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Supplemental Information

Empowering Reentrant Projections from V5 to V1 Boosts Sensitivity to Motion

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Supplemental data

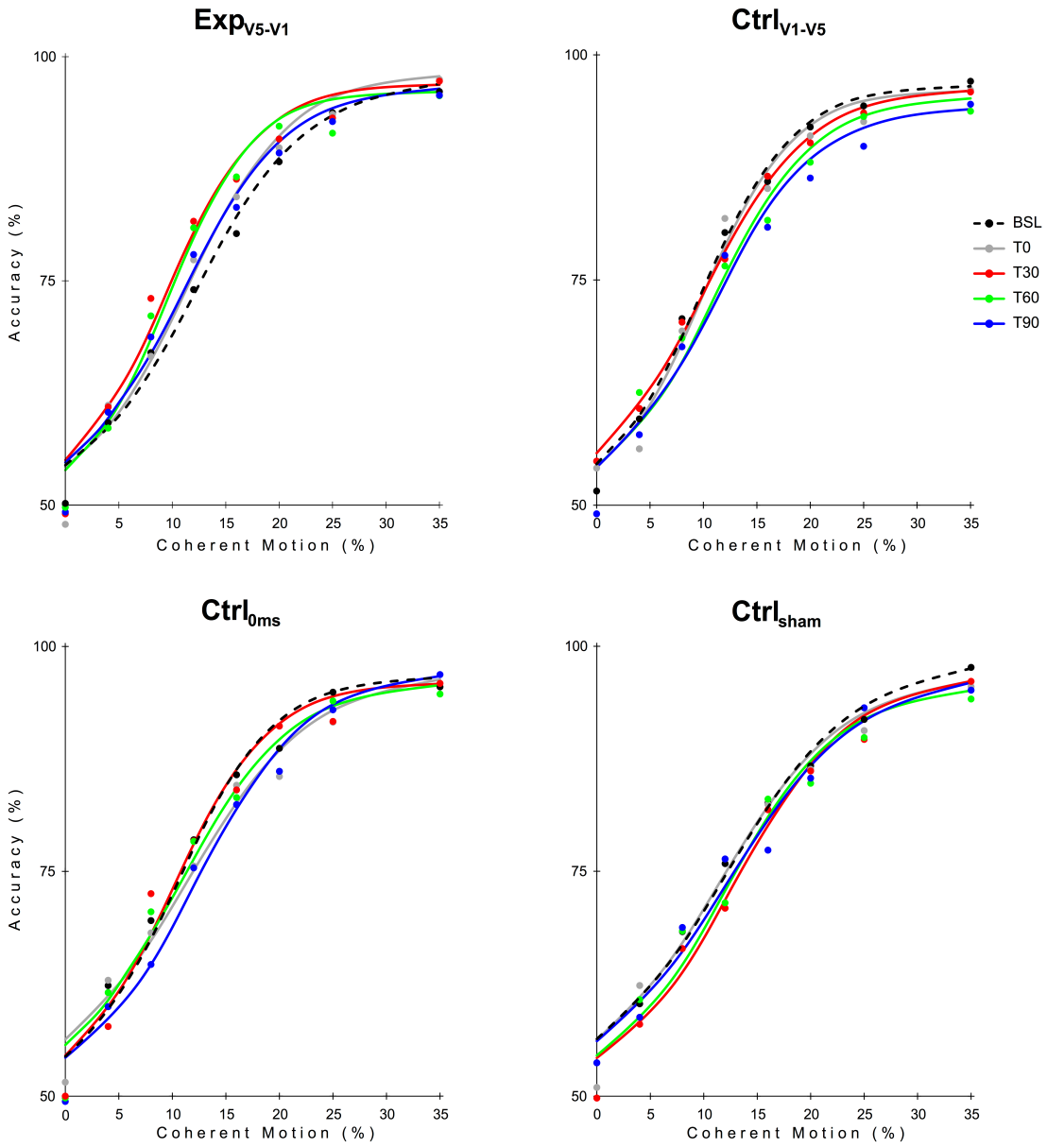

Figure S1. Related to Figure 3; Curve fitting and groups' performance. Sigmoid curve fits and averaged data points for each group and each time. See Figure 3 for detailed information.

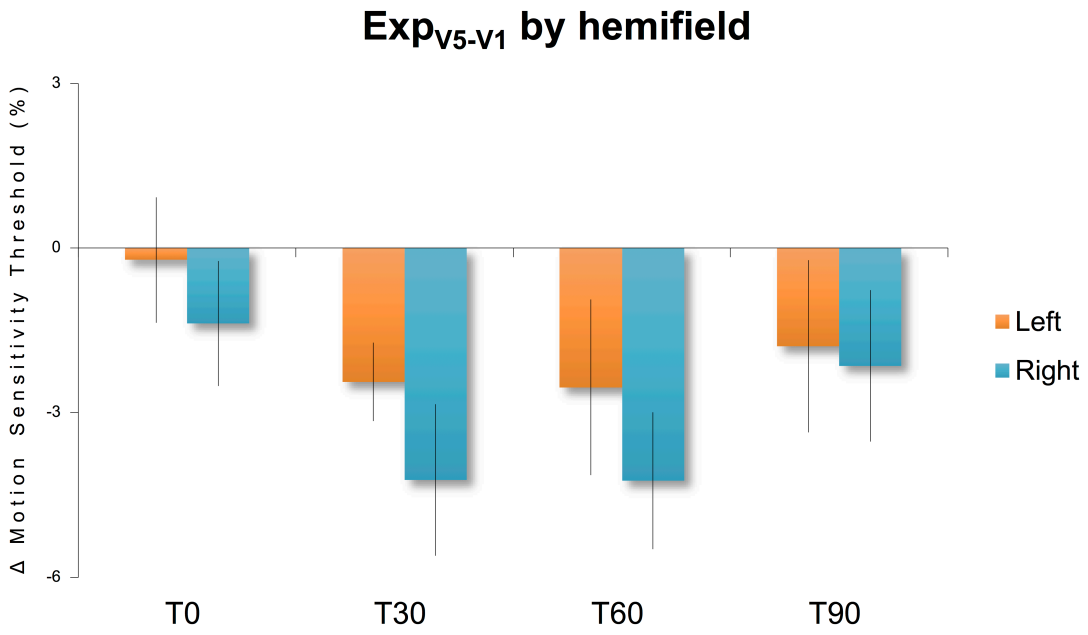

Figure S2. Related to Figure 2; ccPAS-induced changes in visual motion sensitivity for stimuli occurring in the left and right hemifields of the $Exp_{V5\text{-}V1}$ group. Error bars denote ±1 s.e.m. Similar changes in motion sensitivity threshold were found in the two hemifields. This is not surprising because our ccPAS protocol included stimulation of lateralized left V5 but central V1. Indeed a TMS coil positioned 2 cm above the inion is likely to stimulate V1 over both hemispheres. It should also be noted that neurons in V5 (and in neighboring motion-sensitive areas like the medial superior temporal area) possess large receptive fields covering the contralateral visual field and spreading up to 10 degrees across the ipsilateral visual field [S1-S3]. Therefore, it is likely that our ccPAS protocol may have recruited a bilateral cortical network with aftereffects spread across both hemifields. To test for any possible hemifield specific effect we presented lateralized rather than central motion stimuli (see Fig. 1). We did not observe any significant difference in performance as a function of hemifield (no main effect of Hemifield, nor interaction with this condition in the experimental as well as in the control groups; all $p > 0.1$; See also Table S1). Rather, the $Exp_{V5\text{-}V1}$ group showed a similarly enhanced performance in global motion perception for both left (LHF) and right (RHF) visual hemifields, with only a slight trend by visual inspection for a better performance over the right hemifield. The idea that $Exp_{V5\text{-}V1}$ ccPAS may have activated a bilateral V5-V1 pathway is well in keeping with the known transmission time of the circuit. Indeed, it is likely that during ccPAS activation of left V5 spreads interhemispherically through the homologue right V5 and reaches the right V1 within a fast transmission time (as early as 4 ms for interhemispheric transfer [S4,S5] and as early as 5-10 ms for V5-V1 [S6,S7]). This is coherent with the possibility of inducing associative plasticity between right V5 and V1 (that was centrally stimulated by the second TMS pulse in the $Exp_{V5\text{-}V1}$ ccPAS protocol). Additionally, instead of the interhemispheric spreading of stimulation during ccPAS induction, spreading of excitation during the expression phase of plasticity could have occurred between the two hemispheres.

Table S1. Motion Sensitivity Threshold (%)						
		BSL	**T0**	**T30**	**T60**	**T90**
$Exp_{V5\text{-}V1}$	L Hf	13.05	12.84	10.61	10.51	11.26
	R Hf	13.58	12.21	9.36	9.34	11.44
$Ctrl_{V1\text{-}V5}$	L Hf	10.88	10.49	9.50	11.62	12.17
	R Hf	9.08	9.36	10.41	11.05	10.32
$Ctrl_{0ms}$	L Hf	10.37	13.40	10.20	12.33	10.93
	R Hf	10.23	10.60	9.60	9.44	13.33
$Ctrl_{Sham}$	L Hf	10.38	10.92	10.67	11.76	12.84
	R Hf	13.84	12.63	14.16	13.13	12.82

Table S1. Related to Figure 2. ccPAS-induced changes in visual motion sensitivity for stimuli occurring in the left and right hemifields for the $Exp_{V5\text{-}V1}$ and each control group.

Supplemental Experimental Procedures

Participants

Thirty-two healthy volunteers (11 male, 21 female; mean age ± SD: 22.31 ± 4.22 years) were recruited for the study. They were right-handed by self-report and naive as to the purpose of the study. All participants gave written informed consent before taking part in the study, which had been approved by the University of Essex Research Ethics Committee.

Motion direction discrimination task

Stimuli were generated and presented using MATLAB and the Psychophysics Toolbox extensions [S8-S10]. They were presented on an 18-inch CRT monitor (ViewSonic G90fB, ViewSonic Corporation, Walnut, CA) with a resolution of 1280 x 1024 pixels and a refresh rate of 85 Hz. A chin rest was used to keep the viewing distance at 57 cm. Every stimulus consisted of 400 white dots (6 pixels each) moving within a square region subtending 12.8 x 12.8 degrees of visual angle, which could be on the left or on the right side of a white fixation cross (20 x 20 pixels) located in the centre of the screen on a grey background. The inner border of the square region was 2.2° to the side of the fixation spot. Half of the trials were randomly presented in the left and half in the right visual hemifield.

In each trial, dots moved with a different level of motion coherence (0, 4, 8, 12, 16, 20, 25, 35, 50 or 80%) leftward or rightward. Motion coherence was expressed as the percentage of dots that were moving in the signal direction. For example, in the 0% coherence trials all the dots moved randomly, in the 80% coherence trials, 320 dots (80%) moved coherently towards leftwards or rightwards, while the remaining 80 dots (20%) were each given a randomly selected direction of motion. Each dot moved at a speed of 4.5°/sec.

The task was a two-alternative forced choice. After each trial participants were asked to make un-speeded responses by pressing the left arrow or the right arrow key to indicate the perceived global direction of motion. Each trial began with a fixation cross appearing in the middle of the screen for 500 ms, followed by the stimulus, the duration of which was 400 ms (see Fig. 1B). A task block consisted of 160 trials: 4 trials x 2 directions (left/right-ward coherent direction of motion) x 2 hemifields (left/right hemifield presentation) x 10 coherence levels. Each session consisted of 4 blocks, for a total of 640 trials and it lasted approximately 13 minutes.

Experimental design

Participants were randomly assigned to four different groups according to the cortico-cortical Paired Associative Stimulation (ccPAS) protocol they would undergo. After having familiarized themselves with the task and achieving a stable performance on the motion task in a training session, participants performed their baseline session (BSL) before undergoing their assigned ccPAS protocol. Participants performed the motion direction discrimination task again, immediately (T0), 30 (T30), 60 (T60) and 90 (T90) minutes after the ccPAS.

ccPAS protocol

ccPAS was delivered by means of a Magstim BiStim^2 machine (Magstim Company, UK) via two 50 mm figure-of-eight coils. 90 pairs of stimuli were continuously delivered at a rate of 0.1 Hz for ~15 min [S11-S13], each pair of stimuli consisted of two monophasic transcranial magnetic pulses. The pulses were triggered remotely using a computer that controlled both stimulators. Left V5 and central V1 were stimulated using established procedures [S6,S7,S14-S18]. To target left V5, the coil was centered 3 cm dorsal and 5 cm lateral to the inion, corresponding to the average functionally localized scalp position where perception of moving phosphenes and disruption of motion perception can be elicited by TMS. The coil was held tangentially to the scalp with the handle pointing upwards and laterally at 45° angle to the sagittal plane. To target V1, the coil was centered 2 cm dorsal to the inion, corresponding to the scalp position where phosphenes in the center of the visual field are typically elicited. From this position it is expected that V1 of both hemispheres is recruited during stimulation. The handle was held tangentially to the scalp and pointed downwards at an angle of 120° clockwise. For both areas intensity of TMS was set at 70% of the maximum stimulator output.

The ccPAS protocol was manipulated in four different groups of participants:

Experimental group ($EXP_{V5\text{-}V1}$). The first pulse was given to V5 followed by another pulse, delivered to V1 with an ISI of 20 ms. This ISI was selected in accordance with the average timing of V5-V1 interactions reported by Pascual-Leone & Walsh [S6] and Silvanto and colleagues [S7] and corresponds to the optimal timing at which V5 exerts a physiological effect on V1. Thus, this ISI was critical to repeatedly activate presynaptic and postsynaptic neurons in reentrant V5-V1 connections in a way that is consistent with spike timing-dependent plasticity (STDP), i.e. a form

of synaptic plasticity meeting the Hebbian principle and predicting that synapses are potentiated if the presynaptic neuron fires repeatedly before the postsynaptic neuron [S19-S20]. Thus, ccPAS in the $EXP_{V5\text{-}V1}$ group was aimed at strengthening re-entrant connections from V5 to V1.

Control group 1 ($Ctrl_{V1\text{-}V5}$, control for direction). In this control group we switched the direction of the associative pulses: the first pulse was given to V1 and the second pulse to V5 at the same ISI as the experimental condition (20 ms). The $Ctrl_{V1\text{-}V5}$ group controlled for direction dependent effects, i.e. we verify that any effect as found in the $Exp_{V5\text{-}V1}$ group is the result of enforced feedback connections (V5 to V1) and should not be found when feedforward connections (V1 to V5) are instead stimulated.

Control group 2 ($Ctrl_{0ms}$, control for timing). In this group both pulses were delivered simultaneously (ISI = 0 ms). According to the Hebbian principle [S19-S22], a synapse will increase its efficiency if it persistently takes part in firing the postsynaptic target neuron. However, if two neurons fire at the same time, then one cannot have caused, or taken part in causing the other to fire. Thus, although neural interactions may occur during simultaneous TMS pairing [S23], no net STDP is expected. This ccPAS condition therefore controlled for timing dependent effects, i.e. we verify that any effect as found in the $Exp_{V5\text{-}V1}$ group is timing dependent and not provoked merely by a consistent stimulation pairing of the targeted areas.

***Control group 3 ($Ctrl_{sham}$*, control for *unspecific effects)**:* stimulation in this group was identical to that of the $EXP_{V5\text{-}V1}$ group except for the fact that the TMS coils were tilted at 90 degrees so that no TMS pulses were effectively applied throughout the ccPAS session.

Statistical analysis

By presenting several different levels of coherent motion, we could observe a sigmoid distribution of correctly perceived coherent motion as a function of the degree of coherence. We fitted the data with a logistic function y=a/(1+exp(-(x-b)/c)) and defined the motion sensitivity threshold as the coherence level at which the direction was correctly perceived 75% of the times. We used motion sensitivity threshold as our dependent variable to assess the impact of ccPAS in the 4 groups.

To assess the effect of ccPAS on motion sensitivity threshold we performed an overall mixed ANOVA with STIMULATION ($Exp_{V5\text{-}V1}$, $Ctrl_{V1\text{-}V5}$, $Ctrl_{0ms}$, $Ctrl_{Sham}$) as a between subject factor, and HEMIFIELD (LEFT, RIGHT) and TIME (BSL, T0, T30, T60, T90) as within subject factors. In order to readily compare performance across the 4 groups ($Exp_{V5\text{-}V1}$, $Ctrl_{V1\text{-}V5}$, $Ctrl_{0ms}$, $Ctrl_{Sham}$) as a function of time (T0, T30, T60 and T90), variations in motion sensitivity threshold were baseline corrected such that the values obtained in the performance at each time after the stimulation were subtracted from the value obtained in the performance at baseline. In this way, any negative value reflects enhancement in performance, while positive values reflect reduction in performance, compared to baseline values. To validate our comparison approach we evaluated whether baseline differed across groups. A mixed ANOVA with STIMULATION ($Exp_{V5\text{-}V1}$, $Ctrl_{V1\text{-}V5}$, $Ctrl_{0ms}$, $Ctrl_{Sham}$) as a between subject factor and HEMIFIELD (LEFT, RIGHT) as within subject factor did not reveal any significant difference among the baselines of the 4 groups ($F_{3,28}$=1.05, p=0.39). T-tests (one-tailed, as directionality of the effects was predictable based on our theoretical assumptions) were Bonferroni corrected for multiple comparisons as a function of time (4 comparisons) and group (3 comparisons).

In the main parametric analyses we found that the $Exp_{V5\text{-}V1}$ group was the only to show the expected decrease in motion sensitivity threshold at T30 and T60. Although motion sensitivity threshold was normally distributed, we additionally performed Bonferroni-corrected non-parametric analyses in view of the relatively low sample size. These analyses substantially replicated the effects detected with parametric analyses as reported in the following. When comparing post-ccPAS performance relative to baseline values, we found that only the $Exp_{V5\text{-}V1}$ group showed a significant change over time (Friedman ANOVA: $\chi2(4) = 19.5$, $p = 0.003$), with significant lower motion sensitivity threshold detected at T30 and T60 (Wilcoxon tests: all $p < 0.023$), but not at T0 or T90 (all $p > 0.25$). No change over time was found in the other groups (all Friedman ANOVAs with $p > 0.11$). Baseline-corrected motion sensitivity threshold values in the 4 groups differed at T30 and T60 (Kruskal-Wallis ANOVA: all $\chi2(3) > 11.51$, all $p < 0.023$) but not at T0 or T90 (all Kruskal-Wallis ANOVAs with $p > 0.24$). In particular, these threshold values were lower for the $Exp_{V5\text{-}V1}$ group relative to the $Ctrl_{V1\text{-}V5}$ (Mann-Whitney Test: all $p < 0.0035$) and $Ctrl_{Sham}$ (all $p < 0.0095$) at both time points. Moreover, relative to the $Ctrl_{0ms}$ group, the $Exp_{V5\text{-}V1}$ group presented significantly lower threshold values at T30 ($p = 0.018$) and marginally significantly lower values at T60 ($p = 0.069$).
The statistical results reported in the main ANOVA were also substantially replicated using other fittings (i.e., Hill equation).

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