Imaging Anxiety in Parkinson's Disease

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CONTENTS

CHAPTER 1

General Introduction

Parkinson's disease

Parkinson's disease is a neurodegenerative disease first described by James Parkinson in 1817. It is characterised by selective degeneration of dopaminergic neurons in the *pars compacta* of the substantia nigra in the midbrain and by eosinophilic cytoplasmic inclusions in the surviving neurons of the central nervous system [1,2]. These inclusions, called Lewy bodies, are mainly composed of aggregated misfolded forms of alpha-synuclein. It is hypothesized that these histopathological phenomena appear first in other structures (e.g. vagal nerve nucleus, olfactory bulb, raphe nuclei) and subsequently spread to the *pars compacta* of the substantia nigra before the onset of the clinical symptoms. It would then gradually spread to the cortex [3]. It has been recently shown that even at early disease stages, similar pathological changes can occur in other organs, including the skin, colon, and salivary glands, suggesting that Parkinson's disease is a multisystem disease [4].

Parkinson's disease is the second most frequent neurodegenerative disorder after Alzheimer's disease. Its incidence and prevalence have doubled in the past two decades. Approximately 6.1 million people were affected worldwide in 2016, according to the Global Burden of Disease Study 2016 [5]. The World Health Organization (WHO) estimated over 8.5 million individuals with Parkinson's disease in 2019. WHO also suggested that, in 2019, Parkinson's disease would result in 5.8 million disability adjusted life years, an increase of 81% since 2000, and would cause 329 000 deaths, an increase of over 100% since 2000. Parkinson's disease does not exclusively affect older people. The age of onset for almost 25% of affected individuals is before the age of 65 and, for 5–10%, it is before the age of 50 [2]. The disease classically begins, on average, between 58 and 62 years old and is more frequent in men than in women, with a sex-ratio of 1.5 [6].

For some people, a prodromal period might precede the onset of Parkinson's disease. It is characterized by symptoms such as hyposmia, sleep disorders, constipation, or neuropsychiatric disorders. The prodromal symptom with the highest risk of conversion to Parkinson's disease is the idiopathic Rapid Eye Movement (REM) sleep behaviour disorder. New evidence suggests that the prodromal period can start up to 20 years before the onset of motor symptoms [2].

Parkinson's disease is diagnosed clinically, based on patient history and neurological examination. It is classically considered that the diagnosis is made after 50-60% loss of dopaminergic cells. The International Parkinson and Movement Disorder Society's diagnostic criteria for Parkinson's disease guides the clinicians in establishing the diagnosis [7]. Parkinson's disease is characterised by motor symptoms, namely bradykinesia associated with rigidity or/and rest tremor. These symptoms must have a beneficial response to dopaminergic therapy. Absolute exclusion criteria and red flags must be

systematically assessed [7]. Moreover, Parkinson's disease is also characterised by non-motor symptoms such as dysautonomic (hypersialorrhea, orthostatic hypotension, bladder disorders, etc.), sensory-painful, sleep, and cognitive symptoms (notably attentional and executive disorders) as well as behavioural disorders (depression, anxiety, apathy, hallucinations, psychosis, etc.) [1]. These non-motor symptoms contribute substantially to the disability of affected individuals and the loss of quality of life [8]. After several years, motor and non-motor fluctuations as well as complications due to medication appear and lead to difficulties in treatment management. Finally, the disease progresses to a motor and/or non-motor decline [1].

The management of motor symptoms in Parkinson's disease is based on symptomatic treatments with dopaminergic supplementation (levodopa, dopaminergic agonists), exercise and deep brain stimulation. The management of non-motor signs is based on specific drugs and non-drug treatments depending on the symptoms. The treatment of Parkinson's disease is thus personalized and adapted to the symptoms of each patient [1,2]. So far, there is currently no treatment to stop or slow down the progression of the disease. In 2023, more than 60 "disease modifying treatments" clinical trials (phase I, II or III) were underway worldwide [9].

In this work, we choose to focus on anxiety symptoms which are frequent and disabling non-motor symptoms in Parkinson's disease.

Anxiety in Parkinson's disease

Among the Parkinson's disease related neuropsychiatric disorders, anxiety is one of the most frequent. The prevalence of anxiety disorders in Parkinson's disease ranges from 25 to 43% [10] with a lifetime point prevalence estimated to 31%, higher than reported in the general population or medically ill patients without Parkinson's disease [11]. To compare, anxiety disorders currently affect 4% of the world's population with 301 million people worldwide affected in 2019 [12]. In France, the lifetime prevalence of anxiety disorders was estimated at 21,6% (18-65 years old) [13] and in the Netherlands at 28,6% (18-75 years old) [14]. As in the general population, several subtypes of anxiety have been described in Parkinson's disease, such as generalized anxiety, agoraphobia, social phobia, panic attack and not otherwise specified (NOS) anxiety (Figure 1) [15,16]. These different subtypes often appear concomitantly forming "multiple anxiety disorders". The co-existence of "multiple anxiety disorders" is probably artificial and could be the consequence of an imperfect definition of the spectrum of anxiety disorders by the *Diagnostic and Statistical Manual of mental disorders, 5th edition* (DSM-5). Interestingly, NOS anxiety disorders could be the most frequent subtype of anxiety disorders in Parkinson's disease [17]. Patients diagnosed with NOS anxiety experience clinically significant anxiety that does not meet DSM-5 criteria [18]. NOS anxiety has a mean prevalence of 14.9% in Parkinson's

disease. It refers to anxiety related to Parkinson's disease symptoms such as fluctuating anxiety (non-motor fluctuations), fear of falling or driving, social phobia related to potentially embarrassing symptoms (drooling, dysarthria, …), anxiety related to withdrawal of dopaminergic medication or to wearing-off of medication in patients with fluctuations, panic-like disorder related to "OFF" periods, anxiety of dyskinesia and even anxiety related to cognitive symptoms. Finally, adverse effects of dopaminergic medication can also generate significant anxiety as in patients with impulse control disorders, dopaminergic dysregulation syndrome, or hallucinations [16,18,19].

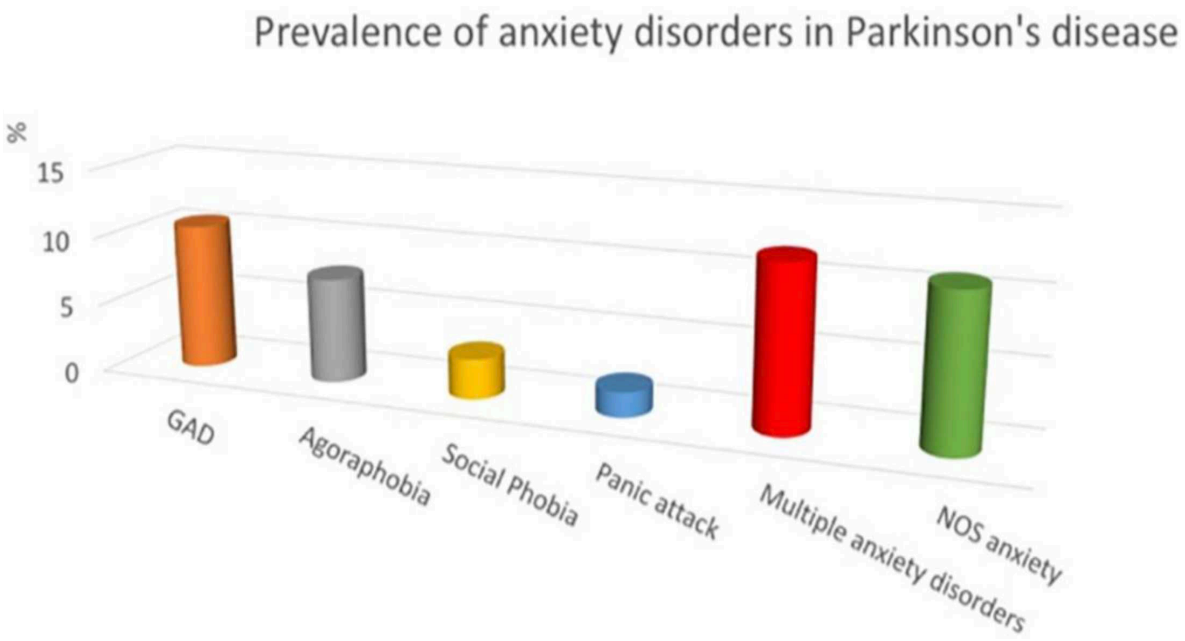

Figure 1. Prevalence of anxiety disorders subtypes in Parkinson's disease.
Abbreviations: GAD = generalized anxiety disorder; NOS = not otherwise specified. Adapted from Dujardin et al, 2020 [16]

Anxiety in Parkinson's disease is more frequent in women and in people with a previous history of anxiety. It is associated with worse motor symptoms, increased functional disabilities, and reduced quality of life [15]. Anxiety symptoms in patients with Parkinson's disease are also associated with motor fluctuations. In a cross-sectional observational study, patients with motor fluctuations suffered more from generalized anxiety disorders than those without. Interestingly, most anxious patients with motor fluctuations reported that anxiety symptoms had no specific temporal relationship with specific motor states. In cases for which a relationship was reported, symptoms were almost always related to 'off' periods and not to 'on' or "on with dyskinesia" periods. This study highlighted that the relationship between anxiety and motor fluctuations in Parkinson's disease is more complex than can be explained by wearing off phenomena of levodopa only [20]. Several studies confirmed these findings [10,21,22]. Other specific symptoms of Parkinson's disease have been associated with anxiety such as freezing of

gait, imbalance and walking disabilities or cognitive disorders, even at an early stage of the disease [23–25]. Therefore, it is now clear that anxiety symptoms have a major impact on the motor and non-motor symptoms of Parkinson's disease. In this manuscript, we will refer to this specific anxiety by using the term of "Parkinson's disease related anxiety".

If anxiety symptoms in Parkinson's disease are related to the pathophysiology of the disease and differ from anxiety in the general population, an important challenge was to identify the best way to assess and diagnose these disorders. Several non-specific scales have been used for this purpose [26]. The Parkinson Anxiety Scale (PAS) has been developed in 2014 to specifically assess Parkinson's disease related anxiety [27] and is recommended in clinic and research.

Another challenge in Parkinson's disease is to find ways to manage and treat anxiety symptoms [28,29]. The use of dopaminergic treatments (levodopa, dopaminergic agonists, and enzymatic inhibitors) can help to improve anxiety symptoms by reducing motor symptoms and motor fluctuation but have no direct effect on anxiety. Similar as in the general population, pharmacological treatments are currently used in order to reduce anxiety in Parkinson's disease such as benzodiazepines (diazepam, clonazepam, buspirone), selective serotonin reuptake inhibitors (fluoxetine, sertraline, etc.), selective noradrenaline reuptake inhibitors (venlafaxine, duloxetine), tricyclic antidepressants (amitriptyline, nortriptyline, etc) or miscellaneous antidepressant drugs (agomelatine, mirtazapine) [30]. No study has specifically evaluated the efficacy of pharmacological treatment for anxiety in PD. Only few studies assessed the efficacy of pharmacological treatment for mixed anxiety and depression symptoms and failed to show any improvement according to a meta-analysis [31–33]. Some treatments, such as buspirone, seem to be slightly effective but are very poorly tolerated [33]. Parkinson's disease related anxiety can also be generated by side effects of treatment. Therefore, non-pharmacological treatment could be a preferable alternative to reduce anxiety symptoms in Parkinson's disease. Psychotherapy, mindfulness yoga, and sensory focused exercise have been suggested to be effective in reducing anxiety symptoms in Parkinson's disease patients without any side-effect [33]. A recent randomized controlled trial assessed the efficacity of cognitive behavioural therapy (CBT), compared to a clinical monitoring only, to reduce anxiety symptoms in Parkinson's disease patients suffering from clinically significant anxiety. Firstly, the authors found a reduction of anxiety symptoms in both groups meaning that assessing and managing anxiety, regardless of the way, lead to improvement of the symptoms. Secondly, they found that CBT was more effective than clinical monitoring only to reduce anxiety symptoms in patients with Parkinson's disease which was especially the case for episodic anxiety and avoidance behaviour [34,35]. Even if for some authors there is not enough evidence about treatment of anxiety in Parkinson's disease to make recommendations [32], others recently suggested a complex algorithm to manage these symptoms [36].

Anyway, a better knowledge of the underlying mechanisms of anxiety in Parkinson's disease is necessary to better diagnose and manage them. For this purpose, we firstly define the terms "fear", "anxiety" and "anxiety disorders".

From fear to anxiety disorders

The distinction between fear and anxiety is unclear. One popular distinction is that fear occurs in response to a specific object while anxiety does not have a specific eliciting stimulus [37,38]. According to the DSM-5, fear is the emotional response to real or perceived imminent threat (e.g. the need to escape from a dangerous situation), whereas anxiety is anticipation of future threat (e.g. anxiety about failing a job interview). Even though these two states overlap, they also differ. Fear is associated with surges of autonomic arousal necessary for fight or flight, thoughts of immediate danger, and escape behaviours. Anxiety is associated with muscle tension and vigilance in preparation for future danger as well as cautious or avoidant behaviours [39,40]. So far, no official definition of fear and anxiety exists.

In this work, we define fear as an emotion which leads to an alert state to cope with a real or supposed threat. It is a survival mechanism. It can lead to somatic reactions such as muscle tension, sweating, tremor, and psychological reactions such as fear, tension, nervousness or worry. These reactions refer to anxiety. According to *Tovote et al.* [41], fear and anxiety elicit defensive behavioural responses that have evolved to enable the organism to avoid or reduce harm and thus ensure its survival. In humans, excessive fear and/or chronic anxiety are major burdens on both affected individuals and, because of their high prevalence, society in general.

Distinction between fear, anxiety and anxiety disorders is also unclear. According to the DSM-5, anxiety disorders include disorders that share features of excessive fear and anxiety and related behavioural disturbances. The anxiety disorders differ from one another in the objects or situations that induce fear, anxiety, or avoidance behaviour, and the associated cognitive ideation. While the anxiety disorders tend to be highly comorbid with each other, they can be differentiated by examination of the situations that are feared or avoided and the content of the associated thoughts or beliefs. Anxiety disorders differ from developmentally normative fear or anxiety by being excessive or persisting beyond developmentally appropriate periods. They differ from transient fear or anxiety, often stress-induced, by being persistent (e.g., typically lasting 6 months or more). Individuals with anxiety disorders typically overestimate the danger in situations they fear or avoid [39,40,42].

In adults, DSM-5 classically divides the anxiety disorders in specific phobia, social anxiety disorder (social phobia), panic disorder, agoraphobia, generalized anxiety disorder, substance/medication-induced anxiety disorder, anxiety disorder due to another medical condition, other specified anxiety disorder and unspecified anxiety disorder. Obsessive-compulsive disorders and post-traumatic stress

disorders are no more considered as anxiety disorders according to the DSM-5 [42]. To develop novel strategies to alleviate the burdens of anxiety disorders, neuroscientists are studying the neural substrates and mechanisms that underlie fear and anxiety in both animal models and humans.

Underlying mechanisms of fear and anxiety in normal brain

The limbic system has long been considered involved in emotions and more particularly in fear and anxiety. At this time, the limbic system referred to a neural system including the septo-hippocampal system, the Papez circuit, the prefrontal cortex, and forebrain structures [43]. Today, the limbic system refers to the above-mentioned structures plus the amygdala, insula, cingulum, and hypothalamus, which are organized together in networks involved in behaviours and emotions processing. More particularly, the amygdala is considered an interface between external sensory stimuli and motor, behavioural and cognitive responses linked to fear and anxiety [44].

A. The amygdala: a central hub for regulation of fear and anxiety

The amygdala is a bilateral structure located in the dorsomedial part of the temporal lobe. It is involved in different functions, including emotional processing, and has a pivotal role in memory-related, feeding, and appetitive behaviours [45]. It is composed of heterogeneous nuclei, defined primarily by their distinct cytoarchitecture, neurotransmitters, and connectivity patterns [46]. Different atlases exist but the most clearly identified amygdala subnuclei are the lateral (LA), the basolateral (BLA) and the central (CeA) nucleus, respectively. The LA receives inputs from visual, auditory, and somatosensory (including pain) systems [45]. The BLA receives afferents from the prefrontal, the anterior cingulate and parietal cortex and from the thalamus. It plays a role in acquisition and expression of the fear conditioning as well as the control of emotions (Figure 2). The BLA and the LA project efferences to the CeA and to an intermediate area of GABAergic interneurons described as the medial intercalated cells' nucleus (mITC) [45,47]. The CeA is involved in somatic and behavioural responses to fear and anxiety as well as in pain, cognition, motivation, and different functions related to food consumption. This subnucleus includes lateral (CeL), capsular (CeC) and medial (CeM) subdivisions. It receives inputs from the BLA/LA/mITC and from the ventral tegmental area, locus coeruleus, nucleus tractus solitarius, periaqueductal grey area, bed nucleus of the stria terminalis (BNST), subthalamic nucleus, substantia nigra and dorsal raphe nucleus. The CeA projects outputs to the hypothalamus and brainstem nuclei, such as BNST, ventral tegmental area, parabrachial nucleus, nucleus tractus solitarius, dorsal vagal complex, periaqueductal grey area, and substantia nigra [45,47,48]. Figures 1 summarizes the amygdala connections.

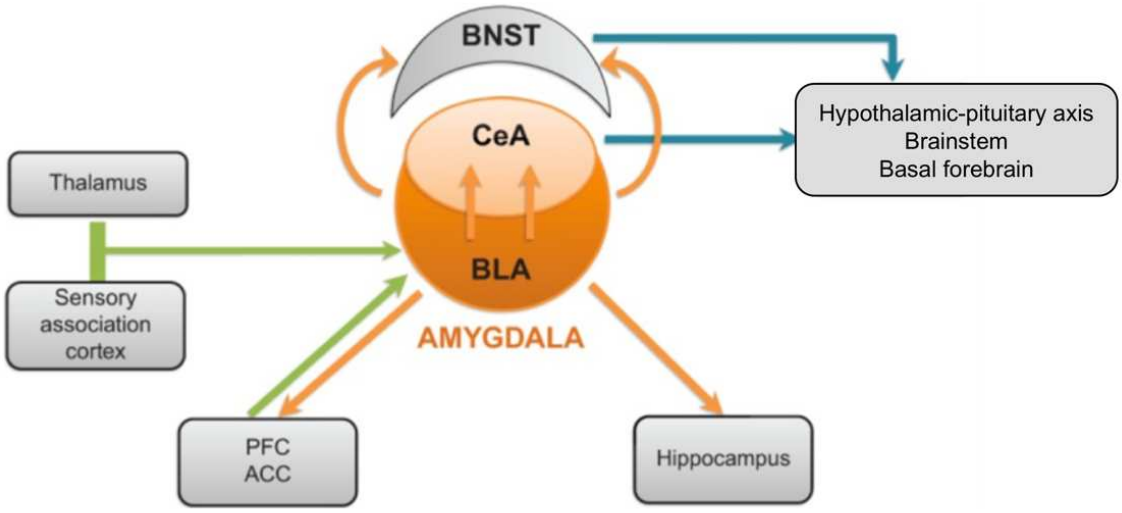

Figure 2. Connectivity between amygdala and other brain structures
Abbreviations: ACC = anterior cingulate cortex; BLA = Basolateral amygdala; BNST = Bed nucleus of stria terminalis; CeA = Central amygdala; PFC = prefrontal cortex. Adapted from Gauthier and Nuss, 2015 [47]

B. Other subcortical structures involved in fear and anxiety

Among the subcortical areas cited before, some could play a particular role in fear and anxiety regulation. The locus coeruleus/subcoeruleus would be involved in the genesis of panic, stress, and motor response to fear [49]. The periaqueductal grey area would be involved in response to aversive stimuli. The dorsal periaqueductal grey area could be involved in flight and defence while its ventral part would be involved in immobilization and prostration [50].

Dysfunction of amygdala and, more precisely, dysfunction of the interconnexion between the amygdala and the BNST could be involved in anxiety disorders pathophysiology in the general population [51–53]. Classically, together the BNST and the CeA, and sometimes the nucleus accumbens shell, are considered as an entire structure named the central extended amygdala which is known to be involved in processing of stressful stimuli [54]. Anther limbic and hormonal structure has been involved in anxiety symptoms, the hypothalamus. It could activate the adrenergic system and release peptides and hormones in response to stress factors [55]. Finally, the septo-hippocampal system would be involved in the recognition of familiar situations, in behaviours of avoidance and apprehension of danger and would be part of a circuit involved in the learning and memory of fear [56,57].

These different structures are connected and organized in functional circuits. It is hypothesized that fear and anxiety would be regulated by different circuits that may be in disbalance in anxiety disorders.

C. The fear circuit: fear processing and regulation

The underlying mechanisms of anxiety disorders in the general population have been explored by studies in animals and humans. A complex functional network including some of the previously mentioned structures and the cerebral cortex has been described in animal studies and named “the fear circuit”. This circuit could be involved in fear conditioning and fear processing [41].

In humans, functional imaging studies have validated the existence of such a circuit, centred on the amygdala, with connections to cortical areas, such as the medial prefrontal cortex (PFC), the anterior cingulate cortex (ACC), the insula, and subcortical structures, such as the striatum hippocampus and thalamus (Figure 3). Some authors also included parts of the temporal and parietal cortex [58–60].

The fear circuit is involved in fear and anxiety processing. This functional circuit would be activated by stressful stimuli and anxiety inducing situations to trigger an emotion and a somatic reaction. In theory, a chronic overactivation of this circuit could lead to anxiety disorders [60]. In a systematic imaging review, the authors found patterns of hyperactivation in the fear circuit structures across different anxiety and stress disorders. These dysfunctions also differed among the type of anxiety disorders and, interestingly, a greater involvement of the fear circuit was reported in panic disorder and phobia [61]

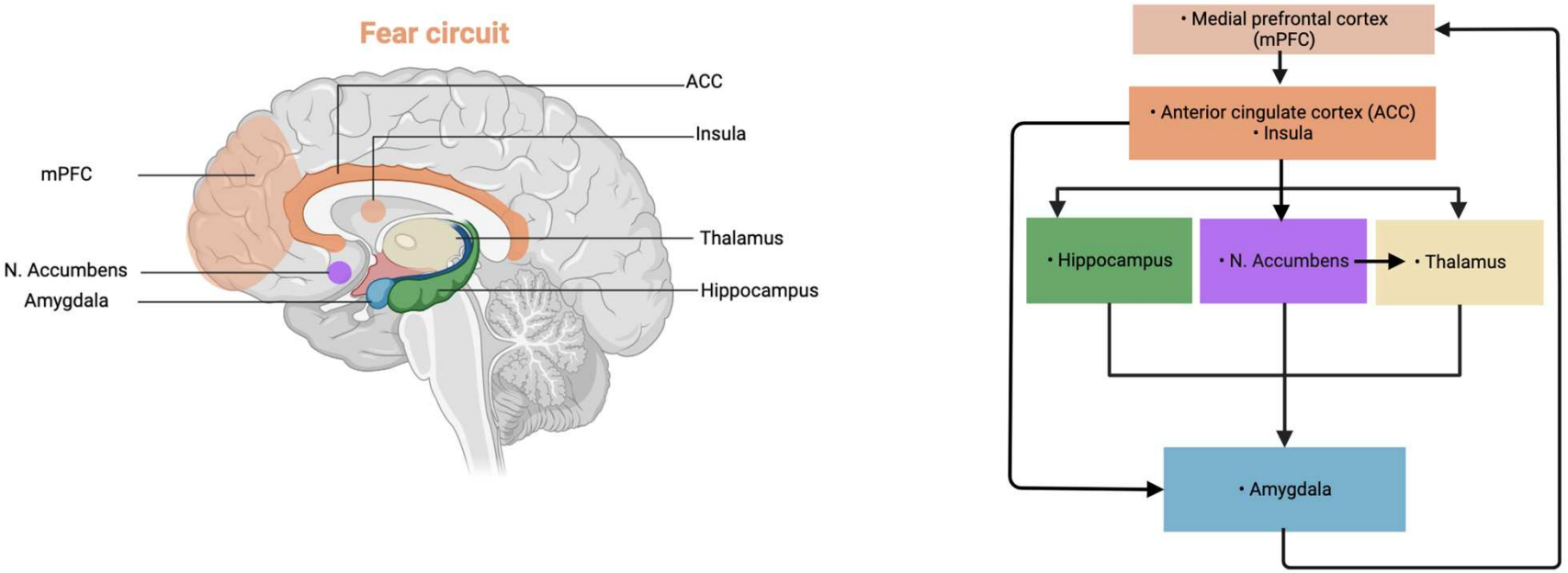

Figure 3. The schematic representation of the fear circuit in humans. *Adapted from* [62]

D. The cortico-striato-thalamo-cortical circuit: basal ganglia and emotion

The basal ganglia are involved in movement control but also in associative learning, planning, working memory, and emotion through several functional cortico-striato-thalamo-cortical circuits (Figure 4) [63]. The motor circuit involves the motor and supplementary motor cortices, the posterolateral part of the putamen, the posterolateral external globus pallidus (GPe) and internal globus pallidus (GPi), the dorsolateral subthalamus nucleus and the ventrolateral thalamus and would be involved in motor control. The associative loop connects the dorsolateral PFC with intermediate areas of the GPe, GPi, subthalamus nucleus and the thalamus and would be involved in cognitive control. Finally, the limbic loop connects the anterior cingulate and OFC cortices with ventromedial regions of the basal ganglia and the thalamus and is involved in emotion and behaviour control. Several studies showed that dysfunction of this limbic loop in neurological and psychiatric diseases (Parkinson's disease, obsessive-compulsive disorder) could be associated with neuropsychiatric symptoms such as depression and anxiety [64–66].

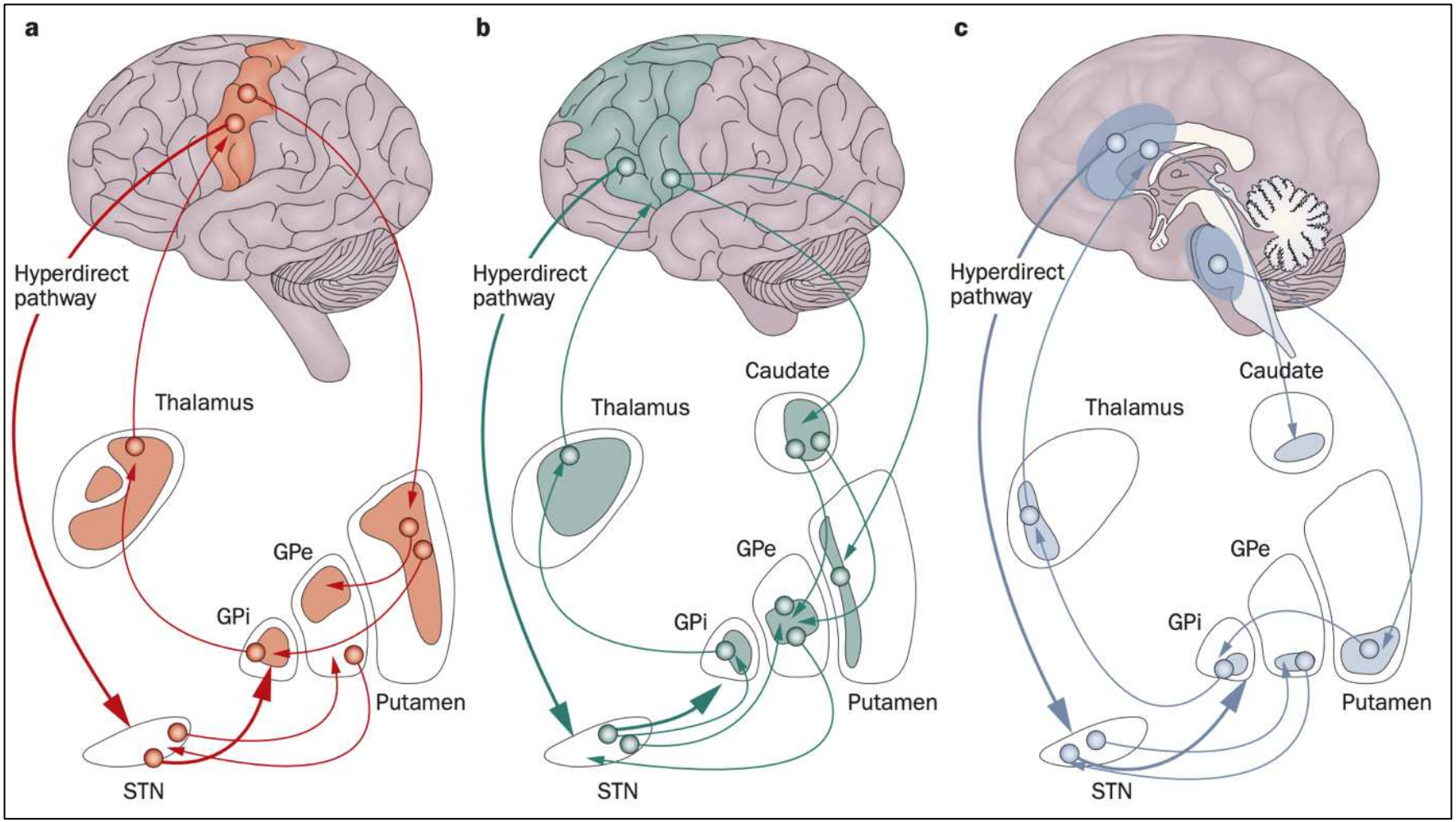

Figure 4. The cortico-striato-thalamo-cortical circuits with a. the motor loop, b. the associative loop and c. the limbic loop. *Figure adapted from Volkmann et al, 2010* [64].
Abbreviations: GPe = external globus pallidus; GPi = internal globus pallidus; STN = subthalamus nucleus.

E. Neurotransmitters in fear and anxiety regulation

Fear and anxiety processing is also modulated by several neurotransmission systems and hormones. The gamma-aminobutyric acid (GABA) system plays a significant role in modulation of the subnuclei of the amygdala, notably with the medial intercalated cells' nucleus and within the CeA subnucleus, as well as in modulation between amygdala and medial PFC. It exerts an inhibitory function between these structures in order to modulate the fear reaction to stressful stimuli [47].

Anxiety and fear are often associated with somatic manifestations, such as tachycardia, gastro-intestinal disorders, nausea, palpitations, dizziness, restlessness, nervousness, fatigue, muscle tension, sweating, sleep disorders, etc. These manifestations could be mainly mediated by the norepinephrine system of the locus coeruleus/subcoeruleus complex. The connections between locus coeruleus/subcoeruleus complex and BLA could play a main role in modulating these somatic manifestations but also in generating anxious behaviour and anxiety disorders [67–69]. The locus coeruleus/subcoeruleus complex plays also a role of mediation with the hypothalamic-pituitary-adrenal axis and some authors argue for a neuroendocrine modulation of anxiety. Thus, the hypothalamic corticotropin-releasing hormone neurons would play a significant role in controlling anxiety-like and stress-induced behaviours [70,71]. Furthermore, endogenous locus coeruleus inputs of corticotropin-releasing hormone from the amygdala could induce anxiety-like behaviours. Thus, locus coeruleus and amygdala also play a role in this neuroendocrine regulation of anxiety and fear [67,71,72]. Some authors also discussed the role of several hormones in anxiety, including neurosteroids, gut peptides, neuropeptides and hormonal signals derived from fatty acids [70]. Among these hormones, recent studies highlighted the role of oxytocin, a neuropeptide synthesized by the hypothalamus, in anxiety disorders. This hormone is known to play a role in human social behaviour, social cognition, anxiety, mood, stress modulation and fear learning and extinction. Anxiolytic effects of oxytocin have been found in preclinical and clinical studies, possibly related to its neurobehavioral mechanisms (social cognition, fear learning, and extinction) and its modulation of other neurotransmitter and neuroendocrine systems (hypothalamic-pituitary-adrenal axis, serotoninergic, and GABAergic systems) [73,74].

The serotoninergic system also plays a role in psychiatric manifestations and especially in anxiety and mood disorders. Serotonergic neurons are located in the dorsal and median raphe nuclei, part of the reticular formation in the brainstem. The expression of anxiety-like behaviours could be supported by the interaction between both dorsal and median raphe nuclei with the dorsal hippocampus. Serotonin (5-hydroxytryptamine, 5-HT) receptors have been detected in basal forebrain, amygdala and BNST. In preclinical studies, activation of the 5-HT receptors enhanced fear and anxiety. It is suggested that 5-HT might interact with other neurotransmission systems, such as glutamatergic or dopaminergic systems, but its role in anxiety disorders is not clearly understood yet. It could also be involved in an

inhibitory circuit of anxiolytic processes dependent on corticotropin-releasing hormone. However, 5-HT receptors are common therapeutic targets in anxiety disorders and the serotonin reuptake inhibitors (selective or not) are often used to treat anxiety symptoms [75–77].

Finally, dopamine could also play a role in fear and anxiety. In a recent review [78], the authors explained that excitation of the meso-cortico-limbic pathway, originating from dopaminergic neurons of the ventral tegmental area, would be relevant for the development of anxiety. The amygdala could be an essential component of the neural circuitry of conditioned fear. The dopamine D2 receptor-signalling pathway connecting the ventral tegmental area to the BLA could modulate fear and anxiety, whereas neural circuits in the midbrain tectum underlie the expression of innate fear. In this review, it was stated that dopamine could mediate conditioned fear by acting at the rostral levels of the brain and regulate unconditioned fear at the midbrain level. In another study, the authors stated that the activity of dopaminergic system could be modulated by other neurotransmitters, such as glutamatergic neurons from the medial PFC, GABAergic fibbers from the nucleus accumbens and the ventral pallidum and cholinergic fibbers from the pedunculopontine nucleus and the latero-dorsal tegmental nucleus. Thus, changes in the mesolimbic, mesocortical and nigrostriatal dopaminergic system may influence anxiety-like behaviour [79]. According to other authors, the dopaminergic activity of the nucleus accumbens could play a role in fear and anxiety regulation by modulating the glutamatergic activity in PFC [80]. Therefore, the nucleus accumbens would have a central role in the dopaminergic regulation of anxiety in the brain.

These findings are mainly from animal studies. Other methods are necessary to explore the underlying mechanisms of anxiety in humans, such as neuroimaging methods which are powerful tools for this purpose.

Neuroimaging to explore anxiety mechanisms

In the last decades, improvement in brain imaging techniques made it possible to better explore the underlying mechanisms of neurodegenerative and psychiatric disorders. Molecular imaging, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging, measure brain activity. Magnetic resonance imaging (MRI) is largely used in clinic and research to assess neurodegenerative and psychiatric disorders. It allows structural (T1 and diffusion tensor imaging (DTI)) as well as functional (resting-state functional MRI (rs-fMRI)) analyses but also detection of specific biomarkers of diseases. Recently, high field MRI scanners such as 7-Tesla MRI, allowed a more precise assessment of the brain structure and functioning using the sequences and methods mentioned before. New insights of research are opening thanks to these new methods [81].

A. Imaging studies in anxiety disorders

Several neuroimaging studies explored the underlying mechanisms of anxiety disorders in the general population. Some results were associated to anxiety disorders in general, others to some anxiety subtypes.

In studies using structural imaging methods, anxiety disorders were associated with a reduction of grey matter volume in the amygdala and the hippocampus and with a lower cortical thickness in the ACC, the OFC, the inferior and middle frontal cortices, and the temporal lobe [82]. In a recent systematic review, anxiety disorders were associated with a reduced cortical thickness in the medial OFC and the ventromedial PFC and an increased cortical thickness in the insula, the temporo-parietal areas and the ACC. Interestingly, these changes occurred more on the left than the right hemisphere. The authors also highlighted that the high variability of the findings across the studies makes interpretation difficult [83]. In a recent review of DTI studies, anxiety disorders were associated with alterations within the connectivity of the cingulum and the uncinate fasciculus, a major pathway between the amygdala and the OFC, with a reduced fractional anisotropy [84].

In functional neuroimaging studies (fMRI, PET and SPECT), anxiety disorders were associated with an increased activity in the amygdala, insula and dorsal anterior cingulate and reduced activity in activity of the PFC and OFC [82,85]. Increased amygdala reactivity was restored with common treatments (pharmacological and psychological therapies). Therefore, the authors stated that reduction of neural activity in the amygdala may be a final common pathway for successful therapeutic interventions [85]. Regarding the functional networks, anxiety disorders were associated with abnormalities in resting-state networks such as a decreased functional connectivity (FC) of the default-mode network and an

increased FC in the salience and the sensory-motor network [86]. Moreover, several reviews highlighted disruptions in the fronto-limbic, fear and salience networks [82].

Finally, the underlying mechanisms seem to differ between the subtypes of anxiety. Generalized anxiety disorder was associated with an altered PFC-amygdala FC, a reduced activity in the culmen and altered FC within the salience, default-mode, and central executive networks [86,87]. Panic disorders were associated with alterations within the connectivity of the cingulum and an increased FC within the sensory-motor network, and social anxiety disorders with increased activity of parietal and occipital regions and reduced connectivity between parietal and limbic and executive network regions [88].

In general, anxiety disorders seem to be associated with grey-matter, white-matter, and functional alterations within the fear circuit and especially the fronto-limbic structures. However, these alterations would be not enough to develop anxiety symptoms and anxiety could also be provoked by other or additional mechanisms.

B. Imaging anxiety in Parkinson's disease

Despite the frequency of anxiety in PD, only few studies focused on the underlying mechanisms of anxiety in Parkinson's disease. In these studies, anxiety was often one symptom among other non-motor or neuropsychiatric symptoms. Moreover, in most of these studies, no validated scale was used to assess anxiety symptoms in Parkinson's disease. Finally, only few studies specifically focused on Parkinson's disease related anxiety.

In PET/SPECT studies, several metabolic alterations within structures involved in the fear circuit were found. Anxiety symptoms were associated with dopaminergic depletion in the striatum [89,90], reduced activity in the PFC [91] and serotoninergic dysfunctions in the thalamus [92]. In a systematic review, the authors associated anxiety, depression, and apathy with reduced serotoninergic, dopaminergic, and noradrenergic activity in the OFC, ACC, amygdala, striatum, and thalamus [93].

In fMRI studies, anxiety symptoms were associated with reduced functional connectivity between the striatum and the OFC and with increased functional connectivity between amygdala and prefrontal, temporal and parietal cortices in Parkinson's disease patients [94–96].

A longitudinal structural MRI study brought out a correlation between worsening of anxiety symptoms over 18 months and the volume of the ACC and reduction in the cortical thickness of the precuneus in patients with Parkinson's disease [97]. Other authors found a correlation between the severity of anxiety symptoms and reduction of the volume of the left amygdala in Parkinson's disease patients. These authors stated that the amygdala could be a central structure involved in Parkinson's disease related anxiety [98].

Although these studies are in line with the hypothesis of an involvement of the fear circuit and the basal ganglia circuit in Parkinson's disease related anxiety, many limitations remain in interpreting the

results. Only correlation analyses between anxiety severity, according to non-validated scales in Parkinson's disease patients, and imaging parameters were performed while the patients had no significant anxiety disorders. Therefore, these findings needed to be confirmed with further studies.

Objectives and hypotheses

The objectives of this work were:

(1) To better understand the underlying mechanisms of Parkinson's disease related anxiety, using multimodal MRI approach including structural, functional and diffusion methods.

We hypothesize that Parkinson's disease related anxiety might be associated with structural and functional alterations within the fear circuit as well as within the limbic cortico-striato-thalamo-cortical circuit. Moreover, we hypothesize that imbalance between these two circuits with domination of the fear circuit may be implied in the pathophysiology of anxiety in PD, and that overlapping structures that are part of these two circuits play a pivotal role in this.

(2) To decipher the mechanisms by which cognitive behavioural therapy (CBT) is effective in reducing anxiety symptoms in Parkinson's disease [35], using functional MRI analyses.

We hypothesize that CBT can restore the balance between the fear circuit and the limbic cortico-striato-thalamo-cortical circuit by strengthening the functional connectivity within the limbic circuit.

(3) To validate our previous findings with ultra-high-field (7-Tesla) MRI data.

Outline of this thesis

In **Chapter 2**, we address research aim (1) by comparing clinical, cognitive, and imaging variables between patients with Parkinson's disease with anxiety and without anxiety. We first focused on changes in the amygdala and fear circuit. We compared structural imaging data such as cortical thickness and volume, shape and texture of amygdala and functional connectivity of amygdala between the two groups.

The systematic review in **Chapter 3** addresses aim (1) by providing details and reviewing of neuroimaging data (structural, functional, and metabolic) associated with Parkinson's disease related anxiety. We conducted a systematic review and included 18 imaging studies focussing on anxiety in Parkinson's disease. This review allowed us to set the main hypotheses about the underlying mechanisms of Parkinson's disease related disorders.

In **Chapter 4**, we work towards research aim (1) by comparing structural connectivity using DTI within the fear circuit and the limbic circuit between anxious and non-anxious patients with Parkinson's disease. So far, no such study had been performed. We compared DTI parameters, such as fractional anisotropy and mean diffusivity, between anxious and non-anxious patients with Parkinson's disease. We also performed regressions between anxiety severity, according to the PAS-total score, and these parameters.

In **Chapter 5**, we collaborated with the neurophysiology research team of the Lille University to address aim (1) by analysing the electroencephalography (EEG) functional connectivity changes associated with Parkinson's disease related anxiety. Spectral and functional connectivity characteristics were compared between Parkinson's disease patients with and without anxiety.

Chapter 6 addresses aims (1) and (2) by comparing functional connectivity changes after cognitive behavioural therapy (CBT) for anxiety symptoms in patients with Parkinson's disease. This is an ancillary study of the clinical trial that showed the efficacy of CBT to improve Parkinson's disease related anxiety versus control group. Patients with Parkinson's disease and significant anxiety were included and divided into two groups: patients with CBT and clinical monitoring and a control group with clinical monitoring only. We compared the changes in functional connectivity within and between the two anxiety circuits mentioned before and the common resting-state functional networks between the two groups across the time (baseline and after the intervention). We also performed regression analyses

between these changes in functional connectivity and severity of anxiety according to the PAS-total score.

In **Chapter 7**, we address research aim (1) and (3) by focusing on the involvement of the thalamus and the thalamic subnuclei in the underlying mechanisms of anxiety in Parkinson's disease. The thalamus is one of the overlapping structures between the two anxiety circuits. Using ultra high-field (7-T) MRI scans, we extracted the mean volume of these structures and compared it between patients with Parkinson's disease with anxiety, without anxiety and healthy controls. We also performed regression analyses between the severity of anxiety according to the PAS-total score and the volume of the thalamus and the thalamic regions.

Chapter 9 summarizes and discusses the main findings. In this chapter, the strengths, and limitations of this work as well as the new insights and further projects are stated.

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CHAPTER 2

Anxiety in Parkinson's Disease is Associated with Changes in the Brain Fear Circuit

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Summary of the chapter

This study launched our work on anxiety in Parkinson's disease (PD). There were previous studies on anxiety in PD, but they used non-specific scales, often mixing depression and anxiety symptoms or included participants without clinically significant anxiety. More specific studies were thus needed to decipher the underlying mechanisms of PD-related anxiety. According to the literature, we assumed the volume of the amygdala could be reduced in PD patients with anxiety. By consequence, we decided to investigate PD-related changes in the amygdala and the fear circuit.

Using the dataset of an observational bi-centre (Lille and Maastricht) cohort of non-demented PD patients (CogPhenoPark2), we compared the demographic, clinical and cognitive characteristics of PD patients with and without clinically significant anxiety, according to their score at the Parkinson Anxiety Scale. We compared both groups using structural imaging data such as cortical thickness and volume, shape and texture of amygdala and functional connectivity of amygdala. In anxious PD patients, we found changes in the shape and texture of the left amygdala, suggesting remodelling or local atrophy, but not in the volume compared with non-anxious PD patients. We also found changes in the functional connectivity of the amygdala and of the salience network. Our results suggest that PD-related anxiety could induce structural and functional changes in the fear circuit.

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) 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.

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 behavioural 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, 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.

Materials and methods

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].

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].

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 T1-weighted (3D-T1w) images were acquired with a magnetization-prepared gradient echo sequence (voxel size: 1 x 1 x 1 mm3; 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 T2*-weighted EPI sequence (voxel size: 3 x 3 x 3 mm3; 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.

Structural MRI analysis

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.

Volume, shape and texture of amygdala

Amygdala were manually segmented on 3D-T1w images with MRICRON software to bring out any atrophy. Anatomical borders were defined by a radiologist and according to literature data [14]. Volumes were recorded in mm3 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-T1w 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 seven second-order texture parameters detailed in Supplementary Table 1.

Functional MRI analysis

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://fmriprep.readthedocs.io). At the end of this procedure, an individual quality control was performed. CONN Toolbox [18] was then used for: i) Gaussian kernel 6mm 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).

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.

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.

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.

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).

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.

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.

Results

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 Figure 1).

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).

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).

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%)	**2.84 (1.23; 6.58); p = 0.013**[a]	
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%)	**3.21 (1.31; 7.85); p = 0.009**[a]	
Left (n = 48)				
Clinical variables				
LEDD (mg/day)	937.36 (±494.38)	732.53 (±578.12)	**0.02**[a]	
Antidepressant use (n = 17)	13 (38.24%)	4 (4.76%)	**12.38 (3.66; 41.91); p < 0.0001**[a]	
Anxiolytic use (n = 12)	10 (29.41%)	2 (2.38%)	**17.08 (3.5; 83.33); p < 0.0001**[a]	
MDS-UPDRS part 1 (0–4) [§]				
1.3. Depressed mood	1 (0–4)	0 (0–4)	**0.001**[a]	
1.4. Anxious mood	2 (0–4)	0 (0–4)	**< 0.0001**[a]	
1.7. Night-time sleep problems	2 (0–4)	2 (0–4)	**0.03**[a]	
1.8. Daytime sleepiness	2 (0–4)	2 (0–4)	0.26	
1.9. Pain and other sensations	2 (0–4)	1 (0–4)	**< 0.0001**[a]	
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. Lightheadeness on standing	1 (0–3)	0 (0–3)	0.28	
1.13. Fatigue	2 (0–4)	1 (0–4)	**0.03**[a]	
MDS-UPDRS part 3 (/132)	30.2 (±14.9)	28.1 (±12.0)	0.54	
Hoehn & Yahr stage (0–5) [§]	2 (1–5)	2 (0–4)	**0.003**[a]	
PAS total (/48)	14.79 (±4.69)	3.69 (±2.87)	**< 0.0001**[a]	
Part A (/20)	9.47 (±4.32)	2.85 (±2.87)	**< 0.0001**[a]	
Part B (/16)	2.38 (±2.26)	0.42 (±0.85)	**< 0.0001**[a]	
Part C (/12)	2.94 (±2.32)	0.43 (±0.85)	**< 0.0001**[a]	
HAMD total (/54)	8.7 (±5.2)	4.5 (±3.6)	**< 0.0001**[a]	
HARS total (/56)	11.4 (±5.8)	5.3 (±4.2)	**< 0.0001**[a]	
Cognitive variables	A+ group (n = 34)	A- group (n = 84)	Uncorrected p-value	FDR-corrected p-value
Overall efficiency				
MDRS score (/144)	136 (±5.90)	138 (±6.62)	0.024	0.096
Attention and working memory				
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	**0.008**[a]
SDMT: number in 90 s	35.35 (±10.57)	44.71 (±11.91)	0.00009	**0.001**[a]
Executive functions				
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
Episodic memory				
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
Language				
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	**0.005**[a]
Visuospatial functions	10.71 (±3.26)	11.95 (±2.55)	0.08	0.128
Benton Judgment of Line Orientation				

Table 1. Demographic, clinical and cognitive variables: group comparisons (Parkinson's disease patients with (A+) and without (A-) anxiety).

Interpretation: a = FDR-corrected p-value < 0.05, § = described as median and range.

Abbreviations: 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.

Structural analyses

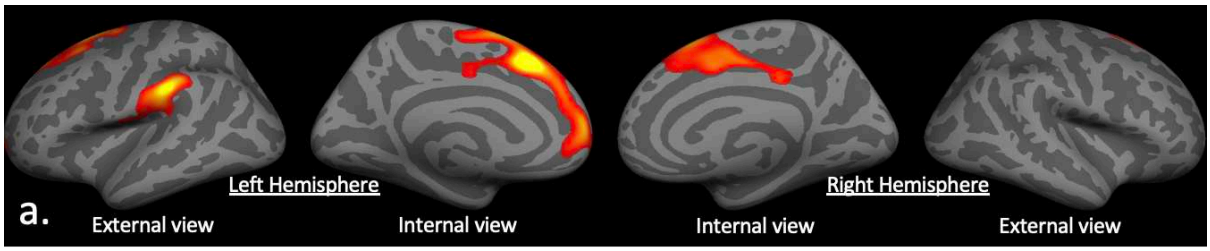
All following analyses were adjusted for sex, disease stage and centre.

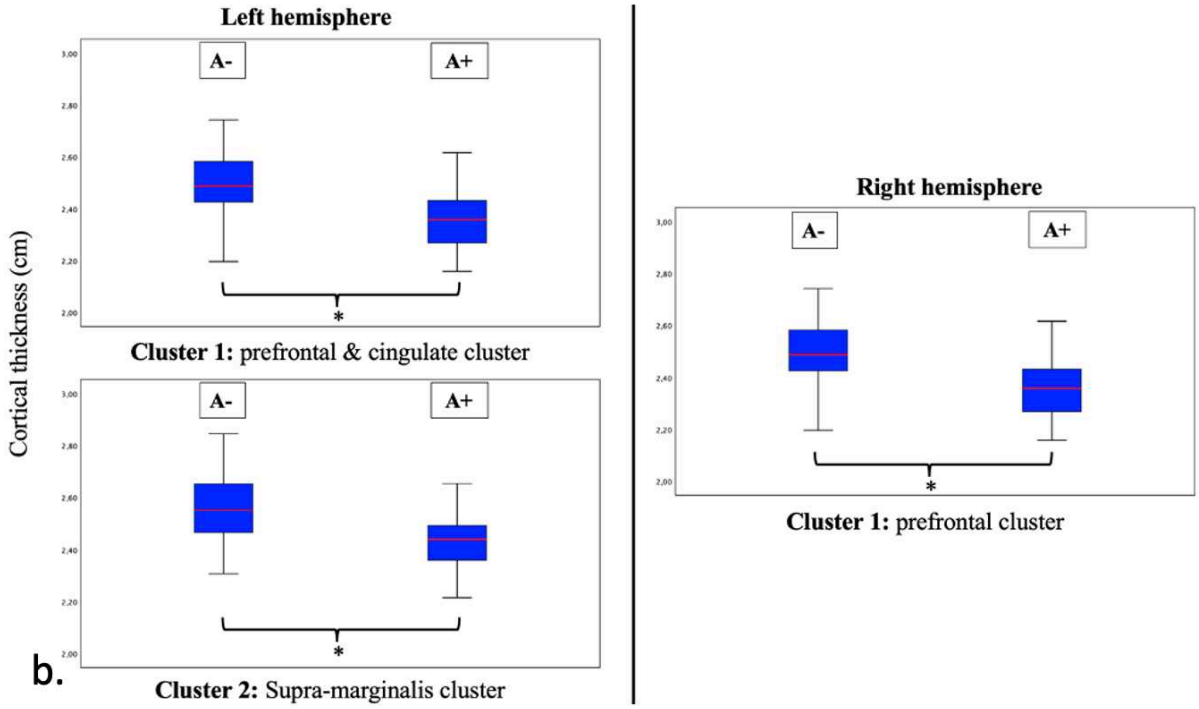
Cortical thickness

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

Volume, shape, and texture of amygdala

There was no significant group difference for the volume of amygdala (A+/A-, left: 1201 mm3 / 1284mm3; right: 1210 mm3 / 1273 mm3). Shape analysis revealed several remodelling 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) (Figure 2).





	Cluster	Size (mm²)	T-max	Talairach coordinates X	Y	Z	Location
Right hemisphere	1	2060.59	6.320	10.5	19.9	55.1	**Superior frontal gyrus** Inferior frontal gyrus (oper) Inferior frontal gyrus (tri) Middle frontal gyrus
Left hemisphere	1	4986.41	6.901	-7.4	23.3	41.5	**Superior frontal gyrus** Cingulate (anterior) gyrus Cingulate (posterior) gyrus
	2	1703.03	5.406	-60.6	-27.8	29.7	**Supra-marginalis gyrus**

c.

Figure 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.*

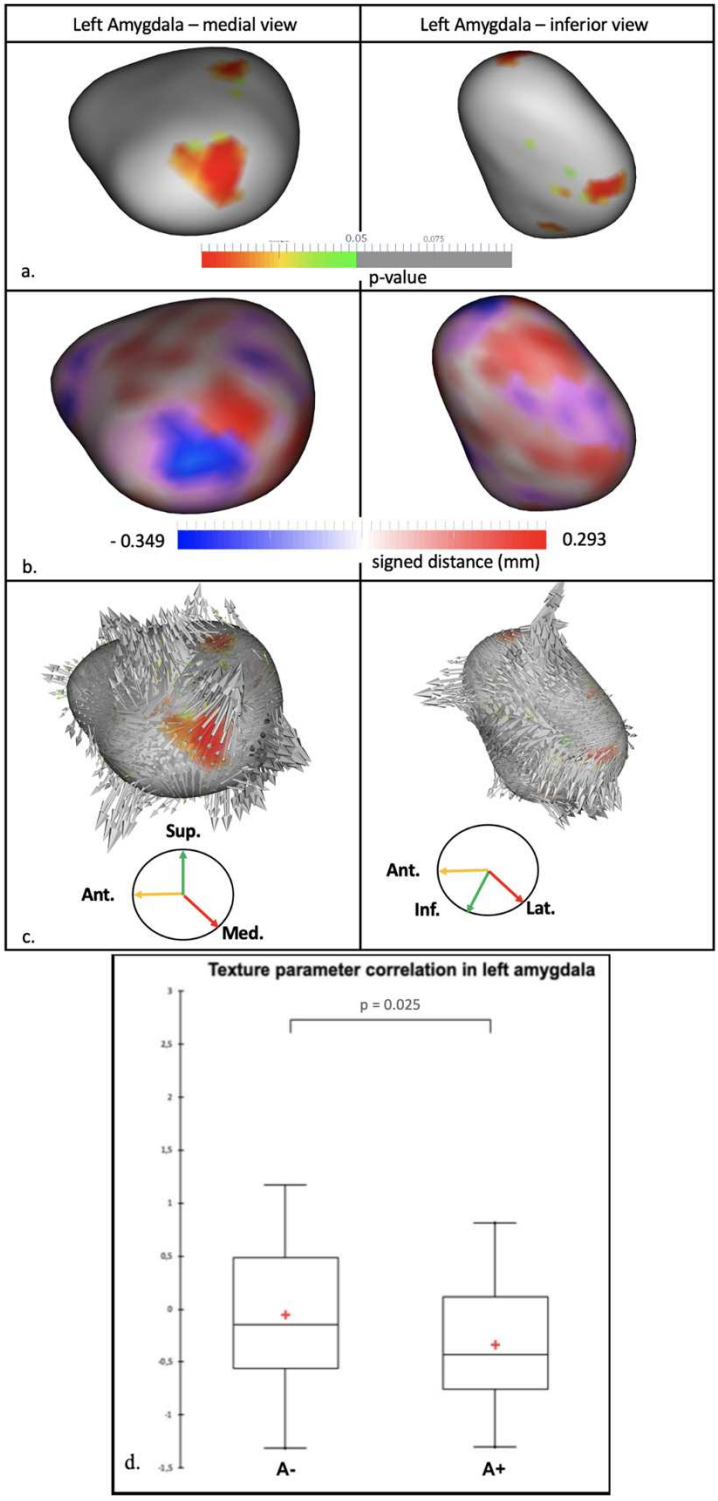


Figure 2. Anatomical changes of left amygdala in A+ compared to A- patients in PD, adjusted by center, sex and Hoehn-Yarh stage.

Shape analysis: (a)statistical map, (b) signed distance map (mm) and (c) vertex map showing significative 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.

Functional analyses

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 Figure 2 and Supplementary Table 3).

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.

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

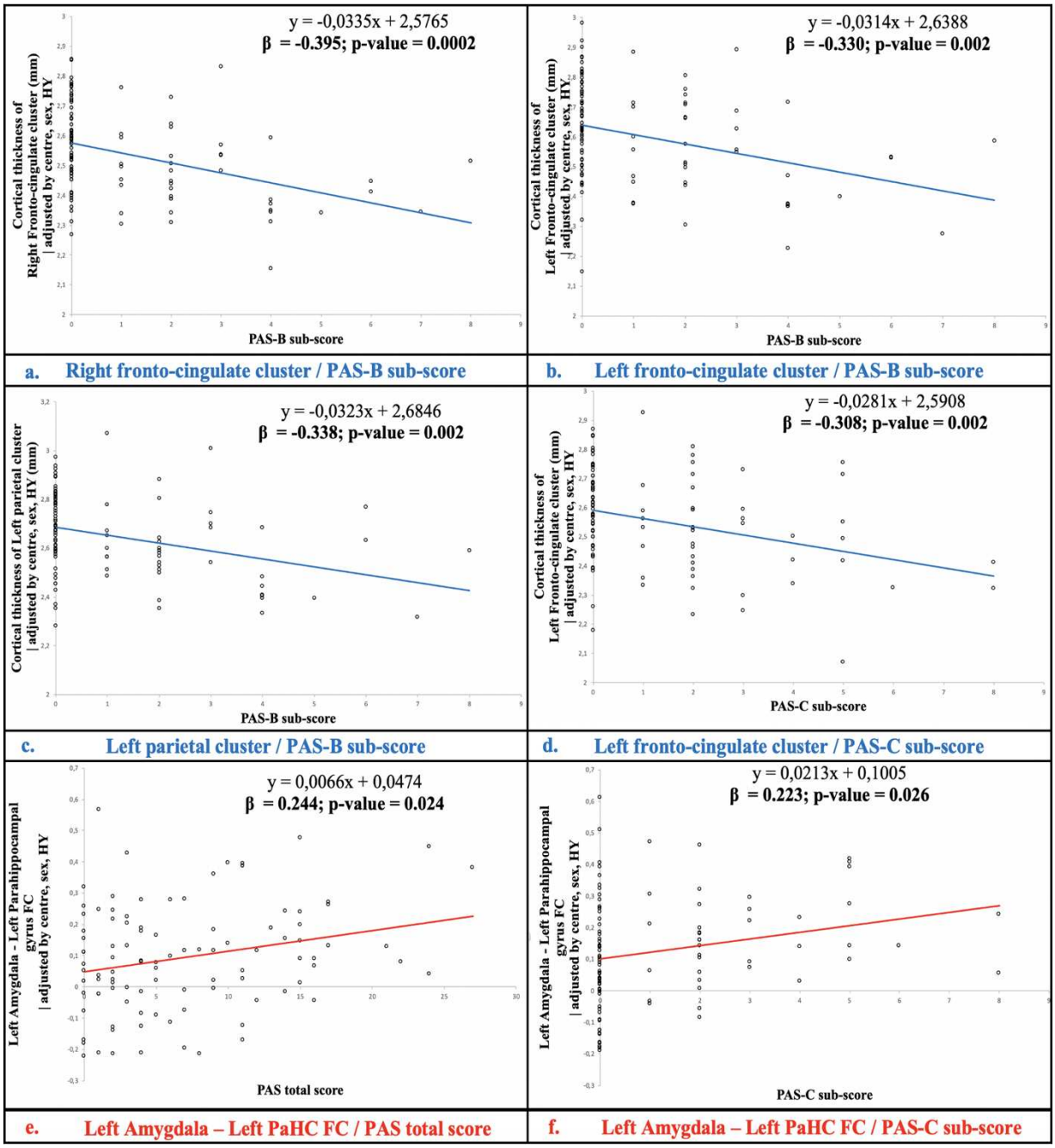


Figure3. Regression of the PAS score and sub-scores with the mean cortical thickness of the right fronto-cingulate (a), the left fronto-cingulate (b, d), the left parietal cluster (c) and with the functional connectivity values between the left amygdala and left parahippocampal cortex (e, f).

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

Discussion

The present study sought to identify anatomical and functional markers of PD-related anxiety. We observed reduced cortical thickness of the bilateral fronto-cingulate and left parietal regions, and anatomical changes of the left amygdala in PD patients with anxiety, with several remodelling 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.

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 remodelling area at the medial and postero-lateral sides of the left amygdala in the “A+” group. Moreover, this structural remodelling 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.

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 behaviour (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].

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 behaviour. 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 worse 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.

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].

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 Figure 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 reduce the effect of anxiety. Regarding depression, only few patients had clinically relevant depressive symptoms and we considered that such correction would have distort 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.

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 physiopathology of the disease.

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SUPPLEMENTARY DATA

Supplementary material

Cortical thickness

The cortical thickness was automatically calculated for each patient using the FreeSurfer 5.3 software (https://surfer.nmr.mgh.harvard.edu/) from fMRIPrep tool [1]. Internal (grey matter / white matter) and external (grey matter / cerebrospinal fluid) cortical surfaces were segmented; the cortical thickness was measured by plotting the normal at all points between the two surfaces, and a Gaussian smoothing of 10 mm was applied. In order to eliminate any segmentation error, each surface was inspected visually and manually corrected if necessary.

Segmentation of amygdala

Firstly, a random sample of 40 amygdala were segmented blindly of the A+/A- status by two investigators (GC and GK – i.e. the first author and an expert in neuroimaging) as a training step. After discussion and according to literature data [2,3], the anatomical borders of the amygdala on 3D-T1w images were defined by these two ratters in order to fix the errors and discrepancies. Finally, the amygdala of all the patients were segmented by GC and a decision of the radiologist (GK) was required if there was any doubt. Volumes were recorded in mm3 and normalized to the patient's total intracranial volume estimated by FreeSurfer 5.3 software. A FreeSurfer semi-automatic segmentation was also performed on this sample of 40 amygdala. As the volume of amygdala using the manual segmentation (1.25 cm3) was closer to the real size of the amygdala (1.24 cm3) than using the semi-automatic one (1.12 cm3) according to the post-mortem histological study [4], the manual segmentation was chosen for the present study. These discrepancies could also be due to the fact that the amygdala mask of FreeSurfer has been developed from MRI of healthy subjects and not PD patients.

Texture analyses

Feature	Description	Formula
First-order statistics		
Mean	Mean value of the image grey level	
SD	Standard deviation of grey level values	
Kurtosis	Kurtosis is a measure of whether the data are heavy or light-tailed relative to a gaussian distribution.	$\mathrm{Kurt} = \mathrm{E}\left[\left(\frac{\mathrm{X}-\mathrm{Mean}}{\mathrm{SD}}\right)^4\right]$
Skewness	Quantification of the lack of symmetry: zero for a symmetric distribution and negative values for left-skewed data.	$\mathrm{Skew} = \mathrm{E}\left[\left(\frac{\mathrm{X}-\mathrm{Mean}}{\mathrm{SD}}\right)^3\right]$
Second-order statistics		
Homogeneity	Uniformity of texture intensity (a measure of the closeness of the distribution of elements in the co-occurrence matrix).	$\mathrm{Homogeneity} = \sum_{i=0}^{G-1}\sum_{j=0}^{G-1}\{P(i,j)\}^2$
Contrast	Degree to which the texture intensity levels differ between voxels or local intensity variation. Will favor contributions from p (i, j) away from the diagonal.	$\mathrm{Contrast} = \sum_{i=0}^{G-1}\sum_{j=0}^{G-1}\{i-j\}^2 . P(i,j)$
Entropy	Degree of uncertainty (measure of randomness).	$\mathrm{Entropy} = -\sum_{i=0}^{G-1}\sum_{j=0}^{G-1} P(i,j).\log\left(P(i,j)\right)$
Correlation	Degree of mutual dependency between pixels.	$\mathrm{Correlation} = \sum_{i=0}^{G-1}\sum_{j=0}^{G-1}\frac{\{i,j\}.P(i,j)-\{\mu_x,\mu_y\}}{\sigma_x.\sigma_y}$
Variance	A high weight is given to elements that are different from the average value.	$\mathrm{Variance} = \sum_{i=0}^{G-1}\sum_{j=1}^{G-1}(i-\mu)^2 P(i,j)$
Sum average	Relationship between occurrence of pairs with lower intensity values and occurrence of pairs with higher intensity values.	$\mathrm{SumAvg} = \sum_{I=0}^{2G-2} i.P_{x+y}(i)$
Inverse difference moment	Gets small contributions from non-homogeneous areas (i#j). The result is a low InvDiff value for non-homogeneous images and a relatively higher value for homogeneous images.	$\mathrm{InvDiff} = \sum_{i=0}^{G-1}\frac{P(i,j)}{1+(i-j)^2}$

Supplementary Table 1. Texture features description and formulas.

G: number of grey levels used; i: intensity value of a neighbour voxel; j: intensity value of a reference pixel; P(i,j): probability of the appearance of the (i,j) pair in the co-occurrence matrix; μis: mean value of P; Px and Py: marginal probabilities; $\sigma_x . \sigma_y$*: STD of Px and Py, respectively;* $P_{x+y} = \sum_{i=0}^{G-1}\sum_{j=1}^{G-1} P(i,j)$*, i+j =k for k=0,1….G-1 [5].*

Functional analysis: preprocessing and quality control

Common preprocessing steps, including co-registration, normalization, unwarping, noise component extraction, segmentation, skull stripping, were performed using fMRIprep 1.2.5 (https://fmriprep.readthedocs.io). At the end of this procedure, an individual quality control report was generated by fMRIprep toolbox. This report indicated the mean threshold of two quality criteria for each patient as suggested by Power [6]. The DVARS (D for time derivative and VARS for signal variance) indexed the rate of change of the BOLD signal throughout the brain at each data frame. The FD (Framewise Displacement) indexed the movement of the head from one frame to another. A threshold (T) was defined for these two criteria as

$$T = Mt + 2\sigma$$

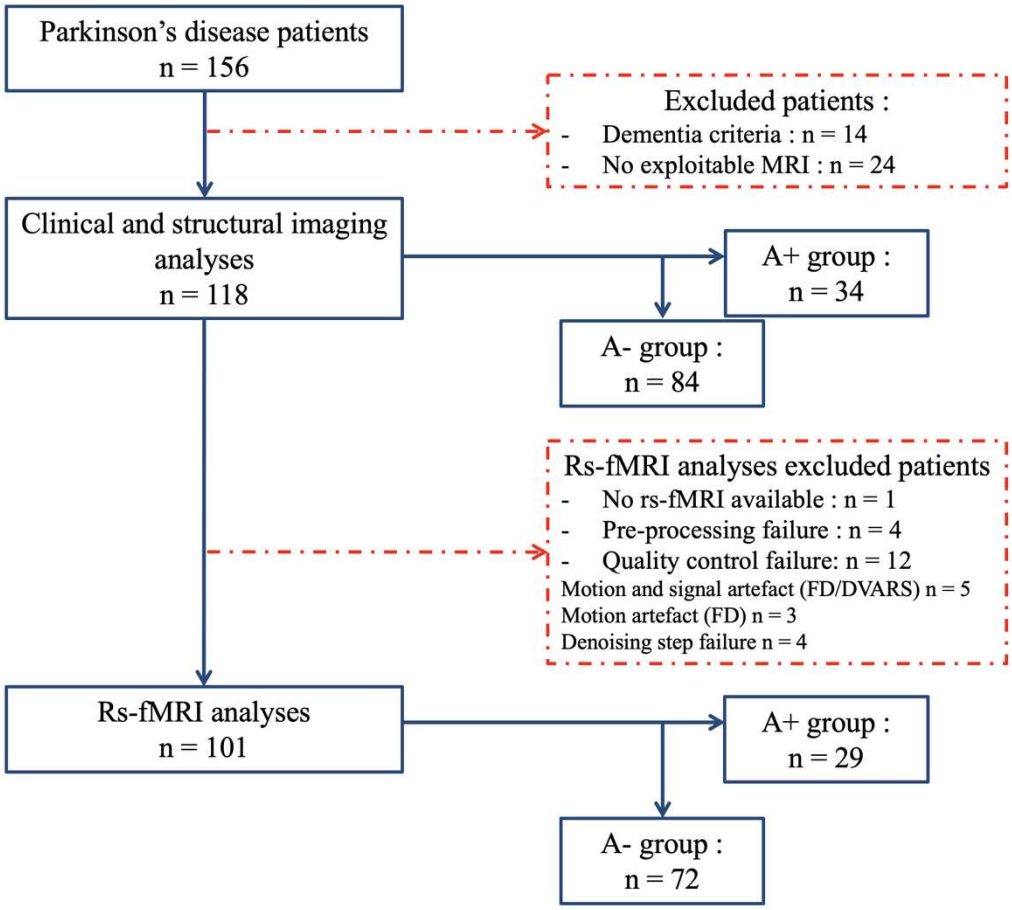
Mt was the average of the mean thresholds of all patients and σ the associated standard deviation (Mt(DVARS): A+ = 1.68 (σ 0.33); A- = 1.81 (σ 0.86) / Mt(FD): A+ = 1.13 (σ 1.76); A- = 2.25 (σ 2.25), no significant difference). When the mean threshold of a patient was higher than T, he was excluded from the functional analysis. Using CONN Toolbox [7] denoising steps were processed: 1) Gaussian kernel 6mm smoothing. 2) Confound regression step by applying linear regression in order to remove motion, physiological and other artefactual effects from BOLD signal. A principal component analysis was performed within tissue classes of interest. We extracted 5 components for white matter and cerebrospinal fluid masks each, in addition to motion estimates and their temporal derivatives [8]. These masks were derived from the segmentation of each subject's structural image. 3) Band-pass filter of 0.008 Hz – 0.09 Hz. At the end of all these steps, 17 patients have been excluded from the rs-fMRI analyses: 1 for no rs-fMRI, 4 after failure of preprocessing steps in fMRIprep toolbox and 12 after quality control and denoising steps failure (5 for motion and signal artefacts (FD/DVARS), 3 for motion artefacts only (FD) and 4 for denoising step failure).

MRI acquisition details

Before the MRI scan, the patient was informed to stay awake, with eyes closed, quiet and to not move during the rs-fMRI acquisition (10min). Using a microphone, the MRI technologist checked if the patient was awake and understood well the instructions. Just after the functional sequence, the MRI technologist checked a second time if the patient was still awake by asking them some questions. There was no video monitoring.

Supplementary results

Population



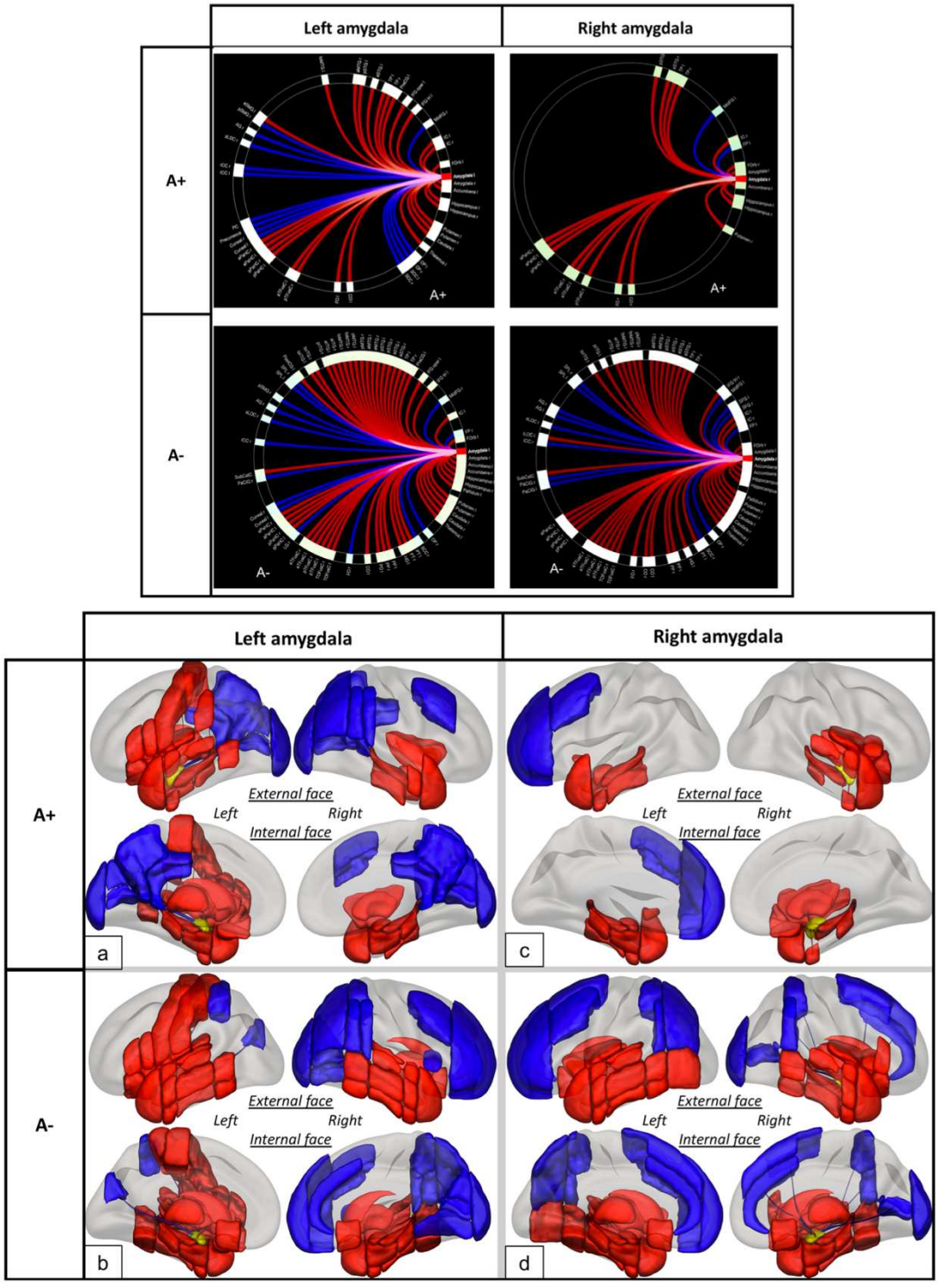
Supplementary Figure 1. Flow chart of the study

Functional connectivity

Demographic variables	A+ group (n = 29)	A- group (n = 72)	OR (CI 95%); p-value
Age (years)	65.7 (± 6.2)	64.0 (± 8.6)	0.59
Women (n = 31)	15 (51.7%)	16 (22.2%)	**3.8 (1.5; 9.4); p = 0.004***
Hand dominance (right, n = 86)	24 (85.7%)	62 (88.6%)	0.7 (0.2; 2.5); p = 0.74
Formal education (years)	12.1 (± 4.3)	12.5 (± 3.5)	0.43
Illness duration (years)	9.9 (± 7.6)	7.1 (± 4.5)	0.21
First motor side			
Left *(n = 38)*	16 (64.0%)	22 (35.5%)	**6.1 (2.3; 16.1); p = 0.02***
Clinical variables			
LEDD (mg/day)	950.7 (± 503.1)	721.0 (± 613.5)	**0.007***
Antidepressant use (n = 17)	12 (41.4%)	3(4.2%)	**16.2 (4.1; 64.0); p < 0.0001***
Anxiolytic use (n = 12)	10 (34.5%)	2 (2.8%)	**18.4 (3.7; 91.3); p < 0.0001***
MDS-UPDRS part 1 (0 - 4) §			
1.3. Depressed mood	1 (0 – 4)	0 (0 – 2)	**< 0.001***
1.4. Anxious mood	2 (0 – 4)	0 (0 – 3)	**< 0.0001***
1.7. Night-time sleep problems	3 (0 – 4)	1 (0 – 4)	**0.003***
1.8. Daytime sleepiness	2 (0 – 4)	2 (0 – 4)	0.35
1.9. Pain and other sensations	2 (0 – 4)	1 (0 – 4)	**< 0.0001***
1.10. Urinary problems	1 (0 – 4)	1 (0 – 4)	0.13
1.11. Constipation problems	1 (0 – 4)	0 (0 – 4)	0.10
1.12. Lightheadeness on standing	1 (0 – 3)	0 (0 – 3)	0.17
1.13. Fatigue	2 (0 – 4)	1 (0 – 4)	**0.002***
MDS-UPDRS part 3 (/132)	28.4 (± 12.5)	27.1 (± 11.4)	0.66
Hoehn & Yahr stage (0 – 5) §	2 (1.5 – 3)	2 (0 – 3)	**0.02***
PAS total (/48)	15.2 (± 4.9)	3.6 (± 3.3)	**< 0.0001***
Part A (/20)	9.7 (± 4.5)	2.8 (±2.8)	**< 0.0001***
Part B (/16)	2.4 (± 2.3)	0.4 (± 0.8)	**< 0.0001***
Part C (/12)	3.0 (± 2.3)	0.4 (± 0.8)	**< 0.0001***
HAMD total (/54)	9.2 (± 5.3)	4.2 (± 3.6)	**< 0.0001***
HARS total (/56)	12.0 (± 5.9)	5.2 (± 4.3)	**< 0.0001***
Cognitive variables	A+ group (n = 34)	A- group (n = 84)	Uncorrected p-value / FDR-corrected p-value
Overall efficiency			
MDRS score (/144)	136.3 (± 5.6)	138.3 (± 6.1)	0.06 / *0.192*
Attention and working memory			
WAIS-R forward digit (/14)	7.2 (± 2.3)	7.8 (± 2.1)	0.15 / 0.267
WAIS-R backward digit (/14)	5.0 (± 2.0)	5.9 (± 1.7)	0.018 / 0.096
SDMT: number in 90 s	37.2 (± 8.6)	44.8 (± 11.7)	< 0.001 / **0.006***
Executive functions			
TMT (time B/time A)	2.8 (± 0.9)	2.4 (± 0.6)	0.034 / 0.136
Stroop: interference index	2.0 (± 0.7)	1.8 (± 0.5)	0.19 / 0.304
Phonemic fluency: words in 60 s	11.9 (± 4.3)	13.6 (± 4.9)	0.23 / 0.335
Alternating fluency: words in 60 s	10.6 (± 3.9)	12.0 (± 5.0)	0.25 / 0.308
Episodic memory			
HVLT Learn 1 (/12)	6.2 (± 1.9)	6.5 (± 1.8)	0.33 / 0.377
HVLT Learn total (/36)	24.6 (± 4.2)	26.2 (± 4.4)	0.11 / 0.220
HVLT delayed recall (/12)	8.9 (± 2.1)	9.1 (± 2.4)	0.33 / 0.352
HVLT recognition hits (/12)	11.2 (± 0.9)	11.3 (± 1.0)	0.45 / 0.450
HVLT number of intrusions	1.8 (± 1.9)	1.4 (± 2.0)	0.24 / 0.320
Language			
Boston Naming Test (/15)	12.0 (± 2.4)	12.9 (± 2.0)	0.07 / 0.190
Semantic fluency: animals in 60s	17.1 (± 4.7)	20.7 (± 5.9)	0.005 / **0.04***
Visuospatial functions			
Benton Judgment of Line Orientation	10.5 (± 3.3)	12.2 (± 2.5)	0.07 / 0.16

Supplementary Table 2. Demographic, clinical and cognitive variables: rs-fMRI subgroup comparisons (Parkinson's disease patients with (A+) and without (A-) anxiety).

** = 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.*



Supplementary Figure 2. Functional connectivity connectogram (up) and 3D display (down) of left (a-b) and right (c-d) amygdala with cerebral ROIS in Parkinson's disease patients with (a-c) and without anxiety (b-d).
A+ = patient with anxiety; A- = patient without anxiety; yellow = amygdala used as seed; blue = negatively-correlated with amygdala ROI; red = positively-correlated with amygdala ROI; ROI = region of interest.

Seed	ROI	F score	Uncorrected p-value	FDR-corrected p-value
Right amygdala	**Negative associations:**			
	Left superior (anterior) temporal gyrus	-3.12	**0.0024***	0.250
	Medial frontal cortex	-2.47	**0.0151***	0.786
	Positive associations:			
	Right supplementary motor area	2.10	**0.0385***	0.976
Left amygdala	**Positive associations:**			
	Left (anterior) supra-marginalis gyrus	2.46	**0.0156***	0.727
	Left parahippocampal gyrus	2.21	**0.0297***	0.727
	Negative associations:			
	Right middle (posterior) temporal gyrus	-2.20	**0.021***	0.727
	Right superior (anterior) temporal gyrus	-2.00	**0.031***	0.727

Supplementary Table 3. Inter-group comparisons of amygdala functional connectivity patterns between patients with and without anxiety in Parkinson's disease, adjusted by sex, centre and Hoehn-Yahr stage

** = p-value < 0.05; FDR = false discovery rate; ROI: region of interest*

Regression analyses

Mean cortical thickness of the significant clusters																
	Model 1						Model 2									
	B	95% CI	SE B	β	p-value	R^2	B	95% CI	SE B	β	p-value	ΔR^2	R^2	p-value
R. Frontal cluster						0.016						0.067	0.083	0.072
Constant	2.55	2.42; 2.69	0.068		<0.001		2.563	2.43; 2.69	0.066		<0.001			
Centre	-0.021	-0.081; 0.038	0.030	-0.072	0.480		0.001	-0.060; 0.059	0.030	-0.001	0.989			
Sex	-0.038	-0.102; 0.025	0.032	-0.121	0.237		-0.058	-0.21; 0.006	0.032	-0.183	0.074			
H-Y stage	0.0003	-0.059; 0.060	0.030	0.001	0.990		0.020	-0.040; 0.079	0.030	0.065	0.514			
PAS total	-	-	-	-	-		**-0.007**	**-0.012; -0.002**	**0.002**	**-0.287**	**0.009**	0.13	0.15	**0.003***
R. Frontal cluster						0.016								
Constant	2.55	2.42; 2.69	0.068		<0.001		2.576	2.450; 2.703	0.064		<0.001			
Centre	-0.021	-0.081; 0.038	0.030	-0.072	0.480		-0.029	-0.085; 0.026	0.028	-0.100	0.300			
Sex	-0.038	-0.102; 0.025	0.032	-0.121	0.237		-0.083	-0.147; -0.019	0.032	-0.264	0.011			
H-Y stage	0.0003	-0.059; 0.060	0.030	0.001	0.990		0.022	-0.034; 0.079	0.028	0.074	0.439			
PAS-B	-	-	-	-	-		**-0.033**	**-0.051; -0.016**	**0.009**	**-0.395**	**0.0002**	0.041	0.069	0.129
L. Frontal cluster						0.028								
Constant	2.614	2.463; 2.765	0.076		<0.001		2.625	2.476; 2.774	0.075		<0.001			
Centre	-0.053	-0.120; 0.014	0.034	-0.160	0.119		-0.034	-0.102; 0.033	0.034	-0.104	0.316			
Sex	-0.025	-0.096; 0.046	0.036	-0.071	0.484		-0.043	-0.114; 0.029	0.036	-0.120	0.244			
H-Y stage	-0.011	-0.078; 0.055	0.034	-0.033	0.739		0.006	-0.062; 0.073	0.034	0.017	0.864			
PAS total	-	-	-	-	-		**-0.006**	**-0.011; 0.0001**	**0.003**	**-0.226**	**0.040**	0.091	0.12	**0.013***
L Frontal cluster						0.028								
Constant	2.614	2.463; 2.765	0.076		<0.001		2.638	2.493; 2.784	0.073		<0.001			
Centre	-0.053	-0.120; 0.014	0.034	-0.160	0.119		-0.061	-0.125; 0.004	0.032	-0.182	0.064			
Sex	-0.025	-0.096; 0.046	0.036	-0.071	0.484		-0.067	-0.140; 0.006	0.037	-0.190	0.070			
H-Y stage	-0.011	-0.078; 0.055	0.034	-0.033	0.739		0.009	-0.056; 0.074	0.033	0.028	0.777			
PAS-B	-	-	-	-	-		**-0.031**	**-0.051; -0.012**	**0.010**	**-0.330**	**0.002**	0.087	0. 115	**0.016***
L. Frontal cluster						0.028								
Constant	2.614	2.463; 2.765	0.076		<0.001		2.591	2.445; 2.737	0.073		<0.001			
Centre	-0.053	-0.120; 0.014	0.034	-0.160	0.119		-0.042	-0.106; 0.022	0.032	-0.127	0.197			
Sex	-0.025	-0.096; 0.046	0.036	-0.071	0.484		-0.028	-0.097; 0.040	0.034	-0.080	0.411			
H-Y stage	-0.011	-0.078; 0.055	0.034	-0.033	0.739		0.015	-0.051; 0.081	0.033	0.045	0.651			
PAS-C	-	-	-	-	-		**-0.028**	**-0.046; -0.010**	**0.009**	**-0.308**	**0.002**	0.050	0. 069	0.13
L. Parietal cluster						0.020								
Constant	2.668	2.518; 2.819	0.076		<0.001		2.680	2.532; 2.828	0.075		<0.001			
Centre	-0.034	-0.101; 0.032	0.034	-0.104	0.310		-0.014	-0.082; 0.053	0.034	-0.043	0.679			
Sex	-0.027	-0.098; 0.044	0.036	-0.076	0.456		-0.046	-0.117; 0.026	0.036	-0.130	0.207			
H-Y stage	-0.023	-0.090; 0.043	0.033	-0.070	0.486		-0.005	-0.072; 0.062	0.034	-0.014	0.887			
PAS total	-	-	-	-	-		**-0.006**	**-0.012; -0.001**	**0.003**	**-0.248**	**0.024**	0.096	0.116	**0.016***
L. Parietal cluster						0.020								
Constant	2.668	2.518; 2.819	0.076		<0.001		2.693	2.549; 2.838	0.073		<0.001			
Centre	-0.034	-0.101; 0.032	0.034	-0.104	0.310		-0.042	-0.106; 0.022	0.032	-0.127	0.194			
Sex	-0.027	-0.098; 0.044	0.036	-0.076	0.456		-0.070	-0.143; 0.003	0.037	-0.198	0.060			
H-Y stage	-0.023	-0.090; 0.043	0.033	-0.070	0.486		-0.002	-0.067; 0.062	0.033	-0.007	0.939			
PAS-B	-	-	-	-	-		**-0.032**	**-0.052; -0.013**	**0.010**	**-0.338**	**0.002**	0.038	0.058	0.21
L. Parietal cluster						0.020								
Constant	2.668	2.518; 2.819	0.076		<0.001		2.653	2.504; 2.803	0.075		<0.001			
Centre	-0.034	-0.101; 0.032	0.034	-0.104	0.310		-0.027	-0.093; 0.039	0.033	-0.082	0.416			
Sex	-0.027	-0.098; 0.044	0.036	-0.076	0.456		-0.029	-0.099; 0.041	0.035	-0.082	0.414			
H-Y stage	-0.023	-0.090; 0.043	0.033	-0.070	0.486		-0.006	-0.074; 0.061	0.034	-0.018	0.857			
PAS-C	-	-	-	-	-		**-0.018**	**-0.037; 0.0001**	**0.009**	**-0.203**	**0.050**			

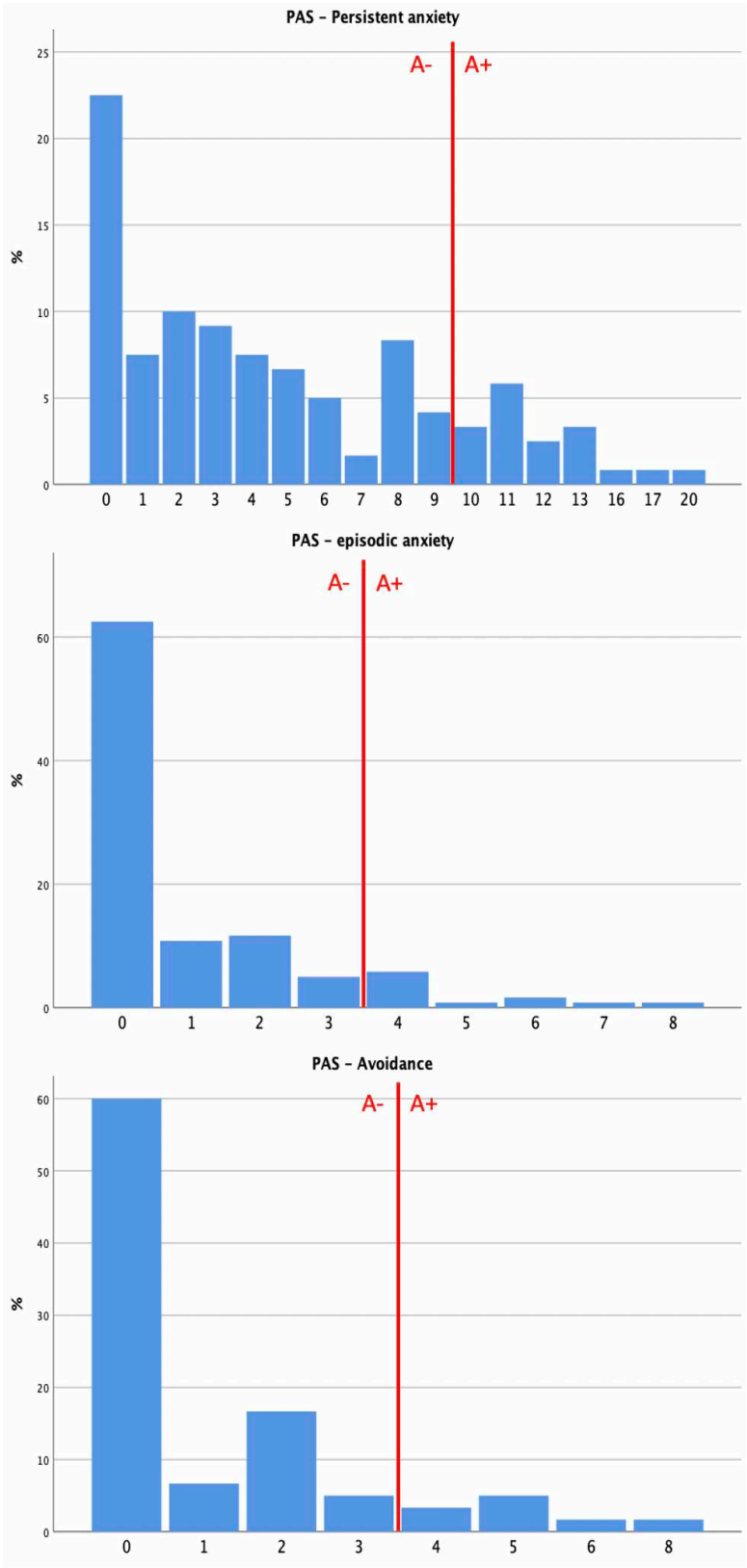
Connectivity values of the significant FC differences														
	Model 1						Model 2							
	B	95% CI	SE B	β	p-value	R^2	B	95% CI	SE B	β	p-value	R^2	ΔR^2	p-value
R. amyg – aSTGl						0.016						0.051	0. 067	0.153
Constant	0.088	-0.116; 0.292	0.103		0.392		0.090	-0.109; 0.290	0.101		0.372			
Centre	-0.001	-0.073; 0.071	0.036	-0.002	0.983		0.004	-0.067; 0.074	0.036	0.011	0.918			
Sex	-0.042	-0.119; 0.035	0.039	-0.112	0.284		-0.079	-0.161; 0.003	0.041	-0.211	0.060			
H-Y stage	0.020	-0.055; 0.096	0.038	0.054	0.597		0.040	-0.036; 0.116	0.038	0.107	0.296			
PAS-B	-	-	-	-	-		**-0.026**	**-0.048; -0.003**	**0.011**	**-0.251**	**0.024**	0.047	0.069	0.140
R. amyg- MedFC						0.022								
Constant	0.106	-0.107; 0.319	0.107	-0.062	0.325		0.109	-0.100; 0.318	0.105		0.304			
Centre	-0.022	-0.097; 0.053	0.038	-0.099	0.555		-0.029	-0.103; 0.045	0.037	-0.079	0.441			
Sex	-0.039	-0.120; 0.042	0.041	-0.080	0.339		-0.045	-0.125; 0.034	0.040	-0.116	0.259			
H-Y stage	-0.031	-0.110; 0.047	0.040	-	0.431		-0.014	-0.093; 0.065	0.040	-0.035	0.732			
PAS-C	-	-	-	-	-		**-0.022**	**-0.042; -0.002**	**0.010**	**-0.224**	**0.030**	0.058	0.062	0.182
L. amyg; aSMGl						0.005								
Constant	-0.004	-0.170; 0.162	0.084		0.961		-0.006	-0.168; 0.156	0.082		938			
Centre	0.009	-0.049; 0.068	0.029	0.033	0.754		0.015	-0.043; 0.072	0.029	0.052	0.613			
Sex	0.016	-0.047; 0.079	0.032	0.053	0.611		0.022	-0.040; 0.083	0.031	0.071	0.488			
H-Y stage	0.003	-0.059; 0.064	0.031	0.008	0.934		-0.013	-0.074; 0.049	0.031	-0.041	0.686			
PAS-C	-	-	-	-	-		**0.019**	**0.003; 0.034**	**0.008**	**0.247**	**0.017**	0.047	0.127	**0.010***
L. amyg; pPaHCl						0.080								
Constant	0.104	-0.096; 0.304	0.101		0.304		0.048	-0.154; 0.250	0.102		0.640			
Centre	0.093	0.022; 0.163	0.036	0.264	0.010		0.111	0.041; 0.182	0.036	0.317	0.002			
Sex	-0.037	-0.113; 0.039	0.038	-0.097	0.334		-0.011	-0.089; 0.066	0.039	-0.030	0.773			
H-Y stage	-0.033	-0.107; 0.041	0.037	-0.088	0.373		-0.050	-0.124; 0.024	0.037	-0.133	0.179			
PAS total	-	-	-	-	-		**0.007**	**0.001; 0.012**	**0.003**	**0.244**	**0.024**	0.047	0.127	**0.011***
L. amyg; pPaHCl						0.080								
Constant	0.104	-0.096; 0.304	0.101		0.304		0.102	-0.094; 0.297	0.099		0.306			
Centre	0.093	0.022; 0.163	0.036	0.264	0.010		0.099	0.030; 0.168	0.035	0.282	0.006			
Sex	-0.037	-0.113; 0.039	0.038	-0.097	0.334		-0.031	-0.106; 0.043	0.038	-0.081	0.411			
H-Y stage	-0.033	-0.107; 0.041	0.037	-0.088	0.373		-0.050	-0.125; 0.024	0.037	-0.133	0.179			
PAS-C	-	-	-	-	-		**0.021**	**0.003; 0.040**	**0.009**	**0.223**	**0.026**	0.045	0.049	0.299
L. amyg; pMTGr						0.004								
Constant	0.097	-0.095; 0.289	0.097		0.318		0.099	-0.089; 0.288	0.095		0.298			
Centre	0.0001	-0.067; 0.068	0.034	0.001	0.996		-0.005	-0.072; 0.061	0.034	-0.016	0.874			
Sex	0.011	-0.062; 0.084	0.037	0.030	0.771		0.005	-0.067; 0.077	0.036	0.015	0.886			
H-Y stage	-0.020	-0.091; 0.051	0.036	-0.056	0.582		-0.004	-0.076; 0.067	0.036	-0.012	0.904			
PAS-C	-	-	-	-	-		**-0.019**	**-0.037; -0.001**	**0.009**	**-0.219**	**0.036**			

Supplementary Table 4. Hierarchical multiple regression analyses between PAS score and sub-scores and significant clusters of cortical thickness reduction and differences of FC in anxious compared with non-anxious Parkinson's disease patients.

*Abbreviations: 95% CI = 95% confidence interval of B coefficient; Δ R2 = change in explained variance between model 1 and model 2; FC = functional connectivity; H-Y = Hoehn-Yahr; L. = left; PAS = Parkinson Anxiety Scale; R. = right; R2 = explained variance by model; SE B, standard error of B coefficient. In bold the multiple regression model parameters of the coefficient of interest; * = p-value of the model 2 < 0.05.*

ROI: amyg = amygdala; MedFC = Medial frontal cortex; pMTGr = right middle temporal gyrus, posterior division; pPaHCl = left parahippocampal cortex, posterior division; aSMGl = left supramarginal gyrus, anterior division; aSTGl = left superior temporal gyrus, anterior division; aSTGr = right superior temporal gyrus, anterior

Limitations



Supplementary Figure 3. Histogram illustrating the distribution of the PAS sub-scores : the majority of patients are outside the borderline threshold (red line).

Abbreviations: A+: patients with anxiety; A-: patients without anxiety; PAS: Parkinson Anxiety Scale.

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CHAPTER 3

Neuroimaging of Anxiety in Parkinson's Disease: a Systematic Review

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Summary of the chapter

After showing that alterations in the fear circuit were involved in PD-related anxiety, we conducted a systematic review on neuroimaging of anxiety in PD in order to generate new hypotheses and increase our knowledge of the underlying mechanisms of PD-related anxiety.

This systematic review reinforced the role of changes in the fear circuit in the occurrence of PD-related anxiety but also revealed the role of the limbic cortico-striato-thalamo-cortical circuit. While the fear circuit would be overactivated in PD patients with anxiety, the limbic circuit would be underactivated. This imbalance might be due to a hypodopaminergic state of the limbic circuit and may explain the high prevalence of anxiety symptoms in PD patients. This was our main hypothesis along this thesis.

Abstract

The aim of this systematic review was 1) to identify the brain regions involved in anxiety in Parkinson's disease (PD) based on neuroimaging studies, and 2) to interpret the findings against the background of dysfunction of the fear circuit and limbic cortico-striato-thalamocortical circuit. Studies assessing anxiety symptoms in PD patients and using magnetic resonance imaging (MRI), positron emission tomography (PET) or single-photon emission tomography (SPECT) were included. The severity of anxiety was associated with changes in the fear circuit and the cortico-striato-thalamo-cortical limbic circuit. In the fear circuit, a reduced GMV of the amygdala and the anterior cingulate cortex (ACC), an increased functional connectivity (FC) between the amygdala and orbitofrontal cortex (OFC) and hippocampus, between the striatum and the medial prefrontal cortex (PFC), temporal cortex and insula, and a reduced FC between the lateral PFC and the OFC, hippocampus and amygdala were reported. In the cortico-striato-thalamo-cortical limbic circuit, a reduced FC was reported between the striatum and ACC, a reduced dopaminergic and noradrenergic activity in striatum, thalamus and locus coeruleus, and a reduced serotoninergic activity in thalamus. To conclude, anxiety is associated with structural and functional changes in both the hypothesized fear and the limbic cortico-striato-thalamocortical circuits. These circuits overlap and may well constitute parts of a more extensive pathway, of which different parts play different roles in anxiety. The neuropathology of PD may affect these circuits in different ways, explaining the high prevalence of anxiety in PD and also the associated cognitive, motor and psychiatric symptoms.

Introduction

Fear is an universal emotion that triggers a state of alertness in response to a real or perceived threat. It may lead to a psychological and physiological state called anxiety, and become a pathological symptom when the manifestations of anxiety are deleterious for the daily life of the subject, such as occurs when the response is exaggerated, prolonged or occurs after exposure to inadequate stimuli. . Anxiety is among the most frequent non-motor symptoms in PD. The prevalence of anxiety in PD is 31%, which is higher than reported in community or other medically ill patients [1]. While anxiety is a frequent worsening factor of the disease and is associated with lower quality of life [2–4], the underlying mechanisms remain largely unknown.

The fear circuit and the limbic cortico-striato-thalamocortical circuits play a parallel role in fear and anxiety. The fear circuit involves the amygdala and the anterior cingulate cortex (ACC), the medial prefrontal cortex (mPFC), the insular cortex, the hippocampus and the striatum [5–7]. The limbic cortico-striato-thalamo-cortical circuit involves the PFC, the basal ganglia and the thalamus [8]. In PD patients, alteration of these circuits such as dopaminergic, noradrenergic and serotoninergic neurodegeneration may explain the high prevalence of anxiety [9].

Several studies have explored the neural correlates of anxiety in PD using anatomical (MRI) and functional (positron emission tomography (PET), single-photon emission computed tomography (SPECT), functional MRI (fMRI)) neuroimaging. Although some systematic reviews of neuroimaging studies focusing on non-motor symptoms in PD have been performed [9–11], none focused specifically on anxiety.

The aim of this systematic review was 1) to identify the brain regions involved in anxiety in PD patients based on the results of neuroimaging studies, and 2) to interpret the findings against the background of dysfunction of the fear and the limbic circuits.

Methods

The protocol for this systematic review was registered in PROSPERO and followed the PRISMA guidelines [12, 13] (PROSPERO-ID CRD42020158980). A literature search in PubMed/Medline, PsychINFO and the Cochrane Library was performed using these search terms: (Parkinson's disease OR Parkinson) AND (Anxiety) AND ((imaging) OR (MRI) OR (PET) OR (SPECT) OR (fMRI) OR (functional MRI)). The search was conducted across the entire time span until January 8 2020 and resulted in a total of 382 articles. Further information about data selection and inclusion criteria are detailed in Supplementary Methods S1.

A quality assessment to assess risk of bias in individual studies has been performed using nine quality criteria, following the approach of Wolters et al. [14]. More information about data extraction and

quality assessment are detailed in Supplementary Methods S2. The data selection, quality assessment and data extraction were performed by two authors independently (G.C. and M.G.) and discrepancies were discussed until consensus was reached.

Imaging data were summarized in three tables: anatomical, functional or metabolic differences. In each table, the localizations of these changes were identified according to their peak-coordinates in MNI (Montreal Neurological Institute) space. The main changes were considered relevant according to their frequency and reproducibility between all the studies. No statistical test was used for this systematic review. Relevant changes were reported on figures representing cortical or subcortical structures to summarize and to better visualize these changes.

Results

Research results

Eighteen imaging studies met the inclusion criteria and were included in this systematic review. These consisted of four anatomical MRI studies [15–18], four functional MRI studies [19–22], eight neurotransmitters/transporters imaging studies [23–30] and two metabolic imaging studies [31, 32]. No CT study was found. The flow chart of the study selection procedure is displayed on Supplementary Results S3. Taken together, the included studies comprised 1840 participants (1470 PD patients and 370 healthy controls (HCs)). Demographics characteristics are reported in Table 1. According to the quality assessment, 12 out of 18 studies received a score of "good" [15–20, 22, 23, 26, 29, 30, 32], and 6 received a score of "moderate" [21, 24, 25, 27, 28, 31]. Further information about this quality assessment can be found in the Supplementary Methods S2.

Study	Size	Age (y)	Gender (M/F)	Education (y)	Disease duration (y)	LEDD (mg/day)	UPDRS-III	Anxiety scale	Depression scale	Cognition (MMSE/MoCa*)
Anatomical imaging studies										
Oosterwijk et al[15]								BAI	BDI	
PD	115	63.9 (±11.0)	71/44	–	3.6 (±4.5)	164.5 (±290.2)	24.7 (±11.3)	11.7 (±8.3)	11.0 (±7.5)	28.4 (±1.5)
Ma et al[18]								HAMA	HAMD	
aPD	8	65.75 (±8.41)	2/6	11.88 ± 4.05	8.88 (±5.74)	593.16 (±293.77)	30.75 (±11.06)	17.63 (±3.11)	10.50 (±3.21)	28.00 (±3.34)
naPD	33	65.27 (±9.09)	17/16	13.52 ± 2.73	7.67 (±4.11)	401.28 (±246.05)	28.09 (±10.62)	6.45 (±3.17)	6.64 (±2.91)	28.42 (±1.35)
Vriend et al[16]								BAI	BDI	
PD	110	64.6 (±10.3)	66/44	–	3.3 (±3.6)	436.4 (±332.7)	24.9 (±10.4)	12.3 (±8.3)	10.2 (±7.1)	28.4 (±1.5)
Wee et al[17]								HADS-A	GDS	
PD	73	65.19 (±7.99)	56/17	11.03 (±3.21)	4.85 (±3.10)	595.88 (±398.99)	18.42 (±8.20)	4.53 (±3.37)	2.81 (±2.82)	26.42 (±2.91)*
Functional imaging studies										
Zhang et al[22]								SAS	SDS	
PD	36	62.98 (±6.61)	30/6	–	6.73 (±4.21)	928.46 (±132.82)	20.86 (±10.81)	31.42 (±4.67)	32.53 (5.85)	28.94 (±1.19)
Wang et al[21]								HAMA	HAMD	
aPD	15	71.33 (±5.27)	10/5	12.13 (±2.72)	4.27 (±3.44)	454.03 (±262.34)	24.80 (±9.90)	15.00 (±3.21)	8.93 (±2.34)	27.53 (±2.03)
naPD	33	69.48 (±6.03)	24/9	11.48 (±3.95)	4.43 (±3.00)	441.48 (±291.27)	22.73 (±10.82)	6.61 (±3.34)	4.24 (±3.39)	28.09 (±1.70)
HC	19	66.21 (±3.51)	10/9	10.58 (±3.24)	NA	NA	NA	1.79 (±1.62)	1.47 (±0.90)	28.79 (±1.03)
Dan et al[19]								STAI	BDI	
PD	27	64.9 (±7.9)	15/12	13.5 (±2.7)	11.1 (±3.7)	1306.1 (±616.7)	14.4 (±7.1)	(S) 38.7 (±9.4) (T)41.8 (±8.9)	10 (±4.8)	26 (±2.2)*
Wang et al[20]								HAMA	HAMD	
aPD	18	71.74 (±5.16)	12/6	12.84 (±2.95)	3.76 (±3.23)	450.17 (±252.08)	24.05 (±8.92)	15.47 (±3.01)	9.26 (±2.64)	27.79 (±1.87)
naPD	45	66.17 (±8.11)	34/11	11.52 (±3.56)	3.94 (±2.87)	373.95 (±306.93)	21.52 (±10.59)	5.93 (±3.42)	3.83 (±3.19)	28.24 (±1.57)
HC	24	65.33 (±4.65)	10 /14	10.79 (±2.92)	NA	NA	NA	2.33 (±2.04)	1.54 (±2.06)	–
Neurotransmitter/transporter imaging studies										
Bayram et al[30]										
PD-L	154	60.3 (±9.86)	88/66	15.4 (±3.07)	0.52 (±0.53)	–	21.6 (±8.38)			
PD-R	213	62.3 (±9.59)	147/66	15.6 (±3.10)	0.55 (±0.54)	–	19.9 (±8.77)	Not shown	Not shown	Not shown
HC	113	60.8 (±12.2)	65/48	16.3 (±3.04)	NA	–	NA			
Joling et al[29]								BAI	BDI	
PD	127	64.91 (±10.98)	84/43	–	2.55 (±2.90)	161.77 (±274.78)	23.02 (±10.68)	11.50 (±8.32)	8.00 (±9.00)	Not shown
Picillo et al[28]								STAI	GDS	
PD	405	61.20 (±9.8)	264/140	15.56 (±2.98)	–	–	20.25 (±8.93)	65.35 (±18.47)	2.29 (±2.37)	–
HC	187	60.24 (±11.2)	121/66	16.12 (±2.9)	–	–	NA	57.03 (±14.33)	1.28 (±2.08)	–
Ceravolo et al[27]								HAMA	HAMD	
PD	44	68.1(±7.9)	–	–	13.7 (±11.7)	–	17.9 (±7.7)	3 (±3.6)	4.1 (±5.0)	26.9 (±1.5)
Erro et al[26]								HADS-A	BDI	
aPD	9	58.7 (±9.4)	4/5	–	14.9 (±3.5)	–	15.5 (±5.7)	≥7	5.9 (±7.3)	28.2 ± 0.9
naPD	25	59.5 (±8.3)	18/7	–	16.2 (±3.1)	–	13.3 (±6.1)	<7	7.5 (±6.8)	27.4 (±2.2)
Moriyama et al[25]						–		BSPS		
sad_PD	12	50.5 (±11.3)	9/3	–	7.1 (±3.8)	–	34.7 (±16.1)	56.5 (±11.3)	–	–
nosad_PD	20	52.5 (±12.8)	15/5	–	9 (±6.2)		31.7 (±12.2)	25.7 (±14.2)	–	–
Weintraub et al[24]								STAI	POMSd	
PD	76	62.8 (±10.8)	57/19	15.1 (±2.9)	7.5 (±5.5)	–	–	(S)37.5 (±9.0) (T) 37.0 (±7.6)	6.0 (±7.4)	–
Remy et al[23]								STAI	BDI	
PD	20	58.15 (±8.1)	14/6	–	4 (±2.2)	501.9 (±415.6)	23.8 (±8.95)	41.3 (±12.65)	12.3 (±4.75)	–
Metabolic imaging studies										
Wang et al[31]								HAMA	HAMD	
aPD	13	68.31 (±5.71)	9/4	10.62 (±2.33)	3.85 (±2.72)	297.88 (±185.29)	21.31 (±10.04)	14.08 (±3.04)	5.46 (±3.26)	29.15 (±0.99)
naPD	15	64.13 (±8.95)	8/7	10.67 (±2.87)	2.44 (±2.65)	190.83 (±256.28)	15.60 (±9.24)	5.33 (±3.11)	5.13 (±2.90)	28.93 (±1.22)
HC	15	63.33 (±4.62)	8/7	10.00 (±3.16)	NA	NA	NA	1.87 (±1.85)	4.53 (±2.23)	29.00 (±0.85)
Huang et al[32]								BAI	BDI	
PD	26	66.5 (±1.4)	16/10	17.8 (±0.6)	5.5 (±0.7)	–	Not shown	12 (1–35)	8 (1–21)	29 (26–30)
HC	12	67.4 (±2.0)	7/5	17.2 (±1.1)	NA	NA	NA	2 (0–10)	3 (0–10)	30 (30–29)
Total										
PD	1470	63.40 (±5.26)	1114/356	13.55 (±2.43)	6.26 (±4.47)	510.13 (±363.23)	20.91 (±4.87)	NA	NA	NA
HC	370	63.89 (±2.93)	221/149	14.44 (±3.88)	NA	NA	NA	NA	NA	NA
Total	1840									

Table 1. Demographic and clinical characteristics of the participants in the studies included in the systematic review.

Abbreviations: aPD = PD with anxiety; BAI = Beck anxiety inventory; BDI = Beck depression inventory; BSPS = brief social phobia scale; GDS = geriatric depression scale; HADS-A = hospital anxiety scale; HAMA = Hamilton anxiety rating scale; HAMD = Hamilton depression rating scale; HC = healthy controls; LEDD = levodopa equivalent daily dosage; MMSE = mini mental state examination; MoCA =Montreal cognitive assessment; NA = not applicable; naPD = PD without anxiety; PD = Parkinson's disease; POMSd = profile of mood state – depression; SAS = self-rating anxiety scale; (S) = state; SDS = self-rating depression scale; STAI= Spielberg state-trait anxiety inventory; (T) = trait; UPDRS = unified Parkinson's disease rating scal.

Anatomical MRI studies

The four anatomical MRI studies together included 329 PD patients. None included HCs. All were based on 3 Tesla MRI T1-weighted scans. Three studies used voxel-based morphometry (VBM) to analyse GMV and one used structural covariance analyses to analyse structural connectivity. Two studies compared PD patients with and without anxiety (aPD and naPD), and two studies correlated the severity of anxiety to anatomical changes. The studies used three different scales for the assessment of anxiety: the Beck anxiety inventory (BAI) [33], the Hamilton rating scale for anxiety (HAMA) [34], and the hospital anxiety and depression scale, anxiety subscale (HADS-A) [35].

In studies using VBM, higher anxiety scores, as measured with the BAI, were associated with a reduced volume of the bilateral anterior cingulate cortex, the left amygdala, the bilateral precuneus and the bilateral cerebellar tonsils. There were negative correlations between the BAI and structural covariance of the left striatum and right caudate, and the left striatum and bilateral prefrontal cortex (PFC). The results are displayed in Table 2.

Studies	Size	Anxiety scale	Outcome	Analyze software	Localization	MNI coordinates *x/y/z*	Statistic values
Oosterwijk et al[15]					Negative correlation		*z* Scores
PD	115	BAI	Structural covariance	Multiple regression	l. DCN	−13/15/9	5.36
					r. caudate	12/18/14	5.33
				SPM	r. DCN	13/15/9	4.71
					r. vlPFC	51/30/−4	5.48
					l. DCP	−28/1/3	4.79
					r. caudate	10/16/14	
					l. NA	−9/9/−8	
					r. caudate	10/16/12	
					l. dlPFC	−48/20/40	
Ma et al[18]							*z* Scores
aPD	8	HAMA	GMV (VBM)	Comparisons (ANOVA)	r. tonsil/lobule VIII	34.5/−48/−43.5	2.92
naPD	33			SPM	l. tonsil	−40.5/−46.5/−43.5	2.76
Vriend et al[16]				Multiple regression			T-values
PD	110	BAI	GMV (VBM)	FreeSurfer, SPM	l. amygdala	−24/0/−29	2.91
Wee et al[17]							*z* Scores
PD	73	HADS-A	GMV (VBM)	Multiple regression	l. precuneus	−18/−63/36	3.69
					l. ACC	−8/23/28	3.70
				SPM	r. precuneus	12/−55/36	3.73
					r. ACC	8/30/15	3.36

Table 2. Anatomical imaging studies of PD-related anxiety.

Abbreviations: aPD = PD patients with anxiety; BAI = Beck anxiety inventory; GLM = generalized linear model; HADS-A = hospital anxiety scale; HAMA = Hamilton rating scale for anxiety; MNI = Montreal Neurological Institute; naPD = PD patients without anxiety; PD = Parkinson's disease; SPM = statistical parametric mapping; VBM = voxel-based morphometry.

ROI: ACC = anterior cingulate cortex; DCN = dorsal caudate nucleus; DCP = dorsal-caudate putamen; dlPFC = dorsolateral prefrontal cortex; IC = insular cortex; IFG = inferior frontal gyrus; l. = left; NA = accumbens nucleus; PCC = posterior cingulate cortex; preCG = precentral gyrus; r. = right; SFG = superior frontal gyrus; SMG = supramarginal gyrus, vlPFC = ventrolateral prefrontal cortex.

Functional MRI studies

The four functional MRI studies included 217 participants of which 174 PD patients and 43 HCs. In all studies, 3 Tesla resting-state functional MRI (rs-fMRI) and T1-weighted scans were performed. In all studies, voxel-level seed-based analysis was performed and in one an additional ROI-level analyses was performed. Functional connectivity strength between an identified seed and the whole brain was performed in three studies, while in one study the amplitude of low-frequency fluctuations (ALFFs) in the whole brain was analysed, corresponding to the functional activity. In two studies aPD, naPD and HC were compared, while in two studies the severity of anxiety was correlated with functional changes. No study was found using diffusion tensor imaging (DTI). Three different anxiety rating scales were used: the HAMA, self-rating anxiety scale (SAS) [36] and the Spielberg state-trait anxiety inventory (STAI) [37].

In aPD patients, higher ALFFs were reported in the right cerebellum (region IX and VIII) and the right orbitofrontal cortex (OFC) than naPD or HC. Increased anxiety was associated with a stronger functional connectivity between the amygdala and the OFC, parietal cortex (more specifically the superior parietal lobule, precuneus, and angular gyrus), and the medial temporal cortex. Moreover, there was stronger functional connectivity between the OFC and temporal cortex, between the striatum and temporal cortex, and between the striatum and the cingulate cortex. Increased severity of anxiety severity was associated with a lower functional connectivity between the amygdala and the dorsolateral prefrontal cortex (dlPFC), between the striatum and the OFC, and between the OFC and dlPFC. The results are displayed in Table 3.

Studies	Size	Anxiety scale	Outcome	Analyze software	Localization	MNI coordinates x/y/z		Statistic values	
Zhang et al[22]	36	SAS	Weighted degree	Correlations (GLM)	Anxiety			T-values	
PD			and FC strength	SPM, RESTplus	FC l. amygdala.	−21/0/−12			
			(BOLD signal)		l. AG	−54/−63/33		6.15	
					l. SPL	−36/−69/48		5.54	
					l. cuneus	−9/−87/6		5.25	
					r. IFG	42/36/9		−5.74	
					l. STG	−63/−33/12		−5.39	
Wang et al[21]	15	HAMA	ALFF methods	Comparisons (ANCOVA)	aPD ≥ naPD			*z* score	
aPD	33			SPM	r. cereb.IX	9/−42/−51		4.07	
naPD	19			rs-fMRI data analyses toolkit	r. cereb.VIII	18/−72/−42		4.40	
HC					r. OFC	33/51/9		4.44	
					aPD ≥ HC				
					r. cereb.VIII	21/−72/−42		4.24	
					r. OFC	27/48/3		4.11	
					r + l. medulla	6/−42/−51		4.24	
Dan et al[19]	27	STAI	FC strength	Multiple regression	Anxiety	Left	Right	T-values	
PD			(BOLD signal)	Software "CONN" (Matlab)	FC OFC	−5/37/−18	8/36/−18	Left	Right
					Amyg.	−23/−1/−17	27/1/−18	3.73	4.19
					Hipp.	−25/−21/−10	29/−20/−10	4.35	3.81
					ParaHipp.G	−21/−16/−21	25/−15/−20	5.38	7.36
					FC iMTG	–	57/−37/−1		
					OFC	–	8/36/−18	ns	3.94
					Amyg.	–	27/1/−18	ns	4.9
					Hipp.	–	29/−20/−10	ns	4.55
					ParaHipp.G	–	25/−15/−20	ns	3.95
					FC SMC	−39/−6/51	41/−8/52		
					OFC	−36/31/−12	18/48/−14	−5.02	−4.04
					FC dlPFC	−5/54/−7	8/52/−7		
					Amyg.	−23/−1/−17	27/1/−18	−4.26	−5.18
					TP	−40/15/−20	–	−4.38	ns
					OFC	−36/31/−12	–	−5.01	ns
Wang et al[20]	18	HAMA	FC strength	Comparisons (ANCOVA)	aPD ≥ naPD			*z* Values	
aPD	45		(BOLD signal)	SPM	FC l. putamen	−24/4/2			
naPD	24				r. OFC	13/18/60		−3.130	
HC					FC r. putamen	28/5/2			
					l. OFC	−6/63/−3		−3.744	
					r. cereb.	51/−63/−48		−5.199	
					r. precuneus	0/−45/72		−3.981	
					r. IC	39/−9/−6		4.713	
					l. TP	−39/−3/−15		4.343	
					l. MOG	−42/−87/−3		3.162	
					l. caudate	−15/15/18		3.976	
					r. MCC	12/−6/33		3.208	
					aPD ≥ HC				
					FC l. putamen	−24/4/2			
					l. ACC	−12/36/3		−4.136	
					FC r. putamen	28/5/2			
					l. OFC	−6/39/−9		−3.490	
					r. paraCL	6/−24/75		3.590	
					l. paraCL	0/−30/63		3.755	

Table 3. Functional imaging studies of PD-related anxiety.

Abbreviations: ALFF = amplitude of low-frequency fluctuations; aPD = PD patients with anxiety; HAMA = Hamilton anxiety rating scale; HC = healthy controls; FC = functional connectivity; GLM = generalized linear model; ns = not significative; MNI = Montreal Neurological Institute; naPD = PD patients without anxiety; PD = Parkinson's disease; SAS = self-rating anxiety scale; SPM = statistical parametric mapping; STAI = Spielberger state-trait anxiety inventory

ROI: ACC = anterior cingulate cortex; AG = angular gyrus; amyg. = amygdala; cereb = cerebellum; dlPFC = dorsolateral prefrontal cortex; hipp. = hippocampus; IC = insular cortex; IFG = inferior frontal gyrus; iMTG = inferior middle temporal gyrus; l. = left; MCC = middle cingulate cortex; MOG = middle occipital gyrus; OFC = orbitofrontal cortex; paraCL = paracentral lobule; r. = right; SMC = sensorimotor cortex; SPL = superior parietal

Neurotransmitters/neurotransporters studies

The eight neurotransmitters/neurotransporters imaging studies together included 1292 participants, of which 1105 PD patients and 187 HCs. In six studies, the dopamine transporter (DAT) BR in the striatum was analysed using 99mTc-TRODAT-1 SPECT (2 studies) or 123I-FP-CIT SPECT (4 studies). In one study, the DAT and noradrenaline transporter (NAT) BR were analysed using 11C-RTI-32 PET. In another study, the DAT and serotonin transporter (SERT) BR were analysed using 123I-FP-CIT SPECT. In five studies, aPD patients were compared with naPD patients or HCs. Eight studies correlated the severity of anxiety with changes in the BR. Five different anxiety scales were used: the STAI, the BAI, the HAMA, the Brief Social Phobia Scale (BSPS) [38] and the HADS-A.

In the Increased anxiety in PD was associated with reduced DAT binding in the bilateral caudate, the left putamen, the bilateral thalamus, bilateral amygdala and the left locus coeruleus. Increased anxiety was also associated with reduced NAT in the left caudate, the bilateral thalamus, the bilateral amygdala and the left locus coeruleus, as well as with reduced SERT in the bilateral thalamus. Two studies focused specifically on social anxiety disorders [25, 27]. Both reported that severity of social anxiety was associated with increased DAT binding in the striatum, bilaterally. The results are displayed in Table 4.

Metabolic imaging studies

The two metabolic imaging studies included 81 participants, of which 54 PD patients and 27 HCs. In these studies, the cerebral glucose metabolism was analysed using 18FDG-PET. In one study, aPD patients were compared with naPD patients and HCs. The other one correlated the severity of anxiety with metabolic changes. Two different anxiety scales were used: the HAMA and the BAI.

Increased anxiety was associated with reduced cortical FDG metabolism in the OFC, dlPFC, ventrolateral PFC (vlPFC) and the cingulate cortex as well as reduced striatal FDG metabolism (bilateral caudate and right putamen). The results are displayed in Table 4.

Studies	Size	Anxiety scale	Imaging/outcome	Analyze software	Localization	MNI coordinates *x/y/z*	Statistic values
Neurotransmitter/transporter imaging (PET/SPECT) studies							
Bayram et al[30]	154	STAI	^{123}I-FP-CIT SPECT	Correlation: mixed model	PD-L	Not available in this study	r-Values
PD-L	213		Striatal DAT BR	SAS	STAI-state		(*P*-values)
PD-R	113				r. caudate		−0.11 (0.039)
HC					l. caudate		−0.19 (<0.001)
					l. putamen		−0.13 (0.006)
					STAI-trait		
					r. caudate		−0.13 (0.029)
					l. caudate		−0.20 (<0.001)
					l. putamen		−0.14 (0.004)
					No correlation with PD-R and HC		
Joling et al[29]	127	BAI (affective subscale)	^{123}I-FP-CIT SPECT	Multiple regression	$BAI_{affective}$		β-Values
PD			Striatal DAT	SPM	ROI level		(*P*-value)
			Extrastriatal	Software "FMRIB"	r. thalamus	–	−0.203 (0.019)
			SERT BR		VBM		T-values
					l. thalamus	−14/−24/0	−4.11
Picillo et al[28]	405	STAI	^{123}I-FP-CIT SPECT	Multiple regression	STAI-trait subscale		β-Values
PD	187		DAT BR	Software "Hermes" and "Pmod"			(*P*-value)
HC					r. caudate	Not available in this study	−1.536 (0.009)
Ceravolo et al[27]	44	HAMA	^{123}I-FP-CIT SPECT	Partial correlations	HAMA	Not available in this study	r-Values
PD			DAT BR	SPSS			(*P*-value)
					r. caudate		0.311 (<0.05)
					l. caudate		0.323 (<0.05)
					r. putamen		0.356 (<0.05)
					l. putamen		0.309 (<0.05)
Erro et al[26]	9	HADS-A	^{123}I-FP-CIT SPECT	Comparisons (*t* test), multiple regression	aPD ≥ naPD	Not available in this study	*P*-values
aPD	25		V3″ value	SPM, ImageJ	Lower V3″ in		
naPD					r. caudate		0.007
					l. caudate		0.001
					l. putamen		0.001
					Correlations		β-coefficient (*P*-value)
					HADS-A		
					r. caudate		−0.47 (0.01)
Moriyama et al[25]	11	BSPS	^{99m}Tc-TRODAT-1 SPECT	Comparisons (*t* test), partial correlations	Correlation	Not available in this study	r-Values
sad_PD	21		DAT BR	SPSS	BSPS		(*P*-value)
nosad_PD					l. caudate		0.43 (0.02)
					l. putamen		0.43 (0.02)
					Comparisons: no difference		
Weintraub et al[24]	76	STAI	^{99m}Tc-TRODAT-1 SPECT	Pearson correlations	STAI	Not available in this study	r-Values
PD			DAT BR	*StatS* package			(*P*-value)
					l. ant. Putamen		−0.24 (0.04)

Remy et al[23]	20	STAI	^{11}C-RTI-32 PET	Correlation with GLM	STAI		*z* Values
PD			DAT and NAT BR	SPM	l. Ve. striatum	−18/10/8	−2.72
					l. caudate	−12/14/14	−2.34
					l. LC	−6/−30/−18	−2.70
					r. thalamus	16/−10/16	−2.55
					l. thalamus	−6/−8/12	−2.38
					l. amygdala	−22/0/−10	−2.10
					r. amygdala	−24/4/−14	−2.06
Metabolic imaging studies							
Wang et al[31]	13	HAMA	^{18}FDG-PET	Comparisons (*t* test)	aPD ≥ naPD		*z* Scores
aPD	15		Cerebral glucose metabolism	SPM	r. OFC	8/62/18	−3.15
naPD	15				l. OFC	−4/40/−24	−2.82
HC					aPD ≥ HC		
					b. dACC	0/50/16	−3.90
					r. OFC	8/62/22	−3.35
					r. dlPFC	8/54/36	−2.94
					r. SMC	60/6/46	−2.83
					l. SMC	−6/8/70	−3.24
					r. SA	8/12/−18	−3.52
					r. putamen	14/14/0	−3.43
					l. caudate	−12/14/6	−2.89
					l. OFC	−10/38/−30	−3.04
					r. vlPFC	24/10/38	−3.11
					naPD ≥ HC		
					r. SMC	50/−2/52	−4.08
					l. SMC	−10/8/66	−3.61
Huang et al[32]	26	BAI	^{18}FDG-PET	Multiple regression	BAI		*z* Values
PD	12		Cerebral glucose metabolism	SPM	r. caudate	12/10/2	−3.60
HC					l. caudate	−10/10/4	−3.50

Table 4. Metabolic imaging studies of PD-related anxiety.

Abbreviations: aPD = PD patients with anxiety; BAI = Beck anxiety inventory; BR = biding rate; BSPS = brief social phobia scale; DAT = dopamine transporter; FDG = fluorodeoxyglucose; GLM = generalized linear model; HADS-A = hospital anxiety depression scale, anxiety subscale; HAMA = Hamilton anxiety rating scale; NAT = noradrenaline transporter ; naPD = PD patients without anxiety; PD = Parkinson's disease; PD-L = PD patients with left limbs dominantly affected; PD-R = PD patients with right limbs dominantly affected; sad = social anxiety disorder; SBR = striatal binding ratio; SERT = serotonin transporter; STAI = state-trait anxiety inventory; SPECT = single photon emission computerized tomography; SPM = statistical parametric mapping; PET = positron emission tomography; V3" value = specific-to-non-displaceable binding ratio; VBM = voxel-based morphometry.

ROI: ant. = anterior; dACC = dorsal anterior cingulate cortex; dlPFC = dorsolateral prefrontal cortex; l. = left; LC = locus coeruleus; r. = right; SA = subgenual area; SMC = premotor cortex and supplementary motor cortex; Ve = ventral; vlPFC = ventrolateral prefrontal cortex.

Discussion

This review aimed to delineate the brain regions involved in anxiety in PD as identified by studies using three types of approaches: anatomical, functional, and metabolic imaging. It revealed that several structures were implied in the pathophysiology of fear. Both anatomical and functional changes occurred in the amygdala, the PFC, the ACC, and the striatum corresponding both to the fear and the limbic cortico-striato-thalamocortical circuits. A reduced dopaminergic and noradrenergic binding rate occurred in the striatum, the amygdala, the thalamus, and the locus coeruleus, and a reduced serotoninergic binding in the thalamus.

The fear circuit is altered in PD patients with anxiety

This review found evidence of anatomical and functional alterations in the fear circuit in PD-related anxiety. Anatomical and functional changes in the amygdala and a dopaminergic as well as noradrenergic binding rate reduction were associated with anxiety severity [16, 19, 22, 23]. The amygdala is the central hub of the fear circuit, commonly separated in three nuclei: the centromedial (CeA), the basolateral (BLA) and the superficial (SupA) nucleus. The BLA is the input nucleus and receives afferent inputs from the PFC, the ACC, the hippocampus, the thalamus, and the brainstem nuclei. It projects to the CeA, the bed nucleus of stria terminalis and the striatum. The CeA is the output nucleus of the amygdala and projects to the brainstem nuclei and the hypothalamus [6, 39] (Figure 1.a.). Hence, an imbalance between the BLA and CeA, with functional dominance of the BLA could contribute to the occurrence of anxiety symptoms. This review also brought out anatomical and functional changes in the PFC and the ACC. In the fear circuit, theses cortices are postulated to be involved in the cognitive regulation of emotion while the hippocampus is involved in emotional memory and contextual fear reaction [6]. Other studies also showed that the ventral striatum, the ACC, and the insular cortex could play a crucial role in encoding aversive contextual information and in controlling negative motivation to execute avoidance behaviour in response to aversive cues and anticipation of consequence. It was reported that these structures had major inputs form amygdala [40, 41]. Their dysfunction could be associated with impaired voluntary emotion regulation and lower ability to inhibit intrusive negative thoughts. So, it could lead to a disturbance of attentional resources and lower executive performance in anxious PD patients [42, 43]. Functional changes between the hippocampus and amygdala could lead to dysfunction in emotional memory and promote negative thoughts or resurgence of erratic emotional memories. However, dysfunction of the fear circuit is not the only mechanism that can explain the high prevalence of anxiety in PD.

Changes in basal ganglia circuits are involved in PD-related anxiety

The central factor in the neuropathology of PD is dysfunction of the basal ganglia. A hypodopaminergic state of the limbic cortico-striato-thalamocortical circuit has been associated with behavioural and psychiatric symptoms in PD, such as anxiety [8, 44]. This circuit connects the ACC, the mPFC and brainstem nuclei with the basal ganglia such as the striatum, the pallidum, the subthalamic nucleus (STN) and the thalamus in order to modulate mood and behaviour (Figure 1.a.). In this review, functional changes of the striatum were associated with the severity of anxiety. Moreover, anxiety was associated with a reduced dopaminergic, noradrenergic, and serotoninergic binding rate in the structures involved in the limbic cortico-striato-thalamocortical circuit such as the striatum, the locus coeruleus and the thalamus. Erro et al. [26] proposed that cognitive and behavioural dysfunctions observed in PD patients might reflect a sequential process of dopamine depletion occurring in the striatum. The relationship between anxiety and hypo-dopaminergic state in the striatum may be mediated by disruption of dopaminergic cortico-striato-thalamocortical circuit [26]. In this circuit, the mediodorsal thalamus is an especially important relay between the basal-ganglia and the mPFC/ACC, but it also brings sensory input to the BLA and more generally to the fear circuit [6, 8]. The locus coeruleus is the main noradrenergic centre in the brain. Remy et al. [23] postulated that anxiety in PD could implicate thalamo-cortical interactions under the control of the noradrenergic innervation originating in the locus coeruleus [23]. These findings are consistent with the hypothesis of a hypo-catecholaminergic and hypo-serotoninergic state of the limbic circuit in PD patients with anxiety. It is thus postulated that the neuropathology of PD itself could affect the pathophysiology of the fear circuit.

The neuropathology of PD increases the risk of anxiety

In this review, anxiety in PD was associated with anatomical and functional changes in both the fear circuit and the limbic cortico-striato-thalamocortical circuits. We assume that the neuropathology of PD could affect the fear circuit in different ways. First, there is an important overlap between the fear and the limbic circuit. The anatomical separation between these circuit seems artificial. They must be seen as two parts of a bigger limbic circuit (Figure 1.b.). Dysfunction of the basal-ganglia and the hypo-dopaminergic state due to PD could affect the proper function of the limbic circuit. It could promote an over-activation of the fear circuit, altering fear processing, as well as an under-activation of the limbic cortico-striato-thalamocortical circuit, altering the cognitive and behavioural long-term adaptation to fear. Secondly, dysfunction of these circuits may occur simultaneously or successively in the course of the disease. In this review, anxiety was associated with reduced dopamine, catecholamine and serotonin in the thalamus and in the locus coeruleus. These structures are both closely connected to the two circuits [6]. On the one hand, the mediodorsal thalamus is directly

connected to the BLA and brings sensory input to the fear circuit [6, 39, 43]. It is also probably connected to the striatum in the fear circuit, but we didn't find any confirmation in literature (Figure 1.a.). On the other hand, lesions of brainstem nuclei, such as lesions of the locus coeruleus or the raphe nucleus occur early in the course of PD [45, 46] and could promote dysfunction of both the cortico-striato-thalamocortical circuit and the fear circuit, in parallel or successively. The early impairment of these nuclei could therefore promote anxiety symptoms. It could explain the high prevalence of anxiety and its associated symptoms in PD. Finally, other structures have been identified to be involved in fear and anxiety disorders but have not been studied in PD such as the ventral tegmental area (VTA), the STN, the periaqueductal grey matter, the raphe nuclei, or the parabrachial nuclei [47]. The alterations in limbic circuits in the included studies could also indirectly reflect neuropathological dysfunction of these structures due to the pathology of PD.

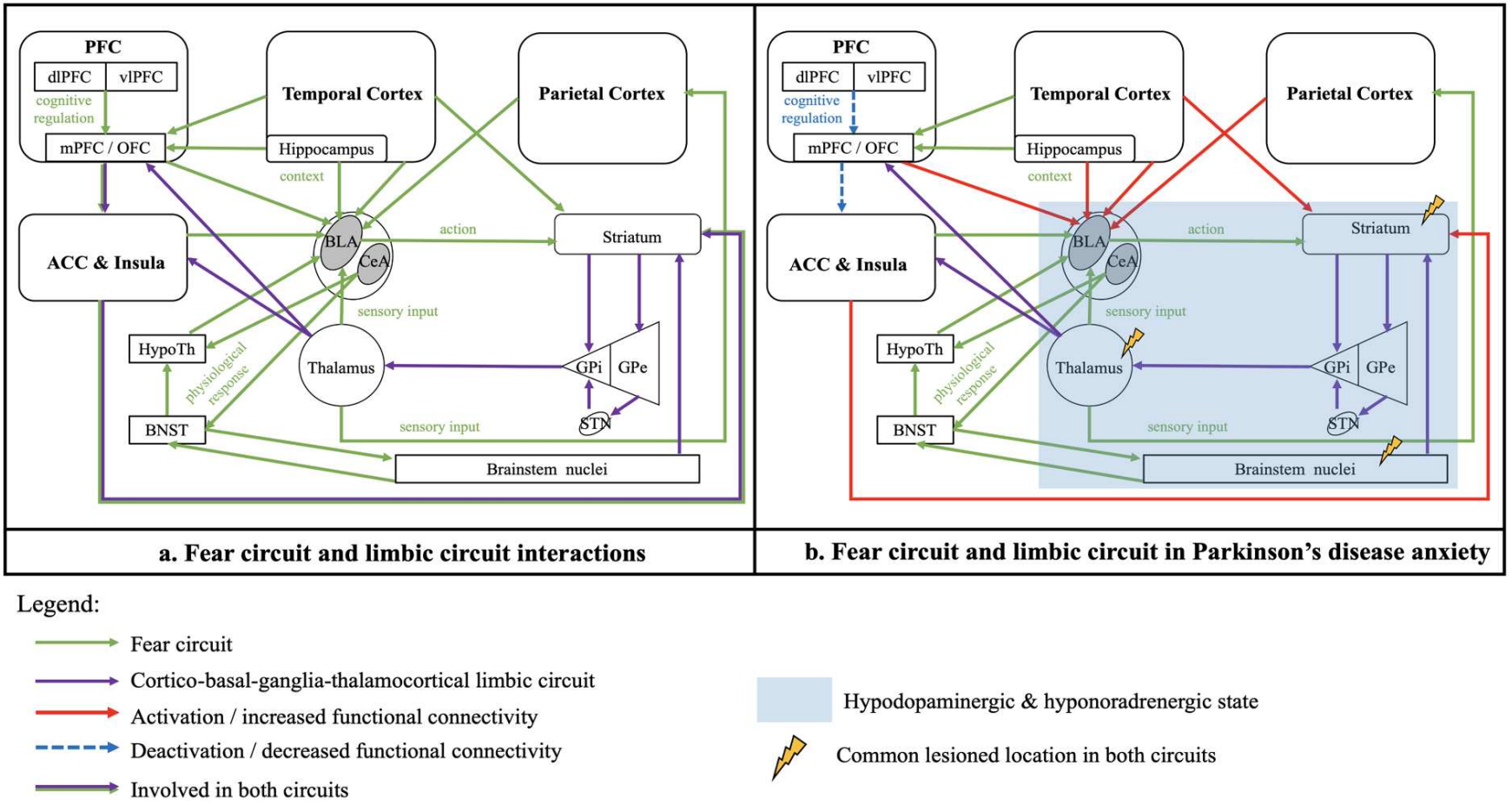

Figure 1. Schematic representation of the fear circuit and cortico-striato-thalamocortical limbic circuit in human brain:
a. Interactions model in normal brain as reported in the literature [6, 8, 39];
b. Fear and limbic circuit changes in Parkinson's disease anxiety found in this systematic review. *The blue area corresponds to the structures with a reduced receptor binding for dopamine and noradrenaline but no information on structural and functional connectivity changes for the basal ganglia and thalamus has been reported in these studies. So it does not mean that there is no change.*
Abbreviations: ACC = anterior cingulate cortex; BLA = basolateral nucleus of amygdala; BNST = bed nucleus of the stria terminalis; CeA = centromedial nucleus of amygdala; GP(e/i) = globus pallidus (external / internal); HypoTh = hypothalamus; OFC = orbitofrontal cortex; (m/dl/vl)PFC = (medial / dorsolateral / ventrolateral) prefrontal cortex; STN = subthalamic nucleus.

Anxiety, depression, and apathy: a “non-motor triad”

In addition to studies focusing on the imaging of anxiety, studies addressing the border area of anxiety, depression and apathy may also shed light on the neurocircuitry of anxiety. While not the focus of our search, depression and apathy are commonly associated with anxiety. Some authors suggested that these three neuropsychiatric manifestations would constitute a behavioural “non-motor triad” in PD [48]. On the one hand, several studies demonstrated that dysfunction of the cortico-striato-thalamocortical limbic circuit (OFC, ACC, and limbic part of basal ganglia) is implied in the pathophysiology of apathy, depression, and anxiety. These suggest that a more widespread meso-cortico-limbic dopaminergic denervation (OFC, DLPFC, cingulate cortices, the left ventral striatum, and the right amygdala), is involved in the pathogenesis of apathy and depression [49]. Moreover, another study stressed the importance of degeneration of serotonergic structures within the limbic system in this “non-motor triad”, that is already present at the beginning of the disease. The severity of anxiety in apathetic PD patients was linked to a serotonergic disruption within the bilateral ACC, without a prominent role of dopaminergic degeneration [48]. In our review, one study also showed that the severity of depression, apathy and anxiety was associated with a loss of dopamine and noradrenaline innervation in the locus coeruleus and the limbic system (ACC, thalamus, amygdala, and ventral striatum) [23]. In another systematic review, the authors confirmed that not only mesolimbic dopaminergic but also mesolimbic serotonergic and noradrenergic lesions play a major role in the mechanisms of these three psychiatric symptoms [9]. On the other hand, several studies showed differences in the underlying mechanisms of depression, apathy, and anxiety. In neurotransmitter imaging studies, these three symptoms were associated with a reduced dopaminergic innervation in the striatum, notably the ventral striatum, but several studies showed a specific reduction in the caudate nucleus in anxious PD patients [23, 26, 50]. Zhang et al. reported a positive correlation between the functional connectivity of the left parahippocampal gyrus and the severity of depressive symptoms in PD while the severity of anxiety was positively correlated to the functional connectivity between the parahippocampal gyrus and the left amygdala. The functional networks associated with depression and anxiety were also different [19][22]. Recently, a study using voxel-based morphometry and diffusion tensor imaging showed that de novo apathetic PD patients (with or without depression) had microstructural alterations in the medial cortico-striatal limbic system (striatum, ACC, medial frontal cortex, thalamus, midbrain). There was no microstructural alteration correlated with symptoms of anxiety [46]. These studies point out that considering the pathophysiology of anxiety independently of depression and apathy is difficult but that they might have distinct underlying mechanisms. They also highlight the fact that further appropriate studies are needed to decipher these mechanisms.

Strengths and limitations

In our review, we strictly followed the PRISMA guidelines for systematic reviews. We did not include the terms 'electroencephalography' or 'magnetoencephalography' in our search strategy since this was not considered within the scope of our review. In a post-hoc exploratory search, no study used these methods to specifically explore the pathophysiology of anxiety in PD. However, such studies could usefully extend the understanding of the pathophysiology of anxiety in PD.

Anxiety is usually not an isolated symptom. It is often associated with depression, apathy and/or cognitive decline. It is thus difficult to determine the pathophysiology of anxiety independently of these other neuropsychiatric symptoms (see section 4.4). The mean cognitive scores (MMSE or MoCA) of the patients in the included studies are provided in the Table 1 and reveal no cognitive decline in our sample. However, there were limitations related to the included studies. All studies were cross-sectional, which implies that it was not possible to make conclusions about temporal or causal relations. Moreover, there may be alterations in other structures than those we focused on, such as the VTA and STN. Further studies are needed to identify the involvement of the latter and other structures in PD related anxiety. Other limitations of the included studies were inclusion of patients with subclinical anxiety symptoms, the use of non-validated clinical rating scales for anxiety, lack of separation of different anxiety diagnoses, and lack of correction for covariables. Finally, the lack of a healthy control group in some of the included studies is also a limitation.

Conclusion

In this review, anxiety symptoms were associated with alterations of the limbic cortico-striato-thalamocortical circuit and the fear circuit. In PD, dysfunction of basal-ganglia and brainstem nuclei could lead to alteration in both circuits explaining the high prevalence of anxiety in Parkinson’s disease and the motor, behavioural and cognitive symptoms associated [3]. Further studies are needed to better understand the pathophysiology of this symptom.

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SUPPLEMENTARY MATERIAL

Supplementary data S1

Research protocol: Data selection and inclusion criteria

For this systematic review, we included studies (a) with PD-patients that assessed anxiety symptoms, (b) including any method of neuroimaging such as structural or functional MRI or metabolic imaging (PET, SPECT), (c) categorically comparing PD-patients with and without anxiety on imaging parameters, or correlating imaging parameters with severity of anxiety as a continuous variable (d) whose outcome parameters were either grey-matter volume (GMV), the blood-oxygen-level-dependent (BOLD) signal, or the binding-rate (BR) values. Any study design was eligible for inclusion. Also, both studies performing whole brain seed-based analyses or region of interest (ROI) analyses were included. The exclusion criteria were: (a) review articles reporting no original data or preclinical studies, (b) studies focusing on other diseases than PD or on anxiety in general, (c) studies without assessment of anxiety, (d) studies without neuroimaging.

Eligibility was determined independently by two researchers (G.C. and M.G.). Firstly, duplicates were removed from the research results and followed by screening of the title and abstract. Discrepancies were resolved in a consensus meeting and a third specialist (A.L.) was consulted if no consensus was reached.

Supplementary data S2

Quality assessment procedure

Two authors (G.C. and M.G.) extracted data. The following information was extracted from each study: the first author, year of publication, journal name, sample size, method of neuroimaging, the anxiety scale used, the statistical methods, the imaging data pre-processing and processing methods, patient characteristics, including age, gender, duration of PD, education, medication and cognitive function. Peak coordinates and effect size measures of the regions with a significant difference in imaging data were also collected if possible. Some publications did not report peak coordinates because of the study design.

To assess risk of bias in individual studies we used nine quality criteria, following the approach of Wolters et al. [1]: (a) patient demographics; (b) imaging procedure; (c) spatial normalization method; (d) determination of ROIs; (e) reproducibility of the analyzes; (f) statistical tests used to substantiate the results; (g) correction for the multiple testing problem; (h) figures and tables; (i) quality control measures (Table S2.1). Studies could score 0, 0.5 or 1 point for each item. An overall score of ≥7.5 was considered as good quality, 4–7.5 as moderate quality, and ≤4 as poor quality [1]. The quality

assessment results are detailed on Table S2.2 and Figure S2.1. It was performed by two researchers (G.C. and M.G.) and discrepancies were discussed until consensus was reached. If no consensus could be reached, a third specialist was consulted (A.L.).

The risk of bias across studies could not be assessed formally because the number of included studies was small for each imaging type and/or because the study design was very heterogeneous (comparisons or correlation, different anxiety scale, different metabolic imaging). Moreover, due to this heterogeneity, it was not possible to perform a meta-analysis as we intended initially. Instead, we decided to present a systematic review including peak coordinates and effect size measures.

a. Did they give a full description of the study participants?
b. Did they give a full description of the imaging type and acquisition?
c. Did they specify the spatial normalization procedure, including the atlas or template which is used to match the images to?
d. Did they specify how the regions of interest were determined?
e. Did they provide enough detail to reproduce the analyses?
f. Are all the empirical claims supported by a specific statistical test?
g. Did they describe and account for the multiple testing problem?
h. Do the figures and tables stand on their own?
i. Are the quality control measures documented?

Table S2.1. Nine quality assessment criteria according to A.F. Wolters et al, 2018 [1]

Study	a	b	c	d	e	f	g	h	i	Total	
Anatomical											
Oosterwijk et al., 2018	1	1	1	1	1	1	1	1	1	9	
Ma et al., 2018	1	1	1	1	1	1	0.5	1	0.5	8	
Vriend et al., 2016	1	1	1	1	1	1	1	1	1	9	
Wee et al., 2016	1	1	1	1	1	1	1	1	0	8	
Functional											
Zhang et al., 2019	1	1	1	1	1	1	1	1	0	8	
Wang et al., 2018	1	1	1	0	1	1	1	1	0	7	
Dan et al., 2017	1	1	1	1	1	1	1	0.5	0	7.5	
Wang et al., 2017	1	1	1	1	1	1	1	1	0	8	
Metabolic											
Bayram et al., 2019	1	1	1	0.5	1	1	1	1	0	7.5	
Joling et al., 2018	1	1	1	1	1	1	1	1	0	8	
Picillo et al., 2017	1	1	1	0.5	1	1	0.5	1	0	7	
Wang et al., 2017	1	1	1	0.5	1	1	0	1	0	6.5	
Ceravolo et al., 2013	1	1	0.5	1	1	1	0.5	1	0	7	
Huang et al., 2013	1	1	1	0.5	1	1	1	1	0	7.5	
Erro et al., 2012	1	1	1	1	1	1	0.5	1	0	7.5	
Moriyama et al, 2011	1	1	0.5	1	1	1	0	1	0	6.5	
Weintraub et al., 2005	0.5	1	0.5	0.5	1	1	0.5	1	0	6	
Remy et al., 2005	1	1	1	1	1	1	1	1	0	8	

Table S2.2. Quality assessment of the studies as good (green), fair (yellow) or poor (red) quality.
0 = poor criteria; 0.5 = moderate criteria; 1 = good criteria; QC = quality control.

Figure S2.1. Quality assessment according to A.F. Wolters et al, 2018 [1] nine criteria.
An overall score of ≥7.5 was considered as good (green), 4-7.5 as fair (orange) and ≤4 as poor (red) quality.

Supplementary results S3

Flow chart of the review

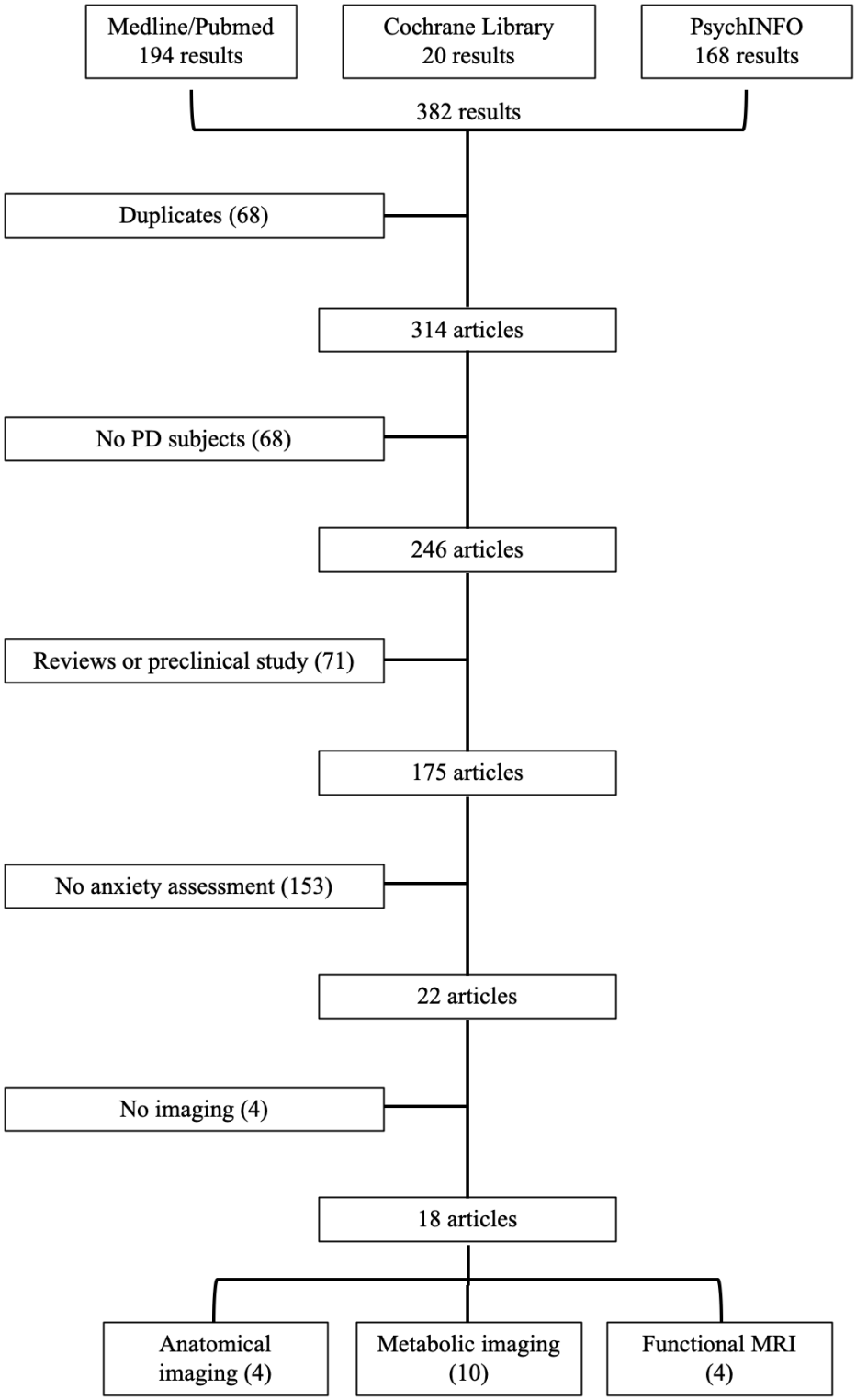

Figure S2.1. Flow chart and research procedure of the systematic review. *PD = Parkinson's disease.*

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CHAPTER 4

Anxiety in Parkinson's Disease is Associated with Changes in Brain Structural Connectivity

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Summary of the chapter

In a previous study, we identified functional connectivity and structural changes within the fear circuit in PD patients with anxiety. Thereafter, our systematic review showed that the limbic circuit is also involved in the mechanism of PD-related anxiety. Therefore, we aimed to identify if PD-related anxiety was associated with changes in structural connectivity between the main parts of these two circuits. Using diffusion tensor imaging (DTI) we compared connectivity parameters, such as fractional anisotropy (FA) and mean diffusivity (MD), between PD patients with and without clinically significant anxiety from the same observational cohort as in Chapter 2. We also performed regression analyses between changes of these parameters and anxiety severity according to the PAS-total score. We found that PD-related anxiety was associated with a reduced FA between the orbito-frontal cortex and the striatum within the limbic circuit. We also found both reduced and increased FA within the fear circuit such as between the insula and the amygdala and the anterior cingulate cortex, accumbens nucleus and thalamus, respectively. These changes could correspond to microstructural alterations within the two circuits. This reinforce the view that PD-related anxiety is also linked to white matter structural alterations within the anxiety-related brain circuits.

Abstract

Background: Anxiety in Parkinson's disease (PD) has been associated with grey-matter changes and functional changes in anxiety-related neuronal circuits. So far, no study has analysed white-matter (WM) changes in patients with PD and anxiety.

Objective: The aim of this study was to identify WM changes by comparing PD patients with and without anxiety, using diffusion-tensor imaging (DTI).

Methods: 108 non-demented PD patients with (n= 31) and without (n = 77) anxiety as defined by their score on the Parkinson Anxiety Scale participated. DTI was used to determine the fractional anisotropy (FA) and mean diffusivity (MD) in specific tracts within anxiety-related neuronal circuits. Mean FA and MD were compared between groups and correlated with the severity of anxiety adjusted by sex, center, Hoehn & Yahr stage, levodopa equivalent daily dosage and Hamilton depression rating scale.

Results: Compared to patients without anxiety, PD patients with anxiety showed lower FA within the striato-orbitofrontal, striato-cingulate, cingulate-limbic, and caudate-thalamic tracts; higher FA within the striato-limbic and accumbens-thalamic tracts; higher MD within the striato-thalamic tract and lower MD within the striato-limbic tract.

Conclusion: Anxiety in PD is associated with microstructural alterations in anxiety-related neuronal circuits within the WM. This result reinforces the view that PD-related anxiety is linked to structural alteration within the anxiety-related brain circuits.

Introduction

Anxiety is among the most frequent neuropsychiatric symptoms in Parkinson's disease (PD) with an average point prevalence of 31%[1]. However, the underlying mechanisms remain uncertain. Recent neuroimaging studies showed that anxiety in PD may be associated with an imbalance between two neuronal circuits[2,3]. The fear circuit, involved in fear processing, could be over-activated in PD patients with anxiety. This circuit involves the amygdala, anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), insular cortex, hippocampus and striatum[4–6]. In addition, the limbic cortico-striato-thalamo-cortical anxiety circuit, a dopaminergic circuit involved in the control of emotions, could be under-activated. This circuit involves the mPFC, the orbitofrontal cortex (OFC), the ACC, the ventral part of the basal ganglia (accumbens nucleus, pallidum, caudate, subthalamic nucleus) and the thalamus[7,8]. These findings were based on MRI measures of functional connectivity and grey matter (GM) volume. White matter (WM) abnormalities have also been associated with motor symptoms and disease severity[9], cognitive decline[10] and depression[11] in PD. To date, no study has explored WM changes associated with PD-related anxiety. Diffusion tensor imaging (DTI) is a common method for exploring structural connectivity and WM changes as integrity along WM fibres through parameters such as the fractional anisotropy (FA) and the mean diffusivity (MD). These parameters are indices of axonal and myelin integrity. Changes in these parameters could thus reflect microstructural alterations in the brain[10,11].

In PD, a recent DTI study showed that reduced FA and increased MD in fronto-occipital, insular, thalamic and callosal regions were associated with cognitive decline[10]. A recent systematic review also reported microstructural changes (ie. reduced FA and increased MD) in specific limbic structures such as prefrontal regions, in depressed PD patients compared to non-depressed PD patients and healthy controls[11]. In non-PD anxious patients, DTI abnormalities have been described, specifically a reduced FA in the uncinate fasciculus, a tract between limbic structures, namely the amygdala and the orbitofrontal cortex, and in the cingulum [12,13]. These structures are part of the fear circuit.

DTI may thus help to decipher the underlying mechanisms of cognitive and behavioural symptoms in PD.

The aim of this study was to identify microstructural changes between structures involved in the fear and the limbic circuits in PD patients with anxiety compared to PD patients without anxiety, using DTI parameters such as FA and MD.

We hypothesized that FA would be reduced and MD increased in PD patients with anxiety in the anxiety-related neuronal circuits reflecting a higher level of microstructural alteration and dopaminergic neuronal degeneration.

Material and methods

Population

One-hundred and fifty-six PD patients were enrolled from two movement disorders clinics in Lille (France) and Maastricht (The Netherlands) between March 2013 and August 2014[14]. All the patients met the PD diagnostic criteria from the United Kingdom Parkinson's Disease Society Brain Bank and Movement Disorders Society clinical diagnostic criteria for PD [15]. Patients with other neurological disorders or moderate to severe dementia (Movement Disorders Society criteria for Parkinson's disease dementia[16]) were excluded.

Age, sex, duration of formal education, disease duration, history of PD or psychiatric disorders were recorded.

Non-motor symptoms, motor symptoms and disease severity were assessed using the Movement Disorder Society Unified Parkinson Disease Rating Scale (MDS-UPDRS) part I, MDS-UPDRS part IIII and Hoehn-Yahr staging[17], respectively. The levodopa equivalent daily dosages (LEDD) were calculated, and the use of antidepressant and anxiolytics treatments reported.

The Parkinson Anxiety Scale (PAS)[18], the Hamilton Depression Rating Scale (HAMD) [19] and the Lille Apathy Rating Scale (LARS)[20] were used to assess anxiety, depression and apathy, respectively.

Vascular risk factors and cerebral WM changes (hypersignals) were assessed to control for potential confounding bias. Diabetes, hypertension, hypercholesterolemia, tobacco use, cerebral infarcts, arteriopathy and total vascular risk factors (at least one of the reported factors) were reported. Cerebral WM changes were assessed using Fazekas scores for periventricular (P), deep (D) and total (P+D) changes. Additional information on this study group is detailed in the original paper[14]. Assessments were all performed when the patients were in the ON-drug state.

Written informed consent was obtained from all participants after full information of the procedure. The study was approved by the institutional ethics committees of both participating institutions (Lille: CPP Nord-Ouest IV, 2012-A 01317-36; Maastricht: METC AZM/UM 12-3-064).

Characterization of anxiety

Patients were divided into two groups, one with and the other without 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 "with anxiety" if they had a score above the defined 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 behaviour) >3)[18].

Imaging data acquisition

Patients were scanned at two sites using identical 3-Tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) with identical software versions and MR sequences. The imaging protocol included an anatomical three-dimensional T1-weighted (3D-T1w) sequence [voxel size = 1 × 1 × 1 mm3, repetition time (TR) = 7.2 ms, echo time (TE) = 3.3 ms, matrix size = 256 × 256 × 176 voxels, flip angle = 9°] and a diffusion tensor imaging (DTI) sequence [voxel size = 2 × 2 × 2 mm3, TR = 13,000 ms, TE = 55 ms, matrix size = 128 × 128 × 66 voxels, flip angle = 90°, 64 gradient directions at b = 1,000 s/mm2]. To correct B0 field inhomogeneity-induced distortion, two non-diffusion-weighted images (b = 0 s/mm2) with opposite phase-encoding directions were also collected[21]. For quality control, all images were visually inspected by a board-certified neuroradiologist (GK).

DTI data preprocessing

DTI data were first corrected for eddy currents and geometrical/signal distortions[22]. Eddy current artifacts were corrected using the eddy_correct function in the FMRIB Software Library. Then, the distortion field, inherent to echo planar images (EPI) in the phase encoding direction and responsible for geometric and signal artifacts, was calculated using a pair of spin echo EPI scans with opposite phase encoding directions[21]. The “epiunwarp” function in the Computational Morphometry Toolkit (CMTK 3.2.25) was used to estimate the distortion field and applied it to the DTI data.

DTI analysis

A complete brain parcellation including 91 cortical areas and 15 subcortical areas from the FSL Harvard-Oxford Atlas was used to define regions of interest (ROI) in MNI-space[23] through FreeSurfer (version 5.3) [24] procedure. A non-linear registration was performed to transform these ROIs to the patient-space using the tool ANTs (https://github.com/ANTsX/ANTs). According to our hypotheses, five subcortical bilateral ROIs, the nucleus accumbens, amygdala, caudate, putamen and thalamus, as well as eight cortical ROIs, the caudal ACC (cACC), rostral ACC (rACC), caudal middle frontal gyrus (cMFG), rostral middle frontal gyrus (rMFG), superior frontal gyrus (SFG), insula, lateral fronto-orbital cortex (lForb), medial fronto-orbital cortex (mForb), were selected. The ROIs were visually inspected for each individual, and then dilated (one voxel in each direction) by masking with the lateral ventricles and brain mask.

Fiber tracking between the cortical and subcortical ROIs was performed using a probabilistic streamline tractography, as implemented in MRtrix software[25,26]. Fibre pathways were generated by randomly seeding a starting subcortical ROI and tracking until the fibre reached the ending cortical or subcortical ROI to ensure symmetrical fibre tracking (maximum number of harmonics was set to 6, maximum

number of fibres =5000, FA cutoff =0.1, curvature =60 degrees). Fibers leaving the WM mask were terminated and discarded. Fibres obtained from the tractography solutions were then reduced to core fibre tracts by removing false positive using linear fascicle evaluation (LiFE)[27]. Next, fibres greater than 3 standard deviations away from the mean spatial position of the core fibre (Mahalanobis distance) and fibres greater or smaller than 3 standard deviations in size were removed.

Among the created tracts, those that are known to be involved in unrelated non-mental functions, such as the corticospinal tract end the longitudinal fasciculus, or those whose procedure failed were excluded from further analyses. The selected tracts are called “WM specific tracts”, in this study.

To estimate the integrity of each WM specific tract, FA and MD maps were computed for each subject using MRTrix process[25,26]. FA represents a common measurement used in DTI studies ranging from 0 = isotropic movement of water molecules (e.g. cerebrospinal fluid) to 1 = anisotropic movement of water molecules (e.g. fibre tracts). It means that diffusion of molecules is allowed in only one direction. Inversely, MD, describing the average mobility of water molecules, will be higher in the cerebrospinal fluid (approx. 3 × 10−3 mm2/s) than in WM (approx. 5 × 10−4 mm2/s). The mean FA and MD values were then calculated for each patient and each tract.

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 for DTI data. The normality of distribution was assessed using a Kolmogorov-Smirnov tests.

Demographic and clinical data

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 SPSS-IBM, version 26 (SPSS, Chicago).

Comparison analyses

The mean FA and MD values of each specific tract were compared between the two groups using an ANCOVA procedure with centre, sex, Hoehn-Yahr stage, LEDD and HAMD score as covariates. We ensured that all comparisons met the assumptions of ANCOVA procedure.

Regression analyses

Hierarchical multiple regression analyses were performed to examine the relationship between the PAS score and the mean FA and MD values of each specific tract. Centre, sex, Hoehn-Yahr stage, LEDD and HAMD score were set as nuisance regressors in the first block (model 1) of all regression models whereas PAS score (independent variable) was separately added to the second block (model 2) of the model. We ensured that all models met the assumptions of multiple regression analyses, including normality of the residuals, multicollinearity, and homoscedasticity.

Results

Population

After exclusion of patients for dementia (n=14), refusal or contraindication to have an MRI (n=22), unusable 3D-T1w (major motion artefact – n = 2), no available DTI (n = 1) or unusable DTI after quality control (n = 9), 108 participants were included in the present study, 31 with anxiety and 77 without anxiety.

Demographic and clinical variables

The anxious patients tended to be more frequently female, had a more advanced disease stage, were using a higher LEDD, and more frequently used antidepressants and anxiolytics (Table 1). Logically, PAS total score and sub-scores as well as the HAMD total were higher in the anxious than in the non-anxious group. There was no between-group difference regarding vascular risk factors and cerebral WM changes. The results are detailed in Table 1.

Demographic variables	anxious group ($n = 31$)	non-anxious group ($n = 77$)	OR (CI 95%); p
Age (y)	65.91 (±6.30)	64.19 (±8.74)	0.48
Women ($n = 36$)	14 (42.42%)	20 (25.97%)	2.35 (0.98; 5.62); 0.052
Hand dominance (right, $n = 101$)	26 (83.87%)	67 (87.01%)	0.97 (0.28; 3.37); 0.67
Formal education (y)	12.13 (±4.19)	12.60 (±3.60)	0.37
Illness duration (y)	9.29 (±7.45)	8.04 (±4.94)	0.57
Clinical variables			
LEDD (mg/day)	944.46 (±511.47)	740.31 (±588.12)	**0.028***
Antidepressant use ($n = 17$)	12 (38.71%)	4 (5.19%)	**11.53 (3.34; 39.81); <0.0001***
Anxiolytic use ($n = 12$)	9 (29.03%)	2 (2.60%)	**15.34 (3.08; 76.30); <0.0001***
MDS-UPDRS part 3 (/132)	38.77 (±13.18)	28.06 (±11.87)	0.73
Hoehn & Yahr stage (0–5)§	2 (1–3)	2 (0–4)	**0.010***
PAS total (/48)	14.42 (±4.39)	3.66 (±4.35)	**<0.0001***
Part A (/20)	9.32 (±4.35)	2.81 (±2.92)	**<0.0001***
Part B (/16)	2.35 (±2.24)	0.43 (±0.87)	**<0.0001***
Part C (/12)	2.74 (±2.32)	0.43 (±0.84)	**<0.0001***
HAMD total (/54)	8.87 (±5.36)	4.68 (±3.68)	**<0.0001***
LARS total	–21.81 (±9.23)	–23.91 (±10.35)	0.058
Vascular risk factor			
Total vascular risk factor	25 (80.65%)	58 (75.32%)	0.553
- Diabetes	4 (12.90%)	5 (6.49%)	0.275
- Hypertension	11 (35.48%)	18 (23.38%)	0.199
- Hypercholesterolemia	8 (25.81%)	16 (20.78%)	0.570
- Tobacco use	2 (6.45%)	2 (2.60%)	0.324
- Cerebral infarcts	3 (9.68%)	1 (1.30%)	0.070
- Arteriopathy	1 (3.23%)	5 (6.49%)	0.671
Fazekas score§			
- Periventricular (P)	1 (0–2)	1 (0–2)	0.238
- Deep (D)	1 (0–2)	1 (0–2)	0.799
- Total (P+D)	2 (0–4)	2 (0–4)	0.345

Table 1. Demographic and clinical variables: group comparisons (Parkinson's disease patients with and without anxiety).

** = p-value < 0.05, § = described as median and range; CI = confidence interval; HAMD = Hamilton Depression Rating Scale; LARS = Lille Apathy Rating Scale; LEDD = Levodopa Equivalent Daily Dosages; MDS-UPDRS = Movement Disorder Society Unified Parkinson Disease Rating Scale; OR = Odds Ratio; PAS = Parkinson Anxiety Scale.*

MRI analyses

Specific tracts creation

After the processing steps, 60 tracts were created bilaterally. Of these, 18 bilateral tracts (i.e. 36 tracts) were included in the analyses: the accumbens-amygdala tract, the accumbens-thalamus tract, the accumbens-insula tract, accumbens-lateral OFC, accumbens-medial OFC, accumbens-right ACC, amygdala-putamen, amygdala-thalamus, amygdala-insula, amygdala- lateral OFC, amygdala-medial OFC, amygdala-right ACC, caudate-left OFC, caudate-medial OFC, caudate-right ACC, putamen-lateral OFC, putamen-medial OFC and thalamus-caudate tract.

Comparison analyses

In the anxious group, the mean FA value was lower within the left striato-OFC (accumbens-mOFC, caudate-mOFC, putamen-mOFC), left striato-cingulate (accumbens-rACC), left cingulate-limbic (amygdala-rACC) and right striato-thalamic (caudate-thalamus) tracts. It was higher within the right

striato-limbic (accumbens-insula) and right striato-thalamic (accumbens-thalamus) tracts compared with the non-anxious group (Table 2).

In the anxious group, the mean MD value was higher within the right striato-thalamic (caudate-thalamus) tract and lower within the right striato-limbic (accumbens-insula) tract compared with the non-anxious group (Table 3).

Regression analysis

There was no association between the severity of anxiety and the FA or MD mean values within the tracts studied.

Comparison of FA mean values between anxious and non-anxious PD patients				
Specific tracts	PD with anxiety	PD without anxiety	F	FDR *p*
Left Tracts				
L. Accumbens-Amygdala	0.269 (±0.021)	0.274 (±0.025)	2.651	0.05
L. Accumbens-Insula	0.291 (±0.020)	0.296 (±0.023)	1.762	0.16
L. Accumbens-lOFC	0.260 (±0.020)	0.264 (±0.022)	2.667	0.05
L. Accumbens-mOFC	**0.260 (±0.023)***	**0.264 (±0.025)**	**4.180**	**0.012**
L. Accumbens-rACC	**0.263 (±0.028)***	**0.264 (±0.027)**	**3.496**	**0.016**
L. Accumbens-Thalamus	0.307 (±0.025)	0.311 (±0.029)	1.553	0.222
L. Amygdala-Insula	0.273 (±0.023)	0.283 (±0.023)	1.259	0.32
L. Amygdala-lOFC	0.249 (±0.018)	0.256 (±0.019)	1.917	0.13
L. Amygdala-mOFC	0.260 (±0.018)	0.266 (±0.021)	2.262	0.07
L. Amygdala-Putamen	0.274 (±0.021)	0.281 (±0.026)	1.363	0.28
L. Amygdala-rACC	**0.269 (±0.019)***	**0.272 (±0.020)**	**3.955**	**0.012**
L. Amygdala-Thalamus	0.295 (±0.023)	0.303 (±0.024)	1.173	0.35
L. Caudate-lOFC	0.292 (±0.019)	0.296 (±0.022)	2.234	0.07
L. Caudate-mOFC	**0.310 (±0.023)***	**0.312 (±0.023)**	**2.997**	**0.035**
L. Caudate-rACC	0.305 (±0.025)	0.304 (±0.023)	2.508	0.06
L. Putamen-lOFC	0.267 (±0.017)	0.271 (±0.021)	2.237	0.07
L. Putamen-mOFC	**0.282 (±0.020)***	**0.285 (±0.023)**	**3.610**	**0.016**
L. Thalamus-Caudate	0.366 (±0.023)	0.373 (±0.023)	0.568	0.75
Right Tracts				
R. Accumbens-Amygdala	0.263 (±0.017)	0.264 (±0.020)	0.660	0.68
R. Accumbens-Insula	**0.263 (±0.016)**	**0.259 (±0.024)***	**3.260**	**0.034**
R. Accumbens-lOFC	0.236 (±0.019)	0.239 (±0.018)	1.504	0.33
R. Accumbens-mOFC	0.237 (±0.022)	0.237 (±0.019)	1.969	0.20
R. Accumbens-rACC	0.238 (±0.023)	0.235 (±0.022)	1.665	0.31
R. Accumbens-Thalamus	**0.292 (±0.026)**	**0.290 (±0.021)***	**3.346**	**0.034**
R. Amygdala-Insula	0.278 (±0.016)	0.284 (±0.024)	0.996	0.53
R. Amygdala-lOFC	0.260 (±0.015)	0.262 (±0.021)	0.804	0.62
R. Amygdala-mOFC	0.261 (±0.014)	0.262 (±0.018)	0.773	0.62
R. Amygdala-Putamen	0.282 (±0.015)	0.285 (±0.024)	1.336	0.37
R. Amygdala-rACC	0.270 (±0.015)	0.270 (±0.018)	1.563	0.33
R. Amygdala-Thalamus	0.319 (±0.012)	0.321 (±0.021)	1.427	0.35
R. Caudate-lOFC	0.274 (±0.019)	0.273 (±0.024)	2.339	0.17
R. Caudate-mOFC	0.263 (±0.023)	0.263 (±0.023)	2.127	0.19
R. Caudate-rACC	0.265 (±0.020)	0.266 (±0.022)	2.061	0.19
R. Putamen-lOFC	0.289 (±0.016)	0.292 (±0.023)	1.076	0.53
R. Putamen-mOFC	0.281 (±0.016)	0.283 (±0.020)	0.987	0.53
R. Thalamus-Caudate	**0.343 (±0.021)***	**0.346 (±0.019)**	**6.091**	**0.0003**

Table 2. Comparison of the mean values of Fractional Anisotropy (FA) in specific DTI tracts between Parkinson's disease (PD) patient with and without anxiety.

Abbreviations: FDR = false discovery rate; mOFC = medial fronto-orbital cortex; lOFC = lateral fronto-orbital cortex; L. = left; R. = right; rACC = rostral anterior cingulate cortex.

*Interpretation: Bold = significant difference; * = Lower FA between the two groups.*

Comparison of MD mean values between anxious and non-anxious PD patients				
Specific tracts	PD with anxiety	PD without anxiety	F	FDR *p*
Left Tracts				
L. Accumbens-Amygdala	0.0010 (±0.0001)	0.0010(±0.0001)	2.215	0.285
L. Accumbens-Insula	0.0010 (±0.0001)	0.0009(±0.0001)	1.102	0.597
L. Accumbens-lOFC	0.0010 (±0.0001)	0.0010(±0.0001)	1.811	0.356
L. Accumbens-mOFC	0.0010 (±0.0001)	0.0010(±0.0001)	1.811	0.356
L. Accumbens-rACC	0.0010 (±0.0001)	0.0010(±0.0001)	1.669	0.356
L. Accumbens-Thalamus	0.0010 (±0.0001)	0.0010(±0.0001)	0.978	0.597
L. Amygdala-Insula	0.0010 (±0.0001)	0.0010(±0.0001)	2.981	0.182
L. Amygdala-lOFC	0.0011 (±0.0001)	0.0011(±0.0001)	1.661	0.356
L. Amygdala-mOFC	0.0011 (±0.0001)	0.0011(±0.0001)	1.473	0.390
L. Amygdala-Putamen	0.0011 (±0.0001)	0.0010(±0.0001)	2.378	0.285
L. Amygdala-rACC	0.0010 (±0.0001)	0.0010(±0.0001)	1.489	0.390
L. Amygdala-Thalamus	0.0010 (±0.0001)	0.0010(±0.0001)	1.410	0.392
L. Caudate-lOFC	0.0009 (±0.0001)	0.0009(±0.0001)	0.901	0.597
L. Caudate-mOFC	0.0009 (±0.0001)	0.0009(±0.0001)	0.625	0.710
L. Caudate-rACC	0.0009 (±0.0001)	0.0009(±0.0001)	0.966	0.597
L. Putamen-lOFC	0.0010 (±0.0001)	0.0010(±0.0001)	0.945	0.597
L. Putamen-mOFC	0.0010 (±0.0001)	0.0010(±0.0001)	0.784	0.657
L. Thalamus-Caudate	0.0009 (±0.0001)	0.0009(±0.0001)	0.646	0.710
Right Tracts				
R. Accumbens-Amygdala	0.0011 (±0.0001)	0.0011(±0.0001)	0.588	0.912
R. Accumbens-Insula	**0.0010 (±0.0001)**	**0.0011(±0.0001)***	**3.331**	**0.044**
R. Accumbens-lOFC	0.0011 (±0.0001)	0.0011(±0.0001)	0.706	0.894
R. Accumbens-mOFC	0.0011 (±0.0001)	0.0011(±0.0001)	0.239	0.963
R. Accumbens-rACC	0.0011 (±0.0001)	0.0011(±0.0001)	0.377	0.944
R. Accumbens-Thalamus	0.0010 (±0.0001)	0.0010(±0.0001)	2.375	0.208
R. Amygdala-Insula	0.0011 (±0.0001)	0.0010(±0.0001)	1.125	0.878
R. Amygdala-lOFC	0.0011 (±0.0001)	0.0011(±0.0001)	0.562	0.912
R. Amygdala-mOFC	0.0011 (±0.0001)	0.0011(±0.0001)	0.917	0.881
R. Amygdala-Putamen	0.0011 (±0.0001)	0.0011(±0.0001)	1.062	0.878
R. Amygdala-rACC	0.0010 (±0.0001)	0.0010(±0.0001)	0.780	0.881
R. Amygdala-Thalamus	0.0010 (±0.0001)	0.0010(±0.0001)	0.443	0.944
R. Caudate-lOFC	0.0010 (±0.0001)	0.0010(±0.0001)	2.003	0.259
R. Caudate-mOFC	0.0010 (±0.0001)	0.0010(±0.0001)	1.696	0.389
R. Caudate-rACC	0.0010 (±0.0001)	0.0010(±0.0001)	2.098	0.259
R. Putamen-lOFC	0.0010 (±0.0001)	0.0010(±0.0001)	0.797	0.881
R. Putamen-mOFC	0.0010 (±0.0001)	0.0010(±0.0001)	0.904	0.881
R. Thalamus-Caudate	**0.00099 (±0.0001)***	**0.00098(±0.0001)**	**3.810**	**0.033**

Table 3. Comparison of the mean values of Mean Diffusivity (MD) in specific DTI tracts between Parkinson's disease (PD) patient with and without anxiety.

Abbreviations: FDR = false discovery rate; mOFC = medial fronto-orbital cortex; lOFC = lateral fronto-orbital cortex; L. = left; R. = right; rACC = rostral anterior cingulate cortex.

*Interpretation: Bold = significant difference; * = Higher mean MD between the two groups.*

Discussion

Anxiety is a common non-motor symptom in PD. It is associated with functional and GM changes in neuronal anxiety-related circuits. So far, WM changes related to anxiety were not explored in PD. In this study, PD patients with anxiety had a lower FA within left striato-OFC, left striato-cingulate, left cingulate-limbic and right striato-thalamic tracts as well as a higher FA within right striato-limbic and right striato-thalamic tracts, a higher MD within the right striato-thalamic tract and a lower MD within the right striato-limbic tract compared with the non-anxious group. These results suggest microstructural changes and potential neuronal degeneration in anxiety-related brain circuits. The results are summarized in Figure 1.

Microstructural alteration of anxiety-related circuits

FA depicts the summative direction of the diffusion which provides a prominent vector while MD indicates the rate of molecular diffusion[28]. The meaning of FA and MD changes is still debated. FA reduction would refer to the level of disorganization of fibres going into different directions, rather than being organized in a clear pathway, going into the same direction which is the case with high FA. MD would be more specific to axonal changes[29]. Moreover, a reduced FA with increased MD has been associated with degeneration and axonal damage in the WM[28]. Recent studies suggested that DTI parameters would represent axonal and myelin integrity indices. Changes of these parameters could reflect microstructural alterations and axonal or myelin degeneration[10,11]. In these studies, any change (increase or decrease) of these parameters was associated with microstructural alteration.

We found a reduced FA within striato-OFC, striato-cingulate and striato-thalamic tracts and increased MD within striato-thalamic tract. These tracts are involved in the limbic anxiety circuit[7,8] (Figure 1). These results support the hypothesis of neuronal microstructural disorganization of the limbic anxiety circuit in anxious PD patients. This could be associated with the known dopaminergic neuronal degeneration in this neural circuit.

We also found reduced FA within cingulate-limbic tract, increased FA within striato-limbic and striato-thalamic tracts and reduced MD within striato-limbic tract. These tracts are involved in the fear circuit[4–6]. These results also support the involvement of neuronal microstructural disorganization of the fear circuit in anxious PD patients. This increased FA in parts of the fear circuit could reflect a compensatory mechanism for the disorganization in the limbic anxiety circuit. This limbic neuronal disorganization would lead to WM organization within the fear circuit.

In our study, changes in the two involved circuits are opposite, with likely higher FA/lower MD in the fear circuit and lower FA/higher MD in the limbic anxiety circuit. In a recent systematic review, we discussed the substantial overlap between the fear and limbic circuits. The anatomical separation

between these circuits may appear artificial but both circuits have already been described and validated independently [4–8]. They can be considered as two parts of a larger limbic circuit. The alteration of several neurotransmission systems (dopamine, serotonin, norepinephrine) due to PD and the resulting dysfunction of the basal ganglia loops could explain the underactivity of the limbic circuit which is involved in cognitive control of emotions [2]. It could promote an hyperactivation of the fear circuit, altering fear processing, as well as an hypoactivation of the limbic circuit, altering the cognitive and behavioural long-term adaptation to fear. The imbalance between these two overlapping circuits could partly explain the high prevalence of anxiety in PD compared with non-PD patients in whom only fear circuit changes are involved in anxiety [12,13]. These hypotheses are also supported by other independent study groups [3,30]. In the present study, we suggest that the differences of FA and MD between both circuits support the hypothesis that anxiety in PD could result from an imbalance between the fear and the limbic anxiety circuit [2,31]. It reveals that anxiety in PD patients is not only associated with GM and functional connectivity changes in anxiety-related circuits but also with microstructural alteration of the WM tracts themselves and structural connectivity changes. Moreover, alterations in WM could also be related to GM changes.

In non-PD anxious patients, DTI abnormalities have been only described within the fear circuit [12,13]. The results of this study are also consistent with our hypothesis that anxiety in PD patients is a distinct disorder from anxiety in general population. They are also in line with our hypothesis that anxiety in PD is not only a dopaminergic state but also involve extra-striatal structures. So far, only cognitive behavioural therapy was proven effective in reducing anxiety symptoms in PD. CBT was shown to increase functional connectivity between the frontal cortex and striatum, thus strengthening cognitive control over anxiety and restoring the balance between the anxiety and the fear circuit[32]. There is as yet no evidence for the efficacy of any medication in treating these symptoms[33]. A better understanding of the underlying mechanisms of anxiety disorders in PD may facilitate the development of novel therapeutics.

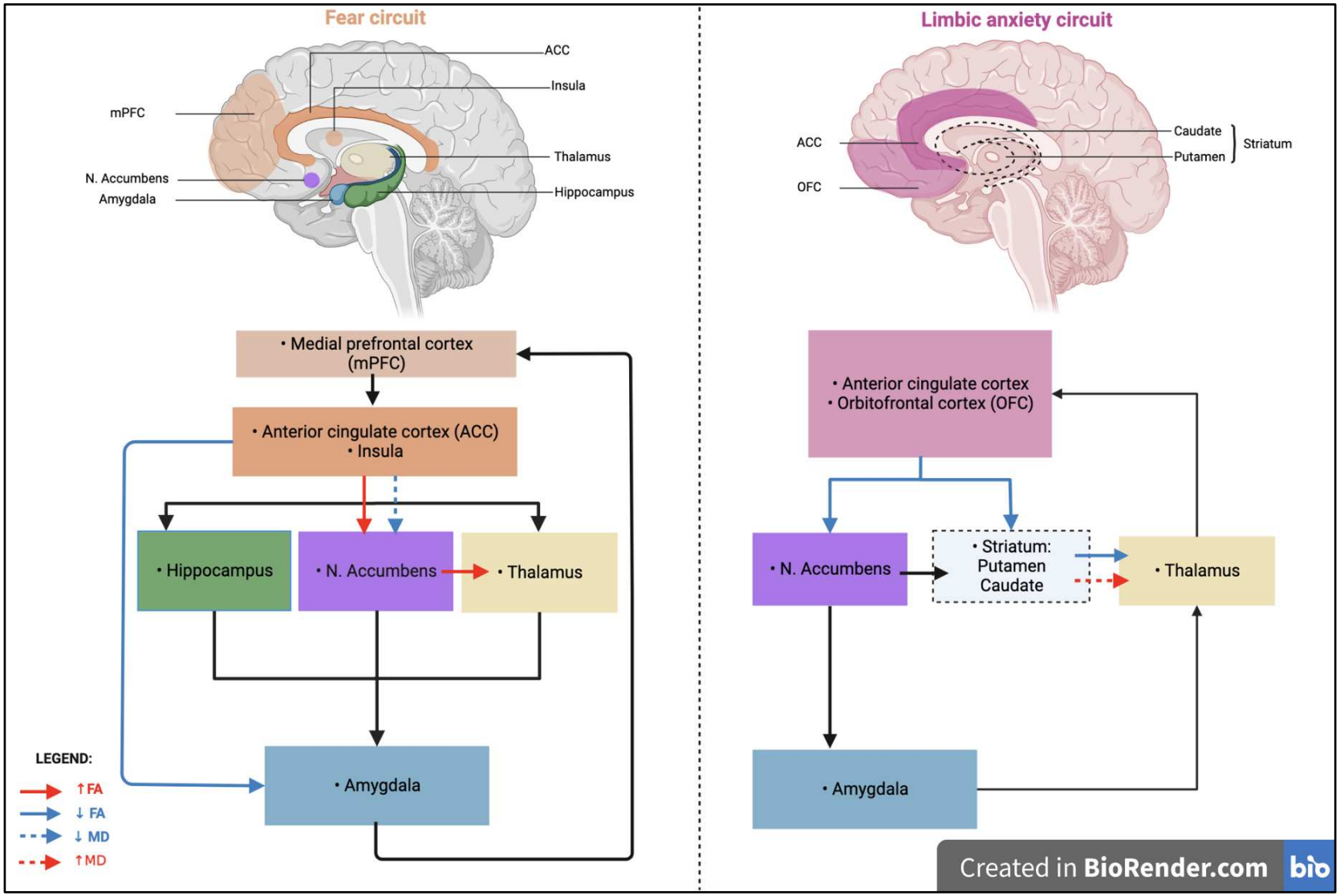

Figure 1. Graphical representation of microstructural alteration in specific tracts of the fear circuit and the limbic anxiety circuit in Parkinson's disease patients with anxiety.

Abbreviations: FA = fractional anisotropy; MD = mean diffusivity.

Strengths and limitations

This is the first study to analyse changes in DTI parameters in PD patients in relation with anxiety. We included a large cohort of patients (n = 108) who underwent 3-Tesla MRI scans and standardized clinical evaluation in two sites (Lille and Maastricht). We also compared patients with and without clinically significant anxiety and correlated anxiety symptoms severity to imaging data. However, our study had some limitations. Firstly, the patients were considered to have clinically significant anxiety symptoms according to their score at the PAS. They did not have a formal diagnosis of specific anxiety disorder according to diagnostic criteria (DSM). However, the PAS has demonstrated high sensitivity and specificity for diagnosing anxiety disorders in PD[18]. Secondly, the lack of a healthy control group did not enable us to determine which findings are specific to PD and which findings are not specific and associated with anxiety in general. But the aim of the present study was not to compare anxiety and healthy controls but to compare patients with and without PD-related anxiety. The non-anxious PD patients were considered as the control group. Finally, some structures that may also play a role in anxiety, have not been included in the analyses such as the sub-thalamic nucleus, the ventral tegmental area, the bed nucleus of stria terminalis and other brainstem nuclei (locus coeruleus, raphe nuclei). The 3-Tesla MRI did not allow us to clearly identify these structures. Future studies using 7-Tesla MRI may be necessary to analyse these structures.

Conclusion

In this study, we found changes in structural connectivity in the two neuronal circuits involved in anxiety in PD patients. These results support our earlier hypothesis that anxiety in PD could result from an imbalance between the fear and the limbic anxiety circuits[2,31]. Moreover, it reveals that anxiety in PD patients is not only associated with GM and functional changes in anxiety-related circuits but also with microstructural alteration of the WM tracts themselves and structural connectivity changes.

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CHAPTER 5

Anxiety in Parkinson's Disease: a Resting-State High Density EEG Study

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Summary of the chapter

In a previous study, we used resting-state functional MRI and showed that functional connectivity changes in the anxiety-related brain circuits were involved in the underlying mechanisms of PD-related anxiety. In the present study, we used another approach for assessing brain functional connectivity - functional electroencephalography (EEG) analysis - to reinforce the implication of dysfunctions of these circuits. We compared the EEG power spectrum and functional connectivity characteristics in PD patients with and without clinically significant anxiety in the dataset of the cohort “CogPhenoPark2”.

We found a reduction of the relative power in the alpha1 frequency band in the prefrontal cortex and a stronger functional connectivity between the left insula and several fronto-cingulate and temporal regions highlighting an important role of these structures, involved in the fear circuit, in the underlying mechanisms of Parkinson’s disease related anxiety. These results reinforce the role of functional changes within the fronto-limbic pathways, such as the fear circuit, in PD-related anxiety.

Abstract

Objective: to identify markers of Parkinson's disease (PD) related anxiety, using high density electroencephalography (hd-EEG).

Methods: 108 patients participated in the study. They were divided into two groups: with and without clinically relevant anxiety, according to their score at the Parkinson Anxiety Scale. Resting-state hd-EEG was recorded. Spectral and functional connectivity characteristics were compared between the two groups.

Results: Thirty-three patients (31%) had significant anxiety symptoms. In the spectral analysis, the relative power in the alpha1 frequency band in the right prefrontal cortex was lower in patients with anxiety than without. Functional connectivity analysis showed a stronger connectivity between the left insula and several regions of the right prefrontal cortex in patients with anxiety than in those without.

Conclusion: This study shows the pivotal role of the insula and frontal cortex in the pathophysiology of anxiety in PD and extends the results of previous studies using magnetic resonance imaging or positron emission tomography imaging.

Introduction

Parkinson's disease is characterized by motor but also non-motor symptoms. Among the latter, anxiety is frequent with a mean prevalence of about 31% [1]. While several methods have been used to decipher the underlying mechanisms, those mechanisms remain largely unknown. Structural magnetic resonance imaging (MRI) studies have shown correlations between the severity of anxiety and the volume of the amygdala as well as with cortical thickness of the bilateral frontal cortex, precuneus and anterior cingulate cortex ([2], [3], [4]). Task-based and resting-state (rs) functional MRI studies have identified disturbances in the fronto-limbic networks, resulting in limbic and paralimbic hyper-reactivity in emotion processing areas, such as the amygdala and the insula. Another outcome was an aberrant activation in regions known to regulate emotional reactivity, such as the dorsolateral prefrontal and the cingulate cortex ([5], [6], [7], [4]). Using positron emission tomography (PET), [8] showed that anxiety in PD was associated with an hypometabolism of the prefrontal cortex, suggesting a loss of voluntary control of emotion.

Apart from the amygdala, all these studies pointed out the predominant role of different cortical regions, such as the prefrontal cortex. Electroencephalography (EEG) provides a direct measure of the neural activity of the cortex and consequently appears as an appropriate method to identify markers of anxiety disorders. Furthermore, with a temporal resolution in the order of a millisecond, EEG assesses the fast and lagged spontaneous brain activity in time and frequency enabling the characterization of intrinsic neurocognitive networks. Up to now, EEG has not been used to explore the cortical basis of anxiety disorders in patients with PD. [9] have compared EEG patterns in non-parkinsonian subjects suffering from social phobia and healthy controls (HC). They reported a decrease in absolute and relative power in the delta, theta and slow beta frequency bands as well as a power increase in the intermediate beta band in the patient group. Moreover, trait anxiety scores correlated positively with the dominant alpha frequency. In terms of functional connectivity, a higher connectivity in the theta band in non-parkinsonian subjects with generalized anxiety relative to HC group during rest was described by [10].

The aim of this study was to identify changes associated with PD-related anxiety by comparing EEG spectral patterns and functional resting-state networks in patients with and without anxiety disorders.

Material and Methods

Study population

The data came from a previously published cross-sectional observational study [11] and were used in (Carey et al., 2020) for the identification of MRI markers of PD-related anxiety. One hundred and fifty-six patients were recruited from two movement disorders clinics in Lille (France) and Maastricht (The

Netherlands). PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank diagnostic criteria [12]. Dementia and any other neurological disorders were exclusion criteria.
The Movement Disorder Society Unified Parkinson Disease Rating Scale (MDS-UPDRS) was used to assess the severity of motor and non-motor symptoms. Disease severity was evaluated by Hoehn and Yahr stage.
The levodopa equivalent daily dosages (LEDD) were calculated [13] and the use of antidepressant and anxiolytics treatments reported. Anxiety was assessed using the Parkinson Anxiety Scale (PAS) [14], depression by the Hamilton Depression Rating Scale (HAMD) [15], apathy by the Lille Apathy Rating Scale (LARS) [16] and overall cognition by the Mattis dementia rating scale (MDRS) [17].
Patients were categorized as having (Anx+) or not (Anx-) clinically relevant anxiety symptoms according to their score at the PAS. Patients were affected to the Anx+ group if their score in one of the three subscales of the PAS was above the cut-off values that were fixed at 9 for persistent anxiety (PAS_A), 3 for episodic anxiety (PAS-B) and 3 for avoidance behaviour (PAS_C).
After information of the procedure, written informed consent was obtained from the participants. The study was approved by the institutional ethics committees (Lille: CPP Nord-Ouest IV, 2012-A 01317-36, Maastricht: NL56176.068.16)."

EEG Acquisition and preprocessing

The patients were explored when they were in on-drug condition using a 128-channel EEG system (ANT Software BV, Enschede, the Netherlands) with a 10-minute eyes-closed resting-state protocol. Electrodes were set up according to the 10/05 system and signal was sampled at a frequency of 512 Hz.
The BrainVision Analyzer software (BrainProducts GMBH, Gilching, Germany) was used for signal preprocessing with the following steps: averaging to the common reference, ocular artefacts correction and 50-Hz filtering. Each recording was visually checked to remove periods of drowsiness. Muscle activity was removed by applying a 90 µV threshold. Finally, a 4-second epoch segmentation was performed.

EEG analysis

In order to explore group differences in the EEG patterns, two methods were used: spectral decomposition and functional connectivity analysis. Spectral power analysis using Fast Fourier Transformation with a 2-second duration and 50% overlap, was applied on the scalp signals using the EEGLAB toolbox [18]. Absolute powers were evaluated in six frequency bands: delta (1-4 Hz), theta (4-7 Hz), alpha1 (8-10.5 Hz), alpha2 (10.5-13 Hz), beta1 (13-20 Hz) and beta2 (20-30 Hz) and averaged for

each EEG channel. Based on these absolute power values, relative powers were estimated by normalizing each value by the sum of the powers in the all the considered frequency bands. Thereafter, only relative powers were considered for comparisons.

Two other measures were also considered: the peak of the background rhythm, defined as the frequency having the maximum power of the alpha rhythm on the posterior electrodes P3, P4 and Oz [19], and the spectral ratio between the power in the alpha and theta frequency bands.

The electrodes were organized, by hemi-sphere, into 5 regions of interest (ROI): frontal, central, parietal, occipital, and temporal, according to the cortical projections of the scalp electrodes in the Talairach space [20].

Functional connectivity between cortical areas was measured at the source level. The inverse problem was solved, using the weighted Minimum Norm Estimate (wMNE) method [21], using an average template, available in the Brainstorm toolbox [22], in order to reconstruct the temporal dynamics of the cortical regions. The source signals were then projected on the 68 ROIs of the Desikan-Killiany atlas [23]. Matrices of functional connectivity between the reconstructed sources were computed in the different frequency bands via the Phase Locking Value (PLV) method [24] using the EEGNET toolbox [25]. This measure quantifies the interaction between two oscillatory signals through the estimation of the phase relationships.

Statistical analyses

Demographic and clinical data

Demographic and clinical data were compared using the chi-square test for the qualitative variables and using two sample t-tests or Mann-Whitney tests, depending on normality of the distribution, for the quantitative variables.

All variables that were significantly different between the Anx+ and Anx- groups, except those related to anxiety, were used as co-variates in the following analyses.

Spectral powers

Between-group comparisons of the relative spectral analysis values, global and by hemi-sphere lobes, were performed using analysis of covariance (ANCOVA).

Multiple comparisons corrections were applied using false discovery rate method. For each case, global values and lobes values, the six p-values obtained for the six considered frequency bands were adjusted.

All the analyses were performed with the XLStat software (Addinsoft. XLSTAT 2019: Data Analysis and Statistical Solution for Microsoft Excel. Paris, France).

Connectivity

Hypothesis tests on functional connectivity matrices were based on the network-based statistics (NBS) Toolbox from [26]. This toolbox implements two different analysis methods. Both were considered in this study. The first is the NBS method that enables rejection of the null hypothesis at the network level while controlling for the family-wise error rate (FWER). Statistical significance is estimated for subsets of mutually connected network nodes in topological rather than physical space. The NBS method comprises four steps. Firstly, the t-statistic for each individual edge in the connectivity network is calculated. For the experiments in this study, a threshold t-score of 3 was chosen. Secondly, a primary component forming threshold ($p<0.05$) is applied to identify edges displaying differences. Thirdly, sub-threshold edges were assessed for mutual connections forming clusters in topological space. Fourthly, a test with 5000 random permutations was applied to compute statistical significance for all previously identified network component.

The second analysis method in the NBS toolbox, the false discovery rate (FDR) [27], is more sensitive to focal effects involving single, isolated connections. It enables rejection of the null hypothesis at the level of specific connections, while controlling for the false discovery rate. In our study, 5000 permutations were used and significance threshold set to $p<0.05$.

In both analyses and as previously, the co-variates were introduced in the design matrix. The Brain Connectivity Toolbox (BCT) [28] was used to create three-dimensional graph visualizations, which represented below p-threshold connection pairs surviving multiple comparison correction.

Results

Demographic and clinical characteristics

EEG data were available for 108 patients. Their demographic and clinical characteristics are described in Table 1.

Gender, Hoehn & Yahr stage as well as the Mattis dementia rating scale were considered as co-variates.

	Patients without anxiety (n = 75)	Patients with anxiety (n = 33)	p-value
Demography			
Center: Lille (n = 57)	36 (48%)	21 (63.64%)	0.363
Maastricht (n = 51)	39 (52%)	12 (36.36%)	
Age (Years)	64.86 ± 8.65	65.93 ± 7.01	0.533
Education Duration (Years)	12.49 ± 3.58	11.73 ± 3.56	0.307
Sex-ratio (Male/Female)	2.95	1.06	**0.032**
Clinic			
Disease duration (Years)	7.83 ± 5.08	9.76 ± 7.26	0.173
Hoehn & Yahr stage	2 (0 – 3)	2 (2 – 3)	**0.002**
MDS-UPDRS part 1			
1.3. Depressed mood	0 (0 – 4)	1 (0 – 4)	**0.001**
1.4. Anxious mood	0 (0 – 4)	2 (0 – 4)	< **0.0001**
1.7. nighttime sleep problems	2 (0 – 4)	2 (0 – 4)	**0.03***
1.8. Daytime sleepiness	2 (0 – 4)	2 (0 – 4)	0.26
1.9. Pain and other sensations	1 (0 – 4)	2 (0 – 4)	< **0.0001**
MDS-UPDRS part 3 (/132)	27.16 ± 11.91	30.97 ± 12.46	0.134
Hamilton depression rating scale (/54)	4.48 ± 3.48	8.97 ± 5.58	< **0.001**
Lille apathy rating scale (/36)	−26.33 ± 6.18	−22.36 ± 7.49	**0.004**
Parkinson anxiety scale total (/48)	3.69 ± 2.87	14.79 ± 4.69	< **0.0001**
Part A (/20)	2.85 ± 2.87	9.47 ± 4.32	< **0.0001**
Part B (/16)	0.42 ± 0.85	2.38 ± 2.26	< **0.0001**
Part C (/12)	0.43 ± 0.85	2.94 ± 2.32	< **0.0001**
Mattis Dementia rating scale (/144)	138.43 ± 4.82	136.03 ± 5.44	**0.016**
Medications			
LEDD (mg / day)	706.98 ± 515.62	960.70 ± 553.80	**0.023**
Antidepressants (n = 18)	5 (6.67%)	13 (39.39%)	< **0.001**
Anxiolytics (n = 12)	3 (4.00%)	9 (27.27%)	**0.001**

Table 1: Demographic and clinical characteristics of the patients included in the study, separated into two groups: patients without anxiety disorders and patients with anxiety disorders.
MDS-UPDRS: Movement Disorders Society-Unified Parkinson's Disease Rating Scale; LEDD: Levodopa Equivalent Daily Dose.

Spectral analysis

The topography of the relative power in the different frequency bands is depicted in Figure 1.

The global relative spectral power in the different frequency bands is presented in Table 2 and showed no significant difference between groups. The cortical topography of the relative spectral power observed in each group is depicted in Figure 1 and shows a global decrease in the alpha band and an increased in the beta2 and delta bands for the Anx+ group, possibly more marked in the anterior part of the right hemisphere. Regional analyses confirmed a significant decrease of alpha1 power in the right frontal region ($p_{uncorrected}$=0.04,$p_{corrected}$=0.042) (Figure 2). The posterior background rhythm was at a peak frequency of 8.63 ± 1.19 Hz for the Anx- group and 8.55 ± 1.20 Hz for the Anx+ group (p = 0.75).

There was no significant group difference regarding the spectral ratio between the alpha and theta bands (p = 0.11). Multiple regression analyses were carried out to examine associations between the alpha1 power in the right frontal region and the PAS scores and sub-scores. They revealed a significant association with avoidance behaviour (PAS_C, ß= 0.20, p = 0.035). There was a trend toward a

significant association for episodic anxiety (PAS_B, ß = 0.15, p = 0.06), while no associations were found for persistent anxiety (PAS_A, p = 0.62) and the total scale (p = 0.18).

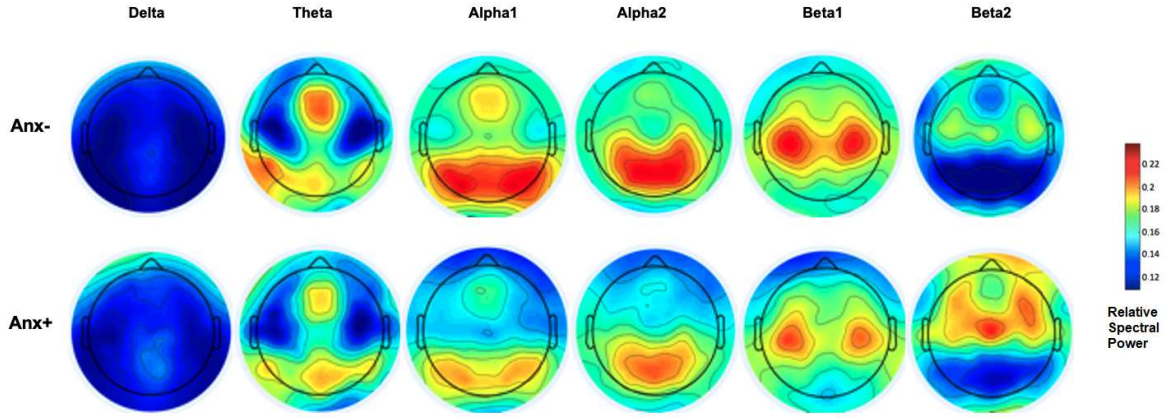

Figure 1: Topographical representation of the relative spectral power at the scalp level for each frequency band according to the group: Parkinson's disease patients with (Anx+) and without clinically relevant anxiety (Anx-).

	Patients without anxiety	Patients with anxiety	p-value
Delta	0.11±0.02	0.12±0.04	0.37
Theta	0.20±0.06	0.20±0.07	0.96
Alpha1	0.19±0.05	0.17±0.05	0.13
Alpha2	0.12±0.03	0.11±0.02	0.32
Beta1	0.19±0.03	0.18±0.03	0.50
Beta2	0.16±0.03	0.18±0.05	0.28

Table 2. Comparison of the global relative spectral power in the different frequency bands between the two patient groups.

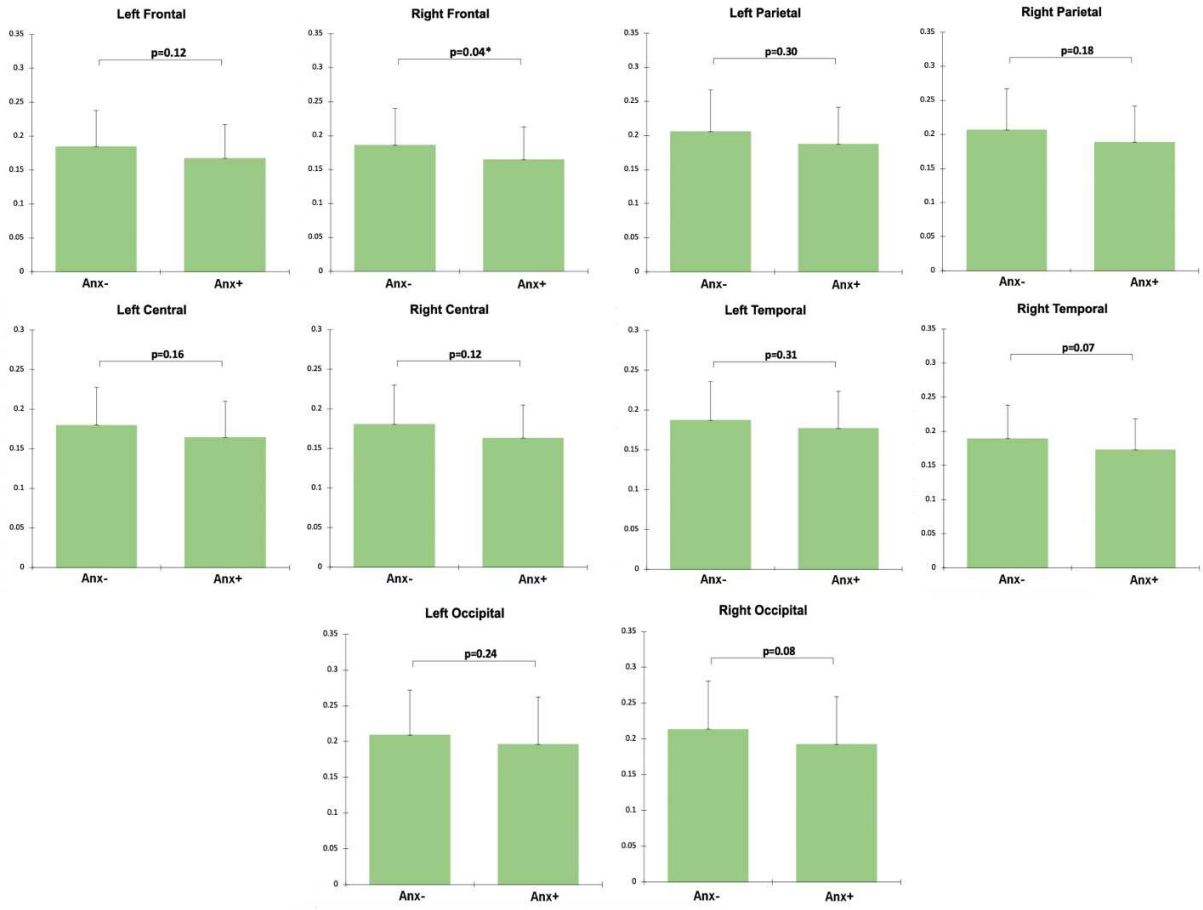

Figure 2. Spectral relative powers distribution in the alpha1 band in the two patients' groups. Parkinson's disease patients with (Anx+) and without clinically relevant anxiety (Anx-).

Functional connectivity

Global Network level

The NBS method analysis revealed no significant between-group differences when comparing the complete network connectivity matrices in the different frequency bands.

Regional level

The FDR method revealed significantly stronger connections involving the left insula in the Anx+ group than in the Anx- group (Table 3).

In the theta band, this between-group difference concerned connections of the left insula with the right frontal, including the rostral middle, the pars opercularis, the pars triangularis and the pars orbitalis regions and the cingulate cortices. In the alpha band, it concerned the connection linking the left insula with the right isthmus cingulate cortex. In the beta2 band, a single connection linking the left insula and the rostral middle frontal cortex was implicated.

We also looked for connections that showed a trend towards a significant difference between the two groups, by considering connections with t-values close to those described above. This identified the

connection between the left insula and right isthmus cingulate cortex in the beta1 and beta2 bands as well as the connection between the left insula and the right rostral middle frontal cortex, in the alpha2 and beta1 bands. New regions also appeared, thus, in the theta band, connections linking the left insula to the right precentral and the right temporal lobes as well as the right fusiform appeared stronger in the Anx+ group. This last connection appeared stronger also in the alpha2 band.

Complete description of these results is summarized in Table 3 with the corresponding t-values and illustrated in Figure 3.

Region 1	Region 2	t values
Theta band		
Left insula	**Right rostral middle frontal**	**3.94**
Left insula	**Right pars opercularis**	**3.52**
Left insula	**Right pars triangularis**	**3.38**
Left insula	**Right pars orbitalis**	**3.15**
Left insula	**Right isthmus cingulate**	**3.76**
Left insula	*Right precentral lobe*	*2.95*
Left insula	*Right temporal lobe*	*3.05*
Left insula	*Right fusiform*	*3.02*
Alpha1 band		
Left insula	**Right isthmus cingulate**	**3.93**
Alpha2 band		
Left insula	**Right isthmus cingulate**	**4.27**
Left insula	*Right rostral middle frontal*	*3.10*
Left insula	*Right fusiform*	*3.08*
Beta1 band		
Left insula	*Right rostral middle frontal*	*3.10*
Left insula	*Right isthmus cingulate*	*3.00*
Beta2 band		
Left insula	**Right rostral middle frontal**	**3.85**
Left insula	*Right isthmus cingulate*	*3.03*

Table 2: Connections between the cortical regions of the Desikan-Killiany atlas for which significant or with trend significance (shaded lines) differences (Anx+ > Anx-) were found by the false discovery rate method.
Anx+: Parkinson's disease patients with clinically relevant anxiety and Anx-: Parkinson's disease patients without clinically relevant anxiety.

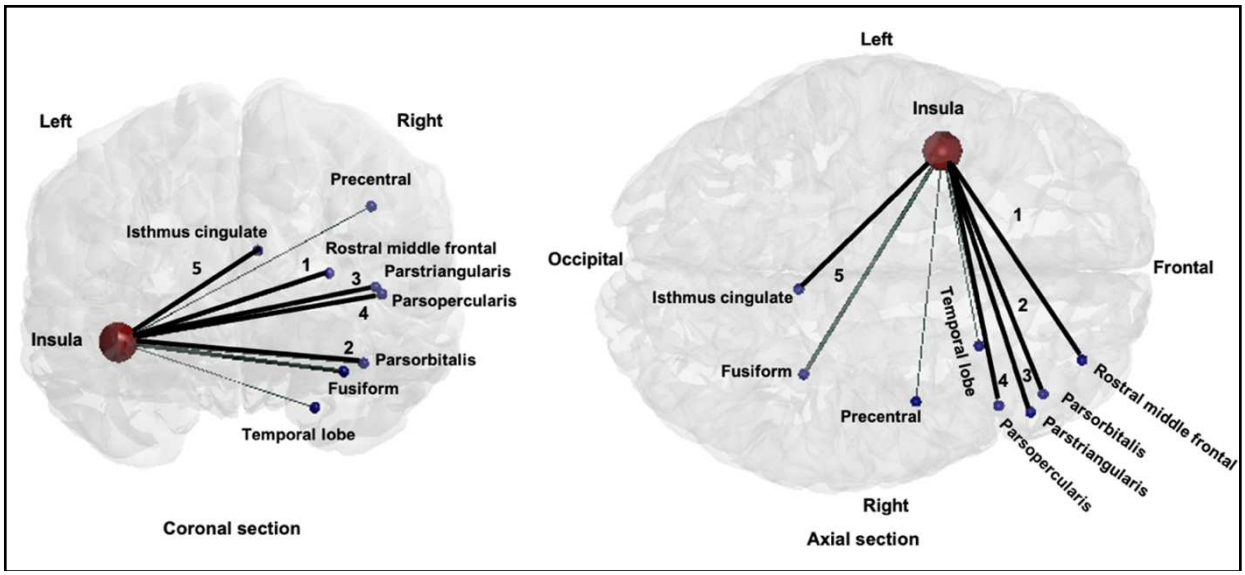

Fig. 3 Functional connectivity comparison results between Parkinson's disease patients with (Anx+) and without clinically relevant anxiety (Anx-).

Connections quoted 1 to 5 (in bold) were statistically significantly stronger in Anx+ group in at least one of the considered bands (theta, alpha and beta). Connections not in bold showed a non-significant trend towards being stronger in the Anx+ group. (See Table 3 for t-values of the connections).

Anx+: Parkinson's disease patients with clinically relevant anxiety and Anx-: Parkinson's disease patients without clinically relevant anxiety.

Discussion

Main outcomes

The objective of this study was to identify EEG signatures of PD-related anxiety. Two approaches were considered. The first one used signal spectral decomposition while the second one investigated functional connectivity. The findings of the spectral analysis show that anxiety is associated with changes in the alpha activity in the right frontal cortex, and the connectivity analysis shows that the functional connectivity between the left insula and the areas of the right frontal and cingulate cortex is increased in the theta, alpha and beta-2 band. Using MRI on the same study population [4], we found cortical thinning in the prefrontal, parietal left cingulate cortices in the group of PD patients with anxiety. We also found a deformation in the left amygdala in this group as well as a higher connectivity between the fear circuit and the salience network and a lower connectivity between the left frontoparietal attentional and language networks. The results of our EEG study seem to be in line with these MRI results and bring insights about the mechanisms of anxiety in PD.

Alpha activity changes

The main outcome of the spectral analysis was a statistically significant decrease of the relative power in the alpha1 band in the right frontal region in the Anx+ group. Figure 1 reveals that this decrease seems to be rather global, with a predominance in the right lobe, in the alpha bands (alpha1 and 2),

while a power increase in the delta band was also observed for this group. Activity in the alpha frequency band is the most common component of the human brain activity [29]. It is modulated by sensory, motor and cognitive processes [30]. At rest, alpha activity is generally low in the frontal region. It increases with increased cognitive load [31]. Cognitive impairment is generally associated with a decrease in resting-state alpha power [32].

Regarding anxiety in general, very few studies used EEG to explore it and the reported results were poorly validated [33]. Nevertheless, some studies have reported an increase of the alpha oscillations in anxious individuals [34] or in patients with anxiety disorders ([9], [35]). This was interpreted as a manifestation of hyperarousal in anxious subjects. However, these data are from studies with small numbers of young participants, very short period of recording in the resting-state condition, with a limited number of electrodes. The decrease in alpha power observed in the Anx+ group cannot be related to lower cognitive efficiency in this group since this factor was controlled. Moreover, despite a slight between-group difference at the Mattis dementia rating scale score, mean scores were high and dementia was an exclusion criterion. Several studies showed that the alpha power, particularly in the frontal regions, was modulated by attention. More specifically, an increase in alpha power could reflect the efficacy of attentional filtering when irrelevant information is inhibited and cognitive resources focused by internally oriented attention (top-down) ([36], [37]). In the Anx+ group, the reduced alpha power in the frontal region could reflect the lower efficiency of this process. Due to altered representation of real or potential threats in their environment, anxious PD patients may allocate more attention to externally bottom-up stimulation leading to a change in the distribution of alpha power.

Besides spectral power analyses, EEG rhythm modifications were also analyzed through the ratio between the alpha and theta bands and the peak of the posterior background rhythm. No between-group difference was observed. This supports the hypothesis that the alterations observed in anxious patients are not the result of a general brain slowing or a global cognitive deficit since changes in these parameters were reported as markers of cognitive decline and conversion to dementia in PD [38].

Increase of the left insula's functional connectivity

In terms of functional connectivity, the network-based analysis did not reveal any specific difference in terms of clusters between the two groups while the FDR analysis, more suitable to detect local differences in single connections, brought out significant differences in the different frequency bands. All involved the left insula (Table 2 and Figure 2). In the theta band, connections between the left insula and the lateral prefrontal cortex (rostral middle, pars opercularis, pars triangularis and pars orbitalis) and the isthmus cingulate cortex were stronger in the Anx+ group. In the alpha bands, the connection between the left insula and the isthmus cingulate cortex was stronger in this group and, in the beta2 band, the connection between the left insula and the rostral middle frontal cortex (dorso-lateral

prefrontal cortex) was stronger. These results suggest that the left insula acts as a hub around which connections are strengthened in the Anx+ group. The insula plays a key role in anxiety since it conveys interoceptive information to medial prefrontal cortex [39]. It is part of a network involving the anterior cingulate cortex, the medial prefrontal cortex, the amygdala and other limbic structures [40]. The increased connectivity of the insula with the frontal cortex observed here in anxious PD patients could reflect a change in emotional information processing with more attention allocated to visceral changes caused by anxiety, contributing to its maintenance [41] and altered regulation of emotion by the prefrontal cortex [42]. The insula and the prefrontal cortex are densely interconnected with the amygdala [43]. Here, functional connectivity analysis was limited to the cortical areas, since localization of sub-cortical sources, while possible, remains the subject of many reservations. Nevertheless, the reported results are in line with the increased connectivity between the fear and salience circuits, described in rs-fMRI on the same study population [4]. Changes in this functional circuit were also reported by Wang et al using PET-FDG.

There was also an increased connectivity between the insula and the posterior cingulate cortex in several frequency bands in the Anx+ group. This region, with the precuneus to which it is very close, is involved in self-awareness [44]. Through its connections with the hippocampus, it can play a role in anxiety via learning / memory mechanisms or the establishment of avoidance behaviours [45]. The increased connectivity observed here may be a marker of the maintenance of avoidance behaviours that characterize anxiety in PD [46].

Strengths and limitations of the study

The study was carried out with well-established protocols and techniques in terms of signal acquisition, preprocessing and processing. The analysis scheme combined two different and complementary approaches, scalp signal spectral decomposition and source-connectivity analysis, for a full understanding of the changes and the results are consistent. The resting-state protocol, in addition to allow comparison with studies using different brain imaging methods, limits the influence of fatigue and does not require participation of the subject. Moreover, the combination of wMNE for the inverse problem resolution and sources localization and the PLV, as a similarity measure for connectivity quantification, reported as the most appropriate in for group comparison study [47]. Furthermore, the PLV is often told to be sensitive to volume conduction because this technique does not remove zero-lag connectivity. Moreover, it has recently been reported that PLV-based functional networks are significantly correlated with fMRI networks during resting state, while it was not the case for methods that remove zero-lag connections such as the PLI. Indeed, all zero-lag connections are not spurious.

Nevertheless, our study has several limitations. Firstly, among the 108 patients, only 33 had clinically relevant anxiety. The sample size of the two patient groups was thus unbalanced and this may have

hampered the statistical analyses. A solution could have been the artificial matching of patients by randomly reducing the number of the patients in the Anx- group. We did not adopt this solution because our distribution (33 Anx+/75 Anx-) was close to the usually reported prevalence (31%) of anxiety in PD [1]. Additionally, the sample sizes were sufficient for most statistical methods and special attention was paid to the existence of outliers in the different analyses. Secondly, anxiety was here considered globally without distinction of the different anxiety subtypes (general anxiety, phobias, PD-specific anxiety disorders, etc). It is possible that there are specific markers for each subtype and further studies are needed to identify them. Thirdly, although our analyses were adjusted on several demographic and clinical variables, our patient groups differ in terms of severity of the depressive symptoms, apathy and medication status. We decided to not adjust on these variables in order to not excessively reduce the statistical power. Moreover, due to the overlap between depression and anxiety symptoms, it is quite logical that patients with anxiety had higher scores at the HAMD. Nevertheless, the scores were quite low and only few patients had clinically relevant depression. The higher score at the LARS in the Anx+ group could also be explained by an influence of anxiety symptoms. Indeed, avoidance behaviour may limit initiatives, social phobia or PD-specific anxiety disorders may limit social interactions. The interactions between both syndromes are unknown and should be further explored.

Regarding medication, the Anx+ group had a more severe PD as shown by the significantly higher Hoehn & Yahr stage and lower score on the Mattis dementia rating scale. A higher LEDD may thus be required to bring their motor scores back to the same level as the Anx- group. Patients in the Anx+ group took more frequently benzodiazepine. This could have influenced their EEG spectrogram (increased frontal beta powers caused by rapid rhythms induced by the treatment and slight decrease of alpha powers in posterior regions ([48]). However, the changes observed here did not correspond to this pattern.

Lastly, considering the connectivity analysis, only static patterns were estimated while one of the main advantages of EEG exploration, compared with imaging explorations, is its higher temporal resolution. EEG offers the possibility to dynamically measure the connectivity states. Although this approach seems to be more suitable for task-based explorations, it can be an interesting approach for further analyses to characterize the temporal course of the connections.

Conclusion

This study was the first to investigate the mechanisms of PD-related anxiety using high density resting state EEG. The reported results provide new insights as supplements to those already described using other modalities, mainly rs-fMRI, and show that EEG could be a relevant modality to explore these disorders. However, in view of the lack of validated results about the modifications of EEG patterns in

these disorders in general and the significance levels of the results of the current study, it seems imperative to confirm these outcomes with further works and by recruiting patients with a larger panel of anxiety disorders. The used data were from a cohort, originally set up to study cognition and some anxiety disorders sub-types could have been not sufficiently represented.

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CHAPTER 6

Cognitive Behavioural Therapy for Anxiety in Parkinson's Disease Induces Functional Brain Changes

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Summary of the chapter

In 2021, *Moonen et al.* published the results of a randomized, controlled clinical trial demonstrating the efficacy of cognitive behavioural therapy (CBT) to reduce anxiety symptoms in PD patients compared with clinical monitoring only. In this article, which is an ancillary study of this clinical trial, we aimed to decipher the mechanisms by which CBT improves anxiety symptoms in PD patients, using functional MRI.

We found that functional changes occurred after CBT, associated with anxiety improvement, between the structures involved in the fear circuit and the limbic circuit and within the resting state functional networks. Our results suggest that CBT could improve anxiety in PD by restoring the balance between these two circuits and by reinforcing the cognitive control of emotion.

These results reinforce our hypothesis of an imbalance between the fear and the limbic circuits at the origin of PD-related anxiety. These also highlight that a non-pharmacological therapy, such as CBT, can induce functional changes in the brain in order to relieve anxiety symptoms in PD. This open new insights about non-pharmacological ways to treat non-motor symptoms in PD.

Abstract

Background: Cognitive Behavioural Therapy (CBT) reduces anxiety symptoms in patients with Parkinson's disease (PD). The objective of this study was to identify changes in functional connectivity in the brain after CBT for anxiety in patients with PD.

Methods: Thirty-five patients with PD and clinically significant anxiety were randomized over two groups: CBT plus clinical monitoring (10 CBT sessions) or clinical monitoring only (CMO). Changes in severity of anxiety symptoms were assessed with the Parkinson Anxiety Scale (PAS). Resting-state functional brain MRI was performed at baseline and after the intervention. Functional networks were extracted by an Independent Component Analysis (ICA). Functional connectivity (FC) changes between structures involved in the PD-related anxiety circuits, such as the fear circuit (involving limbic, frontal and cingulate structures) and the cortico-striato-thalamo-cortical limbic circuit, and both within and between functional networks were compared between groups and regressed with anxiety symptoms changes.

Results: Compared to CMO, CBT reduced the FC between the right thalamus and the bilateral orbitofrontal cortices and increased the striato-frontal FC. CBT also increased the fronto-parietal FC within the central executive network (CEN) and between the CEN and the salience network. After CBT, improvement of PAS-score was associated with an increased striato-cingulate and parieto-temporal FC, and a decreased FC within the default-mode network and between the dorsal attentional network and the language network.

Conclusion: CBT in PD-patients improves anxiety symptoms and is associated with functional changes reversing the imbalance between PD-related anxiety circuits and reinforcing cognitive control on emotional processing.

Introduction

Parkinson's disease (PD) is characterized by both motor and non-motor symptoms. Anxiety is one of the most common neuropsychiatric symptoms in PD, with an average point prevalence around 31% [1]. Anxiety is associated with increased motor disability and a reduced quality of life [2,3]. Recently, we showed that a cognitive behavioural therapy (CBT) module tailored to treat anxiety in PD patients was more effective than clinical monitoring only (CMO) in reducing symptoms of anxiety [4]. In a systematic review, we reported that PD-related anxiety was associated with structural and functional changes in the limbic cortico-striato-thalamo-cortical (CSTC) circuit and the fear circuit and hypothesized that anxiety in PD would be due to an imbalance between these two circuits [5]. Moreover, a recent study suggested that anxiety in PD patients would be associated with a reduced cognitive control of emotional processes [6]. Hence, the reduction of anxiety symptoms induced by CBT could result from restoring the balance between these two circuits and reinforcing cognitive control on emotion.

The aim of the present study was to identify functional changes in the brain occurring after CBT for anxiety in PD patients. We hypothesized that CBT would induce functional changes in the PD-related anxiety circuits, with also FC changes both within and between networks.

Materials and methods

Study design

This study is a prospective, open, randomized controlled trial. PD patients with anxiety were randomized to either 'CBT plus clinical monitoring' (the intervention group) or 'CMO' (control group). All participants underwent standardized clinical, cognitive, and behavioural assessment as well as MRI scanning (except in case of contra-indications such as deep brain stimulation leads or claustrophobia) at baseline and at the end of the intervention. The duration of the intervention was approximately 10–12 weeks. For more details on the design, we refer to Mulders et al [7].

Study population

Patients were recruited among outpatients of the movement disorders clinics in Maastricht University Medical Centre, Maastricht (the Netherlands) and University Hospital of Lille (France). Patients included in this study had a diagnosis of idiopathic PD according to the Queens Square Brain Bank diagnostic criteria [8], were between 35 and 80 years old, had clinically relevant anxiety symptoms, defined as a score on the Parkinson Anxiety Scale (PAS) subscale for persistent anxiety (PAS-A) >9 and/or a score on the avoidance subscale (PAS-C) >3 [9], and were not receiving any other current psychological treatment for anxiety (psychopharmacotherapy was allowed if a stable dose was used at least two months prior to participation. During the trial the dosage should not be changed. Medication

use and mental health care were tracked throughout the study). They were on a stable dose of antiparkinsonian medication for at least one month and provided informed consent. Patients with other neurological disorders, dementia or severe cognitive impairment operationalized as a Montreal Cognitive Assessment (MOCA) score < 24 [10], contra-indications for MRI, major depressive disorder or abuse of alcohol, drugs or benzodiazepines were excluded.

At baseline, demographic and clinical variables were collected including sex, age, years of formal education, year of PD onset, side of onset, type and dose of antiparkinsonian medication and other medication including psychopharmacotherapy. Motor symptoms and disease severity were respectively assessed during “ON'' phases (in case of motor and non-motor fluctuations) with the Movement Disorder Society – unified Parkinson’s disease rating scale (MDS-UPDRS) part 3 and the Hoehn & Yahr staging system [11]. Depression was assessed using Hamilton depression rating scale (HDRS) [12] (See published design [7]).

The study is carried out in compliance with the Helsinki Declaration. The local ethics committee of Maastricht University Medical Centre (July 2016) and Lille University Hospital (September 2016) have approved the study protocol. Written informed consents was obtained from all participants. The study is registered at the ClinicalTrials.gov database under registration number NCT02648737, as well at FoxTrialFinder under ID number 004701 (See published design7).

Assessment of anxiety

The PAS, a scale specifically developed to detect and measure anxiety in PD patients, was used to assess anxiety symptoms at baseline (t = 0) and post-intervention (t = 1). It is insensitive for motor and depressive symptoms and has subsections for persistent anxiety (PAS-A), episodic/situational anxiety (PAS-B), and avoidance behaviour (PAS-C) [9].

CBT and clinical monitoring

The CBT consisted of 10 weekly individual sessions of 60–75 min, tailored to the preferences and needs of each patient. All patients received clinical monitoring which involved an information sheet about anxiety and a phone call by an independent psychologist one month after baseline assessment to inquire about current anxiety symptoms. More details are reported in the study design paper [7].

Imaging acquisition

Patients were scanned at baseline and post-intervention with both sites using identical 3T Philips Achieva MRI scanners (Philips Healthcare, Best, The Netherlands) with identical software versions and MR sequences. High-resolution anatomical T1-weighted (T1w) images were acquired using a 3D

inversion recovery MP-RAGE sequence (231 sagittal slices, no gap, TR/TE/flip angle = 12ms/3.3ms/9°, matrix 384x384, field of view 240x240mm, voxel size 0.63×0.63×0.65mm). Resting-state functional MRI (rs-fMRI) was performed using echo-planar imaging (40 axial slices, no gap, TR/TE/flip angle = 2400ms/30ms/90°, matrix 64x64, field of view 192x192mm, voxel size 3x3x3mm). The total MRI scan took about 45min. Patients were scanned on "ON-dopamine medication state".

Functional analyses

Functional analyses were performed using CONNv18 toolbox in MATLAB (SPM12) [13].

Preprocessing

rs-fMRI data were preprocessed using the CONNv18 toolbox (https://web.conn-toolbox.org/fmri-methods/preprocessing-pipeline). More details are provided in the Supplementary data, section Ia.

ROI-based comparisons

A Brodmann atlas, created from the Talairach one [14], was used to define the cortical regions-of-interest (ROI). The FSL Harvard-Oxford Atlas was used to define the subcortical ROIs [15]. Based on the aforementioned atlases, the following twenty structures involved in the PD-related anxiety circuits were defined: the amygdala, striatum (caudate nucleus, putamen, nucleus accumbens), thalamus, the prefrontal cortex (lateral, medial, orbito-frontal), cingulate gyrus (anterior and posterior), parietal cortex (superior parietal lobule), temporal cortex (pole temporal, temporal gyri) and insular cortex [5]. Functional connectivity was computed by Pearson correlating time series data between every pair of ROI, resulting in 20x20 FC matrices.

Identification of functional networks: ICA

Group Independent Component Analyses (ICA) were performed to identify common functional networks in patients using group-level ICA approach with CONN toolbox. Twenty independent components have been identified. Among these components, the common functional resting-state networks were identified using a cross-correlation based on existing standardized templates (Human brain project) [13]: the default-mode network (DMN), the dorsal attentional network (DAN), the salience network, the central executive network (CEN), the sensorimotor network (SMN), the language network (LN), the visual network (VN). Each ICA component was divided into regions of interest (ROI) according to a standard anatomical atlas ((Human brain project) [13]. The process is defined in Supplementary Figure 1. Functional connectivity was computed by Pearson correlating time series data between every pair of ROI, resulting in 113x113 FC matrices.

Correlation between FC matrices and change in anxiety scores

For each patient, the change in PAS-total score was calculated, corresponding to the difference in score between baseline (BL) and post-intervention (PI) (ΔPAS = PASBL – PASPI). The FC matrices were extracted for each patient and each session. A variable indicating the change in FC was calculated for each patient (ΔFC = FCPI – FCBL) and associated with the ΔPAS-total using multiple regression analyses.

Statistical analyses

The significance threshold was set at $p < 0.05$ and corrected for multiple comparisons (FDR - False Discovery Rate) when necessary.

Analyses 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. The normality of distribution was assessed using a Kolmogorov-Smirnov test. Categorical data were compared with Chi2 and quantitative data with t-tests in case of normally distributed data and Mann-Whitney tests otherwise. A repeated mixed ANOVA test was performed to compare the PAS total score at baseline and after the intervention between the two groups (CMO and CBT). All these statistical tests were performed using SPSS, version 26 (SPSS, Chicago).

Functional MRI analyses

All the functional analyses were adjusted for time (in days) between BL and PI session and centre. The ROI-based analysis consisted of comparing the FC matrices between the two groups and across the time [13]. Generalized linear models with permutation inference were calculated first to identify significant FC values for each group and each session and secondly to compare these connections between the groups and sessions [16]. Repeated mixed ANOVA tests with permutation inferences were then performed to compare the connectivity values longitudinally between groups [13].

Hierarchical multiple regression analyses were performed to examine the relationship between PAS and FC, using SPSS version 26. Centre and time (in days) between BL and PI sessions were set as nuisance regressors in the first block (model 1) of all regression models, whereas PAS score (independent variable) was separately added to the second block of the model (model 2). FC was set as dependent variable. We ensured that all models met the assumptions for multiple regression analyses, including normality of the residuals, multicollinearity, and homoscedasticity.

Data availability

Data supporting the findings of this study are available from the principal investigator (AFGL), upon reasonable request.

Results

Demographic and clinical characteristics

Among the 49 patients included in the clinical study, fourteen were excluded (13 due to the absence of an MRI scan mainly due to claustrophobia and one after preprocessing failure). Thirty-five patients were thus included in the analyses: 17 in the CBT group and 18 in the CMO group (Figure 1). All participants were right-handed except for 2 participants in the CMO group and 1 ambidexter participant in the CBT group. There were no between-group differences regarding demographic variables and baseline clinical scores. Time between BL and PI was significantly longer in the CBT than in CMO group (p = 0.001 – Table 1).

In the repeated mixed ANOVA test, the PAS total score was reduced in both groups after the intervention but significantly more in the CBT group (F-score = 4.58, p-value = 0.04 – Supplementary Figure 2). These results were in line with the clinical study[4].

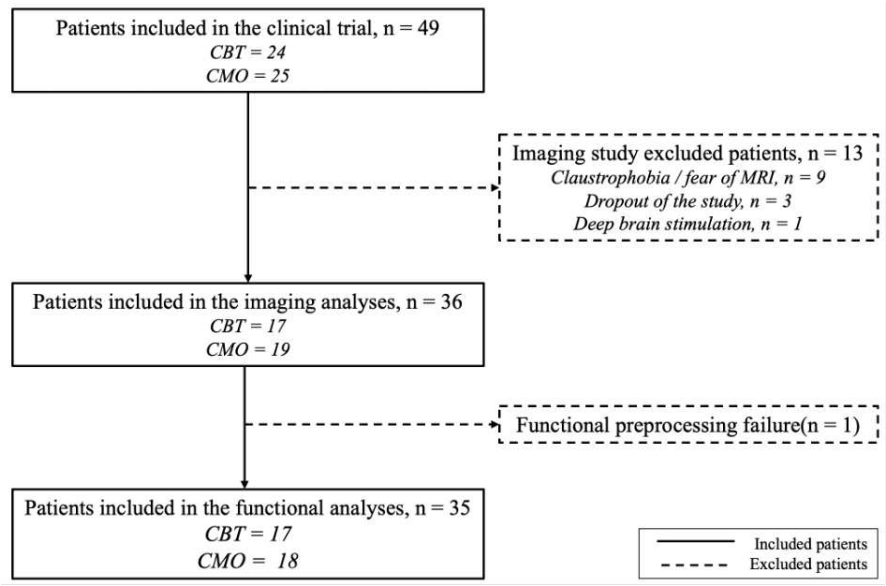


Figure 1. Flow chart of the study.
Abbreviations: CBT = cognitive behavioural therapy; CMO = clinical monitoring only group.

BASELINE	*CBT group* (*n* = 17)	*CMO group* (*n* = 19)	*p*
	Demographic variables		
Age *(y)*	62.8 (±7.8)	64.5 (±8.9)	0.15
Female *(men/women ratio)*	0.89	0.73	0.77
Clinical center:			0.43
Maastricht (n = 13)	5 (29%)	8 (42%)	
Lille (n = 23)	12 (71%)	11 (58%)	
Right hand dominance *(n = 33)*	16 (94%)	17 (90%)	0.49
Formal education *(y)*	13.5 (±3.4)	14.2 (±4.0)	0.59
Illness duration *(y)*	7.4 (±5.8)	4.8 (±4.2)	0.18
First motor side *(right, n = 19)*	10 (59%)	9 (56%)	0.88
LEDD total *(mg/day)*	592.6 (±374.0)	770.3 (±583.6)	0.40
Antidepressant use *(n = 8)*	4 (24%)	4 (22%)	0.99
Benzodiazepine use *(n = 7)*	3 (18%)	4 (21%)	0.99
	Baseline (BL) clinical variables		
PAS			
Part A. Persistent anxiety (/20)	13.2 (±2.4)	13.7 (±2.67)	0.62
Part B. Episodic anxiety (/16)	6.4 (±3.8)	4.4 (±2.8)	0.06
Part C. Avoidance (/12)	5.1 (±3.0)	4.11 (±2.7)	0.33
Total score (/48)	25.0 (±6.9)	22.7 (±6.3)	0.27
Hamilton DRS *(/54)*	11.4 (±4.6)	10.8 (±4.6)	0.66
MoCA *(/30)*	26.9 (±2.1)	25.9 (±2.9)	0.40
MDS-UPDRS part III total *(/108)*	22.9 (±10.3)	27.6 (±10.8)	0.19
Hoehn & Yahr stage *(0–5)*	2 (1–3)	2 (2-3)	0.36
Time baseline – post intervention (days)	121.9 (±30.3)	94.6 (±36.5)	**0.001***

Table 1. Demographic variables, baseline clinical scores and time between baseline and post-intervention comparisons between patients with CBT and patient with CMO.

*Abbreviations: * = p-value < 0.05; ** = no statistical analyses performed (see clinical study for the longitudinal analyses, Moonen et al.. Mov Disord 2021); BL = baseline; CBT = cognitive behavioural therapy; CMO = clinical monitoring only; DRS = depression rating scale; LEDD = levodopa equivalent daily dosages; MDS-UPDRS = Movement Disorder Society unified Parkinson's disease rating scale; MoCA = Montreal cognitive assessment; PAS = Parkinson anxiety scale.*

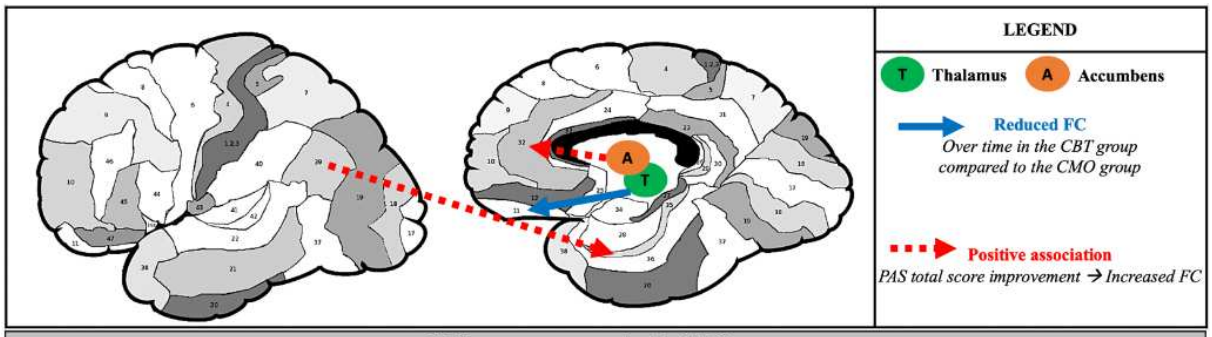
PD-related anxiety circuits

Functional connectivity analysis

After intervention, there was a significant reduction of FC between the right thalamus and the bilateral OFC in the CBT group compared to the CMO group (FDR p-value = 0.027) (Figure 2).

Correlation of FC with anxiety scores

After CBT, improvement of PAS-total score was associated with increased FC between the right nucleus accumbens and the right dorsal anterior cingulate cortex (dACC) and between the right angular cortex and the right perirhinal area (of the medial temporal gyrus) (Figure 2). Details are also provided on Supplementary Table 1.



CBT group compared with CMO group				
Seed (BA) (x, y, z)	***ROI (BA) (x, y, z)***	***T-score***	***Unc. p-value***	***FDR p-value***
Right thalamus (11, -18, 7)	Left orbitofrontal cortex (11) – (-16, 45, -29) Right orbitofrontal cortex (11) – (18, 46, -29)	-3.51 -3.39	0.0014 0.0014	0.027 0.027

Regression analyses between Δ PAS and Δ FC							
Δ PAS scores	**CBT**			**CMO**			**Interpretation**
	R^2	**p-value**	**Beta**	**R^2**	**p-value**	**Beta**	
Right Accumbens – left dorsal Anterior Cingulate Cortex (32)							
PAS total	0,586	**0,012***	0,761	0,377	0,095	0,344	**Effect of CBT: positive association**
Right Angular Coxtex (39) – right perirhinal area (35)							
PAS total	0,551	**0,019***	0,706	0,184	0,432	0,306	**Effect of CBT: positive association**

Figure 2. Representation of the induced functional connectivity changes after cognitive behavioural therapy in Parkinson's disease related anxiety circuits and corresponding tables of statistical results.
Abbreviations: BA = Brodmann area; CBT = cognitive behavioural therapy; CMO = clinical monitoring only; ΔPAS = $PAS_{baseline} - PAS_{post\text{-}intervention}$; FC = functional connectivity; PAS = Parkinson anxiety scale; Unc. = uncorrected. (image credits: www.fmriconsulting.com/brodmann/)

Resting-state functional networks

Functional connectivity analysis

There was a significantly increased fronto-parietal FC within the CEN and striato-frontal FC within the language network in the CBT group compared with the CMO group.

There was also a significantly greater fronto-parietal FC between the CEN and the salience network in the CBT group compared with the CMO group (Table 2 and Figure 3).

Correlation of FC with anxiety scores

After CBT, improvement of PAS-total score was associated with a significantly decreased frontal FC within the DMN as well as an increased fronto-temporal FC and a decreased temporo-caudate FC between the DAN and the language network (Table 3).

Network	ROI / Network 1 Localization, Brodmann area (BA), MNI coordinates (x, y, z)	ROI / Network 2 Localization, Brodmann area (BA), MNI coordinates (x, y, z)	T-score	FDR*p*
		Differences within networks		
CEN	L. inferior parietal lobule, BA 40, (–50, –47, 52)	L. anterior PFC, BA 10, (–4, 60, 30)	**3.29**	0.044
LN	R. caudate (13, 8, 25)	L. anterior PFC, BA 10, (–22, 61, –15)	**3.65**	0.024
		L. orbito-frontal gyrus, BA 11, (–10, 64, –16)	**3.64**	0.024
		Differences between networks		
	CEN L. superior frontal gyrus, BA 6, (–12, 8, 61)	Salience network L. inferior parietal lobule, BA 40, (–53, –38, 41)	**4.11**	0.031

Table 2. Comparisons of ROIs extracted from ICA analyses within and between common functional networks after cognitive behavioural therapy for anxiety in Parkinson's disease compared with clinical monitoring only. *Coordinates in Montreal Neurological Institute (MNI) space. Only significant results are provided in this table.*
Abbreviations: BA = Brodmann area; CEN = central executive network; FDR = false discovery rate; L. = left; LN = language network; PFC = prefrontal cortex; R. = right; ROI = region of interest; Unc. = uncorrected.

Network / ROI 1 *Localization,* *Brodmann area (BA),* *coordinates (x, y, z)*	Network / ROI 2 *Localization,* *Brodmann area (BA),* *coordinates (x, y, z)*	PAS-total		
		R^2	*Std.*β	*p*
	Within network changes			
DMN L. inferior frontal gyrus, pars orbit., BA 47, (–39, 41, –14)	DMN R. anterior PFC BA 10, (3, 57, –11)	0.59	–0.43	0.007
	Between network changes			
DAN R. middle frontal gyrus, BA 6, (27, –7, 58)	LN R. inferior temporal gyrus BA 20, (50, –2, –44)	0.89	0.26	<0.0001*
DAN R. fusiform gyrus, BA 37, (48, –59, –10)	LN L. caudate BA 34, (27, 9, –17)	0.74	–0.81	0.0004

Table 3. Regression analyses between ΔFC and ΔPAS-total in ROIs extracted from ICA analyses within and between common functional networks after cognitive behavioural therapy for anxiety in patients with Parkinson's disease. *Coordinates in Montreal Neurological Institute (MNI) space. Only significant results are provided in this table. No significant change in CMO group.*
*Abbreviations: * = significant change in both CBT and CMO groups; BA = Brodmann area; CBT = cognitive behavioural therapy; CEN = central executive network; CMO = clinical monitoring only; DAN = dorsal attentional network; L. = left; LN = language network; R. = right; ROI = region of interest; Std.β = standardized beta score.*

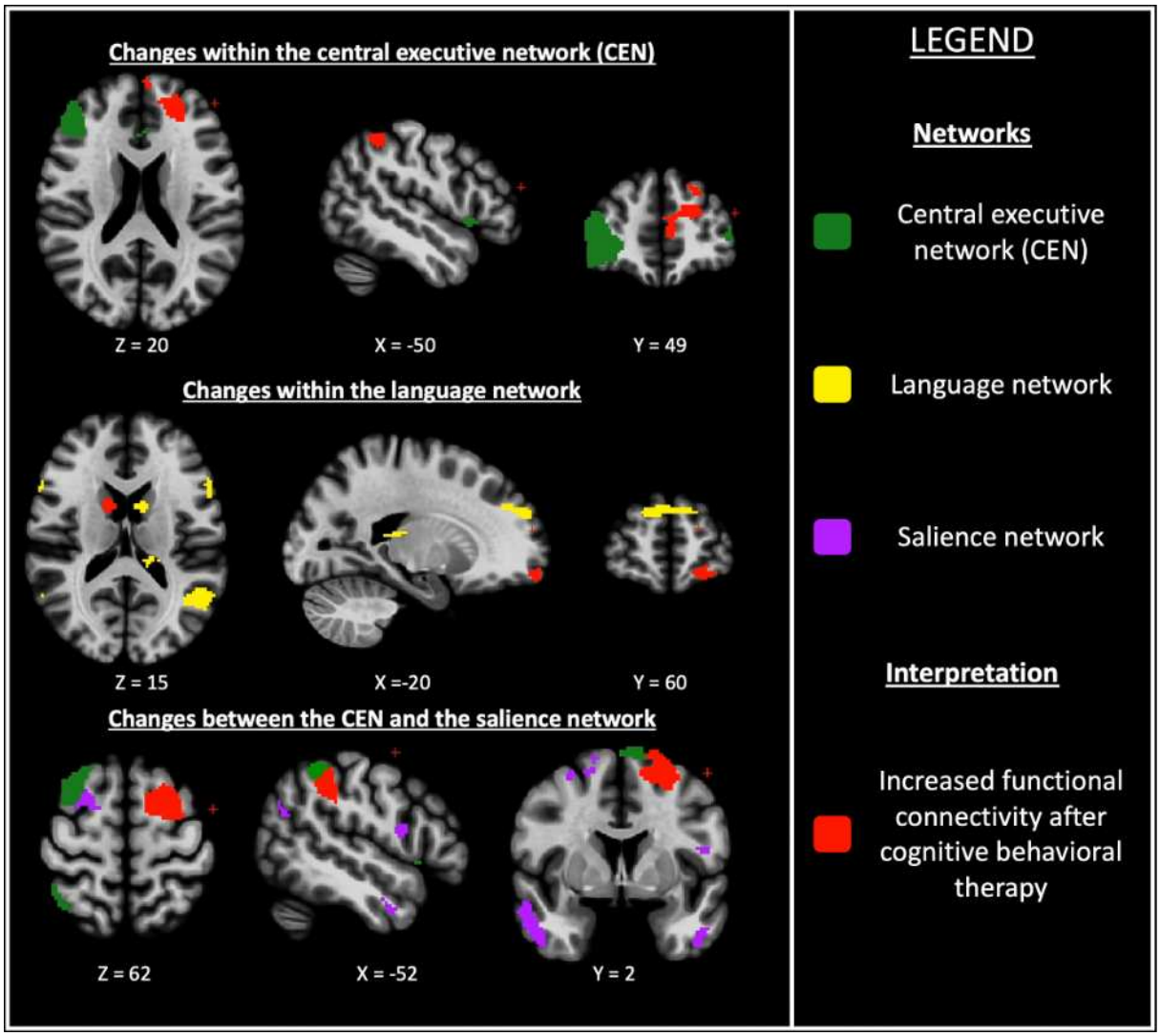


Figure 3. Changes within and between resting-state functional networks in cognitive behavioural therapy group compared with clinical monitoring only group for anxiety in Parkinson's disease.
Abbreviations: Axial, sagittal and frontal view; (z, x, y) = coordinates in the MNI space (Montreal Neurological Institute).

Discussion

CBT is an effective treatment to reduce symptoms of anxiety, especially for situational anxiety and avoidance behaviour [4]. This analysis showed that a reduction of anxiety symptoms after CBT is associated with changes of FC in the PD-related anxiety circuits and both within and between functional networks. FC between the right thalamus and the bilateral OFC was reduced to a greater degree in the CBT group compared to the CMO group and the improvement of anxiety after CBT was associated with increased striato-cingulate and parieto-temporal FC. Moreover, the fronto-parietal FC within the CEN, the striato-frontal FC within the language network and the fronto-parietal FC between the CEN and the salience network were higher in the CBT group compared with the CMO group. The improvement of anxiety after CBT was associated with a decreased FC within the DMN as well as between the language

network and the DAN. Therefore, CBT-induced reduction of anxiety in anxious PD patients is mediated by functional connectivity changes.

CBT reverses the imbalance between PD-related anxiety circuits

In PD, the striatal dopaminergic depletion leads to reduced activity in the cortico-striato-thalamocortical (CSTC) circuits, including the limbic one. Dysfunction of this limbic loop has been associated with psychiatric symptoms, such as anxiety. This circuit connects the anterior cingulate cortex (ACC), the medial PFC and brainstem nuclei with the basal ganglia such as the nucleus accumbens, the pallidum, the subthalamic nucleus (STN) and the thalamus in order to modulate mood and behaviour[17,18]. The fear circuit involves the amygdala and the ACC, the medial PFC, the insular cortex, the hippocampus, and the striatum. The fear circuit is involved in fear processing, while the limbic CSTC circuit is more involved in emotional and behavioural adaptations to fear [5]. In anxiety, the limbic CSTC circuit is mostly under-activated while the fear circuit is over-activated. We recently proposed that anxiety in PD could be due to this imbalance between these two circuits [5].

In the present study, we showed that CBT induces an increased FC between the nucleus accumbens and the PFC and between the caudate and the dACC. These are parts of the limbic CSTC circuit which could be reactivated by CBT in anxious PD patients. This would be in line with our earlier hypothesis of imbalance between the limbic CSTC circuit and the fear circuit. Moreover, the FC between the thalamus and the OFC was lower in the CBT group than in the CMO group. Scarce studies of CBT for anxiety disorders in non-PD patients reported that the improvement of anxiety symptoms after CBT was associated with a lower activity of the OFC and the thalamus [19,20]. A systematic review addressing the neurobiological basis of emotional processing in PD patients showed that the pathway between the thalamus and the OFC was involved in emotion recognition as well as the processing of intense emotional stimuli [21]. It was hypothesized that difficulties in emotion recognition in PD patients may arise from reduced dopaminergic input from structures that have close interconnections with OFC, such as the caudate nucleus [21]. Moreover, in PD the reduced dopaminergic projections to the frontal cortex, including the OFC, may prevent a disinhibition of the amygdala. This may lead to an inappropriate response to intense emotional stimuli [21]. In PD, anxiety symptoms could be associated with an imbalance between overactivity of the thalamus-OFC pathway and a reduced dopaminergic state between the PFC/OFC and striatal structures. By reducing the thalamo-orbitofrontal FC and increasing the striato-prefrontal and striato-cingulate FC, namely the limbic CSTC circuit, CBT could restore the balance between these circuits. This would reduce the abnormal representation of non-anxious stimuli that are wrongly interpreted as anxiogenic, and thus modulate the processing of intense emotional reaction. Reduction of thalamo-orbitofrontal activation would be a general effect of CBT on

anxiety symptoms while reactivation of the limbic CSTC circuit could specifically act on PD-related anxiety. Finally, we did not find any CBT-induced changes in the fear circuit.

CBT reinforces the cognitive control on the emotional processing

Firstly, the fronto-parietal FC within the CEN was increased in the CBT group compared with the CMO group. The CEN, which includes the dorsal lateral PFC, the inferior parietal lobule, and the anterior cingulate cortex, is involved in coordination of multiple domains of cognitive control such as attention, working memory, planning, and motor and behavioural inhibition [22,23]. In a recent study, Micco et al. reported that anxiety in PD-patients was associated with a decreased fronto-parietal FC within the CEN [6]. In our study, by restoring the fronto-parietal FC within the CEN, CBT could improve cognitive control. Moreover, CBT induced an increased fronto-parietal FC between the CEN and the salience network. The salience network mainly includes the insular and cingulate cortices but also parts of the parietal and frontal cortices. It is therefore involved in "bottom-up" attentional processing and may cause hypervigilance in case of insufficient filtering of the captured stimuli [24]. Micco et al. reported disruptions in the salience network in PD patients with anxiety symptoms, with both a decreased and an increased FC within the ACC and insula. They also found a reduced FC between the salience network and the CEN at disease onset in patients with PD and anxiety symptoms. The authors hypothesized that an abnormal interplay within and between limbic and executive areas may impair the filtering role of the salience network over external and internal stimuli, leading to anxiety symptoms. Thus, abnormal interconnection between the salience network and the CEN may decrease the ability to modulate behavioural, as was also shown in anxious non-PD patients [6]. In our study, we found the opposite in the CBT compared with the CMO group. Hence, CBT could restore the FC between the CEN and the salience network in anxious PD-patients. By increasing cognitive control on the emotional process, it could reduce anxiety symptoms in PD patients. Besides, we found disrupted connections between the DAN and the language network, with an increased fronto-temporal FC and decreased temporo-caudate FC. The DAN, which includes the inferior parietal sulcus, the frontal eye fields, the visual cortex, and the temporal cortex, is involved in working memory, spatial attentional function, flexible coordination of cognitive control and decision-making processes [25]. CBT could induce fronto-temporal FC changes in order to modulate the cognitive control of emotions. Moreover, the reduced temporo-caudate FC, which is also part of the fear circuit, could reflect its reduced activity after CBT. These results seem to be in line with our previous findings but as no previous work has studied the DAN activity in anxious PD patients, further studies are needed. Finally, we found that improvement of anxiety symptoms was associated with a reduced FC within the DMN. A similar decreased activity in the DMN has been described in anxious PD patients [6].

Both interventions induced changes in FC

The clinical study showed that even though CBT was more effective than CMO in improving situational anxiety and avoidance behaviour, improvement was also observed in the CMO group4. In our study, in both groups, improvement of the PAS-total score was associated with increased parieto-cingulate and striato-cingulate FC and decreased temporo-insular FC. We suggest that both interventions are able to improve anxiety symptoms. Even a simple clinical monitoring was able to slightly improve anxiety symptoms, and this induced slight FC changes. Detecting, diagnosing, and offering support for anxiety in PD patients is thus essential.

Strengths and limitations

This study has several strengths and limitations. A strength is that this is the first study to explore the neural bases of changes induced by CBT in PD patients with anxiety. Moreover, this study was done in the context of a randomized controlled trial with a control group. Finally, in order to validate the results, several statistical methods have been performed. Only the significant results for all the methods were considered for this paper. A limitation is the small sample size that could have reduced the statistical power and increase the chance of type II errors. However, our sample size is higher (n=35) than that usually observed in the literature on neuroimaging of CBT [19,20,26–29]. Secondly, the control group is not really a placebo group, since clinical monitoring may also be seen as an intervention. Clinical monitoring has been recommended as a control situation when exploring the clinical effectiveness of a new or adjusted psychotherapeutic intervention [7,30]. Thirdly, we choose to not introduce the improvement of depressive symptoms after the intervention as a nuisance factor. The CBT was tailored for anxiety symptoms and the PAS-score is insensitive for depressive symptoms [9]. In our study, the severity of depressive symptoms was assessed by the HDRS-score. At baseline this score was low in both groups. Moreover, this scale includes items assessing anxiety. Correcting our analyses by the change in the HDRS-score would cancel a great part of the effects due to anxiety improvement. There is also a frequent co-morbidity between anxiety and depression. These symptoms are often intermingled and separating them would distort reality. Fourth, we excluded 10 patients from the imaging analyses (and 3 patients dropped out the clinical and imaging study) for lack of MRI scan at the 2 sessions (claustrophobia n=9 and deep brain stimulation n=1). We ensured that the excluded patients had no differences at baseline by comparing them with included patients using Chi2 and Mann-Whitney tests. They were no differences (Supplementary Table 2). Then, as mentioned before, the CSCT circuit is classically divided in limbic, motor, and associative loop. We did not compare these three loops in the same analyse. A further work would be interesting to better understand the effect of CBT on the entire CSTC circuit. Finally, the OFC is classically susceptible to imaging artifacts. As

mentioned in the supplementary data, the EPI double-echo sequence was not available for all the patients. We processed a distortion correction using the Susceptible Distortion Correction included in the CONN toolbox pipeline.

Conclusion

In this study, CBT induced cerebral changes in anxious PD patients that were associated with symptom reduction. CBT can restore the imbalance between PD-related anxiety circuits and thus reinforce cognitive control of emotional processing, leading to improvement of anxiety symptoms in PD patients. Our study also revealed that, in PD patients, the limbic CSTC circuit is more accessible to modulation by CBT than the fear circuit.

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SUPPLEMENTARY MATERIAL

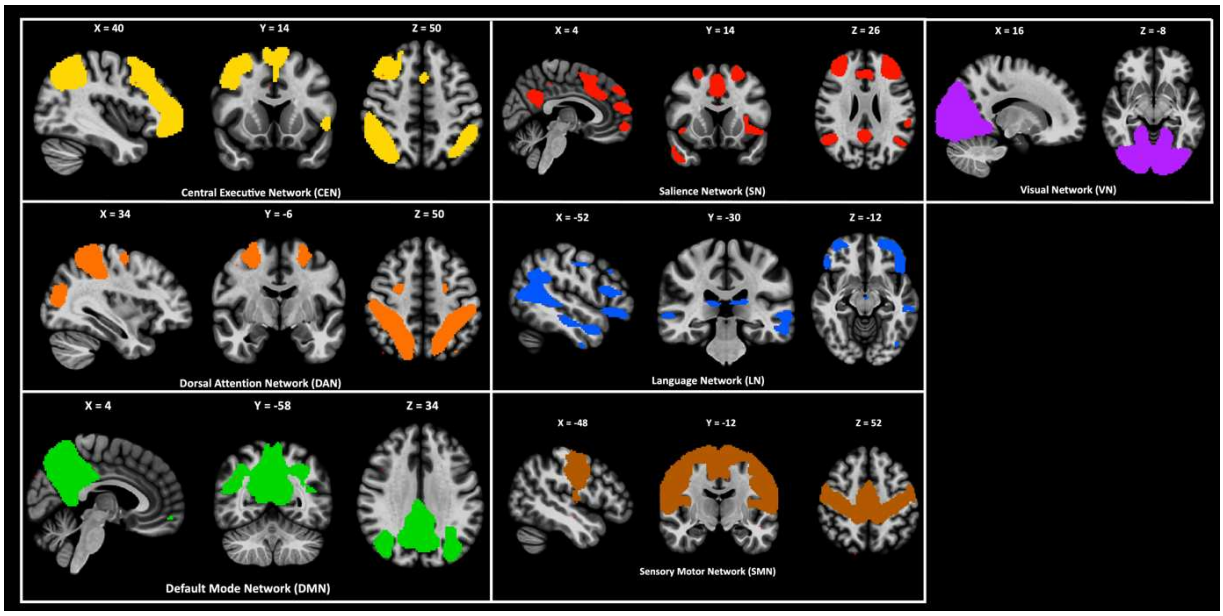
Materials and methods

Preprocessing steps using CONN toolbox

Rs-fMRI data were preprocessed using the CONNv18 toolbox (https://web.conn-toolbox.org/fmri-methods/preprocessing-pipeline).

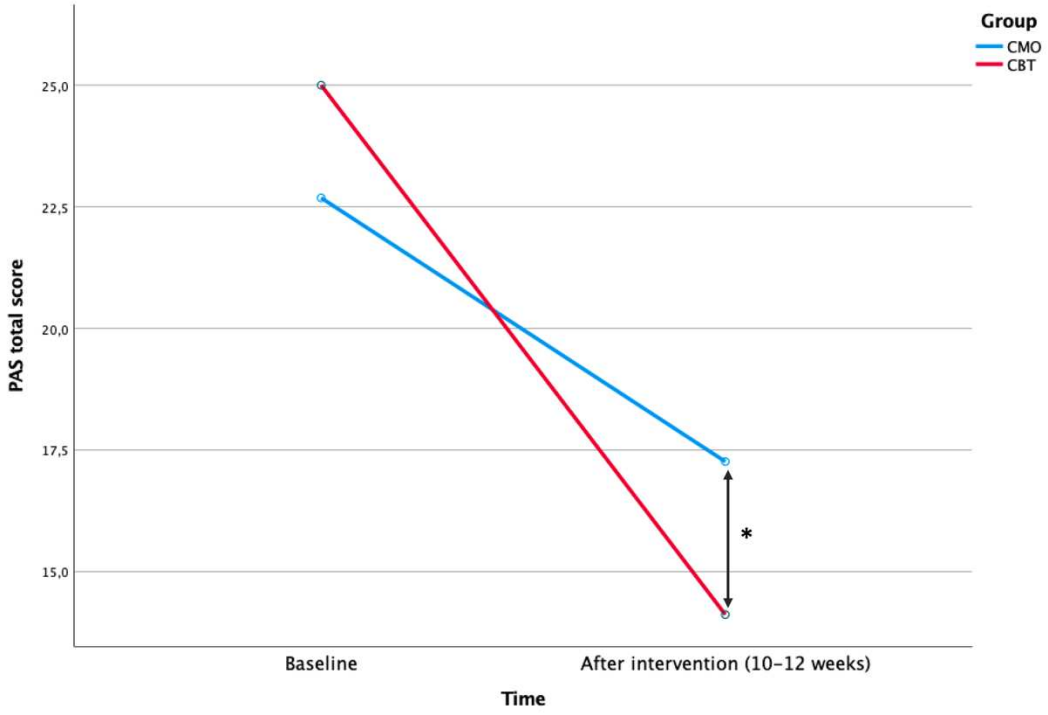
The first 3 volumes were discarded before slice-timing correction with the fourth volume as a reference. The remaining volumes were realigned and corrected for head motion, using linear registration. As the EPI double-echo sequence was not available for all the patients, distortion correction has been processed using the Susceptible Distortion Correction included in the CONN toolbox pipeline. Two 'denoising' steps were performed. Firstly, linear/quadratic trends (to account for scanner drift), and translation and motion parameters were regressed out from BOLD signals. Secondly, an outlier detection step was based on the 'scrubbing' method. Head motion–induced displacements were computed using 6 translation and rotation measures from the linear registration. We computed the sum of the absolute values of the 6 measures for each frame. Time frames with a displacement of more than 5 standard deviation were flagged as potential outliers16. A separate nuisance regressor was generated that matched the length of rs-fMRI time series, containing a value of 1 at the location of the contaminated frame and 0 elsewhere. Moreover, principal component analysis (PCA) was performed in the time domain on the voxel time series separately for white matter and cerebrospinal fluid masks segmented by SPM software (SPM12, fil.ion.ucl.ac.uk/spm/software/spm12/). The top 5 principal components for each tissue type were extracted. Thus, scrubbing and tissue confounds were regressed out from each BOLD signals. Finally, a band-pass filter of 0.008 Hz – 0.09 Hz was applied and smoothing with a 6mm Gaussian kernel was performed only for voxel-based analysis.

ICA analysis



Supplementary Figure 1. Representation of the seven functional resting-state networks identified after the group Independent Component Analyses using the CONN Toolbox. *For each network: sagittal, frontal and axial view; (x, y, z) = coordinates in the MNI space (Montreal neurological institute)*

Results



Supplementary Figure 2. Representation of the repeated mixed ANOVA comparing the PAS total score at baseline and after the intervention between CBT and CMO group : reduction of anxiety symptoms in both group and especially in the CBT group.

*Abbreviations: CBT = Cognitive Behavioural Therapy; CMO = Clinical Monitoring Only; * = p-value < 0.05*

Δ PAS scores	CBT			CMO			Interpretation
	R^2	p-value	Beta	R^2	p-value	Beta	
Right Accumbens – right dorsal Anterior Cingulate Cortex (32)							
PAS total	0,456	0,055	0,617	0,524	**0,019***	0,455	Effect of CMO: positive association
Right Accumbens – left dorsal Anterior Cingulate Cortex (32)							
PAS total	0,586	**0,012***	0,761	0,377	0,095	0,344	**Effect of CBT: positive association**
Right Angular Coxtex (39) – right ventral Posterior Cingulate Cortex (23)							
PAS total	0,486	**0,04***	0,661	0,561	**0,011***	0,607	Effect of both CMO and CBT: positive association
Right Angular Coxtex (39) – right perirhinal area (35)							
PAS total	0,551	**0,019***	0,706	0,184	0,432	0,306	**Effect of CBT: positive association**
Right Insular Cortex (13) – Left Temporal Pole (38)							
PAS total	0,585	**0,012***	-0,552	0,533	**0,017***	-0,645	Effect of both CMO and CB: negative association

Supplementary Table 1. Multiple linear regression between ΔPAS (pre-post) and ΔFC (post-pre) adjusted by center and time between Baseline and Post-intervention MRIs (days) in CBT and CMO groups for anxiety in Parkinson's disease.

Only significant results are presented in the table.

Abbreviations: CBT = Cognitive Behavioural Therapy; CMO = Clinical Monitoring Only; FC = Functional Connectivity; PAS = Parkinson Anxiety Scale.

*Interpretation: * = significant difference; $ = Significant correlation but no effect of PAS (effect of other co-variable); Positive association between ΔPAS and ΔFC means that an improvement of PAS is associated with an increased FC after the intervention; Negative association between ΔPAS and ΔFC means that improvement of PAS is associated with a decreased FC after the intervention.*

Groups		Excluded	Included	p-value
Center	**Maastricht**	4	13	0.99
	Lille	6	23	
Intervention group	**CMO**	4	19	0.48
	CBT	6	17	
Sex	**Women**	4	20	0.48
	Men	6	16	
Education (years)		21.1	13.8	0.512
Disease duration (years)		5.1	6.1	0.738
Baseline PAS-A		13.0	13.4	0.638
Baseline PAS-B		6.1	5.4	0.453
Baseline PAS-C		4.5	4.6	0.84
Baseline PAS total		23.9	23.8	0.852
Baseline HAMD		11.3	11.1	0.728
Baseline LARS		-25.9	-25.1	0.729
Baseline MOCA		26.6	26.4	0.957
Baseline MDS-UPDRS part3		30.8	25.4	0.393
Baseline Hoehn & Yarh		2 (1-3)	2 (1-3)	0.793

Supplementary Table 2. Comparison of baseline demographic and clinical data between included and excluded patients, from the original cohort, in the study.

Abbreviations: CBT = cognitive behavioural therapy; CMO = clinical monitoring only.

CHAPTER 7

Reduced thalamic nuclei volume may contribute to anxiety in Parkinson's disease: a cross-sectional 7-tesla MRI study

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Article submitted to "Movement Disorders" – Under review

Summary of the chapter

After showing, using 3-Tesla MRI scans, that i) PD related anxiety is associated with anatomical, structural connectivity and functional connectivity changes within the fear and limbic circuits, ii) there is an imbalance between these two circuits, and iii) cognitive behavioural therapy can reduce anxiety by restoring this balance, we aimed to confirm these findings using high-field 7-Tesla MRI scans. We also aimed to understand how this imbalance between these two circuits could explain the high prevalence of anxiety symptoms in PD. These two circuits involve overlapping structures, such as the thalamus, the striatum and brainstem nuclei. We hypothesised that the hypodopaminergic state, the atrophy and the underactivation of the limbic circuit due to PD lesions could also alter these overlapping structures, contributing to alterations in the anxiety circuits and to this imbalance.

TRACK-PD is a longitudinal, on-going, observational study including PD patients without dementia and healthy controls. All patients undergo a 7-T MRI scan and clinical evaluation at baseline, 2-year and 4-year follow-up. We worked on the baseline dataset in order to challenge our working hypotheses. In the present study, we focused on one of the overlapping structures of the two circuits, the thalamus. We compared the volume of the thalamus and thalamic subregions in PD patients with and without clinically significant anxiety and healthy controls. We explored the links between the severity of anxiety according to the Parkinson Anxiety Scale and the volume of thalamus and thalamic subregions through regression analyses. We found that a smaller volume of the anterior and the mediodorsal regions of the thalamus was associated with a higher anxiety severity in PD. These regions are known to be involved in cognitive and emotional processes and were already associated with anxiety in the general population. Both are also involved in the two anxiety-related circuits. Therefore, these findings reinforce our hypothesis that lesions in overlapping structures of the anxiety-related circuits could lead to anxiety symptoms in PD patients.

Abstract

Background: Parkinson's disease (PD) related anxiety occurs frequently and may be associated with imbalance between anxiety-related circuits. While the thalamus is a common region of these circuits, its role in PD-related anxiety was not explored so far.

Objective: To identify changes in volume of the thalamus and its subnuclei in patients with PD-related anxiety.

Methods: Cognitively intact PD patients (n=105) were divided into two groups based on their score on the Parkinson anxiety scale (PAS): 31 PD patients had anxiety (Anx-PD) and 74 did not have anxiety (non-Anx-PD). Fourty-five healthy controls were also included. All participants underwent 7-Tesla MRI scanning. Using an automatic segmentation pipeline, the volume of the thalamus and its subnuclei were extracted for each dataset. These were compared between the groups and regressed on the PAS score.

Results: The volume of the thalamus or its sub did not significantly differ between groups. However, in anxious patients, a higher severity of anxiety was strongly associated with a smaller volume of the right mediodorsal thalamic subregion, more specifically the right mediodorsal magnocellular nucleus and the right mediodorsal parvocellular nucleus ($R = 0.63$, $ß_{PAS} = -0{,}546$, $p\text{-value}_{model} = 0.007$ and $R = 0.60$, $ß_{PAS} = -0{,}547$, $p\text{-value}_{model} = 0.016$, respectively).

Conclusion: A reduced volume of the dorsomedial thalamus, an overlapping structure between the anxiety related circuits, is associated with a higher severity of PD-related anxiety and may explain its high prevalence in the disease.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by both motor and non-motor symptoms. Anxiety is one of the most common neuropsychiatric symptoms in PD, with an average point prevalence around 31% (1). PD-related anxiety is associated with increased motor disability and a reduced quality of life (2). While the potential involvement of various anatomical structures in PD-related anxiety was suggested (3–5), findings are inconsistent and in need of confirmation.

Recent studies suggested that PD-related anxiety could result from an imbalance between the anxiety-related circuits, i.e. the fear circuit and the limbic anxiety circuit (6,7). In PD, striatal dopamine depletion could lead to reduced activity in the limbic anxiety circuit while the fear circuit would become relatively overactivated (6).

The limbic anxiety circuit, involved in emotional and behavioral adaptations to fear, connects the anterior cingulate cortex, the medial prefrontal cortex (PFC) and brainstem nuclei with the nucleus accumbens, the pallidum, the subthalamic nucleus and the thalamus to modulate mood and behavior (8). The fear circuit, involved in fear processing, connects the amygdala to the anterior cingular cortex, the medial PFC, the insular cortex, the hippocampus, the striatum and the thalamus (9,10).

The overlap between these two circuits mainly concerns the striatum (i.e. nucleus accumbens), brainstem nuclei (i.e. locus coeruleus, ventral tegmental area, substantia nigra) and the thalamus (6). In a previous study, we reported that changes in functional connectivity between the orbitofrontal cortex and the thalamus were induced after cognitive behavioral therapy for PD-related anxiety suggesting that thalamic dysfunction could be associated with these symptoms (11). So far, the role of the thalamus in the development of anxiety in PD was never explored. This can be helped by high-field imaging, namely the use of the recent high-field imaging atlas of the thalamic subregions (12,13).

The aim of this study was to identify anatomical changes in the volume of the thalamus and the thalamic subnuclei in patients with PD-related anxiety compared to patients without anxiety, using 7-Tesla MRI. We hypothesized that the volume of the thalamic subnuclei could be smaller in patients with PD-related anxiety compared to patients without anxiety, especially the subnuclei connected to the PFC and the orbitofrontal cortex. We did not expect any differences between PD-patients without anxiety and healthy controls.

Material and methods

Study design

Data came from the TRACK-PD study (14). In this on-going longitudinal observational study, non-demented PD patients and healthy controls (HC) are included, with a 4-years follow-up. All data were collected at Maastricht University Medical Centre. A 7-Tesla brain MRI as well as demographical, clinical, cognitive, and neuropsychiatric information were recorded. In the current study, we only focused on the baseline dataset. The study design is published previously and detailed by Wolters et al (14). Ethical approval was provided by the local medical ethical committee and the study has been registered (Dutch Trial Register, NL7558). Written informed consent was obtained from all participants.

Study Population

In this study, patients diagnosed with PD in the last three years were recruited among outpatients of the Movement Disorder Clinic of Maastricht University Medical Centre and collaborating hospitals. In addition, other media, such as websites, social media, and patient meetings were used to recruit patients. HC participants were recruited through advertisements in the hospital and university. Participants included in this study were diagnosed with PD by a neurologist based on Postuma et al. diagnostic criteria (15), had a score equal or higher than 24 on the Montreal Cognitive Assessment (MoCA) at baseline (16), were able to read and understand Dutch, were 18 years of age or older and provided written informed consent. Participants with advanced cognitive impairment, defined as a score of < 24 on the MoCA, or a diagnosis of dementia according to the criteria of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM 5), at baseline, or diagnosed with a neurodegenerative disease other than PD, or with any contra-indications for a 7-T MRI scan (such as claustrophobia, permanent make-up or the presence of incompatible metallic devices in their body) were excluded. These exclusion criteria were also in place for the HC group.

Demographic and clinical variables were recorded including age, sex, handedness, disease duration, and the total levodopa equivalent daily dose (LEDD). Motor symptoms and disease severity were respectively assessed during “ON'' phases with the Movement Disorder Society – unified Parkinson’s disease rating scale (MDS-UPDRS) part three and the Hoehn & Yahr staging system (17). Depressive symptoms were assessed with the ‘Beck Depression Inventory’ (BDI) (18), overall cognition with the MoCA and non-motor symptoms with MDS-UPDRS part one. More details are available in the published protocol (14).

Anxiety assessment

The Parkinson anxiety scale (PAS), a scale specifically developed to detect and measure anxiety in PD patients, was used to assess anxiety symptoms. It is insensitive to motor and depressive symptoms and has subsections for persistent anxiety (PAS-A), episodic/situational anxiety (PAS-B), and avoidance behavior (PAS-C) (19). A PAS-total score was calculated by summing the three subscores. PD-patients were considered to have significant PD-related anxiety if they had a score above the cut-off in at least one of the three subparts of the scale: PAS-A >9, PAS-B >3, or PAS-C >3. Three groups were made: 1) PD-patients with anxiety (Anx-PD), 2) PD-patients without anxiety (non-Anx-PD), 3) healthy controls (HC).

Imaging acquisition

Participants were scanned on a 7T MRI scanner (*Magnetom, Siemens, Erlangen, Germany*) equipped with a Nova Medical 32-channel head coil. Dielectric pads were applied to enhance the signal in the temporal brain regions. Cardiac and respiratory physiological signals were measured synchronized with the scan start. For this study, a whole-brain MP2RAGE (Magnetization Prepared 2 Rapid Acquisition Gradient Echoes) acquisition was used with an acquisition time of 10:57 min, resulting in a T1-weighted image and a quantitative T1map (more details in the published protocol (14)).

Thalamus and thalamic subnuclei segmentation

After dicom to nifti conversion, the MP2RAGE datasets were bias field corrected and aligned to the ACPC line using MIPAV software. The MP2RAGE structural brain MRI scans were processed using FreeSurfer (version 6.0.0) (https://surfer.nmr.mgh.harvard.edu/) for segmentation of subcortical structures including the whole thalamus (20). A first visual quality control was performed for each segmentation. The pipeline of Iglesias et al. (12) was used to segment the thalamus into 25 nuclei (Figure 1). To make the analysis more powerful, we grouped these 25 nuclei into five different subregions per hemisphere: anterior, lateral, ventral, intralaminar/medial and pulvinar according to previous studies (Figure 1) (21). A visual quality control was performed for all segmentations to identify outliers.

The mean volume of the whole thalamus and each of the thalamic subnuclei (in mm^3) were extracted bilaterally. The mean volumes of each subregion were obtained by adding the corresponding thalamic subnuclei's volumes.

Group	Abbrev.	Nucleus
Anterior	AV	Anteroventral
Lateral	LD	Laterodoral
	LP	Lateral posterior
Ventral	VA	Ventral anterior
	VAmc	Ventral anterior magnocellular
	VLa	Ventral lateral anterior
	VLp	Ventral lateral posterior
	VPL	Ventral posterolateral
	VM	Ventromedial
Intralaminar / medial	CeM	Central medial
	CL	Central lateral
	Pc	Paracentral
	CM	Centromedian
	Pf	Parafascicular
Medial	Pt	Paratenial
	MV-re	Reuniens (medial ventral)
	MDm	Mediodorsal medial magnocellular
	MDl	Mediodorsal lateral parvocellular
Pulvinar	PuA	Pulvinar anterior
	PuM	Pulvinar medial
	PuL	Pulvinar lateral
	Pul	Pulvinar inferior

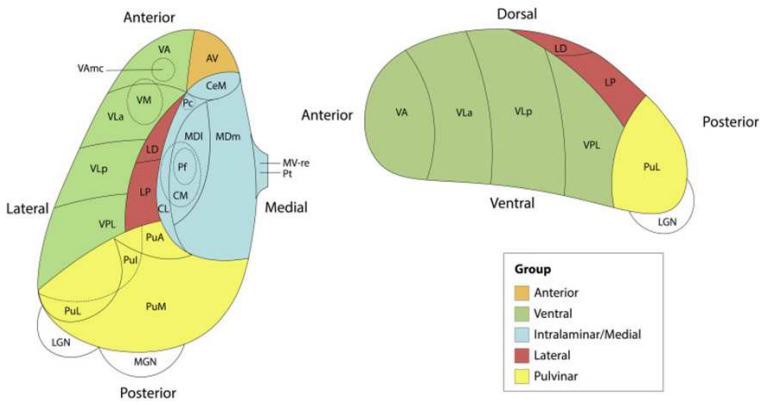

Figure 1. Schematic representation of the thalamic subnuclei and the five subregions. *Figure adapted from Weeland et al. 2022* (21).

Statistical analyses

The significance threshold was set at p-value < 0.05 and corrected for multiple comparisons (FDR - False Discovery Rate) when necessary. 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. The normality of distribution was assessed using a Kolmogorov-Smirnov test.

Descriptive analyses

Categorical data were compared with Chi^2 tests and quantitative data with ANOVA tests, between the three groups, using SPSS, version 29 (SPSS, Chicago).

Imaging analyses

First, the volumes of the whole thalamus and of the thalamic subregions were compared between the three groups using ANCOVA models. According to the literature, age and sex are associated with the volume of the thalamus (21,22), especially in PD patients (23). Moreover, according to our previous studies, anxiety symptoms are associated with depressive symptoms and sex (5,24). Finally, the mean volume of thalamus had to be normalized by the total brain volume. Therefore, in this ANCOVA model, age, sex, depressive symptoms (BDI-score) and total brain volume were set as covariables.

Secondly, hierarchical multiple regression analyses were performed to examine the relationship between the PAS-total score and the mean volume of the subregions. Age, sex, depressive symptoms (BDI-score) and total brain volume were set as nuisance regressors in the first block (model 1) of all regression models, whereas PAS-total score (independent variable) was separately added to the second block of the model (model 2). The mean volume of the analyzed subregion was set as dependent variable. We ensured that all models met the assumptions for multiple regression analyses, including normality of the residuals, multicollinearity, and homoscedasticity.

In case of significant differences, the same analyses were performed for each subnucleus of the corresponding subregion.

Results

Population and descriptive analysis

Among the 151 participants included in TRACK-PD study, only one was excluded because of MRI refusal. Among the 105 PD patients, 31 (29.5%) had clinically significant anxiety while 74 (70.5%) had not. Among the 45 healthy controls, 8 (17.8%) had significant anxiety, according to their score on the PAS. The BDI total score was higher in the Anx-PD group than in the other groups as were the scores on the MDS-UPDRS 1.1 (cognitive disorders), 1.3 (depressive mood), 1.4 (anxiety state) and 1.5 (apathy) items. The results are detailed in Table 1.

Variables	Anx-PD (n=31)	Non-Anx-PD (n=74)	HC (n=45)	p-value
Demographic variables				
Sex (women)	10 (32.3%)	22 (29.7%)	14 (31.1%)	0.97
Age (years)	62.9 (± 9.19)	62.1 (± 8.13)	60.7 (± 8.07)	0.48
Dominant hand (right)	28 (90.3%)	62 (83.8%)	42 (93.3%)	0.27
Disease duration	1.64 (± 0.84)	1.61 (± 0.76)	NA	0.54
Side onset (right)	12 (46.2%)	39 (55.7%)	NA	0.40
LEDD	467.0 (± 261.53)	373.2 (± 209.88)	NA	0.094
Cognitive variable				
MoCA Total	27.7 (± 1.56)	28.0 (± 1.68)	27.7 (± 1.56)	0.57
Clinical variables				
MDS-UPDRS 1				
1-1 Cognitive disorders	1 (0-2)	0 (0-2)	NA	**0.02**
1-3 Depressive mood	0 (0-3)	0 (0-2)	NA	**0.008**
1-4 Anxious state	1 (0-2)	0 (0-2)	NA	**<0.0001**
1-5 Apathy	0 (0-2)	0 (0-1)	NA	**<0.001**
MDS-UPDRS part 3	15.8 (± 4.87)	20.5 (± 6.99)	NA	0.51
Hoehn & Yahr stage	2 (1-2)	2 (1-3)	NA	0.64
PAS total score	15.55 (± 4.04)	5.70 (± 3.40)	5.98 (± 4.35)	**<0.001**
PAS-A: persistent anxiety	8.00 (± 2.89)	3.86 (± 2.48)	3.71 (± 2.60)	**<0.001**
PAS-B: episodic anxiety	4.10 (±1.87)	0.96 (± 0.99)	1.33 (± 1.64)	**<0.001**
PAS-C: avoidance behavior	3.45 (± 1.93)	0.88 (±0.86)	0.93 (±1.27)	**<0.001**
Depression score (BDI)	11.9 (± 5.71)	6.0 (± 3.59)	3.51 (±3.60)	**< 0.001**

Table 1. Descriptive analyses between Parkinson's disease patients with anxiety (Anx-PD), without anxiety (non-Anx-PD) and healthy controls (HC).

Abbreviations: BDI = Beck depression inventory; LEDD = levodopa equivalent daily dose; MoCA = Montreal cognitive assessment; PAS = Parkinson anxiety scale.

Bold = significant difference (p-value < 0.05).

Comparison of thalamic volume and thalamic subregions volumes

There were no between-group differences in the mean volume of the whole thalamus and the thalamic subregions (Table 2).

ROI	Anx-PD (n = 31)	non-Anx-PD (n = 74)	HC (n = 45)	F-score	FDR p-value
Whole Thalamus					
Right Thalamus (mm^3)	5658.0 (±705.5)	5579.1 (±580.2)	5444.7 (±615.4)	2.70	0.20
Left Thalamus (mm^3)	5548.5 (±750.8)	5563.5 (±559.0)	5367.2 (±648.5)	0,11	0,29
Thalamic subregions: right hemisphere					
Anterior region (mm^3)	118.1 (±19.0)	115.7 (±21.3)	117.8 (±23.5)	1.03	0.36
Lateral region (mm^3)	121.1 (±25.3)	118.1 (±20.4)	124.9 (±25.4)	2.5	0.20
Ventral region (mm^3)	2512.9 (±365.3)	2520.9 (±302.4)	2455.7 (±310.9)	1.59	0.25
Intralaminar-medial region (mm^3)	1323.7 (±178.3)	1311.6 (±152.8)	1268.5 (±133.0)	2.04	0.20
Pulvinar (mm^3)	1552.3 (±193.3)	1512.9 (±171.9)	1477.9 (±181.4)	2.26	0.20
Thalamic subregions: left hemisphere					
Anterior region (mm^3)	104.6 (±15.8)	101.0 (±21.7)	97.7 (±21.0)	1.13	0.39
Lateral region (mm^3)	122.5 (±31.5)	123.7 (±29.5)	131.8 (±29.3)	0.75	0.47
Ventral region (mm^3)	2403.8 (±357.9)	2420.5 (±266.5)	2316.4 (±291.9)	1.96	0.29
Intralaminar-medial region (mm^3)	1275.2 (±188.5)	1274.2 (±144.9)	1212.9 (±140.8)	3.00	0.29
Pulvinar (mm^3)	1642.4 (±231.6)	1644.1 (±185.7)	1608.3 (±239.5)	1.18	0.39

Table 2. Comparison of the mean volume of the thalamus and the thalamic subregions between Parkinson's disease patients with anxiety (Anx-PD), without anxiety (non-Anx-PD) and healthy controls (HC) adjusted by sex, age, total brain volume and depressive symptoms according to BDI score.
Abbreviations: BDI = Beck depression inventory; FDR = false discovery rate.

Multiple regression analyses

There was no significant association between the PAS-total score and the thalamus or thalamic subregions' volume in the HC group nor in the non-Anx-PD groups or in all PD-patients (Anx-PD group and non-Anx-PD group).

In the Anx-PD group, there was a strong and significant negative association between the volume of the right intralaminar-medial subregion and the PAS-total score (R = 0.80, FDR p-value$_{model}$ < 0.001, ß$_{PAS}$ = -0,330, p-value$_{PAS}$ = 0.02) and between the left anterior subregion and the PAS-total score (R = 0.73, FDR p-value$_{model}$ = 0.002, ß$_{PAS}$ = -0,407, p-value$_{PAS}$ = 0.01) (*Table 3*).

The volume of the subnuclei involved in the intralaminar-medial subregion were analyzed post-hoc, i.e. the central medial, central lateral, paracentral, centro-median and parafascicular nuclei for the intralaminar subregion and the paratenial, medial ventral reuniens, mediodorsal magnocellular and mediodorsal parvocellular nuclei for the medial subregion (Figure 1) [20].

The anterior region corresponded to the anteroventral nucleus in the thalamic atlas.

In Anx-PD group, the volumes of the right MDm and left MDl had a strong negative association with the PAS-total score (R = 0.63, $ß_{PAS}$ = -0,546, $p\text{-value}_{model}$ = 0.007 and R = 0.60, $ß_{PAS}$ = -0,547, $p\text{-value}_{model}$ = 0.016, respectively) (Table 4 and Figure 2). There were no significant associations in the HC, non-Anx-PD groups.

ROI	F-score	FDR $p\text{-value}_{model}$	R	Beta $_{PAS}$	$p\text{-value}_{PAS}$
Thalamic subregions: right hemisphere					
Anterior region	1.91	0.13	0.53	-0.154	0.43
Lateral region	2.49	0.07	0.58	0.134	0.47
Ventral region	**6.30**	**0.001***	0.75	-0.120	0.43
Intralaminar-medial region	**9.09**	**<0.001**	**0.80**	**-0.330**	**0.02**
Pulvinar	5.26	0.002*†	0.72	-0.124	0.44
Right Thalamus	**9.45**	**<0.001***	0.81	-0.179	0.19
Thalamic subregions: left hemisphere					
Anterior region	**5.79**	**0.002**	**0.733**	**-0.407**	**0.01**
Lateral region	2.23	0.08	0.31	-0.026	0.89
Ventral region	**5.61**	**0.002***	0.72	-0.087	0.58
Intralaminar-medial region	**8.53**	**<0.001***	0.79	-0.151	0.28
Pulvinar	**4.40**	**0.006***	0.68	-0.056	0.74
Left Thalamus	**7.92**	**<0.001***	0.78	-0.106	0.46

Table 3. Hierarchical multiple regression between the PAS-total score and the volume of the thalamic subregion in Parkinson's disease patients with anxiety adjusted by age, sex, total brain volume and BDI score.

Abbreviations: BDI = Beck depression inventory; FDR = false discovery rate; PAS = Parkinson anxiety scale.

*Bold = significant difference (p-value < 0.05); † = effect of the variable sex (and not PAS); * = effect of the variable total brain volume (and not PAS).*

ROI (/TIV)	F-score	FDR p-value$_{model}$	R	Beta $_{PAS}$	p-value$_{PAS}$
Thalamic subnuclei: right hemisphere					
MDm: Mediodorsal medial magnocellular nucleus	**7.78**	**<0.001**	**0.78**	**-0.398**	**0.01**
MDl: Mediodorsal lateral parvocellular nucleus	**4.52**	**0.006**	**0.69**	**-0.394**	**0.02**
Thalamic subnuclei: left hemisphere					
Antero-ventral nucleus	**5.79**	**0.002**	**0.733**	**-0.407**	**0.01**

Table 4. Hierarchical multiple regression between the PAS-total score and the volume of the thalamic subnuclei involved in the intralaminar-medial subregion in Parkinson's disease patients with anxiety adjusted by age, sex, total brain volume and BDI score.

Abbreviations: BDI = Beck depression inventory; PAS = Parkinson anxiety scale; TIV = total intracranial volume. Bold = significant difference (p-value < 0.05)

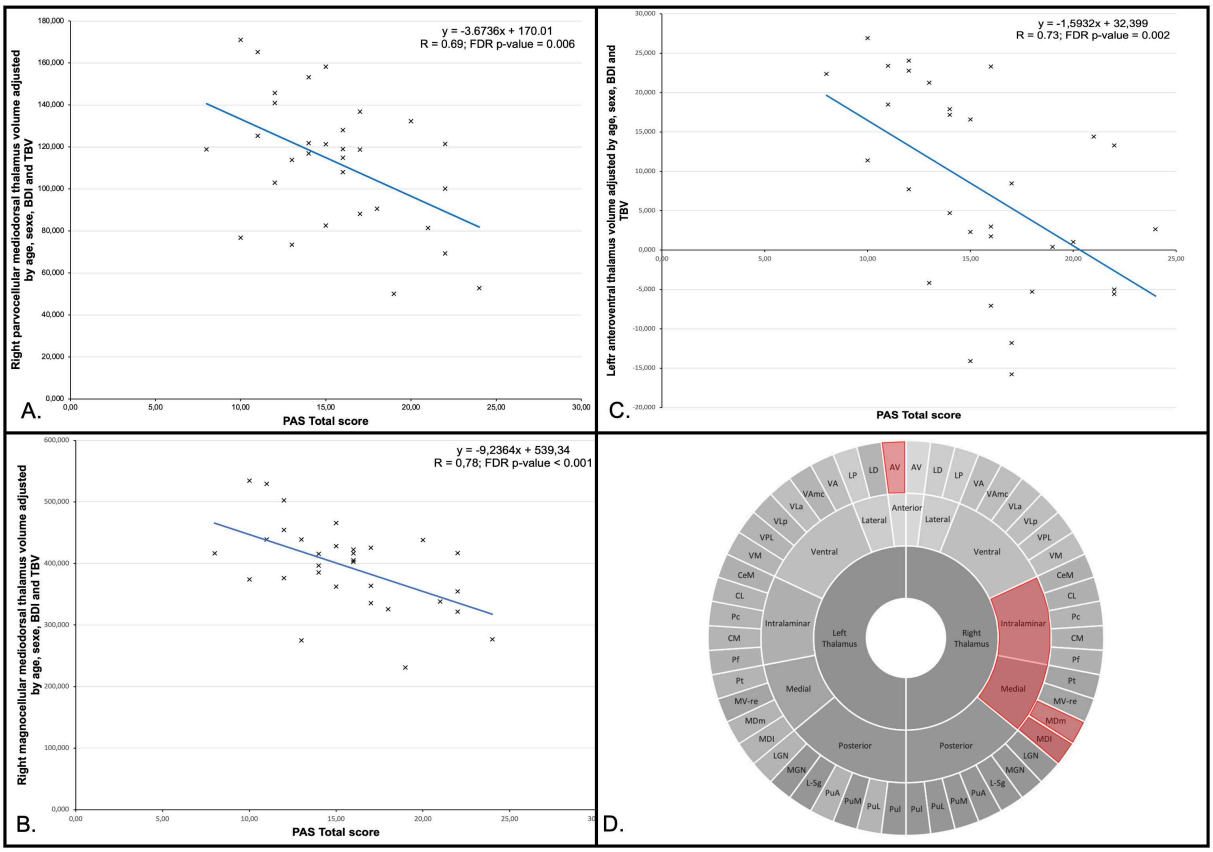

Figure 2. Scatterplot of the regression analyses between the right parvocellular mediodorsal thalamus (A), the right magnocellular mediodorsal thalamus (B) and the left anteroventral thalamus (C) volumes adjusted by age, sex, Beck depressive inventory (BDI) and total brain volume (TBV) and the Parkinson anxiety scale (PAS) total score in Parkinson's disease (PD) patients with anxiety. Schematic illustration of reduced thalamic nuclei (in red) associated with PD-related (D) adapted from Chibaatar et al (25)**.**

Abbreviations: AV = anteroventral; LD = laterodorsal; LP = lateral posterior; VA = ventral anterior; VAmc = ventral anterior magnocellular; VLa = ventral lateral anterior; VLp = ventral lateral posterior; VPL = ventral posterolateral; VM = ventromedial; CeM = central medial = CL = central lateral; Pc = paracentral; CM = centromedian; Pf = parafascicular; Pt = paratenial; MV-re = medial ventral reuniens; MDm = mediodorsal medial magnocellular; MDl = mediodorsal lateral parvocellular; LGN = lateral geniculate; MGN = medial geniculate; L-Sg = limitans suprageniculate; PuA = pulvinar anterior; PuM = pulvinar medial; PuL = pulvinar lateral; PuI = pulvinar inferior; FDR = False discovery rate.

Discussion

This study aimed to identify anatomical differences in thalamic volume and its subnuclei between PD patients with anxiety and those without anxiety, using 7-Tesla MRI scanning. We found no differences in thalamic volume or its subregions between PD patients with or without anxiety, and healthy controls. However, in PD patients with anxiety, a higher severity of anxiety was associated with a smaller volume of the anterior thalamic subregion and the right mediodorsal thalamic subregion, more specifically the right mediodorsal magnocellular nucleus and the right mediodorsal parvocellular nucleus.

Anxiety severity in PD is associated with a smaller anteroventral thalamus

The anteroventral thalamus (AVT) is known to be part of an associative-cognitive grouping of nuclei with the laterodorsal, mediodorsal, reuniens and parateniens nuclei and involved in learning, memory, cognition, and emotion regulation (26). It is a significant part of the limbic system. More specifically, the AVT is structurally connected to the nucleus accumbens, hypothalamus, hippocampus, amygdala, temporal cortex, orbitofrontal cortex, medial PFC and anterior cingulate cortex as well as the midbrain (Figure 3) (27). Therefore, this nucleus can be considered to be part of the fear circuit. Structural alteration of anterior thalamus and its connections has been associated with anxiety-like behaviour in mice (28), while a reduced volume of the anterior thalamus has already been linked with anxiety symptoms in humans (26). Reduced volume of the anterior thalamus was also found in other neuropsychiatric symptoms such as obsessive-compulsive disorder and depression (21,25). Interestingly, in a recent study, acute ischemic stroke involving the anterior thalamus but not the other thalamic regions would lead to long term anxiety symptoms (29). Therefore, these different findings suggest that lesioning the anterior thalamus could lead to neuropsychiatric symptoms and especially anxiety in the general population. In PD patients, changes in the volume of thalamic nuclei have been correlated with the diagnosis and the progression of the disease or with symptoms such as freezing of gait, rapid eye movement sleep behavioural disorders (RBD) or cognitive disorders. However, the results of these studies were inconsistent since a smaller thalamic volume was correlated with disease progression, the presence of RBD and mild cognitive impairment (30–32) while a bigger thalamic volume was reported in case of freezing of gait (33). It is also suggested that PD patients have a bigger volume of the AVT at the onset of the disease compared with healthy controls (34). Therefore, the thalamus, namely the AVT subnucleus, is involved in PD but its exact role remains uncertain. In our study, severity of anxiety symptoms in PD patients was associated with a smaller AVT volume. Since the AVT is part of the fear circuit, its atrophy could play a role in the inappropriate fear processing in PD-related anxiety.

Anxiety severity in PD is associated with a smaller mediodorsal thalamus

The mediodorsal thalamus (MDT) is the largest nuclear structure of the medial thalamus. It is classically described with several subdivisions such as the magnocellular MDT and the parvocellular MDT (35). The MDT is mainly structurally connected with the PFC, limbic structures, and basal ganglia. These connections differ between the magnocellular and the parvocellular MDT. The magnocellular MDT has connections with the ventromedial PFC, the orbitofrontal cortex and the anterior cingular cortex, and receives afferents from the amygdala, the parahippocampal cortex, the midbrain, the brainstem, the substantia nigra, the ventral striatum and the ventral pallidum. The parvocellular MDT relates to the dorsolateral PFC and the anterior cingular cortex and receives afferents from the brainstem, the substantia nigra, the dorsal caudate and the rostral pallidum (Figure 3) (35,36). Recently, an "emotional pathway" was described in primates between the amygdala, the orbito-frontal cortex and the MDT that could refer to the fear circuit. Moreover, it has been shown that the MDT is involved in a cortico-striatal-thalamic loop involved in psychiatric symptoms such as anxiety disorders (37,38). Animal studies showed that lesioning the MDT in rats or in mice leads to structural and functional alterations in the PFC as well as in the amygdala and enhances anxiety-like behaviours suggesting that MDT alteration could lead to anxiety disorders (39,40). In humans, reduced volume of the MDT was associated with occurrence of anxiety symptoms (26) and other neuropsychiatric disorders such as depression and obsessive-compulsive disorders (21,25). In PD patients, studies found that reduced volume of the MDT would be involved in cognitive disorders and behavioural symptoms such as hallucinations and depression (41–43). In a recent study it was reported that, compared to healthy controls, the volume of the MDT was smaller in PD patients at the onset of the disease (34). Similar changes were described in Lewy bodies disease, suggesting a possible involvement of MDT atrophy in other synucleinopathies (44).

In our study, the severity of anxiety in anxious PD patients was strongly associated with a smaller volume of the magnocellular MDT and the parvocellular MDT. These results are consistent with previous studies from our group. Indeed, we reported a reduced functional connectivity between the orbitofrontal cortex and the thalamus, increased functional connectivity between the amygdala and the thalamus and changes in structural connectivity between the striatum and the thalamus in PD-related anxiety (6,11,24). MDT atrophy in PD could alter the connection between the thalamus and the PFC, the anterior cingular cortex and the orbitofrontal cortex leading to loss of function in the anxiety-related circuits. This could alter the cognitive control of emotion within the PFC and the limbic structures. Furthermore, it could inappropriately activate the emotional/fear processes, i.e. the fear circuit, leading to anxiety symptoms.

Mediodorsal and Anteroventral thalamus: interfaces between the anxiety-related circuits

As mentioned before, the AVT is involved in the fear circuit while the MDT is part of both the fear and the limbic circuits. These nuclei are interconnected highlighting again that these two anxiety-related circuits overlap (27). A previous study suggested that the functional interaction between the MDT dopaminergic receptors and the PFC glutamatergic (NMDA) receptors may be partially involved in anxiogenic-like behaviours in rats (45). We mentioned before that MDT atrophy occurred in PD and in anxiety disorders while AVT atrophy was mainly found in anxiety disorders. We speculate that neuronal loss due to PD would alter the cortico-striatal circuits by inducing atrophy in their structures. It could lead to MDT atrophy and then alteration and dysfunction of the fear circuit. This could promote AVT atrophy and worsen the dysfunction in the fear circuit resulting in anxiety symptoms. Therefore, these shared structures in the pathophysiology of both PD and anxiety disorders could explain the high prevalence of PD-related anxiety (Figure 3). However, the involvement of other structures such as the striatum, the ventral tegmental area or the brainstem nuclei may also be essential and still need to be explored. Further studies are needed to confirm this hypothesis.

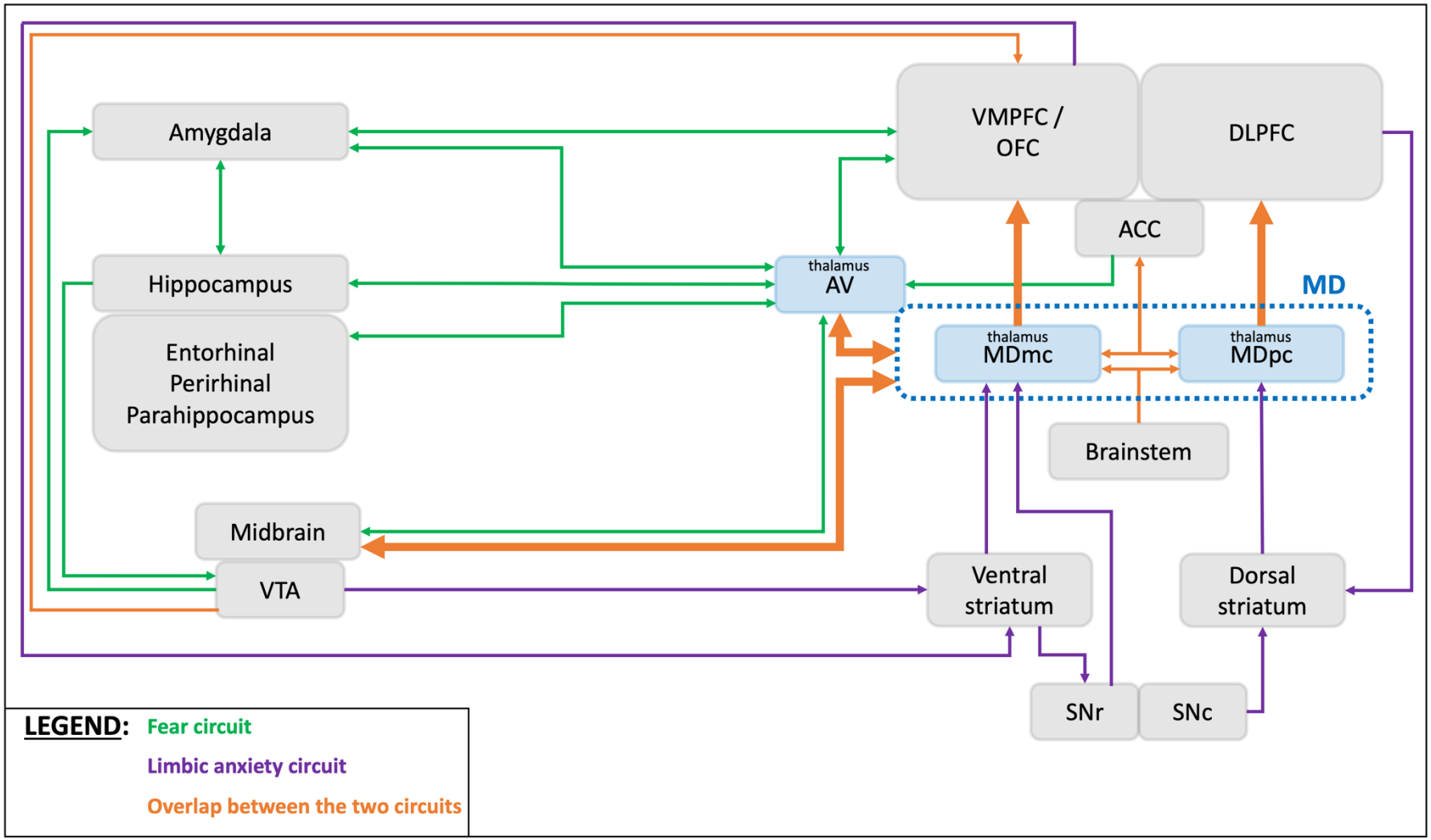

Figure 3. Schematic representation showing the structural connectivity of the anteroventral thalamus (AV), mediodorsal magnocellular thalamus (MDmc) and the mediodorsal parvocellular thalamus (MDpc), adapted from Pergola et al. and Nelson (36,46)**.**

Abbreviation: ACC = anterior cingular cortex; DLPFC = dorsolateral prefrontal cortex; MD = mediodorsal thalamus; OFC = orbitofrontal cortex; SNc = SNr = substancia nigra pars compacta; SNr = substancia nigra pars reticulata; VMPFC = ventromedial prefrontal cortex; VTA = ventral tegmental area.

Strength and Limitations

Strengths of this study are the large sample of participants with both PD patients and healthy controls, all scanned with high-field 7-Tesla MRI. So far, no study has used high-field MRI to study anxiety in PD with this number of well-characterized patients. There are however also some limitations. We used a probabilistic and automated atlas to segment the thalamic subnuclei that is less accurate compared to manual segmentation. However, all segmentations were visually inspected, and we followed common methods validated in other studies (21). Moreover, in this study, we only focused on the volume of the thalamus and thalamic subnuclei/subregions. It would be interesting to include other methods such as functional MRI, diffusion tensor imaging or iron content, and other structures such as striatum, ventral tegmental area or brainstem nuclei. Finally, we only focused on cross-sectional data while TRACK-PD is a longitudinal cohort, with an ongoing follow-up. Further studies are needed to better understand the progression of anxiety symptoms with imaging features and to determine risk factor for triggering or worsening anxiety in PD.

Conclusion

In PD patients with anxiety, a higher severity of anxiety was associated with a smaller volume of the left AVT and the right MD, specifically the right magnocellular MDT and the right parvocellular MD. Specific thalamic alteration in PD could promote the imbalance between the anxiety-related circuits potentially explaining the high prevalence of PD-related anxiety. Further studies are necessary to understand the role of other structures such as brainstem nuclei.

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CHAPTER 8

General Discussion

During this thesis, we used a multimodal MRI approach and ultra-high-field (7-Tesla) MRI to better understand the pathophysiology of Parkinson's disease (PD) related anxiety and to decipher the mechanisms by which CBT is effective in reducing anxiety symptoms in PD. We found that PD-related anxiety may result from an imbalance between the fear circuit and the limbic cortico-striato-thalamo-cortical circuit. The changes in both circuits could explain the high prevalence of anxiety in PD. Indeed, on the one hand, because of the hypodopaminergic state due to PD-related brain lesions and neuronal degeneration, the limbic circuit is underactivated leading to reduced cognitive control of emotions. On the other hand, the fear circuit is overactivated leading to inappropriate fear reaction even for slight stimuli. Moreover, these two circuits show overlap since the striatum, thalamus, and brainstem nuclei (i.e. ventral tegmental area, locus coeruleus, subthalamic nucleus, etc) are part of both circuits. Alterations in these overlapping structures could play a significant role in the imbalance between the two circuits. They are probably key structures to decipher the interactions between both circuits, and to determine whether alteration in one circuit may drive alterations in the other. For example, PD-related changes in the limbic circuit could enhance dysfunction of the fear circuit and explain why anxiety is so frequent in PD. Furthermore, cognitive behavioural therapy (CBT) is effective in reducing anxiety symptoms in PD patients (1). In this thesis, we found that CBT induces functional changes in anxiety-related circuits. CBT increases functional connectivity between the frontal cortex and the striatum and parietal cortex, thus strengthening cognitive control over anxiety symptoms and restoring the imbalance between the two circuits (Chapter 6). This finding reinforces our hypothesis of an imbalance between the fear circuit and the limbic circuit at the origin of PD-related anxiety. So far, this work was the first to specifically study the mechanisms of anxiety in PD using multimodal neuroimaging. Some of our results were in line with the literature and some were new findings.

Changes in the fear circuit have been reported in anxiety disorders in general, i.e. in patients not suffering from PD. The fear circuit is reported to be overactivated, leading to increased somatic and psychological fear reactions (2–4). Several imaging studies confirmed structural and functional alteration of parts of this circuit, notably the amygdala, hippocampus, medial PFC, OFC, and temporal cortex, in patients with anxiety disorders in the general population (5–7). The amygdala is commonly considered the key structure in anxiety disorders (8,9). In our study, we found slight structural and functional alterations of the amygdala, but this did not appear as central in the occurrence of PD-related anxiety. Moreover, we also found structural and functional changes in the striatum, the thalamus, the dorsolateral PFC, and the ACC associated with PD-related anxiety. These structures are part of the limbic circuit which could be underactivated in anxious PD patients leading to less cognitive control of emotions and enhancing the inappropriate activity of the fear circuit. Alterations of the limbic circuit were not reported in previous studies on anxiety disorders, but they were reported in

other neuropsychiatric disorders such as obsessive-compulsive disorders or Tourette syndrome (10,11). In PD, dysfunction of the cortico-striato-thalamo-cortical circuits is commonly considered as responsible of the motor symptoms. Namely, alterations in the limbic circuit are associated with neuropsychiatric symptoms in general but have never been specifically associated with PD-related anxiety (12,13). Our work is the first to describe such an association. In a previous review, *Thobois et al.* already found structural and metabolic changes in the OFC, PFC, ACC, amygdala, and thalamus associated with depression and anxiety in PD (14). In a systematic review on neuroimaging of PD-related anxiety, *Perepezko et al.* found similar results as ours and also assumed an imbalance between the fear and the limbic circuits (15). In early untreated PD patients with anxiety, *de Micco et al.* found functional imbalance between the common functional networks leading to less cognitive control of emotions (16). Therefore, our findings are in line with previous works, and the imbalance hypothesis we suggested has been reinforced by recent studies. However, some questions remain unsolved in understanding the link between these two circuits and further analysis are needed.

In this work, we mainly focused on cortical and subcortical structures, but some were not included in the analyses. Similar as our analysis regarding the thalamus (in Chapter 7), it could be interesting to also analyse the subnuclei of the amygdala and the BNST to specifically explore the extended amygdala. Moreover, we did not analyse the nucleus accumbens, an important structure of the striatum. Semi-automatic segmentation methods already exist, and 7-Tesla high field imaging could make it possible to identify the amygdala subnuclei (17). Using 7-Tesla MRI, it is also possible to manually segmentate the BNST and the nucleus accumbens (18–20). To understand the interactions between the two circuits, focusing on the overlapping structures is also necessary. We started with the thalamus (see Chapter 7) but other structures need to be examined such as the brainstem nuclei. In Chapter 1 and Chapter 7 we mentioned that the ventral tegmental area, the substantia nigra and the locus cœruleus/subcœruleus may be involved in the pathophysiology of fear and anxiety through their connections with the amygdala and play a role in PD-related anxiety (12,21–23). These structures are involved in both the fear circuit and the limbic circuit (2,12). Therefore, identifying their alteration could be essential to understand the link between the two circuits and their role in PD-related anxiety. The small volume of these structures was a limitation during this work and made it impossible to segmentate them using current anatomical sequences. High-field 7-Tesla MRI and new imaging sequences could make it possible to identify neuromelanin-related signal changes in catecholaminergic nuclei such as the locus coeruleus, ventral tegmental area and substantia nigra and help to identify and analyse them. Changes in neuromelanin signal have been considered as a promising MRI biomarker in PD (24–27). This has never been explored in PD-related anxiety and could help to better understand the involvement of these brainstem nuclei in its occurrence. Susceptibility-weighted T2 sequences, such as transverse

relaxation rate (R2*) and quantitative susceptibility mapping (QSM), have been used to identify iron deposition in brain which is considered as a potential marker of neuronal degeneration in PD (28–30). These sequences could help to identify neuronal degeneration in subcortical or brainstem structures even at early stage of the disease and provide biomarker of anxiety symptoms in PD. Finally, as explained in Chapter 1, hormonal system and hypothalamic-pituitary-adrenal axis alteration also play a role in anxiety disorders (31–34). Yet, some studies suggested that the hypothalamic-pituitary-adrenal axis is dysregulated in neurodegenerative diseases such as PD (35–37). Notably, Lewy bodies were found in the hypothalamus of PD patients (38). One study reported that psychological stress and changes in hypothalamic-pituitary-adrenal axis were present in early untreated PD patients (39). Further studies are needed to understand if and how hypothalamic-pituitary-adrenal axis alteration could play a role in the mechanisms of PD-related anxiety.

Another limitation to overcome is the heterogeneity of manifestations of both PD and anxiety disorders. As mentioned in Chapter 1, there are different subtypes of anxiety disorders. The high variability of symptoms and imaging results was already discussed and made difficult to decipher underlying mechanisms of anxiety disorders in previous reviews (7). Symptoms of PD also differ a lot across the patients and different motor and non-motor subtypes have been described. However, there is currently no consensus on a subtyping system (40). We also mentioned that not otherwise specified anxiety can specifically occur in PD patients, underlining the need to precisely identify anxiety symptoms in PD. In Chapter 2, we reported significant clusters of atrophy in the fronto-cingulate and parietal cortex as well as increased functional connectivity between the temporal cortex and amygdala in episodic anxiety and avoidance behaviour but not in persistent anxiety (41). We also found that CBT may reduce anxiety symptoms especially in episodic anxiety and avoidance behaviour rather than in persistent anxiety (1). Finally, in a recent study, *Dissanayaka et al.* showed the specific characteristics of atypical anxiety in PD such as not otherwise specified anxiety, fear of falling, fluctuating anxiety symptoms. Anxiety symptoms in PD are thus highly variable and future work need to consider this variability.

In our results, the asymmetry of imaging findings was surprising. Indeed, we mainly found reduced cortical thickness in the left frontal, temporal and parietal cortices (Chapter 2), structural changes in the left amygdala (Chapter 2), changes in the structural connectivity between the left nucleus accumbens and the left medial OFC, the left nucleus accumbens and the left anterior cingulate (ACC), the left amygdala and the left ACC, the left striatum and the left medial OFC (Chapter 4) and functional changes of the connectivity between the left insula, left amygdala, left caudate, left PFC and left OFC (Chapters 2, 5, 6). In addition, the results involving the thalamus were lateralized on the right side such

as the reduced right mediodorsal thalamic subnuclei volume (Chapter 7), changes in the structural connectivity between the right thalamus and the right striatum (Chapter 4) and in the functional connectivity between the right thalamus and bilateral OFC (Chapter 6). These results were not associated with the patient hand preference, the side of disease onset nor the most symptomatic side. Some authors hypothesized that the side of disease onset could predict some non-motor symptoms (42–44) but these results are inconsistent. Some authors reported that anxiety symptoms worsen more over time in patients with predominantly left-sided motor symptoms (42), while the same team reported that right-sided motor symptoms are a risk factor for more severe neuropsychiatric symptoms following deep brain stimulation of the subthalamic nuclei, suggesting a higher left hemispheric vulnerability (44). A recent longitudinal study suggested that early untreated left hemibody PD patients did not significantly differ from those with right hemibody PD regarding avoidance behaviours, apathy, anxiety and depression (43). Therefore, this issue remains unsolved. However, an alternative hypothesis could come from the new brain-first body-first pathophysiology concept of PD recently suggested by *Borghammer et al* (45). According to this concept, Lewy body diseases (e.g. PD or Lewy body dementia) could be classified in body-first or brain-first subtypes. The body-first subtype would start with Lewy body deposition in the enteric neuronal system before propagating to the brainstem which may be associated with specific premotor symptoms such as REM-sleep disorders or dysautonomic failure. Then, the Lewy bodies would spread to the brain in a bilateral way leading to bilateral PD symptoms, bilateral brain lesions and a more severe phenotype with faster cognitive symptoms progression. The brain-first subtype would start with Lewy bodies deposition in the olfactory bulb, amygdala and brainstem and may be less or not associated with premotor symptoms. Then, the Lewy bodies would spread to the brain unilaterally leading to unilateral PD symptoms and a slower progression of the disease. We suggest that the unilateral results in our studies could reflect this concept. We state that anxiety and neuropsychiatric symptoms could occur early in the course of the brain-first subtype PD and explain the asymmetric brain changes in PD-related anxiety. Further studies are needed to explore this new hypothesis and to determine whether anxiety symptoms could help to predict PD progression.

Prediction of onset or progression of PD is a research topic of high importance. Identifying predictive factor such as demographical, clinical, behavioural, and cognitive features, biological markers or imaging characteristics would help to identify prodromal PD-patients and their subtype of progression, which may in the future allow treatment with a disease-modifying medication and thus slow the progression (46). Anxiety disorders or changes in pre-existing anxiety frequently occur before PD onset or at early stage of the disease (14,47,48). Research criteria for prodromal PD include depression and anxiety as a “prodromal marker” with a median positive likelihood ratio of 1.6 and a median negative

likelihood ratio of 0.88 (49,50). Therefore, a better understanding of PD-related anxiety mechanisms could help to understand how and if anxiety is really a prodromal marker of PD. In association with other prodromal symptoms such as REM-sleep disorders, dysautonomia, depression, apathy or cognitive decline, our imaging findings could help to identify new biomarkers in this purpose. Further longitudinal studies on prodromal PD patients are needed.

In conclusion, we found evidence that PD-related anxiety results from an imbalance between the anxiety circuits. The fear circuit, known to be involved in fear processing, is overactivated in PD-related anxiety; while the limbic cortico-striato-thalamo-cortical circuit, known to be altered in PD, is underactivated. Moreover, we provided a biological hypothesis for the working mechanism of CBT for anxiety in PD. By increasing the activity of the limbic circuit, CBT could provide more cortical control over the fear circuit, reducing the imbalance between these circuits and improving anxiety symptoms. We also started to show alterations in the overlapping structures between these two circuits, opening an avenue to explore how both circuits interact and determine if this interaction could explain the high prevalence of anxiety symptoms in PD patients. However, some issues remain unsolved. New conceptual approaches, such as the brain/body first concept, and technological advances, such as high field 7-Tesla MRI, will be helpful for meeting these challenges. Therefore, a better understanding of the pathophysiology of PD-related anxiety would lead to better monitoring and managing these symptoms in order to improve the quality of life of PD-patients.

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CHAPTER 9

Summary

Anxiety is a frequent and debilitating non-motor symptom in Parkinson's disease (PD) with a high point prevalence of 31%. It is associated with increased motor symptoms and reduced quality of life of patients with PD. Therefore, understanding and managing this symptom is of great importance for patients, clinicians, and researchers. So far, the underlying mechanisms of PD-related anxiety are largely unknown. During this thesis, we used a multimodal MRI approach and ultra-high-field (7-Tesla) MRI to better understand the pathophysiology of PD-related anxiety and to decipher the mechanisms by which cognitive behavioural therapy (CBT) is effective in reducing anxiety symptoms in PD.

Chapter 1 introduces the background of the thesis by defining anxiety, anxiety disorders and PD. The current concepts about the pathophysiology of anxiety disorders in the general population and the avenues for research on PD-related anxiety are presented. Our objectives and hypotheses are stated at the end of this introduction.

Chapter 2 is the first study of this thesis that launched our work on anxiety in Parkinson's disease (PD). Based on literature showing that the severity of anxiety in PD is correlated with the volume of the amygdala, we assumed that the volume of the amygdala could be reduced in PD patients with anxiety compared to PD patients without anxiety. By consequence, we decided to investigate PD-related changes in the amygdala and the fear circuit. Using the dataset of a cross-sectional observational bi-centre (Lille and Maastricht) cohort of non-demented PD patients (CogPhenoPark2), we compared the demographic, clinical and cognitive characteristics of PD patients with (n= 34) and without (n=84) clinically significant anxiety, according to their score at the Parkinson Anxiety Scale (PAS). We compared structural imaging data such as cortical thickness and volume, shape and texture of amygdala and functional connectivity (FC) of amygdala between the two groups using ANOVA and generalized linear models. Compared with non-anxious PD patients, we found changes in the shape and texture of the left amygdala in anxious PD patients, suggesting remodelling or local atrophy. However, there was no significant difference in the volume of the amygdala. We also found a positive correlation between the severity of anxiety symptoms (total score at the PAS) and the FC between the amygdala and the parahippocampal cortex, as well as an increased FC within the salience network in anxious PD patients. Our results suggested that PD-related anxiety was associated with structural and functional changes in the fear circuit.

In **Chapter 3**, we conducted a systematic review of studies focusing on neuroimaging of anxiety in PD with the aim to generate new hypotheses and increase our knowledge of the underlying mechanisms of PD-related anxiety. Studies assessing anxiety symptoms in PD patients and using magnetic resonance imaging (MRI), positron emission tomography (PET) or single-photon emission tomography (SPECT)

were included. We followed the PRISMA guidelines to conduct this review. Eighteen studies met the inclusion criteria and were included in this systematic review: four structural MRI studies, four functional MRI studies, eight neurotransmitters/transporters imaging studies and two metabolic imaging studies. The severity of anxiety was associated with changes in the fear circuit and the cortico-striato-thalamo-cortical limbic circuit. In the fear circuit, a reduced volume of the amygdala and the anterior cingulate cortex (ACC), an increased FC between the amygdala and orbitofrontal cortex (OFC) and hippocampus, between the striatum and the medial prefrontal cortex (PFC) temporal cortex and insula, as well as a reduced FC between the lateral PFC and the OFC, hippocampus and amygdala were reported. In the cortico-striato-thalamo-cortical limbic circuit, a reduced FC was reported between the striatum and ACC, a reduced dopaminergic and noradrenergic activity in striatum, thalamus, and locus coeruleus, and a reduced serotoninergic activity in the thalamus. These findings reinforced the role of the fear circuit alterations in PD-related anxiety but also showed the involvement of the limbic cortico-striato-thalamo-cortical circuit, so called the limbic circuit in this thesis. We suggested there could be an imbalance between these two overlapping circuits at the origin of PD-related anxiety, with relative overactivity of the fear circuit in relation to the limbic anxiety circuit. This was our main hypothesis along this work. In the subsequent chapters, we aimed to confirm and validate this hypothesis.

Chapter 4 is a comparative study from the same cross-sectional observational cohort as in Chapter 2 (CogPhenoPark2). In this study, we aimed to identify if PD-related anxiety was associated with changes in structural connectivity between the fear circuit and the limbic circuit. Using diffusion tensor imaging (DTI), we compared connectivity parameters, such as fractional anisotropy (FA) and mean diffusivity (MD), in PD patients with (n=31) and without (n=77) clinically significant anxiety using ANOVA models. We also performed regression analyses between these parameters and anxiety severity as measured with the PAS-total score. Within the limbic circuit we found that PD-related anxiety was associated with a reduced FA between the orbito-frontal cortex and the striatum. We also found both reduced and increased FA within the fear circuit for instance between the insula and the amygdala and the anterior cingulate cortex, accumbens nucleus and thalamus, respectively. These changes could correspond to microstructural alterations within the two circuits. These results reinforced our main hypothesis.

Chapter 5 is also an ancillary study of the observational cohort "CogPhenoPark2". In this study, we used another approach for assessing brain FC - functional electroencephalography (EEG) analysis - to study the implication of dysfunctions of these circuits. We compared the EEG power spectrum and FC characteristics in PD patients with (n=33) and without (n=75) clinically significant anxiety according to the PAS-total score. The spectral analysis revealed that the relative power in the alpha1 frequency band in the right prefrontal cortex was lower in patients with anxiety than without. FC analysis showed a

stronger connectivity between the left insula and several fronto-cingulate and temporal regions in anxious PD-patients. This suggests an important role of these structures, which are involved in the fear circuit, in the underlying mechanisms of PD- related anxiety. These results reinforced the role of functional changes within the fronto-limbic pathways, such as the fear circuit, in PD-related anxiety.

In **Chapter 6**, we conducted an ancillary study to the randomized, controlled clinical trial previously published by *Moonen et al*., demonstrating the efficacy of cognitive behavioural therapy (CBT) to reduce anxiety symptoms in PD. In this study, we aimed to decipher the mechanisms by which CBT improves anxiety symptoms in PD patients, using functional MRI. We compared changes in FC between anxious PD patients treated by CBT (n= 17) or having clinical monitoring only (CMO) (n= 18) across the time between the baseline and the post-intervention (10-12 weeks) sessions. We performed repeated mixed ANOVA models. Anxiety severity was assessed by the PAS total score. Compared to CMO, CBT reduced the FC between the right thalamus and the bilateral orbitofrontal cortices and increased FC between the striatum and the PFC. CBT also increased FC between the PFC and the parietal cortex within the central executive network (CEN) and between the CEN and the salience network. After CBT, improvement of PAS total score was associated with an increased FC between the striatum and the ACC and the parietal and the temporal cortices as well as a decreased FC within the default-mode network and between the dorsal attentional network and the language network. We concluded that functional changes between the structures involved in the fear circuit and the limbic circuit and within the resting state functional networks are induced by CBT and are associated with improvement of anxiety. These results strongly reinforced our hypothesis of an imbalance between the fear and the limbic circuits at the origin of PD-related anxiety. These also highlight that a non-pharmacological therapy, such as CBT, can induce functional changes in the brain able to relieve anxiety symptoms in PD.

In **the Chapter 7** we worked on the TRACK-PD cohort dataset for the first time in this thesis. TRACK-PD is a longitudinal, on-going, observational study including PD patients without dementia (n=105) and healthy controls (n=45). All patients underwent a 7-T MRI scan and clinical evaluation at baseline, 2-year and 4-year follow-up. We worked on the baseline dataset in order to challenge our working hypotheses. In the present study, we focused on one of the overlapping structures of the two circuits, the thalamus. We compared the volume of the thalamus and thalamic subregions in PD patients with (n= 31) and without (n=74) clinically significant anxiety and healthy controls (n=45) using ANOVA models. We explored the links between the severity of anxiety according to the PAS total score and the volume of thalamus and thalamic subregions by way of regression analyses. We found that a smaller volume of the anterior and the mediodorsal regions of the thalamus was associated with a higher

severity of anxiety in PD. These regions are known to be involved in cognitive and emotional processes and were already associated with anxiety in the general population. Both are also involved in the two anxiety-related circuits. Therefore, these findings reinforced our hypothesis that lesions in overlapping structures of the anxiety-related circuits could lead to anxiety symptoms in PD patients.

Finally, in **the Chapter 8**, we discuss our overall results and issues that remain unsolved. For example, it was not possible to analyse some structures such as the subnuclei of the amygdala, the nucleus accumbens or some brainstem nuclei (i.e. ventral tegmental area, substantia nigra, locus cœruleus). We also discuss the asymmetry of the results in our studies and how our results could help to identify biomarkers of progression or onset of PD. Further studies are needed to answer these remaining questions.

CHAPTER 10

Résumé

(Short abstract- French version)

L’anxiété dans la maladie de Parkinson (MP) est un symptôme non-moteur fréquent et invalidant dont la prise en charge est difficile. La faible connaissance des mécanismes impliqués est une limite à sa prise en charge. L’objectif de ce travail était d’identifier les mécanismes sous-jacents de l'anxiété liée à la MP, via une approche IRM cérébrale multimodale.

Une revue systématique de la littérature portant sur les données d’imagerie dans l’anxiété liée à la MP a d’abord été réalisée, permettant de générer de premières hypothèses. Ensuite, plusieurs études incluant des analyses en IRM cérébrale structurale et fonctionnelle ont été menées chez des patients atteints de MP et présentant ou non une anxiété cliniquement significative. Nos analyses se sont focalisées sur le circuit de la peur, connu pour être impliqué dans les troubles anxieux, et le circuit cortico-striato-thalamo-cortical limbique, connu pour son implication dans les symptômes psycho-comportementaux de la MP.

Nos résultats suggèrent que l’anxiété liée à la MP serait la conséquence d’un déséquilibre fonctionnel et structural entre ces deux circuits. Certaines structures communes, comme le thalamus, le striatum ou les noyaux du tronc cérébral, pourraient être des zones clés dont l’altération pourrait expliquer la forte prévalence de ces troubles dans la MP. D’autres travaux s'appuyant notamment sur les avancées technologiques en imagerie et sur de nouveaux concepts concernant la physiopathologie de la MP, seront nécessaires pour répondre à ces questions.

CHAPTER 11

Résumé substantiel

(Summary- French version)

La maladie de Parkinson (MP) est une maladie neurodégénérative fréquente, invalidante et caractérisée par une triade de signes moteurs, la bradykinésie, la rigidité et le tremblement de repos. Elle est également caractérisée par des signes non-moteurs, parmi lesquels on retrouve notamment des troubles du sommeil, des troubles dysautonomiques, des troubles cognitifs et des troubles psychocomportementaux tels que l'apathie, la dépression, l'anxiété, les hallucinations ou les idées délirantes. L'anxiété dans la MP est un symptôme non-moteur fréquent avec une prévalence estimée à 31% des patients. Il s'agit également d'un symptôme invalidant, associé à une aggravation des signes moteurs et une altération de la qualité de vie des patients. Actuellement, la prise en charge de ces troubles est difficile, ce qui en fait un enjeu majeur pour les patients et les cliniciens. Seule la thérapie cognitive et comportementale (TCC) a montré récemment une efficacité dans la réduction de ces troubles. Cependant, les mécanismes physiopathologiques de l'anxiété liée à la MP restent mal compris. Leur meilleure compréhension permettrait ainsi leur meilleure prise en charge. L'imagerie cérébrale et notamment l'imagerie par résonnance magnétique (IRM) permet l'analyse précise des différentes structures et circuits impliqués dans la physiopathologie des maladie neurodégénératives et des maladies psychiatriques. Ainsi, de nombreux travaux ont déjà été menés en IRM cérébrale sur la compréhension des mécanismes sous-jacents aux troubles anxieux dans la population générale ou des mécanismes impliqués dans la MP. Cependant, peu de travaux se sont spécifiquement intéressés aux mécanismes de l'anxiété dans la MP. Jusqu'à présent, les études réalisées sur le sujet évaluaient l'anxiété de manière non spécifique sans caractériser l'anxiété cliniquement significative ou en confondant les mécanismes de l'anxiété avec ceux de la dépression.
Le principal objectif de ce travail de thèse était de mieux caractériser les mécanismes impliqués dans l'anxiété liée à la MP, en utilisant une approche multimodale en IRM à l'aide de séquences anatomiques, en tenseur de diffusion (DTI) ou fonctionnelles de repos. Un second objectif était de décrypter les mécanismes par lesquels la TCC est efficace pour réduire les symptômes d'anxiété dans la MP à l'aide d'analyses en IRM fonctionnelle de repos. Enfin, un troisième objectif était de confirmer nos résultats précédents à l'aide de techniques d'imagerie de très haut champs magnétique (IRM 7-Tesla).

Notre hypothèse initiale était que l'altération des amygdales cérébrales pourrait être à l'origine de l'anxiété liée à la MP, à l'instar de travaux précédents décrivant une association entre la sévérité des symptômes anxieux dans la MP et l'atrophie de l'amygdale gauche. Notre première étude incluait des patients atteints de MP avec ou sans anxiété cliniquement significative et bénéficiant d'une IRM cérébrale 3-Tesla. Cette étude a permis d'identifier des altérations anatomiques et fonctionnelles au sein de l'amygdale cérébrale mais également de manière plus large au sein de régions corticales préfrontales, cingulaires et pariétales. Ces différentes régions font partie d'un circuit neuronal plus

large, centré sur l'amygdale et connu pour être impliqué dans la physiopathologie des troubles anxieux en population générale, le circuit de la peur. Ce circuit est donc connu pour intervenir dans le traitement des émotions et notamment de la peur face à des stimuli anxiogènes. Ainsi, nous concluions que l'anxiété dans la MP était associée à des modifications au sein du circuit de la peur.

Nous avons ensuite mené une revue systématique de la littérature portant sur les travaux de neuroimagerie, IRM cérébrale ou imagerie métabolique (TEP, SPECT ou DAT-scan), dans l'anxiété liés à la MP. Sur la base des 18 études inclues dans cette revue, nous confirmions l'atteinte des structures participant au circuit de la peur mais mettions également en évidence des atteintes anatomiques et fonctionnelles au sein de la boucle limbique du circuit cortico-striato-thalamo-cortical, connu pour être impliqué dans le contrôle cognitif des émotions et dans la survenue des troubles psychocomportementaux liés à la MP. Ainsi, les données de cette revue systématique permettaient de formuler l'hypothèse d'un déséquilibre anatomique et fonctionnel entre ces deux réseaux à l'origine des troubles anxieux liés à la MP. Il existerait ainsi une hyperactivation du circuit de la peur, entrainant une réaction anxieuse importante même face à des stimuli faiblement menaçant, et une hypoactivation du circuit limbique, liée aux lésions cérébrales entraînées par la MP, à l'origine d'un plus faible contrôle cognitif de la peur. Cette l'hypothèse principale est le fondement de l'ensemble de cette thèse. Les études ultérieures que nous avons menées, ont cherché à confirmer et renforcer cette hypothèse.

Nous avons ainsi cherché à identifier des modifications de connectivité anatomique entre les différentes structures impliquées dans le circuit de la peur et le circuit limbique chez les patients atteints de MP anxieux. A l'aide de séquences DTI en IRM, nous avons pu comparer les données de connectivité anatomique, c'est à dire la fraction d'anisotropie (FA) et la diffusivité moyenne (MD), au sein de ces circuits entre des patients atteints de MP avec ou sans anxiété cliniquement significative. Cette étude a mis en évidence des modifications de FA et de MD au sein des deux circuits. On retrouvait une diminution de la FA au sein du circuit limbique, notamment entre le cortex fronto-orbitaire et le striatum et des modifications de FA au sein du circuit de la peur, notamment une diminution de FA entre le cortex insulaire et l'amygdale et une augmentation entre le cortex cingulaire antérieur et le noyau accumbens. Ces résultats rendaient compte d'altération microstructurales et renforçaient l'hypothèse d'un déséquilibre entre ces deux circuits dans l'anxiété liée à la MP.

Ensuite, nous avons mené une étude sur des données de connectivité fonctionnelle à partir de d'enregistrement d'électroencéphalographie de repos. Ici encore, nous avons comparé la puissance du signal (analyse spectrale) et la connectivité fonctionnelle chez des patients atteints de MP avec ou sans anxiété cliniquement significative. Cette étude a permis de mettre en évidence une réduction de la puissance relative dans la bande de fréquence alpha 1 au sein du cortex préfrontal et une connectivité fonctionnelle accrue entre le cortex insulaire gauche et plusieurs régions corticales fronto-cingulaires et temporales chez les anxieux comparativement aux non anxieux. Ces structures sont impliquées dans

le circuit de la peur et ces résultats renforçaient donc l'hypothèse de l'implication de ce circuit dans les mécanismes de l'anxiété liée à la MP.

Dans l'étude suivante, nous avons cherché à comprendre si la réduction des symptômes anxieux entrainée par la TCC était associée à des modifications de connectivité fonctionnelle au sein ou entre les deux circuits mentionnés. Ainsi, nous avons réalisé une étude ancillaire à l'essai clinique contrôlé et randomisé qui a révélé l'efficacité de la TCC sur la réduction des symptômes anxieux dans la MP. Des patients atteints de MP et d'anxiété cliniquement significative étaient inclus et randomisés dans deux groupes : 1) TCC et suivi habituel ou 2) suivi habituel uniquement. Chaque patient avait bénéficié d'une IRM cérébrale 3-Tesla avant puis 10 à 12 semaines après l'intervention. Les modifications en IRM fonctionnelle de repos au sein des circuits de la peur et limbique, en fonction du temps, ont été comparées entre le groupe TCC et le groupe contrôle. Nous avons mis en évidence des modifications fonctionnelles après TCC, en lien avec l'amélioration des symptômes anxieux, notamment entre les structures impliquées dans les circuits de la peur et limbique ainsi qu'au sein des réseaux fonctionnels de repos. Ces résultats suggèrent que la TCC pourrait améliorer les symptômes anxieux dans la MP en réduisant le déséquilibre fonctionnel entre les circuits de la peur et limbique. A nouveau, ces résultats renforçaient notre hypothèse principale.

Enfin, la dernière étude présentée dans ce travail de thèse se base sur les données de la cohorte TRACK-PD, une cohorte longitudinale incluant des patients atteints de MP et des contrôles sains, bénéficiant d'évaluations cliniques, cognitives et comportementales ainsi que d'IRM cérébrales de très haut champs magnétique (7-Tesla) à l'inclusion, à 2 ans et à 4 ans de suivi. Nos études précédentes nous ont permis d'identifier plusieurs structures communes au circuit de la peur et au circuit limbique : le thalamus, le striatum et les noyaux du tronc cérébral. Nous suggérons que l'exploration spécifique de ces structures nous permettrait de comprendre les interactions qui existent entre ces deux circuits et si ces interactions pourraient expliquer la forte prévalence de l'anxiété dans la MP. Dans cette étude, nous avons comparé le volume du thalamus et des noyaux thalamiques chez des patients atteints de MP avec anxiété et sans anxiété cliniquement significative et des contrôles sains. Nous avons mis en évidence que la sévérité de l'anxiété liée à la MP était associée à une réduction du volume du noyau dorsomédial et du noyau antérieur du thalamus. Ces deux régions sont connues pour être impliquées dans les deux circuits de l'anxiété. Leur altération dans l'anxiété liée à la MP confirme d'une part l'implication des deux réseaux mais permet de suggérer que l'interaction entre ces réseaux pourraient être altérée, ce qui favoriserait l'apparition de l'anxiété dans la MP.

En conclusion, ce travail de thèse a permis d'évoquer l'hypothèse d'un déséquilibre structural et fonctionnel entre le circuit de la peur et le circuit limbique comme mécanisme physiopathologique de l'anxiété liée à la MP. Cependant, l'analyse de certaines structures communes aux deux circuits reste à

poursuivre, notamment le striatum et les noyaux du tronc cérébral. Elle permettra de mieux appréhender les interactions entre ces circuits et leur rôle dans la survenue de l'anxiété liée à la MP. De plus, l'hétérogénéité des symptômes anxieux mais aussi des phénotypes de MP rend l'interprétation de ces mécanismes difficile. La recherche de mécanismes physiopathologiques distincts entre les différents types d'anxiété semble nécessaire. Enfin, la place des troubles anxieux comme symptômes prodromaux dans la MP reste débattue. Ainsi, des études sur des cohortes longitudinales permettront d'identifier si l'anxiété et certains marqueurs en imagerie seront capables de prédire soit le début soit le profil de progression de la MP sur la base de ces symptômes non moteurs.

CHAPTER 12

Samenvatting

(Dutch version)

Angst is een frequent en invaliderend niet-motorisch symptoom bij de ziekte van Parkinson (ZvP), met een hoge puntprevalentie van 31%. Angst is geassocieerd met ernstiger motorische symptomen en een verminderde kwaliteit van leven van patiënten met de ziekte van Parkinson. Een beter begrip en betere behandeling van deze dit symptoom is daarom van groot belang voor patiënten, artsen en onderzoekers. Tot nu toe zijn de onderliggende mechanismen van parkinsongerelateerde angst slechts gedeeltelijk bekend. Tijdens ons onderzoek hebben we een multimodale MRI benadering en ultra-high-field (7-Tesla) MRI gebruikt om de pathofysiologie van parkinsongerelateerde angst beter te begrijpen en om de mechanismen te achterhalen waarmee cognitieve gedragstherapie (CGT) effectief is in het verminderen van angstsymptomen bij de ZvP.

Hoofdstuk 1 introduceert de achtergrond van dit proefschrift door angst, angststoornissen en de ZvP te definiëren. De huidige opvattingen over de pathofysiologie van angststoornissen in het algemeen en de mogelijkheden voor onderzoek naar parkinsongerelateerde angst worden gepresenteerd. Onze doelstellingen en hypothesen vindt u aan het einde van deze inleiding.

Hoofdstuk 2 is de eerste studie van dit proefschrift waarin gefocust wordt op de rol van de amygdala. Gebaseerd op de literatuur gingen we ervan uit dat het volume van de amygdala verminderd zou kunnen zijn bij parkinsonpatiënten met angst. Met behulp van de dataset van een crosssectioneel observationeel bi-center (Lille en Maastricht) cohort van niet-dementerende parkinsonpatiënten (CogPhenoPark2) vergeleken we de demografische, klinische en cognitieve kenmerken van parkinsonpatiënten met (n= 34) en zonder (n=34). =84) klinisch relevante angst, als gemeten met de totaalscore op de *Parkinson Anxiety Scale* (PAS). We vergeleken structurele beeldgegevens zoals de dikte van de cortex en het volume, de vorm en textuur van de amygdala, alsook de functionele connectiviteit van de amygdala tussen de twee groepen met behulp van ANOVA en gegeneraliseerde lineaire modellen. Bij angstige parkinsonpatiënten vonden we veranderingen in de vorm en textuur van de linker amygdala, wat duidt op her modellering of lokale atrofie, maar niet in het volume vergeleken met niet-angstige parkinsonpatiënten. We vonden ook een positieve correlatie tussen de functionele connectiviteit tussen de amygdala en de parahippocampale cortex en de PAS-totaalscore en een verhoogde functionele connectiviteit binnen het saliencenetwerk bij angstige parkinsonpatiënten. Onze resultaten suggereerden dat parkinsongerelateerde angst geassocieerd is met structurele en functionele veranderingen in het angstcircuit.

In **Hoofdstuk 3** hebben we een systematische review uitgevoerd van neuroimaging onderzoeken van angst bij de ZvP om nieuwe hypothesen te genereren en onze kennis van de onderliggende mechanismen van parkinsongerelateerde angst te vergroten. Studies waarin angstsymptomen bij

parkinsonpatiënten werden onderzocht en waarbij gebruik werd gemaakt van magnetische resonantie imaging (MRI), positron emissie tomografie (PET) of single-photon emissie tomografie (SPECT) werden geïncludeerd. We hebben de PRISMA-richtlijnen gevolgd om deze beoordeling uit te voeren. Achttien onderzoeken voldeden aan de inclusiecriteria en werden opgenomen in deze systematische review: vier structurele MRI-onderzoeken, vier functionele MRI-onderzoeken, acht beeldvormende onderzoeken naar neurotransmitters en/of transporteiwitten en twee metabole beeldvormende onderzoeken. De ernst van de angst was geassocieerd met veranderingen in het 'fear' circuit en het cortico-striato-thalamo-corticale limbische circuit. In het angstcircuit is er sprake van een verminderd volume van de amygdala en de anterieure cingulaire cortex (ACC), een verhoogde functionele connectiviteit tussen de amygdala en orbitofrontale cortex (OFC) en de hippocampus, tussen het striatum en de mediale prefrontale cortex (PFC), temporale cortex en insula, en een verminderde functionele connectiviteit tussen de laterale PFC en de OFC, hippocampus en amygdala werden gerapporteerd. In het cortico-striato-thalamo-corticale limbische circuit werd een verminderde functionele connectiviteit gerapporteerd tussen het striatum en ACC, een verminderde dopaminerge en noradrenerge activiteit in striatum, de thalamus en de locus coeruleus, en een verminderde serotonerge activiteit in de thalamus. Deze bevindingen versterkten de rol van de veranderingen in het angstcircuit bij parkinsongerelateerde angst, maar tonen ook de betrokkenheid aan van het limbische cortico-striato-thalamo-corticale circuit. Wij suggereerden dat er een disbalans zou kunnen bestaan tussen deze twee overlappende circuits, met relatieve overactiviteit van het fear ciruit ten opzichte van het limbische angstcircuit die aan de basis liggen van parkinsongerelateerde angst. Dit was onze belangrijkste hypothese. In de navolgende hoofdstukken probeerden we deze hypothese te bevestigen en te valideren.

Hoofdstuk 4 is een vergelijkende studie uit hetzelfde cross-sectionele observationele cohort als in Hoofdstuk 2 (CogPhenoPark2). In deze studie wilden we onderzoeken of parkinsongerelateerde angst geassocieerd was met veranderingen in de structurele connectiviteit tussen het fear circuit en het limbische angstcircuit. Met behulp van een MRIC techniek die we 'diffusion tensor imaging' (DTI) noemen vergeleken we connectiviteitsparameters, zoals fractionele anisotrope (FA) en gemiddelde diffusiviteit (mean diffusity, MD), tussen parkinsonpatiënten met (n=31) en zonder (n=77) klinisch relevante angst met behulp van ANOVA. We hebben ook regressieanalyses uitgevoerd tussen deze parameters en de ernst van de angst als gemeten met de PAS-totaalscore. Binnen het limbische angstcircuit vonden we dat parkinsongerelateerde angst geassocieerd was met een verminderde FA tussen de orbitofrontale cortex en het striatum. Ook vinden we zowel verminderde als verhoogde FA binnen het angstcircuit, zoals tussen respectievelijk de insula en de amygdala en de voorste cingulaire

cortex, accumbens-nucleus en thalamus. Deze veranderingen kunnen overeenkomen met microstructurele veranderingen binnen de twee circuits.

Hoofdstuk 5 is ook een aanvullende studie van het observationele cohort “CogPhenoPark2”. In deze studie hebben we een andere benadering gebruikt voor het beoordelen van de functionele connectiviteit van de hersenen, namelijk functionele elektro-encefalografie (EEG) -analyse, om disfunctie van angstcircuits te onderzoeken. We vergeleken het EEG-powerspectrum en de functionele connectiviteitskenmerken bij parkinsonpatiënten met (n=33) en zonder (n=75) klinisch relevante angst. In de spectraalanalyse was de relatieve power in de alfa1-frequentieband in de rechter prefrontale cortex lager bij patiënten met angst dan bij patiënten zonder angst. Functionele connectiviteitsanalyse toonde een sterkere connectiviteit tussen de linker insula en verschillende fronto-cingulaire en temporale regio's. Dit bevestigt, een belangrijke rol van deze structuren bij angstregulatie en parkinsongerelateerde angst. Deze resultaten bevestigen de rol van functionele veranderingen binnen de fronto-limbische routes, zoals het angstcircuit, bij PD-gerelateerde angst.

In **Hoofdstuk 6** hebben we een analyse uitgevoerd op onze gerandomiseerde, gecontroleerde klinische studie die de effectiviteit van cognitieve gedragstherapie (CGT) bij het behandelen van angstsymptomen bij parkinsonpatiënten anntoont, eerder gepubliceerd door *Moonen et al.*. In deze studie wilden we met behulp van functionele MRI de mechanismen onderzoeken waarmee CGT de angstsymptomen bij parkinsonpatiënten verbetert. We vergeleken functionele connectiviteitsveranderingen bij angstige parkinsonpatiënten die werden behandeld met cognitieve gedragstherapie (n= 17) en die van patiënten die alleen klinische monitoring (n= 18) kregen voor en na de (10-12 weken). We hebben “repeated mixed measures ANOVA” uitgevoerd. De ernst van de angst werd beoordeeld aan de hand van de PAS-totaalscore. Bij patiënten die met CBT behandeld werden verminderde de functionele connectiviteit tussen de rechter thalamus en de bilaterale orbitofrontale cortex en verhoogde de functionele connectiviteit tussen het striatum en de PFC. CBT verhoogde ook de functionele connectiviteit tussen de PFC en de pariëtale cortex binnen het centrale executieve netwerk (CEN) en tussen de CEN en het salience-netwerk. Na CGT was verbetering van de PAS-totaalscore geassocieerd met een verhoogde functionele connectiviteit tussen het striatum en de ACC en de pariëtale en temporale cortex, en tevens was er een verminderde functionele connectiviteit binnen het default-mode netwerk en tussen het dorsale aandacht netwerk (dorsal attention network) en het taalnetwerk. We concludeerden dat CBT leidde tot veranderingen in functionele connectiviteit tussen de structuren die betrokken zijn bij het angstcircuit en het limbische circuit en binnen het default-mode network. Deze veranderingen waren geassocieerd met verbetering van de angstklachten,. Deze resultaten versterkten onze hypothese dat parkinsongerelateerde angst het

gevolg is van een disbalans tussen de angst en de limbische circuits. Ook laten onze bevindingen zien dat niet-farmacologische therapie, zoals CGT, eveneens functionele veranderingen in de hersenen kan veroorzaken die leiden tot verlichting van angstsymptomen bij de ZvP.

In **Hoofdstuk 7** hebben we met de TRACK-PD cohortdataset gewerkt. TRACK-PD is een longitudinaal, observationeel onderzoek bij parkinsonpatiënten zonder dementie (n=105) en gezonde controles (n=45). Alle patiënten ondergingen een 7-T MRI-scan en klinische evaluatie op baseline, en na 2 en 4 jaar follow-up. We hebben met de baseline data gewerkt om onze hypothese verder te onderzoeken. In deze analyse concentreerden we ons op een van de overlappende structuren van de twee angstcircuits, namelijk de thalamus. We vergeleken het volume van de thalamus en thalamische subregio's bij parkinsonpatiënten met (n= 31) en zonder (n=74) klinisch relevante angst- en gezonde controles (n=45) met behulp van ANOVA. We onderzochten de verbanden tussen de ernst van angst als gemeten met de PAS-totaalscore en het volume van de thalamus en thalamische subregio's door middel van regressieanalyses. We vonden dat een kleiner volume van de voorste en mediodorsale gebieden van de thalamus geassocieerd was met een hogere ernst van angst bij de ZvP. Het is bekend dat deze regio's betrokken zijn bij cognitieve en emotionele processen en werden eerder al in verband gebracht met angstklachten in de algemene bevolking. Beiden structuren zijn ook betrokken bij de twee angstgerelateerde circuits. Deze bevindingen zijn opnieuw een bevestiging van onze hypothese dat disfunctie in overlappende structuren van de angstgerelateerde circuits zou kunnen leiden tot angstsymptomen bij parkinsonpatiënten.

Ten slotte bespreken we in **hoofdstuk 8** de betekenis en impact van onze resultaten en enkele punten die momenteel nog onopgelost blijven. Zo was het om praktische redenen binnen de termijn van dit proefschrift niet mogelijk om een aantal andere structuren die betrokken zijn bij angstklachten te onderzoeken, zoals de subnuclei van de amygdala, de nucleus accumbens of sommige hersenstamkernen (bijvorobeeld het ventrale tegmentum, de substantia nigra en de locus cœruleus). We gaan ook in op de asymmetrie van onze resultaten, die overigens ook in ander onderzoek gerapporteerd worden en hoe onze resultaten kunnen helpen bij het identificeren van biomarkers voor het begin of progressie van de ziekte van Parkinson. Verder onderzoek is nodig om deze resterende vragen te beantwoorden.

CHAPTER 13

Impact & Valorisation

Better management of anxiety symptoms in patients with Parkinson's disease (PD) is a major issue. A large survey and an expert meeting report revealed that finding approaches to reduce stress and anxiety in people with PD was considered as the second most important priority, after reducing balance problems and falls, by patients, researchers, and clinicians (1,2). Our research has impact for the clinical management of anxiety in PD, as well as for research.

Clinical impact

We state that getting more insight in the pathophysiology of PD-related anxiety, could help to identify new or additional potential targets for treatment. In our studies we found that dysfunction of two neuronal circuits, i.e. the fear circuit and the limbic anxiety circuit, is involved in PD-related anxiety.

In a randomised clinical trial evaluating the effect of CBT added to clinical monitoring to reduce anxiety symptoms in PD, anxiety symptoms were significantly reduced after CBT. Interestingly, anxiety symptoms were also reduced after a clinical monitoring only (3). In the Chapter 6, we showed that CBT significantly reduced anxiety symptoms in PD by inducing functional connectivity changes such as reduced functional connectivity between the right thalamus and the bilateral orbitofrontal cortices and increased functional connectivity between the striatum and the prefrontal cortex (PFC), between the striatum and the anterior cingulate cortex (ACC) and between the parietal and the temporal cortices. The latter may be involved in increasing cognitive control over anxiety symptoms. Besides, slight changes in functional connectivity were also observed after clinical monitoring only, such as increased functional connectivity between the striatum and the ACC and reduced functional connectivity between the insula and the temporal cortex. Moreover, these findings suggest that even a simple monitoring of anxiety, including diagnosis, information on the symptoms and advice to manage them, is enough to induce brain changes. Here, we showed that non-pharmacological treatment can induce brain changes and must be considered to reduce PD-related anxiety.

Finally, as PD-related anxiety is a frequent and disabling non-motor symptoms reducing the quality of life, its early identification would improve its management. With our findings, we could identify MRI markers of PD-related anxiety in order to predict onset or progression of anxiety symptoms in PD. That could be useful to better monitor, prevent and treat PD-related anxiety.

Research impact

PD-related anxiety is a complex and heterogeneous non-motor symptom that involves many structures and pathways. In this thesis, we mainly focused on structural and functional imbalance between the fear circuit and the limbic anxiety circuit. We stated that the fear circuit is overactivated while the limbic circuit is underactivated. Using standard brain MRI methods, we gained better insight in the underlying

mechanisms of PD-related anxiety, but for a comprehensive view of the complete pathways and connections, other structures need to be considered as well. Moreover, it is necessary to investigate if clinical, cognitive, and psychiatric features or imaging markers are associated with the course of anxiety symptoms in PD and can help to predict their onset or progression. The use of high-field 7-Tesla brain MRI methods and longitudinal cohorts are necessary to answer these remaining questions. At Maastricht University, the TRACK-PD cohort, an ongoing longitudinal study including PD patients and healthy controls, may help tackling these issues. The participants undergo complete clinical assessments and 7-Tesla MRI scans at baseline, 2-year and 4-year follow-up (4).

In follow-up studies we will explore the role of brain structures that have been implied in anxiety, but have not been extensively studied before. We will be focusing on structures such as the subnuclei of the amygdala, the bed nucleus of stria terminalis (BNST), the nucleus accumbens. Using high-field 7-Tesla MRI with 3D-T1 weighted MPRAGE sequences, it will be possible to precisely segmentate these structures to compare their structural and functional characteristics in PD-related anxiety. Moreover, the TRACK-PD protocol involves neuromelanin and magnetic susceptibility (T2*) sequences. As mentioned earlier, the neuromelanin relative signal, considered as a novel biomarker in PD, will allow to study the signal of the locus cœruleus/subcœruleus, the ventral tegmental area and the substantia nigra. The magnetic susceptibility sequences are used to identify the iron deposition in cortical, subcortical and brainstem structures. It is considered a biomarker of neuronal degeneration and could provide new information about neuronal degeneration in these structures in PD-related anxiety. These different analyses are planned after the end of the PhD work (post-doctoral position of 6 months). As revealed by our systematic review, other avenues are also to be explored. Thus, we mentioned that hormonal dysfunction and alteration of the hypothalamic-pituitary-adrenal axis could be associated with neurodegenerative disorders and anxiety disorders. Therefore, studying these alterations in PD-related anxiety could be interesting to complete our knowledge of the pathophysiology of anxiety in PD and possibly find novel therapeutic targets to reduce anxiety symptoms in PD. So far, this has not been done yet. Manual and automatic segmentation of the hypothalamus and its subunits are possible using 7-Tesla MRI (5) and these analyses could help to complete our hypotheses of imbalance between the neuronal anxiety circuits with, maybe, imbalance in hormonal regulation of anxiety.

The next step is to better understand the evolution of anxiety in the course of the disease. So far, no predictive factor of PD-related anxiety onset or worsening has been identified. Using the longitudinal dataset of TRACK-PD, it will be possible to identify PD patients with improvement, worsening, stabilisation, or onset of clinically significant anxiety symptoms and to associate clinical, cognitive, behavioural and imaging predictive factors. All the baseline clinical and imaging data could be

associated with the evolution of anxiety symptoms at follow-up. This would help to better predict the evolution of these symptoms and eventually the progression of PD. The TRACK-PD study is still ongoing, and this kind of analyses could be done at the end of the follow-up. Apart from this, in this thesis we studied anxiety in PD patients with dopaminergic treatment after several years of diagnosis (+/- 5 years). Extending our work to early untreated PD patients may help to identify early MRI markers of PD-related anxiety progression. Therefore, we will work on the FAIR-PARK-2 cohort (6). This cohort includes early untreated PD patients at the baseline assessment. They underwent a 3-Tesla MRI scan that could be used to set the structural characteristics of the cortical and subcortical structures related to anxiety in PD at this stage of the disease. This work is ongoing as part of supervision of a Master's student in the University of Lille. Finally, as mentioned in the general discussion (Chapter 8), considering anxiety as a premotor symptom of PD is still debated. If it was the case, it is essential to determine how it could help to predict the onset and the progression of the disease. In France, the longitudinal cohort RDB-France includes patients diagnosed with idiopathic REM-sleep behavioural disorders undergoing clinical, psychological, and cognitive assessment every year until the conversion in Lewy bodies disease (i.e. PD or Lewy bodies dementia). More than 400 patients have already been included. Analysing these clinical data could make it possible to identify if anxiety symptoms could be a predictive factor of conversion in Lewy bodies disease in patients with REM-sleep behavioural disorders.

To conclude, when I started this research, the underlying mechanisms of PD-related anxiety were largely unclear. We stated the implication of an imbalance between two anxiety-related neuronal circuits. The fear circuit, involved in fear processing, is overactivated, while the limbic cortico-striato-thalamo-cortical circuit, involved in cognitive control of emotion and altered in PD, is underactivated. Our different studies reinforced this hypothesis and suggested that alteration of overlapping structures could be involved in the interaction between these neuronal circuits. The use of high field 7-Tesla MRI will provide new findings in understanding the alteration of these circuits. This work opens new avenues to better understand the underlying mechanisms of PD-related anxiety and that will hopefully lead to a better way of managing it.

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Curriculum Vitae

Guillaume Carey was born on July 24th, 1991, in Lesquin, France. In 2009, he succeeded his "Baccalauréat mention Très Bien" at Saint-Paul high-school in Lille, France. He started his medical studies at the School of Medicine of the Catholic University of Lille in 2010. He passed the national medical examen and started his residency and training in Neurology at the Lille University Medical Centre (CHU de Lille) in 2015. He started his research project on anxiety in Parkinson's disease and brain imaging under the supervision of Prof. Kathy Dujardin in 2016 and graduated his Master degree in 2018 at the University of Lille. Thereafter, he worked on "imaging and Gamma-Knife thalamotomy for essential tremor" for his medical thesis under the supervision of Dr. Nicolas Carrière at the University of Lille and graduated as a neurologist in October 2019. From November 2019 to April 2020, he experienced a full-time research stay in Maastricht University under the supervision of Prof. Albert Leentjens and Prof. Kathy Dujardin to prepare the PhD project. From November 2020, he got a position of clinical fellow in Neurology (chef de clinique universitaire assistant hospitalier) at the Neurology department of the Lille University Medical Centre under the supervision of Prof. Luc Defebvre. Alongside this clinical activity, he started his PhD project under the international co-supervision of Prof. Albert Leentjens (Maastricht University) and Prof. Kathy Dujardin (University of Lille). Throughout his clinical fellowship he continued and completed the research described in this thesis.

Guillaume will continue his research project at Maastricht University during a postdoctoral mobility from November 2024 to April 2025. Then, he will start working as a neurologist and researcher at University of Reims Champagne-Ardenne and Reims University Medical Centre in order to apply for a position of lecturer in Neurology (maître de conférence universitaire praticien hospitalier).

List of Publications

First author:

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