Decision on your PeerJ submission: "A genetic manipulation of motor neuron excitability does not alter locomotor output in Drosophila larvae" (#2014:10:2891:0:1:REVIEW)

PeerJ <peer.review@peerj.com> Thu, Nov 20, 2014 at 12:53 AM
Reply-To: PeerJ <peer.review@peerj.com>
To: Erin McKiernan <emck31@gmail.com>

PeerJ

Thank you for your submission to PeerJ. I am writing to inform you that in my opinion as the Academic Editor for your article, your manuscript "A genetic manipulation of motor neuron excitability does not alter locomotor output in *Drosophila* larvae" (#2014:10:2891:0:1:REVIEW) requires a number of major revisions before we could accept it for publication.

The comments supplied by the reviewers on this revision are pasted below. My comments are as follows:

Editor's comments

We have now received expert reviews of your manuscript from 3 reviewers. Your paper is considered to be well written and generally thought to employ a valid experimental approach. However, each of the reviewers raised serious criticisms, focussed around the interpretation of the negative findings of the manuscript. As such, while it is possible that the submission may be acceptable for publication it will first require major revision. Please address the comments of the reviewers, with particular regard to the testing of the hypotheses raised in the Discussion. The Discussion as it stands is extensive and would benefit from a more focussed approach that directly addresses the data generated and the most likely explanations of the divergence between your data and the literature. Testing of at least one of these focussed hypotheses would considerable strengthen the submission. Validation of the techniques employed in the manuscript will be required. Please note that unless the reviewer’s criticisms are comprehensively addressed, with inclusion of new experimental data, the paper is unlikely to be acceptable for publication in PeerJ.

Please be aware that we consider these revisions to be major, and your revised manuscript will probably have to be re-reviewed.

If you are willing to undertake these changes, please submit your revised manuscript

(with any rebuttal information*) to the journal within 60 days.

*** Resubmission checklist:**

When resubmitting, in addition to any revised files (e.g. a clean manuscript version, figures, tables, which you will add to the "Primary Files" upload section), please also provide the following two items:

1. A rebuttal Letter: A single document where you address all the Editor and reviewers' suggestions or requirements, point-by-point.
2. A 'Tracked Changes' version of your manuscript: A document that shows the tracking of the revisions made to the manuscript. You can also choose to simply highlight or mark in bold the changes if you prefer.

Accepted formats for the rebuttal letter and tracked changes document are: docx (preferred), doc, or PDF.

As you previously uploaded a single manuscript file for your initial submission you will need to upload any primary high resolution image and table files separately if you have not already done so.

Melinda Fitzgerald
Academic Editor for PeerJ

Reviewer Comments

Reviewer 1 (Andreas Prokop)

Basic reporting

Insufficient validation of used tools (see below)

Experimental design

Good design, but insufficient use of complementary strategies (see below).

Validity of the findings

Lack of tool validation (see below).

Comments for the author

This paper is well written with impressive knowledge of the background of the topic.

The experimental strategy and data presented are of high quality and support the statements the authors make. However, unfortunately, this work is not ready for publication.

First, the authors have not provided good controls which would convince me that the experimental manipulation used was functional. In the Discussion a list of possible further tests is given that could and should have been tested within the presented work. Finally, there is almost exclusive mentioning of synaptic input to the MNs but little mentioning of synaptic output and potential compensation at the NMJ, although there is a huge body of work about homeostasis at the Drosophila NMJ. Certainly, this would not explain timing issues of MN activity, but the amplitude of responses and provide validation that the used tools are functional. Easy tests can be performed to image NMJs and their receptor clusters, and also miniature analyses could have been provided.

Second, the authors provide an impressive and knowledgeable list of published data in the Discussion, but most of them point out that their own findings are unusual and unexpected, even including experiments using the exact same genetic tools. Their own experimental data are therefore conflictive and require a more rigorous validation to make this a convincing manuscript. Many other tools for complementary approaches to manipulate Drosophila neurons are available and, in my view, restricting to one single and uncontrolled manipulation is insufficient.

In conclusion, I do unfortunately not feel that this paper is ready for publication because it does not yet make a valid scientific statement.

Minor comments:

l. 74-6: EKI needs a more thorough explanation either in the introduction or results section.

l. 162ff.: The MN1-Ib nomenclature is precise, yet little used. Certainly, the statement in the Discussion that the old nomenclature describes embryonic predecessors of the larval MN neurons is misleading. To make this article easier accessible, please, provide the traditional names at least once to explain what neurons your are using. Similarly, for muscle 1, please, mention the Bate nomenclature which is more precise.

l. 168ff.: Authors should consider to take out statements about non-included larvae since they do not provide helfpul information and are even confusing (I am not clear about whether 2 of four or 2 of six larvae were discarded). Take out similar statements in l. 193f. and l. 207.

l. 168ff: Please, indicate that you measure postsynaptic responses in muscles and not the neurons themeselves; please, state what technique was used (intracellular recordings? one/two electrode? patch?)

l. 181-90: The authors should consider giving the information about the physiological differences between aCC and RP2 together with similar information at the beginning of the first results section.

Reviewer 2

Basic reporting

No comment

Experimental design

No comment

Validity of the findings

In this manuscript by McKiernan [redacted] an FLP/FRT system is used to drive the expression of transgenes encoding dominant negative alleles of two Drosophila K channel subunits: ether-a-go-go and Shaker. The activity of muscles innervated by these motor neurons versus muscles that were innervated by unaffected motor neurons is then measured.
Surprisingly, they found no difference in motor activity between affected muscles and controls. The authors then propose and discuss several scenarios that could account for the observed result.
Although this result was unexpected and therefore potential interesting there is no attempt to experimentally test at least some of their hypotheses. In addition, the authors may want to consider the possibility that although this genetic manipulation has been shown to alter neuronal excitability, may not be effective to see a difference in muscle activity in their experimental paradigms.
In my opinion, this paper in its actual version raises more problems than it can solve and therefore it does not represent a sufficient advancement of the field to warrant publication at this stage.
The authors should consider the possibility to test some of their hypotheses and only then a revised version of the manuscript may be re-considered for publication.

Reviewer 3

Basic reporting

Figures 6A and 6B do not correlate with the description.

Experimental design

In the present manuscript authors investigate a possible effects of altering MN intrinsic properties on the rhythmic motor behavior in Drosophila larvae.

I have some concerns about the experimental design of this work:

1. Mosaic expression of EKI in MN1-Ib and MNISN-Is in created mosaic animals should be demonstrated.
2. Altered excitability of MN1-Ib and MNISN-Is in created mosaic animals should be demonstrated. For the best control, excitability of motor neurons and motor activity of muscle innervated by these neurons should be recorded from the same animal.
3. It would be good to check crawling behavior of not dissected mosaic larvae to avoid the possible effects of larval preparation on the muscle activity.
4. Although authors found no effect of changing motor neurons excitability on spontaneous motor behavior, they have planned several experiments for possible explanation. Authors could direct more efforts toward identification of possible mechanisms in this study.

Validity of the findings

It would be good to have recordings from more than two animals in experiments described in Fig. 6

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