# Sucrose responsiveness and behaviour in honey bees *(Apis mellifera* L.)

**Thesis**

**for the degree of**

**Dr. rer. nat.**

**at the Technical University of Berlin**

**Faculty VII**

**Institute of Ecology**

**presented by**

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**Berlin, June 2001**

**D 83**

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**Date of exam**: 24 September 2001

# Acknowledgements

First of all, I would like to thank my advisor, Prof. Dr. Joachim Erber, for the topic of my dissertation and for his excellent supervision. I very much appreciate the numerous discussions we had on different aspects of my work, and his valuable advise. I am grateful to Prof. Dr. Robert Page for his support and his helpful suggestions, particularly with regards to statistical questions. I would like to thank Dr. Wolfgang Blenau for his ideas and comments on my thesis, and Achim Buchholz for his continuous technical support. Special thanks to Stephan Haupt for his help with the electrophysiological recordings. I am very grateful to all the members of Prof. Dr. Erber's lab for their support and help with the experiments, particularly Marcus Barnert, Sigrid Wiese, Stephanie Baumgarten, Annegret Weiß, Karin Grandy, Johannes Kisch and Lore Grohmann. I am indebted to Dr. Tanya Pankiw from Prof. Dr. Page's lab for her help with the high- and low-strain bees, and to Dr. Uli Müller and Ulf Thomas of the Free University, Berlin, for their help with the PKA activity measurements. This thesis was supported by the Sonderforschungsbereich 515 "Mechanisms of developmental and experience-dependent neural plasticity" of the Deutsche Forschungsgemeinschaft.

# Abstract

In dieser Arbeit wird bei der Honigbiene (*Apis mellifera* L.) analysiert, wie sich die individuelle Empfindlichkeit für Zuckerwasserreize auf verschiedene Formen der Verhaltensplastizität auswirkt. Die Versuche klären insbesondere die Rolle des Belohnungsreizes Zuckerwasser beim assoziativen und nicht-assoziativen Lernen auf. In den Untersuchungen werden Bienen verschiedenen Genotyps, verschiedenen Alters und mit verschiedener Sammelrolle verwendet. Die Funktion von potentiellen endogenen Modulatoren und von intrazellulären Signalmolekülen auf die Zuckerwasserempfindlichkeit wird analysiert.

Die individuelle Empfindlichkeit für Zuckerwasserreize wird mit Hilfe des Rüsselreflexes oder durch Ableitungen am Proboscismuskels M17 bestimmt. Die Versuche zeigen, daß Pollensammlerinnen empfindlicher für Zuckerwassereize als Nicht-Pollensammlerinnen sind, Bienen der genetischen Linie „high pollen hoarding“ sind empfindlicher als solche der Linie „low-pollen hoarding“. Ältere Sammlerbienen sind empfindlicher als Jungbienen. Die individuelle Zuckerwasserempfindlichkeit bestimmt beim taktilen und olfaktorischen assoziativen Lernen in starkem Maße den Verlauf von Akquisition, Extinktion und Diskriminierung. Dabei ist die Zuckerwasserperzeption an der Proboscis für das Lernniveau entscheidender als die an der Antenne. Auch beim nicht-assoziativen Lernen determiniert die individuelle Zuckerwasserempfindlichkeit den Grad und den Verlauf von Habituation und Sensitisierung. Liganden für biogene Amin-Rezeptoren können die Zuckerwasserreaktion modulieren. Die Injektion von Oktopamin und Tyramin erhöht die Zuckerwasserempfindlichkeit, während Dopamin und der Dopamin-Rezeptor-Agonist 6,7-ADTN funktionell antagonistisch wirken. Die Aktivität der PKA im Antennallobus korreliert mit der Zuckerwasserempfindlichkeit. Bienen mit niedriger Empfindlichkeit zeigen eine signifikant niedrigere PKA-Aktivität als Bienen mit hoher Zuckerwasserempfindlichkeit.

Die hier vorgestellten Experimente zeigen, daß die Zuckerwasserempfindlichkeit einer Biene alle untersuchten Formen des Lernens entscheidend beeinflußt. Damit ist ein wichtiger Faktor identifiziert worden, der durch biogene Amine moduliert werden kann und der Unterschiede der Verhaltensplastizität bei Bienen erklärt.

# Summary

This work shows the effects of individual sucrose responsiveness on two forms of associative and non-associative learning in the honey bee. In addition, the effects of genotype, foraging role and age on sucrose responsiveness, associative learning and discrimination are presented. It is tested whether myograms of the proboscis muscle 17 can determine sucrose responsiveness more accurately than behavioural tests. It is further analysed whether the three biogenic amines octopamine, tyramine and dopamine, and the dopamine receptor agonist ADTN can modulate sucrose responsiveness. In search of neuronal correlates for sucrose responsiveness the activity of cAMP-dependent protein kinase (PKA) in the antennal lobes of bees with different sucrose responsiveness is studied.

Sucrose responsiveness varies between individuals of different genotypes, foraging roles or ages. Associative tactile and olfactory learning is strongly affected by individual sucrose responsiveness. Bees with high sucrose responsiveness show a higher acquisition level and less extinction than individuals with low sucrose responsiveness. Genotype or foraging role have no separate effects on associative learning or extinction. Age has an additional effect on acquisition, which cannot be explained by differences in sucrose responsiveness. Discrimination depends on acquisition and is thus indirectly related to sucrose responsiveness. Genotype has a separate effect on discrimination in foragers but not in preforagers. The short sucrose stimulation of the antenna during associative tactile learning only has a week effect on the learning performance. The sucrose concentration offered to the proboscis as reward strongly determines the level of acquisition and extinction. Discrimination is best when antenna and proboscis are stimulated with a low sucrose concentration. The degree of non-associative habituation and sensitisation is strongly determined by individual sucrose responsiveness. Bees with high sucrose responsiveness demonstrate weaker habituation and stronger sensitisation than bees with low sucrose responsiveness. The sucrose concentration of the habituating or sensitising stimuli strongly affects habituation and sensitisation. Age affects habituation but not sensitisation. Sucrose responsiveness can be exactly determined by myograms from the proboscis muscle 17, which differs in its spike rate when the antennae of a bee are stimulated with different sucrose concentrations, even when the bee responds with proboscis extension to all sucrose concentrations offered.

Modulation of sucrose responsiveness by biogenic amines is possible. Octopamine and tyramine injections result in a dose-dependent increase in sucrose responsiveness. Dopamine

and ADTN decrease sucrose responsiveness in a dose-dependent manner. This modulation of sucrose responsiveness is reversible. Sucrose responsiveness covaries with PKA activity in the antennal lobes. Bees with high sucrose responsiveness show higher PKA activity than bees with low sucrose responsiveness. Thirty minutes after feeding, PKA activity is higher than 90 min after feeding.

These findings show that individual sucrose responsiveness is a very good indicator of the "physiological state" of a bee and can explain a large part of individual differences in non-associative and associative learning. The neuronal mechanisms regulating individual sucrose responsiveness are not yet known, but PKA activity in the antennal lobes and biogenic amines appear to be involved in these processes.

# Zusammenfassung

Diese Arbeit beschreibt den Einfluß der individuellen Zuckerwasserreaktion auf verschiedene Parameter des nicht-assoziativen und assoziativen Lernens. Außerdem werden die Einflüsse von Genotyp, Sammelrolle und Alter auf die Zuckerwasserreaktion sowie auf assoziatives Lernen und Diskriminierung untersucht. Es wird getestet, ob elektrophysiologische Ableitungen am Proboscis-Muskel 17 eine genauere Bestimmung der Zuckerwasserreaktion ermöglichen als Verhaltenstests. Die Wirkung der drei biogenen Amine Oktopamin, Tyramin und Dopamin sowie des Dopamin-Rezeptor-Agonists ADTN auf die Zuckerwasserreaktion wird überprüft. Auf der Suche nach neuronalen Korrelaten für die Zuckerwasserreaktion wird untersucht, ob es einen Zusammenhang zwischen Zuckerwasserreaktion und Aktivität der cAMP-abhängigen Proteinkinase (PKA) gibt.

Die Reaktion auf Zuckerwasser variiert zwischen Individuen unterschiedlichen Genotyps, zwischen Bienen, die Pollen oder Nektar sammeln und in Abhängigkeit vom Alter. Assoziatives taktiles und olfaktorisches Lernen werden stark durch die individuelle Reaktion auf Zuckerwasser beeinflußt. Bienen, die sehr empfindlich sind für Zuckerwasser, zeigen bei einer gegebenen Zuckerwasserbelohnung ein höheres Akquisitionsniveau und eine schwächere Extinktion als Bienen, die weniger empfindlich sind für Zuckerwasser. Genotyp und Sammelverhalten haben keinen zusätzlichen Einfluß auf das Lernen. Junge Stockbienen lernen schlechter als Sammlerinnen. Dies liegt zum einen daran, daß sie weniger empfindlich sind für Zuckerwasser. Zum anderen beeinflußt das Alter der Bienen die Beziehung zwischen Zuckerwasserreaktion und Lernverhalten in bislang ungeklärter Weise. Die Diskriminierung hängt von der Akquisition ab und ist somit indirekt abhängig von der Zuckerwasserreaktion. Der Genotyp beeinflußt die Diskriminierung in Sammlerinnen, jedoch nicht in jungen Stockbienen. Beim assoziativen Lernen wird zunächst die Antenne, dann die Proboscis mit der belohnenden Zuckerlösung berührt. Während die kurze Stimulation der Antenne kaum Einfluß auf das Lernverhalten hat, bestimmt die Zuckerwasserkonzentration, mit der die Proboscis stimuliert wird, in starkem Maße, wie gut gelernt und wie schnell vergessen wird. Die Diskriminierung ist am besten bei geringer Belohnung, d. h., wenn Antenne und Proboscis mit niedrig konzentriertem Zuckerwasser stimuliert werden.

Auch das nicht-assoziative Lernen ist in starkem Maße abhängig von der individuellen Reaktion auf Zuckerwasser. Bienen mit hoher Empfindlichkeit für Zuckerwasser habituieren langsamer und zeigen eine stärkere Sensitisierung als Bienen, die weniger empfindlich für Zuckerwasser sind. Die Konzentration des Zuckerwassers, das als habituierender oder

sensitisierender Stimulus verwendet wird, bestimmt ebenfalls in entscheidendem Maße den Verlauf von Habituation und Sensitisierung. Das Alter der Bienen beeinflußt die Habituation aber nicht die Sensitisierung. Die Empfindlichkeit für Zuckerwasser kann durch elektrophysiologische Ableitungen am Proboscis-Muskel 17 (M17) exakt bestimmt werden. Wird die Biene an der Antenne mit verschiedenen Zuckerwasserkonzentrationen gereizt, so ändert sich mit der Zuckerwasserkonzentration auch die Aktivität des M17. Selbst wenn die Biene auf alle gebotenen Konzentrationen mit dem Rüsselreflex reagiert, so zeigt sie doch eine konzentrationsabhängige M17-Aktivität.

Die individuelle Zuckerwasserreaktion kann kurzfristig durch biogene Amine moduliert werden. Die Injektion von Oktopamin oder Tyramin erhöht reversibel und dosisabhängig die Zuckerwasserreaktion, während die Injektion von Dopamin oder ADTN die Reaktion auf Zuckerwasser dosisabhängig und reversibel erniedrigt. Zuckerwasserreaktion, Sättigungsgrad und PKA-Aktivität im Antennallobus stehen in engem Zusammenhang. Bienen mit hoher Zuckerwasserreaktion zeigen eine höhere PKA-Aktivität als Bienen mit niedriger Reaktion auf Zuckerwasser. Dreißig Minuten nach dem Füttern ist die PKA-Aktivität höher als 90 Minuten nach dem Füttern.

Diese Befunde zeigen, daß die individuelle Zuckerwasserreaktion ein ausgezeichneter Indikator für den physiologischen Zustand einer Biene ist und einen großen Teil an individuellen Lernunterschieden erklären kann, sowohl im assoziativen Lernen als auch im nicht-assoziativen Lernen. Die neuronalen Mechanismen der Regulation der Zuckerwasserreaktion sind noch nicht aufgeklärt, aber PKA-Aktivität im Antennallobus und biogene Amine sind offenbar an der Regulation der Zuckerwasserreaktion beteiligt.

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# Formulae

(1) Discrimination index (DI):

$$DI = \frac{(Ext\ CS+) - (Ext\ CS-)}{(Ext\ CS+) + (Ext\ CS-)}$$

Ext CS+ extinction scores of conditioned responses

Ext CS- extinction scores of responses to the alternative stimulus.

(2) Modulation index (MI):

$$MI\ (t) = \frac{GRS_t - GRS_0}{GRS_t + GRS_0}$$

0 = initial time

t = time relapsed since feeding.

GRS = gustatory response scores

# Abbreviations

ADTN: 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide

cAMP: cyclic adenosine monophosphate

cGMP: cyclic guanosine monophosphate

CES: central excitatory state

CS: conditioned stimulus (general)

CS+: conditioned pattern or odour used in experiments presented here

CS-: alternative test pattern or test odour used in experiments presented here

DI: discrimination index

GRS: gustatory response score

JH: juvenile hormone

M17: muscle 17

MI: modulation index

PER: proboscis extension response

PKA: cAMP-dependent protein kinase

PKG: cGMP-dependent protein kinase

QTL: quantitative trait locus

UR: unconditioned response

US: unconditioned stimulus

VUM neuron: ventral unpaired medium neuron

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# 1 Introduction

## *1.1 General introduction*

Honey bees have fascinated generations of scientists not only with their flexible division of labour in colonies of thousands of individuals but also with their great behavioural plasticity and their learning abilities. The different behavioural decisions of honey bees are very complex and only slowly are we beginning to understand some of them. In all the years, little attention has been paid to individual differences of honey bees, particularly in laboratory experiments. Recently, however, it was suggested that these differences can be a very useful tool for the analysis and understanding of complex relationships.

This work will focus on differences in sucrose responsiveness and their effects on various forms of associative and non-associative learning. The individuals trained differ with respect to genotype, foraging behaviour and age. A method for determining sucrose responsiveness by muscle recordings will be presented and artificial modulation of individual sucrose responsiveness by biogenic amines will be demonstrated. Finally, the relationship between sucrose responsiveness and the activity of a protein kinase which is involved in the formation of memory will be shown.

## *1.2 The honey bee as laboratory animal*

Honey bees have caught the attention of researchers for centuries for many reasons. They are fast and elegant flyers and learn quickly how to locate and handle flowers of different colours, shapes and odours. They also demonstrate great plasticity of behaviour in laboratory experiments, which have the advantage of offering controllable conditions. Honey bees are comparatively easy to train to stimuli of a variety of modalities (for a review see Menzel and Müller 1996). In a colony, honey bees demonstrate an extremely flexible system of division of labour (Winston 1987, Seeley 1995). This plasticity of behaviour and the underlying neuronal mechanisms are of particular interest to the neurobiologist. The aim of this work is to combine different aspects of the division of labour and of associative and non-associative learning behaviour in honey bees by individual sucrose responsiveness.

## *1.3 Age-dependent division of labour in a honey-bee colony*

Honey bees show a distinctive division of labour throughout their life time, which has been hypothesised to lead to greater ergonomic efficiency (Oster and Wilson 1978). One form

of division of labour is shown by the three different groups of sterile workers, drones and the queen. A second aspect of division of labour in honey bees takes the form of temporal polyethism, where individuals switch tasks depending on their ages. Very young bees work in the centre of the nest, cleaning and repairing cells, tending brood or the queen, receiving incoming nectar or building new combs. Older bees work in the periphery of the nest, guarding the entrance or removing dead corpses from the hive as so-called "undertakers". At the age of about three weeks, bees begin their scouting and then foraging activities outside the nest (Winston 1987, Seeley 1995). But not all of the bees performing different jobs are of different ages. The third form of division of labour becomes apparent in same-aged bees. Whereas some preforaging bees guard the hive entrance, other bees of the same age act as undertakers. Some foragers, for example, only collect pollen, others solely forage for nectar (Fewell and Page 2000). Many bees, however, collect both pollen and nectar. A small number of bees collect propolis, and some bees forage for water. All of these bees are similarly-aged foragers (Seeley 1995).

Many researchers have been fascinated by this system of complex interactions between individuals and the plasticity of division of labour, which is continually adjusted to the colony requirements and environmental conditions, and several theories aim to explain the division of labour in honey-bee colonies. The "foraging-for-work" model of Tofts and Franks (1992) explains the decision of individuals to perform a certain task solely by their location. If a honey bee is in the location where a certain task is required, it will perform that task. As young bees live closer in the centre of the nest, they will tend the brood and clean the cells. Older bees move to the periphery and act as guards or foragers. There is no developmental component in age polyethism according to this theory. Only the time spent in a colony decides which task is performed by an individual. However, this theory received direct experimental contradiction by Calderone (1995) and Calderone and Page (1996) who clearly showed that age polyethism is coupled with a developmental process. Bees of different ages performed different tasks, even when they were introduced to the same colony at the same time.

The activator-inhibitor model (Huang and Robinson 1992) assumes that behavioural development is speeded by an activator or delayed by an inhibitor. The hypothesised activator is juvenile hormone (JH), because JH titres have repeatedly been shown to correlate with the tasks bees perform (Robinson et al. 1989, 1992, Huang et al. 1991). However, a decisive experiment by Sullivan et al. (2000) recently demonstrated that even in bees without JH, behavioural development was speeded when older bees were removed from the colony, so

that JH is not likely to be the hypothesised activator. The inhibitors are supposed to be produced by older individuals to delay behavioural development in younger bees (Huang and Robinson 1992). The only inhibitory substance acting on behavioural development found so far is queen mandibular pheromone, which can delay foraging behaviour (Pankiw et al. 1998). But this substance is only produced by the queen.

The most generally recognised model of age-dependent division of labour has so far been the response-threshold model (Page and Robinson 1991, Robinson 1992). The idea behind this theory is that each individual has response thresholds for certain stimuli which are associated with certain tasks and, therefore, elicit specific behaviour. Response thresholds to stimuli change with age so that an older bee has a different probability of performing a certain task than does a younger bee. An example of response-threshold regulated behaviour is colony defence. Sensitivity to alarm pheromone increases with age (Robinson 1987a), and older bees were shown to have a higher probability of defending a colony than younger bees (Breed et al. 1990).

## *1.4 Division of foraging labour*

Foraging behaviour demonstrates an even finer division of labour among sub-groups of honey bees. Bees start to forage when they are about two to three weeks old and collect pollen, nectar, water or propolis. Whereas foraging for water and propolis has been studied relatively little, foraging for pollen and nectar has received more attention. A most interesting question with regard to the division of labour is how bees "decide" whether to collect pollen or nectar. Several parameters have been shown to increase or decrease pollen foraging activity in a colony. Adding pollen to a colony, for example, reduces pollen foraging activity (Fewell and Winston 1992, Dreller 1999). An increase in pollen foraging activity is achieved by increasing the amount of brood in the colony (Dreller 1999), by adding brood pheromone (Pankiw et al. 1998) or by increasing the space for cells in the colony (Fewell and Winston 1992, Dreller et al. 1999). Besides these environmental effects, the decision to forage for pollen or nectar also has a strong genotypic aspect. Fewell and Page (1993) found different probabilities to collect pollen or nectar in foragers of three different colonies with unrelated queens. Two strains of honey bees which were selected for the amount of stored pollen over several generations significantly differed in their foraging behaviour (Hellmich et al. 1985, Calderone and Page 1988, Page and Fondrk 1995, Page et al. 1998, Fewell and Page 2000). Bees of the "high-pollen-hoarding" strain had a higher probability of collecting pollen than

bees of the "low-pollen-hoarding" strain, which collected more nectar. Recently, bees of these genetic lines were also shown to differ systematically in their responses to gustatory stimuli (Page et al. 1998, Pankiw and Page 1999).

## *1.5 The role of sucrose stimuli*

In a natural context, the perception and evaluation of sucrose stimuli affects all sorts of behavioural decisions made by bees because sucrose is the main sugar in nectar (Beutler 1935). A feeder offering a highly-concentrated sucrose solution attracts more bees than a feeder with diluted sucrose solution (von Frisch 1927). Honey-bee foragers vary in their probability of performing a recruitment dance depending on the sucrose concentrations offered (von Frisch 1965, Raveret-Richter and Waddington 1993, Seeley 1995). The crop-load of a forager also depends on the concentration of the sucrose. The higher the sugar concentration of a nectar source, the greater is the load size of a forager (von Frisch 1965, Pflumm 1969, Schmid-Hempel et al. 1985, Nuñez and Giurfa 1996). The time between foraging bouts decreases with increasing sucrose concentration of a nectar source (Pflumm 1969). Crop emptying rates in foraging honey bees (Roces and Blatt 1999) and the transfer rate of sucrose solution during trophallaxis also depend on sucrose concentration (Tezze and Farina 1999).

But the different forms of behaviour are not only affected by the sucrose concentrations offered. Individual bees differ in their perception and evaluation of sucrose stimuli. Responsiveness to sucrose can be easily measured. If the antenna of a bee, its tarsae or its mouthparts are touched with a droplet of sucrose solution, the bee reflexively extends its proboscis – the proboscis extension response (PER) is performed (Kuwabara 1957). The individual sucrose response threshold was defined by Page et al. (1998) as the lowest sucrose concentration which a bee can discriminate from water. Minnich (1932) and Marshall (1935) were among the first to show that individuals differ in their behavioural responses to water and to sucrose. Using the proboscis extension paradigm, sucrose responsiveness of a great number of individuals can easily be measured. This measure, however, is rather rough, because it yields an "all-or-none" response. To measure accurately the intensity of proboscis extension, myograms of muscle 17 (M17, Snodgrass 1984), which is involved in all phases of the proboscis motor programme, i. e. extension, licking and retraction (Rehder 1987), appear very helpful. The number of M17 spikes has been shown to be an effective measure for the quantification of response strength of proboscis extension (Rehder 1987, Smith and Menzel

1989, Braun and Bicker 1992, Hammer 1993, Hammer et al. 1994). The aim of this work was to measure individual sucrose responsiveness not only behaviourally but also by means of recordings from M17. It is particularly interesting to analyse whether bees can differentiate between different sucrose concentrations above their individual response threshold, even though they might show proboscis extension in response to several sucrose concentrations (Experiment 7).

Unfortunately, the phenomenon of individual differences in sucrose responsiveness has received little attention since the first experiments by Minnich (1932) and Marshall (1935), although individual differences in sucrose responsiveness can have strong effects on behavioural parameters such as foraging behaviour (Page et al. 1998, Pankiw and Page 2000) or associative learning (Scheiner et al. 1999).

## *1.6 Honey-bee learning*

### 1.6.1 Associative learning

#### *1.6.1.1 General observations*

Associative learning is an essential component of the foraging behaviour and of the dance communication in honey bees. The memory of honey bees is well adapted to their learning tasks while foraging. Bees can, for example, learn to discriminate alternative colours after only one sucrose reward lasting for 100 ms (Erber 1975), a behaviour which seems to be well suited to foraging bouts, when an individual forager flies from flower to flower, receiving short rewards while visiting.

Under the controlled conditions of the laboratory, honey bees still show a very plastic behavioural repertoire and can be conditioned to various stimuli. In proboscis extension conditioning, an appetitive stimulus, such as sucrose solution, serves as an unconditioned stimulus (US), which is first applied to the antennae. If the sucrose solution is high enough, the bee reflexively extends its proboscis in expectation of food (Kuwabara 1957), which is the unconditioned response (UR). In a typical learning protocol, a neutral stimulus, such as an odour, is presented to the bee shortly before sucrose is applied to the antennae. If the bee shows proboscis extension, it is allowed to drink a small volume of sucrose solution as reward. A few pairings of the reward and the odour, which has become the conditioned stimulus (CS), suffice for the bee to form associations. When the animal perceives the odour the next time, it will respond to it by proboscis extension – the conditioned response (Erber 1980, Bitterman et al. 1983). Instead of odours, tactile cues can serve as conditioned stimuli.

Regardless of the stimuli involved, an important requirement for associative learning is the contiguity between CS and US. Only when the CS shortly precedes the US will learning be successful in the honey bee (Menzel et al. 1993). Bees have excellent learning capacities for visual stimuli, for odours and for tactile stimuli.

#### *1.6.1.2 Visual learning*

Visual stimuli play a major part in the orientation of foragers, guards and scouts. Visual learning abilities have been studied extensively, demonstrating that honey bees are capable of learning and discriminating a great variety of different visual forms and patterns (Wehner 1967, Horridge 1994, Horridge and Zhang 1995, Lehrer et al. 1995, Ronacher 1998, Giurfa et al. 1999, Horridge 2000) and of different colours (Menzel 1967, 1968, Meineke 1978, Kevan and Giurfa 1996). Discrimination depends on the specific visual stimuli. Even in the laboratory, associative visual learning in the honey bee was demonstrated (Erber and Schildberger 1980).

#### *1.6.1.3 Olfactory learning*

Odours are experienced by honey bees throughout their lifetime and facilitate orientation and communication within the hive and the orientation and allocation of foraging patches and nest sites. Olfactory learning and memory has been extensively studied by many groups (for a review see Menzel and Müller 1996). Classical olfactory conditioning has not only been used to study the nature of learning and memory formation but also as a window into the molecular mechanisms underlying learning and memory formation. Honey bees easily learn to associate an odour with a sucrose reward (Erber 1980, Bitterman et al. 1983). A single pairing of an odour and a reward can induce a stable memory for several days (Menzel 1999). Three pairings of odour and reward often result in a life-long memory of that odour (Menzel 1999). Conditioning bees to odours in the laboratory is a useful paradigm to compare the associative learning performance of individual bees. The increasing number of conditioned responses during the acquisition phase and the extinction of memory, which can be measured in unrewarded tests, are good indicators of the learning performance of individuals. In addition, olfactory discrimination can be easily tested by comparing responses to a conditioned odour with those to a novel odour. Classical olfactory conditioning was employed in this study to analyse the role of sucrose responsiveness on learning in different groups of bees (Experiments 2 and 3).

#### *1.6.1.4 Tactile learning*

As honey bees spend a large part of their life in the dark hive, they are likely to extensively use tactile cues for orientation and communication. Honey-bee workers have the capability of discriminating fine details of texture which they use in building comb and preparing the insides of cells (Martin and Lindauer 1966). Foragers use tactile cues to discriminate between different flowers and between different parts of the same flower to find their way to nectar and pollen (Kevan and Lane 1985, Kevan 1987). For these purposes, the honey-bee antennae are densely covered with mechanosensory sensilla in addition to olfactory and contact-chemosensory sensilla (Esslen and Kaissling 1976, Kevan 1987). Honey bees scan objects within the range of their antennae with frequent, short-lasting antennal contacts (Erber et al. 1993b). The nature of these antennal scanning movements has been carefully analysed by Pribbenow and Erber (1996). The ability of bees to learn and discriminate tactile surface structures of flower petals was demonstrated by Kevan and Lane (1985), their ability to learn and discriminate different surface structures was shown by Martin (1965) and Mühlen (1987). Honey bees learn easily the surface of tactile objects which are associated with a sucrose reward (Erber et al. 1998, Scheiner et al. 1999) and can distinguish between objects presented to the right or left antenna (Erber et al. 1997, Scheiner et al. 2001). They can discriminate between different sizes of objects, different forms and different surface structures (Erber et al. 1998). Under free-flying conditions, tactile learning is slower than olfactory learning. In the laboratory, tactile learning is as effective as olfactory learning (Erber et al. 1998). In addition, bees learn the location of a tactile object within the range of their antennae even without receiving a reward (Erber et al. 1997, Erber et al. 2000). Bees can also be conditioned operantly to associate antennal motor activity and positional information with sucrose rewards (Kisch and Erber 1999). And not only the antennae can be conditioned operantly. It is possible to condition the activity of a single antennal muscle operantly. This muscle is innervated by a single motoneuron whose action potentials correlate 1:1 with the muscle potentials (Erber et al. 2000). These studies show the usefulness of tactile learning protocols not only for behavioural analyses but also for the study of the neuronal mechanisms underlying these forms of learning.

Tactile learning is a form of operant conditioning, which differs from classical olfactory learning in several parameters (see below). It offers the opportunity to analyse the acquisition of tactile stimuli, the extinction of conditioned responses in unrewarded tests and the discrimination between different tactile patterns. To study general effects of sucrose responsiveness on associative learning it seems suitable to use different learning paradigms.

In this work, the tactile conditioning paradigm of Erber et al. (1998) was employed for several learning experiments studying the effects of differences in sucrose responsiveness (Experiments 1, 2 and 3) and of different stimulation sites (Experiment 4) on associative learning. Results were compared with classical olfactory conditioning (Experiments 2 and 3).

#### *1.6.1.5 Classical or operant conditioning?*

Olfactory conditioning in restrained honey bees, where an odour is blown at the antennae of the bee and paired with a sucrose reward, is generally regarded as a form of classical conditioning (Kuwabara 1957, Bitterman et al. 1983, Menzel et al. 1991). The only operant component in this paradigm is the proboscis extension of the bee in expectation of food and the licking of the sucrose solution.

Tactile conditioning in restrained honey bees, in contrast, requires more operant action on part of the animal. In this paradigm, the bee is not only required to extend its proboscis in expectation of the sucrose reward. It also has to actively scan the tactile stimulus, such as a small plate with a certain grating, in order to learn its structure and to discriminate it from an alternative tactile stimulus (Erber et al. 1997, 1998).

Unfortunately, invertebrate literature lacks a consensus of researchers on definitions for many different learning phenomena, and popular olfactory conditioning in *Drosophila*, for example, was considered to represent as many as three different forms of learning, including classical and operant learning (Abramson 1997). According to the criteria of Gormezano and Kehoe (1975), which are based on the nature of the conditioned response, both the typical olfactory learning paradigm for honey bees (Bitterman et al. 1983) and the tactile learning paradigm for honey bees (Erber et al. 1998) are regarded as different forms of classical conditioning. In their definition, olfactory conditioning involves a "conditioned stimulus – conditioned response" relationship, were the conditioned stimulus does not elicit the unconditioned response prior to training, and the conditioned response emerges from the same effector system as the unconditioned response. Tactile conditioning, in contrast, shows an "instrumental approach behaviour". Some approach to the conditioned stimulus (scanning of tactile pattern) is necessary to receive the unconditioned stimulus (Gormezano and Kehoe 1975).

In this work, olfactory learning will be regarded as a form of classical conditioning, because this has been generally recognised (Bitterman et al. 1983, Menzel et al. 1990). Tactile conditioning, in my view, displays a form of operant conditioning, because many more operant components are involved in this learning paradigm than are involved in olfactory

conditioning. So far, there has been no classification to organise the various procedures used to measure associative learning in invertebrates, based on training variables such as stimulus intensity, inter-stimulus interval and pattern of reinforcement (Abramson 1997). Therefore, two different forms of learning such as tactile and olfactory learning must not be compared directly. Nevertheless, some general observations on the responses of the bees in those learning paradigms can help to identify general mechanisms underlying associative learning.

#### *1.6.1.6 Learning of pollen and nectar foragers*

Pollen and nectar foragers do not only differ in their foraging behaviour. When individuals of both behavioural groups were conditioned to tactile patterns (Scheiner et al. 1999), pollen foragers learned the tactile stimuli better than nectar foragers. They reached a higher level of acquisition and showed stronger resistance to extinction than nectar foragers. The reason for this behavioural difference is that pollen foragers were more responsive to sucrose than nectar foragers, and bees with high sucrose responsiveness learned better than bees with low sucrose responsiveness.

For the experiments in this work two strains of honey bees that differ genetically in their foraging behaviour were used. The “high- ”and “low-pollen-hoarding” strain bees of Page and Fondrk (1995) were selected over 14 generations for the amount of pollen they store in the colony. High-strain bees mainly collect pollen. Low-strain bees mainly collect nectar. In addition, the two strains systematically differ in their sucrose responsiveness. High-strain bees are more responsive to sucrose than low-strain bees (Page et al. 1998). The interesting questions were a) whether high-strain bees would show better acquisition and less extinction than low-strain bees because they are more responsive to sucrose, b) whether the relationship between sucrose responsiveness and several learning parameters would be the same for the two strains, c) whether pollen and non-pollen foragers in each strain would differ in their sucrose responsiveness and learning similar to wild-type foragers and d) whether genotype has separate effects on learning performance (Experiment 1).

As individual sucrose responsiveness is affected by the foraging behaviour of the bees (Pankiw et al. 2001), sucrose responsiveness and tactile learning was tested in young bees that had presumably not begun to forage yet and were therefore called “preforagers”. For this experiment too, high- and low-strain bees were used. Preforagers of both strains with known sucrose responsiveness were trained to tactile patterns and to odours to see whether the same rules which apply to operant tactile learning also apply to classical olfactory conditioning (Experiment 2). To further compare the relationship between sucrose responsiveness and

learning between classical olfactory conditioning and operant tactile conditioning, wild-type pollen and non-pollen foragers were conditioned using both paradigms (Experiment 3).

#### *1.6.1.7 Different stimulation sites*

When bees forage for nectar or approach an artificial sucrose feeder, they first contact the nectar or sucrose solution with their antennae and then with the proboscis, before they "decide" whether to collect that material or not. It is unclear whether the antennal sucrose input or the proboscis input has more weight on the "decision" to learn a certain floral source or feeding site. Experiment 4 was designed to measure the behavioural role of antennal and proboscis sucrose input in associative tactile PER learning. Associative tactile learning was studied in the form that antennae and proboscis were always stimulated with sucrose, but the antennae were sometimes stimulated with a different sucrose concentration than the proboscis.

#### *1.6.1.8 Learning and reversal learning*

Under natural conditions, a pollen or nectar forager must be able to switch between different food sources when appropriate, and indeed, reversal learning has often been observed in the honey bee (von Frisch 1967, Menzel 1969, Meinecke 1978, Ben-Shahar et al. 2000, Chandra et al. 2000). Reversal learning assays can be very helpful for the comparison of different groups of bees. When differences in learning behaviour are very small in the initial learning phase, they often become more pronounced during reversal learning (Scheiner et al. 1999, Chandra et al. 2000). For that reason, pollen and non-pollen foragers of a wild-type colony were studied for learning and reversal learning of tactile and olfactory stimuli (Experiment 3).

### 1.6.2 Non-associative learning

Non-associative forms of learning change the behaviour of an animal as a result of the exposure to a stimulus. If the response decreases as a result of repeated stimulation, the animal may have habituated rather than undergone motor fatigue or sensory adaptation (Carew 1987, Menzel et al. 1991, Kandel et al. 1996). Honey bees, for example, show habituation of the PER when their antennae are repeatedly stimulated with a low sucrose concentration while the bees are not allowed to lick sucrose (Erber 1980, Braun and Bicker 1992). Even long-term habituation of the proboscis extension response has been demonstrated in the honey bee (Bicker and Hähnlein 1994). On the other hand, a strong or very salient

stimulus may enhance responsiveness to a certain stimulus (Carew 1987, Dudai 1989, Menzel et al. 1991, Kandel et al. 1996). The honey bee can be sensitised by antennal stimulation with a high sucrose concentration. As a result, the responsiveness of the bee to a following odour or visual stimulus is enhanced (Erber 1981, 1984).

Individual sucrose responsiveness was shown to strongly affect associative tactile PER learning (Scheiner et al. 1999). As associative and non-associative learning share many similarities (Hawkins and Kandel 1984), it is conceivable that individual sucrose responsiveness also affects non-associative learning. Experiment 5 analysed the effect of individual sucrose responsiveness on the degree of habituation and sensitisation to sucrose stimuli. In addition, the effects of different sucrose concentrations used as habituating or sensitising stimuli were studied. The effect of age on non-associative habituation and sensitisation was tested in young wild-type preforagers of different ages (Experiment 6).

## *1.7 Biogenic amines*

In the honey bee, biogenic amines act as transmitters, neurohormones or neuromodulators and affect sensory mechanisms, central states and behavioural responses (Bicker 1999, Baumann et al. in press). The various biogenic amines have different effects on a wide range of behavioural responses. Octopamine injections into the input regions of the mushroom bodies, for example, facilitate olfactory conditioning and memory retrieval (Menzel et al. 1990), and injection of octopamine into the mushroom bodies or the antennal lobes can substitute for the sucrose reward in associative olfactory learning (Hammer and Menzel 1998). Octopamine also enhances nestmate recognition (Robinson et al. 1999). Generally, octopamine seems to have an arousing effect on the nervous system (Bicker and Menzel 1989, Braun and Bicker 1992, Erber et al. 1993a, Roeder 1999). The behavioural functions of tyramine, the metabolic precursor of octopamine, have not been studied as extensively as those of octopamine. But some experiments indicate that tyramine can enhance responsiveness like octopamine (Braun and Bicker 1992). The effects of dopamine are often very different and sometimes functionally antagonistic to those of octopamine. Local injections of dopamine into the median protocerebrum or into the central complex selectively inhibit the retrieval of learned information (Menzel et al. 1991), although they do not block acquisition. Dopamine can also interfere with the processing of olfactory cues or the motor programme of proboscis extension (Menzel et al. 1990). 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (ADTN) shows a high affinity to dopamine-sensitive

binding sites in membrane preparations of bee brains (Blenau et al. 1995) and to a heterologously expressed honey-bee dopamine receptor (AmDOP1, Blenau et al. 1998). Like dopamine, ADTN can stimulate cAMP synthesis (Blenau et al. 1998). Behavioural effects of ADTN are little studied (Blenau and Erber 1998). The effects of octopamine, tyramine, dopamine and ADTN on sucrose responsiveness were therefore studied in Experiment 8 of this work.

## *1.8 Sucrose responsiveness, PKA and learning*

In the last few years there has been increasing evidence that changes in synaptic connections are the neural substrate for behaviourally-induced plasticity (Milner et al. 1998). In this context, different protein kinases have been shown to act as mediators of phosphorylation processes. In honey bees, cAMP-dependent protein kinase (protein kinase A, PKA) plays a pivotal role in the formation of long-term memory (Fiala et al. 1999, Müller 2000). A primary candidate for a molecular substrate of learning is the cAMP cascade (Hammer 1997). Consistent with this hypothesis, octopamine, cAMP and sucrose rewards activate PKA (Hildebrandt and Müller 1995). So far, sucrose responsiveness and its modulation have mainly been analysed in behavioural assays. To understand the underlying mechanisms and the signalling cascades involved in physiological properties of sucrose responsiveness and its modulation, it is necessary to find biochemical correlates. As sucrose responsiveness is affected by octopamine (see 3.14.1), and PKA activity can also be modulated by octopamine (Hildebrandt and Müller 1995), the hypothesis was that the mechanisms regulating sucrose responsiveness and PKA activity might be related. This work analysed the activity of PKA in bees with different sucrose responsiveness (Experiment 9).

# 2 Materials and Methods

## *2.1 Experiment 1 Sucrose responsiveness and tactile learning in high- and low-strain foragers*

### 2.1.1 Intention

This experiment was conducted to analyse the relationship between sucrose responsiveness and associative tactile learning in foragers of the high and low strains. Bees of these genetic strains differ systematically in their sucrose responsiveness. High-strain bees are more responsive to water and sucrose than low-strain bees (Page et al. 1998). Therefore, high-strain bees should show a better learning performance than low-strain bees, because bees with high sucrose responsiveness show better acquisition and less extinction than bees with low sucrose responsiveness (Scheiner et al. 1999). If there are learning differences between the strains, it can be determined if these differences are due solely to differences in sucrose responsiveness, or if there are other genetic differences that also affect learning performance.

A second aim of this experiment was to compare pollen and non-pollen foragers within each strain with respect to discrimination and the relationship between sucrose responsiveness and different tactile learning parameters. It is known that pollen foragers are generally more responsive to water and sucrose (Page et al. 1998) and that they perform better in tactile learning assays (Scheiner et al. 1999). Therefore, pollen foragers should show a better acquisition than non-pollen foragers, regardless of genotype. With this experiment the effect of genotype and foraging role on learning performance can be partitioned.

### 2.1.2 Preparation of the bees

These experiments were conducted in the autumn of 1998 at the University of California, Davis. Honey bees were derived from the “high”- and “low”-pollen-hoarding strains of Page and Fondrk (1995). These strains were selected for the amount of pollen stored by colonies. When raised in a common colony, foragers of the high strain are more likely to collect pollen, while those of the low strain tend to return with nectar. The bees were derived from a single high-pollen-strain queen and a single low-pollen-strain queen. The queens were instrumentally inseminated with one drone each. Frames with pupae were removed from their colonies and placed in an incubator maintained at 34 °C. Newly emerged adults were removed daily and marked with different coloured paints (Enamel, Testors, Rockford, USA). They were then introduced into a common, full-sized host colony, which contained a naturally

mated queen. The hive containing high- and low-strain bees was located in an outdoor flight cage. Pollen and non-pollen foragers were collected at artificial feeding sites offering pollen, 30 % or 40 % sucrose solution. Bees were placed in small glass vials and soon cooled in a refrigerator maintained at 5 °C until they showed first signs of immobility. They were then mounted in brass tubes (Figure 1) with a strip of adhesive tape attached between head and thorax and another one over the abdomen as described in Erber et al. (1998). Subsequently, the eyes of the bees were occluded with black paint (Lucas acrylic paint) to block visual inputs. The bees were then fed with a droplet of 40 % sucrose solution, which approximately amounts to 5 µl. The experiments started after a one-hour interval to ensure that all the bees were in a similar physiological condition.

### 2.1.3 Measuring of sucrose responsiveness

At the start of the behavioural experiments, sucrose responsiveness of individuals was tested. All the bees were tested for their proboscis extension response (PER) to antennal stimulation with the following sucrose concentrations: 1 %, 1.6 %, 2.5 %, 4 %, 6.3 %, 10 %, 16 %, 25 % and 40 % (weight/volume), which corresponds to a logarithmic series of approximately 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6. The sucrose concentrations were presented in ascending order, because the order of sucrose presentations does not affect sucrose responsiveness (see Experiment 7). Prior to each sucrose stimulation, all bees were stimulated with water to control for sensitisation. For each bee it was recorded whether it responded to the stimulation with water or increasing sucrose concentrations with proboscis extension. The inter-trial interval was 2 min. The total number of proboscis responses to the first water and the 9 sucrose stimulations is the "gustatory response score (GRS)" of a bee. This gustatory response score is an estimate of the individual sucrose response threshold, because bees normally begin responding when a concentration of sucrose offered exceeds their threshold and then continue to respond to all subsequent increasing sucrose concentrations.

### 2.1.4 Conditioning

For tactile conditioning (Figure 1), small rectangular copper plates (3 × 4 mm) were used. In these plates either vertical grooves (conditioned stimulus, CS+) or horizontal grooves (alternative test stimulus, CS-) were engraved (wave length of grooves: 450 µm, width of the grooves: 150 - 190 µm, depth of the grooves 30 - 40 µm). For details see Erber et al. (1998).


**Figure 1 Tactile conditioning. A: The mounted bee scans the tactile pattern with its antennae for 3 s. No proboscis extension occurs. B: Proboscis extension is elicited by applying a droplet of sucrose solution to the antennae. Subsequently, the bee can drink from that sucrose solution for 1 s. C: When the bee has learned the pattern, it shows proboscis extension when scanning the tactile pattern.**

At the start of the learning experiments, all bees were tested for their spontaneous responses to the tactile targets. Whenever a bee responded spontaneously to either pattern, it was excluded from the experiment. The number of spontaneous responses was very small and was not statistically analysed. The acquisition phase consisted of 10 trials (Figure 2). In each trial the CS+ (vertical pattern) was presented for 3 s before proboscis extension was elicited by applying a droplet of 30 % sucrose solution to either antenna, and the bee was allowed to drink from that sucrose droplet for about 1 s. This amounts to approximately 0.2 µl sucrose solution for each bee. As all bees were given the same time for drinking the sucrose solution, it is assumed that all the bees received the same volume of reward.

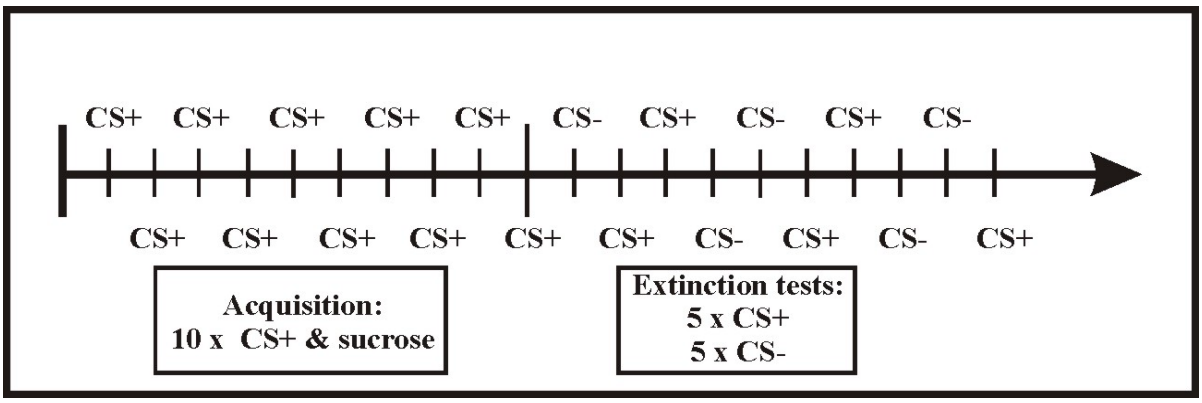


**Figure 2 Sequence of acquisition and extinction trials in Experiment 1. After ten acquisition trials in which the conditioned stimulus (CS+) was presented with a sucrose reward, extinction of conditioned responses and of responses to the alternative stimulus (CS-) was measured in 5 unrewarded tests for each stimulus. During extinction, the test stimuli were presented in pseudo-randomised order.**

Conditioned proboscis extensions were recorded. The "acquisition score" of a bee is defined as the total number of conditioned responses. The conditioned responses were tested after each of the 10 conditioning trials. After the acquisition phase, extinction of conditioned responses to the CS+ and responses to the alternative stimulus, CS- (pattern with horizontal

grating) were measured in 5 unrewarded tests for each stimulus. Bees that discriminated well did not respond to the alternative tactile stimulus. The test stimuli were applied in pseudo-randomised order (Figure 2). The "extinction scores" for CS+ or for CS- are defined as the total number of responses to the CS+ and to the CS-, respectively. The inter-trial interval was 5 min throughout the experiment. If a bee touched the tactile target with its proboscis, the plate was subsequently cleaned with 70 % ethanol and water. To further avoid contamination of the plates with olfactory or gustatory stimuli from the antenna of a bee, the plate with the vertical grating used in the extinction tests was different from that used in the conditioning trials but had the same pattern.

### 2.1.5 Statistics

For graphic display, sucrose concentrations were transformed to their $Log_{10}$ values. To compare the sucrose-concentration response curves of different groups, linear regression functions ($f(x) = a + b * x$) of the arcsine-square root transformed proportion of bees showing proboscis extension on the different sucrose concentrations were calculated (Sigma Plot 2000). The slope coefficients and intercepts of these functions were compared using two-tailed t-tests or two-tailed Welch's t-tests. Sucrose responsiveness as measured by gustatory response scores was compared between groups using two-tailed Mann-Whitney U-tests. For the acquisition and extinction curves bees were grouped according to their gustatory response scores. Some of the groups were only represented by few individuals. Therefore, bees with certain gustatory response scores were pooled: GRS ≤ 4: low sucrose responsiveness, GRS 5-8: intermediate sucrose responsiveness, GRS ≥ 9: bees with high sucrose responsiveness. To analyse the relationship between gustatory response scores and acquisition scores, between gustatory response scores and extinction CS+ or extinction CS- scores and between acquisition scores and extinction CS+ or extinction CS- scores, Spearman rank correlations were calculated (SPSS 9.0). To compare these relationships between different groups, linear regressions ($f(x) = a + b * x$) were calculated (Table 1, SPSS 9.0). The slope coefficients of these regressions are indicators of the rates of change in the different relationships. The corresponding intercepts demonstrate the initial response level. The slope coefficients and the intercepts of these regressions were compared between different groups using two-tailed t-tests or two-tailed Welch's t-tests. Only when the intercepts of two groups did not differ from zero were they not compared.

| regression | slope coefficients | intercept |
|---|---|---|
| of the proportion of bees showing proboscis extension on the different sucrose concentrations | rate of increase in responsiveness with increasing sucrose concentrations | initial level of responsiveness |
| of acquisition scores on gustatory response scores | rate of increase in acquisition scores with increasing sucrose responsiveness | initial level of acquisition |
| of extinction CS+ scores in gustatory response scores | rate of increase in conditioned responses with increasing sucrose responsiveness | initial level of extinction CS+ |
| of extinction CS- scores on gustatory response scores | rate of increase in extinction CS- scores with increasing sucrose responsiveness | initial level of extinction CS- |
| of extinction CS+ scores on acquisition scores | rate of increase in extinction CS+ scores with increasing acquisition scores | initial level of extinction CS+ |
| of extinction CS- scores on acquisition scores | rate of increase in extinction CS- scores with increasing acquisition scores | initial level of extinction CS- |

**Table 1 Different regressions used to compare different groups of bees and their relationships between sucrose responsiveness, acquisition and extinction.**

Discrimination within groups was tested by comparing extinction CS+ scores with extinction CS- scores using two-tailed Wilcoxon-tests. Bees which responded more often to the conditioned stimulus than to the alternative stimulus showed discrimination. To compare discrimination of different groups, discrimination indices (DI) were calculated as follows and compared between different groups using two-tailed Mann-Whitney U-tests.

$$DI = \frac{(Ext\ CS+) - (Ext\ CS-)}{(Ext\ CS+) + (Ext\ CS-)}$$

where DI = discrimination index

Ext CS+ = extinction CS+ scores

Ext CS- = extinction CS- scores.

## *2.2 Experiment 2: Sucrose responsiveness, and tactile and olfactory learning in high- and low-strain preforagers*

### 2.2.1 Intention

This series of experiments was designed to determine the relationship between sucrose responsiveness and different learning parameters in young bees of the high and low strains prior to them becoming foragers. Two learning paradigms were used: operant tactile learning and classical olfactory learning. Young hive bees of the two genetic strains differ in their sucrose responsiveness (Pankiw and Page 1999). They are important to study, because their sucrose responsiveness and learning behaviour are not confounded by foraging behaviour,

providing a test of the relationships of genotype and sucrose responsiveness that is independent of foraging experience. This experiment also gave the opportunity to compare operant tactile and classical olfactory learning with regard to the relationship between sucrose responsiveness and different learning parameters.

### 2.2.2 Preparation of the bees

The experiments were conducted at the University of California, Davis, in the summer of 1999. The high- and low-pollen-hoarding strains of Page and Fondrk (1995) were used. This time, preforaging bees of the two genetic strains were used, because their sucrose responsiveness was not affected by foraging behaviour. The bees were derived from the same sources as those used in Experiment 1. Newly emerged high- and low-strain bees were marked with paint and introduced into a commercial colony. Bees were captured between 6 and 12 days of age, individually mounted in small holders, and prepared for the learning assays as described for Experiment 1. Individual responsiveness to sucrose was then measured and compared as described in Experiment 1.

### 2.2.3 Conditioning

For the learning experiment, bees were chosen on the basis of their gustatory response scores to generate a broad distribution of gustatory response scores for regression analyses. The learning protocol for tactile learning was the same as that described for Experiment 1. For olfactory learning, citral was used as CS+ and carnation oil as CS-. Each bee was placed in front of a tube of 0.5 cm diameter, through which a constant airstream was blown at its antennae. The odours were added to the airstream by opening a valve of a channel that contained a piece of cellulose soaked with either 2 µl of citral or 4 µl of carnation oil. In a conditioning trial, the valve was opened for 3 s. During the last second, the sucrose stimulus was applied first to the antennae and then to the proboscis. While the bee was licking the sucrose it could still smell the odour. Conditioning to odours basically followed the same paradigm as tactile conditioning, which was described for Experiment 1. All bees were tested for their spontaneous responses to citral (CS+) and carnation (CS-) at the start of the learning experiments. Whenever a bee responded spontaneously to either odour, it was excluded from the experiment. The number of spontaneous responses was very small and was not statistically analysed. During acquisition, citral was blown at the antennae of a bee and paired with the sucrose reward as described for tactile learning (Experiment 1). After the acquisition

phase, extinction and responses to the alternative odour carnation were measured similar to Experiment 1 (Figure 2).

### 2.2.4 Statistics

Basically, the same statistics were applied as described for Experiment 1 (see 2.1.5). Individual data points on the extinction curves were compared between the strains using two-tailed Fisher-Exact-tests.

## *2.3 Experiment 3: Sucrose responsiveness, and tactile and olfactory learning in wild-type foragers*

### 2.3.1 Intention

This series of experiments was designed to analyse the relationship between sucrose responsiveness and different learning parameters in wild-type foragers. Pollen and non-pollen foragers were either operantly conditioned to tactile patterns or classically conditioned to odours. Pollen foragers are generally more responsive to water and sucrose than non-pollen foragers (Page et al. 1998). For that reason, they show better tactile acquisition than non-pollen foragers (Scheiner et al. 1999). The expectation for this experiment was that pollen and non-pollen foragers would differ in their sucrose responsiveness but not in their relationship between sucrose responsiveness and different learning parameters. Despite the different protocols, tactile learning shares important characteristics with olfactory learning. Therefore, in olfactory learning there should be a similar relationship between sucrose responsiveness and different learning parameters as in tactile learning. Although operant tactile learning must not be directly compared with classical olfactory learning, similar rules should apply. In addition, these experiments analysed the relationship between learning and reversal learning.

### 2.3.2 Preparation of the bees

This experiment was conducted in the spring of 1999 together with Marcus Barnert at the Technical University, Berlin, Germany. Bees were derived from a wild-type *Apis mellifera* colony. Returning foragers were individually collected at the hive entrance, which was temporarily blocked by a wire mash. Pollen foragers were identified by their loads of pollen attached to the hind legs. To ensure the same foraging role of the test bees, only returning pollen foragers with large pollen loads were taken, because they were less likely to also collect nectar (Fewell and Page 2000). Returning foragers without filled pollen baskets

were classified as non-pollen foragers. All bees were taken from the same colony. Bees were placed in small glass vials and soon cooled in a refrigerator maintained at 5°C until they showed first signs of immobility. They were then mounted as described for Experiment 1, and their eyes were occluded with black paint to block visual inputs. The bees were fed with one droplet of 30 % sucrose solution, which approximately amounts to 5 µl. The experiments started after a one-hour interval to ensure that all the bees had adapted to the new situation and were in a similar physiological condition.

### 2.3.3 Measuring of sucrose responsiveness

Sucrose responsiveness was tested as described for Experiment 1, apart from the fact that a different series of sucrose concentrations was used. This time, the following sucrose concentrations were offered in ascending order: 0.1 %, 0.3 %, 1 %, 3 %, 10 %, 30 %, (weight/volume), which approximately corresponds to the following logarithmic series of –1, -0.5, 0, 0.5, 1 and 1.5. As before, all the bees were stimulated with water before a sucrose stimulus was applied. For each bee it was recorded whether it responded to the stimulation with water or increasing sucrose concentrations with proboscis extension. The total number of responses is the gustatory response score of a bee (see 2.1.3).

### 2.3.4 Conditioning

Conditioning experiments started directly after measuring sucrose responsiveness. For tactile conditioning the same plates were used as those described for Experiment 1 (see 2.1.4). For olfactory conditioning the same odours were used as those described for Experiment 2 (see 2.2.3). Bees responding spontaneously to either pattern or either odour were excluded from the further experiment. The course of the acquisition phase and that of the extinction phase were the same as those described for Experiment 1. This time, however, the acquisition phase consisted of only 6 trials. Extinction was measured 5 times for each pattern or odour, as described before (Experiments 1 and 2). After the extinction tests, reversal learning was tested. This time, the bees were conditioned to the pattern or odour which had served as the alternative stimulus (CS-) in the first part of the experiment. Bees were conditioned 6 times and extinction was afterwards measured for each pattern/odour 5 times.

### 2.3.5 Statistics

Sucrose responsiveness was displayed and compared as described for Experiment 1 (see 2.1.5), but a different series of sucrose concentrations was used (see 2.3.3). For the acquisition and extinction curves, bees were grouped according to their gustatory response scores similar to Experiment 1. Because a different series of sucrose concentrations was used in Experiment 3, gustatory scores were pooled as follows: GRS ≤ 2: low sucrose responsiveness, GRS 3-4: intermediate sucrose responsiveness, GRS ≥ 5: high sucrose responsiveness. The relationships between sucrose responsiveness, acquisition, extinction CS+ and extinction CS- were analysed as described for Experiment 1 (Table 1). The relationships between gustatory response scores and reversal acquisition, reversal extinction CS+ and reversal extinction CS- were compared in the same way. Discrimination and the relationships between sucrose responsiveness, acquisition and extinction were analysed as described for Experiment 1 (see 2.1.5).

## *2.4 Experiment 4: The effect of stimulation site on tactile learning*

### 2.4.1 Intention

This experiment was conducted to analyse the effect of different sucrose concentrations offered to antennae and proboscis during associative tactile PER learning. When bees visit flowers, they usually first test the nectar with their antennae. If the quality of the nectar is sufficient, the bees then extend their probosces to imbibe the nectar and fill their crops. The assumption is that the bees learn to associate a certain flower type with the nectar reward. Bees were conditioned to a tactile pattern under laboratory conditions to test whether the short antennal stimulation or the longer proboscis stimulation with sucrose has a stronger effect on the tactile associative learning performance. Antennae and probosces were stimulated with different sucrose concentrations. Acquisition, extinction of conditioned responses and discrimination between the conditioned pattern and an alternative pattern were analysed.

### 2.4.2 Preparation of the bees

These experiments were conducted in the summer of 1998 at the Technical University Berlin, Germany. Bees were derived from a wild-type *Apis mellifera* colony. For the conditioning experiments only pollen foragers were used. They were identified, captured and mounted for conditioning as described for Experiment 3 (see 2.3.2).

### 2.4.3 Conditioning

To minimise differences in acquisition due to differences in sucrose responsiveness (Scheiner et al. 1999), only bees that extended their probosces in response to antennal stimulation with water were used for conditioning. For tactile conditioning, the same tactile plates and basically the same protocol as described for Experiment 1 (see 2.1.4) were used. However, this time bees were conditioned 5 times to the plate with the vertical pattern. Extinction of conditioned responses and responses to the alternative horizontal pattern were tested 3 times each. The inter-trial interval was always 5 min. During conditioning the concentrations of sucrose solutions applied to antennae and probosces were varied to analyse the influence of antennal and proboscis stimulation on associative learning. Table 2 shows the four groups of bees that were conditioned.

| experimental group | sucrose at antenna | sucrose at proboscis |
|---|---|---|
| 1 | 1.6 % | 1.6 % |
| 2 | 30 % | 1.6 % |
| 3 | 1.6 % | 30 % |
| 4 | 30 % | 30 % |

**Table 2: Summary of the groups of bees which were stimulated with different sucrose concentrations at antenna and proboscis in Experiment 4.**

### 2.4.4 Statistics

The percentage of bees showing the conditioned response was calculated for acquisition and extinction curves (Figure 24). The number of bees showing the conditioned response at the end of the conditioning trials was compared between the groups using two-tailed Fisher-Exact-tests. The relationship between extinction CS+ and acquisition scores was analysed using Spearman rank correlation coefficients. To compare the relationship between acquisition and extinction of different groups, linear regressions of extinction CS+ scores on acquisition scores were calculated (Table 1). The slope coefficients and intercepts were compared using two-tailed t-tests or Welch's t-tests. To test for discrimination, extinction CS+ scores were compared with extinction CS- scores using two-tailed Mann-Whitney U-tests. Discrimination indices (DI) were calculated for each group and compared as described for Experiment 1 (see 2.1.5). Linear regressions of extinction CS- scores on acquisition scores were also calculated and compared as described for Experiment 1 (Table 1).

## *2.5 Experiment 5: Sucrose responsiveness and non-associative learning with different sucrose stimuli*

### 2.5.1 Intention

This experiment was designed to analyse the role of individual sucrose responsiveness in non-associative habituation and sensitisation and to study the effect of different sucrose concentrations used as habituating/sensitising stimuli on non-associative habituation and sensitisation. Individual sucrose responsiveness and the sucrose concentration used as reward strongly affect associative PER learning (Scheiner et al. 1999). As associative PER learning and non-associative habituation and sensitisation share many similarities, the hypothesis was that 1) individual sucrose responsiveness should also play an important role in habituation and sensitisation and 2) the sucrose concentration of the habituating/sensitising stimulus also has a huge effect on the degree of habituation/sensitisation.

### 2.5.2 Preparation of the bees

These experiments were conducted together with Sigrid Wiese at the Technical University, Berlin, Germany, in the early summer of 2000. Bees were derived from a wild-type *Apis mellifera* colony. Returning bees without filled pollen baskets were captured at the hive entrance, which was blocked by a wire mash. Bees were mounted and prepared for the experiments as described for Experiment 3 (see 2.3.2), apart from the fact that this time the eyes were not occluded with black paint.

### 2.5.3 Measuring of sucrose responsiveness, habituation and sensitisation

Sucrose responsiveness was measured as described in Experiment 3 (see 2.3.3). Habituation started 10 s after the antennae were touched with the highest sucrose solution. A low-concentrated sucrose solution (Table 3) was applied to both antennae 30 times with an inter-stimulus interval of 10 s. After the last habituation trial, sensitisation started. This phase consisted of 5 antennal stimulations with a high sucrose concentration (Table 3), using the same inter-stimulus interval as before. The total number of proboscis responses of each bee was counted for each phase of the experiment and represents its “habituation” score and its “sensitisation” score, respectively. To analyse the effect of the sucrose concentration used as habituating or sensitising stimulus, different sucrose concentrations were used. Table 3 shows the different sucrose concentrations used as habituating or sensitising stimuli.

| experimental group | habituating stimulus (sucrose concentration) | sensitising stimulus (sucrose concentration) |
|---|---|---|
| 1 | 0.1 % | 30 % |
| 2 | 1 % | 30 % |
| 3 | 10 % | 30 % |
| 4 | 1 % | 3 % |
| 5 | 1 % | 10% |

**Table 3 Sucrose concentrations used as habituating or sensitising stimuli in Experiment 5.**

### 2.5.4 Statistics

For graphic display and the analysis of habituation and sensitisation, individuals were grouped according to their gustatory response scores as described for Experiment 3 (see 2.3.5) to compensate for the great variation of numbers in some groups. Spearman rank correlation coefficients were used to test whether gustatory response scores correlated with habituation or sensitisation scores. Habituation scores demonstrate the degree of habituation, sensitisation scores the degree of sensitisation. These scores were compared between groups with different GRS or between groups which were stimulated with different sucrose concentrations using two-tailed Mann-Whitney U-tests.

## *2.6 Experiment 6: Sucrose responsiveness and non-associative learning in young bees of different ages*

### 2.6.1 Intention

This experiment was designed to study sucrose responsiveness and non-associative learning in young bees of different ages. Sucrose responsiveness was measured in 1-hour-old bees, 4-hour-old bees, 1-day-old bees and 5-day-old bees. Subsequently, bees were habituated with 1 % sucrose and afterwards sensitised with 30 % sucrose. In the high- and low- strain bees of Page and Fondrk (1995), sucrose responsiveness was shown to increase with age (Pankiw and Page 1999). The expectation was therefore that young wild-type bees of different ages should also differ in their sucrose responsiveness. As sucrose responsiveness affects the degree of habituation and sensitisation (see 3.10), young bees of different ages should also differ in their habituation and sensitisation.

### 2.6.2 Preparation of the bees

This experiment was conducted at the Technical University, Berlin, Germany in the early spring of 2000. Bees from a wild-type *Apis mellifera* colony were used. Brood frames

were placed in an incubator maintained at 34 °C. Emerging brood was brushed off the frame every hour. Bees were colour-coded (Enamel, Testors, Rockford, USA) and transferred into cages in groups of 100 - 200 bees. The following age groups were tested: 1 h, 4 h, 1 d, 5 d. The 1-hour-old bees were mounted immediately after emergence. The 4-hour-old bees were placed in a cage after emergence and were mounted one hour prior to the experiment. The 1-day-old bees and the 5-day-old bees were restored to the colony after marking and sampled from the frames either 1 day or 5 days after emergence. They were mounted 1 hour before the start of the behavioural experiments.

### 2.6.3 Measuring of sucrose responsiveness, habituation and sensitisation

Sucrose responsiveness was measured as described for Experiment 3 (see 2.3.3). The same habituation and sensitisation phases were used as described for Experiment 5 (see 2.5.3). However, in this experiment, all bees were habituated with 1 % sucrose and sensitised with 30 % sucrose.

### 2.6.4 Statistics

The same statistics were applied as described for Experiment 5 (see 2.5.4), apart from the fact that in this experiment different age groups were compared, which had all been stimulated with the same sucrose concentrations.

## *2.7 Experiment 7: Sucrose responsiveness and M17 activity*

### 2.7.1 Intention

The aim of this experiment was to find a physiological correlate for sucrose responsiveness. The activity of muscle 17 (M17), which is responsible for proboscis extension, was measured in bees whose antennae were stimulated with different sucrose concentrations. The hypothesis was that even when no proboscis extension is observable, bees would respond differently to different sucrose concentrations. This should become apparent in recordings from M17. A higher sucrose concentration should result in a greater M17 activity than a lower sucrose concentration.

### 2.7.2 Preparation of the bees

This experiment was conducted in the summer of 2000 at the Technical University Berlin, Germany. Returning pollen foragers with big pollen loads were captured at the hive

entrance and immobilised and mounted as described for Experiment 3 (see 2.3.2). In addition, the heads of the bees were dorsally fixed with a 1:1 mixture of bees' wax and colophony. This was necessary for the insertion of the electrodes and the recordings. Then bees were fed with 30 % sucrose until they did not show proboscis extension to a 30 % sucrose solution.

### 2.7.3 Muscle recordings

One hour after feeding, a bee was placed in a Faraday cage for recordings. To record extracellularly from M17 the recording electrode (tungsten microelectrode 1-1.5 MΩ, World Precision Instruments, Inc., Sarasota, USA) was inserted into the bees' head, close to the median margin of the compound eye. The indifferent electrode was placed in the contralateral eye, because this position provided the optimal signal-to-noise ratio (Rehder 1987). The experimental apparatus is shown in Figure 3.

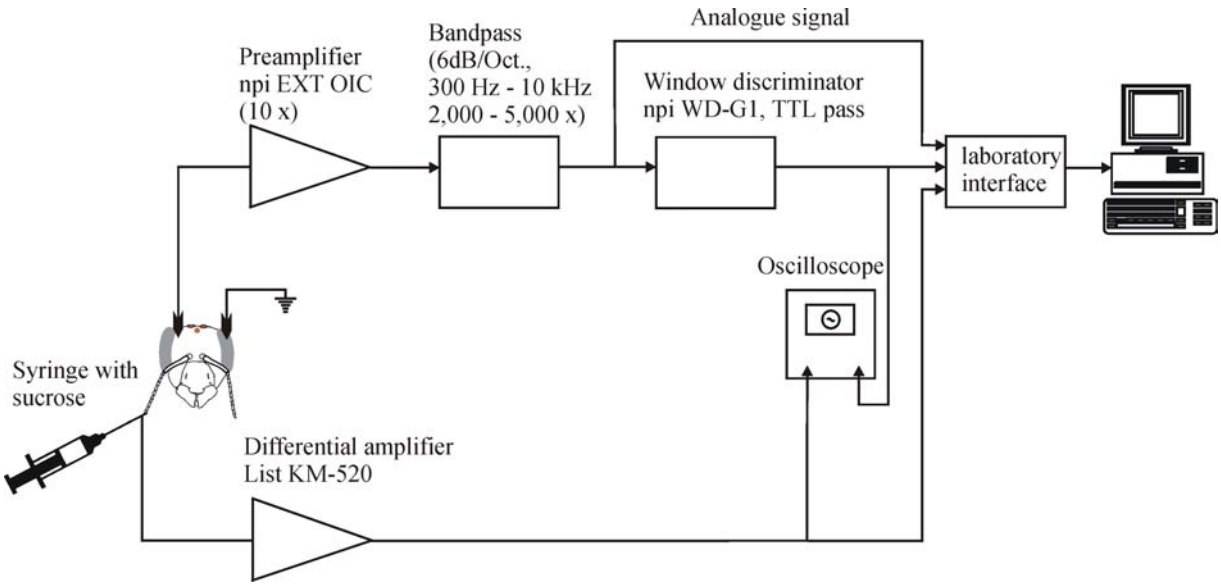


**Figure 3 Experimental apparatus for recordings from muscle 17.**

The signal of the recording electrode was pre-amplified 10 times with an EXT 01C amplifier (npi electronic GmbH) and 2,000-5,000 times amplified using an npi DPAZF amplifier (bandpass 6 dB/Octave, 300 Hz – 10 kHz). This signal was fed into one input of the micro 1404 laboratory interface (Cambridge Electronic Design) and into a window discriminator (npi, WD-G1). The output of the window discriminator was fed into another input of the laboratory interface and into one channel of an oscilloscope. The signal from the syringe was 1,000 times amplified in a List KM-520 differential amplifier. This signal was fed into the second channel of the oscilloscope and into one input of the laboratory interface. The

laboratory interface was connected to a PC. The soft ware “Spike 3 for Windows” was used to analyse the data.

### 2.7.4 Behavioural assay

Bees were stimulated for 20 ms with water or sucrose solutions of different concentrations in five blocks of five stimulations each. The inter-stimulus interval was 2 min. Between each block of stimulations a 10-min interval was inserted in which no recordings or stimulations took place. In each block, bees were stimulated with one of the following stimuli: water, 0.1 %, 1 %, 10 % and 30 % sucrose solution. The order of stimuli changed from block to block and between individuals. The number of M17 spikes occurring after antennal stimulation with water or sucrose was counted for 5 s, 10 s and 20 s. The relative results for the three durations were very similar. For statistical analyses the 5 s interval was used.

### 2.7.5 Statistics

The mean number of spikes following antennal stimulation with water or sucrose were calculated for figures (SPSS 9.0). Spearman rank correlations were estimated for the relationships between M17 activity, the different sucrose concentrations and the probability of proboscis extension (SPSS 9.0).

## *2.8 Experiment 8: Modulation of sucrose responsiveness by biogenic amines*

### 2.8.1 Intention

Biogenic amines play an important role in the neuromodulation of insects. They have been shown to act differently on the motor and learning behaviour of honey bees (Baumann et al. in press). This experiment aimed to analyse the effect of different biogenic amine receptor ligands on sucrose responsiveness. As octopamine was shown to facilitate olfactory conditioning and memory retrieval (Menzel et al. 1990), the hypothesis was that octopamine should increase sucrose responsiveness, because bees with high sucrose responsiveness learn better than bees with low sucrose responsiveness (Scheiner et al. 1999). Tyramine could have a similar effect, because it induces similar behavioural changes as octopamine (Braun and Bicker 1992) and it is the metabolic precursor of octopamine. Dopamine should decrease sucrose responsiveness, because it acts inhibitory on different behaviours (Menzel et al.

1990). ADTN should have similar effects to those of dopamine, because it also binds to a characterised honey-bee dopamine receptor (Blenau et al. 1998). The effects of the different ligands were tested 30 min and 90 min after injection into the thorax to measure the time scale of possible effects.

### 2.8.2 Preparation of the bees

These experiments were done together with Stephanie Baumgarten in the late summer of 1999. Returning non-pollen foragers were caught at the hive entrance and mounted as described for Experiment 3. In addition, the heads of the bees were fixed in a straight position with a 1:1 mixture of bees' wax and colophony for later injection. To maximally equalise their condition with regard to nutrition, they were all fed with 30 % sucrose until they did not show proboscis extension to 30 % sucrose the evening before the experiments started. Two hours prior to the experiment each bee was fed again with 3 droplets of 30 % sucrose solution, which approximately amounts to 15 µl. Responsiveness to sucrose was measured as described for Experiment 3 (see 2.3.3). After measuring responsiveness to sucrose, 1 µl of the neuroactive substances shown in Table 4 or ringer solution (270 mM NaCl, 3.2 mM KCl, 1.2 mM CaCl2, 10 mM MgCl, 10 mM morpholinopropansulfonic acid, pH 7.4) was injected into the thorax of each bee. Substances were dissolved in ringer solution which included $10^{-2}$ M ascorbic acid.

| **substances** | **concentrations** |
|---|---|
| tyramine | $10^{-2}$ M, $10^{-3}$ M |
| octopamine | $10^{-2}$ M, $10^{-3}$ M |
| ADTN | $10^{-2}$ M, $10^{-3}$ M, $10^{-4}$ M |
| dopamine | $10^{-1}$ M, $10^{-2}$ M, $10^{-3}$ M |

**Table 4 Concentrations of neuroactive substances which were injected into the thorax of bees in Experiment 8.**

Sucrose responsiveness was tested again 30 min and 90 min after the injection. The neuroactive substances were injected in fairly high concentrations, because it was unclear how much of the substances injected into the thorax would reach the neuronal centres involved in the perception and evaluation of sucrose stimuli and in the motor response. The different concentrations of the neuroactive substances were chosen to test for dose-dependent effects.

### 2.8.3 Statistics

Sucrose responsiveness was measured as gustatory response scores for the first water and all following sucrose stimulations. Therefore, the maximum score was 7. For statistical analysis, a modulation index (MI) was calculated as follows:

$$MI(t) = \frac{GRS_t - GRS_0}{GRS_t + GRS_0}$$

where 0 = initial time
t = time relapsed since feeding
GRS = gustatory response scores

A positive index marks an increase in sucrose responsiveness. A negative index implies a decrease in sucrose responsiveness. The modulation indices of different groups were compared with two-tailed t-tests.

## *2.9 Experiment 9: PKA activity in bees with different sucrose responsiveness*

### 2.9.1 Intention

Protein kinase A (PKA) plays an important role as mediator of phosphorylation processes. In the honey bee, it is involved in the formation of long-term memory (Fiala et al. 1999, Müller 2000). There are some conspicuous similarities between the modulation of PKA activity and the modulation of sucrose responsiveness. The injection of octopamine, which leads to an increase in sucrose responsiveness (see 3.14.1), increases the PKA activity in the antennal lobes (Hildebrandt and Müller 1995). Stimulation of the antennae with sucrose, which leads to a short-term increase in sucrose responsiveness, also leads to a short-term increase in PKA activity (Hildebrandt and Müller 1995).

Looking for neuronal correlates for sucrose responsiveness and its plasticity, the following experiment was to analyse the effects of different sucrose responsiveness on PKA activity in the antennal lobes of honey bees. As sucrose responsiveness can be modulated by starvation, PKA activity was measured at two time points after feeding.

### 2.9.2 Preparation of the bees

This experiment was conducted in the summer of 2000. Returning non-pollen foragers were caught at the hive entrance, immobilised and mounted as described for Experiment 3 (see 2.3.2). The eyes, however, were not occluded. Bees were fed with 30 % sucrose solution

until they did not show proboscis extension to antennal stimulation with that sucrose solution 1 hour before measuring of sucrose responsiveness started. Then, responsiveness to water and sucrose was measured by touching both antennae with the following stimuli: water, 0.1 %, 1 %, 10 % and 30 % sucrose. For the following parts of the experiment, only bees which already responded to water ("high sucrose responsiveness") and those that only responded to 30 % sucrose or did not respond at all ("low sucrose responsiveness") were chosen. Six groups of bees were separated as shown in Table 5. In the control group, sucrose responsiveness was measured two times, whereas PKA activity was not measured. In the groups in which PKA activity was to be measured, the heads of the bees were cut off shortly after measuring of sucrose responsiveness (groups 1 and 4) or 30 min after feeding (groups 2 and 5). The heads were immediately placed in liquid nitrogen. The heads were subsequently subjected to incomplete lyophylization (3.5 h at –20 °C, 0.05 mbar, Hildebrandt and Müller 1995) and stored in liquid nitrogen over night.

| group | sucrose responsiveness | experiment |
|---|---|---|
| 1 | high (bees respond to $H_2O$) | PKA activity measured 30' after feeding |
| 2 | high (bees respond to $H_2O$) | PKA activity measured 90' after feeding |
| 3 | high (bees respond to $H_2O$) | control: GRS measured 30' and 90' after feeding |
| 4 | low (bees respond to 30%) | PKA activity measured 30' after feeding |
| 5 | low (bees respond to 30%) | PKA activity measured 90' after feeding |
| 6 | low (bees respond to 30%) | control: GRS measured 30' and 90' after feeding |

**Table 5 Experimental groups in Experiment 9. GRS – gustatory response scores.**

### 2.9.3 Preparation of antennal lobes

The next day, heads were fixed onto a metal block using Tissue Teck. The head capsule was opened and one antennal lobe of each bee was dissected under constant liquid nitrogen cooling and transferred into a 100 µl glass capillary containing 10 µl extraction buffer (50 mM Tris-HCl, pH 7.5, 2 mM ethylene glycoltetraacetic acid and 10 mM mercaptoethanol), cooled by liquid nitrogen. The antennal lobes were homogenised with a metal pistil on the surface of the frozen extraction buffer and stored in liquid nitrogen till the start of the phosphorylation.

### 2.9.4 Phosphorylation assay

PKA activity was determined with phosphatase inhibitor (I-1), which was purified from bovine brain and used as the specific substrate (Hildebrandt and Müller 1995). Samples in the capillaries were thawed and immediately added to a 10 µl phosphorylation mixture.

This mixture contained 1 µCi ($\gamma$-$^{32}$P) adenosine triphosphate (5,000 Ci/mmol, 10 µM adenosine triphosphate, 20mM magnesium chloride ($MgCl_2$), 2 mM ethylene glycoltetraacetic acid and 10 mM mercaptoethanol in 50 mM Tris-HCl, pH 7.5 and an aliquot of the heat-stable *Apis* PKA-specific substrate protein I-1, which was boiled for 2 min before use. After incubation for 30 s at room temperature, reactions were stopped by adding 5 µl sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (500 mM Tris-HCl, pH 6.8, 5 % mercaptoethanol, 5 % SDS, 20 % glycerol and 0.1 % bromphenol blue). After SDS-PAGE (T = 12.5 %, C = 2.7 %) and autoradiography, the autoradiographs were scanned and density of I-1 bands was determined using NIH Image. All values of each gel were related to the mean value of a gel to allow comparisons.

### 2.9.5 Statistics

Relative PKA activity was compared between bees with high or low sucrose responsiveness using two-tailed t-tests. PKA activity measured at different time points was compared within groups using two-tailed t-tests. The sucrose concentrations were transformed to their $Log_{10}$ values for the graphic display of responsiveness to the different sucrose concentrations 30 min and 90 min after feeding.

# 3 Results

## *3.1 Experiment 1: Sucrose responsiveness and tactile learning in high- and low-strain foragers*

### 3.1.1 Sucrose responsiveness in high- and low-strain foragers

Genotype and foraging role strongly affected sucrose responsiveness. High- and low-strain foragers showed increasing responsiveness to increasing sucrose concentrations (Figure 4), but sucrose responsiveness differed between the two strains of bees and between pollen and non-pollen foragers within each strain.

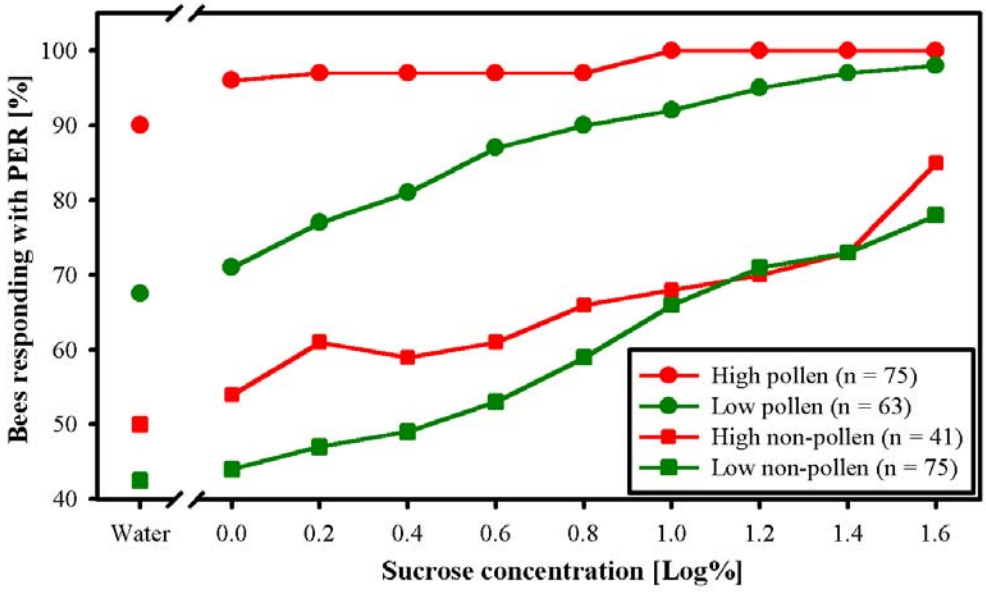


**Figure 4 Responsiveness to water and increasing sucrose concentrations in foragers of the high and low strains. The abscissa represents the sucrose concentrations of the gustatory stimuli and the ordinate the percentage of bees responding with proboscis extension (PER). Number of bees tested (n) is indicated.**

High-strain bees were more responsive to water and low sucrose concentrations than low-strain bees (Figure 4). Their gustatory response scores, which represent the degree of responsiveness, were significantly higher than those of low-strain foragers (Figure 26A, $z = 4.23$, $n_{\text{high-strain foragers}} = 116$, $n_{\text{low-strain foragers}} = 135$, $p < 0.001$, two-tailed Mann-Whitney U-test). To compare the different sucrose-concentration response curves, linear regressions were calculated for the proportion of bees extending their probosces to increasing sucrose concentrations. The slope of this regression (Table 6) was significantly steeper for low-strain bees than it was for high-strain bees (for pollen foragers: $t = 10.05$, $df = 136$, $p < 0.001$, two-tailed t-test; for non-pollen foragers: $t = 2.87$, $df = 62$, $p < 0.01$, two-tailed Welch's t-test). But the intercept of this regression was significantly higher for high-strain foragers than for

low-strain foragers (for pollen foragers: $t = 15.56$, $df = 180$, $p < 0.001$; for non-pollen foragers: $t = 5.37$, $df = 60$, $p < 0.001$, two-tailed Welch's t-test). This demonstrates a stronger increase in sucrose responsiveness with increasing sucrose concentrations in low-strain bees, while high-strain bees were initially more responsive than low-strain bees and showed a smaller increase in responsiveness with higher concentrations of sucrose.

Within each strain, pollen foragers were more responsive to water and low sucrose concentrations than non-pollen foragers, which is demonstrated by higher gustatory response scores (Figure 27A, B), for high strain: $z = 5.55$, $n_{\text{pollen foragers}} = 75$, $n_{\text{non-pollen foragers}} = 41$, $p < 0.001$; for low strain: $z = 4.81$, $n_{\text{pollen foragers}} = 63$, $n_{\text{non-pollen foragers}} = 75$, $p < 0.001$, two-tailed Mann-Whitney U-test). The slope of the linear regression on the sucrose-concentration response curve (Table 6) was significantly steeper for non-pollen foragers than it was for pollen foragers (for high strain: $t = 6.02$, $df = 44$, $p < 0.001$, two-tailed Welch's t-test; for low strain: $t = 3.30$, $df = 136$, $p < 0.01$, two-tailed t-test). The intercept was significantly higher for pollen foragers than for non-pollen foragers in each strain (for high strain: $t = 18.78$, $df = 60$, $p < 0.001$; for low strain: $t = 22.63$, $df = 161$, $p < 0.001$, two-tailed Welch's t-test). These findings show that pollen foragers were more responsive to water and low sucrose concentrations than non-pollen foragers, while the increase in sucrose responsiveness with higher sucrose concentrations was stronger in non-pollen foragers.

### 3.1.2 Tactile acquisition and extinction in high- and low-strain foragers

In high- and low-strain foragers, sucrose responsiveness strongly affected the performance during acquisition and extinction. Genotype or foraging role had no separate effects. Individuals with low sucrose responsiveness (GRS ≤ 4) learned only poorly (Figure 5) and showed strong extinction of responses to the conditioned pattern (CS+, Figure 6), while bees with high sucrose responsiveness (GRS ≥ 9) learned well and showed weak extinction. In the low strain, bees with intermediate sucrose responsiveness (GRS 5 - 8) reached a fairly high level of acquisition and showed weak extinction, while in the high strain, individuals with the same sucrose responsiveness learned poorly and showed strong extinction. However, this group was only represented by three individuals.

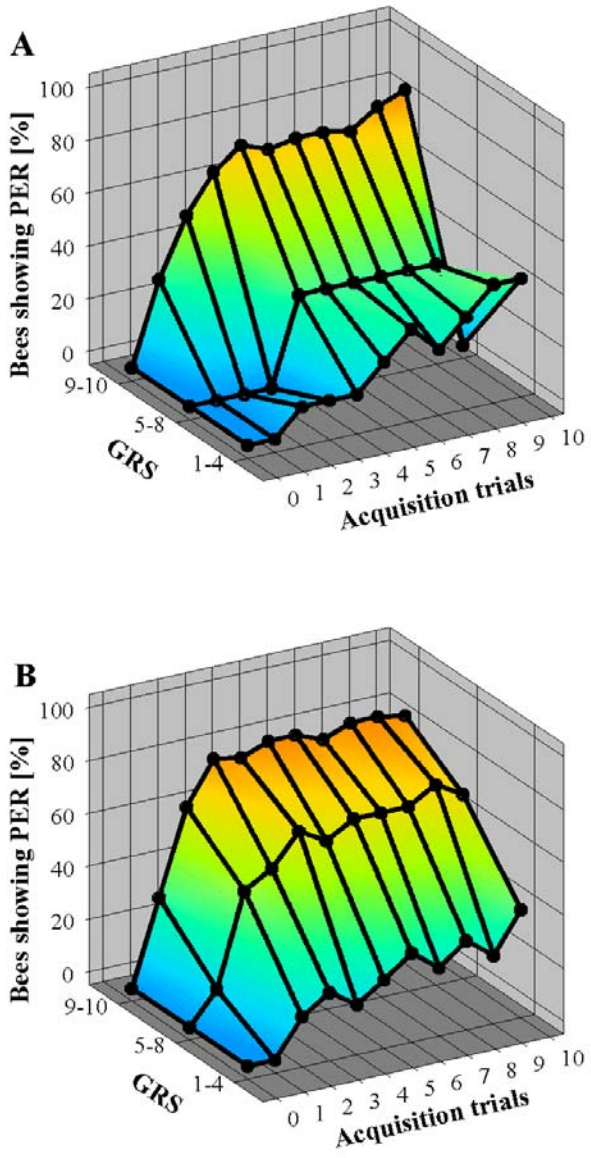


**Figure 5 Tactile acquisition curves of foragers of the high (A) and low (B) strains. Pollen and non-pollen foragers were pooled, because foraging role had no effect on acquisition. Some of the groups with different gustatory response scores (GRS) were combined to compensate for the great variation in numbers in some groups. Bees with GRS ≤ 4 showed low sucrose responsiveness. Individuals with GRS 5 - 8 displayed intermediate sucrose responsiveness. Bees with GRS ≥ 9 had a high sucrose responsiveness. The x-axis represents the acquisition trials, the y-axis the grouped GRS and the z-axis the percentage of bees responding with proboscis extension. Number of bees: high strain: GRS 1 - 4: n = 9, GRS 5 - 8: n = 3, GRS 9 - 10: n = 55. Low-strain: GRS 1 - 4: n = 14, GRS 5 - 8: n = 17, GRS 9 - 10: n = 50.**

The degree of acquisition and extinction is described by acquisition and extinction scores (see 2.1.5). Gustatory response scores correlated positively with acquisition scores and extinction CS+ scores in both strains (Table 7). The higher the GRS of a bee, the higher were its acquisition and extinction CS+ scores. This implies that bees with high sucrose responsiveness learned well and showed weak extinction, while those with low sucrose responsiveness learned poorly and showed strong extinction. The relationships between

sucrose responsiveness and acquisition and between sucrose responsiveness and extinction of conditioned responses can be described by significant linear transfer functions (Table 8, Table 9, respectively).

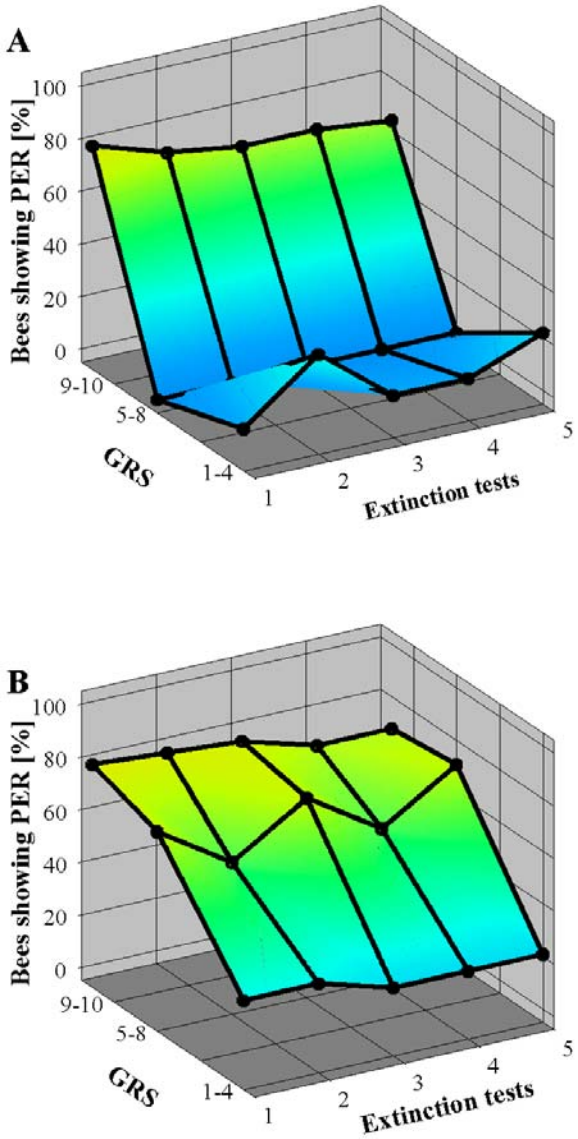


**Figure 6 Pooled extinction CS+ curves of pollen and non-pollen foragers of the high (A) and low (B) strains. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees responding with proboscis extension. Number of bees as in Figure 5.**

There were no significant differences between the strains in the slope coefficients of the linear regressions of acquisition scores on GRS (Table 8, for slope coefficient: $t = 0.29$, $df = 147$, $p < 0.05$, two-tailed t-test) or of the regressions of extinction CS+ scores on GRS (Table 9, $t = 0.07$, $df = 147$, $p > 0.05$, two-tailed t-test). The intercepts of both regressions were not different from zero in either strain (Table 8, Table 9, respectively). This shows that high- and

low-strain foragers did not differ in their relationship between sucrose responsiveness and acquisition or extinction. Extinction of conditioned responses correlated with acquisition (Table 7) in both strains. Bees which showed good acquisition demonstrated weak extinction. Individuals with low acquisition scores showed strong extinction. The slopes of the linear regressions of extinction CS+ scores on acquisition scores (Table 10) did not differ significantly between the strains ($t = 0.63$, $df = 147$, $p > 0.05$, two-tailed t-test). The intercepts of both groups (Table 10) were not different from zero.

In the low strain, GRS correlated positively with acquisition scores and extinction CS+ scores (Table 7) in both pollen and non-pollen foragers. Pollen foragers did not differ from non-pollen foragers in their slope coefficients of the regressions of acquisition scores on GRS (Table 8, $t = 0.33$, $df = 72$, $p > 0.05$, two-tailed Welch's t-test) or of the regressions of extinction CS+ scores on GRS (Table 9, $t = 0.33$, $df = 79$, $p > 0.05$, two-tailed t-test). The intercepts of the respective regressions were not different from zero in both groups of low-strain foragers (Table 8, Table 9, respectively). These findings suggest that foraging role did not affect the relationship between sucrose responsiveness and acquisition or extinction in the low strain. In high-strain pollen foragers, GRS did not correlate with acquisition or extinction CS+ scores (Table 7), probably because there was little variation in the gustatory response scores of these bees. In non-pollen foragers of the high strain, sucrose responsiveness correlated positively with acquisition and extinction CS+ scores (Table 7).

Acquisition scores correlated with extinction CS+ scores in pollen and non-pollen foragers of both strains (Table 7). The slope coefficients of the corresponding linear regressions (Table 10) did not differ between high- and low-strain foragers ($t = 0.07$, $df = 147$, $p > 0.05$, two-tailed t-test) or between pollen and non-pollen foragers in each strain (Table 10, high strain: $t = 0.10$, $df = 27$, $p > 0.05$, two-tailed Welch's t-test; low strain: $t = 0.38$, $df = 79$, $p > 0.05$, two-tailed t-test). The intercepts of the respective regressions were not different from zero. These findings show that good learners showed weak extinction, whereas poor learners showed strong extinction, regardless of foraging role.

### 3.1.3 Tactile discrimination in high- and low-strain foragers

Genotype affected discrimination of tactile patterns in foragers. Both high- and low-strain foragers responded significantly more often to the conditioned pattern (CS+, Figure 6) than to the alternative pattern (CS-, Figure 7) and thus demonstrated tactile discrimination (high strain: $z = 5.18$, $n = 68$, $p < 0.001$, low strain: $z = 5.33$, $n = 81$, $p < 0.001$, two-tailed Wilcoxon-test).

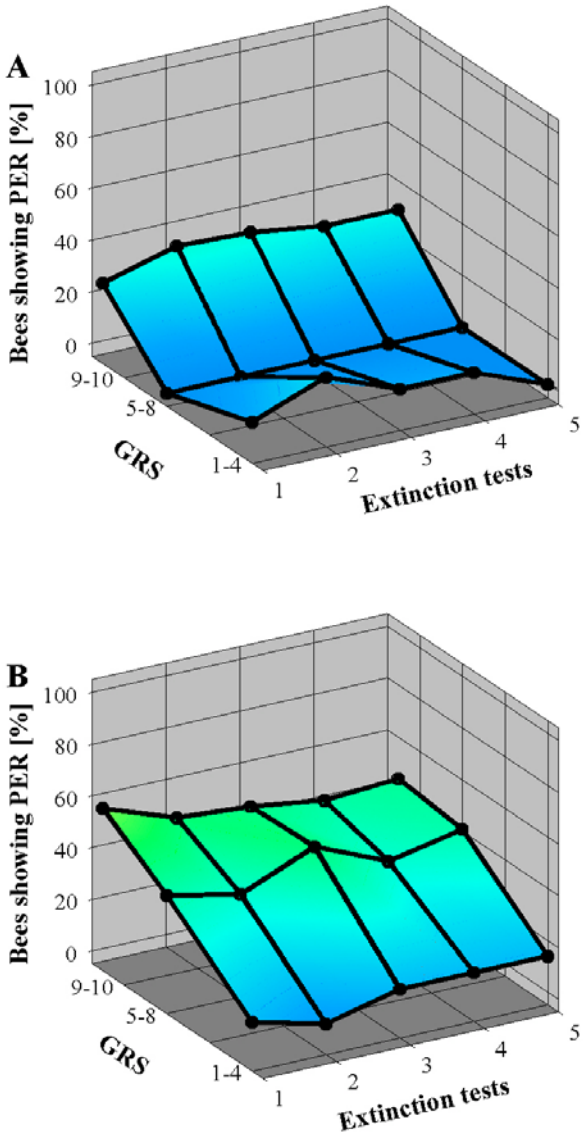


**Figure 7 Pooled extinction CS- curves of pollen and non-pollen foragers of the high (A) and low (B) strains. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction CS- tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees responding with proboscis extension. Number of bees as in Figure 5.**

Discrimination indices were calculated to compare discrimination between the different groups (Table 23). The discrimination indices of pollen and non-pollen foragers were pooled, because the two groups did not differ significantly (Figure 35A, B). High-strain foragers had a significantly higher discrimination index than low-strain foragers (Figure 34), demonstrating better tactile discrimination. This could be a result of differences in sucrose responsiveness, because extinction CS- scores correlated positively with sucrose responsiveness and acquisition (Table 7). Bees with high sucrose responsiveness learned better but responded more often to the alternative pattern than bees with low sucrose responsiveness, and high-strain foragers were significantly more responsive to sucrose than

low-strain foragers (see 3.1.1). However, the high-strain foragers did not differ significantly from low-strain foragers in their acquisition or extinction CS+ scores (acquisition: $z = -0.56$, extinction: $z = -0.45$, $p > 0.05$, two-tailed Mann-Whitney U-test). These findings suggest that genotype affected discrimination independent of sucrose responsiveness. The relationship between extinction CS- scores and GRS could not be compared between high- and low-strain foragers, because there was no significant linear transfer function for high-strain foragers. The relationship between extinction CS- and acquisition could be compared between foragers of the two strains. The slope coefficients of the linear regressions of extinction CS- scores on acquisition scores (Table 10) did not differ between the strains ($t = 0.98$, $df = 147$, $p > 0.05$, two-tailed t-test) or between pollen and non-pollen foragers within each strain (high strain: $t = 1.00$, $df = 65$, $p > 0.05$, two-tailed Welch's t-test; low strain: $t = 0.33$, $df = 80$, $p > 0.05$, two-tailed t-test). All intercepts did not differ from zero. Therefore, good learners, which were mostly bees with high sucrose responsiveness, discriminated poorly, regardless of genotype or foraging role. Poor learners, which had a low sucrose responsiveness, discriminated better. In addition, genotype had a direct effect on discrimination, which was independent of sucrose responsiveness.

## *3.2 Experiment 2: Sucrose responsiveness, and tactile and olfactory learning in high- and low-strain preforagers*

### 3.2.1 Sucrose responsiveness in high- and low-strain preforagers

Genotype affected sucrose responsiveness in bees without foraging experience. Responsiveness to sucrose increased with increasing sucrose concentrations in preforagers of both strains. But high-strain preforagers were generally more responsive to water and to the different sucrose concentrations than low-strain preforagers (Figure 8). The slopes of the regressions on the sucrose-concentration response curves (Table 6) were not different between high- and low-strain preforagers ($t = 1.52$, $df = 522$, $p > 0.05$, two-tailed Welch's t-test), but the intercept of the high-strain preforagers was significantly higher than that of the low-strain preforagers ($t = 4.92$, $df = 561$, $p < 0.001$, two-tailed t-test), indicating a higher initial responsiveness. High-strain preforagers had significantly higher GRS than low-strain preforagers (Figure 26B, $z = 2.47$, $p < 0.05$, $n_{high} = 259$, $n_{low} = 304$, two-tailed Mann-Whitney U-test). These data confirm that selection for pollen-hoarding behaviour resulted in a correlated change in water/sucrose responsiveness that is expressed in bees without foraging experience.

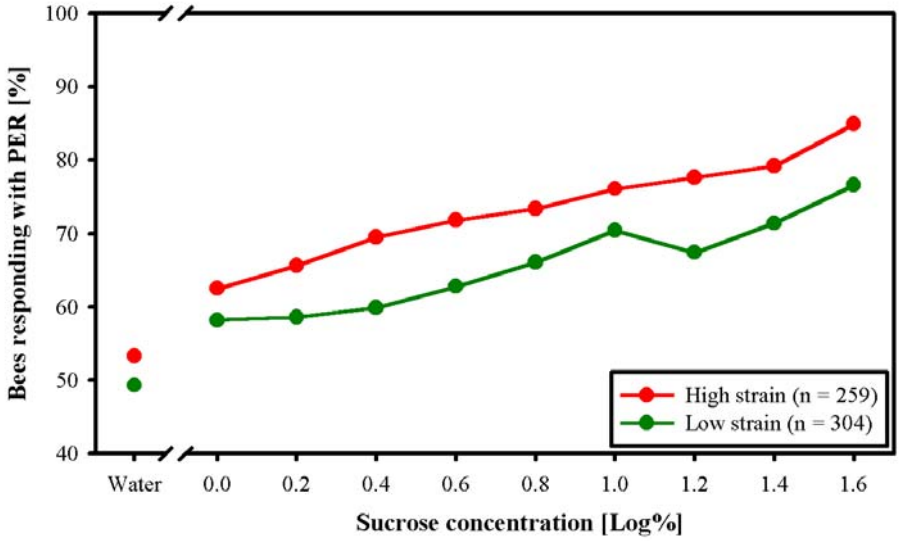


**Figure 8 Responsiveness to water and increasing sucrose concentrations in preforagers of the high and low strains. The abscissa represents the sucrose concentrations of the gustatory stimuli, the ordinate the percentage of bees showing the proboscis extension response (PER). Number of bees tested (n) is indicated.**

### 3.2.2 Tactile acquisition and extinction in high- and low-strain preforagers

Sucrose responsiveness determined the level of acquisition and extinction in high- and low-strain preforagers. In contrast to foragers, high- and low-strain preforagers with low sucrose responsiveness (GRS ≤ 4) showed no learning at all (Figure 9), whereas bees with intermediate sucrose responsiveness (GRS 5-8) learned fairly well. About 80 % of bees with high sucrose responsiveness (GRS ≥ 9) learned the tactile pattern, regardless of genotype. In both high- and low-strain preforagers, acquisition scores correlated positively with GRS (Table 11). Significant linear transfer functions of acquisition scores on GRS were found for high- and low-strain preforagers (Table 12). The slope coefficients and intercepts of these regressions did not differ significantly between high- and low-strain preforagers (for slope coefficient: $t = 0.16$, $df = 74$, for intercept: $t = 0.09$, $df = 70$, $p > 0.05$, two-tailed Welch's t-test), demonstrating that the relationship between sucrose responsiveness and tactile acquisition was similar in both strains.

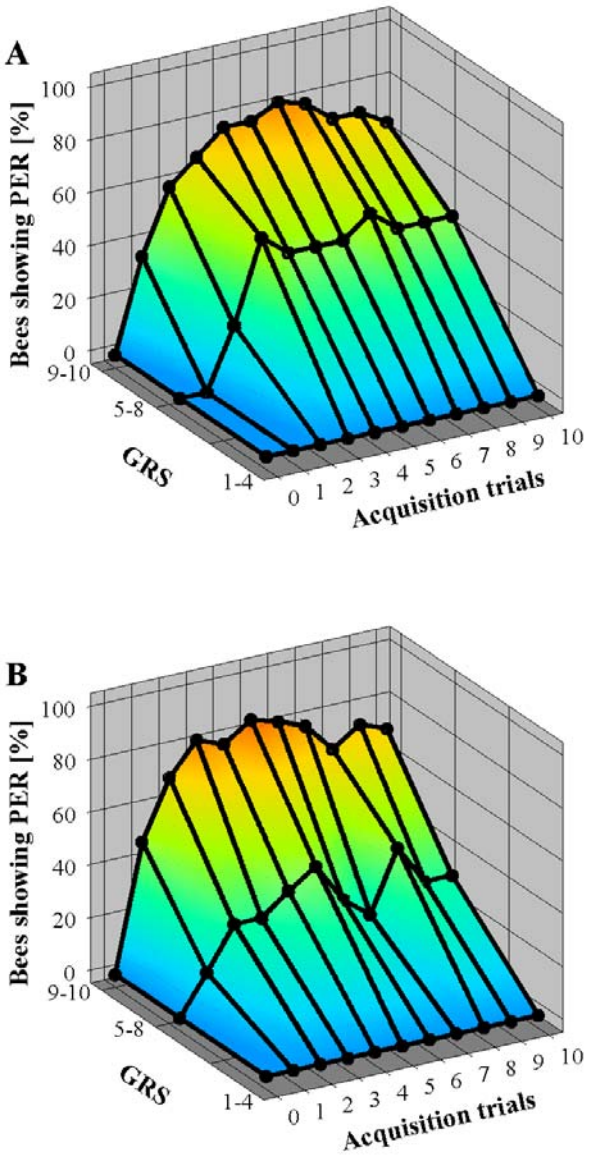


**Figure 9 Tactile acquisition curves of high- (A) and low-strain preforagers (B). Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the acquisition trials, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension. Number of bees: high-strain: GRS 1 - 4: n = 3, GRS 5 - 8: n = 13, GRS 9 - 10: n = 34. Low-strain: GRS 1 - 4: = 10, GRS 5 - 8: n = 13, GRS 9 - 10: n = 27.**

Extinction CS+ scores correlated with GRS in low-strain preforagers but not in high-strain preforagers (Table 11). However, Figure 10 shows that in both strains bees with low sucrose responsiveness (GRS ≤ 4) demonstrated strong extinction, while individuals with high sucrose responsiveness (GRS ≥ 9) showed weak extinction. In addition, there were only few individuals with low GRS among the high-strain preforagers.

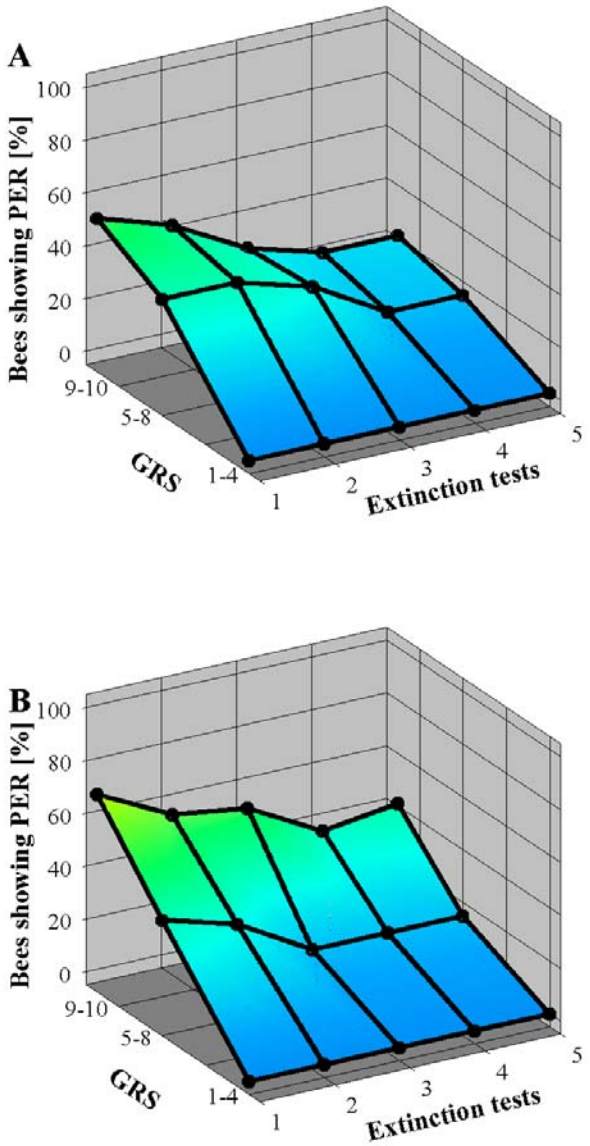


**Figure 10 Extinction CS+ curves after tactile learning in preforagers of the high (A) and low (B) strains. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction CS+ tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing the proboscis extension response (PER). Number of bees as in Figure 9.**

Extinction CS+ scores correlated positively with acquisition scores in both strains (Table 11). The slopes of the linear regressions of extinction CS+ scores on acquisition scores (Table 14) did not differ significantly between high- and low-strain preforagers ($t = 1.20$, $df = 80$, $p > 0.05$, Welch's t-test). The intercepts of both groups did not differ from zero (Table 14), suggesting that in both strains, "good" learners (i.e. bees with high acquisition scores) continued responding during the extinction tests, while bees with low acquisition scores showed a strong decay of conditioned responses during the extinction tests. These findings

imply that genotype had no effect on the relationships between sucrose responsiveness, tactile acquisition and extinction CS+ in preforagers.

### 3.2.3 Tactile discrimination in high- and low-strain preforagers

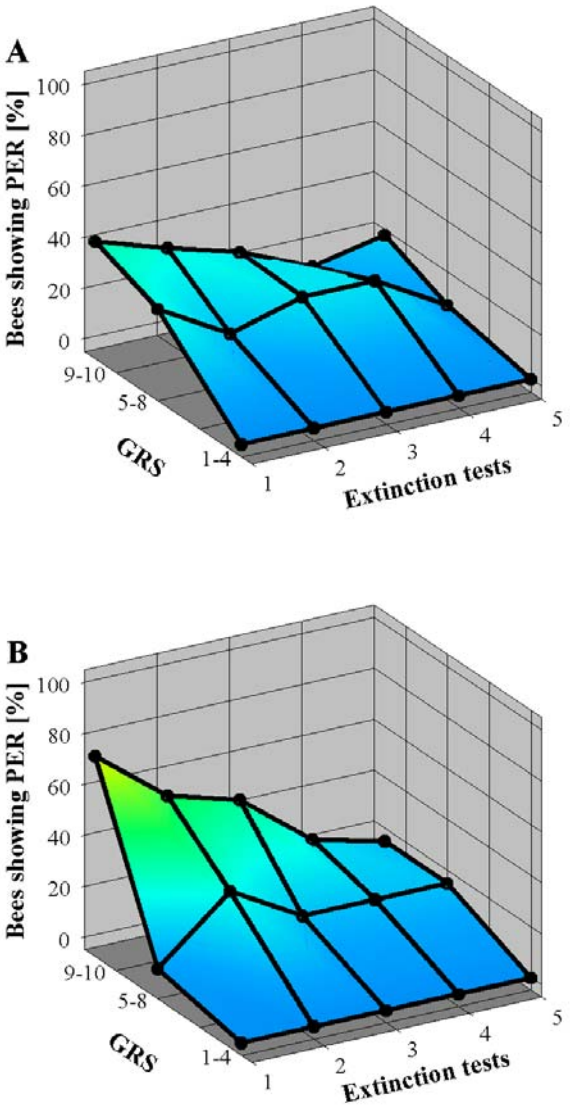


**Figure 11 Extinction CS- curves after tactile learning in high- (A) and low-strain (B) preforagers. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction CS- tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees responding with proboscis extension (PER). Number of bees as in Figure 9.**

Genotype had no effect on discrimination in preforaging bees. High- and low-strain preforagers discriminated equally well between the conditioned pattern (CS+, Figure 10) and the alternative pattern (CS-, Figure 11). Discrimination indices were calculated to compare tactile discrimination between the two groups of preforagers (Table 23). The preforagers of both strains did not differ significantly in their discrimination indices (Figure 34B) and

therefore in their discrimination. Extinction CS- scores correlated significantly with GRS in the low strain but not in the high strain (Table 11). In both strains, extinction CS- scores correlated positively with acquisition scores (Table 11). Bees with high acquisition scores discriminated poorly and had high extinction CS- scores, regardless of genotype. There were no significant differences in the slopes of the regressions of extinction CS- scores on acquisition scores (Table 14) between high- and low-strain preforagers ($t = 1.20$, $df = 80$, $p > 0.05$, Welch's t-test). The intercepts were not significantly different from zero in both groups. This suggests that the two strains exhibit the same functional relationships between sucrose responsiveness, acquisition and discrimination.

### 3.2.4 Olfactory acquisition and extinction in high- and low-strain preforagers

Sucrose responsiveness had large effects on olfactory acquisition (Figure 12) and extinction CS+ (Figure 13) in preforagers of the two strains. High- and low-strain preforagers with high sucrose responsiveness (GRS ≥ 9) reached a high level of acquisition and responded frequently to the conditioned odour (CS+) during the extinction tests. Bees with low sucrose responsiveness (GRS ≤ 4) showed poor acquisition in the high strain, no acquisition in the low strain and strong extinction in both strains. Low-strain preforagers with intermediate sucrose responsiveness (GRS 5 - 8) appeared to learn better and to show less extinction than high-strain preforagers with the same sucrose responsiveness. In high- and low-strain preforagers, olfactory acquisition and extinction CS+ scores correlated positively with GRS (Table 11), and acquisition scores correlated positively with extinction CS+ scores (Table 11). The higher the sucrose responsiveness of a bee, the higher were its olfactory acquisition and extinction CS+ scores, regardless of genotype.

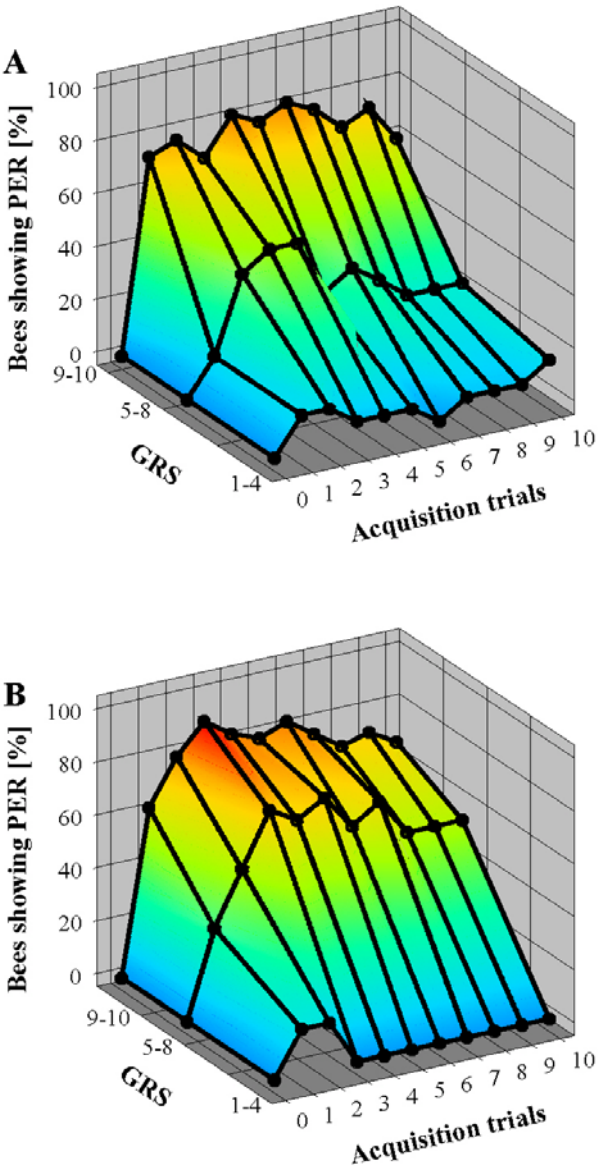


**Figure 12 Olfactory acquisition curves of high- (A) and low-strain (B) preforagers. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the different acquisition trials, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees: high strain: GRS 1 - 4: n = 14, GRS 5 - 8: n = 14, GRS 9 - 10: n = 22. Low strain: GRS 1 - 4: n = 6, GRS 5 - 8: n = 15, GRS 9 - 10: n = 29.**

Significant linear transfer functions of acquisition and extinction CS+ scores on GRS and of extinction CS+ scores on acquisition scores were calculated (Table 12 - Table 14). The slopes of these regressions were not significantly different between preforagers of the two strains (for acquisition on GRS: $t = 0.15$, $df = 91$, $p > 0.05$, Welch's t-test; for extinction CS+ on GRS: $t = 0$, $df = 90$, $p > 0.05$, two-tailed Welch's t-test; for extinction CS+ on acquisition scores: $t = 1.56$, $df = 98$, $p > 0.05$, two-tailed t-test) and all intercepts did not differ from zero. These findings indicate the same relationships between sucrose responsiveness, olfactory acquisition and extinction CS+ in high- and low-strain preforagers.

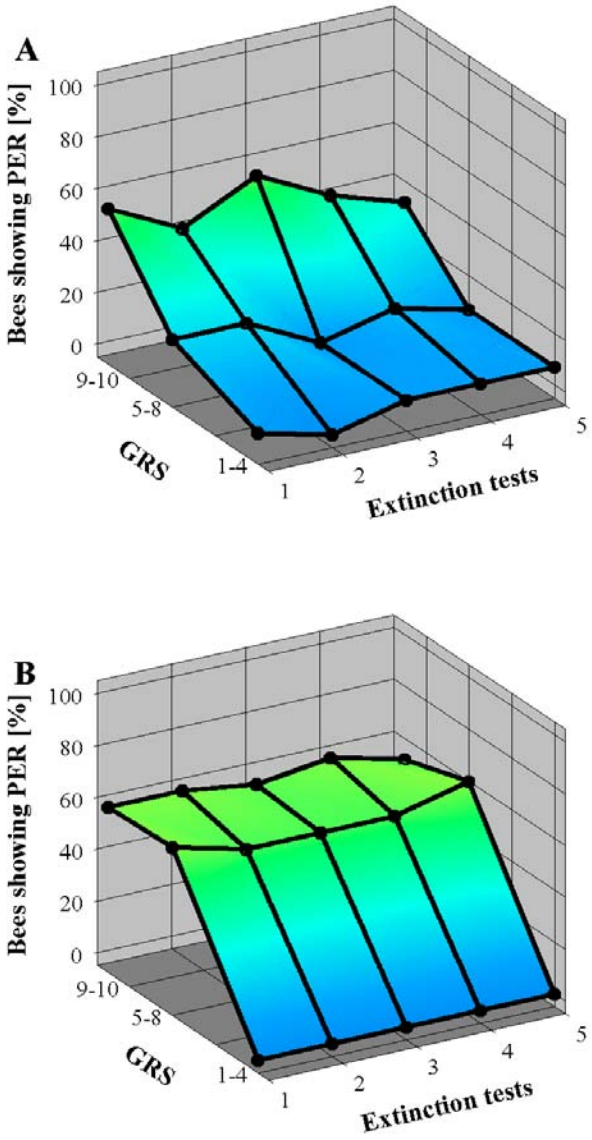


**Figure 13 Extinction CS+ curves after olfactory learning in preforagers of the high (A) and low (B) strains. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees responding with proboscis extension (PER). Number of bees as in Figure 12.**

### 3.2.5 Olfactory discrimination in high- and low-strain preforagers

Preforagers of both strains discriminated equally well between the conditioned odour and the alternative odour. Few bees responded to the untrained odour (CS-, Figure 14). Both high- and low-strain preforagers had significantly higher extinction CS+ scores than extinction CS- scores and thus showed olfactory discrimination (high strain: $z = 3.76$, $n = 50$, $p < 0.001$, low strain: $z = 4.82$, $n = 50$, $p < 0.001$, two-tailed Wilcoxon-test). Discrimination indices were calculated to compare discrimination between strains (Table 23) High-strain

preforagers did not differ from low-strain preforagers in their olfactory discrimination index (Figure 34C).

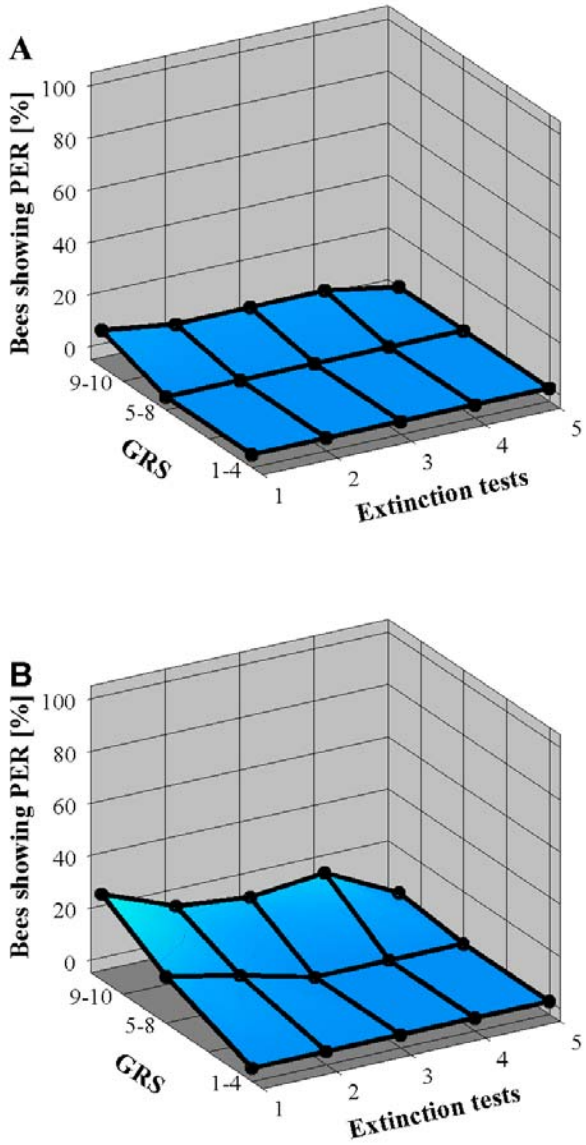


**Figure 14 Extinction CS- curves after olfactory learning in high- (A) and low-strain (B) preforagers. Bees were grouped according to their sucrose responsiveness (see Figure 5). The x-axis represents the extinction CS- tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees as in Figure 12.**

Apparently, genotype did not affect discrimination of odours in preforagers. Bees with low or intermediate GRS basically did not respond to the alternative odour. Low-strain preforagers with high sucrose responsiveness initially responded significantly more often to the alternative odour than high-strain preforagers ($n_{high\ strain} = 34$, $n_{low\ strain} = 22$, $p < 0.05$, two-

tailed Fisher-Exact-test), but from the second extinction CS- test onwards, high- and low-strain preforagers with high sucrose responsiveness did not differ. Extinction CS- scores correlated positively with GRS in the low strain but not in the high strain (Table 11). But in the high strain very few bees responded to the alternative odour. In both strains, extinction CS- scores correlated positively with acquisition scores (Table 11), indicating that bees with good acquisition discriminated less well than "poor" learners. This relationship could not be compared between high- and low-strain preforagers, because there no linear regression of extinction CS- scores on acquisition scores was found in high-strain preforagers.

## *3.3 Experiment 3: Sucrose responsiveness, and tactile and olfactory learning in wild-type foragers*

### 3.3.1 Sucrose responsiveness in wild-type foragers

Wild-type pollen foragers demonstrated a higher responsiveness to water and sucrose than wild-type non-pollen foragers (Figure 15). Both groups of foragers showed increasing responsiveness to increasing sucrose concentrations as is demonstrated by significant positive slopes of linear regressions on the sucrose-concentration response curves (Table 6).

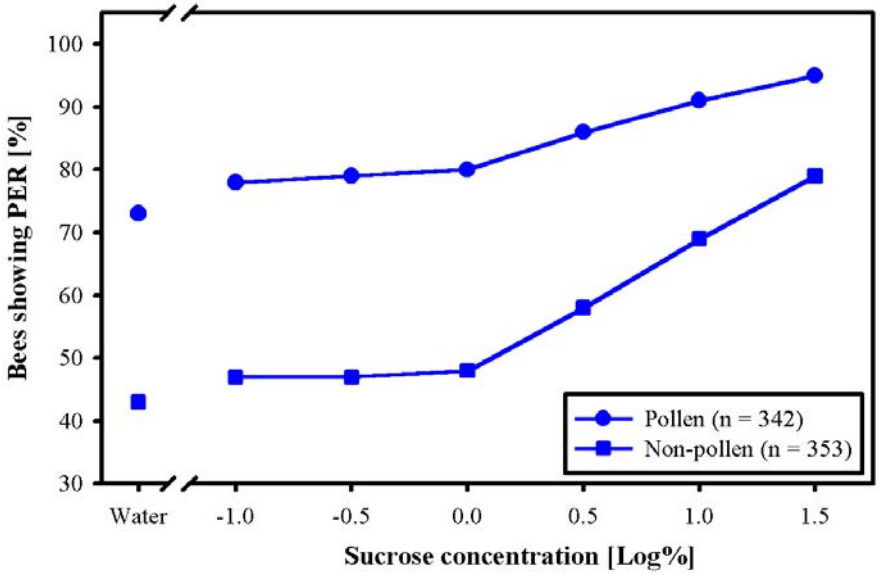


**Figure 15 Responsiveness to water and increasing sucrose concentrations in wild-type pollen and non-pollen foragers. The abscissa represents the sucrose concentrations of the gustatory stimuli, the ordinate the percentage of bees showing the proboscis extension response (PER). Number of bees tested (n) is indicated.**

The slopes of these regressions were not significantly different between pollen and non-pollen foragers ($t = 1.03$, $df = 590$, $p > 0.05$, two-tailed Welch's t-test), which implies the same

increase in responsiveness with increasing sucrose concentrations. But pollen foragers had a significantly higher intercept ($t = 11.31$, $df = 693$, $p < 0.001$, two-tailed t-test), suggesting that pollen foragers were initially more responsive than non-pollen foragers. Pollen foragers had significantly higher GRS than non-pollen foragers (Figure 27C) and thus demonstrated a higher degree of responsiveness to sucrose.

### 3.3.2 Tactile acquisition and extinction in wild-type foragers

Sucrose responsiveness determined the level of acquisition and extinction of conditioned responses in wild-type pollen and non-pollen foragers. Regardless of foraging role, bees with low sucrose responsiveness (GRS ≤ 2) showed poor acquisition (Figure 16) and strong extinction (Figure 17), bees with intermediate sucrose responsiveness (GRS 3 - 4) learned slightly better, whereas more than 80 % of the bees with high sucrose responsiveness (GRS ≥ 5) learned the conditioned tactile pattern.

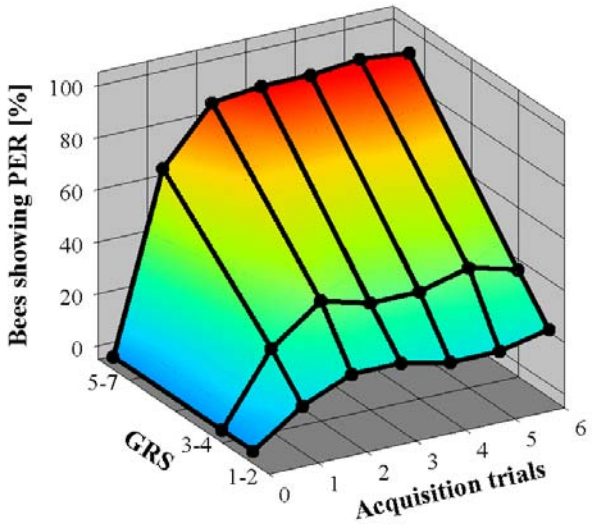


**Figure 16 Tactile acquisition curves of wild-type foragers. Pollen and non-pollen foragers were pooled, because foraging role had no effect on tactile acquisition. Bees were grouped according to their gustatory response scores (GRS). Bees with GRS ≤ 2 showed low sucrose responsiveness, those with GRS 3 - 4 showed intermediate sucrose responsiveness, and bees with GRS ≥ 5 displayed high sucrose responsiveness. Note that the series of sucrose concentrations used in this experiment is different from that in Experiments 1 and 2. The x-axis represents the acquisition trials, the y-axis the grouped gustatory response scores and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested: GRS 1 - 2: n = 24, GRS 3 - 4: n = 22, GRS 5 - 7: n = 64.**

Acquisition scores and extinction CS+ scores correlated positively with GRS for wild-type pollen and non-pollen foragers (Table 15). The slopes of the linear regressions of tactile acquisition scores on GRS (Table 16) or of extinction CS+ scores on GRS (Table 17) did not differ between pollen and non-pollen foragers (acquisition on GRS: $t = 1.03$, $df = 108$, $p >$

0.05, extinction CS+ on GRS: t = 0.67, df = 108, p > 0.05, two-tailed t-test). The intercepts were not different from zero in both groups.

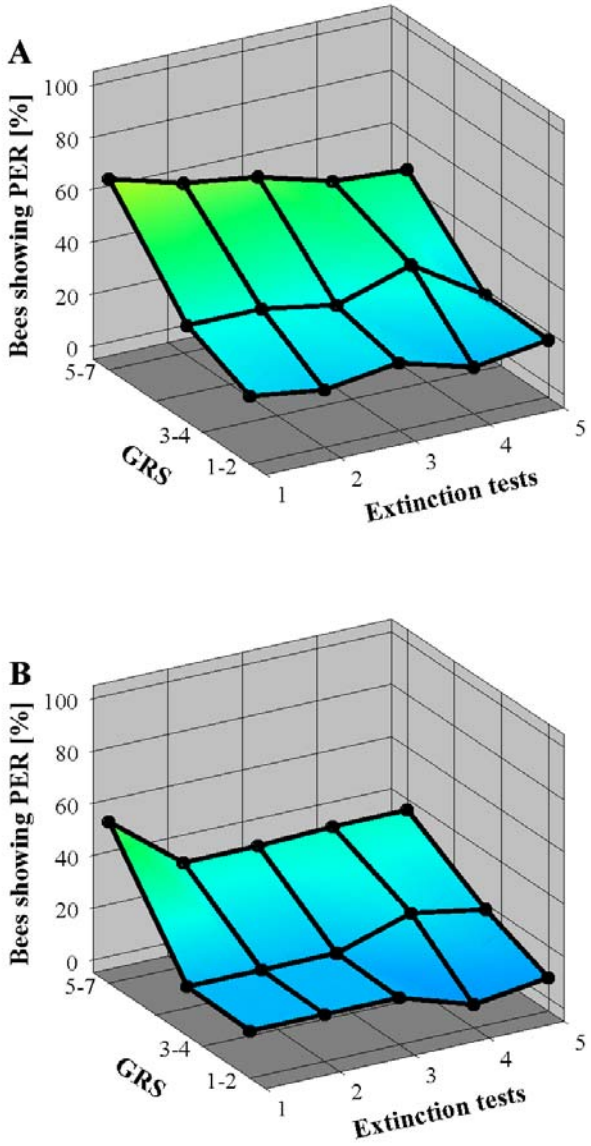


**Figure 17 Pooled tactile extinction CS+ (A) and extinction CS- (B) curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension. Numbers of bees as in Figure 16.**

This shows that pollen and non-pollen foragers with high sucrose responsiveness learned well and showed a slow decay of conditioned responses during the extinction tests, while those with low sucrose responsiveness learned poorly and showed strong extinction. Pollen and non-pollen foragers did not differ in their relationships between sucrose responsiveness, tactile acquisition and extinction of conditioned responses. Extinction CS+ scores correlated positively with acquisition scores (Table 15). Interestingly, the slope of the linear regression

of extinction CS+ scores on acquisition scores (Table 18) was significantly steeper for pollen foragers than for non-pollen foragers ($t = 2.30$, $df = 108$, $p < 0.05$, two-tailed t-test), the intercepts were not different from zero in both groups, which demonstrates that non-pollen foragers with a given acquisition score showed stronger extinction than pollen foragers with the same acquisition score.

### 3.3.3 Tactile discrimination in wild-type foragers

Discrimination of tactile patterns was not affected by foraging role. The groups with low or intermediate sucrose responsiveness responded rarely to the alternative pattern (CS-), while bees with high sucrose responsiveness responded frequently (Figure 17B). Both pollen and non-pollen foragers had higher extinction CS+ scores than extinction CS- scores (pollen foragers: $z = 3.86$, $n = 55$, $p < 0.001$, non-pollen foragers: $z = 2.52$, $n = 55$, $p < 0.05$, two-tailed Wilcoxon-test) and thus demonstrated discrimination of tactile surface structures. To compare discrimination of pollen and non-pollen foragers, discrimination indices were calculated (Table 23). Tactile discrimination indices did not differ between pollen and non-pollen foragers (Figure 36A), showing that foraging role did not affect tactile discrimination. Extinction CS- scores correlated positively with GRS and acquisition scores (Table 15). The slopes of the corresponding regressions (Table 17) of extinction CS- scores on GRS or on acquisition scores did not differ between pollen and non-pollen foragers (for GRS: $t = 0.30$, $df = 100$, $p > 0.05$, for acquisition: $t = 1.31$, $df = 96$, $p > 0.05$, Welch's t-test). All the intercepts did not differ from zero. These findings imply that bees with high sucrose responsiveness showed good acquisition but discriminated poorly, while bees with low sucrose responsiveness learned poorly but discriminated better, regardless of foraging role.

### 3.3.4 Reversal tactile acquisition and extinction in wild-type foragers

After the first extinction phase, pollen and non-pollen foragers were conditioned a second time, this time to the pattern which had served as alternative pattern in the first acquisition phase (pattern with horizontal grooves). Foraging role had no effect on reversal tactile acquisition.

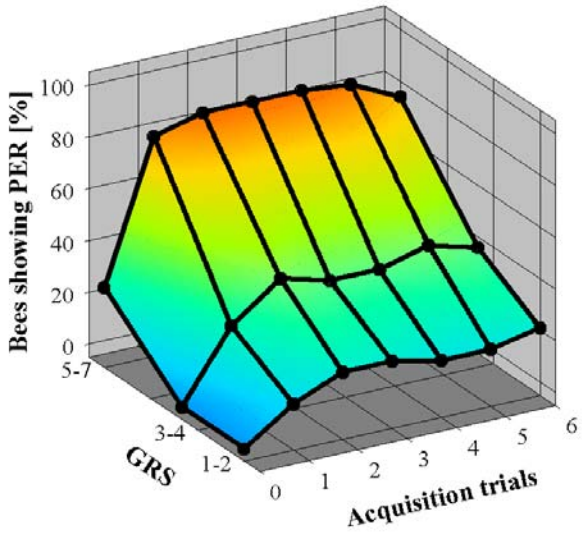


**Figure 18 Pooled reversal tactile acquisition curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the reversal acquisition trials, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested as in Figure 16.**

Regardless of foraging role, bees with low sucrose responsiveness showed poor reversal tactile acquisition (Figure 18) and strong extinction (Figure 19A), whereas more than 80 % of the bees with high sucrose responsiveness learned the now conditioned tactile pattern and showed weak extinction. In pollen and non-pollen foragers, reversal acquisition scores correlated significantly with GRS (Table 19). Bees with high sucrose responsiveness had higher reversal tactile acquisition scores than bees with lower sucrose responsiveness. Pollen foragers did not differ from non-pollen foragers in the slope of the linear regression of reversal acquisition scores on GRS (Table 20, $t = 0.89$, $df = 108$, $p > 0.05$, two-tailed t-test), the intercepts of both groups were not different from zero, which indicates the same relationship between GRS and reversal acquisition in both groups of foragers. For non-pollen foragers, a significant linear regression of reversal extinction CS+ scores on GRS was found, for pollen foragers not (Table 21). Reversal extinction CS+ scores strongly correlated with reversal acquisition scores in both pollen and non-pollen foragers (Table 19). The slopes of the corresponding linear regressions (Table 22) were not significantly different between pollen and non-pollen foragers ($t = 1.50$, $df = 100$, $p > 0.05$, two-tailed Welch's t-test). The intercepts were not different from zero in both groups. Taken together, these findings indicate that pollen and non-pollen foragers did not differ grossly in their relationships between GRS, reversal acquisition and reversal extinction CS+. The reversal learning phase supports the data gained in the first learning phase. Regardless of foraging role, bees with good reversal

acquisition showed weak extinction, while bees with poor reversal acquisition showed strong extinction.

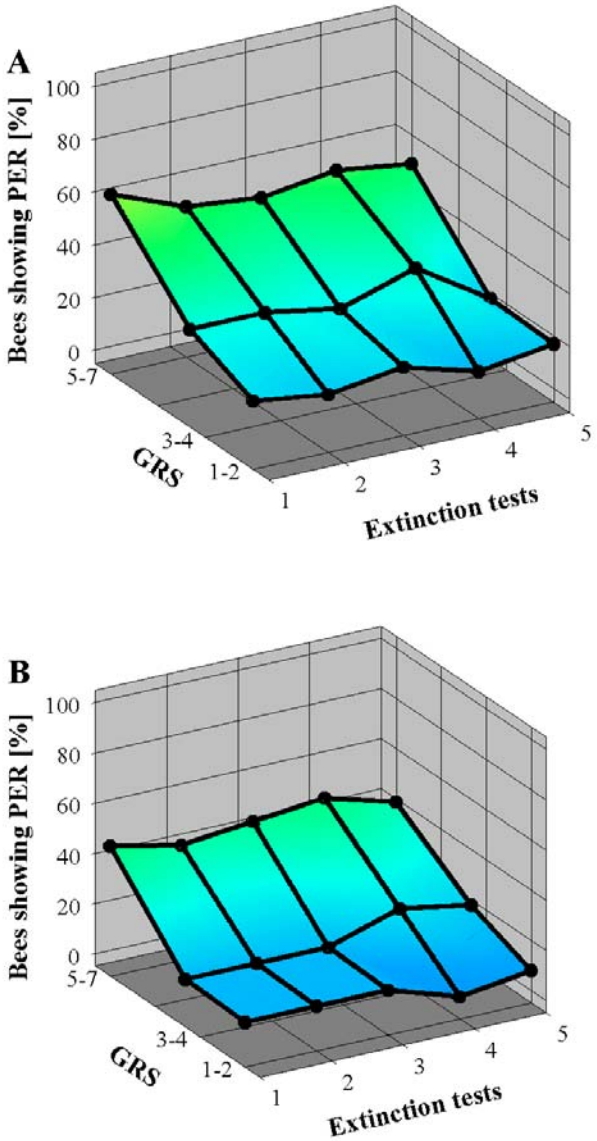


**Figure 19 Pooled tactile reversal extinction CS+ (A) and extinction CS- (B) curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested as in Figure 16.**

### 3.3.5 Tactile discrimination after reversal learning in wild-type foragers

Tactile discrimination after reversal acquisition was as good as after the first acquisition phase. Foraging role affected the transfer functions of extinction CS- scores on reversal acquisition scores. Bees with low or intermediate sucrose responsiveness responded rarely to the alternative vertical pattern (although this pattern had been used as CS+ during the first acquisition phase), while about 40 % of the bees with high sucrose responsiveness

responded (Figure 19B). Pollen and non-pollen foragers discriminated significantly between the now conditioned horizontal pattern and the now alternative vertical pattern (pollen foragers: $z = 1.97$, $n = 55$, $p < 0.05$, non-pollen foragers: $z = 2.20$, $n = 55$, $p < 0.05$, two-tailed Wilcoxon-test). Discrimination indices of pollen and non-pollen foragers (Table 23) did not differ significantly ($z = 1.16$, $n_{pollen} = 37$, $n_{non\text{-}pollen} = 32$, $p > 0.05$, two-tailed Mann-Whitney U-test). Reversal extinction CS- scores correlated significantly with GRS and reversal tactile acquisition scores in non-pollen foragers (Table 19). In pollen foragers, however, reversal extinction CS- scores only correlated with reversal acquisition scores (Table 19). Foragers with high reversal acquisition scores discriminated less well than bees with low reversal acquisition scores. The slopes of the linear regressions of reversal extinction CS- scores on reversal acquisition scores (Table 22) were significantly steeper for pollen foragers than for non-pollen foragers ($t = 2.39$, $df = 108$, $p < 0.05$, two-tailed t-test). The intercepts of both groups did not differ from zero. This shows that after reversal learning, pollen foragers with a given acquisition score were more responsive to the now alternative vertical pattern than non-pollen foragers with the same acquisition score. This is well in line with the finding that after the first tactile acquisition phase, pollen foragers showed less extinction to the conditioned vertical pattern than non-pollen foragers. Pollen foragers appear to keep responding to a stimulus once trained longer than non-pollen foragers.

### 3.3.6 Comparison of the two tactile learning phases

Tactile learning and reversal learning basically showed the same phenomenon: sucrose responsiveness determined the level of acquisition, extinction and discrimination to a large extent. GRS correlated positively with acquisition (Table 16) and extinction CS+ scores (Table 20) in pollen and non-pollen foragers. The slope coefficients of the corresponding regressions (Table 17 and Table 21, respectively) did not differ between the two learning phases (acquisition scores on GRS: pollen foragers: $t = 0.56$, $df = 102$, $p > 0.05$, two-tailed Welch's t-test, for non-pollen foragers: $t = 0.53$, $df = 108$, $p > 0.05$, two-tailed t-test; extinction CS+ scores on GRS: pollen foragers: $t = 0.85$, $df = 101$, $p > 0.05$, two-tailed Welch's t-test, non-pollen foragers: $t = 0.47$, $df = 108$, $p > 0.05$, two-tailed t-test). All the intercepts did not differ from zero. Regardless of foraging role, individuals with high sucrose responsiveness showed strong tactile learning and reversal learning and weak extinction. Bees with low sucrose responsiveness learned poorly and demonstrated strong extinction of conditioned responses. Extinction CS+ scores correlated positively with acquisition scores in both learning phases (Table 15, Table 19, respectively). The slopes of the corresponding

regressions (Table 18 and Table 22, respectively) did not differ between the first and the second acquisition phases (pollen foragers: $t = 0.62$, $df = 108$, $p > 0.05$, two-tailed t-test, non-pollen foragers: $t = 0.50$, $df = 54$, $p > 0.05$, two-tailed Welch's t-test). The intercepts did not differ from zero in both groups. This implies that the first acquisition phase did not affect reversal acquisition or extinction. In both phases of the experiment, the relationships between sucrose responsiveness, tactile acquisition and extinction of conditioned responses were similar. Pollen and non-pollen foragers significantly discriminated between the conditioned pattern and the alternative pattern in both phases of the experiment, although the pattern which served as CS+ in the first learning phase was used as alternative pattern in the second part of the experiment. The discrimination indices did not differ between the two phases (pollen foragers: $z = 1.144$, $n = 55$, $p > 0.05$, non-pollen foragers: $z = 0.898$, $n = 55$, $p > 0.05$, two-tailed Wilcoxon-test). Extinction CS- scores correlated positively with sucrose responsiveness and acquisition in pollen and non-pollen foragers after the first learning phase (Table 15). After reversal learning, sucrose responsiveness correlated with extinction CS- scores only in non-pollen foragers, whereas acquisition correlated with extinction CS- in both groups of foragers (Table 19). The slopes of the regressions of extinction CS- scores on acquisition scores (Table 18 and Table 22, respectively) did not differ between the two learning phases (pollen foragers: $t = 0.86$, $df = 108$, $p > 0.05$, non-pollen foragers: $t = 0$, $df = 108$, $p > 0.05$, two-tailed t-test). The intercepts were not different from zero. In both learning phases, pollen foragers with a given acquisition score responded more often to the vertical pattern than non-pollen foragers with the same acquisition score, although this pattern had served as CS+ in the first acquisition phase and served as CS- in the reversal acquisition phase.

### 3.3.7 Olfactory acquisition and extinction in wild-type foragers

Similar to tactile learning, sucrose responsiveness determined the level of olfactory acquisition (Figure 20) and extinction (Figure 21A). Foraging role had no separate effect.

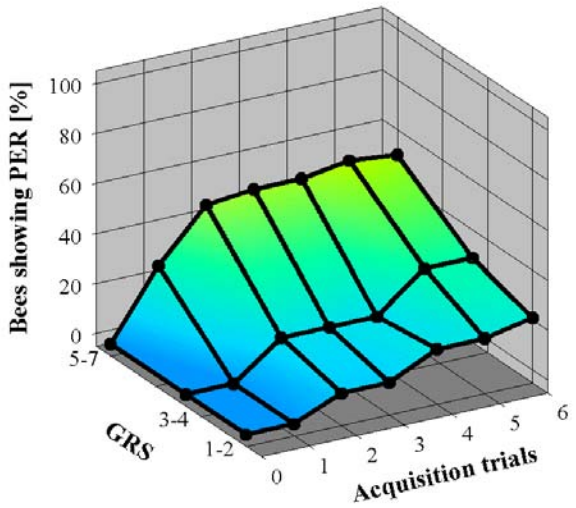


**Figure 20 Pooled olfactory acquisition curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the acquisition trials, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees with proboscis extension (PER). Number of bees: GRS 1 - 2: n = 24, GRS 3 - 4: n = 18, GRS 5 - 7: n = 68.**

The acquisition level was generally not very high. Bees with low and intermediate sucrose responsiveness learned poorly (Figure 20) and showed strong extinction (Figure 21A), while individuals with high sucrose responsiveness learned comparatively well (Figure 20) and showed little extinction (Figure 21A). Olfactory acquisition and extinction CS+ scores correlated positively with GRS for pollen and non-pollen foragers (Table 15). The slopes of the regressions of acquisition scores on GRS (Table 16) and of extinction CS+ scores on GRS (Table 17) did not differ between pollen and non-pollen foragers (for acquisition scores on GRS: $t = 1.14$, $df = 92$, $p > 0.05$, for extinction scores on GRS: $t = 0$, $df = 88$, $p > 0.05$, two-tailed Welch's t-test). All intercepts did not differ from zero. Extinction CS+ scores significantly correlated with acquisition scores in pollen and non-pollen foragers (Table 15). The slopes of the respective regressions (Table 18) did not differ between pollen and non-pollen foragers ($t = 1.54$, $df = 108$, $p > 0.05$, two-tailed t-test). The intercepts of both groups did not differ from zero. Therefore, pollen and non-pollen foragers did not differ in the relationships between sucrose responsiveness, acquisition and extinction in olfactory learning. Regardless of foraging role, bees with high sucrose responsiveness showed high acquisition and weak extinction, whereas bees with low gustatory response scores showed low acquisition and strong extinction.

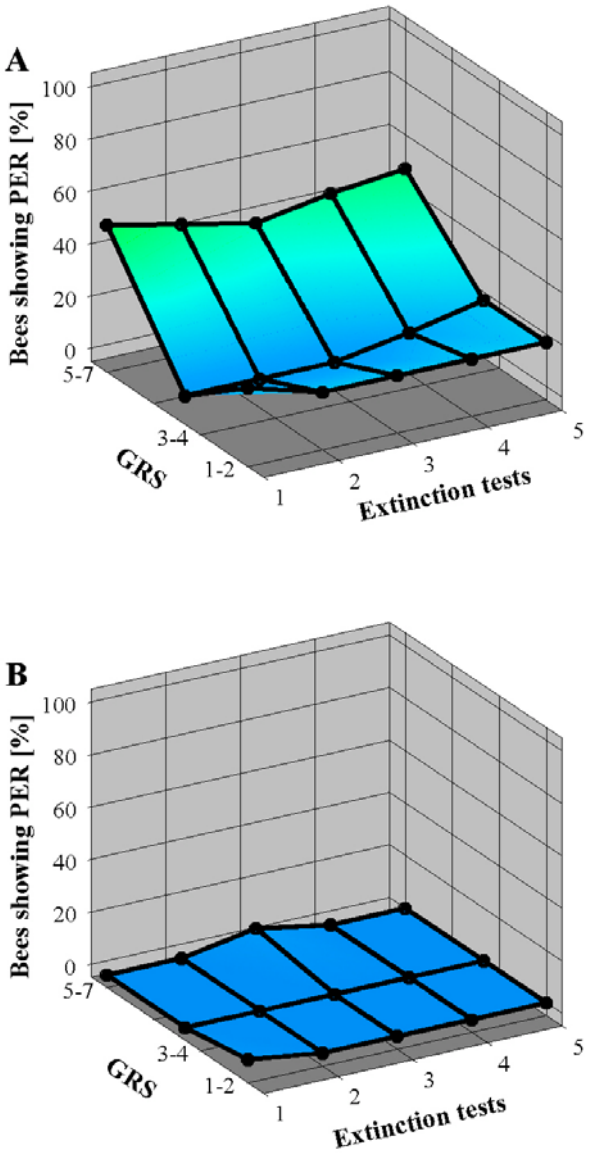


**Figure 21 Pooled olfactory extinction CS+ (A) and extinction CS- (B) curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested as in Figure 20.**

### 3.3.8 Olfactory discrimination in wild-type foragers

Olfactory discrimination was excellent in pollen and non-pollen foragers and was not affected by foraging role. Pollen and non-pollen foragers basically did not respond to the alternative odour (Figure 21B). Probably for that reason extinction CS- scores and acquisition scores did not correlate significantly with GRS (Table 15). Pollen and non-pollen foragers had significantly higher extinction CS+ scores than extinction CS- scores (pollen foragers: $z = 4.74$ $n = 55$, $p < 0.001$, non-pollen foragers: $z = 4.32$, $n = 55$, $p < 0.001$, two-tailed Wilcoxon-test), which also demonstrates significant discrimination of odours. Olfactory discrimination

indices (Table 23) did not differ significantly between pollen and non-pollen foragers (Figure 36, $z = 1.20$, $n_{pollen} = 29$, $n_{non\text{-}pollen} = 24$, $p > 0.05$, two-tailed Mann-Whitney U-test).

### 3.3.9 Reversal olfactory acquisition and extinction in wild-type foragers

Similar to the first olfactory learning phase, reversal acquisition and extinction were strongly affected by sucrose responsiveness in pollen and non-pollen foragers of the wild type. Individuals with high sucrose responsiveness reached a comparatively high level of reversal acquisition (Figure 22) and demonstrated little extinction (Figure 23A). Bees with low or intermediate sucrose responsiveness did not learn well (Figure 22) and showed strong extinction (Figure 23A).

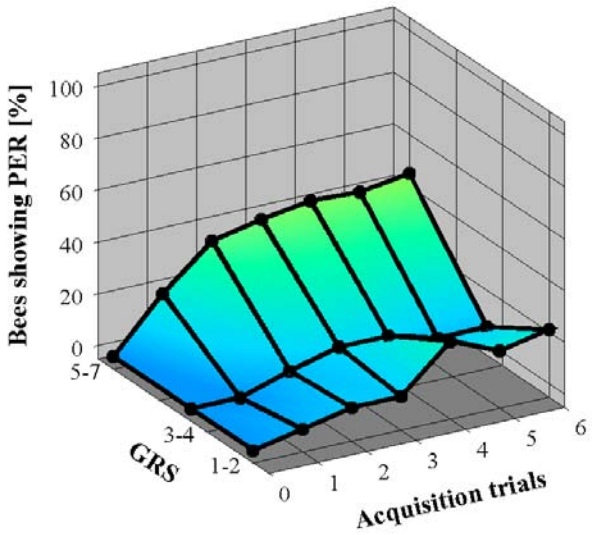


**Figure 22 Pooled reversal olfactory acquisition curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the reversal acquisition curves, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested as in Figure 20.**

In pollen and non-pollen foragers, reversal olfactory acquisition scores correlated positively with GRS (Table 19). Individuals with high sucrose responsiveness had a higher level of reversal acquisition than bees with low sucrose responsiveness. The slopes of the corresponding regressions of reversal acquisition scores on GRS (Table 20) did not differ significantly between the two groups of foragers ($t = 0.43$, $df = 89$, $p > 0.05$, two-tailed Welch's t-test). The intercepts did not differ from zero. This indicates that foraging role had no effect on the relationship between sucrose responsiveness and reversal acquisition. Reversal extinction CS+ scores correlated with GRS in non-pollen foragers but not in pollen foragers (Table 19).

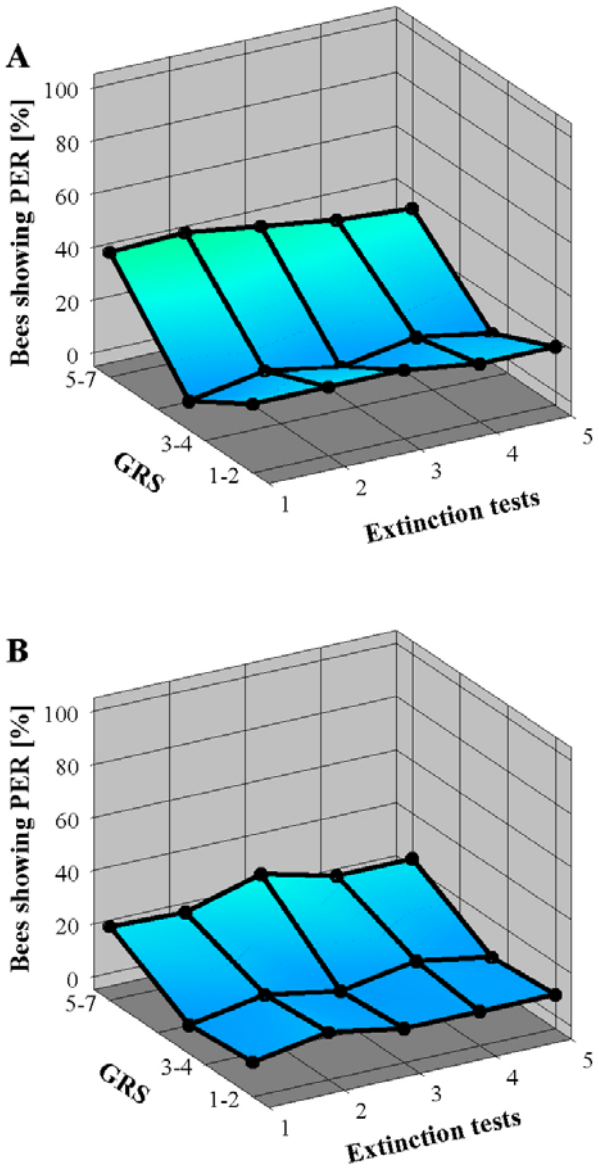


**Figure 23 Pooled reversal olfactory extinction CS+ (A) and CS- (B) curves of wild-type pollen and non-pollen foragers. Bees were grouped according to their sucrose responsiveness (see Figure 16). The x-axis represents the extinction tests, the y-axis the grouped gustatory response scores (GRS) and the z-axis the percentage of bees showing proboscis extension (PER). Number of bees tested as in Figure 20.**

In both pollen and non-pollen foragers, reversal extinction CS+ correlated significantly with reversal olfactory acquisition scores (Table 19). The slope coefficients of the corresponding regressions (Table 22) were not different between pollen and non-pollen foragers ($t = 1.40$, $df = 97$, $p > 0.05$, two-tailed Welch's t-test). The intercepts of both groups did not differ from zero. Regardless of foraging role, bees with good reversal olfactory acquisition showed a slow decay of conditioned responses during the following unrewarded tests, while bees with low reversal acquisition scores showed fewer conditioned responses during the extinction tests.

### 3.3.10 Olfactory discrimination after reversal learning in wild-type foragers

Olfactory discrimination after reversal learning, which was affected by foraging role, was not as good as after the first acquisition phase. After reversal olfactory conditioning, bees with low or intermediate sucrose responsiveness responded rarely to the alternative odour. But about 20 % of the bees with high sucrose responsiveness responded. Non-pollen foragers had a significantly higher discrimination index (Table 23) than pollen foragers ($z = 2.82$, $n_{pollen} = 29$, $n_{non\text{-}pollen} = 24$, $p < 0.01$, two-tailed Mann-Whitney U-test) and thus demonstrated better discrimination. Reversal extinction CS- scores correlated with sucrose responsiveness in non-pollen foragers but not in pollen foragers (Table 19). In both pollen and non-pollen foragers, extinction CS- scores after reversal learning correlated with reversal olfactory acquisition scores (Table 19). The slopes and intercepts of the corresponding regressions (Table 22) did not differ between the two groups (for slope: $t = 1.30$, $df = 100$, $p > 0.05$, for intercept: $t = 1.53$, $df = 94$, $p > 0.05$, two-tailed Welch's t-test). Pollen and non-pollen foragers with high reversal acquisition scores discriminated poorly, whereas foragers with low reversal acquisition scores discriminated better and responded less often to the alternative odour.

### 3.3.11 Comparison of the two olfactory learning phases

During both olfactory learning phases, sucrose responsiveness determined the level of acquisition and extinction. Gustatory response scores correlated positively with acquisition scores in both learning phases (Table 15 and Table 19, respectively). The slope coefficients of the regressions of acquisition scores on GRS did not differ between the two learning phases (pollen foragers: $t = 0.889$, $df = 108$, $p > 0.05$, non-pollen foragers: $t = 0.51$, $df = 108$, $p > 0.05$, two-tailed t-test). The intercepts did not differ from zero. Extinction of conditioned responses correlated with sucrose responsiveness in pollen and non-pollen foragers after the first acquisition phase (Table 15), but only in non-pollen foragers after reversal learning (Table 19). The slope of the regression of extinction CS+ scores on gustatory response scores did not differ for non-pollen foragers between the first and the second learning phases ($t = 0.149$, $df = 108$, $p > 0.05$, two-tailed t-test). The intercepts were not different from zero at both times. Extinction CS+ scores correlated positively with acquisition scores in pollen and non-pollen foragers in both learning phases (Table 15 and Table 19, respectively). The slopes of the corresponding regressions did not differ between the two learning phases (pollen foragers: $t = 0.22$, $df = 108$, $p > 0.05$, non-pollen foragers: $t = 0.28$, $df = 108$, $p > 0.05$, two-

tailed t-test). The intercepts were not different from zero. Olfactory discrimination was significantly poorer after reversal learning compared to the first learning phase. The discrimination indices of pollen and non-pollen foragers were significantly smaller after reversal learning (pollen foragers: $z = 4.21$, $n = 55$, $p < 0.001$, non-pollen foragers: $z = 2.94$, $n = 55$, $p < 0.01$, two-tailed Wilcoxon-test). Extinction CS- scores did not correlate with sucrose responsiveness or acquisition in the first learning phase (Table 15), probably because most of the bees did not respond to the alternative odour. In the second learning phase, extinction CS- scores correlated positively with GRS in non-pollen foragers (Table 19) and with acquisition scores in non-pollen and pollen foragers (Table 19). These findings show that discrimination was better after the first learning phase than after the second learning phase, indicating that bees did not stop responding to the CS+ (citral) from the first phase of the experiment when it was presented as CS- in the second phase. In addition, non-pollen foragers discriminated significantly better than pollen foragers after reversal learning, although there were no differences in the discrimination of the two groups after the first acquisition phase.

## *3.4 Experiment 4 The effect of stimulation site on tactile learning*

### 3.4.1 The effect of stimulation site on acquisition and extinction

The sucrose concentration applied to the proboscis strongly affected acquisition and extinction of conditioned responses, whereas antennal sucrose stimulation played a minor part. In all four groups, the percentage of bees showing the conditioned PER increased with increasing number of acquisition trials, but the sucrose concentration applied to the proboscis determined the level of acquisition and extinction (Figure 24). Bees which had been stimulated with different sucrose concentrations at the antenna (A) but which had been rewarded with the same sucrose concentration at the proboscis (P) did not differ in the level of acquisition (A: 1.6 % P: 1.6 % vs. A: 30 % P: 1.6 %: $p > 0.05$, A: 30 % P: 30 % vs. A: 1.6 % P: 30 %: $p > 0.05$, $n = 40$ in each group, two-tailed Fisher-Exact-test). The short antennal sucrose stimulation did not determine how well a bee learned, as long as the sucrose concentration of the reward offered to the proboscis remained constant. If, however, the sucrose concentration offered to the proboscis varied, while the sucrose concentration presented to the antenna remained constant, the level of acquisition differed between the groups (A: 1.6 % P: 1.6 % vs. A: 1.6 % P: 30 %: $p < 0.05$, A: 30 % P: 30 % vs. A: 30 % P: 1.6 %: $p < 0.01$, two-tailed Fisher-Exact-test).

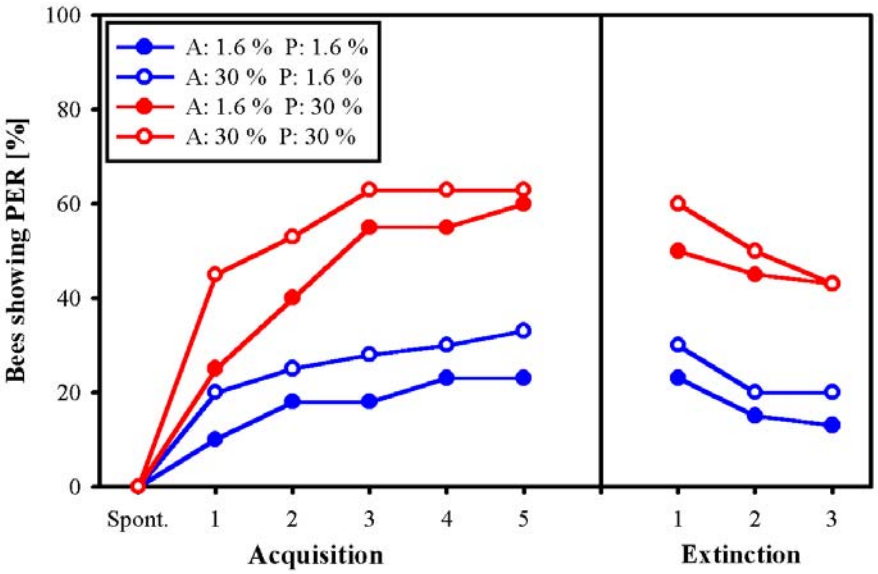


**Figure 24 Acquisition and extinction curves of bees which were stimulated with different sucrose concentrations at antennae and proboscis. The abscissa represents the acquisition and extinction trials and the ordinate the percentage of bees showing the proboscis extension response (PER). In each group 40 bees were conditioned. All of the bees showed the same sucrose responsiveness.**

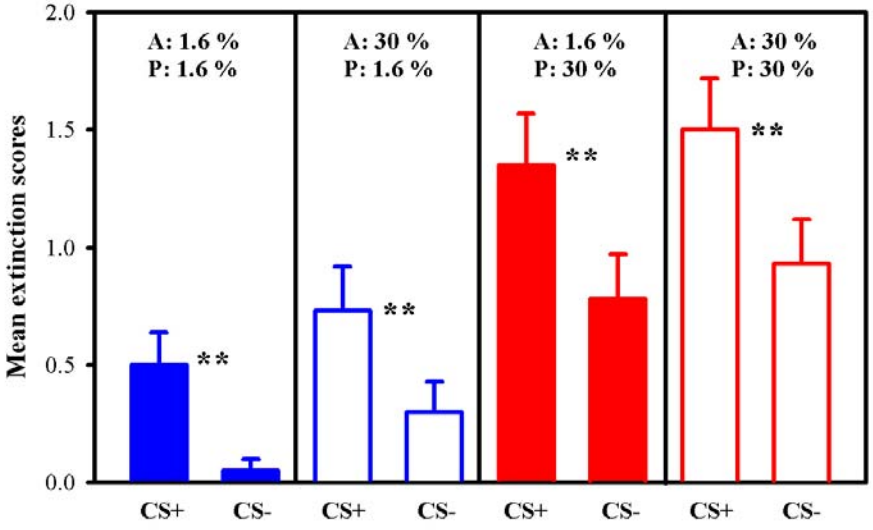


**Figure 25 Mean extinction scores of responses to the conditioned vertical pattern (CS+) and to the alternative horizontal pattern (CS-) in bees which had been conditioned with different sucrose concentrations at antenna and proboscis. Means and standard errors of the means are shown. Significant differences between responses to the CS+ and those to the CS- are indicated by asterisks (**: $p < 0.01$, two-tailed Wilcoxon-test). Number of bees tested as in Figure 24.**

Bees which could imbibe 30 % sucrose but were stimulated with 1.6 % sucrose at the antenna learned very well, whereas bees which were stimulated with 30 % sucrose at the antenna and

with 1.6 % sucrose at the proboscis only learned poorly. The level of extinction was determined by acquisition in all four groups.

### 3.4.2 The effect of stimulation site on discrimination

Discrimination strongly depended on acquisition and was therefore indirectly affected by stimulation site. All four groups of bees demonstrated good discrimination between the conditioned pattern and the alternative pattern by responding significantly more often to the conditioned pattern (Figure 25). To directly compare discrimination of the four groups, discrimination indices were calculated (Table 23). The discrimination index of the group A: 1.6 % P: 1.6 % was significantly higher than were the indices of the groups A: 1.6 % P: 30 % and A: 30 % P: 30 % (Figure 38). Bees that received the smallest reward (A: 1.6 % P: 1.6 %) learned only poorly but discriminated best. The higher the reward, the better was acquisition but the poorer became discrimination.

## *3.5 General comparison of sucrose responsiveness*

Sucrose responsiveness strongly depended on genotype (Figure 26), foraging role (Figure 27) and age (Figure 28). High-strain foragers, which are more likely to collect pollen, were more responsive to water and different sucrose concentrations than low-strain foragers, which have a higher probability of collecting nectar (Figure 26A). Preforagers of the high strain had higher gustatory response scores than preforagers of the low strain (Figure 26B). As these bees had no foraging experience, this difference clearly demonstrates an effect of genotype on sucrose responsiveness. Foraging role also affected sucrose responsiveness (Figure 27). Regardless of whether high-strain bees (Figure 27A), low-strain bees (Figure 27B) or wild-type foragers (Figure 27C) were analysed, pollen foragers had always higher gustatory response scores than non-pollen foragers. These findings demonstrate a clear relationship between foraging behaviour and sucrose responsiveness.

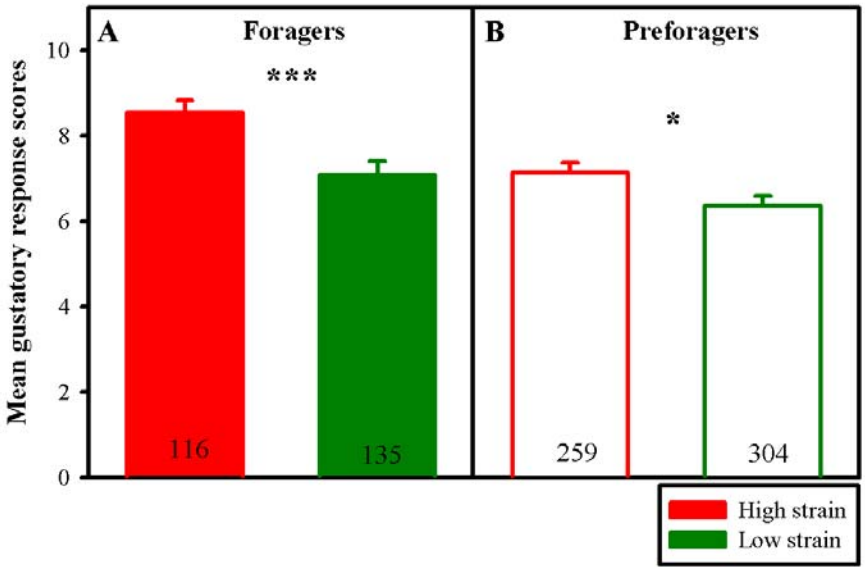


**Figure 26 The effect of genotype on sucrose responsiveness. Mean gustatory response scores of high- and low-strain bees. A: Foragers. B: Preforagers. Means and standard errors of the of means are shown. Significant differences between the strains are marked with asterisks (*: $p < 0.05$, ***: $p < 0.001$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

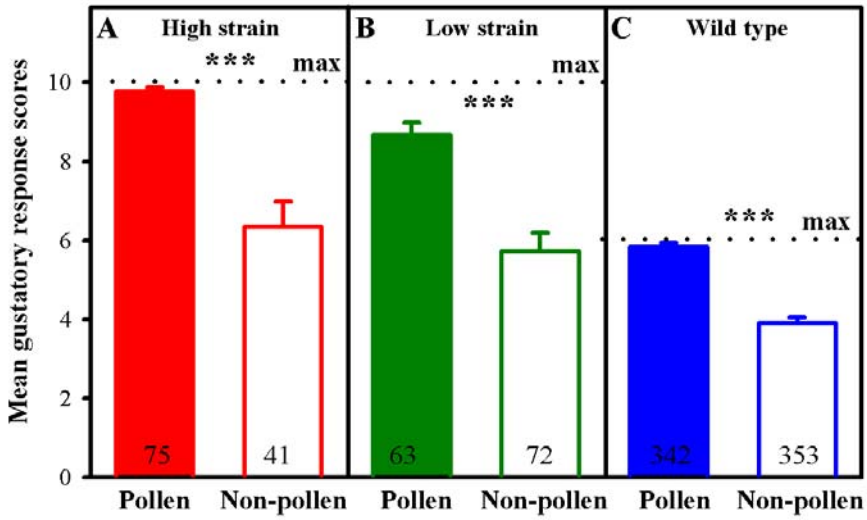


**Figure 27 The effect of foraging role on sucrose responsiveness. Mean gustatory response scores of pollen and non-pollen foragers. A: High-strain foragers. B: Low-strain foragers. C: Wild-type foragers. Means and standard errors of the means are shown. Significant differences are marked with asterisks (***: $p < 0.001$, two-tailed Mann-Whitney U-test). Gustatory response scores were measured with different sucrose concentrations in wild-type bees vs. bees of the two genetic strains. Therefore, the maximum gustatory response scores (max.) are indicated. Number of bees tested is shown.**

The effect of age on sucrose responsiveness (Figure 28) can be seen in the high strain and in the wild type. In the high strain, preforagers had significantly lower gustatory response scores than foragers (Figure 28A). In the low strain, there is no significant difference between foragers and preforagers (Figure 28B). In wild-type bees, preforagers not older than 1 day had significantly lower GRS than foragers (Figure 28C), while 5-day-old preforagers did not differ significantly from foragers (Figure 28C).

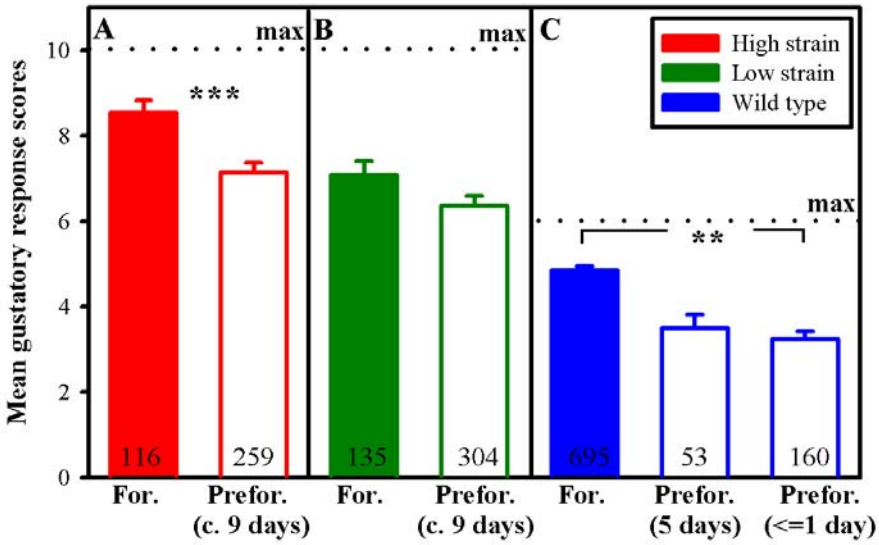


**Figure 28 The effect of age on sucrose responsiveness. Mean gustatory response scores of preforagers (Prefor.) of different ages and of foragers (For.). A: High-strain bees. B: Low-strain bees. C: Wild-type bees. Means and standard errors of the means are shown. Significant differences are marked with asterisks (**: $p < 0.01$, ***: $p < 0.001$, two-tailed Mann-Whitney U-test). As GRS were measured with different sucrose concentrations in wild-type bees vs. bees of the two genetic strains, the maximum GRS are indicated (max.). Number of bees tested is indicated.**

In both genetic strains, pollen foragers showed a higher degree of sucrose responsiveness and displayed significantly higher gustatory response scores than preforagers (high strain: $z = 6.15$, $n_{\text{pollen foragers}} = 75$, $n_{\text{preforagers}} = 259$, $p < 0.001$, low strain: $z = 4.32$, $n_{\text{pollen foragers}} = 63$, $n_{\text{preforagers}} = 304$, $p < 0.001$, two-tailed Mann-Whitney U-test). Non-pollen foragers, in contrast, did not differ from preforagers in their GRS (high strain: $z = 1.06$, $n_{\text{non-pollen foragers}} = 41$, $n_{\text{preforagers}} = 304$, low strain: $z = 1.39$, $n_{\text{non-pollen foragers}} = 72$, $n_{\text{preforagers}} = 304$). In wild-type bees, too, pollen foragers had significantly higher GRS than either group of preforagers (bees not older than 1 day: $z = 11.99$, $n_{\text{pollen foragers}} = 342$, $n_{\text{preforagers} \leq 1\text{d}} = 160$, $p < 0.001$, 5-day-old bees: $z = 7.47$, $n_{\text{preforagers 5d}} = 53$, $p < 0.001$, two-tailed Mann-Whitney U-test). Non-pollen foragers of the wild type did not differ from 5-day-old wild-type bees ($z = 1.16$, $n_{\text{preforagers 5d}} = 53$, $p < 0.01$, two-tailed Mann-Whitney U-test) but had significantly higher GRS than bees not older than 1 day ($z = 2.88$, $n_{\text{non-pollen foragers}} = 353$, $p < 0.01$, two-tailed Mann-Whitney U-

test). These findings show that in both genetic strains and in the wild type, pollen foragers were more responsive to sucrose than preforagers, whereas non-pollen foragers did not differ from preforagers, implying a complex relationship between sucrose responsiveness, age and division of foraging labour.

## *3.6 General comparison of acquisition in associative PER learning*

In all groups of bees, acquisition scores correlated positively with GRS (high- and low-strain foragers: Table 7, high- and low-strain preforagers: Table 11, wild-type foragers: Table 15). Bees with high sucrose responsiveness showed better acquisition than bees with lower sucrose responsiveness. Genotype and foraging role did not affect the relationship between sucrose responsiveness and acquisition. Age affected this relationship only in low-strain bees.

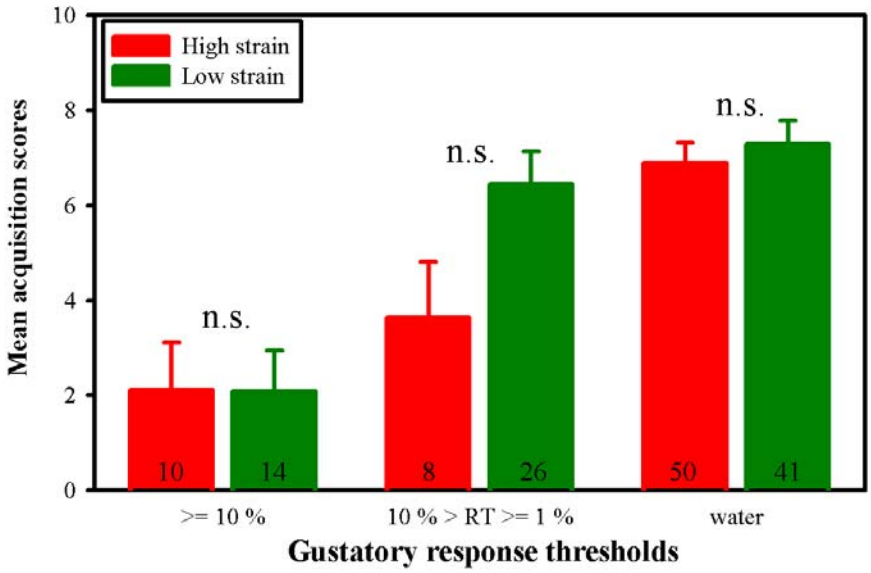


**Figure 29 The effect of genotype on the relationship between sucrose responsiveness and acquisition in high- and low-strain foragers. Sucrose responsiveness was grouped differently this time for easy comparisons with wild-type bees. (RT = response threshold. In this experiment, RT indicates the lowest sucrose concentration that elicits proboscis extension. RT ≥ 10 % corresponds to GRS 1 - 4; 10 % > RT ≥ 1 % corresponds to GRS 5 - 9 and RT = water corresponds to GRS 10). Means and standard errors of the means are shown. The maximum acquisition score is 10. Groups did not differ significantly in their acquisition scores (p > 0.05, two-tailed Mann-Whitney U-test), which is indicated by "n.s". Number of bees tested is indicated.**

Genotype had no effect on the relationship between sucrose responsiveness and acquisition. High- and low-strain foragers with different sucrose responsiveness did not differ in their acquisition scores (Figure 29). Preforagers of the two strains with different sucrose

responsiveness also did not differ in their tactile acquisition (Figure 30A) or in their olfactory acquisition scores (Figure 30B).

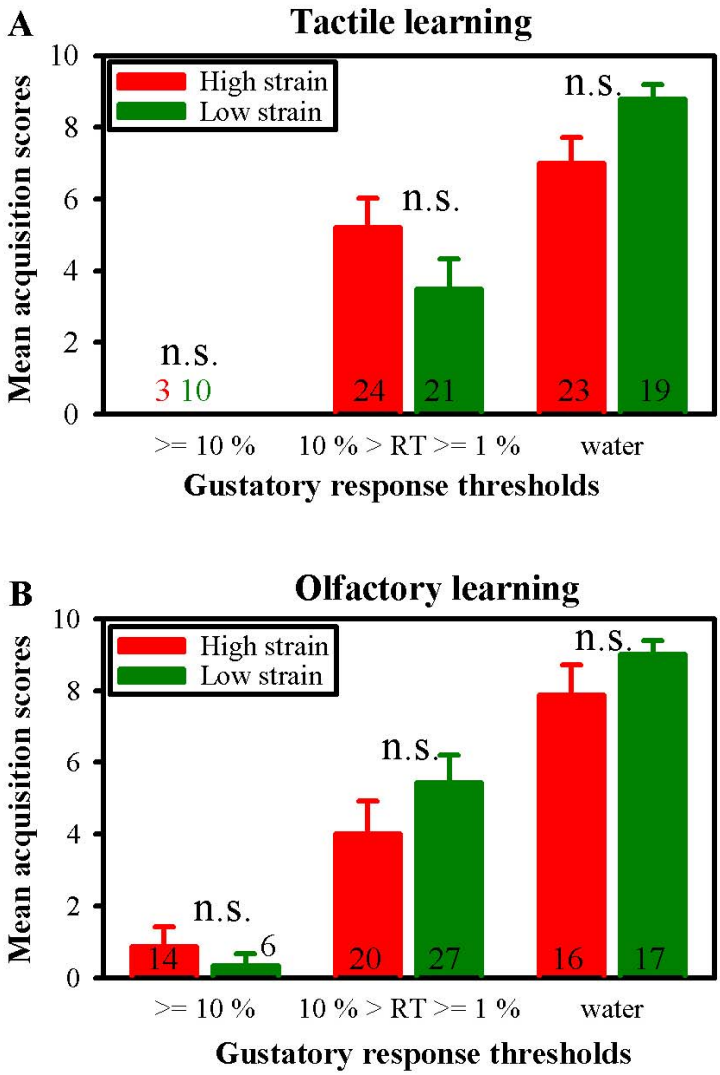


**Figure 30 The effect of genotype on the relationship between sucrose responsiveness and tactile (A) or olfactory (B) acquisition. Bees were grouped according to their response threshold (see Figure 29). Means and standard errors of the means are shown. The maximum acquisition score is 10. The two strains did not differ significantly in their acquisition scores ($p > 0.05$, two-tailed Mann-Whitney U-test), which is indicated by "n.s". The number of bees tested is indicated.**

Foraging role also did not affect the relationship between sucrose responsiveness and acquisition. High-strain pollen foragers with different sucrose responsiveness did not differ in their acquisition scores from high-strain non-pollen foragers with the same sucrose responsiveness (Figure 31A). The same is true of the low strain (Figure 31B). Wild-type pollen foragers with different sucrose responsiveness mostly did not differ in their acquisition scores from wild-type non-pollen foragers with the same sucrose responsiveness (Figure 32).

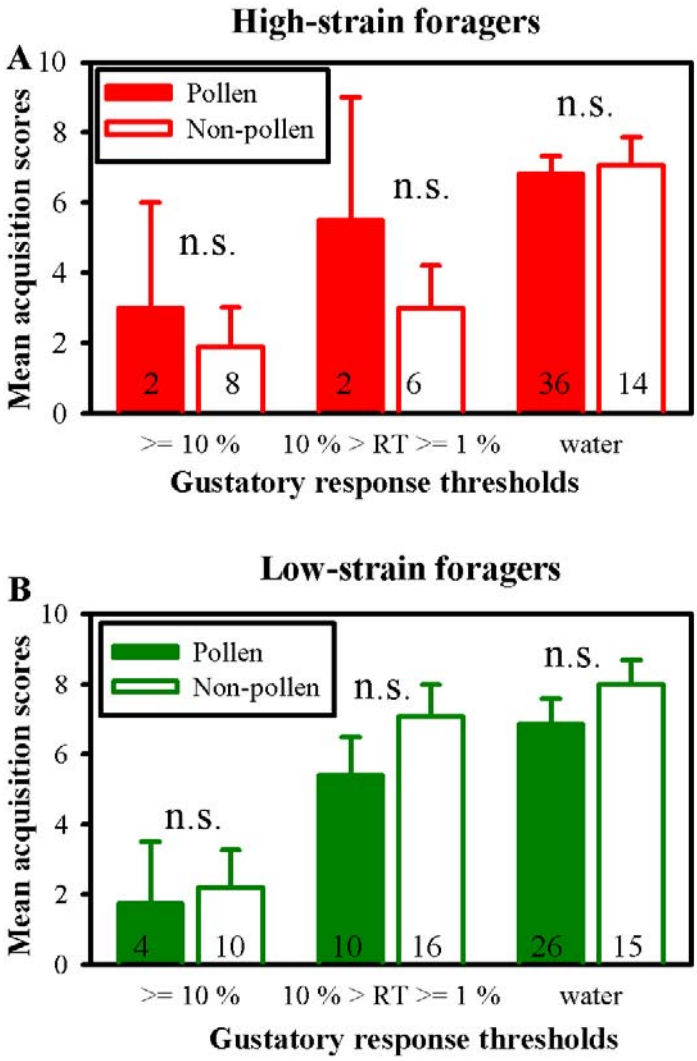


**Figure 31 The effect of foraging role on the relationship between sucrose responsiveness and acquisition in high- (A) and low-strain foragers (B). Bees were grouped according to their sucrose response threshold (see Figure 29). Means and standard errors of the means are shown. The maximum acquisition score is 10. Pollen foragers did not differ from non-pollen foragers in their acquisition scores ($p > 0.05$, two-tailed Mann-Whitney U-test), which is indicated by "n.s.". Number of bees tested is shown.**

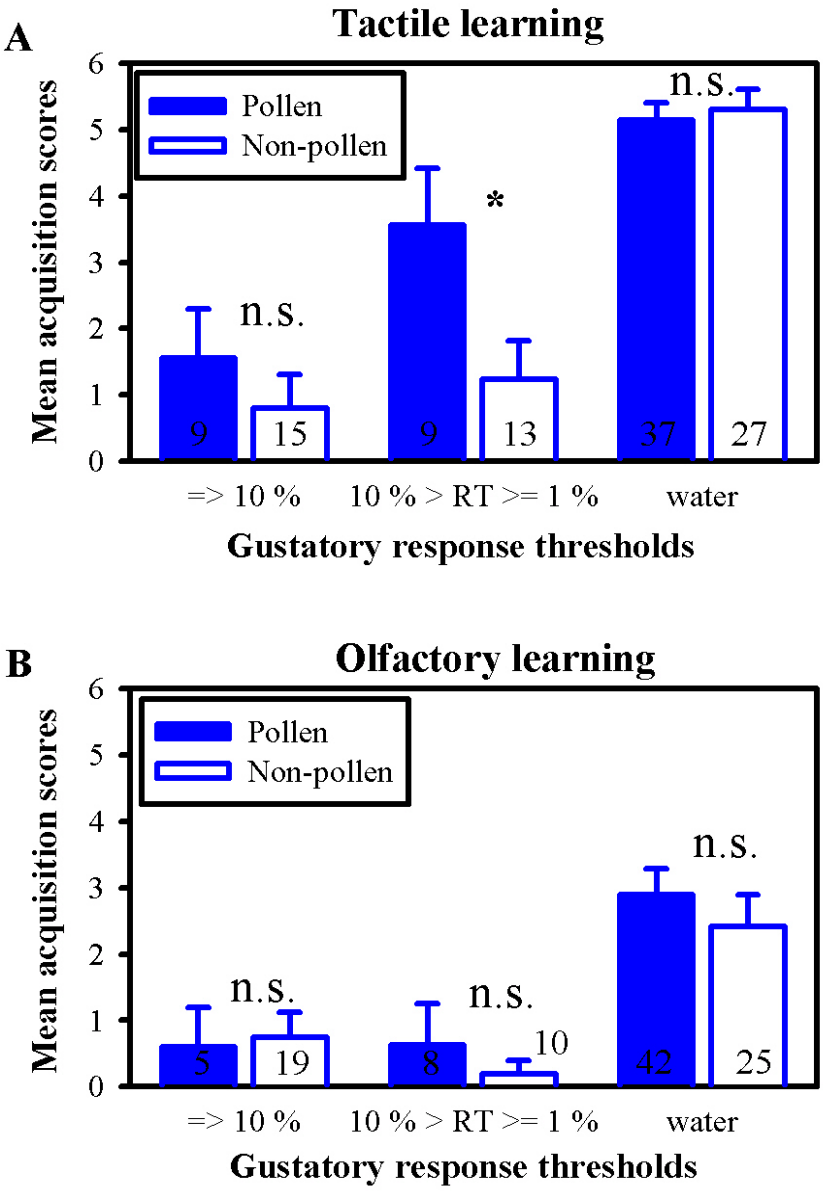


**Figure 32 The effect of foraging role on the relationship between sucrose responsiveness and tactile (A) or olfactory (B) acquisition in wild-type foragers. Bees were grouped according to their sucrose response threshold (RT, see Figure 29). RT ≥ 10 % corresponds to GRS ≤ 2, 10 % > RT ≥ 1 % corresponds to GRS 3 - 4, RT = water corresponds to GRS 5 - 7. Means and standard errors of the means are shown. The maximum acquisition score is 6. The only significant difference in acquisition scores is shown between pollen and non-pollen foragers with intermediate response thresholds in tactile acquisition ($p < 0.05$, two-tailed Mann-Whitney U-test). In all of the other groups, pollen foragers did not differ from non-pollen foragers in their acquisition scores ($p > 0.05$, two-tailed Mann-Whitney U-test), which is indicated by "n.s.". The number of bees tested is indicated.**

Age affected the relationship between sucrose responsiveness and acquisition in low-strain bees (Figure 33B), but not in high-strain bees (Figure 33A). Low-strain preforagers with low or intermediate sucrose responsiveness learned poorer than low-strain foragers with the same sucrose responsiveness. Low-strain preforagers with high sucrose responsiveness did not differ in their acquisition scores from low-strain foragers with the same sucrose responsiveness. In the high strain, none of the three groups with different sucrose

responsiveness differed in their acquisition scores between foragers and preforagers. In preforagers and foragers of both genetic strains, acquisition scores strongly correlated with sucrose responsiveness (Table 7 and Table 11).

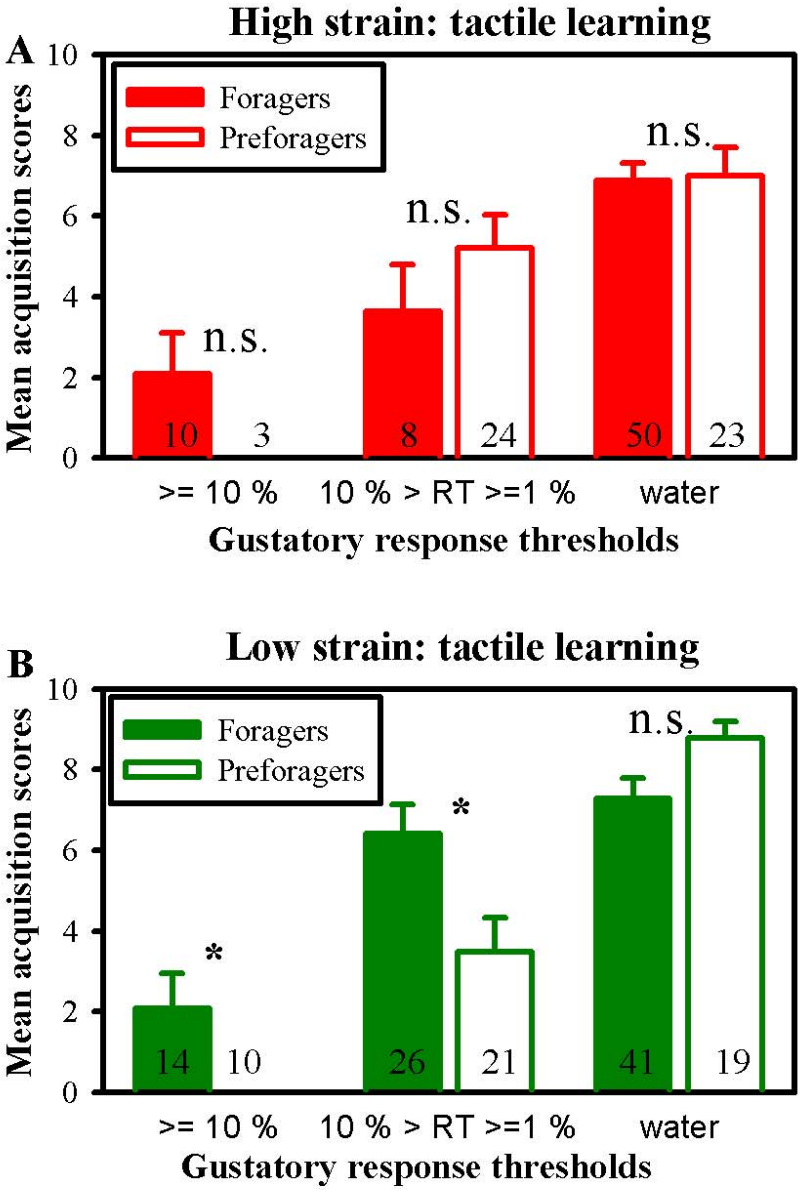


**Figure 33 The effect of age on the relationship between sucrose responsiveness and acquisition in high- (A) and low-strain foragers (B). Bees were grouped according to their sucrose response threshold (see Figure 29). Means and standard errors of the means are shown. The maximum acquisition score is 10. Significant differences are indicated by an asterisk (*:p < 0.05, two-tailed Mann-Whitney U-test). Otherwise, differences were not significant ("n. s."). The number of bees tested is indicated.**

The slopes of the linear regressions of acquisition scores on gustatory response scores (Table 8 and Table 12, respectively) did not differ significantly between foragers and preforagers of the high strain ($t = 1.42$, $df = 76$, $p > 0.05$, two-tailed Welch's t-test) and the intercepts were

not different from zero, indicating a similar relationship between sucrose responsiveness and acquisition. Low-strain preforagers had a significantly steeper slope than low-strain foragers ($t = 2.06$, $df = 129$, $p < 0.05$, two-tailed t-test), but also a negative intercept which was significantly lower than that of low-strain foragers ($t = 2.47$, $df = 121$, $p < 0.05$; two-tailed Welch's t-test). This implies that low-strain foragers with low or intermediate sucrose responsiveness learned better than low-strain preforagers with the same sucrose responsiveness, while low-strain foragers with high sucrose responsiveness did not differ in their acquisition from low-strain preforagers with the same sucrose responsiveness.

## *3.7 General comparison of extinction in associative PER learning*

Whereas genotype and foraging role had no effect on extinction, age had a strong effect. Extinction of conditioned responses correlated positively with acquisition in all groups (high- and low-strain foragers: Table 7, high- and low-strain preforagers: Table 11, wild-type foragers: Table 15). Bees with high acquisition scores showed higher extinction scores than bees with low acquisition scores. Genotype did not affect this relationship in foragers (see 3.1.2) or preforagers (see 3.2.2 and 3.2.4). Foraging role also had no effect on this relationship in high- and low-strain foragers (see 3.1.2). In the wild type (see 3.3.2 and 3.3.7), pollen foragers did not differ from non-pollen foragers in most phases of the tactile learning paradigm. Because GRS correlated with acquisition scores in all groups, and extinction scores correlated with acquisition scores in all groups, it follows from the law of transitivity that extinction scores correlate with GRS, which was found in most groups. In some groups of bees, no such correlation was found, probably because of a reduced variation in GRS. Age affected extinction of conditioned responses. Low-strain preforagers showed stronger extinction than low-strain foragers with the same acquisition scores. The slope of the linear regression of extinction CS+ scores on acquisition scores was significantly steeper for low-strain foragers than for preforagers ($t = 2.00$, $df = 128$, $p < 0.05$, two-tailed Welch's t-test). In the high strain, the difference in the slope coefficients of preforagers and foragers was not quite significant ($t = 1.80$, $df = 116$, $p = 0.075$, two-tailed t-test). The difference in extinction of conditioned responses between foragers and preforagers is particularly striking in bees with high sucrose responsiveness. In both genetic strains, preforagers with high sucrose responsiveness had significantly lower extinction scores than foragers with the same sucrose responsiveness (high strain: $z = 3.98$, $n_{foragers} = 55$, $n_{preforagers} = 34$, $p < 0.001$, low strain: $z = 3.25$, $n_{foragers} = 50$, $n_{preforagers} = 27$, $p < 0.01$, two-tailed Mann-Whitney U-test). Preforagers

with intermediate sucrose responsiveness did not differ from foragers in their extinction scores (high strain: $z = 1.24$, $n_{foragers} = 10$, $n_{preforagers} = 3$, $p > 0.05$, low strain: $z = 1.81$, $n_{foragers} = 14$, $n_{preforagers} = 10$, $p > 0.05$). Preforagers with low sucrose responsiveness had significantly lower extinction scores than foragers in the low strain ($z = 2.42$, $n_{foragers} = 17$, $n_{preforagers} = 13$, $p < 0.05$), but not in the high strain ($z = 1.23$, $n_{foragers} = 3$, $n_{preforagers} = 13$, $p > 0.05$).

## *3.8 General comparison of discrimination in associative PER learning*

The effects of different parameters on discrimination were less clear in most experiments. Genotype affected discrimination in foragers but not in preforagers. Foraging role had no effect on discrimination after one learning phase but affected the relationship between sucrose responsiveness and discrimination after reversal learning (see 3.3.5 and 3.3.10). Age affected discrimination in the high strain but not in the low strain. In bees with uniform sucrose responsiveness discrimination was significantly better when bees received a low sucrose concentration than when they were rewarded with a high sucrose concentration.

Discrimination between the conditioned pattern or odour and the alternative pattern or odour was very good in all groups of bees. Genotype affected discrimination strongly in foragers but not in preforagers (Figure 34). High-strain foragers had a significantly greater discrimination index after tactile learning than low-strain foragers (Figure 34A), while high-strain preforagers did not differ in their discrimination index from low-strain preforagers after tactile (Figure 34B) or olfactory learning (Figure 34C). However, the number of bees tested was smaller in preforagers than in foragers. Greater numbers of individuals may have detected significant differences in the discrimination indices of high- and low-strain preforagers. Foraging role did not affect discrimination after one learning phase but had an effect on discrimination after reversal learning. After one acquisition phase, pollen foragers did not differ from non-pollen foragers in their discrimination indices in both genetic strains (Figure 35) and in the wild type (Figure 36). For discrimination after reversal learning see (3.3.5 and 3.3.10).

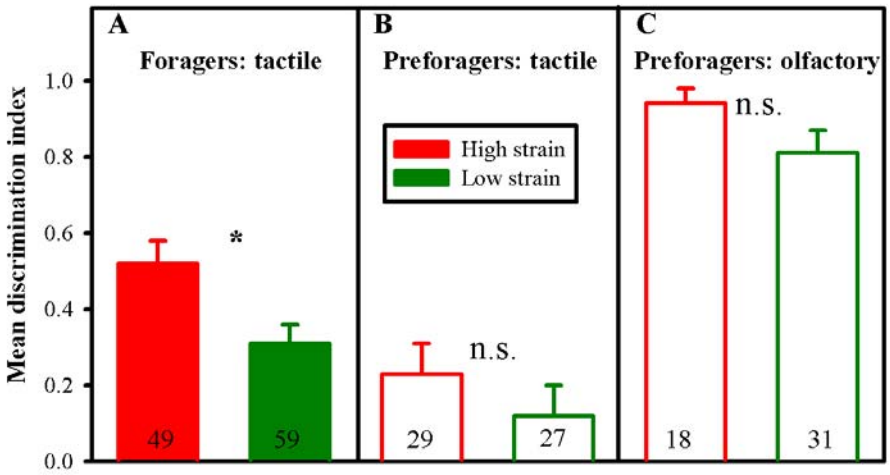


**Figure 34 Effect of genotype on discrimination indices in foragers after tactile learning (A), and in preforagers after tactile (B) or olfactory (C) learning. Means and standard errors of the means are shown. The only significant difference between the groups is indicated by an asterisk (*: $p < 0.05$, two-tailed Mann-Whitney U-test). Otherwise, groups did not differ significantly ("n. s."). Number of bees tested is indicated.**

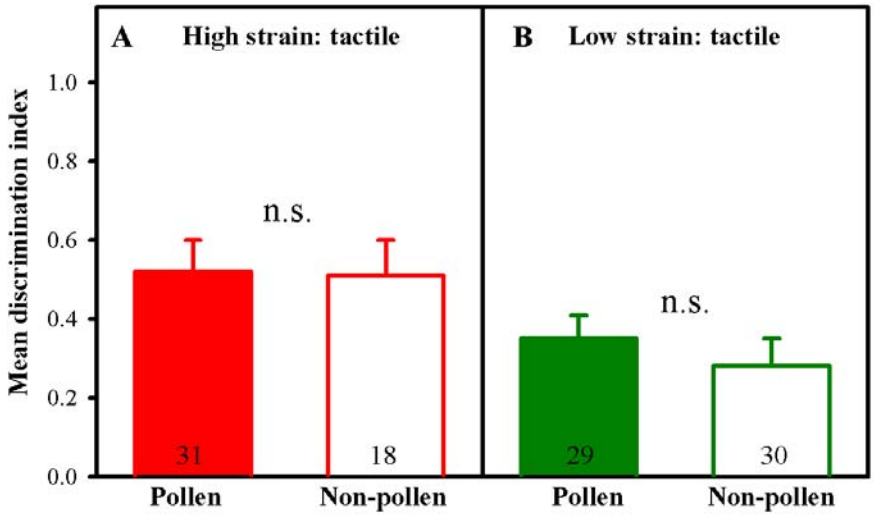


**Figure 35 The effect of foraging role on discrimination indices in foragers of the high (A) and low (B) strains. Means and standard errors of the means are shown. There were no significant differences between the groups ("n.s.": $p > 0.05$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

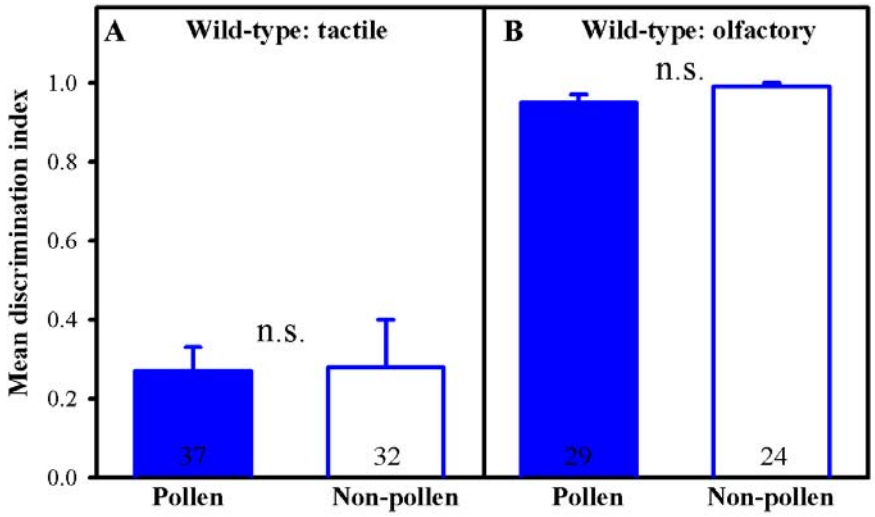


**Figure 36 The effect of foraging role on discrimination in wild-type foragers after tactile (A) or olfactory (B) learning. Means and standard errors of the means are shown. There were no significant differences between groups ("n.s.": p > 0.05, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

Age had a strong effect on discrimination in the high strain, but no effect in the low strain. High-strain foragers had a significantly higher discrimination index than high-strain preforagers (Figure 37A). Low strain foragers did not differ significantly from low-strain preforagers in their discrimination index (Figure 37B).

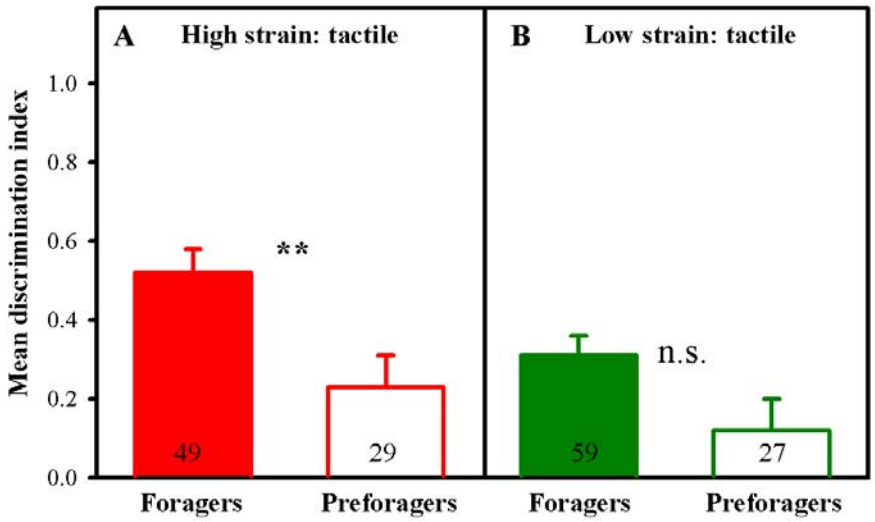


**Figure 37 The effect of age on the discrimination indices of high- (A) and low-strain bees (B). Means and standard errors of the means are shown. Significant differences are indicated by asterisks (**: p < 0.01, "n.s.": p > 0.05, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

An interesting effect of the sucrose concentration used as reward on discrimination becomes apparent in Experiment 4. Bees which had been stimulated with a high sucrose concentration at antenna and proboscis during associative tactile learning discriminated significantly poorer than bees which had been stimulated with a low sucrose concentration at the proboscis and a high or a low sucrose concentration at the antenna (Figure 38). This implies that bees which are rewarded with a high sucrose concentration learn better but discriminate less well than bees which receive a low-concentrated sucrose solution during conditioning.

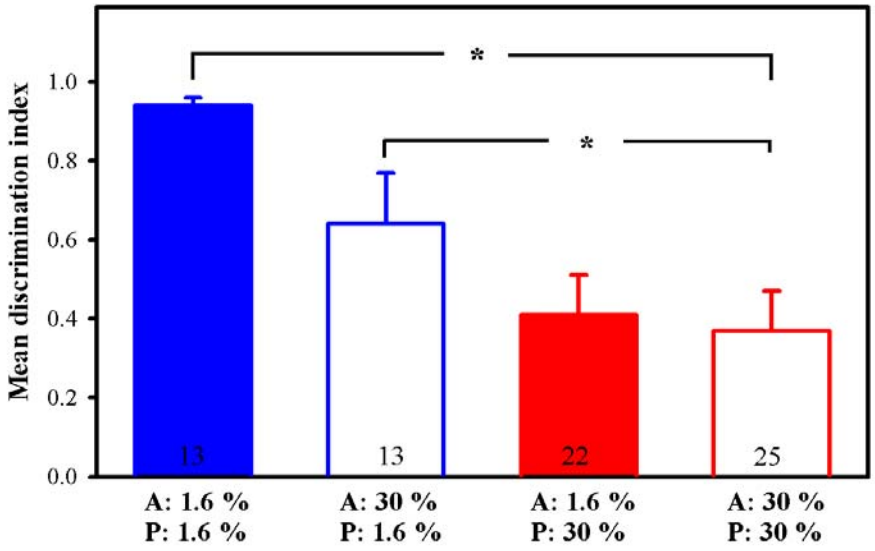


**Figure 38 Mean discrimination indices of bees with uniform sucrose responsiveness which had been stimulated with different sucrose concentrations at antenna and proboscis. Means and standard errors of the means are shown. Significant differences between groups are marked by an asterisk. (*: $p < 0.05$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

In all experiments, bees with high acquisition scores demonstrated poorer discrimination than bees with low acquisition scores, implying that "good learners" showed stronger generalisation in the unrewarded tests than "poor learners".

## *3.9 Operant tactile learning vs. classical olfactory learning*

A direct comparison of tactile and olfactory learning is not appropriate, because the two learning paradigms differ in many respects (see 1.6.1.5). Nevertheless, some general observations can be made. Gustatory response scores correlated positively with acquisition and extinction CS+ scores in operant tactile and classical olfactory learning, regardless of age or genotype. In both learning paradigms, bees with high sucrose responsiveness showed good

acquisition and weak extinction, while those with low sucrose responsiveness did not learn well and showed strong extinction.

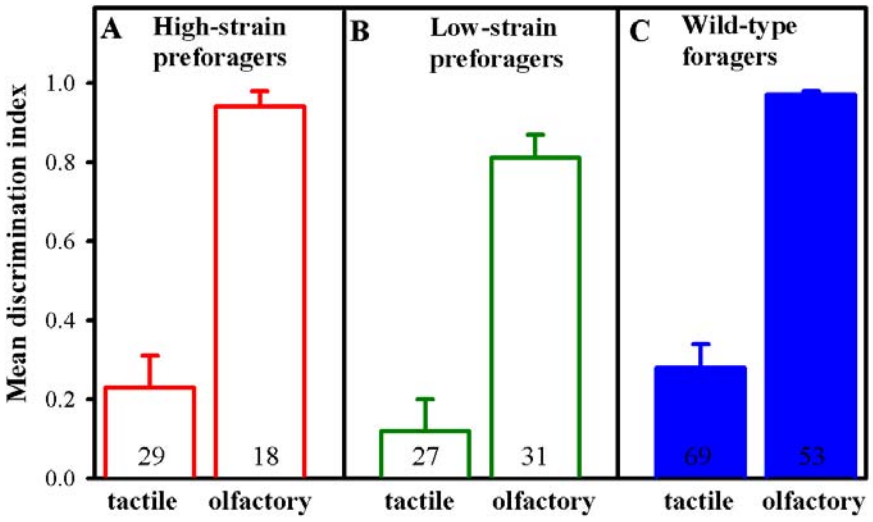


**Figure 39 Mean discrimination indices of high-strain (A), low-strain (B) and wild-type (C) bees. Means and standard errors of the means are shown. Direct comparisons of the discrimination indices between the different learning protocols are not appropriate. Number of bees is indicated.**

Preforagers and foragers discriminated the tactile and olfactory stimuli well (Figure 39). In tactile learning, extinction CS- scores correlated positively with acquisition scores in preforagers (Table 11) and foragers (Table 15). Bees with high acquisition scores responded more often to the alternative pattern and thus showed less discrimination than bees with low acquisition scores. In olfactory learning, acquisition scores rarely correlated with extinction CS- scores in preforagers (Table 11) or foragers (Table 15). This is probably due to the fact that olfactory discrimination was so good that most bees did not respond to the alternative odour, regardless of their acquisition scores. Discrimination indices of olfactory learning were much higher than those of tactile learning (Figure 39). But as the salience of the tactile stimuli must not be directly compared to that of the olfactory stimuli used, these results do not allow to draw conclusions about the abilities of bees to discriminate tactile patterns and odours. In the PER learning experiments presented here, citral was better discriminated from carnation oil after learning than the pattern with the vertical grooves from that with the horizontal grooves.

## *3.10 Experiment 5: Sucrose responsiveness and non-associative learning with different sucrose stimuli*

### 3.10.1 Habituation in bees with different sucrose responsiveness

The degree of habituation, which was measured by habituation scores, was strongly affected by individual sucrose responsiveness (Figure 40).

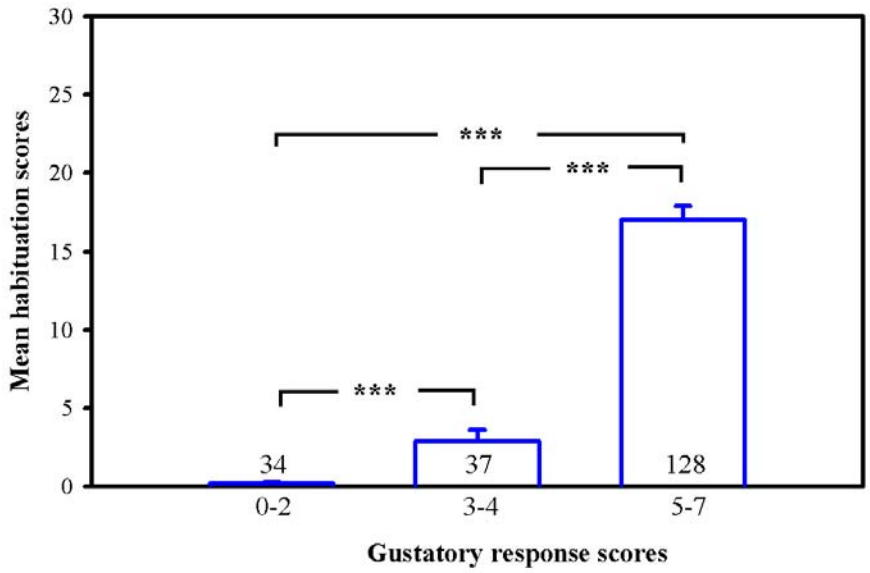


**Figure 40 Mean habituation scores of bees with different sucrose responsiveness. All of these bees were stimulated with 1 % sucrose solution. The abscissa represents the grouped gustatory response scores (see 2.5.4), the ordinate the habituation scores. Means and standard errors of the means are shown. Significant differences between the groups are indicated by asterisks (***: $p < 0.001$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

Sucrose responsiveness was measured in all bees shortly before the habituation trials. Bees with low sucrose responsiveness (GRS ≤ 2) had significantly lower habituation scores than bees with intermediate (GRS 3-4) or high (GRS ≥ 5) sucrose responsiveness (Figure 40), which indicates stronger habituation. Individuals with intermediate sucrose responsiveness had lower habituation scores than bees with high sucrose responsiveness (Figure 40). This relationship between sucrose responsiveness and habituation is also demonstrated by a significant positive correlation between GRS and habituation scores (rho = 0.79, $p < 0.001$, Spearman rank correlation coefficient). Individuals with low sucrose responsiveness showed stronger habituation than bees with high sucrose responsiveness.

### 3.10.2 Sensitisation in bees with different sucrose responsiveness

The degree of sensitisation, measured as sensitisation scores, strongly depended on individual sucrose responsiveness (Figure 41).

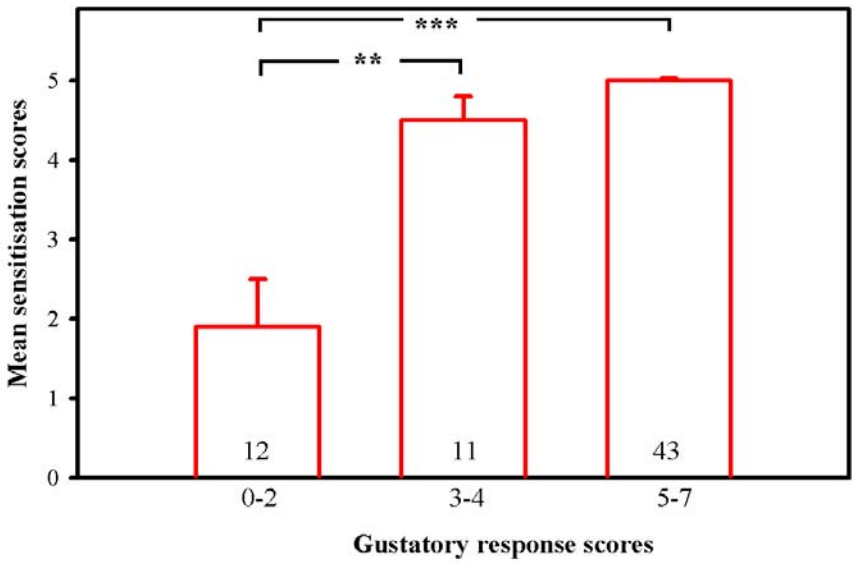


**Figure 41 Mean sensitisation scores of bees with different sucrose responsiveness. All of these bees had been tested for their sucrose responsiveness and habituation to 1 % sucrose solution shortly before the 5 sensitisation trials using 30 % sucrose solution. The abscissa represents the grouped gustatory response scores (see 2.5.4), the ordinate the sensitisation scores. Means and standard errors of the means are shown. Significant differences between the groups are indicated by asterisks (**: $p < 0.01$, ***: $p < 0.001$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

Bees with initially low sucrose responsiveness (GRS ≤ 2) had significantly lower sensitisation scores than bees with intermediate (GRS 3 - 4) or high (GRS ≥ 5) sucrose responsiveness (Figure 41), indicating less sensitisation by 30 % sucrose. Gustatory response scores correlated positively with sensitisation scores (rho = 0.62, $p < 0.001$, Spearman rank correlation coefficient), implying that bees with initially high sucrose responsiveness were more sensitised by a 30 % sucrose solution and therefore showed higher sensitisation scores than bees with low sucrose responsiveness.

### 3.10.3 Habituation with different sucrose stimuli

The sucrose concentration used as habituating stimulus largely determined the degree of habituation (Figure 42). In all groups, GRS measured prior to habituation correlated positively with habituation scores (0.1 %: rho = 0.73, 1 %: rho = 0.77, 10 %: rho = 0.79, $p < 0.001$, Spearman rank correlation coefficient), indicating that bees with high sucrose responsiveness showed less habituation than bees with low sucrose responsiveness. But

habituation scores were generally greater for bees which were stimulated with a high sucrose concentration than for bees which were stimulated with lower sucrose concentrations (Figure 42).

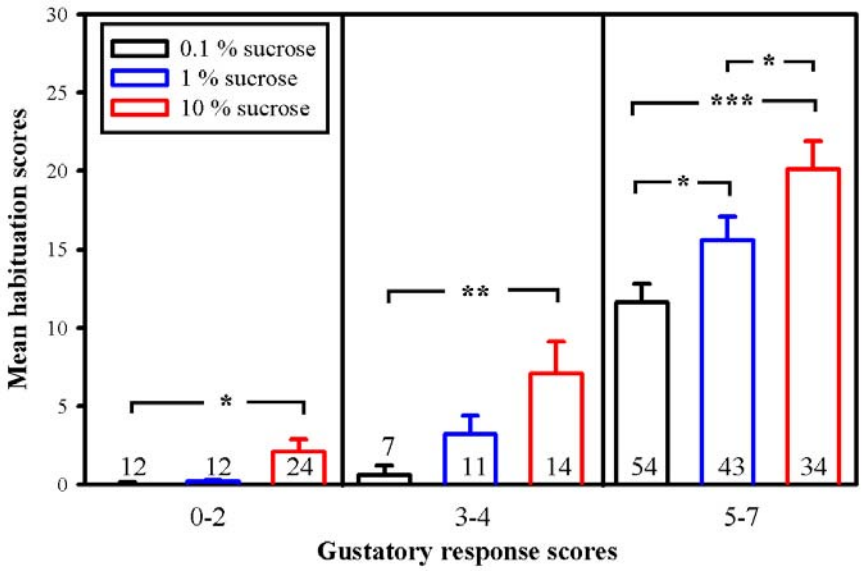


**Figure 42 Mean habituation scores in bees with different sucrose responsiveness which were stimulated with different sucrose concentrations. The x-axis represents the grouped gustatory response scores (see 2.5.4), the y-axis the mean habituation scores. Means and standard errors of the means are shown. Significant differences between the groups are indicated by asterisks (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

Regardless of sucrose responsiveness, bees which were stimulated with a high sucrose concentration showed weaker habituation and therefore higher habituation scores than bees which were simulated with lower sucrose concentrations (Figure 42). This finding suggests that both the individual sucrose responsiveness and the sucrose concentration used as habituating stimulus determine the degree of habituation.

### 3.10.4 Sensitisation with different sucrose stimuli

Similar to habituation, the degree of sensitisation depended on the sucrose concentration used as sensitising stimulus (Figure 43). Gustatory response scores measured at the beginning of the experiment correlated positively with sensitisation scores in all groups (3 %: rho = 0.80, 10 %: rho = 0.73, 30 %: rho = 0.62, $p < 0.001$, Spearman rank correlation coefficient). The degree of sensitisation, however, was higher when bees were stimulated with a high sucrose concentration compared to stimulations with lower sucrose concentrations (Figure 43). Sensitisation scores of bees which were sensitised with 30 % sucrose were significantly higher than those of bees which were sensitised with lower sucrose

concentrations. This findings collectively imply that individual sucrose responsiveness and the sucrose concentration of the sensitising stimulus largely affect the degree of sensitisation.

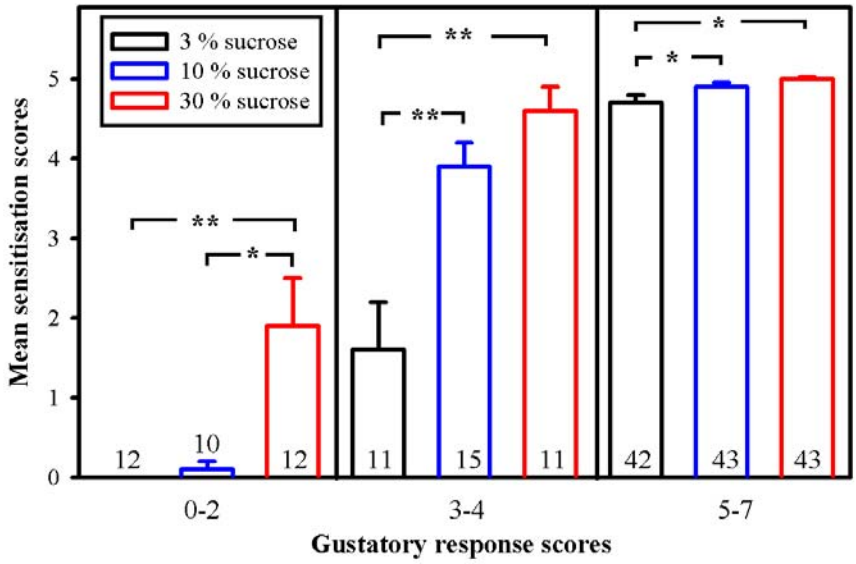


**Figure 43 Mean sensitisation scores in bees with different sucrose responsiveness which were sensitised with different sucrose concentrations. The abscissa represents the grouped gustatory response scores (see 2.5.4), the ordinate the sensitisation scores. Means and standard errors of the means are shown. Significant differences between the groups are indicated by asterisks (*: $p < 0.05$, **: $p < 0.01$, two-tailed Mann-Whitney U-test). Number of bees is indicated.**

## *3.11 Experiment 6: Sucrose responsiveness and non-associative learning in young bees of different ages*

### 3.11.1 Sucrose responsiveness in young bees of different ages

Sucrose responsiveness was not affected by age in young wild-type preforagers. Bees of all the represented age groups responded increasingly to increasing concentrations of sucrose (Figure 44). This is also demonstrated by significant positive slopes of the linear regressions on the sucrose-concentration response curves (Table 6). The different age groups did not differ in the slope coefficients of their regressions (Table 24). However, bees younger than 1 day old and 5-day-old bees had a significantly higher intercept than 1-day-old bees (1 h: $t = 3.00$, $df = 99$, 4 h: $t = 3.88$, $df = 62$, 5 d: $t = 2.91$, $df = 81$, $p < 0.01$, two-tailed Welch's t-test), implying that initial responsiveness changed during the first 5 days of adult life in a complex way. The overall degree of sucrose responsiveness, measured as GRS, did not differ between the different age groups (1 h vs 4 h: $p > 0.05$, 1 h vs 1 d: $p = 0.05$, 1 h vs 5 d: $p > 0.05$, 4 h vs 1 d: $p > 0.05$, 4 h vs 5 d: $p > 0.05$, 1 d vs 5 d: $p > 0.05$, two-tailed Mann-Whitney U- test).

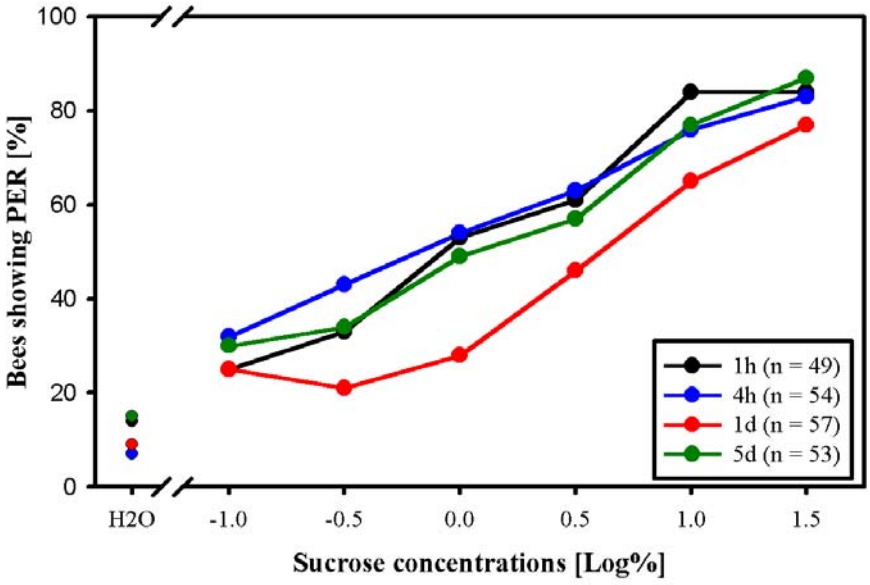


**Figure 44 Sucrose responsiveness of very young wild-type preforagers. The abscissa represents the sucrose concentration, the ordinate the percentage of bees showing the proboscis extension response (PER). Number of bees tested (n) is indicated.**

### 3.11.2 Habituation in young bees of different ages

Age did not affect the degree of habituation in bees with low sucrose responsiveness but in individuals with intermediate or high sucrose responsiveness (Figure 45). In all 4 age groups, GRS correlated positively with habituation scores (1 h: rho = 0.87, 4 h: rho = 0.77, 1 d: rho = 0.84, 5 d: 0.84, $p < 0.001$, Spearman rank correlation coefficient), indicating that bees with low sucrose responsiveness showed stronger habituation than bees with high sucrose responsiveness. Bees of the 4 age groups with low sucrose responsiveness (GRS ≤ 2) did not differ in their habituation scores (Figure 45). However, individuals younger than 1 day old with intermediate (GRS 3-4) or high (GRS ≥ 5) sucrose responsiveness had significantly lower habituation scores than older bees and thus demonstrated stronger habituation (Figure 45).

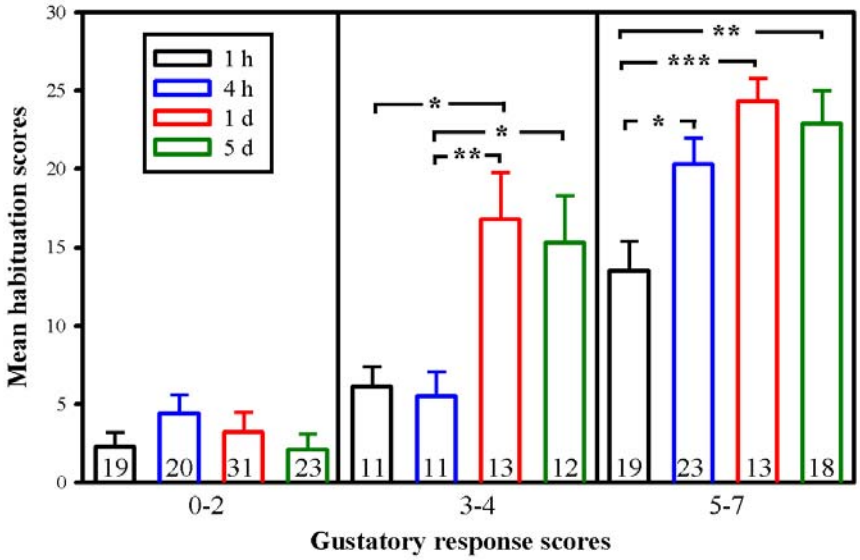


**Figure 45 Mean habituation scores of young wild-type bees of 4 different age groups. The abscissa represents the grouped gustatory response scores (see 2.6.4), the ordinate the mean habituation scores. Means and standard errors of the means are shown. Significant differences between the groups are indicated by asterisks (*: p < 0.05, **: p < 0.01, ***: p < 0.001, two-tailed Mann-Whitney U-test). Number of bees tested is indicated.**

These results suggest that the relationship between sucrose responsiveness and habituation changes with age. However, it must be noted that the different age groups had to be handled in a slightly different way (see 2.6.2), which might have affected their behaviour in these tests.

### 3.11.3 Sensitisation in young bees of different ages

Age had no demonstrable effect on the degree of sensitisation (Figure 46). All of the bees had been tested for their sucrose responsiveness and habituation using 1 % sucrose prior to the 5 sensitisation trials. Sensitisation scores correlated positively with GRS, regardless of age (1 h: rho = 0.49, 4 h: rho = 0.56, 1 d: rho = 0.84, 5 d: rho = 0.66, $p < 0.001$, Spearman rank correlation coefficient). Bees with high GRS demonstrated stronger sensitisation in all age groups than bees with low GRS. Basically, there were no differences in the degree of sensitisation between the different age groups (Figure 46). Only 4-hour-old preforagers showed significantly higher sensitisation scores than 1-day-old preforagers (Figure 46). All the other age groups did not differ significantly. Interestingly, bees with intermediate sucrose responsiveness often reached the maximum sensitisation scores like bees with high sucrose responsiveness.

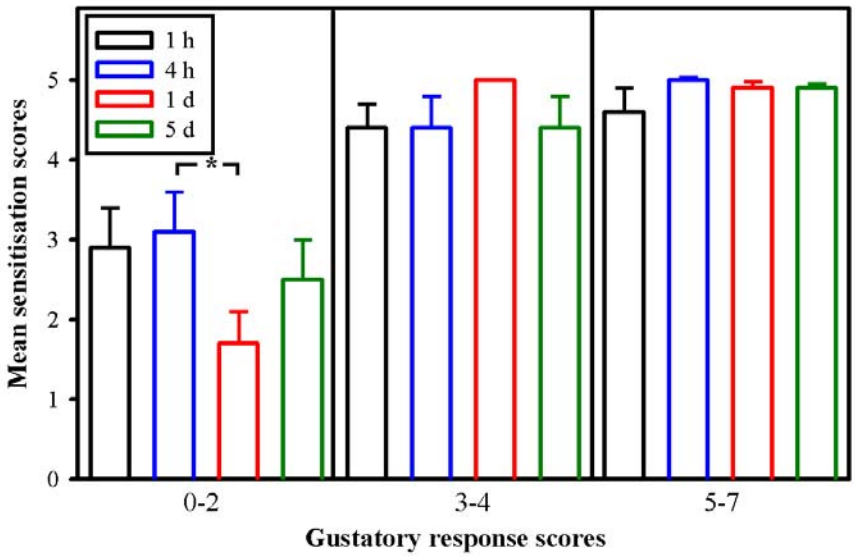


**Figure 46 Mean sensitisation scores of young bees of 4 different age groups. The abscissa represents the grouped gustatory response scores (2.6.4), the ordinate the mean sensitisation scores. Means and standard errors of the means are shown. The only significant difference between the groups is indicated by an asterisk (*: $p < 0.05$, two-tailed Mann-Whitney U-test). Number of bees tested as in Figure 45.**

## *3.12 General comparison of sucrose responsiveness and non-associative learning*

Both habituation and sensitisation were affected by individual sucrose responsiveness and the sucrose concentration of the habituating or sensitising stimuli. Age affected the degree of habituation. Sensitisation was not affected by age. In preforagers of all 4 age groups (Figure 42) and in foragers (Figure 40 and Figure 45), individuals with low sucrose responsiveness showed stronger habituation than bees with intermediate or high sucrose responsiveness. A high sucrose concentration used as habituating stimulus led to weaker habituation than a low sucrose concentration (Figure 42). The relationship between sucrose responsiveness and habituation changed with age. Whereas bees with low sucrose responsiveness had low habituation scores in all 4 age groups tested, individuals with intermediate or high sucrose responsiveness which were younger than 1 day old showed stronger habituation than 1-day-old bees or older bees with the same sucrose responsiveness. Habituation in foragers could not be compared with that in preforagers, because the experiment with the foragers was done under different experimental conditions and at a different time of the year. Sucrose responsiveness and the sucrose concentration of the

sensitising stimulus had independent effects on sensitisation, whereas age had no effect. In all age groups, bees with low sucrose responsiveness were less sensitised by a 30 % sucrose solution than bees with intermediate or high sucrose responsiveness and therefore showed smaller sensitisation scores (Figure 41 and Figure 43). Individuals with intermediate sucrose responsiveness often reached the maximum sensitisation scores and did not differ from bees with high sucrose responsiveness. Bees which were stimulated with a high sucrose concentration demonstrated stronger sensitisation than those which were stimulated with lower concentrated sucrose solutions. Age had no demonstrable effect on sensitisation in the 4 groups of bees tested.

## *3.13 Experiment 7: Sucrose responsiveness and M17 activity*

Activity of M17, measured as number of spikes occurring during the first 5 s after stimulation, increased with increasing sucrose concentration, regardless of whether proboscis extension occurred or not.

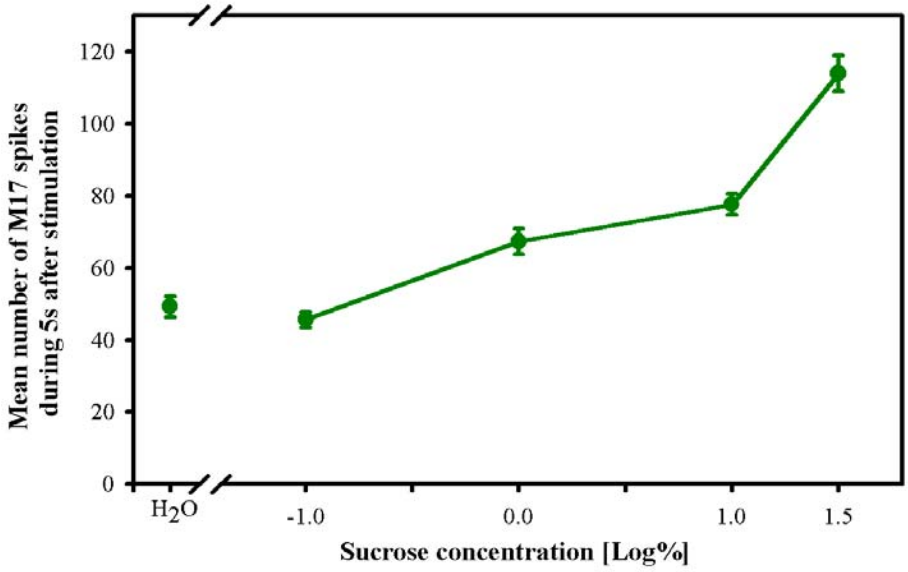


**Figure 47 Activity of M17, the muscle responsible for proboscis extension, after antennal stimulation with different sucrose concentrations. Ten bees were stimulated five times with each sucrose concentration in blocks of five stimulations. The abscissa represents the sucrose concentrations, the ordinate the number of M17 spikes occurring during the first 5 s after stimulation. Means and standard errors of the means are shown.**

Muscle 17 basically did not show any spontaneous activity. Stimulations with different sucrose concentrations which were offered several times and in pseudo-randomised order showed that muscle activity increased with increasing sucrose concentrations (Figure 47). Sucrose concentration correlated positively with number of M17 spikes (rho = 0.38, n = 10, p

< 0.01, Spearman rank correlation coefficient). Even when all the sucrose stimulations resulted in proboscis extension, as was the case in the individual represented in Figure 48, M17 activity increased with increasing sucrose concentration.

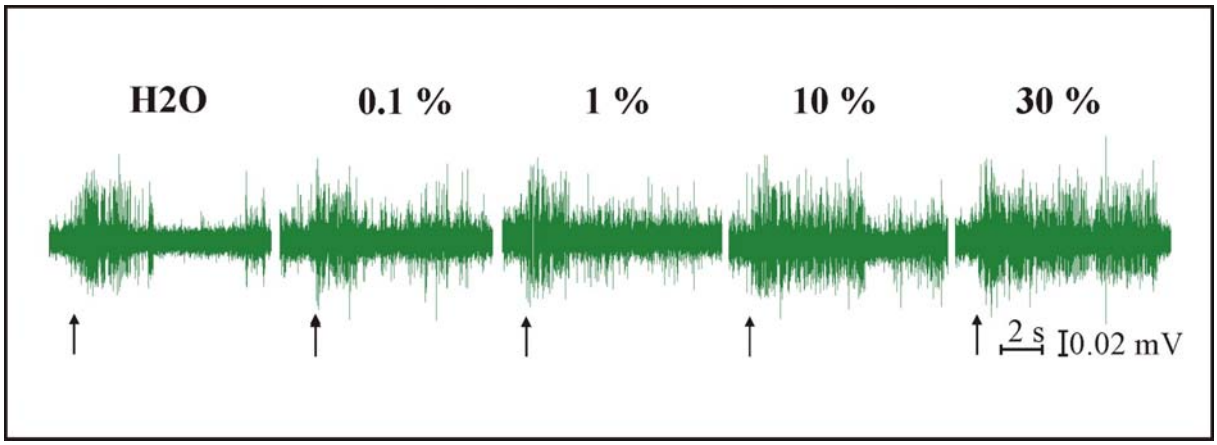


**Figure 48 Muscle 17 activity in one representative individual after antennal stimulation with different sucrose concentrations. This individual responded with proboscis extension to all sucrose concentrations offered. Scale bars are indicated. The arrows point to the begin of the stimulation with water or sucrose. Approximately 5 s following the stimulation are shown.**

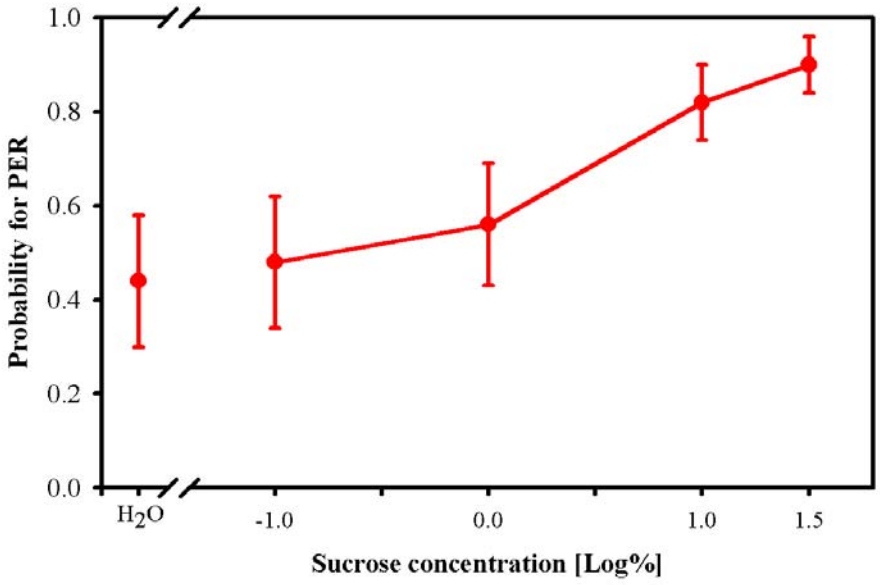


**Figure 49 Probability for proboscis extension following antennal stimulation with water or different sucrose concentrations. The abscissa represents the sucrose concentrations, the ordinate the probability for the proboscis extension response (PER). Means and standard errors of the means are shown for ten bees. Each solution was presented five times in blocks of five stimulations. The order of the solutions differed from block to block.**

The probability for proboscis extension increased with increasing sucrose concentrations (Figure 49, rho = 0.433, $p < 0.01$) and with muscle activity measured during the first 5 s after

stimulation (rho = 0.757, $p < 0.001$, Spearman rank correlation coefficient). These findings present a new method for determining individual sucrose responsiveness exactly and in areas where behavioural tests are not sensitive enough. They demonstrate that bees differentiate between different sucrose concentrations, even though they may show proboscis extension to each or none of them.

## *3.14 Experiment 8: Modulation of sucrose responsiveness by biogenic amines*

### 3.14.1 Octopamine and tyramine

Both octopamine and tyramine increased sucrose responsiveness 30 min after injection. Sucrose responsiveness was measured prior to injection with octopamine, tyramine or Ringer solution, 30 min after injection and 90 min after injection. To compare the effects of different neuroactive substances on sucrose responsiveness, a modulation index (MI) was calculated (see 2.8.3). A positive index shows an increase in sucrose responsiveness in a determined period of time (30 min or 90 min), a negative index shows a decrease in sucrose responsiveness.

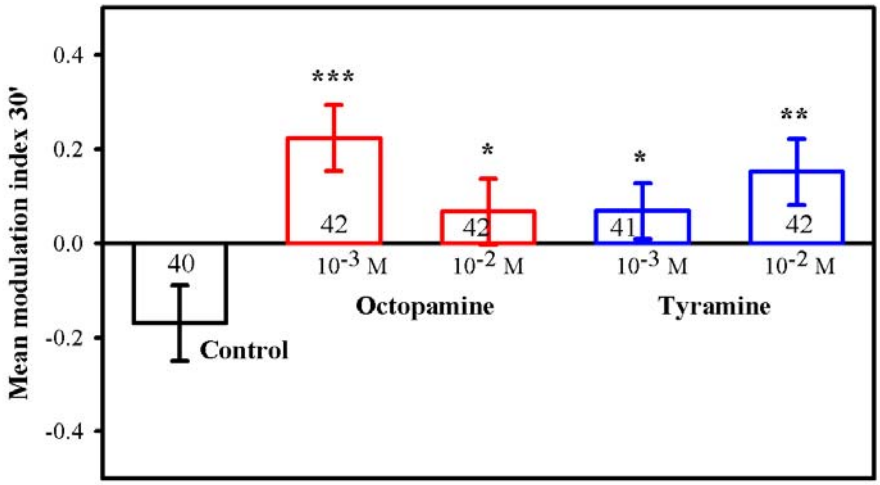


**Figure 50 Modulation indices (see 2.8.3) of octopamine and tyramine 30 min after injection. A positive index marks an increase in sucrose responsiveness, a negative index a decrease. Means and standard errors of the means are shown. Significant differences between the control group and all the other groups are indicated by asterisks (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, two-tailed t-test). Number of bees tested is shown.**

Thirty minutes after injection, the modulation index was significantly higher for bees which had been injected with $10^{-3}$ M and $10^{-2}$ M octopamine or with tyramine in concentrations of

$10^{-3}$ M and $10^{-2}$ M compared to Ringer-injected control bees (Figure 50, Table 25), showing that injection of octopamine or its metabolic precursor tyramine significantly increased sucrose responsiveness over the range of 30 min. The modulation index of bees which had been injected with octopamine in the concentration of $10^{-2}$ M was not different 30 min after injection from that of bees which had been injected with $10^{-3}$ M octopamine (Table 26). The two tyramine concentrations also did not lead to significantly different modulation indices 30 min after injection (Table 26). However, Figure 50 suggests that the effects of octopamine and tyramine were dose-dependent. Therefore, more experiments using a wider variety of concentrations of these biogenic amines are needed to determine whether the effects of octopamine and tyramine on sucrose responsiveness are dose-dependent.

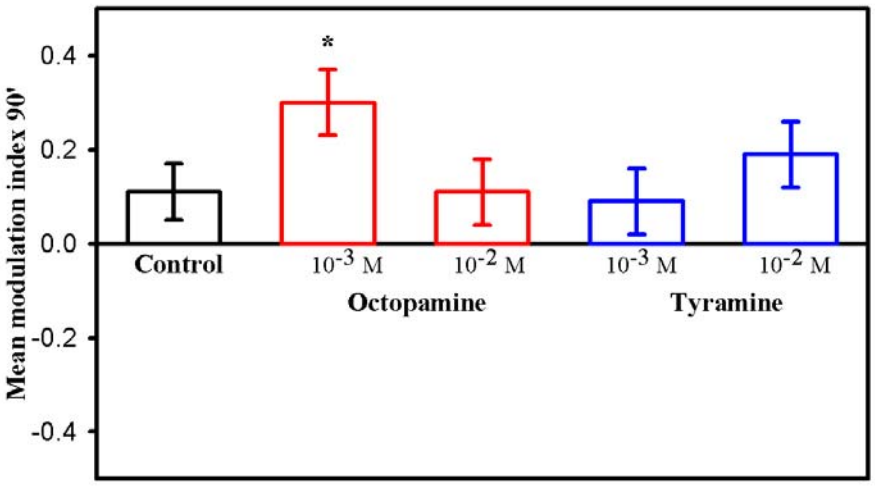


**Figure 51 Modulation indices (see 2.8.3) of octopamine and tyramine 90 min after injection. A positive index marks an increase in sucrose responsiveness, a negative index a decrease. Means and standard errors of the means are shown. The only significant difference between the control group and octopamine $10^{-3}$ M is indicated by an asterisk (*: $p < 0.05$, two-tailed t-test). Number of bees as in Figure 50.**

Ninety minutes after injection, only $10^{-3}$ M octopamine still had an effect on sucrose responsiveness (Figure 51, Table 25). The effects of the two tyramine concentrations and the effect of $10^{-2}$ M octopamine were no longer detectable. The modulation index of control bees was now positive. This could be an indicator of hunger in the bees, because bees increase their sucrose responsiveness when they become hungry (Page et al. 1998). The two different concentrations of octopamine and of tyramine did not lead to significantly different modulation indices (Table 26).

### 3.14.2 Dopamine and ADTN

Dopamine and the dopamine receptor agonist ADTN decreased responsiveness to sucrose (Figure 52). Thirty minutes after injection, bees which had been injected with $10^{-1}$ M dopamine showed a significantly lower modulation index than control bees (Table 25). Injection of dopamine in the concentrations of $10^{-3}$ M or $10^{-2}$ M did not lead to a significant change in sucrose responsiveness 30 min after injection (Table 25). The modulation indices of bees that had been injected with $10^{-3}$ M or $10^{-4}$ M ADTN were significantly lower than was the modulation index of control bees (Table 25). Injection of $10^{-2}$ M ADTN did not lead to a significant change in sucrose responsiveness. Only the highest concentration of dopamine had a significant effect on sucrose responsiveness (Table 25).

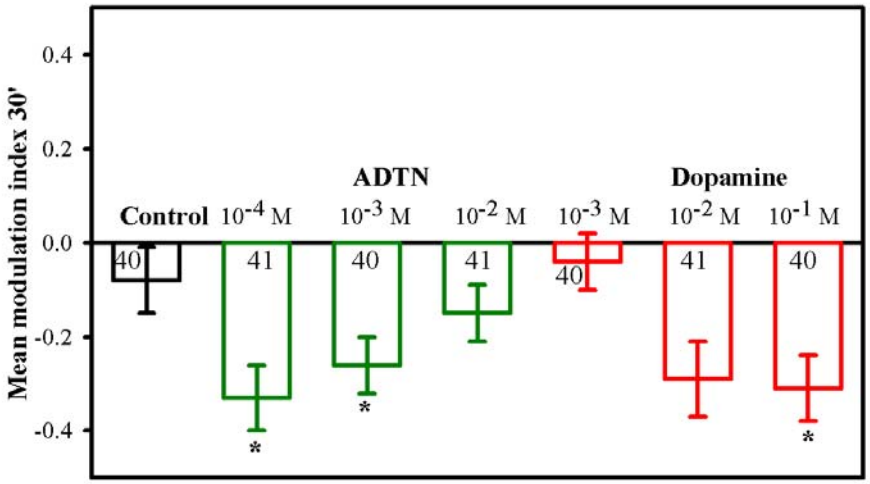


**Figure 52 Mean modulation indices (see 2.8.3) of dopamine and ADTN 30 min after injection. A negative index marks a decrease in sucrose responsiveness. Means and standard errors of the means are shown. Significant differences between the control group and all the other groups are indicated by an asterisk (*: $p < 0.05$, two-tailed t-test). Number of bees tested is indicated.**

Figure 52 strongly suggests that the effects of dopamine and ADTN were dose-dependent. The modulation indices of bees which had been injected with different dopamine concentrations differed significantly (Table 26), the differences in the modulation indices of bees which had been injected with different concentrations of ADTN were not significantly different (Table 26). Ninety minutes after injection, the modulation index of control bees was positive. Whereas the modulation indices of bees which had been injected with dopamine in the concentrations $10^{-1}$ M or $10^{-2}$ M were significantly lower than was the modulation index of control bees (Table 25), an effect of ADTN on sucrose responsiveness was no longer

detectable (Figure 53). The different concentrations of dopamine or ADTN did not result in significantly different modulation indices 90 min after injection (Table 26).

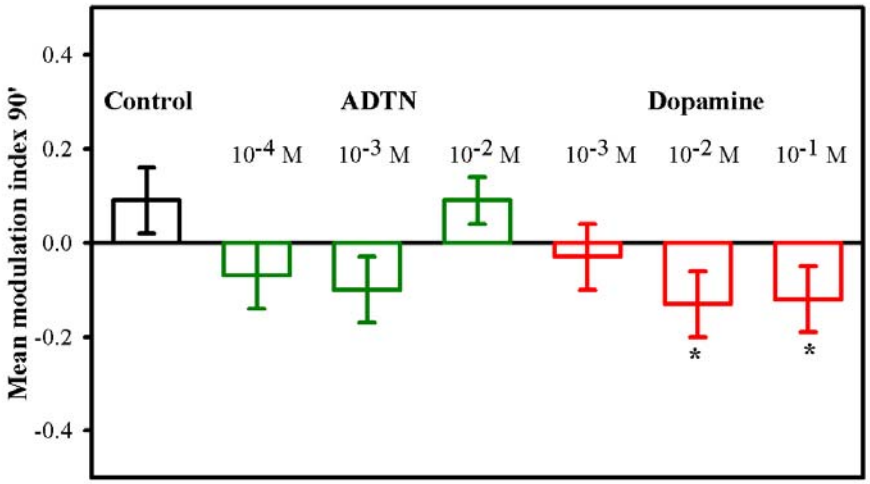


**Figure 53 Modulation indices (see 2.8.3) of dopamine and ADTN 90 minutes after injection. A positive index marks an increase in sucrose responsiveness, a negative index a decrease. Means and standard errors of the means are shown. Significant differences between the control group and all the other groups are indicated by an asterisk (*: $p < 0.05$, two-tailed t-test). Number of bees as in Figure 52.**

## *3.15 Experiment 9: PKA activity in bees with high or low sucrose responsiveness*

PKA activity changed with sucrose responsiveness and time after feeding (Figure 54). Bees with high sucrose responsiveness (see 2.9.2) displayed a significantly higher PKA activity than bees with very low sucrose responsiveness (see 2.9.2), regardless of the time when PKA activity was measured (after 30 min: $t = 3.29$, $df = 45$, $p < 0.01$, after 90 min: $t = 2.70$, $df = 38$, $p < 0.05$, two-tailed t-test). This shows a basal difference in PKA activity in the antennal lobes of bees with different sucrose responsiveness. In addition, 30 min after feeding, PKA activity was significantly higher than 90 min after feeding, regardless of individual sucrose responsiveness (high GRS: $t = 2.15$, $df = 49$, $p < 0.05$, low GRS: $t = 3.59$, $df = 38$, $p < 0.01$, two-tailed t-test).

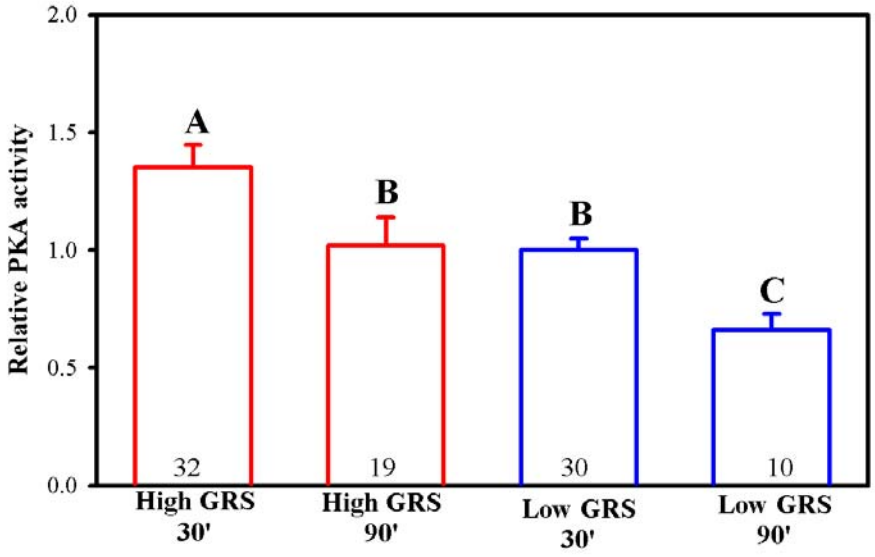


**Figure 54 Relative PKA activity in bees with different gustatory response scores (GRS). Bees with high GRS responded to water, those with low GRS to 30 % sucrose, if at all. The ordinate represents the relative PKA activity in the antennal lobes. Bees could not be directly compared but were normalised to the sum of PKA activity of each gel. Different letters imply significant differences (see text). Number of bees tested is indicated.**

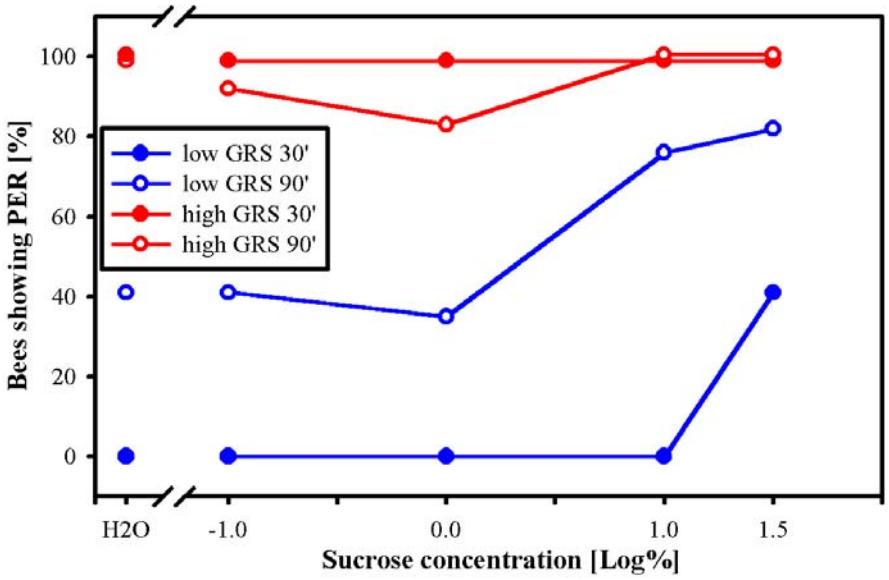


**Figure 55 Sucrose responsiveness in bees measured 30 min and 90 min after feeding. The abscissa represents the sucrose concentrations, the ordinate the percentage of bees responding with proboscis extension. Number of bees: low GRS: 18, high GRS: 12.**

Sucrose responsiveness in bees with low GRS increased over time (Figure 55). Forty per cent of control bees (in which PKA activity was not measured), which initially only responded to 30 % sucrose (or which did not respond at all) responded to water stimulation 90 min after

feeding. Bees with initially high GRS scores did not change their sucrose responsiveness as measured by the occurrence of the PER. These results show that PKA activity in bees with high sucrose responsiveness was higher than that in bees with low sucrose responsiveness. In addition, PKA activity in bees measured 30 min after feeding was higher than that measured 90 min after feeding. These findings imply that there are two mechanisms regulating the PKA activity in the antennal lobes. One regulates the basal activity and the other one regulates short-term modifications, which could depend on the state of satiation.

# 4 Discussion

## *4.1 Sucrose responsiveness*

### 4.1.1 General observations

The number of bees showing proboscis extension increased with increasing concentration of the sucrose solution, regardless of genotype, foraging role or age. This finding supports early studies by Minnich (1932) and Marshall (1935) who showed with the PER paradigm that bees responded in greater numbers to high sucrose concentrations than to water or low sucrose concentrations. Von Frisch (1965) also showed that more bees were attracted to a feeder which was filled with a high sucrose concentration than to a feeder filled with a low sucrose concentration. A comparison of the different experimental groups from Experiments 1, 2, 3 and 6 reveals that genotype, foraging role and age can have great effects on individual sucrose responsiveness.

### 4.1.2 The effect of genotype on sucrose responsiveness

Individual responsiveness to water and different sucrose concentrations was strongly affected by genotype. In both age groups, high-strain bees, which had been selected over many generations to store large amounts of pollen in the colony, were more responsive to water and low sucrose concentrations than low-strain bees, which mainly collect nectar (Figure 26). These results are well in line with earlier findings. Page et al. (1998) were the first to demonstrate that high-strain foragers were more responsive to water and sucrose than low-strain foragers. Since then several other studies have shown that high-strain bees of different age groups or castes were more responsive to water and different sucrose concentrations than low-strain bees (Pankiw and Page 1999, 2000, Fewell and Page 2001, Pankiw et al. 2001). Even high- and low-strain queens and drones, which never forage, differ in their sucrose response thresholds (Pankiw and Page 1999). High- and low-strain foragers also differ in the concentration of nectar they collect. Whereas high-strain bees collect more nectar of low quality, low-strain foragers collect nectar of a higher quality (Page et al. 1998, Pankiw and Page 1999). As high-strain bees also have lower sucrose response thresholds, they appear to have lower acceptance thresholds when foraging.

Taken together, these findings demonstrate a close relationship between genotype, sucrose responsiveness and foraging behaviour. Genotype was shown to place constraints on individual sucrose responsiveness and thus offers the opportunity to search for genetic correlates. Hunt et al. (1995) found a genetic correlate for foraging behaviour and sucrose

concentration of nectar collected. In the above-mentioned high- and low-strain bees of Page and Fondrk (1995) they found two quantitative trait loci (QTL), *pln1* and *pln2*, which affect the probability that a forager will collect pollen or nectar. In addition, one of these QTLs, *pln2*, correlated with the sugar concentration of the nectar collected. Recently, a third QTL, *pln3*, has been shown to also correlate with the sugar concentration of the nectar collected (Page et al. 2000). What makes these QTLs so interesting for sucrose responsiveness is that *pln1* and *pln3* have also been demonstrated to correlate directly with sucrose response thresholds (R. Page, personal communication).

### 4.1.3 The effect of foraging role on sucrose responsiveness

Foraging role had a strong effect on sucrose responsiveness. In bees of the two selected strains and in wild-type bees, pollen foragers were more responsive to water and low sucrose concentrations than were non-pollen foragers (Figure 27). These findings confirm earlier reports of the sucrose responsiveness of pollen and non-pollen foragers from different strains of bees (Page et al. 1998, Scheiner et al. 1999). They show that foraging role and sucrose responsiveness strongly correlate, and not only in genetic strains selected for their foraging behaviour. In fact, sucrose responsiveness of 1-week-old bees was shown to be an excellent predictor of the foraging role the bees adopted two weeks later (Pankiw and Page 2000). Previous foraging experience also affects sucrose responsiveness (Pankiw et al. 2001). When low sucrose concentrations were offered in feeders, bees generally showed lower sucrose response thresholds than when higher sucrose concentrations were offered. Whether previous foraging experience at different pollen or nectar sources can also change foraging roles is not clear. There is some evidence that bees maintain their foraging role for lifetime (Fewell and Page 2001). However, there is also evidence for the changing of foraging roles with changing colony conditions or foraging conditions (Fewell and Winston 1992, Fewell and Page 1993).

### 4.1.4 The effect of age on sucrose responsiveness

The age of bees can strongly affect sucrose responsiveness. Preforagers between 1 day and 5 days of age did not differ significantly in their sucrose responsiveness (see 4.1.4). But foragers were significantly more responsive to water and different sucrose concentrations than preforagers in both selected strains and in the wild type (Figure 28). This finding is directly

supported by earlier experiments of Pankiw and Page (1999), that found a correlation between different ages of bees and their sucrose responsiveness.

An interesting phenomenon is the development of sucrose responsiveness from preforagers to foragers. In the wild type and in the two genetic strains, pollen foragers were more responsive to water and sucrose than preforagers, whereas non-pollen foragers did not differ in their sucrose responsiveness from preforagers. These findings suggest that preforagers which will later in life collect pollen might increase their sucrose responsiveness with age, whereas preforagers which will later forage for nectar might not change their sucrose responsiveness. However, it is most likely that not all of the preforagers will later in life forage, because many bees remain in the hive as a reserve, even when they are of foraging age (Seeley 1995). A correlation between sucrose responsiveness in preforagers and foraging role has been shown by Pankiw and Page (2000). Bees with the highest sucrose responsiveness at 1 week old were more likely to become water and pollen foragers, while those with lower sucrose responsiveness were more likely to collect nectar or return empty from foraging trips.

One reason why sucrose responsiveness is higher in foragers than in preforagers could be that foragers have more contact with dilute sucrose solutions while foraging, whereas young hive bees will feed on the sweet honey stored in the hive. When foraging conditions are poor, foragers have to collect even low-quality nectar in order to save the colony from starvation. Preforagers, however, presumably have no "necessity" to respond to low sucrose concentrations.

So far, the mechanism regulating this age-dependent modulation of sucrose responsiveness is unknown. It seems unlikely that preforagers are generally less sensitive to sucrose than foragers, because even some of the newly emerged bees responded with proboscis extension to antennal stimulation with water or very low sucrose concentrations. Even if the gustatory system were not fully developed in the first days of adult life, as has been shown to be the case for the olfactory system (Masson and Arnold 1987), the differences in sucrose responsiveness between 9-day-old preforagers and foragers could not be explained. It seems more likely that sucrose responsiveness is up-regulated during behavioural maturation by some internal process. Response thresholds to alarm pheromone, for example, also change with age (Robinson 1987a). A conceivable mediator of these changes is juvenile hormone (JH), whose titre in the haemolymph increases with age (Robinson 1987b, Fahrbach and Robinson 1996). In addition, the activity of the *corpora allata*, the site of JH synthesis in the honey bee, is increased when bees are very hungry (Kaatz et al. 1994), and hungry bees

have a higher sucrose responsiveness than satiated bees (Page et al. 1998, Pankiw et al. 2001). It is further conceivable that octopamine is involved in the age-dependent mediation of sucrose responsiveness. Octopamine stimulates the activity of the *corpora allata* (Kaatz et al. 1994, Rachinsky 1994) and induces precocious foraging (Schulz and Robinson 2001). In addition, injections of octopamine strongly increase sucrose responsiveness (see below), and foragers have a higher sucrose responsiveness than preforagers. The relationships between titres of JH, octopamine and sucrose responsiveness, however, have yet to be determined.

An age-dependent development of responsiveness to sugars has not only been observed in the honey bee. In *Drosophila melanogaster* and *Phormia regina*, sugar response thresholds increase with age (Brigui et al. 1990, Stoffolano 1975). In contrast, *Musca autumnalis* females decrease their response thresholds for glucose with age (Stoffolano 1968).

### 4.1.5 Sucrose responsiveness and M17 activity

Most bees show proboscis extension when their antennae are stimulated with a sucrose concentration above their individual response threshold. This, however, is a very coarse behavioural measure. It does not show whether different sucrose concentrations above the individual response threshold are perceived differently or lead to a different intensity of proboscis extension. Recordings from muscle 17, the muscle which is responsible for the extension of the proboscis, proved to be a much more accurate measure of the bee's sucrose responsiveness than the PER. Muscle activity highly correlated with the concentration of the sucrose solution. When bees did not show proboscis extension to several "sub-threshold" sucrose concentrations, they still differentiated between the sucrose concentrations and responded with greater M17 activity to higher sucrose concentrations. Bees with low individual response thresholds, which showed proboscis extension to most sucrose concentrations offered, still discriminated between the different sucrose concentrations, as can be seen in varying M17 activity. The results of the recordings from M17 are well in line with earlier experiments by Braun and Bicker (1992). These authors showed that M17 activity was greater when bees were stimulated with 50 % sucrose than when they were stimulated with water. In addition, they demonstrated that hunger affected M17 activity. The number of M17 spikes evoked by a 5 % sucrose stimulus was higher for hungry animals than for fed animals. Hungry bees, however, usually have a higher sucrose responsiveness than satiated bees (Page et al. 1998, Pankiw et al. 2001) and should therefore place a higher "relevance" to a certain sucrose stimulus.

Recordings from M17 have also proved to be a very sensitive tool for measuring associative and non-associative forms of learning. During extinction after classical conditioning, for example, the responsiveness of the bee to the conditioned stimulus decreases and the proboscis motor responses become weaker, which sometimes leads to incomplete proboscis extension. This "sub–threshold" activity of M17 (Snodgrass 1987) can be recorded and be used as a more exact indicator of retrieval performance than the occurrence of the PER (Rehder 1987, Smith and Menzel 1989). In non-associative sensitisation, the response strength to the sensitising stimulus can be measured accurately by M17 recordings (Hammer et al. 1994). It was shown, for example, that antennal sucrose stimulation evoked the lowest number of M17 spikes, whereas the compound stimulation of antenna and proboscis or the stimulation of the proboscis alone resulted in a greater number of M17 spikes. That way it was also shown that long stimulations of antenna or proboscis lead to greater M17 activity than short stimulations. For compound stimulations of antenna and proboscis, however, no difference between long and short stimulations were found.

These findings demonstrate the usefulness of M17 recordings to measure not only individual sucrose responsiveness very accurately, but to also use M17 recordings as a means to quantify the strength of proboscis extension in non-associative and associative learning experiments. The individual sucrose response threshold of a bee can be defined as the number of M17 spikes leading to full proboscis extension, and the strength of proboscis extension during associative conditioning or during sensitisation or habituation can also be measured as a concrete number of spikes. This could prove very useful for mathematical models on the intensity of rewarding or arousing sucrose stimuli in bees with different sucrose responsiveness. Unfortunately, this paradigm is technically more demanding than measuring the occurrence of proboscis extension and is therefore less suitable for testing great numbers of bees.

### 4.1.6 Conclusions

Taken together, these findings show a close relationship between sucrose responsiveness, foraging role, genotype and age. For parts of this relationship, genetic correlates have been found. The differences in the behavioural responses to sucrose can be measured very accurately by recordings from M17. Even when bees show proboscis extension to all sucrose concentrations offered they can differ in their M17 activity.

The variances in sucrose responsiveness imply differences in the neural processing of gustatory stimuli. So far, no causal relationships have been detected for sucrose

responsiveness and the different factors related to it. Future research will concentrate on the behavioural effects of differences in sucrose responsiveness and the molecular mechanisms underlying its regulation. The effects of sucrose responsiveness on associative and non-associative forms of learning will be discussed in the next sections. The modulation of sucrose responsiveness by endogenous transmitters in the bee brain will be discussed thereafter. Finally, the relationship between sucrose responsiveness and the activity of an important protein kinase will be analysed as a first step towards the discovery of mechanisms regulating sucrose responsiveness.

## *4.2 Associative learning*

### 4.2.1 Acquisition

#### *4.2.1.1 General observations*

It has been shown in several experiments analysing associative learning that the acquisition of stimuli in bees depends on the concentration of the sucrose reward (Loo and Bitterman 1992, Couvillon et al. 1994, Bitterman 1996, Smith 1997, Laloi et al.1999, Scheiner et al. 1999). Bees which are rewarded with a high sucrose concentration learn better than ones that receive a low sucrose concentration. However, even bees which receive the same sucrose concentration as reward differ in their learning behaviour, if they vary in their individual sucrose responsiveness (Scheiner et al. 1999). Entire groups of bees differ in their learning performance because they systematically differ in their sucrose responsiveness. Pollen foragers learn tactile patterns better than nectar foragers simply because they have a higher sucrose responsiveness (Scheiner et al. 1999). Hungry bees learn better than satiated bees (Menzel et al. 1989), presumably because their sucrose responsiveness is higher (Page et al. 1998, Pankiw et al. 2001). Individuals with high sucrose responsiveness learn better than those with low sucrose responsiveness. The assumption is that the difference between the individual sucrose responsiveness and the sucrose concentration of the reward determines the level of acquisition. For bees with high sucrose responsiveness, the magnitude of the reward is subjectively greater than for individuals with low sucrose responsiveness (Scheiner et al. 1999).

The results from Experiments 1, 2 and 3 in this work strongly support this hypothesis. In all groups of bees, sucrose responsiveness positively correlated with acquisition. Individuals with high sucrose responsiveness reached higher levels of acquisition than those with low sucrose responsiveness. The intensity of the unconditioned stimulus, sucrose,

apparently changed with differences in individual sucrose responsiveness. It is an established principle in learning theory and experimental praxis that the intensity of the unconditioned stimulus affects the asymptotic strength of learning (Annau and Kamin 1961). Loo and Bitterman (1992) and Couvillon et al. (1994) gave good examples of this theory when they trained free-flying honey bees with two different sucrose concentrations. Bees learned a target better when they were rewarded with 50 % sucrose than when they were rewarded with 20 % sucrose solution. My experiments provide abundant evidence that bees which receive the same reward differ in their level of acquisition. The reason for this behaviour is to be found in individual differences in sucrose responsiveness. For bees with high sucrose responsiveness the reward subjectively appears to have a larger intensity than for bees with lower sucrose responsiveness. Differences in the level of acquisition in several groups of bees can be explained by systematic differences in sucrose responsiveness.

#### *4.2.1.2 The effect of genotype on acquisition*

Genotype had no direct effect on acquisition (Figure 29). But as high-strain bees have a higher sucrose responsiveness than low-strain bees, they, on average, showed a higher level of acquisition. The relationship between sucrose responsiveness and acquisition did not differ between the two genetic strains, implying that high- and low-strain bees with the same sucrose responsiveness did not differ in their acquisition. In both strains, bees with high sucrose responsiveness learned better than bees with low sucrose responsiveness. These findings do not only demonstrate learning differences in selected strains of bees but can also explain a large part of these differences by genetically determined differences in sucrose responsiveness. It is conceivable that in other experiments in which bees were selected for their learning performance, a selection of sucrose responsiveness took place, which then led to the observed differences in learning behaviour.

Brandes et al. (1988), for example, selected honey bees for their olfactory learning performance. The two genetic strains did not only differ in their time-courses of consolidation from short- into long-lasting memory. They also differed in their behavioural sensitisation caused by sucrose stimulation. Sensitisation was much stronger in “good” learners than in “poor” learners. A possible interpretation of this finding comes from my experiments: “good” learners should have a higher sucrose responsiveness then “poor” learners and should therefore attribute a higher relevance to a sensitising sucrose stimulus. This idea is supported by my experiments on non-associative learning (Experiments 5 and 6, see below), which

show that individuals with high sucrose responsiveness become more sensitised by a given sucrose concentration than bees with low sucrose responsiveness.

Another example of learning differences between different strains of bees is given by Brandes and Menzel (1990). The authors selected strains of honey bees for their performance in classical conditioning to olfactory stimuli and examined their operant visual learning abilities. “Good” and “poor” learners from the strains selected for olfactory conditioning also differed significantly in their visual learning values. This led the two authors to conclude that these strain differences reflect genetic differences in a common learning system rather than task-specific differences in sensory, motor or motivational components of learning. They suggest that genetic selection influences the learning performance of bees by changing their learning/memory system itself and not factors such as motivation or spontaneous response levels to the unconditioned and conditioned stimuli. However, the authors did not measure individual responsiveness to the unconditioned stimulus of “good” and “poor” learners. So, it may be that their selection for “good” and “poor” learners resulted in a selection for high and low responsiveness to sucrose, respectively.

Comparative learning experiments analysing different species and subspecies of the honey bee showed that *Apis cerana* learned the quickest and reached the highest acquisition level, *Apis mellifera lamarckii* learned similarly well, whereas *Apis mellifera ligustica* and *Apis mellifera carnica* reached a lower level of learning (Menzel et al. 1973). The authors suggested that the rewarding sucrose concentration had the same value for all the species or subspecies, because in *Apis cerana* they found no difference in the acquisition level of bees that were rewarded with 2 M (68 %) sucrose solution and those that were rewarded with honey water. In addition, Menzel and Erber (1972) had found no difference in the acquisition of *Apis mellifera* bees which were rewarded with different but fairly high sucrose concentrations (15 % to 60 % sucrose). However, my results strongly suggest that the value of the sucrose reward depends on individual sucrose responsiveness. In bees with uniform sucrose responsiveness, the sucrose concentration of the reward determines the level of acquisition (Scheiner et al. 1999). To show that bees which were rewarded with two similarly high-concentrated sugar solutions did not differ in their acquisition is not enough to demonstrate that bees of different species or subspecies perceive and evaluate a certain sucrose concentration similarly. It could well be that some of the learning variances between the 4 groups of bees were a result of differences in sucrose responsiveness instead of differences in the number of approaches per minute, as suggested by the authors. The number of approaches, regarded as “foraging motivation”, was initially higher in the group with

poorer acquisition, but soon decreased sharply. In the other two groups "foraging motivation" remained at a constant low level throughout the experiment. Future experiments should measure individual sucrose responsiveness before testing the bees in associative or non-associative learning assays to avoid problems in the interpretation of learning differences between different groups of bees.

Another comparative study of classical olfactory learning in Africanised honey bees and European honey bees (Abramson 1997) showed that during the first twenty conditioning trials European *Apis mellifera* showed a much better acquisition than the Africanised bees. The logical conclusion from my experiments is that Africanised honey bees are less responsive to sucrose than European *Apis mellifera* bees and therefore show a poorer learning performance. Whether this is the case can easily be tested experimentally.

Adult individuals of the stingless Uruçu bee show proboscis extension to Uruçu honey, but not to sucrose solutions. They show no associative learning when they are rewarded with Uruçu honey (Abramson et al. 1999). My interpretation of this behaviour is that the response thresholds to honey of these bees are so high that even though they show proboscis extension to Uruçu honey, that stimulus does not suffice to act as reward in associative learning. Interestingly, although Uruçu bees did not show any associative learning, they demonstrated non-associative learning.

#### *4.2.1.3 The effect of foraging role on acquisition*

Foraging role did not affect acquisition directly (Figure 31 and Figure 32). In the two selected strains and in the wild type, pollen foragers learned better than non-pollen foragers, because they were more responsive to sucrose. Pollen foragers did not differ from non-pollen foragers in their relationship between sucrose responsiveness and acquisition. These findings support earlier experiments analysing the tactile learning performance of wild-type foragers (Scheiner et al. 1999). In those experiments, too, pollen foragers only differed from nectar foragers in their acquisition because they had different sucrose response thresholds. When pollen and nectar foragers with uniform sucrose response thresholds were trained, they did not differ in their acquisition. These results show a strong relationship between sucrose responsiveness, foraging behaviour and associative learning performance. In how far the learning activities of pollen and nectar foragers in the field are comparable to laboratory conditioning studies with restrained animals remains to be analysed. But there is some evidence that foraging success is linked to learning abilities of workers (Seeley 1995). In

addition, these findings may be an indicator for causal relationships between individual response thresholds, learning ability and division of labour.

#### *4.2.1.4 The effect of age on acquisition*

Age affected acquisition in two ways. Preforagers were less responsive to sucrose than foragers and therefore showed a poorer tactile acquisition. In addition, age affected the relationship between sucrose responsiveness and tactile acquisition. In preforagers and foragers of the two genetic strains, sucrose responsiveness correlated positively with acquisition. But while foragers with low sucrose responsiveness showed some learning, preforagers with the same low sucrose responsiveness did not learn at all. The preforagers tested were in their second week of life. When 1-day-old bees of the two strains were analysed for their tactile or olfactory learning behaviour using the same protocol, they did not demonstrate any associative learning, although they showed proboscis extension to the unconditioned sucrose stimulus (personal observation). These findings show an age-dependent development of associative learning, which seems to be related to a change in sucrose responsiveness.

Olfactory learning studies on Africanised honey bees (Abramson et al. 1999) demonstrate a similar age effect on learning. Whereas adult bees of that species can be conditioned to odours, 1-day-old bees were unable to learn in an associative PER learning assay. But 1-day-old bees showed proboscis extension to antennal sucrose stimulation and could be sensitised.

Ray and Ferneyhough (1997) showed that 1-day-old honey bees did not show olfactory PER learning. Bees between 3 and 10 days of age showed very poor learning. About 70 % of 16-day-old bees, which were observed to guard the nest entrance, and more than 90 % of 24-day-old bees, which actively foraged, learned the conditioned stimulus. Sigg et al. (1997) showed a very poor olfactory learning performance in 2-day-old bees and an increase in learning performance during the first week of adult life. Chandra et al. (2000) demonstrated a difference in latent inhibition learning between bees from inside the hive, which were probably nurses, and those at the hive entrance, which were presumably older bees guarding the nest. Pham-Delègue et al. (1990) also showed an age effect on olfactory conditioning. The learning performance increased in the first 2 weeks of life and reached its maximum at 16 days, when bees usually begin to forage. Afterwards, learning performance decreased, which was probably due to different experimental factors such as satiation, because returning

foragers, which probably had full honey stomachs, were caught at the hive entrance, whereas all the groups of younger bees came from the hive.

One hypothesis for the observed phenomenon that preforagers in their second week of life learned more poorly than foragers is that preforagers had a lower sucrose responsiveness than foragers and will therefore show a poorer acquisition. In addition, preforagers with particularly low sucrose responsiveness showed no associative learning at all, although foragers with the same sucrose responsiveness did. The "ability-to-learn" curve is therefore shifted to higher sucrose responsiveness from preforagers to foragers. One-day-old bees have an even lower sucrose responsiveness than preforagers (Experiment 6). Nevertheless, some bees should still be able to learn. However, several attempts at conditioning 1-day-old bees failed. The hypothesis is that the "ability-to-learn" curve in 1-day-old bees is shifted even further towards higher sucrose responsiveness. It is not only the bees with very low sucrose responsiveness that show no learning in the group of 1-day-old bees, but bees with higher sucrose responsiveness also do not show associative learning, although non-associative learning is successful. In other words, the difference between the individual sucrose response threshold and the sucrose concentration of the reward needs to be very large in young bees to induce associative learning, but becomes smaller with age. This hypothesis suggests that the "attraction" of the sucrose reward is smaller for preforagers than it is for foragers with the same sucrose responsiveness.

The value of the rewarding sucrose stimulus can be enhanced by adding the amino acid glycine (Kim and Smith 2000). Some amino acids have been shown to affect feeding preferences (Inouye and Waller 1984). Under the assumption that amino acids affect the feeding preferences of preforagers and foragers to the same extent, amino acids could be added to the rewarding sucrose solution for preforagers but not for foragers, when the two groups are compared for their learning performance. Learning differences between foragers and preforagers should be reduced, but this remains to be demonstrated.

Several studies suggest that improvements of olfactory learning performance during the first days and weeks of life are a result of rapid increases in the volume of mushroom bodies and glomerular neuropil of the antennal lobes (Whithers et al. 1993, Durst et al. 1994, Whithers et al. 1995, Winnington et al. 1996, Sigg et al. 1997). Early maturation of the antennal lobes was suggested to be a prerequisite for some forms of associative olfactory learning. Whether structural changes in the bee brain are related to age-dependent differences in tactile learning remains to be tested.

It is also possible that experience with the multitude of olfactory, mechanosensory and chemosensory stimuli during adult development may have an effect on the structure of the antennal lobe. Experiments with ablated antennal lobes, which are certainly very difficult to conduct, should give some answer as to whether the learning deficits of young bees are related to different or smaller brain structures. However, preforagers in their second week of life most probably do not differ from 3-week-old foragers in their brain structure. They might rather differ from foragers in the composition of their haemolymph or in the amount of transmitters and hormones. Age-related changes of biogenic amines have, for example, been demonstrated by Taylor et al. (1992) and by Wagner-Hulme et al. (1999). These differences between foragers and younger age groups might affect sucrose responsiveness and associative learning.

#### *4.2.1.5 The effect of stimulation site on acquisition*

In associative PER learning with mounted honey bees, the antenna of an individual is usually touched very briefly with a sucrose solution, while the bee experiences the conditioned stimulus. As soon as the bee extends its proboscis, the sucrose droplet is transferred to the proboscis, and the bee is allowed to drink from the sucrose solution for about 1 second. Experiment 4 analysed the role of antennal and proboscis sucrose perception on associative PER learning.

Interestingly, the concentration of the sucrose solution which was applied to the proboscis determined the level of acquisition, whereas the sucrose concentration applied to the antenna only had a small effect. Bees which were stimulated with a high sucrose concentration at the proboscis showed a higher acquisition asymptote than bees which were stimulated with a low sucrose concentration at the proboscis, while the sucrose concentration offered to the antennae remained constant. In contrast, bees which were stimulated with different sucrose concentrations at the antennae, while the proboscis was touched with a uniform sucrose solution, did not differ in their level of acquisition (Figure 24).

One hypothesis for this behaviour is that if the duration of the sucrose stimulation had an effect on the learning performance, the sucrose stimulation of the proboscis should play a more important role in learning than that of the antenna, because the proboscis was stimulated for a longer period. Hammer et al. (1994) showed in recordings from M17 that longer stimulation of antenna or proboscis resulted in a greater activity of M17 than shorter stimulation. Only when antenna and proboscis were stimulated simultaneously did a longer stimulation not result in a greater number of M17 spikes. Therefore, the fact that sucrose

evaluation of the proboscis determined the level of acquisition could be a result of the longer stimulation *time*.

An alternative hypothesis is that the stimulation *site* determined the learning performance. Proboscis stimulation with the sucrose stimulus appeared to be more important for acquisition than antennal sucrose stimulation. Results from learning studies of Bitterman et al. (1983) support this hypothesis. The authors showed that eliminating the proboscis component of the US lowered the asymptotic level of performance in a classical conditioning experiment considerably, whereas the elimination of the antennal component had no effect. Antennal stimulation alone was not very effective as unconditioned stimulus, although it reliably elicited proboscis extension. If, however, sucrose is only applied to the proboscis during associative learning, conditioning is successful (Takeda 1961).

In an experiment investigating the strength of sensitisation to an odour after sucrose stimulation of the different sites involved in the PER, it was shown that a 1-second sucrose stimulation of the antenna alone evoked significantly fewer M17 spikes than stimulation of the proboscis or compound stimulation of antenna and proboscis (Hammer et al. 1994). This suggests a rather small effect of antennal sucrose stimulation on the motor activity of M17. But after a longer stimulation (3 s), the difference in the number of M17 spikes after stimulation at the different sites disappeared. However, it is not clear whether the intensity of proboscis extension is related to the value of a sucrose stimulus used as unconditioned stimulus and reward.

Whereas sucrose stimulation at the antenna signals a food stimulus, stimulation of the proboscis invariably leads to sucrose uptake and could therefore have more relevance in the internal evaluation of sucrose stimuli. It might well be that antennal sucrose perception and proboscis sucrose perception and evaluation are based on different mechanisms. Abramson et al. (2000) fed and conditioned bees with a sucrose-ethanol solution. Bees drank readily low concentrations of ethanol in a sucrose solution. However, most bees did not extend their probosces at antennal stimulation with 95 % ethanol in sucrose. If the antennae were stimulated with a low concentration of ethanol in sucrose but 95 % ethanol in sucrose was applied to the proboscis, the bees even drank 95 % ethanol in sucrose, as long as their antennae did not detect it. This implies that the sucrose evaluation of antennal inputs might differ from that of proboscis inputs. Further support for this hypothesis comes from learning studies using sodium chloride as US. Surprisingly, when bees were stimulated with a 1 M sodium chloride solution at the antenna, they showed proboscis extension. If they were rewarded with a sodium chloride solution at the proboscis during operant tactile conditioning,

they did not show any learning. If, however, the proboscis was stimulated with 30 % sucrose solution, while the antenna was stimulated with a sodium chloride solution, the learning performance was nearly as good as when antenna and proboscis were stimulated with the sucrose solution (personal observation).

These data show that although individual sucrose responsiveness measured at the antenna determines the level of acquisition in associative PER learning (4.2.1.1), the sucrose concentration applied to the proboscis during a learning experiment has a much greater effect on the level of acquisition than the sucrose concentration applied to the antennae. This finding implies that sucrose responsiveness measured at the antenna can serve as an excellent indicator of the "physiological" state of a bee, but the proboscis sucrose input during conditioning is more important for the level of learning than the antennal input.

#### *4.2.1.6 Acquisition and reversal acquisition*

In my experiments, bees showed fast reversal learning of tactile and olfactory stimuli. Pollen and non-pollen foragers basically did not differ in their learning behaviour between the first acquisition phase and reversal acquisition. In both learning phases, sucrose responsiveness determined the level of acquisition in pollen and non-pollen foragers similarly. Bees with high sucrose responsiveness showed good acquisition and good reversal learning, whereas bees with low sucrose responsiveness performed poorly in both learning phases. This finding, in which both learning and reversal learning basically did not differ, is supported by earlier experiments using the same protocol (Scheiner et al. 1999). However, it is in contrast to findings of Menzel (1985) that reversal learning is strongly retarded after 5 or 10 conditioning trials and the suggestion of Rescorla (1988) that the unconditioned stimulus loses its power as a reinforcer after extended training. The learning paradigms used in the different experiments, however, are probably not comparable and more studies comparing a wider range of experimental conditions are needed to investigate this phenomenon in detail.

In my experiments, the alternative test stimulus from the first part of the learning experiment (horizontal pattern or carnation odour) served as conditioned stimulus during reversal learning. The conditioned stimulus from the first learning phase (vertical pattern or citral) became the alternative test stimulus in reversal learning. Both odours and both tactile patterns were learned equally well. This, however, does not prove that the salience of the two olfactory stimuli and the two tactile stimuli was the same, because the vertical pattern and citral were always conditioned first. It is rather surprising that the two odours and the two tactile patterns were learned equally well, given that the test stimulus from the first

conditioning phase should elicit latent inhibition when used as conditioned stimulus in the second learning phase (Lubow 1997, Chandra et al. 2000). As no latent inhibition was observed, it might be that the lack of reward during the extinction tests after the first acquisition phase did not serve as a negative signal associated with the test stimulus.

The most important result of the comparison of the first acquisition phase and reversal learning is that sucrose responsiveness determined the level of acquisition in both phases. As both groups of foragers did not differ in their acquisition between the two learning phases, the sucrose stimuli which served as reward during the first acquisition phase did not have a strong effect on reversal learning.

#### *4.2.1.7 Acquisition in operant tactile and in classical olfactory learning*

Although the two learning paradigms are too different to be compared directly, some general observations seem appropriate. In both operant tactile learning and classical olfactory learning, sucrose responsiveness determined the asymptote of the acquisition function. Regardless of genotype or foraging role, bees with high sucrose responsiveness showed a higher level of acquisition than bees with low sucrose responsiveness. These findings support the assumption that individual sucrose responsiveness is generally a strong determinant of the learning performance in PER conditioning, regardless of the conditioned stimuli involved or the learning paradigm. However, this hypothesis has still to be tested for other forms of associative learning, such as visual learning.

#### *4.2.1.8 Conclusions*

Taken together, these findings demonstrate that acquisition in associative PER learning strongly depends on individual sucrose responsiveness, which is affected by genotype, foraging role, age and other factors such as nutrition. Regardless of whether bees were operantly trained to tactile patterns or classically conditioned to odours, learning differences between bees of different genetic strains, between pollen and non-pollen foragers, and between bees of different ages can to a large extent be explained by differences in individual sucrose responsiveness. This makes it very important to test bees for their gustatory responsiveness prior to learning experiments or to compensate for differences in sucrose responsiveness. It also shows that sucrose responsiveness is a good behavioural indicator of the physiological state of a bee. The phenomenon that differences in response thresholds for appetitive stimuli affect acquisition is not unique to honey bees, but has been shown for other insect species (Brigui et al. 1990, Fois et al. 1999), supporting the hypothesis

that learning, like division of labour, is affected by individual differences in response thresholds to stimuli associated with the conditioned stimulus or task. In bees with uniform sucrose responsiveness, acquisition is strongly affected by the sucrose concentration applied to the proboscis and little affected by the sucrose concentration applied to the antenna.

### 4.2.2 Extinction of conditioned responses

#### *4.2.2.1 General observations*

Many bees ceased responding to the conditioned stimulus with repeated unrewarded tests. In all groups, the extinction of responses to the conditioned pattern or odour was strongly influenced by acquisition. Bees with high acquisition scores showed less extinction than bees with low acquisition scores. Genotype and foraging role did not have a direct effect on extinction, but age independently affected extinction.

#### *4.2.2.2 The effect of genotype on extinction*

Genotype did not affect the extinction of conditioned responses directly. In high- and low-strain bees, individuals with high acquisition scores displayed less extinction than those with low acquisition scores. The relationship between acquisition and extinction of conditioned responses did not differ between bees of the two genetic strains in tactile or olfactory learning. But high-strain bees, on average, showed less extinction than low-strain bees, because they had a higher sucrose responsiveness. Individuals with high sucrose responsiveness, in turn, demonstrated a high level of acquisition. Whether long-term memory is different in high-and low-strain bees, needs to be tested. However, preliminary data from "wild-type" bees (personal observation) suggest a role for sucrose responsiveness in long-term memory.

#### *4.2.2.3 The effect of foraging role on extinction*

Foraging role did not have a direct effect on the extinction of conditioned responses after one acquisition phase. Pollen foragers showed less extinction than non-pollen foragers, because they were more responsive to sucrose, and GRS correlated positively with acquisition and extinction CS+ scores. Individuals with high sucrose responsiveness showed stronger acquisition and less extinction than bees with lower sucrose responsiveness. Pollen foragers did not differ from non-pollen foragers in their relationship between sucrose responsiveness and acquisition or extinction, regardless of genotype. These results are supported by earlier experiments analysing the tactile learning performance of wild-type foragers (Scheiner et al.

1999) and suggest that workers with different foraging roles do not differ in the formation of short-term memory. They imply that although pollen and nectar foragers have to learn different types of flowers, the basic mechanisms underlying acquisition and storage of information are the same. Division of foraging labour and differences in associative learning and memory formation are related to sucrose responsiveness.

Interestingly, after reversal tactile learning, pollen foragers differed from non-pollen foragers in their relationship between sucrose responsiveness and extinction. Pollen foragers with a given sucrose responsiveness showed stronger extinction than non-pollen foragers, although they did not differ from non-pollen foragers during reversal acquisition. After olfactory reversal learning, the relationship between sucrose responsiveness and extinction also differed between pollen and non-pollen foragers. In non-pollen foragers, sucrose responsiveness and extinction CS+ scores correlated. In pollen foragers, however, no correlation was found. These findings imply that the sucrose rewards during the first conditioning phase might have had a different effect on pollen foragers than on non-pollen foragers. But further studies are needed to analyse the extinction of pollen and non-pollen foragers in reversal learning assays.

#### *4.2.2.4 The effect of age on extinction*

Age affected extinction. Particularly preforagers with high sucrose responsiveness showed more extinction than foragers with the same sucrose responsiveness. This is due to the fact that preforagers differed from foragers in their relationship between sucrose responsiveness and extinction. However, at this point it is not possible to determine whether the observed differences in the extinction of preforagers and foragers are related to foraging experience or age. The results imply that foragers are more persistent in their responses to learned stimuli than preforagers – a behaviour which could be related to foraging experience. Gerber et al. (1996), for example, demonstrated that foraging experience can affect learning behaviour in the laboratory. But the observed phenomenon could also be related to an age-dependent difference in the evaluation of sucrose stimuli in the context of associative learning such as was demonstrated for acquisition.

Similar results were found in *Drosophila*, where flies of different ages differed in extinction, although not in acquisition (Brigui et al. 1990). Flies were conditioned to suppress proboscis extension when their tarsi touched a sucrose stimulus by applying either an electroshock or quinine chlorhydrate to the legs. Middle-aged flies (30 days old) and old flies (50 days old) did not differ in acquisition but in extinction. Middle-aged flies showed faster

extinction than old flies. As flies differ from bees in their behavioural development and in their age-related change in sucrose responsiveness, direct comparisons are not appropriate. However, the middle-aged flies behaved similar to the preforaging bees, and the older flies showed similar behavioural patterns as foragers of the honey bee. Very young flies (7 days old) differed from both groups, as is the case in honey bees (see above).

Ben-Shahar et al. (2000) showed that nurse bees went extinct to a "punished" odour during reversal learning faster than normal-aged foragers. Nurse bees also showed more rapid extinction to a negatively reinforced odour than same-aged precocious foragers. These data show that differences in extinction cannot be traced back exclusively to behavioural role or age.

#### *4.2.2.5 Conclusions*

Extinction of conditioned responses is mainly affected by acquisition, which, in turn, is set by sucrose responsiveness. For that reason, extinction should not be analysed separately from acquisition. Genotype did not affect extinction independent of sucrose responsiveness. Foraging role had no separate effect on extinction after one acquisition phase. Preforagers showed stronger extinction than foragers with similar sucrose responsiveness. It is conceivable that this difference in short-term memory is affected by foraging behaviour, which might require a different form of memory than that displayed in young hive bees. But the differences between preforagers and foragers could also be age-dependent, as has been shown to be the case in other studies of honey bees, and flies of different ages. These findings show that sucrose responsiveness is the decisive factor not only for acquisition but also for multiple-trial induced memory in the minutes' range after conditioning.

### 4.2.3 Discrimination

#### *4.2.3.1 General observations*

All groups of bees discriminated significantly between the conditioned tactile pattern and the alternative pattern or between the conditioned odour and the alternative odour. They responded significantly more often to the conditioned stimulus than to the alternative stimulus. Even after reversal learning, when conditioned stimulus and alternative stimulus were exchanged, bees showed significant discrimination.

Acquisition strongly affected discrimination in all groups. Bees with high acquisition scores showed little discrimination, whereas individuals with low acquisition scores discriminated well. "Poor" learners usually have a low sucrose responsiveness, while "good"

learners show a high sucrose responsiveness. Therefore, discrimination is indirectly affected by sucrose responsiveness. This was, however, not always reflected directly by significant positive correlations between GRS and extinction CS- scores.

There has been good evidence that antennal scanning behaviour of honey bees affects tactile discrimination abilities (J. Erber, personal communication). Bees that discriminated between two tactile patterns differed in their antennal scanning behaviour from those that did not discriminate. Most bees scanned large areas of the conditioned pattern with movements of the flagellum, when it was novel to them. After a few conditioning trials, only small areas of the conditioned pattern were scanned. Bees which showed discrimination also scanned large areas of the alternative test pattern. Individuals which did not discriminate between the two patterns scanned only small areas of both the conditioned pattern and the alternative pattern. In addition, Kisch (2001) showed a correlation between frequency of antennal contacts and discrimination. Bees with low spontaneous scanning frequency discriminated better after a learning experiment than bees with high initial antennal scanning frequency. There is some evidence for a correlation between sucrose responsiveness and antennal scanning behaviour. Bees with high sucrose responsiveness show a higher contact frequency ventrally in the range of two minutes than bees with lower sucrose responsiveness (Kisch 2001).

These factors collectively suggest that bees with high sucrose responsiveness appear to be more "active" during conditioning but are less likely to discriminate between rewarded tactile stimuli and unrewarded test stimuli, presumably because they pay less attention to details. Individuals with low sucrose responsiveness, in contrast, seem less "active" but are very "accurate" in their choice behaviour. Whether these behavioural differences are related to foraging strategies remains to be tested. Genotype affected discrimination in foragers. Foraging role had an effect on discrimination after reversal learning, and age affected discrimination in high-strain bees.

#### *4.2.3.2 The effect of genotype on discrimination*

High-strain foragers discriminated significantly better than low-strain foragers (Figure 34), suggesting an effect of genotype on discrimination which is independent of sucrose responsiveness. These results are supported by data of Benatar et al. (1995) who showed that sensitisation and discrimination seem not to be genetically linked. When the authors selected strains of bees for "good" or "poor" discrimination in an olfactory differential conditioning assay, they chose drones with a very fast acquisition. They found no difference in sensitisation to odours in the worker progeny but differences in discrimination between a

rewarded odour and a negatively reinforced odour. I assume that the authors only used bees that were very responsive to sucrose and therefore demonstrated good acquisition and strong sensitisation (see below). Because they differed in discrimination, sensitisation and sucrose responsiveness appear not to be genetically linked to discrimination.

High- and low-strain preforagers did not differ in their discrimination in my experiments. However, this could be related to the smaller sample sizes compared to foragers. A greater number of preforagers tested may have detected significant differences in the discrimination of high- and low-strain preforagers. Alternatively, the difference in discrimination between high- and low-strain bees could only be present in foragers and be related to differences in the development of high- and low-strain bees.

#### *4.2.3.3 The effect of foraging role on discrimination*

The effect of foraging role on discrimination is less clear. In the two selected strains (Figure 35) and in the wild type (Figure 36) pollen foragers did not differ from non-pollen foragers in their discrimination indices after one acquisition phase. This finding supports the assumption that foraging role does not affect discrimination and is supported by earlier experiments comparing the discrimination of wild-type workers with different foraging roles (Scheiner et al. 1999). After reversal learning, however, foraging role seemed to have an effect on discrimination: non-pollen foragers showed the tendency to discriminate better than pollen foragers. After reversal tactile learning in wild-type foragers, the slope of the regression of extinction CS- scores on acquisition scores was significantly steeper for pollen than for non-pollen foragers, indicating that pollen foragers with a certain acquisition score responded more often to the alternative pattern than corresponding non-pollen foragers and thus showed less discrimination. After reversal olfactory learning, the discrimination index of non-pollen foragers was significantly higher than that of pollen foragers. So far, little is known about the memory of pollen and non-pollen foragers. It could be that the differences in the discrimination of pollen and non-pollen foragers are very small, so that they only become apparent in an experiment with several learning and reversal learning phases, where small effects add up. Alternatively, pollen and non-pollen foragers could differ in the course of memory retrieval, so that pollen foragers might show less discrimination after reversal learning, because they keep responding to the first conditioned stimulus longer than non-pollen foragers. More experiments with several learning and reversal learning situations are needed to test whether non-pollen foragers generally discriminate better than pollen foragers.

#### *4.2.3.4 The effect of age on discrimination*

Age had a strong effect on discrimination in high-strain bees but not in low-strain bees (Figure 37). High-strain foragers discriminated significantly better than high-strain preforagers. Low-strain foragers did not differ from low-strain preforagers in their discrimination index. The reason for this difference in discrimination is unclear. Additional experiments with wild-type bees and bees of the two genetic strains of several age groups should be conducted to determine the nature of this phenomenon.

#### *4.2.3.5 Discrimination after learning and after reversal learning*

Interestingly, tactile discrimination after reversal acquisition was as good as after the first learning phase in pollen and non-pollen foragers. This result is rather surprising, because the conditioned stimulus and the alternative stimulus were changed. It demonstrates a quick adjustment of the bees to the new situation. Discrimination of odours, in contrast, was significantly poorer after reversal acquisition than after the first acquisition phase, implying a longer memory of the odour that was first learned. This difference between operant tactile and classical olfactory learning is probably related to the differences in the discrimination of olfactory and tactile cues, which will be discussed in the next section.

#### *4.2.3.6 Discrimination in operant tactile and classical olfactory conditioning*

In my experiments, olfactory discrimination differed from tactile discrimination. Bees discriminated better between citral and carnation than between the pattern with vertical grooves and that with horizontal grooves (Figure 39). However, a direct comparison of discrimination between the two learning protocols is not appropriate. Tactile and olfactory learning differ in many respects. The two forms of learning involve different stimuli, receptors, interneurons and brain structures. Tactile learning requires the active scanning of the object on part of the bee. During olfactory learning, the odour is blown at the antennae, while the bee remains stationary.

Menzel (1990) points out that tactile stimuli generally have less salience than olfactory stimuli. However, this has not been demonstrated by my experiments. Mühlen (1987) also showed that bees discriminated very well between different forms in a walking apparatus. In addition, Erber et al. (1997) and Scheiner et al. (2001) showed that bees can very well discriminate between positions of tactile targets. When an individual is trained to a tactile target presented to one antenna, the bee will not respond if the target is presented to the

contralateral antenna. Tactile discrimination itself strongly depends on the nature of the stimuli (Erber et al. 1998).

Discrimination between different odours also varies considerably (Takeda 1961, Vareschi and Kaissling 1970, Vareschi 1971, Menzel et al. 1993, Pelz et al 1997, Vickers et al. 1998), and visual learning and discrimination strongly depend on the individual stimulus quality (Menzel 1967, Lehrer 1999, Maddess et al. 1999, Horridge 2000). Generally, tactile cues might be more relevant for the orientation within the hive, whereas odours and visual stimuli might be more easily learned in association with food.

Taken together, these findings demonstrate that bees can very well discriminate tactile structures and odours. It is inappropriate to compare the two learning paradigms directly, but a general observation can be made. In both learning paradigms, "good" learners did not discriminate well, whereas "poor" learners discriminated very well. As acquisition is strongly affected by sucrose responsiveness, a bee with high sucrose responsiveness is less likely to discriminate as well as a bee with low sucrose responsiveness.

#### *4.2.3.7 The effect of stimulation site on discrimination*

Discrimination was better when proboscis and antenna were stimulated with a low sucrose concentration than when they were stimulated with a high sucrose concentration (Figure 38). This finding is well in line with earlier results of Scheiner et al. (1999) showing that discrimination was best when bees with uniform sucrose responsiveness received a low-concentrated sucrose solution as reward. In my experiments, discrimination directly correlated with acquisition. Bees which received the smallest reward (i.e. low sucrose concentration at antenna and proboscis) showed the poorest acquisition but the best discrimination. The biological relevance of this phenomenon is unclear. The behaviour of the bees in the learning experiments might be related to their foraging strategies. Some bees tend to switch easily between different nectar or pollen sources and thus demonstrate great generalisation, whereas other bees stay with the same flower type and thus demonstrate good discrimination. The decision of whether to stay with one flower type or to switch to a different type was shown to depend on reward volume (Greggers and Menzel 1993, Fülöp and Menzel 2000). My hypothesis is therefore that bees decide whether to switch to a different flower type on the basis of their sucrose responsiveness. If the current food source is of a low quality, bees with high sucrose responsiveness might stay, because for them the food is still attractive. Individuals with low sucrose responsiveness might switch more easily to a different flower type, because for them the same nectar source might be unattractive. Similar

to associative reward learning, the difference between the individual sucrose response threshold and the nectar concentration of a food source might be decisive for the foraging strategy.

#### *4.2.3.8 Conclusions*

Taken together, these results show that acquisition strongly affected discrimination. Bees with high sucrose responsiveness or bees which were rewarded with a high sucrose concentration at the proboscis showed strong acquisition and consequently poor discrimination. Individuals with low sucrose responsiveness or ones that were rewarded with low sucrose concentrations at the proboscis discriminated very well. Genotype affected discrimination in foragers but not in preforagers. Age affected discrimination in high-strain bees but not in low-strain bees. Foraging role affected discrimination after reversal learning but not after the first learning phase. Bees discriminated the odours better than the tactile patterns. These findings show the complexity of discrimination in honey bees and the diversity of factors determining it.

## *4.3 Non-associative learning*

### 4.3.1 Effect of sucrose responsiveness and different sucrose stimuli on habituation

Habituation was strongly affected by individual sucrose responsiveness and the sucrose concentration of the habituating stimulus. Bees with low sucrose responsiveness demonstrated stronger habituation than bees with high sucrose responsiveness (Figure 40). This finding is directly supported by earlier studies, which, however, did not take into account individual differences in sucrose responsiveness. Braun and Bicker (1992) showed that hungry bees habituate more slowly than satiated bees. As hungry bees have a higher sucrose responsiveness than satiated bees (Page et al. 1998, Pankiw et al. 2001), differences in the rate of habituation might well have been related to differences in sucrose responsiveness. The reason why habituation was stronger in bees with low sucrose responsiveness than in bees with high sucrose responsiveness is presumably the smaller difference between individual sucrose response threshold and concentration of the habituating stimulus. This means that bees with similar sucrose responsiveness should habituate faster to a low sucrose concentration than to a high sucrose concentration. My experiments demonstrate that the sucrose concentration of the habituating stimulus indeed affected habituation. Regardless of

individual sucrose responsiveness, a high sucrose concentration resulted in weak habituation. Low sucrose concentrations resulted in strong habituation (Figure 42). This finding is supported by earlier studies of Braun and Bicker (1992). The authors showed that bees habituated faster to tap water and 5 % sucrose solution than to 50 % sucrose solution. Both results support the dual-process theory, according to which the degree of habituation is inversely correlated to stimulus intensity (Petrinovich 1984).

There is now some evidence that in preforagers of the two selected strains of Page and Fondrk (1995) individual sucrose responsiveness also affects the course of habituation. High-strain bees, which always show a higher sucrose responsiveness than low-strain bees, habituated more slowly and showed stronger sensitisation than low-strain bees (R. Page, personal communication).

The mechanisms regulating sucrose responsiveness and habituation are not clear. However, it is conceivable that biogenic amines could be involved in that regulation. It has been shown that bees which had been fed with tyramine or the formamidine chlordimeform, an octopaminergic receptor agonist (Evans 1985), needed more trials for habituation than untreated bees (Braun and Bicker 1992). My experiments demonstrate that octopamine and tyramine increase sucrose responsiveness (see 4.5.1) and that bees with higher sucrose responsiveness need more trials for habituation than bees with low sucrose responsiveness. These findings collectively suggest a relationship between sucrose responsiveness, biogenic amines, and non-associative learning.

### 4.3.2 Effect of sucrose responsiveness and different sucrose stimuli on sensitisation

Both individual sucrose responsiveness and the sucrose concentration of the sensitising stimuli affected sensitisation. Bees with high sucrose responsiveness measured at the beginning of the experiment responded more often to the sensitising sucrose solution than bees with low sucrose responsiveness (Figure 41). This finding is well in line with the results from my habituation experiments. For bees with high sucrose responsiveness the “value” of the sensitising sucrose solution seems to be greater than for bees with low sucrose responsiveness. Therefore, in bees with uniform sucrose responsiveness a high sucrose concentration should induce stronger sensitisation than a low sucrose concentration. My experiments directly support this assumption (Figure 43). These results are well in line with several studies showing that the strength of sensitisation depends on the quality of the

sensitising stimuli (Marcus et al. 1988). A higher sucrose concentration implies a stronger salience for bees with similar sucrose responsiveness than a low sucrose concentration and results in stronger sensitisation.

In my sensitisation experiments, most bees responded with proboscis extension to the first sensitising stimulus. Several stimulations with the sensitising sucrose stimulus did not enhance the sensitisation effect but led to a decrease in responses, similar to that observed in habituation. Particularly a low sucrose concentration elicited rather habituation than sensitisation effects. This finding is supported by an earlier experiment by Menzel et al. (1989) who showed that three sensitisation trials did not enhance the sensitisation effect but rather reduced it and by experiments of Hammer et al. (1994), who showed that multiple sensitisation trials reduced sensitisation. It fits well into the dual-process theory, which suggests that sensitisation with multiple stimulations first grows and then decays (Petrinovich 1984). However, there are also experiments showing that five successive sucrose stimulations led to stronger sensitisation of visual neurons than one stimulation (Erber 1984). More than five stimulations, however, also led to a decrease in sensitisation to visual stimuli. In addition, the inter-trial interval in that experiment was with 1 min much longer than that used in my experiments.

### 4.3.3 Effect of age on habituation and sensitisation

Age affected habituation but not sensitisation. The young hive bees of the different age groups did not differ in their sucrose responsiveness but in their relationship between sucrose responsiveness and habituation. Bees with low sucrose responsiveness did not differ in their habituation between the different age groups. But bees younger than 1 day old with intermediate or high sucrose responsiveness showed stronger habituation than older bees with the same sucrose responsiveness (Figure 45). It might be that these differences in habituation are related to the slightly different handling procedures of the different age groups. However, the phenomenon that young bees (4 - 7 days old) need fewer trials for habituation than older bees (8 - 10days old) has also been demonstrated by Guez et al. (2001). In addition, sucrose responsiveness differs between foragers and preforagers (Pankiw and Page 1999, see 4.1.4). Because habituation is strongly affected by individual sucrose responsiveness (see 4.3.1), foragers should, on average, show weaker habituation than preforagers, but direct evidence is still needed.

Neural correlates of age-dependent changes in habituation have not been described so far. It can be assumed that the modulation of sucrose responsiveness and habituation is related

to changing titres of biogenic amines during behavioural development (Taylor et al. 1992, Wagner-Hulme et al. 1999) but there is no experimental evidence so far.

Age had no demonstrable effect on sensitisation in my experiments, which, however, could be related to the fact that most bees with intermediate or high sucrose responsiveness reached maximum sensitisation scores. Therefore, it would have been difficult to detect small differences between the groups. It appears necessary to address this question in a new experiment in which bees of different age groups (preferably with greater age differences) are sensitised with lower sucrose concentrations to detect possible differences in sensitisation. The hypothesis for such an experiment is that older bees should become more sensitised by a given sucrose concentration than younger bees, because they have a higher sucrose responsiveness, and sensitisation might change with development similar to habituation.

## *4.4 Comparison of associative and non-associative learning*

Responsiveness to gustatory stimuli determined the course of associative and non-associative forms of learning in the honey bee. Bees with high sucrose responsiveness showed good acquisition, little extinction, and poor discrimination in associative learning. In non-associative learning, individuals with high sucrose responsiveness demonstrated strong sensitisation and weak habituation. The assumption is that the greater the difference between the individual response threshold to sucrose and the sucrose concentration of the unconditioned stimulus, the greater is the salience of the unconditioned sucrose stimulus. A great salience of the US then results in good acquisition, weak extinction and poor discrimination but also in slow habituation and great sensitisation. My experiments strongly suggest a correlation between associative and non-associative learning with respect to sucrose responsiveness. Further evidence for this hypothesis comes from experiments by Brandes et al. (1988). The authors found that bees displaying “good” associative learning showed stronger sensitisation to an odour than “poor” associative learners. “Good” learners, however, were presumably bees with high sucrose responsiveness, whereas “poor” learners probably had a low sucrose responsiveness.

A correlation of sensitisation and associative learning was also found in the blowfly *Phormia regina*. In this fly, sucrose stimulation of either labellum or prothoracic tarsi induces behavioural modifications which Dethier et al. (1965) called “central excitatory state” (CES), and which is comparable to sensitisation. Flies normally do not respond to water stimulation with proboscis extension, but they will respond with proboscis extension to a water stimulus

when they were sensitised with sucrose shortly before. Nelson (1971) showed that blowflies can even be classically conditioned to respond to different gustatory stimuli using the proboscis extension response (PER). McGuire and Hirsch (1977) succeeded in selecting blowflies for their performance in such an associative learning paradigm. Flies with a "high" or a "low" learning performance were selected. Tully et al. (1982) and McGuire (1983) demonstrated that CES scores highly correlated with PER conditioning scores. Flies which were easily sensitised by sucrose also learned well to associate water or saline with a sucrose reward. This finding does not only indicate a genetic link (pleiotropy or linkage) between non-associative and associative forms of learning but also implies that sucrose responsiveness might be that link. Although the proboscis extension paradigm for flies is slightly different from that of bees, it is conceivable that flies which showed a strong sensitisation and a "high" conditioning performance had a high sucrose responsiveness, whereas flies of the "low" conditioning line, which showed poor sensitisation, also had a lower sucrose responsiveness.

Vargo and Hirsch (1985) selected *Drosophila* for their performance level of sucrose-induced CES. However, whereas in one experiment only a "high CES performance" line but no "low CES performance" line was successfully selected, in another experiment, only a "low CES performance" line was selected. Evidence for a genetic link between associative and non-associative learning comes from *Drosophila* learning mutants. Many of the associative learning mutants also express deficiencies in habituation and sensitisation (Davis 1996). In the *turnip* mutant, the effects of associative learning and of habituation both map to the same genetic region (near *carnation*) (Duerr and Quinn 1982). The abnormalities of the *amnesia* mutant in sensitisation and associative memory map to a locus near *carnation*. In *Aplysia*, parallel findings have also indicated that non-associative and associative conditioning have in part a common molecular cascade (Carew 1987), which, if it applies genetically, might be a basis for pleiotropy. These findings are all well in line with the suggestion by Hawkins and Kandel (1984) that non-associative and associative learning share many similarities and basic mechanisms and differ rather quantitatively than qualitatively.

However, there have also been a number of results contrasting this idea. Whereas honey-bee foragers with different sucrose responsiveness can be trained in associative and non-associative learning assays, 1-day old bees fail to learn associatively but can be sensitised and habituated (personal observation). Adult Africanised honey bees can be conditioned to odours, 1-day-old Africanised bees fail to learn odours in the same paradigm, although they show non-associative learning (Abramson et al. 1999). Adult stingless Uruçu bees can be sensitised but will not learn associatively (Abramson et al. 1999). These findings imply

different mechanisms for non-associative and associative learning. Alternatively, non-associative learning might be less "sensitive" for sucrose responsiveness than associative learning, so that the difference between individual sucrose response threshold and sucrose concentration of the US needs to be larger for associative learning than for non-associative learning to induce successful learning.

A further difference between non-associative and associative learning is the effect of multiple trials on learning performance. My experiments and earlier experiments by Erber (1984), Menzel et al. (1989) and by Hammer et al. (1994) show that multiple sensitisation trials diminish the effect of sensitisation rather than increase it, whereas multiple conditioning trials lead to a more robust memory (Menzel 1999). Menzel et al. (1999) argue that modulators involved in sensitisation and in reinforcement during associative conditioning are pharmacologically separable in the honey bees. When they injected octopamine into reserpinised bees, it rescued olfactory conditioning but did not rescue sensitisation. My results, in contrast, show that octopamine, when injected in excess of intrinsic octopamine, increases sucrose responsiveness (see 4.5.1) and should thus indirectly lead to a faster acquisition in associative PER learning, to a stronger sensitisation and to a slower course of habituation.

At this point, without having the neuronal mechanisms of associative and non-associative learning at hand, it is impossible to determine to what degree non-associative and associative learning processes are related and in what ways they differ. The analyses of the interactions between neurons, synapses, glia cells, transmitters, modulators and second messengers are very complex. It is certain, however, that there are many similarities between non-associative and associative learning. Sucrose responsiveness is a key factor. Future research will, hopefully, separate the different mechanisms underlying both forms of learning and the effects of transmitters, neuromodulators and neurohormones in the different brain structures. The study of transmitters and signalling cascades involved in sucrose responsiveness, non-associative and associative learning is a first step towards the understanding of the biochemical basis of these forms of behavioural plasticity.

## *4.5 Modulation of sucrose responsiveness by biogenic amines*

### 4.5.1 Octopamine and tyramine increase sucrose responsiveness

Both octopamine and its metabolic precursor tyramine increased sucrose responsiveness in the range of minutes. Thirty minutes after injection, bees which were

injected with octopamine or tyramine in different concentrations showed significantly higher gustatory response scores than prior to injection. The effects of octopamine and tyramine appeared dose-dependent. Ninety minutes after injection, most of these modulatory effects were no longer detectable, demonstrating the reversible modulation of sucrose responsiveness.

These findings are supported by a number of earlier studies on the effects of biogenic amines on the elicitation of the PER which show that octopamine increased responsiveness to gustatory stimuli and water vapour (Mercer and Menzel 1982, Bicker and Menzel 1989, Menzel et al. 1990, Erber et al. 1993). Fully satiated bees do not show proboscis extension when their antennae are stimulated with sucrose solution. Injection of octopamine, however, restores the PER in fully satiated bees (Menzel et al. 1990). Reserpine depletes the bee nervous system of monoamines. In an experiment by Braun and Bicker (1992) 30 % of the reserpinised bees showed no longer the proboscis extension response, the rest displayed lower response amplitudes than controls. Injection of tyramine or octopamine into reserpinised bees restored the PER and strongly increased spike activity of M17 compared to control bees or bees in which reserpine treatment did not lead to a loss of the PER. In addition, both octopamine and tyramine increased the number of trials necessary for habituation of the PER (Braun and Bicker 1992), indicating an increase in sucrose responsiveness, because bees with high sucrose responsiveness need more trials for habituation than bees with low sucrose responsiveness (see 3.10.1). Feeding of octopamine also reduced the age of first foraging (Schulz and Robinson 2001). Because sucrose responsiveness increases with age (foragers have a higher sucrose responsiveness than preforaging bees), octopamine might be involved in the age-dependent regulation of sucrose responsiveness.

Octopamine does not only increase responsiveness to different stimuli in honey bees. In several other insects, too, octopamine increases cellular responses by acting on sensory receptors, interneurons, motoneurons or muscles (Roeder 1999). A stimulating effect of octopaminergic agonists on feeding behaviour was also demonstrated for the blowfly (Long and Murdock 1983).

How octopamine and tyramine increase responsiveness to sucrose in the honey bee is not known. Firstly, the amines could directly modulate the sensitivity of sucrose receptor neurons. In locust muscle cells, for example, octopamine was shown to affect currents maintaining the resting membrane potential and the $Na^+/K^+$ pump via mediation through PKA (Walther and Zittlau 1998). Secondly, the transmitters could act indirectly on the sucrose-mediating pathway by affecting the metabolism. Octopamine, for example, is known to

control the energy metabolism of insects by increasing the release of lipids from the fat body and enhancing glycolysis (Evans 1985). Octopamine or tyramine could also affect sucrose responsiveness via the cAMP pathway. It has been shown by Hildebrandt and Müller (1995) that injection of octopamine into the antennal lobes increases PKA activity. Bees with high sucrose responsiveness showed a higher PKA activity in the antennal lobes than bees with low sucrose responsiveness (see below). It is most likely that sucrose responsiveness is not modulated by only one of the suggested mechanisms but probably by all of them.

### 4.5.2 Dopamine and ADTN decrease sucrose responsiveness

The effects of dopamine on the elicitation of proboscis extension are different from those of octopamine or tyramine. Injection of dopamine or the dopamine receptor agonist ADTN led to a dose-dependent decrease in sucrose responsiveness 30 min after injection. Ninety minutes after injection, the effects were mostly vanished.

There are only few studies on the effects of dopamine on the elicitation of sucrose-induced PER. Blenau and Erber (1998) showed that injection of dopamine into the α-lobes of the mushroom bodies decreased the rate of proboscis extension in response to water vapour, which is in line with my findings that dopamine reduces gustatory responsiveness, although water vapour is perceived by olfactory receptors and not by contact-chemosensory receptors. Mercer and Menzel (1982) showed that dopamine injection did not affect the elicitation of sucrose-induced PER. This was also true when dopamine was applied onto the antennal lobes (Macmillan and Mercer 1987) and when dopamine was injected into the protocerebrum (Menzel et al. 1988). Presumably, the sucrose concentrations which were used in those experiments were too high to detect individual changes in sucrose responsiveness, because most bees respond to high sucrose concentrations with proboscis extension. Alternatively, their experiments might show that the application and injection sites chosen have nothing to do with sucrose responsiveness. Braun and Bicker (1992) demonstrated that the injection of octopamine or its metabolic precursor tyramine restored the PER in reserpinised bees, whereas dopamine had no such effect. It is not surprising that injection of dopamine into reserpinised bees did not result in restoration of the reflex, because dopamine should decrease sucrose responsiveness even further, as it did in my experiments. This could also explain why dopamine, in contrast to octopamine, did not rescue acquisition in reserpinised bees (Menzel et al. 1999) or why dopamine, when injected into the mushroom bodies, reduced the conditioned PER in memory and retrieval tests (Bicker and Menzel 1989).

So far, nothing is known about the mechanisms by which dopamine or ADTN modulate sucrose responsiveness. Similar mechanisms as those suggested for octopamine or tyramine are conceivable. It has been shown, for example, that injection of dopamine into the Kenyon cells of the mushroom bodies increases PKA activity like octopamine (Müller 1997).

### 4.5.3 Other factors modulating sucrose responsiveness

In addition to biogenic amines, there are other factors modulating sucrose responsiveness. The state of satiation, for example, affects sucrose responsiveness. Hungry bees are more responsive to water and sucrose than satiated bees (Menzel et al. 1989, Page et al. 1998, Pankiw et al. 2001). Bees fed with low sucrose concentrations for 24 hours were more responsive to sucrose than bees fed with a high sucrose concentration for the same time (Pankiw et al. 2001). Brood pheromone increases sucrose responsiveness (Pankiw and Page 2001). Weather and season affect sucrose responsiveness (Scheiner et al. 2000). A short-term modulation of sucrose responsiveness is demonstrated by crop filling. Pankiw et al. (2001) compared the sucrose responsiveness of water foragers that had just landed on a water feeder with that of water foragers that had imbibed water for several seconds. Bees were more responsive to sucrose before they consumed water, although the water did not have any nutritional value.

### 4.5.4 Conclusions

These data demonstrate a great plasticity of sucrose responsiveness on different time scales. Crop-filling changes sucrose responsiveness in the range of seconds. Biogenic amine receptor ligands, foraging experience or nutrition modulate sucrose responsiveness in the range of minutes to hours, whereas pheromones have longer-lasting effects on sucrose responsiveness. In addition, genetic selection can modulate sucrose responsiveness over many bee generations. The different forms of modulation probably have different underlying mechanisms.

### 4.5.5 Neural correlates for the modulation of sucrose responsiveness

It has been shown that sucrose responsiveness can be modulated by a great variety of factors. How this modulation is regulated and which brain structures are involved is less clear. The pathway mediating the PER to sucrose consists of contact-chemoreceptors on the antennae and the proboscis, which project into the suboesophageal ganglion and terminate in close apposition to both motor neurons and premotor neurons involved in the movement of

the proboscis (Rehder 1989). Modulation of sucrose stimuli could take place at three different levels.

Antennal gustatory receptors receive the sensory information and first evaluation of sucrose responsiveness takes place at this level. Modulation of sucrose responsiveness might also take place here, for example through the action of neuromodulators. It has been shown for the silkmoth *Antheraea polyphemus*, for example, that octopamine injection can modulate the responses of antennal pheromone receptors (Pophof 2000).

The next level of the evaluation of gustatory stimuli are interneurons in the brain processing gustatory antennal information. A good example comes from the VUMmx1 (ventral unpaired median) neuron (Hammer 1997). This neuron responds to antennal sucrose stimulation with long-lasting excitation. It has its cell body in the suboesophageal ganglion and projects into the mushroom bodies, the antennal lobes and the protocerebral lobes. The VUMmx1 neuron was shown to mediate the reward in classical olfactory conditioning (Hammer 1993). Depolarisation of VUMmx1 substitutes for the sucrose reward during olfactory learning. It is assumed that activation of this neuron following a sucrose reward modulates the response properties of a great number of interneurons in the brain by releasing octopamine. Local injections of transmitters or neurohormones into the close vicinity of the VUMmx1 neuron might change its properties.

Modulation of sucrose responsiveness could also take place at the level of the motor system. Muscle 17 is responsible for proboscis extension. Injection of dopamine into the thorax was observed to change the motor pattern of this muscle (W. Blenau, personal communication). In reserpinised bees, injection of dopamine into the brain rescued the motor pattern of the proboscis extension response (Menzel et al. 1999). But it is also possible that sucrose responsiveness is modulated simultaneously at the three levels of the nervous system discussed above.

## *4.6 Sucrose responsiveness and PKA activity*

PKA activity in the antennal lobes of bees with high sucrose responsiveness was significantly higher than that of bees with low sucrose responsiveness (Figure 54). Thirty minutes after feeding, PKA activity was lower than 90 min after feeding. This implies that the regulation of sucrose responsiveness and satiation is related to PKA activity in the antennal lobes but presumably involves different mechanisms.

There are some implications that PKA activity, sucrose responsiveness and associative learning might correlate and share some of the physiological pathways: sucrose responsiveness is higher in bees which show a good learning performance than in "poor" learners. PKA was also shown to be involved in the formation of memory. Multiple-trial conditioning induces profound prolongation of PKA activity in the antennal lobes (Müller 2000). Bees in which PKA activity was inhibited showed disruption of their long-term memory (Fiala et al. 1999). A *Drosophila* mutant of the catalytic sub-unit of PKA (*DC*0) exhibits poor learning (Skoulakis et al. 1993, Milner et al. 1998). A mutant of the regulatory sub-unit of PKA (*PKA-RI*) shows disrupted classical conditioning (Goodwin et al. 1997).

Octopamine injected into the antennal lobes or the mushroom bodies is able to substitute for the US in associative olfactory learning (Hammer and Menzel 1998). Moreover, octopamine injections into the antennal lobes lead to an increase in PKA activity (Hildebrandt and Müller 1995). Sucrose stimulation of the antennae also increases PKA activity in the antennal lobes (Hildebrandt and Müller 1995). However, the molecular mechanisms downstream from PKA have not yet been identified.

Empty bees have higher sucrose responsiveness than filled bees (Page et al. 1998, Pankiw et al. 2001) and show better associative learning (Menzel et al. 1989). Interestingly, PKA activity in the antennal lobes of bees 90 min after feeding was lower than in bees 30 min after feeding. This implies that the relationship between PKA activity and sucrose responsiveness changes with time after feeding. However, in these experiments, only the antennal lobes were analysed. It is urgently necessary to study PKA activity of bees with different sucrose responsiveness in other brain structures involved in sucrose perception and learning, such as the mushroom bodies, in order to separate different regulatory mechanisms. It might well be that PKA activity in the mushroom bodies works opposite to that measured in the antennal lobes. Taken together, these findings suggest a relationship between PKA activity, sucrose responsiveness and associative learning.

Interestingly, similar results have been found in *Drosophila* larvae (Sokolowski 1998, Sokolowski and Riedl 1999). Some larvae move long distances while feeding on yeast paste and are therefore called "rovers". Others move relatively short distances and are accordingly called "sitters". Being a sitter or rover involves the expression of a cGMP-dependent protein kinase (protein kinase G, PKG). Rovers were shown to have higher PKG enzyme activity levels than sitters. PKG is assumed to play a role in chemoreception and therefore in the "foraging" behaviour of rovers and sitters (Sokolowski 1998), and there is evidence that cGMP signalling is involved in the transduction of different taste qualities (Kinnamon and

Margolskee 1996). Being a sitter or rover also depends on the larval medium. In the absence of food, sitters behave like rovers. Honey bees with high sucrose responsiveness, which have a high PKA activity in the antennal lobes, are more likely to be pollen foragers. Individuals with low sucrose responsiveness, which display a lower PKA activity, are more likely to forage for nectar. In addition, the two groups of bees differ in their learning behaviour. Whether the activity of PKA in the honey bee or of PKG in the flies is causally linked to feeding and foraging behaviour has yet to be determined.

## *4.7 General conclusions*

The experiments described in this work have demonstrated that differences in individual sucrose responsiveness can explain many differences in the non-associative and associative learning behaviour of honey bees. Individuals were shown to differ in their sucrose responsiveness because of their genotype, foraging role or age. But sucrose responsiveness is also affected by other parameters (Figure 56). How sucrose responsiveness is regulated is not clear, but several neuroactive substances were shown to affect sucrose responsiveness, and PKA activity differed in bees with high or low sucrose responsiveness.

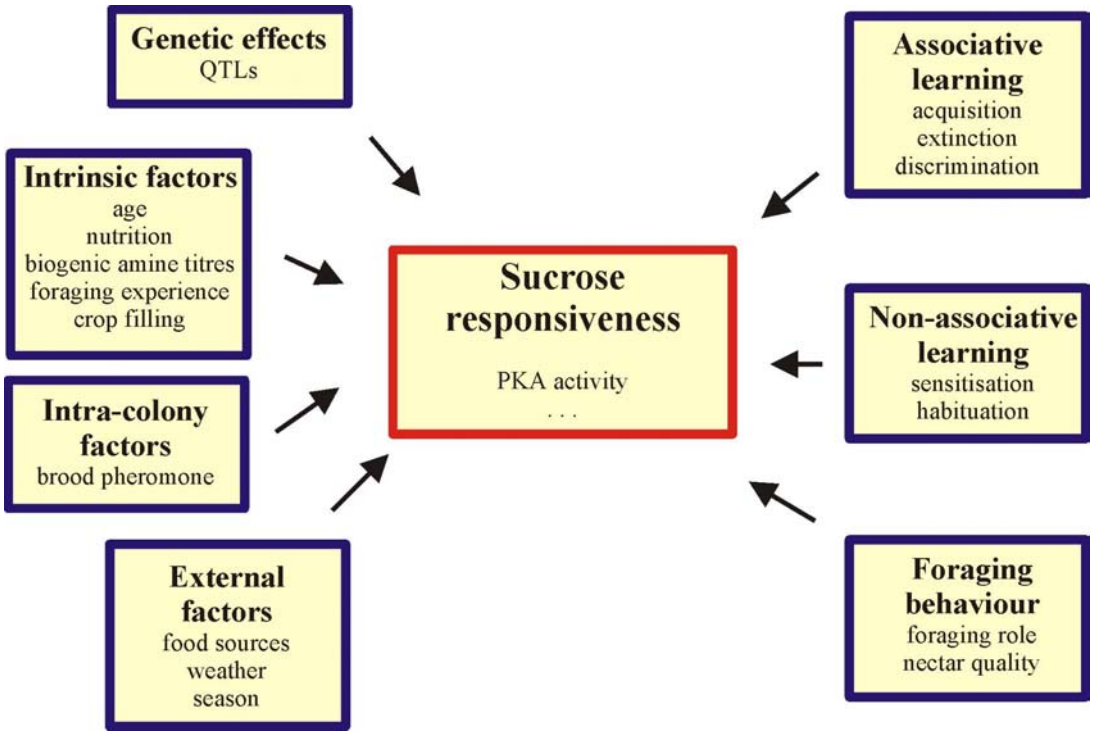


**Figure 56. Survey of the effects of sucrose responsiveness and parameters affecting sucrose responsiveness. Note that the arrows do not imply causal relationships.**

Individual sucrose responsiveness appears to be a very good indicator of the "general physiological state" of a bee. Recent experiments have demonstrated that responsiveness to sucrose correlates positively with responsiveness to pollen and with phototactic responsiveness (personal observation, J. Erber, personal communication). Individuals with high responsiveness to sucrose were more responsive to pollen and had a higher phototactic responsiveness than bees with low sucrose responsiveness. These findings imply that measuring of sucrose responsiveness is a very effective way of determining the "physiological" state of a bee.

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# 6 Appendix

| type of bees | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| high-strain pollen foragers | 75 | 0.82 | **< 0.001** | 0.96 ± 0.010 (**p < 0.001**) | 0.03 ± 0.01 (**p < 0.001**) |
| high-strain non-pollen foragers | 41 | 0.89 | **< 0.001** | 0.54 ± 0.02 (**p < 0.001**) | 0.16 ± 0.02 (**p < 0.001**) |
| all high-strain foragers | 116 | 0.92 | **< 0.001** | 0.75 ± 0.01 (**p < 0.001**) | 0.09 ± 0.01 (**p < 0.001**) |
| low-strain pollen foragers | 63 | 0.96 | **< 0.001** | 0.74 ± 0.01 (**p < 0.001**) | 0.17 ± 0.01 (**p < 0.001**) |
| low-strain non-pollen foragers | 75 | 0.98 | **< 0.001** | 0.42 ± 0.01 (**p < 0.001**) | 0.23 ± 0.01 (**p < 0.001**) |
| all low-strain foragers | 138 | 0.99 | **< 0.001** | 0.58 ± 0.007 (**p < 0.001**) | 0.20 ± 0.01 (**p < 0.001**) |
| high-strain preforagers (6-12 days old) | 259 | 0.97 | **< 0.001** | 0.92 ± 0.01 (**p < 0.001**) | 0.14 ± 0.01 (**p < 0.001**) |
| low-strain preforagers (6-12days old) | 304 | 0.92 | **< 0.001** | 0.85 ± 0.01 (**p < 0.001**) | 0.12 ± 0.01 (**p < 0.001**) |
| wild-type pollen foragers | 342 | 0.91 | **< 0.01** | 1.15 ± 0.02 (**p < 0.001**) | 0.11 ± 0.02 (**p < 0.01**) |
| wild-type non-pollen foragers | 353 | 0.87 | **< 0.01** | 0.83 ± 0.02 (**p < 0.001**) | 0.14 ± 0.03 (**p < 0.01**) |
| wild-type preforagers 1 hr | 49 | 0.94 | **< 0.01** | 0.81 ± 0.03 (**p < 0.001**) | 0.28 ± 0.04 (**p < 0.01**) |
| wild-type preforagers 4 hrs | 54 | 1.00 | **< 0.001** | 0.82 ± 0.01 (**p < 0.001**) | 0.22 ± 0.01 (**p< 0.001**) |
| wild-type preforagers 1 day | 57 | 0.90 | **< 0.01** | 0.66 ± 0.04 (**p < 0.001**) | 0.25 ± 0.04 (**p < 0.01**) |
| wild-type preforagers 5 days | 53 | 0.97 | **< 0.001** | 0.79 ± 0.02 (**p < 0.001**) | 0.26 ± 0.02 (**p < 0.001**) |

**Table 6 Parameters of linear regressions (f(x) = a + b * x) on sucrose concentration and proportion of bees showing proboscis extension in foragers and preforagers of two genetic strains and in wild-type foragers. (n – number of individuals, $r^2$ proportion of observed variation in total sample that can be explained by the regression line, p in column 4 – level of significance for regression, p in columns 5 and 6 - probability that regression parameters a and b are not different from zero.**

| type of bees | n | rho | p |
|---|---|---|---|
| **acquisition scores – gustatory response scores** | | | |
| high-strain pollen foragers | 40 | 0.243 | > 0.05 |
| high-strain non-pollen foragers | 28 | 0.575 | **< 0.01** |
| all high-strain foragers | 68 | 0.495 | **< 0.001** |
| low-strain pollen foragers | 40 | 0.454 | **< 0.01** |
| low-strain non-pollen foragers | 41 | 0.469 | **< 0.01** |
| all low-strain foragers | 81 | 0.419 | **< 0.001** |
| **extinction CS+ scores – gustatory response scores** | | | |
| high-strain pollen foragers | 40 | 0.293 | > 0.05 |
| high-strain non-pollen foragers | 28 | 0.410 | **< 0.05** |
| all high-strain foragers | 68 | 0.391 | **< 0.001** |
| low-strain pollen foragers | 40 | 0.359 | **< 0.05** |
| low-strain non-pollen foragers | 41 | 0.340 | **< 0.05** |
| all low-strain foragers | 81 | 0.300 | **< 0.01** |
| **extinction CS- scores – gustatory response scores** | | | |
| high-strain pollen foragers | 40 | 0.104 | > 0.05 |
| high-strain non-pollen foragers | 28 | 0.466 | **< 0.05** |
| all high-strain foragers | 68 | 0.294 | **< 0.05** |
| low-strain pollen foragers | 40 | 0.399 | **< 0.05** |
| low-strain non-pollen foragers | 41 | 0.287 | > 0.05 |
| all low-strain foragers | 81 | 0.296 | **< 0.01** |
| **extinction CS+ scores – acquisition scores** | | | |
| high-strain pollen foragers | 40 | 0.552 | **< 0.001** |
| high-strain non-pollen foragers | 28 | 0.783 | **< 0.001** |
| all high-strain foragers | 68 | 0.661 | **< 0.001** |
| low-strain pollen foragers | 40 | 0.731 | **< 0.001** |
| low-strain non-pollen foragers | 41 | 0.743 | **< 0.001** |
| all low-strain foragers | 81 | 0.732 | **< 0.001** |
| **extinction CS- scores – acquisition scores** | | | |
| high-strain pollen foragers | 40 | 0.556 | **< 0.001** |
| high-strain non-pollen foragers | 28 | 0.659 | **< 0.001** |
| all high-strain foragers | 68 | 0.592 | **< 0.001** |
| low-strain pollen foragers | 40 | 0.651 | **< 0.001** |
| low-strain non-pollen foragers | 41 | 0.694 | **< 0.001** |
| all low-strain foragers | 81 | 0.657 | **< 0.001** |

**Table 7 Spearman rank correlation coefficients for correlations between gustatory response scores, acquisition scores, extinction CS+ and extinction CS- scores in high- and low-strain foragers. Rho = correlation coefficient. N = number of individuals tested. P = the significance level for correlation.**

| type of bees | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| high-strain pollen foragers | 40 | 0.07 | > 0.05 | 0.31 ± 3.80 (p > 0.05) | 0.65 ± 0.39 (p > 0.05) |
| high-strain non-pollen foragers | 28 | 0.27 | **< 0.01** | 0.41 ± 1.53 (p > 0.05) | 0.58 ± 0.19 (**p < 0.01**) |
| all high-strain foragers | 68 | 0.13 | **< 0.001** | 0.26 ± 1.37 (p > 0.05) | 0.63 ± 0.15 (**p < 0.001**) |
| low-strain pollen foragers | 40 | 0.17 | **< 0.01** | 0.13 ± 2.17 (p > 0.05) | 0.68 ± 0.24 (**p < 0.01**) |
| low-strain non-pollen foragers | 41 | 0.33 | **< 0.01** | 0.51 ± 1.40 (p > 0.05) | 0.78 ± 0.18 (**p < 0.01**) |
| all low-strain foragers | 81 | 0.23 | **< 0.001** | 0.65 ± 1.19 (p > 0.05) | 0.69 ± 0.14 (**p < 0.001**) |

**Table 8 Parameters of linear regressions (f(x) = a + b * x) of acquisition scores on gustatory response scores for tactile learning in high-and low-strain foragers. Abbreviations as in Table 6.**

| type of bees and regression | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| **extinction CS+scores - gustatory response scores** | | | | | |
| high-strain pollen foragers | 40 | 0.14 | **< 0.05** | -2.43 ± 2.34 (p > 0.05) | 0.59 ± 0.24 (**p < 0.05**) |
| high-strain non-pollen foragers | 28 | 0.13 | > 0.05 | 0.90 ± 0.99 (p > 0.05) | 0.24 ± 0.12 (p > 0.05) |
| all high-strain foragers | 68 | 0.13 | **< 0.01** | 0.44 ± 0.87 (p > 0.05) | 0.30 ± 0.10 (**p < 0.01**) |
| low-strain pollen foragers | 40 | 0.15 | **< 0.05** | 0.09 ± 1.23 (p > 0.05) | 0.36 ± 0.14 (**p < 0.05**) |
| low-strain non-pollen foragers | 41 | 0.15 | **< 0.05** | 0.93 ± 0.91 (p > 0.05) | 0.30 ± 0.12 (**p < 0.05**) |
| all low-strain foragers | 81 | 0.14 | **< 0.001** | 0.72 ± 0.71 (p > 0.05) | 0.31 ± 0.09 (**p < 0.001**) |
| **extinction CS- scores – gustatory response scores** | | | | | |
| high-strain pollen foragers | 40 | 0.03 | > 0.05 | -0.76 ± 2.33 ( p > 0.05) | 0.24 ± 0.24 (p > 0.05) |
| high-strain non-pollen foragers | 28 | 0.08 | > 0.05 | 0.17 ± 0.62 ( p > 0.05) | 0.12 ± 0.08 (p > 0.05) |
| all high-strain foragers | 68 | 0.05 | > 0.05 | -0.01 ± 0.73 (p > 0.05) | 0.15 ± 0.08 (p> 0.05) |
| low-strain pollen foragers | 40 | 0.17 | **< 0.01** | -1.01 ± 1.12 (p > 0.05) | 0.34 ± 0.13 (**p < 0.01**) |
| low-strain non-pollen foragers | 41 | 0.09 | > 0.05 | 0.42 ± 0.90 (p > 0.05) | 0.23 ± 0.11 (p > 0.05) |
| all low-strain foragers | 81 | 0.11 | **< 0.01** | 0.08 ± 0.68 (p > 0.05) | 0.25 ± 0.08 (**p < 0.01**) |

**Table 9 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ scores and extinction CS- scores on gustatory response scores for tactile learning in high-and low-strain foragers. Abbreviations as in Table 6.**

| type of bees and regression | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| **extinction CS+ scores – acquisition scores** | | | | | |
| high-strain pollen foragers | 40 | 0.40 | **< 0.001** | 0.63 ± 0.59 (p > 0.05) | 0.40 ± 0.08 (**p < 0.001**) |
| high-strain non-pollen foragers | 28 | 0.63 | **< 0.001** | 0.46 ± 0.43 (p > 0.05) | 0.47 ± 0.71 (**p < 0.001**) |
| all high-strain foragers | 68 | 0.50 | **< 0.001** | 0.54 ± 0.36 (p > 0.05) | 0.43 ± 0.05 (**p < 0.001**) |
| low-strain pollen foragers | 40 | 0.76 | **< 0.001** | 0.29 ± 0.32 (p > 0.05) | 0.49 ± 0.05 (**p < 0.001**) |
| low-strain non-pollen foragers | 41 | 0.64 | **< 0.001** | 0.29 ± 0.41 (p > 0.05) | 0.46 ± 0.06 (**p < 0.001**) |
| all low-strain foragers | 81 | 0.69 | **< 0.001** | 0.30 ± 0.26 (p > 0.05) | 0.47 ± 0.04 (**p < 0.001**) |
| **extinction CS- scores – acquisition scores** | | | | | |
| high-strain pollen foragers | 40 | 0.27 | **< 0.01** | -0.53 ± 0.61 (p > 0.05) | 0.31 ± 0.08 (**p < 0.01**) |
| high-strain non-pollen foragers | 28 | 0.33 | **< 0.01** | 0.06 ± 0.35 (p > 0.05) | 0.21 ± 0.06 (**p < 0.01**) |
| all high-strain foragers | 68 | 0.29 | **< 0.001** | -0.20 ± 0.35 (p > 0.05) | 0.26 ± 0.05 (**p < 0.001**) |
| low-strain pollen foragers | 40 | 0.45 | **< 0.001** | -0.12 ± 0.44 (p > 0.05) | 0.35 ± 0.06 (**p < 0.001**) |
| low-strain non-pollen foragers | 41 | 0.34 | **< 0.001** | 0.17 ± 0.53 (p > 0.05) | 0.32 ± 0.07 (**p < 0.001**) |
| all low-strain foragers | 81 | 0.39 | **< 0.001** | 0.02 ± 0.34 (p > 0.05) | 0.33 ± 0.05 (**p < 0.001**) |

**Table 10 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ scores and extinction CS- on acquisition scores for high- and low-strain foragers. Abbreviations as in Table 6.**

| type of bees | n | rho | p |
|---|---|---|---|
| **acquisition scores – gustatory response scores** | | | |
| high-strain tactile | 50 | 0.443 | **< 0.01** |
| high-strain olfactory | 50 | 0.643 | **< 0.001** |
| low-strain tactile | 50 | 0.762 | **< 0.001** |
| low-strain olfactory | 50 | 0.543 | **< 0.001** |
| **extinction CS+ scores – gustatory response scores** | | | |
| high-strain tactile | 50 | 0.186 | > 0.05 |
| high-strain olfactory | 50 | 0.521 | **< 0.001** |
| low-strain tactile | 50 | 0.660 | **< 0.001** |
| low-strain olfactory | 50 | 0.347 | **< 0.05** |
| **extinction CS- scores – gustatory response scores** | | | |
| high-strain tactile | 50 | 0.099 | > 0.05 |
| high-strain olfactory | 50 | 0.245 | > 0.05 |
| low-strain tactile | 50 | 0.700 | **< 0.001** |
| low-strain olfactory | 50 | 0.300 | **< 0.05** |
| **extinction CS+ scores – acquisition scores** | | | |
| high-strain tactile | 50 | 0.768 | **< 0.001** |
| high-strain olfactory | 50 | 0.828 | **< 0.001** |
| low-strain tactile | 50 | 0.891 | **< 0.001** |
| low-strain olfactory | 50 | 0.837 | **< 0.001** |
| **extinction CS- scores – acquisition scores** | | | |
| high-strain tactile | 50 | 0.707 | **< 0.001** |
| high-strain olfactory | 50 | 0.288 | **< 0.05** |
| low-strain tactile | 50 | 0.866 | **< 0.001** |
| low-strain olfactory | 50 | 0.492 | **< 0.001** |

**Table 11 Spearman rank correlation coefficients for correlations between gustatory response scores, acquisition scores, extinction CS+ and extinction CS- scores in high- and low-strain preforagers. Abbreviations as in Table 7.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| high-strain bees: tactile acquisition | 50 | 0.24 | **< 0.001** | -3.77 ± 2.47 (p > 0.05) | 1.08 ± 0.28 (**p < 0.001**) |
| low-strain bees: tactile acquisition | 50 | 0.54 | **< 0.001** | -3.53 ± 1.20 (**p < 0.01**) | 1.13 ± 0.15 (**p < 0.001**) |
| high-strain bees: olfactory acquisition | 50 | 0.41 | **< 0.001** | -2.10 ± 1.22 (p > 0.05) | 0.93 ± 0.16 (**p < 0.001**) |
| low-strain bees: olfactory acquisition | 50 | 0.28 | **< 0.001** | -0.96 ± 1.71 (p > 0.05) | 0.89 ± 0.21 (**p < 0.001**) |

**Table 12 Parameters of linear regressions (f(x) = a + b * x) of acquisition scores on gustatory response scores for tactile and olfactory learning in high-and low-strain preforagers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| high-strain bees: tactile extinction CS+ | 50 | 0.05 | > 0.05 | -0.10 ± 0.37 (p > 0.05) | 0.06 ± 0.04 ( p > 0.05) |
| low-strain bees: tactile extinction CS+ | 50 | 0.34 | **< 0.001** | -0.34 ± 0.18 (p > 0.05) | 0.11 ± 0.02 (**p < 0.001**) |
| high-strain bees: olfactory extinction CS+ | 50 | 0.23 | **< 0.001** | -0.26 ± 0.19 (p > 0.05) | 0.09 ± 0.03 (**p < 0.001**) |
| low-strain bees: olfactory extinction CS+ | 50 | 0.11 | **< 0.05** | 0.02 ± 032. (p > 0.05) | 0.09 ± 0.04 (**p < 0.05**) |
| high-strain bees: tactile extinction CS- | 50 | 0.03 | >0.05 | -0.17 ± 1.08 (p > 0.05) | 0.15 ± 0.12 (p > 0.05) |
| low-strain bees: tactile extinction CS- | 50 | 0.35 | **< 0.001** | -1.15 ± 0.52 (p < 0.05) | 0.33± 0.07 (**p < 0.001**) |
| high-strain bees: olfactory extinction CS- | 50 | 0.04 | > 0.05 | -0.12 ± 0.16 (p > 0.05) | 0.03 ± 0.02 (p > 0.05) |
| low-strain bees: olfactory extinction CS- | 50 | 0.08 | **= 0.05** | -0.63 ± 0.58 (p > 0.05) | 0.14 ± 0.07 (**p = 0.05**) |

**Table 13 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ and extinction CS-scores on gustatory response scores for tactile and olfactory learning in high-and low-strain preforagers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| high-strain bees: tactile extinction CS+ | 50 | 0.47 | **< 0.001** | -0.23 ± 0.32 (p > 0.05) | 0.30 ± 0.05 (**p < 0.001**) |
| low-strain bees: tactile extinction CS+ | 50 | 0.75 | **< 0.001** | -0.14 ± 0.20 (p > 0.05) | 0.37 ± 0.03 (**p < 0.001**) |
| high-strain bees: olfactory extinction CS+ | 50 | 0.61 | **< 0.001** | -0.22 ± 0.24 (p > 0.05) | 0.34 ± 0.04 (**p < 0.001**) |
| low-strain bees: olfactory extinction CS+ | 50 | 0.63 | **< 0.001** | -0.24 ± 0.35 (p > 0.05) | 0.44 ± 0.05 (**p < 0.001**) |
| high-strain bees: tactile extinction CS- | 50 | 0.35 | **< 0.001** | -0.22 ± 0.32 (p > 0.05) | 0.23 ± 0.05 (**p < 0.001**) |
| low-strain bees: tactile extinction CS- | 50 | 0.68 | **< 0.001** | -0.13 ± 0.19 (p > 0.05) | 0.30 ± 0.03 (**p < 0.001**) |
| high-strain bees: olfactory extinction CS- | 50 | 0.06 | > 0.05 | -0.03 ± 0.09 (p > 0.05) | 0.02 ± 0.01 (p > 0.05) |
| low-strain bees: olfactory extinction CS | 50 | 0.15 | **< 0.01** | -0.21 ± 0.29 (p > 0.05) | 0.11 ± 0.04 (**p < 001**) |

**Table 14 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ and CS- scores on acquisition scores for tactile and olfactory learning in high-and low-strain preforagers. Abbreviations as in Table 6.**

| type of bees | n | rho | p |
|---|---|---|---|
| **acquisition scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.610 | **< 0.001** |
| pollen olfactory | 50 | 0.479 | **< 0.001** |
| non-pollen tactile | 50 | 0.695 | **< 0.001** |
| non-pollen olfactory | 50 | 0.397 | **< 0.01** |
| **extinction CS+ scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.362 | **< 0.01** |
| pollen olfactory | 50 | 0.343 | **< 0.05** |
| non-pollen tactile | 50 | 0.387 | **< 0.01** |
| non-pollen olfactory | 50 | 0.455 | **< 0.001** |
| **extinction CS- scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.429 | **< 0.01** |
| pollen olfactory | 50 | 0.205 | > 0.05 |
| non-pollen tactile | 50 | 0.436 | **< 0.01** |
| non-pollen olfactory | 50 | -0.201 | > 0.05 |
| **extinction CS+ scores – acquisition scores** | | | |
| pollen tactile | 50 | 0.691 | **< 0.001** |
| pollen olfactory | 50 | 0.817 | **< 0.001** |
| non-pollen tactile | 50 | 0.637 | **< 0.001** |
| non-pollen olfactory | 50 | 0.865 | **< 0.001** |
| **extinction CS- scores – acquisition scores** | | | |
| pollen tactile | 50 | 0.623 | **< 0.001** |
| pollen olfactory | 50 | 0.214 | > 0.05 |
| non-pollen tactile | 50 | 0.629 | **< 0.001** |
| non-pollen olfactory | 50 | 0.139 | > 0.05 |

**Table 15 Spearman rank correlation coefficients for correlations between gustatory response scores, acquisition scores, extinction CS+ and extinction CS- scores in wild-type foragers. Abbreviations as in Table 7.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: tactile acquisition | 55 | 0.42 | **< 0.001** | 0.46± 0.66 (p > 0.05) | 0.70 ± 0.11 (**p < 0.001**) |
| non-pollen foragers: tactile acquisition | 55 | 0.53 | **< 0.001** | -0.77 ± 0.57 (p > 0.05) | 0.86 ± 0.11 (**p < 0.001**) |
| all foragers: tactile acquisition | 110 | 0.50 | **< 0.001** | -0.34 ± 0.42 (p > 0.05) | 0.81 ± 0.08 (**p < 0.001**) |
| pollen foragers: olfactory acquisition | 55 | 0.19 | **< 0.01** | -1.1 ± 1.03 (p > 0.05) | 0.59 ± 0.17 (**p < 0.01**) |
| non-pollen foragers: olfactory acquisition | 55 | 0.18 | **< 0.01** | -0.11 ± 0.53 (p > 0.05) | 0.36 ± 0.11 (**p < 0.01**) |
| all foragers: olfactory acquisition | 110 | 0.20 | **< 0.001** | -0.43 ± 0.49 (p > 0.05) | 0.46 ± 0.09 (**p < 0.001**) |

**Table 16 Parameters of linear regressions (f(x) = a + b * x) of acquisition scores on gustatory response scores for tactile and olfactory learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: tactile extinction CS+ | 55 | 0.16 | **<0.01** | 0.45 ± 0.73 (p > 0.05) | 0.39 ± 0.10 (**p < 0.01**) |
| non-pollen foragers: tactile extinction CS+ | 55 | 0.12 | **< 0.05** | 0.34 ± 0.54 (p>0.05) | 0.29 ± 0.11 (**p < 0.05**) |
| pollen foragers: olfactory extinction CS+ | 55 | 0.08 | **< 0.05** | 0.06 ± 0.90 (p > 0.05) | 0.32 ± 0.15 (**p < 0.05**) |
| non-pollen foragers: olfactory extinction CS+ | 55 | 0.17 | **< 0.05** | 0.01 ± 0.48 (p > 0.05) | 0.32 ± 0.09 (**p < 0.05**) |
| pollen foragers: tactile extinction CS- | 55 | 0.11 | **< 0.05** | 0.12 ± 0.70 (p > 0.05) | 0.31 ± 0.12 (**p < 0.05**) |
| non-pollen foragers: tactile extinction CS- | 55 | 0.14 | **< 0.01** | -0.14 ± 0.46 (p > 0.05) | 0.27 ± 0.09 (**p <0.01**) |
| pollen foragers: olfactory extinction CS- | 55 | 0.03 | > 0.05 | -0.07 ± 0.12 (p > 0.05) | 0.03 ± 0.02 (p > 0.05) |
| non-pollen foragers: olfactory extinction CS- | 55 | 0.03 | > 0.05 | 0.07 ± 0.04 (p > 0.05) | -0.01 ± 0.01 (p > 0.05) |

**Table 17 Parameters of linear regressions (f(x) = a+b*x) of extinction CS+ scores and extinction CS- scores on gustatory response scores for tactile and olfactory learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: tactile Ext CS+ | 55 | 0.57 | **< 0.001** | -0.37 ± 0.40 (p > 0.05) | 0.69 ± 0.08 (**p < 0.001**) |
| non-pollen foragers: tactile Ext CS+ | 55 | 0.38 | **< 0.001** | 0.30 ± 0.32 (p > 0.05) | 0.43 ± 0.08 (**p < 0.001**) |
| pollen foragers: olfactory Ext CS+ | 55 | 0.70 | **< 0.001** | 0.30 ± 0.21 (p > 0.05) | 0.69 ± 0.06 (**p < 0.001**) |
| non-pollen foragers: olfactory Ext CS+ | 55 | 0.81 | **< 0.001** | 0.22 ± 0.14 (p > 0.05) | 0.81 ± 0.05 (**p < 0.001**) |
| pollen foragers: tactile Ext CS- | 55 | 0.33 | **< 0.001** | -0.30 ± 0.47 (p > 0.05) | 0.49 ± 0.10 (**p < 0.001**) |
| non-pollen foragers: tactile Ext CS- | 55 | 0.31 | **< 0.001** | 0.02 ± 0.29 (p>0.05) | 0.33 ± 0.07 (**p < 0.001**) |
| pollen foragers: olfactory Ext CS- | 55 | 0.05 | > 0.05 | 0.02 ± 0.05 (p > 0.05) | 0.02 ± 0.01 (p > 0.05) |
| non-pollen foragers: olfactory Ext CS- | 55 | 0.01 | > 0.05 | 0.01 ± 0.02 (p > 0.05) | 0.01 ± 0.01 (p > 0.05) |

**Table 18 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ scores and extinction CS- scores on acquisition scores for tactile and olfactory learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees | n | rho | p |
|---|---|---|---|
| **reversal acquisition scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.391 | **< 0.01** |
| pollen olfactory | 50 | 0.325 | **< 0.05** |
| non-pollen tactile | 50 | 0.635 | **< 0.001** |
| non-pollen olfactory | 50 | 0.268 | **< 0.05** |
| **reversal extinction CS+ scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.277 | **< 0.05** |
| pollen olfactory | 50 | 0.218 | > 0.05 |
| non-pollen tactile | 50 | 0.498 | **< 0.001** |
| non-pollen olfactory | 50 | 0.420 | **< 0.01** |
| **reversal extinction CS- scores – gustatory response scores** | | | |
| pollen tactile | 50 | 0.244 | > 0.05 |
| pollen olfactory | 50 | 0.389 | < 0.01 |
| non-pollen tactile | 50 | 0.456 | **< 0.001** |
| non-pollen olfactory | 50 | 0.290 | **< 0.05** |
| **reversal extinction CS+ scores – reversal acquisition scores** | | | |
| pollen tactile | 50 | 0.779 | **< 0.001** |
| pollen olfactory | 50 | 0.792 | **< 0.001** |
| non-pollen tactile | 50 | 0.811 | **< 0.001** |
| non-pollen olfactory | 50 | 0.857 | **< 0.001** |
| **reversal extinction CS- scores – reversal acquisition scores** | | | |
| pollen tactile | 50 | 0.737 | **< 0.001** |
| pollen olfactory | 50 | 0.627 | **< 0.001** |
| non-pollen tactile | 50 | 0.551 | **< 0.001** |
| non-pollen olfactory | 50 | 0.598 | **< 0.001** |

**Table 19 Spearman rank correlation coefficients for correlations between gustatory response scores, acquisition scores, extinction CS+ and extinction CS- scores in wild-type foragers during reversal learning. Abbreviations as in Table 7.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: rev. tactile acquisition | 55 | 0.27 | **< 0.001** | 1.37 ± 0.80 (p > 0.05) | 0.60 ± 0.14 (**p < 0.001**) |
| non-pollen foragers: rev. tactile acquisition | 55 | 0.39 | **< 0.001** | -0.45 ± 0.67 (p > 0.05) | 0.77 ± 0.13 (**p < 0.001**) |
| pollen foragers: rev.olfactory acquisition | 55 | 0.08 | **< 0.05** | -0.21 ± 1.07 (p > 0.05) | 0.37 ± 0.18 (**p < 0.05**) |
| non-pollen foragers: rev.olfactory acquisition | 55 | 0.11 | **< 0.05** | 0.17 ± 0.57 (p > 0.05) | 0.28 ± .11 (**p < 0.05**) |

**Table 20 Parameters of linear regressions (f(x) = a + b * x) of acquisition scores on gustatory response scores for tactile and olfactory reversal learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: tactile ext CS+ (2) | 55 | 0.07 | >0.05 | 1.32 ± 0.75 (p > 0.05) | 0.25 ± 0.13 (p > 0.05) |
| non-pollen foragers: tactile ext CS+ (2) | 55 | 0.21 | **< 0.001** | -0.25 ± 0.48 (p> 0.05) | 0.36 ± 0.10 (p > 0.05) |
| pollen foragers: olfactory ext CS+ (2) | 55 | 0.01 | > 0.05 | 1.07 ± 0.96 (p > 0.05) | 0.10 ± 0.16 (p > 0.05) |
| non-pollen foragers: olfactory ext CS+ (2) | 55 | 0.16 | **< 0.01** | -0.08 ± 0.48 (p > 0.05) | 0.30 ± 0.10 (**p < 0.01**) |
| pollen foragers: tactile ext CS- (2) | 55 | 0.05 | > 0.05 | 1.09 ± 0.78 (p > 0.05) | 0.22 ± 0.13 (p > 0.05) |
| non-pollen foragers: tactile ext CS- (2) | 55 | 0.18 | **< 0.01** | -0.52 ± 0.49 (p > 0.05) | 0.27 ± 0.13 (**p < 0.05**) |
| pollen foragers: olfactory ext CS- (2) | 55 | 0.08 | **< 0.05** | -0.19 ± 0.77 (p < 0.05) | 0.27 ± 0.13 (**p < 0.05**) |
| non-pollen foragers: olfactory ext CS- (2) | 55 | 0.03 | > 0.05 | 0.16 ± 0.32 (p > 0.05) | 0.08 ± 0.07 (p > 0.05) |

**Table 21 Parameters of linear regressions (f(x) = a+b*x) of extinction CS+ scores and extinction CS- scores on gustatory response scores for tactile and olfactory reversal learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees and experiment | n | $r^2$ | p | a = $\bar{x}$ ± SEM | b = $\bar{x}$ ± SEM |
|---|---|---|---|---|---|
| pollen foragers: tactile ext CS+ (2) | 55 | 0.57 | **< 0.001** | -0.21 ± 0.40 (p > 0.05) | 0.62 ± 0.08 (**p < 0.001**) |
| non-pollen foragers: tactile ext CS+ (2) | 55 | 0.55 | **< 0.001** | -0.05 ± 0.25 (p> 0.05) | 0.47 ± 0.06 (**p < 0.001**) |
| pollen foragers: olfactory ext CS+ (2) | 55 | 0.66 | **< 0.001** | 0.31 ± 0.22 (p > 0.05) | 0.67 ± 0.07 (**p < 0.001**) |
| non-pollen foragers: olfactory ext CS+ (2) | 55 | 0.83 | **< 0.001** | 0.10 ± 0.13 (p > 0.05) | 0.79 ± 0.05 (**p < 0.001**) |
| pollen foragers: tactile ext CS- (2) | 55 | 0.49 | **< 0.001** | -0.50 ± 0.44 (p > 0.05) | 0.60 ± 0.08 (**p < 0.001**) |
| non-pollen foragers: tactile ext CS- (2) | 55 | 0.27 | **< 0.001** | 0.05 ± 0.31 (p > 0.05) | 0.33 ± 0.08 (**p <0.001**) |
| pollen foragers: olfactory ext CS- (2) | 55 | 0.39 | **< 0.001** | 0.52 ± 0.24 (**p < 0.05**) | 0.45 ± 0.08 (**p < 0.001**) |
| non-pollen foragers: olfactory ext CS- (2) | 55 | 0.33 | **< 0.001** | 0.08 ± 0.16 (p > 0.05) | 0.32 ± 0.06 (**p < 0.001**) |

**Table 22 Parameters of linear regressions (f(x) = a + b * x) of extinction CS+ scores and extinction CS- scores on acquisition scores for tactile and olfactory reversal learning in wild-type foragers. Abbreviations as in Table 6.**

| type of bees and experiment | n | DI ± SEM |
|---|---|---|
| high-strain pollen foragers: tactile learning | 31 | 0.52 ± 0.08 |
| high-strain non-pollen foragers: tactile learning | 18 | 0.51 ± 0.09 |
| all high-strain foragers: tactile learning | 49 | 0.52 ± 0.06 |
| low-strain pollen foragers: tactile learning | 29 | 0.35 ± 0.06 |
| low-strain non-pollen foragers: tactile learning | 30 | 0.28 ± 0.07 |
| all low-strain foragers: tactile learning | 59 | 0.31 ± 0.05 |
| high-strain preforagers: tactile learning | 29 | 0.23 ± 0.08 |
| low-strain preforagers: tactile learning | 27 | 0.12 ± 0.08 |
| high-strain preforagers: olfactory learning | 18 | 0.94 ± 0.04 |
| low-strain preforagers: olfactory learning | 31 | 0.81 ± 0.06 |
| wild-type pollen foragers: tactile learning | 37 | 0.27 ± 0.06 |
| wild-type pollen foragers: reversal tactile learning | 42 | 0.19 ± 0.07 |
| wild-type non-pollen foragers: tactile learning | 32 | 0.28 ± 0.12 |
| wild-type non-pollen foragers: reversal tactile learning | 29 | 0.32 ± 0.14 |
| wild-type pollen foragers: olfactory learning | 29 | 0.95 ± 0.02 |
| wild-type pollen foragers: reversal olfactory learning | 33 | -0.03 ± 0.12 |
| wild-type non-pollen foragers: olfactory learning | 24 | 0.99 ± 0.01 |
| wild-type non-pollen foragers: reversal olfactory learning | 21 | 0.50 ± 0.14 |
| wild-type bees: A: 1.6% P: 1.6% | 13 | 0.94 ± 0.06 |
| wild-type bees: A: 30% P: 1.6% | 13 | 0.64 ± 0.14 |
| wild-type bees: A: 1.6% P: 30% | 22 | 0.41 ± 0.10 |
| wild-type bees: A: 30% P: 30% | 25 | 0.37 ± 0.10 |

**Table 23 Discrimination indices (DI) of foragers and preforagers of the high- and low strains and of wild-type foragers.**

| comparison of groups | t-value | df | p | test |
|---|---|---|---|---|
| 1h vs. 4h | 1.51 | 50 | > 0.05 | Welch's t-test |
| 1h vs. 1d | 0.52 | 104 | > 0.05 | t-test |
| 1h vs 5d | 0.38 | 82 | > 0.05 | Welch's t-test |
| 4h vs 1d | 0.61 | 58 | > 0.05 | Welch's t-test |
| 4h vs 5d | 1.64 | 59 | > 0.05 | Welch's t-test |
| 1d vs 5d | 0.27 | 86 | > 0.05 | Welch's t-test |

**Table 24 Comparison of slopes of linear regressions (f(x) = a + b * x) for arcsine square-root transformed proportion of bees showing PER at increasing sucrose concentrations in young bees of different ages. P – significane level for the test.**

| substance | df 30’ | t-value 30’ | p-value 30’ | df 90’ | t-value 90’ | p-value 90’ |
|---|---|---|---|---|---|---|
| OA $10^{-2}$M | 76 | 2.25 | **< 0.05** | 80 | 0.00 | > 0.05 |
| OA $10^{-3}$M | 77 | 3.73 | **< 0.001** | 80 | 2.04 | **< 0.05** |
| Tyr $10^{-2}$M | 78 | 3.12 | **< 0.01** | 80 | 0.82 | > 0.05 |
| Tyr $10^{-3}$M | 77 | 2.35 | **< 0.05** | 79 | 0.30 | > 0.05 |
| DA $10^{-1}$M | 78 | 2.29 | **< 0.05** | 79 | 2.18 | **< 0.05** |
| DA $10^{-2}$M | 79 | 1.95 | > 0.05 | 79 | 2.26 | **< 0.05** |
| DA $10^{-3}$M | 78 | 0.44 | > 0.05 | 78 | 1.31 | > 0.05 |
| ADTN $10^{-2}$M | 78 | 0.70 | > 0.05 | 79 | 0.09 | > 0.05 |
| ADTN $10^{-3}$M | 77 | 2.02 | **< 0.05** | 78 | 1.85 | > 0.05 |
| ADTN $10^{-4}$M | 79 | 2.53 | **< 0.05** | 79 | 1.75 | > 0.05 |

**Table 25 Comparison of modulation indices between control bees and bees which were injected with different neuroactive substances. OA – octopamine, Tyr – tyramine, DA – dopamine, p - significane level for the test.**

| comparison of groups | index | t-value | df | p |
|---|---|---|---|---|
| OA $10^{-2}$M vs $10^{-3}$M | DI 30’ | 1.59 | 79 | > 0.05 |
| Tyr $10^{-2}$M vs $10^{-3}$M | DI 30’ | 0.90 | 81 | > 0.05 |
| OA $10^{-2}$M vs $10^{-3}$M | DI 90’ | 1.83 | 82 | > 0.05 |
| Tyr $10^{-2}$M vs $10^{-3}$M | DI 90’ | 1.06 | 81 | > 0.05 |
| DA $10^{-1}$M vs $10^{-2}$M | DI 30’ | 0.20 | 79 | > 0.05 |
| DA $10^{-1}$M vs $10^{-3}$M | DI 30’ | 2.91 | 78 | **< 0.01** |
| DA $10^{-2}$M vs $10^{-3}$M | DI 30’ | 2.49 | 74 | **< 0.05** |
| ADTN $10^{-2}$M vs $10^{-3}$M | DI 30’ | 1.37 | 77 | > 0.05 |
| ADTN $10^{-2}$M vs $10^{-4}$M | DI 30’ | 1.96 | 79 | > 0.05 |
| ADTN $10^{-3}$M vs $10^{-4}$M | DI 30’ | 0.76 | 78 | > 0.05 |
| DA $10^{-1}$M vs $10^{-2}$M | DI 90’ | 0.02 | 79 | > 0.05 |
| DA $10^{-1}$M vs $10^{-3}$M | DI 90’ | 1.00 | 78 | > 0.05 |
| DA $10^{-2}$M vs $10^{-3}$M | DI 90’ | 0.94 | 79 | > 0.05 |
| ADTN $10^{-2}$M vs $10^{-3}$M | DI 90’ | 1.95 | 70 | > 0.05 |
| ADTN $10^{-2}$M vs $10^{-4}$M | DI 90’ | 1.85 | 80 | > 0.05 |
| ADTN $10^{-3}$M vs $10^{-4}$M | DI 90’ | 0.19 | 79 | > 0.05 |

**Table 26 Comparison of modulation indices between different concentrations of neuroactive substances. Df – degrees of freedom, p – significane level for the test.**

# Publications

Parts of this work have been published in:

**Journals**

Scheiner R, Page RE, Erber J. 2001. Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains. Behav Brain Res 120:67-73.

Scheiner R, Page RE, Erber J. In press. The effects of genotype, foraging role and sucrose perception on the tactile learning performance of honey bees (*Apis mellifera* L.). Neurobiol Learn Mem.

**Abstracts**

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# Curriculum Vitae

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|---|---|
| **Name**: | Ricarda Scheiner |
| **Address**: | Prieborner Str. 04, 12526 Berlin, Germany |
| **Date of birth**: | 21.07.1972 |
| **Place of birth**: | Berlin |

**Education**

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|---|---|
| 1979 - 1987 | Oberschule Berlin-Treptow |
| 1987 - 1991 | Gymnasium Theresienschule Berlin |
| | Degree: "Abitur" |
| 10/1991 - 08/1998 | Technical University Berlin |
| | Studies in Biology and English (teacher training) |
| 26.05.1998 | University degree "1st Staatsexamen" |

**Research Experience**

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|---|---|
| 10/1997 – 01/1998 | Diploma thesis ("Staatsexamensarbeit") with the title "Comparative studies on the tactile learning behaviour of pollen and nectar foragers of the honey bee (*Apis mellifera*)" |
| 06/1998 until present | PhD student in the laboratory of Prof. Dr. J. Erber, TU Berlin, Institute of Ecology |

**Awards**

Katharina Heinroth Award 1999 of the Society of Naturalists, Berlin (Gesellschaft Naturforschender Freunde zu Berlin)

**Other**

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|---|---|
| 09/1993 - 05/1994 | Foreign language assistant at Greenock Academy, Greenock, Schottland |