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Circadian regulation of glutamate release pathways shapes synaptic throughput in the brainstem nucleus of the solitary tract (NTS).

Forrest J Ragozzino, BreeAnne Peterson, Ilia Nicholas Karatsoreos, and James Henry Peters
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Marian H Lewandowski (Referee #2)

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(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 Peters,

Re: JP-RP-2023-284370 "Circadian regulation of glutamate release pathways shapes synaptic throughput in the brainstem nucleus of the solitary tract (NTS)." by Forrest J Ragozzino, BreeAnne Peterson, Ilia Nicholas Karatsoreos, and James Henry Peters

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 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.

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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,

David Wyllie
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.

- An ethics approval number and more details on euthanisation are needed. You must start the Methods section with a paragraph headed Ethical Approval. A detailed explanation of journal policy and regulations on animal experimentation is given in Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818). A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: https://physoc.onlinelibrary.wiley.com/hub/animal-experiments. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015). The Methods section must contain details of the anaesthetic regime: anaesthetic used, dose and route of administration and method of killing the experimental animals.

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

In summary:

- If n â‰¤ 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.

- 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:

Autonomic reflexes that are mediated by the vagal nerve are under strong circadian regulation, but the underlying physiological mechanisms for the rhythms are not well-understood. This study uses patch-clamp methods in an ex vivo preparation to examine the mechanisms of circadian regulation of transmission from the vagal afferents onto neurons in the brainstem nucleus of the solitary tract (NTS). Two important changes are observed which, interestingly, have opposite dependences with the light-dark cycle. The first is a higher frequency of spontaneous glutamate release events that causes NTS neurons to have a higher basal firing rate during light, while the second is a higher afferent-evoked firing rate for NTS neurons during dark that is due to reduced membrane conductance. The authors suggest a number of potential physiological functions that could be associated with these opposing changes in basal versus evoked transmission.

The manuscript has been reviewed by two expert reviewers, both of whom were very positive about the significance of the study. The reviewers also raised no significant objections around the quality or interpretation of the experiments. In my own analysis, I found one issue around the error bars in the figures and the error values in the main text. I could not find information to indicate whether they reflect standard deviations (SDs) or standard errors. They should all be SDs, and this should clearly indicated in the Methods and the first time the convention is used in the results. The authors should address this and the other minor points raised by the reviewers.

Senior Editor:

Your manuscript has been assessed by two expert referees and a Reviewing Editor. As you will read, each are very positive about this work and suggest only a relatively few changes/clarifications. Please pay particular attention to you Statistics Policy and report SD (not SEM) and provide precise P values unless less than 0.0001. In addition, you will need to provide a Statistics Summary Document with the revised submission. Thanks you for submitting this work to The Journal of Physiology.

REFEREE COMMENTS

Referee #1:

In this study, the authors examine circadian regulation of neurotransmitter release in the brain stem nucleus of the solitary tract. They observe strong circadian rhythmicity of spontaneous quantal glutamatergic neurotransmission onto NTS neurons. Where highest rate of release during the light phase and lowest in the dark. Interestingly, this circadian oscillation of spontaneous release was sufficient to regulate action potential firing. They also observe that the regulation of evoked release was completely the opposite where it was enhanced through the night and diminished during the day. The presynaptic changes that regulate evoked release were also coupled with alterations in conductance.

Overall, I do not have much to add to this work. The authors could have validated that the fluctuations they see are indeed due to circadian rhythms by testing them in a mutant (e.g. CLOCK mice). However, I strongly believe this is beyond the scope of the current work. I believe this is an extremely important study and to my knowledge it is the first of its kind. Synaptic transmission is typically monitored in short time frames, often in response to brief stimulation patterns and responses are followed for up to a maximum of a few hours. This study therefore opens up a new avenue focusing on long term oscillation and/or stability of neurotransmission patterns in an intact circuit. Therefore, it has implications beyond the NTS. Authors may want to comment on this in the discussion. In particular, given the proposed roles of spontaneous neurotransmission in homeostatic plasticity, its long-term rhythmicity may also impact synaptic strength.

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Referee #2:

The reviewed manuscript deals with very topical issues related to the mechanisms of biological rhythms. Novel findings question the dominance of one oscillator, giving evidence for the involvement of SCN-independent, endogenous neuronal and non-neuronal clocks, located in multiple brain structures and throughout the body. One of these structures, which is the subject of research by Peters' group, is the nucleus of the solitary tract (NTS) in the brainstem. The authors, using ex vivo patch clamp electrophysiology methods, studied the circadian profile of neurotransmission activity in the NTS and its synaptic connection. They showed a clear circadian spontaneous secretion of glutamate, which is the reason for the day-night activity of action potentials generated in the postsynapse. They also determined diurnal changes in postsynaptic membrane conductance that promote circadian neurotransmitter secretion and synaptic communication under changing conditions of light and dark. In my opinion, the obtained results have a great impact on chronobiology research, in particular on understanding its neural mechanisms. And considering that the NTS receives information via the vagus nerve from peripheral organs to ensure vital body functions (nutrition, energy balance and other autonomic regulation), the presented results are of great importance for understanding the physiology of our body. The originality of the presented studies lies in the fact that they are the first electrophysiological studies of NTS and its connections. They complement previous research, especially by the group of Chrobok et al., 2020, which the authors often refer to.

I have no comments on the research methodology, analysis of results and their clear presentation. The discussion is conducted objectively and carefully, based on the results obtained earlier, with an indication of those elements that require further in-depth research.

Minor remarks

The author should carefully follow the reference:

Lines 568 and 640 are repeated lines 662 and 665.

Also release probabilities (Pr) are marked by the author sometimes with an apostrophe (lines 478, 493) and once without (line 472).

END OF COMMENTS

Confidential Review **12-Jan-2023**

JOURNAL OF PHYSIOLOGY

MANUSCRIPT: JP-RP-2023-284370

TITLE: “Circadian regulation of glutamate release pathways shapes synaptic throughput in the brainstem nucleus of the solitary tract (NTS).”

AUTHORS: Forrest J. Ragozzino, BreeAnne Peterson, Ilia Nicholas Karatsoreos, and James Henry Peters

RESPONSE TO THE REVIEWERS / EDITORIAL COMMENTS:

We are thrilled with feedback from the reviewers and decision by the editors. Thank you for the careful review and support of this line of investigation. We have noted our replies to the specific comments below and the associated changes in the manuscript.

REFEREE COMMENTS

Referee #1:

In this study, the authors examine circadian regulation of neurotransmitter release in the brainstem nucleus of the solitary tract. They observe strong circadian rhythmicity of spontaneous quantal glutamatergic neurotransmission onto NTS neurons. Where highest rate of release during the light phase and lowest in the dark. Interestingly, this circadian oscillation of spontaneous release was sufficient to regulate action potential firing. They also observe that the regulation of evoked release was completely the opposite where it was enhanced through the night and diminished during the day. The presynaptic changes that regulate evoked release were also coupled with alterations in conductance.

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Thank you for the supportive comments and appreciation of the work. We have now added text in the discussion section addressing the final sentences in the second paragraph. These additional discussion points can be found on Page 22, lines 444-446 and Page 25, lines 519-521 of the revised manuscript.

***

Referee #2:

The reviewed manuscript deals with very topical issues related to the mechanisms of biological rhythms. Novel findings question the dominance of one oscillator, giving evidence for the involvement of SCN-independent, endogenous neuronal and non-neuronal clocks, located in multiple brain structures and throughout the body. One of these structures, which is the subject of research by Peters' group, is the nucleus of the solitary tract (NTS) in the brainstem. The authors, using ex vivo patch clamp electrophysiology methods, studied the circadian profile of neurotransmission activity in the NTS and its synaptic connection. They showed a clear circadian spontaneous secretion of glutamate, which is the reason for the day-night activity of action potentials generated in the postsynapse. They also determined diurnal changes in postsynaptic membrane conductance that promote circadian neurotransmitter secretion and synaptic communication under changing conditions of light and dark. In my opinion, the obtained results have a great impact on chronobiology research, in particular on understanding its neural mechanisms. And considering that the NTS receives information via the vagus nerve from peripheral organs to ensure vital body functions (nutrition, energy balance and other autonomic regulation), the presented results are of great importance for understanding the physiology of our body. The originality of the presented studies lies in the fact that they are the first electrophysiological studies of NTS and its connections. They complement previous research, especially by the group of Chrobok et al., 2020, which the authors often refer to.

I have no comments on the research methodology, analysis of results and their clear presentation. The discussion is conducted objectively and carefully, based on the results obtained earlier, with an indication of those elements that require further in-depth research.

Minor remarks

The author should carefully follow the reference:

Lines 568 and 640 are repeated lines 662 and 665.

Apologies, it is not clear to us what is indicated with the comment above. We have checked for repeated references and did not find any. Please advise and we are happy to update the manuscript.

Also release probabilities (Pr) are marked by the author sometimes with an apostrophe (lines 478, 493) and once without (line 472).

Thank you for pointing this out. All instances of P_r are now made uniform in the text.

EDITORIAL:

- 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.

This has now been provided for Dr. Ragozzino and uploaded with the resubmission.

- An ethics approval number and more details on euthanisation are needed. You must start the Methods section with a paragraph headed Ethical Approval. A detailed explanation of journal policy and regulations on animal experimentation is given in Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818). A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: https://physoc.onlinelibrary.wiley.com/hub/animal-experiments. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015). The Methods section must contain details of the anaesthetic regime: anaesthetic used, dose and route of administration and method of killing the experimental animals.

Attention to these issues in the above paragraph have now all been directly addressed in the revised manuscript.

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The Abstract Figure is now included in the revised submission and was made using the available access to BioRender (thank you!). The Abstract Figure Legend is included in the main draft of the manuscript in the Figure Legends section.

-"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:

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In summary:

- If n >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.

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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.

These issues have been addressed. Please let us know if there are any other details we need to attend to.

Thank you.

Dear Dr Peters,

Re: JP-RP-2023-284370R1 "Circadian regulation of glutamate release pathways shapes synaptic throughput in the brainstem nucleus of the solitary tract (NTS)." by Forrest J Ragozzino, BreeAnne Peterson, Ilia Nicholas Karatsoreos, and James Henry Peters

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Yours sincerely,

David Wyllie
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

The authors have addressed most of the minor concerns previously raised. One concern raised by a prior referee does still need to be addressed. Two of the references show up twice in the reference list -- the second listing of the two references are in lines 678 and 681 of the revised manuscript.

Senior Editor:

Thank you for revising your manuscript which I am delighted to accept for publication. You will see that there is still the issue with two references being duplicated in the reference list, but I think this can be dealt with at the Proof stage. Buijs, Scheer, et al correctly appears at Line 582 but is repeated, erroneously, at Line 678. Similarly, Konturek et al correctly appears at

Line 656 but is repeated, erroneously, at Line 681. Each easily corrected at Proof stage. Thank you for submitting this work to The Journal of Physiology.

1st Confidential Review **24-Feb-2023**