HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2020 December 12.

Published in final edited form as:

Neuroscience. 2019 November 21; 421: 31–38. doi:10.1016/j.neuroscience.2019.10.003.

Temporal Dynamics of Corticocortical Inhibition in Human Visual Cortex: A TMS Study

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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 to 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.

Keywords

paired-pulse; transcranial magnetic stimulation; phosphenes; occipital; inter-stimulus interval; cortical inhibition

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CONFLICT OF INTEREST STATEMENT

Dr. Taylor reports having received grant support from Neuronetics, unrelated to the content of the current study. None of the other authors have any potential conflicts of interest to disclose.

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 (Kammer, 1998; Kastner et al., 1998; Boroojerdi et al., 2000; Gerwig et al., 2005; Silvanto et al., 2007, 2017; Cattaneo et al., 2011). 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 et al., 1996b), 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 (Ziemann et al., 1996a; Di Lazzaro et al., 2000, 2005). 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 (McDonnell et al., 2006; Florian et al., 2008).

Similar patterns of net inhibition and facilitation have been observed in prefrontal (Oliveri et al., 2000b) and parietal cortices (Oliveri et al., 2000a). 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 (Ray et al., 1998; Sparing et al., 2005; Kammer and Baumann, 2010). 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., 2019) 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., 2019) 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., 2019) 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).

EXPERIMENTAL PROCEDURES

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 h of the study.

This sample was independent from the sample reported in our previous work (Khammash et al., 2019). 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., 2019).

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., 2019).

Transcranial magnetic stimulation (TMS)

TMS followed a similar procedure to our previous work (Khammash et al., 2019). Monophasic posterior-anterior TMS was delivered with a figure-eight coil (model MCB70, 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 × 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., 2019). 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., 2019). 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.

Phosphene quantification

Phosphenes were quantified according to our previously published tracing method (Khammash et al., 2019). 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 × 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.

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 ×100 stimulator. The experimenter controlled the progression through the line-by-line protocol. The minimum inter-trial interval was 10 s to ensure that each trial was independent.

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, 5 ms), medium (10, 15 ms), and long (50, 100, 200 ms) 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 0.017 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).

RESULTS

Phosphene threshold

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

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 <0.05) 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 (Fig. 1).

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%: $F_{3,57} = 8.87$, $\varepsilon = 0.50$, $p = 0.002$; 75%: $F_{3,57} = 4.86$, $\varepsilon = 0.61$, $p = 0.016$). 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: $t(19) = 3.70$, $p = 0.002$, medium: $t(19) = 3.79$, $p = 0.001$ and long: $t(19) = 2.90$, $p = 0.009$) (Fig. 2A). At 75% CS intensity, phosphene suppression was much smaller overall and was only statistically significant at long ISIs ($t(19) = 2.72$, $p = 0.014$), but not at the short ($t(19) = 2.02$, $p = 0.058$) or medium ($t(19) = 0.16$, $p = 0.878$) intervals, with some subjects displaying no change and others showing facilitation of phosphenes (Fig. 2B).

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 0.182 and 0.824).

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 et al., 2000b) and parietal cortices (Oliveri et al., 2000a). 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 (Sparing et al., 2005; Kammer and Baumann, 2010). 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 (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. (2005) reported significant facilitation of phosphene detection across ISIs ranging from 2 to 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 and 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 dissappeared 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., 2019). 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, conditionining stimulus intensities ranging from

15 to 30% of TS amplitude significantly suppressed visual evoked activity regardless of interstimulus interval, while intensities ranging from 60 to 90% of TS amplitude enhanced visual evoked activity independent of interstimulus interval (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 interstimulus intervals 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 et al., 1996a). Even if the effect of increasing the interstimulus interval 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 to 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 (Fig. 2B), 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% conditioning intensity 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 suband 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; Sparing et al., 2002, 2005; Khammash et al., 2019). 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 a 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.

ACKNOWLEDGEMENTS

Funding: This work was supported by the University of Michigan, the Claude D. Pepper Older Americans Independence Center [grant number P30AG024824], and the Boledovich Schizophrenia Research Fund. D.K. collected the data. D.K. and S.K.M. performed the analyses. D.K., S.K.M., T.A.P., S.F.T., and M.S. wrote the manuscript.

Abbreviations:

BA	Brodmann Area
CS	conditioning stimulus
GABA	gamma-Aminobutyric acid
ICF	intracortical facilitation
ISI	interstimulus interval
LICI	long-interval cortical inhibition
MEP	motor evoked potential
NMDA	*N*-methyl-D-aspartate
ppTMS	paired-pulse transcranial magnetic stimulation

SICI	short-interval intracortical inhibition
TMS	transcranial magnetic stimulation
TS	test stimulus
VCI	visual cortex inhibition

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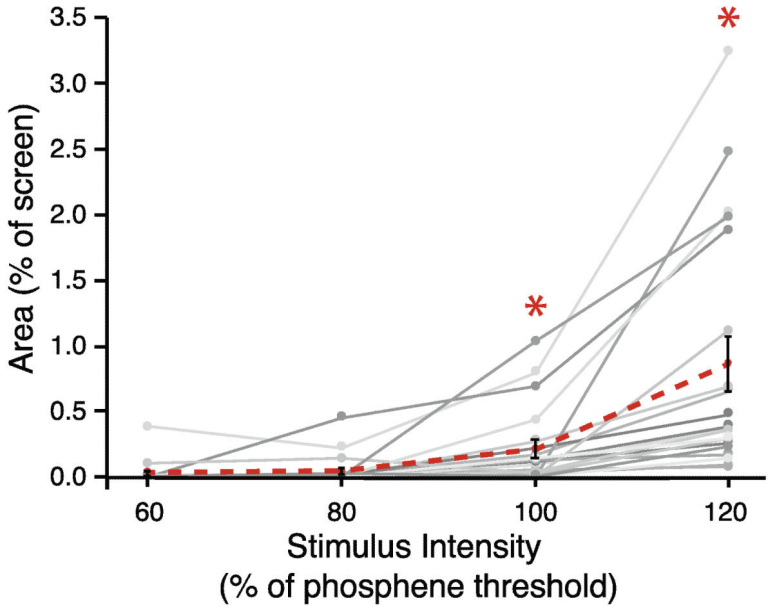

Fig. 1.

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 ($p < 0.05$).

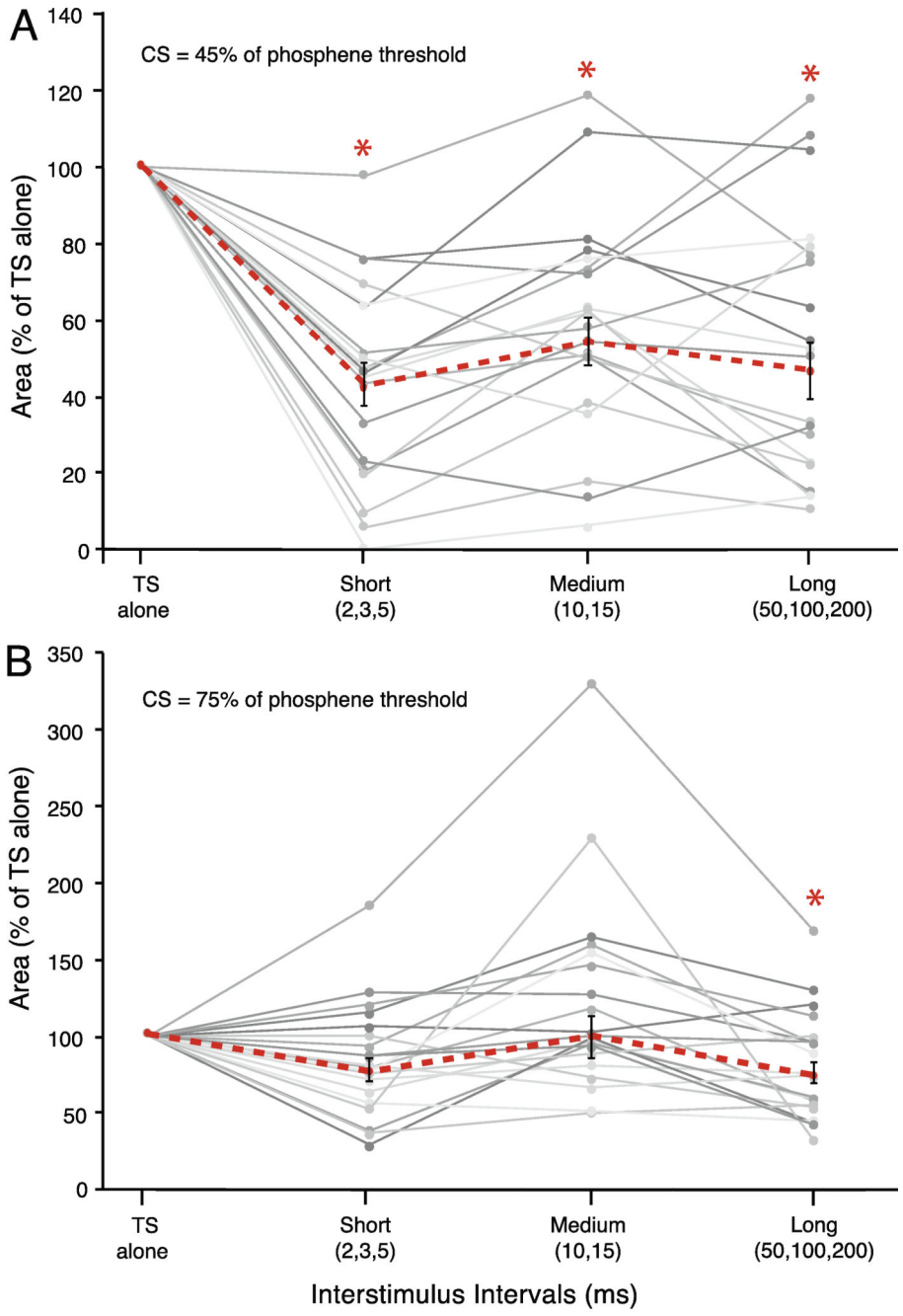

Fig. 2.

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.

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