The Journal of Physiology

Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults

Lucas B R Orssatto, Gabriel Fernandes, Anthony Blazevich, and Gabriel S. Trajano
DOI: 10.1113/JP283708

Corresponding author(s): Lucas Orssatto (l.betdarosaorssatto@qut.edu.au)

The referees have opted to remain anonymous.

Review Timeline:

Submission Date:	11-Aug-2022
Editorial Decision:	05-Sep-2022
Revision Received:	23-Sep-2022
Accepted:	07-Oct-2022

Senior Editor: Richard Carson

Reviewing Editor: Mathew Piasecki

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Dr Orssatto,

Re: JP-RP-2022-283708 "Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults" by Lucas B R Orssatto, Gabriel Fernandes, Anthony Blazevich, and Gabriel S. Trajano

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

The reports are copied at the end of this email. Please address all of the points and incorporate all requested revisions, or explain in your Response to Referees why a change has not been made.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

Authors are asked to use The Journal's premium BioRender (https://biorender.com/) account to create/redraw their Abstract Figures. Information on how to access The Journal's premium BioRender account is here: https://physoc.onlinelibrary.wiley.com/journal/14697793/biorender-access and authors are expected to use this service. This will enable Authors to download high-resolution versions of their figures. The link provided should only be used for the purposes of this submission. Authors will be charged for figures created on this premium BioRender account if they are not related to this manuscript submission.

I hope you will find the comments helpful and have no difficulty returning your revisions within 4 weeks.

Your revised manuscript should be submitted online using the links in Author Tasks Link Not Available.

Any image files uploaded with the previous version are retained on the system. Please ensure you replace or remove all files that have been revised.

REVISION CHECKLIST:

- Article file, including any tables and figure legends, must be in an editable format (eg Word)

- Abstract figure file (see above)

- Statistical Summary Document

- Upload each figure as a separate high quality file

- Upload a full Response to Referees, including a response to any Senior and Reviewing Editor Comments;

- Upload a copy of the manuscript with the changes highlighted.

You may also upload:

- A potential 'Cover Art' file for consideration as the Issue's cover image;

- Appropriate Supporting Information (Video, audio or data set https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#supp).

To create your 'Response to Referees' copy all the reports, including any comments from the Senior and Reviewing Editors, into a Word, or similar, file and respond to each point in colour or CAPITALS and upload this when you submit your revision.

I look forward to receiving your revised submission.

If you have any queries please reply to this email and staff will be happy to assist.

Yours sincerely,

Richard Carson
Senior Editor
The Journal of Physiology

REQUIRED ITEMS:

-Author photo and profile. First (or joint first) authors are asked to provide a short biography (no more than 100 words for one author or 150 words in total for joint first authors) and a portrait photograph. These should be uploaded and clearly labelled with the revised version of the manuscript. See Information for Authors for further details.

-You must start the Methods section with a paragraph headed Ethical Approval. If experiments were conducted on humans confirmation that informed consent was obtained, preferably in writing, that the studies conformed to the standards set by the latest revision of the Declaration of Helsinki, and that the procedures were approved by a properly constituted ethics committee, which should be named, must be included in the article file. If the research study was registered (clause 35 of the Declaration of Helsinki) the registration database should be indicated, otherwise the lack of registration should be noted as an exception (e.g. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.). For further information see: https://physoc.onlinelibrary.wiley.com/hub/human-experiments

-Your manuscript must include a complete Additional Information section

-Please upload separate high-quality figure files via the submission form.

-Your paper contains Supporting Information of a type that we no longer publish. Any information essential to an understanding of the paper must be included as part of the main manuscript and figures. The only Supporting Information that we publish are video and audio, 3D structures, program codes and large data files. Your revised paper will be returned to you if it does not adhere to our Supporting Information Guidelines

-A Statistical Summary Document, summarising the statistics presented in the manuscript, is required upon revision. It must be on the Journal's template, which can be downloaded from the link in the Statistical Summary Document section here: https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics

-A Data Availability Statement is required for all papers reporting original data. This must be in the Additional Information section of the manuscript itself. It must have the paragraph heading "Data Availability Statement". All data supporting the results in the paper must be either: in the paper itself; uploaded as Supporting Information for Online Publication; or archived in an appropriate public repository. The statement needs to describe the availability or the absence of shared data. Authors must include in their Statement: a link to the repository they have used, or a statement that it is available as Supporting Information; reference the data in the appropriate sections(s) of their manuscript; and cite the data they have shared in the References section. Whenever possible the scripts and other artefacts used to generate the analyses presented in the paper should also be publicly archived. If sharing data compromises ethical standards or legal requirements then authors are not expected to share it, but must note this in their Statement. For more information, see our Statistics Policy.

-Please include an Abstract Figure. The Abstract Figure is a piece of artwork designed to give readers an immediate understanding of the research and should summarise the main conclusions. If possible, the image should be easily 'readable' from left to right or top to bottom. It should show the physiological relevance of the manuscript so readers can assess the importance and content of its findings. Abstract Figures should not merely recapitulate other figures in the manuscript. Please try to keep the diagram as simple as possible and without superfluous information that may distract from the main conclusion(s). Abstract Figures must be provided by authors no later than the revised manuscript stage and should be uploaded as a separate file during online submission labelled as File Type 'Abstract Figure'. Please ensure that you include the figure legend in the main article file. All Abstract Figures should be created using BioRender. Authors should use The Journal's premium BioRender account to export high-resolution images. Details on how to use and access the premium

account are included as part of this email.

-Please include a full title page as part of your article (Word) file (containing title, authors, affiliations, corresponding author name and contact details, keywords, and running title).

EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your manuscript to JP. It has been assessed be 2 experts within the field and although there is clear collective enthusiasm for this work there are a number of comments to address before it can be considered for publication. I also have a number of suggestions I hope the authors can consider:

Please clarify total n and justification of reduced n. This is particularly important to the field if it is a result of low MU yield.

The old were non-sarcopenic, was this part of the screening process? If so, please add brief details on the definition used.

Please state the reference number for ethical approval.

"Additional data supporting this claim includes evidence that peak discharging rates are maintained with ageing in well-used muscles (e.g., hand muscles, quadriceps, and triceps surae) but decline markedly in lesser used muscles (e.g., hamstrings and tibialis anterior)" - it is unclear how the level of muscle use is justified. Put simply, is there any evidence to suggest quadriceps are typically activated more often than tibialis anterior? This seems at odds with the description of TA several sentences prior. Furthermore, is it the case that quadriceps discharge rate is largely maintained with age? Please clarify and cite.

I would encourage the authors to further explore the differential response of TA and soleus as this is a particularly interesting finding. Although far from a direct anatomical count, the aged soleus is one of the few (if not only) muscles to appear to preserve the number of motor units with age (see Dalton et al doi.org/10.1002/mus.20984), largely unseen in other muscles. I will let the authors decide on the relevance of this.

REFEREE COMMENTS

Referee #1:

Orssatto and colleagues investigated facilitation-inhibition control of the estimated PICs in young and older adults. The work builds on their previous findings that older adults exhibit smaller estimates of PIC strength. The results suggested that older adults are able to similarly amplify estimates of PICs with a remote muscle contractions to that of younger adults, however, their ability to decrease PIC strength via reciprocal inhibition seems to be attenuated.

The manuscript tackles an important topic and improves our understanding of the neural underpinnings of the ageing process. The study is very-well designed and methodologically sound. The authors are also commended on data transparency.

I have several comments that I hope the authors may use when revising the manuscript

.

Lines 18-22: I believe the last point of the 'Key points' section is too speculative and not directly related to the data presented. I suggest sticking to what your data shows directly in this section. Whilst some speculation might be acceptable, this should, in my opinion, be succinct and constrained to the Discussion.

Introduction

I believe some attention should be given to the mechanism by which a remote muscle contraction increases serotonin secretion. On page 5, line 15 a mere statement of 'belief' is provided without any reference, though I think this is provided at a later section.

P5, line 15: reciprocal inhibition is stated twice whereas I believe the first one should probably say 'vibration of the antagonist tendon/muscle'.

Methods

P6, line 3: Is the disparing number of participants to account for potentially smaller yield of MUs in older individuals? If so, I suggest you clarify that.

I realise that the sample population was the same/similar as in a prior study. However, I do believe the basic information such as sex and mean age of the participants should be listed here, thus not requiring the reader to dig out the other paper.

P7, line 2: It is unclear what the duration and intensity of the remote contraction is based on. Would a weaker/stronger and shorter/longer duration of a contraction be more/less effective?

P7, line 11: How often were repeats needed? Some data here would be reassuring.

Is 2 mins a sufficient rest period for the effects of prior contractions to dissipate? If so, what was this based on?

P7, line 18: Was this fully randomised or block randomised (e.g. participant 1 started without, but participant 2 with vibration, etc.)?

P7, line 19: Was the vibration device hand held? If so, how was the same pressure ensured?

P8: Was there a particular reason for a 20% contraction in Experiment 2, but a 30% contraction in Experiment 3? Note that I don't think this disparity changes the interpretation of the results, but it does seem somewhat odd to have different contraction levels. Given the same pool of participants, the same contraction level would have allowed a direct comparison of facilitatory and inhibitory responses.

P10, line 4: Whilst again, unlikely to change the interpretation, it would be useful for the reader to make a note that polynomial functions (and in particular 5th order) likely over smooth the data, resulting in some level of fit error (at least compared to the support vector regression approach; Beauchamp et al. 2022 J Neural Eng).

P10, line 5-6: 'a low recruitment threshold', I believe you mean 'lower than...' here. Unless you provide specific cut-off points (which would, in my opinion, be erroneous) units may merely be classified as that of a lower/higher recruitment threshold than another unit.

P10, line11-12: Given your citation of Hassan et al. 2020, I am surprised you seemingly did not specify the criterion for the derecruitment time difference. Any particular reason why only the recruitment time difference was considered?

Results

It would be good to report a strength difference between the groups in the interest of comparison with other studies.

P20, line 8: It might be my personal preference, but I find it rather odd to not have the basic information on the motor unit population before delving into the results of delta F. I would suggest this be moved somewhere towards the beginning of the Results section.

P20, line 10: presumably 'older' should be 'younger' here.

Discussion

The discussion is generally well written, but what the authors seemingly fail to consider is the potential for a ceiling effect when it comes inhibition control. As the authors will be aware, the delta F method estimates not only neuromodulatory input, but likely also the extent of ionotropic inhibition. Thus, lower delta F estimates that older adults exhibit might be a reflection of greater inhibition compared to younger at baseline. As such, an additional inhibitory input might result in less of a further decrease in delta F.

Referee #2:

This manuscript provides important new results about control of excitability of motor output and its alterations with aging. The authors estimated "PIC facilitation-inhibition" in the TA and SOL muscles during submaximal triangle-shaped contractions, using the standard method of assessment of persistent inward currents, ΔF. They show that regardless of age, ΔF increased in the SOL and TA after a handgrip contraction. In contrast, decreases in ΔF were found in the SOL in response to antagonist tendon vibration. However, in the TA, ΔF decreased less in older adults in response to antagonist tendon vibration. They suggest that this difference in older adults signifies an impaired ability to deactivate PICs in response to reciprocal inhibition with age. I suggest a few minor revisions, mainly to explain what other mechanisms may be responsible the observed difference.

1. Please define "facilitation-inhibition control". It is mentioned throughout the abstract and conclusion but would be helpful to clearly define this in the introduction for the reader.

2. The authors should consider re-phrasing the final key point, as the interpretation seems to be an over-statement. For instance, another possibility is that inhibition is already higher in older adults, since estimates of PICs have been shown to be lower in this population. Thus, when vibration is given, there is less of an inhibitory effect.

3. Have the authors considered to what degree the observed changes in peak discharge or recruitment torque contribute to the reported differences in ΔF? Changes in the ascending phase of discharge of the control units may impact ΔF estimates, but can we be certain the changes in peak discharge and recruitment are monoaminergic driven?

4. The authors should explain the rationale behind the different length and intensity of contractions across experiments. While this is not a fundamental flaw, it should be explained.

5. Page 23, lines 28-29: Vandenberk and Kalmar show correlation in this paper - consider revising this to reflect what they did. The subthreshold CPN stim used to measure reciprocal inhibition was correlated with estimations of PICs.

6. Page 24, line 16: the H-reflex is a response - consider just saying "H-reflexes"

END OF COMMENTS

EDITOR COMMENTS

Reviewing Editor:
Thank you for submitting your manuscript to JP. It has been assessed be 2 experts within the field and although there is clear collective enthusiasm for this work there are a number of comments to address before it can be considered for publication. I also have a number of suggestions I hope the authors can consider:
Authors: Thank you for giving us the opportunity to review our manuscript and make improvements based on the editor's and reviewers' suggestions. Point-by-point responses and changes to the manuscript are presented below.

Please clarify total n and justification of reduced n. This is particularly important to the field if it is a result of low MU yield.
Authors: Thank you for your suggestion. A reduced n for young adults was chosen as we expected to identify fewer motor units from the soleus and tibialis anterior of older adults because they are expected to have fewer motor units, due to age-related motor neurone loss (denervation and reinnervation cycle) (Hepple & Rice, 2016), and thicker fat tissue between the muscle and skin, which could reduce the number of yield motor units (Oliveira et al., 2022). However, we ultimately identified a similar number of motor units per participant between age groups. We believe that the greater proportion of lower-threshold motor units and the increased motor unit size (and therefore bigger action potential amplitudes) in older adults facilitated the decomposition of motor units in this population.

To address this suggestion, rationale for the recruitment of a different number of participants per group has been added into the Methods section, and the Supplementary material 2 has been converted into Table 1 in the manuscript, clearly describing the median number of motor units for each group and condition used in Experiments 1 and 2:

Methods (Page 6, lines 10-14):

> *"More older adults were recruited due to the potential for a smaller motor unit yield due to age-related motor neurone loss (denervation and reinnervation cycle) (Hepple & Rice, 2016). Thicker fat tissue between the muscle and electrodes was also expected, which could potentially reduce the motor unit yield (Oliveira et al., 2022)."*

Results (Page 11, lines 13-28):

> *"In Experiment 1, 201 soleus motor units for 21 older adults and 110 soleus motor units for 14 young adults were identified by the decomposition algorithm that could be matched before and after the control condition, resulting in 78 and 64 test units, respectively. 187 soleus motor units were matched before and after the handgrip*

contraction for 19 older adults and 101 for 12 young adults, resulting in 79 and 43 test units, respectively. 326 tibialis anterior motor units for 16 older adults and 262 for 15 young adults were matched before and after the control condition, resulting in 154 and 132 test units, respectively. 253 tibialis anterior motor units for 14 older adults and 222 for 13 young adults were matched before and after the control condition, resulting in 110 and 116 test units, respectively. In Experiment 2, 97 soleus motor units for 15 older adults and 60 motor units for 8 young adults were matched between control and vibration conditions, resulting in 42 and 35 test units respectively. For, 246 tibialis anterior motor units for 13 older adults and 215 motor units from 13 young adults were matched between control and vibration conditions, resulting in 143 and 147 test units, respectively. Motor units sample median and interquartile range per experiment, group, and conditions are presented in Table 1."

Table 1. Total and test motor unit sample medians (interquartile range) for each group and condition used in Experiments 1 and 2.

Experiment 1				
Group	Older adults		Young adults	
Condition	Control	Handgrip	Control	Handgrip
Soleus motor units	8 (7, 10)	8 (6, 11)	7 (5, 9)	8 (7, 9)
Soleus test units (ΔF)	3 (2, 4)	2 (2, 7)	5 (3, 5)	3 (2, 4)
Tibialis anterior motor units	21 (16, 26)	17 (14, 23)	17 (13, 23)	17 (14, 22)
Tibialis anterior test units (ΔF)	10 (7, 14)	10 (6, 10)	8 (5, 13)	8 (5, 13)
Experiment 2				
Group	Older adults		Young adults	
Soleus motor units	6 (5, 7)		5 (4, 8)	
Soleus test units (ΔF)	3 (1, 4)		2 (1, 5)	
Tibialis anterior motor units	19 (15, 26)		18 (11, 20)	
Tibialis anterior test units (ΔF)	11 (7, 14)		14 (5, 17)	

Note: The presented numbers represent the quantity of motor units tracked over time (before and after each condition) in Experiment 1 and tracked between conditions (vibration and control) in Experiment 2.

The old were non-sarcopenic, was this part of the screening process? If so, please add brief details on the definition used.

Authors: Sarcopenia assessment was not part of the screening process, but it was used to characterise our participants. Sarcopenia status was screened using the European

Working Group on Sarcopenia in Older People (EWGSOP2) recommendations. This information has been added into the methods section, as follows (Page 6, lines 20-25):

> *"Sarcopenia status was screened according to the European Working Group on Sarcopenia in Older People (EWGSOP2) recommendations (Cruz-Jentoft et al., 2019). No participants had low handgrip strength (< 27 kg for men or < 16 kg for women) and low appendicular skeletal muscle mass (< 20 kg for men and < 15 kg for women), indicating they were not sarcopenic. The data related to sarcopenia assessment can be found in our previous study (Orssatto et al., 2021a)."*

Please state the reference number for ethical approval.
Authors: The ethical approval reference number (1900000634) has been stated in the manuscript (Page 6, Line 26).

"Additional data supporting this claim includes evidence that peak discharging rates are maintained with ageing in well-used muscles (e.g., hand muscles, quadriceps, and triceps surae) but decline markedly in lesser used muscles (e.g., hamstrings and tibialis anterior)" - it is unclear how the level of muscle use is justified. Put simply, is there any evidence to suggest quadriceps are typically activated more often than tibialis anterior? This seems at odds with the description of TA several sentences prior. Furthermore, is it the case that quadriceps discharge rate is largely maintained with age? Please clarify and cite.
Authors: We are suggesting that quadriceps and triceps surae muscles are more overloaded in normal daily tasks than hamstrings and tibialis anterior because of their antigravitational role. Yes, we are suggesting that there is evidence that discharge rate is maintained with age in hand muscles, quadriceps, and triceps surae, but not in tibialis anterior and hamstrings, as presented in our recent meta-analysis (Orssatto et al., 2022). We have clarified this rationale in the manuscript, as follows (Page 18, lines 13-17):

> *" Additional data supporting this claim includes evidence that peak discharge rates are maintained with ageing in well-used (e.g., hand muscles) and gravity-loaded muscles (e.g., quadriceps and triceps surae) but decline markedly in lesser used/loaded muscles (e.g., hamstrings and tibialis anterior) (Orssatto et al., 2022a)."*

Orssatto, L. B. R., Borg, D. N., Pendrith, L., Blazevich, A. J., Shield, A. J., & Trajano, G. S. (2022). Do motoneuron discharge rates slow with aging? A systematic review and meta-analysis. Mechanisms of Ageing and Development, 203(February), 111647. https://doi.org/10.1016/j.mad.2022.111647

I would encourage the authors to further explore the differential response of TA and soleus as this is a particularly interesting finding. Although far from a direct anatomical count, the aged soleus is one of the few (if not only) muscles to appear to preserve the number of motor units with age (see Dalton et al doi.org/10.1002/mus.20984), largely unseen in other muscles. I will let the authors decide on the relevance of this.
Authors: Thank you for your suggestion. The discussion exploring the preservation of

function in soleus compared to tibialis anterior has been expanded (Including the suggested reference), as follows (Pages 18 and 19, lines 8-33 and 1-2):

> *"The dissimilar response between soleus and tibialis anterior may speculatively be explained by their different functional roles in daily tasks. Both muscles are active during daily living activities, such as upright standing and locomotion (e.g., gait) (Soames & Atha, 1981; Masani et al., 2013),* ***However, soleus is a propulsive muscle and serves an anti-gravity role, implying that motor units are active for longer and produce greater cumulative force than not propulsive flexor muscles (e.g., tibialis anterior) during daily living activities.*** *Additional data supporting this claim includes evidence that peak discharge rates are maintained with ageing in well-used (e.g., hand muscles) and gravity-loaded muscles (e.g., quadriceps and triceps surae) but decline markedly in lesser used/loaded muscles (e.g., hamstrings and tibialis anterior) (Orssatto et al., 2022a). Also, the estimated number of motor units in soleus seems preserved with ageing, again suggesting selective preservation in this muscle (Dalton et al., 2008). In fact, studies show that disuse can aggravate the deleterious effects of ageing on the nervous system, while trained older adults show a substantial preservation of neural function (Aagaard et al., 2010; Mcgregor et al., 2011; Unhjem et al., 2016; Hvid et al., 2018; Orssatto et al., 2020). In addition, between-muscle effects of ageing on muscle spindles and sensory afferents should be considered when interpreting our data. Although no direct comparison between soleus and tibialis anterior exists, reductions in muscle spindle diameter have been observed in aged deltoid and extensor digitorum, although not in quadriceps or biceps brachii, and decreases in the number of intrafusal fibres in deltoid have also been detected (Kararizou et al., 2005). It is therefore possible that ageing differently affects soleus and tibialis anterior muscle spindles and sensory afferents, and this might be confirmed in future experiments. Interestingly, passive ankle plantar and dorsiflexion detectibly influenced cortico-spinal (motor evoked potential/maximal compound action potentials) responses in tibialis anterior in young adults but not in older adults, while it remained unchanged in soleus in both groups (Škarabot et al., 2020). These data support the assertion that soleus and tibialis anterior afferent and/or efferent pathways might be differently affected by ageing. Regardless, further direct comparisons between soleus and tibialis anterior are required to elucidate the mechanisms underpinning the dissimilar effects of ageing on PIC inhibitory control between them."*

REFEREE COMMENTS

Referee #1:

Orssatto and colleagues investigated facilitation-inhibition control of the estimated PICs in young and older adults. The work builds on their previous findings that older adults exhibit smaller estimates of PIC strength. The results suggested that older adults are able to similarly amplify estimates of PICs with a remote muscle contractions to that of

younger adults, however, their ability to decrease PIC strength via reciprocal inhibition seems to be attenuated.
The manuscript tackles an important topic and improves our understanding of the neural underpinnings of the ageing process. The study is very-well designed and methodologically sound. The authors are also commended on data transparency.
I have several comments that I hope the authors may use when revising the manuscript
Authors: Thank you for reviewing our manuscript and providing valuable recommendations. All comments have been addressed and changes are presented below and red coloured in the manuscript.

Lines 18-22: I believe the last point of the 'Key points' section is too speculative and not directly related to the data presented. I suggest sticking to what your data shows directly in this section. Whilst some speculation might be acceptable, this should, in my opinion, be succinct and constrained to the Discussion.
Authors: Thank you for your suggestion, the last key point has been rewritten to reduce speculation, as follows (Page 3, lines 18-21):

> *"Our data from lower-threshold motor units during low-force contractions suggest that PIC facilitation is preserved with ageing in soleus and tibialis anterior. However, the effect of reciprocal inhibition on the contribution of PICs to motor neurone discharge seems reduced in tibialis anterior but preserved in soleus."*

Introduction
I believe some attention should be given to the mechanism by which a remote muscle contraction increases serotonin secretion. On page 5, line 15 a mere statement of 'belief' is provided without any reference, though I think this is provided at a later section.
Authors: The following sentence has been rewritten aiming to clarify the mechanisms by which a remote muscle contraction increases serotonin release onto motor neurones of other muscles in the Introduction (Page 4, lines 9-18):

> *"It has also been suggested that serotonergic projections to the spinal cord vary their activity in proportion to motor output (Jacobs et al., 2002). Thus, the stronger PIC facilitation observed in higher intensity activities (Orssatto et al., 2021b) could theoretically result from a greater serotonergic release onto the motor neurones (Lee & Heckman, 1999, 2000; Heckman et al., 2005). However, descending serotonergic projections are highly diffuse throughout the spinal cord (Heckman et al., 2008), so activation of a specific muscle triggers excitation across diverse muscle groups, including those not involved in the desired tasks (Heckman et al., 2008; Wei et al., 2014). For example, it has been shown that a remote contraction with a leg muscle group triggers a serotonin-mediated increase in motor neuronal gain in hand muscles (Wei et al., 2014).".*

Also, References have been added to support the mentioned statement, as follows (Page 5, lines 15-18):

"In the present study, the responses of PICs to i) a remote handgrip contraction, which is believed to diffusely increase serotonergic release motor neurones (Heckman et al., 2008; Wei et al., 2014), and ii) vibration of the antagonist muscle's tendon, which induces reciprocal inhibition (Pearcey et al., 2022), were estimated in soleus and tibialis anterior of young and older adults (…)"

P5, line 15: reciprocal inhibition is stated twice whereas I believe the first one should probably say 'vibration of the antagonist tendon/muscle'.
Authors: Thank you for pointing out this mistake, which has been corrected (Page 5, line 17), as follows:

"ii) vibration of the antagonist muscle's tendon, which induces reciprocal inhibition, (…)"

Methods
P6, line 3: Is the disparing number of participants to account for potentially smaller yield of MUs in older individuals? If so, I suggest you clarify that.
Authors: Thank you for your suggestion. A reduced n for young adults was chosen as we expected to identify fewer motor units from the soleus and tibialis anterior of older adults because they are expected to have fewer motor units, due to age-related motor neurone loss (denervation and reinnervation cycle) (Hepple & Rice, 2016), and thicker fat tissue between the muscle and skin, which could reduce the number of yield motor units (Oliveira et al., 2022). However, we ultimately identified a similar number of motor units per participant between age groups. We believe that the greater proportion of lower-threshold motor units and the increased motor unit size (and therefore bigger action potential amplitudes) in older adults facilitated the decomposition of motor units in this population.

To address this suggestion, rationale for the recruitment of a different number of participants per group has been added into the Methods section, as follows (Page 6, lines 10-14):

"More older adults were recruited due to the potential for a smaller motor unit yield due to age-related motor neurone loss (denervation and reinnervation cycle) (Hepple & Rice, 2016). Thicker fat tissue between the muscle and electrodes was also expected, which could potentially reduce the motor unit yield (Oliveira et al., 2022)."

I realise that the sample population was the same/similar as in a prior study. However, I do believe the basic information such as sex and mean age of the participants should be listed here, thus not requiring the reader to dig out the other paper.
Authors: mean age and sex for the young and older adults have been reported, as follows (Page 6, lines 5-10):

"Data from 17 young adults (8 women, 29 ± 5 years, dorsiflexion peak torque 41 ± 14 N·m, and plantar flexion peak torque 156 ± 47 N·m) and 25 older adults (14 women, 70 ± 4 years, dorsiflexion peak torque 29 ± 7 N·m, and plantar flexion peak torque 85 ± 32 N·m) were analysed. The same participants were tested in a previous study where these characteristics and additional participant data were reported (Orssatto et al., 2021a)."

P7, line 2: It is unclear what the duration and intensity of the remote contraction is based on. Would a weaker/stronger and shorter/longer duration of a contraction be more/less effective?

Authors: This is a good question and we probably do not have a definitive answer for it. We conducted several pilot trials in our laboratory to find a contraction intensity that could be sustained for at least 30 s, before reductions in the force level was observed. The highest intensity that could be sustained for 30 s for all the pilot tested individuals was 40% of maximal force. In addition, we found that this method was effective for increasing delta F (from tibialis anterior in young adults) in another study developed by our group (unpublished data). However, it remains unclear what the optimal remote contraction method is to assess PIC facilitation in humans. We believe our study will be the starting point for the development of future investigations trying to answer this question. This information has been included in the Methods section, as follows (Page 7, lines 19-21):

"These parameters were based on pilot testing, which showed that 40% was the highest contraction intensity that could be universally sustained during 30 s."

P7, line 11: How often were repeats needed? Some data here would be reassuring.

Authors: Participants were well familiarised to the triangular contractions and only a few trials were excluded and repeated (<10%). Trials with error were interrupted to avoid unnecessary muscle contractions and to shorten the testing time, and files were not saved. Unfortunately, the exact number of excluded trials and repeats have not been recorded. Therefore, we do not have numbers to present regarding this question and we hope the reviewer will understand this limitation.

Is 2 mins a sufficient rest period for the effects of prior contractions to dissipate? If so, what was this based on?

Authors: We understand the reviewer's concern. We presented data, in Experiment 1, showing that ΔF values are similar between repeated triangular contractions with a ~45 s rest interval (control condition). ΔF was similar in soleus (mean difference = -0.10 (-0.28, 0.09) pps, ICC = 0.968 (0.944, 0.982), and SEM = 0.219 pps) and tibialis anterior (mean difference = -0.05 (-0.21, 0.11) pps, ICC = 0.946 (0.901, 0.971), SEM was 0.372 pps). Therefore, if ~45 s is sufficient for the effects of a prior contraction (with the same muscle) to dissipate, we believe the same could be assumed for handgrip remote contractions. Indeed, the duration of remote-contraction-related ΔF facilitation is an

important research question that should be explored in future studies.

P7, line 18: Was this fully randomised or block randomised (e.g. participant 1 started without, but participant 2 with vibration, etc.)?
Authors: The conditions were randomised, prior to the commencement of the study (from 1 to 30) using three distinct blocks (from 1 to 10, 11 to 20, and 21 to 30) for each group. For example, to ensure balance between conditions and muscles, 10 pieces of papers were used for condition (5 each) and another 10 pieces for muscle (5 each), for each block and age group. The randomisation procedure was repeated, independently, for each block (1, 2, and 3) and group (young and older adults). Participants were allocated to each condition sequence based on their testing order (participant #). See example for the randomisation, used in Experiment 2, below:

Block	Participant #	Young	Older
1	1	Starts with vibrator	Starts with vibrator
	2	Starts without vibrator	Starts with vibrator
	3	Starts without vibrator	Starts without vibrator
	4	Starts with vibrator	Starts with vibrator
	5	Starts without vibrator	Starts without vibrator
	6	Starts with vibrator	Starts without vibrator
	7	Starts without vibrator	Starts with vibrator
	8	Starts without vibrator	Starts with vibrator
	9	Starts with vibrator	Starts without vibrator
	10	Starts with vibrator	Starts without vibrator
2	11	Starts without vibrator	Starts with vibrator
	12	Starts without vibrator	Starts without vibrator
	13	Starts with vibrator	Starts with vibrator
	14	Starts with vibrator	Starts without vibrator
	15	Starts without vibrator	Starts without vibrator
	16	Starts with vibrator	Starts with vibrator
	17	Starts with vibrator	Starts without vibrator
	18	Starts without vibrator	Starts with vibrator
	19	Starts with vibrator	Starts without vibrator
	20	Starts without vibrator	Starts with vibrator

P7, line 19: Was the vibration device hand held? If so, how was the same pressure ensured?
Authors: The vibrator was firmly strapped onto the participant's leg using the device's straps. However, we understand that not quantifying the amount of pressure applied on the skin was a limitation. This information has been added to the Methods section, as follows (Page 8, lines 9-11):

"The vibrator device was firmly strapped onto the leg and no discomfort was reported by the participants. However, the amount of pressure applied on the skin was not quantified."

P8: Was there a particular reason for a 20% contraction in Experiment 2, but a 30% contraction in Experiment 3? Note that I don't think this disparity changes the interpretation of the results, but it does seem somewhat odd to have different contraction levels. Given the same pool of participants, the same contraction level would have allowed a direct comparison of facilitatory and inhibitory responses.
Authors: We chose the contraction intensities according to each experiment's hypothesis. There is evidence that ΔF can increase with a higher contraction intensity (from 10 to 20 to 30% of MVC) in soleus (Orssatto, et al., 2021). Therefore, we knew that ΔF obtained during 20% contractions would provide a clear opportunity for increases in ΔF, if they occur (Experiment 1), while the ΔF obtained during 30% contractions would have significant opportunity to be reduced (avoiding a possible floor effect) in case of a possible inhibitory effect (Experiment 2). Previous studies have shown that ΔF obtained from 20% contractions increase to acute neuromodulatory intervention in young (Udina et al., 2010) and chronic exercise in older adults (Orssatto, et al., 2022). Also, ΔF from 30% contractions reduce with reciprocal inhibition in soleus and tibialis anterior in young adults (Pearcey et al., 2020).

In addition, we imposed different contraction durations for each intensity so that the rate of torque rise during the ramps was consistent between experiments. This was used to ensure the participants were familiar with the rate of torque increase used for both tasks.

The following sentences have been added into the methods session and Figure 1 legend.
Methods (Page 7, lines 11-16)

"20% of peak torque was used because it is known that ΔF obtained at this intensity has not reached a ceiling, so it would be possible to observe intervention-dependent increases. Previous studies reported increases in ΔF, from 20% of peak torque contractions have been reported both in tibialis anterior of young adults (Udina et al., 2010; Orssatto et al., 2021b) and soleus of older adults (Orssatto et al., 2022b)."

Methods (Page 8, lines 1-3)

"30% of peak torque was used because i) soleus ΔF obtained at this intensity has potential to be reduced (Orssatto et al., 2021b), and ii) reductions in soleus and tibialis anterior ΔF in response to reciprocal inhibition have been observed in young adults at this intensity (Pearcey et al., 2022)."

Figure 1.

"In both experiments, PICs were estimated during submaximal ramp-shaped contractions with rate of torque rise and decline of 2%/s. The contraction intensities

were 20% of peak torque (20-s total duration) and 30% of peak torque (30-s total duration) for Experiments 1 and 2, respectively."

Orssatto, L. B. R., Mackay, K., Shield, A. J., Sakugawa, R. L., Blazevich, A. J., & Trajano, G. S. (2021). Estimates of persistent inward currents increase with the level of voluntary drive in low-threshold motor units of plantar flexor muscles. Journal of Neurophysiology, 125(5), 1746–1754.

Orssatto, L. B. R., Rodrigues, P., Philips, K. M., Blazevich, A. J., Borg, D. N., Souza, T. R. de, Sakugawa, R. L., Shield, A. J., & Trajano, G. S. (2022). Intrinsic motor neurone excitability is increased after resistance training in older adults Lucas. SportRxiv.

Pearcey GEP, Khurram OU, Beauchamp JA, Negro F & Heckman CJ (2022). Antagonist tendon vibration dampens estimates of persistent inward currents in motor units of the human lower limb. bioRxiv.

Udina, E., D'Amico, J., Bergquist, A. J., & Gorassini, M. A. (2010). Amphetamine increases persistent inward currents in human motoneurons estimated from paired motor-unit activity. Journal of Neurophysiology, 103(3), 1295–1303. https://doi.org/10.1152/jn.00734.2009

P10, line 4: Whilst again, unlikely to change the interpretation, it would be useful for the reader to make a note that polynomial functions (and in particular 5th order) likely over smooth the data, resulting in some level of fit error (at least compared to the support vector regression approach; Beauchamp et al. 2022 J Neural Eng).

Authors: Thank you for your suggestion. A note regarding the adopted polynomial function has been added onto the limitations section, as follows (Page 19, lines 9-18):

"We used a 5th order polynomial function to smooth the instantaneous discharge rate data. This approach has been originally and extensively used to calculate ΔF with the paired-motor unit analysis technique (Gorassini et al., 2002; Udina et al., 2010; Vandenberk & Kalmar, 2014; Trajano et al., 2020; Orssatto et al., 2021b, 2021a; Mesquita et al., 2022), which allows our ΔF values to be compared with other studies. However, polynomial functions (in particular 5th order) may sometimes over-smooth the data, resulting in a level of fit error, when compared to the support vector regression approach for example (Beauchamp et al., 2022). Although this would unlikely change the interpretation of our results, caution is warranted when comparing the results of studies using different instantaneous discharge rate smoothing methods."

P10, line 5-6: 'a low recruitment threshold', I believe you mean 'lower than...' here. Unless you provide specific cut-off points (which would, in my opinion, be erroneous) units may merely be classified as that of a lower/higher recruitment threshold than another unit.

Authors: Thank you, this has been corrected in the manuscript and now reads as follows (Page 9, line 24):

"Motor units with a lower recruitment threshold (…)"

P10, line11-12: Given your citation of Hassan et al. 2020, I am surprised you seemingly did not specify the criterion for the derecruitment time difference. Any particular reason why only the recruitment time difference was considered?

Authors: This is an interesting point. Hassan et al. 2020 suggested that pairs with 1.5 s de-recruitment time difference between test and control units may overestimate ΔF values. We believe this possible overestimation could be more relevant for studies with a between-subject rather than within-subject design. Our study adopted a repeated-measures (within-subject) design and only included motor units tracked over time. Thus, if ΔF was overestimated, then it would have occurred both in baseline and intervention conditions. Therefore, we, believe that any possible overestimation of ΔF absolute values would not change the ability of the method to detect changes over time or between conditions when the ΔF from the same units are analysed. Also, adopting this criterion would likely reduce the number of analysed motor units (and participants), which could reduce statistical power. Note that recent studies investigating the effects of reciprocal inhibition on ΔF (Mesquita et al., 2022; Pearcey et al., 2022) have not adopted this criterion. Also, Pearcey et al. (2022) obtained ΔF values (soleus ~2.9 and tibialis anterior ~4.8 pps) and mean differences (~0.54 pps) similar to our study. Therefore, adopting this criterion would make it difficult to compare our results with theirs.

For instance, we re-analysed data from 8 random participants (4 each group) from Experiment 1 (soleus) and Experiment 2 (tibialis anterior) using the <1.5-s derecruitment difference criterion. In experiment 1, the after-before handgrip emmeans difference slightly changed from 0.37 to 0.43 pps (without and with the criterion, respectively). The total number of test units (i.e., ΔF values) reduced 36%. In Experiment 2, The vibrator-control emmeans difference slightly changed from -0.55 to -0.64 pps in young and from -0.41 to -0.34 pps in older adults (without and with the criterion, respectively). Also, the total number of test units (i.e., ΔF values) reduced 32%.

We are confident that adding this criterion would not be beneficial for our analyses (reducing sample size) and would unlikely change the interpretation of our data. The use of this criterion will be further explored by future studies in our laboratory.

Mesquita, R. N. O., Taylor, J. L., Trajano, G. S., Škarabot, J., Holobar, A., Gonçalves, B. A. M., & Blazevich, A. J. (2022). Effects of reciprocal inhibition and whole-body relaxation on persistent inward currents estimated by two different methods. The Journal of Physiology, 600(11), 2765–2787. https://doi.org/10.1113/jp282765

Pearcey, G. E. P., Khurram, O. U., Beauchamp, J. A., Negro, F., & Heckman, C. J. (2022). Antagonist tendon vibration dampens estimates of persistent inward currents in motor units of the human lower limb. BioRxiv.

Results

It would be good to report a strength difference between the groups in the interest of comparison with other studies.

Authors: Thank you for your suggestion. Strength measurements have now been reported with the participants' characteristics, as follows (Page 6, lines 5-10):

> *"Data from 17 young adults (8 women, 29 ± 5 years, dorsiflexion peak torque 41 ± 14 N·m, and plantar flexion peak torque 156 ± 47 N·m) and 25 older adults (14 women, 70 ± 4 years, dorsiflexion peak torque 29 ± 7 N·m, and plantar flexion peak torque 85 ± 32 N·m) were analysed. The same participants were tested in a previous study where these characteristics and additional participant data were reported (Orssatto et al., 2021a)."*

Orssatto, L. B. R., Borg, D. N., Blazevich, A. J., Sakugawa, R. L., Shield, A. J., & Trajano, G. S. (2021). Intrinsic motoneuron excitability is reduced in soleus and tibialis anterior of older adults. GeroScience, 43(6), 2719–2735. https://doi.org/10.1007/s11357-021-00478-z

P20, line 8: It might be my personal preference, but I find it rather odd to not have the basic information on the motor unit population before delving into the results of delta F. I would suggest this be moved somewhere towards the beginning of the Results section.

Authors: Thank you for your suggestion. The "motor unit identification" subsection has been moved toward the beginning of the Results section. Also, the Supplementary material 2 has been converted into "Table 1" and added into the "motor unit identification" subsection, connecting all the information regarding the number of yielded motor units, as follows (Page 11, lines 13-28):

> *"In Experiment 1, 201 soleus motor units for 21 older adults and 110 soleus motor units for 14 young adults were identified by the decomposition algorithm that could be matched before and after the control condition, resulting in 78 and 64 test units, respectively. 187 soleus motor units were matched before and after the handgrip contraction for 19 older adults and 101 for 12 young adults, resulting in 79 and 43 test units, respectively. 326 tibialis anterior motor units for 16 older adults and 262 for 15 young adults were matched before and after the control condition, resulting in 154 and 132 test units, respectively. 253 tibialis anterior motor units for 14 older adults and 222 for 13 young adults were matched before and after the control condition, resulting in 110 and 116 test units, respectively. In Experiment 2, 97 soleus motor units for 15 older adults and 60 motor units for 8 young adults were matched between control and vibration conditions, resulting in 42 and 35 test units respectively. For, 246 tibialis anterior motor units for 13 older adults and 215 motor units from 13 young adults were matched between control and vibration conditions, resulting in 143 and 147 test units, respectively. Motor units sample median and interquartile range per experiment, group, and conditions are presented in Table 1."*

Table 1. Total and test motor unit sample medians (interquartile range) for each group and condition used in Experiments 1 and 2.

Experiment 1				
Group	Older adults		Young adults	
Condition	Control	Handgrip	Control	Handgrip
Soleus motor units	8 (7, 10)	8 (6, 11)	7 (5, 9)	8 (7, 9)
Soleus test units (ΔF)	3 (2, 4)	2 (2, 7)	5 (3, 5)	3 (2, 4)
Tibialis anterior motor units	21 (16, 26)	17 (14, 23)	17 (13, 23)	17 (14, 22)
Tibialis anterior test units (ΔF)	10 (7, 14)	10 (6, 10)	8 (5, 13)	8 (5, 13)
Experiment 2				
Group	Older adults		Young adults	
Soleus motor units	6 (5, 7)		5 (4, 8)	
Soleus test units (ΔF)	3 (1, 4)		2 (1, 5)	
Tibialis anterior motor units	19 (15, 26)		18 (11, 20)	
Tibialis anterior test units (ΔF)	11 (7, 14)		14 (5, 17)	

Note: The presented numbers represent the quantity of motor units tracked over time (before and after each condition) in Experiment 1 and tracked between conditions (vibration and control) in Experiment 2.

P20, line 10: presumably 'older' should be 'younger' here.
Authors: Thank you. This has been corrected in page 11, line 14.

Discussion
The discussion is generally well written, but what the authors seemingly fail to consider is the potential for a ceiling effect when it comes inhibition control. As the authors will be aware, the delta F method estimates not only neuromodulatory input, but likely also the extent of ionotropic inhibition. Thus, lower delta F estimates that older adults exhibit might be a reflection of greater inhibition compared to younger at baseline. As such, an additional inhibitory input might result in less of a further decrease in delta F.
Authors: Thank you for your valuable input. This brief discussion has been added into the manuscript as follows (Page 18, lines 2-7):

> *"Another mechanism that should be considered is a possible ceiling effect related to inhibition control. The pattern of the inhibitory commands is altered with ageing (Butchart et al., 1993; Kido et al., 2004; Hortobágyi et al., 2006) and may contribute to the lower ΔF observed in older adults at baseline (Hassan et al., 2021; Orssatto et al., 2021a). As such, additional inhibitory input might have reduced the opportunity*

for further decrease in ΔF in tibialis anterior of older adults."

Referee #2:
This manuscript provides important new results about control of excitability of motor output and its alterations with aging. The authors estimated "PIC facilitation-inhibition" in the TA and SOL muscles during submaximal triangle-shaped contractions, using the standard method of assessment of persistent inward currents, ΔF. They show that regardless of age, ΔF increased in the SOL and TA after a handgrip contraction. In contrast, decreases in ΔF were found in the SOL in response to antagonist tendon vibration. However, in the TA, ΔF decreased less in older adults in response to antagonist tendon vibration. They suggest that this difference in older adults signifies an impaired ability to deactivate PICs in response to reciprocal inhibition with age. I suggest a few minor revisions, mainly to explain what other mechanisms may be responsible the observed difference.
Authors: Thank you for your time reviewing our manuscript and valuable suggestions. Changes in the manuscript have been red coloured and are presented below.

1. Please define "facilitation-inhibition control". It is mentioned throughout the abstract and conclusion but would be helpful to clearly define this in the introduction for the reader.
Authors: Thank you for your suggestion. "Facilitation-inhibition control" has been defined in the Abstract and Introduction, as follows:
Abstract (Page 2, lines 2-4):

"A well-coordinated facilitation-inhibition control of motor neuronal persistent inward currents (PICs) via diffuse neuromodulation and local inhibition is essential to ensure motor units discharge at required times and frequencies."

Introduction (Lines Page 4, lines 24-27):

"Thus, a well-coordinated facilitation-inhibition control of PICs via diffuse activation (i.e., facilitation) and local deactivation (i.e., inhibition) is essential to ensure motor units discharge at desired times and frequencies, allowing normal motor behaviour (Heckman et al., 2008)."

Also, the whole manuscript has been checked and updated for consistency.

2. The authors should consider re-phrasing the final key point, as the interpretation seems to be an over-statement. For instance, another possibility is that inhibition is already higher in older adults, since estimates of PICs have been shown to be lower in this population. Thus, when vibration is given, there is less of an inhibitory effect.
Authors: Thank you for your suggestion, the last key point has been rewritten based on what our data shows, as follows (Page 3, lines 18-21):

"Our data from lower-threshold motor units during low-force contractions suggest that PIC facilitation is preserved with ageing in soleus and tibialis anterior. However, the effect of reciprocal inhibition on the contribution of PICs to motor neurone discharge seems reduced in tibialis anterior but preserved in soleus."

3. Have the authors considered to what degree the observed changes in peak discharge or recruitment torque contribute to the reported differences in ΔF? Changes in the ascending phase of discharge of the control units may impact ΔF estimates, but can we be certain the changes in peak discharge and recruitment are monoaminergic driven?
Authors: Thank you. We ran repeated-measures correlations between changes in ΔF with changes in peak discharge rates and recruitment thresholds. In Experiment 1, small correlations were observed between ΔF and peak discharge rates for soleus and tibialis anterior. No correlation was observed between ΔF and recruitment threshold. In Experiment 2, a small correlation was observed between ΔF and peak discharge rate for soleus, and a large correlation for tibialis anterior. No correlation was observed between ΔF and recruitment threshold. This information has been added onto the manuscript, as follows:

Page 10, lines 30-33:

"Repeated-measures correlations were used to determine the association between changes in ΔF and changes in discharge rates and recruitment thresholds in both experiments 1 and 2 (Bakdash & Marusich, 2017). Correlation magnitude was interpreted as: r < 0.1 trivial; 0.1 – 0.3 small; 0.3 – 0.5 moderate; 0.5 – 0.7 large; 0.7 – 0.9 very large; and > 0.9 nearly perfect. (…) The repeated-measures correlation coefficients were determined with the rmcorr package (Bakdash & Marusich, 2017)."

Page 13, lines 5-7:

"A small correlation was observed between changes in ΔF and peak discharge rate for soleus [r = 0.22 (0.11, 0.31)] and tibialis anterior [r = 0.21 (0.13, 0.28)]."

Page 13, lines 17-19:

"A trivial correlation was observed between changes in ΔF and recruitment threshold for soleus [r = 0.12 (0.02, 0.23)], while no correlation was observed for tibialis anterior [r = 0.03 (-0.05, 0.10)].

Page 14, lines 15-17:

"A small correlation was observed between changes in ΔF and peak discharge rate for soleus [r = 0.22 (0.00, 0.43)] and a large correlation for tibialis anterior [r = 0.57 (0.49, 0.64)]."

Page 14, lines 23-25:

"No correlation was observed between changes in ΔF and recruitment thresholds for soleus [r = 0.05 (-0.18, 0.27)] or tibialis anterior [r = -0.07 (-0.19, 0.04)]."

4. The authors should explain the rationale behind the different length and intensity of

contractions across experiments. While this is not a fundamental flaw, it should be explained.

Authors: We chose the contraction intensities according to each experiment's hypothesis. There is evidence that ΔF can increase with a higher contraction intensity (from 10 to 20 to 30% of MVC) in soleus (Orssatto, et al., 2021). Therefore, we knew that ΔF obtained during 20% contractions would provide a clear opportunity for increases in ΔF, if they occur (Experiment 1), while the ΔF obtained during 30% contractions would have significant opportunity to be reduced (avoiding a possible floor effect) in case of a possible inhibitory effect (Experiment 2). Previous studies have shown that ΔF obtained from 20% contractions increase to acute neuromodulatory intervention in young (Udina et al., 2010) and chronic exercise in older adults (Orssatto, et al., 2022). Also, ΔF from 30% contractions reduce with reciprocal inhibition in soleus and tibialis anterior in young adults (Pearcey et al., 2020).

In addition, we imposed different contraction durations for each intensity so that the rate of torque rise during the ramps was consistent between experiments. This was used to ensure the participants were familiar with the rate of torque increase used for both tasks.

The following sentences have been added into the methods session and Figure 1 legend.

Methods (Page 7, lines 11-16)

> *"20% of peak torque was used because it is known that ΔF obtained at this intensity has not reached a ceiling, so it would be possible to observe intervention-dependent increases. Previous studies reported increases in ΔF, from 20% of peak torque contractions have been reported both in tibialis anterior of young adults (Udina et al., 2010; Orssatto et al., 2021b) and soleus of older adults (Orssatto et al., 2022b)."*

Methods (Page 8, lines 1-3)

> *"30% of peak torque was used because i) soleus ΔF obtained at this intensity has potential to be reduced (Orssatto et al., 2021b), and ii) reductions in soleus and tibialis anterior ΔF in response to reciprocal inhibition have been observed in young adults at this intensity (Pearcey et al., 2022)."*

Figure 1.

> *"In both experiments, PICs were estimated during submaximal ramp-shaped contractions with rate of torque rise and decline of 2%/s. The contraction intensities were 20% of peak torque (20-s total duration) and 30% of peak torque (30-s total duration) for Experiments 1 and 2, respectively."*

Orssatto, L. B. R., Mackay, K., Shield, A. J., Sakugawa, R. L., Blazevich, A. J., & Trajano, G. S. (2021). Estimates of persistent inward currents increase with the level of voluntary drive in low-threshold motor units of plantar flexor muscles. Journal of Neurophysiology,

125(5), 1746–1754.

Orssatto, L. B. R., Rodrigues, P., Philips, K. M., Blazevich, A. J., Borg, D. N., Souza, T. R. de, Sakugawa, R. L., Shield, A. J., & Trajano, G. S. (2022). Intrinsic motor neurone excitability is increased after resistance training in older adults Lucas. SportRxiv.

Pearcey, G. E. P., Khurram, O. U., Beauchamp, J. A., Negro, F., & Heckman, C. J. (2022). Antagonist tendon vibration dampens estimates of persistent inward currents in motor units of the human lower limb. BioRxiv.

Udina, E., D'Amico, J., Bergquist, A. J., & Gorassini, M. A. (2010). Amphetamine increases persistent inward currents in human motoneurons estimated from paired motor-unit activity. Journal of Neurophysiology, 103(3), 1295–1303. https://doi.org/10.1152/jn.00734.2009

5. Page 23, lines 28-29: Vandenberk and Kalmar show correlation in this paper - consider revising this to reflect what they did. The subthreshold CPN stim used to measure reciprocal inhibition was correlated with estimations of PICs.

Authors: The reviewer is right. The sentence has been amended and the paragraph has been reorganised, as follows (Page 17, lines 5-15):

> *"(…). Nonetheless, our results are consistent with recent human experiments showing: i) comparable ΔF reductions of 0.54 ± 0.09 pps in both tibialis anterior (effect size g = 0.49) and soleus (g = 0.26) using a similar protocol of antagonist tendon vibration (Pearcey et al., 2022); ii) similar ΔF reductions of 0.33 pps (9.8%) in gastrocnemius medialis of young adults after reciprocal inhibition was induced by electrical stimulation of the common peroneal nerve (Mesquita et al., 2022); iii) inverse correlation between the level of reciprocal inhibition (induced with stimulations to the common peroneal nerve, below motor unit threshold) and ΔF in soleus (Vandenberk & Kalmar, 2014); and iv) artificial activation of inhibitory reflex by sural nerve stimulation reduced the initial steep increases in discharge rates in tibialis anterior motor neurones during ramped contractions, which is likely modulated by PICs (Revill & Fuglevand, 2017). (…)"*

6. Page 24, line 16: the H-reflex is a response - consider just saying "H-reflexes"

Authors: Thank you, this has been amended in page 17, line 31.

Dear Dr Orssatto,

Re: JP-RP-2022-283708R1 "Facilitation-inhibition control of motor neuronal persistent inward currents in young and older adults" by Lucas B R Orssatto, Gabriel Fernandes, Anthony Blazevich, and Gabriel S. Trajano

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

NEW POLICY: In order to improve the transparency of its peer review process The Journal of Physiology publishes online as supporting information the peer review history of all articles accepted for publication. Readers will have access to decision letters, including all Editors' comments and referee reports, for each version of the manuscript and any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

The last Word version of the paper submitted will be used by the Production Editors to prepare your proof. When this is ready you will receive an email containing a link to Wiley's Online Proofing System. The proof should be checked and corrected as quickly as possible.

Authors should note that it is too late at this point to offer corrections prior to proofing. The accepted version will be published online, ahead of the copy edited and typeset version being made available. Major corrections at proof stage, such as changes to figures, will be referred to the Reviewing Editor for approval before they can be incorporated. Only minor changes, such as to style and consistency, should be made a proof stage. Changes that need to be made after proof stage will usually require a formal correction notice.

All queries at proof stage should be sent to TJP@wiley.com.

Are you on Twitter? Once your paper is online, why not share your achievement with your followers. Please tag The Journal (@jphysiol) in any tweets and we will share your accepted paper with our 23,000+ followers!

Yours sincerely,

Richard Carson
Senior Editor
The Journal of Physiology

P.S. - You can help your research get the attention it deserves! Check out Wiley's free Promotion Guide for best-practice recommendations for promoting your work at www.wileyauthors.com/eeo/guide. And learn more about Wiley Editing Services which offers professional video, design, and writing services to create shareable video abstracts, infographics, conference posters, lay summaries, and research news stories for your research at www.wileyauthors.com/eeo/promotion.

* IMPORTANT NOTICE ABOUT OPEN ACCESS *

To assist authors whose funding agencies mandate public access to published research findings sooner than 12 months after publication The Journal of Physiology allows authors to pay an open access (OA) fee to have their papers made freely available immediately on publication.

You will receive an email from Wiley with details on how to register or log-in to Wiley Authors Services where you will be able to place an OnlineOpen order.

You can check if you funder or institution has a Wiley Open Access Account here: https://authorservices.wiley.com/author-resources/Journal-Authors/licensing-and-open-access/open-access/author-compliance-tool.html.

Your article will be made Open Access upon publication, or as soon as payment is received.

If you wish to put your paper on an OA website such as PMC or UKPMC or your institutional repository within 12 months of publication you must pay the open access fee, which covers the cost of publication.

OnlineOpen articles are deposited in PubMed Central (PMC) and PMC mirror sites. Authors of OnlineOpen articles are permitted to post the final, published PDF of their article on a website, institutional repository, or other free public server, immediately on publication.

Note to NIH-funded authors: The Journal of Physiology is published on PMC 12 months after publication, NIH-funded authors DO NOT NEED to pay to publish and DO NOT NEED to post their accepted papers on PMC.

EDITOR COMMENTS

Many thanks for providing such a detailed response. As you will see, both reviewers are content that all comments and recommendations have been addressed.

REFEREE COMMENTS

Referee #1:

I would like to thank the authors for taking the time to respond to my comments. I have no further comments or concerns.

Referee #2:

The authors have done an excellent job in revising and I have no further concerns. The results are intriguing.

1st Confidential Review **23-Sep-2022**