PREreview of OA Week Live-streamed Neuroscience preprint JC

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Abstract

This is a review of the bioRxiv preprint "Sex Differences in Aggression: Differential Roles of 5-HT2, Neuropeptide F and Tachykinin" by Andrew N Bubak, Michael J Watt, Kenneth J Renner, Abigail A Luman, Jamie D Costabile, Erin J Sanders, Jaime L Grace, and John Swallow. DOI: https://doi.org/10.1101/407478 This review was compiled from discussion points raised during a PREreview live-streamed preprint journal club as part of Open Access Week, 2018. The event details can be found here and the collaborative Etherpad showing all the journal club notes can be found here.

In addition to those named as authors above, the participants who wished to be acknowledged for their contributions to this review are as follows: Dariusz K. Murakowski, Tim Koder, and Daniela Saderi.

Overview and take-home messages:

The study looked at the differential changes in behaviour following manipulations of both gene expression and the local environment of the stalk-eyed fly, in particular socialization, following a 7-day period of isolation. The genes of interest are all related to Serotonin signalling - a neurotransmitter linked to aggression in both invertebrates and vertebrates (perhaps in opposite ways). The authors found that 5-HT2 receptors played an inhibitory role in regulating the *initiation* of aggressive behaviours using siRNA knockdown experiments. In contrast, higher *escalation* of aggressive behaviours was observed following application of 5-HT, which the authors speculate was linked to increase Tk expression. Notably both decreased 5-HT2 receptor expression and increased Tk expression were observed in socially isolated males, who were also more aggressive in both measures suggesting that these two parallel pathways might confer a survival advantage under these environmental conditions. The authors did not observe these effects in female stalk-eyed flies, but it is unclear whether this results from different aggression pathways, or is a reflection of the specific behavioural assays used to assess aggression in this study.

Positive remarks:

The journal club participants were surprised that such a ubiquitous neurotransmitter can have such disparate effects across organisms and sexes, and led to the question of how we are able to create such different behaviours using a single molecule. Participants were particularly intrigued by the finding that different sub-types of 5-HT receptors exert opposing effects according to the context. This led to questions about differences in sub-type affinity and any potential impact on sex differences of expression. Furthermore, it was an interesting finding that there were sex-specific effects on aggression after isolation; in particular that size differences affect the aggressive behaviour of the male fly vs. a lack of aggressive behaviour regardless of size or rearing for females. We know so little about the associated receptors: where and how much they are expressed. There is still so much to investigate. This is one great aspect of this paper.

Constructive feedback:

Visualisation / Presentation

- How many people know how cool the stalk-eyed fly is? A simple photo and high-speed video recordings would be a really useful addition to the paper. Furthermore, it would be very nice to see a video that shows how the behaviour changes after the manipulations.
- It was difficult to visualize the behavioural types (initiation, high-intensity, etc.). Therefore, a picture of each type would have been helpful for the reader, together with additional behavioural descriptions. As above, a video/picture would say a thousand words.

Background / Structure

- The introduction was difficult to follow at the beginning. It would be useful to reveal what is known from the field and discuss how that related to this organism.
- It would be really useful to add to the introduction some of the ideas that were in the discussion, for example, the aims and justifications for using this species.
- Every piece of data was treated with the same importance. It would be useful to break the data apart a little to emphasize the important results, and place other less important results in an Appendix/Supplemental Figure/Table. It is difficult to parse out the importance at present, so this would help increase the clarity of the paper.
- Although Table 2 was useful to help summarize the findings of this manuscript, a model diagram/visual would be helpful, particularly to bring these findings into context with what is known in other invertebrates and vertebrates. This could be placed at the end of the manuscript, but referred to in the introduction also (re: what is already known in the literature).
- In line with the above, it would be helpful if each result can be contrasted with a species that is already known in order to give context. Furthermore, when different organisms were mentioned, the connection and comparison between them was not always clear. The referential data changed species from one section to the next, causing some confusion. Therefore, it may be helpful to focus on one or two species for clarity.

Technical Questions / Controls

- It seems possible that the test for aggression is only ethnologically relevant for males. Is there a preferred test of aggression for sex difference, or are there other conditions, for example maternality-related aggression tests, that would be able to pull out differences in the effects of manipulations

for females? It would be useful to be able to generalize across aggressive behaviours, otherwise it is important to state in the discussion that the results apply to this prototype of behaviour specifically. In other words, the authors did not observe these aggressive effects in female stalk-eyed flies, but it is unclear whether this results from different aggression pathways, or is a reflection of the specific behavioural assays used to assess aggression in this study. This should be commented on in the discussion.

- For the forced fight paradigm, specifically the siRNA and 5-HTP experiments, were the opponents socially reared? Furthermore, were the opponents familiar with each other? In some species, aggression differs toward familiar and non-familiar conspecifics, so this could potentially change the outcome of the fight.
- Have the authors considered, and/or tested, whether a change in the amount of movement may have caused some of these differences in aggression? Having the video footage might help us understand this more. It is possible that some lower level behaviours are being missed that would be captured by video analyses.
- Distinction between aggressive bout initiation vs the intensity of the individual experience was a very interesting element and the manuscript would have benefited from emphasizing this more. This is a curious finding, and we would have liked to know more. The high speed videoing may also help with this and could allow the incorporation of machine learning/AI to help distinguish between these two types of behavioural responses. An example of this type of software would be 'deeplabcut' (Mathis et al. Nat Neurosci, 2018).
- If the 5-HT receptors are similar between *Drosophila* and the stalk-eyed fly, could reagents (particularly immunohistochemistry) be used in the stalk-eyed fly to look at receptor sub-type localization/co-localization, and protein levels? If so, this might help the authors compare their results with the *Drosophila* literature.
- More specific antagonists/agonists for these receptors are needed as the pharmacology is limited.

Questions for the authors:

- The manuscript could have benefited from more details on where we stand before and where we are after this preprint. There is a lot of data that can be seen as conflicting across different model system and it would be useful to discuss this more. Furthermore, fundamental questions like "What is serotonin doing?" and "How do you reconcile what happens in invertebrates with other findings in vertebrates?" would be valuable additions to this manuscript. Can we really start to parse out some of these elements on how we can have multiple different effects with the same neurotransmitter? It would be really interested to know what the different evolutionary roles of aggression are in these model and non-model organisms. Might this underlie some of the differences?
- By looking at different receptor sub-types, were the authors trying to address behavioural differences between specific populations of neurons, or were they trying to suggest that serotonin is having a global influence on aggression? If so, this would suggest that serotonin acts in specific neurons to modulate different aspects of aggression depending on which population of neurons it is acting in. Maybe this is something the authors could comment on, or build upon for future experiments.
- Some of the figures show changes in the levels of neuropeptides for the females either after isolation or after treatments, but these changes are not significant. It would be interesting if the authors could expand upon possible minor effects these changes may cause and if they might be affecting other behaviours, even if the changes are not drastic enough to be significant.
- What is the affinity of 5-HT to the different sub-types of receptors? If this fact is known, perhaps the authors can include it in the discussion.
- How does increased aggression elevate 5-HT2 in the stalked-eyed fly?
- Could you make a fake opponent that a fly might engage or retreat from, even if there is a large difference in size? The authors might be able to pull apart different responses by doing this. Alternatively, the

authors could place the flies up against a mirror, and see if they fight themselves.
- We wondered whether aggression behaviours would happen across sexes when 5-HT receptors are manipulated, or whether the fly would show increased mating behaviours with the other sex due to their increased aggressive behaviours. The authors may wish to comment on this in the discussion.
- Is there a difference in 5-HT2 (or other) expression between males and females following 5-HT2 siRNA? [see Fig. 4 and 5].
- The manuscript would benefit from showing how many flies were tested at each stage, how many were lost from the data due to behavioural abnormality, injection failure, death etc? This would increase transparency about such aspects and lead to greater reproducibility and therefore credibility (note: this is a general point for scientific practices rather than a specific point about this manuscript).
- Is it possible to elucidate a causal role for Tk and/or NPF by injecting/expressing them directly?

Minor comments:

- **Title:** the authors may consider modifying the title to emphasize the key questions that are being addressed by this work. We feel this would increase the clarity and impact of this work.
- **Introduction:** it might be useful to reduce its length by grouping the information for the reasons to use the stalk-eyed fly. In lines 107-126, the authors demonstrate that the stalk-eyed fly can be a useful modeI to detect the conflict (i.e, initiation, escalation and termination). In lines 151-171, the authors demonstrate that the stalk-eyed fly can be an ideal model for examining inset aggression in a sex-dependent manner. It might be beneficial to place the information about NPY and sex difference in aggression (lines 127-171) in the discussion instead.
- **Introduction:** More links to other animals and humans would be useful. In lines 132-135: there is a link with mice; though the statement may be the wrong way around because reference 2 covers flies not mice, and reference 22, mice but not flies.
- **Line 242:** First use of NPFr should say "NPF receptor". If abbreviated, NPFr should be consistent throughout the text (see Lines 333, 334 later in the text).
- **Materials and Methods, Line 253:** this paragraph appeared to be more relevant for the results section than for the methods section.
- **Line 262:** the reference to Fig. 1 is apparently incorrect.
- **Line 277:** it would be beneficial to include more details about the siRNA procedure. Ideally the authors should make the sequence public and also comment on how many siRNAs were used to investigate this question. A table or list of all siRNAs used in this study could be added to the text.
- **Line 279:** more information on the cDNA would be useful.
- It would be useful to just show the absolute number of initiations in each case (rather than subtracting the number in the vehicle condition). The data are very convincing so it would be great to see all of it!
- **Fig. 1:** Simply using a color panel to illustrate the groups as females and males would be sufficient, rather than repetitively using a caption. Same suggestion for Fig. 3.
- **Fig. 1-7:** When there are less than 50 observations, it would be beneficial to see the individual points for each of the bar charts, similar to what is shown in the paired line plots.
- **Fig. 5D:** This figure is not described nor mentioned in the text (lines 411-416).
- **Fig. 8:** The "inhibitory" arrow is unclear. In particular, does it mean that 5-HT2 activation inhibits NPF signaling, or does it mean that a *reduction* in 5-HT2 activation inhibits NPF signaling?
- **Discussion:** This research was very insect-centric. More information about the relationship of the results to mammals, and the relationship to human health etc., would be appreciated.
- **Discussion:** The manuscript would benefit from the addition of more information about the 5-HT1A and 5-HT2 receptor in other organisms during aggression.
- **Discussion, Line 521:** Why does the isolation protocol for *Drosophila* differ from the one used in this work? The reasons for not using the same protocol could be stated.

Survey results:

As part of the live-streamed journal club, we conducted a short survey to allow all participants to contribute their overall impressions of the preprint. Below are the results of each question.

Question #1:

In your opinion, how interesting is the research?

- Not interesting
- Quite interesting
- Very interesting

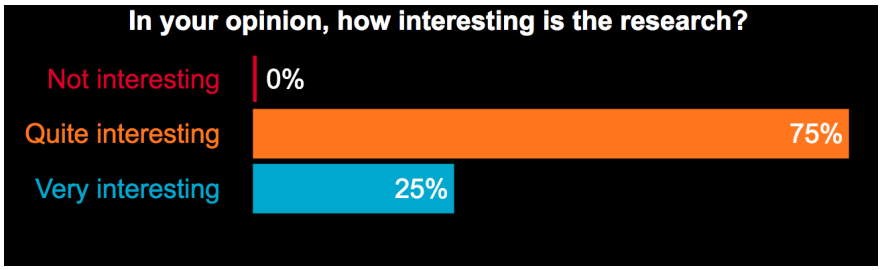

Figure 1: Survey question #1 (n=12 participants)

Question #2:

Which statement best describes the methods?

- Methods are incomplete or poorly written. There are insufficient replicates, and/or there are no (or incorrect) statistical analyses
- Methods are well written with enough detail for the overall procedure to be understood. Statistical significance is calculated
- Methods are complete and written with enough detail to be replicated, including reagents and strains used (if appropriate). Statistical significance is calculated using appropriate tests

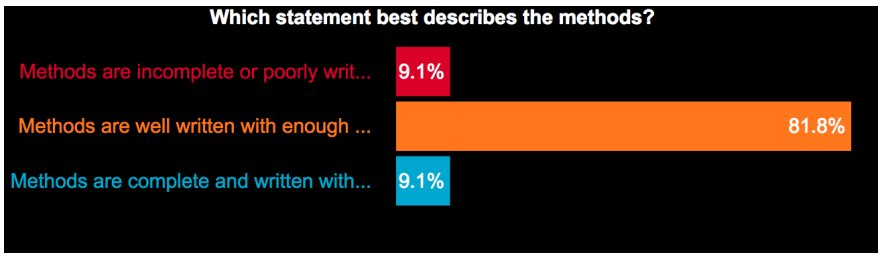

Figure 2: Survey question #2 (n= 11 participants)

Question #3:

Were the figures understandable without having to read the whole manuscript text?

- No, the figures/tables and/or legends were difficult to understand without reading the whole manuscript
- Some of the figures/tables and/or legends were understandable without reading the whole manuscript
- Yes, all of the figures/tables and/or legends were understandable without reading the whole manuscript

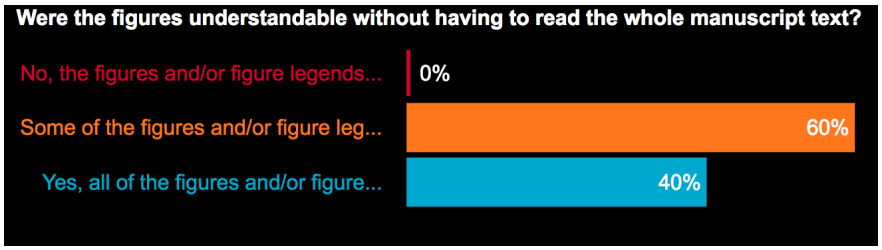

Figure 3: Survey question #3 (n= 10 participants)

Question #4:

Do the results support the conclusions?

- No, the results do not support any of the conclusions
- The results support some of the conclusions
- Yes, the results support all of the conclusions

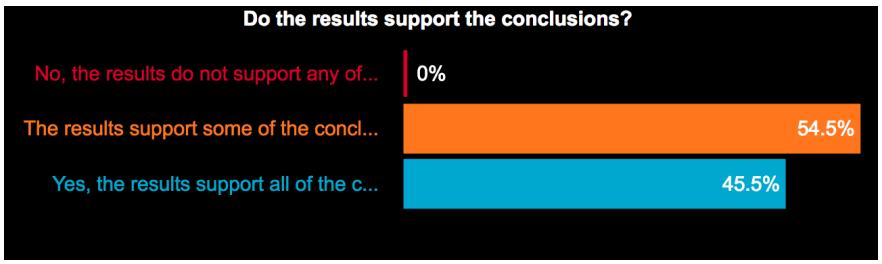

Figure 4: Survey question #4 (n=11 participants)