

## Conditioned responding to magnetic fields by honeybees

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Accepted February 25, 1985

**Summary.** Individual honeybees were trained in two experiments to come for sucrose solution to a target set on a shelf before an open laboratory window. On some visits, the target was presented in the ambient geomagnetic field, and on other visits in a field modified in the vicinity of the target by passing a direct current through a coil under the shelf. The target contained 50% sucrose when it was in one of the two fields and 20% sucrose when it was in the other. Tested subsequently with a pair of targets, one in the ambient field, one in the modified field, and both containing tap water, the animals significantly preferred the target in the field in which they had been given the 50% sucrose during training. Four modified fields, produced with different coils and currents, were discriminated equally well from the ambient field, and performance was as good when the 50% sucrose was given in the ambient field as when it was given in the modified field. Data are provided also to illustrate the excellent discriminative performance attainable when two targets are presented on each training visit – one in a modified field, the other in the ambient field – and choice of one is rewarded with 50% sucrose while choice of the other is punished with mild electric shock. Our results show that foragers attend to magnetic stimuli at the feeding site and that discriminative training techniques are appropriate for the study of magnetoreception and its mechanism in honeybees.

### Introduction

Studies of orientation in honeybees have revealed four reproducible effects of magnetic fields on their behavior. (1) small, systematic errors in the

direction with respect to gravity given by forager bees dancing on a vertical surface – a phenomenon known as missweisung or residual misdirection (von Frisch 1967) that fades away in zero magnetic fields (Lindauer 1977); (2) orientation of horizontal dances towards the cardinal points of the magnetic compass in the absence of visual cues (Lindauer and Martin 1972); (3) magnetic orientation of comb-building by new swarms (Lindauer and Martin 1972); and (4) use of periodic information in the geomagnetic field for circadian orientation (Martin and Lindauer 1977; partially replicated by Gould 1980). Sensitivities to magnetic field direction of 2–9° and to magnetic field intensity of 1–10 nT (nanoTesla = gamma or 10<sup>-5</sup> Gauss) have been inferred from these results (Lindauer 1977; Martin and Lindauer 1977; Gould et al. 1980).

Magnetic orientation experiments with honeybees have several drawbacks. First, they depend on large numbers of observations, and the data generally exhibit considerable scatter (e. g. De Jong 1982). Second, time lags of from 40–60 min to days or weeks occur before responses to magnetic field changes are detected. Third, comb-building and circadian rhythm experiments depend on the responses of very large groups of bees. Towne and Gould (in press) have suggested that to advance our understanding of the magnetic sense of honeybees, the magnetic field must be experimentally manipulated, with each relevant parameter of the field controlled separately. To this suggestion we are inclined to add a further one (based on the drawbacks of orientation experiments) which is that the measure of behavior used should detect the responses of individual bees to magnetic field stimuli directly.

Fortunately, the honeybee is a highly suitable subject for such studies and, inspired by the early work of von Frisch, a variety of efficient training techniques have been developed that can be used

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for the analysis of its discriminative capacities (Menzel 1968; Klosterhalfen et al. 1978; Couvillon and Bitterman 1980, 1982; Sigurdson 1981; Bitterman et al. 1983; Couvillon et al. 1983; Menzel and Bitterman 1983). The technique used here was the following: On some visits to the laboratory, a forager was offered a drop of 50% sucrose solution on a target situated in one of two magnetic fields – either the ambient Hawaiian field or a field that was modified in the vicinity of the target. On other visits, a drop of 20% sucrose solution was offered on a target situated in the alternative field. Then there was a 10-min test with two targets – one in the ambient field, the second in the modified field, and each containing a drop of tap water – during which contacts with the targets were measured. A recent experiment of this kind with targets differing in color and odor showed a clear preference for the one that had previously contained the 50% sucrose solution (Couvillon and Bitterman 1984).

### Materials and methods

The subjects were 40 honeybees, *Apis mellifera*, pretrained individually to fly from the hive to the laboratory and drink to repletion from a large drop of 50% sucrose solution on a target that was set on a shelf, 40 cm wide and 40 cm deep, before an open window. An animal was selected at random from a group of foragers at a feeding platform equipped with a large jar of 12%–15% sucrose solution, carried in a matchbox to the laboratory, set down on the target, permitted to drink its fill of the 50% solution (during which time it was marked with a spot of colored lacquer), and then allowed to fly to the hive. Typically, the animal returned to the laboratory of its own accord in a few minutes and continued thereafter to shuttle back and forth between the hive and the laboratory as long as food was available. If the marked bee did not return to the laboratory after the first placement, it was picked up again at the feeding platform (where it usually could be found), carried back to the laboratory in the matchbox, and set down on the target once more. This procedure was repeated until the bee returned to the laboratory of its own accord.

The target was a covered petri dish of clear plastic, 5.5 cm in diameter, which was sprayed with flat gray paint. It was washed and replaced after each visit from a pool of identical targets in order to randomize extraneous stimuli. On each visit, the target occupied one of two positions on the shelf, 20 cm apart on a line parallel to the opening of the window, with position varying from visit to visit in balanced quasi-random order. The ambient geomagnetic field at each position could be modified by passing a direct current of stated amperage through a 100-turn coil mounted directly beneath the shelf, its center coinciding with that of the target. In the first experiment to be reported, with a single group of eight subjects, the coil was 15 cm in diameter and the current 0.6 A. In the second experiment, four groups of eight subjects each were trained, one with the 15-cm coil and 0.6 A as in the first experiment, a second with the 15-cm coil and 0.4 A, a third with a coil that was 1 cm in diameter and a current of 0.6 A, and a fourth with the 1-cm coil and 0.4 A. The training in each case was with the ambient field

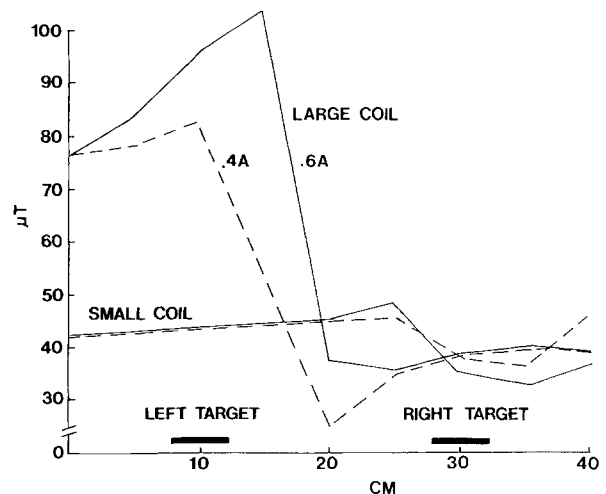


Fig. 1. Total intensity in microTesla ( $\mu\text{T}$ ) along a 40-cm line through the magnetic fields encountered with 0.4 or 0.6 A through the large coil on the left or the small coil on the right. The two target-positions are indicated

vs the field as modified by one of the coil-current combinations. For half the animals in each group, 50% sucrose was offered in the ambient field and 20% in the modified field, while the opposite relation obtained for the remaining animals.

In the pretraining, 50% sucrose was presented under the field condition (ambient or modified) to be associated with 50% sucrose in the discriminative training. The discriminative training itself consisted of 10 visits, on each of which a single target with sucrose solution of the appropriate concentration was presented, five times in the ambient field and five times in the modified field. An alternating sequence of the two fields was used, with position varying quasi-randomly as in the pretraining. On the test visit, two fresh targets were presented for a period of 10 min, one target in the ambient field at one position and a second target in the altered field at the other, with position balanced across subjects and conditions; that is, of the four subjects trained with 50% sucrose in the ambient field, two found the ambient field on the left and two found it on the right, and of the four subjects in each group that were trained with 50% sucrose in the modified field, two found the modified field on the left and two found it on the right. In the test, both targets contained tap water (unacceptable to the animals), which was sampled repeatedly. Responses to each of the targets (defined as direct contacts) were recorded on event-counters that printed the number of responses in successive 30-s intervals. At the conclusion of the test, the bee was destroyed, and the experiment was continued with a new bee chosen from among the foragers at the feeder.

In Fig. 1, total intensity (measured with a Develco three-axis fluxgate magnetometer) along a line through the two target-positions is plotted for four of the eight modifications of the ambient field that were employed: lower and higher current in the large coil beneath the left target and the same for the small coil beneath the right target. The fields in the region of the targets, whose positions also are indicated in the figure, are characteristic of dipole field anomalies. The pattern arises because the symmetrical field produced by the coil adds to the background field on one side and subtracts from it on the other. We had little to guide us in the choice of the coils and currents selected for trial except the idea that it might be best in exploring for evidence of learning about magnetic stimuli to produce several anomalies different in peak intensity and in pattern.

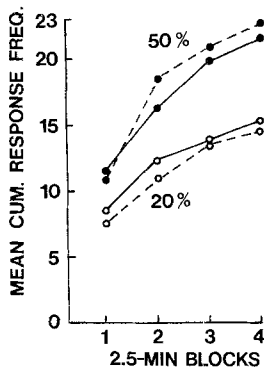


Fig. 2. Mean cumulative frequency of response to each of the two targets presented on the 10-min test visit in Experiment 1 (broken lines) and Experiment 2 (unbroken lines). The 50% curves show responses to the target in the field in which 50% sucrose had been given during training, while the 20% curves show responses to the target in the field in which 20% sucrose had been given. In the test, both targets contained tap water

## Results

Bees find a lower sucrose concentration unacceptable for a time after having been fed a higher concentration, and on visits to 20% sucrose, which always followed 50% sucrose, our animals showed a familiar pattern of disturbance before settling down to uninterrupted feeding (Bitterman 1976; Couvillon and Bitterman 1984). On the test trial, the disturbance was more marked and prolonged. The animal would taste the water on one of the targets, fly up, return to the same target or go to the other, fly up again, and so forth, with further contacts becoming progressively less frequent as the test continued. Both targets were visited repeatedly, but there was a clear preference for the target in the field in which the 50% sucrose had previously been found. In Fig. 2, test performance is plotted in terms of the mean cumulative number of contacts with each of the two targets, one in the old 50% field and the other in the old 20% field. The broken lines are for the results of Experiment 1 and the unbroken lines for the results of Experiment 2. The agreement between the results of the two experiments, which were conducted several months apart and in different laboratory locations, is noteworthy.

A *t*-test based on the data of Experiment 1 shows the preference for the old 50% field to be statistically significant ( $t = 2.79$  with 7 d.f.,  $P = 0.0269$ ). The test and its outcome are reported at the suggestion of a reviewer who thought that it would be more familiar to readers than analysis of variance. The more extensive analysis shows, not only a significant field effect ( $F = 46.09$ , 1 and 6 d.f.,  $P = 0.0005$ ), but a significant interaction

between fields and positions ( $F = 35.44$ , 1 and 6 d.f.,  $P = 0.001$ ). This interaction reflects the fact that the preference for the old 50% field was stronger when it was on the right than when it was on the left – for the two subjects that failed to show a preference for the old 50% field (which all the rest did), the old 20% field was in the preferred position. Analysis of variance shows also that the tendency for the over-all rate of responding to decline over the four 2.5-min time-blocks is significant ( $F = 16.22$ , 3 and 18 d.f.,  $P < 0.0001$ ), but that the tendency for response to the target in the old 20% field to decline more rapidly is not (for the interaction between fields and time-blocks,  $F = 2.78$ , 3 and 18 d.f.,  $P > 0.05$ ).

A *t*-test based on the data of Experiment 2 shows a significant preference for the old 50% field ( $t = 3.48$  with 31 d.f.,  $P = 0.0015$ ). Analysis of variance shows, not only a significant preference for the old 50% field ( $F = 13.30$ , 1 and 16 d.f.,  $P = 0.0022$ ), but, again, a significant interaction between fields and positions ( $F = 8.00$ , 1 and 16 d.f.,  $P = 0.0121$ ). The interaction was somewhat smaller in this experiment (which was done at a different laboratory window) and opposite in direction – the preference for the old 50% field was greater when it was on the left than when it was on the right; of the nine animals that failed to show a preference for the old 50% field, the 20% field was on the left for eight. The decline in over-all rate of responding over the four 2.5-min time-blocks is significant ( $F = 70.04$ , 3 and 48 d.f.,  $P < 0.0001$ ), and the decline is significantly greater in the old 20% field than in the old 50% field (for the interaction between fields and blocks,  $F = 3.80$ , 3 and 48 d.f.,  $P = 0.0159$ ). The results for all 32 animals are pooled in the curves for Experiment 2, because none of the interactions of the main variables is significant: Preference for the target in the old 50% field was no better when it was the modified field than when it was the ambient field ( $F < 1$ ), no better for one coil than for the other ( $F = 1.90$ , 1 and 16 d.f.,  $P > 0.05$ ), no better for one intensity than for the other ( $F < 1$ ), and no better for any of the four possible coil-intensity combinations than for any other ( $F < 1$ ). In each case, there was the same substantial, if not overwhelming, preference for the target in the field in which the animals had previously been fed the sucrose of higher concentration.

## Discussion

While these experiments are of interest primarily because they demonstrate the usefulness of

discriminative training techniques for the study of magnetic sensitivity in honeybees, they are of interest also because they provide the first evidence that honeybees pay attention to magnetic fields during foraging. Unlike bee dance experiments, these experiments were with changes in the magnetic field that were restricted to the feeding area, that were correlated with the acceptability of the foods encountered in the laboratory but not with events occurring between visits to the laboratory, and that were experienced only for short periods (1–2 min/visit). In contrast, magnetic influences on the vertical and horizontal dances of honeybees appear to be unrelated to events experienced during foraging for two reasons. First, the experimental manipulations take place in the hive, where dancing occurs, rather than where the bees feed. Second, although the animals conceivably might translate directional information from a variety of sources available during foraging into a magnetically oriented horizontal dance, the magnetic directions given by foragers dancing on horizontal surfaces are unrelated to the directions of the food sources they have visited (Towne and Gould, in press).

A rather unexpected outcome of our factorial experiment is that the different modifications of the ambient field produced by the two coils and the two currents – especially those produced by the two coils – were equally discriminable. The possibility that even the least discriminable of the modifications is large enough to permit the best performance the technique is capable of yielding can be rejected on the basis of the much better performance obtained with differences in color and odor (Couvillon and Bitterman 1984). It seems more reasonable to suggest that the fields produced by both coils are difficult to discriminate and equally so, perhaps because of a tradeoff between field intensity and the areal extent of the anomaly produced by each coil. From this point of view, better performance might be expected with a marked change of field strength in the immediate vicinity of the target. In experiments designed to provide a detailed map of the honeybee's magnetosensory capabilities, it would be possible with a simple proximity detector to change a large uniform field surrounding the target in a prescribed manner only when the bee is near the target.

As yet, however, we are not satisfied that we have hit upon the most efficient training technique, and we are exploring a variety of others, of which it may be sufficient now to give one example. Suppose that a bee is trained with two targets on every visit, as was true only on the test visit in the

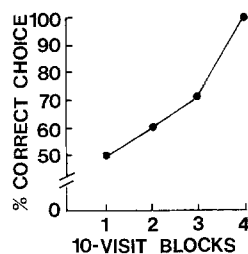


Fig. 3. Percentage of correct choice by a single bee in each of four blocks of 10 visits to a pair of targets, one which was in a modified field and provided 50% sucrose solution, and a second which was in the ambient field and shocked the animal when it landed

experiments already reported. One target is in the ambient field and the other is in the modified field, with the positions of the two fields interchanged from visit to visit in quasi-random fashion. The target in the modified field contains 50% sucrose solution from which the animal is permitted to drink to repletion, while the target in the ambient field is so wired that the animal receives a mild (5 VAC) shock when it lands (both targets are identical and interchangeable, of course). Figure 3 shows the performance of a single forager trained in this way with the modified field produced by the smaller coil and higher current (Fig. 1). In the first block of 10 visits, the percentage of correct initial choice (choice of the safe target in the modified field) was only 50, but by the fourth block of 10 visits (which actually occurred on the following day, since the animal was locked out for the night after the third block) every choice was correct ( $P = 1/2^{10}$ ). One advantage of this technique is that single animals yield statistically reliable evidence of discrimination; another is that animals, once trained, can be tested repeatedly in systematically varied magnetic fields for the purpose of making threshold determinations. We are planning also to try to work with harnessed bees (Bitterman et al. 1983), which permits better control of stimulation, although there are some hints in the vertebrate literature that sensitivity to magnetic fields may be limited to moving animals (Kreithen and Keeton 1974; Bookman 1977).

Plausible hypotheses about magnetoreceptor mechanisms in honeybees are based either on some form of electrical induction (e.g. Jungerman and Rosenblum 1980) or on small particles of magnetite detected in the front third of the abdomen (Gould et al. 1978). Towne and Gould (in press) argue against an induction-based mechanism in honeybees, and Kirschvink (1981) has shown that the magnetically oriented horizontal dance of honeybees is compatible with physical constraints on a

magnetoreceptor based on single-domain particles of magnetite, but neither mechanism has yet been demonstrated or excluded experimentally. With the development of conditioning techniques for the study of magnetoreception, a variety of direct tests of the two principal hypotheses can be contemplated. The magnetite hypothesis suggests, for example, that sensitivity to change in intensity will decrease rapidly at background intensities above or below that of the geomagnetic field, and that discrimination will fail completely in zero fields or at intensities above 500 microTesla (Kirschvink and Walker, in press), while the induction hypothesis suggests that sensitivity will remain constant over a wide range of background intensities. Work with time-varying fields also should be instructive. The sensitivity of an induction-based system in an alternating field must depend on frequency (the Faraday effect), but a magnetite-based system should show no such dependency. In fact, the rotation time (35 ms, Kirschvink and Gould 1981) calculated for magnetic particles suspended in a typical viscous medium should produce failure of discrimination at frequencies beyond 20–40 Hz. Although our experiments do not provide very much information about the magnetosensory capacity of honeybees, they set the stage for a detailed analysis of that capacity and of its underlying mechanism.

*Acknowledgements.* We thank Dr. Joseph L. Kirschvink for advice. This work was supported by grant BNS 83-17051 from the National Science Foundation.

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