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A Multicontrast Approach for Comprehensive Imaging of Substantia Nigra

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Abstract

We characterize the contrast behavior of substantia nigra (SN) in both magnetization transfer (MT) imaging, which is believed to be sensitive to neuromelanin (NM), and susceptibility weighted imaging (SWI). Images were acquired with a MT prepared dual echo gradient echo sequence. The first echo was taken as the MT contrast image and the second was used to generate the SWI image. SN volumes were segmented from these two types of images using a thresholding method. The spatial and signal characteristics of the extracted SWI and MT volumes were compared. Both images showed the presence of SN but the volumes of the SN identified in the two are spatially incongruent. The MT volume was more caudal than the SWI volume and with only a 12% overlap between the two volumes. Considering the SN volumes in each hemisphere separately, the average distances between the centers of mass of the volumes from the two types images are 5.1 ± 1.1 mm and 4.1 ± 1.2 mm, respectively. The frequency offsets (homodyne filtered phase/echo time) for the volumes derived from MT (NM) images and SWI images are 0.09 ± 0.32 radians/s and -1.12 ± 0.57 radians/s ($p < 0.0001$), respectively. The MT contrasts for the two volumes are 0.16 ± 0.02 and 0.10 ± 0.03 ($p < 0.001$), respectively. Our results indicate that the two contrasts are sensitive to different portions of the SN, with MT seeing the more caudal portion of the SN than SWI, likely due to variations of NM and iron content in the SN. Despite the small overlap, these regions are complementary. Our results provide a new understanding of the contrast

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behavior of the SN in the two imaging approaches commonly used to image it and indicate that using both may yield a more comprehensive visualization of the SN.

Keywords

susceptibility weighted imaging; iron; magnetization transfer; neuromelanin; substantia nigra

1. INTRODUCTION

The substantia nigra (SN) consists of two histologically distinct regions, the SN pars reticulata (SNr) and the SN pars compacta (SNpc). The SN plays a prominent role in many cognitive functions including novelty processing (Schiffer et al., 2012) and reward based learning (Guitart-Masip et al., 2012). Furthermore, degeneration of the SN is a hallmark of the progression of a number of neurodegenerative diseases including Parkinson's disease (PD) (Braak et al., 2011). Characteristic effects of PD in the SN include the loss of neuromelanin (NM) generating dopaminergic neurons in the SNpc (Fearnley and Lees, 1991) and deposition of iron throughout the SN (Dexter et al., 1991; Zecca et al., 2004). It has been hypothesized that increased iron deposition is the primary cause of neuronal death in the SN (Becker et al., 1995) while other studies have suggested that increased iron deposition in PD is a byproduct of and not a primary cause of neuronal death (Galazka-Friedman et al., 1996; Uitti et al., 1989). While the cause of neuronal death in the SN is in contention, there is consensus that increased iron deposition is related to decreased NM in the SN and an MR sequence sensitive to iron and NM can enhance the study of the progression of PD.

In PD patients, much of the work focusing on imaging, delineation, and segmentation of the SN using MRI has concentrated on imaging changes in iron content and NM. This is due in part to the difficulty in identifying differences in SN volume between PD and control groups using traditional T₁- and T₂-weighted sequences (Oikawa et al., 2002). In contrast, studies using sequences potentially sensitive to NM (Kashihara et al., 2011a; Mukai et al., 2013; Ogisu et al., 2013; Sasaki et al., 2006) and iron (Gelman et al., 1999; Gorell et al., 1995; Gupta et al., 2010; Peran et al., 2007) were able to identify differences between PD and control groups.

NM is a macromolecule and imaging macromolecules using standard MR sequences is difficult due to their short T₂ (Henkelman et al., 2001). The first published study to image NM in the SN used a T₁-weighted turbo spin echo sequence (TSE) (Sasaki et al., 2006). Incidental magnetization transfer (MT) effects, associated with the refocusing pulses in the TSE sequence, were thought to be the primary source of NM-sensitive contrast in the study. Subsequent attempts to image NM in the SN applied the TSE sequence or slightly modified versions of the TSE sequence to image the SN in PD (Kashihara et al., 2011a, b; Matsuura et al., 2013; Mukai et al., 2013). After the MT effect was shown to be responsible for NM-sensitive contrast (Nakane et al., 2008), others used explicit MT effects, generated by applying MT preparation pulses (Ogisu et al., 2013) or fat saturation pulses (Schwarz et al.,

2011), to image SN. Further work correlated MT sensitive signal with NM in the SN (Kitao et al., 2013) and locus coeruleus (Keren et al., 2009).

MT-based NM MRI (NM-MRI) images have significant T_1 -weighting and minimal T_2 -weighting. While NM containing structures are easily delineated using NM-MRI, structures that do not contain NM exhibit little contrast over the background. For visualizing structures such as the red or subthalamic nuclei, which contain large amounts of iron and little NM, a method with significant T_2 -weighting needs to be used. Furthermore, the delineation of these structures is important as they provide landmarks that can be used for localizing the SN. The most successful methods utilizing T_2 sensitive contrast to image the SN are susceptibility weighted imaging (SWI) (Haacke et al., 2004) and those focusing on mapping the transverse relaxation rate, R_2 . These methods are successful because iron is paramagnetic and generates magnetic field inhomogeneities, which cause spins to dephase and thereby lead to changes in the phase map as well as maps of transverse relaxation rates. SWI derives an iron-sensitive image by applying post-processing techniques to the phase map.

Multiple studies have shown an increase in R_2 in the SN for PD groups over controls (German et al., 1992; Gorell et al., 1995; Peran et al., 2010; Peran et al., 2007) while another study found no change in R_2 between early stage PD, late stage PD, and control groups (Aquino et al., 2014). Other studies using SWI have shown that the concentration of brain iron correlates with unified Parkinson's disease rating scale (UPDRS) score (Zhang et al., 2010) or differentiated PD from atypical Parkinsonian disorders (Gupta et al., 2010), also recent work has used T_2 -weighted methods at high magnetic field strengths (7T) to image nigrosome 1 within the SN (Blazejewska et al., 2013; Cho et al., 2011; Schwarz et al., 2014). In addition, inconsistencies in the definition of SN and its subcomponents between the studies increase the difficulty in comparing and interpreting results across studies. As an example of this inconsistency, see the following works (Du et al., 2011; Martin et al., 2008; Ulla et al., 2013). These inconsistencies are a confounding factor in the development of useful early-stage biomarkers for diagnosis of PD (Schwarz et al., 2013).

To date, no study has comparatively studied the contrast behavior of the SN in MT-based NMMRI and SWI images and compared the resultant delineations of the SN in the two types of images. The present study aims to fill this void. Results from this study indicate the two contrasts are sensitive to different portions of the SN, with MT-based NM-MRI seeing more caudal portions of the SN than SWI. Elucidation of these SN volumes could help resolve the above mentioned discrepancies in the literature and remove a confounding factor in the development of early-stage biomarkers for the diagnosis of PD.

2. MATERIALS AND METHODS

2.1 Sequence Modification and Image Acquisition

All MR data were acquired on a Siemens 3T Trio (Siemens Medical Solutions, Malvern, PA) using a 12-channel receive only head coil. Images were acquired from a volunteer group consisting of 11 healthy young adults (6 males and 5 females), aged 30.3 ± 5.3 years.

A 2D dual gradient echo (GRE) sequence was modified to have an MT preparation pulse placed prior to the excitation pulse. The TE and bandwidth of the first echo was chosen to have minimal T_2^* weighting and to emphasize MT effects ($TE_1 = 2.68$ ms; bandwidth = 470 Hz/pixel) while the TE of the second echo was chosen to have an echo time (TE_2) of 20 ms similar to that commonly used for SWI, and a bandwidth (420 Hz/pixel). Imaging was performed with the following parameters: TR = 465 ms, 12 contiguous slices, 312×384 imaging matrix, FOV = 162×200 mm², voxel size = $0.5 \times 0.5 \times 3.0$ mm³, 7 measurements, FA = 40°, MT pulses (FA = 300°, 1.5 kHz off-resonance, 10 ms duration) with a total scan time of 16 minutes and 16 seconds. The 7 measurements were registered before averaging to reduce motion artifacts.

Prior to the imaging with the dual-echo MT-based NM-MRI/SWI sequence, whole brain anatomical images were acquired with an MPRAGE sequence ($TE / TR / TI = 3.02 / 2600 / 800$ ms, FA = 8°, voxel size = $1.0 \times 1.0 \times 1.0$ mm³). On the MPRAGE images, the scan slices of dual-echo NMMRI/SWI sequence were prescribed perpendicular to the dorsal edge of the brain stem, covering the SN.

2.2 NM-MRI Image Processing

Imaging data were analyzed with FMRIB Software Library (FSL) (Jenkinson et al., 2002; Jenkinson and Smith, 2001; Smith et al., 2004) and MATLAB (The Mathworks, Natick, MA). The SN was segmented from the NM-MRI images using the procedure described in (Chen et al., 2014) and detailed below.

1. Reference ROIs (circles with diameter in 6 mm) were placed in the tissues surrounding the SN in 4 consecutive slices, starting from the most inferior slice showing the MT contrast. Each slice had 2 ROIs, flanking both the left and right SNs.
2. The mean signal intensity, I_{MEAN} , and standard deviation, I_{SD} , were calculated for the reference ROIs and a threshold of the mean plus three times the standard deviations was created ($T = I_{REF} + 3SD_{REF}$).
3. Voxels with intensities greater than T were classified as SN.
4. The opening operator was applied to the SN mask to remove isolated voxels with intensities above the threshold. Then the closing operator was used to fill in holes in the SN mask.

For characterization of MT contrast (MTC), MTC was calculated as $|I_{SN} - I_{REF}| / I_{REF}$ where I_{SN} and I_{REF} represent the mean signal intensity of the SN and a reference region, respectively. Typical reference ROIs are shown in Fig. 3.

2.3 SWI Image Processing

Magnitude images of the multiple measurements of the second echo (SWI) were registered using the spatial registration parameters derived for the first echo and averaged. Next, phase maps from each of the first echo measurements were unwrapped (Langley and Zhao, 2009), registered and averaged. In addition, phase maps from each of the second echo measurements were unwrapped (Langley and Zhao, 2009), registered and averaged. The

average phase map from the second echo was subtracted from the first echo and the resultant phase map was filtered using a high-pass homodyne filter (filter size = 55×55) to eliminate low-frequency phase variations. This filter removes objects larger than 4 mm (Haacke et al., 2004). The phase mask was generated by scaling and thresholding the corresponding homodyne filtered phase image and then multiplied to the magnitude image from the second echo to produce the SWI image.

The SWI images were segmented using an approach similar to the MT-based NM-MRI segmentation described above. In contrast to the segmentation of MT-based NM-MRI images, voxels in SWI images with signal intensities less than the mean of a reference region minus three standard deviations ($I_{\text{voxel}} < I_{\text{REF}} - 3SD_{\text{REF}}$) were taken as part of the SN. The SN in T₂-weighted images were segmented using a similar procedure.

2.4 Comparison Measures for Segmentation

The overlap, denoted O , between the SN volumes derived from the MT-based NM-MRI and SWI images, respectively, was calculated using,

$$O = \frac{(SN_{MT} \cap SN_{SWI})}{(SN_{MT} \cup SN_{SWI})} \quad [1]$$

In Eq. [1], SN_{MT} and SN_{SWI} denote the volumes from the segmentation of the SN from the MT-based NM-MRI and SWI images respectively. The operators $\cap$ and $\cup$ denote the intersection and union operations, respectively.

The centers of mass for all SN volumes were estimated using `fsstats` in FSL.

3. RESULTS

The SN was seen in both MT-based NM-MRI and SWI images. Fig. 1 illustrates the discrepancy of the SN from the two contrasts. The most inferior slice in the MT-based NM-MRI image containing a region of the SN (Fig. 1A) shows little susceptibility-weighted contrast in the same region (Fig. 1B). In contrast, in a caudal slice, the SN (typically defined in SWI studies as a hypointense band between the cerebral peduncle and the red nucleus) is easily delineated in SWI images (Fig. 1D). The same region, however, exhibits negligible contrast in the MT-based NM-MRI image (Fig. 1C).

Both contrasts provide a similarly sized estimate of SN volume (NM-MRI: $792.2 \pm 164.1 \text{ mm}^3$; SWI: $667.2 \pm 114.6 \text{ mm}^3$; $p=0.07$). However, there is little overlap between the two estimates. Considering the two hemispheres separately, the average distance between the centers of mass was $5.1 \pm 1.1 \text{ mm}$ and $4.1 \pm 1.2 \text{ mm}$ for the left and right sides, respectively. Fig. 2A and 2B display the center of masses for left and right components of the SN volumes in MNI152 space, respectively.

The mean overlap across all subjects between the estimated SN volumes is $12.6 \pm 4.6\%$. Slice-by-slice analysis shows that the overlap increases the slice moves rostrally from the most inferior slice showing NM-sensitive contrast in the SN. This increase is illustrated in Fig. 2C, where slice 1 denotes the most caudal slice showing the SN in the MT-based NM-

MRI image and slices 2-4 are increasingly more rostral from slice 1. A slice with a large overlap (overlap=0.224) is illustrated in Fig. 3(B-iv) and a slice showing minimal overlap (overlap= 0.025) is shown in Fig. 4(B-iv).

The mean frequency offsets (homodyne filtered phase/TE) are displayed in Fig. 5A (NM-MRI SN volume: 0.09 ± 0.32 radians/s; SWI SN volume: -1.12 ± 0.57 radians/s; $p < 0.001$). Since the frequency offset (phase) is sensitive to iron, we are able to infer that the SWI SN volume has higher iron content than the NM-MRI SN volume. One-way analysis of variance (ANOVA) testing of the frequency offsets across different slices of for the two SN volumes revealed similar frequency offset values across slices in the NM-MRI SN volume ($p = 0.887$) and SWI SN volume ($p = 0.143$).

The mean MTC values for SN volumes derived from the two contrasts are displayed in Fig. 5C. The mean MTC values for the SN volume derived from SWI and NM-MRI images are 0.10 ± 0.03 and 0.16 ± 0.02 , respectively ($p < 0.001$). ANOVA tests for the median MTC values across the slices for the two SN volumes indicate similar MTC values across slices for MT-based NM-MRI ($p = 0.16$) and SWI ($p = 0.60$) SN volumes.

T₂-weighted (from unprocessed SWI images) and SWI images gave similar SN volumes (SWI: 667.2 ± 114.6 mm³; T₂: 619.2 ± 57.7 mm³; $p = 0.04$). Negligible difference was seen in the centers of mass for the T₂-weighted and SWI SN volumes (0.70 ± 0.34 mm and 0.77 ± 0.56 mm for the left and right hemispheres, respectively). Additionally, a large overlap (0.742 ± 0.046) was seen in SN volumes from T₂-weighted and SWI images, respectively, while a small overlap (0.116 ± 0.048) was seen in SN volumes from T₂-weighted and MT-based NM-MRI images), respectively.

4. DISCUSSION

In this work, we presented a multi-contrast approach to image the SN using MT-based NM-MRI and SWI. The approach combines the sensitivity to NM, associated with the MT preparation pulse, and sensitivity to iron, associated with SWI. The results indicate that the SN volumes derived from the MT-based NM-MRI and SWI images are spatially incongruent with only a 12% overlap between them. Furthermore, the median MTC and frequency offset vary significantly between the SN volumes derived from the two images.

The source of NM-sensitive contrast is still under debate, with some papers attributing it primarily to MT-effects (Chen et al., 2014; Ogisu et al., 2013) while others attributing it to T₁ effects (Sasaki et al., 2006). The necessity of using MT to generate NM sensitive contrast is illustrated in Fig. 6. However, it is possible that MT effects are not solely responsible for generation of the contrast since the SN appears as hyperintense in the brain stem. If NM-sensitive contrast were primarily due to MT-effects, the SN should appear hypointense in MT-based NM-MRI images. As melanin is paramagnetic (Enochs et al., 1997), the T₁ of the bound pool could be much shorter than that of the free pool. If that is the case, the loss of saturation from spins with a short T₁ could explain the hyperintensity seen in the NM-MRI SN volumes. Hence, the source of NM-sensitive contrast could be a combination of MT-

effects and bound water T_1 -effects, the latter associated with the paramagnetic nature of NM.

One possible explanation for the spatial incongruence between the SWI and MT-based NM-MRI SN volumes may arise from the blooming artifacts in the phase map. Such artifacts would be symmetric along the slice direction and frequency shifts due to susceptibility may cause shifts in the slice direction (Schafer et al., 2009). However, we found that such a shift to be rather small with it having a minimal effect on the center of mass of T_2 -weighted and SWI SN volumes as well as a large overlap between the two volumes. Furthermore, little overlap was seen between T_2 -weighted and NM-MRI SN volumes as well as between SWI and NM-MRI SN volumes. Therefore we posit that the discrepancy in SN location as seen in SWI/ T_2 -weighted images with the SN as seen in MT-based NM-MRI images is primarily due factors other than blooming artifacts.

The discrepancies in location, morphology, and signal characteristics of the two SN volumes led us to conjecture that different substructures of the SN are delineated by MT-based NM-MRI and SWI. Histological studies have shown that the SNpc contains low concentrations of ferritin while the SNr contains elevated concentrations of ferritin and is prominently seen when stained for ferritin (Snyder and Connor, 2009). The histological results suggest that SWI identified portion of SN corresponds to the SNr since the frequency offsets are significantly larger in the SWI SN volume than the NM-MRI SN volume. Furthermore, other histological studies have shown that the SNpc contains higher concentrations of NM than the SNr (Damier et al., 1999). The accordance between MTC and frequency offsets from the NM and SWI SN volumes with those from (Damier et al., 1999) and (Snyder and Connor, 2009) seems to indicate that MT-based NM-MRI and SWI are primarily sensitive to the SNpc and SNr, respectively. However, additional histological work and studies involving diffusion tensor imaging-based fiber tractography with the estimated SN volumes as seed points may provide additional evidence for our conjecture.

In addition to the present method, which uses structural information to delineate and segment the SN, connectivity information from diffusion tensor imaging can be used to parcel the SN as done in a previous study (Menke et al., 2010). In that study, the authors used a T_1 map to segment the SN and then subdivided it into two compartments, an internal compartment and external compartment, based on the projections of fiber tracts from seed points in the initial SN mask (Menke et al., 2010). They found that the external compartment contained connections to the motor cortex and ventral thalamus. The authors interpreted this region to be the SNr. The internal compartment contained connections to the posterior striatum, globus pallidus, prefrontal cortex, and anterior thalamus, which the authors' interpreted to be the SNpc. Their connections were consistent with histological studies showing the dopaminergic neurons in the SNpc projecting into the globus pallidus, subthalamic nucleus, striatum, prefrontal cortex and anterior thalamic nuclei. The neurons in the SNr project to the motor cortex and ventral thalamic nuclei and receive projections from the striatum, external globus pallidus, and subthalamic nucleus (Beckstead et al., 1979; Haber, 2003). The SN volumes derived from the SWI and MT-based NM-MRI images occupy similar spatial locations and orientations as external and internal compartments from the tractography-based study (see Fig. 7 for the SWI and NM-MRI SN volumes for a single

subject displayed in MNI152 space). Incorporation of connectivity information will enhance our understanding of the SN volumes derived from the each contrast.

While our interpretation of the signal characteristics in the two SN volumes agrees with histological studies and a connectivity-based study, our study does have certain limitations. First, the sequence is 2D and contains a small number of slices with gaps, preventing us from performing quantitative susceptibility mapping (QSM). A 3D acquisition would be beneficial in terms of increased SNR and allow for QSM, but its long scan times may lead to practical limitations. Specifically, in clinical populations with motor disorders, the increased sensitivity to motion will lead to lower quality images. Despite the increased sensitivity to motion afforded by the 3D acquisition, studies have examined the susceptibility of the SN (Bilgic et al., 2012; Lim et al., 2013; Lim et al., 2014; Lotfipour et al., 2012). Second, a simple thresholding algorithm was used to segment the SN in both types of images. While this segmentation method is sufficient, improvements in segmenting the SN can be made by using a more sophisticated segmentation method, such as a method based on an active contour algorithm (Kass et al., 1988).

Aside from MTC and frequency offset, other quantitative measures can be used to quantify NM or iron in the SN volumes. For example, the use of the magnetization transfer ratio would offer a more quantitative measure of MT than MTC. However such a ratio would also require a separate scan thereby doubling the time necessary in the scanner. For clinical patients, especially those with neurodegenerative or motor disorders, both inter-scan motion and motion between scans will play a confounding role. MTC was chosen as the metric used to indirectly estimate NM concentrations to remove time constraints, increase clinical applicability, and mitigate potential motion artifacts.

While phase is sensitive to paramagnetic iron species; it is also sensitive to inhomogeneities in the magnetic field, filtering, structure shape (Walsh and Wilman, 2011), orientation of neuronal fibers (Lee et al., 2010), myelin content (Duyn et al., 2007), and the orientation of the brain with respect to the main magnetic field (B_0) (Schafer et al., 2009). However, the aforementioned non-iron factors should not significantly affect the phase given that the SN is a grey matter nucleus and the relative proximity of the SN volumes. MT effects could account for the difference in phase values between the two SN volumes; however, in data (not shown here) obtained experimentally, minimal MT effects were found in the high pass filtered phase map leaving iron content as the probable cause for this difference. Recent work correlated iron content with phase (Haacke et al., 2007; Haacke et al., 2010; Hopp et al., 2010) with the latter using X-ray fluorescence to establish a relationship between phase and iron content. Additionally, there are conflicting results as to the relationship between R_2^* , which is also used as a measure of iron content, and phase. Walsh and Wilman (Walsh and Wilman, 2011) affirmed a relationship between R_2^* and phase in individual structures. On the other hand, (Yan et al., 2012) found no statistically significant correlation between R_2^* and phase in seven structures in the brain, including the SN.

The presented approach is beneficial in that other structures, namely the red nucleus (RN) and subthalamic nucleus (STN) are visible in the unprocessed second echo and resultant SWI image. In the corresponding image from the first echo (NM-MRI image), the echo

time was chosen to minimize T_2^* effects. In images from the first echo, these structures are not easily delineated and only the grey matter, white matter, and NM-containing structures and substructures are visible. In addition to degeneration of NM-containing structures, patients with PD have been found to have increased iron deposition in the RN (Lewis et al., 2013; Wang et al., 2013), increased red nucleus volume (Colpan and Slavin, 2010), and an overall decrease in STN volume (Lewis et al., 2013). Hence, the dual contrast method will allow for more comprehensive study of affected structures in PD.

In conclusion, the discrepancies in location, morphology, and signal characteristics of the two SN volumes suggest an approach combining MT-based NM-MRI and SWI will lead to comprehensive delineation of the SN. The discrepancy in the location between the two SN volumes was illustrated by the low overlap between the estimated SN volumes from the two techniques (approximately 12%). Furthermore, each SN volume contained statistically significant differences in MTC and frequency offsets. The proposed technique combines benefits of the MT-based approach to image NM and SWI approach to image iron thereby presenting an opportunity to improve the study of pathogenesis for PD and other neurodegenerative disorders affecting the SN.

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Highlights

- Dual contrast magnetic resonance imaging of the substantia nigra (SN)
- Magnetization transfer (MT) imaging and susceptibility weighted imaging (SWI) used
- The two SN volumes identified are spatially incongruent with a 12% overlap
- The two SN volumes have differing signal characteristics

Fig. 1.

A comparison of the NM-MRI and SWI images. (A) & (B) display the NM-MRI and SWI images for an inferior slice. (C) & (D) display images for a slice containing the red nucleus and substantia nigra.

Fig. 2.

A plot of the centers of mass for the left (shown in **A**) and right (shown in **B**) components of the SWI SN mask (black) and NM-MRI SN mask (red). Projections of the centers of mass to the x - y plane are marked by 'x'. A histogram of the overlap of the two SN volumes is shown in (**C**). Slice 1 denotes the most caudal slice showing the SN using the NM-MRI image and slices 2-4 move rostral from that slice. [Color displayed in online version].

Fig. 3.

A schematic showing the SN volumes and reference regions of interest used in the segmentation procedure. (A) A view from the MT-based NM-MRI image. A zoomed in view of the SN from the MT-based NM-MRI image is shown in (B) with yellow circles denoting reference ROIs. (C) The left SN volume (blue) and right SN volume (red) overlaid. (D) A view from the SWI image. A zoomed in view of the SN from the SWI image is shown in (E) with yellow circles denoting reference ROIs. (F) The left SN volume (blue) and right SN volume (red) overlaid. [Color displayed in online version].

Fig. 4.

A comparison of the SN volumes estimated from the two contrasts. (A) A view from the NM-MRI image. A zoomed in view of the SN from the NM-MRI image is shown in (B) and the MT-based NMMRI (blue) and SWI (red) SN masks are overlaid on the SN in (C). (D) A view from the SWI image. A zoomed in view of the SN from the SWI image is shown in (E) and the MT-based NM-MRI (blue) and SWI (red) SN masks are overlaid on the SN in (F). [Color displayed in online version].

Fig 5.

A comparison of the MTC and angular frequency estimates from the SN volumes derived from MT-based NM-MRI (grey) and SWI (white). The mean MTC and mean frequency offsets for both SN volumes and average of the median angular frequency values for both SN volumes are displayed in (A) and (B), respectively. In (A) and (B), * denotes statistical significance with $p < 0.001$.

Fig. 6.
Comparison of images acquired without MT preparation pulses (**A**) and acquired with MT preparation pulses (**B**).

Fig. 7.

A. An axial (**A**), coronal (**B**) and sagittal (**C**) images from the MP-RAGE sequence with SN volumes overlaid. The red voxels denote the SWI SN volume and the blue voxels show the MT-based NM-MRI SN volume. [Color displayed in online version].