Chapter 14

Discrimination of Low-Frequency Magnetic
Fields by Honeybees: Biophysics and
Experimental Tests

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Introduction

It has been shown repeatedly over the past 20 years that honeybees are able to detect weak, earth-strength magnetic fields. Table I shows a summary of these known geomagnetic effects on honeybee behavior, as well as the independent attempts to replicate them. We know of no attempts to replicate these effects that were not eventually successful (some apparently took practice). Towne and Gould (1985) provide a thorough and critical review of this literature prior to 1985 (effects 1–4 in Table I). Of these unconditioned responses, the horizontal dance experiment of

TABLE I
Summary of Magnetic Effects on Honeybee Behavior

Effect	Original reports	Similar replications
1. Misdirection in the waggle dance influenced by weak magnetic fields	Lindauer and Martin (1968, 1972), Martin and Lindauer (1977)	Hepworth et al. (1980), Towne and Gould (1985), Kilbert (1979)
2. Dances on horizontal comb align with points of magnetic compass	Lindauer and Martin (1972), Martin and Lindauer (1977)	Brines (1978); Gould et al. (1980) (also see Kirschvink, 1981)
3. Magnetic orientation of comb building	Lindauer and Martin (1972), Martin and Lindauer (1973)	De Jong (1982), Towne and Gould (1985)
4. Time sense of bees influenced by geomagnetic variations	Lindauer (1977)	Partially by Gould (1980)
5. Extinction test conditioning experiment	Walker and Bitterman (1985)	Kirschvink and Kobayashi-Kirschvink (1991)
6. Two-choice threshold conditioning experiment	Walker and Bitterman (1989a)	This report
7. Small magnets on anteriordorsal abdomen interfere with conditioning experiments	Walker and Bitterman (1989b)	No attempts reported
8. Pulse remagnetization converts north-seeking into south-seeking bees	Kirschvink and Kobayashi-Kirschvink (1991)	No attempts reported

Lindauer and Martin (1972) and Martin and Lindauer (1977) (effect 2 in Table I) has proven to be particularly easy to replicate.

In a series of elegant papers, Walker and Bitterman (1985, 1989a, b) and Walker et al. (1989) have shown that individual foraging honeybees can be trained to discriminate weak magnetic anomalies superimposed against the background geomagnetic field (effects 5–7 in Table I). Given the proper experimental situation, honeybees learn to discriminate magnetic cues in a fashion similar to visual cues (Walker et al., 1989). Unlike many other experiments demonstrating the effect of magnetic fields on animals, the Walker-Bitterman experiments are amazingly simple but

powerful. Clear magnetic effects can be obtained from small numbers of animals or even individuals, and discrete responses are easy to record electronically. These factors led us to try to replicate the extinction test reported initially by Walker and Bitterman (1985), which succeeded surprisingly well (Kirschvink and Kobayashi-Kirschvink, 1991).

Walker and Bitterman's (1989*a*) measurement of the threshold sensitivity of the bees to a small static anomaly superimposed upon the background field is the most dramatic experimental result to date from their conditioning experiments. By starting with a moderately strong anomaly in a two-choice training paradigm, and by reducing the amplitude of the anomaly in small exponential steps, the threshold sensitivity could be determined by the point at which the bees were no longer able to discriminate correctly. Of nine bees run through the procedure, the median threshold was 250 nanotesla (nT, 0.6% of the background field in Hawaii), whereas their best bee lost the ability to discriminate in fields below 25 nT (0.06%). Similar, but less direct, estimates of the magnetic sensitivity of bees were obtained from both the misdirection and circadian rhythm experiments (effects 1 and 4 in Table I; reviewed by Towne and Gould [1985]). This astounding sensitivity, however, is not biologically unreasonable for a magnetite (Fe_3O_4)-based sensory system. Estimates for the number of discrete sensory organelles per bee, based on the measured magnetic moments, are on the order of several million (Gould et al., 1978; Kirschvink, 1981). Several analyses have shown that the ultimate sensitivity of such an array will improve by the square root of the number of receptors, and that nanotesla level sensitivity is not implausible (Kirschvink and Gould, 1981; Yorke, 1981; Kirschvink and Walker, 1985).

Two of the experiments listed in Table I have a direct bearing on the nature of the magnetic sensory receptors in the honeybee. First, Walker and Bitterman (1989*b*) found that small magnetized wires glued to the anteriordorsal abdomen interfered with the ability of the bees to discriminate magnetic anomalies, whereas copper wires had no effect. Magnetic wires in other locations similarly had no effect, and the experiments were done double-blind. The anteriordorsal abdomen is the site of magnetite biomineralization discovered by Gould et al. (1978), and thus should be the site of any magnetite-based magnetoreceptors. Second, Kirschvink and Kobayashi-Kirschvink (1991) were on occasion able to elicit magnetic North-seeking behavior in bees trained to visit a simple T-maze. A short magnetic pulse with a peak amplitude of 100 mT (stronger than the coercivity of most biogenic magnetites) was able to convert this North-seeking exit response into a South-seeking one. This same experiment works on the magnetotactic bacteria (Kalmijn and Blakemore, 1978; Diaz-Ricci et al., 1991) and is a unique fingerprint of a ferromagnetic compass receptor.

A major question of importance to the electric power industry concerns whether the 50- and 60-Hz magnetic fields generated by electric power lines can influence living organisms, particularly at the low field strengths typically encountered. Some physicists have suggested recently that this is impossible (e.g., Adair, 1991); unfortunately, they ignore the well-known process of magnetite biomineralization in animals. Viewed from the perspective of a flying honeybee choosing between two of Walker and Bitterman's (1989*a*) targets, it is clear that its discriminative choice is not made with a purely static stimulus. During the choice period, the bee flies in and out of the magnetic anomaly and experiences field changes that are at effective frequen-

cies of a few cycles per second. Hence, it is clear that the honeybee magnetic sense must have some response at low frequencies.

In this paper we present two biophysical models of magnetite-based magnetoreceptors, and compare their expected frequency response from a theoretical perspective. We then describe our attempts during the past two years to modify the Walker-Bitterman extinction and two-choice procedures to measure experimentally the honeybee's sensitivity to low frequency magnetic fields.

Biophysical Models of the Honeybee Receptor

Unfortunately, the ultrastructure of an in situ magnetite-based sensory organelle has not been studied in any animal, largely because the volume density of magnetite in most tissue samples isolated magnetically is only a few parts per billion. This low volume density of the receptors is not terribly surprising, as only one receptor cell with a 1-μm-long magnetosome chain could provide a whale with a superb magnetic compass sense. Even in tissue samples containing measurable amounts of magnetite,
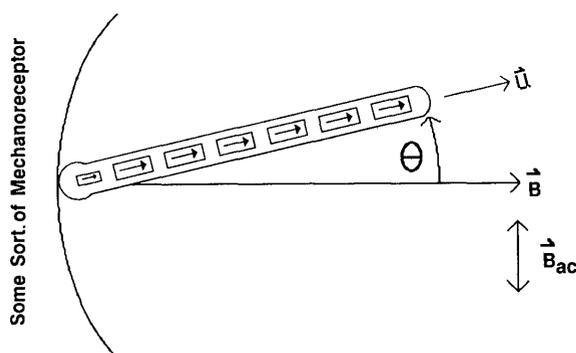

Figure 1. Schematic diagram of a possible magnetite-based magnetoreceptor in honeybees. The magnetic/thermal energy ratio of the magnetosome chain is 6, the chain structure is 1 μm in length and 50 nm in diameter, and it is in a fluid 100 times more viscous than water. The arrow labeled B is the direction of the static geomagnetic background field, and the oscillating field, B_{ac}, is perpendicular to this. The contrasting model discussed in the text would have a single spherical magnetosome 0.12 μm in diameter rather than the magnetosome chain.

the TEM search is at best a needle-in-the-haystack operation. However, magnetite crystals extracted from the ethmoid region of tuna and salmon have been studied intensively (Walker et al., 1984; Kirschvink et al., 1985; Mann et al., 1988; Walker et al., 1988). These particles form linear chains of magnetically single-domain crystals virtually identical to the chains of magnetosomes present in the magnetotactic bacteria. Like these bacteria, the salmon crystals also have their crystallographic {111} alignment parallel to the chain direction, which maximizes the net magnetic moment of the chain (Mann et al., 1988). Neurons and cells containing primary cilia are also present in these tissues (Walker et al., 1988), suggesting that the receptor is something like a modified hair cell (e.g., Kirschvink and Gould, 1981). Unfortunately, the only published attempt to locate cellular iron in the honeybee (Kuterbach et al., 1982) focused on the ventral abdomen rather than the locus of magnetite biomineralization in the anteriordorsal abdomen.

Two simple "end-member" models for a magnetite-based magnetoreceptor are worth considering here. The first is that of a long magnetosome chain suspended in a viscous medium and connected to a suitably modified hair cell mechanoreceptor, as

sketched diagrammatically in Fig. 1; the second is to assume that the magnetite is a sphere attached somehow to a mechanoreceptor. The long chain would have a maximum viscous drag, whereas for the sphere this would be at a minimum. If we assume that the magnetosome chain is free to pivot around its fixed end, and the sphere around its center, and that both are critically overdamped by viscous forces (e.g., the low Reynolds number intracellular environment described by Purcell [1977]) so that inertial terms can be neglected, the equations of motion for both models are similar to that of a forced, damped torsional pendulum. The equation is then:

$$C\theta' + \mu B \sin(\theta) = \mu B_{ac} \cos(\theta) \cos(\omega t) \tag{1}$$

where C is the coefficient of rotational friction about the end of the magnetosome chain or through the center of the sphere, θ is the angle between the static background field and the magnetosomes, θ' (or $d\theta/dt$) is the angular velocity, μ is the total magnetic moment of the receptor, B is the strength of the background (geomagnetic) field, B_{ac} is peak amplitude of an alternating magnetic field aligned perpendicular to B, ω is the angular frequency of the alternating field, and t is time. Although this is a first-order equation, it does not have closed-form solutions for $\theta(t)$ due to the presence of the nonlinear $\sin(\theta)$ and $\cos(\theta)$ terms. However, a close approximation to the correct solution can be found easily by the following approach. In the case where θ is small, $\sin(\theta)$ and $\cos(\theta)$ are approximately θ and 1, respectively. Eq. 1 then becomes linear, and the solution for long times becomes

$$\theta(t) = \theta_{max} \cos(\omega t + E) \tag{2}$$

where

$$\theta_{max} = \frac{\mu B_{ac}}{\sqrt{\mu^2 B^2 + C^2 \omega^2}} \tag{3}$$

and

$$\tan(E) = \frac{-\omega C}{\mu B} \tag{4}$$

For our purposes, the phase delay (E) between the applied frequency and the response is not important. Although this solution works for small θ, if the value of B_{ac} is much larger than B, θ_{max} may become much larger than its maximum possible value of 90°. In the low frequency limit where ω approaches zero, θ_{max} should reduce simply to the arctangent of B_{ac}/B. This modification also works for low values of θ because Arctan(θ) is also θ in this limit. Indeed, numerical solutions of Eq. 1 show that Arctan(θ_{max}), with θ_{max} as given in Eq. 3, is a close approximation to the maximum amplitude of the exact solution of Eq. 1 for all ranges of θ between $-\pi/2$ and $\pi/2$.

We next need to put specific values on the quantities μ and C, which would be appropriate for our honeybee models. For the magnetic moment, μ, Kirschvink (1981) noted that the accuracy of the dance orientation data in varying strength background fields published by Martin and Lindauer (1977) followed closely the Langevin function, and the least-squares comparison of this function with the dance data yields an estimate of the magnetic to thermal energy ratio in the geomagnetic field of ~6. If we take the geomagnetic field at 50 μT (or 0.5 gauss), this yields a

moment for the average honeybee magnetoreceptor of 5×10^{-13} emu (5×10^{-16} Am^2).

The coefficient of rotational friction depends on the size and shape of the magnetic structure which is free to move, as well as the viscosity of the surrounding medium. In the salmon, many of the magnetosome chains were on the order of a micrometer in length (Mann et al., 1988), hence a reasonable approximation for the magnetosome chain model is a right circular cylinder 1 μm in length, with a length/diameter ratio of 20. Sadron (1953) has derived the general expression for the coefficient of rotational friction for motion through the center of a right circular cylinder. It is given by

$$C = \frac{2}{3} \eta V \frac{1}{r(p)} \tag{5}$$

where p is the length/diameter ratio for the cylinder, η is the viscosity of the liquid, and V is the volume of the cylinder. The function $r(p)$ is a shape parameter, the full expression for which is given by Sadron (1953). For our model magnetoreceptor, however, the chain rotates about a fixed end rather than around an axis through its center. In this situation, the coefficient of rotational friction should be exactly half of that produced by rotation of a 2-μm-long cylinder about its center, with a length/diameter ratio of 40. The similar coefficient for the sphere is given by $6\eta V$ (Sadron, 1953).

The viscosity of the medium surrounding the magnetosome chains is more difficult to estimate, as it is not yet known where they are located. The first approximation is to assume that it is similar to cellular protoplasm. Hence, we use the estimate of Keith and Snipes (1974) in their study of the molecular motion of a small spin-label molecule, tempone. They found that typical bacterial viscosities were ~10 times greater than water, and that eukaryotic cells (from protists, plants, and humans) were all similar at ~100 times higher than water. Using this larger estimate for the viscosity, the constant of rotational friction, C, for the long magnetosome chain is 1.8×10^{-13} (cgs), versus 6.5×10^{-15} for the sphere.

It is useful to compare the amplitude of the induced rotations predicted by Eq. 1 with the magnitude of the rotational Brownian motions produced by thermal agitation, which are given by the square root of $(kT/\mu B_{total})$. Even though some signals are below thermal noise at an individual receptor, they may still be detected by suitable averaging across the large numbers of independent organelles inferred to be present (Kirschvink and Gould, 1981).

Fig. 2 shows the maximum amplitude of the angle θ as a function of frequency, ω, for varying values of B_{ac} in a background geomagnetic field, B, of 50 μT for both models, with the heavy dots indicating the portions of the curves below thermal noise. In all cases, the response is largest around 0 Hz, dropping off at higher frequencies. Due to increased viscous drag, this drop-off in the 0–100-Hz range is far more pronounced in the linear magnetosome chain model than in the spherical model. Obviously, a magnetoreceptor employing a single spherical magnetosome would have a much broader frequency response than a linear chain model.

This difference leads to a testable prediction concerning relative threshold sensitivity. From the work of Walker and Bitterman (1989a) we know that at least one bee was able to discriminate a 25-nT static anomaly in the presence of the

background geomagnetic field. According to Eq. 1, at 0 Hz this corresponds to an angular deflection of only 0.029° for either the magnetosome chain or spherical models. (Although this is well below the r.m.s. thermal noise of 23°, the effective noise for an array of 10^6 independent receptors should be reduced by a factor of 10^3, to ~0.023°.) If we assume that the receptors are responding to the angular displacements, we can make estimates for the relative thresholds for each model as a function of frequency. For the linear magnetosome chain model, a 60-Hz magnetic field of 6.5 μT peak amplitude is required to produce the same 0.029° deflection as did the 0-Hz, 25-nT field, or a 2,600-fold reduction in sensitivity. On the other hand, for the spherical model a 60-Hz field of only 240 nT yields this same deflection, for a factor of ~10 reduction in sensitivity. Repeating these calculations for a viscosity only 10 times that of water changes the 60-Hz threshold estimates to 650 and 35 nT
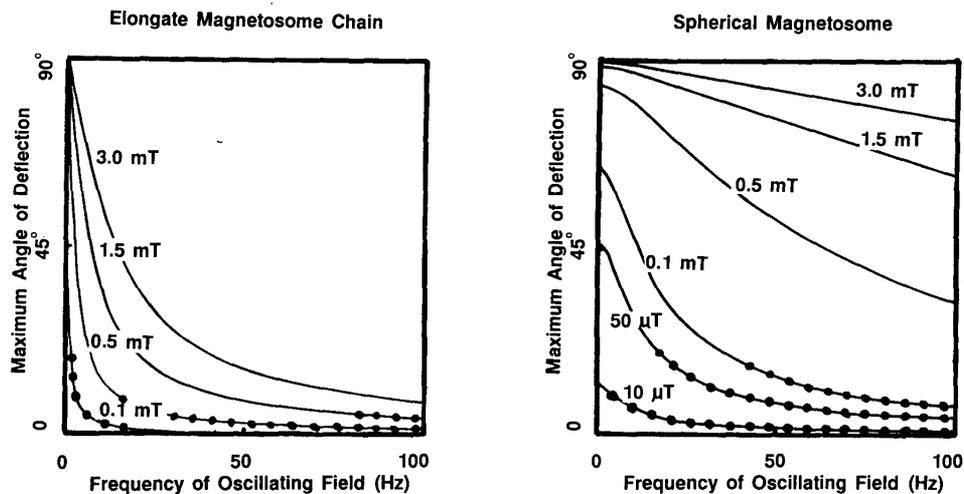

Figure 2. Maximum angular deflections for the magnetoreceptor models as a function of frequency and amplitude of the alternating field component, B_{ac}. The background geomagnetic field is taken at 50 μT, with the alternating field aligned perpendicular to it. Numbers on each curve give the peak amplitude of the oscillating field component in millitesla (1 mT = 10 G). Dotted portions of the curves indicate those regions where the maximum deflection angle is less than the r.m.s. angular deviation produced by thermal noise, given by the square root of $(kT/\mu B_{total})$.

for the magnetosome chain and sphere models, respectively. Obviously, it should be possible to distinguish between these two models by suitable behavioral experiments along the lines of Walker and Bitterman (1989a).

Attempts to Condition Honeybees to Alternating Magnetic Fields

Of the various conditioning experiments developed by Walker and Bitterman, the extinction test (Walker and Bitterman, 1985) and the two-choice procedure (Walker and Bitterman, 1989a) can be adapted most easily to test for sensitivity to alternating magnetic fields. These attempts are described next.

Extinction Test

The protocol and equipment used for this experiment was identical to that used by Walker and Bitterman (1985) as modified slightly by Kirschvink and Kobayashi-Kirschvink (1991). To summarize, individual honeybees labeled distinctively with nail polish were trained to feed from a drop of sucrose solution placed on a single horizontal target in the center of a North-facing window box. Once an animal learned to return on her own, we began a discriminative training series of 10 visits, with the strength of the sugar solutions alternating between 20 and 50% on successive visits, concluding with 50% on the 10th visit. After experiencing 50% sugar, the bees behave as if the 20% is somewhat aversive, hence providing a reasonable reward (S+) and punishment (S−) distinction. During this training interval, either the S+ (50%) or S− (20%) condition was paired with an oscillating magnetic anomaly (peak amplitude 1.5 mT, 1 Hz). After the 10th visit we conducted the extinction test, in which the central target was removed and replaced with two targets separated by ∼15 cm, one of which (either on the east or west) was then paired with the magnetic anomaly. Although the other target had no anomaly, it was equipped with a similar double-wrapped coil with antiparallel currents yielding the same ohmic heating effect. The coils are described fully by Kirschvink and Kobayashi-Kirschvink (1991), and are designed to produce an anomaly that is focused sharply on the target. The double wrapping ensures that, although only one target of the pair produces an anomaly, both produce the same heating and other side effects (e.g., Kirschvink, 1992). The targets without a magnetic anomaly contained a drop of Pasadena tap water, which is aversive to most living things. Upon its return for the 11th visit, the bee would typically hover for a few seconds before landing at one of the targets to taste the liquid. Upon finding only water, the animal would usually fly up and choose again. A simple computer program was used to record the bee's choice (east or west) and the time of contact to the nearest 0.1 s during a 10-min interval. A video tape record of the training period and the extinction test was also kept, and after the test the bee was caught and frozen to prevent her return during the next experiment. Additional details are as described by Kirschvink and Kobayashi-Kirschvink (1991).

This extinction test protocol can be run in four combinations, as either the S+ or S− sugar levels can be paired with the anomaly, and during the extinction test the anomaly can be located under either the east or west target. We therefore ran eight bees through this test, two for each combination, in order to balance fully all such effects.

Three things can be tested for in these data: (1) a preference for the targets associated with the magnetic field environments that were paired with the 50% sucrose (S+) during the previous discriminative training, (2) a position preference for either the east or west targets, and (3) a direct preference (or aversion to) the target associated with the magnetic anomaly, independent of whether or not it had been used as the S+ or S− stimulus. The diagrams of Fig. 3 show the data from this experiment averaged according to these groups, after normalizing the response from each bee for the total number of contacts during the 10-min intervals (thereby giving each animal unit weight in the analysis), along with 2-sigma errors around their mean values.

We were surprised at the results. Unlike the same experiment run with a static magnetic anomaly, there was no suggestion that the bees had learned to associate the presence or absence of the oscillating magnetic anomaly with the S+ or S− sugar ($P > 0.1$ for the F-ratio test of Fig. 3 *A*). On the other hand, there was a highly significant preference for the bees to go toward the target with the magnetic anomaly, whether or not it had been paired with the S+ or S− stimulus in the earlier discriminative training ($P < 0.01$, Fig. 3 *C*). Although the bees were able to detect the presence of the oscillating anomaly, they did not learn its association with strong or weak sugar! We therefore focused our efforts on the two-choice experiment reported by Walker and Bitterman (1989*a*), which, in addition to providing more powerful control of the behavior, could perhaps be used to measure thresholds as a function of frequency. ·
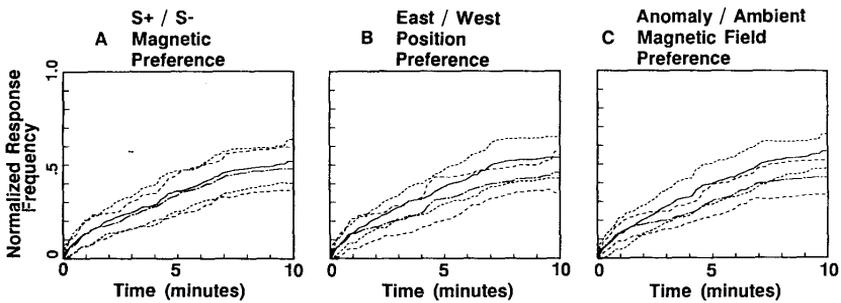

Figure 3. Normalized, averaged results from the 1-Hz extinction test experiment, broken into the three groups described in the text. For the S+/S− group in *A*, the solid line shows the average contact frequency of the bees to the target which had the magnetic field (ambient or anomaly) that was paired previously with the S+ condition. The two short-dashed lines above and below this line indicate the 2-sigma error boundaries, arbitrarily calculated for plotting purposes at 6-s intervals over the 10-min course of the experiments. Similarly, the S− mean values are plotted with the dot-dashed line, with the longer-dashed lines showing the errors. *B* shows these results averaged so as to test for an east/west position preference, the solid line (and short-dashed errors) indicating the west preference, and the dot-dashed line (and longer-dashed errors) representing the east. Finally, *C* shows the data averaged to test for a preferential attraction to the magnetic anomaly (solid curve and short-dashed errors) or the ambient field (dot-dashed curve with longer-dashed errors). Results from the F-ratio ANOVA with 1 and 14 d.f. for testing the significance of separation of the curves in each case are as follows: *A*: (S+, S−, σ) = (0.521, 0.479, 0.116), $F = 0.516$, $P > 0.1$; *B*: (E, W, σ) = (0.461, 0.539, 0.111), $F = 1.936$, $P > 0.1$; *C*: (field, no field, σ) = (0.569, 0.431, 0.092), $F = 8.923$, $P < 0.01$.

The Walker-Bitterman Two Choice Experiment

Fig. 4 shows the general layout of for this experiment. Two targets are placed on a shaded, vertical window separated by ~15 cm. Each target is centered on one of the double-dipole, double-wrapped coil assemblies described above. In an initial pretraining episode, a painted honeybee is trained over six visits to feed on 50% sucrose from alternate targets consistently paired with the magnetic anomaly; during this time the bee is prevented from visiting the nonmagnetic target. After the last pretraining visit, the bee would return to find both targets open, requiring her to make a choice. If she

landed on the target paired with the field (S+), she was rewarded with 50% sucrose; otherwise, she encountered tap water paired with a mild electric shock (3 V, 1,000-Hz square wave), forcing her to go to the other target. In the original Walker-Bitterman protocol, the bee's first choice for each visit was recorded either by having it break the beam of an IR photocell, or by electrically completing a circuit between the copper landing platform and the sugar or water solution. Between visits, the food wells in both targets were rinsed and filled with the proper solutions for the next visit, and the position of the anomaly (and hence the S+ target) was changed in a quasi-random order from visit to visit. Thus, long strings of correct choices would indicate preference for the anomaly associated with the magnetic field, the chance
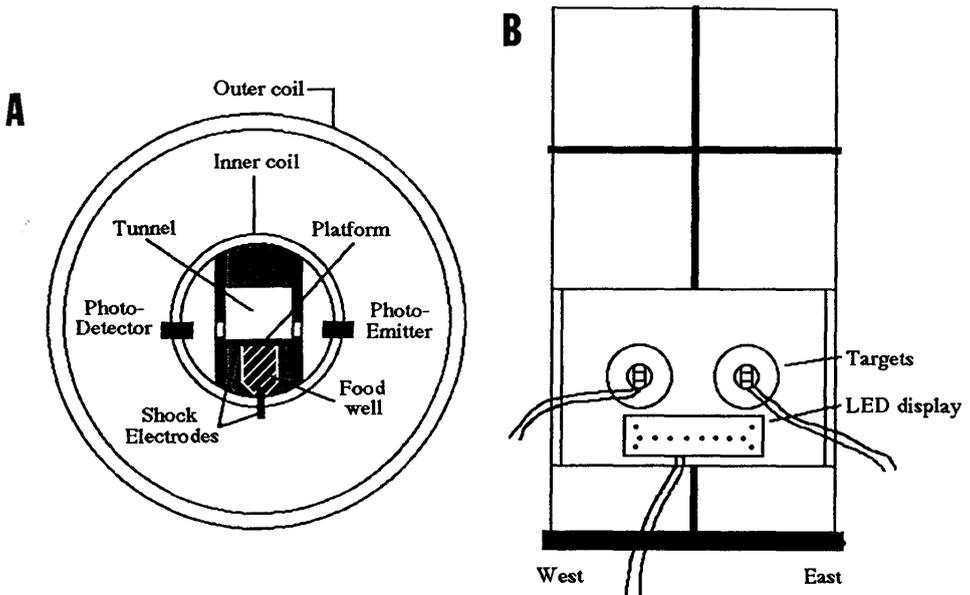

Figure 4. Experimental setup for the two-choice experiment (similar to that of Walker and Bitterman, 1989a). (A) Schematic drawing of individual targets. The coil designs are given by Kirschvink and Kobayashi-Kirschvink (1991). (B) Arrangement of targets on a north-opening window. The LED panel was dark until after the bee made its first choice, whereupon it displayed information concerning the location of the anomaly, the bee position, and the current visit number.

probability of which would decrease in a binomial fashion with the length of the string. In their measurements of the threshold sensitivity, Walker and Bitterman (1989a) adopted a practical criterion of six correct choices in a row, or seven out of eight (chance probabilities 0.016 and 0.035, respectively). For each bee and field setting, they report the number of trials for the animal to reach one of these criteria. Our Monte-Carlo simulations of their published data confirm the highly nonrandom nature of their results with static fields.

Although the ability of Walker and Bitterman's bees to discriminate fields successfully, and their failure to do so at low field levels, is clear evidence for a remarkable magnetic sensitivity, we were bothered by one aspect of their original experiment. When the bee returns at the start of each visit, the targets differ in one

nonmagnetic aspect: one has water, and the other has 50% sucrose. In their experiments in Hawaii, bees never displayed the ability to distinguish these liquids except by direct taste (Abramson, 1986; Walker and Bitterman, 1989*a*). Much of our effort at replicating this experiment was aimed at eliminating or minimizing this difference, and attempting to bring the entire experiment under the control of a dedicated microcomputer. Our first attempts were done in Pasadena during the summer of 1989, and we built circuits to switch the magnetic field position properly and to detect the contact of the bee with the sugar solution using operational amplifiers. We tried several variations from the standard protocol, including using 50% sugar in both targets and relying on shock alone to drive the bee from the S− target after an incorrect choice. Our initial results using static magnetic fields were never as clear as those from Hawaii, unless we reverted to the 50%/0% protocol, which again required the tedious washing of the targets and replacing the solutions after each visit. However, several of our bees that reached criterion performance with the S− water failed subsequent control experiments in which the electric circuit for the anomaly was interrupted. Our best guess at the time was that somehow the Pasadena bees were able to smell the presence of water, as they often landed on the wrong target for a few seconds without triggering the computer.

These experiments were continued during the Spring and Summer of 1990 in Fukuoka, Japan, initially with results disappointingly similar to those in Pasadena. We discovered that after many visits the bees would learn to taste the water quickly without registering a response on the computer, and that this was due to an insulating film which built up on the copper landing platforms. Smell was not involved, as this happened even when a slight inward draft was present. Urban smogs in both Pasadena and Fukuoka apparently were much more corrosive than the atmosphere in Honolulu, Hawaii; the Hawaiian protocol also used the IR photocells as a backup check on the bee's first choice. Our final target design used in Fukuoka avoided this problem by covering the platform with gold foil. We also minimized the problem of switching the solutions by making many interchangeable liquid wells out of 10-ml plastic cubetts which could be plugged into the base of each target. Several interchangeable wells for both sugar and water were necessary, as we discovered that the bees could eventually recognize subtle differences in the cubetts. We also tried to watch the bee as often as possible during her choice procedures to determine whether or not she was cheating, and after strings of successful discrimination choices we usually did no-current control tests. We sometimes allowed bees to continue responding to a stimulus after reaching the Walker-Bitterman criterion, as a test for control over their behavior.

Fig. 5 shows the results from an individual bee. After the six initial pretraining visits and four of the two-choice visits that were paired with a static anomaly (2.2 mT), the bee reached criterion performance of seven out of eight (chance probability 0.035) as shown in Fig. 5 *A*. At this point, a no-current control experiment was run from the 13th through the 68th visits, with only random results as shown in Fig. 5 *B*. From the 69th trial, the magnetic anomaly was reconnected with a 1-Hz sinusoidally oscillating field with peak amplitude of 2.2 mT. After 30 additional visits, the bee produced a string of 12 correct choices in a row (chance probability 0.00024) as shown in Fig. 5 *C*. After the 110th trial, the frequency was increased to 10 Hz, whereupon the bee abruptly lost the magnetic discrimination, choosing the west

target six times in a row. After this, she made 9 out of 10 correct choices (chance probability 0.011; Fig. 5 *D*) before the end of the experiment.

Fig. 6 is a summary diagram of results from bees run following this protocol, showing the number of trials to criterion as a function of the frequency of the magnetic anomaly. The *, #, and @ symbols indicate that the bee achieved chance probabilities $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, and lines connecting symbols at different frequencies show the three cases where the bee returned long enough for us to shift her to higher frequencies. All bees tested at 0 Hz reached the
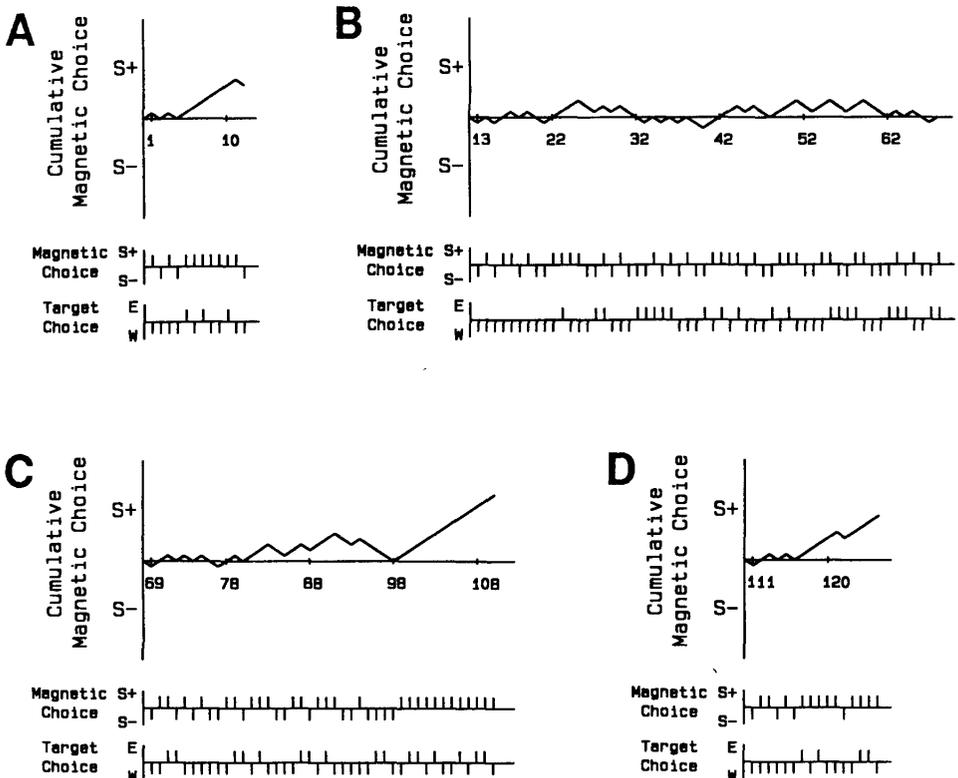

Figure 5. Data from an individual honeybee trained to respond to various frequencies as outlined in the text. (*A*) Testing to a static, 2.2-mT magnetic anomaly. (*B*) Control experiment, exactly as in *A*, but with the circuit interrupted. (*C*) 1-Hz sinusoidally oscillating field, peak amplitude 2.2 mT. Note the string of 12 correct choices in a row near the end of this experimental condition. (*D*) 10-Hz sinusoidally oscillating field, 2.2-mT peak field.

criterion specified by Walker and Bitterman (1989*a*) with a similar distribution of trials. Hence, this replicates their results for this experiment with relatively strong static magnetic anomalies; we have not yet attempted to replicate their measurements of threshold sensitivity. Of nine bees tested at 60 Hz, two did not condition within 32 and 44 visits (shown by the & symbol), whereas the seven others reached criterion performance or better within 20 visits. One bee tested at 80 Hz did not reach criterion within 50 visits.

Although the magnetic effects are clear and significantly nonrandom in our data, we have the subjective impression that we still have not gained proper control of the animal's behavior, particularly at higher frequencies. We allowed several animals that had reached or exceeded criterion performance early in the training sequence to continue visiting the feeders, and found that they often would switch to another pattern of behavior: for example, consistently choosing one target, or simply returning to the last target from which they had been fed.

During the summer of 1991 we focused our efforts on gaining better control of this behavior and improving the experimental design, but so far with only limited success. We now use IR detectors to sense the bee's presence, and computer-controlled solenoid valves with a gravity feed system to add water or 50% sucrose to the feeders after the bee has made her choice. These changes allow both targets to remain identical except for the location of the magnetic anomaly during the choice procedure, and permits the experiment to be run totally by computer control for long
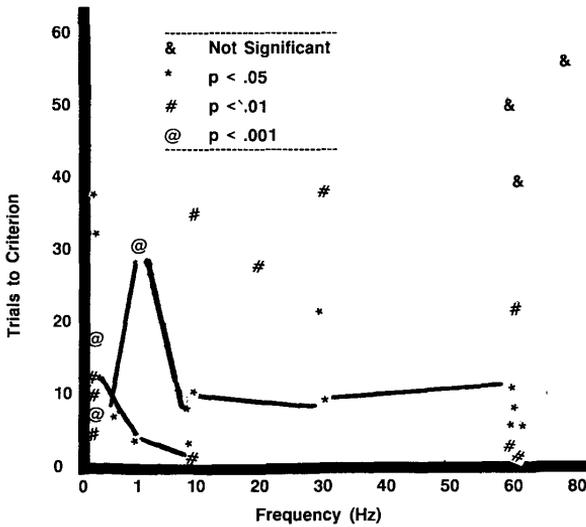

Figure 6. Results from the Fukuoka two-choice conditioning experiments as a function of frequency of the applied field. Solid lines connect responses for an individual bee which reached criterion and was shifted to higher frequency.

intervals. (Operator attention is still necessary, however, as the bee will sometime bring recruits from the hive; the computer has no method of telling one bee from another or destroying the interlopers.) We experimented with the use of an air puff to punish an S− visit, physically blowing the bee out of the target and a meter or so beyond the window, but the learning was not any better. It appears that any deviation from the strict Walker and Bitterman (1989a) protocol does not improve control of the behavior.

Discussion

It is clear that it will be necessary to gain better control of the honeybee's behavior in low frequency oscillating magnetic fields before attempts can be made to place quantitative experimental constraints on their threshold sensitivity. This display of a magnetic response, but the failure to learn it properly, was apparent both in the extinction test and in the two-choice paradigm. It is also similar to the results for

honeybees trained to visit a simple T-maze described by Kirschvink and Kobayashi-Kirschvink (1991), and the failure of stationary honeybees to learn magnetic cues (Walker et al., 1989). Our results to date are broadly consistent with predictions of the physical model outlined above, but without the threshold information more quantitative constraints cannot be placed on it.

Although the natural geomagnetic field does have oscillations in the extremely low and ultra-low frequency range, the amplitude of these signals is usually on the order of a few hundred nanotesla at most, with frequencies confined largely to <10 Hz (e.g., Samson, 1987). The largest low frequency stimuli measured by the bee would be those produced by the motions of the bee relative to either the geomagnetic field or a static anomaly (left frozen in magnetite-bearing rocks by a lightning strike, for example). Hence, there is no *a priori* reason to suspect that the honeybee would use the low frequency information present in the natural geomagnetic field, other than that of the diurnal variation mentioned earlier. Although the low frequency information is being provided to the bee by the receptors, the animals may be responding to it instinctively rather than through a learning process.

The physical model outlined here does have an interesting implication for an experiment reported by Gould et al. (1980). They exposed bees to a strong, 60-Hz oscillating magnetic field in an attempt to scramble the direction of the single-domain crystals. However, no reduction in the magnetic alignment of the horizontal dance was apparent between the control and treated groups. Gould et al. (1980) concluded from this that the receptor was not composed of single-domain magnetite, and suggested that superparamagnetic particles might be involved as the receptor. This was in disagreement with the analysis of Kirschvink (1981), who found that the horizontal dance data of Martin and Lindauer (1977) supported a single-domain receptor. Our simple model of the honeybee receptor outlined here suggests an alternative interpretation for the Gould et al. (1980) experiment, as the 60-Hz frequency they used was clearly slow enough to allow the magnetosome chains to follow the direction of the applied field. If this were the case, the magnetosomes within the chain would not be remagnetized and no change in their behavior would be expected. If repeated using higher frequency fields, the effect anticipated by Gould et al. (1980) should appear, and would help to place constraints on the geometry of the honeybee magnetoreceptor.

Acknowledgments

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