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Magnetic analysis of human brain tissue

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Fourteen samples of human hippocampal tissue were resected during amygdalo-hippocampectomies performed on patients suffering from Mesial Temporal Lobe Epilepsy (MTLE). In addition, eight tissue samples from the hippocampus, cortex basalganglia, cerebellum and leptomeninges were resected from cadavers during routine autopsy and were not chemically fixed. All samples were preserved in liquid nitrogen and magnetic properties were measured at 77K and 273K. Measurements indicate that there are no systematic variations in magnetic particle concentrations or magnetic properties between MTLE patients and non-pathologic tissue from the cadavers. The presence of superparamagnetic particles can be inferred due to differences in the saturation remanence acquired at 77K and 273K. This is a further indication that biogenic magnetite and/or maghemite present in the human brain likely is not primarily associated with geomagnetic field sensing as it is known to occur in other organisms.

Keywords: brain, epilepsy, hippocampus, magnetite

Introduction

Magnetic iron biomineralization is known to occur in a number of organisms ranging from bacteria to vertebrates and in a variety of mineral forms (e.g. Blakemore, 1975; Webb *et al.*, 1990; Bazylinski *et al.*, 1993; Kobayashi & Kirschvink, 1995; Walker *et al.*, 1997). In 1992 particles of biogenic magnetite (Fe_3O_4) were discovered in magnetic extracts of human brain tissue removed from cadavers (Kirschvink *et al.*, 1992). Subsequent studies showed that these particles are not the result of contamination or chemical alteration of the tissue after death (Dobson & Grassi, 1996).

In many organisms, these magnetic particles (mainly ferrimagnetic magnetite) have been shown to be responsible for magnetic field sensing – particularly geomagnetic field sensing (e.g. Frankel, 1984; Walker and Bitterman, 1989; Kirschvink *et al.*, 1997; Walker *et al.*, 1997). The discovery of magnetite in the human brain led to experiments examining the response of epileptic patients to magnetic field stimulation (Dobson *et al.*, 1995; Fuller *et al.*, 1995). The results of these experiments showed that there was an evoked electrical response in the hippocampus recorded by electroencephalography but it was not determined whether this was due to anomalous concentrations of magnetite in the epileptogenic focus.

In order to test for systematic variations in magnetic particle concentration, size and magnetic properties in epileptics vs. non-epileptics, and to examine variations between human brain tissue resected during surgery and tissue resected post-mortem, the magnetic properties of 22 samples of brain tissue were subjected to magnetic analysis techniques.

Methods

In order to determine whether biogenic magnetic material is present in the tissue samples, the isothermal remanent magnetization (IRM) of the tissue was measured. This entails the exposure of a tissue sample to applied magnetic fields of progressively increasing strength. After each field

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application, the magnetization of the sample is measured (in the absence of the applied field). The magnetization that remains in the sample is the remanent magnetization. As more magnetic particles in the tissue become aligned parallel to the applied fields of increasing strength, the magnetization of the sample increases until saturation occurs. At this point, all magnetic particles are aligned with a component of their magnetization parallel to the applied field direction.

IRM is due to the presence of magnetized particles in the tissue and the remanent magnetization is not affected by diamagnetic tissue or paramagnetic materials in the tissue such as heme proteins. While paramagnets will align with an applied field, upon removal of the field the magnetization is lost due to the randomizing effect of thermal fluctuations on the magnetization vectors. In magnetic materials (such as ferrimagnetic magnetite) these thermal energies are overcome by quantum mechanical coupling of electron spins, allowing them to preserve a magnetization once the field is removed.

Human hippocampal tissue samples from 14 patients suffering from Mesial Temporal Lobe Epilepsy (MTLE) aged from 14 to 47 were resected during amygdalohippocampectomies performed at the University Hospital in Zurich (Dept. of Neurosurgery). All tissue samples were immediately sealed in acid-washed, quartz glass vials in the operating theater and submerged in liquid nitrogen in order to prevent chemical changes due to the death of the tissue, following the methods of Dobson and Grassi (1996).

In addition, eight human brain tissue samples were resected during routine autopsy from non-pathologic cadavers, aged 55 to 83 years. The samples were from the hippocampus, cortex basalganglia, cerebellum and leptomeninges. None of the samples were chemically fixed and all were obtained 12–30 hours post-mortem from the University Hospital in Zurich (Dept. of Pathology), sealed in acid-washed vials and submerged in liquid nitrogen.

Each quartz glass vial had been soaked in a 30% Hydrochloric acid (HCl) solution for at least 24 h. and then washed with distilled water. The samples were then placed in quartz glass sample holders for measurement of magnetic properties. The holders also were cleaned with HCl for at least 24 h. and then rinsed with distilled water. The tissue samples were packed into the holders with cellophane (found to be non-magnetic in separate measurements) to prevent movement during the measurements. Before the tissue was measured in the quartz glass holders, the incremental IRM acquisition of the holders themselves, along with the cellophane packing material, was measured at 77K (liquid nitrogen temperature). After measuring the holder and the cellophane, the tissue was placed in the holder and measured in the same manner. The contribution of the quartz glass holders and packing cellophane could then be subtracted from the overall IRM signal (holder, cellophane and tissue) and the IRM of the tissue alone was determined.

The acquisition of IRM of the samples was measured both at 77K and at 273K in order to access the contribution of superparamagnetic particles to the IRM signals. The samples were exposed to DC magnetic fields in stepwise increments up to one Tesla (T) at 77K using an Oxford Instruments water-cooled electromagnet. After each step, the remanent magnetisation was measured in zero-field with a 2G Enterprises Superconducting QUantum Interference Device (SQUID) magnetometer. After the final 1T magnetisation step, the samples were step-wise demagnetised at 77K using an AF Schoensted demagnetiser. After each demagnetization field, the sample was measured with the magnetometer in order to generate demagnetization curves. Afterwards these samples were allowed to warm to room temperature. After all samples were completely demagnetised a second IRM was given – this time at 273K. The samples were magnetised up to 1T, using the same steps as those used for the low temperature measurements. The samples were again step-wise demagnetised at 273K and measured with the magnetometer.

All samples were weighted prior to measuring in order to allow the calculation of the mass concentration of magnetic minerals in the tissues. Methods outlined in Dobson and Grassi (1996) were employed to control for possible sources of contamination due to airborne dust, post-mortem chemical changes and cauterisation effects.

Results

IRM acquisition

IRM acquisition curves measured from 15 tissue samples resected from living epileptic patients reveal clear evidence of the presence of ferrimagnetic material. In all samples the magnetisation from the tissue easily can be distinguished from the background signal produced by the quartz glass holder. Most of the tissue samples have a magnetisation several times larger than that of the holder alone.

Saturation magnetisation is reached in the samples by 200–250 mT, indicating a dominance of particles with low magnetic coercivity (Figure 1a). This saturation field is consistent with previous studies of human brain tissue (Kirschvink *et al.*, 1992; Kobayashi & Kirschvink, 1995; Dunn *et al.*, 1995; Dobson & Grassi, 1996) and with the presence of biogenic magnetite and/or maghemite.

Comparison of these curves with the IRM acquisition curves measured from the eight non-pathologic hippocampi resected from cadavers, shows the same magnetic characteristics observed in the MTLE tissue. The saturation fields again are between 200 and 300 mT and the shape of the acquisition curves indicates the presence of low coercivity magnetic phases (Figure 1b).

Calculation of magnetic particle concentrations

Calculations of the concentration of magnetic minerals were performed assuming that the material responsible for the magnetisation is biogenic magnetite. As maghemite has similar magnetic properties, the calculated concentrations would be only slightly different if the presence of that mineral represented some fraction of the overall magnetisation in the tissues (magnetite and maghemite are the only magnetic biominerals which have been found in examinations of brain tissue so far – Kirschvink *et al.*, 1992; Kirschvink *et al.*, 1995; Dunn *et al.*, 1995; Dobson *et al.*, 1998; Schultheiss-Grassi *et al.*, 1998). We also have assumed that the particles are single domain (SD) or smaller as only SD particles or smaller have been observed in TEM micrographs (Kirschvink *et al.*, 1992). For these reasons a saturation magnetisation value of 476 Am^{-1} (while that of maghemite is 426 Am^{-1}) was used.

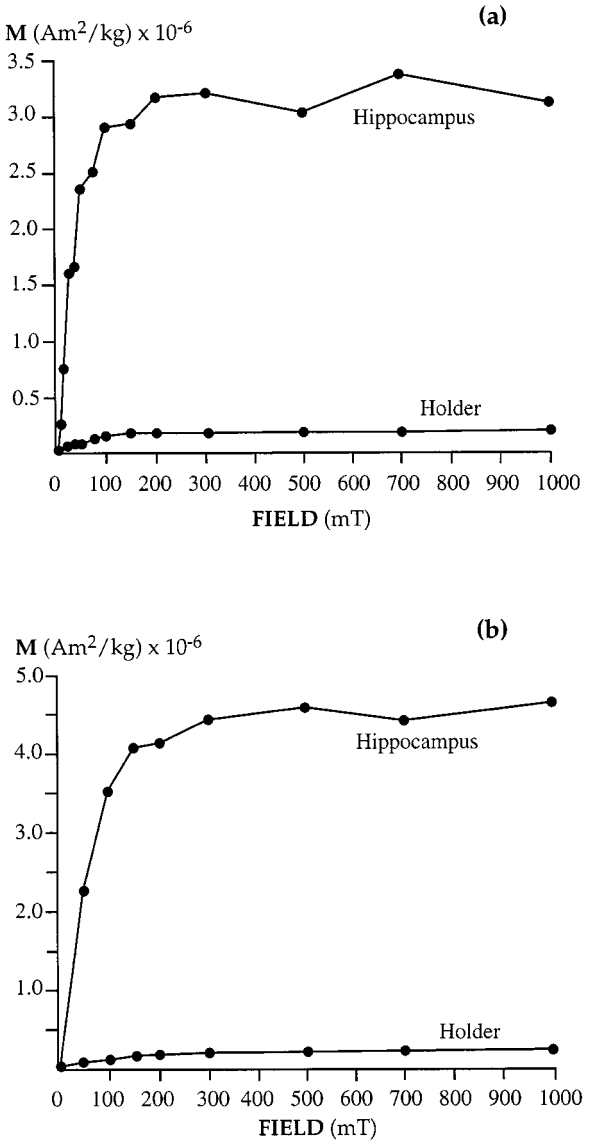

Figure 1. IRM acquisition curves showing both the holder's contribution and the contribution of the hippocampus after subtraction of the holder signal for (a) hippocampal tissue resected from MTLE patient HL and (b) hippocampal tissue resected from a cadaver (96/624).

The saturation remanence values for all hippocampal tissue samples were measured at 77K. The results are shown in Tables 1 and 2. The concentrations of magnetite in the hippocampi of non-pathologic cadaver tissue are broadly in the same range as the concentrations measured in the hippocampi resected from MTLE patients. This is a preliminary indication that this type of pathology doesn't play a role in the formation (and growth) of magnetite and/or maghemite.

Little can be said about variations in the magnetite concentration of tissue samples from regions of the brain other than the hippocampus (Table 3). The concentrations show a large amount of variation and too few tissue samples were available to examine general trends in the data.

IRM/AF demagnetization analysis

Additional magnetic properties of the tissue samples were analysed by examining alternating field (AF) demagnetisation behaviour. The IRM acquisition curve, when plotted together with the AF demagnetisation curve, can yield information on the

Table 1. Concentrations of magnetic material present in the hippocampi of MTLE patients measured at 77K. Calculations assume that magnetite is responsible for the magnetization in these samples.

Patient	Tissue Weight (g)	J_{rs} (Am^2/kg)	Conc. (ng/g) 77K
UW	1.591	1.24E-06	26.9
AK	1.635	1.85E-07	4.00
MS	1.679	1.15E-06	25.0
PH	1.281	2.59E-06	56.3
HD	0.461	3.95E-06	85.9
HL	0.624	3.32E-06	72.2
UR	1.083	7.87E-07	18.9
SH	1.131	1.37E-06	29.8
BL	1.388	1.17E-06	25.4
VH	1.225	1.41E-05	248
SA	1.217	1.38E-06	30.0
AH	0.425	2.02E-06	43.8
OZ	1.800	6.20E-07	13.5
KK	1.492	1.10E-05	240

Table 2. Concentrations of magnetic material present in the hippocampi of eight cadavers measured at 77K. Calculations assume that magnetite is responsible for the magnetization in these samples.

Cadaver #	Tissue Weight (g)	J_{rs} (Am^2/kg)	Conc. (ng/g) 77K
96/245	1.554	1.10E-06	23.9
96/268	1.814	7.55E-06	164
96/425	1.200	1.28E-06	27.8
96/515	1.527	2.70E-06	58.7
96/549	1.669	7.69E-07	16.7
96/624	1.126	4.55E-06	98.9
96/674	0.911	3.81E-06	82.8
97/025	0.889	1.46E-06	31.7

Table 3. Measured Wohlfarth ratios and median coercivities for human brain tissue samples

Sample	Wohlfarth Ratio	Median Coercivity (mT)
UW	0.39	25
MS	0.32	46
PH	0.30	36
HL	0.35	28
96/549 (hippocampus)	0.27	38
96/674 (cortex)	0.25	18
96/674 (cerebellum)	0.25	30
97/025 (basalganglia)	0.27	28
97/025 (cerebellum)	0.25	34

packing geometry of magnetic particles present in the sample (Moskowitz *et al.,* 1989). By examining the point where the two curves cross on the y-axis (the Wohlfarth ratio) it is possible to assess whether the particles are close enough to each other to permit magnetostatic interactions. Samples with a Wohlfarth ratio below 0.5 are magnetically interacting as magnetostatic interactions facilitate demagnetization but hinder IRM acquisition. The results of all IRM acquisition curves plotted together with the AF demagnetisation curves reveal that all samples have a Wohlfarth ratio below 0.5 (Figure 2 and Table 4). This suggests that the particles are magnetically interacting, meaning that they are likely present in the sample in clusters.

Superparamagnetic particle analysis

The IRM acquisition curves of tissue samples resected from three cadavers measured at 77K and at 273K show differences in the saturation remanence values (Table 4). This difference likely is due to the presence of superparamagnetic particles in the tissue indicating that a range of particle sizes is present.

Superparamagnetic particles are magnetic particles (in this case, they are likely ferrimagnetic magnetite and/or maghemite) which are too small to preserve a remanent magnetization at room temperature due to the effects of thermal agitation. Though the electron spin moments are quantum mechanically coupled, the spins are constantly flipping on a time scale much shorter than the measurement time. This time scale is defined by the relaxation time equation:

$$\tau = \frac{1}{f} \exp\left(v \frac{\bullet M_s \bullet H_a}{2kT}\right)$$

where f is the frequency factor, v is the grain volume, M_s is the spontaneous magnetization, H_a is the anisotropy field, k is Boltzmann's constant and T is the temperature in degrees Kelvin (kT represents the thermal energy at temperature T). From this relationship, it can be seen that the relaxation time is proportional to grain volume and inversely proportional to temperature:

$$\tau \propto \frac{v}{T}$$

As thermal energy is removed from the system by cooling to 77K, a fraction of these particles will have their magnetization 'blocked in' and the magnetization will remain stable (i.e. the spins will not flip) on time scales longer than the measurement time. They then will contribute to the remanent magneti-

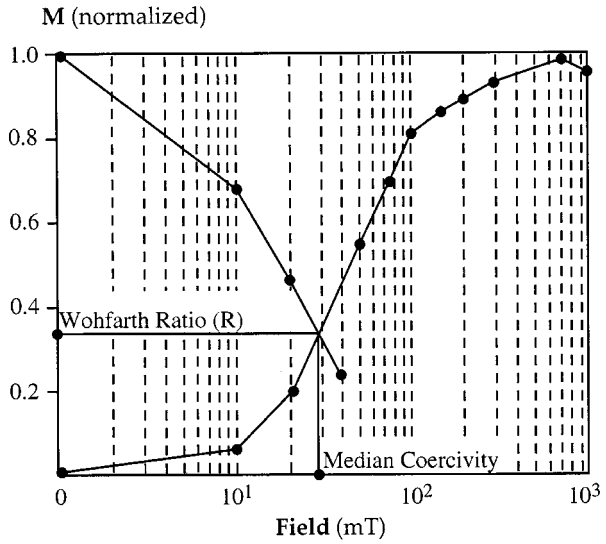

Figure 2. IRM acquisition and AF demagnetization curves for a sample of human hippocampal tissue from patient HL.

Table 4. Calculated magnetite concentrations based on saturation remanence at 77K and 273K. The difference in concentration is due to the unblocking of superparamagnetic particles at higher temperatures rather than an actual decrease in magnetite concentration. This gives an indication of the contribution of superparamagnetic particles to the IRM signal at 77K.

Sample	Conc. (ng/g) 77K	Conc. (ng/g) 273K
96/549 (hippocampus)	16.7	8.80
96/624 (hippocampus)	98.9	65.6
96/674 (leptomeninges)	79.7	79.1
96/674 (cerebellum)	14.2	15.2
96/674 (cortex)	69.8	54.3
96/674 (hippocampus)	82.8	59.8
97/025 (cerebellum)	16.8	16.7
97/025 (basalganglia)	12.1	4.4
97/025 (cortex)	14.9	12.7

zation. Differences in the saturation remanent magnetization at 77K and 273K therefore indicate the presence of superparamagnetic particles in the tissue. This can also explain some of the variation in the calculated concentrations as well as the differences between these concentrations and those reported by Kirschvink *et al.* (1992). Warming of the sample in the measuring space of the magnetometer will cause the unblocking of different proportions of superparamagnetic particles in different samples since the measurement time varies to some degree as does the concentration of superparamagnetic particles in different samples.

It should be noted that the calculated concentrations are 'apparent' concentrations and, as there are superparamagnetic particles present, they are temperature dependent. An increase in temperature will remove the contribution of some superparamagnetic particles from the IRM signal. This results in an apparent decrease in the concentration of magnetite in the tissue at higher temperatures. In fact, the superparamagnetic magnetite is still in the tissue but is no longer considered in the calculation of magnetite concentration. This behaviour is due to the presence of a spectrum of particle sizes and hence blocking temperatures. These concentrations, even those calculated at 77K then, are lower limits and the actual magnetite concentration could be higher if there are magnetite particles in the tissue that are superparamagnetic at 77K.

In addition to ultrafine-grained magnetite, another common iron biomineral found in human brain tissue is superparamagnetic – the ferrihydrite cores of the iron storage protein ferritin. It is important to determine whether or not the magnetization of the ferrihydrite cores in ferritin may be contributing to the overall magnetization of the tissue samples. Mössbauer studies of human ferritin have determined that, due to its ultrafine particle size (~8 nm diameter), the ferrihydrite core is superparamagnetic above 50K on timescales of 10^{-8} sec. (St. Pierre *et al.*, 1986). At 77K then, these particles would behave as paramagnets and do not contribute to the remanent magnetization.

Discussion

The main purpose of this study, was to assess the presence of magnetic material in the human brain and to examine the possibility of systematic variations in magnetic properties and concentrations with brain region and in epileptic vs. non-epileptic patients. The results of the magnetic analyses of hippocampal tissue resected from epileptic patients suffering from MTLE, as well as samples resected from different non-pathologic brain regions from cadavers, reveal magnetic properties and concentrations consistent with those reported recently (Kirschvink *et al.*, 1992; Dobson and Grassi, 1996).

No systematic increase or decrease in magnetic particle concentration was observed when comparing hippocampal tissue of MTLE patients with non-pathologic tissue from cadavers. This is an indication that, although there is evidence that magnetic fields may evoke epileptiform discharges in MTLE patients (Dobson *et al.*, 1995; Fuller *et al.*, 1995), magnetic material does not appear to be responsible for this process. There is some evidence, however, of systematic variation of the Wohlfarth ratio between the MTLE patients and the non-pathologic tissue from the cadavers, with the MTLE patients all having ratios above 30% and the cadavers all having ratios below 30%. This *may* indicate a slightly different particle configuration in the MTLE patients when compared to normal tissue and warrants further investigation.

The presence of superparamagnetic particles in the tissue can be inferred from the increase in saturation IRM values, when measured at 77K compared to measurements at 273K. These particles are ferrimagnetic but are of very small grain size and consequently have very short relaxation times. The presence of significant amounts of superparamagnetic particles in brain tissue is another indication that the magnetite/maghemite may not be employed for geomagnetic field sensing in humans as particles of this size are ineffective geomagnetic field sensors.

It can be seen from Table 4, however, that not all regions of the brain contain a significant amount of superparamagnetic particles. There is some evidence for systematic variation in the superparamagnetic fraction. Only the basalganglia and the hippocampus appear to have significant amounts of particles in this size range, although there is a small amount observed in the cortex. There is no measureable superparamagnetic component in the crebellum. Again, this variation, though seen in only a small number of samples, should be investigated further.

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