Supplementary information

Driving associative plasticity in premotor-motor connections through a novel paired associative stimulation based on long-latency cortico-cortical interactions

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Supplementary Methods

Participants

Twenty-eight right-handed healthy volunteers took part in this study after providing written informed consent. Participants were assigned to one of the three ll-ccPAS group, namely the PMv-to-M1 group (5 females, mean age ± SD: 25.4 y ± 2.5; N = 12), the SMA-to-M1 group (4 females, mean age ± SD: 25.7 y ± 2.3; N = 12) and the Sham group (9 females, mean age ± SD: 23.8 y ± 1.8; N = 12). Data of one participant in the Sham group could not be analyzed due to a technical failure in the acquisition phase, thus the final sample in this group was of 11 participants. Eight participants in the PMv-to-M1 group were also tested on a separate session (interval between sessions: median value ± standard deviation = 32 ± 60 days; minimum = 19 days) in the SMA-to-M1 group (seven participants) or in the Sham group (one participant). There is no evidence of TMS-induced metaplastic effect over such a prolonged period and therefore we assumed no carry over effect of one session over another [e.g. 1, 2]. Direct comparisons of MEPs in participants tested in more than one protocol suggest no effect of order as shown by a series of Mann-Whitney U tests computed across time points in the target muscle FDI (all $p > .31$) and control muscle ADM (all $p > .08$). None of the participants reported adverse reactions or discomfort related to TMS and all of them were naïve as to the aims of the experiment. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki (2013) and approved by the Bioethics Committee of the University of Bologna (2.6/07.12.16).

Experimental procedures

TMS was administered using two 50-mm butterfly-shaped iron-branding coils connected to two independent Magstim 200 stimulators (Magstim, UK), delivering single monophasic waveform pulses. The same stimulators and coils were used for the dsTMS and ll-ccPAS protocols.

dsTMS protocol

In all groups, we assessed long-latency PMv-to-M1 interactions using the dsTMS protocol [3, 4]. In each of the 5 dsTMS blocks (pre-A, pre-B, T0, T20, T40) we collected 25 TS trials (single-pulse TMS over the left M1) and 25 CS-TS trials (paired-pulse TMS, with TS over the left M1 preceded by a CS over the left PMv with an ISI of 40 ms). TS and CS-TS trials were pseudo-randomly intermixed and separated by an inter-trial interval of 7.5-8.5 s. In each trial, the TS simultaneously induced MEPs in the relaxed right FDI (target) and ADM (control) hand muscles. MEPs were recorded using Ag/AgCl electrodes placed in a belly-tendon montage and a Biopac MP-35 (Biopac, USA) electromyography. EMG activity was band-pass filtered (30–500 Hz), acquired at a sample rate of 5 kHz and stored for offline analyses.

The left M1 was identified as the optimal scalp position to elicit MEPs of maximal amplitude in the resting FDI muscle. The intensity of the TS was set in order to elicit a MEP of ~1 mV amplitude in the target FDI muscle. Such intensity was adequate to induce stable MEPs also in the control ADM muscle. The left PMv was identified using a neuronavigation system as reported below. CS intensity for PMv stimulation was set at 90% of the individual resting motor threshold (rMT), defined as the minimum stimulator output intensity that induced MEP with > 50 μV amplitude in 5 out of 10 consecutive trials [5].

TS intensity (mean ± S.D.: 69% ± 13 of the maximum stimulator output; $F_{3,31} = .52$, $p = .67$; $\eta_p^2 = .05$) and CS intensity (36% ± 5 of the maximum stimulator output; $F_{3,31} = .78$, $p = .52$; $\eta_p^2 = .07$) were comparable across the three groups.

ll-ccPAS

In all groups, we administered 90 pairs of TMS pulses at a rate of 0.1 Hz for 15 min [1, 2, 6-9]. In the two active ll-ccPAS groups (i.e., in the PMv-to-M1 and the SMA-to-M1 groups) in each pair, a first pulse was administered either over the left PMv or the SMA (according to the participant's group assignment), and the second pulse was administered over the left M1 with an ISI of 40 ms, so to activate long-latency connections between the two regions [3, 4]. The first and second pulses of each pair were set at an intensity equal to the CS (90% rMT) and TS (~1 mV MEPs criterion) of the dsTMS protocol. The very same stimulation parameters were adopted in the Sham group, however in this group the coils were held perpendicularly so that no current was induced in the brain.

Brain localization

For both dsTMS and ll-ccPAS protocols, coil positions were identified using established methods [1-4] as detailed below. The left M1 was identified functionally as the FDI motor hotspot. To target M1, the coil was held at 45° to the sagittal midline inducing a posterior-to-anterior current direction in the brain [10].

The left PMv was identified using the SoftTaxic neuronavigation system (EMS, Italy). Skull landmarks (nasion, inion, and two pre-auricular points) and about 90 points providing a uniform representation of the scalp were digitized by means of the Polaris Vicra digitizer (Northern Digital INC, Ontario, CA). An individual estimated magnetic resonance image (MRI) was obtained for each subject through a 3D warping procedure fitting a high-resolution MRI template with the participant's scalp model and craniometric points. This procedure ensures a global localization accuracy of ~5 mm [11]. The targeted an anterior sector of the PMv at the border with the posterior part of the inferior frontal gyrus using the following Talairach coordinates: x = −54, y = 10, z = 24. The coil was placed at ~45° to the midline to induce a ventro-lateral to medio-posterior current [3, 4, 12].

The SMA was stimulated 4 cm anterior to the vertex on the sagittal midline [4, 14] with the coil handle pointing forward to induce an anterior-posterior current [15].

The scalp locations that corresponded best to left M1, left PMv and SMA coordinates were identified and marked with a pen. Then, the SofTaxic Navigator system was used to estimate the projection of all targeted scalp positions on the brain surface, confirming correct coil placement for all the sites. Across the three groups, the estimated Talairach coordinates for the left M1 were (mean ± S.D.): x = –33.2 ± 6.1, y = –16 ± 7.5, z = 56.7 ± 5.6; for the left PMv were: x = –53.8 ± 1.9, y = 9.6 ± 1.2, z = 23.6 ± 1. In the SMA-to-M1 group, SMA coordinates were: x = –4.9 ± 3.5, y = 3.5 ± 6.4, z = 63.1 ± 2.7.

Supplementary Results

Peak-to-peak MEP amplitudes from the FDI and the ADM muscles were automatically extracted from EMG signals using a custom Matlab code (v. 2016b; MathWorks, USA) and measured in mV. Trials showing EMG activity 100 ms prior to TMS were discarded from further analysis (6.7%). Mean MEP amplitude in each block was transformed using the formula $Log_{10}(value+1)$ to address lack of normality in a few conditions [15]. We computed a CS-TS modulation index as the difference between MEPs obtained in the CS-TS and TS trials [4]. The index looked sufficiently normal to carry out parametric testing by visual inspection and statistic test of normality (Kolmogorov-Smirnov test: all $p > .20$) and was therefore analyzed using a Protocol x Time x Muscle ANOVA. Post-hoc analysis was conducted using the Duncan test to correct for multiple comparisons. Statistical analyses were performed using the STATISTICA software (v. 12; StatSoft Inc., USA).

As reported in the main text, the ANOVA yielded to 3-way interaction. We observed no significant change over time in the Sham ll-ccPAS group (Table S1) either in the FDI (all $p > .41$), nor the ADM (all $p > .15$).

	FDI - CS-TS modulation index $\log(MEP_{CS\text{-}TS}+1) - \log(MEP_{TS}+1)$					ADM - CS-TS modulation index $\log(MEP_{CS\text{-}TS}+1) - \log(MEP_{TS}+1)$				
	pre-A	pre-B	T0	T20	T40	pre-A	pre-B	T0	T20	T40
Sham ll-ccPAS	–0.06 (0.06)	–0.07 (0.07)	–0.07 (0.06)	–0.06 (0.06)	–0.06 (0.05)	–0.05 (0.04)	–0.03 (0.04)	–0.03 (0.03)	–0.04 (0.05)	–0.03 (0.04)

Table S1. Mean (standard deviation) values of the modulation index.

In contrast, the two active groups showed significant changes over time (see Figure 1 in the main text) with a reduction in the FDI CS-TS modulation index at T0 (PMv-to-M1 ll-ccPAS) or T20 (SMA-to-M1 ll-ccPAS). To ensure these changes purely reflected changes in premotor-motor interactions, not accompanied by changes in M1 excitability, we conducted a control analysis on MEPs induced by the TS alone (single-pulse trials; Table S2). Data were not normally distributed and distribution could not be ameliorated using data transformation. Therefore, we analyzed these MEPs using Friedman ANOVAs. The analyses showed no significant change in FDI MEPs in either the PMv-to-M1 group ($Chi^2_4 = 3.27$, $p = .51$) nor the SMA-to-M1 group ($Chi^2_4 = 7.13$, $p = .13$).

	FDI MEPs induced by TS alone					ADM MEPs induced by TS alone				
	pre-A	pre-B	T0	T20	T40	pre-A	pre-B	T0	T20	T40
PMv-to-M1	0.32 (0.03)	0.33 (0.04)	0.34 (0.05)	0.31 (0.06)	0.31 (0.05)	0.26 (0.09)	0.25 (0.09)	0.24 (0.10)	0.24 (0.01)	0.24 (0.09)
SMA-to-M1	0.31 (0.05)	0.31 (0.05)	0.35 (0.06)	0.34 (0.08)	0.32 (0.08)	0.19 (0.11)	0.19 (0.08)	0.21 (0.12)	0.20 (0.09)	0.19 (0.10)
Sham	0.31 (0.05)	0.33 (0.06)	0.34 (0.07)	0.35 (0.06)	0.34 (0.06)	0.30 (0.12)	0.29 (0.10)	0.32 (0.13)	0.34 (0.11)	0.33 (0.13)

Table S2. Mean (standard deviation) log-transformed MEP amplitudes during single-pulse trials.

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