Introduction

Sensory systems transfer information about external stimuli into ordered neuronal excitation. Through a process of evolutionary optimization, they have adapted to fulfill this task with minimal effort, that is, with as few receptors as possible. Therefore perception can be considered as a filter process, where only a part of the actual information is picked up by the sensory systems. For example the somatic system only encodes the strength and position of a contact to our skin, without encoding the cause for the contact. Likewise the optical receptors in the retina break down the continuous spectrum of light into the differential activity of a few receptors. The multidimensional nature of olfactory stimuli, in turn, does hardly allow for such an optimization. A seemingly unlimited number of different odorants exist having only one thing in common, the fact that they are volatile. As a result, in the genome of many animals, odorant receptors constitute the biggest gene family. How those many receptors and the subsequent processing of olfactory information in the olfactory system finally lead to odor perception is still not fully understood.

Our model organism, the honeybee, has been shown to have an excellent sense of olfaction. To honeybees, odors can convey a large amount of information, like food quality (von Frisch, 1963), readiness of conspecifics for mating (Ayasse *et al.*, 2001), and are even used for social communication (Abdalla and Cruz-Landim, 2001). Depending on the situation, their olfactory system has to enable them to accomplish two important, but opposing tasks. The first task is that of successful odor identification and subsequently that of odor discrimination. Honeybees guarding their hives identify incoming nestmates by their hive-specific smell and attack those whose smell is only slightly different (Ruther *et al.*, 2002; Dani *et al.*, 2005). Bees have also been shown to be able to differentiate between odors which differ only in the ratio of their compounds (Wright *et al.*, 2005; Ditzen *et al.*, 2003). The second and opposite task is that of odor generalization. Most odors, like that of flowers, consist of many different components (Dudareva *et al.*, 2004). The smell of two flowers of the same species but located on different soils or visited during different time points can vary (Dudareva and Pichersky,

2000). To recognize the common theme behind them and to consider them as equivalent is a prerequisite for successful foraging. How can those two tasks be accomplished?

General structure of olfactory systems

Olfactory systems across animal phyla show many similarities: Odors bind to olfactory receptors (ORs), which are located on the dendrites of olfactory sensory neurons (OSNs). ORs are G protein coupled seven trans-membrane domain proteins (Mombaerts, 1996; Clyne *et al.*, 1999; Vosshall *et al.*, 1999). In general, OSNs have been shown to express one olfactory receptor (OR) and the axons of all OSNs expressing the same OR converge onto the same glomerulus (Vosshall *et al.*, 2000; Gao *et al.*, 2000; Ressler *et al.*, 1994; Vassar *et al.*, 1994), though recently exceptions to this rule have been found (Mombaerts, 2004; Goldman *et al.*, 2005; Nezlin and Schild, 2005). Single OSNs have been shown to encode a broad spectrum of different odorants and odor concentrations (Hallem *et al.*, 2004; Akers and Getz, 1993; Getz and Akers, 1993; Getz and Akers, 1994; Sato *et al.*, 1994; Malnic *et al.*, 1999).

The glomeruli are the sites of synaptic interactions of the major neuron types involved in olfaction. Here the above mentioned ONSs converge onto inhibitory local interneurons (LNs) and second order olfactory neurons (Gascuel and Masson, 1991; Pinching and Powell, 1971). The LNs interconnect between several glomeruli and have been shown to shape both temporal and spatial response characteristics of the output neurons (Yokoi *et al.*, 1995; Margrie *et al.*, 2001; Sachse and Galizia, 2002).

Another feature common in the olfactory systems in most animals is the convergent-divergent way in which olfactory information is relayed. Odors bind to receptors located in the dendrites of a large number of olfactory sensory neurons. These converge onto a much smaller number of olfactory glomeruli. Divergent connections then the transfer olfactory information to higher order centers (Hildebrand and Shepherd, 1997).

As described above, there is a high degree of similarity in the organization of the olfactory systems between animals, even if they belong to different phyla. Nevertheless, many differences exist. In different species, the number of olfactory glomeruli can vary

substantially (vertebrates: dog=5000, rabbit=2000, mouse=1000; insects: drosophila=50, honeybee=160, locust=1000)(Hildebrand and Shepherd, 1997). Even more, the innervation patterns can differ between species. While in most olfactory systems the majority of the output neurons are uniglomerular, in species as different as the zebrafish and the locust only multiglomerular PNs have been identified.

Neurons and neurotransmitters of the honeybee olfactory system

The most numerous type of neurons in the honeybee AL are the olfactory sensory neurons (OSNs). For the honeybee, Esslen and Kaissling estimated that the dendrites of 60000 OSNs reside in each antenna (Esslen and Kaissling, 1976). Their axons travel along the antennal nerve into the AL, where they converge onto approximately 160 olfactory glomeruli(Flanagan and Mercer, 1989). The antennal nerve splits into 6 branches, four of which (T1-T4) enter the AL. While two of those tracts, namely T1 and T3 innervate a large proportion of the AL (70-80 glomeruli), T2 and T4 each innervate only 7 glomeruli (Galizia *et al.*, 1999a; Arnold *et al.*, 1985; Flanagan and Mercer, 1989). One OSN generally innervates a single glomerulus (Mobbs, 1982; Brockmann and Brückner, 1995) and within this glomerulus the innervations are mostly restricted to the outer core (Flanagan and Mercer, 1989). Though final evidence is lacking, studies suggest acetylcholine to be the neurotransmitter in OSNs (Scheidler *et al.*, 1990).

The glomeruli form a single layer around the AL (Flanagan and Mercer, 1989) and are interconnected by approximately 4000 local interneurons (LINs) (Witthöft, 1967). Two major groups of LNs can be distinguished: The majority of the LNs show a heterogeneous morphology. They densely branch in glomerulus and additionally have sparse ramifications in several other glomeruli. The second class of LNs homogeneously innervates a large number of glomeruli. While approximately 20% of the LNs show GABA immunoreactivity, the transmitter of the remaining LNs is unknown (Schäfer and Bicker, 1986), but the existence of histaminergic neurons in the AL (Bornhauser and Meyer, 1997) and the inhibitory function of histamine on AL neurons suggests that histamine may indeed be another transmitter of LN acitivity.

After processing in the AL, approximately 800 (Hammer, 1997) the projection neurons (PNs), convey this information to two higher order brain centers: the mushroom bodies (MBs) and the lateral horn (LH) (Bicker, 1993; Abel *et al.*, 2001d). Both uni- and pluriglomerular PNs have been shown to exist (Abel *et al.*, 2001d), differing in the number of glomeruli they innervate. Their projections run along several tracts. The lateral antenno-cerebralis tract (l-ACT) carries the axons of uniglomerular PNs stemming from glomeruli which are innervated by the T1 antennal nerve tract. The medial antenno-cerebralis tract (m-ACT) carries the axons of uniglomerular PNs originating in glomeruli sub served by the other three antennal nerve tracts (Bicker *et al.*, 1993). A third tract, the medio-lateral antenno-cerebralis tract mostly carries pluriglomerular PNs (Abel *et al.*, 2001b). While the m-ACT first projects to the MBs and then to the LH, the l-ACT does the reverse. Histochemical studies showed acetyl choline esterase immunoreactivity within the m-ACT and taurine immunoreactivity in l-ACT PNS (Kreissl and Bicker, 1989; Schäfer *et al.*, 1988). Within the MBs, PNs have been shown to innervate the lip region of the calyx (Abel *et al.*, 2001c), which shows a strong AChE immunoreactivity (Kreissl and Bicker, 1989). Unlike the other two tracts, the ml-ACT, which shows immunoreactivity to GABA (Schäfer and Bicker, 1986), does not project to the MBs but directly to the LH.

Imaging olfactory systems

The general notion that odors are encoded in the combined activity of many different neurons soon showed the limit of single cell recordings. Therefore techniques had to be developed which allow to record from several neurons in parallel. In a pioneering work, Freeman recorded simultaneously from up to 64 electrodes placed into the olfactory bulb of rabbits (Freeman, 1991). Though subsequently multielectrode recordings have proven to be a powerful tool for studying olfaction the number of units from which can be recorded simultaneously (eg. the spatial resolution) is always limited. The development of imaging techniques and their use in olfactory research have overcome this limitation and have greatly increased our knowledge about olfaction. The first methods used, like c-fos or 2-deoxyglucose stainings still had two strong limitations: They had an extremely low temporal resolution and only one single odor could be tested

per animal. Nevertheless they showed that in both the vertebrate OB and the insect AL odors are encoded in mosaics of activated glomeruli (reviewed by (Galizia and Menzel, 2001). Since then the development of new dyes has progressed considerably. While voltage sensitive dyes have an extremely high temporal resolution, this comes at the cost of a weak signal to noise ratio. Calcium reporters have been widely used in different imaging approaches. They achieve a good signal to ratio and offer a reasonable temporal resolution.

In the honeybee, treatment of the AL with membrane permeant calcium sensitive dyes stains several neuron populations, with a dominant contribution of the OSNs (Galizia *et al.*, 1998). This method allows for the visualization of spatiotemporal odor evoked across glomerular activity patterns (Joerges *et al.*, 1997). These patterns have been shown to be both odor and species specific (Galizia *et al.*, 1999b). Recent methodological progress now allows for more specific staining of distinct neuron populations (Sachse and Galizia, 2003).

Odor coding in the antennal lobe

It is common belief that odors are encoded in patterns of across fiber activity and several studies suggest that the identity of the active neurons encodes the identity of the perceived odor. This theory is often termed identity coding. Nevertheless, the exact function of the AL network is subject to debate. Three general hypotheses have evolved: The first hypothesis suggests that odor responses are dominated by the OSN activity and pass relatively unchanged through the AL (Wang *et al.*, 2003; Ng *et al.*, 2002). The second hypothesis, in turn, suggests a contrast enhancing role to the AL network. According to this theory, the inhibitory connections within the AL function like a contrast enhancer on the neural odor representations, thereby sparsening the AL output (Sachse and Galizia, 2002; Sachse and Galizia, 2003). The third hypothesis states exactly the opposite. It suggests that odor processing in the AL network results in broadened response PN profiles as compared to the OSNs (Wilson *et al.*, 2004).

In addition, a totally different theory, often termed temporal coding, exists. Following this theory, odors are encoded by slow temporal patterns of oscillating PN ensemble activity. During stimulation with an odor a local field potential (LFP)

oscillating at 20-30Hz has been observed in the MBs of different species (Laurent *et al.*, 1996; Stopfer *et al.*, 1997). This LFP has been suggested to arise from the synchronous activity of LNs (Wehr and Laurent, 1999) and it can be blocked by local application of the chloride channel antagonist picrotoxin to the AL. According to Laurent *et al.* different PNs synchronize with the LFP at different time points during odor stimulation and odors are encoded by the temporal sequence and identity of these PNs (Laurent *et al.*, 1996). In the honeybee abolishing the LFP with picoinjections of picrotoxin into the AL impairs odor discrimination between similar but not between dissimilar odors, suggesting that the PN-LFP phase lock is necessary for fine odor discrimination (Stopfer *et al.*, 1997).

Many different studies conducted in different species show that OSNs increase their responses with increasing odor concentration (Sachse and Galizia, 2003; Ng *et al.*, 2002; Wang *et al.*, 2003; Wachowiak *et al.*, 2004; Spors and Grinvald, 2002b; Rubin and Katz, 1999; Cinelli *et al.*, 1995; Friedrich and Korsching, 1997). For identity and temporal coding, different ways have been proposed in which odor concentration is encoded in the PN activity. The first theory suggests that single PNs respond to increasing concentrations with increasing activity(Wang *et al.*, 2003; Sachse and Galizia, 2003). *Stopfer et al.*(Stopfer et al., 2003) in turn suggest that odor concentrations are encoded by continuous changes in PN ensembles.

Little is known about odor processing beyond the AL. The MB KCs have been shown to be involved in olfactory learning (Zars *et al.*, 2000; Gerber *et al.*, 2004). Upon odor stimulation they respond with very sparse, non overlapping bursts of activity (Perez-Orive *et al.*, 2002; Stopfer *et al.*, 2003). These properties suggest the KCs to be the ideal mediator of odor identification and discrimination.

In this work, we selectively stained uniglomerular honeybee l-ACT PNs (Sachse and Galizia, 2002) and investigated their activity as response to odors presented at different concentrations. To make our work comparable to the perceptual similarity matrix, we choose the same set of 16 hydrocarbons used to establish the perceptual similarity matrix. We show how PNs simultaneously encode odor identity and concentration. Comparing our results with the behavioral generalization matrix, we conclude that perceived odor similarity is a direct function of the degree of overlap of PN

activity in the AL. As a last conclusion, we show how PNs redundantly encode odors, separating them according to their chemical properties in a putative olfactory space.