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Pool size ratio of the substantia nigra in Parkinson's disease derived from two different quantitative magnetization transfer approaches

Paula Trujillo^{1,2}, Paul E. Summers¹, Alex K. Smith³, Seth A. Smith^{4,5}, Luca T. Mainardi⁶, Sergio Cerutti⁶, Daniel O. Claassen², and Antonella Costa¹

¹Department of Neuroradiology, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122, Milan, MI, Italy

²Department of Neurology, Vanderbilt University Medical Center, 1500 21st Avenue South, Suite 3000, 37232, Nashville, TN, USA

³Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), Nuffield Department of Clinical Neuroscience, University of Oxford, Oxford OX3 9DU, UK

⁴Vanderbilt University Institute of Imaging Science, Vanderbilt University, 1161 21st Avenue South, Medical Center North, AA-1105, 37232, Nashville, TN, USA

⁵Department of Biomedical Engineering, Vanderbilt University, 5824 Stevenson Center, 37232, Nashville, TN, USA

⁶Department of Electronics, Information and Bioengineering, Politecnico di Milano, Piazza Leonardo da Vinci 33, 20133, Milan, MI, Italy

Abstract

Purpose—We sought to measure quantitative magnetization transfer (qMT) properties of the substantia nigra pars compacta (SNc) in patients with Parkinson's disease (PD) and healthy controls (HCs) using a full qMT analysis and determine whether a rapid single-point measurement yields equivalent results for pool size ratio (PSR).

Methods—Sixteen different MT-prepared MRI scans were obtained at 3T from sixteen PD patients and eight HCs, along with B1, B0 and relaxation time maps. Maps of PSR, free and macromolecular pool transverse relaxation times (T_2^f , T_2^m) and rate of MT exchange between pools (k_{mf}) were generated using a full qMT model. PSR maps were also generated using a

Corresponding author: Paul E. Summers, Department of Neuroradiology, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122, Milan, MI, Italy, paul.summers@ieo.it.

ORCID: 0000-0002-5085-1095

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

single-point qMT model requiring just two MT-prepared images. qMT parameter values of the SNc, red nucleus, cerebral crus and grey matter were compared between groups and methods.

Results—PSR of the SNc was the only qMT parameter to differ significantly between groups ($p<0.05$). PSR measured via single-point analysis was less variable than with the full MT model, provided slightly better differentiation of PD patients from HCs (area under curve 0.77 vs. 0.75) with sensitivity and specificity of 0.87, and was better than transverse relaxation time in distinguishing PD patients from HCs (area under curve 0.71, sensitivity 0.87 and specificity 0.50).

Conclusions—The increased PSR observed in the SNc of PD patients may provide a novel biomarker of PD, possibly associated with an increased macromolecular content. Single-point PSR mapping with reduced variability and shorter scan times relative to the full qMT model appears clinically feasible.

Keywords

Magnetization transfer; Neuromelanin; Parkinson's disease; Substantia Nigra

1. Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the loss of pigmented dopaminergic neurons in the substantia nigra pars compacta (SNc) [1, 2]. Motor symptoms, especially slowness of movement and rigidity, are associated with SNc neuron cell death [2]. Neurodegenerative disorders involving other, distinct pathological processes (e.g. progressive supranuclear palsy) can phenotypically mimic PD in its early clinical manifestation, but go on to have different clinical trajectories. As such, there is a need for non-invasive biomarkers of PD with adequate sensitivity and specificity to guide differential diagnosis, quantify disease severity, and further, offer insight into prognosis, and treatment response.

Neuromelanin (NM) is a dark pigment particularly concentrated in the SNc [3] that is relatively diminished in PD patients compared to age-matched healthy subjects [1, 4]. Neuromelanin-sensitive MRI (NM-MRI) techniques [5] have shown notable contrast between the SNc and surrounding brain tissues, with a number of studies indicating that NM-MRI may be used to detect alterations to SNc morphology, even in early stages of PD [6–15]. Moreover, a direct comparison between post-mortem NM-MRI and neuropathology findings [16] found NM-MRI signal intensity in the SNc to be closely associated with the quantity of NM-containing neurons. However, the mechanism by which the presence of NM might give rise to signal hyperintensities on MRI, and the nature of the relationship between the loss of hyperintensity with advancing PD severity is still unclear.

NM-MRI is typically based on a T1-weighted turbo spin-echo (TSE) pulse sequence [5]. The knowledge that such multi-slice TSE sequences are subject to incidental magnetization transfer (MT) weighting associated with the extended train of refocusing pulses [17], and observations that MT preparation of NM-MRI pulse sequences increases the contrast between the SNc and surrounding brain tissue have led several authors to credit MT as the primary source of NM-MRI contrast [18, 19]. Extending this result, others [9, 20–23] have

used explicit MT effects, generated by applying MT preparation pulses to 3D gradient echo pulse sequences (that have little intrinsic MT weighting), to establish contrast between NM-containing structures and their surroundings, with advantages over T1-weighted TSE-based acquisitions in terms of resolution and scan-time. Moreover, sequences with an explicit MT contrast preparation pulse have shown higher sensitivity in detecting NM containing structures in the brain than those relying on incidental MT effects [19, 21].

Outside NM-MRI, the use of the MT effect in clinical practice has largely been limited to enhancing vascular contrast in MR angiography [24], and to the semi-quantitative measurement of the ratio (MTR) between MT-prepared and non-prepared image acquisitions [25]. The MTR combines the effects of all of the mechanisms that contributing to MT into a single semi-quantitative index. In the SN, the MTR has been found to be reduced in both early and late phases of PD [26–30], but the results vary widely both in the values and the variation obtained. Moreover, in terms of understanding the mechanisms of NM-MRI contrast, MTR is hampered by the fact that it reflects the collective impacts of several inter-related physico-chemical properties, including the macromolecular content [29], as well as vendor-specific choices regarding MT and excitation RF pulse characteristics. By using quantitative MT (qMT) methods [31–33] we have sought to decouple these factors, a process that has been demonstrated to generate scanner independent indices of tissue physiology that may prove more reproducible [34, 35] and have a physical meaning in quantifying SNc degeneration in PD.

Pulsed saturation-based qMT involves acquiring multiple images with preparation pulses at different RF offsets (and saturation powers) in order to describe the Z-spectrum (also referred to as MT-spectrum), and derive quantitative indices of tissue structure, such as the macromolecular-to-free pool size ratio (PSR), the rate of MT exchange between pools, and the longitudinal and transverse relaxation times for each pool. Although pulsed-saturation qMT offers several indices of tissue physiology, doing so reliably within a clinically feasible scan time is challenging. A number of methods for obtaining some or all of the qMT-derived indices are available in the literature [36–39], but we will focus our attention on the previous work of Yarnykh et al [40], which suggests concentrating on the derivation of a single qMT parameter such that a single MT prepared acquisition and a reference measurement are sufficient for the calculation [34, 35, 40]. Using this approach, PSR maps can be obtained in clinically relevant scan times, making it a valuable approach for the SN.

While several studies have evaluated the clinical utility of qMT imaging in other neurological disorders, particularly multiple sclerosis [34, 35], the application of qMT in patients with PD, has not yet been reported. Thus, the aim of the present study was to compare the MT properties of the SNc in PD patients and healthy controls (HCs) using a full qMT analysis, and determine whether a rapid single-point measurement yields equivalent results for the PSR.

2. Materials and Methods

2.2 Subjects

The study involved 16 subjects with PD (13 males, age range: 48–72 years old (63 ± 6.7 years); disease duration: 2–10 years (7 ± 3 years); Hoehn and Yahr stage: 1–3), and 8 age-matched healthy controls (HC) (3 males, age range: 51–72 years old (59 ± 7.3 years)). All subjects gave written informed consent for the study, which was approved by the local institutional review board.

The diagnosis of PD was made according to the UK Parkinson Disease Brain Bank criteria. The disease stage was determined using the Hoehn and Yahr scale. All patients completed a neurological examination assessing motor and cognitive function (Unified Parkinson Disease Rating Scale (UPDRS) in the on-medication state, and Montreal Cognitive Assessment (MoCA)).

2.3 Image acquisition

All participants underwent brain MRI, including qMT, B0, B1, T1 and T2 mapping and NM-MRI on a 3T MRI scanner (Achieva, Philips Medical Systems, Best, The Netherlands) with a 32-channel head coil. All images were acquired as oblique axial slices covering the midbrain oriented perpendicular to the floor of the fourth ventricle (Fig. 1a), with a $216 \times 180 \times 21$ mm³ field of view, and 3 mm slice thickness. Specific scan details are described below.

The qMT data (Fig. 1b) were acquired using a 3D MT-prepared SPGR sequence [41] with a multi-shot EPI readout, EPI factor = 5, TR/TE = 47 / 9 ms, $\alpha = 10^\circ$, SENSE factor = 2, acquired / reconstructed with resolution $1.0 \times 1.0 / 0.75 \times 0.75$ mm², 4 signal averages, and MT weighting achieved using a 20-ms, single-lobed sinc-gauss pulse at eight frequency offsets ($\omega = 1, 1.5, 2, 2.5, 8, 16, 32, 100$ kHz) and two powers ($\alpha_{MT} = 600^\circ$ and 900°). Image acquisition time for the complete set was approximately 8 min.

B0 and B1 maps were acquired using 3D SPGR sequences at lower in-plane resolution but with the same slice thickness as the above qMT sequences. B0 maps were obtained using the dual-TE phase-difference method [42] with TR/TE1/TE2 = 50 / 5.8 / 8.1 ms, $\alpha = 25^\circ$, acquisition / reconstruction resolution = $1.5 \times 1.5 / 0.96 \times 0.96$ mm². B1 maps were obtained using the actual flip-angle imaging method [43] with TR1/TR2/TE = 30 / 130 / 5.7 ms, $\alpha = 60^\circ$, acquisition / reconstruction resolution = $2.0 \times 2.0 / 0.96 \times 0.96$ mm². Acquisition times were 1 min 12 s for the B0 map, and 1 min 29 s for the B1 map.

T1 mapping was performed using a multiple flip angle SPGR-based acquisition with TR/TE = 20/5.3 ms, $\alpha = 5, 10, 15, 20, 25, 30^\circ$, acquisition / reconstruction resolution = $1.26 \times 1.26 / 0.96 \times 0.96$ mm². T1 maps (T_1^{obs}) were reconstructed by fitting the Ernst equation with B1 correction [44]. T2 mapping was performed using a multiple echo TSE acquisition with TR/TR = 2000 / 40, 60, 80, 100, 120 ms, $\alpha = 90^\circ$, acquisition / reconstruction resolution = $1.0 \times 1.0 / 0.75 \times 0.75$ mm², from which quantitative T2 maps (T_2^{obs}) were calculated using

the scanner manufacturer-supplied software. Acquisition times were 51 s, and 3 min for the T1 and T2 maps, respectively.

Finally, we acquired a NM-MRI scan (Fig. 1c) consisting of a T1-weighted TSE sequence with the manufacturer's default on-resonance MT preparation pulses (TR/TE = 670/12 ms, echo train length = 4, acquisition / reconstruction resolution = $0.75 \times 0.75 / 0.5 \times 0.5 \text{ mm}^2$, NSA = 4, acquisition time = 3 min 50 s).

2.4 Image processing

Prior to data fitting, all images were co-registered using the FLIRT package (FSL v5.0.2.1, FMRIB, Oxford, UK) [45]. Following co-registration, images were cropped to an area around the midbrain. All remaining steps in the fitting processes were performed in MATLAB (R2015a, Mathworks, Natick, MA). The signal intensities of the MT-weighted images were normalized to the intensity of the reference image ($\omega = 100 \text{ kHz}$ where MT-related saturation is considered negligible), and the nominal offset frequency and RF amplitudes were corrected using B0 and B1 maps, respectively.

Full-fit analysis—The qMT parameters in the full-fit analysis were estimated on a voxel by voxel basis through multiparametric fitting to the two-pool full-fit qMT model described in [33]. The two-pool MT model contains six independent parameters: the longitudinal and transverse relaxation times of the free (f) and macromolecular (m) pools ($T_1^f, T_1^m, T_2^f, T_2^m$), the macromolecular-to-free pool size ratio ($\text{PSR} = M_0^m / M_0^f$, where $M_0^{f,m}$ are the equilibrium magnetization of each pool), and the rate of MT exchange from the macromolecular pool to the free pool (k_{mf}). The exchange rate in the other direction can be calculated as $k_{fm} = k_{mf} * \text{PSR}$. It has been shown that the signal dependence on T_1^m is weak [46], therefore it was fixed at 1 s. The T1 values obtained from the T1 mapping (T_1^{obs}) were used to estimate the parameter T_1^f , as described by [46]. The four remaining parameters (T_2^f, T_2^m, PSR and k_{mf}) were estimated by fitting the qMT data to the model. Cerebrospinal fluid was excluded from the fitting by masking out the voxels with T_1^{obs} higher than 2.5 s and/or T_2^{obs} higher than 0.25 s.

The quality of fit was estimated in a manner similar to [33], by computing the root mean squared difference between the experimental and fitted data at each voxel (σ). The σ map was created for each subject and the median value of its histogram was determined to assess the subject-wise quality of fit.

Single-point-fitting analysis—The PSR can be estimated using a single-point approach [40] that requires a single MT-weighted image, a reference image without saturation, and complementary T1, B0, and B1 maps. The results of the full-fit analysis showed that k_{mf} , T_2^m and the ratio T_2^f / T_1^f exhibit relatively constant values across tissues [33], suggesting they can be fixed for a single-point model where the PSR is the only free parameter. The single-point fitting process was performed using the qMT data acquired with $\omega = 2 \text{ kHz}$, and $\alpha_{\text{MT}} = 900^\circ$, according to method described in [40] with the fixed values for k_{mf} , T_2^f / T_1^f , and

T_2^m being based on the median value of their histograms obtained from the full-fit analysis ($k_{mf} = 10$ Hz, $T_2^f/T_1^f = 0.018$, and $T_2^m = 10$ μ s). To assess the effect of the fixed parameters on the parametric PSR map in PD and HC populations, we also performed a series of PSR reconstructions independently varying the values of k_{mf} , T_2^f/T_1^f , and T_2^m over ranges chosen to cover their possible physiological variability, while keeping the others fixed at their chosen values for the above single fit analysis.

For comparative purposes, the MTR maps were calculated as:

$$\text{MTR} = 1 - M/M_0$$

where M is the MT-weighted image acquired with $\omega = 2$ kHz and $\alpha_{\text{MT}} = 900^\circ$ (same data used for the single-point PSR estimation), and M_0 is the reference image ($\omega = 100$ kHz where MT-related saturation is considered negligible).

Regions of interest—Bilateral regions of interest (ROIs) in the SNc were segmented from the NM-MRI images using a thresholding method similar to [12, 21, 22] in the 3D Slicer software package (version 4.3.1, <http://www.slicer.org>). In brief, a reader, blinded to the clinical status of the subjects, first defined circular (4 mm diameter) background ROIs in the cerebral crus (CC) on the left and right sides. This was repeated for three consecutive slices, in which the SN was visible. For each slice, a binary map was defined as the voxels in the midbrain with signal intensity greater than $\text{MN}_{\text{CC}} + 3 \times \text{SD}_{\text{CC}}$, where MN_{CC} and SD_{CC} are the mean and standard deviation for the background ROI located in the cerebral crus on the corresponding slice and side. ROIs for the SNc were then defined on the binary map (Fig. 1d). SNc segmentation was performed carefully to include only the regions of hyperintensity NM-MRI image to avoid partial volume effects.

In NM-MRI images, the SNc exhibits a distinct hyperintensity relative to the neighboring white matter including the CC as well as other grey matter structures of the midbrain, including the red nuclei. In order to investigate the possible roles of qMT parameters and relaxation times in determining NM-MRI contrast, we defined two additional ROIs: one for the red nucleus (RN) which is iron-rich but lacks NM, and one for tissues outside the brainstem having PSR values matching those of the SNc. The ROI for the RN was manually defined on the T2 maps delimiting a hypointense circular area posterior-medial to the SNc (Fig. 1e). The ROI for the tissues with matching PSR was segmented semi-automatically using the PSR map by applying thresholds (range: $[\text{mean} - 2 \times \text{SD}, \text{mean}]$ of the PSR values for the SNc on each slice), excluding the brainstem and limiting the result to the two largest connected clusters (in 3D). The resulting tissue mask were visually confirmed by an expert neuroradiologist (AC) to almost exclusively involve grey matter of the amygdala, hippocampus, parahippocampus and medial temporal cortex (Fig. 1f), and therefore are referred to as a grey matter (GM) ROI, though in fact they encompass only grey matter having PSR values in the lower half of the range of values seen in the SNc. The previously designed ROI for the CC was taken to be representative of white matter.

The ROIs were used to sample the T_1^{obs} and T_2^{obs} maps, and parametric maps obtained from the qMT fitting. To alleviate the effect of outlying voxels, median values (instead of mean) were measured for each ROI.

2.5 Statistical Analysis

Statistical analyses were performed with the JMP statistical package, (version 10.0, SAS Institute, Inc., Cary, NC, USA). To determine agreement between the full-fit and single-point methods, the median PSR value for each tissue ROI was determined on the corresponding PSR map for each subject, and these were compared using Spearman correlation coefficient and Bland–Altman plots. Differences between groups or methods were assessed using the Mann–Whitney U test. The threshold level of statistical significance was set at $p < 0.05$. Receiver operating characteristics (ROC) analysis was performed to assess the diagnostic accuracy of parameters that provide statistically significant differences between PD and HC groups. The 95% confidence intervals (CI) for sensitivity and specificity were calculated according to the Clopper–Pearson method. In order to quantify the contrast between the SNc and surrounding tissues in the PSR maps, the contrast-to-noise-ratio between the SNc and the CC was calculated for each PSR map as:

$$CNR_{SN} = \frac{(MN_{SNc} - MN_{CC})}{SD_{CC}}$$

where MN_{SN} and MN_{CC} correspond to the mean PSR value of the SNc and CC, respectively, and SD_{CC} corresponds to the standard deviation of the CC.

3. Results

3.2 Full-fit analysis

Representative MT data from full-fit acquisition illustrating the changing contrast with frequency offset and flip angle of the MT pre-pulse can be seen in Fig. 2a. The Z-spectra for regions of interest in the SNc (Fig. 2b, red) and the CC (Fig. 2b, blue) are shown in Fig. 2c.

The PSR maps obtained with the full-fit method provided clear differentiation between white and grey matter, with PSR values being lower in grey matter than in white matter, and offering good visualization of the SNc. The k_{mf} and T_2^m maps exhibited relatively uniform values across tissues and did not provide notable contrast for the SNc compared to surrounding brain tissue. The T_2^f values estimated from the MT data were consistently lower than the independently measured T_2^{obs} , and neither allowed a clear visualization of the SNc. The relative difference between tissues for T_2^f/T_1^f was smaller than for the parameter T_2^f alone. The generally low differences in k_{mf} , T_2^f/T_1^f , and T_2^m between tissues suggest that a single set of fixed values for these parameters may be adequate for use in single-point PSR mapping. An example of the four parametric maps obtained with the full-fit analysis along with maps of T_2^f/T_1^f and T_1^{obs} is shown in Fig. 3.

The histograms for the cropped regions of the parametric maps across subjects exhibited unimodal distributions (Fig. 3). The forms of the PSR histograms were similar for HC and PD subjects, with median values of 0.1018 and 0.1068, respectively. The k_{mf} histograms differed slightly between HC and PD patients with median values of 10.88 Hz for the HC, and 10.36 Hz for the PD. The averaged T_2^f/T_1^f histograms also showed a small difference between groups with median values of 0.0178 and 0.0186 for the HC and PD, respectively. The T_2^m histograms were similar between groups, yielding median values of 9.98 μ s for the HC, and 9.92 μ s for the PD. The small differences between groups in the PSR, k_{mf} , T_2^f/T_1^f , and T_2^m histogram median values were not statistically significant according the Mann–Whitney U test ($p>0.05$ in all cases).

For the individual subjects, the median quality of fit for the full-fit analysis, was in the range of 0.0029–0.0048, indicating a good quality fit [33]. The σ maps and histogram showed a slightly left skewed distribution without visible associations with white and grey matter structures or tissue boundaries (Online Resource 1).

3.3 Single-point fitting analysis

The single-point PSR maps showed a clearer distinction between white matter and grey matter, and higher contrast between SNc and CC than the full-fit PSR map ($\text{CNR}_{\text{full-fit}}=0.89\pm0.40$; $\text{CNR}_{\text{single-point}}=1.77\pm0.53$) (Fig. 4).

The impact of the individual constraining parameters on the single-point fit estimates of PSR for the SNc and the CC for is illustrated in the Online Resource 2. All three parameters affected the PSR results, with k_{mf} having the greatest effect. Relative to the estimates of PSR obtained using the chosen fixed values, lower k_{mf} values resulted in overestimation of the PSR while higher k_{mf} values resulted in an underestimation of PSR. Notably, values of k_{mf} below 4 Hz resulted in poor or no PSR contrast, while k_{mf} higher than 10 Hz, resulted in clear contrast between tissues, and nearly constant contrast between the SNc and CC.

Over the range of T_2^f/T_1^f and T_2^m values used in the simulations, the bias in the PSR values relative to the value estimated using the fixed parameter set was less than $\pm 10\%$, and changed smoothly and in parallel for both the SNc and CC, and the contrast between the SNc and CC was again nearly constant.

In the comparison between full-fit and single-point estimates of the PSR, we found a significant correlation between PSR values obtained by the two methods ($\rho=0.93$, $p<0.0001$) (Fig. 5a). The Bland-Altman analysis showed a small bias between the PSR values obtained by the full-fit and single-point methods (0.0015; single-point > full-fit) (Fig. 5b). When comparing the results obtained using the two methods by means of the Mann–Whitney U test, there were no significant differences between the methods, but the single-point estimates of the PSR presented lower variability than the full-fit results (Fig. 5c).

Lastly, we observed a significant overall correlation between the single-point PSR and the MTR values ($\rho=0.87$, $p<0.0001$). However, when examining the correlation between the PSR and MTR values for specific ROIs, we found a significant correlation only in the GM,

but no correlation in the SNc (GM: $\rho=0.68$, $p<0.001$; RN: $\rho=0.29$, $p=0.19$; CC: $\rho=0.22$, $p=0.29$; SNc: $\rho=0.02$, $p=0.90$) (Online Resource 3).

3.4 Comparison of quantitative parameters between tissue ROIs and groups

Table 1 summarizes the qMT and relaxometry results obtained for the various ROIs. In summary, the highest PSR values were seen in the white matter of the CC and the lowest in the GM. The PSR values in the RN were slightly lower than those of the CC and greater than those of the SNc, which had values nearly midway between those of the GM and CC. The longitudinal relaxation times (T_1^{obs}) were shortest in the CC and RN with slightly longer values seen in the SNc and longest in the GM. From shortest to longest, the transverse relaxation times (T_2^{obs}) followed the sequence RN, SNc GM and CC.

In the comparisons of qMT parameters between the PD and HC groups, we found significant differences ($p<0.05$) only for the PSR of the SNc in both the full-fit analysis (PD: 0.128 ± 0.012 vs. HC: 0.116 ± 0.009 ; $p=0.032$) and single-point fitting (PD: 0.127 ± 0.008 vs. HC: 0.119 ± 0.006 ; $p=0.023$) (Fig. 6a).

The ROC analysis for the ability of PSR of the SNc to distinguish the PD and HC groups showed an area under the curve (AUC) of 0.75 (95% CI=[0.55, 0.95]), and 0.77 (95% CI=[0.57, 0.96]) for the full-fit and single-point analyses, respectively (Fig 6b). For the full-fit, a cut-off value of PSR=0.124 provided a diagnostic accuracy (discriminating PD from HC) of 0.71, while for the single-point a cut-off value of PSR=0.121 provided a diagnostic accuracy of 0.79. The resulting sensitivity and specificity for the full-fit approach were 0.62 (95% CI=[0.35,0.85]) and 0.87 (95% CI=[0.47, 0.99]) respectively, while for the single-point approach the sensitivity was slightly higher at 0.75 (95% CI=[0.48, 0.93]) and the specificity unchanged 0.87 (95% CI=[0.47, 0.99]).

Amongst the relaxation time values, only T_2^{obs} of the SNc distinguished the PD and HC groups (PD: 66.88 ± 4.06 vs. HC: 70.49 ± 2.82 ; $p=0.047$). In the ROC analysis for the ability to distinguish the PD and HC groups, the T_2^{obs} of the SNc showed an AUC of 0.71 (95% CI=[0.50, 0.92]), with a cut-off value of 71.4 ms providing a diagnostic accuracy of 0.75, with a sensitivity of 0.87 (95% CI=[0.62, 0.98]) and specificity of 0.5 (95% CI=[0.16, 0.84]). The MTR values were not significantly different between PD and HC groups ($p>0.35$) for all tissue ROIs.

4. Discussion

Amongst the parameters obtained in characterizing the SNc by fitting the full qMT model, we found only PSR to provide significant differentiation between PD and HC groups. The single-point fit to a reduced set of qMT data provided PSR maps with lower variability, higher CNR, and slightly improved the differentiation between PD and HC groups. This is reflected in a slight increase in the AUC (0.75 and 0.77 for full-fit and single-point, respectively) and moderate sensitivity (0.87) and specificity (0.87). In this small cohort, the performance of both single-point or full-qMT model PSR was better than that of the separately acquired transverse relaxation time (T_2^{obs}) in distinguishing PD patients from

HCs (AUC=0.71, sensitivity=0.87, and specificity=0.5). The significantly higher PSR values throughout the SNc seen in the PD group relative to HCs must be considered preliminary however, in light of the rather small sample size of this study.

As a first study of qMT in PD, it is important to recognize that we have examined only a small number of patients with relatively mild PD symptoms. The observed difference in relaxation times between PD and HC groups is consistent with widely reported progressive reductions in relaxation times in the SNc of PD patients [58–61] that are generally attributed to an overall increase of iron content throughout the SN [68, 69] or an increase in the amount of iron bound to NM [70]. The lack of significant differences in qMT values other than an increase in PSR between HC and PD groups accompanied by a small but insignificant increase in MTR on the other hand, is at odds with prior reports of reduced MTR in the SNc of PD patients [27, 29, 47, 48]. Reductions in MTR and PSR have been attributed to inflammation and neuronal death through an increase in the absolute volume of the free pool, as well as myelin damage and elimination from the site of injury, and have been well-reported in multiple sclerosis lesions and demyelination models [34, 35]. Unlike the white matter typically affected by multiple sclerosis however, the dopaminergic neurons that degenerate in PD are highly branched and relatively poorly myelinated [50]. Thus, demyelination is likely to play a minor role in the evolution of PD. Moreover, whereas demyelination is tied to the damage and progressive loss of the macromolecular structure of myelin, PD is characterized by accumulation of α -synuclein aggregates that accompanies and likely predates neuronal death, and of NM in the extracellular space as neuronal death progresses [54]. While intraneuronal NM can protect neurons by binding toxins and redox active metals, insoluble NM is persistent in the extracellular space [1, 55] and can contribute to the activation of microglia, triggering of neuroinflammation and neurodegeneration. PSR mapping seeks to reflect the concentration of macromolecules in isolation from the dependencies on T1 [40] and exchange times that affect MTR measurements. We suggest that in our patient cohort, inflammation and neuronal death have been sufficiently limited to allow the accumulation of macromolecules to increase PSR without bringing about changes in other qMT parameters, while MTR remained relatively unchanged due to its dependency on relaxation times. This would involve two novel processes that remain to be demonstrated: that qMT parameters, and PSR in particular, do not follow a monotonic course as PD progresses; and that changes in PSR differs from those of MTR under some circumstances. The proposed progression of PSR and MTR would be consistent with reports of increases in PSR [51, 52] and reductions in MTR [53] for brain regions altered by Alzheimer disease, another disease characterized by macromolecule accumulation even prior to onset of neuronal death. Notably, qMT parameters other than PSR, including exchange rate and relaxation time ratios that did not show significant differences between HC and PD patients in our cohort, have been seen to correlate with Alzheimer disease progression [62, 63]. Further study is needed to verify our findings of increased PSR in mild PD, as well as to determine the evolution of qMT parameters including PSR, MTR and relaxation times over the full course of PD in order to establish whether they have a role in PD diagnosis and characterization.

As regards measurement of PSR, our results suggest that single-point PSR mapping provides an alternative to fast but more difficult to interpret MTR and to longer full model qMT

measurements. Nevertheless, it is important to note some practical limitations of the single-point method. The choice of the fixed model parameters (T_2^f/T_1^f , T_2^m , and particularly k_{mf}) affect the values of PSR obtained at fitting, and we note that the values of k_{mf} and T_2^f/T_1^f derived from our full-model analysis differed from those reported in a previous study on healthy younger subjects [23]. This may be a result of incorporating both patients and healthy controls in the derivation of the histograms for the present study, or the older age of the subjects relative to the prior work. Additional studies may be needed to obtain more accurate estimates of these parameters for specific anatomic regions. Similarly, PSR estimation relies on the accuracy of the T1 mapping, and thus any error in T_1^{obs} can propagate into the PSR estimates. The single-point PSR mapping has potential for clinical applications due to its time efficiency, and a number of variants [36–39], exist that may provide greater time-efficiency or other advantages over the pulsed saturation approach used herein. We forego discussion of their relative merits as the potential diversity of T1 and PSR mapping strategies suggests a need for careful standardization of implementations and processing techniques for PSR mapping that goes beyond the scope of the present work.

Part of our motivation for investigating the qMT properties of the SNc lay in seeking to understand the mechanism behind the hyperintensity of NM-containing structures in NM-MRI, a technique that has attracted interest as a potential means of quantifying neuronal loss in the SNc of patients with PD [16]. In particular, apart from the locus coeruleus, which also contains NM, both white matter and other grey matter structures of the brainstem, including the RN, appear hypointense relative to the SNc in NM-MRI. The TE, TR and flip angles typically used in both the TSE and 3D-SPGR based NM-MRI are consistent with T1-weighted imaging [64]. With the T1 of the SNc being intermediate between those of WM (higher T1) and GM (lower T1) however, as reported in [18, 65] T1 values alone cannot account for the contrast seen in NM-MRI images. Thus a further contrast mechanism must be involved. Several observations have led to the suggestion of an association between MT and NM-MRI contrast [18, 20, 22]. Consistent with previous qMT studies [33, 40], our results show PSR and MTR values follow the same trend between healthy brain tissues (low in GM, intermediate in SNc and higher in RN and WM), reflecting the important role of PSR in determining MTR. We propose, that the combination of shorter T1 relative to GM, and lower MTR (associated with lower PSR) compared to the CC and RN, explain the particular appearance of the SNc in NM-MRI and the noted enhancement obtained when applying MT [20–23]. Corrupting the words of empirical scientist Goldilocks, the SNc is “not too hot, not too cold - just right” in terms of T1, “not too high and not too low – just right” in terms of PSR (and more generally MTR) to yield a unique hyperintensity in NM-MRI.

Amongst the images acquired for the full qMT model fitting, the 3D SPGR images with MT pulses at offset frequencies between 1 kHz and 8 kHz we observed areas of hyperintensity in the location of the SNc (Fig. 1b), similar to those seen in TSE based NM-MRI (Fig. 1c). This contrast was achieved incidentally, in much the same way that NM-MRI sequences have developed to date. Our findings that PSR and T1 both appear to play a role in determining NM-MRI contrast, together with in-vitro measurements [66] may permit modeling of the NM-MRI signal, and thus to optimization of the choice of MT and T1

weighting for maximizing NM-MRI contrast. To date, many NM-MRI analyses have focused on measuring decreases in SNc volume [9, 10, 12, 67] as an indicator of loss of NM containing neurons. Due to the small size of the SNc, such volume measurements are very sensitive to slice positioning and image resolution. Moreover, if neuronal loss is distributed in the SNc, volume loss will only become observable when there is compaction of the depleted SNc or sufficient loss of neurons along the margins of the SNc to change its contour. Thus, volume measures from NM-MRI do not necessarily reflect decreases in neuron density within the SNc. PSR, as metric of the local macromolecular concentration provides a different vision of the tissue involved in PD that may be less sensitive to these shortcomings of NM-MRI. Further studies are required to determine whether the PSR can provide a robust metric of SNc degeneration.

The primary limitation of this study is that our sample size was relatively small (16 PD, and 8 HC), and our PD group did not include late stage patients (i.e. Hoehn and Yahr stage > 3). As noted above, the concurrent pathological processes in PD evolve over the course of PD, and complicate interpretation of individual tissue parameters in isolation. Therefore, we cannot generalize the increase of PSR and absence of changes to other qMT parameters to the full spectrum of PD progression. Equally, our hypotheses about the basis for an increase in PSR in early PD, and about the roles of T1 and PSR in determining NM-MRI contrast, remain to be tested in a larger population of PD patients, including those in later stages of the disease. Nonetheless, our results support the use of the single-point pulsed saturation method for measuring PSR, and suggest it may be of interest for early PD, but do not preclude the possible use of other forms of parametric imaging for a more complete view of PD. Further, we have not attempted to limit the effects of partial volume effects on qMT values obtained from the SNc. In defining the SNc ROIs, we adapted an approach previously used in several NM-MRI studies [12, 21, 22]; nonetheless the sensitivity of small structures such as the SNc to partial volume and motion should be considered in defining ROIs in future studies. As well, in the above discussion we have considered the structures adjacent to the brainstem having PSR values that match those of the SNc as being representative of grey matter. Visual inspection showed that the resulting ROI was almost exclusively limited to grey matter, though not inclusive of all grey matter structures. As such, the above comments are best attributable to grey matter with PSR values matching that of the SNc, consistent with our use of this PSR-matched ROI in assessing whether PSR alone is a dominant factor in determining NM-MRI contrast, but should be kept in mind for generalization to all grey matter.

5. Conclusion

Of the qMT parameters examined, PSR was the only one to significantly differentiate PD patients from HC with a modest area under the curve at ROC analysis. An increased PSR, possibly associated with an increased macromolecular content in the SNc was seen in the patients with PD. The particular combination of PSR and T1 values of the SNc likely contribute to determining the contrast seen in NM-MRI scans. Given the small number of subjects examined and lack of biochemical validation in the present study, however, these hypotheses remain to be established. We further demonstrated the feasibility of performing

rapid PSR mapping in human SN in-vivo using the single-point approach with reduced variability, higher CNR, and shorter scan times relative to the full qMT model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CC	Cerebral Crus
GM	Grey Matter
MT	Magnetization Transfer
MTR	MT Ratio
NM	Neuromelanin
PD	Parkinson's disease
PSR	Pool Size Ratio
qMT	quantitative MT
RF	Radio Frequency
RN	Red Nucleus
SNc	Substantia Nigra pars compacta
TSE	Turbo Spin Echo

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Fig. 1.

a) Sagittal T1w image showing the orientation of the axial images passing through the midbrain at the level of the SNc ; b) axial MT-weighted image ($\omega=2$ kHz, $\alpha_{MT}=900^\circ$) (arrows); c) axial NM-MRI showing the SNc (arrows). d-f) Definition of regions of interest (ROIs) for: d) cerebral crus (CC, blue) and substantia nigra (SNc, red) segmented from the NM-MRI images using a threshold-based method; e) red nucleus (RN, yellow) as manually-defined according to hypointensity on the T2 maps; f) grey matter (GM, green) segmented semi-automatically by applying thresholds to the PSR map and extracting the largest connected clusters

Fig. 2.

Representative qMT data from the full-fit acquisition. a) MT-weighted images cropped around the SN. b) ROIs indicating the SNc (red) and the CC (blue), c) their corresponding Z-spectra. The dots correspond to the experimental data while the dashed and solid lines represent the fitted data for $\alpha_{MT}=600^\circ$ and $\alpha_{MT}=900^\circ$, respectively.

Fig. 3.

Example of the parametric maps and the histograms obtained with the full-fit analysis: a-b)

PSR, c-d) k_{mf} , e-f) T_2^f , and g-h) T_2^m . Also shown are the derived quantitative maps and

histograms for: i-j) the ratio T_2^f/T_1^f , and k-l) T_1^{obs} . All histograms are normalized to the total number of voxels, and computed with 200 bins of width: a) 0.0015; b) 0.2440 s⁻¹; c) 0.498

ms; d) 0.0995 μs; e) 0.00023; f) 0.0122 s

Fig. 4.

Examples of NM-MRI (a, d), and PSR maps obtained with the full-fit (b, e), and single-point (c, f) methods in a healthy subject (top), and a patient with PD (bottom). c) The SNc showed intermediate to low PSR (black arrowhead), while areas of grey matter such as those in the mesial temporal lobe exhibited slightly lower PSR values (black arrow). White matter structures were typically characterized by high PSR values, as exemplified by white matter of the temporal lobe (white arrowheads and short white arrows), and the cerebral crus and cortico-spinal tracts (long white arrows)

Fig. 5.

Comparison between full-fit and single-point estimates of PSR. a) Scatter plots with linear regression (dashed line) inclusive of all tissue types and both subject groups. b) Bland–Altman plots (the difference (single-point - full-fit) vs. mean of the two measurements) of the PSR estimates; the mean difference was 0.0015 (solid line), and with limits of agreement $[-0.022, 0.025]$ (mean difference ± 1.96 SD; dotted lines). c) Box plots comparing the full-fit and single-point PSR estimates illustrate the similarity of mean values between techniques and narrow distribution of the values for the single-point estimates.

Fig. 6.

a) The PSR values for the SNc were significantly different between patients with PD and HCs for both the full-fit and single-point strategies. b) In ROC analysis, the single-point strategy yielded a slightly larger AUC relative to the full-fit (0.77 vs 0.75).

Table 1

Mean $\pm$ standard deviation of the quantitative estimates for the HC and PD groups.

ROI	Group	Full-fit				Single-point		Relaxometry	
		PSR	k_{mf} (s ⁻¹)	T_2^f (ms)	T_2^m (μ s)	PSR	MTR	T_1^{obs} (s)	T_2^{obs} (ms)
CC	HC	0.169 $\pm$ 0.016	8.60 $\pm$ 0.85	21.76 $\pm$ 2.43	11.51 $\pm$ 0.65	0.167 $\pm$ 0.012	0.443 $\pm$ 0.015	1.177 $\pm$ 0.126	91.64 $\pm$ 4.52
	PD	0.169 $\pm$ 0.022	9.03 $\pm$ 1.98	22.86 $\pm$ 5.71	11.41 $\pm$ 0.83	0.171 $\pm$ 0.013	0.445 $\pm$ 0.016	1.135 $\pm$ 0.098	90.89 $\pm$ 5.62
SNc	HC	0.116 $\pm$ 0.009	10.05 $\pm$ 1.95	23.03 $\pm$ 2.33	10.36 $\pm$ 0.46	0.119 $\pm$ 0.006	0.390 $\pm$ 0.017	1.283 $\pm$ 0.130	70.49 $\pm$ 2.82
	PD	0.128[*] $\pm$ 0.012	10.23 $\pm$ 2.45	22.32 $\pm$ 1.66	10.38 $\pm$ 1.12	0.127[*] $\pm$ 0.008	0.398 $\pm$ 0.012	1.195 $\pm$ 0.106	66.88[*] $\pm$ 4.06
RN	HC	0.155 $\pm$ 0.028	9.37 $\pm$ 1.42	19.17 $\pm$ 2.64	10.32 $\pm$ 0.78	0.157 $\pm$ 0.022	0.433 $\pm$ 0.019	1.190 $\pm$ 0.190	62.47 $\pm$ 3.62
	PD	0.157 $\pm$ 0.020	9.36 $\pm$ 1.78	18.69 $\pm$ 2.05	10.32 $\pm$ 1.16	0.157 $\pm$ 0.012	0.430 $\pm$ 0.017	1.127 $\pm$ 0.098	59.66 $\pm$ 4.42
GM	HC	0.095 $\pm$ 0.006	11.31 $\pm$ 1.31	25.75 $\pm$ 1.58	9.73 $\pm$ 0.28	0.101 $\pm$ 0.005	0.370 $\pm$ 0.013	1.445 $\pm$ 0.115	83.32 $\pm$ 2.28
	PD	0.099 $\pm$ 0.010	10.54 $\pm$ 1.70	26.62 $\pm$ 2.50	9.85 $\pm$ 0.42	0.101 $\pm$ 0.008	0.368 $\pm$ 0.019	1.405 $\pm$ 0.104	85.72 $\pm$ 2.71

* Significantly different between HC and PD (p<0.05).