Quantification of iron in the non-human primate brain with diffusion-weighted magnetic resonance imaging

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Abstract

Pathological iron deposits in the brain, especially within basal ganglia, are linked to severe neurodegenerative disorders like Parkinson's disease. As iron induces local changes in magnetic susceptibility, its presence can be visualized with magnetic resonance imaging (MRI). The usual approach, based on iron induced changes in magnetic relaxation (T2/T2*), is often prone, however, to confounding artifacts and lacks specificity. Here, we propose a new method to quantify and map iron deposits using water diffusion MRI. This method is based on the differential sensitivity of two image acquisition schemes to the local magnetic field gradients induced by iron deposits and their cross-term with gradient pulses used for diffusion encoding. Iron concentration could be imaged and estimated with high accuracy in the brain cortex, the thalamus, the substantia nigra and the globus pallidus of macaques, showing iron distributions gate iron presence in normal aging subjects (Aquino et al., 2009; Reese et al., 2003). Hence, in the presence of iron deposits, the measured Apparent Diffusion Coefficient (ADC), as evidenced dephasing and an increase in transverse relaxation (overall increase of R2/R2* relaxation) (Milton et al., 1991; Antonini et al., 1993; Schenker et al., 1993; Corell et al., 1995; Brass et al., 2006). Several MRI methods have been developed based on this effect to detect and quantify iron in the brain (Milton et al., 1991; Antonini et al., 1993; Schenker et al., 1993; Corell et al., 1995; Brass et al., 2006; Haacke et al., 2005; Hardy et al., 2005; Wallis et al., 2008; Péran et al., 2009; Aquino et al., 2009; Deistung et al., 2013; Sedlacik et al., 2014; Jensen et al., 2009) investigate iron presence in normal aging subjects (Aquino et al., 2009; Sedlacik et al., 2014) and in patients with neurodegenerative diseases, such as PD (Wallis et al., 2008; Péran et al., 2009; Graham et al., 2000) or traumatic brain injury (Raz et al., 2011). However, these methods of quantification suffer from the fact that iron induced BMS effects are not the unique source of signal phase shifts and R2 changes in tissues (Deistung et al., 2013; Sedlacik et al., 2014). On the other hand, the BMS effects induced by iron are responsible for small local magnetic field gradients, which in the context of diffusion MRI, produce significant cross-terms with the programmed gradient pulses inserted for diffusion encoding, resulting in an underestimation of the measured Apparent Diffusion Coefficient (ADC), as evidenced with the decrease of ADC observed after administration of UltraSmall Particle Iron Oxide (USPIO) in the liver (Zhong et al., 1991; Does et al., 1999). Contrary to the R2/R2* effect which cannot be fully reversed, this effect on the ADC can be eliminated when using diffusion MRI sequences immune to effects of local magnetic field gradients, such as sequences made of “bipolar” gradient pulses (BPG) instead of the usual “monopolar” gradient pulses (MPG) (Zhong et al., 1998; Song et al., 1999; Reese et al., 2003). Hence, in the presence of iron deposits...
a direct comparison of diffusion images acquired with the BPG and MPG sequences could reveal the presence of local field gradients, and, thus, the presence of iron. In this study we have developed this concept to quantify iron deposits. Measurements have been done in the brain of non-human primates of various ages and compared with estimated endogenous iron concentration in the cortex, thalamus and basal ganglia (substantia nigra and globus pallidus). Measurements were also obtained after the injection of USPIOs to increase brain iron content in a controlled manner. Those USPIO results suggest the potential of the approach for perfusion and functional MRI studies.

Theory

Iron particles create local magnetic field gradients which cause a signal reduction in the tissue through static spin dephasing (gradient-echo sequences) and diffusion (gradient-echo and spin-echo sequences). In the presence of such local gradients the measured ADC can be artfactually decreased when using usual (monopolar) diffusion MRI sequences (Zhong et al., 1991; Does et al., 1999; Kennan et al., 1995; Kiselev, 2004). This effect results from the presence of cross-terms between the local background gradients induced and the applied diffusion-encoding pulsed gradients. This ADC decrease may appear counterintuitive, but is well explained by the nonlinear relationship between the diffusion signal attenuation with the b value: the negative cross-term contribution to increase the signal level (decrease in local effective b value) is higher than positive cross-term contribution which decreases the signal level (increase in local effective b values). Assuming the distribution of negative and positive cross-terms is approximately equal, this asymmetry in the effect on the signal level results in an over- or underestimation of the ADC depending on the measurement (diffusion) time and the variance of the local gradients (Zhong et al., 1991), and increases with the iron particle intrinsic relativity and concentration, [Fe], so that the diffusion signal attenuation, S, becomes:

$$ S = S_0 \exp\left(-b \cdot \left(1 - \xi_{\text{Fe}}\right) \cdot ADC\right) \equiv S_0 \exp\left(-b \cdot ADC^*\right) \tag{1} $$

with

$$ ADC^* = \left(1 - \xi_{\text{Fe}}\right) \cdot ADC \tag{2} $$

$S_0$ is the signal at $b = 0$. Ignoring iron effects, fitting of diffusion MRI data with Eq. (1) would lead to ADC with $ADC^* \propto ADC$. When using a bipolar (BPG) gradient pulse sequence the effect of cross-terms disappears ($\xi_{\text{Fe}} = 0$), so that the ADC is correctly estimated. BMS related effects on relaxation $R2/R2^*$ are included in $S_0$, which contains an implicit $exp(-TE/R2)$ term ($TE = $ echo time).

A limitation of Eq. (1), however, is that diffusion is described by a single ADC which does not adequately reflect water diffusion behavior in tissues. Diffusion in tissues, in particular brain tissues, is not free and therefore molecular displacements do not follow a Gaussian distribution. As a result, signal attenuation plots of ln(S) versus b value are curved and do not follow a straight line, even in the absence of BMS effects, as would be expected from Eq. (1). Several models have been proposed to explain this curvature effect. One empirical way to describe this curvature (and the deviation from Gaussian diffusion) is to develop the second order term:

$$ S = S_0 \exp\left[-b \cdot \left(1 - \xi_{\text{Fe}}\right) \cdot D + K \cdot b \left(1 - \xi_{\text{Fe}}\right) \cdot D^2/6\right] \tag{3} $$

$$ \equiv S_0 \exp\left[-b \cdot D' + K \cdot (bD')^2/6\right] $$

with $D' = (1 - \xi_{\text{Fe}}) \cdot D$, where D is now the intrinsic diffusion coefficient when $b$ reaches 0 and K is called kurtosis (related to the 4th moment of the molecular displacement in the narrow pulse regime). D can be directly estimated from Eq. (3) using a BPG sequence ($\xi = 0$), so that the iron related parameter can be obtained (estimating $D'$ from Eq. (3) with a MPG sequence) as:

$$ \xi_{\text{Fe}} = 1 - D'/D. \tag{4} $$

Materials and methods

Non-human primates

Four male rhesus monkeys (Macaca mulatta), (7 yo/11.3 kg, 13.5 yo/10.5 kg, 9 yo/7.4 kg, 13 yo/8.6 kg) were housed individually or paired, with a 12:12 h light–dark cycle. The study was conducted in accordance with the European convention for animal care (86–406) and the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Animal studies were approved by the institutional Ethical Committee (CETEA protocol #10-003).

MRI acquisition

Series of images were acquired on a 3 T MRI scanner (Siemens Tim Trio, Erlangen, Germany) using a 4-channel phased-array transmit-receive coil with a diffusion-weighted echo-planar imaging (EPI) sequence. The number of image acquisitions and the parameter of the sequences were set to maintain the total examination time within acceptable limits to maintain the status of the anesthetized animals stable. The MPG sequence parameters were: TR/TE = 89/3000 ms, FOV = 128 mm, matrix = 64 × 64, 15 slices of 2-mm thickness in the axial direction, and $b = 0, 200, 600, 1000, 1400, 1800, 2200, 2600$, and 3000 s/mm$2$. The same parameters were used for the BPG sequence (twice refocused spin-echo sequence) except for the bipolar gradient pulses (Song et al., 1999; Reese et al., 2003). All BPG pulses had an equal duration (9.4 ms separated by a 9.4-ms interval) to fully cancel cross-terms and the 2 gradient pairs were separated by a 16-ms interval. For the MPG and BPG sequences gradient pulses were applied simultaneously on $X, Y$, and $Z$ axes (gradient vector = $[1, 1, 1]$), as diffusion anisotropy effects were not relevant to this study. Each acquisition was repeated 6 times for averaging in order to increase signal to noise ratio (SNR). A 3D MPRAge $(TR/TE = 2200/3.2$ ms, FOV = 154 mm, matrix = $192 \times 192$, 104 slices of 0.8 mm thickness in the sagittal direction, resulting in 0.8-mm isotropic resolution) was also used to obtain T1-weighted reference anatomical images.

Phantom experiment

The equivalence of the MPG and BPG sequences in terms of quantitative diffusion quantification (as their gradient pulse design is different) was validated using a phantom at room temperature (20–22 °C). The phantom (2-cm-diameter plastic syringe) was filled with cyclohexane (Sigma-Aldrich Chimie, Lyon, France). Regions of interest (ROIs) for measurements were placed on five slices in the center of the syringe (Fig. 1a).

In vivo experiments

The choice of non-human primates was motivated not only by the possibility to use USPIO contrast agents, but also by the similarity in brain anatomical structure and the presence of iron deposits. Monkeys were positioned in a sphinx position using an MR-compatible stereotactic frame. For anesthesia induction, monkeys received an intramuscular injection of ketamine and xylazine (15 mg/kg + 1.5 mg/kg, respectively). Sedation was maintained with continuous intravenous (i.v.) infusion of...
ketamine (15 mg/kg/h i.v.). Atropine (0.02 mg/kg i.m., Aguetant, France) was injected 10 min before induction, to reduce salivary and bronchial secretions. Muscle blocking agent (cisatracium, 0.15 mg/kg bolus i.v. followed by continuous i.v. infusion at a rate of 0.18 mg/kg/h, GlaxoSmithKline, France) was used to avoid artifacts related to potential movements during MRI acquisition. Intravenous hydration was ensured by a mixture of glucose (5%) and normal saline (0.9%) and (100 ml of 5% glucose with 250 ml of normal saline; Fresenius Kabi, France) at a rate of 10 ml/kg/h i.v. All the monkeys were intubated and mechanically ventilated (Aestiva/5 MRI, General Electric Healthcare). Physiological monitoring (Maglife, Schiller, France) included heart rate, non-invasive blood pressure (systolic blood pressure, diastolic blood pressure, mean blood pressure), oxygen saturation (SpO2), respiratory rate, end-tidal CO2 (EtCO2), cutaneous temperature. Body temperature was kept constant during the experiments with an MR compatible heating system (Bruker, Germany).

A USPIO contrast agent (Feraheme, Amag Pharmaceuticals, Inc., Cambridge, MA) was injected intravenously 4 times (2.5 mg/kg every 15 min), resulting in a cumulative dose of 10 mg/kg. The MPG and BPG sequences were acquired before USPIO injection and 5 min after the 4th injection.

The diffusion signals from each animal were measured individually in ROIs (Fig. 1b) located in the substantia nigra (SN), globus pallidus (GP), thalamus (Th) and cortex (Cortex) drawn using MRicrò software (Rorden and Brett, 2000), avoiding cerebrospinal fluid filled spaces (visible on the b = 0 diffusion images and the anatomical T1-weighted images).

Data analysis

Traditionally, MRI images have been quantitatively processed using fitting algorithms (such as the Levenberg-Marquardt algorithm) which provide estimates of parameters according to a given linear or non-linear signal model (Gill and Murray, 1978). Diffusion MRI has been no exception, especially to deal with non-Gaussian diffusion and IVIM (intravoxel incoherent motion) MRI. Instead of fitting the signal data with the diffusion model of Eq. (3) using the standard iterative (fitting) search approach, we directly derive the parameters by comparing the raw signal data acquired at all b values with those of a database of simulated signals built once-for-all for the complete study using an exhaustive set of parameter combinations. A distance is calculated between the measured signal attenuation profile, S(b) and each simulated signal, Sdb, of the database (raw signals were normalized to S(b = 0) = 1000 to have a single database for the whole study). The parameter combination, P, giving the shortest distance, dP, is deemed to represent the searched parameter estimates. This approach not only alleviates the issues of local minima and sensitivity to initial values of the iterative approach, but is also much more efficient (hence, faster) in terms of computing requirements, as only a simple distance needs to be calculated while the iterative method requires many complex calculus elements (such as those present in Eq. (3)) to be performed for each iteration. The distance was defined as the mean square error (Park et al., 2003):

\[ d_i = \frac{1}{n} \sum_b \left( S(b) - S_{db}(b) \right)^2 \]  

where n is the number of b values. The smallest \(d_i\) value, \(d_{min}\), was kept as an index of the matching goodness between the best simulated signals and the measured signals.

An adaptive database was built with the following parameter ranges for the monkey experiments: 980 ≤ S0 ≤ 1020 (step 10, signal was normalized to 1000), 0.0 ≤ K ≤ 0.40 (step 0.05), 0.42 ≤ K ≤ 1.8 (step 0.02) and 0.1 × 10^{−4} ≤ D(Do) ≤ 25 × 10^{−4} (step 0.1 × 10^{−4}). The database, thus, consisted in 98,750 simulated signals taking into account all parameter combinations and b values. For the phantom experiment, we used a more accurate database for D (step 0.05 × 10^{−4}) with identical ranges and steps in the others, resulting in 197,105 signals.

Parameters were estimated not only in the selected ROIs, leading to \(\xi_{ROI}\) according to Eq. (4), but also on a voxel-by-voxel basis to get parametric maps of \(\xi_{ROI}\).

Finally, maps of R2 changes, \(\Delta R2\), following the USPIO injection were also calculated from the changes of S0 estimated from the BPG images:

\[ \Delta R2 = \ln \left( \frac{S_{\text{post}}}{S_{\text{pre}}} \right) / TE \]  

where \(S_{\text{pre/post}}\) are the S0 values before and after USPIO injection. Those maps were obtained only to allow a qualitative comparison with \(\Delta \xi_{ROI}\) maps, as TE was not optimized for detection of R2 effects. All codes for the analysis were implemented on MATLAB (The MathWorks, Natick, MA, USA). After checking the equivalence of the MPG and BPG sequences to evaluate diffusion parameters (Supplemental Fig. S1) and to further increase robustness, especially at voxel level, parameters D and K were first estimated using MPG data, then D′ was estimated using MPG data, fixing K to the value obtained with the BPG data.

Fig. 1. Region of interest (ROI) locations (red) in the phantom (a) and in the substantia nigra (SN), globus pallidus (GP), thalamus (Th) and the cortex, of the monkey brain (Monkey B) (b).
Cross-validation

In order to cross-validate our MRI results we estimated the iron concentration in the substantia nigra and the globus pallidus using a relationship between age and iron concentration established from histological measurements in rhesus monkeys (Hardy et al., 2005): [Fe]SN = 11.1 × age (years) – 18.5; [Fe]GP = 13.1 × age (years) + 106, where [Fe] is the iron concentration (μg/g-ww).

Statistical analysis

Standard deviation/error (SD/SE) calculation methods, which are based on Jacobian matrix and/or variance–covariance matrix (Bates and Watts, 1988; Weisberg, 2005), have been proposed and discussed for evaluating the results in the nonlinear least square fitting, however, the best one has not been determined yet. Here, with the exhaustive search approach, SD for each parameter was determined from the two best one has not been determined yet. Here, with the exhaustive search approach, SD for each parameter was determined from the two best ones (Kruskal–Wallis one-way ANOVA, Mann–Whitney U test and Wilcoxon signed-rank test). The relationship between δ[Fe] and the estimated iron concentration was tested using a linear regression. All statistical analysis was performed using MedCalc (MedCalc Software, Ostend, Belgium) with alpha level = 0.05.

Results

Phantom

As expected, the measurements of the diffusion coefficient of cyclohexane with the MPG and BPG sequences were identical (1.355 ± 0.041 and 1.355 ± 0.061 × 10−3 mm2/s, respectively) despite their different gradient pulse designs, and in agreement with the literature (20 °C, 1.345 × 10−3 mm2/s) (Tofts et al., 2000). The value for δ[Fe] was 0.000 ± 0.003 and the δ[Fe] maps showed only noise, without any iron distribution pattern, as expected (Fig. 2a).

In vivo experiments

Plots of ln(S) versus b (Fig. 2b) show that the signal remained above noise level in all regions (average SNR at b = 3000 s/mm2 was 20 for both BPG and MPG signals with a minimum value of 14 after USPIO injection). Typical maps from the BPG sequence (Monkey B) are shown in Fig. 3 revealing good contrast between anatomical structures. Asymmetry for D and K in white matter between left and right hemispheres reflects diffusion anisotropy. Estimated diffusion parameters (BPG sequence) for each animal are shown in Table 1. Mean values for D and K in white matter between left and right hemispheres were smaller than in the alkane phantom signal level remained well above noise level even at high b values. No significant correlations between δ[Fe] and D and K (Fig. 4b and c) (R2 = 0.05 and 0.006, p = 0.6 and 0.8, respectively) and no correlation between D, iron content and age (Fig. 4b and d). The δ[Fe] maps at the slices including SN and GP indicated high iron concentration, which are corresponding to the ROI measurement results and the previous physiological findings [11] in both regions (Fig. 5).

Upon USPIO injection δ[Fe] increased dramatically and significantly over baseline values (Wilcoxon rank sum test, p = 0.0001), as expected (Δδ[Fe] = 24.5 ± 2.94, 19.9 ± 3.89, 30.7 ± 7.32, and 26.0 ± 5.13 in SN, GP, Th and Cortex, respectively) (Table 2) in all regions, with the increase in the thalamus being the highest in 3 animals. On the Δδ[Fe] maps, the highest values were also observed in the cortex and the thalamus, indicating higher concentrations of the USPIO agent, in accordance with previous results showing that such regions maintain a high metabolism during anesthesia (Itoh et al., 2005) (Fig. 6). AR2 maps showed similar patterns (Fig. 7), especially in the cortex (except in frontal poles which are subject to signal dropout at the bone/air interface). AR2 changes were milder in the basal ganglia than the cortex (Fig. 5c).

\[
\begin{align*}
[\text{μg/g-ww}] &= 5197.3 \times \delta[\text{Fe}] - 371.5 \quad (R^2 = 0.79, p = 0.003), \\
\text{whereas there were no significant correlations between } & \delta[\text{Fe}], \text{D and K} \\
\text{and no correlation between } D, \text{iron content and age (Fig. 4b and d).} \\
\text{The } \delta[\text{Fe}] \text{ maps at the slices including SN and GP indicated high iron concentration, which are corresponding to the ROI measurement results and the previous physiological findings [11] in both regions (Fig. 5).}
\end{align*}
\]
SN, GP, Th and Cortex, respectively) reflecting changes in $\Delta[Fe]$. Overall correlation between $\Delta R^2$ and $\Delta[Fe]$ was significant ($p = 0.01$) (Fig. 7).

A higher sensitivity to T2/T2* should be expected, however, when using gradient-echo sequences with optimized TEs.

**Discussion**

Diffusion MRI has been shown to be exquisitely sensitive to subtle changes occurring in tissue microstructure, especially in the brain (Le Bihan et al., 1986, 2012). Here, we show that diffusion MRI can also be applied for the detection and quantification of iron deposits at concentrations probably as low as a few tens of µg/g-ww. The possibility to estimate the iron load in tissues, especially basal ganglia, noninvasively is an important result. It has been reported that the iron accumulation in the brain may lead to PD or other neurodegenerative disorders (Grifths and Crossman, 1993; Grifths et al., 1999; Berg and Youdim, 2006; Brass et al., 2006; Graham et al., 2000; Hallgren and Sourander, 1960). On the other hand, the iron load in the basal ganglia is known to normally increase with aging (Aquino et al., 2009; Sedlacik et al., 2014; Hallgren and Sourander, 1958). Thus, an accurate estimate of iron content is necessary to establish cut-off values between normal and potentially pathology-leading conditions, especially in elderly populations. In our study we could successfully show statistically significant differences in $\xi_{[Fe]}$ between SN and GP, in line with known differences in iron content ([Fe]$_{GP}$ < [Fe]$_{SN}$) (Hardy et al., 2005), as low as 138 [µg/g-ww]. $\xi_{[Fe]}$ was very low in the cortex suggesting a very low concentration of iron. Although there is a report (Dexter et al., 1991) of iron presence at low concentration in the human cerebral cortex (60.4 [µg/g-ww]), it has not been reported in the monkey brain.

To achieve this result we built upon the sensitivity of diffusion MRI measurements to local magnetic field gradients, through cross-terms with the applied diffusion-encoding gradient pulses, a feature which is generally considered more as a source of artifacts. Hence, efforts have been made in the past to mitigate such effects, for instance with the use of bipolar gradient pulse sequences which can be made insensitive

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>D ($\times 10^{-4}$ mm$^2$/s)</th>
<th>K</th>
<th>d$_{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey B</td>
<td>5.5 ± 0.06</td>
<td>8.9 ± 0.29</td>
<td>7.6 ± 0.04</td>
</tr>
<tr>
<td>Monkey Y</td>
<td>1.39 ± 0.02</td>
<td>1.31 ± 0.04</td>
<td>0.84 ± 0.02</td>
</tr>
<tr>
<td>Monkey T</td>
<td>6.9 ± 0.04</td>
<td>6.9 ± 0.16</td>
<td>6.9 ± 0.08</td>
</tr>
<tr>
<td>Monkey N</td>
<td>1.17 ± 0.02</td>
<td>1.45 ± 0.03</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>7.6 ± 0.29</td>
<td>6.5 ± 0.08</td>
<td>6.9 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>1.11 ± 0.04</td>
<td>1.05 ± 0.02</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6.9 ± 0.04</td>
<td>6.9 ± 0.14</td>
<td>7.6 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.97+/−0.03</td>
<td>1.21+/−0.03</td>
<td>0.91+/−0.01</td>
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**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>SN</th>
<th>GP</th>
<th>Th</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey B</td>
<td>9.1 ± 1.02</td>
<td>11.2 ± 4.07</td>
<td>1.3 ± 0.22</td>
<td>3.3 ± 0.90</td>
</tr>
<tr>
<td>Monkey Y</td>
<td>20.4 ± 1.52</td>
<td>17.3 ± 7.34</td>
<td>35.3 ± 0.30</td>
<td>30.0 ± 1.41</td>
</tr>
<tr>
<td>Monkey T</td>
<td>8.7 ± 0.46</td>
<td>11.6 ± 1.34</td>
<td>0.0 ± 0.97</td>
<td>2.3 ± 0.77</td>
</tr>
<tr>
<td>Monkey N</td>
<td>27.4 ± 0.65</td>
<td>23.3 ± 6.30</td>
<td>37.3 ± 1.54</td>
<td>29.3 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>9.2 ± 2.61</td>
<td>10.8 ± 1.49</td>
<td>1.4 ± 1.45</td>
<td>3.4 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>25.1 ± 4.11</td>
<td>23.1 ± 2.39</td>
<td>29.4 ± 2.33</td>
<td>26.0 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>10.1 ± 0.70</td>
<td>13.0 ± 0.98</td>
<td>0.0 ± 0.66</td>
<td>2.3 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>25.0 ± 1.06</td>
<td>15.8 ± 1.72</td>
<td>21.0 ± 1.15</td>
<td>18.8 ± 0.86</td>
</tr>
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Fig. 4. Relationship between the parameters ($ξ_{[Fe]}$, $D$ and $K$) and the estimated iron concentration (based on Hardy et al., 2005). (a) Significant correlation between $ξ_{[Fe]}$ and iron concentration. A linear regression gives $ξ_{[Fe]} \times 10^{-2}$ = 5197.3 × $ξ_{[Fe]}$ – 371.5 (standard error in slope and intercept: 1077.7 and 113.7, respectively) ($R^2 = 0.79, p = 0.003$). No significant relationships between $D$ (b) and $K$ (c), and iron concentration ($R^2 = 0.047, p = 0.607$ and $R^2 = 0.0062, p = 0.853$, respectively). (d) There was no correlation of $D$ with age ($R^2 = 0.018, p = 0.749$).

Fig. 5. Anatomical T1-weighted image and $ξ_{[Fe]}$ maps including the substantia nigra and globus pallidus in the four monkeys. Regions with high $ξ_{[Fe]}$ values indicate a high iron concentration.
to background gradients. Here, we capitalize on the possibility to turn on or off this sensitivity local background gradients by switching between a monopolar and bipolar gradient pulse sequence. This trick makes it possible to independently estimate the iron induced background gradient parameter, $\xi_{[Fe]}$, although local water diffusion may vary a lot in space and time depending on tissues and pathology, especially with an iron overload (Moos and Morgan, 2004; Hino et al., 2013). This remarkable feature is a very important advantage for the diffusion MRI method compared to other methods based on $R2/R2^*$ measurements (Wallis et al., 2008; Sedlacik et al., 2014; Graham et al., 2000), as relaxivity effects specifically arising from the iron presence cannot be turned off and remain entangled with those induced by tissue composition and pathology. It should be noted that both the $R2/R2^*$ and the diffusion effects would in theory increase with the field strength, $B_0$, of the MRI scanners, as magnetic susceptibility scales with $B_0$, as long as magnetic saturation has not been reached in the iron particles (Deistung et al., 2013). Based on this feature, it has been proposed to more specifically estimate iron content from $R2^*$ measurements made at two different field strengths (e.g. 0.5 and 1.5 T) (Bartzokis et al., 1993), but this is obviously impractical in clinical or even preclinical practice as this means using two different MRI scanners.

The sensitivity of the measured ADC to local iron deposits might perhaps explain some discrepancies in the literature, with reports of high (Scherfler et al., 2013), normal (Péran et al., 2010; Du et al., 2011; Zhan et al., 2012), or low (Sener, 2002) ADC values in basal ganglia, especially in the substantia nigra of patients with PD. When using a MPG diffusion MRI sequence, the ADC may be underestimated in the presence of iron deposits, and BPG sequences must be preferred. An important point of the new approach is that variations of ADC with age or pathology are intrinsically eliminated through the use of the $D'/D$ ratio, while it is not possible to eliminate pathological variations in $R2/R2^*$ for iron content determination. Of course, although $\xi_{[Fe]}$ reflects the presence of local background gradients, whatever their origin, and not necessarily the presence of iron. However, sources of local magnetic field inhomogeneities, like those induced by iron deposits, are rather well known. One may consider local interfaces between brain tissues, bone and air cavities which have large differences in magnetic susceptibility, but their effect on the measured diffusion coefficients has been shown to be negligible (Clark and Le Bihan, 2000). Indeed, in the cerebral cortex $\xi_{[Fe]}$ was very low and the regions with high $\xi_{[Fe]}$ were not in the vicinity of those interfaces, except perhaps near the frontal and petrous bones (Fig. 3) where $d_{min}$ was high, suggesting poor fitting. The other well-known source of local field gradients is the blood in the vessels containing deoxyhemoglobin which is paramagnetic (Kennan et al., 1995; Ogawa et al., 1993).
Although this effect is at the origin of the BOLD fMRI method, which exploits the sensitivity of gradient echo sequences (and to a lesser degree spin-echo sequences) to the resulting changes in $R^2$* relaxivity, its contribution to the diffusion MRI signal is expected to be very small (Pampel et al., 2010), at least compared to the iron effects observed in this study. Indeed, during brain activation, water diffusion has been shown not to increase (as expected from a local field gradient model when the deoxyhemoglobin concentration decreases), but to decrease (Le Bihan et al., 2006; Tsurugizawa et al., 2013), the ADC increase observed at very low b values (Song et al., 1999) has a completely different origin, the increase in blood flow being captured through the Intra Voxel Incoherent Motion (IVM) effect (Le Bihan et al., 1988). Furthermore, the endogenous values found for $\xi$ across brain regions do not at all reflect known variations in blood volume, with $\xi_{\text{Fe}}$ in thalamus much lower than in SN or GP. The difference in $\xi_{\text{Fe}}$ observed between SN and GP rather reflects known differences in iron concentration (Fig. 5) (Hardy et al., 2005). It should be noted that a linear correlation between $\xi_{\text{Fe}}$ and iron concentration has been empirically verified in this study, but the exact relationship has yet to be established and is not necessarily linear (Zhong et al., 1991), as suggested by the non zero intercept we have found. This is not a trivial matter, as hypotheses have to be made on the nature of the iron deposits. In the meantime, an absolute determination of iron concentration will require calibration, as exemplified in Fig. 4a. We conducted this work with a clear translational medicine perspective, in non-human primate models. The macaque brain shares neuroanatomical homologies with the human brain, with iron accumulation in basal ganglia as demonstrated by both MRI and histological studies (Tani et al., 2011; François et al., 1981). Furthermore, the method has the potential to evaluate iron deposits in other organs than the brain, notably the liver. Systemic iron overload, even mild, is considered as an important cofactor of various disorders, and the assessment of body iron stores is often evaluated in the liver, either from biopsies or from MRI images based on the $R^2$* approach.

The situation changes dramatically upon injection of a USPIO agent, with $\xi_{\text{Fe}}$ rising significantly in all regions, especially the cortex and the thalamus, which have high blood volumes and, hence, higher USPIO concentrations (Qiu et al., 2012; Varalayay et al., 2013). During anesthesia with ketamine the metabolism in the thalamus in rhesus monkey can be about 4 times higher than that in the cortex (Itoh et al., 2005). Indeed, effects of USPIOs are much larger than those of endogenous deoxyhemoglobin. Iron contrast agent injection is becoming a standard over BOLD for fMRI experiments performed in animals, especially non-human primates (Leite et al., 2002; Zhao et al., 2009; Autio et al., 2011; Mandeville, 2012). The present approach has the potential to detect and quantify variations in blood volume in the cortex upon brain activation status, through variations in $\xi_{\text{Fe}}$ reflecting variations in USPIO concentration.

An important methodological point in our study is that we have switched for the standard fitting approach, which analyzes MRI and especially diffusion MRI data, to an exhaustive search approach using a unique database. The standard, iterative approach, indeed suffers from some pitfalls, notably its instability in the estimated parameters, especially when the number of parameters to estimate increases and the data become noisy. Improvements have been suggested (Hahn et al., 2013; Yuan and Zhang, 2013), but results may depend on the presence of local minima and the initial values set for each parameter to estimate. With the new approach there is no requirement for initial values and minima between raw and simulated signals are search over the whole ranges of parameter values. Although, it was not the aim of this study to evaluate the gain in calculation speed over the iterative approach, the exhaustive search approach has also a clear advantage in calculating efficiency (and analysis time), because only simple quadratic distances have to be calculated. The complex simulated signals are determined in advance, once-for-all for the whole study, even if the database is large. In the future, the approach may benefit from some tuning in the definition (and the resulting size) of the database (ranges and steps for the values for each parameters, which can be adaptive, with finer steps near the range centers), to get the best compromise between accuracy and computing speed. The database will also likely be slightly different with human subjects, as water diffusion is a little higher than in the monkey brain. Overall, we think that the potential of this new approach to process MRI data is very high, extending well outside of diffusion MRI.

**Conclusion**

Diffusion MRI is sensitive to the presence of iron deposit in tissues and we have shown that it can be used to quantify iron and get maps of iron content in the macaque brain with good accuracy. The method will benefit clinical investigations on the effect of iron overload in some brain structures which have been suspected to induce neurodegenerative disorders, such as PD. Furthermore, diffusion MRI can also be used in the context of fMRI to quantify USPIOs when administered as blood pool contrast agents to estimate variations in the local blood volume induced by brain activation. The method will probably benefit from ultra-high field MRI systems (Tani et al., 2011). Finally, a new procedure was introduced to estimate diffusion parameters from a database, a significant shift from the usual data fitting approach.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.08.049.

**References**


