Exploring Learning and Locomotion Deficits in a *Drosophila* Seizure Model

Madison Lee[1]; Anthony Lilienthal[2]; Krishna Madhav Nukala[2]; J. Robert Manak, Ph.D.[2,3]

[1]Los Altos High School, [2]Dept. of Biology, [3]Dept. of Pediatrics, The University of Iowa 52242

Research Objective

To investigate the effects of isoform-specific *prickle* mutations on the neurological dysfunction of *prickle* larvae, mainly short-term memory and movement

Introduction

Prickle encodes two adult protein isoforms of the Planar Cell Polarity (PCP) complex, and mutations in both isoforms have been associated with neurological disorders. For instance, when the *prickle-spiny-legs* (*sple*) isoform is mutated, the fly exhibits seizures and locomotor defects that mimic those found in human *PRICKLE* patients (Ehaideb et al., 2016). Conversely, when the *prickle-prickle* isoform (*pk*) is mutated, preliminary data reveals widespread neurodegeneration in the mutant brains, and *pk/pk* mutants show a pronounced reduction in lifespan. Finally, when a mutation affects the entire gene (*pk-sple13*) intermediate phenotypes between *sple/sple* and *pk/pk* are observed.

While individuals with *PRICKLE* mutations can present with seizures, they also have an increased likelihood of presenting with an autism spectrum disorder (ASD) that includes intellectual disability (i.e., learning and memory deficits) (Paemka et al., 2013). Preliminary data in the Manak laboratory has shown that adult *sple* mutants, in addition to manifesting seizures, show learning deficits (Figure 1a). Therefore, we wanted to determine whether locomotor or learning deficits were also manifest in earlier life stages of development of the *sple* mutants (i.e., larvae; Fig. 1b).

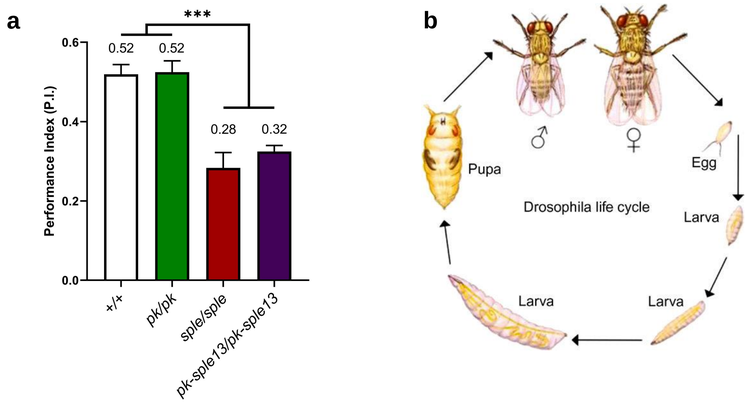


Figure 1: Adult *Drosophila prickle* mutants show a decreased ability to learn. a) The Performance Index (P.I.) indicates how well the adult fly learns to associate smell with an electric shock. *pk/pk mutants* showed no change when compared to controls; however, both the *sple/sple and pk-sple13/pk-sple13* mutants show a significant decrease in short-term learning ability.
n = 225 adult flies each (assayed in 3 batches of 75). *** p < 0.001. One Way ANOVA
b) Illustration of the *Drosophila* life cycle.

Methods and Materials

Genotypes (All outcrossed to a w^{1118} background)

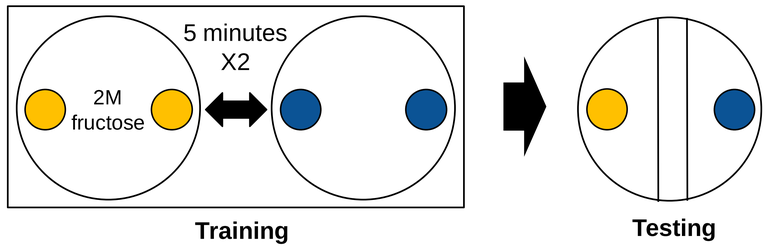
- *WT* (+/+)
- pk^{sple1}/pk^{sple1} (*sple/sple*) – null mutation of the *spiney-legs* isoform, seizure-prone
- pk^{pk1}/pk^{pk1} (*pk/pk*) – null mutation of the *prickle* isoform, exhibits neurological degeneration and shortened lifespan
- $pk^{pk\text{-}sple13}/pk^{pk\text{-}sple13}$ (*pk-sple13/pk-sple13*) – null mutation for the *prickle* gene

Larvae Crawling Assay

1. L3 larvae were individually placed onto an 85 mm 2.5% agarose plate. 30 seconds of active crawling were recorded to track the larval movement. (Canon High Definition Vixia HFM31 Camcorder with zoom [resolution 1920 × 1080] for higher resolution). Motion was assessed using a Manual Tracking plugin of FIJI software

Larval Olfactory Learning Assay

1. L3 larvae were trained on 2M fructose agarose plates with 2 caps of either 1:100 OCT or 1:25 MCH (shown below) for 5 minutes.
2. The larvae were then transferred onto an agarose plate without fructose with 2 caps of the opposite scent for 5 minutes.
3. Steps 1 and 2 were repeated
4. Larvae were immediately placed in the center of the testing plate, which contained 1 cap of each scent. After 5 minutes, the position of each larvae was recorded.
5. The performance index was then calculated.



Results

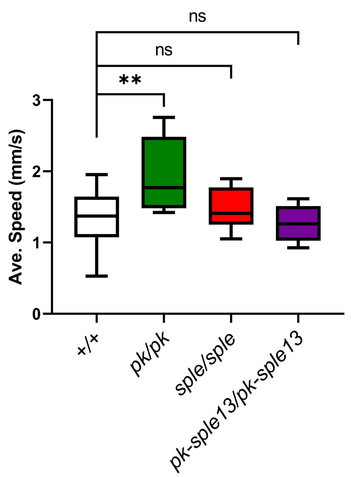


Figure 2: *pk* mutant larvae, but not *sple* mutant larvae, show an increase in locomotor speed. Note the wide variance of velocities for the *pk/pk* mutants in comparison to the other isoforms. However, the median speed for *pk/pk* is higher than the other isoforms and shows a statistically significant increase in velocity. Notably, the *sple/sple* mutant velocities are not significantly different than control, suggesting that the *sple* mutant larvae do not have a locomotor defect.
n = 12 for +/+ larvae, 10 for *pk/pk* larvae, 11 for *sple/sple* larvae, and 10 for *pk-sple13/pk-sple13* larvae.
* p < 0.05, ** p < 0.01. One-way ANOVA

Results

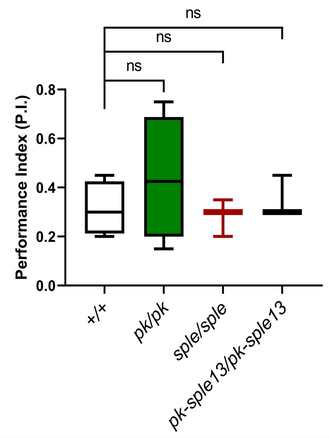


Figure 3: *Drosophila* L3 *sple* mutant larvae do not exhibit learning deficits. Preliminary data reveals that *sple* mutant larvae can associate a scent with an attractive stimulus (fructose) similar to controls. This suggests the *sple* mutation does not drastically impact the ability of *Drosophila* larvae to form and retain short-term memories. n = 30 for all lines. Kruskal-Wallis test.

Conclusions

- *sple* mutant larvae do not show obvious locomotor defects as assessed by the crawling assay.
  - Notably, these data suggest that *sple* mutants only show locomotor defects as adults.
- *sple* mutant larvae do not show learning deficits as assessed by the larval olfactory learning assay.
  - Similar to the locomotor assay results, *sple* mutants only show learning deficits as adults.

Future Directions

- Increase the sample size for each strain for both experiments.
- Explore automating movement tracking to standardize tracking and allow for assessing more detailed tracking parameters.
- Employ the learning assays with an aversive rather than attractive stimulus (NaCl or Quinone) to determine whether learning ability differs between control and mutants.

Acknowledgements

- This work was supported by a grant from the Stead Family Department of Pediatrics, Carver College of Medicine to JRM.
- I would like to thank Dr. Manak and everyone in the Manak lab for their time, help, and input over this summer.

References

1. Ehaideb, S. N., Wignall, E. A., Kasuya, J., Evans, W. H., Iyengar, A., Koerselman, H. L., Lilienthal, A. J., Bassuk, A. G., Kitamoto, T., & Manak, J. R. (2016). Mutation of orthologous *prickle* genes causes a similar epilepsy syndrome in flies and humans. *Annals of Clinical and Translational Neurology*, *3*(9), 695–707. https://doi.org/10.1002/acn3.334
2. Tao, H., Manak, J. R., Sowers, L., Mei, X., Kiyonari, H., Abe, T., Dahdaleh, N. S., Yang, T., Wu, S., Chen, S., Fox, M. H., Gurnett, C., Montine, T., Bird, T., Shaffer, L. G., Rosenfeld, J. A., McConnell, J., Madan-Khetarpal, S., Berry-Kravis, E., … Bassuk, A. G. (2011). Mutations in prickle orthologs cause seizures in flies, mice, and humans. *The American Journal of Human Genetics*, *88*(2), 138–149. https://doi.org/10.1016/j.ajhg.2010.12.012
3. Eaideb, S. N., Iyengar, A., Ueda, A., Iacobucci, G. J., Cranston, C., Bassuk, A. G., Gubb, D., Axelrod, J. D., Gunawardena, S., Wu, C.-F., & Manak, J. R. (2014). *prickle* modulates microtubule polarity and axonal transport to ameliorate seizures in flies. *Proceedings of the National Academy of Sciences*, *111*(30), 11187–11192. https://doi.org/10.1073/pnas.1403357111
4. Apostolopoulou, A. A., Widmann, A., Rohwedder, A., Pfitzenmaier, J. E., & Thum, A. S. (2013). Appetitive associative olfactory learning in *drosophila* larvae. *Journal of Visualized Experiments*, (72). https://doi.org/10.3791/4334
5. Scherer, S., Stocker, R. F., & Gerber, B. (2003). Olfactory learning in individually assayed *drosophila* larvae. *Learning & Memory*, *10*(3), 217–225. https://doi.org/10.1101/lm.57903
6. Paemka, L., Mahajan, V. B., Skeie, J. M., Sowers, L. P., Ehaideb, S. N., Gonzalez-Alegre, P., Sasaoka, T., Tao, H., Miyagi, A., Ueno, N., Takao, K., Miyakawa, T., Wu, S., Darbro, B. W., Ferguson, P. J., Pieper, A. A., Britt, J. K., Wemmie, J. A., Rudd, D. S., … Bassuk, A. G. (2013). Prickle1 interaction with synapsin I reveals a role in autism spectrum disorders. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0080737