Semaphorin Directs Axon Traffic in the Fly Olfactory System

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The convergence of olfactory sensory axons that express the same receptor onto specific glomeruli is a common organizing principle in animal olfactory systems. In this issue of *Neuron*, two beautiful studies in *Drosophila* by Lattemann et al. and Sweeney et al. show that Semaphorin repulsion regulates interactions between olfactory receptor neurons to help axons find their correct targets.

Representation of sensory information in the nervous system relies on precise patterns of connectivity between pre- and postsynaptic neurons. The representation of smell in the olfactory system is an exquisite example of such high-precision neural connectivity. The seminal discovery of olfactory receptors by Buck and Axel (1991) paved the way for a molecular genetic dissection of wiring specificity in this system. *Drosophila* has proven to be an excellent model in which to study mechanisms of olfactory development because of (1) the strong similarities between the organization of fly and mammalian olfactory circuits, (2) the reduced numerical complexity—*Drosophila* has orders of magnitude fewer olfactory receptor neurons, receptors, and glomeruli than mice, and (3) the powerful mosaic genetic approaches that allow the visualization and manipulation of neurons in the circuit with very high (often single-cell) resolution (Hallem and Carlson, 2004; Komiyama and Luo, 2006). In addition, the recent publication of nearly complete olfactory receptor (OR) expression and olfactory receptor neuron (ORN) connectivity maps has laid the foundation for an unprecedented exploration of the molecular mechanisms that mediate connection specificity in the fly olfactory system (Couto et al., 2005; Fishilevich and Vosshall, 2005).

One common feature of insect and mammalian olfactory systems is the striking convergence of ORN axons that express the same receptor onto discrete glomeruli in the fly antennal lobe or mammalian olfactory bulb, where they meet and synapse with dendrites of second-order neurons—projection neurons in *Drosophila* or mitral cells in mammals (Komiyama and Luo, 2006). How is this precise convergence of olfactory axons achieved? Evidence from genetic studies in mice suggests that the ORs themselves contribute to the convergence of like ORN axons but that other instructive cues are also involved. In contrast, fly ORs do not regulate guidance and targeting (Dobritsa et al., 2003), raising the question of what molecules are involved in these processes in *Drosophila*. While several factors have been implicated in ORN axon targeting to the fly antennal lobe, including Dscam (for Down's syndrome cell adhesion molecule; Hummel et al., 2003), N-Cadherin (Hummel and Zipursky, 2004), and the POU transcription factor Acj6 (for abnormal chemosensory jump 6; Komiyama et al., 2004), it is clear that many additional instructive forces remain to be identified.

In this issue of *Neuron*, two groups have independently converged on an important role for the transmembrane Semaphorin-1a (Sema-1a), and its receptor PlexinA in contributing to the fidelity of ORN axon targeting. Semas and Plexins comprise large evolutionarily conserved families of guidance cues and receptors that are well known for mediating a range of predominantly repulsive effects on axons in the developing nervous system (Pasterkamp and Kolodkin, 2003). Semas and Plexins have been implicated in many guidance and targeting functions, including the regulation of axon fasciculation, influencing steering decisions, sorting axons into distinct zones, and contributing to the specificity of motoneuron target selection (Pasterkamp and Kolodkin, 2003).

To identify additional regulators of ORN connectivity, Lattemann et al. (2007) (this issue of *Neuron*) performed a mosaic genetic screen where flies with mutations specifically in the ORNs were created and examined for defects in targeting. Genetic mapping and complementation testing identified *sema-1a* as one of the genes that when mutated leads to characteristic and robust axon misprojection phenotypes in some classes of ORNs. Antibody staining revealed strong expression of Sema-1a in developing ORNs, which are housed in distinct sensory sensilla found in two separate peripheral structures, the third antennal segment (AT) and the maxillary palps (MP). Intriguingly, examination of Sema-1a expression in the antennal lobe revealed that developing glomeruli express different levels of Sema-1a, ranging from undetectable to high-level expression, and that often, neighboring glomeruli express different levels. Could this differential expression account for the differential sensitivity of specific classes of ORNs to loss of *sema-1a* function? Furthermore, could the differences in Sema-1a levels on neighboring glomeruli underlie the class specific segregation of ORN axons?

The authors went on to test these ideas by conducting an extensive series of loss- and gain-of-function genetic experiments. Using the MARCM (mosaic analysis with a repressible cell marker) system (Lee and Luo, 1999) to generate clones of mutant ORNs, the authors examined the targeting of many specific classes of ORNs and attempted to correlate mutant phenotypes with Sema-1a expression levels. *sema-1a* mutations led to disruptions in about half of the 22 ORN classes examined and affected the targeting of ORN axons originating from both the AT and MP. Two types of defects were observed: axons either spread beyond their normal glomeruli boundaries (type 1) or reached their appropriate targets but also accumulated in ectopic locations in relative proximity to the correct glomerulus (type 2). In addition, MP ORN axons were sometimes observed to innervate targets outside of the antennal lobe. Further phenotypic analysis of pairs of neighboring glomeruli revealed that the ectopic type 2 axon accumulations do not invade nontarget glomeruli but rather form new "glomerulus-like" structures. Comparison of the observed differences in Sema-1a expression levels between different glomeruli with the class-specific ORN axon-targeting defects did not reveal a simple predictive relationship between the two; however, the fact that equalizing Sema-1a expression levels in adjacent glomeruli that normally express different levels leads to defective targeting supports the idea that the differences in relative Sema-1a levels contribute to ORN wiring specificity.

Given these mutant phenotypes, the authors next asked where and how *sema-1a* exerts its effects on ORN axons and found strong evidence indicating that *sema-1a* acts nonautonomously to influence neighboring axons. First, single-cell MARCM clones did not reveal the strong defects observed in the larger clones and second, reverse MARCM analysis (a mosaic technique in which wild-type axons are visualized in a background where neighboring unlabeled axons are mutant) resulted in targeting defects comparable to those observed in large MARCM clones, an observation arguing that Sema-1a acts as a ligand for neighboring ORN axons. Genetic interaction experiments in which *sema-1a* and *plexinA* gene dose were partially reduced resulted in similar defects to those observed in *sema-1a* mutants, suggesting that PlexinA is likely to be the relevant Sema-1a receptor in this context, and that axon-axon interactions among in-growing ORNs play an important role in controlling target selection.

In the second study, Sweeney et al. (2007) (this issue of *Neuron*) were interested in exploring the role of axon-axon interactions between ORNs in contributing to the establishment of the olfactory map. The Luo lab had previously obtained evidence for such a role in their analysis of ORN targeting phenotypes in *acj6* mutants, where they found that *acj6* function in certain classes of ORNs is required for appropriate target selection of other classes (Komiyama et al., 2004). Furthermore, genetic studies in mice also support the proposition that ORN axon-axon interactions are important for regulating the convergence of ORN axons onto specific glomeruli (Feinstein and Mombaerts, 2004). However, neither the cellular context of these putative axon-axon interactions nor the surface molecules involved in mediating such interactions were known. These unanswered questions provided the motivation for the current study.

To begin to address these questions, the authors took advantage of two important aspects of the organization and development of the peripheral olfactory sensory organs in the fly. First, as noted above, the third antennal segments and the maxillary palps are spatially segregated, allowing for a certain degree of independent manipulation of the ORNs that reside in these distinct structures. Second, the authors demonstrate in a time course analysis that AT ORN axons arrive at the antennal lobe well in advance of the MP ORN axons (~12 hr) and furthermore that the entry points of MP and AT ORN axon bundles into the antennal lobe are spatially distinct. To determine whether the AT ORN axons might influence the subsequent targeting of MP ORN axons, the authors performed a series of clever genetic ablation experiments that capitalized on the opportune observation that *smoothened* mutant clones occasionally result in loss of one or both third antennal segments or maxillary palps. In the absence of both third antennal segments (and both populations of AT ORN axons), MP ORN axons were frequently mistargeted, invading areas normally occupied by AT ORN axons. This finding suggests that AT ORN axons could restrict the growth of MP ORN axons by repulsion. In contrast loss of both MPs did not affect targeting of AT ORN axons.

Having established that AT ORN axons provide targeting information to the MP ORN axons, the authors took a candidate gene approach as well as an unbiased genetic screen using a library of transgenic *UAS* RNAi lines to independently identify Sema-1a and PlexinA as likely mediators of AT/MP axon-axon interactions. Expression analysis of Sema-1a and PlexinA revealed that both are present on MP and AT ORN axons as they enter the antennal lobe. To assess the functional requirement for *sema-1a*, the authors next performed a series of mosaic genetic experiments including MARCM and reverse MARCM analyses similar to those conducted by Lattemann et al. (2007), although with a greater emphasis on MP ORNs. Phenotypes of AT ORNs in *sema-1a* mutants include spreading beyond normal glomeruli boundaries for certain ORN classes as well as ectopic class-specific ORN axon accumulations, while MP ORN axons exhibited extra-antennal lobe terminations as well as mistargeting within the antennal lobe, phenotypes largely consistent with those described by Lattemann and colleagues. Reverse MARCM and single-cell clone experiments again support a predominantly non-cell-autonomous function for *sema-1a*, while genetic interaction experiments using *UAS-plexinA* RNAi transgenes bolster the argument that PlexinA acts as a receptor for *Sema-1a* during ORN axon targeting; here it should be noted that significant

differences in the phenotypes between *sema-1a* and *plexinA* are observed, raising the possibility that there could be non-*sema-1a*-dependent roles for *plexinA* in olfactory wiring. Furthermore, it is important to point out that although the most likely interpretation of these findings is that PlexinA acts cell autonomously on axons that receive Sema-1a signal, the inability to analyze *plexinA* by MARCM (*plexinA* is on the fourth chromosome where MARCM is not possible) counsels caution in definitively ascribing PlexinA's site of action.

In a final set of experiments, Sweeney et al. (2007) developed an ingenious method to independently manipulate AT and MP ORNs to further define the cellular mechanism of Sema-1a action. By generating mutant clones in both of the ATs and either zero, one, or both MPs, coupled with unilateral severing of MPs, the authors established unambiguously that *sema-1a* is required specifically on AT ORN axons to regulate the targeting of ipsilateral MP ORN axons within the antennal lobe, while *sema-1a* is required earlier in the MP ORN axons to regulate axon entry into the antennal lobe, in a manner akin to that proposed for Sema-1a-mediated control of axon defasciculation during motor axon guidance in the fly embryo (Pasterkamp and Kolodkin, 2003).

Although these two studies strongly suggest that Sema-1a and PlexinA influence ORN axon targeting by mediating axon-axon repulsion, neither study provides proof that Sema-1a acts as a repellant; other possible mechanisms exist. However, in light of the observed mutant phenotypes and the well-documented repulsive functions of Semas in general and specifically of Sema-1a in the fly embryo, repulsion seems the most likely mechanism. Together the elegant genetic experiments in these two reports support a major role for Sema-1a and PlexinA in controlling connection specificity in the antennal lobe and highlight the importance of axon-axon interactions in organizing complex neural circuits.

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A Neural Protection Racket: AMPK and the $GABA_B$ Receptor

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The cellular energy-sensing kinase AMPK is known to be activated in neurons in response to metabolic insults, but the downstream consequences have been unclear. A study by Kuramoto and colleagues in this issue of *Neuron* favors the idea that AMPK activation is neuroprotective, and suggests a potential mechanism for this effect involving phosphorylation of the $GABA_B$ receptor.

The nervous system accounts for a high proportion of total body energy turnover, and neurons are particularly vulnerable to energy deficits due to their rather inflexible metabolism and poor capacity to store nutrients. It is therefore not surprising that 5′AMP-dependent protein kinase (AMPK), part of a signaling system that is a central player in the maintenance of energy balance at both the cellular and whole body levels, should be highly