Odorant Receptors on Axon Termini in the Brain

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In most sensory systems, peripheral neurons project axons to precise locations in the brain to create an internal representation of the external world. In mammals, individual olfactory sensory neurons express only one of ~1000 odorant receptor genes, such that neurons are functionally distinct (*1*). Cells expressing a given receptor are randomly dispersed within a broad but circumscribed zone in the sensory epithelium. Spatial order is achieved by the convergence of like axons at precise glomeruli in the olfactory bulb, the first relay for olfactory information in the brain (*2–4*). The identity of an olfactory percept may therefore be encoded by spatial patterns of glomerular activity in the bulb.

How does a random distribution of sensory neurons in the epithelium resolve to a highly ordered topographic map in the bulb? A role for odorant receptors in guidance is suggested by genetic experiments demonstrating that alterations of receptor sequences perturb the sensory map (*5*). These observations suggest that the odorant receptor will be expressed both on dendrites and axons.

We tested this prediction by generating specific antibodies against two odorant receptors that reveal the sites of receptor expression on sensory neurons. Antibodies were raised against extracellular and cytoplasmic epitopes of the mouse odorant receptors MOR28 and MOR11-4. These antibodies recognize receptor protein in detergent-solubilized membrane fractions (fig. S1, A and B) and in histologic sections from olfactory sensory epithelium. Antibodies directed against both epitopes of MOR28 stain an identical subpopulation of neurons randomly distributed in the ventral zone of the olfactory epithelium (Fig. 1, A and B). The frequency and spatial pattern of these cells are typical of neurons that express MOR28, as revealed by in situ hybridization (*6*). We observed intense staining in both the dendritic knob, the locus of odor binding, and in perinuclear compartments likely to correspond to the endoplasmic reticulum and Golgi apparatus. A similar pattern was observed with an antibody directed against a second receptor, MOR11-4 (Fig. 1C). We also stained epithelia from genetically altered mice in which the MOR28 locus had been modified so that cells expressing MOR28 also expressed tau-βGal (MOR28-IRES-tau-lacZ). In these mice, all βGal^+ cells also stain with antibodies to MOR28. No staining was observed in another genetically altered strain that bears a homozygous deletion of MOR28 (*6*).

If the odorant receptor also serves as a guidance molecule, we would expect it to be localized at the axon terminus. We therefore stained sections through the olfactory bulb with antibodies against both epitopes in MOR28. Both antibodies stained only two identical glomeruli, one medial and one lateral in the ventral aspect of the bulb (Fig. 1, D and E). Staining was observed only within the glomerulus and the fascicles immediately adjacent to it but not in more proximal axon shafts. A single lateral glomerulus at a more dorsal position was stained with antibodies to MOR11-4 (Fig. 1F).

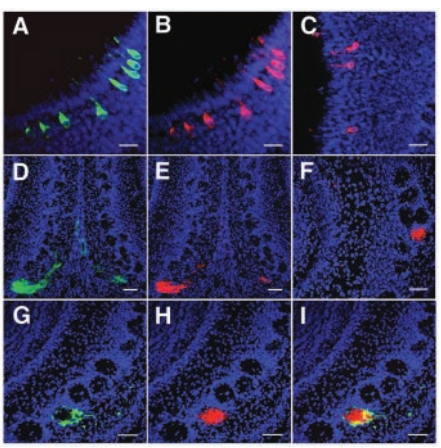

Fig. 1. Odorant receptor protein is expressed on both dendrites and axons of olfactory sensory neurons. (**A** to **C**) Staining of mouse olfactory epithelium with (A) rabbit anti-MOR28 extracellular epitope, (B) guinea pig anti-MOR28 cytoplasmic epitope, and (C) guinea pig anti–MOR11-4 cytoplasmic epitope. Scale bars, 10 μm. (**D** to **F**) Staining of mouse olfactory bulb with (D) rabbit anti-MOR28 extracellular epitope, (E) guinea pig anti-MOR28 cytoplasmic epitope, and (F) guinea pig anti–MOR11-4 cytoplasmic epitope. Only the medial glomeruli of MOR28 are shown in (D) and (E). Scale bars, 100 μm. (**G** to **I**) Staining of MOR28-IRES-tau-LacZ heterozygous mouse olfactory bulb with (G) goat anti-βGal and (H) rabbit anti-MOR28 extracellular epitope. (I) A merged image of (G) and (H). All mice used were 10 days old. Scale bars, 100 μm.

We next demonstrated that the glomeruli stained with antibody to MOR28 were innervated by neurons that express the MOR28 receptor. Sections through the olfactory bulb of mice bearing the MOR28-IRES-tau-lacZ allele were examined with antibodies against both βGal and MOR28 (Fig. 1, G to I). These experiments revealed that the glomeruli stained by antibody to MOR28 also received the tau-βGal^+ fibers. The mice analyzed were heterozygous for the MOR28-IRES-tau-lacZ allele. Thus, although all fibers within the glomerulus stained with antibody to MOR28, only half expressed βGal and appeared to segregate. Segregation of fibers from differentially modified alleles within a single glomerulus has been reported, but the mechanism remains elusive (*7*).

In other sensory systems, peripheral neurons project to the central nervous system in an ordered manner, such that spatial relations in the periphery are maintained in the brain. In contrast, olfactory neurons are randomly distributed in the periphery, but their axons project to precise loci in the bulb. The observation that odorant receptors are expressed both on dendrites and on axon termini supports a mechanism in which the receptor functions as a guidance molecule that recognizes positional cues elaborated by the bulb. In this manner, the olfactory neuron is endowed with an identity that dictates both the set of odors to which it responds and the glomerular target it innervates.

References and Notes

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Supporting Online Material

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Materials and Methods
Fig. S1
References and Notes

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