Journal of Psychology and Behavioral Science
December 2015, Vol. 3, No. 2, pp. 36-44
ISSN: 2374-2380 (Print), 2374-2399 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jpbs.v3n2a5
URL: http://dx.doi.org/10.15640/jpbs.v3n2a5

Drosophila Melanogaster Tone Interval and Substrate Discrimination Employing Classical Conditioning

Brianna D'Elia[1], Alexa Decker[2], Amanda Disney[3], Claudia Ko[4] & Julian Paul Keenan[5]

Abstract

It is speculated that perceiving or differentiating two combined tones (i.e., an interval) is not an evolutionarily evolved trait in *Drosophila melanogaster*. However, in humans and other advanced mammals, recognizing two simultaneously presented auditory stimuli is often thought to be a critical component of verbal linguistic communication. It is not known when tone interval differentiation evolved (or if there has been independent evolution) or if genes exist in insects that might translate into complex interval recognition abilities. Here, we examined *Drosophila melanogaster* to determine if they are able to discriminate between a consonant and dissonant tone paired with different substrates employing a classical conditioning paradigm. Previous research has indicated that sucrose is an unconditioned stimulus (UCS+) that elicits a positive (e.g., approach) response. Conversely, caffeine (UCS-) elicits a negative response. Following 2 conditioning trials of 5 minutes each, the organisms were tested for successful learning. White noise was included as a control.A significant interaction was discovered such that successful conditioning occurred when caffeine was paired with a dissonant interval and sucrose was paired with a consonant interval (but only larvae). Furthermore, conditioning in larvae had a slight but significant correlation in adults. Further research should focus on molecular genetic mechanisms and the role of individual differences in genomics.

Keywords: Drosophila Melanogaster, Tone Interval, and Classical Conditioning

1.1 Being a widely studied species in genetics, *Drosophila melanogaster*have the potential to be a particularly important organism in basic cognitive psychological research. Because they are non-mammalian, non-vertebrae, they are often overlooked in terms of basic cognitive or behavioral research. However, for close to a century *Drosophila* have been of high importance in the study of human diseases such as Alzheimer's, Schizophrenia, and Depression as well as normal behavior such as memory and learning(Boekhoff-Falk & Eberl, 2014; Reza et al., 2013; van Alphen & van Swinderen, 2013).In many ways *Drosophila* are well suited for extrapolating to humans in terms of behavior because they have similar biological components to humans,suchas synapses, synaptic vesicles, mitochondria, microtubules, etc. (Gan, Lv, & Xie, 2014).

Successful auditory communication is critical for Drosophilas'reproductive success, specifically during courtship. During courtship and mating, *Drosophila* employ wing movement for communication between one and other(Murthy, 2010; Riabinina, Dai, Duke, & Albert, 2011).

---

[1] Cognitive Neuroimaging Laboratory, The Department of Biology and Molecular Biology, Montclair State University.
[2] Cognitive Neuroimaging Laboratory, The Department of Biology and Molecular Biology, Montclair State University.
[3] Cognitive Neuroimaging Laboratory, The Department of Biology and Molecular Biology, Montclair State University.
[4] Cognitive Neuroimaging Laboratory, The Department of Biology and Molecular Biology, Montclair State University.
[5] Cognitive Neuroimaging Laboratory, The Department of Biology and Molecular Biology, Montclair State University, 132 Science Hall, Montclair State University Montclair, NJ 07043. Phone: (862) 596-0448, keenanj@mail.montclair.edu

Courtship allowsthe male and female to attain the ability to communicate via auditory stimuli therefore enabling them to detect multiple sensory cues at simultaneously (Albert & Göpfert, 2015; Bretman, Westmancoat, Gage, & Chapman, 2011; Maguire, Lizé, & Price, 2015a) and auditory detection is a highly important factor in the female's ability to successfully mate with the male(Morley, Steinmann, Casas, & Robert, 2012).

During mating the nervous system is filtering out the stimuli that are not pertinent(Kim, Lee, Lim, & Han, 2013) while auditory exposure increases neural activation (Ghaemi, Rezai, Iyengar, & Selvaganapathy, 2015). Males may employ multiple auditory signaling attempts during mating (Saleem, Ruggles, Abbott, & Carney, 2014) and females appear to be capable of detecting cues such as the male's directionality, which is in part a determining whether the male is experienced or inexperienced in intercourse(Morley et al., 2012; Saleem et al., 2014).However, audition is not limited to mating. For example, auditory signaling is utilized during flight(Fuller, Straw, Peek, Murray, & Dickinson, 2014). When the *Drosophila*'s antennae are employed they become more stabilized, especially when flying through narrow regions(Fuller et al., 2014) .

In humans and other advanced mammals, recognizing two simultaneously presented auditory stimuli is often thought to be a critical component of verbal linguistic communication(see Bowling & Purves, 2015; but see McDermott, Lehr, & Oxenham, 2010).Multisensory integration has also been studied in both humans and *Drosophila*(Duistermars & Frye, 2010; Mahoney, Holtzer, & Verghese, 2014). For example, *Drosophila* use an abundance of multisensory integration throughout everyday endeavors such as identifying and tracking odors(Duistermars & Frye, 2010). When multisenorystimuli areemployed, it has been found that there the auditory cortex in the brain is critical in integration(Foxe et al., 2002), though it works in concert with other areas (King & Walker, 2012; Peelle & Sommers, 2015; Ursino, Cuppini, & Magosso, 2014). Additionally, mice are in fact better at dealing with paired audiovisual stimuli than the stimuli presented alone emphasizing the positive effect of two stimuli presented together rather than separately (Siemann et al., 2014, for negative effects in humans see Mahoney et al., 2014).

Auditory signals convey different information using differences in rhythm (e.g., inter-pulse interval), amplitude/volume, timbre (i.e. wave complexity), as well as a number of other factors including pitch (i.e, frequency). These factors are important in both human and non-human communication including Drosophila (Albert & Göpfert, 2015). Presenting two tones of two different pitches simultaneously is commonly referred to as an interval and is important to understanding both human language and musicality including individual brain differences(Gaab, Keenan, & Schlaug, 2003; Keenan, Thangaraj, Halpern, & Schlaug, 2001). Tones are often presented together in different frequencies (e.g., pitch intervals) and some of these are perceived as pleasant, or in harmony (consonant) while other intervals are perceived as dissonant (for review, Moore, 2002; Tramo, Cariani, Delgutte, & Braida, 2001). In a study performed on the brainstem in mammals, it was discovered that consonant and dissonant tones were already encoded at sensory-level processing levels. In fact, it was found that the brainstem responds very well to consonant tones to distinguish between "pleasant" (consonant) and "unpleasant" (dissonant) music (Bidelman & Krishnan, 2009; Cousineau, McDermott, & Peretz, 2012). According to Minati, consonance assists with early auditory processing because there was a greaterhemodynamic response in the brain when presented with consonant tones compared to dissonant tones (Minati et al., 2009).

There is specific neural circuitry involved in the performance of auditory communication in *Drosophila melanogaster*. First, the antenna of the *Drosophila* works as an "ear"(Eberl & Boekhoff-Falk, 2007). The Johnston's organ is the primarysource in the antenna that auditory signals pass through(Göpfert & Robert, 2002; Matsuo et al., 2014). When examining the antennae, there aresensilla, which are the hair-like covering of the antenna that are involved in odor sensing(Menuz, Larter, Park, & Carlson, 2014). Furthermore, within the *Drosophila*'s auditory processing, there aremushroom bodies located in the brain of the *Drosophila*, which are often involved in neuronal processing. This cranial component may assist*Drosophila*in learning and memory (Ito et al., 1998). There are different levels of strength in learning and memory among *Drosophila* due to their genetic diversity(Reza et al., 2013). For example, research on olfactory learning and odor recognition has been studiedwith *Drosophila* melanogaster and demonstrates findings in relation to biological functionality, the auditory system, and identification of neuronal components(de Belle & Heisenberg, 1994; Menuz et al., 2014; Schwaerzel et al., 2003; Yao, Ignell, & Carlson, 2005).

Schwaerzel employed olfactory learning to condition *Drosophila*. The use of sugar was employed as a reward and electric shock was used as a punishment, which demonstrated that when *Drosophila* are conditioned using olfactory learning they are able to equate an unconditioned stimulus with a specific odor(Schwaerzel et al., 2003; Schwaerzel, Jaeckel, & Mueller, 2007).Amazingly,*Drosophila* larvae were shown to have traces of odor memory therefore, showing that when conditioned or introduced multiple times to a specific odor they were able to retain a specific memory of it(Mishra, Chen, Yarali, Oguz, & Gerber, 2013). Drosophila can be conditioned both at larval an adult stages of life. Furthermore,it was found that the larval DL2a and the adult PPL2 DA cell clusters are highly correlated. This suggests that *otd*, which is necessary for *Drosophila*'s brain survival, is a selector gene that's variancein expression among DA neurons might contribute to differences in function(Blanco, Pandey, Wasser, & Udolph, 2011).

Numerous studies have been performed that demonstrate that (in *Drosophila*)learning at the larval stage effects them as adults(Gerber et al., 2004; Hariharan et al., 2014; Huang, Ng, & Jackson, 2015). For example, when consuming sugar,*Drosophila* were found toregain working memory that later evolved into long-term memory particularly in relation to food(Yamagata et al., 2015). As small and undeveloped as *Drosophila*larvae are, they are able to have a high level of olfactory memory exemplifying that larvae are able to be conditioned similarly to adults(Mishra et al., 2013).*Drosophila* are not limited to olfactory training, as it was discoveredthat sensory neurons that were conditioned on larvae can be carried on to adulthood(Gerber et al., 2004).Additionally, Blanco's study suggested that there is a cell lineage between larvae and adults. Through his findings it can be proposed that performance as larvae can foresee adult behavior (Blanco et al., 2011). Therefore, we sought to examine tone interval recognition in Drosphilia melanogaster. To our knowledge, this is the first study of its kind. We also sought to determine if interval learning interacted with substrate (i.e., food) in a controlled environment.

Methods

2.1 *Materials:*

There were 180*Drosophila* melanogaster; Standard Stock (Carolina Biological) used throughout the duration of this experiment. 30g of US Biological LB Agar Miller (Powder) was used as a plate medium. 0.4g of Sigma-Aldrich Caffeine was used as the negative unconditioned stimulus and 0.4g of Levulose Fructose Reagent Grade was used as the positive unconditioned stimulus. Standard culture vials were used to store the *Drosophila*. We prepared these vials with Deionized water, Formula 4-24 Instant Drosophila Medium Blue, and standard netting. All vials were placed in their assigned laboratory tube racks. Additionally, four petri dishes were used in the larvae experiment as storage containers for the *Drosophila* larvae that were waiting to be conditioned and tested. Two specialized petri dishes (Carolina Biological Item #746619) with standard earphones glued to the top of each lid were used throughout the duration of both larvae and adult phases of the experiment. A first generation iPod touch was used to elicit the proper tones at 700Hz within each part of the experiment.

2.2 *Procedure:*

2.2.1 Intervals

Tones were computer generated sine tones using Cakewalk Sonar III (Gibson) and Audacity (Shareware). Two intervals were chosen, a major 7th for dissonance and a major 5th for consonance(Moore, 2002; Tramo et al., 2001). The tone intervals were mixed into a single channel as described above bit presented as dual channels.

2.2.2 Initial Breeding

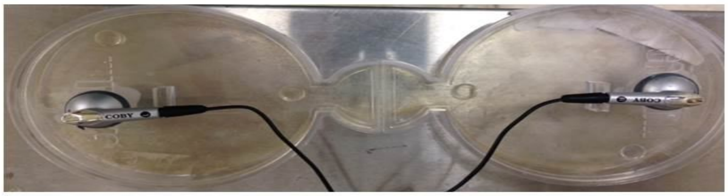
Drosophila Melanogaster; Standard Stock (Carolina Biological), were initially placed in standard culture vials with a mix of Formula 4-24 Instant Drosophila Medium Blue. A small piece of plastic mesh was placed into the Instant Drosophila Medium Blue and the adult male and female *Drosophila* were transferred into the tube. They were left to breed in a 25 Degree Celsius Incubator for 7-10 days and checked on regularly to see if any larvae had been produced.

2.2.3 Larvae Experiment

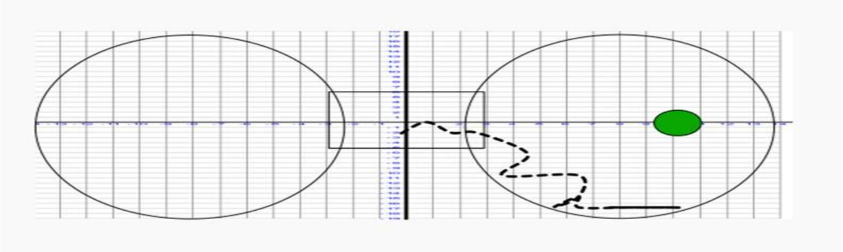
The initial experiment was performed on the *Drosophila* larvae. Once the breeding had occurred, 20 larvae were randomly selected and put in a covered petri dish. Next, the petri dishes were prepared with a basic agar plate (Biological LB Agar Miller). A specialized petri dish was used for this experiment (Carolina Biological Item #746619) which is basically a 'choice chamber' such that two petri dishes are connected in the middle (see Figure 1).

Caffeine was used as the negative unconditioned stimulus (UCS-) and associated with either a consonant or dissonant tone pairing. Sucrose was used as the positive unconditioned stimulus (UCS+) and associated with either a consonant or dissonant tone pairing. Lastly, there was a control that did not have any unconditioned stimuli and only a neutral tone was played (white noise).

A



B



To condition the *Drosophila*, we used caffeine and associated it with either a consonant or dissonant tone pairing. First, in one dish of the choice chamber a 5mL transfer pipet was cut to about one inch long and held in place by a scissor in the middle right hand side of the petri dish. Then, the agar was poured into the petri dish. .10g of caffeine was mixed with 10mL of agar and pipetted into the 1-inch tube. The twenty larvae were then taken out of the tube and put into a separate petri dish waiting to be conditioned. The larvae were conditioned for two sets of five minutes. After the first set of five minutes all twenty larvae were taken out and placed in the petri dish again. They were left to rest for one minute then placed back into the specialized petri dish and conditioned again for another five minutes. Following conditioning all larvae were housed individually.

2.2.4 Testing

The poured petri dishes contained agar only as we were testing to see if conditioning occurred. Two larvae were placed into the middle of the petri dish. Every 15 seconds the positions of the larvae were recorded. After the five minutes had elapsed the *Drosophila* were placed into their assigned tubes and put in a tube rack. This was done for all twenty larvae. After all twenty were tested the tubes were put in the incubator at (see above).

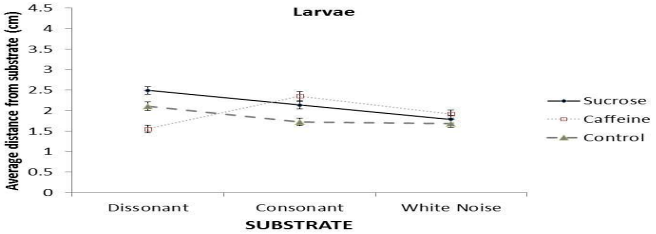
2.2.5 Adults

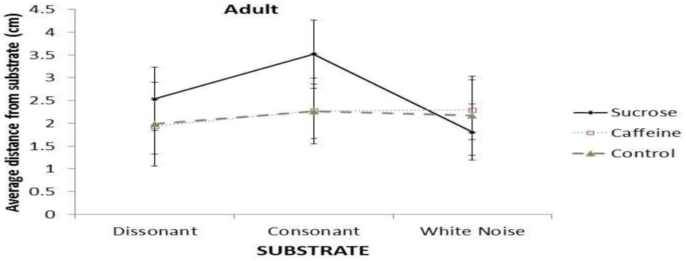
The second part of the experiment was performed on the adult *Drosophila* that matured from the previous experiment. Once each of the five batches had matured they were taken out and tested. The *Drosophila* were taken two at a time and tested the same way as the larvae were. The *Drosophila* was not conditioned in this part of the experiment. They were only tested to determine if they retained their conditioning from their larval stages. The same exact procedure as the larvae part of the experiment was used in this part.

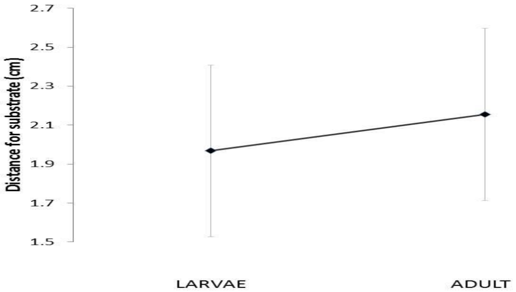
Results

3.1 There were two main sets of findings examined for significance. The first was the interaction between interval and substrate and the second was the correlation between larvae and adult on conditioning. Supplementary findings were also examined. Here we wished to replicate the previous finding that sucrose served as a UCS+ and caffeine served as a UCS- as well as determine if dissonance and consonance influenced behavior. A total of 180 larvae were to be tested, however, 13 died between conditioning and testing leaving a total of 167 larvae. Of these, 114 successful reached adulthood. In terms of larvae across the timepoints (i.e., each timepoint was used as a data point), the average overall distance from the substrate was 1.97 cm (SD=.39). For the adults the average was 2.13 (SD=.32). The difference between these was significant (t=4.84, p<.000001).

This indicated that overall the larvae were in closer proximity to the substrate than the adults. We first looked at an overall model using a Univariate Analysis of Variance. We tested both the larvae and the adults separately under this model. The Independent variables were Interval (consonant, dissonant, white noise) and Substrate (caffeine, sucrose, none). Interactions were analyzed prior to main effects. We first analyzed the data of the larvae. It was found that there was a significant interaction for the (F(4, 158)=3.36, p<.011; Figure 2)







We followed up on this interaction (in larvae) and found that in the dissonant condition, there were significant differences across substrate (F(2,55)= 6.74, p<.002). Caffeine was significantly closer to the substrate when compared to sucrose (t(36)=3.55, p<.001) and the control (t (37)=2.61, p<.013). In the consonant condition, the opposite trend was found (F(2,52)=2.46, p>.096).

This trend was such that the larvae in the caffeine group was significantly further away (t(33)=2.33, p<.026) from white noise. Caffeine did not differ from sucrose (t(33)>.63, p>.54) and sucrose did not differ from white noise (t(38)=1.71, p>.095).However, in the white noise condition, there were no significant differences (F(2,51)=.30, p>.74). Taken together, it seemed that the major effect is on caffeine's interaction with intervalsuch that caffeine and dissonance are preferred to caffeine and consonance. To confirm this, in the larvae only, we compared consonance vs dissonance only in the caffeine group. It was found there was a significant difference (t(32)=2.77, p< .001).

We followed up on the larvae analyses by examining the adults (Figure 3). There was no significant interaction (F (4,118)=.42, p>.79). There was also no main effect for interval (F(2,118)=1.74, p>.18) and there was no main effect for substrate (F(2,118)=.39, p>.68). To test if larvae conditioning influenced adult condition we employed a linear regression. It was found that there was a significant relationship between the two (F (1,165)=5.52, B=.42, Beta= .17, p<.02). The correlation between them was not strong (r=.17) indicating a small, yet significant predictive value between larvae and adult. These data suggest that there may be a relationship between larvae conditioning and adult conditioning (Figure 4).

Discussion

4.1 Surprisingly, it was found that tone interacted with substrate. When dissonance was combined with caffeine, there appeared to be a preference. Furthermore, when sucrose was compared with consonance, there was also a preference.This showed that the UCS- was preferred more than the UCS+ however, only in the presence of dissonance. The larvae compared to adults spent more time closer to the substrate, which indicated increasedconditioning when compared to the adults. In the consonant condition the opposite was found and the larvae were farther away from the substrate in the caffeine condition compared to the sucrose. The adults were analyzed across their substrate/tone pairings and no significance was found. Finally, it appears that conditioning in larvae is retained in adults.

The first finding, which was the interaction between interval and substrate, was not predicted. However, these datademonstrate that tone recognition is highly complex in *Drosophila*, and there are a myriad of variables and phenomena that influence behavior from duetting (LaRue, Clemens, Berman, & Murthy, 2015) to rivalry (Maguire, Lizé, & Price, 2015b) to basic underlying neural mechanisms (Pézier, Jezzini, Marie, & Blagburn, 2014). We consider this but a very preliminary investigation, and there are many nuances (Kavlie et al., 2015) that need investigation. However, we see this as a first step in understanding consonance, dissonance, and substrate.

The correlation between larvae and adult on conditioning showed a significant relationship therefore supporting previous studies (Gerber et al., 2004; Hariharan et al., 2014; Huang et al., 2015). This could indicate that, when consuming sugar, *Drosophila* can regain working memory that later evolves into long-term memory(Yamagata et al., 2015). This may also contribute to the findings that *Drosophila* are able to discriminate between different odors and stimuli and additionally can recollect the different preferences they had towards them(Das et al., 2014; Mishra et al., 2013; Schwaerzel et al., 2003, 2007).In fact, this can support the fact that just like humans, *Drosophila*'s auditory and visual stimuli deteriorate as they get older(Chan, Pianta, & McKendrick, 2014) as there were no significant differences found in the adults. Even though the correlation was not strong between both larvae and adult, there was a significant predictive value determining that conditioning *Drosophila* as larvae has some effect on them as adults. This data can support Blanco's study that suggested that larvae and adults had cell lineage between them(Blanco et al., 2011).

In addition, this study can contribute to the many studies that have been performed on multisensory integration of *Drosophila* (Duistermars & Frye, 2010; Mahoney et al., 2014). Our study may support the Mahoney study which demonstrated how the pairing of two stimuli could be ineffective as well (Mahoney et al., 2014). In our current study we did not isolate olfaction and taste, therefore one cannot distinguish between these variables. Further interactions may be discovered when these variables are parsed. Some may note a number of potential improvements. First, there was the issue of mortality. Future studies should also employ natural stimuli such as wing movement for communication between one and other(Murthy, 2010; Riabinina et al., 2011). Keeping this in mind one could, also attempt testing one larvae/adult fruit fly at a time to see if testing two at a time had a significant effect on their performance. Further, we could also separate the flies by gender and see if that has a significant effect on the results as well. Future studies will be performed to study the genetics of this interaction between substrate and interval.Being that long term memory neuronal pathways and circuitry were studied in relation to long-term memory in *Drosophila* our study may be able to contribute to these findings(Bouzaiane, Trannoy, Scheunemann, Plaçais, & Preat, 2015; Krüttner et al., 2015). These future studies may demonstrate why some *Drosophila* prefer certain substrate/tone pairings compared to others. In the future we would also like to exam both larvae and adults to compare the retention rate of the conditioning. This will demonstrate the significance of conditioning in adults compared to the non-conditioned ones in our experiment.

References

Albert, J. T., & Göpfert, M. C. (2015). Hearing in Drosophila. *Current Opinion in Neurobiology*, *34C*, 79–85. http://doi.org/10.1016/j.conb.2015.02.001

Bidelman, G. M., & Krishnan, A. (2009). Neural correlates of consonance, dissonance, and the hierarchy of musical pitch in the human brainstem. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(42), 13165–13171. http://doi.org/10.1523/JNEUROSCI.3900-09.2009

Blanco, J., Pandey, R., Wasser, M., & Udolph, G. (2011). Orthodenticle is necessary for survival of a cluster of clonally related dopaminergic neurons in the Drosophila larval and adult brain. *Neural Development*, *6*, 34. http://doi.org/10.1186/1749-8104-6-34

Boekhoff-Falk, G., & Eberl, D. F. (2014). The Drosophila auditory system. *Wiley Interdisciplinary Reviews. Developmental Biology*, *3*(2), 179–191. http://doi.org/10.1002/wdev.128

Bouzaiane, E., Trannoy, S., Scheunemann, L., Plaçais, P.-Y., & Preat, T. (2015). Two independent mushroom body output circuits retrieve the six discrete components of Drosophila aversive memory. *Cell Reports*, *11*(8), 1280–1292. http://doi.org/10.1016/j.celrep.2015.04.044

Bowling, D. L., & Purves, D. (2015). A biological rationale for musical consonance. *Proceedings of the National Academy of Sciences*, 201505768. http://doi.org/10.1073/pnas.1505768112

Bretman, A., Westmancoat, J. D., Gage, M. J. G., & Chapman, T. (2011). Males use multiple, redundant cues to detect mating rivals. *Current Biology: CB*, *21*(7), 617–622. http://doi.org/10.1016/j.cub.2011.03.008

Chan, Y. M., Pianta, M. J., & McKendrick, A. M. (2014). Older age results in difficulties separating auditory and visual signals in time. *Journal of Vision*, *14*(11). http://doi.org/10.1167/14.11.13

Cousineau, M., McDermott, J. H., & Peretz, I. (2012). The basis of musical consonance as revealed by congenital amusia. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(48), 19858–19863. http://doi.org/10.1073/pnas.1207989109

Das, G., Klappenbach, M., Vrontou, E., Perisse, E., Clark, C. M., Burke, C. J., & Waddell, S. (2014). Drosophila learn opposing components of a compound food stimulus. *Current Biology: CB*, *24*(15), 1723–1730. http://doi.org/10.1016/j.cub.2014.05.078

de Belle, J. S., & Heisenberg, M. (1994). Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. *Science (New York, N.Y.)*, *263*(5147), 692–695.

Duistermars, B. J., & Frye, M. A. (2010). Multisensory integration for odor tracking by flying Drosophila: Behavior, circuits and speculation. *Communicative & Integrative Biology*, *3*(1), 60–63.

Eberl, D. F., & Boekhoff-Falk, G. (2007). Development of Johnston's organ in Drosophila. *The International Journal of Developmental Biology*, *51*(6-7), 679–687. http://doi.org/10.1387/ijdb.072364de

Foxe, J. J., Wylie, G. R., Martinez, A., Schroeder, C. E., Javitt, D. C., Guilfoyle, D., … Murray, M. M. (2002). Auditory-somatosensory multisensory processing in auditory association cortex: an fMRI study. *Journal of Neurophysiology*, *88*(1), 540–543.

Fuller, S. B., Straw, A. D., Peek, M. Y., Murray, R. M., & Dickinson, M. H. (2014). Flying Drosophila stabilize their vision-based velocity controller by sensing wind with their antennae. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(13), E1182–1191. http://doi.org/10.1073/pnas.1323529111

Gaab, N., Keenan, J. P., & Schlaug, G. (2003). The effects of gender on the neural substrates of pitch memory. *Journal of Cognitive Neuroscience*, *15*(6), 810–820. http://doi.org/10.1162/089892903322370735

Gan, G., Lv, H., & Xie, W. (2014). Morphological identification and development of neurite in Drosophila ventral nerve cord neuropil. *PloS One*, *9*(8), e105497. http://doi.org/10.1371/journal.pone.0105497

Gerber, B., Scherer, S., Neuser, K., Michels, B., Hendel, T., Stocker, R. F., & Heisenberg, M. (2004). Visual learning in individually assayed Drosophila larvae. *The Journal of Experimental Biology*, *207*(Pt 1), 179–188.

Ghaemi, R., Rezai, P., Iyengar, B. G., & Selvaganapathy, P. R. (2015). Microfluidic devices for imaging neurological response of Drosophila melanogaster larva to auditory stimulus. *Lab on a Chip*, *15*(4), 1116–1122. http://doi.org/10.1039/c4lc01245c

Göpfert, M. C., & Robert, D. (2002). The mechanical basis of Drosophila audition. *The Journal of Experimental Biology*, *205*(Pt 9), 1199–1208.

Hariharan, R., Hoffman, J. M., Thomas, A. S., Soltow, Q. A., Jones, D. P., & Promislow, D. E. L. (2014). Invariance and plasticity in the Drosophila melanogaster metabolomic network in response to temperature. *BMC Systems Biology*, *8*(1), 2. http://doi.org/10.1186/s12918-014-0139-6

Huang, Y., Ng, F. S., & Jackson, F. R. (2015). Comparison of Larval and Adult Drosophila Astrocytes Reveals Stage-Specific Gene Expression Profiles. *G3 (Bethesda, Md.)*. http://doi.org/10.1534/g3.114.016162

Ito, K., Suzuki, K., Estes, P., Ramaswami, M., Yamamoto, D., & Strausfeld, N. J. (1998). The organization of extrinsic neurons and their implications in the functional roles of the mushroom bodies in Drosophila melanogaster Meigen. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *5*(1-2), 52–77.

Kavlie, R. G., Fritz, J. L., Nies, F., Göpfert, M. C., Oliver, D., Albert, J. T., & Eberl, D. F. (2015). Prestin is an anion transporter dispensable for mechanical feedback amplification in Drosophila hearing. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *201*(1), 51–60. http://doi.org/10.1007/s00359-014-0960-9

Keenan, J. P., Thangaraj, V., Halpern, A. R., & Schlaug, G. (2001). Absolute pitch and planum temporale. *NeuroImage*, *14*(6), 1402–1408. http://doi.org/10.1006/nimg.2001.0925

Kim, Y.-C., Lee, H.-G., Lim, J., & Han, K.-A. (2013). Appetitive learning requires the alpha1-like octopamine receptor OAMB in the Drosophila mushroom body neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(4), 1672–1677. http://doi.org/10.1523/JNEUROSCI.3042-12.2013

King, A. J., & Walker, K. M. M. (2012). Integrating information from different senses in the auditory cortex. *Biological Cybernetics*, *106*(11-12), 617–625. http://doi.org/10.1007/s00422-012-0502-x

Krüttner, S., Traunmüller, L., Dag, U., Jandrasits, K., Stepien, B., Iyer, N., … Keleman, K. (2015). Synaptic Orb2A Bridges Memory Acquisition and Late Memory Consolidation in Drosophila. *Cell Reports*, *11*(12), 1953–1965. http://doi.org/10.1016/j.celrep.2015.05.037

LaRue, K. M., Clemens, J., Berman, G. J., & Murthy, M. (2015). Acoustic duetting in Drosophila virilis relies on the integration of auditory and tactile signals. *eLife*, *4*. http://doi.org/10.7554/eLife.07277

Maguire, C. P., Lizé, A., & Price, T. A. R. (2015a). Assessment of rival males through the use of multiple sensory cues in the fruitfly Drosophila pseudoobscura. *PloS One*, *10*(4), e0123058. http://doi.org/10.1371/journal.pone.0123058

Maguire, C. P., Lizé, A., & Price, T. A. R. (2015b). Assessment of rival males through the use of multiple sensory cues in the fruitfly Drosophila pseudoobscura. *PloS One*, *10*(4), e0123058. http://doi.org/10.1371/journal.pone.0123058

Mahoney, J. R., Holtzer, R., & Verghese, J. (2014). Visual-somatosensory integration and balance: evidence for psychophysical integrative differences in aging. *Multisensory Research*, *27*(1), 17–42.

Matsuo, E., Yamada, D., Ishikawa, Y., Asai, T., Ishimoto, H., & Kamikouchi, A. (2014). Identification of novel vibration- and deflection-sensitive neuronal subgroups in Johnston's organ of the fruit fly. *Frontiers in Physiology*, *5*, 179. http://doi.org/10.3389/fphys.2014.00179

McDermott, J. H., Lehr, A. J., & Oxenham, A. J. (2010). Individual Differences Reveal the Basis of Consonance. *Current Biology*, *20*(11), 1035–1041. http://doi.org/10.1016/j.cub.2010.04.019

Menuz, K., Larter, N. K., Park, J., & Carlson, J. R. (2014). An RNA-seq screen of the Drosophila antenna identifies a transporter necessary for ammonia detection. *PLoS Genetics*, *10*(11), e1004810. http://doi.org/10.1371/journal.pgen.1004810

Minati, L., Rosazza, C., D'Incerti, L., Pietrocini, E., Valentini, L., Scaioli, V., … Bruzzone, M. G. (2009). Functional MRI/event-related potential study of sensory consonance and dissonance in musicians and nonmusicians. *Neuroreport*, *20*(1), 87–92. http://doi.org/10.1097/WNR.0b013e32831af235

Mishra, D., Chen, Y.-C., Yarali, A., Oguz, T., & Gerber, B. (2013). Olfactory memories are intensity specific in larval Drosophila. *The Journal of Experimental Biology*, *216*(Pt 9), 1552–1560. http://doi.org/10.1242/jeb.082222

Moore, B. C. J. (2002). Interference effects and phase sensitivity in hearing. *Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences*, *360*(1794), 833–858. http://doi.org/10.1098/rsta.2001.0970

Morley, E. L., Steinmann, T., Casas, J., & Robert, D. (2012). Directional cues in Drosophila melanogaster audition: structure of acoustic flow and inter-antennal velocity differences. *The Journal of Experimental Biology*, *215*(Pt 14), 2405–2413. http://doi.org/10.1242/jeb.068940

Murthy, M. (2010). Unraveling the auditory system of Drosophila. *Current Opinion in Neurobiology*, *20*(3), 281–287. http://doi.org/10.1016/j.conb.2010.02.016

Peelle, J. E., & Sommers, M. S. (2015). Prediction and constraint in audiovisual speech perception. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *68*, 169–181. http://doi.org/10.1016/j.cortex.2015.03.006

Pézier, A., Jezzini, S. H., Marie, B., & Blagburn, J. M. (2014). Engrailed alters the specificity of synaptic connections of Drosophila auditory neurons with the giant fiber. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *34*(35), 11691–11704. http://doi.org/10.1523/JNEUROSCI.1939-14.2014

Reza, M. A., Mhatre, S. D., Morrison, J. C., Utreja, S., Saunders, A. J., Breen, D. E., & Marenda, D. R. (2013). Automated analysis of courtship suppression learning and memory in Drosophila melanogaster. *Fly*, *7*(2), 105–111. http://doi.org/10.4161/fly.24110

Riabinina, O., Dai, M., Duke, T., & Albert, J. T. (2011). Active process mediates species-specific tuning of Drosophila ears. *Current Biology: CB*, *21*(8), 658–664. http://doi.org/10.1016/j.cub.2011.03.001

Saleem, S., Ruggles, P. H., Abbott, W. K., & Carney, G. E. (2014). Sexual experience enhances Drosophila melanogaster male mating behavior and success. *PloS One*, *9*(5), e96639. http://doi.org/10.1371/journal.pone.0096639

Schwaerzel, M., Jaeckel, A., & Mueller, U. (2007). Signaling at A-kinase anchoring proteins organizes anesthesia-sensitive memory in Drosophila. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(5), 1229–1233. http://doi.org/10.1523/JNEUROSCI.4622-06.2007

Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., & Heisenberg, M. (2003). Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(33), 10495–10502.

Siemann, J. K., Muller, C. L., Bamberger, G., Allison, J. D., Veenstra-VanderWeele, J., & Wallace, M. T. (2014). A novel behavioral paradigm to assess multisensory processing in mice. *Frontiers in Behavioral Neuroscience*, *8*, 456. http://doi.org/10.3389/fnbeh.2014.00456

Tramo, M. J., Cariani, P. A., Delgutte, B., & Braida, L. D. (2001). Neurobiological foundations for the theory of harmony in western tonal music. *Annals of the New York Academy of Sciences*, *930*, 92–116.

Ursino, M., Cuppini, C., & Magosso, E. (2014). Neurocomputational approaches to modelling multisensory integration in the brain: a review. *Neural Networks: The Official Journal of the International Neural Network Society*, *60*, 141–165. http://doi.org/10.1016/j.neunet.2014.08.003

van Alphen, B., & van Swinderen, B. (2013). Drosophila strategies to study psychiatric disorders. *Brain Research Bulletin*, *92*, 1–11. http://doi.org/10.1016/j.brainresbull.2011.09.007

Yamagata, N., Ichinose, T., Aso, Y., Plaçais, P.-Y., Friedrich, A. B., Sima, R. J., … Tanimoto, H. (2015). Distinct dopamine neurons mediate reward signals for short- and long-term memories. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(2), 578–583. http://doi.org/10.1073/pnas.1421930112

Yao, C. A., Ignell, R., & Carlson, J. R. (2005). Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *25*(37), 8359–8367. http://doi.org/10.1523/JNEUROSCI.2432-05.2005

Figure Captions

6.1

Figure 1: The specialized Petri Dish (A) was obtained from Carolina Biological. It served as a basic choice chamber. The covers were modified to accept dual headphones that played tones at 65db. The path of the organisms were recorded every 15 seconds. A sample path is presented from a single fly is shown (B).

Figure 2: A significant interaction was found between the two independent variables of substrate and tone interval (p<.011). It was found that distance to the substrate (e.g., the *Drosophila* demonstrated a preference) was closer when the interval was dissonant and the substrate was caffeine. Likewise, the distance was closer when sucrose was paired with consonance. However, no main effects were found in the larvae (p's>.05).

Figure 3: In adults, there were no significant findings. Unlike the larvae, there was no preference found across the conditions and all the interaction and all main effects were found to be non-significant (all p's>.05).

Figure 4: A significant correlation was found in the condition between larvae and adults (p<.02). The correlation was small (r=.17) but predictive.