

Optogenetic confirmation of transverse-tubular membrane excitability in intact cardiac myocytes

Marina Scardigli, Michal Pasek, Lorenzo Piantini, Chiara Palandri, Emilia Conti, Claudia Crocini, Marina Campione, Leslie M Loew, Antoine de Vries, Daniel Pijnappels, Francesco Pavone, Corrado Poggesi, Elisabetta Cerbai, Raffaele Coppini, Peter Kohl, Cecilia Ferrantini, and Leonardo Sacconi

DOI: 10.1113/JP285202

Corresponding author(s): Leonardo Sacconi (sacconi@lens.unifi.it)

The following individual(s) involved in review of this submission have agreed to reveal their identity: Fabien Brette (Referee #2)

Review Timeline:

Submission Date:	26-Jun-2023
Editorial Decision:	24-Jul-2023
Revision Received:	18-Dec-2023
Accepted:	17-Jan-2024

Senior Editor: Natalia Trayanova

Reviewing Editor: Wolfgang Linke

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 Sacconi,

Re: JP-RP-2023-285202 "Optogenetic confirmation of transverse-tubular membrane excitability in intact cardiac myocytes" by Marina Scardigli, Michal Pasek, Lorenzo Piantini, Chiara Palandri, Emilia Conti, Claudia Crocini, Marina Campione, Leslie M Loew, Antoine de Vries, Daniel Pijnappels, Francesco Pavone, Corrado Poggesi, Elisabetta Cerbai, Raffaele Coppini, Peter Kohl, Cecilia Ferrantini, and Leonardo Sacconi

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 we are pleased to tell you that it is potentially acceptable for publication following satisfactory major revision.

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

The referee reports are copied at the end of this email.

Please address all the points raised and incorporate all requested revisions or explain in your Response to Referees why a change has not been made. We hope you will find the comments helpful and that you will be able to return your revised manuscript within 9 months. If you require longer than this, please contact journal staff: jp@physoc.org. Please note that this letter does not constitute a guarantee for acceptance of your revised manuscript.

Your revised manuscript should be submitted online using the link in your Author Tasks: Link Not Available. This link is accessible via your account as Corresponding Author; it is not available to your co-authors. If this presents a problem, please contact journal staff (jp@physoc.org). Image files from the previous version are retained on the system. Please ensure you replace or remove any files that are being revised.

If you do not wish to submit a revised version of your manuscript, you must inform our journal staff (jp@physoc.org) or reply to this email to request withdrawal. Please note that a manuscript must be formally withdrawn from the peer review process at one journal before it may be submitted to another journal.

TRANSPARENT PEER REVIEW POLICY: 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 Editors' comments and referee reports, for each version of the manuscript, as well as any author responses to peer review comments. Referees can decide whether or not they wish to be named on the peer review history document.

ABSTRACT FIGURES: Authors are expected to use The Journal's premium BioRender account to create/redraw their Abstract Figures. Information on how to access this account is here: <https://physoc.onlinelibrary.wiley.com/journal/14697793/biorender-access>.

This will enable Authors to create and download high-resolution figures. If authors have used the free BioRender service, they can use the instructions provided in the link above to download a high-resolution version suitable for publication.

The link provided should only be used for the purposes of this submission. Authors will be charged for figures created on this account if they are not related to this manuscript submission.

LANGUAGE EDITING AND SUPPORT FOR PUBLICATION: If you would like help with English language editing, or other article preparation support, Wiley Editing Services offers expert help, including English Language Editing, as well as translation, manuscript formatting, and figure formatting at www.wileyauthors.com/eeo/preparation. You can also find resources for Preparing Your Article for general guidance about writing and preparing your manuscript at www.wileyauthors.com/eeo/prepresources.

REVISION CHECKLIST:

Check that your Methods section conforms to journal policy: https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#methods.

Check that data presented conforms to the statistics policy: https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics.

Upload a full Response to Referees file. To create your 'Response to Referees': copy all the reports, including any comments from the Senior and Reviewing Editors, into a Microsoft Word, or similar, file and respond to each point, using font or background colour to distinguish comments and responses and upload as the required file type.

Please upload two versions of your manuscript text: one with all relevant changes highlighted and one clean version with no changes tracked. The manuscript file should include all tables and figure legends, but each figure/graph should be uploaded as separate, high-resolution files.

You may also upload:

- 'Potential Cover Art' for consideration as the issue's cover image
- Appropriate Supporting Information (Video, audio or data set: see https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#supp).

We look forward to receiving your revised submission.

If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

Natalia Trayanova
Senior Editor
The Journal of Physiology

REQUIRED ITEMS FOR REVISION

- Your manuscript must include a complete [Additional Information section](#)

â€"Please upload separate high-quality [figure files](#) via the submission form.

-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

-Papers must comply with the Statistics Policy https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics

In summary:

-If $n \leq 30$, all data points must be plotted in the figure in a way that reveals their range and distribution. A bar graph with data points overlaid, a box and whisker plot or a violin plot (preferably with data points included) are acceptable formats.

-If $n > 30$, then the entire raw dataset must be made available either as supporting information, or hosted on a not-for-profit repository e.g. FigShare, with access details provided in the manuscript.

-'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

-All relevant 'n' values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

-The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

-Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.

-Statistics Summary Document completed appropriately upon revision

-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 section(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 file, as well as the figure legend text within the main article file. 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.

EDITOR COMMENTS

While both reviewers found your paper interesting and a good fit for the Journal, they raised several concerns, which should be addressed in full in your revision. Concerns include potential alterations in TT properties and medium composition due to the experimental procedures, e.g., can it be excluded that the APs generated are mainly based on the inward current created by the optogenetic channel and not by Na currents? Control measurements have been suggested, which the authors should consider.

REFeree COMMENTS

Referee #1:

The paper by Scardigli and colleagues employs patch-clamp, optogenetic methods and optical measurements of action potentials to demonstrate that isolated t-tubules are excitable. To isolate t-tubules they use an osmotic approach that leads to disruption of t-tubules from the surface membrane resulting in intracellular t-tubule vesicles that can be excited optically.

The results presented are interesting and the authors used highly sophisticated methods. The manuscript is well written and edited.

Major comments:

Obviously, decoupled t-tubules form some kind of intracellular vesicles or tubules and their lumen contains extracellular solution. The composition of the latter is critical to generate physiological driving forces for various ions and finally for generation of action potential. How is this maintained after several excitations? Do these vesicles contain the full set of transporters and channels such as NKA or NCX?

How can the authors exclude that the APs in TT are mainly driven by the depolarizing optogenetic channels. INa-channels in TT are probably not high enough to trigger an AP under physiological condition.

Did the authors apply drugs such as ICa,L-blockers, INa-Blockers or IK-blockers to demonstrate which channels are involved in formation of TT-APs?

Did the authors perform immunofluorescent labelling of various ion channels in TT?

Figure 4: In how many TT was it possible to initiate an AP using electrical stimulation?

Atrial TT structure including axiale tubules and potential relevance for atrial fibrillation should at least be discussed: doi: 10.1152/ajpheart.00284.2011; doi: 10.1172/JCI88241

Referee #2:

Scardigli et al., present a study which shows that cardiac transverse tubules are intrinsically excitable in ventricular myocytes. This provides insight in the field of cardiac physiology where this has been never experimentally shown before, just suspected. By developing an optical platform, t-tubules can be activated using random-access multi-photon microscopy in cardiac myocytes containing light activated ion channels. Computer modelling add values to the experimental work.

The impact is not groundbreaking but it adds to our present knowledge of t-tubules and confirm the current view.

The paper is well written, the study design is innovative and the experimental data are robust. The data support the conclusion.

I have only minor comments:

The authors propose that low excitability of t-tubules can be due to change in Na⁺ conductance after detubulation (top of page10). They use the word "currents" instead of conductance which is confusing. One might expect an increase of excitability with an increase of Na⁺ currents. However, the authors relate it not to NaV1.5 but other channels (conductance) like stretch activated, late or background. I suggest rewording the sentence.

Albeit computer modelling confirms this hypothesis (Figure 5D), an alternative explanation is missing. It is possible that Na⁺ concentration in the vacuolated t-tubules is changed because of the presence of NCX which is still active (see Despa et al, 2003, Biophysical Journal, 85(5) 3388-3396). Depending on the experimental protocol, it may decrease Na⁺ concentration in the vacuole therefore reducing the driving force for NaV1.5 current.

On the same topic, the legend of Figure 5C is not accurate. It indicates that using light it is possible to trigger AP but it is not the case on the figure 5C. Please modify either the legend or the representative traces.

Methods: the authors used Ca buffer (EGTA) and CaCl₂ in the different pipette solution to clamp bulk intracellular calcium. Please provide info on the calcium concentration in the cell, using maxchelator:

<https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/>

There are few typos in the manuscript for example INTRODUCTION, missing space before reference, please double check.

END OF COMMENTS

Confidential Review

26-Jun-2023

Dear Prof. Trayanova,

We hereby submit our revised manuscript 'Optogenetic confirmation of transverse-tubular membrane excitability in intact cardiac myocytes' (JP-RP-2023-285202) for reconsideration at the Journal of Physiology.

The comments of the reviewers were extremely useful and constructive, and we addressed them by performing new experiments, re-analysing datasets, as well as revising the manuscript and figures accordingly.

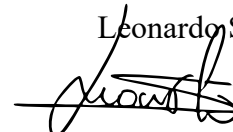
Kindly find attached the revised version of the manuscript with all edits marked in yellow, as well as our point-by-point response to the reviewers.

We hope that our revised manuscript now fulfils the criteria for publication in the Journal of Physiology and look forward to a positive response.

Thank you for your kind consideration.

Firenze, December 18, 2023

Leonardo Sacconi

A handwritten signature in black ink, appearing to read 'Leonardo Sacconi', written over a horizontal line.

EDITOR COMMENTS

While both reviewers found your paper interesting and a good fit for the Journal, they raised several concerns, which should be addressed in full in your revision. Concerns include potential alterations in TT properties and medium composition due to the experimental procedures, e.g., can it be excluded that the APs generated are mainly based on the inward current created by the optogenetic channel and not by Na currents? Control measurements have been suggested, which the authors should consider.

We thank the editor for emphasizing these important aspects. By conducting additional experiments using the patch clamp technique and performing a fresh analysis of T-tubular action potential amplitudes, we can confirm that action potentials are generated by voltage-dependent channels with an activation threshold. This finding rules out the possibility that the AP-like upstroke is provoked by ChR2-induced inward currents.

REFEREE COMMENTS

Referee #1:

The paper by Scardigli and colleagues employs patch-clamp, optogenetic methods and optical measurements of action potentials to demonstrate that isolated t-tubules are excitable. To isolate t-tubules they use an osmotic approach that leads to disruption of t-tubules from the surface membrane resulting in intracellular t-tubule vesicles that can be excited optically. The results presented are interesting and the authors used highly sophisticated methods. The manuscript is well written and edited. Major comments:

Obviously, decoupled t-tubules form some kind of intracellular vesicles or tubules and their lumen contains extracellular solution. The composition of the latter is critical to generate physiological driving forces for various ions and finally for generation of action potential. How is this maintained after several excitations? Do these vesicles contain the full set of transporters and channels such as NKA or NCX?

We thank the reviewer for underling this point. From a methodological perspective, we cannot 'clamp' T-tubule luminal ion concentrations after T-tubule detachment. Therefore, we carried out T-tubule detachment (by formamide shock) on quiescent myocytes, and all our observations are based on a strictly limited experimental window. These aspects are more fully explained and discussed in the revised manuscript (from line 326 to 350).

How can the authors exclude that the APs in TT are mainly driven by the depolarizing optogenetic channels. I_{Na} -channels in TT are probably not high enough to trigger an AP under physiological condition.

We thank the reviewer for highlighting this fundamental aspect. A fresh analysis of TT responses to stimulation show a clear bimodal distribution of silent and AP-generating TT (see new Figure 4). This can best be explained by a mechanism where TT action potentials generation is driven by voltage-dependent ion channels with an activation threshold, rather than generation of action potential-like upstrokes by ChR2-induced inward currents. This finding is in line with the uniform distribution of I_{Na} density that we have found across the sarcolemma (Figure 1D).

This conclusion is further supported by conducting new patch clamp experiments on single cardiomyocytes, where the effect of ChR2-induced photocurrent on membrane potential was evaluated in the presence or absence of Na^+ and Ca^{2+} channel blockers (new Figure 4D). The clear binary response, present in cells with intact threshold-dependent excitation mechanisms is absent in cells whose depolarisation relies simply on photocurrents. The novel analysis and the new experiments are presented and discussed in the revised manuscript (from line 291 to 315).

Did the authors apply drugs such as $I_{Ca,L}$ -blockers, I_{Na} -Blockers or I_K -blockers to demonstrate which channels are involved in formation of TT-Aps?

We conducted a new series of patch clamp experiments on isolated cardiomyocytes using $I_{Ca,L}$ - and I_{Na} -blockers to dissect the impact of ChR2 current on membrane potential variation. The idea of employing the same approach during optical measurements is very intriguing and logical; however, due to the low efficiency associated with optogenetic stimulation of TT action potentials (approximately 10 %), obtaining solid statistical power for these population would require a large number of cells and, consequently, a large number of mice. Considering that Na^{2+} -related inducibility of TT action potentials has already been demonstrated (see previous point), we found these measurements to be in contrast with the ethical assessment of the 3R principles.

Did the authors perform immunofluorescent labelling of various ion channels in TT?

A vast number of immunofluorescent studies have described TT distribution of key membrane proteins involved in excitation–contraction coupling. These studies describe the localization (in mouse) of $NaV1.5$ (Vermij *et al.*, 2020) and $CaV1.2$ (Gadeberg *et al.*, 2017), K^+ currents, e.g. $Kir2.1$

(Clark *et al.*, 2001) as well as background currents such as those carried by NCX (Gadeberg *et al.*, 2017) or NKA (Bossuyt *et al.*, 2009). However, the locally resolved quantification of immunofluorescence data in ventricular myocytes is challenged by the complex geometry of the t-system. Thus, the validated functional approach based on patch-clamp recordings was adopted here to study the general distribution of ion flux pathways using formamide-induced detubulation. By comparing the loss of cell capacitance (a read-out of membrane area) with the loss of membrane currents following detubulation, the fraction of individual membrane currents within the TT were calculated.

Figure 4: In how many TT was it possible to initiate an AP using electrical stimulation?

We previously found that in detubulated cells, approximately 60% of TT do not generate action potentials upon electrical stimulation (Sacconi *et al.*, PNAS 2012). This matches the fraction of TT that cannot be stained with an extracellularly applied membrane dye such as Di-3-ANEPPDHQ (Fig. 1A). For individual TT, we used electrical stimulation to assess whether a specific TT was indeed electrically uncoupled from the surface after formamide exposure (if so – field stimulation did not elicit an AP). This was done to rule out that that action potentials during optical stimulation could have been propagated from the cell surface.

Atrial TT structure including axiale tubules and potential relevance for atrial fibrillation should at least be discussed: doi: 10.1152/ajpheart.00284.2011; doi: 10.1172/JCI88241

Peculiarities of TT structure found in atrial cardiomyocyte are now highlighted in the discussion (lines 378-384).

Referee #2:

Scardigli *et al.*, present a study which shows that cardiac transverse tubules are intrinsically excitable in ventricular myocytes. This provides insight in the field of cardiac physiology where this has been never experimentally shown before, just suspected. By developing an optical platform, t-tubules can be activated using random-access multi-photon microscopy in cardiac myocytes containing light activated ion channels. Computer modelling add values to the experimental work. The impact is not groundbreaking but it adds to our present knowledge of t-tubules and confirm the

current view. The paper is well written, the study design is innovative and the experimental data are robust. The data support the conclusion. I have only minor comments:

The authors propose that low excitability of t-tubules can be due to change in Na⁺ conductance after detubulation (top of page 10). They use the word "currents" instead of conductance which is confusing. One might expect an increase of excitability with an increase of Na⁺ currents. However, the authors relate it not to NaV1.5 but other channels (conductance) like stretch activated, late or background. I suggest rewording the sentence.

Thanks for the comment: the word "currents" instead of "conductance" was misleading. The text has been substantially modified also in line with other comments on this issue raised by the reviewers (lines 326-350).

Albeit computer modelling confirms this hypothesis (Figure 5D), an alternative explanation is missing. It is possible that Na⁺ concentration in the vacuolated t-tubules is changed because of the presence of NCX which is still active (see Despa et al, 2003, Biophysical Journal, 85(5) 3388-3396). Depending on the experimental protocol, it may decrease Na⁺ concentration in the vacuole therefore reducing the driving force for NaV1.5 current.

Yes, the increase in TT g_{Na,b} simulated in Fig S5 (ex. Fig 5D) has a phenomenological significance but does not reflect a unique molecular mechanism. Indeed, we agree with the reviewer that the role of electrogenic transporters (such as NCX and NKA) is fundamental in the maintenance of myocyte Na⁺ homeostasis and the electrical activity at the t-tubules. This results in a complex scenario that is now more fully described in the discussion section (lines 326-350). Thank you!

On the same topic, the legend of Figure 5C is not accurate. It indicates that using light it is possible to trigger AP but it is not the case on the figure 5C. Please modify either the legend or the representative traces.

Many thanks – Figure 5 has been now significantly modified to address other comments.

Methods: the authors used Ca buffer (EGTA) and CaCl₂ in the different pipette solution to clamp bulk intracellular calcium. Please provide info on the calcium concentration in the cell, using maxchelator: <https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/>

The calcium concentration in the cell has been calculated and reported in the manuscript (lines 179 and 187).

There are few typos in the manuscript for example INTRODUCCION, missing space before reference, please double check.

Typos and missing space before reference are fixed. Thank you.

References

- Berry RG, Despa S, Fuller W, Bers DM & Shattock MJ. (2007). Differential distribution and regulation of mouse cardiac Na⁺/K⁺-ATPase alpha1 and alpha2 subunits in T-tubule and surface sarcolemmal membranes. *Cardiovascular research* **73**, 92-100.
- Bondarenko VE, Szigeti GP, Bett GC, Kim SJ & Rasmusson RL. (2004). Computer model of action potential of mouse ventricular myocytes. *Am J Physiol Heart Circ Physiol* **287**, H1378-1403.
- Bossuyt J, Despa S, Han F, Hou Z, Robia SL, Lingrel JB & Bers DM. (2009). Isoform specificity of the Na/K-ATPase association and regulation by phospholemman. *The Journal of biological chemistry* **284**, 26749-26757.
- Clark RB, Tremblay A, Melnyk P, Allen BG, Giles WR & Fiset C. (2001). T-tubule localization of the inward-rectifier K(+) channel in mouse ventricular myocytes: a role in K(+) accumulation. *The Journal of physiology* **537**, 979-992.
- Despa S & Bers DM. (2007). Functional analysis of Na⁺/K⁺-ATPase isoform distribution in rat ventricular myocytes. *American journal of physiology Cell physiology* **293**, C321-327.
- Gadeberg HC, Kong CHT, Bryant SM, James AF & Orchard CH. (2017). Sarcolemmal distribution of I(Ca) and I(NCX) and Ca(2+) autoregulation in mouse ventricular myocytes. *American journal of physiology Heart and circulatory physiology* **313**, H190-H199.
- Horvath B, Hezso T, Kiss D, Kistamas K, Magyar J, Nanasi PP & Banyasz T. (2020). Late Sodium Current Inhibitors as Potential Antiarrhythmic Agents. *Front Pharmacol* **11**, 413.
- Moench I & Lopatin AN. (2014). Ca(2+) homeostasis in sealed t-tubules of mouse ventricular myocytes. *Journal of molecular and cellular cardiology* **72**, 374-383.
- Pasek M, Simurda J & Christe G. (2006). The functional role of cardiac T-tubules explored in a model of rat ventricular myocytes. *Philosophical transactions Series A, Mathematical, physical, and engineering sciences* **364**, 1187-1206.
- Swift F, Tovsrud N, Enger UH, Sjaastad I & Sejersted OM. (2007). The Na⁺/K⁺-ATPase alpha2-isoform regulates cardiac contractility in rat cardiomyocytes. *Cardiovascular research* **75**, 109-117.

- Thomas MJ, Sjaastad I, Andersen K, Helm PJ, Wasserstrom JA, Sejersted OM & Ottersen OP. (2003). Localization and function of the Na⁺/Ca²⁺-exchanger in normal and detubulated rat cardiomyocytes. *Journal of molecular and cellular cardiology* **35**, 1325-1337.
- Vermij SH, Rougier JS, Agullo-Pascual E, Rothenberg E, Delmar M & Abriel H. (2020). Single-Molecule Localization of the Cardiac Voltage-Gated Sodium Channel Reveals Different Modes of Reorganization at Cardiomyocyte Membrane Domains. *Circulation Arrhythmia and electrophysiology* **13**, e008241.
- Ward ML, Williams IA, Chu Y, Cooper PJ, Ju YK & Allen DG. (2008). Stretch-activated channels in the heart: contributions to length-dependence and to cardiomyopathy. *Prog Biophys Mol Biol* **97**, 232-249.
- Zaglia T, Pianca N, Borile G, Da Broi F, Richter C, Campione M, Lehnart SE, Luther S, Corrado D, Miquerol L & Mongillo M. (2015). Optogenetic determination of the myocardial requirements for extrasystoles by cell type-specific targeting of ChannelRhodopsin-2. *Proc Natl Acad Sci U S A* **112**, E4495-4504.

Dear Dr Sacconi,

Re: JP-RP-2023-285202R1 "Optogenetic confirmation of transverse-tubular membrane excitability in intact cardiac myocytes" by Marina Scardigli, Michal Pasek, Lorenzo Piantini, Chiara Palandri, Emilia Conti, Claudia Crocini, Marina Campione, Leslie M Loew, Antoine de Vries, Daniel Pijnappels, Francesco Pavone, Corrado Poggesi, Elisabetta Cerbai, Raffaele Coppini, Peter Kohl, Cecilia Ferrantini, and Leonardo Sacconi

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

TRANSPARENT PEER REVIEW POLICY: 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 Editors' comments and referee reports, for each version of the manuscript, as well as 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 (or similar) version of the manuscript provided will be used by the Production Editor 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 thoroughly checked and corrected as promptly as possible.

Authors should note that it is too late at this point to offer corrections prior to proofing. Major corrections at proof stage, such as changes to figures, will be referred to the Editors for approval before they can be incorporated. Only minor changes, such as to style and consistency, should be made at 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 30,000 followers!

Yours sincerely,

Natalia Trayanova
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. You can 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.

The Corresponding Author 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 order.

You can check if your 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>.

EDITOR COMMENTS

Reviewing Editor:

Ethics Concerns:

I do not see ethical concerns with this paper.

Comments to the Author:

Thank you for revising this paper in accordance with both reviewer requests.

REFEREE COMMENTS

Referee #1:

The authors addressed my comments convincingly and I have no further suggestions.

Referee #2:

No further comments.

1st Confidential Review

18-Dec-2023
