RESPONSES TO REVIEWERS – ROUND 2

Dear Dr. Guidali,

Thank you for your thorough reply to the reviewers and recommender. I have received positive responses from all three of our reviewers and ask you reply to the few remaining comments. Please reply with a point-by-point response to the reviewers and recommender, accompanied by a track-changes updated manuscript. Our anonymous reviewer requests you revisit Dr Oberman's suggestion of extending your inter-pulse interval and asks you explicitly state that the MEPs will be recorded with the EEG cap on using the same conditions as was used for the TEPs.

With regard to your response to my comments, I have a few minor issues I'd like to be addressed:

Q1: *I appreciate that power analyses have been performed on concrete hypotheses, and that the more exploratory analyses have been removed from your Stage 1. However, there are multiple mentions of connectivity in the abstract and introduction which may be confusing to the reader as there are no planned connectivity analyses in the Stage 1. These should also be removed, and the connectivity only discussed in the Stage 2 if exploratory analyses are performed. The only reason we allow the mention of exploratory analyses in a Stage 1 is if there is a particular design component which is only selected to support an exploratory analysis, therefore requiring an explanation for readability. Along these lines, the analysis examining the modulation of the N100 following PASLTP should also only be mentioned in the Stage 2.*

Resp: Thank you for this clarification. We have now carefully read the manuscript and removed mentions of connectivity and possible exploratory analysis at the time of Stage 2 submission.

Q2: *Could you also outline the conclusions you could draw if your hypotheses are not upheld in the final column of the Study Table? You have most of this information in the "Interpretation given different outcomes" column. For example, for H0, what does it mean to the field if you are unable to replicate typical impact of PASLTP and PASLTD protocols on MEP? Investigation of TEPs in this case is highly interesting as modulation impact may be more obvious in this measure.*

Resp: We have now updated the **Study Table** with the conclusions we will draw if our hypothesis is not confirmed. In detail, we state that:

H0: 'If **H0** is not confirmed, it will suggest that our PAS_{LTP} and/or PAS_{LTD} protocols do not induce plastic changes detectable at a corticospinal level. This evidence would argue the effectiveness of PAS protocols, at least at the population level and on MEPs. Nevertheless, such finding will not *a priori* exclude the absence of effects on TEPs – and thus the ineffectiveness of our protocol, given the evidence that MEPs and TEPs could frame different facets of motor system excitability (Biabani, Fornito, Coxon, Fulcher, & Rogasch, 2021;

Guidali et al., 2023). Hence, we will still explore TEPs (i.e., **H1-H4** hypothesis) and set up the discussion of our results accordingly.'

H1: 'Firstly, if **H1** is not confirmed, previous evidence found on PAS_{LTP}-induced modulations (i.e., Costanzo et al., 2023) will not be confirmed and replicated. Secondly, this would suggest that P30 and/or P60 might not be reliable measures for detecting PAS-induced LTP/LTD.'

H2: 'If **H2** is not confirmed, the role of the N100 as a marker of PAS_{LTD} effects within the motor system will be critically discussed and framed within available literature on this TEP component and related confounding factors (e.g., somatosensory/auditory artifacts).'

H3: 'If **H3** is not confirmed, we can assume that M1-PAS plastic effects have a longer duration, extending beyond twice the time of the protocol administration. This information could then be useful to better characterize the temporal profile of LTP-/LTD-induced plasticity by PAS protocols and inform future studies that require the exploitation of such plastic effects for wider time windows.'

H4: 'If **H4** is not confirmed, our results will not corroborate previous studies indicating that M1-TEP components after 50-60 ms (i.e., P60) are influenced by reafferent processing (e.g., Gordon, Desideri, Belardinelli, Zrenner, & Ziemann, 2018; Petrichella, Johnson, & He, 2017). This evidence could then be useful to inform study designs in which M1-TEPs are planned to be exploited as plasticity markers within the motor system, informing on the spurious modulation of supra-threshold stimulation – and the reafferent processing – on the recorded signal.'

Furthermore, we have carefully read the whole **Study Table** to avoid repetition in the information reported in the different columns. The same was done for the '**Sample size estimation**' section (pp. 8-10), in which information already present in the **Introduction** (and in the **Study Table**) about the expected outcomes was repeated, mining the overall readability of the paragraph and making this section unnecessarily wordy.

Q3: *Finally, please also define TEP (i.e. TMS evoked potentials) in the abstract prior to the first use of the acronym.*

Resp: Done.

Reviewed by anonymous reviewer 1, 08 Dec 2023 16:31

The authors have addressed my comments. I only have a couple of suggestions based on the comments of Dr. Veniero and Dr. Oberman.

Q1: *Comment 3 by Dr. Veniero. The authors should also mention that the MEPs will be recorded with the EEG cap on and with the same conditions as the TEPs.*

Resp: We thank the Reviewer for the suggestion. We have now rephrased p. 12 as follows: 'During TMS-EMG blocks, TMS will be delivered with the EEG cap on and with the same conditions (i.e., noise masking applied) and parameters of TMS-EEG recordings – see **TMS** and **EMG recording** for further details.' On p. 14, we have highlighted that 'considering the aim of TMS-EMG blocks (i.e., **H0)**, MEPs will be recorded only at 110% rMT' and, on pp. 15-16, we added that: 'During TMS-EMG, participants will also have noise masking to keep all recording conditions constant between EMG and EEG blocks.'

Q2: *Comment 2 by Dr. Oberman. The authors should consider Dr. Oberman's suggestion. The ISI the authors plan to use is short. See, for instance, https://doi.org/10.1016/j.brs.2023.02.009, section 3.2. TMS threshold determination.*

Resp: Following the suggestion of the Reviewer, we have decided to adopt a longer inter-pulse interval (jittered between 3000 and 4000 sec instead of 2000 and 2300 sec) and record a smaller number of trials for each TMS-EEG condition (120 instead of 150). We believe this is a reasonable trade-off, that will allow us to avoid potential additive effects and, at the same time, to complete **T1** assessment within 20 min from the end of PAS administration (i.e., the ideal temporal window to detect PAS aftereffects with the protocols adopted in our study). Notably, 120 trials for each condition are sufficient to obtain reliable TEPs (Hernandez-Pavon et al., 2023; Kerwin, Keller, Wu, Narayan, & Etkin, 2018). Hence, we have rephrased the **Experimental Procedure** paragraph (p. 12): 'TMS-EEG blocks will consist of 120 trials each. (…) In all the recording blocks acquired before and after PAS, the inter-pulse interval will be randomly jittered between 3000 and 4000 ms'.

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