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Sun compass neurons are tuned to migratory orientation in monarch butterflies

Tu Anh Thi Nguyen, M. Jerome Beetz, Christine Merlin and Basil el Jundi

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Original submission: 1 December 2020
Revised submission: 22 January 2021
Final acceptance: 27 January 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-2988.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this manuscript Nguyen and colleagues provide an excellent examination of the neural basis of celestial navigation in migratory and non-migratory monarch butterflies. Elecrophysiological recordings of tangential neurons that project from the bulb to the central body lower (TL neurons) confirmed previous results in monarchs that these cells respond to different celestial cues. TL neurons were selected by responsiveness to polarized UV light, and the majority responded to a green and/or UV spot, simulating different spectral properties of sunlight. In many of the metrics used, migratory and non-migratory butterflies did not differ in their TL neuron responses, suggesting that these celestial cues are relevant to butterflies in both states. Intriguingly, TL neurons in migratory butterflies appear to be more narrowly tuned in response to a sun stimulus (green light spot) than the cells in the nonmigratory animals. When the green spot was rotated around the butterflies, TL neurons in migratory butterflies exhibited greater sensitivity in front, which could aid in keeping these animals flying south. Together these results suggest that TL neurons not only provide directional inputs into the butterfly compass (CL1/E-PG neurons), but also exhibit some different responses based on migratory state.

The paper is clearly written, figures are elegant, and the results are convincing. I find the focus on one cell type extremely helpful. Although I appreciate the challenge of conducting electrophysiological recordings in the absence of genetic markers, studies that report data from many cell types (and their necessarily small sample sizes) make extracting meaningful conclusions difficult. This manuscript advances our understanding of insect navigation by starting to tackle a broader question of how the navigation circuit may modulate behavior based on internal state. More broadly, this manuscript makes important contributions to understanding neuroethology generally identifying one way that nervous systems can lead to behavioral plasticity. I have only minor suggestions detailed below.

Data availability: It would be nice to supply the raw data in a freely available format in addition to the Matlab format.

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Line 69, 73: Replace “/” with “,”.
Line 70: Delete “supplier”.

Line 80: Delete “the” before Texas A&M.
Line 81: Delete “the” before recordings
Line 95: If there is space, it would be nice to briefly describe the staining procedures.
Line 97: Specify green and UV LEDs.
Line 102: The authors mention 4 arms, 2 of which are attached to LEDs. How is the position of the LEDs relative to each other determined? I would specify that the LEDs are placed at 30 deg elevation here rather than only mentioning in line 111.
Line 110: How were the angular velocities for the polarized light and unpolarized light spots selected?
Line 128: Provide the definition of modulation strength here.
Line 434: Are the recordings shown in Figure 1 c all from the same neuron? This is implied but I think could be more explicit if it is the case.

Fig 1b (bottom): As drawn, it appears that the green LED and UV LED are at different altitudes. Perhaps the drawing can indicate the 30 degree altitude?
Figures 1e, 2c,d, 3b,c, 4b, S4b,d: The figures would be improved by showing individual data points in addition to the interquartile range.

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

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Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This work tries to show a key to the neural processing for seasonal migration by demonstrating physiological properties determined by intracellular-recording in both migratory and non-migratory butterflies. The study focuses on polarization-sensitive neurons transferring light information from the bulb to the lower division of the central body (TL neurons). These neurons in both forms encode skylight cues: polarized blue light, green and UV light spot. The authors newly found that the TL neurons in migratory butterfly show the sharp tuning curve and the higher angular sensitivity to the preferred direction when the light spot is green. This physiological feature to the green spot encoding the sun position might important for keep to fly toward the preferred direction in migratory butterflies.

This work could be the first finding the key to neural processing for migration based on sky compass navigation. The methods and statistical analysis are basically appropriate. The flow of the story; describing brief features encoding skylight cues in the neurons, and comparing physiological features in polarization sensitivity and responses to non-polarized green and UV light spot, is logical and leads the readers to the authors' final opinion. All figures appear nicely arranged. I, therefore, think that this work fits interests of many readers, especially to researchers studying animal navigation in neuroethology, visual ecology and behavioural ecology. While reading the manuscript, I concerned the following three points: pooling data of different TL subtypes, interpretation of background activity and sensitivities to green and UV light spot. I hope that my comments to these points written below would help to improve the description of this work.

The authors pool all data even though the data seems from different subtypes of TL neurons (L254-256). The reason why the authors pooled data from TL subtypes for analysis should be explained more because ideally this type of work is based on a single identified neuron type with a clear correlation between physiological properties and the morphological features. The data shows variations to un-polarized light (green and UV light) in polarization-sensitive TL neurons (L160-165 Result) and most recordings are with co-labelled several TL subtypes (L254-255 Discussion). In the case, it is quite difficult to show the correlation between the physiological feature and the morphology but not find any physiological difference among TL subtypes (L255). I think that variation of responses to the un-polarized light might due to two possibilities; either TL subtypes or exist of physiological subclasses in a single morphological type. I suppose that this work focuses on processed information delivered from the bulb to the central body and how different the information between migratory and non-migratory butterfly is rather than how a single type of neuron processes the skylight information.

Background activity may contain some artefact, I suppose. When we perform intracellular recording by sharp electrodes, we critically consider the side effects of the technique. Not best recording with low resistance of electrodes (50 M Ω or so) often induces higher background activity and bigger responses to the stimuli by leaking from a big whole by penetration into the cell, and many co-labelling even though physiological features (good size of action potentials and get the stable recording) look OK. Of course, I don't completely deny that the higher background is the real physiological feature, which is already in the manuscript. But it would be important to mention the possibility of side effect.

I suppose that high responsiveness and modulation strength to UV light is not only based on the spectral sensitivity of UV-receptor (L250-252). Of course, one possibility is that UV receptor has higher absolute sensitivity because a paper predicting spectral sensitivities of photoreceptors in Pieris rapae shows that sensitivity is different among spectral classes (Stavenga & Arikawa, JCP

A :197,373-385 (2011)). But the integration of ommatidial arrays and the spectral distribution over the retina must contribute to the responses to UV and green light spot. For me, the UV-sensitive receptive field is wider than the green-sensitive one (Fig1C) in migratory butterflies. This means that more arrays of ommatidia containing UV receptor contribute to the response comparing the green receptor. In migratory butterfly, a smaller number of ommatidial array feed green light information (which makes tuning curve higher) as the sun position than in non-migratory butterfly. Considering light intensity which sounds relatively strong (1.4x10^14 photons/cm2/s), the acceptance angle of one ommatidium (reference no 15) and the inter ommatidial angle of compound eye (probably about 1degree), the light spot can stimulate not only on-axis ommatidium but also surrounding ommatidia. This would induce the higher responsiveness and modulation strength if the neurons integrate several ommatidial arrays containing UV receptor.

Minor comments.

L181: Is that true? This is probably just the result of statistical analysis. Fig2b right plots appear quite similar between the two forms and it is not easy to find out how different between them. Could you please add a bit more explanation in that respect, but not only the result of the statistical analysis?

L212: Please mention that this line describes responses to green light spot.

L251-2: This discussion about the reason why the responses to the UV light is different from that to the green light is too simple. Please discuss other possibilities as I mentioned above.

L255: The line “as we often-co-labelled several TL subtypes” should be mentioned in the result. Please show some raw data of the labelling at least as supplementary materials otherwise it is difficult to judge the quality of your data.

Fig. 2b middle plot: Add the result of statistical analysis

Decision letter (RSPB-2020-2988.R0)

05-Jan-2021

Dear Dr el Jundi:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers’ comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions”, click on "Create a Revision”. Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

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If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

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It is a condition of publication that you make available the data and research materials supporting the results in the article. Please see our Data Sharing Policies (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Your manuscript has now been reviewed by two experts in your field, both of whom felt it well written and of general interest and scientific importance. Reviewer 1 has only minor comments, but I agree with their suggestion that all the raw data should be available in an open access format for readers who do not have access to Matlab. Reviewer 2 has a few comments about the interpretation of the data which seem important to discuss. Their primary concern is the possible effect of pooling responses from different TL subtypes. My interpretation of their comment here is that it is possible different subtypes are responding in migratory/non-migratory individuals, and those subtypes have different sensitivities which differentially affect the pooled signal. As with the reviewer's other comments, I don't think this issue necessarily affects the general conclusions of the paper, but they do seem like important and interesting details to discuss more fully.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Minor comments.

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L255: The line “as we often-co-labelled several TL subtypes” should be mentioned in the result. Please show some raw data of the labelling at least as supplementary materials otherwise it is difficult to judge the quality of your data.

Fig. 2b middle plot: Add the result of statistical analysis

Author's Response to Decision Letter for (RSPB-2020-2988.R0)

See Appendix A.

Decision letter (RSPB-2020-2988.R1)

27-Jan-2021

Dear Dr el Jundi

I am pleased to inform you that your manuscript entitled "Sun compass neurons are tuned to migratory orientation in monarch butterflies" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Professor Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Comments to Author:
I thank the authors for addressing the remaining reviewers concerns, and congratulate them on a very nice paper. The submission (and datdryad submission) did not seem to include the excel file mentioned in the author's response. Please ensure this is included before publication.

Appendix A

We thank both reviewers for their helpful comments. We have responded below to all comments of the reviewers. The comments of the reviewers are written in black, our responses in green. In addition, we uploaded a version of the manuscript, in which all changes made in the main text are highlighted in yellow. The line numbers in the response-to-reviewer letters refer to the manuscript version with tracked changes.
To address to the reviewers' suggestions, our main text had to exceed the 10-page limitation of the Journal. We therefore had to move the subsection "Statistics" of the methods to the supplementary materials (together with the new section "Histology and imaging") and refer to it on lines 144-145.

Referee 1:

Data availability: It would be nice to supply the raw data in a freely available format in addition to the Matlab format.
We agree with the reviewer and have added a version of our analysis script as txt-file. In addition, the raw data are now provided as CSV files.

Add "also" after "but".
Changed accordingly (line 6).

Perhaps define TL neurons a little more broadly as a tangential neuron before the first appearance of the acronym. As written it could seem abrupt to readers not familiar with insect central complex neuron types.
We have added the term ***"tangential neuron"*** to the sentence (line 40).

Delete "following".
Deleted on line 50.

Replace "/" with ",".
Replaced "/" on lines 69 and 73 by ",".

Delete "supplier".
Deleted on line 69.

Delete "the" before Texas A&M.
Done (line 79).

Delete "the" before recordings
Deleted (line 80).

If there is space, it would be nice to briefly describe the staining procedures.
We agree with the reviewer that a description of the staining procedure should be included in the manuscript. However, due to space limitations of the Journal (max. 10 printed pages), we have included a description of the staining procedure as supplementary material (please, see supplementary material). We are referring to the supplementary information in the main text on line 94.

Specify green and UV LEDs.
We have now clarified at the beginning of the section "Visual stimulus" which type of LEDs we have used during our experiments (lines 101-102).

The authors mention 4 arms, 2 of which are attached to LEDs. How is the position of the LEDs relative to each other determined? I would specify that the LEDs are placed at 30 deg elevation here rather than only mentioning in line 111.

We set the green and UV LEDs at the arms opposite to each other. Our aim was to set them perpendicular to the polarization angle. This arrangement allowed us to present the green or UV light cue in combination with the polarization stimulus (the polarization angles are arranged perpendicular to the sun in nature) and to test the cue hierarchy between polarized light and the green/UV light cue in central-complex neurons. This arrangement is important for an ongoing project in our lab where we are comparing the cue hierarchy in central-complex neurons to the cue hierarchy tested behaviorally. This arrangement has not any relevance for this study here (where we presented single cues to the animal). We therefore believe that the explanation of the stimulus arrangement would rather confuse than help a reader. However, we are stating in the text now that the LEDs were set on two arms, 180° to each other (lines 106-108).

As suggested by the reviewer, we have moved the elevation information to lines 107-108.

How were the angular velocities for the polarized light and unpolarized light spots selected?

We chose the angular velocity based on the preexisting literature (Heinze and Reppert, 2011). We have added this now to the text on lines 115-116.

Provide the definition of modulation strength here.

We have added on lines 136-138: ***"The modulation strength of each neuron, which contains information about the response strength to a light cue (a higher modulation value indicates a stronger response), was calculated in 20° bins according to [17]."***

Are the recordings shown in Figure 1 c all from the same neuron? This is implied but I think could be more explicit if it is the case.

Example spike trains were taken from different TL-neurons. We have clarified this in the figure descriptions on lines 437-438: ***"Intracellular recordings from different TL neurons show the responses to polarized light (left), the green (middle) and the UV light spot (right)."***

Fig 1b (bottom): As drawn, it appears that the green LED and UV LED are at different altitudes. Perhaps the drawing can indicate the 30 degree altitude?

We agree with the reviewer that the perspective illustration of the UV LED was misleading. In the new version of Fig. 1, we have adjusted the UV stimulus and have indicated the elevation of the stimulus. In addition, we are now mentioning in the fig. legend that both light cues were presented at an elevation of 30° (line 436).

Figures 1e, 2c,d, 3b,c, 4b, S4b,d: The figures would be improved by showing individual data points in addition to the interquartile range.

We added the individual values as grey dots to the figures 1e, 2c,d and 3b,c. As the individual data points in figures 4b and S4b,d are visualized in 4a and S4a,c, respectively, we decided that an additional plotting of these data is not necessary.

Referee 2:

The authors pool all data even though the data seems from different subtypes of TL neurons (L254-256). The reason why the authors pooled data from TL subtypes for analysis should be explained more because ideally this type of work is based on a single identified neuron type with a clear correlation

between physiological properties and the morphological features. The data shows variations to un-polarized light (green and UV light) in polarization-sensitive TL neurons (L160-165 Result) and most recordings are with co-labelled several TL subtypes (L254-255 Discussion). In the case, it is quite difficult to show the correlation between the physiological feature and the morphology but not find any physiological difference among TL subtypes (L255). I think that variation of responses to the un-polarized light might due to two possibilities; either TL subtypes or exist of physiological subclasses in a single morphological type. I suppose that this work focuses on processed information delivered from the bulb to the central body and how different the information between migratory and non-migratory butterfly is rather than how a single type of neuron processes the skylight information.

We agree with the reviewer that a subdivision into subtypes of TL neurons would have been the ideal solution but, as correctly stated by her/him, the co-labeling of several TL neurons in our experiments (see new figure S1) made it impossible to define the exact neuron subtype. As correctly stated by the reviewer, our main goal was to compare compass TL neurons between migratory and non-migratory monarch butterflies but not to characterize the functionality of the input stage of the central complex. Even if different subtypes of TL neuron have different functionalities in monarch butterflies, this could affect our study only if we had a bias in the ratio of recorded TL2/TL3 neurons between migratory and non-migratory butterflies. However, the non-existence of any difference in the general tuning characteristics between migratory and non-migratory TL neurons suggests that this is not the case. We believe that this suggests that the difference in the observed tuning widths is due to the behavioral form. However, as we agree with the reviewer that we cannot fully exclude this possibility, we added, in addition to the lines 255-258 in the discussion, in the methods on lines 94-99: ***"During cell injections, we often co-labeled several TL subtypes (figure S1). We were therefore not able to define from which TL subtype (TL2a/b or TL3, [16]) exactly our recordings were obtained. The similarity in the general tuning characteristic between migratory and non-migratory TL neurons suggests that, even if different TL subtypes may have different functional roles in monarch butterflies, this did not affect our comparisons between migratory and non-migratory TL neurons."***

Background activity may contain some artefact, I suppose. When we perform intracellular recording by sharp electrodes, we critically consider the side effects of the technique. Not best recording with low resistance of electrodes (50 MΩ or so) often induces higher background activity and bigger responses to the stimuli by leaking from a big whole by penetration into the cell, and many co-labelling even though physiological features (good size of action potentials and get the stable recording) look OK. Of course, I don't completely deny that the higher background is the real physiological feature, which is already in the manuscript. But it would be important to mention the possibility of side effect.

We fully agree with the reviewer, that leaking currents during recordings possibly contribute to a higher background activity/modulation strength. To exclude this possibility, we analyzed if the background activity correlated with the electrode resistance for 29 of our 34 recordings (for five recordings, we did not record the electrode resistance). The electrode resistance did not correlate with the observed background activity (p=0.997; Spearman test). We therefore conclude that the influence of leaking currents did most likely not affect the background activities. In the new version of the manuscript, we have added this analysis as supplementary figure S1b. In addition, we are stating in the main text on lines 127-130: ***"As the recording quality can affect the observed background activity (a lower electrode resistance can lead to more spikes/s), we ensured that the background activity did not correlate with the electrode resistance (figure S1b).***

I suppose that high responsiveness and modulation strength to UV light is not only based on the spectral sensitivity of UV-receptor (L250-252). Of course, one possibility is that UV receptor has higher absolute sensitivity because a paper predicting spectral sensitivities of photoreceptors in Pieris rapae shows that sensitivity is different among spectral classes (Stavenga & Arikawa, JCP A :197,373-385 (2011)). But the integration of ommatidial arrays and the spectral distribution over the retina must contribute to the responses to UV and green light spot. For me, the UV-sensitive receptive field is wider than the green-sensitive one (Fig1C) in migratory butterflies. This means that more arrays of ommatidia containing UV receptor contribute to the response comparing the green receptor. In migratory butterfly, a smaller number of ommatidial array feed green light information (which makes tuning curve higher) as the sun position than in non-migratory butterfly. Considering light intensity which sounds relatively strong (1.4x10ˆ14 photons/cm2/s), the acceptance angle of one ommatidium (reference no 15) and the inter ommatidial angle of compound eye (probably about 1degree), the light spot can stimulate not only on-axis ommatidium but also surrounding ommatidia. This would induce the higher responsiveness and modulation strength if the neurons integrate several ommatidial arrays containing UV receptor.

We are thankful to the reviewer for this comment. Due to the length limitation of the Journal (10 printed pages), we unfortunately, cannot discuss this point in much detail. However, we added the possibility of a large integration of UV information in the discussion on lines 252-254: ***"This indicates that the UV photoreceptors in the butterflies' eye are either more sensitive than the green photoreceptors or that the UV light information is integrated over a larger array of ommatidia than the green light information."***

L181: Is that true? This is probably just the result of statistical analysis. Fig2b right plots appear quite similar between the two forms and it is not easy to find out how different between them. Could you please add a bit more explanation in that respect, but not only the result of the statistical analysis?

We agree that this difference requires a bit more explanation. The statistical difference (Mardia-Watson-Wheeler test) arises because the mean preferred tunings, even though not-significantly different from a uniform-distribution (Rayleigh test), are slightly biased between the migratory and non-migratory TL neurons. While most migratory TL neurons have a preferred tuning in the left visual field of the animal, the preferred directions of the non-migratory TL neurons are biased towards the right visual field of the animal. We have added this information to the results on lines 179-181: ***"However, the distribution of the UV preferred directions differed significantly between the two forms (figure 2b, right plots) because there were more migratory ones tuned to the left and more non-migratory ones tuned to the right visual field of the animals."***

We agree with the reviewer that this has most likely no biological meaning but shows the weakness of our current repertoire of available statistics for circular data.

Please mention that this line describes responses to green light spot.

Done according to the reviewer's suggestion (line 213).

This discussion about the reason why the responses to the UV light is different from that to the green light is too simple. Please discuss other possibilities as I mentioned above.

Please, see our comment above. We have added the suggested point on lines 252-254.

L255: The line "as we often-co-labelled several TL subtypes" should be mentioned in the result. Please show some raw data of the labelling at least as supplementary materials otherwise it is difficult to judge the quality of your data.

We agree with the reviewer that this aspect should not only be mentioned in the discussion. However, as we did not find a reasonable section where this information fitted in the results, we have decided to mention it in the methods on lines 95-99. An additional figure has been added to the supplementary materials (figure S1), which shows two examples of traced TL neurons.

Fig. 2b middle plot: Add the result of statistical analysis

We used the Mardia-Watson-Wheeler test for Fig. 2b. However, the low n-size of the preferred directions to the green light cue (5 vs. 8) does not allow a robust statistical analysis of the distribution. We added a comment in the figure legend for fig. 2 (line 458-460): ***"Due to the low sample size, a statistical analysis of the distributions was not performed for the preferred directions to the green light."***