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Outline of a Theory of Olfactory Processing and its Relevance to Humans

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Introduction

Two traditional beliefs about olfaction are that the system is poorly understood and that it functions poorly in humans. An outline of a theory of olfactory processing has in fact been emerging for many years (Rall and Shepherd, 1968; Stewart *et al.*, 1979; Haberly, 1985; Lancet, 1986; Buck and Axel, 1991; Shepherd, 1991; Imamura *et al.*, 1992; Mori and Shepherd, 1994; Mombaerts, 2004; Wilson, 2004). I will first briefly update the theory based on evidence from many recent studies, a consensus that is not widely appreciated because it is multilevel, multidisciplinary and cross-phylogenetic. I will then consider new evidence that evolution has produced in humans an excellent overall sense of smell and, combined with taste and somatosensation and other inputs, the best sense of flavor in the animal world. This should give a new emphasis to the importance of these senses for sensory physiology, human nutrition, and human evolution. Our focus will be on mammals, while recognizing the cross-phylogenetic application of many of the basic principles (Hildebrand and Shepherd, 1997).

The primitives of smell are odor molecule determinants

In order to understand any part of nature, one must have both experimental data and a theory for interpreting the data and predicting new data. A comprehensive theory of olfaction to serve these ends must start with agreement on what are the fundamental sensory elements, the sensory primitives, that are processed by the brain pathways (Shepherd, 1991). A key advance in solving this problem came with the introduction of testing homologous chemical series, which revealed that cell responses in the olfactory bulb are sensitive to one-carbon differences between stimulating molecules (Imamura *et al.*, 1992).

These results indicated that odor primitives consist of the minimum of differences between individual odor molecule types, e.g. one carbon atom, a different functional group. This fit with a prediction that such differences would function like epitopes (determinants) in immune system molecules, except that they would consist not of multiple amino acid residues in a large protein molecule but rather single differences within a single molecule. The terms ‘odotope’, ‘olfactophore’ or the noncommital term ‘odor determinant’ were suggested for these within-molecule features.

Receptor cells encode odor molecule determinants

What is the mechanism by which a determinant on an odor molecule is transduced (mapped, encoded) into a differential response in the brain? The first step was by Sato *et al.* (1994), who found with Ca^{2+} imaging of blotted epithelium that receptor cells show the same type of systematic changes in their responses to homologous series as olfactory bulb cells.

By this time the key breakthrough by Buck and Axel (1991) had taken place showing the large gene family of putative odor receptors, followed by the finding by Chess *et al.* (1994) that a given receptor cell is likely to express only a single receptor gene. The interaction between an odor molecule and a given receptor was therefore the crucial step in olfactory transduction. It was predicted that, in analogy with the interactions of other G protein coupled receptors with small ligand molecules, this interaction would take place within a binding pocket within the plane of the surface membrane (Shepherd and Firestein, 1991). This prediction was tested computationally by molecular modeling methods (Shepherd, 1994; Singer and Shepherd, 1994; Pilpel and Lancet, 1999; Singer, 2000; Floriano *et al.*, 2000; Araneda *et al.*, 2000, 2004), and by several types of sequence analysis and data mining (Singer *et al.*, 1995, 1996; Glusman *et al.*, 2000; Man *et al.*, 2004), all of which have supported the hypothesis. The models give insight into results from expression systems that have begun to give experimental data on odor ligand-odor receptor interactions (Krautwurst *et al.*, 1998; Zhao *et al.*, 1998; Malnic *et al.*, 1999; Katada *et al.*, 2003).

These combined experimental and theoretical studies suppport the hypothesis that the fundamental bits of information in smell (functional group, carbon chain length, shape, etc.) are the determinants of the odor molecules, and that they are transduced from the sensory into the neural domain by differential activation of subsets of amino acid residues within the binding pockets of different odor receptors.

Olfactory glomeruli encode odor molecule determinants in odor maps (odor images)

According to the current evidence in the mammal, all the fibers from a given subset of sensory neurons converge onto two matching glomeruli in the olfactory bulb (Vassar *et al.*, 1994; Ressler *et al.*, 1994; Mombaerts *et al.*, 1996). This convergence means that the response of each glomerulus is an amplified version of the responses of the subset of individual receptor cells.

The precise mechanism for subset targeting of the glomeruli is a topic of intense current interest (see review by Mombaerts, 2004). However, evidence for the functional patterns of activation of the glomeruli in mammals has been accumulating since 1975 (Sharp *et al.*, 1975), by a variety of methods (summarized in Xu *et al.*, 2000). Tests of the same homologous series as in the receptor expression studies show that one carbon differences produce distinguishably different patterns. The 2DG and fMRI methods show that this involves shifts in global patterns within the glomerular layer (Leon and Johnson, 2003; Xu *et al.*, 2003); microscopic observations of the dorsal olfactory bulb show that these shifts involve selective activation of neighboring glomeruli (Mori *et al.*, 1999; Belluscio and Katz, 2001). Of particular interest are the patterns for odor mixtures; these appear to be more circumscribed than for single odors, suggesting a pharmacology of agonist and antagonist interactions at the receptor level (Shepherd and Firestein, 1991; Oka *et al.*, 2004).

The activity pattern may be termed an ‘odor map’ or ‘odor image’, representing the information in the ‘odor object’, just as a ‘visual image’ represents the information in a ‘visual object’. A key challenge for current studies is to understand how these images represent the world of odor molecules in two-dimensional neural space as the basis for smell perception, just as retinal images become the basis for visual perception. The images evolve during stimulation, adding a time dimension to the representation.

The odor image is processed by microcircuits to produce a context-enriched output to the olfactory cortex

In the visual system the visual image in the retina is subjected to processing by microcircuits that enhance the contrast in order to encode only the most salient features of the pattern. Within the olfactory bulb the odor image is also subjected to processing by microcircuits. First are intra and interglomerular operations that enhance salient features of the odor maps. There is a general consensus that periglomerular cells provide for a type of surround inhibition of output from neighboring glomeruli (Mori and Shepherd, 1994). This surround may be extensive and complex, and may include excitatory as well as inhibitory actions (Aungst *et al.*, 2003).

Best understood are the self and lateral inhibitory interactions between mitral/tufted cells and granule cells. Because of the long extensions of the M/T secondary dendrites, the output of a M/T cell to the olfactory cortex is reflective of the larger integrative context of its MRR in relation to surrounding glomerular units (Shepherd, 1991). The microcircuits also generate synchronized patterns of impulse firing, which aid in the encoding of the images. The membrane properties and functional organization of the microcircuits are subjects of intensive current investigation (cf. Schoppa and Westbrook, 2002).

The olfactory cortex functions as a content-addressable memory system

The basic circuit for olfactory cortex defines essential features of a canonical cortical circuit (Shepherd, 2004a). It was early proposed that the properties of the olfactory basic circuit enable it to function as a content addressable memory (CAM), which enables it to associate new odor stimuli with memory traces of previous stimuli to subserve odor recognition (Haberly, 1985; Wilson, 2004). The microcircuit organization containing long association fibers is similar to that of the cortical area involved in the processing of faces. It has been suggested that a profitable strategy for analysing the neural mechanisms involved in recognition of odor images should be to draw on the vast literature on neural mechanisms involved in recognition of visual images (Shepherd, 1991; Wilson, 2004).

Olfactory cortex is now one of the most studied areas in olfaction. A widespread overlapping type of connectivity has been shown, consistent with a CAM. However, a useful rule in studies of neural circuits is never to accept ramdonness as an organizing principle because it discourages doing experiments to test for specificity. Following this rule, recent studies indicate clustering of M/T cell projections (Zou *et al.*, 2001) and topographical ordering (Illig and Haberly, 2003).

Olfactory cortex contains areas that project directly or indirectly through the thalamus to the neocortex, for conscious perception of odors, and areas that project to limbic areas, for emotional and behavioral responses to odors such as feeding and mating. These areas are relatively neglected, and represent an exciting next frontier for olfactory research.

Perception of smell and flavor are neocortical higher cognitive functions

The neocortical areas for conscious smell include the insula and the medial and lateral orbitofrontal cortex. The perception of flavor is a multisensory modality, involving smell, taste and touch (and even vision); active flavor also involves motor control of the jaw, tongue and pharynx. Because the sensory integration does not occur until the neocortex, flavor is actually a higher cortical function. A recent fMRI study has shown how perception of flavor involves cortical areas beyond those involved in taste and smell alone (Small *et al.*, 2004). When you add language to the way we describe flavor, flavor becomes a uniquely human higher cortical function. It is therefore important to reconsider the traditional belief that human smell perception is poor compared with other mammals.

The importance of smell and flavor for human evolution and behavior

All of the main processing steps described above for the rodent are believed to occur also in the human. However, the recent findings that humans have only some 350 functional olfactory receptor genes compared with some 1100 in the mouse seem consistent with the traditional belief that human smell is less acute than in other animals. However, psychophysical studies show that the detection thresholds for some odors of homologous series are equivalent in humans and subhuman primates with those in rats and dogs (Laska *et al.*, 2000). This has suggested that an evaluation of animal olfactory performance in general and human performance in particular must take into account a wider range of possible factors (Shepherd, 2004b).

These factors would include the following. First is the extent of odor space for each species and the nature of the odor objects within it. Second is how external odors access the olfactory receptor cells. This includes passage of inhaled air in orthonasal olfaction through a complex air filter system in most mammals compared with more direct access in humans. Third is how retronasal odors released from ingested foods and liquids access and stimulate the olfactory receptor cells. This is a key to understanding the contribution of odors, together with taste, somatosensation and other sensory modalities, as well as motor activity, to the generation of flavor, which we postulate is crucial for humans.

Beyond these peripheral regions is the role of central brain processing of flavor. Despite appearing to decrease in size during vertebrate evolution, the olfactory areas in humans are in fact relatively large. As already mentioned, when humans perceive flavor they recruit additional cortical regions (Small *et al.*, 2004). Finally, language, a unique human capacity, is intimately involved in our discrimination and assessment of flavors. It thus may be postulated that flavor perception, in which smell plays a dominant role, is a higher cortical function that is most highly developed in humans. As any gourmet chef knows, cognitive effort is required in order to make flavor discriminations that are critical to human cuisines.

Summary

The traditional belief that we have a poor understanding of neural mechanisms underlying smell needs to be replaced by the emerging consensus on a series of essential steps. Olfactory receptors respond differentially and in an apparent systematic fashion to molecular features of the odor world. These features are encoded by activity patterns in the glomerular layer, which function as images representing the odor world. The odor images are processed successively by microcircuits, to provide the basis for the detection and discrimination of smell. The odor images, combined with taste, somatosensation, even vision and hearing, and together with motor manipulation, provide the basis for the perception of flavor, a higher cognitive function uniquely developed in humans with the help of language. The traditional belief that humans have a poor sense of smell needs to be replaced by the recognition that humans show excellent performance on many sensory tests. A full appreciation of human smell capabilities will require a deeper understanding of the many factors that combine with the repertoire of olfactory receptor genes to give humans what may be hypothesized to be a unique sense of flavor in the animal world.

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