5 General Discussion

The major aim of the presented thesis was to investigate the influence of retrieval on a consolidated memory. For this approach the numberof retrieval trials was varied and the retention of memory was tested subsequently. In honeybees the formation of LTM depends on protein synthesis (Wüstenberg et al., 1998; Friedrich et al., 2004) and can therefore be inhibited with blockers of protein synthesis. Thus, in the studies presented here retrieval-induced protein synthesis-dependent consolidation processes were analyzed. For this, the two translation inhibitors emetine and anisomycin 'were applied at the time of retrieval. I demonstrated that depending on the number of retrieval trials reconsolidation of the acquisition memory or consolidation of the extinction memory was induced.

Retrieving the acquisition memory with one CS-only trial

In chapter II it has been demonstrated that one CS-only trial applied 24 h after acquisition led to no significant change in CRs in a series of single tests in comparison to the non-retrieved groups (chapter II). Application of emetine in a concentration of 10 mM (low dose) 30 min before or one hour after the retrieval led to no significant changes of the CRs at a retention test 24 h later (chapter I, chapter II, chapter III). The low dose is sufficient to impair consolidation of a three trial memory (Friedrich et al., 2004).

Application of a mixture of the protein synthesis inhibitors anisomycin and emetine, each in a concentration of 10 mM, one hour before the first retrieval also revealed no protein synthesis-dependent process. Nevertheless, if the mixture was applied one hour after the first retrieval a reduced retention level was detectable after three days (chapter I). Omission of the first retrieval before injection of the inhibitors led to no memory impairment. This demonstrates that the acquisition memory is not susceptible to protein synthesis inhibitors 24 h after conditioning. Furthermore, the observed impaired retention after the first retrieval in conjunction with the inhibitors can not be related to an unspecific effect of the inhibitors. Therefore, it can be concluded, that one CS-only trial returns the memory to a labile state, which requires protein synthesis to be re-stabilized. This process is called reconsolidation. This result was confirmed by the

application of emetine in a concentration of 20 mM (high dose) one hour after the first retrieval. A reduced retention level was observable two days later. Thus, reconsolidation of an olfactory memory in honeybees is less vulnerable to protein-synthesis inhibitors than consolidation of the acquisition memory. This emphasizes that reconsolidation is not a repetition of all processes involved in consolidation. In several studies it has been demonstrated that different brain regions or different molecules are involved in consolidation than in reconsolidation (Alberini, 2005; Dudai & Eisenberg, 2004). For example, protein synthesis and C/EBPb, a transcription factor, are required for inhibitory avoidance consolidation in the hippocampus of rats, but not for reconsolidation (Taubenfeld et al., 2001). In gerbils it has been observed that protein synthesis is required in the auditory cortex for consolidation but not for reconsolidation of a tone discrimination task (Kraus et al., 2002).

A further difference between consolidation and reconsolidation is the time window of sensitivity to consolidation inhibitors. In chicks the sensitive period for interference with the reconsolidation process of passive avoidance memory following retrieval is shorter than after training (Anokhin et al., 2002). That reconsolidation occurs faster than consolidation was also shown in rodents ((Gordon & Spear, 1973); (Gordon, 1977); (Gordon, 1977) in (Nader, 2003). The data presented here do not confirm these results. In honeybees inhibition of the consolidation process has been induced by the application of anisomycin one hour after training, but also by an injection of emetine or the mixture of anisomycin and emetine 30 min before conditioning (Wüstenberg et al., 1998; Friedrich et al., 2004). An inhibition of the reconsolidation process could be demonstrated by injection one hour after the first retrieval, but not by an injection one hour before the first retrieval (chapter I).

As demonstrated in chapter I, application of the high dose of emetine led to a significant memory impairment in comparison to the control group two days after the first retrieval. However, this significant difference was not observed on the fourth day after retrieval. As discussed in detail in chapter I, this phenomenon is probably due to an additionally induced extinction process by the multiple retention tests. Nevertheless, whether memory impairment after inhibited reconsolidation is long-lasting or transient in bees cannot be finally decided upon by the data presented here. A transient impairment of the memory would refer to a retrieval deficit during retention test, while a long-lasting impairment could also be due to a storage deficit. Both transient and long

lasting impairments have been seen after inhibited reconsolidation in rodents. In a contextual fear paradigm in mice, retrieval of the memory in conjunction with injection of anisomycin resulted in a transient impairment. Three weeks after retrieval the memory has been spontaneously recovered (Lattal & Abel, 2004). On the other hand in cued fear conditioning anisomycin in conjunction with a retrieval resulted in amnesia which lasted at least for 20 days (Duvarci & Nader, 2004).

Retrieving the acquisition memory with two CS-only trials

Two CS-only trials applied 24 h after acquisition led to a significant extinction in CRs in a series of single tests in comparison to the non-retrieved groups. This series of single tests revealed that CRs decrease in the next two hours following retrieval and recover slowly over the following two days. But the recovery is not significant. The decrease in CRs was inhibited by application emetine in a low dose 30 min before the retrieval trials. (Chapter II). Extinction is regarded as new learning about an inhibitory CS-noUS association (Bouton, 1993; Bouton, 2004). The presented results support this hypothesis, since formation of long-term memory is thought to dependent on protein synthesis. Hence two CS-only trials induce a protein synthesis-dependent extinction memory. Similar results were reported for aversive paradigms in crabs (Pedreira & Maldonado, 2003), snail (Sangha et al., 2003b) and rats (Berman & Dudai, 2001).

If the US presentation during acquisition was prolonged a protein synthesis-dependent extinction memory was induced by two CS-only trials. In contrast, shortening the US presentation resulted in a protein synthesis-independent extinction memory. The reason for this difference is not understood. One possible explanation could be that the mismatch between expectation and experience at the CS-only trials increases with the length of the US presentation. This difference seems to influence the induction of a protein synthesis-dependent consolidation of the extinction memory (Chapter III).

Retrieving the acquisition memory with five CS-only trials

Five CS-only trials applied 24 h after acquisition led to a significant extinction in CRs in a series of single tests in comparison to non-retrieved groups. The CRs increased significantly in the next four hours following retrieval. Over the subsequent two days the retention level remained stable. Despite the spontaneous recovery from extinction in the retrieved groups, the response level was significantly reduced in comparison to the

non-retrieved groups. Application of emetine in a low dose 30 min before the five CS-only trials inhibited the increase of CRs following the extinction procedure (chapter II). Spontaneous recovery from extinction is defined as the reappearance of the acquisition memory. Since the application of emetine led to a reduced spontaneous recovery, I concluded that during the retrieval session the acquisition memory was returned to a labile state and required protein synthesis to consolidate again. This resembles the concept of reconsolidation. Therefore, I supposed that reconsolidation underlies spontaneous recovery. In contextual fear conditioning in mice a similar observation was made (Lattal & Abel, 2004). Animals subjected to a long context exposure 24 h after training showed a reduction of CR during the retrieval session. CRs of saline-treated mice recovered on the next day, but CRs of anisomycin-treated animals showed no recovery and were significantly reduced in comparison to the saline-treated group.

It is unknown why the reconsolidation process after one CS-only trial is less vulnerable to interference in comparison with the induced process after five CS-only trials. As discussed above, after one CS-only trial a memory impairment can be seen by inhibition with the high dose of emetine or with the mixture of inhibitors one hour after retrieval but neither with the low dose nor with the mixture of inhibitors one hour before the retrieval trial

In the above discussed results the five CS-only trials were applied with an inter-trial interval (ITI) of 10 min. As shown in chapter I, five CS-only presentations with an ITI of 24 h also induced a significant reduction of the CRs. As mentioned above a single CS presentation did not result in a significant extinction. Since there is a clear extinction process over the multiple retention tests, I suppose that with an ITI of 24 h the animals learn about the CS-noUS association. It is unknown, whether at any point between the extinction sessions spontaneous recovery occurs If this extinction learning is protein synthesis-dependent is also unknown. That extinction learning can be independent from protein synthesis was shown for mice in contextual fear-conditioning and in a spatial learning paradigm. The extinction sessions in both cases were spread over several days (Lattal & Abel, 2001).

A protein synthesis-independent extinction process has been presented in chapter III. Shortening of the US duration during acquisition led after two CS-only trials to a significant extinction, which could not be inhibited by the applied translation inhibitor. However, the same protocol with longer US presentations during training resulted in

protein synthesis-dependent extinction. As discussed in chapter III, a possible explanation for this result is the different discrepancy between the expectation and the experience during the retrieval. Trial based learning models propose that a decrease in CR develops when the strength of the CS present on a conditioning trial "overpredicts" the magnitude of the US that actually occurs on the trial (Schwartz, 1984). Therefore, the discrepancy between expectations and experience is larger for bees that have received a long US presentation than for bees that have experienced a short US presentation. I proposed that this difference is the 'force' to switch between the induction of a protein synthesis-dependent extinction memory and a protein synthesis independent formation of an extinction memory. It is conceivable that the extinction memory induced by five CS-only presentations with an ITI of 24 h is independent from protein synthesis, since I demonstrated that one CS-only trial under none of the tested conditions in this study resulted in protein-synthesis dependent extinction. By following the above described hypothesis a possible explanation is that the mismatch between experience and expectation is too small after only one CS-only trial to induce protein-synthesis extinction.

To summarize, this thesis provides first insights into the different protein synthesis-dependent processes which can be caused by retrieval of a consolidated appetive olfactory memory in honeybees. I demonstrated that the induction of reconsolidation of the acquisition memory or the consolidation of extinction memory depends on the number of the retrieval trials. Furthermore, the results lead to the formation of the hypothesis that the degree of discrepancy between expectations and experience is at least influencing the induction of protein synthesis-dependent processes.