5 Outlook

Finally I would like to suggest some experiments, which can easily be performed as most of them require techniques that are already established in the laboratory. Most of them are simple and have been suggested many times before. Yet, they still have not been performed. Others might be new. I present and emphasize them, to stimulate future work challenging our proposed hypothesis and thereby shedding light on the mechanisms of olfactory learning.

5.1 Monitoring of the PER

The preparation of the animal for an imaging experiment is a complicated procedure. Once the necessary skills for a successful recording are acquired, experimenters often hesitate to elaborate this technique even further. However, in my case I would have gained power in persuasion by providing direct evidence for learning. It is not only of importance in case one does not find learning related neural changes. Although reaching up to 80%, it is always only a proportion of animals which learns. Separating learners from non-learners with an *a priori* defined criterion would allow to enhance the preciseness of the analysis of learning induced plasticity.
Monitoring the muscle extensor muscle M17 (Rehder, 1987) has been done before during elaborate electrophysiological experiments (Finke et al., 2003;Hammer, 1993;Mauelshagen, 1993) and has been reported to be possible in preliminary imaging preparations (Faber, 1999). In case an intact M17 causes too strong movement artefacts. Another alternative could be the M20, responsible for movements of the glossa, which is smaller and might cause less movement artefacts than the strong M17.

5.2 Staining different cells

Staining populations of neurons by backfilling is widely used in different systems (Gelperin and Flores, 1997; Delaney et al., 2001; Macleod et al., 2002; Sachse and

Galizia, 2002; Macleod et al., 2003; Sachse and Galizia, 2003). There is no technical reason which argues against the possibility to stain the m- as well as mlACTs comparable to our experiments. Moreover, Szyska has already shown that it is also possible to record calcium signals from PN axon terminals in the MB. PNs were anterogradely stained by injecting Fura-2 dextran or Calcium Green-1 dextran *via* was into l-ACT PN soma clusters in the dorso-medial part of the ALs.
Therefore, all PN neurons can be imaged at both, although not in parallel, the AL and their output regions, the LP and MB. Imaging experiments will help to clarify firstly, which neurons are subject to learning induced modulation, and secondly at what level this modulation is manifested, the AL or the MB.
An interesting and challenging idea is the following: supposed the results of Faber are reliably reproducible (Faber et al., 1999b), the backfilling method with dextranes should be combined with the unspecific AM-ester staining. Measuring the two dyes in parallel has been established recently (Sachse and Galizia, 2002b). The odor responses derived from the unspecific staining could potentially provide a control, as these have been shown to increase following learning.

5.3 Pharmacology

Once learning induced changes are demonstrated, it is inevitable to verify the hypothesized dependency of learning on octopaminergic input by the reward pathway. Experiments described above should be repeated with either reserpine or mianserin treated animals. Both drugs are known to interfere with the octopaminergic system (Braun and Bicker, 1992;Farooqui et al., 2003;Menzel et al., 1999). Self-evidently, results shown by Faber et al. should be repeated with equally pharmacologically treated animals (Faber et al., 1999a).

5.4 The sugar or water response

I argued that the sugar response is not representing an input by the reward pathway. I tried to investigate this further by depleting octopamine with reserpine. It turned out

that the signal quality was strongly affected by the necessary carrier of reserpine, DMSO. Responses were rather small in both, DMSO and reserpine-DMSO treated animals. Moreover, it seemed as if DMSO affected the integrity of the cell membranes, resulting in difficulties in glomeruli identification. These experiments should be repeated by using an octopamine blocker like mianserin instead of reserpine.

I performed preliminary experiments during which I intended to examine possible PN responses to octopamine. To this end, I combined pressure injection of octopamine into the AL and calcium imaging of lACT PNs. In general, both techniques can be performed in parallel. During injection movement artefacts were too strong to analyze the calcium signals. Due to time restrictions I did not follow this project. However, comparing calcium responses before and after injection should be possible. If our hypothesis be of great interest to see whether pairing of an odor stimulation with an octopamine injection changes the odor response, which has been shown to lead to learning in behavioural experiments.

5.6 Behavioural experiments and the role of the AL and MB

I argued that memories in MB and the AL might have different functions. Whereas the MB is responsible for discriminative learning, the AL is involved in absolute learning and generalization. Odors paired with octopamine injection have been shown to induce a memory in both, the MB and the AL. First of all I hypothesize injections in the AL result in higher generalization rates to other odors than the conditioned one. Secondly, very similar odors should be better discriminated when differentially paired with octopamine injections in the MB than into the AL.