Olfaction: Diverse Species, Conserved Principles

Review

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Olfaction is a vitally important sense for all animals. There are striking similarities between species in the organization of the olfactory pathway, from the nature of the odorant receptor proteins, to perireceptor processes, to the organization of the olfactory CNS, through odor-guided behavior and memory. These common features span a phylogenetically broad array of animals, implying that there is an optimal solution to the problem of detecting and discriminating odors.

Introduction

It is difficult for humans, being so visually oriented, to appreciate the fundamental importance of chemical sensitivity to life. The ability to detect and respond in an adaptive manner to chemical signals serves as the primary window to the sensory world for most species of animals. Chemical sensitivity is present even in the simplest of the extant life forms: bacteria, slime molds, and protozoans. Indeed, all living cells are "irritable" to chemicals, and this predisposition of cells to be perturbed by chemicals presumably led to the eventual evolution of specific receptor proteins to detect chemical signals and, ultimately, to specific chemosensory organs and systems such as the olfactory systems that are the focus of this review. The fundamental importance of olfaction to life and health was recognized by the award of the 2004 Nobel Prize in Physiology or Medicine to Drs. Linda Buck and Richard Axel for their pioneering discovery of olfactory receptor proteins and the understanding of olfactory organization that these groundbreaking findings allowed.

What is olfaction? In terrestrial vertebrates and insects we frequently think of olfaction as the chemosensory modality dedicated to detecting low concentrations of airborne, volatile chemical substances. Yet fish and aquatic crustaceans, while they do not encounter airborne, volatile odorants, possess sensory systems that are anatomically similar to the olfactory systems of land-based animals. "Odors" for these aquatic animals are sapid molecules in solution; olfaction therefore is not necessarily the detection of volatile molecules in air. Taste-receptor cells in catfish are as sensitive, and respond to the same molecular species, i.e., amino acids, as do their olfactory counterparts (Caprio, 1977); olfaction therefore is not necessarily the detection of chemical stimuli at low concentrations. What then is really unique about the chemosensory modality that we call olfaction? This fundamental question still awaits a clear functional answer. As research on other species has led to questions about the fundamental nature of olfaction, animal models allow us to perform experiments designed to answer those questions.

In this review we take a broad view of olfaction, trying to look through species-dependent differences in an attempt to reveal broad principles of olfactory organization. We conclude that notwithstanding mechanistic differences between species, the general principles of olfactory organization are shared by many animals. Citations are intended to be representative, not exhaustive. The reader is also referred to excellent earlier reviews (e.g., Hildebrand and Shepherd, 1997; Gelperin, 1999; Laurent, 2002).

Nature of Odorants

Deciphering odor signals presents a common challenge to all animals. Odor signals serve to communicate in a diverse array of informationally demanding behavioral contexts. Environmental odors help the animal locate desirable items (food, water, nesting sites, etc.) as well as dangers to avoid (fire, etc.). Odors emanating from other species of organisms, known collectively as allelochemics (Whittaker and Feeny, 1971), control prey localization, homing, symbiotic associations, territorial marking, predator deterrence and avoidance, metamorphosis and growth, and pollination, to name just a few examples. Odors of conspecific origin, known collectively as pheromones (Shorey, 1976), act far beyond their now well-known role as sex attractants. For example, recognition pheromones denote the identity of individuals, social status, social group, and place; aggregation pheromones mediate feeding, sex, and aggression; dispersion pheromones maintain individual spacing and minimize predation; and reproductive pheromones trigger courtship displays and postures. In addition to these "triggering" functions, pheromones also serve "priming" functions, in which the stimulus additionally or alternately initiates longer-term changes in the recipient animal rather than just eliciting immediate, overt responses (Vandenburg, 1983).

As well as signaling diverse and complex messages, odor signals themselves are often very complex. Many classes of molecules fall within the theoretical limits of molecular size and type for olfactory signal function. These limits expand further when one considers that for aquatic species odors can travel by bulk flow in aqueous media. The information content of the signal is enhanced by the fact that real-world odors are rarely, if ever, single compounds. Rather, they are complex mixtures of compounds, where related signals can contain many of the same components in different ratios. Single chemical compounds can elicit physiological and behavioral responses, but complete biological activity often requires stimulation with complex, multicomponent mixtures of chemical compounds. Even insect pheromones, once thought to be prototypical "silver bullet"

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Figure 1. Odor Plumes Are Highly Discontinuous in Space and Time

Photograph of an odor field in a flume flowing from right to left past the antennule of a lobster. The "odorant" is a fluorescent dye illuminated by a laser sheet that penetrates the water mass in the plane of the organ. Note the scale of the discontinuities relative to the size of the olfactory organ, the tuft of sensilla on the distal end of the antennule (arrow). Unpublished photograph courtesy of M. Koehl.

odorants, as single compounds can attract male moths to females, turn out to be mixtures. For example, cabbage looper moths fly upwind in response to major components of the natural pheromone blend, but at least six other components of the female's pheromone gland are required to evoke the full behavioral response in male moths (Bjostad et al., 1984). Indeed, it is often only subtle differences in the blend ratios of insect pheromones that keep sympatric species isolated.

Most animals experience odor signals intermittently (Dethier, 1987), adding to the challenge of deciphering the signal. Only microbes and the smallest of eukaryotes experience stimulus spread that is dominated by diffusion and thus increases or decreases continuously in concentration. All larger animals experience turbulent air or water flow, where local currents and eddies perturb stimulus clouds emanating from point sources, resulting in highly discontinuous odor plumes (Koehl et al., 2001). As a result, olfactory receptor organs are only intermittently exposed to the stimulus as the animal moves through the medium, or the medium moves over the animal (Figure 1). The specific parameters of the plume structure are medium dependent and presumably contribute to shaping the dynamic aspects of olfactory sensitivity in different animals.

The fact that all species share these challenging aspects of deciphering odorant signals may be the driving force behind the striking organizational similarities (described below) that are found between the olfactory systems of phylogenetically diverse species.

Olfactory Receptor Genes

All animals recognize the vast array of odorants they encounter using G protein-coupled receptors (GPCRs), seven-transmembrane domain proteins that activate G protein-based signaling cascades when activated by their ligands. In 1991 Linda Buck and Richard Axel discovered a large, diverse family of GPCRs expressed in the rat olfactory epithelium (Buck and Axel, 1991) and proposed that they function as odorant receptors (ORs). Their work, along with the availability of numerous completely sequenced genomes, opened the door for a flood of subsequent molecular and bioinformatic studies of olfactory receptors. Mammals, birds, fish, and amphibians have large numbers of olfactory receptor genes in the same family as Buck and Axel's originally identified ORs, while invertebrate species have similar, but independently expanded families of chemosensory GPCRs. Additional, independently expanded GPCR families appear to be responsible for pheromone detection in vertebrates.

Table 1. Number of Genes and Pseudogenes in Selected Odorant Receptor Gene Families

	Genes	Pseudo-genes	% ORs Intact	Reference
Human	388	414	48%	(1)
Chimpanzee	450	450	50%	(2)
Other great apes	n.d.	n.d.	70%	(3)
Old World monkeys	n.d.	n.d.	70%	(3)
New World monkeys (except howler)	n.d.	n.d.	82%	(3)
Howler monkey	n.d.	n.d.	69%	(3)
Lemur	n.d.	n.d.	81%	(3)
Mouse	1200	300	80%	(4)
Rat	1430	640	69%	(5)
Dog	1070	230	82%	(6)
Dolphin	n.d.	n.d.	very few	(7)
Chicken	78	476	14%	(8)
Frog (*X. tropicalis*)	410	478	46%	(8)
Pufferfish	44	54	43%	(8)
Zebrafish	98	35	74%	(8)
Fruit fly	62	1	98%	(9)
Mosquito	79	n.d.	n.d.	(10)
Nematode worm	1100*		n.d.	(11)

Most numbers are approximate and represent lower bound estimates of gene numbers, as genome assemblies are incomplete, and draft assemblies can contain sequencing errors that mean some intact genes appear as pseudogenes. Many gene families have been reported in several studies; for brevity, a single representative report is cited (in general, more recent reports are chosen as they are based on best available genomic data); apologies are due to other authors. n.d.: not determined/reported. * Note that the large chemosensory GPCR family in worms can be thought of as being responsible for the combined senses of smell and taste, separate senses in more complex animals but difficult to distinguish in simple organisms.

(1) Niimura and Nei, 2003; (2) Gilad et al., 2005; (3) Gilad et al., 2004; (4) Young et al., 2003; (5) Rat Genome Sequencing Project Consortium, 2004; (6) Quignon et al., 2003; (7) Freitag et al., 1998; (8) Niimura and Nei, 2005; (9) Robertson et al., 2003; (10) Hill et al., 2002; (11) Robertson, 2001.

Although similar gene families are responsible for odorant detection in multiple species, the OR families vary between species on a detailed level. This is not unexpected given that different sets of chemical signals are important to different species, and likely reflects evolutionary adaptation to new environmental niches, for example utilization of novel food sources. Humans have almost 400 functional olfactory receptors (Niimura and Nei, 2003), far fewer than the ~1200 (mice) or ~1430 (rats) found in rodent genomes (Rat Genome Sequencing Project Consortium, 2004; Young et al., 2003). Table 1 further demonstrates the great variability between species in OR family size. Olfactory receptors form one of the largest gene families known: in the

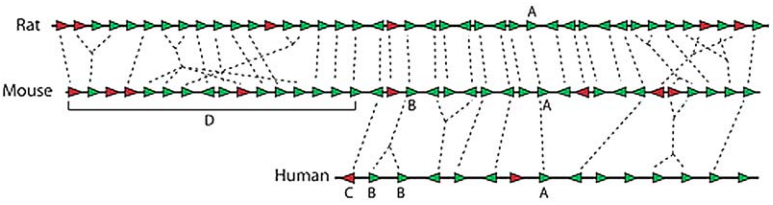

Figure 2. Chemosensory Receptor Gene Families Are Evolving Dynamically

Orthologous regions of rat chromosome 3p11-q11, mouse chromosome 2B and human chromosome 9q33 contain olfactory receptor gene clusters and are depicted horizontally (not to scale). Arrowheads represent intact genes (green) and pseudogenes (red) and point in the direction of transcription of the gene. Dotted lines join orthologous groups of genes that likely derive from the same gene in the most recent common ancestor. OR families have been shaped by several evolutionary processes, as exemplified in these clusters by genes marked with letters: (A) Some genes remain as one-to-one orthologs in all three genomes and may represent functional equivalents. (B) Local duplication events mean that a single gene in one species can have multiple equivalents in another species. Post-duplication sequence changes might allow the new copy to recognize novel odorants. (C) Some genes suffer inactivating mutations and become pseudogenes. (D) Deletions of one or more genes mean, for example, that some rodent receptors lack human equivalents.

extreme case of rats, they comprise about 6% of functional genes in the genome, underscoring the importance of olfaction to the species. It is worth noting that a subset (probably a small subset) of the genes in this family may have taken on nonolfactory functions, in addition to or instead of playing a role in olfaction. Good evidence exists that at least one human OR, hOR17-4, functions in the testis as well as the nose, responds to the chemical bourgeonal, and allows sperm to undergo chemotaxis toward bourgeonal sources (Spehr et al., 2003).

The large variation in OR gene family size between species provides a fascinating entry-point to study evolving genomes. Cross-species comparisons of chemosensory gene families show that many lineage-specific duplications have vastly increased gene numbers, while lineage-specific deletions and inactivating mutations can also reduce functional repertoire size (Robertson, 1998; Young et al., 2002) (e.g., Figure 2), a combination of processes known as birth-and-death evolution. The net result of frequent gene duplications and deletions during evolution is that as well as variability in OR family size, many individual OR genes either do not have a functional equivalent at all in other species, or have multiple equivalents even in closely related species, perhaps with subtle sequence and functional differences between copies. Such evolutionary plasticity demonstrates the remarkable capability of the genome for evolutionary innovation, but makes functional inferences about individual receptors difficult to draw across species.

Once a gene duplication has occurred, the resulting copies can follow several evolutionary paths. If the extra copy is functionally redundant, one copy may suffer an inactivating mutation and be lost from the functional repertoire. If a new gene duplicate acquires sequence changes that allow it to recognize novel, useful odorant ligands, there might be selective pressure to retain the new, changed sequence ("positive selection"). Conversely, other genes might be subject to "purifying selection," where changes in their sequence would eliminate useful ligand-recognition capabilities and would not be tolerated. Several studies have found evidence for weak positive selection acting on some olfactory and chemosensory receptors in mammals, fish, and nematodes (Ngai et al., 1993; Gilad et al., 2000; Hughes and Hughes, 1993; Thomas et al., 2005) although it is not known whether the putatively selected amino acid changes correlate with novel functional capabilities. An additional process, gene conversion, where similar sequences undergo nonreciprocal exchange, has been observed to act in OR clusters (Sharon et al., 1999). Gene conversion could combine existing sequence variants in novel ways, resulting in altered ligand binding capabilities.

The genomes of many species contain a large number of olfactory receptor pseudogenes, in addition to functional ORs. Many of these pseudogenes were once functional genes that have suffered inactivating mutations. The human genome, for example, contains just under 400 apparently functional olfactory receptor genes and a similar number of OR pseudogenes (Glusman et al., 2001). The proportion of intact OR genes and pseudogenes varies greatly between species (see Table 1) and may reflect how much each species has relied on olfaction for survival and reproduction during recent evolutionary time. A reduced need for sophisticated olfactory abilities may result in a relaxation of the selective pressure that normally eliminates inactivating mutations from the gene pool, and thus an accumulation of pseudogenes. Some authors have noted that the decline of the olfactory receptor gene family in some primates coincided with the acquisition of trichromatic vision, and suggest that better visual abilities made olfaction partially redundant (Gilad et al., 2004). However, given the breadth of tuning of many mammalian receptors, and the fact that most subfamilies of ORs (as defined by the mouse repertoire) do contain functional human members, it has been suggested that humans may be capable of detecting almost as many odorants as mice despite having a third as many receptors, but may lack the subtle variants needed to discriminate similar odors (Zhang and Firestein, 2002). The wealth of bioinformatic data now available on OR family size and composition in various species has unfortunately far outstripped our ability to correlate these data with functional differences: it is technically difficult to compare olfactory detection and discrimination abilities fairly between species. In addition, some differences in olfactory ability are undoubtedly due to factors other than variation in the OR gene family, such as differences in surface area of the olfactory epithelium or processing of signals in the brain.

Olfactory Receptor Expression and Protein Function

A remarkable feature of the mammalian olfactory system is that each olfactory neuron in the epithelium appears to transcribe only one allele of only one of the many hundreds of functional receptor genes (Chess et al., 1994;

Malnic et al., 1999; Serizawa et al., 2000). This organizing principle has been demonstrated (but perhaps not proven beyond doubt: Mombaerts, 2004) by a number of elegant mouse experiments and is assumed to be true in other mammals, although evidence would be difficult to obtain in less experimentally tractable species. The "singular expression" regime ensures that responses from different receptors are segregated in different responding cells, thus allowing discrimination between different odorants. Axons of neurons expressing the same olfactory receptor converge at a limited number of locations in the olfactory bulb in the brain, integrating signals from functionally identical neurons. These organizing principles are also observed in the fruit fly, *Drosophila melanogaster*, with some difference in the details: as well as one (or occasionally two) receptors chosen from among the gene family, most fly neurons express a second, broadly transcribed receptor, OR83b (Larsson et al., 2004). Such evolutionary conservation demonstrates the importance of these organizing principles. In flies it seems that upstream transcriptional regulatory sequences are sufficient to specify in which neurons receptors are expressed (e.g., Goldman et al., 2005), whereas in mammals a given receptor seems to be expressed in a random subset of neurons in one of four zones of the olfactory epithelium (Ressler et al., 1993). Nematode worms (*Caenorhabditis elegans*) do not follow the same expression regime: each of the worm's 32 chemosensory neurons expresses multiple genes of the ~1100-member family (Troemel et al., 1995). Each worm neuron is thus likely to recognize a much broader range of odorants than a typical mammalian neuron. Given its similarly sized receptor gene repertoire, worms may be able to detect a similar number of odorants as mammals, despite the limited number of chemosensory cells available.

Animal studies have been successful in some cases in determining which odorant ligand a given receptor recognizes. The ligands for almost all of the fruit fly odorant receptors have been determined by knocking out one receptor gene expressed in a recognizable neuron, expressing in its place a receptor gene to be queried, and then measuring that neuron's response to various odorants (Hallem et al., 2004). The broad principles emerging from this experiment are that receptors vary widely in their breadth of tuning (some receptors recognize only one of the odorants tested and others respond to multiple odorants); odorants vary in the number of neurons they activate; and individual receptors can mediate both excitatory and inhibitory responses, depending on the odorant. One receptor that seems not to follow these principles is the broadly expressed fly receptor, OR83b, that, when expressed alone, does not respond to any odorants tested (Elmore et al., 2003). Instead, it appears to heterodimerize with other fly receptors and enhance their ligand responsiveness (Neuhaus et al., 2005). Other approaches have also been used to determine receptor-ligand pairings, for example coupling expression in heterologous cell lines with functional assays (Krautwurst et al., 1998) and measuring ligand responsiveness of cells isolated from the olfactory epithelium before using RT-PCR to determine which receptor genes they express (Malnic et al., 1999). Similar principles emerge from these studies. However, due to the dynamic evolution of this gene family described above, it is difficult to identify single clear orthologous equivalents of these receptors in anything but the most closely related species. Along with the fact that a small number of amino acid changes in the receptor can result in a change in ligand binding (Katada et al., 2005), it may be difficult to apply what has been learned about the ligand partners of individual receptors to other genes or species.

Comparative studies on individuals from the same species may also be informative. The ability to detect some odorants can vary between individuals, as can the threshold with which some odorants can be detected. Examples include human variation in the ability to detect urinary asparagus metabolites (Lison et al., 1980) and variation between mouse strains in the ability to detect isovaleric acid (Griff and Reed, 1995). Identification of the underlying genetic differences causing such variation may in the future elucidate additional receptor-ligand pairings. However, defining single genes responsible for functional variation will be difficult in the face of the fact that OR genes tend to be arranged in large clusters in the genome and because neighboring ORs tend to be closely related in sequence. A number of obvious candidate polymorphisms have been identified, but none has yet been tied to a phenotype. For example, at least 26 human ORs and 10 nematode worm chemosensory receptors harbor polymorphisms where one allele appears intact and the other a pseudogene (Menashe et al., 2003; Stewart et al., 2005). Copy number polymorphisms of ORs have also been described (Trask et al., 1998), with unknown phenotypic consequences.

Olfactory Receptor Cell Morphology and Turnover

The general morphology of the cells containing the olfactory receptor proteins is strikingly similar among species and systems. Olfactory receptor cells are always primary bipolar neurons in which the dendritic membrane terminates in an array of filamentous processes assumed to increase the surface area for stimulus capture. The axon typically extends without synapsing to the central nervous system. The dendritic processes are primarily of ciliary origin in most animals. Comparing the morphology of olfactory receptor cells in vertebrates and insects, and even "olfactory-like" receptor cells in the nematode amphid organ demonstrates this point (Figure 3). Although overall morphology is conserved, the fine details of olfactory receptor cell morphology can be adaptive, i.e., they can vary in a habitat-dependent rather than a species-dependent manner. Olfactory receptor cells in crustaceans that live in terrestrial environments, such as the giant robber crab show morphology that is more insect-like than it is similar to that of marine crustaceans (Stensmyr et al., 2005).

Presumably in response to the common and necessary stress of environmental exposure, olfactory receptor cells have evolved the ability to turn over and replace themselves throughout the life of the animal. Olfactory receptor cells in all vertebrates are characterized by cycles of birth, maturation, and death (Graziadei and Monti-Graziadei, 1978). This turnover is remarkable given that the olfactory receptor cells are neurons, cells that are not generally considered to undergo neurogenesis in adults. The same pulse-labeling technique used

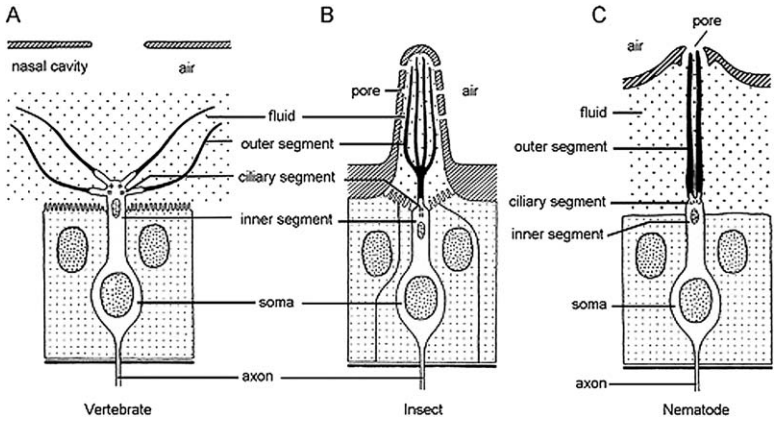

Figure 3. Comparison of Primary Chemoreceptor Neurons in Vertebrates, Insects, and Nematodes

Diagrams compare primary chemoreceptor neurons in the vertebrate olfactory epithelium (A), an insect olfactory sensillum (B) and the nematode amphid organ (C), drawn to appear the same size. Note that all are small, bipolar neurons that terminate in a ciliary arbor and send their axon without branching to the CNS. From Ache, 1991.

to document turnover in vertebrates shows that olfactory receptor neurons in the anterior tentacles (olfactory organs) of snails also turn over (Chase and Rieling, 1986), as do olfactory receptor cells (aesthetascs) in the crustacean olfactory organ (the antennule). In the lobster olfactory organ, receptor cells move from birth, to maturation, to senescence distally along the length of the antennule as the animal grows and adds new segments to the organ (Steullet et al., 2000). Functional constancy in three such phylogenetically diverse groups of animals argues that turnover is a common adaptive property of olfactory receptor cells.

Olfactory Signal Transduction

In addition to expressing GPCRs, olfactory receptor cells also transduce the odor signal by coupling it to one or more downstream effector molecules. As noted earlier, GPCRs couple to downstream effectors through heteromeric GTP binding proteins and intracellular second messengers. Recent evidence suggests that the elementary response is extremely small, i.e., that the ligand-bound receptor has a low probability of activating even one G protein molecule due to the relatively short dwell time of the odorant on the receptor (Bhandawat et al., 2005). Olfactory second messengers generally target ion channels that, when activated, alter the cell's membrane potential and generate a graded, voltage dependent response that gives rise to all-or-none electrical signals (action potentials or "spikes"). The action potentials propagate to the central nervous system (CNS) with a frequency that is proportional to the magnitude of the graded change in membrane potential.

Two main intracellular signaling pathways are used in olfactory neurons, utilizing cyclic nucleotides and phosphoinositide-derived signals. These pathways seem to operate in a diverse range of species, with no clear evolutionary trend in the use of one signaling cascade over the other. Cyclic nucleotide signaling is best understood in vertebrate olfactory receptor neurons (Figure 4A). The target of cyclic nucleotide signaling in these cells is the olfactory cyclic nucleotide-gated ion channel (Zufall et al., 1994), activation of which allows calcium entry into the cell that secondarily activates a calcium-activated chloride current in a two-step activation cascade. The latter current generates much of the excitatory receptor potential (Reisert et al., 2003). Cyclic nucleotide signaling also appears to operate in olfactory transduction in nematodes (*Caenorhabditis elegans*, Komatsu et al., 1999), and arthropods (lobster, Boekhoff et al., 1994). The role of phosphoinositide signaling in chemosensory transduction is less well established than that of cyclic nucleotide signaling, and is perhaps best understood in mediating excitation of crustacean olfactory receptor cells (Figure 4B). There, the target of phosphoinositide signaling is a calcium-sensitive presumptive lobster homolog of the TRP family of ion channels (Bobkov and Ache, 2005). Odorants activate both the PLC- and the PI3K-mediated arms of this signaling cascade, allowing the channel to be targeted directly by 3-phosphoinositides, and/or indirectly via gating of extracellular calcium from an associated plasma membrane $InsP_3$ receptor (Munger et al., 2000). Phosphoinositide signaling has also been implicated at least indirectly in olfactory transduction in other, phylogenetically diverse species, including nematodes (Colbert et al., 1997), insects (Boekhoff et al., 1990), fish (Bruch and Teeter, 1989), and mammals (Spehr et al., 2002).

It remains to be determined whether individual olfactory receptor cells use both cyclic nucleotide and phosphoinositide signaling cascades since there is no a priori need for the receptor cell to utilize multiple signaling cascades to encode the magnitude of receptor binding. When coupled to different receptors or to different sites on the same receptor in a ligand-specific manner, however, multiple signaling cascades could allow the cell to integrate responses to complex odorants, with potentially important consequences for odor coding. Evidence that olfactory receptor cells utilize both signaling pathways in arthropods (lobster, Boekhoff et al., 1994) and mammals (rat, Spehr et al., 2002) suggests that signaling through paired intracellular signaling pathways may serve a fundamental role in olfactory transduction, although one that still needs to be investigated.

Perireceptor Processes in Olfaction

Activation and adaptation of olfactory receptor cells can be influenced by mechanical and biochemical events in the vicinity of the olfactory receptor cell. These so-called perireceptor processes therefore need to be considered essential components of olfaction (Carr et al., 1990a). While the relative importance of perireceptor processes in olfaction is still not fully understood, these processes

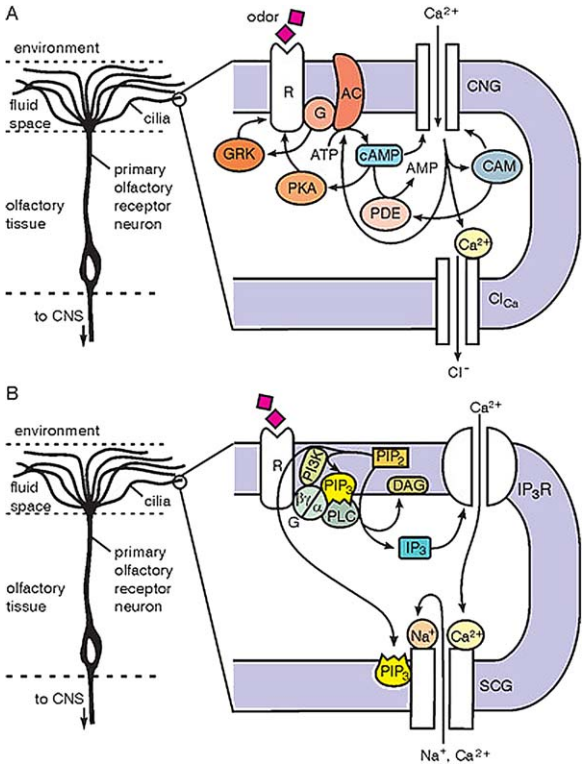

Figure 4. Two Intracellular Signaling Cascades Implicated in Chemosensory Transduction

(A) Diagram of cyclic nucleotide signaling in the transduction compartment (olfactory cilia) of vertebrate olfactory receptor neurons. Odorant molecules bind to a receptor protein (R) coupled to an olfactory specific G_s-protein (G) and activate a type III adenylyl cyclase (AC), increasing intracellular cAMP levels. cAMP targets an olfactory-specific cyclic-nucleotide gated ion channel (CNG), a nonselective cation channel that increases intracellular calcium and secondarily activates a calcium-activated chloride channel thought to carry the majority of the transduction current. Other, regulatory pathways are also shown. (B) Diagram of phosphoinositide signaling as currently understood in the transduction compartment (outer dendrite) of lobster olfactory receptor neurons. Odorant molecules bind to a receptor protein (R) coupled to a G_q-protein and activate both phospholipase-C (PLC) and phosphoinositide 3-OH kinase (PI3K) to generate diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), and phosphatidylinositol 3,4,5-trisphosphate (PIP_3), respectively, from phosphatidylinositol 4,5-bisphosphate (PIP_2). We assume, therefore, that PIP_3 in concert with "release" of extracellular calcium from a plasma membrane IP_3 receptor (IP_3R), also known to be expressed in the transduction compartment, target a lobster homolog of a transient receptor potential channel, a nonselective cation channel that is modulated by both sodium and calcium (SGC) and that has been shown to carry the majority of the transduction current. Details of these pathways vary in other receptor cells and other species.

appear to be conserved in diverse species, suggesting that they indeed are fundamentally important to odor recognition.

In addition to the odor signal itself being intermittent, active processes often gate access of the odor stimulus to the receptor cells. This phenomenon is epitomized by sniffing in mammals, a process that has an integral effect on odor processing in the olfactory bulb and lateral hypothalamus (Macrides and Chorover, 1972) and that optimizes the perception of odor intensity in humans (Laing, 1985). Sniffing, however, is only one of a diverse range of active processes that gate access of stimulus to olfactory organs. Snakes flick their tongue, periodically bringing airborne volatiles to the vomeronasal organ (Kubie and Halpern, 1975). Salamanders actively ventilate their olfactory and vomeronasal receptor cavities at 1–2 Hz (Kauer and Shepherd, 1977). Octopus (Chase and Wells, 1986) and cyclosmate fish like flounders (Døving and Thommesen, 1977) actively pump water through their siphons or nasal chamber, respectively. Moths fan their wings to enhance air penetration through their olfactory sensilla (Loudon and Koehl, 2000) and decapod crustaceans "flick" their olfactory organ (Schmitt and Ache, 1979). The ubiquity of active gating suggests that time-locked intermittency is somehow fundamental to odor recognition and discrimination, and that the dynamic properties of downstream elements in the transduction cascade as well as the kinetics of synaptic interactions in the CNS may be tuned to such intermittency.

Olfactory cilia do not project into the environment directly but instead project into a fluid-filled compartment that in turn contacts the environment. This holds even for aquatic animals where desiccation and/or osmotic challenge would not necessarily be a problem. The composition of the fluid bathing the receptor cells is actively regulated and can contain enzymes, buffers, and other molecules capable of interacting with and potentially modifying the chemical signal (Pelosi, 1996). Best known of these are so-called odorant binding proteins (OBPs), small soluble dimeric proteins that bind hydrophilic odorants. OBPs have been characterized in human olfactory mucus (Briand et al., 2002), as well as in most terrestrial animals, including elephants (Lazar et al., 2002), sheep, pigs, cows, rats, frogs, insects (Pelosi and Maida, 1990), and snails (Chase and Tolloczko, 1985). The common molecular properties and the occurrence of OBPs in such a phylogenetically diverse range of terrestrial animals suggest they played an important role in the terrestrialization of olfaction. OBPs in moths and rats are not homologous, suggesting the common molecular properties of at least these OBPs evolved convergently (Pevsner et al., 1988). Given the homology of mammalian OBPs to the lipocalin superfamily of proteins (Tegoni et al., 2000), it has often been assumed that OBPs serve to bind and transport hydrophobic ligands through the aqueous perireceptor environment. However, OBPs have also been proposed to serve other functions, including serving as molecular filters to specify and perhaps facilitate stimulus access to receptors (Vogt et al., 1990). Another family of proteins, pheromone binding proteins (PBPs), may help deliver volatile pheromone compounds. Some OBPs and PBPs are similar in sequence, suggesting that members of the OBP family may serve as molecular filters for pheromones rather than for general odors (Pelosi, 2001). However, the function of PBPs/OBPs remains unclear.

The perireceptor fluid of phylogenetically diverse species also contains degradative enzymes that could deactivate the odor stimulus (Carr et al., 1990b). An esterase (Vogt et al., 1985) and an aldehyde oxidase (Rybczynski et al., 1989) that rapidly degrade pheromones have been identified in the perireceptor fluid or 'lymph' of insects. Ectonucleotidases in the lobster olfactory organ progressively dephosphorylate adenosine nucleotides (feeding cues) into nonstimulatory adenosine (Trapido-Rosenthal

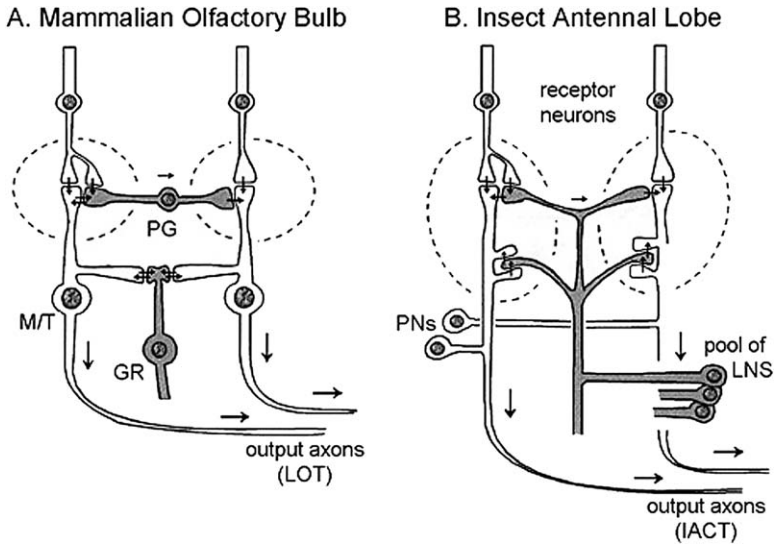

Figure 5. Comparison of the First Olfactory Relay in Insects and Mammals

(A) Diagram of the mammalian olfactory bulb showing receptor cells contacting mitral/tufted (M/T) and periglomerular (PG) cells in glomerularly-organized neuropil (dashed circles), creating parallel output pathways in the lateral olfactory tract (LOT), transected by two levels of lateral inhibitory connections, one formed by the PG cells and the other by granular (GR) cells. (B) Diagram of the insect antennal lobe drawn in the same format as A showing essentially the same overall organization of the projection neurons (PNs) and various types of local interneurons (LNs). Adapted from Christensen and White, 2000.

et al., 1987). The mammalian olfactory epithelium contains many catabolic enzymes that could degrade various classes of odorants, including an olfactory-specific homolog of uridine diphosphate-glucuronosyl transferase (Dahl, 1988) and several isoforms of cytochrome P450 enzymes. It is still unclear whether such degradative enzymes act rapidly to terminate the signal and therefore alter its dynamic properties, or whether they serve to maintain the perireceptor space over longer time periods by minimizing background stimuli and/or removing potentially toxic environmental compounds.

The Olfactory Neuronal Pathway

While in a few animals, most notably molluscs, the olfactory organ is associated with a peripheral nerve net or plexus that allows for some degree of integration in the afferent signal, the primary olfactory afferents in most animals project without synapsing to the CNS. The target of the primary afferents, the first synaptic relay, has a strikingly conserved organizational plan, even in molluscs (Chase and Tolloczko, 1986). The analogy of the first olfactory relay in vertebrates, the olfactory bulb, and the equivalent structure in arthropods, the antennal lobe in insects and the olfactory lobe in crustaceans, was noted by early neuroanatomists (Bellonci, 1883). To see the organizational similarity emerge, however, one has to "look through" the characteristic differences in the organization of vertebrate and invertebrate neuropil (Christensen and White, 2000). The mammalian olfactory bulb is laminarly-organized with neurons integral to the neuropil, the synaptic-containing region of the bulb (Figure 5A), while in insects, the antennal lobe lacks laminar organization, and its neurons are peripheral to the neuropil, with synapses confined to the glomeruli (Figure 5B). These differences notwithstanding, the primary olfactory afferents in both mammals and arthropods converge into glomerularly-organized neuropil where they branch profusely and terminate on both projection neurons and local interneurons, with complex, serial reciprocal synapses (Tolbert and Hildebrand, 1981; Pinching and Powell, 1971). The projection neurons take the output of one or a few (depending on the species) glomeruli directly to the next synaptic level, the olfactory cortex in mammals and the lateral protocerebrum and the corpora pedunculata in arthropods. Local interneurons create at least two levels of lateral connectivity across the afferent fiber-projection neuron throughput pathway. While most of the olfactory glomeruli tend to be morphologically uniform the modified glomerular complex in mammals (Teicher et al., 1980) and the macroglomerular complex in some insects (Matsumoto and Hildebrand, 1981) are greatly enlarged and process input from pheromone receptors.

This rather striking anatomical conservation suggests that the first olfactory relay plays a fundamentally important functional role in odor signal detection. We are only just beginning to understand odor signal processing, although new molecular and imaging approaches to studying the CNS are poised to increase rapidly our knowledge of the central neural substrates for molecular recognition (Zou et al., 2001). Molecular studies have revealed that in mammals (Mombaerts et al., 1996), fish (Dynes and Ngai, 1998), and insects (Vosshall et al., 2000; Wang et al., 2003), spatially distributed receptor cells in the periphery that express the same olfactory receptor protein converge on one, or a small number of glomeruli on each side of the brain, suggesting that massive convergence of functionally similar input is fundamental to how odor information is processed at the first synaptic relay and therefore has been conserved in evolution.

Spatial patterning of odorant-evoked glomerular activity appears in animals as diverse as rodents (Rubin and Katz, 1999; Belluscio and Katz, 2001), zebrafish (Friedrich and Korsching, 1997; 1998) and insects (Joerges et al., 1997; Vickers et al., 1998), suggesting constancy in the overall strategy used to process odor information at this level of the olfactory pathway. Single odorants in all three groups of animals elicit complex spatial activity patterns in which most glomeruli respond to multiple stimuli and all stimuli elicit unique spatial patterns of activity across the population of glomeruli. This suggests that, as with the receptors themselves, detailed information about the molecular structure of the

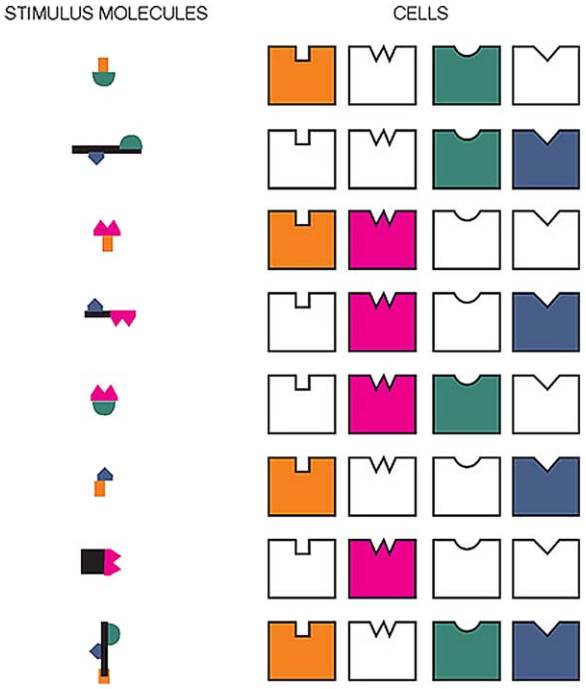

Figure 6. Combinatorial Coding

Diagram of hypothetical stimulatory molecules with some shared as well as some different molecular features (left) and the different patterns of neural activity each molecule would elicit across a hypothetical population of four receptor cells each expressing one receptor protein tuned to a particular molecular feature (right). The cells that are activated by each molecule are colored. Note that many molecules activate multiple receptors, and each molecule generates a unique pattern of activity across the population of receptor cells. Adapted from Malnic et al., 1999.

odorant is combinatorially encoded in the pattern of activity across neural elements (glomeruli). Further, in rodents (Uchida et al., 2000; Meister and Bonhoeffer, 2001), fish (Friedrich and Korsching, 1997) and insects (Rodrigues, 1988; Sachse et al., 1999), molecular features of the odorant molecules (e.g., carbon chain length) appear to map across the glomerular field to form what is known as a chemotopic map. While the degree of chemotopy is relatively coarse, and there is no evidence that the map is used for odorant discrimination per se, the presence of even coarse chemotopy in such diverse animals suggests that at least the neural connections are laid down according to similar developmental rules.

It remains to be determined whether the cellular mechanisms underlying the apparent constancy of the overall strategy have also been conserved, but at least one mechanism of intraglomerular processing, presynaptic afferent inhibition (PAI), is found in multiple species. The terminals of the primary afferent fibers are contacted by inhibitory local interneurons, the anatomical substrate for PAI, in animals as diverse as mammals (Hayar et al., 2004) and lobsters (Wachowiak et al., 1997). Interestingly, PAI in lobsters, turtles, and rodents is mediated by paired neurotransmitters (Wachowiak et al., 2002) although the nature of the transmitters and the cellular mechanism through which they act differ between species. PAI in lobsters results from decreased membrane potential mediated by ionotropic GABA and histamine receptors (Wachowiak et al., 1997), while in turtles (Wachowiak and Cohen, 1999) and mice (Wachowiak et al., 2005) it results from suppression of Ca^{2+} influx into the presynaptic terminal mediated by metabotropic GABA and dopamine receptors.

The odor signature is not necessarily static and may include information shaped by dynamic processes that we are only beginning to understand. Stimulus-specific patterns of activity in the output elements of the first olfactory relay (mitral/tufted cells in vertebrates and projection neurons in arthropods) change over hundreds of msec, i.e., during the course of odor stimulation. These changes have been observed in the output elements of insects (Laurent et al., 2001), salamanders (Cinelli et al., 1995), turtles (Lam et al., 2000), and zebrafish (Friedrich and Laurent, 2001). This conservation suggests that odor coding is achieved not only by instantaneous discharge patterns but also by the sequence of change in these patterns over time. The nature of these so-called "slow" temporal changes, at least in zebrafish (Friedrich and Laurent, 2001), is to decorrelate or 'sharpen' initially similar activity patterns, possibly making them easier to discriminate by reducing the degree of overlap between activity patterns evoked by closely related stimuli.

Most animals studied also show fast, coherent network oscillations or oscillatory field potentials at the first synaptic relay. These oscillations were seen in some of the earliest studies of the mammalian olfactory bulb (Adrian, 1942) and have since been observed in other vertebrates (Delaney and Hall, 1995; Gray, 1994), as well as in the analogous structures in molluscs (Tank et al., 1994), crustaceans (Sandeman and Sandeman, 1998), and insects (Laurent, 1997), providing further evidence for constancy in the overall strategy used to process odor information at the first olfactory relay. Whether these local circuit oscillations or the underlying coherent neural activity play a fundamental role in odor discrimination is still being resolved. Evidence from animals as diverse as molluscs (Teyke and Gelperin, 1999) and insects (Stopfer et al., 1997; Heinbockel et al., 1998) suggests that one possible role may be to contribute to the fine temporal discrimination required to distinguish molecularly similar odorants.

Odor representations in the first olfactory relay also change over time periods of minutes in association with repetitive sampling of the odor environment, as would occur, for example, with sniffing. The output of projection neurons in the locust antennal lobe decreases markedly and synchronizes across the population of these cells on repetitive stimulation of the antenna with odor (Stopfer and Laurent, 1999). Once established, the effect endures for several minutes. The effect is odor specific in that it generalizes only to chemically related odorants, and appears to reflect some sort of non-associative, short term memory in the underlying circuitry. Such fast odor learning improves the reliability of odor responses in a simulated locust antennal lobe network, suggesting that it might serve to improve the signal-to-noise ratio of the odor signal with repetitive sampling (Bazhenov et al., 2005). Interestingly, similar changes in response to repetitive stimulation also occur in the output of the vertebrate olfactory bulb (zebrafish, Friedrich and Stopfer, 2001), suggesting that this aspect of olfactory information processing, too, generalizes across species.

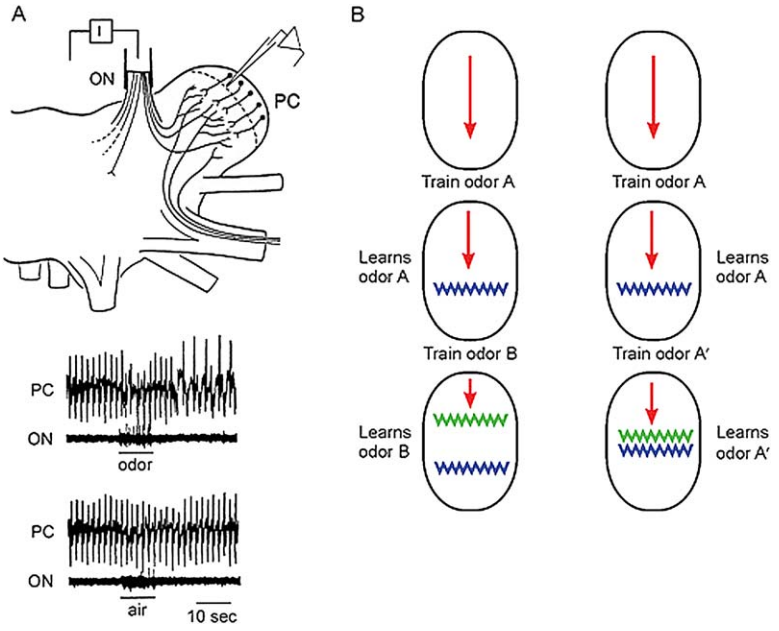

Figure 7. Dynamical Structure of Neural Activity in the First Olfactory Relay of the Slug Is Implicated in Odor Discrimination and Memory

(A) Top: Diagram of the cerebral ganglion of a slug showing a recording electrode in the protocerebral lobe (PC) and a stimulating electrode on the olfactory nerve (ON, shown here cut for electrical stimulation but normally intact). Bottom: recordings of neural oscillations from the preparation diagrammed in (A) showing responses to potato odor (upper pair of traces) versus clean air (lower pair of traces). Note the odor-induced changes in the waveform and frequency of the PC lobe oscillations. (B) Diagram (repeated) of the surface of the PC lobe showing the direction of odor-evoked waves of neural activity that spread across the lobe (red arrows) and bands of greatest activity (zigzag lines). Left column: Training animals to two dissimilar odorants (A and B) leads to two spatially separated bands of activity in the lobe. Right column: Training to two similar odorants (A and A′) leads to the formation of two closely spaced bands of activity. (A) Adapted from Gelperin and Tank, 1990. (B) Adapted from Gelperin, 1999.

Odor Quality Coding

Knowing how the molecular identity of an odor is coded by the nervous system is fundamental to understanding olfaction. There is strong consensus that odorants are coded in a combinatorial manner (Figure 6). This long-standing idea received strong support from recent evidence that individual mammalian olfactory receptor cells expressing a single, identified receptor protein can be activated by multiple different odorants, and that individual odorants activate multiple receptor cells expressing different receptor proteins (Malnic et al., 1999). Similar findings for the olfactory receptors of insects (Hallem et al., 2004), and fish (Luu et al., 2004) suggest that the use of combinatorial coding has been conserved in the evolution of olfaction. It has been proposed that combinatorial coding works in concert with its counterpart strategy, coding by "labeled lines" (neurons dedicated to a particular odorant) to encode complex odor mixtures in the lobster olfactory pathway (Derby, 2000). Labeled line coding is "expensive" in requiring dedicated neural space, but could serve to detect stimuli of especially strong adaptive value, e.g., detection of key pheromone components. If so, it would not be unreasonable to expect labeled lines to operate within an overall combinatorial coding strategy for odors.

Olfactory receptor cells can have two (bipolar) modes of signaling, excitation and inhibition, adding another degree of freedom to the combinatorial code. Odors inhibit as well as excite olfactory receptor cells in phylogenetically diverse species including molluscs (squid, Lucero et al., 1992), arthropods (insects, de Bruyne et al., 2001; lobsters, McClintock and Ache, 1989), fish (Kang and Caprio, 1995), amphibians (frogs, Sanhueza et al., 2000), and mammals (rats, Duchamp-Viret et al., 1999). The cellular mechanism(s) that mediate opponent input are still being explored, but as noted earlier this phenomenon can be explained in at least some species by ligand-directed activation of opposing intracellular signaling pathways.

Olfactory-Mediated Behavior and Odor Memory

Because all animals must adapt to changing environments, it is not surprising that plasticity is a hallmark of odor-mediated behavior, ranging from simple alterations in levels of responsiveness such as sensitization and habituation, to imprinting, to more complex forms of associative leaning. Even complex associative behavior such as food aversion transcends broad species differences. Food aversion is the long-term retention of experience gained from a single association between ingestion and subsequent illness, and has a strong olfactory component (Capaldi et al., 2004). Food aversion has been observed in coyotes and rats in the context of bait shyness (Garcia et al., 1974), in blue jays who learn not to consume toxic monarch butterflies (Brower and Glazier, 1975), and in terrestrial slugs where a single meal of carrot followed by exposure to an irritant causes the slug to reduce its preference for carrots (Gelperin, 1975). Even in slugs, food aversion displays all the classical characteristics of aversion in mammals and jays, including rapid onset, the need for only a single pairing of the conditioned (CS) and unconditioned (US) stimulus, a long CS-US interval, persistence without reinforcement, association restricted to a specific CS, and enhancement through co-association with odor cues.

Odor-dependent associative learning appears to involve changes in the first olfactory relay in diverse species, as observed for simpler, non-associative odor memory in locusts and zebrafish. Early odor experience in rat pups, for example, dramatically enhances later behavioral responses to the familiar odor and is accompanied by learning-dependent physiological and morphological changes in the olfactory bulb (Coopersmith and Leon, 1984). Molluscs can also be behaviorally

conditioned to odors in a similar manner (*Limax,* Gelperin, 1986) providing a useful experimental model to study such conditioning. The brain can be conditioned after it has been isolated from the slug using the same paradigm used to condition the intact animal (Teyke and Gelperin, 1999), allowing direct access to the altered neural circuitry for detailed biochemical and biophysical analyses of the causal mechanisms of odor-guided behavior, including odor memory.

In slugs, odor memory has been associated with fast, coherent network oscillations (oscillatory field potentials) that spread as propagating waves of hyperpolarization and depolarization across the first olfactory relay, the protocerebral lobe (Kimura et al., 1998a). These waves have been proposed to parcel out the neural space to optimize storage of odor memory representations during odor learning (Gelperin, 1999). The oscillatory activity can be modified by associative odor training: injection of an activity-dependent marker into the protocerebral lobe following associative odor training labels a specific band of cells or 'hot spot' as the waves move across the surface of the lobe for each odor learned (Kimura et al., 1998b). The labeling is specific to odor learning because it does not occur after exposure to odor alone nor to the aversive stimulus alone (Figure 7). Such spatial segregation of learned odor representations is strikingly reminiscent of glomerular sites of odor memory modification in the mammalian olfactory bulb (Johnson and Leon, 1996) and the honeybee antennal lobe (Faber et al., 1999), possibly leading to interesting testable general predictions as to how oscillatory dynamics may influence the acquisition and storage of odor memory (Ermentrout et al., 2004).

Overview

There are striking similarities between species in the organization of the olfactory pathway from the nature of the odorant receptor proteins, to perireceptor processes, to the organization of the olfactory CNS, through odor-guided behavior and memory. These common organizational features span a phylogenetically broad array of animals and together serve to define olfaction. Such conservation also implies that there is an optimal solution to the problem of detecting and discriminating odors. Either this solution evolved relatively early and was subsequently retained in evolution or, more likely, animals convergently evolved the same or similar solutions to the problem of odor detection and recognition. Either way, the biological strategy for odor recognition should be worth emulating in applications like the design of biosensors to detect chemicals of importance in medicine, biosafety and biodefense. Just like for the olfactory system, biosensors need to detect multicomponent chemical signatures in the complex, dynamic backgrounds that form real-world odor environments. This is just one example of how we can learn and benefit from a broad, phylogenetic approach to understanding the sense of smell.

The phylogenetic conservation seen in the organization of the olfactory system also supports the continued use of animal models to investigate the sense of smell in humans. Insight into the principles and mechanisms of olfaction has come from a diverse array of animal models, each with its own advantages and disadvantages for study. As the large number of genomes presently being sequenced and those likely to be sequenced in the next few years (Greenspan, 2005) become available, the potential of diverse animal models to contribute to our understanding of the sense of smell will inevitably increase. As noted by Dethier (1981), animal studies can tell us not only about those species but also about ourselves, provided we are careful to avoid the dual pitfalls of anthropomorphism and zoomorphism, and that even knowing the extent to which chemical sensing in animals differs from that in humans cannot help but reveal something about ourselves.

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