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Intrinsic Activity of Odorant Receptors Guides Sensory Map Formation

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Olfactory sensory neurons innervate the olfactory bulb in stereotyped patterns according to the odorant receptors they express. A study by Nakashima et al. (2013) in this issue demonstrates that the odorant receptor's level of intrinsic activity – in the absence of activating odorant – influences the guidance of olfactory axons to their targets.

Olfactory sensory neurons (OSNs) express just one odorant receptor (OR) from a repertoire of over 1000 OR genes (DeMaria and Ngai, 2010). OSNs expressing the same OR are distributed across large areas of the sensory epithelium and project their axons to common and spatially invariant sites – called glomeruli – in the olfactory bulb. Individual glomeruli receive innervation only from OSNs expressing the same OR; the spatial representation of ORs in the bulb forms the anatomical basis of the olfactory sensory map and reflects a remarkable feat of pattern formation during development. Targeting of olfactory axons along the olfactory bulb's dorsal-ventral and medial-lateral axes occurs through OR-independent mechanisms (DeMaria and Ngai, 2010). In contrast, "receptor swaps" in which the coding region of one OR gene is replaced with the coding region of another OR cause shifts in glomerular position along the anterior-posterior (AP) axis of the bulb (Wang et al., 1998). A tantalizing hypothesis emerging from these receptor swap experiments posits that the OR not only receives sensory information from small volatile molecules in the environment, but also from axon guidance cues that determine where in the bulb the OSN's axon projects. This model, as appealing as it may seem, has yet to receive compelling experimental support.

If the OR does not function as an axon guidance receptor per se, perhaps it sets the responsiveness or "gain" of the OSN to classical axon guidance cues through its level of activity. But how? ORs are unlikely to encounter their cognate odorants in utero. Like other G protein-coupled receptors, in the absence of ligand ORs are intrinsically active, existing in equilibrium between an active and inactive state (Rosenbaum et al., 2009). Given the sequence diversity of ORs, it is not hard to imagine a similar diversity in the level of OR intrinsic activity based on each receptor's unique physical properties. Initial support for this model came from a demonstration that perturbations in cAMP signaling – the second messenger pathway employed in olfactory sensory transduction – altered the projection of OSN axons along the AP axis of the olfactory bulb (Imai et al., 2006): decreased cAMP signaling led to aberrant projections toward the anterior bulb, whereas increased cAMP signaling led to projections posterior to the location of the normal glomerulus. Imai et al.

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(2006) further demonstrated that expression of Neuropilin1, a receptor for the repulsive axon guidance cue Semaphorin 3A, is regulated by cAMP (via protein kinase A) in developing OSNs, neatly tying together the OR and axon guidance. Direct evidence that activity of unliganded receptor influences OSN axon guidance, and does so in an OR-specific way, was nonetheless lacking.

To address this issue directly, Nakashima et al. (2013) created a panel of β2-adrenergic receptor (β2-AR) mutants with varying levels of intrinsic activity (agonist-induced activity was unaltered). They then engineered mice to express either wild type or mutant β2-ARs in lieu of OR, and asked where OSNs expressing these receptors innervate the olfactory bulb. Consistent with the studies of Imai et al. (2006), OSNs expressing receptors with reduced activity formed glomeruli in more anterior locations relative to the wild type β2-AR, and those expressing a receptor with higher activity formed glomeruli in more posterior locations. Expression of Neuropilin1 also correlated with receptor activity levels (as expected); the expression of PlexinA1, another axon guidance cue receptor, showed an inverse relationship (also as expected). But do these results accurately reflect the behaviors of native OSNs expressing endogenous ORs? To answer this question, Nakashima et al. measured in heterologous cells the intrinsic unliganded activities of 30 ORs and found that OSNs projecting to the anterior bulb express ORs that show on average low levels of intrinsic activity, whereas posterior-projecting OSNs express ORs with a high average level of intrinsic activity. In addition, a receptor swap in which a “low activity” OR was replaced with a “high activity” OR resulted in a posterior shift in the location of the target glomerulus. Together the data support the hypothesis that the level of OR intrinsic activity influences the AP position of the OSN’s glomerulus.

The results of Nakashima et al. touch on several related aspects of OSN differentiation. OSNs sequentially express two closely related Gα isoforms, $G\alpha_s$ in immature neurons and $G\alpha_{olf}$ in mature OSNs, both of which activate adenylyl cyclase. Could this developmental switch reflect different roles of these two Gα isoforms during OSN maturation? Again using cell based assays, Nakashima et al. demonstrate that unliganded β2-AR and selected ORs couple more efficiently to $G\alpha_s$ than they do to $G\alpha_{olf}$. In vivo, conditional knockout of $G\alpha_s$ causes mistargeting of OSN axons and down-regulation of Neuropilin1 expression; in contrast, axon targeting and Neuropilin1 expression are unaffected in a $G\alpha_{olf}$ knockout (Nakashima et al., 2013). Thus, $G\alpha_s$ appears to mediate intrinsic OR activity in immature OSNs to regulate expression of certain axon guidance receptors and ultimately the global targeting of OSN axons along the olfactory bulb’s AP axis.

It is also interesting to consider the mechanisms underlying the final sorting of OSN axons once they arrive at the appropriate location in the bulb. Odor-evoked neuronal activity is mediated by the cyclic nucleotide-gated (CNG) channel, which subserves membrane depolarization and calcium entry in response to elevations in cAMP; such neuronal activity is not required for proper targeting of most OSNs during development (Lin et al., 2000). Once the axons have found their initial targets, however, CNG-dependent activity is required for their final sorting to individual glomeruli through the regulated expression of specific cell adhesion molecules (Serizawa et al., 2006). Expression of these cell adhesion molecules is not affected in the $G\alpha_s$ conditional knockout, but is dependent on $G\alpha_{olf}$ and the CNG channel (Nakashima et al., 2013; Serizawa et al., 2006), which are up-regulated late in embryogenesis as OSNs mature (Nakashima et al., 2013). OR activity may therefore exert different effects by virtue of the downstream signaling components available at a given stage of the cell’s differentiation. Considering that unliganded OR couples inefficiently to $G\alpha_{olf}$ (Nakashima et al., 2013), it remains a bit of a puzzle as to how OR might be activated at the final sorting stage, at least in the absence of sensory stimulation in utero – perhaps this

sorting occurs only in postnatal life, when OSNs receive sensory input and activity-dependent refinement of OSN projections is known to occur (Zou et al., 2004).

The studies of Nakashima et al. focused mainly on mechanisms of axon guidance and sorting during development. However the olfactory epithelium is one of the few sites in the nervous system that undergoes active regeneration of neurons over the lifetime of the animal. Do adult-born OSNs use similar mechanisms as their embryonic predecessors to wire up the olfactory bulb and maintain the sensory map? For now this remains an open question, although transcriptome analysis by deep sequencing (RNA-Seq) on immature and mature OSNs purified from adult mice may provide some hints: $G\alpha_s$ but not $G\alpha_{olf}$ is enriched in immature neurons, and $G\alpha_{olf}$ and CNG channel subunits are up-regulated in mature OSNs (Magklara et al., 2011). It is possible, then, that adult-born OSNs use OR activity to regulate different stages of olfactory axon guidance, similar to the strategy used during embryogenesis.

Work over the past two decades has established the importance of the OR not only in recognizing the multitude of chemical cues in the olfactory sensory world, but also in shaping the precise connectivity from olfactory epithelium to olfactory bulb. The OR is also a key player in a negative feedback loop that silences other OR genes in order to enforce the “one receptor, one neuron” rule of OR gene expression (Serizawa et al., 2003). It will be interesting to determine the role of OR intrinsic activity – and the relevant downstream signaling pathways – in this and other gene regulatory networks in the developing olfactory system.

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