

SHORT COMMUNICATION
**ATTACHED MAGNETS IMPAIR MAGNETIC FIELD
DISCRIMINATION BY HONEYBEES**

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Evidence that honeybees respond to magnetic fields comes from both orientation (Lindauer & Martin, 1968, 1972; Towne & Gould, 1985) and conditioning experiments (Walker & Bitterman, 1985; Walker *et al.* 1988a). Perhaps because suitable behavioural methods have not yet been developed, the effects obtained are not very large. They are, however, reliable enough to permit evaluation of the ferromagnetic transduction hypothesis (Ising, 1945; Lowenstam, 1962; Gould *et al.* 1978; Kirschvink & Walker, 1985), to which the discovery of large numbers of single-domain particles of magnetite in the anterodorsal abdominal region of honeybees lends credence (Gould *et al.* 1978), and which alone among the various transduction hypotheses that have been proposed (Kirschvink & Gould, 1981; Kirschvink & Walker, 1985; Yorke, 1981; Kalmijn, 1974; Jungerman & Rosenblum, 1980; Leask, 1977; Korall & Martin, 1987) predicts that discrimination can be abolished by magnets attached in the vicinity of those particles (Jungerman & Rosenblum, 1980; Kirschvink & Walker, 1985). Free-flying honeybees were trained in two experiments to discriminate a local anomaly in the ambient magnetic field. Animals carrying small pieces of magnetized steel wire glued to the anterodorsal abdomen failed in the task, but untreated animals and control animals carrying small pieces of nonmagnetic wire succeeded. The results imply that the magnetite crystals in the anterodorsal abdomen play a critical role in magnetoreception by honeybees.

Individual foragers from our own hives were trained to shuttle back and forth between the hives and a small wooden shed adjacent to the laboratory. Set 14 cm apart on a table in the shed were two identical targets, 5 cm in diameter, each containing a food well so constructed that a shock could be delivered when the proboscis made contact with liquid in the well (Abramson, 1986). Directly beneath the table and centred on the targets were two pairs of coils, each pair consisting of an inner (4 cm diameter, 50 turns) and an outer (10 cm diameter, 8 turns) coil. One of the pairs (the left on some visits and the right on others, in balanced quasirandom order) was energized to produce a localized, vertically oriented magnetic dipole anomaly with a radius of 5 cm and a peak intensity of 350 micro-

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Tesla (μT ; field measured with a Develco 9200C model three-axis fluxgate magnetometer). The anomaly was superimposed on the uniform background Hawaiian field (total intensity $38 \mu\text{T}$, inclination 38° , declination $11^\circ 16' \text{E}$). The well of the target in the ambient field (designated S-) contained an unacceptable saline solution, contacts with which were punished with shock on all visits after the first on which an error was made. (The shock was set initially at 2 V and increased in 1 V steps to 4 V with each successive initial error.) The well of the target in the anomalous field (S+) contained 50% sucrose solution which the animal drank to repletion on each visit (whether upon correct initial choice or after some experience with S-). An observer recorded the initial choice made on each visit.

In experiment 1, three groups of bees were trained for 16 visits. There was a magnetic group of eight subjects, each carrying a 1–2 mm length of magnetized stainless-steel wire glued to the anterodorsal surface of the abdomen; a control group of eight subjects carrying nonmagnetic pieces of copper wire instead; and an untreated group of eight subjects. The pieces of stainless-steel wire (0.36 mm diameter) were magnetized by a unidirectional magnetic field pulse with a peak intensity of 100 mT. Copper wires of the same length and gauge were attached to control animals. The subjects were chosen at random from groups of foragers at a feeding station providing 10–12% sucrose solution and were anaesthetized by chilling for wire attachment. The wings of the immobilized animal were held apart with padded nonmetallic forceps and the wire attached with rubber cement in the dorsal midline as close to the front of the abdomen as possible, a location chosen on the basis of the study of Gould *et al.* (1978) and further information provided by J. L. Kirschvink. The animal was then marked with coloured lacquer, returned to the feeder where it was fed some 50% sucrose solution, and permitted to resume normal foraging for at least 1 day before testing. Tagged subjects were found at the feeder up to 9 days after the treatment. The attached steel or copper wires could be distinguished easily by their colour in experiment 1.

As Table 1 shows, the performance even of the untreated group in experiment 1 was not spectacularly good – the mean proportion of correct choices in the 16 visits was only about 0.66 – but there was clear evidence of discrimination. Every untreated animal made more than eight correct choices ($P = 0.0039$), and the same was true of the control animals, but the performance of only four of the magnetic animals exceeded the chance expectation ($P > 0.05$). The median test showed the performance of the control animals to be significantly better than that of the

Table 1. *Mean proportion of correct choices and its standard error (S.E.) for each group in each experiment*

Group	Experiment 1			Experiment 2		
	<i>N</i>	Mean	S.E.	<i>N</i>	Mean	S.E.
Untreated	8	0.66	0.02	8	0.68	0.02
Control	8	0.63	0.03	16	0.63	0.02
Magnetic	8	0.58	0.04	10	0.55	0.02

magnetic animals ($P = 0.0385$), with no significant difference between the untreated animals and the controls ($P > 0.05$).

Experiment 2 differed from the first experiment in only one important respect: the observer did not know which were magnetic and which were control animals until the conclusion of the experiment, because the wires attached to the magnetic and control animals had been made indistinguishable from each other with a coating of coloured lacquer. It was found after the experiment that 10 magnetic animals and 16 control animals had completed the 16 scheduled training visits. Each piece of wire to be used was assigned to a bee according to a randomized key in the possession of a member of our laboratory not involved in the work. Each bee was given an identification number and marked uniquely with spots of coloured lacquer to permit decoding of the data when the experiment was finished. Of 80 wires prepared, 78 were attached to animals successfully. Of these tagged animals, we attempted to train 44 subsequently found at the feeder.

As Table 1 shows, the results of the second experiment were very much the same as those of the first. All eight untreated animals ($P = 0.0039$) and 13 of the 16 control animals ($P = 0.0105$), but only four of the 10 magnetic animals ($P > 0.05$), made more than eight correct choices in the 16 visits. The median test again showed significantly better performance in the control animals than in the magnetic animals ($P = 0.0208$), with no significant difference between the untreated animals and the controls ($P > 0.05$). The differential effectiveness of the two treatments is indicated by another measure; the number of animals whose training was begun but that did not return for the prescribed number of training visits. The number of such animals increases when shock for error is used in an effort to sharpen performance in a difficult discrimination task and may in fact be taken as an index of the difficulty of the task. Of 21 animals selected at random that turned out to be carrying copper wire, only five failed to return for 16 visits, as compared with 13 of 23 animals carrying magnetic wire ($P = 0.0279$).

These results are not only consistent with the ferromagnetic transduction hypothesis but imply strongly that the magnetite crystals found in the anterodorsal abdomen play a critical role. The results of dance experiments with honeybees (Lindauer & Martin, 1968) suggest that the magnetoreceptor system is disabled in fields of greater intensity than $500 \mu\text{T}$, such as are found within 1–2 mm of the magnetized wires we used (Walker *et al.* 1988*b*). Because the field decays rapidly to $10 \mu\text{T}$ (20 % of earth-strength) at a distance of 5 mm from the wires, it seems reasonable to conclude that the magnetoreceptors must have been very near the wires, whose position was dictated by the locus of the magnetite. It should be noted that there is precedent for our work. Magnetic attachments to the heads of pigeons, which also contain magnetite (Walcott *et al.* 1979), have been found to impair homing (Keeton, 1972), although not to prevent it entirely, perhaps because other cues were available (Walraff, 1983). Experiments like ours, in which better-than-chance performance is possible only on the basis of magnetic-field stimuli, must await evidence of conditioned response to magnetic fields, which has been difficult to demonstrate in pigeons (Ossenkopp & Barbeito, 1978).

Magnetite particles useful for magnetoreception can be recognized easily when they are concentrated in small volumes of tissue (Kirschvink, 1983; Walker *et al.* 1985). Such concentrations have been found in widely divergent animals (Walker *et al.* 1984, 1988*a,b*; Kirschvink *et al.* 1985), of which tuna (Walker, 1984) and salmon (Quinn, 1980) as well as pigeons and honeybees are known to respond to magnetic-field stimuli. It follows from the hypothesis of magnetite-based magnetoreception, which is supported by our results, that magnetosensory capabilities may exist in species for which there is as yet no behavioural evidence of any magnetic sensitivity.

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References

- ABRAMSON, C. I. (1986). Aversive conditioning in honeybees. *J. comp. Psychol.* **100**, 108–116.
- GOULD, J. L., KIRSCHVINK, J. L. & DEFFEYES, K. S. (1978). Bees have magnetic remanence. *Science* **201**, 1026–1028.
- ISING, G. (1945). Die physikalische Möglichkeit eine tierischen orientierungsinnes auf Basis der Erdrotation. *Arkiv. for Matematik, Astronomi o. Fysik* **32A**, 1–23.
- JUNGERMAN, R. L. & ROSENBLUM, B. (1980). Magnetic induction for the sensing of magnetic fields – An analysis. *J. theor. Biol.* **87**, 25–32.
- KALMIJN, A. J. (1974). The detection of electrical fields from inanimate and animate sources other than electric organs. In *Electroreceptors and Other Specialized Receptors in Lower Vertebrates, Handbook of Sensory Physiology*, vol. III/3 (ed. A. Fessard), pp. 147–200. Berlin, Heidelberg, New York: Springer-Verlag.
- KEETON, W. T. (1972). Effects of magnets on pigeon homing. In *Animal Orientation and Navigation* (ed. S. R. Galler, K. Schmidt-Koenig, G. J. Jacobs & R. E. Belleville), pp. 579–594. Washington DC: US Government Printing Office.
- KIRSCHVINK, J. L. (1983). Biogenic ferrimagnetism: a new biomagnetism. In *Biomagnetism: An Interdisciplinary Approach* (ed. S. Williamson, G.-L. Romani, L. Kaufman & I. Modena), pp. 501–531. New York: Plenum Press.
- KIRSCHVINK, J. L. & GOULD, J. L. (1981). Biogenic magnetite as a basis for magnetic field detection in animals. *Biosystems* **13**, 181–201.
- KIRSCHVINK, J. L., JONES, D. S. & MACFADDEN, B. J. (eds) (1985). *Magnetite Biomineralization and Magnetoreception in Organisms: A New Biomagnetism*. New York: Plenum Press.
- KIRSCHVINK, J. L. & WALKER, M. M. (1985). Particle-size considerations for magnetite-based magnetoreceptors. In *Magnetite Biomineralization and Magnetoreception in Organisms: A New Biomagnetism* (ed. J. L. Kirschvink, D. S. Jones & B. J. MacFadden), pp. 243–254. New York: Plenum Press.
- KORALL, H. & MARTIN, H. (1987). Responses of bristle field sensilla in *Apis mellifica* to geomagnetic and astrophysical fields. *J. comp. Physiol.* **161**, 1–22.
- LEASK, M. J. M. (1977). A physicochemical mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature, Lond.* **267**, 144–145.
- LINDAUER, M. & MARTIN, H. (1968). Die Schwereorientierung der Bienen unter dem Einfluss des Erdmagnetfeldes. *Z. vergl. Physiol.* **60**, 219–243.
- LINDAUER, M. & MARTIN, H. (1972). Magnetic effects on dancing bees. In *Animal Orientation and Navigation* (ed. S. R. Galler, K. Schmidt-Koenig, G. T. Jacobs & R. E. Belleville), pp. 559–567. Washington DC: US Government Printing Office.

- LOWENSTAM, H. A. (1962). Magnetite in denticle capping in recent chitons (Polyplacophora). *Geol. Soc. Am. Bull.* **73**, 435–438.
- OSSENKOPP, K.-P. & BARBEITO, R. (1978). Bird orientation and the geomagnetic field: A review. *Neurosci. Biobehav. Rev.* **2**, 255–270.
- QUINN, T. P. (1980). Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *J. comp. Physiol.* **137**, 243–248.
- TOWNE, W. F. & GOULD, J. L. (1985). Magnetic field sensitivity in honeybees. In *Magnetite Biomineralization and Magnetoreception in Organisms: A New Biomagnetism* (ed. J. L. Kirschvink, D. S. Jones & B. J. MacFadden), pp. 385–406. New York: Plenum Press.
- WALCOTT, C., GOULD, J. L. & KIRSCHVINK, J. L. (1979). Pigeons have magnets. *Science* **205**, 1027–1029.
- WALKER, M. M. (1984). Learned magnetic field discrimination in yellowfin tuna, *Thunnus albacares*. *J. comp. Physiol.* **155**, 673–679.
- WALKER, M. M., BAIRD, D. L. & BITTERMAN, M. E. (1988a). Failure of stationary but not of flying honeybees to respond to magnetic field stimuli. *J. comp. Psychol.* (in press).
- WALKER, M. M. & BITTERMAN, M. E. (1985). Conditioned responding to magnetic fields by honeybees. *J. comp. Physiol.* **157**, 67–71.
- WALKER, M. M., KIRSCHVINK, J. L., CHANG, S.-B. R. & DIZON, A. E. (1984). A candidate magnetic sense organ in the yellowfin tuna, *Thunnus albacares*. *Science* **244**, 751–753.
- WALKER, M. M., KIRSCHVINK, J. L., PERRY, A. & DIZON, A. E. (1985). Detection, extraction, and characterization of biogenic magnetite. In *Magnetite Biomineralization and Magnetoreception in Organisms: A New Biomagnetism* (ed. J. L. Kirschvink, D. S. Jones & B. J. MacFadden), pp. 155–166. New York: Plenum Press.
- WALKER, M. M., QUINN, T. P., KIRSCHVINK, J. L. & GROOT, C. (1988b). Production of single-domain magnetite throughout life by sockeye salmon, *Oncorhynchus nerka*. *J. exp. Biol.* **140**, 51–63.
- WALRAFF, H. G. (1983). Relevance of atmospheric odours and geomagnetic field to pigeon navigation: what is the 'map' basis? *Comp. Biochem. Physiol.* **76A**, 643–663.
- YORKE, E. D. (1981). Sensitivity of pigeons to small magnetic field variations. *J. theor. Biol.* **89**, 533–537.

