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Structural integrity of midbrain nuclei in tremor-dominant and non-tremor Parkinson's disease

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Abstract

Background

The reason for clinical variability between tremor-dominant and non-tremor Parkinson's Disease (PD) patients is still unclear. Post-mortem evidence suggests that some of this variability may be explained by differences in neurodegeneration patterns in the substantia nigra (SN) and retro-rubral area (RRA). The aim of this study is to in vivo relate patterns of neurodegeneration in the SN and RRA to PD subgroups and resting tremor.

Methods

Using high-resolution diffusion tensor imaging scans of 71 subjects (38 tremor-dominant, 10 non-tremor and 23 healthy controls), we test whether fractional anisotropy (FA) values in the SN and RRA differ between PD subgroups and healthy controls. Circular regions of interest were manually drawn by two raters in sub-regions of the SN, in the RRA, and in the cerebral peduncles as control areas.

Results

FA values for the different type of regions [region of interest (SN posterior and RRA) or control region (cerebral peduncles)] did not differ between PD patients and healthy controls ($p = 0.400$). This was the same between tremor-dominant, non-tremor and healthy controls ($p = 0.306$). When solely looking at the SN and RRA, there were no non-specific FA decreases in PD patients compared to healthy controls in both the SN ($p = 0.090$) and the RRA ($p = 0.174$). No correlation was found between resting tremor scores and FA values for the RRA ($r = 0.064$, $p = 0.648$).

Conclusions

Our findings question whether FA values can be used as a consistent proxy for structural integrity. Other promising measures, like free-water, may provide more reliable measures of neurodegeneration patterns in PD patients.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by bradykinesia (slowness of movement), rigidity and (resting) tremor; an uncontrollable rhythmic movement of body parts. Surprisingly, resting tremor is only seen in about 75% of PD patients, while others never develop this symptom. This led to the classification of PD patients into two subtypes; tremor-dominant and non-tremor patients (Jankovic et al., 1990; Lewis et al., 2005). In addition to resting tremor, tremor-dominant PD patients and non-tremor PD patients also show a difference in disease progression; tremor-dominant PD patients show less cognitive decline and a slower overall disease progression (Jankovic et al., 1990). The reason for this clinical variability is unclear.

Post-mortem evidence suggests that a difference in the pattern of neurodegeneration of dopaminergic midbrain nuclei between tremor-dominant and non-tremor PD patients may explain variability between groups. There is less neurodegeneration in the substantia nigra (SN) of tremor-dominant PD patients compared to non-tremor PD patients (Paulus & Jellinger, 1991), while this pattern is reversed in the retro-rubral area [RRA (Hirsch et al., 1992)]. In vivo evidence, however, is still lacking. Increased neurodegeneration in the RRA in the tremor-dominant group may relate to the occurrence of resting tremor in these patients. According to the dimmer-switch model of tremor, cell death in the RRA causes dopamine depletion in the pallidum, which triggers resting tremor episodes by causing pathological activity in the striato-pallidal circuit (Helmich et al., 2011).

Diffusion tensor imaging (DTI) has shown to be a promising method for studying abnormalities in grey matter areas; previous research has shown that DTI measurements were able to monitor cell degeneration in the SN of a murine PD model (Boska et al., 2012). Furthermore, fractional anisotropy (FA) is shown to be reduced in the SN (especially in the caudal and posterior part) of PD patients compared to healthy controls (Yoshikawa et al., 2004; Chan et al., 2007; Vaillancourt et al., 2009; Péran et al., 2010; Du et al., 2012). FA is modulated by a lot of factors, like for example myelination and the number of axons (Jones et al., 2012). Although FA values are therefore hard to interpret, it is suggested that a decreased FA in the SN of PD patients represents neurodegeneration in this area.

This study uses high resolution DTI to investigate whether there are differences in structural integrity of midbrain nuclei between tremor-dominant PD patients, non-tremor PD patients, and healthy controls. We try to replicate previous findings by testing the hypothesis that PD patients show a lower FA in the SN posterior compared to healthy controls due to cell degeneration in this area. Furthermore, we test the new hypothesis that PD patients show a lower FA in the RRA compared to healthy controls.

More specific, we hypothesize that the FA in the SN posterior is higher (less cell degeneration) in the tremor-dominant PD group compared to the non-tremor PD group and that the FA in the RRA is lower (more cell degeneration) in the tremor-dominant PD group compared to the non-tremor PD group. Furthermore, we examine the relationship between the structural integrity of the RRA and resting tremor severity. Based on previous work, we hypothesize that resting tremor severity is negatively correlated to the FA value in the RRA of the tremor-dominant group (Helmich et al., 2011).

Methods

Subjects and inclusion

This study was part of a larger project which explored structural and molecular cerebral differences between tremor-dominant and non-tremor PD. In this project, 44 tremor-dominant PD patients, 10 non-tremor PD patients and 25 healthy control subjects participated. Out of these, 38 tremor-dominant PD patients, 10 non-tremor PD patients and 23 healthy control subjects were included for this study. Participants were excluded from further analysis if they did not return for the second session (*n*=5) or if their dataset was not complete (*n*=3). The non-tremor group is small, since it represents an ongoing data collection. It is therefore only included as a preliminary analysis.

Recruitment of PD patients was done through their neurologists at the Neurology department of the Radboud University Nijmegen Medical Centre. Only patients with idiopathic PD (according to the UK Brain Bank criteria for PD) were included. Patients' disease severity had to be rated as mild to moderate (Hoehn and Yahr 1-3).

Patients were included in the tremor-dominant group if they did have a clinical resting tremor [defined as Unified Parkinson's Disease Rating Scale (UPDRS) resting tremor score for one arm of ≥ 1 point and a history of tremor]. Patients were included in the non-tremor group if they did not have resting tremor of both arms and legs (UPDRS resting tremor score = 0).

Exclusion criteria were as follows: Use of dopaminergic therapy without a clear clinical response of non-tremor symptoms, presence of contraindication for Domperidone, severe head tremor or dyskinesia, co-medication associated with elongated QT time, medication that can influence uptake speed of levodopa, neurological or psychiatric co-morbidity, any general MRI exclusion criteria, cognitive dysfunction [Mini-Mental State Examination (MMSE) < 24], pregnancy, or an age younger than 25 years. Participant characteristics are shown in Table 1.

Procedure

All participants visited the Donders Institute for Brain, Cognition, and Behavior twice. All patients were measured while off of dopaminergic medication (i.e., at least 12 hours after intake of the last dose of their dopaminergic medication, and 30 hours for dopamine agonists) from the evening before testing.

At each visit, general disease severity was quantified with the UPDRS. Disease severity was quantified at the start and end of each session, resulting in an OFF and ON state measurement. After UPDRS quantification, patients received medication. This could either be placebo (OFF state) or 250 mg Levodopa-Benserazide (ON state). The order of the ON and OFF sessions was counter-balanced. At least 30 minutes after intake of the medication (to ensure full medication uptake), MRI measures were performed. Participants underwent various MRI scans including DTI. DTI scans were made during one of the two visits. Cognitive function was also measured during one of the two visits using MMSE. Finally, general disease severity was quantified again with the UPDRS. The rater of the UPDRS was blinded to the medication state of the PD patients (ON state or OFF state).

DTI acquisition

DTI data were acquired on a 3T MRI system (Siemens, 32-channel head coil) using the DTI RESOLVE sequence (Cohen-Adad, 2012). Acquisition parameters were as follows: repetition time = 2200 ms, echo time = 69 ms, b values = 0-1000 s/mm2, diffusion gradient directions = 34, FOV = 220x220 mm, matrix = 220x220, slice thickness = 1.8 mm (with no gap), and slice number = 14. AutoAlign Head LS was used to place the DTI slab in the same orientation for all participants. The top slice was placed approximately beneath the splenum and rostrum part of the corpus callosum.

DTI analysis

Preprocessing

To increase the signal to noise ratio, a new denoising method called LPCA was used, which has shown to remove noise in multi-directional diffusion weighted imaging data (Manjón et al., 2013). This method makes use of a decomposition which is based on an overcomplete local principal component analysis (Manjón et al., 2013). Further processing steps were performed using tools within the FSL 5.0.9 software package (Jenkinson et al., 2012). Motion and eddy current distortion correction was performed using MCFLIRT (Jenkinson et al., 2002), and FA maps were calculated using DTIFIT.

To compare FA values in midbrain nuclei between groups, circular regions of interest (ROI) were manually drawn in the DTI data. ROI were drawn by two independent raters and were drawn according to guidelines on which both raters agreed on. ROI were drawn blinded to group.

Table 1. Demographics

	Healthy controls (*n* = 23)	Tremor-dominant PD (*n* = 38)	Non-tremor PD (*n* = 10)	Group *P*-values
Male/female, *n*	12/11	22/16	5/5	0.859
Age, years	62.0 (9.8)	61.3 (10.3)	58.6 (9.8)	0.665
Hoehn & Yahr stage	-	2.2 (0.5)	2.0 (0.5)	0.310
UPDRS total OFF condition	-	42.1 (16.5)	34.5 (15.7)	0.201
UPDRS non tremor OFF condition	-	27.6 (13.2)	33.0 (15.0)	0.267
UPDRS resting tremor OFF condition	-	10.1(3.1)	0.0 (0.0)	< 0.001

Age, Hoehn & Yahr stage and UPDRS scores are represented by mean ± standard deviation. *P*-value of age results from a 3x2 ANOVA with factors group and age. *P*-value of gender results from a Chi-square test of independence of group and gender. *P*-values of Hoehn & Yahr stage and UPDRS scores result from independent t-test between the tremor-dominant and non-tremor group.

First, two separate slices of the DTI slab were selected for the SN and the RRA. These slices were selected using B0 images. Such images do not have diffusion weighting and can be considered as T2-weighted images. The red nucleus (RN), which is clearly visible on B0 images, was used to aid in consistently selecting the SN and RRA slice. In the SN, cell loss in PD is most prominent and is seen in an earlier stage in the caudal part of the SN (Fearnley et al., 1991; Damier et al., 1999). The most caudal part of the SN is just inferior to the most caudal part of the RN. The SN slice was therefore defined as the most superior slice containing the SN and not or faintly containing the RN. In the RRA, cell loss in PD is most prominent at the level of the RN (Damier et al., 1999). The RRA slice was therefore defined as one slice superior to the SN slice.

In the SN slice, ROI were drawn in the anterior, middle and posterior segment of the SN. In addition, ROI were drawn in the cerebral peduncles as control area (Figure 1). In the RRA slice, ROI were drawn in the RRA and again in the cerebral peduncles as control area (Figure 2). Control areas were included to show that differences in FA values for the SN posterior and RRA between groups were specific for these regions.

ROI in the SN slice were three voxels in diameter. The voxel size was 0.98x0.98x1.8 mm, which resulted in ROI of 12.2 mm^3. This size was the same for the control areas in the RRA slice. ROI in the RRA itself were five voxels in diameter, which resulted in ROI of 33.93 mm^3. We chose to make these ROI bigger to make sure the area was fully covered, since the precise location is both larger and more difficult to locate.

ROI were drawn in the B0 image by defining the center of each ROI using a custom written Matlab 2012b script. After a rater clicked on the center of a ROI, a circle of abovementioned size was automatically inserted. The inserted circles were shown both in the B0 as well as in the FA image. ROI were drawn according to the following guidelines:

ROI SN slice

First, the anterior circle was placed in the anterior part of the left SN, while making sure to stay in the mesencephalon. Second, the posterior circle was placed in the posterior part of the left SN, while making sure to stay in the mesencephalon. There was some space left between the posterior border of the mesencephalon and the posterior ROI since the SN does not fully reach till the posterior border (Damier et al., 1999). In addition, nigrosome-1 (sub-region in the posterior part of the SN showing the most cell degeneration) is not located directly at the posterior border. Third, the middle circle was placed between the previous two circles, while keeping the distances between the three circles as equal as possible. It was made sure that the circles did not overlap. These three steps were then repeated on the right SN. Fourth, ROI were drawn in the cerebral peduncles. The vertical coordinate of the center was the same as the vertical coordinate of the center of the middle SN ROI. The FA map was used to confirm that the control ROI were actually in the cerebral peduncles, which is the hyperintense area on the FA map. ROI were drawn again if the ratio of the distance between the anterior and middle SN circle and the distance between the middle and the posterior SN circle was lower than 0.9 or higher than 1.1. Figure 1 shows the ROI in the SN slice.

ROI RRA slice

First, reference circles were placed in the centre of the RN. Second, ROI were drawn in the RRA. The horizontal coordinate of the center of the RRA was equal to the most lateral part of the reference circle in the RN. The RRA was placed as close as possible to the RN while making sure not to include the RN in the ROI. Third, ROI were drawn in the cerebral peduncles. The vertical coordinate of the center was equal to the most anterior part of the reference circle in the RN. Again, the FA map was used to confirm that the control ROI were actually in the cerebral peduncles. ROI were drawn again if the ratio of the distance between the RN and the RRA left and the distance between the RN and the RRA right was lower than 0.9 or higher than 1.1. Figure 2 shows the reference circles and ROI in the RRA slice.

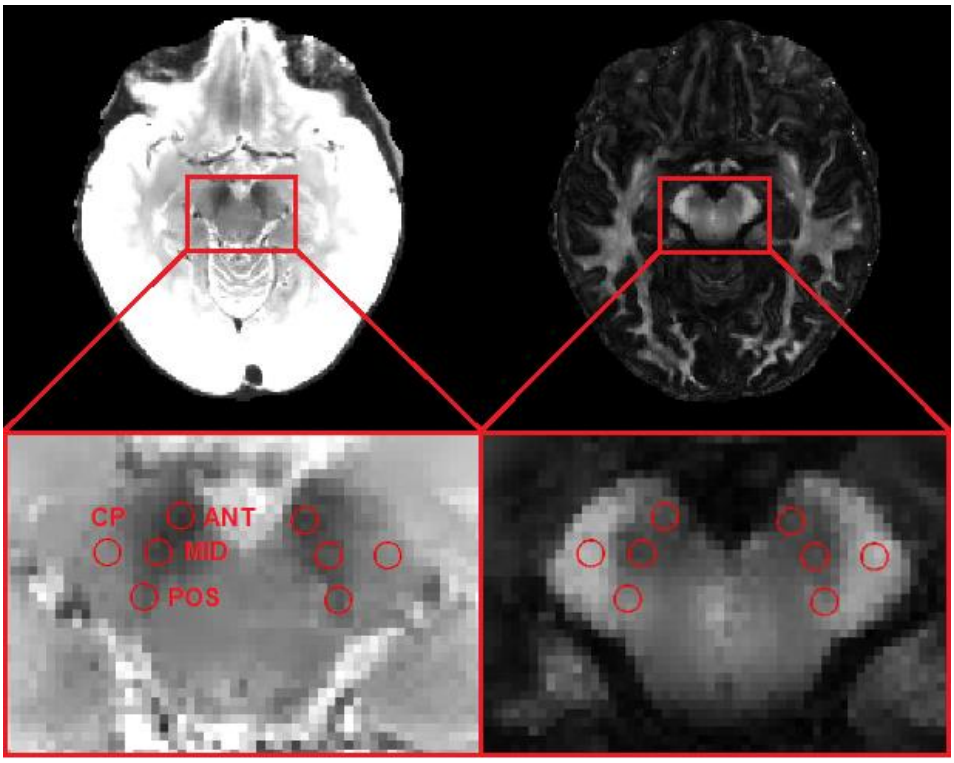

Figure 1. ROI in the SN slice. Left: B0 image showing the ROI for the anterior (ANT), middle (MID) and posterior (POS) substantia nigra and the control regions in the cerebral peduncles (CP). Right: Fractional anisotropy image showing the same ROI.

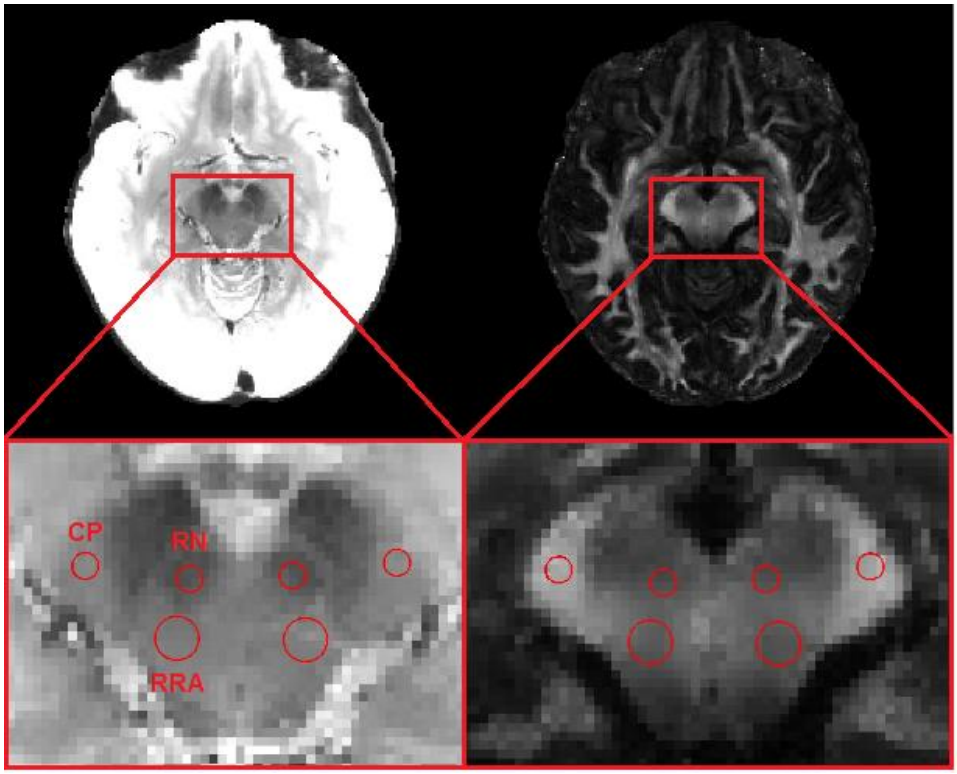

Figure 2. ROI in the RRA slice. Left: B0 image showing the reference circles in the red nuclei (RN), ROI for the retro-rubral area (RRA) and the control regions in the cerebral peduncles (CP). Right: Fractional anisotropy image showing the same ROI.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 23 and JASP 0.8.0.0.

Demographics

Age was compared between groups using a 3x2 ANOVA with factors group and age. Gender distribution was compared between groups using a Chi-Squared test of independence of group and gender. Hoehn and Yahr stage and UPDRS scores were compared between PD subgroups using independent sample t-tests**.**

DTI

Intraclass correlation coefficients were used to examine the inter-rater reliability between both raters. It was asessed whether there were differences in ROI locations between groups using a 3x2 ANOVA with factors group and location (horizontal and vertical coordinates of the ROI).

First, FA values were compared between PD patients and healthy controls. Group x region and group x region x brain area interactions, and group differences were examined using a 2x2x2 repeated measures ANOVA. The between subject factor was group (tremor-dominant and healthy control) and the repeated measures factors were region (region of interest and control region), and brain area (SN and RRA). Age was included as covariate. Significant interactions were examined using post-hoc tests. In case of insignificant interactions, a 2x2 repeated measures ANOVA was performed with group as between subject factor (tremor-dominant and healthy control) and region of interest (SN posterior and RRA) as the repeated measures factor. This allowed us to test for non-specific FA decreases in the regions of interest, since control regions are now not taken into account.

Second, the same analyses with all three groups (tremor-dominant, non-tremor and healthy control) as between subject factor was performed to test the specific hypothesis that PD tremor subgroups show different patterns of neurodegeneration. This analysis was included to show the preliminary results of the incomplete non-tremor group.

Finally, it was examined whether there was a significant correlation between UPDRS resting tremor score and FA values for the RRA using Pearson's correlation coefficient.

Results

Demographics

Demographics are shown in Table 1. As supposed, there only was a significant group difference in UPDRS resting tremor score OFF condition between the tremor-dominant and non-tremor PD group.

DTI: ROI locations

The inter-rater reliability between both raters was assessed using two-way random, absolute agreement, average-measures intraclass correlation coefficients [ICC (McGraw & Wong, 1996)]. This was done to assess the degree that rater 1 and 2 provided agreement in their ratings of the ROI locations across subjects. The ICC values of the horizontal and vertical coordinates of all ROI were in the excellent range [ICC > 0.77 (Cicchetti, 1994)], with a minimum ICC of 0.95 for all coordinates (mean ICC = 0.987, all *p*-values < 0.001). The high ICC indicates that raters had a high degree of agreement and suggests that ROI were placed similarly across raters.

To assess whether there were differences in ROI locations between groups, a 3x2 ANOVA with factors group and location (horizontal and vertical coordinates of the ROI) was performed. For both raters separately, there was no significant effect of group on horizontal and vertical coordinates of the ROI (all *p*-values > 0.05). This implies that locations of the ROI did not differ between groups.

DTI: tremor-dominant and healthy control

Figure 3 shows FA values for the various ROI of the tremor-dominant and the healthy control group. A 2x2x2 repeated measures ANOVA was performed to test the hypothesis that FA values for the SN posterior and RRA are decreased in PD patients compared to healthy controls. By including region as a repeated measures factor in this analysis, we tested whether this decrease was specific for the regions of interest.

There was no significant interaction between group and region, $F(1,58) = 0.718$, $p = 0.400$. This implies that FA values for the different type of regions [region of interest (SN posterior and RRA) or control region (cerebral peduncles)] did not differ between PD patients and healthy controls. There also was no significant interaction between group, region and brain area, $F(1,58) = 0.583$, $p = 0.448$. This implies that FA values for the different type of regions per brain area did not differ between groups. There was a trend, but no significant main effect of group, $F(1,58) = 3.045$, $p = 0.086$, implying that for each region, FA values did not differ between groups. The trend does imply that FA values of PD patients are decreased in all regions, but this did not reach significance.

Because the abovementioned interactions were not significant, we also explored whether there was a group effect or an interaction between group and regions of interest solely (SN posterior and RRA). There was no significant interaction between group and regions of interest, $F(1,58) = 0.321$, $p = 0.573$, implying that FA values for the different regions of interest did not differ between groups. There was a trend, but no significant main effect of group, $F(1,58) = 3.906$, $p = 0.053$. This implies that FA values for the SN posterior and RRA did not differ between groups when we did not take the control regions into account.

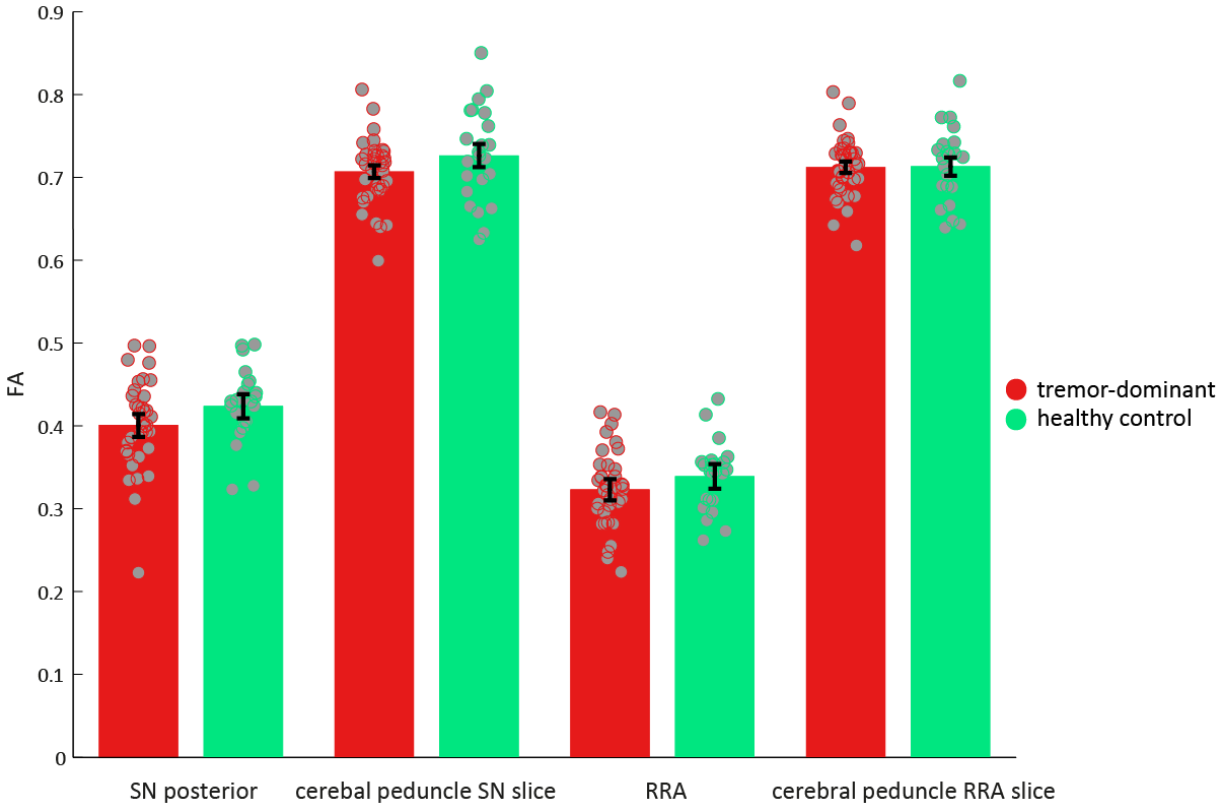

Figure 3. FA values for the SN posterior, RRA and cerebral peduncles of the tremor-dominant and healthy control group. Mean FA values of the SN posterior, RRA and cerebral peduncles are shown including error bars (representing the standard error of the mean) and individual data points. FA values for the different type of regions (interest or control) did not differ between groups.

The trend does imply that FA values in the SN posterior and RRA of PD patients are decreased, but this did not reach significance. Since we did not take into account the control regions here, it also implies that the decrease in FA is not specific for the regions of interest, but that there might be a difference over the entire brain.

Post-hoc independent t-tests were performed to look at FA values in the SN posterior and the RRA separately. Post-hoc independent t-tests did not show significant differences in FA values of the SN posterior $t(59) = -1.724$, $p = 0.090$ and RRA $t(59) = -1.375$, $p = 0.174$ between both groups. There was, however, a trend towards decreased FA values in the SN posterior of PD patients.

DTI: tremor-dominant, non-tremor and healthy control

Figure 4 shows FA values for the various ROI of the tremor-dominant, non-tremor, and healthy control group. A 3x2x2 repeated measures ANOVA was performed to test the hypothesis that PD tremor subgroups show different patterns of neurodegeneration in the SN posterior and RRA. Again, by including the control regions in this analysis, we tested whether those patterns were specific for the regions of interest.

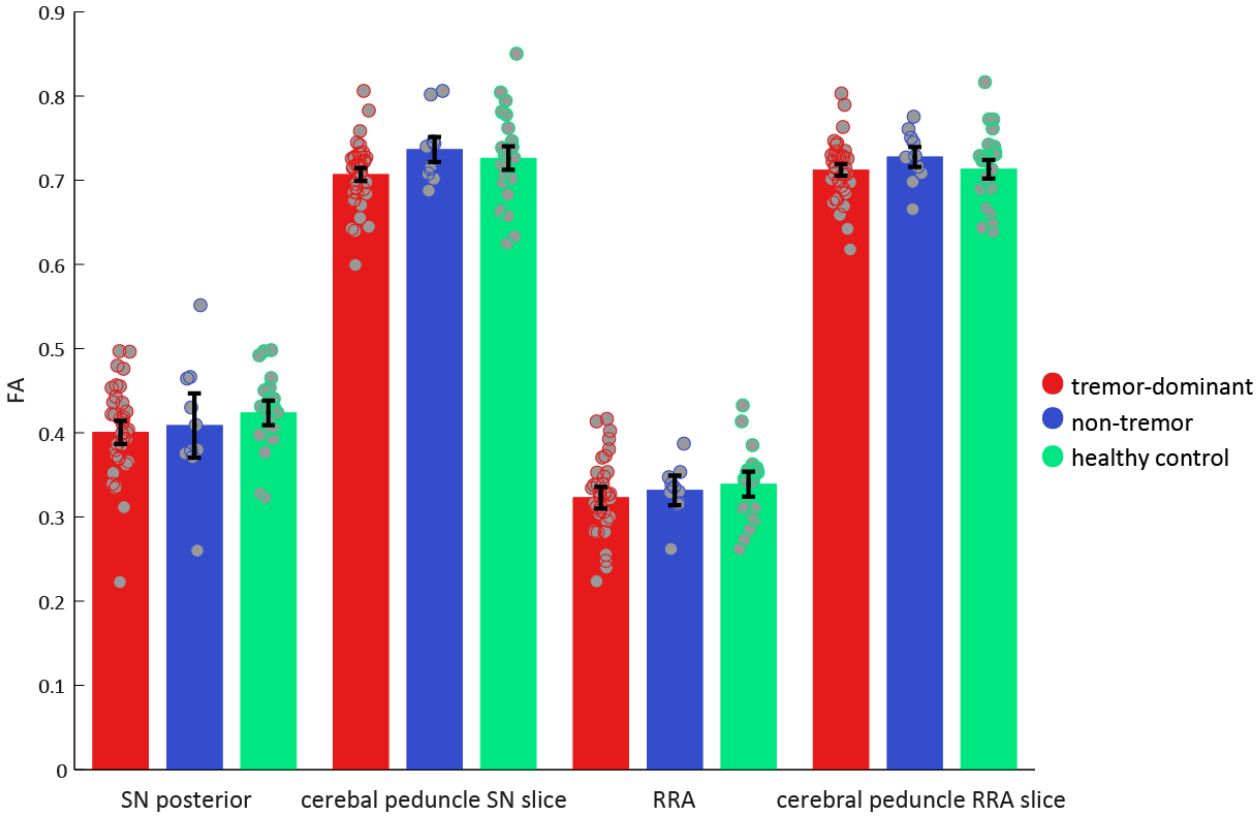

Figure 4. FA values for the SN posterior, RRA and cerebral peduncles of the tremor-dominant, non-tremor, and healthy control group. Mean FA values of the SN posterior, RRA and cerebral peduncles are shown including error bars (representing the standard error of the mean) and individual data points. FA values for the different type of regions (interest or control) did not differ between groups.

There was no significant interaction between group and region, $F(2,67) = 1.204$, $p = 0.306$. This implies that FA values for the different type of regions [region of interest (SN posterior and RRA) or control region (cerebral peduncles)] did not differ between the tremor-dominant, non-tremor and healthy control group. There also was no significant interaction between group, region and brain area, $F(2,67) = 0.482$, $p = 0.620$. This implies that FA values for the different type of regions per brain area did not differ between groups. There was no main effect of group, $F(2,67) = 1.703$, $p = 0.190$, implying that for each region, FA values did not differ between groups.

Because the abovementioned interactions were not significant, we also explored whether there was a group effect or an interaction between group and regions of interest solely (SN posterior and RRA). There was no significant interaction between group and regions of interest, $F(2,67) = 0.189$, $p = 0.828$, implying that FA values for the different regions of interest did not differ between groups. Furthermore, there was no main effect of group, $F(2,67) = 1.830$, $p = 0.168$. This implies that FA values for the SN posterior and RRA did not differ between groups when we did not take the control regions into account.

Correlation of FA with tremor score

We used Pearson's correlation coefficient to test the hypothesis that resting tremor severity was negatively correlated to the FA value in the RRA. There was no correlation between UPDRS resting tremor score in OFF condition and FA values for the RRA of the tremor-dominant group, $r = 0.064$, $p = 0.648$.

Discussion

This study uses high resolution DTI to investigate whether we can find differences in structural integrity of the SN posterior and RRA between tremor-dominant PD patients, non-tremor PD patients, and healthy controls in vivo. This study tries to replicate earlier findings on decreased FA values for the SN posterior of PD patients compared to healthy controls, and is the first one examining FA values for the RRA. In addition, this study is the first one comparing FA values of tremor-dominant and non-tremor PD patients.

In contrast to our hypotheses, we could not replicate the significant decrease in FA values for the SN posterior between PD patients and healthy controls. FA values of the SN posterior and RRA did not differ between groups when we only took those regions of interest into account. There was, however, a trend towards a decreased FA in the regions of interest in PD patients compared to healthy controls. This implies there is a trend towards decreased FA values in the SN posterior and RRA in PD patients, but this decrease in FA is not specific for the regions of interest.

Also in contrast to our hypotheses, FA values did not differ for the different type of regions between tremor-dominant, non-tremor PD patients and healthy controls. FA values of the SN posterior and RRA did not differ between the three groups when we only took the regions of interest into account. The analysis including all three groups was included to show preliminary results, but lacks power due to the small sample size of the non-tremor group.

Finally, UPDRS resting tremor scores in OFF condition and FA values for the RRA of the tremor-dominant group did not correlate.

If FA values do represent structural integrity, our results are not able to confirm the direction of the post-mortem study suggesting there is less neurodegeneration in the SN of tremor-dominant PD patients compared to non-tremor PD patients (Paulus & Jellinger, 1991). Even though our results are in the same direction as the previous post-mortem findings by Hirsch et al., (1992) -suggesting there is more neurodegeneration in the RRA of tremor-dominant PD patients compared to non-tremor patients- group differences of the analyses including the PD subgroups were not significant. We were also not able to relate FA values for the RRA to resting tremor scores. However, this does not necessarily disprove our previous hypothesis since our main analysis shows a definite lack in power.

Our results are not in line with previous studies which did find decreased FA values for the SN of PD patients compared to healthy controls (Yoshikawa et al., 2004; Chan et al., 2007; Vaillancourt et al., 2009; Péran et al., 2010; Du et al., 2012). There are various possibilities for why there is this discrepancy in results. Technical aspects and anatomical variability are suggested to be main factors contributing to a discrepancy in results of FA studies in PD (Schwarz et al., 2013). Our ROI, however, were placed in the caudal and posterior part of the SN, a region which is shown to have the highest level of neurodegeneration (Fearnley et al., 1991; Damier et al., 1999). Futhermore, we used high-resolution scans with low noise levels and relative small voxels compared to previous studies. This increases the precision of drawing the ROI and increases the precision of calculating the FA values in the ROI.

One limitation of this study might be that we did not distinguish the SN pars compacta from the SN reticulata, while neurodegeneration in the SN of PD patients mostly occurs in the SN pars compacta. However, this is not likely to have influenced our results, since a previous study which carefully distinguished the SN pars compacta from the SN reticulata was unable to find a FA decrease in PD patients in both separated regions of the SN (Menke et al., 2010). Another weakness in this study is that we used control regions which are prone to other types of microstructural changes which influence FA values, like myelination levels. In addition, it is not ruled out that the cerebral peduncles do not show any structural changes related to PD. Other control regions could therefore be more reliable. While the effect was also insignificant when ignoring the control regions and henceforth did not influence the negative FA results, we do note that other control regions, like the occipital or parietal cortices (Planetta et al., 2016), should be used in future studies to examine disease-specific differences in midbrain nuclei of PD patients.

However, this study is not the first to be unable to replicate the decreased FA values for the SN of PD patients compared to healthy controls (Menke et al., 2010; Focke et al., 2011; Schwarz et al., 2013). A recent meta-analysis notes that FA values of the control groups in the original studies are questionably high, noting a possible problem with some of these measurements. When studies with these unusually high FA values in the control groups are excluded, this results in an overall non-significant FA decrease in the SN of PD patients compared to healthy controls in all studies (Schwarz et al., 2013).

We suggest that decreased FA values may not represent neurodegeneration. It is still unclear what FA values represent exactly. FA values are highly sensitive to microstructural changes, but are not specific to a specific kind of change. Our results show that there are trend level FA decreases in the PD group when taking both the regions of interest and control regions into account. The reason for the trend level FA decrease in the control regions may represent other microstructural changes than the trend level FA decrease in the regions of interest. Therefore, decreased FA values may also represent other changes besides neurodegeneration.

The replication problem of FA studies in PD was addressed in a recent study by Metzler-Baddeley et al., (2012), who note that decreased FA values are biased by free-water (water molecules without a directional dependence). The combination of diffusion properties of free-water and diffusion properties of water in the brain may lead to lower FA values in PD patients. Since FA values represent the combination of diffusion properties of both free-water and water in the brain, FA values may not be sensitive enough to show neurodegeneration. We would consider FA as a proxy for neurodegeneration, because more neurodegeneration leads to more free-water, and therefore may lead to lower FA values. Measuring free-water itself would be a better and more direct measure of neurodegeneration.

By using a bi-tensor analysis model (which separates diffusion properties of free-water and of water in the brain) instead of the single tensor analysis model we (and previous FA studies) used, this bias can be overcome (Ofori et al., 2015; Planetta et al., 2016). By using a bi-tensor model, free-water maps itself, and FA values which are corrected for free-water can be processed. Free-water values are shown to be increased in the SN posterior of PD patients compared to healthy controls (Ofori et al., 2015; Ofori et al., 2015b; Planetta et al., 2016). Furthermore, free-water corrected FA values for the SN did not differ between groups (Planetta et al., 2016). It is therefore suggested that free-water values may be a good follow-up step in determining neurodegeneration levels in PD (Lehericy et al., 2017).

FA also seems to be a bad proxy for neurodegeneration since it cannot be related to other indirect measures of neurodegeneration levels. A previous study tried to validate reduced FA measures in the SN of PD patients by using dopamine transporter imaging. They did not find a correlation between FA values for the SN and radioligand uptake in the striatum, implying that FA does not represent disease severity (Harper et al., 2011). In addition, previous studies tried to relate disease duration and disease severity (UPDRS scores) to FA values, but this did not result in significant correlations (Du et al., 2012; Schwarz et al., 2013). In contrast, free-water values were able to track changes in neurodegeneration; free-water values have been shown to increase in PD patients during a follow-up measurement after a year, while this was not the case for healthy controls (Ofori et al., 2015b). This shows evidence that free-water values might indeed be a better and more direct measure compared to FA values.

Conclusion

We found only trend level differences in FA values for the SN posterior and RRA between PD patients and healthy controls. Even though previous studies found significant FA decreases in the SN of PD patients, our results are in line with multiple studies which were not able to replicate previous results. The meta-analysis of all FA studies in the SN of PD patients finds no significant decrease in FA. This in combination with the indirect nature of FA as a possible proxy for structural integrity is cause to question its further use in research into neurodegeneration patterns. This does mean that another in vivo biomarker of structural integrity is needed to answer our remaining research question: Are there differences in the patterns of neurodegeneration in the SN posterior and RRA between tremor-dominant and non-tremor PD subgroups? To answer this research question and to continue the search for a PD biomarker, we recommend that the same comparisons should be made using free-water values. Hopefully this would allow us to examine the neurodegeneration patterns of the SN posterior and RRA in vivo, and to relate these to PD tremor subgroups and to resting tremor.

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