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Comparison of the Attraction Index of Male and Female Drosophila Melanogaster to Varying Odorant Substances

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Abstract: This study aims at investigating the differences if any, in the olfactory discrematory ability of wild type drosophila and mutated Or83b type and also if these differences exists between male and females of both species. Insect and mammalian olfactory systems are strikingly similar. Therefore, *Drosophila* can be used as a simple model for olfaction. The olfactory system has evolved the capacity to recognize and discriminate an inordinate number of chemically distinct odors that signal the presence of food, predators, or mating partners. Most organisms including humans rely on their olfactory system to detect and analyze olfactory cues in the environment, cues that are subsequently utilized in the context of behavior. Several works have been done on the olfactory system of the insect drosophila, attraction of various strain of drosophila to different odors but no work has been done to investigate sexual differences in this attraction to odorants. In this research we try investigating differences in the sensitivity of the olfactory receptors of male and female drosophila by measuring their attraction index to odors (yeast). O83b mutants and ORR wild type flies were used. By behavioral analysis, using the attraction index as a measure of sensitivity of the olfactory receptors, we were able to show that the female flies have a higher attraction index to odorant than the males but this difference is not significant statistically as indicated by the p value.

Keywords: Insects, mammalian, odour, olfactory receptor, Or83b mutants

INTRODUCTION

Insect and mammalian olfactory systems are strikingly similar. Therefore, *Drosophila* can be used as a simple model for olfaction (Rubin *et al*., 2000). The olfactory system has evolved the capacity to recognize and discriminate an inordinate number of chemically distinct odors that signal the presence of food, predators, or mating partners. The initial steps in odor detection involve the binding of a volatile odor to Odorant Receptor (OR) proteins displayed on ciliated dendrites of specialized Olfactory Sensory Neurons (OSNs) that are exposed to the external environment. The OR genes that mediate odor detection in *Drosophila* fruit flies are expressed in subpopulations of OSNs (Clyne *et al*., 1999; Dobritsa *et al*., 2003; Elmore *et al*., 2003; Gao and Chess 1999; Vosshall *et al*., 1999, 2000). Fruit flies are attracted by a diversity of odors that signal the presence of food, potential mates, or attractive egg-laying sites (Mattias *et al*., 2004). Most organisms including humans rely on their olfactory system to detect and analyze olfactory cues in the environment, cues that are subsequently utilized in the context of behavior. Odorants are recognized by Olfactory Sensory Neurons (OSNs), which are located in the olfactory epithelia of vertebrates or in the dendrites of olfactory sensory cells within the sensilla on the antennal surface of insects (Buck and Axel, 1991; Carlson, 2001; Chess *et al*., 1992; Vosshall *et al*., 2000). The olfactory system of the genetic model organism, the fruit fly *Drosophila melanogaster*, has been the focus of numerous investigations. *Drosophila* has two pairs of olfactory organs, the antennae and the maxillary palps. Each antenna contains about 1200 OSNs housed in a total of 410 olfactory sensilla covering the antenna, while the maxillary palp has about 120 OSNs and 60 olfactory sensilla (Vosshall *et al*., 2000). ORs are expressed following a conserved pattern in *Drosophila* as well as in mammals. Every OSN typically expresses only one type of OR (as well as the ubiquitous Or83b,). However, a given OSN can also co-express up to three conventional ORs determining a specific molecular response profile along with the Or83 co-receptor. OSNs expressing the same type of OR, converge to a single glomerulus in the Antennal Lobe (AL), which represents the first olfactory neuropil in the insect brain (Couto *et al*., 2005; Fishilevich and Vosshall, 2005). While the functional organization of the olfactory system in vertebrates and insects shows clear

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similarities, the atypical heteromeric and topological design of the ORs in *Drosophila* appears to be insect-specific. Almost all OSNs express a chaperon receptor, called Or83b. Or83b is highly conserved in many insect species (Dahanukar *et al*., 2005) and it is also possible to functionally exchange Or83b with orthologous Or83b proteins from other insects (Hill *et al*., 2002; Jones *et al*., 2005; Krieger *et al*., 2003; Pitts *et al*., 2004). In recent studies, it was shown that Or83b is a nonselective cation channel that interacts directly with the endogenous ligand-tuned OR (Sato *et al*., 2008). In addition, Or83b is needed for the functional integration of receptor proteins in the dendritic part of the OSNs within the sensillum shaft (Larsson *et al*., 2004; Neuhaus *et al*., 2005).

Several studies have been done on the olfactory system of the insect drosophila, attraction of various strain of drosophila to different odors but no work has been done to investigate sexual differences in this attraction to odorants.

In this study we try investigating differences in the sensitivity of the olfactory receptors of male and female drosophila by measuring their attraction index to odors (yeast).

MATERIALS AND METHODS

Flies used: Flies were of the Oregon-R wild-type strain, the Or83b mutants, kept in mass culture and maintained in the laboratory at 25°C and a 12/12 h light/dark cycle and flies were kept in the local fly facilities at 25°C, 60-70% relative humidity and a 14/10 h light/dark cycle. Daily, flies were transferred into a fresh food bottle where they could lay eggs until the following day when they were transferred to a fresh bottle once again (Sawin-McCormack *et al*., 1995). As olfactory stimuli, we used yeast (purity: 99.5%) and distilled water as control.

Experimental setup:

Dual choice test: Here we built a transparent chamber about 12 cm diameters, 6 cm height with a gauze cover for ventilation. Two glass cuvettes of diameter 1.3 cm and height 3 cm were be filled with:

- Yeast (odorant)
- Distilled water (control)

Fifteen male and female flies were introduced into different chambers, respectively. The whole set up was replicated ten times for both male and female for different strains of flies used and allowed for 12-14 h. Thereafter, the flies were made to sleep using anesthetic ether and the number of flies attracted to the yeast, water, those that were neutral and dead flies were counted and recorded, respectively.

Data analysis and statistics: Attraction index was calculated by subtracting the number of flies attracted to the water from those attracted to yeast and dividing it with the total number of flies including those in the neutral zone minus dead flies.
Attraction index in (A) and (B):

$$(T - C) / (T + C + NR - D)$$

where,
A = Yeast
B = Water
T = Number attracted to yeast
C = Number attracted to water
NR = Number in neutral zone
D = Dead flies

The result were subjected to student t test to compare the attraction index for both male and female flies in the various strains used and a p value of less than 0.05 was considered statistically significant.

RESULTS

The attraction indexes in male and female flies were calculated as shown below.

Attraction index in the wild type male drosophila is given by Attraction index in (A) and (B):

$$(T - C) / (T + C + NR - D)$$

The Table 1 to 4 shows the number of observations made. Attraction index in the wild type male drosophila is given by Attraction index in (A) and (B):

$$(T - C) / (T + C + NR - D)$$

where,
A = Yeast
B = Water
T = Number attracted to yeast
C = Number attracted to water
NR = Number in neutral zone
D = Dead flies

$$= (87-18/87+18+25) -20$$
$$= +0.6$$

Attraction index in the Or83b mutant male drosophila is given by Attraction index in (A) and (B):

$$(T - C) / (T + C + NR - D)$$

where,
A = Yeast
B = Water
T = Number attracted to yeast
C = Number attracted to water

Table 1: ORR male strain

Events/sets	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Yeast	10	8	9	9	10	8	7	9	8	9	87
Water	0	3	1	0	0	3	3	1	5	2	18
Neutral	3	2	4	3	2	2	5	4	0	0	25
Dead	2	2	1	3	3	2	0	1	2	4	20
Total	15	15	15	15	15	15	15	15	15	15	150

Table 2: Or83B male strain

Events/sets	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Yeast	4	2	4	0	2	4	3	0	1	4	24
Water	6	6	1	8	8	7	1	10	3	4	54
Neutral	5	4	6	5	5	4	7	4	10	5	55
Dead	0	3	4	2	0	0	4	1	1	2	17
Total	15	15	15	15	15	15	15	15	15	15	150

Table 3: ORR female strain

Events/sets	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Yeast	10	14	8	13	9	8	10	9	12	14	107
Water	2	0	4	2	0	1	0	3	2	0	14
Neutral	1	0	2	0	3	4	1	2	1	0	14
Dead	2	1	1	0	3	2	4	1	0	1	15
Total	15	15	15	15	15	15	15	15	15	15	150

Table 4: Or83B female strain

Events/sets	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Yeast	5	0	5	4	2	4	5	1	5	5	36
Water	6	2	5	5	6	1	5	1	8	6	45
Neutral	4	8	2	1	4	5	2	8	2	4	40
Dead	0	5	3	5	3	5	3	5	0	0	29
Total	15	15	15	15	15	15	15	15	15	15	150

NR = Number in neutral zone
D = Dead flies

= 24-54 / (24+54+55) -17
= -0.3

Attraction index in the wild type female drosophila is given by Attraction index in (A) and (B):

(T - C) / (T + C + NR - D)

where,
A = Yeast
B = Water
T = Number attracted to yeast
C = Number attracted to water
NR = Number in neutral zone
D = Dead flies

= 107-14 / (107+14+14) -15
= 0.8

Attraction index in the Or83b mutant female drosophila is given by Attraction index in (A) and (B):

(T - C) / (T + C + NR - D)

where,
A = Yeast
B = Water
T = Number attracted to yeast
C = Number attracted to water
NR = Number in neutral zone
D = Dead flies

= 36-45 / (36+45+40) -29
= -0.1

DISCUSSION

When we introduced male and female flies separately into the chamber containing yeast as the odorant and distilled water as control, more female flies were attracted to the odorant for both the wild type and Or83b mutants, as indicated by their attraction index in the bar chart above. Where attraction index of 1 corresponds to complete attraction of all flies into the odorant and attraction index of 0 corresponds to anosmic flies. Negative values indicate an aversive response. The male and female Or83b mutants showed aversive behavior towards the odorant while the wild types in both cases showed an attractive behavior towards the odorant. Differences were seen in the attraction of male and female drosophila to the odorant as indicated by their attraction index. Female flies of both the wild type and Or83b mutants were more attracted to the odorant than the male flies, but when the data were analyzed for statistical significance using two-tailed Student t-tests, with SPSS, the differences in the attraction index of male and female flies were found to be statistically insignificant considering the p value. Whereas the differences in the attraction index of the wild type and Or83b mutant was found to be statistically significant. It has been shown by cellular, physiological and behavioral analysis that Or83b is essential for olfaction in Drosophila. Or83b is an atypical member of the OR gene family because it is highly conserved across insect species and is expressed in a large number of olfactory sensory neurons with different odor specificities. Hence, Or83b mutant antennae show no odor-evoked potentials to odorants that elicit robust responses in wild-type antenna (Dobritsa *et al*., 2003; Elmore *et al*., 2003; Hallem *et al*., 2004). This explains the attraction index for both male and female flies of Or83b mutants compared to the wild types as seen on the bar graph.

The number and sensitivity of ORs expressed in a given OSN is an important determinant of the coding logic of the olfactory system. (L'Etoile and Bargmann, 2000; Troemel *et al*., 1995; Wes and Bargmann, 2001), each receptor functions independently within a neuron to recognize ligands that activate the chemosensory neuron to elicit either attractive or aversive behavioral responses (Troemel *et al*., 1997). The Or83b mutants for both male and female flies showed aversive response to the odorant while the wild types elicit an attraction response to the odorant. This agrees with the work of Dobritsa *et al*. (2003), Elmore *et al*. (2003) and Hallem *et al*. (2004). Though differences exist in the attraction of male and female flies to the odorant, the difference is statistically insignifant.

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REFERENCES

Buck, L. and R. Axel, 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell, 65: 175-187.

Carlson, J.R., 2001. Functional expression of a drosophila odor receptor. Proc. Natl. Acad. Sci. USA, 98: 8936-8937.

Chess, A., L. Buck, M.M. Dowling, R. Axel and J. Ngai, 1992. Molecular biology of smell: Expression of the multigene family encoding putative odorant receptors. Cold Spring Harb. Symp. Quant. Biol., 57: 505-516.

Clyne, P.J., C.G. Warr, M.R. Freeman, D. Lessing, J. Kim and J.R. Carlson, 1999. A novel family of divergent seven-transmembrane proteins: Candidate odorant receptors in drosophila. Neuron, 22: 327-338.

Couto, A., M. Alenius and B.J. Dickson, 2005. Molecular, anatomicaland functional organization of the drosophila olfactory system. Curr. Biol., 15: 1535-1547.

Dahanukar, A., E.A. Hallem and J.R. Carlson, 2005. Insect chemoreception. Curr. Opin. Neurobiol., 15: 423-430.

Dobritsa, A.A., G. Van Der, N.W. Van, C.G. Warr, R.A. Steinbrecht and J.R. Carlson, 2003. Integrating the molecular and cellular basis of odor coding in the drosophila antenna. Neuron., 37: 827-841.

Elmore, T. Elmore, R. Ignell, J.R. Carlson and D.P. Smith, 2003. Targeted mutation of a drosophila odor receptor defines receptor requirement in a novel class of sensillum. J. Neurosci., 23: 9906-9912.

Fishilevich, E. and L.B. Vosshall, 2005. Genetic and functional subdivision of the drosophila antennal lobe. Curr. Biol., 15: 1548-1553.

Gao, Q. and A. Chess, 1999. Identification of candidate drosophila olfactory receptors from genomic DNA sequence. Genomics, 60: 31-39.

Hallem, E., A. Hallem, M.G. Ho and J.R. Carlson, 2004. The molecular basis of odor coding in the drosophila antenna. Cell, 117(7): 965-979.

Hill, E.S., M. Iwano, L. Gatellier and R. Kanzaki, 2002. Morphology and physiology of the serotonin-immunoreactive putative antennal lobe feedback neuron in the male silkmoth bombyx mori. Chem. Senses., 27: 475-483.

Jones, W.D., T.A. Nguyen, B. Kloss, K.J. Lee and L.B. Vosshall, 2005. Functional conservation of an insect odorant receptor gene across 250 million years of evolution. Curr. Biol., 15: 119-121.

Krieger, J., O. Klink, C. Mohl, K. Raming and H. Breer 2003. A candidate olfactory receptor subtype highly conserved across different insect orders. J Comp. Physiol. Neuroethol. Sens. Neural. Behav. Physiol., 189: 519-526.

Larsson, M.C., A.I. Domingos, W.D. Jones, M.E. Chiappe, H. Amrein and L.B. Vosshall, 2004. Or83b encodes a broadly expressed odorant receptor essential for drosophila olfaction. Neuron, 43: 703-714.

L'Etoile and C.I. Bargmann, 2000. Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron, 25(3): 575-586.

Mattias, C. L., A.I. Domingos, W.D. Jones, M.E. Chiappe, H. Amrein and L.B. Vosshall, 2004. Or 83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron, 43: 703-714.

Neuhaus, E.M., G. Gisselmann, W. Zhang, R. Dooley, K. Stortkuhl and H. Hatt, 2005. Odorant receptor heterodimerization in the olfactory system of Drosophila melanogaster. Nat. Neurosci., 8: 15-17.

Pitts, R.J., A.N. Fox and L.J. Zwiebel, 2004. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector Anopheles Gambiae. Proceedings of the National Academy of Sciences USA, 101: 5058-5063.

Rubin, G.M., M.D. Yandell, J.R. Wortman, G.L.G. Miklos, C.R. Nelson, I.K. Hariharan, M.E. Fortini, P.W. Li, R. Apweiler, W. Fleischmann, *et al*., 2000. Comparative genomics of the eukaryotes. Science, 287: 2204-2215.

Sato, K., M. Pellegrino, T. Nakagawa, L.B. Vosshall and K. Touhara, 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature, 452: 1002-1006.

Sawin-McCormack, E.P., M.B. Sokolowski and A.R. Campos, 1995. Characterization and genetic analysis of drosophila melanogaster photo behavior during larval development. J. Neurogenet., 10: 119-135.

Troemel, E.R., B.E. Kimmel and C.I. Bargmann, 1997. Reprogramming chemotaxis responses: Sensory neurons define olfactory preferences in C. Elegans Cell, 91(2): 161-169.

Troemel, E.R., J.H. Chou, N.D. Dwyer, H.A. Colbert and C.I. Bargmann, 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans. Cell, 83(2): 207-218.

Vosshall, L.B., A.M. Wong and R. Axel, 2000. An olfactory sensory map in the fly brain. Cell, 102(2): 147-159.

Vosshall, L.B., H. Amrein, P.S. Morozov, A. Rzhetsky and R. Axel, 1999. A spatial map of olfactory receptor expression in the drosophila antenna. Cell, 96(5): 725-736.

Wes, P.D. and C.I. Bargmann, 2001. C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature, 410(6829): 698-701.