THE FLOREY

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15 July 2021

Prof Lian-Sheng Ma
Science editor
World Journal of Psychiatry

Dear Prof Ma,

Re: Submission of revised manuscript (ID 00614755)

Thank you for obtaining a review of our manuscript and the opportunity for submission of a revised manuscript.

We provide our detailed responses to the review suggestions below and have attached the revised manuscript with track changes.

Sincerely,

Terence Y Pang, PhD
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On behalf of co-authors Prof Gary Hime and Ms Sophie Stonehouse.

Response to Science editor's review:

Summary of the Peer-Review Report: The reviewer raised a great deal of issues regarding to this review. Important comments include: authors should give limitations of using invertebrates for psychiatric disorder research; the current review should not just be literature review, but also critical analysis of current researches; further describe how SZ, bipolar disorder models are created in Drosophila. The questions raised by the reviewers should be answered;

Response: Thank you for your summary review. We have edited the manuscript to include a discussion of the limitations of using invertebrates for neuropsychiatric research, comment on the shortcomings of the current research, and briefly discuss the approaches to modelling SZ/BD genetic endophenotypes in Drosophila.

Author's responses to reviewer comments:

1. *In the Introduction, reader gets a sense that every psychiatric disorder is inherited, there are non-heritable psychiatric disorders and I would advise authors to make that distinction clear in the introduction.*

Response: It was not our intention to suggest that every psychiatric disorder is inherited. As suggested, we have edited the Introduction (Page 6-7) to clarify that certain disorders (schizophrenia/bipolar disorder) have higher reported heritability compared to others (alcohol use disorders, major depressive disorders), albeit based on a limited number of studies and populations. We clearly state that epigenetic inheritance is likely to have very limited contribution to the latter conditions.

2. *One cannot do away with mice as model organism, it would be great if both non mammalian model and mammalian models are used for psychiatric research. Authors would be aware that most preclinical drug testing is done is rodents, so one has to use rodents for those kinds of work. Its not clear whether authors mean Drosophila can be used for basic research/ primary screening of drugs or preclinical research.*

Response: We apologise for this misunderstanding; it was not our intention to suggest that rodent models could or should ever be replaced by drosophila or c elegans. We have clarified this statement (Page 8) by indicating that invertebrate research should be complementary to rodent preclinical studies by focussing on the molecular/epigenetic modifications shared with rodents/humans. We hope that readers should at least be aware of the possibility for addressing the relevant biochemical questions in these alternative species.

3. *The authors only give advantages of Drosophila as model organism. Granted a long of neuroscience concepts have been discovered from drosophila studies, but to study complex neuropsychiatric disorders such as Schizophrenia what are the limitations.*

Response: We wholeheartedly agree with this statement that there are significant limitations to attempting to studying neuropsychiatric disorders in invertebrate models. This is discussed in the references cited [94-97] and we have further clarified the limitations of using Drosophila to study complex neuropsychiatric disorders (Page 30-31) to avoid potential misunderstandings.

4. *Authors cite an example of how temperature variations lead to changes in gene expression in Drosophila sperm and oocytes. And there is an increase in transposons during this stress. The authors claim that some disorders such as Bipolar disorder, schizophrenia (SZ) etc may be due to changes in transposon activity. If there are references, those should be cited to show association between transposons Bipolar disorder, schizophrenia (SZ). Unlike lower animals or plants, human do not have active transposons that contribute to variation or disease. And can Drosophila can be used to model Bipolar disorder, schizophrenia (SZ) has not been reported. Hence this argument is not persuasive at all.*

Response: We would like to clarify that this point was raised in reference to the c elegans literature, and not Drosophila. Included in our original text was a statement that "The role of repetitive elements in human health and disease is still unclear" [Lines 2-6, Page 12] and that "At the present time, there are also no available rodent models of abnormal repetitive element expression" [Lines 6-7, Page 12]. We point out that any relevance of transposons in general human health and disease remains speculative at this point. As per the reviewer's suggestion, we elaborated by adding specific references to schizophrenia studies that reported a link to retrotransposons (Page 12) but also flag to readers that the evidence is limited, and independent verification requires further research.

5. *On page 12 and 13, authors describe the mechanism of heat shock proteins and the associated histone H4 levels and with oxidative stress. Does this also affect neurons in Drosophila? Authors need to clarify.*

Response: Thank you for this question. There has indeed been a report of HSPs and their regulation of Drosophila neuronal function through modulation of their resilience to oxidative stress (improved) and lifespan (increased) (Liao, 2008). We have stated (Page 14-15) that this is consistent with the C elegans evidence but flagged that there is scope for further research in this regard.

6. *Authors claim that PTSD leads to changes in miRNAs, these are associations and they may affect neuronal genes, but can this change be transmitted transgenerational?*

Response: Thank you for this question and we agree that it is an important point to clarify so we have provided commentary on this in the manuscript [Pages 19-20]. The collective evidence based on different mouse models of paternal PTSD/stress have indicated that paternal exposures influence progeny behaviour (Dietz, Biol Psychiatry 2011) and changes to paternal sperm miRNAs are also

associated with altered gene expression in the offspring brains (Gapp Nat Neurosci 2014). Consistent with those independent findings, our own studies expanded upon those by demonstrating transgenerational effects of paternal corticosterone-treatment (modelling chronic stress) on progeny behaviour, together with altered the levels of sperm miRNAs and associated changes to the expression levels of imprinted genes such as Igf2 in the hippocampus of two generations of progeny.

7. *Can the authors describe how SZ, bipolar disorder models are created in Drosophila, how are the flies verified that they exhibit characteristics of SZ/PTSD/bipolar disorder. Once these diseases can be modelled in Drosophila, only then can you study these diseased flies for generations. I would strongly advise authors to cite the paper that have shown SZ/PTSD/bipolar disorders successfully modelled in Drosophila.*

Response: In accordance with the clarification requested above (Point #3), we have clarified that Drosophila are limited to modelling the genetic features of neuropsychiatric disorders, and not the complex spectrum of behavioural characteristics. However, the environmental factors associated with these disorders such as stress can certainly be modelled (as summarised in the Table we provided) resulting in certain epigenetic responses and adaptations, which then have the potential to be inherited across generations (subject to further investigations and validation). Again, it is not our intention to claim that these complex human conditions are accurately or comprehensively modelled in simple organisms. But Drosophila are definitely an avenue to explore basic molecular pathologies.

8. *As I understand in the Table 1 and Table 2, the last column shows the "Potentially relevant psychiatric conditions" in humans, but after the first two studies, the others are in mice or other animals. Since the aim of this review is highlighting use of Drosophila as model for Psychiatric conditions, they should restrict to relevant human conditions. Since many of these stresses may not induce "Potentially relevant psychiatric conditions", it would be best to remove these last two columns. The Table can just highlight the stresses that can cross over to F2, or F3 generations and have the epigenetics mechanism.*

Response: We accept the reviewer's perspective and suggestion. However, removing this information greatly diminishes the value of the Tables, which serve to consolidate common epigenetic modifications between human, rodent, and Drosophila/C elegans in a single location (we are not aware of similar tables in the published literature). This enables readers to appreciate what is currently known (but also how little is known, as was apparent to the reviewer). We suggest an alternative to deleting that information is to clarify in the Table titles that the collated information is also relevant to mammalian preclinical models.

9. *Epigenetic modifications identified by transgenerational studies of Caenorhabditis elegans relevant to psychiatry section illustrates several studies which show corelation between epigenetic*

changes and behavior, but it is not clear if these behaviours are transgenerational (Kim et al.,2016 and Heller et al., 2016). If C.elegans has proven to be efficient model, how is Drosophila model better than C.elegans needs to be explained.

Response: We take this opportunity to clarify that it is not our intention to suggest that Drosophila or C.elegans are superior to the other. We are simply highlighting the molecular and epigenetic aspects that feature in transgenerational inheritance observed in both organisms (as the two most commonly used invertebrate models in basic research) that may be relevant to mammalian models (prompting further research by readers hopefully).

10. *Authors should also give limitations of using invertebrates for psychiatric disorder researcher and highlight gaps and experimental means by which the limitations can be overcome. The review should not just be literature review, but also critical analysis of current research along with its limitations.*

Response: Thank you for making this fair point. We acknowledge the significant limitation of simple organisms not being able to model the complex nature of neuropsychiatric disorders. We provided this within the Conclusion section [Page 40-41], edited the phrasing to tone down the statements, but also re-hash the potential of combining simple organism studies with clinical and rodent model research.