The interaction effect of covariates on longitudinal resting-state functional connectivity in Parkinson's disease

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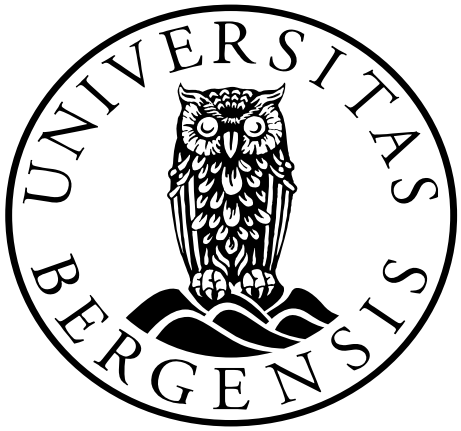

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Abstract

Parkinson's disease is a neurodegenerative disorder, characterized by several motor and non-motor symptoms. It is heterogeneous, and much is still unknown about the mechanisms behind symptomology and the progression of the disease. Resting-state fMRI has been proposed as an important tool to investigate alterations of functional connectivity as possible biomarkers of the disease in the brain. Findings from studies are inconsistent. No standardized approach to conduct research exists, and studies vary in resting-state fMRI acquisition, methodology and covariates measured. The current study aims to investigate the interaction effect of covariates on longitudinal alterations of functional connectivity for people with Parkinson's disease. Covariates chosen were age, gender, levodopa medication (ON/OFF and LEDD values), UPDRS-III and MoCa scores. Data was extracted from Parkinson's Progression Markers Initiative, an open database aimed at investigating progressive biomarkers of Parkinson's disease. The current sample included 40 subjects with Parkinson's disease, who completed three resting-state scans each, during a period of five years. Independent component analysis was used to analyze data. Results revealed a main effect of scan, with alterations of connectivity at the second scan compared to the first, and at the third scan compared to the first. Medication as an ON/OFF measure had a significant interaction effect on alterations found. One prominent finding was the increased connectivity between salience, central executive and default mode network. Alterations of functional connectivity in resting-state fMRI measures for subjects with Parkinson's disease could be influenced by levodopa medication, suggesting that it should be implemented in future research.

Keywords: Parkinson's disease, rs-fMRI, longitudinal, functional connectivity, covariates

Sammendrag

Parkinsons sykdom er en nevrodegenerativ sykdom, og karakteriseres av motoriske og ikke-motoriske symptomer. Sykdommen er heterogen, og kunnskapen om mekanismene bak symptomene og progresjonen er begrenset. «Resting-state fMRI» har blitt foreslått som et nyttig verktøy for å undersøke potensielle biomarkører i form av endringer av funksjonell konnektivitet i hjernen. Funn fra studier avviker fra hverandre. Bakgrunnen for dette kan være at det er forskjeller i hvordan forskningen blir utført. Det finnes ikke en standardisert protokoll for gjennomførelsen av studier, og det er forskjeller i datainnsamling, analysemetoder, og hvilke variabler som blir inkludert. Målet med denne studien er å undersøke interaksjonseffekten av kovariater på funksjonell konnektivitet hos personer med Parkinsons sykdom. Disse kovariatene er alder, kjønn, levodopa medisin (ON/OFF, og LEDD verdier), UPDRS-III og MoCa skårer. Studien er longitudinell, og inkluderer 40 deltakere med tre gjennomførte skanninger over en fem års periode. Datagrunnlaget kommer fra Parkinson's Progression Markers Initiative, en åpen database med mål om å undersøke progressive biomarkører i hjernen for Parkinsons sykdom. Resultatene viste en hovedeffekt av skanning, da det ble funnet endringer i funksjonell konnektivitet for den andre skanningen sammenlignet med den første, og den tredje skanningen sammenlignet med den første. Videre ble det funnet en signifikant interaksjonseffekt av medisin som en ON/OFF variabel, på endringer av funksjonell konnektivitet. Et av funnene indikerer økt konnektivitet mellom salience, central executive og default mode network. Funnene fra denne studien indikerer at bruk av levodopa medisin påvirker funksjonell konnektivitet, og kan være relevant å inkludere i fremtidige «resting-state fMRI» studier på Parkinsons sykdom.

Preface

I reached out to Kjetil Vikene as a possible supervisor for my thesis, as I was interested in writing about Parkinson's disease, and I read his work on the matter. He presented some suggestions on possible topics, sparking an interest in resting-state fMRI research.

Throughout the last year, I have gained ample knowledge about functional neuroimaging, data processing, as well as on how to interpret the results. During the spring semester of 2021, I was granted a student scholarship, and was given the opportunity to work on an ongoing project Kjetil Vikene is currently conducting. As part of this project, I participated in the collection of resting-state data on subjects with Parkinson's disease, giving me valuable experience on the collection of functional neuroimaging data. I was further invited to weekly meetings with the Re:State research group, where I had the opportunity to learn about ongoing projects within the group, and present my own progress on my thesis.

I would like to thank my supervisor Kjetil Vikene for impeccable supervising throughout the year, as well as for always being engaged and involved in my thesis. I would further like to thank my co-supervisor Katarzyna Kazimierczak for helping me with the data analysis, as well as reading through my thesis and providing great feedback. Thank you to the members of the Re:State group, for welcoming me to the group, and always being available for any questions I might have had. I would also like to thank the group for involving me in activities outside the office, as being a part of the group has been a great experience.

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Parkinson's disease (PD).

PD is, second to Alzheimer's, considered to be the most common neurodegenerative disorder, and usually has its onset during the age of 65-80. Both environment, as well as genetics have been identified as possible risk factors, with the most important being age, as PD increases in prevalence after the age of 50 (Sveinbjornsdottir, 2016). PD is characterized by motor symptoms such as bradykinesia (slow movements), rest tremor and muscular rigidity. Non-motor symptoms often appear before motor symptoms and include sleep problems, emotional issues and cognitive impairment (Sveinbjornsdottir, 2016). Motor symptoms are believed to be caused by the loss of dopaminergic cells in the substantia nigra, pars compacta, located in the brainstem. The brainstem forms connections to the basal ganglia, mainly the dorsal putamen, considered important for motor functions. Another disease characteristic is the collection of abnormal α-Synuclein proteins called Lewy bodies, in the brain, which could be a biological marker for the neurodegeneration of the disease (Kalia & Lang, 2015). At present time, it is not possible to make an affirmative diagnosis without confirming the presence of Lewy bodies, which can only be done post-mortem (Filippi et al., 2019). Neurological assessment based on motor symptoms is the most common tool used to make a diagnosis. The Unified Parkinson's Disease Rating Scale (UPDRS) has been one of the most common assessments to evaluate the severity of symptoms, and progression of PD. It was revised in 2008 by the Movement Disorder Society, and their scale MDS-UPDRS is more commonly used, measuring both motor and non-motor symptoms (Evers et al., 2019). About two-thirds of people diagnosed with PD experience pain, a non-motor symptom that is caused by motor problems, such as dystonia (uncontrolled movements) and muscle cramping (Ford, 2010). The neurodegeneration is progressive, and as the symptoms worsen, life quality can be significantly reduced, affecting daily activities. Little is known about how PD progresses in the brain. Braak et al. (2003) has suggested a dual-hit

hypothesis (Braak et al., 2003). It proposes that the initiation of Lewy bodies is caused by an unknown bacteria or virus in the gut and nasal cavity, spreading in stages via the vagus nerve, which connects the brain and gut, and the olfactory tract (Rietdijk et al., 2017). There is confirmed presence of Lewy bodies in both of these tracts, supporting the hypotheses. From there it spreads to the central nervous system, and eventually to the substantia nigra. However, critics of the hypothesis point out that Lewy bodies might not be as strongly linked to clinical symptoms as this hypothesis claims. In addition, there is evidence that not everyone follows the proposed stages (Rietdijk et al., 2017). PD is highly heterogeneous, with multiple subgroups being classified, however, there is not a clear consensus on these groups. The most common subgroups are tremor-dominant and non-tremor dominant groups (Kalia & Lang, 2015). Cognitive impairment is an important non-motor symptom, and people newly diagnosed with PD that are also diagnosed with mild cognitive impairment (MCI), have a higher risk of developing Parkinson's disease dementia (PDD). Research has demonstrated that people with PD are more likely to develop dementia than others in general (Weil et al., 2018). Ten years after diagnosis about 50% of people with PD will develop PDD, and after 20 years this percentage increases to 80%. The development of dementia, and the progression of both non-motor and motor symptoms, are important reasons as to why people with PD need placements in nursing homes (Sveinbjornsdottir, 2016). Much is still unknown about the causes and subgroups of PD, making treatment difficult, and allowing the disease to progress, challenging everyday life.

Pharmacologic treatment is usually based on dopamine, with the most common being levodopa. Levodopa is a precursor of dopamine, which is transformed into dopamine in the brain, predominantly aimed to treat the motor symptoms of PD (Sveinbjornsdottir, 2016). As there are several parkinsonian medications, the levodopa equivalent daily dose (LEDD) can be calculated to find the sum of each medication someone is taking, and can be used both

clinically and in research (Julien et al., 2021). The aim is to treat the symptoms, not the cause of the disease, and as the disease progresses, resistance to treatment increases (Sveinbjornsdottir, 2016). Usually, medication is most effective at the beginning of the course of treatment. As the symptoms evolve, subjects need higher and more frequent doses. Worsening of symptoms is no longer caused by dopaminergic dysfunction, and therefore patients respond poorly to treatment (Armstrong & Okun, 2020). The long duration of levodopa treatment can lead to levodopa-induced dyskinesia (LID). LID is a motor complication caused by long-term use of the medicine, occurring in 40% av people with PD after four years (Espay et al., 2018). Clinically, LID may cause several movement issues such as chorea (involuntary movements) and dystonia (uncontrollable muscle contractions), usually appearing in the upper body or face. The exact underlying mechanisms of LID are not clear (Pandey & Srivanitchapoom, 2017).

Functional magnetic resonance imaging (fMRI)

fMRI is based on the same psychics as structural MRI, however, whereas structural MRI examines anatomical information of the brain, fMRI is believed to indirectly measure neural activity in the brain. This is done by exploiting the blood oxygenation level dependent (BOLD) signal, a hemodynamic response that occurs as a reaction to neuronal activity (Amaro & Barker, 2006). Hemoglobin in the blood has different magnetic properties while saturated with oxygen, compared to when it is low on oxygen. As neurons in the brain are active, the affiliated areas increase their uptake of oxygen (creating deoxyhemoglobin). The body naturally responds to this by increasing the blood flow to these brain areas, increasing the amount of oxygen in the hemoglobin (oxyhemoglobin). Deoxyhemoglobin is paramagnetic, whereas oxyhemoglobin is diamagnetic. This creates local magnetic disturbances which the magnet in the MRI machine detects, providing the basis behind the BOLD signal (Amaro & Barker, 2006).

The BOLD signal can be used to measure neural activity in the brain over time. Anatomically, the brain is divided into regions, or areas. Researchers have been trying to identify different functions these areas could have and attempted to label the entire brain based on the function of each area. However, it has become clear that brain areas are not independent regions, and that each area could have multiple functions (Pessoa, 2014). In other words; assigning individual brain areas to specific cognitive functions, as well as psychological disorders, seems unlikely. Furthermore, interconnectivity between different brain areas have been discovered, which has introduced the concept of networks. When simultaneous variation in neural activity is registered over time in multiple brain areas, it is the basis of network analysis (van den Heuvel & Hulshoff Pol, 2010). This interconnectivity between brain areas, as well as networks, is thought to have a functional value. This is called functional connectivity, defined as the temporal pattern of neural activation across areas of the brain that are not anatomically connected (van den Heuvel & Hulshoff Pol, 2010). Functional connectivity has been suggested to be important for cognitive processes in the brain. Interestingly, this would mean that there is no "hub" in the brain that controls all cognitive processes, instead, cognition occurs due to interconnectivity across the brain (van den Heuvel & Hulshoff Pol, 2010). How this interconnectivity contributes to cognitive processing is not fully known, as research is still ongoing (Fox et al., 2005).

Functional neuroimaging has been important in increasing the understanding of activity in the brain. Traditionally, experiments were conducted utilizing task-related fMRI, where subjects perform different cognitive tasks while in the scanner. The experimental design for this is commonly a block, or event-related design, both consisting of a task condition in which subjects perform the cognitive task, and a rest condition. These two conditions are then compared. If there is any activity in the task condition not appearing in the rest condition, it is thought to reflect brain activity specific to the cognitive task performed.

Such measurements of brain activation could increase the knowledge of which brain areas are involved in implicit cognitive functions (DeDora et al., 2016). Research has demonstrated that brain areas important for a given cognitive task increase in activity, whilst there is a decrease in activity in less relevant areas. In tasks associated with working memory and attention, there is increased activity in frontal and parietal cortical regions. Decreased activity appears in, among others, medial prefrontal and posterior cingulate cortex, modulated by task difficulty, meaning that as the task gets more difficult, activity decreases further (Fox et al., 2005).

Resting-state fMRI

In 1995, Biswal et al. (1995) published a fMRI study where subjects were instructed to not engage in any cognitive tasks while in the scanner, with the aim to investigate signal fluctuations in the "resting" brain (Biswal et al., 1995). Since then, it has become increasingly common to scan subjects when they are not actively performing a task. Subjects are often instructed to lie still, either with eyes closed or open, often with a fixation cross on a screen in the latter option, and to not think of anything specifically. Brain at rest form networks in the form of low frequency, spontaneous fluctuations of the BOLD signal, and the measure of this activity is called resting-state fMRI (rs-fMRI) (van den Heuvel & Hulshoff Pol, 2010). Research using rs-fMRI has identified several resting-state networks, summarized by Tessitore et al. (2019). They reported the most frequently investigated networks, in healthy people without a neurodegenerative disorder. These include default mode (DMN), central executive (CEN), sensorimotor (SMN/SM), salience (SN), dorsal attention (DAN), auditory (AN) and visual (VIS) network (Tessitore et al., 2019). The DMN primarily consists of precuneus and posterior cingulate, as well as areas within the bilateral inferior parietal and ventromedial prefrontal cortex (Filippi et al., 2019). DMN is a task-negative network, as it exhibits increased activity when the brain is at rest, and decreases in activity when the brain is actively engaged in cognitive tasks. It could therefore represent a baseline state of the brain,

with a function of its own (Raichle et al., 2001). Alterations of the DMN have been implicated in the research of neurodegenerative processes, often demonstrated in studies on Alzheimer's. Studies consistently report reduction of functional connectivity within the DMN-network, specifically in posterior DMN, compared to healthy controls (Hohenfeld et al., 2018). Other resting-state networks include salience network, consisting of dorsal anterior cingulate cortex and bilateral insulae, which detects the most important information in a given situation and responds to it (Tessitore et al., 2019), and central executive network (CEN), consisting of mesiofrontal areas, parietal cortex and dorso-lateral frontal areas, important for functions of executive control (Tessitore et al., 2019). Yet to be mentioned is the basal ganglia network (BG), which, in addition to the putamen, consists of caudate nucleus (which together with the putamen makes up the striatum) and globus pallidum, as well as substantia nigra and subthalamic nucleus. BG further includes the thalamus and cerebral cortex (Nelson & Kreitzer, 2014). In addition to being important for motor function, it has been proposed that dysfunction in the basal ganglia contributes to non-motor PD symptoms, and is nonetheless recognized as an important network in the neurodegeneration of the disease (Nelson & Kreitzer, 2014).

Several analytic methods are being applied to rs-fMRI data, which can loosely be divided into two categories; model-driven, dependent on prior selected regions of interest, or data-driven (Rosazza et al., 2012). Model-driven methods include seed-based connectivity, whereas data-driven methods include independent component analysis (ICA), regional homogeneity (ReHo), and amplitude of low-frequency fluctuation (ALFF), more refined as fALFF (fractional amplitude of low-frequency fluctuation). Seed-based connectivity, ICA and ReHo belong to the temporal domain, focusing on changes in signal over time, whereas ALFF and fALLF are frequency methods, extracting information from the spectral component of the BOLD signal (Azeez & Biswal, 2017). ReHo and ALFF/fALLF are believed to extract neural

activity changes in local regions of the brain, whereas ICA and seed-based analysis extract maps of network connectivity across the brain (Yang et al., 2020)

Activity despite the absence of cognitive stimuli is interesting, as it represents some of the fundamental activity in the brain. With rs-fMRI, it could be possible to find a baseline of activity, identifiable across subjects. This could be used to find biomarkers of different diseases in the brain, such as PD (Specht, 2020). Rs-fMRI is non-invasive and compared to task-based fMRI less demanding for the participant, useful for groups of people struggling to perform cognitive tasks due to cognitive impairment (Rosazza et al., 2012). In case that rs-fMRI could be used as a tool to disclose biomarkers of PD in the brain, it could be a great aid in clinical settings, helping to diagnose people and track their progression of the disease (Hohenfeld et al., 2018).

Previous research

To summarize the most important findings in rs-fMRI in PD, Hohenfeld et al. (2018) performed a meta-analysis of current literature, including 2492 PD subjects, with 1685 healthy controls, across 68 studies. Most common findings involve alterations of functional connectivity in the basal ganglia and sensorimotor areas, both important regions for motor symptoms. Other implicated areas in the brain include the thalamus and mesolimbic areas, the latter being important for the dopaminergic pathway. Alterations in the DMN have also been suggested, although the literature on this is not as clear for PD as it is for Alzheimer's (Hohenfeld et al., 2018). Further, they argue that whether alterations involve decreased or increased connectivity is not certain, as an overall consensus on the direction cannot be found in the literature. It does however seem that decreased connectivity in the posterior putamen for PD subjects is the most consistent finding across studies (Tessitore et al., 2019). There is evidence suggesting increased connectivity between sensorimotor cortex and posterior putamen early in the disease, and then decreased connectivity between anterior putamen and

the midbrain as the motor symptoms worsen (Filippi et al., 2019). Brain areas belonging to SMN, including postcentral gyrus, and other areas such as bilateral inferior parietal lobule seem to be involved in the pathology of PD as well (Tessitore et al., 2019). Research on PD has further revealed alterations of functional connectivity in the cerebellum, an area important for motor control. It has been speculated whether increased connectivity in cerebellum works as a compensatory mechanism to counterbalance for the altered connectivity in basal ganglia (Wu & Hallett, 2013). Research investigating this connection includes a study by Wu et al. (2009), who acquired rs-fMRI from 22 PD subjects with an average disease duration of four years, in both ON/OFF medication state, comparing them to healthy controls. Increased ReHo activity was found in bilateral cerebellum for PD subjects off medication, and after administration of levodopa, there was decreased ReHo in that same area, suggesting that levodopa normalized the activity. Further, as subjects were off medication there was decreased ReHo activity in the bilateral putamen, however, once they were on medication this activity was increased (Wu, Long, et al., 2009). Authors argue that cerebellum influences basal ganglia through connected circuits, and that the increased activity found in their study supports the research suggesting that cerebellum compensates for the decreased basal ganglia activity (Wu, Long, et al., 2009). A similar study was conducted by Festini et al. (2015), with a sample of 25 PD subjects in a mild-to-moderate stage of the disease. They found that both within and between-network connectivity in cerebellum was increased whilst subjects were off medication, compared to when they were on medication. Increased connectivity in cerebellum was associated with improved cognitive and motor performance, further arguing for the presence of a compensatory mechanism (Festini et al., 2015).

Cognitive impairment

Wolters et al. (2019) conducted a meta-analysis aiming at improving the understanding of the networks and patterns in the brain involved in the non-motor symptom

cognitive impairment. The analysis included studies comparing cognitively impaired PD subjects with either people with PD without cognitive impairment, or healthy controls. Overall, it included 932 PD subjects, with 289 being cognitively unimpaired, 222 having MCI and 68 subjects having PDD. Cognitive impairment was assessed with either Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCa). Their analysis concluded that alterations in DMN are the most common finding in PD subjects who are cognitively impaired, compared to those who are not (Wolters et al., 2019). This is consistent with the involvement of DMN in other neurodegenerative disorders, such as Alzheimer's, frontotemporal dementia and Huntington's disease. However, comments on the quality of this meta-analysis have been made, with Wang et al. (2019) emphasizing the problem with pooling together studies on people with MCI and PDD, and defining them as the same subgroup. MCI does not always lead to PDD, and they should be compared separately (Wang et al., 2019). They further point to issues with not differentiating between subjects being ON/OFF medication, arguing that they should have made subgroups based on medication criteria. These factors could contribute to the results, making them exploratory, and not final conclusions (Wang et al., 2019). Individual studies have differentiated subjects based on medication status, including Gorges et al. (2015), who conducted a study with 31 PD subjects who were all on medication at the time of rs-fMRI acquisition, with an average of disease duration of six years. Their study consisted of 17 cognitively impaired subjects, with six of these having PD-associated dementia. In line with the meta-analysis, they found decreased functional connectivity in DMN for cognitively impaired PD subjects, compared to PD subjects classified as cognitively unimpaired (Gorges et al., 2015). However, Amboni et al. (2015) found decreased connectivity in DMN in PD subjects with and without cognitive impairment, compared to healthy controls, suggesting that altered connectivity in DMN is common for PD subjects in general. Included in their study was 21 PD subjects with MCI,

excluding people with PDD, and 21 PD subjects without cognitive impairment, all scanned whilst being on medication. Disease duration was on average six years (Amboni et al., 2015). Interestingly, there is some evidence suggesting that DMN alterations could vary depending on duration of PD, with one study finding that people with shorter duration of PD before MCI onset exhibited decreased connectivity in DMN (Shin et al., 2016). People with longer disease durations had decreased connectivity in medial frontal areas, though not in the DMN. Out of 59 PD-MCI subjects, 15 subjects had an average of five months disease duration, whilst for the remaining 43 subjects average disease duration was two years, all on medication at the time of rs-fMRI acquisition. Authors argue that alterations of connectivity in DMN could be dependent on disease duration (Shin et al., 2016).

UPDRS-III scores

UPDRS is one of the most common assessments of the severity and progression of PD, raising the question of how much of alterations in functional connectivity in rs-fMRI can be explained by these scores. A positive connection between altered connectivity and UPDRS would indicate that the alterations could be associated with symptomology and disease progression. Part III of the scale, the motor examination, is commonly used to explore correlation with connectivity measures. Wu et al. (2009) conducted a resting-state study comparing alterations of functional connectivity with UPDRS-III scores in a sample of 22 PD subjects and 22 healthy controls. PD subjects had a disease duration with an average of four years, and they were scanned both whilst being on and off medication. Compared to healthy controls, PD subjects had decreased connectivity in supplementary motor area (SMA) and left putamen, as well as increased connectivity in left cerebellum and left parietal cortex, whilst being off medication (Wu, Wang, et al., 2009). Once PD subjects were on medication, this pattern of alterations was normalized as it matched the healthy controls (Wu, Wang, et al., 2009). Correlations between functional connectivity and UPDRS-III demonstrated a

significant improvement in scores when subjects were on medication, illustrating how alterations in motor areas are influenced by the disease progression (Wu, Wang, et al., 2009). UPDRS-III scores does not always have a positive correlation with functional connectivity. Hou et al. (2016) compared two groups of PD subjects, 18 with early disease onset and 18 with late disease onset, to healthy controls. Both groups had an average disease duration of 16 months, and all subjects were drug-naïve at the time of rs-fMRI acquisition (Hou et al., 2016). Findings showed that there were alterations of functional connectivity in both groups that were negatively correlated with UPDRS-III scores, suggesting that there were no differences in the two groups based on disease progression and severity. Authors argue that other factors such as compensatory mechanisms could explain the results (Hou et al., 2016).

Age and gender

Age is one of the main risk factors for PD, and as for gender, men are more frequently diagnosed with PD than women, with a ratio of 3:2 (Kalia & Lang, 2015). Differentiating between gender and age of PD subjects in studies could therefore be important, as they could influence alterations of connectivity in the brain. One study examined the association between PD symptoms and age and gender, in a database with over 600 PD subjects (Szewczyk-Krolikowski et al., 2014). They ran comparisons (t-test/regression) between several demographic variables and clinical characteristics, and found older age to be predictive of PD severity. Authors further found that being male could affect disease progression more severely than being female. Their study is not a rs-fMRI study, nevertheless, their statistical tests indicate an influence of age and gender on symptoms and disease progression (Szewczyk-Krolikowski et al., 2014). One resting-state study accounting for age and gender is Li et al. (2016), comparing 23 people with PD, with an average disease duration of seven years, with healthy controls. In addition, use of medication was accounted for, as all the PD subjects went off their medication 12 hours or more prior to scanning. Their findings revealed

alterations of ReHo in functional connectivity in visual cortex and motor network for the PD group, however none of the covariates, including age and gender, could explain the differences between the two groups (Li et al., 2016). De Micco et al. (2019) conducted another study examining the effect of gender on functional connectivity. Protocol included newly diagnosed (less than two years from symptom onset) PD subjects, who were all drug-naïve during rs-fMRI acquisition. After two years, neurological assessment was conducted to investigate if motor impairment was predicted by functional connectivity at baseline. For males, and not females, alterations in SMN predicted disease severity at the follow-up assessment (De Micco et al., 2019). Neither males nor females exhibited motor complications due to treatment at the two year follow up. However, the authors argue that a longer time period between baseline and follow-up could be beneficial, both to confirm their results, as well as to investigate if there are gender differences later on, as the disease progresses (De Micco et al., 2019).

Dopaminergic PD medication

Several of the studies mentioned demonstrate how PD medication potentially influences alterations of functional connectivity, as whether connectivity is increased or decreased is dependent on medication status during rs-fMRI acquisition. The interaction of PD medication was further examined by Ballarini et al. (2018) in 31 PD subjects with an average disease duration of 11 years, scanning them both on and off medication. Results demonstrated increased connectivity in sensorimotor areas such as pre- and postcentral gyrus while subjects were off medication, a finding that was normalized when subjects were on medication, compared to healthy controls (Ballarini et al., 2018). Normalized connectivity due to medication could potentially be a pattern, as it is demonstrated in other studies as well (Wu, Long, et al., 2009; Wu, Wang, et al., 2009). Further, Bell et al. (2015) conducted a study investigating the effect of dopaminergic medicine on the striatum in 39 PD subjects,

compared to 40 healthy controls. PD subjects were scanned twice, once while being off medication, and once after administration of medication. Average disease duration was seven years (Bell et al., 2015). Findings included decreased connectivity across the posterior striatum when subjects were scanned off medication. After administration of dopaminergic medication, PD subjects had increased connectivity across posterior striatum. However, PD subjects scanned while being on medication had decreased connectivity in posterior striatum when being compared to the control group. Their findings could indicate that medication does not normalize connectivity compared to healthy controls. Rather it improves the decreased connectivity in the altered area (Bell et al., 2015). Further, adding LEDD values as a variable could elaborate on the influence medication has on functional connectivity. Krajcovicova et al. (2012) used LEDD values as a covariate, examining its effect on connectivity in the DMN. Rs-fMRI was acquired from 18 cognitively unimpaired PD subjects whilst they were on medication, with an average LEDD value of 696 mg/day, and average disease duration of four years. Authors discovered a correlation effect between the LEDD values and increased connectivity in DMN, specifically in left posterior cingulate cortex (PCC) (Krajcovicova et al., 2012). Findings further revealed that when LEDD values were excluded from the analysis, there were no significant differences between healthy controls and PD subjects in functional connectivity. Authors argue that due to their findings being levodopa dose-dependent, it should be further investigated in other studies as well (Krajcovicova et al., 2012).

Longitudinal studies

PD is progressive, and therefore longitudinal studies could be essential to acquire knowledge about the brain activity occurring long term as the disease develops, both compared to activity earlier in the course of the disease, as well as to healthy controls. Manza et al. (2016) included 62 newly diagnosed PD subjects, some on medication, whilst others were off, from the Parkinson's Progression Markers Initiative (PPMI) database. They found

that at baseline, connectivity in basal ganglia changed. Specifically, it decreased in functional connectivity between the midbrain (substantia nigra) and the anterior putamen for subjects with more severe motor symptoms. This finding was maintained during the follow-up a year later, when scans from 11 of the PD subjects were compared with the baseline scans for the rest of the PD sample (Manza et al., 2016). Included in their study were assessments of cognitive abilities at baseline, and they found that decreased cognitive performance was associated with increased connectivity between anterior cingulate cortex (ACC) and the dorsal caudate. During the follow up, correlation between cognition and increased functional connectivity was not found, however the test scores for cognitive abilities did not change significantly (MoCa scores) (Manza et al., 2016). Zeng et al. (2017) conducted a study with 23 PD subjects, comparing baseline ReHo activity with a follow-up two years later. At the time of rs-fMRI acquisition the PD subjects were off medication, and at baseline, average disease duration was 10 years. At the two year follow up, decreased ReHo was found in DMN, left cerebellum and sensorimotor cortex (SMC), more specifically pre- and postcentral gyrus, and increased ReHo in supplementary motor area (SMA), bilateral hippocampus and bilateral temporal gyrus (Zeng et al., 2017). Interestingly they further found a positive correlation between UPDRS scores and activity in left cerebellum, as the ReHo in cerebellum decreased as motor symptoms worsened. Authors argue that this demonstrates how the progression of PD leads to loss of efficiency in DMN and SMC, as well as a reduced compensatory role for the cerebellum (Zeng et al., 2017). Focusing more specifically on cognitive impairment, Dubbelink et al. (2014) conducted a study over a three-year period comparing the follow up with baseline for 36 PD subjects. Average disease duration was 10 years, and subjects were on medication at the time of scanning. At baseline, for PD subjects, there was decreased functional connectivity for several areas, such as post- and precentral gyrus, middle, superior and inferior occipital gyrus and cuneus. During the follow up three

years later, this connection decreased further, which was associated with a decline in cognitive functions (Dubbelink et al., 2014). These brain areas are commonly identified as areas belonging to the visual and sensorimotor network. Interestingly, this implicates that other networks in the brain, and not exclusively the DMN, could be important for cognitive decline in PD (Dubbelink et al., 2014). Tuovinen et al. (2018) investigated the connection between motor issues and longitudinal alterations of functional connectivity, comparing 16 PD subjects at baseline with a follow up 1.5 years later. Subjects were in the early or moderate disease stage, and they were scanned while being off medication. At the follow up, compared to baseline, PD subjects showed alterations in several areas, including increased connectivity between cingulumMid and post- and precentral gyrus (SMN), and decreased connectivity between cerebellum and caudate (BG). Compared to controls, there was hyperconnectivity, i.e. increased connectivity, within cerebellum both at baseline and follow up (Tuovinen et al., 2018). In line with previous studies that find hyperconnectivity within cerebellum, authors argue that this is compensating for alterations in the basal ganglia. The decreased connectivity within the SMN correlated with UPDRS-III scores, suggesting its role in motor impairments in the disease (Tuovinen et al., 2018). Progression of alterations of functional connectivity in areas related to motor impairment was further examined by Hu et al. (2015). By using fALFF, they conducted a study with 17 PD subjects, with an average of four years disease duration, comparing baseline with a follow up two years later. Subjects were on medication at both scanning sessions. At the follow up, increased fALFF values were found in middle temporal gyrus (MTG) and middle occipital gyrus (MOG), as well as decreased fALFF values in, among others, right cerebellum posterior lobe, right thalamus, left inferior parietal lobe (IPL), left pre- and postcentral gyrus (Hu et al., 2015). As most of these regions belong to the sensorimotor circuit, known to be important for motor symptoms

including bradykinesia and akinesia, these findings were in line with previous research (Hu et al., 2015).

Inconsistencies across studies

Drawing overall conclusions on alterations of functional connectivity, as well as on the progression of alterations as PD progresses is challenging, as both the disease and the literature is quite heterogenous. Currently, no standardized acquisition and analysis methods exist to perform rs-fMRI studies, which has contributed to non-consistent results. Across studies, there are methodological differences, i.e. analysis, and different protocols while subjects are in the scanner (Griffanti et al., 2016). Quality criteria varies as well, with differences related to variables such as PD subgroups, symptoms, disease duration, and medication status during rs-fMRI acquisition (P. Pan et al., 2017). Further, no complete agreement exists on which brain areas belong to which resting-state network, and some networks can vary in definition, and include different brain areas across studies. An example of this is how the CEN frequently gets referred to as frontoparietal network (FPN), when the two largely consist of the same brain regions, described to have similar functions (Sherman et al., 2014). Inconsistent findings question how strong the reproducibility of the resting-state functional connectivity studies of PD are. Badea et al. (2017) investigated reproducibility by comparing their own dataset of 27 PD subjects (16 healthy controls) with a dataset containing scans from 40 subjects (20 with PD, 20 healthy controls) and the PPMI dataset with 91 PD subjects and 18 healthy controls. Findings revealed that across these datasets, alterations of functional connectivity were not reproducible (Badea et al., 2017). Further, authors argue that the non-reproducibility is due to disease heterogeneity, and not technical differences. However, two of the datasets included subjects scanned while being on medication, and in the third dataset subjects were off medication. Authors concluded that this did not influence the results (Badea et al., 2017). Considering there are studies that have found alterations of

functional connectivity to be dependent on medication status (Ballarini et al., 2018; Krajcovicova et al., 2012), medication could still have had an effect on their findings. Nonetheless, their study highlights the issues with reliability within rs-fMRI research. In summary, if alterations of functional connectivity could be reproducible across rs-fMRI studies, it could represent important biomarkers of the disease. Longitudinal studies have been proposed to be valuable in providing information on such biomarkers, and their progression in the brain.

Aim of the current study

In the current study, analysing longitudinal rs-fMRI data, the aim is to investigate different covariates that could have an interaction effect on alterations of functional connectivity in PD. Covariates include age, gender, medication, UPDRS-III scores, as well as MoCa scores. The study is twofold; one in which medication is added as a categorical variable (ON/OFF medication), the other were medication is added as a continuous variable based on the LEDD values for each subject. The study is further longitudinal, including three scanning sessions of rs-fMRI acquisition (first, second and third scan). Whole-brain connectivity is assessed, which is why ICA is chosen as the analysis method. The research question of the current study is to explore if there is a main effect of scan, i.e. alterations of functional connectivity across scanning sessions, and if any of the chosen covariates have a significant interaction effect on possible alterations of functional connectivity.

Material and methods

Data was retrieved from the Parkinson's Progression Markers Initiative (PPMI). PPMI was started in 2010, and it is a comprehensive database collected through cooperation between scientists, participants and sponsors. This cooperation is international, and rs-fMRI data is collected across institutions in Europe and the US. The aim is to investigate progressive biomarkers for people with PD, to be used for clinical purposes (Marek et al.,

2018). The database includes a range of neurological imaging data, biological data and neurological assessments. Extracted information used in the current study is demographic variables, resting-state scans acquired at multiple timepoints, and neurological assessments covering motor and non-motor symptoms (Parkinson's Progression Markers Initiative; https://www.ppmi-info.org/).

Participants

Forty-six participants (30 males) were included in the study. All subjects were diagnosed with PD within the last two years of the first scanning. Six participants were excluded due to incomplete rs-fMRI data, leaving the complete number to be 40 (27 males). No healthy control subjects were included in the current study, due to the lack of longitudinal rs-fMRI data in the PPMI database for healthy controls. All subjects were assessed with the Movement Disorders Society-Unified Parkinson´s Disease Rating Scale Part III (UPDRS-III), the motor examination part of the scale, as well as the Montreal Cognitive Assessment (MoCa), an evaluation of cognitive impairment. Information on demographic and clinical data, as well as neurological assessment, is presented in Table 1. Subjects were either ON/OFF medication during rs-fMRI acquisition, depending on their use of levodopa medication at each scanning point. Twenty-four subjects were on medication during the first scan, 35 during the second scan, and at the last scan all 40 subjects were on medication. Subjects off medication were either not on medication treatment, or they had not been taking any medication for more than six hours before scanning.

Further, the LEDD value was calculated for subjects on medication, for each of the three scans. Subjects were on different levodopa medications, and they were all extracted from the PPMI database, and compared with the dates of the rs-fMRI recordings to find the approximate dose they were on the day of scanning.

Table 1.
Characteristics of PD subjects

	Age, years	MoCa	UPDRS-III	Med ON/OFF	LEDD
rs-fMRI1	61.2 (±10.2)	27.0 (±2.9)	17.8 (±9.9)	24/16	185.4 (±211.4)
rs-fMRI2	62.3 (±10.2)	27.2 (±2.6)	20.8 (±9.9)	35/5	312.3 (±212.3)
rs-fMRI3	64.3 (±10.1)	27.4 (±3.3)	22.8 (±12.6)	40/0	496.0 (±275.9)

Note. N = 40 (13 females). Values presented are mean ± standard deviations.
Abbreviation: Med; Medication.

Image acquisition

fMRI resting-state scans were acquired using an EPI-sequence at 3T scanners (Siemens TrioTim/Prisma Fit). Subjects were scanned three times between 2012 and 2017. The time period between the first and second scan was one-two years, with an average of one year, and one-three years from the second scan to the last scan, with an average of two years. Scanning lasted 8 minutes and 24 seconds, and subjects were told not to fall asleep, to keep their eyes open and not think about anything in particular. A total of 210 volumes were collected with echo time (TE) of 25ms and a repetition time (TR) of 2.4 seconds. A flip angle of 80 degrees, slice thickness was 3.3mm, voxel size 3.29x3.29. In the current study only complete datasets containing all 210 volumes have been included (Parkinson's Progression Markers Initiative; https://www.ppmi-info.org/).

Preprocessing

The data were preprocessed using Statistical Parametric Mapping (SPM12; Welcome Trust Centre for Imaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Preprocessing steps included realignment (0.9 quality, 5 mm smoothing kernel, registered to first image with 2nd degree B-spline), unwarping (using 7x7), resliced to mean image, normalized to ICBM-template (with 2mm^3 voxel size), co-registering to anatomical volumes, and smoothing with Gaussian kernel (6 mm^3 voxels).

Independent component analysis

For the resting-state connectivity analysis, Group ICA of functional MRI Toolbox was used (GIFT v3.0b; http://icatb.sourceforge.net) to do spatial group ICA. ICA works by breaking down the data into independent components. These components can either be associated with neurological anatomical systems in the brain, or they can represent noise. The latter is excluded in the analytic process, until one is left with components that are anatomically and functionally plausible (Fox & Raichle, 2007). For the ICA analysis, the steps in GIFT Toolbox setup in two recent studies in PD using this toolbox was followed (See (Díez-Cirarda et al., 2018; Kim et al., 2017) for more details). Rs-fMRI data across all subjects were decomposed into independent components using the Infomax algorithm repeated 20 times in ICASSO, selecting intra-cluster similarity of values >0.8. Minimum description length approach was used for each subject. Before data reduction, voxel-wise variance-normalization steps were applied, followed by a first-step subject-specific data reduction to 120 IC´s, with a second step group-data reduction to 100 IC´s using expectation maximization algorithm. Back reconstruction (GICA) obtained subject-specific maps and time courses, and final components were not scaled to preserve the signal as a per cent change in signal. IC´s were hand classified (Griffanti et al., 2017) and 48 components were identified as meaningful. These IC´s were distributed across eight networks; 10 in visual network (VIS), 10 in sensorimotor network (SM), two in auditory network (AUD), 10 in central executive network (CEN), seven in default mode network (DMN) and two in salience network (SN). Furthermore, basal ganglia was clearly parcellated into individual posterior and anterior putamen, caudate nucleus and thalamus, a total of four IC´s, and these were grouped as a basal ganglia network (BG). In addition, three components in cerebellum (CER) were found.

Components in DMN were found to be medial frontal cortex, left angular gyrus, two components of right angular gyrus, cingulum posterior, precuneus and precuneus/superior

parietal. CEN included middle frontal cortex, left inferior frontal gyrus, left supramarginal gyrus, supramarginal gyrus, posterior insula, right rolandic operculum inferior, right rolandic operculum, frontal middle orbital cortex, frontal inferior orbital cortex and superior parietal junction. BG consisted of posterior and anterior putamen, caudate nucleus and thalamus. Components in SN were found to be anterior insula and anterior cingulate cortex, whilst components in AUD included superior temporal gyrus and middle temporal gyrus. Included in SM was primary somatosensory cortex, right and left precentral gyrus, supplementary motor area (SMA), superior medial frontal cortex, superior frontal cortex, middle cingulum/supplementary motor area (SMA), middle cingulum, and left and right precentral/motor. VIS consisted of middle occipital cortex, inferior occipital cortex, two components of superior occipital cortex, two components of lingual gyrus, cuneus, calcarine cortex, middle temporal gyrus and fusiform. CER included cerebellum 6, cerebellum 4, 5, 6 and vermis 4 and 5.

MANCOVA

The MANCOVAN-toolbox (v1.0) integrated in GIFT-toolbox was used to compare scanner times (one, two and three), modelling interactions with covariates. In the design matrix setup, subjects were grouped over scans and the covariates age, gender, medication, MoCa and UPDRS-III were added. Age was defined as a categorical variable, whereas gender, MoCa and UPDRS-III were defined as continuous variables. Gender should have been a categorical variable, but, the MANCOVAN-toolbox would not show the interaction between gender and scan when gender was added as a categorical variable, as both had less than, or equal to three levels, meaning that the gender covariate does not change over scans. This is due to the choice of grouping subjects according to scan time, which is a way to set up the MANCOVAN-toolbox to be able to compare over scans. Instead of removing gender from the analysis, it was added as a continuous variable. In the first analysis, medication was

categorical (ON/OFF), then continuous (LEDD values) in the second analysis. The analysis was run as a univariate ANCOVA, (i.e. analysis of variance with covariates). In the setup analysis, the networks DMN, CEN, BG, AUD, VIS, SM, SN and CER, as well as their associated components, were added. *P*-value of 0.01 was set as significance threshold, based on the false discovery rate (fdr) threshold criteria. Connectogram was chosen to display the MANCOVAN-toolbox results.

Results

Main effect of scan

A main significant effect of scan was found at the second scanning session compared to the first (2) – (1) ($p < 0.01$), and at the third scanning session compared to the first (3) – (1) ($p < 0.01$). There was no significant effect of scan for the third scanning session compared to the second (2) – (3).

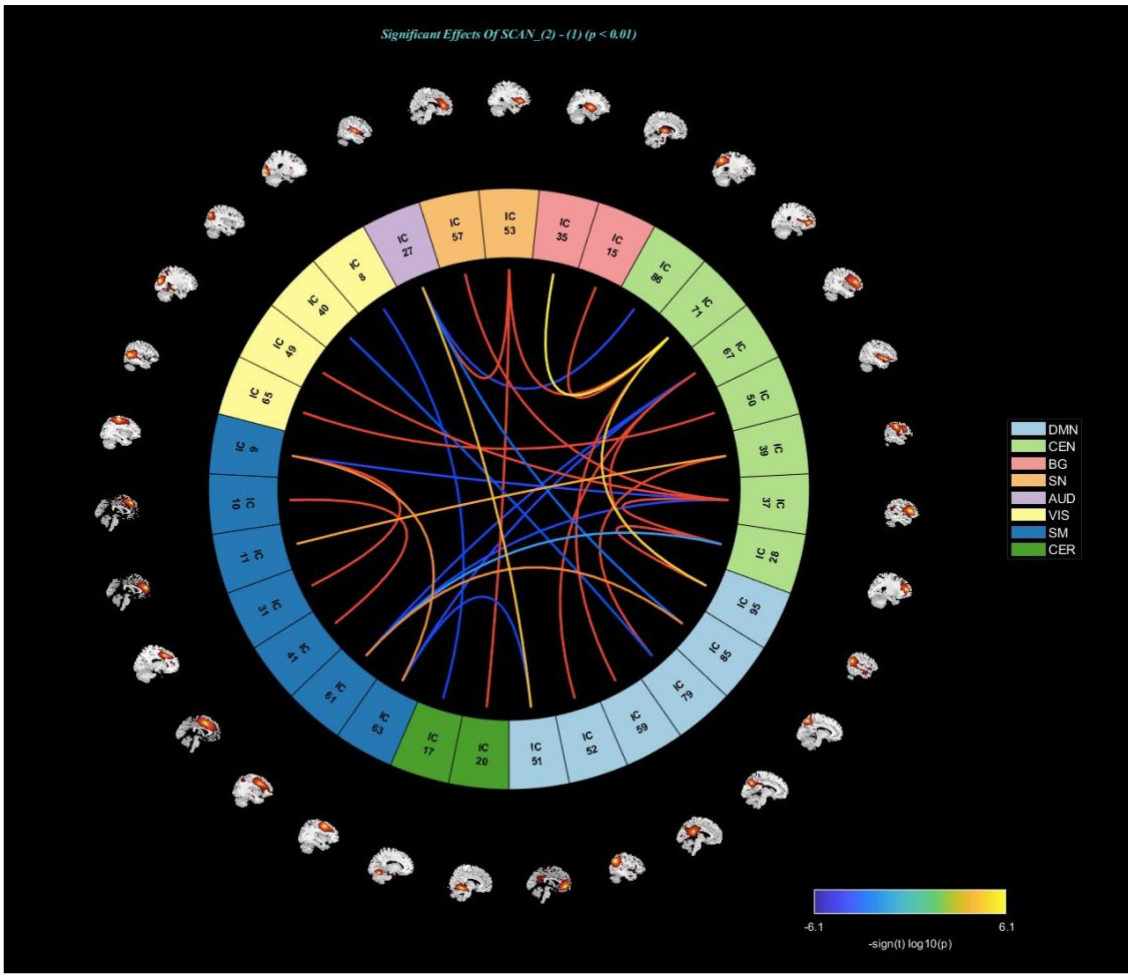

Figure 1: Main effect of scan ($p < 0.01$, fdr). Connectogram displays alterations of functional connectivity at the second scan compared to the first. Blue connections between components indicate decreased connectivity, yellow connections indicate increased connectivity.

Comparing the second scan to the first, see Figure 1, there was increased connectivity between posterior putamen (35), and thalamus (15) and frontal middle orbital cortex (71), as well as between frontal middle orbital cortex (71), and anterior insula (53), right angular gyrus (95) and cingulum posterior (59). Increased connectivity was further found between right rolandic operculum (67) and both right and left angular gyrus (95, 52), between posterior insula (50) and middle temporal gyrus (65), and between left inferior frontal gyrus (37), and anterior cingulate cortex (57), superior occipital cortex (49) and middle frontal cortex (28). Left supramarginal gyrus (39) had increased connectivity with precuneus (79) and superior medial frontal cortex (11), precuneus/superior parietal (85) with left precentral/motor (61), and medial frontal cortex (51) with superior temporal gyrus (27). Within the SM, increased

connectivity was found between right precentral/motor (63) and left precentral gyrus (9), between middle cingulum/SMA (41) and SMA (10), and between superior frontal cortex (31) and left precentral gyrus (9). Anterior insula (53) had increased connectivity with superior temporal gyrus (27) and vermis 4,5 (20).

Decreased connectivity was found between superior temporal gyrus (27), and superior parietal junction (86) and precuneus/superior parietal (85), between right rolandic operculum (67) and both left and right precentral/motor (61, 63), as well as between left inferior frontal gyrus (37), and left precentral gyrus (9) and left precentral/motor (61). The latter also had decreased connectivity with middle frontal cortex (28). Precuneus (79) had decreased connectivity with superior occipital cortex (40), cerebellum 6 (17) with middle occipital cortex (8), and right precentral/motor (63) with medial frontal cortex (51).

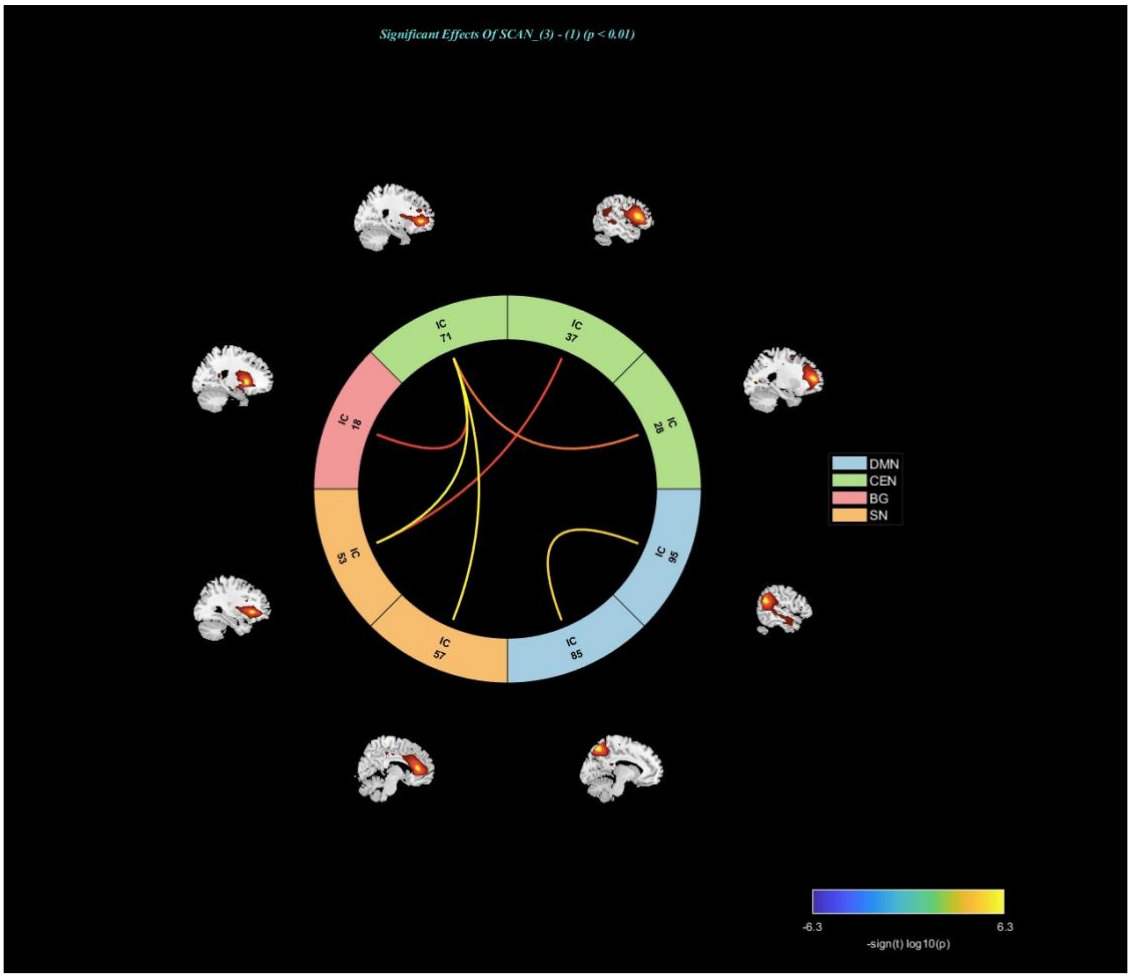

Figure 2: Main effect of scan ($p < 0.01$, fdr). Connectogram displays alterations of functional connectivity at the third scan compared to the first. Yellow connections between components indicate increased connectivity.

At the third scan compared to the first, see Figure 2, there was increased connectivity between frontal middle orbital cortex, (71) and anterior putamen (18), anterior insula (53), anterior cingulate cortex (57) and middle frontal cortex (28). Increased connectivity was further found between anterior insula (53) and left inferior frontal gyrus (37), as well as between right angular (95) and precuneus/superior parietal (85).

Interaction effect of covariates

Age, gender, UPDRS III scores and MoCa scores did not have a significant interaction effect with scanning sessions. Medication as a continuous variable in the second analysis, based on LEDD values, did not have a significant interaction effect either. There was a significant interaction effect of medication (MED) on scan in the ON/OFF analysis, at the

second scanning session compared to the first, SCAN X MED [(1) – (0) X (2) – (1)] (p < 0.01), and at the third scanning session compared to the first, SCAN X MED [(1) – (0) X (3) – (1)] (p < 0.01). There was not a significant interaction effect of medication at the third scanning session compared to the second, SCAN X MED [(1) – (0) X (2) – (3)].

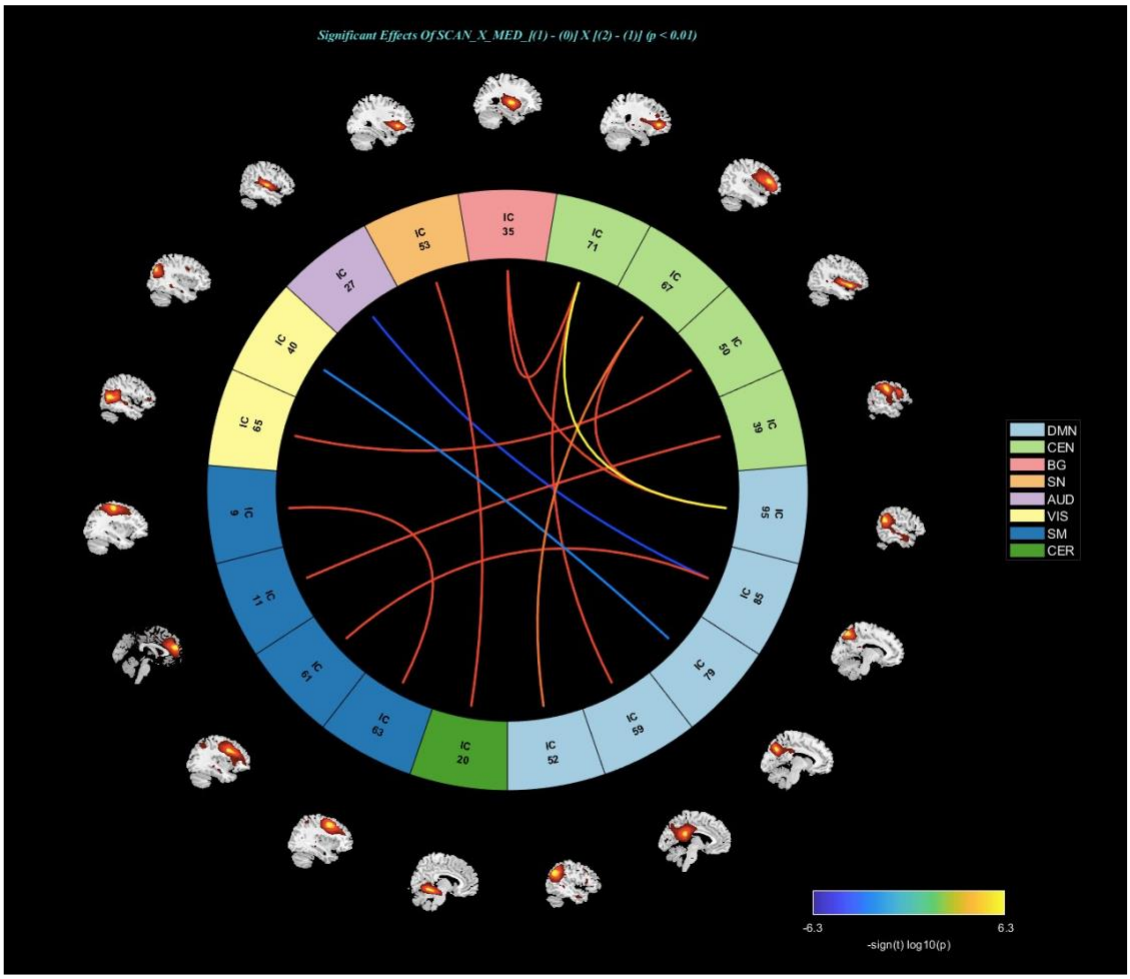

Figure 3: Interaction effect of medication (ON/OFF) (p < 0.01, fdr). Connectogram display the interaction of medication on alterations of functional connectivity at the second scan compared to the first. Blue connections between components indicate decreased connectivity, yellow connections indicate increased connectivity.

At the second scan compared to the first, see Figure 3, medication had a significant interaction effect on the increased connectivity between posterior putamen (35), and frontal middle orbital cortex (71) and right angular (95), between frontal middle orbital cortex (71) and both right angular (95) and cingulum posterior (59), as well as between right rolandic

operculum (67), and right and left angular gyrus (95, 52). Medication further had a significant interaction effect on the increased connectivity between posterior insula (50) and middle temporal gyrus (65), between left supramarginal gyrus (39) and superior medial frontal cortex (11), between precuneus/superior parietal (85) and left precentral/motor (61), as well as on the increased connectivity between right precentral/motor (63) and left precentral (9). Vermis 4, 5 (20) had increased connectivity with anterior insula (53), which medication further had a significant interaction effect on.

Medication had a significant interaction effect on the decreased connectivity found between precuneus/superior parietal (85) and superior temporal gyrus (27), and between precuneus (79) and superior occipital cortex (40).

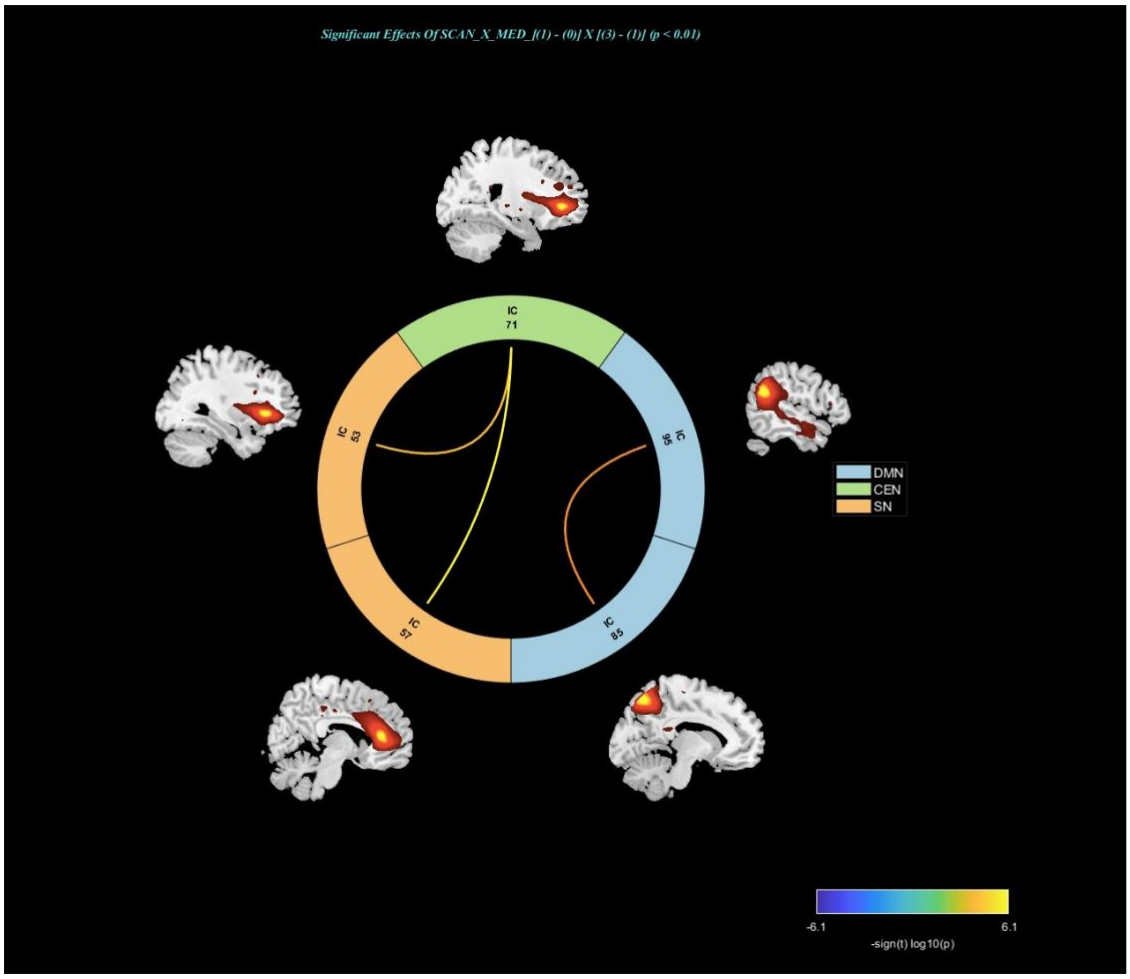

Figure 4: Interaction effect of medication (ON/OFF) (p <. 0.01, fdr). Connectogram displays the interaction of medication on alterations of functional connectivity at the third scan compared to the first. Yellow connections between components indicate increased connectivity.

At the third scan compared to the first, see Figure 4, medication had an interaction effect on the increased connectivity between frontal middle orbital cortex (71), and anterior cingulate cortex (57) and anterior insula (53), as well as on the increased connectivity between right angular (95) and precuneus/superior parietal (85).

Discussion

Data was extracted from the PPMI database to conduct a longitudinal rs-fMRI study of alterations of functional connectivity in people with PD. Included in the current study was three scanning sessions conducted over five years, to examine if any of the chosen covariates would effect functional connectivity measures over time. Results revealed a main significant

effect of scan at the first scan compared to the second, and at the second compared to the first, highlighting alterations in functional connectivity. At the first scan compared to the second, connectivity was increased both within and between networks, mostly in brain areas related to CEN, DMN, SN and SM. Decreased connectivity was found exclusively between networks, mainly between SM, CEN and DMN. No significant alterations of connectivity occurred at the third scan compared to the second, as no significant main effect of scan was found. At the third scan compared to the first, there was a main significant effect of scan, with increased connectivity between CEN, SN and BG, and within DMN.

Neither age, gender, UPDRS III scores or MoCa scores had significant interaction effects on functional connectivity across the scanning sessions. Medication measured in LEDD values did not have a significant interaction effect on scan either. There was a significant interaction effect between medication (ON/OFF) and scan (point). At the first scan compared to the first, medication had an interaction effect mainly on the increased connectivity between BG, CEN, DMN and SM, as well as within SM. Medication had an interaction effect on the increased connectivity between CEN and SN, and within DMN at the third scan compared to the first as well. No interaction effect was found at the third scan compared to the second. To answer to the research question, there were alterations of functional connectivity found across scanning sessions in PD subjects, and use of PD medication as an ON/OFF measure had a significant interaction effect on these alterations.

When comparing the third scanning session with the second, there was no significant main effect of scan, or a significant interaction effect for medication and scan. No alterations of functional connectivity in any of the networks were statistically significant. Considering the alterations at the third scan compared to the first, this finding is unexpected. One possible explanation could be the use of medication. At the first scanning session, subjects had recently been diagnosed with PD, and therefore had recently started with levodopa-

medication. Use of medication could therefore significantly influence connectivity in the first few years, and perhaps normalize connectivity as subjects are taking it for a longer period of time. This cannot be confirmed in the current study, as the PD subjects are not compared to healthy controls. Therefore, it remains as a suggestion, and future research on this could possibly give more answers. Further, several of the subjects that were off medication at the first scan had started on medication at the time of the second scan (see table 1). This could have contributed to the alterations of connectivity found at the first scan compared to the second, and could arguably explain the absence of significant alterations of connectivity later in the disease, at the third scanning session compared to the second.

Compared to previous longitudinal PD studies, mainly focusing on studies comparing follow-up scans to baseline amongst PD subjects, few studies report similar findings as the results from the current study. Included in the current findings were increased connectivity between middle cingulum/SMA and SMA at the first scan compared to the second, a finding present in the study conducted by Tuovinen et al. (2018) as well. Their subjects were in the early or moderate disease stage, with follow-up scan being 1.5 years later, compatible with the second scan in the current study. Further, comparing PD subjects with healthy controls at the follow-up, they found decreased connectivity between rolandic operculum and both pre- and postcentral gyrus (Tuovinen et al., 2018). This finding occurred in the current study as well, at the second scan compared to the first, in PD subjects. However, there were some dissimilarities, mainly that their study found hypoconnectivity, i.e. decreased connectivity, within the SMN, which correlated with UPDRS-III scores, arguing that the hypoconnectivity is related to motor symptoms (Tuovinen et al., 2018). In the current study, there was hyperconnectivity within this network. Medication did have an interaction effect on this hyperconnectivity, whereas Tuovinen et al. (2018) acquired their rs-fMRI data when their PD subjects were off medication. If the hyper connectivity in SM is dependent on levodopa

medication, this could explain some of the dissimilarities between studies. On the other hand, in the study conducted by Hu et al. (2015) decreased fALFF values were found in left pre- and postcentral when their follow-up was compared to baseline, and their PD subjects were on medication at the time of rs-fMRI acquisition (Hu et al., 2015). Elaborating on the general inconsistent findings in PD research, the differences found between these studies could be due to differences in analytical methods used and medication status during rs-fMRI acquisition. Preferably, the current study should be compared to another longitudinal PD study, using ICA, with the same covariates, examining the same interaction effects. To my knowledge, no such studies exist. In doing this, it could further allow for reproducibility between studies to be examined. The current study includes three scanning sessions, while previously mentioned studies include two sessions, baseline and follow-up. As connectivity have been measured with one more rs-fMRI scan in the current study, this could have contributed to the dissimilar findings, and future longitudinal study could benefit from including more than two scanning sessions. These arguments could also extent to findings in cerebellum. Research suggest a compensatory mechanism of the cerebellum for decreased connectivity in basal ganglia (Wu & Hallett, 2013). Results from the current study does not confirm this. Increased connectivity was found between vermis 4 and 5 (CER) and anterior insula (SN) when comparing the second scan to the first, as well as decreased connectivity between cerebellum and superior temporal gyrus (AUD), though none of these alterations indicate a connection to basal ganglia. One could speculate if this is caused by use of levodopa. Several studies find increased connectivity while subjects are off medication, and more normalized connectivity once subjects are on medication (Festini et al., 2015; Wu, Long, et al., 2009). In the current study, there are not separate medication conditions, instead medication was entered as a covariate in the analysis. Findings indicate that medication does have an interaction effect on

connectivity. Future studies, using the same method and including the same covariates, would be beneficial to further elaborate on this interaction.

Another interesting finding is that in the analysis where medication was a continuous covariate, i.e. based on LEDD values, it did not have a significant interaction effect with scanning timepoint. This finding suggest that being ON/OFF levodopa influences functional connectivity more than the exact LEDD values subjects were on at the time of rs-fMRI acquisition. Previous literature is not clear on the matter. Krajovicova et al. (2012) found that enhanced connectivity in the DMN was dependent on their PD subjects being on higher LEDD values, whereas in the longitudinal study conducted by Boon et al. (2020), alterations of connectivity in frontoparietal network and dorsal attention network (DAN) related to executive dysfunction were not affected by adding LEDD values as a covariate (Boon et al., 2020; Krajcovicova et al., 2012). Further research on LEDD values would be useful.

In addition to LEDD values, neither age, gender, MoCa scores nor UPDRS-III scores had a significant interaction effect on scan, indicating that none of these covariates influenced alterations of functional connectivity in the brain. As the current study does not compare PD subjects with healthy subjects, this does not provide evidence on not affecting PD dysfunction, it rather indicates that, compared to the first scan, it does not influence the longitudinal alterations occurring at the second or third scan. Older age has been found to be a predictor of PD severity (De Micco et al., 2019), however considering the sample recently diagnosed with PD in the current study, the lack of significant interaction could be due to the relative young subjects. Further, there is an uneven distribution of men and females in the sample, as it mainly consists of men, which could explain why gender does not have an interaction effect on either of the scanning sessions. Not all studies find that age and gender have an influence on functional connectivity (Li et al., 2016), which could explain why there were no interaction effects in the current study either. UPDRS-III scores did not have an

interaction effect on alterations of functional connectivity either, which could indicate that disease progression and severity did not influence functional connectivity. Studies find different effects of this covariate, as positive and negative correlations with connectivity measure have been found (Hou et al., 2016; Wu, Wang, et al., 2009). All subjects in the current study were quite recently diagnosed with PD, and the study examines connectivity from the first scan and up to five years later at the last scanning session. Scores not having a significant interaction effect with functional connectivity could be due to the PD symptoms, as well as disease progression, not having sufficiently evolved yet for the results to be statistically significant.

Alterations of connectivity in DMN has been implicated in PD, as well as in neurodegeneration in general (Hohenfeld et al., 2018). One previous longitudinal PD study found decreased connectivity within DMN at follow-up, compared to baseline (Zeng et al., 2017). In the current study, there were no alterations of connectivity within DMN at the second scan compared to the first, instead increased connectivity was found between precuneus/superior parietal and right angular gyrus, when comparing the third scan to the first. This alteration of connectivity was further found when comparing the third scan to the first scan when medication was introduced as a covariate, indicating that the use of levodopa could influence connectivity. In the longitudinal study by Zeng et al. (2017) subjects were off medication during rs-fMRI acquisition, which could explain the different results (Zeng et al., 2017). Furthermore, another finding in the current study was that MoCa scores did not have a significant interaction effect on scanning point, indicating that cognitive decline did not influence functional connectivity. Considering the extensive literature implicating DMN in cognitive impairment, not just in PD, but in other neurodegenerative diseases such as Alzheimer's, this finding is unexpected (Hohenfeld et al., 2018). Multiple studies suggest that alterations in DMN could be a biomarker for cognitive decline in PD (Wolters et al., 2019).

Previous research has indicated that use of medication could interact with connectivity in DMN, as decreased connectivity was found when both cognitively impaired and unimpaired PD subjects were on medication at the time of rs-fMRI acquisition (Amboni et al., 2015; Gorges et al., 2015). This cannot explain the findings in the current study. However, out of the 40 PD subjects included, the majority of them have normal MoCa scores across all three scanning sessions, indicating that they are cognitively unimpaired (see table 1). MoCa score above 26 is considered as cognitively unimpaired (Milani et al., 2018). Lack of a significant interaction effect of MoCa on functional connectivity could be due to the sample not including enough subjects who were cognitively impaired. In addition, in the current study, subjects with differences in cognitive status are pooled together into the same group. As Wang et al. (2019) pointed out, the sample should be divided into separate subgroups based on cognitive impairment (Wang et al., 2019). Considering this, the current sample selection is not sufficient to investigate cognitive decline. Further, subjects, independent of their PD, are in an age group that could experience cognitive decline anyway, and the probability for this increases as they get older. Some studies suggest that DMN is implicated in normal aging, not unexpectedly considering its vital role in dementia research (Ferreira & Busatto, 2013). Grady et al. (2016) conducted a study comparing 45 younger (18-29 years) and 39 older (between 60-83 years) subjects, with no history of neurodegenerative diseases, finding reduced functional connectivity in DMN in the older group (Grady et al., 2016). As the sample in the current study has a relative young mean age throughout the scanning sessions (see table 1), it could explain why there were no findings of decreased connectivity in DMN. Another study has further examined decreased connectivity in DMN in normal aging, conducting a study with 2878 non-demented subjects, with a mean age of 66 years (Zonneveld et al., 2019). Included in their findings was decreased connectivity in DMN, and the authors argue that this could be relevant for studies on neurodegeneration as it provides information on how the

normal brain ages. This also stresses the importance of including age-matched healthy control group in future research, to be able to exclude the possibility of findings being a result of aging (Zonneveld et al., 2019). To summarize, several factors could explain the lack of decreased connectivity within, and between, the DMN in the current study.

Findings from the current study included alterations of connectivity between components of AUD and VIS, and CEN, DMN and CER, at the second scanning session compared to the first. Medication as a covariate interacted with some of these alterations, including the increased connectivity between middle temporal gyrus (VIS) and posterior insula (CEN), as well as the decreased connectivity between superior occipital cortex (VIS) and precuneus (DMN), and between superior temporal gyrus (AUD) and precuneus/superior parietal (DMN). VIS and AUD are not amongst the most implicated networks in PD research (Hohenfeld et al., 2018). However, some research has been done. Gratton et al. (2019) conducted a comprehensive study with 107 non-demented PD subjects, compared to 46 healthy controls, examining the extent of functional connectivity alterations in PD, in whole-brain networks. Rs-fMRI acquisition was done while subjects were off medication, and PD subjects had an average disease duration of six years (Gratton et al., 2019). Compared to healthy controls, PD subjects had alterations between thalamic, cerebellar and sensorimotor networks. Further, decreased connectivity was found between sensory cortical networks, including visual and auditory networks, and sensorimotor networks. Correlation analysis was conducted between these alterations and clinical behaviour to examine if they contributed to symptomology of PD. Alterations in sensorimotor and cerebellar networks were correlated with both cognitive and motor performance (Gratton et al., 2019). The correlations between visual and auditory networks and symptomology were not statistically significant. However, authors argue that it is beneficial to study whole-brain network connections instead of focusing on a few regions in the brain, as alterations of functional connectivity in PD occur

across the entire brain (Gratton et al., 2019). Another study examined whole-brain connectivity, comparing 107 PD subjects with 58 healthy controls, scanning PD subjects while they were on medication (de Schipper et al., 2018). Average disease duration was nine years. Findings included increased connectivity within medial and lateral visual networks, as well as in sensorimotor areas, for the PD subjects compared to healthy controls. Decreased connectivity was found mainly in occipital and frontal parts of the brain. Authors argue that the increased connectivity found in the PD group could either be a result of disease-related processes, or be part of the compensatory mechanisms suggested to be occurring due to dysfunction in other areas of the brain (de Schipper et al., 2018). There are networks more implicated in PD research than VIS and AUD. Nevertheless, the mentioned studies demonstrate how whole-brain connectivity could potentially be important to symptomology or progression of the disease, and further research is beneficial.

One prominent finding in the current study was the interaction between CEN, SN and DMN. At the second scan compared to the first, as well as at the third scan compared to the first, there was increased connectivity between several brain areas belonging to these networks, specifically between CEN and DMN, and CEN and SN. Further, increased within-network connectivity was found in DMN at the third scan compared to the first. Medication had an interaction effect on much of the increased connectivity between networks, as well as the within-network connectivity in DMN. Research suggests that SN operates as a switch between CEN and DMN (Menon, 2011). These three networks have been identified as important for cognitive function, as well as dysfunction. DMN is deactivated during cognitive tasks, whereas both CEN and SN are activated. As the SN is crucial for redirecting attention to the most important attentional cues, it has been proposed that SN contributes to CEN being engaged when there is something important to focus on, and that it downregulates the DMN (Menon, 2011). Evidence of this was found in a study by Sridharan et al. (2008), who first

conducted task-related fMRI experiments in 18 participants (without PD) to examine the interaction between the networks in response to cognitive stimuli, and then a rs-fMRI experiment examining the response in a task-free condition. Results firstly confirmed that deactivation of the DMN followed the activation of SN and CEN. Further, authors found that both right fronto-insular cortex (rFIC) and ACC in SN had a peak latency earlier than brain regions belonging to CEN and DMN (Sridharan et al., 2008). Findings suggest that rFIC of SN is responsible for switching between the networks, being an outflow hub, as there was direct or indirect connections between rFIC and areas of CEN and DMN. This finding was present during task-related fMRI, and then replicated in the resting-state experiment. Authors further argue that it could be an important feature of cognitive control, and that the SN contributes to the activation and deactivation of the two other networks (Sridharan et al., 2008). The switching-mechanism has been suggested to be important in different psychiatric disorders, such as autism and schizophrenia, as well as PD (Menon, 2011). Putcha et al. (2015) conducted a resting-state study investigating the interaction between CEN, DMN and SN in a PD group. Included in their study was 24 PD subjects, with average disease duration of five years, all scanned while being on medication, and 20 healthy controls. Findings showed that for the PD subjects, there was a positive interaction between CEN and DMN, as well as a negative interaction between SN and CEN, compared to the control group (Putcha et al., 2015). Authors argue that this opposite interaction pattern could be important for PD pathology, as adequate cognitive performance is dependent on a regular connectivity pattern between these networks. Positive coupling between CEN and DMN could indicate issues in the suppression of DMN activation, a dysfunction found in both Alzheimer's and schizophrenia as well (Putcha et al., 2015). It could explain the increased connectivity between CEN and DMN in the current study, indicating network dysfunction as a result of PD pathology. Further, medication had an interaction effect on the connectivity, while subjects in

the Putcha et al. (2015) study were on medication at the time of scan. PD medication could influence the altered connectivity pattern. Altered interaction between CEN and DMN was found in a study by Tessitore et al. (2017) as well. The aim of their study was to investigate whether alterations of functional connectivity would predict impulse control disorders (ICD) in PD subjects. Eighty-five drug naïve PD subjects were included at baseline, with one year average disease duration. Three years later, 15 of them had developed ICD. Authors found a positive coupling between CEN and DMN at baseline for PD subjects who later developed ICD, arguing that increased connectivity between CEN and DMN predicts the development of ICD (Tessitore et al., 2017). Their findings cannot explain the alterations of connectivity found in the current study, as it does not investigate ICD among PD subjects. Instead, it further elaborates on the importance of the CEN-DMN connection to PD symptomology, urging for more research to be conducted.

Left inferior frontal gyrus (CEN) is an area implicated in alterations of connectivity found in the current study. At the second scan compared to the first, results showed increased functional connectivity between left inferior frontal gyrus and anterior cingulate cortex (SN), middle frontal cortex (CEN) and superior occipital cortex (VIS), as well as decreased connectivity with areas in SM. Increased connectivity was further found between left inferior frontal gyrus and anterior insula (SN) at the third scan compared to the first. Medication did not have an interaction effect on any of these alterations. It has been proposed that the inferior frontal gyrus, or cortex, is specifically sensitive to levodopa, as it could be involved in the mechanisms behind LID (Cerasa et al., 2015). Inferior frontal cortex is part of the prefrontal cortex, and is believed to be involved in motor control inhibition (Yoo et al., 2019). Cerasa et al. (2015) conducted a study comparing 24 PD subjects with, and without LID, examining possible alterations of functional connectivity in the inferior frontal cortex. Average disease duration was seven years for subjects with LID, and five years for subjects without. Subjects

were scanned twice, once being off medication and the other after levodopa administration. Their findings revealed increased functional connectivity between right inferior frontal cortex and right putamen for the subjects with LID when subjects were on medication (Cerasa et al., 2015). However, Cerasa et al. (2015) argue that the increased connectivity between these areas not necessarily arise due to levodopa medicine, as it could be a consequence of the movement issues caused by LID. They investigated this by conducting a follow-up analysis using transcranial magnetic stimulation (TMS), which revealed that the inferior frontal cortex is sensitive to levodopa, confirming their previous finding (Cerasa et al., 2015). In the current study, no interaction was found between the left inferior frontal gyrus and putamen. PD subjects in the current sample were recently diagnosed, and have taken levodopa-medication for a short period of time. As LID is caused by long-term use (Espay et al., 2018), this could explain the lack of interaction with putamen. Cerasa et al. (2015) used a seed-based analysis, including areas in SM, CER and BG as regions of interest (ROI). In the current study, left inferior frontal gyrus interacts with areas in SN, CEN and VIS. As Cerasa et al. (2015) did not include ROIs in either of these networks in their study, dissimilarities between studies could be caused by this. Findings from Cerasa et al. (2015) were not confirmed by Herz et al. (2016), who instead found no interaction of LID in the connectivity between inferior frontal cortex and putamen. Included in their study was 12 subjects with LID, and 10 without, who were scanned whilst being on medication. Subjects with LID had an average disease duration of seven years, whereas the average for subjects without LID was six years. Results revealed decreased functional connectivity between primary sensorimotor cortex and putamen for the PD subjects with LID, compared to subjects without (Herz et al., 2016). Authors argue that this altered connectivity did not significantly distinguish the two groups. However, it predicted severity of LID, suggesting its relevance to PD pathology. Herz et al. (2016) compared their findings to the study by Cerasa et al. (2015), arguing that their results are not

necessarily contradictory. It could be due to methodological differences, further illustrating how inconsistencies in PD research related to methods used (Herz et al., 2016). Yoo et al. (2019) examined the connection between LID and cerebellum, as cerebellum is involved in motor control. Authors acquired rs-fMRI data from a drug-naïve PD sample at baseline, and followed them for the next several years. After five years, 25 PD subjects had developed LID and 26 had not developed LID. Average disease duration was respectively 14 and 18 months at baseline. Rs-fMRI data acquired at baseline was then compared between the two groups. Included in their findings was increased functional connectivity between cerebellum and left inferior frontal gyrus for the PD subjects who developed LID (Yoo et al., 2019). Results are interesting considering hyperconnectivity in cerebellum has been a common finding in PD research. Authors argue that increased connectivity between cerebellum and prefrontal cortex could compensate for issues with motor control for PD subjects, and that inferior frontal gyrus is important for LID pathology (Yoo et al., 2019). Conclusions on LID pathology cannot be drawn from the current study, as there was no assessment of LID amongst the subjects. Nevertheless, results found demonstrate increased connectivity between left inferior frontal gyrus and areas in SN, CEN and VIS, suggesting that it could be important to include these networks in further studies of LID.

Increased connectivity was found between frontal middle orbital cortex (CEN) and several other brain areas in the current study. At the second scan compared to the first, there was increased connectivity between frontal middle orbital cortex and components of CEN, SN and BG, such as posterior putamen, which medication had an interaction effect on. At the third scan compared to the first, frontal middle orbital cortex had further increased connectivity with other components of CEN, BG, and SN. When medication was added as a covariate, it interacted with the increased connectivity between frontal middle orbital cortex and components of SN. Frontal middle orbital cortex is part of the orbitofrontal cortex,

located in the prefrontal cortex (Dixon et al., 2017). It has been suggested that levodopa increases reduced connectivity in prefrontal cortex, as well as between prefrontal cortex and other areas of the brain (Filippi et al., 2019). This is shown in the study by Wu et al. (2009), who found that ReHo activity in prefrontal cortex increased after levodopa administration, compared to the decreased connectivity found in the off-medication state (Wu, Long, et al., 2009). Medication interacted with much of the increased connectivity between frontal middle orbital cortex and other brain areas found in the current study, indicating that the area is sensitive to levodopa. Both frontal middle orbital cortex and inferior frontal cortex are located in prefrontal cortex, and as studies suggest that the latter is important in LID pathology, this could be applicable for orbitofrontal cortex as well (Dixon et al., 2017).Alterations of connectivity in orbitofrontal cortex have further been implicated in PD subjects with depression (Hohenfeld et al., 2018). Luo et al. (2014) conducted a resting-state study using ALFF, including 29 PD subjects without depression, comparing them to 30 PD subjects with depression. Subjects had an average disease duration of two years, and were drug-naïve at the time of rs-fMRI acquisition. Authors found increased ALFF values in left orbitofrontal cortex for the PD group with depression, compared to the group without (Luo et al., 2014). The prefrontal cortex is believed to be important for regulation of emotions, and research has suggested that this area is crucial for development of depressive symptoms. The increased ALFF values found support this notion (Luo et al., 2014). Orbitofrontal cortex has been shown to be involved in the pathology behind pain in PD as well. Polli et al. (2016) found decreased fALFF values in left orbitofrontal cortex for PD subjects with persistent pain. Their sample included 80 PD subjects, 40 with pain, all scanned while being on medication. Average disease duration was 10 years (Polli et al., 2016). In the current study there is no assessment of either depression nor pain. Conclusions on the increased connectivity of the frontal middle orbital cortex and its relation to PD symptomology can therefore not be drawn.

However, it does suggest a possible connection between alterations found, and non-motor PD symptoms.

Right angular gyrus (DMN) was involved in several of the alterations of connectivity found in the current study. At the second scanning session, compared to the first, there was increased connectivity between right angular gyrus and components of CEN. Further, comparing the third scan to the first, there was increased connectivity between right angular gyrus and precuneus/superior parietal (DMN). Medication had an interaction effect on some of this connectivity. Further, after medication was added as a covariate, increased connectivity was found between right angular gyrus and posterior putamen (BG). Angular gyrus is located in the inferior parietal lobule. Its function is not determined, however it has been suggested that it is involved in reading comprehension, semantic processing and attention and spatial cognition (Seghier, 2013). Further, there is evidence suggesting the involvement of right angular gyrus in PD dysfunction (Tahmasian et al., 2017). Choe et al. (2013) conducted a resting-state study with seven drug-naïve PD subjects, as well as 22 PD subjects who were taking medication, scanned whilst being off medication. Average disease duration was respectively six months and three years. The two groups were compared to healthy controls. Results showed increased ReHo activity in angular gyrus for both PD groups, which is compatible with the current study. However, they could not make conclusion about its relevance to PD pathology (Choe et al., 2013). Tahmasian et al. (2017) conducted a meta-analysis of 28 resting-state studies, which included PD subjects both on and off medication at the time of rs-fMRI acquisition. Authors found that across studies, there was increased connectivity in the bilateral inferior parietal lobe while subjects were off medication, and decreased connectivity in the right inferior parietal lobe while subjects were on medication, compared to healthy controls (Tahmasian et al., 2017). Alterations of connectivity in this area being dependent on the medication state is interesting, considering

how medication had an interaction effect on the connectivity in right angular gyrus in the current study. Tahmasian et al. (2017) discuss how alterations of this area could be a potential biomarker for PD. Arguably, it could be a form of compensatory mechanism for the basal ganglia, due to the area being important for sensorimotor integration (Tahmasian et al., 2017). In the current study, at the second scanning session compared to the first when adding medication as a covariate, there was increased connectivity between right angular gyrus and posterior putamen. Increased connectivity could therefore be a compensating mechanism for dysfunction in the basal ganglia. Increased ReHo activity in bilateral inferior parietal lobules in PD subjects compared to healthy controls was found in another meta-analysis (P. L. Pan et al., 2017). Meta-analysis consisted of 10 resting-state studies, including 212 PD subjects, all scanned while being off medication. This could suggest that increased connectivity in inferior parietal lobes is independent of medication status. Authors argue that this area is important for cognitive performance, and that the increased ReHo activity is compensating for cognitive decline in PD subjects (P. L. Pan et al., 2017). The increased connectivity found between right angular gyrus and several other brain areas, such as posterior putamen, in the current study could therefore be compensating for altered connectivity related to PD dysfunction. Future studies should examine this areas importance to symptomology and progression.

Limitations

The current study had several limitations. The current sample consists of 40 subjects, which could be too few to draw any conclusions from. The database from the PPMI is comprehensive, however, 40 subjects were selected as they completed three rs-fMRI scanning sessions. Further, there was a skewed number of females and males in the sample, with the majority being male. As gender was one of the covariates being investigated, the uneven ratio could explain why gender did not have a significant interaction effect on functional connectivity. In addition, gender was entered as a continuous variable in the analysis. As

gender is a categorical variable, and should not be measured as if it is continuous, this could be responsible for the lack of a significant interaction effect found. One of the main limitations of the current study was the lack of a healthy control group. Therefore, it is not possible to conclude that alterations found are exclusive for PD subjects. Including healthy controls could further exclude the possibility of the longitudinal alterations occurring due to normal aging. Future research should implement a control group. Another limitation with the current study is that there was not an equal amount of years between each scanning session for all subjects, and results cannot make conclusions on when alterations of connectivity occur. Subjects were on several other medications in addition to PD medication, both for psychical and psychological reasons. The possible interaction effect of these is not investigated in the current study. As PD medication had a significant interaction effect on connectivity, it would be beneficial to investigate the effect of other medication in future studies. Further, included in the information from PPMI was the month and year for starting/ending on PD medications, however, information on the date was missing. In some cases, subjects ended one medication and started another one the same month as their rs-fMRI scanning. As LEDD values are based on this information, there could be deviations in the exact dosage subjects were on, as the exact date is not known. Moreover, the PPMI database contains assessments for depression, anxiety and sleep, such as Geriatric Depression Scale (GDS). One limitation with the current study is that these were not included. One of the brain areas implicated in the alterations of connectivity found in the current study has been suggested to be important for depression (orbitofrontal cortex). Future research could benefit from including these, along with other covariates. Moreover, statistical analysis was not performed between the covariates and functional connectivity. It would be beneficial to conduct correlations in future studies, to investigate the statistical relationship between interactions found.

Conclusion

In summary, results from the current study elaborate on longitudinal alterations of functional connectivity occurring across scans in PD subjects. Findings further suggest that the use of PD medication influences alterations of functional connectivity measures over time. Accounting for levodopa medication could be important in future studies. Findings from the current study further elaborate on the inconsistent findings in rs-fMRI research. Several of the measured covariates did not have a significant interaction effect on connectivity. Moreover, alterations of connectivity found were compatible with some previous studies, and not compatible with others. Findings does however illustrate that accounting for covariates in future studies is important. Other covariates than the ones chosen in the current study could be included. An example of this is disease duration, as results from different studies indicate that some alterations of functional connectivity occur earlier in the disease course than others. Further, one cannot conclude on the relevance to PD pathology from these findings. Several of the areas implicated in alterations of connectivity could be important in both motor and non-motor symptoms, such as pain and depression, as well as in LID. Future studies on the relevance of increased or decreased connectivity is important. Overall, more longitudinal studies could be valuable to examine progressive alterations of functional connectivity in PD subjects, if rs-fMRI is to be used as a tool for investigating biomarkers.

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Appendix A

Table A.1
Component overview

Network	Component	Name	Peak MNI Coordinates X	Y	Z
AUD	27	Superior Temporal Gyrus	-55.5	-19.5	6.5
	100	Middle Temporal Gyrus	60.5	-29.5	0.5
BG	13	Caudate Nucleus	9.5	19.5	4.5
	15	Thalamus	-10.5	-17.5	4.5
	18	Anterior Putamen	-24.5	8.5	1.5
	35	Posterior Putamen	-27.5	-8.5	3.5
CEN	28	Middle Frontal Cortex	-27.5	47.5	12.5
	37	Inferior Frontal Gyrus (Right)	-53.5	15.5	15.5
	39	Supramarginal Gyrus (Left)	-57.5	-23.5	32.5
	45	Supramarginal Gyrus	59.5	-40.5	32.5
	50	Posterior Insula	-46.5	10.5	-7.5
	54	Rolandic Operculum Inferior (Right)	55.5	14.5	6.5
	67	Rolandic Operculum (Right)	51.5	18.5	15.5
	71	Frontal Middle Orbital Cortex	24.5	40.5	0.5
	83	Frontal Inferior Orbital Cortex	-32.5	40.5	-7.5
	86	Superior Parietal Junction	-30.5	-63.5	42.5
CER	17	Cerebellum 6	-12.5	-64.5	-16.5
	20	Vermis 4 5	8.5	-49.5	-11.5
	23	Cerebellum 4 5 6	7.5	-48.5	-21.5
DMN	51	Medial Frontal Cortex	-2.5	48.5	-1.5
	52	Angular Gyrus (Left)	-49.5	-56.5	26.5
	59	Cingulum Posterior	-2.5	-41.5	25.5
	68	Angular Gyrus (Right)	44.5	-62.5	40.5
	79	Precuneus	-9.5	-67.5	-34.5
	85	Precuneus / Superior Parietal	-8.5	-68.5	55.5
	95	Angular Gyrus (Right)	53.5	-58.5	26.5
SM	3	Primary Somatosensory Cortex	-51.5	-8.5	29.5
	7	Precentral Gyrus (Right)	37.5	-25.5	63.5
	9	Precentral Gyrus (Left)	-38.5	-22.5	53.5
	10	Supplementary Motor Area	2.5	38.5	39.5
	11	Superior Medial Frontal Cortex	1.5	52.5	16.5
	31	Superior Frontal Cortex	17.5	23.5	41.5
	41	Middle Cingulum / Supplementary Motor Area	1.5	13.5	45.5
	58	Middle Cingulum	2.5	-16.5	47.5
	61	Precentral / Motor (Left)	-46.5	17.5	-29.5

	63	Precentral / Motor (Right)	45.5	8.5	43.5
SN	53	Anterior Insula	-35.5	20.5	-2.5
	57	Anterior Cingulate Cortex	-4.5	36.5	9.5
VIS	8	Middle Occipital Cortex	-18.5	-92.5	-0.5
	22	Lingual Gyrus	8.5	-72.5	-4.5
	29	Cuneus	0.5	-79.5	26.5
	30	Calcerine Cortex	3.5	-82.5	8.5
	40	Superior Occipital Cortex	43.5	-72.5	30.5
	46	Lingual Gyrus	-11.5	-54.5	1.5
	49	Superior Occipital Cortex	32.5	-76.5	16.5
	65	Middle Temporal Gyrus	50.5	-61.5	8.5
	84	Inferior Occipital Cortex	-42.5	-66.5	-3.5
	94	Fusiform	-30.5	-46.5	-12.5

Note. Abbreviation: MNI; Montreal Neurological Institute.