The Neuromuscular Effects of Modulating Acetylcholine Neurons in Fruit Flies Using Optogenetics

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Introduction

- Cholinergic neurons are essential for voluntary muscle contractions in humans.
- Parkinson's, Alzheimer's, LEMS, and MG are just few of many diseases and disorders with issues in cholinergic neurons.
- Faulty cholinergic neurons can eventually lead to paralysis in humans. On the other hand, excessive activation of cholinergic neurons can cause muscle spasms and involuntary contractions.
- In this study, we dug into the detailed implications of how fruit fly movement would be affected by acutely activating and inactivating cholinergic neurons.
- By using optogenetics, we are able to study different neurotransmitters noninvasively while the subject is still alive, providing insight on the roles of different neurotransmitters in humans.

Results

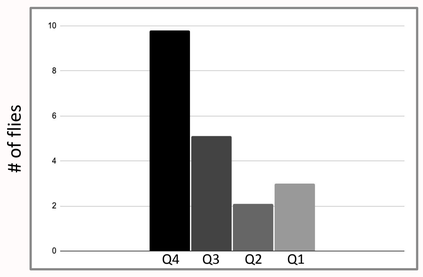


Fig. 1: Average number of UAS 41752 parental control flies per quadrant.

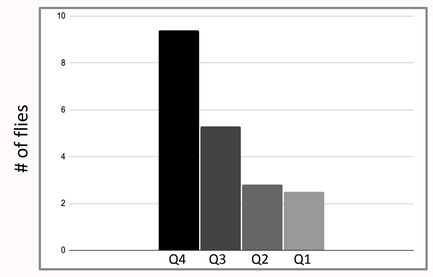


Fig. 2: Average number of UAS 55136 parental control flies per quadrant.

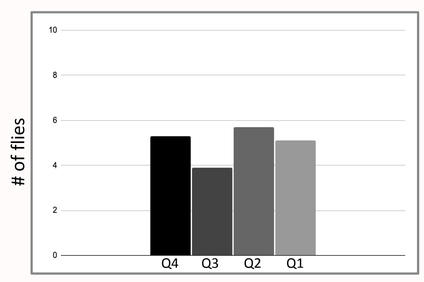


Fig. 3: Average number of ACh-Off flies per quadrant. (p<0.00001 by ANOVA test)

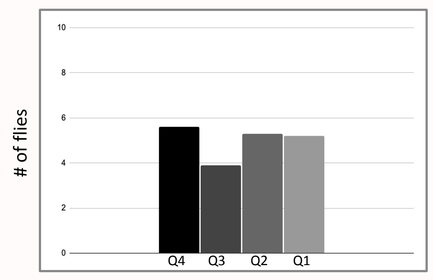
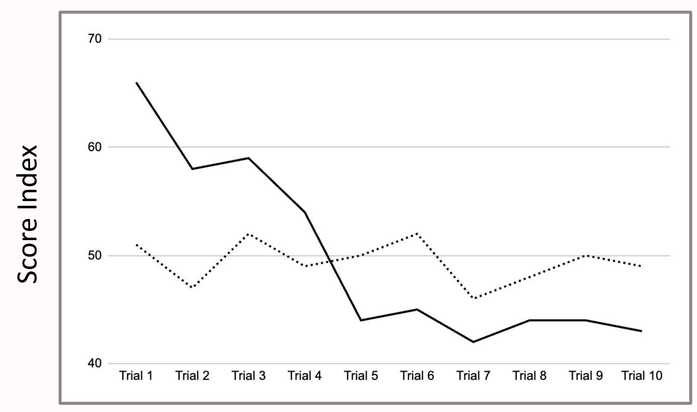


Fig. 4: Average number of ACh-On flies per quadrant.(p<0.00001 by ANOVA test)



Despite average score index being about the same, the trial values progressively decreased in the ACh-On cross, as opposed to the fairly similar values in the ACh-Off cross

Fig. 5: Comparison of ACh-Off and ACh-On score indexes by trial

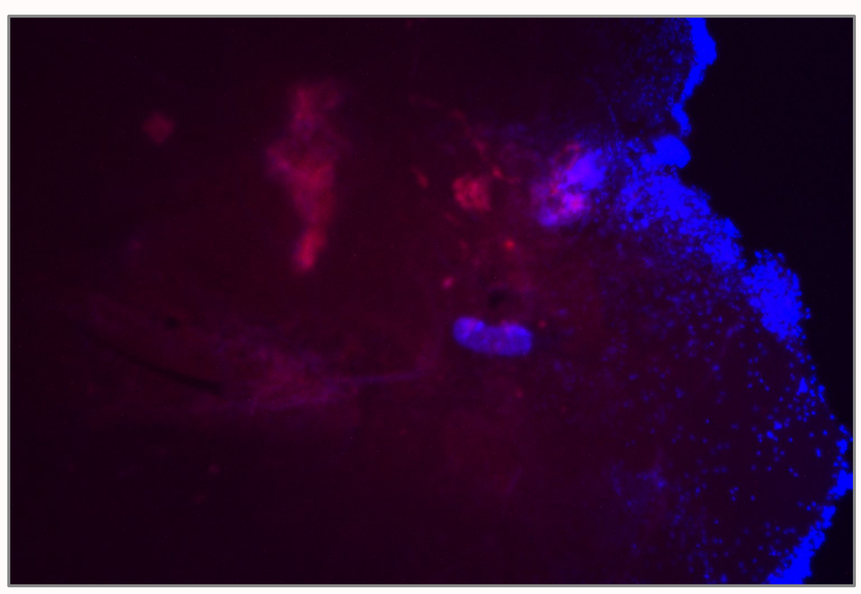


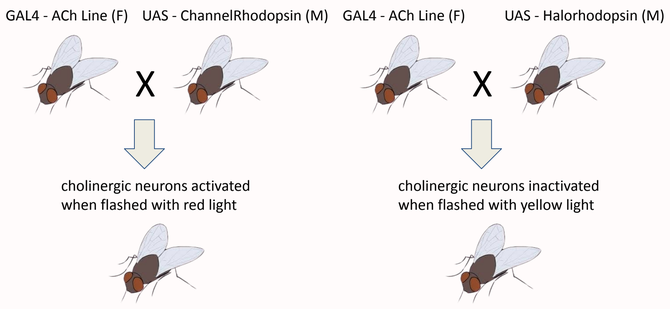
Fig. 6: Overlay image of the fluorescence and DAPI image of a dissected fly brain

Discussion/ Conclusions

- Both inactivation and activation of cholinergic neurons showed signs of impaired locomotion.
- As shown in figure 3, we found that when ACh neurons were turned off, flies noticeably had trouble reaching higher quadrants in the assay.
- This is easily explained since turning acetylcholine off will interrupt synapses in voluntary muscle movement.
- Similarly, figure 4 reports ACh-On flies to have displayed the same trends.
- Interestingly, ACh-On flies were seen to have progressively decreased in movement. We suspect that the constant activation of ACh neurons are causing them to be on the refractory period more often.
- As such, acutely turning on cholinergic neurons portrayed signs of developing muscle fatigue, rather than a complete muscle failure, seen in the ACh-Off cross.
- Conclusively, this study exhibits the significant impact acetylcholine has on fruit fly movement.
- Our research shows the ACh has similar functions in fruit flies as it does in humans, demonstrating great prospect in the use of drosophila organisms to study ACh and its effect on human behavior.

Methods

The most important aspect of the project was setting up the genetic cross using the GAL4/UAS system. The GAL4/UAS system is a genetic tool used to control gene expression in model organisms like Drosophila. It involves two main components: the GAL4 protein, a transcriptional activator driven by a specific promoter to control where and when it's expressed, and the UAS (Upstream Activation Sequence), which is a DNA sequence placed upstream of a gene of interest. When organisms carrying GAL4 and UAS constructs are crossed, the offspring express the gene of interest in a controlled manner, wherever GAL4 is active. This system allows researchers to study gene function, trace neural circuits, and model diseases with high specificity and versatility.



Channelrhodopsin and halorhodopsin are light-sensitive proteins used in optogenetics to control neuronal activity in Drosophila with precision. Channelrhodopsin, derived from algae, is a light-gated ion channel that, when activated by red light, allows positive ions to flow into neurons, depolarizing them and inducing neuronal firing. Conversely, halorhodopsin, derived from archaebacteria, is activated by yellow light and pumps chloride ions into neurons, hyperpolarizing them and inhibiting neuronal activity. By genetically engineering flies to express these proteins in specific neurons, researchers can use light to precisely manipulate neural circuits and study behaviors, neural pathways, and underlying mechanisms of neurological processes.

Climbing Assay

The climbing assay was created by taping two clear vials (totol 19cm) together and dividing it into four equally sized quadrants. The highest quadrant was labeled Q4 and the lowest was Q1.
Each test was run with 20 flies and 10 trials under closely monitored conditions. All lights in the lab were turned off and only the select color flashlight was shone on the tube from the midpoint of the tube. Once the assay was set up, we tapped down all the flies for about 5 seconds and gave them 20 seconds to climb the assay. At the 20 second mark, we recorded how many flies were in each quadrant and calculated a score index. The score index is calculated by multiplying the number of flies in each quadrant by the quadrant number and summing the products. Each trial, the flies were given a 40 second refractory period.

References

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