BioMetals 1999, **12**: 77–82

Magnetic iron biomineralization in rat brains: effects of iron loading

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Received 23 June 1998; accepted for publication 7 August 1998

Isothermal remanent magnetization was measured in 14 Wistar and five Porton rat brains. Results indicate that magnetic iron biominerals are present in most of the samples and the formation of these minerals in the rat brain is influenced by transfusion and dietary iron loading when compared to control samples. The high level of consistency in the concentrations and the lack of magnetic material in several of the measured samples indicates that a genetic mechanism may be responsible for magnetic iron biomineralization in the rat brain. Comparison with human studies indicates that extrapolation of the results of rat studies of electromagnetic field bioeffects may not be accurately extrapolated to humans in all cases.

Keywords: magnetite, maghemite, brain, bioelectromagnetics

Introduction

Biogenic magnetite (Fe_3O_4) is present in a large variety of organisms ranging from bacteria to humans (For review see: Kirschvink *et al*., 1985; Webb *et al*., 1990; Kobayashi & Kirschvink, 1995). Since the discovery of ferrimagnetic magnetite in the human brain (Kirschvink *et al*., 1992), few advances have been made in determining the mechanisms controlling the formation of this material in humans or its role in the central nervous system (CNS). While many organisms use biogenic magnetite as a mechanism for geomagnetic field sensing, in the human brain magnetic particles do not appear to be optimally configured for such a purpose (Dobson *et al*., 1995; Dunn *et al*., 1995). Though the presence of biogenic magnetite and/or ferrimagnetic maghemite (Fe_2O_3) recently has been confirmed in humans (Dobson *et al*., 1995; Dunn *et al*., 1995; Dobson & Grassi, 1996), the origin and function of magnetite in brain tissue remains speculative.

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With growing concern over the possible health effects of environmental magnetic field exposure (e.g. Hocking *et al*., 1996; Repacholi *et al*., 1997), magnetite-facilitated models have proven to be some of the most robust and physically plausible of the many proposed interaction mechanisms (Kirschvink, 1992; Kirschvink, 1996; Dobson & St. Pierre, 1996; Kirschvink & Neeson, 1997). As these models present one of the more plausible mechanisms for interactions of humans with environmental magnetic fields and as rats are used extensively in laboratory studies of bioelectromagnetic effects, it is important to determine whether this material is present in rat brains. A recent pilot study indicates that not all rat brain tissue contains magnetic material (Dobson & Schultheiss-Grassi, 1998). This clearly has implications for the extrapolation of the results of studies of laboratory rats to humans.

In order to address the issues of the presence of magnetite in rat brains and to possibly shed light on its origins and its relationship to iron intake, magnetic analyses were carried out on several populations of iron-loaded (both dietary and transfused) and control rats.

Methods

Brains were removed from four populations of Wistar rats and two populations of Porton rats and magnetic analyses were performed. The four populations of Wistar rats were treated in the following manner:

- Group GB1 was fed 3% carbonyl iron mixed with rat chow diet for 8 weeks.
- Group GB2 was fed 3% carbonyl iron mixed with rat chow diet for 8 weeks then changed to a low iron synthetic diet for 3 days. During the three-day low iron diet phase, this group had their bile ducts blocked.
- Group GB3 was fed 3% carbonyl iron mixed with rat chow diet for 8 weeks then changed to a low iron synthetic diet for 3 days. During the three-day low iron diet phase, this group had a sham operation in which the rat was sliced open but the bile duct was not blocked.
- Group GB4 was fed 3% carbonyl iron mixed with rat chow diet for 8 weeks then changed to a low iron synthetic diet for 3 days. No operations were performed on these rats.

These rats exhibited slowed growth and reduced weight gain when compared to non-iron-loaded Wistar rats.

The two populations of Porton rats were treated in the following manner:

- Group TR5 received intraperitoneal injections of rat blood every two weeks of life and were sacrificed at 10 months (TR5) and 12 months (TR6) of age. Rat TR8 was transfused until the age of 12 months, then the transfusion regime was ceased and the rat was sacrificed at 16 months of age.
- Group CR5 was a control group fed normal rat chow and sacrificed at 10 months (CR5) and 12 months (CR6) of age.

The iron-loaded Porton rats exhibited a loss of body weight and deterioration in appearence. In all iron-loaded Porton rats, the liver and spleen were enlarged compared to control rats. The dietary iron-loaded group also exhibited signs of anaemia whereas the other groups did not (for summary, see Chua-anusorn, 1997).

Brain extraction was carried out by wrapping the rat head in cellophane wrap (found to be non-magnetic in separate analyses) and then dipping the wrapped head in a plastic beaker containing liquid nitrogen until thoroughly frozen. The wrapped head was placed between the jaws of a vice which was covered in several layers of cellophane. The two outside layers covering the jaws of the vice were replaced before each use. The frozen head was split open by tightening the jaws of the vice. The head was then thawed on the laboratory bench wrapped in cellophane to protect the sample from airborne magnetic contamination.

Initially, a surgical scalpel was used to extract the brains but during magnetic analysis it became evident that the ferromagnetic blade was contaminating the samples. To solve this problem, a spatula was constructed from a sheet of high grade aluminium and was washed and placed in an ultrasonic bath before each use. The brain was removed from the rat by 'scooping' it out of the skull with the aluminium spatula when the head had partially thawed – approximately 30 minutes after immersion in liquid nitrogen.

After extraction, the brain was placed immediately in a fresh piece of cellophane and re-frozen in liquid nitrogen. The brain, wrapped in cellophane, was then placed in a plastic vial that had been washed with deionised water. This plastic vial was placed in a plastic cooler containing freezer blocks and 250 to 500 grams of dry ice for transport from the preparation laboratory to the magnetometer. The brains were immediately transported to the magnetics laboratory (about 100m away from the preparation facility) where the measurements were carried out.

All brain tissue samples were transferred to cylindrical quartz crystal glass holders (r=19mm, h=9.5mm) for measurement. These sample holders have a glass cap fitted over the open end of the cylinder. Each holder was soaked in 1M HCl for at least three days prior to measurement and was then washed in deionised water before use. During this procedure the brain was only handled with the aluminium spatula and a pair of plastic tweezers, both of which were cleaned using a Medisafe ultrasonic cleaner before each brain removal. In all cases, whole brains were used for anaylsis and the magnetization was corrected for brain mass.

In order to control for any magnetic signal from the quartz sample holder, the isothermal remanent magnetisation (IRM) acquisition of the holder was measured before each sample by the following procedure:

The sample holder containing a piece of cellophane wrap but no brain tissue was demagnetised by a 2G Enterprises alternating field demagnetiser at 130 mT. The sample holder was exposed to an incrementally increasing applied magnetic field up to 1 tesla (T) using a MMPM9 Pulse Magnetiser. After administering each magnetising pulse the magnetic moment of the sample holder was measured on a 2G SQuID magnetometer interfaced to a P.C. for data acquisition.

After completing the measurement of the blank quartz glass sample holder and cellophane, the brain was transferred into the piece of cellophane in the sample holder using ultrasonically cleaned, plastic tweezers. The cellophane was used to prevent movement during the measurement process. During the transfer of the brain into the quartz sample holder, the tissue was exposed to the open air for less than one minute – this limits the possibility of airborne contamination (Dobson & Grassi, 1996). The procedure for measuring the IRM acquisition outlined above was repeated, this time with the brain wrapped in cellophane in the quartz sample holder. The magnetic moment of the brain for each measurement step was determined by subtracting the magnetic moment of the sample holder/cellophane from the magnetic moment of the brain and sample holder.

In addition, the saturation remanent magnetisation (SIRM) due to the brain was determined as follows:

$$M_{SB} = M_{SHB} - M_{SH}$$

where M_{SB} is the saturation remanent magnetisation of the brain, M_{SHB} is the saturation remanent magnetisation of the holder plus the brain and M_{SH} is the saturation remanent magnetisation of the holder only. The saturation remanent magnetisation was taken to be the average of the magnetisation after exposure to fields greater than 500 mT. All measurements were made at room temperature.

The standard measurement procedure was to measure the IRM acquisition of the holder then the holder plus the brain in one measurement session. Some variations on this standard procedure were used to determine possible sources of contamination. For brain GB2.1 from the population GB2, after the measurement of IRM acquisition of the tissue, the sample was left for two weeks in the freezer and the IRM acquisition re-measured. The same test was done on brain GB2.2 from the same population but this sample was left for two months before the IRM acquisition of both the holder and brain was measured. These contamination tests gave an extreme worst-case indication of the level of potential magnetic contamination on the holder. Airborne contamination of the tissue itself would be negligible as it is only exposed for less than one minute.

The measured SIRM of the sample enables the concentration of magnetite in the brain tissue to be calculated. To determine this concentration it must be assumed that any ferromagnetic material present is magnetite. This assumption is consistent with the observed magnetic properties as well as with earlier studies (Kirschvink *et al*., 1992; Dunn *et al*., 1995; Dobson *et al*., 1995; Dobson & Grassi, 1996).

Results

The IRM acquisition of a total of nineteen rat brains was measured. Of these, the first four brains (Group GB1) were found to be contaminated by ferromagnetic material in the scalpel used to extract the brain. The contamination was likely due to abrasion of the blade when it came into contact with the skull and frozen brain material. Data from these samples were not used and further discussion will not include these samples.

For three of the remaining fifteen brains (GB2.2, GB3.2 and GB4.1) no difference was observed between the IRM acquisition of the holder and the IRM acquisition of the holder and brain. This is an indication that no magnetic material was present in these rat brains (Figure 1a). Also, two samples (GB2.3 and TR6) had a remanent magnetisation for the brain and holder that was less than the remanent magnetisation from the holder only. This is a likely indication that some form of magnetic contamination may have been present on the holder which came off between the two measurements. The difference between the holder saturation remanent magnetisation and the brain plus holder saturation remanent magnetisation was -1.19×10^{-6} e.m.u. for brain GB2.3 and -3.73×10^{-6} e.m.u. for brain TR6. Both these differences are larger than the measured magnetisation for any of the other brains that had not been contaminated by the scalpel.

Of the two brains from the group GB4 used to test for possible contamination sources, GB4.1 exhibited no evidence of magnetic contamination after two months (Figure 1a). GB4.2 showed a slight increase in saturation remanent magnetization (Figure 1b).

IRM analysis of the ten remaining brains (excluding the first four contaminated samples and five samples which contain no magnetic material) revealed the presence of magnetic material (Figure 1c) and magnetite concentrations were calculated for these samples (Table 1).

The concentration of magnetite in brains CR5 and CR6 is considerably lower than the other brain samples (mean = 5.48 ng/g). The mean concentration of magnetite in the iron-loaded brains is 16.49 ng/g (SD = 6.93).

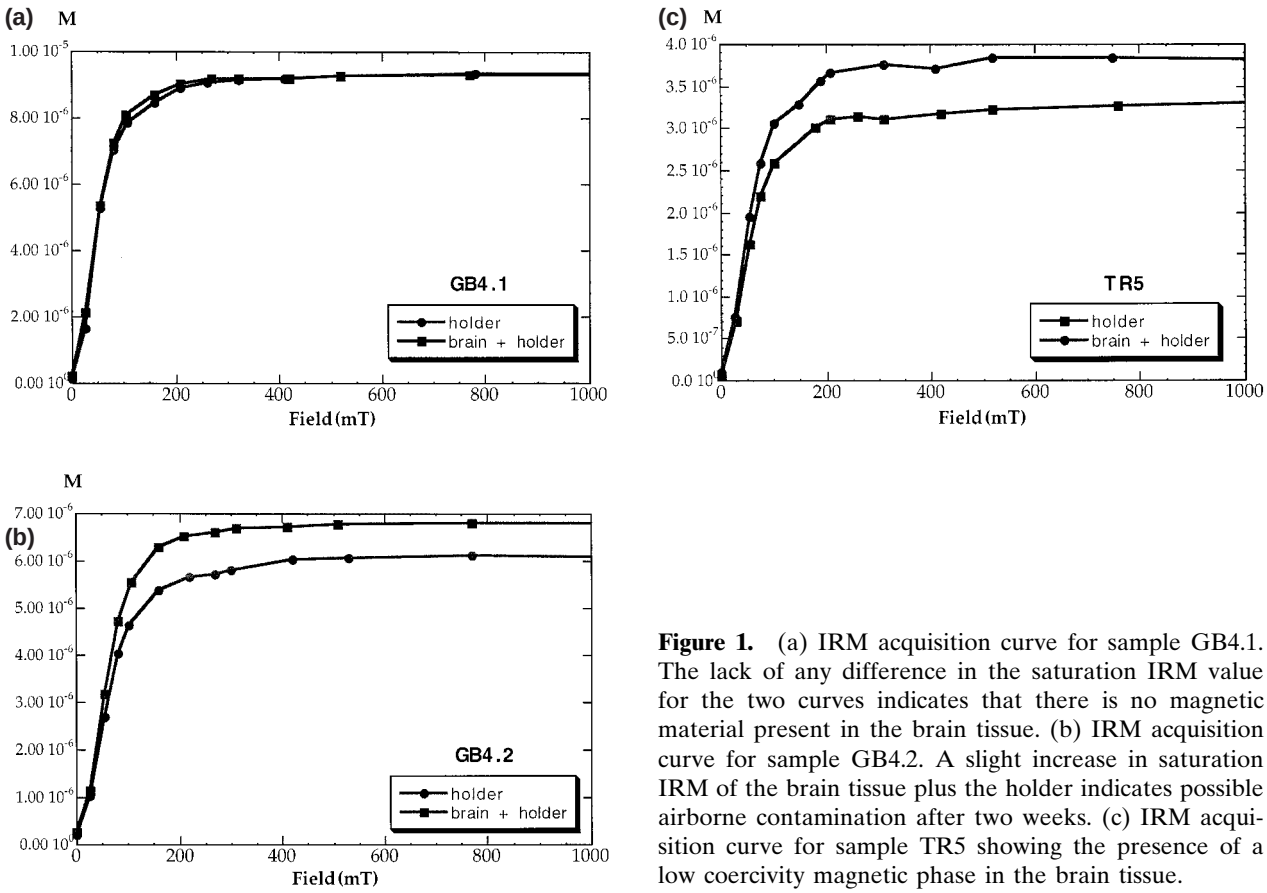

Figure 1. (a) IRM acquisition curve for sample GB4.1. The lack of any difference in the saturation IRM value for the two curves indicates that there is no magnetic material present in the brain tissue. (b) IRM acquisition curve for sample GB4.2. A slight increase in saturation IRM of the brain tissue plus the holder indicates possible airborne contamination after two weeks. (c) IRM acquisition curve for sample TR5 showing the presence of a low coercivity magnetic phase in the brain tissue.

Table 1 Calculated concentrations of magnetite in samples of rat brains. The concentrations were calculated using a saturation remanent magnetisation for pure magnetite of 46 Am^2kg^{-1}.

Sample Name	Sample Weight (grams)	SIRM ($G\ cm^3\ g^{-1}$)	Concentration (ng magnetite per g tissue)
GB2.1	1.0308	9.39E-07	20.41
GB2.2	0.8438	0	0
GB2.3	0.8192	0	0
GB3.1	0.7775	5.45E-07	11.86
GB3.2	1.1372	0	0
GB3.3	0.7781	1.24E-06	26.86
GB3.4	1.0879	6.16E-07	13.40
GB4.1	1.2074	0	0
GB4.1 (re-measure)	1.2074	0	0
GB4.2	1.2680	5.55E-07	12.06
GB4.2 (re-measure)	1.2680	8.05E-07	17.49
GB4.3	0.6038	6.77E-07	14.71
TR5	0.9533	5.92E-07	12.87
TR6	1.0389	0	0
TR8	0.8304	8.62E-07	18.74
CR5	0.8482	2.70E-07	5.86
CR6	0.6495	2.35E-07	5.10

Discussion and conclusions

Results of the IRM acquisition experiments give an indication that iron loading may lead to enhanced formation of magnetic iron biomineral phases in the brain when compared to the control group. Magnetite concentrations did not vary with the mechanism of iron loading. The small sample size of the control group, however, means that this result must be considered preliminary and bears further study. This could give an indication of the mechanism of formation of biogenic magnetite in brain tissue and the pathways of iron transport to the brain.

This study and a previous study (Dobson & Schultheiss-Grassi, 1998) reveal that the concentration of magnetite in rat brains differs from the concentrations in human brains in two ways. The first is the considerably higher level of consistency of detected levels of magnetite in rat brains in comparison to human brains. The measured levels of magnetite in human brains varies from a few ng/g in the cerebral cortex to over 100 ng/g in the meninges (Kirschvink, 1992) and between 4 ng/g and 85.9 ng/g in the human hippocampus (Dobson & Grassi 1996). This is likely due to inhomogeneities in the distribution of magnetic material in the human brain which are averaged out when the whole rat brain is measured and the fact that some of the human tissue samples were measured at 77K and include a superparamagnetic contribution. The measured concentration of magnetite in rat brains from this study is, on average, lower than levels in human brain tissue, although some areas of the human brain have comparable levels.

The second difference is that *all* human brain samples analyzed thus far have been reported to contain magnetite and/or maghemite. The three rat brain samples that had no remanent magnetisation did not differ from the other brains in terms of sample preparation, so it is reasonable to assume that no magnetic material is present in these brains. This is consistent with a previous preliminary report (Dobson & Schultheiss-Grassi, 1998) and could indicate that, rather than being a biproduct of a natural biochemical process, a genetic mechanism may control iron biomineralization. That would explain the fact that some rats do not produce this material while others do. Clearly the presence of rat brain samples containing no magnetic material indicates that extrapolation of laboratory studies of the effects of electromagnetic fields (EMF) on rats may not be an accurate proxy for humans. Larger studies of the magnetic properties of rat brains used in EMF/health effect assessment experiments appears warranted.

Acknowledgements

We would like to thank Mr. Greg Black, Dr. Tim St. Pierre and Miss Wanida Chua Anusorn for supplying the rat heads for this project. We also thank Dr. Z.X. Li for use of the 2G SQuID. This work is supported by the Australian Research Council Small Grants Scheme and The University of Western Australia.

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