J Comp Physiol A (2001) 187: 891–899
DOI 10.1007/s00359-001-0260-z

ORIGINAL PAPER

Yehuda Ben-Shahar · Gene E. Robinson

Satiation differentially affects performance in a learning assay by nurse and forager honey bees

Accepted: 15 October 2001

Abstract When not satiated prior to training, there were no differences between foragers and nurse honey bees in the acquisition of an appetitively based conditioned response in an olfactory associative learning assay, but when satiated foragers showed faster acquisition than did nurses. Satiation-related differences between foragers and nurses were more a function of behavioral state than age, because satiated precocious foragers also showed faster acquisition rates than did satiated nurse bees, despite their similar ages. Tests of sucrose responsiveness and retention of conditioned responses indicate that the observed performance differences between nurses and foragers were more likely due to differential sensitivity of sensory and motor processes related to satiation rather than differences in cognitive ability.

Keywords *Apis mellifera* · Associative learning
Division of labor · Proboscis extension reflex ·
Hymenoptera

Abbreviations *CS* conditioned stimulus · *ITI* inter-trial interval · *PER* proboscis extension response · *US* unconditioned stimulus

Introduction

Honey bee workers perform different jobs as they age. Typical adult bee behavioral development involves tending the brood and queen as a "nurse" bee during the first 7–10 days of adult life, performing other tasks in the hive for the next week or so, and then shifting to foraging for the final 1–3 weeks of life (Winston 1987). Foraging is a particularly challenging cognitive task (Fahrbach and Robinson 1995; Chittka et al. 1999), involving multimodal sensory integration, sun compass navigation, dance communication, and associative learning to increase foraging efficiency. Foragers perform very well in a variety of laboratory associative learning assays (e.g., Menzel and Muller 1996; Menzel 1999), and this is thought to reflect the fact that learning to associate a sugar reward with a specific floral odor, color, or texture can result in more efficient foraging in nature.

Honey bees show many changes in physiology and neural functioning during behavioral development. These include changes in exocrine gland activity (Winston 1987), body weight, oxygen consumption (Harrison 1986), hormone levels, brain chemistry and structure (Robinson 1998), and gene expression in the brain (Kucharski et al. 1998; Toma et al. 2000). Some of these changes are known to enable the bee to perform certain tasks more efficiently at different stages of life, an advantage that is associated with systems of division of labor that feature specialization (Oster and Wilson 1978). Because foraging is thought to be the most cognitively demanding task performed by honey bees, it has been suggested that bees might also be expected to show an increase in cognitive abilities during behavioral development (Fahrbach and Robinson 1995). This speculation was influenced by the discovery that honey bee behavioral development also is associated with an increase in the neuropil volume of the mushroom bodies (Withers et al. 1993), a brain region involved in insect learning and memory.

The hypothesis that there is an increase in cognitive abilities during honey bee behavioral development has been tested in several studies by comparing the performance of nurse bees and foragers on well-established laboratory olfactory associative learning assays. This

G.E. Robinson
Neuroscience Program, University of Illinois
at Urbana-Champaign, IL 61801,USA

Y. Ben-Shahar (✉) · G.E. Robinson
Department of Entomology,
University of Illinois at Urbana-Champaign,
IL 61801, USA
E-mail: yehudab@life.uiuc.edu
Tel.: +1-217-2440895
Fax: +1-217-2443499

assay is based on the reflexive proboscis extension response (PER) to sucrose stimulation of the antennae (e.g., Menzel 1999). The results of comparative analyses of nurse bees and foragers have been variable. Bhagavan et al. (1994) showed no differences between nurses and foragers in the rate at which a conditioned response was acquired. Ray and Ferneyhough (1999) reported that foragers showed faster acquisition than nurses and similar results were obtained by Pham-Delegue et al. (1990). Pankiw and Page (1999) showed that response thresholds to sucrose decreased with increasing worker age; lower response thresholds were correlated with better acquisition, suggesting that any differences in performances that might exist are related to changes in sensitivity not cognitive abilities. Ben-Shahar et al. (2000) did not find differences in acquisition between nurses and foragers but did detect differences in the reversal (Ferguson et al. 2001) of a conditioned response. One possible explanation for these contradictory results is that there was variation among the studies in one or more factors that influence how a bee performs in the learning assay.

One factor that might influence performance in an appetitive learning assay is how satiated bees are when they are being conditioned. Satiation state is known to affect conditioned responses in bees and other animals, especially when the unconditioned stimulus is an appetitive one (Capaldi and Myers 1978; Menzel et al. 1989; Croy and Hughes 1991; Braun and Bicker 1992). The possibility that satiation state exerts differential effects at different stages of behavioral development has not been examined before, for bees or any other animal. It is plausible that such satiation-related performance differences may reflect basic physiological differences between bees that perform different tasks. We studied this by determining the effects of satiation on the rate of acquisition of a conditioned response by nurse bees and foragers. To better understand the nature of any satiation-related effects on acquisition, we also determined the effects of satiation on both the retention of the conditioned response and sensitivity to sucrose, the unconditioned stimulus.

Materials and methods

Bees

We used European honey bees, *Apis mellifera*, which in North America are derived from a mix of European subspecies. All bees were maintained according to standard beekeeping techniques at the University of Illinois Bee Research Facility. Bees were identified according to established methods (e.g., Robinson 1987). Foragers were identified as returning bees with either clearly visible pollen loads on their hind legs or distended abdomens (bearing either nectar or water). Nurses were identified as bees that repeatedly inserted their heads into cells with larvae.

Experimental colonies

Three different types of colonies were used in this study: typical ($N=1$), triple-cohort ($N=2$), and single-cohort ($N=1$). All colonies were unrelated to each other, and were each derived from a naturally mated queen. The typical colony had about 40,000 bees.

Triple-cohort colonies were made by sequentially introducing cohorts of 1-day-old (0–24 h) bees into a small hive at weekly intervals for 3 weeks ($n=\sim600$/cohort). The bees in each colony were derived from a naturally mated queen. Each triple-cohort colony had a laying queen (unrelated to the bees), one honeycomb frame of honey and pollen, and one empty frame for the queen to lay eggs in. One-day-old bees were obtained by removing frames with sealed brood from a typical colony in the field and placing them in an incubator (33°C) until adults emerged. Each 1-day-old bee was marked with a spot of paint (Testor's PLA) on the thorax prior to its introduction to the colony. Bees in triple-cohort colonies established similarly show an age at onset of foraging that is not different than what is seen in more typical colonies (Giray and Robinson 1994). Triple-cohort colonies were used in this study for convenience; their small size allowed them to be kept on a rooftop very close to the laboratory used for the learning assays, minimizing transport time between the field and lab. Foragers were collected from the oldest cohort at ~3 weeks of age, and nurses were collected at ~1 week of age.

The single-cohort colony was made similarly to the triple-cohort colonies, but with ~1,000 1-day-old bees. The absence of older bees induces precocious behavioral development in 5–10% of the bees in a single-cohort colony (Huang and Robinson 1992). As a result, it is possible to sample from a single-cohort colony normal-age nurses and precocious foragers that are the same age. We collected age-matched nurses and precocious foragers when they were 5–13 days-old. Testing normal age nurses and foragers from typical and triple-cohort colonies and normal age nurses and precocious foragers from a single-cohort colony allowed us to distinguish between effects of chronological age and current behavioral state.

Acquisition and retention assays

Standard methods were used (Bitterman et al. 1983; Bhagavan et al. 1994; Hammer and Menzel 1995). Bees were collected individually in glass vials and anesthetized by chilling on ice. Similar numbers of bees from each test group (i.e., nurses and foragers) were collected on each day of training to control for possible effects of environmental variation. Once anesthetized, each bee was harnessed in a plastic tube that allowed free movement of antennae and proboscis (Bitterman et al. 1983). Bees were then allowed to recover from the anesthesia for 30 min. Pretraining procedures were different in each experiment and are described below. Bees were kept at room temperature in the vicinity of the testing arena until tested. Once bees recovered from cold anesthesia they were tested for sucrose responsiveness by stimulating their antennae with sugar solution. Bees that failed to respond were excluded from the study.

Sugar and salt solutions were used as unconditioned stimuli (US) and odors (1-hexanol and geraniol; Sigma Chemical. St. Louis, Mo.) were used as conditioned stimuli (CS). To expose bees to odors, two 2-ml glass syringes were prepared with a small piece of filter paper soaked with 1.5 μl of the undiluted odor. The US was delivered by touching both antennae with a droplet of 1.25 mol l^{-1} sucrose and then feeding the bee with 0.4 μl of the solution, once she extended her proboscis. As soon as the bee began to extend its proboscis to odor alone, we omitted the antennal component of the US and delivered the sucrose directly to the proboscis. Punishment was delivered by touching both antennae with a droplet of 3 mol l^{-1} NaCl (Ferguson et al. 2001). The conditioning phase consisted of a total of six forward-pairing odor exposures (conditioning trials), three with each odor. Each of the odors was coupled with either a reward (A+) or a punishment (B–). The odor presentations were done in a pseudo-random fashion (ABBABA).

Odor delivery was as in Ben-Shahar et al. (2000). In short, in each conditioning trial the tested bee was placed in the training arena under a weak airflow (suction of a laboratory hood) for 35 s before the odor was delivered. This was done to habituate the tested bee to the mechanical component of the odor presentation.

Odor delivery was controlled by a computer and was timed for 5 s. The computer signaled the experimenter to deliver either a reward or a punishment to the bee beginning 2 s after the onset of odor. Each bee received such a conditioning trial every 6–10 min (six to ten bees were trained per day, three to five bees from each group). Although the inter-trial interval (ITI) between US presentations varied, there were no systematic differences in ITI between nurses and foragers on any given day. The ITIs in this study are in the range that is known to induce long-term retention of a learned odor (Gerber et al. 1998). Bees were kept outside the training arena between conditioning trials to prevent odor exposure.

The criterion for a response was the full extension of the proboscis at the onset of odor delivery prior to touching the with either the reward or punishment solution (PER). Acquisition rates were calculated based on the number of correct responses.

Retention tests were conducted as follows. After the six conditioning trials, bees were fed $\sim$5 µl of 1.25 mol l^{-1} sucrose and left harnessed in a dark chamber at room temperature. They were tested 24 h later by exposing them first to a single puff of odor A (CS+) without the sucrose reward. Then they were exposed to a puff of odor B (CS–) without the salt punishment. PER was recorded in the same manner as for acquisition. Bees were tested in the same order as they were trained during conditioning trials.

Satiation state

Nurses and foragers were either satiated or unsatiated prior to the onset of training. Cold anesthetized bees were allowed to recover for 30 min prior to feeding. Satiation was achieved by stimulating one of the antennae with a cotton stick soaked with 1.25 mol l^{-1} sucrose. Once the bee extended her proboscis she was allowed to feed from the cotton until the proboscis was retracted. We used two or three rounds of stimulation/satiation until bees stopped extending the proboscis in response to antennal stimulation. All bees showed a noticeably distended abdomen after cessation of satiation, indicating presence of food in their crop. Based on measurements from ten randomly selected bees, bees stopped extending their proboscis when they ingested 46.5 ± 8.2 µl (mean ± SD) of sugar syrup. Satiated bees in our experiments thus had a well-filled, but not completely filled, crops (Winston 1987). The number of antennal stimulations we used is not likely to induce habituation, although we did not actually test for this (Gerber et al. 1998). All unsatiated bees were fed 0.5 µl of 1.25 mol l^{-1} sucrose. Training of satiated or unsatiated bees began 30 min after feeding ended.

Experiments

Experiment 1: unsatiated bees

We compared acquisition and retention for nurses and foragers that were unsatiated. Nurses ($n = 22$) and foragers ($n = 21$) were of typical ages, 5–7 days and > 21 days old, respectively, collected from triple-cohort colony 1. This experiment served to provide "baseline" data similar to the results of experiments conducted in Ben-Shahar et al. (2000).

Experiment 2: unsatiated and satiated bees

We compared acquisition and retention for nurses and foragers that were either satiated or unsatiated. Testing bees from both groups during the same session, we compared unsatiated and satiated nurses ($n = 16$ per group) and unsatiated and satiated foragers ($n = 14$ and 15, respectively). Nurses were 5–7 days old and foragers > 21 days old, and were from triple-cohort 2. This experiment provided direct comparative data on the effects of satiation.

Experiment 3: effects of age and current occupation on the satiation effect

Results of experiment 2 showed differences in acquisition between satiated and unsatiated animals in each behavioral group. Experiment 3 was performed to determine whether nurses are more sensitive to the effects of satiation than foragers or vice versa. We also tested whether satiation-related differences between nurses and foragers were more closely associated with differences in age or current behavioral state. Nurses ($n = 29$) and precocious foragers ($n = 23$) were 6–8 days old, collected from a single-cohort colony. We also tested nurses and foragers from triple-cohort colony 2 ($n = 38$ and 35, respectively) and from a typical colony ($n = 21$ and 22, respectively). The nurses and foragers from the triple-cohort colony were 5–8 days old and > 21 days old, respectively. The nurses and foragers from the typical colony were of unknown age, but were assumed to be similar in age to those from the triple-cohort colony (Giray and Robinson 1994). All bees were satiated prior to testing. Both acquisition and retention were tested, as in experiments 1 and 2.

Experiment 4: effect of satiation on sucrose responsiveness

Nurses and foragers ($n = 16$ and 16, respectively) were collected from triple cohort colony 2 when they were 8 days old and

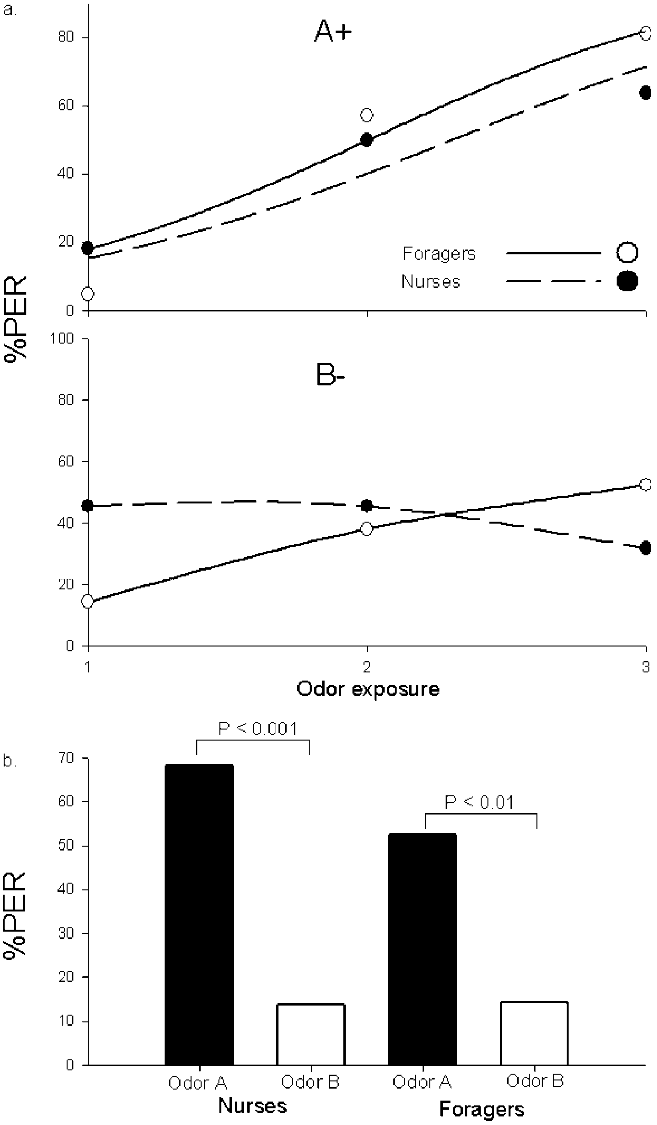

Fig. 1 Acquisition (**a**) and retention (**b**) of conditioned response to odors A+ and B– by unsatiated nurses and foragers in a three-trial test. *% PER* = the percentage of bees showing the proboscis extension reflex. $n = 22$ nurses and 21 foragers. Results of statistical analyses for acquisition tests in text

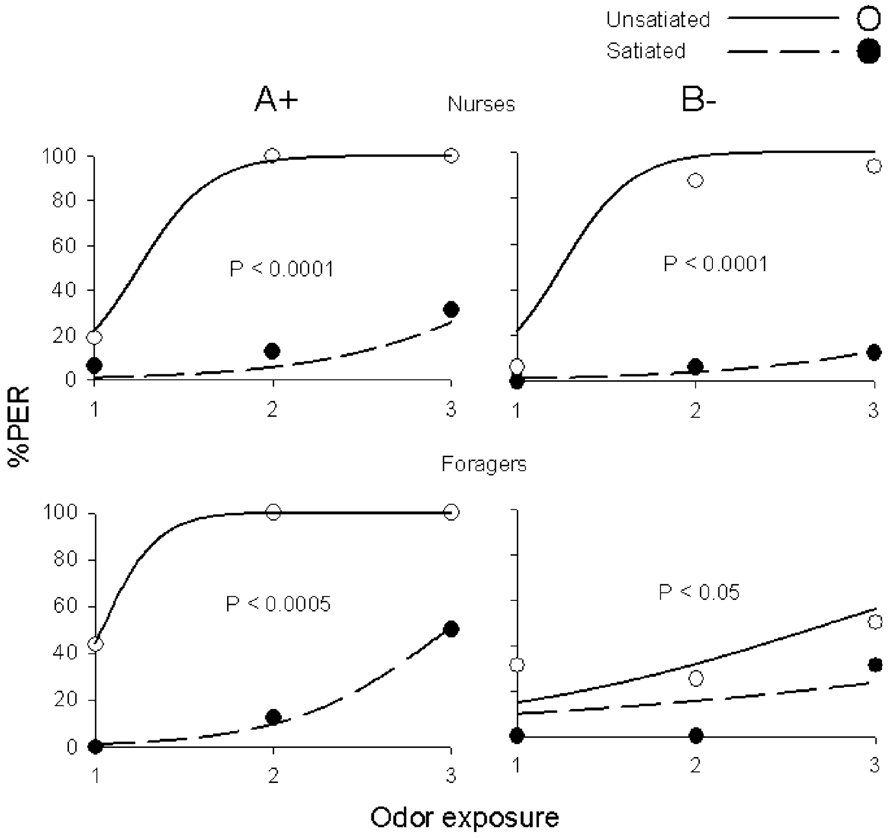

Fig. 2 Effects of satiation on acquisition of conditioned response to odors A+ (rewarded) and B– (punished) by nurses and foragers. Nurses: $n = 16$ satiated and unsatiated bees; foragers: $n = 14$ satiated and 15 unsatiated bees

> 21 days old, respectively. They were all satiated prior to testing, and then not fed any more for the duration of the experiment. Responses to 1.25 mol l^{-1} sucrose were measured as the percentage of bees that exhibited PER in response to antennal stimulation, just prior to the onset of satiation (Pre-S) and 10 min, 30 min, 90 min, and 120 min post-satiation. Bees were tested in the same order at each time point. Between sessions they were kept outside the training arena at room temperature. The sucrose concentration used here was the same one used in all other experiments. This experiment was conducted to determine whether satiation affected sensitivity to sucrose similarly to its effects on acquisition.

Experiment 5: effect of post-acquisition satiation on retention

Unsatiated bees were trained to respond to odor A; any bee that did not show the conditioned response after three trials was excluded from this experiment (3 out of 36 bees were excluded). The rest were maintained as described above for 24 h. A retention test was performed as in experiments 1–3, except half of the bees were satiated (with 1.25 mol l^{-1} sugar) just prior to testing and half were not. This experiment was performed only with foragers because in experiment 3 they showed better performance than did nurses under satiation conditions ($n = 17$ satiated and 16 unsatiated individuals).

Statistical analyses

Growth curve analysis (with a logistic regression model; PROC GENMOD, SAS Version 6.12) was used to determine differences in rates of acquisition between behavioral groups. This test functions as a repeated measurement analysis of variance, and is adjusted to individual performance by blocking the data for each bee (Hardy and Field 1988; Ben-Shahar et al. 2000; Hartz et al. 2001). We analyzed performance results separately for each component of the test (A+, B–) as was done in similar studies of bees and mammals for discrimination during associative learning (Ferguson et al. 2001; Fournier et al. 2001). Results of each regression analysis are presented in the text as the Δ regression coefficient (±SE). For each experiment the model created two logistic regression lines that best fitted the learning curves of each behavioral group, and tested whether the difference between the slopes (Δ regression coefficient) was significantly different than 0. When the difference in slope is statistically significant, the difference between learning rates is statistically significant. The figures show regression curves of each test group with its corresponding data points.

Results from retention tests were analyzed with χ^2 analysis. Treatment effects were assessed with direct comparisons within an experiment. Results from sugar responsiveness tests also were analyzed with χ^2 analysis. Fisher exact tests were used when one category contained < 5 data points.

Results

Experiment 1: unsatiated bees

There were no significant differences in acquisition between nurses and foragers that were trained when they were not satiated, for either A+ or B– (Fig. 1a; A+: Δ regression coefficient = 0.19 ± 0.26, NS; B–: Δ regression coefficient = 0.26 ± 0.21). There also were no significant differences between nurses and foragers for retention (Fig. 1b). Both nurses and foragers responded significantly more frequently to odor A (which was A+ during conditioning) than B (B–).

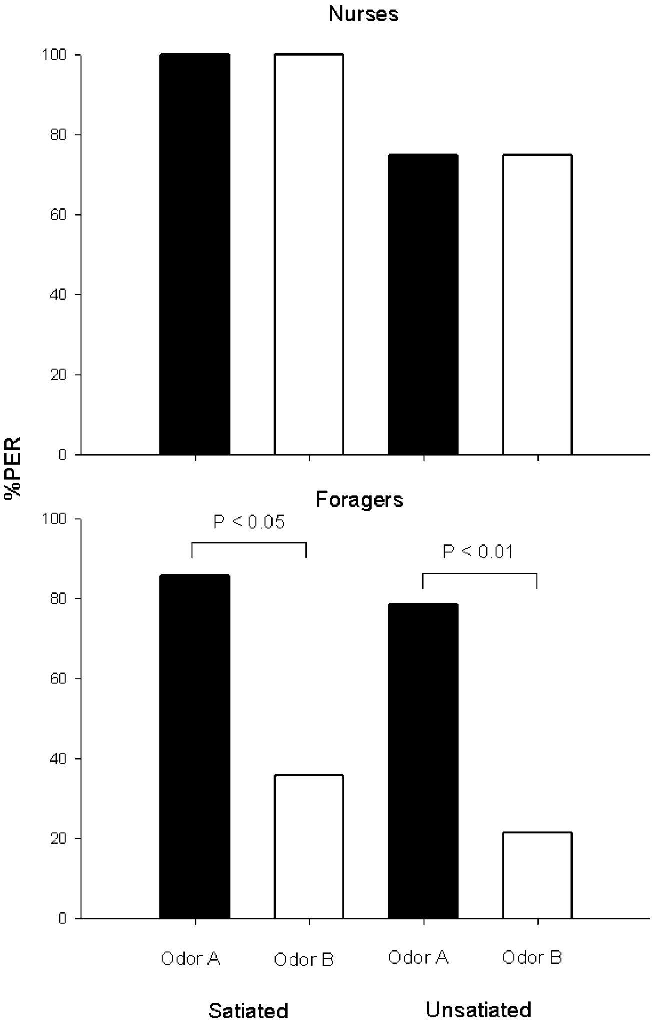

Fig. 3 Effects of satiation on retention of conditioned response to odors A+ and B– by nurses and foragers. Bees in these tests were the same ones used in the acquisition tests in Fig. 2

Experiment 2: unsatiated and satiated bees

Satiation slowed acquisition rates for both nurses and foragers (Fig. 2). For A+, satiated individuals showed significantly slower rates of acquisition than unsatiated animals (nurses: Δ regression coefficient = 3.30 ± 0.70, $P < 0.001$; foragers: Δ regression coefficient = 4.27 ± 1.17, $P < 0.005$). For B–, a satiation effect also was observed;this was much less pronounced in foragers than in nurses (B–; nurses: Δ regression coefficient = 2.15 ± 0.42, $P < 0.001$; foragers: Δ regression coefficient = 0.48 ± 0.24, $P < 0.05$).

Foragers responded significantly more frequently to odor A than odor B in retention tests (Fig. 3), under both satiated and unsatiated conditions. There was no significant ($P > 0.05$) effect of satiation on performance by foragers in the retention tests. Nurse bees did not show significantly different responses to A and B in retention tests, under either satiated or unsatiated conditions.

Experiment 3: effects of age and current occupation on the satiation effect

Direct comparisons revealed that foragers showed significantly faster acquisition rates than did nurse bees when they were satiated (Fig. 4). This difference was seen for normal-age foragers from a typical colony (Δ regression coefficient = 0.48 ± 0.19, $P < 0.02$) and a triple-cohort colony (Δ regression coefficient = 0.3 ± 0.26, $P < 0.007$). Precocious foragers also showed significantly faster acquisition rates than did nurses, even though they were the same age (Δ regression coefficient = 1.06 ± 0.40, $P < 0.008$).

One difference between precocious foragers and normal-age foragers was detected (Fig. 4). Precocious foragers showed a significantly increased rate of response to B– relative to nurses (Δ regression coefficient = 0.77 ± 0.31, $P < 0.02$) but normal-age foragers did not ($P > 0.05$).

As in experiment 2, satiated foragers again responded significantly more frequently to odor A than odor B in retention tests (Fig. 5). Nurses did so also in two out of three cases, in contrast to the results of experiment 2. However, nurses responded significantly more frequently to odor B than did foragers in two out of three cases, suggesting less retention of the odor discrimination, which is consistent with the results of experiment 2.

Experiment 4: effect of satiation on sucrose responsiveness

No differences between nurses and foragers were observed in responsiveness to sucrose when tested prior to satiation (Fig. 6). Ten minutes after satiation, there was a significantly lower percentage of nurses responding to the sucrose stimulus than foragers. There were no other significant differences between satiated nurses and foragers at any of the other time points.

Experiment 5: effect of post-acquisition satiation on retention

Satiation just prior to retention testing had a significant effect on performance (Fig. 7), even though both satiated and unsatiated bees showed similar rates of acquisition the previous day. Satiated bees responded significantly less often than did unsatiated bees.

Discussion

It is well known that satiation decreases acquisition of a conditioned response in an appetitive assay, for bees and other animals (Capaldi and Myers 1978; Menzel et al. 1989; Capaldi et al. 1982; Croy et al. 1991; Braun and Bicker 1992). Our results indicate that satiation also can exert differential effects on individuals depending on

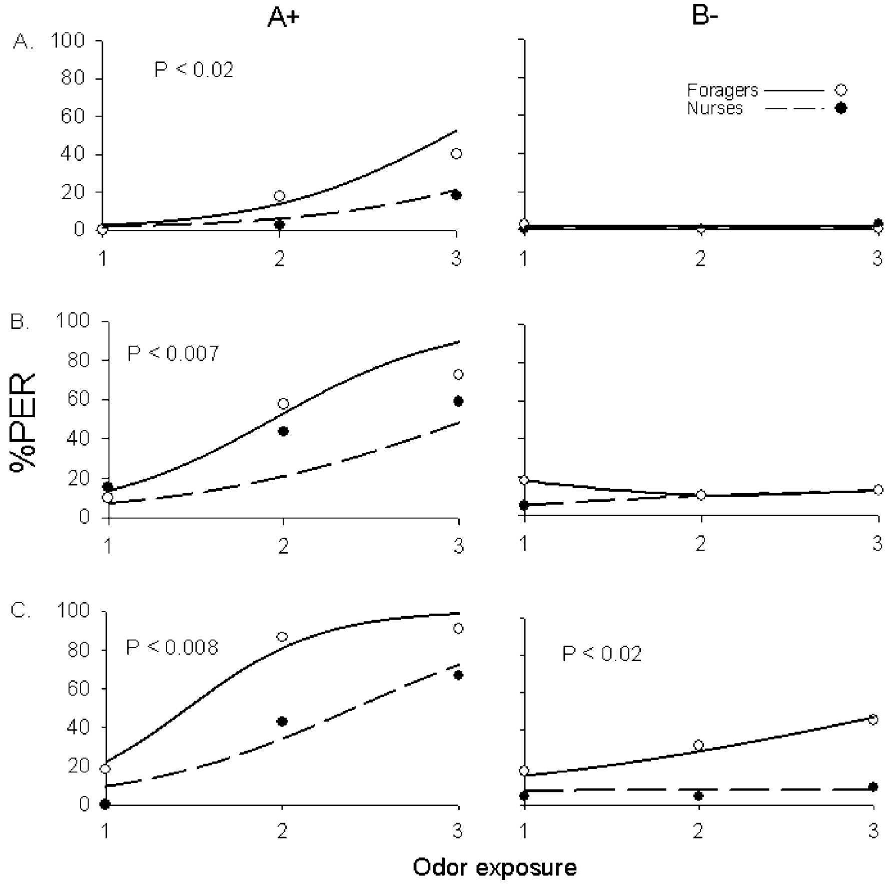

Fig. 4A–C Effects of age or current behavioral state on the satiation effect. **A** Nurses and foragers of unknown age from a typical colony ($n=21$ and 22, respectively); **B** 5- to 8-day-old nurses and >21-day-old foragers from a triple-cohort colony ($n=38$ and 35, respectively); **C** 6- to 7-day-old nurses and precocious foragers from a single-cohort colony ($n=29$ and 23, respectively)

their current behavioral state. Satiation decreased acquisition performance more strongly in nurses than in foragers. This resulted in measurable differences in performance during acquisition tests between the two groups that were not detected when they are not satiated.

Previous studies have shown genetic differences in sucrose response thresholds and in performance on assays similar to the one used here (Pankiw and Page 1999; Ferguson et al. 2001; Pankiw et al. 2001). Colonies used in our study were derived from naturally mated queens, which results in a genotypically diverse worker population. We think it is unlikely that the differential effects of satiation on nurses and foragers reported here can be solely the consequence of genotypic differences between workers independent of behavioral state, for two reasons. First, the differences between nurses and foragers were detected in four different colonies, each unrelated to the others. Second, in three out of four cases our experiments were conducted with colonies in which bees underwent typical behavioral development, with nursing occurring early in life and foraging later in life. Under these circumstances, bees of all genotypes were likely to be represented in our samples of nurses and foragers. While there may also have been genotypic differences in the effects of satiation that were not quantified in this study, the major effects reported here appear to be related closely to behavioral state.

The relative insensitivity to satiation shown by foragers in our study may explain why previous studies comparing the performance of nurse and forager honey bees in learning assays yielded variable results. Ray and Ferneyhough (1999) reported that foragers had faster acquisition than nurses, using a pre-training protocol that appears to be more similar to our satiation conditions. In contrast, Bhagavan et al. (1994), Ferguson et al. (2001), and Ben-Shahar et al. (2000) reported no differences in acquisition between nurses and foragers, using a protocol in which bees were not satiated prior to training. We showed differences in acquisition between nurses and foragers under satiation conditions but no differences when unsatiated. There were general differences in acquisition between the bees in experiments 1 and 2, which were performed in different years, at different times of year, and with bees from different genetic sources. These factors can affect performance in this assay (Gerber et al. 1996; Ray and Ferneyhough 1997; Pankiw and Page 1999).

It is not known why foragers are less sensitive than nurses to the effect of satiation in an appetitive-learning assay. One possibility is that this is related to the fact that food hoarding by a colony of honey bees is at least partially decoupled from the "personal" hunger status of the foragers. Foragers collect food when it is available in the environment and not only when they are hungry (Seeley 1995), so perhaps they are relatively resistant to

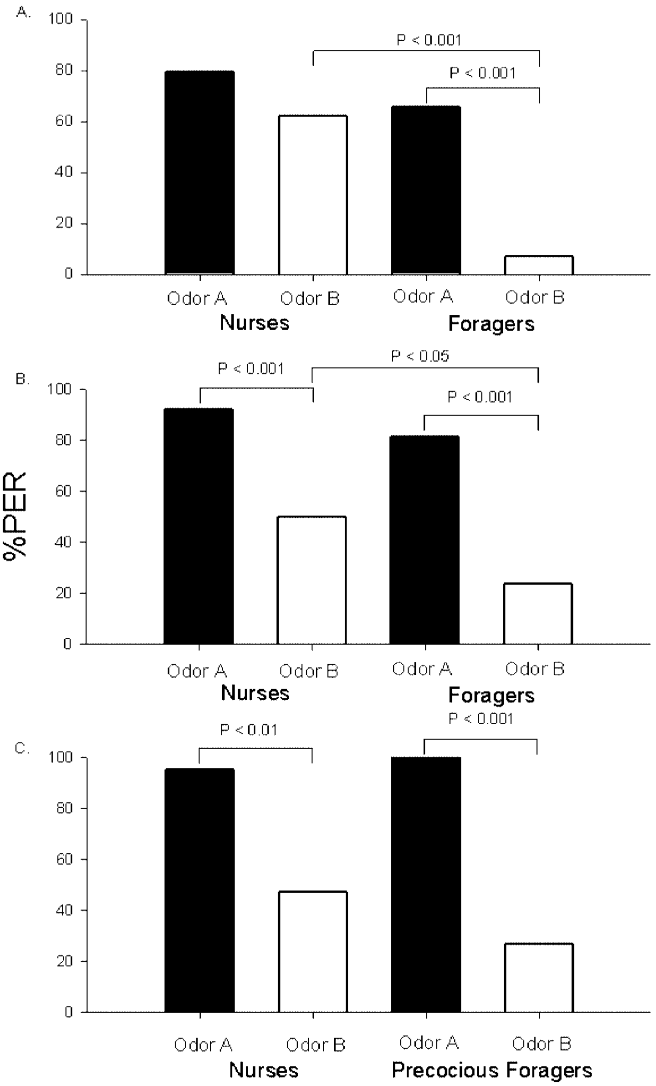

Fig. 5A–C Effects of age or current behavioral state on retention of conditioned response to odors A+ and B– by nurses and foragers satiated at the time of conditioning. Bees in these tests were the same ones used in the acquisition tests in Fig. 4

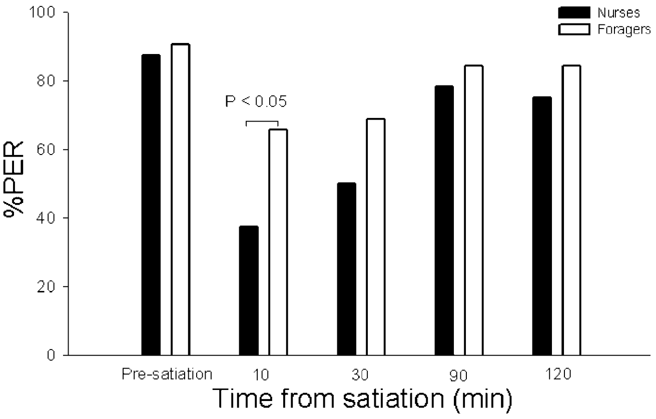

Fig. 6 Effects of satiation on response thresholds to 1.25 mol l^{-1} sucrose, measured by the percentage of bees showing the proboscis extension reflex in response to antennal stimulation. There were no significant differences in % PER except for 10 min post-satiation

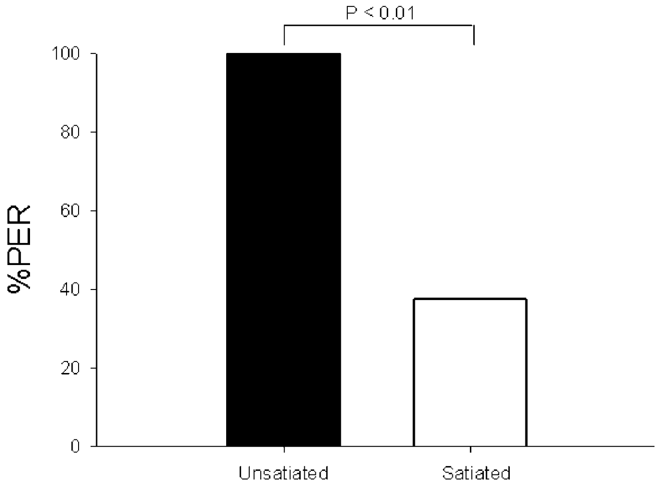

Fig. 7 Effects of satiation on retrieval of a learned odor. To conduct this experiment, foragers were trained to respond to odor A (when unsatiated); any bee that did not show the conditioned response after three trials was excluded from this experiment. Those that did respond were maintained under normal conditions for 24 h, with half of them satiated just prior to retention tests and the other half not. n = 16 satiated and 17 unsatiated bees

the effect of satiation. Another, non-mutually exclusive, explanation is that foraging experience causes bees to be less sensitive to the observed effects of satiation. Rats trained to make an association when hungry retained this association when satiated (Capaldi et al. 1982). Perhaps this is what happens when honey bees forage (Menzel 1999). If so, the effects of foraging experience would appear to be rapid, because precocious foragers also are less sensitive to satiation than nurses, and they may have taken only a few foraging trips prior to their capture (discussed in the following paragraph). At the physiological level, perhaps nurses and foragers differ in the regulation of how much sugar passes from the crop, a storage organ, to the midgut, where digestion and absorption occurs, thus causing differences in their satiation states. A lower concentration of sugar in the hemolymph of honey bees leads to increased passage of sugar from the crop to the midgut (Roces and Blatt 1999).

Precocious foragers showed a higher response to B–during acquisition than did normal age foragers (Fig. 4c), as in Ben-Shahar et al. (2000) – these apparent “errors” in response are known as “generalization” (Smith 1993). Foragers, like nurses, generalized more than did normal age foragers in their response to both the rewarded and punished odors. This effect was not influenced by satiation state. These results suggest that foraging experience causes bees to generalize less. This suggestion is based on the assumption that precocious foragers have less foraging experience than do normal age foragers. Precocious foragers were likely collected on their 1st or 2nd day of foraging, while normal age foragers may have been foraging for at least several days before they were collected. Other effects of natural foraging experience on performance in this laboratory assay have been shown (Gerber et al. 1996). The possibility that foraging experience influences the tendency to generalize in an olfactory conditioning assay could be examined with bees with precisely controlled amounts of foraging experience. Differences in generalization were also observed in experiment 2 (Fig. 2) between satiated and unsatiated animals, especially in nurses. The exact mechanisms that contribute to the generalization phenomenon are not known. Perhaps it is in part due to learning but it also occurs when more trials are given. Since it was mostly apparent in specific behavioral groups, and under a specific physiological state, it would appear to be a true biological difference.

Satiation inhibited at least two different components of the behavioral response in this learning assay, the response to the US and the conditioned response itself. Responsiveness to sucrose (the US) was lower in satiated animals. Nurses and foragers did not show a difference in sucrose responsiveness when unsatiated, but did when satiated. These findings are consistent with previous results with bees showing that changes in sucrose response thresholds are strongly associated with changes in performance in an associative learning assay (Pankiw and Page 1999; Scheiner et al. 1999). However, a comparison of the time-course of the sucrose responsiveness and acquisition tests suggests that a decrease in responsiveness to sucrose in response to satiation is not the only factor mediating the satiation-induced decrease in acquisition. Acquisition tests were conducted over a period of 1 h, while the satiation effect on sucrose responsiveness did not appear to persist beyond 30 min.

The second component of the behavioral response that was inhibited by satiation was the conditioned response. Satiated animals showed lower responses in a retention test than did unsatiated animals, even though tests were conducted with individuals that were in the same state of satiation when the conditioned response was first acquired on the day before. Additional evidence for the transitory nature of the inhibitory effects of satiation comes from the observation that there were no differences in performance on the retention tests, regardless of whether the bees were conditioned when they were either satiated or unsatiated. Both groups had the same, high, percentage of responses to the odors, and both were able to discriminate between the two odors.

One intriguing observation was that bees showed higher response levels in retention tests than even at the end of the acquisition trials. It has been reported that animals can show learning without actually performing during the acquisition phase of an associative learning test (Kane et al. 1997). *Drosophila melonogaster* flies genetically manipulated to be deficient in protein kinase C (PKC) showed lower acquisition performance than wild-type flies but were not different from them in long-term retention. It is not known whether the PKC pathway is involved in the results we report here for bees. Our findings suggest that the inhibitory effects of satiation involve effects on the proboscis extension reflex itself rather than on the formation of the association. This is probably a process that is independent of long-term consolidation, which in bees and flies is protein synthesis dependent and usually longer than the 24-h retention test that was used here (Wittstock et al. 1993; Wustenberg et al. 1998; Dubnau et al. 2001).

By carefully controlling satiation state, we showed that the division of labor in a honey bee colony is associated with differences in performance on an appetitive learning assay. The differences detected thus far, however, appear to be related more to differential effects on sensory and motor programs rather than differences in cognitive ability between nurses and foragers.

Acknowledgements We thank A. Ross for invaluable technical assistance, and M. Giurfa, S.N. Beshers, G. Bloch, M.M. Elekonich, D.J. Schulz, C.W. Whitfield, and two anonymous reviewers for helpful comments on the manuscript. This research was supported by a grant from the NIH to G.E.R. All experiments described in this paper were done in compliance with the current laws of the United States.

References

Ben-Shahar Y, Thompson CK, Hartz SM, Smith BH, Robinson GE (2000) Differences in performance on a reversal learning test and division of labor in honey bee colonies. Anim Cognit 3:119–125

Bhagavan S, Benatar S, Cobey S, Smith BH (1994) Effect of genotype but not of age or caste on olfactory learning performance in the honey bee, *Apis mellifera*. Anim Behav 48:1357–1369

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

Braun G, Bicker G (1992) Habituation of an appetitive reflex in the honeybee. J Neurophysiol 67:588–598

Capaldi ED, Myers DE (1978) Resistance to satiation of consummatory and instrumental performance. Learn Motiv 9:179–201

Capaldi ED, Davidson TL, Myers DE (1982) Resistance to satiation: reinforcing effects of food and eating under satiation. Learn Motiv 12:171–195

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361–377

Croy MI, Hughes RN (1991) The influence of hunger on feeding behaviour and the acquisition of learned foraging skills by the fifteen-spined stickleback, *Spinachia spinachia* L. Anim Behav 41:161–170

Dubnau J, Grady L, Kitamoto T, Tully T (2001) Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory. Nature (Lond) 411:433–434

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

Ferguson HJ, Cobey S, Smith BH (2001) Sensitivity to a change in reward is heritable in the honeybee, *Apis mellifera*. Anim Behav 61:527–534

Fournier C, Kourrich S, Soumireu-Mourat B, Mourre C (2001) Apamin improves reference memory but not procedural memory in rats by blocking small conductance Ca^{2+}-activated K^{+} channels in an olfactory discrimination task. Behav Brain Res 121:81–93

Gerber B, Geberzahn N, Hellstern F, Klein J, Kowalski O, Wüstenberg D, Menzel R (1996) Honey bees transfer olfactory memories established during flower visits to a proboscis extension paradigm in the laboratory. Anim Behav 52:1079–1085

Gerber B, Wüstenberg D, Schütz A, Menzel R (1998) Temporal determinants of olfactory long-term retention in honeybee classical conditioning: non monotonous effects of the training trial interval. Neurobiol Learn Mem 69:71–78

Giray T, Robinosn GE (1994) Effects of intracolony variability in behavioral development on plasticity of division of labor in honey bee colonies. Behav Ecol Sociobiol 35:13–20

Hammer M, Menzel R (1995) Learning and memory in the honeybee. J Neurosci 15:1617–1630

Hardy ICW, Field SA (1988) Logistic analysis of animal contests. Anim Behav 56:787–792

Harrison JM (1986) Caste-specific changes in honeybee flight capacity. Physiol Zool 59:175–187

Hartz SM, Ben-Shahar Y, Tyler M (2001) Logistic growth curve analysis in associative learning data. Anim Cognit 4:185–189

Huang Z-Y, Robinson GE (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. Proc Natl Acad Sci USA 89:11726–11729

Kane NS, Robichon A, Dickinson JA, Greenspan RJ (1997) Learning without performance in PKC-deficient *Drosophila*. Neuron 18:307–314

Kucharski R, Maleszka R, Hayward DC, Ball EE (1998) A royal jelly protein is expressed in a subset of Kenyon cells in the mushroom bodies of the honey bee brain. Naturwiss 85:7:343–346

Menzel R (1999) Memory dynamics in the honeybee. J Comp Physiol A 185:323–340

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

Menzel R, Hammer M, Sugawa M (1989) Non-associative components of conditioning in honeybees. In: Erber J, Menzel R, Pflueger HJ, Todt D (eds) Neural mechanisms of behavior. Thieme, Stuttgart, pp 221

Oster GF, Wilson EO (1978) Caste and ecology in social insects. Princeton University Press, Princeton

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 187:293–301

Pankiw T, Waddington KD, Page RE Jr (2001) Modulation of sucrose response thresholds in honey bees (*Apis mellifera* L.): influence of genotype, feeding, and foraging experience. J Comp Physiol A 185:207–13

Pham-Delegue M-H, De Jong R, Masson Claudine (1990) Effet de l'âge sur la réponse conditionnée d'extension du proboscis chez l'abeille domestique. C R Acad Sci Paris Ser III 310:527–532

Ray S, Ferneyhough B (1997) Seasonal variations of proboscis extension reflex conditioning in the honey bee (*Apis mellifera*). J Apic Res 36:108–110

Ray S, Ferneyhough B (1999) Behavioral development and olfactory learning in the honeybee (*Apis mellifera*). Dev Psychobiol 34:21–27

Robinson GE (1987) Regulation of honey bee age polyethism by juvenile hormone. Behav Ecol Sociobiol 20:329–338

Robinson GE (1998) From society to genes with the honey bee. Am Sci 86:456–462

Roces F, Blatt J (1999) Haemolymph sugars and the control of the proventriculus in the honey bee *Apis mellifera*. J Insect Physiol 45:221–229

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 185:207–213

Seeley TD (1995) The wisdom of the hive: the social physiology of honey bee colonies. Harvard University Press, Cambridge, Mass.

Smith BH (1993) Merging mechanism and adaptation: an ethological approach to learning and generalization. In: Papaj DR, Lewis AC (eds) Insect learning: ecological and evolutionary perspectives. Chapman and Hall, New York, pp 79–125

Toma DP, Bloch G, Moore D, Robinson GE (2000) Changes in *period* mRNA levels in the brain and division of labor in honey bee colonies. Proc Natl Acad Sci USA 97:6914–69149

Winston ML (1987) The biology of the honey bee. Harvard University Press, Cambridge, Mass.

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

Wittstock S, Kaatz HH, Menzel R (1993) Inhibition of brain protein synthesis by cycloheximide does not affect formation of long-term memory in honeybees after olfactory conditioning. J Neurosci 13:1379–1386

Wustenberg D, Gerber B, Menzel R (1998) Long- but not medium-term retention of olfactory memories in honeybees is impaired by actinomycin D and anisomycin. Eur J Neurosci 10:2742–2745