative forced choice orientation discrimination task of two gabors. The gabors had one of five different contrast intensities, modulated to increase or decrease task difficulty. This will be controlled for by thresholding participants at the fourth level of contrast (12.5), based on their ability to detect the gabor tilted right or left away from vertical 80% of the time, in each visual field. The difficulty of right/left tilt detection will be held constant across contrasts. Thus, performance increments or decrements is a function of contrast intensity, and should be better for levels at the thresholded contrast (12.5) and above, and decrease, with decreasing contrast. The aims of the Experiment 1 (Occipital tRNS stimulation) and Experiment 2 (Parietal tRNS stimulation are threefold:

1) Is task performance modulated by tRNS intensity independently, across contrast intensity, or is there an interaction of these factors, and furthermore does stimulating a cortically distal area (parietal stimulation) potentially involved in neural recruitment by the task also modify task performance or does it act as a reliable control site for occipital paradigms?
2) Does task performance align with the mechanisms predicted by stochastic resonance as advocated by previous studies of transient tRNS?
3) Is cortical excitability measured by phosphene threshold a reliable measure to control for state-dependency, as a measure of individual subjects' variability which can control for potential variances in the data due to individual subject differences?

Here we designed an experiment to explore the same experimental paradigm across a wide range of stimulus and stimulation intensities within the same participant population. We use low-level stimuli based on increasing levels of contrast intensity, which have been shown to expressly increase the neuronal responses in V1 areas in a linear manner (Boynton et al., 1999). This limits potential confounds present in motion-discrimination tasks found in previous studies (Abrahamyan et al., 2011). Additionally, it allows us to discretely investigate a well-studied system and its properties of linearity regarding neural response. If, as proposed by the mechanism of stochastic resonance, subthreshold contrasts are amplified and pushed above the threshold for detection by tRNS, we will see a disruption of a linear increase in performance, resulting in an interaction between the stimulus properties and stimulation properties. Principally, in stochastic resonance, it is proposed that the noise added to the system has

beneficial effects only for subthreshold contrasts, and potentially detrimental effects for supra-threshold contrasts, resulting in an “inverse U” pattern of behavioral accuracy, as outlined in Chapter 1 and discussed regarding properties of TMS in Chapter 2. However, if tRNS is simply additive, we hypothesize that while there might be one intensity that is more beneficial than another, a main effect of tRNS will be present, but this would not be in line with predictions under stochastic resonance. Thus, while we expect an increase of performance with increasing contrast intensity (a main effect of contrast intensity), what we consider a potential proof of a stochastic resonance effect would be an interaction between the two independent variables, most notably an increase of performance accuracy at subthreshold stimulus intensity levels. Furthermore, we propose that individual differences in cortical excitability may play a role in influencing behavioral outcomes. With cortical excitability measures in the occipital field through induction of phosphenes and the determination of individual phosphene thresholds, it is plausible to determine if participants with lower cortical excitability may respond differentially to tRNS than those with medium or high excitability.

Materials and Methods

Participants – Experiment 1: Occipital Stimulation

17 participants (7 male, 10 females, mean age: 24.18 years [SD: ± 3.06 years]) with normal or corrected-to-normal vision participated in the study. Participants reported no history of neurological or psychiatric illness and were not taking any medications for the duration of the experiment. Participants and were also screened for TMS/tES contraindications prior to participation. Participants were selected based on their ability to detect the presence of phosphenes induced by occipital TMS. Before and after each stimulation session, participants completed a form detailing any short-term side-effects, and were screened with the Mini-mental state examination (MMSE; Folstein et al 1975). All participants gave informed consent to participate in the study, which was approved by the ethics committee of the University of Trento.

Participants – Experiment 2: Parietal Stimulation (Control Study)

19 participants (7 males, 12 females), mean age: 25.74 years [SD: ± 5.97 years]). All

participants in Experiment 2 were informed, consented, and screened for neurological/psychiatric illness and stimulation contraindications in the same way as participants in Experiment 1. Participants filled out the same questionnaires to assess before and after short term side effects as participants in Experiment 1. Participants in Experiment 2 differed in that they were not assessed for cortical excitability based on phosphene induction, so participants in this group were not stimulated with TMS, they performed the experiment only with tRNS.

Experimental Setup

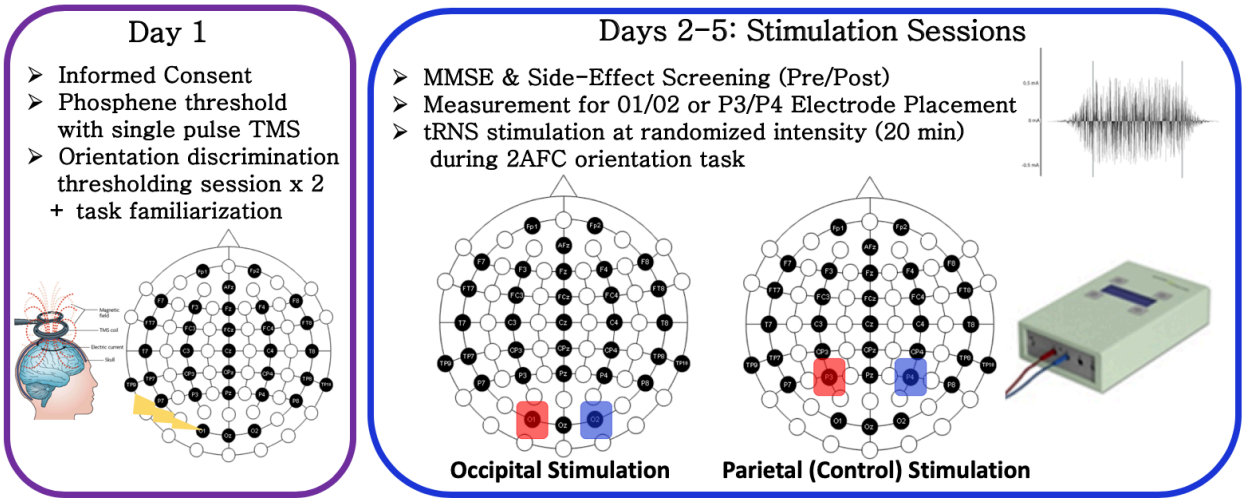


Figure 1. Experimental protocol. On day 1 participants participants in Experiment 1 and 2 were first screened for contraindications of stimulation. Following participant consent, participants were then thresholded for the orientation discrimination task. For Experiment 1 ***only*** (**Occipital experiment**), participants were also thresholded for individual phosphene levels with single pulse TMS. On days 2-5, in both experiments, participants either received Occipital Hf-tRNS or Parietal hf-tRNS for the duration of the experimental session while performing the orientation discrimination task. Presentation of contrast intensities (1.56, 3.125, 6.25, 12.5, 25). were randomized across each block. The order of hf-tRNS intensity (Sham, .250, .500, .750.) stimulation delivery for each day was randomized prior to subject participation to prevent order confounds of stimulation intensity.

Task Setup

Experiment 1: Occipital Stimulation

The experiment was run in a dimly lit room using a chin-rest to ensure participants' heads would remain as still as possible at a distance of 57 cm from the monitor throughout the duration of each run. Stimuli were presented on a 22 inch (~55.8 cm) Samsung 2233RZ LCD monitor with the screen set to a resolution of 1680 x 1050 at a refresh rate of 120 Hz. The experiment was presented via Matlab R2010B (The Mathworks, Massachusetts) and ASF: A Simple

Framework, an add-on to the Psychophysics toolbox on a Dell desktop computer running Windows 7.

Experiment 2: Parietal Stimulation (Control Study)

The task set-up of Experiment 2 was identical to Experiment 1.

TMS Setup

Experiment 1: Occipital Stimulation

Single pulse TMS was delivered to the left visual cortex (V1) using a 70 mm figure-8-coil connected to a Magstim Rapid2 stimulator (Magstim Co., UK). Previously acquired magnetic resonance image scans were used in co-registering the head position of each subject in space in order to determine and average point of stimulation for each subject. Scans of each participant were high-resolution, T1-weighted (magnetization-prepared rapid gradient echo sequence (MPRAGE) with 176 slices, in-plane resolution 256 × 224, 1 mm isotropic voxels, using Generalized Autocalibrating Partially Parallel Acquisition (GRAPPA) with acceleration factor of 2, time to repeat (TR) = 2700 ms, time to echo (TE) = 4.18 ms, time to inversion (TI) = 1020 ms, flip angle = 7°) with a MedSpec 4-T head scanner (Bruker BioSpin, Ettlingen, Germany) equipped with an 8-channel array head coil (USA Instruments, Aurora, Ohio, USA).

Experiment 2: Parietal Stimulation

The main goal of Experiment 2 was to determine that the input level of stimulation (electrode placement) by tRNS in a cortically different location could not produce the same effects, ensuring our protocol was relevant to results obtained from the occipital lobe stimulation only.

Phosphene Session

To determine a measure of cortical excitability, we measured the phosphene threshold for each subject. This was done by stimulating area left V1 corresponding to the right visual field. Each participant's LV1 was determined by moving the coil over the left occipital area until a phosphene was reported at least 3 out of 5 times, similar to the method used to measure MEPs thresholds in the motor cortex (Borsook et al., 2012). We used a standard figure-of-eight, 70mm Magstim coil with the handle of the TMS coil pointed leftward tangentially to the

skull. Pulses were applied intermittently with a separation of at least 6 seconds in between each pulse. We started stimulation at a point marked on a cloth swimmers cap at a point of 10% above the inion (OZ) and 5% total circumference to the left of OZ (approximate position of O1) of individual head measurements based on the 10-20 EEG Electrode Placement system (Jasper, 1958). We continued with discrete movements in a 4cm by 4cm grid until a reliable phosphene location was determined. This procedure took place in a dimly lit room, while subjects fixated on the screen with a mock set-up of the experiment (darkened to prevent additional light), and indicated the presence of phosphenes (Yes/No), their location and size (using the mouse on the screen) and luminosity (scale of 1-10, 10 extremely bright). Average stimulation intensity to induce a phosphene for each participant was determined during this session by incrementally increasing TMS intensity from a starting value of 50% of the maximum stimulator output. We increased stimulator output by 1% until subjects consistently reported visual phosphenes in the manner indicated above (3 of 5 pulses created phosphene detection). This location was marked on a reconstructed 3D MRI scan with the Brainsight neuronavigation system (Rogue Research Inc., Montreal Canada), (see **Fig. 2)**. Average maximum stimulation intensity across participants was 63.12% maximum stimulation output (SD: ± 6.86%, range = 50-73%). The phosphene intensities for each subject were recorded to determine if participants' cortical excitability may predict behavioral impact of the tRNS.

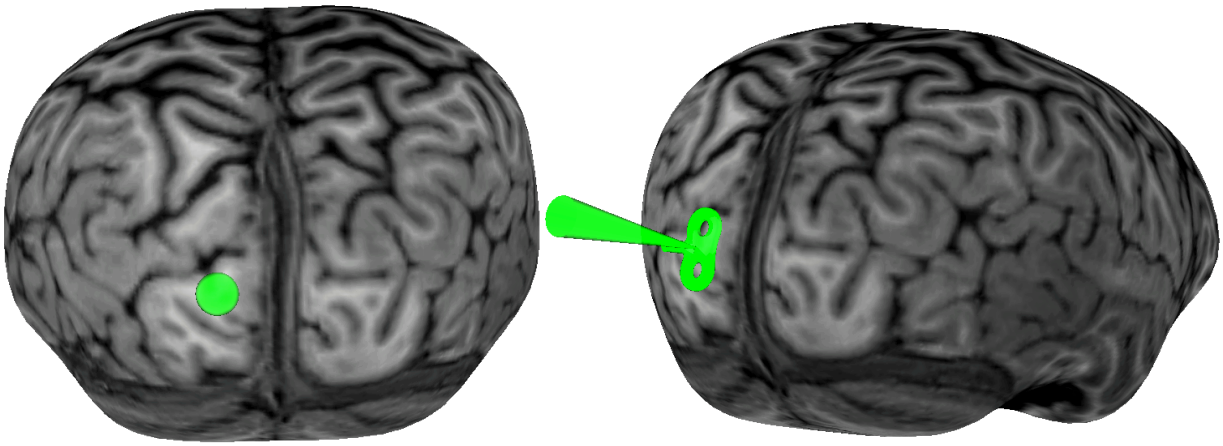


Figure 2. Average location of stimulation point on a representative subject. Average Talairach coordinates (Talairach & Tournoux, 1989) yielded an approximate stimulation point on the cortex at X= -12.28 (SD=4.67), Y= -99.9 (SD=3.80), Z= -4.18 (SD=11.04), (MNI Coordinates: X=-11.68 (SD=4.98), Y=-104.90 (SD=4.62), Z=.20(12.06), which corresponds to area LVF V1.

Psychophysics Session

Experiment 1: Occipital Stimulation

Participants completed a minimum of 2 runs of a thresholding procedure based on García-Pérez (2000) in order to determine the minimum tilt away from vertical discriminated with 80% accuracy at a 12.5% level of contrast. The first of these sessions was used to familiarize the participant with the task, while the second session value was used to obtain a more accurate orientation threshold. If the thresholding session resulted in values that were more than 3 points away from the initial value, a third session was completed. This thresholding session was then completed each day of experimental sessions that followed, prior to tRNS stimulation. This was deemed as a way to determine if participants performance at threshold increased or decreased, and to adjust the difficulty of the task accordingly. It is now well-known that tRNS can effectively promote learning, especially in repeated applications (Fertonani et al., 2011). Therefore, re-thresholding was implemented in our design in order to prevent a ceiling effect confound and ensure the task remained difficult between experimental sessions. This threshold was thus performed each day, and the lowest obtained threshold up to that point (even if it was obtained on a different day) was used to prevent the task being easier from one stimulation session to the next.

Experimental Sessions

Experiment 1: Occipital Stimulation

Participants took part in a 2 alternative forced choice (2AFC) orientation discrimination task over 4 different days, one for each different stimulation intensity of tRNS (including sham) (**see Fig. 3**). hf-tRNS (101-640Hz) was delivered by NeuroConn DC Stimulator-Plus (NeuroConn GmbH, Ilmenau, Germany), through two 5x7 ($35cm^2$) standard electrodes uniformly covered with Ten20 paste (Weaver and Company, Aurora, USA). Electrodes were applied bilaterally to position O1 and O2 as based on the international 10-20 system (Jasper, 1958), (see **Fig. 1**). Measurements were marked on the participants scalp with washable marker through a cloth cap at the beginning of each session so that stimulation point was visible on the scalp and allowed accurate centralized placement of the electrodes. Non-conductive rubber bands were used to secure electrodes in place and help in impedance reduction.

Subjects were blind to stimulation condition, and the machine was placed behind the subjects so that they could not see the stimulator screen. Stimulation was applied for the duration of the task (20 min, fade in/fade out 8 seconds, 0mA offset). Impedance was measured before the start of each stimulation session for each subject, ensuring a level below 15Ω before stimulation was commenced. During sham condition, the machine was turned on, measurements of electrodes took place as normal, impedance levels were checked, and stimulation was applied for 15 seconds before being turned off, to replicate as closely as possible true stimulation sessions and to ensure adequate subject blinding. Each stimulation session was separated by a minimum of 24 hours to reduce any potential crossover effects of different stimulation intensities, and order was randomized to prevent order effects.

Subjects were told to maintain fixation for the duration of each trial on a centralized fixation dot. Each trial began with a fixation dot, followed by the presentation of two tilted peripheral Gabors (diameter = 4.98 degrees of visual angle [DVA]; see **Fig.3)**, the centers of which were presented simultaneously 5.01 DVA to the left and right of (and 2.51 DVA below) a central fixation dot (diameter=0.5 DVA) for a period of 30ms, to afford the same task set up as the main TMS experiment.

The Gabors were presented at 5 different contrast levels tilted left or right away from the vertical under 4 different tRNS intensities, resulting in a factorial structure of 2 (left / right) × 5 (1.56% / 3.12% / 6.25% / 12.5% / 25%) × 2 (oriented left / oriented right) × 4 (Sham, .250mA, .500mA, .750 mA). After the Gabors disappeared, a horizontal line appeared to the left or right of the fixation dot, indicating on which of the two previously presented Gabors a subject should make an orientation judgment. The fixation dot turning green signaled to the participant they should respond. Response collection lasted for a total of 3 seconds and was terminated if the participant made a response (see **Fig 3**). Participants were instructed to make the orientation judgment with the index fingers of the left and right hand using a mouse (left finger for leftward orientation, right finger for rightward orientation). If a response was not made within the 3 seconds time window, the response was marked as incorrect and the experiment proceeded to the next trial.

Stimulation of different intensities took place on different days separated by a minimum of 24 hours to prevent crossover effects. Participants were re-thresholded for the orientation discrimination task each stimulation session to prevent learning effects. Stimulation lasted a

total of 20 min.

Experiment 2: Parietal Stimulation

All experimental sessions of Experiment 2 were identical to experimental sessions of Experiment 1 (Occipital Stimulation), with the exception that the two 5x7 standard electrodes with Ten20 paste were applied to position P3 and P4 as identified by individual measurements of each participant for each session, according to the 10-20 system of electrode placement (see **Fig. 1**).

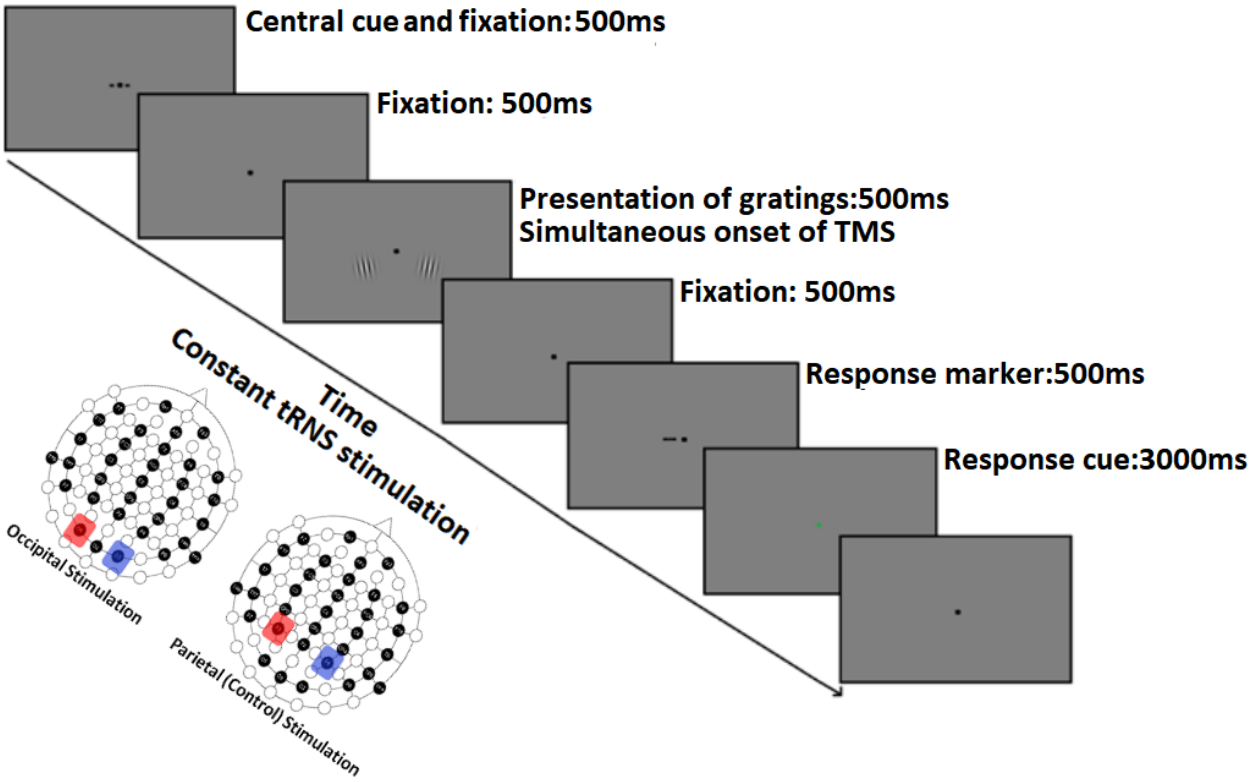


Figure 3. Example trial of the orientation discrimination task. After perceiving two tilted Gabor patches, participants were cued toward which stimulus they should respond. After a short delay, the fixation dot turned green, signaling participants to make the response. Participant responses terminated the trial. All contrast intensities were presented in randomized order each day, with a total of 96 trials per condition. Each experimental session used only one level of tRNS stimulation intensity (Sham, .250, .500, .750). The experiment took place in a dimly lit room, and participants maintained fixation for the duration of the session, while their head was placed in a head-rest to prevent movement.

Data Analysis

All behavioral and statistical analyses were performed using MATLAB and SPSS. Mauchley's test of Sphericity was performed, and when violated, Greenhouse-Geisser corrections of degrees of freedom and resultant p-values were reported accordingly. All values are corrected for Bonferroni multiple comparisons.

Behavioral Performance

Experiment 1 and 2:

Obtained percent correct data were collapsed over left/right tilt orientation of the Gabor stimulus. The percent correct data for the tRNS studies we examine in this chapter were then combined across left and right visual fields as, contrary to unilateral stimulation by TMS in the left occipital lobe in the experiment in **Chapter 2**, electrodes in the tRNS studies conducted here are applied bilaterally (occipital and parietal), and predict a global response difference irrespective of visual field. This is evidenced by non-field specific results of bilateral hemispheric stimulation as applied in a similar paradigm by other research groups exploring this paradigm, (van der Groen et al., 2018). Furthermore, tRNS is not polarity specific. This resulted in subjects participating in a total of 20 different conditions, with 96 trials each. The resultant percent correct data were normalized using an arcsine transformation as in previous analysis of TMS data in **Chapter 2**, and submitted to a 2-way repeated measures ANOVA computed with factors tRNS intensity x contrast level to determine if performance differed according to stimulation intensity, contrast intensity, or an interaction of the two independent factors. Post-hoc 2x5 ANOVAs comparing performance at each TMS intensity across contrast levels were computed to further elucidate any main effects or interactions. Paired t-tests using corrections for multiple comparisons were computed where appropriate based on the results of these tests, comparing relative performance in the Sham to tRNS condition/s to determine drivers of interactions or main effects.

Reaction Time Performance

Reaction times for all tRNS intensities by contrast intensities were submitted to a 2-way RM ANOVA to determine whether reaction time influenced, or was influenced by either the main independent variables or an interaction between the two. In the case of significant results

in the data analyses, planned comparisons between significant levels will be scrutinized to determine if the effect was truly due to tRNS manipulation, or if the results can be explained by differences by the time it took participants to make a decision. This allowed us to determine if difficult trials were considered more carefully than easier trials when a behavioral performance boost or reduction was present, to elucidate the possible confound of a speed-accuracy trade off.

Effect of Cortical Excitability on Performance

We measured each subject's cortical excitability in the occipital lobe by measuring the max stimulator output it took to induce a phosphene. This allowed us to consider the possibility that subjects' differences in cortical excitability may result in differential effects of tRNS intensity, and act as a potential future marker that will allow us in the future to predetermine which tRNS intensity is most beneficial for participants based on individualized methods. Phosphene threshold measures recorded prior to the start of the experimental sessions were divided into three equal groups (range: 50-73% max stimulator output; Mean= 63.11, SD=6.86). The transformed phosphene range values consisted of low (50-60), medium (61-66), and high threshold (67-73) of the max stimulator output, divided into equivalent groups (low=5, medium=6, high=6) per condition. We reanalyzed the performance data to account for PT threshold (low, medium, high) in a RM mixed model with contrast intensity and tRNS intensity as within-subjects variables, and PT threshold as a between subjects' factor.

Results: Experiment 1 (Occipital Stimulation)

Behavioral Results

The main 2-way ANOVA between tRNS levels and contrast Intensity showed no main effect of tRNS ($F_{(3, 48)}$=.208, p=.891) and a significant main effect of contrast Intensity ($F_{(4, 64)}$=290.577, p<.000). Crucially, participants' performance appeared affected by differing tRNS intensities only at specific contrast levels ($F_{(12,192)}$=1.906, p=.036), (see **Fig. 4**)

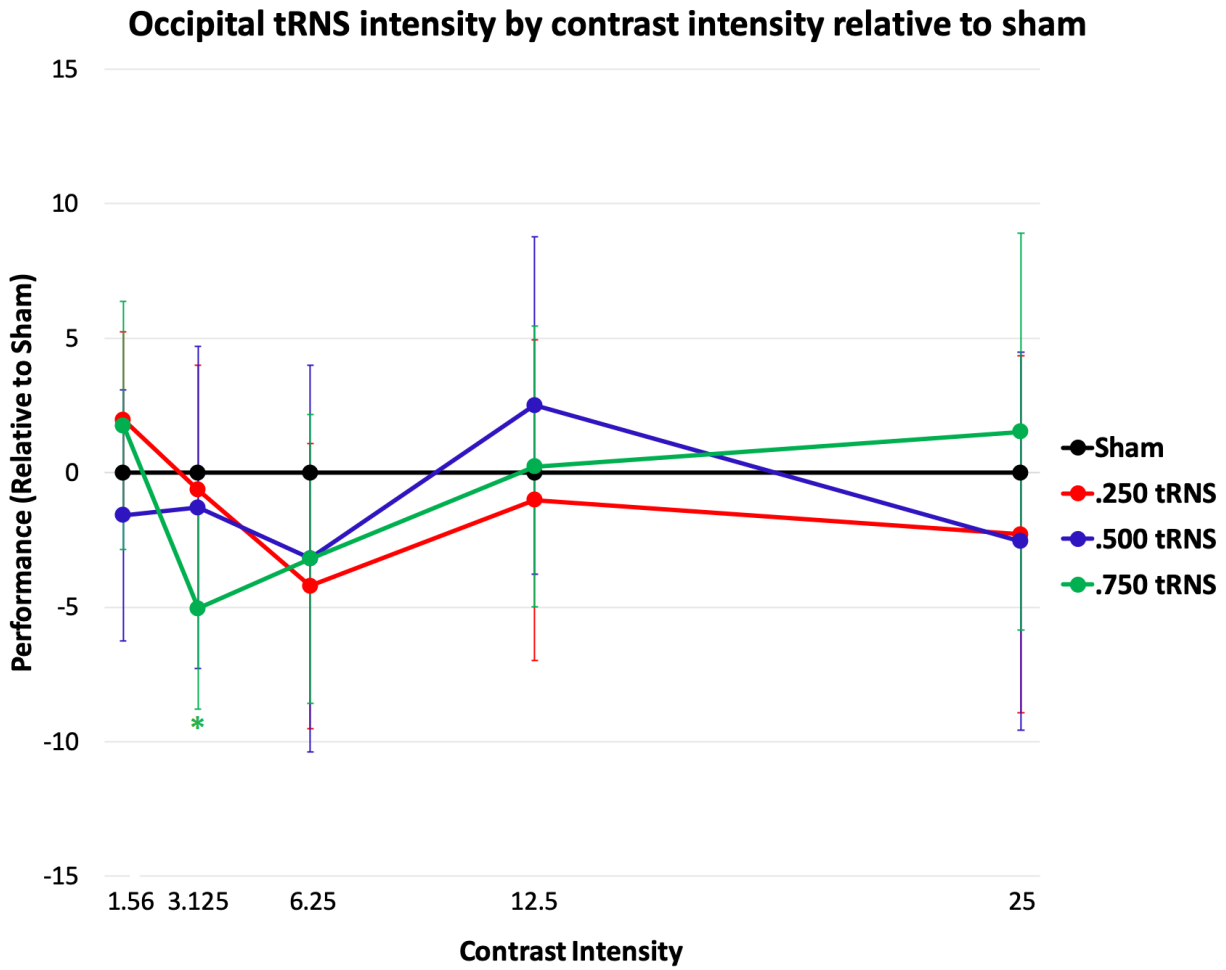


Figure 4. Normalized percent correct data were subtracted from sham condition for each tRNS intensity (.250, .500, .750) to obtain delta values of performance benefit or reduction, indicated in this graph. This shows the relative Δ value across all tRNS intensities by contrast, resulting in a significant performance difference only for tRNS .750 at contrast 3.125, t(16)=2.850, p=.012. All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.

2x5 ANOVA RM analyses

2x5 ANOVAs were computed to determine the likely driver of the interaction effect seen in the main analysis. All analyses showed a main effect of contrast intensity at each level of tRNS stimulation compared to sham (.250mA tRNS: ($F_{(4,64)}$ =163.978, p<.000); 500mA tRNS: $F_{(4,64)}$=223.436, p< .000); 750mA tRNS: ($F_{(2.738,43.814)}$=196.389, p<.000). Sham compared to .250mA tRNS showed no main effect of tRNS ($F_{(1,16)}$=.460, p=.507), or significant interaction between tRNS and contrast intensity ($F_{(4, 64)}$= 1.420, p=.238). Similarly, comparing Sham tRNS TO .500mA tRNS showed no main effect of tRNS ($F_{(1,16)}$= .281, p=.603), and no significant interaction effect between tRNS and contrast intensity (F(4,64) = 1.119, p=.356). However,

when comparing Sham tRNS to .750mA tRNS, we found no main effect of tRNS intensity ($F(1,16)=.259, .618$), but the analysis did show a trend towards significance for the interaction between tRNS intensity and contrast intensity ($F(4,64)=2.407$, $p=.058$), the likely driver of the interaction in the main ANOVA, that indicated a decrease only at specific subthreshold contrast levels for the .750mA tRNS condition compared to sham.

Due to the effects shown in the 2x5 ANOVAs we computed post-hoc paired t-tests comparing Sham to tRNS intensities across contrasts above and below the contrast threshold for tRNS at .750mA, with an adjusted p-value of .0125 for multiple comparisons. Results of the paired t-test for contrast level 3.125, showed that indeed, at tRNS level .750mA at stimulation caused a significant performance decrement (Mean = 71.57, SD=8.77) compared to Sham (Mean=76.61, SD=6.95; $t_{(16)}=2.850$, $p=.012$), but no other contrasts (1.56, 3.125, 25) were statistically significant. These comparisons show that tRNS negatively impacted subjects' performance by 5.03 percent (SD=7.28), only for one level of tRNS (.750mA) relative to Sham performance.

Reaction Time Analysis

Reaction time analyses were performed across all tRNS intensities by contrast intensities to determine if reaction times could predict the differences seen in the performance data above. The RM ANOVA showed that reaction time significantly decreased with increasing contrast intensity ($F_{(1.308,\ 20.920)}=11.981$, $p=.001$), indicating that as contrast of the visual stimuli increased, participants responded faster. RT performance did not differ as a function of tRNS intensity ($F_{(3,48)}=.278$, $p=.841$), and there was no significant interaction effect of RT performance for tRNS intensity by contrast intensity ($F_{(3.616,\ 57.864)}=.942$, $p=.506$). Pairwise comparisons indicated that performance was faster for contrast 3.125 (Mean=321.09ms, SE=44.96), contrast 6.25 (Mean=310.00ms, SE=41.67ms), contrast 12.5 (Mean=306.44, SE=43.87ms), and contrast 25 (Mean=308.79ms, SE=44.54ms), compared to contrast 1.56 (Mean=370.46ms, SE=45.42ms). Differences between all other contrasts were non-significant (see **Fig. 5**).

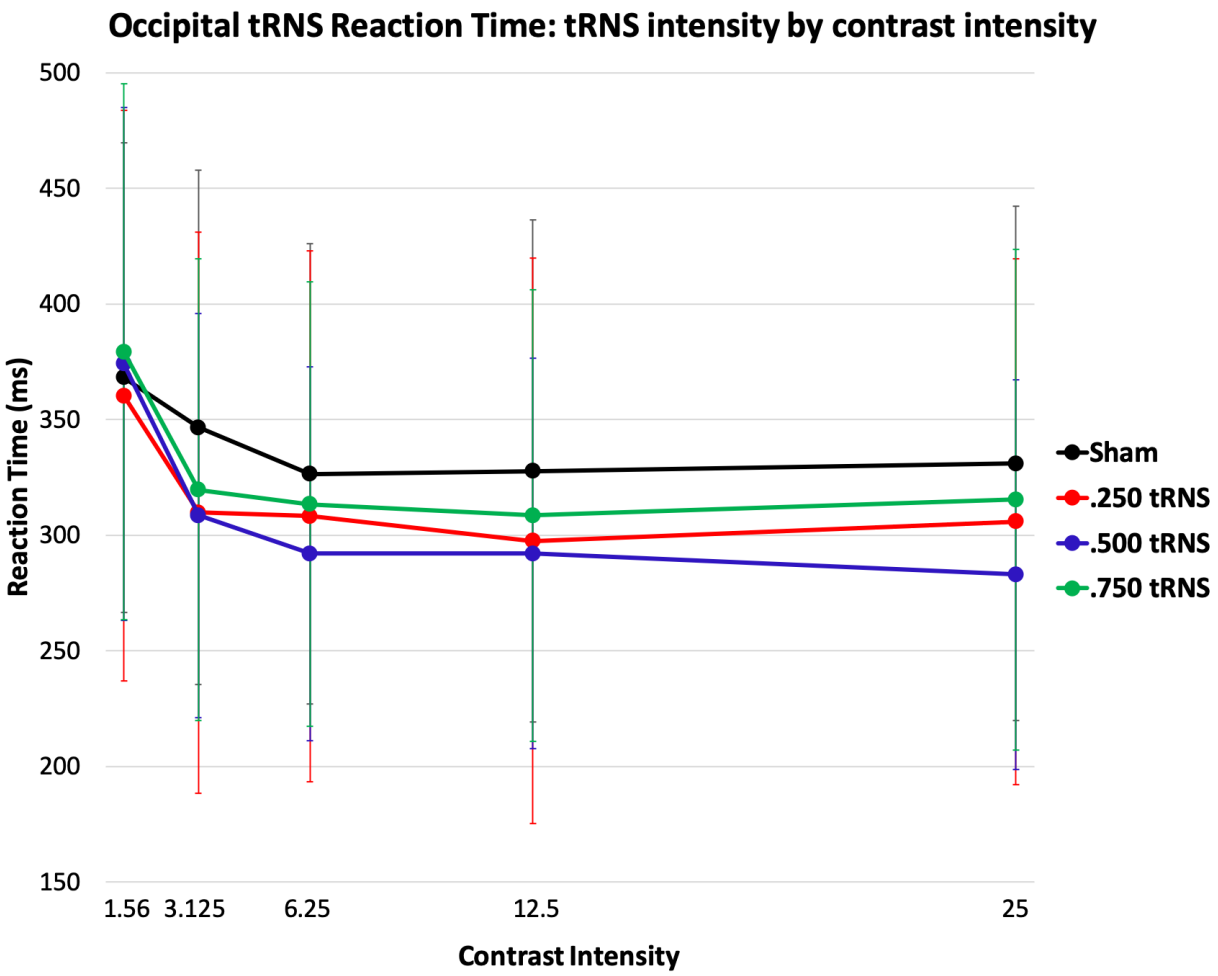


Figure 5. Reaction time analysis of Occipital tRNS stimulation. RT were averaged across each contrast intensity (1.56, 3.125, 6.25, 12.5, 25) for each participant, at each tRNS intensity (Sham, .250, .500, .750), and submitted to the 2way RM ANOVA. This graph shows the results of the effects of stimulation at each contrast level, and did not differ by tRNS intensity, nor is there any interaction between contrast intensity and tRNS intensity, but did significantly differ as a function of contrast intensity, such that subjects were significantly faster at responding for all contrasts compared to RT at contrast 1.56, regardless of tRNS intensity. All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.

Effects of Cortical Excitability in a tRNS paradigm

The 2-way RM mixed model with PT threshold as a between subjects factor showed similar results to the performance data above. Namely, it revealed a significant effect of contrast intensity ($F_{(4,56)}$=337.306, p<.000), and no main effect of tRNS ($F_{(3,42)}$=1.48, p= .930). There was, as in the original analysis, a significant interaction effect of tRNS by contrast intensity ($F_{(12,168)}$=1.936, p=.033). Similarly, we found an interaction between contrast intensity and Phosphene Group ($F_{(8,56)}$=128.354, p=.025). The three way interaction between tRNS, contrast

intensity, and phosphene threshold was however insignificant ($F_{(24,168)}$=39.126, p=1.153, p=2.93), (see **Fig. 6**).

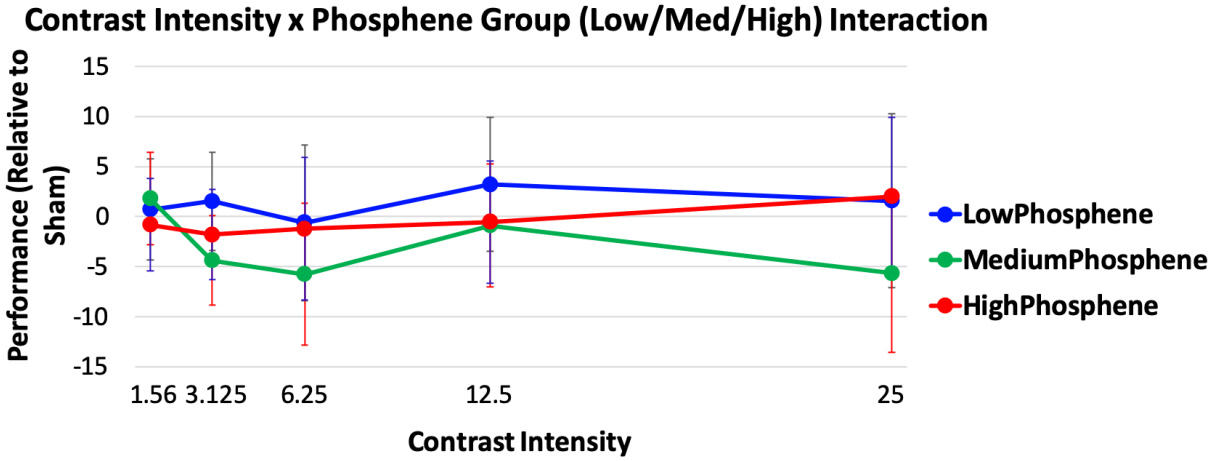
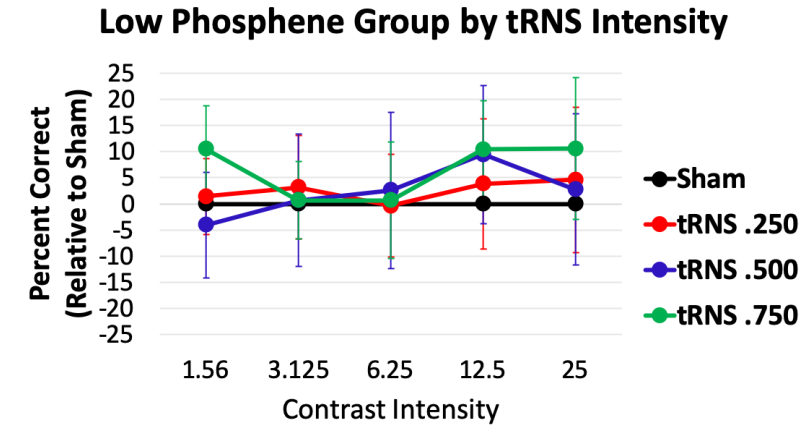
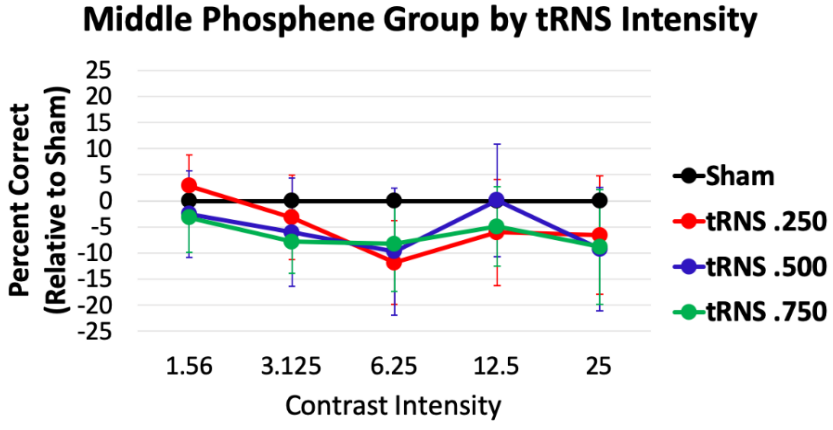


Figure 6a. The interaction effect of contrast intensity by phosphene group (Low, Medium, High), showed that participants behavioral performance was modulated differentially according to varying levels of cortical excitability, as measured by percent stimulator output required to produce phosphenes in the occipital lobe. Namely, for the Medium group, participants performance appeared to worsen at specific subthreshold, as well as suprathreshold levels of stimulation, but not at the lowest contrast, or at sham contrast. All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.





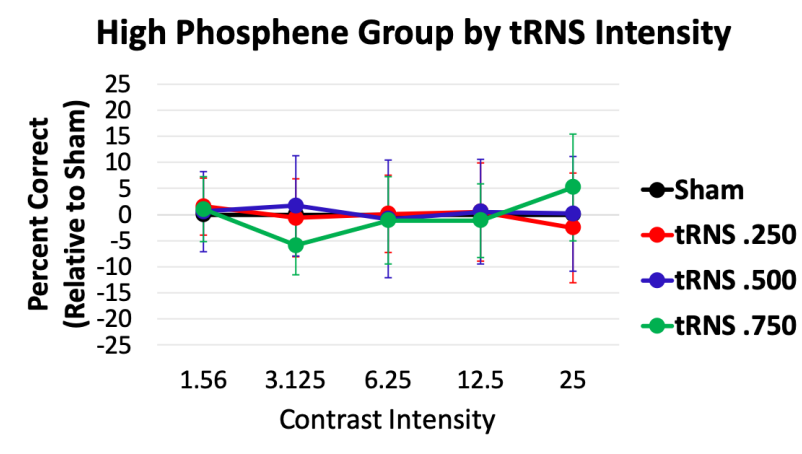


Figure 6b. Phosphene Group (Low, Medium, High) by tRNS intensity. There was no significant interaction by tRNS and Phosphene group, such that no one tRNS stimulation intensity was more or less beneficial for one phosphene group over the other. Therefore, we cannot at this time make conclusive results regarding cortical excitability and optimal tRNS parameters. All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.

Results: Experiment 2 (Parietal Stimulation)

Behavioral Results

The main 2-way ANOVA between tRNS levels and contrast Intensity showed a significant main effect of contrast Intensity ($F_{(2.316, 41.683)}$=163.414, p < .000), no main effect of tRNS ($F_{(3, 54)}$=.454, p=.716), and no significant interaction effect between tRNS intensity and contrast ($F_{(12, 216)}$=1.135, p=.333), (see **Fig. 7**). Post hoc tests were not computed due to non-significance of main effects or interactions.

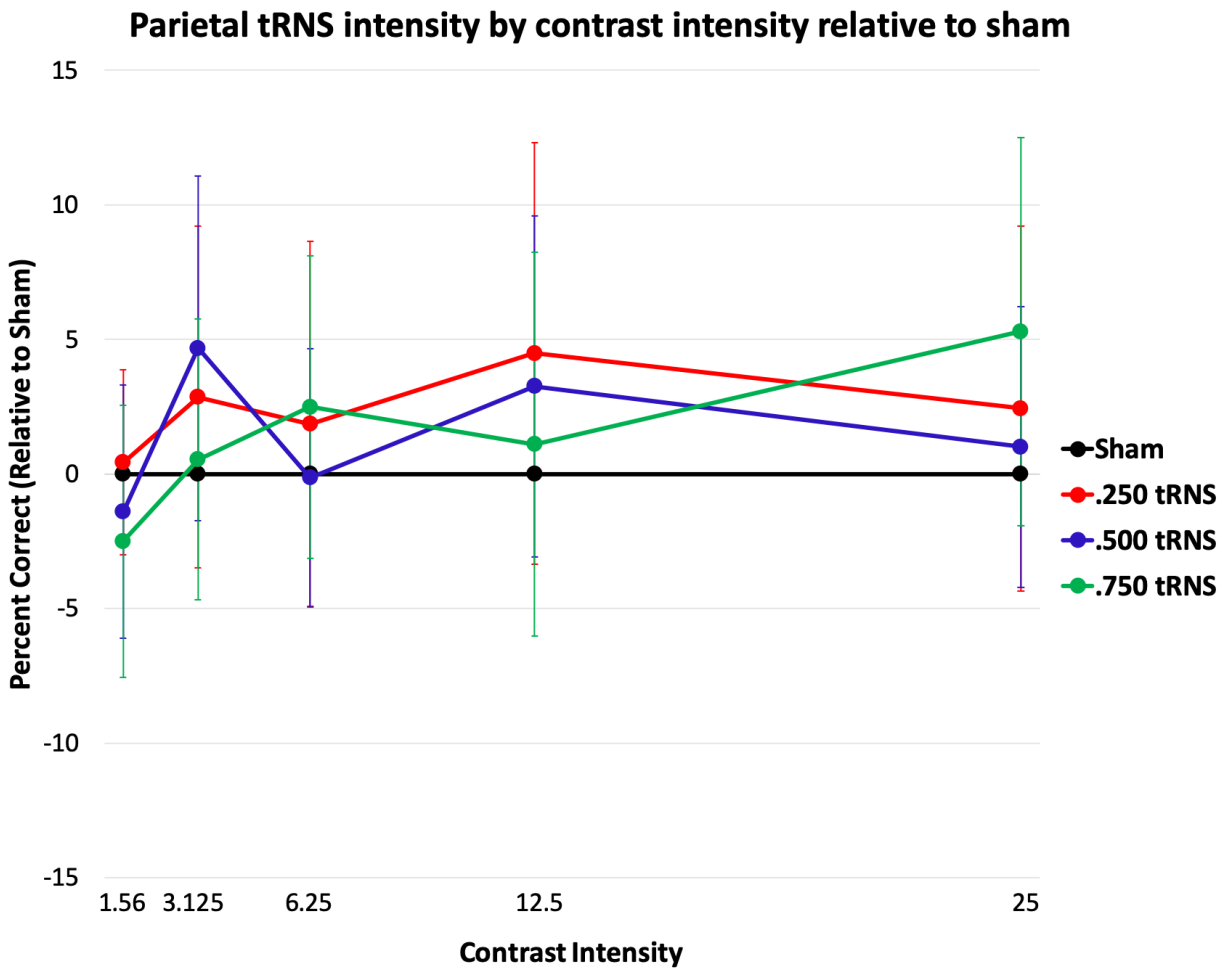


Figure 7. Normalized percent correct data were subtracted from sham condition for each tRNS intensity (.250, .500, .750) to obtain delta values of performance benefit or reduction, indicated in this graph. This shows the relative Δ value across all tRNS intensities by contrast. All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.

Reaction Time Analysis

Reaction times were averaged across each contrast intensity (1.56, 3.125, 6.25, 12.5, 25) for each participant, at each tRNS intensity (Sham, .250, .500, .750), and submitted to the 2way RM ANOVA (see **Fig. 8**). Results of the ANOVA showed that reaction time significantly decreased with increasing contrast intensity ($F_{(1.419,\ 25.533)}$=18.884, p<.000), indicating that as contrast of the visual stimuli increased, participants responded faster. Mean RT performance did not differ as a function of tRNS intensity ($F_{(3,54)}$=.275, p=.843), and there was no significant interaction effect of RT performance for tRNS intensity by contrast intensity ($F_{(4.155,\ 74.790)}$ =.692, p=.758). Pairwise comparisons indicated that performance was faster for contrast 3.125

(Mean=299.07ms, SE=34.37), contrast 6.25 (Mean=288.46ms, SE=30.59ms), contrast 12.5 (Mean=278.57, SE=30.19ms), and contrast 25 (Mean=275.18ms, SE=31.58ms), compared to contrast 1.56 (Mean=347.36ms, SE=41.06ms). The only other pairwise comparison that was statistically significant showed that participants also responded significantly slower at contrast 3.125 than to the highest-level contrast (25).

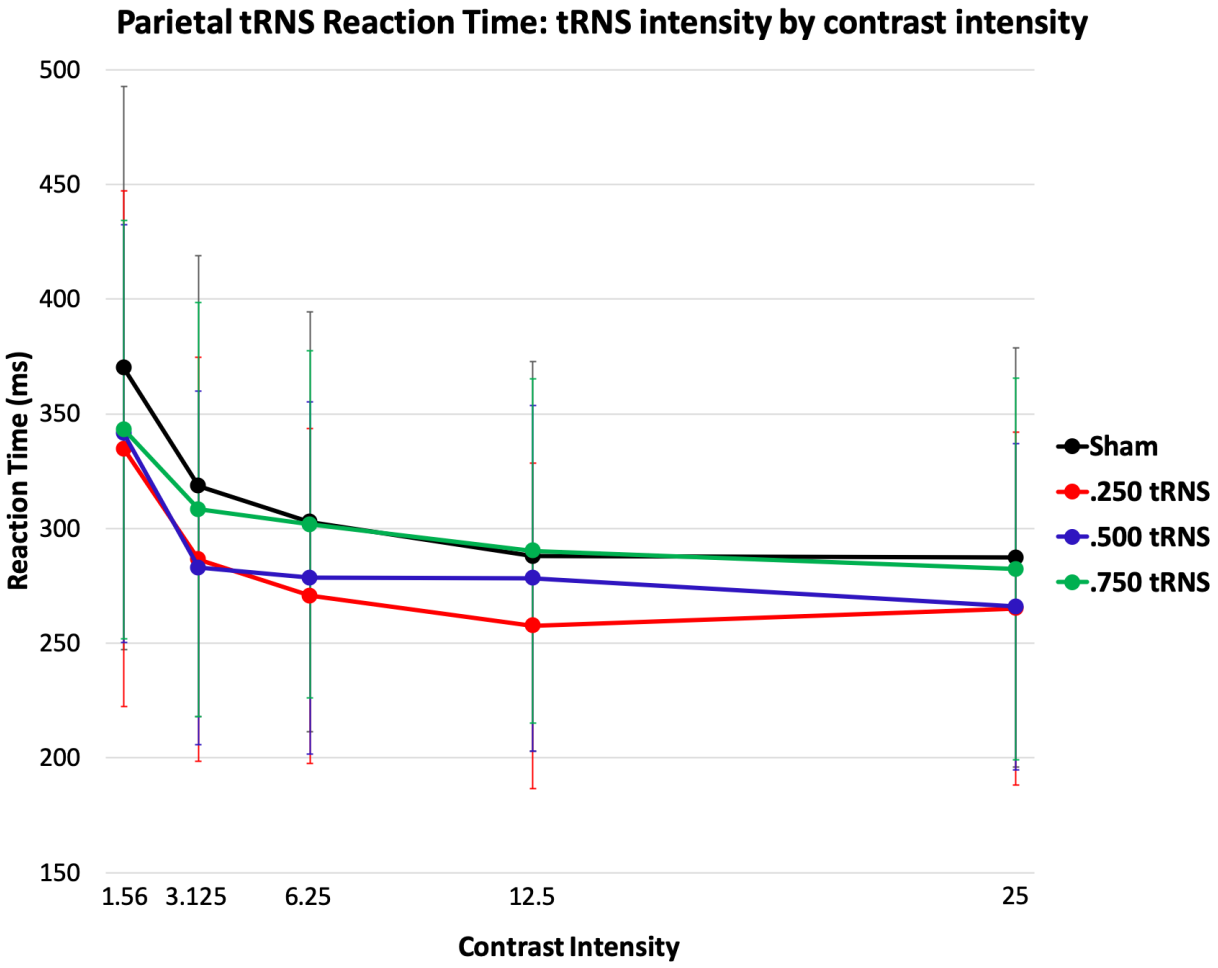


Figure 8. Reaction time analysis of Parietal tRNS stimulation at each contrast level. This graph shows that reaction time did not differ by tRNS intensity, nor is there any interaction between reaction times for contrast intensity and tRNS intensity, but did significantly differ as a function of contrast intensity, such that subjects were significantly faster at responding for all contrasts compared to contrast 1.56. Pairwise comparisons corrected with the Bonferoni correction for multiple comparisons noted only one other significant difference, that RT performance was significantly slower at the second contrast level (3.125) compared to the highest contrast level (25). All error bars indicate 95% confidence intervals for each contrast intensity at each tRNS intensity.

Discussion

Occipital Stimulation

In the primary study of interest on tRNS in the occipital lobe, we employed a multi-session experimental protocol that allowed us to examine the effects of different tRNS intensities (Sham, .250mA, .500mA, .750mA) across a wide range of contrast intensities that were either subthreshold (1.56, 3.125, 6.25), at threshold (12.5), or suprathreshold (25). This was done in order to delineate how this method may influence performance independently, as a function of tRNS intensity, or, as proposed under the theory of stochastic resonance, as an interaction of the stimuli properties (contrast intensity) and tRNS intensity. Our results showed that at subthreshold contrast level 3.125, participants performance significantly decreased relative to Sham, and these effects could not be accounted for by analysis of reaction time differences.

We expected the primary results of the RM ANOVA based solely on behavioral results to indicate, a performance improvement at one of these subthreshold levels to interact with stimulation intensity of tRNS (van der Groen and Wenderoth, 2016; van der Groen et al., 2018), boosting performance, by pushing subthreshold stimuli above the level for conscious detection with the addition of “helpful noise”. What our experiment demonstrated was indeed an interaction, but with an effect in the opposite direction proposed under the hypothesis, and to the results found in the TMS experiment (Chapter 2), as well as those previously discussed in the literature (van der Groen and Wenderoth, 2016; van der Groen et al., 2018; Pavan et al., 2019). We explored the effects of reaction time data to control for participants responding in a way that indicated a speed accuracy trade-off. This was not evident from our results, which indicated that subjects performed across all conditions of the task at a similar rate according, regardless of intensity of the session. This finding is in the opposite direction predicted by the theory of stochastic resonance, as we find in our study that noise added to the cortex significantly impaired detection (performance) at subthreshold levels instead of a performance benefit. When accounting for reaction time differences, the only significant differences noted by the RM analysis of the reaction time data showed that participants spent more time reacting to subthreshold stimuli at contrast level 1.56 compared to any of the other contrast levels, and

there were no significant differences in reaction time performance between any other levels. Therefore, they responded similarly to each of the other contrasts, with the only difference being their performance in accuracy at one of these levels. Therefore, this indicates that the results were a true effect of stimulation interaction with stimulus contrast, making it consequentially implausible that effects were due to a speed-accuracy trade-off.

Interestingly, recent literature has begun to explore the effects of the short-term transient effects of tRNS in relation to behavior, but conclusions have been apparently contradictory, however, what is interesting is that response of the cortex to stimulation seems to strongly depend and stimuli features and stimulation intensities as well as on cortical site of stimulation (van der Groen and Wenderoth, 2016; van der Groen et al., 2018; Pavan et al., 2019). Similarly, while some tasks explore results between groups, others focus on how the technique differs across multiple factors within the same subject, and therefore very different conclusions can be drawn, based on the fact that we know that individual differences and susceptibility to stimulation of different intensities can have very different effects from subject to subject in other forms of brain stimulation (Siebner et al 2009, Wagner et al 2007, Janssen et al. 2014; Stewart 2001, Sandrini et al. 2011, Boroojerdi et al., 2002). The main goal of the current study was to determine the transient effect of tRNS under a variety of conditions both related to the stimulus properties associated with visual recruitment (contrast intensity) as well as stimulation properties related to relative intensity applied to the cortex. Secondly, we aimed to determine if these potential effects vary and can be explained by the properties of stochastic resonance (Moss et al., 2004). Particularly, we purposely employed the use of low-level visual stimuli (Gabors) to which neurons in V1 are typically sensitive, with the goal to specifically characterize local effects of the tRNS mechanism on the cortical area, whereby in normal circumstances we see a linear increase of performance with increasing contrast intensity (Boynton et al., 1999). Thereby, we can see how tRNS influences the modification (by amplifying or reducing) neural activity that is generally linear. Simplified paradigms such as these are essential to basic research, allowing future researchers and clinicians to better understand the repercussions of one technique, paradigm, or montage over another. While tRNS is currently starting to be widely used in both theoretical paradigms and clinical paradigms, we still know very little regarding its effects on the system.

We further divided our subjects in three groups based on their phosphene thresholds based off of literature that proposed differential results due to levels of cortical excitability and the balance between excitation and inhibition (Krause et al., 2013), and we proposed that performance results might differ for participants with low, medium, or high phosphene thresholds. Specifically, we proposed that this could be a mitigating factor that may explain potentially differential results across these groups, modifying the overall effect of stimulation intensity. Critically, we hypothesized that it could be the basis of contradictory findings of previous studies, since others have not implemented a way to measure the state of the brain prior to stimulation in tRNS studies. We therefore attempted to control for this factor by measuring each subject's cortical excitability in the occipital lobe (level of max stimulator output that resulted in consistent phosphene production) at the start of the experiment (Krause et al., 2014). We used phosphene threshold as a between subject factor in a mixed model GLM to determine potential differences based on low, medium, or high cortical excitability. However, we found that cortical excitability did not predict the impact of tRNS on behavior. There was however, a significant interaction effect of cortical excitability level and perception at different contrast intensities. In particular, while the "Low" and "High" phosphene groups did not seem to differ much in their performance relative to sham, the "Medium" phosphene group showed similar results to "Low" and "High" phosphene groups at both the lowest contrast (1.56) and threshold, but seemed to perform worse at both other subthreshold contrasts (3.125; 6.25), and the suprathreshold contrast (25).

The work by van der Groen et al, in 2018, in which they measured performance on a motion-coherence task with a similar range of stimulation frequencies to the frequencies used in our own paradigm (Sham, .250mA, .375 mA, .500 mA, and .750 mA), found effects echoing a likely effect under the stochastic resonance principle, mainly, an increase of performance at .250mA (and .375mA that did not survive correction for multiple comparisons, but was nonetheless, subthreshold). This performance seemed stochastic in nature as it crucially only occurred for subjects in the "below threshold" performance group, and followed in the "inverse" U shape of performance, with increasing tRNS intensity after these effects causing performance decrements, concordant with the stochastic resonance model. However, the data from our own study do not corroborate these results. There are several aspects that differ between our study and the van den Groen and colleagues 2018 study that should be considered. Firstly, the

sessions were substantially different in their paradigm. tRNS was applied for only 20 trials, then turned off for 20 trials, and all trials across all intensities took place within one session, with an average of 90 minutes per participant. This could be potentially problematic, because the effects of specific tRNS intensities applied within the same hour and a half could have a potential carry-over effects on one another, and this was our main motivation for separating our sessions across multiple days. Another notable difference was that all of our subjects run through all conditions, whereas in van der Groen et al. (2018), participants were divided in a between groups paradigm. We believe this is an important consideration to take into account when studying the basic effects of a mechanism, as it may work on different subjects in a variety of unpredictable ways. Despite the similarities in our stimulation paradigms (bilateral occipital stimulation and tRNS intensities), several other aspects of this experiment were different from our own, potentially accounting for differences in the results. Since our primary site of interest was V1, we centered our electrodes (an average) of 3.5cm up, and respectively 2.8cm to the left and to the right of the inion, to center the electrode over O1 and O2 as measured by the 10-20 system of electrode placement. While van der Groen and Wenderoth (2018) noted an average electrode placement of 3.5 cm above inion (similar to our placement), but 6.5 centimeters to the left and right of the inion, significantly further out than our electrode placement, and their electrodes were much more focal in intensity delivery, with measurements of 4x4, as opposed to our electrode size of 5x7. As mentioned, the focality can have a significant impact on the strength of electrical signals which reach the cortex, and thus, the differences in results between their paradigm and ours are not directly comparable.

Due to significant differences between our experimental paradigm and that of van der Groen and Wenderoth (2016), it is briefly important to note that while our results are not comparable due to unilateral stimulation in their experiment compared to bilateral stimulation in our experiment. We initially planned our experiment before their experiment was published, however, upon viewing the results of their study, we took this into consideration for the design of our own. Specifically, we were influenced by their findings of significant results due to unilateral stimulation of to the occipital cortex, at intensities below 1mA, an initial interest of ours as well. Because their subthreshold group of subjects showed facilitatory performance enhancement at .500mA and 1mA, with the majority of subthreshold participants benefiting the most at .750mA, we hypothesized that this could be not only an interesting range for us to

pursue, but also elucidate whether these effects could be replicated in a bilateral stimulation experiment. Hence, decided to explore potential significance in this range. Their results were in direct contradiction to the results obtained from our experiment, but, as stated, may be due to a significant difference in the montage set-up between our experiment and theirs, and also significantly differs from the results of the study of van der Groen et al., (2018) noted above. In the 2018 study by van der Groen et al. also controlled for unilateral versus bilateral effects of tRNS, but in this case, did not find results that supported their previous study, yielding non-significant effects for tRNS in a unilateral montage. The designs of the 2016 and 2018 study were however, used different task paradigms. It is interesting that in one case (van der Groen and Wenderoth, 2016) the low-level stimulus paradigm (visual discrimination of Gabors comparable to our experiment) found significant effects with a unilateral montage, while in van der Groen et al. (2018), they used a motion discrimination paradigm, and found no evidence of stochastic resonance with the same montage set-up. Our experiment explores a complimentary combination between the two, and attempted to explore how bilateral stimulation, specifically, contributes to perception low-level stimuli (Gabors), and if this can be attributed to the stochastic resonance theory at the intensities that showed significance in the 2016 study. Interestingly, our results show that bilateral hf-tRNS delivered at an intensity of .750 mA impaired performance for stimuli at a subthreshold contrast. This raises the possibility we either missed any potential enhancement effects because we did not test higher ranges of intensities of stimulation (hence we missed the chances to see the classical U-shaped curve response) or, more likely, the significant decrease in performance at subthreshold contrast 3.125 worked as further disruption to an already extremely noisy, subthreshold, stimulus. Notably, at .750 mA the experiment by Van der Groen et al. (2018) also indicated a decrease in performance for the subthreshold level of 6% coherence, albeit the decrement was not judged statistically significant, and was in contrast to their findings of statistically significant enhancement at the same coherence level with .250mA stimulation, a finding our study did not support.

The results of our experiments should however be extended to explore a broader range of tRNS intensities, as a new study (Pavan et al., 2019) showed that bilateral stimulation in a motion coherence paradigm resulted in significant performance benefits when bilateral tRNS was applied at 1.5mA. Pavan et al. also considered potential cross-over stimulation effects as in our experimental paradigm, but their stimulation intensity levels included a much higher

range. This is of particular interest to extending the parameters of our own study in the future, explored in a within-subjects paradigm, to determine if results are comparable for a v1 task. This is a particularly relevant follow-up considering we implemented intensities in only the lower half of the stimulation range based off of significant results found for these specific stimulation intensities of studies published prior to the implementation of our experiment. As noted, it is not only important to consider the range in which previous studies have found significant effects, especially regarding research of new techniques like tRNS. Unfortunately, due to significant time constraints due to the fact that the within-subject paradigm that we use requires 5 sessions per subject, follow up analyses regarding the higher ranges of stimulation intensities were unable to be explored, but we acknowledge that this follow-up is fundamental. This is made evident by the results of Pavan et al. (2019), which also indicated significant results that followed an inverse U model of stochastic resonance, but only when they applied the control condition following their initial experimental paradigm that included intensities up to 1.5mA. In their first experiment, they did not find a significant decrement in performance for their parameters. However, in their secondary experimental paradigm, they included ranges up to 2.25mA, at which point they noticed the marked decrease in performance accuracy at suprathreshold intensities, and evidence for stochastic resonance. This being said, their experimental paradigm was also a motion detection paradigm, and it is of interest to determine whether these results can be replicated in a simple low-level discrimination task which primarily recruits V1, instead of the likely recruitment of both V5 and V1 in the motion paradigm experiments.

Previous papers have indicated a substantial benefit using bilateral occipital stimulation directly adding “noise” to the cortex (Herpich et al., 2019), equivalent to adding noise directly to the visual stimulus itself in the absence of tRNS (van der Groen et al., 2018; Pavan et al., 2019), however, these results do not translate to the effects that we see in our experiment. In fact, we witness a significant performance decrement at one sub-threshold contrast level in particular (3.125) at tRNS intensity of .750mA. While there is a consistent significant effect of contrast across the occipital studies, such that with increasing contrast, participants performance improves, this was expected and intuitive, as when visual stimuli are clearer on their own, they are easier to perceive, thus resulting in increased performance benefits, as the threshold for detection is lower with improved signal to noise ratio.

Another note of particular interest to future studies is that the length of time the stimulation applied can also interact with cortical excitability generally assumed to be produced by this technique. The study by Moliadze et al. 2012, showed that, instead of an increase of performance at low-intensity tRNS, a performance impairment at .400mA if the stimulation extended longer than 20 minutes. These results however, cannot fully explain the observed behavior in our experiment, as participants were stimulated for no more than 20 minutes under our experimental paradigm, but did in fact show a performance decrement. We did not, however, measure cortical excitability immediately following our experimental sessions, so currently, we cannot say whether our results were due to an unexplained reversal at the stimulation intensity in our study that produced the performance reductive results (.750mA, as opposed to .400mA reduction in cortical excitability in Moliadze et al.'s 2012 study) at any time point in our paradigm. However, implementing such a measurement could provide further insight into our contradictory results. The conflicting results between previous studies and our own indicate the continued need for clarification using even more consistent paradigms, as it is distinctly possible that even within the same cortical area, neurons that are recruited for motion-tasks require different levels of tRNS intensity than neurons recruited for simple low-level discrimination tasks.

Furthermore, the results of the occipital study indicate that cortical excitability does not seem to correlate with performance at certain tRNS intensities, and cannot therefore be considered a reliable method of differentiating between possible subgroups that benefit from different levels of tRNS stimulation. However, the necessity to determine a method of accounting for individual differences remains an important one. Such factors (i.e. skull thickness, montage placement) in future studies should be taken into account and studied in a within subjects design, as this could lead to determining critical features that set subjects at an equal "pre-stimulation" brain state. This is evidenced by the fact that it is possible to set participants undergoing TMS stimulation to a relatively similar state by using stimulation intensities that are dependent on their own cortical excitability. Doing so reduces the potential "wash-out" effect, and TMS methodologies employing these parameters have gone a long way towards allowing us to delineate results in the field of TMS. Such factors, as yet undetermined but equally important, could potentially exist for tRNS, and result in studies that are more

controlled on the individual levels, and reduce confounds of this type between research groups exploring this paradigm.

Finally, how do we know what is the “optimal level” of tRNS intensity, if we fail to explore the full range of intensities in paradigms that are consistent? 1mA intensity of tRNS may be beneficial for the motor cortex or in visual motion discrimination paradigms, but detrimental for the low-level discrimination tasks in the occipital cortex. As in Stewart et al., (2001) motor thresholds and phosphene thresholds determined with TMS, excellent indicators of the cortical excitability underlying their respective areas, do not correlate. Even at .750mA we find a decrement when the signal-to-noise ratio is not sufficiently strong. Therefore, the parameters even between areas of the cortex that are relatively close may also respond to tES stimulation in different manners which are not yet accounted for. Alternatively, it is necessary to consider the time course of application may also influence the relative effects, as evidenced by a study by Moliadze et al, 2012, where a reverse effect was determined when stimulation was applied after 20 minutes up through 90 minutes post-tRNS, resulting in inhibitory effects instead of excitatory effects normally seen by tRNS stimulation. These effects, compared in a within-subjects design accounted for individual subject differences, as TMS intensity was reduced compared to each subjects individual baseline measure of cortical excitability. Therefore, time course, intensity, stimulus properties, and level of cortical excitability (among other parameters) are necessary to investigate when implementing this technique in neurotypical or atypical populations. Particularly, if the technique is used over a long period of time in a rehabilitative setting, we need to be especially aware of how the mechanism interacts with the cortex, so that we promote beneficial therapeutic effects, and do not induce decrements. Such studies that have employed tRNS therapeutically (Herpich et al., 2019; Campana et al., 2014; Camilleri et al., 2016) are promising, but effects need to be explored across cortical areas, as they may differ fundamentally in the amount of stimulation intensity required. These future directions are yet to be explored, but with the aforementioned clinical benefits, it is a promising technique for patient populations.

Parietal Stimulation

The results from our control experiment in comparison to bilateral occipital stimulation show that using parietal stimulation with bilateral tRNS did not result in a significant change in performance at any tRNS intensity relative to sham condition. Additionally, there was no interaction between tRNS intensity and level of contrast, which further supports that the addition of "noise" introduced by tRNS in the bilateral stimulation of the occipital cortex working by adding noise at the input of the area of the cortex that deals with these particular low-level stimuli (Gabors), but does not extend to cortical areas further away from the site of interest. Instead, these results suggest that while tRNS is generally considered to be less focal than TMS, electrode placement is paramount, and the parietal stimulation paradigm was an excellent control mechanism to determine the effects we were seeing in the occipital lobe were truly due to differences in occipital and parietal (control) stimulation. This is even more relevant given that we chose a control stimulation site (parietal P3/P4) relatively close to our actual targeted stimulation point, and which have shown to have a significant effect on an orientation discrimination task across multiple tRNS sessions (Conto' et al., 2018), indicating the direct involvement of the area in perceptual learning. While tRNS stimulation has been shown to provide an increased benefit over time in perceptual learning paradigms across neurotypical and atypical subjects (Fertonani et al., 2011; Pirulli et al., 2013; Camilleri et al, 2016; Campana et al., 2014; Moret et al., 2018; Tyler et al. 2018; Herpich et al., 2019), it usually requires more than one session to do so. This is further explored by a recent study in our own group which showed parietal stimulation over 4 sessions aided in sustained performance benefit on an orientation discrimination task, (Conto' et al., 2018), but since our primary interest was not perceptual learning, but how the intensities of the stimulation themselves interact with the stimuli, we also corrected for this possibility of perceptual learning in both this parietal stimulation control task and the main occipital stimulation experiment, by re-thresholding participants each day to keep the level of difficulty constant. While we have not seen it employed as a method to correct for preventing task improvement in other studies in this particular field, other studies (besides the recently published study by Pavan et al., 2019) in the field have also not used multi-session paradigms, such that all of their data was usually collected within one day, and it is not likely that learning could occur so quickly. Due to this potential confound specific to the extended length of our paradigm compared to others, we believed re-

thresholding for left/right tilt discrimination at the fixed contrast (12.5) could be a useful method to prevent potential ceiling affects due to any possible learning effects that are generally associated with multi-session application of this technique. Under the scope of the current thesis it is not our primary goal of interest to explore perceptual learning, because we would like to first clarify the transient effects of the method before looking towards its benefits of long-term application, for which there already exists significant evidence pointing towards therapeutic potential. It is possible that with repeated sessions of the same intensity over several days, these results could be different, but, as it is beyond the scope of this experiment, it remains to be clarified.

In conclusion, for the parietal control condition, we do not see evidence of distal effects of parietal stimulation on behavioral performance in our experimental paradigm. The results of the experiment, in direct comparison to the occipital experiment, indicated that indeed the effects we witness in the occipital stimulation on task performance are due to modulation of the occipital cortex, and do not translate task-irrelevant regions, when perceptual learning is controlled for. Thus, the tRNS related performance reduction we see in the main experiment seems to be conclusively related to the influence of transient effects of occipital stimulation with tRNS, and its consequent interaction with low-level stimuli properties.

General Conclusions

Current evidence shows that simplistic models of NIBS effects on the brain cannot account for the full range of effects evident in the literature, as also demonstrated by our results. One way to account for this is that the visual system utilizes all internal and external available sources to enhance signal to noise ratios in order to perceive stimuli that surround us. One plausible explanation for how our brain, a non-linear system, does this, is to incorporate even low-level sources of external signal present in randomly fluctuating noise to boost perception through the mechanism of stochastic resonance. NIBS techniques have been posited to be an external source of noise that could be helpful to the system when the intensity of stimulation is below threshold, such that NIBS and stimulus dependent input interact in a way that boosts below threshold performance. This is visible in the interaction effects seen in our experiments between stimulation intensity and stimulus intensity in our TMS and tRNS experiment, both of which showed that the behavioral impact is not necessarily as linear as previously believed, and that performance was dependent on both properties interacting to determine measured behavioral outcomes. Though the outcomes differed by stimulation type, this type of interaction

was present in both techniques. While we have likely identified an optimal source of additive noise for TMS, for tRNS the results show the need for extension along the line of input intensity.

The results of the tRNS study do not demonstrate conclusively that tRNS enhances signal in a stochastic manner, albeit we only tested performance with low intensity tRNS. Results from our experiment showed that tRNS delivered at a low stimulation intensity (.750mA compared to the frequently used 1mA), actually reduces behavioral performance at subthreshold levels of stimulus intensity. This is in line with other studies (Moliadze et al., 2012), which showed performance detriment instead of enhancement.

In the TMS experiment, behavioral results in the hemifield contralateral to stimulation did receive a boost at subthreshold contrast levels for low-level stimulation, in support of the stochastic effect seen in all the previous studies, but without a significant performance drop proposed by the predominant model of stochastic resonance that is typically implied in these studies (Schwarzkopf et al., 2011; Perini et al. 2012). However, differences in experimental paradigms could account for the discrepancies between studies and further experiments will help clarify these differences. Results from our study lend additional support to the model of activity-driven adaptive stochastic resonance proposed by Wenning and Obermeyer (2003) and supported by Funke et al.'s (2007) physiology studies in anesthetized cats. Due to conflicting results in the previous literature, and with the results of our own tRNS experiment, we conclude that the full spectrum of response to tRNS needs to be explored by extending the parameters both of stimulus and stimulation intensities for this modality of non-invasive brain stimulation. The results of our studies, in conclusion, point to evidence of a stochastic resonance effect, but the full model of stochastic resonance, which can best explain these effects of the interactions between internal and external input, remains to be determined.

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