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11. The coil system consisted of two different coils arranged orthogonally (*7*). Each coil measured 2.27 m on a side and was constructed in accordance with the four-coil design of Merritt *et al.* (*26*). Turtles were restricted to an area in the center of the coil defined by a horizontal circle of radius 25 cm and a vertical area of about 5 cm; in this region, calculated (*26, 27*) and measured deviations from perfect field uniformity were less than 1%. For details about the coil, arena, and tracking system, see *Science* Online (www.sciencemag.org/cgi/content/full/294/5541/364/DC1).
12. Methods were described in detail in (*7*). Briefly, hatchlings were collected from their nests on the night when they would otherwise have emerged. Each was tethered in the arena and permitted to swim for 10 to 30 min in the field of the natal beach (inclination 57.5°, intensity 47 μT) toward a dim light in the east, a process that serves to set the initial offshore magnetic heading of the turtles (*28*). The light was then turned off and the field immediately changed to one of the three experimental fields (see below). After an acclimation period of 3 min, a computer monitored the direction toward which each turtle swam in darkness under the new field condition (*7*). Each turtle was tested only once under one of the three field conditions; no more than four turtles from the same nest were tested in any given field. The field used to approximate magnetic conditions near northern Florida had an inclination of 59.3° and a total intensity of 49.1 μT (as assessed by five independent measurements with a Schoenstedt digital fluxgate magnetometer, model DM-2220 R). The field used to approximate conditions in the northeastern gyre had an inclination of 59.1° and an intensity of 45.2 μT; the field simulating the southern border of the gyre had an inclination of 16.7° and an intensity of 31.0 μT. The experimental fields were selected on the basis of estimates provided by the International Geomagnetic Reference Field (IGRF) model, 1995 revision, for July and August 1995 (when the data were collected) using latitude 29.0°N, longitude 80.0°W for northern Florida; 43.0°N, 20.0°W for the northeastern gyre region; and 10.0°N, 39.0°W for the southern gyre boundary. The field measured within the arena was within ±0.4° (inclination) and ±0.4 μT (intensity) of the IGRF estimates for each target location. The IGRF declination estimates for the target locations were −5.2° for northern Florida, −10.4° for the northeastern gyre, and −18.7° for the southern gyre. Experiments were conducted in Boca Raton, Florida (declination estimate = −4.6°).
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20. An interesting speculation is that some of the variation in responses of different turtles to the same field (Fig. 1) might reflect diversity in genes affecting factors such as which fields elicit responses or which direction a turtle swims when a given field is encountered. By producing offspring with variable responses, adult turtles might increase the likelihood that some progeny will survive even if Earth's field changes significantly.
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Neuroanatomy of Magnetoreception: The Superior Colliculus Involved in Magnetic Orientation in a Mammal

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The neural substrate subserving magnetic orientation is largely unknown in vertebrates and unstudied in mammals. We combined a behavioral test for magnetic compass orientation in mole rats and immunocytochemical visualization of the transcription factor c-Fos as a marker of neuronal activity. We found that the superior colliculus of the Zambian mole rat (*Cryptomys anselli*) contains neurons that are responsive to magnetic stimuli. These neurons are directionally selective and organized within a discrete sublayer. Our results constitute evidence for the involvement of a specific mammalian brain structure in magnetoreception.

Behavioral studies have provided abundant evidence for magnetic compass orientation among vertebrates, but its sensory and neural basis remains enigmatic (*1, 2*). A few electrophysiological studies have addressed the involvement of a specific brain structure in the processing of magnetic information (*3–9*). This method, however, has a particular drawback: It does not allow systematic screening of neuronal activities in the central nervous system. Therefore, well-aimed electrophysiological studies cannot be conducted in the absence of a known receptor site. Here, we investigated magnetoreception by combining two established methodological approaches: a behavioral test designed to assess magnetic compass orientation in mole rats (*10, 11*) and immunocytochemical visualization of the transcriptional regulatory protein c-Fos as a marker of neuronal activity, a neuroanatomical technique used extensively in sensory research (*12–14*).

We detected the evoked expression of c-Fos in order to map neuronal activities that had been entrained either by active orientation via the magnetic compass or by changes in the ambient magnetic field. Experimental animals built nests in an unfamiliar arena [i.e., performed a magnetically based spatial orientation task (*15*)] under different test conditions (*16*). Controls (used also to assess basal levels of c-Fos expression) were of two types: (i) untreated animals freely moving within a familiar home area, and (ii) animals resting or sleeping in a shielded magnetic field. We focused on neuronal activities in the superior colliculus (SC), a prominent subcortical sensorimotor integrator that plays an important role in orientation to diverse stimuli (*17–19*). The unique intrinsic circuitry of the SC (*20*) may serve to integrate magnetic information with multimodal sensory and motor information. Magnetic stimuli thus may directly elicit orientation responses via initiation of activity in the premotor efferent collicular pathways.

The SC in all of the experimental and control animals displayed a symmetrical

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bilateral distribution of c-Fos immunoreactivity (*21*) (Fig. 1, A to F). The density and distribution pattern of immunoreactive cells, however, differed markedly between animals subjected to different experimental conditions [Figs. 1 and 2; Web figs. 1 to 3 (*22*)]. In control animals resting or sleeping in the shielded magnetic field, basal expression of c-Fos was very low and only a few randomly scattered immunoreactive neurons were observed [Fig. 1F; Web fig. 1F (*22*)]. In control animals active in their home cages, c-Fos expression always remained moderate; labeled cells were more numerous than in the SC of resting animals, but were still randomly scattered and devoid of any obvious pattern of alignment [Fig. 1E; Web fig. 1E (*22*)]. In contrast, the SC of experimental animals invariably showed large numbers of heavily immunostained cellular nuclei, arranged in tangentially oriented bands aligned with collicular layers or sublayers [Fig. 1, A to D; Web fig. 1, A to D (*22*)].

In both the outer sublayer of the intermediate gray layer (InGo) and the deep gray layer (DpG), the density of immunopositive cells was increased significantly in all experimental groups, irrespective of experimental conditions [Web figs. 2 and 3 (*22*)]. Density differences between experimental groups were, however, much less pronounced and mostly statistically insignificant. The distribution pattern of labeled cells was common to all experimental groups (Fig. 1, A to D). Therefore, increased neuronal activity in those two layers is likely an unspecific novelty response to the unfamiliar environment (information input from nonmagnetic sensory and motor systems likely accounted for the activation).

In contrast, in the inner sublayer of the intermediate gray layer (InGi), both the density and distribution pattern of immunoreactive cells correlated significantly with physical properties of the magnetic field (Figs. 1 and 2). This sublayer is distinguished by rather irregularly distributed cells and by the frequent occurrence of large, dark multipolar neurons in Nissl- or Klüver-Barrera–stained sections [Web fig. 4 (*22*)]. In animals building their nests in the constant magnetic field, very strong but focal immunoreactivity was detected in the mediorostral part of the SC [Fig. 1A; Web fig. 1A (*22*)]. The extent of the labeling was about 300 to 600 μm × <1000 μm (rostrocaudal × mediolateral dimension). Tightly packed, darkly labeled neurons were distributed within the InGi in a patchy manner, forming two or three clusters. Animals building their nests in the periodically changing magnetic field exhibited comparatively weaker staining [Fig. 1, B and C; Web fig. 1, B and C (*22*)]. The area of labeled neurons, however, was significantly larger, spanning almost completely the InGi throughout the rostral half of the SC (about 1200 μm × 2000 μm, in rostrocaudal × mediolateral dimension). More widely spaced immunopositive cells formed five or six clusters that were less compact and consisted of a smaller number of labeled cells. Finally, in animals building their nests in the shielded magnetic field, only a few scattered immunoreactive cells were found in the InGi [Fig. 1D; Web fig. 1D (*22*)].

Neuronal activation within the InGi was related to the presence of a perceptible magnetic field of about the strength of Earth's field. Changes in the polarity of the magnetic field led to the activation of increasing numbers of collicular compartments, but activation within individual compartments was less pronounced, as indicated by both lower intensity of immunostaining and lower density of immunoreactive cells.

Because mole rats use a polarity compass for orientation (*10*), one would expect that the change of field polarity stimulates their magnetosensory system. We therefore expected increases in c-Fos expression pro-

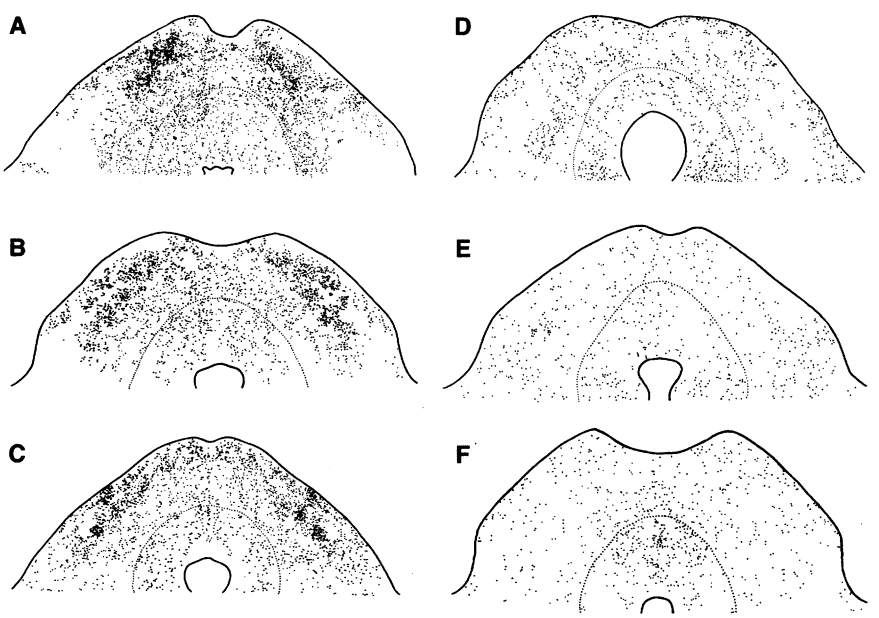

Fig. 1. Characteristic distribution patterns of c-Fos–immunoreactive neurons in the SC of Zambian mole rats subjected to different experimental conditions. (**A** to **D**) Nesting in unfamiliar circular arena: (A) natural (constant) magnetic field; (B and C) experimental magnetic field, the horizontal component of which was manipulated every 5 min (B) and every second (C); (D) shielded magnetic field. (**E**) Movement within home cage, natural magnetic field. (**F**) Inactivity, shielded magnetic field. Each dot represents a single labeled neuronal nucleus.

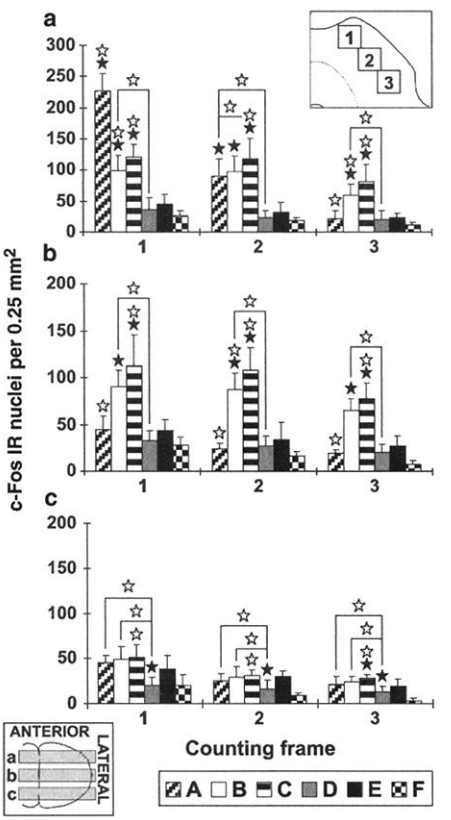

Fig. 2. Mean numbers of c-Fos–immunoreactive (IR) neurons (±SEM) in the InGi (*30*). Density counts are from the rostral (**a**), middle (**b**), and caudal (**c**) parts of the SC; see Fig. 1 for coding of experimental conditions A to E. Significant differences ($P < 0.05$) are indicated by stars. Solid stars indicate comparison with control group E; open stars indicate comparison with adjacent experimental group on the right or with another experimental group when so indicated. Upper right inset shows position of individual counting frames; lower left inset shows approximate levels of counting.

portional to the frequency of polarity changes. However, this was not the case: In trials with changing polarity, the intensity of expression decreased, whereas the area involved expanded. We propose the following explanation for this phenomenon: (i) The presence of a magnetic field of a given polarity, rather than a change of polarity, represents a relevant stimulus; (ii) neurons of individual collicular compartments respond only to magnetic fields with a distinct range of polarity; and (iii) compartments responding to different field polarities (or, under natural conditions, to different orientations of the animal toward the polarity) are distributed systematically within the InGi. Such an arrangement can account for spatial and temporal segregation of neuronal activities when magnetic field polarity is periodically manipulated, and thus it can account for periodicity of excitatory influence on neurons of individual compartments. It seems likely that, under such conditions, only smaller numbers of neurons per compartment could attain a level of activity above the threshold required for immunocytochemical detection. Our findings thus suggest that, as in the case of other sensory modalities (*20*), the magnetosensory input is also organized in a topographical map of external sensory space within the mole rat SC. Experiments with immobilized animals are needed to provide direct evidence for the existence of such a magnetotopic map.

Our data show that the SC of mole rats contains populations of neurons that are responsive to magnetic stimuli and that it is involved in the neural processing of magnetic information. As such, our work offers experimental evidence that a specific brain structure serves neural processes underpinning magnetic compass orientation in mammals. Our experiments also have methodological implications. Detection of immediate early gene expression may be useful for identifying neurons that have been activated by magnetic stimuli. Such a method could be used to screen for neuronal activities throughout the central nervous system.

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15. We have shown (*10*, *11*) that recognition of an unfamiliar space is coupled with retrieval of available scattered nesting materials or food items and building of a nest or food cache, apparently as a reference point for further spatial orientation. In the absence of any constant external cues but the magnetic field, these naturally blind animals significantly prefer to position their nests in the southeast sector of the arena. When magnetic north is turned by means of Helmholtz coils, mole rats change the nest position accordingly (*11*). Subsequent experiments (*10*) have yielded further evidence for the magnetically based orientation and showed that the magnetic compass of mole rats is a polarity (i.e., not inclination) compass. The preference for building a nest in the southeast direction is an inborn, spontaneous species-specific trait. After the animals become familiar with their (relatively small) home cages or mazes, they rely on kinesthetic and olfactory orientation.
16. Twenty-four adult Zambian mole rats (*Cryptomys anselli*, Bathyergidae, Rodentia) of both sexes were examined. All the experiments were performed on freely moving animals that had been kept in sibling pairs to avoid social stress through isolation. The animal husbandry and all of the experimental procedures were approved by an institutional animal care and use committee. Sixteen animals were used in arena experiments. They were released in a circular plastic arena (diameter 82 cm) filled with a thin layer of horticultural peat as litter, scattered slips of tissue paper as nest material, and randomly distributed pieces of carrots as food. Animals were allowed to build their nests and food caches for 1 hour. Immediately afterward, the animals were anesthetized and killed. Arena experiments were performed under three test conditions: (i) in the natural geomagnetic field (magnetic north = 360°, intensity = 47 μT, inclination = +66°; eight animals); (ii) in an experimental magnetic field, the horizontal component of which was turned by 120° either every 5 min or every second (field polarity was manipulated by a pair of Helmholtz coils, magnetic north = 360° or 240°, intensity = 47 μT, inclination = +66°; four and two animals, respectively); and (iii) in an experimentally shielded magnetic field (with an arena placed in a Mu-metal chamber, magnetic north = 360°, intensity = 300 nT, inclination = +66°; two animals). Six untreated animals freely moving within their home cages (i.e., in a familiar area) under conditions of the natural geomagnetic field were used as controls. Two other control animals were kept under conditions of the shielded magnetic field for 14 days (their home cage was placed in a Mu-metal chamber). They were anesthetized and killed after a period of inactivity, such as rest or sleep.
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20. The inputs from various functional systems (visual, auditory, somatosensory, striatonigral, and cerebellar) are confined to various (sub)layers of the SC (*23*, *24*) and are organized as superimposed topographical maps of the external sensory space or as corresponding representations of eye, head, or pinna movements (*17–19*, *23–25*). Integration of sensory and motor information takes place in compartmentalized (*26*, *27*), multimodal (*17–19*, *23–25*), deeper layers of the SC. Compartmental (modular) architecture of these layers facilitates spatial segregation of afferents related to sensory and motor systems and, in turn, their integration on dendritic shafts of efferent neurons situated in characteristic positions relative to the compartmental (modular) matrix (*28*). The latter neurons are the cells of origin of premotor collicular pathways that terminate in many motor areas of the brainstem and the spinal cord and mediate spatially appropriate orientation responses.
21. Animals were deeply anesthetized with diethyl ether and then transcardially perfused with heparinized saline followed by fixative [4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4]. Perfused animals were decapitated and their brains were dissected, postfixed for 4 hours in the same fixative, and transferred to sucrose buffer (30% sucrose in PB) for cryoprotection. Before sectioning, the brains were embedded in sucrose-gelatin [30% sucrose, 10% gelatin (300 bloom) in distilled water]. The gelatin blocks were fixed in sucrose-paraformaldehyde solution (30% sucrose, 4% paraformaldehyde in PB) and trimmed, and the brains were sectioned on a cryotome in the coronal plane at a thickness of 60 μm. Free-floating sections were incubated in 0.3% H_2O_2 for 30 min, rinsed in phosphate-buffered saline, and then incubated for 12 hours at room temperature in primary polyclonal rabbit antibody to c-Fos (*29*). The following procedures involved incubation for 90 min in the secondary antibody, incubation for 2 hours in avidin-biotin-peroxidase complex, and incubation in 0.05% diaminobenzidine solution for 5 min followed by addition of 660 μl of 3% H_2O_2 per 100 ml of solution for 2 min. After stopping the reaction, sections were rinsed in PB, mounted on chromalum-coated slides, dried overnight, and coverslipped. Control sections were incubated with normal rabbit serum or with primary antibody preabsorbed with native peptide (0.1 μg/ml), both of which prevented all nuclear staining. Every fourth section was stained for cresyl violet and used for general orientation. Other Nissl- and Klüver-Barrera–stained, serially sectioned mole rat brains were available for comparative purposes.
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30. Numbers of c-Fos–immunoreactive neurons were quantified in the InGo, InGi, and DpG within the rostral, middle, and caudal parts of the SC. At 100× magnification, the number of immunopositive neuronal nuclei in a standard-size frame (0.5 mm by 0.5 mm for the InGi; 0.25 mm by 0.25 mm for the InGo and the DpG) was counted blind with image analysis software. Within a single section, c-Fos–positive nuclei were counted bilaterally in the dorsomedial, central, and ventrolateral parts of each mentioned layer. Counts were averaged over three representative sections, over the two sides of the brain, and across animals within the different experimental groups. Comparisons between groups were statistically analyzed using the Duncan multiple range test with a 0.05 level of significance.

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