The Journal of Physiology

Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice.

Jean-Baptiste Cavin, Preedajit A Wongkrasant, Joel A Glover, Onesmo Begira Balemba, Wallace K. MacNaughton, and Keith A Sharkey
DOI: 10.1113/JP284171

Corresponding author(s): Keith Sharkey (ksharkey@ucalgary.ca)

The following individual(s) involved in review of this submission have agreed to reveal their identity: Ken D O'Halloran (Referee #1); Gemma Mazzuoli-Weber (Referee #3)

Review Timeline:

Submission Date:	24-Nov-2022
Editorial Decision:	20-Dec-2022
Revision Received:	16-Jan-2023
Accepted:	17-Jan-2023

Senior Editor: Harold Schultz

Reviewing Editor: Michel Neunlist

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 Sharkey,

Re: JP-RP-2022-284171 "Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice." by Jean-Baptiste Cavin, Preedajit A Wongkrasant, Joel A Glover, Onesmo Begira Balemba, Wallace K. MacNaughton, and Keith A Sharkey

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 3 expert referees and we are pleased to tell you that it is acceptable for publication following satisfactory 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 4 weeks. If you require longer than this, please contact journal staff: jp@physoc.org.

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,

Harold D Schultz
Senior Editor
The Journal of Physiology
https://jp.msubmit.net
http://jp.physoc.org
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London, EC1R 3AW
UK
http://www.physoc.org
http://journals.physoc.org

REQUIRED ITEMS:

-Please check your supporting information files. We have a note that they may need updating?
See our Supporting Information Guidelines for permissible types of supporting information.

-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.

-The Reference List must be in Journal format

-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

-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

-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

Reviewing Editor:

All reviewers recognize the scientific quality of the study and the added value of the additional experiments performed.

The authors are to be congratulated for this important study.

Senior Editor:

Thank you for submission of your revised manuscript to the Journal of Physiology. The revision is a major improvement and deemed worthy for publication in the Journal with no further revision. However, the authors will need to submit the required Statistical Summary Document before it can be accepted for publication.

REFEREE COMMENTS

Referee #1:

Thank you for submitting your manuscript to The Journal of Physiology. All issues relating to ethics and welfare are appropriately described in the manuscript.

Referee #2:

The authors have significantly revised their manuscript. They have added additional data which has greatly strengthened the study. The authors have addressed all reviewer concerns/comments adequately.

Referee #3:

General comment

I would like to make my compliments to the authors who have done a pioneering work and a very meaningful study in the field of neurogastroenterology. This study is describing, with state of the art live-cell confocal imaging, enteric jejunal and colonic neuronal activity, in terms of intracellular calcium trafficking in response to different stimuli such as KCl, veratridine, DMPP and luminal nutrients. The experiments have been with different mice reporter (Wnt1-, ChAT- and Calb1-GCaMP6). Results (very nice figures and explicative video material) show that not only the neuronal responses, but also their patterns and kinetics are dependent on the level of the intestinal wall distention. Mechanosensitive channels, in particular BK channels, regulating intracellular calcium level, are likely to be involved in this mechanism. The text is clear, good written, without redundancy. The discussion is well structured, linear, the main pitfall are argued well.

Thus, the authors demonstrated that the distention level of the intestine regulates the excitability of the enteric neurons, a novel concept and a very important piece of information to understand the physiology of the intrinsic neuronal circuits regulating motility.

_____

END OF COMMENTS

Confidential Review **24-Nov-2022**

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

Department of Physiology & Pharmacology
Health Sciences Centre, Room 2037

Telephone: (403) 220-4601
Email: ksharkey@ucalgary.ca

January 16, 2023

Harold Schultz
Senior Editor
Journal of Physiology

Dear Dr. Schultz:

Thank you for the provisional acceptance of our manuscript entitled "Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice" for publication in the Alimentary Section of *The Journal of Physiology*.

We were delighted that the reviewers found the paper to be of merit with no further revision and we are grateful for the opportunity to publish our work in the *Journal.*

As requested, we have revised the manuscript to add all the supporting data into the paper. This has resulted in 2 additional Figs, and revision to some of the others and their respective figure legends (shown on the marked file). We have added a graphical abstract, the statistical summary and the first author's biosketch and photos. In addition, we revised the references to be in the correct format and we corrected a minor mislabelling on a 4 of the supplementary video files (uM, instead of um).

We are requesting to be allowed one supporting information file for the design of the imaging chamber and the associated 3D printer files. By including these purely technical files we allow for others to reproduce our work using similar equipment.

Finally, we are very pleased also to submit two potential cover art images (same image in different colours).

Thank you for accepting our paper for publication in *The Journal of Physiology*.

With best wishes

Yours sincerely,

Keith Sharkey.

Keith A. Sharkey, Ph.D., CAGF, FCAHS
Professor

Dear Dr Sharkey,

Re: JP-RP-2023-284171R1 "Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice." by Jean-Baptiste Cavin, Preedajit A Wongkrasant, Joel A Glover, Onesmo Begira Balemba, Wallace K. MacNaughton, and Keith A Sharkey

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. 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 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,

Harold D Schultz
Senior Editor
The Journal of Physiology
https://jp.msubmit.net
http://jp.physoc.org
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London, EC1R 3AW
UK
http://www.physoc.org
http://journals.physoc.org

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

Thank you for your final edits to the manuscript. The supporting information files are fine. Congratulations on a very detailed and insightful study.

30-Nov-2021

JP-RP-2021-282464 The Journal of Physiology Decision Letter

Dear Dr Sharkey,

Re: JP-RP-2021-282464 "Intestinal distension orchestrates neuronal activity in the enteric nervous system of adult mice" by Jean-Baptiste A Cavin, Joel A Glover, Wallace K. MacNaughton, and Keith A Sharkey

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by three Referees and the reports are copied below.

I regret to say that the manuscript has not been accepted for publication.

Some positive comments were made on the manuscript. Unfortunately, they did not outweigh the more serious criticisms which led the Reviewing Editor to recommend rejection.

I am sorry to have to pass on this disappointing news, and hope it will not discourage you from making future submissions of new work to The Journal of Physiology.

However, we believe your manuscript is worthy of further consideration and suggest that you transfer your manuscript to Physiological Reports (https://physoc.onlinelibrary.wiley.com/hub/journal/2051817X/aims-and-scope/read-full-aims-and-scope), a peer-reviewed, open access, interdisciplinary journal, jointly owned by the American Physiological Society and The Physiological Society.
To transfer your manuscript to Physiological Reports, the corresponding author must send authorization within 120 days of receipt of this letter. Please use this link Transfer to Physiological Reports to send an authorization email to transfer your manuscript. If your manuscript does not require additional peer review, the editors of Physiological Reports will aim to give you an initial decision within 3 working days. In fact, >80% of transferred submissions are accepted for publication. Please note, of course, that we cannot guarantee final acceptance.

I hope you will take advantage of the opportunity to allow the editors of Physiological

Reports to evaluate your manuscript.

You may be able to publish Open Access with no direct cost to yourself. You can check your eligibility here https://secure.wiley.com/openaccess?

Yours sincerely,

Editor-in-Chief
The Journal of Physiology
https://jp.msubmit.net
http://jp.physoc.org
The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London, EC1R 3AW
UK
http://www.physoc.org
http://journals.physoc.org

****

EDITOR COMMENTS

Reviewing Editor:

Thank you for submitting your work to the Journal of Physiology. The manuscript has been reviewed by 2 expert referees. In this instance, while one referee found merit in your work, particularly the novel and integrated experimental design, another referee found serious concerning elements in the manuscript which upon review I concur with. In its current state, we are unable to proceed further with review of the manuscript for The Journal. At present, the manuscript represents an important methodological tool to explore ENS integration in response to various mechanical and chemical signals, this may be of interest as a preliminary methods paper in our sister journal Physiological Reports but the findings in the paper are too preliminary to be of high impact to warrant publication in The Journal of Physiology. In particular, the lack of identification of the specific neuron populations that are activated or inactivated during distention is of concern and a major gap of the study. In addition, while the authors speculate on the nature of the link between distention and Ca2+ regulation, this would need to be experimentally explored in some detail in order for the paper to be considered of sufficient impact.

****

REFEREE COMMENTS

Referee #1:

Comments for Author (Required):

Thank you for submitting your manuscript to The Journal of Physiology. There are some minor but important revisions to the text required pertaining to animal ethics and welfare.

Please start the Methods section with the sub-heading "Ethical approval". Thank you for providing full details of ethical approval, source of the animals and general housing conditions.

Please include the concentration of isoflurane used to induce deep anaesthesia. Please also include detail of the carrier gas.

Referee #2:

General comments:

The general aim of this study was to evaluate the changes in neuronal excitability (to KCl, veratramine, nutrient infusion) in response to distension. The authors utilized calcium imaging techniques in mice expressing GCaMP6s specifically in neurons (Wnt1-GCaMP mice) or IPANs (Calbindin1-GCaMP mice).

The rationale for the study is well thought out and the experiments that have been performed to test the hypothesis are appropriate. The novel techniques used to examine the hypothesis are eloquent and highly innovative. The findings are mostly discussed well, and appropriate references have been included. No major flaws are noted. The findings of this study will advance the field by providing important information regarding how neurons respond to distension. There are however some relatively minor concerns that need to be addressed to improve the overall quality of this study. These are outlined in the specific comments below.

Specific comments:

Methods:

- p.7, line 7. Does nicardipine do anything to this type of Ca2+ signaling in enteric neurons? Since L-type calcium channels can be activated by stretch/distention it's important to clarify this.

- p.7, description of preparation. It would be helpful to include "a piece of intestine" in the illustration in chamber design or could more easily refer to Fig. 1A in addition to this.

Results:

- p.13, line 17. It should be stated what veratridine is and why it was used specifically. Also, why was 75mM chosen as the concentration for KCl? Is this an increase to 75mM or is it an additional 75mM added to the solution? Does this alter the osmolarity or mucosal activity in any way that might affect the neuronal behavior? Are similar effects seen at a lower concentration such as 60mM? Please clarify.

- Fig. 1 and throughout. It is difficult to see the dashed red line, perhaps the authors could use a different color or thicken the line to make it more visible.

- Fig. 3. How were the three different responses in proximal colon summarized in panel B? Were they added together? If so, it would be nice to have a summary of each type of activity independently as a supplemental figure/data point. Is there any correlation between the type of response and the distance from the cecum?

- Fig. 3 onwards. Why was KCl chosen over veratridine? A sentence indicating why now only looking at KCl responses would be a nice addition.

- Fig. 4C. At least one additional experiment should be carried out (currently n=2) for statistical evaluation ("virtually identical...compared to Fig. 3" is not quantitative or definitive).

p.21, last line and Fig. 6 legend. There are different values indicated for the average speed of the calcium wave propagating in the circumferential direction (i.e., 480 vs. 470 um.min-1).

- Fig. 7 and Table 2. It would be good if the authors were able to complete at least one additional experiment evaluating distension with nutrient administration since this is currently n=3 but n=4 for TTX and n=8 for control.

- Supplemental video files. In videos where veratridine or TTX are used, um is used instead of uM. Please correct. This also needs to be corrected for TTX in Figs. 4 and 7.

Discussion:

- General comment. The discussion is very well written but it would be good if the authors could include a short section speculating how these changes in neuronal activity with

distention translate to motility, absorption, etc. and why these changes may occur (are they more beneficial or detrimental?). A statement relating the findings to what occurs in patients with chronic constipation that have distended colons for example would be a valuable addition and a great way to translate this work to a focus for clinical studies.

Overall, this is an elegant study that has approached the topic in a novel and innovative fashion. The conclusions presented are valid based on the experimental evidence provided. This study adds to our knowledge and understanding of how mechanical changes are detected and regulated.

Referee #3:

I have some difficulties with this paper, including in the way the work is described. The authors make comparisons to empty segments. What do they mean by empty? Are the segments actually empty? Or do they mean undistended? If they mean undistended, that is the term they should use. If they mean empty in some other sense, this should be defined.

They also refer to the intestine with normal luminal contents. What do they mean? Do they mean containing oxygenated Krebs solution? This is not the normal content of the jejunum or the colon (for one thing, the lumen is anoxic in vivo). If they mean containing Krebs solution, this is what they should say. If they mean something different by "normal luminal contents", this should be defined.

They refer to "differences in neuronal intracellular Ca2+ homeostasis" (page 13). The study is hardly done under conditions in which homeostatic controls, in a physiological sense, are exerted by the organ. I suggest removing homeostatic.

In Fig 1C there are provided "Representative traces of neuronal Ca2+ fluorescence changes in the myenteric plexus". Are these traces from individual neurons, or averaged traces each from a region of myenteric plexus, as suggested by the description? It looks like there are about 7 traces for KCl. The description of Fig 2A has the same ambiguity. I apologize if this is made clear somewhere else in the manuscript. It would assist to have it in the figure legend.

Responses to KCl peak at 2 minutes in some cases, and sometimes last as long as 4 minutes. It is quite a long response to a high concentration of KCl, 75 mM, that neurons would not normally encounter. Perhaps the authors could consider whether there might be depolarization block, and failure of synaptic transmission.

Responses to veratridine, a toxin that blocks Na channel inactivation, causing a prolonged inward current and increased intracellular Ca, lasted over 5 minutes in some cases. The authors might comment on whether this may compromise neuronal function, including

activation of intrinsic sensory neurons and neuro-neuronal communication.

It would be valuable to know how long KCl or veratridine was present in the bath, whether they were flushed out, what concentrations they achieved and how the concentrations varied over time.

What was the composition of the Krebs' solution. Was it oxygenated on its way to the lumen?

The authors conclude that they have discovered that intestinal distension in the small and large intestines affects most of the neurons in the ENS by locally modulating their levels of intracellular Ca. This is written as if modulating intracellular Ca is the primary effect of distension. The authors might be encouraged to discuss whether there may be steps between distension and Ca responses, for example the release of active substances such as hormones from the mucosa or the release of neurotransmitters.

There is inadequate description of the nutrient solution, which is described as containing (in g per 100mL) 2 protein, 1.4 lipids, 7.4 sugar. Which sugar? Is this an absorbable sugar? Is it a sugar that is detected by sweet taste receptors in the jejunum or colon? What is the protein? What are the lipids? This information might help with the interpretation of the data of Table 2.

The authors state that "our study invites the development of more integrated research approaches to study how the ENS responds to the complex mechanical and chemical inputs it receives from the intestinal lumen". This would be more convincing if the neurons that responded were identified, for example as motor neurons, interneurons or putative intrinsic sensory neurons.

Kunze has described enteric neurons that are inhibited by mechanical forces (Kunze, J. Physiol 526: 375-385,2000).

In order to generate intraluminal distension, the authors transiently interrupted the withdrawal from the anal output, while keeping the oral input flowing. When the distension attained the desired amount (by visual estimation), the withdrawal on the anal end was resumed. Relaxation was attained by interrupting the perfusion flow while keeping the withdrawal on. It would be very important to know the luminal pressures achieved, their time-resolved changes and how the pressure relate to physiologically observed intraluminal pressures.

****