

## General Discussion

The lip region of the median calyx included in the mushroom body (MB) of the honeybee (*Apis mellifera*) is a central neuropil in relation with the processing of olfactory signals, which are perceptible via the antennae. Glomerular activity patterns produced by the antennal lobe (AL) project in one case directly into the median calyx. In the other case, olfactory information is passed via the lateral protocerebrum (LP) to the median calyx (see figure 1). The role of the LP is to a large extent unknown. Within the MB olfactory information is integrated with other modalities, therefore the MB has an outstanding position on the way to building a multimodal memory.

An open question answered by this work was, whether the MBs are necessary for mere olfactory learning, or whether memory traces as found in the AL (Grünbaum & Müller 1998) are sufficient for storing information about relevant odors. Chapter II and III of this work indicate, that the mere discrimination achievement done by the ALs (Joerges et al. 1997) is not sufficient to associate odors with a special meaning (prediction of a reward). Both chapters show that described learning problems are only solvable by transferring and comparing olfactory information between sides. This transfer occurs on the level of the MBs. Transfer of information that may occur between the ALs is not sufficient to sum up information from both antennae. In contrast, the involvement of the MBs in simple olfactory learning tasks seems to be bigger than assumed before. The release of the proboscis extension response (PER) is not only caused by recognizing an odor in the ALs, it also depends on the recognition on a higher processing level, involving directly or indirectly the MB.

Afterwards, the development of higher order learning paradigms as it has been done in chapter I seems not to be promising for investigating the role of the MBs within the olfactory learning in the honeybee, because it is not possible to produce bees without MBs as it is possible in the fruit-fly (*Drosophila melanogaster*) (Heisenberg et al. 1985, de Belle & Heisenberg 1994, Conolly et al. 1996). Nevertheless one has to assume that MBs also play an important role within the framework of these experiments, because olfactory information has to be transferred in complex olfactory learning as well as in mere elemental olfactory learning.

**Chapter I: Successive reversal learning**

Results show a kind of higher order learning. They show the plasticity of the olfactory memory because bees learn to invert their response to a former learned odor. Beyond that, the whole learning process seems to change. Therefore bees solve a learning problem of the same kind faster if it occurs a second or a third time.

Honeybees had to develop the ability to modify olfactory memories, because of their foraging behavior. Food sources used by bees change their amount of reward during a season also within their circadian rhythm. Thus it is necessary for a bee colony to react to the decreasing amount of nectar of a certain plant and to redirect the foraging emphasis to a more worthwhile food source. This ability is not only based on the evaluation of a food source by a dancing bee but also on the willingness of a single foraging bee to change the kind of flower visited. This willingness to use new food sources is increased if the amount of reward of the former flower visited decreases. The memory becomes more open for new or changed information, while a successful foraging bee keeps visiting its flower species. This plasticity is not or at least not only regulated by communication between individuals, but a characteristic of the honeybee brain visible in adequate learning tasks. Whether this might be due to local changes in certain brain regions cannot be questioned by this work, because these experiments were not resumed on ablated or somehow treated bees.

With respect to the results of these experiments bees do not seem to learn an abstract rule as they may do in learning symmetry (Giurfa et al. 1996) or during categorical learning of sameness or difference (Giurfa et al. 2001). It is more likely to think of bees becoming faster in changing their memory or inverting faster the meaning of an odor than animals that were not confronted with this kind of task in former conditioning phases. It may well be some kind of attention playing a major role. Honeybees may change the state of their behavioral plasticity if they expect changes to the former learned values of odorants. This has never been investigated, because it is difficult to determine the state of attention or expectation on the level of an individual bee. But it may well be, that the attention to a modality, which can be learned associatively by a honeybee, is represented in a particular neuronal subset as it has been postulated for humans (Baer & Fuhrer 1982). To interfere with such a neuronal correlate could be an approach to shed light into this mere speculative acceptance. Nevertheless, with respect to the findings of chapter I, one cannot conclude anything about neuronal mechanisms or correlates involved in this learning phenomenon.

## Chapter II: Side-specific patterning

These experiments are based on the idea that olfactory information is exchanged between brain sides mainly on the level of the MBs. This would mean, if a learning problem is only solvable by the use of identical information given to both antennae, it is likely to think that the capacity of both MBs rises if they can act as a functional unit. Local cooling experiments implied that MBs influence one another during single side learning (Menzel et al. 1974, Erber et al. 1980), whenever the MB input region of the side opposite to the stimulated antenna has been cooled. Therefore one assumes that olfactory learning in the honeybee needs a bilateral symmetrical alignment of olfactory information between hemispheres. The transferred information is therefore not evaluated before the transfer but at the input region of the other brain side. In a non-linear learning paradigm as negative patterning (NP) the complexity of the learning task is transferred more inaccurately than in logic, linear problems as positive patterning (PP). If a discriminating problem is symmetrically transferred to the other MB depends therefore on the comprehensibility of the paradigm, thus, if it is classified as clear or misleading. Whether bees are able to solve NP if they lost one antenna cannot be answered by this study. It is possible that information coming from a destroyed AL is totally different from the absence of information coming from a non-stimulated antenna.

These experiments show impressively that discrimination between A, B and AB is not only a question of similarity. In both kinds of patterning honeybees are confronted with identical stimuli. It is therefore unlikely that the interconnecting of the AL which causes a different glomerular pattern for AB than the simple sum of the patterns for A and B (Sachse & Galizia 2002) is sufficient to form something like a configuration or a unique cue (see discussion of chapter II) which would make it possible to solve NP. It rather seems that basic processes beneath the mere elemental learning are added on the level of the MBs, leading to a successful discrimination. Extensive studies prefer mathematically a unique cue over a configural processing for NP (Deisig et al. 2003) but the effect of the glomerular network of the AL, leading to excitation patterns, seems not to be sufficient to form such a unique cue. This hypothesis shall be investigated in future works by the use of different sensory modalities within patterning problems. Thus, the neuronal representations of the different compound components lead to no interference within the ALs because these different inputs do not converge on the level of the AL.

### Chapter III: Olfactory learning in hydroxyurea (HU) treated bees

Honeybees are able to combine different olfactory information coming from both antennae (Sandoz & Komischke *unpubl.*). In addition they can separate ambiguous olfactory information between sides (Sandoz & Menzel 2001). Those experiments and this work show that the honeybee brain normally does not fulfill two differential conditionings given to separated antennae at the same time. In that case, most of the individuals learn only one of the problems. Different olfactory information between sides therefore impedes learning. In untreated bees, both hemispheres are equivalent. This leads to the same number of animals learning on the right side as learning on the left side. A different case is ablated bees, where one median calyx of the MB is missing. Distinct differential conditionings can-not be learnt on any side by these animals. Single side learning of differential conditioning is possible (Malun et al. 2002). Here lies another hint that MB of both hemispheres normally acts as a functional unit during olfactory learning. If this unit gets out of equilibrium, olfactory information of both sides can no longer be integrated. If olfactory information on both sides is identical, ablated bees show no difference in learning compared to untreated bees. The loss of a median calyx does therefore not restrain olfactory learning per se, but, above all, the integrative transfer of olfactory information between the MBs.

Data of experiments presented here show also a deficit of the ablated side compared to the intact hemisphere. If a separation of ambiguous olfactory information is included in the learning problem, the intact brain side is able to ignore information coming from the ablated brain side. The learning performance of the intact side is in this case as good as the performance of hemispheres of untreated bees. The ablated side is not able to solve a differential conditioning in this experimental design. There are two possible reasons:

1. The ablated side is not able to prevent transfer from the intact side and therefore sums up information that makes the fulfillment of the task impossible.
2. An isolated ablated brain side is not able to solve a differential conditioning because the median calyx is necessary to do so.

Both possibilities cannot be separated on the basis of the available dataset. To solve this question experimentally, hemispheres have to be artificially separated. This seems to be impossible at the moment, because separation of hemispheres would cause massive damages to the bee brain.