

Summary:

This thesis describes olfactory learning in honeybees (*Apis mellifera*). The first two chapters describe complex forms of learning; Second-order conditioning (SOC) and Context-dependent learning (CDL). The third chapter focuses on sleep behavior.

1) To show SOC we used classical conditioning of the proboscis extension response (PER) in honeybees using odors as conditioning stimulus (CS) and sugar as US. We showed that bees can learn an odor (C) even without an US, but only if C was paired with a previously rewarded odor (A). In other words, bees respond to the new odor C after 5 times A-US pairing followed by 5 times C-A pairing (A-US → C-A). By changing the sequence of odors (A-US → A-C) we could not demonstrate an effective SOC. Furthermore not only the sequence of odors, but also the sequence of training is important. Fewer bees show PER to C, after the sequence C-A → A-US in comparison to the sequence A-US → C-A. These results helped us to understand conditions critical for SOC.

2) In context dependent learning experiment we could simultaneously test several combinations of contexts and study properties of the brain by extracellular recordings. We showed that bees can learn to differentiate contexts in relatively short time (<1h). Bees showed better learning when two contexts were presented together as opposed to single context. Extracellular recordings from alpha lobe of mushroom bodies showed that the neuronal firing changed sharply between hot and cold temperature contexts. Neurons responded more strongly to the rewarded context as compared to unrewarded context, but the response towards rewarded odor was weaker compared to other odors.

3) Sleep-like behavior in bees has been observed before but its role in learning and memory has not been studied. Described here is a technique which allowed monitoring of sleep combined with olfactory learning tasks. We found that, like many animals, bees also slept more during night compared to daytime and the patterns of antennal movements (sleep indicators) were different during sleep and wake period. Learning experiments showed that bees that were conditioned to odors slept lesser compared to unconditioned bees. Sleep depriving bees with shaking did not affect acquisition learning but affected extinction learning.