Recommender's comments = black
Authors' comments = blue

PCI-RR Stage 1 Triage decision

Dear Dr. McIntosh,

Thank you for your constructive feedback. We have updated the manuscript according to your comments and address each point below.

Yours sincerely,

Grace Edwards, Mica Carroll, and Chris Baker

The Introduction is generally well-written but I see three main ways in which it could be strengthened: (1) when reviewing prior studies, especially the study that provides the target effect for replication (Ghin et al, 2018), it would be useful to give some quantitative details (e.g. sample size, effect size etc); (2) the review seems to be restricted to tRNS interventions, but it would be good to consider also the evidence regarding other NIBS interventions at MT+ on motion processing; (3) I would like to see a clearer rationale for why this particular effect has been targeted – if the aim is to select an effect just to validate the effect of tRNS, then is this particular effect the best candidate? Or if the interest is more specifically in motion processing, then why? (Also note, elsewhere in Introduction, keep the conceptual distinction between replication and reproduction clear.)

According to the Recommender's suggestions, we have edited in the introduction to include 1) quantitative details of the Ghin et al., 2018 findings, 2) other NIBS evidence for hMT+ modulation, 3) a clearer rationale for reproducing contralateral motion processing impact following hf-tRNS targeted at hMT+, and 4) how we separate reproduction versus replication.

From p.5 of manuscript:

"We propose to reproduce and extend Ghin et al.'s (2018) findings by combining Experiments 1 and 2 in their paper. In Experiment 1, the authors employed a within-subjects design with three types of tES (cathodal tDCS, anodal tDCS, hf-tRNS) and sham stimulation. Each participant (n = 16) underwent each type of stimulation over the course of four, non-consecutive sessions in which the active electrode was placed over left hMT+

and the reference electrode was placed over Cz. The authors applied stimulation during a motion direction task (i.e. online stimulation) which required participants to determine the overall direction of random dot kinematograms (RDK's), or fields of moving dots, during an eight alternative forced choice task. RDK's were either shown to the left or right of a fixation dot in the middle of the screen. An adaptive maximum likelihood procedure (MLP) was used to determine the coherence threshold of the stimuli in each trial until the participants performed at 70% accuracy within 18 minutes (Grassi and Soranzo, 2009). The authors found that only the hf-tRNS condition lowered the coherence threshold in the right visual field, contralateral to the active electrode placed over the left hMT+ (ipsilateral > contralateral = 10.51%). Anodal and cathodal tDCS had no impact on performance.

To verify that the effects of Experiment 1 were location-specific, Ghin et al. (2018) stimulated two control sites in Experiment 2. In the first control (n = 12), the active electrodes were placed over Cz and the left forehead. This control examined if stimulation over Cz alone affected motion direction discrimination in Experiment 1. In the second control (n = 12), the active electrodes were placed over Cz and the left primary visual cortex (V1) to determine if visual cortex stimulation was sufficient to improve motion direction discrimination. No significant effects on performance in the motion direction discrimination task were found in either control (forehead: ipsilateral > contralateral = 1.17%; V1: ipsilateral > contralateral = 1.58%), apparently confirming the hypothesis that the observed effects of stimulation were specific to hMT+. However, the authors did not directly compare the hMT+ condition with the V1 or forehead control conditions.”

From p.4 of manuscript:

"The motivation to reproduce the hf-tRNS effect in Ghin et al. (2018) is reinforced by the well-supported hypothesis that hMT+ is specific to contralateral global motion processing (Strong et al., 2017; Ajina et al., 2015; Braddick et al., 2001). Many NIBS studies have investigated the effects of stimulation over hMT+ on motion processing and have found reliable effects on neural activity and task performance (e.g. Antal et al., 2004; Antal et al., 2012; Campana et al., 2016; Pavan et al., 2019). Combined, studies demonstrate that stimulation over hMT+ has consistent effects. For example, Antal et al. (2004) tested the impact of tDCS (anodal and cathodal) on hMT+, V1, and the motor cortex during a visuomotor coordination task and a motion direction discrimination task. The authors found that only a-tDCS over hMT+ improved performance on these tasks. TMS over hMT+ has also been shown to modulate motion processing. For example, Laycock et al. (2007) found that single pulse TMS to hMT+ 158 ms after stimulus onset of a motion direction discrimination task disrupted task performance. Similarly, McKeefry et al. (2008) found that repetitive TMS over hMT+ and V3A caused deficits in speed discrimination, while TMS over adjacent areas and V1 did not. Finally, Campana, Maniglia, and Pavan (2013) found that repetitive TMS over hMT+ reduced the duration of dynamic and static motion after-effect.

Motion processing is predominantly lateralized, which enables the comparison of stimulation on the left and right visual fields as a within session control. A tDCS study found that c-tDCS over left hMT+/V5 reduced a noisy signal while a-tDCS boosted a weak signal in the contralateral visual field only during a motion coherence task (Battaglini,

Noventa, and Casco, 2017). Furthermore, repetitive TMS over left hMT+ impaired performance in a multiple object tracking task in the contralateral visual field only (Chakraborty et al., 2021). Due to strong theoretical support and evidence across NIBS techniques for the highly reliable effects of stimulation over hMT+, our study provides an ideal test for the reproducibility of hf-tRNS effects."

From p.6 of manuscript:

"In order to implement some additional controls, we propose to reproduce the contralateral impact of hf-tRNS over left hMT+, rather than perform an exact replication. In contrast to an exact replication, we include only a selection of the original conditions, and have introduced extra within-subject stimulation controls in our design."

(4) Hypothesis 1 is that tRNS will cause a differential reduction of contralateral motion coherence threshold, greater than that in either control condition. Rather than propose a repeated-measures ANOVA with planned comparisons, it would be more targeted to specify the exact comparisons of interest and focus upon these (the full ANOVA can be added in subsequent exploration if you wish). The dependent variable of interest for each treatment condition would seem to be the subtraction of contralateral from ipsilateral thresholds, and your critical prediction that this difference will be significantly larger in tRNS than in both control conditions. This seems to suggest that you should perform two independent t-tests to make these comparisons. Because both outcomes would need to be significant to support your hypothesis, no correction for multiple comparisons would be required; see e.g. Rubin 2021). (It is even possible that your theory predicts the conjunction of three effects: significant difference between hemifields in tRNS condition, and significantly greater inter-hemifield difference in tRNS condition as compared with each of the control conditions.)

We have now specified our exact comparisons of interest and subsequently target these comparisons to power our design. Specifically our three targeted effects are: 1) the difference between contralateral and ipsilateral motion coherence threshold for the hMT+ targeted hf-tRNS condition, 2) the difference between contralateral and ipsilateral motion coherence threshold for hMT+ targeted hf-tRNS versus the difference between contralateral and ipsilateral motion coherence threshold for hMT+ targeted sham tRNS, 3) the difference between contralateral and ipsilateral motion coherence threshold for

hMT+ targeted hf-tRNS versus the difference between contralateral and ipsilateral for forehead targeted hf-tRNS.

We edited the stated comparisons of interest in the introduction, method (*Participants, Statistical analysis,* and *Expected results)*, and Study Design Template.

Introduction edit, from p.7 of manuscript:

"We have three main hypotheses: 1) We hypothesize that we will reproduce the facilitatory effect of hf-tRNS to hMT+ on contralateral global motion processing in comparison to ipsilateral global motion processing. 2) We expect the facilitation for contralateral in comparison to ipsilateral motion processing will be larger for hMT+ in comparison to sham, and 3) the facilitation for contralateral in comparison to ipsilateral motion processing will be larger for hMT+ in comparison to forehead stimulation.

We also will perform an exploratory analysis to examine if an increased overlap between the predetermined hMT+ targeted stimulation coordinates and neural response to processing lateralized motion predicts an increased impact of hMT+ targeted stimulation."

Methods edits:

Participants*, from p.8 of manuscript:*

"We will use Bayes Factors to determine evidence for or against each of our three hypotheses, namely:

1) evidence for or against a difference between contralateral versus ipsilateral motion coherence thresholds following hf-tRNS to hMT+.
2) evidence for or against the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ targeted hf-tRNS versus the

difference between contralateral versus ipsilateral motion coherence thresholds following sham hf-tRNS.

3) evidence for or against the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ targeted hf-tRNS versus the difference between contralateral versus ipsilateral motion coherence thresholds following forehead targeted hf-tRNS.

A maximum of 42 participants will take part in the study, determined by the largest number of participants needed for the three effect sizes of interest using the data from Ghin et al., (2018). We performed three power analyses on the three comparisons of interest (See Appendix B). In each power analysis, the standard deviation is based on the Ghin et al., (2018) between-subjects design, providing a conservative estimate of variance for our within-subjects design. We therefore predict 42 participants to be the maximum number of participants necessary to provide evidence for or against our hypotheses in our within-subjects design. In order to maintain counterbalancing across stimulation conditions, we will analyze our data after every six participants are collected, determining if we have sufficient evidence for or against our three hypotheses (See Statistical Analysis in Methods). Only when we have evidence for or against the hypotheses will we stop collecting data, otherwise we will continue to 42 participants. Participants will only be excluded from analysis if there is an equipment failure, or if the participant fails to attend all sessions. Participants will not be excluded due to outliers as the counterbalancing of the three stimulation sessions may lead to spurious outliers as a result of learning. The participants will be screened for normal or corrected-to-normal visual acuity and the ability

to meet a 70% threshold during the constant thresholding procedure (see Appendix C). This study was approved by the Ethics Committee of the NIH. We will obtain written informed consent from each participant before taking part in the study, for which they will receive monetary compensation."

Statistical analysis, from p.15 of manuscript:

"We will perform three comparisons using Bayes Factors (Good, 1979; Kass and Raftery, 1995; Morey, Romeijn, and Rouder, 2016; Rouder et al., 2009; Dienes, 2011; Teichmann et al., 2022; Wagenmakers, 2007):

1. evidence for or against a difference between contralateral versus ipsilateral motion coherence thresholds following hf-tRNS to hMT+.
2. evidence for or against the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ targeted hf-tRNS versus the difference between contralateral versus ipsilateral motion coherence thresholds following sham hf-tRNS.
3. evidence for or against the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ targeted hf-tRNS versus the difference between contralateral versus ipsilateral motion coherence thresholds following forehead targeted hf-tRNS.

We will use a one-sided Bayes Factor test with a half-Cauchy prior for the alternative hypotheses, width of 0.7071 (Boayue et al., 2020; Keysers, Gazzola, and Wagenmakers,

2020). This prior was motivated by the small to medium effect sizes of Ghin et al., (2018). We will use the Bayes Factor function developed by Teichmann et al., 2022 within Matlab. We will have a Bayes Factors cut-off of 6 or above as substantial evidence for our alternative hypothesis (Wetzels et al., 2011). Evidence for the null will be accepted with Bayes Factors smaller than 1/6.

In an exploratory analysis, we will compare individual simulated current flow over hMT+ to individual functional maps for contralateral motion processing. We will use the percentage overlap between simulated current flow and contralateral motion processing regions as a predictor for behavioral outcome following hf-tRNS over hMT+. Specifically, we expect a positive correlation between overlap of simulated e-field current flow and lateralized motion processing with hf-tRNS stimulation impact in contralateral versus ipsilateral motion coherence thresholds. Support for or against the hypothesized correlation will be determined using Bayes Factors (Wetzels and Wagenmakers, 2012; See Table 1)"

Expected results, from p.16 of manuscript:

"We expect to find a reduction in coherence threshold for hf-tRNS stimulation to the left hMT+ in the contralateral right visual field in comparison to ipsilateral left visual field. We also expect the facilitation of contralateral in comparison to ipsilateral motion coherence threshold to be larger for hMT+ targeted hf-tRNS in comparison to hMT+ sham tRNS and for hMT+ targeted hf-tRNS in comparison to forehead targeted hf-tRNS."

Please see the study design template in the edited manuscript (pages 18-21).

(5) If that is a correct formulation of your Hypothesis 1, then your targeted effect size of interest should correspond specifically to that effect, rather than (as at present) simply to the hemifield difference within the tRNS condition.

Following the Recommender's comments in points 4 and 5, we have run three power analyses targeting our effects of interest. We will then use Bayes Factors to determine evidence for or against each of our hypotheses. The smallest effect size provides the stopping point of our data collection (n=42). In each power analysis, the standard deviation is based on the between-subjects design of Ghin et al., providing a conservative estimate. We expect reduced variance in our within-subjects design. We therefore predict 42 participants to be the maximum number of participants necessary to provide evidence for or against our hypotheses in our within-subjects design. In the *Participants* and *Statistical analysis* sections of the methods, we describe our use of Bayes Factors, and we outline all three power analyses in Appendix B.

See edits to *Participants* and *Statistical Analysis* in response to comment 4.

*Appendix B: Power analyses**, from p.23 of manuscript:***

"We performed three power analyses on our three comparisons of interest:

First, we examined contralateral versus ipsilateral motion coherence thresholds following hf-tRNS to hMT+. Specifically, we used a true mean of 28.75% related to the contralateral motion coherence threshold, null hypothesis mean of 39.26% related to the ipsilateral motion coherence threshold, and standard deviation of 17.56% from Experiment 1 of Ghin et al. (2018). With a power of 0.9 and type I error rate of 2%, we calculated the need for 34 participants.

Second, we examined the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ targeted hf-tRNS versus the difference between contralateral versus ipsilateral motion coherence thresholds following sham hf-tRNS. We used a true mean of 10.51% related to the difference in contralateral and ipsilateral motion

coherence threshold for hMT+ targeted hf-tRNS, null hypothesis mean of 2.59% related to the difference in contralateral and ipsilateral motion coherence threshold for hMT+ targeted sham tRNS, and standard deviation of 14.8% from Experiment 1 of Ghin et al (2018). With a power of 0.9 and type I error rate of 2%, we calculated the need for 42 participants.

Finally, we examined the difference between contralateral versus ipsilateral motion coherence thresholds following hMT+ hf-tRNS versus the difference between contralateral versus ipsilateral motion coherence thresholds following forehead targeted hf-tRNS. We used a true mean of 10.51% related to the difference in contralateral and ipsilateral motion coherence threshold for hMT+ targeted hf-tRNS, null hypothesis mean of 1.17% related to the difference in contralateral and ipsilateral motion coherence threshold for forehead targeted hf-tRNS, and standard deviation of 8.44% from Experiment 1 of Ghin et al (2018). With a power of 0.9 and type I error rate of 2%, we calculated the need for 12 participants.

These power analyses indicate 42 participants as our stopping point for data collection."

(6) Your effect size estimate is drawn directly from Ghin et al (2018) (although as noted above you may need to adjust precisely which effect size you are estimating). It is possible that this effect is an upwardly-biased estimate, considering that it is a small-n (n=16) study that has been published with a positive finding (see e.g. Button et al, 2013). If so, would it be advisable to be more conservative in setting your effect size of interest? One strategy would be to define the smallest expected effect size, for instance by estimating the lower bound on prior relevant effects in the literature. Ideally, you would draw your smallest expected effect size from consideration of more than a single prior study, although I understand that this may not be possible if there is only one directly relevant study. An alternative strategy is to define the smallest effect size that would be of theoretical interest, and to target that (see Dienes 2019; or for related discussion Lakens, 2022). By powering your study for a conservative or smallest interesting effect size, you would increase the potential informativeness of a null result. Of course, this is likely to mean that you may need to increase the sample size.

Following the Recommender's comments, we ran three power analyses on our three effects of interest and have selected the one with the smallest effect size as our stopping point for data collection. We examined the previous literature on hf-tRNS targeted at hMT+ to give us further examples of lateralized stimulation effects, and therefore potential a lower bound of the difference between ipsilateral and contralateral stimulation effects. The two most relevant tRNS studies using hf-tRNS at 1.5 mA over predefined anatomical coordinates of hMT+ (Campana et al., 2016; Pavan et al., 2019) were performed using bilateral hMT+ stimulation, examining the effect of stimulation on motion presented at fixation. Due to the bilateral tRNS, no contrast of visual field contralateral or ipsilateral to stimulation could be performed, therefore limiting our ability to use these data in a power analysis. We hesitate to make claims on effect sizes following other stimulation methods as stimulation impact is highly variable between stimulation types, and stimulation protocols (van der Groen et al., 2022).

(7) The second hypothesis is based on a correlational relationship. If this is a critical hypothesis, then you similarly need to motivate your smallest effect size of interest, and show how much power your study has to detect it.

The correlational relationship between stimulation e-field and lateralized motion activity within the cortex is an exploratory analysis. To our knowledge, this analysis has not been performed before. If we are unable to reproduce the stimulation effects from Ghin et al. (2018), we may need to determine how well we targeted hMT+ using the predetermined coordinate approach. We have highlighted this analysis as exploratory in the main text and study design template.

(8) Make sure that you state the significance threshold for each test, and whether it is one- or two-tailed. You can choose any level of power that you wish, but your combination of power and alpha will constrain the pool of eligible journals in which you might choose to place your Stage 2 manuscript, if it receives recommendation. For a list of PCI_friendly journals, and criteria,
see https://rr.peercommunityin.org/about/pci_rr_friendly_journals. You are not obliged to publish your work in a journal at all; I am just making sure that you appreciate how your design choices may affect the pool of potential journals if you do.

Thank you for highlighting the criteria of the PCI_friendly journals. We updated our power analyses to one-tailed *t*-tests at an alpha of 0.9 and a $p<0.02$. Furthermore, we now include the Bayes Factor cut-off of >6.

(9) Appendix C reports threshold estimation method for your MLP. I think that this may be sufficiently central to the the experiment that it should be fully described within the main Methods.

Appendix C is now in the Methods section of our manuscript (pages 10-11).

(10) Make sure that you have considered and clearly stated all relevant exclusion criteria (at trial and participant level). Also consider whether your design has (or needs) any critical manipulation checks/outcome-neutral criteria. These are criteria that must be satisfied in order for your experiment to be deemed capable of testing the hypothesis of interest (which might include the absence of floor or ceiling effects, the presence of positive control effects, or other essential quality checks orthogonal to the main hypotheses.)

Participants' performance will be thresholded to 70% correct within every session, overcoming concerns with ceiling and floor effects. Due to potential learning effects across session, positive controls per participant are unlikely to occur, meaning we consider our stimulation effects at the group level after counterbalancing across subjects. Every trial will impact the thresholding procedure, if the subjects fail to respond (e.g. too slow or attentional lapse), the MLP will treat the trial as having an incorrect response.

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