Apis mellifera bees acquire long-term olfactory memories within the colony

Mariana Gil and Rodrigo J. De Marco†‡

Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, C1428EHA, Buenos Aires, Argentina

Author for correspondence (rjdm02@yahoo.com.ar)

Present address: Freie Universität Berlin, Fachbereich Biologie/Chemie/Pharmazie, Institut für Biologie, Neurobiologie, König-Luise-Strasse 28-30, 14195 Berlin, Germany

Early studies indicate that Apis mellifera bees learn nectar odours within their colonies. This form of olfactory learning, however, has not been analysed by measuring well-quantifiable learning performances and the question remains whether it constitutes a ‘robust’ form of learning. Hence, we asked whether bees acquire long-term olfactory memories within the colony. To this end, we used the bee proboscis extension response. We found that within-the-nest bees do indeed associate the odour (as the conditioned stimulus) with the sugar (as the unconditioned stimulus) present in the incoming nectar, and that the distribution of scented nectar within the colony allows them to establish long-term olfactory memories. This finding is discussed in the context of efficient foraging.

Keywords: Apis mellifera; olfactory learning; proboscis extension response (PER); conditioning; nectar foraging

1. INTRODUCTION
Already Karl von Frisch (1946) has reported evidence indicating that Apis mellifera bees learn nectar-related olfactory cues within their colonies. This form of learning, however, has not been analysed by measuring well-quantifiable learning performances. Instead, it has been inferred from the ensuing choice behaviour of the animals (Von Frisch 1946, 1965). Thus, if bees acquire specific olfactory memories within the colony, the question remains whether these are short- or long-term memories (Menzel 1999). This distinction might have important implications for both foraging and pollination. We asked whether bees acquire long-term olfactory memories within the colony. To this end, we took advantage of the classical olfactory conditioning of the bee proboscis extension response (PER conditioning; Takeda 1961; Bitterman et al. 1983). Our experimental approach was straightforward; we first presented a group of foragers with scented sugar solution during a flowering-like foraging period. Next, within-the-nest bees were randomly collected from the hive and tested for long-term olfactory memories derived from the odour diluted in the offered reward. We then found that both foragers and younger bees had already established long-term olfactory memories, even when they had never foraged on the training feeder. The relevance of this finding is discussed in the context of efficient foraging.

2. MATERIAL AND METHODS
A colony of Apis mellifera ligustica (Spinola) bees (without a queen) was obtained from a larger colony (henceforth, original hive) placed indoors in a one-frame observation hive. Foragers were marked and trained to collect 1.8 M scented (i.e. 50 μl of pure 1-nonanol) sucrose solution from an artificial feeder placed 15 m away from the hive. The feeder offered 50 μl of sugar solution per minute during four different foraging periods distributed over four successive days (one foraging period per day). Each foraging period began at 9.00–11.00 h and lasted approximately 270 min. Twenty-four hours after the end of the latest foraging period, unmarked bees (i.e. animals that had never foraged on the training feeder; henceforth, test bees) were randomly collected from the inner observation hive. A second group of bees (henceforth, control bees) was simultaneously collected from the original hive (i.e. descendents of the same queen). Bees from this colony had never foraged on the training feeder. Animals from both groups were restrained in metal harnesses. Each animal could freely move its antennae, mandibles and proboscis (Bitterman et al. 1983). Once fixed in the harnesses, they were placed in racks in a dark humidified chamber. On the evening following capture, they were fed up to satiation (unscented 1.8 M sucrose solution) and kept inside the chamber until tested.

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3. RESULTS
An increasing number of marked foragers collected a total amount of 43 ml of sugar solution during the 4 successive foraging days (18 h). Test bees were presented with the CS via the nectar crops of the marked foragers. We then compared the learning performances of two different groups of animals (test bees and control bees) that had been exposed, or not, to the scented 1.8 M sucrose solution previously offered at the feeder. Figure 1 shows the % PE obtained for each group. Results show a higher % PE for the test bees (67.6% and 2.2% for the test bees and the control bees, respectively; G(1) = 105.4, p < 0.0001, n = 192, G-test).

4. DISCUSSION
We found that test bees (see above) showed a high percentage of CRs (figure 1). According to the temporal dynamics of memory formation after PER conditioning (Menzel 1999), they exhibited already consolidated olfactory memories. Hence, our results indicate that within-the-nest bees associate the odour (CS) with the sugar (US) present in the incoming nectar and that the distribution of scented nectar within the colony allows them to establish a robust form of learning under natural conditions. In a honeybee colony, 75% of the whole population corresponds to young bees involved in different within-the-nest tasks (i.e. food-receivers, nurse and guard bees; Seeley 1995). According to the observed percentage (68%) of CRs, it is reasonable to assume that both foragers and younger bees acquired long-term olfactory memories, though the relative proportions of both groups remain unknown and their particular learning performances cannot be compared. Indeed, both foragers and younger bees learn olfactory cues under controlled laboratory conditions (Ray & Ferneyhough 1999; Ichikawa & Sasaki 2003).

It has been recently reported that trophallaxis, the exchange of liquid food by mouth (Wilson 1971), allows bees to learn nectar scents and leads to long-term olfactory memories under controlled laboratory conditions, i.e. bees associate the odour (CS) with the sugar (US) present in the nectar they receive by means of trophallaxis (Gil & De Marco 2005). Trophallaxis most likely also underlies acquisition of long-term olfactory memories within the colony, mainly because of two reasons: (i) the nectar collected in the field is rapidly distributed among colony members via trophallaxis (Wilson 1971) and (ii) bees inexorably perceive nectar odours during trophallaxis (Gil & De Marco 2005). Moreover, olfactory conditioning in honeybees strongly depends upon CS–US contingency (Bitterman et al. 1983; Menzel 1999). Thus, if that were the case, analysis must still be done upon the effects of CS and US intensity (Gil & De Marco 2005) on the olfactory learning occurring within the colony. Furthermore, both the rate and the duration of the whole CS–US stimulation provided by a given nectar source might determine the strength of the association achievable at the colony level. In addition, the area where most trophallaxes occur is relatively large and contains other bees, so that foragers and receivers need to search for a partner, usually antennating several other bees before a partner is found (Seeley 1995). Interestingly, unfamiliar odours present in the mouthparts of a possible partner decrease the occurrence of trophallaxis (Gil & Farina 2003). Moreover, familiar odours seem to elicit trophallaxes when foragers face increased resource uncertainty (De Marco & Farina 2003). If the perception of nectar odours affects the occurrence of trophallaxis, both foragers and receivers might benefit from learned odours in searching for a transfer partner, eliciting trophallaxis or even avoiding it. This might have important implications in the organization of foraging within the colony. According to this hypothesis, however, the effect of a given CS must be closely related to its relative prevalence inside the nest (as calculated from the relative intake rates of the different types of nectar being simultaneously exploited by the colony) in order to provide a given group of foragers and receivers (experiencing an increasing cohesion based on the type of nectar they exchange) with the necessary plasticity to gradually ‘move’ their interactions into a different group.

Although the colony simultaneously exploits different flower species, individual bees tend to forage on a single flower species (Von Frisch 1965; Seeley 1995; Chittka et al. 1999). This kind of flower fidelity seems to improve individual foraging strategies by reducing search and handling times (Heinrich 1975; Keban & Baker 1983). Presumably, both the recognition of specific flowers (which involves learning) and their manipulation are sharpened during flower fidelity, enhancing the rate of nectar gathering (Heinrich 1975). Indeed, bees not only exhibit flower fidelity, but also benefit from olfactory (and visual) long-term memories acquired during foraging in order to optimize their choices (Von Frisch 1965; Chittka et al. 1999; Menzel 1999). Olfactory memories established within the colony might play a critical role during the early development of flower fidelity. This means, for instance, that currently unemployed foragers (and even non-experienced foragers) might benefit from a highly prevalent olfactory CS present
within the colony in order to elicit their later foraging bouts. According to this hypothesis, the higher the rate of encounter with the rewarded CS the higher the probability of flying out to search for the prospective nectar source. This might, in turn, enhance the rate of nectar gathering during flowering, particularly in the case of flower species in which flowering begins abruptly and diminishes (Rathcke & Lacey 1985). Moreover, the flowering periods of most species of plants do not allow closing species-specific foraging fidelity and colonies must track different blossoms throughout the season (Heinrich 1975; Seeley 1995). It would be interesting to investigate how these olfactory memories are integrated into the context of the foraging task, especially when two or more combinations of CS–US stimuli are simultaneously perceived within the colony. This might have important implications for the study of foraging and pollination.

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