CHAPTER 1

Olfaction and the Chemical Senses

1.1 Synopsis

This chapter presents an introduction to the thesis. My goal has not been to make this review exhaustive, but rather to place the thesis in a broad functional context, and expand on topics where I felt I had a contribution to make or those where substantial progress has been made recently. The interested reader is pointed to excellent reviews in Laurent (1996, 1997, 1999), Hansson (Ed.) (1999), Wehr (1999), MacLeod (1999) and Laurent et al. (2001).

I purposefully go back and forth between vertebrate and insect model systems, for the evidence suggests that both are strikingly analogous in structure and function, and furthermore, because I am primarily concerned with those common aspects which are fundamental to olfactory computations.

The chapter begins with an exposition of the virtues of the olfactory system as a model system for pattern recognition, followed by a description of the demands put on olfaction by behavior. I then compare the strategy evolved by the olfactory system with that of other chemosensory systems. This section includes a note on potential shortcomings of a widely cited theoretical method to estimate neural information and compute the optimal sparseness of neural representations. I then describe the stimulus space and compare it with that of other sensory systems. That leads to a discussion of the computational problems posed by the stimulus-output requirements. I then argue for the benefits of insects as a model system for olfaction, and provide a brief anatomy of the system.

I then proceed sequentially through the various stages of olfactory processing, from getting odorants to receptors and the nature of olfactory sampling, through olfactory receptors, the antennal lobe and olfactory bulb, the mushroom bodies and pyriform cortex and beyond, with particular emphasis on the processing leading up to and including the insect antennal lobe, the focus of the experimental findings of this thesis. At each stage, I focus on the computational role served by the underlying anatomy and physiology. Of particular interest may be a discussion of non-classical receptive fields

in olfaction and a proposed model for a larger role than has previously been suggested for activity-dependence in the establishment of the connectivity patterns between receptor neurons and glomeruli.

Toward the end, I discuss a little explored aspect of olfaction, that of stimulus variance across space and time, and propose a speculative novel hypothesis for the role of glomerular convergence of receptor neurons expressing the same receptor gene. I end with a discussion of functional differences between the immune and olfactory systems, intra-extra-body counterparts in chemodetection.

1.2 Olfaction as a model system for information processing and pattern recognition

This thesis presents a set of experiments aimed at uncovering a biological solution to the computational problem of information processing for pattern recognition. The choice of olfaction is not coincidental: it is one of the simplest general-use pattern recognition systems in the brain (Hendin et al., 1994) –there are, of course, biological pattern recognition systems outside the brain, and we will explore architectural differences between the olfactory and immune systems in §1.20. This relative simplicity stems from the nature of the olfactory task. In systems such as the visual and auditory systems, where the patterns to be recognized require complex computational processes of reconstruction to convert the raw data from the receptors into a construct that is invariant enough from one exposure to a stimulus to the next so that recognition may take place, recognition cannot occur until several synapses downstream of receptor neurons. To illustrate this, picture the visual system recognizing a given combination of pixels turning on; the probability of that combination repeating itself would be extremely small, since small changes in eye direction, position, angle or illumination would change which photoreceptors are active even if the object observed was exactly the same.

For that reason, visual object recognition operates not on specific combinations of individual pixels, but rather on complex constructs involving several layers of processing leading from pixels to lines on to phase-invariant lines and all the way to faces and other objects. Something similar is true for hearing, a sense used to recognize complex sequences of the single notes that are detected by individual hair cells in the ear. This means that, in both of these systems, it is difficult to address the questions of recognition before achieving a satisfactory understanding of the nature of the representations and how they are developed as signals make their way up the visual and auditory hierarchies. In olfaction, in contrast, the patterns to be detected are not complicated sequences, nor are they complex reconstructions far removed from the raw data gathered by receptors. On the contrary, the chemical signature of an odorant given by what receptor types it binds pretty much defines the pattern to be encoded and recognized. Memory areas are just two synapses downstream of olfactory receptors. This is not meant to belittle the recognition process —we will study at great length some of the difficulties inherent in obtaining invariance to the natural variability in the stimuli—, but rather that the recognition process is not far removed from the periphery and thus may be studied without the need to treat inputs as black boxes or wait until sensory physiologists 'work their way up there.'

1.3 Brief history of the beginnings of research in olfaction

Despite these advantages, research on olfaction has been rather slow in comparison to that on vision and hearing, modalities more crucial to survival of *Homo sapiens* (Vosshall et al., 2000). This cannot be attributed to lack of a good beginning, since Lord Adrian published a neurophysiological investigation of the olfactory tract of fish in 1938 (Adrian and Ludwig, 1938), a full 21 years before Hubel and Wiesel's pioneering paper on receptive fields of single neurons in the cat's striate cortex (Hubel and Wiesel, 1959). Adrian seems to have attracted few early followers, though: when he

published a paper on olfactory discrimination in 1951 (Adrian, 1951), the only reference cited was his own pioneering electrophysiological study of the olfactory bulb and pyriform cortex in the anesthetized hedgehog and the cat (Adrian, 1942). Before that, olfactory research appears to have been limited to anatomical and cytological descriptions (Golgi, 1875; Cajal, 1890; Retzius, 1892, 1897; Gray, 1924), and behavioral investigations. Adrian's ground-breaking studies paved the way for much of olfactory research in the following 60 years. He demonstrated the existence of oscillations in breathing animals and their dependence on odor stimulation (Adrian, 1942). Moreover, remarkably, he demonstrated the existence of spatiotemporal coding in the olfactory bulb, the clustering of these spatio-temporal responses according to chemical similarity of the odorants and the relative invariance of these patterns over a wide range of concentrations (Adrian, 1951). He further hypothesized that these temporal differences might be caused by varying rates of air flow in different regions of the olfactory epithelium or by differences in the solubility of different odorants in water, a prediction that remains untested to this day.

Despite the relative lack of data on olfaction compared to, say, vision[1], research in olfaction has undergone a boom in the last decade[2]. But this sensory system that was among the last to come to the attention of the bulk of the neurophysiological community (Adrian, 1942) has also been around the longest.

1. A search in ISI's SciSearch yields 1,399 papers for the term 'olfactory' for 2000, compared to 10,035 for 'visual.'

2. A search in ISI's SciSearch/ Web of Science yields 242 papers for 'olfaction' for 2000, compared to 167 in 1995 and 17 in 1990, a 14-fold increase in the 10-year period, a percentage gain 50% greater than that of papers with the keyword 'vision', for example, which experienced a 9-fold increase (3005-2139-321) in the same period –a control to ensure the effect was not due to non biological uses of the word 'vision' used the word 'visual' and found the same 9-fold ratio: 10,035/1,166 over the 10-year period.

1.4 Olfaction's place in evolution

For humans, who use vision as their primary window on the world and hearing as their primary mode of communication, olfaction is a rather little used sense, as demonstrated by the relatively normal lives led by so-called *anosmic* people —people who cannot smell. But for much of the animal kingdom, olfaction is the primary means of exploration and communication. Compared to vision, olfaction has the advantage that it works in the dark. Unlike hearing, it does not take moving parts to emanate an odor. It has a longer range than both touch and taste, both contact senses.

More importantly, olfaction evolved sooner than any other sense (Dethier, 1990), 500 million years ago (Hara, 1994). It is thus the most ubiquitous of all senses. There is good reason for this: olfaction requires the least amount of hardware. In its simplest form, it entails nothing more than the process of intra- and inter-cellular communication: a ligand binding a receptor. It thus constitutes a natural extension of such basic protein-protein interactions, if not a precursor of cell-to-cell communication altogether (Hölldobler and Wilson, 1994). It is therefore not surprising to learn that organisms all the way from prokaryotes to mammals, passing through nematodes and insects, all possess olfactory systems.

Biologists were recently surprised with the discovery that, in nematodes as well as in mammals, the family of olfactory receptor genes is large and may encode as many as 1000 seven transmembrane domains proteins (Buck and Axel, 1991; Levy et al., 1991; Ben-Arie et al., 1994; Troemel et al., 1995; Sengupta et al., 1996; Robertson, 1998; Zozulya et al, 2001) –a figure that puts it as using 3% of the mammalian genome and a full 5% of the worm genome (The Genome Sequencing Consortium, 1998). But, viewed in another light, that is still substantially less than the number of receptors used to detect messengers originating in the organism; given that living beings are chemical machines and that they survive by interacting with their surroundings, it is perhaps not surprising that a substantial fraction of the set of genes coding for receptors be devoted to detecting chemical

signals from the outside. We will see later, though (§1.14), that the strategy used by the olfactory system in the selection of receptors is very different from most other processes of chemical detection in the brain.

Possibly by virtue of forming part of the earliest sensory system, cells of the olfactory cortex send and receive information from more brain regions than any other sensory system (Gesteland, 1992). This underscores its importance, but also the potential for future exploration: many of these brain regions are not yet physiologically characterized for odor inputs. In particular, the psychophysical literature has confirmed the popular notion that odor-evoked memories tend to be highly emotional, vivid, specific, rare and relatively old (Herz and Cupchik, 1992, 1995). This leads to the natural question: what is olfaction used for?

1.5 Odor-mediated behavior

Any system is best understood if one knows what its function is. In biology, knowing the function of a system helps one keep in mind what one is trying to explain. The same is true of the olfactory system. This section presents a brief overview of the formidable tasks that are faced by the olfactory system, in the manner of a description of 'features' included, as well as a brief mention of a function commonly presumed present which does not appear to be among olfaction's capabilities.

Firstly, the olfactory system has three essential interrelated roles in feeding behavior: it must be able recognize nutritious foods, it must help recognize poisonous substances, and it must help the animal locate sources of attractive smells. Detection of food odors elicits stereotyped antennal scanning behavior in the cockroach, *Periplaneta americana*, a nocturnal scavenger. This olfactory scanning consists of circular antennal movements at ~1.5–7 Hz, after which both antennae point in the direction of the odor source for 1–2 seconds. The animal then orients toward and approached

the odor source (Chee-Ruiter and Laurent, 1995). We will discuss some of the cues that insects may use in locating an odor source in §1.10.3. The grasshopper, *Melanoplus sanguinipes*, is attracted to the odors of its host plants, wheat, ryegrass, sorghum and alfalfa (Hopkins and Young, 1990; see Hartlieb and Anderson, 1999, for a review on insect odor-mediated behaviors). Dogs are capable of detecting concentrations of certain nitroaromatic compounds as low as 500 parts per trillion (Williams et al., 1998).

Second, olfaction is used for navigation. Salmon, for example, are *anandromous* fish: they grow up in fresh water, migrate to feed in salt water, and then come back to mate in fresh water; the reverse can also happen: eels of the Atlantic Ocean are *catadromous*: they spend their lives in fresh and salt water but breed in the sea. Remarkably, although some straying occurs, most salmon surviving the ocean journey —even though separated from other salmon of their own stock—are able to find their own stream at about the same time as their cohorts, years after departure. Salmon often "test" rivers other than their own, entering the estuary or lower river and then retreating to the sea and moving farther along the coast (Steelquist, 1992). Sockeye salmon use a combination of visual and olfactory cues to return to their natal area (Ueda et al., 1998). Salmon are *imprinted* with the odor of their natal areas during a critical period in development (Morin et al., 1987). Astonishingly, dogs can correctly determine the direction of a trail of 20-minute-old footsteps within 3-5 seconds after encountering it and sniffing only 5 footsteps (Thesen et al., 1993). This remarkable skill has been speculated to employ concentration discrimination (Thesen et al., 1983), although this remains to be demonstrated. Ants encountering a trail leading from a nest to a food source midway cannot tell which direction is which initially, but can do so after walking for a short distance on the trail (Brun, 1914), and may use an odor gradient existing along the trail with the highest concentration near the nest (Bossert and Wilson, 1963, reviewed in Schöne, 1984). Animals may also use temporal and spatial fluctuations in odor intensity to derive information about the direction and distance to an odor source (Murlis et al, 1992; Mafra-Neto and Cardé, 1994; Gomez and Atema, 1996); see §1.10.3.

Feeding and navigation are just two examples of a more general capability: that of recognizing a chemical environment. The same skill can be used by a mother and her calf to recognize each other (Kallquist and Mossing, 1982) and for many other purposes. More importantly, the olfactory system is able to perform such recognition both innately (see Chapter 3; Simpson and White, 1990; Tabuchi et al., 1991; Matsumoto and Mizunami, 2000) and through learning (von Frisch, 1967).

In addition to recognizing a chemical environment, there is evidence that Tiger salamanders (*Ambystoma tigrinum*) generalize a conditioned response to olfactory cues chemically similar to the odor they were trained with (Rusell Mason and Stevens, 1981), suggesting that the olfactory system preserves some notion of chemical similarity between similar compounds. Experiments disrupting neuronal synchronization have also shown that the discrimination among chemically similar odors in bees is more labile to disruption than that among chemically distinct ones (Stopfer et al., 1997).

Animals also exhibit more complex olfactory-mediated behaviors, such as so-called olfactory scene analysis and the learning and identification of abstract relationships. For example, hamsters preferentially remember or value the top scent of a scent over-mark (Johnston and Borade, 1998). What cues do they use to do this? Johnston and Borade (1998) showed that overlap or apparent occlusion are necessary for hamsters to identify the top over-mark, suggesting that these mammals use regions of overlap and the spatial configuration of scents to evaluate over-marks. In a very elegant recent experiment, Giurfa, Srinivasan and colleagues (Giurfa et al., 2001), showed that bees can learn the concept of sameness or difference between two visual or olfactory stimuli and then transfer the learned association, between sameness or difference and a reward, across modalities.

There is behavioral evidence that the olfactory system solves what is known as the blind source separation problem: separating out odors originating at different sources. *Limax maximus*, a mollusk, can discriminate two food odors from separate sources separated by 1 cm (Hopfield and Gelperin, 1989). In humans, delays of 200 to 400 ms between two odors presented monorhinically or

dichorhinically (through one or two nostrils) elicited detection of components (Rouby and Holley, 1995). We will return to this in §1.13.

The capacity to identify components in mixtures of more than three components coming from the same source, in contrast, does not appear to be an ability of humans (Laing et al., 1983; Laing and Francis, 1989; Laska and Hudson, 1992) with or without attention deployed to individual components (Laing and Glemarec, 1992). The same is true of insects, except for some (Smith and Cobey, 1994), but not all (Smith, 1998), binary mixtures. In fact, the presence of one odor can sometimes mask or suppress the perception of a second one (Bell et al., 1987). The olfactory system is therefore not analogous to a gas chromatography or spectroscopy system whereby odors are separated into their components, but rather, odors in mixtures appear to generally blend to form a new odor with few of the characteristics of constituent odors. Whether this is due to the difficulty of the problem or the lack of sufficient adaptive value for such a skill, or both, remains an open question. I suspect there is adaptive value in identifying each blend as a distinct odor, multiplying the information content in the chemical signature of an environment in a combinatorial fashion, even when individual components may be common to many objects or environments. But this is not to say that both abilities, that of assigning a unique identity to a blend and that of identifying the ingredients in a mixture, could not have evolved in concert.

Lastly, the detection of chemical signals has a role in intra-species communications between individuals. That is what we turn our attention to in the next section, after a brief mention of the timescale of odor perception.

1.5.1 How fast is odor perception?

Laing and Macleod (1992) employed psychophysical procedures to determine human recognition

times to three matched intensity levels of the odorants n-butanol, (+)-limonene and propionic acid. A computer controlled the delivery of the odorants from an air dilution olfactometer and measured recognition times. The mean times recorded with the odorants were significantly different and ranged between 680–867 ms. Laing and his colleagues (1994) showed that a time separation of 400 msec between presentation of two odors from separate sources is sufficient to allow significant discrimination of the order in which they were presented. Claims that odors from separate sources presented simultaneously are perceived sequentially (Laing et al., 1994), however, have, I believe, proven unfounded. [3]

1.6 Pheromones, the accessory olfactory system and chemical communication

Once a system evolves the capacity to sense chemical signals from the environment, it is but a small step to evolve a system capable of sending its own chemical signals for detection by other

3. The correlation between order presented and order perceived found by Laing et al. (1994) may be due to the extreme time differences of 400 msec and not true for intermediate values. It is not surprising that presenting an odor almost half a sec before another yields two sequential percepts. Even if the correlation is significant for intermediate values, the odors were presented at different concentrations to generate suppression of one odor by another. That does not seem to be a good condition to test whether two equally perceptible components of a mixture are perceived sequentially. It is perhaps not surprising that a stronger odor is perceived earlier than a faint one, which is the second conclusion of the paper: that the suppressant odor is perceived first. If they wanted to test whether two odors presented at the same time are processed serially, they needed to test whether the order in which they are perceived is significantly different from random (50%-50%). They did not show this, although presumably they have the data. Even if it turned out to be significantly different, it would not be surprising unless the odor concentrations were matched for intensity, or better, for detection latency when presented alone.

individuals. The molecules used for such chemical communication take the name of *pheromones*, from the Greek *pherein,* to transfer, and *hormon,* to excite.

Pheromones have evolved in all animal phyla (Pantages and Dulac, 2000). Fish release an alarm pheromone when disturbed that causes other fish to flee (von Frisch, as cited in Agosta, 1992). The pheromone is carried in large alarm-system cells on the skin of the fish. These cells are fragile, and rupture upon injury, discharging the pheromone into the water. Simply scaring a fish will not discharge these cells, but damage to only a small area of the skin of a single fish is capable of causing fright in an entire school. Honeybees release an alarm pheromone too when disturbed and fan their wings to disperse the signal to their nest mates (Agosta, 1992). Male moths are sexually attracted by bombykol, a pheromone released by female moths. Other pheromones bear messages such as "the queen is in the hive and all is well," "produce more sex hormone," "we are under attack!" and "I am pregnant" (Agosta, 1992). Some species, such as ants and honeybees, use as many as thirty different pheromones to coordinate the activities of their complex communities. It is pheromones that guide ants along their trails, and the importance of chemical signals in the process can be easily demonstrated by crossing an ant's antennae, a procedure which will leave the ant confused and unable to follow the trail in its normal zigzag motion (Agosta, 1992). Bethe (1898, 1900) took an ant from one nest, deodorized it with alcohol and water, and then dipped it in a juice obtained by crushing the bodies of antes of another species. If the ant was placed in its own nest, it was immediately killed, but if placed in the nest of the ants whose odor it bore, it was accepted, although later, when the artificial odor wore off and the ant's own scent became apparent, it was sometimes attacked. Fabre showed that if a female Oak Eggar or Banded Monk moth was placed under a glass, males paid no attention to her, but went straight to a twig on which the female had previously perched at the other side of the room (Moncrieff, 1967, p. 340). He also reported that the smell of a serpent arum flower, which exhales a horrible stench of putrid flesh for two days, attracts hordes of insects, most of which will die engulfed in the capsule after hours of swarming and rolling, unable to resist

the flower's lure, even though they are not ovipositing or feeding, nor are they prisoners (Moncrieff, 1967, pp. 337-338). Other species, such as the *Temnochila chlorodia* beetle, are attracted to the pheromone of their prey. Yet other species imitate pheromones of others to their advantage: bolas spiders mimic moth sex attractant to capture moths (Eberhard et al., as cited in Agosta, 1992). Orchids of the genus *Ophrys* broadcast scents that imitate the sex pheromones of insects resembled by their flowers, hoaxing bees and wasps to attempt copulating with the flowers and impregnating them with pollen in the process (this is successful in part because the male bees emerge earlier than female bees, and the orchids are ready for pollination when there are many males and few females) (Agosta, 1992). Birds are believed to use pheromones too, but evidence for them remains incomplete.

In humans, Darwin showed more than a century ago that an infant with its eyes closed will turn toward its mother, and more recent experiments have shown that infants will display a preference for his or her mother over other mothers. There is also evidence for a chemical attractant that guides human sperm to the egg for fertilization, and about twenty different olfactory receptors have been discovered in sperm tissue (Parmentier et al., 1992, cited in Agosta, 1992). Axillary sweat from women synchronizes other women's menstrual cycles, and axillary sweat from men regularizes women's menstrual cycles. The cause of this striking phenomenon, initially observed in college dormitories by Martha K. McClintock at Harvard University (reviewed in Agosta, 1992), was disputed for a long time, until the chemosensory nature of the cues was pinpointed by an elegant experiment by George Preti and colleagues showing that sweat placed on the lips of women who had never seen or otherwise been in contact with the donors had an effect on the menstrual cycle of the recipient, while controls with no sweat had none (reviewed in Agosta, 1992). Two aromatic compounds also found in musk and civet are produced by bacteria in human sweat of both men and women — although their concentration is higher in males— and constitute the basis for much of today's perfume industry (Agosta, 1992).

Pheromones vary in chemical composition. Some of them are pure substances, while others are complex blends. In the aggregation pheromone system of the desert locust, *Schistocerca gregaria*, six different aromatic compounds that elicit electrophysiological activity in the olfactory epithelium have been identified. Similarly complex odors facilitate individual or group recognition in mammals, e.g., in territorial marking with urine or feces.

In many reptiles and all nonprimate mammals, pheromonal signals, carrying social and sexual information, are processed by the sensory cells in the vomeronasal organ (VNO) of the nose and their central connections in the brain. Together, they receive the name of *accessory olfactory system*. The accessory olfactory system is separate from the main olfactory system and differs from it both in physiology and function. Until recently, it was believed that in some primates, including man, the VNO made a transitory appearance during embryological development but disappeared before birth (Agosta, 1992). More recently, however, an examination of a large number of adult humans showed the VNO, also called Jacobson's organ, present in every one of them (Watson, 2000). The exploration of this most recently discovered human sense, which appears not to influence conscious perception but may well affect behavior subconsciously (Watson, 2000), has only just begun. In a report published this past summer, Savic et al. (2001) used PET to show that women smelling an androgen-like compound activate the hypothalamus, while men, in contrast, activate the hypothalamus when smelling an estrogen-like substance. A role for the hypothalamus in pheromonal processing appears consistent with the subconscious nature of human pheromonal perception (see also Sobel et al., 1999), and may help explain why its effects, such as the synchronization of menstrual cycles described above, appear so surprising to us.

Airborne odorant molecules cannot efficiently enter the dead-end passage containing the VNO. In snakes, the tongue delivers molecules collected from the air and nearby objects to ducts at the entrance to the VNO. In mammals, molecules are transported into the VNO by saliva.

Surgical ablation of the VNO in rodents has been shown to profoundly impair pheromone-induced behaviors such as mating and territorial defense and to perturb associated neuroendocrine responses, such as male testosterone surge and female oestrus cycle (Halpern, 1987; Wysocki, 1989). Pheromone signals ultimately result in activation of centers of the ventromedial hypothalamus involved in reproductive and aggressive responses (reviewed in Wysocki, 1989).

Mammalian VNO neurons use at least three different families of molecular receptors (Pantages and Dulac, 2000; Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997), each composed of 50–100 genes and unrelated to the family of receptors of the main olfactory system. Two of these families are related to each other and to the family of taste receptor genes encoding receptors for bitter tastants; the third is unrelated to the rest. VNO receptors' detection threshold for their corresponding pheromones can be remarkably low, near 10^{-11} M, placing these neurons among the most sensitive chemodetectors in mammals (Leinders-Zufall et al., 2000). VNO neurons show highly selective tuning properties and their tuning curves do not broaden with increasing concentrations of ligand, unlike those of receptor neurons in the main olfactory epithelium (Leinders-Zufall et al., 2000).

Why has such molecular diversity of vomeronasal receptors emerged? The recent analysis of VNO response to pheromonal stimuli directly demonstrates that natural sources of pheromones, such as urine, activate large subsets of sensory neurons (Holy et al., 2000). However, in sharp contrast to the combinatorial mode proposed for olfactory recognition in which specific odorants are recognized by multiple and overlapping populations of olfactory receptor neurons (Buck, 2000), individual pheromonal compounds seem to activate distinct subsets of VNO neurons (Leinders-Zufall et al., 2000). The lack of promiscuity in the VNO neuronal response implies that multiple subpopulations of VNO neurons function as independent chemosensors for many still uncharacterized pheromonal cues (Pantages and Dulac, 2000). Despite the fact that each pheromone activates a distinct subset of

neurons, though, each neuron expresses multiple receptor types (Martini et al., 2001). As we will see below, this is analogous to taste, where multiple receptor types, all binding compounds that are perceived as bitter, are expressed by the same neurons.

Before we concentrate on olfaction for the rest of the thesis, we stop to consider the two remaining chemical senses (§1.7 and §1.8), compare them to olfaction (§1.9) and then consider the nature of the inputs to the olfactory system (§1.10).

1.7 The trigeminal system in the olfactory epithelium

No treatise on olfaction would be complete without at least a brief mention of the fact that the olfactory epithelium contains another chemosensory system in the form of *trigeminal nerve receptors*. The fifth cranial or trigeminal nerve (which is the largest cranial nerve and carries the sensory nerves responsible for the face, teeth, mouth, most of the scalp, as well as the motor nerves of the muscles of mastication) provides a second set of nerve endings which are responsible for tactile, pressure, pain and temperature sensations in the areas of the mouth, eyes and nasal cavity. A number of chemical trigeminal stimulants produce effects described as hot, cold, tingling or irritating. For example, 'leavo-menthol' produces the trigeminal feeling of cold at moderate concentrations and 'hot' at high concentrations in the nasal cavity. This type of sensory description is often not just limited to the areas of the nose, mouth and eyes, but also occurs on skin areas not served by the 5th cranial nerve (especially, the genitalia) and thus such stimulants may affect a variety of nerve endings (Leffingwell, 1999). Similarly camphor, which possesses markedly more aroma than menthol, also produces the 'cold' sensation via interaction with trigeminal receptors. Other commonly encountered trigeminal stimulants include the chemicals allyl isothiocyanate (mustard, mustard oil), capsaicin (hot chili powder, mace spray) and diallyl sulfide (onion). The trigeminal sense is relatively

unexplored at present –a search in SciSearch for ‘trigeminal receptor’ yielded but 7 results, and one for trigeminal sense yielded none. We do know that about 70% of all odors are said to stimulate the trigeminal nerve, although, in general, the latter is several times less sensitive than olfactory receptors (Ohloff, 1994).

1.8 Taste

No review of the chemical senses would be complete without a mention of taste, yet another chemical sense that animals are endowed with, and one closely related to olfaction. Taste is the sensory system devoted primarily to a quality check of food to be ingested. Although aided by smell and visual inspection, the final recognition and selection relies on chemoreceptive events in the mouth. There is no life form known that neglects to check its intake using chemoreception (Lindemann, 2001, an excellent review of the topic, from which this section draws heavily). A human baby can already distinguish sweet and bitter and express pleasure for sweet taste but displeasure for bitter taste at only a few days old (Ganchrow et al., 1991). Taste research has seen notable advances in the last few years.

Already in worms, like the nematode *Caenorhabditis elegans*, different cells are involved in olfaction (the detection of airborne molecules) and taste (the detection of soluble attractants and repellants) (Pierce-Shimomura et al., 2001). In the fruitfly *Drosophila melanogaster*, for example, taste sensations are mediated by nerve cells whose sensory dendrites are contained in 'hairs' found on the body surface. Other taste neurons, found on the proboscis (also called the labellum or labial palps), but also the legs, anterior wing margins, and three discrete patches of sensilla in the gustatory tract within the head, express a family of 70 G-protein-coupled receptors (GPCRs) encoded by 62 genes named GR (Clyne et al., 2000; Scott et al., 2001; Dunipace et al., 2001; Robertson, 2001).

A surprising observation is that a subset (four so far) of the gustatory receptor genes examined are expressed in a subset of olfactory receptor neurons in the antennae, the primary olfactory organ in *Drosophila* (Robertson, 2001). These neurons were not previously identified as expressing any of the odorant receptors (Voshall et al, 2000). The neurons expressing one of these genes project axons to a pair of glomeruli in the antennal lobe, rather than to the suboesophageal ganglion (Scott et al., 2001). Thus, they apparently behave as additional odorant receptors (Robertson, 2001).

Vertebrate taste receptor cells are not neurons

In contrast, the taste receptor cells of vertebrates are not neurons, but originate from the epithelial covering of the body (Stone et al., 1995). Vertebrate taste cells are small bipolar cells. To connect to the oral space, they send a thin dendritic process to the epithelial surface. The cells occur either singly or densely packed in taste buds, where up to 100 form a functional unit. Although taste buds also occur abundantly on the body surface and barbels of some fish, all vertebrates have taste buds in the oral epithelium, typically on tongue, palate and pharynx. The marker molecule gustducin, a taste-specific G protein (MacLaughlin et al., 1992), shows additional 'taste cells' in the nasal mucosa (Zancanaro et al., 1999) and in the stomach (Höfer et al., 1996). Each chemoreceptive area of the human tongue responds to each of the qualities of sweet, sour, salty and bitter taste. Only minor differences in subjective thresholds were noted across area (Hänig, 1901; Lindemann, 1999).

A single taste receptor cell expresses many taste receptor genes

Two families of G-coupled transmembrane proteins have recently been identified as mammalian taste receptors (Adler et al., 2000; Chandrashekar et al., 2000; Matsunami et al., 2000). One of

them has two receptors, two of which, T1R2 and T1R3, have been shown, using a heterologous expression system, to combine to function as a sweet receptor, recognizing sweet-tasting molecules as diverse as sucrose, saccharin, dulcin, and acesulfame-K (Nelson et al., 2001). The other family, T2R, codes for 40-80 receptors with different molecular specificities but all expressed in the same group of receptor cells: those responsible for bitter taste detection (Adler et al., 2000; Chandrashekar et al., 2000). Each of the bitter taste receptor cells expresses more than one type of (but not all) T2Rs (Adler et al., 2000). Calcium imaging of taste bud neurons confirmed that only a subset of bitter taste neurons respond to any particular chemical (Caicedo and Roper, 2001). The detection threshold for sugars is roughly 0.1 M, more than five orders of magnitude greater than observed for bitter compounds (Lewcock and Reed, 2001).

The practical consequences of recent efforts to understand the taste receptors are considerable. Based on binding-site structure, advanced techniques of drug design are expected to allow the construction of taste ligands that activate or inhibit a receptor protein, thereby enhancing or inhibiting a specific taste. Thus it might become possible to expand the already huge commercial market for artificial sweeteners into other taste qualities. This would be beneficial in many ways. For example, aged people often have a general decline of taste function (Stevens et al., 1995) and need taste enhancement to once again enjoy their food. And an organic enhancer of sodium taste would be a great help for patients on a low-sodium diet (Lindemann, 2001).

Recordings from the sensory nerve fibers and from the soma of their neurons have consistently revealed that some nerve fibers are specialists, but many are generalists, carrying responses to more than one taste quality (Lundy and Contreras, 1999). A simple 'labeled line' design, where each fiber responds to just one of the qualities, to bitter only or to sour only, is not evident, as many fiber are broadly tuned with respect to taste ligands. These generalist fibers carry responses to salty and sour, to glutamate and sucrose, and so on. Similarly, many taste receptor cells, too, are generalists,

as responses to taste qualities are randomly and independently distributed, varying in intensity across cells (Gilbertson et al., 2001). Given such distributed responses, a part of the information about individual tastants must be buried in the quorum of the receptor cells and the 'across-fiber pattern' of the sensory nerve (Erickson, 2000). All of these properties in the organization of the information processing are strikingly reminiscent of the olfactory system, and are particularly noteworthy given the large differences in the structures used in both systems. We will encounter such an evolutionary convergence once again when we discuss the olfactory systems of evolutionarily distant phyla. The broad tuning of olfactory and taste receptors has been claimed to be optimal: Zhang and Sejnowski (1999, but see [4-5]) maintain that for stimuli space of dimensionality three or higher, more information per neuron (although less information per spike) can be coded by broader tuning and thus lead to maximal resolution using optimal decoding.

The bipolar taste cells have two obviously important specializations: microvilli in contact with the oral cavity and synapses with sensory nerve fibers. Taste receptor proteins are mounted on the microvilli, acting as molecular antennas listening into the chemical environment. On binding taste molecules, taste cells fire action potentials, by means of voltage-gated Na^+, K^+ and Ca^{2+} channels (Avenet and Lindemann, 1987; Roper, 1993; Lindemann, 1996). A local increase in Ca^{2+} concentration is needed for synaptic activation (and hence nerve excitation), and transient rises in the cytosolic Ca^{2+} concentration were observed by fluorescence imaging in taste cells responding to bitter and sweet agents (Akabas et al., 1988), while amino acids triggered either increases or decreases of the Ca^{2+} signal (Zviman et al., 1996; Hayashi et al., 1996). In turn, this process activates synapses and thus causes excitation of the nerve fibers. These carry the signal to the brain stem, where central taste processing begins (Lindemann, 2001).

A number of transmitters have been found within taste buds, but those released by taste cell synapses have been difficult to identify. Noradrenaline and acetylcholine seem to be secreted by nerve

fibers and modulate the responses of taste cells (Herness et al., 1999). Serotonin is thought to act as a paracrine agent between taste cells. Secreted by one cell and modulating the taste response of a neighboring cell, this agent mediates local signal processing within a taste bud (Delay et al., 1997; Herness and Chen, 1997). Glutamate is a strong candidate for a mainstream afferent transmitter secreted by taste cell synapses (Caicedo et al., 2000; Lawton et al., 2000).

Drosophila taste receptor neurons show axonal targeting to stereotypically different regions of the suboesophageal ganglion in larvae and adults, although these brain targets are rather diffuse, pos-

4. There are a number of caveats to note regarding Zhang and Sejnowski's (1999) result. By their use of Fisher information, Zhang and Sejnowski assume that the mean firing rate is a continuous and differentiable function of the encoded stimulus. But this is not necessarily the case. In fact, the responses of retinal ganglion cells, for example, are not properly described by a firing probability that varies continuously with the stimulus. Instead, these neurons elicit discrete firing events that may be the fundamental coding symbols in retinal spike trains (Berry et al., 1997; see also Wehr et al., 1996 for a similar demonstration in the olfactory system). The difficulties of defining a continuous stimulus space are most clearly evident in olfaction. The fact that tuning curves may not be continuous or even well defined, though, renders the method of Zhang and Sejnowski unusable, but does not make the question meaningless: sparseness is an important coding parameter of any representation, independently of whether the stimulus space and the firing rates are continuous or not (see, for example, Pérez-Orive et al., in press).

As Zhang and Sejnowski note, their method applies only to optimal estimation algorithms, for uncorrelated neurons or neurons with weakly correlated noise, and for radially symmetrical tuning curves.

Furthermore, for dimensionality greater than 2 and large tuning widths, Zhang and Sejnowski's result has limited usefulness: it places a lower bound on the error that gets asymptotically close to zero —a lower bound to begin with.

Moreover, their method is only valid for tuning widths that are small relative to the size of the stimulus space. This unstated restriction is most evident in the limit of infinite tuning width: Zhang and Sejnowski's bound for the error is lowest for this case, and yet in reality error rates are at their maximum for that case, for an infinitely wide tuning curve provides zero discriminability. The method is thus unable to estimate optimal tuning widths for stimulus dimensionality greater than 2 (continues in 5)

sibly overlapping, and not nearly as discrete as the glomeruli of the antennal lobe (Robertson, 2001).

1.8.1 Why do vertebrates possess separate gustatory and olfactory systems?

If the difference between taste and olfaction in vertebrates were, as in *C. elegans*, that the former detects soluble molecules and the latter detects airborne ones (Pierce-Shimomura et al., 2001), then how does one explain the fact that fish have both? For this reason, vertebrate olfaction is defined as chemical information transmitted to the central nervous system (CNS) by neurons through cranial nerve I, while chemical information detected by specialized epithelial cells and transmitted to the CNS by cranial nerve VII (facial), IX (glosopharyngeal), or X (vagal) is termed gustation

5. The Cramer-Rao lower bound on the mean squared error of estimation used by Zhang and Sejnowski bounds the error rate *given the amount of information present in the encoding variable chosen*. But nothing guarantees that Zhang and Sejnowski's choice, neuronal firing rates during a time window Tau, are the optimal encoding variable or the one used by the brain. With 1 or few spikes per perceptual event per neuron, mean firing rate may constitute a comparatively poor source of information. Perhaps spike timing, an analog quantity, is a better way to go to optimize estimation.

Zhang and Sejnowski's theoretical results suggest that the accuracy of a 2-D code should be unaffected by the width of the tuning curves. Nevertheless, multiple parallel maps, exhibiting neuronal tuning with different widths, are universal in sensory systems, even when they do not exist at the sensory periphery (Konishi, 1986; Lewis and Maler, 2001). Maps with greater tuning widths have been found to result in equal accuracies of estimation for some parameters, greater accuracies for others and smaller accuracies for others still (Lewis and Maler, 2001).

Finally, it must also be noted that although Zhang and Sejnowski suggested the Fisher information per neuron increases with increasing tuning width for stimulus dimensionalities greater than 2, they showed that Fisher information per spike always decreases with increasing tuning width. If energetic considerations prevail, the latter could be more relevant.

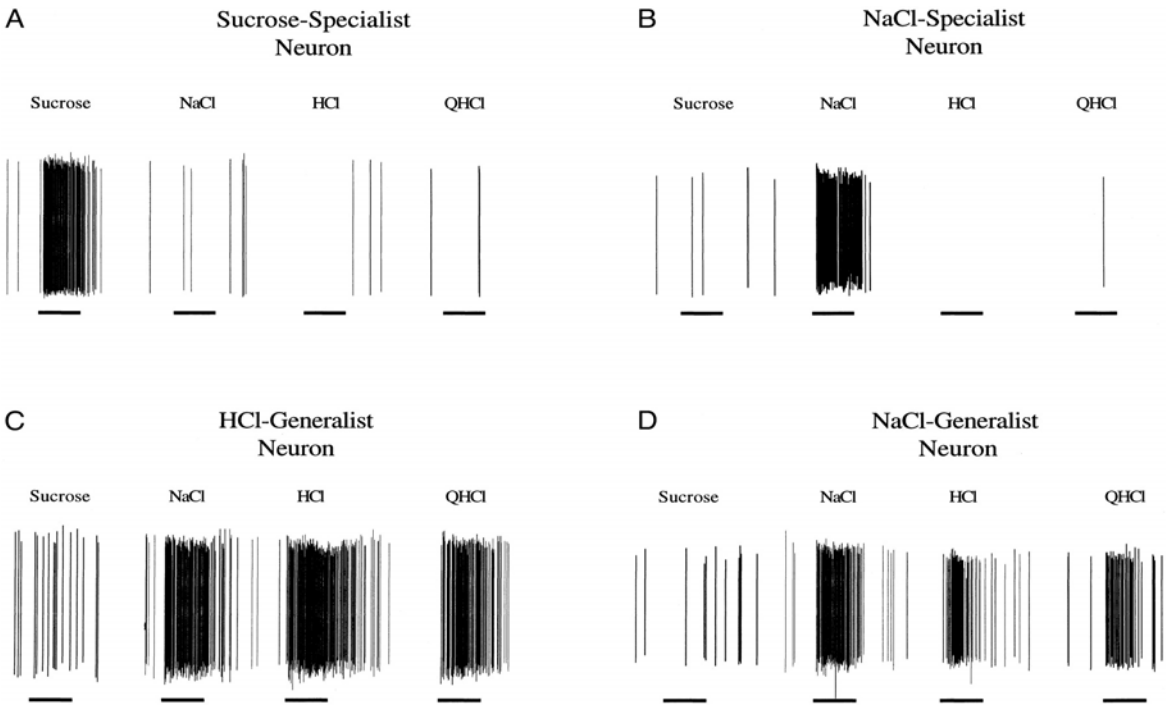

Figure 1.1. Specialist and generalist coding in taste neurons. Each neuron was tested for its sensitivity to 4 chemicals: 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl. The solid black bar below each spike record represents the duration of stimulus application (15 s) (reproduced from Lundy and Contreras, 1999).

or taste (Hara, 1994). While this makes the definition unambiguous, it leaves unanswered the question of why two separate and apparently nonhomologous systems evolved. If fish were the descendants of land vertebrates, it could be hypothesized that olfaction originally evolved for the detection of volatiles in land animals, and taste evolved for the detection of soluble molecules, and that olfaction was later adapted to detect soluble molecules in fish. But our current understanding of vertebrate evolution maintains that the original vertebrate precursors were aquatic (Encyclopaedia Brittannica Online, 2001).

A second possibility is that gustation evolved to sense ingested molecules, while olfaction evolved to sense the surroundings. There are two problems with this hypothesis. The first is that external taste buds are common in fish: the yellow bullhead (*Ictalurus natalis*), for example, has taste buds

on its entire body surface (Hara, 1994). This problem could be dismissed if this was a secondary adaptation not present in the original vertebrates. The second problem, though, is that even if their locations in the body are different, it is not clear why the same original system could not be co-opted to a different location.

Interestingly, in *Drosophila*, the 33 amino acid signature motif characteristic of the GR gustatory gene family is present but somewhat diverged in 33 of the 60 members of the family of *Drosophila* odorant receptor (DOR) genes. The DOR genes, however, possess additional conserved motifs not present in the GR genes and define a distinct family (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999, 2000). Indeed, the gustatory receptors are an extraordinarily divergent family, of which the odorant receptors are in fact just a single branch among many (Robertson, 2001). This great divergence hints at great antiquity, and indeed five genes have been found that form three lineages within the gustatory family in the nematode *Caenorhabditis elegans* genome, indicating that the superfamily predates the nematode—arthropod divergence (Robertson, 2001). These observations suggest that the putative gustatory and olfactory receptor gene families may have evolved from a common ancestral gene (Scott et al., 2001). Consistent with a common origin, in insects, both types of receptors may be found side-by-side, not localized to different organs as in vertebrates (Schneider, 1963).

In agreement with the large size of the family of taste receptor genes, in fish, taste appears to respond to a wide spectrum of compounds (Kotrschal, 2000). So the size of the stimulus space is unlikely to be the critical distinction between olfaction and taste. In contrast, the difference between them appears to lie in their behavioral outputs. Whereas stimulation of the taste systems alone triggers reflexes, complex, conditional or conditioned behaviors occur only when the olfactory system is intact (Kotrschal, 2000). Thus, our responses to different tastes are to a large degree hardwired — thus the ease with which terms adapted from taste, such as sour and sweet, are co-opted for other

meanings, meaning which are constant across cultural barriers. Descriptions of odors, on the other hand, have a much greater cultural or experience-dependent component, and are therefore impractical as descriptors outside their specific realm.

Finally, olfaction appears to work at low thresholds, designed for remote sensing, while taste appears to operate with higher thresholds and designed mainly for close-distance discrimination (Kotrschal, 2000).

1.8.2 Interactions between olfaction and taste

There are several senses in the mouth. Thermal, touch and pain sensations in the mouth are unambiguous. Olfactory stimuli, however, can have two different origins: orthonasal, when sniffed, or retronasal, to the nasal cavity from the mouth through chewing and swallowing. Our brains interpret these as smell or flavor depending on whether chemosensation is accompanied by touch sensations in the nose, caused by sniffing, or by touch sensations in the mouth, caused by eating or drinking, respectively (Bartoshuk and Beauchamp, 1994). Retronasal olfactory sensations can be localized to the mouth by providing a tactile cue in the mouth: if a tube leading to a chocolate reservoir is placed in the mouth of a subject who then chewed on tasteless odorless gum, the subject perceived the gum to become chocolate when the odorant is turned on (Bartoshuk and Beauchamp, 1994).

1.9 On the nature of the diverse chemical senses

1.9.1 The practicality of labeled lines when the behavioral significance of stimuli is fixed

In essence, VNO neurons, insect neurons that respond to pheromones and project to the macroglomerular complex (MGC), and specialist taste receptors appear to function as labeled lines, each neuron's activity carrying the signal of one particular signal having been detected. Their properties are hard-wired and very different from those of the distributed codes that the main olfactory system employs, and so we will not consider the accessory olfactory system for most of this thesis. This hard-wired character and specificity of receptors is made possible by two factors. First, the specificity and constancy of the signals to be detected has given the pheromonal system, for example, the time to evolve receptor molecules extremely sensitive and exquisitely tuned to the corresponding pheromones. Second, the fixed behavioral meaning of pheromonal signals, as well as the relatively constant biological value of food substances, has allowed taste and the VNO to establish hard-wired connectivity patterns between the periphery and central brain structures. When biology had a limited set of molecules to map onto a set of limited behavioral outcomes, as in the case of pheromonal signals or the five primary tastes, it has favored a labeled line design, with each receptor cell type signaling a distinct behavioral message –not necessarily a unique chemical identity, as witnessed by the large variety of molecules that taste bitter and activate the same group of receptor neurons.

Of course, the simplicity of such a design means that if the meaning of a stimulus changes, evolution must change the system. Indeed, even in closely related species, distinct differences in taste sensory performance may be noted, which seem to match the nutritional 'needs' of a species. Receptor specificity appears to have changed in evolution with the availability of food ingredients (Lindemann, 2001). Such an evolutionary fine-tuning, I argue, may have been necessary due to the relative lack of plasticity of taste compared to olfaction.

1.9.2 The synthetic nature of olfaction?

The main olfactory system, however, cannot afford that luxury: it must be able to recognize *any* odorant that experience may bring upon it, under a variety of conditions and *with any possible behavioral significance*. Much has been said about the former, to the point that this character of olfaction has received a name: its *synthetic* nature, the name given to the presumed need for olfaction to recognize essentially any combination of odorants (Laurent, 1999). This presumed ability awaits experimental demonstration, though (Laurent, 1999); it is unclear to me that we are able to distinguish between the smells of any arbitrary number of different piles of garbage, to use a possible olfactory analog of visual random dot patterns. In fact, electroantennogram recordings paired with gas chromatography (GC), gas chromatography-mass spectroscopy (GC-MS) and a coupled gas chromatograph-electroantennographic detector (GC-EAD) suggest that the olfactory system of the locust responds to only a small fraction of the compounds present in plant volatiles (Njagi and Torto, 1996). The poor ability of human subjects to discriminate among related odor mixtures is even more notorious (Laska and Hudson, 1992). Perhaps it is simply our lack of reliance on olfaction in our daily lives that masks what would otherwise be obvious shortcomings in our ability to discriminate compounds using our noses.

1.9.3 The plastic nature of olfaction

The flexibility of the olfactory system as its hallmark trait, though, has gone comparatively unnoticed. Just as much as the large number of potential odorants, it is precisely the fact that olfactory stimuli have no intrinsic biological meaning (Mombaerts, 1999b) and thus the plastic nature of the association between olfactory stimuli and behavioral significance, as evidenced by the prevalence of olfac-

tory learning (von Frisch, 1967), that makes a labeled line approach, where each neuron may group many signals *with the same behavioral significance* together, like bitter taste neurons do, impractical. How could the system decide which two signals to group together under the same labeled line (which receptors to express in the same neuron, for example) if the significance of each of the two is subject to plasticity, and thus could end up being the same or different depending on experience? In this, the olfactory system shares a generalist task with its visual and auditory counterparts –although in those systems, too, specialist subsystems exist for the detection of specific behaviorally relevant stimuli, such as bug detectors in frogs (Marr, 1970).

We turn our attention to the computational problem faced by the generalist olfactory system in §1.11. Before that, we take a look at the inputs that olfactory systems count with in order to face the formidable challenges posed by their task.

1.10 What constitutes an odorant?

1.10.1 The stimulus space

Odorants are small, generally volatile compounds with molecular weights less than 300 Daltons. The number of different odorant molecules has been guessed to be over 400,000 (Mori and Yoshihara, 1995) —I am aware of no rigorous quantification of this figure. Yet most odors in the natural world are complex blends of these compounds. Each odor, whether monomolecular or a blend, generally evokes a singular percept, leading to an astronomical number of possible smells.

Not all small volatile compounds evoke an odor percept. In order to elicit one, a compound must be able to traverse an aqueous interface (see §1.13) and bind one or more olfactory receptors with a detectable affinity. Thus the existence of odorless small compounds, of which water is perhaps the

most obvious example.

1.10.2 Smells versus images and sounds

Much has been said to the effect that, unlike vision and hearing, olfaction's stimulus space is highly multi-dimensional (Weyerstahl, 1994). What do we mean by the dimensionality of the stimulus space? This is a question more complex than would appear at first sight, so we will build up in complexity gradually. Firstly, a distinction must be drawn between the dimensionality of the stimulus space, independent of the biology, and the dimensionality of the first neural representation of the stimuli. Take color vision, for example: the color of *one isolated monochromatic ray* of light can be uniquely defined by a single number, the frequency of the light. Thus, the dimensionality of the physical color space for *a single ray* is two: frequency and intensity or power. In reality, though, any given point in a visual scene can reflect multiple wavelengths. This means that in reality, the physical stimulus space is much higher dimensional: for each 'pixel', the stimulus space has as many dimensions as quantized levels of light there are in the visible spectrum, since each point in an image is characterized by an intensity for each of those frequencies. To sense color, though, the visual system of humans employs four types of photoreceptors —three cones and one rod—, the activation of each of which is independent from the other due to their different absorption-wavelength functions. This means that only four numbers of information are captured by the visual system about any point in the visual field, thus the input neural representation *of each pixel*[6] in the visual system is four-dimensional. Of these, one is interpreted as luminance or brightness, which leaves our visual system with

6. In reality, the maps of different types of photoreceptors have different tiling densities. Thus, these maps do not have equal spatiotemporal resolutions: the luminance channel's acuity is by far superior to those of the other two (see 6). This is reflected in our behavioral abilities: we have a much lower threshold for detecting lack of focus in luminance than in color (Wandell, 1995).

three dimensions for color[7,8].

In olfaction, in contrast, taking into account a single antenna or nostril, each stimulus is given by a vector in n-dimensional space, where n is at least the number of olfactory receptor types, and possibly the number of receptor neurons if differences in the activation of similar receptor neurons in different locations of the epithelium are preserved downstream of the epithelium (see *Spatial codes in olfaction?*, below). With the number of receptor genes currently estimated at 86 for rats, over 150 for humans (Mombaerts, 1999) and 57 for Drosophila (Vosshall et al, 2000), the number of receptor types is thus substantially larger in olfaction than in vision, and thus olfaction's stimulus space is considerably larger than trichromatic color space. Why, then, does olfaction employ so many more receptors than color vision? Part of the answer lies in the fact that the chemical universe of molecules is nowhere as tidy and linear as the physical universe of electromagnetic radiation: while the color of a monochromatic ray of light can be described by a single number, that of its frequency, describing the chemical structure of a single molecule is more complicated. Furthermore, while the light absorption properties of photoreceptors allow for a complete specification of a visible wavelength by the relative photon absorption levels of two to four (Land, 1977) receptor types with overlapping wavelength-absorption curves, no three receptors are known whose relative affinities for different odorants uniquely specify any odorant.

But is the comparison between the number of molecular photoreceptor types and the number of molecular olfactory receptors a good reflection of the size of the stimulus space for vision and olfac-

7. All four photoreceptor types are employed for a range of brightness levels we call mesoscopic vision. Under bright illumination conditions, only the three cone types contribute to the signal. In scotopic (dark) conditions, only the rods are sensitive enough to respond (Wandell, 1995).

8. The visual representation, of course, changes throughout visual processing. The present description applies to the initial signal in the retina. Downstream, light signals get converted into two color-opponent maps and one luminance map (Wandell, 1995).

tion? I will argue it is not. It is certainly true that olfaction's stimulus space has a higher dimension than *color* space: a color perceived by a human can be completely specified with three numbers, while an odor perceived by one would presumably require ~150. Color space plus luminance is a valid description of visual space for systems that do not form an image, i.e., for the vision associated with the luminance and/or chromatic information in a single point in space, such as that associated with a simple ocellus or ommatidium. But we do not recognize a face by analyzing the color of a single pixel. Rather, visual recognition is the process of identifying a pattern in an image. For each eye, an image is the specification of color and luminance for each location in the retinal surface, or the activities of 5 million cones and 100 million rods (Wandell, 1995). Even if we restrict ourselves to the number of retino-ganglion axons leaving through the optic nerve, there are 1.5 million of those (Wandell, 1995). So the input space of visual cortex is closer to 1.5 million-dimensional than it is to three-dimensional.

The computational nature of any spatial olfactory maps

Olfaction, on the contrary, has many receptor types, but exhibits a very important difference with respect to vision: as far as we know, noses do not form olfactory images (but see §1.19.2). In other words, because of the nature of the transmission of odorants, the spatial distribution of odorant on the olfactory epithelium is not *directly* related to the location of odor sources in the outside world, in contrast with the way in which photons on the retina are directly informative about the location of the objects they come from. As a consequence, the spatial map in the olfactory epithelium does not seem to be preserved in the next layer of olfactory processing. Rather, as we will see in more detail in §1.15.1, a process of remarkable convergence takes place that collects the signals from the all the olfactory receptor neurons expressing the same olfactory receptor type in the same pair of glomeruli in the olfactory bulb (antennal lobe in insects). This means that the dimensionality of olfactory

space is much smaller than that of visual space: if there is no spatial map of receptors, the dimensionality of the output of the olfactory epithelium is only twice the number of olfactory receptor genes (once for each nostril or antenna). In this sense, olfaction appears more similar to hearing than to vision, in that no spatial map of the input is present in the periphery, and any spatial map must be computationally reconstructed from the comparison of the signals arriving in each hemisphere or from a temporal reconstruction of signals collected at different places or different times.

1.10.3 Stimulus dynamics: The nature of odor plumes

So far, we have described olfactory stimuli in any one moment in time. But olfactory systems do not operate on single moments in time, but rather on a time continuum. In most olfactory environments, the simple diffusion of odorant molecules is a negligible means of dispersing odorants (Hopfield, 1991; Murlis et al., 1992). Odors of distant objects are brought to the nose by wind (Hopfield, 1991). Odorant molecules leaving an object follow the path of the air packet into which they evaporate (Hopfield, 1991). This packet already contains odors from upwind objects. The packet will slowly mix with odors from nearby packets, due to microturbulence in the air (Hopfield, 1991). As a result, the odor plume is increasingly mixed with odors from other parts of the environment as time increases. Thus, the stimulus at the nose due to distant objects contains mixtures of odors from many sources, whose relative contributions are constantly changing (Hopfield, 1991). A passive detector placed away from a source experiences intermittent odor pulses lasting from a few milliseconds to more than a second, with interpulse intervals between several 100 ms and minutes (Laurent, 1999). What useful information is there in these temporal fluctuations? Moore and colleagues have shown they carry information on the size, direction and distance of the odor source (Moore et al., 1989; Murlis et al., 1992). The intensity of the odor of a nearby object varies strongly as the wind direction changes. A distant object, in contrast, has an odor plume that is more contorted, broader and weaker, and its

strength will vary less with fluctuations in the wind direction (Hopfield, 1991). The relative timescale of variation also contains information about distance (Hopfield, 1991). Jelle Atema's group at the Boston University Marine Program in Woods Hole, MA, further showed that these fluctuations carry information about upstream obstacles to flow in aquatic environments (Dittmer et al., 1996), and extended these one-sensor results through the identification and statistical analysis of dispersal patterns in a turbulent odor plume using a pair of sensors separated by the 3 cm distance of lobster lateral antennules (Grasso, Basil and Atema, personal communication).

Do animals *use* the information in the temporal fluctuations in olfactory stimuli, though? There is behavioral evidence to suggest they do (reviewed by Murlis et al, 1992): moths flying upwind to a pheromone source will fly faster and straighter upwind, and locate sources more frequently if the plumes are either turbulent or mechanically pulsed than if they are continuous and narrow (Mafra-Neto and Cardé, 1994). Furthermore, lobster chemoreceptor cells show maximum stimulus intensity discrimination when stimulated with odor steps of ~200 milliseconds and showed clear responses at even the shortest pulse durations used (50 msec), demonstrating that they resolve odor peak onsets within the time window corresponding to the 4-5 Hz frequency of olfactory sampling as well as the rapid fluctuation in odor concentration common in natural odor plumes (Gomez and Atema, 1996). Under repetitive stimulation conditions in an aquatic environment, flicker-fusion frequency (that at which two pulses become indistinguishable from one) and synchronization with the stimulus pulse train were concentration dependent: performance rates above 1 Hz became poorer both with increasing pulse amplitude and frequency (Gomez et al., 1999). Flicker fusion frequency was 3 Hz for 100 mmol/l pulses and 2 Hz for 1000 mmol/l pulses. Individual cells showed differences in their stimulus pulse following capabilities (Gomez et al., 1999). These individual differences may form a basis for coding temporal features of an odor plume in an across-fiber pattern (Gomez et al., 1999). Temporal resolution is substantially better in a land animal: cockroach olfactory sensory neurons are able reliably to follow 25 ms pulses of the pure odorant 1-hexanol and 50 ms pulses of the complex

odor blend coconut oil (Lemon and Getz, 1997).

Having examined the nature of the inputs to the olfactory system, what is olfaction's task? What kind of processing shall we expect to turn inputs into outputs usable by behavior? This is what we now turn our attention to.

1.11 Olfaction: The computational problem

Having summarized the range of behaviors that olfaction serves and the nature of the inputs it operates on, we are ready to extract from them the essence of the computational problem at hand, the first level of analysis of any information processing task in Marr's scheme (Marr, 1982). This is important because the nature of the computations that underlie perception depends more on the computational problems that have to be solved than upon the particular hardware in which their solutions are implemented. This becomes particularly obvious when considering the similarities in the principles at work in the olfactory systems of species with hardware as diverse as insects and mammals (§1.12).

First and foremost, olfaction is a process that produces, from raw olfactory receptor activation maps, a description that is useful to the animal. A process may be thought of as a mapping from one representation to another (Marr, 1982). Having examined the nature of the input representation (§1.10), we now ask how that representation must be transformed in order to serve the purposes of odor-mediated behaviors. What is the output of olfaction?

Clearly, the output of olfaction depends on the behaviors it must guide, and thus must vary from species to species (see Marr, 1982). There are likely to be important similarities, though. The first function that the olfactory system needs to perform is to segment the olfactory inputs to separate

individual olfactory sources, or objects (Hopfield, 1991). This is necessary because the olfactory environment is rarely devoid of noise in the form of multiple odor sources, which must be distinguished from one another if a source is to be identified. Hopfield and colleagues have proposed several algorithms by which this can be done (Hopfield, 1991; 1995; 1999; Hendin et al., 1994): by analyzing the fluctuations in concentration common to all odors traveling from the same source, components corresponding to the same source could be grouped together and separated from other sources or noise in the environment. There is indeed behavioral evidence that *Limax maximus*, a mollusk, can discriminate two food odors from separate sources separated by 1 cm but not two odors if they originate at the same location (Hopfield and Gelperin, 1989). Moths can discriminate between a pheromone and an antagonist as long as the sources are 1 mm apart (Fadamiro et al., 1999). In humans, delays of 200 to 400 ms between two odors presented monorhinically or dichorhinically (through one or two nostrils) gave significant increases in the frequency of detection of components, whereas synchronous mixtures favored the perception of a single blended odor (Rouby and Holley, 1995).

In addition to source separation, the olfactory system needs to eliminate background contaminants for successful recognition, and in the case of simple mixtures of 2-3 known components, it is sometimes able to separate these components, a computational problem in and of itself (Hopfield, 1999).

For each 'olfactory object' (source or component), these are the basic outputs that must be computed by most olfactory systems:

Familiarity: The system must establish whether the odor has been experienced in the past, or whether it is in the presence of a novel odor. Even beyond the problem of specifying an algorithm to arrive at this, this problem is hard to define given the arbitrary nature of what is to be considered sufficiently dissimilar from any previously experienced odor so as to warrant classification as 'novel' (see §1.11.1). Even in the absence of identification, judgment of novelty (vs. familiarity) can aid an

animal by putting it on the alert in the presence of something unusual.

Identification: Related to familiarity, identification consists in arriving at identifying information for an odor for cases in which a percept has been experienced previously. I will show that single projection neurons in the antennal lobe of the locust contain substantial information about odor identity in Chapter 4. Importantly, identification needs to be invariant to concentration (at least to some degree), given that a given odor will seldom be encountered at exactly the same concentration twice. Two ways in which the olfactory system of the locust addresses this problem will form the subject of Chapters 7 and 8. Identification (and consequently encoding) in humans seems to involve at least two different representations: a verbal one, and a nonverbal one stored in the right hemisphere (Ilmberger et al., 2001). Even though identification could in principle be performed concurrently with the assessment of familiarity, the latter appears to precede identification in human sensory systems, as evidenced by the tip-of-the-tongue phenomenon, also called semantic retrieval failure, in which subjects report familiarity with an object before being able to name it or provide other identifying information (Brown, 1991).

Association: Association is intimately related to identification, and in fact probably constitutes the method of identification for neurobiological sensory systems, but is distinct in its scope from it. Identification entails associating one unique identifying character with a percept, such as the name of an odor; association, in contrast, entails associating a host of percepts to the eliciting percept, such as times and places of previous occurrences, visual appearance of the odorant, etc. In reality, identification is probably given by a subset of the associations, which are not instantaneous or simultaneous, so that some associations may take longer than others. Association is key for adaptive behavior, allowing contingencies with predictive value based on previous experience to guide behavior.

Valence: Valence is a special case of association: the association of a percept with positive or neg-

ative reinforcement value. These associations, or value systems, play a key role in shaping behavior, especially in early development when more subtle and complex behavioral plans are absent and behavior is guided by the immediate urges given by positive and negative reinforcement. I will show that particular odors have an innate valence for locusts in Chapter 3.

Intensity: Intensity is a perceptual description, created by the brain, related to the physical concentration of an odorant. The two are not synonymous, though; changing the concentration of an odorant, for example, can lead the percept to change in odor quality and even in valence rather than in intensity (Alcorta, 1991; Ayyub et al., 1990). Intensity judgments are used in evaluating the direction of a trail (Schöne, 1984) and also carry information on the distance to a source (Moore et al., 1989). I will look at the coding of intensity in projection neurons in the antennal lobe of the locust in Chapter 7.

Direction to source: The direction to an odor source is not present directly in the stimulus but rather must be computed by the brain, using, for example, time arrival or concentration differences between both nostrils or olfactory appendages (von Békésy, 1964) or spatio-temporal fluctuations in concentration during successive samplings (Moore et al., 1989). Many olfactory-guided behaviors, including foraging and navigation, require an assessment of the direction to the source of an odor perceived.

Distance to source: Like direction, the distance to an odor source is not carried explicitly by odors and must be computed using the parameters above, plus knowledge of the concentration at the source, when available.

Needless to say, some animals will require specific additional outputs from their olfactory systems. Golden hamsters, for example, will require an output specifying whether a particular scent overmark is on top (Johnston and Borade, 1998).

1.11.1 The computational role of learning in olfaction

Olfaction is an extremely plastic sense, from the very beginning. Rabbit pups, for whom the period of parental care is particularly brief due both to the risks of predation, which forces them into a closed nursery burrow while their mother forages for food, and to the short inter-litter period (26 days), show a preference for food in the diet by their pregnant mothers (Hudson and Distel, 1997). Human fetuses also learn odors from their pregnant mother's diet (Schaal et al., 2000).

The advantage of a learning system is obviously adaptability. Let us consider exactly what needs to happen during learning.

Perhaps the hardest aspect of the pattern recognition problem is the fact that the boundaries around the patterns to be recognized are somewhat arbitrary. In other words, who's to tell the olfactory system that ethyl acetate at a concentration of 10^{-6} should be recognized as the same attractive odor as ethyl acetate as 10^{-5} at low concentration, but that that very odor at a concentration of 10^{-1} should be avoided, and that an intermediate concentration should be recognized as neither of those two? And yet that's exactly what the behavior of unconditioned flies shows toward most odorants (Ayyub et al., 1990; Alcorta, 1991; Acebes and Ferrús, 2001). It must be remembered, then, that the function of olfactory identification is *not* to *reconstruct* the exact nature of the odorant, but rather, to *classify* it as pertaining to the *closest or most likely* class of olfactory memories (using the term to mean odor templates learned or innate), or as a novel odorant altogether. The problem is the same for learned odors: what the process of encoding a novel odor must do is to *imprint* the energy landscape, in the sense of Hopfield's energy function, with a valley leading from the representations of stimuli *similar* to the one being learned to the representation of the stimulus being learned, which will henceforth act as an attractor. Exactly how wide such a valley should be, or how dissimilar an odor

can be before avoiding recognition, is not known. I will address this important topic in Chapter 9, showing that the width of the valley itself is plastic and subject to the influence of experience.

1.12 Convergent evolution? Insects as a model system

The brain is an immensely complex system. To makes things even more difficult, the brain is not a neat modular machine, but rather, each area usually receives feedback from the very brain regions to which it projects, making the isolated study of individual neurons or even brain regions rather limiting (Koch and Laurent, 1999). Luckily, evolution has provided us with a way out —or a way in, so to speak: complex organisms were not created *ab initio*, but rather gradually through a procession of evolutionary steps. Unfortunately, the original ancestors are usually not available to us anymore: every creature alive today has been evolving for the same amount of time: since the beginning of life. Some species, though, appear to have evolved less complexity over evolutionary time than others. And, while biological complexity is very difficult to define (Koch and Laurent, 1999), there are some objective parameters that correlate intuitively with some notion of complexity or at least with our ability to monitor a system's activity. While mapping out the complete connectivity of the nematode *C. elegans* (White et al., 1984) might not get us anywhere close to a complete understanding of its nervous system, it probably brings us closer than not knowing it. And it is certainly harder to obtain such a connectivity map for a brain with billions of neurons than it is for one with 302. Even if you disagreed with the notion that complexity scales in some way with the number of neurons, an experimental reality is that, with the current limitations in our ability to record simultaneously from large numbers of neurons, the number of neurons about whose activity we are ignorant increases with the number of neurons in the brain, giving small brains a practical advantage for the neurophysiologist.

Just how simple a brain do we want to tackle first? Ideally, one that is as simple as possible without losing the computational principles at work in more complex brains. A unicellular organism counts with chemodetection, but clearly no nervous system and no real olfactory system. Perhaps the next obvious candidate would be *Caenorhabditis elegans* , since it can smell and has the advantage that the connectivity of every neuron in its nervous system is known. But the worm has so few cells compared to the number of genes in its genome that it has evolved an entirely different computational strategy, one that expresses up to 20 receptor types in the same receptor neuron (Bargmann and Horvitz, 1991; Colbert and Bargmann, 1995; Troemel et al., 1995, 1999) and thus different from the one-receptor-type-per-neuron doctrine that appears to hold for so-called higher animals (see §1.14). *C. elegans* also differs from higher organisms in many other respects: it has a single neuron expressing each receptor type, and lacks glomeruli, for example. Therefore, even if it may prove a useful model to understand the cellular mechanisms at work in olfactory receptor neurons, the worm is not a satisfactory model system for the vertebrate olfactory system as a whole.

Insects have received a substantial amount of study over the past century or so. Their behavioral repertoire is wide enough to make them more adaptable than most any man-made machine. And yet with a locust brain comprising 360,000 neurons in a volume of 6 mm^3, compared to 100 billion in the 1350 cm^3 of a human brain, it seems like an awfully good place to start. Now, if insect brains were completely unrelated to human brains, they might be easier to comprehend, but that would still not bring us anywhere closer to understanding our brains. While the understanding of insect olfaction would constitute a worthy pursuit in itself, both for intellectual and practical reasons —insects are agricultural pests, disease vectors and are responsible for pollination and for the production of honey—, it is made all the more fascinating by the remarkable parallels between the structure and function of the olfactory systems of insects and vertebrates. It is to this common design that we now turn our attention for a brief overview before we examine each stage of olfactory processing in detail.

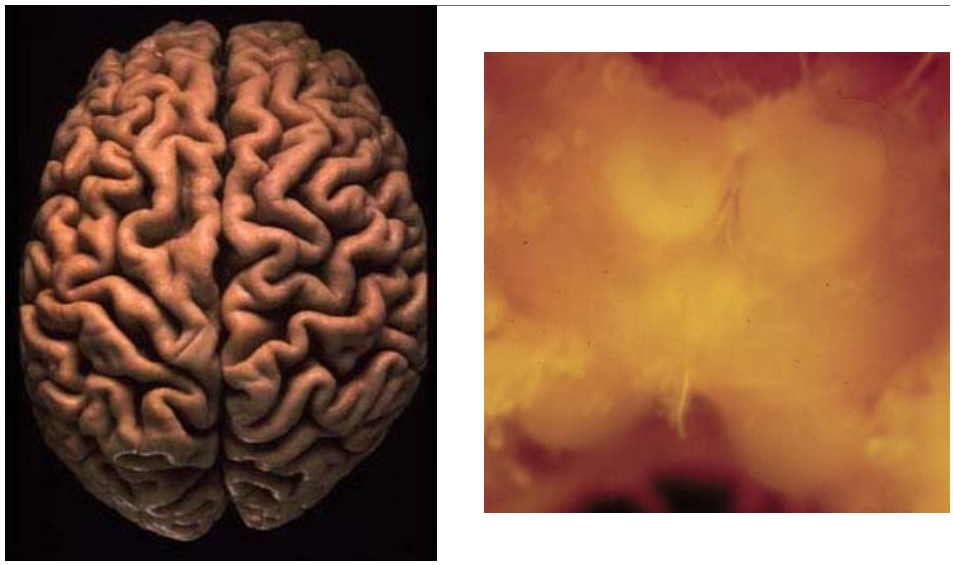

Figure 1.2. A human brain is made of 100 billion neurons and has a volume of 1350 cm^3; a locust brain is composed of 360,000 neurons and is a mere 6 mm^3 (human brain photo courtesy of Virtual Hospital, University of Iowa; locust micrograph by the author).

1.12.1 A brief anatomy of the olfactory system

In both insects and vertebrates, there is massive convergence from receptor neurons to the next processing layer (the vertebrate olfactory bulb [OB] and the insect antennal lobes [AL]) and massive divergence again from there to memory areas. Receptor neurons in both insects and vertebrates are likely to express a single or very few odorant receptor genes (Mombaerts, 1999; Vosshall et al., 2000).

The second processing relays of the olfactory systems of insects, crustaceans, and most vertebrates feature glomeruli, discrete structures of neuropil that have been described as one of the most distinctive structures in the brain (Shipley and Ennis, 1996). Each glomerulus receives projections from receptor neurons expressing the same type of odorant receptor genes (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Vosshall et al., 2000). Even more strikingly, perhaps,

the proteins responsible for glomerular structure, glycoproteins expressed in glial cells, are antigenically very similar and comparable in size in insects and mammals (Krull et al., 1994; Gascuel et al., 1996). These growth inhibitory extracellular matrix molecules (e.g., tenascin and chondroitin sulfate proteoglycans [CSPGs]) have been localized around glomeruli, and it has been suggested that these molecules form a macromolecular wall that restricts axon growth to glomeruli (Gonzalez et al., 1993; Gonzalez and Sliver, 1994; Kafitz and Greer, 1998). Interestingly, these molecules are also responsible for the distinctive patterns of neuropil in rat barrel cortex (whisker somatosensory cortex) (Tolbert, 2000), another structure where the targets of a discrete number of sensory inputs are well separated from each other (see §1.15.1). These second processing relays of both insects and vertebrates have excitatory projection neurons (vertebrate mitral/tufted cells and insect projection neurons [PNs]) and local inhibitory neurons. In addition, mollusks, arthropods and chordates exhibit odor-evoked oscillatory synchronization, whose function has been an enigma for over half a century (Adrian, 1942).

Vertebrate mitral/tufted cells and insect PNs distribute information about odors to several structures in their respective brains. Among those brain targets, two are possibly comparable between phyla (Strausfeld and Hildebrand, 1999). In mammals, the piriform cortex is the main recipient of inputs from the OB and, like the bulb, shows odor-evoked fast oscillations (Ketchum and Haberly, 1993). Mitral/tufted cells also project to other areas of the cortex, including the entorhinal cortex, which sends axons to the hippocampus. Both cortical regions may have parallels in insects. In neopterans, all PNs extend an axon ipsilaterally into a prominent area of the forebrain called the superior lateral protocerebrum and lateral horn (Hornberg et al., 1988). A subset of these PNs provides axon collaterals to the calyces of the mushroom bodies, which are paired, lobed centers consisting of many thousands of intrinsic neurons (Kenyon cells) arranged approximately parallel to each other. Mushroom bodies have been implicated in olfactory learning and memory (Heisenberg, 1998). As in olfactory cortex (Ketchum and Haberly, 1993), neurons postsynaptic to AL PNs, in a region of each

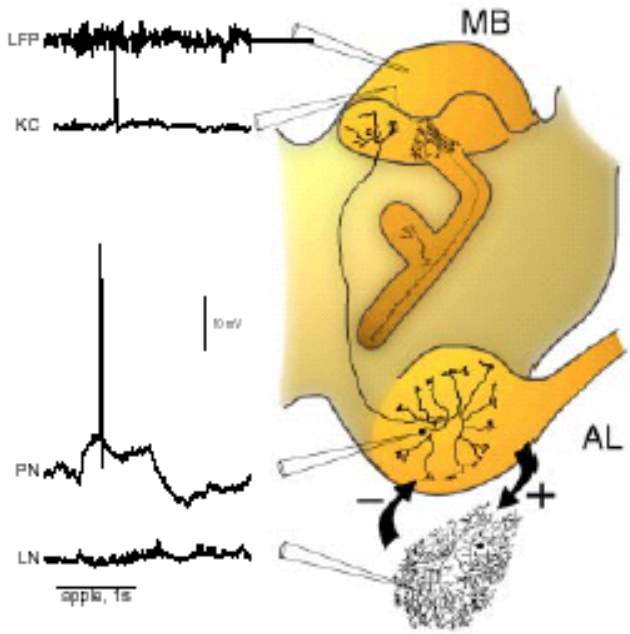

Figure 1.3. The locust olfactory system. Receptor afferents project from the antenna (not shown) to the antennal lobe (AL). The AL consists of projection neurons (PNs) which are spiking, excitatory cells with discrete glomerular arborization patterns, and local neurons (LNs) which are nonspiking, inhibitory cells with global arborization patterns. Both cell types, as well as Kenyon cells (KC), experience odor-evoked membrane potential oscillations as shown in representative intracellular recordings at left in response to apple odor (each cell recorded separately in different animals and aligned to the 1s odor pulse, indicated by solid bar). Projection neurons project to the mushroom body, where odors evoke oscillations in the local field potential (LFP, here lowpass filtered at 50 Hz). Kenyon cells project to the α- and ß-lobes of the mushroom body. Cobalt fills courtesy of G. Laurent. From Wehr (1999).

mushroom body called the calyx, exhibit synchronous activity (Laurent and Naraghi, 1994; Pérez Orive et al., unpublished). Axons from the calyx project to the lobes of the mushroom body, which, like the mammalian hippocampus (Morris et al., 1982), is involved in place-memory functions (Mizunami et al., 1998) and context-specific sensory filtering (Strausfeld and Hildebrand, 1999).

Do these similarities in organization reflect common origins or convergent evolution imposed by the common function of olfactory systems across phyla? The apparent lack of homology between olfac-

tory receptor gene families in insects and mammals, as well as the important differences in olfactory systems of intermediate species, suggest that convergent evolution is responsible (Strausfeld and Hildebrand, 1999). The last word, however, has not been said; molecular studies of the genes involved in the rest of the olfactory system will be of great value in this endeavor.

Figure 1.4. The locust, *Schistocerca americana*.

Having settled on insects as our model system, we need to settle on a particular species for our experiments. My choice of the American locust, *Schistocerca americana*, was largely historical and owed itself to the pioneering studies by Gilles Laurent and his laboratory. The locust is particularly suitable for studies of a generalist olfactory system because of its polyphagous nature (Lee and Bernays, 1988; Bernays and Lee, 1988). There are two distinct advantages of the locust over other insects for its use in electrophysiological investigations: it is a relatively large insect, making surgery easier, and it possesses relatively large neurons, making electrophysiology comparatively easy and allowing us to hold intracellular recordings of single neurons for hours at a time.

Having described the nature of the computations that the olfactory system must perform and the nature of the inputs it counts with for this purpose, and having explained the great similarities between insect and vertebrate systems that allow us to draw on both literatures for the description of the principles at work, the next few sections describe the actual nature of the system, the way we understand it at present.

1.13 Getting odorants to and away from receptors

1.13.1 The active and pulsed nature of olfactory sampling

The olfactory system is not a passive system, and does not rely only on the natural variations in odor concentration. In vertebrates, the sampling of olfactory space is pulsed due to a process known as sniffing (Freeman, 1978; Gray and Skinner, 1988), although dogs can inhale continuously if they are following a trail while running (Steen et al., 1996). In insects, a similar pulsed sampling process can occur due to systematic flicking of the antennae (Mellon, 1997). It has been consistently found that the inhalation and exhalation processes in dogs are complex and are modified by the behavioral task that the dog is performing (reviewed in Kauer and White, 2001), suggesting that the natural statistics of odor plumes are further complicated by the complex sampling mechanisms inherent in sniffing.

1.13.2 The olfactory epithelium is covered by an aqueous mucosa

Before odorant molecules reach olfactory receptors, they must first cross an aqueous interface. In vertebrates, the olfactory epithelium is covered by the nasal mucosa, which is 5–30 micrometers

deep depending on the species and living environment (Menco, 1980). In insects, olfactory receptor neurons (ORNs) are covered by the sensillar lymph, which lies below the cuticular walls of the sensilla.

1.13.3 The rate of air flow through the nose influences olfactory responses differentially for odorants of different sorptions

Different odorants sorb to and cross the mucosa at different rates (Mozell and Jagodowicz, 1973). In the bullfrog, a specific odorant's sorption rate interacts with the rate of airflow across the mucosa to produce varying amplitudes of response in the olfactory nerve (Mozell et al., 1991). This occurs because, when a high-sorption odorant has a low airflow rate, the odorant molecules sorb to the mucosa before moving very far along it. Only a small portion of the epithelium is involved in the response, which is small. When the same odorant flows at a high airflow rate, it spreads across a larger mucosal area before sorbing, so the response is larger. When a low-sorption odorant flows quickly, it moves past the mucosa without sorbing so the epithelial response is small. When the same low-sorption-odorant flows slowly, it has time to sorb across the mucosa and the response is larger (Mozell et al., 1991).

1.13.4 The bilaterality of olfactory sampling

The olfactory system counts with more than just time to look at statistical fluctuations in the distribution of odor plumes: invariably, from insects to mammals, it counts with two spatially separate sensors. Von Békésy (1964) has reported an amazing precision of localization of odor sources by humans. Within an angle of 65 degrees in either direction from the median plane, experienced

experimental subjects could localize an odor source 8 cm away from the nose to within 7-10 degrees. Closing one of the nostrils impaired performance severely (von Békésy, 1964). Using an odor delivery system with tubes connecting into a subject's nostrils, von Békésy (1964) described two processes that could be responsible for such accuracy: a simultaneous process with two sensors and a time interval measurement. For simultaneous measurement, a concentration difference of 5-10% was enough to localize the odor to the side of the nostril that received the higher concentration. In the time interval process, differences of only 0.3 ms in the arrival time of the odor at the right and left nostrils were enough to determine with side the odor came from. Not that the abilities of a single sensor location should be underestimated: even with one nostril plugged, though, a shark can sniff out pieces of food, moving the front part of its body from side to side (Hara, 1971). Bees orient using simultaneous sampling with both antennae or successive sampling depending on the steepness of the odor gradient (Schöne, 1984). Wasps and dung beetles with only one antenna can follow an odor gradient upward (Murr-Danielczick, 1930; Otto, 1951).

1.13.5 Flow rate through each human nostril is differentially regulated and contributes to differential sensitivity to different odorants

In addition to forming separate spatial olfactory images, both nostrils generate chemically distinct filters on the environment, further differentiating the information the brain gets from each. It has long been known that the flow of air is greater into one nostril than into the other because there is a slight turbinate swelling in one (Kayser, 1895; Principato and Ozenberger, 1970; Hasegawa and Kern, 1977). The nostril that takes in more air switches from the left to the right one and back again every few hours (Bojsen-Muller and Fahrenkrug, 1971).

This difference in airflow between the nostrils, combined with the differential dependence on flow rate of responses to odorants of different sorptions described above, causes each nostril to be opti-

mally sensitized to different odorants, so that each nostril conveys a slightly different olfactory image to the brain (Sobel et al., 1999).

1.13.6 The aqueous mucosa provides the olfactory system with invariance to volatility

The olfactory system faces a seemingly formidable challenge in reconstructing the concentrations of olfactory objects in the world, as well as the composition of simple mixtures, given that different substances have different volatilities, and thus the relationship between the concentration found in the gas entering the nose or surrounding an antenna on the one hand, and the concentration at the source on the other, will vary for each substance. We will see in Chapter 2, however, that the system has solved this problem with astounding elegance and simplicity.

1.13.7 The aqueous mucosa concentrates odorants

In land vertebrates, in addition to providing invariance to volatility, the aqueous environment may serve to provide an aqueous environment for the biochemistry of ligand binding. Providing a medium for odorant removal is likely to be another important reason to have an aqueous environment surrounding receptors (Pelosi, 1994). An aqueous layer also serves to concentrate odorants: calculation of partition coefficients using vapor pressures and solubilities shows that the concentrations in grams per liter for all but the most volatile of hydrophobic odorants are actually higher in the aqueous layer than in air by two to four orders of magnitude (Amoore and Buttery, 1978). These cannot be the mucosa's only function, however, since olfactory mucosa are also present in fish (Wehr, 1999). The aqueous mucosa may well serve a protective role for the ORNs, the most

exposed neurons in the entire nervous system: an aqueous layer prevents the receptors from coming in direct contact with potentially toxic substances (Wehr, 1999).

1.13.8 Odorant binding proteins (OBPs)

In insects, most of the sensillar lymph is constituted by a family of odorant-binding proteins (OBPs) and pheromone-binding proteins (PBPs) (Wehr, 1999) secreted by non-neuronal support cells (Kim et al., 1998). OBPs have been found in many species, including numerous vertebrates. In vertebrates, OBPs are members of the lipocalin transport family (Flower, 1996). Lipocalins typically function as carriers of hydrophobic molecules. Invertebrate OBPs, in contrast, constitute a unique family of low molecular weight, chemosensory-specific proteins with six conserved cystein residues (Kim et al., 1998). These show no homology with the vertebrate OBP family (Pelosi and Maida, 1995).

Odorants have been shown to bind directly to these proteins in both mammals and insects (Vogt and Riddiford, 1981; Pelosi et al., 1982; Pevsner et al., 1985; Pevssner et al., 1990; Du and Prestwich, 1995). Vertebrate OBPs bind odorants at the interface of a dimer (Bianchet et al., 1996) while insect OBPs bind ligands as monomers (Sandler et al., 2000). Binding experiments have been performed on bovine and pig OBPs, and have indicated a broad specificity for medium sized hydrophobic compounds, often of green or floral origins (reviewed in Pelosi, 1996).

The *Drosophila* genome contains at least 32 members of this gene family, rivaling the number of odorant receptors in this species (Kim and Smith, 2001). Unlike mammals, whose olfactory cilia are bathed in a common overlying fluid, most arthropods, including insects, have compartmentalized their olfactory neurons into sensilla (Kim et al., 1998). This compartmentalization provides the opportunity to independently regulate the composition of the fluid bathing the olfactory neuron dendrites. Indeed, in *Drosophila*, the identified OBP family members have surprisingly low sequence

similarity and are expressed in different, overlapping zones of chemosensory sensilla (Kim et al., 1998).

One of the *Drosophila* OBPs, *lush*, is required for normal olfactory avoidance behavior responses to a small subset of chemically related odorants (Kim et al., 1998), suggesting that OBPs participate in determining the chemical specificity of olfactory neurons in *Drosophila*. This result illustrates the critical importance of OBPs in olfaction, but it does not address the precise role played by OBPs, since a failure in any of the steps required to get an odorant to receptors would impair behavioral responses to the odorant. Remarkably, expression of a moth pheromone binding protein (not normally expressed in *Drosophila*) under control of the *lush* promoter causes an abnormal repulsion by moth pheromone in transgenic flies (Kim and Smith, 1997), although the concentrations of moth pheromone required are a million times higher than those required by moths (D. P. Smith, personal communication). This result suggests that individual sensilla constitute labeled lines to specific valences, and thus to behavioral reactions to particular odorants.

Moth pheromone-binding protein members of the same family have been shown to bind directly to pheromone with chemical selectivity *in vitro* (Du and Prestwich, 1995) and have been localized to trichodeal sensilla (known to sense pheromones), whereas general OBPs were localized to the generalist basiconic sensilla (Kaissling, 1986; Steinbrecht et al., 1992; Maida et al., 1993; Laue et al., 1994). Moth pheromone-binding OBPs have been found to be expressed in species that are not pheromone responsive too, though, showing that OBPs are not sufficient to confer chemical sensitivity (Zhang et al., 2001). OBPs have also been shown to be expressed in the pheromonal gland of the cabbage armyworm, which has no chemosensory structure (Jacquin-Joly et al., 2001).

What is the function of odorant binding proteins?

In addition to being present in high concentrations in the perireceptor space, OBPs also have a rapid turnover: complete replacement over 48 hours (Vogt et al., 1989). This must represent a large expenditure of energy, which surely is offset by a selective advantage. The facts from the previous section suggest that OBPs may be involved in the solubilization or transport of hydrophobic molecules across aqueous layers. This would be consistent with their influence on chemical selectivity of sensilla, since only hydrophobic odorants that bind an OBP might make it in sufficient concentration to the ORNs and be detected. Since airborne odorants are typically hydrophobic (Wehr, 1999), solubilization to concentrate odorants in the sensillum lymph is indeed very important for odorants to reach receptors. In vertebrates, however, the concentrations of OBPs are too low by an order of magnitude to affect odorant concentrations around receptors (Pelosi, 1994).

A second potential function for OBPs is to provide a means to detect small odorants that might prove difficult for a 7-transmembrane-receptor protein to bind, such as ammonia (NH_3). We know that ammonia is detected by mammals and insects alike (Meijerink et al.,2001). These molecules may prove too small for the pockets of a membrane-associated protein, and it is possible that biology has circumvented this limitation in a manner similar to the way in which other small gaseous molecules are bound in the circulatory system: by using proteins associated with a group, such as the haeme group, that changes conformation in the presence of particular gaseous molecules. On the other hand, quaternary ammonium compounds have been shown to inhibit voltage-activated Shaker K+ channels (Choi et al., 1993), and could be detected that way, and many small gaseous molecules, such as poisonous carbon monoxide, are odorless despite the potential behavioral advantage of detecting it (at least for modern-day humans), suggesting that the olfactory system has not quite circumvented the difficulties associated with detecting small gaseous molecules. More importantly, recent experiments have shown that the ligands for OBPs are larger molecules (Vincent

et al., 2000; Ramoni et al., 2001). 1-octen-3-ol, a typical component of bovine breath and in general of odorous body emanations of humans and animals, has been shown to be the natural ligand for bovine OBP (Ramoni et al., 2001). The recent structural characterization of porcine OBP binding properties (Vincent et al., 2000) has shown that a high degree of hydrophobicity coupled to a molecular mass between 160 and 200 daltons is the main requirement for a ligand to fit the b-barrel cavities of OBP, irrespective of the chemical class, substituents, and molecular structure. Furthermore, biology seems to have circumvented the difficulty of detecting small molecules, such a NaCl, with seven transmembrane (7TM) receptors by employing other membrane-bound receptors, such as the salty taste receptor, a 4TM receptor (Lindemann, 2001).

A third hypothesis (Pelosi, 1994) for the elusive function of OBPs, based on the belief that OBPs' affinities (K_D's of 0.1 to 20 mM) for odorants were poor relative to those of olfactory receptors, suggested that OBPs will bind odorants mostly at high concentration, which in turn suggested that OBPs may act as buffers, keeping odorants' concentration at intermediate values. In this sense, they would act to increase the dynamic range in which changes in odor concentration can be detected, much like the retina acts via multiple mechanisms to keep responses similar across an astoundingly wide range of light intensities. Olfactory receptors' affinities have recently turned out to be of the same order of magnitude, though (Pelosi, 1994). More importantly, experiments done with insect antennal sensilla appear to point in the opposite direction. When the sensillum lymph in *A. polyphemus* was replaced with saline solution, the electrophysiological response to the specific sex pheromones was greatly reduced; normal sensitivity was then restored by the specific purified PBP, but also by bovine serum albumin (Van der Berg and Ziegelgerber, 1991). These results suggest that the function of insect PBP is to concentrate the pheromone, increasing the sensitivity rather than buffering its concentration. In mice, too, the absolute sensitivity of ORNs was much higher in intact epithelium than in experiments using dissociated cells: cells in intact epithelium consistently responded to nanomolar odor concentrations (Ziesmann et al., 2001), suggesting the extracellular

environment contributes to heighten sensitivity rather than to buffer.

Mediating odorant removal constitutes another possible function of OBPs (reviewed in Pelosi, 1994). In vertebrates, the mucosa is constantly being discarded. In insects, however, the sensillum wall prevents this, and thus active mechanisms of degradation must be involved (Pelosi, 1996). The rapid turnover of OBPs suggests that the possibility that they either remain bound to odorant molecules for long periods and thereby mediate odorant removal, or else are permanently modified by binding to odorants and must be replaced after losing functionality.

More recent evidence, however, points to a role of vertebrate OBPs in the VNO rather than the olfactory epithelium (Pelosi, 2001). The elements of evidence towards this view include[9]

– OBPs are structurally similar to pheromone-binding proteins of urine, saliva and vaginal discharge; several subclasses of OBPs have been identified in the same animal species, each best related to a particular group of PBPs;

– OBPs are secreted by glands of the respiratory region of the nasal epithelium; from this area they are translocated to the VNO, but not to the olfactory mucosa; some OBPs are also synthesized in the VNO.

Volatile pheromones are able to activate the enzymatic cascade in the VNO leading to production of cAMP, while a PBP found in the urine of rats binds dissociated VNO membranes and leads to activation of the IP3 signaling cascade (Krieger et al., 1999): this observation would exclude the

9. The finding that olfactory receptors functionally expressed in cells not synthesizing OBPs are still able to respond to odors (Zhao et al., 1998; Wetzel et al., 2001), contrary to early assertions (Pelosi, 2001), does not suggest OBPs do not play a role in the vertebrate olfactory epithelium: on the contrary, recent evidence suggests that intact epithelium is significantly more sensitive to odorants than dissociated receptor neurons (Ziesmann et al., 2001).

requirement of OBPs, at least for one of the two transduction mechanisms active in the neurons of the VNO (Pelosi, 2001).

A final and very interesting role has been recently advanced for bovine OBP (bOBP) by Ramoni et al. (2001), who found that the natural ligand for bOBP is 1-octen-3-ol, a typical component of bovine breath and in general of odorous body emanations of humans and animals, as well as a chemoattractant for mosquitos and many insect species. They thus suggested that bOBP might be used by bovines to remove parts of 1-octen-3-ol from the breath flowing through the nasal cavities and to make them less appealing for several insect species. This would result in a general decrease of the number of insect bites and furthermore might partially protect the animal from parasitosis and infectious diseases carried by these insect vectors.

1.13.9 Olfactory degrading enzymes (ODEs)

Degrading enzymes distinct from OBPs have also been found in olfactory mucosa and sensillar lymph, with very high activity. Olfactory forms of cytochrome P-450, a degrading enzyme of broad specificity first described in the liver, have been found (Dahl, 1988; Ding and Coon, 1988; Nef et al., 1989; Ding et al., 1991), as have enzymes which further detoxify the products of P-450 (Longo et al., 1988; Lazard et al., 1990; Rama-Krishna et al., 1992). These proteins show activity levels in the olfactory system equal to or higher than those in the liver. The functions of these proteins have been speculated to involve protection against toxic odorants as well as removal of odorants to prevent continuous sensory responses. After all, the stimuli for vision and hearing only reach their target organs for as long as they are being emitted from the source, and even gustatory stimuli eventually get ingested, but odorants coming from transient olfactory stimuli would persist in the nasal mucosa or sensillar lymph were it not for an active removal or degradation process. Such a removal process

is particularly important given the importance of dynamic concentration information in guiding olfactory-mediated behavior (see §1.10.3). To investigate the role of ODEs in signal termination, Maida et al. (1995) correlated electrophysiological responses of the moth olfactory epithelium to pheromone with the activity of pheromone-degrading esterase across individuals. While the esterase activity was found to vary over two orders of magnitude, responses to the pheromone retained the same shape and amplitude, ruling out a role for it in signal termination.

1.14 A broad array of generalist sensors: Olfactory receptors

1.14.1 Olfactory receptor (OR) genes

Vertebrate olfactory receptor genes make up a huge family

Until recently, the molecular transducers responsible for odor detection were unknown. Although much remains yet unknown, the last decade has seen a revolution in our understanding of olfactory receptor (OR) genes spearheaded by Richard Axel's laboratory at Columbia University and a small army of postdoctoral fellows in his lab, now in their own laboratories around the country.

This explosion derived from the initial isolation of OR genes from the rat (Buck and Axel, 1991) using an experimental design based on three assumptions (Mombaerts, 1999). First, ORs were likely G-protein coupled receptors, and these are generally seven-transmembrane (7TM) proteins. This first and most critical assumption was based on biochemical evidence that had implicated G proteins in olfactory signal transduction (Pace et al., 1985; Sklar et al., 1986; Jones and Reed, 1989). The 7TM superfamily includes rhodopsin, dopaminergic, adrenergic, muscarinic and other receptor types (Dohlman et al., 1991). Second, ORs are likely members of a multigene family of a considerable size, because of the immense variety of chemicals that can be discriminated by the

olfactory system. Third, ORs are likely expressed selectively in olfactory receptor neurons (ORNs). Buck and Axel designed a series of degenerate PCR primers based on conserved regions of 7TM proteins, and amplified a multigene family from cDNA of olfactory epithelium. They then demonstrated by Northern blot analysis that members of this family are expressed only in the olfactory epithelium of the rat. Stuart Firestein and his colleagues then used an adenovirus vector system to overexpress a putative mammalian odorant receptor in the rat olfactory epithelium and measured elevated physiological responses to octanal and some related odorants (Zhao et al., 1998).

Screening rat genomic libraries suggested that the OR gene family has 500-1000 genes (Buck, 1992), making it the largest family in the mammalian genome (Mombaerts, 1999). Sequence divergence in this family is highest in transmembrane domains 3 to 5, which are believed to be involved in ligand binding in other 7TM proteins (Kobilka, 1992), suggesting, as expected, that different receptor genes bind with odorants of widely varying chemical structure. A given OR gene will typically cross-hybridize with a few others (Mombaerts, 1999). These sets of similar genes are called subfamilies. Because OR genes are intronless, genomic DNA has been successfully used to create primers to clone OR genes in multiple vertebrate species (Mombaerts, 1999). The genes form clusters throughout the genome, just like all gene superfamilies (Mombaerts, 1999).

There is a high frequency of pseudogenes in the human OR repertoire

A recently reported enigma is the high frequency of pseudogenes in the human OR repertoire (Mombaerts, 1999c): Because of frameshifts, non-sense mutations, and deletions, between 38 and 76% of the 500 to 750 OR-like sequences do not appear to encode full-length polypeptides (Mombaerts, 1999b). By contrast, no pseudogenes have been reported among 200 OR sequences in mouse and rat (Mombaerts, 1999b), and in other vertebrate species, OR pseudogenes are also

scarce. This raises the interesting issue of whether the pseudogenes contribute to perceptual diversity in the human population, with individuals having different pseudogenes (Mombaerts, 1999b). The massive degeneration of the human OR repertoire may be related to our inferior sense of smell relative to other species. Perhaps less selective pressure was exerted on the OR repertoire during the evolution of *Homo sapiens*, who apparently came to rely more on the visual and auditory senses (Mombaerts, 1999b). Analogously, the relative lack of pseudogenes in other species pinpoints the importance olfaction and the huge diversity of receptor genes has in those species.

Insect olfactory receptor genes appear not to be homologous to their vertebrate counterparts

Difference cloning, along with searches in the recently completed *Drosophila* genome, yielded a family of 57 7TM genes which are expressed in the third segment of each of its antennae (Clyne et al., 1999b; Gao and Chess, 1999; Vosshall et al., 1999; Adams et al., 2000; Rubin et al., 2000). Two recent experiments showed that this family indeed codes for functional odorant receptors. Stortkuhl and Kettler overexpressed the *Or43a* gene in the fly antenna and tested for an increase in odor response *in vivo*. *Or43a* is normally expressed in circa 15 olfactory receptor neurons (ORNs) of the antenna, but Stortkuhl and Kettler were able to drive its expression in a high fraction of the approximately 1,200 antennal neurons by using the GAL4/UAS system. They then found a concomitant elevation in antennal response to a subset of odors, as measured by electroantennograms (EAGs), which are extracellular recordings of the receptor potentials of populations of neurons. Stortkuhl and Kettler found that overexpression of the *Or43a* gene conferred increased response to cyclohexanol, cyclohexanone, benzaldehyde, and benzyl alcohol, each of which contains a six-member carbon ring with a single attached polar group. Responses to several other tested odorants, including some others containing six-member rings, were unaffected. In a second experiment, Vetzel and col-

leagues (2001) showed that heterologous expression of the *Or43a* gene in oocyte cells conferred oocytes responsiveness to cyclohexanol, cyclohexanone, benzaldehyde, and benzyl alcohol.

Interestingly, the family shows no homology with the vertebrate family of OR genes. The insect family as a whole is extremely divergent and exhibits from 17% to 26% amino acid identity. However, each of the genes shares short common motifs in fixed positions that define these sequences as highly divergent members of a gene family. As in vertebrates, analysis of the sequence of all 57 receptors reveals the existence of discrete subfamilies whose members exhibit significantly higher sequence identity, ranging from 40% to 60% The *Drosophila* OR genes are widely dispersed in the genome and most exist as single genes that distribute on each of the *Drosophila* chromosomes, although a few are in clusters of two or three genes (Vosshall et al., 2000). Given the high level of divergence shown by the OR gene families, however, ancient similarities may be hard to come by, and the last word in terms of arthropod and vertebrate olfactory receptor homology may be yet to come.

1.14.2 Olfactory receptor neurons (ORNs)

The number of ORNs substantially exceeds the number of ORs

The ‘nose’ of an insect is the third segment of each of its antennae, which bears olfactory sensilla housing olfactory receptor neurons (ORNs) that supply axons to discrete islets of neuropil called olfactory glomeruli (Strausfeld and Hildebrand, 1999). A locust antenna has 50,000 olfactory receptor neurons (Leitch and Laurent, 1996). In humans, each of the two nostrils is about 2.5 square centimeters containing in total approximately 50 million primary sensory receptor cells (Lefingwell, 1999). In dogs, the number is close to one billion. This number, tens of thousands to hundreds of thousands of times larger than that of inner hair cells, for example, surely serves a purpose. We will

explore this in §1.19.

Each ORN expresses a single OR gene

The olfactory system seems to have gone to great lengths to ensure that each ORN expresses a single OR gene. Two mechanisms are used to this end.

First, each ORN transcribes a single OR gene, both in mammals (Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994; Malnic et al., 1999) and in insects (Vosshall et al., 2000).

Second, allelic inactivation of odorant receptor genes ensures that only a single allele of an OR gene is expressed in each ORN (Chess et al 94). Whether the paternal or maternal allele is inactivated is chosen independently, apparently at random, in each ORN (Serizawa et al, 2000; Ebrahimi et al., 2000).

We will explore a novel hypothesis for the reason for this apparent selective pressure for ORNs representing a single chemical signature in §1.19.

Selectivity for odotopes grants ORs high specificity for some features and broad tolerance for others

Odorant receptors are thought to work by forming a pocket which binds epitopes in odorant molecules (odotopes). Thus, they provide a signature of 3-D structure of the odorant molecules, and are capable of discriminating enantiomers (Kraft and Frater, 2001). The response of a given OR type across different molecular odorants has been found to be highly specific for some molecular features and highly tolerant for others (Fujimura et al, 1991; Araneda et al., 2000; Wetzel et al., 2001).

This combination of wide and narrowly tuned detectors sensitive to features common to several molecules allows the olfactory system to be able to perform fine discrimination of thousands of odors (Araneda et al., 2000).

Bilateral symmetry in the early olfactory system

Wes and Bargmann (2001) recently showed that *C. elegans* odor discrimination requires bilateral asymmetric diversity in olfactory neurons. The same is true of taste neurons in *C. elegans* (Pierce-Shimomura et al., 2001). In insects and mammals, though, asymmetry in the olfactory bulb may be limited to fine structure. Experiments using optical imaging to assay the olfactory bulb's responses to odorants in mice, patterns of activated glomeruli were bilaterally symmetric and consistent in different individual mice, but the precise number, position, and intensity of activated glomeruli in the two bulbs of the same individual and between individuals varied considerably (Belluscio and Katz, 2001). In the honey bee, calcium imaging showed bilateral symmetry in the activation of the antennal lobes (Galizia et al., 1998). This symmetry held true for all odors tested, irrespective of their role as pheromones or as environmental odors, or whether they were pure substances or complex blends.

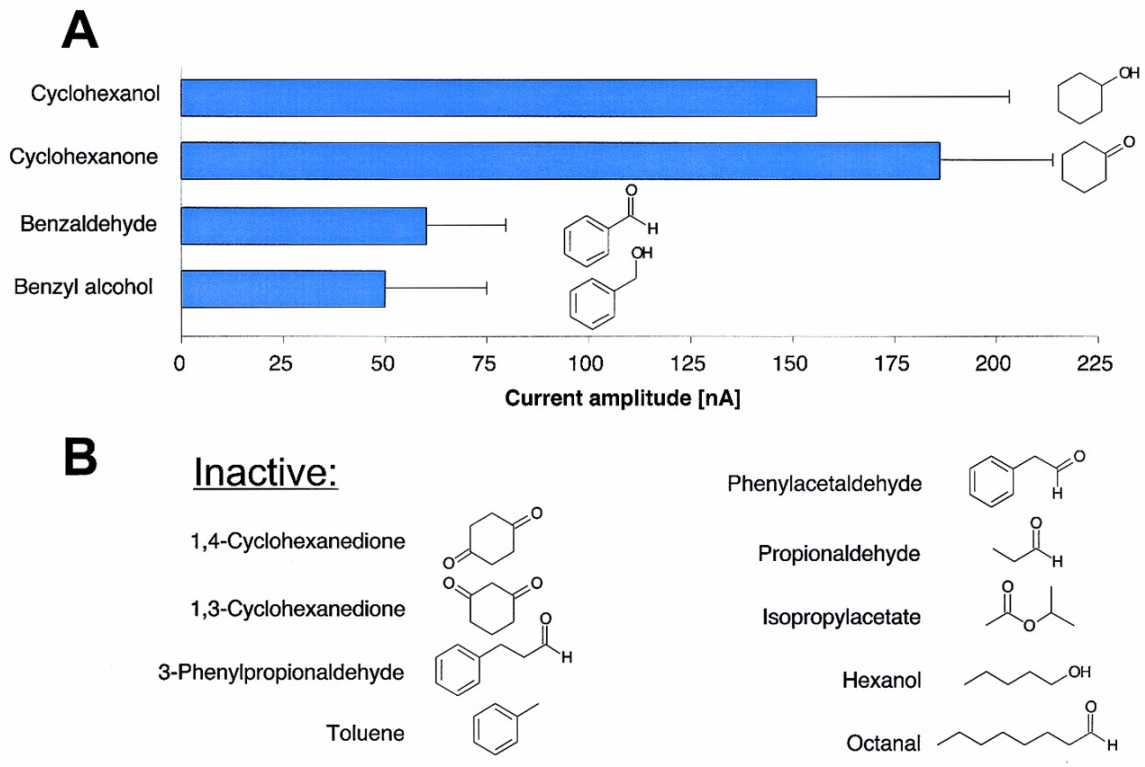

Figure 1.5. (A) Diagram showing the structure and the activity of the compounds tested on oocytes injected with Drosophila OR43a. The stimulatory action of agonists is presented as the peak amplitude of the induced currents (mean +- SE). The odor concentration was 1 mM. Only cyclohexanol, cyclohexanone, benzyl alcohol, and benzaldehyde were active as agonists at the Or43a. (B) Structures of compounds that were inactive at millimolar concentration at the OR43a (from Wetzel et al., 2001).

A topographic map of OR gene expression: Each OR is expressed apparently randomly within one zone of the olfactory epithelium

Both in mammals (Ngai et al., 1993; Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994) and in the fly (Vosshall et al., 1999;Vosshall et al., 2000; de Bruyne et al., 2001), each receptor gene is expressed in an apparently random subset of a zone within the olfactory epithelium, and, conversely, each receptor neuron expresses only one or a small fraction of the receptor genes.

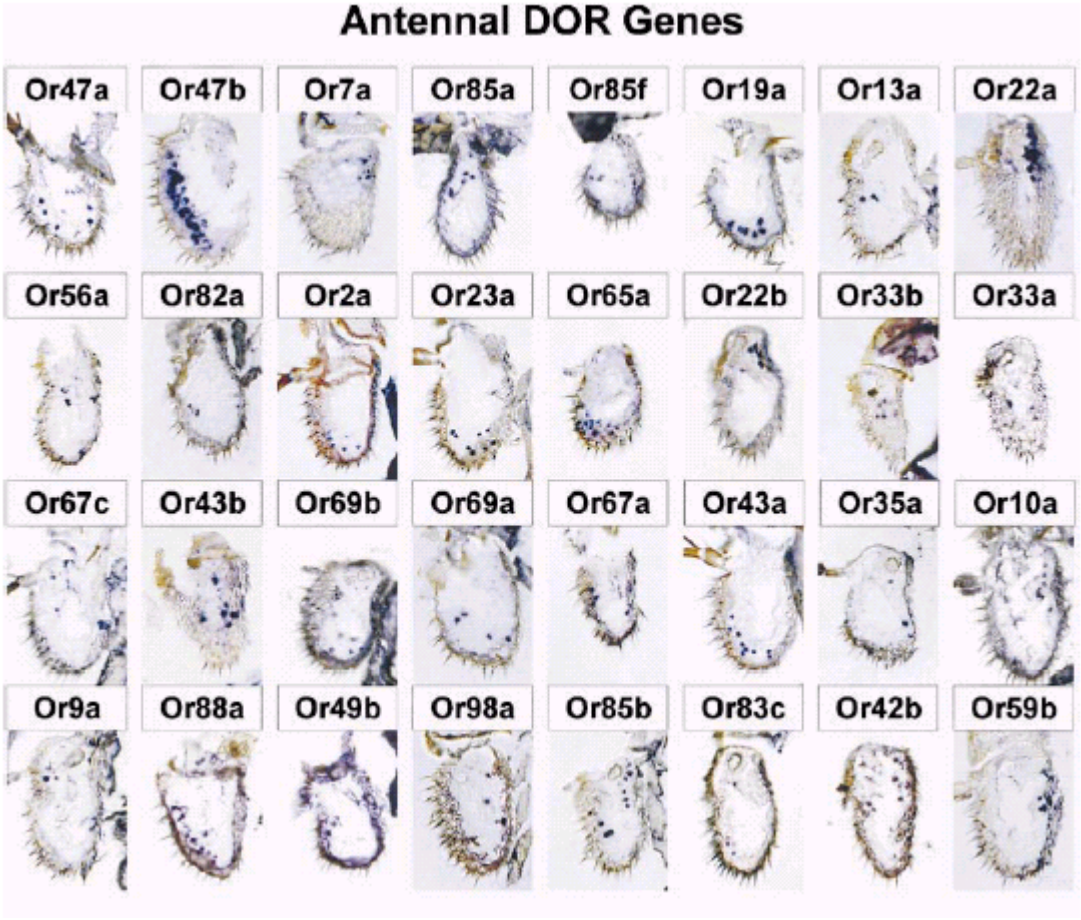

Figure 1.6. Each odorant receptor is expressed in a subset of odorant receptor neurons in the fly (from Vosshall et al., 2000).

Individual ORNs can show either excitatory or inhibitory responses to odors

ORNs exhibit multiple modes of response dynamics: an individual neuron can show either excitatory or inhibitory responses, and can exhibit different modes of termination kinetics, when stimulated with different odors (de Bruyne et al., 2001). In *Drosophila*, there are 16 ORN classes combined in ste-

reotyped configurations within seven functional types of basiconic sensilla. One sensillum type contains four ORNs and the others contain two neurons, combined according to a strict pairing rule (de Bruyne et al., 2001).

A case for non-classical receptive fields in olfaction

As had previously been shown for the lobster (Ache, 1994), Breer and colleagues have shown that different odorants activate different second messenger signaling pathways in the rat: one using cAMP and the other using IP3 and diacylglycerol (Breer and Boekhoff, 1991; Schandar et al., 1998). Brunet et al. (1996) showed, however, that targeted disruption of both alleles of the mouse olfactory cyclic nucleotide-gated cation channel eliminates extracellular electrophysiological responses to odorants that activate *either* of the two pathways. Despite the contention that these results are difficult to reconcile (Schandar et al., 1998), an interpretation is readily available if one considers the nature of non-classical receptive fields (those in which a stimulus causes a response detectable only in the presence of other stimuli), that have now been well demonstrated in vision. Taken together, these results suggest that IP3-activating odorants cause only suppressive or modulatory responses in ORNs, so their effect is not noticeable unless the cAMP pathway is also activated. Indeed, some odorants have been shown to suppress the inward current in newt olfactory receptor cells, by a mechanism that is distinct from inhibition and adaptation (Kurahashi et al., 1994). Suppression may sharpen the odorant specificities of single cells (Kurahashi et al., 1994). Given that most natural odorants are complex blends likely to contain components activating both pathways, an inhibitory or modulatory response may well be as important as an excitatory one in facilitation discrimination between odors.

Primary odors?

In vision, the concept of primary colors —the minimum set of colors necessary to create the percept of any color— has proved extremely useful, both from a theoretical standpoint, because knowing that vision depends on 3 to 4 receptor types is crucial to understanding color vision, and from a practical standpoint, because it has allowed the development of television using only 3 color signals (RGB) and of printers using only 4 ink colors (CMYK). In olfaction, in contrast, the concept has proved less fruitful, if only because the number of olfactory receptor types is so much greater than of photoreceptors. Nevertheless, attempts have been made to find primary colors via genetics, by finding inheritable specific anosmias —an increased olfactory threshold for specific compounds (Amoore, 1977). This is a tricky business, though: some anosmias could be due to regulator genes rather than receptor genes, and many receptor types could go unnoticed due to redundancy. In any case, we are still in the infancy of the mapping of the receptive fields, or set of agonists, for each OR.

How many odorant molecules are needed for behavioral detection? How many ORNs must be activated?

Like the visual system, the olfactory system both is remarkably sensitive and is able to operate over a huge dynamic range, possibly the entire available physical range. For the skunk odorant, for example, only about 40 receptor cells in the human nose need be stimulated by no more than nine molecules each to give a detectable odor sensation (chemoreception, Encyclopaedia Brittannica Online, 2001). Some insects have been reported to be capable of detecting single molecules (Kaissling and Thorson, 1980). At the other end of the scale, animals often discriminate odors at the physical maximum of concentration, given by saturated vapor —inside a flower for a bee or against a potential mate's behind for a dog, for example (Laurent, 1999).

Temporal resolution of olfactory receptor neurons

Adaptation and disadaptation rates determine the temporal response properties of sensory receptor cells. In olfaction, temporal filter properties of receptor cells are as yet poorly understood. In the lobster, antennular chemoreceptor cells recover from a 5–s adapting pulse after time intervals ranging from 1 to 60 s. After complete adaptation by the adapting pulse, individual cells recovered at different rates. After 1 s, a third of the cells respond with a mean response of 3 spikes/cell, representing approximately 20% recovery. Mean full recovery is within 25 s, with a time constant of 14 s, independent of stimulus concentration (Gomez and Atema, 1996b). A study in frog ORNs, on the contrary, found that the duration of adaptation increased with increasing concentration of the adapting pulse (Reisert and Matthews, 2000). The duration of adaptation has been found to increase with increasing durations of exposure (Getchell and Shepherd, 1978; Strausfeld and Kaissling, 1986).

The bandwidth of olfaction

In a provoking recent review, Gilles Laurent (2000) challenged readers to imagine reading an article with their noses: although possible in principle (one might learn to assign odors or concentrations to words or letters), the rate at which information could be conveyed appears to be low. Olfaction seems to be poor at following many or rapidly varying signals, he claimed, and is as such a low-bandwidth sense. Given humans' lack of reliance of olfaction, though, it might be more appropriate, in order to estimate olfaction's bandwidth, to think of a task that olfactory-guided animals might be adept at. Dogs, for example, are able to rapidly follow a track using odor cues: they are thus 'reading' the direction in which to go (an analog time-varying quantity requiring many bits for its digital representation) at every step of the way (Thesen et al., 1993). Dogs' ability to track on the fly is all

the more impressive when judged by the frequency with which my co-pilot fails to read a (visual) map in time to make the right turn in an unfamiliar environment.

Due to the difficulties involved in generating predictable and controllable natural odor stimuli, the stimuli used in the great majority of experiments on olfaction, unfortunately, including the ones described in this thesis, lack temporal structure: they usually consist of single pulses provided with relatively constant intensity from a nearby source. A step in the right direction has recently been made by two studies that used more natural odor sources that generate temporally varying plumes (Vickers et al., 2001; Stopfer and Laurent, unpublished).

It is true that sniffing usually occurs on a relatively slow time scale (Laurent, 1999), but it is not clear that olfaction is impervious to temporal fluctuations on a timescale faster than that of sniffing — vision is of course sensitive to temporal fluctuations faster than the frequency of eye blinks. Even though at least some insects produce stereotypic antennal movements in response to odor stimulation (Chee-Ruiter and Laurent, 1995), their olfactory systems clearly do not require those movements in order to respond to odors (Laurent and Naraghi, 1994; Laurent and Davidowitz, 1994; Laurent, Wehr and Davidowitz, 1996; Wehr and Laurent, 1996; Stopfer et al., 1997; MacLeod et al., 1998; this thesis). Lemon and Getz's data (1997) suggests that the olfactory system of the cockroach is indeed capable of updating its representations in response to stimulus changes every few tens of seconds, a timescale comparable to that of the visual system (Meister and Berry, 1999). Whether these rapid variations are present in natural odor plumes and whether they are behaviorally relevant remains to be ascertained. Electroantennogram (EAG) recordings in the presence of natural odor plumes in the field have already shown that insect receptors are capable of responding to temporal structure in the plumes that provides relevant information on distance to the source up to a frequency of at least 5 Hz (Murlis et al., 2000)[10]. The 5 Hz figure must be considered a minimum, given that the EAG is a very coarse measure of receptor activation and that responses at a

finer timescale of individual receptor neurons might be averaged away in it. Thus, it is important to remember that even if odor identity does not vary very rapidly in the field (even this is questionable for a bee rapidly flying from one flower to a neighboring one), this environmental constancy does not apply to the rapid fluctuations in concentration given by the spatiotemporal structure of odor plumes, and it may well be precisely these that occupy the bulk of the olfactory system's bandwidth. In summary, a characterization of the bandwidth of olfaction must await until we understand the nature of the information that the olfactory system recovers from natural olfactory stimuli.

Fetal ORNs respond to odors with no selectivity

Rat olfactory receptor neurons begin to differentiate from stem cells on day E10 of embryonic life in the rat. By day E16, the receptor epithelium is well populated and receptor neurons respond to odors. However, they were not selective. Each cell responded to nearly all of the substances in the stimulus set. The first synaptic connections between receptors and mitral cells are established on day E18. The olfactory marker protein appears first in the receptors on the same day. By day E21, single unit responses changed dramatically: the cells became selective, responding to about half of the substances in the set used (Gesteland et al., 1982).

The epithelial map of expression and patterning of genes encoding ORs can develop in mice lacking olfactory bulbs, suggesting retrograde influences of the bulb on the epithelium are not required (Sullivan et al., 1995). ORNs degenerate within 5-14 days of neuronal age in a bulbectomized adult rat, suggesting the olfactory bulb is necessary for prolonged survival of ORNs, as is the case in other

10. This timescale would allow a temporal representation of 4 cycles/stimulus in projection neurons in the antennal lobe of the locust (see below). The period in between bursts of detectable odor 'packets' is highly variable (Murlis et al., 2000), however, and longer interburst periods would allow longer temporal representations.

sensory systems (Schwob et al., 1992).

1.15 Noise reduction, analog to digital conversion and decorrelation: The convergence to the insect antennal lobe and the vertebrate olfactory bulb

1.15.1 Glomeruli: Converting a spatial code into an identity code

In insects, the antennal lobes (ALs), the structures immediately downstream of ORNs, exhibit an array of structurally and functionally identifiable glomeruli (Rospars and Hildebrand, 1992; Vickers et al. 1998; Galizia et al., 1998) from which classes of projection neurons (PNs) send axons to distributed nuclei in the forebrain or protocerebrum (Homberg et al., 1988). In vertebrates, the analogous structure is termed the olfactory bulb, and also exhibits glomeruli.

Glomeruli are compartments of neuropil constrained by glial cells to a location in the bulb or antennal lobe. Glial cells accomplish this by expressing tenascin-like molecules on their cell surface during the period of glomerulus formation. These molecules repel growing neurites of many AL neurons *in vitro* (see Hildebrand et al., 1997 for a review), and may constrain neuropil growth within glomeruli.

Similar molecules are involved in the formation of *barrels* in the rat primary somatosensory cortex, where inputs from each whisker are compartmentalized into units called barrels, which are the flat equivalent of glomeruli in the cortical surface.

By virtue of this compartmentalization, glomeruli convert a spatial code, embedded in the position of afferents, into an identity code, which carries information in the identity of the neurons activated. This would only be meaningful if there was indeed a spatial code in the afferents to the antennal

lobes and olfactory bulb. In 1994, Vassar and colleagues found that there is. The key to it lies in the projection pattern of olfactory receptor neurons to the antennal lobes and olfactory bulbs.

1.15.2 The convergence of like olfactory receptor neurons: Noise reduction?

ORNs expressing the same OR project to the same glomerulus

Using in situ hybridization with five different receptor probes, Vassar et al. (1994) demonstrated that axons from neurons expressing a given receptor converge on at most a few glomeruli within the olfactory bulb of the rat. Moreover, they found that the position of specific glomeruli is bilaterally symmetrical, and constant in different individuals. Each glomerulus receives converging inputs from about 3000 ipsilateral neurons (Meisami, 1979, 1989).

In Drosophila, ORNs, which are located within sensory hairs, send projections to one of 43 glomeruli within each antennal lobe of the brain (Laissue et al., 1999; Stocker, 1994). Drosophila ORNs expressing the same receptor project to the same one or two glomeruli in both the ipsilateral and contralateral antennal lobe (Vosshall et al., 2000). The sorting zone (SZ) region of the antennal nerve of the moth *Manduca sexta* comprises a glia-rich domain just outside the antennal lobe of the brain. During development, ingrowing olfactory receptor neuron (ORN) axons abruptly change their trajectories upon encountering this domain, lose association with their neighbors, and exit in large fascicles of axons destined for particular glomeruli.

Glomeruli have indeed been shown to be functional units in the encoding of odors in the input to the olfactory bulb and antennal lobe: 2-deoxyglucose, voltage-sensitive dyes and calcium imaging studies have all shown it is common to see a glomerulus respond in its entirety in response to an odor (Kauer and Cinelli, 1993; Galizia and Menzel, 2001).

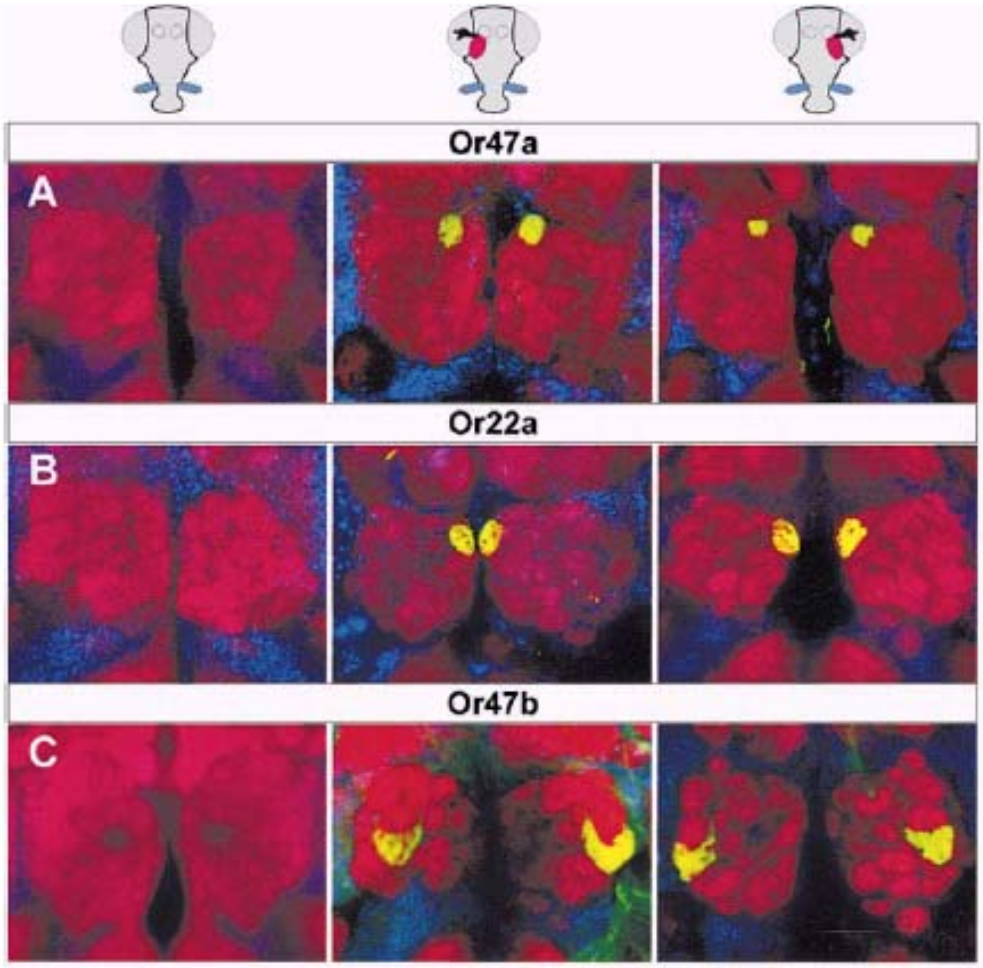

Figure 1.7. The set of receptor neurons expressing any given OR projects both ipsi- and contralaterally to 1-2 pair(s) of bilaterally symmetrical glomeruli in the fly *Drosophila melanogaster*. Cells expressing GFP under the control of different OR promoters project to different glomeruli. Bilateral deafferentiation resulted in complete loss of ORN staining in the antennal lobe (left). Unilateral deafferentiation of left (center) or right antenna (right) show labeling in both antennal lobes (from Vosshall et al., 2000).

The common wisdom is that convergence of like receptors to the same glomeruli achieves an increase of the signal to noise ratio through the averaging away of uncorrelated noise. We will discuss this further in §1.19.2.

Regarding summation of signals across afferents, we do know that the *Drosophila* mutant *gigas,* which establishes more synapses than normal, is attracted to concentrations of ethyl acetate to

which sibling controls are indifferent (Acebes and Ferrus, 2001). In addition, the intensity of responses is augmented at both attractive and repulsive odorant concentrations with respect to that of controls.

Are all inputs to a glomerulus equal? There is evidence to suggest they are not. In the mouse, input to a single glomerulus shows a dynamic range much greater than that reported for single neurons (Wachowiak and Cohen, 2001).

How do ORNs know where to project? A larger role for activity-dependence than previously suspected

The development of the complex connectivity pattern between ORNs and glomeruli is a flourishing field of study (see Mombaerts, 2001 for a recent review). Below, I summarize some exciting recent developments and put forth a novel proposal for an expanded role of activity dependence in shaping the connectivity pattern between epithelium and olfactory bulb.

Briefly, how ORNs know where to project is currently unknown. It has been argued that odorant receptor proteins are involved in the process of axon guidance to the bulb (Mombaerts et al., 1996; Wang et al., 1998; Mombaerts, 2001). The evidence for this is fourfold: 1. OR mRNA is present in ORN axons (Vassar et al., 1994), although data on OR protein expression in the axons is lacking; 2) mutations and deletions of the coding region of an OR gene lead to disruptions in the pattern of projection of the corresponding ORN to the bulb (Wang et al., 1998); 3) swapping one OR gene for another leads the ORNs to project to a third glomerulus that is neither the donor or the target (Mombaerts et al., 1996; Wang et al., 1998); 4) the projection patterns of ORNs and the odor response patterns of glomeruli are largely conserved across different individuals of the same species (Vassar et al., 1994; Galizia et al., 1998; Galizia and Menzel, 2001).

More recently, the developmental pattern has been shown to be more heterogeneous across the OR ensemble. In a study that shows the magnificent power of molecular biology used intelligently, Zheng et al. (2000) showed that, in mice rendered practically anosmic (Brunet et al., 1996; Parent et al., 1998) by a mutation in a cyclic nucleotide-gated channel, OCNC1, the projection pattern of ORNs expressing the P2 OR remain comparable to those in the wildtype, but that of ORNs expressing the M72 OR becomes more diffuse, terminating in additional glomeruli. When additional crosses were used to generate mice with both OCNC1-positive and OCNC1-negative ORNs expressing M72 in an OCNC1-negative background for ORNs expressing the rest of the ORs, axons from channel-positive and channel-negative neurons expressing the same receptor terminated in distinct glomeruli in the bulb. Importantly, OCNC1-negative axons converged mostly to glomeruli rather than projecting diffusely, and these glomeruli were close to the targets in the wild-type. Zheng and colleagues concluded that glomerulization per se and axonal pathfinding to a restricted area of the bulb are not dependent on OCNC1, and that neural activity subsequently refines the connectiviy pattern. In a follow-up study, Potter and colleagues showed that M72-expressing ORN axons occupy a large surface area of the bulb postnatally and coalesce into a single protoglomerulus only later in development, at a reproducible stage (Potter et al., 2001).

In a related truly beautiful and groundbreaking recent study, Zhao and Reed (2001) exploited the same phenomenon used by Zheng et al., X inactivation, to generate a mosaic mouse, half of whose cells expressed a wild-type copy of OCNC1 and half of which expressed a mutant, inactivated copy together with a reporter gene. X inactivation is a natural phenomenon through which one of the two chromosomes in any female gets inactivated for transcription. The mosaic mouse allowed Zhao and Reed to study the effects of odorant-induced activity on competition, by setting up a situation in which cells which differ only in whether they exhibit odor-induced activity or not compete with each other for innervation of glomeruli. In male mice hemizygous OCNC1-deficient mice, whose neurons do not compete with OCNC1-wild-type neurons, the epithelium and olfactory bulb was found to be

morphologically normal. The normality in the face of a lack in odor-induced activity prompted Zhao and Reed to predict that the epithelium of heterozygous females would consist of a mosaic of wild-type and mutant ORNs. To their surprise, however, they found that the epithelium of adults consisted apparently entirely of wild-type neurons. In contrast, the epithelium of neonatal animals consisted of both mutant and wild-type neurons. Zhao and Reed hypothesized that homozygous OCNC1-deficient females would result in a noncompetitive situation and show a mosaic of mutant and wild-type neurons, and found that, indeed, roughly equal numbers of patches of mutant and wild-type neurons are visible. The clusters were not mutually exclusive, but were intermingled in the epithelium and in each glomerulus of the bulb.

Even more interestingly, unilateral naris occlusion shortly after birth led to a recovery of the mutant population, such that at 40 days to 4 months afterwards the epithelium had both mutant and wild-type neurons, much like the hemizygous male's. The unoccluded hemisphere —ORNs project ipsilaterally to the bulb— showed a depletion of mutant neurons. Zhao and Reed concluded that ORNs compete with each other for scarce resources potentially provided by target downstream neurons, and that odor-mediated neural activity gives a neuron an advantage in that competition. When all neurons are subject to the same lack of neural activity, no type is preferentially impaired. When one group of ORNs has a selective disruption of neural activity, that group of neurons is disadvantaged in the competition.

Why did Zhao and Reed see a depletion in mutant neurons with reduced odorant-induced activity while Zheng et al. did not? The difference may lie in the fact that Zhao and Reed's neurons faced competition from odorant-induced-activity-positive neurons expressing *each* of the 1000 OR's in the mouse repertoire, while Zheng et al.'s faced competition only from odorant-induced-activity-positive neurons expressing the M72 OR. This means that Zheng et al's OCNC1-deficient neurons were not at a relative disadvantage with respect to other neurons in any glomeruli but the site of projection of

wild-type M72. This may explain both why the channel-negative neurons survived and why they projected to a different glomerulus than the wildtype.

There is a further apparent contradiction between Zhao and Reed's findings and Zheng et al's. Zhao and Reed report that without competition, male hemizygous OCNC1-deficient mice have normal epithelium and bulb. Zheng et al., on the other hand, report that M72-expressing ORNs have abnormal projection patterns in an OCNC-1 deficient background. I presume that given that Zhao and Reed's mice had all ORNs equally labeled, though, it would be hard for them to notice an additional glomerulus in the projection pattern of a particular OR type. Alternatively, if they can indeed see M72's projection pattern to be normal, M72 may be protected by a lack of its ligand in their laboratory's olfactory environment, a ligand that may be present in Zheng et al's laboratory. This could be tested by analyzing whether M72's projection pattern is affected in Zhao and Reed's female mice (whose OCNC-1-negative neurons do face competition, but only in the face of olfactory stimulation).

Zhao and Reed's findings warrant a major reevaluation of the olfactory development literature far beyond their paper's claims. The demonstration, firstly, that ORN axons compete for survival, secondly, that this survival is activity dependent, and thirdly, that activity plays a permissive role only in a competitive situation, as shown by the fact that OCNC1-negative neurons are unaffected in olfactory-deprived hemispheres, suggests a larger role for activity-dependence in the establishment of the projection pattern of ORNs to the bulb. Importantly, it provides an alternative explanation for the previous finding that deletion or substitution of an OR gene affects the corresponding ORNs' projection pattern (Mombaerts et al., 1996; Wang et al., 1998): rather than owing to the lack of the OR gene products in the axons, the abnormalities could be due to the corresponding alteration in odor-induced activity of the neurons. This possibility had been downplayed after the discovery that practically anosmic mice developed normal projection patterns for P2-expressing ORNs (Belluscio et al., 1998; Lin et al., 2000). These mice, however, faced a uniform down-regulation of activity in all

ORNs, though, and thus would presumably not have been affected by the activity-dependence demonstrated by Zhao and Reed, that surfaces only upon competition with ORNs exhibiting normal activity. A small remnant of odorant-induced activity, as indeed observed in the so-called “anosmic” mutants, particularly among newborns (Belluscio et al., 1998; Zhao and Reed, 2001), could be enough to bring the competiiton-based mechanisms observed by Zhao and Reed into play. Indeed, the small remnant of activity left in OCNC-1-negative neurons in hemizygous males was enough to prevent the odorant activity-dependent phenotype of OCNC-1 +/- heterozygous females. Furthermore, ORN activity or lack thereof was not assayed by single-cell recordings, but only indirectly via electro-olfactograms (EOG). The generality of the conclusions stemming from the analysis of the projections of P2-expressing ORNs is further put in doubt by the discovery that the development of ORN projection patterns is heterogeneous for different ORs (Zheng et al., 2000; Potter et al., 2001), and that inferences about the lack of a requirement for odor-induced activity have been generally based on analysis of one or two ORs (e.g., Lin et al., 2000).

Zhao and Reed’s paper allows a further important conclusion to be drawn. Because olfactory deprivation led to the elimination of any competitive advantage of neurons with a wild-type OCNC1 allele, constitutive activity, such as the wave-like activity found in the retina that guides visual development (Stellwagen and Shatz, 2002), cannot be responsible for the development of ORN axons —discarding one of three modes of OCNC1 action proposed by Zheng et al. (2000). At least two reasons come to mind to explain this difference between the visual and olfactory systems. First, since neighbor relations in the olfactory epithelium do not correlate with functional proximity, as opposed to the visual, auditory and somatosensory epithelium, wave-like spontaneous bursts of activity propagating through gap-junctions cannot generate a meaningful wiring pattern in which similar inputs wire together. Second, unlike the mammalian visual system, which remains in darkness during development due to eyelid closure and the poverty of the womb as a visual environment, olfactory responsiveness has been demonstrated at early embryonic stages in the rat (Gesteland et al., 1982),

rabbit (Hudson and Distel, 1997) and human (Schaal et al., 2000). Natural stimuli are thus available to guide olfactory development since early on.

An activity-dependent competitive scenario for the establishment of ORN-glomerular projection patterns is attractive from several standpoints. Firstly, it would obviate the need for a large set of axon guidance molecules to interact with the set of 1000 OR proteins which have herefore been postulated as axon guidance cues. With the set of ORs already constituting 1 in every 30 genes in the mouse and human genomes, the need for an additional set of receptors for them might prove embarrasingly expensive for our as-of-recent smaller-than-expected genome (Venter et al., 2001; Lander et al., 2001). Secondly, activity-dependent competition is the norm in the development of other senses, and has been demonstrated particularly thoroughly in the visual system. The demonstration that the same mechanisms are at work in the olfactory system would prove satisfyingly parsimonious. Thirdly, simple mathematical models of activity-dependent unsupervised learning have been shown to maximize the difference between the responses of different mitral cells in a model of adult neurogenesis of granule cells (Cecchi et al., 2001). The same class of models (see below) could be used to demonstrate how simple competitive rules could lead to ORNs expressing different ORs survive differentially in different glomeruli starting out with broad projection patterns that are pruned via differential survival, as seen experimentally (Potter et al., 2001). Finally, as noted by Zhao and Reed (2001), an activity-dependent projection scheme would prove evolutionarily helpful, since a mutation in an OR gene, which caused a change in its odor-mediated activity pattern, would automatically and simultaneously eliminate the corresponding ORNs' projection to their old target and lead them to assemble into a novel and unique glomerulus.

Towards an activity-dependent model for the generation of the ORN-glomerular mapping

How would activity regulate the formation of the projection map seen experimentally and ensure that ORNs expressing different ORs end up projecting to different glomeruli? I propose a model with a) initially broad projections of ORNs across the bulb (observed by Potter et al., 2001), b) slight initial inhomogeneities across different areas of the bulb in connection numbers or strength of ORNs expressing any given OR type, such as could be generated by a gradient of neurotrophins, c) winner-take-all competition between ORNs expressing different receptor genes such that only ORNs with similar patterns of activity survive in the projection to any one glomerulus, as observed empirically by Zhao and Reed (2001), and thus each glomerulus eventually receives input only from ORNs expressing one OR type, and d) winner-take-all competition between glomeruli for ORNs expressing any given OR type, such that ORNs expressing any given OR type will project to only one glomerulus (or one in each of *n* separate maps, such as observed for the medial and lateral bulb). The latter competition, which need be OR-specific, may be implemented via granule-cell-mediated inhibition, whose specificity could derive from STDP in the synapses between granule cells: the winning glomerulus, due to its larger synaptic efficacy, would result in earlier firing than the rest and would thus precede spikes in granule cells, but all other M-T cells would fire later, leading to strengthening of the inhibitory synapses to them, eventually leading to the retraction of the corresponding ORN projections, due to a decreased ability to make the postsynaptic cell fire.

Importantly, the initial heterogeneities in connection strengths or numbers do not need to be different for ORNs expressing each OR type: a spatially distributed projection map is ensured nevertheless because once ORNs for one OR type take over one glomerulus, the winner-take-all competitive mechanism between ORNs with different activity patterns will have pruned the synapses of ORNs expressing other ORs and thus forcing to project to another glomerulus. Alternatively, the initial gradient could be a double opposing gradient, such that different relative affinities of

each receptor for each of the two guiding molecules would lead to a different spot of maximal attraction (Ofer Mazor, personal communication). This model requires that most every receptor type bind to some degree to each of the guiding molecules, something that would only be expected if there are conserved regions across all receptor types.

The consequence of this set of assumptions is a diagonalization of the OR-glomerulus matrix, as described for adult neurogenesis of granule cells (Cecchi et al., 2001), via the following sequence of events: 1) all ORNs being with broad projection patterns across much of the bulb, 2) ORNs with the strongest odor-evoked activity win over competition for the glomerulus to which they project most strongly due to initial inhomogeneities, while a winner-take-all competitive mechanism between glomeruli mediated by lateral inhibition prunes out projections of those ORNs to all other glomeruli, 3) the process in (2) is repeated for ORNs expressing the next strongest driven OR, with the constraint that a glomerulus that has already been dominated by ORNs expressing a different OR are not available for further colonization, and so on until all ORNs expressing a given OR project to a unique glomerulus.

The model makes predictions that can be tested experimentally. First, it predicts that ORNs expressing different ORs with the same odorant specificities will project to the same glomeruli. Second, it predicts that odor exposure during early development will guide the order in which ORNs expressing each OR type will take over given glomeruli. Thus, changing such early exposure through the introduction of exogenous odorants *in utero* should lead to a change in the order in which the projection patterns of ORNs expressing individual ORs crystallize into a unique glomerulus. Such changes in the temporal sequence of development for ORNs expressing each OR may or may not lead to a change in the final mapping, depending on the specificity of the initial bias present in genetic chemical gradients for different ORs.

The final word is not said, and the picture is likely to include a combination of genetic cues and activ-

ity-dependent modifications. It is too early to say the degree to which activity-dependent competitive mechanisms shape olfactory development. It is also too late to say they don't.

Multiglomerular projection patterns of locust ORNs

In contrast to mammals and flies, all single locust ORNs stained have been found to project to several (~2-6) glomeruli in the antennal lobe (Hansson et al., 1996). The total number of glomeruli in *Schistocerca gregaria* has been found to be over 1000 (Hansson et al., 1996), substantially more than in the fly or bee brains. Combined with the fact that locust projection neurons arborize in several glomeruli as opposed to a single one for most other species studied (see §1.15.3), this suggests that a single glomerulus in other species may be analogous to a group of several glomeruli in the locust. Whether glomeruli innervated by any one PN are isofunctional or not remains to be tested.

1.15.3 The anatomy of the vertebrate olfactory bulb and insect antennal lobe

The insect antennal lobe is composed of two main classes of neurons: spiking excitatory output neurons called projection neurons (PNs) and inhibitory local neurons (LNs). In the locust, PNs number about 830, fire action potentials and arborize in several glomeruli. In other insect species as well as in mammals, PNs arborize in a single glomerulus. Locust LNs number 300 or so and are non-spiking, althoug this is not generally the case in other species. PNs respond to a subset of odors; LNs appear to be tuned more broadly —less sparsely— both in time and in odor space.

The existence of two major tracts between the primary and secondary olfactory processing areas is a common neural organization of olfactory systems, both in vertebrates and insects. In the honey-

bee, functional subdivisions of PNs have been described (Mueller et al., 2001): morphologically distinct subpopulations of PNs projecting to different regions of the mushroom bodies were seen to have different coding properties. lACT neurons appeared to code odors by spike rate differences, have broader response profiles to different odors, and convey the information rapidly. Neurons in the mACT tract coded odors by latency differences, had more specific response profiles, and conveyed the information with a delay. Thus more general information about the olfactory stimulus might reach the mushroom bodies first via lACT neurons, and then mACT neurons add more specific odor information.

In vertebrates, the architecture is functionally very similar, although somewhat more complex. The projection neurons are mitral and tufted cells. These neurons are similar in many ways, so they are often lumped together as mitral/tufted cells. In mammals, both send their primary dendrite to a single glomerulus. In amphibians, fish and reptiles, mitral cells can receive input from several glomeruli through multiple apical dendrites (Herrick, 1931). Both cell types are excitatory and project to the piriform cortex via the lateral olfactory tract. In addition to mitral and tufted cells, periglomerular cells also receive input from glomeruli, including ORNs, mitral/tufted cells. Their axons, which ramify in other glomeruli, are GABAergic but have been shown to have an excitatory action on each other and on mitral/tufted cells. Such GABA-mediated excitation has been described elsewhere (Cherubini et al., 1991; Michelson and Wong, 1991), and is believed to come about through an inverted chloride potential across postsynaptic cell membranes. The inhibitory cells in the bulb are termed granule cells. They make dendrodendritic synapse with other granule cells and with the basal dendrites of mitral/tufted cells, which can extend laterally for up to 1 mm in rats. Finally, there is a little known small population of short axon cells (Scott et al., 1987).

Recently, a precise intrabulbar connection of tufted cells was shown to link glomeruli in the medial and lateral sides of the bulb that receive input from ORNs expressing the same OR type (Lodovichi

et al., 2001). As this projection terminates in the granule cell layer, which provides inhibitory input to the external tufted cells, this finding reveals that a set of mutually inhibitory connections link isofunctional glomeruli in the medial and lateral maps of olfactory receptors.

It has been argued that the olfactory bulb is analogous to primary sensory cortices in other senses (Johnson et al., 2000), while piriform cortex is analogous to associations cortices (see below).

1.15.4 Odors are represented by overlapping assemblies of PNs

Because individual odorant receptors respond to a variety of odorants, and potentially also because individual PNs sample from several glomeruli, PNs respond to a variety of odorants (Laurent and Davidowitz, 1994; Wehr and Laurent, 1996; Laurent et al., 1996; this thesis). Thus, odors are said to be coded in a combinatorial or distributed code, with the identity of an odor at any time encoded in the set of active PNs.

1.15.5 Oscillatory inhibition: Analog to digital conversion

A striking characteristic of neurons in the insect antennal lobe and the vertebrate olfactory bulb is their tendency to respond to odor stimulation with oscillations in their membrane potential and their firing rates. Since their original discovery in the hedgehog (Adrian, 1942, 1950), these oscillations have been found in the cells themselves or in the local field potential of many vertebrate species: fish (including rainbow trout, salmon, char), amphibians (frog, toad), reptiles (iguana, caiman, snakes and turtle), birds (including albatross, duck, vulture, pigeon and others), and mammals (rat, rabbit, cat, dog, monkey and human)[11].

In insects, the oscillations were first described in the locust (Laurent and Dawidowitz, 1994) and have since been shown in the honeybee (Stopfer et al., 1997), moth (Wu et al., 1995), wasp and cockroach (Stopfer et al., 1999). Oscillations have also been found in the olfactory system of molluscs (Gelperin and Tank, 1990).

Oscillatory synchronization is not exclusive to the olfactory system. It has been described in the visual cortex of cat and monkey (Eckhorn et al., 1988; Gray et al., 1989, 1990, 1992; Engel et al., 1990; Livingstone, 1996), motor cortex (Murthy and Fetz, 1992, 1996, 1996b), and hippocampus (Whittington et al., 1995).

In the locust, this oscillatory synchronization involves different PNs in different epoques of the response, and is also odor dependent (Laurent and Davidowitz, 1994; Laurent et al., 1996). The oscillations require fast (20 Hz) periodic GABA inhibition by local neurons in the antennal lobe (MacLeod and Laurent, 1996), which do not fire action potentials but do produce Calcium-like spikelets. When the oscillatory synchronization was disrupted by injecting a GABA antagonist, picrotoxin, in the antennal lobe, the ability of bees to discriminate between chemically similar odorants was impaired (Stopfer et al., 1997). The ability to discriminate between chemically unrelated odorants was not impaired.

By dividing responses in discrete cycles of the oscillations, the system may be effectively digitizing the signals, converting analog time into a digital series of vectors, where each element represents one PN.

When I embarked on the investigations described in this thesis, the functional role of oscillatory synchronization was unknown. Chapter 5 is devoted to a series of explorations that begin to address this issue.

11. From 33 references, the originals of which are cited in Wehr (1999), pp.37-38.

1.15.6 Temporal patterning of responses: Decorrelation

Projection neurons in the antennal lobe and mitral cells in the olfactory bulb respond to odors with temporally complex patterns of excitation and inhibition (Kauer, 1974; Laurent and Davidowitz, 1994). For some neurons, the response patterns can even change from one oscillation cycle to the next (Wehr and Laurent, 1996); most neurons, however, show a high correlation in the timescale of hundreds of milliseconds in their response properties (see Chapter 4). These changes mean that, over the entire PN assembly, the set of PNs that fires changes as the response dynamics unfold.

What these response dynamics, which occur in response to static (non changing) stimuli, buy for the animal remains unclear. Friedrich and Laurent (2001) have shown that in fish, the responses for chemically similar odorants get decorrelated as the response evolves in time. What is most remarkable about their results, though, is that while the dynamics in the antennal lobe amplified initial differences between the representations of chemically similar odorants, the variance across trials decreased slightly. In other words, unless the stimuli were *exactly* identical across multiple trials, we can assume that the dynamics suppressed the differences between stimuli that were very close together, or that had acquired a common functional meaning, while amplifying differences between stimuli that were farther apart, or that had different functional meanings. How the system manages to retain this sensitivity to small differences in stimulus space without simultaneously increasing intertrial variabiilty remains a mystery. The response sparseness, on the other hand, remained constant in time. Functionally, the system may be remapping chemical odorant space into a representation space that is more regularly populated, a process that can ease odor classification by increasing the distance between closest neighbors in stimulus space. A caveat of these experiments, though, is that only three trials were presented for each odor/cell pair, so intertrial variability may have been hard to assess, and could in any case not be representative of steady-state

responses, since the first few trials of any odor presentation are known to evoke responses different from later ones in the locust (Stopfer and Laurent, 1997). A second caveat is that the neurons were not recorded simultaneously but rather over many animals.

1.16 Sparsifying, digital decoding, intermodal association and learning: The divergence to the insect mushroom bodies and the vertebrate piriform cortex

1.16.1 The insect mushroom bodies

PNs in insects project to Kenyon cells (KCs) in the mushroom bodies, so called because of their shape, and continue on to synapse on the lateral protocerebral lobe. Kenyon cells project on to α- and ß-lobe neurons, which feed back onto Kenyon cells, forming a loop. Very little is known about how information processing continues beyond the mushroom bodies.

Kenyon cells show a long after-spike-hyperpolarization period and exhibit sparse response patterns to odors, both in time and in odor space: they respond with one or few spikes and only to one or few odors, although there appears to be some heterogeneity across the population (Pérez-Orive et al., 2002). KCs can exhibit high reliability across multiple presentation of the same stimulus (Pérez-Orive et al., 2002).

Kenyon cells exhibit non-linearities which are consistent with a role in coincidence detection across PN inputs (Pérez-Orive et al., 2002). Furthermore, their membrane potential oscillates at a fixed offset to PN membrane voltage oscillations, and are systematically inhibited during part of each oscillation cycle. Thus, it is conceivable that their spikes happen in response to synchronized spikes only, and that the KC assembly provides a readout of which PN combinations are active and synchronized at any given cycle of the oscillations.

An intriguing observation is that Kenyon cells of adult Drosophila exhibit synchronous oscillation of intracellular calcium concentration, with a mean period of approximately 4 min (Rosay et al., 2001).

The mushroom bodies have been implicated in learning and memory both through lesions and genetic studies. Chemical ablation of the mushroom bodies leads to total loss of olfactory learning (de Belle and Heisenberg, 1994). More subtle manipulations have shown a requirement for mushroom body signaling during memory retrieval, but not during acquisition or consolidation (McGuire et al., 2001; Dubnau et al., 2001). The mushroom bodies have also been implicated in the perception of odor attractiveness, but not aversiveness (Want et al., 2001). In other words, blocking the output of Kenyon cells does not interfere with memory acquisition, which suggests that memories are encoded upstream of KCs, possibly in ORN-PN and/or PN-KC synapses.

The mushroom bodies also receive multimodal inputs, including visual and tactile inputs in addition to olfactory ones (Strausfeld and Li, 1999; Li and Strausfeld, 1999).

1.16.2 The mammalian piriform cortex

As opposed to other senses, which project to cortex through the thalamus, mammalian mitral/tufted cells project directly to pyramidal cells in piriform cortex. Piriform cortex, also called paleocortex, is a three-layered structured simiar to reptilian cortex, in contrast to the six-layered neocortex of all other primary sensory areas. Layer 1 consists of pyramidal apical dendrites, afferent mitral cell fibers and intrinsic cortico-cortical collaterals. Layer 2 contains pyramidal cell bodies, and layer 3 has basal dendrites of pyramidal cells, deep pyramidal cell bodies, and multipolar neurons. The set of cortico-cortical collaterals, or association pathway, has been likened to the recurrent feedback chatacteristic of artificial neural network models. Deep within the piriform cortex lies the endopiriform nucleus, a large group of multipolar cells.

Piriform and endopiriform efferents target cortical rather than nuclear structures. Extensive projections from the endopiriform nucleus extend to most basal forebrain areas including the piriform cortex, entorhinal cortex, insular cortex, orbital cortex, and all cortical amygdaloid areas. The perirhinal cortex, olfactory tubercle, and most subdivisions of the hippocampal formation receive light projections. Projections are highly distributed spatially within all target areas. Efferent axons from the endopiriform nucleus are unmyelinated and give rise to boutons along their entire course rather than arborizing locally. Efferents from the endopiriform nucleus lack the precise laminar order of those from the piriform cortex, and provide a heavy caudal to rostral pathway that is lacking in the cortex (Behan and Haberly, 1999).

Pyramidal cells in piriform cortex project widely to many areas of cortex, including areas involved in high-order function (Johnson et al., 2000). This has lead Johnson et al. (2000) to compare piriform cortex with association cortices in other sensory modalities, rather than with primary cortices. Within piriform cortex, pyramidal cells' arbors are highly distributed with no regularly arranged patchy concentrations like those associated with the columnar organization in other primary sensory areas (i.e., where periodically arranged sets of cells have common response properties, inputs, and outputs) (Johnson et al., 2000). A lack of columnar organization is also indicated by a marked disparity in the intrinsic projection patterns of neighboring injected cells (Johnson et al., 2000).

1.17 Beyond

Beyond piriform cortex, our knowledge is only sketchy.

Neuronal responses in rat orbitofrontal cortex are more likely to reflect associations between simultaneously trained odors than between odors that predict similar responses (Alvarez and

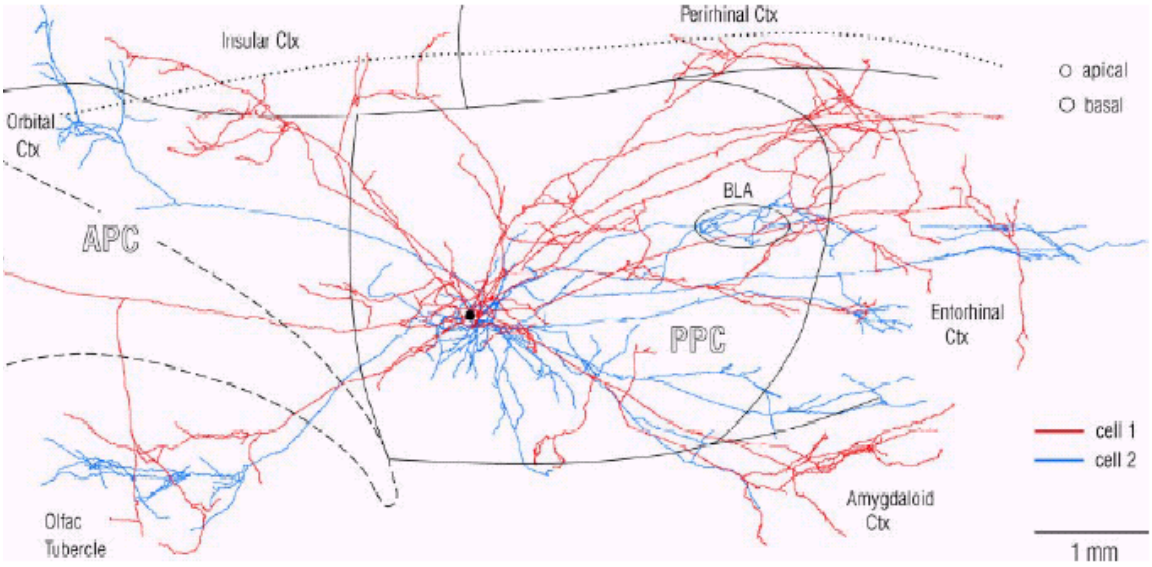

Figure 1.8. Pyramidal cells in piriform cortex project widely over large regions of cortex. Association (cortico-cortical) axons from a pair of neighboring superficial pyramidal cells in posterior piriform cortex. Note the minimal overlap of the two axonal arbors outside the ;1 mm diameter local collateral region that surrounds SP somata. The arborizations from the second cell (*blue*) in the orbital cortex (*top left*) and basolateral amygdala (*BLA, oval*) are deep to piriform cortex. The *black spot* indicates the position of the cell bodies. The *circles* at *top right* denote typical diameters of pyramidal cell dendritic trees at the depths where they are contacted by association fibers (proximal apical dendrites in layer Ib and basal dendrites in layer III). The borders of piriform cortex and the insular-perirhinal border are indicated by *solid lines*; the *dashed line* outlines the lateral olfactory tract; the *dotted line* is the rhinal sulcus. From Johnson et al.,

Eichenbaum, 2001).

The parahippocampus has been involved in the discrimination of the odor of individuals in hamsters, which use scent-marking (Petrulis et al., 2000).

Single-unit activity of 15% of the neurons recorded in the human amygdala was positively correlated with perceived odor unpleasantness (Buchanan et al., 2001).

Finally, hemispheric asymmetries have been found in olfactory recognition of artificial, but not natural, odors (Ilmberger et al., 2001).

1.18 Neuromodulators and learning

The field of olfactory learning and neuromodulators is outside the scope of this introduction, but I will point out two outstanding series of studies (see also reviews by Hasselmo and Bower, 1993; Hasselmo, 1995).

An octopaminergic neuron mediates unconditioned stimuli in the bee brain

In a beautiful study, Martin Hammer showed that, in the honeybee, an identified octopaminergic neuron mediates the effect of the unconditioned stimulus (US) in learning, and showed that you could replace the US by injection of octopamine (Hammer, 1993; Hammer and Menzel, 1995).

Oxytocin mediates attachment in the mammalian brain

Oxytocin is a neural mediator of attachment, both to sexual partners (Shapiro et al., 1991; Winslow et al., 1993; Williams et al., 1994; Insel et al., 1997) and for infant-mother relations (Nelson and Panksepp, 1998).

Oxytocin induces recognition acting on the olfactory bulb

Oxytocin induces preservation of social recognition and the induction of maternal behavior in rats by activating receptors in the olfactory bulb (Yu et al., 1996, 1996b; Dluzen et al., 2000).

1.19 Olfaction: A sense of variance

As we saw in §1.10.3, even though most of olfactory research has gone into unraveling how the olfactory system encodes the chemical identity of odors, it is perhaps in the encoding and decoding of spatiotemporal patterns of odor identity and intensity that most of the complexities of olfaction lie. Many odor-driven behaviors, such as the search for a mate in moths, depend on the analysis of chemically simple but physically complex signals that allow orientation as well as detection (Laurent, 1999).

1.19.1 Evaluating variance takes time…

If the olfactory system extracts information out of fluctuations in the absolute and relative intensities of different odorants, it must compare intensities over time and/or space. Do responses of projection neurons in the antennal lobe of the locust correlate better to differences in odor intensity than to absolute concentrations? This important question remains unanswered, although the means to answer it are now at our disposal.

1.19.2 …or space and convergence: Olfactory images and spatial codes in olfaction?

Alternatively, fluctuations could be evaluated spatially, as the differences between activation of identical receptors in different locations of the epithelium. Let us examine this possibility, hitherto largely unexplored. The olfactory epithelium of a dog has a surface area larger than that of the retina (Adrian, 1951). Furthermore, unlike in the visual, auditory and somatosensory systems, the spatial coordinates of olfactory receptors do not correlate with their physiological properties. Also differently

from vision, hearing and touch, the projection patterns of olfactory receptors do not preserve neighbor relationships between source and target. It has been argued that this may reflect the fact that olfaction is not a spatial sense (Zheng et al., 2001). If olfaction was a non-spatial sense, though, why have such a large sensory surface? The conventional explanation suggests that this redundancy, coupled with the convergence of olfactory receptor neurons (ORNs) expressing the same receptor genes onto the same glomeruli in the olfactory bulb of vertebrates or the antennal lobe of insects, is there to sum over similar inputs and reduce noise (Laurent, 1999). Yet there is no a priori reason why a large organ should be more sensitive to smells than a small one (Adrian, 1951). While it is certainly plausible that summing across receptors is indeed what convergence in glomeruli accomplishes, the lack of evidence for it does not warrant the widespread assumption that this is the case. In the visual system, convergence of many rods onto a single retinoganglion cell is effective because a) the physics of light transmission ensures that different photoreceptors will receive different inputs corresponding to different points in the visual field, and b) noise in the photoreceptors is indeed the limiting factor for light detection (Meister and Berry, 1999). There is no compelling evidence to my knowledge, however, that olfactory receptor neurons are both sufficiently noisy and sufficiently uncorrelated that such a summation would indeed help much. Individual ORNs, in fact, appear to have basal firing rates more than five times lower than photoreceptors (compare Lemon and Getz, 1997, with Meister and Berry, 1999). It is noteworthy that in the auditory system, there are only about 15,000 hair cells in each ear (SFN, 1994), only 3500 of which are inner hair cells, less than 1/10,000th of the number of odorant receptor cells in humans and less than 1/200,000th of that number in dogs. Hecht, Schlar and Perrine demonstrated in the 40's that human subjects can reliably detect single photons (Boroditsky, 1999; Wandell, 1995), thus showing that light can also be detected reliably without the benefit of summation across receptor neurons. In other words, biology does not require a large number of identical cells in order to obtain a trustworthy signal: sensory neurons are in general reliable detectors. Furthermore, even if ORNs turn out to be affected by sub-

stantial uncorrelated noise, it is unclear that this could not be averaged away without convergence of like receptors onto the same targets. Odor responses of Kenyon cells, for example, are more reliable than those of their PN inputs, even if each PN has a different 'tuning curve.'

But a large epithelial surface will certainly provide a screen on which the pattern of excitation can be mapped in greater detail (Adrian, 1951). In fact, the nose has a complex structure that seems designed to ensure that the stimulus is distributed inhomogeneously over the epithelium (Adrian, 1951). Rather than *summing* over inputs to *reduce* variance (Laurent, 1999), convergence may perhaps be *estimating variance* across the olfactory epithelium, extracting information from an olfactory *image*.

Is there any plausible biophysical implementation of the computation of the variance in the activity of inputs? Indeed, stochastic resonance has been shown to increase neuronal firing rates as a function of noise levels, i.e., as a function of the variance in the signal (Douglass et al., 1993; Gammaitoni et al., 1998). Although in the past these mechanisms have been proposed to use noise to amplify signals, they could potentially be exploited to measure noise *per se*.

This new hypothesis is consistent with both the one cell – one gene expressed finding and the allelic exclusion exhibited by olfactory receptor genes. If the responses of individual ORNs were homogenous across the epithelium, there would be little incentive to force the expression of a single allele – indeed, cells in the VNO express multiple receptors (Martini et al., 2001): the discrimination of odors can be done regardless of whether the 'bases' of the vectorial representation of odors represent pure odotopes or a more complex chemical signature—; if, on the contrary, activation was diverse across the epithelium and the olfactory system extracted information from the analysis of *differences* across ORNs, then there would be an advantage to having the detectors be homogeneous: otherwise, differences in the spatial pattern of activation of one receptor would be multiplexed and confused with differences in the spatial pattern of activation of another receptor expressed in the same

cells, differences that need not always be aligned. If proved correct, then, this hypothesis could serve to explain the evolutionary reasons behind (i) the one cell – one gene expression pattern, (ii) allelic exclusion, (iii) the nonlocalized distribution of ORNs expressing the same receptor gene, (iv) the large surface of the olfactory epithelium and (v) convergence of cells expressing the same receptor type onto the same glomeruli.

How could one test such a hypothesis? It would predict that differential stimulation of two ORNs (or groups of ORNs) expressing the same receptor type would excite some mitral cells (or insect PNs) in the glomerulus the ORNs innervate more than identical stimulation of both. In contrast, the traditional summing hypothesis predicts that stimulation of additional receptors will always lead to additional excitation of all mitral cells or PNs in the corresponding glomerulus, and thus stimulation with a strong stimulus to both groups of ORNs would lead to more excitation of than stimulation of one group of ORNs with a high concentration and stimulation of the other group with a low concentration. The test would need to be careful to stimulate only one receptor type to ensure that stimulating an additional site affects only the sum and difference of excitation of receptors of the same type, and does not introduce a confound of additional receptor types stimulated. This is particularly important given the uneven distribution of receptor types across the epithelium. Second, the test should measure the activity of PNs or MCs innervating the glomerulus targeted by the receptor type stimulated, to measure the direct action of convergence onto the glomerulus, and not indirect effects due to bulb or AL dynamics. Lastly, the test should be exhaustive, since the coexistence of summation and variance estimation may occur in different mitral cells or PNs.

While this hypothesis is entirely speculative at this point, its possibility should serve to focus efforts to demonstrate the role of glomerular convergence rather than assume it to be the simplest alternative.

1.20 Architectural differences with the immune system

It is of considerable interest to compare the olfactory system with biology's other major chemosensory system, the immune system, to find similarities and differences in the strategies they evolved, speculate on the functional reasons behind them, and draw on our knowledge of one to make predictions for the other.

1.20.1 Output requirements and specificity of response

The output requirement of the immune system is very different from that of the olfactory one: while olfaction needs only to recognize chemical signals, but not act on the signals themselves, the immune system has to mount an attack on them. The immune system is much more ambitious, just like the Star Wars Missile Defense system is more ambitious than a simple attack identification system. The immune system's need to provide a target-specific attack mechanism requires selectively amplifying molecules (antibodies) and cells that bind the targeted intruding molecule and kill intruding and infected cells.

This difference in output requirements dictates a difference in sensing strategies. The broad specificity of olfactory receptors allows the olfactory system to be sensitive to a huge variety of stimuli with a much smaller set of receptors. For the immune system, instead, broad specificity is not a desirable property. If the system goes awry and specificities become too broad, autoimmune disease ensues. Killing only the intended target, and not benign self cells, using broad specificities would require having the killing dependent on the binding of a particular *combination* of antibodies or T-cells. Such a combinatorial detection capability could be hard to implement given the physical constraints of getting a large number of different T-cells (or even antibodies) to bind the same, potentially small, intruding molecule.

1.20.2 Different mechanisms to generate diversity of specificities

A second, and related, difference between both systems lies in the mechanisms of diversity generation. The complexity of the odorant receptor repertoire is estimated in mouse and rat at 2000 genes, or about 3 percent of the genome, surpassing that of the immunoglobulin and T cell receptor genes combined (Mombaerts, 1999b). While the immune system has developed a sophisticated combinatorial mechanism to generate diversity, this does not appear to be the case with chemosensation, which uses a fixed (and large) repertoire of genes instead. Why? In particular, once one system appeared in evolutionary history, why was it not co-opted for a second function, since biology has shown to co-opt genes with much greater ease than that with which it evolves new ones? I believe the answers can be found by two converging lines of analysis.

The tradeoff between specificity and a small receptor set

First, could either of the mechanisms of diversity generation work in the other system? Because antibodies and T-cells cannot afford broad specificities (see above), a much larger diversity of molecules is needed to recognize as large a set of antigens. Furthermore, while a low-affinity receptor might provide enough signal for accurate odor detection and recognition, the immune system's task requires much more than simple detection and recognition. It is not enough to detect the presence of a foreign antigen; each and every antigen-bearing molecule or cell that binds the receptor of an activated B-cell needs to be attacked. The immune system's effectiveness in doing this depends directly on antibodies' affinity for an antigen, and an ellaborate set of processes involving amplification of activated cells, somatic hypermutation and the germinal center have evolved to maximize antibodies' affinity for the antigens that activate a B-cell. Thus, every second cell generated during

the process of hypermutation has a different receptor (Janeway and Travers, 1994). Clearly, it would be immensely costly in genome size, if not impossible, to achieve such a large repertoire of high affinity receptors with a fixed set of genes. Specificity of response calls for a more ellaborate system of diversity generation.

Could immunoglobulins work as odor detectors? Clearly, they could be expressed on the surface of olfactory receptor neurons; several members of the immunoglobulin superfamily are membrane-associated. Whether antibodies can evolve to bind small volatile molecules is an open question, but they are known to bind small molecules. Would the large repertoire of specificities of the immune system work in the olfactory system? Possibly not: each odorant receptor gene acts as a labeled line to the brain, not only to glomeruli in the mammalian olfactory bulb and insect antennal lobes, but also beyond into the insect protocerebrum (Wong et al., 2002; Marin et al., 2002), and it is unclear that the benefits of increased dimensionality of the olfactory representation offset the costs at increasingly large number of such labeled lines. Indeed, a large fraction of the members of the human odor receptor family are pseudogenes, suggesting that, at least in a species with little reliance on olfactory-mediated behavior as humans, there is not much selective pressure for an increased set of odor receptors. A gene family evolved from immunoglobulins could have developed broader specificities, though; MHC molecules are one such family. The critical question, then, is which came first: olfactory receptor genes or immunoglobulins. ORs came first, the reason for the evolution of immunoglobulins is apparent. If, on the other hand, immunoglobulins preceded ORs, then perhaps that indicates a fundamental inability of immunoglobulins to evolve affinity for small organic molecules or to develop broad-tuned response profiles.

The evolutionary history of the immune and olfactory systems

Let us take a brief look at the history of both systems. How old is the immune system? Although even primitive creatures such as sponges have means of distinguishing self components from non-self components, cellular immunity does not appear until worms or starfish, none of the complement system appears until arthropods, and immunoglobulins and lymphocytes do not appear but in vertebrates (Steiner, 1996; Encyclopaedia Brittannica Online, 2001) —although immunoglobulin-like domains have been found with functions different from antigen recognition in *C. elegans* and *Drosophila* (Steiner, 1996). Analysis of genetic diversity suggests the divergence from the ancestral immunoglobulin took place some 200,000,000 years ago. Based on paleontological evidence, this is about the same as the time at which amphibians are thought to have diverged from the main vertebrate line. It is not until one reaches the level of the terrestrial vertebrates—amphibians, reptiles, birds, and mammals—that a complete immune system with thymus, spleen, bone marrow, and lymph nodes becomes evident and that both IgM and IgG antibodies are made. Antibodies of the IgA class are only found in birds and mammals, and IgE antibodies are confined to mammals.

Olfactory receptors, instead, appear to be much older, and a complete olfactory system is present in insects as well as vertebrates, with simpler membrane-bound chemodetection systems present in worms, yeast and even bacteria (see §1.4). The evidence is thus consistent with the fact that immunoglobulins evolved much later than odorant receptors to fulfill a function that odorant receptors could not have performed themselves.

Hereditary response profiles?

Note that the differences in the diversity-generation mechanisms dictates a secondary difference: while odor-response profiles are largely hereditary, immune response profiles have both a genetic

and random components. Whether this difference was a driving force for the evolutionary differences or a consequence is debatable: it is advantageous to both systems to preserve specificities selected for in the past, as it is advantageous to both systems to have a diverse set of specificities in a population.

1.20.3 Monospecificity is common to lymphocytes and olfactory receptor cells

As opposed to taste receptor cells, which can express several receptor genes in the same cells (Adler et al., 2000; Chandrashekar et al., 2000; see §1.8), olfactory receptor neurons and lymphocytes exhibit monospecificity: each cell expresses a single type of receptor out of the diverse array in the population of cells. In the immune system, the advantages for this are clear: clonal selection can thus selectively amplify responses to one antigen without simultaneously upregulating responses to another. In the olfactory system, the reasons are as of yet less clear (but see §1.19.2 for a hypothesis). Perhaps the lessons from the immune system can help. We know that new olfactory receptor cells, as well as new granule cells in the olfactory bulb, which exhibits monospecificy of glomeruli as well, are born throughout life. Further, persistent exposure to an odorant can selectively upregulate responses to it both at the behavioral (Rabin, 1988; Wysocki et al., 1989; Laska and Hudson, 1991; Nevitt et al., 1994; Dittman et al., 1997; Moller et al., 1998; Hudson and Distel, 1998) and peripheral neural (Wang et al., 1993; Yee and Wysocki, 2001) levels, even causing the detectability of odorants for which a person was originally anosmic. Finally, although the question was asked in the context of development rather than plasticity, there is recent evidence that odorant-induced activity upregulates survival of olfactory receptor neurons (Zhao and Reed, 2001). It is thus not unreasonable to predict that some sort of clonal selection may be at work in the olfactory system, selectively enhancing the representation of common odorants by upregulating the survival of cells that respond to them.