

Supplementary Materials for:

Driving Hebbian plasticity over ventral premotor-motor projections transiently enhances motor resonance

By Emilio Chiappini, Sonia Turrini, Marco Zanon, Mattia Marangon, Sara Borgomaneri, & Alessio Avenanti

Participants: Sample size computation and demographics

A power analysis computed using GPower 3.1, setting $\alpha=0.05$ and power=0.95 and assuming a medium effect size f of 0.27 [S1] for repeated measures within-between design with 3 groups and 3 measurements suggested a total sample size of 45 participants. To account for potential technical failures, we recruited 48 right-handed healthy volunteers (26 females; mean 24 ± 3 years) with normal or corrected-to-normal visual acuity. None reported contraindication to TMS [S2,S3].

Participants were randomly assigned to one of three experimental groups. Groups were compared to ensure they were matched by age, gender, and corticospinal excitability by performing χ^2 and one-way ANOVAs (Table S1).

	Group			Statistical comparison
	ccPAS _{PMv→M1}	ccPAS _{M1→PMv}	ccPAS _{Sham}	
Gender (M / F numerosity)	9 / 7	8 / 8	5 / 10	$\chi^2=1.732, p=0.42$
Age (years)	24.9 \pm 3.2	24.3 \pm 4.4	24.4 \pm 2.6	$F_{2,44}=0.26, p=0.77$
rMT (% maximum stimulator output)	41% \pm 6%	39% \pm 7%	40% \pm 7%	$F_{2,44}=0.48, p=0.62$
SI_{1mV} (% maximum stimulator output)	70% \pm 17%	66% \pm 15%	69% \pm 15%	$F_{2,44}=0.25, p=0.78$

Table S1. Demographic characteristics and motor excitability of participants assigned to the three experimental groups. Data are expressed as Mean \pm S.D. (except for the measure of gender).

Assessment of motor resonance

Each trial lasted a total of 7000 ms and began with a first screen of the duration of 1000 ms depicting a fixation cross, followed by a stimulus screen of 3000 ms displaying either the fixation cross (FIX trials) or a video clip (AO trials), and ended up with a blank screen of 3000 ms.

In AO trials, each clip began with a static hand for 1200 ms, followed by the hand's movement that was interrupted after 1800 ms. One complete abductive/adductive movement lasted about 1 second, the finger moved on the horizontal plane of the hand, and lift-and-displace movements were avoided to generate movements requiring the maximum activity of the abductive muscles of the considered fingers. Each video consisted of 45 frames, which remained on the screen for 67 ms each, providing a fluid perception of movement.

All the frames of the displayed stimuli were presented on a white background. The perpendicular intersection of two black lines of 2 degrees of visual angle composed the fixation cross. Videos were inscribed in a virtual square that subtended a visual arc of 13.8 degrees per side.

Each session (PRE, T0, T20) consisted of 2 blocks of ~3 minutes each, for a total of 20 FIX trials and 32 AO trials – including 16 abductive/adductive movements of the index finger (IND) and 16 abductive/adductive movements of the little finger (LIT). In each block, 10 FIX and 16 AO (8 IND and 8 LIT) trials were presented in two separate sub-blocks whose order was counterbalanced across participants.

In every trial (AO and FIX), a single biphasic TMS pulse was delivered over the left primary motor cortex (M1) to induce motor-evoked potentials (MEPs) simultaneously in the right first dorsal interosseous (FDI) and abductor digiti minimi (ADM). TMS was administered at five randomized intervals between 1400 and 2200 ms after the beginning of the stimulus screen. This timing ensured that TMS was always administered during the movement in the AO condition (from 200 to 1000 ms after the movement onset) and that the TMS intertrial interval was 7000 ± 800 ms.

Coil positioning during ccPAS

The active ccPAS configurations adopted in $ccPAS_{PMv \rightarrow M1}$ and $ccPAS_{M1 \rightarrow PMv}$ reproduced that of previous studies [S4–S6] that successfully manipulated PMv-M1 connectivity. PMv stimulation was performed using a monophasic device and the coil was oriented to induce a current flow in the neural tissue directed toward the M1 site (Figure S1), in keeping with prior work [S4–S6]. However, unlike previous studies that used monophasic devices to stimulate M1, a biphasic TMS device was used in this study. These stimulators generate biphasic pulse waveforms. To address this difference, we oriented the coil so that the first and less effective phase of the pulse waveform induced an anterior-to-posterior current in the brain (shown in Figure S1). In contrast, the second and most effective phase of the pulse waveform induced a posterior-to-anterior current in the brain (not shown in Figure

S1), thus matching the current direction applied in previous studies that used monophasic stimulators [S4–S6]. This adjustment was shown to be effective in inducing ccPAS behavioral modulations in another study conducted by our group [S1]. Also, we found similar physiological effects when directly comparing MEPs collected during the application of ccPAS_{PMv→M1} protocols performed with biphasic or monophasic stimulators [S7].

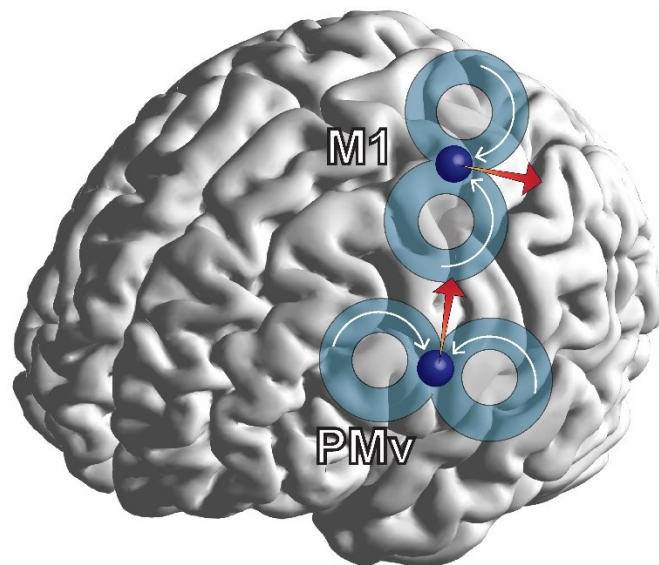


Figure S1 – schematic representation of coil position over M1 and PMv during the ccPAS. White arrows represent the current flow in the coils and red arrows represent the induced current flow in the neural tissue. For the M1 coil, connected to the biphasic device, the first phase of the pulse is represented. However, the second phase (not represented) is the most effective in activating the neural tissue and flows in the opposite direction.

Data analysis

MEP amplitude was automatically extracted as the peak-to-peak amplitude in the time window between 15 and 60 ms after TMS using a custom Matlab script. MEPs were excluded if, within the 100 ms window preceding the TMS pulse, the EMG deviated by more than 2 SD from the mean of the same block, either in terms of (i) mean rectified amplitude (to detect deviant tonic activity) or (ii) peak-to-peak amplitude (to detect deviant phasic activity). Additionally, minimal MEPs with amplitudes < 0.03 mV were excluded from the analysis since they did not clearly emerge from the EMG background activity in a few participants (0% and 0.06% of the FDI and ADM MEPs, respectively). Overall, 7% of MEPs were removed across muscles and conditions.

Supplementary Results

No change in corticospinal excitability following ccPAS

As reported in the main text, there was no change in M1 corticospinal excitability following ccPAS, as evident in MEPs during the FIX condition across the three sessions (Table S2).

	FDI			ADM		
	PRE	T0	T20	PRE	T0	T20
ccPAS _{PMV→M1}	0.99 ±0.27	1.02 ±0.37	0.96 ±0.37	0.83 ±0.63	0.78 ±0.59	0.82 ±0.57
ccPAS _{M1→PMV}	1.06 ±0.40	1.09 ±0.27	1.14 ±0.33	0.77 ±0.49	0.73 ±0.49	0.81 ±0.55
ccPAS _{SHAM}	1.13 ±0.27	1.06 ±0.26	1.19 ±0.32	1.13 ±0.71	1.16 ±0.78	1.19 ±0.71

Table S2. ccPAS x Session x Muscle. Raw MEP values (Mean ±S.D.) expressed in mV and recorded in the FIX trials.

References

- [S1] Fiori F, Chiappini E, Avenanti A. Enhanced action performance following TMS manipulation of associative plasticity in ventral premotor-motor pathway. *NeuroImage* 2018;183:847–58. <https://doi.org/10.1016/j.neuroimage.2018.09.002>.
- [S2] Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmüller J, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clinical Neurophysiology* 2021;132:269–306. <https://doi.org/10.1016/j.clinph.2020.10.003>.
- [S3] Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology* 2015;126:1071–107. <https://doi.org/10.1016/j.clinph.2015.02.001>.
- [S4] Buch ER, Johnen VM, Nelissen N, O’Shea J, Rushworth MFS. Noninvasive Associative Plasticity Induction in a Corticocortical Pathway of the Human Brain. *J Neurosci* 2011;31:17669–79. <https://doi.org/10.1523/JNEUROSCI.1513-11.2011>.
- [S5] Johnen VM, Neubert F-X, Buch ER, Verhagen L, O’Reilly JX, Mars RB, et al. Causal manipulation of functional connectivity in a specific neural pathway during behaviour and at rest. *eLife* 2015;4:e04585. <https://doi.org/10.7554/eLife.04585>.
- [S6] Sel A, Verhagen L, Angerer K, David R, Klein-Flügge MC, Rushworth MFS. Increasing and decreasing interregional brain coupling increases and decreases oscillatory activity in the human brain. *Proc Natl Acad Sci USA* 2021;118:e2100652118. <https://doi.org/10.1073/pnas.2100652118>.
- [S7] Turrini S, Fiori F, Chiappini E, Santarnecchi E, Romei V, Avenanti A. Gradual enhancement of corticomotor excitability during cortico-cortical paired associative stimulation. *Sci Rep* 2022;12:14670. <https://doi.org/10.1038/s41598-022-18774-9>.