Empowering reentrant projections from V5 to V1 boosts sensitivity to motion.

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Summary

Evidence from macaques [1] and humans [2,3] has shown that back-projections from extrastriate areas to the primary visual area (V1) determine whether visual awareness will arise. For example, reentrant projections from the visual motion area (V5) to V1 are considered to be critical for awareness of motion [2,3]. If these projections are also instrumental to functional processing of moving stimuli [4-8], then increasing synaptic efficacy in V5-V1 connections should induce functionally relevant short-term plastic changes, resulting in enhanced perception of visual motion. Using transcranial magnetic stimulation (TMS), we applied a novel cortico-cortical paired associative stimulation (ccPAS) protocol to transiently enhance visual motion sensitivity and demonstrate both the functional relevance of V5-V1 reentrant projections to motion perception, and their plasticity. Specifically, we found that ccPAS aimed at strengthening reentrant connectivity from V5 to V1 (but not in the opposite direction) enhanced the human ability to perceive coherent visual motion. This perceptual enhancement followed the temporal profile of Hebbian plasticity [9-18] and was observed only when an optimal timing of 20 ms between TMS pulses [2,3,5,6] was used, not when TMS pulses were delivered synchronously. Thus, plastic change is critically dependent on both the direction and timing of connectivity; if either of these requirements was not met, perceptual enhancement did not take place. We therefore provide novel causal evidence that V5-V1 back-projections, instrumental to motion perception, are functionally malleable. These findings have implications for theoretical models of visual awareness and for the rehabilitation of visual deficits.

Results

Using a novel ccPAS protocol by means of TMS based upon Hebbian principles [9,10], we tested whether temporarily increasing reentrant connectivity from V5 to V1, considered to be crucial for visual motion [1-8], enhances perceptual sensitivity to motion.

We repeatedly activated the neural pathway between V5 and V1 in 32 healthy volunteers assigned to 4 ccPAS conditions, in which 90 paired TMS pulses over V5 and V1 were administered at 0.1Hz frequency [13-19] (see Supplemental Experimental Procedures). The directionality and timing of the stimulation between V5 and V1 were manipulated across groups, resulting in 1 experimental and 3 control groups.

The experimental group received V5-to-V1 ccPAS ($Exp_{V5\text{-}V1}$). During ccPAS, the first TMS pulse was administered to V5 followed by another pulse to V1. The inter-stimulus interval (ISI) was set at 20 ms, corresponding to the average time for V5 stimulation to exert an effect over V1 processing [2,3], i.e., the optimal timing for the activation of V5-V1 back-projections underlying visual motion perception [2-6]. The specific ISI used was critical to create sequential pre- and post-synaptic activity in the V5-V1 pathway. This is essential for the occurrence of spike timing-dependent plasticity (STDP) [10-12], a form of synaptic plasticity that meets the Hebbian principle that synapses are potentiated if the pre-synaptic neuron fires repeatedly before the post-synaptic neuron [9,10]. Thus, ccPAS in the $EXP_{V5\text{-}V1}$ group was aimed at strengthening re-entrant connections from V5 to V1. Control group 1 received V1-to-V5 ccPAS with 20ms ISI, thus controlling for the directionality of the connectivity ($Ctrl_{V1\text{-}V5}$). Control group 2 received simultaneous V5-V1 ccPAS with 0ms ISI, thus controlling for timing

($Ctrl_{0ms}$). Finally, control group 3 received V5-to-V1 ccPAS with 20ms ISI in sham mode, controlling for nonspecific TMS effects ($Ctrl_{sham}$).

To test the effect of ccPAS on visual perception, participants performed a motion coherence discrimination task before (i.e. at baseline, BSL) and immediately after the ccPAS phase (T0) and after 30, 60 and 90 minutes (T30, T60, T90; see Fig. 1A). The motion coherence discrimination task consisted of a two-alternative forced-choice where participants had to report the direction of coherent motion (leftward or rightward) for 10 different magnitudes of motion coherence ranging from 0 (random motion) to 80% coherence (Fig. 1B,C). For each experimental condition and time we determined the motion sensitivity threshold, calculated as the minimum percentage of motion coherence necessary to discriminate the coherent direction of the moving dots with an accuracy of 75% (see Supplemental Experimental Procedures).

The experiment used a 5 x 2 x 4 design with Time (BSL, T0, T30, T60, T90) and HemiField (Left, Right) as within group conditions and Experimental manipulation ($Exp_{V5\text{-}V1}$, $Ctrl_{V1\text{-}V5}$, $Ctrl_{0ms}$, $Ctrl_{sham}$) as a between groups condition.

A 5 x 2 x 4 mixed-factors analysis of variance (ANOVA) showed a main effect of time ($F_{4,112}$ = 2.51, p = 0.046) suggesting that motion sensitivity threshold changed as a function of testing time. Crucially, there was an interaction between Time and Experimental manipulation ($F_{12,112}$ = 2.51, p = 0.006) suggesting that any modification of motion sensitivity threshold depended on the specific ccPAS condition. No other main effects or interactions were significant (all p > 0.1). As clearly reported in Fig. 2, only the experimental group ($Exp_{V5\text{-}V1}$) showed motion

sensitivity enhancements, as evidenced by significant threshold shifts towards lower levels of motion coherence between 30 and 60 minutes following the ccPAS phase, before returning towards baseline values (see also Fig. 3 and FigS1). Bonferroni-corrected t-tests indicate that participants assigned to $Exp_{V5\text{-}V1}$ are more sensitive to visual motion (lower motion sensitivity threshold) at T30 ($p = 0.003$) and T60 ($p = 0.048$) relative to baseline. Moreover, Bonferroni-corrected t-tests comparing $Exp_{V5\text{-}V1}$ versus all the other groups confirmed the greater sensitivity of the $Exp_{V5\text{-}V1}$ group at T30 ($Exp_{V5\text{-}V1}$ vs. $Ctrl_{V1\text{-}V5}$: $p = 0.008$; $Exp_{V5\text{-}V1}$ vs. $Ctrl_{0ms}$: $p = 0.034$; $Exp_{V5\text{-}V1}$ vs. $Ctrl_{sham}$: $p = 0.003$) and T60 ($Exp_{V5\text{-}V1}$ vs. $Ctrl_{V1\text{-}V5}$: $p = 0.006$; $Exp_{V5\text{-}V1}$ vs. $Ctrl_{0ms}$: $p = 0.046$; $Exp_{V5\text{-}V1}$ vs. $Ctrl_{sham}$: $p = 0.025$). Perceptual enhancement in the $Exp_{V5\text{-}V1}$ group was similar across hemifields as suggested by the non-significance of the triple interaction (see FigS2).

None of the control groups showed a similar increase in performance after ccPAS ($Ctrl_{V1\text{-}V5}$: all $p > 0.19$; $Ctrl_{0ms}$: all $p > 0.12$; $Ctrl_{sham}$: $p > 0.53$), suggesting that perceptual boosting was specifically determined by the ccPAS manipulation when stimulation directionality (from V5 to V1) and timing (20ms) met the physiological constraints of reentrant connectivity [2-3]. This pattern of results was substantially replicated when using non-parametric tests (see Supplemental data).

Discussion

Repetitive paired stimulation, evoking sequential pre- and post-synaptic activity in interconnected neurons, induces Hebbian associative plasticity, prompting those synaptic connections to transiently strengthen [9-12]. Previous TMS studies have shown that similar

synaptic strengthening can be induced in the human motor system over two interconnected motor areas through ccPAS administered at an optimal ISI [12-19]. These studies have shown that the ISI at which one targeted region (e.g. the premotor cortex) exerts a physiological effect on an anatomically connected second region (i.e., the motor cortex) is also the ISI at which ccPAS can induce Hebbian-like cortico-cortical connection changes (e.g., 6-8 ms for premotor-motor circuits [compare 15,16 with 20,21]). Such ccPAS studies have supported the notion of STDP by showing a causal and directional change of influence of the first over the second targeted region [16,19]. However, little is known about the impact on behavior of such an experimental increase in synaptic efficiency and no study to date has tested ccPAS protocols over the visual system.

Seminal studies in animals have provided *in vitro* and *in vivo* evidence of Hebbian plasticity in the visual system [10,22,23]. Our study goes beyond previous animal evidence by providing the first demonstration that directly fostering Hebbian plasticity in a cortical visual circuit has an impact on behavior. We demonstrated for the first time that ccPAS over two interconnected visual regions with an ISI consistent with evoking pre- and post-synaptic activity necessary for STDP [2-6] affects visual perception. In particular, we showed that stimulation aimed at increasing synaptic efficacy in back projections from V5 to V1 transiently boosted visual motion sensitivity. Such perceptual enhancement was evident for at least 60 minutes and its time course resembled that of Hebbian-like physiological effects observed in animal studies as well as in studies using ccPAS over the human motor system [10-19].

Our findings provide causal evidence that short-term synaptic strengthening of reentrant V5-V1 connections can enhance motion perception. This supports the view that reentrant

connectivity from higher-order to early visual areas subserves integrative visual functions [1-8,24]. Animal studies have shown that suppression of V5 in the visual system weakens V1 responses to moving bar stimuli, in particular when stimuli have low salience [25], which suggests a top-down amplification mechanism in the processing of visual motion. This mechanism is also thought to promote visual awareness of motion [1,26,27] and TMS studies in humans have provided causal evidence of the role of V5-V1 backward connectivity on motion visual awareness as probed by TMS-induced visual phosphenes [2,3]. However, evidence indicates that backward connectivity is important also for efficient processing of actual moving stimuli [4-7], even when motion stimuli are not consciously perceived [5]. This suggests that the top-down gain control function of backward connections [6,25] is not limited to subserving awareness [2,3] and reflects a general principle of visual cortical information processing [6,8,24]. Remarkably, our study is the first to directly show that synchronous stimulation of V5 and V1 aimed at strengthening backward connections improves the perceptual processing of coherent motion. Notably, we specifically tested for a novel account of the functionality of reentrant projections, namely the plasticity of the V5-V1 circuit, by manipulating its pre- and post-synaptic nodes according to the Hebbian rule as implemented through this novel ccPAS protocol. The most immediate consequence of this novel intervention approach is that participants in the experimental group ($Exp_{V5\text{-}V1}$) experienced an enhanced perception of motion coherence. In contrast, none of the participants in the control groups (including $Ctrl_{V1\text{-}V5}$ controlling for directionality of the stimulation) improved their perception at any testing time following the TMS application, when compared to their pre-TMS BSL measure.

One may wonder why no change in performance was detected following ccPAS in the $Ctrl_{V1\text{-}V5}$ group. In principle, reversing the order of the stimulation (i.e., first TMS pulse over V1, second over V5) would strengthen feedforward rather than backward connectivity in the network. Our findings suggest that backward more than feedforward connections are amenable to plastic boosting of visual perception, which is in keeping with their top-down modulatory role [1-8,24,25]. However, it should be noted that the ISI of the ccPAS was selected based on the timing of causal interactions that V5 exerts over V1 [2,3] and thus, other ISIs may be effective for modulating perceptual function via changes in feedforward connectivity. Visual tasks strongly relying on bottom-up processes may be particularly sensitive to manipulations of feedforward connectivity [28].

It might be worth noting that during $Exp_{V5\text{-}V1}$ ccPAS, the stimulation of V5 may not only induce orthodromic activation of backward V5-to-V1 connections, but also antidromic activation of feedforward V1-to-V5 connections. Thus, one may consider the possibility that during $Exp_{V5\text{-}V1}$ ccPAS, stimulation of V1 could re-activate the same feedforward connections and this repeated pairing may also contribute to the observed plastic effect. Indeed, studies have shown that repeated TMS pairing over the same region can induce STDP [29]. However, such induction is selective for very short ISIs (~1.5 ms) [30] making unlikely it played a major role in the plastic effects we detected. While our study supports the hypothesis of Hebbian strengthening of V5-V1 backward connections, future studies are needed to elucidate the possible contribution of additional mechanisms underlying ccPAS aftereffects.

In sum, our study suggests that ccPAS can enhance visual perception of motion in participants

where the V5-V1 circuit is critically manipulated by repeatedly pairing pre- and post-synaptic nodes in the direction and timing that are optimal for strengthening these reentrant connections. This provides a novel mechanistic insight into the circuit and computational basis of visual perception, by providing causal evidence of its malleability, and demonstrating that this strictly depends on the timing and directionality of the repeated ccPAS manipulation.

This new demonstration of the malleability of the network governing visual processing paves the ground for future exploration of brain mechanisms responsible for integrative visual functions. While our off-line ccPAS procedure addressed the basic features of associative plasticity in the cortical network for motion perception, future investigations might use a state-dependent approach [31-33] and pair ccPAS with specific motion directions in order to boost direction-specific perceptual tuning. Our study may also have implications for understanding more general mechanisms of perceptual learning [34], and fine-tuning interventional approaches aimed at enhancing perception, for example by combining training and neuromodulation strategies. However, physiological evidence indicates that ccPAS aimed at strengthening a given pathway may also induce weakening of non-stimulated pathways [19]. Thus, future studies are needed to understand the impact of such neural changes on behavior, as in principle the ccPAS protocol may be useful but also detrimental depending on the stimulated pathway and the task at hand.

We have probed the effects of associative plasticity on the motion perception reentrant network. There has been no attempt in the previous literature to explore this aspect of motion perception. Currently, it is not obvious whether and how our ability to make sense of

motion signals depends on the capacity of the circuit to adapt to the environment. Here we specifically shed light on the mechanisms by which reentrant connections become functionally adaptive. This has important implications for the way we perceive, conceptualize, interpret and learn motion patterns, from simple to more complex spatio-temporal structures. Our study may have implications for the recovery of abilities that have been lost as a result of disorders such as stroke, as it suggests possible therapeutic interventions aimed at enhancing motion perception, and sensory processing in general.

Conclusions

We have enhanced motion coherence perception for an extended period through the application of the ccPAS protocol. This enhancement was critically dependent on mimicking the temporal features of Hebbian plasticity, by exactly pairing the nodes of the network subserving motion perception in the right direction and at the right time. The effects we observed are the result of a plastic modification of the circuit and not a mere interference with the circuit. As such, they provide novel mechanistic insights in the way the circuit functions. These findings have implications for theoretical models of visual perception as well as for the rehabilitation of visual deficits through non-invasive brain stimulation. Moreover, this novel protocol provides a novel perspective on current models of perceptual learning and its potential underlying neurophysiology.

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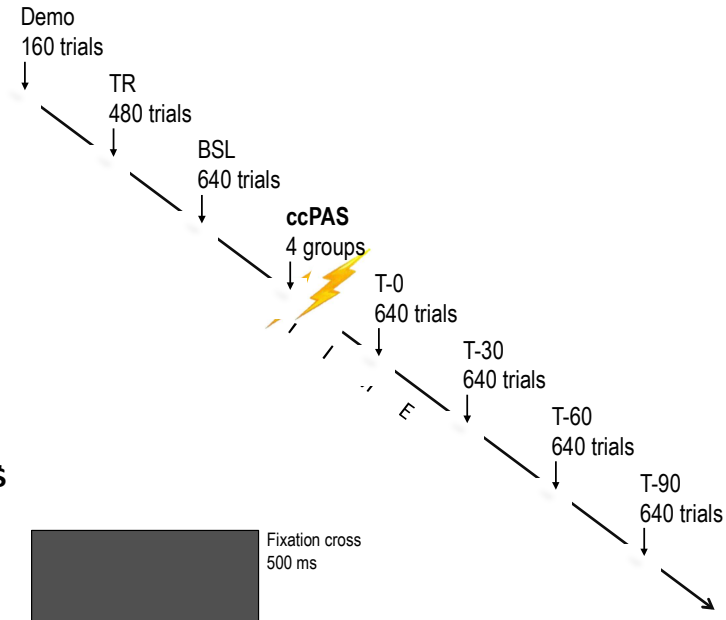
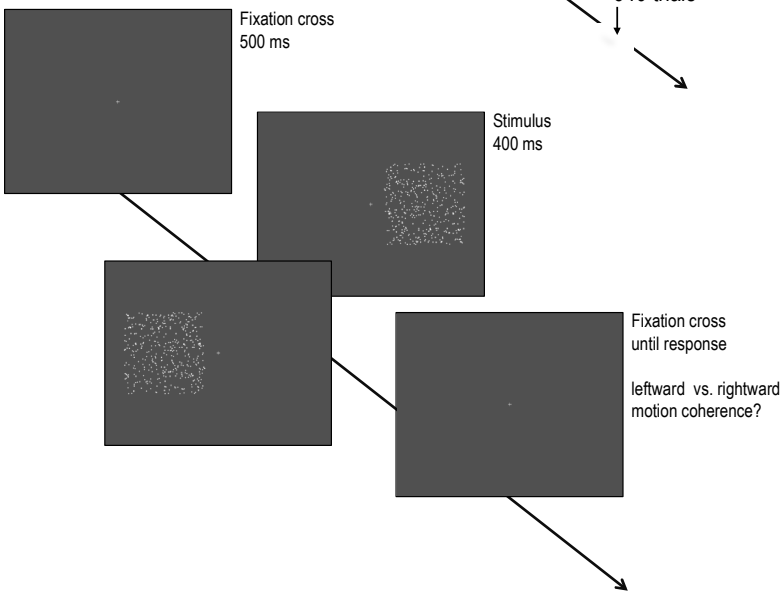
Figures

A.


Demo
160 trials
TR
480 trials
BSL
640 trials
ccPAS
4 groups
T-0
640 trials
T-30
640 trials
T-60
640 trials
T-90
640 trials

B.$


Fixation cross
500 ms
Stimulus
400 ms
Fixation cross
until response
leftward vs. rightward
motion coherence?

C.$

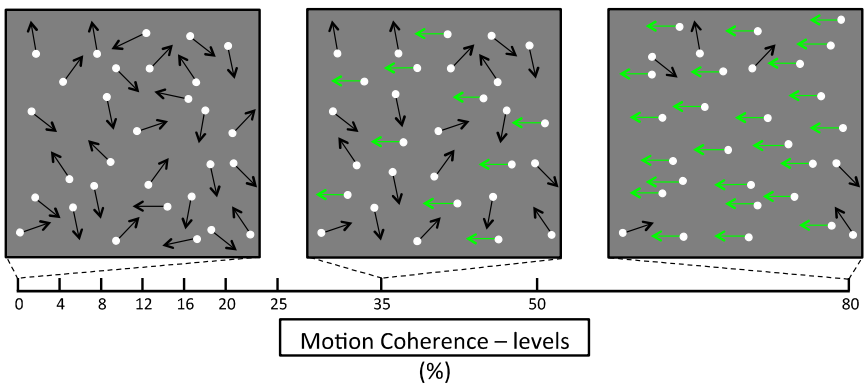

0
4
8
12
16
20
25
35
50
80
Motion Coherence – levels
(%)

Figure 1. A. Timeline of the experiment. For each participant the experiment began with a preparation phase composed by a demo block (Demo) aimed to familiarize with the basic mechanisms of the motion coherence task and a training session (TR) of 3 blocks, performed to allow the participant to reach a stable performance level before the actual experiment. This preparation phase was followed by a baseline session (BSL). After the BSL measurement, participants were randomly assigned to one of four groups, therefore undergoing either the experimental or one of the three control ccPAS protocols. Participants had to perform the same task immediately (T0), 30 (T30), 60 (T60) and 90 (T90) minutes following ccPAS protocol. One session consisted of 4 blocks of 160 trials each. **B. Task sequence.** Each trial consisted of a white central fixation cross displayed alone for 500 ms followed by a
dot motion coherence stimulus displayed for 400 ms. Here, a single frame of the motion coherence stimulus used in the study is depicted. The motion coherence of the stimulus varied across trials and it could appear either on the left or on the right side of the cross. To indicate that a response was required, the dot motion stimulus disappeared and the cross remained. A new trial started as soon as the participant pressed the response key on a keyboard indicating whether the coherent motion was perceived moving leftwards (left arrow) or rightwards (right arrow), regardless of the side of presentation. **C. Stimuli.** Schematic representation of the stimuli used to test the coherence threshold. The coherent motion display contains a set of 400 moving dots, a fixed proportion of which are moving in a coherent direction (except for 0% motion coherence condition), while the remainder moved in randomly chosen directions. Coherence of the motion ranged from 0% to 80%, distributed in ten levels (represented on the line below). When the proportion (“coherence level”) is high,

task difficulty is low. The coherence threshold is the minimal percentage of dots moving in the same direction needed for the participant to accurately perceive (75% of accuracy) the predominant motion direction. The left panel represents a schematic trial with 0% coherence as all the dots are moving randomly. The central panel represents a trial with 35% coherence in the leftward direction. The right panel represents a trial with 80% coherence in the leftward direction. The arrows illustrate the motion direction of each dot. Green arrows represent the directions of signal dots, black arrows represent the directions of noise dots.

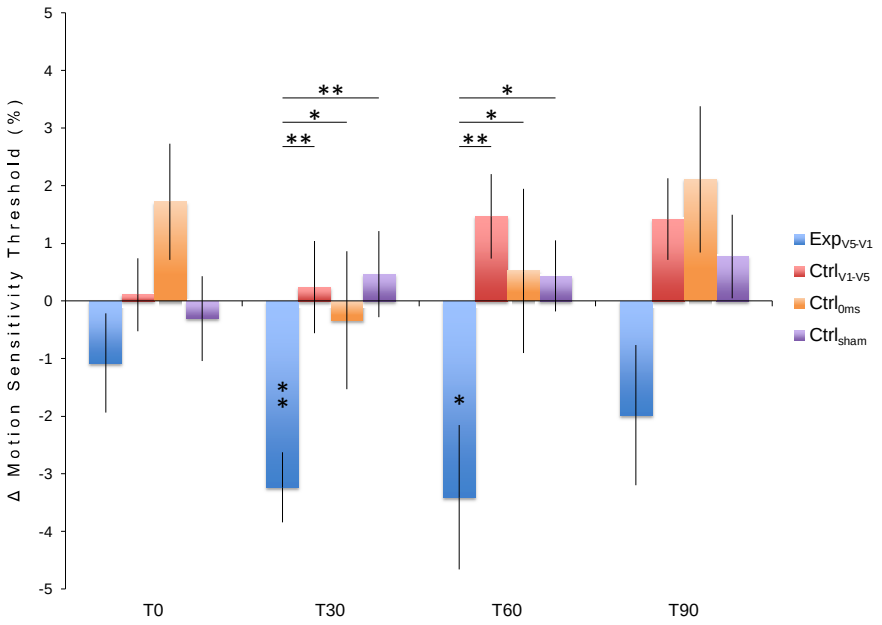


Figure 2 Changes in visual motion sensitivity induced by cortico-cortical paired associative stimulation (ccPAS). Only participants assigned to the experimental group ($Exp_{V5\text{-}V1}$; ccPAS: direction V5-V1, ISI 20ms) showed a reduction of motion sensitivity threshold (baseline corrected) at 30 and 60 min after ccPAS, indicating enhanced visual motion sensitivity. Participants in control group 1 ($Ctrl_{V1\text{-}V5}$; ccPAS: direction V1-to-V5, ISI 20ms); control group 2 ($Ctrl_{0ms}$; ccPAS: simultaneous V5-V1 stimulation, ISI 0ms) and control group 3 ($Ctrl_{sham}$; V5-to-V1 sham stimulation, ISI 20ms) showed no significant changes in motion sensitivity threshold over time. Error bars denote ±1 s.e.m. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$). See also Figure S2 and Table S1.

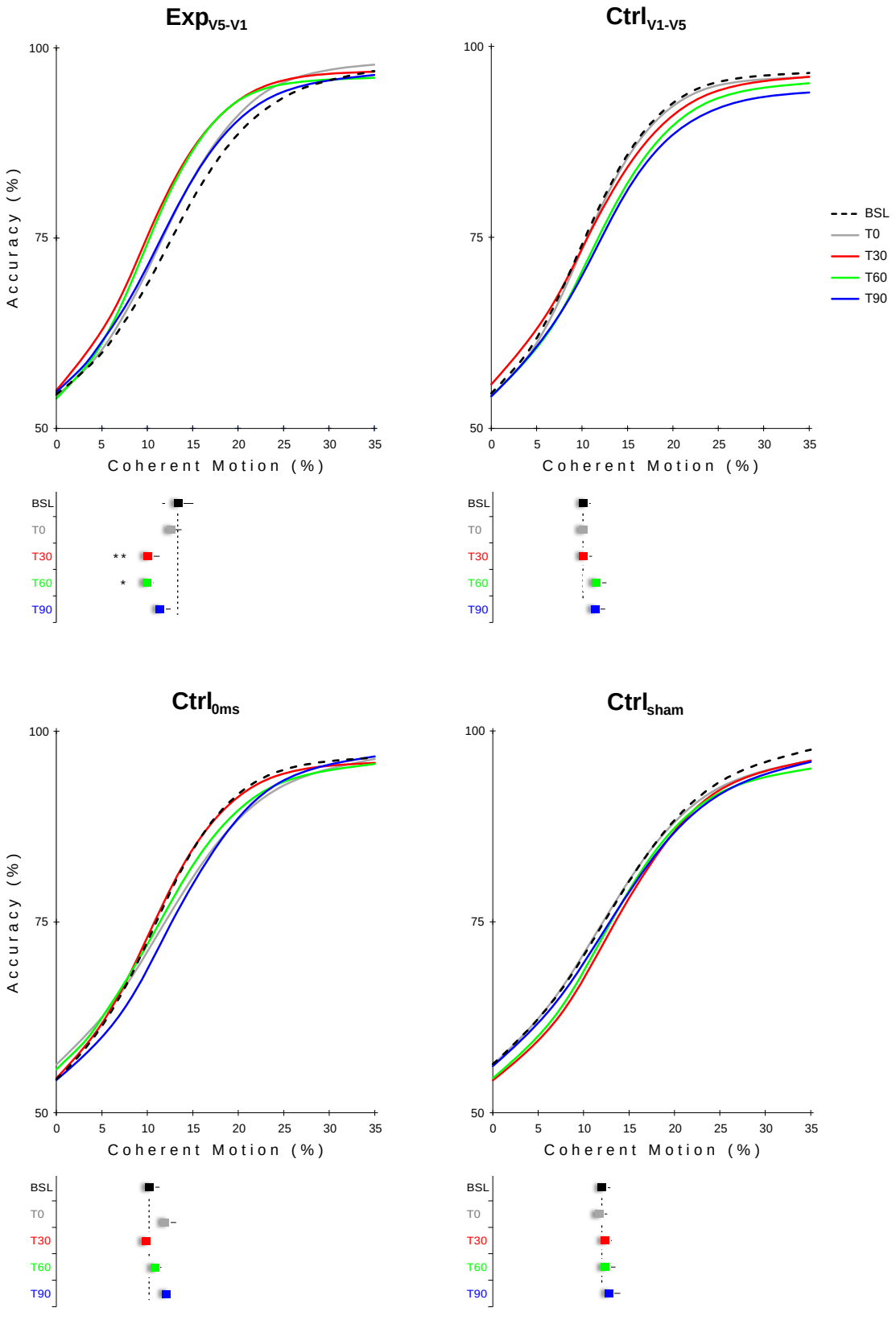

Exp V5-V1
Ctrl V1-V5
Ctrl 0ms
Ctrl sham
Accuracy (%)
Coherent Motion (%)
100
75
50
0
5
10
15
20
25
30
35
BSL
T0
T30
T60
T90
**
*

Figure 3. Curve fitting and groups' performance. Sigmoid curve fits (upper subpanels) and participants' average performance (lower subpanel) are plotted for each group as a function of time before and after the ccPAS protocol has been applied. The black dotted line represents the baseline session (BSL), grey, red, green, and blue lines represent task performance at 0 (T0), 30 (T30), 60 (T60) and 90 (T90) minutes after the end of the ccPAS protocol respectively. The motion sensitivity threshold was determined by taking the percentage of coherent motion where the logistic function had a value of 75% of correct responses. The motion sensitivity threshold represents the percentage of coherent motion necessary to discriminate the coherent direction of the moving dots with an accuracy of 75%. Below each graph, the averaged motion sensitivity threshold (and standard error) across participants, in each of the four groups, are plotted for each session. Only in the $EXP_{V5\text{-}V1}$ group is there a significant TMS-induced decrease in the motion sensitivity threshold, at T30 and T60 relative to BSL, as indicated by the asterisks (* $p < 0.05$, ** $p < 0.01$). This reduction shows an enhancement in sensitivity to the global motion task. See also FigS1 for a representation of averaged data points for each group and each time.

Supplemental data

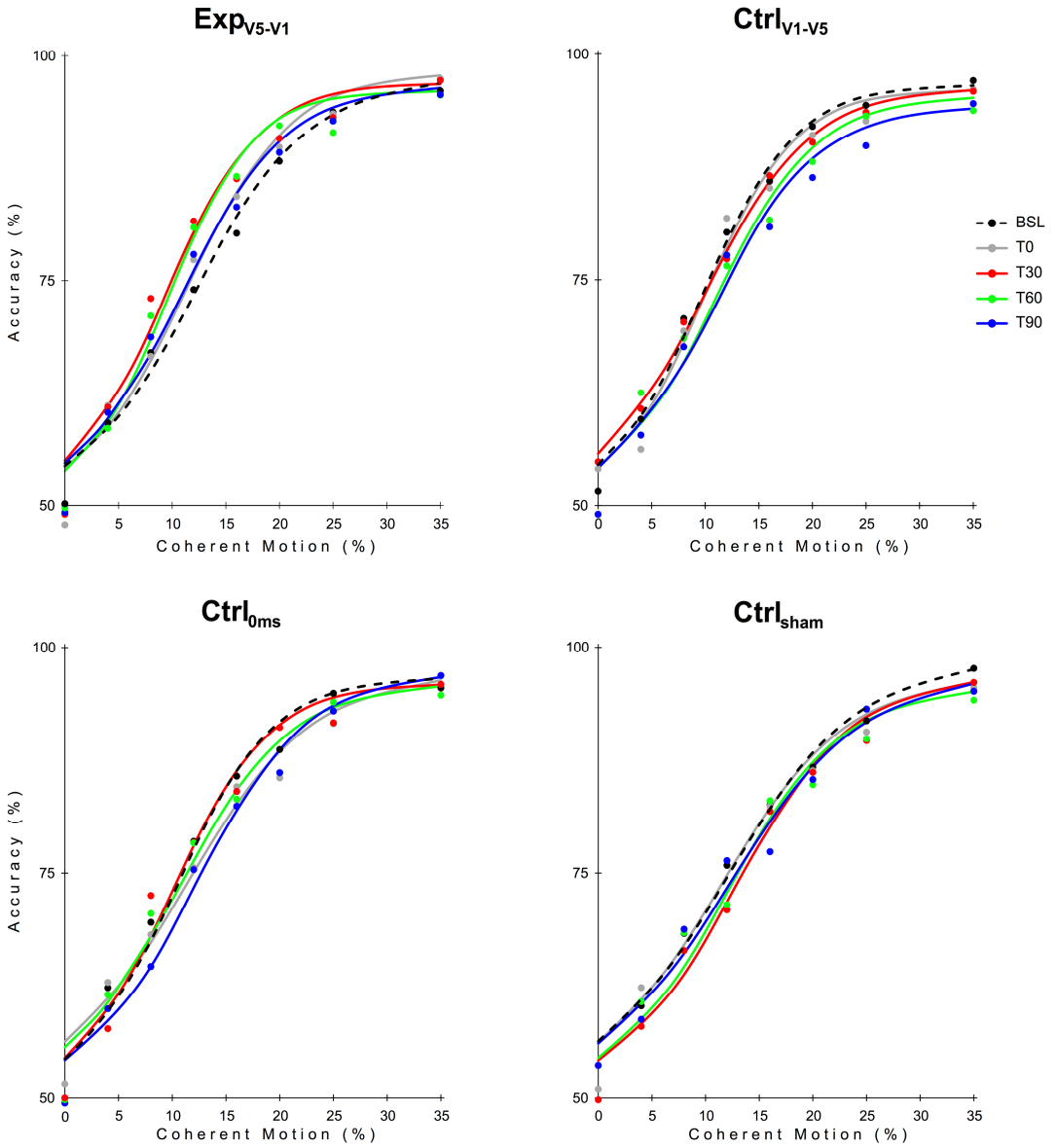


Figure S1. Related to Figure 3; Curve fitting and groups' performance. Sigmoid curve fits and averaged data points for each group and each time. See Figure 3 for detailed information.

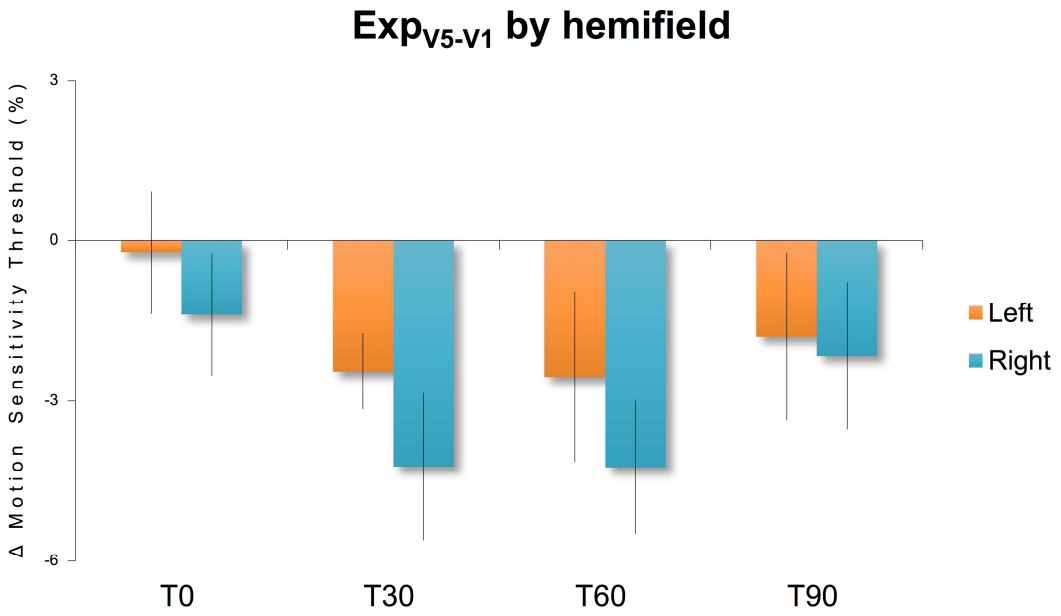


Figure S2. Related to Figure 2; ccPAS-induced changes in visual motion sensitivity for stimuli occurring in the left and right hemifields of the $Exp_{V5\text{-}V1}$ group. Error bars denote ±1 s.e.m. Similar changes in motion sensitivity threshold were found in the two hemifields. This is not surprising 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-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
$Ctrl_{V1-V5}$	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-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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