The cell lysates were incubated sequentially with pre-immune sera, polyclonal rabbit antibodies to human IL-1β, or anti-p20 ICE and protein A-agarose. After extensive washes, the adsorbed proteins were eluted by boiling the beads in SDS-PAGE sample buffer and analyzed by PAGE and autoradiography.

13. The ICE homologs and p35 were expressed in E. coli under the control of a pl promoter. ICE-1 (7) and CPI32a (8) contain a polyhistidine linker and were purified by IMAC. p35 was purified for ICE-2. p35 was purified by chromatography on Q-Sepharose, Mono-Q, and Superdex-75.

14. Colorimetric assays were performed in 96-well plates by incubating recombinant ICE, N-acetyl-Tyr-Val-Asp-p-nitroanilide (AcYVAD-pNA) substrate for ICE and N-acetyl-Tyr-Val-Ala-Asp-p-nitroanilide (AcYVAD-pNA) for ICE-2, which were used, respectively.


16. Proteins were separated on gels containing 10 to 20% gradient of acrylamide (Integrated Separations Systems). Native or denatured rabbit anti-ICE serum was used as a marker for autoradiography.


18. Complementary DNA encoding the p32 form of ICE was subcloned into a pBluescript II KS (Stratagene) derivative under the control of the bacteriophage T7 promoter. We constructed a second plasmid with the p35 gene inserted upstream of the ICE gene, creating a bicistronic message for expression in E. coli.

19. The plasmid pHS2P35V1+ contains p35 under the control of the Drosophila Hsp70 promoter in a pBluescript-based (Stratagene) plasmid vector (2). This promoter is constitutively transcribed upon transfection in SF-21 cells and can be stimulated further by heat shock (2,3). Plasmid pHS3P35V1+ is identical to pHS2P35V1+ except that it contains p32 ICE instead of p35. SF-21 cells or TN-368 cells (5 x 10^6 in 6 x 10^-5 per 35-mm dish) were transfected with plasmid DNA with the use of Lipofectin (Bethesda Research Laboratories). At 20 hours posttransfection, the cells were heat-shocked for 30 min at 42°C. The media were removed to 12 hours after heat shock, the cells were resuspended in 500 μl of phosphate-buffered saline containing 0.04% trypan blue, and viable cells were counted with a hemocytometer. Transfections were repeated in triplicate and viable cells in four grids of the hemocytometer were counted for each replicate.

20. S. Seshagiri, unpublished results. SF-21 or TN-368 cells were transfected with pBluescript, pHS3P35V1+, and pHS2P35V1+ and total DNA was isolated, electrophoresed through an agarose gel, and visualized by ethidium bromide staining.


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**TECHNICAL COMMENTS**

Honeybees and Magnetoreception

Chin-Yuan Hsu and Chia-Wei Li (1) propose that magneitite particles in the fat body of honeybees are part of a magnetic field sensory system used for navigation. They make the following statements that we think are in error or may have other interpretations.

1) Honeybees contain iron "granules" only in the fat body. Iron granules have been described in honeybee midgut as well as the fat body (2). They are mineral concretions in vacuoles derived from the rough endoplasmic reticulum (RER) and form in response to iron in pollen in the diet.

2) Iron in honeybee fat body occurs in particles 7.5 nm in diameter. We have identified iron in holoferritin in the RER of many insects. Holoferritin has dense cores 7.5 nm in diameter (3,4), the size of the particles mentioned by Hsu and Li. The dense masses in the iron granules look like hemosiderin degradation products. At least Li reported iron-containing structures in other insects (5). Hsu and Li make no mention of ferritin or hemosiderin.

3) The fat body is innervated. Their scanning electron micrograph [figure 2B in (1)] illustrates innervation of a fat body shows a filamentous structure resembling a trachea. Hsu and Li do not mention the tracheae and tracheoles commonly observed on the surface of the fat body–oocyst complex, or how they may be distinguished from nerves in scanning electron micrographs. Innervation of insect fat body has not been described. Should the honeybee fat body–oocyst complex prove an exception, it may be related to the control of wax secretion rather than to magnetotaxis. The transmission electron micrograph of a nerve ending in a fat body [figure 2C in (1)] shows the synaptic vesicles appropriate for an excitatory nerve rather than the sensory nerve required for a magnetotactic organ.

4) Nerve endings in the fat body are stimulated through a transducer mechanism. We could find no structure corresponding to the proposed transducer complex needed to convert intracellular stress to extracellular nerve action potentials.

5) The granules are attached to cytoskeletal elements. They are not a form of hemo-activated part of the transducer complex. Honeybee vacuoles have no special attachment to cytoskeletal filaments (Fig. 1). Their bounding membranes resemble those of the rest of the RER. Preparations without osmication to preserve the cytoskeleton show filaments between mouse pancreas lamellate ER (6), but not around honeybee iron-containing vacuoles. Iron concretions are abundant throughout honeybee fat body, as would be expected...
ed for an insect subject to iron overload from the ingestion of iron-rich pollen, a major component of their diet. Bees on a pollen-poor diet lack fat body granules, but continue to contain some holoferritin (2). We found that the fat body of foraging honeybees contained vacuoles at all stages forming from the RER and the nuclear envelope, as would be expected if iron sequestration is a response to iron in the diet.

It appears to us that Hsu and Li may have misinterpreted their observations of honeybee fat body. In particular, they do not discuss the role of ferritin and the general function of insect fat body in iron metabolism (3). Holoferritin- and iron-containing granules are present in many insect tissues, especially when dietary iron is high or experimentally elevated. In honeybees the appearance of such granules is consistent with diet and involvement of the fat body with ferritin and its degradation products.

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Studies of honeybee magnetoreception that provide anatomical and biophysical constraints on the insect receptor system contradict the conclusions of Hsu and Li in their report about iron granules in the abdominal trophocytes of honeybees (1). First, studies using ultrasensitive superconducting quantum-interference device magnetometers and transmission electron microscopy of extracts indicate that magnetite in honeybees is located primarily in the anterior, dorsal region of the abdomen, not in each abdominal segment as are the iron granules. This includes crystals of both single-domain (2) and superparamagnetic (3) size, as well as ordered sheets containing arrays of 15- to 20-nm superparamagnetic particles with electron diffraction patterns of magnetite (4).

Second, because large numbers of closely interacting superparamagnetic particles will display net magnetic behavior typical of larger grains (5), the magnetite particles postulated by Hsu and Li should have been detected by low-temperature magnetic warming experiments (3). The volume of superparamagnetic magnetite ($4.4 \times 10^{-13}$ ml) observed to be in each granule (1) should produce a moment of $1.1 \times 10^{-15}$ Am$^2$ in these experiments. Because the total volume of the iron granules per bee is $2.5 \times 10^{-5}$ ml (6) and each granule has a volume of $1.13 \times 10^{-13}$ ml (1), each bee should have a total of about $2.2 \times 10^8$ iron granules. This should produce a moment of $2.3 \times 10^{-7}$ Am$^2$, compared to the much lower measured value of $2 \times 10^{-9}$ Am$^2$ (3). Hence, we doubt that the crystalline material identified by Hsu and Li (1) is magnetite.

Third, behavioral experiments with small magnetized wires glued at various locations on the surface of free-flying bees have explicitly tested the hypothesis that ferromagnetic magnetoreceptors are located in the anterior, dorsal region of the abdomen (7). Magnets positioned in this location impair the ability of the bees to respond to magnetic cues in discriminative choice experiments, whereas nonmagnetic control wires, or magnets positioned elsewhere (7), have no effect. Hence, we find no evidence to support the conclusion of Hsu and Li (1) that the ventral abdominal trophocytes could function as magnetoreceptors in honeybees.

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Hsu and Li (1) conclude that some of the iron-containing granules present in the trophocytes of the honeybee abdomen contain superparamagnetic crystallites of magnetite. However, earlier studies by Kuterbach et al. (2) and by Hsu and Li (3) did not produce detectable electron diffraction patterns from these granules. Hsu and Li (1) do not present any electron diffraction pattern from the imaged crystallites. Their evidence for the presence of magnetite is based on Fourier transform optical analysis of the lattice fringes observed in their high-resolution transmission electron micrograph images [figure 1, B and C, in (1)]. Attempts to index the optical diffraction pattern from figure 1D in their report are necessarily crude; nevertheless, I have been unable to make the entire pattern consistent with a single crystal pattern from magnetite. Assuming that the electron microscope was accurately calibrated, relatively strong diffraction maxima with a spacing of 0.24 to 0.25 nm are evident. Although these maxima were indexed as 113,311, this single d-spacing is by itself insufficient to positively identify magnetite, much less distinguish between magnetite and other ferrimagnetics, maghemite, or hematite.

That Hsu and Li did not obtain any electron diffraction pattern, despite the demonstration of lattice fringes, requires explanation. For a highly ordered crystal structure, the information in the sample that is converted into an image of the lattice fringes is the same as that which can be transformed into an electron diffraction pattern by an alternative arrangement of lens currents in the electron microscope. Hsu and Li estimate that the volume of "superparamagnetic magnetite" (that is, the crystalline domains) per granule is $4.4 \times 10^{-13}$ cm$^3$, which is equivalent to a sphere more than 200 nm in diameter. Such a quantity of magnetite might be expected to provide ample material to produce a strong electron diffraction pattern. I readily obtained characteristic magnetite single crystal electron diffraction patterns from individual 40-nm magnetosomes in plastic-embedded sections of the magnetotactic bacterium Magnetospirillum magnetotacticum (unpublished results). The random arrangement of the individual crystallites within the honeybee iron granule should lead to the production of a typical powder pattern.

The formation of the honeybee iron granules by aggregation of 7.5-nm electron-
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