A TMS Paradigm to Measure Visual Cortical Inhibition and Its Application to Aging

by

Dalia Khammash

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in the University of Michigan
2022

Doctoral Committee:

Professor Thad A. Polk, Chair
Assistant Professor Taraz G. Lee
Professor Stephan F. Taylor
Assistant Professor Michael Vesia

Dalia Khammash
daliak@umich.edu
ORCID iD: 0000-0002-1896-3863
© Dalia Khammash 2022

Dedication

To mom and dad, for your endless love and support, and to the memory of my nan-nan, who showed me how to always look on the bright side of life.

Acknowledgements

First and foremost, I'd like to thank my faculty advisor, Dr. Thad Polk. Throughout graduate school he has always been my biggest advocate. He encouraged me to try new things, showed enthusiasm about my work, and consistently provided critical feedback to help me grow as a scientist. I consider myself to be extremely fortunate and cannot imagine having a better mentor. In addition to being an excellent role model for how to be a good, ethical researcher, Thad has also helped me build essential skills including how to mentor others, how to effectively collaborate, and how navigate the grant writing process. I received two NIH grants (F31 & F99/K00) during my graduate training, neither of which would have been possible without his expertise and guidance. Thad is also just an all-around great person, who genuinely cares about the happiness and wellbeing of each of his students. He cultivated a sense of community in the lab, a community that I am grateful to be a part of. I only hope that I'll live up to Thad when I find myself in a mentor position of my own!

I'd also like to thank my secondary mentor, Dr. Mike Vesia, for taking me under his wing. Mike was a pleasure to collaborate with; he was always upbeat and positive, accommodating, and consistently provided critical feedback on my TMS work. He was also tremendously

supportive through all of our project setbacks (including but not limited to: building moves, floods, equipment malfunctions, and COVID shutdowns).

I would like to thank my committee members, Dr. Stephan Taylor and Dr. Taraz Lee for their support. Dr. Taylor collaborated on all three studies in this dissertation and played an essential role in making these projects the best that they could possibly be. Dr. Lee challenged me by asking excellent questions pertaining to these projects. He forced me to think critically and made me a stronger and more thorough researcher.

I'd also like to thank both Dr. Bill Gehring, and again Dr. Polk, for modelling how to be excellent teachers. It was a pleasure teaching with both of them. I learned how to use various teaching techniques to meet the needs of a range of students, as well as how to effectively convey information to non-expert audiences. I went from being shy and unconfident my first term teaching, to really growing into the role and enjoying every class. I ultimately received an outstanding GSI award from the Psychology department. I attribute this success to my work with both Thad and Bill, and thank them for all that they taught me.

I'd also like to thank Dr. Cindy Lustig and Dr. John Jonides whose 400 level undergraduate classes piqued my interest in cognitive neuroscience and were instrumental in my decision to pursue graduate school. I am also thankful for Cindy's support in pursuing alt-ac careers and her helpful recommendations for professional development.

I'd also like to thank my friends and colleagues. Dr. Molly Simmonite entertained my research

questions at any hour of the day and was always up for a food adventure (tacos in Tijuana, anyone?). I'm also grateful for my collaborations with Katy, who provided never-ending motivation and support, and challenged me to keep learning and improving. I'm also thankful for Karthik, who helped me improve my skills in R, and mentored me during my career search. I'd also like to acknowledge many others who enriched my graduate experience, including Abbey, Violet, Kaitlin, Pia, Shreya, Kristin, Madison, Tyler, Hyesue, Lauren and EunSeon. Last but not least, I'd like to thank my family. My mom (Christine) and dad (Mustafa) for their love and support. Trey, for being an amazing partner and best friend, and for providing encouragement when I needed it most. And Mizzo, for providing life advice, mantras to live by, and for always making me laugh.

Table of Contents

List of Tables

List of Figures

Abstract

The neurotransmitter GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the adult human brain. Deficits in GABA-mediated inhibitory function are associated with various motor and psychiatric disorders and may also play a role in age-related cognitive decline. However, there are limited ways of probing GABA and inhibitory function in vivo. While Magnetic Resonance Spectroscopy (MRS) can assess the concentration of GABA, this measure may not be indicative of local inhibitory functioning. Instead, techniques such as paired-pulse Transcranial Magnetic Stimulation (ppTMS) are often used to probe local GABA-mediated inhibitory function. However, ppTMS is almost exclusively used in motor cortex due to the ease-of-measurement and sensitivity of the resulting muscle twitch response. The ability to investigate inhibitory function outside of motor cortex would require adaptation of this technique for use in other cortical regions. While TMS of the motor cortex elicits muscle-twitches, stimulation of primary visual cortex elicits short-lived visual percepts called phosphenes. Previous studies were unable to elicit inhibitory phenomena in visual cortex using phosphenes (Kammer & Baumann, 2010; Ray et al., 1998; Sparing et al., 2005), likely due to a less sensitive measurement system and non-optimal stimulation parameters derived from motor cortex. The objective of this dissertation is to implement and test a new tracing paradigm as a method of measuring the phosphene response, as well as apply this new technique in the realm of aging research.

In the first study of this dissertation, I developed a reliable method of assessing cortical inhibition in visual cortex using phosphenes and ppTMS. Specifically, I demonstrated that a phosphene-tracing procedure can capture subtle increases or decreases in phosphene size. I also determined the proper conditioning stimulus intensity setting (i.e. the magnitude of the first stimulus in each pulse pair) to elicit the strongest inhibitory response.

In the second study, I utilized the ppTMS phosphene-tracing paradigm developed in the first study to explore the temporal dynamics of local inhibitory and facilitatory networks in visual cortex. I determined that certain time intervals between pulse-pair stimuli do not elicit the same response (i.e. inhibition versus facilitation) in visual cortex as it does in motor cortex. Together, the first and second studies pinpoint the optimal paired pulse parameters for measuring cortical inhibition in visual cortex, and suggest that these parameters differ from what is optimal in motor cortex.

In the final study, I conducted a multimodal investigation using ppTMS and MRS to probe both inhibitory function and GABA in the same group of older and younger adults. I determined that ppTMS measures of visual inhibitory activity are reduced with age. Furthermore, I found that MRS measures of GABA and ppTMS measures of cortical inhibition are significantly positively correlated in primary visual cortex. This pattern of results is different from what is observed in primary sensorimotor cortex, where $GABA_A$-mediated inhibitory function remains stable (Bhandari et al., 2016), and ppTMS and MRS measures are uncorrelated (Cuypers et al., 2021; Dyke et al., 2017; Tremblay et al., 2013).

Together, this dissertation demonstrates the feasibility of using ppTMS to assess corticocortical inhibition in a brain region outside of motor cortex. It also provides further insight

into how GABAergic signaling changes with age in primary visual cortex, as well as highlights that age-related changes in GABAergic signaling differ in different brain regions.

Chapter 1 : Introduction

GABA is the brain's primary inhibitory neurotransmitter. It is important in regulating the balance of excitation and inhibition in neuronal circuits, the stability of which is necessary to maintain proper brain function. GABAergic imbalances have been implicated in many prevalent health conditions including motor disorders such as amyotrophic lateral sclerosis (Foerster et al., 2013) and Parkinson's Disease (Błaszczyk, 2016), behavioral-control disorders like gambling (Mick et al., 2017), psychiatric mood disorders like bipolar disorder (Brady et al., 2013) and schizophrenia (de Jonge et al., 2017), and in concussions (Friedman et al., 2017). Declines in inhibitory function with age have also been hypothesized to play a role in age-related cognitive decline (Hasher & Campbell, 2020; Marenco et al., 2018; Porges et al., 2017; Simmonite et al., 2019).The ability to probe inhibitory function in vivo is therefore important for increased understanding of the underlying pathophysiology of these conditions.

Unfortunately, there are limited methods for noninvasively measuring cortical inhibition in humans. One common method is Magnetic Resonance Spectroscopy (MRS), which assesses the concentration of various neurochemicals, including GABA, in a precisely defined region of the brain. However, there are drawbacks to using MRS, including the concern that this measure of concentration may not reflect local activity/function. Paired-pulse Transcranial Magnetic Stimulation (ppTMS), on the other hand, is able to probe local GABA-mediated inhibitory function through the stimulation of a focal region near the cortical surface (Kujirai et al., 1993). However, ppTMS is almost exclusively used in motor cortex because stimulating motor cortex

results in an overt, easily-measured response: muscle twitches in the hand, the magnitude of which can be assessed by mounting surface electromyography electrodes on the skin above target muscles. In order to investigate inhibitory function in regions outside of motor cortex, it is necessary to adapt this ppTMS paradigm for use in other brain areas. Guided by previous TMS work in motor cortex (reviewed below), **Study 1** and **Study 2** of this dissertation detail the development of a ppTMS method of assessing cortical inhibition in visual cortex by measuring TMS-induced visual disturbances called phosphenes in lieu of peripheral muscle contractions. **Study 3** then investigates the relationship between this new measure of visual cortical inhibition and MRS-derived measures of primary visual GABA, as well as changes in inhibitory function associated with aging.

1.1 Basic principles and mechanisms of TMS

1.1.1 Equipment

TMS is a non-invasive tool that utilizes magnetic fields to stimulate localized regions of the cortex. Magnetic stimulators consist of a capacitor, a discharge switch, and a stimulating coil. The capacitor stores an electric charge, often of 1-3 kV (Riehl, 2008). When the switch is triggered and the circuit closes, current flows into the stimulating coil which generates a time-varying magnetic field. When the coil is held over the head, the magnetic field pulse induces an electric field in the cortex (up to ~140 V/m (Riehl, 2008)), which leads to the stimulation of neurons. Using a graphical user interface on the stimulator device, an operator is able to control the length of time current is able to flow through the coil, the stimulation intensity, the direction of current flow through the coil (posterior-anterior or anterior-posterior), and the length of time between pulses.

There are several types of coils used in research and in clinical settings, the shape and size of which affect the spatial distribution of the induced electric field (Hallett, 2007). The studies presented in this dissertation utilize a figure-of-eight coil, which is ideal for the activation of small, focal brain areas (Ueno & Sekino, 2021). This is because in figure-of-eight coils, current in one 'wing' passes in a clockwise direction, while current in the other passes in a counterclockwise direction. A superposition of the two currents occurs in the central segment of the coil, resulting in the maximum field peak at the intersection of the two coil wings. This allows for figure-of-eight coils to focally stimulate superficial cortical regions of up to 2-3cm in depth (Deng et al., 2014). Deeper cortical, subcortical, and limbic areas are not stimulated (Lu & Ueno, 2017).

1.1.2 The neuronal response to magnetic stimulation

At rest, the membrane potential of a neuron is approximately -70 millivolts. When subjected to an electric field, charge accrues at the cell membrane. If the electric field is of sufficient strength, local hyper- or depolarization will occur (Koponen et al., 2018). Interestingly, the waveform of the TMS coil current and the waveform of the membrane potential change are approximately congruent (Peterchev et al., 2011). For example, if a full-sine ('biphasic') waveform is delivered, the temporal shape of the membrane potential change will also manifest as a single cycle of a sine wave. If instead the coil current is switched off after only the first quarter of a sine wave (which is termed 'monophasic' stimulation, and is the type used in the studies that make up this dissertation), the membrane potential change will similarly mirror this shape.

1.1.3 Selectivity of neuronal activation

Not all neurons subjected to the TMS-induced electric field will be activated. One of the most important factors in determining which neurons are more likely to fire is their physical orientation with respect to the electric field. Neurons that are oriented parallel to the coil (and are therefore perpendicular to the magnetic field and parallel with the electric field) are preferentially activated (Klomjai et al., 2015; Tofts, 1990). Among neurons in this plane, certain neuronal features are associated with a decrease in threshold, especially larger fiber diameters, which further increase the likelihood of stimulation (Pashut et al., 2011). In addition to neuronal orientation and features, the intensity of the magnetic stimulation plays a large role in dictating which subpopulations of neurons will be activated. For example, inhibitory interneurons in the motor cortex have a lower firing threshold than excitatory neurons (Chen et al., 1998; Schäfer et al., 1997). Therefore, greater electric field strengths are required for recruitment of excitatory neurons.

1.2 Examining cortical physiology with TMS

1.2.1 Calibrating the TMS dosage in motor cortex: resting motor threshold

To account for the inherent variability in physiology between individuals, TMS experiments often use subject-specific stimulation dosages. In motor cortex, this dosage is calibrated by calculating a parameter called resting motor threshold, which is based on the minimum stimulation intensity required to elicit a response of a certain magnitude (in this case, a muscle contraction in the contralateral hand). Thus, resting motor threshold is a reflection of an individual's cortical excitability, where a lower stimulation threshold suggests greater cortical excitability. The intensities of paired pulse stimuli delivered to motor cortex are therefore relative to an individual's resting motor threshold.

1.2.2 Short-interval cortical inhibition (SICI)

ppTMS is one of the most common TMS methods to probe inhibitory function. It is almost exclusively used over motor cortex. In ppTMS, a suprathreshold test stimulus is preceded by a subthreshold conditioning stimulus. While the supratheshold test stimulus is strong enough to induce motor activity and evoke a muscular response, the weaker subthreshold conditioning stimulus is only strong enough to activate or 'prime' local cortical neurons. The state induced by the conditioning stimulus is theorized to reflect the net influence of local facilitatory and inhibitory interneuron populations converging on the pyramidal output neuron at the time of test stimulus delivery (Ziemann, Rothwell, et al., 1996). One ppTMS protocol used to measure inhibitory function in motor cortex is called short-interval intracortical inhibition, or SICI, which is typically elicited when there is a subthreshold (~70–90% of resting motor threshold) conditioning stimulus, and a short interstimulus interval of 2-5 ms between the conditioning stimulus and the test stimulus (Kujirai et al., 1993). This paradigm leads to a reduction in the amplitude of the muscular response evoked by the test stimulus. Pharmacological evidence demonstrates that the reduction in the evoked response is mediated by $GABA_A$ receptor sub-types (Di Lazzaro et al., 2000; Ziemann, Lönnecker, et al., 1996). The vast majority of studies investigating this inhibitory process have been conducted over the motor cortex, because the motor-evoked potential response is overt and easily measured with surface electromyography. Few other cortical regions yield a readily measurable output, making objective measurement challenging and the mechanisms of action more poorly understood.

1.2.3 TMS outside of motor cortex

Several 'readouts' other than a muscular response have been explored to examine cortical physiology with TMS. Electroencephalography (EEG) can be used to record evoked responses by TMS in areas outside of motor cortex (Fitzgerald et al., 2009; Ilmoniemi et al., 1997). However, such approaches have not been completely validated and are characterized by technological challenges associated with TMS artifacts. Another approach has been to study phosphenes, short-lived visual percepts that are sometimes experienced when TMS pulses are applied to the occipital cortex. Measuring phosphenes depends upon a subject noticing and reporting on them, and prior work has not been able to demonstrate inhibitory phenomena with ppTMS paradigms (Sparing et al., 2005). The variable results to date may reflect the subjective nature of reporting increases or decreases in phosphene size. Alternatively, the variable results may indicate different cortical physiology in the visual cortex compared to motor cortex. The latter cannot be ruled out given the common use of stimulation parameters derived from studies of motor cortex that are not optimized for visual cortex.

Studies assessing inhibitory function in the motor cortex rely upon changes in an electromyography signal that are independent of participant perception. The test stimulus response is recorded on a continuous scale, generally to the nearest millivolt. The inhibitory effect of the conditioning stimulus in SICI can be inferred as the percent change in the response between conditioned and unconditioned trials. In the visual cortex, the perceptual manifestation of such brain responses (i.e. phosphenes) are used as a proxy. However, phosphenes are often quantified using subjective reports of characteristics such as brightness, clarity, color, shape, and location in the visual field (Boroojerdi et al., 2000; Cattaneo et al., 2011; Samaha et al., 2017) or manual responses indicating presence or absence (Taylor et al., 2010). Such scales present

challenges in objectively measuring a continuous phenomenon with any level of reliability. A more promising approach involves tracing of the evoked phosphene (Elkin-Frankston et al., 2010; Kammer, 1998; Kammer et al., 2005; Kastner et al., 1998; Marg & Rudiak, 1994). A tracing approach offers potential increased sensitivity to detect graded inhibitory phenomena such as SICI. Therefore, **Study 1** focused on developing a reliable tracing method of eliciting and measuring phosphenes, and determined whether ppTMS could be used to elicit SICI-like inhibitory phenomena in occipital cortex.

As noted previously, stimulation parameters that are optimal for motor cortex may not be optimal for sensory, premotor and association cortices. This problem is highlighted by the comparison of cortical thresholds. Resting motor threshold is a common parameter measured in motor cortex to assess cortical excitability. It is commonly defined as the lowest stimulus intensity able to produce peak-to-peak motor-evoked potential amplitudes of at least 50μV on five out of ten trials (Rothwell et al., 1999). A parallel parameter in the visual cortex is the phosphene threshold, defined as the lowest stimulus intensity that produces phosphenes on a specific proportion of stimuli (Abrahamyan et al., 2011). However, despite similarities in their definition, there is little to no correlation between resting motor threshold and phosphene threshold (Gerwig et al., 2003; Stewart et al., 2001; Terhune et al., 2015). Furthermore, anodal and cathodal transcranial direct-current stimulation (tDCS) applied to the motor cortex modulates motor-evoked potential amplitude, but it does not modulate phosphene threshold when applied to the visual cortex (Brückner & Kammer, 2016). The lack of correlation between phosphene threshold and resting motor threshold, and the fact that tDCS affects the two cortical regions in different ways, indicates that motor cortex stimulation parameters are likely not transferable to the visual cortex. Therefore, as part of **Study 1,** I additionally used phosphene

tracing to generate single pulse TMS input-output curves for each subject across two separate sessions. During each session, SICI was also assessed across varying conditioning stimulus intensities to identify optimal parameters specific to the visual cortex.

1.2.4 Temporal dynamics of ppTMS

In motor cortex, the effect of the subthreshold conditioning stimulus upon corticospinal neuron excitability depends on the interval between the conditioning and test pulses. When a subthreshold (~70–90% of resting motor threshold) conditioning stimulus is delivered 1–5 ms prior to the test stimulus, motor evoked potential amplitudes are reduced. This net inhibitory state is referred to as short-interval intracortical inhibition (SICI, as discussed above) (Kujirai et al., 1993), and is thought to result from fast-acting $GABA_A$ receptor sub-types that hyperpolarize the corticospinal neuron prior to the delivery of the test stimulus (Di Lazzaro et al., 2000, 2005; Ziemann, Lönnecker, et al., 1996). When the interstimulus interval is between 7 and 20 ms, the conditioning stimulus increases the amplitude of the motor evoked potential elicited by the test stimulus, resulting in intracortical facilitation (ICF) (Kujirai et al., 1993). ICF is theorized to result from stronger excitatory N-methyl-d-aspartate (NMDA) glutamate receptors overwhelming weaker inhibitory $GABA_A$ receptors, leading to a net facilitation of the response (Schwenkreis et al., 1999). When the interstimulus Interval is between 50 and 200 ms, motor evoked potential amplitudes are again reduced. This phenomenon is known as long-interval cortical inhibition (LICI) (Nakamura et al., 1997), and has been pharmacologically demonstrated to be mediated by the slower-acting $GABA_B$ receptors (Florian et al., 2008; McDonnell et al., 2006).

Prefrontal (Oliveri, Rossini, et al., 2000) and parietal (Oliveri, Caltagirone, et al., 2000) cortices demonstrate similar patterns of net inhibition and facilitation as primary motor cortex.

However, in visual cortex a SICI-like effect is absent. Earlier investigations have shown that a conditioning stimulus ranging from 60% to 130% of phosphene threshold across inter-stimulus intervals of 1–20 ms increase the perception of phosphenes (Kammer & Baumann, 2010; Ray et al., 1998; Sparing et al., 2005). One explanation for this may be that a different balance between inhibitory and excitatory mechanisms exists in visual cortex compared to motor cortex.

Therefore, using the reliable quantitative phosphene tracing method developed in **Study 1** as well as the conditioning intensity determined to be optimal for inhibiting the phosphene response, **Study 2** aimed to assess local facilitatory-inhibitory dynamics across inter-stimulus intervals that induce SICI, LICI, and ICF in motor cortex. Understanding how both conditioning stimulus intensity and inter-stimulus interval affect the phosphene response is necessary in order to select optimal parameters for probing inhibitory and excitatory networks in the visual cortex in both healthy and clinical populations.

1.3 Basic principles of MRS

MRS is performed using the same equipment as conventional MRI, and can be used to assess the local concentration of various neuro-metabolites, including GABA, in a ~3cm^3 voxel in the brain. MRS primarily utilizes the signal of the hydrogen protons on each of the metabolites in order to generate spectral data. Because proton nuclei are in different chemical environments that are specific to the metabolite, this leads to different metabolites exhibiting different resonance frequencies. And because their frequency distributions are unique and highly reproducible, it is possible to identify which peaks in the spectral data belong to which metabolites. In this dissertation we are primarily interested in the GABA peak, which appears at 3ppm (Figure 1). The amount of GABA is then measured by fitting a gaussian curve to this peak of interest, and then measuring the area under the curve.

Multiple scanning sequences can be used to acquire MRS GABA estimates. One of the most popular is MEGA-PRESS due to its ability to isolate the GABA signal from that of other metabolites (Mescher et al., 1998). MEGA-PRESS consists of a sequence of edit-ON and edit-OFF acquisition conditions, each of which consist of slightly different radio frequency pulses. These different radio frequency pulses differentially affect metabolite resonances, leading to the generation of two separate spectrums. The resulting edit-ON and edit-OFF spectra can then be subtracted to produce a difference spectrum that more clearly displays GABA's resonant peak. Other scanning sequences do not utilize this interleaved sequence, resulting in a less reliable measure of GABA.

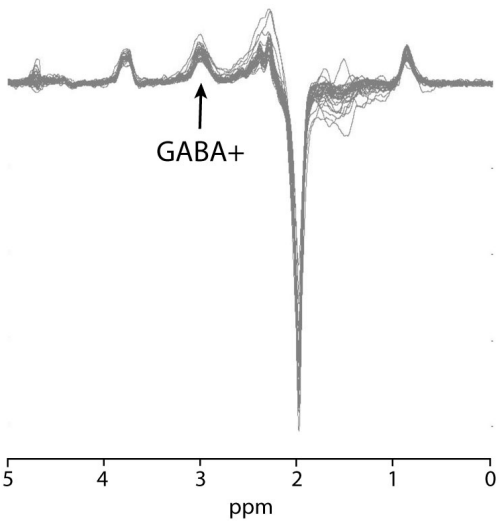

Figure 1. Example MEGA-PRESS spectra of several participants, with GABA+ peak identified. ppm = parts per million.

While ppTMS reflects synaptic GABAergic activity (Di Lazzaro et al., 2000; Ziemann, Lönnecker, et al., 1996), MRS-derived estimates of GABA are thought to reflect levels of ambient extracellular GABA and cytoplasmic GABA in the cell body (Stagg, 2014; Stagg, Bachtiar, et al., 2011; Stagg, Bestmann, et al., 2011).

1.4 Relationship between TMS-measures and MRS-derived GABA+

In motor cortex, the relationship between various TMS metrics of inhibition and MRS-derived GABA has been extensively explored. However, studies have found that measures of resting motor threshold (Dyke et al., 2017), average size of motor-evoked potential amplitude at threshold (Dyke et al., 2017), linear slope of the single pulse input-output curve (Dyke et al., 2017), 2.5 ms (Stagg, Bestmann, et al., 2011) and 3ms (Dyke et al., 2017; Tremblay et al., 2013)

SICI, 10ms and 12ms ICF (Dyke et al., 2017), and 100ms (Tremblay et al., 2013) and 150ms (Stagg, Bestmann, et al., 2011) LICI are not correlated with local GABA concentration. It is therefore hypothesized that in motor cortex, MRS estimates of GABA are not reflective of GABAergic synaptic transmission.

The relationship between GABA and TMS measures of inhibitory function has been largely unstudied outside of primary motor cortex due to the lack of other easily-measurable outputs. Though visual cortex stimulation yields phosphenes, the absence of a validated measurement system with enough sensitivity to detect graded responses resulted in phosphene threshold being one of the few commonly assessed TMS metrics in that brain region. Phosphene threshold is typically assessed using binary decisions of presence vs absence of phosphenes after each pulse (Abrahamyan et al., 2011). Multiple studies have explored the relationship between phosphene threshold and MRS measures in primary visual cortex, with inconclusive results. While Terhune et al. (Terhune et al., 2015) found a significant negative association between phosphene threshold and local glutamate but not GABA levels, Rafique et al. (Rafique & Steeves, 2020) found no relationship between phosphene threshold and either of these measures. However, the relationship between visual short-interval inhibition and primary visual cortex GABA has not yet been explored. Therefore, **Study 3** aims to fill this gap by utilizing the phosphene-tracing paradigm and appropriate stimulation parameters for 2 ms short-interval inhibition derived in **Studies 1 and 2** in order to explore whether MRS measures of visual GABA availability are reflective of visual cortical inhibitory activity.

1.5 Changes in GABAergic signaling with age

1.5.1 GABA levels

Changes in GABA concentration estimates with age have been quantified in several brain regions using MRS. A recent meta-analysis demonstrated a non-linear relationship between age and GABA concentration in frontal GABA. It found that GABA tended to increase in early-life, plateau in early adulthood, and then gradually decrease for the remainder of the lifespan (Porges et al., 2021). While similar age-related reductions have been observed in other regions as well (Chamberlain et al., 2021; Gao et al., 2013; Hermans et al., 2018; Lalwani et al., 2019; Marenco et al., 2018; Simmonite et al., 2019), it is clear that reductions in GABA are not uniform across the brain. For example, there are mixed results as to whether age-related reductions in GABA concentration occur in early visual areas (Hermans et al., 2018; Maes et al., 2018; Pitchaimuthu et al., 2017; Simmonite et al., 2019). **Study 3** therefore additionally investigates whether age-differences in GABA are observed in left primary visual cortex.

1.5.2 Cortical inhibition

Several studies have investigated age-related changes in motor SICI with mixed results. While some studies found a decrease in SICI with age (Marneweck et al., 2011; Peinemann et al., 2001), others found an increase (Kossev et al., 2002; McGinley et al., 2010; A. E. Smith et al., 2009), or no significant changes at all (Cirillo et al., 2010; Oliviero et al., 2006; Rogasch et al., 2009). Additionally, a meta-analysis of 11 such studies determined that only a slight but statistically insignificant reduction in motor SICI occurs with advancing age (Bhandari et al., 2016). However, because age-related changes in the brain are not uniform, it is unclear how corticocortical inhibition is affected in other regions of the brain. Therefore, using the method of

assessing visual cortical inhibition developed in **Studies 1 and 2**, in **Study 3** I also explored the relationship between age and inhibitory activity in primary visual cortex.

Together, the three studies in this dissertation detail a novel TMS-based method for investigating inhibition in visual cortex, and inform the relationship between this new metric and MRS-derived estimates of primary visual GABA levels. They also provide novel insight into how inhibitory function in this brain region changes with age. A better understanding of the role that age-related changes in inhibitory function play in aging could be an important step in designing interventions—for example, pharmacological compounds that act on GABA function—to slow or reverse age-related behavioral impairments.

Chapter 2 : Probing Short-latency Cortical Inhibition in the Visual Cortex with Transcranial Magnetic Stimulation: A Reliability Study

2.1 Abstract

Background: Transcranial magnetic stimulation (TMS) is a non-invasive method to stimulate localized brain regions. Despite widespread use in motor cortex, TMS is seldom performed in sensory areas due to variable, qualitative metrics.

Objective: Assess the reliability and validity of tracing phosphenes, and to investigate the stimulation parameters necessary to elicit decreased visual cortex excitability with paired-pulse TMS at short inter-stimulus intervals.

Methods: Across two sessions, single and paired-pulse recruitment curves were derived by having participants outline elicited phosphenes and calculating resulting average phosphene sizes.

Results: Phosphene size scaled with stimulus intensity, similar to motor cortex. Paired-pulse recruitment curves demonstrated inhibition at lower conditioning stimulus intensities than observed in motor cortex. Reliability was high across sessions.

Conclusions: TMS-induced phosphenes are a valid and reliable tool for measuring cortical excitability and inhibition in early visual areas. Our results also provide appropriate stimulation parameters for measuring short-latency intracortical inhibition in visual cortex.

2.2 Introduction

Transcranial magnetic stimulation (TMS) is commonly used to assess cortical excitability and intracortical function. In the motor cortex, the motor evoked potential (MEP) provides an overt, graded response which is easily quantified with surface electromyography. In contrast, stimulating visual cortex often evokes the experience of seeing light (a phosphene), but that response is covert and difficult to quantify objectively. Recently, phosphene tracing was used to quantify the induced phosphene (Elkin-Frankston et al., 2010). However, doing so required a complicated laser tracking system. Further, the reliability of tracing has yet to be established, a critical step in the expanded use of visual TMS in healthy and clinical populations.

Paired-pulse TMS (ppTMS) has also been used to assess local inhibitory function in healthy and clinical populations in motor cortex (Kujirai et al., 1993) and adjacent somatosensory cortex (Oliveri, Caltagirone, et al., 2000). However, the validity of ppTMS as a marker of short-interval intracortical inhibition in visual cortex is questionable. The relatively few studies in visual cortex have failed to reduce phosphene perception at interstimulus intervals and conditioning stimulus (CS) intensities that elicit short-interval cortical inhibition (SICI) in sensorimotor cortex (Kammer & Baumann, 2010; Ray et al., 1998; Sparing et al., 2005). Whether the absence of comparable inhibition in visual cortex is methodological or reflects distinct functional mechanisms is unknown. The probabilistic phosphene perception used in previous studies may be a less sensitive measure of inhibition compared to the graded magnitude of the motor evoked response. Further, unique intracortical dynamics in visual cortex could alter the relative recruitment of inhibitory and facilitatory mechanisms at CS intensities optimized to induce SICI in motor cortex (60-90% of phosphene threshold). For example, ppTMS studies in cat visual cortex show inhibition is restricted to CS intensities <60% across a range of inter-

stimulus intervals associated with SICI and intracortical facilitation in motor cortex (Moliadze et al., 2005).

The current brief communication reports on the reliability and validity of a simple phosphene tracing approach in the assessment of stimulus input-output curves. We also demonstrate that short-latency intracortical inhibition can be observed in visual cortex, provided that parameters optimized for this brain region are used.

2.3 Methods and Materials

Thirty healthy adults (10 male, mean age: 20.4±2.7, age range: 18-29) provided informed consent. The experimental protocol was approved by the Institutional Review Board of the University of Michigan Medical School (IRBMED). Seven subjects who did not report phosphenes were excluded. The remaining 23 subjects completed two 2-hour identical sessions at least one week apart.

Each session involved tracing phosphenes elicited by occipital TMS. The optimal occipital stimulation site was determined using a 3x3 cm grid (see Fig. 2a for procedure). The site eliciting the most intense and consistent phosphenes was defined as the occipital "hotspot". Hotspot phosphene threshold (PT) was defined as the intensity that induced phosphenes on 5/10 stimuli.

Occipital recruitment curves were generated from 90 randomized single pulses ranging from 60-150% PT (10% increments; 9 repetitions at each intensity). Intracortical inhibition was assessed using 10 randomized paired stimuli across a range of CS intensities (30, 45, 60, 75, 90, 105, 120, and 150% of PT) at an inter-stimulus interval of 2ms. Test stimulus (TS) intensity was fixed at 120% of PT to elicit a stable phosphene so as to be sensitive to any relative change induced by the CS (Ilić et al., 2002; Kujirai et al., 1993).

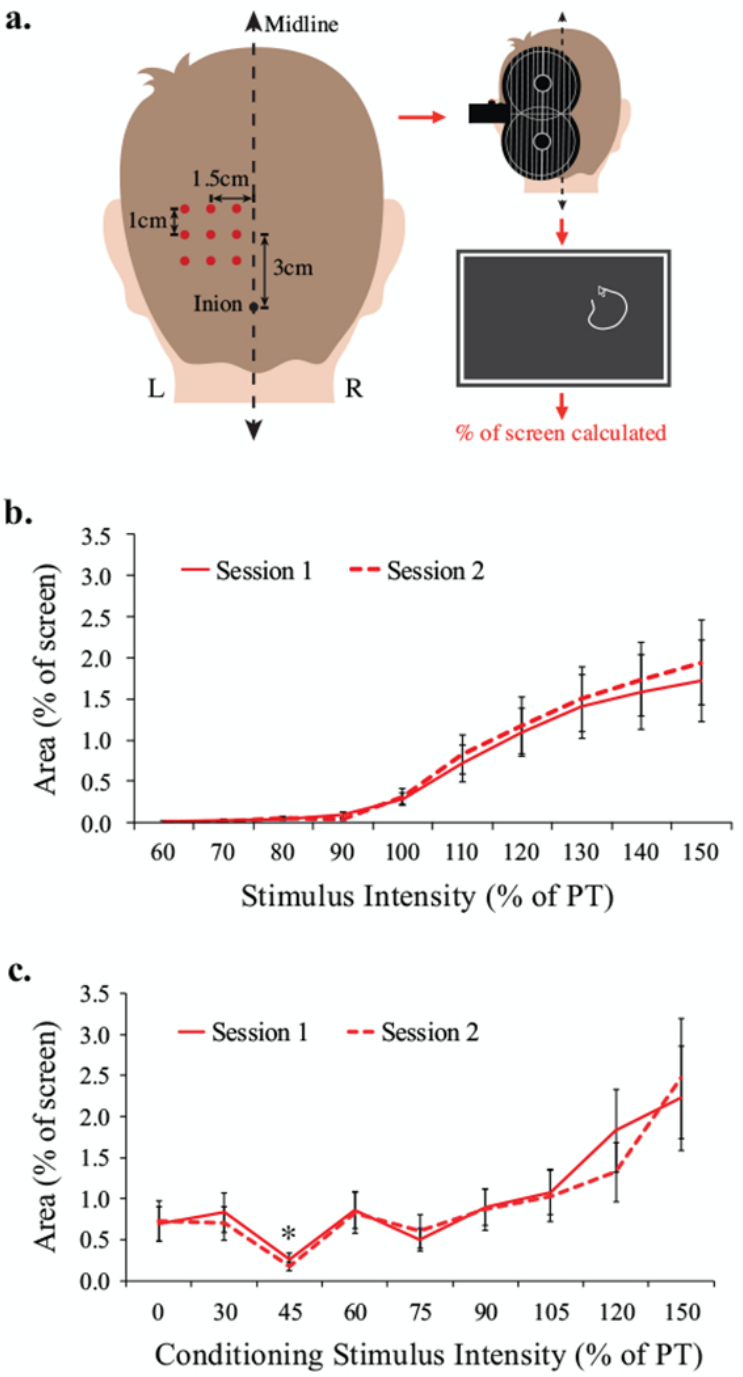

Figure 2 a. Grid placement over the visual cortex to elicit phosphenes through transcranial magnetic stimulation (TMS). Stimulation targets were spaced 1cm apart, with the center of the grid 3cm dorsal and 1.5 cm left-lateral of the inion. Stimulator output was initially set to 60% of maximum stimulator output and each target was stimulated three times (monophasic, lateral-medial induced current, MagPro X100 with MagOption stimulator and MC-B70 butterfly coil). If no phosphenes were induced, intensity was increased in increments of 10% until phosphenes were reported. If phosphenes were reported at one or more locations, the site reported to generate the brightest most consistent phosphenes was selected as the target "hotspot". At the hotspot, stimulation intensity was decreased by 2% of stimulator output until no longer reported, then incremented by 1% until the phosphene threshold (PT) was reached. PT was defined as the lowest stimulator output at which at least 5 out of 10 stimuli induced a phosphene. If no phosphenes were reported at any of the 9 locations with 100% of stimulator output, the participant was excluded from further participation (n=7). The TMS coil was held at 90° to the midline. The BrainSight™ frameless stereotactic neuronavigation system was used for accuracy of targeting and trajectory. Subjects traced phosphenes on screen after each trial and area was calculated. **b.** Phosphene area as a function of stimulator intensity during single-pulse TMS. Error bars indicate standard error of the mean. **c.** Phosphene size as a function of conditioning stimulus intensity during paired-pulse TMS. Error bars indicate standard error of the mean. Asterisk denotes significant inhibition.

For both the single-pulse and paired-pulse TMS protocols, participants used a mouse to draw the shape of the perceived phosphene on a 46" computer screen in a darkened room. The size of each phosphene was calculated as a percentage of the computer screen. Recruitment curves were generated by plotting phosphene area as a function of stimulus intensity. For paired-pulses, we tested whether a preceding CS reduced the response to the TS relative to when no CS was presented. The CS effect was expressed as a percentage of unconditioned phosphene area.

2.4 Results

2.4.1 PT

PT was 67.1±11.5% and 64.7±9.5% of maximum stimulator output for Session 1 and 2 respectively (t_{22} =1.03, p=0.32). PT was significantly positively correlated across sessions (r_{21}=.44, p=.03).

2.4.2 Recruitment Curves

Phosphene input-output curves exhibited a sigmoidal shape (Fig. 1b). Data points from the linear region of the sigmoidal curve (90-150% of phosphene threshold) were selected for analysis. The data from the three lowest intensities (60-80% of phosphene threshold) were omitted from further analysis as they were all clearly subthreshold and almost never elicited a phosphene. A two-way repeated-measures ANOVA with a Greenhouse-Geisser correction indicated a main effect of Intensity on phosphene size ($F_{6,132}$=12.04, ε=0.21, p=.001). There were no differences in phosphene size across Session ($F_{1,22}$=.73, p=.403) and Session did not interact with Intensity ($F_{6,132}$=.53, ε=0.59, p=.692). Paired t-tests indicated significant increases in phosphene area with each increment across the linear portion of the curve (90-130% of PT; all p's <.05). The slopes (r^2=.82, n=23, p<.001) and intercepts (r^2=.80, n=23, p<.001) across the two sessions were highly correlated indicating high relative reliability. There was no significant

difference in standard error of the estimates across session (t_{22}=.69, p=.50) indicative of high absolute reliability.

2.4.3 Paired-Pulse

CS intensities above 110% PT increase, rather than decrease, phosphene size in motor cortex (Kujirai et al., 1993) and were omitted from the analysis of inhibition. A two-way repeated-measures ANOVA with a Greenhouse-Geisser correction revealed a main effect of CS Intensity on phosphene size ($F_{6,132}$=9.23, ε=0.28, p=.001). There were no differences across Session ($F_{1,22}$=.06, p=.81) and Session did not interact with CS Intensity ($F_{6,132}$=.80, ε=0.41, p=.477) (Fig. 1c). Modified Bonferroni (Keppel, 1991) corrected paired t-tests indicated significant suppression of phosphene area at the 45% CS intensity (p=.017). No other CS intensity caused significant suppression of phosphene area.

2.5 Discussion

This study demonstrates the absolute and relative reliability of tracing TMS-induced phosphenes to assess graded change in visual cortex excitability. Additionally, the sigmoidal shape and graded increase in phosphene size observed from 100% PT to ~130% PT is evidence of a lawful relationship between stimulation energy and elicited response, similar to the input-output properties of corticospinal neurons in motor cortex.

The current study also demonstrates the validity of using phosphene tracing in assessing visual cortex inhibition. Phosphene area was significantly inhibited when the TS was preceded by a CS with an intensity of 45% PT. This CS intensity is lower than the intensity that typically elicits SICI in sensorimotor cortex (60-90% PT), but is consistent with ppTMS studies in cat visual cortex (Moliadze et al., 2005). Furthermore, the emergence of inhibition at low intensities that dissipate with increasing CS intensity is consistent with sensorimotor models of SICI that

include a low-threshold inhibitory pathway and a high-threshold excitatory pathway (Ilić et al., 2002).

Overall, our results suggest that our phosphene tracing paradigm is an effective way to quantify graded changes in visual cortical excitability. Further, we demonstrate that intracortical inhibition can be observed in visual cortex, at relatively low CS intensity.

Chapter 3 : Temporal Dynamics of Corticocortical Inhibition in Human Visual Cortex: a TMS Study

3.1 Abstract

Paired-pulse transcranial magnetic stimulation (ppTMS) has been used extensively to probe local facilitatory and inhibitory function in motor cortex. We previously developed a reliable ppTMS method to investigate these functions in visual cortex and found reduced thresholds for net intracortical inhibition compared to motor cortex. The current study used this method to investigate the temporal dynamics of local facilitatory and inhibitory networks in visual cortex in 28 healthy subjects. We measured the size of the visual disturbance (phosphene) evoked by stimulating visual cortex with a fixed intensity, supra-threshold test stimulus (TS) when that TS was preceded by a sub-threshold conditioning stimulus (CS). We manipulated the inter-stimulus interval (ISI) and assessed how the size of the phosphene elicited by the fixed-intensity TS changed as a function of interval for two different CS intensities (45% and 75% of phosphene threshold). At 45% of threshold, the CS produced uniform suppression of the phosphene elicited by the TS across ISIs ranging from 2-200 ms. At 75% of threshold, the CS did not have a significant effect on phosphene size across the 2-15 ms intervals. Intervals of 50-200 ms exhibited statistically significant suppression of phosphenes, however, suppression was not uniform with some subjects demonstrating no change or facilitation. This study demonstrates that the temporal dynamics of local inhibitory and facilitatory networks are different across motor and visual cortex and that optimal parameters to index local inhibitory and facilitatory

influences in motor cortex are not necessarily optimal for visual cortex. We refer to the observed inhibition as visual cortex inhibition (VCI) to distinguish it from the phenomenon reported in motor cortex.

3.2 Introduction

Transcranial magnetic stimulation (TMS) (Barker et al., 1985) is a non-invasive technique commonly used to study sensory and motor cortex physiology. In visual cortex, the dual ability of TMS to induce visual disturbances (so-called phosphenes) or suppress visual perception has been used to assess cortical function under varying circumstances in healthy and clinical populations (Boroojerdi et al., 2000; Cattaneo et al., 2011; Gerwig et al., 2005; Kammer, 1998; Kastner et al., 1998; Silvanto et al., 2007, 2017). In particular, the dependency of TMS effects upon brain activity at the time of stimulation has provided insight into the interaction between subpopulations of neurons in early visual areas that represent task-relevant and task-irrelevant information from the visual field (Silvanto et al., 2017). However, the intracortical mechanisms that allow neuronal responses to be facilitated or suppressed from early to later visual cortical areas are not well understood.

Paired-pulse TMS is used to evaluate local corticocortical connections (Kujirai et al., 1993) by comparing the TMS-evoked behavior elicited by a single suprathreshold test stimulus (TS) alone to the TMS-evoked behavior when the state of the same population of neurons is conditioned by a preceding magnetic stimulus. It is theorized that the state induced by the conditioning stimulus (CS) reflects the net influence of local facilitatory and inhibitory interneuron populations converging on the pyramidal output neuron at the time of TS delivery (Ziemann, Rothwell, et al., 1996), although this hypothesis has not been directly tested in humans. In motor cortex, a key determinant of the effect of the CS upon corticospinal neuron

excitability is the interval between the conditioning and test pulses. A subthreshold (~70-90% of motor threshold) CS occurring 1-5 ms prior to the TS yields decreased motor evoked potential (MEP) amplitudes reflective of a net inhibitory state known as short-interval intracortical inhibition (SICI) (Kujirai et al., 1993). This inhibitory phenomenon is thought to be dominated by fast-acting gamma-Aminobutyric acid (GABA)-A receptor sub-types at the time of TS delivery that hyperpolarize the corticospinal neuron (Di Lazzaro et al., 2000, 2005; Ziemann, Lönnecker, et al., 1996). At intervals between 7-20 ms, the same subthreshold CS intensity increases the amplitude of the MEP elicited by the TS, and is referred to as intracortical facilitation (ICF) (Kujirai et al., 1993). ICF is thought to result from stronger excitatory N-methyl-D-aspartate (NMDA) glutamate receptors overwhelming weaker inhibitory GABA-A receptors, leading to a net facilitation of the response (Schwenkreis et al., 1999). Longer intervals between 50 and 200 ms yield decreased MEP amplitude, a phenomenon known as long-interval cortical inhibition (LICI) (Nakamura et al., 1997). Pharmacological studies indicate that LICI is mediated by the slower-acting GABA-B receptors (Florian et al., 2008; McDonnell et al., 2006).

Similar patterns of net inhibition and facilitation have been observed in prefrontal (Oliveri, Rossini, et al., 2000) and parietal cortices (Oliveri, Caltagirone, et al., 2000). However, in visual cortex, phosphene perception is enhanced by a CS ranging from 60 to 130% of phosphene threshold across interstimulus intervals of 1-20 ms (Kammer & Baumann, 2010; Ray et al., 1998; Sparing et al., 2005). The absence of a SICI-like effect in visual cortex appears to reflect a different balance between inhibitory and excitatory mechanisms in visual compared to motor cortex. We recently demonstrated a SICI-like suppression of phosphene area in early visual cortex with a 2 ms interstimulus interval and a CS intensity of 45% of threshold, much lower than past work. Similar to studies in motor cortex, we found that the effects of

conditioning intensity on response size are non-linear. Phosphene size did not increase as conditioning intensity increased, as demonstrated by the fact that 45% conditioning intensity evoked greater suppression than both higher and lower conditioning intensities (e.g. 30% and 60%). Non-linear responses have also been reported in visual cortex when single pulse TMS is primed by a task-related visual stimulus (Schwarzkopf et al., 2011). For paired-pulse paradigms, the lower CS intensity required for the suppression of single unit activity (Moliadze et al., 2005) and phosphene area (Khammash et al., 2019a) at relatively low CS intensities (15-30% of threshold) for short interstimulus-intervals suggests a local shift in the threshold of excitatory and inhibitory activity. One possibility is that excitatory activity induced by visual afference may interact with the effect of the CS to shift the ratio of excitatory and inhibitory activity elicited at a given CS intensity in an alert, conscious human (Moliadze et al., 2003). The presence of phosphene suppression at relatively lower CS intensities in cat (Moliadze et al., 2005) and human visual cortex (Khammash et al., 2019a) supports the hypothesis that an SICI-like intracortical inhibitory phenomena does exist in visual cortex. However, the temporal dynamics of this paired-pulse TMS state dependency is unknown.

The current study combined paired-pulse TMS and a reliable, quantitative phosphene tracing method (Khammash et al., 2019a) to assess local facilitatory-inhibitory dynamics across inter-stimulus intervals (ISIs) that induce SICI (2, 3 and 5 ms), LICI (10 and 15 ms), and ICF (50, 100 and 200 ms) in motor cortex. Understanding the ways in which CS intensity and ISI affect the phosphene response will make it possible to select optimal parameters for probing the pharmacology and functional significance of inhibitory and excitatory networks in the occipital lobe. It would also open the door to future studies of neuropsychiatric conditions that are

associated with abnormal inhibitory or excitatory processing in visual cortex (e.g., schizophrenia, bipolar disorder).

3.3 Experimental Procedures

3.3.1 Participants

Twenty-eight healthy adults (19 females, nine males, mean age 20.68 ± 2.9 years, range 18-29 years) participated in the present study, which was approved by the University of Michigan's Medical Institutional Review Board (IRBMED). All subjects provided informed consent. All subjects were screened for contraindications to TMS prior to participating. Subjects had no history of psychiatric or neurological disorder, alcohol or drug abuse, traumatic brain injury, brain surgery, implanted metal in the head, or seizures. Additionally, no CNS-active medications were allowed within 48 hours of the study.

This sample was independent from the sample reported in our previous work (Khammash et al., 2019a). Similar to the previous study, all subjects were naïve and had never been exposed to TMS-induced phosphenes. Participants were therefore required to learn what phosphenes are, how to see them, and how to report them using our tracing protocol. We previously demonstrated this system to have high test-retest reliability with no significant difference in phosphene recruitment curves and thresholds across two sessions (Khammash et al., 2019a).

Of the 28 subjects in this study, 8 (~29%) did not report phosphenes and were thus excluded from the study. The proportion of individuals who did not report phosphenes is consistent with our previous work, where 7 out 30 (~23%) participants failed to report phosphenes even after a detailed description and testing across a range of stimulus intensities and occipital targets (Khammash et al., 2019a).

3.3.2 Transcranial magnetic stimulation (TMS)

TMS followed a similar procedure to our previous work (Khammash et al., 2019a). Monophasic posterior-anterior TMS was delivered with a figure-eight coil (model MC-B70, MagVenture Inc, Atlanta, GA) coupled with a MagPro X100 stimulator with Option (MagVenture Inc., Atlanta, GA) over the left primary visual cortex. The Brainsight™ stereotactic guidance system (Rogue Research Inc, Montréal, QC) was used to mark stimulation targets and to ensure accuracy of trajectory. The coil was held tangentially to the scalp with the handle at 90° to midline.

In order to find the optimal site to elicit phosphenes, a virtual 3 x 3 grid of targets spaced 1 cm apart were placed over the left occipital lobe using Brainsight™. The center of the grid was located 1.5 cm lateral and 3 cm dorsal of the inion. Each target was stimulated three times at 60% of maximum stimulator intensity. After each stimulation, participants verbally reported whether or not a phosphene was evoked. If phosphenes were not observed at any of the nine grid points, the intensity was increased in increments of 10% up to 100% (the stimulator's maximum output). In individuals that reported seeing phosphenes, the site that most reliably elicited phosphenes was selected and this hotspot was used for the remainder of the session. Phosphene threshold was then determined at the hotspot by decrementing stimulator output in increments of 2% until phosphene probability fell below 5 out of 10 trials and then incrementing stimulator output by 1% until the 5 out 10 threshold was reached or exceeded.

Single and paired pulses were delivered over the phosphene hotspot. Each participant was administered 10 single pulses at each of the following intensities: 60, 80, 100, and 120% of the phosphene threshold. Additionally, 20 paired pulses were delivered at each of the following ISIs: 2, 3, 5, 10, 15, 50, 100, and 200 ms. Of the 20 paired pulses at each interval, 10 included a CS

intensity of 45% of phosphene threshold, which was previously shown to be optimal for inhibiting the phosphene response in visual cortex (Khammash et al., 2019a). The remaining 10 paired pulses included a CS intensity of 75% of phosphene threshold, an intensity optimal for inhibiting MEPs in motor cortex (Kujirai et al., 1993), but that did not produce significant inhibition or facilitation of phosphenes when previously tested at an ISI of 2 ms (Khammash et al., 2019a). The TS intensity was fixed at 120% of phosphene threshold. For analysis, phosphene sizes at intervals that induce SICI (2, 3, 5 ms), ICF (10, 15 ms), and LICI (50, 100, 200 ms) in motor cortex were averaged and referred to as short, medium, and long ISIs, respectively.

3.3.3 Phosphene quantification

Phosphenes were quantified according to our previously published tracing method (Khammash et al., 2019a). Participants were seated in front of a projector screen. A projector behind the participant projected a custom LabView (National Instruments Co., Austin, TX) display at a size of 102 cm x 57 cm. To minimize ambient light, room lights were turned off and windows blacked out. The only ambient light originated from the projector behind the participant. After each TMS stimulus, participants used the mouse to trace the location and size of observed phosphenes in their visual field. Participants were instructed to first move the mouse to the location of the phosphene, during this phase no trace was generated. Once in the correct location participants were instructed to press the "enter" key to initiate the tracing of the phosphene. The participant's trace appeared as a white outline on a black background and appeared in real-time as the mouse was moved across the screen. If participants made an error during the tracing of the phosphene by either tracing in the wrong location or tracing an inaccurate shape they could press the "escape" key to reset the display and start again. Once participants were satisfied that their trace reflected the correct location and area of the

phosphene, participants were instructed to press the "enter" key again to signify the end of phosphene tracing. Once the participants ended the trial the screen reset in preparation for the next trial. If a phosphene was not observed, participants were instructed to leave the screen blank and press the "enter" key to signify the end of tracing on that trial. Phosphenes were quantified by calculating their size as a percentage of the projected screen area.

3.3.4 Experimental design and procedure

The experiment consisted of a single session. Prior to determination of the phosphene hotspot participants were oriented to the tracing paradigm and instructed to trace the outline of any observed phosphene that immediately followed stimulation. Phosphenes were described as short-lived visual disturbances that often manifest as a fuzzy spot or a spot of colored or white light in the visual field. Following the orientation, the phosphene hotspot and threshold were determined, followed by the single and paired pulse stimulation trials. Single and paired pulses were intermixed to create a set protocol that was administered to all subjects. The intensity and single/paired pulse nature of the stimulus on a given trial was pseudorandomized and was controlled using the line-by-line protocol function of the MagPro X100 stimulator. The experimenter controlled the progression through the line-by-line protocol. The minimum inter-trial interval was 10 seconds to ensure that each trial was independent.

3.3.5 Statistical analysis

Single pulse Stimuli

The average phosphene size traced at each of the single pulse intensities (60, 80, 100, and 120% of phosphene threshold) was calculated and averaged across participants. T-tests, corrected for multiple comparisons using the Bonferroni correction, were conducted between

each successive stimulus intensity to determine the effect of increasing stimulus intensity upon phosphene size.

Paired pulse Stimuli

For each of the two CS intensities (i.e. 45% and 75% of phosphene threshold), the average phosphene size at each of the ISIs (2, 3, 5, 10, 15, 50, 100, 200 ms) was calculated and averaged across participants. Intervals were grouped into short (2, 3, 5ms), medium (10, 15ms), and long (50, 100, 200ms) ISI based on common conventions in motor cortex (Kujirai et al., 1993; Nakamura et al., 1997).

A one-way repeated-measures analysis of variance (ANOVA) with Interval as a within-subject factor (TS alone, short, medium, long) was performed for the 45% CS intensity. The Greenhouse-Geisser method was used to correct for sphericity. Planned comparisons were then conducted to compare the unconditioned (0% CS intensity) and conditioned phosphene size at the short, medium, and long intervals to identify significant suppression or facilitation. The same analysis was repeated for the 75% CS intensity. The Bonferroni method was used to correct for multiple comparisons using an adjusted alpha level of .017 per test.

All single and paired pulse measures were analyzed according to the raw, non-normalized data, the means and standard deviations of which are displayed in Table 1.

Stability of Measures

To measure the consistency of the effects over time and to assess whether there were changes due to fatigue or adaptation to the stimuli, paired *t*-tests were performed comparing phosphene size in each condition (i.e. single pulses and each interval grouping for both

conditioning intensities) during the first vs. last half of the session. The stimulation protocol was set such that trials of each condition were evenly mixed throughout the session (i.e. 5 trials during the first half, 5 trials during the last half).

Table 1. The mean and standard deviation of phosphene area (expressed as a % of the screen) for single and paired pulse conditions

Paradigm	**Condition**	**Phosphene Area (% of Screen)**	
		Mean	**Standard Deviation**
Single Pulse (% of PT)	60%	0.024	0.086
	80%	0.045	0.109
	100%	0.213	0.294
	120%	0.862	0.944
Paired Pulse – 45% Conditioning Intensity	Short ISI (2–5 ms)	0.370	0.461
	Medium ISI (10–15 ms)	0.469	0.543
	Long ISI (50–200 ms)	0.403	0.553
Paired Pulse – 75% Conditioning Intensity	Short ISI (2–5 ms)	0.653	0.726
	Medium ISI (10–15 ms)	0.853	0.898
	Long ISI (50–200 ms)	0.633	0.672

3.4 Results

3.4.1 Phosphene threshold

Average phosphene threshold was 64±8% of maximum stimulator output.

3.4.2 Single pulse

Consistent with our past work, paired *t*-tests demonstrated that the area of reported phosphenes exhibited a positive relationship with stimulus intensity, significantly increasing (p's<.05) with each incremental increase in stimulator intensity after 80% of phosphene

threshold. As expected, phosphenes were rarely elicited at 60% and 80% of phosphene threshold (Figure 3).

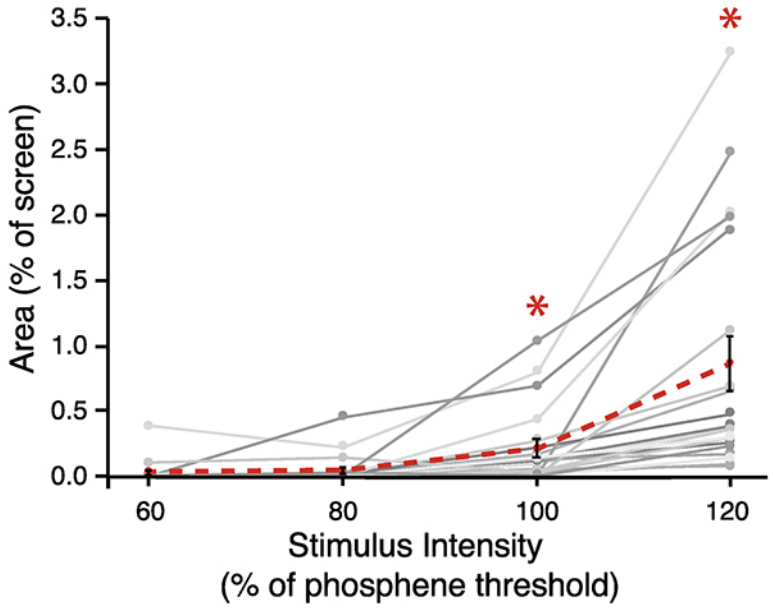

Figure 3. Spaghetti plot of phosphene area as a function of single-pulse stimulus intensity. Each subject is shown as a separate trendline. The dashed line represents the group average. Error bars provided represent the standard error of the mean for the group. * beside the label on the x-axis indicates significantly greater phosphene size than was observed at the preceding intensity ($p < 0.05$).

3.4.3 Paired pulse

The one-way repeated-measures ANOVAs for the 45% CS intensity and the 75% CS intensity both revealed a significant effect of ISI on phosphene size (45%: $F_{3,57} = 8.87$, $\varepsilon = .50$, $p = .002$; 75%: $F_{3,57} = 4.86$, $\varepsilon = .61$, $p = .016$). Paired *t*-tests indicated that a 45% CS intensity resulted in significant suppression of phosphene size, compared to unconditioned size, across all three ISIs (short: $t(19) = 3.70$, $p = .002$, medium: $t(19) = 3.79$, $p = .001$ and long: $t(19) = 2.90$, $p = .009$) (Figure 4A). At 75% CS intensity, phosphene suppression was much smaller overall and was only statistically significant at long ISIs ($t(19) = 2.72$, $p = .014$), but not at the short ($t(19) = 2.02$, $p = .058$) or medium ($t(19) = 0.16$, $p = .878$) intervals, with some subjects displaying no change and others showing facilitation of phosphenes (Figure 4B).

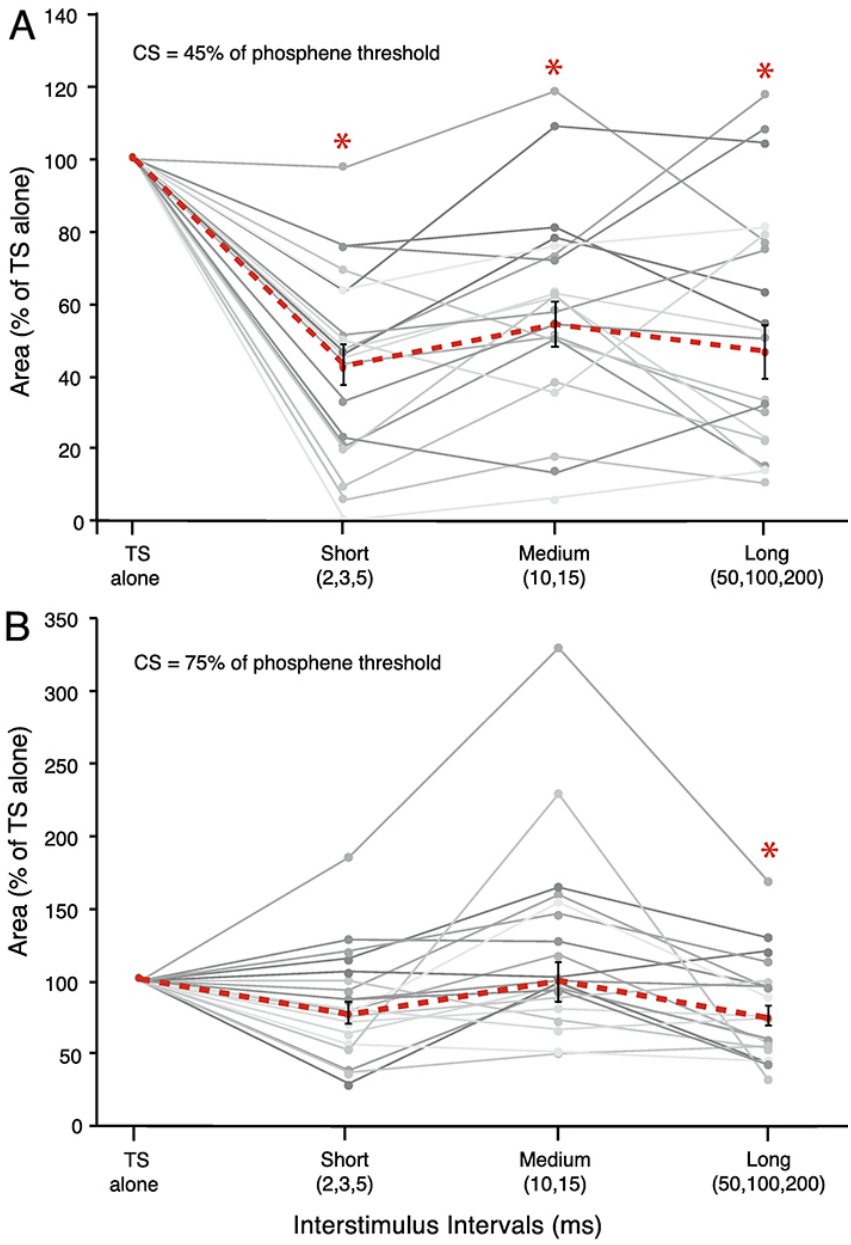

Figure 4. Spaghetti plots of phosphene area as a function of conditioning-test stimulus inter-stimulus interval for (A) a conditioning stimulus of 45% and (B) a conditioning stimulus of 75%. The data was normalized for each subject by dividing the mean phosphene size at each condition by the mean phosphene size when the test stimulus (TS) is delivered alone. Each subject is shown as a separate trendline. The dashed line represents the group average. Error bars provided represent the standard error of the mean for the group. * beside the label on the x-axis indicates significantly smaller phosphene compared to the TS. For the 75% conditioning stimulus intensity, one participant reported particularly large conditioned phosphenes and was classified as an outlier. Removal of this participant did not change the pattern of results.

3.4.4 Stability of measures

Overall, phosphene size was stable within a given condition across the session. Paired *t*-tests indicated no significant difference between the first and last half of the session for all four single pulse intensities and the short, medium, and long paired-pulse intervals for both tested conditioning intensities (all *p*-values between .182 and .824).

3.5 Discussion

The present study used paired-pulse TMS to investigate the temporal dynamics of local facilitatory-inhibitory networks in the visual cortex. Most importantly, we demonstrate the persistence of conditioned phosphene suppression across short (2-5 ms), medium (10-15 ms) and long (50-200 ms) ISIs at a low (45% of threshold) CS intensity. We also observed statistically significant suppression of average phosphene size at long ISIs with a relatively stronger (75% of threshold) CS. However, there was significant heterogeneity in the phosphene response under these conditions and many subjects did not experience a reduction in phosphene size.

The ISIs employed here represent intervals associated with a mix of inhibitory and facilitatory phenomena in the motor cortex at a fixed CS intensity: SICI (intervals of 1-5 ms), ICF (10-15 ms) and LICI (50-200 ms) (Kujirai et al., 1993; Nakamura et al., 1997). Similar patterns of net inhibition and facilitation have been observed in prefrontal (Oliveri, Rossini, et al., 2000) and parietal cortices (Oliveri, Caltagirone, et al., 2000). However, the current results are consistent with past work that demonstrates that the flip from inhibition to facilitation across the 1-20 ms ISI range is absent in visual cortex (Kammer & Baumann, 2010; Sparing et al., 2005). Importantly, the current work is the first observation of persistent inhibition in visual cortex across ISIs associated with SICI and ICF in motor cortex. Past work in visual cortex has solely reported facilitation-like phenomena across ISIs associated with both SICI and ICF in

motor cortex. For example, Kammer and Baumann (Kammer & Baumann, 2010) observed a consistent decrease in phosphene threshold across 2-20 ms ISIs when a threshold TS was preceded by a CS of 100% of threshold. Sparing et al. (Sparing et al., 2005) reported significant facilitation of phosphene detection across ISIs ranging from 2-12 ms for CS intensities ranging from 90-100% of phosphene threshold. The facilitatory effect disappeared with CS intensities ranging from 60-80% of phosphene threshold. Similarly, we failed to observe a CS effect on phosphene detection at 75% of phosphene threshold across the 2-15 ms range of ISIs.

The current study did not assess CS intensities over the 90-100% of phosphene threshold range that lowered phosphene threshold (Kammer & Baumann, 2010) and enhanced phosphene detection (Sparing et al., 2005). However, consistent with current and past work, we have previously demonstrated phosphene area suppression at 45% of phosphene threshold that disappeared at intensities of 60-90% of threshold before eventually producing facilitation at intensities greater than 105% of threshold at a static 2 ms ISI (Khammash et al., 2019a). The emergence of persistent phosphene suppression at low CS intensities independent of ISI, and the eventual transition to facilitation at near-threshold stimulation threshold intensities, are both consistent with past work in cat visual cortex (Moliadze et al., 2005). In cat visual cortex, CS intensities ranging from 15-30% of TS amplitude significantly suppressed visual evoked activity regardless of ISI, while intensities ranging from 60-90% of TS amplitude enhanced visual evoked activity independent of ISI (Moliadze et al., 2005). Further, within each CS intensity range there was little relation between conditioning-TS interval (2-30 ms) and the suppression/enhancement of visual evoked activity elicited by the CS (Moliadze et al., 2005). Taken together, past and current work suggest that visual cortical inhibitory/excitatory dynamics

across ISIs of 2-20 ms are dependent on CS intensity, not the relative interval between the conditioning and test stimuli.

Visual cortex and motor cortex are fundamentally different, given the afferent nature of the former and efferent nature of the latter. One possibility is that the relatively lower CS intensities needed to elicit phosphene suppression reflect a state-dependent shift in the inhibitory/excitatory balance of the neurons subjected to the magnetic stimulus. In visual cortex, the effect of the CS may interact with excitatory/inhibitory activity induced by visual afference related to the environment (Moliadze et al., 2003, 2005). The activation of visual cortex neurons by the CS in the darkened environment devoid of an extrinsic task-related stimulus may have triggered a noise suppression mechanism in visual cortex that in turn reduced the response to the suprathreshold TS. As CS intensity is increased from 45% to 75% of threshold the higher levels of noise induced by the CS may have been more difficult to suppress such that the TS arrived at a time of greater depolarization in the underlying cortex.

The relatively lower CS intensity to induce net inhibition in visual cortex is also consistent with cytoarchitectural differences between the early visual and primary motor cortical areas. Brodmann Area (BA) 17, which encompasses primary visual cortex, contains ~50% more GABA-immunoreactive cells relative to BA 4, which encompasses motor cortex (Hendry et al., 1987). In contrast to GABA-ergic cells, the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), NMDA and kainate glutamatergic receptors is relatively similar across the two areas. The emergence of inhibition at relatively lower CS intensities in visual (~45% of phosphene threshold) compared to motor (~70-90% of motor threshold) cortex (Kujirai et al., 1993) may simply reflect the greater proportional excitation of GABAergic projections by the CS over visual cortex. The greater propensity to recruit more densely situated

GABA projections acting on visual cortical output neurons may also explain the persistence of a net inhibitory state at ISIs of 10-15 ms. Pharmacological studies suggest that motor cortical ICF is mediated by NMDA receptors (Ziemann et al., 1998) and is reduced by positive modulators of GABA-A receptor function (Ziemann, Lönnecker, et al., 1996). Even if the effect of increasing the ISI to 5-20 ms in visual cortex is assumed to increase facilitatory recruitment in visual cortex as in motor cortex, facilitation may be masked by the greater recruitment of GABAergic interneurons and still produce a net inhibition. The net facilitatory state may only emerge at higher stimulation intensities as a result of a plateaued GABAergic recruitment or the temporal summation of the near-threshold CS and TS.

In addition to a simple probabilistic explanation, the emergence of inhibition in visual cortex at relatively lower CS intensities may reflect a differential distribution of GABAergic cells across cortical layers. A significant proportion of the GABA-immunoreactive cells in BA 17 reside in the granular layer, providing a potentially strong influence over cells in this layer receiving visual afferents from the lateral geniculate nucleus (Hendry et al., 1987). In contrast, the densest population of GABA-immunoreactive cells in BA4 are found in Layer II (Hendry et al., 1987), relatively more distant from the Betz cells projecting from Layer V to the internal capsule and spinal cord. As a result, the effect of GABA-A receptor influence on motor efferents may be relatively weaker given the smaller number of GABA-immunoreactive neurons in Layers III and V of motor cortex. Considering the cytoarchitectural differences across visual and motor cortex and the lack of pharmacological studies of visual paired pulse TMS, we suggest that the phenomenon in the visual cortex be referred to as ‘visual cortex inhibition’ or ‘VCI’, to distinguish it from the SICI phenomenon in motor cortex.

Relatively little work in visual cortex has investigated the effect of longer ISIs, associated with LICI in motor cortex. The only long-interval paired pulse study in visual cortex to date employed two subthreshold stimuli (Ray et al., 1998), a significant deviation from the two suprathreshold stimuli used to elicit LICI in motor cortex (Nakamura et al., 1997). We observed statistically significant phosphene area suppression elicited by a suprathreshold stimulus using two different subthreshold CS intensities (45% and 75% of phosphene threshold) at ISIs ranging from 50-200 ms. The suppression of phosphene area at 45% of phosphene threshold is consistent with relatively greater recruitment of low threshold inhibitory networks in visual compared to motor cortex. For the 75% CS (Figure 4B), though the inhibitory effect was statistically significant, many subjects experienced no change in or facilitation of the phosphene elicited by the TS. The more variable phosphene response suggests greater heterogeneity in the inhibitory-excitatory ratio across individuals under these parameters. Thus, we cannot assert that a 75% CS reliably produces inhibition in a given individual. In motor cortex, the CS at short (2-5 ms) and long (100-200 ms) ISIs act upon the same late I-waves (Nakamura et al., 1997). However, pharmacological studies have shown that LICI is dependent upon slower mechanisms mediated by metabotropic GABA-B receptors (Florian et al., 2008). Whether the long-interval effects in visual cortex are mediated by the same GABA-B receptor mechanism is unknown. Future work could employ a suprathreshold CS to establish whether inhibition persists at both sub- and suprathreshold intensities.

One important limitation of our study is that not all subjects reported seeing phosphenes. Specifically, of our 28 participants, 8 did not report phosphenes (~29%). This percentage of non-reporters is consistent with other studies that elicited phosphenes using TMS (Boroojerdi et al., 2000; Khammash et al., 2019a; Sparing et al., 2002, 2005). It is possible that these individuals

simply had phosphene thresholds that exceeded the stimulator's maximum output, or that they remained unsure as to what to look for in their visual field despite the brief experimental orientation. We cannot rule out the possibility that their necessary exclusion removed an important source of physiological variance from the data.

Another limitation is the inability to draw direct comparisons between TMS measurements in visual and motor cortex due to inherent differences between the measured response to TMS. In the current study, we quantified phosphene threshold as the minimal stimulator intensity at which a subject identifies phosphene in their visual field. However, the effect of the CS in the paired pulse paradigm was quantified by measuring the change in the area of the evoked phosphene when a fixed TS was delivered alone versus when it was preceded by the CS. We chose to measure phosphene area because it is a continuous measure which appears more sensitive to the manipulation of our paired pulse paradigm. While phosphene threshold may more closely correspond to motor threshold (e.g. the minimal stimulator intensity at which an MEP can reliably be differentiated from background noise), the change in phosphene area used to quantify the effect of the CS on the volume of cortex that interacts with the TS might not as closely correspond to MEP amplitude. Increases or decreases in phosphene area likely reflect an increase/decrease in the area of visual cortex that is excitable by the TS. On the other hand, MEP amplitudes are typically measured from an individual distal muscle (e.g. first dorsal interosseous, abductor pollicis brevis) and thus reflect changes in excitability within the cortical representation of the specific muscle. A truly parallel measure in motor cortex would require MEP measurement across multiple muscles at once to assess the extent of activation, an approach not typically employed when assessing SICI or LICI in motor cortex.

Overall, the present results show that TMS-induced phosphene tracing can be used to measure cortical inhibition in the visual cortex. Together, our past and current work identify significant differences in the net effects of inhibitory and facilitatory intracortical networks that limit the validity of the typical motor cortex-based paradigms. The identification of parameters that can reliably elicit inhibition in visual cortex opens the door to studying inhibitory processing in the visual cortex of both healthy participants and in populations who may have inhibitory abnormalities.

Chapter 4 : The Effects of Age on Inhibition in Visual Cortex: A Multimodal TMS-MRS Study

4.1 Abstract

Deficits in neural inhibition associated with the neurotransmitter γ-aminobutyric acid (GABA) play a role in several health conditions and may be important in age-related cognitive and sensorimotor decline. Magnetic resonance spectroscopy (MRS) and paired-pulse transcranial magnetic stimulation (ppTMS) are two methods of probing GABAergic neurotransmission in vivo. While MRS can estimate local GABA levels, ppTMS is used to measure $GABA_A$ receptor-mediated inhibitory activity in motor cortex. We recently demonstrated that ppTMS can also be used to assess inhibitory activity in primary visual cortex (V1) through the objective measurement of phosphenes, short-lived visual percepts elicited by occipital stimulation. Here, we utilize MRS and ppTMS to investigate the effect of age on inhibition in V1. Twenty younger (18-28 years) and eighteen older (65-84 years) adult participants completed both an MRS session to assess left V1 GABA concentration and a ppTMS session to assess left V1 inhibitory activity. The TMS-based measure of inhibitory activity was significantly reduced in older compared to younger adults. While the uncorrected MRS measure of left V1 GABA+ was reduced in older adults, tissue-corrected GABA+ measures were unchanged with age. Furthermore, TMS-measured inhibitory activity and tissue-corrected GABA+ were significantly and positively correlated with each other. These results demonstrate that age differences in inhibitory processing extend beyond resting GABA levels and also include differences in inhibitory activity. They also demonstrate the feasibility of using ppTMS to assess inhibitory activity

outside of motor cortex. Finally, they provide important confirmation of the functional relevance of MRS-based measures of GABA in V1.

4.2 Introduction

Magnetic Resonance Spectroscopy (MRS) and paired-pulse transcranial magnetic stimulation (ppTMS) are common, non-invasive techniques used to measure GABAergic mechanisms. Studies using MRS and ppTMS in primary motor cortex (M1) have suggested that each measure reflects different aspects of GABAergic neurotransmission, with MRS assessing local GABA *concentration,* and ppTMS assessing GABAergic *activity*. Specifically, spectroscopy measurements are thought to primarily capture ambient extracellular GABA and cytoplasmic GABA in the cell body, because vesicular pools of GABA in axon terminals are often associated with macromolecules, which may prevent identification on an MRS spectrum (Stagg, Bachtiar, et al., 2011; Stagg, Bestmann, et al., 2011). Specific ppTMS protocols in M1 on the other hand, can be used to measure local $GABA_A$ (Ziemann, Lönnecker, et al., 1996) or $GABA_B$-mediated inhibitory function (McDonnell et al., 2006) by quantifying the physiological state of intracortical synapses in a localized region of the cortex. Therefore, because both MRS and ppTMS are assessing different aspects of GABAergic transmission, together they can generate a more complete picture of how disease and aging affect different aspects of GABAergic signaling.

Many studies have used MRS to quantify age-related differences in GABA levels. While several such studies have reported reductions in GABA with age across various cortical areas (Chamberlain et al., 2021; Gao et al., 2013; Hermans et al., 2018; Lalwani et al., 2019; Marenco et al., 2018; Porges et al., 2021; Simmonite et al., 2019), there are mixed results as to whether age-related reductions in GABA concentration occur in early visual areas (Hermans et al., 2018; Maes et al., 2018; Pitchaimuthu et al., 2017; Simmonite et al., 2019).

Often, ppTMS measures of inhibition are limited to M1 due to its objectively measurable output. Stimulation of M1 can elicit a measurable motor evoked potential (MEP) in a hand muscle with skin surface electrodes. Measured MEP amplitudes can be used as an assay for the relative contributions of localized GABA-mediated inhibitory networks in M1 using a well-established ppTMS protocol called short interval intracortical inhibition (SICI) (Kujirai et al., 1993). Pharmacological studies indicate that this SICI effect in M1 is mediated by $GABA_A$ receptors, since positive allosteric modulators of $GABA_A$ (e.g. benzodiazapines) increase the measured inhibitory effect (Di Lazzaro et al., 2005; Ziemann, Lönnecker, et al., 1996).

Our group recently demonstrated that ppTMS can be used to assess inhibition in primary visual cortex (V1) by measuring participant-traced phosphenes, short-lived visual disturbances that result from occipital stimulation (Khammash et al., 2019a, 2019b). In this paradigm, a decrease in the conditioned phosphene size compared to the unconditioned phosphene size reflects greater excitability of visual inhibitory networks. We demonstrated high test-retest reliability of this method and determined the optimal ppTMS parameters for this SICI-like paradigm in V1. Here, we use this paradigm to explore the relationship between age and V1 inhibitory activity. Age-related changes in SICI have previously been investigated in M1 with mixed results (Age-related decrease: (Marneweck et al., 2011; Peinemann et al., 2001); Age-related increase: (Kossev et al., 2002; McGinley et al., 2010; A. E. Smith et al., 2009); No change with age: (Cirillo et al., 2010; Oliviero et al., 2006; Rogasch et al., 2009)). However, a meta-analysis of 11 studies that investigated the effects of aging on motor neurophysiology yielded a slight, but insignificant, reduction in motor SICI with age (Bhandari et al., 2016).

The present study assesses GABA levels (measured via MRS) and cortical inhibition (measured via ppTMS) in the left V1 of healthy older and younger adults. We investigate whether

GABA levels and cortical inhibition in V1 change with age. To our knowledge, this is the first study to examine aging effects with ppTMS in V1. We also explore the relationship between MRS measures of V1 GABA and TMS measures of visual cortical inhibition.

4.3 Methods

4.3.1 Participants

29 younger (15 men and 14 women, mean age 23.0 ± 3.0 years, range = 18-28 years) and 49 older (21 men and 28 women, mean age 70.7 ± 4.7 years, range = 65-84 years) right-handed adults participated in the current study. All participants took part in the Michigan Neural Distinctiveness (MiND) study (Gagnon et al., 2018). Prior to enrollment, all subjects were screened for contraindications to TMS and MRI (Rossi et al., 2021). All subjects were free of psychiatric or neurological disorders, traumatic brain injury, implanted metal in the body, history or family history of seizures, use of CNS-active medications, and a history of drug or alcohol abuse. Additional exclusion criteria for the MiND study included hearing aids, color blindness, motor control problems, non-native English speakers, and drinking more than a moderate amount of alcohol (defined as an average of >4 drinks per week). All participants provided informed consent and the Institutional Review Board of the University of Michigan approved the study protocols (HUM00050673, HUM00103117).

Of the 78 subjects who participated in the present study, GABA was successfully estimated in all but three subjects. However, phosphenes could not be evoked in three younger adults and 16 older adults even after testing at a range of stimulus intensities and V1 targets. The proportion of younger adults who did not report phosphenes (~10%) was lower than in our previous work investigating the phosphene response in a young population (Khammash et al., 2019a, 2019b). In addition, three younger and nine older adults had a phosphene threshold too

high to run our protocol based on the limits of our TMS machine. The TMS data of one younger and five older adult subjects were also excluded for not meeting reliability criteria for inclusion (see *Phosphene Data Quality Control*). Lastly, one older and one younger subject withdrew from the study, and one younger adult was excluded due to a vasovagal syncope event resulting from TMS stimulation. Therefore, the TMS dataset consisted of 18 older adults and 20 younger adults, and our MRS dataset consisted of 47 older adults and 28 younger adults.

4.3.2 Transcranial Magnetic Stimulation

A MagPro X 100 stimulator (MagVenture Inc., Atlanta, GA) with a figure-eight coil (MC-B70, MagVenture Inc, Atlanta, GA) was used to deliver posterior-anterior single-pulse and paired-pulse TMS over the left occipital cortex. A frameless stereotactic neuronavigation system was used to mark scalp locations and to ensure accuracy of stimulation location and trajectory during testing (Brainsight 2 Rogue-Research Inc., Montreal, Canada). Participants sat in a dark room and were instructed to fix their gaze on the center of a large 188cm (L) x 107cm (W) wall-projected screen situated 120cm away in the vertical plane. This screen displayed a custom LabView (National Instruments Co., Austin, TX) program that participants could use to trace perceived phosphenes using a mouse and keyboard interface. Prior to beginning the TMS protocol, research personnel verbally explained to participants that stimulation over the occipital lobe may result in brief visual disturbances called phosphenes. Participants were told that phosphenes were subtle and short-lived, and that they could appear anywhere in the visual field. Participants were also told that the sensation may differ from person to person, with most reporting either dull flashes of light or a focal ‘fuzzy’ spot. In order to discourage response bias, research personnel also emphasized that most— but not all— participants see phosphenes.

The method described in Khammash et al. (Khammash et al., 2019a, 2019b) was used to determine the optimal stimulation location and intensity for visual cortex stimulation. First, BrainSight™ was used to generate a 3 x 3 grid of targets over the left occipital lobe. Grid points were 1cm apart and the center was placed 3cm anterior and 1.5cm left-lateral of the inion. The coil was pressed tangentially against the scalp, with the handle angled 90° to the midline. Starting at 60% of the maximum stimulator intensity, each grid target was stimulated three times. After each stimulation, the participant reported whether or not they perceived a phosphene. If no phosphenes were evoked at any of the 9 grid targets, the stimulator intensity was increased by 10% and all targets tested again at the new intensity. This process was repeated until a tested intensity evoked phosphenes at one or more locations. If more than one location evoked phosphenes, the location that generated the largest most consistent phosphenes was selected as the hotspot. At this hotspot, the phosphene threshold was calculated by incrementing or decrementing the stimulator by first 2%, and then fine-tuning by 1% increments, until the intensity that elicited phosphenes on 5 out of 10 trials was achieved.

Participants received 10 single pulses at stimulation intensities of 60, 80, 100, 120, and 140% of phosphene threshold. We previously demonstrated that paired-pulses with a conditioning stimulus of 45% of phosphene threshold delivered 2ms before a 120% test stimulus significantly inhibits phosphene size compared to when the test stimulus is delivered alone (Khammash et al., 2019a, 2019b). Participants were therefore administered 10 paired-pulses with these parameters (i.e. conditioning stimulus at 45% of phosphene threshold, test stimulus at 120% of phosphene threshold, 2ms interstimulus interval). Phosphenes elicited by this paired-pulse stimulation were then compared to phosphenes elicited by the same test stimulus (120% of phosphene threshold) but without a preceding conditioning stimulus (i.e., single-pulse).

Phosphene quantification was performed using our previously validated tracing method (Khammash et al., 2019a, 2019b). For each trial, if the TMS stimulus evoked a phosphene, participants were instructed to move the mouse to the screen location where the phosphene appeared and outline the shape of the perceived phosphene with the mouse cursor. If a TMS stimulus did not evoke a phosphene, the participant was instructed to press the "enter" key twice, which resulted in a blank screen being saved. Phosphene traces were then quantified by calculating their area as a percentage of the projected screen's area. Phosphenes were additionally quantified in terms of their frequency by calculating the percentage of phosphene-present trials for each condition for each subject.

4.3.3 Magnetic Resonance Spectroscopy

GABA edited MR spectra were collected in a left primary visual cortical voxel using a MEGA-PRESS (Mescher et al., 1998)(Mescher et al., 1998) sequence with the following acquisition parameters: TR = 1800 ms; TE = 68 ms (TE1 = 15 ms, TE2 = 53 ms); 256 transients (128 ON interleaved with 128 OFF) of 4096 data points; spectral width = 5 kHz; frequency selective editing pulses (14 ms) applied at 1.9 ppm (ON) and 7.46 ppm (OFF); FOV = 240 × 240 mm; voxel size = 30 × 30 × 30mm. The acquisition time for this voxel was approximately 8.5 min. The left primary visual voxel was placed using a template and was positioned as close to the cortical surface as possible in order to overlap with the TMS-targeted region. Figure 5 shows the voxel placement for participants that have both TMS and MRS data.

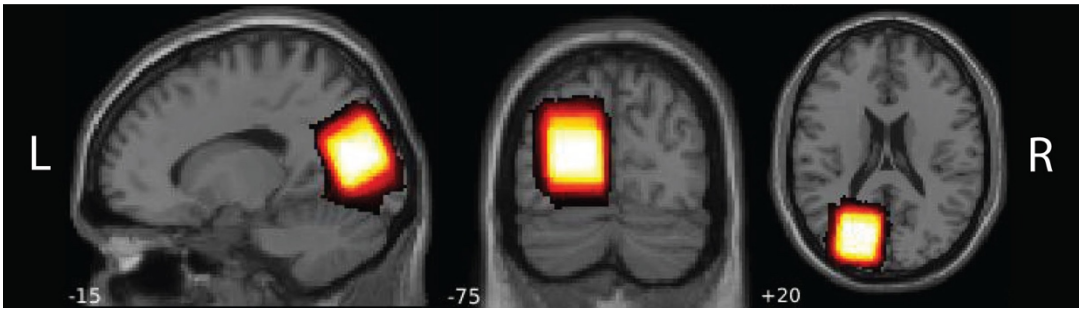

Figure 5. Left primary visual MRS voxel locations for participants with both TMS and MRS data (N = 38). Brighter coloration (white) indicates the greatest overlap, while darker coloration (red) indicates the least overlap.

4.3.4 Data Preprocessing

Spectral Fitting and GABA estimation

The MATLAB toolbox Gannet 3.0 (Edden et al., 2014) was used to estimate GABA concentration in the left primary visual MRS voxel. Spectral registration was used to frequency- and phase-correct time-resolved data. Time-resolved data was filtered with 3Hz exponential line broadening and zero-filled by a factor of 16. The 3-ppm GABA peak in the difference spectrum was modeled using a five-parameter Gaussian model between 2.19 and 3.55 ppm. GABA was quantified relative to water, which was fit using a Gaussian-Lorentzian model, and is expressed in institutional units (i.u.). GABA levels substantially differ across tissue type (i.e. grey matter, white, cerebrospinal fluid), and voxel composition varies across subjects (Harris, Puts, & Edden, 2015). Additionally, water relaxation times differ between tissues (Harris, Puts, & Edden, 2015). Therefore, voxel composition was calculated for each participant by co-registering each subject's anatomical image to their MRS voxel, and then performing segmentation of the anatomical image using SPM12 Segmentation. These tissue composition calculations were then used to generate tissue-corrected GABA estimates using methods described by Harris et al. (Harris, Puts, Barker, et al., 2015). All analyses were performed using both uncorrected and tissue-corrected

GABA estimates. Because the GABA peak in the GABA-edited spectrum includes macromolecules, we will use the term GABA+ (i.e., GABA + macromolecules) when describing the results, in keeping with previous reports (e.g., (Edden et al., 2014)).

Phosphene data quality control

One concern with self-reported phosphenes is the propensity for some participants to respond the way they think they are supposed to, rather than based on their true experiences. Despite reducing demand characteristics by telling participants that it is not abnormal if one does not see phosphenes, the concern remains that some individuals' reports of phosphenes may not be accurate. We therefore established objective quantitative criteria with which to determine if a subjects' data is reliable or should be excluded from analysis. In our previous studies, we demonstrated that phosphene size scales linearly with stimulus intensity for single pulses ranging from 80% to 140% of phosphene threshold (Khammash et al., 2019a, 2019b), much like MEPs scale with stimulus intensity in motor cortex. Consequently, for each participant in the current study, we assessed phosphene size as a function of stimulus intensity by using regression to fit a linear model to their data from the single-pulse 80, 100, 120, and 140% of phosphene threshold conditions. For each model, if stimulus intensity was a significant predictor of phosphene size, we judged the associated participant's responses to be genuine. If not, we excluded their TMS data from further analysis. Single-pulse stimulus intensity did not predict phosphene size in five older adults and one younger adult.

4.3.5 Statistical Analysis

All statistical analyses were conducted using R (version 1.2.5042).

Phosphene Threshold

Average phosphene thresholds were calculated for the combined sample of older and younger adults as well as within each group separately. Age differences in phosphene threshold were assessed with a two-sample *t*-test.

Four logistic regression analyses were conducted to determine whether uncorrected GABA+, tissue-corrected GABA+, the voxel grey matter percentage, or age group were significant predictors of whether a participant reports phosphenes. These regression analyses included participants that reported phosphenes but had too high of a phosphene threshold to perform the experimental protocol.

Single-pulse TMS data

For each subject, single-pulse recruitment curves were derived from the single-pulse intensities (60, 80, 100, 120, and 140% of phosphene threshold) in two ways: 1) By averaging the phosphene size (as a % of the screen size) traced at each intensity, and 2) Using frequency of phosphenes at each intensity (i.e. the % of the 10 trials that evoked a phosphene). A two-way repeated-measures analysis of variance (ANOVA) with Condition as a within-subject factor (60, 80, 100, 120, and 140% of phosphene threshold) and Age Group as a between-subject factor (older adults, younger adults) was conducted on the phosphene size data derived from single-pulse stimulation. Sphericity was corrected using the Greenhouse-Geisser method. The same analysis was also performed on the phosphene frequency data derived from single-pulse stimulation. Planned comparisons with a Bonferroni-adjusted alpha level of 0.025 were conducted between each successive stimulus intensity to determine the effect of increasing stimulus intensity upon both phosphene size and phosphene frequency in the whole sample.

Age differences in Cortical inhibition

In two independent groups of young healthy participants, we previously demonstrated that a conditioning stimulus that is 45% of the phosphene threshold was optimal for reducing the size of phosphenes elicited by the test stimulus (120% of phosphene threshold) relative to the single pulse condition (Khammash et al., 2019a, 2019b). One objective of the present study was to determine whether this inhibitory effect was reduced in older compared to younger adults. We therefore tested for age differences in this effect, using both phosphene size and phosphene frequency as dependent measures. Consistent with our past work, the control (single-pulse) condition consisted of a 120% of phosphene threshold test-stimulus, with a 0% (i.e. absent) conditioning stimulus. The paired-pulse condition, intended to induce inhibition of the phosphene response, consisted of the same 120% of phosphene threshold test-stimulus, preceded by a 45% of phosphene threshold conditioning stimulus.

To analyze the phosphene area data, we used a linear mixed effects model (LMEM) which could accommodate repeated measures and participant-level variability (as a random effect). R's "lme4" package was used to fit and analyze the linear mixed effects model (Bates et al., 2015). In this model, phosphene size as a percentage of the drawing screen was used as the outcome measure, which included 20 observations per subject (10 single-pulse and 10 paired-pulse). Age group (young vs. old), stimulus condition (single vs. paired-pulse), and an age group × stimulus condition interaction term were included in the model as fixed effects. Participant was included as a random effect (varying intercept). The model was specified as follows: PhospheneSize ~ AgeGroup + StimulusCondition + AgeGroup:StimulusCondition + (1|Participant). To reduce skewness of the response variable and ensure normality of residuals, an arcsinh transformation was applied to the

dataset. An arcsinh transformation is preferable to a log transformation for phosphene size data, as sometimes a stimulus may not elicit a phosphene at all, and ln(0) is undefined.

To determine whether participants also experience a reduction in phosphene frequency in this paradigm, as well as whether this effect changes with age, we conducted a two-way repeated-measures analysis of variance (ANOVA) with stimulus condition as a within-subject factor (single-pulse vs. paired-pulse) and age group as a between-subject factor (young vs. old). We opted for an ANOVA, rather than another LMEM, because phosphene frequency was derived by calculating the percentage of phosphene-present trials, yielding only one observation per subject per condition.

Age differences in GABA+

Age differences in both uncorrected and tissue-corrected MRS-derived GABA+ were assessed using two pooled variances t-tests; $p<.05$ was considered statistically significant.

Tissue Composition

The average tissue composition of the left primary visual MRS voxel was calculated in younger and older adults in both the whole sample as well as in the subsample of participants with TMS data. Age differences in grey matter, white matter, and cerebrospinal fluid percentages were assessed with two-sample t-tests.

Relationship between cortical inhibition and GABA

The average phosphene size and phosphene frequency in the single-pulse (no conditioning stimulus) and paired-pulse (conditioning stimulus at 45% of phosphene threshold) conditions was calculated for each participant. A size-based index of visual cortical inhibition was calculated for each participant using the following formula:

$$1 - \frac{avg.conditioned\ size}{avg.unconditioned\ size}.$$

Similarly, a frequency-based index of visual cortical inhibition was also calculated for each participant:

$$1 - \frac{conditioned\ frequency}{unconditioned\ frequency}.$$

Zero-order Pearson correlations and partial correlations controlling for age were conducted to assess the relationship between size-derived metrics of visual cortical inhibition, and both uncorrected and tissue-corrected left V1 GABA+. The same analyses were also performed using the frequency-derived metric of visual cortical inhibition. Pearson correlations examining the inhibition-GABA+ relationship were also performed separately within each age group using both size and frequency metrics of inhibition.

4.4 Results

4.4.1 Phosphene threshold

Average phosphene threshold for the combined sample of older and younger adults was 66.4 ± 10.7% of maximum stimulator output. Average phosphene threshold was 68.3 ± 8.6% and 64.6 ± 12.3% of maximum stimulator output within the older and younger groups, respectively. T-tests showed no significant difference in phosphene threshold between older and younger adults ($t(36) = -1.08$, $p = 0.289$). Figure 6 shows the V1 hotspot locations for all 38 subjects in MNI space.

Logistic regression analyses indicated that neither uncorrected GABA+ (b = 0.15 (1.25), $p = 0.905$), tissue-corrected GABA+ (b = 0.43 (1.01), $p = 0.670$), nor the voxel grey matter percentage (b = 5.78 (6.16), $p = 0.349$) significantly predicted whether or not an individual reported phosphenes. However, age group was a significant predictor (b = 1.51 (0.69), $p = 0.028$), such that younger adults were 4.5x more likely to report phosphenes than older adults.

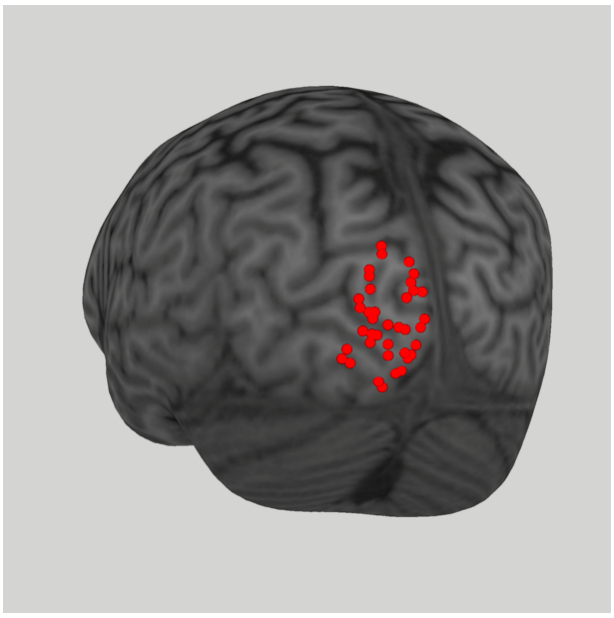

Figure 6. MNI locations of TMS phosphene hotspots in left V1. N = 38 subjects.

4.4.2 Single-pulse TMS data

The two-way repeated-measures ANOVA revealed a significant main effect of stimulus intensity on phosphene size ($F(1.43,48.53) = 23.28$; $p < 0.0001$). There were no differences in phosphene size with age ($F(1,34) = 0.12$; $p = 0.73$), and age did not interact with stimulus intensity ($F(1.43,48.53) = 0.32$; $p = 0.65$; Figure 7a). Paired t-tests indicated that phosphene size was positively associated with stimulus intensity, significantly increasing (p's < .05) with incremental increases in stimulus intensity between 80-140% of phosphene threshold (Figure 7b). This finding is consistent with our past work (Khammash et al., 2019a, 2019b). The second two-way repeated-measures ANOVA revealed a significant main effect of stimulus intensity on phosphene frequency ($F(2.79,94.73) = 190.49$; $p < 0.0001$). There were no differences in phosphene frequency with age ($F(1,34) = 0.28$; $p = 0.60$), and age did not interact with stimulus intensity ($F(2.79,94.73) = 0.76$; $p = 0.51$; Figure 8a). Paired t-tests demonstrated that phosphene frequency was also positively associated with stimulus intensity, significantly increasing (p's <

.05) with incremental increases in stimulus intensity between 60-120% of phosphene threshold (Figure 8b). Phosphene frequency tended to plateau after 120% of phosphene threshold. The means and standard deviations of raw, non-normalized data are displayed in Table 2.

Table 2. The mean and standard deviation of phosphene area and phosphene frequency in older and younger adults, for single and paired pulse conditions.

Paradigm	**Condition**	**Phosphene Area (% of Screen)**				**Phosphene Frequency (% of Trials)**			
		Mean Older	**SD Older**	**Mean Younger**	**SD Younger**	**Mean Older**	**SD Older**	**Mean Younger**	**SD Younger**
Single pulse (% of phosphene threshold)	**60%**	0.020	0.045	0.030	0.067	10.000	17.489	6.167	9.052
	80%	0.013	0.022	0.035	0.064	12.222	19.268	15.389	18.823
	100%	0.499	1.096	0.225	0.270	47.778	32.769	53.944	25.466
	120%*	0.903	1.042	0.691	0.730	81.543	18.570	82.500	18.317
	140%	1.540	1.777	1.608	1.770	86.736	16.691	87.500	17.434
Paired pulse (conditioning stimulus % of phosphene threshold)	**0%***	0.903	1.042	0.691	0.730	81.543	18.570	82.500	18.317
	45%	0.702	0.918	0.316	0.356	64.444	19.470	66.000	25.629

*Single-pulse 120% and paired-pulse 0% conditioning stimulus conditions are equivalent. Values are derived from the same set of 10 trials.

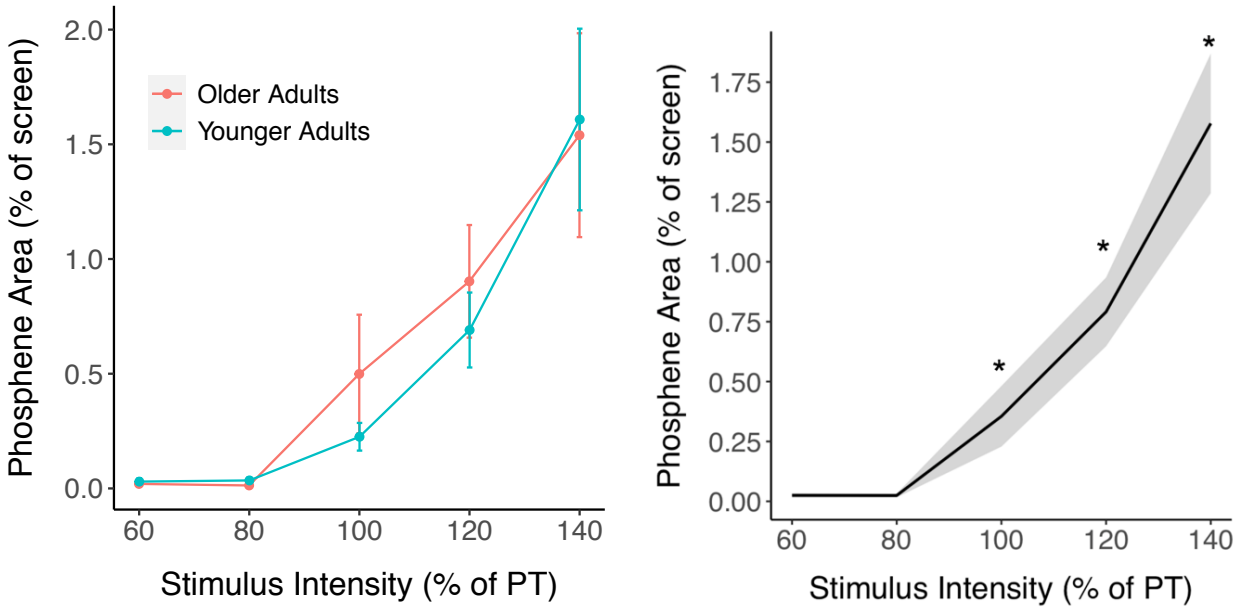

Figure 7. a.) Phosphene area as a function of single-pulse stimulation intensity for both older and younger adults. Points represent mean phosphene size at each intensity condition. Error bars represent the standard error of the mean. **b.)** Mean phosphene area as a function of single-pulse stimulation intensity for the whole sample. An asterisk (*) suggests that a given stimulus intensity produces significantly larger phosphenes than the previous intensity ($p < 0.05$). The error ribbon represents the standard error of the mean.

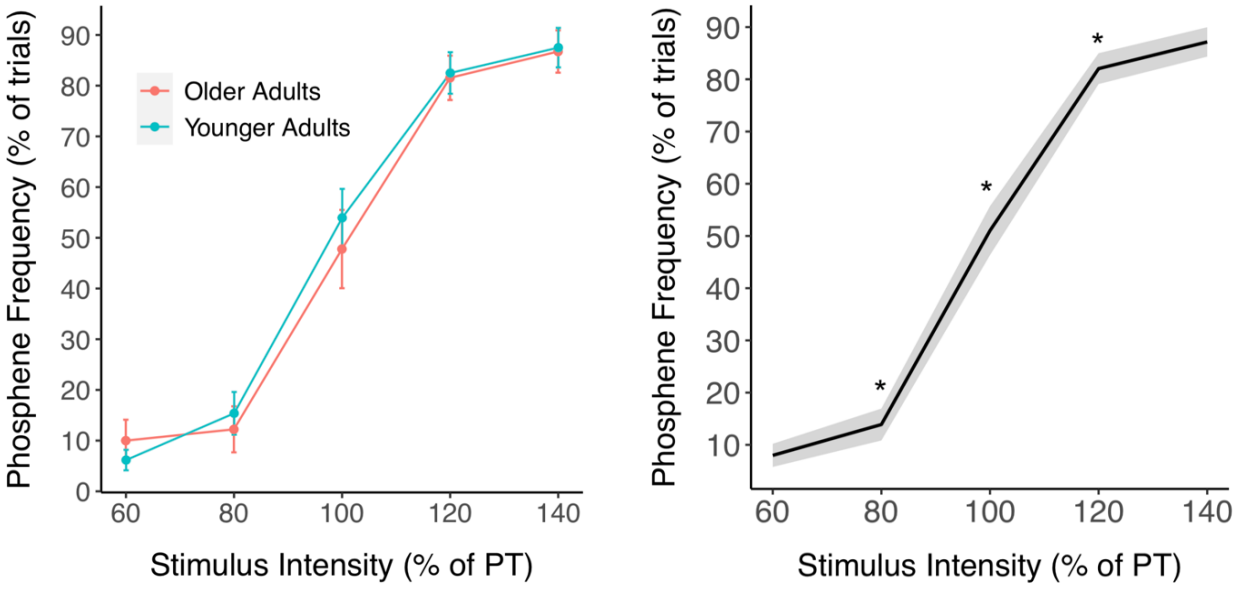

Figure 8. a.) Phosphene frequency as a function of single-pulse stimulation intensity for both older and younger adults. Points represent mean frequency at each intensity condition. Error bars represent the standard error of the mean. **b.)** Mean phosphene frequency as a function of single-pulse stimulation intensity for the whole sample. An asterisk (*) suggests that a given stimulus intensity elicits phosphenes on a significantly larger proportion of trials than the previous intensity ($p < 0.05$). The error ribbon represents the standard error of the mean.

4.4.3 Age differences in Cortical Inhibition Based on Paired-Pulse TMS

Results of the LMEM indicate that younger adults experienced a greater reduction in phosphene size in response to the conditioned stimulus compared to older adults, reflecting decreased visual cortical inhibition with age (Figure 9a, 9b). The model yielded a significant interaction of age and condition (paired-pulse vs. single-pulse) (beta = -0.19, t = -2.79, p = .005), along with a main effect of condition that approached significance (beta = -0.10, t = -1.96, p = .051). There was no main effect of age on phosphene size ((beta = -0.002, t = -0.02, p = .984). We also performed the same analysis while including the MRS voxel grey matter percentage as a nuisance covariate, and the age x condition interaction remained significant, $p = 0.023$.

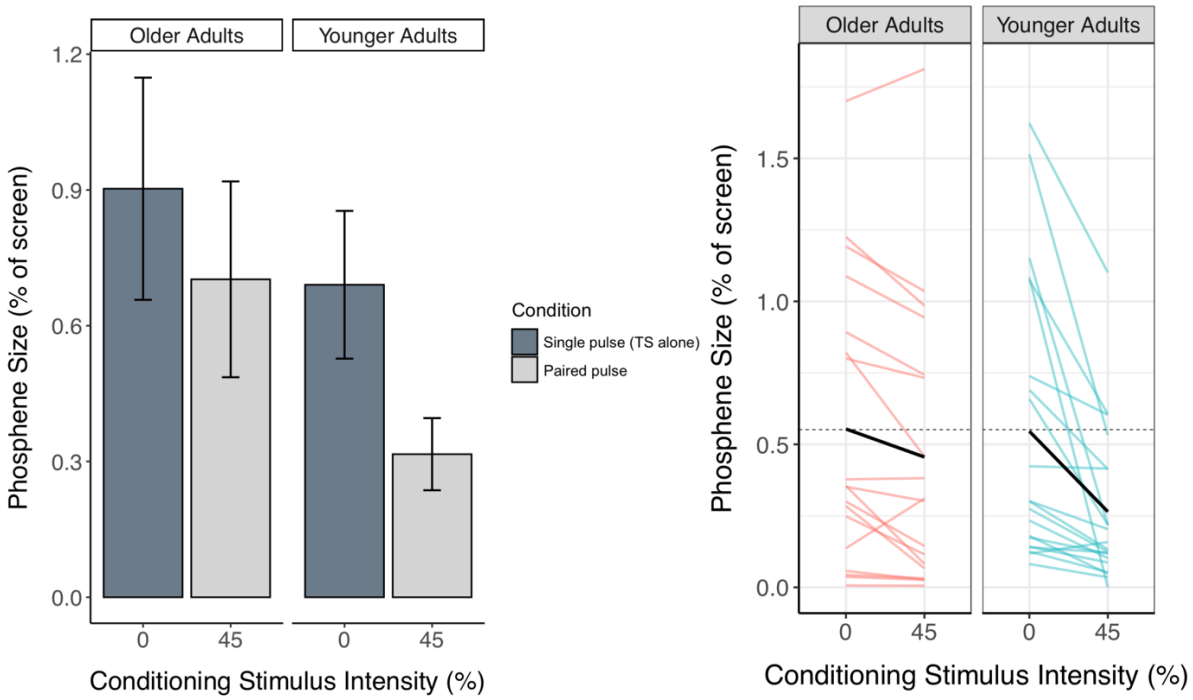

Figure 9. a.) Bar graph of phosphene size as a function of condition for older and younger adults. Bars represent the average size of phosphenes elicited during either the single-pulse (no conditioning stimulus) or paired-pulse (conditioning stimulus at 45% of phosphene threshold) condition. N = 18 older adults, 20 younger adults. Error bars denote the standard error of the mean. **b.)** Average phosphene size as a function of condition for each subject. A linear mixed effects model was built with age and condition as fixed effects, and with participant as a random effect. Each subject is represented by a separate line. Steeper negative slopes indicate stronger inhibition of phosphene size resulting from the conditioning stimulus. The black solid lines indicate the fixed effect slopes of the two age groups. The dotted horizontal line represents the model intercept.

The two-way repeated-measures ANOVA for phosphene frequency revealed a significant main effect of condition (paired-pulse vs. single-pulse) ($F(1,36) = 27.10$; $p < 0.0001$). There were no differences in phosphene frequency with age ($F(1,36) = 0.05$; $p = 0.83$), and age did not interact with condition ($F(1,36) = 0.01$; $p = 0.93$; Figure 10).

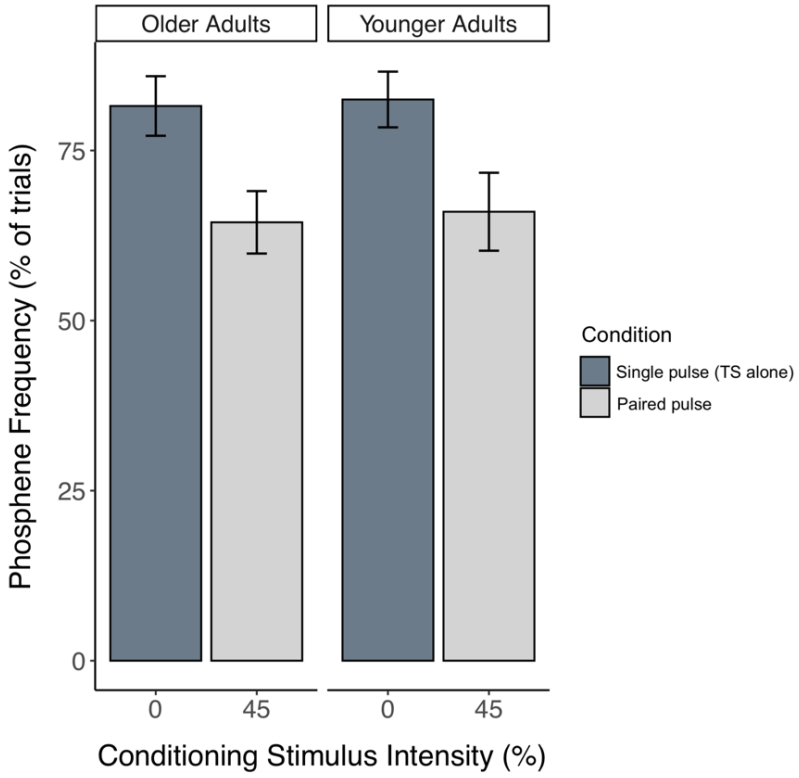

Figure 10. Bar graph of phosphene frequency as a function of condition for older and younger adults. Bars represent the average frequency of phosphenes elicited during either the single-pulse (no conditioning stimulus) or paired-pulse (conditioning stimulus at 45% of phosphene threshold) condition. N = 18 older adults, 20 younger adults. Error bars denote the standard error of the mean.

4.4.4 Age differences in GABA+

Uncorrected GABA+ levels were significantly higher in younger adults than older adults ($t(73) = -2.29$, $p = 0.025$). However, tissue-correction eliminated this difference between age groups ($t(73) = -0.66$, $p = 0.514$; Figure 11). The means and standard deviations of both uncorrected and tissue-corrected GABA+ levels are presented in Table 3. When these analyses

are repeated in only the subset of participants with TMS data, the same pattern of results is observed (uncorrected GABA+: Y > O, $p = 0.015$; tissue-corrected GABA+: n.s., $p = 0.486$).

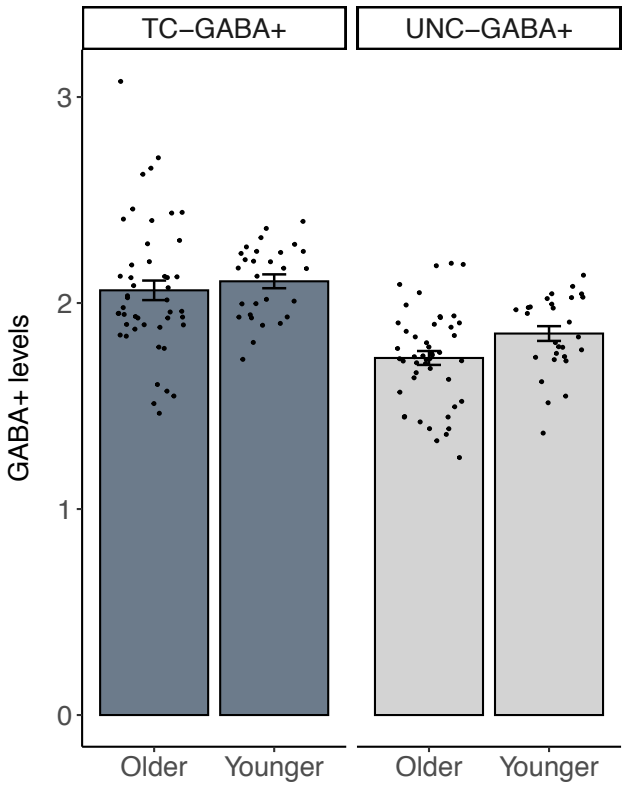

Figure 11. Bar graph of both tissue-corrected and uncorrected left V1 GABA+ levels in older and younger adults. Bars represent average GABA+ levels for each group. Dots represent observations from individual subjects, N = 47 older adults, 28 younger adults. Error bars denote the standard error of the mean.

Table 3. The means and standard deviations of MRS-derived estimations of H2O-referenced uncorrected and tissue-corrected GABA+ in left V1 of older and younger adults. All values in institutional units (i.u.).

	Older Adults (N = 47)		**Younger Adults (N = 28)**	
	Mean	**SD**	**Mean**	**SD**
GABA+	1.733	0.231	1.852	0.190
TC-GABA+	2.062	0.325	2.106	0.178

4.4.5 Tissue Composition

The average tissue composition of the left primary visual MRS voxel in both the whole sample of younger and older adults, as well as the subsample with TMS data are displayed in Table 4. In both analyses, younger adults had greater grey matter percentages and lower cerebrospinal fluid percentages than older adults (all p's < .001). There were no age-differences in white matter percentages (p's > .05).

Table 4. Tissue composition of the left primary visual MRS voxel both in older and younger adults.

Tissue Type	All participants with MRS data			Subset of participants with MRS and TMS data		
	Younger Adults (N = 28)	**Older Adults (N = 47)**	**Differences**	**Younger Adults (N = 20)**	**Older Adults (N = 18)**	**Differences**
Grey Matter	47.6% ± 3.6%	41.8% ± 4.0%	Y > O, p < .001	47.9% ± 3.1%	42.5% ± 3.6%	Y > O, p < .001
White Matter	45.5% ± 4.0%	46.0% ± 4.2%	n.s., p = .617	45.3% ± 3.6%	45.4% ± 4.4%	n.s., p = .969
Cerebrospinal Fluid	6.9% ± 3.0%	12.1% ± 4.5%	O > Y, p < .001	6.7% ± 3.3%	12.1% ± 4.1%	O > Y, p < .001

4.4.6 Relationship between cortical inhibition and GABA+

Using the phosphene size-derived metric of visual cortical inhibition

Across all participants, a significant positive correlation was found between visual cortical inhibition measured using ppTMS and tissue-corrected V1 GABA+ levels measured using MRS ($r(35) = 0.49$, $p = 0.002$; Figure 12a). This correlation remained significant when partial correlations were used to control for age ($r(34) = 0.48$, $p = 0.003$). Assessed within each age group separately, size-derived visual cortical inhibition was significantly positively associated with tissue-corrected GABA+ in older adults ($r(16) = 0.52$, $p = 0.028$). In younger adults, the relationship between these measures was positive but was only marginally significant ($r(17) = 0.40$, $p = 0.093$).

A positive relationship between size-derived visual cortical inhibition and uncorrected V1 GABA+ levels also approached significance ($r(35) = 0.30$, $p = 0.070$; Figure 12b). Size-derived visual cortical inhibition was not significantly associated with uncorrected GABA+ in either age group when analyzed separately (older ($r(16) = 0.21$, $p = 0.393$; younger ($r(17) = 0.33$, $p = 0.173$).

We repeated these analyses excluding one outlier participant (Cook's Distance > 1, the pink dot on the bottom left of Figures 12a and 12b). This older adult experienced strong facilitation of the phosphene response in the paired-pulse condition, resulting in a negative index of visual cortical inhibition with a large magnitude. The pattern of data remained the same after excluding this participant, with the same analyses remaining significant/insignificant.

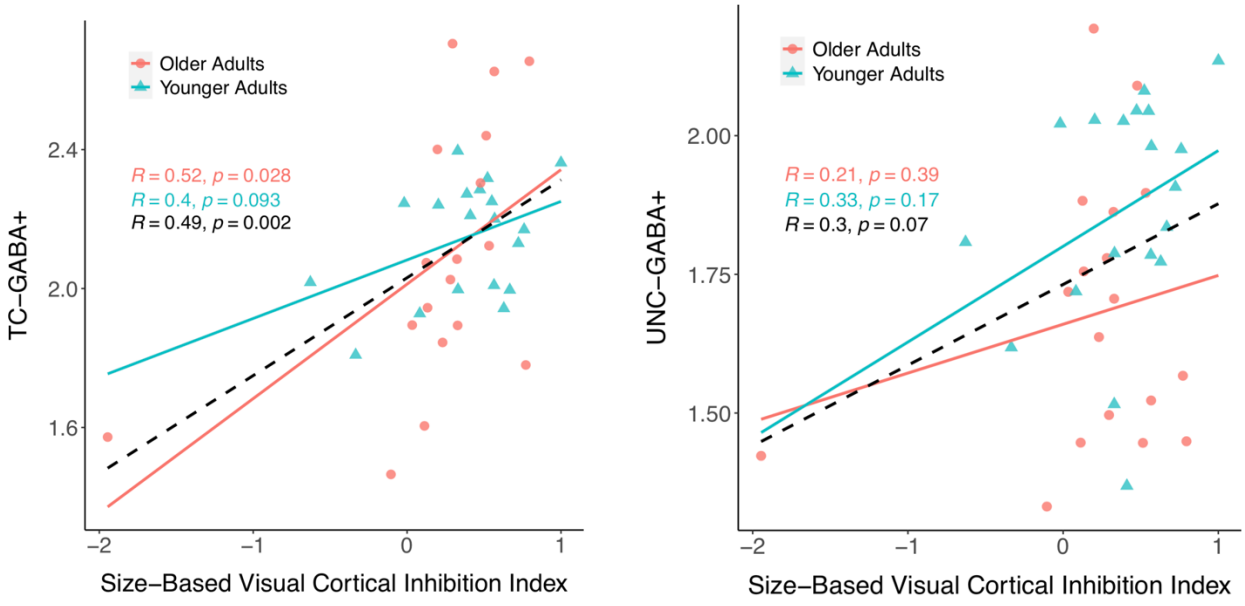

Figure 12. Scatterplots of the relationship between the TMS phosphene size-based metric of visual cortical inhibition and tissue-corrected GABA+ **(a)**, and uncorrected GABA+ **(b)** for each age group. The dotted black lines represent the correlation for the whole sample. N = 18 older adults, 20 younger adults.

Using the frequency-derived metric of visual cortical inhibition

Across all participants, no significant relationship was observed between the frequency-derived metric of visual cortical inhibition and either tissue-corrected ($r(35) = 0.18$, $p = 0.293$; Figure 13a) or uncorrected GABA+ levels ($r(35) = 0.11$, $p = 0.516$; Figure 13b). The relationship between frequency-derived visual cortical inhibition and GABA+ levels was similarly not significant within the older adult group (tissue-corrected GABA+: $r(16) = 0.15$, $p = 0.554$; uncorrected GABA+: $r(16) = -0.06$, $p = 0.806$), or younger adult group (tissue-corrected GABA+: $r(17) = 0.30$, $p = 0.219$; uncorrected GABA+: $r(17) = 0.28$, $p = 0.238$) alone.

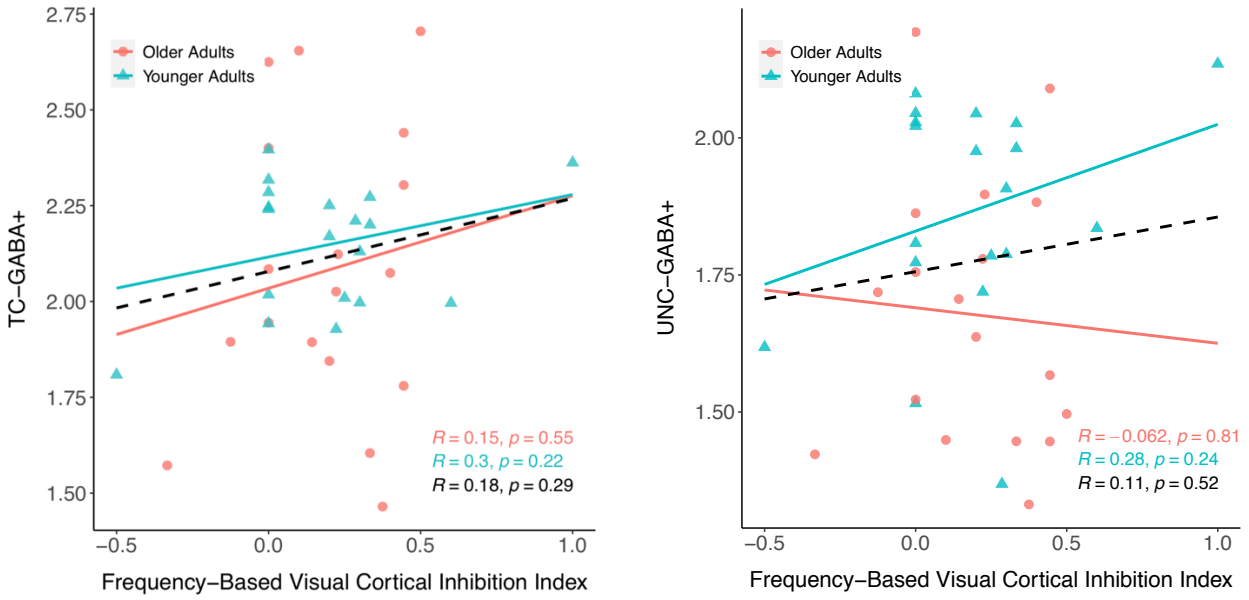

Figure 13. Scatterplots of the relationship between the TMS phosphene frequency-based metric of visual cortical inhibition and tissue-corrected GABA+ **(a)**, and uncorrected GABA+ **(b)** for each age group. The dotted black lines represent the correlation for the whole sample. N = 18 older adults, 20 younger adults.

4.5 Discussion

The present study utilized a multimodal approach to explore age differences in TMS-measured inhibitory function and MRS-derived GABA+ in V1, as well the relationship between

these measures. We demonstrated three main findings. 1) Uncorrected GABA+ levels in left V1 are lower in older compared to younger adults, but the age effect is eliminated by tissue correction. 2) Older adults exhibit reduced visual cortical inhibition compared to younger adults. 3) Greater inhibitory activity in left V1 is associated with higher tissue-corrected GABA+ levels.

4.5.1 Examining cross-sectional age differences in MRS-derived V1 GABA+

While older adults exhibited reduced uncorrected GABA+ compared to younger adults, there were no age differences in tissue-corrected GABA+. These findings extend previous studies showing age-differences in uncorrected (Simmonite et al., 2019) but not tissue-corrected occipital GABA+ (Maes et al., 2018). However, others have demonstrated age-related decreases (Hermans et al., 2018), or even increases (Pitchaimuthu et al., 2017) in occipital tissue-corrected GABA+.

As the brain ages, it atrophies. Brain weight declines ~5% per year after the age of 40 (Svennerholm et al., 1997), with the shrinkage rate increasing after the age of 70 (Scahill et al., 2003). This age-related loss of neurons results in the average MRS voxels of older adults having a lower fraction of gray matter tissue and a larger fraction of cerebrospinal fluid than younger adults (Maes et al., 2018). Because the distribution of GABA differs across tissue types, with cerebrospinal fluid containing negligible amounts (Harris, Puts, & Edden, 2015), this shift in tissue composition has profound effects on MRS-derived GABA+ estimates. This likely explains why the correction for tissue composition resulted in the disappearance of age differences in V1 GABA+. These results imply that although levels of GABA associated with V1 gray matter may not decline with age, overall levels of GABA do, largely because of reductions in gray matter volume.

4.5.2 Investigating cross-sectional age differences in visual cortical inhibition

Older adults exhibited weaker visual cortical inhibition than younger adults, as evidenced by a smaller reduction in conditioned phosphene size compared to unconditioned phosphene size. We do not believe that this age-related reduction in visual cortical inhibition can be explained by cortical atrophy, as there were no significant differences in phosphene size between age groups, and our results did not change even after accounting for voxel grey matter percentage in our analysis. However, no age-differences in visual cortical inhibition were observed using frequency of phosphene-present trials as a metric. Potential reasons for this are twofold. Though previous studies have utilized phosphene frequency as a metric of neuronal excitability (Cattaneo et al., 2011), it has less granularity compared to phosphene size. For example, a binary determination of presence versus absence offers no insight into whether phosphenes that were present were reduced on conditioned trials. Second, because we only had one frequency observation per subject per condition (i.e. a ratio of phosphene-present to total number of trials, rather than a repeated measure of size), this analysis likely suffered from a lack of power.

Motor cortical inhibition, as assessed by SICI, does not significantly change with age (Bhandari et al., 2016). It is unsurprising that cortical inhibition may show age differences in some brain regions but not others, as age-related changes are not uniform and affect some regions more than others (Scahill et al., 2003; Trollor & Valenzuela, 2001). These changes can in part be explained by age-related changes in gene expression (Kumar et al., 2013; S. M. Smith et al., 2020). Gene expression levels can sometimes be associated with chronological age (Kumar et al., 2013), including genes associated with the GABAergic neurotransmission system. For example, GABA is synthesized by two co-expressed isoforms of the same enzyme, called

glutamic acid decarboxylase (GAD). GAD65 is located in the axon terminals, and synthesizes GABA that is bound for release into the synaptic terminal (Feldblum et al., 1995; Soghomonian & Martin, 1998). This pool of phasically active vesicular GABA plays a role in inhibitory synaptic transmission (Bell et al., 2021). The other isoform, GAD67, is localized in the cell body and maintains the basal pool of GABA (Feldblum et al., 1995; Soghomonian & Martin, 1998). GAD67-synthesized GABA is tonically active and modulates inhibition via extrasynaptic receptors (Bell et al., 2021; Soghomonian & Martin, 1998). However, in the human visual cortex, the developmental trajectories of these two enzymes are quite different. While levels of GAD67 remain fairly constant across the lifespan, levels of GAD65 decline with advancing age (Pinto et al., 2010). This would translate to similar levels of basal GABA across the lifespan (which is what MRS is hypothesized to assess), yet a decrease in the axonal pool of GABA with age (which is hypothesized to influence ppTMS measures of inhibition) (Pinto et al., 2010; Stagg, 2014). Our results are consistent with these age-related differences in GABA synthesis, as we observed no age-differences in tissue-corrected V1 GABA+, but did observe age-related declines in visual inhibitory activity. Furthermore, while GAD65 declines with age in V1, it appears to remain relatively stable in M1 (Pandya et al., 2019), offering another complementary explanation as to why age-related reductions in ppTMS measures of cortical inhibition may be observed in V1, but not M1.

4.5.3 Assessing the relationship between visual cortical inhibition and MRS-derived V1 GABA+

We observed a significant relationship between the phosphene size-derived metric of visual cortical inhibition and tissue-corrected V1 GABA+ levels. Interestingly, no relationship

between GABA+ and SICI has been observed in M1 (Cuypers et al., 2021; Dyke et al., 2017; Tremblay et al., 2013).

It is hypothesized that the pool of GABA measured by MRS may not reflect the contributions of vesicular GABA that is utilized in synaptic transmission (Dyke et al., 2017; Stagg, Bachtiar, et al., 2011; Stagg, Bestmann, et al., 2011; Tremblay et al., 2013). Our results therefore may reflect some key physiological differences between V1 and M1. The mean areal density of $GABA_A$ receptors is highest in the visual cortex, with a maximum in V1, while the lowest densities are in motor cortex (Zilles & Palomero-Gallagher, 2017). We hypothesize that due to this increased density of $GABA_A$ receptors, basal GABA in V1 may have a greater influence on cell excitability than in M1, thus leading to a positive association between MRS-measures of basal GABA and TMS-measured inhibitory activity.

One limitation of this study is that some subjects do not see phosphenes. The absence of phosphenes could be related to many factors, including individual differences in neuron orientation in V1. TMS preferentially activates neurons that are oriented perpendicularly to the plane of the coil's magnetic field, and some individuals may have a greater proportion of V1 neurons that go unstimulated. Some subjects simply may have had trouble identifying phosphenes. It is possible that with more training time, a higher proportion of subjects would see phosphenes (Kammer & Baumann, 2010). It is also possible that many of the older adults, who were disproportionately affected by the inability to detect phosphenes, have a phosphene threshold higher than the MagVenture's maximum output. Age-related cortical atrophy leads to an increase in the brain-to-scalp distance. Because magnetic field strength diminishes with distance, higher stimulation intensities are needed to achieve the same degree of tissue stimulation. Studies of M1 indicate that brain-to-scalp distance is a significant determinant of

motor threshold (McConnell et al., 2001). Regardless, the exclusion of individuals that did not see phosphenes or had too high a phosphene threshold— especially the disproportionate number of older adults— may have removed an important source of variability from our TMS dataset and artificially deflated average phosphene thresholds.

Another limitation is the poor spatial resolution of MRS, which is quite coarse (~3cm^3). The inability to collect GABA+ from a smaller acquisition volume limits our ability to closely match the exact area of neuronal tissue being activated by TMS.

Lastly, though several pharmacological studies have been conducted to understand the mechanisms underlying SICI, to our knowledge no studies have explored the mechanisms underlying visual ppTMS. We therefore cannot unambiguously attribute the observed inhibitory effects of paired pulse stimulation to the action of GABA and $GABA_A$ receptors.

4.5.4 Conclusion

The present study provides evidence for age-related reductions in inhibition in V1. We also confirm that MRS-derived measures of V1 GABA+ are functionally relevant, as they are significantly and positively correlated with TMS-measured visual cortical inhibition. This work opens the door to future research investigating whether age-related changes in visual cortical inhibition are related to age-related changes in functional brain activity and behavior. The ability to probe inhibitory function outside of M1 is important for increased understanding of inhibitory deficits hypothesized to play a role in age-related cognitive decline and in prevalent neurological and psychiatric disorders.

Chapter 5 : General Discussion

5.1 Summary

In this dissertation I demonstrated the feasibility of using ppTMS to probe cortical inhibition in visual cortex. I also used this method to explore how GABAergic signaling changes with age in this region. It has been known for some time that short-interval intracortical inhibition (SICI) ppTMS paradigms can be used to assess motor cortical $GABA_A$ergic function in healthy and clinical populations. Early evidence that similar inter-pulse intervals and conditioning stimulus amplitudes over parietal cortex could suppress perception of tactile stimulation further heightened interest in SICI-like protocols (Koch et al., 2006; Meehan et al., 2008). However, previous work in visual cortex raised questions regarding the translation of motor cortex principles to visual cortex (Brückner & Kammer, 2016; Gerwig et al., 2003; Stewart et al., 2001). For example, SICI-like protocols employing conditioning stimulus intensities at 90% of phosphene threshold produced strong facilitation of phosphene detection across a 2-12ms range of inter-pulse intervals (Sparing et al., 2005), while comparable parameters in motor cortex typically produce inhibition.

In **Study 1** (Khammash et al., 2019a)**,** I used ppTMS to investigate the optimal stimulation parameters to elicit SICI-like inhibition of phosphenes at short inter-stimulus intervals. I also used single pulse TMS to assess the validity and reliability of tracing as a method of phosphene measurement. First, I demonstrated in two independent sessions that phosphenes traced on a computer screen spanning the visual field consistently scale with increasing single pulse stimulation intensity. Phosphenes have been demonstrated to be stable and to have high

test-retest reliability (Siniatchkin et al., 2011; Stewart et al., 2001); however there was no routine, reliable method for their measurement. Based on our results, I believe that phosphene tracing is such a method. Although the phosphene response depends upon self-report, the area of phosphene traces exhibits high absolute and relative reliability. Single pulses over the visual cortex result in a sigmoidal stimulus-response curve, with increased stimulus intensity leading to larger observed phosphenes. Though other studies have also used tracing as a method of phosphene measurement (Elkin-Frankston et al., 2010), the reliability and validity of the approach was not assessed.

This lawful relationship between stimulation intensity and response magnitude is well documented in the motor cortex as well, where increased intensity leads to larger motor evoked potentials (Hallett, 2000). The reliability of stimulus-response curves in the motor cortex has been assessed across different muscles in the hand and forearm and indicate good test-retest reliability (Malcolm et al., 2006). These stimulus response curves are often used as a measure of motor cortical excitability. Our results therefore open the door to the use of traced phosphene size as a reliable index of visual cortex excitability.

In addition to the single pulse results, our paired pulse results also demonstrated robust SICI-like inhibition of phosphene amplitude similar to that observed in motor cortex. However, reduced phosphene amplitude was greatest with a conditioning stimulus intensity of 45% of phosphene threshold, as opposed to the ~70-80% of motor threshold that elicits similar effects in motor cortex. These findings suggest that there are similar inhibitory intracortical networks in both motor and visual cortex, but that the thresholds may differ. At higher conditioning stimulus intensities, similar to those employed in motor cortex, the recruitment of lower threshold

inhibitory neurons may be tempered by greater recruitment of higher threshold facilitatory intracortical networks that mask the inhibitory influence of the conditioning stimulus.

Overall, the suppression of phosphene area in early visual cortex with a 2 ms inter stimulus interval and a CS intensity of 45% of PT supports the hypothesis that a SICI-like intracortical inhibitory phenomena does exist in visual cortex, but with different stimulation parameter requirements. However, the temporal dynamics of this paired-pulse TMS state dependency remained unknown. In **Study 2** (Khammash et al., 2019b), I therefore combined ppTMS with the reliable, quantitative phosphene tracing method tested in Study 1 to assess primary visual facilitatory-inhibitory dynamics across inter-stimulus intervals that induce SICI, LICI, and ICF in motor cortex. I demonstrated that short (2-5 ms), medium (10-15 ms), and long (50-200 ms) intervals all resulted in significant suppression of phosphene size compared to the control size. This is different than what is observed in motor cortex, where medium interstimulus intervals elicit a facilitation of the response (Kujirai et al., 1993). This shows that the temporal dynamics of local inhibitory and facilitatory networks are different across motor and visual cortex.

Together, Studies 1 and 2 provide a better understanding of the ways in which CS intensity and inter-stimulus interval affect the phosphene response. This allows for the selection of optimal parameters for probing the pharmacology and functional significance of inhibitory and excitatory networks in the occipital lobe.

In **Study 3** (Khammash et al., *in preparation*), I conducted a multimodal investigation using ppTMS and MRS to explore GABAergic changes with age. I showed that both ppTMS measures of visual cortical inhibition and MRS measures of GABA are reduced in older relative to younger adults, although once GABA estimates are corrected for tissue composition

(proportion of grey matter, white matter, and cerebrospinal fluid), age-related differences in GABA are eliminated. In this study I also demonstrated that MRS-derived measures of GABA and ppTMS-based measures of cortical inhibition are significantly positively correlated in V1. This is different from what is observed in multimodal studies of primary sensorimotor cortex, where ppTMS and MRS measures are uncorrelated (Cuypers et al., 2021; Dyke et al., 2017; Tremblay et al., 2013). I hypothesize that this may be due to significant physiological differences between early visual and motor cortex, especially in $GABA_A$ receptor density.

Together, these studies provide evidence that phosphene tracing is a reliable method of measuring the TMS response in visual cortex, and that ppTMS can be used to assess cortical inhibition in a brain region outside of motor cortex. This research also provides a deeper understanding of how GABAergic signaling changes with age in V1. These findings also confirm that MRS-derived measures of GABA in primary visual cortex are functionally relevant.

5.2 Limitations & Future Directions

One limitation of these studies is that they are based on samples that may not represent the general population. Younger adults were largely recruited from the University of Michigan, and thus have a higher education level as well as a likely-higher socioeconomic status than the average young adult. Older adults from Study 3 were recruited from the Michigan Neural Distinctiveness (MiND) study in Ann Arbor, Michigan, one of the most highly educated metropolitan areas in the country. Thus, the education level and socioeconomic status of the older adult sample was similarly higher relative to the general population. Furthermore, because the MiND study excluded individuals with certain health issues or were under a certain performance threshold for mobility and cognition (Gagnon et al., 2018), the sample of older adults used in Study 3 had greater cognitive abilities and were in better general health than the

average older adult. This disparity should be taken into consideration when attempting to generalize these results to the larger population. That said, the fact that I did observed significant age differences in my ppTMS measure of cortical inhibition despite the fact that my older group was high functioning suggests that these differences are probably very robust.

Another limitation of the studies presented here is that not all subjects report phosphenes. For example, in Study 3, 10% of the younger adults and 33% of the older adults did not experience phosphenes. An additional 10% of younger adults and 31% of older adults only reported phosphenes at a threshold too high to conduct the study protocol. All in all, a significant portion of our sample (especially older adults) was excluded on this basis, which may have removed an important source of variability from our dataset. Kammer and Baumann (Kammer & Baumann, 2010) demonstrated that more extensive training can promote subjects' ability to see phosphenes. Thus, in future studies, it may be beneficial to conduct an additional 'pre-study' session devoted to phosphene training in order to decrease the percentage of non-reporting subjects. Additionally, there is some variability in the maximum voltage rating of capacitors utilized in different stimulator brands. Future research may consider using a stimulator with a higher voltage rating capacitor then the MagVenture, which would allow researchers to capture data from subjects with higher phosphene thresholds.

Another limitation of the studies presented in this dissertation is that we cannot unambiguously attribute short-interval ppTMS-induced visual cortical inhibition to the action of GABA and $GABA_A$ receptor activity. A full understanding of the mechanisms underlying the observed SICI-like inhibition of phosphene area would require pharmacological studies involving $GABA_A$ agonists and antagonists, similar to what has been done when investigating motor cortical SICI (for review, see (Ziemann, 2008)). To the best of my knowledge, no such

studies have been conducted in visual cortex. Further pharmacological studies could additionally explore whether long-interval (50-200 ms) ppTMS-induced visual cortical inhibition can be attributed to the action of $GABA_B$ receptor activity as has been shown in motor cortex.

Future research could also expand on the analyses in this dissertation by exploring the relationship between MRS measures and additional TMS measures of excitability and inhibition in visual cortex, including phosphene threshold, the maximal slope of the single pulse phosphene input-output (recruitment) curve, and ppTMS-elicited inhibition at a wider range of interstimulus intervals (e.g. 10, 15, 50, 100, 200 ms). Because imbalances between excitation and inhibition are hypothesized to play a role in many neurological conditions, future multimodal TMS-MRS studies of the visual cortex should also assess the concentration of additional neurometabolites such as glutamate/glutamine (Glx). Three exploratory questions might include: (1) Is there a relationship between either phosphene threshold or input-output slope (both measures of excitability) and V1 Glx? (2) Is V1 GABA also associated with visual cortical inhibition at ppTMS intervals of 50-200ms? (3) At ppTMS intervals of 10-15 ms, an interval which in motor cortex is thought to capture activity from a combination of excitatory NMDA glutamate receptors and inhibitory $GABA_A$ receptors (Schwenkreis et al., 1999), is visual cortical inhibition associated with a ratio of V1 GABA/Glx? Together, additional pharmacological and multimodal MRS-TMS studies will allow for a deeper understanding of both the underlying mechanisms as well as the relative influence of different pools of neurotransmitters (e.g. cytoplasmic vs vesicular) on these V1 TMS metrics.

Lastly, future research could explore the relationship between ppTMS measures of cortical inhibition and behavior. Declines in GABA with age have been hypothesized to play a role in age-related cognitive decline (Marenco et al., 2018; Porges et al., 2017; Simmonite et al.,

2019). For example, Porges et al. (Porges et al., 2017) found that frontal MRS-derived GABA levels were positively associated with performance on the Montreal Cognitive Assessment, a well-validated tool that assesses performance across various cognitive domains including attention, working memory, verbal memory, naming, and fluency. Additionally, Simmonite et al. (Simmonite et al., 2019) found that better performance on a set of 11 fluid processing tasks was associated with higher MRS-derived GABA levels in primary visual cortex. Using ppTMS to explore the relationship between cortical inhibition and behavior may help shed light on whether age-related changes in cognition may be related to differences in both GABA *activity* as well as *levels*. A limited number of studies have related measures of motor SICI to age-related changes in behavior (e.g. (Heise et al., 2013)), however similar studies have not been conducted using a ppTMS metric of visual cortical inhibition.

In conclusion, the studies that make up this dissertation demonstrate that the use of a phosphene-tracing paradigm and appropriate TMS stimulation parameters allow for the investigation of cortical excitability and inhibition in V1. They also provide novel insights into how inhibitory function in this brain region changes with age, as well as the nature of the relationship between ppTMS-measures of visual cortical inhibition and MRS-derived GABA. This research therefore opens the door to future investigations of age-related changes in inhibitory phenomena in V1. A better understanding of the role that GABAergic changes play in aging is the first step in designing interventions—for example, pharmacological compounds that act on GABA function—that can slow or reverse age-related behavioral impairments.

References

Abrahamyan, A., Clifford, C. W. G., Ruzzoli, M., Phillips, D., Arabzadeh, E., & Harris, J. A. (2011). Accurate and rapid estimation of phosphene thresholds (REPT). *PloS One*, *6*(7), e22342. https://doi.org/10.1371/journal.pone.0022342

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet*, *325*(8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bell, T., Stokoe, M., & Harris, A. D. (2021). Macromolecule suppressed GABA levels show no relationship with age in a pediatric sample. *Scientific Reports*, *11*(1), 722. https://doi.org/10.1038/s41598-020-80530-8

Bhandari, A., Radhu, N., Farzan, F., Mulsant, B. H., Rajji, T. K., Daskalakis, Z. J., & Blumberger, D. M. (2016). A meta-analysis of the effects of aging on motor cortex neurophysiology assessed by transcranial magnetic stimulation. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *127*(8), 2834–2845. https://doi.org/10.1016/j.clinph.2016.05.363

Błaszczyk, J. W. (2016). Parkinson's Disease and Neurodegeneration: GABA-Collapse Hypothesis. *Frontiers in Neuroscience*, *10*, 269. https://doi.org/10.3389/fnins.2016.00269

Boroojerdi, B., Bushara, K. O., Corwell, B., Immisch, I., Battaglia, F., Muellbacher, W., & Cohen, L. G. (2000). Enhanced Excitability of the Human Visual Cortex Induced by

Short-term Light Deprivation. *Cerebral Cortex*, *10*(5), 529–534. https://doi.org/10.1093/cercor/10.5.529

Brady, R. O., McCarthy, J. M., Prescot, A. P., Jensen, J. E., Cooper, A. J., Cohen, B. M., Renshaw, P. F., & Ongür, D. (2013). Brain gamma-aminobutyric acid (GABA) abnormalities in bipolar disorder. *Bipolar Disorders*, *15*(4), 434–439. https://doi.org/10.1111/bdi.12074

Brückner, S., & Kammer, T. (2016). No Modulation of Visual Cortex Excitability by Transcranial Direct Current Stimulation. *PLOS ONE*, *11*(12), e0167697. https://doi.org/10.1371/journal.pone.0167697

Cattaneo, Z., Pisoni, A., Papagno, C., & Silvanto, J. (2011). Modulation of Visual Cortical Excitability by Working Memory: Effect of Luminance Contrast of Mental Imagery. *Frontiers in Psychology*, *2*. https://doi.org/10.3389/fpsyg.2011.00029

Chamberlain, J. D., Gagnon, H., Lalwani, P., Cassady, K. E., Simmonite, M., Seidler, R. D., Taylor, S. F., Weissman, D. H., Park, D. C., & Polk, T. A. (2021). GABA levels in ventral visual cortex decline with age and are associated with neural distinctiveness. *Neurobiology of Aging*, *102*, 170–177. https://doi.org/10.1016/j.neurobiolaging.2021.02.013

Chen, R., Tam, A., Bütefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., & Cohen, L. G. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *Journal of Neurophysiology*, *80*(6), 2870–2881. https://doi.org/10.1152/jn.1998.80.6.2870

Cirillo, J., Rogasch, N. C., & Semmler, J. G. (2010). Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. *Experimental Brain Research*, *205*(1), 57–68. https://doi.org/10.1007/s00221-010-2332-1

Cuypers, K., Hehl, M., van Aalst, J., Chalavi, S., Mikkelsen, M., Van Laere, K., Dupont, P., Mantini, D., & Swinnen, S. P. (2021). Age-related GABAergic differences in the primary sensorimotor cortex: A multimodal approach combining PET, MRS and TMS. *NeuroImage*, *226*, 117536. https://doi.org/10.1016/j.neuroimage.2020.117536

de Jonge, J. C., Vinkers, C. H., Hulshoff Pol, H. E., & Marsman, A. (2017). GABAergic Mechanisms in Schizophrenia: Linking Postmortem and In Vivo Studies. *Frontiers in Psychiatry*, *8*, 118. https://doi.org/10.3389/fpsyt.2017.00118

Deng, Z.-D., Lisanby, S. H., & Peterchev, A. V. (2014). Coil Design Considerations for Deep Transcranial Magnetic Stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *125*(6), 1202–1212. https://doi.org/10.1016/j.clinph.2013.11.038

Di Lazzaro, V., Oliviero, A., Meglio, M., Cioni, B., Tamburrini, G., Tonali, P., & Rothwell, J. C. (2000). Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *111*(5), 794–799.

Di Lazzaro, V., Oliviero, A., Saturno, E., Dileone, M., Pilato, F., Nardone, R., Ranieri, F., Musumeci, G., Fiorilla, T., & Tonali, P. (2005). Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. *The Journal of Physiology*, *564*(Pt 2), 661–668. https://doi.org/10.1113/jphysiol.2004.061747

Dyke, K., Pépés, S. E., Chen, C., Kim, S., Sigurdsson, H. P., Draper, A., Husain, M., Nachev, P., Gowland, P. A., Morris, P. G., & Jackson, S. R. (2017). Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. *Neuroimage*, *152*, 360–370. https://doi.org/10.1016/j.neuroimage.2017.03.011

Edden, R. A. E., Puts, N. A. J., Harris, A. D., Barker, P. B., & Evans, C. J. (2014). Gannet: A Batch-Processing Tool for the Quantitative Analysis of Gamma-Aminobutyric Acid–Edited MR Spectroscopy Spectra. *Journal of Magnetic Resonance Imaging : JMRI*, *40*(6), 1445–1452.

Elkin-Frankston, S., Fried, P. J., Pascual-Leone, A., Rushmore III, R. J., & Valero-Cabré, A. (2010). A Novel Approach for Documenting Phosphenes Induced by Transcranial Magnetic Stimulation. *Journal of Visualized Experiments : JoVE*, *38*. https://doi.org/10.3791/1762

Feldblum, S., Dumoulin, A., Anoal, M., Sandillon, F., & Privat, A. (1995). Comparative distribution of GAD65 and GAD67 mRNAs and proteins in the rat spinal cord supports a differential regulation of these two glutamate decarboxylases in vivo. *Journal of Neuroscience Research*, *42*(6), 742–757. https://doi.org/10.1002/jnr.490420603

Fitzgerald, P. B., Maller, J. J., Hoy, K., Farzan, F., & Daskalakis, Z. J. (2009). GABA and cortical inhibition in motor and non-motor regions using combined TMS-EEG: A time analysis. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(9), 1706–1710. https://doi.org/10.1016/j.clinph.2009.06.019

Florian, J., Müller-Dahlhaus, M., Liu, Y., & Ziemann, U. (2008). Inhibitory circuits and the nature of their interactions in the human motor cortex a pharmacological TMS study. *The Journal of Physiology*, *586*(2), 495–514. https://doi.org/10.1113/jphysiol.2007.142059

Foerster, B. R., Pomper, M. G., Callaghan, B. C., Petrou, M., Edden, R. A. E., Mohamed, M. A., Welsh, R. C., Carlos, R. C., Barker, P. B., & Feldman, E. L. (2013). 3T MR Spectroscopy Reveals an Imbalance between Excitatory and Inhibitory Neurotransmitters in Amyotrophic Lateral Sclerosis. *JAMA Neurology*, *70*(8), 1009–1016. https://doi.org/10.1001/jamaneurol.2013.234

Friedman, S. D., Poliakov, A. V., Budech, C., Shaw, D. W. W., Breiger, D., Jinguji, T., Krabak, B., Coppel, D., Lewis, T. M., Browd, S., & Ojemann, J. G. (2017). GABA alterations in pediatric sport concussion. *Neurology*, *89*(21), 2151–2156. https://doi.org/10.1212/WNL.0000000000004666

Gagnon, H., Simmonite, M., Cassady, K., Chamberlain, J. D., Freiburger, E., Lalwani, P., Kelley, S., Foerster, B., Park, D. C., Petrou, M., Seidler, R. D., Taylor, S. F., Weissman, D. H., & Polk, T. A. (2018). Michigan Neural Distinctiveness (MiND) project: Investigating the scope, causes, and consequences of age-related neural dedifferentiation. *BioRxiv*. https://doi.org/10.1101/466516

Gao, F., Edden, R. A. E., Li, M., Puts, N. A. J., Wang, G., Liu, C., Zhao, B., Wang, H., Bai, X., Zhao, C., Wang, X., & Barker, P. B. (2013). Edited magnetic resonance spectroscopy detects an age-related decline in brain GABA levels. *NeuroImage*, *78*, 75–82. https://doi.org/10.1016/j.neuroimage.2013.04.012

Gerwig, M., Kastrup, O., Meyer, B.-U., & Niehaus, L. (2003). Evaluation of cortical excitability by motor and phosphene thresholds in transcranial magnetic stimulation. *Journal of the Neurological Sciences*, *215*(1–2), 75–78. https://doi.org/10.1016/s0022-510x(03)00228-4

Gerwig, M., Niehaus, L., Kastrup, O., Stude, P., & Diener, H. C. (2005). Visual Cortex Excitability in Migraine Evaluated by Single and Paired Magnetic Stimuli. *Headache: The Journal of Head and Face Pain*, *45*(10), 1394–1399. https://doi.org/10.1111/j.1526-4610.2005.00272.x

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature*, *406*(6792), 147–150. https://doi.org/10.1038/35018000

Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron*, *55*(2), 187–199. https://doi.org/10.1016/j.neuron.2007.06.026

Harris, A. D., Puts, N. A. J., Barker, P. B., & Edden, R. A. E. (2015). Spectral-Editing Measurements of GABA in the Human Brain with and without Macromolecule Suppression. *Magnetic Resonance in Medicine*, *74*(6), 1523–1529. https://doi.org/10.1002/mrm.25549

Harris, A. D., Puts, N. A. J., & Edden, R. A. E. (2015). Tissue correction for GABA-edited MRS: Considerations of voxel composition, tissue segmentation, and tissue relaxations. *Journal of Magnetic Resonance Imaging*, *42*(5), 1431–1440. https://doi.org/10.1002/jmri.24903

Hasher, L., & Campbell, K. L. (2020). Inhibitory Theory: Assumptions, Findings, and Relevance to Interventions. In A. Gutchess & A. K. Thomas (Eds.), *The Cambridge Handbook of Cognitive Aging: A Life Course Perspective* (pp. 147–160). Cambridge University Press. https://doi.org/10.1017/9781108552684.010

Heise, K.-F., Zimerman, M., Hoppe, J., Gerloff, C., Wegscheider, K., & Hummel, F. C. (2013). The aging motor system as a model for plastic changes of GABA-mediated intracortical inhibition and their behavioral relevance. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(21), 9039–9049. https://doi.org/10.1523/JNEUROSCI.4094-12.2013

Hendry, S. H., Schwark, H. D., Jones, E. G., & Yan, J. (1987). Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *7*(5), 1503–1519.

Hermans, L., Leunissen, I., Pauwels, L., Cuypers, K., Peeters, R., Puts, N. A. J., Edden, R. A. E., & Swinnen, S. P. (2018). Brain GABA Levels Are Associated with Inhibitory Control Deficits in Older Adults. *Journal of Neuroscience*, *38*(36), 7844–7851. https://doi.org/10.1523/JNEUROSCI.0760-18.2018

Ilić, T. V., Meintzschel, F., Cleff, U., Ruge, D., Kessler, K. R., & Ziemann, U. (2002). Short-interval paired-pulse inhibition and facilitation of human motor cortex: The dimension of stimulus intensity. *The Journal of Physiology*, *545*(1), 153–167.

Ilmoniemi, R. J., Virtanen, J., Ruohonen, J., Karhu, J., Aronen, H. J., Näätänen, R., & Katila, T. (1997). Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. *Neuroreport*, *8*(16), 3537–3540. https://doi.org/10.1097/00001756-199711100-00024

Kammer, T. (1998). Phosphenes and transient scotomas induced by magnetic stimulation of the occipital lobe: Their topographic relationship. *Neuropsychologia*, *37*(2), 191–198. https://doi.org/10.1016/S0028-3932(98)00093-1

Kammer, T., & Baumann, L. W. (2010). Phosphene thresholds evoked with single and double TMS pulses. *Clinical Neurophysiology*, *121*(3), 376–379. https://doi.org/10.1016/j.clinph.2009.12.002

Kammer, T., Puls, K., Erb, M., & Grodd, W. (2005). Transcranial magnetic stimulation in the visual system. II. Characterization of induced phosphenes and scotomas. *Experimental Brain Research*, *160*(1), 129–140. https://doi.org/10.1007/s00221-004-1992-0

Kastner, S., Demmer, I., & Ziemann, U. (1998). Transient visual field defects induced by transcranial magnetic stimulation over human occipital pole. *Experimental Brain Research*, *118*(1), 19–26. https://doi.org/10.1007/s002210050251

Keppel, G. (1991). *Design and analysis: A researcher's handbook, 3rd ed*. Prentice-Hall, Inc.

Khammash, D., Simmonite, M., Polk, T. A., Taylor, S. F., & Meehan, S. K. (2019a). Probing short-latency cortical inhibition in the visual cortex with transcranial magnetic stimulation: A reliability study. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2019.01.013

Khammash, D., Simmonite, M., Polk, T. A., Taylor, S. F., & Meehan, S. K. (2019b). Temporal Dynamics of Corticocortical Inhibition in Human Visual Cortex: A TMS Study. *Neuroscience*, *421*, 31–38. https://doi.org/10.1016/j.neuroscience.2019.10.003

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and Rehabilitation Medicine*, *58*(4), 208–213. https://doi.org/10.1016/j.rehab.2015.05.005

Koch, G., Franca, M., Albrecht, U.-V., Caltagirone, C., & Rothwell, J. C. (2006). Effects of paired pulse TMS of primary somatosensory cortex on perception of a peripheral

electrical stimulus. *Experimental Brain Research*, *172*(3), 416–424. https://doi.org/10.1007/s00221-006-0359-0

Koponen, L. M., Nieminen, J. O., Mutanen, T. P., & Ilmoniemi, R. J. (2018). Noninvasive extraction of microsecond-scale dynamics from human motor cortex. *Human Brain Mapping*, *39*(6), 2405–2411. https://doi.org/10.1002/hbm.24010

Kossev, A. R., Schrader, C., Däuper, J., Dengler, R., & Rollnik, J. D. (2002). Increased intracortical inhibition in middle-aged humans; a study using paired-pulse transcranial magnetic stimulation. *Neuroscience Letters*, *333*(2), 83–86. https://doi.org/10.1016/s0304-3940(02)00986-2

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., Wroe, S., Asselman, P., & Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *The Journal of Physiology*, *471*, 501–519.

Kumar, A., Gibbs, J. R., Beilina, A., Dillman, A., Kumaran, R., Trabzuni, D., Ryten, M., Walker, R., Smith, C., Traynor, B. J., Hardy, J., Singleton, A. B., & Cookson, M. R. (2013). Age associated changes in gene expression in human brain and isolated neurons. *Neurobiology of Aging*, *34*(4), 1199–1209. https://doi.org/10.1016/j.neurobiolaging.2012.10.021

Lalwani, P., Gagnon, H., Cassady, K., Simmonite, M., Peltier, S., Seidler, R. D., Taylor, S. F., Weissman, D. H., & Polk, T. A. (2019). Neural distinctiveness declines with age in auditory cortex and is associated with auditory GABA levels. *NeuroImage*, *201*, 116033. https://doi.org/10.1016/j.neuroimage.2019.116033

Lu, M., & Ueno, S. (2017). Comparison of the induced fields using different coil configurations during deep transcranial magnetic stimulation. *PLOS ONE*, *12*(6), e0178422. https://doi.org/10.1371/journal.pone.0178422

Maes, C., Hermans, L., Pauwels, L., Chalavi, S., Leunissen, I., Levin, O., Cuypers, K., Peeters, R., Sunaert, S., Mantini, D., Puts, N. A. J., Edden, R. A. E., & Swinnen, S. P. (2018). Age-related differences in GABA levels are driven by bulk tissue changes. *Human Brain Mapping*, *39*(9), 3652–3662. https://doi.org/10.1002/hbm.24201

Malcolm, M. P., Triggs, W. J., Light, K. E., Shechtman, O., Khandekar, G., & Gonzalez Rothi, L. J. (2006). Reliability of motor cortex transcranial magnetic stimulation in four muscle representations. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *117*(5), 1037–1046. https://doi.org/10.1016/j.clinph.2006.02.005

Marenco, S., Meyer, C., van der Veen, J. W., Zhang, Y., Kelly, R., Shen, J., Weinberger, D. R., Dickinson, D., & Berman, K. F. (2018). Role of gamma-amino-butyric acid in the dorsal anterior cingulate in age-associated changes in cognition. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(11), 2285–2291. https://doi.org/10.1038/s41386-018-0134-5

Marg, E., & Rudiak, D. (1994). Phosphenes induced by magnetic stimulation over the occipital brain: Description and probable site of stimulation. *Optometry and Vision Science: Official Publication of the American Academy of Optometry*, *71*(5), 301–311. https://doi.org/10.1097/00006324-199405000-00001

Marneweck, M., Loftus, A., & Hammond, G. (2011). Short-interval intracortical inhibition and manual dexterity in healthy aging. *Neuroscience Research*, *70*(4), 408–414. https://doi.org/10.1016/j.neures.2011.04.004

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E., & George, M. S. (2001). The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: A replication in healthy adults comparing two methods of assessing the distance to cortex. *Biological Psychiatry*, *49*(5), 454–459. https://doi.org/10.1016/s0006-3223(00)01039-8

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research*, *173*(1), 86–93. https://doi.org/10.1007/s00221-006-0365-2

McGinley, M., Hoffman, R. L., Russ, D. W., Thomas, J. S., & Clark, B. C. (2010). Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. *Experimental Gerontology*, *45*(9), 671–678. https://doi.org/10.1016/j.exger.2010.04.005

Meehan, S. K., Legon, W., & Staines, W. R. (2008). Paired-pulse transcranial magnetic stimulation of primary somatosensory cortex differentially modulates perception and sensorimotor transformations. *Neuroscience*, *157*(2), 424–431. https://doi.org/10.1016/j.neuroscience.2008.09.008

Mescher, M., Merkle, H., Kirsch, J., Garwood, M., & Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine*, *11*(6), 266–272. https://doi.org/10.1002/(sici)1099-1492(199810)11:6<266::aid-nbm530>3.0.co;2-j

Mick, I., Ramos, A. C., Myers, J., Stokes, P. R., Chandrasekera, S., Erritzoe, D., Mendez, M. A., Gunn, R. N., Rabiner, E. A., Searle, G. E., Galduróz, J. C. F., Waldman, A. D., Bowden-

Jones, H., Clark, L., Nutt, D. J., & Lingford-Hughes, A. R. (2017). Evidence for GABA-A receptor dysregulation in gambling disorder: Correlation with impulsivity. *Addiction Biology*, *22*(6), 1601–1609. https://doi.org/10.1111/adb.12457

Moliadze, V., Giannikopoulos, D., Eysel, U. T., & Funke, K. (2005). Paired-pulse transcranial magnetic stimulation protocol applied to visual cortex of anaesthetized cat: Effects on visually evoked single-unit activity. *The Journal of Physiology*, *566*(3), 955–965. https://doi.org/10.1113/jphysiol.2005.086090

Moliadze, V., Zhao, Y., Eysel, U., & Funke, K. (2003). Effect of transcranial magnetic stimulation on single-unit activity in the cat primary visual cortex. *The Journal of Physiology*, *553*(2), 665–679. https://doi.org/10.1113/jphysiol.2003.050153

Nakamura, H., Kitagawa, H., Kawaguchi, Y., & Tsuji, H. (1997). Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *The Journal of Physiology*, *498 (Pt 3)*, 817–823.

Oliveri, M., Caltagirone, C., Filippi, M. M., Traversa, R., Cicinelli, P., Pasqualetti, P., & Rossini, P. M. (2000). Paired transcranial magnetic stimulation protocols reveal a pattern of inhibition and facilitation in the human parietal cortex. *The Journal of Physiology*, *529 Pt 2*, 461–468.

Oliveri, M., Rossini, P. M., Filippi, M. M., Traversa, R., Cicinelli, P., Palmieri, M. G., Pasqualetti, P., & Caltagirone, C. (2000). Time-dependent activation of parieto-frontal networks for directing attention to tactile space. A study with paired transcranial magnetic stimulation pulses in right-brain-damaged patients with extinction. *Brain: A Journal of Neurology*, *123 (Pt 9)*, 1939–1947.

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neuroscience Research*, *55*(1), 74–77. https://doi.org/10.1016/j.neures.2006.02.002

Pandya, M., Palpagama, T. H., Turner, C., Waldvogel, H. J., Faull, R. L., & Kwakowsky, A. (2019). Sex- and age-related changes in GABA signaling components in the human cortex. *Biology of Sex Differences*, *10*, 5. https://doi.org/10.1186/s13293-018-0214-6

Pashut, T., Wolfus, S., Friedman, A., Lavidor, M., Bar-Gad, I., Yeshurun, Y., & Korngreen, A. (2011). Mechanisms of Magnetic Stimulation of Central Nervous System Neurons. *PLoS Computational Biology*, *7*(3), e1002022. https://doi.org/10.1371/journal.pcbi.1002022

Peinemann, A., Lehner, C., Conrad, B., & Siebner, H. R. (2001). Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. *Neuroscience Letters*, *313*(1), 33–36. https://doi.org/10.1016/S0304-3940(01)02239-X

Peterchev, A. V., Murphy, D. L., & Lisanby, S. H. (2011). Repetitive Transcranial Magnetic Stimulator with Controllable Pulse Parameters. *Journal of Neural Engineering*, *8*(3), 036016. https://doi.org/10.1088/1741-2560/8/3/036016

Pinto, J., Hornby, K., Jones, D., & Murphy, K. (2010). Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. *Frontiers in Cellular Neuroscience*, *4*. https://www.frontiersin.org/article/10.3389/fncel.2010.00016

Pitchaimuthu, K., Wu, Q., Carter, O., Nguyen, B. N., Ahn, S., Egan, G. F., & McKendrick, A. M. (2017). Occipital GABA levels in older adults and their relationship to visual perceptual suppression. *Scientific Reports*, *7*(1), 14231. https://doi.org/10.1038/s41598-017-14577-5

Porges, E. C., Jensen, G., Foster, B., Edden, R. A., & Puts, N. A. (2021). The trajectory of cortical GABA across the lifespan, an individual participant data meta-analysis of edited MRS studies. *ELife*, *10*, e62575. https://doi.org/10.7554/eLife.62575

Porges, E. C., Woods, A. J., Edden, R. A. E., Puts, N. A. J., Harris, A. D., Chen, H., Garcia, A. M., Seider, T. R., Lamb, D. G., Williamson, J. B., & Cohen, R. A. (2017). Frontal Gamma-Aminobutyric Acid Concentrations Are Associated With Cognitive Performance in Older Adults. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging*, *2*(1), 38–44. https://doi.org/10.1016/j.bpsc.2016.06.004

Rafique, S. A., & Steeves, J. K. E. (2020). Assessing differential effects of single and accelerated low-frequency rTMS to the visual cortex on GABA and glutamate concentrations. *Brain and Behavior*, *10*(12), e01845. https://doi.org/10.1002/brb3.1845

Ray, P. G., Meador, K. J., Epstein, C. M., Loring, D. W., & Day, L. J. (1998). Magnetic Stimulation of Visual Cortex: Factors Influencing the Perception of Phosphenes. *Journal of Clinical Neurophysiology*, *15*(4), 351.

Riehl, M. (2008, January 24). *TMS stimulator design*. Oxford Handbook of Transcranial Stimulation. https://doi.org/10.1093/oxfordhb/9780198568926.013.0003

Rogasch, N. C., Dartnall, T. J., Cirillo, J., Nordstrom, M. A., & Semmler, J. G. (2009). Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *107*(6), 1874–1883. https://doi.org/10.1152/japplphysiol.00443.2009

Rossi, S., Antal, A., Bestmann, S., Bikson, M., Brewer, C., Brockmöller, J., Carpenter, L. L., Cincotta, M., Chen, R., Daskalakis, J. D., Di Lazzaro, V., Fox, M. D., George, M. S., Gilbert, D., Kimiskidis, V. K., Koch, G., Ilmoniemi, R. J., Lefaucheur, J. P., Leocani, L.,

… Hallett, M. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *132*(1), 269–306. https://doi.org/10.1016/j.clinph.2020.10.003

Rothwell, J. C., Hallett, M., Berardelli, A., Eisen, A., Rossini, P., & Paulus, W. (1999). Magnetic stimulation: Motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalography and Clinical Neurophysiology. Supplement*, *52*, 97–103.

Samaha, J., Gosseries, O., & Postle, B. R. (2017). Distinct Oscillatory Frequencies Underlie Excitability of Human Occipital and Parietal Cortex. *Journal of Neuroscience*, *37*(11), 2824–2833. https://doi.org/10.1523/JNEUROSCI.3413-16.2017

Scahill, R. I., Frost, C., Jenkins, R., Whitwell, J. L., Rossor, M. N., & Fox, N. C. (2003). A Longitudinal Study of Brain Volume Changes in Normal Aging Using Serial Registered Magnetic Resonance Imaging. *Archives of Neurology*, *60*(7), 989–994. https://doi.org/10.1001/archneur.60.7.989

Schäfer, M., Biesecker, J. C., Schulze-Bonhage, A., & Ferbert, A. (1997). Transcranial magnetic double stimulation: Influence of the intensity of the conditioning stimulus. *Electroencephalography and Clinical Neurophysiology*, *105*(6), 462–469. https://doi.org/10.1016/s0924-980x(97)00054-4

Schwarzkopf, D. S., Silvanto, J., & Rees, G. (2011). Stochastic resonance effects reveal the neural mechanisms of transcranial magnetic stimulation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(9), 3143–3147. https://doi.org/10.1523/JNEUROSCI.4863-10.2011

Schwenkreis, P., Witscher, K., Janssen, F., Addo, A., Dertwinkel, R., Zenz, M., Malin, J. P., & Tegenthoff, M. (1999). Influence of the N-methyl-D-aspartate antagonist memantine on human motor cortex excitability. *Neuroscience Letters*, *270*(3), 137–140.

Silvanto, J., Bona, S., & Cattaneo, Z. (2017). Initial activation state, stimulation intensity and timing of stimulation interact in producing behavioral effects of TMS. *Neuroscience*, *363*, 134–141. https://doi.org/10.1016/j.neuroscience.2017.09.002

Silvanto, J., Muggleton, N. G., Cowey, A., & Walsh, V. (2007). Neural adaptation reveals state-dependent effects of transcranial magnetic stimulation: State-dependent effects of TMS. *European Journal of Neuroscience*, *25*(6), 1874–1881. https://doi.org/10.1111/j.1460-9568.2007.05440.x

Simmonite, M., Carp, J., Foerster, B. R., Ossher, L., Petrou, M., Weissman, D. H., & Polk, T. A. (2019). Age-Related Declines in Occipital GABA are Associated with Reduced Fluid Processing Ability. *Academic Radiology*, *26*(8), 1053–1061. https://doi.org/10.1016/j.acra.2018.07.024

Siniatchkin, M., Schlicke, C., & Stephani, U. (2011). Transcranial magnetic stimulation reveals high test-retest reliability for phosphenes but not for suppression of visual perception. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *122*(12), 2475–2481. https://doi.org/10.1016/j.clinph.2011.05.003

Smith, A. E., Ridding, M. C., Higgins, R. D., Wittert, G. A., & Pitcher, J. B. (2009). Age-related changes in short-latency motor cortex inhibition. *Experimental Brain Research*, *198*(4), 489–500. https://doi.org/10.1007/s00221-009-1945-8

Smith, S. M., Elliott, L. T., Alfaro-Almagro, F., McCarthy, P., Nichols, T. E., Douaud, G., & Miller, K. L. (2020). Brain aging comprises many modes of structural and functional

change with distinct genetic and biophysical associations. *ELife*, *9*, e52677. https://doi.org/10.7554/eLife.52677

Soghomonian, J. J., & Martin, D. L. (1998). Two isoforms of glutamate decarboxylase: Why? *Trends in Pharmacological Sciences*, *19*(12), 500–505. https://doi.org/10.1016/s0165-6147(98)01270-x

Sparing, R., Dambeck, N., Stock, K., Meister, I. G., Huetter, D., & Boroojerdi, B. (2005). Investigation of the primary visual cortex using short-interval paired-pulse transcranial magnetic stimulation (TMS). *Neuroscience Letters*, *382*(3), 312–316. https://doi.org/10.1016/j.neulet.2005.03.036

Sparing, R., Mottaghy, F. M., Ganis, G., Thompson, W. L., Töpper, R., Kosslyn, S. M., & Pascual-Leone, A. (2002). Visual cortex excitability increases during visual mental imagery—A TMS study in healthy human subjects. *Brain Research*, *938*(1), 92–97. https://doi.org/10.1016/S0006-8993(02)02478-2

Stagg, C. J. (2014). Magnetic Resonance Spectroscopy as a tool to study the role of GABA in motor-cortical plasticity. *NeuroImage*, *86*, 19–27. https://doi.org/10.1016/j.neuroimage.2013.01.009

Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011). What are we measuring with GABA magnetic resonance spectroscopy? *Communicative & Integrative Biology*, *4*(5), 573–575. https://doi.org/10.4161/cib.4.5.16213

Stagg, C. J., Bestmann, S., Constantinescu, A. O., Moreno Moreno, L., Allman, C., Mekle, R., Woolrich, M., Near, J., Johansen-Berg, H., & Rothwell, J. C. (2011). Relationship between physiological measures of excitability and levels of glutamate and GABA in the

human motor cortex. *The Journal of Physiology*, *589*(Pt 23), 5845–5855. https://doi.org/10.1113/jphysiol.2011.216978

Stewart, L. M., Walsh, V., & Rothwell, J. C. (2001). Motor and phosphene thresholds: A transcranial magnetic stimulation correlation study. *Neuropsychologia*, *39*(4), 415–419. https://doi.org/10.1016/s0028-3932(00)00130-5

Svennerholm, L., Boström, K., & Jungbjer, B. (1997). Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. *Acta Neuropathologica*, *94*(4), 345–352. https://doi.org/10.1007/s004010050717

Taylor, P. C. J., Walsh, V., & Eimer, M. (2010). The neural signature of phosphene perception. *Human Brain Mapping*, *31*(9), 1408–1417. https://doi.org/10.1002/hbm.20941

Terhune, D. B., Murray, E., Near, J., Stagg, C. J., Cowey, A., & Cohen Kadosh, R. (2015). Phosphene Perception Relates to Visual Cortex Glutamate Levels and Covaries with Atypical Visuospatial Awareness. *Cerebral Cortex (New York, NY)*, *25*(11), 4341–4350. https://doi.org/10.1093/cercor/bhv015

Tofts, P. S. (1990). The distribution of induced currents in magnetic stimulation of the nervous system. *Physics in Medicine and Biology*, *35*(8), 1119–1128. https://doi.org/10.1088/0031-9155/35/8/008

Tremblay, S., Beaulé, V., Proulx, S., de Beaumont, L., Marjańska, M., Doyon, J., Pascual-Leone, A., Lassonde, M., & Théoret, H. (2013). Relationship between transcranial magnetic stimulation measures of intracortical inhibition and spectroscopy measures of GABA and glutamate+glutamine. *Journal of Neurophysiology*, *109*(5), 1343–1349. https://doi.org/10.1152/jn.00704.2012

Trollor, J. N., & Valenzuela, M. J. (2001). Brain ageing in the new millennium. *The Australian and New Zealand Journal of Psychiatry*, *35*(6), 788–805. https://doi.org/10.1046/j.1440-1614.2001.00969.x

Ueno, S., & Sekino, M. (2021). Figure-Eight Coils for Magnetic Stimulation: From Focal Stimulation to Deep Stimulation. *Frontiers in Human Neuroscience*, *15*. https://www.frontiersin.org/article/10.3389/fnhum.2021.805971

Ziemann, U. (2008, January 24). *Pharmacology of TMS measures*. Oxford Handbook of Transcranial Stimulation. https://doi.org/10.1093/oxfordhb/9780198568926.013.0013

Ziemann, U., Chen, R., Cohen, L. G., & Hallett, M. (1998). Dextromethorphan decreases the excitability of the human motor cortex. *Neurology*, *51*(5), 1320–1324.

Ziemann, U., Lönnecker, S., Steinhoff, B. J., & Paulus, W. (1996). The effect of lorazepam on the motor cortical excitability in man. *Experimental Brain Research*, *109*(1), 127–135.

Ziemann, U., Rothwell, J. C., & Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of Physiology*, *496 (Pt 3)*, 873–881. https://doi.org/10.1113/jphysiol.1996.sp021734

Zilles, K., & Palomero-Gallagher, N. (2017). Multiple Transmitter Receptors in Regions and Layers of the Human Cerebral Cortex. *Frontiers in Neuroanatomy*, *11*. https://www.frontiersin.org/article/10.3389/fnana.2017.00078