Introduction

Sensory systems are the gateway between an animal's environment and its nervous system. The capacity of sensory systems to transduce physical stimuli into receptor potentials and further into action potentials forms the foundation of the internal neuronal representation of the animal's environment. The perception based on these internal representations differs qualitatively from the physical properties of the stimuli because the nervous system extracts only certain pieces of information from each stimulus while ignoring others. This information is then interpreted in the context of the brain's intrinsic structure and previous experience. Our perception of the world critically depends on the stimuli which can be decoded by our different sensory systems. The human acoustic system for example can transduce frequencies between 16 and 20,000Hz. Thus our acoustic experience is devoid of all higher frequency sounds, i.e. ultrasounds which are for example emitted by bats in order to navigate within their environment and to locate prey. Understanding how the nervous system creates internal representations of the external environment therefore requires knowledge about the stimuli perceived by the sensory systems and the features extracted from them.

Sensory systems of different modalities can be distinguished based on the physical properties of the stimuli transduced by their receptors into electrical activity. The photoreceptors of visual systems are activated by electromagnetic waves of specific wavelengths, the mechanosensory cells of vertebrate acoustic systems are activated by mechanic stimulation through pressure waves of certain frequencies, and the olfactory receptors expressed by sensory neurons of the olfactory system are activated by thousands of volatile organic compounds with diverse chemical structures and properties (Breer, 2003). This list already reveals a fundamental difference distinguishing the olfactory system from other sensory systems: Unlike visual or acoustic stimuli where electromagnetic waves or pressure waves can be sorted along a linear scale according to their frequencies, the dimensions of the 'odorant space' are still poorly defined (Kauer and White, 2001). The two odors D-carvone and L-carvone e.g., which are structurally as similar to each other as a right hand is to a left hand, to humans smell like peppermint and caraway while hydrogen cyanide and

benzaldehyde are structurally very different from each other yet both smell to humans like almond. Depending on their source odorous molecules can signal food, home, mate or predator to an animal. Despite recent advances little is known about which features of an odor make it capable of interacting with and subsequently activating a particular olfactory receptor (Araneda et al., 2000;Gaillard et al., 2002;Katada et al., 2005;Luu et al., 2004;Spehr et al., 2003). The number of substances which are odorous to humans and hence are capable of interacting with olfactory receptors, has been estimated to be around 400,000 (Mori and Yoshihara, 1995). How is the perception of this large variety of signals organized?

Olfactory systems are remarkably similar across different phyla (Hildebrand and Shepherd, 1997). In the periphery, i.e. in the nose of mammals or on the antennae of insects, odors interact with olfactory receptors (ORs), which are expressed in olfactory receptor neurons (ORNs). The ORNs' axons project to a first relay station, the vertebrate olfactory bulb (OB) or its insect analogue the antennal lobe (AL). Within the OB/AL the ORNs converge on spherical subunits called glomeruli. Glomerular structures have been found in virtually all olfactory systems studied (Hildebrand and Shepherd, 1997) suggesting that they play a fundamental role in processing of olfactory information. Within the glomeruli the ORN form a network with relay neurons, the vertebrate mitral cells and the insect projection neurons (PNs), and with inhibitory local neurons, vertebrate periglomerular, and granule cells or insect local neurons (LNs).

The role of the OB/AL network in odor processing is still a matter of debate. Particularly in insects opposing models exist. Two recent studies in *Drosophila melanogaster* suggest that the AL merely serves as a relay station where the information encoded in the ORNs is faithfully transmitted to the PNs (Ng et al., 2002;Wang et al., 2003). In contrast, another study also done in *Drosophila* concluded that the response pattern of the ORNs is broadened by the AL network at the level of the PNs (Wilson et al., 2004). A third model has been proposed based on work done in the honeybee where the suggested role of the inhibitory AL network is the sharpening of the broadly tuned response profiles of the ORNs (Linster et al., 2005;Sachse and Galizia, 2002).

Advancing our understanding of odor processing within the glomerular network requires knowledge about the information available to it. The first recordings from single insect ORNs were perfomed with tungsten electrodes inserted into single sensilla by Boeckh (1962) and Schneider and co-workers (1964). Around the same time the earliest single-cell recordings from vertebrate olfactory epithelium were done (Gesteland et al., 1965). Since then a considerable amount of data on ORN physiology has been collected. It could be shown that ORNs are activated by different odors and that the same odor can activate different ORNs (de Bruyne et al., 2001;Duchamp-Viret et al., 1999;Firestein et al., 1993;Friedrich and Korsching, 1998;Wachowiak et al., 2002b;Wachowiak and Cohen, 2001). Thus a picture of an across ORN code for odors arose. It has been suggested that for an odor to be activating, ORNs required the presence of certain molecular features and the absence of others while they were able to tolerate others (Araneda et al., 2000;Fuss and Korsching, 2001). ORNs were shown to be highly selective at naturally occurring odor concentrations (Bichao et al., 2005;Rostelien et al., 2005;Stensmyr et al., 2003b) and some ORNs were even shown to be enantioselective (Stranden et al., 2003).

It is the ORNs and the ORs expressed in these neurons that determine which of the odors in an animal's olfactory environment will be transduced into electrical signals and hence made available for further processing and the creation of internal representations. However, in order to fully understand how the information encoded in the ORN activity is further processed by higher brain centers, it would be ideal to know the coding capacities of the entire ORN population of a model olfactory system. This model system should be representative of olfactory systems in general yet be of manageable size. Additionally, the possibility of identifying the same ORNs and higher order neurons across animals would greatly facilitate a systematic investigation.

The olfactory system of *Drosophila melanogaster* fulfills these criteria with the additional advantage of a range of genetic tools available. The *Drosophila* olfactory system receives input from 1320 ORNs. These are housed in sensilla on two different appendages: the majority of the ORNs (1200) is found on the main olfactory organ, the third antennal segment (funiculus) and the remaining ORNs (120) are housed on the maxillary palps (Stocker, 1994). The antennal sensilla can be grouped into

morphological subtypes (Shanbhag et al., 1999;Venkatesh and Singh, 1984). Basiconic sensilla which can be subdivided in large and small basiconics, generally house two ORNs with exception of a large basiconic sensillum type housing four ORNs. Coeloconic sensilla house two ORNs and trichoid sensilla house between one and three ORNs (Stocker, 1994). Basiconic and coeloconic sensilla and their respective ORNs have been classified based on their physiological response properties to a diagnostic set of odors (de Bruyne et al., 1999;de Bruyne et al., 2001;Yao et al., 2005). Sensilla belonging to a physiological class are restricted to a particular spatial domain on the antennal surface (de Bruyne et al., 2001). Although certain types are intermingled, each is compartmentalized by particular spatial boundaries (de Bruyne et al., 2001).

In 1999 the olfactory receptor genes of *Drosophila* were identified and characterized (Clyne et al., 1999;Gao and Chess, 1999;Vosshall et al., 1999). Just like their vertebrate counterparts (Buck and Axel, 1991) they form a large gene family of 7-transmembrane G-protein coupled receptors (7TM GPCRs) with little sequence homology among each other and with those of other phyla (Clyne et al., 1999;Vosshall et al., 1999). While in rats and mice the OR family comprises approximately a 1000 members, the *Drosophila* OR family only consist of 60 genes coding for 62 olfactory receptors, 46 out of which have been shown to be expressed in the adult fly (Couto et al., 2005;Fishilevich and Vosshall, 2005;Goldman et al., 2005). It could also be shown that, in most cases, individual ORNs express only a single OR (Buck and Axel, 1991;Lewcock and Reed, 2004;Serizawa et al., 2003;Vosshall et al., 2000). Exceptions to this rule exist both in mammals and in *Drosophila* (Dobritsa et al., 2003;Fishilevich and Vosshall, 2005;Goldman et al., 2005;Larsson et al., 2004;Rawson et al., 2000).

In *Drosophila* Or83b is co-expressed with other ORs in all maxillary palp ORNs and in 70% to 80% of antennal ORNs (Larsson et al., 2004). In contrast to ‘conventional’ ORs, Or83b is highly conserved across insect species (Krieger et al., 2003). In Or83b null mutants, dendritic localization of the co-expressed conventional ORs is perturbed (Larsson et al., 2004). Furthermore, Or83b could be shown to form heterodimers with conventional ORs, highly increasing their functionality in a heterologous expression system (Neuhaus et al., 2005). The results obtained so far all

point to a general function of Or83b. On the other hand, co-expression of conventional ORs also exists (Couto et al., 2005;Dobritsa et al., 2003;Fishilevich and Vosshall, 2005;Goldman et al., 2005;Rawson et al., 2000). Most data on co-expressed ORs is morphological (Couto et al., 2005;Fishilevich and Vosshall, 2005). A physiological study on co-expressed Or22a and Or22b showed that only Or22a is responsible for the odor-responsiveness of the ORNs expressing both ORs (Dobritsa et al., 2003). Goldman et al. (2005) on the other hand showed that Or85e and Or33c co-expressed in maxillary palp ORNs were both functional each contributing to the odor-evoked response of the ORNs expressing them. It remains to be determined if in other cases co-expression of ORs results in modulation of the respective ligand-response profile as suggested by Fishilevich and Vosshall (2005).

Still, those ORNs expressing the same set of ORs project their axons to the same glomerlus (Couto et al., 2005;Fishilevich and Vosshall, 2005;Gao et al., 2000;Goldman et al., 2005;Vosshall et al., 2000). In mice each ORN class projects to two glomeruli, one in the lateral and one in the medial hemisphere of the bulb. Matching the number of approximately 1000 ORs mice have around 2000 glomeruli (Mombaerts et al., 1996). In *Drosophila* 49 glomeruli have been mapped and identified (Couto et al., 2005;Kondoh et al., 2003;Laissue et al., 1999). These numbers clearly illustrate the numerical advantage of *Drosophila* over e.g. mice as olfactory model system. Furthermore, there is considerable data on the innervation patters of the PN to higher brain centers, namely the mushroom body and the lateral horn (Marin et al., 2002;Tanaka et al., 2004;Wong et al., 2002).

Through misexpression of ORs in an ORN devoid of its endogenous OR in *Drosophila* it could be shown that ORs are responsible for normal olfactory function of ORNs (Dobritsa et al., 2003). The same experimental approach made it possible to establish a receptor to neuron map by linking a physiological response profile to an OR (Hallem et al., 2004). Furthermore, it was demonstrated that ORs are responsible for the signaling mode of an ORN, i.e. excitation or inhibition, which can both be mediated by the same OR, the response dynamics and the spontaneous frequency (Hallem et al., 2004).

Although ORs are largely responsible for the physiological response of ORNs they are not the only determining factors. Olfactory binding proteins[1] (Pophof, 2002;Pophof, 2004;Xu et al., 2005), ORNs housed within the same sensillum (Dobritsa et al., 2003), co-receptors (Dobritsa et al., 2003;Larsson et al., 2004;Neuhaus et al., 2005) and receptor associated G-proteins (Shirokova et al., 2005) have also been suggested to influence the response characteristics of an ORN. Thus the input to the olfactory system is provided by the information encoded within the odor response profile of an entire ORN including all auxiliary mechanisms and cells. The set of odors which either activates or inactivates an ORN has been called its molecular receptive range (MRR) (Mori et al., 1992;Mori and Shepherd, 1994;Mori and Yoshihara, 1995). Determining the input to a model olfactory system therefore requires establishing the MRR of its entire ORN population.

The entire ORN population of an olfactory system can be further divided into subpopulations of ORNs expressing the same OR and thus showing the same MRR. As described above, ORNs expressing the same OR innervate the same glomerulus. The glomerulus has been shown to be a functional unit (Wachowiak et al., 2004) and has been suggested to play a major role in olfactory coding. Thus ORN populations expressing the same OR can be considered to represent the 'input channels' of the olfactory system. Characterizing these input channels thus requires to measure population responses. Functional imaging allows for recordings from populations of identified neurons and has been successfully applied in studying the olfactory system. Calcium dyes for example have been manually introduced into ORNs of mice to study how odors of different chemical structures and concentrations are coded across the OB (Fried et al., 2002) as well as to examine the functional organization of sensory input to individual glomeruli (Wachowiak et al., 2004). Selective labeling of honey bee projection neurons with a calcium (Ca^{2+}) sensitive dye has been used to study the role of inhibition in odor processing within the AL (Sachse and Galizia, 2002) and to examine the coding of odor intensity (Sachse and Galizia, 2003).

In *Drosophila* genetically encoded indicator dyes exist, thus functional imaging is a rather non-invasive technique as the dyes do not have to be manually introduced.

[1] A large, diverse family of proteins, which has been suggested (among others) to be involved in solubilization of odors but whose function in olfactory signal transduction remains unclear.

There are two groups of protein-based dyes currently employed: those based on a pH sensitive mutation of GFP linked to synaptobrevin which report the release of transmitter into the synaptic cleft (Miesenbock et al., 1998). These dyes have been used to study how olfactory information is represented successively at the level of the ORNs, LNs and PNs (Ng et al., 2002) and to visualize how the representation of olfactory information is changed after olfactory conditioning (Yu et al., 2004). The other dyes have one or two GFP moieties linked to calmodulin which acts as a sensor for intracellular Ca^{2+} concentration, which is dependent on neuronal activity (GCaMP, Cameleon) (Miyawaki et al., 1999;Nakai et al., 2001). GCaMP has previously been used to study odor-evoked patterns in the AL at the level of ORNs and PNs (Wang et al., 2003). Cameleon2.1 expressed in PNs was employed to study odor-evoked activity patterns in the AL and in the calyx of the mushroom bodies (Fiala et al., 2002).

While signals recorded with synaptopHluorin are clearly linked to synaptic transmission they are also limited to it. Sensors of intracellular Ca^{2+} concentration on the other hand are less clear in the exact source of their signal, e.g. voltage operated calcium channels or intracellular stores (Augustine et al., 2003) but at the same time they are more universal. The activity of ORNs can be measured at two different levels within the living animal: in the dendrites and cell bodies on the antenna, the place where sensory transduction takes places, and at the axonal endings in the glomerulus where sensory information is transmitted to higher-order neurons. Both processes involve changes in Ca^{2+} concentration while release of synaptic vesicles only occurs at the axonal endings in the glomerulus. Thus, employing a calcium sensor gives much more possibilities when examining ORN physiology within the living animal.

The aim of this study was to characterize *Drosophila* olfactory receptor neurons functionally as a step towards determining the MRRs of the entire Drosopila ORN population. Furthermore, I was interested in a possible influence of the inhibitory AL network on the ORN responses. I wanted to establish the MRR of an exemplary ORN population in great detail, i.e. characterize the chemical identity of the odors belonging to the MRR as well as their potency as ligands. Both pieces of information were thought of as the basis for identifying odotopes common to activating ligands. Additionally I wanted to validate the method and test its general applicability.

To this end I chose an exemplary ORN population, namely those expressing the olfactory receptor protein Or22a, which at the beginning of the experiments was suspected to be the ab3a cell as classified by de Bruyne et al. (2001). Thus, some putative ligands of this ORN population were already known. This had the advantage of enabling me to validate the results obtained with Ca^{2+} imaging by comparing them to results obtained by a different technique, i.e. electrophysiology.

The specificity of a glomerular response has been taken as being determined by the specificity of the OR expressed by the ORNs innervating it (Friedrich and Korsching, 1997;Friedrich and Korsching, 1998;Ng et al., 2002;Wang et al., 2003). However, previous studies have shown that both in vertebrates and invertebrates presynaptic inhibition of ORNs exists, changing their activity (Aroniadou-Anderjaska et al., 2000;Ennis et al., 2001;Murphy et al., 2005;Wachowiak et al., 2002a;Wachowiak et al., 2005;Wachowiak and Cohen, 1998;Wachowiak and Cohen, 1999) potentially altering the MRR. Thus, I established the MRR of Or22a expressing ORNs at the level of sensory transduction on the antenna and at the level of sensory transmission in the AL to see if both MRRs were indeed identical. Moreover, I applied ionotropic GABA agonists and antagonists to reveal evidence for presynaptic inhibition.

Establishing the MRR of Or22a expressing ORNs was done in a two-step process: Initially, I tested the responses to a panel of 104 odors at a high concentration (10^{-2} [vol/vol]) employing an automated stimulus application system. 39 of the odors tested were found to be activating. Odors activating an olfactory receptor are conceptually equivalent to drugs activating a receptor. In pharmacology, dose-response curves are a means of measuring drug-receptor interactions and are the standard method for comparing the potencies of various compounds that interact with a particular receptor (Silverman, 1992). Therefore I subsequently determined the dose-response curves of each of the activating odors.

The dose-response curves allowed me to determine the potency of the odors belonging to the Or22a MRR. These potencies made it possible to deduce common odotopes of Or22a activating odors. Odotopes have been defined as those parts of an odor molecule putatively responsible for its interaction with a particular receptor

(Mori and Shepherd, 1994). Identification of such odotopes potentially furthers our understanding of receptor ligand interactions. If the 3D-structure of the receptor is unknown as in case of *Drosophila* ORs, odotopes common to odors activating the same OR provide information about possible binding sites within the receptor. To this end I collaborated with Daniel Baum from the Zuse Institute Berlin (ZIB) who developed an algorithm for superpositioning 3D molecular structures (Baum, 2005).

Finally, I started the characterization of another OR, namely Or47b, which is expressed in ORNs housed in trichoid sensilla (Hallem et al., 2004). ORNs housed in trichoid sensilla are of particular interest as hardly anything is known about their physiology to date (Clyne et al., 1997;Hallem et al., 2004;Xu et al., 2005)