Conclusions

The goal of this thesis was to understand how odor information is encoded and transformed within the AL of *Drosophila melanogaster*. I found that the input-output relationship of each glomerulus is modulated by the activity of the AL network in an odor dependent manner. PN responses are shaped by a global inhibitory network involving GABA neurotransmission and by glomerulus specific connections. These glomerulus specific connections are presumably both inhibitory and excitatory. These results give further insight about the complexity of the AL network and the information processing taking place in it.

In **Chapter I**, I characterized the information flow in the AL network by analyzing glomerular responses of olfactory sensory neurons (OSNs), two non-overlapping populations of GABAergic local neurons (LNs) and projection neurons (PNs) to three odors over a wide range of concentrations.

OSNs and PNs responses were clearly glomerular, as would be expected from the uniglomerular arborization pattern of OSNs and most PNs in *Drosophila* (Stocker, 1994; Vosshall et al., 2000). OSNs responded to odor stimulation with calcium increases and decreases. Both PN and OSN responses were temporally complex, and the dynamic of the responses was odor and glomerulus dependent and conserved across animals. Odor responses were concentration dependent in OSNs and PNs, and in general, higher concentrations activated more glomeruli. These results are in agreement with previous data obtained from electrophysiological and imaging studies both in OSNs and PNs (de Bruyne et al., 2001; Wang et al., 2003; Sachse and Galizia, 2003; Hallem et al., 2004; Wilson et al., 2004; Pelz et al., 2006) and confirm the combinatorial spatio-temporal character of odor representations in the first stage of the insect olfactory system.

I also performed a detailed functional characterization of two genetically and anatomically distinct populations of GABAergic LNs (LN1 and LN2). In both LN populations odor evoked activity patterns were not limited by glomerular boundaries, but were spatially structured, although LN1 and LN2 project to all AL glomeruli. These non-glomerular spatial activity patterns were odor specific, stereotyped across animals and different for LN1 and LN2. LN1 responses patterns were distributed over larger areas of the AL than LN2 response patterns. Moreover LN1 and LN2 differed in their response dynamics. LN1 responded with monophasic and rather homogeneous calcium transients, while LN2 responses were

temporally complex for some odors in some AL areas, combining phases of calcium increase and decrease. LN responses were also concentration dependent, although the dynamic range was in some cases narrower than that of OSNs and PNs. The functional differences between LN1 and LN2 go along with their anatomical dissimilarities: while LN1 present focalized innervations in the core of the glomeruli, LN2 extend arborizations over the whole glomerular volume. Thus, it is likely that neurons from the two populations have different connectivity patterns with OSNs and PNs and play different roles in odor processing. The existence of at least two subpopulations of functionally different local neurons is in agreement with the complex modulations observed at the PN level (see below).

To test whether the representation of odor information is changed between OSNs and PNs I analyzed the differences between OSN and PN responses (referred to as activity transfer function) across odors in each glomerulus. This approach has the advantage of avoiding direct comparisons between responses from different neurons types. Direct comparisons between neuron populations should be avoided for two main reasons. First, the relationship between calcium signals and spiking frequency might differ for different neurons and neuronal compartments. Second, signal transmission between OSNs and PNs might be non-linear because it depends, among others, on the synaptic strength and convergence ratio between neuron populations.

By comparing the activity transfer functions across odors, I could show that PN responses are modulated (amplified or suppressed) in an odor and glomerulus specific manner. Interestingly, this modulation was independent from the global input to the AL network, which suggests that glomerulus-specific interactions must be involved.

Since modulation of the PN response profiles must be mediated by neurons innervating multiple glomeruli, I tried to establish whether this task could involve LN1 and LN2. However, I found no general correlation between LN activity and PN response modulation. The incompatibility between measured LN responses and PN modulations might result from the functional heterogeneity within each of the two LN populations. Moreover, it is likely that other populations of LNs or multiglomerular PNs not measured here could be involved, such as recently described excitatory LNs (Shang et al., 2007).

In **Chapter II**, I analyzed the glomerular representation of monomolecular odors and their mixtures at the OSN and PN level.

I asked the question whether such mixtures are represented in the AL as the linear combination of their components or whether interactions between the components might

influence the representation of the mixtures. Interactions between the components can occur both at the level of the OSNs, where different odors can antagonize or potentiate each other. Alternatively, the representation of a mixture can be modified by the AL network, such that the representation of the mixture deviates from the linear combination of the components' representations.

How should mixtures be represented in the AL if no interactions between the components occur? Considering the properties of the ligand-receptor binding, and the fact that OSNs expressing one and the same odorant receptor project to single glomeruli, it is possible to predict how an odor mixture should be represented if its components do not interact. Following this approach, I could show that mixture interactions are rare at the OSN level. At the PN level, however, mixture interactions are common, but only in the form of mixture suppression. I thus concluded that in the AL of *Drosophila* odor mixture representation is modified by the network, such that the representations of the components are partially suppressed when presented as a mixture. I could also show that mixture suppression at the PN level occurs in an odor- and glomerulus-dependent manner and is not correlated with the global input to the AL. The degree of mixture suppression in some glomeruli was correlated positively and negatively with OSN input to other glomeruli, thus suggesting that glomerulus-specific connections modulate the degree of mixture suppression.

In an attempt to obtain more details about the mechanisms underlying mixture processing I used a pharmacological tool to manipulate the AL network. Application of PTX, an antagonist of ionotropic $GABA_A$ receptors, revealed that even the representations of monomolecular odors are subject to global inhibition mediated by GABA. The inhibitory component acting on the responses was strongest during the first 500 ms of the response, unveiling a fast inhibitory input, which antagonized a phasic component of odor responses.

Since the effect of the global inhibitory network was stronger with increasing input to the AL, and since the input to the AL increases with stimulus complexity (i.e. number of components in a mixture), it could be speculated that mixture suppression is generated by the global inhibitory network. However, the glomerulus and odor specific interaction profile found in the PNs cannot be explained by the global inhibitory network alone. In addition to the inhibitory input from the global network, each glomerulus might receive lateral excitatory or inhibitory input (Fig. 2.5C, Chapter II). It has been recently shown that each glomerulus in *Drosophila* receives lateral excitatory input, besides the excitatory input from its corresponding OSNs (Olsen et al., 2007). This lateral excitatory input saturates fast with increasing input. Therefore, odor and glomerulus specific mixture suppression could result from the interplay

between global inhibitory and lateral excitatory input if the strength of global inhibition and lateral excitation increased differentially in different glomeruli with increasing input to the AL. Additionally, glomerulus specific inhibitory input could also contribute to the modulation of PN responses. In honey bees, heterogeneous inhibitory LNs have been described (Fonta et al., 1993), and have been proposed to mediate glomerulus-specific modulations (Sachse and Galizia, 2002; Linster et al., 2005). In *Drosophila*, LNs innervating only a few glomeruli have been described which could underlie glomerulus-specific interactions (Wilson and Laurent, 2005).

How do these results fit in the current view of the AL network function? I found that the input- output relationship in each glomerulus is not only defined by the input provided by the OSNs, but also by input from a global inhibitory network, which inhibits all glomeruli with a strength proportional to the global input to the AL, and input from a glomerulus specific network, which modulates the PN activity in an odor and glomerulus-specific manner. These combined modulatory inputs result in suppression and enhancement of PN responses, i.e. a glomerulus and odor specific change in the input-output relationship (gain). The function of that gain control mechanism could be to decorrelate the input and output activity in the AL and thereby decrease the similarity between odor representations. A similar mechanism to increase odor discriminability has been proposed by Wilson and Laurent (2005). In other models, decorrelation mechanisms based on temporal patterning of PN/MC activity have been proposed (Friedrich and Laurent, 2001; Laurent, 2002; Lei et al., 2004).

The results of a decorrelation mechanism based on glomerulus-specific inhibition and excitation are illustrated in Figure 3.1A. Let us consider a simplified situation with two odors, X and Y, which evoke strong responses in one OSN class (OSN 2) and weak responses in two different OSNs classes (OSN 1 and OSN 3). The glomerular representations of these odors at the OSN level are similar and dominated by glomerulus 2 (i.e. the glomerulus innervated by OSN 2). However, at the PN level, the distance between the glomerular representations of X and Y would increase, because the input-output relationship in glomerulus 2 (PN 2) would differ for the different odors.

Spatial decorrelation mechanisms based on sharpening of PN/MC response profiles through lateral inhibition, have been proposed in rabbit (Yokoi et al., 1995) and bees (Sachse and Galizia, 2002; Sachse and Galizia, 2003; Linster et al., 2005). However, in such models suppression of weak responding glomeruli might decrease the distance between representations of similar odors (Fig. 3.1B).

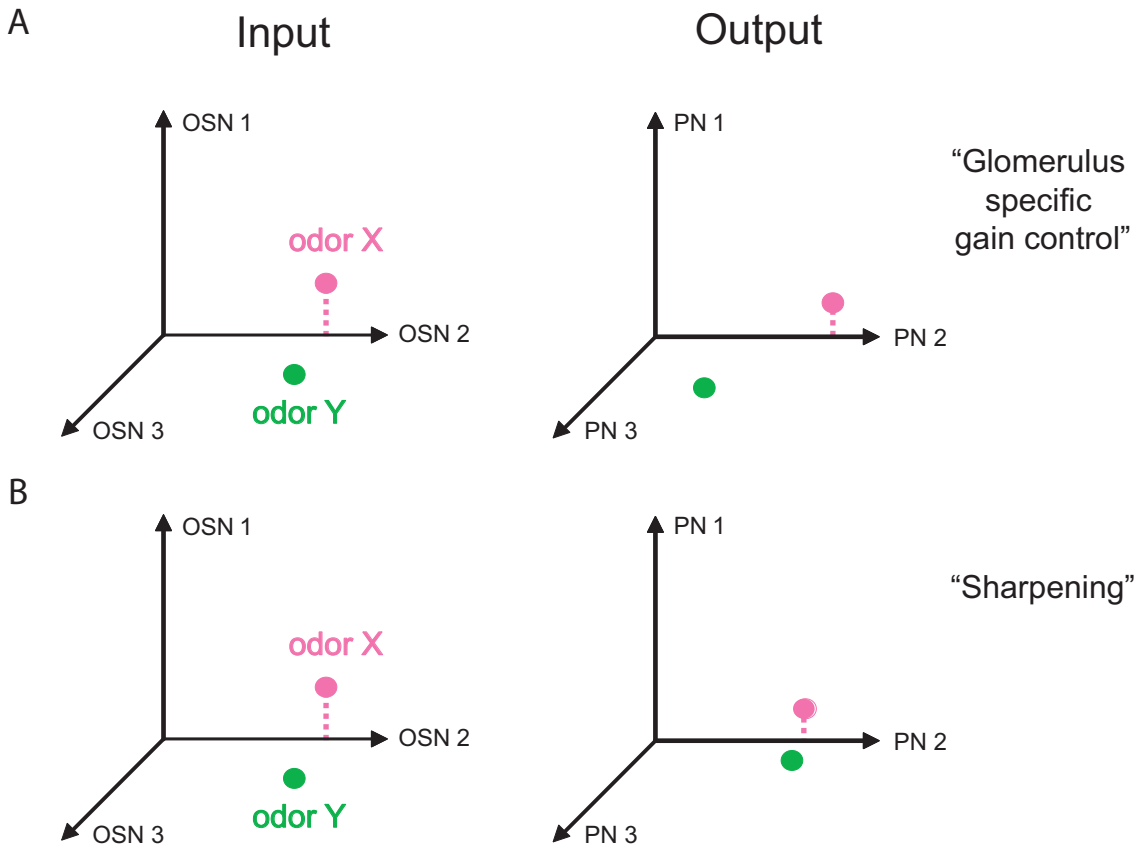

Figure 3.1 - Transformation of odor representations in the AL - Two models

Odors X and Y are represented in a simplified receptor space defined by the responses of OSNs 1, 2 and 3. In model A, the AL network controls the gain in a glomerulus specific manner, and thus, the distances between the representations of X and Y in the PN space increases. In model B, the AL network sharpens the responses, increasing the response difference between strong and weak glomeruli for each odor, but decreasing the distance between the representations of the two odors in the PN space.

Why is decorrelation of odor representations important? Decorrelation of odor representations in PNs might provide the basis of odor discrimination in downstream networks. The mushroom body Kenyon cells, for example, which are one target of PNs (Wong et al., 2002; Marin et al., 2002; Jefferis et al., 2007), encode odors in highly sparse and decorrelated activity patterns (Perez-Orive et al., 2002; Wang et al., 2004; Szyszka et al., 2005). In contrast to PNs, odor activated Kenyon cell populations are sparse and highly odor specific. Similar odors activate dissimilar Kenyon cell populations. Encoding odors with sparse and dissimilar Kenyon cell population might facilitate the formation of odor specific memory traces in the mushroom body (Heisenberg, 2003). Since less similar patterns of activated PNs will generate less overlapping Kenyon cell response patterns, the decorrelation of PN responses in the AL might facilitate the discrimination of similar odors.