Mu rhythm and corticospinal excitability capture two different frames of motor resonance: a TMS/EEG co-registration study

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Supplementary Results

Mu rhythm. To check whether EEG modulations occurred only in the left hemisphere – i.e., over C3 and CP3 electrodes, contralateral to the observed/moving hand – or extended over the right hemisphere, we also extracted mu-ERD from C4 and CP4 and analyzed these data by adding the factor Hemisphere into the same RM ANOVA reported in the main text. This Condition x Hemisphere ANOVA showed only a main effect of Condition ($F_{3,57}$ = 7.05, p < 0.001, η_p^2 = 0.271), and no main effect of, or interaction with, the factor Hemisphere (all p ≥ 0.48; see Figure S1). Post-hoc tests showed that Action Observation (–0.52 µV ± 0.62) entailed a stronger mu-ERD than Static Observation (–0.27 µV ± 0.39; p < 0.001) and Rest (–0.09 µV ± 0.31; p = 0.02), which did not differ from one another (p = 0.68). Similarly, Action Execution (–0.39 µV ± 0.36) showed stronger mu-ERD than Static Observation (p = 0.017) and Rest (p = 0.02). Mu-ERD was comparable during Action Execution and Action Observation (p = 0.65). These findings support the notion that observing actions performed by others engages the motor system bilaterally.

Figure S1. Mu rhythm from the four experimental conditions (Rest, Action Execution, Static Observation and Action Observation) in the two hemispheres (left vs right). Bar plot represents the averaged signal from electrodes C3-CP3 (left hemisphere) and C4-CP4 (right hemisphere) before the TMS pulse. The four experimental conditions are plotted on the x-axis, while the y-axis depicts mu rhythm desynchronization (µV²). Mu desynchronization is stronger during Action Execution and Action Observation compared to the remaining conditions, but there are no significant differences between the two hemispheres. Left hemisphere is depicted in yellow while right hemisphere is depicted in light blue.

Beta rhythm. To investigate the possible involvement of the beta rhythm, we extrapolated values from a specific time window (-1 to -0.35 sec) over left central electrodes (FC3, C3) for those beta frequencies showing the strongest activity (19-28 Hz). Data strongly violated normality and were therefore analyzed using a non-parametric Friedman ANOVA with Condition (Rest, Action Execution, Static Observation, Action Observation) as the within-subjects factor. The analysis was significant (Chi^2 = 14.40, p = 0.002; Figure S2). A follow-up analysis conducted with Wilcoxon tests showed that beta desynchronization during Action Execution (–0.57 µV ± 1.8) was stronger than in all the other conditions, namely Action Observation (–0.11 µV ± 0.3; p = 0.028), Static Observation (–0.13 µV ± 0.65; p = 0.008) and Rest (–0.02 µV ± 0.15; p = 0.011),

which in turn did not differ from one another (all $p \geq 0.17$). Thus, while action execution was associated with both mu- and beta-ERD, action observation was associated only with mu-ERD, but not consistent beta-ERD.

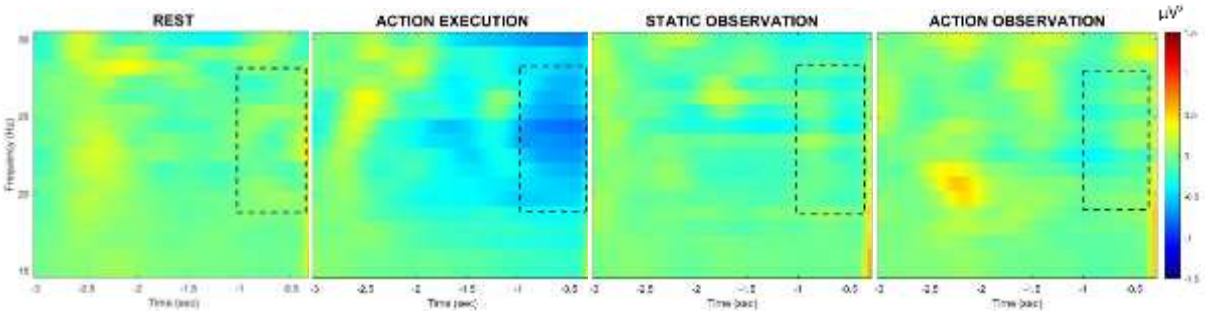

Figure S2. Beta rhythm oscillations from the four experimental conditions (Rest, Action Execution, Static Observation and Action Observation). Time-frequency (TF) plots represent motor cortex activity before the TMS pulse, averaged from electrodes C3 and FC3. Time (seconds) is plotted in the x-axis, while the y-axis depicts Frequency (Hz). The color bar on the right side ranges from -1.5 to +1.5 μV^2 of mu power, where blue indicates desynchronization and red indicates synchronization. The dashed rectangles outline both the frequencies (19-28 Hz) and the time-window (-1 to -0.35 sec) selected for statistical analysis. Beta desynchronization is clearly visible over central electrodes of the contralateral cortex in Action Execution but not in the other conditions.

Relation between Mu rhythm and MEPs during Action Observation. To further test the robustness of the main finding that MEPs and mu rhythms are not associated within the Action Observation condition, we carried out a series of additional analyses.

First, we tested whether the lack of a significant relation could be due to extreme high or low values. We thus performed a LMM and trial-by-trial correlations between MEPs and mu-ERD after discarding extreme values in both indices (i.e., absolute values ≥ 3 SD from the mean). We also performed a correlation between averaged MEPs and mu-ERD values after discarding those participants with extreme mean values. In all these analyses, we confirmed the lack of a significant association (all $p \geq 0.29$). Moreover, given that some participants exhibited very small / inconsistent MEPs in several trials (i.e., s6, s18, s19, s20), we performed a LMM and trial-by-trial correlations without those subjects and further confirmed the lack of a significant relation (all $p \geq 0.47$).

Second, to test for non-linear relations between between mu-ERD and MEPs, we carried out additional LMM analyses by including the factor mu-ERD following quadratic, cubic or quartic transformation. We found that none of these transformed values of mu-ERD predicted MEP amplitudes (all $p \geq 0.47$), neither by themselves nor in interaction with the factor Muscle (all $p \geq 0.68$). Similarly, trial-by-trial correlations between transformed mu-ERD values and MEPs were not significant (all $p \geq 0.78$).

Third, we tested whether alternative ways of calculating these indices of motor resonance could reveal any relation between them. We performed two LMMs in which the average MEPs recorded during Static (LMM1) or Rest (LMM2) were subtracted from single-trial MEPs recorded during Action Observation, specifically during observation of a movement congruent with the recorded muscle. These variants of MEP indices (i.e., MEP_{static} and MEP_{rest}) were entered into two separate models as the dependent variable, with Muscle (FDI, ADM) entered as a fixed within-subjects factor, mu variations entered as a covariate and subject variability as a random effect. Results of both LMM1 and LMM2 showed no significant effects (all $p \geq 0.24$). Similarly, performing correlation analyses between mu-ERD and the variants of MEP indices used in LMM1 (MEP_{static}) and LMM2 (MEP_{rest}) showed no significant correlations, neither using averaged trials nor the trial-by-trial data (all $p \geq 0.23$).

Lastly, the relation between MEPs (congruent - incongruent) and mu rhythm was also analyzed in each participant separately. As shown below in Figure S3, none of participants exhibited a consistent relationship between TMS and EEG motor resonance signatures (all $p \geq 0.08$).

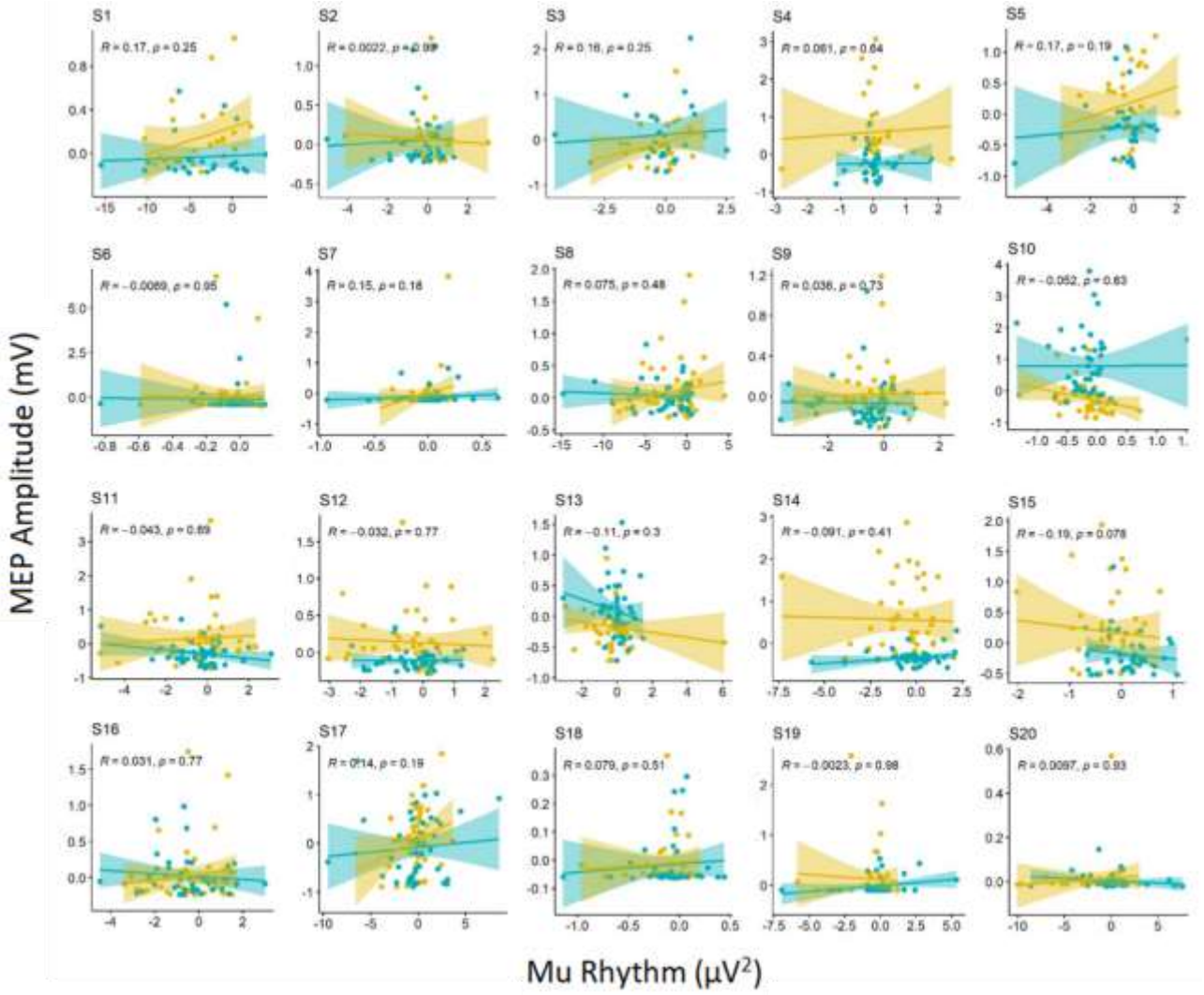

Figure S3. Correlation between MEP facilitation from FDI/ADM muscles and mu-ERD related to index finger/little finger movement observation, respectively, at a single-subject level. Each dot represents a single trial. MEP facilitation is expressed as the difference between congruent and incongruent movements. Data from the FDI and ADM muscles are depicted in blue and yellow, respectively.