In vivo detection of lateral-ventral tier nigral degeneration in Parkinson’s disease

Daniel E Huddleston1,*, Jason Langley2, Jan Sedlacik5, Kai Boelmans4, Stewart A Factor1, and Xiaoping P Hu3

1Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA
2Center for Advanced Neuroimaging, University of California Riverside, Riverside, CA, USA
3Department of Bioengineering, University of California Riverside, Riverside, CA, USA
4Department of Neurology, Julius-Maximilians University, Würzburg, Germany
5Department of Neuroradiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Abstract

Objective—To use a novel region of interest to measure neuromelanin-sensitive MRI contrast changes in the lateral-ventral tier of substantia nigra pars compacta in Parkinson’s disease (PD).

Background—Histopathological studies of PD have demonstrated both massive loss of melanized dopamine neurons and iron accumulation in the substantia nigra pars compacta. Neurodegeneration is most profound in the lateral-ventral tier of this structure. We have previously shown in both healthy controls and individuals with PD that neuromelanin-sensitive MRI and iron-sensitive MRI contrast regions in substantia nigra overlap. This overlap region is located in the lateral-ventral tier.

Experimental Design—Exploiting this area of contrast overlap for region of interest selection, we developed a semi-automated image processing approach to characterize the lateral-ventral tier in MRI data. Here we apply this approach to measure magnetization transfer contrast, which corresponds to local neuromelanin density, in both the lateral-ventral tier and the entire pars compacta in 22 PD patients and 19 controls.

Results—Significant contrast reductions were seen in PD in both the entire pars compacta (p = 0.009) and in its lateral-ventral tier (p = 0.0002); in PD contrast was significantly lower in the lateral-ventral tier than in the entire pars compacta (p = 0.0008).
**Conclusion**—These findings are the first *in vivo* evidence of the selective vulnerability of this nigral subregion in PD, and this approach may be developed for high impact biomarker applications.

**Keywords**
Neuromelanin; MRI; Substantia Nigra; Pars Compacta; Iron; Biomarkers; Humans; Image Processing; Dopaminergic Neurons

**Introduction**

Fearnley and Lees famously identified the lateral-ventral tier of substantia nigra pars compacta (SNc) as selectively vulnerable to the loss of melanized dopamine neurons in PD in 1991 (Fearnley and Lees, 1991). This distribution of neuronal vulnerability in PD is consistent with both prior (German, et al., 1989; Gibb and Lees, 1991; Hassler, 1938) and subsequent (Damier, et al., 1999) pathologic studies. Other histopathology studies found substantia nigra pars reticulata (SNr) rich in iron in controls and SNc as a site of iron accumulation in PD (Morris and Edwardson, 1994; Sian-Hulsmann, et al., 2011; Wypijewska, et al., 2010). Different MRI pulse sequences have been developed that are sensitive either to iron, e.g. T2-weighted MRI sequences, or to neuromelanin (Sasaki, et al., 2008). Neuromelanin-sensitive MRI (NM-MRI) uses magnetization transfer (MT) effects to generate hyperintense signal sensitive to neuromelanin (Chen, et al., 2014). At 3 Tesla this signal colocalizes with areas of neuromelanin containing neurons in radiologic/histologic correlation studies of SNc and locus coeruleus (LC) (Keren, et al., 2015; Kitao, et al., 2013; Lee, et al., 2016). These studies suggest that NM-MRI contrast corresponds to the local density of neuromelanin containing neurons. Neuromelanin sensitive signal is seen in NM-MRI data acquired using pulse sequences with incidental MT effects, such as turbo spin echo (TSE) (Sasaki, et al., 2006), or in MRI pulse sequences with explicit MT preparation pulses (Chen, et al., 2014; Nakane, et al., 2008). However, NM-MRI approaches based on explicit MT contrast (MTC) exhibit high test-retest reproducibility (Langley, et al., 2016a) and also generate more contrast and deposit less energy than incidental approaches (Chen, et al., 2014).

Recent work found iron deposition localized to the lateral-ventral tier of the NM-MRI defined SNc in PD and controls (illustrated in Fig. 1). Importantly, the spatial extent of T2-weighted hypointensity in the NM-MRI defined SNc is significantly increased in PD patients as compared to controls in the lateral-ventral tier (Langley, et al., 2016c). This observation provides the basis for a strategy to select this biologically important region of interest (ROI). Based on this finding in our previous study (Langley, et al., 2016c), here for the first time we report the use of a lateral-ventral SNc common space PD population mask to study PD effects in this selectively vulnerable SNc subregion *in vivo*. An image processing approach with automated ROI selection (Langley, et al., 2016b) is also applied in this work, enhancing the potential for clinical and translational development of candidate biomarkers. The aim of this work is to apply these methods to detect PD neurodegeneration associated changes in MTC in the lateral-ventral SNc. We hypothesize that MTC in the lateral-ventral SNc will be significantly reduced in PD patients as compared to older
controls, and that MTC will be lower in the lateral-ventral SNc than in the entire SNc in PD. In vivo markers of PD pathology in this selectively vulnerable SNc subregion could have high impact clinical and translational applications.

**Materials and Methods**

**Participants**

22 PD patients were recruited from the Emory Movement Disorders Clinic, and 19 control subjects were recruited from the Emory Alzheimer’s Disease Research Center. All research was conducted under a protocol approved by the Emory Institutional Review Board and with informed written consent obtained in accordance with the Declaration of Helsinki. PD diagnosis was established based on the U.K. Brain Bank Criteria (Lees, et al., 2009), and the Unified Parkinson’s Disease Rating Scale Part III (UPDRS-III) motor assessment was performed by a movement disorders neurologist. To minimize motion artifact during scanning and to improve patient comfort, PD patients continued their regular medication regimen without interruption prior to neurological examination and MRI scanning. Cognition was assessed using the Montreal Cognitive Assessment (MOCA), a battery of PD non-motor symptoms was assessed using the Non-motor Symptoms Questionnaire (NMSQ), and REM sleep behavior disorder (RBD) symptoms were assessed using the RBD Symptoms Questionnaire (RBD-SQ) (Chaudhuri, et al., 2006; Stiasny-Kolster, et al., 2007). Clinical characteristics of participants are presented in Table I. Disease duration data was available for 21 of the 22 PD subjects. A separate, previously described cohort of 54 participants with PD (aged 65.4 ± 8.4 years, ON UPDRS-III: 22.7 ± 10.3, disease duration = 12.3 ± 7.3 years) was recruited for MRI scanning at University Medical Center – Eppendorf (UKE) in Eppendorf, Germany (Langley, et al., 2016c). This study was also conducted under a protocol approved by the local ethics board and informed written consent was obtained in accordance with the Declaration of Helsinki.

**MRI Acquisition: NM-MRI Cohort**

The 41 participants at Emory underwent MRI scanning on one of two Siemens Trio 3 T MRI scanners (Siemens Medical Solutions, Malvern, PA, USA) at Emory University with a 12 channel receive-only head coil. NM-MRI data was acquired using a 2D gradient echo pulse sequence with MTC preparation (Chen, et al., 2014; Langley, et al., 2016c). Parameters: echo time = 2.68 ms, repetition time = 337 ms, 15 contiguous slices, slice thickness 3mm, in plane resolution 0.39 × 0.39 mm², 416 × 512 imaging matrix, 162 × 200 mm² field of view, 7 measurements, flip angle = 40°, 470 Hz/pixel bandwidth, and MTC pulses (300°, 1.2 kHz off resonance, 10 ms duration), scan time 16 minutes 17 seconds. Images were also collected using a T₁ magnetization-prepared rapid gradient echo (MP-RAGE) sequence for registration from subject space to common space. Parameters: echo time = 3.02 ms, repetition time = 2600 ms, inversion time = 800 ms, flip angle = 8°, voxel size = 1.0 × 1.0 × 1.0 mm³.

**MRI Acquisition: T₂ Cohort**

The 54 participants with PD at UKE underwent scanning with a 3T scanner (Skyra, Siemens Medical Solutions, Erlangen, Germany) with a 20 channel receive only coil to generate a
lateral-ventral SNC mask. T2-weighted data were acquired using a 3D gradient echo pulse sequence with the following parameters: echo time = 20 ms, repetition time = 50 ms, 56 contiguous slices, 384 × 288 imaging matrix, 229 × 172 mm2 field of view, 1 average, flip angle = 17 degrees, and 50 Hz/pixel bandwidth. Images were also collected using a T1 MP-RAGE sequence for registration from subject space to common space (echo time = 2.46 ms, repetition time = 1900 ms, inversion time = 900 ms, flip angle = 0°, voxel size = 0.94 × 0.94 × 0.94 mm3.

**Entire SNC Mask**

A common space neuromelanin SN population mask was developed in a separate cohort of 11 healthy controls and reported previously (Langley, et al., 2016b). This mask, based on hyperintense NM-MRI contrast in SN, was used to determine the NM-MRI defined (entire) SNC.

**Creation of Lateral-ventral SNC Common Space Mask**

Image processing was done using FMRIB Software Library (FSL; Oxford, UK) version 5.0 and custom scripts in MATLAB version R2015a. First an iron sensitive SN mask was created using the T2-weighted images from the UKE cohort as follows. The iron sensitive SN was defined to be the hypointense region between the red nucleus and cerebral peduncle and segmented by J.L. with a previously reported method (Langley, et al., 2016b; Schwarz, et al., 2013). After segmentation, T2-weighted SN volumes were transformed into MNI space as follows using a method similar to one described previously (Langley, et al., 2016b). A linear transformation from T2 space to T1 space was derived using FMRIB’s Linear Image Registration Tool (FLIRT; 6 degrees of freedom; cost function: correlation ratio) and individual iron sensitive SN masks were transformed from T2 space to the same individual’s T1 image using the derived linear transformation. After the transformation there were no discernable differences in the location of the ventricles in the T1-weighted image with those in the T2-weighted image.

The T2-weighted SN masks in T1 space were then transformed into T1 MNI 152 common space (MNI-152) using FMRIB’s Nonlinear Image Registration Tool (FNIRT). This transformation was derived by first aligning brain extracted T1 images with the MNI-152 brain extracted image using an affine transformation with FLIRT. This was followed by nonlinear transformation with FNIRT (cost function: sum-of-squared differences; degrees of freedom: 12) from individual subject T1 space to MNI-152 space. T2-weighted SN population masks were then generated by averaging the left and right SN volumes across all subjects, and these masks were in turn thresholded at a 0.6 level, meaning that if at least 60% of subjects shared a voxel it was included in the mask. The mask was then binarized.

In order to generate the lateral-ventral SNC mask the region of overlap between the aforementioned entire SNC mask and the T2-weighted SN population mask was selected as follows. The entire SNC mask was binarized in MNI 152 space, and this mask was then multiplied by the binarized T2-weighted SN mask. This overlap region was thresholded at a level of 0.6 and binarized. Fig. 1 shows the overlap regions localized using this process in controls and PD, illustrating that these overlap regions are located in the lateral-ventral SNC.
Langley, et al., 2016c). In order to select the ROI most relevant to PD effects a lateral-ventral SNc PD population mask was generated using T2-weighted MRI data from PD subjects. This lateral-ventral SNc common space mask was used to define the lateral-ventral SNc ROI for analyses in this work.

NM-MRI Image Processing

NM-MRI image processing steps to discard motion degraded measurements prior to offline registration were done using a previously reported method (Chen, et al., 2014). MTC was calculated in MATLAB using the process described in (Langley, et al., 2016a). To measure MTC in the lateral-ventral SNc and in the entire SNc, the ROI masks for these structures were transformed from MNI-152 to individual space using a method analogous to a previously reported approach (Langley, et al., 2016b) as follows. Each participant’s T1-weighted image was first brain extracted and then aligned with the MNI brain extracted image using an affine transformation (FLIRT). Next FNIRT was used to carry out a nonlinear transformation between individual subject T1 space and common space. Then this transformation was inverted and the ROI (lateral-ventral SNc common space mask or entire SNc common space mask) was transformed back to T1 subject space. For each research participant the processed NM-MRI image was registered to the brain extracted T1-weighted image and transformed using FLIRT into T1-space. The transformed lateral-ventral SNc mask and entire SNc mask were then each used to select these ROIs in the NM-MRI data and mean MTC in each ROI was determined for each participant.

Statistical Analysis

Chi-square analysis of categorical variables and analysis of variance (ANOVA) among continuous variables were performed to assess for baseline differences in demographics between groups. Group means for baseline clinical characteristics (UPDRS-III, MOCA, RBD-SQ, NMSQ) were compared using ANOVA. Normality of MTC distributions was confirmed for both the lateral-ventral tier and entire SNc ROIs by Q-Q plots. Group mean MTC values for each ROI were compared controlling for potential confounders (age and race) using analysis of covariance. A paired samples t-test was used to compare MTC in lateral-ventral SNc vs. entire SNc in the PD patients. Statistical analysis was done using IBM SPSS Statistics 24 except for receiver operating characteristic (ROC) analysis which was done using GraphPad Prism 6.0.

Results

PD and control groups were compared for baseline differences as shown in Table I. Race and age were significantly different between groups. As expected, the PD group showed significantly higher levels of motor parkinsonism as assessed by UPDRS-III, REM sleep behavior disorder symptomatology as measured by the RBD Symptoms Questionnaire (RBD-SQ), and also showed a significantly higher burden of a variety of non-motor symptoms as measured by the Non-motor Symptoms Questionnaire (NMSQ). There was no significant difference between groups in cognition as measured by the Montreal Cognitive Assessment (MOCA). Group comparisons of MTC in the entire SNc (as defined by the NM-MRI SNc mask) and lateral-ventral SNc (as defined by the control overlap mask) are
presented in Fig. 2. Significant reductions in MTC were observed in both ROIs in the PD group as compared to controls. In the PD group MTC was significantly lower in the lateral-ventral SNc ROI than in the entire SNc ROI (Fig. 2C). With one tailed Pearson correlation (hypothesizing an inverse relationship) analysis, neither MTC in the entire SNc \( (r = -0.24, p = 0.14) \) nor MTC in the lateral-ventral SNc \( (r = -0.20, p = 0.18) \) showed statistically significant correlation with UPDRS-III score. ROC analysis of MTC in the entire SNc in PD and controls found an area under curve (AUC) of 0.71 \( (SE=0.082 ; 95\% CI: 0.54 \text{ to } 0.87 ; p = 0.025) \) and in the lateral-ventral SNc revealed an AUC of 0.78 \( (SE = 0.072 ; 95\% CI: 0.64 \text{ to } 0.92 ; p = 0.0024) \). The ROC curves are shown in Fig. 3.

**Discussion**

In this study we show *in vivo* evidence of neuromelanin loss (as measured by NM-MRI) specifically in the region of SNc exhibiting iron deposition as measured by T2-weighted MRI. Because this region is localized to the lateral-ventral tier of SNc, the highly significant decrease in contrast in this region in PD patients is consistent with known histopathology in this nigral subregion (Damier, et al., 1999; Fearnley and Lees, 1991; German, et al., 1989; Hassler, 1938). Specifically, the finding that MTC is significantly lower in the lateral-ventral SNc than in the entire SNc in PD indicates that this approach is sensitive to the selective vulnerability of this SNc subregion in PD.

Because the ROI selection approach applied here is based on the overlap between neuromelanin-sensitive and iron-sensitive MRI contrasts it represents a promising new tool to investigate nigral PD biology *in vivo*. SNc neurodegeneration in PD is associated with massive loss of neuromelanin (NM) (Zecca, et al., 2002), and the neurodegeneration-associated release of NM into the extracellular space has been shown to set off a neuroinflammatory cascade, which in turn causes iron accumulation in microglia and neurons (Urrutia, et al., 2013; Urrutia, et al., 2014). Extracellular NM granules activate microglia (Viceconte, et al., 2015; Zecca, et al., 2008) and release toxic iron species. The activated microglia then scavenge NM granules and iron to become laden with iron themselves, leading to local iron accumulation (Viceconte, et al., 2015; Zucca, et al., 2015). Numerous MRI studies using iron-sensitive pulse sequences have found *in vivo* evidence of iron accumulation in substantia nigra in PD (Du, et al., 2012; Langkammer, et al., 2016; Langley, et al., 2016c). A future radiologic/histologic correlation study of post-mortem PD and control brains from individuals monitored longitudinally with a multimodal MRI approach is planned in order to confirm the presence of a PD-related neuroinflammatory response with associated iron accumulation and neuromelanin loss in this selectively vulnerable SNc ROI.

Approximately 50% of melanized dopamine neurons in SNc have been lost by the time of PD diagnosis (Fearnley and Lees, 1991). Therefore, an imaging approach capable of accurately measuring this fundamental feature of PD may be developed as part of a strategy for prodromal detection. Because the lateral-ventral SNc is the most profoundly affected SNc subregion in PD, it is likely damaged early in the disease course during the prodromal period. This is supported by the observation that in asymptomatic patients with incidental Lewy bodies nigral cell loss is confined to the lateral-ventral tier (Fearnley and Lees, 1991).
The prodromal or “pre-motor” period of PD concludes with the emergence of the cardinal motor symptoms of 4–6 Hz rest tremor, rigidity, bradykinesia and postural instability, and clinical diagnosis is based on these features (Lees et al., 2009). The loss of melanized dopamine neurons in the SNc is also believed to be the substrate for these motor symptoms in PD and the basis for their responsiveness to levodopa (Dauer and Przedborski, 2003). The UPDRS-III motor score quantifies directly observed motor parkinsonism, and the lack of correlation between the UPDRS-III score and mean MTC values in either SNc ROI may be due to a non-linear relationship between these measures. A limitation of the current study was the lack of available OFF-medication UPDRS-III scores for the PD group, and future studies will include both OFF- and ON-medication assessment of parkinsonism. However, one of the central issues underlying the need for improved PD biomarkers is that clinical phenotype, including the motor deficits of PD, reflects only later stage aspects of the disease process. Therefore, biomarkers that capture pathology spanning both the prodromal and symptomatic periods are needed. Because half of melanized nigral neurons are lost by the time of clinical diagnosis, it is likely that for a period of years prior to the onset of motor symptoms this NM-MRI approach may detect prodromal changes in PD. This inference also suggests that NM-MRI measures will not have a linear relationship with motor phenotype throughout the disease course. The present results indicate that MTC in the SNc and its lateral-ventral tier are promising candidates for further development for the detection and study of prodromal PD.

Neuroimaging tools are also needed to assist differential diagnosis among PD, atypical parkinsonian syndromes and normal aging effects. The regional selectivity of PD for the lateral-ventral SNc is relatively specific, and differential effects on the nigral subregions are noted in multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and normal aging (Fearnley and Lees, 1991). Aging is associated with preferential neuronal loss in the dorsal SNc. MSA affects the lateral-ventral tier, but is associated with 21% greater cell loss in dorsal SNc than PD. PSP has a greater effect on the medial SNc than the lateral SNc (Fearnley and Lees, 1991). Primarily due to a lack of tools to effectively parcellate SNc, no human imaging studies thus far have measured the regionally selective effects of these conditions within SNc as a potential means to distinguish them. The results of this study support the capability of this approach to detect disease effects in the lateral-ventral tier vs. the entire SNc. Further development of this promising approach is therefore needed to investigate the differential effects of aging and atypical parkinsonism on the nigral subregions in vivo.

Although NM-MRI was first reported in 2006 to detect PD effects in SNc and LC (Sasaki et al., 2006), technical challenges have slowed its widespread translational application. The most prominent challenge posed by the initially reported turbo spin echo (TSE) sequence is its reliance upon incidental MT effects, which are associated with multi-slice imaging (Dixon et al., 1990), to generate NM-MRI contrast. Recently MT was shown to be the basis for NM-MRI contrast (Langley et al., 2015), and an approach using explicit MT effects generates higher MTC in SN and LC than TSE-based approaches (Chen et al., 2014). Furthermore, the use of explicit MTC also allows decreased energy deposition (using a decreased flip angle MT preparation pulse) which prevents scans from exceeding the scanner’s specific absorption rate safety limit. This is a translationally important issue since.
energy deposition from the TSE-based approach results in aborted scans and inconsistent datasets (Chen, et al., 2014; Schwarz, et al., 2011). Thus, these improvements can be leveraged in larger scale investigations of PD biology to measure nigral degeneration in vivo.

The use of a cross-sectional cohort of PD patients with some variability in disease duration and motor deficits likely contributed to variability in SNc MRI measures seen in the PD group. Individual variation in brain structures independent of PD may have contributed to variability in both study groups. We are currently conducting a larger longitudinal study to address these issues and seek to identify sensitive and specific NM-MRI biomarkers for PD. Longitudinal study of PD with this MRI approach will allow assessment of within-subject changes and may identify biomarkers for high impact applications such as monitoring disease progression and response to treatment in neuroprotective therapeutics trials. A future study is also planned in advanced stage PD patients with the aim of imaging lateral-ventral tier degeneration longitudinally until the time of death, followed by correlation of MRI with histology. Because SNc neurodegeneration begins long before symptom onset, application as part of a screening algorithm to identify very early/prodromal PD for subject selection in neuroprotection trials is another high impact goal for this technology.

**Conclusion**

In summary, we identified a region in the lateral-ventral SNc prone to iron accumulation. We transformed this ROI to NM-MRI images and measured MTC in this nigral subregion. We found a reduction of SNc MTC in both the entire structure and specifically in its lateral-ventral tier ROI in PD. Furthermore we found that MTC is significantly lower in the lateral-ventral SNc than in the entire SNc in PD. These results represent robust in vivo detection of lateral-ventral nigral pathology in PD. Because the lateral-ventral tier undergoes profound, early neurodegeneration in PD, lateral-ventral SNc MTC is a promising candidate biomarker for early-stage PD and warrants further development for translational applications.

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Figure 1. NM-MRI / T2-weighted contrast overlap regions in SNc in PD and controls
Group data from recent work (Langley, et al., 2016c) in PD and controls showing the NM-MRI defined SNc (colored regions). The red/yellow colors identify the portion of SNc with T2-weighted hypointensity, indicating the presence of iron. The scale indicates that the probability of overlap between NM-MRI and T2-weighted contrasts approaches 1 in the yellow regions. The extent of T2-weighted hypointensity in SNc is significantly increased in PD as compared to controls, consistent with iron accumulation known to occur in SNc in this disease (Morris and Edwardson, 1994; Sian-Hulsmann, et al., 2011; Wypijewska, et al., 2010).
Figure 2. Comparison of mean NM-MRI MTC in controls (blue) and PD (red) using the entire SNC ROI (A) and the lateral-ventral SNC ROI (B).

Significant disease effects are detected using both the entire SNC ROI (Mean ± SEM: Controls = 0.189 ± 0.00477 ; PD = 0.167 ± 0.00665 ; p = 0.009) and the lateral-ventral SNC ROI (Mean ± SEM: Controls = 0.174 ± 0.00537 ; PD = 0.143 ± 0.00685 ; p = 0.0002). In the PD group MTC is significantly lower (p=0.0008) in the lateral-ventral SNC ROI than in the entire SNC ROI (C). The whiskers on the plots represent the 5th to 95th percentile values for each ROI in each group.
Figure 3. Receiver operating characteristic (ROC) curve for MTC in the entire SNC and in the lateral-ventral SNC in PD (n=22) and controls (n=19)

AUC = area under curve.
Table 1

Clinical characteristics of study groups

<table>
<thead>
<tr>
<th>Group Characteristic</th>
<th>Control</th>
<th>PD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>19</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>% Female</td>
<td>47.4%</td>
<td>40.9%</td>
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</tr>
<tr>
<td>Age</td>
<td>71.3 ± 1.2</td>
<td>60.4 ± 1.8</td>
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<tr>
<td>Race: Caucasian</td>
<td>15 (78.9%)</td>
<td>22 (100%)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>4 (21.1%)</td>
<td>0 (0%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Disease Duration</td>
<td>-</td>
<td>6.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Levodopa Equivalents</td>
<td>-</td>
<td>768.8 ± 90.4</td>
<td></td>
</tr>
<tr>
<td>UPDRS-III ON Medication</td>
<td>3.3 ± 0.7</td>
<td>18.7 ± 2.3</td>
<td>&lt; 10^-6</td>
</tr>
<tr>
<td>MOCA</td>
<td>27.5 ± 0.5</td>
<td>27.1 ± 0.6</td>
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</tr>
<tr>
<td>RBD-SQ</td>
<td>2.3 ± 0.4</td>
<td>4.7 ± 0.6</td>
<td>0.002</td>
</tr>
<tr>
<td>NMSQ</td>
<td>3.4 ± 0.7</td>
<td>10.5 ± 0.9</td>
<td>&lt; 10^-6</td>
</tr>
</tbody>
</table>

Values for age, disease duration, levodopa equivalents, UPDRS-III, MOCA, RBD-SQ, and NMSQ scores are expressed as mean ± standard error. Baseline group differences in age and race were addressed by controlling for these variables in the main analysis.