Results 2

A preliminary molecular receptive range of Or47b expressing ORNs

The second receptor I studied was Or47b which is expressed in about 50 ORNs along the distal edge of the antenna (Bhalerao et al., 2003;Vosshall et al., 2000). Their axons innervate glomerulus VA1lm (Bhalerao et al., 2003;Wang et al., 2003) which is a large, banana shaped glomerulus at the ventral lateral edge of the AL (Vosshall et al., 2000), at the entry point of the antennal nerve (Kondoh et al., 2003). Or47b is in many regards different from Or22a. Whereas Or22a is expressed in ORNs housed in large basiconic sensilla (Dobritsa et al., 2003;Hallem et al., 2004), Or47b is expressed in ORNs housed in trichoid sensilla (Bhalerao et al., 2003;Hallem et al., 2004). Each of the receptors is in a way sexually dimorphic: there is a larger number of ORNs expressing Or22a in females than in males (Dobritsa et al., 2003) while ORNs expressing Or47b innervate VA1lm (Bhalerao et al., 2003;Couto et al., 2005;Fishilevich and Vosshall, 2005) which is one of two glomeruli enlarged in males (Kondoh et al., 2003). The spontaneous firing frequency which is determined by the olfactory receptor (Hallem et al., 2004) is rather low in ORNs expressing Or22a (4Hz) (de Bruyne et al., 2001) and about 15 times higher in ORNs expressing Or47b (60Hz) (Hallem et al., 2004). Because of all these differences, examining the response properties of ORNs expressing Or47b greatly expands the picture about the input to the *Drosophila* olfactory system, in particular as no (activating) ligand has been found so far (Hallem et al., 2004;Wang et al., 2003) for this ORN population.

When I started with the characterization of ORNs expressing Or47b hardly anything was known about them. Because of their expression along the distal edge of the antenna they were suspected to innervate trichoid sensilla (de Bruyne et al., 2001;Vosshall et al., 2000) which has been proven to be the case by now (Hallem et al., 2004). However, even to date there is still only scarce data on the physiology of trichoid sensilla (Clyne et al., 1997;Hallem et al., 2004;Xu et al., 2005). Before starting a characterization of any class of olfactory receptor neurons it is important to have at least one activating ligand in order to have a reference odor for the animal's responsive state. Thus, I started by testing the odors employed by Clyne et al. (1997)

which were found to elicit a response in trichoid sensilla. Those odors were cis-vaccenyl acetate, 4-methylcyclohexanol, and E2-hexenal. E2-hexenal was found to elicit an increase in fluorescence intensity upon presentation which was highly reproducible within an animal (Figure 18A and 18B). As E2-hexenal is a C6 aldehyde I tested all other C6 odors included in the panel of odors tested on Or22a expressing ORNs (for molecular structures of odors tested see Figure 19). Responses were elicited by E2-hexen-1-ol, 1-hexanol, Z3-hexen-1-ol, and hexanal and were highly reproducible across animals (Figure 18B right). As shown in Figure 18C the majority of odors eliciting a change in fluorescence intensity were inactivating, i.e. they led to a decrease in fluorescence intensity. The decrease in fluorescence upon presentation of an inactivating odor was not uniform across odors: the responses varied in the maximal amplitude as well as in the time courses (Figure 18C and 18D). Hexanal for example evoked a negative off response, i.e. a Ca^{2+} decrease beginning after stimulus offset. A detailed comparison of time courses evoked by different odors is, however, not possible as the responses also varied in their response amplitude. The only activating odor, i.e. the only odor eliciting an increase in fluorescence intensity, was E2-hexenal. The response to this odor was dose-dependent as can be seen in Figure 18C and 18D.

In contrast to ORNs expressing Or22a where the signal quality remained stable for up to 100 minutes the signal in ORNs expressing Or47b usually deteriorated rather fast despite the state of the animals still being good (i.e. the animals were still moving, the antennae were dry, and the brain transparent). This made it difficult to test a larger number of odors within an animal. As no solution could be found for this problem and due to time constraints the data set is based on a lower number of odors than for ORNs expressing Or22a. However, the results presented here provide a sound basis for further experiments.

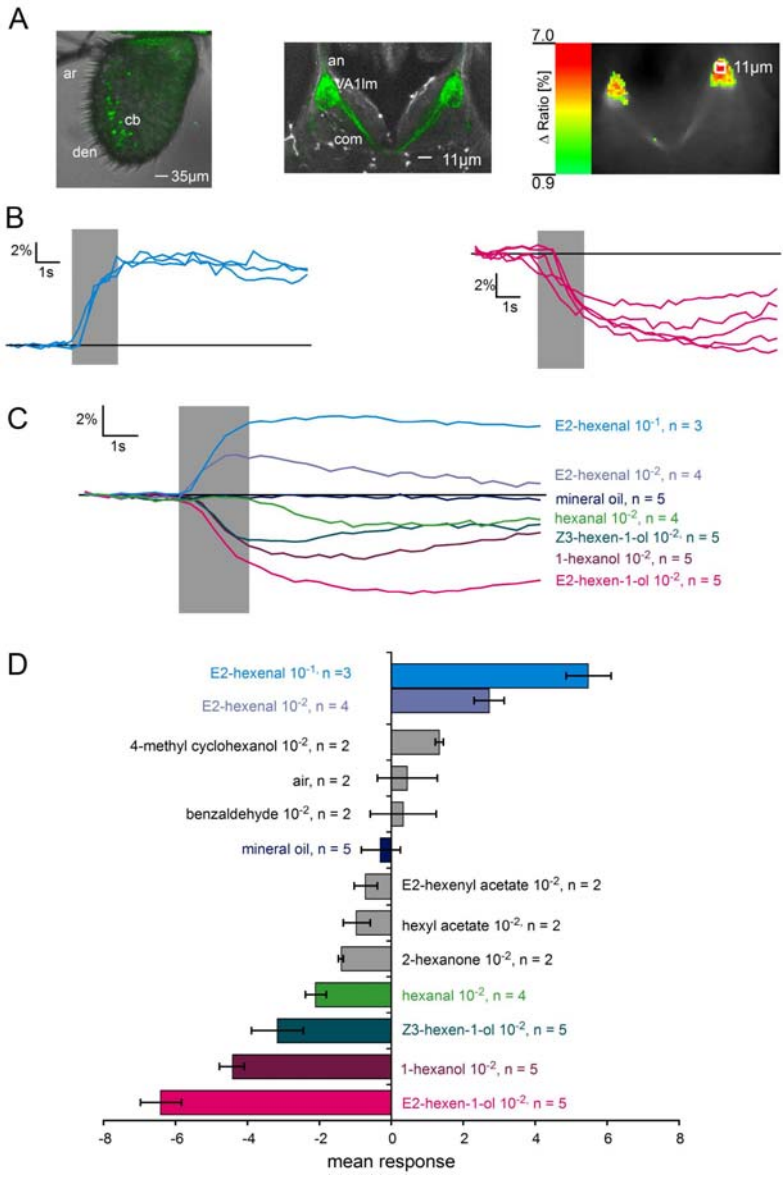

Figure 18 Preliminary molecular receptive range of ORNs expressing Or47b. A Confocal images of ORNs expressing CD8-GFP and GFP under control of the Or47b promotor. Left: antenna, ar, arista; cb, cell body; den, dendrites. Middle: antennal lobe (AL), an, antennal nerve; VA1lm, glomerulus innervated by ORNs expressing Or47b; com, comissure formed by collaterals projecting to the contralateral AL. Orientation of the AL as in the preparation employed in this study. Right: False color-coded image of a response to E2-hexenal 10^{-1} [vol/vol] indicating area from which responses were calculated. **B** Responses are reproducible within and across animals. Grey bar indicates stimulus presentation. Y-axis shows ΔRatio [%]. Left: Time traces of repeated presentations of E2-hexenal at a concentration of 10^{-1} [vol/vol] within the same animal measured in the AL over a time course of 22 minutes with other odors presented in between, interstimulusinterval 2 minutes. Right: Time traces of responses to presentations of E2-hexen-1-ol at a concentration of 10^{-2} [vol/vol] in 5 different animals. **C** Responses to different odors presented at a concentration of 10^{-2} [vol/vol] with exception of E2-hexenal which was also presented at a concentration of 10^{-1} [vol/vol]. Odors were only presented once in each animal unless stated otherwise. Time traces are averages of air n =2, benzaldehyde n = 2, E2-hexen-1-ol n = 5, E2-hexenal 10^{-2} [vol/vol] n = 4, E2-hexenal 10^{-1} [vol/vol] n = 3, hexyl acetate n = 2, 4-methyl cyclohexanol n = 2, 1-hexanol n = 5, hexanal n = 4, hexyl acetate n = 2, 2-hexanone n = 2, Z3-hexen-1-ol n = 5, mineral oil n = 5 animals. Grey bar indicates stimulus presentation. Y-axis shows ΔRatio[%]. **D** Average response elicited by all odors tested in ORNs expressing Or47b. Bars show mean, error bars indicate SEM. N as in C.

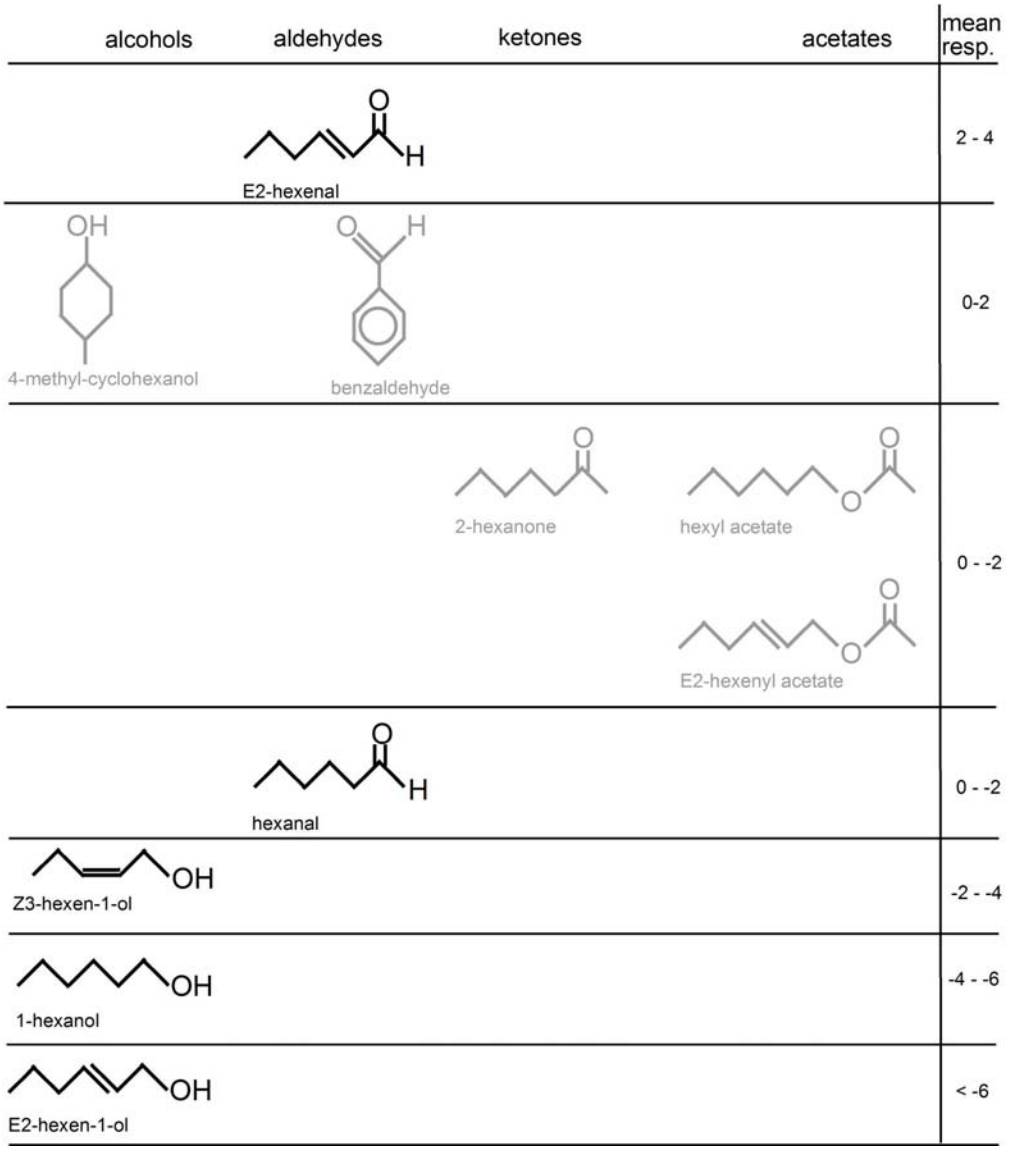

Figure 19 Chemical structures of odors tested on ORNs expressing Or47b. Molecules indicated in black elicit a response, either activating or inactivating. Molecules depicted in grey are inactivating. Across columns odors are sorted according to their chemical class. Within columns odors are sorted according to the average mean response (mean resp.) elicited by them (indicated to the right).