Badre, D., and Wagner, A.D. (2007). Neuropsychologia *45*, 2883–2901.

Beauregard, M., Levesque, J., and Bourgouin, P. (2001). J. Neurosci. *21*, RC165.

Cunningham, W.A., Raye, C.L., and Johnson, M.K. (2004). J. Cogn. Neurosci. *16*, 1717–1729.

Eippert, F., Veit, R., Weiskopf, N., Erb, M., Birbaumer, N., and Anders, S. (2007). Hum. Brain Mapp. *28*, 409–423.

Levesque, J., Eugene, F., Joanette, Y., Paquette, V., Mensour, B., Beaudoin, G., Leroux, J.M., Bourgouin, P., and Beauregard, M. (2003). Biol. Psychiatry *53*, 502–510.

Lieberman, M.D., Eisenberger, N.I., Crockett, M.J., Tom, S.M., Pfeifer, J.H., and Way, B.M. (2007). Psychol. Sci. *18*, 421–428.

Ochsner, K.N., and Gross, J.J. (2005). Trends Cogn. Sci. *9*, 242–249.

Ochsner, K.N., and Gross, J.J. (2008). Curr. Dir. Psychol. Sci. *17*, 153–158.

Ochsner, K.N., Bunge, S.A., Gross, J.J., and Gabrieli, J.D. (2002). J. Cogn. Neurosci. *14*, 1215–1229.

Ochsner, K.N., Ray, R.D., Cooper, J.C., Robertson, E.R., Chopra, S., Gabrieli, J.D., and Gross, J.J. (2004). Neuroimage *23*, 483–499.

Pandya, D.N., and Yeterian, E.H. (1990). Prog. Brain Res. *85*, 63–94.

Ray, R.D., Ochsner, K.N., Cooper, J.C., Robertson, E.R., Gabrieli, J.D., and Gross, J.J. (2005). Cogn. Affect. Behav. Neurosci. *5*, 156–168.

Stuss, D.T., Gow, C.A., and Hetherington, C.R. (1992). J. Consult. Clin. Psychol. *60*, 349–359.

Wager, T.D., Davidson, M.L., Hughes, B.L., Lindquist, M.A., and Ochsner, K.N. (2008). Neuron *59*, this issue, 1037–1050.

Drosophila Olfaction: The End of Stereotypy?

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Recent work has demonstrated substantial wiring and functional stereotypy in the fly olfactory system. In this issue of *Neuron*, Murthy et al. demonstrate that in the mushroom body, a site of olfactory associative learning, this initial peripheral stereotypy gives way to functionally nonstereotyped circuits.

In the study of the circuit basis of behavior, it is often interesting to examine the extent to which genetically specified connectivity may underlie species-specific behavior. Equally interesting (and perhaps even more challenging) is the circuit basis of differences in the behavior of individuals within a species, which may represent experience-dependent processes such as learning. Fruit flies are being used to address both of these issues as they have a range of innate and learned behaviors. Because both types of behaviors coexist in the same individual, it is conceivable that the nervous system will include stereotyped as well as nonstereotyped elements. The latter could result from differences in neuronal connectivity or more subtle differences in synapse function, raising the possibility that anatomically identical circuits could produce different behavioral outputs. Both scenarios, differences in connectivity or function, prompt the question of where along the path from sensory input to motor output interindividual differences lie. In the present issue of *Neuron*, Murthy et al. (2008) identify the first nonstereotyped element in the olfactory system of *Drosophila*.

During the last decade great insight has been gained into the structure and function of the first two relays of the olfactory system in *Drosophila* (Figure 1). Perhaps the defining feature of these results has been the demonstration of extensive anatomical and functional stereotypy. This stereotypy first becomes evident in the invariant projection of each type of olfactory receptor neuron from the antenna to specific glomeruli in the antennal lobe, which produces an invariant spatial map of odor space (Vosshall et al., 2000; Couto et al., 2005; Fishilevich and Vosshall, 2005). Within each antennal lobe glomerulus, olfactory receptor neuron axons form connections with the dendrites of a specific group of projection neurons (PNs), the principal output cells. In addition both excitatory and inhibitory local neurons connect multiple glomeruli.

The wiring of olfactory receptor neurons and PNs is under precise genetic control (reviewed by Jefferis and Hummel, 2006) such that there appears to be a hard-wired transfer of information across each glomerulus that may be required for innate olfactory behavior. Consistent with this, olfactory responses of PNs are highly stereotyped (Ng et al., 2002; Wang et al., 2003; Wilson et al., 2004). On leaving the antennal lobe, PNs send axons to the lateral horn, where they form highly stereotyped axon terminals. On their way to the lateral horn, PNs also send axon collaterals to the mushroom body calyx. The mushroom body is composed of some 2500 neurons called Kenyon cells (KCs). While there is consensus about the anatomical stereotypy of PN axonal arborizations in the lateral horn (Marin et al., 2002; Wong et al., 2002), a unified image has not yet emerged for the synapses of PNs on the KCs. Although initial studies were inconclusive, more recent reports show a significant level of stereotypy in PN-KC projections (Tanaka et al., 2004; Jefferis et al., 2007; Lin et al., 2007); this stereotypy can be described as a zonal bias for the termination site of PN axons and KC dendrites. At the functional level, Wang et al. (2004) used Ca^{2+}

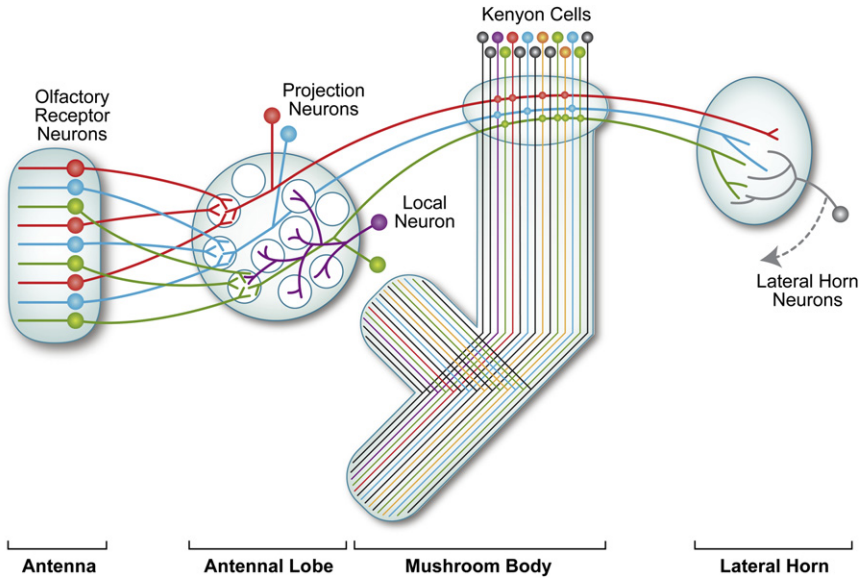

Figure 1. Schematic Representation of the Olfactory System of *Drosophila*
Olfactory receptor neurons in the antennae project their axons to specific glomeruli in the antennal lobe. There they form synaptic contacts with projection neurons (PNs) and local neurons. PNs then project to the lateral horn (LH) neuropile where they contact LH neurons. On their way to the LH, PNs send axonal collaterals to the calyx of the mushroom body where they form synapses with Kenyon cells (KCs).

imaging to show that the cell body positions of KCs responding to two odors (ethyl acetate and octanol) were reproducible and largely segregated across flies, suggesting at least some level of functional stereotypy.

To study the stereotypy of KC responses to odors, Murthy et al. (2008) used in vivo whole-cell patch-clamp recordings. Because at present there is no way of labeling the same single KC in different individuals, a more indirect approach was taken. A fly line was used in which GFP is reproducibly expressed in a group of 23 cells under the control of an enhancer trap element called NP7175-Gal4; the number is approximate because expression levels are somewhat graded—in some cases one can see more cells; in others, fewer. However these cells always have both their axons and dendrites closely grouped together in spatially reproducible positions.

Responses to a panel of odors were recorded from one cell from each of 27 different individuals. On statistical grounds, if each of the 23 GFP+ cells present in each fly has the same odor response in every individual—complete functional stereotypy—then a large number of the recorded cells will respond identically to the panel of odorants. In fact, more than 18 of the 27 recordings should correspond to pairs or triplets of the same identifiable cell. On the other hand, if there is no functional stereotypy at all, then a much lower number of the cells sampled from different individuals will show identical responses.

To gain an intuitive understanding of the statistical argument, it may be helpful to consider the following simple situation. Given 27 bags containing 23 sweets each, if all 621 sweets (23 × 27) are of a different color and you blindly pick one sweet from each bag, you would get 27 different sweets. This corresponds to the situation of nonstereotyped KCs. Now imagine that the 27 bags each contains one green sweet, one red, one blue, and so on. After picking one sweet from each bag you will certainly get a few sweets of the same color (most likely the ones you like the least!). This represents the situation of complete stereotypy. Note that even though you can't look inside the bags, you can still infer something about their contents from the distribution of sweets that you pick.

The comparison of spiking responses from 27 GFP+ cells identified only one pair with an identical response profile to the different odors tested, which is much lower than the number expected if KCs are uniquely identifiable. This initial result immediately suggests that the KCs labeled by NP7175-Gal4 are very unlikely to have unique functional identities, but the authors go to some length to demonstrate that this conclusion is statistically sound. First they looked at control recordings from GFP− cells that had their somata next to GFP+ cells. When comparing both samples, they found two cell pairs that showed identical odor responses. There therefore seems to be no evidence that the 27 GFP+ cells contain an unusual number of functionally identical neurons. Interestingly, the response probability among GFP− cells was double that of their GFP+ counterparts, highlighting a clear difference between these two groups of KCs.

A potential caveat of this result is that KC spiking responses are very sparse and therefore very sensitive to experimental noise. In order to get more robust results, a similar analysis based on subthreshold responses was conducted. Although spikes are presumed to be the most important measure of KC output, subthreshold responses are likely to reflect more directly the integrated input of each KC and might therefore be more likely to reveal any latent stereotypy. In fact, the subthreshold responses indicated that the 27 GFP+ cells were not more similar among themselves than they were to the GFP− control cells. In line with these observations, when Murthy et al. carried out a cluster analysis, GFP+ neurons did not cluster separately from GFP− cells.

Could the lack of stereotypy in KC responses be due to interindividual variability in the synaptic input from PNs? In agreement with previous reports (Ng et al., 2002; Wang et al., 2003; Wilson et al., 2004), recordings from PNs showed that both spiking and subthreshold responses from different individuals were highly stereotyped. Nevertheless, the convergence of several PNs onto a KC might produce less stereotyped responses, as small variations add up. This possibility was explored by modeling KC input by linear summation of PN responses; the authors took care to ensure that the PN input used was at least as variable as recordings from PNs in different animals. The result was that different model KCs receiving input from the

same types of PNs always clustered together. This indicates that interindividual PN variability is not high enough to prevent the identification of identical KCs.

Having concluded that these KCs are not functionally identical across animals, the authors investigate why this might be the case. They consider two options: variable synaptic weights and variable connectivity. They use their KC model to argue that differences in synaptic strengths between PNs and KCs cannot explain the observed variability in KC responses and therefore propose a model where PN-KC connectivity varies.

Murthy et al. (2008) present the first compelling evidence that nonstereotyped and stereotyped elements coexist in the olfactory system of *Drosophila*. While these are certainly novel results, it is important not to extrapolate them to the entire KC population. As the authors themselves point out, the cells they have analyzed are (essentially by design) a select group and therefore not necessarily representative of all KCs: they project only to the α/β lobes of the mushroom body, they are among the last-born KCs, and they have a response probability to odors of less than half of that of neighboring cells. For example, it is conceivable that the stereotypy in KC responses and KC-PN connectivity might be less marked in late-born cells that integrate into an already partially formed olfactory circuit. More generally we suspect that it will be very helpful to record from several Gal4 lines labeling different groups of KCs. The very fact that most of the control GFP− cells studied by Murthy et al. were immediate neighbors of the marked GFP+ cells means that they may not be representative of the mushroom body as a whole. These experiments, which were designed to test for the functional existence of stereotypy down to the level of the individual cell, cannot argue against the existence of stereotypy at the level of groups of cells: e.g., that cells of group A are more likely to respond to an odor than cells of group B. This distinction between zonal stereotypy and individual cell stereotypy may well explain the apparent contradiction of these new results with the imaging data of Wang et al. (2004) that showed some degree of functional stereotypy in KCs.

In addition the authors came to the conclusion that differences in synaptic weight cannot account for the observed differences in PN-KC connectivity. While this conclusion proceeds logically from their modeling, it is also pointed out in the paper that a number of factors were not taken into account when constructing model KCs (differences in release probability across time within each odor response, nonlinear integration by KCs, the ability of synapses to facilitate or depress, and the amount of inhibitory input on each KC). If all these factors are taken into consideration, we wonder if variations in synaptic weight without variations in connectivity might still be sufficient to explain the observed absence of KC stereotypy.

In summary, Murthy et al. have identified the end of stereotypy for one road in the olfactory system. Taking the existing anatomical and functional data together, it seems that there is likely to be a biased random connectivity between PNs and KCs. This may lead to some functional stereotypy in KCs even if this does not translate into functionally identical individual neurons among the 2500 KC population. More generally a lack of individually identifiable neurons in the mushroom bodies would seem very consistent with their extensively studied role in learning and memory (reviewed by Keene and Waddell, 2007). For example, if synaptic plasticity during life extensively alters the downstream connections of KCs, there would be little point in genetically specifying their input connectivity. In contrast anatomical data have indicated a very stereotyped projection map in the other higher olfactory center, the lateral horn, which is implicated in innate olfactory behavior (e.g., Heimbeck et al., 2001). It will be instructive to compare what level of connectional and functional stereotypy is present in that structure.

Neuroscientists at large often contrast hard-wiring of the nervous systems of invertebrates like flies and nematodes with more flexible and more redundant wiring in mammalian brains. As Drosophilists, we take some exception to this characterization, pointing out for example the number of different muscles in a mouse that are reliably innervated by well-defined classes of motor neuron; in this sense there may be more stereotypy in the mouse nervous system than the fly. Now however we can also point out that for at least one part of the olfactory system, every fly has a mind of its own.

REFERENCES

Couto, A., Alenius, M., and Dickson, B.J. (2005). Curr. Biol. *15*, 1535–1547.

Fishilevich, E., and Vosshall, L.B. (2005). Curr. Biol. *15*, 1548–1553.

Heimbeck, G., Bugnon, V., Gendre, N., Keller, A., and Stocker, R.F. (2001). Proc. Natl. Acad. Sci. USA *98*, 15336–15341.

Jefferis, G.S., Potter, C.J., Chan, A.M., Marin, E.C., Rohlfing, T., Maurer, C.R., Jr., and Luo, L. (2007). Cell *128*, 1187–1203.

Jefferis, G.S.X.E., and Hummel, T. (2006). Semin. Cell Dev. Biol. *17*, 50–65.

Keene, A.C., and Waddell, S. (2007). Nat. Rev. Neurosci. *8*, 341–354.

Lin, H.H., Lai, J.S., Chin, A.L., Chen, Y.C., and Chiang, A.S. (2007). Cell *128*, 1205–1217.

Marin, E.C., Jefferis, G.S.X.E., Komiyama, T., Zhu, H., and Luo, L. (2002). Cell *109*, 243–255.

Murthy, M., Fiete, I., and Laurent, G. (2008). Neuron *59*, this issue, 1009–1023.

Ng, M., Roorda, R.D., Lima, S.Q., Zemelman, B.V., Morcillo, P., and Miesenbock, G. (2002). Neuron *36*, 463–474.

Tanaka, N.K., Awasaki, T., Shimada, T., and Ito, K. (2004). Curr. Biol. *14*, 449–457.

Vosshall, L.B., Wong, A.M., and Axel, R. (2000). Cell *102*, 147–159.

Wang, J.W., Wong, A.M., Flores, J., Vosshall, L.B., and Axel, R. (2003). Cell *112*, 271–282.

Wang, Y., Guo, H.F., Pologruto, T.A., Hannan, F., Hakker, I., Svoboda, K., and Zhong, Y. (2004). J. Neurosci. *24*, 6507–6514.

Wilson, R.I., Turner, G.C., and Laurent, G. (2004). Science *303*, 366–370.

Wong, A.M., Wang, J.W., and Axel, R. (2002). Cell *109*, 229–241.