**Research project title:** **Exogenous manipulation of parietal-motor connectivity in aging**

Word count: 6945 characters

**Theoretical background**

Brain changes throughout the lifespan can result in either functional improvements or decline. In the aging process, alterations in the connectivity of the cortical motor system are associated with impairments in motor control, which in turn affect daily activities and independence (Draganski, Lutti, & Kherif, 2013). Therefore, the development of non-invasive tools to target the plasticity of cortical connections appears as a promising approach to enhance the efficiency of the motor system and improve the quality of life in older individuals.

Recent advancements in transcranial magnetic stimulation (TMS) have enabled the induction of plastic changes in brain connectivity through a technique called cortico-cortical paired-associative stimulation (ccPAS) (Koch et al., 2013; Veniero et al., 2013). By coupling the stimulation of two interconnected brain areas, ccPAS can induce Hebbian-like associative plasticity between these areas. Previous studies have demonstrated that ccPAS over the posterior parietal cortex (PPC) and the primary motor cortex (M1) strengthens the influence of PPC over M1 (Koch et al., 2013; Veniero et al., 2013). However, most of the ccPAS research has focused on young adults. Testing ccPAS over the aging motor system is potentially interesting because older individuals may exhibit reduced connectivity strength and plasticity (Turrini et al., 2023). Nevertheless, neurostimulation of motor regions in aging may lead to larger behavioral improvements in older adults due to their greater potential for enhancing less efficient processes such as motor functions (Zimerman et al., 2013), provided that the stimulated network is intact (Turrini et al., 2023).

**Aims and Hypotheses**

The aim of this project is to investigate the neurophysiological and behavioral markers associated with changes in the strength of PPC-to-M1 connectivity induced by ccPAS over PPC and M1 in young and older adults. Our hypotheses are as follows:

1) At baseline, older adults will exhibit reduced PPC-to-M1 connectivity, correlating with impaired motor performance;

2) The ccPAS protocol will improve performance in both age groups, with larger effects observed in older adults with greater PPC-to-M1 connectivity strength at baseline.

**Methods**

***Participants, sample size and justification of the sample size:*** To achieve a statistical power of 90% (two-tailed α=0.05), a sample size of 32 participants is necessary (Koch et al., 2013). Therefore, we will test 16 young healthy volunteers (~20-30 years old) and 16 older healthy volunteers (~65-80 years old), all free from contraindications to TMS.

***Tools:*** TMS will be performed using a Magstim Bistim2 stimulator equipped with 2 focal coils. A neuronavigation system will assist in identifying the left PPC and M1 on the participant’s scalp.

To assess the strength of PPC-M1 connectivity, we will record motor-evoked potentials (MEPs) induced by a test TMS pulse over M1 preceded by a conditioning pulse over PPC (or alone as a control) following established procedures (Koch et al., 2007).

To assess motor performance, we will employ the 9-hole peg test (9-HPT) which measures skillful visually-guided object grasping and manipulation and a visuomotor tracking task (VMT) requiring tracking a target pathway formed by 2 lines on a computer screen while monitoring finger position with an accelerometer. These tests have been shown to relate to PPC functioning (Breveglieri et al., 2023). The completion time of two tasks will be recorded.

As control tasks, a 4-choice reaction time (cRT, requiring participants to release a key in response to the appearance of a number from 1 to 4 on a screen) will be used.

***Procedure:*** The procedure will consist of 2 counterbalanced sessions. In one session, a ccPAS protocol will be administered to enhance PPC-to-M1 connectivity (experimental session). This will involve administering 90 paired TMS pulses at a 0.1-Hz frequency. Each pair will consist of a TMS pulse over the PPC followed by a pulse over M1, with an inter-stimulus interval (ISI) of 4 ms. This ISI is optimal for activating PPC-to-M1 projections (Koch et al., 2007) and would therefore induce sequential pre-synaptic activity in the PPC and post-synaptic activity in M1, which is essential for Hebbian plasticity to occur (Caporale & Dan, 2008). The other session will involve an ineffective M1-to-PPC ccPAS protocol (control session). In this session, the order of each pair will be reversed to prevent the enhancement of PPC-to-M1 connectivity. For both sessions, we will assess neurophysiological measures of PPC-to-M1 connectivity strength (MEPs) and behavioral measures (9-HPT, VMT, cRT) at baseline, immediately after ccPAS (T0), and at 30, 60, and 90 minutes post-ccPAS (T30-T90). Before starting the two sessions, participants will familiarize themselves with the three tasks, and neurophysiological preparation, including individual calibration of stimulation parameters and localization of PPC and M1 sites, will be conducted.

***Statistical analyses:*** We will conduct mixed factors ANOVAs to analyze MEP-based indices of PPC-M1 connectivity strength with Age-group (young, older participants), Session (experimental, control), and Time (baseline, T0, T30, T60, T90) as factors. Similar ANOVAs will be used to analyze response times and accuracy in three behavioral tasks (9HTP, VMT, cRT). We will perform correlational analyses between neurophysiological and behavioral measures at baseline and following ccPAS using Pearson’s coefficients.

***Declaration of commitment to request ethical approval:*** We have already obtained ethical approval from the Bioethical Committee (May 2018) for ccPAS procedures in aging. However, we will submit an updated request tailored to this project involving PPC-M1 stimulation.

**Expected results and implications**

We expect that older participants will show reduced performance and strength of connectivity compared to younger participants at baseline, with poorer performance associated with reduced PPC-to-M1 connectivity strength. We expect that ccPAS will strengthen PPC-to-M1 connectivity in both groups. Moreover, if older participants benefit more from stimulation of motor areas, as shown in previous studies (Zimerman et al., 2013), we expect the behavioral gain induced by ccPAS will be relatively greater in the aging population, correlating with preserved connectivity strength. We expect no changes following ineffective ccPAS.

This study will highlight the malleability and functional relevance of PPC-to-M1 connectivity in both young and older humans. It will provide insights into whether and to what extent age-related differences exist in the physiological and behavioral sensitivity to the exogenous manipulation of PPC-to-M1 connectivity. The findings may have implications for the clinical application of neurostimulation in pathological conditions that typically occur later in life, such as stroke and dementia.

-Riferimenti bibliografici e piano delle attività formative

Word count: 3480 characters

**References**

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Koch, G., Fernandez Del Olmo, M., Cheeran, B., Ruge, D., Schippling, S., Caltagirone, C., & Rothwell, J.C. (2007), Focal stimulation of the posterior parietal cortex increases the excitability of the ipsilateral motor cortex. *Journal of Neuroscience, 27(25), 6815–6822.* <https://doi.org/10.1523/JNEUROSCI.0598-07.2007>

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Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L.G., & Hummel, F.C. (2013). Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. *Annals of Neurology, 73(1), 10–15*. <https://doi.org/10.1002/ana.23761>

**Plan of activities**

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

***Training activities:*** readings, discussions with the supervisor, direct involvement in lab meetings, attendance of lectures and workshops, manuscript revision; activities aimed at acquiring: 1) theoretical knowledge related to cognitive neuroscience of aging, neuroplasticity, behavioral and neurophysiological evaluation of motor functions; 2) skill for designing and conducting scientific research projects, data analysis and use of advanced TMS protocols; 3) writing and oral communication skills for scientific dissemination; 4) skills for translation of scientific knowledge into the development of novel rehabilitation programs.

***Timing of activities:***

Literature search, Design & piloting: from 11/23 to 01/24

Data collection & analysis: from 01/24 to 08/24

Dissemination: from 05/24 to 10/24

PI supervision: from 11/23 to 10/24

***Feasibility of the project:*** the project is highly feasible and involves low risks. The supervisor and his research group have acquired extensive expertise on the methods and have already conducted several studies using ccPAS. The main procedures have been already approved by the Bioethical committee. All the tools and research materials have been already acquired. Based on previous studies we predict mid/large effect size, therefore an adequate sample can be acquired in less than 7-8 months. We have already developed contacts with local associations (e.g., Università della Terza Età) to recruit older participants.