Cortico-cortical paired associative stimulation highlights asymmetrical communication between rostral premotor cortices and primary motor cortex

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Supplementary Methods

Participants

We tested 60 young adult volunteers (mean age: 23.2 y, standard deviation: 2.2, range: 19-29; 19 males and 41 females), divided into four groups (N = 15 each) in which we administered different cortico-cortical paired associative stimulation (ccPAS) protocols: $ccPAS_{PMv\text{-}M1}$ (22.20 y ± 2.11; 4 male and 11 female), $ccPAS_{M1\text{-}PMv}$ (22.27 y ± 1.53; 5 male and 10 female), $ccPAS_{SMA\text{-}M1}$ (24.00 y ± 2.24; 5 male and 10 female) and $ccPAS_{M1\text{-}SMA}$ (24.40 y ± 2.13; 5 male and 10 female). All participants were right-handed according to the Edinburgh Handedness Inventory [1], had normal or corrected-to-normal eyesight, were unaware of the goal of the experiment, and had no contraindications to TMS [2]. All experimental procedures were carried out in accordance with the 1964 Helsinki Declaration and its amendments[3] and were approved by the University of Bologna's Department of Psychology "Renzo Canestrari" Ethical Committee and the Bioethics Committee. There were no adverse responses or TMS-related discomfort reported by subjects or observed by experimenters. Behavioral data collected in this study are part of a larger study in which participants performed an imitation task. The results of the imitation tasks will be reported elsewhere.

Choice reaction time (cRT) task

Before (Pre), immediately after (T0) and 30 minutes after the end of the ccPAS protocols participants performed a cRT task. They were seated approximately at 80 cm from the screen, with the right index and middle finger pressing two keys labelled as “1” and “2”, respectively. Each trial began with an irrelevant visual stimulus (a resting hand) presented for 1628 ms. After 500 ms from

the irrelevant stimulus onset, the imperative stimulus (numbers “1” or “2”) appeared for 68 ms on the center of the screen. Participants were instructed to lift the index finger when presented with number “1”, and lift the middle finger when presented with number “2”. They were instructed to lift the appropriate finger as soon as they saw the imperative stimulus, and subsequently replace the finger on the same key. An inter-trial fixation cross was displayed for a random interval (2-3 s). We collected 40-trials for each block.

ccPAS protocol and electrophysiological recording

All ccPAS protocols employed the same stimulation parameters. The ccPAS protocol consisted of 90 pairs of pulses (~15 min) administered over a premotor area (either ventral premotor cortex, PMv, or supplementary motor area, SMA) and the primary motor cortex (M1) at a rate of 0.1 Hz[4–11]. The coil position to target the left M1 was identified functionally, as the hotspot to induce MEPs of maximal amplitude in the relaxed right FDI.

The left M1 coil was positioned tangentially to the scalp and at a 45-degree angle to the midline, resulting in a posterior-anterior current flow, optimal for M1 stimulation[12]. In line with previous dual coil and ccPAS investigations[4-8], the left PMv coil was positioned tangentially to the scalp, causing a current flow in the brain directing toward the M1 coil. The SMA coil was positioned to induce a current pointing toward the M1 site[13].

In each experimental group, we varied the relative order of stimulation of each area that compose the ccPAS protocol. In the $ccPAS_{PMv\text{-}M1}$ group, PMv stimulation always preceded that over M1: on each pair the first pulse was delivered over PMv and the second pulse delivered over M1; in the $ccPAS_{M1\text{-}PMv}$ group, the order was reversed. In the $ccPAS_{SMA\text{-}M1}$ group, SMA stimulation always preceded that over M1; in the $ccPAS_{M1\text{-}SMA}$ group, the order was reversed. In all groups, in each TMS pair the second pulse was delivered 8 ms after the first pulse, matching the interstimulus interval (ISI) found to recruit short-latency connections between both PMv and SMA, and the M1[5,14–16].

The intensity of the pulse on PMv or SMA was set at 90% of the individual’s resting motor threshold (rMT), defined as the minimum stimulator output intensity necessary to induce MEPs ~50 μV in 5 out of 10 consecutive trials[17] in the relaxed first dorsal interosseous (FDI). The intensity of the pulse on M1 was adjusted to evoke MEPs with an amplitude of ~1 mV[4,5,9,10,18]. Pulses delivered during the ccPAS were triggered remotely using a custom MATLAB script (MathWorks, Natick, USA).

Since M1 stimulation during ccPAS was set at a suprathreshold intensity, we were able to record a MEP elicited by each of the 90 paired stimulations, thus allowing us to monitor online changes in corticospinal excitability[6–8]. MEPs were recorded from the right FDI by means of surface Ag/AgCl electrodes placed in a belly-tendon montage. A Biopac MP-35 (Biopac, USA)

electromyograph was used to acquire EMG signals (band-pass filter: 30–500 Hz; sampling rate: 20 kHz).

Neuronavigation

The left PMv and the SMA locations were identified using the SofTaxic Navigator System (Electro Medical System, Bologna, IT). Firstly, skull landmarks (nasion, inion, and two preauricular points) and 80 points providing a uniform representation of the scalp were digitized in all participants using a Polaris Vicra digitizer (Northern Digital). A 3D warping process fitting a high-resolution MRI template to the participant's scalp model and craniometric points generated an individual estimated magnetic resonance image (MRI) for each participant. To locate the left PMv we adopted Talairach coordinates determined by averaging previously reported coordinates[19–23] and used in our prior ccPAS studies[4–8]: x = –52; y = 10; z = 24. To identify SMA we adopted Talairach coordinates based on the metanalysis by Mayka et al.[24]: x = –2; y = -7; z = 55.

The Talairach coordinates corresponding to the projections of the left PMv, SMA and left M1 scalp sites onto the brain surface were automatically estimated by the SofTaxic Navigator from the MRI-constructed stereotaxic template, mean and standard deviation of the four groups are reported in Table S1 and the hot-spot of the subjects are depicted in Figure 1b in the main text.

			M1			**PMv**		
			x	**y**	**z**	**x**	**y**	**z**
Group	***ccPAS PMv-M1***	Mean	-30.29	-21.47	60.34	-54.59	9.54	23.70
		SD	6.65	5.91	3.28	1.48	0.72	1.63
	ccPAS M1-PMv	Mean	-28.49	-18.48	60.92	-54.04	9.90	24.52
		SD	4.84	6.01	4.48	1.19	1.05	1.10
			M1			**SMA**		
			x	**y**	**z**	**x**	**y**	**z**
	ccPAS SMA-M1	Mean	-32.97	-20.57	59.87	-2.97	-7.04	62.68
		SD	5.10	4.46	4.82	1.36	1.04	2.44
	ccPAS M1-SMA	Mean	-31.07	-19.87	59.09	-3.92	-6.30	64.44
		SD	5.34	5.28	4.86	1.46	1.74	2.58

Table S1 – Talairach coordinates of the stimulated sites across the four groups.

Data analysis

Using a MATLAB script, MEPs were collected by extracting peak-to-peak EMG amplitude (in mV) in a time window of 60 ms, beginning 15 ms after the second TMS pulse. We excluded the MEPs recorded in trials showing a muscular preactivation defined as trials with an EMG activity

deviating more than 2SD from the participant's rectified mean in a time window of 100 ms before the TMS pulse eliciting the MEP. MEPs were averaged after being divided into 6 epochs of 15 trials each. Mean MEPs were analyzed with a GLM with within-subjects factor Epoch (6 levels) and between-subject factors Area (2 levels: PMv and SMA) and Direction (2 levels: Forward and Reverse). Post-hoc analysis was carried out using the Duncan's post-hoc test (see main text and Figure 1c).

As an index of behavioral performance, the Inverse Efficacy was computed as the ratio between mean reaction times and accuracy for each participant. This index was then submitted to a GLM within-subjects factor Session (3 levels) and between-subject factors Area (2 levels: PMv and SMA) and Direction (2 levels: Forward and Reverse). Results are reported in main text.

To explore the relationship between behavioral improvement and the modulation of corticospinal excitability observed during ccPAS (see main text Fig.1c) a GLM was ran with behavioral improvement following ccPAS (IE at T0 – IE at pre) as dependent variable and the full factorial combination of the categorical variable Area and Direction and the continuous variable MEP size modulation (MEP amplitude at last Epoch – MEP amplitude at first Epoch) as predictors. The results are reported in main text.

Supplementary Results

Relationship between physiological and behavioral correlates of ccPAS

The GLM revealed a Direction*MEP interaction ($F_{1,52} = 4.27$; $p = 0.044$; $\eta_p^2 = 0.76$) in the context of a non-fully significant model ($F_{7,52} = 1.31$; $p = 0.26$; $\eta_p^2 = 0.15$; $_{adj}R^2 = 0.036$). Thus, a second model was ran narrowing the predictors to Direction (forward, reverse) and MEP modulation (last-first Epoch), and resulting in a significant model ($F_{3,56} = 2.99$; $p = 0.038$; $\eta_p^2 = 0.14$; $_{adj}R^2 = 0.092$) driven by the Direction*MEP interaction ($F_{1,56} = 6.39$; $p = 0.014$; $\eta_p^2 = 0.10$). Specifically, the extent of physiological modulation significantly predicted behavior in the forward ($\beta = 58.65$; $p = 0.004$; $\eta_p^2 = 0.14$; see main text Fig.1b), but not in the reverse ccPAS groups ($\beta = 2.91$; $p = 0.77$; $\eta_p^2 = 0.002$). These results indicate that greater ccPAS-induced MEP facilitations predicted reduced performance gains in the cRT task, exclusively for the two forward groups. Thus, increasing PMv or SMA input to M1 led to reduced behavioral improvement following forward ccPAS.

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