Multimodal MRI evaluation of parkinsonian limbic pathologies

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ABSTRACT

Previous multimodal magnetic resonance imaging (MRI) studies of parkinsonian syndromes have focused primarily on motor-related basal ganglia structures. The present study investigated MRI changes in nonmotor-related limbic structures in 35 Parkinson's disease, 16 multiple system atrophy parkinsonian subtype, 17 progressive supranuclear palsy, and 37 control subjects. Mean diffusivity (MD), fractional anisotropy, transverse relaxation rate (R2*), quantitative susceptibility mapping, and volume measurements were obtained from the amygdala, hippocampus, and nucleus accumbens (NAc) to examine differences between groups and to test for associations with clinical scores. Compared with controls, Parkinson's disease subjects had lower NAc volume; multiple system atrophy parkinsonian subtype subjects had higher NAc transverse relaxation rate; and progressive supranuclear palsy subjects had higher amygdala and hippocampus MD and lower hippocampus fractional anisotropy ($p\text{'s} \leq 0.008$). Among parkinsonian subjects, amygdala and hippocampus MD associated positively with Unified Parkinson's Disease Rating Scale nonmotor and activities of daily living scores ($p\text{'s} \leq 0.005$). Together, these findings support the inclusion of limbic structures in future MRI studies of parkinsonian syndromes.

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1. Introduction

Parkinson's disease (PD), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP) are parkinsonian syndromes with overlapping motor features. Tremors, rigidity, bradykinesia, and postural instability are signs and symptoms found in all three (McFarland, 2016). Nonmotor symptoms such as mood disturbances and cognitive changes are also prevalent among PD, MSA, and PSP patients (Averbeck et al., 2014; O'Sullivan et al., 2010; Zhang et al., 2017). Magnetic resonance imaging (MRI) has been used to investigate the neural underpinnings of parkinsonian motor and nonmotor symptoms (Pyatigorskaya et al., 2014). Previous multimodal MRI studies, however, have focused primarily on motor-related structures of the basal ganglia (Cochrane and Ebmeier, 2013; Wang et al., 2016). Few have used MRI to examine parkinsonian limbic structure pathologies beyond volume changes in PD (Camicioli et al., 2003; Carriere et al., 2014; Junque et al., 2005).

Postmortem studies have demonstrated that parkinsonian syndrome neuropathologies extend beyond the basal ganglia to affect limbic structures such as the hippocampus, amygdala, and nucleus accumbens (NAc) (Bertrand et al., 2004; Jellinger, 2008; Kalaitzakis et al., 2009; Papp and Lantos, 1994; Piao et al., 2001; Yokota et al., 2010). These structures exhibit characteristic aggregates of α-synuclein in PD and MSA (Bertrand et al., 2004; Kalaitzakis et al., 2009; Papp and Lantos, 1994; Piao et al., 2001) and neurofibrillary tangles of tau in PSP (Jellinger, 2008; Yokota et al., 2010). Diffusion tensor imaging (DTI) and susceptibility MRI have demonstrated possible sensitivities to these and other neuropathologic changes in a manner complementary to structural MRI (Cochrane and Ebmeier, 2013; Wang et al., 2016). In parkinsonian syndrome studies, the DTI measurements of mean diffusivity (MD) and fractional anisotropy (FA) have been used to reflect gray matter changes in microstructural integrity (Cochrane and Ebmeier, 2013); the apparent transverse relaxation rate (R2*) and quantitative susceptibility mapping (QSM) have revealed susceptibility differences reflective of tissue iron accumulation (Du et al., 2016; Wang et al., 2016).

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Previous studies of motor structures have shown further that DTI and susceptibility MRI changes are associated with worsening motor function and increased levodopa-equivalent dosages (Barbagallo et al., 2016; Schwarz et al., 2018). It presently is unknown, however, whether such changes in limbic structures are related to changes in nonmotor functions. Semiquantitative histological assessments of PD, MSA, and PSP patients have shown that anxiety and cognitive impairment are not associated with localized cortical or subcortical proteinopathy (Asi et al., 2014; Jellinger, 2013; Prediger et al., 2012; Wakabayashi and Takahashi, 2004). Moreover, clinical studies have suggested that mood disturbances may be secondary to the stress of chronic disability (Hemmerle et al., 2012; Walsh and Bennett, 2001). This conclusion may be misleading because currently there is a lack of comprehensive in vivo assessments of limbic structure pathologies in parkinsonian patients.

Thus, to address this overall question, the primary hypothesis of the present study was that MD, FA, R2*, and QSM would reveal limbic structure changes among parkinsonian syndromes distinct from volume deviations. The secondary hypothesis was that MRI measurements demonstrating significant limbic structure changes would be associated with nonmotor symptoms.

2. Methods

2.1. Study subjects

This study included 68 parkinsonian subjects and 37 age-matched controls for a total of 105 individuals. Of the parkinsonian subjects, 35 were diagnosed with PD, 16 with MSA (parkinsonian subtype; MSA-P), and 17 with PSP. Parkinsonian subjects were recruited from a tertiary movement disorder clinic, whereas controls were recruited from the spouse population and surrounding community. All subjects belonged to a longitudinal case-control cohort that was established in 2012 as part of the NINDS-sponsored Parkinson's Disease Biomarker Program (PDBP). Parkinsonian syndrome diagnoses were made by a movement disorder specialist according to published criteria (Gilman et al., 2008; Goetz et al., 2007; Litvan et al., 1996). All PD subjects had a history of adequate response to levodopa or other dopaminergic therapies and a history of asymmetrical symptom onset; MSA-P subjects had a history of significant autonomic and/or urinary dysfunction; and PSP subjects had a history of postural instability and vertical gaze palsy (or slowness). Both MSA-P and PSP subjects had a history of inadequate response to levodopa treatment. Diagnoses were confirmed by postmortem pathology in 13 of the 68 parkinsonian syndrome subjects (7 PD, 2 MSA-P, and 4 PSP).

All controls demonstrated a Mini−Mental State Examination score ≥24, were screened for neurological and neuropsychiatric disorders through a study questionnaire, and were cleared of neuropathologic findings upon later review of their MRIs. All subjects or their legal guardians were willing and able to provide written informed consent. Both control and parkinsonian subjects were excluded if they had any condition that would preclude an MRI examination, a history of cerebrovascular disease, and/or renal or liver failure. History of and treatment for mood disorders and/or cognitive impairment were not exclusion criteria because these were features of interest in the present study. In all, 13 subjects (7 PD, 3 MSA-P, and 3 PSP) had present or past history of antidepressant use; 7 subjects (5 PD, 1 MSA-P, and 1 PSP) were on medications for cognitive impairment.

For parkinsonian subjects, disease duration was defined as the time from the date of first diagnosis to study visit date. Disease severity was assessed using Hoehn and Yahr staging (Hoehn and Yahr, 1967). Levodopa equivalent daily dosage (LEDD) was calculated using previously published criteria (Tomlinson et al., 2010). In all subjects, the Montreal Cognitive Assessment (MoCA) was used to evaluate global cognitive status, the Hamilton Anxiety Rating Scale (HAM-A) to assess anxiety levels, and the Hamilton Depression Rating Scale (HAM-D) to evaluate depression levels. All subjects also were administered the Unified Parkinson's Disease Rating Scale Parts I, II, and III (UPDRS-I, UPDRS-II, and UPDRS-III). UPDRS-I assesses nonmotor symptoms experienced by patients, whereas UPDRS-II and UPDRS-III evaluate motor symptoms experienced by patients in activities of daily living (ADL) and motor signs observed by trained personnel, respectively. Neurobehavioral assessments and MRI scans were performed while parkinsonian subjects were in a medication "on" state because only 26 of the 68 parkinsonian subjects were able to undergo examination in a medication "off" state. Collection of all clinical and imaging measurements was approved by the institutional review board at the Pennsylvania State Milton S. Hershey Medical Center.

2.2. MRI image acquisition and postprocessing

Brain MRIs from all study subjects were obtained using a 3T MR imaging system (Magnetom Trio; Siemens, Erlangen, Germany). T1-weighted images were acquired using a magnetization-prepared rapid gradient echo sequence with the following parameters: repetition time (TR)/echo time (TE) = 1540/2.34 ms, field of view (FoV) = 256 × 256 mm, matrix = 256 × 256, slice thickness = 1 mm (no gap), and slice number = 176. T2-weighted images were acquired using a fast spin-echo sequence with TR/TE = 2500/316 ms and the same resolution parameters as the T1-weighted images. DTI parameters were as follows: TR/TE = 8300/82 ms, b-value = 1000 s/mm^2, diffusion gradient directions = 42, 7 b = 0 scans, FoV = 256 × 256 mm, matrix = 128 × 128, slice thickness = 2 mm (no gap), and slice number = 65. T2*-weighted images (for QSM and R2* maps) were acquired using a multiple-gradient-echo sequence: six echoes with TEs ranging from 7 to 47 ms at an equal interval of 8 ms, TR = 54 ms, flip angle = 20°, FoV = 256 × 256 mm, matrix = 256 × 256, slice thickness = 1.5 mm (no gap), and slice number = 64.

DTI images were processed using DTIPrep (NIRAL, UNC-Chapel Hill, North Carolina), where intersection and intervolume correlation analysis, eddy currents, and motion artifact correction were performed for quality control before estimation of MD and FA maps. For R2* and QSM, 6 magnitude images taken from the multiple gradient echo were aligned by affine registration and then averaged to generate a mean magnitude image to correct for potential head motion. R2* maps were generated from a voxel-wise, nonlinear, Levenberg-Marquardt algorithm to fit the monoexponential function $s = s_0 e^{-TE \times R2*}$ using an in-house MATLAB (MathWorks, Natick, Massachusetts) tool. QSM maps were generated using morphology-enabled dipole inversion with a nonlinear formulation (Liu et al., 2012, 2013).

2.3. Region of interest segmentation

Regions of interest (ROIs) for the hippocampus, amygdala, and NAc were defined on a study-specific T1-weighted template. To reduce partial volume effects after automatic segmentation in the lower resolution DTI and susceptibility MRI images, template ROIs were reduced by 1 voxel along structure boundaries. ROIs for each subject were defined from template ROIs using AutoSeg (http://www.nitrc.org/projects/autoseg/) with a warping option from Advanced Normalization Tools software (ANTs; http://stnava.github.io/ANTs) (Fig. 1). DTI and susceptibility MRI images then were registered to individual T2-weighted images using ANTs. The resultant transformations were applied to MD, FA, R2*, and QSM maps using B-spline interpolation to bring maps and segmented ROIs into the same space (Fig. 1). Segmentation results were inspected visually at each slice for misalignments and manually

adjusted by a blinded investigator. MD, FA, R2*, and QSM values were calculated from mean intensities across each ROI. Volume measurements were normalized by dividing by total intracranial volume (sum of gray matter, white matter, and cerebrospinal fluid) to yield a percentage of total intracranial volume measurement. For each modality, right- and left-side values were compared using two-sided *t*-tests to test for possible hemispheric asymmetries. Because there were no hemisphere differences in any ROI (amygdala, hippocampus, or NAc) using any MRI modality (MD, FA, R2*, QSM, or volume), subsequent group comparisons used measurements that were the average of the right- and left-side values.

2.4. Statistical analysis

2.4.1. Group comparisons

Differences in sex frequency were assessed by the chi-square test. Age and disease duration were compared by one-way analysis of variance. Clinical scores (MoCA, HAM-A, HAM-D, and UPDRS) were compared by one-way analyses of covariance (ANCOVA) with adjustments for age and sex. Group differences in MRI measurements (MD, FA, R2*, QSM, volume) for each ROI were assessed by pairwise, one-way ANCOVAs with adjustments for age and sex. Statistical significance was defined by the Bonferroni method as $p < 0.0083$ (0.05/6) to adjust for 6 pairwise comparisons.

2.4.2. MRI associations with clinical scores

We also explored whether MRI measurements showing group differences from controls were associated with MoCA, HAM-A, HAM-D, and UPDRS scores among parkinsonian subjects. In linear models, MoCA, HAM-A, HAM-D, and UPDRS-I, UPDRS-II, and UPDRS-III were explained separately by MRI measurements, age, sex, and disease duration. The strength of the association was determined by the coefficient estimate for each MRI measurement after individually introducing them into an age-, sex-, and disease duration−controlled model. Statistical significance was defined by the Bonferroni method as $p < 0.0021$ (0.05/24) to adjust for the number of tested associations. All statistical analyses were performed using R, version 3.4.2 (R Core Team, Vienna, Austria).

3. Results

3.1. Demographics and clinical data

Table 1 shows the demographic and clinical data for study subjects. As expected, there was no difference in age among groups because this was part of our overall design. There also was no difference in sex among groups, although there were more females than males in the MSA-P group. Therefore, we adjusted for sex in later group comparisons. Disease duration did not differ among the parkinsonian groups. MoCA scores were similar among control, PD, and MSA-P subjects but were significantly lower in PSP subjects. UPDRS-II scores in PD subjects were significantly lower than those in PSP subjects. Control subjects had an average UPDRS-III score of 7.9. This is below a recent population estimate of the average UPDRS-III score for aged adults without parkinsonian syndrome diagnoses (12.5 ± 9.8) (Keezer et al., 2016).

3.2. Group differences in MRI measurements

3.2.1. Comparisons with controls

Mean MRI measurements and group differences are presented in Fig. 2 and Supplemental Table 1. Compared with controls, PD subjects had lower NAc volume ($p = 0.006$); MSA-P subjects had higher NAc R2* values ($p = 0.003$); and PSP subjects had higher amygdala ($p = 0.001$) and hippocampus ($p < 0.001$) MD and lower hippocampus FA ($p = 0.008$) values. Differences from controls that did not survive correction for multiple comparisons were higher amygdala R2* ($p = 0.011$) and hippocampus MD ($p = 0.040$) values in PD subjects; lower NAc volume ($p = 0.009$) and hippocampus FA ($p = 0.019$) values in MSA-P subjects; and higher NAc MD ($p = 0.027$) and hippocampus R2* ($p = 0.035$) values in PSP subjects. QSM measurements were not significantly different in the
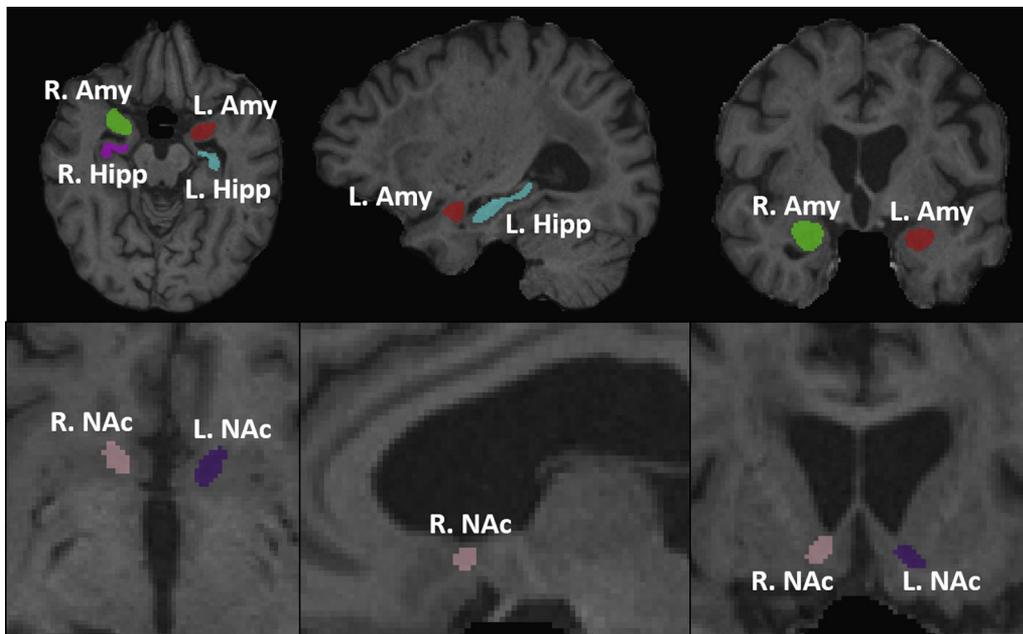

Fig. 1. Representative images of segmentation results. Representative autosegmentation results of the amygdala (Amy), hippocampus (Hipp), and nucleus accumbens (NAc). Axial (left), sagittal (center), and coronal (right) slices illustrating regions of interest of the amygdala and hippocampus (top row) and NAc (bottom row).

amygdala, hippocampus, or NAc in parkinsonian subjects compared with controls ($p > 0.050$).

3.2.2. Comparisons among parkinsonian syndromes

Amygdala ($p = 0.004$) and hippocampus ($p < 0.001$) MD values were higher in PSP subjects than MSA-P subjects. Hippocampus R2* values were higher in MSA-P subjects than in PSP subjects ($p = 0.003$). Group differences that did not survive correction for multiple comparisons were higher hippocampus MD ($p = 0.014$) and lower hippocampus FA ($p = 0.036$) values in PSP subjects than in PD subjects; higher hippocampus R2* values in MSA-P subjects than in PD subjects ($p = 0.018$); higher hippocampus volume ($p = 0.037$), higher NAc R2* ($p = 0.040$), and lower NAc FA ($p = 0.033$) values in MSA-P subjects than in PSP subjects. Among parkinsonian syndrome subjects, there were no differences in QSM measurements in any limbic structure.

3.3. MRI associations with clinical scores

Associations between the 4 MRI measurements (amygdala MD, hippocampus MD, hippocampus FA, and nucleus accumbens R2*) demonstrating group differences from controls and MoCA, HAM-A, HAM-D, and UPDRS scores among parkinsonian syndrome subjects are shown in Table 2. Notably, amygdala MD values were associated positively with UPDRS-I ($\beta = 26.91$, $p = 0.003$) and UPDRS-II ($\beta = 47.63$, $p = 0.002$) scores. Hippocampus MD values also were associated positively with UPDRS-I ($\beta = 11.56$, $p = 0.005$) and UPDRS-II ($\beta = 19.83$, $p = 0.003$) scores. Of these, only the association between amygdala MD and UPDRS-II survived the correction for multiple comparisons. Hippocampus FA and NAc R2* values did not demonstrate associations approaching significance with any clinical score.

4. Discussion

This is the first study to focus on limbic structure pathologies in parkinsonian syndrome subjects using multimodal MRI. The

findings reveal that PD, MSA-P, and PSP subjects have distinct patterns of DTI and R2* changes in the hippocampus, amygdala, and NAc that may reflect underlying differences in disease neuropathology that are not apparent in volume estimations. The associations between amygdala and hippocampus DTI measurements and UPDRS-I and UPDRS-II scores suggest that these measurements in particular are relevant to parkinsonian symptoms. Together, these results support the inclusion of limbic structures in future MRI-based research on parkinsonian syndrome pathologies.

4.1. Parkinsonian limbic structure MRI findings

4.1.1. PD MRI findings

Previous studies of hippocampal volume in PD have yielded mixed findings. Camicioli et al. (2003) reported lower volumes in PD patients that were associated with cognitive impairment, whereas Tanner et al. (2017) found volume loss to be associated with disease duration. On the other hand, in combined volume and DTI assessments of the hippocampus in PD, both Carlesimo et al. (2012) and Yao et al. (2016) found no volume changes, but higher MD. The results from the present study are in agreement with the latter findings, suggesting that microstructural alterations are discernable by MD when macrostructural differences are not present.

The lower NAc volume we found in PD subjects matches the previous finding of NAc atrophy by Carriere et al. (2014). It is known that PD patients exhibit higher frequencies of impulse control disorders (ICDs) (Averbeck et al., 2014). It is unclear, however, whether ICD behaviors in PD, such as compulsive eating, compulsive shopping, hypersexuality, and pathologic gambling, are attributable entirely to dopaminergic medication effects (to which they are strongly associated) or perhaps due to pathologic involvement of limbic structures such as the NAc (Weintraub et al., 2010). The trend for higher amygdala R2* values in PD is interesting for the same reason, as both the NAc and amygdala are known to mediate functions related to ICDs such as motivation processing, emotion processing, and reward-seeking behaviors (Baxter and Murray, 2002; Ikemoto and Panksepp, 1999; Stuber et al., 2011). The present study, however, did not incorporate an adequate measure of ICDs to suggest that lower NAc volume and higher amygdala R2* values were reflective of underlying neuropathologic substrates for Parkinson's ICDs. In follow-up analyses, NAc volume and amygdala R2* were not associated with dopamine dysregulation syndrome subscores of the UPDRS-III after controlling for sex, age, and disease duration. Dopamine dysregulation syndrome subscores also were not associated with LEDD or with NAc volume and amygdala R2* after controlling for LEDD. Future histologic and MRI studies may examine NAc and amygdala changes in relation to more comprehensive measures of ICDs to test whether associations between pathology and behavior exist.

4.1.2. MSA-P MRI findings

MSA-P subjects exhibited both higher R2* values and lower volumes in the NAc. Beyond the postmortem findings by Spokes et al. (1979) of NAc dopamine depletion, pathologic involvement of the NAc in MSA has not been reported previously. Indeed, Papp and Lantos (1994) found the NAc to be spared of the oligodendroglial inclusion characteristics of MSA in a comprehensive assessment of brain structures. The results here are intriguing because MSA patients are not known to exhibit the impulsive-compulsive behaviors seen in PD and PSP (Averbeck et al., 2014; O'Sullivan et al., 2010). Although ICDs may not be prevalent in MSA, symptoms such as depression, anxiety, and apathy are frequent (Ceponiene et al., 2016). It has not been determined yet

Table 1
Demographics and clinical scores

	Controls	PD	MSA-P	PSP	p
No. of subjects	37	35	16	17	-
Sex, female/male[a]	14/23	11/24	10/6	4/13	0.100
Age[b]	70.4 ± 7.8	71.0 ± 7.4	66.4 ± 8.2	72.5 ± 9.7	0.152
Disease duration[b]	-	3.4 ± 3.6	4.1 ± 3.3	3.3 ± 2.9	0.745
LEDD (mg/d)[c]	-	706.5 ± 426.0	752.3 ± 279.4	558.8 ± 282.1	0.264
H & Y[c]	0.4 ± 1.0	2.5 ± 1.2	3.4 ± 1.1	3.2 ± 1.0	<0.001
MoCA[c]	25.0 ± 2.4	22.5 ± 4.2	23.9 ± 2.6	19.8 ± 4.7	<0.001
HAM-A[c]	4.5 ± 4.6	9.0 ± 6.5	11.1 ± 8.9	8.4 ± 5.7	0.001
HAM-D[c]	2.6 ± 3.1	6.7 ± 5.0	8.1 ± 7.5	5.2 ± 5.0	<0.001
UPDRS-I[c]	4.1 ± 4.5	11.8 ± 8.2	11.9 ± 8.4	10.8 ± 6.7	<0.001
UPDRS-II[c]	1.4 ± 4.3	14.9 ± 13.3	22.6 ± 13.5	26.0 ± 12.7	<0.001
UPDRS-III[c]	7.9 ± 13.2	37.3 ± 27.3	50.5 ± 19.1	41.8 ± 26.5	<0.001

Data represent sums or mean ± standard deviation.
Key: LEDD, levodopa equivalent daily dose; MoCA, Montreal Cognitive Assessment; MSA-P, multiple system atrophy (parkinsonian type); HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; H & Y, Hoehn and Yahr stage; PD, Parkinson's disease; PSP, progressive supranuclear palsy; UPDRS-I, II, III, Unified Parkinson's Disease Rating Scale parts I, II, and III.

[a] Group differences using the chi-square test.
[b] Group differences using one-way analysis of variance.
[c] Group differences using one-way analysis of covariance with adjustments for age and sex.
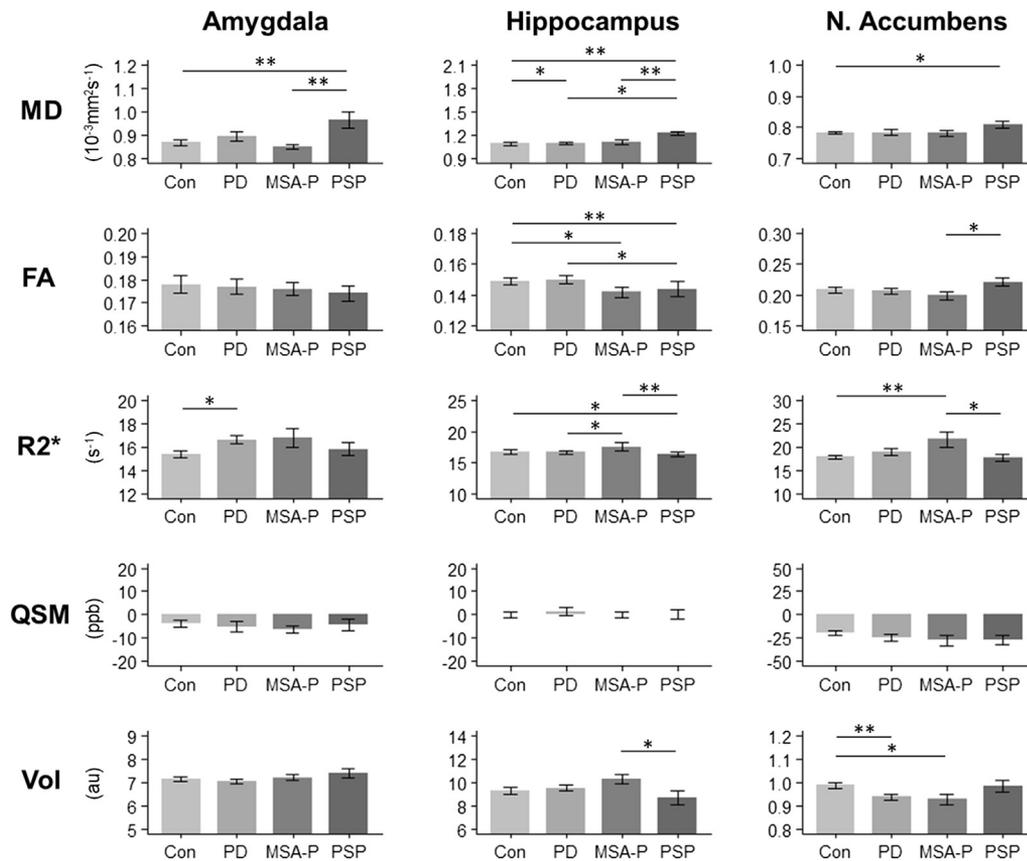
Fig. 2. MRI measurements by limbic structure. Group comparisons of MD, FA, R2*, QSM, and volume in the amygdala, hippocampus, and NAc. Bars represent mean ± standard deviation. * indicates $p < 0.05$. ** indicates significance after Bonferroni correction at $p < 0.0083$. Abbreviations: au, arbitrary unit; Con, controls; FA, fractional anisotropy; MD, mean diffusivity; MSA-P, multiple system atrophy (parkinsonian type); N. Accumbens, nucleus accumbens; PD, Parkinson's disease; ppb, parts per billion; PSP, progressive supranuclear palsy; QSM, quantitative susceptibility mapping; R2*, transverse relaxation rate; Vol, volume.

whether NAc changes in MSA are related to such neuropsychiatric presentations.

Amygdala R2* values also were higher in MSA-P patients, although the difference was nonsignificant. Nevertheless, the finding may reflect actual pathology because a recent postmortem study of 35 MSA brains revealed that neuronal inclusions were present in all sampled amygdalae (Cykowski et al., 2015). Moreover, higher amygdala R2* values may be related to autonomic dysfunction in MSA (Gilman et al., 2008). The central nucleus of the

Table 2
Associations between MRI measurements and clinical scores among all subjects

Associations	Amy MD		Hipp MD		Hipp FA		NAc R2*	
	β[a]	p[b]	β	p	β × 10	p	β	p
MoCA	−6.30	0.182	−2.41	0.254	6.22	0.086	0.15	0.147
HAM-A	8.41	0.277	1.83	0.597	−6.71	0.261	−0.22	0.203
HAM-D	7.47	0.231	1.56	0.577	−6.37	0.185	−0.13	0.362
UPDRS-I	26.91	0.003	11.56	0.005	−12.26	0.094	−0.43	0.039
UPDRS-II	47.63	**0.002**	19.83	0.003	−30.32	0.012	−0.26	0.468
UPDRS-III	61.98	0.034	29.78	0.022	−39.48	0.083	−0.05	0.941

Bold indicates significance after Bonferroni correction at $p < 0.0021$.
Key: Amy, amygdala; FA, fractional anisotropy; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; Hipp, hippocampus; MD, mean diffusivity; MoCA, Montreal Cognitive Assessment; NAc, nucleus accumbens; UPDRS-I, II, III, Unified Parkinson's Disease Rating Scale parts I, II, and III; R2*, transverse relaxation rate.
[a] MRI measurement coefficient estimates in age- and sex-controlled linear models.
[b] Significance of the coefficient in age- and sex-controlled linear models.

amygdala is known to modulate the autonomic nervous system through the hypothalamus (Ressler, 2010). Whether pathology in the amygdala contributes to autonomic dysfunction in MSA is unknown and needs to be investigated further.

4.1.3. PSP MRI findings

Prior DTI studies of gray matter structure changes in PSP have reported higher MD and lower FA values in the striatum compared with controls (Piattella et al., 2015). In the present study, we demonstrated that similar changes also occur in the amygdala and hippocampus. The hippocampal MD and FA findings in particular may be related to memory impairments known to occur in PSP (Litvan et al., 1989). Consistent with this hypothesis, PSP subjects in our cohort demonstrated significantly lower MoCA scores compared with PD and MSA subjects despite comparable disease durations.

4.1.4. Possible proteinopathy-related sensitivities of DTI and R2*

Few studies have examined possible associations between protein pathology, and tissue and cellular changes in limbic structures of parkinsonian subjects. Using MRI, our study detected proteinopathy-related differences that future neuropathology comparisons may examine. DTI measurements were more sensitive to changes in PSP, a tauopathy, whereas R2* was more sensitive to changes in PD and MSA-P, both synucleinopathies. This pattern suggests that DTI measurements, particularly MD, may better reflect microstructural changes in tauopathies, whereas R2* may better reflect changes in α-synucleinopathies.

It is also noteworthy that limbic structure differences found in R2* were not seen in QSM. We believe that this is not due to a lack of QSM sensitivity, as significant differences in the substantia nigra were found between PD and control subjects using a similar cohort (Du et al., 2018). As a cleaner measurement of susceptibility, QSM is a more selective measure of iron (Wang and Liu, 2014). Therefore, the R2* changes observed here may reflect microstructural changes more so than differences in iron deposition. We observed a similar result in our recent MRI-neuropathology correlation study of the substantia nigra, where R2* was associated strongly with α-synuclein burden but not with Perls' stain for iron (Lewis et al., 2018). Because R2* captures both susceptibility and the transverse relaxation rate, it is possible that measurements unrelated to iron content are influenced strongly by local cell structure properties (Wang and Liu, 2014).

4.2. Clinical relevance of MRI findings in limbic structures

In the present study, the respective positive and negative associations of hippocampus MD values with nonmotor scores (UPDRS-I) were expected because it is known that the hippocampus is involved in nonmotor cognitive functions such as memory and learning. As Albouy et al. (2013) previously reviewed, studies also have shown that the hippocampus may interact with the striatum in the acquisition and consolidation of motor-related memory. The positive association of hippocampus MD values with ADL (UPDRS-II) scores is consistent with a possible hippocampal role in motor function.

The positive association of amygdala MD values with nonmotor scores fits the current understanding of the nonmotor functions of the amygdala in mediating the processing of emotions (Baxter and Murray, 2002). The association of amygdala MD values with ADL scores suggests that the amygdala also may have an effect on movement. A possible pathway by which this may occur is through regulation of mood. Anxiety and depression are known to influence ADL mobility, motor symptoms, and quality of life among parkinsonian syndromes (Kuhn et al., 1996).

Alternatively, these associations may be explained by overall disease progression. The relationships between MRI measurements and UPDRS-I, UPDRS-II, and UPDRS-III scores may be due to parallel processes of nonmotor and motor deterioration, and not clinical manifestations of limbic pathologies. Nonetheless, the present study suggests that limbic contributions to overall disease phenotype are worth exploring. Future studies may employ a more comprehensive battery of neuropsychiatric tests to align limbic pathologies with specific behavioral deficits.

4.3. Limitations and future directions

There were limitations in the present study that present opportunities for future investigation. First, of the 68 parkinsonian subjects included in the study, 13 had pathology-confirmed diagnoses. Future studies of limbic structures from this study cohort will update analyses with additional postmortem confirmations. Second, groups were imbalanced in both number and gender proportions, a limitation that was addressed by adjusting for age and gender in our analyses. Third, segmentation of small structures, such as the NAc, continues to be challenged by technological limitations. Accurate delineation of the hypothalamus, which we ideally would have included here, is presently limited in 3T MRI (Makris et al., 2013). Future developments in image acquisition and processing capabilities will improve on analyses of these small structures. Finally, future studies would benefit from additional neuropsychiatric measurements, such as those assessed by the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's

Disease–Rating Scale (QUIP-RS) (Weintraub et al., 2012). QUIP-RS scores would allow future studies to test directly whether NAc R2* and volume changes are reflective of impulsive-compulsive behaviors.

4.4. Conclusions

This study of PD, MSA-P, and PSP subjects demonstrates that DTI and R2* reveal distinct pathologies in the amygdala, hippocampus, and NAc. These findings support the inclusion of limbic structures in future MRI-based research efforts. As DTI and R2* are not capable of describing exact microstructural changes, future neuropathology studies may examine the MRI differences observed here to enhance our understanding of parkinsonian disease pathology and progression.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.12.019.

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