The Effect of Bang-Sensitive Mutations on Olfactory Learning In *Drosophila* Larvae

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Abstract

The goal of this experiment is to examine the effects of bang-sensitive mutations on *Drosophila* larvae's associative learning. This was tested through a double-blind, olfactory associative learning assay. It was found that the bang-sensitive mutants have abnormalities in olfactory associative learning. However more research is needed to determine the source of this abnormality.

Introduction

Larvae were chosen as test subjects because of their simplicity and the youth of the field. *Drosophila* larvae are easy to grow, harvest and manipulate. On a neurological level the number of olfactory neuron receptors in a larvae is 21 pairs and the number of gustatory neuron receptors is about 80 pairs. When compared to adult *Drosophila*, which have 1300 pairs of olfactory neuron receptors and 650 pairs of gustatory neuron receptors, the larvae are neurologically much simpler. The larvae also have a simplified sensory network, with three major sensory organs: the dorsal organ, the ventral organ and the terminal organ (see Figure 1).

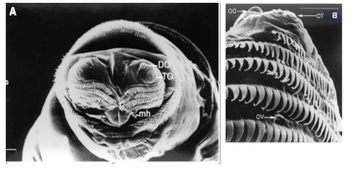

Figure 1. The major sensory organs on a third instar larva's head. Panel A shows an anterior view of the larval head and Panel B shows a ventral view. DO (dorsal organ) controls gustatory and olfactory functions, TO (terminal organ) and VO (ventral organ) both control gustatory functions.[1]

Bang-sensitive mutations in adult *Drosophila* cause the adults to have seizures when traumatized with intense vibrations, or electrical stimulation. The seizures that mutant adult *Drosophila* experience has been compared to the seizures that an epileptic patient may experience. Bang-sensitive *Drosophila* are known to have neurological abnormalities. This gives rise to the hypothesis that bang-sensitive mutants may express learning deficiencies as larvae in an associative, olfactory/appetitive assay.

Experimental Method

The experimental procedure was modified from Scherer et. al. Three to four larvae were trained on an agar plate by exposing them to two different attractive olfactory cues (amyl acetate and 1-octanol) while simultaneously exposing them to a negative or positive reinforcement (quinine sulfate or fructose). Fructose acts as a positive reinforcer, and should train the larvae to have a higher preference toward the associated odor. Quinine sulfate acts as a negative reinforcer and should train the larvae to have a lower preference to the associated odor. Both *sda* and wild-type larvae were trained in the same way. Two treatments were administered: one to build a preference toward amyl acetate and one to build preference to 1-octanol. Only third instar larvae were used (see Figure 2).

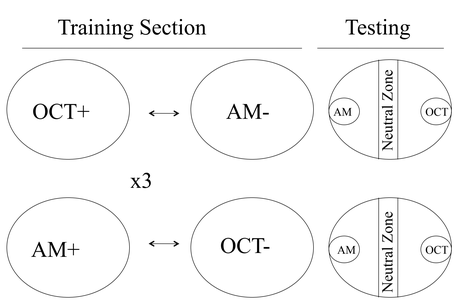

Figure 2. The training and testing processes. AM stands for amyl acetate, OCT stands for 1-octanol, - represents quinine sulfate reinforcement and + represents fructose reinforcement

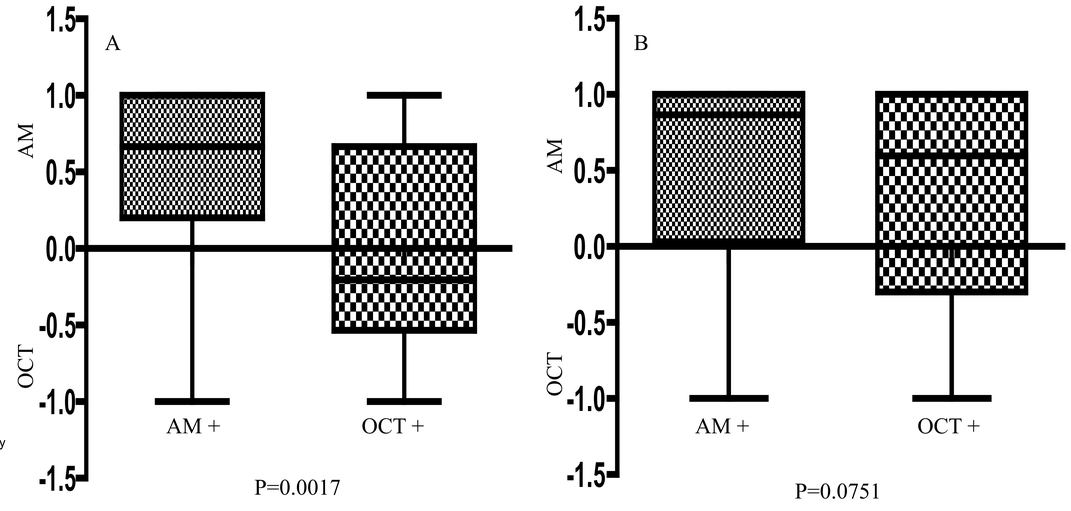

Figure 3. Box and whiskers plot of the data gathered from the experiment. Graph A shows the box and whiskers plot for the Pref(AM) for the wild-type larvae, while Graph B shows the Pref(AM) for the *sda* larvae. Pref(AM) is the preference toward AM the larva have after treatment.

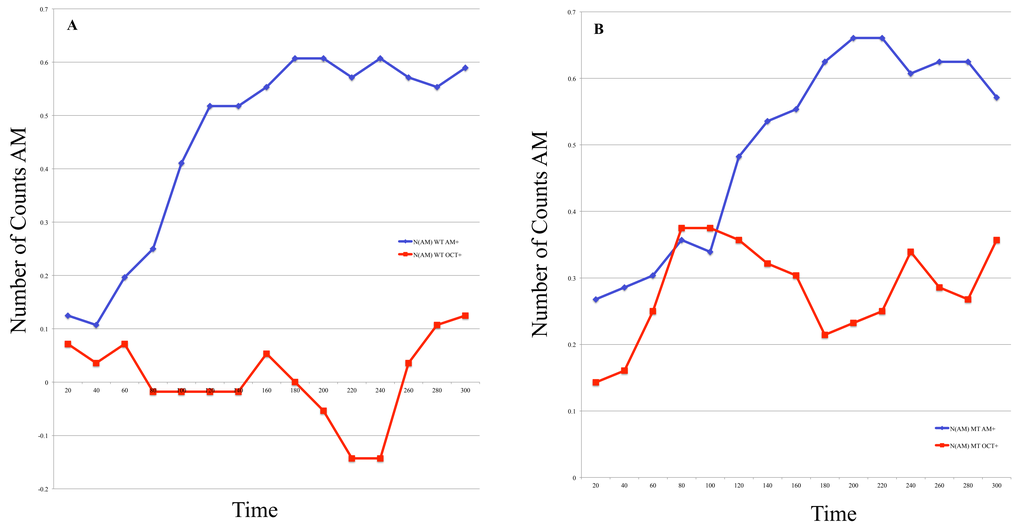

Figure 4. Graph A shows the Num(AM) calculated from the raw data for the tested wildtype larvae, while Graph B shows the Num(AM) calculated from the data collected from the *sda* larvae. These graphs indicate that *sda* larvae were less responsive to the treatment.

Experimental Method Continued

To test for associative learning, after training each larva was placed on a new agar plate with a 7mm neutral zone down the center and each of the olfactory cues on opposite sides. The larva was placed in the center of the plate and was allowed to freely roam for 5 minutes. Every 20 seconds the position of the larva was recorded. A preference value was taken for every larva and a number count value was taken for each larva's position at every time point.

Results and Discussion

As seen in Figure 3, Graph A, there is a statistically significant difference between the preference toward amyl acetate for each treatment of the wild-type larvae, which indicates learning. However there was no significant difference in the preferences toward either odor in the mutant larvae, shown in Graph B, which indicates that learning did not occur.

Although the data show that the there is an abnormality in the *sda* larvae's learning capabilities we can not conclusively state that the larva are learning deficient. If the mutant larvae were learning deficient we would expect a Pref(AM) value to be around zero. By examining Figure 4, we can also see that both the mutant and the wild-type larvae show a higher preference to amyl acetate. If the larvae were truly learning deficient we would expect graph B in Figure 4, to show more random movements. From the data and the experimental protocol we cannot determine whether the source of the abnormality is behavioral or sensory. It may be necessary to re-evaluate the dilution of the amyl acetate to ensure the preference between the two cues are the same. Also, another assay can be developed and performed which does not involve the olfactory system. For example the visual/appetitive assay from Gerber et al. could be used.

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[1] Scherer S, Stocker RF, Gerber B (2003) Olfactory learning in individually assayed *Drosophila* larvae. *Learn Mem 10:217–225*

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