GENERAL CONCLUSIONS

The major aim of the present thesis was to investigate how the local circuits in the honeybee AL shape the olfactory code. To this end, I selectively optically recorded the output of the olfactory glomeruli, which represents the processed odor information. Comparison of these odor-induced output patterns with the compound signals, which mainly reflect the afferent input to the AL (i.e. RN responses), allowed me to draw conclusions about the role of the AL network involved in the odor processing.

How are odors represented in the output neurons compared to the afferent input? What do the two both processing levels have common and what are the differences? The studies of the present thesis showed that, like the afferent input patterns, individual glomeruli have highly predictable output responses from individual to individual (CHAPTER I). Consequently, both the input and output neurons innervating a particular glomerulus are genetically determined. Moreover, the odor representations in the dominantly responding glomeruli are very well correlated between both processing levels: Glomeruli with a strong RN input, also revealed a strong output response. The strong RN responses become even higher within the AL, based on the high convergence of RNs onto each PN, leading to an improved sensitivity on the PN level (CHAPTER IV). Contrary to the strongly-activated glomeruli, those which get a weak or intermediate RN input revealed a decreased sensitivity, leading to reduced or even no responses in their PNs (CHAPTER I, IV). This indicates specific inhibitory interactions within the AL. Thus, the strong glomerular responses are further emphasized by the specific reduction of weaker-activated glomeruli, leading to an odor- and glomerulus-specific contrast-enhancement of the spatial odor representations. Moreover, the input signals from the RNs are transformed into temporal complex glomerular output patterns (CHAPTER I). PN responses to odors consisted of both excitatory and inhibitory phases. Inhibitory responses occurred as either a calcium-concentration decrease during stimulus application or as a rebound calcium increase after stimulus offset (i.e. off-response) due to the release from inhibition. The response polarity differed for PNs depending on both the glomerulus innervated and the odor used as stimulus, and was conserved among individuals. Since PNs were spontaneously active, an inhibitory response corresponds to a reduction in their firing frequency. Thus, it is likely that these inhibitory responses are also part of the olfactory code and may represent a further coding channel, which would extend the coding capacities at the output level. Since the temporal resolution is substantially reduced in calcium imaging studies as compared to electrophysiological recordings, the temporal structure of the PN responses in terms of spike frequency or the existence of oscillations could not be described precisely. Future experiments combining electro- and opto-physiological recordings will help to complete our picture of the temporal modifications of afferent input by the AL network.

How do local circuits of the honeybee AL transform the RN responses into temporal complex and contrast-enhanced representations of odors at the output level? Using pharmacological tools such as the chloride channel blocker picrotoxin (PTX), the inhibitory interactions within the AL could be investigated further (CHAPTER I, III). The results showed that there are at least two separate inhibitory circuits within the AL, of which only one is PTX sensitive. The PTX sensitive network is a global network and effects a global gain control mechanism by globally inhibiting all PNs. This network is probably realized by the homogeneous LNs, which distribute their branches homogeneously over the whole AL. Consequently, this morphological type of LNs should be GABAergic. Indeed, the number of homogeneous LNs corresponds very well to the number of GABA-immunoreactive LNs in the honeybee AL, which strongly supports the assumption that homogeneous LNs are involved in a GABAergic global network.

The second inhibitory network is glomerulus-specific and probably accomplished by the majority of LNs (heterogeneous LNs), which densely innervate one particular glomerulus and diffusely branch in other glomeruli. Its transmitter could be histamine, which has been shown to act as an inhibitory transmitter in the honeybee AL (CHAPTER II). This specific network connects glomeruli with overlapping response profiles and thus leads to the effect that the strongest-activated glomerulus inhibits its 'functional' neighbors, resulting in the contrast-enhancement as mentioned above. Moreover, these specific connections are responsible for the inhibitory and off-responses in some glomerular outputs. It is easy to identify the inhibitory input if the response is pushed below baseline. In contrast, it is not trivial to differentiate between an originally weak response and a decreased response due to an inhibitory input. At first this led to the impression that the specific inhibitory connections exists only between glomeruli which have a minimum distance between their glomerular response profiles, but not between glomeruli with very similar responses, nor between those with completely different responses (CHAPTER I). However, further studies using binary odor mixtures revealed inhibitory mixture interactions, which were strongest for binary mixtures of odorant molecules with neighboring chain length (CHAPTER III). Since these mixture suppressions were not reduced but were rather intensified by PTX, they probably derived from glomerulus-specific computations. Thus, in addition to glomeruli with only slight overlapping response profiles, specific inhibitory connections probably also exist between glomeruli with very similar responses. The latter connections were not visible as inhibitory responses, since the inhibitory input was not strong enough to exceed the similarly strong RN input, which initially led to an underestimation of the number of glomerulus-specific inhibitory connections. These findings show that the mapping of specific inhibitory connections is just beginning. Future experiments using the respective antagonists to histamine or increasing the number of components in odor mixtures will help to

identify further specific inhibitory connections within the AL to map the complete glomerulus-specific network.

Are the reported odor processing properties of the AL network of biological relevance for the honeybee? Based on the strong convergence of the primary afferents onto only a few PNs and due to the existence of two separate inhibitory networks, the AL improves several important features of olfactory coding. Firstly, the AL circuits transform the broadly-tuned RN responses into globally-modulated and contrast-enhanced odor representations in the output neurons (CHAPTER I). This contrast-enhancement improves the discrimination of chemically closely related odorant molecules and thus allows the honeybee to discriminate, for example a specific flower's odor from a vast array of other flower fragrances. Secondly, the glomerulus-specific computations led to inhibitory mixture interactions (CHAPTER III). Since most naturally-occurring odors are not pure substances, but complex blends consisting of an enormous number of volatile compounds, this mechanism may ensure sufficient coding capacities to discriminate between them. Finally, the AL network increases odor discrimination at very low odor concentrations, improves the concentration-invariance for odor quality detection and optimizes a reliable representation of odor intensity in its overall excitation (CHAPTER IV). Forager honeybees have to find nectar sources and must learn the odors of good crops while sitting in the middle of the highly concentrated blossom. Since odors occur in plumes at varying concentrations, the bee must detect the previously encountered odor over a wide intensity range even at very low concentrations to find the reliable nectar source again. Thus the olfactory system has the important task of extracting the odor quality from its occurring intensity, while information about the intensity should not be lost and could be used as an indicator of the odor source.

To conclude, the studies in my thesis reveal that already at the level of the first olfactory brain center, the AL, a substantial part of odor processing takes place, which provides the neural substrate for the accomplishment of essential behavioral tasks in a honeybee's life. The processed spatio-temporal activity patterns of the output neurons must be 'read out' by higher processing centers, such as the mushroom bodies or the lateral protocerebrum, in order to have a meaning for the animal and to manifest a behavioral response. However, so far it is unknown how and which parameters of the glomerular output responses to odors are read out by subsequent stages of processing. Future experiments dedicated to these higher-order brain centers will help to complete the processing path of the olfactory information to make progress towards the elucidation of the olfactory code.