

Is your memory Shot? Investigating the Role of Cytoskeletal Proteins in Learning

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Probing the molecular mechanisms of learning and memory. Can knocking down the cytoskeletal cross-linker Shot prevent learning in *Drosophila*?

***Drosophila* are a simple model organism, capable of learning, easy to keep, with many homologous and analogous genes to investigate.**

Shot is a cytoskeletal crosslinking protein:

- Allelic to the gene Kakapo
- Binds actin and microtubules [1]
- Important for steering growth cones to their targets [2]

Why care about growth cones?

Growth cones steering is necessary for proper synaptogenesis [3], i.e. learning!



Image from <http://flybase.org/tmp-shared/reports/FBim0000474.png>

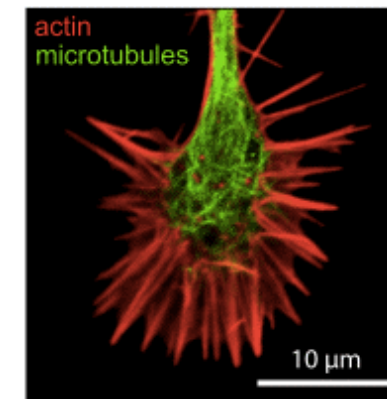


Image from Koch et al. 2012 [4]

Results:

Hypothesis: Both control types will be able to learn and display scent-food associations after a starvation period. Knocking down Shot will impair fly ability to learn these scent-stimulus pairings.

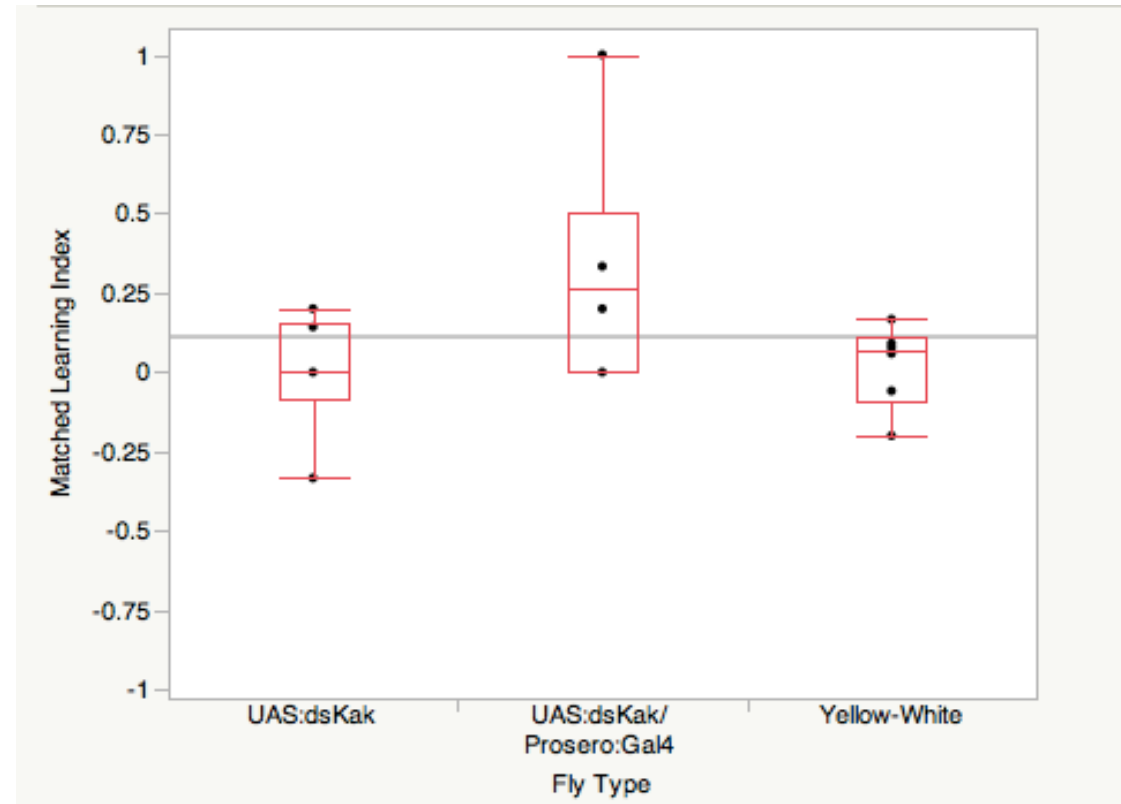


Figure 1: Aggregated learning index for Yellow-White flies, UAS-dsKak flies, and UAS:dsKak/Prospero:Gal4 flies. Higher values would represent a greater success in moving in the direction of the paired scent. No difference was observed between groups, but more surprisingly, no learning effects were observed either.

Methods:

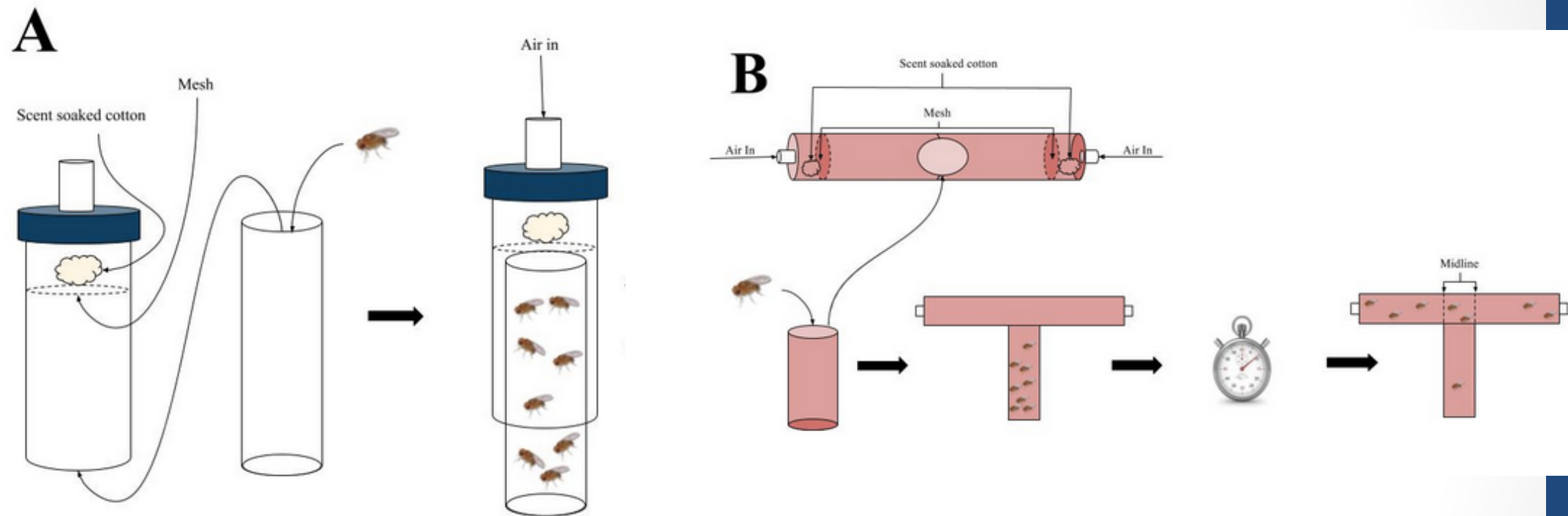


Figure 2. Schematics depicting conditioning paradigm and learning assay.

In A: Flies starved 24 hrs -> Flies placed in tube with neutral scent ->

Waiting period with clean air -> Flies placed in tube with preferred scent and sugar plate.

In B: Flies placed in T-Maze apparatus -> Neutral and Preferred scents blown from opposite direction

-> Flies chemotax (move) towards preferred scent -> Flies counted, index determined.

In Conclusion:

No flies assayed showed effects of learning. Further investigation needed to answer this question.

Future Directions:

Future endeavors may need to more thoroughly explore options in apparatus construction and scent delivery in order to see optimal effects. Success of genetic manipulations should also be determined through genetic (blotting) or cell biological (dissection, dissociation, and antibody staining) methods. Because this experiment was ultimately unsuccessful, the question remains unresolved and invites further explorations.

References:

Image used:

<http://flybase.org/tmp-shared/reports/FBim0000474.png>

Methods figure produced by Theresa Steele.

1. Applewhite, D.A., Grode, K.D., Keller, D., Zadeh, A.D., Slep, K.C., and Rogers, S.L. (2010). The Spectraplakins Short Stop Is an Actin-Microtubule Cross-Linker That Contributes to Organization of the Microtubule Network. *Molecular Biology of the Cell* 21, 1714–1724.
2. Geraldo, S., and Gordon-Weeks, P.R. (2009). Cytoskeletal dynamics in growth-cone steering. *Journal of Cell Science* 122, 3595–3604.
3. Haydon, P.G., McCobb, D.P., and Kater, S.B. (1987). The regulation of neurite outgrowth, growth cone motility, and electrical synaptogenesis by serotonin. *J. Neurobiol.* 18, 197–215.
4. Koch, D., Rosoff, W.J., Jiang, J., Geller, H.M., and Urbach, J.S. (2012). Strength in the Periphery: Growth Cone Biomechanics and Substrate Rigidity Response in Peripheral and Central Nervous System Neurons. *Biophysical Journal* 102, 452–460.

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