ORIGINAL PAPER

Evidence for associative learning in newly emerged honey bees (*Apis mellifera*)

Andreas Behrends · Ricarda Scheiner

Abstract The honey bee is a model organism for studies on the neural substrates of learning and memory. Associative olfactory learning using sucrose rewards is fast and reliable in foragers and older hive bees. However, researchers have so far failed to show any significant learning in newly emerged bees. It is generally argued that in these bees only part of the brain structures important for learning are fully developed. Here we show for the first time that newly emerged honey bees are capable of associative learning, if they are sufficiently responsive to sucrose. Responsiveness to sucrose, which can be measured using the proboscis extension response (PER), increases with age. Newly emerged bees are on average very unresponsive to sucrose. We show that if newly emerged bees displaying a PER to 10% sucrose or lower sucrose concentrations are conditioned to an odour, they show significant associative learning and early long-term memory. Nevertheless, the level of acquisition is still lower than in foragers. The general assumption that newly emerged honey bees are incapable of associative learning must therefore be reconsidered. Further, our study suggests that an age-dependent increase in responsiveness to rewarding stimuli is directly related to the development of early learning abilities. The decisive influence of responsiveness to rewarding stimuli in associative learning of newly emerged bees has far reaching consequences for studies on the development of associative learning capabilities in insects and vertebrates.

Introduction

The honey bee has a high capacity for learning and memory and can associate environmental stimuli like colours and odours with a nectar source fast and reliably (von Frisch 1967; Menzel and Müller 1996; Giurfa 2004). A well-established method to measure associative learning in bees under laboratory conditions is the conditioning of the proboscis extension response (PER) (Kuwabara 1957). Bees can be trained effectively to different stimuli (Bitterman et al. 1983; Menzel and Müller 1996; Erber et al. 1998; Giurfa 2004). Most of these experiments involved experienced worker bees. Only few experiments were conducted with younger hive bees or even with newly emerged bees. Studies by Ray and Ferneyhough (1997) and by Morgan et al. (1998) suggest that newly emerged bees have no significant associative learning capacity. This is not trivial, since metamorphosis to the adult is already completed in the capped brood before the bees emerge. Nevertheless, newly emerged bees strongly differ in their gene expression profiles from older bees (Whitfield et al. 2006), which might be related to their learning capacities. After 1–2 days of life the bees already start to conduct social tasks in the colony. More complex brains like those of young human infants, in contrast, display evidence for early learning (Bower 1974; Kirkham et al. 2002). In *Drosophila melanogaster*, behavioural adaptations to sensory inputs (e.g. avoidance response to benzaldehyde) were shown to develop in early adulthood (Devaud et al. 2003). In *Aplysia*, habituation and dishabituation occur in early juvenile stages (Rankin and Carew 1987). Shock-avoidance

A. Behrends · R. Scheiner (✉)
Institut für Ökologie, Technische Universität Berlin,
FR 1-1, Franklinstr. 28/29, 10587 Berlin, Germany
e-mail: Ricarda.Scheiner-Pietsch@TU-Berlin.de

behaviour was also shown to develop with age in young mice (Nagy et al. 1977). It is generally assumed that the poor learning performance in newly emerged bees is related to the ongoing development of the antennal lobe, which is an important neuropile for olfactory learning (Masson and Arnold 1984; Morgan et al. 1998). Sigg et al. (1997) showed that volumetric increases of the antennal lobe glomeruli temporally correlate with activity-dependent improvement in learning performance. However, the onset of significant learning performance in other studies is not detected before 5 days of adult life (Maleszka and Helliwell 2001) and seems to be delayed compared to the rapid growth of the antennal lobes (Masson and Arnold 1984). This suggests that additional factors contribute to the poor learning and memory performance of newly emerged bees. A number of studies on young worker bees (Ray and Ferneyhough 1997; Morgan et al. 1998; Maleszka and Helliwell 2001) investigated external factors that have an effect on the development of associative learning capacity (e.g. juvenile hormone titres, social structure of the colony, queen pheromone). In contrast to our study, those experiments did not control for individual responsiveness to sucrose. The sucrose responsiveness of honey bee workers, however, increases with age (Pankiw and Page Jr 1999), and associative learning performance strongly depends on responsiveness to sucrose (Scheiner et al. 1999, 2001a, 2001b, 2001c, 2003, 2005). Bees that are highly responsive to sucrose show a better learning performance than bees that are less responsive to sucrose. Hence, low sucrose responsiveness could be an important factor limiting the associative learning performance of young bees when they are conditioned with sucrose. Habituation of the PER, a form of non-associative learning, also correlates with sucrose responsiveness in newly emerged bees and foragers (Scheiner 2004).

In the present study we investigated for the first time the olfactory acquisition performance of newly emerged bees with known sucrose responsiveness.

Methods

Bees

During July and August 2007 combs with capped brood were incubated at 80% humidity and 34°C. Newly emerged bees were brushed off the comb every 24 h. Randomly selected bees were collected in glass vials after they had rested for 15 min.

After collection in glass vials bees were cooled at 4°C in the refrigerator until they showed first signs of immobility. Subsequently, they were mounted in metal tubes and restrained with one tape between head and thorax and one over the abdomen. Afterwards, bees rested for 1 h in a humidified chamber before behavioural experiments were conducted. Data for sucrose responsiveness and acquisition performance of experienced forager bees were taken from Behrends et al. (2007). These bees were handled according to the same learning protocol and had been collected directly from brood combs out of the hive.

Sucrose responsiveness

Sucrose responsiveness was measured using the PER. Each bee was stimulated at the tip of her antennae with water and the following sucrose concentrations 0.1, 0.3, 1, 3, 10, 30% (weight/volume; molarity of a 30% sucrose solution = 0.88). The inter-test interval was 2 min at minimum to prevent sensitisation effects. At each stimulation, it was recorded if the bee showed the PER. The gustatory response score (GRS) was used as a measure of sucrose responsiveness and comprises the total number of PERs over the stimulation series. The GRS ranges between 0 and 7, where 7 indicates the highest level of responsiveness (bee responded to all stimulations) (Scheiner 2004).

Olfactory conditioning

Before conditioning, bees were tested for a spontaneous response to the conditioned stimulus carnation. Two microlitres of the odour were applied to a piece of filter paper that was placed in a 20-ml syringe. The syringe was placed in front of the bee that was moved into a constant neutral air stream approximately 8 s before odour stimulation. The bee remained in the air stream approximately 8 s after stimulation with the odour. The inter-trial interval was 5 min. Only bees that did not respond spontaneously to the odour were conditioned to carnation. During each conditioning trial, approximately 5 ml of air filled with odour were applied to the bee's antennae. While the bee experienced the odour, the PER was elicited by applying a droplet of 30% sucrose solution to her antennae. When the bee showed proboscis extension, she was allowed to drink approximately 1 µl of sucrose solution. For each conditioning trial, it was recorded whether the bee showed a conditioned PER. Bees were conditioned six times with carnation. The acquisition score was calculated as an overall measure of learning performance. It comprises the total number of conditioning trials that resulted in a conditioned PER. The scale was 0–5, because bees that responded in the first conditioning trial were discarded from the further experiment. At 24 h after conditioning, it was tested whether the conditioned newly emerged bees still showed the conditioned PER in a single test using the CS odour.

Unpaired controls

To exclude effects of pseudo-conditioning, unpaired controls were used. Bees in this group were either stimulated with the odour or with sucrose, but the two stimuli were never applied together or close to each other. However, each bee in this group received the same number of odour and sucrose stimulations and feeding stimulations as bees in the conditioning group.

Statistical analyses

For graphic display of GRS and acquisition scores means and standard errors of the means (SEM) were calculated (SPSS 15.0). The learning curves show the percentage of bees displaying PER during conditioning. Comparisons of GRS and acquisition scores were performed using two-tailed Mann–Whitney U-tests. Spearman rank correlations were calculated to analyse correlations between GRS and acquisition scores. T-Tests were used for one sample comparisons (SPSS 15.0). Comparisons between conditioned bees and unpaired controls for their responses to the conditioned stimulus in each conditioning trial were analysed by two-tailed Fisher Exact Tests (GraphPad InStat 3.06). All tests were two-tailed. When we compared the GRS, acquisition scores or the number of conditioned responses between conditioned newly emerged bees, unpaired newly emerged controls and foragers, we applied the corresponding Bonferroni correction (Miller 1981) to the level of significance: the new alpha level to which the probabilities extracted from the respective tests were compared was 0.017 in order to detect a significant difference with a confidence level of 5%.

Results

Sucrose responsiveness

Pilot experiments showed that newly emerged bees with a GRS <2 reliably fail to show the PER to the unconditioned sucrose stimulus. Therefore, only bees with a GRS ≥2 were used for the learning assays. These bees only constituted a subset of 22% of the total number of newly emerged bees tested for PER (Fig. 1), because sucrose responsiveness was generally very low (mean GRS of all newly emerged bees = 1.68 ± 0.093 SEM). In the group of foragers, in contrast, 74% of the bees tested for PER could be examined for olfactory conditioning (Fig. 1; mean GRS of all foragers: 3.65 ± 0.226 SEM). Half of the newly emerged bees (GRS ≥2) were subsequently used for olfactory conditioning and the other half served as unpaired controls. GRSs did not
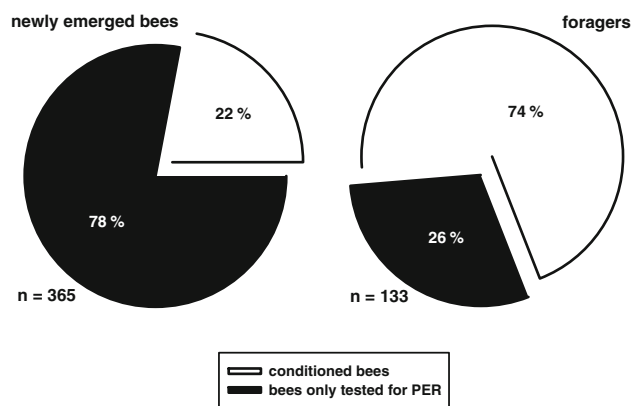
Fig. 1 Percentage of newly emerged bees and foragers that were used in olfactory conditioning. Only 22% of newly emerged bees could be used for the conditioning assay and as unpaired controls. The criterion for the learning assay was a GRS of ≥2, indicating sufficient responsiveness to sucrose. In contrast to the newly emerged bees, 74% of the foragers could be used for olfactory conditioning. For details on statistics see text. The number of bees tested is shown
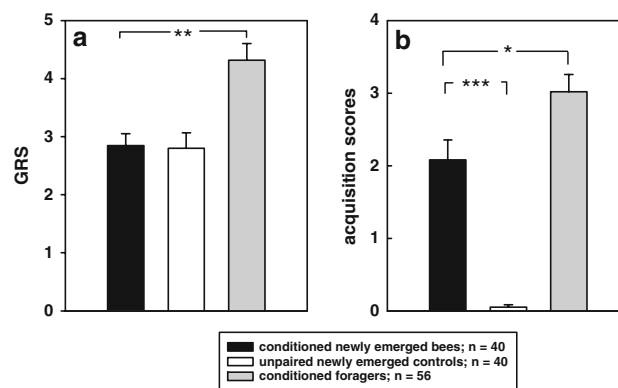

Fig. 2 Sucrose responsiveness and acquisition scores of newly emerged bees in olfactory conditioning, unpaired controls, and foragers. **a** Mean gustatory response scores (GRS) and standard errors of the means. **b** Mean acquisition scores and standard errors of the means. GRS of newly emerged bees tested for olfactory learning and those serving as unpaired controls did not differ. But conditioned newly emerged bees displayed significantly higher acquisition scores than unpaired controls. Foragers displayed significantly higher GRS and significantly higher acquisition scores than conditioned newly emerged bees. For statistics see text. *Asterisks* indicate significant differences between groups (*$P ≤ 0.017$; **$P ≤ 0.01$; ***$P ≤ 0.001$; two-tailed Mann–Whitney U-test). The number of bees tested is shown

differ between the two groups (Fig. 2a; two-tailed Mann–Whitney U-test; $z = 0.411$, $n_{\text{conditioned newly emerged}} = 40$, $n_{\text{unpaired controls}} = 40$, $P = 0.681$). This is important, because GRS are a decisive factor for associative learning in honey bees (Scheiner et al. 2004). Foragers tested for acquisition performance were significantly more responsive to sucrose than newly emerged bees (Fig. 2a; two-tailed Mann–Whitney U-test; $z = 2.772$; $n_{\text{foragers}} = 56$, $n_{\text{newly emerged}} = 40$, $P = 0.006$).

Acquisition and memory

Interestingly, newly emerged bees selected for the learning assay displayed significant olfactory learning (Fig. 2b; two-tailed one-sample T-test; $df = 39$, $P \leq 0.001$). Unpaired controls, in contrast, did not show any olfactory PER learning (Fig. 2b; two-tailed one sample T-test; $df = 39$, $P = 0.160$). The acquisition scores of conditioned bees were significantly higher than those of the unpaired control group (Fig. 2b; two-tailed Mann–Whitney U-test; $z = 6.813$; $n_{\text{conditioned newly emerged}} = 40$; $n_{\text{unpaired controls}} = 40$; $P \leq 0.001$). Foragers displayed significantly higher acquisition scores in the conditioning assay than newly emerged bees (Fig. 2b; two-tailed Mann–Whitney U-test; $z = 2.511$; $n_{\text{foragers}} = 56$; $n_{\text{newly emerged}} = 40$; $P = 0.012$). The learning curve of newly emerged bees is displayed in Fig. 3. In each trial, conditioned newly emerged bees responded significantly more often to the conditioned odour than bees of the unpaired control (two-tailed Fisher Exact Test; $n_{\text{unpaired controls}} = 40$; $n_{\text{conditioned newly emerged}} = 40$; P 1st trial = 1.0; P 2nd trial—6th trial ≤ 0.001), which demonstrates associative learning. Unlike in foragers, conditioned responses declined after the third trial in newly emerged bees (Fig. 3; two-tailed Fisher Exact Test; $n_{\text{foragers}} = 56$; $n_{\text{newly-emerged}} = 40$; P-values: 1st trial = 1.0, 2nd trial = 0.049, 3rd trial = 0.835, 4th trial = 0.093, 5th–6th trial ≤ 0.001). Only one-quarter of the bees showed conditioned PER till the last trial (Fig. 4, group "cond. PER in last trial"), displaying a learning curve resembling that of foragers (compare with Fig. 3). But three quarters of the bees sharply decreased in
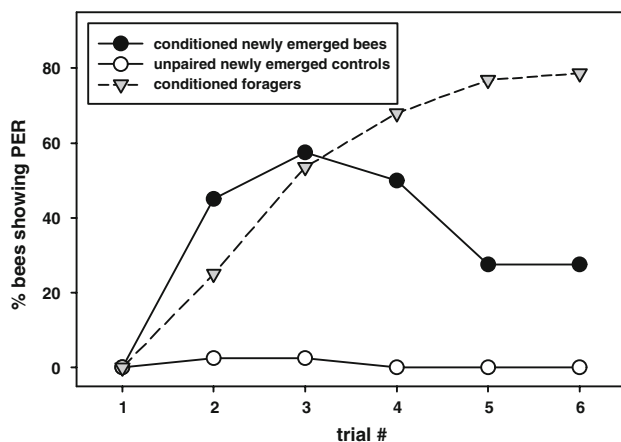
Fig. 3 Learning curves of newly emerged bees in olfactory conditioning, of unpaired controls and of foragers. The percentages of bees responding with PER to the conditioned odour carnation are displayed for each acquisition trial. In each trial, conditioned newly emerged bees displayed significantly more often the PER than unpaired controls (for statistics see Table 1). Compared to conditioned newly emerged bees, foragers displayed significantly more conditioned responses in the 5th and 6th trials (for statistics see Table 1). Number of bees tested in each group is the same as in Fig. 2
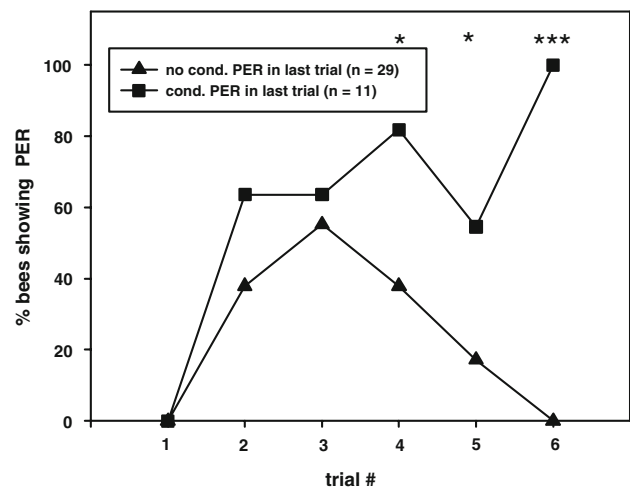
Fig. 4 Learning curves differ between bees responding in the last conditioning trial with PER and those that did not. Conditioned bees were divided according to their response in the last conditioning trial. Bees that responded in the last conditioning trial ("cond. PER in last trial") displayed a learning curve with mostly increasing conditioned responses with increasing trials. Conditioned bees which did not respond with conditioned proboscis extension in the last conditioning trial ("no cond. PER in last trial") displayed a sharp decrease in conditioned PER after the third trial. *Asterisks* indicate significant differences (*$P \leq 0.05$; ***$P \leq 0.001$; two-tailed Fisher Exact Test)

their conditioned PER after the third trial (Fig. 4, group "no cond. PER in last trial"). The two subgroups of conditioned newly emerged bees differed significantly from each other in the last three trials (two-tailed Fisher Exact Test; $n_{\text{cond PER in last trial}} = 11$; $n_{\text{no cond. PER in last trial}} = 29$, 4th trial: $P = 0.031$, 5th trial: $P = 0.042$, 6th trial: $P \leq 0.001$). Nevertheless, both subgroups displayed the PER to the unconditioned stimulus sucrose throughout the whole conditioning procedure. Sucrose responsiveness did not correlate with acquisition scores in newly emerged bees (Fig. 5; Spearman rank correlation; $\rho = 0.168$; $n = 40$, $P = 0.299$), but in the group of foragers (Spearman rank correlation; $\rho = 0.667$, $n = 56$, $P \leq 0.001$). This implies different relationships between sucrose responsiveness and acquisition in newly emerged bees and foragers.

At 24 h after conditioning, 50% of all newly emerged bees trained in olfactory conditioning still displayed the conditioned PER and thus demonstrated early long-term memory.

Discussion

Our results show for the first time that newly emerged bees can build a stable association of a sucrose reward with an odour. But a certain level of sucrose responsiveness is required for forming this association. If newly emerged bees are not pre-selected based on their sucrose responsive-
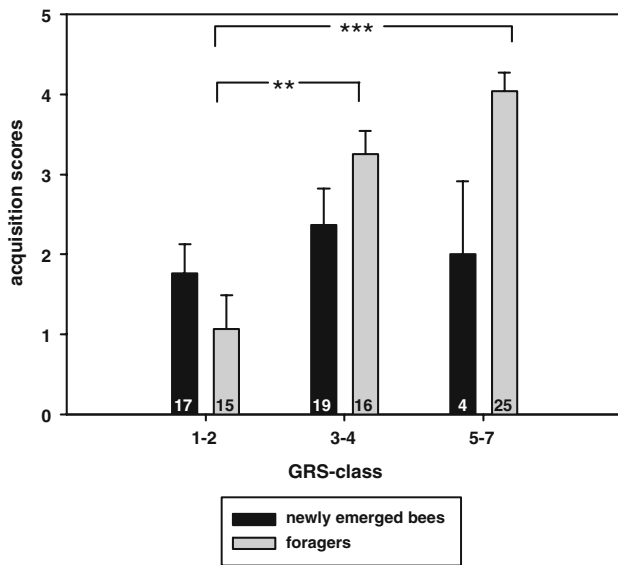
Fig. 5 Relationship between sucrose responsiveness and acquisition performance of newly emerged bees and foragers. The *x*-axis displays classes of gustatory response scores (GRS classes) of bees tested for olfactory conditioning. The mean acquisition scores and standard errors of the means are shown on the y-axis. Whereas in foragers acquisition scores correlated with GRS, newly emerged bees did not show this correlation (for details on statistics see text). Significant differences in the acquisition scores of foragers in different GRS classes are indicated by *asterisks* (**$P \leq 0.01$; ***$P \leq 0.001$; two-tailed Mann–Whitney *U*-test). Number of bees tested in each group is shown

Table 1 Comparison of bees showing conditioned PER in each conditioning trial between conditioned newly emerged bees and unpaired controls or foragers from Behrends et al. (2007)

Conditioning trial no	Conditioned newly emerged bees versus unpaired controls	Conditioned newly emerged bees versus foragers from Behrends et al. (2007)
1	$P = 1.000$	$P = 1.000$
2	$P \leq 0.001$	$P = 0.142$
3	$P \leq 0.001$	$P = 0.996$
4	$P \leq 0.001$	$P = 0.254$
5	$P \leq 0.001$	$P \leq 0.001$
6	$P \leq 0.001$	$P \leq 0.001$

ness, most of them (about 80%) will not show any associative PER learning. Even of the bees responding to 10% sucrose or to lower sucrose concentrations, only a subset displayed the conditioned responses till the end of the training session. Newly emerged bees displayed a lower acquisition in olfactory learning than forager bees, which becomes particularly apparent in the last three conditioning trials. The learning capacity, however, exists and the brain structures involved in learning also appear to be sufficiently developed. However, the degree of development might be different between individuals, which could have led to the

fact that some newly emerged bees performed reasonably well in the tests while others quit showing the conditioned PER after few trials. These individual differences in learning behaviour could be related to differences in gene expression. A recent study of Whitfield et al. (2006) shows a relationship between gene expression profiles and ongoing brain maturation during the first 8 days after eclosion. In their study, for example, newly emerged bees displayed different gene expression profiles than bees older than 3 days. Individual differences in gene expression among newly emerged bees and their relationship to behavioural differences are currently under investigation in our laboratory. Another factor that seems to account for behavioural differences is brood temperature, which was shown to have a distinct effect on the development of learning capacities independent of sucrose responsiveness (Tautz et al. 2003).

Surprisingly, GRSs did not correlate with acquisition scores in newly emerged bees. In the group of foragers and in most foragers and hive bees from previous studies sucrose responsiveness and acquisition correlated (Scheiner et al. 1999, 2001a, 2001b, 2001c, 2003, 2005). The only other group in which we did not find such a correlation is aged worker bees with long foraging duration (Behrends et al. 2007). The possible reasons for this finding are currently under study. An important aspect of this issue is certainly that we could hardly condition any bees with a higher GRS. A correlation between responsiveness to the unconditioned stimulus and associative, olfactory learning performance was recently also found in aversive learning (Vergoz et al. 2007). In that study, the sting extension reflex in response to an electric shock was conditioned to the delivery of an odour. This shows that the responsiveness to the unconditioned stimulus in these very different paradigms is a decisive factor for successful associative learning. Earlier experiments on the habituation of the PER (Scheiner 2004) revealed that even newly emerged bees display the correlation between to sucrose responsiveness and non-associative habituation.

Our data prove the generally accepted idea that newly emerged bees are incapable of associative learning wrong. The apparent absence of learning ability can be explained by a general low responsiveness to olfactory and gustatory stimuli, which is related to immature olfactory and gustatory systems (Masson and Arnold 1984; Morgan et al. 1998; Pankiw and Page Jr 1999). Responsiveness to sucrose has been shown to increase with age in bees (Pankiw and Page Jr 1999), and sucrose responsiveness is a decisive determinant of associative and non-associative learning performance (Scheiner et al. 1999, 2001a, 2001b, 2001c, 2003, 2005). The insensitivity for the reward in associative learning might also explain poor learning performance of young individuals of other animal species (Nagy et al. 1977; Rankin and Carew 1987; Devaud et al.

2003). Our experiments suggest that in learning experiments, responsiveness to rewarding stimuli has to be controlled for before conclusions about learning capacities between groups can be drawn. But the olfactory system is also not fully developed in newly emerged bees (Masson and Arnold 1984; Morgan et al. 1998) and experiments analysing the role of odour intensity and odour sensitivity in associative olfactory learning of newly emerged should be conducted.

In our experiments, 50% of the conditioned newly emerged bees displayed early long-term memory 24 h after conditioning. This level of conditioned responses is similar to that obtained by pre-foraging hive bees of approximately 1 week of age (50–55%, data from Scheiner et al. 2003) or of nurse bees and foragers trained to a tactile stimulus (37–60%, R. Scheiner, unpublished data). Compared with the low level of acquisition achieved by newly emerged bees, the level of retention 24 h after conditioning is thus surprisingly high. A better performance of the bees in the memory test than during conditioning might be related to different satiation levels. At 24 h after conditioning, bees might have been less satiated than directly after emergence, so that bees which had not shown the conditioned response during conditioning (although they showed the PER at antennal sucrose stimulation and imbibed a small volume of sucrose solution) showed the conditioned PER 24 h later in expectation of food. Ray and Ferneyhough (1997) did not detect any long-term memory after 24 h in bees younger than 5 days which were conditioned to odours. But the low memory performance in their experiments might have been a consequence of insufficient responsiveness to the sucrose reward and thus poor acquisition in the newly emerged bees they tested. Maleszka and Helliwell (2001) reported retention levels of 65–70% in 5-day-old bees 24 h after olfactory conditioning, but they did not test newly emerged bees. Our results show that newly emerged bees can build a reliable and stable association of an odour and a sucrose reward, indicating that even newly emerged bees have fully developed mechanisms for memory consolidation and transition from short-term memory to long-term memory (for review see Menzel and Müller 1996). Borsellino et al. (1970) showed in a maze test that adult mealworm beetles can even recall experiences which they had acquired prior to metamorphosis. Honey bee larvae, however, which were exposed to an unrewarded odour, did not exhibit any significant behavioural adaptations to that odour in a PER-conditioning paradigm or in an olfactometer after emergence (Sandoz et al. 2000). This suggests that in honey bees there is no transfer for olfactory memory contents from the pupae to the adult status. But certainly more experiments are needed to validate this hypothesis.

The development of visual and olfactory learning capacities of honeybees in the field is experience-dependent and starts with the first orientation flights (for review see Fahrbach and Robinson 1995). In addition, the brain structures associated with learning and memory in honeybees display a high plasticity depending on the social tasks the bees perform. Neuropiles in the mushroom bodies, for example, increase in volume when worker bees that previously engaged in hive duties switch to foraging (Withers et al. 1993). Interestingly, even very young bees are able to undergo a precocious development as early as 4 days of age, when the colony lacks older worker bees or in single-cohort colonies (Fahrbach and Robinson 1996; Behrends et al. 2007). This suggests a rapid structural change in important brain centres, which accompany the high plasticity of behaviour. It is intriguing to speculate that the experience of newly emerged bees during conditioning already improved their performance in the 24 h memory test by affecting the structure of brain centres involved in learning and memory, such as the antennal lobes and mushroom bodies.

Our data suggest that newly emerged bees are able to acquire and store reward-related information in an associative manner. But these experiments are only a first step in understanding the memory formation in newly emerged bees. Numerous studies on the details of memory formation, for example on the different forms of long-term memory and extinction, on gene expression and on protein synthesis in newly emerged bees are required to fully grasp this aspect of honey bee behaviour.

References

Behrends A, Scheiner R, Baker N, Amdam GV (2007) Cognitive aging is linked to social role in honey bees (*Apis mellifera*). Exp Gerontol 42:1146–1153

Bitterman ME, Menzel R, Fietz A, Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). J Comp Psychol 97:107–119

Borsellino A, Pierantoni R, Schieti-Cavazza B (1970) Survival in adult mealworm beetles (*Tenebrio molitor*) of learning acquired at the larval stage. Nature 225:963–964

Bower TGR (1974) Development of infant behaviour. Br Med Bull 30:175–178

Devaud JM, Acebes A, Ramaswami M, Ferrús A (2003) Structural and functional changes in the olfactory pathway of adult *Drosophila* take place at a critical age. J Neurobiol 56:13–23

Erber J, Kierzek S, Sander E, Grandy K (1998) Tactile learning in the honeybee. J Comp Physiol [A] 183:737–744

Fahrbach SE, Robinson GE (1995) Behavioral development in the honey bee: toward the study of learning under natural conditions. Learn Mem 2:199–224

Fahrbach SE, Robinson GE (1996) Juvenile hormone, behavioral maturation, and brain structure in the honey bee. Dev Neurosci 18:102–114

Giurfa M (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. Naturwissenschaften 91:228–231

Kirkham NZ, Slemmer JA, Johnson SP (2002) Visual statistical learning in infancy: evidence for a domain general learning mechanism. Cognition 83:35–42

Kuwabara M (1957) Bildung des bedingten Reflexes von Pavlovs Typus bei der Honigbene. J Fac Sci Hokkaido Univ Zool 13:458–464

Maleszka R, Helliwell P (2001) Effect of juvenile hormone on short-term olfactory memory in young honeybees (*Apis mellifera*). Horm Behav 40:403–408

Masson C, Arnold G (1984) Ontogeny maturation and plasticity of the olfactory system in the Workerbee. J Insect Physiol 30:7–14

Menzel R, Müller U (1996) Learning and memory in honeybees: from behavior to neural substrates. Annu Rev Neurosci 19:379–404

Miller RG (1981) Simultaneous statistical inference. Springer, New York, pp 6–8

Morgan SM, Butz Huryn VM, Downes SR, Mercer AR (1998) The effects of queenlessness on the maturation of the honey bee olfactory system. Behav Brain Res 91:115–126

Nagy ZM, Thaller K, Mazzaferri TA (1977) Acquisition and retention of a passive-avoidance task as a function of age in mice. Dev Psychobiol 10:563–573

Pankiw T, Page RE Jr (1999) The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). J Comp Physiol [A] 185:207–213

Rankin CH, Carew TJ (1987) Development of learning and memory in *Aplysia*. II. Habituation and dishabituation. J Neurosci 7:133–143

Ray S, Ferneyhough B (1997) The effects of age on olfactory learning and memory in the honey bee *Apis mellifera*. Neuroreport 8:789–793

Sandoz JC, Laloi D, Odoux JF, Pham-Delègue MH (2000) Olfactory information transfer in the honeybee: compared efficiency of classical conditioning and early exposure. Anim Behav 59:1025–1034

Scheiner R (2004) Responsiveness to sucrose and habituation of the proboscis extension response in honey bees. J Comp Physiol [A] 190:727–733

Scheiner R, Erber J, Page RE Jr (1999) Tactile learning and the individual evaluation of the reward in honey bees (*Apis mellifera* L.). J Comp Physiol [A] 185:1–10

Scheiner R, Weiß A, Malun D, Erber J (2001a) Learning in honey bees with brain lesions: how partial mushroom-body ablations affect sucrose responsiveness and tactile antennal learning. Anim Cogn 3:227–235

Scheiner R, Page RE Jr, Erber J (2001b) 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 Jr, Erber J (2001c) The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). Neurobiol Learn Mem 76:138–150

Scheiner R, Page RE Jr, Erber J (2004) Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). Apidologie 35:133–142

Scheiner R, Müller U, Heimburger S, Erber J (2003) Activity of protein kinase A and gustatory responsiveness in the honey bee (*Apis mellifera* L.). J Comp Physiol [A] 189:427–434

Scheiner R, Kuritz-Kaiser A, Menzel R, Erber J (2005) Sensory responsiveness and the effects of equal subjective rewards on tactile learning and memory of honeybees. Learn Mem 12:626–635

Sigg D, Thompson CM, Mercer AR (1997) Activity-dependent changes to the brain and behavior of the honey bee, *Apis mellifera* (L.). J Neurosci 17:7148–7156

Tautz J, Maier S, Groh C, Rossler W, Brockmann A (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc Natl Acad Sci USA 100:7343–7347

Vergoz V, Roussel E, Sandoz JC, Giurfa M (2007) Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. PLoS ONE 2:288

von Frisch K (1967) The dance language and orientation of bees. Harvard University Press, Cambridge

Whitfield CW, Ben-Shahar Y, Brillet C, Leoncini I, Crauser D, Leconte Y, Rodriguez-Zas S, Robinson GE (2006) Genomic dissection of behavioral maturation in the honey bee. Proc Natl Acad Sci USA 103:16068–16075

Withers GS, Fahrbach SE, Robinson GE (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364:238–240