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

Dear Editor,

cortico-cortical paired associative stimulation (ccPAS) is a transcranial magnetic stimulation (TMS) protocol designed to mimic neurostimulation patterns capable of inducing spike-timing-dependent plasticity (STDP). This protocol, based on the Hebbian principle, entails coupling of pre- and post-synaptic activity through TMS, targeting two interconnected brain areas. By tailoring stimulation parameters to the characteristics of the target pathway, ccPAS can modulate its connectivity strength [1–3] and induce functional changes [4,5]. Understanding the behavioral and physiological impact of ccPAS manipulation over different networks is key to developing clinical interventions.

We have recently demonstrated that ccPAS over the ventral premotor cortex (PMv) and primary motor cortex (M1) induces changes in motor-evoked potentials (MEPs) [3–7]. Forward PMv→M1 ccPAS, with subthreshold PMv conditioning, followed by suprathreshold M1 stimulation 8 ms later ($ccPAS_{PMv\text{-}M1}$), led to a gradual corticospinal excitability (CSE) increase during protocol administration, reflecting a progressive efficacy increase of excitatory PMv inputs to M1 via STDP [7]. Conversely, reversed stimulation order during ccPAS ($ccPAS_{M1\text{-}PMv}$), expected to hinder connectivity between the two nodes [1], tended to decrease CSE [6]. While ccPAS has been applied to other premotor-motor networks [8], it remains unclear whether similar time-specific bidirectional effects characterize networks other than the PMv-M1.

To clarify this issue, here, we compared 4 different ccPAS protocols targeting two premotor-motor circuits, i.e., the PMv-M1 and the supplementary motor area (SMA)-M1 pathways, and tested their physiological and behavioral effects (Fig. 1A). In 60 healthy adults, we administered 90 paired-pulses at a 0.1-Hz frequency, employing an 8-ms inter-stimulus interval (ISI), appropriate for both PMv-M1 and SMA-M1 cortico-cortical interactions [1,9]. We tested different ccPAS configurations, following the factorial combination of the targeted premotor Area (PMv; SMA) and stimulation Direction ("forward" premotor-M1; "reverse" M1-premotor), resulting in 4 groups of 15 participants each: $ccPAS_{PMv\text{-}M1}$, $ccPAS_{SMA\text{-}M1}$, $ccPAS_{M1\text{-}PMv}$, and $ccPAS_{M1\text{-}SMA}$ (Fig. 1B; Supplementary Methods). We examined i) the online effect on MEPs recorded during the ccPAS protocol; ii) the impact of ccPAS on a choice reaction time (cRT) task before (pre), immediately (T0) and 30 minutes after the end of the ccPAS (T30) (Fig. 1A); and iii) the relationship between the observed neurophysiological and behavioral effects.

We divided the 90 MEPs collected during the administration of ccPAS into 6 consecutive epochs, each containing 15 MEPs. Subsequently, we analyzed MEP amplitudes with an Area*Direction*Epoch general linear model (GLM), which revealed a significant 3-way interaction ($F_{5,280} = 5.12$; $p < 0.001$; $\eta_p^2 = 0.08$). Post-hoc analyses emphasized distinct patterns between the two targeted networks (see Fig. 1C for post-hoc comparisons). When targeting the PMv-M1 pathway, we observed strong bidirectional effects, with a gradual CSE increase during $ccPAS_{PMv\text{-}M1}$ and a decline during $ccPAS_{M1\text{-}PMv}$ (Epoch-1 vs. 6 comparisons: all $p \leq 0.001$). In contrast, targeting the SMA-M1 circuit produced an increase in excitability regardless of the stimulation direction (Epoch-1 vs. 6 comparisons: all $p \leq 0.05$). Indeed, the $ccPAS_{PMv\text{-}M1}$, $ccPAS_{SMA\text{-}M1}$, and $ccPAS_{M1\text{-}SMA}$ groups exhibited a comparable linear increase in MEPs, with no significant difference across time points (all $p \geq 0.18$), while the $ccPAS_{M1\text{-}PMv}$ group displayed the opposite pattern, differing significantly from all other groups at the end of the protocol (all $p \leq 0.03$).

Our findings point to dissociable features of the two targeted premotor-motor networks. Manipulating the PMv-M1 pathway with the same 8-ms ISI induced modulations of CSE with a comparable magnitude but opposite directions depending on the order of the paired TMS pulses during ccPAS [6]. This suggests that excitatory interactions that govern PMv influences on M1 [7] can be enhanced or decreased by modulating the strength of the PMv input to M1, supporting the idea of a symmetrical organization and temporal features of the pathways between PMv and M1. Conversely, our study points to the distinctiveness of the SMA-M1 network, showing no such bidirectional modulations using the same 8-ms ISI. Paired-stimulation during $ccPAS_{SMA\text{-}M1}$ relied on an excitatory influence of SMA over M1 [8,9] and our study supports the idea that the protocol gradually enhanced excitatory SMA input to M1. Interestingly, we show a similar increase in CSE during $ccPAS_{M1\text{-}SMA}$ using the 8-ms ISI, whereas a prior study reported non-significant facilitation with a 10-ms ISI and inhibition with a 15-ms ISI during $ccPAS_{M1\text{-}SMA}$ [8]. Together with the evidence that TMS of the M1 elicits activity peaking at about 30 ms over the medial frontal cortex [10], these findings suggest that M1-SMA interactions could occur in a different timeframe compared to other directional pathways tested in this study. Reduced CSE following $ccPAS_{M1\text{-}SMA}$ with a longer-latency (15-ms) ISI [8] aligns with the principles of STDP if one assumes M1-SMA interactions to recruit a slower, likely indirect, circuit. In contrast, shorter ISIs are found to be either ineffective (ISI = 10 ms [8]) or facilitatory (ISI = 8 ms; this study). This indicates that distinct mechanisms govern the interactions within the M1→PMv and M1→SMA networks as observed during reverse ccPAS: while $ccPAS_{M1\text{-}PMv}$ acts by progressively weakening excitatory PM→M1 projections [11], resulting in a decreased CSE [6], $ccPAS_{M1\text{-}SMA}$ induces a progressive increase in CSE, which may suggest that enhancing M1 input to SMA may either progressively attenuate SMA inhibitory influences on CSE or potentiate excitatory influences.

At the behavioral level, the Area*Direction*Time GLM on inverse efficacy (IE, i.e., reaction times divided by task accuracy) index showed a general improvement in performance over time ($F_{2,112} = 16.47$; $p <$

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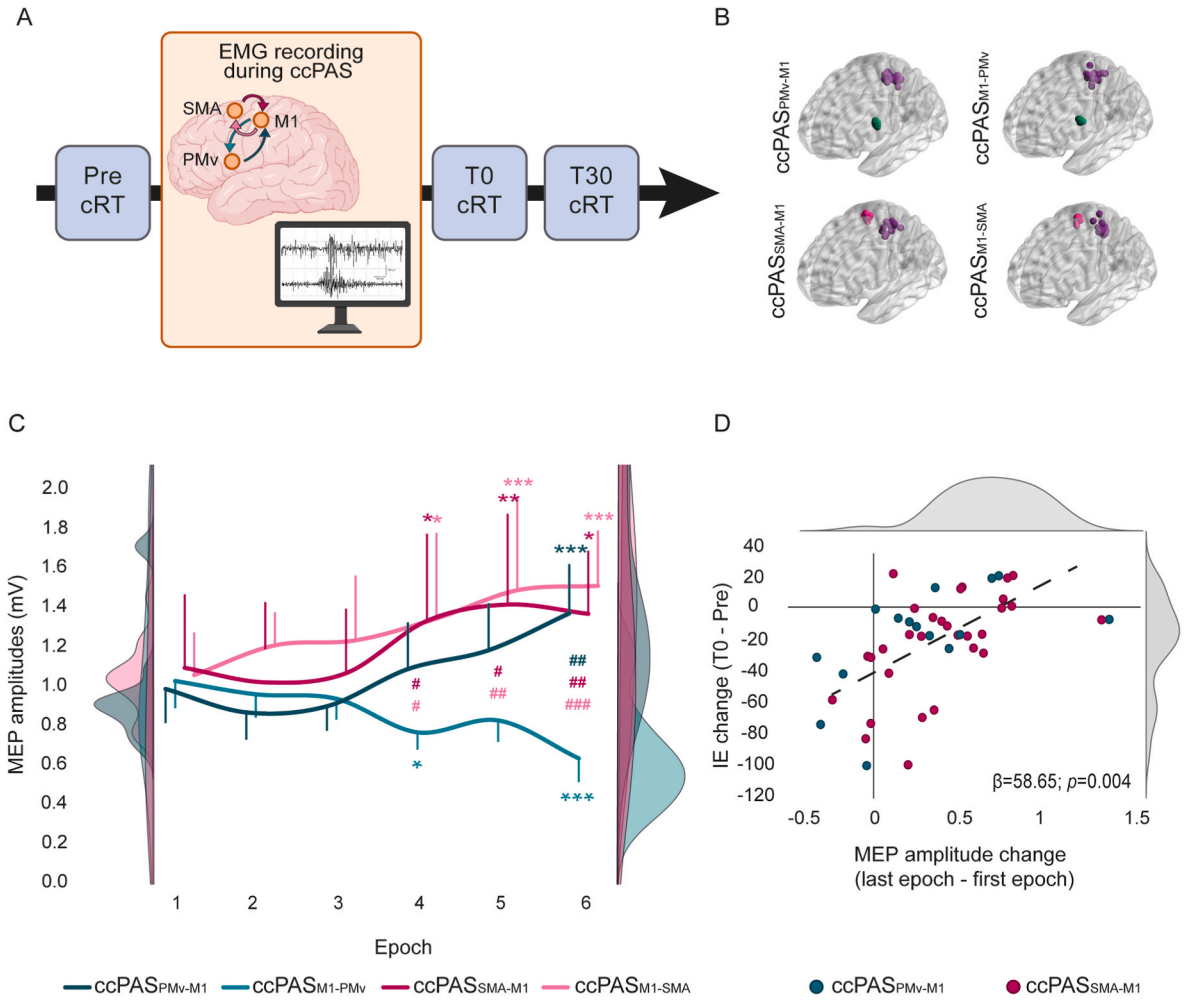

Fig. 1. (**A**) Experimental design. (**B**) Individual stimulation sites were reconstructed onto a standard template using BrainNEt after MNI space conversion. Purple, green, and pink dots represent M1, PMv, and SMA stimulation sites, respectively. (**C**) MEP amplitudes during the four ccPAS protocols merged in 6 epochs of 15 MEPs each. Asterisks and hashtags represent differences relative to Epoch 1 ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$) and between-group differences relative to $ccPAS_{PMv\text{-}M1}$ ($^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, $^{\#\#\#}p \leq 0.001$). Error bars represent standard error; (**D**) Relation between MEP amplitude changes (last minus first epoch) and behavioral changes (IE-T0 minus IE-pre) in the forward groups. Green and pink dots represent $ccPAS_{PMv\text{-}M1}$ and $ccPAS_{SMA\text{-}M1}$ participants, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

0.0001; $\eta_p^2 = 0.23$), but no influence of the targeted Area (all $p > 0.99$) or ccPAS Direction (all $p \geq 0.35$). Nonetheless, a further GLM tested the relation between behavioral and physiological changes and revealed that greater ccPAS-induced MEP facilitation predicted reduced performance gains in the cRT task, but exclusively for the two forward groups ($ccPAS_{PMv\text{-}M1}$, $ccPAS_{SMA\text{-}M1}$; $F_{1,56} = 6.39$; $p = 0.014$; $\eta_p^2 = 0.10$; $_{adj}R^2 = 0.092$; Fig. 1D; see Supplementary Material). According to previous premotor-M1 ccPAS studies, forward ccPAS configurations increase connectivity within the targeted circuit [1–3,8] while simultaneously decreasing connectivity in parallel and competing pathways to M1 [3]. Assuming this, we speculate that both $ccPAS_{SMA\text{-}M1}$ and $ccPAS_{PMv\text{-}M1}$ would entail reduced connectivity between the dorsal premotor cortex and M1, whose interaction is crucial for cRT performance, accounting for the observed relationship between increased SMA-M1/PMv-M1 connectivity and reduced gains in cRT.

In sum, our ccPAS results highlight different physiological mechanisms underlying premotor-motor pathways. The PMv-M1 network exhibits opposite plastic effects (i.e., excitatory and inhibitory) depending on paired-stimulation order [7], while the SMA-M1 shows enhanced excitability, irrespective of stimulation direction.

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CRediT authorship contribution statement

Naomi Bevacqua: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Sonia Turrini:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Francesca Fiori:** Investigation,

Methodology, Software, Writing – review & editing. **Chiara Saracini:** Formal analysis, Writing – review & editing. **Boris Lucero:** Resources, Writing – review & editing. **Matteo Candidi:** Supervision, Writing – review & editing. **Alessio Avenanti:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2024.01.001.

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