Introduction

The goal of this thesis was to understand how odor information is encoded by different neuron populations in the antennal lobe (AL) of *Drosophila melanogaster* and how odor representations are modified by the AL network. In order to investigate these questions, I have optically recorded odor representations in four different AL neuron types, analyzed differences and similarities in the glomerular response profiles at the input and output of the AL network, and pharmacologically manipulated the network. The presented results reveal that odor processing in the AL of *Drosophila* involves modulation of odor representations through glomerulus-specific connections.

Chemical senses

All living forms need to interact with their environment in order to survive. This interaction implies the detection of changes in the environment, and the ability to react to these changes. Sensory systems are in charge of probing the environment and providing the information required to produce the appropriate behavioral responses. Sensory systems range in complexity from single receptor proteins in unicellular organisms, to the highly developed visual, auditory, tactile and chemosensory systems of vertebrates and invertebrates. Chemosensory systems are probably the most ancient kind of sensory system, and the functionality of a living cell is indivisible from its capacity to sense and react to chemical stimuli present in its environment. At the single cell level the detection of chemical cues is achieved by membrane receptors activated by chemical stimuli of all kinds: pH, gases (NO), small hydrocarbons (glucose), macromolecules (proteins), etc. Along evolution, and with the emergence of complex multicellular organisms, two types of chemical-detecting organs have emerged: gustatory and olfactory systems. While gustatory systems deal with the detection of chemicals by contact with its source (e.g. the piece of chocolate in your mouth), olfactory systems detect chemical signals released by sources that might be far away (e.g. the neighbor's barbecue). In this thesis I focused on the olfactory system of *Drosophila melanogaster*.

From odor space to odor perception

For terrestrial animals, the olfactory world consists of thousands of volatile molecules, called odors. The complexity of the olfactory environment arises from several factors. 1) Biologically relevant odors are very diverse in molecular structure, ranging from short chain aliphatic molecules, like butanol, to terpenes and nitrogenated compounds, like limonene and indole. 2) Natural odor sources generally emit mixtures of many odors (Jordan et al., 2001; Pichersky and Gershenzon, 2002), and even mixtures with the same components but different component-ratios can have a different biological meaning (e.g. pheromones of different moth species, Linn, Jr. and Roelofs, 1989). 3) Odors concentration can span many orders of magnitude, and odors can be detected and identified over a wide range of concentrations (Kaissling, 1996; Pelz et al., 1997). 4) The temporal structure of olfactory stimuli is usually complex due to air turbulences, and the information contained in such odor plumes is of biological relevance (Vickers, 2006). The olfactory systems of terrestrial animals have evolved to detect and analyze, categorize and discriminate odors from this highly complex olfactory world.

Along the evolution of olfactory systems, a common anatomical organization and common functional principles have prevailed, possibly through evolutionary convergence (Hildebrand and Shepherd, 1997; Eisthen, 2002). Odor information coding is generally based on combinatorial rules. In this way a huge repertoire of odors can be encoded with a limited amount of coding "units", i.e. classes of olfactory sensory neurons [OSN, OSNs expressing the same odorant receptor gene (Or) constitute an OSN class]. OSNs are arranged at the interface with the environment (nasal epithelium in mammals, antennae in invertebrates) and project axons to a first olfactory center, the olfactory bulb (OB) in vertebrates and the AL in invertebrates (Hildebrand and Shepherd, 1997; Wilson and Mainen, 2006). In general, single odors activate several OSN classes and each OSN class can detect several odors (de Bruyne et al., 2001; Breer, 2003). Thus odor information is encoded in the ensemble of activated OSN classes. All OSNs from the same class converge to the same functional unit in the AL/OB (Mombaerts et al., 1996; Vosshall et al., 2000; Fishilevich and Vosshall, 2005). In these functional units - called glomeruli - OSNs make synapses with output neurons of the AL/OB, projection neurons (PN) and mitral cells (MC) respectively, and with local neurons (LN). The ratio of convergence between OSNs and PNs/MCs differs between species, for example a convergence of 1000:1 is found in rodents (Shepherd et al., 2004) while a convergence of

30:1 is found in *Drosophila* (Gerber and Stocker, 2007). Convergence at this level is thought to amplify signals and to reduce the signal-to-noise ratio (Wilson and Mainen, 2006). Most PNs/MCs receive input in only one glomerulus. In contrast, LNs innervate several glomeruli in the vertebrate OB and most glomeruli in the invertebrate AL (Stocker, 1994; Aungst et al., 2003; Shang et al., 2007), and have been shown to modulate glomerular activity (Christensen et al., 1993; Aungst et al., 2003).

PNs/MCs transfer integrated odor information to higher order brain areas, the mushroom bodies and lateral protocerebrum in insects and the olfactory cortex in vertebrates (Zou et al., 2001; Jefferis and Hummel, 2006). The divergent-convergent connectivity between PNs/MCs and their target neurons is thought to underlie the fundamental alteration of odor representations found in the mushroom body/olfactory cortex. While odors activate broadly overlapping neuron ensembles in the AL/OB, odors are encoded in a sparse way by the mushroom body intrinsic Kenyon cells (Perez-Orive et al., 2002) and the pyramidal cells of the olfactory cortex. (Zou et al., 2005). The common anatomical organization and functional principles that can be found across many phyla seem to be well suited to provide the brain with the required olfactory information to solve complex behavioral tasks, which are based on odor discrimination and generalization. While the anatomical features of the olfactory system are fairly well understood, knowledge about many functional aspects of odor information coding and processing is still limited.

Olfaction is a highly synthetic sense. Although animals in nature rarely encounter pure odors, odor mixtures emerging from the same source are usually perceived as single objects: banana smells like banana, and not like a mixture with more than twenty compounds. In fact, humans are not able to distinguish more than 3 compounds from a 5 component mixture (Livermore and Laing, 1996). Moreover, odor quality perception is not linearly related with any single physical parameter of odor stimuli. Even odor intensity, which is rather easy to quantify, can affect the perceived odor quality in a non-linear way. While isopentyl acetate smells like banana over a wide range of concentrations, certain sulfur compounds (thiols) smell sweet at low concentrations and repulsive at high concentrations (Firestein, 2001). Furthermore, odor discrimination depends strongly on previous experience (Wilson and Stevenson, 2003). All these factors might explain the controversy regarding the synthetic or analytic nature of odor perception. Attempts to understand how odors are encoded in the brain using behavioral or psychophysical approaches are limited by the lack of an objective measure for perceived odor identity (Chandra and Smith, 1998; Deisig et al., 2001; Wiltrout et al., 2003; Kay et al., 2005). When bees or rats are trained to associate a reward with an odor mixture and show a

conditioned response when tested with the mixture components, is that an indication that the identity of the components is conserved in the mixture percept? Or is it just an indication of the similarity between the components and their mixture? The use of physiological tools to complement behavioral experiments is fundamental to investigate how the olfactory system transforms odor evoked combinatorial activity pattern at the OSN level into single odor percepts.

The olfactory system of *Drosophila*: Anatomy and physiology

The olfactory system of the fruit fly *Drosophila melanogaster* presents three main advantages for the study of odor processing: first, in spite of its reduced scale, it maintains many similarities with other insect and vertebrate olfactory systems; second, many genetic tools are available which allow the manipulation of specific cell populations; and third, the knowledge base about its anatomical and physiological properties increases almost on a daily basis.

Fruit flies possess two olfactory appendages, the third antennal segment and the maxillary palp, where the dendrites of the OSNs are housed in olfactory sensilla (Stocker, 2001). Four morphological sensilla types have been described on the third antennal segment (large and small basiconic, trichoid, coeloconic and intermediate) containing a total of ~1200 OSNs. One sensillum type (basiconic) has been found in the maxillary palps with a total of ~120 OSNs (Shanbhag et al., 1999). Single-sensillar recordings have been used to characterize the response profiles of the OSNs of different sensillum types (de Bruyne et al., 1999; de Bruyne et al., 2001; Yao et al., 2005). OSNs classes are found in stereotyped combinations within the sensilla giving rise to functional sensillum classes in the maxillary palps and the antennae (de Bruyne et al., 1999; de Bruyne et al., 2001; Couto et al., 2005).

The Or family of *Drosophila* is one of the smallest described so far, with only 62 members (Clyne et al., 1999; Vosshall et al., 1999), each encoding a seven-trans-membrane-domain odorant receptor protein (OR). *Drosophila* ORs share no primary sequence similarity with vertebrate ORs, and it is not clear whether they belong to the family of the G-protein coupled receptors (GPCR), because its membrane topology seems to differ from that of most GPCRs (Bargmann, 2006; Benton et al., 2006). Almost no information is available about the second messenger cascades coupled to the ORs in the fruit fly, but they might share some features with vertebrate second messenger cascades, including the second messenger IP_3 and the G-protein subunits G_q and G_o (Smith, 2007). With few exceptions, each OSN expresses two

ORs, a OSN class-specific OR which defines the spontaneous activity and response properties of the OSN (Dahanukar et al., 2005), and a second, non-canonical receptor (OR83b) which acts as a co-receptor or chaperone for the class-specific receptor (Larsson et al., 2004). In some cases, OSNs have been shown to express genes that are members of the gustatory receptor family, such as Gr21a and Gr63a, which mediate CO_2 detection (Jones et al., 2007; Kwon et al., 2007). Unlike in the mammalian sensory system, odorant receptor proteins in *Drosophila* are not involved in axon guidance of the OSN axons to their corresponding glomeruli in the AL (Mombaerts, 2006; Jefferis and Hummel, 2006).

The AL of *Drosophila* is divided in ~50 glomeruli, and each of them is usually innervated by a single OSN class and by 3-5 PNs (Stocker, 1994; Laissue et al., 1999; Vosshall et al., 2000). The total number of PNs has been calculated in ~150, and most of these innervate only one glomerulus (Stocker, 1994). PN somata are located in three clusters, dorsal-anterior, ventral and lateral to the AL, and PN axons leave the AL trough 3 tracts, the outer, medial and inner antennocerebral tracts (Wong et al., 2002). Axons leaving the AL through the inner antennocerebral tract project into the mushroom body calyces, while the axons leaving the AL through the medial and outer antennocerebral tracts innervate the lateral protocerebrum (Wong et al., 2002). PN projections to the mushroom bodies and lateral protocerebrum are stereotyped across animals (Wong et al., 2002; Marin et al., 2002; Jefferis et al., 2007).

The number of AL LNs has been estimated in ~100 (Ng et al., 2002). Most *Drosophila* LNs described so far innervate all AL glomeruli, but their innervation patterns within the glomeruli are not uniform: some LNs arborize homogeneously over the entire glomerulus and others show spot-like innervation (Wilson and Laurent, 2005; Shang et al., 2007; Okada, personal communication, see Chapter I). Some described LNs innervate only a few glomeruli (Wilson and Laurent, 2005), but, unlike in honeybees (Fonta et al., 1993), no LNs with dense innervation in one glomerulus and sparse innervation in others have been found. Both GABAergic and cholinergic LNs have been described in *Drosophila* (Ng et al., 2002; Shang et al., 2007).

In the last years, a lot of information has been gathered about the physiological properties of the OSNs in *Drosophila*. OSNs respond to odors with temporally complex increases or decreases in spike frequency and intracellular calcium concentration. Broadly and narrowly tuned OSNs have been described (Hallem and Carlson, 2006), for example OSNs expressing the receptor proteins OR22a and OR22b are activated by a broad panel of chemically similar and dissimilar compounds (Pelz et al., 2006), while OSNs expressing OR49b are activated only by few aromatic compounds (out of a panel of over 100 odors) (Hallem and Carlson,

2006). However, the definition of broadly and narrowly tuned receptors depends strongly on the odors tested and the chosen odor concentration. OSN responses have been previously measured both in the antennae and in the AL and only subtle differences have been found between the dendritic response profiles in the antenna and axonal response profiles in the glomeruli of the AL (Pelz et al., 2006). Since most PNs in *Drosophila* have uniglomerular projections, the combinatorial representation of odor information is conserved at the PN level, and PNs also respond to odor stimulation with temporally complex changes in spike frequency and calcium concentration (Fiala et al., 2002; Wilson et al., 2004). However, the AL is not a simple relay station, and the response profile of the PNs is modified as compared to the OSN response profile (Wilson et al., 2004; Shang et al., 2007; Olsen et al., 2007). The modification of glomerular response profiles probably involves the activity of LNs (Shang et al., 2007). Although a lot of information has been gathered in recent years, the transformation of odor representations within the AL network is far from being understood.

Experimental approach

I used odors in different concentrations and combinations to address the question of odor representation and processing in the AL of *Drosophila* using a physiological approach.
In **Chapter I**, I compared the concentration-response dependencies in different populations of AL neurons in identified glomeruli. In **Chapter II**, I analyzed the representation of odor mixtures at the input and output of the AL network. The results of both chapters are discussed in the context of the current knowledge about odor processing in the insect AL.