The Role of the Substantia Nigra in predicting longitudinal non-motor changes in Parkinson's Disease

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Adelaide Medical School

The University of Adelaide

By Benjamin Ellul

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Abstract

Parkinson's Disease (PD) can be characterised by non-motor symptoms resulting from loss of dopamine found in the substantia nigra (SN). Declines in SN's innervation throughout basal ganglia mediates the non-motor symptoms of PD through its feedforward output to the cortex, yet the relationship between SN volume loss and subsequent prediction of baseline non-motor symptoms and change over time hasn't been established. Our study aims to address these changes as predicted by baseline SN volume at baseline. SN Volumes were gathered by manually tracing the nuclei of interest on T2 MRI images and were compared to cognitive/mood data made available through the Parkinson's Progressive Markers Initiative (PPMI). Within a PPMI subset, greater SN Volume loss was present at baseline in PD patients compared to controls. Cognitive performance within the PPMI subset showed no significant deficits in the PD cohort compared to controls and showed no significant deficits between groups over time. Mood dysfunction in the PPMI subset was significantly higher in the PD cohort than in controls at baseline and this difference continued over time. Linear regressions that controlled for covariables of age, gender and education were run and showed that the SN Volume at baseline wasn't a significant predictor of either longitudinal cognitive decline or mood dysfunction. Future work will continue to assess this predictive nature of SN volume as this would allow earlier identification for those who are risk of cognitive/mood dysfunction, allowing non-therapeutic interventions to alleviate these symptoms and improve overall quality of life for PD patients.

Introduction

Parkinson's Disease (PD) is a neurodegenerative disease that currently affects around 1 out of 350 Australians[1]. With life expectancy continuing to rise, the prevalence of PD is expected to double by 2030[2]. PD is characterised by cardinal motor symptoms (tremor, rigidity, akinesia or bradykinesia and postural instability), the onset of which are necessary for PD diagnosis[3,4] , but PD also manifests in a number of non-motor symptoms, including cognitive decline and mood dysfunctions, which have been consistently reported amongst previous literature[5-7.] In PD, cognitive decline is shown through reduced memory performance and executive functioning ability, which have been demonstrated to worsen over time[8]. Additionally, neuropsychiatric symptoms known as mood dysfunctions have been reported in PD, with approximately 30-40% of PD patients having significant depressive symptoms[9,10] as well as 40% of PD patients showing high rates of anxiety[11,12]. These non-motor symptoms of PD have demonstrated to have important consequences for quality of life and daily functioning, as studies have associated the prevalence of these symptoms with increased carer burden and increased risk of admission into care homes[13].

PD is characterised pathologically by the death of dopaminergic cells (neurons that release the neurotransmitter dopamine (DA)) found within the dorsal region of the substantia nigra, known as the substantia nigra pars compacta (SNc), a nucleus within the brainstem which plays a critical role in the modulation of motor movement[14,15] . The reduction of dopaminergic output from the SNc affects action selection and inhibition of the basal ganglia, a group of subcortical nuclei responsible primarily for motor control and decision making[16]. The basal ganglia is comprised of two pathways, a direct and indirect pathway responsible for the promotion and inhibition of motor activity respectively[17]. In the direct pathway, the dopaminergic projections from the SNc promote the activation of the striatum, in turn, inhibiting activation of the globus pallidus internal (GPi) and substantia nigra pars reticula (SNr). This decreased inhibitory output from the GPi/SNr feeds forward and promotes activation of the thalamus, which promotes cortical activation[15,17].

Conversely, in the indirect pathway, activation of the striatum feeds forwards to the globus pallidus external (GPe) and then to the subthalamic nucleus (STN). Striatal activation in the indirect pathway inhibits the external GPe, and this causes disinhibition of the STN due to inhibitory output form the GPe to STN. As a result, the STN promotes the release of glutamate on the GPi/SNr, driving increased activation of these nuclei, decreasing both thalamic and cortical activation, inhibiting motor movement [16,17]. In the indirect pathway, striatal output is impacted directly by the DA input from the SNc and can be seen in Figure 1. Information from the basal ganglia is relayed from the output nuclei, GPi/SNr, to various cortical areas, including the primary motor cortex and the prefrontal cortex (PFC)[18].

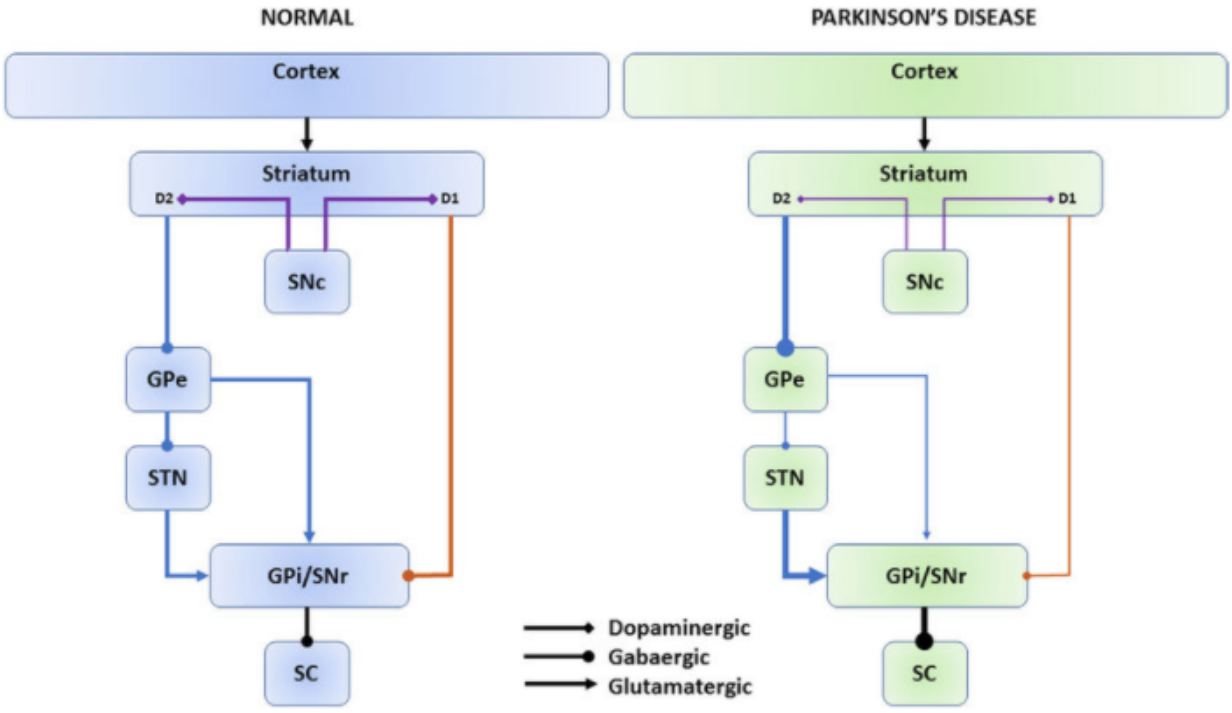

Figure 1: A schematic diagram showing the circuitry of the basal ganglia and how the indirect pathway is affected in PD. SC, superior colliculus; thickness of the arrows refers to strength of firing rate[19].

As PD is pathologically characterised by a loss of DA in the SNc, dopaminergic depletion leads to reduced inhibitory direct pathway output, and increased excitatory indirect pathway output onto the GPi/SNr, resulting in greater thalamic and cortical inhibition (Figure 1)[19]. This DA loss in PD can also affect the cognitive and mood symptoms due the feedforward connections from the basal ganglia to the cortex and limbic system[20]. Specifically, in studies that assessed the cortical co-activation on functional neuroimaging, specific cortical regions were displayed to have received feedforward communication from the basal ganglia, including primary and supplementary motor cortices, the dorso-lateral prefrontal cortex (PFC) (decision making), the amygdala (emotional regulation) and the hippocampus (memory)[18]. Loss of input to these specific cortical areas highlight the prevalence of the non-motor symptoms of PD, namely the PFC for the cognitive decline and amygdala for the mood dysfunctions that have been reported in prior literature[5-12]. As the basal ganglia is mediated via DA input, this loss of DA in PD could explain the onset of these non-motor symptoms.

As evident from prior literature, DA input facilitates the activation of cortical areas responsible for cognitive functioning[21,22]. Anatomically, the PFC contains a large number of DA receptors and is highly sensitive to DA binding[23]. Due to the anatomical distribution of the brainstem DA projection towards the PFC as well as the GABAergic input from the dorso-lateral striatum within the basal ganglia, this provides a basis for DA's influence on working memory and executive decision making, explaining how decreased DA levels in PD may be associated with decreased memory performance[23,24] and demonstrates that normal levels of DA appear necessary for optimal cognitive performance[24-27].

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Previous findings have also highlighted that dopamine has implications for the regulation of mood [28,29]. Studies have shown that DA loss is a factor of mood dysfunctions seen in individuals[30-32]. In studies of depression and anxiety, PET imaging studies have shown significantly lower DA

transporter binding and striatal activation compared with healthy subjects[27]. Moreover, a PET study demonstrated that PD patients who showed increased levels of anxiety and depression after receiving deep brain stimulation, a surgical treatment for PD, expressed a greater cortico-limbic dopaminergic denervation than patients who do not[33]. Anatomically, the amygdala is highly innervated by DA projections for activation regulation, as well as convergent fibres from the ventral striatum, which could explain why loss of DA in PD is accompanied by mood dysregulation and overall dysfunction[20,34]. These findings highlight the importance of monitoring DA levels in PD patients, as these levels may be indicative of decline in cognitive and increase of mood dysfunctions seen in PD.

Currently, assessing the levels of DA is difficult in vivo, which, in turn, makes predicting the onset of the non-motor symptoms of PD rather challenging. Although PET imaging of dopaminergic markers has improved in recent years[35] it is prohibitively expensive, not widely available and requires the use of invasive radioactive tracers[36]. Thus, it has been suggested that SN Volume, as measured by T2 MRI, could be a proxy marker of DA loss, as with greater signal loss in the SN, indicating volume loss, which in turn indicates loss of DA neurons.

To date, the relationship between the volume of the SN and cognitive and mood symptoms of PD, particularly how predictive baseline SN volume is of the change over time in these non-motor symptoms, has not been established. Establishing this relationship may aid in the strengthening the prognosis of the non-motor symptoms in PD and their trajectory from a single, baseline measurement.

Thus, this study aims to assess whether baseline measures of SN volume, cognitive function and mood differ between the PD cohort and those in the control group and investigate the trajectory of change in cognition/mood over a 5-year period. It is hypothesised that cognition function and mood

will be negatively over time as a result of volume loss in the SN, predicted through DA loss in the SN, measured via SN volume. Understanding this relationship is significant as it could lead towards the improvements in predicting the trajectory of these non-motor symptoms in PD, allowing earlier identification for those who are risk of cognitive/mood dysfunction, allowing non-therapeutic interventions to alleviate these symptoms and improve overall quality of life for PD patients.

Methodology

Data Extraction

Participants for the current study were drawn from the Parkinson's Progressive Markers Initiative (PPMI) database[37] and consisted of 69 PD patients and 34 controls who at the time of PD diagnosis (or recruitment for the control cohort) underwent baseline T2 MRI scans. Participants in the control cohort were all above the age of 30 years on and had no first degree relative with PD[37]. Collection of the data was originally conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines and was approved by the local ethics committees of each of the participating sites. For the current word, access to this data was provided through application[37]. The PPMI database contains data for a number of cognitive performance and mood measures, which were taken at baseline (time of diagnosis or recruitment), as well as annually for a 5-year period. The specific cognitive and mood measures are displayed in Table 1.

Table 1: Cognitive and Mood Measures and their specific domains assessed

Name of Measure	Aspect of cognition/mood measured
Hopkins Verbal Learning Test (**HVLT)**	Assessment of verbal learning and memory (recognition and recall)[38]
Letter Number Sequencing (**LNSPD)**	Assess incremental demand of working memory[39]
Sematic Fluency **(SFT)**	Determine sematic fluency by measuring production of words to fit a given category[40]
Geriatric Depression Scale **(GDS)**	Assessment of depressive symptoms in an older adult population
State Trait Anxiety Index **(STAI)**	Describes trait levels of Anxiety

For the analysis of the cognitive/mood data, the scores from the three cognitive tests that the participants completed annually were summed into a singular score that captured the overall cognitive performance of the participants at each time point. This was done by converting the test scores from each cognitive test to z-scores and these z-scores were averaged to form a single 'cognitive beta-score'. The use of this combined cognitive score was a good representative of general cognitive performance. A systematic review in 2012 reported that testing overall performance is enhanced by utilizing measures that assess attention span, recognition and recall and verbal fluency, and these aspects were of cognition were tested between the HVLT, LNSPD and SFT measures[41]. In parallel with the cognitive data, mood test results were also converted to z-scores and averaged, to form a 'mood beta-score'. The use of this combined mood score gives a good overall measurement of neuropsychiatric function, as previous DA-mediated findings have been linked to the prevalence of depression and anxiety in individuals, which have been assessed through the GDS and STAI[42].

Mapping the volumes of Brainstem Nuclei

For all PPMI subjects, a non-contrast enhanced T2 weighted brain MRI using at least 1.5 Tesla scanner and a non-contrast enhanced 3D volumetric T1 weighted brain MRI were performed at baseline. T1 weighted images were acquired with the MPRAGE sequence with parameters including: slice thickness of <1.5mm with no interslice gap, voxel size = 1 x 1 x 1.2mm^3 and acquired matrix size = 256 x 256 x 170-200 [37]. Using an imaging program *'FSLeyes'*, blank overlay masks were created on top of these T2 weighted MRI scans. After selecting the most appropriate horizontal slice and contrast and brightness of the brainstem that optimised the view of the nuclei, masks were edited by manually tracing the SN, the Red Nucleus (RN), as well as the overall midbrain itself, and these tracings were filled into the masks (*Figure 2*). The SN, RN and midbrain were chosen for mapping as this would allow a series of nuclei ratios to be created, namely SN:RN and SN:midbrain. As opposed to using the SN signal on its own, these ratios allowed for individual

differences between each participant to be accounted for. In addition, the RN was chosen for mapping as the A10 dopaminergic projections from the RN are spared in PD[43-46] , even though the RN physically neighbours the SN.

A total of 619 subjects (423 PD and 196 healthy controls) are included in the baseline PPMI dataset[37]. However, for the current analysis, MRI scans from a subset of 103 particpiants (69 patients and 34 controls) were selected for analysis. This subset was chosen as all participants had been scanned on a 1.5 tesla MPRAGE scanner.

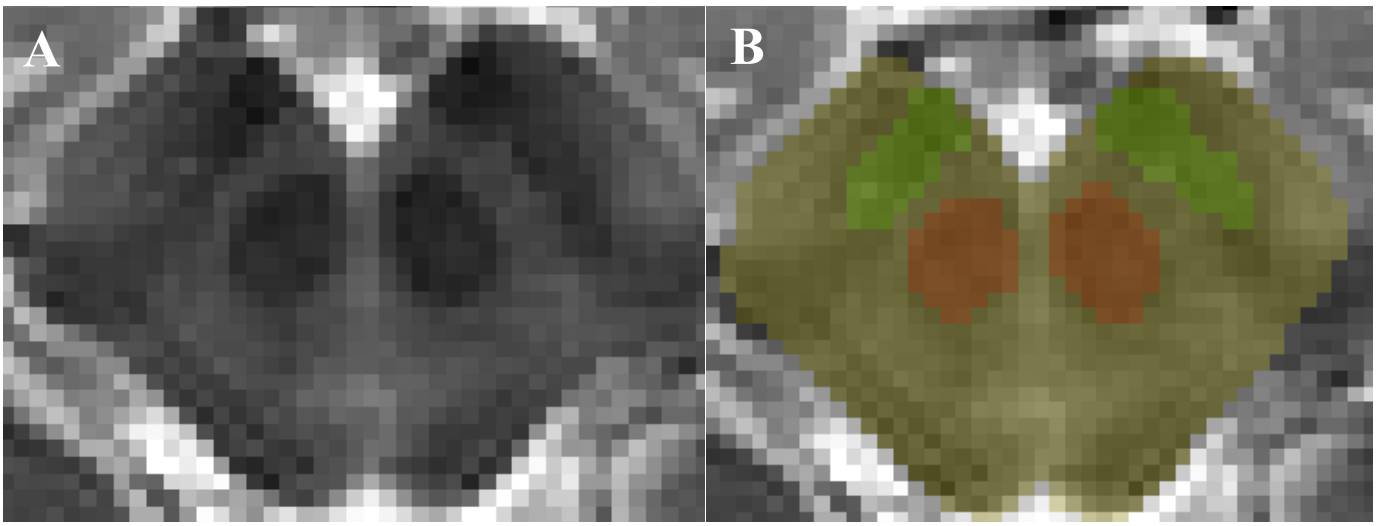

Figure 2: Manual mask creating mapping of the brainstem's nuclei.
A. T2 weighted MRI of the brainstem **B**. The same T2 MRI with the following regions mapped: midbrain *(yellow),* red nuclei *(red)* and the substantia nigra *(green).*

Mapping Criterion

Due to the nature of manual tracing of the midbrain nuclei, these measurements can be quite subjective across different raters. In order to maximise the reliability of the traced masks, the masks were created individually by two raters and were compared. The inter-rater reliability was calculated and, in cases of extreme variance between the raters (where voxel difference > 30), specific cases were re-mapped independently and corrected (SN:midbrain - $r = 0.712$, SN:RN - $r = 0.435$. As the correlation coefficient of the SN:midbrain ratio was much stronger than the SN:RN

ratio's coefficient, all quantitative analysis of the data was restricted to the use of the SN:midbrain ratio.

Quantitative Analysis

Given the high correlation between the SN:midbrain data, scores were averaged between the raters. To analyse the baseline SN volume loss between the two groups, a Welch two samples t-test was conducted. To investigate the cognitive and mood trajectories, multiple regression models were run to test whether group membership predicted baseline cognitive function and mood, and the rate of change seen over a 5-year time period. To assess the predictive nature of SN volume on cognitive/mood performance, linear regressions were executed to analyse how influential the SN volume is in predicting cognitive/mood outcomes. All quantitative analyses were conducted using *'RStudio'*.

Results

PPMI Data Demographics: Cognitive and Mood Dataset

Demographic information for both PD patients and healthy controls for the entire PPMI database is reported in Table 2. The groups were similar with regards to age, gender and years of education (table 2).

Table 2: Subject demographics of PD patients and healthy controls

	PD patients		Healthy Controls			
	N	**Mean ± SD**	**N**	**Mean ± SD**	**t-Test (df)**	***P***
Age (years)	423	(61.66 ± 10.17)	196	(60.82 ± 10.19)	-0.91(335)	.364
Education (years)	423	(15.60 ± 3.05)	196	(16.04 ± 3.05)	1.92(389)	.055
Gender	423		196		0.29 (377)	0.772
Males	146	23.60%	70	35.71%		
Females	277	44.75%	126	64.29%		

Note: Within the PPMI Database, gender was coded as "1 = Male' and "2 = Female" after being refined from three gender codes.

PPMI Imaging Subset Demographics: Cognitive and Mood Dataset

Demographic information for both PD patients and healthy controls within the subset of PPMI participants used for the imaging subset is reported in Table 3. The groups were similar with regards to age, gender and rates of education (table 3).

Table 3: Subject demographics of PD patients and healthy controls within the Subset

	PD patients		**Healthy Controls**			
	N	**Mean ± SD**	**N**	**Mean ± SD**	**t-Test (df)**	***P***
Age (years)	69	(61.42 ± 9.47)	34	(59.65± 11.56)	-0.77 (55)	0.443
Education (years)	69	(16.45 ± 2.21)	34	(16.76 ± 2.47)	0.63 (59)	0.53
Gender	103		103		-0.24 (66)	0.81
Males	45	65.22%	23	67.65%		
Females	24	34.78%	11	32.35%		

Note: Within the PPMI Database, gender was coded as "1 = Male' and "2 = Female" after being refined from three gender codes.

Cognitive and Mood Trajectory within the Full Dataset

Within the full PPMI dataset, cognitive performance was significantly lower in PD patients than in controls at baseline (*t(1,359) = -4.151, p < 0.001)* and significantly decreased over time after controlling for age, gender and education *(coefficient = -0.278, t(4, 551) = -3.381, p < 0.001)* (*figure 3).*

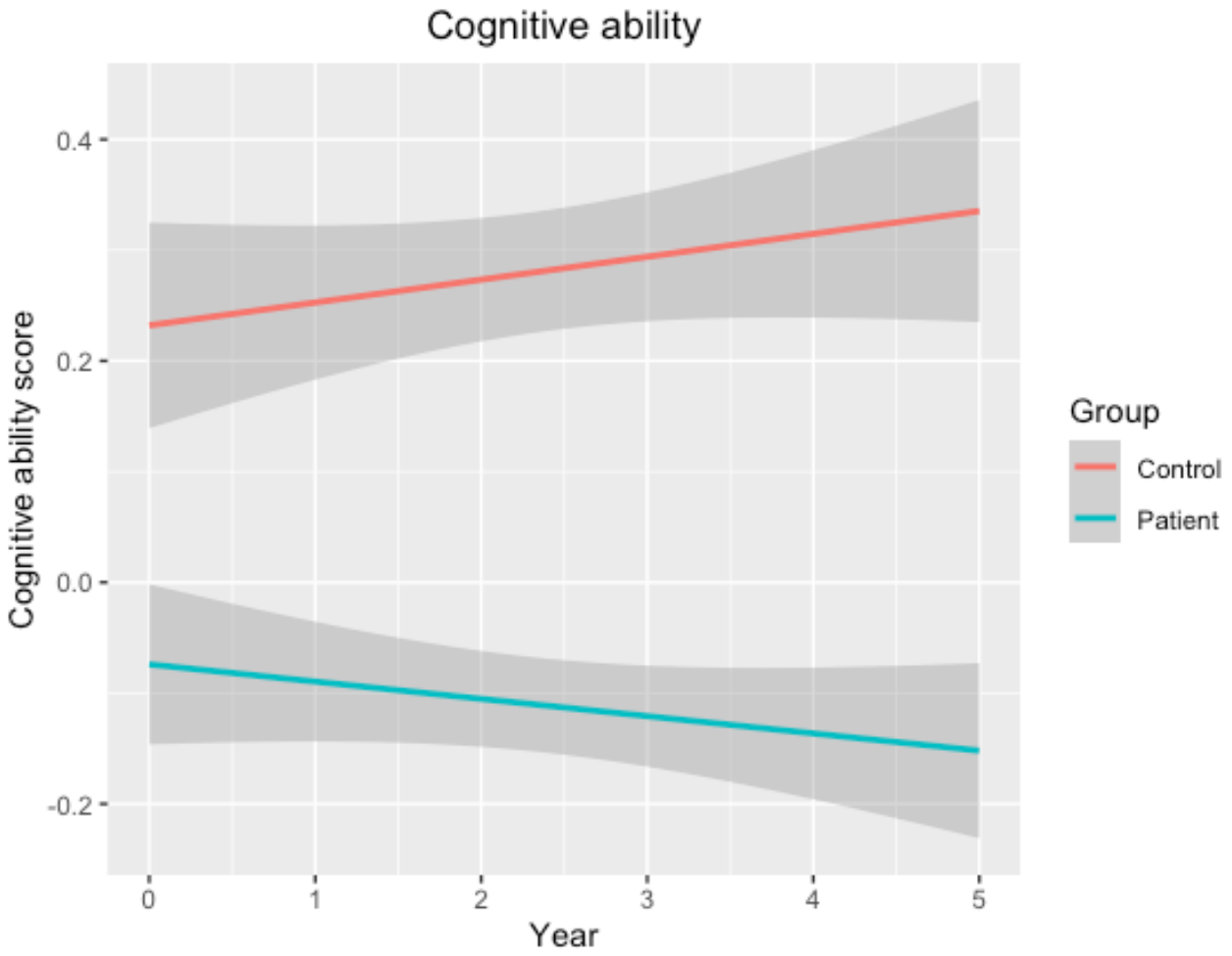

Figure 3: Cognitive performance in PD was **lower** than that of controls at baseline (*p < 0.001*) and decreased at a faster rate in the patient group compared to controls (*p<0.001)*.

Within the full dataset, mood dysfunction was significantly higher in PD than in controls at baseline *(t(1,425) = 6.267, p < 0.001*) and increased over time at significantly faster rate in PD compared to controls after controlling for age, gender and education *(coefficient = .489, t(4, 553) = 5.537, p < 0.001)* (*figure 4).*

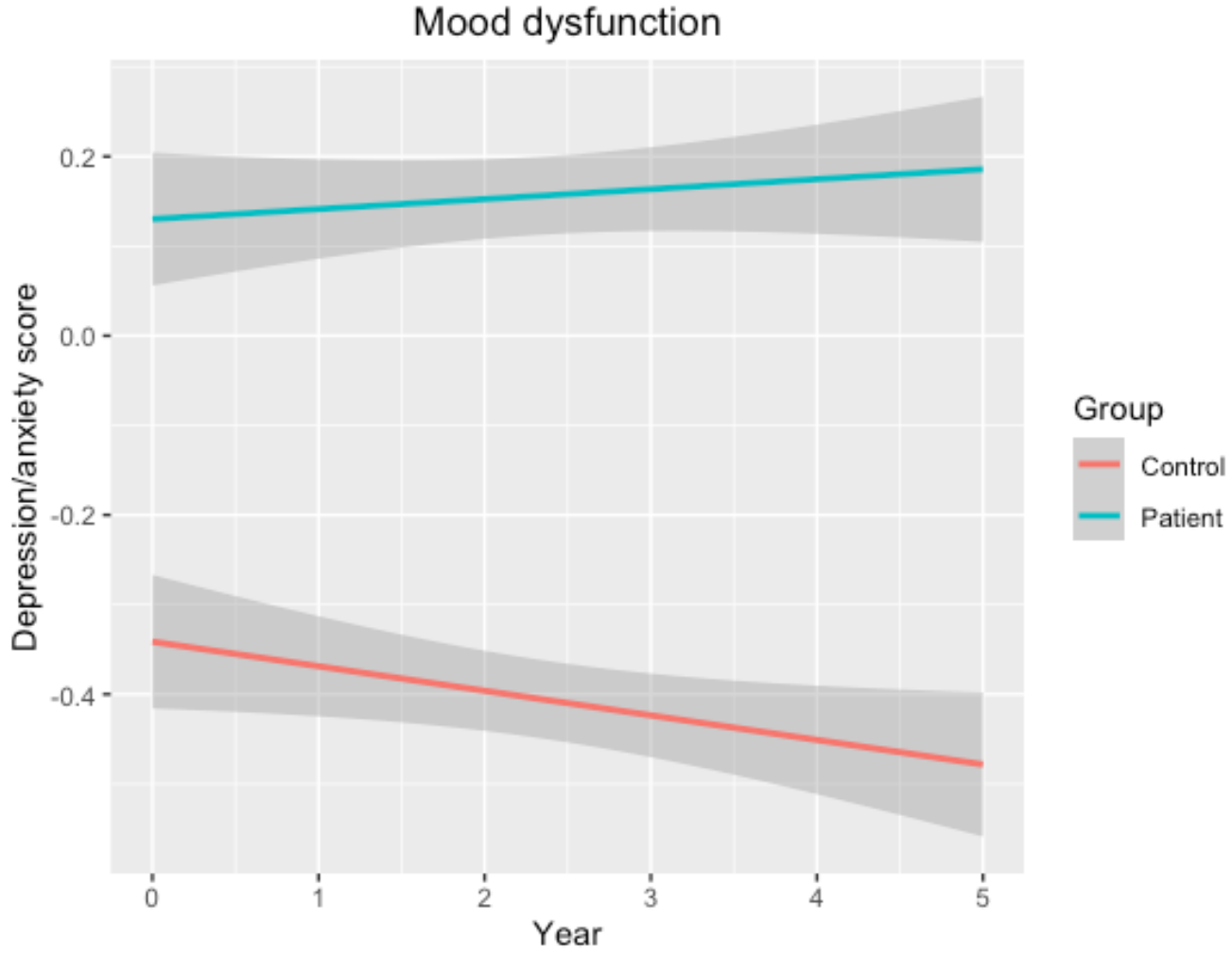

Figure 4: Mood dysfunction in PD was **greater** than that of controls at baseline *(p < 0.001*) and continued to increase a faster rate in PD compared to controls (*p < 0.001)*

Cognitive and Mood Trajectory for Imaging Subset

Given that MRI scans were only available on a restricted portion of the sample, the above analyses were repeated within the 103 participants that had both MRI scans and cognitive/mood data. Within this sample, the SN:Midbrain ratio was significantly higher in PD patients than in controls *[(t) = -6.05, $p < 0.001$]*, indicating greater MRI signal loss, and, in turn, greater SN volume loss, in the PD group (*figure 5).*

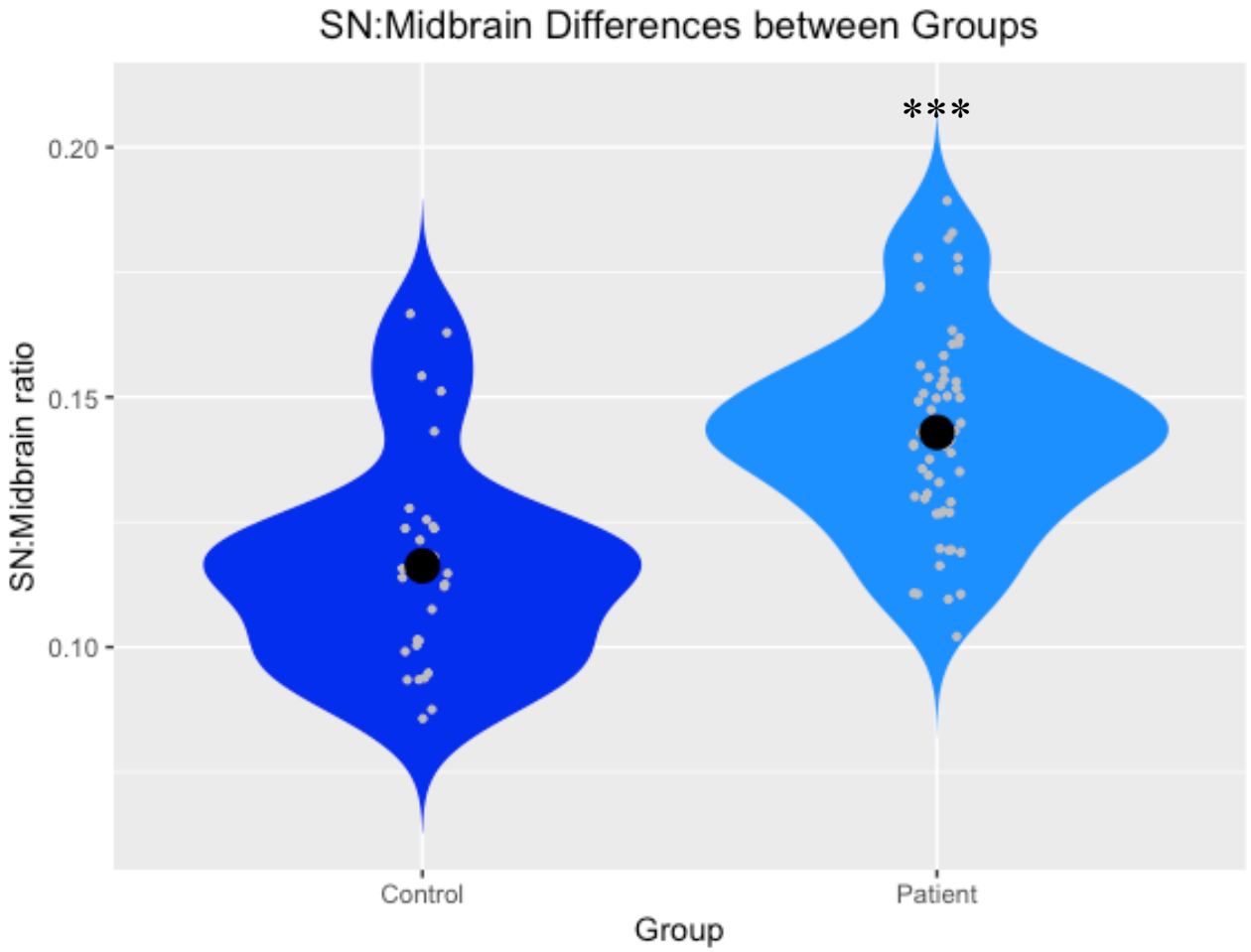

Figure 5: SN/midbrain ratio comparison between groups. The SN/midbrain ratio of the Control group (dark blue) was significantly **lower** than the patient group (light blue) (*$p < 0.001$).*

Within the imaging sample, cognitive performance did not significanty differ between PD patients and controls (t(1,53) = 1.09; p = 0.27) at baseline. Similarly, change in cognitive function over the 5-year period did not differ in PD patients compared to healthy controls after controlling for age, gender and education *(coefficient = 0.065, t(4, 84) = 1.56, p = 0.33) (figure 6)*.

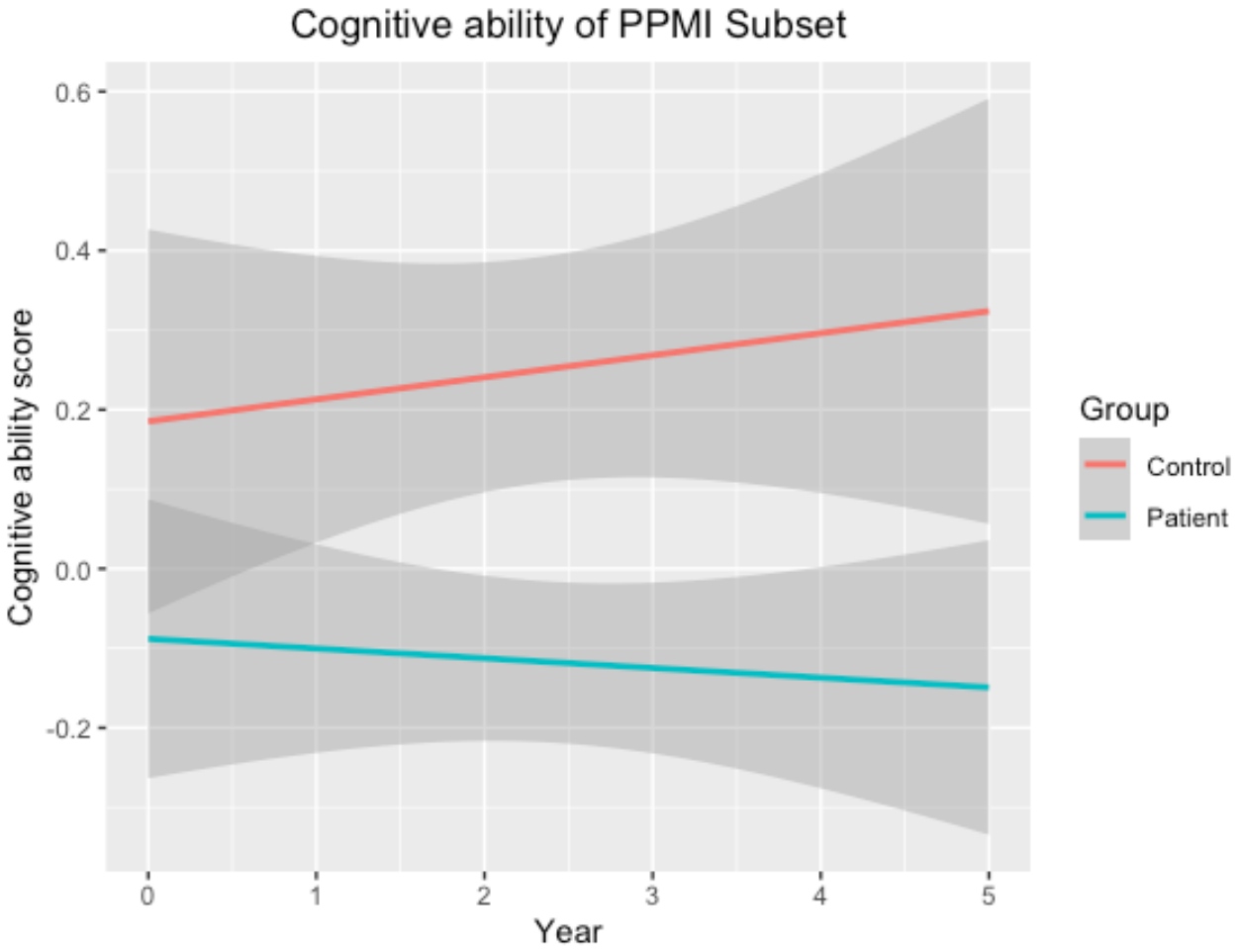

Figure 6: Cognitive performance in PD showed no statistical difference to that in the control group at baseline *(p = 0.27*) and did not differ significantly over time in PD patients compared to controls (*p = 0.33)*.

In the subset of participants who had MRIs, mood dysfunction was significantly higher in PD than in healthy controls at baseline (t (1,81) = -2.21, p = 0.029). Over time, the rate of change in mood dysfunction continued to be significantly greater in PD patients than in healthy controls after controlling for age, gender and education (*coefficient = 0.069, t(4, 84) = -0.776, p = 0.28) (figure 7).*

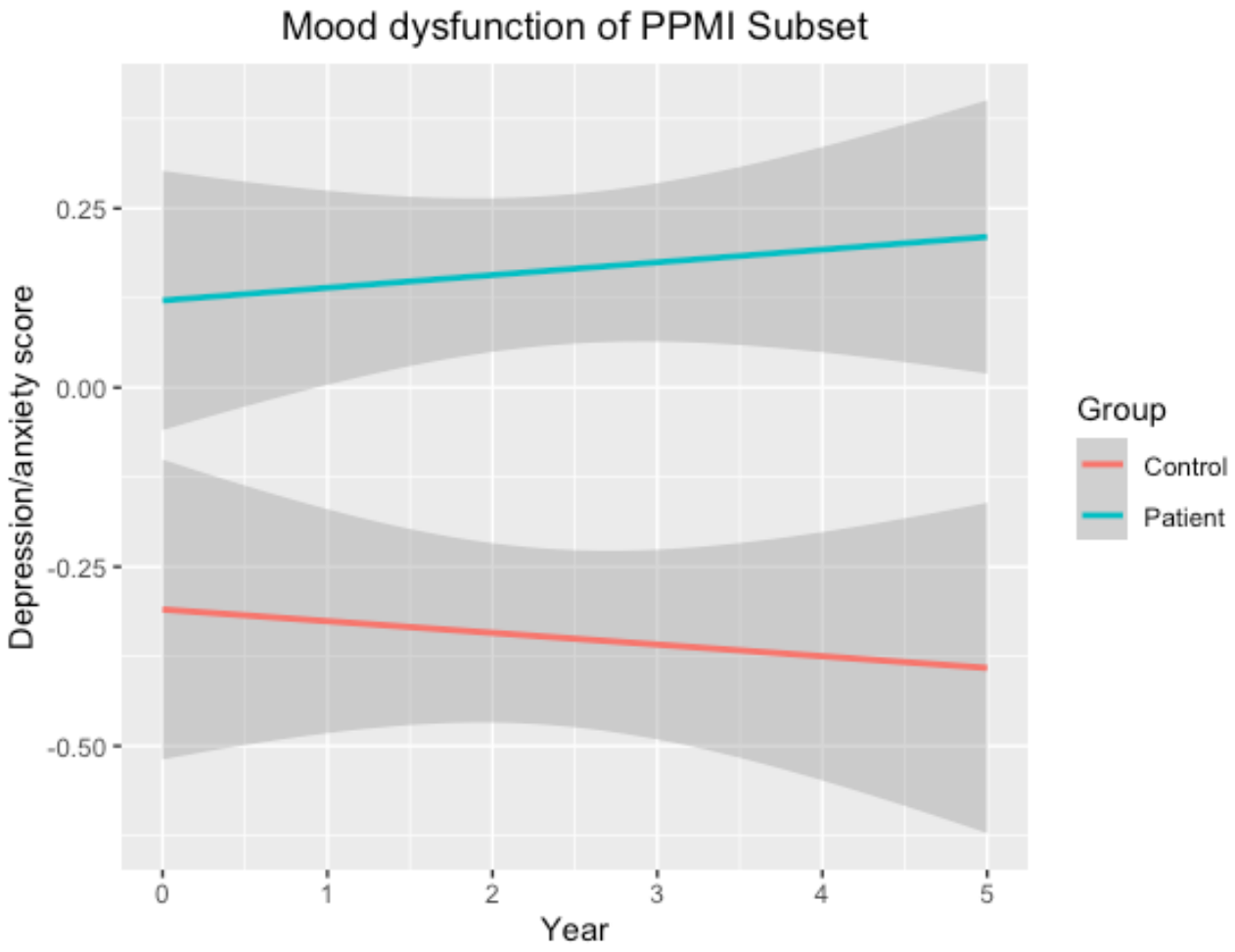

Figure 7: Mood dysfunction in PD was **greater** than that of controls at baseline *(p = 0.029*) but did not differ over time in PD patients compared to controls (*p = 0.28).*

Ability of SN volume to predict baseline cognitive performance/mood dysfunction and change over time

The regression coefficients for the ability of SN:midbrain to predict baseline cognitive performance/mood dysfunction, as well as change over time, are reported in the tables below, along with relevant covariates. Table 4 assessed baseline measurement, whereas table 5 utilised the 'beta score'.

Table 4: Baseline regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes.

	N	R^2	F Statistic (df)	Slope Coefficient	T Statistic	P -Value
Baseline Cognitive Performance	**90**	**0.19**	**5.637 (5,90)**			**<0.001**
Age				-0.02	-2.43	*
Gender				0.48	2.85	**
Education				0.11	3.12	**
Diagnosis				-0.30	-1.47	/
SN:Midbrain				4.59	1.08	/
Baseline Mood Dysfunction	**90**	**0.045**	**1.903 (5,90)**			**0.10**
Age				-0.015	-1.58	/
Gender				0.04	0.25	/
Education				-0.03	-0.92	/
Diagnosis				0.30	1.29	/
SN:Midbrain				3.51	0.72	/

Note: */ = 'no significance between groups', "*" 0.05, "**" 0.01.*

Table 5: Longitudinal regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes.

	N	R^2	F Statistic (df)	Slope Coefficient	T Statistic	P -Value
Beta Cognitive Performance	**84**	**0.0095**	**1.171 (5,84)**			**0.33**
Age				-0.018	-2.07	*
Gender				0.12	0.66	/
Education				0.017	0.45	/
Diagnosis				0.18	0.86	/
SN:Midbrain				-6.76	-1.56	/
Beta Mood Dysfunction	**84**	**0.014**	**1.259 (5,84)**			**0.29**
Age				0.011	1.32	/
Gender				-0.011	-0.07	/
Education				-0.0067	-0.19	/
Diagnosis				0.36	1.85	/
SN:Midbrain				-3.079	-0.78	/

Note: */ = 'no significance between groups', "*" 0.05,*

Results of the multiple linear regressions indicated that at baseline, there was a collective significant effect between the covariables and cognitive performance *($F(5,90) = 5.637$, $R^2 = .19$, $p < .001$),* however, there was no significant effect on mood dysfunction *($p = 0.1$).* The individual predictors of baseline cognition were examined further and indicated that age (*$t = -2.43$, $p = .05$),* gender *($t=2.85$, $p = .01$)* and education *($t=3.12$, $p = .01$)* were significant predictors in this model. Results of the multiple linear regressions of the change in scores over time indicated that there was no collective significant effect between the covariables and cognitive performance *($p = .33$)* and mood dysfunction *($p = .29$).* Examining the individual predictors of beta cognition further, it was

indicated that age (*t = -2.07, p = 0.05)* was the only significant predictor in this model. Apart from this, no other variables were significant predictors of change in cognition or mood over time.

In the above regression models, the whole dataset was used, with diagnosis included as a covariate. In order to probe the effect of diagnosis on the predictive value of the SN:midbrain ratio, a linear regression was also run in just the PD group, with age, gender and years of education included as covariates (Tables 6 and 7). These regressions were additionally run in just the control cohort (Tables 8 and 9). Full tables of all regression model output in the specific cohorts can be found in the appendix.

Results of the multiple linear regressions in the PD group indicated that at baseline, there was a collective significant effect between the covariables and cognitive performance *(F(4,59) = 9.316, R^2 = .35, p <.001),* however, there was no significant on mood dysfunction *(p = .93).* The individual predictors of baseline cognition were examined further and indicated that age (*t = -3.79, p =.001),* gender *(t=2.33, p = .05)* and the SN:midbrain ratio *(t= 2.41, p = .05)* were significant predictors in this model. Results of the multiple linear regressions of the change in scores over time in PD indicated that there was no collective significant effect between the covariables and cognitive performance *(p =.088)* and mood dysfunction *(p = .73).* Examining the individual predictors of beta cognition further, it was indicated that age (*t = -2.22, p = 0.05)* was the only significant predictor in this model. Apart from this, no other variables were significant predictors of change in cognition or mood over time.

Results of the multiple linear regressions in the control group indicated that at baseline, there was a collective significant effect between the covariables and mood dysfunction *(F(4,27) = 7.639, R^2 = .46, p <.001),* however, there was no significant on cognitive performance *(p = .327).* The individual predictors of baseline mood dysfunction were examined further and indicated that education (*t = -3.09, p =.01)* and the SN:midbrain ratio *(t= 3.06, p = .01)* were significant

predictors in this model. Results of the multiple linear regressions of the change in scores over time in the control group indicated that there was no collective significant effect between the covariables and cognitive performance *(p =.15)* and mood dysfunction *(p = .68).* In these models no variables were significant predictors of change in cognition or mood over time in the control group.

Discussion

The study aimed to investigate the predictive nature of the SN volume on the longitudinal non-motor symptoms of PD, assessed by highlighting difference in the non-motor symptoms between the PD group and healthy controls and investigated if these differences could be predicted by SN volumes. In the overall PPMI dataset, cognitive performance was lower in PD than in controls and rates of mood dysfunction in PD were greater than the control and results remain similar with change over time. Within the PPMI subset, baseline cognitive performance showed no difference between groups, however baseline mood dysfunctions were greater in PD compared to control. Over time, cognitive performance and mood dysfunction within the PPMI subset showed no significant differences. After executing regression models, the SN volume was only a significant predictor of baseline cognition in the PD group and baseline mood dysfunction in the control group. Beyond this, the SN volume overall was not a significant predictor of the longitudinal non-motor symptoms of PD.

In the PPMI subset, cognitive performance did not differ between the two groups at baseline and over time. These results were not hypothesized and are inconsistent with literature, which demonstrated significant cognitive decline in PD compared to controls[47,48]. This discrepancy may be accounted for by the small number of subjects in the subset. The results showed that among the total PPMI cohort, cognition differs between groups, however, the subset didn't reflect this, suggesting this subset wasn't a good representation of the overall data. Future work may wish to try and capture this representation in a better way. Literature shows that visuospatial and executive functioning aspects of cognition are more likely to show changes in PD[49] and deficit in memory and language aren't often reported[50]. This could be probed further in future by utilizing tests that assess these specific domains of cognition and ultimately, the utilisation of a single cognition score wasn't ideal and may explain why we saw these results.

The PPMI subset also demonstrated baseline differences in mood dysfunction between PD and control groups. Higher mood dysfunctions in PD than in controls has been reported throughout literature and our results are consistent with these findings[51]. According to the Braak hypothesis, there is a caudal to rostral spread of DA loss pathology from the brainstem to the cortex in early PD[52]. This suggests that pathology within brainstem nuclei, such as the amygdala (responsible for emotional regulation) is more prevalent in early PD than that of areas responsible for cognition (pre-frontal cortex). This provides explanation to why we saw baseline differences in mood, but not in cognition in the PPMI subset. Furthermore, previous findings have highlight that the prevalence of depressive symptoms in PD is significantly greater than all other psychiatric disorders[53]. Therefore, the use of the single mood score scores may not have been a good representation over overall mood dysfunction, and future work may need to be investigate levels of depression independently to better capture the mood dysfunctions of PD.

The SN/midbrain ratio of the control group was significantly lower than the patient group. This greater ratio in the PD group indicates greater signal loss of DA on MRI, and in turn, reflects greater loss in SN volume. These results validate this methodology of assessing SN integrity as a means of comparing differences between PD patients and healthy controls, however, future work could further strength the capturing of SN volume with the use of correlating volumes to DaTScans, single-photon emission computed tomography (SPECT) imaging technique that helps visualize dopamine transporter levels in the brain[54], whose data was available through the PPMI.

As opposed to what was hypothesised, after running the linear models for both baseline and longitudinal cognitive/mood data in the PPMI subset, SN:midbrain was not a significant predictor. Additionally, besides for predicting baseline cognitive performance, there was no significant evidence showing that the overall linear models significantly predicted the outcomes through the

influence of the covariables. Though the SN:midbrain was not a significant predictor of cognitive and mood performance, it was the most influential covariable at baseline and longitudinally.

All four regression models displayed adjusted R^2 values that showed that less than 20% of the variance (at best) in cognitive/mood data in all cases could be explained by the covariables, indicating that the models didn't sufficiently fit the data. This could be improved by increasing to sample size of the subset or through controlling for specific covariables. Linear regressions models were refined and repeated to only account for those each cohort respectively within the PPMI subset.

Looking at the cognitive regressions, SN:midbrain ratio was a significant predictor of baseline cognition, but not of the change over time only within the PD cohort only and not of the controls. This indicates that real time SN volumes (as they were taken at baseline) were able to predict the cognitive performance in PD patients at the time of their cognitive testing. This highlights a weakness to this study, as the MRIs were only taken at baseline, unlike the cognitive tests which were executed annually. The baseline SN:midbrain ratio wasn't predictive of the change in cognition over time and may need to be further investigated with annual MRIs to assess how SN volumes changed with respect to changes in cognition. Focusing on the mood regressions of the control group only, SN:midbrain ratio was a significant predictor of baseline mood dysfunction, in the control group. However, this was not the same within the PD group. This suggests that the SN:midbrain ratio was not a good predictor of mood dysfunctions in PD at baseline, nor the change over time.

Our results suggest that the changes in cognition and mood could better explained by other factors. Literature has stated that the time-of-day appears to affect cognitive performance of older patients who have suffered a neurological defect (minor stroke)[55] and has also found that age is the biggest

risk for cognitive decline and difficulty with mood regulation[56]. These findings can be seen throughout our results which show that the age was the most significant predictor of these non-motor changes over time compared to all other covariables, including the SN:midbrain ratio. This highlights a number of factors besides SN volumes that may better predict cognitive performance and mood dysfunction and future work may seek to assess their predictive nature of the change in cognition and mood over time.

As highlighted throughout, non-motor symptoms are highly prevalent in PD and have demonstrated to have a number of factors that decrease the overall quality of life of PD patients. It is of continuing importance to predict the onset and trajectory of these non-motor symptoms of PD and our results show that the volume of SN may have selective benefits in predicting the cognitive performance and mood dysfunction at baseline, future work will continue to be predict this trajectory.

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Appendix

Table 6: Baseline regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes in the PD Group

	N	R^2	F Statistic (df)	Slope Coefficient	T statistic	P -Value
Baseline Cognitive Performance	**59**	**0.35**	**9.316 (4,59)**			**< 0.001**
Age				-0.04	-3.79	***
Gender				0.5	2.33	*
Education				0.056	1.23	/
SN:Midbrain				13.29	2.41	*
Baseline Mood Dysfunction	**59**	**0.05**	**0.217 (4,59)**			**0.93**
Age				-0.012	-0.77	/
Gender				0.052	0.19	/
Education				0.023	0.39	/
SN:Midbrain				-2.7	-0.39	/

Note: */ = 'no significance between groups', "*" 0.05, "***" 0.001*

Table 7: Longitudinal regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes in the PD group.

	N	R^2	F Statistic (df)	Slope Coefficient	T Statistic	P -Value
Beta Cognitive Performance	**55**	**0.071**	**2.136 (4,55)**			**0.088**
Age				-0.03	-2.22	*
Gender				0.077	-0.29	/
Education				-0.111	-1.81	/
SN:Midbrain				-3.427	-0.53	/
Beta Mood Dysfunction	**55**	**0.03**	**0.496 (4,55)**			**0.73**
Age				0.018	1.21	/
Gender				-0.008	-0.029	/
Education				-0.019	-0.28	/
SN:Midbrain				-2.70	-0.38	/

Note: */ = 'no significance between groups', "*" 0.05*

Table 8: Baseline regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes in the Control Group

	N	R^2	F Statistic (df)	Slope Coefficient	T statistic	P -Value
Baseline Cognitive Performance	**27**	**0.028**	**1.229 (4,27)**			**0.327**
Age				-0.012	-0.62	/
Gender				0.47	1.21	/
Education				0.16	1.97	/
SN:Midbrain				-2.41	-0.26	/
Baseline Mood Dysfunction	**27**	**0.46**	**7.639 (4,27)**			**<0.001**
Age				-0.006	-0.44	/
Gender				0.094	0.32	/
Education				-0.18	-3.09	**
SN:Midbrain				21.5	3.06	**

Note: */ = 'no significance between groups', "**" 0.01.*

Table 9: Longitudinal regression coefficients and their accompanying statistical test results for the proposed covariables on cognitive and mood outcomes in the Control group.

	N	R^2	F Statistic (df)	Slope Coefficient	T Statistic	P -Value
Beta Cognitive Performance	**25**	**0.10**	**1.811 (4,25)**			**0.15**
Age				-0.038	-1.96	/
Gender				0.56	1.46	/
Education				0.18	2.23	/
SN:Midbrain				-10.5	-1.14	/
Beta Mood Dysfunction	**25**	**0.06**	**0.57 (4,25)**			**0.68**
Age				0.0012	0.066	/
Gender				0.11	0.28	/
Education				0.047	0.58	/
SN:Midbrain				-10.7	-1.14	/

Note: */ = 'no significance between groups'*