Magnetic Properties of Iron Oxides in the Human Globus pallidus

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Abstract
Several types of iron oxides can be found in the various parts of the human brain. These can be highlighted in the light microscopy and using scanning or transmission mode of the electron microscopy. Some of them are non-magnetic, some, on the contrary, display magnetic response. It is not clear which kind of magnetic particles are accumulated in the human brain as inorganic deposits. Light microscopy, electron microscopy and sensitive Superconducting Quantum Interference Device (SQUID magnetometer) were used in order to detect iron deposits and their magnetic response in the samples extracted from the Globus pallidus of the human brain. Electron microscopy reveals a presence of the single crystals of hematite (α-Fe₂O₃) of the size up to 1000 nm in the samples extracted from G. pallidus because of the diffractograms characteristic for the hexagonal unit cell; this mineral offers basically a diamagnetic response. The temperature dependence of the magnetic susceptibility allows a classification of the samples into three groups: mostly diamagnetic I, prevailing paramagnetic III, and an intermediate class II. The bulk samples exhibit a long-range magnetic ordering with magnetic hysteresis evidenced not only at low temperature but also at the room temperature. The recorded magnetic functions refer either to the presence of magnetite (Fe₃O₄), or maghemite (γ-Fe₂O₃). Iron oxides and oxidohydroxides found as inorganic deposits in the human brain can result from interaction between iron and microenvironment in the form of polysaccharides of glycoconjugates. They display magnetooactivity characteristic for magnetite and/or maghemite.

Keywords: Iron; Brain; Globus pallidus; Superconducting Quantum Interference Device (SQUID magnetometry); Polysaccharides

Abbreviations: Ft-H: Heavy Chain of Ferritin; Ft-L: Light Chain of Ferritin; RSO Mode: Reciprocating Sample Option Mode; SEM: Scanning Electron Microscopy; SQUID Magnetometry: Superconducting Quantum Interference Device Magnetometry; TEM: Transmission Electron Microscopy; ZFCM/FCM: Zero Field Cooled Magnetization/Field Cooled Magnetization

Introduction
Iron, as the most abundant transition metal in the human brain, occurs with uneven distribution. Highest concentration of iron can be observed in areas associated with motor function (Globus pallidus, putamen, Substantia nigra) than in other areas of the human brain [1,2]. Therefore, the actual role of iron accumulation in brain pathobiology is an active area of investigation nowadays [3]. It is evident that amount of iron in brain gradually grows with age. Accumulation of iron in basal ganglia is probably associated with neuronal death leading to Alzheimer disease, Parkinson disease, epilepsy, Huntington disease, dementia with Lewy bodies, and multiple sclerosis [4-8]. It was reported that iron participates in redox reactions, and catalyzes the formation of reactive oxygen species responsible for oxidative stress and damaging processes [9-11].

Iron in the human brain can be found mostly in the form of ferritin, hemosiderin (a product of ferritin breakdown) and other bioinmineralized oxidohydroxides and oxides such as hematite, magnetite, and maghemite [12,13]. Ferritin, as an iron storage nonheme-protein with diameter up to 12 nm, consist of inorganic core (6 nm) formed of ferrihydrite and minor portion of magnetite and hematite that is enclosed by two types of polypeptides [14]. The role of the heavy (Ft-H) form polypeptide coat of ferritin is to catalyze Fe(II) to Fe(III) ions whereas the light (Ft-L) polypeptide coat promotes the formation of ferritin iron core [15,16]. The amount of Ft-H and Ft-L polypeptide coats changes during ageing and the magnetic properties of ferritin can also alter.

In terms of the response to the magnetic field, three different classes of species were identified in the brain: The diamagnetic matrix (organic tissues, myelin, oxyhemoglobin), paramagnetic deoxyhemoglobin, paramagnetic/superparamagnetic ferritin and hemosiderin along with ferrimagnetic magnetite or maghemite [17-19].

In our previous work we studied iron distribution and magnetic properties in the human spleen and in G. pallidus of the human brain and their relationships to glycoconjugates [20,21]. The aim of this study is to investigate the magnetic properties of these iron deposits by the Superconducting Quantum Interference Device (SQUID) and asset the effect of glycoconjugates microenvironment.

Materials and Methods
Samples
Postmortem brain tissue sections from Globus pallidus externus were routinely obtained during the autopsy to prepare tissue sections for the pathology diagnosis at Department of pathology, Comenius University, Bratislava. Tissues were taken from individuals without clinical findings of any motor abnormalities, iron metabolism, movements involving limbs, face, and tongues (Table 1). All procedures were conducted in accordance with the Declaration of Helsinki.

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During the sample preparation, special attention was paid to avoid manipulations with magnetizable instruments. Fresh, soft tissues were dried in vacuum and the resulting samples were obtained in a form of powder that was used for magnetic measurements.

**Light microscopy**

The samples of 2 × 2 cm size were fixed in 10% formaldehyde for 24 h and embedded in paraffin blocks, cut by microtome to 5 μm thin sections and mounted on gelatin-coated slides. Sections were stained for general morphological purposes by Perls’ method (Table 2). Tissue sections were then covered by cover glass. Measurements by inductively coupled plasma mass spectrometry before and after the fixation process with formaldehyde solution showed that no leakage of tissue iron into the buffer solution had occurred [22].

Immunohistochemistry of human samples was performed on 10 μm paraffin embedded sections. Tissue sections were pretreated for 20 min with Antigen unmasking solution (Vector Laboratories, California) and for 1 min with 100% formic acid (Sigma-Aldrich, Germany). Sections were blocked with 1% BSA (Sigma-Aldrich, Germany) and incubated overnight with anti-ferritin light chain antibody ab110017 (Abcam, Cambridge, UK) at 4°C in the blocking solution. All sections were incubated with a biotinylated secondary antibody at room temperature for 1 h, and then treated with avidin-biotin peroxidase-complex for 60 min. The staining was visualized using Vector VIP kit (Vector Laboratories, California, USA) (Table 2). Tissue sections were then covered by cover glass and the samples were examined under the light microscope Eclipse E50i (Nikon, Japan).

**Transmission electron microscopy (TEM) and electron diffraction**

The samples investigated in light microscopy were removed from slide, prepared in powder form and cut by ultramicrotome for transmission electron microscopy investigation. Sample were fixed in solution of glutar (di) aldehyde (SERVA, Heidelberg, Germany) for 2 h and buffered by phosphate (pH 7.2-7.4). The samples were then washed by phosphate buffer and post fixed by OsO4, for 1 h in 0.0393 mol dm$^{-3}$ solution buffered by phosphate, pH 7.2-7.4. After dehydration the tissue by alcohol, samples were embedded into Durcupan ACM (Fluka AG, Busch, Switzerland) as recommended by the manufacturer and cut by ultramicrotome (Reichert, Wien, Austria). The thickness of samples was 200 nm. Noncontrasted ultrathin sections were mounted on nickel grids and investigated by transmission electron microscope JEOL 840B (Jeol, Japan) with acceleration voltage of 150 kV. In order to determine the iron oxide phase chemical analysis EDX KEVEX 3205-1200 (Kevex, Valencia, USA) was applied. For phase identification was used International Centre for Diffraction Data (ICDD).

**SQUID magnetometry**

The SQUID apparatus (Quantum Design, MPMS-XL7) was used for measurements of the magnetic moment of the specimen in the RSO (reciprocating sample option) mode of detection. Powder-like lyophilized samples were weighed (10-30 mg) into gelatin-made sample holders. The centering was done at $B=0.1 \text{ T}$ and $T=5 \text{ K}$. For the susceptibility measurements at the applied field $B$, $T=0.1 \text{ T}$, temperature varied between 2 K and 300 K. Magnetization measurements were conducted at $T_c=2.0 \text{ K}$ and 4.6 K at the applied field of up to $B=7.0 \text{ T}$. The measured signal was converted to the mass magnetization and/or mass magnetic susceptibility which are presented in SI units ($1 \text{ T}^1 \text{ kg}^{-1}=A \text{ m}^3 \text{ kg}^{-1}$). Actual magnetic data is presented: Diamagnetic signal of the organic tissue is subtracted neither for susceptibility nor for magnetization. The susceptibility was measured first (virgin sample), then magnetization and magnetic hysteresis loop, and finally when cooling in the zero field, the ZFCM/FCM experiments were carried out. As the recorded signal of the specimen is small, there is a hardware problem when passing from the paramagnetic to the diamagnetic response on heating: the system is frustrated in fitting to the equation of a perfect dipole and the regression fit is rather bad. This may cause some artificial discontinuities on the recorded data. For instance, the sample Ia possesses the measured magnetic moment $+9.4 \times 10^{-7} \text{ (cgs and emu)}$ at $T=8.2 \text{ K}$ and $-5.3 \times 10^{-7} \text{ at } T=8.5 \text{ K}$.

**Results**

**Optical microscopy**

Sections immunoreacted with ferritin showed two various structures in sample Ia. The first structure was ferritin positive glial cells. Ferritin immunostaining was present mainly on microglia and perivascular leukocytes in the human Globus pallidus. It is widely accepted that ferritin represents excellent marker of microglial cells.

In Figure 1 we demonstrated that ferritin was present in different stages of microglial activation and senescence, anti-ferritin antibody recognized reactive microglia (Figure 1A), hypertrophic microglia (Figure 1B), and dystrophic microglia (Figure 1C). The antibody did not recognize neurons or other types of glial cells. The second structure was a population of round, non-sharp-edged deposits of regular shape. The size of these ones ranged from 10 μm to 20 μm (Figure 1D). These deposits corresponded to iron detected by Perls’ staining. Ferritin distribution revealed an irregular pattern with distinctive foci.

Perls’ reaction revealed a population of blue round deposits of Fe(III) in sample Ia (Figure 2). The size Fe(III) round deposits in diameter ranged from 10 μm to 20 μm. Turnbull reaction for Fe(II) detection revealed very fine granular blue deposits located sporadically around glial cells (not shown).

**Electron microscopy**

Selective electron diffraction in TEM showed the presence of well crystalline material of various structure with lattice parameters $a=0.503 \text{ nm}, c=1.375 \text{ nm}$ corresponding to hematite in sample Ia. Some particles

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**Table 1:** Characteristics of the samples extracted from the Globus pallidus.

<table>
<thead>
<tr>
<th>Sample classification</th>
<th>Age at decease</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Post-mortem interval in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>69</td>
<td>F</td>
<td>heart failure</td>
<td>10</td>
</tr>
<tr>
<td>Ib</td>
<td>52</td>
<td>M</td>
<td>gastritis</td>
<td>11</td>
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<td>F</td>
<td>heart failure</td>
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<tr>
<td>Ilib</td>
<td>66</td>
<td>M</td>
<td>nephritis</td>
<td>9</td>
</tr>
<tr>
<td>IIIa</td>
<td>53</td>
<td>M</td>
<td>cirrhosis</td>
<td>7</td>
</tr>
<tr>
<td>IIIb</td>
<td>73</td>
<td>M</td>
<td>fat embolus</td>
<td>8</td>
</tr>
</tbody>
</table>

*M: Male; F: Female

**Table 2:** Immunohistochemical reagents used in this study.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Interpretation of staining reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perls’ method</td>
<td>Fe(III) ions, blue color</td>
</tr>
<tr>
<td>Anti-Ferritin Light Chain antibody (polyclonal) Abcam ab110017</td>
<td>Synthetic peptide: C-GEYLFERLTLKDHD, corresponding to C-terminal amino acids 163-175 of Human Ferritin Light Chain</td>
</tr>
</tbody>
</table>

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were irregular, non-homogeneous, solid punctuate, sporadically exhibit hexagonal shape. The shape of smaller particles was more regular; the size varied around 2 µm. The shape of larger bumpy particles was more irregular (Figure 3). Sometimes the bumpy particles exhibit less ordered structure. The size of these particles was up to 6 µm. Small deposits of unknown origin can be seen on the surface of some particles.

**SQUID magnetometry**

Temperature dependence of the mass magnetic susceptibility $\chi$ (taken at $B=0.1$ T) is shown for three representative samples in Figure 4. The inverse susceptibility and the product function $\chi T$ are plotted. The data reveals three groups of samples. Each class is represented at least by two examples; the data for the second member of each class were forwarded to Electronic supplementary material.
Figure 3: Human brain, Globus pallidus sample Ia. Irregular micrometer-sized particle (left) with diffraction pattern corresponding to hematite-α-Fe₂O₃ (right). Transmission electron microscopy, scale bar-1 µm.

Figure 4: Classification of samples according to the magnetic susceptibility (virgin samples at B=0.1 T): A class I-sample Ia, B class II-sample IIa, C class III-sample IIIa.
Class I contains dominating diamagnetic species with an admixture of paramagnetic entities registered at low temperature. The product function $\chi T$ vs. $T$ develops according to a straight line with a negative slope.

Class III, on the other hand, displays paramagnetism over the whole temperature range (2-300 K). The $\chi T$ vs. $T$ function is linear, but with a positive slope. The diamagnetic signal of the organic tissues is superimposed by much stronger paramagnetic (or ferromagnetic) signal of the admixture present in the sample.

Class II is intermediate between class I and III. The overall magnetoactivity corresponds to paramagnetism; the $\chi T$ vs. $T$ function displays a complex behavior. The susceptibility decays almost to the zero at the room temperature.

The $\chi T$ product function in the interval $T=150$-250 K has been fitted by a straight line $\chi T = C + a T$ and the data are presented in Table 3.

The ferro-/ferrimagnetic nanoparticles exposed to a strong magnetic field (5-7 T) could orient themselves along an easy axis so that the subsequent susceptibility measurements in the longitudinal field could reflect the ordered sample with prevailing paramagnetism. Notice, the RSO mode of the operation means that the sample oscillates with defined frequency (4 Hz) within the detection coil so that the corresponding force supports the ordering of nanoparticles.

### Magnetization data for class I

The magnetization measurement conducted for the representative class I compound is shown in Figure 5. This is caused by the superposition of the Langevin function weighted by the amount of the paramagnetic (ferro/ferrimagnetic) fraction $w_{PF}$ and the constant negative contribution from the diamagnetic tissue $\chi_{dia}$. The magnetization curve can be analyzed by using the equation

$$M_{max}(B,T) = w_{PF} \times \left[ M_{a} \times (N_{a} \mu_{a} \mu_{0}) \times \left( \coth \left( \frac{B}{T} \right) - 1 \right) \right] + (1-w_{PF}) \times \left( \frac{\chi_{dia}}{\mu_{0}} \right) B_{int} \quad (1)$$

For the argument

$$x = \mu_{B} \left( \frac{B_{int} + B_{ext}}{k_{B} T} \right) \quad (2)$$

Here $\mu$-magnetic moment in units of Bohr magneton, $M_{a}$ -the molar mass of iron, $w_{PF}$ -mass fraction of the paramagnetic species; physical constants adopt their usual meaning. The internal magnetic field $B_{int} = W \times M$ is expressed by the Weiss field constant $W$ and it causes the appearance of the remnant magnetization and the magnetic hysteresis. These equations need be solved by an iterative procedure.

The calculated magnetization curve for the sample under study is in a satisfactory agreement with the experimental data and the retrieved parameters are listed in Table 3. In this measurement the admixed paramagnetic signal is strong enough and masks the diamagnetic

<table>
<thead>
<tr>
<th>Sample classification</th>
<th>$T=150$-250 K</th>
<th>$T=2$ K</th>
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<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Sex</td>
</tr>
<tr>
<td>Ia</td>
<td>69</td>
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<tr>
<td>Ib</td>
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<td>66</td>
<td>M</td>
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<tr>
<td>IIIa</td>
<td>53</td>
<td>M</td>
</tr>
<tr>
<td>IIIb</td>
<td>73</td>
<td>M</td>
</tr>
</tbody>
</table>

*SI units: Curie constant $C$ [m$^3$ kg$^{-1}$ K]; temperature-independent term $a$ and $x_{dia}$ [m$^3$ kg$^{-1}$]; magnetic moment per particle $\mu_{B} \mu_{0}$ [J T$^{-1}$]; Weiss-field constant $W$ [J T$^2$ kg$^{-1}$]; remnant magnetization $M_{R}$ [J T$^{-1}$ kg$^{-1}$].

Table 3: Susceptibility and magnetization parameters for representative samples.

![Figure 5: Left: field evolution of the mass magnetization for class I sample Ia; solid line-fitted. Centre and right: ZFCM/FCM experiments.](image-url)
background at $T=2.0$ K. There is some remnant magnetization $M_R$, Weiss field $W$, and the retrieved magnetic moment is $\mu=4.3 \mu_B$.

The sample was heated up to the room temperature and then cooled to 2 K at the zero fields. Then small magnetic field was applied (5 mT and/or 10 mT) and the magnetization data was taken on the heating up to the room temperature in the settle mode (ZFCM experiment). The data was acquired during the cooling phase with the same applied field (FCM experiment). These records are also presented in Figure 5. The two curves merge approximately at 100 K which is the paramagnetic region. Notice an anomaly at ca 40 K that is below the expected solidus-solidus transition for the oxygen contaminated sample. With the doubled field the magnetization data is approximately doubled that confirms a reproducibility of these measurements.

The hysteresis loops are visualized in Figure 6. With increasing temperature the paramagnetic signal decays and diamagnetic background starts to dominate. Consequently the profile of the magnetization curve alters from the S-shape to the Z-one. Above $T>100$ K the hysteresis loop stays almost unchanged.

**Magnetization data for class III**

For the class III compound (that is paramagnetic at $B=0.1$ T in the whole temperature region) the situation is different as shown by magnetization measurements in Figure 7. The virgin magnetization curve differs from that measured after several exposures to a strong magnetic field when the magnetic nanoparticles are subjected to an ordering.

Also the ZFCM/FCM experiments were done for this sample. It is registered that the devitrification point (when the FCM/ZFCM curves coincide) is not below 300 K which means that the Curie temperature $T_C$ lies above 300 K. A feature around 50 K is characteristic for the presence of magnetite but this could be due to the phase transition for solid oxygen present in the sample.

The search for the presence of magnetic hysteresis of the class III types was positive, and the recorded representative curves are displayed in Figure 8. The hysteresis loop survives until the room temperature. Again one can register a switch of the S- to the Z-shaped magnetization curves on the heating. It is registered that the hysteresis loops possess a complex structure, especially at higher temperature, that indicates a multicomponent character of the sample. They, however, keep a perfect symmetry on field reversal. Thus, individual features are not artifacts of the particular measurement (hardware limitations, noise).

The measurements for $T=300$ K have been repeated and identical hysteresis loop was recorded. In these records the SQUID response passes through zero when the system fails in fitting to the ideal dipole function. Those artifact points with the regression fit coefficient <$0.8$ have to be removed. After subtraction of an overall linear diamagnetic signal ($M_{dia}=-17.2 \times 10^{-3}$ J T$^{-1}$ kg$^{-1}$) the coercive field becomes 9 mT at the room temperature. In an analogous treatment $B_c=10$, 12 and 13 mT for $T=150, 100$, and 70 K, respectively.

**Magnetization data for class II**

Magnetization data for the class II sample is presented in Figure 9.
Figure 7: Left: field evolution of the mass magnetization for class III (virgin) sample IIIa; solid line-fitted; Centre: field treated sample; Right: ZFCM/FCM experiments.

Figure 8: Hysteresis curves for the class III (sample IIIa); bottom-right-after subtraction of the diamagnetic signal and removal of artifact points.
The mass magnetization passes through a maximum at 2 K and then it decays in accordance with the prevailing diamagnetic response at the higher fields. The ZFCM/FCM intercept exceeds 300 K so that there is indication that the magnetic hysteresis will survive to the room temperature.

Indeed, the sample belonging to class II exhibits the magnetic hysteresis as shown in Figure 10. The complexity manifests itself not only on the course of the magnetic susceptibility (and/or the product function $\chi_T$), but also in the profile of the magnetization curves. As evident, the Z-shaped high-field part possesses an S-shaped low-field insert; just this insert escapes on the heating. The coercivity again depends on temperature: in the series of $T=5$, 10, 20, 50, 100, 200 and 300 K the coercive field varies as $B_c=73$, 24, 17, 9, 22 (12), 20 (10) and 18 (8) mT where values in parentheses refer to the loop after subtracting the diamagnetic background.

Temperature evolution of the remnant magnetization $M_r$ is given in Figure 11. It is seen that this property decreases with increasing temperature, the fact that matches expectation. Unlike the expectation, the $M_r$ values are higher for the class II sample at low temperature.

Returning to the Table 3, clear difference among the three classes is represented by the Weiss field constant at $T=2.0$ K: $W<0.5$ for class-I, $W=1.0$ for class-II, and $W>1.5$ for class III (in the given units). Just this parameter characterizes the long-range ordering. The temperature-independent susceptibility term is $\alpha<0$ for class I and II, whereas $\alpha>0$ holds true for class III samples.

Discussion

Iron deposits are found in places with high metabolic activity around glial cells and are physiologically accumulated depending on age [23,24]. Iron is the metal that can form ferrimagnetic compounds with oxygen and/or sulfur. However, sulfide minerals have only been reported accompanying bacterial activity [15]. Our findings of iron depositions near glial cells agree with results of other studies [25-27].

Magnetic properties of iron compounds depend greatly on the ligand-based environment. Ferritin as an iron storage globule displays a superparamagnetic behavior and in its inorganic core it contains about 4500 atoms of Fe(III) [28]. Ferromagnetic deposits of iron oxides in the form of nanocrystals of magnetite in the human brain were observed for the first time by Kirschvink et al. [19].

The complexity of the samples under study can be enumerated as follows. Each sample is unique and there is no chance for its reconstruction or synthesis and thus only small amounts should be used for any destructive investigative methods. The mass of the sample depends upon the degree of lyophilization and the sample behaves unlike to inorganic salts that can be grained to a fine powder and eventually fix by some additives or as pellets. The mass loss caused lyophilization leads to a higher concentration of iron deposits in the sample under investigation and a reduction of the diamagnetic background given by water and tissues.

Each sample contains magnetic particles of different sizes, compositions and counts. The iron-oxide minerals that are antiferromagnetic at bulk possess uncompensated spins at the surface the contribution of which increases with the decreasing size. Thus the overall paramagnetic response can originate in the ferritin, magnetite (Fe₃O₄), maghemite ($\gamma$-Fe₂O₃) as well as nanoparticles of hematite ($\alpha$-Fe₂O₃), FeO, FeOOH and other minerals along with some other compounds of transition metals (like Cu(II)) and dioxygen itself. The accumulation of these species could be different in the different part of the brain and depends upon disease and age.

Identification of the hematite nano/microparticles in the human brain by electron microscopy is rather surprising since just this mineral is missing among deposits in a huge group of animals. However, the hematite, weakly-magnetic in bulk at the room temperature, is the thermodynamically most stable iron-oxide mineral and this could appear as the end-product on the human aging. Particles of hematite smaller than 8 nm display superparamagnetic behavior at the room temperature [29].

Samples of human Globus pallidus from the view of magnetic susceptibility may be classified into three groups-dominating diamagnetism with some paramagnetic impurities (class I),
Figure 10: Hysteresis curves for the class II (sample IIa); diamagnetic signal not subtracted.

Figure 11: Temperature dependence of the remnant magnetization. A) Class I (sample Ia) (B) Class II (sample IIa) (C) Class III (sample IIIa).
paramagnetic and diamagnetic with much stronger paramagnetic and/or ferromagnetic components (class III), and an “intermediate” group (class II). A classification according to the temperature evolution of the magnetic susceptibility or the product function $\chi T$ is somewhat arbitrary, since this can be influenced by the applied field and the sample treatment (exposure to high magnetic fields).

In all classes the ZFCM/FCM experiments confirm a rather high devitrification temperature: ca 100 K for class I, and $T_C > 300$ K for class II and class III samples. Nevertheless, the magnetic hysteresis survives for all three classes until the room temperature with different remnant magnetization (lowest for class I, highest for class II). Temperature dependence of the remnant magnetization for class II and class III shows a similar course. This data does not correspond to the presence of $\alpha$-Fe$_2$O$_3$, Cu(II), Cr(III), Fe(III)/Fe(II), ferritin or other paramagnetic species. Moreover, FCM/ZFCM measurements do not match the presence of ferritin because of absence the fingerprint feature (a maximum at the ZFCM curve) at $T = 10$ K [30]. In addition, FCM/ZFCM measurements show features at around 50 K that are characteristic for the presence of magnetite [31]. The Verwey (charge ordering) phase transition at 120 K, characteristic for the magnetite, is seen neither on the susceptibility curve nor on the ZFCM curve; this supports the presence of maghemite.

To this end, the remnant magnetization, coercive field and ZFCM/FCM profiles indicate a presence of magnetite or maghemite. Notice, however, the distribution of magnetite in the human brain. From these results it can be concluded that magnetite is not formed spontaneously from ferritin, but through a biologically controlled processes.

Hysteresis curves of the class III sample reveal a variation of the coercivity with temperature: On temperature elevation $T = 2$, 5, 20, 50, and 70 K the coercive field varies in a non-systematic way as $B = 7, 11, 4, 5, 17$ mT. Between $T = 100-300$ K, however, the shape of the hysteresis loop becomes very complex and the coercive field is problematic to read off. The first cross with the zero magnetization occurs at 50, 41, and 5, and 17 mT. Between $T = 100-300$ K, however, the shape of the hysteresis curve becomes very complex and the coercive field is problematic to read off. The first cross with the zero magnetization occurs at 50, 41, and 5, and 17 mT. Between $T = 100-300$ K, however, the shape of the hysteresis curve becomes very complex and the coercive field is problematic to read off. The first cross with the zero magnetization occurs at 50, 41, and 5, and 17 mT. Between $T = 100-300$ K, however, the shape of the hysteresis curve becomes very complex and the coercive field is problematic to read off. The first cross with the zero magnetization occurs at 50, 41, and 5, and 17 mT.

The complexity of the hysteresis loops (especially for class III sample) may be caused by a multicomponent composition of the samples containing deposits of several magnetic and non-magnetic iron oxides ($\alpha$-Fe$_2$O$_3$, $\gamma$-Fe$_2$O$_3$, $\delta$-Fe$_2$O$_3$) of different size, shape, and count [34].

Variations of the magnetic properties of brain tissue can have several different biophysical origins-ferritin accumulation [35], the presence of metal deposits [16,22], various structure of ferritin iron core during ageing [36-39] and interaction between iron and the surrounding environment [40]. The organic components of the cell-lipids, proteins and polysaccharides can influence the biomineralization process by acting as a general surface for accumulation. Two models have been proposed to explain the interactions between iron(III) and (poly)saccharides. Results of this interaction are various iron oxyhydroxides such as hematite, ferrihydrite, magnetite, goethite, akaganéite or iron metal.

**Conclusion**

Deposits of iron oxides in the *Globus pallidus* of the human brain are visible under optical microscope as objects of regular and or/irregular shape and giving sharp diffraction patterns in the transmission mode of electron microscopy confirming their crystallinity. The SQUID magnetometry shows that the magnetization curves deviates from an ideal Langevin function due to the dominating diamagnetism of organic tissue. Owing to different portion of magnetic particles (nano/microcrystals of Fe$_3$O$_4$ and/or $\gamma$-Fe$_2$O$_3$) the hysteresis curves detected even at the room temperature display a complex course. Based on magnetic susceptibility, the samples can be classified into three groups: Class I with dominating diamagnetism, class III with prevailing paramagnetism, and the intermediate class II. This classification is supported also by the Weiss field parameter that characterizes the average long-range ordering.

**Acknowledgments**

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