

## Selective Imaging of the Receptor Neuron Population in the Olfactory Bulb of Zebrafish and Mice

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### Convergence of the olfactory projections allows analysis of receptor repertoires in the olfactory bulb

The neuronal processing of odors takes place in several types of neurons, including the sensory neurons, projection neurons and interneurons. To understand the neuronal representation of odors and eventually the encoding of those odors it is important to selectively measure the contributions of the different neuron populations to odor-induced neuronal activity. We are analyzing the odor responses of the population of olfactory receptor neurons in two experimental systems, zebrafish and mouse. Odor responses are measured in the receptor neuron terminals within the olfactory bulb. Thus, the response properties of many different odorant receptors can be visualized simultaneously by optical imaging of neuronal activity in the olfactory bulb, since olfactory receptor neurons expressing the same odorant receptor converge onto common neuropil structures in the olfactory bulb, the glomeruli (cf. Korsching, 2002). This transition from presumably stochastic expression of odorant receptor genes in scattered olfactory receptor neurons within the sensory epithelium to an ordered map in the olfactory bulb (Figure 1) allows a unique view on the size and composition of the receptor repertoires that are activated by particular odorants.

### Selective analysis of odor-induced presynaptic activity

We have introduced activity indicator dyes selectively into olfactory receptor neurons by local application in the nose. We use the voltage-dependent dye ANEPPQ (Friedrich and Korsching, 1998) and the calcium indicator dye CalciumGreen (Friedrich and Korsching, 1997), both of which distribute in the whole cell, up to and including the axon terminals in the glomerular layer of the olfactory bulb. By this means we have analyzed the tuning properties of major receptor populations selectively in the presynaptic compartment of glomeruli (Friedrich and Korsching, 1998; Fuss and Korsching, 2001; Fried *et al.*, 2002).

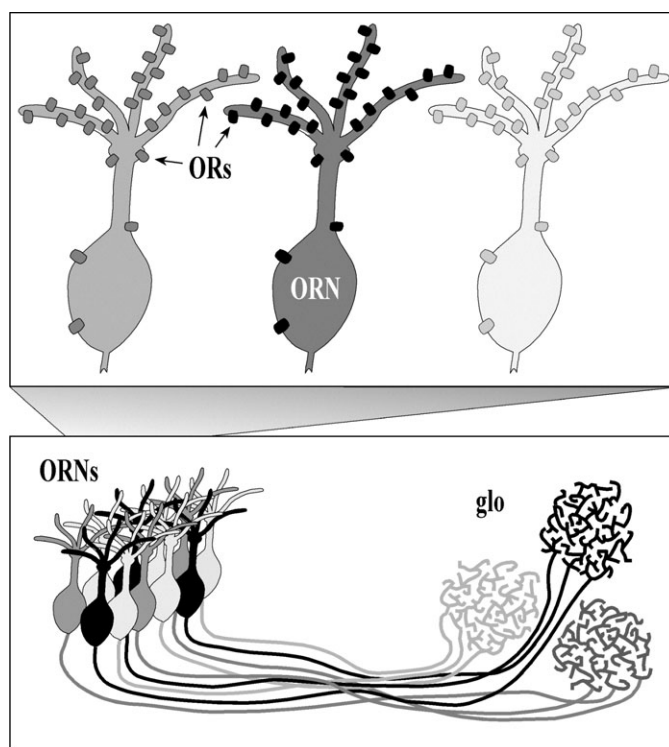
### Chemotopic representation of odorants in the olfactory bulb

We report that responses to different chemical groups of odors are segregated within subregions of the zebrafish olfactory bulb. Amino acids—important feeding stimuli—are represented in the anterio-lateral olfactory bulb, whereas nucleotides elicit responses in the posteriolateral olfactory bulb and bile acids are represented medially. Pheromone responses are localized in central and medial regions. Amino acid responses are found in microglomerular structures reminiscent of the termination areas of microvillous olfactory receptor neurons in the mammalian accessory olfactory system. On the other hand, pheromone responses were detected in standard size glomeruli such as those formed by terminals of ciliated receptor neurons in the mammalian main olfactory system.

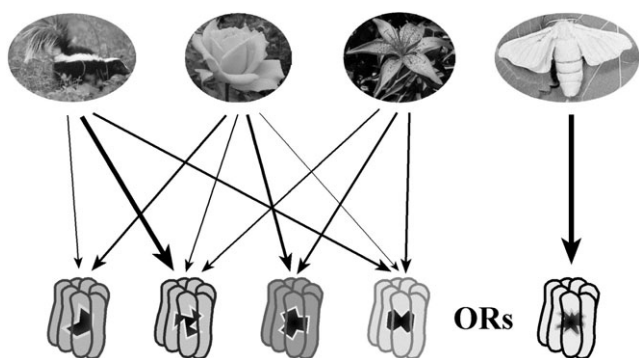
### Odorant features may be either required, tolerated or prohibitive

We have performed a detailed analysis of the chemical tuning of the amino-acid-responsive microglomeruli. We report that the amino acid head region is required to elicit a response, i.e. neither amines nor carboxylic acids of equivalent chain length function as odorants in the fish system (interestingly, both groups of chemicals are very strong odorants for air-breathing vertebrates).

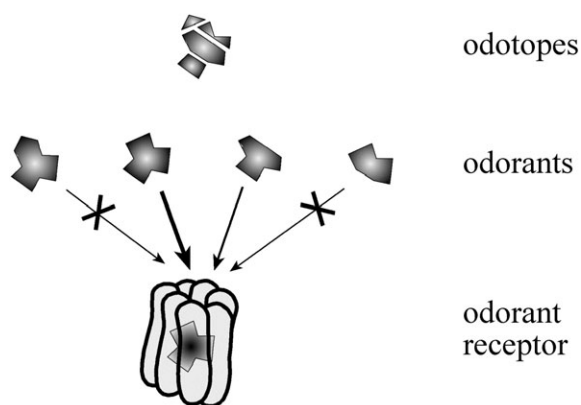
Using neutral amino acids of various side chain lengths as stimuli we find that—barring few exceptions—most odorant receptors react stronger to longer side chain amino acids, even extending beyond the size-range present in the 20 proteinaceous amino acids. Different microglomeruli are differently tuned to side chain length, so that new odorant receptors are recruited to the response pattern even with additions as small as a single methylen group. As predicted from



**Figure 1** Monogenic expression and convergence of odorant receptors. The top panel shows a schematic representation of three olfactory receptor neurons (ORNs), each expressing a different odorant receptor (OR): the one neuron—one receptor concept. The bottom panel depicts the convergence of same receptor-expressing neurons on single glomeruli (glo) in the olfactory bulb.



**Figure 2** Two coding strategies for odors. Common odors and odors: combinatorial representation by activation of a characteristic subset of odorant receptors—a unique ‘finger print’ for each odorant. Pheromones: mono-specific activation of a single odorant receptor—labeled line concept.



**Figure 3** The combinatorial model of odorant/receptor binding. Even a simple odorant may consist of several odotopes. A particular receptor requires a particular combination and positioning of odotopes for maximal activation. Odotopes may be not tolerated (triangle), tolerated (twin triangle) or even required (rectangle).

these differences in tuning we find that each concentration of each amino acid elicits a unique response pattern not matched by any other combination of chain length and concentration.

When testing the recruitment of odorant receptors by polar and charged amino acids (serine, threonine, and ornithine, lysine, respectively) we observe characteristic differences in the response patterns. Some receptors are only activated by neutral amino acids, some only by basic amino acids, and some are activated by both. There exist also receptors that tolerate both neutral and polar amino acids, but none that specifically require polar amino acids.

## Mammalian aldehyde receptors are oligo-specific

In another project we have analyzed the tuning properties of a major mammalian odorant receptor population using the same method of high resolution calcium imaging (Fried *et al.*, 2002). We show that eighty different odorant receptors projecting to the dorsal olfactory bulb of mouse respond to high concentrations of aliphatic unbranched aldehydes with limited specificity. Different ensembles of ~10–20 receptors encode any particular aldehyde at low stimulus concentrations with high specificity. Pronounced differences in affinity were observed within the aldehyde receptor repertoire. Again, a unique response pattern of activated glomeruli is observed for each chain length and (non-saturating) concentration.

## Concept of odorant/receptor interaction

We conclude that odorant detection is combinatorial, i.e. requires several receptors even for relatively simple odors such as amino acids and aldehydes, but may be mono-specific for pheromones (Figure 2). Furthermore, we find that individual odorant receptors require the presence of some molecular features, the absence of others, and tolerate still other molecular features. Thus, odorant receptors appear not to be simple ‘feature detectors’ but to detect particular combinations of molecular features—odotopes (Figure 3).

## Current work

An interesting application of our imaging method will be the study of odor responses of genetically labeled glomeruli. Several mouse lines expressing green fluorescent protein (GFP) under the control of a specific odorant receptor promoter are available. We are currently investigating the compatibility of the CalciumGreen signal detection with the GFP labeling in such a mouse line (Feinstein and Mombaerts, 2004).

To address receptor neurons sharing an identified receptor and to address interneurons and projection neurons separately we are characterizing suitable promoter regions and using them to drive expression of genetically encoded calcium dyes as indicators of odor-induced neuronal activity.

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