Supplementary material

Enhanced action performance following TMS manipulation of associative plasticity in ventral premotor-motor pathway

Fiori F, Chiappini E, Avenanti A

Supplementary Results

1. Physiological changes induced by cortico-cortical Paired Associative Stimulation (ccPAS)

All ccPAS protocols involved 90 pairs of transcranial magnetic stimulation (TMS) pulses administered over the left ventral premotor cortex (PMv) and the left primary motor cortex (M1). In the two active protocols (PMv-to-M1 and M1-to-PMv ccPAS), suprathreshold TMS pulses over the left M1 induced motor responses in the right hand. This allowed us to record motor-evoked potentials (MEPs) and thus assess motor excitability during the ccPAS protocol. MEPs induced by the paired stimulation were recorded in the right first dorsal interosseous (FDI) of a subsample of participants (N = 12 participants in the $Exp_{PMv\rightarrow M1}$ group and N = 9 participants in the $Ctrl_{M1\rightarrow PMv}$ group). Peak-to-peak MEP amplitudes induced by the 90 paired stimuli were measured in mV, and MEPs were grouped into 6 epochs of 15 MEPs each (epoch 1: MEP 1-15; epoch 2: MEP 16-30; epoch 3: MEP 31-45; epoch 4: MEP 46-60; epoch 5: MEP 61-75; and epoch 6: MEP 76-90) and averaged. Mean MEP amplitudes were analyzed using a two-way ANOVA with ccPAS ($Exp_{PMv\rightarrow M1}$ and $Ctrl_{M1\rightarrow PMv}$) as the between-subjects factor and Epoch (1-6) as the within-subjects factor.

Fig. S1 displays changes in MEP amplitudes during the administration of the ccPAS protocol. The ANOVA of mean MEP amplitudes showed a significant main effect of ccPAS ($F_{1,19} = 13.35$, $p = .002$, $\eta_p^2 = .41$). This effect was qualified by a significant ccPAS x Epoch interaction ($F_{5,95} = 3.78$, $p = .004$, $\eta_p^2 = .17$), indicating that there was a gradual increase in MEPs over time in the $Exp_{PMv\rightarrow M1}$ group, whereas no similar

effect of time was observed in the $Ctrl_{M1\rightarrow PMv}$ group. Newman-Keuls post-hoc comparisons showed that, relative to epoch 1 (i.e., the initial phase of the ccPAS protocol associated with the first 15 paired stimuli), the $Exp_{PMv\rightarrow M1}$ group showed marginally larger MEPs at epoch 4 (p = .058) and significantly larger MEPs at epochs 5 and 6 (all p < .005; red marks in Fig. S1). In the $Exp_{PMv\rightarrow M1}$ group, the last epoch showed an average MEP enlargement of +36% relative to the first epoch. No similar increase was detected in the $Ctrl_{M1\rightarrow PMv}$ group, which showed no significant change in MEPs across epochs (all p > .29). The two groups were comparable at epochs 1 (p = .31), non-significantly different at epochs 2 and 3 (all p > .09) and significantly different in the last three epochs (all p < .05; black marks in Fig. S1).

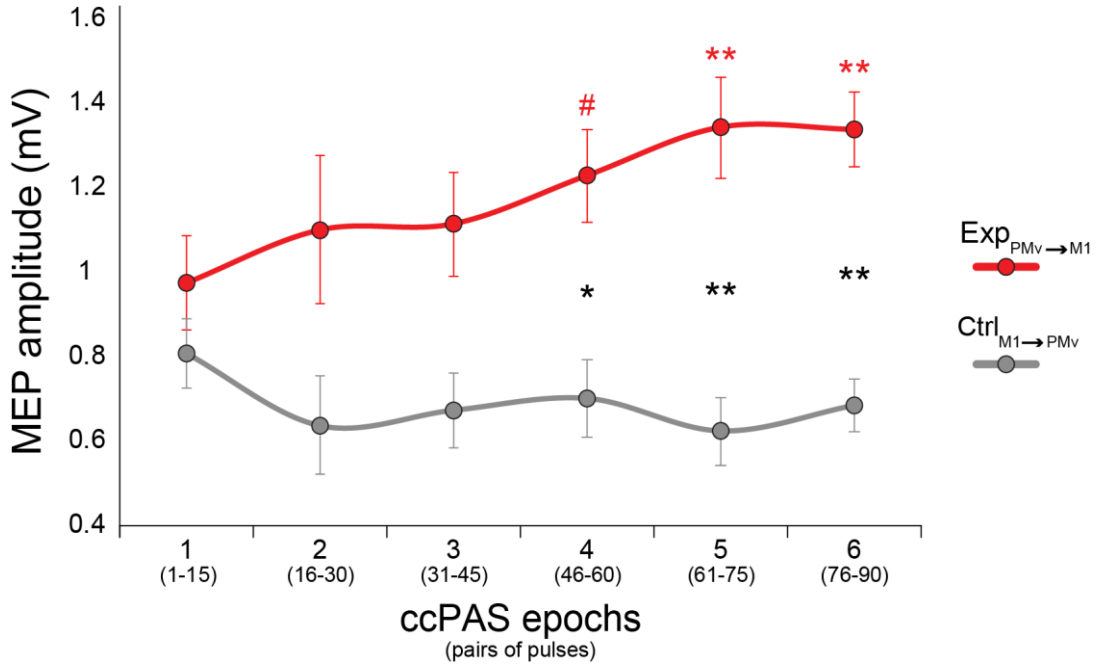

Figure S1. MEPs during ccPAS. In the $Exp_{PMv\rightarrow M1}$ group there was a gradual increase in motor excitability, whereas no similar effect was observed in the $Ctrl_{M1\rightarrow PMv}$ group. Asterisks and hash marks indicate post-hoc comparisons (# p = .06, * p < .05; ** p < .01). Error bars denote s.e.m.

It is important to note that, during PMv-to-M1 ccPAS ($Exp_{PMv\rightarrow M1}$ group), the cortico-cortical volley elicited by each PMv stimulation (first pulse of each TMS pair) is supposed to spread to M1

immediately before / during the stimulation of M1 (second TMS pulse), thus producing convergent activation of M1 neurons. Our findings allow us to conclude that repeating this convergent activation over time – in the $Exp_{PMv \rightarrow M1}$ group – results in a gradual increase of MEP amplitude. While this increase indexes an enhancement of motor excitability, it is unclear whether such an increase reflects plastic effects occurring at the level of cortico-cortical connections, or M1 corticospinal neurons, or both. Indeed, we only assessed MEPs induced by paired stimulation of PMv and M1; that is, all MEPs evoked by M1 stimulation were also affected by the conditioning effect of PMv stimulation. Because our focus was on behavioral effects, we did not include a control condition (i.e., a single-pulse stimulation of M1 to record unconditioned MEPs) interleaved with the protocol's paired stimulation. Indeed, such control stimulation could potentially interfere with ccPAS efficacy, by reducing the coherence of the PMv-to-M1 stimulation which is essential for STDP to occur. Although our study does not clarify the precise level at which plastic effects occur, our data allow us to preliminarily conclude that PMv-to-M1 ccPAS induces a consistent increase in motor excitability that is already apparent during the stimulation protocol.

2. Behavioral changes induced by ccPAS

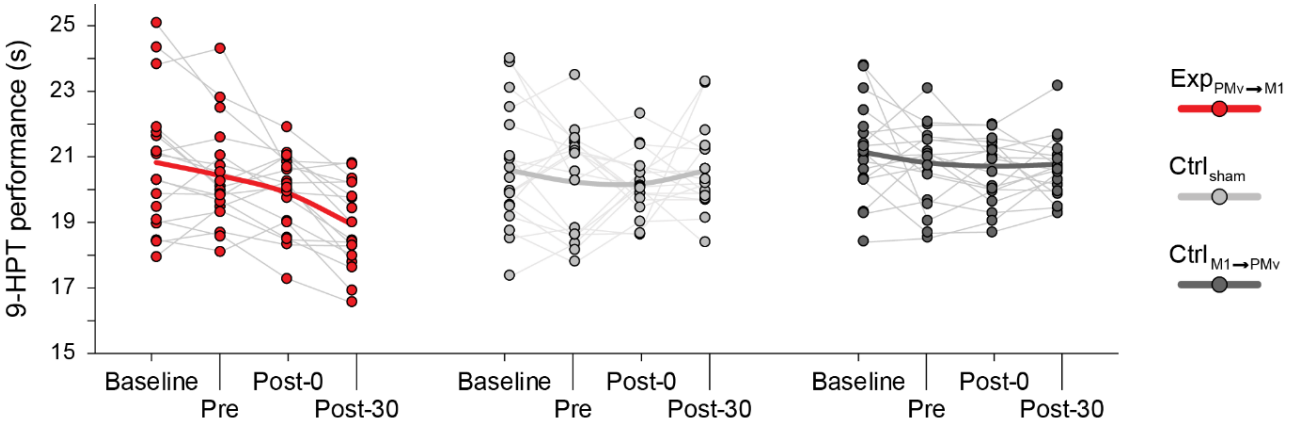

Figure S2. Performance on the experimental task. For each group, individual participants' changes in 9-HPT execution time are displayed across the four sessions.

3. Correlation between MEPs and behavioral measures

In two correlation analyses, we found no significant relationship between behavioral improvements in the $Exp_{PMv \rightarrow M1}$ group and changes in MEP amplitudes during the administration of the PMv-to-M1 ccPAS protocol. Behavioral improvements were indexed as changes in 9-HPT performance at Post-0 and Post-30 relative to Baseline. In a first analysis, changes in MEPs were indexed as the difference in mean MEP amplitudes in the last epoch relative to the first epoch. In a second analysis, changes in MEPs were indexed as slope of the increase in MEP amplitudes across the 6 epochs. No significant correlations were detected either in the first analysis (Post-0: $r = –.11$, $p = .73$; Post-30: $r = .11$, $p = .72$) or the second analysis (Post-0: $r = .06$, $p = .86$; Post-30: $r = .23$, $p = .47$). It should be considered that these correlations were computed on a subsample of participants, and it would be appropriate to increase sample size in future studies to clarify whether behavioral and physiological effects induced by ccPAS reflect a single mechanism or distinct mechanisms.