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Susceptibility Contrast in High Field MRI of Human Brain as a Function of Tissue Iron Content

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Abstract

Magnetic susceptibility provides an important contrast mechanism for MRI. Increasingly, susceptibility-based contrast is being exploited to investigate brain tissue microstructure and to detect abnormal levels of brain iron as these have been implicated in a variety of neuro-degenerative diseases. However, it remains unclear to what extent magnetic susceptibility-related contrast at high field relates to actual brain iron concentrations. In this study, we performed susceptibility weighted imaging as a function of field strength on healthy brains *in vivo* and post-mortem brain tissues at 1.5T, 3T and 7T. Iron histology was performed on the tissue samples for comparison. The calculated susceptibility-related parameters R_2^* and signal frequency shift in four iron-rich regions (putamen, globus pallidus, caudate, and thalamus) showed an almost linear dependence ($r=0.90$ for R_2^* ; $r=0.83$ for phase, $p<0.01$) on field strength, suggesting that potential ferritin saturation effects are not relevant to susceptibility-weighted contrast for field strengths up to 7T. The R_2^* dependence on the putative (literature-based) iron concentration was 0.048 Hz/Tesla/ppm. The histological data from brain samples confirmed the linear dependence of R_2^* on field strength and showed a slope against iron concentration of 0.0099 Hz/Tesla/ppm dry-weight, which is equivalent to 0.05 Hz/Tesla/ppm wet-weight and closely matched the calculated value *in vivo*. These results confirm the validity of using susceptibility-weighted contrast as an indicator of iron content in iron-rich brain regions. The absence of saturation effects opens the way to exploit the benefits of MRI at high field strengths for the detection of iron distributions with high sensitivity and resolution.

Keywords

Phase; Ferritin; Relaxation; susceptibility; brain; iron

INTRODUCTION

Over the last two decades there has been considerable interest in the use of MRI to provide a measure of brain iron concentration (for reviews, see Haacke et al., 2005; Schenck and Zimmerman, 2004). MRI techniques that are sensitive to the magnetic susceptibility effects induced by iron (Haacke et al., 2004; Ogg et al., 1999) are potentially valuable for the study

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of diseases with abnormal iron distributions, such as Huntington's disease (HD), Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), and Friedreich's ataxia (FA) (Bartzokis and Tishler, 2000; Brass et al., 2006; Chen et al., 1993; Helpert et al., 2004; Jack et al., 2007; Kosta et al., 2006; Neema et al., 2007; Waldvogel et al., 1999). Previous studies have relied on the effect of iron on a number of MR parameters, including T_1 , T_2 , T_2^* , and resonance frequency (Hardy et al., 2005; House et al., 2007; Ogg and Steen, 1998; Peters et al., 2007; Schenck, 1995).

With the advent of high field MRI, the potential of MRI for the study of brain iron has received increased attention (Abduljalil et al., 2003; Duyn et al., 2007). Apart from the overall sensitivity gains available with high field MRI, the effect of magnetic susceptibility on image contrast in T_2^* -weighted imaging increases with field strength, potentially allowing improved sensitivity and resolution (Duyn et al., 2007; Li et al., 2006). However, despite these potential gains, there is an important unresolved issue that hampers the widespread application of high field MRI to the measurement of brain iron. This issue relates to uncertainties about the precise relationship between brain iron levels and MRI parameters that depend on magnetic susceptibility such as T_2^* relaxation time and resonance frequency shift, and the potential dependence of this relationship on field strength.

In MRI of heart and liver at 1.5 T, an almost linear relationship was found between the R_2^* (i.e. $1/T_2^*$) relaxation rate and the iron concentration determined from chemical measurements (Anderson et al., 2001; Wood et al., 2004). In brain, direct correlation between tissue R_2^* or frequency shifts with iron levels derived from chemical measurements are missing. A number of studies have compared MRI susceptibility-related measures with putative iron levels derived from literature values, most notably the authoritative study of Hallgren and Sourander (1958). Based on this approach, a study at 1.5 T found an almost linear relationship between MRI frequency shift and iron concentration in gray matter regions (Ogg et al., 1999). A study at 3 T focusing on the iron-rich basal ganglia demonstrated that this linearity also holds for R_2^* (Gelman et al., 1999). On the other hand, for R_2 , there is some data available based on direct correlation with iron chemical measurement; this work found a nearly linear relationship (Hardy et al., 2005; House et al., 2007; Vymazal et al., 1996a).

The linear relationship between R_2^* and iron concentration found at 1.5 T and 3 T is also expected to hold at high field, although this remains to be investigated. A caveat is that the R_2^* increase with field might level off at high field strengths, for example due to saturation of the iron magnetization in ferritin, an important storage protein for brain iron (Dietrich and Bradley, 1988; Schenck, 1995). Although one study has suggested some evidence of ferritin saturation (Bizzi et al., 1990), this has not been confirmed by more recent studies (Bartzokis et al., 1994; Gelman et al., 1999; Schenck, 1995; Vymazal et al., 1996a).

The goal of the current study was to extend the previous investigations into the relationship between R_2^* and iron concentration to high field strengths, and to directly correlate susceptibility-related MRI measures with histological data obtained from the same tissue. Determination of a precise relationship between R_2^* and iron concentration at high field strength may provide high sensitivity and high resolution measurements of brain iron.

MATERIALS AND METHODS

MRI Data Acquisition

MRI experiments were performed on three separate GE Signa MRI scanners (General Electric Medical Systems, Milwaukee, WI) with field strengths of 1.5, 3 and 7 T, and using 8- (1.5 and 3 T) or 32-channel (7 T) detector arrays (Nova Medical, Wilmington, MA). For the 7 T scanner,

which had only 16 receiver channels, signals from the 16 coils closest to the brain region under study were acquired.

Nine normal volunteers (female/male = 4/5) with mean age = 31 ± 5 (mean $\pm$ standard deviation) participated in the study and each completed three scan sessions (1.5, 3 and 7 T) under a human subjects protocol approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health. Foam pads were inserted into the space between the subject's head and the interior lining of the MRI detector arrays to minimize head motion. To allow determination of T_2^* and frequency shifts, axial T_2^* -weighted gradient echo (GRE) images were acquired at several echo times (TEs) at each field strength (Table 1). All GRE data were acquired with repetition time (TR) = 1000 ms, flip angle = 60° , bandwidth = 32 kHz, field-of-view (FOV) = 240×180 mm and matrix size 256×192 , resulting in an in-plane resolution of 0.94×0.94 mm². At 7 T, a slice thickness of 1 mm was used while at 1.5 T and 3 T, the slice thickness was increased to 2 mm to increase the signal-to-noise ratio (SNR). Multiple echo data were acquired in separate measurements. The total scan time was 13 minutes (for 3 T and 7 T). At 1.5 T, two excitations (NEX = 2) were summed in order to increase the SNR, so the total scan time at 1.5 T was 26 minutes. First-order flow compensation was applied to all imaging gradients. 16 slices were acquired throughout the brain with inter-slice gaps equal to 2 mm (for 1.5 T and 3 T) or 3 mm (for 7 T). At 7 T, real-time second-order shimming was performed during each scan to compensate for field fluctuations related to the respiratory cycle (van Gelderen et al., 2007). A summary of the main differences in acquisition parameters used at different field strengths is listed in Table 1.

To enable alignment of data between acquisitions at the various field strengths, a 3D Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE)-based T_1 -weighted scan was also performed for each subject and field strength. The following parameters were used (the range refers to differences between field strengths): TR = 5.9 – 7.5 ms; TE = 2.8 – 3.2 ms; inversion time (TI) = 725 – 1200 ms; flip angle = 6° – 9° ; slice thickness = 1 – 2 mm; bandwidth = 32 kHz; FOV = 240×180 mm, matrix size = 256×192 , which resulted in the same in-plane resolution as the corresponding GRE images. The MP-RAGE scan time was about 14 minutes for each session. The center of the 3D slab for each subject was carefully selected to match the center slice of the T_2^* -weighted GRE scan. This greatly facilitated an accurate spatial registration between GRE images and MP-RAGE images.

Image Reconstruction

Images were reconstructed by using phase-sensitive combination of the individual coil data (de Zwart et al., 2002) using the GRE data acquired at the shortest TE as the coil sensitivity reference. These reference data were spatially low-pass-filtered to an in-plane resolution of 1.5 cm. After coil signal combination, a complex-valued pixel intensity was obtained, from which the phase and magnitude were derived. Quantitative R_2^* images were then calculated from the GRE images by fitting an exponential function to the data from the four different echo times. Phase images were corrected for remaining macroscopic susceptibility effects (not compensated for by the magnetic field shimming procedure) by estimating their magnitude and distribution from a polynomial fit to the phase data. The polynomial order should be high enough to describe the whole background phase change. On the other hand, a higher order polynomial function model requires a larger number of data points in order to give an accurate fit and also it is more time consuming. Therefore, prior tests were performed using fourth through twelfth order polynomial function to fit the phase data. An optimal eighth order polynomial function was determined and applied to the phase data for all the subjects in this study. The 2D polynomial functions were used on a slice-by-slice basis, taking into account any 2π phase wrap present in the data. The latter was done by first taking the spatial derivative of the phase data. The fitted macroscopic phase was then subtracted from the original phase

data (Duyn et al., 2007). The procedure is demonstrated in Figure 2. Figure 2A shows an original wrapped phase image in the basal ganglia area of one slice and its fitted phase image is shown in Figure 2B. Then the fitted macroscopic phase was subtracted from the original phase data and result is shown in Figure 2C, in which most background phase has been removed. Some pixels have values beyond or below the range $[-p, p]$ show black and white spots in the image, respectively. Those out-of-range pixels could be corrected after folding their values into $[-p, p]$ range hence the unwrapped phase image is obtained (Figure 2D). After all the unwrapped phase images were calculated, the frequency map for each individual at each field strength was then calculated by a linear fit to the phase values (in radians) from the corresponding four TEs, resulting in frequency maps in Hz.

ROI Selection and Registration

To determine the relationships between MRI T_2^* or frequency shift and iron concentration, we focused on four brain regions that are relatively rich in iron. This was done to minimize secondary contributions from other sources of susceptibility such as myelin. Regions of Interest (ROIs) were selected from the MP-RAGE images for each subject and each magnetic field strength (Figure 1). The ROIs included: Putamen (PU), Caudate nucleus (CN), Globus pallidus (GP) and Thalamus (TH). The ROIs were manually drawn in both brain hemispheres in each slice which cut through the above brain regions. While selecting each ROI, care was taken to avoid apparent large vessels and cerebral spinal fluid (CSF). The ROIs were registered to the R_2^* and phase images by using the transformation matrix that had been obtained from the registration between the GRE and MP-RAGE images using the registration algorithm implemented in SPM5 in Matlab (version 7.0, the MathWorks, Inc., MA). The frequencies and R_2^* values were averaged within each ROI for each individual at each field strength. The mean frequency of CSF in the lateral ventricle (LV) was used as a reference. The frequency shift of each ROI was directly calculated from the difference between the mean frequency in that ROI and the reference.

Brain Tissue Analysis

Formalin-fixed whole brain specimens from two males (ages = 66 and 70, respectively) who had died of non-neurological causes were also scanned at 1.5T, 3 T and 7 T using a 3D gradient echo sequence before being sampled for further chemical analysis. The acquisition parameters used for MRI of the brain specimen were: TR = 63 ms, flip angle = 10° , bandwidth = 32 kHz, FOV = 179×179 mm, matrix size = 256×256 , and slice thickness = 0.7 mm, resulting in an isotropic resolution of 0.7 mm. T_2^* -weighted GRE data at five different TEs were acquired at each field strength (see Table 1 for a summary of the data acquisition parameters). At each field strength, four measurements were averaged to improve SNR and allow accurate determination of T_2^* values. The images were processed in the same way as the images of healthy subjects. Because the brain tissues were immersed in fomblin during scanning, no signal could be measured outside of the brain tissue as a reference for comparing the phase values among different field strengths. Therefore, no analysis of the MRI phase was performed for the brain tissue study. After scanning, the brain specimens were sectioned into coronal slabs about 10 mm thick. For each ROI, one to four tissue samples of approximately $2 \times 2 \times 2$ mm³ were excised for the purpose of chemical analysis. These tissue samples were sealed and sent to West Coast Analytical Service Inc (Santa Fe Springs, CA) for iron quantification using inductively coupled plasma-mass spectrometry.

RESULTS

In vivo Study

Figure 3 shows the GRE images (A) and R_2^* maps (B) of a representative subject collected at three different field strengths. Large vessels appear dark and CSF appear bright in the GRE

images and this contrast is reversed in the R_2^* images. Iron-rich areas (PU, CA, TH, and GP) are more hypo-intensive compared to the other areas in the GRE images (i.e. brighter in the R_2^* maps). As expected, the R_2^* values increase with the field strength (note that different scales are used in the R_2^* maps for maximum display contrast). The dependence of R_2^* on field strength is shown in Figure 4 both for individual subjects (A) and for group averaged data (B). In all four ROIs, the mean R_2^* values increase roughly linearly with the field strength (up to 7 Tesla). The two-tailed Pearson's correlation coefficient r equals 0.97 for PU, 0.95 for caudate CA, 0.98 for TH, and 0.97 for GP. These coefficients were significant ($p < 0.01$) for all ROIs. At 1.5 T, the R_2^* value in GP is slightly higher than in PU, CA and TH, and this difference becomes larger as the field strength increases. For example, the R_2^* difference between GP and TH is 7.5 Hz at 1.5 T and 18.2 Hz at 3 T. At a field strength of 7 T, this difference increases to 47.2 Hz, which is about 5-fold greater than that at 1.5 T. In other words, the slope of the R_2^* change as a function of field strength is different among ROIs (PU: 6.22 Hz/T, CA: 6.08 Hz/T, GP: 11.57 Hz/T, TH: 4.34 Hz/T). The group averaged R_2^* values across all subjects at different field strengths for different ROIs are listed in Table 2.

A similar analysis was performed for the phase images. Figure 5 shows a comparison of the phase images (zoomed area in Figure 3) at three different field strengths for a representative subject. As expected, fine structures and large vessels become more visible at higher field strength. The iron-rich areas appear hypo-intense compared to the nearby white matter areas. The CSF is not as clearly distinguishable as that in the GRE images or R_2^* maps. Figure 6 shows the frequency shifts as a function of field strength for (A) all the individuals and (B) the group averaged data. Compared to the corresponding R_2^* variation, the relative frequency shift variation among subjects is greater. A roughly linear increase of the frequency shift with field strength was observed in the four ROIs ($r = 0.90$ for PU, 0.89 for CA, 0.87 for TH, and 0.83 for GP). These coefficients were significant ($p < 0.01$) for all ROIs. In the group averaged data (Figure 6B), the frequency shifts in GP and CA show a greater increase with the field strength than those in PU and TH (Table 2).

From the empirical formulae reported by Hallgren and Sourander (Hallgren and Sourander, 1958) based on 81 subjects, the iron concentration in PU, CA and GP can be calculated for each individual using the following equations:

$$\text{Putamen: } y = 146.2 \cdot \{1 - \exp(-0.04x)\} + 4.6 \quad (1)$$

$$\text{Caudate nucleus: } y = 96.6 \cdot \{1 - \exp(-0.05x)\} + 3.3 \quad (2)$$

$$\text{Globus pallidus: } y = 214.1 \cdot \{1 - \exp(-0.09x)\} + 3.7 \quad (3)$$

where, y is the nonheme iron in parts per million (ppm) fresh weight and x is the subject's age in years.

Figure 7A shows the relationship between R_2^* in the three ROIs in all the subjects and the corresponding iron concentration at different field strengths. The iron concentration in each ROI was calculated using Equations (1)–(3) based on each subject's age. At all field strengths, R_2^* shows a clear linear increase with iron concentration ($r = 0.79$ at 1.5 T, 0.87 at 3 T and 0.92 at 7 T, all significant, $p < 0.01$). These results indicate a strong correlation between R_2^* and iron concentration. The best-fit lines to the data are given by: $y = 0.058x + 1.075$ (at 1.5 T), $y = 0.120x + 1.412$ (at 3 T) and $y = 0.319x + 1.979$ (at 7 T), where x is the iron concentration

in ppm and y is the R_2^* in Hz. In contrast, no such a tightly linear relationship between frequency shift and iron concentration has been found in this study at all the three field strengths (Figure 7B).

In Figures 3, 5 and 6 it is apparent that the rates of change of R_2^* and frequency with field strength differ among the four ROIs studied. It is interesting to know how these rates of change in different ROIs correlate with iron concentrations. This correlation (calculated from a linear fit of the values at three different field strengths) is shown in Figure 8. The R_2^* rate of change has a very clear linear increase with the iron concentration (Figure 8A), which indicates a close correlation between the R_2^* rate of change and the iron concentration ($r = 0.884$, $p < 0.01$). The best-fit line to the data is given by: $y = 0.048x + 0.159$, where x is the iron concentration in ppm, and y is the R_2^* rate of change with field strength in Hz/Tesla. In contrast, the rate of change of frequency shift with field strength is not tightly correlated with the iron concentration (Figure 8B). Some regions have very high frequency shift rates of change even though they have a low iron concentration.

***In vitro* Study**

The iron concentration measured in each sample is listed in Table 3, which covers the ROIs: PU, CA, GP, TH, and corpus callosum (CC) (only for brain tissue A). Two to four samples were cut from each ROI depending on the ROI volume in the tissue. Based on the histology data, the iron concentration (in ppm) in the CA is lower than that in the GP and PU, but much higher than that in the TH, which is consistent with the empirical formulae (Eq. (1)–(3)). The R_2^* values of different ROIs as a function of field strength are shown in Figure 9A. At all three field strengths, the R_2^* values in the GP is greater than those in the PU, CA and TH. For all the ROIs, the R_2^* increases linearly with field strength ($r = 0.99$ for all the ROIs, $p < 0.05$). The rate of change of R_2^* with field strength as a function of iron concentration is shown in Figure 9B. A clear linear increase with iron concentration is found ($r = 0.93$, $p < 0.05$). The line of best fit is given by: $y = 0.0099x + 1.71$, where x is the iron concentration in ppm, dry weight, and y is the rate of change of R_2^* in Hz/Tesla.

DISCUSSION

There are major advantages in using high magnetic fields for MRI of the human brain, among which the most important are the gain in sensitivity and increase in MRI contrast. In particular, the fact that T_2^* contrast increases dramatically with magnetic field strength makes it possible to characterize anatomical structure at a high resolution for both normal and pathological brain. In general, the T_2^* -weighted contrast arises mainly from the magnetic susceptibility differences between the tissue compartments. These tissue susceptibility differences may have many sources, such as iron deposition, deoxyhemoglobin, myelin content, and tissue microstructure. This mechanism of T_2^* contrast provides a potential tool to measure the iron concentration of human brain *in vivo*, especially in the most iron-rich areas.

Although R_2^* relaxation is a complicated process, with possible contributions that are non-linear with field strength, the results presented here suggest a nearly linear field dependence of R_2^* in the four iron-rich brain regions studied. Similar results have been reported in a recent study (Peters et al., 2007). The rates of change of R_2^* in Peters' paper are 6.1 ± 0.4 Hz/T for caudate nucleus and 7.9 ± 0.3 Hz/T for putamen, respectively. This result is close to our finding (Table 2). This suggests that there are minimal saturation effects of the iron magnetization in ferritin which constitutes a large portion of the iron in brain tissue (Haacke et al., 2005; Morris et al., 1992; Schenck and Zimmerman, 2004). The iron in ferritin is known to be antiferromagnetic. One of the properties of small antiferromagnetic crystals is that they saturate at much lower magnetic field strengths than paramagnetic substances do. In an early report, a theoretical calculation for fully loaded ferritin (4500 iron atoms), according to Neel's theory

(Neel, 1961), predicts that saturation will begin at around 1.0 T at body temperature. However, a number of recent studies (Bartzokis et al., 1994; Schenck, 1995; Vymazal et al., 1996a) have reported a strong linear correlation between the relaxation rate and the field strength (1.0 T and up) in either ferritin solutions or iron-rich brain areas. No saturation effect has been observed. The size of the ferritin core may be a factor since the field strength needed for saturation depends inversely on crystal size. Thus, only when the iron content of the particle is sufficiently high will it lead to magnetic saturation at lower field strengths.

The number of iron atoms stored within a ferritin particle (called loading factor) could be another factor affecting the transverse relaxation rate in the tissue. It is possible that a given amount of iron could exert different relaxation effects, depending on how it is distributed among the ferritin particles. However, a study that measured relaxation times at field strengths from 0.05 T to 1.5 T in solutions of ferritin with loading factors from 90 to 3600 iron atoms per particle found a linear increase of the transverse relaxation rate with field strength (Vymazal et al., 1996b). Moreover, the slope per iron atom was approximately the same in all samples, which indicates that the field dependence of the transverse relaxation rate may be used as a measure of ferritin-bound iron, regardless of loading factor.

In the present study, we showed a linear relation between R_2^* and iron concentration in the basal ganglia of healthy human volunteers at all three field strengths (Figure 7A). Moreover, not only R_2^* but also the rate of change of R_2^* with field increases linearly with iron concentration in the basal ganglia (Figure 8A). The slopes obtained from a linear fit of rate of change of R_2^* (in Hz/Tesla) against iron concentration (in ppm) are 0.048 Hz/Tesla/ppm for the *in vivo* data. A similar calculation was performed with the *in vitro* data to verify this linear relationship. From the *in vitro* brain tissue data, a slope of 0.0099 Hz/Tesla/ppm was obtained. One should note that for the *in vivo* data, the iron concentration was measured using fresh tissue weight (Hallgren and Sourander, 1958), while for the *in vitro* data, the iron concentration was measured using dry weight. A previous study reports that the dry to fresh weight ratios of tissues are between 0.18 to 0.22, which varies among different brain regions such as cortex, thalamus and cerebellum (Stedman and Spyrou, 1998). This approximate 1:5 dry to fresh weight ratio explains quite well the difference in slope between *in vivo* and *in vitro* results in our study. This consistent result indicates that the rate of change of R_2^* with magnetic field strength could serve as a useful index to quantify the iron concentration in the brain.

The frequency shift also shows more or less a linear increase with the magnetic field strength (Figure 6). Although for some individuals in certain brain regions the increase in frequency shift with field strength appears to flatten off at higher fields, the group average shows a good linear relationship between the frequency shift and the magnetic field strength at all the four ROIs (Figure 6B), therefore, no prominent saturation effect is observed. However, there remain major challenges in using frequency shift as an indicator of iron concentration, and this is evident from Figure 7B and 8B. The relatively large variation in frequency shifts between ROIs across subjects is probably attributed to the fact that the phase is not only affected by the magnetic susceptibility of a tissue region, but also its geometry and orientation in the main magnetic field. This could explain the lack of correlation between frequency shift and iron content (Figure 7B, 8B) and indicates a problem with using frequency shifts as an indicator of iron content. For proper interpretation of phase data, corrections of or accounting for geometric variations will be necessary. Ideally, one would like to derive true susceptibility maps from the phase data. This is an area of active research in this and other laboratories (Haacke et al., 2005; Marques and Bowtell, 2005; Shmueli et al., 2008). A further caveat to using either R_2^* or frequency shifts to quantify brain iron content are confounding contributions of other susceptibility sources such as deoxyhemoglobin and myelin (Duyn et al., 2007). Although an excellent correlation between T_2^* and iron content was observed in this and previous studies (Anderson et al., 2001; Gelman et al., 1999), this does not exclude the contribution of other

sources. For example, blood volume might co-localize with iron in the regions studied, leading to a bias in the slope of the R_2^* -iron content relationship. These confounding sources could play a larger role in other brain regions with lower iron content.

CONCLUSIONS

MRI susceptibility effects, as measured indirectly via R_2^* and resonance frequency shift, increase linearly with field strength in iron-rich regions of the basal ganglia, confirming a recent study and suggesting that ferritin saturation effects are only minimally, if at all, present at field strengths up to 7 T. Furthermore, in these same regions, R_2^* depends linearly on iron concentration, at all field strengths studied. This was true for *in vivo* data using literature iron values, and *in vitro* tissue sample with measured iron values. This suggests that the dramatic susceptibility contrast available with 7 T MRI could be exploited to quantitatively study iron accumulations in the human brain with high sensitivity and resolution.

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■ PU ■ CA ■ GP ■ TH ■ CSF

Figure 1. Four iron-rich ROIs and a ROI containing Cerebrospinal fluid (CSF) were drawn on the MPRAGE images at 1.5 T, 3 T and 7 T. The ROIs are: Putamen (PU), Caudate Nucleus (CA), Globus Pallidus (GP) and Thalamus (TH).

Figure 2.

Illustration of the phase unwrapping procedure. (A) The original wrapped phase image in basal ganglia area at 7 T, TE = 23.2 ms. (B) The fitted macroscopic phase image using an eighth-order polynomial function. (C) Subtraction of fitted phase background from the original wrapped phase. (D) Unwrapped phase image after trim the pixel values into range $[-p, p]$.

Figure 3. Comparison of the Gradient Echo images (A) and the calculated R_2^* images (B) at different field strengths. The figure shows the images of one subject (TR = 1000 ms, flip angle = 60° , resolution = $0.94 \times 0.94 \text{ mm}^2$, TE = 57.0/35.9/30.0 ms for 1.5/3/7 T, respectively). 7 T images have a much better SNR and show finer structural details. R_2^* values (Hz) increase with field strength (Note that different scales are used for each field strength for better demonstration). The box in (A) indicates the zoomed-in region illustrated in Figure 5.

Figure 4.

(A) Individual subject R_2^* values as a function of field strength in the four ROIs (refer to Figure 1). The different line colors indicate the different subjects. (B) Group averaged R_2^* values against field strength in the four ROIs. The error bars indicate the standard errors.

Figure 5. Comparison of phase maps at different field strengths (basal ganglia area, see box in Figure 3). Greater phase contrast was observed in the higher field strength images. The phase value is shown in units of Hertz, with respect to the phase value of CSF in the lateral ventricle at the corresponding field strength.

Figure 6.

(A) Individual frequency shifts as a function of field strength in the four ROIs (refer to Figure 1). The different line colors indicate the different subjects. (B) Group averaged frequency shifts against field strength in the four ROIs. Error bars indicate the standard error.

Figure 7.

R_2^* and frequency shift (B) change as a function of iron concentration at field strengths of 1.5, 3, and 7 T, respectively. The dots are the data points from each ROI in each individual subject; the solid lines indicate the linear fits. The iron concentration was calculated from experimental equations (1) – (3) with respect to tissue wet weight.

Figure 8.

R_2^* (A) and frequency shift (B) rates of change with field strength as a function of iron concentration. The dots are the data points from each ROI in each individual subject; the solid line indicates the linear fit. The iron concentration was calculated from experimental equations (1) – (3) with respect to tissue wet weight.

Figure 9.

(A) R_2^* value as a function of field strength in each ROI for the two brain tissue specimens. The different line colors indicate the different ROIs. The solid lines and dotted lines represent different brain tissues, respectively. Note the ROI CC (corpus callosum) is only measured in brain tissue A. (B) R_2^* rate of change with field strength as a function of iron concentration in the brain tissues. The dots are the data points from each ROI in the two brain tissues; the solid line indicates the linear fit. The iron concentration was measured with respect to tissue dry weight. It has different scale from Figures 7 and 8 (see discussion for details).

Table 1
Summary of the main data acquisition parameters for T₂*-weighted images at each field strength (B₀).

B ₀	in vivo			in vitro		
	1.5 T	3 T	7 T	1.5 T	3 T	7 T
TE (ms)	7.3	7.5	9.6	5.0	6.0	5.0
	23.9	21.7	16.4	15.0	16.0	16.0
	40.5	35.9	23.2	30.0	26.0	26.0
	57.0	50	30	60.0	36.0	36.0
				70.0	46.0	46.0
NEX	2	1	1	4	4	4
Slice thickness (mm)	2	2	1	0.7	0.7	0.7
Slice spacing (mm)	2	2	3	n/a ^J	n/a ^J	n/a ^J
Total scan time (min)	26	13	13	180	180	180

^J A 3D gradient echo sequence was used in the brain tissue experiment.

Table 2
Group averages of $R_2^* \pm$ standard error (Hz) and frequency shift $\pm$ standard error (Hz) at different field strengths for *in vivo* data in different ROIs.

	R_2^*	Frequency shift							
		PU	CA	GP	TH	PU	CA	GP	TH
1.5 T	16.7±0.3	15.7±0.4	22.8±1.3	15.3±0.2	1.0±0.1	1.9±0.1	1.7±0.3	0.9±0.2	
3 T	25.2±0.9	25.5±0.9	39.5±1.4	21.3±0.8	2.2±0.3	3.9±0.2	3.1±0.3	2.0±0.4	
7 T	50.7±2.0	49.3±2.6	86.2±3.2	39.0±1.0	5.0±0.4	8.0±0.7	7.2±0.8	5.0±0.4	
Slope (Hz/T)	6.2±1.0	6.1±1.3	11.6±2.0	4.3±0.4	0.7±0.2	1.1±0.4	1.0±0.4	0.7±0.2	
r^I	0.97	0.95	0.97	0.98	0.90	0.89	0.83	0.87	

^I r represents the two-tailed Pearson's correlation coefficient. All the coefficients are significant at a level $p < 0.01$.

Table 3

The Iron concentrations measured in several brain tissue specimens*.

ROI	Iron (ppm)	
	Brain Tissue A	Brain Tissue B
PU	636.0	1034.5
CA	528.0	718.5
GP	1090.0	1125.0
TH	151.5	235.0
CC	59.0	/

* The iron contents were measured using inductively coupled plasma-mass spectrometry at West Coast Analytical Service Inc (Santa Fe Springs, CA). The detection limit for iron is about 0.05 parts per million (ppm or $\mu\text{g/g}$, dry weight).