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Journal of Visualized Experiments
One Alewife Center, Suite 200
Cambridge, MA 02140

Dear Editors:

Thank you for considering our manuscript titled “Quantitative Analysis of Neuronal Dendritic Arborization Complexity in *Drosophila*” for publication in *Journal of Visualized Experiments (JoVE)*. We would like to thank you for a prompt and comprehensive review. We would also like to thank the Scientific Review Editors for very helpful comments on our manuscript.

Below is a point-by-point summary of how we have addressed the Scientific Review Editors' concerns and revised the manuscript, accordingly.

Comment [A1]: Please thoroughly revise your manuscript for grammar. Several sentences cannot be understood.

Revisions: We have checked grammar and corrected errors accordingly.

Comment [A2]: Which tissues? In 3.5 the body was flattened but no tissues were extracted. Presumably the brain is extracted and then sectioned. Please describe all tissue processing steps to ensure proper imaging.

Revisions: “tissue” is removed. Changed to “larva body wall”.

Comment [A3]: Mention all the necessary microscopy settings, e.g. excitation and emission wavelengths. How are the various da neuron classes identified?

Revisions: Follow instructions for imaging of confocal microscope according to the laboratory's specific system. We deleted subtitle 4 due to the set-ups of confocal microscope vary in different labs. You may please see the change in 4 (deleted). Imaging processing.
We used the da-neuron specific GAL4 lines. The GFP was co- expressed in class IV da neurons specifically, which can be visualized directly by microscope.

Comment [A4]: Are the images processed at all before this step? Is any window and leveling performed? How do you determine the ends of the dendrites?

Revisions: These were the Z-projection images from confocal microscope and can be directly analyzed by tracing neurites. There might be some short branches cut off because of the limited number of Z-projection images. This method was the relative measurement of dendrites length compare to the controls. So we chose the endpoints of the selected path in the image visuals.

Comment [A5]: Unclear what this means.

Revisions: The sentence was reworded to “the dendrite path was traced until the endpoints of the selected path in the visible images”. Added a new sentence "Some dendrites were ended farther from the starting points, in which the pathway was divided into smaller segments. The segments were combined to provide a completed path for the tracer".

Comment [A6]: How do you determine the tip ends consistently?

Revisions: The dendrite path was traced until the endpoints of the selected path in the visible image. Some dendrites were ended farther from the starting points, in which the pathway was divided into smaller segments. The segments were combined to provide a completed path for the tracer. After tracing path, the endpoints were connected by the Freehand selections in Fiji ImageJ program window.

Comment [A7]: How is a path defined? Is it the length between two nodes? OR is it the path between the cell body and the dendrite tips meaning that 1 path can include multiple branches?

Revisions: The path was defined between the cell body and the dendrite tips and one path can include multiple branches, such as the primary arbors are the dendrites from the neuron cell body; the secondary arbors were branches from the primary and so on with the tertiary, quaternary and quinary arbors. The structure of dendrite branching was separated depending on the levels of branches. For instance, primary% was the number of branches divided by total number of branching, and so on.

Comment [A8]: Please mention all statistical test performed and sample sizes.

Revisions: Added statistical test performed and sample sizes.

Comment [A9]: Please mention all statistical test performed and sample sizes.

Revisions: Added statistical test performed and sample sizes.

Comment [A10]: Were all estimates based on just one sample per cohort? This study is unacceptable if that is the case and given the major concerns expressed by the peer reviewers we will not be able to accept this. If more than one samples were used please report the standard deviations.

Revisions: Group samples were tested. Sample size and the standard deviations were added.

Comment [A11]: Were all estimates based on just one sample per cohort? This study is unacceptable if that is the case and given the major concerns expressed by the peer reviewers we will not be able to accept this. If more than one samples were used please report the standard deviations.

Revisions: Group samples were tested. Sample size and the standard deviations were added.

Comment [A12]: Please mention all statistical test performed and sample sizes.

Revisions: added statistical test performed and sample sizes.

Comment [A13]: Unclear what this means.

Revisions: Dendrite length was the average of all dendrite lengths in all measured da neuron.

Comment [A14]: Per neuron?

Revisions: Yes, per neuron.

Comment [A15]: Dendrite length per neuron?

Revisions: No. Dendrite length was the average of all dendrite lengths in all measured da neurons.

Comment [A16]: Define 1,2,3,4 here.

Revisions: 1. Primary arbor. 2. Secondary arbor. 3. Tertiary arbor. 4. Quaternary arbor.

Comment [A17]: Please use an arrow to point to it. The low line weight makes it hard to see.

Revisions: An arrow is added.

Comment [A18]: Please remove the tradename Excel from the figure.

Revisions: "Excel" is removed.

Comment [A19]: How is a path defined? Is it the length between two nodes? OR is it the path between the cell body and the dendrite tips meaning that 1 path can include multiple branches?

Revisions: The path was defined between the cell body and the dendrite tips and one path can include multiple branches, such as the primary arbors were the dendrites from the neuron cell body; the secondary arbors were branches from the primary and so on with the tertiary, quaternary and quinary arbors. The structure of dendrite branching was separated depending on the levels of branches. For instance, primary% was the number of branches divided by total number of branching, and so on.

Comment [A20]: This isn't really a schematic. The information can simply be presented in the text somewhere. The only graphical component is already present in figure 1. Please consider deleting this figure.

Revisions: Fig 6 is deleted.

Comment [A21]: Please mention the sample sizes for each fly cohort.

Revisions: Sample size is added.

Comment [A22]: Define * here and mention the significance levels.

Revisions: added "p<0.05 was defined as the statistically significant".

Comment [A23]: Please thoroughly discuss the critical steps, troubleshooting, and limitations.

Revisions: The critical step was to remove as many tissues from larva body as possible to fully expose the da neurons for imaging and analyses. There might be some short branches cut off

because of the limited number of Z-projection images. As this method is the relative measurement of dendrites length in comparison with the controls, a large number of images for each group of da neurons should be taken to increase coverage areas of Z-projection images.

Comment [A24]: Please cite a reference.

Revisions: References are added.

Comment [A25]: Please cite a reference.

Revisions: References are added.

Comment [A26]: Please define this.

Revisions: Confusing text was removed.

Comment [A27]: Unclear.

Revisions: Confusing text was removed.

Comment [A28]: needs a reference.

Revisions: Added references¹⁵.

Comment [A29]: Unclear why the method was not applied to other classes of neurons and presented here along with results if you can claim that it “can” be applied.

Revisions: Revised the sentence to remove unsupported claim.

Comment [A30]: Reference?

Revisions: Revised the sentence to remove unsupported claim.

We have uploaded the revised manuscript file in word format and the high quality versions of revised figures in tiff file format to ManuscriptCentral in accordance with the *JoVE* publication requirements during the resubmission process.

Thank you once again for a very helpful and comprehensive review. We believe that our revised manuscript has been improved with the Editors’ suggestions and sincerely hope that it is now appropriate for publication in *JoVE*.

Best regards

Sincerely,

A handwritten signature in black ink, appearing to be 'Airong Li'.

Airong Li, MD PhD
Assistant Professor of Neurology