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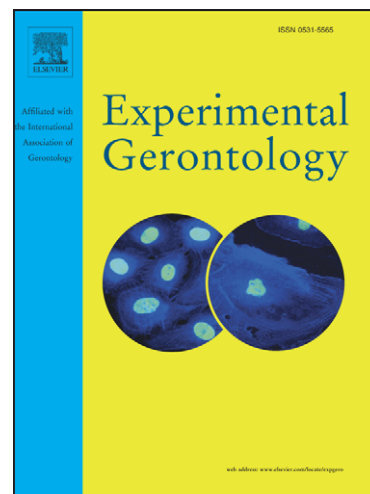
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Cognitive aging is linked to social role in honey bees (*Apis mellifera*)

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Introduction

Central nervous system (CNS) aging is observed in many species despite vast differences in CNS structure and organismal longevity (Gower and Lamberty, 1993; Yeoman and Faragher, 2001; Grotewiel et al., 2001; Driscoll et al., 2006; Mery, 2007). Over the last decades, vertebrate and invertebrate models have contributed to fundamental insights into the processes of normal brain aging (Finch, 2002; Keller, 2006) and of age-related disorders of the CNS (Maccioni et al., 2001; Chung et al., 2003, Reddy, 2006). In the most-studied systems to date, CNS aging is linked strongly to the age of adult animals. Therefore, although the plasticity of senescence in the brain is a topic of much interest, we lack models of extreme plasticity – i.e., in which CNS aging is at least partly decoupled from chronological age.

An interesting case of plasticity in aging is found in worker honey bees, a caste of largely sterile females that perform all of the non-reproductive social tasks in the honey bee society. They normally shift from nest tasks (nursing, cleaning and comb construction) to foraging duties (collecting nectar, pollen and water) after approximately 18-28 days of adult life (Winston, 1987). The foraging stage consists of several phases. The early phases are associated with selective growth of specific brain neuropiles (Menzel et al., 1994; Robinson et al., 1989) and an increase in the net rate of forage uptake (Dukas and Visscher, 1994). The later phases of foraging, however, appear to be associated with increased rates of aging, including development of mechanical senescence (Cartar, 1992), immunosenescence (Amdam et al., 2005), and accumulation of oxidative damage in the optic lobes (Seehuus et al., 2006). Foragers experience increased mortality risks, and most workers die after 1-2 weeks of foraging activity (Visscher and Dukas, 1997). Moreover, the higher energetic expenditure in foraging honey bees (Crailsheim et al., 1996; Suarez et al., 1996) leads to faster physiological exhaustion, partly due to depletion of carbohydrate reserves (Neukirch, 1982). Yet, the age when bees start foraging is variable within a range from 5 to >200 days (reviewed by Amdam and Omholt, 2003; Sekiguchi and Sakagami, 1966), and it is highly amendable to social factors: foraging onset can be postponed, accelerated or reversed by changes in colony demography, and some patterns of physiological aging respond correspondingly (Amdam et al., 2005).

It is unclear if CNS function is negatively affected in aging bees, but key aspects of CNS function such as associative learning can be quantified under controlled laboratory conditions, e.g. by olfactory conditioning of the proboscis extension response (Bitterman et al., 1983; for review see Menzel and Müller, 1996). A bee will reflexively extend her proboscis if her antennae are touched with sucrose solution of sufficient concentration, (Kuwabara, 1957). This gustatory response can be paired with an odor stimulus, and after few conditioning trials the bee extends her proboscis at stimulation with odor alone (Erber, 1980; Bitterman et al., 1983). Measurements of honey bee gustatory responsiveness are commonly used (for review see Scheiner et al., 2004), but have not been applied to bees of advanced chronological age or as a function of foraging duration. Associative learning in worker bees correlates strongly with gustatory responsiveness (Scheiner et al., 1999, 2001a, b, c, 2003, 2005). Individuals with high gustatory responsiveness show higher acquisition rates than those with low responsiveness. This association is related to the individual evaluation of the sucrose reward. Thus, if workers that differ in gustatory responsiveness receive equal subjective rewards (i.e. a bee with low responsiveness receives a highly concentrated sucrose solution and a bee with high responsiveness is rewarded with a comparatively low-concentrated sucrose solution), they do not differ in their associative learning performance (Scheiner et al., 2005).

In this study we analyse gustatory responsiveness and associative olfactory learning in honey bee workers, who were of different chronological age and performed different social tasks. We use experimental colonies of same-aged bees (single cohort colonies, Robinson et

al. 1989) and reverted foragers (socially reversed colonies, Robinson et al. 1992a). We combine these setups with behavioral observations to obtain workers of known age, known social role and known duration of foraging. Bees are tested for gustatory responsiveness and for associative olfactory acquisition. After acquisition, we test how accurate the bees learned the conditioned odor (“generalization test”) by presenting them an alternative odor that the bees had not experienced before. Our data document cognitive senescence of honey bees in olfactory acquisition, but at the same time show improved discrimination. The deficits in acquisition suggest that CNS aging can at least partly be decoupled from chronological age.

Materials and Methods

Preparation of bees

Experiments testing the effect of social role, chronological age and foraging duration on gustatory responsiveness, olfactory acquisition and generalization were conducted at the Technical University of Berlin. To obtain workers of known age, brood combs were placed in an incubator at 33°C and 70% humidity. Newly emerged worker bees (0-24 hours old) were collected and marked on the abdomen with paint (Testors™) to identify age. Two single cohort colonies were established with approximately 1,500 marked bees, a queen and brood. Foraging activity was observed daily during peak foraging hours (from noon to about 5 p.m., depending on weather conditions). When bees returned from presumably their first foraging flight, they received an additional paint mark on their thorax. For laboratory assays, nurse bees and foragers were collected from inside the hives in the morning, before foraging activity started. Bees without a second paint mark, who had intact wings and hairs on their thorax and who inserted their heads into cells with larvae were considered nurse bees. Bees with two paint marks were considered foragers. Thereby, workers of known chronological age, known social role, and known foraging duration could be collected.

Bees were collected over a period of 5 weeks. Their chronological age ranged between 15 and 57 days, and for analysis both foragers and nurse bees of corresponding chronological age were grouped according to the number of days that the foragers had been working in the field (6-13 and > 15 days). The mean chronological age of each these groups and the standard errors (SEM) were: nurses $_{6-13 \text{ days}} = 20$ days, SEM 0.28; nurses $_{>15 \text{ days}} = 38$ days, SEM 1.8; foragers $_{6-13 \text{ days}} = 20$ days, SEM 0.24; foragers $_{>15 \text{ days}} = 32$ days, SEM 0.66. Thus, for both nurse bees and foragers, the workers in the >15 days group were on average chronologically older than the bees in the 6-13 group ($Z_{\text{foragers}} = -1.445$; $p_{\text{foragers}} \leq 0.001$, $Z_{\text{nurse bees}} = -14.008$; $p_{\text{nurse bees}} \leq 0.001$ two-tailed Mann Whitney U Test). Further, the foragers that had worked in the field for > 15 days were chronologically younger than the corresponding nurse bees ($Z = -4.427$; $p \leq 0.001$; two-tailed Mann Whitney U Test).

Experiments testing the effect of foraging onset on gustatory responsiveness, olfactory acquisition and generalization were conducted at Arizona State University. Colony setups were equal to those of the reversion described by Amdam et al. (2005). In short, workers were separated by foraging experience (experienced foragers vs. all other workers including nurse bees), and new colonies were thereafter made up entirely of experienced foragers, queen and brood. In this social context, division of labor is re-established within few days, as some of the foragers revert to nurse tasks (Robinson et al., 1992a). Workers were of diverse and unknown chronological age and foraging duration, and thus the factors that allow cognitive aging to be studied (see above) were not controlled for. However, the purpose was not to study effects of reversion on olfactory acquisition and generalization of odors, but rather to determine the role of foraging onset per se. Thus we asked whether behavioral effects measured after foraging onset were linked to foraging duration specifically, or whether the event of foraging onset alone induced irreversible processes that lead to changes in learning behavior. To answer this question, continuing foragers and reverted nurses (former foragers)

were sampled 5, 9 and 13 days after reversion. This interval after reversion was chosen to assure full effect of reversion manipulation on the worker bees (Amdam et al., 2005).

For both experiments, bees were collected from the hive and placed in individual glass vials. They were then transferred to a refrigerator maintained at 4°C until they reduced their movements. They subsequently were restrained in individual metal holders: fixed with tape between head and thorax and over the abdomen to prevent stinging (Bitterman et al., 1983). After restraining, the bees were left in a humidified chamber for 1h.

Measuring of gustatory responsiveness

To measure gustatory responsiveness, the proboscis extension response (PER) was used. Each bee was stimulated at the tip of her antennae with water and with 6 sucrose solutions of following concentration order: 0.1%; 0.3%; 1%; 3%; 10%; 30%. 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. A gustatory response score (GRS) was used as a measure of gustatory responsiveness (Scheiner et al., 2004). This score comprises the total number of proboscis extension responses over the stimulation series with water and sucrose solutions. The GRS, thereby, is reported on a scale from 0-7, where 7 indicates the highest level of responsiveness (bee responded to all stimulations, Scheiner et al., 2004).

Olfactory conditioning

Only workers that responded to at least 30% sucrose ($GRS \geq 1$) were used for conditioning. Before conditioning, bees were tested for a spontaneous response to the conditioned stimulus carnation oil and to the alternative odor cineole. Two μ l of each odor 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 seconds before odor stimulation. The bee remained in the air stream approximately 8 seconds after stimulation with the odor. The inter-trial interval was 5 min. Only bees that did not spontaneously respond to either odor were conditioned to carnation. During each conditioning trial, approximately 5 ml of air filled with odor were applied to the bee's antennae. While the bee experienced the odor, 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 a small volume (approximately 1 μ l) of sucrose solution. For each conditioning trial, it was recorded whether the bee showed a conditioned PER. Bees were conditioned 6 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.

Afterwards, their responses to the alternative odor cineole and to the conditioned odor carnation were tested once with an inter-test interval of 5 minutes to analyze generalization of odors. This assay allowed us to draw conclusions about the accuracy of learning to the conditioned stimulus: i.e., in pure associative learning a bee should only respond to the conditioned odor and not to an alternative odor that is only offered in a test *after* conditioning.

Statistical analysis

For graphic display, means and standard errors of GRS and acquisition scores were calculated. The responses to the conditioned odor carnation and those to the alternative odor cineole in the tests following conditioning were shown as percentage of bees that expressed the PER. Spearman rank correlations were calculated to evaluate the relative effects of age and social task on GRS and acquisition scores (SPSS 14.0). Comparisons of GRS and acquisition scores were performed using the two-tailed Mann Whitney U Tests (SPSS 14.0). Generalization of odors was analyzed on the basis of PER to the conditioned odor carnation

and the unconditioned odor cineole. PERs to the unconditioned odor cineole were compared with two-tailed Fisher's Exact Tests (GraphPad InStat 3.06).

Results

Effects of age and social role on GRS

Gustatory responsiveness did not correlate with age ($\rho = 0.032$, $p = 0.487$, $n = 461$), but with social role of the workers ($\rho = 0.103$, $p \leq 0.05$, $n = 461$; Spearman rank correlation). Foragers displayed a higher gustatory responsiveness than nurse bees (Fig. 1). Notably, workers that had foraged for 6-13 days had significantly higher GRS than same-aged nurse bees (see Fig. 1 for statistics). This association shows for the first time that foragers are more responsive to gustatory stimuli primarily because of their social role and not because of their age.

Effects of age and social role on associative olfactory learning

In foragers, chronological age (15-57 days) correlated negatively with acquisition scores ($\rho = -2.67$, $p \leq 0.05$, $n = 79$). Chronologically older foragers performed less well in acquisition than younger foragers. Nurse bees, in contrast, did not show a correlation between chronological age and acquisition ($\rho = -0.017$, $p = 0.88$, $n = 88$; Spearman rank correlation).

In addition, duration of foraging affected olfactory acquisition in foragers (Fig. 2, $\rho = -0.31$, $p \leq 0.01$; Spearman rank correlation). Foragers that had foraged for > 15 days performed significantly less well in olfactory conditioning than foragers that had foraged for 6-13 days (see Fig. 2 for statistics). Nurse bees of the same chronological age (i.e., collected at the same time-points as the bees that had foraged for 6-13 days and > 15 days, respectively), in contrast, did not show a comparable correlation ($\rho = -0.04$, $p = 0.714$; $n = 88$; Spearman rank correlation). Thus, we found no significant difference in the acquisition performance of the two age groups of nurse bees (see Fig. 2 for statistics). These results demonstrate a clear difference in the progression of cognitive aging in foragers relative to nurse bees.

As expected (Scheiner et al., 1999, 2001a, b, 2005), individuals with high gustatory responsiveness generally performed better in olfactory acquisition than bees with low responsiveness. In all groups, apart from the group of foragers with the longest foraging duration, acquisition scores correlated positively with GRS (see Fig. 3 for statistics). Foragers that had foraged for >15 days, on the other hand, showed a significant decline in learning performance albeit being highly responsive to gustatory stimuli (Fig. 3).

Effects of age and social role on odor generalization

Forager bees did not differ from same-aged nurse bees in their response level to the conditioned odor carnation in the test after conditioning (see Fig. 4 for statistics). In addition, foragers with long foraging duration did not differ in their response level from foragers with short foraging duration, nor did respective nurse bee groups differ from each other (see Fig. 4 for statistics). Our data thus show that the difference in acquisition scores between foragers with long foraging duration (> 15 days) and those with shorter foraging duration (6-13 days) was mainly due to differences in the speed of acquisition. This is because the level of acquisition was similar in the two groups of foragers, but the overall learning performance measured as acquisition scores differed.

Foragers with long foraging duration (> 15 days) showed significantly less generalization between the conditioned odor carnation and the alternative test odor cineol (see Fig. 4 for details on statistic). This result implies that although foragers with long foraging duration learn slowly, they learn the conditioned odor more accurately than those that have been foragers for a shorter period of time.

Effect of reversion on gustatory responsiveness and associative learning

There was no correlation between social role and gustatory responsiveness after reversion ($\rho = -0.065$, $p = 0.677$; Spearman rank correlation): i.e., worker bees that reverted from foraging to nest tasks did not differ in GRS from bees that continued to forage ($Z = -0.42$, $p = 0.67$; two-tailed Mann Whitney U Test). These data show that GRS do not respond to further shifts in behavior after bees have initiated foraging.

Olfactory acquisition, in contrast, correlated significantly with social role after reversion (Fig. 5; $\rho = 0.314$, $p \leq 0.05$; Spearman rank correlation). Reverted nurses (former foragers) had higher acquisition scores than the continuing foragers (see Fig. 5 for statistics). This result shows that foraging onset per se does not trigger a physiological decline that ultimately leads to cognitive impairment. Instead, the result is consistent with a reduction of cognitive aging by reversal.

Reverted nurses did not differ in their response levels to the conditioned odor carnation from continuing foragers in the test after conditioning ($p_{\text{carnation}} = 1.0$; $n_{\text{rev. foragers}} = 19$; $n_{\text{cont. foragers}} = 24$; two-tailed Fisher's Exact Test). As before, the differences in acquisition scores were mainly due to differences in the learning speed. Reverted nurses also did not differ in their response levels to the alternative odor cineole from continuing foragers and thus showed no effect of reversion on generalization of odors ($p_{\text{cineole}} = 1.0$).

Discussion

Our data show that associative olfactory acquisition performance of honey bee workers declines in bees with long duration of foraging, while discrimination of odors improves. Interestingly, the impairment of olfactory acquisition is not a simple function of chronological age but of social task. Chronological age had no effect per se on gustatory responsiveness and learning performance. Instead, social role had a complex influence on different parameters of associative learning. Whereas foragers that had foraged for at least two weeks showed a reduced acquisition performance, same-aged nurse bees that had performed tasks inside the hive did not show a decline in associative learning. The level of conditioned responses after training was similar between foragers with long foraging duration (> 15 days) and those with shorter foraging duration (6-13 days). However, the acquisition scores differed, which indicates that the learning differences between these two groups are mainly related to the speed of acquisition, with foragers with long foraging duration being slower in acquisition. Our results contrast a recent study on aging by Rueppell et al. (2007), where the authors failed to detect aging deficits in honey bee associative olfactory PER-learning. However, Rueppell et al. did not control for behavioral function, i.e. they did not know whether workers were nurse bees, foragers or bees performing other tasks. Another difference between the studies is that the Rueppell et al. used honey bee colonies with typical age structure, whereas we used single cohort colonies. Although individuals in single-cohort colonies display typical patterns of division of labor after few days, the same chronological age of the colony members may bias the selection of tasks among individuals by genetic factors (for review see Robinson 1992b).

Neukirch (1982) detected a relationship between increased foraging activity, decreased lifespan, and impaired glycogen synthesis in old forager bees. Deficits in longevity and glycogen production rates were not a consequence of chronological age, but were related to the high activity of the forager bees. Furthermore, a study by Tofilski (2000) indicates that nectar foragers reduce their food collection activity with age. A decline in foraging performance was detected as early as 5 days of foraging. It is not clear to what extent our laboratory findings on associative olfactory learning can be transferred to the behavior of nectar foragers in the field, yet our results suggest that foragers would not only reduce their

foraging activity but also their speed of learning new food sources. This prediction is testable in aged foragers under free-flying conditions.

Interestingly, gustatory response scores correlated positively with acquisition scores in our experiments in all groups except the group that had foraged for >15 days. Normally, acquisition performance in honey bees is accompanied by corresponding differences in gustatory responsiveness (Scheiner et al., 1999, 2001a, b, c, 2003, 2005). Our experiments indicate that this relationship between sensory responsiveness and associative learning performance is different in worker bees with long foraging duration, and it is conceivable that effects of aging on learning performance in honey bees involve impairment in higher cognitive processing. A similar association of high sucrose responsiveness but impairment in learning was detected recently in honey bees challenged with a pathogen (deformed wing virus, Iqbal and Müller, 2007).

Our results from the reversion of social task show that it is not the event of foraging onset alone that triggers irreversible effects on learning behavior. It is rather the state of being a forager that leads to task-related decline in olfactory acquisition. Our results further imply that by reversion of social role deficits in olfactory acquisition can be compensated for. The next step would be to test this possibility, and to search for physiological correlates in the brain (e.g. proteins and metabolites) that change with foraging activity but can also be reversed to nurse bee levels by reversal of social role. A recent study by Amdam et al. (2005) revealed that circulating hemolymph hormone titers, vitellogenin protein concentrations and immunity can be reversed to levels characteristic of nurse bees. Ultimately, it may become possible to identify the general mechanisms and to reduce aging-related deficits in learning performance.

Our results do not only show a decline in acquisition of foragers with long duration of foraging, but detect at the same time less generalization in these bees. This is an interesting phenomenon, because it shows that aging is a complex process that may reduce some brain functions while enhancing others. Workers with long foraging duration apparently learn the details of a conditioned odor more exactly than those with short foraging duration. Our result is in accordance with vertebrate literature, suggesting that animals of different ages can compensate behavioral deficits involving different compartments of the brain (Cabeza et al., 2002; Madden DJ et al., 2004). In addition, our data may be explained by a complex relationship between acquisition and discrimination, suggesting that fast learners show more generalization after conditioning than bees that learn more slowly.

Previously, progressive behavioral senescence has been demonstrated in the fly *Drosophila melanogaster* (for review see Grotewiel et al., 2005), the nematode *Caenorhabditis elegans* (Murakami and Murakami, 2005), rats (Zyzak et al., 1995), primates (Price et al., 1991) and humans (Perlmutter et al., 1981). This phenomenon appears to reflect a general pattern of life history in these species, but the progressive nature of their aging makes it difficult to separate effects of chronological aging from those of physiological aging. Our data of the honey bee show that cognitive aging does not have to be a strict function of chronological age. In bees, the social role of the animal is the decisive factor that translates into complex patterns of cognitive aging. Together with the fully sequenced honey bee genome (Honeybee Genome Sequencing Consortium, 2006) our findings provide a solid justification of future use of honey bees to understand mechanisms that can alter age-related cognitive dysfunction in animals.

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Figure legends

Figure 1

Gustatory responsiveness of bees with different behavioral roles. The abscissa displays the duration of foraging in the group of foragers and the respective nurse bees of same chronological age. Note that the nurse bees never foraged. The ordinate shows gustatory response scores. Means and standard errors of the means are shown. Foragers with short foraging duration (6 – 13 days) were significantly more responsive to gustatory stimuli than same-aged nurse bees. This difference is marked by an asterisk ($Z = 2.41$, $p \leq 0.05$, $n_{\text{foragers 6-13 days}} = 133$; $n_{\text{nurses 6-13 days}} = 148$; $n_{\text{foragers >15 days}} = 64$; $n_{\text{nurses >15 days}} = 116$; two-tailed Mann Whitney U Test).

Figure 2

Acquisition scores of foragers and same-aged nurse bees of different age groups. The abscissa displays the duration of foraging in the group of foragers and the respective nurse bees of the same chronological age. Note that the nurse bees never foraged. The ordinate shows acquisition scores. Foragers who had foraged for more than 15 days performed significantly less well than foragers who had foraged for 6-13 days ($Z = 2.72$; $p \leq 0.01$, $n_{\text{foragers > 15 days}} = 23$; $n_{\text{foragers 6-13 days}} = 56$; two-tailed Mann Whitney U Test). Nurse bees of the same chronological age groups as the foragers did not differ in their learning performance ($Z = -0.369$, $p = 0.712$, $n = 88$). Foragers with >15 days of foraging duration did not differ from same-aged nurse bees ($Z = -0.906$; $p = 0.365$; $n_{\text{foragers > 15 days}} = 23$; $n_{\text{nurses > 15 days}} = 37$) and foragers with 6-13 days of foraging duration did not differ from respective nurse bees ($Z = -1.2$; $p = 0.23$; $n_{\text{foragers 6-13 days}} = 56$; $n_{\text{nurses 6-13 days}} = 51$) Asterisks mark the significant difference (**: $p \leq 0.01$).

Figure 3

Acquisition scores of foragers with different duration of foraging and nurse bees of the same chronological age groups divided in low (1-3) and high (5-7) GRS-classes. The bees were conditioned to carnation odor with sucrose as reward. The ordinate shows the acquisition score reflecting the overall degree of acquisition. Foragers have significantly higher acquisition scores after foraging for 6-13 days than bees that had foraged > 15 days. This difference is very pronounced in the high GRS-class ($Z = -3.64$, $p \leq 0.001$, $n_{\text{forager 6-13}} = 25$; $n_{\text{forager >15}} = 14$; two-tailed Mann Whitney U Test). It is not significant in the groups of bees with low GRS ($Z = -1.82$; $p = 0.069$; $n_{\text{foragers 6-13}} = 25$; $n_{\text{foragers >15}} = 6$). Nurse bees did not show any significant difference, either in the high or low GRS-class. Asterisks mark the significant difference (***) $p \leq 0.001$. Both age groups of nurse bees and foragers that foraged 6-13 days displayed positive correlations between GRS and acquisition scores (nurses 6-13 days: $\rho = 0.49$, $p \leq 0.001$, $n = 51$; nurses >15 days: $\rho = 0.57$, $p \leq 0.001$, $n = 37$; foragers foraging 6-13 days: $\rho = 0.67$, $p \leq 0.001$, $n = 56$; Spearman rank correlation). Only foragers that foraged for > 15 days did not show such a correlation (foragers foraging >15 days: $\rho = 0.40$, $p = 0.061$, $n = 23$).

Figure 4

Responses to the conditioned odor carnation after conditioning (A) and to the unconditioned odor cineole (B) of nurse bees and foragers with different foraging durations. The bars display the percentage of bees that responded with PER to each odor. Foragers that foraged for > 15 days did not respond to cineole at all and differed significantly from all other groups (foragers foraging >15 days vs. foragers foraging 6-13 days: $p \leq 0.05$; foragers foraging >15 days vs. nurses >15 days: $p \leq 0.05$; foragers foraging >15 days vs. nurses 6-13 days: $p \leq 0.01$; $n_{\text{foragers foraging >15 days}} = 23$; $n_{\text{foragers foraging 6-13 days}} = 56$; $n_{\text{nurses >15 days}} = 37$; $n_{\text{nurses 6-13 days}} = 51$; two-tailed Fisher's Exact Test).

Asterisks mark the significant differences (* $p \leq 0.05$; ** $p \leq 0.01$). Response to the conditioned odor carnation did not differ between roles (foragers foraging 6-13 days vs. same-aged nurses: $p = 0.065$; foragers foraging >15 days vs. same-aged nurses: $p = 0.58$) or between age groups (nurses >15 days vs. nurses 6-13 days: $p = 0.83$; foragers foraging >15 days vs. foragers foraging 6-13 days: $p = 0.78$, two-tailed Mann Whitney U Test).

Figure 5

Acquisition scores and GRS of different behavioral phenotypes after reversion. Continuing foragers and reverted nurses had the same chronological age and were sampled 5, 9 and 13 days after reversion. The acquisition score / GRS are displayed on the abscissa. While continuing foragers and reverted nurses did not differ in gustatory responsiveness, the reversion had a strong influence on learning performance. Reverted nurses displayed higher acquisition scores than continuing foragers ($Z = -2.03$, $p \leq 0.05$, $n_{\text{cont. foragers}} = 24$; $n_{\text{rev. foragers}} = 19$; * $p \leq 0.05$; two-tailed Mann Whitney U Test).

Fig1

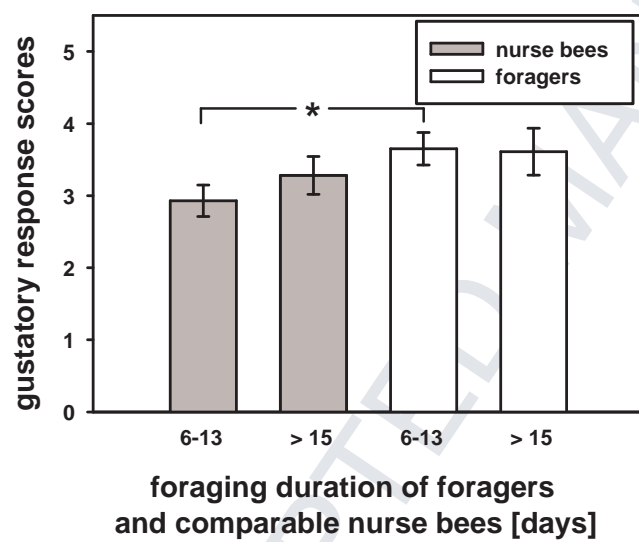


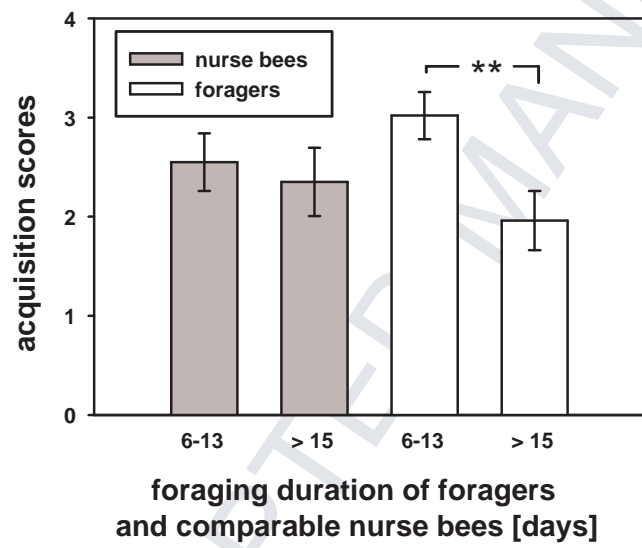
Fig2

Fig3

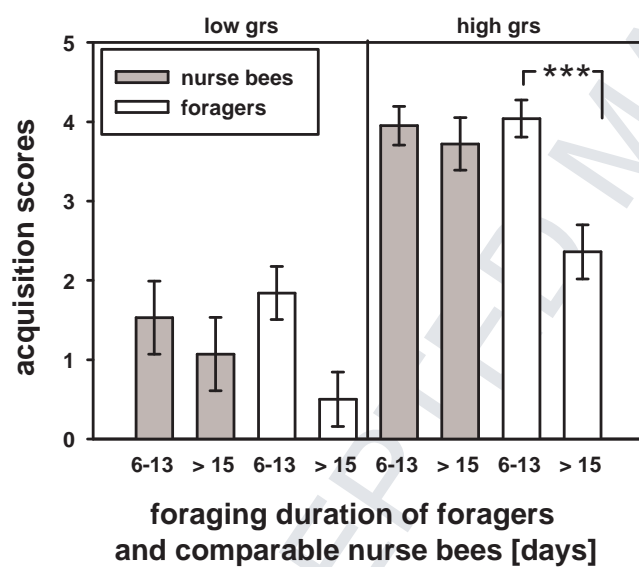


Fig4A

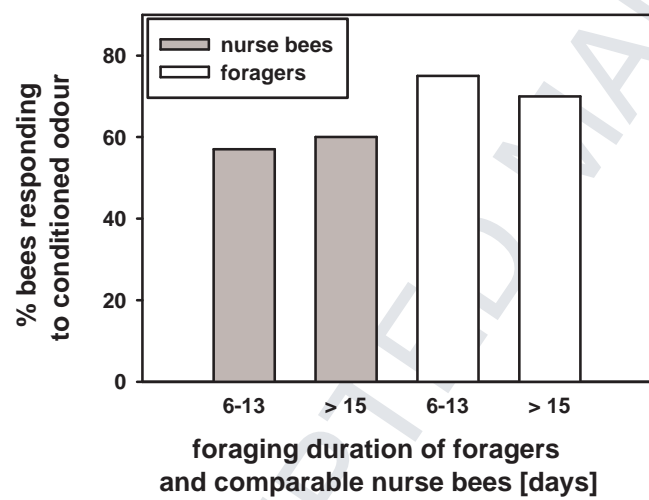


Fig4B

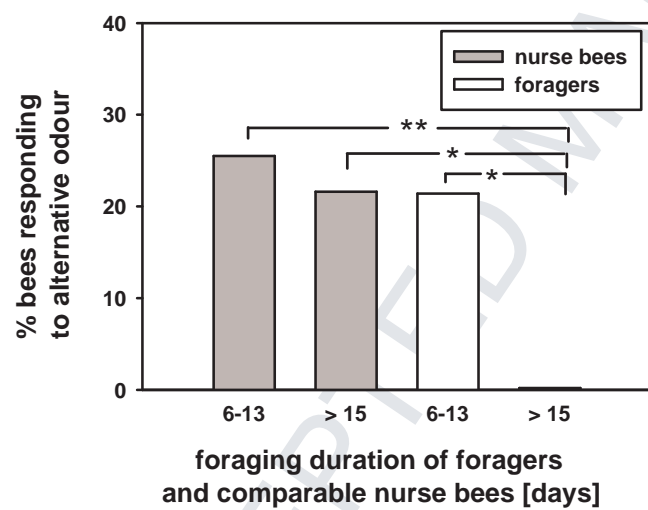


Fig5

