Selective glycinergic input from vGluT3 amacrine cells confers a suppressed-by-contrast trigger feature in a subtype of M1 ipRGCs in the mouse retina

Seunghoon Lee, Minggang Chen, Yuelin Shi, and Z. Jimmy Zhou
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The following individual(s) involved in review of this submission have agreed to reveal their identity: R. Lane Brown (Referee #1)

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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.)

1st Editorial Decision 11-May-2021

Dear Professor Zhou,

Re: JP-RP-2021-281717 "The Suppressed-by-contrast Trigger Feature of a Non-image-encoding Ganglion Cells in the Mouse Retina" by Seunghoon Lee, Minggnag Chen, Yuelin Shi, and Z. Jimmy Zhou

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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.

Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

The referees were enthusiastic and agreed that the paper makes a significant contribution to the field. They made some useful suggestions for revision that would improve the presentation and should be straightforward to address. I agree with the second referee's suggestion to use letters rather than numbers to identify the sub-populations of M1 ipRGCs but leave this to the authors' discretion.

Please reformat your manuscript to conform to the Journal's style.

The main findings are solid and speak for themselves. I would encourage the authors to consider revising the abstract to take advantage of the more generous word limits afforded by the Journal to describe the main findings in more measured language that more closely reflects the data. For example, what does it mean to say that "... the suppressed-by-contrast trigger feature ... is a fundamental receptive field property associated with image-forming vision"? The data don't really support the notion that transient inhibition is a defining feature of image-forming vision. Similarly, the claim that the "glycinergic circuit endowed M1-1s with the capability to discriminate between contrast and uniformity" seems to overstate the findings. The paper doesn't really define the difference between contrast and uniformity, nor does it present any quantitative data that demonstrates that the GCs make such a distinction.

The title also could be modified to be less buzzy and more descriptive of the main findings. For example, something like, "Selective glycinergic input from vGLuT3 amacrine cells defines two subpopulations of M1 ipRGCs in the mouse retina", seems more descriptive and would likely improve visibility of the work in literature searches.

Senior Editor:

Congratulations on an interesting MS. We hope you can attend to the referees comments quickly. Please pay particular attention to re-writing the abstract, title and you may want to add a small diagram to bring together the circuitry.

REFEREE COMMENTS

Referee #1:

Intrinsically photosensitive retinal ganglion cells (ipRGCs), which mediate circadian photo

entrainment and many other non-visual light-dependent functions, were discovered almost 20 years ago. Although it has been previously reported that the M1 subtype of ipRGCs receives rod- and cone-driven synaptic input, the presynaptic circuitry remains virtually unknown. In this manuscript, Lee et al. use an elegant combination of electrophysiological methods to identify the

vGluT3 cells as a presynaptic partner of a subpopulation of M1 ipRGCs and demonstrate that these ipRGCs respond to light in a "suppressed-by-contrast" fashion, which is reminiscent of RGCs mediating visual perception. The experiments described are convincing, and the manuscript is well-written.

Elegant electrophysiology experiments using both optogenetic activation of vGluT3 cells and paired whole-cell recordings demonstrate that vGluT3 cells provide presynaptic glycinergic inhibition of a subpopulation of M1 ipRGCs. Using a combination of stimulus type and pharmacology, the authors demonstrate that this inhibitory input gives rise to a suppressed-by-contrast feature in the ipRGC receptive field, a characteristic only previously seen in conventional RGCs.

Major comments:

When stimulated with high intensity light, similar to that used in these experiments, previous reports have shown a faster rise, sharper peak, and faster decay of the melanopsin-activated currents. The melanopsin-activated currents shown in this manuscript seem to have somewhat different kinetics. Can this be explained by the order of stimulation (for instance, are the currents at 0 mV elicited in response to optogenetic stimulation measured first such that the subsequent currents measured at - 70 mV be desensitized or run-down?

Minor comments:

1. Some wording is missing from the first sentence on page 5.

Referee #2:

The authors have identified two types of synaptic input profiles to M1 ipRGCs. M1-1 cells receive glycinergic synaptic input that results in a suppressed by contrast response, while M1-2 cells do not. The authors provide strong evidence of a direct, glycinergic input onto M1-1 cells when the cells are stimulated with a small spot. The authors correlate the presence of this glycinergic input with other morphological and physiological properties. This is in and of itself

interesting as very little correlation has been reported amongst the varied M1 cell properties (Emanuel et al., 2017 Cell Reports; Lee et al., 2019 Cell Reports). Moreover, the findings of a more complex receptive field structure in M1 cells is unexpected given the current understanding of their role as luminance detectors dominated, in photopic conditions, by the melanopsin response, which integrates intensity across broad spatial and temporal scales. Overall this work will be of high interest to those who study ipRGCs, retinal circuitry, and non-image forming vision. The work is convincing and well-done. I have only minor comments.

Given that there are already M1 and M2 cells, the naming scheme is a bit cumbersome (M1-2 versus an M2 cell) and may cause confusion. I would suggest considering M1a and M1b instead to keep the numbers for the main subtypes and the lettering system for the subsets within subtypes. While in the end this is up to the authors, it is worth considering the long term utility of this naming scheme. If diversity in other subtypes is eventually found, then the lettering scheme could be applied to those as well.

How prevalent is each subtype? Are they both seemingly equally present when targeting ipRGCs for recording? A sense of this would be helpful.

The authors state that there is no regional concentration of the M1-1 cells, but they do not explicitly rule this out because there could be a mixed populations of M1-1 and M1-2 cells in one hemisphere/quadrant and just M1-2 cells in another. The language here can simply be softened a little.

The authors reference Figure 1E showing that the slow inward current is resistant to glutamatergic blockers in both M1-1 and M1-2 cells, but then only show an example from an M1-1. Either add these data or say data not shown.

For the Sholl analysis in Figure 6F, why measure pixel density and not the standard number of crossings?

Do the authors know whether there is a difference in the central projections of these two populations? This is likely beyond the scope of the current study, but is worth commenting on in the discussion, including what the utility of this feature of the M1-1 cells might be.

END OF COMMENTS

Confidential Review 31-Mar-2021

The authors greatly appreciate the efforts, comments, and constructive suggestions of the editors and referees. The following are point-by-point responses.

Comments from Reviewing Editor:

The referees were enthusiastic and agreed that the paper makes a significant contribution to the field. They made some useful suggestions for revision that would improve the presentation and should be straightforward to address. I agree with the second referee's suggestion to use letters rather than numbers to identify the sub-populations of M1 ipRGCs but leave this to the authors' discretion. Please reformat your manuscript to conform to the Journal's style.

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Comments from Senior Editor:

Congratulations on an interesting MS. We hope you can attend to the referees comments quickly. Please pay particular attention to re-writing the abstract, title and you may want to add a small diagram to bring together the circuitry.

Response:

We have changed the title to: “Selective glycinergic input from vGluT3 amacrine cells confers a suppressed-by-contrast trigger feature in a subpopulation of M1 ipRGCs in the mouse retina.”

We renamed the two M1 subpopulations as M1a and M1b.

We revised the abstract to clarify the points raised by the reviewing editor**.**

Comments of Referee #1:

Intrinsically photosensitive retinal ganglion cells (ipRGCs), which mediate circadian photo entrainment and many other non-visual light-dependent functions, were discovered almost 20 years ago. Although it has been previously reported that the M1 subtype of ipRGCs receives rod- and cone-driven synaptic input, the presynaptic circuitry remains virtually unknown. In this

manuscript, Lee et al. use an elegant combination of electrophysiological methods to identify the vGluT3 cells as a presynaptic partner of a subpopulation of M1 ipRGCs and demonstrate that these ipRGCs respond to light in a "suppressed-by-contrast" fashion, which is reminiscent of RGCs mediating visual perception. The experiments described are convincing, and the manuscript is well-written.

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Major comments:

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Minor comments:

Some wording is missing from the first sentence on page 5.

Response:

Major comments:

This is a very good point. In early experiments, we indeed first used intense blue light to optogenetically activate vGluT3 cells, followed by white light stimulation to map receptive field. As the reviewer speculated, blue light significantly distorted subsequent white light-evoked current responses recorded at -70 mV (although white light-evoked inhibitory synaptic currents at 0 mV quickly recovered and remained stable even after intense blue light illumination). Therefore, in all subsequent experiments (including all results shown in the figures), we always applied optogenetic stimulation after recording white light-evoked visual responses. This stimulation sequence allowed us to obtain more stable and physiological responses to both white and blue light in a single recording episode. We believe the seemingly slow kinetics of melanopsin-mediated responses detected under our recording condition was likely due to the white light intensity used. In fact, the kinetics of our melanopsin-mediated currents (Fig.2) closely resembled that of other reported studies using similar white light intensity (e.g., Fig1.H, 2nd and 3rd trace, Emanuel et.al, Cell Report, 2017). It is also worth pointing out that there is large variation in kinetics and sensitivity of melanopsin currents across M1 cells (Hattar et.al, Science, 2002, and Milner and Do, Cell, 2017). We now further clarified these points in Methods and Results sections.

Minor comment:

The sentence on page 5 is now broken down into two shorter sentences.

Comments of Referee #2:

The authors have identified two types of synaptic input profiles to M1 ipRGCs. M1-1 cells receive glycinergic synaptic input that results in a suppressed by contrast response, while M1-2 cells do not. The authors provide strong evidence of a direct, glycinergic input onto M1-1 cells when the cells are stimulated with a small spot. The authors correlate the presence of this glycinergic input with other morphological and physiological properties. This is in and of itself interesting as very little correlation has been reported amongst the varied M1 cell properties (Emanuel et al., 2017 Cell Reports; Lee et al., 2019 Cell Reports). Moreover, the findings of a more complex receptive field structure in M1 cells is unexpected given the current understanding of their role as luminance detectors dominated, in photopic conditions, by the melanopsin response, which integrates intensity across broad spatial and temporal scales. Overall this work will be of high interest to those who study ipRGCs, retinal circuitry, and non-image forming vision. The work is convincing and well-done. I have only minor comments.

Given that there are already M1 and M2 cells, the naming scheme is a bit cumbersome (M1-2 versus an M2 cell) and may cause confusion. I would suggest considering M1a and M1b instead to keep the numbers for the main subtypes and the lettering system for the subsets within subtypes. While in the end this is up to the authors, it is worth considering the long term utility of this naming scheme. If diversity in other subtypes is eventually found, then the lettering scheme could be applied to those as well.

Response: This is an excellent suggestion. Fully agree.

How prevalent is each subtype? Are they both seemingly equally present when targeting ipRGCs for recording? A sense of this would be helpful.

Response: We randomly recorded from M1 cells and encountered 80 M1a and 73 M1b (Fig.1H), suggesting an M1a:M1b ratio around 1:1 among GFP-labeled M1 cells in this mouse line. We now added comments in both Results and Discussion to give a sense of the prevalence.

The authors state that there is no regional concentration of the M1-1 cells, but they do not explicitly rule this out because there could be a mixed populations of M1-1 and M1-2 cells in one hemisphere/quadrant and just M1-2 cells in another. The language here can simply be softened a little.

Response: Agree. Our paired recordings showed that M1a and M1b cells coexist as neighbors in randomly chosen retinal regions. We did not intend to infer from these data any specific topographic distribution of M1a and M1b cells. We have now revised our description of this finding in Results and Discussions.

The authors reference Figure 1E showing that the slow inward current is resistant to glutamatergic blockers in both M1-1 and M1-2 cells, but then only show an example from an M1-1. Either add these data or say data not shown.

Response: We added “data not shown” for M1b cells.

For the Sholl analysis in Figure 6F, why measure pixel density and not the standard number of crossings?

Response: While standard Sholl analysis is effective in describing dendritic complexity using the number of dendritic crossings, we believe it could miss some special morphological features specific to the M1 subtypes, such as differences in dendritic complexity due to total dendritic length, even if dendritic branching points remain similar. We felt that dendritic density more effectively depicts dendritic complexity in terms of dendritic length and geometry, and that it may also shed light on the observed difference in melanopsin current amplitude between M1a and M1b.

Do the authors know whether there is a difference in the central projections of these two populations? This is likely beyond the scope of the current study, but is worth commenting on in the discussion, including what the utility of this feature of the M1-1 cells might be.

Response: We have now expanded the discussion of potential central projection patterns of M1a and M1b cells and discussed potential functional utilities of the sbc property in regulating non-image-forming and image-forming vision, as well as pupillary reflex.

Dear Dr Zhou,

Re: JP-RP-2021-281717R1 "Selective glycinergic input from vGluT3 amacrine cells confers a suppressed-by-contrast trigger feature in a subtype of M1 ipRGCs in the mouse retina" by Seunghoon Lee, Minggang Chen, Yuelin Shi, and Z. Jimmy Zhou

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Yours sincerely,

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EDITOR COMMENTS

Reviewing Editor:

No further comments. Congrats on a nice paper.

REFEREE COMMENTS

Referee #1:

The authors have addressed all of the concerns and suggestions of both Reviewers, and the manuscript is now suitable for publication.

Referee #2:

The authors have addressed my previous concerns. This work will be of interest to many in the retinal circuitry and ipRGC communities, as well as those that study M1-dependent behaviors such as circadian photoentrainment and the pupillary light reflex.

END OF COMMENTS

1st Confidential Review **17-Jun-2021**