



Effects of Sleep Deprivation on Spatial Learning in *Drosophila*



OBJECTIVE

Sleep is an integral function in all organisms and is known to play a part in multiple physiological roles. Benefits of healthy sleep have been observed and documented to a high degree. The effects of sleep deprivation are just as extensive, yet they have not received such experimentation and study. The objective of these experiments is to observe the effects of reduced or impaired sleep on certain learning functions exhibited in the fruit fly *Drosophila*. The goal of this research is to identify what functions are impaired by lack of sleep and eventually find ways of reversing the negative effects of sleep deprivation.

BACKGROUND

It is well documented that impaired sleep can result in decreased cognitive function. The fruit fly *Drosophila* is an excellent model to study sleep and learning. They are easy to genetically modify, have very short developmental periods, and display sleep patterns very similar to those in most mammals. Consolidated sleep has been proven necessary to establish optimal learning in *Drosophila*, and this research aims to observe this relationship.

METHODS

- The main group of study is wild-type *Cs* flies. Flies are kept on a 12hr day-night cycle at a temperature of 24.6°C and fed a yeast and agar mixture. The genetic variant *rut1* is used as a comparison and control group due to its documented spatial learning defects.
- The DAM2 Activity Monitor was used to track the activity of each fly and excel functions were used to translate this data to minutes slept per hour. This is based on previous research describing the relation of fly activity to wakefulness.
- Sleep deprivation was achieved using a sleep-nullifying apparatus (SNAP) that takes advantage of the negative geotaxis observed in *Drosophila*. The fly is forced to the bottom of the tube every 10 seconds where it must ascend the walls of its confinement, only to be knocked back down – not allowing the fly to sleep at the top as it would prefer.
- The learning assay, or Heat Maze, is designed using a visual cue and a target quadrant. The visual cue is synced with the target and the time it takes to reach the target is recorded. The target is moved around an arena every 3 minutes. There are 10 trials at 10 different positions for each experiment.

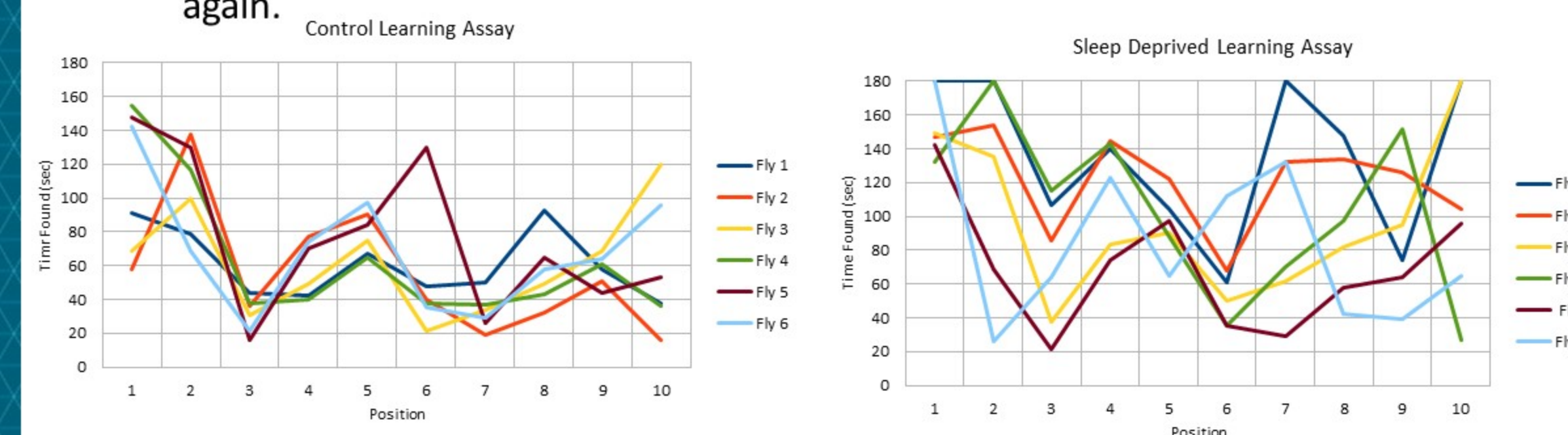


Natalie Gardner and Dr. Matthew Thimgan
Department of Biological Sciences

RESULTS

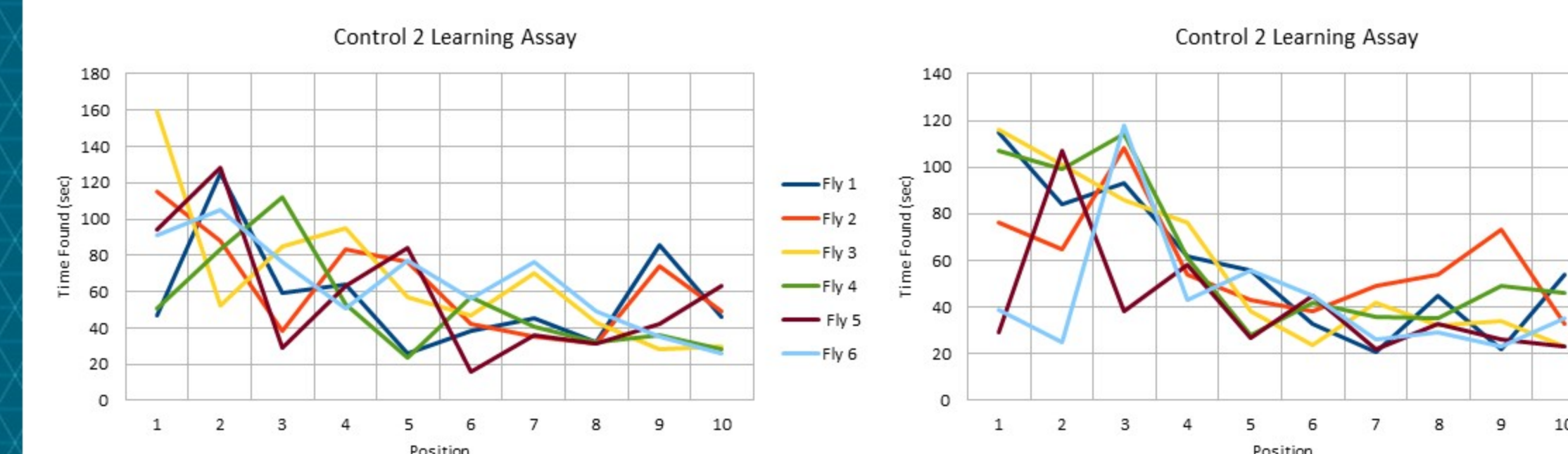
Based on the following results, it was concluded that *Drosophila* exhibit decreased spatial learning after a night of sleep deprivation.

The following set of experiments used the fly as its own control. Its learning was observed after a night of normal sleep. After being deprived of its sleep the following night, its learning was measured again.



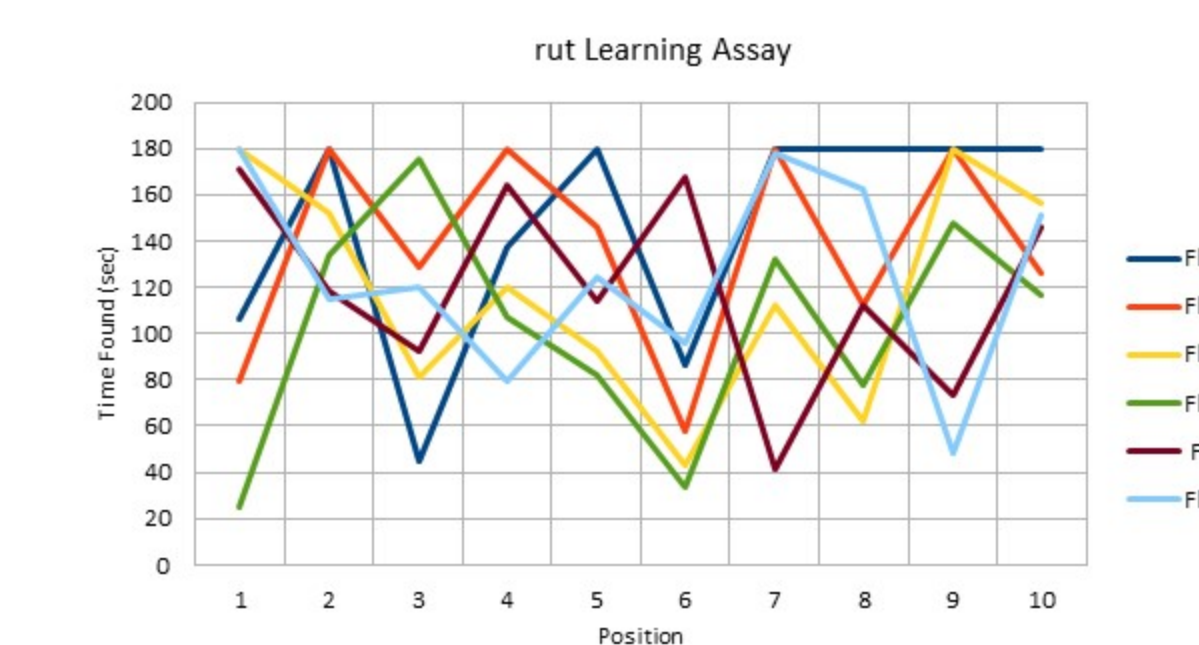
With a p-value < 0.05, we concluded there was a significant difference between the control and experimental groups. The control had an average of 64.4 seconds to reach the target while the experimental average was 101.8 seconds.

A second control group was used to provide data as to what a fly can achieve the second time through after a night of normal sleep. Since sleep plays a role in memory consolidation, we hypothesized that the fly would perform better the second time if allowed natural sleep.

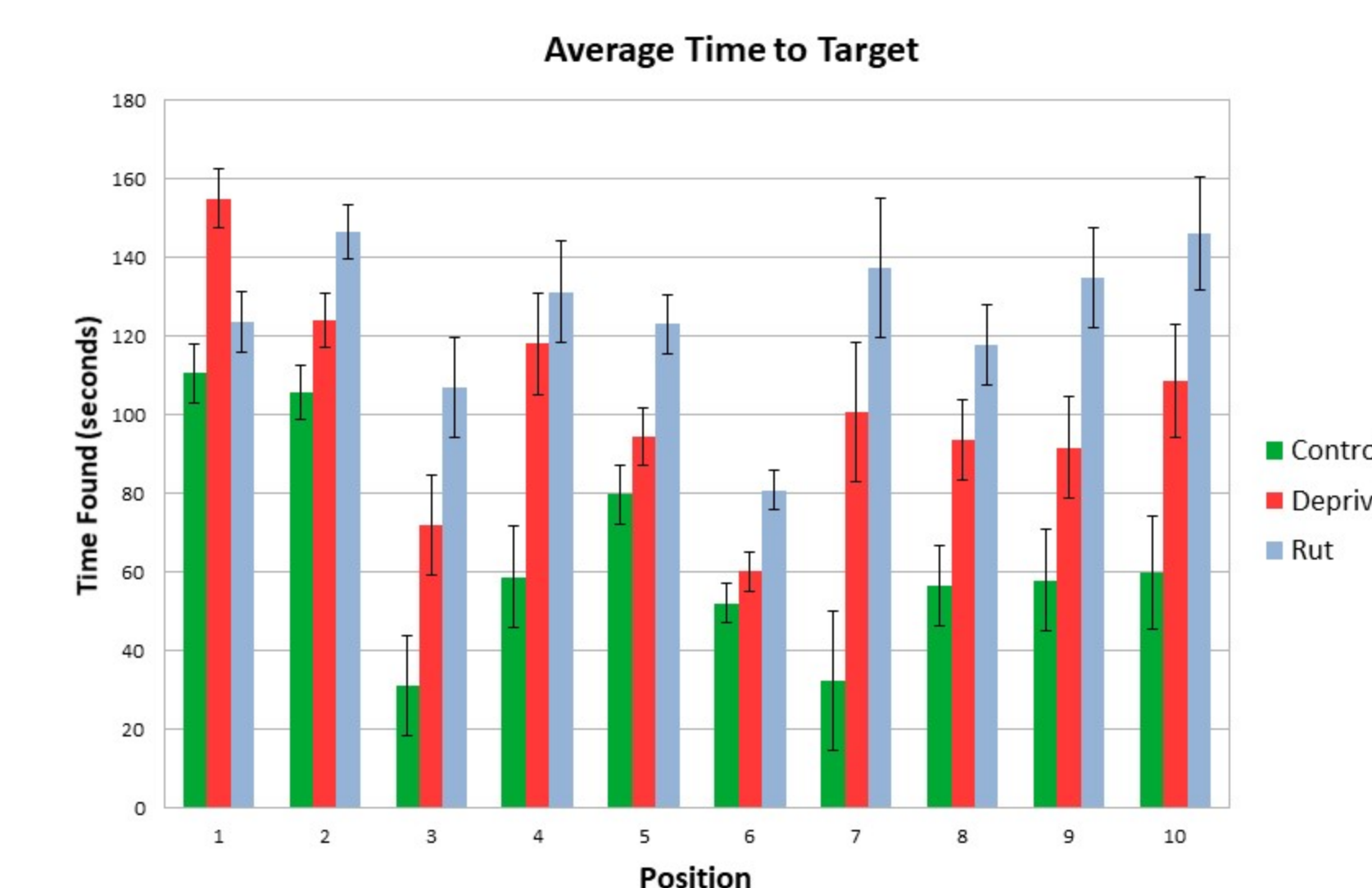


A T-test showed no statistically significant difference between the first and second runs, but, on average, the same fly was 6 seconds faster completing the trials the second time. There was a significant difference between the second run of the control compared to the sleep deprived group. The average of the unpaired control was 54 seconds compared to the experimental average of 101.8 seconds.

The genetic variant *rut1* was also used as a method of comparison. Based off previous research, these variants have a genetic mutation that negatively impacts their spatial learning capabilities. Therefore, we hypothesized that there would be similarities between their results and those of the experimental group.



After a night of natural sleep, *rut1* produced an average of 125 seconds to find its target. This is an increase compared to the experimental group, but a T-test showed no significant difference between the results of *rut* and the sleep deprived flies.



SUMMARY

These data provide evidence that sleep deprivation has a negative impact on spatial learning in *Drosophila*. Previous studies have shown that wakefulness does not damage functions related to performance in this learning assay, meaning poor performance is not related to any sleep deprivation-induced sensory impairments. With this in mind, we obtain the following information:

- Sleep deprived flies performed worse in our spatial learning assay compared to flies that had a healthy night of sleep, and a statistically significant difference was found between the two groups.
- Flies with adequate sleep on both nights performed better on their second time through the heat maze compared to the first time – increasing the significance of the experimental group's poor performance the second time through.
- Sleep deprived flies were found to be, on average, not significantly different from the *rut1* variants who have proven spatial learning deficits.

Further research could involve other factors in this experiment besides time. Video analysis software can be used to track the fly's movement to obtain information such as speed, distance traveled, and percent time spent in the target. Another potential improvement could be an additional stimulus when the temperature changes that signals the fly when it should relocate.

This research only concludes that extended wakefulness negatively impacts spatial learning in fruit flies. Further investigation could find the mechanisms by which this occurs and possible ways to reverse the negative effects of sleep deprivation.

ACKNOWLEDGEMENTS

- Van Dongen HP, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*. 2003 Mar 15;26(2):117-26. doi: 10.1093/sleep/26.2.117. Erratum in: *Sleep*. 2004 Jun 15;27(4):600. PMID: 12683469.
- Dissel S. *Drosophila* as a Model to Study the Relationship Between Sleep, Plasticity, and Memory. *Front Physiol*. 2020 May 28;11:533. doi: 10.3389/fphys.2020.00533. PMID: 32547415; PMCID: PMC7270326.
- Cirelli C, Bushey D. Sleep and wakefulness in *Drosophila melanogaster*. *Ann N Y Acad Sci*. 2008;1129:323-9. doi: 10.1196/annals.1417.017. PMID: 18591491; PMCID: PMC2715168.
- Levin LR, Han PL, Hwang PM, Feinstein PG, Davis RL, Reed RR. The *Drosophila* learning and memory gene *rutabaga* encodes a Ca²⁺/Calmodulin-responsive adenylyl cyclase. *Cell*. 1992 Feb 7;68(3):479-89. doi: 10.1016/0092-8674(92)90185-f. PMID: 1739965.
- Seugnet L, Suzuki Y, Vine L, Gottschalk L, Shaw PJ. D1 receptor activation in the mushroom bodies rescues sleep-loss-induced learning impairments in *Drosophila*. *Curr Biol*. 2008 Aug 5;18(15):1110-7. doi: 10.1016/j.cub.2008.07.028. PMID: 18674913; PMCID: PMC2603029.
- Krishna Melnattur, Leonie Kirszenblat, Ellen Morgan, Valentin Millitchin, Blake Sakran, Denis English, Rushi Patel, Dorothy Chan, Bruno van Swinderen, Paul J Shaw. A conserved role for sleep in supporting Spatial Learning in *Drosophila*. *Sleep*, Volume 44, Issue 3, March 2021, zsa197, <https://doi.org/10.1093/sleep/zsa197>
- Berry JA, Cervantes-Sandoval I, Chakraborty M, Davis RL. Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting. *Cell*. 2015 Jun 18;161(7):1656-67. doi: 10.1016/j.cell.2015.05.027. Epub 2015 Jun 11. PMID: 26073942; PMCID: PMC4671826.
- lyengar A, Imoehl J, Ueda A, Nirschl J, Wu CF. Automated quantification of locomotion, social interaction, and mate preference in *Drosophila* mutants. *J Neurogenet*. 2012 Sep;26(3-4):306-16. doi: 10.3109/01677063.2012.729626. Epub 2012 Oct 29. Erratum in: *J Neurogenet*. 2019 Dec;33(4):218. PMID: 23106154; PMCID: PMC3613147.

