Summary

Chapter I - The question of whether or not neural activity patterns recorded in the olfactory centres of the brain correspond to olfactory perceptual measures remains unanswered. To address this question, I studied olfaction in honeybees *Apis mellifera* using the olfactory conditioning of the proboscis extension reaction (PER). I conditioned bees to odours and tested generalisation responses to different odours. Sixteen odours were used, which varied both in their functional group (primary and secondary alcohols, aldehydes and ketones) and in their carbon-chain length (from six to nine carbons).The results obtained by presentation of a total of 16 x 16 odour pairs to more than 2000 animals show that (i) all odorants presented could be learned, although acquisition was lower for short-chain ketones; (ii) generalisation varied depending both on the functional group and the carbon-chain length of odours trained; higher generalisation was found between long-chain than between short-chain molecules and between groups such as primary and secondary alcohols; (iii) for some odour pairs, cross-generalisation between odorants was asymmetric; (iv) a putative olfactory space could be defined for the honeybee with functional group and carbon-chain length as inner dimensions; (v) perceptual distances in such a space correlate well with physiological distances determined from optophysiological recordings of antennal lobe activity. I conclude that functional group and carbon-chain length are inner dimensions of the honeybee olfactory space and that neural activity in the antennal lobe reflects the perceptual quality of odours.

Chapter II - The overshadowing effect has long attracted attention of researchers interested in the learning and processing of sensory compounds. Overshadowing occurs when a subject conditioned with a binary compound of two stimuli responds significantly more to one component at the expense of the other. The mechanism of overshadowing is still unclear. Within-mixture, inhibitory interactions between components as well as differential, independent processing of compound components have been proposed to account for it. Here I studied overshadowing in honeybees conditioned to associate odour mixtures with sucrose solution (PER). I trained bees with 6 odorants in all possible 15 binary combinations. For each group trained with a mixture (*OVS* group), two control groups were trained with each component (*Ctrl A* and *Ctrl B*). Recording the performance of more than 2700 bees showed that overshadowing depended on the odour combination and the amount of conditioning trials (1 or 3 trials). Two factors derived from the control groups accounted for overshadowing: 1) asymmetric cross-generalization between odorants, due to bees responding more to odorant B after learning odorant A than in the reversed situation, and 2) differences in single odorant

learning. Asymmetric cross-generalization contributed more to the overshadowing effect after 1 as well as after 3 conditioning trials. After 3 trials, weighting between both factors became more similar. Thus, no within-mixture interactions were necessary to describe odorant dominance upon compound learning in honeybees. Asymmetric cross-generalization between components, a parameter that has been rarely considered in studies on compound processing, is a critical factor for predicting overshadowing.

Chapter III – The neuronal correlates for overshadowing and asymmetric cross-generalization are not known. Here, I studied both effects in the olfactory modality using binary odour mixtures. I made the attempt to find out if these phenomenons are encoded in the olfactory network of the antennal lobes of the honeybee by investigating neuronal activation in the projection neurons using *in vivo* calcium-imaging techniques while presenting odours to the antennae of the animals. Because of a high interconnectivity between AL neurons, information processing within the AL is thought to shape the odour representation by inhibitory synapses and plasticity within the AL after conditioning could accentuate specific inhibitory connections. These network properties could be the fundament for neuronal correlates of overshadowing and asymmetric cross-generalization. I measured neuronal activation patterns of honeybees trained to 2-hexanol, hexanal and the mixture (2-hexanol/hexanal) as well as of naïve animals responding to stimulation with 2-hexanol, hexanal and their mixture. The honeybees were conditioned to associate odours and odour mixtures with sucrose solution (PER), before the animals were used for calcium imaging experiments. In unrewarded tests to the single odours and their mixture the behavioural control groups showed clear asymmetrical cross-generalization between the single odours after single odour conditioning and an overshadowing effect with hexanal as the dominant and 2-hexanol as the dominated odour after mixture conditioning. The imaging experiments revealed neither a neuronal correlate for asymmetric cross-generalization nor for an overshadowing effect. Conditioning did not change overall-activity intensity of the neuronal activation patterns in the AL, it did not change the odour induced glomeruli activation pattern and the changes in activation intensity of individual glomeruli did not follow a common pattern, they in- or decrease their activity independently. Moreover, I was not able to find asymmetrical changes in the glomerular activation patterns for 2-hexanol and hexanal after conditioning to these odours and no indications why hexanal should overshadow 2-hexanol after conditioning to their mixture.