General Conclusions

The major goal of my thesis was to investigate anatomical and functional principles of odor encoding in projection neurons underlying olfactory learning and olfactory-guided behaviors in the honeybee.

For this purpose, I investigated the response profiles and connectivity of single projection neurons which are important components of the olfactory pathway conveying olfactory information onto higher-order brain centers. I applied intracellular recordings and 3-D reconstruction and registration techniques extending the 'Atlas of the Honeybee Brain'. The correlation of these functional and anatomical features allowed me to draw conclusions about odor encoding at two processing levels, the projection neuron input site at the level of the antennal lobes and the output site at the level of the mushroom bodies. Furthermore, I took a closer look on the differences of elemental structures within the microcircuitry of the projection neuron output region in groups of animals which had experienced different odors in a particular behavioral contexts. In my thesis I thus related the anatomical and functional principles of odor encoding in projection neurons to the relevant type of olfactory-guided behavior of the honeybee.

An intriguing question is why projection neurons are organized in parallel tracts, the lACT and mACT. Is this anatomical organization functionally relevant representing two parallel coding pathways which convey different aspects of olfactory information onto higher-order brain centers? Do they track different odor-coding strategies and how is this related to their morphological features? The studies in this thesis (**Chapter II**) showed that, both types of projection neurons (m- and lACT neurons) encode odors according to their uniglomerular

arborizations and convey the olfactory information through different tracks to presumably different output regions. Their olfactory responses are highly reliable from trial to trial which means that their encoding strategy is definite. It appeared, that they generalized odors which are similar considering their functional group independent of their hydrocarbon backbone (**Chapter II**). This effect needs to be elucidates further by applying multidimensional analysis regarding the temporal dynamics of odor responses elicited by functionally similar odors.

Both, the m- and lACT neurons responded to odors in a phasic-tonic temporal response pattern but with different response latencies. This has been extending previous work by Mueller et al. (2002) supporting the hypothesis of different strategies encoding one and the same olfactory stimulus.

Knowing the response properties for single compound odors, it was crucial to test the responses of the same individual neurons to mixtures of these single compound odors. I expected to reveal whether odor-complexity in terms of odor mixtures might be differentially encoded by the m- and lACT projection neurons.

The results of my studies show that odor mixtures elicited different responses in the projection neurons depending on their projection pathway, e.g. the m- or lACT tract (**Chapter II**). mACT projection neuron odor mixture responses were highly predictable from the knowledge about their response to the most effective component of the mixture whereas lACT odor mixture responses were not. Thus, I conclude that they represent two olfactory processing channels which allow encoding different features of one and the same odor as for example represented by the mixture.

Responses to odor mixtures of the mACT neurons followed the most effective component model. Considering a tertiary mixture their responses to the elemental components of the mixture varied in strength. The single compound eliciting the highest response strength was reflected in the response to the mixture. Thus,

the mACTs appear to identify information about single compound odors within a mixture which might enable them to extract odor-identity information. The lACTs, on the other hand showed suppressive odor mixture responses which might indicate the detection of odor mixtures implying identification of odor-complexity. This hypothesis needs to be further confirmed but the results of my thesis present evidence for such functional divergence.

Is such functional divergence just mirroring coding processes at the level of the antennal lobe? Or in other words, does the antennal lobe microcircuitry determine this dual encoding strategy?

Performing recordings from local interneurons with the same set of single compound odors and odor mixtures, inhibitory interaction within the antennal lobe could be investigated further (**Chapter II**). The results showed that there are separate inhibitory networks either inhibiting all PNs globally, or locally in a glomerulus-specific manner. Since some local interneurons showed differential odor mixture responses in comparison to the projection neurons (synergistic effects) the global network is presumably realized by the homogeneous local interneurons which are homogeneously distributed over the entire antennal lobe. The local interneuron odor mixture responses resembling either those of m- or lACT projection neurons (hypoadditivity and suppression) might be caused by a local network inhibiting the projection neurons in a glomerulus-specific manner. This is probably accomplished by the heterogeneous local interneurons, which densely innervate one specific glomerulus and diffusely branch into several other glomeruli.

Thus olfactory information arising from the antennal lobe network is divided into two categories: odor-identity and odor-complexity (mixtures), which are conveyed via two parallel pathways onto the second-order relay station, the mushroom body.

Is this dual odor coding reflected in the topography of the projection neuron terminal arborization pattern? Do the projection neurons target different subsets

of postsynaptic Kenyon cells which "read out" the relevant information about the identity and the complexity of odors? Composing single projection neurons within a spatial reference map, the Atlas of the Honeybee Brain (**Chapter I**), the spatial distribution of their axon terminal (boutons) arborization pattern could be determined which allowed me to draw conclusions about the olfactory information read out by the Kenyon cells (**Chapter II**). The results showed that, in general, the lACT boutons appear to be segregated from mACT boutons. Since clawed class II Kenyon cells are assumed to be exclusively driven by the lACTs they might play a dominant role in extracting information about the complexity of an odor. mACT boutons among themselves are determinate in their topography but exhibit glomerulus-specific arborization densities. This might reflect the fact that odor-identity is extracted by the projection neurons and conveyed onto different subset of Kenyon cells. Since the class I Kenyon cells among themselves exhibit dendritic arborizations in spatially distinct subregions, they might inherit glomerulus-specific olfactory information.

Is such connectivity pattern between presynaptic boutons and KC postsynaptic spines forming so-called microglomeruli complexes in the MB lips subject to plasticity? One virtue of honeybee biology and behavioral development is the possibility of manipulating age- and task-related olfactory experience within their natural environment. Structural changes within their brains could be thus correlated to ontogenetical and behavioral history of olfactory experience (**Chapter III**). I found a surprising induction of degrading compensatory structural plasticity in the MB lip boutons caused by manipulations of age-related sensory perception. Projection neurons appeared to have altered their arborization pattern which was presumably compensated by strengthening synaptic efficacy thus resulting in an increase in bouton volume.

Is this an indication for a possible reorganization of the entire mushroom body lip microcircuitry? And how would such reorganization be related to the dual coding strategy of projection neurons and to the different behavioral tasks which

worker honeybees perform during their live? The Atlas of the Honeybee Brain (**Chapter I**) provides an opportunity to answer these questions. The implementation of a 4^{th} dimension with respect to developmental and behavioral maturation would allow investigation of the evolution of functional and anatomical principles accordant to the honeybee's life. Further investigations are needed to answer these questions in detail. Such experiments should combine electrophysiological recordings, anatomical tracing and behavioral manipulations. They would enrich our understanding about functional and anatomical principles of odor encoding in relation to the elaborate behavior of the honeybee.

Furthermore, the distinct odor encoding strategies of m- and lACTs could be tested by recording the odor responses to differently overlapping stimuli. This could be achieved by either different ratios of the elemental components within a mixture or by consecutive stimulation with different odors exhibiting overlapping sequences. Since lACTs showed suppressive odor mixture responses the threshold of odor-complexity encoding could be determined by using ratio-dependent or overlapping stimuli. The suppression of lACTs triggered by the stimulation with a multicompound mixture might be diminished if the ratio of the elemental components is shifted more towards a single compound odor or if the overlap between consecutive stimulation with different odors decreases.

The question whether olfactory experience might alter neural physiological properties could be investigated by single-cell recordings of bees which experienced different olfactory stimulation or performed different behavioral tasks. Since mACTs appear to extract odor-identity whereas lACTs odor-complexity the hypothesis would imply that lACT responses alter in relation to the honeybee's olfactory experience. The functional relevance of m- and lACTs in relation to olfactory experience could be tested by 'disconnecting' one of the two tracts and by applying specific learning tasks. Bees with for example simply a functioning mACT could be trained to learn the discrimination between olfactory mixtures and their odor components. Positive patterning in which bees

have to learn that two elements are both non-reinforced when presented alone (A-,B-), while their compound is reinforced (AB+) would be impaired which in turn would support the assumption that the lACTs play a major role in odor mixture encoding.

To conclude, the studies of my thesis reveal that different odor encoding strategies arise from the network properties within the antennal lobe which is reflected in terms of the axon terminal topography within the mushroom lips. This topography in turn is variable and seem to be influenced by particular olfactory experience essential for the accomplishment of behavioral tasks in a honeybee's life and at different ages. Thus, to extend our understanding of olfactory information processing in the honeybee brain, physiological, anatomical and behavioral techniques need to be combined using a common morphological framework, the Atlas of the Honeybee Brain.