Research Project: **From physiology to behavior: Online changes in corticomotor excitability during premotor to motor cortico-cortical paired associative stimulation predict action control capabilities**

Theoretical background

Cortico-cortical paired associative stimulation (ccPAS) represents an effective method of transcranial magnetic stimulation (TMS) for inducing associative plasticity among interconnected brain regions in humans (Borgomaneri et al., 2023). This technique operates on the Hebbian principle of spike-timing-dependent plasticity. The ccPAS protocol involves the repetitive application of pairs of TMS pulses across two interconnected brain sites, utilizing an optimal interstimulus interval between pulses. This interval ensures that each pair of pulses activates pre-synaptic neurons in the first site, leading to the spread of activation reaching the second site containing post-synaptic neurons, either immediately before or simultaneously with the administration of a pulse over that site. This pre- and post-synaptic coupling mirrors neural stimulation patterns crucial for achieving STDP, thereby augmenting or attenuating the strength of the neural pathway connecting the stimulated brain areas. Numerous studies have demonstrated that ccPAS induces alterations in both functional and effective connectivity within the targeted networks, alongside behavioral effects across various domains such as motor, visual, and executive functions (Borgomaneri et al., 2023; Chiappini et al., 2020; Romei et al., 2016). These findings suggest the potential utility of ccPAS for investigating and modulating behavior following plastic 're-wiring' of the human connectome. Importantly, prior studies have mainly addressed the physiological and behavioral effects of ccPAS separately, without delving into how plastic changes unfold during the protocol. Moreover, there hasn't been clarification on whether the physiological changes happening "online" during ccPAS administration could predict the behavioral impacts. Specifically, our study aims to explore whether the application of a ccPAS protocol targeting premotor to motor cortices can influence the excitability of the corticospinal system and whether these alterations might forecast a subsequent enhancement in action control facing emotional stimuli. Notably, the interplay between the pre-supplementary motor cortex (pre-SMA) and the primary motor cortex (M1) is believed to be pivotal for action control in an emotional context (Sagaspe et al., 2011). Nonetheless, essential evidence to support this notion is still lacking.

Aims and Hypotheses

The current project seeks to explore the physiological changes occurring "online" during ccPAS administration. Additionally, our objective is to determine whether these plastic physiological changes induced by ccPAS in premotor-motor regions can forecast improved performance in a subsequent task requiring motor control, where the interaction between premotor and motor areas is deemed critical.

Methods

Participants: sample size and justification of the sample size

A power analysis based on previously published studies (Romei et al., 2016) indicates that a sample size of 15 healthy participants is necessary to achieve a statistical power of > 95% (2-tailed = 0.05). Thus, 30 healthy volunteers will be tested in two experiments (see the Procedure section).

Tools

To boost functional connectivity we will use an innovative TMS protocol called ccPAS, able to induce spike-timing-dependent-plasticity (Romei et al., 2016). Motor-evoked potentials will be collected to offer a measure of the online changes in corticospinal excitability. To measure action inhibition we will use a widely used paradigm called Stop Signal Task that we have widely employed in our previous works (Battaglia et al., 2022a, 2022b). In this task, participants are requested to respond to a go stimulus (i.e., discriminating an arrow orientation). However, sometimes, the go stimulus is followed by a stop signal (represented by either an emotional or a neutral stimulus) that requires participants to withhold the ongoing action. To measure the participant's performance, the stop-signal reaction time (SSRT), an index of reactive inhibition, will be computed. Estimated SSRT values will give the measure of the duration of the inhibitory process, with a lower value indicating a more efficient action control.

Procedure

In Experiment 1, the connectivity between the supplementary and the motor cortex (SMA-M1) will be boosted while in Experiment 2 we will boost the connectivity between the left and right motor cortices (M1-M1). During the ccPAS administration, M1 stimulation will be performed using suprathreshold TMS intensity. Thus, on each paired stimulation we induced a motor-evoked potential (MEP) in the relaxed contralateral first dorsal interosseous (FDI), allowing us to track the emergence of changes in corticomotor excitability during protocol administration. Before and after the ccPAS participants will be asked to perform the Stop Signal Task.

Statistical analyses

Peak-to-peak amplitudes of the 90 MEPs induced by M1 stimulation in the FDI muscle in the two ccPAS groups will be analysed. The Analysis of variance (ANOVA) will be used to investigate differences within and between groups. Post-hoc analyses will be conducted with Newman-Keuls test, and the significance threshold will be set at $p < 0.05$. Correlation analysis will be conducted between MEPs amplitudes and the SSRT.

Declaration of commitment to request ethical approval

All procedures have been already approved by the Bioethical Committee at UNIBO (2020), Prot. 126991 del 1/7/2020.

Expected results and Implications

If the functional connectivity between the SMA and M1 is crucial for inhibiting actions in response to emotional stimuli, then strengthening their connections should lead to better performance in emotional trials compared to neutral ones (i.e., reduced SSRT following SMA-M1 ccPAS) and this behavioral improvement could be related to MEPs modulation during the SMA-M1 ccPAS. This would enhance participants' ability to regulate action inhibition in emotional contexts. Conversely, we do not anticipate any performance changes after enhancing the connectivity between the left and the right M1. Although changes in MEPs are expected, such physiological effects would be unrelated to performance changes. The significant scientific contribution will lie in identifying the neural network underlying the interaction between actions and emotions. This understanding opens avenues for improving our ability to control actions, particularly when emotional stimuli typically hinder cognition, through our innovative TMS protocol designed to enhance the function of the action

control network. Furthermore, the lack of behavioral inhibition is a characteristic of various psychiatric disorders, and deciphering its neural dynamics could pave the way for modifying these maladaptive mechanisms.

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Plan of activities

Research environment: the proposed project will be carried out at the Center for studies and research in Cognitive Neuroscience in Cesena.

Project activities: literature review to acquire relevant theoretical knowledge and to define stimulation parameters and behavioral procedures, recruitment of participants, execution of a pilot study to assess experimental duration and participant's compliance, data collection and analysis, writing of a draft of the main findings to be submitted to a scientific journal and research dissemination at national/international congresses.

Training activities: readings, discussions with the supervisor, direct involvement in lab meetings, attendance of lectures and workshops, revision of manuscripts; activities aimed at acquiring: 1) theoretical knowledge about key models and thematic areas related to cognitive neuroscience of action control; 2) skill for designing and conducting scientific research projects, data analysis and use

of non-invasive brain stimulation procedures; 3) writing and oral communication skills for scientific dissemination.

Timing of activities: literature search designing and piloting (Oct 2024 – Dec 2024); Data collection and analysis (Jan 2025 – July 2025); Dissemination (June 2025 – Oct 2025).

Feasibility of the project: the project is highly feasible and involves low risks. The supervisor have acquired extensive expertise on the methods and have already conducted several studies using TMS. Procedures have been already approved by the ethical committee at UNIBO. All the tools and research materials have been already acquired. Based on previous studies we predict mid/large effect sizes; therefore, an adequate sample can be acquired in less than 7 months.

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