Context cues eliminate retroactive interference effects in honeybees *Apis mellifera*

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Summary

Free flying honeybees were trained successively on two different tasks of landmark-based spatial memory. On both task 1 and task 2, the goal was at a consistent distance and direction from a cylindrical landmark. The colours of the landmarks differed for the two tasks. The target direction from the landmark in task 2 was opposite to that in task 1. The context in which task 2 took place was either the same as the task-1 context or different: being a short distance away, having different surrounding landmarks, and a different colour on the training table. After each task, the bees were tested on task 1 in the task-1 context (test 1 and test 2). If task 2 had the same context as task 1, the bees performed at chance on test 2. If task 2 had a different context, performance on test 2 was unaffected, remaining as good as on test 1. Contextual cues thus guide memory retrieval, and prevent any confusions about which response (that appropriate for task 1 or for task 2) to perform.

Key words: bees, context, interference, response competition, landmark, spatial memory.

Introduction

Foraging honeybees can learn many things (Menzel and Giurfa, 2001) and thus have many memories. In foraging, they use multiple memories to navigate (Cheng, 2000; Collett and Collett, 2002). They must retrieve the right memory at the right time. Context is thought to help both learning and memory retrieval (Collett et al., 2003). By context is meant a suite of cues surrounding the time and place in which a task takes place. It includes the physical surroundings and the route a bee has taken to get there, outdoors (honeybees: Collett and Kelber, 1988; Collett et al., 1997) and indoors (honeybees: Collett and Baron, 1995; Collett et al., 1996; bumblebees: Colborn et al., 1999; Fauria et al., 2000, 2002), the time of day (Koltermann, 1969, 1971; Wahl, 1932), and motivational state (going out to forage *vs* heading home with forage; Dyer et al., 2002). Under some circumstances, the bee can readily learn diametrically conflicting patterns of responding in two different contexts (Colborn et al., 1999), but not under other conditions (Fauria et al., 2002). In particular, if the two tasks (in the two contexts) are learned in succession, little interference between tasks is found. But if the two tasks are learned simultaneously, much interference is found (Fauria et al., 2002).

In recent work, we created situations in which honeybees exhibited confusions as to which memory to use, or response competition. Cheng and Wignall (submitted) presented two tasks in succession to honeybees (task 1 and task 2), and tested for performance on task 1 after both tasks (test 1 and test 2).

A large performance decrement on test 2, to chance levels, was found if, and only if, task 2 had a directly conflicting response to task 1. Cheng and Wignall concluded that response competition was a major cause of the interference effect, rather than memory erasure or suppression. Both memories (for task 1 and task 2) were intact, but the bee was uncertain as to which memory to exhibit on the test 2.

These experiments examined whether a change of context for task 2 can serve to eliminate this performance deficit. Honeybees were tested outdoors under seminatural conditions. Their task was to search in a particular direction near a single landmark set on a table. In the two tasks, the landmark colour differed, as did the target direction. Crucially, bees either learned task 2 in the same context as task 1, or in a different context. Tests (on task 1) took place in the task-1 context. The second, different context was only a short distance from the first context, and thus did not differ greatly. Nevertheless, landmarks near the two contexts were quite different, and the test tables in the two contexts differed in colour. If context is associated with the task learned, and serves as a retrieval cue, a change in context in task 2 should eliminate ambiguity about which response to exhibit in each context. This should in turn prevent any response competition from arising. Bees for whom the context for the two tasks were identical should behave at chance on test 2, replicating the results of K. Cheng and A. Wignall (manuscript submitted for publication).

Materials and methods

Animals

Free flying honeybees (*Apis mellifera* L.) were maintained in a hive kept outdoors at Macquarie University. Potential subjects came to a feeding station near the test table offering sugar water. Experimental animals were recruited from the feeding station. Each subject was individually trained and tested, and was painted for identification. Stranger bees that were not subjects but that occasionally found their way to the experimental table were captured and removed for the duration of the day's experimentation.

Set Ups

For each experiment, two contexts were created a short distance apart; the distance had to be short in order to lure the bees from one context to the other. Experimentation took place just outside a house, and directions (left, right, near, far) are described as viewed from the door of the house. Although close, the two locations differed greatly in surrounding landmarks (Fig. 1). Location 1, used in both experiments, was in between two very large and tall coniferous trees, each less than 1 m apart. The other location was right by the wall of the house. In experiment 1, it was to the right of the door, ~120 cm away from location 1 (measuring from nearest edges). In experiment 2, the second location was a little farther away, ~220 cm from location 1, to the left of the door, to provide some variation across experiments. Experiment 1 used the same table, measuring 90.5×75.5×70.5 cm high, for both contexts; it was moved between tasks. The length of the table ran left–right between the trees, the length of the table ran near–far by the wall. The table was covered by a gridded cardboard (5.2 cm squares drawn lightly in pencil) marked with wiggly lines made with a felt-tipped pen. The markings allowed the bees to stabilise their flights in searching over the table. The cardboard was in turn covered by clear plastic (from Lincraft Pty, Macquarie Centre, North Ryde, Australia; transparent to all wavelengths of light including those in the ultraviolet range) and secured with clips to the ends of the table. The colours of the cardboard and markings differed in the two contexts, white and yellow, respectively, in one, and yellow and pink, respectively, in the other. All combinations of locations and table coverings were used.

Experiment 2 used two different tables at the two locations. The table used in experiment 1 was used, also at location 1. It was covered with the white cardboard. The table by the wall of the house measured 68.5×60.5×71 cm high, with the long axis perpendicular to the wall. It was covered with yellow cardboard. Each context always had the same colour of cardboard because this factor did not produce any significant effects in experiment 1. Both tables stayed in place during the experiment. But for each task, only the table on which the bees were being trained was set up with cardboard; the unused table was left bare.

Landmarks were cylinders covered with light green (task 1) or light blue (task 2) cardboard paper (9 cm in diameter and height). The target for the bees was a 10.4 cm red cardboard

Fig. 1. Photograph of experimental locations used for contexts. Location 1 was used in both experiments. Location 2, the location of the other context, differed between experiments. At location 2, experiment 1, the table shown is not the correct size. The table shown at location 1 was also used for the other context in experiment 1 (moved between tasks). Location 2, experiment 1 was by a similar wall with windows, like location 2, experiment 2. None of the tables have the coverings used for experimentation. See Materials and methods for further description.

square laminated in clear plastic. The landmark was centred at an intersection of grid lines. The cardboard was one unit away, making its centre 11.1 cm away from the edge of the landmark. A translucent bottle cap (2.5 cm diameter, 0.5 cm high) holding the reward (two parts sugar to three parts water) stood on the centre of the target cardboard. During pre-training, a white cardboard circle (3.5 cm in diameter) was placed under the target bottle cap; this was removed by the time task 1 began. Across trials during training, the set up on the table was translated on the table. Thus, the target–landmark spatial relation remained constant, but the target location in an Earth-based framework changed. This forced the animals to use the landmark–target array for exact localisation.

Design and analysis

The two experiments had the same basic design. Each animal was trained on two different landmark tasks. In task 1, the landmark was green. In task 2, the landmark was blue, and the target was on the opposite side to where it was in task 1. After each task, the bee was tested on task 1, in the task-1 context (test 1 and test 2). The crucial factor (between-subjects) was the relation between the contexts for task 1 and task 2 (sameness of task contexts: either the same or different). Other between-subjects factors that entered into analysis (mixed analysis of variance) in experiment 1 included target location in task 1 (to the right or to the left of the landmark), the context location of task 1, and the table covering for task 1 (white or yellow). Other between-subjects factors that entered into analysis in experiment 2 were target location in task 1 and the

context location of task 1. Results concerning these other between-subjects factors will not be reported, as they produced no significant statistical effects. Test was a within-subject factor. Alpha level was set at $P=0.05$.

Procedure

In pre-training, the landmark–target array was at first set up in one place on the table. The feeder at the feeding station was briefly removed and replaced by a bottle cap full of sugar water. When a bee landed on the bottle cap and began imbibing, it was moved (with the cap) to the test table and placed on the target red cardboard. Once a bee had found the reward three times unassisted, the set up was moved randomly around the table from trial to trial. After two such trials, the white circle was removed from underneath the lid. A further three trials were given before training began.

During training, the target bottle cap was filled with 15 µl of sugar water for a trial. The bee would empty the feeder and fly from the table. The training array was moved to a new position on the table, and the target was refilled with another 15 µl of sugar water. The bee was free to find the reward again, which counted as a second training trial. A maximum of four feeding bouts were allowed per visit; on the fourth trial, the target bottle cap was full of sugar water, and the bee drank her fill and flew home. Most of the time, a subject bee would partake in the maximum number of trials allotted on a visit. On each task, each bee was given 10 trials of training.

On a test, four fresh pieces of 10.4 cm red cardboard were placed on four sides of the green (task 1) landmark, each with an empty bottle cap on it. Tests were 2 min long, timed from the arrival of the bee at the table. After test 1, task 2 began immediately. During each 5 s period of the test, marked by a beeping stopwatch, the experimenter scored whether the bee hovered over or landed on each cardboard. In scoring the data, a location was given 1 point for a hover, and 2 points for a landing. The dependent variable was the score for the target divided by the total score, equivalent to the proportion of searching at the target, with chance level at 0.25. The total score over all four areas, reflecting the amount of searching, was also noted and analysed.

Two experimenters tested this method for reliability, scoring a number of tests independently. One of the scorers was blind to the training condition of the bee. Over all locations and all tests, scores from the two scorers correlated (Pearson product moment) 0.92. The test method thus proved reliable.

Experiment 1 had 32 animals, but only data from animals whose test-1 performance exceeded 0.30 ($N=18$) were used for analysis. Experiment 2, which provided a fuller replication, had 32 animals who scored >0.30 on test 1. Bees whose performance did not meet this criterion (nine of them) were excluded, and replaced by another.

Results

Experiment 1

An analysis of variance was first conducted on the data of

all animals, including all counterbalancing factors (target location in task 1, context location of task 1, table covering for task 1). This analysis produced no significant effects. A second analysis was then conducted on the data of animals whose task-1 performance exceeded 0.30 ($N=18$). Sameness of task contexts, target position in task 1, and test were factors in the analysis. Results shown in Fig. 2 indicate that performance on test 2 dropped to near chance level when the contexts of the two tasks were the same, but performance remained good when the contexts of the two tasks were different. The analysis of variance found a significant main effect of test ($F_{1,14}=10.33$, $P=0.006$) and, importantly, a significant interaction of test with sameness of task contexts ($F_{1,14}=5.43$, $P=0.035$). Distribution of searching over all locations are shown in Table 1. For bees that learned the two tasks in the same context, performance on test 2 was distributed evenly across all locations, a pattern found by K. Cheng and A. Wignall (manuscript submitted for publication) as well. For bees that learned two tasks in different contexts, the proportion of searching in the opposite location (appropriate location for task 2) seemed to increase on test 2. But this result is not replicable with a larger sample (see results for experiment 2 and Table 2).

The total score on a test, reflecting the amount of searching, is shown in Fig. 3 for the bees whose test-1 performance exceeded 0.30. The amount of searching dropped on test 2 in bees who learned the two tasks in different contexts. This was confirmed with an analysis of variance with the same factors (sameness of task contexts, target position in task 1, and test), which found a significant main effect of test ($F_{1,14}=12.12$, $P=0.004$) and a significant interaction of test with sameness of task contexts ($F_{1,14}=6.52$, $P=0.023$). On test 2, bees that learned the tasks in different contexts were observed to fly frequently from one context to the other. In sum, if the context changed in task 2, performance continued to be good, but amount of searching on test 2 decreased. If task-2 context is the same as task-1 context, the amount of searching was maintained on test 2, but performance was near chance level.
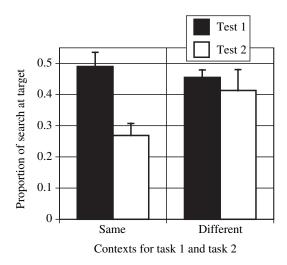

Fig. 2. The proportion of searching at the target in experiment 1, out of searching at four locations (mean ± S.E.M.). Chance level is 0.25.

Table 1. *Searching behaviour of bees in experiment 1*

Location	Contexts	Test 1	Test 2
Target	Same	0.490±0.045	0.269±0.038
Opposite	Same	0.135±0.048	0.256±0.038
Other 2	Same	0.375±0.042	0.475±0.047
Target	Different	0.455±0.024	0.413±0.067
Opposite	Different	0.142±0.028	0.233±0.053
Other 2	Different	0.402±0.020	0.354±0.045

Data shown are the mean ± S.E.M. of the proportion of searching at the target location, the opposite location, and the other two locations in experiment 1. The opposite location was the target location for task 2. The scores for the other two locations have been added together. Contexts refer to whether the contexts for task 1 and task 2 were the same or different.

Table 2. *Searching behaviour of bees in experiment 2*

Location	Contexts	Test 1	Test 2
Target	Same	0.412±0.019	0.280±0.019
Opposite	Same	0.180±0.082	0.257±0.020
Other 2	Same	0.408±0.028	0.463±0.019
Target	Different	0.430±0.023	0.404±0.039
Opposite	Different	0.159±0.021	0.172±0.026
Other 2	Different	0.411±0.016	0.423±0.037

Data shown are the mean ± S.E.M. of the proportion of searching at the target location, the opposite location, and the other two locations in experiment 2. The opposite location was the target location for task 2. The scores for the other two locations have been added together. Contexts refer to whether the contexts for task 1 and task 2 were the same or different.

Experiment 2

Experiment 2, with a larger subject pool, replicated the pattern of results found in experiment 1. On the proportion of searching at the target (Fig. 4), performance on test 2 dropped to near chance level if the contexts for the two tasks were the same. Performance on test 2 was maintained if task 2 had a different context from task 1. An analysis of variance found significant main effects of sameness of task contexts ($F_{1,24}=6.21$, $P=0.020$) and test ($F_{1,24}=12.57$, $P=0.002$), and, importantly, a significant interaction of sameness of task contexts and test ($F_{1,24}=5.66$, $P=0.026$). Full search distributions are shown in Table 2. If the two tasks were learned in the same context, searching on test 2 was evenly distributed across all locations. If the contexts for the two tasks were different, performance on test 2 resembled performance on test 1. If errors are distributed randomly across the three non-target locations, searching at the opposite location should be about half as much as searching at the other two locations combined. On both tests, searching at the opposite location to the target amounted to slightly less than half of searching at the other two locations. This pattern might be expected because the other two locations are closer to the target location than the opposite location is.
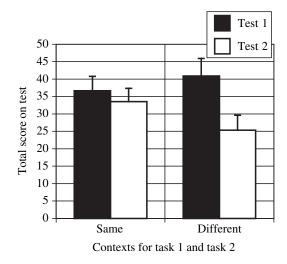

Fig. 3. The amount of searching, measured by total score on a 2 min test, in experiment 1 (mean ± S.E.M).

Total scores, reflecting the amount of searching on a test (Fig. 5) again dropped if the two task contexts were different, but remained at similar levels if the two task contexts were the same. An analysis of variance found significant main effects of sameness of task contexts ($F_{1,24}=7.29$, $P=0.013$) and tests ($F_{1,24}=21.53$, $P<0.001$), and a significant interaction of sameness of task contexts and tests ($F_{1,24}=26.54$, $P<0.001$). On test 2, bees that learned the tasks in different contexts were observed to fly frequently from one context to the other.

Discussion

The results of the experiments are clear and consistent. Bees were trained in two different landmark tasks in succession. The target response (direction with respect to the landmark) conflicted in the two tasks. When task 2 took place in the same context as task 1, the bees responded at chance on test 2, replicating findings of K. Cheng and A. Wignall (manuscript submitted for publication). When the contexts for the two tasks were different, task-2 training did not affect the level of performance on test 2 at all. After learning task 2, the bees performed as well on test 2 as they did on test 1, which took place immediately after task-1 training.

Task 2 in a different context did have one notable effect on behaviour on test 2. The total amount of time that the bees searched around the landmark decreased significantly. This result has a ready explanation. Because task 2 took place in a different context, the bees were attracted to the location of that context, thus resulting in less searching at the task-1 context, where test 2 took place. This is probably response competition at another level. The bees were unsure as to which context to visit. Recent history favoured the task-2 context, but cues for reward (the table covering, the landmark, and the red cardboards) were found at the task-1 context. More experimentation, however, along the lines of Cheng and Wignall's manipulations (K. Cheng and A. Wignall,
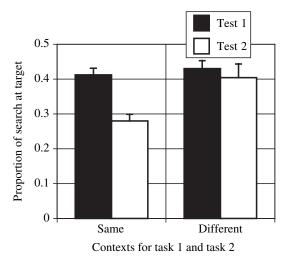
Fig. 4. The proportion of searching at the target in experiment 2, out of searching at four locations (mean ± S.E.M). Chance level is 0.25.

Fig. 5. The amount of searching, measured by total score on a 2 min test, in experiment 2 (mean ± S.E.M).

manuscript submitted for publication; discussed below) are needed to confirm this explanation.

Turning to the search performance, consider first the decrement on test 2 when the two task contexts were the same. K. Cheng and A. Wignall (manuscript submitted for publication) found such results in several experiments. They also manipulated conditions of training and testing to alleviate this deficit. When task 2 had the same response requirement as task 1 (that is, with the target at the same direction from a differently coloured landmark), no deficits on test 2 were observed. Likewise, when task 2 had a different basis for responding (based on colour rather than position) and thus presented no response conflicts, no interference effects were found. Finally, when the 'wrong' response (the conflicting one appropriate for task 2) was removed as much as possible (by placing the test set up at the appropriate edge of the table), the interference effects, though not completely eliminated, were much reduced. Their interpretation was thus that both memories (for task 1 and task 2) were retained, but the bee exhibited confusion as to which response to perform – response competition in short.

The new results in these experiments, obtained when the task-2 context was changed, show that contextual cues prevent this response confusion or competition from arising. When retested on task 1 in the task-1 context after task 2, the bees were as clear as before task 2 as to which response to perform. The results, together with those of K. Cheng and A. Wignall (manuscript submitted for publication), pinpoint the role of context in the retrieval of appropriate memories, in addition to learning and encoding. They suggest that while the bee encodes many memories, it relies on contextual cues for retrieving the right one to use.

The manipulations of context allow us to identify the contextual cues at play in the experiments. The contexts were near one another, so that the foragers would travel roughly the same global route to the test site. The motivation of the
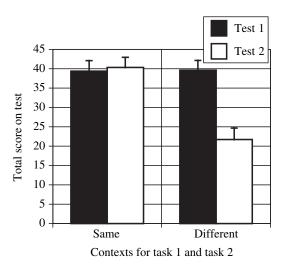
foragers were identical at the two contexts: they were out to gather nectar. The time of training for the two tasks differed, as one followed the other. But this difference favoured the wrong response on test 2, that appropriate for task 2 rather than task 1. What differed across the two contexts most was probably the physical surrounding. The landmarks surrounding the test tables in the two sites differed (Fig. 1), even if the small distance between the two contexts meant some similarities. And the colour on the test table differed in the two contexts. The results strongly implicate surrounding visual cues as contextual cues used for memory retrieval. Bees have been shown to use surrounding contextual cues in tasks with sensorimotor response requirements, both in a lab (Collett and Baron, 1995; Collett et al., 1996) and outdoors (Collett et al., 1997), and in landmark-based search tasks (Collett and Kelber, 1988). Searching in a particular direction from a landmark is another task that is similarly context sensitive. Whereas bees in the studies of Collett and colleagues learned the two tasks in alternation, the bees in this study learned the two tasks in two blocks. Context thus probably helps retrieval no matter what the learning arrangements.

Context also plays a role in vertebrate memory retrieval, and retrieval failures have been implicated in many cases of performance failure (Bouton, 1993; Kraemer and Golding, 1997; Zentall, 1997; Roberts 1998; Bouton et al., 1999). Aside from contextual cues located in the current spatial surround, Bouton and colleagues have proposed that the passage of time itself creates contextual change, and can serve as a contextual cue. They were referring to absolute time elapsed since a memorial episode, rather than cyclic (e.g. circadian) time. Circadian time is known to serve as a contextual cue for memory in vertebrates (e.g. Biebach et al., 1989) and invertebrates (Koltermann, 1969, 1971; Wahl, 1932). We are currently investigating the passage of time as a contextual cue in honeybees.

In animal memory, the role of context in learning and in

organising and retrieving memory might be widespread. Performance failures may often be retrieval failures of some kind. Context helps to alleviate such failure. Understanding the neurophysiological mechanisms that allow contextual cues to help memory retrieval will probably need to wait until we have a better understanding of the neurophysiological basis of memory storage. Given the extant behavioural data, neurophysiolgical accounts of both storage and retrieval may well be closely related.

References

Biebach, H., Gordijn, M. and Krebs, J. R. (1989). Time-and-place learning by garden warblers *Sylvia borin*. *Anim. Behav.* **37**, 353-360.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian conditioning. *Psychol. Bull.* **114**, 80-99.

Bouton, M. E., Nelson, J. B. and Rosas, J. M. (1999). Stimulus generalization, context change, and forgetting. *Psychol. Bull.* **125**, 171-186.

Cheng, K. (2000). How honeybees find a place: lessons from a simple mind. *Anim. Learn. Behav.* **28**, 1-16.

Colborn, M., Ahmad-Annuar, A., Fauria, K. and Collett, T. S. (1999). Contextual modulation of visuomotor associations in bumble-bees (*Bombus terrestris*). *Proc. R. Soc. Lond. B.* **266**, 2413-2418.

Collett, T. S. and Baron, J. (1995). Learnt sensori-motor mappings in honeybees: interpolation and its possible relevance to navigation. *J. Comp. Physiol. A* **177**, 287-298.

Collett, T. S., Baron, J. and Sellen, K. (1996). On the encoding of movement vectors by honeybees: are distance and direction represented independently? *J. Comp. Physiol. A* **179**, 395-406.

Collett, T. S. and Collett, M. (2002). Memory use in insect visual navigation. *Nature Rev. Neurosci.* **3**, 542-552.

Collett, T. S., Fauria, K., Dale, K. and Baron, J. (1997). Places and patterns – a study of context learning in honeybees. *J. Comp. Physiol. A* **181**, 343-353.

Collett, T. S., Fauria, K. and Dale, K. (2003). Contextual cues and insect navigation. In *The Neurobiology of Spatial Behaviour* (ed. K. J. Jeffery), pp. 67-82. Oxford: Oxford University Press.

Collett, T. S. and Kelber, A. (1988). The retrieval of visuo-spatial memories by honeybees. *J. Comp. Physiol. A* **163**, 145-150.

Dyer, F. C., Gill, M. and Sharbowski J. (2002). Motivation and vector navigation in honey bees. *Naturwissenschaften*, **89**, 262-264.

Fauria, K., Colborn, M. and Collett, T. S. (2000). The binding of visual patterns in bumblebees. *Curr. Biol.* **10**, 935-938.

Fauria, K., Dale, K., Colborn, M. and Collett, T. S. (2002). Learning speed and contextual isolation in bumblebees. *J. Exp. Biol.* **205**, 1009-1018.

Koltermann, R. (1969). Lern- und vergessensprozesse bei der honigbiene – aufgezeigt anhand von duftdressuren [Data on processes of learning and forgetting, gained from scent training of honey-bees]. *Z. vergl. Physiol.* **63**, 310-334.

Koltermann, R. (1971). 24-Std-Periodik in der Langzeiterrinerung an Duft- und Farbsignale bei der Honigbiene [Circadian memory rhythm after scent and colour training with honey-bees]. *Z. vergl. Physiol.* **75**, 49-68.

Kraemer, P. J. and Golding, J. M. (1997). Adaptive forgetting in animals. *Psychonomic Bull. Rev.* **4**, 480-491.

Menzel, R. and Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends Cogn. Sci.* **5**, 62-71.

Roberts, W. A. (1998). *Principles of Animal Cognition*. Boston: McGraw-Hill.

Wahl, O. (1932). Neue untersuchungen über das zeitgedächtnis der bienen [New investigations on memory for time in bees]. *Z. vergl. Physiol.* **18**, 709-717.

Zentall, T. R. (1997). Animal memory: the role of 'instructions'. *Learn. Motiv.* **28**, 280-308.