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Bat Head Contains Soft Magnetic Particles: Evidence From Magnetism

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Recent behavioral observations have indicated that bats can sense the Earth's magnetic field. To unravel the magnetoreception mechanism, the present study has utilized magnetic measurements on three migratory species (*Miniopterus fuliginosus*, *Chaerephon plicata*, and *Nyctalus plancyi*) and three non-migratory species (*Hipposideros armiger*, *Myotis ricketti*, and *Rhinolophus ferrumequinum*). Room temperature isothermal remanent magnetization acquisition and alternating-field demagnetization showed that the bats' heads contain soft magnetic particles. Statistical analyses indicated that the saturation isothermal remanent magnetization of brains ($SIRM_{1T\_brain}$) of migratory species is higher than those of non-migratory species. Furthermore, the $SIRM_{1T\_brain}$ of migratory bats is greater than their $SIRM_{1T\_skull}$. Low-temperature magnetic measurements suggested that the magnetic particles are likely magnetite (Fe_3O_4). This new evidence supports the assumption that some bats use magnetite particles for sensing and orientation in the Earth's magnetic field. Bioelectromagnetics 31:499–503, 2010. © 2010 Wiley-Liss, Inc.

Key words: bat; magnetoreceptor; magnetic measurements; migratory; non-migratory

INTRODUCTION

As the only volant mammals, most bats possess an echolocation system to orient and hunt prey in the area around their roosts. However, like birds, bats often fly large distances beyond their typical range in daily life, and some need to migrate hundreds of kilometers between their breeding and wintering roosts each year [Altringham, 1996]. The echolocation system alone is inadequate for orientation and navigation over such long distances: the effective range of echolocation is ~20 meters [Kick, 1982; Lawrence and Simmons, 1982]. Therefore, other potential sensory cues might participate to guide the bats' orientation and navigation in flight.

Recent studies have revealed that bats can orient by using a magnetic compass based on the polarity of magnetic field [Holland et al., 2006; Wang et al., 2007], which suggest that magnetic cues play a key role in the bats' orientation and navigation processes, similar to other animals [Kirschvink et al., 1985]. However, the mechanism of detecting the Earth's magnetic field is unknown. Two biophysical mechanisms are currently hypothesized in animals, one based on a light-dependent mechanism [Schulten et al., 1978; Ritz et al., 2000], the other on biogenic iron-mineral particles [Kirschvink et al., 1985; Kirschvink, 1989; Wiltschko and Wiltschko, 2006]. Recently, a "Kalmijn-Blakemore" pulse-remagnetization experiment indicated that bats may use magnetite particles to perceive the Earth's magnetic field [Holland et al., 2008].

Based on previous studies of other animals, biogenic magnetite particles were identified in animals' heads. For example, the magnetite particles were found in the nasal cavities of salmonid fish [Walker et al., 1997; Diebel et al., 2000] and the upper-beak skin of pigeons [Hanzlik et al., 2000; Fleissner et al., 2007; Tian et al., 2007]. However, there is less evidence about the presence of biogenic magnetic particles in bats. The purpose of the present study is to use sensitive superconducting quantum interference device (SQUID) magnetometers to investigate if biogenic

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magnetic particles (e.g., magnetite) exist in the heads of bats.

MATERIALS AND METHODS

Six species of bats were investigated, including three migratory species and three non-migratory species. Migratory bats included *Miniopterus fuliginosus* (Chiroptera: Vespertilionidae, n = 4), *Chaerephon plicata* (Chiroptera: Molossidae, n = 4), and *Nyctalus plancyi* (Chiroptera: Vespertilionidae, n = 2). Non-migratory bats included *Hipposideros armiger* (Chiroptera: Hipposideridae, n = 5), *Myotis ricketti* (Chiroptera: Vespertilionidae, n = 4), and *Rhinolophus ferrumequinum* (Chiroptera: Rhinolophidae, n = 4).

Animal care and all procedures were approved in accordance with guidelines of the Animal Care and Use Committee of the Chinese Academy of Sciences. Although the perfusion method is usually helpful to clear out the blood influence, our trial test showed that the blood of bats exhibits paramagnetic behavior and has no contribution to remanent magnetization of the measured tissue samples. So, we did not perform perfusion treatments to the bats in this study. The bats were sacrificed by decapitation, and their heads were skinned using non-magnetic titanium blades and forceps. The skinned heads were rinsed several times using ultrapure water (18 MΩ cm resistivity) and further dissected into two parts: soft brain tissues and hard skulls with connective muscles. Samples were immediately freeze-dried to prevent possible biochemical alterations. All sample containers were non-magnetic and cleaned with 2 M HCl before use.

Stepwise acquisition of isothermal remanent magnetization (IRM) at room temperature was conducted on 42 samples using a pulse magnetizer (2G Enterprises, Pacific Grove, CA) in 21 steps up to 1 T. The remanence was measured on a 2G Enterprises cryogenic magnetometer (Model 755R; magnetic moment sensitivity 10^{-12} Am^2) installed in a magnetic-shielded room (with a residual field <300 nT). The saturated IRM acquired at 1 T ($SIRM_{1T}$) was then stepwise demagnetized using a 2G Enterprises alternating field (AF) demagnetizer (Model 2G-600) up to a peak field of 80 mT. The remanence of each sample container and cotton, which was used to fix the sample, was subtracted.

Kruskal–Wallis non-parametric test was used for statistical analyses on measured $SIRM_{1T}$ values by using Statistical Analysis Software (Version 9.1, SAS Institute, Cary, NC). Differences were considered significant at $P \leq 0.05$.

Low-temperature magnetic measurements were performed on one *M. fuliginosus* sample, one *H. armiger* sample, and two *N. plancyi* samples using a MPMS SQUID magnetometer (Model XP-5, Quantum Design, San Diego, CA; magnetic moment sensitivity 5.0×10^{-10} Am^2). Thermal demagnetization of low-temperature saturation isothermal remanent magnetizations ($SIRM_{5K}$), acquired in a field of 5 T at 5 K after "zero-field cooling," was measured. Specifically, samples were cooled from 300 to 5 K in a zero field, and then a $SIRM_{5K}$ was acquired and remanence was measured during warming from 5 to 300 K at intervals of 2.5–5 K. The net remanence at each step was obtained by subtracting the contribution of the used capsule container.

RESULTS AND DISCUSSION

The room temperature IRM acquisition curves and AF demagnetization curves of $SIRM_{1T}$ of migratory and non-migratory bat samples are shown in Figure 1. All measured samples reached saturations by a 200 mT field. The net $SIRM_{1T}$ varied from 0.1 to 2.91×10^{-5} Am^2/kg (mean $(1.12 \pm 0.80) \times 10^{-5}$ Am^2/kg) for brain samples, and 0.13 to 2.17×10^{-5} Am^2/kg (mean $(0.60 \pm 0.52) \times 10^{-5}$ Am^2/kg) for skull samples. AF demagnetization of $SIRM_{1T}$ decays rapidly below 30 mT. This suggests that the remanence carriers in the measured samples are magnetically soft minerals (probably magnetite) [Dunlop and Özdemir, 1997; Hautot et al., 2003].

Comparison of the net $SIRM_{1T}$ values of brain samples ($SIRM_{1T\_brain}$) with skull samples ($SIRM_{1T\_skull}$) is shown in Figure 2. Specifically, for the migratory species, $SIRM_{1T}$ of the brain samples varied from 1.04 to 2.91×10^{-5} Am^2/kg with a mean of $(1.88 \pm 0.78) \times 10^{-5}$ Am^2/kg, while $SIRM_{1T}$ of the skull samples were between 2.65×10^{-6} and 2.17×10^{-5} Am^2/kg with a mean of $(0.64 \pm 0.63) \times 10^{-5}$ Am^2/kg. For the non-migratory species, both $SIRM_{1T\_brain}$ and $SIRM_{1T\_skull}$ were generally lower than 1.0×10^{-5} Am^2/kg (Fig. 2). Statistical analyses on $SIRM_{1T}$ values revealed that (i) the $SIRM_{1T\_brain}$ values of migratory species were significantly higher than those of non-migratory species ($P < 0.05$), but their $SIRM_{1T\_skull}$ values did not differ significantly from those of non-migratory bats; (ii) the $SIRM_{1T\_brain}$ values had no significant interspecies differences within migratory and non-migratory bats; (iii) the $SIRM_{1T\_brain}$ values of migratory bats were also significantly greater than their $SIRM_{1T\_skull}$ values ($P < 0.05$), however, this was not true for the non-migratory bats. Therefore, it is reasonable to speculate that the brains of migratory bats contain a higher concentration of magnetic minerals.

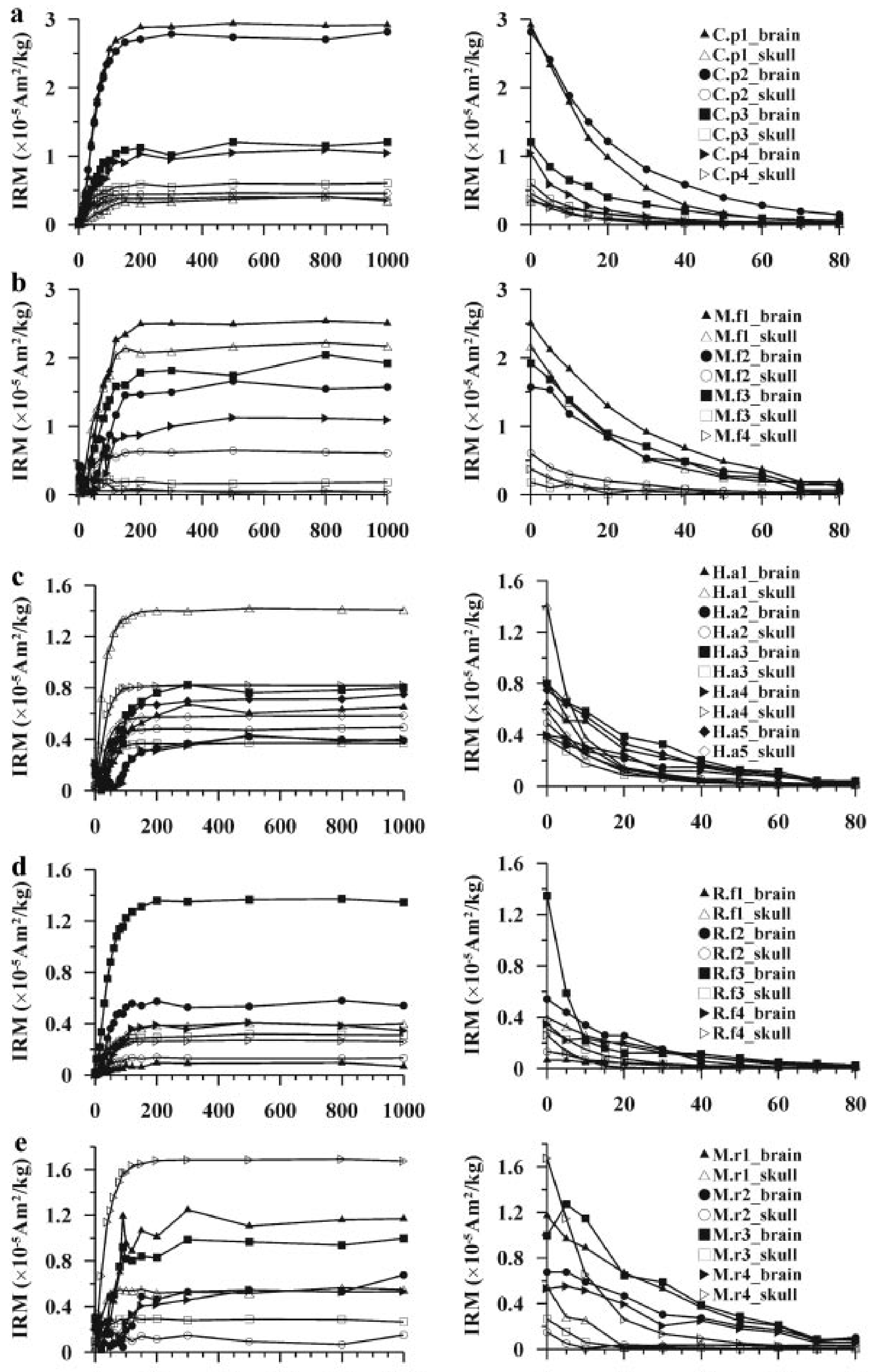

Fig. 1. Room temperature saturation remanent magnetization acquisition curves (left column) and AF demagnetization curves (right column) of five species (migratory species *C. plicata* (**a**) and *M. fuliginosus* (**b**); non-migratory species *H. armiger* (**c**), *R. ferrumequinum* (**d**), and *M. ricketti* (**e**)). Filled and open symbols stand for the brain and skull samples, respectively. Background signals from the sample containers and cotton were subtracted. C. p1, p2, p3, and p4 refer to different individuals of *C. plicata*.

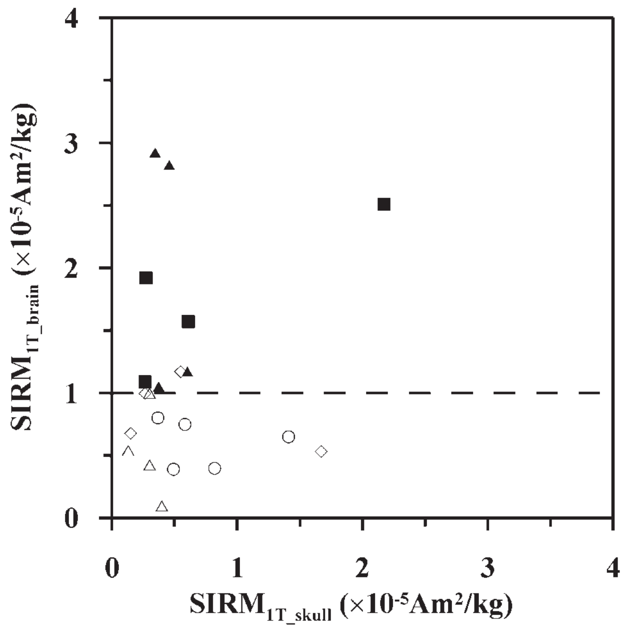

Fig. 2. Comparison of saturation remanent magnetization acquired at a field of 1 T ($SIRM_{1T}$) of 21 measured individual bat samples. $SIRM_{1T\_brain}$ and $SIRM_{1T\_skull}$ refer to brain and skull samples, respectively. Filled and open symbols stand for migratory bats and non-migratory bats, respectively. Filled square, *M. Fuliginosus*; filled triangle, *C. plicata*; open diamond, *M. ricketti*; open triangle, *R. ferrumequinum*; open circle, *H. armiger*.

The demagnetization curves of $SIRM_{5K}$ of brain and skull samples from migratory *M. fuliginosus*, *N. plancyi*, and non-migratory *H. armiger* individuals are shown in Figure 3. The $SIRM_{5K}$ rapidly decayed from 5 to 20 K, which indicates superparamagnetic (SP) particles. With further temperature increases, the remanence slightly drops at around 110–120 K, indicative of the Verwey transition of magnetite.

It has been demonstrated by previous behavioral observations that bats can sense the polarity of the Earth's magnetic field during orientation [Wang et al., 2007] and respond to the pulse remagnetization [Holland et al., 2008]. For some bat species, ocular vision may play a role in homing processes [Williams and Williams, 1967; Serra-Cobo et al., 2000]. However, most bats have relatively poor eyesight and fly under low-light conditions, which result in limited utilization of the light-based magnetoreception. Low-temperature measurements indicated that most magnetite particles lie within the SP grain-size range. Based on this, we assume that they could have similar magnetoreception processes to that of homing pigeons [Beason, 2005; Davila et al., 2005; Wiltschko et al., 2009]. However, how bats sense the magnetic field using those magnetite particles has yet to be well demonstrated.

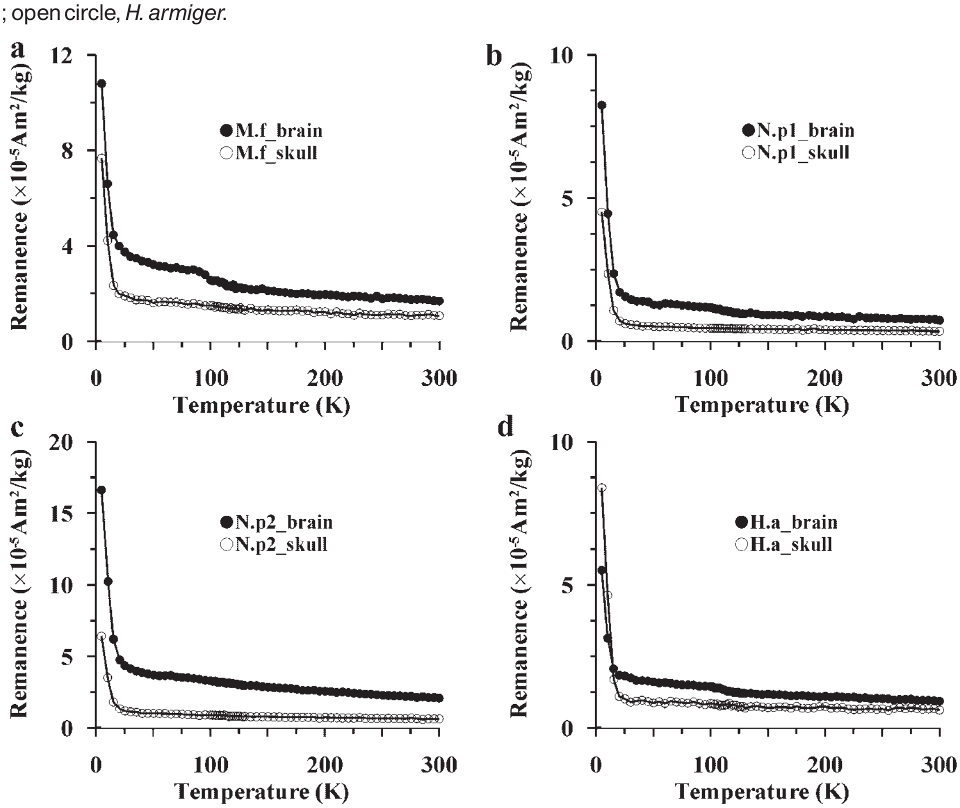

Fig. 3. Thermal demagnetization curves of $SIRM_{5K}$ (acquired in a field of 5 T at 5 K) of the brain and skull samples of migratory species *M. fuliginosus* (**a**), *Nyctalus plancyi* (**b**,**c**), and non-migratory species *H. armiger* (**d**) after zero-field cooling.

CONCLUSIONS

The magnetic analyses in this study have indicated that the brains of the studied bats contain soft magnetic particles (magnetite), which might be used in their magnetoreception. As evidenced by $SIRMs_{1T}$ values, we found that brains of migratory species have significantly higher magnetic particle contents than those of non-migratory species, which may be related to the migratory behavior of these species. In view of electrophysiological and neuroanatomical methods used in the study of magnetoreception in birds, the process of magnetoreception in bats (i.e., how magnetic information is mediated to the nervous system) is a topic that requires further study.

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