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RESEARCH ARTICLE

Retention of long-term memories in different age groups of honeybee (*Apis mellifera*) workers

C. Grüter · M. S. Balbuena · W. M. Farina

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Abstract Honeybees can learn food odors inside the nest during food sharing and use this information during flower choice, dance choice or choice of a trophallactic partner. We investigated for how long odors learned inside the hive are retained by bees of different age groups. In our study, bees retrieved food odors for up to 10–11 days. Our results suggest that olfactory information acquired by young bees while performing in-hive duties can be retrieved when performing foraging duties later in life.

Keywords *Apis mellifera* · Proboscis extension response · Trophallaxis · Olfactory learning

Introduction

Bees learn food odors during foraging or food sharing inside the hive (von Frisch, 1967). This affects decision making in several behavioral contexts, e.g. when deciding to follow a dancer (von Frisch, 1967), unload nectar from a forager (Goyret and Farina, 2005) or land on a food source with a particular odor (Arenas et al., 2008).

C. Grüter · M. S. Balbuena · W. M. Farina (✉)
Grupo de Estudio de Insectos Sociales, IFIBYNE-CONICET, Departamento de Biodiversidad y Biología Experimental Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina
e-mail: walter@fbmc.fcen.uba.ar

C. Grüter
Laboratory of Apiculture and Social Insects, Department of Biological and Environmental Science, John Maynard-Smith Building, University of Sussex, Falmer BN1 9QG, UK

Olfactory information can be transferred to long-term memory. For example, Arenas et al. (2008) found that olfactory memories established inside the hive affects the food choice of foragers for up to 8 days. Even young worker bees conditioned at the age of 5–8 days to floral odors within the colony can retain this odorant information 9 days later (Arenas and Farina, 2008). Both studies treated colonies with large amounts of scented food offered with in-hive feeders. Here, we tested if the same effect can be observed under more natural conditions and in different age groups. We offered relatively small amounts of food to eight foragers at feeders 80 m from the hive. Solution collected by foragers is shared with other hive bees which causes a propagation of odor information inside the colony (Grüter et al., 2006). We measured memory retention in bees of various age groups belonging to colonies that had unrestricted access to natural food sources. Memory retention was tested with the proboscis extension response (PER) assay. The PER assay has been successfully used to measure olfactory memory retention in honeybees in both laboratory and natural conditions (Gerber et al., 1996; Menzel, 1999). If a bee has learned an association between a reward (e.g. sugar solution) and a biologically relevant stimulus like a floral odor, the subsequent reception of the odor alone can cause a conditioned response, the extension of the proboscis (Menzel, 1999).

Materials and methods

Animals

Two colonies of honeybees (*Apis mellifera ligustica*) were housed in two-frame observation hives (H1 and H2).

Colonies had about 4,000 workers, a queen, brood and reserves.

Combs with pre-emerging brood from various hives around the lab were maintained in an incubator (temperature 36°C, relative humidity 55%). On the day of emergence, young bees were color-marked and introduced to H1 and H2. We used different colors every 2 days.

Experimental procedure

We trained eight marked foragers of each colony to two different artificial feeders, 80 m from the hives. The foragers collected 14 ml sucrose scented solution [Linalool (LIO)] per day for 5 days. Only these foragers were allowed to contact and collect the solution. Hence, each colony was treated with 70 ml sucrose solution over 5 days, containing 50 µl pure LIO per liter of sugar solution. H1 was fed with a 2 M solution, H2 was fed with a 0.5 M solution. The differences in molarity can affect associative learning in honeybees (Menzel, 1999) and potentially lead to higher retention levels in H1. However in this paper, we focus only the temporal dynamics of retention.

We tested the PER of bees belonging to three different age or task groups (1) 4–9-day-old bees (nurses responsible for brood care), (2) 12–16-day-old bees (processors responsible for processing liquid food) and (3) foragers (usually >17 days) (Seeley, 1995).

We measured the PER before offering scented solution for the first time (day 0), on days 2 and 5 of the treatment period. Furthermore, we tested bees again 4, 7 (H1), 8 (H2), 10 (H1), 11 (H2), 13 (H1) and 14 days (H2) after the end of the treatment period. Each bee was only tested once for the PER towards the treatment odor. Bees were captured for the PER assay following Grüter et al. (2006). The PER assay was performed according to Grüter et al. (2006) using equipment described in Guerrieri et al. (2005).

Test trials lasted for 46 s and consisted of 20 s of airflow, 6 s of odor (CS) and 20 s of airflow.

Results

Foragers that did not respond to sucrose and foragers responding to air alone were discarded (Grüter et al., 2006). Figure 1 shows that PER values for LIO increased during the feeding period, whereas the PER percentages for 2-octanol (2-OCT; control odor) were generally low.

During the entire experiment, 7.2% responded to both odors ($N = 293$; not shown in Fig. 1). Foragers of a control hive (full sized) were also tested twice for their PER during the experiment.

These foragers showed low PER percentages for the solution odor (LIO) after the treatment period of H1 (6.7%, $N = 15$) and after the treatment period of H2 (4.8%, $N = 21$; Fig. 1).

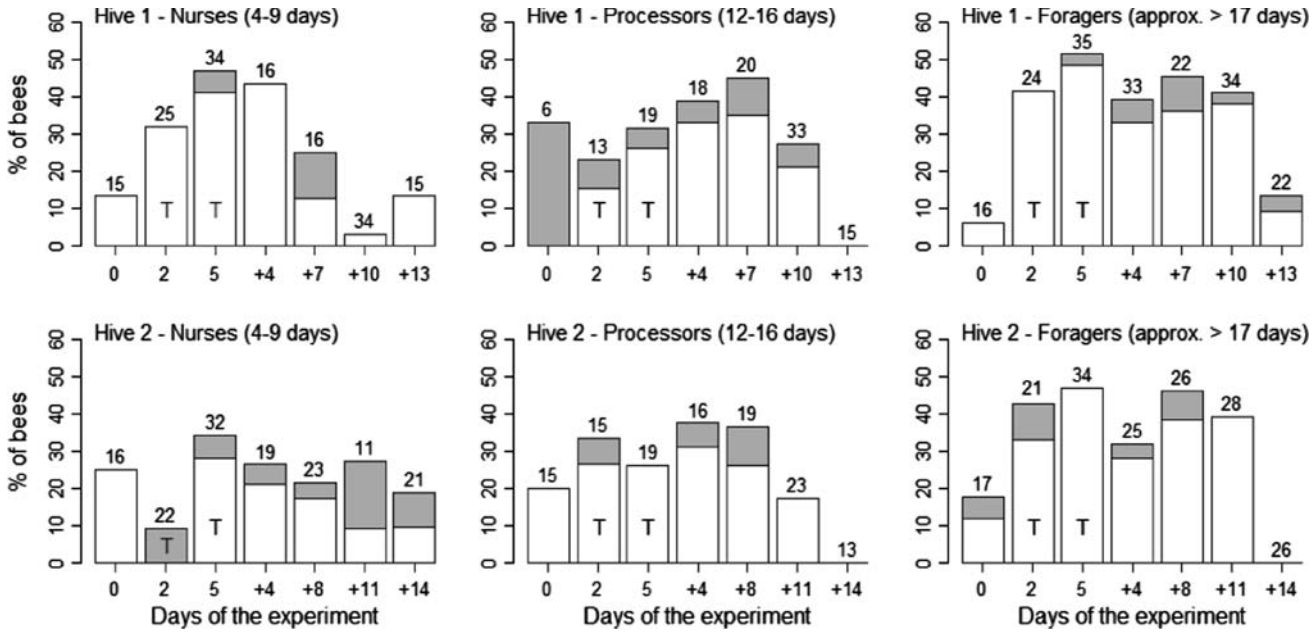

Fig. 1 Proboscis extension response (PER) percentages for the solution odor (*white*) and the control odor (*gray*) for bees of 4–9 and 12–16 days and foragers. The *numbers* below the *x* axis with a (+) indicate how many days after the end of the feeding period the bees were tested. The *bars* representing bees that were captured during the feeding period are indicated with a *T*. The *numbers* above the *bars* represent the number of tested bees

After the feeding period, PER percentages remained at a high level for foragers until 10 (H1) and 11 (H2) days after the end of treatment. During this period, PER frequencies of nurse-aged bees decreased gradually. Ten days (H1) and 11 days (H2) after the feeding period foragers still responded more to LIO than before the feeding at day 0 (G test, H1: $G_{adj} = 6.28$, $df = 1$, $N = 16/34$, $P = 0.012$; H2: $G_{adj} = 4.08$, $df = 1$, $N = 17/28$, $P = 0.043$). There was no difference in the case of nurse-aged bees (Fisher's exact test, H1: $N = 15/34$, $P = 0.22$; H2: $N = 16/11$, $P = 0.63$) and processor-aged bees (Fisher's exact test, H1: $N = 6/33$, $P = 0.57$; H2: $N = 15/23$, $P = 1$).

We found no differences in response levels of LIO compared with 2-OCT before the treatment at day 0 [Mc Nemar tests ($df = 1$); H1; foragers: $\chi^2 = 0$, $P = 1$; processor-aged: $\chi^2 = 0.5$, $P = 0.48$; nurse-aged: $\chi^2 = 0.5$, $P = 0.48$; H2; foragers: $\chi^2 = 0$, $P = 1$; processor-aged: $\chi^2 = 1.33$, $P = 0.25$; nurse-aged: $\chi^2 = 2.25$, $P = 0.13$]. The same was true 10 (H1) and 11 days (H2) after the feeding period for processor-aged and nurse-aged bees (H1; processor-aged: $\chi^2 = 1.78$, $P = 0.18$; nurse-aged: $\chi^2 = 0$, $P = 1$; H2; processor-aged: $\chi^2 = 2.25$, $P = 0.13$; nurse-aged: $\chi^2 = 0$, $P = 1$). However, foragers still responded more to LIO than 2-OCT (H1: $\chi^2 = 8.64$, $P = 0.003$; H2: $\chi^2 = 9.09$, $P = 0.003$).

Discussion

Olfactory learning inside the hive affects behaviors that are important for foraging success (von Frisch, 1967; Goyret and Farina, 2005; Arenas et al., 2008). The results presented in this study suggest that olfactory information learned inside the hive can potentially be retrieved until 10–11 days after learning had occurred. Although foragers consistently showed high PER levels during this period, the PER frequencies in nurse-aged bees gradually decreases, presumably because nurse-aged bees (4–9-day-old) emerged after the feeding period and, therefore, had no contact with the scented solution. The late season in combination with the small amount of scented food we used, prevented an accumulation of scented solution in the hive.

Retention of olfactory memory several days after learning has been shown previously (e.g. Arenas and Farina, 2008; Arenas et al., 2008). Our study was performed in a more natural situation where a relatively small amount of scented solution was collected by foragers of colonies that had accessed to natural food sources. Hence, our bees were exposed to other odors as well.

The bees that had been nurse-age during the feeding period probably switched to processor duties or even foragers duties when we tested colonies 10–11 days after feeding. These results suggest that young bees performing nurse or food processor tasks can potentially remember odors after they switched duty and caste. For example, food processors that learn food odors during unloading of foragers (Farina et al., 2007) could remember these odors when they become foragers. This olfactory information might help foragers to discover food patches if a particular food type is available during several days in the foraging area of the colony. To confirm our hypothesis, future studies should directly test whether flower choices of foragers are affected by olfactory information acquired when performing in-hive duties earlier in life.

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References

Arenas A. and Farina W.M. 2008. Age and rearing environment interact in the retention of early olfactory memories in honeybees. *J. Comp. Physiol.* A **194**: 629–640

Arenas A., Fernández V. and Farina W.M. 2008. Floral scents experienced within the colony affect long-term foraging preferences in honeybees. *Apidologie* **39**: 714–722

Farina W.M., Grüter C., Acosta L.E. and Mc Cabe S. 2007. Honeybees learn floral odors while receiving nectar from foragers within the hive. *Naturwissenschaften* **94**: 55–60

Gerber B., Gerberzahn N., Hellstern F., Klein J., Kowalksy O., Wüstenberg D. and 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

Goyret J. and Farina W.M. 2005. Non-random nectar unloading interactions between foragers and their receivers in the honeybee hive. *Naturwissenschaften* **92**: 440–443

Grüter C., Acosta L.E. and Farina W.M. 2006. Propagation of olfactory information within the honeybee hive. *Behav. Ecol. Sociobiol.* **60**: 707–715

Guerrieri F., Schubert M., Sandoz J-C. and Giurfa M. 2005. Perceptual and neural olfactory similarity in honeybees. *PloS Biol.* **3**: e60

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

Seeley T.D. 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies.* Cambridge, Massachusetts, Harvard University Press. 309 pp

von Frisch K. 1967. *The Dance Language and Orientation of Bees.* Cambridge, Massachusetts, Harvard University Press. 566 pp