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# Odor Coding in Projection Neurons of the Honeybee Brain

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Olfactory stimuli are coded by a spatio-temporal pattern of activity at the primary level of sensory integration, the olfactory bulb in vertebrates and the antennal lobe (AL) in insects (Hildebrand and Shepherd, 1997; Galizia and Menzel, 2000), indicating that multiple glomeruli encode particular chemical compounds as well as odor mixtures. A glomerular activity pattern may be translated into a cross-fiber-activity pattern in relay neurons (projection neurons, PN) enriched by temporal components, e.g. synchrony effects of spike activity (Laurent, 2003). In all studied insects, the inner antenno-cerebral tract (in honeybees, the median antennocerebral tract, m-ACT), connects the mushroom body (MB) and the lateral horn (LH) with the AL. Similarly, axons of the outer antenno-cerebral tract (in bees, the lateral ACT, l-ACT) innervates the LH and MB but in a reverse sequence: first the LH and then the lip region of the MB calyces. A third bundle of axons, called the mediolateral ACT, also innervates the LH and larger parts of the LH, but not the calyces. This third bundle of PNs will not be dealt with here.

In the bee, the anatomy of the PN tracts is rather simple. The PNs of the m- and l-ACT receive input only from single glomeruli in the AL (uniglomerular PNs; Abel *et al.*, 2001). Thus, the MB receives olfactory information only via uniglomerular PNs.

Considering the similar anatomical features of the m- and the l-ACT neurons, we asked: (i) how do they terminate in the lip region of the MB calyx; (ii) how do these neurons differ in their olfactory response properties; and (iii) how is the activity pattern of these neurons translated into activity patterns at their outputs.

We reconstructed >130 intracellularly marked PNs, aiming to analyse axon terminal patterns in the lip region of the MB. PN axons innervate large parts of the MB lips and terminate with up to 12 blebs per PN axon within the dendritic area of one Kenyon cell (KC) (Müller *et al.*, 2002). These axon terminals provide overlapping inputs to KC dendritic areas. The blebs are not evenly distributed, accumulating in some regions of the lip and leaving others empty. Such an organization will lead to different combinations of inputs to the same KC dendritic areas. Since the dendritic branches of neighboring KCs overlap considerably, different PNs still terminate at many different KCs. However, clusters of KCs should receive similar combinations. Axon terminals of m- and l-ACT neurons appear to fully overlap in the lip regions of the calyces, making it likely that a given KC receives input from both types of PNs. Since l-ACT neurons may transmit more general and m-ACT neurons more specific information about the quality of the odor (see below), KCs would extract the relevant information most effectively if they were particularly sensitive to the timing of spike arrival within an odor puff. This is exactly what we find.

Since the PNs of the m- and l-ACT originate in different glomeruli, one might expect different olfactory sensitivity profiles. In fact, the glomeruli served by the two groups of PNs are located in two subdivisions of the AL as defined by the innervation of receptor axons running in four branches of the antennal nerve. Receptor axons of branches 2, 3 and 4 (T2, T3 and T4) go to glomeruli served by dendrites of m-ACT neurons and receptor axons in branch 1 (T1) go to glomeruli served by dendrites of l-ACT neurons (Abel *et al.*, 2001). The anatomical separation in the two groups of PNs may, therefore, reflect a functional separation between groups of receptor inputs. This separation appears not to be related to the distinction between the general odor system and a sex-pheromonal system, because worker bees do not possess a macroglomerular complex. Nevertheless, neurons of the m- and l-ACT tracts may receive differential input from receptors sensitive to general odors or pheromones, e.g. those important for social communication in the colony.

Another possibility would be that the two tracts have overlapping odor sensitivity profiles but convey different aspects of odor stimuli, e.g. odor quality or the temporal structure or intensity of a stimulus. This is exactly what we find. Neurons of the m- and l-ACT overlap in their olfactory sensitivity profiles, but have different time courses of their responses to different odors. Odor-induced responses in m-ACT neurons are temporally highly structured. The latency of excitatory response onset is a consistent and reliable parameter which codes for various odors. Some odors evoke response latencies differing by >400 ms, whereas response variability to stimulus repetition is only ~60 ms. In contrast, neurons of the l-ACT neurons respond to different odorants in a graded fashion with a phasic-tonic spike pattern. This leads to broad response profiles (Müller *et al.*, 2002).

l-ACT neurons receive their uniglomerular input from glomeruli from the T1 AL regions and this was measured by *in vivo* imaging methods (Joerges *et al.*, 1997; Galizia *et al.*, 1999). This gives us the opportunity to compare the intracellular recordings with opto-physiological measurement. The two methods revealed results for three glomeruli. The response spectra determined by the imaging method are broader than those measured by intracellular recording from its output neuron. The greatest differences were found for lime, orange and isoamyl acetate: the respective neuron did not respond, but the imaged glomerulus did. 2-Heptanone elicited the strongest responses in both measurements and the chemically similar odorants 2-octanone and 2-hexanone evoked similar graded responses in both measurements. 1-Hexanol, an alcohol with the same chain length as 2-hexanone, evoked a response from the glomerulus in the imaging experiment, but not in the recording of the respective output neuron. The responses to several odors in the imaging experiments from glomerulus T1-27, that were <50% of the maximal measured signal for a particular animal, corresponded to those measured in l-ACT neurons receiving input from that glomerulus. These same odors did not elicit a measurable response from these neurons. The T1-22 glomerulus responded strongly to geraniol as measured by both methods and responses <50% found in the imaging data also corresponded to weak or failing responses in a neuron originating in this glomerulus. Exceptions in this glomerulus were the responses to 2-heptanone, which only produced a response <50% in the imaging measurement, whereas the neuron shows the strongest response to 2-heptanone. Taken together, the response profile of the output neurons shows a sharper profile than the imaged glomerulus response; a level of 50% of the maximum response measured for one animal to one odor could be a threshold for transferring information to the l-ACT neurons.

On a population level neurons of the two tracts, the m- and the l-ACT, have overlapping response profiles, excluding the possibility that the separation of olfactory receptor neurons (ORNs) projecting to different glomerulus populations involves different classes of olfactory stimuli (e.g. different groups of general odors; general odors versus pheromones; airborne odors versus contact chemoreceptive odors). Such a strategy of sensory coding is reminiscent of, for example, the retinal ganglion cells serving the magnocellular and parvocellular substructure of the lateral geniculate in mammals (Livingstone and Hubel, 1988). Generalized to the olfactory system of the honeybee, different aspects of olfactory stimuli could be coded separately, for example their temporal structure, their intensity fluctuations and the sequence, their evaluation and meaning. Furthermore, since inhibitory interactions at the level of the AL increase with the number of components (Joerges *et al.*, 1997), m-ACT neurons may be more proper to code odor mixtures.

The differences in the latencies could also account for another possibility. The more unspecified but fast-responding l-ACT neurons might just focus the bee's attention onto olfactory stimuli and prepare the MB for processing m-ACT neurons' specific signals. In addition, the l-ACT, as time markers, could contribute more to the temporal code. The beginning of odor stimulation should be processed by a number of immediately responding l-ACT neurons, which may allow the evaluation of different latencies as expressed by m-ACT neurons.

An additional demand could also be solved by such a dual system, namely plasticity underlying associative learning. One subsystem, the l-ACT neurons, might change during learning; the other (m-ACT neurons) might stay stable. Indeed, Faber *et al.* (1999) demonstrated learning-induced increases of activity in those glomeruli from which the l-ACT neurons originate. Thus, l-ACT neurons may play a more prominent role in odor learning.

We imaged the presynaptic terminals (blebs) of the PNs in an attempt to evaluate the spatial and temporal components of the olfactory code as it appears at the input site of the MB (Szyszka *et al.*, 2003; Szyszka *et al.*, unpublished data.). We found that the odor-induced activity patterns consisted of specific distributions of excitatory input to KCs which covered large areas of the lip region, indicating that PNs provide excitatory and inhibitory input to KCs all over the lip region. This finding is constant with both the anatomical and physiological findings reported above. It has not yet been possible to image PNs of the l-ACT and m-ACT separately; therefore we cannot determine whether the blebs of l- and m-ACT neurons differ in their temporal and spatial response patterns. Imaging of the KC responses to odors indicated that their responses were transient and sparse both at the level of spike activity and at the population level. This result is constant with the view presented above that KCs should extract the information from PNs by a combinatorial strategy.

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