µ-XRF and µ-XANES as essential tools to develop a first sound concept for an avian magnetoreceptor

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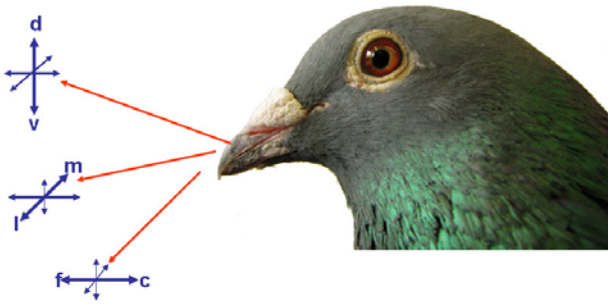

Already in ancient times, animal migration has fascinated as an enigmatic phenomenon, which regularly occurs twice a year and reliably brings birds, turtles, fishes and insects to long distant locations. A "sixth sense", magnetoreception, has been recognized as its driving and controlling reference for more than 50 years. But the respective receptor organ and its functionality remained unknown – until now. The Earth's magnetic field and its natural changes play an important role for the navigation of many organisms over long distances on their migratory routes, but also finding home from nearby places is controlled by the magnetic field. Astonishingly small changes of magnetic field intensity, direction and inclination obviously suffice to serve as magnetic map and compass factors. But still, the neurobiological mechanisms and the magnetophysical principles of the underlying sensory processes are widely unknown. Lately, we have detected a system of sensory dendrites in the upper beak of pigeons, which may be a first sound candidate for a magnetic-field receptor. By means of x-ray analyses, we could develop a novel receptor concept. The decisive progress, achieved by microscopic X-ray fluorescence (µ-XRF) and microscopic X-ray absorption nea- edge structure (µ-XANES) investigations, is the finding that the subcellular iron compartments contain more than one type of magnetic iron minerals, which produce a strong, direction sensitive amplification of the local magnetic field at the receptor membrane. Due to its 3-D-microarchitecture and physico-chemical nature, the dendritic system should be able to separately sense the three vector components of the Earth's local field, simultaneously – allowing birds detecting their geographic position by the magnetic vector, i.e. amplitude, declination and inclination of the local magnetic field, irrespective of the animal's posture or movement and independent from photoreception.

Magnetoreception is the so far least explored sensory system of animals, and consequently many laboratories have invested major efforts into its exploration. The research field was dominated by two hypotheses concerning the basic physical principles: (1) The so-called cryptochrome-based mechanism, where the singlett/triplett relation of the plant pigment cryptochrome is assumed to depend on the parameters of the magnetic field, or (2) the so-called magnetite-based mechanism, where magnetic iron minerals, like magnetite, are supposed to exert a torque like a compass needle or change their position or shape, thus, inducing an opening of membrane receptor channels. Both hypotheses have been tested in manifold behavioural experiments [1]. Obviously, none can explain magnetic orientation, exclusively, but rather both processes might share certain functions of magnetic navigation: chemical processes in retinal photoreceptor cells might serve as a magnetic

compass, magnetic material in nervous tissue might supply the organisms with a magnetic map [2]. But up to now, none of these papers has dealt with the essential physical or receptor physiological principles underlying magnetoreception, none has shown a convincing receptor system down to its subcellular components or magnetic field effects inside the sensory units. Thus, a vast number of papers attributed magnetoreceptive function to any tissue containing magnetite, for example blood cells or blood vessels, brain areas or dead body appendages (like invertebrate mouth parts), irrespective of their sensory capability [1].

For the first time, we have now proposed a structural candidate for the "magnetite-based" magnetoreceptor in the avian beak [3,4]. Interdisciplinary investigations with different neurobiological and physicochemical methods enabled us to develop a concept of the "stimulus conducting system" of this magnetoreceptor [5]. It shows how magnetic field parameters might be transformed into nervous excitation, which features might delimit its working range and dynamics, and which characteristics might explain the specificity and sensitivity of the sensory system. And, based on these findings, we have to insist on a renaming of the so-called "magnetite-based" magnetoreceptor into "iron-mineral-based" magnetoreceptor: X-ray analyses have clearly shown that the avian magnetoreceptor does not only contain magnetite, but that it has additional iron minerals of different size and shape, which are indispensable for a magnetoreceptive function [3].

Histological findings

By means of histological methods (Prussian Blue staining for the localization of iron concentrations, immunohistology to identify the nervous nature of the iron containing structures and electron microscopic analysis of serial ultrathin sections for recognizing the subcellular compartments that contain the iron particles), a complex biological structure in the skin of the upper beak of pigeons could be detected, which is the same in all investigated samples [3,4]:

1. 2x3 dendritic fields: The iron containing dendrites of the median ophthalmic branch of the trigeminal nerve (ROM) are not randomly distributed. They occur in six distinct iron containing regions inside the dermal lining of the upper beak and have a volume of about 150x150x300 μm^3 each. The areas lie in a distance of less than 1 cm along the outer rim of the beak skin. The left and right half of the beak skin exhibit mirror symmetry.
2. Alignment: In both halves of the upper beak skin, each of the 3 iron containing regions consists of dendrites with a nearly perfect axial alignment. The preferred spatial orientation of the axons, which carry the iron-containing dendrites, varies systematically: the caudal areas with fronto-caudal dendrites, the median regions with mainly medio-lateral dendrites, the regions in the tip of the bill with preferably dorso-ventral dendrites. (Few axons matching the other spatial directions are present in all dendritic fields, too.) This architecture corresponds to the 3 dimensions of space.
3. Regularity: The dendrites within an axon bundle display a regular order. For instance, their uniform longitudinal separation is in the order of 80 to 100 µm.
4. Uniaxial anisotropy: Within its tip over a length of about 20 µm, each dendrite contains a uniaxial chain or band of Fe constituents.

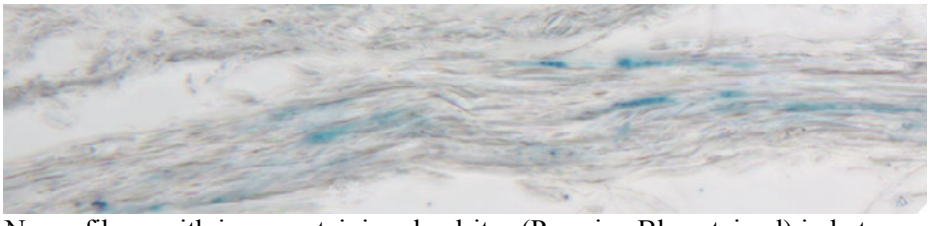

Figure 1.Nerve fibres with iron-containing dendrites (Prussian Blue stained) in between [after 3].

These subcellular Fe elements are of three types:

5. Bullets and platelets: In each dendrite these are about 10 bullets (diameter 1 µm) containing nanocrystals (with a size of 2-4 nm) and about 6-8 parallel platelet chains of 3 to 5 monocrystals, each (1x1x0.1 μm^3). The bullets, which seem to lie preferably at the end of a platelet chain, are attached to the cell membrane by thin filaments that could transduce deformations or displacements into primary receptor potentials via mechanosensory membrane channels.
6. Vesicle: A vesicle of unknown physicochemical nature with a diameter of about 5 µm is found in the middle of each of these dendrites. It is covered by a thin crust (about 0.1 µm thick) of presumably non-crystalline iron containing "scales".

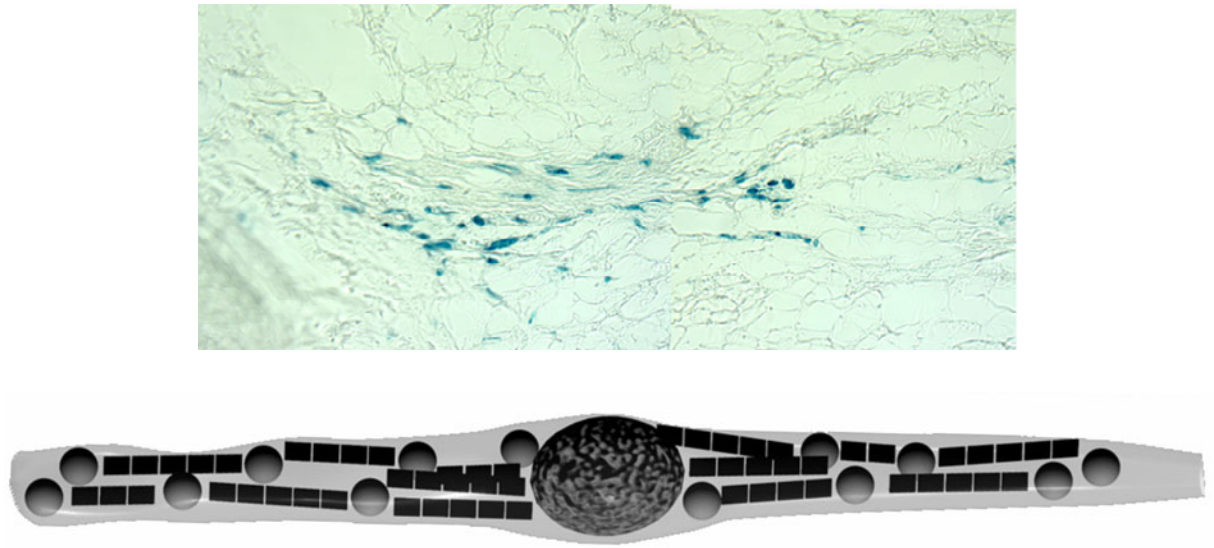

Figure 2. Dendrites (stained with Prussian Blue to show iron, top), and sketch of the subcellurlar structure of a sensory dendrite (bottom) [top after 3, bottom after 5].

Physicochemical findings

This dendritic system of iron-containing endings looks very intriguing and obviously matches prior published data of electrophysiological recordings of nervous action potentials from the ROM, which change their frequency according to modifications of the magnetic field [2]. Now, we need a spatially resolved physico-chemical analysis of this putative magnetoreceptive tissue in birds, a method, which does not destroy the structures nor does not depend on great quantities of material. Only, if we know more than that the structures contain "iron", we can develop a sound hypothesis on the interaction of the iron minerals inside this structure with the Earth's magnetic field. We may then refer to the dendrites in the upper beak as a magnetoreceptor candidate.

Therefore, we have chosen a topographic X-ray analysis of the critical areas in the skin of the upper beak of homing pigeons. We used unstained tissue, embedded in low temperature (45°C) paraffin and cut into 10 µm thick sections in order to include an entire dendrite. PB-stained parallel sections served as a control to locate the proper area of interest. Our X-ray data reliably show the distribution of chemical elements and reveal characteristics of the iron minerals present [3].

1. The µ-XRF measurements of paraffin section series taken from the beak skin of homing pigeons show that the element spectra inside a Fe containing dendrite and outside in the surrounding nervous tissue are clearly distinct. Nervous tissue gives a strong Ca fluorescence signal in contrast to connective tissue. Fe is found within the nervous dendrite as a sharp local

representation. The amount of Fe, determined by a comparison of the absolute count rate with that of a known Germanium film, adds up to $4 \cdot 10^{11}$ Fe atoms which would be equivalent to a mass of 35 pg of pure Fe.

2. The iron content of an entire dendritic field can be estimated by the extrapolation of the µ-XRF data taken at the centre of the field in the analyzed section. The 2-dimensional Fe distribution of the section has a mean radius of approximately 115 µm. From this, the corresponding sphere of a 3-dimensional Fe distribution (see Fig. 3b) leads to an estimation of the Fe content of the entire dendritic field of about $1.5 \cdot 10^{14}$ atoms (equivalent to 14 ng of pure Fe). Taking the Fe content of a single dendrite (35 pg), a value of 400 terminals is estimated for the whole dendritic field. All 6 dendritic fields should contain similar amounts of iron, giving a total of 85 ng of Fe in the dendrites of the skin of the upper beak. This amount of Fe would fill a cube of 22 μm^3.

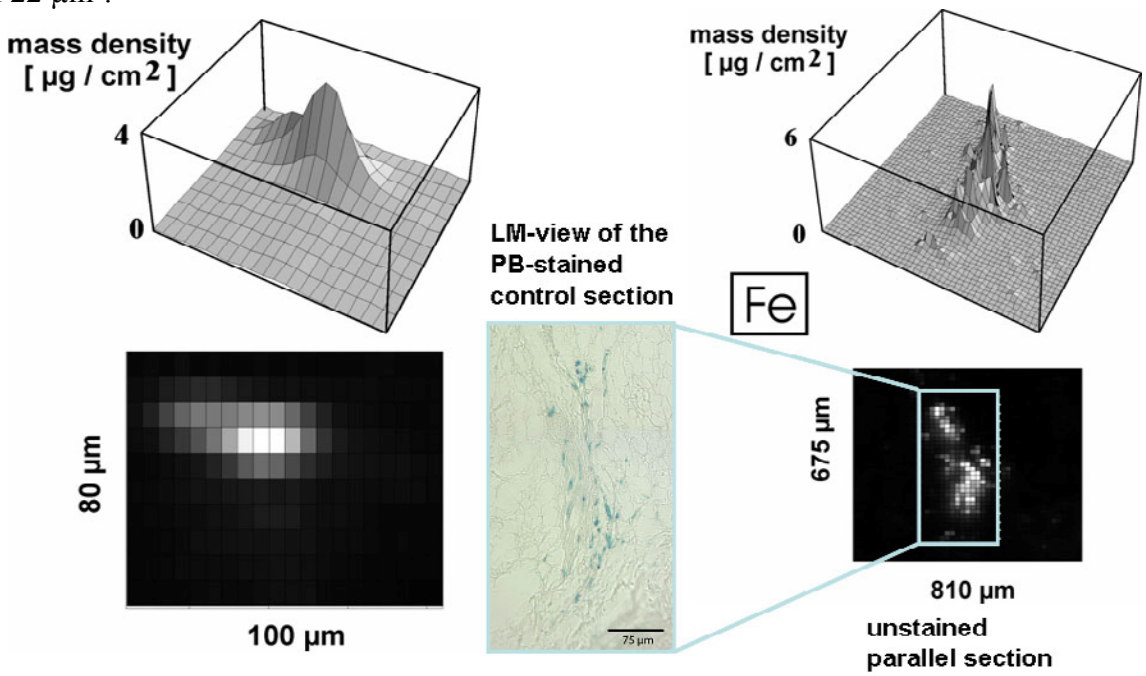

Figure 3: Micro X-ray Fluorescence results of dendrites in the iron-map (left: single dendrite; right: dendritic area; middle: microscopic view of PB-stained control section). [after 3]

Additionally, but not of specific interest for our analysis of the putative magnetoreceptor, is a major amount of iron bound to blood corpuscles (hemoglobin) and involved in the iron metabolism (ferritin) etc.. Therefore, prior bulk measurements of the entire beak or the dermal lining by e.g., SQUID [6] or Mössbauer measurements are mostly based on this "non-dendritic" iron, and may contribute only to a lesser degree to the discussion on the nature of the dendritic iron minerals.

Magnetite was assumed to be the "key molecule" of animal magnetoreception, and deduced from data of magnetic bacteria, a general concept for all metazoan organisms was postulated: a torque of either a chain of magnetite crystals similar in size and shape to the bacteria or of a solid major magnetite rod would trigger sensory receptor potentials whenever the animal is turning relative to the Earth's magnetic field [2]. All structural data published so far could not show such a magnetic compass needle. Our histological findings did not verify these predictions, but we had to find the magnetic material inside the dendrites of the beak.

3. By means of selected area electron diffraction (SAED) measurements in the transmission electron microscope (TEM), we found a magnetite-like powder diffraction pattern in the bullets, as they consist of many tiny nano-sized crystals [4]. But the other two iron-compartments, the

platelets and the encrusted vesicle, could not be investigated by this method. Pilot studies with TEM-EDX-analysis of a platelet lead to an iron-phosphate-mix signal [4], but did not deliver data on the physicochemical feature of these compartments. Only afterwards, we learned about the putative destruction of the subcellular crystals by TEM-analyses.

4. Therefore, avoiding this destruction in the TEM, we performed µ-XANES analyses in the maximum of the iron signal (determined by µ-XRF) of a single dendrite of the pigeon beak [3]. Our data gave a surprising result: they clearly show that magnetite can contribute to the dendritic iron only to a small amount. Evaluated by several reference samples of well-defined iron minerals, we found the fluorescence signal from the pigeon sample following closely that of the reference sample of pure maghemite ($Fe^{3+}{}_2O_3$) nanoparticles (4 nm diameter). Due to the presence of Fe^{2+} in magnetite ($Fe^{3+}{}_2Fe^{2+}O_4$), the characteristic absorption edge for the pigeon is slightly shifted to lower photon energies compared to the signal for maghemite. The XANES signal for the pigeon stays the same after many repetitive scans and also after storing the sample over several months. A detailed analysis of the K-edge position by differentiating the XANES signal and superimposing the magnetite and maghemite reference signal with various weights suggest a mixture of magnetic iron minerals in the pigeon sample in the range of 85 % maghemite and 15 % magnetite. These values coincide with the estimates for the occurrence of platelets and vesicle versus bullets as gained from TEM images. Hence, we propose that the bullets consist of single domain magnetite nanocrystals, while the platelets seem to be maghemite multidomain microcrystals. The crust around the vesicle might well be of maghemite or another iron mineral with a µ-XANES spectrum similar to maghemite. Recent – not yet published - data support this assumption.

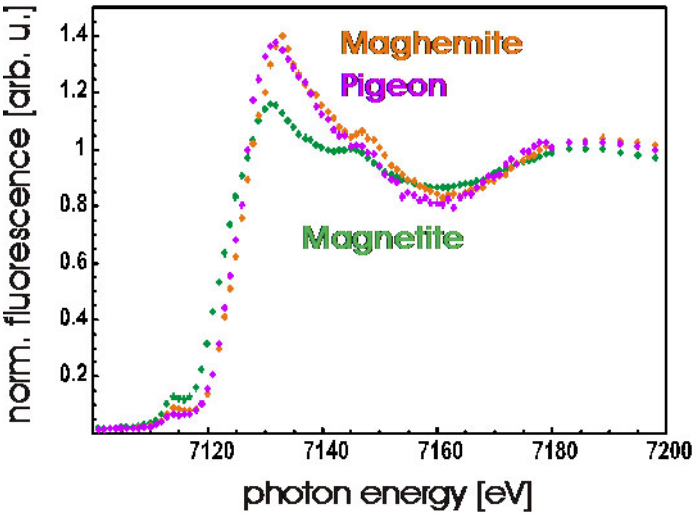

Figure 4. XANES of magnetite and maghemite (pure reference samples) compared to micro-XANES of a dendrite in a pigeon's beak. [after 3]

Therefore, we will need a higher resolution of X-ray analyses, as up to now the diameter of the measuring capillary at DORIS III beamline L is 5 to 15 µm. As the sensory dendrite has a length of about 20 µm and a diameter of 5 µm, we could only integrate over the entire terminal. Future experiments will aim at an analysis of single subcellular compartments, separately, in order to test how the different iron minerals are distributed inside the bullets, platelets and vesicle. The size, shape and arrangement of the magnetic particles will strongly influence the response to an external magnetic field.

Receptor model and biological function

Although we still need many more biophysical details of the micro- and nano-scaled components of proposed magnetoreceptor candidate, we could make a significant step towards understanding the remarkable sensitivity and specificity of this sensory system. The most important result was the detection that iron minerals of different magnetic properties – hard and soft magnetic material - compose the sensory dendrites, an unequivocal finding by means of µ-XANES at HASYLAB [3], which was not possible with any of the previously applied methods. We can now present for the first time a receptor concept for avian magnetoreception [3,5] that has already been verified by

behavioural experiments [1] and mathematical simulations [7]. Here, we summarize our model concept and refer to the cited papers for further details.

The iron containing dendrite in the upper beak probably is the smallest sensory unit of avian magnetoreception with distinct consequences for the adequate stimulus [3,5], which is a certain stimulus of minimal energy, transmitting only a minute selection out of the environmental magnetic field. The knowledge of this "selective sensation" will be decisive for the design and evaluation of future neurophysiological and behavioural experiments.

1. Each dendrite is a sensor with a specific orientation in the magnetic field. The adequate stimulus for this dendrite is the momentary intensity of the parallel component of the local magnetic field vector.
2. The iron-crusted vesicle might serve as a 3D-amplifier of the Earth's magnetic field.
3. The bands of maghemite platelets are exposed to this amplified field, which is diminished according to their distance from the vesicle.
4. Selectively in one direction, the maghemite platelets become maximally magnetized when in parallel to the magnetic field vector. Only then, the magnetic forces at the ends of each platelet band are strong enough for inducing a magnetic moment in the bullets to pull them towards the higher magnetic field.
5. This strain to the membrane could act on mechanosensitive membrane channels. Multiple bullets - attracted simultaneously - will compose the primary receptor potential of a single dendrite.
6. The primary receptor potential of a dendrite correlates in its amplitude to the magnetic flux along its aligning vector component.
7. Only the entity of all dendrites constitutes the sensory system, which provides separate information on the 3 vector components of the Earth's magnetic field.

The peripheral information flow must be composed in the central nervous system to the local field vector including information on its length (field intensity) and direction (inclination and azimuth). This information may well serve as a map factor for magnetic navigation.

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