Magnetic biomineralisation in Huntington’s disease transgenic mice

W Beyhum¹, D Hautot¹,², J Dobson² and Q A Pankhurst¹
¹London Centre for Nanotechnology, University College London, London, WC1E 7HN, UK
²Institute for Science and Technology in Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, ST4 7QB, UK

E-mail: w.beyhum@ucl.ac.uk

Abstract. The concentration levels of biogenic magnetite nanoparticles in transgenic R6/2 Huntington’s disease (HD) mice have been investigated, using seven control and seven HD mice each from an 8 week-old litter and from a 12 week-old litter. Hysteresis and isothermal remnant magnetisation data were collected on a SQUID magnetometer, and analysed using a model comprising dia/paramagnetic, ferrimagnetic and superparamagnetic contributions, to extract the magnetite and ferritin concentrations present. It was found that magnetite was present in both superparamagnetic and blocked states. A larger spread and higher concentration of magnetite levels was found in the diseased mice for both the 8 week-old and 12 week-old batches, compared to the controls.

1. Introduction
Iron plays a crucial role in maintaining the well-being and healthy functioning of organisms [1]. Iron is present in various forms in the human body and brain [1,2]. It is stored primarily in the iron-storage protein ferritin, whose 8 nm diameter cavity can store up to ca. 4500 iron atoms in the form of a ferrihydrite-like mineral [3,4].

Elevated levels of iron have been reported in the brains of patients suffering from a range of neurodegenerative diseases [1]. These diseases include Huntington’s, Parkinson’s and Alzheimer’s disease, as well as a newly diagnosed disease known as Neuroferritinopathy [5]. Further studies of the brain, spleen and other organs, have revealed the presence of the ferrimagnetic iron-oxide magnetite, Fe₃O₄ [2,6]. Thus far, mouse models of Huntington’s disease (HD) have proved to be popular and useful in studying the disease. In that light, HD mice brains were studied using magnetometry techniques in order to detect levels of magnetite present.

2. Experimental
Brain samples from transgenic R6/2 HD mice [7] and their non-HD mice litter-mates were used for the study. Two sets of different age mice, 8 weeks old and 12 weeks old, were used. Each set consisted of seven control (healthy) and seven HD mice. The experiments were conducted under blind conditions and the HD and control mice were only identified upon completion. The samples were taken from the cortex, striatum and hippocampus areas of the mouse brain, where the bulk of the diseased tissue is thought to lie.

Superconducting quantum interference device (SQUID) magnetometry measurements were taken using a Quantum Design MPMS-7. Standard M(H) hysteresis curves were taken from –2 T to 2 T at
Additionally, isothermal remanent magnetisation (IRM) curves were recorded. The SQUID magnetometer uses a sample holder on the end of which is attached a straw (in which the sample is put), which can then be inserted into the device. The samples were freeze-dried and compacted into the straw using two aluminium pistons. This was necessary to ensure that the sample would not move once in the straw. To do this, another straw was taken and a small section the length of the sample cut out the middle. This produced two half straws, which were inserted into the main straw on either end of the sample, essentially wedging the sample in place. For further information and details on the methods used please see reference [8]. All reasonable precautions were taken to ensure that no magnetic contamination came into contact with either the samples or the straws.

At 150 K the M(H) curve for each sample comprises four distinguishable magnetic contributions: superparamagnetic ferritin, superparamagnetic magnetite (corresponding to particles of diameter less than ca. 30 nm), blocked ferrimagnetic magnetite (particles larger than ca. 30 nm), and a linear contribution arising from the diamagnetic tissue and any paramagnetic fractions (such as residual haemoglobin) in the tissue. As the tissue makes up a significantly sized diamagnetic matrix throughout which both magnetite and ferritin are sparsely distributed, a system of non-interacting magnetic particles is assumed. Hence, in keeping with established methods [9], a model consisting of three separate Langevin terms and a linear term was used to fit the M(H) data. Two of the Langevin terms are used to model the magnetite in its blocked and superparamagnetic forms. For blocked magnetite, a mean particle diameter of 30 nm is used in the Langevin. For the superparamagnetic magnetite, a lognormal distribution of particles is assumed. Thus the magnetisation is modelled as a sum of Langdevins weighted by the distribution of particle sizes. The actual concentration of magnetite was found as a parameter of the fit, under the assumption that the saturation magnetisation of magnetite, 95.3 emu/g at 150 K, is to first approximation independent of particle size. Each individual term in the model must necessarily make up a portion of the total magnetisation, hence this parameter is simply a fractional multiplier of the saturation magnetisation – Langevin function product. As the saturation magnetisation of magnetite differs significantly from that of ferritin (at 150 K), it was possible to separate from the fit the abundance of magnetite in the sample. From the IRM data (taken at different temperatures), it was possible to see the presence of the magnetite and ferritin.

3. Results and discussion

Figures 1 and 2 show representative data from the healthy and HD cohorts of transgenic mice, along with least-squares fits to the data. It is notable that there is more magnetite in the HD sample than in the healthy sample, which is evident in the raw data (the upper-left insets in Figures 1 and 2) as a deviation from linearity. Relatively large (30 nm or bigger) magnetite particles are present in both samples, as evidenced by the non-zero hysteresis (illustrated by the ‘∆M curves’ shown as lower-right insets in Figures 1 and 2). In the plot of the HD data, the contributions of both blocked and superparamagnetic magnetite are discerned (Figure 2, dot-dash and dashed lines). In the control data, the contribution of total, unseparated magnetite is shown (Figure 1).

IRM data on the HD sample of Figure 2 is shown in Figure 3. These data confirm the presence of blocked magnetite, which saturates at a characteristic field of ca. 2 - 2.5 kOe. This is best seen in the 300 K curve, which is well above the blocking temperature of ferritin and/or sub 30 nm magnetite. A second magnetic contribution, not evident in the 50 K or warmer IRM curves, appears at 20 K and is clearly present at 5 K. This second contribution does not fully saturate, even in fields of 10 kOe, which is characteristic of magnetic nanoparticles with either (i) very large magnetocrystalline anisotropies or (ii) very small (sub 5 nm) size. In keeping with the M(H) data it is likely that the unsaturated component in this sample is due to the presence of both the ca. 8 nm antiferromagnetic ferrihydrite cores of ferritin (which have a large anisotropy), with perhaps some sub 5 nm magnetite nanoparticles also making a contribution.
Figure 1. Magnetisation versus applied field (M(H) curve) of a control sample. The crosses and solid line are the data and fit respectively (both with the linear components of the magnetisation subtracted). The dashed line is the superparamagnetic magnetite contribution. The dot-dash line is the contribution of blocked magnetite. The top inset is the raw data plot. The bottom inset is a $\Delta M$ plot (difference between the top and bottom branches of the M(H) curve).

Figure 2. M(H) curve of a HD sample. The crosses and the solid line are the data and the fit respectively (both with the linear components of the magnetisation subtracted). The dashed line is the superparamagnetic magnetite contribution. The dot-dash line is the contribution of blocked magnetite. The insets are as those of Figure 1.

Figure 3. IRM curves for an HD sample recorded at: 5 K (▲), 20 K (●), 50 K (♦), 150 K (■) and 300 K (▼).

Figure 4. Magnetite concentrations obtained from the fit of the M(H) curve of each sample for 8 week-old (◊) and 12 week-old (○) mice.
The concentrations of magnetite present in all 28 samples measured are plotted in Figure 4. There is a marked difference in spread and concentration magnitude between the control and diseased samples. Additionally, there seems to be a decrease of magnetite levels in general for 12 week-old mice as compared to 8 week-old mice. The significance and origin of this is still under study.

4. Conclusions
The fits to the M(H) data show the existence of ferritin, and of both blocked and superparamagnetic magnetite. The IRM data confirms the presence of both magnetite and ferritin. The HD samples for both 8 week-old and 12 week-old mice show a larger spread of magnetite concentration, with higher mean concentrations, than their control counterparts. The presence of both blocked and superparamagnetic magnetite suggests a spread of particle sizes. The significance of this and of the larger spread of magnetite in the HD samples is the subject of continued investigation.

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