Introduction

Chemosensory systems, generally divided in olfactory and gustatory systems, present the animal with neural representations of the chemicals in their environment. Most animals use this information for localizing resources such as food and mates, as well as for avoiding toxic, dangerous or simply les profitable environments. Many basic mechanisms and design principles are shared between the olfactory systems of vertebrates and insects (Hildebrand and Shepherd, 1997). Olfactory receptor neurons (ORNs) respond to several odorants and each odorant excites more than one class of ORNs (Duchamp-Viret *et al.,* 1999, Malnic *et al.,* 1999, de Bruyne *et al.,* 1999, 2001). Axons from all ORNs of a single class converge onto spherical units called glomeruli in the olfactory bulb of vertebrates (Mombaerts *et al.,* 1996) or the antennal lobe of insects (Vosshall *et al.,* 2000) and odors elicit spatio-temporal patterns of activation in these primary olfactory centers (Friedrich and Korsching, 1998, Rubin and Katz, 1999, Joerges *et al.,* 1997, Wang *et al.,* 2003, Ng *et al.,* 2003). Therefore, odors are thought to be primarily encoded in a combinatorial way (Galizia and Menzel, 2000, Korsching, 2001). Efforts to understand the principles of the neural encoding of chemical information in the olfactory system are hampered by many problems. Odors are often complex, difficult to handle and their diversity impossible to quantify in simple parameters. Furthermore, it has proven hard to link the synthetic view of spatio-temporal patterns of neuronal activity that underlie an odor percept,

to behavioral responses on the one hand and the properties of individual receptor neurons on the other.

In insects certain odorants are perceived via specialized receptor neurons that provide the brain with unique input. Most notably, pheromone signals in moths are mixtures of a few compounds that each stimulates a single class of ORN (Hansson, 1995). A small set of closely apposed glomeruli in the antennal lobe processes this signal (Christensen and Hildebrand, 2002). Such focal activity has also been observed in the olfactory bulb of zebra fish (Friedrich and Korsching, 1998). Does the olfactory system consist of a homogeneous array of sensors from which an odor percept is extracted by the combined activity of all inputs? Or, are there specific subsets of olfactory stimuli that require special processing by receptors that are 'wired' differently? Carbon dioxide could be an olfactory stimulus that holds such a position across species.

Carbon dioxide is a gas, ubiquitously present in atmospheric air, which the human olfactory system cannot detect at concentrations below 25% (Shusterman, 2002). However, ORNs that are more sensitive to CO_2 have been found in the epithelium of a frog (Coates and Balam, 1990). Several insect species are known to possess specific receptor cells that respond to very small changes in levels of CO_2 just above or below the 0,035% present in atmospheric air (Stange, 1996). It represents a rather general cue that is used in different ways, for example to detect a source of blood by mosquitoes (Bowen, 1991) or to indicate bad ventilation in bee hives (Seeley, 1974). One important advantage in studying the perception of CO_2 compared to other

odorants is that the stimulus can be well defined. For other odorants, concentrations in air depend on questions of volatility, but CO_2 can be easily produced in desired concentrations and measured with various sensors. We recently identified a CO_2 receptive neuron in the antenna of *Drosophila melanogaster* (de Bruyne *et al.*, 2001). In *Drosophila* ORNs are compartmentalized in sensilla which can be categorized into basiconic, coeloconic or trichoid sensilla based on morphology (Shanbhag *et al.*, 1999). The CO_2 sensitive neuron is housed together with three other ORNs in a large basiconic sensillum. The other three ORNs respond to several odorants the way other ORNs on the antenna and maxillary palps do (de Bruyne *et al.*, 2001). To what extent is this CO_2 sensitive neuron like other ORNs, and what is its contribution to the olfactory code? Moreover, the ability to sense this small molecule raises the question whether transduction is comparable to other odorants. What are the concentrations that can be detected and how does the output from this neuron relate to olfactory driven behaviors? The fly is an excellent model system to answer these questions because it is amenable to electrophysiological and behavioral experiments as well as the manipulation of genetically targeted cell classes.

In *Drosophila,* odor detection is thought to be mediated by a family of 61 G-protein coupled receptor genes, the ORs, with many members expressed in ORNs of antennae and maxillary palps (Clyne *et al.,* 1999, Vosshall *et al.,* 1999). Several lines of evidence indicate a role of these receptors in transduction of odors. The *Or43a* receptor, when over expressed in *Drosophila* antennae or in a heterologous cell system, generates odor dependent physiological responses (Störtkuhl and Kettler,

2001, Wetzel *et al.,* 2001). Analyses of mutations that remove *Or22a* (Dobritsa *et al.,* 2003) or *Or43b* (Elmore *et al.*, 2003) show that expression of these receptors is necessary for endowing an ORN class with its typical response properties. The link between receptor gene expression and response properties was further strengthened by expressing several such genes in an olfactory neuron and showing that their responses matched those of the original neuron in which that receptor is constitutively expressed (Hallem *et al*, 2004). An exception to this rule is the *Or83b* receptor, which appears to occur in many ORNs as a second receptor with an as yet unknown function (Vosshall *et al*., 1999). *Drosophila* has another family of chemosensory receptor genes of similar size, the gustatory receptor (GR) genes (Clyne *et al.,* 2000). Several members of this family are expressed in taste hairs of legs and proboscis (Clyne *et al.,* 2000, Scott *et al.,* 2001, Dunipace *et al.,* 2001).

We noted with interest that one of the GR genes, *Gr21a*, shows expression in the antenna and sends axons to a ventrally located glomerulus, the V glomerulus (Scott *et al.* 2001). A conspicuous, ventrally located glomerulus responds to CO_2 in 2DG labeling studies of a blowfly (Distler *et al.,* 1998). This prompted us to investigate the identity of the neurons that express *Gr21a*. We demonstrate that *Gr21a* is expressed in CO_2 sensitive neurons of the antenna. We also show that *Drosophila* flies respond behaviorally to CO_2 concentrations just 3x ambient levels and that the sensitivity of receptor neurons is even lower. We provide first indications of the direct relation between calcium signals in these neurons and the action potential firing rate. In addition, the fact that CO_2 is an easily quantifiable stimulus, exciting only a single

type of ORN, allows us to quantify the relation between responses of sensory neurons and behavioral output.