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How Does the Brain Smell?

Minireview

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Sensory stimuli are detected by specialized receptors in sensory organs, and the signals hence generated flow through multiple and interconnected centers of the brain where they are analyzed and processed into complex sensory perception. Processing of the sensory information requires the coding of sensory inputs into specific patterns of neuronal activity. A common mechanism for sensory information coding is provided by the topographic organization of sensory neurons and their axonal projections, such that sensory centers represent an internal map of the external sensory world: the body surface, the visual world, the frequency of sounds. In that manner, the quality of sensory stimuli is encoded by the spatial coordinates of neuronal activity in high sensory centers of the brain, and the discrimination between sensory signals results from the stimulation of topographically distinct subsets of neurons.

The olfactory system is a molecular analyzer that can discriminate a vast array of odorants with large variety in chemical structure. How does the brain determine which odor has been detected by olfactory sensory neurons? Olfactory discrimination raises a peculiar problem since odorant molecules per se do not convey any spatial information.

Vertebrate and insect olfactory systems display common organizational and functional characteristics. The initial event in odorant detection requires the specific interaction of odorant molecules with odorant receptors expressed on the cilia of olfactory neurons. The vertebrate olfactory epithelium (and the insect antenna) contains several thousand bipolar olfactory sensory neurons, each projecting to one of several glomeruli in the main olfactory bulb (the antennal lobes of insects). Olfactory glomeruli are distinctive bundles of neuropil found in the olfactory system. Their number varies in different species: rodent olfactory bulbs contain several thousand glomeruli; fishes and insects have 10-fold fewer. Glomerular structures result from the convergence in the bulb (or the antennal lobes of insects) of thousands of axon terminals that originate from olfactory sensory neurons and that establish synapses with dendrites of mitral and tufted cells (output neurons in insects) and of various classes of interneurons.

Discrimination between odors requires the specific interaction between odorant molecules and odorant receptors and the precise recognition by the brain of which subset of receptors has been activated by a given odorant. Independent molecular and functional approaches have recently provided a model for the specific recognition of odors by the brain, which involves the translation of odorant quality into arrays of topographically segregated stimuli.

The Molecular Framework of Olfactory Information Coding

The isolation of odorant receptor genes has provided molecular tools to investigate the topographic organization of the olfactory system and to gain access into the neuronal coding of olfactory stimuli in the brain. In mammals, odorant receptors are G-coupled seven transmembrane domain receptors encoded by a family of more than a thousand genes (Buck and Axel, 1991). Convergent lines of evidence strongly suggest that individual olfactory sensory neurons express only 1 or few of the 1000 receptor genes (Ressler et al., 1993; Vassar et al., 1993). In addition, it has been demonstrated in rodents that all neurons expressing the same receptor (and therefore presumably responsive to a small subset of odors), although randomly distributed in domains of the epithelium, project their axons to one or a small number of discrete loci or glomeruli within the olfactory bulb (Ressler et al., 1993, 1994; Vassar et al., 1993, 1994; Mombaerts et al., 1996). The positions of specific glomeruli are topographically fixed, and are conserved in the brains of all animals within a species. These data provide physical evidence that the olfactory bulb defines a two-dimensional map that identifies which of the numerous receptors have been activated within the sensory epithelium. This led to an attractive model of olfactory coding in the brain, according to which discrimination of odor quality would result from the detection by the brain of specific patterns of glomeruli activity.

Testing the Molecular Model by Directly Looking at Odor Signal Processing in the Brain

Molecular approaches, however sophisticated, provide a view of sensory processing that lacks functional perspective. In this regard, a milestone has recently been reached with two independent studies performed in the zebrafish, Danio rerio (Friedrich and Korsching, 1997) and in the honeybee, Apis mellifera (Joerges et al., 1997), describing the optical imaging of glomerular activation after exposure of olfactory sensory neurons to various natural odorants. Ingenious tricks allowed the use of Ca^{2+} indicators to directly monitor glomerular electrical activities in a significant portion of the fish olfactory bulb and the honeybee antennal lobes when various odorant stimuli are presented to the distant olfactory sensory organs. These two publications are of special interest because they confirm the topographic organization of the olfactory system revealed by molecular genetics in rodents and reinforce, but also go beyond, the model of olfactory discrimination predicted from molecular studies.

Zebrafish olfactory sensory neurons were loaded with a Ca^{2+} indicator that distributes throughout the neuronal cell bodies and axon terminal, resulting in presynaptic labeling of the fish olfactory bulb, which was observed in explant preparation of the system. Experiments with honeybees were performed by incubating the entire insect brain in Ca^{2+} indicators, which results in both pre- and postsynaptic labeling. After odors were delivered to the freely moving antennae, the fluorescent changes in the antennal lobes were directly monitored through

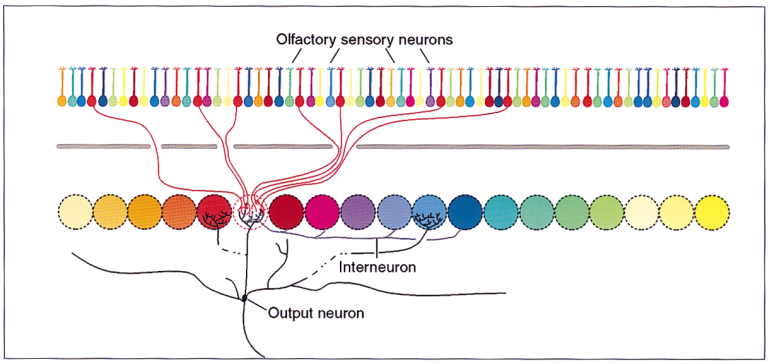

Figure 1. Schematic Representation of Olfactory Sensory Coding in the Olfactory Bulb

Sensory neurons of the vertebrate olfactory epithelium (and the insect antenna) project to one glomerulus of the olfactory bulb (antennal lobe in insect) where they synapse with mitral cells (output neurons in insects). Depending on the animal species, mitral cells send dendrites to one glomerulus or several glomeruli (only one in the main olfactory bulb of mammals). Optical imaging and molecular methods suggest a common model of olfactory discrimination employed by evolutionarily distant animal species: olfactory sensory neurons express one olfactory receptor gene or a few, and all neurons expressing the same receptor project to one glomerulus or a few glomeruli. Intrabulbar circuits involving inhibitory interneurons and lateral inhibition by output cell lateral dendrites participate in the refinement of olfactory signals.

a window cut in the head cuticle, which enables the experimentors to visualize about one fifth of the glomeruli. In both systems, the exposure of olfactory sensory receptor cells to individual odors elicits defined patterns of fluorescence in the target tissue that can be perfectly superimposed with the neuroanatomical structures of glomeruli. Signals generated by each odor display bilateral symmetry in the two halves of the brain and are found in topographically fixed positions that are reproducible in multiple assays of the same animal preparation and conserved in different animals of the same species. These data confirm the results alluded to by earlier electrophysiological and tracing experiments in the bulb (reviewed by Buck, 1996) and directly visualized by molecular studies in rodents (Vassar et al., 1994; Mombaerts et al., 1996), which pointed to the olfactory glomerulus as the functional unit of olfactory perception in the brain and showed that glomeruli sharing the same receptor specificity are found in the same position in different animals.

After exposure of olfactory sensory neurons to a series of individual odorants, the optical imaging of the electrical activity in the brains of the honeybee and the zebrafish shows that each compound activates multiple glomeruli and generates distinct but overlapping activity patterns. The number of glomeruli activated by a given odorant was not directly predictable from the structural complexity of the odorant: a rather simple molecule such as citral activates a very complex pattern of glomerular activity in the honeybee brain, while carnation, which is a blend of several substances, generates a very simple activation pattern. The monitoring of glomerular activity induced by multiple odorants indicates that a given glomerulus participates in the sensory processing of multiple odors but that each odor generates a specific combinatorial pattern of glomerular excitation. Such a combinatorial code is likely to permit the brain to discriminate a number of odorants far exceeding the number of individual glomeruli and olfactory receptor proteins.

It is difficult to compare results obtained in bees and fish with the model of olfactory discrimination deduced from molecular studies in rodents. Olfactory receptor genes have not been isolated in insects, and the pattern of axonal projections from fish olfactory sensory neurons expressing a given receptor gene has not been identified. It is fair to say, however, that data obtained by optical imaging in these two species are entirely compatible with a molecular organization of the fish and the honeybee olfactory systems similar to that hypothesized for rodents (Figure 1): each sensory neuron expresses only one receptor gene or a few receptor genes and hence displays a broad but distinctive tuning, and all neurons expressing the same receptor send their axonal projections to one glomerulus or few glomeruli with topographically fixed positions.

According to a model in which individual neurons express only one odorant receptor gene or a few odorant receptor genes, the broad tuning of olfactory sensory neurons is likely to result from the distinctive affinity of individual receptor molecules for subsets of odorant ligands. When Friedrich and Korsching increased the odorant concentration smelled by the zebrafish, they observed an increase in the number of activated glomeruli that seems a logical consequence of the saturation of specific receptors and the recruitment of receptors of lower affinity. This provides a simple mechanism ensuring the coding of both the odor identity and its relative concentration. In the honeybee's brain, however, Joerges et al. reported identical signal patterns obtained at increasing odorant concentrations. But they also noticed that the odorant concentrations used in their experiments were never saturating. It is possible, therefore, that the discrepancy found between the fish and the honeybee can be attributed to differences in experimental systems. Alternatively, one cannot exclude more profound differences in the olfactory signal processing of the two species including a poor sensitivity of the honeybee olfactory system to odorant concentrations. Thus, evolutionarily distant animal species appear to use a common olfactory coding strategy that provides optimal olfactory discrimination based on the activation by a given odorant of specific combinatorial patterns of glomeruli.

Unraveling the Chemotopic Map of the Brain

In a given animal species, specific classes of odorants are ecologically relevant to indicate the presence of food, danger, or mating partners. The close conservation in a given species of the position of glomeruli activated by a given odor points to the potential importance of the exact coordinates of bulbar electrical activity in providing nearly identical olfactory information to all individuals of the species. Interestingly, Joerges et al.

noticed that the variability in pattern of glomerular activity in honeybees is significantly higher for flowery odors, which are known to induce variable responses in mature individual workers, than for citral, which has an additional role as a pheromone (although not for the animals studied). This variability might be attributed to a lower selective pressure to keep fixed locations for glomeruli associated with flower fragrances compared to potential pheromones, or might result from acquired changes associated with sensory experience and training of individual worker bees.

Fishes, on the other hand, are sensitive to a wide range of waterborne odorants such as amino acids and bile acids. Amino acids constitute a particularly interesting class of odors that are ecologically relevant for the animal in indicating the presence of nutrients. Optical imaging of the entire bulb surface in the zebrafish shows that the lateral portion of the fish olfactory bulb is devoted to detection of amino acids, whereas other portions of the bulb process other physiologically relevant odorants. A first level of organization of the olfactory bulb thus exists that segregates broad categories of odors, possibly according to their physiological significance or their overall chemical structure. A relationship may exist between this gross anatomical and functional segregation of bulb regions and the zones of projection originating from neurons located in the distinct zones of the olfactory epithelium that have been documented both in rodents and fish (Ressler et al., 1993; Vassar et al., 1993; Weth et al., 1996). A detailed analysis of glomerular activity induced by distinct amino acids reveals a second level of organization within the bulb, such that chemically related amino acids activate glomeruli located in the same area of the bulb, while amino acids belonging to different structural classes, such as neutral, acidic, or basic, elicit activity patterns in distinct zones of the bulb. In fact, small glomerular modules with similar response profiles are closely clustered. Thus, a given cluster might be equivalent to a larger size glomerulus displaying specificity for a certain structural class of amino acids. Finally, a third level of bulbar organization might be provided by differential fine tuning of small subsets of glomeruli modules for specific amino acids within a structural group. These data clearly generalize previous electrophysiological data obtained by direct recording of mitral cells that showed a topographical arrangement according to odorant selectivity (Mori et al., 1992).

A partial chemotopic map of the fish olfactory bulb thus emerges, and will hopefully be completed by the analysis of other physiological classes of odors. One may wonder about the extent of the sequence divergence of odorant receptors detecting different amino acids within a structural group, or discriminating between the various types of amino acids or between bile acids and amino acids. Cross-adaptation experiments performed on fish olfactory epithelia (Caprio and Byrd, 1984) indicate that amino acids of similar structures are poorly discriminated, suggesting that receptor molecules with such fine selectivity might be rare and share close sequence homologies with receptors of similar specificity.

The molecular basis for this chemotopic glomerular topography is intriguing. Genetic approaches in mouse showed that the swap of receptor coding sequences on the chromosome has a considerable effect on the choice of the target glomerulus by the olfactory sensory neuron expressing the modified locus (Mombaerts et al., 1996). This finding indicates that the olfactory receptor plays a crucial role in the guidance process, although it is not likely to be the sole determinant in the establishment of the bulb topographic map. In a similar manner, olfactory receptor molecules might be involved in the specification of the fish axon terminals to the appropriate broad region and/or precise glomeruli in the olfactory bulb. It will be of great interest to correlate the three parameters of receptor selectivity, topographical coordinates of the corresponding glomeruli, and sequence similarities.

Odor Detection and Information Processing

The refinement of incoming odorant signals is critical for their roles in the modulation of social and feeding behaviors in bees (Menzel and Muller, 1996). Recording glomerular activity with optical imaging provides a glimpse into integration processes of the crude olfactory signal that occur in the antennal lobe. It appears that the fluorescent patterns elicited by mixtures of odors differ significantly from the arithmetic sum of glomeruli patterns obtained after stimulation with individual odors. In particular, the activity of specific glomeruli appears reduced when activated by a mixture of odors rather than by individual compounds. Such modulatory events that integrate and refine complex odor signaling are probably of critical importance since animals in the wild are likely to be exposed to blends of odorant compounds. More studies will be required to further analyze these early integrative events and to determine the respective roles of inhibitory intrabulbar circuits that have already been documented (Figure 1). Among those are the GABAergic dendrodendritic synapses established between periglomerular and output neurons and the lateral inhibitory contacts established by the long basal dendrites of output neurons on other types of interneurons (Mori, 1987; Mori and Shepherd, 1994; Yokoi et al., 1995).

Direct recordings of complex brain processes by imaging technologies have just opened the door to the analysis of olfactory signal processing. This method holds great potential for future study of synaptic plasticity during development, training, and various physiological contexts, and might finally provide direct access to the tantalizing issue of olfactory sensory processing in higher brain structures leading to complex perception and behavior.

Selected Reading

Buck, L. (1996). Annu. Rev. Neurosci. *19*, 517–544.

Buck, L., and Axel, R. (1991). Cell *65*, 175–187.

Caprio, J., and Byrd, R.J (1984). J. Gen. Physiol. *84*, 403–422.

Friedrich, R.W., and Korsching, S.I. (1997). Neuron *18*, 737–752.

Joerges, J., Kutter, A., Galizia, C.G., and Menzel, R. (1997). Nature *387*, 285–288.

Menzel, R., and Muller, U. (1996). Annu. Rev. Neurosci. *19*, 379–404.

Mombaerts, P., Wang, F., Dulac, C., Chao, S., Nemes, A., Mendelsohn, M., Edmondson, J., and Axel, R. (1996). Cell *87*, 675–686.

Mori, K., and Shepherd, G.M. (1994). Semin. Cell Biol. *13*, 771–790.

Mori, K., Mataga, N., and Imamura, K. (1992). J. Neurophysiol. *67*, 786–789.

Ressler, K.J., Sullivan, S.L., and Buck, L.B. (1993). Cell *73*, 597–609.

Ressler, K.J., Sullivan, S., and Buck, L.B. (1994). Cell *79*, 1245–1255.

Vassar, R., Ngai, J., and Axel, R. (1993). Cell *74*, 309–318.

Vassar, R., Chao, S.K., Sitcheran, R., Nunez, J.M., Vosshall, L.B., and Axel, R. (1994). Cell *79*, 981–991.

Weth, F., Nadler, W., and Korsching, S. (1996). Proc. Natl. Acad. Sci. USA *93*, 13321–13326.

Yokoi, M., Mori, K., and Nakanishi, S. (1995). Proc. Natl. Acad. Sci. USA *92*, 3371–3375.