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Multi-modal Imaging and Cognitive Profiles in de novo Parkinson's Disease

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Summary

1. Introduction

1.1 Parkinson's Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disease, affecting up to 3% of the population above 65 years of age (Poewe *et al.*, 2017).

The neuropathological hallmarks of PD are defined as the gradual accumulation of the misfolded synaptic protein α-synuclein in the nervous system, as well as the neural loss in the substantia nigra pars compacta in the mesencephalon, where the dopaminergic neurons reside, leading to dopaminergic deficiency. The degeneration of these neurons consequently causes a cascade of impairments in several networks connecting the basal ganglia, thalamus and cortical motor regions (Weingarten *et al.*, 2015; Meles *et al.*, 2017). The cardinal motor features of PD are the result of this degenerative process, and are represented by bradykinesia, rest tremor and rigidity (Postuma *et al.*, 2015). The presence of these three symptoms together is referred as to parkinsonism. Although, with the progress made in the understanding of PD, it became clear that PD is not just a movement disorder, but it is also characterized by a multitude of non-motor symptoms, such as cognitive impairment, autonomic dysfunction, sleep disorders, depression and hyposmia (Poewe *et al.*, 2017).

Over the years, researchers have come to a diagnostic classification of PD comprehensive of both motor core features and non-motor symptoms.

In order to have an established diagnosis of PD, clinicians have to go through a three-phase evaluation (Postuma *et al.*, 2015):

1. Assessment of parkinsonism, presence of:
    a. Bradykinesia;
    b. Rigidity;
    c. Rest tremor.
2. Assessment of absolute exclusion criteria such as:
    a. Cerebellar abnormalities;

b. Gaze palsy;
c. Diagnosis of FTD or APP;
d. Parkinsonian features restricted to the lower limbs for more than 3 years;
e. Treatment with a dopamine receptor blocker or a dopamine-depleting agent in a dose and time-course consistent with drug-induced parkinsonism;
f. Absence of observable response to high-dose levodopa despite at least moderate severity of disease;
g. Unequivocal cortical sensory loss, clear limb ideomotor apraxia, or progressive aphasia;
h. Normal functional neuroimaging of the presynaptic dopaminergic system;
i. Documentation of an alternative condition known to produce parkinsonism and plausibly connected to the patient's symptoms.

3. Assessment of supportive criteria for diagnosis such as:
    a. Clear and dramatic beneficial response to dopaminergic therapy (improvement with dose increase; univocal and marked on/off fluctuations);
    b. Presence of levodopa-induced dyskinesia;
    c. Rest tremor of a limb, documented on clinical examination;
    d. Positive results from at least one ancillary diagnostic test having a specificity greater than 80% for differential diagnosis of PD from other parkinsonian conditions.

Together with these criteria, some *red flag* features need to be taken into account. Presence of red flags are defined by:

- Rapid progression of gait impairment requiring regular use of wheelchair within 5 y of onset;
- A complete absence of progression of motor symptoms or signs over 5 or more years unless stability is related to treatment;

- Early bulbar dysfunction, defined as one of severe dysphonia, dysarthria or severe dysphagia within the first 5 y of disease;
- Inspiratory respiratory dysfunction;
- Severe autonomic failure in the first 5 y of disease (orthostatic hypotension, severe urinary incontinence);
- Recurrent falls due to impaired balance within 3y from the onset;
- The presence of disproportionate anterocollis (dystonic in nature) or contractures of hand or feet within the first 10 years;
- Absence of any of the common nonmotor features of disease despite 5 y disease duration;
- Otherwise unexplained pyramidal tract signs;
- Bilateral symmetric parkinsonism throughout the disease course.

1.2 Neuroimaging

Different neuroimaging techniques are widely used in the diagnostic work-up of PD, all with the aim of determine with high accuracy the degree of severity of the pathology, as well as identify the presence of comorbidities. Among these techniques it's possible to find the single-photon emission computerized tomography (SPECT), the ^{18}F-fluorodeoxyglucose positron emitting tomography ([^{18}F]FDG-PET) and the electroencephalography (EEG).

1.2.1 Single-photon emission computerized tomography (SPECT)

Nowadays, SPECT imaging is the most used technique to support the clinical diagnosis of PD, since it allows both the differential diagnosis between PD and similar pathologies and the staging of the disease. In particular, the [^{123}I]Ioflupane Single Photon Emission Computed Tomography ([^{123}I]FP-CIT-SPECT) o DAT-SCAN is the most widely used method. It allows the assessment of DAT dependant Na-Cl channels, these being the presynaptic receptors for dopamine reuptake. DAT availability reflects the dopamine deafferentation within the nigrostriatal pathway, associated with PD symptomatology when the loss of dopamine is 80% in the striatum and 50% in the substantia nigra (Uhl *et al.*, 1994; Bezard *et al.*, 2001; Scherfler *et al.*, 2002). [^{123}I]FP-CIT-SPECT it has also been used as an exploratory marker of serotonergic

neurodegeneration, thanks to its affinity to the serotonin transporter (SERT) (Roselli *et al.*, 2010) in those regions without significant DAT expression, such as the thalamus (Koch *et al.*, 2014; Arnaldi *et al.*, 2015). Indeed, studying SERT availability at thalamus levels, where the highest density of SERT was observed (Takano *et al.*, 2011) provides an indirect measure of the serotonin system, as DAT density in those structures is negligible (Sun *et al.*, 2012).

A typical PD DAT-SCAN image shows a quantitative depletion of DAT starting from posterior putamen and extending, with the progression of the disease, to anterior putamen and caudate nuclei (**Figure 1**), with more severity associated with major DAT loss in the caudate nuclei (Rispoli *et al.*, 2018).

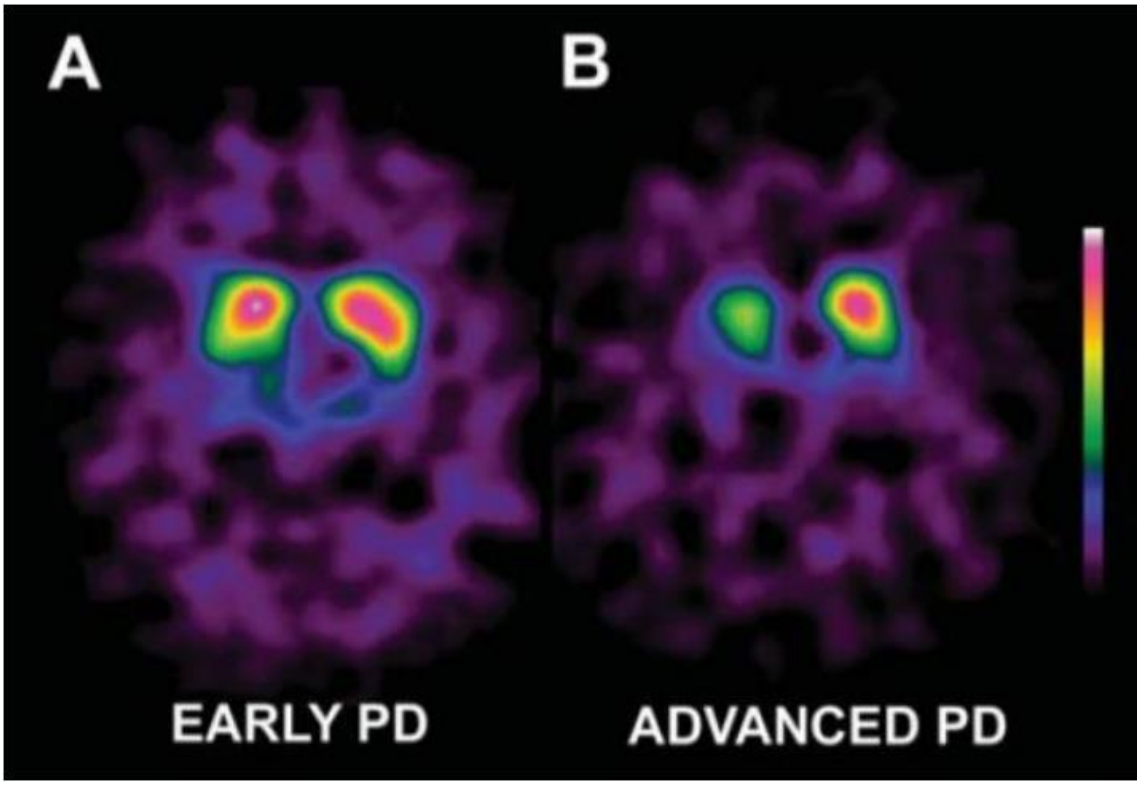

Figure 1*: Impairment of DAT availability in A) early PD (1 year from diagnosis); and B) advance PD (10 years from diagnosis). Figure from Eidelberg D. (2011) Imaging in Parkinson's disease. Oxford University Press.*

1.2.2 ^{18}F-fluorodeoxyglucose positron emitting tomography ([^{18}F]FDG-PET)

Brain PET imaging is prevalently used as a direct index of neuronal activity, local synaptic function, and accompanying biochemical network changes at resting state. It is a radiotracer-based method, for which any isotope that decay by releasing a positron can be used as tracer. The most commonly used tracers include ^{11}C, ^{13}N, ^{15}O and ^{18}F (Eidelberg, 2011). In particular, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET has been developed to identify and measure metabolic abnormalities in PD. While clinically [^{18}F]FDG-PET has been mainly used to differentiate between PD and other neurodegenerative parkinsonism, converging evidence point to a possible role of [^{18}F]FDG-PET also as a prognostic marker and to differentiate different subtypes of PD.

[^{18}F]FDG-PET studies have indeed demonstrated that an abnormal glucose brain metabolism profile is present at early stage of PD, especially in the lentiform nucleus, the thalamus, as well as the lateral frontal, paracentral, inferior parietal, and parietooccipital areas, known as Parkinson's Disease-Related elated Pattern (PDRP) (**Figure 2**). Usually the occipital hypometabolism is more evident in the contralateral hemisphere to tremor side (Eidelberg, 2011).

Moreover, it has been shown that posterior hypometabolism is also associated with cognitive impairment in PD patients. In particular, PD-MCI patients presents hypometabolism among the temporo-parietal junction, precuneus and the inferior temporal cortex (Hosokai *et al.*, 2009; Eidelberg, 2011). Hypometabolism in these areas worsen with the conversion from PD-MCI to PD with dementia (PDD).

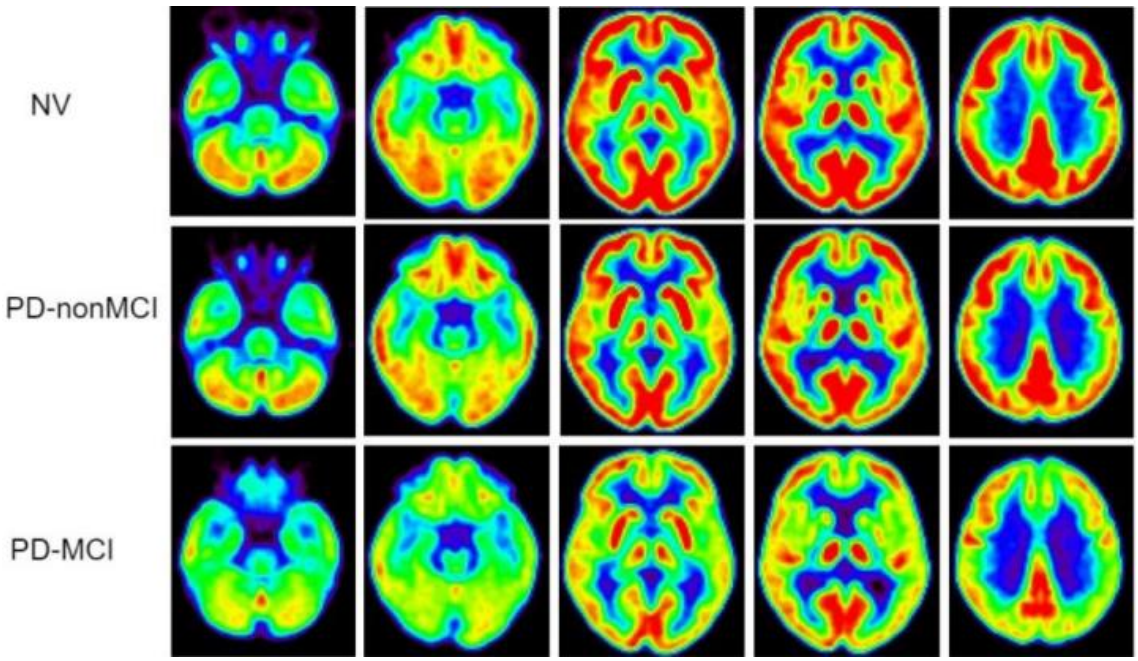

***Figure 2**: [^{18}F]FDG-PET images of healthy controls (NV), PD without Mild Cognitive Impairment (PD-nonMCI) and PD with MCI (PD-MCI). Figure from Eidelberg D. (2011) Imaging in Parkinson's disease. Oxford University Press.*

1.2.3 Electroencephalography (EEG)

By definition, a biomarker has the aim of measuring and assessing normal biological processes, pathophysiologic processes, or pharmacologic response to a therapeutic intervention (Working, 2001). Quantitative biomarkers could be used to identify degenerative processes before conversion to an overt stage of the disorder (Geraedts *et al.*, 2018). EEG could be considered as a good biomarker, since it's a non-invasive, widely available and cheap technique; moreover, it allows to study cortical dysfunction by directly measuring brain activity. In particular, quantitative EEG (qEEG), along with spectral information of cortical rhythms, it also provides data on regional or whole-brain synchronization of brain activity. Early identification of cortical dysfunction could be useful in anticipate possible future clinical deterioration, so to improve patients' management.

To date, however, the relationship between qEEG and its clinical features of PD remains unclear. In fact, so far no consistent pattern of correlation has been found among qEEG and motor impairment (Geraedts *et al.*, 2018). As for cognition, it has been shown that EEG slowing correlates with worst cognitive impairment, defined as

a lower score in global cognition tests (i.e., Mini Mental State Evaluation – MMSE) as well as patients' overall cognitive state.

Also, previous studies have been shown that EEG response is modulated by cholinergic tone (Prichep *et al.*, 2006; Wink *et al.*, 2006). For example, in both healthy aging and in Alzheimer's Disease (Moretti *et al.*, 2004; Babiloni *et al.*, 2006) impairment in cholinergic transmission has been linked the power of the EEG dominant frequency and increases the power of the lower frequencies (Moretti *et al.*, 2008), while in PD, some qEEG features thought to be underpinned by cholinergic impairment have been proposed as risk markers for the development of cognitive deterioration (Arnaldi *et al.*, 2017).

1.3 Cognition

Cognitive impairment in PD represents one of the most common non-motor symptoms of the pathology, and it could be present at early stages as PD-MCI or appear later on in the disease course.

In *de novo* PD patients, some degree of cognitive impairment could be detectable, but not severe enough to compromise activities of daily living.

It has been shown that executive deficits are present at early phases of PD, and the later involvement of posterior cortical impairment leads to the progression from PD-MCI to PDD. However, the pattern of cognitive impairment in PD is variable not just to the extents on which are the affected cognitive domains, but also on which are those domains that became affected first (Martinez-Horta and Kulisevsky, 2019).

A typical neuropsychological pattern of cognitive decline has been described over the years, and it is mainly characterized by frontal-executive deficits (Williams-Gray *et al.*, 2009). More in detail, these executive difficulties present as slowed processing speed, attention and working memory, set-shifting and non-cue facilitate recall. In *de novo* drug-naïve PD patients the aforementioned deficits are to be attributed to the dopamine-mediated dysfunction of the associative circuit of the basal ganglia (Carbon *et al.*, 2004). Although, up to 40% of PD patients also display visuo-spatial deficits, as well as memory and/or language impairment (Muslimović *et al.*, 2005).

The MDS Task Force stated that PD-MCI is a common syndrome among PD patients, and it anticipate the conversion towards PDD (Litvan *et al.*, 2011). Its clinical profile

is mainly heterogenous, but the non-amnestic, single-domain impairment is the most common subtype of PD-MCI.

In 2012, Litvan et al. defined clinical, cognitive and functional criteria to describe PD-MCI syndrome, which are divided in two Levels (Litvan *et al.*, 2012):

- Level I (abbreviated assessment)
    - Impairment on a scale of global cognitive abilities validated for use in PD or
    - Impairment on at least two tests, when a limited battery of neuropsychological tests is performed (i.e., the battery includes less than two tests within each of the five cognitive domains, or less than five cognitive domains are assessed.
- Level II (comprehensive assessment)
    - Neuropsychological testing that includes two tests within each of the five cognitive domains (i.e., attention and working memory, executive, language, memory, and visuospatial);
    - Impairment on at least two neuropsychological tests, represented by either two impaired tests in one cognitive domain or one impaired test in two different cognitive domains;
    - Impairment on neuropsychological tests may be demonstrated by:
      - Performance approximately 1 to 2 SDs below appropriate norms or
      - Significant decline demonstrated on serial cognitive testing or
      - Significant decline from estimated premorbid levels.

2. Thesis Aims and Outline

The overall aim of my PhD was to study the correlations between cognitive functions, neurodegeneration and functional alterations in diffuse projection systems. To this aim we plan to combine formal neuropsychological testing, structural and functional imaging and in-vivo quantitative assessments of the dopaminergic, serotonergic and cholinergic systems.

Through a multi-modal imaging approach, we had the chance to deeply understand neuroanatomical and neurochemical basis of cognition in its whole, in a large cohort of *de novo* Parkinson's Disease (PD) patients.

Our study cohort ranges from 30 to 96 of *de novo* PD patients, who underwent ^{18}F-fluorodeoxyglucose Positron Emission Tomography ([^{18}F]FDG-PET), used as a marker of regional neurodegeneration; [^{123}I]Ioflupane Single Photon Emission Computed Tomography ([^{123}I]FP-CIT-SPECT, as a marker of dopaminergic impairment in the basal ganglia and in the cortex as well as a proxy marker of serotonergic deafferentation in the thalamus; and quantitative electroencephalography (qEEG) recordings, used instead as a marker of cholinergic deafferentation.

We combined these imaging methods with formal neuropsychological testing, a social cognition test and multiple clinical variables.

In this work we aim to answer to three main scientific questions:

i) Does social cognition in *de novo* PD patients have a specific cortical and neurochemical signature?

ii) Is there a mediated effect between diffuse projection systems degeneration and cortical metabolism as well as on regional expression of monoaminergic transmission in the deep grey matter?

iii) Are specific cognitive domains impaired in *de novo* PD patients and are they differently affected by regional metabolism and diffuse projection systems degeneration?

In **Chapter 3** we investigated the topographical and neurochemical bases of Theory of Mind (ToM), which refers to the ability to attribute mental states to others and to predict, describe, and explain behaviour based on such mental states, using multi-tracer molecular imaging and quantitative electroencephalography in a group of 30 drug-naïve, *de novo* PD patients. ToM was assessed using the "Reading the Mind in the Eyes Task" (RMET), while general cognition with the Mini Menta State examination (MMSE).

We found that PD patients performed significantly worse at RMET compared to 60 healthy controls, as well as a significant positive correlation between RMET performance and regional metabolism in the superior temporal gyrus and the insula, and an inverse correlation with [^{123}I]FP-CIT thalamic specific binding ratio values, as expression of serotonin deafferentation. On the other hand, MMSE correlated with qEEG posterior Theta/Alpha power, confirming its independency from social cognition.

In **Chapter 4**, using multi-tracer molecular imaging, we assessed in a cohort of 96 drug-naïve, *de novo* PD patients the association between cortical metabolism and dopaminergic and serotonergic systems deafferentation of either striatum or thalamus, and whether this association was mediated by either striatum or thalamus metabolism. We found that the impact of deep grey matter monoaminergic deafferentation on cortical function is mediated by striatal and thalamic metabolism in this population. We showed a significant direct correlation between bilateral temporo-parietal metabolism and caudate dopaminergic innervation, as well as a significant correlation between prefrontal metabolism and thalamus serotonergic innervation, which were, respectively, mediated by striatal and thalamic metabolism.

In **Chapter 5**, we evaluate the association between neurotransmitter impairment, brain metabolism and cognition in a cohort of 95 drug-naïve, *de novo* PD patients, using [^{18}F]FDG-PET images as a marker of brain glucose metabolism and proxy measure of neurodegeneration, [^{123}I]FP-CIT-SPECT for dopaminergic deafferentation in the striatum and frontal cortex, as well as a marker of serotonergic deafferentation in the thalamus, and quantitative electroencephalography (qEEG) as an indirect measure of cholinergic deafferentation. Patients also underwent a complete neuropsychological

tests battery. We found positive correlations between (i) executive functions and left cerebellar cortex metabolism, (ii) prefrontal dopaminergic expression and working memory, (iii) qEEG slowing in the posterior leads and both memory and visuo-spatial functions.

3. Anatomical and neurochemical bases of theory of mind in *de novo* Parkinson's Disease.

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Abstract

Theory of mind (ToM) deficit is a frequent finding in subjects with neurological and psychiatric conditions. While several brain regions play a role in ToM, to date the contribution of the diffuse projection systems is less understood.

Here, we explored the topographical and neurochemical bases of ToM using multi-tracer molecular imaging and quantitative electroencephalography (qEEG) in a group of 30 drug-naïve, *de novo* Parkinson 's Disease (PD) patients (mean age 73.39±8.93 years, 11 females).

ToM was assessed using the "Reading the Mind in the Eyes Task" (RMET), while general cognition with the MMSE.

We acquired [^{18}F]FDG-PET images (as a marker of regional neurodegeneration), [^{123}I]Ioflupane Single Photon Emission Computed Tomography ([^{123}I]FP-CIT-SPECT, as a marker of dopaminergic impairment in the basal ganglia and in the cortex and as a proxy marker of serotonergic deafferentation in the thalamus), and qEEG recordings (using the Theta/Alpha power ratio as marker of cholinergic deafferentation).

PD presented with a significantly worse RMET score compared to 60 controls (20.7± 5.5 vs. 27.5± 3.0 p=0.001) while there was no difference between the two groups in age, education or MMSE.

The voxel-wise analysis of total RMET score and regional metabolism showed a positive correlation in the superior temporal gyrus and in the insula. Among the proxy markers of dopaminergic, serotonergic and cholinergic deafferentation, ToM presented an inverse correlation with [^{123}I]FP-CIT thalamic specific binding ratio (SBR) values -as a proxy serotonergic marker- which remained significant after correction for [^{18}F]FDG metabolism in the areas associated with ToM. On the other hand, MMSE correlated with qEEG posterior Theta/Alpha power.

These findings point to the presence of a specific cortical and neurochemical signature of ToM in PD, to the independence of ToM from general cognition, and suggest possible therapeutic targets to treat social cognition deficits.

Introduction

Theory of Mind (ToM) is a construct widely explored in cognitive neurology, neuropsychiatry as well as in developmental and clinical psychology. It refers to the ability to attribute mental states to others and to predict, describe, and explain behaviour on the basis of such mental states (i.e. mentalization; Baron-Cohen *et al.*, 1995). ToM deficit has been found to be commonly present in several dementing conditions, albeit with different degrees of severity (Poletti *et al.*, 2012) and in some cases it can represent the first manifestation of a subclinical neurodegenerative process (Pardini *et al.*, 2013). ToM is a heterogeneous construct, spanning both affective and cognitive components and this heterogeneity is underlined by its articulate neural bases (Abu-Akel and Shamay-Tsoory, 2011).

Previous studies showed that the ventromedial prefrontal cortex (Shamay-Tsoory and Aharon-Peretz, 2007), the amygdala (Völlm *et al.*, 2006), the inferior frontal gyrus (Bodden *et al.*, 2010; Dal Monte *et al.*, 2014), and the anterior cingulate cortex (Bodden *et al.*, 2010) are all involved in ToM. Also, the basal ganglia are thought to play a role in mentalizing, with an involvement of the dorsal and ventral striatum in its cognitive and affective components, respectively (Abu-Akel and Shamay-Tsoory, 2011). It has been proposed that also the diffuse monoaminergic and cholinergic projection systems play a role in ToM abilities both in healthy controls (HC) and in subjects with neuropsychiatric conditions (Abu-Akel and Shamay-Tsoory, 2011). Indeed, polymorphisms in genes, such as the dopamine (Zahavi *et al.*, 2016) and serotonin (Homberg and Lesch, 2011) transporters, have been shown to impact on social cognition and mentalizing abilities, and psychoactive drugs have been found to modulate ToM performance (Savina and Beninger, 2007). The diffuse projection systems, moreover, are known to modulate the activity of the fronto-striatal networks involved in cognition often in a non-linear, inverted-U curve relationship, in HC as well as in neurological and psychiatric conditions (Robbins and Arnsten, 2009).

To date, the relative contribution of the degeneration of the diffuse projection systems to ToM impairment remains poorly characterized, thus limiting our understanding of the neural bases of ToM as well as our ability to devise pharmacological strategies for ToM deficits.

We decided to explore the topographic and neurochemical bases of ToM deficits in drug-naïve, *de novo* Parkinson's Disease (PD) patients. *De novo* PD represents a good model to study the neurochemical bases of ToM, given the presence in this population of multiple monoaminergic deficits (Abu-Akel and Shamay-Tsoory, 2011; Chung *et al.*, 2018). ToM impairment has been previously described in subjects with advanced PD (Roca *et al.*, 2010; Bora *et al.*, 2015), in whom the neurodegenerative process is more diffuse, spanning the cortex as well as those components of the basal ganglia usually relatively spared in early PD (i.e. the dorsal striatum) (Pavese and Brooks, 2009).

To this aim we combined ^{18}F-fluorodeoxyglucose Positron Emission Tomography ([^{18}F]FDG-PET) imaging (to explore the topography and severity of neurodegeneration), with [^{123}I]Ioflupane Single Photon Emission Computed Tomography ([^{123}I]FP-CIT-SPECT) data, as well as with indirect markers of cholinergic deafferentation, based on quantitative EEG (qEEG) (see **Table 1** for a summary of the metrics used in the study). Indeed, while [^{123}I]FP-CIT-SPECT is used in clinical practice as a marker of nigrostriatal degeneration, thanks to its affinity to the dopamine transporter (DAT) in the basal ganglia, it has also been used as an exploratory marker of cortical dopaminergic deafferentation (Pilotto *et al.*, 2019) and serotonergic degeneration, thanks to its affinity to the serotonin transporter (SERT) (Roselli *et al.*, 2010) in those regions without significant DAT expression, such as the thalamus (Koch *et al.*, 2014; Arnaldi *et al.*, 2015).

Regarding qEEG, previous studies has shown that the cholinergic tone modulates EEG activity (Prichep *et al.*, 2006; Wink *et al.*, 2006). In healthy aging and in Alzheimer's Disease, for example an impairment in cholinergic transmission has been linked the power of the EEG dominant frequency and increases the power of the lower frequencies (Moretti *et al.*, 2004, 2008; Babiloni *et al.*, 2006), while in PD, some qEEG features underpinned by cholinergic impairment have been proposed as risk markers for the development of cognitive deterioration (Arnaldi *et al.*, 2017).

Table 1: Summary of the surrogate marker metrics used in the study. *Legend: SBR = specific binding ratio.*

	Metric
Serotonin	Thalamus [^{123}I]FP-CIT SBR
Dopamine	Putamen and Caudate [^{123}I]FP-CIT SBR
Cortical Dopamine	Cortical regional [^{123}I]FP-CIT SBR
Acetylcholine	qEEG in the posterior (: P3/O1, P4/O2, T5/O1, T6/O2) and anterior (F3/C3, F4/C4) cortical regions
Brain metabolism	[^{18}F]FDG-PET

Materials and Methods

Patients

In a period of three years' time, we examined 30 consecutive drug-naïve, *de novo* PD patients (age: 73.39±8.93 years; range: 50-81; 11 females and 19 males; education: 11.0±3.54 years) who underwent a full neuropsychological assessment, [^{18}F]FDG-PET, [^{123}I]FP-CIT-SPECT and qEEG recording at the time of the diagnosis. The inclusion criteria were a diagnosis of PD (Postuma *et al.*, 2016), confirmed by evidence of dopaminergic deficit on [^{123}I]FP-CIT-SPECT and by at least two-years of follow-up. The exclusion criteria were use of dopaminergic, cholinergic, or serotonergic therapies, the presence of neuropsychiatric or medical comorbidities, absence of dementia, and lack of informed consent. **Table 2** shows the demographic and clinical characteristics of the PD group.

All subjects were informed about the aim of the study and gave their written consent. The study was approved by the local ethics committee, and it was conducted in respect of the rules of the Helsinki declaration.

Healthy controls

A control group of 60 HC was used for ToM performance (41 males, 19 females, mean age 70.1±10.9 years). Demographic and clinical characteristics are reported in **Table 2**.

All subjects were free from current or past neurological disorders, primary psychiatric disorders, or medical comorbidities as well as from neurological complaints. Medical and neurological examinations were normal.

Table 2: Demographic and clinical characteristics of Parkinson's Disease patients and healthy controls used for comparison of the RMET (M = male; F= female; MMSE = Mini Mental State Examination; RMET: Reading the Mind in the Eyes Test; MDS-UPDRS-III = Movement Disorders Unified Parkinson's Disease Rating Scale, motor section; GDS = 15-item Geriatric Depression Scale; n.s = not significant; n.a = not available). Values are reported as mean ± standard deviation.

	Parkinson's Disease (n=30; mean ± sd)	Healthy Controls (n=60)	p value	Effect size (Cohen's *d*)
Age (yr)	73.39 ± 8.93	70.1 ± 10.9	n.s	d = 0.33
Education (yr)	11 ± 3.54	9.5 ± 5.4	n.s	d = 0.32
Gender (M:F)	19:11	41:19	n.s	n.a
MMSE score	28.0 ± 2.58	29.5 ± 3.9	n.s	d = 0.45
RMET total score	20.7± 5.5	27.5± 3.0	p=0.001	d = 1.53
MDS-UPDRS-III score	20.65 ± 7.6	n.a.	n.a.	n.a
GDS-15 score	2.80 ± 2.90	2.1 ± 3.50	n.s	d = 0.21

ToM assessment

ToM functioning was assessed using the "Reading the mind in the eyes task" developed by Baron-Cohen and colleagues (2001) and revised by Harkness *et al*. (2005), that consists of 36 black and white pictures of the eye region (Baron-Cohen *et al.*, 2001; Harkness *et al.*, 2005). The subject has to recognize the emotional state represented in the picture and choose one among four given words (**Figure 1**); in the revised version, already used in the Italian population (Pardini and Nichelli, 2009), the meaning of each word was classified as positive, negative or neutral. Each item is scored 1 if the answer is correct and 0 if the answer is wrong (range 0–36). For each patient were calculated both a total score (number of correct responses) and separate subscores for positive, negative, and neutral stimuli.

Figure 1: Example of one item of the RMET task.

[^{18}F]FDG-PET acquisition

All PD patients underwent brain [^{18}F]FDG-PET scan, acquired according to the guidelines of the European Association of Nuclear Medicine (Varrone *et al.*, 2009), used as a marker of neurodegeneration.

Subjects fasted for at least six hours. Before radiopharmaceutical injection, blood glucose was checked and was <7.8 mmol/l in all cases. After 10 minutes rest in a silent and obscured room, with eyes open and ears unplugged, subjects were injected with approximately 200 MBq of [^{18}F]FDG via a venous cannula. They remained in the room for 30 minutes after the injection and then moved to the PET room where scanning

started approximately 45 minutes after the injection. A polycarbonate head holder was used to reduce head movements during the scan. Images were acquired by means of a SIEMENS Biograph 16 PET/CT equipment with a total axial field of view of 15 cm and no interplane gap space. Attenuation correction was based on CT. Images were reconstructed through an ordered subset-expectation maximization algorithm, 16 subset and 6 iterations, with a reconstructed voxel size of 1.33×1.33×2.00 mm. Images were then exported as Dicom files and transformed into analyze files for subsequent post-processing.

[^{123}I]FP-CIT-SPECT acquisition

[^{123}I]FP-CIT-SPECT data was acquired by means of a 2-headed Millennium VG camera (G.E. Healthcare). Acquisition started between 180 and 240 minutes after injection of [^{123}I]FP-CIT and lasted 40 minutes. A "'step-and-shoot'" protocol was applied with a radius of rotation < 15 cm, and 120 projections evenly spaced over 360° were generated. Total counts ranged between 2.0 and 2.5 million. The pixel size of the acquisition matrix was 2.4 mm, thanks to an electronic zoom (zoom factor = 1.8) applied in the data collection phase. In the reconstruction phase, also a digital zoom was used and the resulting images were sampled by isotropic voxels with 2.33 mm sides. Projections were processed by means of the ordered subsets expectation maximization (OSEM) algorithm (8 iterations, 10 subsets) followed by post filtering (3D Gaussian filter with full width-half maximum = 8 mm). The OSEM algorithm included a proback pair accounting for collimator blur and photon attenuation. No compensation for scatter was performed. The 2D+1 approximation was applied in the simulation of the space-variant collimator blur, whereas photon attenuation was modelled with the approximation of a linear coefficient uniform inside the skull and equal to 0.11 cm−1. Data were exported in analyse format for further post-processing.

Image laterality

Taking into account the frequent asymmetric clinical and biological presentation of PD, 11 of the [^{18}F]FDG-PET and [^{123}I]FP-CIT-SPECT images were flipped based on the side of the more affected limbs, to have the more affected hemisphere on the right-hand-side of each image. Thus, the right hemisphere in the images represent the More

Affected Hemisphere (MAH), and the left hemisphere in the images represent the Less Affected Hemisphere (LAH).

EEG recording

Quantitative EEG (qEEG) was used as an indirect approach to study the cholinergic system integrity.

EEG recordings were obtained from patients seated in a comfortable chair with eyes closed. EEG electrodes were placed using standard 10–20 EEG electrode positions (Fp1, Fpz, Fp2, F3, Fz, F4, F7, F8, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2, T3, T4). Recordings were referenced to the Fpz electrode, Oz electrode served as ground. Electrode impedances were closely monitored and kept below 5 kOhm. Data were acquired using LTM system (EBNeuro, Florence, IT) at a sampling rate of 512 Hz and a bandpass of 0.3–70 Hz. To monitor eye movements, the horizontal electro-oculogram was simultaneously recorded with the same recording parameters of EEG. The EEG recordings were performed in the late morning to minimize drowsiness. An EEG technician was present during the entire recording session to keep constant the level of vigilance and to monitor signal quality. Standard longitudinal bipolar montage was used for analysis. EEG data were manually analysed off-line to reject artifacts. One minute of artifact-free EEG data was used for further analysis.

[^{18}F]FDG-PET analysis

[^{18}F]FDG-PET images were subjected to affine and nonlinear spatial normalization into Talairach and Tournoux space using SPM12 (Wellcome Department of Cognitive Neurology, London, UK). All the default choices of SPM were followed with the exception of spatial normalization. For this study, the $H_2{}^{15}O$ SPM-default template was replaced by an optimized brain [^{18}F]FDG-PET template as described by Della Rosa and colleagues (Della Rosa *et al.*, 2014). The spatially normalized set of images was then smoothed with a 10-mm isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. A whole-brain voxel-wise correlation between local [^{18}F]FDG uptake and total RMET score was performed in the PD group using a height threshold $p<0.001$ (uncorrected) and a minimum cluster size of 100 voxels for significant clusters. At cluster level, the accepted threshold of

statistical significance was $p<0.05$, family-wise error corrected for multiple comparisons. The [^{18}F]FDG signal in the regions found to correlate significantly with RMET was then normalized over the whole brain and used as a covariate in the following analyses of cortical RMET-related volume of interest (VOI).

[^{123}I]FP-CIT-SPECT: putamen and caudate uptake evaluation as a dopaminergic marker

The reconstructed [^{123}I]FP-CIT-SPECT images were exported in analyze format and processed by the automatic BasGan algorithm version 2 based on a high definition, 3D striatal template, derived from Talairach's atlas (Nobili *et al.*, 2013). Using this approach, an optimization protocol automatically performs fine adjustments in the positioning of blurred templates to best match the radioactive counts and locates an occipital region of interest for background evaluation. Partial volume effect (PVE) correction is included in the process of uptake computation of caudate, putamen, and background. The partial volume effect correction performed by the method consists of an activity assignment in a Talairach-Tornoux atlas-based 3-compartment model of basal ganglia. Background uptake was subtracted by putamen and caudate uptake as follows (caudate or putamen uptake – background uptake)/background uptake, to generate specific to non-displaceable binding ratio (SBR) values.

[^{123}I]FP-CIT-SPECT: thalamus uptake evaluation as serotonergic marker

Beside DAT, [^{123}I]FP-CIT also shows high affinity for the serotonin transporter SERT (KI 9.73), with a DAT/SERT selectivity of 2.8 (Roselli *et al.*, 2010; Pilotto *et al.*, 2019). DAT and SERT display a non-overlapping distribution in the brain: DAT levels are higher in the basal ganglia, whereas SERT is highly expressed in the thalamus. As previously reported, [^{123}I]FP-CIT-SPECT signal in the thalamus represents an indirect marker of SERT availability (Koch *et al.*, 2014; Arnaldi *et al.*, 2015).

Indeed, studying SERT availability at thalamus level~~s~~, where the highest density of SERT was observed (Takano *et al.*, 2011) provides an indirect measure of the serotonin system, as DAT density in those structures is negligible (Sun *et al.*, 2012).

In order to study SERT binding, [^{123}I]FP-CIT-SPECT images were analysed using SPM12 package (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab R2018b (MathWorks, Natick, Massachusetts, USA). A

customize brain [^{123}I]FP-CIT-SPECT template was built from 20 brain MRI and SPECT scans of healthy subjects that are not included in the control group of the present study as previously described (Arnaldi *et al.*, 2015). The SPECT images were then normalized to the MNI space in SPM12 and smoothed (3D gaussian filter with 10 mm full-width at half-maximum) (Pilotto *et al*., 2019). Then regions of interest (ROIs) for the left and right thalamus were created using the WFUPickAtlas toolbox. Background uptake was subtracted from these ROIs as follows (ROI − background uptake)/background uptake, to generate SBR values for each ROI.

[^{123}I]FP-CIT SPECT: cortical uptake as a marker of dopaminergic cortical deafferentation

As previously described (Pilotto *et al.*, 2019), using the normalized, smoothed SPECT images of each subject, the [^{123}I]FP-CIT uptake was extracted from the cortical RMET-related VOI. With this choice, we want to explore the cortical dopaminergic activity just in those regions showing a significant direct correlation between RMET and brain metabolism. Background uptake was subtracted from the cortical clusters as follows (cortical cluster uptake − background uptake)/background uptake, to generate SBR values.

Quantitative EEG: posterior and anterior Theta/Alpha power ratio as a proxy of cholinergic tone

The Fast Fourier Transform (FFT, Welch method), applied to 4-s segments (Tukey window) with 2-s overlaps, was used to compute EEG spectral power for each derivation. The EEG spectrum was then divided into the following frequency bands: delta (2.25-4 Hz), theta (4.25-8 Hz), alpha (8.25-12 Hz), sigma (12.25-16 Hz) and beta (16.25-32 Hz) and relative power was computed for each band as normalized to the total EEG power. We computed the theta/alpha power ratio, both in the posterior (P3/O1, P4/O2, T5/O1, T6/O2) and anterior (F3/C3, F4/C4) cortical regions as indirect proxies of the cholinergic tone.

Statistical analysis

Statistics were performed using IBM SPSS Statistics 25 (Armonk, NY). Univariate statistics (t-tests) were used to compare PD and HC ToM performance. In the PD group parametric correlations were run between total RMET score, the aforementioned molecular imaging and qEEG, as proxy measures of metabolic, dopaminergic, serotonergic, and cholinergic degeneration, respectively.

All analyses were also run using a partial correlation approach correcting for age, MMSE score (Folstein *et al.*, 1983) as well as GDS-15 score (Yesavage, 1988) and the cortical RMET-related VOI normalized over the whole brain.

Moreover, to assess the specificity of the correlations observed between ToM and the different neuroimaging measures, we correlated MMSE score (as a proxy measure of general cognition) with [^{123}I]FP-CIT SPECT and EEG metrics.

Statistical threshold was set at 0.05 and p-values were reported both uncorrected and corrected for multiple comparisons using a false discovery rate (FDR) approach.

Results

ToM performance

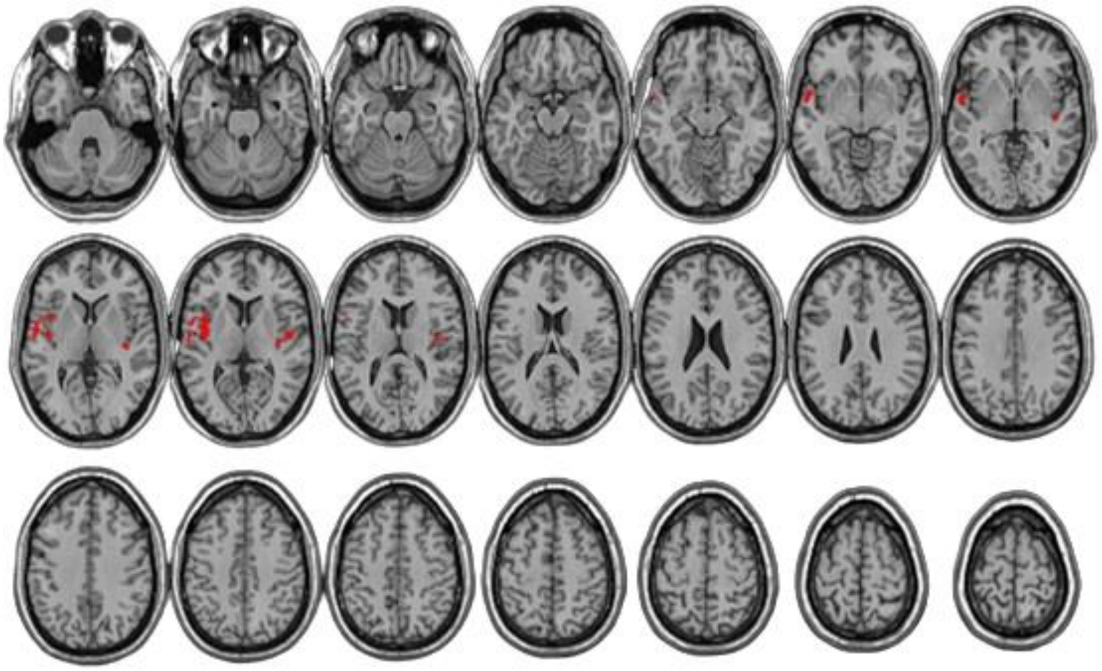
PD patients presented with a significantly worse total RMET score compared to controls (20.7± 5.5 vs. 27.5± 3.0 p=0.001, t=7.6) while there was no difference between the two groups in age, education or MMSE score.

[^{18}F]FDG-PET: ToM and brain metabolism

The voxel-wise correlation between total RMET score and regional metabolism showed a cluster of significant positive correlation focused in the superior temporal gyrus (STG) in the LAH and one focused in the insula in the MAH.

Results are reported in **Figure 2**.

Figure 2: Results of the voxel-wise correlation between total RMET score and regional metabolism. The figure shows a cluster of positive correlation focused on the superior temporal gyrus in the Less Affected Hemisphere (here represented on the left side in neurological convention; coordinates of the peak: -56, 2, -4; p=0.0001, cluster size=329) and a cluster of positive correlation focused in the insula of the More Affected Hemisphere (here represented on the right side in neurological convention; coordinates of the peak: 40, -10, 12; p=0.0001, cluster size=152). Analyses were corrected for age and MMSE score. Significant clusters are shown overlaid on a MRI T-1 image.



[^{123}I]FP-CIT-SPECT: ToM and subcortical serotonergic function

All the analyses performed on [^{123}I]FP-CIT-SPECT data are reported in **Table 3a.**

We observed a significant negative correlation between total RMET score and the LAH thalamus SBR (p=0.004, r= -0.620), while there was no correlation between the total RMET score and the MAH thalamus SBR (p=0.917, r= -0.026).

[^{123}I]FP-CIT-SPECT: ToM and Basal Ganglia dopaminergic function

As reported in **Table 3a** we found no correlation between RMET score and SBR in the putamen and caudate nuclei.

[^{123}I]FP-CIT-SPECT: ToM and dopaminergic function in the cortical RMET-related VOI

There was no significant correlation between SBR of the cortical RMET-related VOI and ToM performance (**Table 3a**).

Table 3a*:* Correlation between total RMET score and [^{123}I]FP-CIT SBR. *Legend: MAH = More Affected Hemisphere; LAH = Less Affected Hemisphere; MMSE= Mini Mental State Examination; GDS= Geriatric Depression Scale*

		MAH (correction for age, hypometabolism*, MMSE and GDS)				LAH (correction for age, hypometabolism*, MMSE and GDS)		
	Mean ± Standard deviation MAH	r values	p values (unc.)	FDR	Mean ± Standard deviation LAH	r values	p values (unc.)	FDR
Thalamus SBR	1.44 ± 0.15	r= -0.026	p= 0.917	p= 0.913	1.45 ± 0.18	r= -0.620	p= 0.004	p= 0.036
Putamen SBR	1.41 ± 0.74	r= 0.064	p= 0.794	p= 0.907	1.91 ± 0.83	r= 0.079	p= 0.746	p= 0.907
Caudate SBR	2.95 ± 0.94	r= 0.180	p= 0.458	p= 0.734	3.38 ± 1.04	r= 0.175	p= 0.475	p= 0.791

*Note: correction for the [^{18}F]FDG uptake values of the clusters found associated with the RMET score reported in *Figure 2*, normalized over the whole brain.

Quantitative EEG: ToM and cholinergic function

As reported in **Table 3b**, we found no correlation between ToM performance and qEEG metrics.

Table 3b: Correlation between total RMET score and qEEG parameters (anterior leads: F3/C3, F4/C4; posterior leads: P3/O1, P4/O2, T5/O1, T6/O2)

	Results after correction for age, hypometabolism*, MMSE and GDS			
	Mean ± sd	r values	p values (unc.)	FDR
Theta/Alpha power ratio; anterior leads	0.99 ± 0.69	r= -0.251	p= 0.331	p=0.791
Theta/Alpha power ratio; posterior leads	0.92 ± 0.88	r= -0.198	p= 0.445	p=0.734

MMSE correlation with serotonergic, dopaminergic and cholinergic proxy measures

We found a negative correlation between MMSE and qEEG metrics, namely the average Theta/Alpha ratio in the posterior leads (r= -0.658, p=0.002). Results are shown in **Table 4a** and **4b**. No other significant correlations were found with serotonergic and dopaminergic indexes.

Table 4a*:* Correlation between MMSE score and [^{123}I]FP-CIT SBR. *Legend: MAH = More Affected Hemisphere; LAH = Less Affected Hemisphere.*

	MAH (correction for age and GDS)			LAH (correction for age and GDS)		
	r values	p values (unc.)	FDR	r values	p values (unc.)	FDR
Thalamus SBR	r= -0.014	p= 0.950	p=0.950	r= 0.167	p= 0.468	p=0.535
Putamen SBR	r= 0.381	p= 0.087	p=0.175	r= 0.225	p= 0.326	p=0.435
Caudate SBR	r= 0.397	p= 0.074	p=0.175	r= 0.458	p= 0.032	p=0.11

Table 4b*:* Correlation between MMSE score and qEEG parameters (anterior leads: F3/C3, F4/C4; posterior leads: P3/O1, P4/O2, T5/O1, T6/O2).

	Results after correction for age and GDS			
	Mean ± sd	r values	p values (unc.)	FDR
Theta/Alpha power ratio; anterior leads	0.99 ± 0.69	r= -0.375	p= 0.103	p=0.206
Theta/Alpha power ratio; posterior leads	0.92 ± 0.88	r= -0.658	p= 0.002	p=0.016

Emotional categories and serotonergic, dopaminergic and cholinergic proxy measures

We found a negative correlation between RMET stimuli with an emotional valence (positive + negative) and LAH thalamus SBR (r= -0.736; p=0.001), while no correlation was found with neutral stimuli. The correlation with the LAH thalamus SBR remained significant also when considering positive and negative emotions separately (r= -0.527, p=0.036; r= -0.653, p= 0.006, respectively).

No other significant correlations were observed between RMET sub-scores and either cholinergic or dopaminergic proxy measures.

Correlation between serotonergic, dopaminergic, cholinergic and regional [^{18}F]FDG metabolism

There was no correlation between normalized [^{18}F]FDG-PET signal of the cortical RMET-related VOI and the serotonergic, dopaminergic and cholinergic metrics used in this study.

Discussion

In this work we evaluated the neural bases of ToM abilities in a group of unmedicated patients with a newly established diagnosis of PD. We showed that performance at an advanced ToM test (i.e., the RMET) is impaired in *de novo* PD patients compared to controls, and that it is directly associated with both cortical metabolic levels in the

superior temporal gyrus and in the insula, as well as with a higher subcortical serotonergic tone.

ToM performance

Our PD group showed a significantly reduced RMET performance compared to HC. Mentalizing deficits have been previously described in subjects with PD and associated with more severe cognitive (Nobis *et al.*, 2017) and motor (Raffo De Ferrari *et al.*, 2015) deficits. The focus of those studies, however, was mainly on the more advanced stages of the disease, in which the severity of neurodegeneration, presence of long-term medication, psychosocial adjustment difficulties and cognitive decline all impacted on ToM performance.

Regarding the early phases of the disease, conflicting results on the presence of ToM deficits have been published. Peron and colleagues (2009) showed no differences between unmedicated PD patients and controls in ToM abilities, while Roca *et al.* (2010) showed a deficit of cognitive but not affective ToM in unmedicated PD patients compared to controls (Péron *et al.*, 2009; Roca *et al.*, 2010). In these studies, however, different recruitment strategies that could explain the heterogeneity of the results were used. Indeed, while our study focused only on the earliest motor phases of the disease, Peron *et al.* (2009) defined early PD based on the lack of cognitive deficits while Roca *et al.* (2010) used a relatively low MMSE score cut-off (24), and no explicit exclusion criteria for psychoactive drug use or history of depression.

The presence of ToM deficits in our PD cohort (i.e., in the early phase of the disease) is in line with findings in other neurodegenerative conditions such as Frontotemporal Dementia (Pardini *et al.*, 2013, Orso *et al.*, 2020*b*), showing a possible role for mentalizing assessment as a tool to monitor cognition early on in neurodegenerative conditions and possibly as an outcome marker for symptomatic therapies clinical trials (Pardini *et al.*, 2015).

ToM and brain metabolism

We showed that RMET performance positively correlated with cortical metabolism in the insula and in the STG. These findings are in line with the role played by the insula

in social emotions (Kanske *et al.*, 2015) and by the inclusion of the STG in the social cognition network (Specht and Wigglesworth, 2018).

Recent years have seen an increase of the awareness of the role played by the insula in a number of processes related with the construct of ToM, such as self-awareness and multi-modal social stimuli processing (Uddin *et al.*, 2017). Indeed, lesions in the insula have been associated with a reduced ToM performance in subjects with stroke (Nott *et al.*, 2019), while subjects with schizophrenia have been shown to present reduced fMRI activity in the insula during ToM tasks. The insula is a cytoarchitectonic complex area, and the peak of the observed correlation falls in the anterior insula. Numerous functional neuroimaging and neuropsychological investigations suggest that this area is strongly activated while experimenting social emotions, such as empathy (Lamm and Singer, 2010) and pain (Wager *et al.*, 2008). Kraus *et al.* (2019) have demonstrated an enhanced reactivity of the anterior insula while presenting personal and emotional pictures, supporting results obtained from research employing emotional visual stimulation (Fusar-Poli *et al.*, 2009; Kraus *et al.*, 2019). Furthermore, taking into account the emotional processing of visual stimuli, Nott *et al.* (2019) demonstrated that lesions in the insula were associated with poorer performance on the RMET. Taken together, these results are in line with the view that the anterior insular region plays a prominent role in emotional processing and social emotions (Kober *et al.*, 2008).

Moreover, converging evidence in different neurological and psychiatric conditions also points to a role for the STG in ToM abilities. In schizophrenia, for example, Koelkebeck and colleagues (2013) showed an association between grey matter volume loss in the superior temporal gyrus and ToM (Koelkebeck *et al.*, 2013); while in stroke, focal damage in the STG was associated with reduced RMET performance (Nott *et al.*, 2019), also in autism STG activations has been associated with abnormalities in face processing (Baron-Cohen *et al*., 1999).

Given the verbal nature of RMET and the role played by the STG in language (Bigler *et al.*, 2007) it is possible that the observed correlation is partially driven by linguistic abilities. Indeed, the role played by linguistic abilities in mentalizing and its mediation of RMET association with neural structure is well known (Dal Monte *et al.*, 2014).

At least to partially compensate for the impact of general cognition status on RMET performance, we corrected all our analysis for the MMSE score. We are aware of the limitations of the partial value of the MMSE as a screening tool in PD, and this should be considered in the interpretation of our findings.

While to our knowledge no studies used [^{18}F]FDG-PET to probe the neural substrate of ToM in PD, to the same aim different groups used fMRI instead. These studies seem to point to an involvement of the orbitofrontal cortex (OFC; Ibarretxe-Bilbao *et al.*, 2009) and the temporal poles (Poletti *et al.*, 2011) as key areas for ToM in PD. Those studies, however, were focused on patients undergoing L-DOPA treatment and in an advanced stage of the disease, and thus are not readily comparable with our findings. A possible unifying explanation is given by the presence of anatomical connections between the insula and the STG, and the areas found in previous studies. Indeed, it has been proposed that focusing on networks rather than single areas could be instrumental to improve the reproducibility of neuroimaging findings across conditions and imaging modalities (Boes *et al.*, 2015). Caution, moreover, is needed when comparing [^{18}F]FDG-PET and fMRI findings, especially taking into account the methodological differences between the two techniques.

ToM and serotonergic function

Using [^{123}I]FP-CIT-SPECT, we showed an inverse association between thalamus SBR and ToM performance in the less affected hemisphere, independent from the metabolic levels. Taking into account the affinity of [^{123}I]FP-CIT not only for DAT but also for SERT, as well as the lack of DAT expression in the thalamus (Roselli *et al*., 2010; Pilotto *et al*., 2019), this inverse correlation suggests that those PD subjects with an increased serotonergic expression present with lower mentalizing abilities.

The association of a serotonergic marker with ToM is in line with the role played by the serotonergic system in social cognition, both in healthy controls and in subjects with neurological or psychiatric disorders (Kiser *et al.*, 2012). Indeed, serotonin is thought to impact our abilities to perceive social stimuli as shown by both tryptophan-depletion studies (Beacher *et al.*, 2011) and acute administration of serotonergic antidepressants (Crockett *et al.*, 2010). Serotonin receptor levels, moreover, have been found to be associated with social cognition and qualitative abnormalities in reciprocal

social interactions, which rely on ToM functioning in subjects with Asperger's syndrome (Murphy, 2006). The distribution of serotonergic innervation, with its robust projections to the prefrontal cortex, is in line with the aforementioned role in social cognition played by serotonin, and represents one of the anatomical substrata of the impact of serotonin on social functioning (Charnay and Léger, 2010).

The correlation between serotonergic function and cognition is not linear and -as with other neurotransmitters such as dopamine and noradrenaline- is thought to follow an inverted U-shape, as shown by the modelling of the impact of serotonin on working memory (Cano-Colino *et al.*, 2014). Evidence for an inverted U-shape on the relationship between serotonin and performance has been shown also in a study of dose-response curves of antidepressants, with a reduction of positive effects on mood shown at the high end of the dose curve for both serotonin selective reuptake inhibitors and for mirtazapine (Furukawa *et al.*, 2019), as well as in a study about the impact of tryptophan intake on cognition, with reduced performance associated with the low- and high-dose ends of the dose/response curve (Hulsken *et al.*, 2013).

Our data point indeed to higher serotonergic expression as a predictor of reduced ToM abilities in *de novo* PD patients. Interestingly, this correlation was independent of cortical [^{18}F]FDG metabolism, suggesting that the serotonergic expression impacts on ToM independently from the extent of cortical damage in early PD. Moreover, in line with the known role played by the serotonergic system in emotions (Canli and Lesch, 2007) the thalamus SBR correlated only with emotionally relevant (positive + negative) and not with neutral stimuli.

Modulation of the serotonergic system could thus represent a reasonable approach to tackle ToM deficits in PD.

ToM and dopaminergic function

We did not find any correlation between surrogate markers of dopaminergic degeneration and ToM. The hypothesis of a possible role for the dopaminergic system in ToM stems from the role played by dopamine in the prediction of events (Schurz and Perner, 2015), including social relevant stimuli (Abu-Akel *et al*., 2011), as well as the frequency of ToM deficits in those disorders associated with a dysregulation of dopaminergic signalling, such as schizophrenia (Koelkebeck *et al.*, 2013). Moreover,

ToM performance in preschool aged children was associated with the polymorphisms of the COMT gene and the dopamine receptor D4 gene (DRD4), suggesting that DRD4 receptors may represent a necessary gene governing the development of ToM (Lackner *et al.*, 2010). Indeed, it is thought that the role played by dopamine in updating mental models of short-term outcomes in social interactions represents the cognitive basis of the role played by the dopaminergic system in mentalizing (Brunet-Gouet and Decety, 2006).

While the aforementioned studies point to a role for the dopaminergic system in the development of ToM, the lack of association between dopaminergic markers and ToM in PD observed in this study, as well as the lack of an impact of dopaminergic therapy on ToM in PD subjects with a more advanced form of the disease (Abu-Akel *et al.*, 2011), suggest the presence of disease-specific mechanism in the development of ToM deficits in PD. Thus, our results are in line with the findings in advanced PD and this increases our confidence in the lack of the role of the dopaminergic system in ToM.

While it would have been of interest to study separately the contribution of the dorsal and ventral striatum to the RMET performance, this is hampered by the limited spatial resolution of [^{123}I]FP-CIT-SPECT. Indeed, dorsal striatum is thought to play a larger role in cognitive rather than in affective ToM (Abu-Akel *et al.*, 2011), and this could explain our results. Future studies, for example based on DAT PET tracers, could explore this aspect.

ToM and cholinergic function

General cognition, evaluated with the MMSE, correlated with an indirect marker of cholinergic deafferentation (i.e., the posterior leads Theta/Alpha ratio), while there was no correlation between our cholinergic marker and ToM. While the presence of an association between general cognition and the cholinergic function is in line with previous studies (Arnaldi *et al.*, 2017), our findings are of relevance regarding the at least partial independence of ToM from other cognitive domains. The relationship between general cognition, and more in detail with executive functions, and ToM is complex. While ToM and executive function maturation are thought to be related in childhood (Wade *et al.*, 2018), their trajectories are not paired over the lifespan (Giovagnoli *et al.*, 2019) and selective deficits of ToM without executive function

abnormalities have been described, for example in the early stages of frontotemporal dementia or in lesion studies (Spikman *et al.*, 2012). Indeed, our results point to a clear difference in the neurochemical bases of general cognition and ToM deficits, at least in PD, thus suggesting possible therapeutic avenues to target selectively these impairments in early PD.

Strength and limitations

This study represents a proof-of-concept investigation of the different roles played by grey matter degeneration and by the serotonergic, dopaminergic and cholinergic diffuse projection systems on ToM abilities in *de novo* PD patients. To our knowledge this is the first study combining multi-tracer molecular imaging and quantitative EEG to shed light on the contribution of multiple diffuse projection systems on the development of ToM deficits. Early PD patients represent a good opportunity to shed light on this research question. Firstly, because the presence of ToM difficulties could contribute to part of their symptomatology, and thus the identification of possible therapeutic targets for this aspect is of clinical relevance. Moreover, early PD patients present with deficits in multiple diffuse projection systems without marked atrophy, thus allowing to dissociate the contribution of cortical dysfunction to cognition from those of different neurotransmitters (Grosch *et al.*, 2016).

We used [^{123}I]FP-CIT-SPECT metrics to evaluate dopaminergic and serotonergic functions. Compared to PET, SPECT has a relatively low spatial resolution, and this thus did not allow to separately study the ventral striatum, i.e., the subdivision of the basal ganglia more involved in social cognition. Future studies, based on the use of different PET tracers, are needed to clarify a possible role for dopaminergic denervation of the ventral striatum in ToM deficits in PD. Furthermore, we did not use a specific serotonergic marker, instead we use the [^{123}I]FP-CIT-SPECT SBR in the thalami, taking advantage of the [^{123}I]FP-CIT affinity for SERT, and also the lack of DAT in the thalami (Roselli *et al*., 2010; Pilotto *et al*., 2019). While this approach has been previously used in several studies (Koch *et al*., 2014; Arnaldi *et al*., 2015), it would be worthwhile to confirm our findings using a SERT-specific PET tracer, that, however, it is not currently approved for clinical use, nor it is widely available. Moreover, while different tests have been proposed to probe ToM, here we focused

only on the RMET, which probes the more affective facets of ToM, thus caution is needed when generalizing these findings to the other components of ToM. Lastly, the lack of [^{123}I]FP-CIT-SPECT data in HC who completed the RMET, due to radioprotection concerns, suggests caution in the generalization of the results. Future studies in HC, focused on the use of MRI-based markers of dopaminergic damage are warranted.

Conclusions

Using a multi-modal and multi-tracer molecular imaging approach and quantitative EEG we were able to point to an independent contribution of the insula and the superior temporal gyrus degeneration, as well as of the serotonergic system on ToM abilities in *de novo* PD patients. These findings point to the presence of a specific cortical and neurochemical signature of ToM in PD and suggest possible therapeutic targets to treat social cognition deficits in this population.

4. Dopaminergic and serotonergic degeneration and cortical metabolism in *de novo* Parkinson's Disease.

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Abstract

Degeneration of the nigrostriatal dopaminergic and the raphe-thalamic serotonergic systems is among the earliest changes observed in Parkinson's Disease. The consequences of those changes on brain metabolism are poorly understood, especially regarding their impact on the cortex.

Using multi-tracer molecular imaging, we assessed in 96 drug-naïve *de novo* Parkinson's disease patients (age 71.9±7.5 years) the association between cortical metabolism and dopaminergic and serotonergic systems deafferentation of either striatum or thalamus, and then we explored whether this association was mediated by either striatum or thalamus metabolism.

We acquired brain [^{18}F]FDG-PET images as a marker of neurodegeneration and [^{123}I]FP-CIT-SPECT as a marker of dopaminergic impairment in the striatum and of serotonergic deafferentation in the thalamus.

We found that [^{123}I]FP-CIT specific-to-non displaceable binding ratio (SBR) and glucose metabolism positively correlated one another in the striatum and thalamus. Using a voxel-wise approach, we observed a direct correlation between temporo-parietal metabolism and caudate dopaminergic innervation, as well as a direct correlation between prefrontal metabolism and thalamus serotonergic innervation. The effect of caudate [^{123}I]FP-CIT SBR values on temporo-parietal metabolism was mediated by caudate metabolic values (percentage mediated 89%, p-value= 0.008), and the effect of thalamus [^{123}I]FP-CIT SBR values on prefrontal metabolism was fully mediated by thalamus metabolic values ($p < 0.001$).

These data shed light on the impact of diffuse projection systems degeneration on cortical metabolism in Parkinson's Disease as well as on their regional specificity, and provide novel evidence on the role played by deep grey matter in this population.

Introduction

The degeneration of diffuse monoaminergic projection systems due to alpha-synuclein accumulation in the brainstem is one of the earliest changes observed in Parkinson's Disease (PD, Dickson *et al.*, 2009).

According to current models, cortical degeneration becomes more evident later on in the disease course compared to brainstem pathology at least in the majority of PD patients. An open question remains regarding the association between cortical and deep grey matter neurodegeneration, on one side, and monoaminergic systems degeneration on the other. This question is of clinical interest, as it relates to the understanding of neurodegeneration progression, and could provide novel therapeutic insights.

Drug-naive, *de novo* PD patients represent a suitable model to study the relationships between monoaminergic degeneration and cortical function, given the presence of monoaminergic system deficits early on in the disease course (Poewe *et al.*, 2017), the relatively modest presence of cortical pathology (Schindlbeck *et al.*, 2020) at this stage of the disease, and the lack of confounding factors from dopaminergic medications use.

Here, we thus decided to study the relationship between the nigrostriatal and raphe-thalamic systems and cortical and deep grey matter degeneration.

The available in vivo evidence regarding the issue of the impact of monoaminergic deafferentation on grey matter metabolism and neurodegeneration, is still patchy and mainly focused on the nigrostriatal dopaminergic (DA) system, with heterogeneous results showing associations between cognitive striatum DA and the limbic system metabolism (Apostolova *et al.*, 2020) or putaminal DA deafferentation and premotor and prefrontal hypometabolism (Berti *et al.*, 2010).

The rationale of the choice of only two among the many different diffuse projection systems is three-fold. Firstly, DA and the raphe-thalamic serotonergic (SE) systems can be studied in vivo using robust approaches with [^{123}I]Ioflupane Single Photon Emission Computed Tomography ([^{123}I]FP-CIT-SPECT) analysis. Secondly, the direct projection of these systems to the striatum and thalamus, associated with their sparse direct cortical projections, allows to easily study both the direct and the indirect effects of monoaminergic deafferentation on striatum and thalamus. Lastly, the extent

of their degeneration in PD is both well characterized from a neuropathological standpoint and highly relevant from a clinical one.

Indeed, while [^{123}I]FP-CIT-SPECT is used in clinical practice as a marker of DA nigrostriatal degeneration, thanks to its high affinity to the dopamine transporter (DAT) in the basal ganglia, it has also been used to study SE neurodegeneration, thanks to its affinity to the serotonin transporter (SERT) (Roselli *et al.*, 2010). In fact, beside DAT, [^{123}I]FP-CIT also shows high affinity for SERT (KI 9.73), with a DAT/SERT selectivity of 2.8 (Roselli *et al.*, 2010; Pilotto *et al.*, 2019). DAT and SERT display a non-overlapping distribution in the brain: DAT levels are higher in the basal ganglia, whereas SERT is highly expressed in the thalamus, where DAT expression is very low. Thus, as previously reported, [^{123}I]FP-CIT-SPECT signal in the thalamus represents an indirect marker of SERT availability (Koch *et al.*, 2014; Arnaldi *et al.*, 2015).

Here we thus combined [^{123}I]FP-CIT-SPECT data with ^{18}F-fluorodeoxyglucose Positron Emission Tomography ([^{18}F]FDG-PET) imaging (used as a proxy marker of neurodegeneration) to assess in drug-naïve, *de novo* PD patients the following open questions:

i) The association between striatal and thalamic metabolism and DA and SE deafferentation;

ii) The association between striatal and thalamic metabolism and DA and SE deafferentation and the cortical metabolic pattern on [^{18}F]FDG-PET;

iii) The possible mediatory role of striatal and thalamic metabolism on the association between the nigrostriatal DA and raphe-thalamic SE pathways integrity on the one side, and cortical metabolism on the other side.

Materials and Method

Patients

In a period of six years, we examined 96 consecutive drug-naïve, *de novo* PD patients who underwent a full neuropsychological assessment, a brain [^{18}F]FDG-PET and [^{123}I]FP-CIT-SPECT imaging in the three months following clinical diagnosis (**Table 1**). The inclusion criteria were a diagnosis of PD (Gelb *et al.*, 1999), confirmed by evidence of DA deficit on [^{123}I]FP-CIT-SPECT and by at least two-year follow-up.

The exclusion criteria were use of dopaminergic or serotonergic medication, the presence of neuropsychiatric or medical comorbidities, and dementia.

At the time of examinations, subjects gave their written consent for use of their anonymized data for research purposes, according to the protocol approved by our institutional review board. The study was conducted in respect of the Helsinki declaration.

Table 1: Demographic and clinical characteristics of Parkinson's Disease group. Values are reported as mean ± standard deviation.

	Parkinson's Disease (n=96)
Age (years)	71.91±7.49
Education (years)	10.86±4.08
Gender (M:F)	59:37
MMSE score	28.07 ± 2.13
MDS-UPDRS-III score	20.61 ± 9.16
GDS-15 score	3.57 ± 2.93

Legend: M = male; F= female; MMSE = Mini Mental State Examination; MDS-UPDRS-III = Movement Disorders Unified Parkinson's Disease Rating Scale, motor section; GDS = 15-item Geriatric Depression Scale.

[^{18}F]FDG-PET acquisition

Patients underwent brain [^{18}F]FDG-PET scan, acquired according to the guidelines of the European Association of Nuclear Medicine (Varrone *et al.*, 2009).

Patients fasted for at least six hours. Before radiopharmaceutical injection, blood glucose was checked and was found to be lower than 7.8 mmol/l in all cases. After 10 minutes in a silent and obscured room, with eyes open and ears unplugged, subjects were injected with approximately 200 MBq of [^{18}F]FDG. The subjects remained in the

room for 30 minutes after the [^{18}F]FDG injection and then moved to the PET room where scanning started around 45 minutes after the injection. A polycarbonate head holder was used to reduce head movements during the scan. Images were acquired using a SIEMENS Biograph 16 PET/CT equipment with a total axial field of view of 15 cm and no interplane gap space. Attenuation correction was based on CT. Data were reconstructed through an ordered subset-expectation maximization (OSEM) algorithm, 16 subset and 6 iterations, with a reconstructed voxel size of 1.33×1.33×2.00 mm.

[^{18}F]FDG-PET images were subjected to affine and nonlinear spatial normalization into Talairach and Tournoux space using SPM12 (Wellcome Department of Cognitive Neurology, London, UK). All the default choices of SPM were followed with the exception of spatial normalization. For this study, an optimized brain [^{18}F]FDG-PET template was used.(Della Rosa *et al.*, 2014) Normalized images were then smoothed with a 10-mm isotropic Gaussian filter. The WFUPickAtlas toolbox was used to create bilateral masks for the caudate, putamen and thalami in MNI space and their mean [^{18}F]FDG counts were computed from those masks and normalized over the whole brain counts.

[^{123}I]FP-CIT-SPECT

[^{123}I]FP-CIT-SPECT data was acquired by means of a 2-headed Millennium VG camera (G.E. Healthcare). Acquisition started between 180 and 240 minutes after injection of [^{123}I]FP-CIT and lasted 40 minutes. A "'step-and-shoot'" protocol was applied with a radius of rotation < 15 cm, and 120 projections evenly spaced over 360° were generated. Total counts ranged between 2.0 and 2.5 million. The pixel size of the acquisition matrix was 2.4 mm, thanks to an electronic zoom (zoom factor = 1.8) applied in the data collection phase. In the reconstruction phase, also a digital zoom was used, and the resulting images were sampled by isotropic voxels with 2.33 mm sides. Projections were processed by means of the OSEM algorithm (8 iterations, 10 subsets) followed by post filtering (3D Gaussian filter with full width-half maximum = 8 mm). The OSEM algorithm included a proback pair accounting for collimator blur and photon attenuation. No compensation for scatter was performed. The 2D+1 approximation was applied in the simulation of the space-variant collimator blur,

whereas photon attenuation was modelled with the approximation of a linear coefficient uniform inside the skull and equal to 0.11 cm−1. The reconstructed [^{123}I]FP-CIT-SPECT images were processed using the BasGan software version 2 based on a high definition, 3D striatal template, derived from Talairach's atlas.(Nobili *et al.*, 2013) Using this approach, an optimization protocol automatically performs fine adjustments in the positioning of blurred templates to best match the radioactive counts, and locates an occipital region of interest for background evaluation. Partial volume effect (PVE) correction is included in the process of uptake computation of caudate, putamen, and background. The partial volume effect correction performed by the method consists of an activity assignment in a Talairach-Tornoux atlas-based 3-compartment model of basal ganglia. Background uptake was subtracted by putamen and caudate uptake as follows (caudate or putamen uptake – background uptake)/background uptake, to generate specific to non-displaceable binding ratio (SBR) values. A single [^{123}I]FP-CIT SBR value was computed for the caudate (representing the mean between the left and right caudate nuclei) and for the putamen (representing the mean between the left and right caudate nuclei).

In order to study SERT binding, a customized brain [^{123}I]FP-CIT-SPECT template was built from 20 brain MRI and SPECT scans of healthy subjects as previously described, (Arnaldi *et al.*, 2015) using SPM 12. The SPECT images of the enrolled patients were then normalized to the MNI space in SPM12 and smoothed (3D gaussian filter with 10 mm full-width at half-maximum) (Pilotto *et al.*, 2019). A single region of interest (ROI) for the thalamus was created using the WFUPickAtlas toolbox, combining the left and right thalamic mask. Background uptake was subtracted from this ROI as follows (ROI − background uptake)/background uptake.

Statistical analysis

Overview

Firstly, we evaluated the association between striatum and thalamus whole brain-normalized [^{18}F]FDG-PET counts and [^{123}I]FP-CIT specific to non-displaceable binding ratio (SBR) values in the same regions. Then, we evaluated the correlation between Caudate (C), Putamen (P) and Thalamus (T) [^{123}I]FP-CIT SBR values and cortical metabolism using a voxel-wise approach. In the resulting regions, whole-brain normalised [^{18}F]FDG counts were computed and labelled as C_{SBR} ROI values, P_{SBR} ROI values and T_{SBR} ROI values, respectively (**Table 2**). Lastly, we performed a mediation analysis between striatum and thalamus [^{123}I]FP-CIT SBR values and C_{SBR}, P_{SBR} and T_{SBR} ROIs values, respectively, using striatum or thalamus [^{18}F]FDG values as mediator's variable.

Table 2: Summary of imaging metrics.

Variable Name	Description
Caudate [^{123}I]FP-CIT SBR	Caudate [^{123}I]FP-CIT uptake / Occipital CIT uptake
C_{SBR} ROI	Voxel-wise correlation between cortical [^{18}F]FDG values and C [^{123}I]FP-CIT SBR values
Putamen [^{123}I]FP-CIT SBR	Putamen [^{123}I]FP-CIT uptake / Occipital CIT uptake
P_{SBR} ROI	Voxel-wise correlation between cortical [^{18}F]FDG values and P[^{123}I]FP-CIT values
Thalamus [^{123}I]FP-CIT SBR	Thalamus [^{123}I]FP-CIT uptake / Occipital CIT uptake
T_{SBR} ROI	Voxel-wise correlation between cortical [^{18}F]FDG values and T[^{123}I]FP-CIT values

Legend: C = Caudate; P = Putamen; T = Thalamus; SBR = specific to non-displaceable binding ratio.

Correlations between striatal and thalamic [^{18}F]FDG and [^{123}I]FP-CIT SBR values

Firstly, to assess the impact of monoaminergic deafferentation of the striatum and thalamus on their metabolism, we run the following partial correlations, correcting for age and MDS-UPDRS-III score:

i) mean [^{123}I]FP-CIT SBR with mean normalized [^{18}F]FDG values both computed in the caudate (values for the left and right caudate combined, as in all following analyses);
ii) mean [^{123}I]FP-CIT SBR with mean normalized [^{18}F]FDG values in the putamen;
iii) mean [^{123}I]FP-CIT SBR with mean normalized [^{18}F]FDG values in the thalamus.

Voxel-wise correlation between cortical [^{18}F]FDG and striatal and thalamic [^{123}I]FP-CIT SBR values

Using Statistical Parametric Mapping 12 (SPM12) software, we run the following voxel-wise analyses on [^{18}F]FDG images, using age and mean MDS-UPDRS-III score as nuisance variables, and restricting our analysis to the cortical mantle, to assess the direct correlation between cortical [^{18}F]FDG and:

i) mean caudate [^{123}I]FP-CIT SBR values, to obtain C_{SBR} ROI;
ii) mean putamen [^{123}I]FP-CIT SBR values , to obtain P_{SBR} ROI;
iii) mean thalamus [^{123}I]FP-CIT SBR values, to obtain T_{SBR} ROI.

We set a height threshold of $p < 0.001$, uncorrected for multiple comparisons at the peak level; at cluster level, the accepted threshold of statistical significance was $p<0.05$, family-wise error corrected for multiple comparisons. We considered only significant clusters containing at least 100 voxels. Lastly, we normalized over the whole brain the [^{18}F]FDG mean values extracted from the three aforementioned ROIs.

Mediation analyses

To explore the possible mediatory role of striatal and thalamic metabolism on the association between striatal or thalamic [^{123}I]FP-CIT SBR and cortical metabolism, we

run the following partial correlations, correcting for age and mean MDS-UPDRS-III score:

i) mean caudate [^{18}F]FDG values with C_{SBR} ROI values;
ii) mean putamen [^{18}F]FDG values with P_{SBR} ROI values;
iii) mean thalamus [^{18}F]FDG values with T_{SBR} ROI values.

Lastly, we used the mediation package of the R software to run the following causal mediation analyses, using a bootstrap approach (1000 permutations) and correcting for age and mean MDS-UPDRS-III score.

i) independent variable: mean caudate [^{123}I]FP-CIT SBR values; mediatory variable: mean caudate [^{18}F]FDG values; dependent variable: C_{SBR} ROI values;
ii) independent variable: mean putamen [^{123}I]FP-CIT SBR values; mediatory variable: mean putamen [^{18}F]FDG values; dependent variable: P_{SBR} ROI values;
iii) independent variable: mean thalamus [^{123}I]FP-CIT SBR values; mediatory variable: mean thalamus [^{18}F]FDG values; dependent variable: T_{SBR} ROI values.

For all statistical analyses p threshold was set at 0.05 unless otherwise specified.

Results

Correlations between striatal and thalamic [^{18}F]FDG and [^{123}I]FP-CIT SBR values

We found a significant positive correlation between caudate [^{123}I]FP-CIT SBR values and [^{18}F]FDG uptake in the bilateral caudate (p=0.001, r=0.331), a significant positive correlation between putamen [^{123}I]FP-CIT SBR values and [^{18}F]FDG uptake in the bilateral putamen (p=0.001, r=0.423), and a significant positive correlation between thalamus [^{123}I]FP-CIT SBR values and [^{18}F]FDG uptake in the bilateral thalami (p=0.001, r=0.541).

Voxel-wise correlation between cortical [^{18}F]FDG and striatal and thalamic [^{123}I]FP-CIT SBR values

Caudate [^{123}I]FP-CIT SBR values showed a significant direct correlation with bilateral posterior temporo-parietal [^{18}F]FDG uptake (**Figure 1-A**, **Table 3**). Also, thalamus

[^{123}I]FP-CIT SBR values showed a direct significant correlation with prefrontal [^{18}F]FDG uptake (**Figure 1-B**, **Table 4**). Instead, we did not find a significant correlation between putamen [^{123}I]FP-CIT SBR values and cortical [^{18}F]FDG uptake.

Figure 1: A) Results of the voxel-wise correlation between Caudate [^{123}I]FP-CIT SBR and cortical [^{18}F]FDG values. **B)** Results of the voxel-wise correlation between Thalamus [^{123}I]FP-CIT SBR and cortical [^{18}F]FDG values.

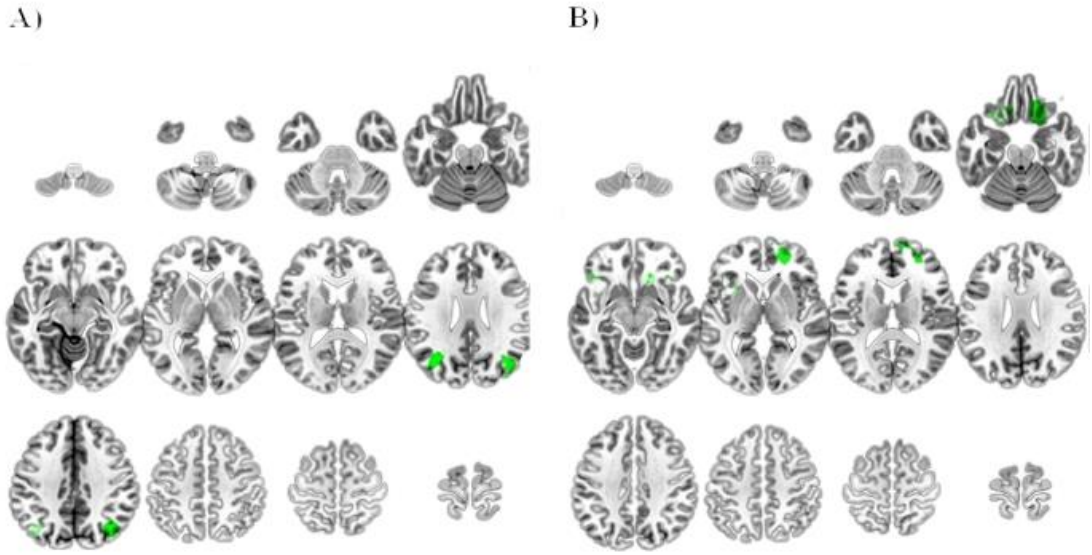


Table 3: Results of the correlation between Caudate [^{123}I]FP-CIT SBR and cortical [^{18}F]FDG values

N. voxel	Coordinates			Laterality	Anatomic Area	T	Z
	X	Y	Z				
631	33.58	-68.26	29.01	Right	Precuneus	4.72	4.46
	33.57	-82.99	25.81	Right	Superior Occipital Gyrus	3.60	3.47
509	-34.81	-67.21	20.74	Left	Middle Temporal Gyrus	4.66	4.41

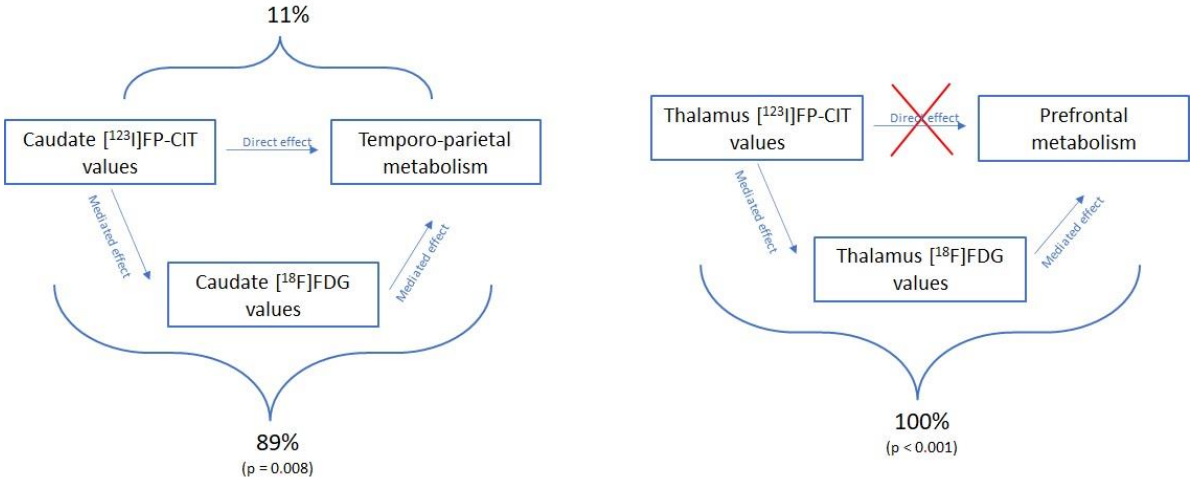
Table 4: Results of the correlation between Thalamus [^{123}I]FP-CIT SBR and cortical [^{18}F]FDG values

N. voxel	Coordinates			Laterality	Anatomic Area	T	Z
	X	Y	Z				
634	15.92	25.8	-9.22	Right	Inferior Frontal Gyrus	4.74	4.47
	15.93	13.11	-14.03	Right	Medial Frontal Gyrus	4.14	3.95
	30.67	21.65	-5.77	Right	Inferior Frontal Gyrus	3.84	3.69
	8.64	37.71	-15.43	Right	Medial Frontal Gyrus	3.35	3.25
710	-19.26	13.11	-12.82	Left	Subcallosal Gyrus	3.60	3.47
	-11.8	24.43	-13.43	Left	Subcallosal Gyrus	3.39	3.28
	-32.48	7.54	6.24	Left	Claustrum	3.38	3.27
585	17.56	42.51	12.2	Right	Medial Frontal Gyrus	4.32	4.11
	24.92	43.98	16.07	Right	Superior Frontal Gyrus	4.19	3.99
	8.27	56.94	18.82	Right	Medial Frontal Gyrus	4.06	3.89
	17.56	53.51	15.05	Right	Superior Frontal Gyrus	3.95	3.79

Mediation analyses

The mediation analysis revealed that the effect of caudate [^{123}I]FP-CIT SBR values on C_{SBR} ROI values was mediated by caudate [^{18}F]FDG uptake (percentage mediated 89%, p=0.008). In the same way, mediation analysis revealed that the effect of thalamus [^{123}I]FP-CIT SBR values on T_{SBR} ROI values was fully mediated by thalamus [^{18}F]FDG uptake (p<0.001), meaning that thalamus [^{123}I]FP-CIT SBR values no longer affects T_{SBR} ROI values after thalamus [^{18}F]FDG uptake has been controlled for. Given the lack of correlation between putamen [^{123}I]FP-CIT SBR values and cortical [^{18}F]FDG uptake, no mediation analysis was run for the putamen. (**Figure 2**)

Figure 2: Results of the mediation analysis.



Discussion

In this study we aimed to assess the association between cortical metabolism and DA and SE deafferentation of either striatum or thalamus, and then to explore whether this association was mediated by either striatal or thalamic metabolism. First, we observed a significant direct correlation between either DA or SE innervation and striatal or thalamic metabolism, respectively. We then observed a significant direct correlation between bilateral temporo-parietal metabolism and caudate DA innervation, as well as a significant correlation between prefrontal metabolism and thalamus SE innervation. Lastly, we observed that the association between DA and SE systems integrity and cortical metabolism was mediated by either striatal or thalamic metabolism.

Correlations between striatal and thalamic [^{18}F]FDG and [^{123}I]FP-CIT SBR values

We found a significant positive correlation between striatal and thalamic [^{18}F]FDG values and [^{123}I]FP-CIT SBR values, meaning that DA expression directly affects striatal metabolic levels and SE expression directly affects thalamic metabolic levels. In animal models of neurodegeneration, dopamine is thought to impact on synapse viability as shown by the association of DA deafferentation with reduction of synapse density, simplification of neurite arborization of striatal neurons (Alberquilla *et al.*, 2020), and a reduction of excitatory neurotransmitters release (Caravaggio *et al.*,

2016). These observations represent a possible biological explanation of the direct correlation observed in our patient group between striatal [^{18}F]FDG uptake and striatal [^{123}I]FP-CIT SBR values.

In PD, data on the association between striatum or thalamus metabolism and DA are quite heterogeneous both regarding the target patient populations and the pattern of the correlations. Eggers and colleagues (Eggers *et al.*, 2014), for example, explored the differences in striatal DA innervation and [^{18}F]FDG metabolism between tremor-dominant and akinetic-rigid types of PD, showing a difference in the strength of the direct association of DA denervation and ventral striatum glucose metabolism between the two clinical subtypes (Eggers *et al.*, 2014). Conversely, Apostolova et al. (Apostolova *et al.*, 2020) and Berti et al. (Berti *et al.*, 2010), failed to report a correlation between striatal [^{18}F]FDG uptake and striatal DA. Some methodological issues could explain such heterogeneous results, including the patients sample size and inclusion/exclusion criteria, as well as the striatum ROI drawing choice. In the present study, to improve the reliability of the results, we used stringent inclusion/exclusion criteria, a large series of consecutive drug-naïve PD patients and adopted a three-dimensional, automatic ROI drawing tool focusing on the whole striatum (rather than on its sub-parts, as in Eggers et al. (Eggers *et al.*, 2014)).

As the DA system, also the SE fibres are known to be altered in PD, and to play a role in grey matter tropism. Indeed, loss of SE neurons in the raphe nuclei is thought to be present early on in PD and to be associated with both motor and non-motor symptoms (Politis and Niccolini, 2015).

SE is known to modulate excitatory amino acid responses and synaptic activity in the thalamus in a non-linear fashion (Eaton and Salt, 1989), and to impact on thalamic metabolism. Indeed, functional MRI data collected during an acute citalopram challenge showed that increased SE availability was associated with an increase of the hemodynamic response in the striatum and thalamus (McKie *et al.*, 2005).

In neurodegenerative diseases, SE fibre loss has been associated with grey matter volume reduction in its projection cortices. In Alzheimer's Disease, for example, serotonin-1A receptor density in the midbrain raphe, assessed with a receptor-specific PET radioligand, was associated with grey matter volume loss in those frontal regions

richer in SE afferents (Kraus *et al.*, 2012), in line with the proposed neurotrophic role of SE (Daubert and Condron, 2010).

These data provide the context to interpret the observed association between thalamic metabolism and SE innervation, thus expanding the findings in the aforementioned studies also to PD, and suggesting that the reported associations between SE and PD symptomatology may be affected by the impact of SE innervation on the thalamus. While this hypothesis needs to be further explored, it is worthwhile to point out that this interpretation is in line with the reported association of tremor (i.e. one of the motor symptoms more often associated with SE dysfunction in PD) (Politis and Niccolini, 2015) and thalamic abnormalities (Duval *et al.*, 2016).

Correlation between caudate [^{123}I]FP-CIT SBR, caudate metabolism and cortical metabolism

We found a significant positive correlation between caudate DA innervation and posterior temporo-parietal cortical metabolism; this correlation, even taking into account differences in cognitive and motor symptoms, was mediated by caudate metabolic levels.

This finding is in line with the role in PD-related cognitive impairment played by both caudate DA loss and posterior cortical dysfunction. Indeed, previous studies showed that cognitive decline in PD is mainly associated with posterior parieto-temporo-occipital [^{18}F]FDG hypometabolism (Wu *et al.*, 2018), both in early PD (Firbank *et al.*, 2017) and in PD-related dementia (Bohnen *et al.*, 2011). Moreover, also caudate DA deficits have been linked with cognitive abnormalities, especially in the earliest phases of the disease (Jokinen *et al.*, 2009), and to be associated with progression to PD-related dementia (Bohnen *et al.*, 2011), and overall with a worse neuropsychological outcome at 4-year follow-up (Pasquini *et al.*, 2018). Lastly, caudate DA level has been associated with cognitive performance in healthy subjects in [^{18}F]Fluordopa PET studies (Vernaleken *et al.*, 2007).

Our results expand previous observations of an association between nigrostriatal degeneration at baseline, posterior cortical atrophy and cognitive impairment in PD after one year from imaging (Sampedro *et al.*, 2019).

Taking into account the spatial distribution of nigro-caudate DA fibers, it is unlikely that the observed association between posterior cortical metabolism and caudate DA is due to a direct effect of DA, also given the lack of correlation between DA cell's loss in the substantia nigra and in the ventral-tegmental area (i.e., the source of cortical DA innervation). It is also unlikely that our observation is due to a temporal synchronization of alpha-synuclein deposition in the cortex and in the substantia nigra, as in current models the temporal pattern of Lewy Bodies deposition in the cortex is significantly delayed and independent from the degree of DA neurons loss in the brainstem (Jellinger, 2009).

The aforementioned studies suggest that a direct effect of nigro-caudate degeneration on cortical metabolism is unlikely and thus point to an indirect effect mediated by other brain structures.

In line with this, here we showed that the correlation between cortical metabolism and nigro-caudate degeneration is mediated by caudate metabolism.

We did not find a correlation between putamen SBR values and cortical [^{18}F]FDG uptake, this suggests that the impairment of nigro-putaminal DA deafferentation is not associated with changes in cortical metabolism at least in the early phases of the disease, in line with the pattern of alpha-synuclein distribution over time (Braak *et al.*, 2003).

Correlation between thalamic [^{123}I]FP-CIT SBR, thalamic metabolism and cortical metabolism

We found a significant positive correlation between thalamic SE innervation and prefrontal metabolism; this correlation, even taking into account differences in cognitive and motor symptoms, was mediated by differences in thalamic metabolism. Previous studies have shown an association between different facets of PD-related symptoms and both SE and prefrontal territories. Theory of mind (ToM), for example, is thought to be mediated by a vast cortical network focused on the prefrontal cortex in different conditions including PD, and it has been shown to be also associated with SE deafferentation in drug-naive PD patients (Bora *et al.*, 2015, Orso *et al.*, 2020*a*). Beside social cognition, also neuropsychiatric symptomatology has been associated with both SE and prefrontal functions in subjects with PD. Depression in subjects with

PD has been associated with reduced activity of the SE system (as shown by reduced levels of SE metabolites in the cerebrospinal fluid and of SE receptors both in PET and post-mortem studies) (Mayeux *et al.*, 1984; Sharp *et al.*, 2008; Politis and Niccolini, 2015) as well as with grey matter loss and functional alterations in the prefrontal cortex (Wen *et al.*, 2016), while similar findings have been reported also for anxiety-spectrum symptomatology (Politis and Niccolini, 2015).

Moreover, the observation of a mediatory role of thalamic metabolism on the association between SE function and prefrontal cortex activity is in line with the known anatomical distribution of the raphe-thalamic SE fibers and with the spatial distribution of the thalamic projections to the cortex (Planetta *et al.*, 2013). Indeed, serotonergic afferents to the thalamus mainly target the midline and intralaminar higher order nuclei (Cropper *et al.*, 1984; Varela, 2014), which in turn are known to present with rich cortical projections, especially to the more anterior and medial cortical regions (Saalmann, 2014). While these findings are focused on the raphe-thalamic SE pathway, future studies are warranted to explore the role of deep grey matter metabolism on the association between cortical function and the other components of the SE system.

Strength and limitations

We used [^{123}I]FP-CIT-SPECT metrics to evaluate dopaminergic and serotonergic functions. Compared to PET, SPECT has a relatively low spatial resolution, and this thus did not allow to separately study mesocortical projections. Furthermore, we did not use a specific serotonergic marker, instead we use the [^{123}I]FP-CIT SBR in the thalami, taking advantage of the [^{123}I]FP-CIT affinity for SERT, and also the lack of DAT in the thalami (Roselli *et al.*, 2010; Pilotto *et al.*, 2019). While this approach has been previously used in a number of studies, (Koch *et al.*, 2014; Arnaldi *et al.*, 2015) it would be worthwhile to confirm our findings using a SERT-specific PET tracer, that, however, it is not currently approved for clinical use nor is widely available.

These limitations are however balanced by the large number of enrolled patients, the lack of confounding factors from drugs and the use of two molecular imaging techniques both acquired for all patients in the same center.

Conclusions

Here, we reported the association between nigro-striatal DA and raphe-thalamic SE function and cortical metabolism in PD and the mediatory effect on this association of deep grey matter metabolism. These data expand on the role played by diffuse projection systems in PD physiopathology.

5. The role of monoaminergic tones and brain metabolism in cognition in *de novo* Parkinson's Disease

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Abstract

Cognitive impairment is frequent in Parkinson's Disease (PD) and several neurotransmitter changes have been reported since the time of diagnosis, although seldom investigated altogether in the same patient cohort.

Our aim was to evaluate the association between neurotransmitter impairment, brain metabolism and cognition in a cohort of *de novo*, drug-naïve PD patients.

We retrospectively selected 95 consecutive drug-naïve PD patients (mean age 71.89±7.53) undergoing at the time of diagnosis a brain [^{18}F]FDG-PET as a marker of brain glucose metabolism and proxy measure of neurodegeneration, [^{123}I]FP-CIT-SPECT as a marker and dopaminergic deafferentation in the striatum and frontal cortex, as well as a marker of serotonergic deafferentation in the thalamus, and quantitative electroencephalography (qEEG) as an indirect measure of cholinergic deafferentation. Patients also underwent a complete neuropsychological test battery. Positive correlations were observed between (i) executive functions and left cerebellar cortex metabolism, (ii) prefrontal dopaminergic expression and working memory ($r=0.304$, $p=0.003$), (iii) qEEG slowing in the posterior leads and both memory ($r=0.299$, $p=0.004$) and visuo-spatial functions ($r=0.357$, $p<0.001$).

In *de novo*, drug-naïve PD patients, the impact of regional metabolism and diffuse projection systems degeneration differs across cognitive domains. These findings suggest possible tailored approaches to the treatment of cognitive deficits in PD.

Introduction

Cognitive impairment is a frequent finding in subjects with Parkinson's Disease (PD) and represents a key determinant of quality of life in this population (Fengler *et al.*, 2017; Goldman *et al.*, 2018; Heinzel *et al.*, 2019).

The main pathological hallmark in the early stage of the disease is the intracellular fibrillization of α-synuclein, along with Lewy-body brainstem pathology (Emre, 2003; Calabresi *et al.*, 2006; Kehagia *et al.*, 2013). Cognitive deficits in PD seem to be due to the uneven degeneration of several neurotransmitters systems, reflecting the complexity of the disease evolution (Kehagia *et al.*, 2013). Drug-naïve, *de novo* PD patients represent a suitable model to study the relationships among monoaminergic degeneration, regional neurodegeneration and cognitive functions, given the presence of monoaminergic system deficits early on in the disease course (Poewe *et al.*, 2017), the relatively modest presence of cortical pathology (Schindlbeck *et al.*, 2020) at this stage of the disease, and the lack of confounding factors from dopaminergic medications use.

In the last decade, several studies have been focused on the relationship between cognition and monoaminergic system degeneration (Kehagia *et al.*, 2010), and found that impaired nigrostriatal dopaminergic innervation in the caudate, anterior putamen, and ventral striatum was directly associated with attention/working memory, executive, and visuospatial functions (Sawamoto *et al.*, 2008; Polito *et al.*, 2012; Niethammer *et al.*, 2013; Chung *et al.*, 2018). Beside dopaminergic degeneration, also reduction of raphe serotonin receptors density and cortical cholinergic receptors, have been correlated both with non-motor symptoms (Politis and Niccolini, 2015) and the severity of cognitive impairment in a cohort of drug-naïve, *de novo* Parkinson's patients (Xu *et al.*, 2012; Kehagia *et al.*, 2013). Also, quantitative EEG (qEEG) studies have consistently shown the slowing down of background rhythm early on in the course of Lewy body diseases, thought to reflect mainly cortical cholinergic deafferentation (Geraedts *et al.*, 2018; Massa *et al.*, 2020).

Another neuroimaging modality, i.e., brain [^{18}F]FDG-PET, can track metabolic changes reflecting both neurodegeneration and synaptic dysfunction in vivo. [^{18}F]FDG-PET studies have indeed demonstrated that an abnormal glucose brain metabolism profile is present at early stage of PD, especially in the lentiform nucleus,

the thalamus, as well as the lateral frontal, paracentral, inferior parietal, and parieto-occipital areas, known as Parkinson's Disease-Related Pattern (PDRP) (Eidelberg *et al.*, 1994). Similarly, Huang et al. (2007) (Huang *et al.*, 2007) described the Parkinson's Disease Cognitive Pattern (PDCP), characterized by relative hypometabolism in frontal and parietal association areas, along with relatively preserved metabolic levels in the cerebellar vermis, the dentate nuclei, correlating with executive functions tasks (Huang *et al.*, 2007, 2008; Meles *et al.*, 2020). From these findings, new interests rose in correlating those patterns with clinical and imaging variables. For example, it has been seen that, using [^{123}I]FP-CIT-SPECT and [^{18}F]FDG-PET, a reduced striatal DAT binding along with an increase in the expression of the PDRP is present at early stage of PD (Peng *et al.*, 2021), as well as a loss within the default mode network metabolism with an increased expression in the PDCP (Schindlbeck *et al.*, 2021).

Based on these findings, and considering that few studies have combined information deriving from a multi-modal approach, we combined [^{123}I]FP-CIT-SPECT (used in the thalamus and the striatum as a marker of serotonergic and dopaminergic degeneration, respectively; as well as a marker of extra-striatal prefrontal cortical dopaminergic expression) (Koch *et al.*, 2014; Arnaldi *et al.*, 2015; Pilotto *et al.*, 2019), [^{18}F]FDG-PET (used as a marker of neurodegeneration, Jack Jr *et al.*, 2018), and quantitative EEG (qEEG, used as an indirect marker of cholinergic deafferentation, Prichep *et al.*, 2006; Wink *et al.*, 2006; Arnaldi *et al.*, 2017), in order to assess the potential impact of dopaminergic and serotonergic deafferentation, as well as of neurodegeneration and cholinergic tone, on the main cognitive domains in drug-naïve, *de novo* PD patients (Litvan *et al.*, 2012).

Materials and Methods

Patients

In a 3-year period, we examined 95 consecutive, drug-naïve, *de novo* PD patients (age: 71.8 ± 7.5 years; range: 50-84; 35 females; education: 10.9 ± 4.0 years) who underwent neuropsychological assessment (Mattioli *et al.*, 2021), brain [^{18}F]FDG-PET, [^{123}I]FP-CIT-SPECT and qEEG recording at the time of the diagnosis. The inclusion criteria were a diagnosis of PD (Gelb *et al.*, 1999), confirmed by evidence of dopaminergic deficit on [^{123}I]FP-CIT-SPECT and by at least two years of follow-up. The main exclusion criteria were use of dopaminergic, cholinergic, or serotonergic medications, as well as of other medication known to affect brain function, such as benzodiazepines, the presence of neuropsychiatric or medical comorbidities, presence of dementia, and lack of informed consent. **Table 1** shows the demographic, clinical and imaging characteristics of the PD group in more detail. Nevertheless, all study procedures were performed before the beginning of any dopaminergic, cholinergic and/or serotonergic medications. Structural imaging was available for all patients to help in the diagnostic process; however, MRI data were not used in the present study as all images were required using different scanners and protocols.

At the time of examinations, subjects gave their written consent for use of their anonymized data to research purposes, according to the protocol approved by our institutional review board. The study was conducted in respect of the Helsinki declaration.

Table 1: Demographic, clinical and imaging characteristics of Parkinson's Disease group. Values are shown as mean ± standard deviation (SD).

	Parkinson's Disease (n=95; mean ± SD)
Age (yr)	71.89 ± 7.53
Education (yr)	10.92 ± 4.06
Gender (M:F)	60:35
MMSE score	28.09 ± 2.13
MDS-UPDRS-III score	20.72 ± 8.79
MCI (Y:N)	46:49
Striatal and thalamic [^{123}I]FP-CIT SBR values	
Caudate [^{123}I]FP-CIT SBR values	2.23 ± 0.75
Putamen [^{123}I]FP-CIT SBR values	1.41 ± 0.57
Thalamus [^{123}I]FP-CIT SBR values	1.24 ± 0.18
Cortical [^{123}I]FP-CIT SBR values	
Prefrontal [^{123}I]FP-CIT SBR values	0.88 ± 0.13
qEEG Alpha/Theta ratio	
Posterior leads	2.17 ± 2.36

Legend: M = male; F= female; MMSE = Mini Mental State Examination; MDS-UPDRS-III = Movement Disorders Unified Parkinson's Disease Rating Scale, motor section; MCI = Mild Cognitive Impairment.

Neuropsychological evaluations and cognitive domain quantification

All subjects underwent a complete neuropsychological evaluation, as previously described (Mattioli *et al.*, 2021). Based on the criteria for the assessment of mild cognitive impairment (MCI) in PD (Litvan *et al.*, 2012), we grouped each tests into

five main cognitive domains (i.e., Executive Functions, Attention and Working Memory, Memory, Language and Visuo-Spatial Functions), then we compute a single score for each of these domains, based on previously published data (Litvan *et al.*, 2012). To this aim, we used a principal component analysis with fixed factors for each domain, using the raw scores of individual tests to represent each of the five domains. The first principal component for each domain was retained for further analyses.

[^{18}F]FDG-PET

[^{18}F]FDG-PET images were acquired as a marker of regional neurodegeneration. Brain [^{18}F]FDG-PET scan were acquired according to the guidelines of the European Association of Nuclear Medicine32, using a SIEMENS Biograph 16 PET/CT hybrid system with a total axial field of view of 15 cm and no interplane gap space. Attenuation correction was based on CT. Data were reconstructed through an ordered subset-expectation maximization (OSEM) algorithm, 16 subset and 6 iterations, with a reconstructed voxel size of 1.33×1.33×2.00 mm. All [^{18}F]FDG PET images were acquired in static mode and then subjected to affine and nonlinear spatial normalization into Talairach and Tournoux space using SPM12 (Wellcome Department of Cognitive Neurology, London, UK). All the default choices of SPM were followed except for spatial normalization. For this study, the $H_2{}^{15}O$ SPM-default template was replaced by an optimized brain [^{18}F]FDG-PET template as described by Della Rosa and colleagues (Della Rosa *et al.*, 2014). The spatially normalized set of images was then smoothed with a 10-mm isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. In order to exclude white matter voxels and to limit the amount of voxels from hypometabolic regions from the reference value of the global mean, we fixed a 'grey matter threshold' of 1.0 instead of the default value of 0.8 (Pagani *et al.*, 2014).

[^{123}I]FP-CIT-SPECT

[^{123}I]FP-CIT-SPECT images were used to study nigro-striatal and raphe-thalamic degeneration as well as prefrontal monoaminergic (i.e, dopaminergic and serotonergic) (Pilotto *et al.*, 2019) innervation.

[^{123}I]FP-CIT-SPECT data was acquired by means of a 2-headed Millennium VG camera (G.E. Healthcare), as previously described (Orso *et al.*, 2020*a*).

The reconstructed [^{123}I]FP-CIT-SPECT images were processed using the BasGan software version 2 based on a high definition, 3D striatal template, derived from Talairach's atlas (Nobili *et al.*, 2013). Partial volume effect (PVE) correction is included in the process of uptake computation of caudate, putamen, and the occipital region background. The partial volume effect correction performed by the method consists of an activity assignment in a Talairach-Tornoux atlas-based 3-compartment model of basal ganglia. Background uptake was subtracted by putamen and caudate uptake as follows (caudate or putamen uptake – background uptake)/background uptake, to generate specific to non-displaceable binding ratio (SBR) values. Partial volume correction, a feature included in the BasGan pipeline, allows to reduce the impact of the limited SPECT spatial resolution of the assessment of midline structures. A single [^{123}I]FP-CIT SBR value was computed for the caudate as well as for the putamen (representing the mean between the left and right nuclei SBR). In order to study SERT binding and prefrontal dopaminergic innervation, a customized brain [^{123}I]FP-CIT-SPECT template was built from 20 brain MRI and SPECT scans of healthy subjects as previously described, (Arnaldi *et al.*, 2015) using SPM12. The SPECT images of the enrolled patients were then normalized to the MNI space in SPM12 and smoothed (3D gaussian filter with 10 mm full-width at half-maximum) (Pilotto *et al.*, 2019). In order to study cortical dopaminergic activity, we created region of interest (ROI) masks for the prefrontal cortex, consisting of Brodmann Areas 10, 11, 46 and 47 in both hemispheres, and for the bilateral thalami, using the WFU Pickatlas toolbox (http://fmri.wfubmc.edu/software/pickatlas, Wake Forest University Health Sciences Medical Center Blvd. Winston-Salem, NC), then we evaluated [^{123}I]FP-CIT-SPECT SBR in these ROIs. Prefrontal cortex SBR was computed as follows: (ROI − background uptake)/background uptake. All data represent a mean value across the two hemispheres.

Quantitative EEG recording and posterior Alpha/Theta power ratio

Quantitative EEG (qEEG) was used as an indirect approach to study the cholinergic system integrity (Prichep *et al.*, 2006; Wink *et al.*, 2006).

EEG recordings were performed in the late morning to minimize drowsiness and obtained from patients seated in a comfortable chair with eyes closed. EEG electrodes were placed using standard 10–20 EEG electrode positions (Fp1, Fpz, Fp2, F3, Fz, F4, F7, F8, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2, T3, T4). Electrode impedances were closely monitored and kept below 5 kOhm. Data were acquired using LTM system (EBNeuro, Florence, IT) at a sampling rate of 512 Hz and a bandpass of 0.3–70 Hz. To monitor eye movements, the horizontal electro-oculogram was simultaneously recorded with the same recording parameters of EEG. An EEG technician was present during the entire recording session to keep constant the level of vigilance and to monitor signal quality. Standard longitudinal bipolar montage was used for analysis. EEG data were manually analyzed off-line to reject artifacts. One minute of artifact-free EEG data was used for further analysis. Segments were two seconds continuous with no overlap. The Fast Fourier Transform (FFT, Welch method), applied to 4-s segments (Tukey window) with 2-s overlaps, was used to compute EEG spectral power for each derivation. The EEG spectrum was then divided into the following frequency bands: delta (2.25-4 Hz), theta (4.25-8 Hz), alpha (8.25-12 Hz), sigma (12.25-16 Hz) and beta (16.25-32 Hz) and relative power was computed for each band as normalized to the total EEG power.

Based on mean EEG spectra, the ratio between alpha (8.25–12 Hz) and theta (4.25–8 Hz) band power (alpha/theta ratio) was computed in the posterior (P3/O1, P4/O2, T5/O1, T6/O2) cortical region as indirect proxies of the cholinergic tone (Prichep *et al.*, 2006; Wink *et al.*, 2006). Considering the right-skewed distribution of the alpha/theta ratio, the logarithmic (log-) transformation was applied in order to approximate a symmetric distribution of data.

We choose to explore the cholinergic tone expressed in the posterior leads since, in PD cohorts, deficits in cholinergic system exhibited mainly in the posterior cortices has been shown to be present at all stages of the disease, including non-demented subjects(Kuhl *et al.*, 1996), and to consequently correlate with slowing at qEEG in the posterior leads (Arnaldi *et al.*, 2017).

Statistical analysis

Statistics were performed using IBM SPSS Statistics 25 (Armonk, NY).

Partial correlations, correcting for age and MDS-UPRS-III score, were used to correlate each cognitive domain score with the [^{123}I]FP-CIT-SPECT SBR, both in the basal ganglia and the prefrontal mask, and qEEG metrics. Statistical threshold was set at 0.05 and p-values, both uncorrected and corrected using a false discovery rate (FDR) approach, were reported.

As for [^{18}F]FDG-PET, using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/), a whole-brain voxel-wise correlation between whole-brain scaled [^{18}F]FDG counts and each cognitive domain score was performed using a voxel height threshold $p<0.001$ (uncorrected) and a minimum cluster size of 100 voxels for significant clusters. Analyses were corrected for age and MDS-UPDRS-III score, the latter used as a marker of disease severity. At cluster level, the accepted threshold of statistical significance was $p<0.05$, family-wise error corrected for multiple comparisons.

Results

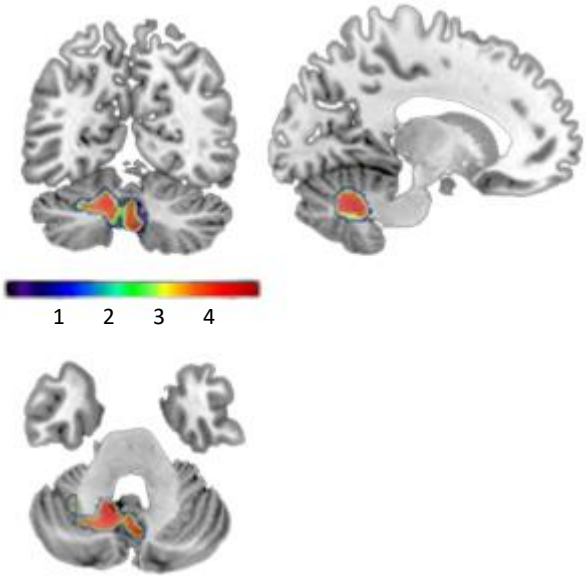
Executive functions

We found a positive correlation between executive functions and brain metabolic levels in the left cerebellar hemisphere, extending in the vermis. Resulting anatomic areas are shown in detail in **Table 2** and **Figure 1**.

Table 2: Anatomic areas resulting from the voxel-wise analysis between Executive Functions and frontal [^{18}F]FDG values. Analyses were corrected for age and MDS-UPDRS-III score.

N. voxel	Coordinates			Laterality	Anatomic Area	T	Z
	X	Y	Z				
624	-11.79	-46.73	-27.01	Left Cerebellum	Anterior Lobe	4.42	4.20
	-1.32	-50.43	-35.53	Left Cerebellum	Posterior Lobe	3.61	3.48

Figure 3: Results of the voxel-wise correlation analysis between executive functions and brain metabolism. Analyses were corrected for age and MDS-UPDRS-III score.



Attention and Working Memory

We found a positive correlation between attention and working memory and prefrontal [^{123}I]FP-CIT SBR values (p = 0.003). Results are shown in **Table 3** and **Figure 2**.

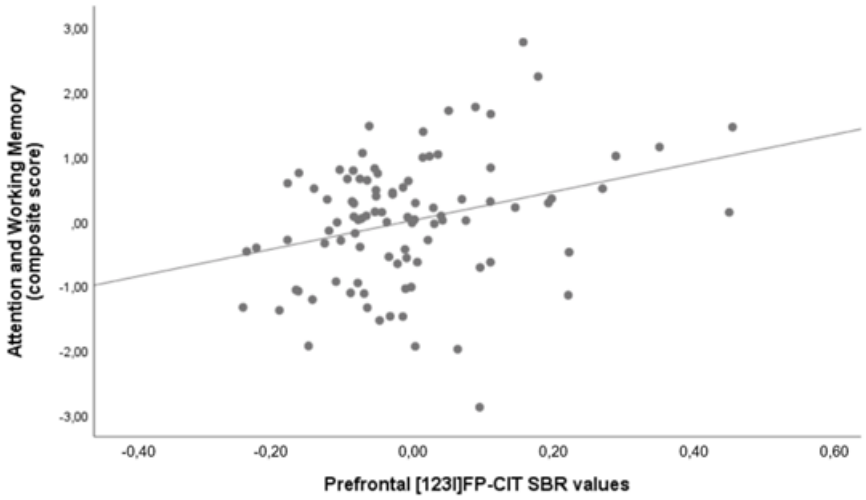
Table 3: Correlation between neuropsychological domain composite scores, striatal and thalamic [^{123}I]FP-CIT SBR values, frontal [^{123}I]FP-CIT SBR values and qEEG Alpha/Theta ratio. Analyses were corrected for age and MDS-UPDRS-III score.

	r values	p values (unc.)	FDR
Executive Functions			
Striatal and thalamic [^{123}I]FP-CIT SBR values			
Caudate [^{123}I]FP-CIT SBR values	-0.174	0.094	0.28
Putamen [^{123}I]FP-CIT SBR values	-0.083	0.432	0.54
Thalamus [^{123}I]FP-CIT SBR values	-0.030	0.779	0.77
Cortical [^{123}I]FP-CIT SBR values			

Prefrontal [^{123}I]FP-CIT SBR values	-0.139	0.186	0.31
qEEG Alpha/Theta ratio			
Posterior leads	0.112	0.112	0.28
Attention and Working Memory			
Striatal and thalamic [^{123}I]FP-CIT SBR values			
Caudate [^{123}I]FP-CIT SBR values	0.194	0.064	0.16
Putamen [^{123}I]FP-CIT SBR values	0.103	0.331	0.55
Thalamus [^{123}I]FP-CIT SBR values	0.070	0.505	0.63
Cortical [^{123}I]FP-CIT SBR values			
Prefrontal [^{123}I]FP-CIT SBR values	**0.304**	**0.003**	**0.015**
qEEG Alpha/Theta ratio			
Posterior leads	-0.005	0.960	0.96
Memory			
Striatal and thalamic [^{123}I]FP-CIT SBR values			
Caudate [^{123}I]FP-CIT SBR values	0.027	0.802	0.92
Putamen [^{123}I]FP-CIT SBR values	0.021	0.843	0.92
Thalamus [^{123}I]FP-CIT SBR values	-0.039	0.711	0.92
Cortical [^{123}I]FP-CIT SBR values			
Prefrontal [^{123}I]FP-CIT SBR values	0.010	0.924	0.92
qEEG Alpha/Theta ratio			

Posterior leads	**0.299**	**0.004**	**0.024**
Language			
Striatal and thalamic [^{123}I]FP-CIT SBR values			
Caudate [^{123}I]FP-CIT SBR values	0.203	0.052	0.08
Putamen [^{123}I]FP-CIT SBR values	0.112	0.288	0.28
Thalamus [^{123}I]FP-CIT SBR values	0.206	0.049	0.08
Cortical [^{123}I]FP-CIT SBR values			
Prefrontal [^{123}I]FP-CIT SBR values	0.176	0.092	0.11
qEEG Alpha/Theta ratio			
Posterior leads	0.226	0.030	0.08
Visuo-spatial			
Striatal and thalamic [^{123}I]FP-CIT SBR values			
Caudate [^{123}I]FP-CIT SBR values	0.159	0.131	0.21
Putamen [^{123}I]FP-CIT SBR values	0.065	0.535	0.66
Thalamus [^{123}I]FP-CIT SBR values	0.043	0.682	0.68
Cortical [^{123}I]FP-CIT SBR values			
Prefrontal [^{123}I]FP-CIT SBR values	0.202	0.054	0.13
qEEG Alpha/Theta ratio			
Posterior leads	**0.357**	**< 0.001**	**< 0.001**

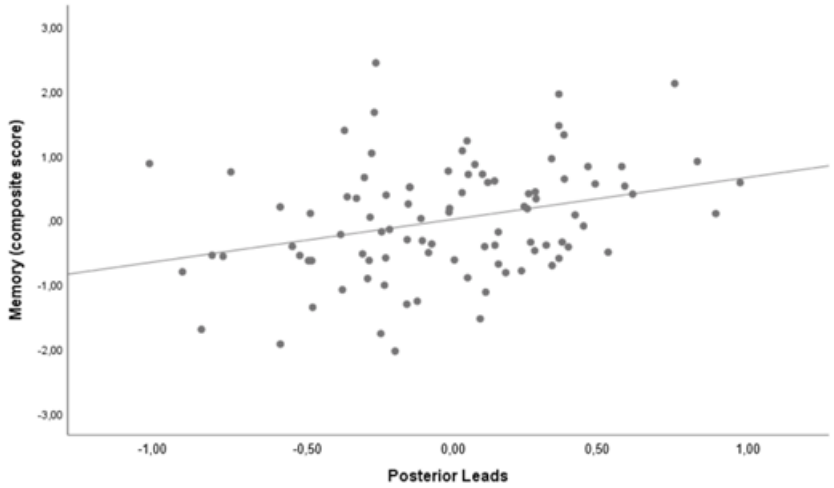
Figure 2: Scatter plot of the correlation between Attention and Working Memory domain and Prefrontal [^{123}I]FP-CIT SBR values. Analysis was corrected for age and MDS-UPDRS-III score.



Memory

We found a positive correlation between memory domain and qEEG alpha/theta ratio ($p = 0.004$). Results are shown in **Table 3** and **Figure 3**.

Figure 3: Scatter plot of the correlation between Memory domain and the qEEG alpha/theta ratio in the posterior leads. Analysis was corrected for age and MDS-UPDRS-III score.



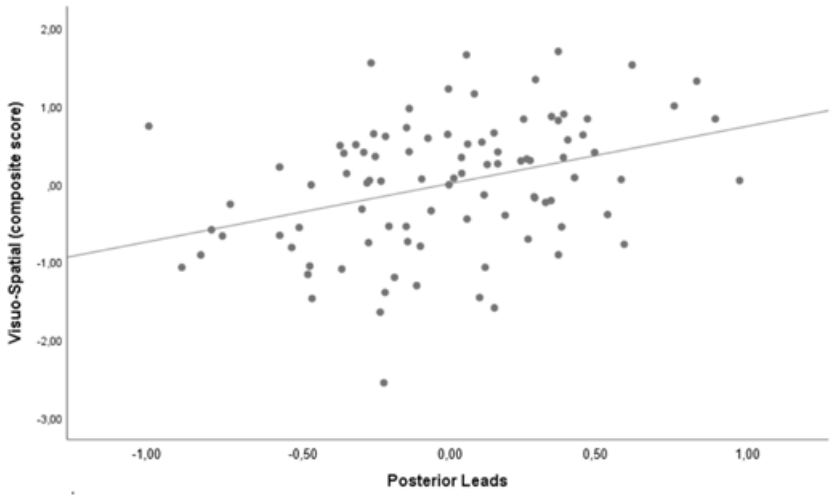
Language

We found a positive correlation between language and qEEG alpha/theta ratio, not surviving correction for multiple comparisons. Results are shown in **Table 3**.

Visuo-spatial functions

We found a positive correlation between visuo-spatial functions and qEEG alpha/theta ratio ($p < 0.001$). Results are shown in **Table 3** and **Figure 4**.

Figure 4: Scatter plot of the correlation between Visuo-spatial domain and the qEEG alpha/theta ratio in the posterior leads. Analysis was corrected for age and MDS-UPDRS-III score.



Discussion

In this study, we tried to assess the impact of cortical and deep grey matter monoaminergic expression, as well as of regional neurodegeneration, on the main cognitive domains in drug-naïve, *de novo* PD patients.

Overall, we found that executive functions directly correlated with metabolic levels in the left cerebellum, while attention and working memory directly correlated with prefrontal dopaminergic expression, while memory and visuo-spatial functions were related to the alpha/theta ratio in the posterior leads at qEEG, reflecting cholinergic expression.

Executive functions

We showed a positive correlation between executive functions and cerebellar glucose metabolism. Over the years there has been an increasing interest in the association between cerebellum and cognition. Grafman et al. (1992) (Grafman *et al.*, 1992) found that deficit in higher-order cognitive functions, such as executive functions, were associated with cerebellum, basal ganglia and frontal regions damage (Grafman *et al.*, 1992). Different theories have been proposed to describe the role played by the cerebellum in cognitive processes. Firstly, the cerebellum is included in a vast network spanning also to the basal ganglia and the prefrontal cortex, structures that are known to play a key role in executive functions (Tyson *et al.*, 2014). Schmahmann & Sherman (1998) (Schmahmann and Sherman, 1998) observed impaired executive functions, deficient planning and abstract reasoning in patients with focal cerebellar injury. Other studies, using different imaging techniques (such as resting state functional connectivity MRI and diffusion MRI) found that the dorsal and ventral attention, frontoparietal, default mode and salience networks all map onto focal areas within the posterior lobe of the cerebellum (Guell *et al.*, 2018; Schmahmann, 2019). In PD, a role for abnormal functional connectivity between the cerebellum and the cerebral cortex has been proposed as a potential substrate for cognitive deficits, also possibly mediated by changes in cholinergic activity (Maiti *et al.*, 2020).

Our findings expand on the observations found within the PDCP (Huang *et al.*, 2007, 2008; Meles *et al.*, 2015), and link cerebellar focal metabolic changes with executive functions.

Attention and Working Memory

Attention and working memory were directly correlated with prefrontal dopaminergic expression. It has been previously shown that dopamine is thought to play a significant role in attentional process, both in healthy subjects and in neuropsychiatric disorders (Kalbitzer *et al.*, 2012, Clark and Noudoost, 2014*a*).

In rats, lesions of the meso-cortical dopaminergic bundles are associated with difficulties in task-switching and in the space exploration, in line with the proposed role of prefrontal dopamine in attentional processes (Iversen, 1977; Nieoullon, 2002).

In healthy controls, prefrontal dopamine levels, as assessed by PET with 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA), it has been shown to correlate with executive functions independently from regional grey matter volumes (Kalbitzer *et al.*, 2012). Also, in subjects with psychiatric conditions, such as attention deficit-hyperactivity disorder and schizophrenia, prefrontal dopamine levels have been found to directly correlate with cognitive performance (Kalbitzer *et al.*, 2012, Clark and Noudoost, 2014*b*). In PD, prefrontal dopamine presents a complex relationship with cognition. In fact, both high and low levels of regional prefrontal dopamine were associated with reduced reaction times, in line with the known U-inverted shape of the dopamine-cognitive performance association (Brück *et al.*, 2005; Picco *et al.*, 2015). Our results broaden previous literature exploring the selective role of cortical dopaminergic expression in attentional processes in *de novo*, drug-naïve PD patients.

Memory, Visuo-spatial functions

We found that cholinergic tone, indirectly expressed by the alpha/theta ratio in the posterior cortical regions, positively correlated with memory and visuospatial functions. The alpha/theta power ratio mirrors the reduction of alpha frequency power and the increase of theta power during wake state at rest (Massa *et al.*, 2020) and reliably, though indirectly, reflects the cholinergic-correlated background slowing in the brain; in particular, it has been associated with cognitive impairment in PD (Massa *et al.*, 2020), as well as with response to acetylcholinesterase inhibitors (Lanctôt *et al.*, 2003).

Among diffuse projection systems, the impact of acetylcholine on cognition is relatively well characterized. A reduction in cholinergic cortical innervation is thought to play a key role in cognitive performance in patients with Alzheimer's Disease (AD) and dementia with Lewy Bodies (DLB) (Hampel *et al.*, 2018) in which cholinergic drugs are currently used to improve cognition.

Cholinergic system deficits in posterior cortices in PD has been found at all disease stages, including non-demented subjects (Kuhl *et al.*, 1996), and to correlate with posterior slowing at qEEG (Arnaldi *et al.*, 2017).

The role of cholinergic system in memory is well known, as acetylcholine is thought to be relevant for the consolidation of memory traces, both in healthy controls and

among different neurodegenerative conditions, including PD (Perry *et al.*, 1985). In particular, nicotinic alpha7 acetylcholine receptors (α7 nAChRs) are highly expressed in the hippocampus and are associated with cholinergic pathways. Their depletion accompanies cognitive decline, specifically memory impairment, observed in different neurodegenerative conditions (Perez-Lloret and Barrantes, 2016). In a cohort of 25 idiopathic PD patients, Lorenz *et al*. (2014) (Lorenz *et al.*, 2014) found a significant correlation between nAchR density loss and the results of CERAD (the Consortium to Establish a Registry for Alzheimer's Disease), suggesting that AchE inhibitors could be useful in the treatment of cognitive impairment in PD (Pagano *et al.*, 2015), since therapy with acetylcholinesterase inhibitors has been shown to improve memory performance and to modulate the memory network in PD patients (Imamura *et al.*, 2008). On the other hand, also muscarinic receptors expression is correlated with cognitive deficits. Antagonists, such as scopolamine, impair the cognitive performance in animal models (Beatty *et al.*, 1986).

As for visuo-spatial functions, they have been associated with cholinergic deficits, assessed using transcranial magnetic stimulation with the short-latency afferent inhibition, showing that a reduced central cholinergic tone was associated with a worse performance at visuo-spatial tests (Manganelli *et al.*, 2009). Interestingly, in PD, visual hallucinations are thought to be supported by visuo-spatial deficits (Manganelli *et al.*, 2009) and, from a pharmacological point of view, respond to an increase of cholinergic tone instead than to antipsychotics (Diederich *et al.*, 2009).

Lack of correlation with deep grey matter metabolism and monoaminergic innervation

We did not find significant correlation between cognitive domains and both cortical and deep grey matter metabolism, nigro-striatal and raphe-thalamic pathway degeneration.

The lack of correlation between cognition, putamen metabolism and nigro-putaminal integrity is not surprising, given both the role of this structure involved mainly in motor control, and its lack of association with cortical metabolism found in previous studies in PD patients (Orso *et al.*, 2021).

We also failed to find a correlation between executive functions and fronto-parietal metabolism, included within the PDCP (Huang *et al.*, 2007). This lack of correlation might be explained when taking into consideration the characteristics of our cohort, composed by drug-naïve, *de novo* PD patients, thus with fairly preserved brain metabolism and executive functions, compared to the more advanced disease stage of patients included in the PDCP studies.

Conversely, the lack of correlation between caudate measures and cognition was partly unexpended. However, it must be noted that the nigro-caudate pathway degenerates later on in the PD course than putamen (Arnaldi *et al.*, 2021), with half of *de novo* PD patients showing a normal dopaminergic innervation of the caudate (Pasquini *et al.*, 2019), especially in absence of frank cognitive decline. Moreover, from imaging perspective, the correlation between nigro-caudate metrics and cognition in early PD is somewhat controversial, with some studies failing to show significant associations (Apostolova *et al.*, 2010).

Regarding the lack of findings in the raphe-thalamic serotonergic pathway, it stems from the cognitive domains explored. Indeed, we have previously shown that alteration in this pathway are associated in PD with theory of mind abilities and not with more conventional cognitive domains (Orso *et al.*, 2020*a*).

Strength and limitations

This study represents a proof-of-concept investigation of the different roles played by regional neurodegeneration and both cortical and deep grey matter monoaminergic expression on cognitive functions in drug-naïve, *de novo* PD patients. To our knowledge this is the first study combining multi-tracer molecular imaging and quantitative EEG to shed light on the contribution of multiple diffuse projection systems on each cognitive domain in PD.

Drug-naïve, *de novo* PD is an interesting model to investigate deficits in multiple diffuse projection systems because the degree of neurodegeneration is still moderate at this stage, while later on in the disease course the severity of damage and the effects of drugs may hamper meaningful conclusions.

Regarding the assessment of cognition, we did not explore a single cognitive construct, such as general cognition, but we systematically explored each cognitive domain using an a-priori model (Litvan *et al.*, 2012).

We did not use a specific serotonergic marker, but the [^{123}I]FP-CIT SBR in the thalami, taking advantage of both the [^{123}I]FP-CIT affinity for SERT and the lack of DAT uptake in the thalami (Roselli *et al.*, 2010; Pilotto *et al.*, 2019). Although this approach has been previously used in a number of studies (Koch *et al.*, 2014; Arnaldi *et al.*, 2015), it would be worthwhile to confirm our findings using a SERT specific PET tracer; however, it is not currently approved for clinical use nor is widely available. Moreover, the cholinergic marker here used (i.e, qEEG) it's an indirect measure of cholinergic activity, thus caution is needed when interpreting qEEG results.

These limitations are however balanced by the large number of enrolled patients, the lack of confounding factors (i.e., drugs) and the use of multi-modal molecular imaging and neurophysiological approach, acquired for all patients in the same center.

Conclusion

We have shown the peculiar neurotransmitter and metabolic correlates of specific cognitive functions in drug naïve, *de novo* PD patients, using a multi-modal approach. Our results suggest possible novel treatment approaches for cognitive decline in *de novo* PD. Future longitudinal studies are warranted to explore changes in each specific cognitive domain.

6. Discussion and Concluding Remarks

Using a multi-modal and multi-tracer molecular imaging approach and quantitative EEG, combined with formal neuropsychological testing, we had the chance to shed the light on the neuroanatomical and neurochemical aspects of cognition in its whole, as well as diffuse projection systems mechanisms, in a large cohort of drug-naïve, *de novo* Parkinson's Disease (PD) patients.

In **Chapter 3** we were able to point to an independent contribution of insula and superior temporal gyrus degeneration and of the serotonergic system on ToM abilities in drug-naïve, *de novo* PD patients. These findings point to the presence of a specific cortical and neurochemical signature of ToM in PD and suggest possible therapeutic targets to treat social cognition deficits in this population.

In **Chapter 4** we reported the association between nigro-striatal dopaminergic, raphe-thalamic serotonergic function and cortical metabolism in PD, as well as the mediatory effect on this association of deep grey matter metabolism. These data expand on the role played by diffuse projection systems in PD physiopathology.

In **Chapter 5** we have shown the peculiar neurotransmitter and metabolic correlates of specific cognitive functions in drug naïve, *de novo* PD patients, using a multi-modal molecular imaging approach. Our results suggest possible novel treatment approaches for cognitive decline in *de novo* PD.

De novo PD is an interesting model to investigate deficits in multiple diffuse projection systems because the degree of neurodegeneration is still moderate at this stage, while later on in the disease course, the severity of damage and the effects of drugs may hamper meaningful conclusions.

Nevertheless, future studies in independent cohorts, as well as on healthy controls, are warranted to explore the relative contribution of monoaminergic and cortical degeneration to motor and cognitive deficits.

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8. Appendix

8.1 Publication during PhD

Biassoni E., Kreshpa W., Massa F., D'Amico F., Bauckneht M., Arnaldi D., Pardini M., Orso B., Girtler N., Brugnolo A., Morbelli S., Tinazzi M., Nobili F., Mattioli P (2023) Right Posterior Hypometabolism in Pisa Syndrome of Parkinson's Disease: A Key to Explain Body Schema Perception deficit? Parkinsonism and Related Disorders, (Pre-print), 105371.

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