Does avian magnetoreception rely on both magnetite and maghemite?

Michael Winklhofer\textsuperscript{1} and Joseph L. Kirschvink\textsuperscript{2}

\textsuperscript{1}Department of Earth and Environmental Sciences, University of Munich, D-80333 München, Germany
\textsuperscript{2}Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena CA 91125, USA

Recently, a putative magnetoreceptor in the upper-beak skin of homing pigeons was chemically characterized using X-ray fluorescence and absorption studies [Fleissner et al., 2007. A novel concept of Fe-mineral based magnetoreception: histological and physiochemical data from the upper beak of homing pigeons. Naturwissenschaften 94 (8), 631-642]. In this short communication we point out that the novel concept propagated is based on conclusions that are not supported by data and, in fact, stand in contradiction to previously published crystallographic and magnetic data.

I. INTRODUCTION

Fleissner and coworkers [1] report on their recent approach to elucidate the nature of the putative magnetoreceptor in homing pigeons. The experimental work was done very carefully and undoubtedly defines a significant progress in the field, with highlights being the reconstruction of the spatial distribution and orientation of the dendritic fields hosting the putative magnetoreceptor cells and the quantification of the amount of iron in these fields using X-ray fluorescence analysis with a spatial resolution of 15 \( \mu m \). Unfortunately, the experimental data presented in the paper do not justify either of the two main conclusions, namely that both magnetite and maghemite are needed for Fe-mineral based magnetoreception, and, more seriously, that the \( \mu m \)-sized electron-opaque platelets are monocrystals of maghemite. These problems are as follows:

II. NON-UNIQUENESS OF XANES DATA

In [1], X-ray near-edge absorption spectroscopy (XANES) was used as a tool to chemically characterize the iron compounds in the dendritic field. The obtained XANES data were fitted to reference spectra of magnetite (\( \gamma - Fe_9/3O_4 \)), maghemite (\( \gamma -Fe_8/3O_4 \), the fully oxidized, slightly less magnetic sister mineral to magnetite), and hematite (\( \alpha -Fe_8/3O_4 \)). On the basis of the fitted spectra, Fleissner et al. [1] concluded that they have unambiguously identified maghemite as the predominating iron mineral in the dendritic fields, which is at least five times, if not ten times more abundant than magnetite.

This conclusion is not justified because the XANES spectrum of maghemite is similar to those of other ferric, but non-magnetic (or only weakly magnetic) Fe-compounds not taken into consideration in [1]. For example, goethite (\( \alpha -FeOOH \)), lepidocrocite (\( \gamma -FeOOH \)), ferrihydrite (\( Fe_{10}O_{14}(OH)_2 \)) and even Fe(III)PO\textsubscript{4} show an equally good fit to the slope of the pigeon spectrum (Fig. 1f in [1]) in the energy window selected in [1] (i.e., 7116-7125 eV) as does the spectrum of maghemite (see Fig. 1 here)! Differences between these ferric iron-mineral phases become apparent only at higher energies in the spectrum (above 7125 eV), as illustrated in Fig. 1 here but not reported in [1]. Despite these differences at higher energies, the unambiguous isolation of a particular mineral of the family of ferric oxides/hydroxides/phosphate in a mixture is difficult (see also [2]).

For a better comparison, we therefore recommend that these other minerals be included as possible components in their curve-fitting comparisons, and that the slope in the whole energy range be fitted. In particular, the spectrum of ferrihydrite (with variable amounts of phosphorous incorporated to capture physiological ferrihydrite, see below) should be used in the curve fitting procedure, as this mineral is found ubiquitously within the cage proteins ferritin or hemosiderin that...
sequester iron, and it is also the mineral precursor to magnetite biomineralization in the teeth of the Polyplacophoran molluscs [3].

In any case, the only conclusion that can be drawn from the XANES data is that pure magnetite can explain at most 20% of the Fe compounds present in the dendritic fields. The predominant ferric iron phase, however, cannot be unambiguously determined in the energy range selected and the fact that only maghemite is strongly magnetic does not imply its presence. At this stage, other candidate phases are equally likely.

III. NO EVIDENCE FOR CRYSTALLINE NATURE OF THE PLATELETS

The authors go so far as to claim that the μm sized electron-opaque platelets arranged in chains or bands in the dendritic fields (Figs. 2c-f in [1]) are monocrystals (crystallographic single domains) of maghemite. However, an electron-opaque region need not be crystalline. In particular, the size of an electron-opaque region need not represent the crystallographic domain size. Selected-area electron diffraction (SAED) analysis would have been the method of choice to demonstrate the single-crystal nature of the platelets and provide the lattice parameter and space group. For, single maghemite crystals of that particle size and shape (1 μm x 1 μm x 0.1 μm) will produce clear and unmistakable, mineral-specific diffraction patterns [26]. Yet, the authors do not support their claim by showing SAED patterns of the structures in question, despite their use of numerous TEM images from which such patterns would have been trivial to obtain.

Showing the corresponding diffractogram would have been even more important in light of the previous paper [4], in which the electron-opaque platelets were reported to be amorphous (i.e., they showed no diffraction rings in SAED) and to be associated with high concentrations of iron and phosphorous. Those characteristics are more typical of physiological ferrirhydrite, which also contains variable amounts of phosphorous but yields characteristically poor diffraction patterns. A similar mistake - misidentifying concentrations of ferrirhydrite for magnetite/maghemite - was made about 10 years ago in the study of iron in the abdomen of honeybees [5]; see discussions by [6, 7, 8].

The conclusion that the platelets are monocrystals of maghemite is also at odds with bulk magnetic measurements [6], which showed that the predominant magnetic phase is superparamagnetic. Judging from the high abundance of platelets relative to clusters in the dendritic fields, we would expect to see a pronounced and stable magnetic remanence (at room temperature) if each platelet (dimensions 1 μm x 1 μm x 0.1 μm) were to represent a single-crystal of maghemite. This is not observed.

IV. WHY THE DISTINCTION BETWEEN MAGNETITE AND MAGHEMITE IS NOT DECISIVE

Unlike conveyed by [1], magnetite and maghemite actually are closely related to one another, both structurally and magnetically (see next Section). From the point of view of crystal chemistry, they are both inverse spinels [27], have similar lattice parameters, and differ only in the oxidation state of iron, with maghemite representing fully oxidized, cation-deficient magnetite, where every ninth Fe cation is substituted by a vacancy to compensate for the charge imbalance. This is why there is a solid-solution series between the two end members, Fe$_{(2+2z/3)}$O$_4$ and Fe$_{(2+3−y)}$O$_4$ (0 ≤ z ≤ 1) and □ denotes a vacancy, see for example [10, 11].

Note that even magnetotactic bacteria (MB) - the model organisms for magnetite biomineralization - do not contain 100% end-member magnetite as can easily be seen by the fact that MB have lower Verwey transition temperatures ($T_v$ ~ 105 − 115 K) than ideal stoichiometric magnetite has ($T_v$ = 125 K). Since $T_v$ decreases with increasing cation deficiency (maghemitization) in magnetite [16], MB contain partially maghemitized magnetite crystals, which reflects the oxidation state of the local environment in which they are grown [17]. Nonetheless we refer to them as magnetite, without making a big deal out of the oxidation state.

V. WHY IRON-BASED MAGNETORECEPTION CAN PERFORM WITH ONE MAGNETIC FE-MINERAL

In their abstract, Fleissner et al. [1] state, “in this paper we show that iron-based magnetoreception needs the presence of both of these iron minerals, . . .”. Again, we find no support for this statement in the published work. In essence, the authors claim that magnetite and maghemite have distinctly different magnetic properties. For example, it is written in [1] that ”The flat quadratic shape [of the electron-opaque platelets] perfectly fits the magnetocrystalline anisotropy of maghemite that coincides with the direction of the cubic axis”. That statement is wrong for two reasons. For one, the postulated quadratic shape of the electron-opaque platelets cannot be taken as crystallographic evidence of a (100) facet, let alone as evidence of maghemite (see Section III). Secondly, both magnetite and magnetite have a negative cubic magnetocrystalline anisotropy constant $K_1$, which means that the preferential axes of the magnetocrystalline anisotropy are the (111) axes (i.e., normal to the faces of an octahedron) and not the crystallographic (100) axes (normal to the faces of a cube) (e.g., [18]). This is also well-known from paleomagnetic studies, where magnetite is often found to be oxidized, but still carries the originally recorded remanence direction. If the sign of $K_1$ were to change during oxidation, the rema-
nence direction would change too, which is not observed.

Whether the magnetic core in the receptor is made up of magnetite or maghemite is not a decisive physiological parameter. The single most important material parameter for Fe-mineral based magnetoreception is the saturation magnetization, \(M_s\), which defines the sensitivity of the receptor to detect minute changes in geomagnetic field strength. The value of \(M_s\) is 480 G for pure magnetite compared to 380 G for pure maghemite (at room temperature), that is, both values are significantly larger than the geomagnetic field strength (0.25-0.6 G) and thus can locally amplify the external field by two orders of magnitude.

While the sign of \(K_1\) for magnetite is negative, different values of \(K_1\) have been reported in the literature, ranging from one-third to 80% the value of magnetite \([18,19]\). Probably, these differences reflect different degrees of vacancy ordering in the maghemite crystals studied. Nevertheless, even with one-third the value of \(K_1\) for magnetite, maghemite is magnetically not much softer than magnetite. Importantly, the value of \(K_1\) is not directly related to magnetic hardness or softness, but rather determines the ease with which a desired hardness (or softness) can be achieved. Coercivity, an actual measure of magnetic hardness, is determined mainly by additional structural features such as lattice defects, grain boundaries, magnetic particle size and shape \([20]\). The platelets in question (if they were to represent defect-free single crystals of magnetite or of magnetite) would behave as soft magnets (easily magnetizeable) primarily because of their dimensions and the resulting shape anisotropy, which overrides the magnetocrystalline anisotropy. We conducted micromagnetic calculations for such platelets and found that a magnetite composition yields a slightly higher susceptibility (i.e., is more easily magnetizeable) than a magnetite composition does, even though maghemite was modelled with a \(K_1\) of one-third the value of \(K_1\) for magnetite. Thus, the higher \(M_s\) value of magnetite compared to maghemite more than outweighs the intrinsically harder character of magnetite and a higher magnetic-field amplification can be realized on the basis of magnetite.

Therefore, the ”novel concept of Fe-mineral-based magnetoreception” \([1,21]\) first and foremost relies on a bimodal distribution of magnetic particle-sizes and, with it, of domain states (superparamagnetic, SP vs multi-domain, MD), but not on the presence of two Fe oxides which have very similar magnetic properties anyway \([28]\). In essence, what has been suggested in \([1]\) and followed up on in \([21,22,23]\) is a hybrid-magnetoreceptor (combining adjacent magnetic elements with different properties, here SP and MD), conceptually similar to the ones that have already been discussed in the literature (e.g. \([24]\), there SP and SD).

VI. CONCLUSIONS

The experimental data presented in \([1]\) do not justify the reinterpretation of the crystallographic results reported in \([3]\), where the \(\mu\text{m}\)-sized electron-opaque platelets were found to be amorphous. The XANES spectra cannot be uniquely attributed to maghemite, but may as well be explained by physiological ferrirhydrite, which is only weakly magnetic, but due to its poorly crystalline (amorphous) nature and high concentration of iron and phosphorous consistent with the results previously reported in \([4]\). The claim that both magnetite and maghemite are needed to realize a biological magnetometer is based on the wrong assumption that maghemite and magnetite are positive-anisotropy and negative-anisotropy cubic materials, respectively. However, they are both negative-anisotropy cubic materials \((K_1 < 0)\) and rather similar in their magnetic properties.

Acknowledgments

We acknowledge funding from the Human Frontier Science Program Organization, HFSP research grant RGP28/2007.

Note

This is an extended version of a comment submitted to Naturwissenschaften on 17 June 2007. A manuscript number was eventually assigned on 23 Jan 2008 (NAWI-08-0022) and the manuscript has since been under review. The complementary diffraction data requested have not been furnished yet.

VII. REFERENCES

[1] G. Fleissner, B. Stahl, P. Thalau, G. Falkenberg, and G. Fleissner, Naturwissenschaften 94, 631 (2007).
[2] P. O’Day, N. Rivera, R. Root, and S. A. Carroll, Am. Mineral. 89, 572 (2004).
[3] J. L. Kirschvink and H. A. Lowenstam, Earth Planet. Sci. Lett. 4, 193 (1979).
[4] G. Fleissner, E. Holtkamp-Rötzler, M. Hanzlik, M. Winklhofer, G. Fleissner, N. Petersen, and W. Wiltschko, J. Comp. Neurol. 458, 350 (2003).
[5] C.-Y. Hsu and C.-W. Li, Science 265, 95 (1994).
[6] H. Nichol and M. Locke, Science 269, 1888 (1995).
[7] J. L. Kirschvink and M. M. Walker, Science 269, 1889 (1995).

[3] J. L. Kirschvink and H. A. Lowenstam, Earth Planet. Sci. Lett. 4, 193 (1979).
Although maghemite and magnetite have almost the same d spacings, which makes their discrimination by means of diffraction difficult, the vacancies in maghemite are often ordered, which gives rise to a tetragonal superstructure, by which maghemite can be identified.

An inverse spinel has (B)[AB]O$_4$ structure, where the tetrahedrally coordinated sites (indicated by round brackets) are occupied by cation B, and the octahedral sites (indicated by square brackets) are occupied by cations A and B. To emphasize the inverse spinel structure, the formula units of magnetite and maghemite can also be written as Fe$^{3+}$[Fe$^{2+}$Fe$^{3+}$]O$_{2-}^4$ and Fe$^{3+}$[Fe$^{3+}$]O$_{2-}^4$, respectively.

Note that despite their similar magnetic properties, magnetite and maghemite have different electronic properties. Maghemite is an isolator, while magnetite is a half-metal, that is, electronic conductivity is spin-polarized and depends on the magnetization direction. Whether a magnetite-based magnetoreceptor can exploit these intriguing electronic properties is a different question. One way of realizing a magnetically gated biological electrical circuit with magnetite was suggested in [25], referred to as "the membrane shortcut model".