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Sensorimotor integration within the primary motor cortex by selective nerve fascicle stimulation

Federico Ranieri, Giovanni Pellegrino, Anna Lisa Ciancio, Gabriella Musumeci, Emiliano Noce, Angelo Insola, Lorenzo Alirio Diaz Balzani, Vincenzo Di Lazzaro, and Giovanni Di Pino
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Calogero Maria Oddo (Referee #1); Zhen NI (Referee #2)

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Dear Dr Ranieri,

Re: JP-RP-2021-282259 "Sensorimotor integration within the primary motor cortex by selective nerve fascicle stimulation" by Federico Ranieri, Giovanni Pellegrino, Anna Lisa Ciancio, Gabriella Musumeci, Emiliano Noce, Angelo Insola, Lorenzo Alirio Diaz Balzani, Vincenzo Di Lazzaro, and Giovanni Di Pino

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Yours sincerely,

Richard Carson
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

I have now received the assessments from two independent reviewers, both of whom see merit in your work. I invite you to respond to their concerns, and also to point out that this study was conduced on a single participant.

In the Methods section, please provide the ethics committee approval reference number.

REFEREE COMMENTS

Referee #1:

The authors present a carefully designed study with the aim to investigate sensorimotor integration in the human cortex. Somatosensory electrical stimuli are delivered via intraneural stimulation of the peripheral nerves in a human amputee that underwent surgical implantation of electrodes in the median and ulnar nerves.

The paper is clear and well written, and deserves attention.

The main requested revision is associated to data availability, recommending to make data (both raw and elaborated data) available in a public open access repository, rather than just the figures and tables reported in the manuscript.

Furthermore, the following minor revisions are suggested:

- Explain why different stimulators (DS7 and STG4008) are used for transcutaneous and invasive stimulation. This is presumably due to different amplitudes required in transcutaneous and intraneural stimulation, but the authors are suggested to explicitly state the reasons underlying the choices related to the experimental apparatus, so that reproducibility is enhanced.

- Please use "k" instead of "K", since in the international system capital "K" stands for Kelvin whereas kilo is lower-case "k" (e.g., "Signal was sampled at 5 kHz" instead of "Signal was sampled at 5 KHz").

- I would suggest using a different acronym for short-latency afferent inhibition instead of SAI, that is commonly used in touch physiology to indicate Slowly Adapting type I (SAI), the tactile afferents terminated by Merkel corpuscles.

- In the caption of Figure 1, please use "150x50 µm2" rather than "150x50 µm".

- Please check the scale of the stimulation range in Figure2B, since the scale of the x-axis should be in nC rather than in µC.

Referee #2:

This is a case study. In an upper limb amputated patient with implanted intraneural multi-channel electrodes, the authors measured the somatosensory evoked potential and short-latency afferent inhibition. It was found that the measurements with sensory stimulation delivered from the intraneural electrodes and those from a traditional external transcutaneous stimulation are comparable. The novel experiment identified the cortical source of the electrophysiological measurements which are very often used in neurological tests. The study was well designed and the manuscript was well written. I only have a few minor comments.

1, A relatively major point is that it should be mentioned and acknowledged that this is a case study. More importantly, it should be discussed how the results (which parts) from a case study may be and may not be applied to the general population.

2, The locations of amputation and electrodes should be described with more details. Both could also be marked on Figure 1.

3, Details of statistical analysis should be mentioned in the method part. Another problem is that it is not clear if a correction for the multiple comparisons (for example, short-latency afferent inhibition with different interstimulus intervals) was considered.

4, The resting motor thresholds in different muscles were measured. It is worth reporting the result and discussing it (comparisons for different muscles on the same side and those for homonymous muscles on intact and amputated sides) although it may be found in previous studies.

5, Similarly, it was not clear how the short-latency afferent inhibition was measured in different muscles (Figure 6). Were they measured in a single experimental session or separately? This point should be discussed as the best location (hotspot) for these muscles with magnetic stimulation could be different on the scalp.

END OF COMMENTS

Confidential Review **13-Aug-2021**

REVIEWING EDITOR:

I have now received the assessments from two independent reviewers, both of whom see merit in your work. I invite you to respond to their concerns, and also to point out that this study was conduced on a single participant.

We are delighted to read that both reviewers appreciate our study and manuscript.
All revisions are highlighted in red colour.

In the Abstract and Introduction of our revised manuscript, we explicitly state that the study was performed on a single participant. Moreover, in the final part of the Discussion, we consider that this is a case study, and that caution should be used towards generalization of results (see also reply to Referee #2, point 1).

In the Methods section, please provide the ethics committee approval reference number.

We added the Ethics Committee approval reference code. We moved the information on ethical approval and informed consent to a paragraph headed "Ethical approval" at the beginning of the Methods section, as per journal policy.

REFEREE #1:

The authors present a carefully designed study with the aim to investigate sensorimotor integration in the human cortex. Somatosensory electrical stimuli are delivered via intraneural stimulation of the peripheral nerves in a human amputee that underwent surgical implantation of electrodes in the median and ulnar nerves.

The paper is clear and well written, and deserves attention.

We thank the reviewer for appreciating our work.

The main requested revision is associated to data availability, recommending to make data (both raw and elaborated data) available in a public open access repository, rather than just the figures and tables reported in the manuscript.

We made datasets (scalp SEP recordings, individual CMAP amplitudes, individual MEP amplitudes and calculated SAI values) publicly available in the FigShare open access repository that can be accessed through the link: https://doi.org/10.6084/m9.figshare.17088857
This information is reported in the Data Availability Statement of our manuscript.

Please note that MRI and original EEG traces cannot be publicly shared as this may represent a risk of sensitive data exposure, because the single participant in the study could be easily identified considering that information on these unique experiments has been divulgated by media in Italy. Moreover, original EEG traces used to analyse SEP activity (about 1h of EEG signal co-recorded from 32 channels) contain much more information (e.g., high frequency signals) than that used for present analysis, and they may be used by the authors for further investigations. However, MRI and EEG datasets are uploaded on the FigShare repository (.nii and Brain Products file format, respectively, compatible with several tools), and they can be made accessible by the authors to other researchers upon reasonable request and agreement on data protection rules. A private link to these data for review purposes is uploaded as .txt file together with the revised manuscript.

Furthermore, the following minor revisions are suggested:

- Explain why different stimulators (DS7 and STG4008) are used for transcutaneous and invasive stimulation. This is presumably due to different amplitudes required in transcutaneous and intraneural stimulation, but the authors are suggested to explicitly state the reasons underlying the choices related to the experimental apparatus, so that reproducibility is enhanced.

For invasive stimulation, we used a specific stimulator (STG4008) because it allows multichannel stimulation (up to 8 channels) with low current intensity (output range: -16 mA to +16 mA) and with a high output resolution (intensity: 2000 nA; time: 20 us), controllable by software in shape, intensity, and duration. This was functional to the entire set of experiments that we performed in our subject for physiological investigations and prosthesis control. On the other side, the DS7 stimulator that we used for transcutaneous

stimulation can deliver higher current intensities and has a lower output resolution (analogue control with continuously variable current between 0-100 mA x 50-2000 us).

The reasons for using a specific device for invasive stimulation have been added in the Methods / "Transcutaneous and invasive nerve stimulation" section (Page 7):
"For invasive stimulation… A dedicated stimulator was chosen to enable multichannel stimulation with low current intensity (output range: -16 mA to +16 mA) and with a high output resolution (intensity: 2000 nA; time: 20 us), freely programmable in shape, intensity, and duration."

- Please use "k" instead of "K", since in the international system capital "K" stands for Kelvin whereas kilo is lower-case "k" (e.g., "Signal was sampled at 5 kHz" instead of "Signal was sampled at 5 KHz").

Sorry for the inaccuracy: corrected.

- I would suggest using a different acronym for short-latency afferent inhibition instead of SAI, that is commonly used in touch physiology to indicate Slowly Adapting type I (SAI), the tactile afferents terminated by Merkel corpuscles.

We thank the Reviewer for alerting us on this issue, and we understand reviewer's point. However, respectfully, we think it is better to maintain the acronym SAI because this is the acronym used for Short-latency Afferent Inhibition in a large amount of existing TMS literature, with which we prefer to remain consistent. However, for sake of clarity, we added the following sentence at the beginning of the section Methods / "Test of short-latency afferent inhibition (SAI)" (Page 9):
"We refer to short-latency afferent inhibition with the acronym SAI consistently with TMS literature, and we alert the reader that in the present study SAI does not refer to slowly adapting type I afferent fibers."

- In the caption of Figure 1, please use "150x50 µm2" rather than "150x50 µm".

Corrected.

- Please check the scale of the stimulation range in Figure2B, since the scale of the x-axis should be in nC rather than in µC.

Thank you for bringing this to our attention. The correct charge unit is indeed nC since charge was calculated as the product of intensity (µA) × duration (ms). Units have been corrected both in the x-axis and in the figure legend.

REFEREE #2:

This is a case study. In an upper limb amputated patient with implanted intraneural multi-channel electrodes, the authors measured the somatosensory evoked potential and short-latency afferent inhibition. It was found that the measurements with sensory stimulation delivered from the intraneural electrodes and those from a traditional external transcutaneous stimulation are comparable. The novel experiment identified the cortical source of the electrophysiological measurements which are very often used in neurological tests. The study was well designed and the manuscript was well written. I only have a few minor comments.

We thank the Reviewer for appreciating our work.

1, A relatively major point is that it should be mentioned and acknowledged that this is a case study. More importantly, it should be discussed how the results (which parts) from a case study may be and may not be applied to the general population.

The Reviewer is absolutely right.
In the Abstract and Introduction of the revised version of our manuscript, we explicitly state that the study was performed on a single participant.
Moreover, we added the following sentences in the final part of the Discussion to acknowledge this point and to present the limits of result generalization (Page 18):
"Since present results come from a case study, we want to point out that the extension beyond our subject of some findings of the work should be done with caution. Indeed, the main finding is that selective sensory fascicle stimulation is able to evoke a detectable activity in S1 and to produce a SAI effect in M1, and this could be employed as a physiological basis for further developments of intraneural stimulation and prosthesis control strategies. However, the more specific results related to the different amount of SAI due to the location of implanted active sites and to the timing of afferent stimulation, as well as SEP morphology, might be individual characteristics and might not be confirmed in the general population."

2, The locations of amputation and electrodes should be described with more details. Both could also be marked on Figure 1.

We added the stump length to the description of the characteristics of amputation reported in the "Participants" paragraph within the Methods section (Page 6):
"(forearm stump length: ~10 cm)"

We added details on the implantation procedure and location of intraneural and perineural electrodes in the "Neural electrodes" paragraph within the Methods section (Page 6):
"Six invasive neural electrodes were implanted through a microsurgery intervention accessing the medial aspect of the middle third of the left arm following the medial edge of the biceps muscle and exposing the ulnar and the medial nerves for about 5 cm along their course (Di Pino et al., 2014). Three electrodes were implanted in each nerve. More in detail, from distal to proximal, two intraneural ds-FILEs and one perineural Cuff electrode were inserted equally spaced at ~10 to ~15 cm above the elbow."

We also labelled the stump and added a scale bar in the representation of the upper limb in Figure 1.

3, Details of statistical analysis should be mentioned in the method part. Another problem is that it is not clear if a correction for the multiple comparisons (for example, short-latency afferent inhibition with different interstimulus intervals) was considered.

We expanded information on statistical analysis and moved it to a separate paragraph headed “Statistical analysis” at the end of the Methods section.
We also updated p values to 3 significant figures and added n values to the Results, as per journal policy.

For a more accurate analysis of selectivity of sensory intraneural stimulation, in addition to reporting analytical values at individual sites in Table 1, we added the result of the comparison between sensory thresholds by intraneural and perineural stimulation, that we also represented in the new Figure 2. Moreover, to provide a more accurate representation of collected data, we included the distributions (boxplots) of test MEP amplitudes in Figure 5 (former Figure 4) and moved the boxplot of IM12_ISI8ms form Figure 6 (former Figure 5) to Figure 5 (former Figure 4).

As for the multiple comparison issue, we originally opted for not applying corrections, and accordingly we presented the results, due to the specific reasons detailed below. This is now clearly stated in the Methods. Nonetheless, we highlight that the majority and most relevant findings of our study (i.e., a significant inhibition by sensory fiber stimulation and a higher SAI by selective sensory fiber stimulation) are associated with very low p values and would survive the most conservative correction approach (Bonferroni).
We agree that caution must be exercised when dealing with multiple comparisons as they increase the likelihood of falsely rejecting the null hypothesis (i.e., type I error), especially when few significant values are detected among several comparisons. On the other hand, conservative correction methods increase the likelihood of failing to detect real differences (i.e., type II error), especially when most comparisons bear significant results. In our study, no corrections for multiple comparison were applied based on the following specific considerations.

1) This is a single case study, and therefore it has an exploratory nature, with a main intrinsic limitation related to the generalizability of some of the results, as the Reviewer also observed at Point 1.
2) Multiple pairwise comparisons of SAI obtained with different afferent stimulation sites (Figures 6A and 7) and interstimulus intervals (Figure 6B) have been performed only after a significant group effect had been detected by non-parametric analysis of variance (Kruskal-Wallis test), i.e., only after a source of variability between groups had been demonstrated.
3) A specific consideration applies to data reported in Figure 5 (SAI at different ISIs for each stimulation site): we now underline that this figure and analysis has a prominent descriptive value and is intended to guide the subsequent analyses; significance levels reported here serve the scope to evaluate the presence of any SAI effect in each stimulation condition (i.e., conditioned vs unconditioned MEP amplitudes). As for all SAI protocols applied to single subjects, we never expected to obtain inhibition surviving Bonferroni corrections, as these measurements are limited by the usually high variability of MEP measurements unless data are pooled together.

We also underline that the entire dataset is made available with our revised manuscript, should the reader opt for another correction approach.

4, The resting motor thresholds in different muscles were measured. It is worth reporting the result and discussing it (comparisons for different muscles on the same side and those for homonymous muscles on intact and amputated sides) although it may be found in previous studies.

Following the Reviewer's suggestion, we added a table (Table 2) summarizing RMT values, TMS intensity and test MEP amplitude for each tested muscle of left and right side in the transcutaneous SAI protocol. We also report in the Discussion (Page 16) that *"…we observed a reduced inhibition of M1 output to stump muscles compared with the contralateral M1… However, in our subject we did not observe an overt imbalance of motor thresholds supporting increased corticospinal excitability in the amputated side; therefore, an alternative explanation is that…"*.
We limited the discussion of cortical excitability findings based on motor thresholds to the extent that this information is functional to the interpretation of SAI results. Since motor threshold is a parameter influenced by the excitability of cortical, subcortical, and peripheral structures and by a high variability, we are not able to make comparisons or further hypotheses based on RMT data from a single measurement.

5, Similarly, it was not clear how the short-latency afferent inhibition was measured in different muscles (Figure 6). Were they measured in a single experimental session or separately? This point should be discussed as the best location (hotspot) for these muscles with magnetic stimulation could be different on the scalp.

We report in the Methods / "Test of short-latency afferent inhibition" (Page 10) that
"In the case of transcutaneous mixed (sensory and motor) nerve stimulation… TMS was targeted to the MFM hotspot on the scalp. MEPs were recorded simultaneously from relaxed BB, MFM and OP…".

We agree that the TMS hotspot is expected to be different for proximal and distal upper limb muscles. However, we chose to co-record MEPs for two reasons: 1) limiting experimental time to ensure subject's compliance; 2) allowing comparison of SAI in different muscles of the same side without introducing further variability resulting from a test-retest procedure. We now describe in the Methods (Page 10) the reasons of our approach and to which extent it could limit the reliability of results:
"MEPs were recorded simultaneously from relaxed BB, MFM and OP, to limit experimental time and test-retest variability. This approach is limited by the suboptimal activation of cortical areas out of the hotspot or by cortico-cortical influences, although mitigated by the overlapping of cortical representation maps of adjacent muscles (Krings et al. 1998 ; Raffin et al. 2015 ; DeJong et al. 2021) and by the presence of a within-test control intrinsic of the SAI measurement (i.e., the ratio of conditioned and unconditioned MEPs)."

In other words, the fact that SAI is expressed as a ratio of conditioned/unconditioned MEPs implicates that, even in the case that the stimulus intensity reaching the hotspot of a muscle different than MFM was slightly below the one needed for 1mV MEPs, it was the same for conditioned and unconditioned MEPs thus not impacting importantly on their ratio.

Dear Dr Ranieri,

Re: JP-RP-2021-282259R1 "Sensorimotor integration within the primary motor cortex by selective nerve fascicle stimulation" by Federico Ranieri, Giovanni Pellegrino, Anna Lisa Ciancio, Gabriella Musumeci, Emiliano Noce, Angelo Insola, Lorenzo Alirio Diaz Balzani, Vincenzo Di Lazzaro, and Giovanni Di Pino

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology, subject to any modifications to the text and/or satisfactory clarification of the Methods section that may be required by the Journal Office to conform to House rules.

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Yours sincerely,

Richard Carson
Senior Editor
The Journal of Physiology

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EDITOR COMMENTS

Reviewing Editor:

Dear Dr Ranieri,

Thank you for addressing the reviewers' concerns. I am satisfied you have adequately attended to the minor amendments requested.

REFEREE COMMENTS

Referee #1:

All the points raised during the review process have been carefully addressed by the authors

Referee #2:

No more comments. My previous questions have been well addressed.

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1st Confidential Review **28-Nov-2021**