4 Discussion

Olfactory information is encoded in spatio-temporal combinatorial patterns of activated glomeruli in vertebrate OBs (Astic and Cattarelli, 1982; Cinelli and Kauer, 1992; Friedrich and Korsching, 1997; Hildebrand and Shepherd, 1997; Johnson et al., 1998; Meister and Bonhoeffer, 2001; Rubin and Katz, 2001; Sharp et al., 1975; Spors and Grinvald, 2002; Wachowiak et al., 2004) and invertebrate ALs (Fiala et al., 2002; Galizia et al., 1999; Joerges et al., 1997; Ng et al., 2002; Sachse et al., 1999). On the other hand, olfactory learning has been described to induce transformations of the odor code already at the level of the first odor-processing level, the vertebrate OB (Brennan et al., 1998; Sullivan and Wilson, 1995; Woo et al., 1987) and the insect antennal lobe (AL) (Daly et al., 2004; Faber et al., 1999a; Yu et al., 2004). These results are without doubt striking indications of an olfactory memory trace. Yet, they raise an unmentioned enigma. How can an animal learn to efficiently respond to a particular odor with an adequate response, if the odor code itself is changing during this process?

Here we report that odor evoked activity in a subpopulation of AL output neurons, uniglomerular PNs, is remarkably resistant to change following a variety of appetitive olfactory learning paradigms. Furthermore, these neurons seem to be necessary for a behavioral response to a learned odor. We discuss the possibility of parallel olfactory processing in the antennal lobe: lACT uniglomerular projection neurons are necessary for reliable odor-coding, while other neurons (such as the multiglomerular projection neurons) may experience memory related plasticity.

4.1 Odor stimulation elicits reliable activity patterns in PNs that are equal across animals

In experimentally naïve animals 1-nonanol, 1-hexanol and 1-octanol elicited the predicted glomerular calcium responses in the PNs that are equal across animals which confirms previous findings (Sachse and Galizia, 2002). PN odor responses

proved to be stable throughout successive odor stimulations. Repeating a single odor up to six times or a set of three different odors up to three times in a randomized order, does not substantially affect the PN odor responses. Small changes throughout the whole experiment are discussed in detail below.

4.2 PN responses to sucrose

Interestingly, not only upon odor but also upon sucrose stimulation, a specific set of glomeruli was activated, which was equal between animals. Responses were only observed upon stimulation of the ipsilateral antenna, neither of the contralateral antenna nor of the proboscis. The absence of responses to stimulation of the proboscis might have been due to injuries during the preparation, as the oesophagus was lifted and stretched. Two previous studies prepared the oesophagus equally (Mauelshagen, 1993; Abel, 1997). While proboscis mediated sucrose responses were also not found in electrophysiological recordings of single PNs, Abel reported responses in single cells in the dorsal lobe. This, together with the clear responses in the PE1 neuron, reported by Mauelshagen, suggests that this preparation does not interfere with the sucrose pathway of the proboscis. Instead, our results might reflect the hypothesized different neuronal representations of proboscis and antennal sucrose processing pathways (Hammer et al., 1994; Sandoz et al., 2002). Sucrose information detected via antennal receptor cells (Haupt, 2004) and receptor cells on the proboscis are processed independently and converge on motorneurons, mediating the PER, which are located in the sub-oesophagial ganglion (Rehder, 1989).

Sucrose responses were dependant on the application method. Presentation of the sucrose by means of a soaked wooden toothpick elicited pronounced PN responses in glomerulus 28. This response was absent when using an Eppendorf pipette as an application device. An odorous substance from the wooden toothpick might have been dissolved in the sucrose water thereby contaminating it. This point is important to mention, as using a wooden toothpick is an established application method for the sucrose reward. It is widely used during PER conditioning, examining odor learning,

identification and discrimination (Guerrieri et al., 2005). Our results strongly favor the use of an odor free application method as has been used in early studies of PER (Bitterman et al., 1983). Throughout the later learning experiments, the sucrose reward was always applied with a 10µl Eppendorf pipette with clean tips.

Are the sucrose responses representing a modulatory input by the reward pathway? If so, the anatomy of the reward pathway (Hammer, 1993) (which is discussed in detail below) suggest that a sugar stimulation alone would be expected to activate either all glomeruli uniformly or none. In contrast, in a pairing condition it is expected that at least all PNs activated by the odor are subject to modulation. However, when comparing the summed responses of separate odor and sucrose stimulations with pairings of odors with sucrose, the responses were not different. Therefore, we might have recorded sensory input rather than a modulatory input by the reward pathway.

PNs responded similarly to water and different sucrose concentrations. The only differences in glomerulus 28 upon stimulation with 30% sucrose, 3% sucrose and water are obviously due to a contamination by the wooden toothpick, as this glomerulus did not respond to sucrose applied with a pipette. This indicates that the observed responses were possibly mediated by hygroreceptors (Yokohari et al., 1982; Yokohari, 1983). From cockroaches it is known that PNs and LNs can be tuned specifically to humidity detection (Nishino et al., 2003). In this respect it is worth mentioning, that a majority of the glomeruli activated by sucrose water/water, namely glomerulus 42, 47, 48 , 49, and 60, have remained silent to a great selection of different odors (Sachse et al., 1999; Sachse and Galizia, 2003). Whether the observed responses are humidity specific and which receptors mediate this response remains to be shown by future experiments.

In conclusion, our preparation allows to record reliable responses to odors and sucrose water/water which indicates intact sensory processing at the level of the AL.

4.3 Animals do learn under the experimental conditions and uniglomerular lACT PNs are necessary for a behavioral response to a learned odor

Olfactory conditioning of animals prepared almost identical as in the imaging experiments led to acquisition in the behavioral experiments. Following conditioning, 65% of the animals responded to the CS+ with a PER when tested on both antennae or unilaterally with the CS+ on the antenna of the intact side. This unambiguously shows that animals learned despite being harnessed in a recording chamber and prepared for imaging. The punctuation of the brain due to dye injection did not affect the motivational status of the animals. Coincidence detection of reward and CS was given and the motor circuits responsible for PER were intact.

An identified neuron, VUMmx1, has been shown to mediate the reinforcement in PER, because forward pairing (but not backward) of an odor CS with an artificial depolarization of the VUMmx1 produces an associative memory (Hammer, 1993). This putatively octopaminergic neuron (Kreissl et al., 1994) converges bilaterally with the CS pathway at the level of the ALs, the MBs and the lateral protocerebrum. Convergence of the CS and the US is crucial for memory formation (Rescorla, 1988). On the basis of the anatomy of the VUMmx1 neuron and several experiments, the ALs, the MBs and the lateral protocerebrum have been postulated as putative convergence sites of the CS and US (Hammer and Menzel, 1995). Indeed, microinjections of the neuromodulator octopamine into the AL alone is sufficient as a substitute for the reinforcing function of sucrose US in PER conditioning (Hammer and Menzel, 1998).

The location of the dye insertion does not disconnect the bilateral reward pathway to the ALs. Therefore, input by the reward pathway in both AL to sucrose stimulation has to be assumed in our experiments. It is difficult to say whether the same holds true for the MB. However, there is no anatomical argument to reason that input to the MBs is vital for the contiguity of CS and US at the level of the AL.

The animals did not respond to the conditioned odor when stimulated only unilaterally ipsilateral to the stained AL. Staining the lACT PNs disconnected a necessary part of the CS odor pathway to third-order olfactory neuropiles. Thus,

odor-coding in uniglomerular PNs appears to be crucial for odor recognition in eliciting an adequate behavioral response to the learned odor.
We therefore conclude that the animals in our imaging experiments did learn and putative modulations of the calcium responses in the AL lACTs PNs due to conditioning should have been observable.

4.4 Following learning lACT PN odor responses remain unmodulated

One and multiple trial absolute conditioning of 1-nonanol known to produce an olfactory memory, did not change the glomerular PN odor responses at any point in time tested subsequent conditioning (one, five and fifteen minutes), neither in response shape nor in overall amplitude. Behavioral studies have shown that the responses (PER) in the first three minutes following a single trial conditioning are dominated by a non-associative component, due to sensitization (Menzel, 1990). In contrast, responses subsequent three trial learning reflect associative memory. Therefore, odor responses in lACT PNs remain unaffected by both, associative and non-associative paradigms. Odor responses in animals which received three trial forward conditioning were expected to be different from the others. Only multiple trial conditioning induces long-term memory and leads to prolonged PKA activity (Müller, 2000b). However, odor responses in this group (group 3+) did not differ from any of the other at any point in time.
The robustness of odor responses to change due to conditioning are supported by the results of differential reinforcement. Differential conditioning to 1-nonanol as the CS+ and either 1-hexanol or 1-octanol as the CS- did not lead to conditioning specific modulations of the odor response to CS+ 1-nonanol. This held true for both, three and five trial conditioning.
Behavioral generalization experiments indicate that conditioning to one odor might alter the responses to other odors (Couvillon et al., 1983; Guerrieri et al., 2005; Linster et al., 2001; Takeda, 1961; von Frisch, 1919). This suggests a possible change in the AL network due to conditioning of one odor. However, we showed that the

glomerular PN responses to other odors than the reinforced did not change either, neither the CS- in a differential conditioning nor the neutral odor in either, differential and absolute conditioning.

These results are corroborated by a multidimensional network perspective similar to previous studies (Sachse and Galizia, 2003; Sandoz et al., 2003). Neural odor representations were depicted in a virtual olfactory space where the response of each glomerulus represents one dimension. This view allows to integrate the odor response of the different 15 measured glomeruli into one odor response in every individual animal. As expected from the glomerular activity patterns the representations of 1-hexanol were more distant to 1-nonanol than to 1-octanol, and the latter two were closest in the multidimensional coding space. In rats, odors that elicit almost identical activity patterns in the olfactory bulb, need differential training to be recognized as different chemicals and are generalized after absolute conditioning (Linster et al., 2002a). This suggests that the border between a generalized odor and a discriminated odor can be modified by training, i.e. that the neural olfactory space is plastic. However, even when differentially trained to 1-nonanol and 1-octanol, with overlapping response patterns and close odor distances, we found no difference between naive and conditioned individuals as for all other conditioning protocols.

A lot is known about odor processing at the level of the AL (Galizia and Menzel, 2001). Each odor is coded in the graded activity of several glomeruli and each glomerulus participates in the code of several odors in the AL (Fiala et al., 2002; Joerges et al., 1997; Wang et al., 2003). The representation of odors is symmetrical in the right and the left antennal lobe (Galizia et al., 1998) and the patterns are equal between individuals (Galizia et al., 1999).

So the straightforward hypothesis was that possible changes should be odor specific and systematically related to their glomerular activity patterns, thus be glomerulus specific. As this was not the case we took a different approach. Neglecting the identity of the glomeruli, we analyzed the number of glomeruli in each animal, that showed an increase/decrease in the odor response following conditioning, as has been done previously for multi-units in electrophysiological recordings in the moth (Daly

et al., 2004). Although a considerable number of changes were observed, the net number of glomeruli per animal that show an increase or a decrease to either CS+, CS- or neutral odor was not influenced by conditioning.
On the basis of our results, which we analyzed with different perspectives, we conclude that odor-coding in uniglomerular lACT PNs at the level of the AL is stable and not modulated by learning. Interestingly, similar results have been obtained for adult mice (Mizrahi and Katz, 2003). The spatial pattern of activity evoked by a trained odor as well as the fine dendritic structure of the underlying neurons remained stable after learning.

4.5 Critical discussion of the results

These results are somewhat contradictive to the results obtained previously in a reduced experimental approach (Weidert, 2003). The study of Weidert was designed to reveal reinforcement specific effects. Therefore, all odors used were randomly assigned as either CS+, CS- or neutral odor. Responses to the CS+ were only modified when tested five minutes following differential conditioning, not at fifteen minutes. In contrast, responses to the CS- and the neutral odor remained unaltered. We wanted to reproduce these results, and were interested in odor specific effects. Therefore, we conditioned group specific odor combinations in each group but kept the general paradigm similar to Weidert.
It is difficult to explain the differences of the two studies. Noteworthy, the results of Weidert cannot yet unambiguously be interpreted as this change was only found in those animals which showed an increase in the response to the CS+ already in the pre-test phase. The biological relevance of such a correlation remains unknown. Furthermore, it cannot be excluded with certainty that the changes are representing a sequence effect due to successive odor stimulation. A control group with animals receiving a similar but unrewarded odor stimulation was not included in the experiments. Finally, slight differences in the injection sites of the dye, although

unintentially, could have resulted in staining of different cells. Effects of staining pluriglomerular PNs, for example, are discussed below.

A rather general critical aspect to consider is the fact that we measured calcium with a low sampling rate of 5 Hz. We cannot exclude changes in fast temporal encoding of the odor, due to, for example, modulations of synchrony between spikes of PNs and their relationship to oscillations (Laurent, 1999). Multi-unit recording in the honeybee has been established by Finke and Schaup (Finke et al., 2005). Their results will help to elucidate this question in the near future. At least on the single cell level our results are supported by electrophysiological recordings, which failed to show a significant increase of PN odor responses following single trial olfactory learning (Abel, 1997).
Another concern is the fact that by staining the neurons we introduced a calcium buffer which might have interfered with intracellular signalling cascades mediated via calcium, thereby preventing any modulatory effects. However, using slightly different calcium indicators, other studies have successfully demonstrated plasticity in calcium signalling and calcium mediated plasticity in vertebrate and invertebrate (Faber et al., 1999b; Faber and Menzel, 2001; Lohmann and Wong, 2005; Mutoh et al., 2005; Szyszka, 2005; Yu et al., 2004). This indicates that calcium sensitive dyes do not interfere with the intracellular signalling cascades to such an extend, which prevents calcium mediated plasticity.
We imaged the glomeruli at the dorsal surface of the AL. Therefore we cannot exclude other glomeruli contributing to plasticity in the representation of odors. However, in a recent study, perceptual distance between odors calculated from behavioral data could be predicted on the basis of activity patterns in the AL of only a slightly larger subset of the dorsal glomeruli (Galizia et al., 1999; Guerrieri et al., 2005). This supports the view that the analyzed glomeruli in our study are sufficient for odor coding.

4.6 Robust PN odor responses albeit plasticity in the AL network due to associative conditioning?

As already mentioned, a considerable number of studies, including a variety of different experimental approaches, ranging from behavior to molecular biology, have shown that the OB and the AL are involved in associative odor learning (Davis, 2004; Menzel, 2001).

In case of the vertebrate literature, the vast majority of studies is dealing with olfactory learning, occurring during sensitive periods of enhanced neural plasticity (Keverne and Brennan, 1996): pheromonal learning in female mice and learning of new-born lamb odors that occur after parturition in sheep. Both examples occur in the adult animal in relation to a 'life event' during which robust olfactory learning is vital for reproductive success of the individual. A third example is that of odor conditioning during a developmentally determined sensitive period, in young rats and rabbit pups (Leon, 1992). The first days of life constitute a period of enhanced learning, during which the only solid changes of the neural representations of an odor at the level of the OB due to learning have been described (Johnson et al., 1995; Johnson and Leon, 1996; Yuan et al., 2002). Periods of enhanced plasticity, most probably under the control of juvenile hormone are known in the honeybee as well. Glomerular growth during the first 4 days of adult life has been associated with the shift to foraging duties and with different tasks, like nectar- and pollen-foraging (Winnington et al., 1996). Furthermore, treatment with the juvenile hormone analog methoprene led to improvements in associative learning performance. This coincides temporally with increases in glomerular volume (Sigg et al., 1997). However, as we were dealing with adult foraging bees, learning during sensitive periods or 'life events' might not provide a suitable framework to discuss our results in.

Instead, how do our results relate to findings which clearly documented learning induced plasticity at the level of the adult insect AL? Using the honeybee, *Apis mellifera*, Faber et al. showed that differential conditioning of two odors led to an increase in the calcium responses in glomeruli that responded to the odor before

conditioning (Faber et al., 1999a). However, while clearly showing that associative learning transforms the measured calcium responses in the AL, we do not know the cellular origin of the signals. In this study, calcium-green-AM was used as a calcium indicator which potentially stained all cells. Thus, it is difficult to identify the cell type where the signals came from, OSN, LN or PNs, uni- or multiglomerular.

In the AL of the moth *Manduca sexta*, Daly et al. recorded spike activity of neuronal ensembles before and after classical conditioning. Following conditioning they observed a net recruitment of responsive neural units across the AL in response to the rewarded odor and a net loss to the unrewarded odor (Daly et al., 2004). Furthermore, they argue that in addition to recruitment, conditioning also induced shifts in the temporal responses of 16% of recorded units. Again, however, the identity of the responding neurons within the AL remained unknown as well as the mechanism that led to the changes in responsiveness.

Although using an aversive and therefore fundamentally different conditioning paradigm in *Drosophila melanogaster*, Yu et al. provided evidence that pairing of an odor with an electric shock causes new projection neuron synapses to respond to the odor along with those normally activated prior to conditioning (Yu et al., 2004). This change in odor responses is odor specific and lasts only for a couple of minutes. They recorded odor responses optically using a fluorescent and transgenically supplied reporter of synaptic activity. Synapto-pHluorin reports the movements of a membrane bound pH-sensitive derivative of GFP during synaptic vesicle release and recycling (Yuste et al., 2000)). They used flies carrying the GH146-GAL4 transgene (Stocker et al., 1997) to drive the expression of UAS-synapto-pHluorin in a sub-population of PNs. However, GH146-GAL4 is expressed in a heterogenous PN population (Marin et al., 2002; Stocker et al., 1997). Firstly, the population is comprised of adPNs (derived from the anterodorsal neuroblast) and lPNs (from the lateral neuroblast) running in the different tracts (inner and outer). Secondly, and more importantly, GH146-GAL4 is expressed in uni- and multiglomerular PNs (Marin et al., 2002; Stocker et al., 1997). Although the anatomy already strongly argues for different physiological properties, in the honeybee it has been shown that the response

characteristics of l-, m- and ml-ACT PNs differ remarkably (Abel et al., 2001; Müller et al., 2002).

Taken together, these studies compellingly demonstrate neural plasticity in the AL. But none of the studies identify neither the mechanism nor the identity of the involved neurons. All these studies have in common that their authors interpret their results as a conditioning related transformation of the neural odor representation. However, we do not know "which" neural representation of the odor is transformed. It is difficult to integrate their views as none of them has been able to relate the signals to a specific neuron identity. For this reason, besides differences in species and conditioning paradigms (appetitive versus aversive) our results are not contradicting the idea of neural plasticity in the AL. Moreover, we can add an important piece of information. Namely, associative conditioning does not effect the odor representation in uniglomerular lACT PNs at the level of the AL in the honeybee. This suffices the need for reliable encoding of internal representations of olfactory information at the first processing level, the AL.

4.7 Changes of odor responses in the AL

Although the responses were not modulated due to conditioning, the odor responses changed over the time course of the experiment. Comparing early and late responses in the beginning and at the end of the experiments revealed significant changes in odor responses that are odor specific. Inhibitory responses were always reduced. PN odor responses in the most prominent glomerulus remained stable, except in glomerulus 28 to 1-hexanol. However, this change was only about 5% of the absolute response in comparison with 23% for the second strongest glomerulus 38. For all odors the second and third strongest glomeruli decreased. The most plausible explanation are adaptation processes or weakening of the animal. These results have an important implication. The applied method is sensitive enough to reliably detect

even small changes in calcium responses and the results discussed above are not due to technical limitations.

A recent study in locusts (Bazhenov et al., 2005) suggests that activity-dependent synaptic facilitation may improve the signal-to-noise ratio for repeatedly encountered odor stimuli. Does the fact that the most prominent glomeruli remain almost unchanged implicate that odor responses become more focussed due to successive odor stimulation? Moreover, does the response increase observed in a single glomerulus 36 to 1-nonanol and 1-octanol indicate non-associative olfactory learning?

This is a very provocative hypothesis and our experimental design does not allow us to speculate about the mechanism nor about the exact cause.

Nevertheless, a variety of studies point toward the intriguing question of the relevance of odor experience of an animal. Successive odor experiences might be accompanied by long lasting changes in their representation in the central nervous system, other than ones simply explained by receptor adaptation. On the behavioral level it has been shown that repeated odor stimulation leads to latent inhibition in a subsequent conditioning experiment (Hammer and Menzel, 1995). Preconditioning, unreinforced exposure to a compound of two odors leads to responses to both after conditioning of only one of the odors (Müller et al., 2000). In the locust repeated odor stimulation resulted in a stimulus specific decrease in intensity of odor responses in PN assemblies while they showed an increase in spike time precision and inter-neuronal oscillatory coherence (Stopfer and Laurent, 1999). Repeated discrete exposures to odors habituates the startle response in Drosophila (Cho et al., 2004). Further experiments are necessary to reveal the role of lACT in non-associative olfactory learning.

4.8 Which neurons are subject to modulation in the AL?

In order to stimulate future experiments we would like to speculate which neurons could be subject to modulation due to conditioning in the AL. What if the activity

pattern of uniglomerular PNs were indeed changing at the level of the AL due to olfactory conditioning, either increased in overall response strength or even more PNs recruited? In both cases, this would unequivocally lead to a change in the response pattern in the Kenyon cells. However this is not the case, as pairing an odor with sucrose induced a pronounced prolongation of odor responses without changing the ensemble of activated Kenyon cells (Szyszka, 2005). Whether these changes are due to pre- or postsynaptic modulations in the MB remains unknown, but importantly due to the necessary synaptic specificity in both cases the induction is required in the MB and not in the AL.

Induction of olfactory memory at the level of the AL has been shown (Hammer and Menzel, 1998). It might be that the memories at the two levels, AL and MB, are fundamentally different as already speculated by Szyska (Szyszka, 2005). Whereas both have been shown to be the sites of associative memory, it is conceivable that the MB is the site of discriminative learning and the ALs for absolute learning. Is there any evidence for this idea?

It is known that the discriminative capabilities of an animal depend on the conditioning paradigm (Giurfa, 2004). If an odor is reinforced animals generalize to other odors, which highly correlates with the similarity in response pattern in the insects AL and the vertebrate OB (Guerrieri et al., 2005; Linster et al., 2002a). Following differential olfactory conditioning, generalization is reduced (Bitterman et al., 1983; Ditzen et al., 2003; Linster et al., 2002b). This cannot be explained with odor coding at the level of the MB. Due to sparse coding in Kenyon cells, overlapping responses to different odors are unlikely (Perez-Orive et al., 2002; Szyszka, 2005; Wang et al., 2001). Learning in the MB may, therefore, account for the ability of insects to reliably discriminate similar odors, whereas learning in the AL would allow for generalization (Szyszka, 2005). It is then conceivable that the behavioral outcome depends on the comparison between the two. Indeed, a recent modelling study (Sivan and Kopell, 2004) hypothesized that activity of neurons in the lateral protocerebrum encodes the odor cluster (generalization), and activity of neurons in the mushroom body encodes the identity of the odor (discrimination).

We demonstrated that odor responses in uniglomerular PNs at the level of the AL remain stable following learning. However, as already mentioned above the PNs comprise a heterogeneous group of neurons, in the honeybee, uniglomerular m- and l-ACT, and the multiglomerular PNs. Recently it has been argued that the different subpopulation might serve different functions (Menzel et al., 2005). It was speculated that lACT might undergo learning related plasticity whereas mACT remain stable. However, the lACT PNs remain stable and the only remaining candidates innervating the dorsal glomeruli, in which Faber et al. reported learning induced increases of activity (Faber et al., 1999a), are the pluriglomerular ml-ACT.

Multiglomerular PNs have been described for several insects species, e.g., cockroaches (Ernst et al., 1977), *Manduca sexta* (Kanzaki et al., 1989), *Lepidoptera* (Anton and Hansson, 1994), and *Drosophila (Stocker et al., 1990)*. In the honeybee multiglomerular PNs differ from uniglomerular PNs running in the m- and lACT, as they do not innervate the calyces of the MBs, but instead project via the ml-ACT to the LP only, or to the LP and to the ring neuropil around the alpha-lobe (Abel et al., 2001). The multiglomerular ml-ACT PNs have been demonstrated to respond to many odors. Interestingly, if the first response to a particular odor was excitatory, the second response was reduced (Abel, 1997a). In another study, multiglomerular PNs have been shown to remain silent to many odors despite innervating a large number of glomeruli (Müller, 2000a). This unreliable response behavior implicates that the multiglomerular PNs are not involved in coding the identity of an odor.
Multiglomerular PNs run perpendicular to the odor pathway in the AL, as they receive convergent input by all glomeruli. If an odor is reinforced, we postulate an increase in the response strength to that particular odor in the multiglomerular PNs. An odor with a similar glomerular response pattern would evoke an increased response in the multiglomerular PNs although not being reinforced. The odor information is transmitted to the PL and the ringneuropile, where it can be compared with odor information processed by the MB. At the same time, responses to uniglomerular PNs remain unaltered due to associative learning in the AL ensuring reliable and precise odor discrimination at the MB.

This idea is consistent with previous findings. The results cited above according plasticity in odor responses (Daly et al., 2004; Faber et al., 1999a; Yu et al., 2004) can all be sufficiently explained with plasticity in multiglomerular PNs. And formally, the neural odor representation is only changed in one of the many parallel odor processing channels. Both, uniglomerular m- and lACT PNs remain stable, which ensures the separate encoding of the neural representations of a variety of aspects of olfactory stimuli, for example their temporal structure, their intensity fluctuations and the sequence, their evaluation and meaning (Menzel et al., 2005).