

# Anxiety in Parkinson's disease is associated with changes in the brain fear circuit

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## Anxiety in Parkinson's disease is associated with changes in the brain fear circuit

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### ABSTRACT

**Background:** Anxiety is frequent in Parkinson's disease (PD) and has a negative impact on disease symptoms and quality of life. The underlying mechanisms remain largely unknown. The aim of this study was to identify anatomical and functional changes associated to PD-related anxiety by comparing the volume, shape and texture of the amygdala, the cortical thickness as well as the functional connectivity (FC) of the fear circuit in patients with and without clinically relevant anxiety.

**Methods:** Non-demented PD patients were recruited, and anxiety was quantified using the Parkinson Anxiety Scale. Structural MRI was used to compare cortical thickness and amygdala structure and resting-state functional MRI to compare FC patterns of the amygdala and resting-state functional networks in both groups.

**Results:** We included 118 patients: 34 with (A+) and 84 without (A-) clinically relevant anxiety. Clusters of cortical thinning were identified in the bilateral fronto-cingulate and left parietal cortices of the A+ group. The texture and the shape of the left amygdala was different in the A+ group but the overall volume did not differ between groups. FC between the amygdala and the whole brain regions did not differ between groups. The internetwork resting-state FC was higher between the "fear circuit" and salience network in the A+ group.

**Conclusion:** Anxiety in PD induces structural modifications of the left amygdala, atrophy of the bilateral fronto-cingulate and the left parietal cortices, and a higher internetwork resting-state FC between the fear circuit and the salience network.

### 1. Introduction

Anxiety is among the most frequent non-motor symptoms in Parkinson's disease (PD) with an average point prevalence of 31% [1]. However, the underlying mechanisms remain poorly understood. In PD patients, studies have shown a negative correlation between the level of anxiety and the volume of the left amygdala, the anterior cingulate cortex (ACC) and precuneus thickness [2,3]. Resting-state functional MRI (rs-fMRI) studies have shown that the severity of anxiety was correlated with increased functional connectivity (FC) between the amygdala and the prefrontal cortex (PFC), as well as the temporal and parietal cortices, and the striatum [4,5]. These studies mostly correlated imaging data with anxiety levels, which probably reflects trait anxiety (i.

e. the individual's tendency to experience anxiety) [6], but they did not compare patients with and without anxiety at the time of assessment, in order to reveal the mechanisms of state anxiety. Nevertheless, these studies suggest dysfunction of the fear circuit.

Based on animal studies, the existence of an anatomo-functional network called the "fear circuit" was postulated [7] whose hub is the amygdala [8]. In humans, the amygdala is known to be an interface between external stimuli and behavioral as well as cognitive responses to anxiety. Functional connections between the amygdala and the anterior cingulate cortex (ACC), the medial prefrontal cortex (mPFC), the insular cortex (IC), the hippocampus and the striatum have been reported, that together form the human "fear circuit" [9].

The aim of this study was to identify anatomical and functional changes associated with PD-related anxiety by comparing the volume,

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**Abbreviations**

3D-T <sub>1</sub> w	3-Dimension T <sub>1</sub> -weighted	LARS	Lille Apathy Rating Scale
A+	patient group with anxiety	LEDD	Levodopa Equivalent Daily Dosages
A-	patient group without anxiety	MDRS	Mattis dementia rating scale
ACC	Anterior Cingulate Cortex	MDS-UPDRS	Movement Disorder Society Unified Parkinson Disease Rating Scale
BJLO	Benton judgment of line orientation test	mPFC	medial prefrontal cortex
BNT	Boston naming test	PAS	Parkinson Anxiety Scale
dIPFC	dorsolateral prefrontal cortex	PFC	prefrontal cortex
FDR	false discovery rate	ROI	region of interest
GLCM	Grey-Level Co-occurrence Matrix	rs-fMRI	resting state functional MRI
HAMD	Hamilton Depression Rating Scale	SDMT	Symbol digit modalities test
HVLT	Hopkins verbal learning test	TMT	Trail Making Test
IC	insular cortex	vIPFC	ventrolateral prefrontal cortex
ICA	Independent Component Analyses	WAIS-R	Wechsler Adult Intelligence Scale - Revised.

shape and texture of the amygdala, the cortical thickness, the FC of amygdala and the internetwork resting state FC of the fear circuit in PD patients with and without clinically relevant anxiety. We assumed that changes in the fear circuit will be observed in patients with anxiety, more specifically we hypothesized a smaller volume of the amygdala, a smaller cortical thickness of the prefrontal, cingulate and insular cortices and a higher FC within the fear circuit, compared to patients without anxiety.

## 2. Materials and methods

### 2.1. Population

This study included 156 consecutive PD patients enrolled from two movement disorders clinics in Lille (France) and Maastricht (The Netherlands) between March 2013 and August 2014. PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank diagnostic criteria. Patients with other neurological disorders, as well as patients with moderate to severe dementia according to the Movement Disorders Society criteria for Parkinson's disease dementia were excluded [10].

Age, sex, duration of formal education, disease duration, history of PD or psychiatric disorders were recorded. The Movement Disorder Society Unified Parkinson Disease Rating Scale (MDS-UPDRS) was used to assess motor (part III), non-motor symptoms (part I) and disease severity (Hoehn-Yahr stage). The levodopa equivalent daily dosages (LEDD) were calculated and the use of antidepressant and anxiolytics treatments reported. Anxiety, depression and apathy were respectively assessed by the Parkinson Anxiety Scale (PAS) [11], the Hamilton Depression Rating Scale (HAMD) and the Lille Apathy Rating Scale (LARS). Each patient had undergone a thorough evaluation of cognitive functions (see Dujardin et al. [12] for details of the procedure). This evaluation assessed the overall cognitive efficiency (Mattis dementia rating scale (MDRS)); attention and working memory (symbol digit modalities test (SDMT), forward-backward digit span subtest of the Wechsler for adults intelligence scale revised (WAIS-R)); executive functions (Trail Making Test (TMT), phonemic and alternating fluency tests); episodic memory (Hopkins verbal learning test (HVLT)); language (Boston naming test (BNT) and animal fluency) and visuospatial functions (Benton judgment of line orientation test (BJLO)).

Written informed consent was obtained from all participants after full information of the procedure. The study was approved by the institutional ethics committees (CPP Nord-Ouest IV, 2012-A 01317–36). Additional information on this study group is detailed in the original paper [12].

### 2.2. Characterization of anxiety

Patients were divided into two groups, one with (A+) and one without (A-) anxiety, according to their score on the PAS, a scale specifically developed to detect anxiety in PD patients. We used the observer-rated version. Patients were considered "A+" if they had a score above the cut-off in at least one of the three subparts of the scale (part A (persistent anxiety) > 9, part B (episodic anxiety) > 3, or part C (avoidance behavior) > 3) [11].

### 2.3. Imaging data acquisition

Patients were scanned at two sites using identical 3T Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) with identical software versions and MR sequences. High-resolution 3D T<sub>1</sub>-weighted (3D-T<sub>1</sub>w) images were acquired with a magnetization-prepared gradient echo sequence (voxel size: 1 x 1 x 1 mm<sup>3</sup>; TR: 7.2 ms; TE: 3.3 ms; matrix size: 172 x 256 x 256 voxels; flip angle: 9°). rs-fMRI was performed with a 10 min T<sub>2</sub>\*-weighted EPI sequence (voxel size: 3 x 3 x 3 mm<sup>3</sup>; TR: 2400 ms; TE: 30 ms; matrix size: 64 x 64 x 40 voxels; flip angle: 90°). Resting-state fMRI using posterior to anterior direction with interleaved acquisition was used. Patients were required to remain quiet, stay awake and close their eyes.

### 2.4. Structural MRI analysis

#### 2.4.1. Cortical thickness extraction

To study potential cortical atrophy, cortical thickness was automatically calculated using FreeSurfer 5.3 software (<https://surfer.nmr.mgh.harvard.edu/>) from fMRIPrep tool [13]. Statistical analysis was implemented in Surf-Stat toolbox (<http://www.math.mcgill.ca/keith/surfstat/>) for MATLAB. The pipeline is detailed on Supplementary material, 1.a.

#### 2.4.2. Volume, shape and texture of amygdala

Amygdala were manually segmented on 3D-T<sub>1</sub>w images with MRI-CRON software to bring out any atrophy. Anatomical borders were defined by a radiologist and according to literature data [14]. Volumes were recorded in mm<sup>3</sup> and normalized to the patient's total intracranial volume estimated by FreeSurfer 5.3 software. The detailed procedure is provided in Supplementary material, 1.b.

To study potential deformations of the amygdala, shape analysis was performed using the spherical harmonic-point discrimination model (SPHARM-PDM) [15].

A texture analysis was performed on the 3D-T<sub>1</sub>w images in order to determine changes in the amygdala. Texture analysis is an image processing method for the quantification of grey levels inside an image

[16]. The procedure is detailed in Betrouni et al. [17]. Here, we compared four first-order and 7 s-order texture parameters detailed in [Supplementary Table 1](#).

## 2.5. Functional MRI analysis

### 2.5.1. Preprocessing and quality control

Common preprocessing steps, including co-registration, normalization, unwarping, noise component extraction, segmentation, skull stripping, slice-timing correction, were performed using *fMRIPrep* 1.2.5 (<https://fmripred.readthedocs.io>). At the end of this procedure, an individual quality control was performed. CONN Toolbox [18] was then used for: i) Gaussian kernel 6 mm smoothing; ii) to remove motion, physiological and other artefactual effects from BOLD signal; iii) Band-pass filter of 0.008 Hz–0.09 Hz (more details in [Supplementary material 1.d](#)).

### 2.5.2. Resting-state functional connectivity of the amygdala

Resting-state FC analysis were performed with CONN. A complete brain parcellation including 91 cortical areas and 15 subcortical areas from the FSL Harvard-Oxford Atlas [19] was used to define both amygdala and the regions of interest (ROI) in MNI-space. The right and left amygdala were defined as seeds and compared to all the other ROIs. The correlation indices between the mean BOLD signal of both amygdala and of the ROIs were calculated for each patient. These correlation indices were then compared in each group and between groups.

### 2.5.3. Independent component analysis and functional network connectivity

Group Independent Component Analyses (ICA) were performed to identify common functional networks in patients using Calhoun's group-level ICA approach with CONN. Forty independent components have been identified. A functional networks atlas from the *Human Connectome Project*, provided by the CONN Toolbox, was used to correlate common healthy functional networks with these forty components. The following networks were identified: default-mode network, left and right frontoparietal network (cognitive control), visual network, sensorimotor network and dorsal attentional network. Moreover, the salience network provided by default from the CONN toolbox was used because the group ICA failed to identify it. Finally, the "fear circuit" was defined using bilateral cortical and subcortical areas from the FSL Harvard-Oxford Atlas including amygdala, striatum, hippocampus, ACC, mPFC and IC [9]. Network masks were extracted. The correlation between the mean BOLD signal of these masks were calculated for each patient and compared in each group and between groups.

## 2.6. Statistical analyses

For all analyses, the statistical significance threshold was set at  $p$ -value  $< 0.05$ . Correction for multiple comparisons (FDR – False Discovery rate) were performed separately for cognitive variables, functional and structural data. The normality of distribution was assessed using a Kolmogorov-Smirnov test.

### 2.6.1. Analysis of clinical data

The numerical variables were described as means and standard deviations, the ordinal variables as median and range and the categorical variables as frequencies and percentages.

Qualitative data were compared using Odds Ratio's and quantitative data using two sample T-tests or Mann-Whitney tests depending on normality of the distribution. These analyses were performed with the Statistical Package for the Social Sciences, version 22 (SPSS, Chicago).

### 2.6.2. MRI analyses

Generalized linear models (GLM) were performed to compare cortical thickness, amygdala volumes and amygdala texture parameters

between groups. Amygdala shape comparison was performed using a MANCOVA procedure. For rs-fMRI analyses, generalized linear models with permutation inference were calculated to identify significant functional connections for each group and to compare these connections between the groups.

### 2.6.3. Regression analysis

Hierarchical multiple regression post-hoc analyses were performed to examine the relationship between the PAS score and sub-scores and the volume and texture of amygdala, mean cortical thickness in the significant clusters and FC values. Center, sex and Hoehn-Yahr stage were set as nuisance regressors in the first block (model 1) of all regression models whereas PAS score or sub-scores (independent variable) were separately added to the second block (model 2) of the model to examine the association between anxiety symptoms and imaging data, adjusted by the effect of center, sex and Hoehn-Yahr stage. We ensured that all models met the assumptions of multiple regression analyses, including normality of the residuals, multicollinearity and homoscedasticity.

## 3. Results

### 3.1. Population

After exclusion of 38 patients for dementia ( $n = 14$ ), refusal or contraindication to have an MRI ( $n = 22$ ) or unusable MRI (major motion artefact –  $n = 2$ ), 118 were involved in the present study, 34 with ("A+") and 84 without ("A-") anxiety (Flowchart in [Supplementary Fig. 1](#)).

### 3.2. Demographic and clinical variables

"A+" patients were more frequently female, with a family history of PD, and more often a left-sided onset of motor symptoms. MDS-UPDRS part I sub-scores of depression, anxiety, sleep disturbances, pain, and fatigue were higher in "A+" group than in the "A-" group and disease stage was more advanced. In the "A+" group, LEDD was higher and antidepressants and anxiolytics were used more frequently ([Table 1](#)).

### 3.3. Cognitive variables

After FDR-correction for multiple comparisons, patients in the "A+" group had lower results at backward digit span and animal fluency test as well as a slower processing speed than in the "A-" group ([Table 1](#)).

### 3.4. Structural analyses

All following analyses were adjusted for sex, disease stage and center.

#### 3.4.1. Cortical thickness

Three clusters of reduced cortical thickness were identified in the bilateral frontal and left parietal regions in the "A+" compared to "A-" group ([Fig. 1](#)). There was no significant difference for the reverse analysis.

#### 3.4.2. Volume, shape and texture of amygdala

There was no significant group difference for the volume of amygdala (A+/A-, left:  $1201 \text{ mm}^3/1284 \text{ mm}^3$ , right:  $1210 \text{ mm}^3/1273 \text{ mm}^3$ ). Shape analysis revealed several remodeling areas located on the medial and postero-lateral sides of the left amygdala. Texture analyses showed a significant group difference for the second-order texture value "correlation", in the left amygdala ( $F = 3.86$ ,  $p = 0.025$ ) ([Fig. 2](#)).

**Table 1**

Demographic, clinical and cognitive variables: group comparisons (Parkinson's disease patients with (A+) and without (A-) anxiety).

Demographic variables	A+ group (n = 34)	A- group (n = 84)	OR (CI 95%); p-value	
Age (years)	65.62 (±7.66)	64.10 (±8.62)	0.37	
Women (n = 36)	16 (47.06%)	20 (23.81%)	<b>2.84 (1.23; 6.58); p = 0.013<sup>a</sup></b>	
Hand dominance (right, n = 101)	29 (85.29%)	72 (85.71%)	0.97 (0.31; 3); p = 0.99	
Formal education (years)	12.12 (±3.96)	12.61 (±3.53)	0.51	
Illness duration (years)	9.59 (±7.82)	8.18 (±4.99)	0.25	
First motor side	20 (58.82%)	28 (33.33%)	<b>3.21 (1.31; 7.85); p = 0.009<sup>a</sup></b>	
<i>Left (n = 48)</i>				
<b>Clinical variables</b>				
LEDD (mg/day)	937.36 (±494.38)	732.53 (±578.12)	<b>0.02<sup>a</sup></b>	
Antidepressant use (n = 17)	13 (38.24%)	4 (4.76%)	<b>12.38 (3.66; 41.91); p &lt; 0.0001<sup>a</sup></b>	
Anxiolytic use (n = 12)	10 (29.41%)	2 (2.38%)	<b>17.08 (3.5; 83.33); p &lt; 0.0001<sup>a</sup></b>	
MDS-UPDRS part 1 (0–4) <sup>§</sup>				
1.3. Depressed mood	1 (0–4)	0 (0–4)	<b>0.001<sup>a</sup></b>	
1.4. Anxious mood	2 (0–4)	0 (0–4)	<b>&lt; 0.0001<sup>a</sup></b>	
1.7. Night-time sleep problems	2 (0–4)	2 (0–4)	<b>0.03<sup>a</sup></b>	
1.8. Daytime sleepiness	2 (0–4)	2 (0–4)	0.26	
1.9. Pain and other sensations	2 (0–4)	1 (0–4)	<b>&lt; 0.0001<sup>a</sup></b>	
1.10. Urinary problems	1 (0–4)	1 (0–4)	0.09	
1.11. Constipation problems	1 (0–4)	0 (0–4)	0.10	
1.12. Lightheadedness on standing	1 (0–3)	0 (0–3)	0.28	
1.13. Fatigue	2 (0–4)	1 (0–4)	<b>0.03<sup>a</sup></b>	
MDS-UPDRS part 3 (/132)	30.2 (±14.9)	28.1 (±12.0)	0.54	
Hoehn & Yahr stage (0–5) <sup>§</sup>	2 (1–5)	2 (0–4)	<b>0.003<sup>a</sup></b>	
PAS total (/48)	14.79 (±4.69)	3.69 (±2.87)	<b>&lt; 0.0001<sup>a</sup></b>	
Part A (/20)	9.47 (±4.32)	2.85 (±2.87)	<b>&lt; 0.0001<sup>a</sup></b>	
Part B (/16)	2.38 (±2.26)	0.42 (±0.85)	<b>&lt; 0.0001<sup>a</sup></b>	
Part C (/12)	2.94 (±2.32)	0.43 (±0.85)	<b>&lt; 0.0001<sup>a</sup></b>	
HAMD total (/54)	8.7 (±5.2)	4.5 (±3.6)	<b>&lt; 0.0001<sup>a</sup></b>	
HARS total (/56)	11.4 (±5.8)	5.3 (±4.2)	<b>&lt; 0.0001<sup>a</sup></b>	
Cognitive variables	A+ group (n = 34)	A- group (n = 84)	Uncorrected p-value	FDR-corrected p-value
<b>Overall efficiency</b>				
MDRS score (/144)	136 (±5.90)	138 (±6.62)	0.024	0.096
<b>Attention and working memory</b>				
WAIS-R forward digit (/14)	7.12 (±2.14)	7.90 (±2.19)	0.06	0.120
WAIS-R backward digit (/14)	4.88 (±1.87)	6.04 (±1.67)	0.001	<b>0.008<sup>a</sup></b>
SDMT: number in 90 s	35.35 (±10.57)	44.71 (±11.91)	0.00009	<b>0.001<sup>a</sup></b>
<b>Executive functions</b>				
TMT (time B/time A)	2.75 (±0.91)	2.47 (±0.71)	0.09	0.131
Stroop: interference index	2.04 (±0.70)	1.75 (±0.47)	0.024	0.077
Phonemic fluency: words in 60 s	11.97 (±4.19)	13.75 (±4.84)	0.06	0.107
Alternating fluency: words in 60 s	10.74 (±3.89)	12.04 (±4.85)	0.12	0.137
<b>Episodic memory</b>				
HVLT Learn 1 (/12)	5.79 (±1.81)	6.64 (±1.92)	0.03	0.080
HVLT Learn total (/36)	24.31 (±4.30)	26.27 (±4.43)	0.04	0.091
HVLT delayed recall (/12)	8.62 (±1.92)	9.04 (±2.69)	0.09	0.120
HVLT recognition hits (/12)	11.32 (±0.88)	11.24 (±1.26)	0.78	0.780
HVLT number of intrusions	1.75 (±2.05)	1.50 (±2.13)	0.37	0.395
<b>Language</b>				
Boston Naming Test (/15)	12.26 (±2.31)	13.00 (±1.96)	0.09	0.111
Semantic fluency: animals in 60s	16.94 (±4.60)	20.71 (±5.91)	0.001	<b>0.005<sup>a</sup></b>
<b>Visuospatial functions</b>				
Benton Judgment of Line Orientation	10.71 (±3.26)	11.95 (±2.55)	0.08	0.128

<sup>a</sup> = FDR-corrected p-value < 0.05, § = described as median and range; CI = confidence interval; HAMD = Hamilton Depression Rating Scale; HARS = Hamilton Anxiety Rating Scale; HVLT = Hopkins verbal learning test; LARS = Lille Apathy Rating Scale; LEDD = Levodopa Equivalent Daily Dosages; MDRS = Mattis dementia rating scale; MDS-UPDRS = Movement Disorder Society Unified Parkinson Disease Rating Scale; OR = Odds Ratio; PAS = Parkinson Anxiety Scale; SDMT = Symbol digit modalities test; TMT = Trail Making Test; WAIS-R = Wechsler for adults intelligence scale revised.

### 3.5. Functional analyses

#### 3.5.1. Functional connectivity of the amygdala

Of the 118 patients, 17 were excluded from the FC analyses after quality control (n = 101). Their demographic and clinical characteristics (presented in [Supplementary Table 2](#)) were similar as the original study population.

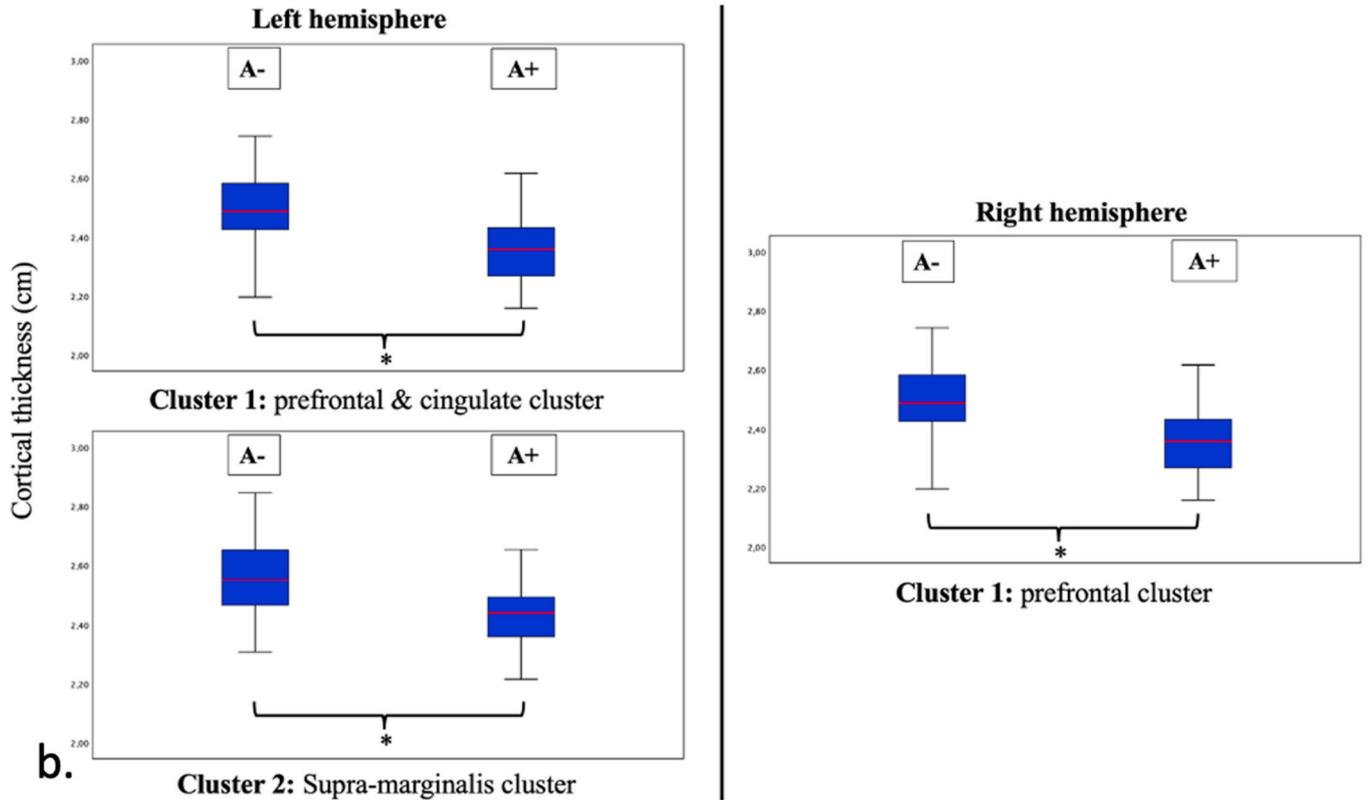
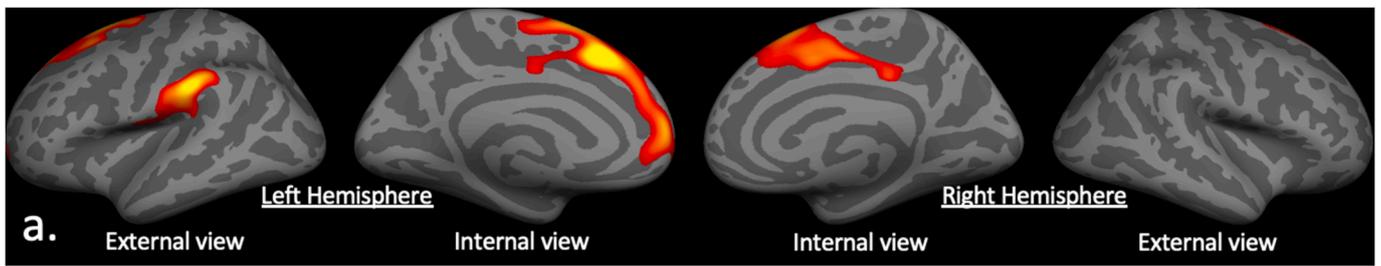
There were fewer functional connections with both amygdala in the "A+" than in "A-" group. However, after FDR-correction, there was no longer any significant difference ([Supplementary Fig. 2](#) and [Supplementary Table 3](#)).

#### 3.5.2. Resting-state functional networks and the fear circuit

In the "A+" group, the FC was significantly higher between the fear circuit and the salience network (F-score = 2.55, FDR-corrected p-value = 0.0375), compared to "A-" group.

#### 3.5.3. Regression analyses

The PAS score was significantly positively related to the FC between the left amygdala-left parahippocampal cortex (p = 0.010). The PAS-B sub-score was significantly negatively related to the mean cortical thickness of the left fronto-cingulate (p = 0.003), right fronto-cingulate (p = 0.013) and left parietal areas (p = 0.016). The PAS-C sub-score was significantly negatively related to the mean cortical thickness of the left fronto-cingulate cluster (p = 0.016) and positively related to the FC



	Cluster	Size (mm <sup>2</sup> )	T-max	Talairach coordinates			Location
				X	Y	Z	
Right hemisphere	1	2060.59	6.320	10.5	19.9	55.1	<b>Superior frontal gyrus</b> Inferior frontal gyrus (oper) Inferior frontal gyrus (tri) Middle frontal gyrus
Left hemisphere	1	4986.41	6.901	-7.4	23.3	41.5	<b>Superior frontal gyrus</b> Cingulate (anterior) gyrus Cingulate (posterior) gyrus
	2	1703.03	5.406	-60.6	-27.8	29.7	<b>Supra-marginalis gyrus</b>

Fig. 1. Cortical thickness analysis.

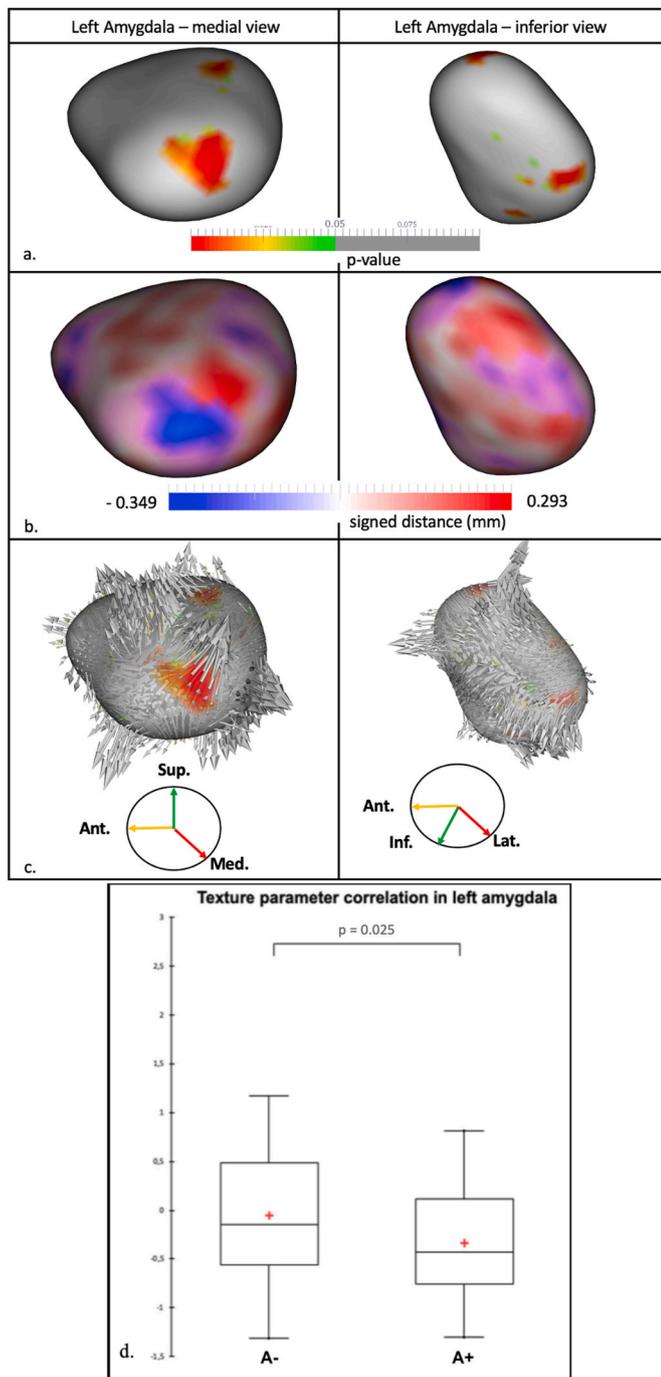
(a) Map of reduced cortical thickness clusters in patients with anxiety compared to patients without anxiety (T-score). (b) Boxplots of cortical thickness comparisons for the four significant clusters of cortical thickness reduction in the Parkinson's disease patients with (A+) and without (A-) anxiety, adjusted by sex. (c) Location and MRI coordinates of cortical thickness atrophy clusters in Parkinson's disease patients with anxiety compared to patients without anxiety.

\* = FDR-corrected p-value < 0.05; **Bold** = T-max gyrus; oper = opercularis; tri. = triangularis.

between the left amygdala-left parahippocampal cortex ( $p = 0.011$ ). There were no other significant associations. (Fig. 3 and Supplementary Table 4).

#### 4. Discussion

The present study sought to identify anatomical and functional markers of PD-related anxiety. We observed reduced cortical thickness



**Fig. 2.** Anatomical changes of left amygdala in A+ compared to A- patients in PD, adjusted by center, sex and Hoehn-Yahr stage.

**Shape analysis:** (a) statistical map, (b) signed distance map (mm) and (c) vertex map showing significant shape differences on the medial and inferior sides of the left amygdala.

**Texture analysis:** (d) distribution of the second-order texture parameter “correlation” in left amygdala between the two groups.

Ant. = Anterior side; Inf. = Inferior side; Lat. = Lateral side; Med. = Medial side; Sup. = Superior side.

of the bilateral fronto-cingulate and left parietal regions, and anatomical changes of the left amygdala in PD patients with anxiety, with several remodeling areas located on the medial and postero-lateral sides of the left amygdala and changes in texture). Moreover, FC between the fear circuit and the salience network was higher in the “A+” group.

#### 4.1. PD-related anxiety is associated with changes in the left amygdala

A negative correlation between the severity of anxiety in PD and the volume of the left amygdala was previously reported [2]. However, we did not find any between group difference in the volume of the amygdala, this volume was not associated with the severity of anxiety symptoms as measured by the PAS. However, shape analyses revealed a remodeling area at the medial and postero-lateral sides of the left amygdala in the “A+” group. Moreover, this structural remodeling of the left amygdala altered the image texture. The lower “correlation” texture parameter in anxious patients may be interpreted as a reduction of the MRI signal consistency. Overall, these results support the role of the amygdala in PD-related anxiety. However, the rather subtle anatomical modifications suggest that the amygdala is not the only structure involved in PD-related anxiety, but more part of a complex system including the “fear circuit” as well as other structures.

#### 4.2. Anxiety in PD is associated with cortical atrophy in the fear circuit

We observed clusters of cortical thinning in the bilateral fronto-cingulate and left parietal cortices. Moreover, the mean cortical thickness of these clusters was negatively associated with the severity of anxiety, especially for episodic anxiety (PAS-B) and avoidance behavior (PAS-C).

As these areas are parts of the fear circuit, their thinning could contribute to disruption of fear processing and thus promote anxiety. In the A+ group, the mPFC, ventrolateral PFC (vlPFC) and dorsolateral PFC (dlPFC) had less cortical thickness. In the fear circuit, the vlPFC would be involved in salience detection and action inhibition whereas the dlPFC would be involved in allocation of attentional resources to salient information and cognitive regulation [20–22]. Authors suggested an impaired voluntary emotion regulation by the lateral PFC along with an increased automatic emotion regulation by the mPFC in PD patients with anxiety [4]. These regions are also involved in more general cognitive processes and related to cognitive deficits in PD [23]. Furthermore, smaller cortical thickness of the cingulate cortex could contribute to intrusive negative thoughts that may underlie anxiety symptoms [3]. It could lead to attentional resources disturbance, as found in this study. The parietal cortex has also been involved in PD-related anxiety (precuneus, supramarginalis cortex) [3]. It would be involved in internal awareness and adaptation after environment changes, which possibly explains the difficulty of anxious individuals diverting attention from their negative thoughts [5,24].

#### 4.3. PD-related anxiety is associated with changes in the FC between networks

The FC between the “fear circuit” and the salience network was significantly increased in the “A+” group. The salience network is involved in stimuli identification in order to adapt behavior. It is an interface between cognition, emotion and somatic manifestations. It is therefore involved in “bottom-up” attentional processing and could lead to hypervigilance in case of insufficient filtering of these stimuli [25]. These results suggest that any event (e.g. changes in habits, unexpected situations, ...) would be disproportionately perceived in “A+” patients. It would then promote anxious manifestations by letting intrusive thoughts and negative emotions to occur. It could worsen anxiety in a vicious circle.

We also observed a trend toward a significant between-group difference of the FC of the amygdala, as well as a positive association between the severity of anxiety and the FC of the left amygdala with the left parahippocampal gyrus, especially for avoidance behavior (PAS-C). Similar associations have been previously reported [5]. Hence, anxiety in PD is associated with a higher activation of the brain fear circuit, led by a higher temporo-amygdala connectivity, which could interfere with other structures.

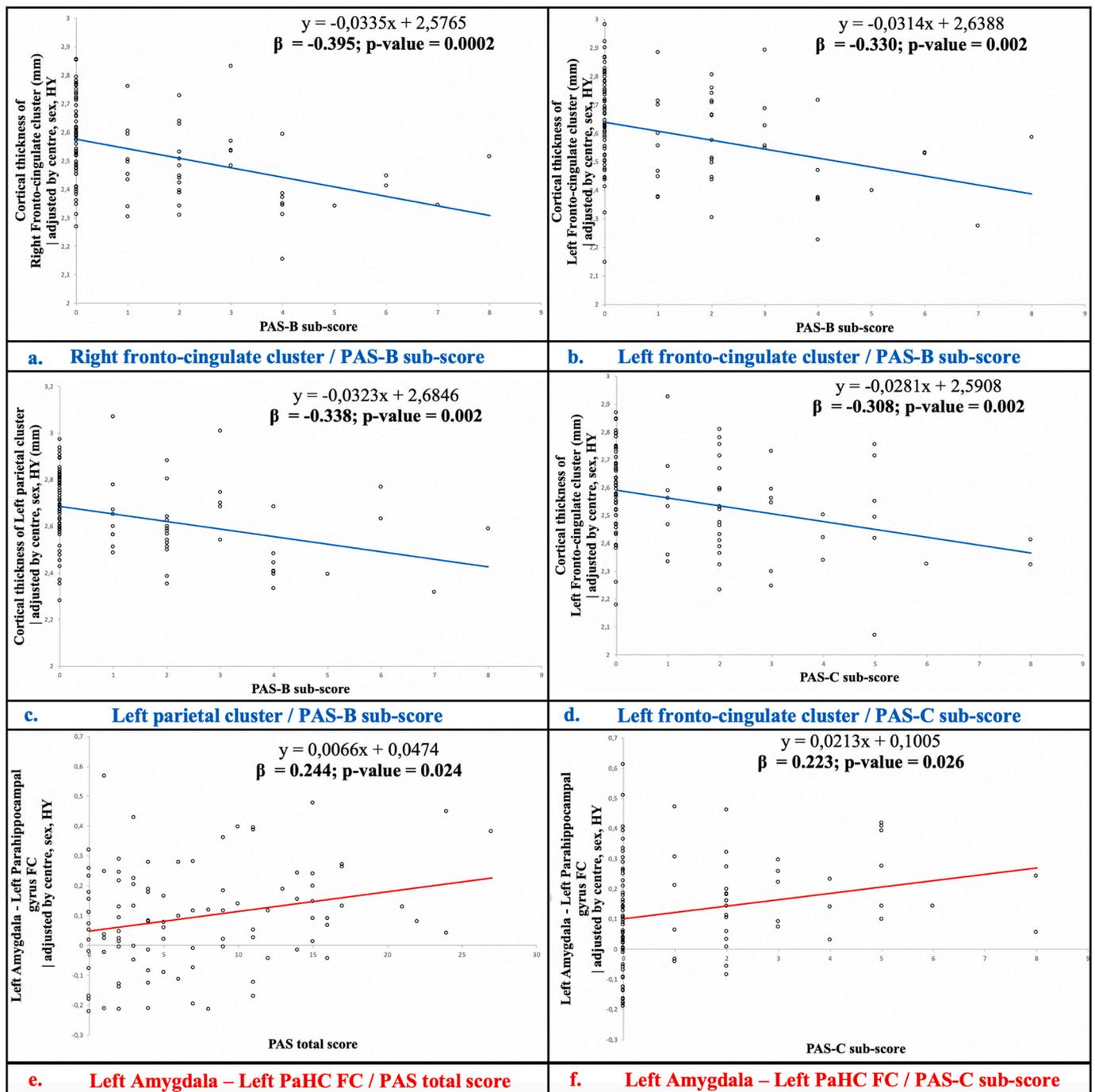


Fig. 3. Regression of the PAS score and sub-scores with the mean cortical thickness of the right fronto-cingulate (a), the left fronto-cingulate (b, d), the left parietal cluster (c) and with the functional connectivity values between the left amygdala and left parahippocampal cortex (e, f).

Abbreviation: HY = Hoehn & Yahr stage; PaHC = parahippocampal cortex; PAS = Parkinson Anxiety Scale.

#### 4.4. Anxiety is associated with clinical and cognitive features in PD

Female gender, left-sided motor symptoms onset, severity of the disease, the presence of other non-motor symptoms and higher drug use have already been associated with PD-related anxiety [26,27]. Only few studies examined cognitive features of PD patients with anxiety. In this study, “A+” patients had lower scores in attention, working memory and language. This is in line with a previous study showing that, in PD, state anxiety predicts performance in these cognitive domains [28]. Anxiety could thus worsen PD-associated cognitive dysfunctions. We hypothesize that by focusing their attention on anxiety-inducing topics, anxious patients would divert attentional resources, leading to less efficient

cognitive control. In return, these cognitive difficulties may increase anxiety. However, the place of cognitive impairment as cause or consequence of anxiety symptoms in PD remains very controversial [29, 30].

#### 4.5. Limitations

Firstly, the “A+” patients were considered to have significant anxiety symptoms according to their score at the PAS but did not have a formal diagnosis of any specific anxiety disorder according to diagnostic criteria. However, the PAS has demonstrated high sensitivity and specificity as a diagnostic test for anxiety disorders in PD [11]. Secondly,

anxiety is a continuous symptom. Interpreting it using a cut-off value could be a potential limitation since a certain proportion of subjects obtained a score close to this threshold (the distribution is shown in [Supplementary Fig. 3](#)). Thirdly, despite between-group differences, statistical analyses were not adjusted on medication status and depression. As both groups had similar severity of motor symptoms, it is highly probable that the difference in LEDD was related to anxiety. Introducing it as a covariate would have reduced the effect of anxiety. Regarding depression, only few patients had clinically relevant depressive symptoms and we considered that such correction would have distorted reality. Finally, the lack of a healthy control group did not enable us to determine which findings are specific to PD and which are for anxiety in general.

## 5. Conclusion

Structural and functional changes in the human brain fear circuit, including the amygdala, the fronto-cingulate and parietal cortex, play a role in anxiety in PD. These changes could also explain associated cognitive features, such as lower attention. However, alterations within the fear circuit are probably not the only mechanism. Further studies are needed to better explain its link with the pathophysiology of the disease.

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## Authors credits

1) Research project: A. Conception, B. Organization, C. Execution;  
 2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;  
 3) Manuscript: A. Writing of the first draft, B. Review and Critique.  
 Guillaume Carey (1A/B/C, 2A/B, 3A); Renaud Lopes (1B/C, 2B/C, 3B); Romain Viard (1C, 2B/C, 3B); Nacim Betrouni (1C, 2B/C, 3B); Gregory Kuchcinski (2B/C, 3B); Quentin Devignes (2B/C, 3B); Luc Defebvre (1A, 2C, 3B); Albert FG Leentjens (1A, 2C, 3B); Kathy Dujardin (1A/B, 2A/C, 3B).

## Trial registration

Clinical Trials Identifier: NCT01792843.

## Data availability

Data supporting the findings of this study are available from the corresponding author, upon reasonable request.

## Ethics

Written informed consent was obtained from all participants after full information of the procedure. The study was approved by the institutional ethics committees (CPP Nord-Ouest IV, 2012-A 01317–36). Additional information on this study group is detailed in the original paper.

## Disclosure of competing interest

No author had conflict of interests related to this study.

G. Carey was employed by Lille University Medical Center and received the EURON mobility grant which was not related to this study.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parkreldis.2020.09.020>.

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