

Supplemental Information:

Strengthening functionally specific neural pathways with transcranial brain stimulation

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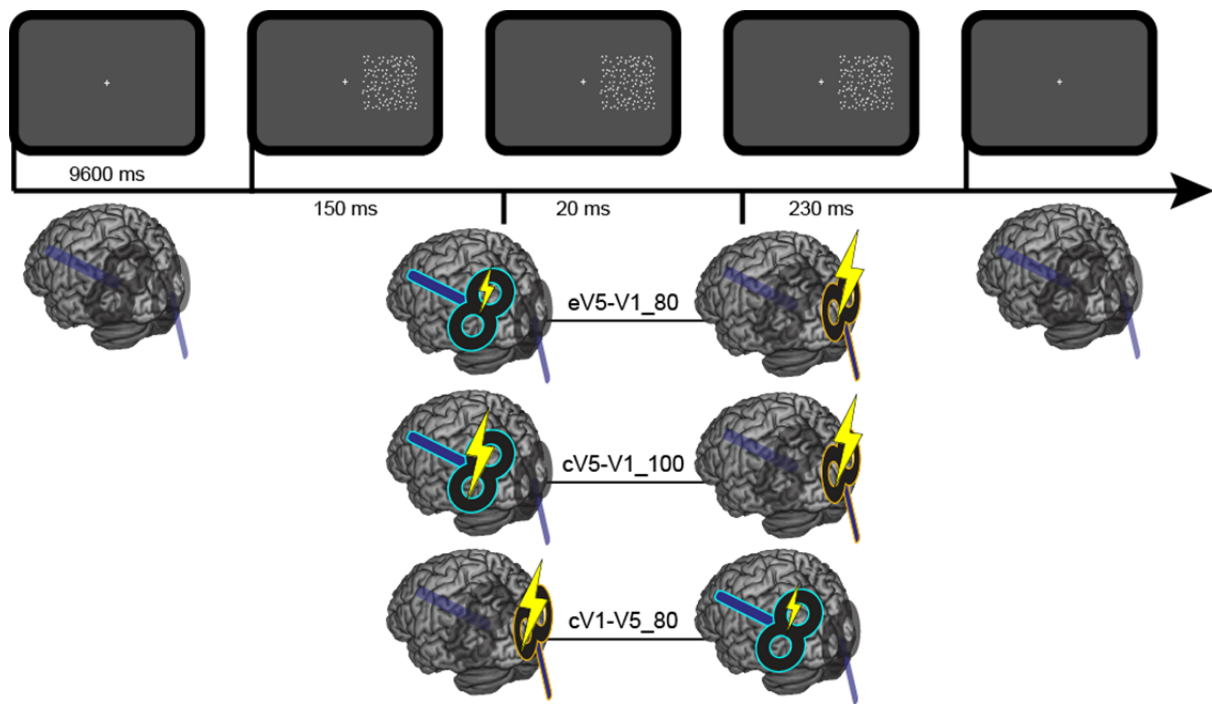


Figure S1. Related to Figure 1. Temporal details of the “function-tuning ccPAS” protocol. A total of 90 pairs of TMS pulses delivered over V5/MT+ and V1/V2 with an inter-pulse interval (IPI) of 20 ms were paired with the presentation of a moving visual stimulus. A fixation cross was displayed for 9600 ms followed by the presentation of a motion stimulus for a period of 400 ms, with 100% dot motion coherence (which could move either leftward or rightward across participants, but always in the same direction within sessions and counterbalanced across participants). A first TMS pulse was delivered at 150 ms from motion stimulus onset, followed by a second TMS pulse 20 ms after. At the end of the motion stimulus presentation the next trial started. No response was required, participants were asked to stare at the fixation cross throughout the ccPAS session, which lasted approximately 15 minutes (90 stimuli at 0.1 Hz). The whole procedure was repeated for 3 sessions separated by at least 24 h, and differing for stimulation directionality and intensity. Specifically, in the experimental condition (eV5_V1_80), TMS was applied first over V5/MT+ at 80% of the individual phosphene threshold (PT) and then, after 20 ms over V1/V2 at 100% of the PT; in the first control condition (vV5_V1-100) TMS was applied over V5/MT+ followed by 20 ms over V1/V2, with intensity of stimulation delivered over both areas at 100% of PT. Finally, we also included a control condition in which V1/V2 stimulation at 100% of PT preceded V5/MT+ TMS at 80% PT.

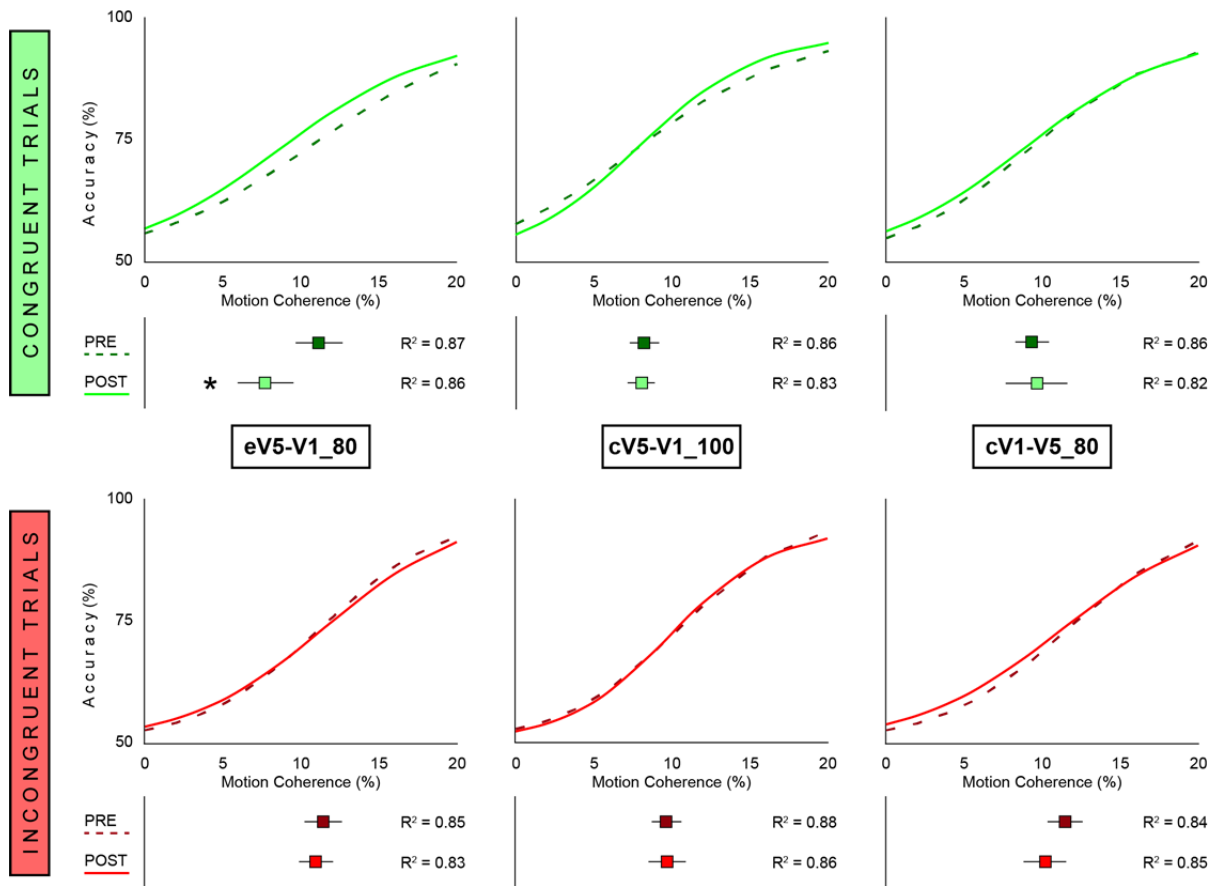


Figure S2. Related to Figure 1. Illustrative curves fitted on the averaged performance of participants in each level of the motion direction discrimination task in the 6 experimental conditions (0-20% motion coherence interval is shown). Squares (and errorbars) below the curves represent participants' average inflexion point value (and s.e.m.) before (PRE) and after (POST) ccPAS; asterisks indicate significant PRE vs. POST comparisons ($p < 0.05$; Bonferroni Corrected; see below). To the right of each square are reported the mean R^2 values of the corresponding curves fitted to obtain the individual inflexion points, indexing the goodness of fit (overall R^2 mean \pm s.d. = 0.85 ± 0.08). **Non-Baseline-corrected analysis:** The repeated measures ANOVA with the factors Session (3), Direction (2) and Time (2) conducted on the inflexion points of the logistic curves fitted on data of the task performance revealed a significant three-way interaction ($F_{2,30} = 3.86$, $p = 0.032$, $\eta_p^2 = 0.2$) and no other main effects or interactions (all p s > 0.14). Motion sensitivity threshold differences within the same factor Direction before ccPAS (PRE) were tested with post-hoc comparisons; analyses revealed no differences (all p s > 0.08), indicating comparable performance in the different sessions before TMS intervention. With the same post-hoc analysis, motion sensitivity threshold differences within

the factor Stimulation and Direction were tested. Results showed no significant comparisons between PRE and POST ccPAS (all p s = 1) except for the congruent direction in the eV5-V1_80 Session [PRE vs. POST (mean \pm s.e.m.); 11.14% \pm 1.51% vs. 7.72% \pm 1.78%, p = 0.017, *Cohen's d* = 0.66], showing a selective enhancement of motion sensitivity threshold occurring only for the direction congruent to the seen stimulus in the ccPAS phase and after the experimental session. **Analyses for possible confounding effects.** A median split of the baseline performance for each of the 3x2 conditions was conducted and contrasts on post-ccPAS gain (POST - PRE) between the groups with low and high baseline values revealed no significant differences (uncorrected t -tests; all T s(14) > 1.10, all p s > 0.29), indicating that performance improvements were not contingent on the baseline ability of the participants. Furthermore, a repeated measures ANOVA with factors Order (3) and Direction (2) on the behavioural gain (POST - PRE) showed no main effects nor interactions (all p s > 0.18), ruling out the possibility that the order of ccPAS sessions played a significant role in the effects reported.

Supplemental Methods

Phosphenes perception screening and induction

Inclusion criteria for the current experiment required taking part in a separate initial screening assessing phosphene perception. Phosphenes' perception was tested on 37 right-handed subjects with no contraindications to TMS as assessed by a screening questionnaire approved by the University of Essex Research Ethics Committee in compliance with the guidelines for non-invasive magnetic brain stimulation for research application [S1]. Phosphene perception thresholds from the V1/V2 and the left V5/MT+ were assessed using a 50 mm figure-of-eight coil, connected to a mono-phasic Magstim BiStim² stimulator (Magstim Co., Whitland). To target V1/V2, the coil was centered 2 cm dorsal to the inion, holding the handle tangential to the scalp and pointing downwards at an angle of $\sim 120^\circ$ clockwise. This location is expected to activate V1/V2 bilaterally. To target left V5/MT+, the coil was centered 3 cm dorsal and 5 cm lateral (left) to the inion, holding the handle tangential to the scalp and

pointing upwards and laterally at an angle of $\sim 45^\circ$ to the sagittal plane. These stimulation sites obtained using craniometric coordinates based on anatomical skull landmarks correspond to the average V1/V2 and V5/MT+ stimulation sites as functionally assessed in previous studies [S3, S4]. We also measured phosphenes in both V1/V2 and V5/MT+ sites, providing a first prompt of correct functional localization (albeit approximate as localization was fixed to craniometrics coordinates). Yet, the craniometric coordinates were determined to represent the best compromise between optimal functional localization and space constraints in coil positioning due to the bulkiness of their geometry. Single TMS pulses were applied with increasing intensity, starting from 30% of the maximum stimulator output (MOS), until participants perceived reliably phosphenes; intensity was then adjusted to evoke phosphenes 3 out of 6 consecutive pulses. Self-reported phosphenes for both V1/V2 and V5/MT+ had to fulfil the following criteria: phosphene perception should be possible both with eyes open and shut; no phosphene perception should be reported in sham pulses; only for V1, coarse retinotopical perception should be achieved, depending on the site of stimulation (i.e. phosphene on the left visual field should be induced only after right occipital cortex stimulation and vice versa). Only 16 subjects (43% of the sample tested) fulfilled the criteria and were therefore eligible for the experiment.

Sample

Based on power analysis (conducted on [S2], actual effect size, $f = 0.22$), with a conservatively estimated effect size $f = 0.1$, (which is a medium-to-large size by convention), $\alpha = 0.05$, and 80% power, a total sample size of 16 participants is suggested. Therefore, 16 healthy volunteers (11 female; mean \pm s.d. age 25.3 ± 7.7 years) were recruited for the study. They were right-handed according to the Edinburgh handedness inventory [S5]; all of them perceived phosphenes evoked by V1/V2 and V5/MT+ TMS; they reported no neurological history; all of them gave written informed consent before taking part to the experimental procedures, which had been approved by the University of Essex Research Ethics Committee.

Task

A motion direction discrimination task was used to determine the global motion perception threshold in every participant at different timepoints. The task was adapted from Romei and colleagues [S2]. It was created and displayed using MATLAB (version 2015a, The MathWorks Inc., Natick, MA) and the Psychophysics Toolbox 3 extensions [S6,S7] and presented on an 18-inch CRT monitor (ViewSonic G90fB, ViewSonic Corp., Walnut, CA) with a resolution of 1280 x 1024 pixels and a refresh rate of 85 Hz. Participant's viewing distance was kept at 57 cm using a chin rest. A stimulus consisted of 400 white (RGB: [255 255 255]) dots (6 pixels each) moving within a squared region subtending 12 x 12 degrees of visual angle, the centre of which was 8° to the right of a white central fixation cross (20 x 20 pixels) on a grey (RGB [80 80 80]) background.

In each trial, dots could move coherently either leftward or rightward with 10 different percentages of motion coherence (0, 2, 4, 6, 9, 12, 16, 20, 35, 80). Motion coherence value indicates the percentage of dots that move in the signal direction. For example, in trials with 0% coherence, each of the 400 dots moved with a randomly selected direction motion (0% of signal, 100% of noise); in trials with 80% of coherence, 320 dots moved coherently towards either left or right (80% of signal), while each of the remaining 80 dots moved in randomly determined directions (20% of noise). Dots moved at a speed of 4.5°/s.

The task was a two-alternative forced choice task. Participants were instructed to always keep the gaze on the fixation cross that was constantly present at the centre of the screen. Each trial began with the fixation cross for 500 ms, then the moving stimulus appeared on its right side for 400 ms. Once the stimulus ended, only the fixation cross persisted and participants had to make an unspeeded response by pressing the left or the right arrow key to indicate which was the perceived global coherent direction of the motion. One task block consisted of 600 trials having 30 repetitions for each of the 10 coherence percentages in 2 possible (right/leftward) directions (30 x 10 x 2). A block lasted approximately 13 minutes.

ccPAS

ccPAS was administered through two 50 mm figure-of-eight coils, connected to a mono-phasic dual pulse Magstim stimulator (Magstim Co., Whitland), consisting of a BiStim² and a 200² module. Coils positioning and orientation were consistent with those adopted for the assessment of phosphenes thresholds (see *Phosphenes induction* section). Function-tuning ccPAS consisted of a combination of motion stimulus presentation and TMS that was delivered at a specific stimulus onset asynchrony (SOA) and were controlled through the E-Prime software (Psychology Software Tools, Pittsburgh, PA). Throughout this phase, the setting was identical to the one of the task, participants were asked to maintain their head still on the chinrest, to keep the gaze on the fixation cross and to watch the stimuli appearing on the screen passively, since no response was required. Stimuli were identical to those presented in the motion direction discrimination task, except that the coherence of the motion that was always at 100% i.e. all the dots were coherently moving in one direction. Each participant underwent 3 sessions of stimulation differing for ccPAS configuration, while the direction of movement was consistent throughout the session and across the sessions, randomly determined and counterbalanced across participants (8 participants were presented with 100% leftward motion, 8 with 100% rightward). Moving stimuli were presented for 400ms. 150ms after the motion stimulus onset, a first TMS pulse was delivered over V5/MT+ (or V1/V2) followed after 20ms by a second TMS pulse delivered over V1/V2 (or V5/MT+). Each set of paired pulses was delivered every 10 seconds (0.1 Hz). A total of 90 motion stimuli were paired with 180 TMS pulses (90 pulses on each of the two target areas). These parameters were selected for the following reasons:

- i. SOA of 150 ms is consistent with the peak of temporal activation course of V1/V2 and V5/MT+ in response to a motion stimulus in which V5/MT+ feeds back the processed information to V1/V2 [S8];
- ii. IPI of 20 ms corresponds to the timing at which MT/V5 exerts a physiological effect on V1/V2 [S3,S9], thus representing a critical timing to optimally activate the pre- and post-synaptic neuronal populations of V5/MT+-V1/V2 connection [S2], and comply with the spike timing-dependent plasticity (STDP) principles [S10-S12];

- iii. 90 is a standard amount of (double) pulses delivered for ccPAS protocols intended to repeatedly activate the cortico-cortical connection and foster the establishment of STDP-like phenomena [S2,S13-S16];
- iv. Stimulation rate of 0.1 Hz (intertrial interval: 10 s) assures no temporal summation effects of TMS pulses per se [S17].

The ccPAS condition varied depending on the session, the order of which was counterbalanced across participants. IPI and TMS intensity were manipulated across the ccPAS sessions.

IPI: STDP phenomena of long-term potentiation (LTP) revealed in cells [S10-S12] and mimicked by ccPAS protocols [S13-S16] depend on the exact timing of the connection, since the pre-synaptic node needs to causally assist the activation of the post-synaptic node to establish associative plasticity. We therefore expected that the IPI of 20 ms was optimal to induce LTP-like phenomena whilst IPI of -20 ms (determining a stimulation of opposite direction) was not [S2,S3,S9].

Intensity: Two intensities were used; subthreshold (80% of phosphene threshold) and suprathreshold (at 100% of phosphene threshold) intensity. The logic was the following: as concurrent presentation of a specific visual motion information changes susceptibility of those neuronal populations to be activated by TMS, we expected that direction-selective effects would be obtained with subthreshold intensity. This is because at the lower intensity, only pre-activated neurons (i.e. those driven by presented motion direction) would be sufficiently affected by TMS and therefore plastic changes would be limited to these neurons. In contrast, at a higher intensity all neurons would be likely activated and therefore direction-selective effects may not be observed (e.g. [S18-S20]).

These expectations seem to be at odds with conventional state-dependent paradigms which purport that TMS will selectively activate the least active population of neurons of the targeted area. Such an expected difference can be attributable to non-linear interactions between neural activation state and TMS intensity (see [S19] for an example of non-linear interactions possibly interpreted in terms of stochastic resonance). In conventional state-dependent protocols, the principle of stimulus adaptation leads neurons to be “less active” and thus less responsive to stimulation. In this case, instead, higher

stimulation intensity is needed to induce facilitatory effects. Here, we expect that the facilitatory nature of subthreshold intensity stimulation will further activate those neurons that have been pre-activated (but not adapted). Yet, as TMS intensity is increased, facilitation may turn into inhibition and thus no benefit of pre-activated neurons should be further observed (for a detailed discussion see [S21]).

Based on these considerations, we expect to have one experimental ccPAS session that, consistent with STDP rules, optimally targets the V5/MT+→V1/V2 pathway for the direction of motion congruent with the motion stimulus observed during ccPAS protocol.

Experimental session (eV5-V1_80): the first pulse was delivered over V5/MT+, the second over V1/V2 at the intensity of 80 and 100% of the phosphene threshold, respectively. This ccPAS condition was expected to potentiate the V5/MT+→V1/V2 reentrant connectivity specifically for the congruent direction of the motion. The subthreshold intensity at which V5/MT+ was stimulated, was intended to facilitate pathways conveying information about the displayed motion direction, while no effect was expected on non-congruent ones, having a higher threshold and being inhibited by the congruent stimuli (e.g. [S18-S20]). Hence, we expected this ccPAS condition to be optimal to enhance the connectivity between the presynaptic node (V5/MT+), and the postsynaptic node (V1/V2) selectively for the neurons coding for the primed direction of motion.

Control session for intensity (cV5-V1_100): identical to the eV5-V1_80 session except for the intensity applied to V5/MT+ stimulation that was at 100% of the phosphene threshold. This stimulation was expected to have no effects on motion perception.

Control session for directionality (cV1-V5_80): identical to the eV5-V1_80 session except for the order of stimulation, i.e. V1/V2 pulse was delivered prior to V5/MT+ pulse. This stimulation was expected to have no effects on motion perception (e.g. [S2]).

Procedure

The experiment was a within-subject design carried out in 3 sessions, separated by at least 1 day (average: 7.9 days). Each session was defined by the specific ccPAS condition that each participant

would undergo, the order of which was randomly determined, but fully counterbalanced. In all the sessions, prior to the beginning of the experiment, phosphene threshold was assessed on the day of the experiment for both V1/V2 and V5/MT+ areas. In line with previous studies [S3] the average phosphene threshold was lower for V1/V2 than V5/MT+ (MOS mean \pm s.d.) $58 \pm 8\%$ and $62 \pm 9\%$ respectively, as shown by a paired 2-tailed t -test ($t_{15} = -4.48$, $p < 0.001$).

Threshold values did not significantly fluctuate across the sessions either for V1/V2 [(session: MOS mean) session1: 59%, session2: 57%, session3: 58%; one-way ANOVA $p = 0.63$] or V5/MT+ [(session: MOS mean) session1: 62%, session2: 61%, session3: 62%; one-way ANOVA $p = 0.65$].

Participant's sensitivity to global motion (in both the congruent direction with respect to the priming stimulus presented during ccPAS and the non-congruent) was tested before (BSL) and 30 minutes after the end of the ccPAS phase. The decision to evaluate the performance 30 minutes after the ccPAS was driven by our previous findings [S2] showing the most notable effects exactly at this latency. In addition, a training block of 200 trials was performed in order to achieve a stable performance before the BSL.

Data handling

Data collected through the task were plotted on a cartesian plane with the X axis representing the motion coherence and the Y axis the percentage of accuracy. As expected based on our previous study [S2], data distribution described a psychophysical curve having a sigmoidal shape roughly ranging between 50 (at 0% of motion coherence; guessing rate) and 100% (at 80% of motion coherence) of accuracy. Therefore, data were well fitted by a nonlinear function modelled on the logistic curve:

$$y = \frac{1}{2} \left(1 + \frac{a}{1 + e^{-\frac{x-b}{c}}} \right)$$

where a determines the value of the upper horizontal asymptote; b represents the value of the point of critical change in the function behaviour at half the way between the lower and the upper asymptotes, named the inflexion point of the curve; c defines the slope.

For each participant, the value of the inflexion points for each block and each motion direction (congruent or incongruent to that presented in the ccPAS phase) was calculated using MATLAB (version 2016b, the MathWorks, Natick, MA), applying the Levenberg-Marquardt algorithm. This value represents the motion sensitivity threshold, intended as the percentage of coherent motion that mathematically describes the change in the global motion perception. The value of the R^2 was calculated to verify the goodness of fit of individual's data-points to the logistic curve. Mean values for each condition are reported in Figure S2.

A factorial ANOVA with Stimulation (eV5-V1_80, cV5-V1_100, cV1-V5_80), Direction (Congruent, non-Congruent) and Time (PRE, POST) on the raw values of the motion sensitivity threshold was performed. Bonferroni corrected post-hoc tests were performed on relevant comparisons (for Results of this analysis, see Figure S1 Legend). To compare the modulatory effect of the ccPAS independently of the BSL values, we calculated a modulation index by subtracting the motion sensitivity values of POST from those of PRE (POST minus PRE ccPAS). Negative values reflect less percentage of coherent global motion necessary to change the perception, thus a performance enhancement, while positive values index a performance decay. Based on previous findings [S2] and on our a priori theoretical assumptions, one-tailed *t*-tests (with Bonferroni correction for multiple comparisons) were performed on the modulation index (i) to compare within the eV5-V1_80 session the modulatory effect of the experimental ccPAS configuration on congruent and incongruent direction of motion, and (ii) to compare in the congruent direction the modulatory effect of eV5-V1_80, cV5-V1_100 and cV1-V5_80 stimulation. The Statistica software (v.12, StatSoft Inc., Tulsa, OK) was used to compute the analyses.

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