Dear Dr. Markram,

Re: JP-RP-2021-281711 "Morphology, Physiology and Synaptic Connectivity of Local Interneurons in the Mouse Somatosensory Thalamus" by Jane Yi and Henry Markram

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

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

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

Yours sincerely,

Katalin Toth
Senior Editor
The Journal of Physiology
EDITOR COMMENTS

Commenting Editor:

Comments to the Author:
This paper by Li and Markram reports the results of a thorough electrophysiological and synaptic investigation of local interneurons of the mouse somatosensory ventral posterior thalamus. The experiments appear to have been expertly carried out and the results are fundamentally novel. This manuscript has been reviewed by two expert reviewers and they provide highly convergent evaluations. They raise a number of relatively minor concerns and the roadmap provided to address them appear to be reasonable.

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REFEREE COMMENTS

Referee #1:

Yi & Markram investigate the sparse population of inhibitory neurons in the mouse somatosensory thalamus. Though known previously to exist, these cells have remained almost entirely unstudied. The authors used acute brain slices to investigate the morphology, physiology, and synaptic connectivity of these cells. They showed that inhibitory ventroposterior thalamus (VPI) cells can inhibit thalamic relay neurons and each other. Physiological experiments are consistent with VPI synapses onto relay cells having both triadic and non-triadic arrangements with medial lemniscal synapses, similar to the wiring within LGN. They also observe both triadic and non-triadic configurations of medial lemniscal synapses for VPI-VPI connections.

The role of inhibitory cells in any thalamic nucleus, including LGN where they have been most studied, is not entirely settled. Investigations outside of LGN are therefore timely. The experiments appear well executed, and the data clearly presented. My comments are fairly limited.

1. Fig.1D is not clearly bimodal. This could simply be a skewed unimodal distribution. One could perform a comparison of the two models (skewed unimodal vs mixture of Normals) or perform another test of bimodality.

2. What fraction of cells had an axon branch that went to the slice edge? This could be a useful way of estimating how much/little of the axon might be truncated during cutting. Is it possible that cells had more expansive arbors outside of the slice? If there are no cut ends of the axon, this is unlikely. The authors could probably address this possibility quite readily. Related 1: How much time passed between the end of the recording and the perfusion of the slice? Could it be that the axons only appear limited because the tracer has had little time to diffuse? Related 2: “Cut” in Figure 1 appears to refer to major dendrites, but this is not made explicit. Please clarify somewhere such as the legend.

3. Why not report the total lengths of dendrite and axon observed? Were the dendrites completely smooth or just sparsely spiny?

4. P.10/Fig.4. Did relative positions of the cells affect the probability of finding a connection (i.e., if the relay cell was medial, lateral, dorsal, or ventral of the inhibitory cell? If the VPI-VPI pairs were more in a M-L or D-V arrangement?). These analyses seem straightforward.
5. Key points summary. "The function of..." The experiments describe physiological (functional) properties of these cells, but I would not say that this paper reveals the function of the cell type.

6. First line of Intro is probably not the right place to introduce the VPi abbreviation. Similarly, one probably should not use the abbreviation at the paragraph's end. At this point the authors are talking about thalamus in general, not VP. Could define the abbreviation in the second paragraph instead.

7. Methods, Animals. Please state exactly how many male mice and how many female mice were used.

8. P.9, "VP interneurons have high input resistances, likely due to their small soma sizes." Many neurons with similarly sized somata have been described and have much lower Rin. This seems unlikely to be the only possible explanation.

9. P.11, "To exclude direct excitation..." Do you mean glutamatergic (as opposed to direct electrical) excitation? Isn't the electrical stimulation activating VPi soma/dendrites, which is why GluR block doesn't eliminate the early IPSC in 6D?

10. Fig.5C,D. The red dots probably need to be enlarged for better visibility.

Referee #2:

Interneurons of the thalamus are one of the least understood cell type in the brain. Any information concerning their structure and function is an important addition to the field. Here Yi and Markram characterize the interneurons in the VP nucleus of mice. The paper contains important and high quality data. I have the following suggestions to enhance the presentation.

Introduction:

Rarity of interneurons in the VP (and in all other nuclei except LGN) is a unique feature of the rodent linage among mammals. This should be clearly spelled out in the Introduction.

Methods:

The authors used 11-33 days old mice. This is a large developmental window. Below 20 days the somatosensory system is immature above 30 days, it can be considered as young adult. Triads undergo complex differentiation during this time. The authors should report any age dependent differences observed (e.g. distribution of the two interneurons types, differences in amplitude or types of synaptic connections, examined, etc.)

Results

1) The authors suggest that "VP interneurons are anatomically well positioned to provide inhibition to all TC neurons in the VP, despite their relatively lower densities as compared to the LGN." If this is true the author should consider citing reference from the rodent VP EM literature on triadic arrangement in this nucleus.

2) The authors should report the number of cells in the text used for the anatomical analysis.

3) VP actually consists of two well- separated nuclei VPM and VPL. VPM contains the well characterized barreloids. The border can be easily discerned even in the images provided. Please report if there is any difference in interneurons or synaptic activity between he two nuclei.

4) Do interneuron dendrites respect nuclear boundaries?

5) The most serious problem is that short term plasticity of the connections described is not quantified. This would be especially important to understand the dynamic nature of the triad, which eluded investigators for long. More specifically, if the ML to IN, ML to relay cells and the IN to relay cell connections display different short term plasticity properties the connection may be specifically tailored to transfer some but not other input frequencies.
Discussion

It should be bear in mind that, outside dLGN, the proportion of INs rodents is as low as shown here for VP. Hence the argument concerning spatial/temporal refinement and lack if INs should be removed, especially if the arrangement of INs in barreloids is not shown.

Figure

For visualization purposes, I recommend to place reconstructed interneurons of both types to the histological image to be able to judge how much area is actually covered by the IN dendritic arbours.

END OF COMMENTS

Confidential Review

27-Apr-2021
To the Journal of Physiology staff, editors, and reviewers,

Thank you for your time and consideration in reviewing our manuscript. Below you will find the authors’ response to the reviewing editor’s and reviewer’s comments and questions.

EDITOR COMMENTS

Reviewing Editor:

Comments to the Author:
This paper by Li and Markram reports the results of a thorough electrophysiological and synaptic investigation of local interneurons of the mouse somatosensory ventral posterior thalamus. The experiments appear to have been expertly carried out and the results are fundamentally novel. This manuscript has been reviewed by two expert reviewers and they provide highly convergent evaluations. They raise a number of relatively minor concerns and the roadmap provided to address them appear to be reasonable.

Authors’ response: We would like to thank the reviewing editor for these comments. Hopefully we have responded to the reviewers’ comments and recommendations to align with the high standards of Journal of Physiology.

REFEREE COMMENTS

Reviewer #1:

Yi & Markram investigate the sparse population of inhibitory neurons in the mouse somatosensory thalamus. Though known previously to exist, these cells have remained almost entirely unstudied. The authors used acute brain slices to investigate the morphology, physiology, and synaptic connectivity of these cells. They showed that inhibitory ventroposterior thalamus (VPI) cells can inhibit thalamic relay neurons and each other. Physiological experiments are consistent with VPI synapses onto relay cells having both triadic and non-triadic arrangements with medial lemniscal synapses, similar to the wiring within LGN. They also observe both triadic and non-triadic configurations of medial lemniscal synapses for VPI-VPI connections.

The role of inhibitory cells in any thalamic nucleus, including LGN where they have been most studied, is not entirely settled. Investigations outside of LGN are therefore timely. The experiments appear well executed, and the data clearly presented. My comments are fairly limited.

1. Fig.1D is not clearly bimodal. This could simply be a skewed unimodal distribution. One could perform a comparison of the two models (skewed unimodal vs mixture of Normals) or perform another test of bimodality.

Authors’ response: We thank Reviewer #1 for raising this point. Our skewed distribution of soma size calls for a test of unimodality/bimodality. This has been completed in the text. We have left the finite mixture model in order to calculate a cut-off value for further analysis that occurs later in the Results: Morphology of VP neurons portion of the text.

2. What fraction of cells had an axon branch that went to the slice edge? This could be a useful way of estimating how much/little of the axon might be truncated during cutting. Is it possible
that cells had more expansive arbors outside of the slice? If there are no cut ends of the axon, this is unlikely. The authors could probably address this possibility quite readily. Related 1: How much time passed between the end of the recording and the perfusion of the slice? Could it be that the axons only appear limited because the tracer has had little time to diffuse? Related 2: "Cut" in Figure 1 appears to refer to major dendrites, but this is not made explicit. Please clarify somewhere such as the legend.

**Authors’ response:** All axon-like branches that could potentially have stemmed from the soma or proximal dendrites have been labeled. We have found there are only a few reconstructions with axons present, and as Reviewer #1 mentioned, the others are likely to not have been filled since axons are usually thinner neurites than dendrites. Therefore, we do not think there are axons that have been cut since they were likely not filled in the first place. Recordings were quite lengthy (between 20-40 minutes) which is normally sufficient for filling a cell; however, these neurons are rather extensive so it is true that 20 minutes may not be enough. Filling success may depend on the type of protocol used, for example passive filling is usually not useful. The quality of the seal for each experiment may also play a part in the effectiveness of filling. All in all, this makes filling axons exceedingly difficult and we are glad to have been able to fill at least one axon structure. We thank Reviewer #1 for pointing out the unclear language in the Figure 1 legend. We have written in the figure legend that cut refers to major dendrites.

3. Why not report the total lengths of dendrite and axon observed? Were the dendrites completely smooth or just sparsely spiny?

**Authors’ response:** Dendrites often had beaded processes with small spine-like branches. We do not think these neurons have classical dendritic spines. We have clarified this further in the text. We have decided not to measure total lengths of neurites (dendrites and axons observed) because we could not be certain that all lengths were filled or complete. Though we have 8 neurons that are “completely” stained, we believe portions of the neuronal morphology are too deep beneath the surface to be resolved or, as pointed out in the Figure 1 legend, neurites that have been cut from their exposure to the slice surface.

4. P.10/Fig.4. Did relative positions of the cells affect the probability of finding a connection (i.e., if the relay cell was medial, lateral, dorsal, or ventral of the inhibitory cell? If the VPi-VPi pairs were more in a M-L or D-V arrangement?)? These analyses seem straightforward.

**Authors’ response:** Reviewer #1 asks an important question with very interesting implications of how interneurons may shape particular somatotopic regions and whether there is a sort of uniform organization. We do not think the relative M-L or D-V position of the post-synaptic neuron will impact the connection probability due to the fact that the interneurons are located in various angles relative to the plane of the slice whether it is a horizontal or coronal orientation. The interneurons are bipolar with arborizations that are the densest in the bipolar axis. Therefore, it is likely that connection probability will increase in this axis. Unfortunately, the vast majority of the patching experiments, particularly for the paired neuron searches, have very faint somatic stains and a formal analysis of this hypothesis is not possible. Furthermore, most paired neuron searches occurred at intersomatic distances of only 100um or less, thus, the results for this particular analysis, even if it is possible, may not be meaningful. There are other high-throughput and fairly impactful methods to tackle this sort of question, particularly in the imaging field. Though whole cell patch clamp provides beautifully detailed data, unfortunately, it is not very high-throughput.

5. Key points summary. "The function of..." The experiments describe physiological (functional) properties of these cells, but I would not say that this paper reveals the function of the cell type.
Authors’ response: We thank Reviewer #1 for this correction, we have changed to this bullet point to, “physiology and structure of...”

6. First line of Intro is probably not the right place to introduce the VPi abbreviation. Similarly, one probably should not use the abbreviation at the paragraph’s end. At this point the authors are talking about thalamus in general, not VP.

Authors’ response: That is true. We have introduced the abbreviations in a following paragraph.

7. Methods, Animals. Please state exactly how many male mice and how many female mice were used

Authors’ response: We have included the exact number of male and female mice used in this study.

8. P.9, "VP interneurons have high input resistances, likely due to their small soma sizes." Many neurons with similarly sized somata have been described and have much lower Rin. This seems unlikely to be the only possible explanation.

Authors’ response: That is indeed true, we have used their electrotonically extensive dendritic structures as a potential reason for our observed high input resistance

9. P.11, "To exclude direct excitation..." Do you mean glutamatergic (as opposed to direct electrical) excitation? Isn’t the electrical stimulation activating VPi soma/dendrites, which is why GluR block doesn’t eliminate the early IPSC in 6D?

Authors’ response: That is correct (glutamatergic as opposed to direct electrical stimulation). And that is correct that the electrical stimulation is activating VPi soma/dendrites or perhaps also TRN axons and that is why the GluR block doesn’t eliminate the early IPSC in Figure 6D. We have found that this contamination of direct electrical stimulation is only an issue when we utilize unnecessarily high stimulation amplitudes or when the electrode is within a few hundred microns from the recording pipettes. We have clarified this further in the text.

10. Fig.5C,D. The red dots probably need to be enlarged for better visibility

Authors’ response: Asterisks have been enlarged.

Reviewer #2:

Interneurons of the thalamus are one of the least understood cell type in the brain. Any information concerning their structure and function is an important addition to the field. Here Yi and Markram characterize the interneurons in the VP nucleus of mice. The paper contains important and high quality data. I have the following suggestions to enhance the presentation.

Introduction:

Rarity of interneurons in the VP (and in all other nuclei except LGN) is a unique feature of the rodent lineage among mammals. This should be clearly spelled out in the Introduction.

Authors’ response: We thank Reviewer #2 for pointing this out, we have clarified this in the text of the introduction.
Methods:

1) The authors used 11-33 days old mice. This is a large developmental window. Below 20 days the somatosensory system is immature above 30 days, it can be considered as young adult. Triads undergo complex differentiation during this time. The authors should report any age dependent differences observed (e.g. distribution of the two interneurons types, differences in amplitude or types of synaptic connections, examined, etc.)

Authors' response: Reviewer #2 raises an important point. We have completed statistical tests to report on any differences due to age in electrophysiological features. Taking our age range into account, we have decided to compare between pre-weaned (<p21) and post-weaned (>=p21) mice. Only one mouse was under p21 (p18) out of the 18 paired neuron recordings we have done; therefore, we did not think it would be worthwhile to perform a statistical test of differences in age for synaptic features. These results have been introduced in the text. We have found that there are no significant differences for the vast majority of features.

Results

1) The authors suggest that "VP interneurons are anatomically well positioned to provide inhibition to all TC neurons in the VP, despite their relatively lower densities as compared to the LGN." If this is true the author should consider citing reference from the rodent VP EM literature on triadic arrangement in this nucleus.

Authors' response: Currently, there is hardly any VP EM literature in rodents specifically. A note-worthy observation is a reconstruction in 1974: in rat, a small dendrite receiving sensory input was found of unknown origin that displayed properties different from those of TC dendrites. The authors asserted that it was possibly a local interneuron dendrite (Spacek & Lieberman, 1974). We have therefore decided to remove the phrase about vesicular release in dendrites in this sentence due to the lack of anatomical EM evidence and since at that point in the manuscript, we had not yet provided physiological evidence for the participation of VP interneurons in thalamic activity.

Spacek, J., & Lieberman, A. R. (1974). Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. Journal of anatomy, 117(3), 487–516.

2) The authors should report the number of cells in the text used for the anatomical analysis

Authors’ response: We have added the numbers of cells used for anatomical analysis in text (soma sizes and numbers of reconstructions).

3) VP actually consists of two well-separated nuclei VPM and VPL. VPM contains the well characterized barreloids. The border can be easily discerned even in the images provided. Please report if there is any difference in interneurons or synaptic activity between he two nuclei

Authors’ response: We have completed statistical tests to compare the difference in electrophysiological features of synaptic activity between the two nuclei based on our affine transformation of slices to atlas images. Our results currently suggest likely no difference of biological significance for electrophysiological features or synaptic activity which could perhaps indicate analogous processes in each of the two nuclei (or perhaps all first order thalamic nuclei). The exact nature of neural computation for thalamic nuclei of various sensory modalities would be an interesting future topic of study.
4) Do interneuron dendrites respect nuclear boundaries?

**Authors’ response:** We have answered this question with Reviewer #2’s final comment (in the “Figure” section) regarding adding a figure of morphologies overlayed within nucleus boundaries. It seems as though interneuron dendrites do not respect nuclear boundaries indicating perhaps a cross-talk between sensory modalities or to higher order thalamic nuclei.

5) The most serious problem is that short term plasticity of the connections described is not quantified. This would be especially important to understand the dynamic nature of the triad, which eluded investigators for long. More specifically, if the ML to IN, ML to relay cells and the IN to relay cell connections display different short term plasticity properties the connection may be specifically tailored to transfer some but not other input frequencies.

**Authors’ response:** We have added short term depression quantifications for IC-VPi, TRN-VPi, ML-VPi, ML-VPi synapse, ML-TC synapse, VPi-VPi, and VPi-TC to be able to visualize our experimental data points rather than in qualitative figures. Reviewer #2’s point of understanding how each connection in the circuit processes input-output relationships is crucial and we have thought about this overarching theme while completing these figures and associated text. We will also describe the figures here and the experimental limitations of doing any further analysis. VPi-VPi or VPi-TC paired recordings: Unfortunately, it is not possible to conclude whether the synaptic connection in these recordings is triadic or non-triadic (or even if the pre-synaptic structure is axonal or dendritic). Therefore, any short term plasticity analysis from these recordings will be assumed to be from all synaptic possibilities. Due to the extreme difficulty and rarity of successfully finding and patching pairs of neurons, we did not risk using multiple trains of differing frequencies, preventing a more comprehensive analysis of plasticity. ML-VPi or ML-TC: In these feedforward inhibition and disinhibition experiments, we were able to physiologically differentiate triadic and non-triadic arrangements. For this reason, we can separately analyze the short term plasticity of these experiments. However, we used trains or pairs of pulses for the sole purpose of distinguishing between ML vs. cortical inputs (which are facilitating rather than depressing). Therefore, in some cases, we do not have complete datasets for a pooled analysis and are rather limited in the forms of analysis that can be completed.

Discussion

It should be bear in mind that, outside dLGN, the proportion of INs rodents is as low as shown here for VP. Hence the argument concerning spatial/temporal refinement and lack if INs should be removed, especially if the arrangement of INs in barreloids is not shown.

**Authors’ response:** Indeed, Reviewer #2 is correct that the proportion of interneurons in other first thalamic nuclei like the MGN is similar. Our belief that interneurons refine spatial and temporal features still stands for these other nuclei as well. To clarify our stance, we have discussed spatial/temporal refinement also in the context of MGN in the discussion.

Figure

For visualization purposes, I recommend to place reconstructed interneurons of both types to the histological image to be able to judge how much area is actually covered by the IN dendritic arbours.
Authors’ response: We have included morphologies overlayed to nuclear boundaries that have been determined by the Allen Institute Mouse Brain Atlas in Figure 1. For the purposes of visualizing scale, we believe this will be much clearer than using histological images.
Dear Dr. Markram,

Re: JP-RP-2021-281711R1 "Morphology, Physiology and Synaptic Connectivity of Local Interneurons in the Mouse Somatosensory Thalamus" by Jane Simko and Henry Markram

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 I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

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

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

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

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I look forward to receiving your revised submission.

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

Reviewing Editor:

The reviewers and myself have examined the revised manuscript and rebuttal and are fully satisfied. This is a nice, thorough and overall very well executed study.

There remains a small point to address pertaining to the terminal procedures, as was raised by the Journal's ethic editor:

- Please include the approval code/number for this particular study.

- Mice were decapitated. Please indicate if the mice were sedated before decapitation. If they were not sedated, please include justification for this method (which is permissible) over other approved methods. For example, it may be that the authors wished to avoid the potential effects of a sedative/anaesthetic agent on subsequent recordings in brain tissue.

REFEREE COMMENTS

Referee #1:

The authors have adequately addressed all of my concerns. The revised version is a wonderful piece of work.

Referee #2:

The authors adequately answered all my concerns and performed the appropriate changes in the ms. I have no further
Referee #3:

Thank you for submitting your manuscript to The Journal of Physiology. Some additional details are required pertaining to animal ethics and welfare.

Please include the approval code/number for this particular study.

Mice were decapitated. Please indicate if the mice were sedated before decapitation. If they were not sedated, please include justification for this method (which is permissible) over other approved methods. For example, it may be that the authors wished to avoid the potential effects of a sedative/anaesthetic agent on subsequent recordings in brain tissue.

_______________________________________________
END OF COMMENTS
To the Journal of Physiology staff, editors, and reviewers,

Thank you for your time and consideration in reviewing our manuscript. Below you will find the authors’ response to the reviewing editor’s and reviewer’s comments and questions.

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Authors’ response: We thank the reviewing editor for these comments and we are excited to hear about our provisional acceptance.

REFEREE COMMENTS

Referee #1:

The authors have adequately addressed all of my concerns. The revised version is a wonderful piece of work.

Authors’ response: Thank you.

Referee #2:

The authors adequately answered all my concerns and performed the appropriate changes in the ms. I have no further comment.

Authors’ response: Thank you.

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Please include the approval code/number for this particular study.

Mice were decapitated. Please indicate if the mice were sedated before decapitation. If they were not sedated, please include justification for this method (which is permissible) over other approved methods. For example, it may be that the authors wished to avoid the potential effects of a sedative/anaesthetic agent on subsequent recordings in brain tissue.
Authors’ response: We have included the animal license number for this study in the text. We have also further explained the decapitation procedure and the rationale for not using anesthesia. Thank you for reviewing our manuscript.
Dear Dr. Markram,

Re: JP-RP-2021-281711R2 "Morphology, Physiology and Synaptic Connectivity of Local Interneurons in the Mouse Somatosensory Thalamus" by Jane Simko and Henry Markram

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology, subject to any modifications to the text and/or satisfactory clarification of the Methods section that may be required by the Journal Office to conform to House rules.

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Yours sincerely,

Katalin Toth
Senior Editor
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NO COMMENTS

2nd Confidential Review 24-Sep-2021