

Vasoactive intestinal peptide as a mediator of the effects of a supergene on social behaviour

Brent M. Horton, Christina M. Michael, Mackenzie R. Prichard and Donna L. Maney

Article citation details

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Review timeline

Original submission: 29 January 2020

Revised submission: 11 March 2020

Final acceptance: 13 March 2020

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

Review History

RSPB-2020-0196.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?

N/A

Is it clear?

N/A

Is it adequate?

N/A

Do you have any ethical concerns with this paper?

No

Comments to the Author

The main findings of this paper are that VIP expression in the AH predicts male song rate during the early breeding stage, and importantly, morph and testosterone do not. During the nestling stage, although WS males still have higher VIP expression in the AH compared to TS males, their song rate is no longer predicted by VIP expression, but rather by testosterone. The relationship between VIP expression in the AH and aggression also holds true for females. For the INF, the more parental morph had higher VIP expression but expression was not correlated with nest provisioning rate.

The strengths of this paper are its clear rationale and methodological approach, which make the findings very convincing and, in my opinion, reliable. The genetically determined morphs in the white-throated sparrow are an excellent model to investigate how specific genes (within the inversion) influence brain function at the level of specific neurons, in this case VIP neurons, and how this is related to behavioral differences in these morphs. The gene encoding VIP is within the inversion of WS morphs and the authors' aim was to examine whether this gene was differentially expressed between morphs and how this related to behavior and circulating hormones.

This paper examines the relationship between VIP expression and behavior through the quantification of 1) VIP mRNA from two brain nuclei, the anterior hypothalamus (AH) and infundibular region (INF), 2) frequency of territorial song and nesting provisioning and 3) circulating sex steroids.

The brain areas that this paper focuses on have well substantiated evidence from other birds to be linked to aggression and parental care and this regional specificity is key for being able to understand how the genetic changes can lead to behavioral differences and ultimately, distinct alternative mating strategies. Furthermore, these findings are important because they are able to provide evidence for how the behavioral differences between morphs are not solely due to differences in testosterone and estradiol, which is often difficult to demonstrate.

This paper is very well written, easy to follow and the statistics and results are clear.

Major comments:

1. Provide an explanation for how the axis for "relative VIP mRNA expression" (Fig. 2) was calculated. In the methods the explanation for quantification from the sections is clear, but then how were these mean pixel values converted to the axis that is shown, in other words, what does the "relative" mean, relative to what? Also, the values in Fig. 3 are the actual mean pixel values for each individual, is this correct?
3. I strongly suggest that you show box plots and the actual data points instead of the bar graphs. I think it is important for readers to be able to see the variability in the data, especially because the findings are related to

Minor comments:

1. State over how many years these birds were collected
2. Mention other brain areas that are known to have VIP expression, either from other bird species or from your own in situ on the white-throated sparrow
3. It would be useful to see the full nissl stained coronal section rather than the traced drawing.

Review form: Reviewer 2

Recommendation

Accept as is

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

This is an exceptionally interesting manuscript that clearly and economically reports its findings. The interpretations are not over or understated. The paper advances our understanding of the neuroendocrine basis of aggression and parenting by making excellent use of a study system that provides the opportunity to compare 2 sexes and 2 morphs in two brain regions. The same neuropeptide VIP is transcribed at greater levels in nucleus associated with aggression and at lower levels in a nucleus associated with parental behavior. The level of expression in males

relates to frequency of song, suggested a basis for individual variation. Several explanation for why VIP might vary are raised including SNPs in a regulatory region of degree of methylation, laying out a path for future research. I have no criticisms.

Decision letter (RSPB-2020-0196.R0)

06-Mar-2020

Dear Dr Maney:

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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It is a condition of publication that you make available the data and research materials supporting the results in the article. 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.

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

For more information please see our open data policy <http://royalsocietypublishing.org/data-sharing>.

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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].

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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,
Dr Sasha Dall
mailto: proceedingsb@royalsociety.org

Associate Editor

Comments to Author:

Both referees have only minor suggestions for revisions, which should be easy to implement. On a more personal note I recommend the authors to keep in mind the general readership of Proceedings B and try to reduce the number of acronyms. For instance, WS and TS could be spelled out throughout the text and maybe the authors see potential for reducing the number of acronyms also at other places.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

The main findings of this paper are that VIP expression in the AH predicts male song rate during the early breeding stage, and importantly, morph and testosterone do not. During the nestling stage, although WS males still have higher VIP expression in the AH compared to TS males, their song rate is no longer predicted by VIP expression, but rather by testosterone. The relationship between VIP expression in the AH and aggression also holds true for females. For the INF, the more parental morph had higher VIP expression but expression was not correlated with nest provisioning rate.

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3. I strongly suggest that you show box plots and the actual data points instead of the bar graphs. I think it is important for readers to be able to see the variability in the data, especially because the findings are related to

Minor comments:

1. State over how many years these birds were collected
2. Mention other brain areas that are known to have VIP expression, either from other bird species or from your own in situ on the white-throated sparrow
3. It would be useful to see the full nissl stained coronal section rather than the traced drawing.

Referee: 2

Comments to the Author(s)

This is an exceptionally interesting manuscript that clearly and economically reports its findings. The interpretations are not over or understated. The paper advances our understanding of the neuroendocrine basis of aggression and parenting by making excellent use of a study system that provides the opportunity to compare 2 sexes and 2 morphs in two brain regions. The same neuropeptide VIP is transcribed at greater levels in nucleus associated with aggression and at

lower levels in a nucleus associated with parental behavior. The level of expression in males relates to frequency of song, suggested a basis for individual variation. Several explanation for why VIP might vary are raised including SNPs is a regulatory region of degree of methylation, laying out a path for future research. I have no criticisms.

Author's Response to Decision Letter for (RSPB-2020-0196.R0)

See Appendix A.

Decision letter (RSPB-2020-0196.R1)

13-Mar-2020

Dear Dr Maney

I am pleased to inform you that your manuscript entitled "Vasoactive intestinal peptide as a mediator of the effects of a supergene on social behavior" 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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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,
Dr Sasha Dall
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
Thank you for the careful and thorough revision and congratulations to a great paper!
Wolfgang Goymann

Appendix A

Response to reviews

Dear Editor,

Thank you for the comments on our manuscript. Please see below for our responses. A version of the manuscript, then the supplemental material, has been appended here with changes tracked.

Associate Editor

I recommend the authors to keep in mind the general readership of Proceedings B and try to reduce the number of acronyms. For instance, WS and TS could be spelled out throughout the text and maybe the authors see potential for reducing the number of acronyms also at other places.

Response: The following abbreviations are now spelled out throughout the main text: WS, TS, AH, INF, STI, and ISH. A few abbreviations remain in the Table and Figures 2 and S1, with definitions in the captions.

We decided to leave “VIP” since it is long to spell out and appears almost 100 times in the text.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

Major comments:

1. Provide an explanation for how the axis for “relative VIP mRNA expression” (Fig. 2) was calculated. In the methods the explanation for quantification from the sections is clear, but then how were these mean pixel values converted to the axis that is shown, in other words, what does the “relative” mean, relative to what?

As described in the main text (second paragraph under “*Labeling and quantification of VIP mRNA*”) and the Supplemental Material (p. 2), we used ImageJ to measure the average gray value for VIP mRNA signal within each region of interest bilaterally in two to four sections containing the region for each bird. For each of these measurements the value was corrected for background by subtracting the gray value of a nearby region with no discernable signal. The values that we analyzed statistically thus represented the absolute value of this difference between the gray value of the region and the background gray value, averaged across region for each bird. We have edited this section of the main text to clarify these methods.

The Y-axis label in Fig. 2 has been changed to “Normalized VIP mRNA Expression”. The method of normalization is described in the figure caption.

Also, the values in Fig. 3 are the actual mean pixel values for each individual, is this correct?

The values in Fig. 3, panels a and e are the gray values for each region, then corrected for background, then averaged within individual. A sentence of explanation has been added to the caption.

3. I strongly suggest that you show box plots and the actual data points instead of the bar graphs. I think it is important for readers to be able to see the variability in the data, especially because the findings are related to

The bar graphs have been replaced with box plots.

Minor comments:

1. State over how many years these birds were collected.

This information was originally stated in the Supplemental Materials and is now repeated in the main text.

2. Mention other brain areas that are known to have VIP expression, either from other bird species or from your own in situ on the white-throated sparrow

A paragraph has been added to the Supplementary Materials explaining that the distribution of VIP mRNA in our material was similar to what has been reported in other avian species, and we have listed several major labeled areas.

3. It would be useful to see the full nissl stained coronal section rather than the traced drawing.

The purpose of the larger drawing is to show the precise locations of the areas of interest relative to major landmarks. The photomicrographs of the Nissl-stained tissue were acquired using a microscope at a magnification that allows one to see the cell groups of interest. Photomicrographs showing the entire section would require substantially lower magnification (not available on a standard microscope) and the cell groups of interest would not be as easy to see.

We have chosen to show higher-magnification photomicrographs of the relevant regions rather than low magnification of the entire area so that readers will be able to see the relevant regions in greater detail. As noted above, a blow-up of the entire section would not add relevant information not already available in the drawing, and would not be as informative with respect to the locations of the regions in relation to major landmarks.