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Kappa opioids inhibit the GABA/glycine terminals of rostral ventromedial medulla projections in the superficial dorsal horn of the spinal cord

Yo Otsu and Karin R. Aubrey
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The referees have opted to remain anonymous.

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Senior Editor: Katalin Toth

Reviewing Editor: Samuel Young

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 Aubrey,

Re: JP-RP-2022-283021 "Characterisation of the inhibitory GABA/glycine projections of rostral ventromedial medulla neurons to the superficial dorsal horn of the spinal cord" by Yo Otsu and Karin R. Aubrey

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 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.

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Yours sincerely,

Katalin Toth
Senior Editor
The Journal of Physiology

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EDITOR COMMENTS

Reviewing Editor:

This study is an excellent characterization study of RVM rostral ventromedial medulla inputs into the superficial dorsal horn. Both reviewers agreed that the authors carried out experiments were technically sound, carefully controlled and thorough. However, the study largely recapitulates many previous published findings. Therefore, the lack of advancement in addressing a biological question is a glaring weakness in the paper and the characterization study alone is not a significant enough advancement. Given that Journal of Physiology is seeking to publish manuscripts that are likely to have a major impact on the field, this manuscript is better suited for another journal such as Physiological Reports.

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REFEREE COMMENTS

Referee #2:

The manuscript by Otsu and Aubrey describes an elegant set of experiments where rostral ventromedial medulla (RVM) neurons were labeled and transfected with optogenetic ChR2, and then descending projections were identified in the superficial dorsal horn (SDH) of the spinal cord. SDH neurons with associated RVM axons were patched and the electrophysiological profile of these neurons was assessed. Overall, the authors found that optogenetic activation of descending RVM neurons evoked exclusively inhibitory GABA/glycinergic currents in SDH neurons. The manuscript was clear, experiments well-designed, and a level of appropriate limitations and conclusions were provided. The data provide important further clarification and classification of modulation of sensory pathways by descending projections from the RVM.

Authors state in abstract "...confirm that RVM inputs onto SDH neurons are exclusively inhibitory...combination of

GABAergic and glycinergic neurotransmission". Although within the authors' data all observed inputs were seen as inhibitory, I would caution against such definitive statements denoting that all RVM inputs to SDH are inhibitory. Even though in this set of experiments the authors did not observe excitatory currents, this does not by necessity preclude their existence. Authors should temper this statement.

Similarly, an important limitation to be noted is that these experiments were conducted in otherwise normal and healthy rats. Is there any existent literature or data regarding models of chronic pain or allodynia suggesting modulation of these descending RVM-SDH pathways? This could be further explored in the discussion.

Authors state "Data from male and female rats were grouped and no sex differences were noted." Were sex differences simply not observed by the investigators under general observation of the data, or were statistical tests employed to evaluate the absence of sex differences followed by subsequent grouping of data? Even in the absence of observed sex-differences, authors may want to consider identifying sex within their figures (eg. Square symbols for males, circle for female - or something to a similar effect).

Referee #3:

The study by Otsu and Aubrey uses a robust optogenetic approach to investigate the synaptic properties of RVM inputs to the SDH. The techniques are appropriate for the scope of questions addressed and the characterization of neuronal properties is strong. However, this reviewer is unclear on the magnitude of advance this study represents.

In their work, the authors exclusively patch LII neurons in acute spinal cord slices and reveal that optogenetic activation of RVM axons / terminals within the slice exclusively produces inhibitory responses in the neurons. As the authors indicate, this is consistent with many previous studies for this region of the spinal cord. They further indicate that the opto-RVM postsynaptic responses recorded are a mixture of GABAergic and glycinergic ionotropic receptor activation, also consistent with previous reports. The authors correctly indicate that the most pressing question related to this system is the potential for complex RVM modulation of spinal cord activity, particularly where "bidirectional control can be achieved by altering the activity of inhibitory RVM inputs in the spinal cord". The current study adds to our understanding of the types of LII neurons innervated by RVM projection, but is unable to add to this important question.

Notably, the authors do a commendable job of characterizing the morphological and firing heterogeneity of LII neurons that received input from RVM projections. However, it is not clear whether or how the activity of RVM inputs can modulate the activity of these neurons in response to excitation. Nor is it clear that the neurons studied represent the most important targets of RVM innervation, which is shown to be in both LI and LIIo.

Both GlyR and GABAA are represented as Cl- permeable receptors. The impact of inhibitory input on SDH neuronal activity can be strongly modulated by changes in KCC2 activity, but this is not discussed in the report. Nor is the contribution of HCO_3^- flux via GABAA receptors considered as a possible factor in the analysis of postsynaptic responses or impact of disease state.

Overall, this study represents a solid characterization of a robust in vitro system for studying RVM input to the SDH and the authors are to be commended on their careful characterization. The work reaffirms many previous studies of this descending pathway. However, this work does not substantially advance to our understanding of the complexities of RVM modulation of pain.

Confidential Review **24-Feb-2022**

Dear Professor Tóth,

We appreciate the reviewers' comments and your assessment that the previous version of our paper titled "Characterisation of the inhibitory GABA/glycine projections of rostral ventromedial medulla neurons to the superficial dorsal horn of the spinal cord" was technically sound, carefully controlled and thorough, but not a significant enough advancement for publication in the Journal of Physiology.

We have now extended the paper to include additional data that investigates for the first time the modulation of this synapse by the opioid receptor agonists. We believe this inclusion, along with modifications suggested by the reviewers, now constitutes a more significant advancement of knowledge.

We have attached the extended manuscript now titled " Kappa opioids inhibit the GABA/glycine terminals of rostral ventromedial medulla projections in the superficial dorsal horn of the spinal cord " for your consideration.

Sincerely,

Karin Aubrey and Yo Otsu

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Thanks to the reviewers for their comments, our responses to the comments are *in italics* below

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EDITOR COMMENTS

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Authors' response: We have now extended the paper to include additional data that investigates for the first time the modulation of this synapse by the opioid receptor agonists. We believe this inclusion constitutes a more significant advancement of knowledge.

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Authors' response: This has been done as suggested

Similarly, an important limitation to be noted is that these experiments were conducted in otherwise normal and healthy rats. Is there any existent literature or data regarding models of chronic pain or allodynia suggesting modulation of these descending RVM-SDH pathways? This could be further explored in the discussion.

Authors' response: The potential links to neuropathic and inflammatory pain are now included in the discussion.

Authors state "Data from male and female rats were grouped and no sex differences were noted." Were sex differences simply not observed by the investigators under general observation of the data, or were statistical tests employed to evaluate the absence of sex differences followed by subsequent grouping of data? Even in the absence of observed sex-differences, authors may want to consider identifying sex within their figures (eg. Square symbols for males, circle for female - or something to a similar effect).

Authors' response: This has been clarified in the methods. In addition, a new supplementary fig 2 has been added to the paper which illustrates the effects of opioid receptor agonists on oIPSCs recorded from female and male rats. Thank you for your review.

Referee #3:

The study by Otsu and Aubrey uses a robust optogenetic approach to investigate the synaptic properties of RVM inputs to the SDH. The techniques are appropriate for the scope of questions addressed and the characterization of neuronal properties is strong. However, this reviewer is unclear on the magnitude of advance this study represents.

Authors' response: We have enhanced the significance of this paper by adding figures 6 and 7 and supplementary figures 1 and 2 which present the first investigation of the opioid modulation of the RVM to SDH synapse. We have now added this data to the paper as and extended the introduction and discussion sections.

In their work, the authors exclusively patch LII neurons in acute spinal cord slices and reveal that optogenetic activation of RVM axons / terminals within the slice exclusively produces inhibitory responses in the neurons. As the authors indicate, this is consistent with many previous studies for this region of the spinal cord. They further indicate that the opto-RVM postsynaptic responses recorded are a mixture of GABAergic and glycinergic ionotropic receptor activation, also consistent with previous reports. The authors correctly indicate that the most pressing question related to this system is the potential for complex RVM modulation of spinal cord activity, particularly where "bidirectional control can be achieved by altering the activity of inhibitory RVM inputs in the spinal cord". The current study adds to our understanding of the types of LII neurons innervated by RVM projection, but is unable to add to this important question.

Notably, the authors do a commendable job of characterizing the morphological and firing heterogeneity of LII neurons that received input from RVM projections. However, it is not clear whether or how the activity of RVM inputs can modulate the activity of these neurons in response to excitation. Nor is it clear that the neurons studied represent the most important targets of RVM innervation, which is shown to be in both LI and LIIo.

Authors' response: We agree that there is still a lot of work to be done to understand the mechanisms of how this descending pathway achieves bidirectional control of sensory inputs in the spinal cord. However, this paper is one of the few to isolate and directly record the synaptic currents generated by activation of RVM projections onto SDH target neurons and provides a comprehensive overview of its signalling. A major strength of this study is that it combines a thorough characterisation of the SDH target neurons' morphological and electrical properties with new data about the quality of descending inhibitory signals into the SDH and its modulation by opioids. As a result, our findings can be linked to previous work exploring SDH neuronal types and organisation.

Because we succeeded in recording from over 70 SDH target neurons (a significant achievement as the input is sparse, ~10% of the >700 SDH neurons tested had an oIPSC response), we are confident that our data includes the most important LII targets of the descending RVM input, including those that have been associated with pain and/or itch behaviours (Nguyen et al., 2022; Francois et al., 2017; Kato et al., 2006). We have included a more comprehensive discussion of the functional relevance of RVM to SDH signalling, and its modulation by opioids, in the final section of the discussion to highlight this. In addition, we have reiterated that LI and the deeper lamina are not addressed in this study in the methods and results sections.

This paper now adds knowledge to our understanding of the organisation of the RVM-spinal cord circuit and the mechanisms by which RVM neurons and opioids modulate neural activity at the spinal level.

Overall, this study represents a solid characterization of a robust in vitro system for studying RVM input to the SDH and the authors are to be commended on their careful characterization. The work reaffirms many previous studies of this descending pathway. However, this work does not substantially advance to our understanding of the complexities of RVM modulation of pain.

Authors' response: Thank you for your comments we hope the revised manuscript with additional experiments exploring the opioid modulation of this RVM to SDH synapse mitigates this concern.

Dear Dr Aubrey,

Re: JP-RP-2022-283021R1-A "Kappa opioids inhibit the GABA/glycine terminals of rostral ventromedial medulla projections in the superficial dorsal horn of the spinal cord" by Yo Otsu and Karin R. Aubrey

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

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Yours sincerely,

Katalin Toth
Senior Editor
The Journal of Physiology

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EDITOR COMMENTS

Reviewing Editor:

The authors have done an excellent job of addressing the previous concerns. The addition of the new data addresses a physiological question which now makes the manuscript appropriate for Journal of Physiology. Supplemental data should be moved to the main text.

REFEREE COMMENTS

Referee #2:

In this revision the authors have addressed all of my initial comments and suggestions. The inclusion of the novel studies further investigating the modulation of the system by opioids provides additional and novel import to their work.

I have no further comments or questions.

1st Confidential Review **08-Jun-2022**