Cortico-cortical paired associative stimulation (ccPAS) over premotor-motor areas affects local circuitries in the human motor cortex via Hebbian plasticity

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Supplemental information

Pilot Study

We used dual coil TMS (dcTMS) on 15 healthy volunteers who did not participate in the main experiment, to investigate the inhibitory/facilitatory sign of cortico-cortical interactions between PMv and M1 at early ISIs, and to get insights into the targeted neural mechanism during ccPAS. Additionally, this pilot study allowed us to select the best ISI at which PMv conditioning influenced M1 excitability, to be used in the ccPAS protocol. To these ends, we administered 36 single pulse TMS (spTMS) trials, where only the left M1 was stimulated, and 54 dcTMS trials, where M1 stimulation was preceded by a conditioning pulse over PMv at 3 different interstimulus intervals (ISIs): 6, 8 and 10 ms (18 MEPs for each ISI). Trial order was randomized. PMv and M1 locations were defined in Talairach coordinates as described in the Neuronavigation paragraph of the main text and were consistent with the regions defined as human PMv and M1 (Mayka et al., 2006). The mean MNI-transformed coordinates (± standard deviation) corresponding to the projections of the left PMv and M1 scalp sites onto the brain surface were x = –57.26 ± 2.48, y = –12.74 ± 1.35, z = 21.46 ± 1.95 for PMv and x = –34.72 ± 3.31, y = –15.95 ± 6.11, z = 63.75 ± 2.74 for M1. PMv was stimulated at 90% of the individual rMT, while M1 was stimulated at SI_{1mV}. MEPs were assessed by measuring peak-to-peak EMG amplitude (in mV). Trials with background EMG activity were excluded from the analysis (4% on average) as described in the main text. The mean MEP amplitude of each dcTMS trial was expressed as the ratio relative to the mean of the 5 nearest spTMS trials (Buch et al., 2011). MEP ratios were analyzed using a repeated-measures ANOVA with ISI (3 levels: 6, 8, 10) as a within-subjects factor.

The ANOVA revealed a marginally significant influence of ISI ($F_{2,28}$ = 3.30, p = 0.051, η_p^2 = 0.19; Figure S1). Bonferroni-corrected one-sample t-tests against 1 showed that dcTMS MEPs at an 8-ms ISI were consistently facilitated relative to spTMS (1.15 ± 0.19 of spTMS trials; p = 0.009; *Cohen's d* = 0.77), whereas MEPs at a 6-ms or 10-ms ISI were not (both $p \geq 0.35$, *Cohen's d* ≤ 0.25).

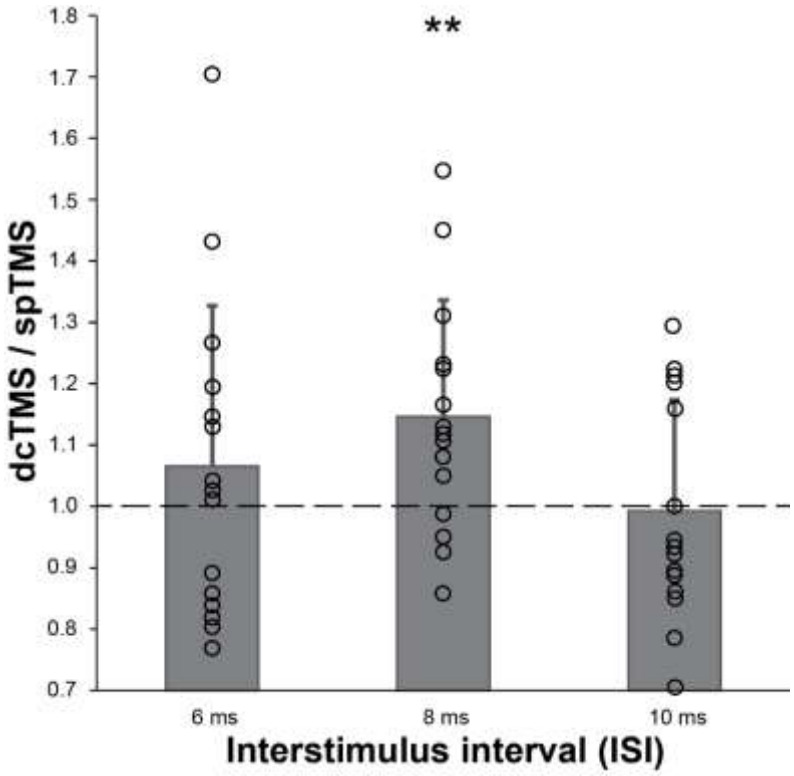

Figure S1. Conditioning effect of left PMv stimulation on left M1 excitability at different interstimulus intervals. Asterisks indicate significant comparisons: ** = $p \leq .01$. Error bars represent 1 standard deviation.

Main study: input-output (IO) curve MEP analysis

We further analyzed the effect of $ccPAS_{PMv \rightarrow M1}$ on the IO curve by entering MEP amplitudes into a Time (2 levels: Baseline and Expression block) x Stimulation Intensity (6 levels: 100%, 110%, 120%, 130%, 140% and 150% of rMT) ANOVA. To reduce skewness and approximate the MEP data to a normal distribution, mean amplitudes in each condition were transformed using the formula Log10(value+1). The ANOVA showed a main effect of Stimulation intensity ($F_{5,115} = 90.96$, $p < .001$; $\eta_p^2 = .80$), qualified by a significant Time x Intensity interaction ($F_{5,115} = 2.72$, $p = .02$; $\eta_p^2 = .11$; Figure S2, panel A). Post-hoc analysis revealed that MEP amplitudes in the Pre and Post blocks were comparable at 100% and 110% rMT intensities (all $p \geq .52$), but were significantly higher in the Post block at 120%-140% rMT (all $p \leq .05$). At 150% rMT, intensity MEP amplitudes, again, did not differ between timepoints ($p = .25$).

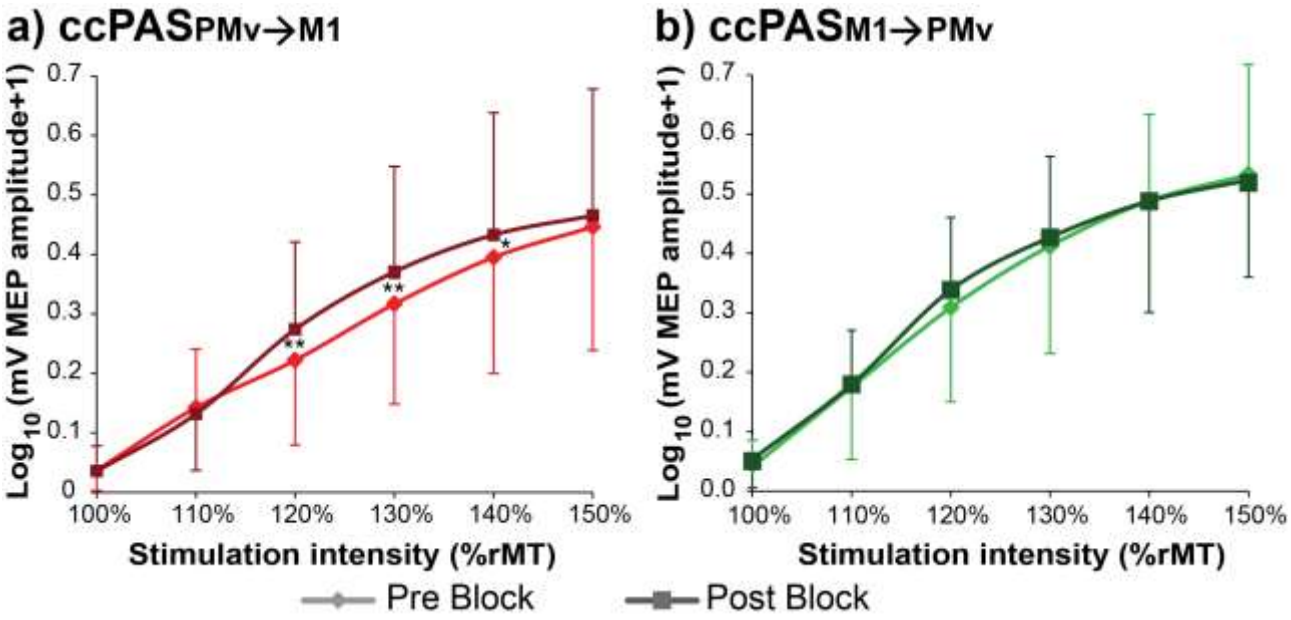

Figure S2. Input-output curve before and after $ccPAS_{PMv \rightarrow M1}$ (panel a) and $ccPAS_{M1 \rightarrow PMv}$ (panel b). Asterisks indicate significant comparisons: * = $p \leq .05$; ** = $p \leq .01$. Error bars represent 1 standard deviation.

We also carried out a Time x Stimulation intensity ANOVA in the $ccPAS_{M1 \rightarrow PMv}$ control group (Figure S2, panel B). The analysis only showed a significant main effect of Intensity ($F_{5,115} = 158.07$, $p < .001$; $\eta_p^2 = .87$), with a gradual increase in MEP amplitudes as intensities increased, but no main effect of Time or Time x Intensity interaction (all $F \leq 1.09$; all $p \geq .31$). This suggests there was no change in M1 corticospinal excitability following $ccPAS_{M1 \rightarrow PMv}$.

Main study: Short intracortical inhibition (SICI) and facilitation (ICF) analysis

In the main analyses reported in the main text, the standard SICI index computed as the ratio between ppTMS and spTMS trials revealed the presence of four statistical outliers (2 per group). In these participants the adopted SICI protocol induced a marked facilitation rather than an inhibition. Hence, these participants were removed from the main analysis on the standard SICI index (Figure 6A and C). However, to ensure the stability of our results, we ran a further analysis including all participants. To minimize the influence of outlier values on our results, in this analysis we computed a modified SICI index as the differences between ppTMS and spTMS trials. The index was normally distributed and therefore was entered into a ccPAS x Time ANOVA. In keeping with the results reported in the main text (Figure 6), we observed a significant

ccPAS x Time interaction ($F_{1,46}$ = 5.33, p = .025, η_p^2 = .10, Figure S3), showing different influences of the $ccPAS_{PMv\rightarrow M1}$ and $ccPAS_{M1\rightarrow PMv}$ protocols on the modified SICI index, with reduction of inhibition following $ccPAS_{PMv\rightarrow M1}$ (in keeping with the main analysis) and, additionally, increased inhibition following the $ccPAS_{M1\rightarrow PMv}$. We interpret this last finding with caution, as the additional analysis included participants for whom the chosen protocol failed to produce inhibition; because the removal of the two outlier participants showing strong facilitation nullifies the effect of increased inhibition following $ccPAS_{M1\rightarrow PMv}$ (Figure 6), we preliminary conclude that while $ccPAS_{PMv\rightarrow M1}$ reduces SICI, the effect of $ccPAS_{M1\rightarrow PMv}$ is either weak or null and further research is needed to draw stronger conclusions regarding the bidirectionality of the ccPAS influences.

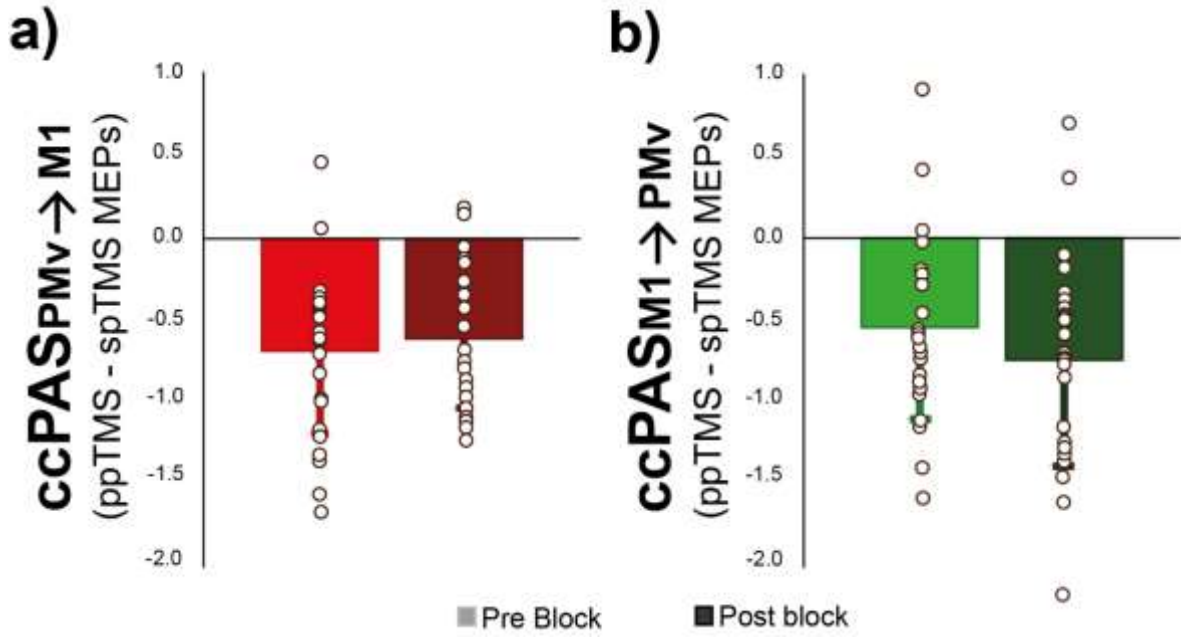

Figure S3. SICI computed as the difference between ppTMS MEPs and spTMS MEPs before and after $ccPAS_{PMv\rightarrow M1}$ (panel a) and $ccPAS_{M1\rightarrow PMv}$ (panel b). Error bars represent 1 standard deviation.

References

Buch, E.R., Johnen, V.M., Nelissen, N., O'Shea, J., & Rushworth, M.F. (2011). Noninvasive associative plasticity induction in a corticocortical pathway of the human brain. *Journal of Neuroscience* **31**, 17669–79.

Mayka, M.A., Corcos, D.M., Leurgans, S.E., & Vaillancourt, D.E. (2006). Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage* **31**, 1453–74.