	Experiment title: Magnetic biomineralisation in the brain	**Experiment number**: MD-6
Beamline: ID-18	**Date of experiment**: from: 08/11/02 to: 11/11/02	**Date of report**: 29/08/03
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Report:

Aim: The aim of this study was to characterise the iron-bearing compounds associated with the disruption of normal iron metabolism in the brain due to Huntington's disease (HD). In the normally functioning brain the iron is almost all sequestered in the storage protein ferritin, in the form of the partially crystallised mineral ferrihydrite ($Fe_5HO_8.9H_2O$). Ferrihydrite is a superparamagnetic antiferromagnet and has a characteristic anhysteretic magnetic response to an applied magnetic field. In our recent magnetometry studies of transgenic HD mice, we have identified at least one additional magnetic material in the diseased brain tissues (not present in the controls) that gives rise to a hysteretic magnetisation curve, the properties of which are consistent with the presence of the magnetic minerals magnetite (Fe_3O_4) and/or its oxidation product, maghemite (γ-Fe_2O_3). It is particularly important to obtain further information on these additional magnetic compounds, as they may hold a key to the neuropathological process itself. For example, magnetite is a stable repository for Fe^{2+} ions; and Fe^{2+} ions have been implicated as catalytic centres for the production of reactive oxygen species in the brain, which could in turn promote pathological collapse of brain biochemicals and proteins. Given the high specificity of the Mössbauer effect in distinguishing the structural and magnetic properties of iron-bearing compounds, and given the very low concentration of these compounds in the brain, our goal was to employ the nuclear forward scattering (NFS) technique to study HD mouse tissue.

Results: After many tests and optimisation of the beamline, a usable count-rate of ca. 0.12 counts/s was obtained on the sample (HD-250) containing the most iron as determined by SQUID magnetometry. The NFS spectrum of HD-250 was measured at 173 K and under a field of 3 T. The spectrum was acquired for 12 hours and led to the spectrum shown in Fig.1. (The same sample was measured in zero field but the quality of the data was poorer by a factor of three and could not be unambiguously analyzed.) The signal-to-noise ratio of the 3 T spectrum was sufficient to detect the presence of magnetic phases, as evidenced by the fast beating observed in the data. The spectrum was fitted between 20 and 160 ns using the *Motif* FORTRAN code; the *R*-factor of the fit was 1.20 and the sum of squares was 170.90. The background value was 13.3 counts per bin (10 channels per bin) and the calculated effective thickness was 1.68. The best fit is also shown in Fig.1 as well as the hyperfine field distribution which is shown in the inset. The hyperfine parameters are listed in Table I and further details about the field distribution are given in Table II.

The spectral analysis revealed two main components: an ordered magnetic phase with a broad distribution of hyperfine fields, corresponding to ca. 60% of the spectral area; and a quadrupole-split paramagnetic-like

phase, accounting for ca. 33% of the spectral area. We attribute these to the HD disruption product, and the normally occurring ferrihydrite, respectively. A very small third phase, accounting for ca. 7% of the spectral area, was also found. Its hyperfine field of 35.3 T is larger to that expected for pure □-iron. Since we believe it is extremely unlikely that □-Fe would appear in the brain tissue, we surmise that this is most likely to be somewhere else in the beam path, such as in the beryllium window where the external magnetic field could be up to 2.5 T. The count-rate for the impurity is less than 0.01 count/s, which may explain why it is not significant in other experiments performed on this beamline.

The field distribution of the majority phase, i.e. the HD disruption product, is bimodal, and has an overall mean value of 18.8 T, which is much less than the ca. 47 T expected for well crystallised magnetite, and the ca. 50 T expected for well crystallised maghemite. This is an intriguing result, implying that there is significant lattice disruption and/or substitutional effects leading to reduced hyperfine fields. The ferritin component, because of the external field of 3 T, shows a Collins-type spectrum. Collins-type spectra are not easily calculated with *Motif*, so it was approximated as a sextet with a small hyperfine field on the order of the applied field. The linewidth of ferritin, 3□$_0$, is consistent with a linewidth of 0.4 mm/s.

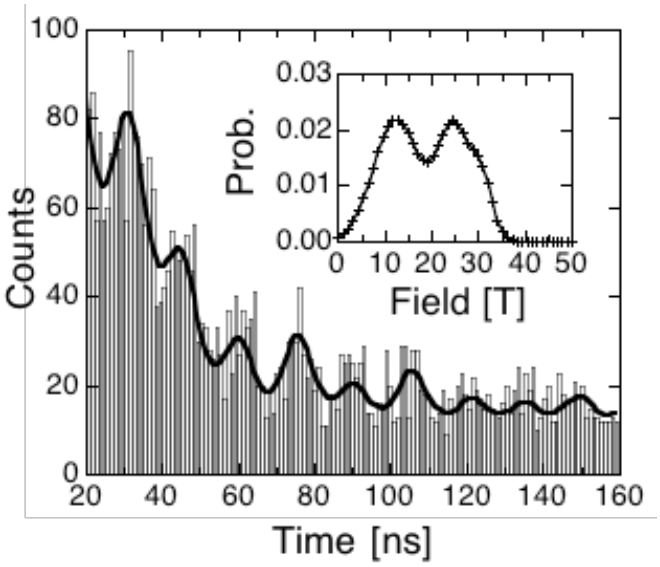

Fig. 1. NFS spectrum of the brain of an HD mouse measured at 173 K and with an external field of 3 T parallel to the beam.

Table I. Hyperfine parameters.

	Ferritin	Magnetite-like	□-iron like
IS, □$_0$	-	same	same
QS, □$_0$	-4.7*	0	0
Field, T	0.44(15)	See Table II	35.3(2)
LW, □$_0$	3.35	1	1
% Area	33.5	59.5(47)	7(1)

The background is 13.3(7), the scaling factor is 7226(210) and the effective thickness is 1.68.
*The EFG direction is defined by two angles which were fitted as ca. 36º and 23º.

Table II. Hyperfine field distribution.

Distribution	Peak I	Peak II	Peak III
Center	30.9(9)	24.7(4)	12.3
Width	2.16	3.6	4.84
Relative Weight	1	3.95	5.6

The distribution has 48 reference points between 0 and 50 T.

Conclusions: A majority magnetic phase, distinct from the normal ferrihydrite material found in healthy brain tissue, has been identified in the HD mouse. Although the statistical quality of the data obtained is not optimal, and not yet sufficient to allow a detailed analysis of the nature of this disruption product, it has been a major accomplishment to be able to measure it at all, given its very low concentration. Nevertheless it is notable that the magnetic signal does not seem to conform to that expected in bulk magnetic minerals.

Future experiments could include iron-57 enrichment of the HD mouse samples (via enriched feedstocks), a better monochromator, and the use of another cryostat to allow for the measurement of a larger sample. This improvement could lead to good quality spectra in 2 to 3 hours and would allow a realistic study of the field and temperature dependence as well as the comparison of diseased samples with control samples.

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