Correspondence

No evidence for a magnetite-based magnetoreceptor in the lagena of pigeons

E. Pascal Malkemper[1], Daniel Kagerbauer[2], Lyubov Ushakova[1], Simon Nimpf[1], Paul Pichler[3], Christoph D. Treiber[4], Martin de Jonge[5], Jeremy Shaw[6], and David A. Keays[1,*]

It is well established that an array of avian species sense the Earth's magnetic field and use this information for orientation and navigation. While the existence of a magnetic sense can no longer be disputed, the underlying cellular and biophysical basis remains unknown. It has been proposed that pigeons exploit a magnetoreceptor based on magnetite crystals (Fe_3O_4) that are located within the lagena [1], a sensory epithelium of the inner ear. It has been hypothesised that these magnetic crystals form a bed of otoconia that stimulate hair cells transducing magnetic information into a neuronal impulse. We performed a systematic high-sensitivity screen for iron in the pigeon lagena using synchrotron X-ray fluorescence microscopy coupled with the analysis of serial sections by transmission electron microscopy. We find no evidence for extracellular magnetic otoconia or intracellular magnetite crystals, suggesting that if an inner ear magnetic sensor does exist it relies on a different biophysical mechanism.

Utilising pigeons as a model system, Dickman and Wu [1,2] have reported that various regions of the brain, including the vestibular nuclei and hippocampus, are activated when exposed to magnetic stimuli. Removal of the lagena demonstrated that this activation is dependent on the structural integrity of the inner ear, leading the authors to suggest that ferrimagnetic particles stimulate lagenar receptors that form the basis of a magnetic sensory apparatus [1]. This hypothesis is attractive, as the lagena (like the utricle and saccule) contains sensory hair cells coupled to dense crystals of calcium carbonate, called otoconia. Moreover, it has been reported that iron is present in the lagenar otoconia of birds [3]. We have further reported the presence of an iron-rich spherical organelle, the cuticulosome, which is found apically in the cuticular plate of hair cells of birds [4,5]. Cuticulosomes are predominantly composed of ferrihydrite nanocrystals and are therefore unlikely to act as torque-based magnetoreceptors [6]; however, they closely resemble structures that are abundant in the cusp epithelia of chitons, which are involved in the biomineralization of magnetite teeth [7].

It is therefore conceivable that cuticulosomes act as an intermediate iron store, supplying precursor material for the formation of extracellular magnetic otoconia. There could be either a discrete subclass of magnetic otoconia that are associated with a specific population of hair cells, or magnetic structures that are interspersed amongst the primary bed of calcium carbonate otoconia (Figure 1A–C). It is challenging to test these ideas as magnetic otoconia cannot be visualized with classical iron-staining methods (for example Prussian Blue), which dissolve the structures of interest. Additionally, iron contamination from non-biological sources is a persistent problem, and high sensitivity is required to identify magnetite crystals within a comparatively large anatomical structure.

For this reason, we performed a high-resolution elemental analysis of

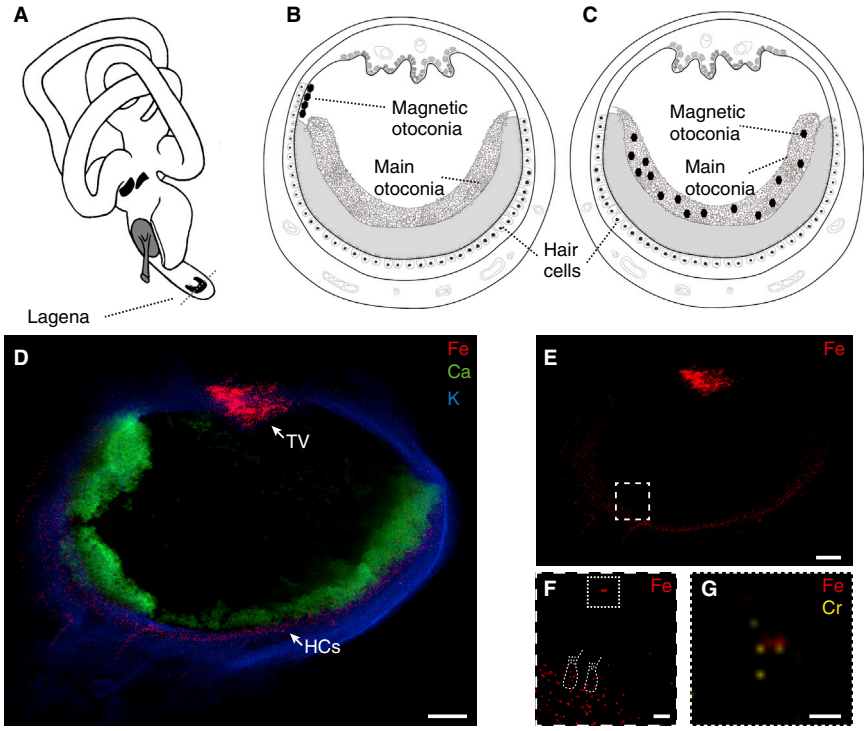

Figure 1. X-ray fluorescence microscopy of serial sections reveals the absence of magnetic otoconia in the pigeon lagena.
(A) Diagram of the pigeon inner ear showing the lagena at the tip of the elongated cochlear duct. The otoconia are shown in black. (B) Diagram illustrating the magnetic otoconia hypothesis. Shown is a schematic cross-section through the lagena at the level indicated by the dashed line in (A). Hypothetical magnetic otoconia could be linked to a specific population of hair cells that are not associated with calcium-carbonate main otoconia. (C) Alternative to the hypothesis presented in (B), magnetic structures could be interspersed within the primary array of calcium carbonate otoconia in the pigeon lagena (based on [3]). (D) Cross-section of the pigeon lagena showing the elemental maps for iron (red), calcium (green), and potassium (blue). The hair cell layer (HCs) and tegmentum vasculosum (TV) are rich in iron. The scale bar shows 50 µm. (E) Iron map of the same lagenar cross-section shown in (D). The scale bar shows 50 µm. (F) Enlargement of boxed region shown in (E), showing the iron-rich hair cell layer (hair cells indicated) and an extracellular iron-rich structure. The scale bar shows 10 µm. (G) Enlargement of the extracellular iron-rich structure boxed in (F). It contains chromium (yellow), indicative of laboratory contamination. The scale bar shows 5 µm.

the pigeon lagena with synchrotron-based X-ray fluorescence microscopy (XFM) coupled with an assessment of serial sections by transmission electron microscopy. XFM relies on the analysis of X-rays emitted from a sample of interest and generates elemental maps with exquisite sensitivity. We exploited a beamline at the Australian Synchrotron for this purpose, which was optimised to detect iron above a concentration of 174 ng iron/cm^2. Critically, this minimal detection limit would permit the identification of a chain of single domain magnetite crystals that could act as a torque-based magnetoreceptor (297 ng iron/cm^2; Figure S1 and supplemental calculations in the Supplemental Information).

We prepared 100 µm thick sections from the apical to basal edges of the lagena (n = 3 birds), which were screened using a beam of 10 keV X-rays. A total of 14 elements were analysed by XFM and X-ray maps were generated for iron, calcium, chromium and potassium (Figure 1D–G and Figure S1). The calcium carbonate otoconia were readily identifiable, as well as the hair cell layer and tegmentum vasculosum, both of which were noted for their high iron content (Figure 1D). The latter observations were expected as it is known that pigeon hair cells express high levels of ferritin and contain cuticulosomes [4,5], and the tegmentum vasculosum is a highly vascularised tissue that is responsible for producing endolymph. In none of the birds analysed did we observe a discrete concentration of extracellular iron particles. In some sections we did observe iron-rich structures within the main otoconia; however, analysis of the chromium spectra revealed that these structures were contaminants, most likely from the preparation of lagenar sections (Figure 1F,G).

Next, we considered the possibility that single domain magnetite crystals may reside intracellularly within the iron-rich hair cell layer in the lagena. To this end, we prepared serial ultrathin sections encompassing the entire sensory epithelium of the lagena for analysis by transmission electron microscopy (TEM). Bright-field TEM imaging of magnetotactic bacteria revealed that a 7,100 x magnification enables the swift identification of electron dense 50 nm size magnetite crystals. Employing this magnification, we screened the complete hair cell layer at regular 30 µm intervals (n = 3 birds, 161 sections, approximately 17,500 hair cells in total) for crystalline structures from the apical to basal edges of the lagena (Figure S2). We did not identify any structures consistent with intracellular magnetite crystals.

In summary, our data do not support the existence of a magnetite-based magnetoreceptor in the lagena of pigeons. We find no evidence for extracellular magnetic otoconia or intracellular single-domain crystalline magnetite in the lagena. We acknowledge that our TEM screen may have failed to identify single superparamagnetic magnetite crystals smaller than 10 nm in size; however, such crystals could not serve as a torque-based magnetoreceptor that would provide information on the polarity of the magnetic field [8]. Moreover, our findings are consistent with that of Zhao *et al.* [9], who performed mass-spectrometric analysis on the lagenar otoconia of pigeons and show a near absence of iron. How can these findings be reconciled with the data that magnetic stimuli cause neuronal activation in the vestibular nuclei of pigeons? It is possible that inner ear magnetoreceptors rely on electromagnetic induction, rather than a magnetite-based mechanism [10]. It has been proposed that the semicircular canals and conductive endolymph may serve as an anatomical circuit for this purpose. As a pigeon moves through a static magnetic field, a voltage could be induced that might be detected by a highly sensitive electroreceptor. Alternatively, it is conceivable that the brainstem vestibular nuclei are involved in the multimodal integration of linear acceleration, gravitational input, and magnetic information that originates from primary magnetoreceptors at an unknown location [2].

SUPPLEMENTAL INFORMATION

Supplemental Information includes experimental procedures, calculations of detection limits and two figures and can be found with this article online at https://doi.org/10.1016/j.cub.2018.11.032.

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[1]Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Campus-Vienna-Biocenter 1, 1030 Vienna, Austria. [2]Atominstitut, TU Wien, Stadionallee 2, 1020 Vienna, Austria. [3]School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK. [4]Centre for Neural Circuits and Behaviour, The University of Oxford, Oxford, UK. [5]Australian Synchrotron, ANSTO, 800 Blackburn Road, Clayton, Victoria 3168, Australia. [6]Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Crawley, WA 6009, Australia.
*E-mail: keays@imp.ac.at