MOVING ALONG THE NON-MOTOR SPECTRUM OF PARKINSON'S DISEASE
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Gwenda Engels
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MOVING ALONG THE NON-MOTOR SPECTRUM OF PARKINSON’S DISEASE

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CHAPTER 1
GENERAL INTRODUCTION
PROLOGUE

Parkinson's disease (PD) is a severely debilitating disease. It affects patients and their caregivers’ life drastically. Knowledge about the disease aids in understanding its progression, and may contribute to a better patient-tailored treatment. While standing on the shoulders of giants, the aim of this thesis is to contribute by looking at the disease from a clinical point-of-view as well as from a neuroscientific point-of-view. Investigating symptom-interactions gives insight into the clinical expression of the disease (Part I of this thesis), and by linking symptoms to neural mechanisms, one might reach a deeper understanding of pathological mechanisms that are at work (Part II of this thesis). In this first chapter, an introduction is provided on which the research questions throughout this thesis are based. This introductory chapter will end with a short overview of the studies that seek an answer to these questions.
CLINICAL EXPRESSION OF PARKINSON’S DISEASE

PARKINSON’S DISEASE

Parkinson’s disease (PD) is the second most common neurodegenerative disease, with an annual incidence of 160 per 100,000 people aged 65 years and over (Ascherio and Schwarzschild, 2016). While the exact cause of the disease is not yet known, neuronal loss within the substantia nigra pars compacta and subsequent dopamine depletion are primarily involved in its cardinal motor symptoms (Schapira, 2009). Risk factors for PD are of genetic or environmental origin (e.g., dairy products and pesticides increase the risk and nicotine and caffeine intake decrease the risk) (Ascherio and Schwarzschild, 2016; Shulman et al., 2011). The clinical diagnosis is based on the presence of bradykinesia together with rest tremor or rigidity (Postuma et al., 2015). However, accuracy of the clinical diagnosis is only 80-90%, since a definite diagnosis of PD can only be determined post-mortem (Hughes et al., 2002). Besides the cardinal motor features, PD patients also experience a broad range of symptoms that are not of motor origin.

NON-MOTOR SYMPTOMS

Examples of non-motor symptoms fall into the following categories: sensory symptoms (e.g. pain), sleep (e.g. insomnia), neuropsychiatric (e.g. dementia, depression), gastrointestinal (e.g. constipation) or autonomic dysfunction (e.g. orthostatic hypotension) (Chaudhuri et al., 2006a; Chaudhuri and Schapira, 2009). Non-motor symptoms as a whole can be more devastating for patients’ quality of life than motor symptoms (Martinez-Martin et al., 2011a). During the last few decades, extensive research into non-motor symptoms has led to an enormous increase in knowledge and awareness about the non-motor side of PD. For this thesis, we will focus on pain and cognitive impairment as non-motor symptoms of PD. Although the symptoms themselves are relatively well-known in clinical practice, the relationship between pain and cognition as well as the relationship with other non-motor symptoms has received relatively little attention. In addition, underlying neural mechanisms are largely unknown.
PAIN

Pain is a common symptom of PD, and has been estimated to occur in about two thirds of PD patients (range 30 to 83%) (Broen et al., 2012; Wasner and Deuschl, 2012). Pain in PD classifies as one of five categories (Ford, 2010): musculoskeletal pain is most prevalent, followed by dystonic, radicular and then central pain. Finally, akathisia as the fifth category is least commonly reported in PD (Broen et al., 2012). An undertreatment of pain in PD has been suggested based on the intake of analgesics compared to the prevalence of pain (Beiske et al., 2009; Lee et al., 2006). However, it remains unclear to what extent clinicians’ evaluation of patients’ pain actually concurs with pain according to patients themselves. Pain threshold and pain tolerance are decreased in PD patients compared to people without PD independent of whether or not a patient actually experiences pain (Conte et al., 2013; Defazio et al., 2008). Pain has been mentioned as one of the preceding symptoms of PD (Lin et al., 2013; Stamey et al., 2008), although pain thresholds appear to be decreasing only after the diagnosis has been established (Mylius et al., 2011). Even when taking peripheral mechanisms into account, central mechanisms play an important role in pain processing in PD (Mylius et al., 2011). In addition, a recent study that investigated which factors contribute to pain experience in a large cohort of PD patients found that the presence of symptoms such as rigidity and poor posture cannot fully account for the presence of pain in PD (Silverdale et al., 2018). In short, the increased pain experience in PD could, at least partly, be the result of disturbed neural mechanisms that are characteristic of the disease itself.

COGNITIVE FUNCTIONING

Cognitive impairment is another frequently occurring non-motor symptom in PD. The spectrum of cognitive impairment ranges from mild cognitive impairment (MCI) in different domains to dementia. Cognitive impairment in PD is most evident in executive, memory and visuospatial domains, although there is a large variability in cognitive impairment between patients, as well as a large variability in how impairment develops within patients (Aarsland et al., 2010; Kehagia et al., 2010, 2012). Ten to twenty percent of patients with newly diagnosed PD meet the criteria for MCI (Litvan et al., 2012). In PD patients without dementia, MCI is present in about one third of patients (Aarsland et al., 2017). After five years from time of diagnosis, prevalence of dementia is estimated
between 15 and 20%. This percentage increases to 46% after 10 years from time of diagnosis (Williams-Gray et al., 2009, 2013). The progression of cognitive impairment coincides with patients’ level of global disability, and impaired cognition is a strong indicator of quality of life. Moreover, a major reduction in quality of life of the caregiver is also present when a partner with PD has dementia (Lawson et al., 2014; Leroi et al., 2012).

NEURAL PROCESSING OF PAIN AND COGNITION
The phenomenon of pain is a healthy reaction to physical damage, resulting in favorable behavior, such as retracting an arm when a hot plate is touched. Pain is a complex neurophysiological and psychological phenomenon entailing several aspects: sensory-discriminatory, cognitive-evaluative, and motivational-affective (Melzack and Casey, 1968). The majority of studies focuses on the presence or intensity of pain, which is captured by the sensory-discriminatory aspect. The cognitive-evaluative and motivational-affective additionally give meaning and valence, or a quality, to the pain experience. All aspects of pain are processed in a vast network of brain areas, many of which are affected by the neuropathology of PD (Tracey and Mantyh, 2007; Wager et al., 2013; Wiech et al., 2008). Although there is no definite consensus on all areas involved in such a pain-network, the core of pain processing seems to occur in a network including basal ganglia, anterior cingulate cortex (ACC), thalamus, amygdala, insula, periaqueductal gray (PAG) and somatosensory cortices (Scherder et al., 2003; Tracey and Mantyh, 2007; Wager et al., 2013). The considerable presence of Lewy bodies in a multitude of areas involved in the pain-network provides a neuropathological explanation for the increase in pain intensity (Scherder et al., 2005). Moreover, pain experience seems to worsen over the course of PD, suggesting that the spread of neuropathology throughout the pain processing network indeed contributes to this disabling symptom (Mylius et al., 2011; Zambito Marsala et al., 2011).

There is considerable overlap between brain networks involved in pain processing, and those involved in cognitive functioning (Moriarty et al., 2011). A direct, behavioral link between cognitive functioning and pain can be interpreted in two ways: on the one hand, the presence of a painful stimulus negatively affects cognitive task-performance. On the other hand, performing a cognitive task attenuates pain (the task functions as
a distraction away from pain) (Wiech et al., 2008). This link can thus be explained by a direct competition for cognitive demands. However, when a task is not concurrently performed (and thus, when pain and cognitive functions are not directly competing), the evidence for an association between pain and cognitive functioning is more ambiguous. For example, although chronic pain patients appear to show cognitive impairment (Berryman et al., 2013; Jongsma et al., 2011; Nadar et al., 2016), this association could be mediated by a third variable such as stress (Hart et al., 2003). In the case of PD, knowledge on the association between pain and cognitive functioning could be valuable: a decrease in cognitive functioning could coincide with a reduction in pain perception (as suggested by Scherder et al., 2005). This would then mean that people with severe cognitive impairment might not communicate severe physical injuries, resulting in inadequate treatment. Besides knowledge on the interaction specifically between pain and cognitive symptoms, one might also consider the interaction between other non-motor symptoms, since all symptoms might somehow be connected.

NETOWORKS AND NEUROIMAGING IN PD

THE BRAIN AS A NETWORK
The human brain is a highly complex and dynamic network from which behavior and thoughts arise. The brain can be represented as a network, as can be any complex system (e.g. social networks). In the case of brain networks, the nodes in the network are brain regions, which are connected through edges. These connections can be based on structural data (e.g. white matter tracts) or functional data (defined as covarying patterns of activity across brain regions). Analyses can be performed on the strength and direction of connections, but also on the architecture of the network (network topology). In this thesis, we will make use of two approaches to functional connectivity: static functional connectivity and dynamic functional connectivity (see Figure 1.1). We define functional network topology based on static functional connectivity and we will use variability of functional connections to investigate dynamic functional connectivity.
Network science, which has its origins in mathematics, has begun to reveal properties of the brain that increase our understanding of how our most complex organ functions (Stam, 2014). One such property is the integration of the network, or the brain’s ability to couple and combine information from different brain areas (Rubinov and Sporns, 2010). A delicate balance between integration (i.e. global communication between subnetworks of the brain via hubs) and segregation (i.e. local communication within these densely connected subnetworks) seems to be seminal for a well-functioning brain (Sporns, 2013). The degree of integration of the brain can be expressed by measures of local and global network efficiency. Studies investigating network topology in PD suggest that network efficiency is reduced (Skidmore et al., 2011), and decreases over time (Olde Dubbelink et al., 2014b). Yet, many other network measures exist that express different characteristics of the network, e.g. modularity, small-worldness or hubness. Hubs are nodes that play a central role in the network (Rubinov and Sporns, 2010). One recent study shows that there is a reorganization of hubs in PD patients compared to healthy controls (Koshimori et al., 2016). This reorganization could point towards hub overload.

Figure 1.1. From a structural MRI-scan (A), nodes of the network (B) are defined. The whole resting-state timeseries as shown in (C) can then either be averaged into one static functional connectivity measure ($D_1$), or functional connectivity can be repeatedly calculated over a number of smaller sliding windows. Variance over these windows ($D_2$) can be used to estimate dynamic functional connectivity.
The hub overload theory postulates that the selective damage to hub regions brought about by a(ny) neurodegenerative process results in a shift of that hub’s load towards another hub. In turn, this hub will show failure because of an overload, leading to a complete network breakdown (Stam, 2014). This theory has found evidence in several diseases, including PD (Boon et al., 2017). It still remains unclear to what extent this theory is associated with clinical symptoms in PD, such as pain or cognitive impairment (Koshimori et al., 2016). Nonetheless, the hub overload theory is interesting because it provides a general mechanism that can be applied to many disorders, and could result in a broad range of functional outcomes, depending on which hubs are affected. In other words, an overloaded hub that is located in prefrontal areas might result in disturbed executive functioning, while an overloaded hub that is located in the insular cortex might result in a disturbed pain experience (Cottam et al., 2018).

RESTING-STATE NETWORKS

This thesis uses functional magnetic resonance imaging (fMRI) data collected during the resting-state to construct functional connectivity. During the resting-state, a subject is awake, keeps their eyes closed and has no specific task. The idea behind the use of data collected during rest is that a neuronal baseline state is reflected, which is specific for that person and at that moment in time. Indeed, consistent patterns of resting-state activity have been found across subjects, the so-called resting-state networks (Damoiseaux et al., 2006). The default mode network (DMN) has received most attention: it is a network consisting of precuneus/posterior cingulate cortex, lateral parietal cortex and mesial prefrontal cortex (Rosazza and Minati, 2011). The DMN is a task-negative network, indicating it is deactivated during performance of a cognitive task. The DMN has been found susceptible to perturbations (e.g. in Alzheimer’s disease or schizophrenia), making it a promising target as a biomarker (Anticevic et al., 2012). Another functionally relevant and well-researched network is the frontoparietal network (FPN), which includes portions of the lateral prefrontal cortex and posterior parietal cortex. The FPN appears to be a key role player in cognitive control as it flexibly changes its connectivity with other networks during cognitive control (Cole et al., 2013; Zanto and Gazzaley, 2013). The DMN and FPN have been mentioned as typical examples of processing networks (which are modular and static) and control networks (which are
flexible and dynamic), respectively (Power et al., 2011). Network characteristics of nodes that belong to either one of these resting-state networks fundamentally differ from one another in their topological role in the network, affirming such a distinction: nodes from the DMN, a typical ‘rich club’ network, show high betweenness centrality or hubness. Nodes from the FPN, a typical ‘diverse club’ network, have a high participation coefficient, indicating that they are involved in many different networks. This property makes diverse club nodes an ideal candidate for functions such as cognitive control (Bertolero et al., 2017).

STATIC AND DYNAMIC FUNCTIONAL CONNECTIVITY STUDIES

Numerous studies have found a disruption in resting-state functional connectivity in PD compared to controls, either with fMRI, magnetoencephalography (MEG) or electro-encephalography (EEG) (Boon et al., 2017; Gao and Wu, 2016; Olde Dubbelink et al., 2013; Stoffers et al., 2008). Again, the DMN as well as the FPN appear to play an important role (Boord et al., 2017; Mohan et al., 2016; Tahmasian et al., 2017; Tessitore et al., 2012; van Eimeren et al., 2009). When investigating connectivity between individual regions, pain in PD has been related to a decreased functional connectivity between right nucleus accumbens and left hippocampus (Polli et al., 2016). Another study found that a disturbance of functional connectivity of the right frontoparietal network was present during painful stimulation in PD patients (Tan et al., 2015). It remains unclear which larger-scale networks are related to pain in PD.

Until recently, research on functional connectivity has been based on the assumption that connections remain stable throughout an fMRI session (Figure 1.1D1). However, temporal fluctuations of connectivity may in fact be a fundamental feature of brain networks (Sizemore and Bassett, 2017). Dynamic functional connectivity (dFC) is an approach to assess these temporal fluctuations by calculating the variability of functional connectivity over a number of sliding windows within each scan (Figure 1.1D2). dFC has been introduced relatively recent, thus its exact behavioral function and neural origin have not yet been firmly established (Hutchison et al., 2013). dFC may have added value in explaining symptoms of PD above and beyond that of static connectivity, as it may detect the brain network’s ability to deal with varying demands from the environment. The dynamics of functional connectivity have been related to severity of
motor symptoms in PD patients (Kim et al., 2017), as well as patients’ performance on an attention task (Madhyastha et al., 2015a). A higher dFC at rest appears to be beneficial for cognitive functioning in PD (Díez-Cirarda et al., 2018; Madhyastha et al., 2015a, 2015b), but the heterogeneity of methods makes it difficult to generalize these results to the population of PD patients and cognition. Moreover, the exact role that major resting-state networks such as the DMN and FPN play remains unclear.

AIM & OUTLINE OF THE THESIS

The aim of this thesis is to provide insight into pain and cognition as non-motor symptoms in Parkinson’s disease. This is performed both from a clinical perspective as well as from a neuroscientific perspective. Therefore, this thesis is divided into two parts. Part I describes the cross-sectional clinical studies: In Chapter 2, we address the basic question of how pain is evaluated by clinicians. First, we compare pain as judged by clinicians to pain as judged by patients, and address the possibility of undertreatment of pain in PD. Second, we assess the inter-rater reliability between neurologists regarding pain in PD. In Chapter 3, we investigate how pain is related to patients’ cognitive impairment. This is done in a cross-sectional sample of PD patients. Cognition is addressed with an extensive battery of neuropsychological tests, and all aspects of pain are included in the analyses. In Chapter 4, we first re-assess the contribution of single non-motor symptoms to quality of life. As a second, exploratory section of that chapter, we address symptom-to-symptom interactions by applying network theory to non-motor symptoms. Part II describes the cross-sectional neuroimaging studies, both of which use resting-state fMRI data: Chapter 5 addresses a possible underlying mechanism of pain in PD by linking pain experience to topology of the brain, both in PD patients and healthy controls. Finally, in Chapter 6 we first investigate whether cognitive functioning is related to dynamic functional connectivity of the resting-state, and second, whether pain and motor severity are related to dynamic functional connectivity. A summary of the main findings is provided in Chapter 7, and Chapter 8 provides a general discussion.
of the results, a critical evaluation of the used methods, and will finish with suggestions for future directions in this field.
PART I
CLINICAL STUDIES
CHAPTER 2
THE CLINICAL EVALUATION OF PAIN IN PARKINSON’S DISEASE
ABSTRACT

Non-motor symptoms have a devastating impact on quality of life in patients with Parkinson’s disease (PD). Pain is such a non-motor symptom and might be underrecognized, and possibly undertreated in clinical practice. Here, we aim to explore whether pain assessment by a clinician corresponds with the pain perception of the patient (Study 1). Additionally, we aim to compare clinical evaluations of patients’ pain between neurologists (Study 2). For Study 1, 57 PD patients filled out pain-related questionnaires, and their medical statuses were checked for pain-related information. For Study 2, 23 patients with pain underwent pain-evaluation by three independent neurologists. We found no significant level of agreement on pain between the patient and the clinician in Study 1. There was an association between the number of patients receiving analgesics and patients’ pain according to the clinician, rather than to the patient. Study 2 showed that agreement was strongest for the item on pain related to response fluctuation, agreement on other types of pain was lower. In conclusion, pain assessment in PD patients in clinical practice can still be improved. Actively inquiring about pain in patients, and guidelines for pain in PD could benefit adequate pain-treatment.
INTRODUCTION

During the past few decades, non-motor symptoms of Parkinson’s disease (PD) have received increasing attention (Chaudhuri et al., 2006a, 2011; Martinez-Martín et al., 2011b). These non-motor symptoms have a large negative impact on patients’ quality of life (Barone et al., 2009). Pain is one of the non-motor symptoms, and appears to be present in about two thirds of PD patients (Broen et al., 2012). Pain in PD may have an additional impact on health-related quality of life (Quittenbaum and Grahn, 2004) and a possible under-treatment of pain in PD has been suggested before (Beiske et al., 2009; Lee et al., 2006).

An undertreatment of pain might be the result of an underrecognition of pain. On the one hand, patients might not recognize and thus not mention their pain as a PD-related symptom, or they might classify it as a side effect of medication. This could then result in a suboptimal pain treatment (Chaudhuri et al., 2006a, 2010). Alternatively, clinicians might not primarily focus on pain because of other PD-related problems, or they might not evaluate the pain consistently. Such an inconsistent evaluation could hinder an optimal pain treatment because of a discrepancy in pain evaluations.

The present study aims to explore how pain in PD patients is evaluated in clinical practice. Because clinical evaluation depends on patients’ as well as clinicians’ evaluation, we will address this question in two parts: in Study 1, we aim to explore the level of agreement between patients and clinicians on pain. In Study 2, we will assess the inter-rater agreement between clinicians on the evaluation of patients’ pain (i.e. location, type of pain, and treatment of pain).

METHODS

The medical ethical committee of the VU Medical Center approved both studies. All participants gave their written informed consent. Participating clinicians in Study 1 were either neurologists or nurse practitioners, all of whom were specialized in PD. In Study 2, only neurologists who were specialized in PD participated.
PARTICIPANTS FOR STUDY 1 AND STUDY 2
Eighty-four patients from regional and academic hospitals participated. Three patients were excluded because of a different diagnosis: One patient had MSA instead of PD, one patient had PSP instead of PD, and one patient had epilepsy. One patient returned their questionnaires without answers. The remaining 80 participants all had a diagnosis of PD according to UK PD Society Brain Bank diagnostic criteria (Hughes et al., 1992). Details regarding all participants will be discussed per study.

STATISTICAL ANALYSES FOR STUDY 1 AND STUDY 2
Statistical analyses were performed in SPSS 22. For Study 1, Cohen’s $\kappa$ was used for inter-rater agreement. We used Fisher’s $\phi$ to test the association between analgesic intake and pain. For Study 2, Fleiss’ $\kappa$ determined inter-rater agreement. Additionally, we calculated percentages of absolute and part agreement. Altman’s Benchmark Scale for Kappa was used to interpret the inter-rater agreement (Sim et al., 2005): a ‘Poor’ strength of agreement is represented by a significant Kappa of $<.20$, ‘Fair’ by a significant Kappa between $.21$ and $.40$, ‘Moderate’ by a significant Kappa between $.41$ and $.60$, ‘Good’ by a significant Kappa between $.81$ and $1.00$.

STUDY 1
QUESTIONNAIRES AND MEDICAL RECORDS
The Non-Motor Symptoms Scale (NMSS) (Chaudhuri et al., 2006b) and the Parkinson’s Well-Being Map (PD-WBM) were administered. Pain was assessed with items in these questionnaires: The NMSS assesses all non-motor symptoms, including pain (Chaudhuri et al., 2006b). Patients assign a score to both ‘Severity’ (range 0-3) and ‘Frequency’ (range 1-4) of each symptom. A total score for per item was obtained by multiplying severity and frequency. For this study, such a score was calculated on the item regarding pain. The PD-WBM is a screening tool developed to improve communication about PD-related symptoms between PD patients and their clinicians. Patients rate PD-symptoms on a scale from 0 (never) to 4 (always). We used the item regarding pain.

Pain as assessed by the clinician was determined in the following way. Patients’ medical records (hardcopy records used in the hospital for patient follow-up) were
screened to assess whether or not pain was mentioned by the clinician (scored as ‘yes’ or ‘no’), up to six months before the date on which questionnaires were filled out.

**STUDY 2**

**PARTICIPANTS AND PROCEDURE**

Each patient consecutively visited three neurologists. On average, patients scored 54.80 ($SD = 24.20$) on a visual analogue scale (VAS) for each visit. Fourteen neurologists examined 23 patients (10 females, 13 males). Mean age was 68.35 years ($SD = 9.31$).

**MATERIALS**

An in-house developed form was used for classifying type of pain (based on Wasner and Deuschl’s taxonomy), location of pain (indicated on a body map) and type of suggested pain treatment (Wasner and Deuschl, 2012). Outcome measures were defined in the following way.

*Type of pain:* In Tier 1 of Wasner & Deuschl’s taxonomy of pain in PD, pain is categorized according to its relationship to PD (i.e. related or not related). In Tier 2, the pain is categorized into either nociceptive, neuropathic or a mix of these two types of pain. Tier 3 further divides the categories of Tier 2 into musculoskeletal, visceral, cutaneous, peripheral, or central (or a mix of these). Tier 4 divides pain further into specific structures and pathology. Here, we scored answers in Tier 4 as either ‘pain related to dopamine response fluctuation’ or ‘pain not related to dopamine response’.

*Location of pain:* The origin of the pain could be indicated on the figure of a human body. These markings were translated into a number representing that specific body part (in total, the body was divided into 18 areas). Any body part that received a marking was counted as ‘one painful area’. The percentage of painful areas on which consensus was reached was calculated in the following way: the number of locations of complete agreement (i.e. all three clinicians indicated the same areas) was divided by the total number of painful body areas (‘one painful area’ as indicated by at least one clinician).
This percentage was averaged over all patients. The total number of locations was defined as any location that was indicated as painful by at least one clinician. The same procedure was repeated for 2 out of 3 clinicians who agreed, and for locations where only one clinician indicated pain.

RESULTS

STUDY 1

PARTICIPANTS

Fifty-seven patients (34 males) participated in Study 1, the mean age was 69.7 years ($SD = 9.2$, 45-92), disease duration was 6.6 years ($SD = 6.2$, 0-30 years). Education was measured by means of the Dutch Verhage-system, which ranges from 1 (not finished primary school) to 7 (finished university). See Table 2.1 for an overview of patients’ educational level. Table 2.2 provides an overview of the presence of non-motor symptoms of patients, per domain of the NMSS. Problems in the domains Sleep/Fatigue and Attention/Memory were most often present. The median for total number of non-motor symptoms in this group was 7.

<table>
<thead>
<tr>
<th>Education</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfinished elementary school</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>6 grades of elementary school</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>7th and 8th grade of primary school</td>
<td>4 (6.9%)</td>
</tr>
<tr>
<td>3 years of lower general secondary education</td>
<td>11 (19.0%)</td>
</tr>
<tr>
<td>4 years of lower general secondary education</td>
<td>19 (32.8%)</td>
</tr>
<tr>
<td>Pre-university education and higher vocational education</td>
<td>12 (20.7%)</td>
</tr>
<tr>
<td>University and equal</td>
<td>8 (13.8%)</td>
</tr>
</tbody>
</table>

Table 2.1. Overview of patients’ educational level (1 missing)
<table>
<thead>
<tr>
<th>NMSS-domain</th>
<th>Absent (%)</th>
<th>Present (%)</th>
<th>Missing data (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardio-vascular</td>
<td>22 (38.6%)</td>
<td>28 (49.1%)</td>
<td>7 (12.3%)</td>
</tr>
<tr>
<td>Sleep, fatigue</td>
<td>3 (5.3%)</td>
<td>46 (80.7%)</td>
<td>8 (14.0%)</td>
</tr>
<tr>
<td>Mood, apathy</td>
<td>11 (19.3%)</td>
<td>39 (68.4%)</td>
<td>7 (12.3%)</td>
</tr>
<tr>
<td>Perception, hallucination</td>
<td>36 (63.2%)</td>
<td>13 (22.8%)</td>
<td>8 (14.0%)</td>
</tr>
<tr>
<td>Attention, memory</td>
<td>6 (10.5%)</td>
<td>44 (77.2%)</td>
<td>7 (12.3%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>16 (28.1%)</td>
<td>35 (61.4%)</td>
<td>6 (10.5%)</td>
</tr>
<tr>
<td>Urinary</td>
<td>9 (15.8%)</td>
<td>33 (57.9%)</td>
<td>15 (26.3%)</td>
</tr>
<tr>
<td>Sexual</td>
<td>20 (35.1%)</td>
<td>18 (31.6%)</td>
<td>19 (33.3%)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>13 (22.8%)</td>
<td>31 (54.4%)</td>
<td>13 (22.8%)</td>
</tr>
</tbody>
</table>

Table 2.2. Presence of non-motor symptoms in participants for Study 1. NMSS = Non-Motor Symptom Scale
LEVEL OF AGREEMENT ON PAIN BETWEEN CLINICIAN AND PATIENT
Thirty-two patients experienced pain (56.0%), as reported by the clinician in the medical record; 44 patients experienced pain according to the questionnaires (76.8%). No significant level of agreement existed on presence of pain as judged by the patient and the clinician ($\kappa = .194; p = .139$).

USE OF ANALGESICS
Twenty patients received analgesics (35.1%), 19 of whom had pain. Fourteen patients received acetaminophen (24.6%), 6 patients received non-steroid anti-inflammatory drugs (NSAIDs, 10.5%), and 3 people received opioid analgesics (5.3%). Three patients received a combination of analgesics. One patient received analgesics without reporting any pain. We found a significant positive association between intake of analgesics and having pain as stated in the medical record ($\phi = .329, p = .020$), but no association between intake of analgesics and self-reported pain ($\chi^2 (1) = 1.178, (\phi = .145, p = .278)$).

PREDICTION OF ANALGESICS BY PAIN
Independent variables of the logistic regression were pain as measured by the NMSS (severity and frequency) and frequency as measured by the PD-WBM. PD-WBM was significantly associated with use of analgesics ($B = .721, p = .005, OR = 2.056$), with 13.6% of variance explained (Nagelkerke’s $R^2 = .136$).

STUDY 2
LOCATION OF PAIN
We found an absolute agreement (all three neurologists) for an average of 52.6% ($SD = 38.2$) of the painful locations, part agreement (2 out of 3 neurologists) for an average of 18.3% ($SD = 26.8$). We found that no agreement existed for an average of 29.2% ($SD = 29.3$).

TYPE OF PAIN AND DIAGNOSIS
Table 2.3 shows agreement on type of pain. Most agreement was found on whether pain fluctuates with PD-medication (Tier 4, Table 2.3), least (significant) agreement was found on whether or not pain is PD-related.
### Table 2.3. Overview of inter-rater agreement on the tiers of Wasner and Deuschl (Wasner and Deuschl, 2012). For strength of agreement, Altman’s benchmark for Kappa was used. Tier 1: Is pain PD related? Tier 2: Is pain nociceptive or neuropathic? Tier 3a: If neuropathic, which type? Tier 3b: If nociceptive, which type? Tier 4: Is pain result of response fluctuation to Parkinson medication? *Only 2 options existed*

<table>
<thead>
<tr>
<th>Tier</th>
<th>Absolute agreement (all neurologists)</th>
<th>Partly agreed (2 out of 3 neurologists)</th>
<th>No agreement</th>
<th>Fleiss’ $\kappa$</th>
<th>p-value</th>
<th>Strength of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1</td>
<td>30.4% (7/23)</td>
<td>60.9% (14/23)</td>
<td>8.7% (2/23)</td>
<td>.258</td>
<td>.003</td>
<td>Fair</td>
</tr>
<tr>
<td>Tier 2</td>
<td>47.8% (11/23)</td>
<td>43.5% (10/23)</td>
<td>8.7% (2/23)</td>
<td>.150</td>
<td>.125</td>
<td>ns</td>
</tr>
<tr>
<td>Tier 3a</td>
<td>52.2% (12/23)</td>
<td>47.8% (11/23)</td>
<td>0</td>
<td>.180</td>
<td>.081</td>
<td>ns</td>
</tr>
<tr>
<td>Tier 3b</td>
<td>78.3% (18/23)</td>
<td>21.7% (5/23)</td>
<td>0</td>
<td>.487</td>
<td>&lt;.001</td>
<td>Moderate</td>
</tr>
<tr>
<td>Tier 4</td>
<td>82.6% (19/23)</td>
<td>17.4% (4/23)</td>
<td>- *</td>
<td>.718</td>
<td>&lt;.001</td>
<td>Good</td>
</tr>
</tbody>
</table>

Table 2.4. Agreement on additional diagnostics and physiotherapy. *2 patients were advised additional diagnostics, 5 were not; ** 11 patients were advised treatment by a physiotherapist and 3 were not.*
TREATMENT AND MEDICATION

Table 2.4 shows agreement on treatment by medication (either analgesic or by adjusting Parkinson-medication), treatment by physiotherapy (yes/no), or additional diagnostics (i.e. further diagnostics are needed before a treatment can be suggested – yes/no). Acetaminophen was the most often prescribed medication (31.7%), followed by non-steroid anti-inflammatory drugs (NSAIDS, 15.9%), anticonvulsants (3.2%) and ‘other’ (1.6%). In all other cases (41.3%), no medication was prescribed.

DISCUSSION

This study explored how pain experience in PD patients was evaluated in clinical practice. In Study 1, we investigated how well clinicians’ and patients’ evaluation of patients’ pain corresponded. In Study 2, we investigated inter-rater agreement on patients’ pain between three neurologists.

STUDY 1

First, we found a poor level of agreement on pain between clinicians and patients: patients indicated pain more often than the clinician. Patients might not communicate pain to their clinician, a phenomenon that has been found for other non-motor symptoms, such as depression (Chaudhuri et al., 2010). PD patients might be less likely to report PD-related pain (Brefel-Courbon et al., 2005), or they might refrain from reporting their pain because they know the pain subsides after dopaminergic medication intake (Brefel-Courbon et al., 2005).

Second, we found that a patient’s intake of analgesics was associated with pain as evaluated by the clinician rather than by the patient. Patients thus receive analgesic treatment for the pain that is known to their clinician. This could either indicate that patients are treated for the pain they feel they should mention to their clinician, for example the most severe pain. It could also mean an under-treatment (Beiske et al., 2009; Nègre-pagès et al., 2008): patients report more pain than clinicians, but are only treated
according to the clinicians’ pain evaluations. This misperception could be resolved by actively questioning the patient about their pain during the clinician’s visit.

**STUDY 2**

Study 2 showed that neurologists did not always agree on all types and treatment of pain. Nonetheless, some agreement existed: the strongest agreement existed on whether pain was related to motor fluctuations, and no agreement was found on whether pain was of nociceptive or neuropathic nature. Agreement was weaker when it regarded treatment of pain. Most agreement was found on whether people should receive physiotherapy and on whether to adjust dopaminergic medication. The undertreatment found in other studies might be related to this lack of agreement: for certain types of pain (e.g. those for which least agreement was reached), undertreatment might be more frequent than for other types of pain (e.g. those for which most agreement was reached). In summary, our findings in Study 2 show that pain assessment and treatment in PD could still be improved, as Beiske and colleagues also stress in their study (Beiske et al., 2009).

**LIMITATIONS**

For Study 1, patients filled out the questionnaires, but not on the same day as the visit on which the clinician’s opinion was based. Pain as stated in the questionnaire could therefore have been different compared to the day of visit. Additionally, no information was available about dopaminergic or analgesic medication intake at the time of the doctor’s visit (Nègre-pagès et al., 2008). For Study 2, we created a score-form for pain based on the review by Wasner and Deuschl (2012). Having such a score-form with limited options for types of pain could also induce a bias: the score form might not completely represent all types or aspects of pain.
CONCLUSION

Knowledge on pain in PD should be more embedded into clinical practice. An active attitude of the clinician regarding pain will hopefully result in better recognition and a more adequate treatment of pain. Our results show that evaluation of pain in PD varies between clinicians, and that patients evaluate their own pain differently from their clinician. As a starting point for individually tailored effective pain treatment, pain evaluation could benefit from guidelines specifically for pain in PD. A PD-specific pain questionnaire has recently been developed (Chaudhuri et al., 2015) and we suggest that this promising new tool should be part of such a guideline.
CHAPTER 3

CLINICAL PAIN AND NEUROPSYCHOLOGICAL FUNCTIONING IN PARKINSON’S DISEASE: ARE THEY RELATED?
ABSTRACT

Pain is an important non-motor symptom of Parkinson’s disease (PD). Brain areas such as the hippocampus and the prefrontal cortex play an important role in the processing of pain. Since these brain areas are also involved in cognitive functioning, e.g. episodic memory and executive functions, respectively, we examined whether a relationship exists between cognitive functioning and spontaneous pain in PD. Forty-eight patients with PD and 57 controls participated. Cognitive functioning was measured by a comprehensive battery of neuropsychological tests. Both the sensory-discriminative aspect as well as the motivational-affective aspect of pain were assessed. Multiple linear regression analyses were performed to assess a relation between cognition and pain. Cognition was neither related to the sensory, nor the affective aspect of pain in our sample of PD patients. Variance in pain measures was primarily explained by symptoms of depression and anxiety. The difference between the affective and the sensory aspect of pain might be due to the neuropathology of PD, which is mainly present in areas processing the affective aspect of pain. Pain treatment might improve when mood is taken into account. We provide several explanations for the lack of an association between pain and cognition.
INTRODUCTION

Non-motor manifestations of Parkinson’s disease (PD) have received increasingly more attention over the past few decades, which is justified considering their impact on everyday life of PD patients (Martinez-Martin et al., 2011a). Pain is one of these non-motor manifestations, which has a large impact on quality of life (Gallagher et al., 2010; Quittenbaum and Grahn, 2004). Pain is estimated to be present in two thirds of PD patients (Broen et al., 2012), and has been reported already at the clinical onset of the disease (Defazio et al., 2008; Nègre-pagès et al., 2008). Additionally, a difference between PD patients and controls has been found in sensitivity to pain: both the tolerance and threshold of pain appear lowered in PD (Zambito Marsala et al., 2011). Thus, painful stimuli might be more painful for PD patients than for controls. The importance for everyday clinical practice is further emphasized when the possibility of an undertreatment of pain in this group was suggested by a study from Beiske and colleagues, who found that only half of the PD patients with pain received analgesics or physiotherapy (Beiske et al., 2009).

Pain is a complex psychological and neurophysiological phenomenon, and is processed in a neural network, involving the lateral and the medial pain system (Kulkarni et al., 2005). The lateral pain system is mainly involved in processing sensory-discriminative aspect (e.g. localization, intensity) of pain. It consists of the spinothalamic tract, which passes through the lateral thalamus and projects mainly towards sensory cortical areas. The medial pain system mainly processes motivational-affective and cognitive-evaluative aspects of pain (e.g. unpleasantness, suffering), and projects through medial thalamic nuclei to cognition- and emotion related areas, e.g. the hippocampus and anterior cingulate cortex (ACC). For a detailed overview of these pain systems, the reader is referred elsewhere (Apkarian et al., 2005; Peyron et al., 2000; Rainville, 2002; Scherder et al., 2003; Willis and Westlund, 1997). The medial pain system appears to be primarily affected by the neuropathology of PD (Scherder et al., 2005).

Pain in PD has been linked to various factors, such as dopaminergic fluctuations (Chaudhuri and Schapira, 2009; Jarcho et al., 2012), depressive symptoms (Ehrt et al., 2009), duration of disease (Zambito Marsala et al., 2011), motor problems (Tinazzi et al., 2006) and cognitive functioning (Scherder et al., 2005). Impairment of cognitive
functioning, in particular executive functioning (EF) and memory, is common in PD patients (Aarsland et al., 2010; Muslimović et al.). In the general population, the relation between cognition and pain has been assessed before. Cognitive functions, such as executive functioning and attention, are thought to be inversely related to pain, implying that less pain coincides with better cognitive functioning. Performance on a cognitive test was related to acutely induced experimental pain in a study of Seminowicz and Davis (Seminowicz and Davis, 2007c). Processing of pain was found to show significant overlap with an attention-specific network, taking away resources for cognitive performance: pain demands attention (Seminowicz and Davis, 2007c). More complex relations have been suggested as well. For example, the degree to which cognitive performance is affected might depend on the difficulty of the task and the intensity of pain (Buhle and Wager, 2010; Seminowicz and Davis, 2007b, 2007a). In a study from Pickering and colleagues it was shown that the interaction between cognition and pain is not restricted to a concurrent cognitive task: a higher score on the Mini Mental State Examination (MMSE, measured off-line) correlated with a higher pain tolerance (assessed by psychophysical mechanical and thermal techniques) in healthy elderly. The authors explain this relation by the ability to centrally integrate the multiple facets of pain (Pickering et al., 2002). The fact that there is a convincing overlap in areas involved in pain processing and areas involved in cognitive performance emphasizes the association between these functions.

Many of the pain processing areas eventually become affected in PD (Braak et al., 2003; Del Tredici et al., 2002). Brainstem areas involved in suppression of pain, such as the periaqueductual grey and locus coeruleus, are affected already in an early stage of the disease. This might result in an increase of pain in PD patients, but when neocortical areas are affected in later stages of the disease, pain might decrease (Scherder et al., 2005). In other words, pain might subside when cognitive impairment progresses in PD. However, if a higher level of pain does remain, and cognitive impairment worsens, patients might not be able to communicate their pain. This would hinder successful treatment. The goal of the present study is to examine the possible relationship between cognitive functioning and pain in PD patients.
METHODS

PARTICIPANTS
Patients were included when they had a diagnosis of PD according to the UK PD Society Brain Bank diagnostic criteria (Hughes et al., 1992). This diagnosis was confirmed by a neurologist. Furthermore, the subjects had no history of cerebral traumata or of psychiatric disorders unrelated to PD (e.g. schizophrenic episodes, major depressive disorder long before the onset of PD), and were able to understand the neuropsychological tests (i.e. MMSE score > 16). Forty-eight PD patients and 57 controls participated in this study. The patient group consisted of more males (40% females) than the control group (67% females) ($\chi^2(1) = 7.702, \ p = .006$). Patients were older ($t(103) = 2.098, \ p = .038$) than controls ($M_{\text{patients}} = 72.9$ years, SD = 9.5; $M_{\text{controls}} = 67.9$ years, SD = 11.9).

EDUCATION
Education was measured on a 5-point rating scale (1 = primary school, unfinished; 2 = primary school; 3 = secondary school; 4 = higher secondary school 5 = higher vocational training or university degree). Level of education was matched between groups (Mann-Whitney $U = 1151.500, Z = -.729 \ p = .466$).

COMORBIDITIES
Comorbidities were recorded. As can be seen in Table 3.1, patients were significantly more often diagnosed with diabetes.

MEDICAL ETHICAL COMMITTEE
This study was approved by the medical ethical committee of the VU medical center. All participants received information about the study before signing an informed consent form.

PAIN
The Colored Analogue Scale (CAS) is a colored version of the visual analogue scale. To test pain intensity, the CAS Intensity was used. Here, subjects indicate how much pain they have on a scale ranging from 'No pain' (light pink) to 'Maximal pain' (dark red).
To test suffering from pain, the CAS Affect was used. The CAS Affect is a similar color-coded scale ranging from ‘No suffering’ to ‘Maximal suffering’. Both versions of the CAS range from 0 to 100. The Faces Pain Scale (FPS) primarily measures pain intensity, but also pain affect (Bieri et al., 1990). The FPS consists of seven pictures of faces, with a pain expression ranging from ‘no pain’ to ‘most severe pain’. Subjects choose a face that represents their pain best. Answer possibilities of the FPS range from 0 to 6. The Number of Words Chosen-Affective (NWC-A) is the affective part of the McGill Pain Questionnaire, and was utilized to investigate the affective aspect of pain (Melzack, 1975; Verkes et al., 1989). Three adjectives, all expressing an increasing amount of pain, are read aloud by the examiner. Subjects have to indicate which of the three adjectives, if any, best represents their pain. Possible scores range from 0 to 15.

**MEMORY**

The Eight Word Test is part of the Amsterdam Dementia Screening test (Lindeboom and Jonker, 1989), and consists of three verbal memory subtests: immediate recall, delayed recall and delayed recognition. In the immediate recall subtest, eight words are read aloud five times by the examiner and each time the participant is required to recall as many words from the list as possible. Maximum score for this subtest is 40. The delayed recall subtest is assessed after approximately 15 minutes. The participant is required to

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Controls Percentage (number)</th>
<th>Parkinson’s disease Percentage (number)</th>
<th>Difference between groups</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac failure</td>
<td>28.1% (16)</td>
<td>29.2% (14)</td>
<td></td>
<td>.015</td>
<td>.901</td>
</tr>
<tr>
<td>Kidney disease</td>
<td>1.8% (1)</td>
<td>0% (0)</td>
<td></td>
<td>.850</td>
<td>.356</td>
</tr>
<tr>
<td>Lung disease</td>
<td>10.5% (6)</td>
<td>4.2% (2)</td>
<td></td>
<td>1.497</td>
<td>.221</td>
</tr>
<tr>
<td>Diabetes*</td>
<td>5.3% (3)</td>
<td>18.8% (9)</td>
<td></td>
<td>4.682</td>
<td>.030*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29.8% (17)</td>
<td>27.1% (13)</td>
<td></td>
<td>.096</td>
<td>.757</td>
</tr>
<tr>
<td>Peripheral arterial</td>
<td>8.8% (5)</td>
<td>14.6% (7)</td>
<td></td>
<td>.869</td>
<td>.351</td>
</tr>
<tr>
<td>Arthritis</td>
<td>12.3% (7)</td>
<td>8.9% (4)</td>
<td></td>
<td>.301</td>
<td>.583</td>
</tr>
</tbody>
</table>

Table 3.1. Comorbidities for both groups; * p < .05.
reproduce as many words from the original set of words as possible. Maximum score for this test is 8. The delayed recognition subtest is assessed immediately after the delayed recall test. The participant is required to recognize the initial set of eight words from a set of 16 words, and indicate which words are new. Maximum for this test is 16. Face Recognition is a subtest of the Rivermead Behavioral Memory Test (RBMT) and measures visual recognition memory (Wilson et al., 1999). Ten pictures of faces are presented to the participant in a fixed order. Approximately three minutes later the participant is asked to recognize the initial set of 10 faces from a set of 20 faces, and indicate which faces are new. Maximum score for this test is 20. Picture Recognition is another subtest of the RBMT, and measures visual recognition memory. Twenty drawings of objects and animals are displayed to the participant in a fixed order. Approximately three minutes later the participant is asked to recognize the initial set of 20 drawings from a set of 40 drawings, and indicate which drawings are new. For both RBMT subtests, number of correct answers minus number of incorrect answers was taken as an outcome measure. Maximum score for this test is 40. The Knox Cube Test was used to assess visual attention and memory (Bornstein, 1983). Subjects are asked to imitate the pattern tapped by the researcher on a wooden model of four small, aligned blocks. The length of the tapped pattern increases, until the subject is unable to reproduce it. The number of correct test trials was recorded as outcome measure. Maximum score for this test is 15.

EXECUTIVE FUNCTIONING

The Digit Span Backward test from the Wechsler Memory Test was used to assess working memory (Wechsler, 1945). Subjects are instructed to repeat a string of verbally presented digits backwards. The total amount of correct responses was used as the outcome measure. Maximum score for this test is 21.

The Key Search test of the Behavioural Assessment of the Dysexecutive Syndrome (BADS) measures planning, monitoring and regulation of behavior (Norris and Tate, 2000). The subject is required to draw the route they would take in the hypothetical situation of trying to find a lost key on a large field. The drawn route is scored according to a predefined set of rules. This score was used as outcome measure. Maximum score for this test is 16. The Rule Shift test of the BADS measures cognitive flexibility and attention (Norris and Tate, 2000). A set of playing cards is shown to the subject. In the
first part, a response pattern is established according to a simple rule (i.e. “Yes” for a red card, “No” for a black card). For the second part, subjects are required to respond differently to the same set of cards. The outcome measure was the number of correct answers. Maximum score for this test is 19. Category Fluency measures verbal ability and executive control: the subject is required to name as many animals within 60 seconds, and as many professions in another trial of 60 seconds (Shao et al., 2014). Total number of mentioned instances served as outcome measure. The Picture Completion test of the Groninger Intelligence Test (GIT) was used to assess visual perception and alertness to detail (Luteijn and Van der Ploeg, 1983). Subjects are asked to complete a partly drawn figure, each with increasing difficulty (i.e. less complete). Number of correct answers was taken as outcome measure. Maximum score for this test is 20.

**ATTENTION**

To assess attention, the Digit Span Forward test from the Wechsler Memory Test was administered (Wechsler, 1945). Subjects are instructed to repeat a string of verbally presented digits. Number of digits per string was increased until the subject was unable to reproduce the string. The total amount of correct responses was used as the outcome measure. Maximum score for this test is 21.

**MOOD**

Depressive symptoms were evaluated with the Beck Depression Inventory (BDI)(Beck et al., 1961). The administered BDI consists of 20 questions, all addressing depressive symptoms, with answer options ranging from no symptoms (0) to severe symptoms (3). Additionally, the depression and anxiety subscales of the Symptom Check List-90 (SCL90) were administered (Derogatis et al., 1976). The subtest Depression consists of 15 symptoms, with answer options ranging from 1 (‘not bothered at all’), to 5 (‘extremely bothered’), the subtest Anxiety addresses 10 symptoms with the same answer options.

**PROCEDURE**

Subjects were recruited through outpatient clinics of hospitals, patient societies and immediate surroundings. Patients and controls were assessed in their own home, and were given the freedom to choose the day and time of assessment. By this, we aimed to
overcome the possibility of suboptimal performance. The order of neuropsychological tests, pain assessments and mood questionnaires was as follows: MMSE, CAS, FPS and NWC-A, Eight words test - immediate recall condition, BADS rule-shifting subtest, BADS key search subtest, Eight words test - long-term recall and recognition condition, Digit Span Forward, Digit Span Backward, RBMT Faces (present faces), Fluency – animals, RBMT Faces - recognition condition, RBMT Pictures – presentation, Fluency – Professions, RBMT Pictures – recognition condition, KNOX cubes, Figure completion test, BDI, SCL90 subscales anxiety and depression. Approximate duration of assessment was 1.5-2 hours.

STATISTICAL ANALYSES
Statistical Package for the Social Sciences (SPSS), version 21 and Lavaan package in R were utilized to perform statistical analyses (Rosseel, 2012). In order to determine whether PD patients and controls differed on pain measures, an Analysis of Covariance (ANCOVA) was conducted with pain as dependent variable, group and sex as factors and age as covariate. Confirmatory factor analysis (CFA) was performed to determine domains in cognitive scores. Four linear multiple regression analyses were performed within the PD group, all with one pain measure as outcome variable and memory, executive functioning and attention performance, mood and sex as predictors. The alpha level was set at 0.05.

COGNITIVE DOMAINS
A factor analysis was performed to reduce the number of predictors in the analysis. The 12 cognitive subtests were entered into a CFA analysis. Two domains were created: the domain Memory entailed three subtests of the Eight-Words Test (immediate recall, delayed recall and recognition), RBMT Face- and Picture Recognition, KNOX and Picture Completion. The domain Executive Functioning was represented by BADS Rule Shift test, BADS Key Search test, Digit Span Backward and Category fluency. The Comparative Fit Index (CFI) of the CFA was .912, and the Root Mean Square Error of Approximation (RMSEA) was 0.097 (p = .009), indicating that domains were indeed measuring the same construct. Digit Span Forward (DSF) served as an individual variable representing attention.
RESULTS

COGNITIVE PERFORMANCE
The average MMSE score for patients ($M = 26.14, SD = 3.57$) was significantly lower than for control subjects ($M = 28.00, SD = 2.09$, Mann-Whitney $U = 896.000, Z = -3.076 p = .002$). Patients performed worse on all neuropsychological subtests (see Table 3.2 for details).

PAIN SCORES
After controlling for age, there was no main effect of group on CAS Intensity ($F(1,100) = 1.905 (1); p = .171$). There was a significant main effect of group on CAS Affect ($F(1,100) = 6.803; p = .010$, partial $\eta^2 = .064$). Additionally, the effect of sex as a factor showed a trend on CAS Affect, with women experiencing more pain then men ($F(1,100) = 3.433; p = .067$, partial $\eta^2 = .033$), there was no significant interaction between these variables ($F(1,100) = .170; p = .681$, partial $\eta^2 = .002$). Patients showed to experience more pain as measured on the FPS than controls after controlling for age ($F(1,100) = 6.191; p = .014$, partial $\eta^2 = .058$). Here, the effect of sex as a factor showed a trend ($F(1,100) = 3.253; p = .074$, partial $\eta^2 = .032$), with women scoring higher on the FPS but without a significant interaction between these variables ($F(1,100) = 2.143; p = .146$, partial $\eta^2 = .021$). There was a main effect of group on NWC-A ($F(1,100) = 15.503; p = .000$, partial $\eta^2 = .134$), as well as an effect of sex as a factor ($F(1,100) = 7.264; p = .008$, partial $\eta^2 = .068$). There was no significant interaction between group and sex ($F(1,100) = .030; p = .864$, partial $\eta^2 = .000$). See Table 3.3 for scores on the pain scales.

ASSOCIATION BETWEEN PAIN AND COGNITION
Four multiple linear regression analyses were calculated to predict pain (as measured by the four different pain measures) based on Memory, EF, attention, sex and Mood for the group of PD patients. No significant regression equation was found for the multiple linear regression to predict outcome on CAS Intensity ($F(5, 36) = 1.355, p = .264$), with an $R^2$ of .158.

The regression model for predicting scores on FPS significantly accounted for approximately 46% of the variance ($R^2 = .463, F(5, 36) = 6.207, p < .001$). However, none
<table>
<thead>
<tr>
<th>Test</th>
<th>Controls</th>
<th>Parkinson's disease</th>
<th><strong>Mann-Whitney U test (Z)</strong></th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>M (SD)</strong></td>
<td><strong>Range</strong></td>
<td><strong>M (SD)</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>MMSE</td>
<td>28.00 (2.09)</td>
<td>21-30</td>
<td>26.14 (3.57)</td>
<td>16-30</td>
</tr>
<tr>
<td>Eight Word Test – immediate recall subtest</td>
<td>32.23 (5.00)</td>
<td>20-40</td>
<td>25.86 (7.94)</td>
<td>4-38</td>
</tr>
<tr>
<td>Eight Word Test – delayed recall subtest</td>
<td>5.81(1.55)</td>
<td>1-8</td>
<td>4.16 (2.37)</td>
<td>0-8</td>
</tr>
<tr>
<td>Eight Word Test – delayed recognition subtest</td>
<td>15.37 (.86)</td>
<td>12-16</td>
<td>14.14 (2.30)</td>
<td>6-16</td>
</tr>
<tr>
<td>Rule Shift test (BADS)</td>
<td>17.16 (3.05)</td>
<td>9-19</td>
<td>14.75(4.17)</td>
<td>6-19</td>
</tr>
<tr>
<td>Key Search test (BADS)</td>
<td>10.61 (4.07)</td>
<td>3-16</td>
<td>8.55 (4.33)</td>
<td>2-16</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>13.28 (2.96)</td>
<td>8-21</td>
<td>11.84 (2.96)</td>
<td>5-19</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>9.00 (3.05)</td>
<td>3-18</td>
<td>7.34 (2.69)</td>
<td>3-14</td>
</tr>
<tr>
<td>Face Recognition (RBMT)</td>
<td>16.88 (3.14)</td>
<td>6-20</td>
<td>14.41 (4.50)</td>
<td>2-20</td>
</tr>
<tr>
<td>Picture Recognition (RBMT)</td>
<td>38.58 (2.57)</td>
<td>26-40</td>
<td>36.55 (5.90)</td>
<td>12-40</td>
</tr>
<tr>
<td>Category Fluency</td>
<td>39.30 (11.85)</td>
<td>6-67</td>
<td>30.48 (13.05)</td>
<td>5-70</td>
</tr>
<tr>
<td>Knox Cube Test</td>
<td>11.14 (1.70)</td>
<td>7-15</td>
<td>10.14 (1.91)</td>
<td>5-14</td>
</tr>
<tr>
<td>Picture Completion test (GIT)</td>
<td>11.51 (3.36)</td>
<td>5-18</td>
<td>9.75 (3.98)</td>
<td>2-19</td>
</tr>
</tbody>
</table>

Table 3.2. Cognitive performance in PD patients and controls. SD = standard deviation; MMSE = Mini-Mental State Examination; BADS = Behavioral Assessment of the Dysexecutive Syndrome; RBMT = Rivermead Behavioral Memory Test; *p < .05, **p < .001
<table>
<thead>
<tr>
<th>PD patients</th>
<th>Controls</th>
<th>Main effect of group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M (SD) Range</strong></td>
<td><strong>M (SD) Range</strong></td>
<td><strong>F-value (df)</strong></td>
</tr>
<tr>
<td>Colored Analogue Scale (CAS) Intensity</td>
<td>25.7 (25.5) 0-95</td>
<td>30.9 (27.1) 0-92</td>
</tr>
<tr>
<td>Faces Pain Scale (FPS)</td>
<td>1.5 (1.3) 0-5</td>
<td>2.2 (1.8) 0-7</td>
</tr>
<tr>
<td>Colored Analogue Scale (CAS) Affect</td>
<td>20.2 (25.0) 0-75</td>
<td>32.0 (27.2) 0-90</td>
</tr>
<tr>
<td>Number of Words Chosen – Affective (NWC-A)</td>
<td>2.0 (2.7) 0-10</td>
<td>4.1 (3.6) 0-13</td>
</tr>
</tbody>
</table>

Table 3.3. Pain scores in PD patients and controls. M = mean; SD = standard deviation; *p < .05, **p < .001.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td><strong>SE B</strong></td>
<td><strong>β</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td>Memory</td>
<td>5.94</td>
<td>7.10</td>
<td>.19</td>
</tr>
<tr>
<td>EF</td>
<td>-5.77</td>
<td>8.15</td>
<td>-.17</td>
</tr>
<tr>
<td>Mood</td>
<td>10.05</td>
<td>5.10</td>
<td>.34</td>
</tr>
<tr>
<td>Attention</td>
<td>-.42</td>
<td>4.96</td>
<td>-.02</td>
</tr>
<tr>
<td>Sex</td>
<td>-4.69</td>
<td>8.48</td>
<td>-.09</td>
</tr>
</tbody>
</table>

Table 3.4. Four multiple linear regression analyses were performed, each with a different pain measure as predicted variable. EF = Executive Functioning; coding for ‘Sex’: 1 = female, 2 = male. B = unstandardized beta; SE B = standard error of the B; $\beta$ = standardized beta; *p < .10, **p < .05, ***p < .001.
of the cognitive measures significantly contributed to the model. Mood and (male) sex showed a trend in predicting pain as measured by FPS. The regression model for CAS Affect accounted for 25.7% of the variance ($F(5) = 2.495, p = .049$). As shown in Table 3.4, none of the cognitive measures contributed to the model. In this model, only Mood significantly predicted scores on CAS Affect. Similar results were found for the regression model predicting scores on the NWC-A ($R^2 = .463, F(5,) = 6.207, p < .001$): None of the cognitive measures were contributing to the model. Mood was the only significant predictor in this model. See Table 3.4 for details.

**DISCUSSION**

To our knowledge, this is the first study to address the association between cognition and pain in PD patients. Although pain intensity did not significantly differ between groups consistently (CAS intensity, FPS), PD patients experienced more pain affect (CAS Affect, NWC-A).

When compared to controls, it has been found that PD patients experience pain more frequently (Broen et al., 2012). However, this has not been found consistently: others report that pain in PD does not differ from pain in the general population (Quittenbaum and Grahn, 2004). Even though Quittenbaum and colleagues did not include an assessment of all aspects of pain, we propose that this inconsistency in findings might be explained by a difference between the aspects of pain: In our sample, patients may have suffered more from pain than controls, whereas the sensory-discriminative aspect was comparable to controls. This discrepancy might be explained by the progression of PD-neuropathology throughout the pain systems: the lateral pain system (sensory-discriminative aspect) remains relatively intact, whereas the medial pain system, involved in the affective aspect of pain, shows more neuropathology (Braak et al., 2003; Scherder et al., 2005).

The main goal of this study was to examine a possible relationship between pain and cognition in PD. We hypothesized that this relationship could be either positive or negative and that specifically a negative relationship would be clinically relevant,
considering the risk of undertreating pain in cognitively impaired patients who might have more difficulty communicating their pain to a physician. Contrary to our hypothesis, cognitive functioning showed no relationship with pain in our sample of PD patients. Symptoms of mood disorders strongly influenced pain experience, as was found in other studies. In healthy elderly, a positive relationship between MMSE scores and pain tolerance has been found previously (Pickering et al., 2002). The relationship between everyday clinical pain and cognitive performance has been researched before, albeit not in Parkinson's disease: for a group of Alzheimer's disease patients, a positive correlation between executive functioning and pain was found. However, the authors emphasized the need to control for symptoms of mood disorders when studying the relation between pain and cognition (Scherder et al., 2008). This was confirmed in a study looking into patients with rheumatoid arthritis, where cognition was negatively correlated to pain (Brown et al., 2002). This correlation disappeared when the authors additionally assessed the mediating effect of depression, indicating the major influence of depression on both pain and cognition.

One possible explanation for the lack of a significant association between pain and cognitive functioning might be that neural functional reorganization takes place to compensate for deficits on cognitive performance. These compensatory processes may result in the activation of other brain regions during tasks that measure memory and executive functioning than in controls. These brain regions may not be as involved in the processing of pain as they are in the controls. An example of this shift in recruited brain regions is found in multiple sclerosis (MS). In a study investigating MS patients, increased and additional activation of frontal and posterior parietal cortices was observed during several attention tasks. These areas were not or only partly activated in controls, during the same tasks (Penner et al., 2003, 2007). In PD, a recent study investigated functional connectivity and cognitive functioning in PD patients (Gorges et al., 2015). The authors concluded that in the beginning of the disease, a state of hyperconnectivity arises, and that a hypoconnectivity is associated with more cognitively impaired patients. They also state that this might be a compensatory mechanism of the brain. A similar mechanism might apply for pain: a decrease in gray matter in pain areas (e.g. ACC, insula) has been found for several chronic pain disorders, and could even be the consequence of chronic pain (May, 2008). A deviant neural functioning as a consequence of the disease (the
compensatory neural mechanism), or of the symptoms themselves (pain) might therefore explain the lack of association between pain and cognition.

Our study confirms that mood disorders are the main contributor to pain experience in PD: symptoms of anxiety and depression were predictive for three out of four pain parameters. This finding is in line with previous research (Bair et al., 2003). The relation between pain and mood could be bidirectional: symptoms of depression or anxiety might increase pain experience, and vice versa, pain might exacerbate symptoms depression and anxiety (Bair et al., 2003). In our sample, NWC-A and CAS Affect showed the strongest relationship with Mood. The FPS and CAS Intensity, both focusing on intensity (sensory-discriminative aspect) of pain showed a weaker association (Perrott et al., 2004). This is an interesting finding, since it strengthens our belief that pain experience consists of several components, all of which should be paid equal attention. Clinically, it also indicates that pain treatment will probably benefit when mood is taken into account.

LIMITATIONS

Several limitations apply to this study. First, regarding cognitive functioning, no patients showed severe cognitive impairment, which reduces the variability in the sample. Subsequently, our hypothesized relationship might not be as pronounced as it would have been when this increased variability would be present in our sample. We chose to exclude patients with a severe cognitive impairment because they are no longer able to fully understand the neuropsychological tests. However, this unfortunately results in a reduction of variability of cognitive functioning. Second, use of pain medication was not recorded. Analgesic medication (evidently) influences pain experience, and therefore might also influence the relationship between cognition and pain. In addition, participants were not required to withhold dopaminergic medication. This might influence our results in two ways. Indirectly, the reduction of (painful) motor symptoms during the ‘on’ period might bring pain relief. Directly, dopaminergic medication has an ameliorating influence on pain experience as it has been shown that hypofunctioning of nigrostriatal dopamine is associated with an increase in pain experience in healthy persons (Hagelberg et al., 2004). Indeed, PD patients show a lowered pain threshold during their ‘off’ period, and the pain threshold returns to a normal level after levodopa
administration (Brefel-Courbon et al., 2005), although this effect has also been found to differ depending on how the patient responds to dopaminergic medication (Lim et al., 2008). The dopaminergic anti-nociceptive effect might therefore also have an influence on the association between pain and cognition in our study. Finally, several clinical and demographical variables have not been taken into account, such as disease duration and severity of the disease. These have been shown to influence pain experience (Valkovic et al., 2015). Regarding the comorbidities, there were more patients with diabetes as comorbidity than controls, which poses the possibility of e.g. encephalopathy.

CONCLUSION

This study suggests that symptoms of mood disorders are an important predictor for the experience of pain in PD patients, probably of more significance than cognitive dysfunction in these patients, since we did not find a significant relationship between pain experience and cognition.
CHAPTER 4

A NETWORK APPROACH TO THE NON-MOTOR SYMPTOMS OF PARKINSON’S DISEASE: AN EXPLORATIVE STUDY
ABSTRACT

Research on the association between non-motor symptoms (NMS) of Parkinson’s disease (PD) and patients’ quality of life (QoL) has given insight into the burden of NMS. Most studies investigate NMS by assessing the contribution of individual symptoms to QoL. However, symptoms could also have an interactive relationship, which might not be fully taken into account when only studying these individual contributions. Recently, a network approach has been developed that treats symptoms as nodes and associations between symptoms as edges in a network, providing the opportunity to investigate the dimensional spectrum of NMS. In the current study, we investigated NMS with both approaches: first, we assessed individual contributions of NMS to QoL. Second, we aimed to assess NMS using a network approach. Seventy PD patients completed questionnaires on NMS and QoL. Our primary analysis shows that the domains Mood and Pain are significant contributors to QoL. Our secondary network analysis suggests that Mood and Sleep play central roles in the NMS-network, and that Mood and Cognition are strongly related. Because of power issues, the generalizability of our explorative results is limited. However, complementary information from the network analysis does suggest that focusing on sleep problems might help both mood and pain symptoms, which negatively affect QoL. Investigating symptoms not only as individual and independent entities but rather as part of a connected network could show how treating one symptom affects other symptoms.
INTRODUCTION

Non-motor symptoms (NMS) of Parkinson’s disease, such as cognitive dysfunctioning, depressive symptoms and pain, play a major role in the disease next to the characteristic motor symptoms. There is ample evidence of the negative association between NMS and quality of life (QoL) (Barone et al., 2009; Martinez-Martin et al., 2011a; Soh et al., 2011). Despite the large body of work investigating NMS and QoL in PD, there is only a small number of NMS consistently showing an association with patients’ QoL, namely depression and cognitive impairment. The fact that different studies show different results might be explained by hidden symptom-to-symptom interactions that complicate the interplay between NMS towards QoL.

A contemporary method adapted from the field of psychology is a network approach (Fried and Cramer, 2017) that treats symptoms as nodes and the relations between symptoms as edges in a network. Network theory has been applied to many areas of research, such as sociology and neuroscience. A major difference between networks in neuroscience and networks in psychology, or in our case neurology, is that the entities that make up the network are not based on physical matter (Fried and Cramer, 2017). To put it differently, a connection between two brain areas can be physically measured, but the association between two symptoms cannot: it is determined by the behavior of those two symptoms with respect to one another, and to other symptoms present in the network at group level. What we can deduct from this type of analysis is which symptoms play a central role in the disease, and thus which symptoms might be most effective to treat in order to improve other symptoms in the network.

The aim of our study is twofold. First, we aim to assess the association of non-motor symptoms with QoL in PD patients, to find which NMS contribute most to QoL in our sample. As a second exploratory analysis, we aim to investigate symptom-to-symptom relationships in our sample by making use of the network approach for NMS, and examine which symptoms play a central role in the NMS-network.
METHODS

PARTICIPANTS AND PROCEDURE
Participants were recruited from local hospitals in the Amsterdam area (The Netherlands). Inclusion criteria were a diagnosis according to the UK Brain Bank criteria, and ability to sign informed consent form and to finish all tests. The study was approved by the medical ethical committee of OLVG Hospital (Amsterdam, The Netherlands). Patients were invited to participate in our study either after their hospital appointment, or at home if so desired. All participants provided written informed consent. All methods were carried out in accordance with the relevant guidelines and regulations. Trained assistants administered the Non-Motor Symptom Scale (NMSS), King’s Parkinson’s Disease Pain Scale (KPPS) and Montreal Cognitive Assessment (MoCA). The Numeric Rating Scale (NRS), EQ-5D-3L and Hospital Anxiety and Depression Scale (HADS) were filled out by participants at a time convenient to them.

QOL
The EQ-5D-3L questionnaire assesses health related QoL with five questions, and three levels per question. Total sum score was used as an outcome measure (Herdman et al., 2011).

NON-MOTOR SYMPTOMS
The NMSS assesses all non-motor symptoms in 30 questions, subdivided into domains: Cardiovascular, Sleep/Fatigue, Mood/Apathy, Perceptual problems, Attention/Memory, Gastrointestinal tract, Urinary, Sexual function, Miscellaneous. Patients are asked to evaluate frequency and severity of each symptom, which is then multiplied (range from 0, indicating no symptoms present, to 12, indicating maximum frequency and severity) (Chaudhuri et al., 2007). The KPPS was used to measure pain (Chaudhuri et al., 2015). The KPPS is developed to capture pain experience specifically in patients with Parkinson’s disease. A Dutch version of the KPPS was not yet available, therefore the English version of this questionnaire was translated into Dutch according to an internationally approved procedure and in close cooperation with the authors of the original publication, rendering a validated Dutch version of the KPPS (Chaudhuri et al.,
Approved bilingual translators performed all translations. A graphical overview of the procedure is depicted in Supplementary Figure S4.1. Additionally, the Numeric Rating Scale (NRS) was used to measure the severity of patients’ pain during the last month on a scale from 0 (no pain) to 10 (worst pain imaginable). The MoCA, a brief cognitive screening tool, was used as an indication of cognitive functioning (Nasreddine et al., 2005). From the HADS, two scores were calculated: one for depression and one for anxiety.

Supplementary Table S4.1 provides an overview of all domains with concomitant Crohnbach’s $\alpha$, included items and how domain scores were calculated. Domains will be indicated with a capital throughout the paper. The seven included domains are Cardiovascular, Sleep, Mood, Cognition, Intestinal, Urinary and Pain.

**STATISTICAL ANALYSIS**

All individual items were converted to z-scores before they were used to calculate a mean for their domain. For our primary analysis, a stepwise forward multiple linear regression was performed to test the association between NMS and QoL, with QoL as dependent variable, and the seven domains as independent variables. QoL was normally distributed, but domain scores were not. Residuals of the regression analysis were normally distributed. Alpha level was set at 0.05. SPSS version 23 was used.

RStudio, version 1.1.383 was used for our secondary analysis. A correlation network was calculated with the `qgraph` package (Epskamp et al., 2012). A regularized partial correlation network was calculated using a graphical LASSO with EBIC model selection (Epskamp et al., 2016). Regularized partial correlation networks estimate a large number of parameters and thus require a large set of participants. The sample on which our network was based is quite small, which makes network estimation unstable. The EBIC hyperparameter $\gamma$ was therefore set to 0 (by default set to 0.5), with a higher $\gamma$ indicating a sparser network (with fewer edges). This low hyperparameter implies a larger risk of false positives, therefore the second part of our analyses is of exploratory nature (Epskamp et al., 2016). The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.
RESULTS

PARTICIPANT CHARACTERISTICS
Seventy patients were included in the final analysis, of whom 34 were female (48.6%). Average age was 71.6 years ($SD = 9.72$); average disease duration was 6.0 years ($SD = 5.6$) and average MoCA score was 22.90 ($SD = 4.58$). See Supplementary Table S4.2 for an overview of medication, and Supplementary Table S4.3 for level of education.

PREDICTORS OF QOL
For our main analysis, the final model included Mood and Pain as significant predictors for QoL. The first model included only Mood, and had an explained variance ($R^2$) of 19.6%. The final model included Mood and Pain, with $R^2$ of 28%. See Table 4.1.

<table>
<thead>
<tr>
<th>$R^2$ of the model</th>
<th>Included variables</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Beta</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>.196</td>
<td>Mood -.620 .162 -.433</td>
<td></td>
<td>$p &lt; .001$</td>
</tr>
<tr>
<td>Model 2</td>
<td>.280</td>
<td>Mood -.537 .158 -.384</td>
<td></td>
<td>$p = .001$</td>
</tr>
<tr>
<td></td>
<td>Pain -.439 .168 -.295</td>
<td></td>
<td></td>
<td>$p = .011$</td>
</tr>
</tbody>
</table>

Table 4.1. Multiple regression analysis, using a stepwise forward model, quality of life was used as dependent variable.

THE NMS NETWORK
As mentioned before, our secondary analysis was of an exploratory nature with a predominantly qualitative interpretation. For our secondary analysis, all seven domains were entered as nodes both in a full correlation network as well as in a regularized partial correlation network. Figure 4.1a shows the full correlation network. In Figure 4.1b, we controlled for spurious connections, and each association was corrected for all other associations in a regularized partial correlation network. Cognition and Mood were most strongly connected in both networks. Correlation coefficients can be found in Supplementary Table S4.4. Figure 4.1c shows centrality measures of each node in from the regularized partial correlations network. Mood and Sleep had the highest betweenness centrality, indicating their central role in the network.
DISCUSSION

With this study, we aimed to investigate NMS of PD with two approaches: first, by examining how each NMS contributes to patients’ QoL quantitatively, and second by examining how NMS are related to each other qualitatively by making use of a network approach. Here, we will elaborate on possible implications of these complementary analyses.

Results from our primary analysis point out that mood and pain both contribute majorly to QoL. These results are in line with earlier findings: depression is a factor consistently associated with QoL in PD (Soh et al., 2011), and although pain has less consistently been associated with QoL (Martinez-Martin et al., 2017), we suggest that the use of the KPPS gives a more robust measure of PD-related pain than other more generic pain measures, resulting in a stronger association with QoL. Additionally, analysis of the NMS-network indicates that mood and sleep play central roles (Figure 4.1c), and

![Network Analysis Diagram](image)
that mood and cognitive problems are tightly linked (Figure 4.1b). Together with the results on our primary analysis, this paper emphasizes the importance of mood problems, both for QoL as well as within the network of NMS. Also, treatment of sleep problems could be beneficial for QoL in PD: improvement of sleep might coincide with a change in pain and mood based on our network analysis (see Figure 4.1b). Longitudinal data should be able to add information on directionality, since network analysis cannot shed light on any causal relations between symptoms: it is an undirected network based on correlations (Fried and Cramer, 2017). Nonetheless, a network approach to symptoms could provide suggestions as to which symptoms to target, i.e. those that play a central role in the network, in order to achieve amelioration of a symptom that might be more difficult to treat. Amelioration would thus follow indirectly.

Because of aforementioned power issues, we would first and foremost like to emphasize that our results should be replicated. Also, the choice of nodes in our network should be considered: the variables that make up the nodes in our network are an aggregate of measures of the same construct (e.g. the node ‘mood’ encompasses symptoms of depression as well as anxiety). This makes the measure on the one hand more robust, but on the other hand reduces specificity. In general, nodes in a network are dependent on the items from the clinical instruments on which they are based. A network might therefore never be ‘complete’ (i.e. include all symptoms of the disease). Nonetheless, although influences that cannot be accounted for will remain in every dataset, such as within-person variability or influence of medication, even a near-complete network of symptoms and its interactions will greatly aid in the treatment of NMS of PD.
Supplementary Figure S4.1: Graphical representation of the translation process. The procedures as specified by the authors of the original paper were used. All steps were completed in close collaboration with the authors of the original paper.
<table>
<thead>
<tr>
<th>Domain</th>
<th>NMSS items</th>
<th>Additional items</th>
<th>Crohnbach's α of whole domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Included: Cardiovascular</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sleep</td>
<td>3, 4, 5, 6</td>
<td>-</td>
<td>.577</td>
</tr>
<tr>
<td>Mood</td>
<td>7, 8, 9 10, 11, 12</td>
<td>HADS anxiety subscore, HADS depression subscore</td>
<td>.824</td>
</tr>
<tr>
<td>Cognition</td>
<td>16, 17, 18</td>
<td>Seven MoCA subscores (inverted, so that higher score indicates more problems)</td>
<td>.625</td>
</tr>
<tr>
<td>Intestinal</td>
<td>19, 20, 21</td>
<td>-</td>
<td>.549</td>
</tr>
<tr>
<td>Urinary</td>
<td>22, 23, 24</td>
<td>-</td>
<td>.709</td>
</tr>
<tr>
<td>Pain</td>
<td>27</td>
<td>KPPS total score, NRS</td>
<td>.723</td>
</tr>
<tr>
<td>Excluded: Miscellaneous</td>
<td>28, 29, 30</td>
<td>-</td>
<td>.238</td>
</tr>
<tr>
<td>Perceptual problems, hallucinations</td>
<td>13, 14, 15</td>
<td>-</td>
<td>.752</td>
</tr>
<tr>
<td>Sexual function</td>
<td>25, 26</td>
<td>-</td>
<td>.815</td>
</tr>
</tbody>
</table>

Supplementary Table S4.1: Overview of in- and excluded domains and their reliability (Crohnbach's α). HADS = Hospital Anxiety and Depression Scale; MoCA = Montreal Cognitive Assessment; KPPS = King's Parkinson’s Pain Scale; NRS = Numeric Rating Scale. All NMS were subdivided into categories according to the domains of the NMSS. We added the domain Pain, and removed the domains Perceptual problems/Hallucinations, Sexual Function and Miscellaneous. Variance was too low for the former two (<25% of non-zero answers), and Crohnbach’s α was low for the latter (indicating low reliability). For Cardiovascular, item 2 was removed because variance was too low (7% of that item had a non-zero answer). Thus, seven domains were included for final analyses: Cardiovascular, Sleep/Fatigue, Mood, Cognition, Intestinal, Urinary and Pain. Second, we added items from the additional questionnaires and tests in the domains: anxiety and depression subscores from the HADS were added to the domain Mood, MoCA-subscores were inverted (with higher scores indicating more problems) and added to the domain Cognition. The sumscores of the KPPS and NRS were added to the domain Pain. All individual items were converted to z-scores before they were used to calculate a mean for their domain.
<table>
<thead>
<tr>
<th>Category</th>
<th>Number (%)</th>
<th>Missing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson-related medication</td>
<td>61 (87.1%)</td>
<td>6 (8.6%)</td>
</tr>
<tr>
<td>Cardiovascular medication</td>
<td>31 (44.3%)</td>
<td>9 (12.9%)</td>
</tr>
<tr>
<td>Respiratory medication</td>
<td>3 (4.3%)</td>
<td>8 (11.4%)</td>
</tr>
<tr>
<td>Neurotrophic medication other than Parkinson-medication</td>
<td>11 (15.7%)</td>
<td>8 (11.4%)</td>
</tr>
<tr>
<td>Pain medication (any type)</td>
<td>41 (58.6%)</td>
<td>6 (8.6%)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>17 (26.6%)</td>
<td></td>
</tr>
<tr>
<td>Opioid</td>
<td>7 (10.9%)</td>
<td></td>
</tr>
<tr>
<td>Antidepressant/Anti-epileptics</td>
<td>6 (9.3%)</td>
<td></td>
</tr>
<tr>
<td>Non-steroid Anti Inflammatory Drugs</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>More than one type</td>
<td>8 (11.4%)</td>
<td></td>
</tr>
<tr>
<td>Other medication</td>
<td>23 (32.9%)</td>
<td>8 (11.4%)</td>
</tr>
</tbody>
</table>

Supplementary Table S4.2. Overview of medication use.

<table>
<thead>
<tr>
<th>Level of education</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary education</td>
<td>10 (14.3%)</td>
</tr>
<tr>
<td>Lower secondary education</td>
<td>26 (37.1%)</td>
</tr>
<tr>
<td>Higher secondary education</td>
<td>14 (20.5%)</td>
</tr>
<tr>
<td>Higher professional education/university</td>
<td>17 (24.3%)</td>
</tr>
</tbody>
</table>

Supplementary Table S4.3: Overview of level of education of participants.
Supplementary Table S4.4: Correlation coefficients per edge in both the correlation network (top) and the regularized partial correlation network (bottom).

<table>
<thead>
<tr>
<th></th>
<th>Cardio-vascular</th>
<th>Sleep</th>
<th>Cognition</th>
<th>Mood</th>
<th>Intestinal</th>
<th>Urinary</th>
<th>Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation coefficients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardio-vascular</td>
<td>-</td>
<td>0.20</td>
<td>0.13</td>
<td>0.18</td>
<td>0.11</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.20</td>
<td>-</td>
<td>0.30</td>
<td>0.37</td>
<td>0.26</td>
<td>0.32</td>
<td>0.36</td>
</tr>
<tr>
<td>Cognition</td>
<td>0.13</td>
<td>0.30</td>
<td>-</td>
<td>0.52</td>
<td>0.33</td>
<td>0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Mood</td>
<td>0.18</td>
<td>0.37</td>
<td>0.52</td>
<td>-</td>
<td>0.38</td>
<td>0.22</td>
<td>0.12</td>
</tr>
<tr>
<td>Intestinal</td>
<td>0.11</td>
<td>0.26</td>
<td>0.33</td>
<td>0.38</td>
<td>-</td>
<td>0.13</td>
<td>-0.01</td>
</tr>
<tr>
<td>Urinary</td>
<td>0.21</td>
<td>0.32</td>
<td>0.28</td>
<td>0.22</td>
<td>0.13</td>
<td>-</td>
<td>0.32</td>
</tr>
<tr>
<td>Pain</td>
<td>0.13</td>
<td>0.36</td>
<td>0.01</td>
<td>0.12</td>
<td>-0.01</td>
<td>0.32</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cardio-vascular</th>
<th>Sleep</th>
<th>Cognition</th>
<th>Mood</th>
<th>Intestinal</th>
<th>Urinary</th>
<th>Pain</th>
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PART II
NEUROIMAGING STUDIES
CHAPTER 5
CLINICAL PAIN IS RELATED TO FUNCTIONAL NETWORK TOPOLOGY IN PARKINSON’S DISEASE: AN FMRI STUDY
ABSTRACT

Pain is an important non-motor symptom in Parkinson’s disease (PD), but its underlying pathophysiological mechanisms are still unclear. Research has shown that functional connectivity during the resting-state may be involved in persistent pain in PD. In the present cross-sectional study, 24 PD patients (both during on and off medication phase) and 27 controls participated. We assessed pain with the colored analogue scale and the McGill pain questionnaire. We examined a possible pathophysiological mechanism with resting-state fMRI using functional network topology, i.e. the architecture of functional connections. We took betweenness centrality (BC) to assess hubness, and global efficiency (GE) to assess integration of the network. We aimed to (1) assess the differences between PD patients and controls with respect to pain and resting-state network topology, and (2) investigate how resting-state network topology (BC and GE) is associated with clinical pain in both PD patients and controls. Results show that PD patients experienced more pain than controls. GE of the whole brain was higher in PD patients (on as well as off medication) compared to healthy controls. GE of the specialized pain network was also higher in PD patients compared to controls, but only when patients were on medication. BC of the pain network was lower in PD patients off medication compared to controls. We found a positive association between pain and GE of the pain network in PD patients off medication. For healthy controls, a negative association was found between pain and GE of the pain network, and also between pain and BC of the pain network. Our results suggest that functional network topology differs between PD patients and healthy controls, and that this topology can be used to investigate the underlying neural mechanisms of pain symptoms in PD.
INTRODUCTION

Parkinson's disease (PD) was for a long time mainly considered a motor disease, with rigidity, bradykinesia and resting tremor as its cardinal symptoms. In recent decades, however, there has been growing interest in the non-motor symptoms of PD (Postuma et al., 2015), which may be equally or more incapacitating than the motor symptoms themselves (Chaudhuri et al., 2006a). Pain is one such non-motor symptom that is present in about two thirds of PD patients (Broen et al., 2012; Defazio et al., 2008), and puts an additional burden on patients' quality of life (Buhmann et al., 2017; Quittenbaum and Grahn, 2004; Shibley et al., 2008). It has been linked to several clinical factors, such as disease progression (Mylius et al., 2011), dopaminergic fluctuation (Brefel-Courbon et al., 2005; Nègre-pagès et al., 2008; Silva et al., 2008), depressed mood, and dyskinesias (Rodríguez-Violante, 2017).

During the past few decades, possible neural substrates of pain have been studied extensively, resulting in a potential network of connected brain areas that is thought to underlie the processing and experience of pain (Tracey and Mantyh, 2007). There is no definite consensus on all areas involved in such a pain network; nonetheless, pain-related regions consistently found across studies include the thalamus, anterior cingulate cortex (ACC), posterior and anterior insula, amygdala, prefrontal cortex (PFC), secondary somatosensory cortex (SII) and the periaqueductal grey (PAG) (Scherder et al., 2003). Wager and colleagues employed a machine-learning-based regression technique (LASSO-PCR) to identify such a network, attempted to predict the presence of physical pain (Wager et al., 2013). The result, a so-called neural pain signature (NPS), was found to predict physical pain with high sensitivity and high specificity. Many of the areas involved in this NPS are congruent with areas previously associated with pain, such as the insula, ACC, PFC, thalamus and SII (Tracey and Mantyh, 2007).

Connectivity analysis of the resting-state, during which no specific task is performed, is based on patterns of co-activation of brain regions, providing insight into how the brain is functionally connected. The characteristics of such resting-state connectivity can be employed to study clinically relevant symptoms, such as cognitive functioning in PD (Olde Dubbelink et al., 2014b) or pain in chronic back-pain patients (Tagliazucchi et al., 2010). Using magnetoencephalography (MEG), an increase in functional connectivity
in PD was found to be associated with the duration and severity of the disease (Stoffers et al., 2008). A recent MRI study, investigating both structural and functional mechanisms of persistent pain in PD, found that resting-state connectivity between the right nucleus accumbens and the left hippocampus was reduced in PD patients with pain, compared to PD patients without pain (Antonini et al., 2018; Polli et al., 2016).

In addition to studying increases and decreases of functional connectivity, modern network science has provided an alternative method of investigating the brain, namely by studying specific characteristics of complex networks. Here, the brain (network) is comprised of brain regions (nodes), which have connections of varying strength between them (edges). In functional connectivity, these edges are based on the extent of co-activation of pairs of brain regions. A consensus in modern network science is that the brain is a cost-effective small-world network, which is both locally integrated (i.e. local clusters of connected nodes), and well connected on a global scale (i.e. connected nodes over longer distances) (see Stam for a review (2014)). A number of measures of network topology, i.e. the architecture of connections, have been proposed (for an overview see Rubinov and Sporns, 2010). Investigating the brain via functional network topology, as opposed to standard activation or simple functional connectivity measures, can provide further insight into the pathological mechanism of pain in PD.

A large body of literature in this field has examined the default mode network (DMN) (Raichle et al., 2001). Alterations of the DMN and its relationship with cognitive functioning have also been linked to disease (e.g. Alzheimer’s disease, epilepsy) (Anticevic et al., 2012; Broyd et al., 2009a). Previous research has shown deviant functioning of the DMN at an early stage of PD (Rektorova, 2014; van Eimeren et al., 2009), as well as in chronic pain patients (Baliki et al., 2014).

In this study, we investigated how both clinical pain and functional network topology (whole brain, the pain network (NPS) and DMN) differ between PD patients and controls. Furthermore, we examined the relationship between the amount of clinical pain and functional pain network topology during resting-state within each group. We hypothesized that subjective pain is related to functional topology of the pain network both in PD patients and in controls. Since dopamine is thought to affect both pain (Jääskeläinen et al., 2001) and resting-state functional connectivity (Kelly et al., 2009), PD patients were assessed both during an ON phase, in which dopaminergic medication
was taken as usual, as well as an OFF phase, in which dopamine levels were low. We hypothesized differences between the ON and OFF phase with respect to both pain and resting-state functional connectivity.

**MATERIALS AND METHODS**

For healthy controls, inclusion criteria were (i) aged 40-75 years (2) provision of written informed consent (3) normal or corrected-to-normal vision, and additionally for patients (4) a diagnosis of PD following UK Brain Bank criteria. Exclusion criteria for all participants were (i) current use of psychotropic medication other than levodopa, dopamine-agonists or other Parkinson-medication, (2) major somatic disorder, (3) current psychiatric diagnosis as established by a psychiatrist, (4) presence of dementia, history of stroke or other neurological diseases (as established by neurologist). An additional screening for dementia was performed using the Montreal Cognitive Assessment (MoCA), a screening tool for cognitive dysfunction (Nasreddine et al., 2005), with a cutoff for dementia according to Biundo and colleagues (2014). Patients were recruited through outpatient clinics. Healthy controls were recruited through advertisement in local newspapers, online advertisement and through participating patients (e.g. spouses, relatives, etc.).

**PROCEDURE**

The study was approved by the medical ethical committee of the VU Medical Center, Amsterdam. Informed consent was obtained from all individual participants included in the study. All methods were carried out accordance with relevant guidelines and regulations.

This study was part of a larger cross-sectional case-control study investigating visual attention, reward and pain in PD. Study size was based on expected learning and attention differences between groups. Note that only procedures concerning this project will be described. Patients visited the hospital twice: on the first visit, the MoCA was administered, and the questionnaires were handed in (filled out just prior to visit).
During the second and third visit, the MRI was performed in either the ON or OFF phase. The same procedure was completed for the controls, with the exception that they underwent only one MRI session. The MRI was planned in the same week as the clinical assessment in almost all patients, but as a rule no later than 60 days after the clinical assessment. For the MRI, patients were invited to the hospital in the afternoon for the ON phase, and in the morning for the OFF phase. ON and OFF phase as the first or second MRI session was counter-balanced across participants. The OFF phase was defined as at least 12 hours of dopaminergic medication overnight withdrawal. One patient took their medication 8.5 hours before the resting-state scan to relieve symptoms.

**PAIN**

Here, we define clinical pain as naturally occurring pain that is not experimentally induced, regardless of origin or type. Ultimately, all pain processing is a neurophysiological phenomenon, irrespective of origin (Garland, 2012). Pain was measured during the ON and OFF phase by means of the Colored Analogue Scale (CAS) for intensity as well as for pain affect: subjects were asked to indicate the intensity of their pain (CAS intensity) and how much they were bothered by their pain (CAS affect) both on a scale ranging from 'None' (light pink, 0) to 'Maximal' (dark red, 100) (McGrath et al., 1996). The Dutch version of the McGill Pain Questionnaire was administered during the first visit to inquire about pain during the previous month (Melzack, 1975). We utilized the total score on the Number of Words Chosen (NWC) part of the McGill pain questionnaire. The NWC consists of three major classes of pain descriptors, which were used by the subjects to specify their pain experience. These classes are of sensory, affective or evaluative nature (van der Kloot et al., 1995). Total score on the NWC was used as score for each subject’s clinical pain experience.

**MRI**

Imaging data were collected with a 3T GE Signa HDxT (General Electric, Milwaukee, WI, USA) at the VU University Medical Center (Amsterdam, The Netherlands). Structural images were acquired with a 3D T1-weighted MP RAGE sequence with the following acquisition parameters: voxel size = 1 mm isotropic, 176 slices, 256 x 256 matrix, repetition time (TR) = 8.2 ms, echo time (TE) = 3.2 ms, flip angle (FA) = 12 degrees,
inversion time (TI) = 450 ms. Resting-state data were acquired using a T2*-weighted echo-planar functional scan: number of volumes = 202, 42 slices, slice thickness = 3.2 mm, matrix size = 64 x 64, TR = 2150 ms, TE = 35 ms, FA = 80 degrees, field of view = 240 mm, total duration 7:12 minutes, voxel size was 3mm with 0.3 mm spacing. For the resting-state scan, subjects were instructed to close their eyes, lie still and avoid falling asleep. Participants’ heads were immobilized using foam pads to reduce motion artifacts.

**PROCESSING OF FMRI DATA**

Data were analyzed using FSL FMRIB software library v5.0.9 (Jenkinson et al., 2012) and custom-built scripts in bash and Matlab, version 2015a (Mathworks, Natick, MA, USA). The following pre-processing steps were taken: 1) images were corrected for head motion (using MCFLIRT), 2) slice-timing correction was applied, 3) non-brain tissue was removed (using Brain Extraction Tool, BET), 4) functional images were registered to subject-space (T1-weighted structural image) using BBR, 5) this image was registered to MNI152 standard space (FLIRT for linear registration with 12 DOF), 6) high-pass filtering above 0.01 Hz was applied, 7) spatial smoothing was performed at 5mm full-width half maximum (FWHM), 8) segmentation of gray and white matter was performed using FAST and SIENAX, 9) the first three volumes of each resting-state scan were discarded to achieve field equilibrium, 10) average motion was calculated as the mean of the absolute head movement over all time series for 6 DOF per individual (three translations and three rotations).

After preprocessing, a resting-state adjacency matrix (representing an undirected weighted network) was reconstructed per subject as follows. First, time series were scrubbed for motion outliers: time points with frame-to-frame displacement >1.5 mm (6 DOF) were excluded from further analyses. The remaining time series of chosen atlas regions (see below) were used to calculate the connectivity matrix using Pearson correlation coefficients between time series of each pair of regions included. A Fisher transformation on these correlation coefficients was used to get normally distributed correlation values. Atlas regions were based on the atlas of Power and colleagues (2011). The default mode network (DMN) was constructed from 58 of these nodes (see (Power et al., 2011) for all atlas regions and specification of the DMN). In order to form a pain network, we additionally used a subdivision of the NPS of Wager and colleagues (2013).
In their paper, Wager and colleagues based their NPS on 32 areas (Wager et al., 2013). Sixteen of these areas had positive predictive weights for physical pain, and the other 16 had negative predictive weight. For the current study, only the areas of the NPS with positive predictive weights were used to form a pain network because the interpretation of a network of positive predictive weights is most straightforward, particularly when making comparisons between patient/control groups and medication sessions. See Figure 5.1 for a visual overview of this 16-node pain network.

![Image of pain network](http://www.nitrc.org/projects/bnv/)

**Figure 5.1.** The pain network, based on the positive predictive weights of the NPS (Wager et al., 2013). Areas shown are: 1 = vermis cerebellum; 2 = anterior/mid insula (right); 3 = superior temporal gyrus; 4 = calcarine gyrus; 5 = ventrolateral thalamus (right); 6 = mid insula (left); 7 = hypothalamus; 8 = ventrolateral thalamus (left); 9 = frontal operculum/temporal pole; 10 = dorsal posterior insula/secondary somatosensory area (left); 11 = dorsal posterior insula (right); 12 = somatosensory area (right); 13 = temporoparietal junction; 14 = dorsal anterior cingulate cortex; 15 = supramarginal gyrus; 16 = inferior parietal lobule. BrainNet Viewer version 1.6 was used for visualization (http://www.nitrc.org/projects/bnv/) (Xia et al., 2013).

**NETWORK ANALYSIS**

The resting-state adjacency matrix was used to calculate network topology. We calculated betweenness centrality (BC) and global efficiency (GE) of the resting-state adjacency matrix. BC measures how central a node lies with respect to the rest of the network, and is based on how many shortest paths pass through it. Nodes with high BC represent hubs of the network (Fornito et al., 2016). GE represents efficiency of the underlying network and is a measure of integration. The Brain Connectivity Toolbox (brain-connectivity-toolbox.net) was used to calculate GE per network, and BC of all nodes in the network.
(with Matlab scripts: efficiency_wei.m and betweenness_wei.m, respectively) (Rubinov and Sporns, 2010). BC was then normalized (z-scored) per participant, and averaged per network. GE and average BC for all 16 pain nodes represented the pain network, GE and BC for the 58 DMN nodes represented the DMN. For the whole brain (264 nodes), only GE was calculated, since calculating BC (or ‘hubness’) of the whole brain is essentially meaningless.

**STATISTICAL ANALYSES**

Mann-Whitney’s *U*-test was performed to investigate the difference between PD and controls on all pain measures, as they were not normally distributed. In order to investigate the differences in pain and network topology, the independent variable ‘group’ (patients ON and OFF, and controls) and dependent variables BC and GE measures, were entered in multivariate analyses of covariance (MANCOVAs), with average motion during the scan as a covariate. Only differences between patients and controls were considered. To investigate the relationship between pain and network topology, a hierarchical stepwise linear regression was performed per group (controls, PD ON, PD OFF), with the BC and GE of the pain network as independent variables, and clinical pain as dependent variable. In each analysis’ first block, average movement during the scan was added as a covariate to account for motion in the scanner, and a forward stepwise method was utilized to investigate the contribution of each separate independent variable. Scores on the NWC served as a dependent variable. The alpha level was set at 0.05, and tested two-sided.

**RESULTS**

**SUBJECTS**

Twenty-four patients and 27 healthy controls participated in this study. See Figure 5.2 for an overview of the inclusion process. Patients and controls were matched for age and sex. Education was measured by means of the Verhage-system, ranging from 1 (unfinished lower education) to 7 (finished scientific education) (Verhage, 1964). Patients had a lower
level of education ($U = 174.50, p = .003$). Though not significantly different, patients had a slightly lower score on the MoCA ($t = 1.9(49), p = .063$). Patients had a higher score on the Beck’s Depression Inventory (BDI) (Yin et al., 2004), indicating more severe symptoms of depression in patients than in controls ($t = -5.107(48), p < .01$). None of the patients experienced dyskinesia during scanning. All participant characteristics are shown in Table 5.1. Details per patient are shown in Table 5.2.

![Flowchart of inclusion process](image)

**Figure 5.2. Flowchart of inclusion process.**

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<th>Patients ($n = 24$)</th>
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Table 5.1. Subject characteristics; LEDD = Levodopa Equivalent Daily Dose; UPDRS = Unified Parkinson’s Disease Rating Scale; MoCA = Montreal Cognitive Assessment; BDI = Beck’s Depression Inventory.
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<td></td>
<td></td>
<td>16.5</td>
<td>2.0</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>#20</td>
<td>53</td>
<td>5.0</td>
<td>615</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td>19.5</td>
<td>1.5</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>#21</td>
<td>72</td>
<td>13.0</td>
<td>1150</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td>16.0</td>
<td>2.0</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>#22</td>
<td>57</td>
<td>1.0</td>
<td>106</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td>18.5</td>
<td>4.5</td>
<td>18</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 5.2. Overview of clinical characteristics per patient. *Wearing off was determined by having 2 or more symptoms that improved with medication-intake, a criterion used by (Martinez-Martin and Hernandez, 2012). One patient took a combination of an NSAID (Ibuprofen) and acetaminophen on a daily basis, all other patients did not any have pharmacological intervention for their pain.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Disease duration (years)</th>
<th>LEDD (mg)</th>
<th>Parkinson medication</th>
<th>Levodopa</th>
<th>DA-agonist</th>
<th>Other</th>
<th>Time to scan since medication (hours)</th>
<th>UPDRS III (before scan)</th>
<th>Duration of pain (years)</th>
<th>Wearing off*</th>
</tr>
</thead>
<tbody>
<tr>
<td>#23</td>
<td>51</td>
<td>6.0</td>
<td>1645</td>
<td>Yes</td>
<td>DA-agonist (Ropinirol)</td>
<td>Amantadine</td>
<td></td>
<td>14.0</td>
<td>2.5</td>
<td>16</td>
<td>5.00</td>
</tr>
<tr>
<td>#24</td>
<td>61</td>
<td>1.5</td>
<td>108</td>
<td>No</td>
<td>DA-agonist (Pramipexol)</td>
<td></td>
<td></td>
<td>27.0</td>
<td>12.0</td>
<td>15</td>
<td>22</td>
</tr>
</tbody>
</table>
PAIN MEASURES

An overview of types of pain present in patients and controls is shown in Table 5.3. Pain types are based on classification according to Ford (2010), with ‘Headache’ as an added category. Scores on pain measures were higher in PD patients than in controls (see Table 5.4). PD patients experienced more chronic pain than controls: 75% of patients had chronic pain compared to 40.7% of healthy controls (Pearson’s $\chi^2(1) = 6.080, p = 0.014$). Scores on the NWC were higher in patients ($M = 11.92, SD = 11.77$) than in controls ($M = 4.83, SD = 6.03$, Mann-Whitney $U = 412.00, p = .010$).

<table>
<thead>
<tr>
<th>Type of pain</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musculoskeletal</td>
<td>16 (66.7%)</td>
<td>9 (33.3%)</td>
</tr>
<tr>
<td>Dystonic</td>
<td>3 (12.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Neuropathic/Radicular</td>
<td>5 (20.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Central</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Akathisia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>2 (7.4%)</td>
</tr>
</tbody>
</table>

Table 5.3. Overview of types of pain according to the pain categories of Ford et al. (2005), ‘Headache’ was added as a category. Multiple types of pain for a single subject were possible.

<table>
<thead>
<tr>
<th></th>
<th>HC vs. PD OFF</th>
<th>HC vs. PD ON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC (M, SD)</td>
<td>PD OFF (M, SD)</td>
</tr>
<tr>
<td>CAS Intensity</td>
<td>4.35 (9.38)</td>
<td>15.87 (22.09)</td>
</tr>
<tr>
<td>CAS Affect</td>
<td>2.81 (6.89)</td>
<td>15.74 (22.49)</td>
</tr>
</tbody>
</table>

Table 5.4. Differences on pain scores between PD (ON and OFF phase) and healthy controls, tested with Mann-Whitney’s U test. PD = Parkinson’s disease; HC = Healthy Controls; CAS = Colored Analogue Scale.
NETWORK TOPOLOGY

Next, we investigated the difference between PD patients (PD ON or PD OFF) and controls on network topology (see Table 5.5). An overview of the findings for each brain network is provided below. GE of the whole-brain network was significantly higher in PD ON versus controls, and showed a trend for higher GE in PD OFF versus controls. BC of the pain network was lower in PD OFF than in controls. GE of the pain network was higher in PD ON than in controls. No differences were found for the DMN, nor were there any differences between patients’ ON and OFF phases.

RELATIONSHIP BETWEEN PAIN AND NETWORK TOPOLOGY

We performed three regression analyses to investigate the relationship between pain and topology of the pain network, i.e. one within each group. As a control analysis, we investigated whether LEDD and functional topology of the pain network were related. During ON, neither BC \((r = -.081, p = .707)\), nor GE \((r = .298, p = .158)\) were related to LEDD. Also, during OFF, neither BC \((r = -.067, p = .754)\), nor GE \((r = -.213, p = .318)\) were related to LEDD. Scores on the NWC were also not related to LEDD \((r = .102, p = .634)\).

Table 5.6 lists the parameters of all three regression analyses. Since we used a forward stepwise method, only significant predictors in addition to the entered covariate are shown. In controls, the final model (Step 3) contained BC and GE of the pain network as predictors for the NWC-scores \((R^2 = .29, F(3) = 3.14, p = .045)\). In PD OFF, the final model (Step 2) contained GE of the pain network as a predictor for NWC-scores \((R^2 = .29, F(2) = 4.22, p = .029)\). In PD ON, none of the predictors were significant predictors for scores on the NWC. For clarity, plots of the regression slopes are shown in Figure 5.3, one for each significant predictor.
<table>
<thead>
<tr>
<th>Network</th>
<th>HC vs. PD OFF</th>
<th></th>
<th>HC vs. PD ON</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC (M, SD)</td>
<td>PD OFF (M, SD)</td>
<td>Difference</td>
<td>HC (M, SD)</td>
</tr>
<tr>
<td>Whole brain</td>
<td>GE 125.31 (60.26)</td>
<td>159.38 (44.05)</td>
<td>F (1, 48) = 3.80; p = .057</td>
<td>125.31 (60.26)</td>
</tr>
<tr>
<td>DMN</td>
<td>BC .02 (.12)</td>
<td>-.01 (.15)</td>
<td>ns</td>
<td>.02 (.12)</td>
</tr>
<tr>
<td></td>
<td>GE .30 (.33)</td>
<td>.37 (.34)</td>
<td>ns</td>
<td>.30 (.33)</td>
</tr>
<tr>
<td>Pain</td>
<td>BC .11 (.23)</td>
<td>-.04 (.26)</td>
<td>F (1, 48) = 4.70; p = .035</td>
<td>.11 (.23)</td>
</tr>
<tr>
<td></td>
<td>GE 11.40 (10.61)</td>
<td>23.74 (37.63)</td>
<td>ns</td>
<td>11.40 (10.61)</td>
</tr>
</tbody>
</table>

Table 5.5. Network measures for all groups and networks. MANCOVAs were performed, with average motion during the scan as a covariate. BC = Betweenness Centrality; GE = Global Efficiency; DMN = Default Mode Network; PD = Parkinson’s disease; HC = Healthy Controls; ns = not significant.
Table 5.6. One linear hierarchical regression was performed for each group (controls, PD ON and PD OFF medication). To control for motion, average motion during scanning was entered at Step 1 as a covariate, after which a forward stepwise method was used to add significant independent variables to the model. BC = Betweenness Centrality; GE = Global Efficiency; ns = not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Step</th>
<th>Independent variables</th>
<th>Unstandardized B</th>
<th>Std. error of B</th>
<th>Standardized B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>Step 1</td>
<td>Average motion</td>
<td>-.055</td>
<td>18.62</td>
<td>-.001</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>Average motion</td>
<td>.203</td>
<td>17.50</td>
<td>.002</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC of pain network</td>
<td>-10.17</td>
<td>4.89</td>
<td>-.39</td>
<td>.049</td>
</tr>
<tr>
<td></td>
<td>Step 3</td>
<td>Average motion</td>
<td>-8.65</td>
<td>16.88</td>
<td>.09</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BC of pain network</td>
<td>-9.99</td>
<td>4.57</td>
<td>.38</td>
<td>.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GE of pain network</td>
<td>-.218</td>
<td>.10</td>
<td>.38</td>
<td>.046</td>
</tr>
<tr>
<td>PD OFF</td>
<td>Step 1</td>
<td>Average motion</td>
<td>-5.43</td>
<td>65.11</td>
<td>.02</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>Average motion</td>
<td>-38.30</td>
<td>57.43</td>
<td>.13</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GE of pain network</td>
<td>.171</td>
<td>.059</td>
<td>.55</td>
<td>.009</td>
</tr>
<tr>
<td>PD ON</td>
<td>Step 1</td>
<td>Average motion</td>
<td>27.32</td>
<td>36.34</td>
<td>.158</td>
<td>ns</td>
</tr>
</tbody>
</table>

Figure 5.3. Plots of separate significant effects of the regression analyses. Left panel (blue): A negative linear association of GE of the pain network with NWC scores for HC. Middle panel (blue): A negative linear association of BC of the pain network with NWC scores for HC. Right panel (green): A positive linear association of GE of the pain network with NWC scores for PD OFF. GE = Global Efficiency; NWC = Number of Words Chosen; BC = Betweenness Centrality; PD = Parkinson’s disease; HC = Healthy controls; PD OFF = PD patients during OFF phase.
DISCUSSION

This study investigated both pain and functional network topology, as well as their interrelatedness in PD. Pain as a symptom of PD has been investigated before, and our results corroborate the increased pain experience in PD compared to controls (Broen et al., 2012; Fil et al., 2013). Pain scores in our patient group were not different when they were on or off medication. Although dopamine could have an ameliorating effect on subjective pain experience (Brefel-Courbon et al., 2005; Hagelberg et al., 2004), this effect has not consistently been found (Dellapina et al., 2011; Flores et al., 2004; Polli et al., 2016).

Our results on functional network topology indicate that PD patients had a higher efficiency of the whole brain off medication (trend-level) as well as on medication (significance-level) compared to healthy controls. Global efficiency of the pain network was also higher compared to controls, but only when PD patients were on medication. Compared to controls, PD patients’ pain network had a lower hubness (BC) when off medication. These results are not in line with previous findings relating to topology of resting-state networks: a decrease in global efficiency of the whole brain network (Skidmore et al., 2011) and a decrease in efficiency of the cortico-basal ganglia motor circuit (Wei et al., 2014) in PD patients off medication has been reported using fMRI. Olde Dubbelink and colleagues show that global efficiency of the functional network assessed with MEG is dynamic across the disease course: de novo patients have global efficiency similar to controls, but global efficiency and local integration decrease with disease progression (Olde Dubbelink et al., 2014b). Our results suggest an increase, not a decrease in (whole brain) efficiency in PD. This could point towards a dynamic mechanism of hub overload: brain disorders, such as AD and schizophrenia, have recently been shown to preferentially affect highly connected hub regions. Certain hubs appear to become overloaded, after which a neural traffic rerouting occurs from the overloaded (provincial) hubs towards connector hubs (Stam, 2014). This process of shifting loads in the hierarchy continues until the most centrally situated hubs are overloaded and the disease has reached a chronic phase. This theory was partly confirmed in Alzheimer’s disease (AD) (Jones et al., 2016), where hub-overload was found before any clinical symptoms became apparent. Similar results were found in glioma and
multiple sclerosis patients, where connectivity between hubs and non-hubs was higher in patients at diagnosis than in controls (Derks et al., 2017; Meijer et al., 2017). Hub-overload could therefore be a central issue in these brain disorders. Applying this theory to PD as a dynamic, progressive disease, our current findings might be understood as follows: patients were in a relatively early stage of the disease, with relatively few symptoms. The reported increase in global efficiency could therefore reflect the early relaying of information. As the disease progresses, such rerouting of connectivity may lead to an overload of the hubs of the brain, which will hypothetically be accompanied by increasing symptom burden. Subsequently, a breakdown of the system could result in further progression of the disease.

We found that higher global efficiency of the pain network was associated with more clinical pain in PD patients off medication. Global efficiency and betweenness centrality were associated with less pain in healthy controls. These findings may indicate group differences as to how pain is reflected in the pain network. Higher global efficiency could indicate a more integrated network (Stam, 2014), or a network in which information can be processed in parallel (Skidmore et al., 2011). Regarding our results on the group of healthy controls this would signify a quickly transferred signal within the pain network, which reflects little clinical pain over the last month. Also, when hubness of the pain network is higher, controls experience less clinical pain. Alternatively, it could suggest an ongoing pathological process: increased efficiency advocates spreading of seizures in epilepsy patients (DeSalvo et al., 2014). Higher efficiency of the pain network in PD could therefore also be seen as pathological, with pain as a clinical manifestation in PD patients. More research is needed to replicate our results and investigate causality of either increased or decreased efficiency and centrality. It should be noted that two of our included patients had an exceptionally high GE of the pain network. One patient had painful polyneuropathy, the other had dystonia, which caused pain. After careful consideration, we chose to keep these patients in the dataset, as we could not determine that these high values of GE were due to artifacts or other technical issues. Instead, we conclude that these subjects represent variation of GE in PD patients with pain.

Even though this study provides insight into a possible underlying mechanism of pain in PD, several issues should be taken into account. Our study focused on neural processing of pain, and did not take any other clinical factors into account that might
influence pain. One such factor is the presence of symptoms of depression, which is known to influence pain experience (Rana et al., 2017). Other examples are age, sex, disease duration and severity of motor symptoms, as well as a possible effect of wearing-off, the loss of response to dopaminergic medication over time. The effect of wearing-off is the quick re-emergence of symptoms after medication intake. Even though our patients had relatively short disease duration, more than half experienced wearing off (see Table 5.2). In addition, the effect of pharmacological subclasses of PD medication could be of influence. Due to the complexity of dopamine-receptor binding for the various subclasses of dopamine medication, this could not be analyzed within the small sample of the current study. Another issue regarding PD medication is the time from last medication intake until MRI-scanning during the ON phase: an average of 2.9 hours remained between medication intake and the resting-state scan. Since the half-life of levodopa-medication is 1-2 hours, this could have influenced our results. A larger group of patients, preferably representing both low and high pain phenotypes, is needed to find independent contributions of these variables in the context of pain and the functional brain network. Future research that includes patients with high pain intensity could also reinforce our results. In this study, we investigated pain as a continuous variable, including subjects with a score of 0. A continuous scale was chosen, as we cannot be sure at which point pain becomes a pathological symptom of PD. A score of 0 might therefore also represent a pathological processing of pain. Moreover, the pain network as we included it might not explain all of patients’ pain experience: we chose to include only positive predictive weights of the NPS (Wager et al., 2013), and might therefore have missed information coming from the subdivision of the NPS of the negative predictive weights. In addition to this, our pain network did not include any areas typical of the brainstem descending modulatory network (including the periaqueductal gray and the rostral ventral medulla), which modulates pain further by facilitation or inhibition at the spinal level (Tracey and Mantyh, 2007; Vanegas and Schaible, 2004). Finally, most neural activation studies into pain are based on groups of healthy and relatively young volunteers, who receive acute pain stimuli. The generalization towards clinical pain in a patient group might therefore reflect a different mechanism as compared to other studies into clinical pain.
To our knowledge, this is the first study that investigates resting-state functional connectivity-derived network measures in PD to study pain. Our main findings are a higher integration of the whole brain in PD patients compared to controls, a higher integration of the pain network in PD patients (on medication) compared to controls, and a lower hubness of the pain network in patients (off medication) compared to controls. Additionally, a positive association was found between clinical pain and hubness of the pain network in patients off medication, but because of low sample size and statistical outliers, these results should be interpreted cautiously. Further (replication) studies are needed to substantiate the nuances of network measures, and to investigate other symptoms in order to gain insight in the entirety of the disease.
CHAPTER 6
DYNAMIC FUNCTIONAL CONNECTIVITY AND SYMPTOMS OF PARKINSON’S DISEASE: A RESTING-STATE FMRI STUDY
ABSTRACT

Research has shown that dynamic functional connectivity (dFC) in Parkinson’s disease (PD) is associated with better attention performance and with motor symptom severity. In the current study, we aimed to investigate dFC of both the default mode network (DMN) and the frontoparietal network (FPN) as neural correlates of cognitive functioning in patients with PD. Additionally, we investigated pain and motor problems as symptoms of PD in relation to dFC. Twenty-four PD patients and 27 healthy controls participated in this study. Memory and executive functioning were assessed with neuropsychological tests. Pain was assessed with the numeric rating scale; motor symptom severity was assessed with the Unified Parkinson’s Disease Rating Scale. All subjects underwent resting-state functional magnetic resonance imaging (fMRI), from which dFC was defined by calculating the variability of functional connectivity over a number of sliding windows within each scan. dFC of both the DMN and FPN with the rest of the brain was calculated. Patients performed worse on tests of visuospatial memory, verbal memory and working memory. No difference existed between groups regarding dFC of the DMN nor the FPN with the rest of the brain. A positive correlation existed between dFC of the DMN and visuospatial memory. Our results suggest that dynamics during the resting state are a neural correlate of visuospatial memory in PD patients. Furthermore, we suggest that brain dynamics of the DMN, as measured with dFC, could be a phenomenon specifically linked to cognitive functioning in PD, but not to other symptoms.
INTRODUCTION

The organization of structural and functional connections of the brain, or brain topology, shapes complex behavior (Park and Friston, 2013; Stam, 2014): cognitive functioning arises from both local and global integration across the whole brain. Local integration, which mainly entails short-range neural connections, subserves specialized cognitive functions. Global integration entails long-range neural connections, and underlies higher cognitive functions (Park and Friston, 2013; Sporns, 2013). Cognitive dysfunctioning has been related to altered brain network topology as measured by resting-state imaging in several disorders, such as in multiple sclerosis (Hawellek et al., 2011; Meijer et al., 2017), schizophrenia (Lynall et al., 2010), and epilepsy (Douw et al., 2011).

Parkinson’s disease (PD) is a neurodegenerative disorder, hallmarked by motor symptoms: rigidity, bradykinesia, tremor, and postural instability. Although clinical diagnosis of PD is based on these motor symptoms, non-motor symptoms play an equally, sometimes even more devastating role in patients’ quality of life (Martinez-Martin et al., 2011b). One such non-motor symptom is cognitive decline, e.g. in the executive and memory domains (Litvan et al., 2012). In line with the abovementioned link between network integration and cognition, cognitive decline in PD has been associated with decreased functional connectivity (Amboni et al., 2015; Olde Dubbelink et al., 2014a) or deviant coupling between subnetworks of the brain (Putcha et al., 2016). Another non-motor symptom is pain (Broen et al., 2012; Choi et al., 2017), a symptom that is present in about two thirds of patients and has been associated with sleep and mood disturbances, as well as severity of motor symptoms (Broen et al., 2012; Defazio et al., 2017). Pain in PD has been linked to a functional disconnection between the hippocampus and nucleus accumbens (Polli et al., 2016).

Connectivity analyses on resting-state functional magnetic resonance imaging (rs-fMRI) have, until recently, been based on the assumption that connections remain stable throughout an fMRI session. However, temporal fluctuations of connectivity may in fact be a fundamental feature of brain networks, particularly in the context of cognitive functioning (Sizemore and Bassett, 2017). Dynamic functional connectivity (dFC) is an approach to assess these temporal fluctuations, by calculating the variability...
of functional connectivity over a number of sliding windows within each scan. dFC may have added value in explaining cognitive functioning above and beyond that of static connectivity, as it may detect the brain network’s ability to deal with varying demands from the environment. Higher dFC (throughout the brain) has been related to better performance in healthy controls in the domains of cognitive flexibility, sustained attention and working memory (Jia et al., 2014). Also, lower dFC of a hub region of the default mode network (DMN), the posterior cingulate cortex (PCC), was associated with worse memory performance in epilepsy patients, suggesting that high dFC is favorable in the memory domain (Douw et al., 2015).

The DMN is a task-negative network (i.e. deactivated during cognitive processes) that appears to be particularly vulnerable to the effects of disease (Broyd et al., 2009b). Dynamics of the DMN appear to be associated with internally-oriented cognition (Kucyi and Davis, 2014). Another network that is involved in cognitive functioning is the frontoparietal network (FPN), which plays a central role in flexibly adapting to the environment (Cole et al., 2013). Dynamics of the FPN have been implicated in cognitive control (Zanto and Gazzaley, 2013). In healthy controls, a lower resting-state dFC between FPN and the DMN was related to a higher cognitive flexibility (Douw et al., 2016). Regarding cognitive functioning in PD, a higher dFC of the dorsal attentional network at rest appears to be beneficial for performance on an attention task (Madhyastha et al., 2015a, 2015b). Although dFC has been suggested to be associated with mild cognitive impairment (MCI) before (Jones et al., 2012), specific cognitive domains have not yet been investigated with respect to dFC in PD.

The relationship between dFC and other symptoms of PD, such as pain or motor symptom severity, has been largely underexplored. One study investigated dynamics of the resting state in PD by determining brain states with a sliding window technique. Two brain states were found: one relatively more integrated and one more segregated brain state. Time spent in the more integrated brain state was correlated with the severity of motor symptoms of PD patients (Kim et al., 2017). Pain in general, i.e. not specifically related to PD, may also depend on dynamic properties of the brain (Kucyi et al., 2013; Kucyi and Davis, 2015). However, this association seems primarily related to the modulation of pain by the cognitive domain of attention, instead of pain itself: a higher tendency to attend to painful stimuli was related to higher dFC.
This study investigates behavioral correlates of brain dynamics during the resting-state in PD. The primary aim is to investigate whether dFC of the DMN and the FPN is a correlate of cognitive functioning. More specifically, we hypothesize that dFC of the DMN with the rest of the brain shows an association with memory functioning and that dFC of the FPN with the rest of the brain shows an association with executive functioning. We will further investigate dFC in PD by assessing a possible difference between PD patients with MCI and those who have intact cognitive functioning. In addition to cognition, we will investigate whether dFC relates to motor problems and pain in PD patients.

MATERIALS AND METHODS

SUBJECTS
Patients were referred through neurologists of outpatient clinics (VU University Medical Center, Amsterdam, The Netherlands; OLVG Hospital, Amsterdam, The Netherlands; Zaans Medical Center, Zaandam, The Netherlands). Healthy controls were recruited through advertisement in local newspapers, online advertisement and through participating patients (e.g. spouses, relatives, etc.). Inclusion criteria were (1) age 40-75 years old (2) ability to provide written informed consent (3) normal or corrected-to-normal vision, and (4) for patients only, a diagnosis of PD following UK Brain Bank criteria. Exclusion criteria for all subjects were (1) current use of psychotropic medication other than levodopa, dopamine-agonists or other Parkinson medication, (2) major somatic disorder, (3) current psychiatric diagnosis as established by a psychiatrist, (4) presence of dementia, history of stroke or other neurological diseases (as stated in their medical status). A screening for dementia was performed using the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), with a cutoff of 21 or lower for dementia according to Biundo and colleagues (2014). Additionally, Beck’s Depression Inventory (BDI) was administered to assess presence of depressive symptoms (Yin et al., 2004). Patients and controls were matched for age and sex. Education was categorized according to the Verhage-system, which runs from 1 (unfinished lower education) to 7 (finished university level) (Verhage, 1964).
The study was carried out in accordance with regulations of the medical ethical committee of the VU University Medical Center (Amsterdam, The Netherlands), which also approved the protocol. All subjects gave written informed consent in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.

PROCEDURE
This project was part of a larger study investigating reinforcement learning, visual attention, and pain in PD. Sample sizes of 24 patients and 24 controls were predetermined for the reinforcement learning task, however for the current study we could include additional subjects who passed the initial screening procedure (see Results). Patients visited the hospital three times and controls twice. On the first day, all subjects underwent clinical assessment including neuropsychological testing, practiced a reward-learning task, and filled out questionnaires.

Patients underwent MRI twice: once with their normal Parkinson medication (ON phase), once without Parkinson medication (OFF phase). Only OFF phase imaging was used for this study in order to reduce the effect of dopaminergic medication. The OFF phase was defined as at least 12 hours of dopaminergic medication withdrawal (overnight). One patient took their medication 8.5 hours before the resting-state scan to relieve motor symptoms. Controls underwent MRI once. Note that only those procedures concerning the current project will be described in the remainder of the text. The MRI was generally planned in the same week as the clinical assessment, but no later than 60 days after clinical assessment.

CLINICAL ASSESSMENT: COGNITION, MOTOR SYMPTOMS AND PAIN
Since neuropsychological testing was performed on a different day than the scanning day, patients took their medication as normal, thus cognition was not tested during the OFF phase.

EXECUTIVE FUNCTIONING
The Stroop Colour Word test was used to investigate interference (Stroop, 1935). The interference measure was calculated by dividing the time needed for Stroop, card II
(colored rectangles) by time needed for Stroop, card III (names of colors written in incongruent colors) (Lansbergen et al., 2007), with a high score indicating a low degree of interference. To test verbal fluency, subjects were asked to generate as many words as possible from a specific semantic category within 1 minute, which was repeated with a different category (categories were first animals, then professions). Subjects were then asked to generate as many words as possible starting with a specific letter, which was repeated twice (letters were D, A and T). Word fluency was the average of both the category and the letter fluency tests. The Digit Span Backwards from the Wechsler Adult Intelligence Scale was administered to test working memory (Wechsler, 1955). Subjects were asked to repeat a series of digits in a reversed order. The number of correctly repeated series was used as working memory measure. The Rule-Shift Cards Test of the Behavioural Assessment of the Dysexecutive Syndrome (BADS) was used to assess mental flexibility (Wilson et al., 1998). Subjects were asked to respond to a set of stimulus cards according to a rule. In the second condition, the rule was changed. Number of mistakes was used to calculate a profile score, which served as a measure for mental flexibility.

MEMORY
Verbal memory was assessed using the Rey Auditory Verbal Learning Test (AVLT). The Dutch version of this test encompasses a list of 15 unrelated words (Saan and Deelman, 1986). In the immediate recall condition, the total number of correct words after 5 trials served as a measure for short-term verbal memory. In the delayed recall condition, the number of correct words after an interval of at least 15 minutes was used as a measure for long-term verbal memory. The Complex Figure of Rey (CFR) was used as an indication of visuospatial memory. Subjects were asked to copy a complex figure. After several minutes, subjects were asked to reproduce the figure without the original (Osterrieth, 1941). Scoring was performed according to a standardized method.

MOTOR FUNCTIONING
The motor part of the Unified Parkinson’s Disease Rating Scale (UPDRS III) was administered during patients’ OFF phase before MRI (Postuma et al., 2015). UPDRS III was always performed by the same person [GE].
**PAIN**

The Numeric Rating Scale (NRS) was administered before commencing the resting-state scan during the OFF phase. Subjects were asked to rate their pain on a scale from 0 – 10 for intensity of pain, with 0 indicating ‘no pain’, and 10 indicating ‘the worst pain ever experienced’.

**MRI**

Imaging data were collected with a 3T GE Signa HDxT MRI scanner (General Electric, Milwaukee, WI, USA) at the VU University Medical Center (Amsterdam, The Netherlands). Structural images were acquired with a 3D T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence with the following acquisition parameters: voxel size = 1 mm isotropic, 176 slices, 256 x 256 matrix, repetition time (TR) = 8.2 ms, echo time (TE) = 3.2 ms, flip angle (FA) = 12 degrees, inversion time (TI) = 450 ms. Resting-state data were acquired using a T2*-weighted echo-planar functional scan: number of volumes = 202, 42 slices, slice thickness = 3.2 mm, matrix size = 64 x 64, TR = 2150 ms, TE = 35 ms, FA = 80 degrees, field of view = 240 mm, total duration 7:12 minutes. For the resting-state scan, subjects were instructed to close their eyes, lie still and avoid falling asleep. The subject’s head was immobilized using foam pads to reduce motion artifacts.

**PROCESSING OF FMRI DATA**

Data were analyzed using FSL FMRIB software library v5.0.9 (Jenkinson et al., 2012) and custom built scripts in bash and Matlab, version 2015a (Mathworks, Natick, MA, USA). The following pre-processing steps were taken: 1) images were corrected for head motion using MCFLIRT (Jenkinson 2002), 2) bottom-up slice-timing correction was applied, 3) non-brain tissue was removed (using Brain Extraction Tool, BET), 4) functional images were registered to subject-space (T1-weighted structural image) using BBR, 5) the T1-weighted structural image was registered to MNI152 standard space (FLIRT for linear registration with 12 DOF), 6) high-pass filtering above 0.01 Hz was applied, 7) spatial smoothing was performed at 5mm full-width half maximum (FWHM), 8) segmentation of gray and white matter was performed using FAST and SIENAX, 9) the first three volumes of each resting-state scan were discarded to achieve field equilibrium.
DYNAMIC FUNCTIONAL CONNECTIVITY

See Figure 6.1 for a schematic overview of calculation of dFC. Time series of 264 atlas regions were used for subsequent analyses, based on the parcellation suggested by Power and colleagues (Power et al., 2011). These time series were scrubbed for motion outliers: time points with framewise displacement >1.5 mm (6 DOF) were excluded from further analyses. Pearson’s correlation coefficients were calculated between remaining time series from all regions of interest (ROIs) of the Power atlas, resulting in a 264x264 matrix per window per subject, with a window length of 60.2 seconds (28 x TR) and a shift of

Figure 6.1. Graphical representation of the calculation of dynamic functional connectivity. Figure 6.1A: Nodes of the default mode network (DMN) are depicted in red; nodes of the frontoparietal network (FPN) are depicted in yellow. See Power et al. (2011) for specification of the networks. Figure 6.1B: A shifting window approach was used to calculate dynamic functional connectivity. The left panel shows that an adjacency matrix was calculated for each shifted window. The standard deviation was then calculated for each connection and normalized for its average strength. The right panel of B shows a matrix of the resulting coefficients of variation for a single subject. Subsequently, an average was calculated for the DMN with the rest of the brain (red rectangle in right panel) and for the FPN with the rest of the brain (yellow rectangle).
10.75 seconds (5 x TR), resulting in 34 sliding windows per subject. The choice of window length was based on earlier studies (e.g. (van Geest et al., 2018b)). The standard deviation for each connection was calculated and normalized for the average of that individual connection, yielding the coefficient of variation of each connection. This resulted in a separate dFC matrix per individual. Subsequently, we looked at dFC between the regions of the DMN (see (Power et al., 2011) for included ROIs) with the rest of the brain, and at dFC between regions of the FPN (see (Power et al., 2011) for included ROIs) with the rest of the brain.

**STATISTICAL ANALYSES**

Statistical analyses were performed in IBM SPSS version 23 (Chicago, IL, USA). Normality of all variables was assessed with Kolmogorov-Smirnov tests and histogram inspection. Cognitive test scores were normally distributed, thus independent samples t-tests were performed for all cognitive measures, and False Discovery Rate (FDR) correction for multiple comparisons was applied with $Q < 0.05$ (Hochberg and Benjamini, 1995). dFC measures were not normally distributed, thus differences in dFC measures were tested using non-parametric tests (Mann-Whitney). To test the association between dFC and cognition, a hierarchical linear regression using a stepwise forward method was performed per cognitive outcome measure that was significantly different between patients and controls. dFC of the DMN was used as a predictor for memory tests. dFC of the FPN was used as a predictor for tests of executive functioning. Separate hierarchical linear regression analyses were also performed with pain and motor symptoms as dependent variables, and dFC of the DMN and FPN as predictors. Residuals were normally distributed for all regression analyses, justifying the use of these parametric tests. In all regression analyses, average motion during the scan (as a variable) was added in the first step to control for a possible confounding effect of motion despite scrubbing high motion data points. Because of a possible relationship between cognitive decline and dynamics and because differences in static functional connectivity have been found in PD-MCI before on measures of static functional connectivity (Baggio et al., 2015), we divided the group of patients into those with MCI and those without MCI, based on patients’ score on the MoCA. We applied a cut-off score of 25 as indicative of MCI (Hoops et al., 2009). Considering the explorative nature of this study, the association between
PD symptoms and dFC was tested with an alpha level set at .05, without correcting for multiple comparisons.

RESULTS

SUBJECTS
Twenty-four patients and 27 healthy controls participated. See Figure 6.2 for a flowchart of in- and exclusion of patients. Characteristics of all subjects are shown in Table 6.1. Patients had a lower level of education than healthy controls ($U = 174.50$, $p = .003$). Though not significantly different, patients had a lower score on the MoCA ($t = 1.9(49)$, $p = .063$). Patients had a higher score on the BDI, indicating more severe symptoms of depression in patients than in controls ($t = -5.107(48)$, $p < .01$).

![Figure 6.2. Overview of in- and exclusion process for subjects.](image)

COGNITIVE PERFORMANCE
Group differences in cognitive performance are summarized in Table 6.2. Patients performed worse on the CFR, and on both immediate and delayed recall of the AVLT. Fewer words were named in the verbal fluency tests by PD patients when compared to healthy controls. Interference score for the Stroop was similar for PD patients as for controls. No difference was found for number of digits correctly repeated backwards.
between PD patients and controls. No difference was found on performance on the BADS between PD patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 27)</th>
<th>Patients (n = 24)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (M, SD)</td>
<td>59.37 (8.54)</td>
<td>63.42 (7.93)</td>
<td>( t(49) = -1.749, p = .087 )</td>
</tr>
<tr>
<td>Education level (Mdn, range)</td>
<td>6 (3)</td>
<td>5 (4)</td>
<td>( U = 174.50, p = .003 )</td>
</tr>
<tr>
<td>Sex</td>
<td>11 females</td>
<td>7 females</td>
<td>( \chi^2(1) = .745, p = .558 )</td>
</tr>
<tr>
<td>Disease duration in years (M, SD)</td>
<td>-</td>
<td>4.08 (3.13)</td>
<td>-</td>
</tr>
<tr>
<td>LEDD in mg (M, SD)*</td>
<td>-</td>
<td>796.29 (616.44)</td>
<td>-</td>
</tr>
<tr>
<td>UPDRS during OFF phase (M, SD)</td>
<td>-</td>
<td>21.08 (8.31)</td>
<td>-</td>
</tr>
<tr>
<td>MoCA (M, SD)</td>
<td>27.89 (1.89)</td>
<td>26.88 (1.92)</td>
<td>( t(49) = 1.90, p = .063 )</td>
</tr>
<tr>
<td>BDI</td>
<td>22.96 (2.24)</td>
<td>30.46 (7.12)</td>
<td>( t(48) = -4.938, p &lt; .001 )</td>
</tr>
</tbody>
</table>

Table 6.1: Subject characteristics; LEDD = Levodopa Equivalent Daily Dose; MoCA = Montreal Cognitive Assessment; BDI = Beck’s Depression Inventory. * Twelve patients received Levodopa monotherapy, all other patients were taking a combination of Levodopa with DA-agonists or MAO-B/COMT- inhibitors.

<table>
<thead>
<tr>
<th></th>
<th>Controls M (SD)</th>
<th>Patients M (SD)</th>
<th>Statistics</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFR</td>
<td>24.37 (4.98)</td>
<td>18.56 (7.32)</td>
<td>( t(45) = 3.17 )</td>
<td>.003*</td>
</tr>
<tr>
<td>AVLT immediate recall</td>
<td>44.48 (11.48)</td>
<td>37.33 (8.28)</td>
<td>( t(49) = 2.52 )</td>
<td>.015*</td>
</tr>
<tr>
<td>AVLT delayed recall</td>
<td>9.26 (3.60)</td>
<td>6.88 (2.54)</td>
<td>( t(46.72) = 2.75 )</td>
<td>.008*</td>
</tr>
<tr>
<td>Fluency</td>
<td>19.70 (3.11)</td>
<td>16.94 (4.04)</td>
<td>( t(49) = 2.74 )</td>
<td>.008*</td>
</tr>
<tr>
<td>BADS Rule Shift</td>
<td>32.44 (10.00)</td>
<td>37.04 (13.61)</td>
<td>( U = 273.5 )</td>
<td>.402</td>
</tr>
<tr>
<td>Digit span Backwards</td>
<td>6.85 (2.10)</td>
<td>6.33 (2.33)</td>
<td>( t(49) = .841 )</td>
<td>.405</td>
</tr>
<tr>
<td>Stroop (interference)</td>
<td>.66 (.10)</td>
<td>.62 (.11)</td>
<td>( t(49) = 1.22 )</td>
<td>.227</td>
</tr>
</tbody>
</table>

Table 6.2: Group differences in cognitive performance. AVLT = Auditory Verbal Learning Test; BADS = Behavioral Assessment of the Dysexecutive Syndrome; CFR = Complex Figure of Rey; * = significant, corrected using the False Discovery Rate
DYNAMIC FUNCTIONAL CONNECTIVITY

No difference was found between controls \((Mdn = .572)\) and patients \((Mdn = .941)\) regarding dFC between the DMN and the rest of the brain \((U = 288.00, p = .497)\). No difference was found between controls \((Mdn = .896)\) and patients \((Mdn = .962)\) regarding dFC between the FPN and the rest of the brain \((U = 318.00, p = .910)\).

ASSOCIATION BETWEEN DYNAMIC FUNCTIONAL CONNECTIVITY AND COGNITIVE PERFORMANCE

As described above, performance on the AVLT (both immediate and delayed recall), CFR, and verbal fluency was lower in patients compared to controls (see Table 6.2). With respect to memory functioning, dFC of the DMN was positively associated with performance on the CFR \((R^2 = .219, F(1,21) = 5.979, p = .023)\). dFC of the DMN was not associated with score on the immediate recall, or with score on the delayed recall of the AVLT. With respect to executive functioning, no association was between the dFC of the FPN and performance on verbal fluency tasks. See Table 6.3 and Figure 6.3 for details.

Additionally, we investigated dFC differences according to presence of MCI in PD patients. PD patients without MCI had a higher dFC of the DMN with the rest of the brain \((U = 23.00, p = .027)\). There was no difference in dFC of the FPN with the rest of the brain with respect to MCI \((U = 46.00, p = .535)\).

<table>
<thead>
<tr>
<th>Network: Cognitive measure:</th>
<th>Unstandardized B</th>
<th>Std. error of B</th>
<th>Standardized Beta</th>
<th>p-value</th>
<th>Effect size ((R^2) change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN CFR</td>
<td>.974</td>
<td>.398</td>
<td>.502</td>
<td>.023*</td>
<td>.219</td>
</tr>
<tr>
<td>AVLT immediate</td>
<td>.464</td>
<td>.491</td>
<td>.212</td>
<td>.356</td>
<td>.039</td>
</tr>
<tr>
<td>AVLT delayed</td>
<td>.214</td>
<td>.151</td>
<td>.317</td>
<td>.171</td>
<td>.039</td>
</tr>
<tr>
<td>FPN Verbal fluency</td>
<td>.405</td>
<td>.267</td>
<td>.305</td>
<td>.145</td>
<td>.083</td>
</tr>
</tbody>
</table>

Table 6.3. Association between dynamic functional connectivity and cognitive functioning. Linear hierarchical regression analyses were performed with a forward stepwise method, per cognitive outcome measure. DMN = Default Mode Network; FPN = Frontoparietal Network; CFR = Complex Figure of Rey; AVLT = Auditory Verbal Learning Test; * = significant association
ASSOCIATION BETWEEN DYNAMIC CONNECTIVITY AND OTHER PD SYMPTOMS

A linear hierarchical regression using a forward method was also performed with motor severity and pain intensity as outcome variables. Again, average movement during the scan was added in the first block to control for the possible confounding effect of motion. dFC of the DMN and FPN was not significantly associated with either motor severity or with pain intensity during the OFF phase. See Table 6.4.

![Graph showing association between dynamic functional connectivity (dFC) of the default mode network (DMN) and performance on the visuospatial memory task (Complex Figure of Rey, CFR) in PD patients.](image)

**Figure 6.3.** A positive association was found between dynamic functional connectivity (dFC) of the default mode network (DMN) and performance on the visuospatial memory task (Complex Figure of Rey, CFR) in PD patients.

<table>
<thead>
<tr>
<th>Network</th>
<th>Symptom</th>
<th>Unstandardized B</th>
<th>Std. error of B</th>
<th>Standardized B</th>
<th>p-value</th>
<th>Effect size ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN</td>
<td>Motor UPDRS III</td>
<td>-.301</td>
<td>.510</td>
<td>-.137</td>
<td>.562</td>
<td>.016</td>
</tr>
<tr>
<td></td>
<td>Pain NRS</td>
<td>-.121</td>
<td>.140</td>
<td>-.198</td>
<td>.398</td>
<td>.034</td>
</tr>
<tr>
<td>FPN</td>
<td>Motor UPDRS III</td>
<td>.239</td>
<td>.250</td>
<td>.216</td>
<td>.350</td>
<td>.042</td>
</tr>
<tr>
<td></td>
<td>Pain NRS</td>
<td>.009</td>
<td>.071</td>
<td>.031</td>
<td>.896</td>
<td>.001</td>
</tr>
</tbody>
</table>

**Table 6.4.** Associations between dynamic functional connectivity, pain and motor symptoms. Linear hierarchical regression analyses were performed with a forward stepwise method, per symptom.
DISCUSSION

The main aim of this study was to investigate a link between cognitive performance and dynamics of the DMN and FPN in PD patients. First, we found that both dFC of the DMN and dFC of the FPN with the rest of the brain did not significantly differ between PD patients and controls. In addition, we report dFC of the DMN with the rest of the brain as a correlate of visuospatial memory in our patient group.

The positive association between dFC and visuospatial memory in PD strengthens the findings of a previous study investigating cognitive functioning in PD, also using a sliding window dFC technique: higher dynamics within the dorsal attention network at rest was found to be predictive for attention performance (Madhyastha et al., 2015a). The association between cognition and dFC has also been investigated in other patient populations: in a group of epilepsy patients, lower dFC of the posterior cingulate cortex (PCC), a key player in the DMN, with the rest of the brain was related to disturbed verbal memory functioning (Douw et al., 2015), suggesting a similar association between dFC and memory functioning as was found in our PD group.

Jones and colleagues (Jones et al., 2012) have suggested before that dFC of the DMN might underlie the cognitive deterioration in people at risk for developing Alzheimer’s disease (AD) patients. Our results established a lower dFC of the DMN with the rest of the brain for patients with MCI. These results hint that Jones’ hypothesis could also hold for PD, and that dFC of the DMN with the rest of the brain is linked to cognitive fitness, or to the preservation of cognitive functioning.

We found no group differences in dFC of either network (i.e. DMN or FPN), even though patients’ cognitive functioning was worse on several tests. This could be due to methodological choices, such as our focus on dFC of the entire FPN and DMN. Consideration of further subdivisions of each network (e.g. posterior DMN vs. anterior DMN) may increase sensitivity to group differences. This is evident in a study of people with autism spectrum disorder, which showed dFC increases in certain subsets of the DMN and decreases in other subsets of the DMN when compared to controls (de Lacy et al., 2017). Another example is the study of Jones and colleagues (Jones et al., 2016), where dwell time in several subnetworks of the DMN was calculated for a group of AD patients, using a sliding window technique for resting-state connectivity. AD patients
spent more time in the anterior subnetwork of the DMN, but less time in the posterior subnetwork of the DMN. Thus, focusing on different subnetworks of the DMN might improve specificity of conclusions on group differences.

Our second aim was to test whether dFC was specific for certain aspects of cognitive functioning, or whether it might also be related to motor symptoms or pain experience. No association was found between dFC and motor symptoms, or dFC and pain. This does not rule out the possibility of an association between dFC and other symptoms of PD: in the recent study by Kim and colleagues, severity of motor symptoms was related to time spent in a specific configuration of sparse interconnectedness, which was calculated using a sliding window technique (Kim et al., 2017). However, our results suggest that cognition is the main correlate of dFC of the DMN specifically. To our knowledge, however, no study has investigated a link between dFC and pain in PD. More research is needed to investigate to what extent dFC might be specifically underlying cognitive functioning as opposed to other symptoms of PD. Future studies could, for example, select patients on motor-subtypes, such as tremor, akinesia, rigidity or loss of balance.

The results of our study indicate that the dFC of the DMN is related to visuospatial memory in PD patients. This association between dFC and cognitive functioning is not only interesting from a clinical point of view, but it also strengthens the notion that dynamic connectivity is linked to brain function (Hutchison et al., 2013; Sizemore and Bassett, 2017). However, some limitations apply. First, the number of subjects included in this study is small, and so results cannot be generalized to the entire population of PD patients. Given the heterogeneity of symptoms and symptom severity in the population of PD patients together with the relatively mild symptoms present in our small sample, one should be careful in extrapolating these results to the entire population of PD patients. Second, factors such as long-term effects of medication and disease duration were not taken into account. Third, cognitive functioning was tested in the ON phase, whereas resting-state data came from the OFF phase. A stronger link might be found if neuropsychological testing would occur in the same medication state as the resting-state scan. Fourth, one should take into account that dynamic connectivity has been introduced quite recently, and its biological correlates are not yet firmly established. Additionally, we report only a correlation between dynamics and cognition
in this study. Longitudinal studies may elucidate whether a decrease of dFC precedes cognitive deterioration in PD or vice versa. Animal and intervention studies, such as pharmacological and brain stimulation studies, could provide evidence for a causal link between brain dynamics and cognitive functions, and determine its exact implications.

CONCLUSION

This cross-sectional study reports dFC as a neural correlate of cognitive functioning in PD: dFC of the DMN with the rest of the brain was associated with better visuospatial memory functioning. This association was not found when motor symptoms or pain were considered, which suggests that dFC of the DMN may be specifically linked to cognitive functioning. This study adds to the understanding of which factors possibly contribute to cognitive functioning in PD.
Chapter 7

Summary

CHAPTER 7
SUMMARY
SUMMARY OF FINDINGS

The main aim of this thesis was to provide more insight into pain and cognition as non-motor symptoms of Parkinson’s disease (PD). This was achieved by studying pain and cognitive functioning of PD patients in clinical practice in (Chapter 2 & Chapter 3), investigating non-motor symptom interactions in (Chapter 4), and exploring underlying neural mechanisms of pain and cognition (Chapter 5 & Chapter 6). A short summary per chapter is provided in this chapter.

PART I: CLINICAL STUDIES

CHAPTER 2: PAIN IS RECOGNIZED AS A SYMPTOM IN PD PATIENTS

Chapter 2 addressed clinical pain assessment in PD. We were interested in how clinician’s pain assessments correspond with the pain perception of the patient. Additionally, we aimed to compare clinical evaluations of patients’ pain between neurologists. Fifty-seven patients filled out questionnaires including pain-related questions, and their medical statuses were checked for pain-related information. In addition, 23 PD patients with pain underwent pain evaluation by three independent neurologists. First, we found no significant level of agreement on pain between the patient and the clinician, yet patients did receive appropriate analgesics, albeit according to the clinician’s evaluation rather than to the patient’s evaluation. Second, interrater agreement between three neurologists was strongest for the item on pain related to response fluctuation, agreement on other types of pain was lower.

CHAPTER 3: THERE IS NO DIRECT ASSOCIATION BETWEEN COGNITION AND PAIN

In Chapter 3, we hypothesized an association between clinical pain and neuropsychological functioning, based on the overlap in brain areas involved in both pain and cognition. We tested neuropsychological functioning of 48 patients with PD and 57 healthy controls, and both the affective as well as the sensory aspect of pain were assessed. PD patients experienced more pain than controls, particularly regarding the affective aspect of pain. Linear regression analyses showed that cognition was not related to pain. Instead, clinical pain was primarily related to symptoms of depression.
and anxiety. The difference between the affective and the sensory aspect of pain might be due to the neuropathology of PD, which is mainly present in areas processing the affective aspect of pain (e.g. the insula). This study underlines that symptoms of depression and anxiety should be considered when pain is treated.

CHAPTER 4: INVESTIGATING NON-MOTOR SYMPTOMS AS A NETWORK PROVIDES NEW INSIGHT INTO SYMPTOM-INTERACTIONS

When looking at how non-motor symptoms (NMS) impact daily life, most studies investigate NMS by assessing the contribution of individual symptoms to quality of life (QoL). However, symptoms could also have an interactive relationship. This interaction might be overlooked when only studying these individual contributions. The aim of Chapter 4 was, first, to assess how NMS impact quality of life, and, second, to investigate NMS by using a network approach. This approach treats symptoms as nodes and associations between symptoms as edges in a network, providing the opportunity to investigate the dimensional spectrum of NMS. Seventy PD patients completed questionnaires on NMS and QoL. The non-motor symptom domains Mood and Pain are significant contributors to QoL. The network analysis suggests that Mood and Sleep play central roles in the NMS-network, and that Mood and Cognition are strongly related. Because of power issues, the generalizability of our results is limited. However, complementary information from the network analysis does suggest that focusing on sleep problems could potentially ameliorate both mood and pain symptoms. Future studies should focus on investigating the presence of causal relationships.

PART II: NEUROIMAGING STUDIES

CHAPTER 5: PAIN IN PD IS RELATED TO THE ARCHITECTURE OF FUNCTIONAL NEURAL CONNECTIONS

Research has shown that functional connectivity during the resting-state is related to persistent pain in PD. Besides measuring the strength of connections, functional topology of the brain (i.e. the architecture of functional neural connections) can also be assessed. Functional topology has been implicated as a correlate of human behavior. In Chapter 5, we aimed to (1) assess the differences between PD patients and controls with respect to pain and resting-state network topology, and (2) investigate how resting-
state network topology is associated with clinical pain in both PD patients and controls. Resting-state activity of 24 PD patients (both on and off medication) and 27 controls was assessed with fMRI. We investigated pain with the colored analogue scale and the McGill pain questionnaire. We calculated the following measures of functional network topology: betweenness centrality (BC) was used to assess hubness, and global efficiency (GE) was used to assess integration of the network. PD patients experienced more pain than controls. GE of the whole brain was higher in PD patients (on as well as off medication) compared to healthy controls. GE of the specialized pain network was also higher in PD patients compared to controls, but only when patients were on dopaminergic medication. BC of the pain network was lower in PD patients off medication compared to controls. We found a positive association between pain and GE of the pain network in PD patients off medication. For healthy controls, a negative association was found between pain and GE of the pain network, as well as between pain and BC of the pain network. Our results suggest that functional network topology differs between PD patients and healthy controls, and that this topology can be used to investigate the underlying neural mechanisms of pain symptoms in PD.

CHAPTER 6: COGNITION IN PD IS RELATED TO THE DYNAMICS OF FUNCTIONAL CONNECTIONS

It has previously been observed that dynamic functional connectivity (dFC) in PD is associated with better attention performance and with motor symptom severity. In Chapter 6, we aimed to investigate dFC of both the default mode network (DMN) and the frontoparietal network (FPN) as neural correlates of cognitive functioning in patients with PD. Additionally, we investigated pain and motor problems as symptoms of PD in relation to dFC. We used the same data as for Chapter 5. Memory and executive functioning were assessed with neuropsychological tests. Pain was assessed with the numeric rating scale; motor symptom severity was assessed with the Unified Parkinson’s Disease Rating Scale. Resting-state dFC was defined by calculating the variability of functional connectivity over a number of sliding windows within each scan. dFC of both the DMN and FPN with the rest of the brain was calculated. Patients performed worse on tests of visuospatial memory, verbal memory and working memory. No difference existed between groups regarding dFC of the DMN nor the FPN with the rest of the
brain. A positive correlation existed between dFC of the DMN and visuospatial memory. Our results suggest that higher dynamics of the DMN with the rest of the brain are beneficial in PD patients, particularly for visuospatial memory. Furthermore, we suggest that brain dynamics of the DMN, as measured with dFC, could be a phenomenon specifically linked to cognitive functioning in PD, but not to other symptoms.
GENERAL DISCUSSION

THE INTERACTION BETWEEN ALL NON-MOTOR SYMPTOMS OF PD SHOULD BE CONSIDERED IN CLINICAL PRACTICE

Part I of this thesis concerned the clinical manifestation of non-motor symptoms in Parkinson’s disease (PD). Pain as a symptom in PD patients seems to be recognized in clinical practice, as was shown in Chapter 2 of this thesis. Underrecognition of pain in PD, as proposed a decade ago (e.g. by Nègre-Pàges and colleagues (2008)) has possibly benefitted from the plethora of investigations into pain as a non-motor symptom in PD. Here, we suggest that this increased awareness should now be applied to any meaningful interactions that non-motor symptoms have on each other: clinicians should look beyond the symptom in question with the help of symptom-networks, for example by noticing to which other symptoms it is connected. For example, the proposed association between pain and cognition, although not found significant in Chapter 3, could be linked by a third symptom. We suggest that more studies should more firmly establish this symptom network. As indicated in Chapter 4, causality of the symptom-network has not yet been established. Indeed, the main conclusion of Chapter 4 is that we can derive a network structure of correlated symptoms from the data. Treatment of symptoms represented in one of the central nodes might therefore as a consequence not necessarily improve the nodes to which it is connected, but could be used to raise awareness for symptom interactions. The use of network analysis for symptoms in mental disorders has only recently gained scientific interest, and applying this network analysis to symptoms of PD has, to our knowledge, not at all been performed to date. Yet, the fact that symptoms co-occur and could even exacerbate each other could, as a first step, create more awareness for symptom interactions in clinical practice.

HETEROGENEITY IN THE DATA

PD is a disease with a clinical syndrome and consists of many different symptoms, but not all of those symptoms are necessarily present in all patients. In our studies, we statistically considered all patients as equal and thus did not apply subtyping. Besides the more well-known postural instability and gait disturbance (PIGD) and tremor dominant (TD) motor subtypes of PD, subtyping based on non-motor symptoms has also shown...
promising results (Sauerbier et al., 2016). Heterogeneity in our data caused by subgroups could potentially have affected our results. For example, when focusing on cognition, two subtypes have been found in PD patients: a subtype characterized by problems in dopaminergic, fronto-striatal and executive functioning in which patients exhibit MCI, and a dementia subtype, characterized by problems in posterior and temporal functioning, where patients experience visuospatial dysfunctioning, the latter of which is better aided with cholinergic medication (Kehagia et al., 2012; Williams-Gray et al., 2009). Although executive functioning and memory were separately assessed in Chapter 3, the underlying pathophysiology of these cognitive subtypes (i.e. primarily dopamine vs. choline system) might also differently affect pain processing. As a result, we might have missed a significant pain-cognition interaction, for example because it of its presence in only one subtype.

**CHANGES IN NETWORK PROPERTIES IN EARLIER STUDIES**

In line with an earlier study (van Geest et al., 2018a) that investigated brain dynamics in patients with multiple sclerosis (MS), we found a relationship between brain dynamics and cognition, but no difference between groups regarding dynamic functional connectivity of the DMN, nor the FPN (Chapter 6). To the best of our knowledge, dFC of the FPN and its relationship specifically with executive functioning has not been investigated before in PD. A hypoconnectivity of static FC of the FPN has been found before, specifically for PD patients with motor subtype PIGD (Vervoort et al., 2016). PD patients with this motor phenotype also have a higher risk of cognitive decline, especially in the executive domain (Arie et al., 2017). In Chapter 6, we did not distinguish between different motor-phenotypes, but this could be an interesting direction to take. Besides a possible group difference, the hypothesized relationship between dFC of the FPN and executive functioning might be more pronounced in the PIGD-subgroup.

Several studies indicate that static functional connectivity of the DMN is actually affected already in the early stages of PD (Baggio et al., 2015; Mohan et al., 2016). Yet, we found no difference between PD and controls regarding network topology based on static FC of the DMN (Chapter 5). For one, this could indicate that, although the strength of connections changes, the architecture that they make up does not. However, it might also be related to other factors: Baggio and colleagues only found an effect on
static FC of the DMN after they divided their patient group into MCI vs. non-MCI (Baggio et al., 2015). This was similar in Chapter 6 regarding *dynamic* FC: no difference was found when all patients were compared to healthy controls, but after dividing the PD group into MCI vs. non-MCI, higher dFC of the DMN was found for PD patients without MCI. Interestingly, the direction of the association was opposite to the study of Baggio and colleagues. This suggests that static FC in the DMN, which was increased in PD patients with MCI and negatively associated with outcomes on a visuospatial task.
(Baggio et al., 2015), might have a different function than dynamic FC in the DMN, which was decreased in PD patients with MCI and positively associated with outcomes on a visuospatial memory task (Chapter 6).

The decrease in whole-brain network efficiency that has been found in other topology studies of PD was not corroborated by our data as described in Chapter 5. We suggest that these inconsistent findings might be due to the mechanism of hub-overload, as proposed by Stam (2014). In short, this theory proposes that overloaded hubs in a network shift their load to other hubs, which in time become overloaded as well. This shift does not necessarily have to lead to, for example, a longer path length (which is used to calculate global efficiency), if the hubs that receive the extra load are ‘on the same level’ as the ones offloading (see Figure 8.1). In other words, a change in connectivity does not necessarily lead to a change in topology measures. When compared to the MEG-study of Olde Dubbelink and colleagues, who found a decrease in global efficiency, our patients had a relatively high levodopa equivalent dose (Olde Dubbelink et al., 2014b). Since dopamine has been found to affect hub-properties in PD (Koshimori et al., 2016), hub organization in our sample could have been disrupted in such a way that efficiency measures were still similar.

**TOPOLOGY OF THE NETWORK UNDERLIES PAIN IN PD**

Global efficiency of the pain network was negatively related to patients’ pain (Chapter 5). This suggests that a less efficient network with longer pathlengths, coincides with more pain in PD patients. Intuitively, a higher efficiency appears advantageous: the network needs fewer steps to convey information, and so spread is more economical. However, the exact meaning of global efficiency with respect to clinical outcome is less clear: when a signal can easily spread throughout the network, this could either be healthy or unhealthy. Let us consider the analogy of communication networks with high global efficiency. Positive phenomena (such as innovations, ideas) as well as negative phenomena (such as rumors) both spread easily in such a network (Barabási, 2015). In our PD data, we assume that correlated timeseries indicate communication (a positive phenomenon), but the content of the message that is conveyed is unknown. In addition, it is unclear at what moment pain becomes pathological or disadvantageous (i.e. when an innovation becomes a rumor).
The concept of temporal fluctuations in brain connectivity is a relatively new concept, and arises from the notion that the brain must constantly update itself and respond to input, making it a dynamic rather than a static organ. Assuming an averaged resting-state functional connectivity measure over the duration of the whole scan to be a full representation of brain connectivity is therefore unlikely (Sizemore and Bassett, 2017). Yet, the question is how to fully capture the dynamics of the brain. Using the standard deviation over a number of sliding-windows, as was performed in Chapter 6, is a simple and straightforward method to achieve a measure of dynamics, and has already shown clinically relevant results in other studies (Douw et al., 2016). An alternative way to achieve insight into how the brain temporally behaves is by investigating configurations over sliding windows (Kim et al., 2017; Madhyastha et al., 2015b) or the dynamics of topology (Yu et al., 2015). Yet, none these methods might fully apprehend the complexity of brain dynamics. In general, the method of sliding windows might only approximate the concept of dynamics, instead of fully grasping it. To use another analogy, it could be compared to the suggestion of movement when viewing a stop-motion. Still, the fact that these measures are regularly being associated with clinical and behavioral measures validates the use of sliding windows to approach brain dynamics.

We found specifically that the dynamics of the DMN during rest are positively related to performance on a visuospatial memory task. Having highly flexible connections might aid in several steps along the way towards successful encoding, consolidation, storage and retrieval. Future studies could focus on how exactly these processes are related to brain dynamics. In addition, it has been suggested that the relative difference between resting-state and task-state dynamics are also related to cognition (Douw et al., 2016; van Geest et al., 2018a). Taking the task vs. rest difference into account might provide even more insight into memory functioning and brain dynamics of the DMN in PD.

In addition to the well-researched rich club (van den Heuvel and Sporns, 2011), the concept of diverse club nodes has recently been introduced (Bertolero et al., 2017). Nodes that belong to the diverse club have high participation coefficient, indicating that they are involved in many different networks. Diverse club nodes are at the topological center of the network. The FPN, being a cognitive control network, has been indicated as such a diverse club network. This network might be prone to be differently engaged
dynamically with different brain areas. In other words, the FPN might show more stable connections with some, and more dynamic connections with other networks. It might be specifically these nuances that underlie cognitive functioning. As a result, averaging dynamics of the FPN with all other nodes in the brain, as was performed in Chapter 6, might result in missing these nuances. In sum, although we have only scratched the surface, investigating the dynamics of the brain in relation to cognitive functioning and cognitive decline could greatly extend our knowledge on this debilitating feature of PD.

METHODOLOGICAL CONSIDERATIONS

The studies in this thesis provide insight into pain and cognition as part of the non-motor spectrum in PD. Several limitations apply, however. Here, we will focus on general limitations and methodological considerations.

CAPTURING PAIN

Clinical pain (i.e. not experimentally induced pain) is a subjective experience and is influenced by a multitude of subjective and objective factors (e.g. sex, age, mood, psychological factors, physical causes and pain-history). Besides the physical aspect of pain (the actual or potential damage to the body), there are major emotional and cognitive components of pain that are responsible for a substantial part of the subjective nature of pain (Melzack and Casey, 1968). Capturing the ‘amount of pain’ in one or two single numbers is thus a delicate matter, and is prone to be influenced by the state of the subject as well as by the method of inquiry. For example, pain was operationalized as a dichotomous variable in chapter 2. Even though this facilitated comparability of pain estimates between patients and clinicians, it cannot capture patients’ whole pain experience. The McGill pain questionnaire, by which a wider range of pain aspects can be obtained, is a more comprehensive method (Chapters 3 & Chapter 5), but this questionnaire requires subjects to have sufficient cognitive capacity to understand and adequately answer its items. Although the instruments used in this thesis have been validated and are often used in research or in clinical practice (e.g. the McGill pain questionnaire, colored analogue scale and numeric rating scale), the complexity of the concept of pain itself and how we measure it should continuously be discussed.
CAPTURING CLINICAL PAIN IN A NEURAL NETWORK

We made use of a pain network in Chapter 5. There are two main concerns about this pain network: first, it is based on a study that investigated small groups of healthy young volunteers, who received acute pain stimuli in order to derive a neural pain signature (Wager et al., 2013). Our population is that of an older group of patients with a neurodegenerative disease, who often experience chronic pain as a symptom. The underlying pain network might, as a consequence, not be completely similar, as was suggested in the discussion of Chapter 3. Second, we chose to include only those brain areas of the neural pain signature that had positive predictive value for physical pain to increase interpretability of results. We propose that future studies add the areas with a negative predictive weight to the pain network, in order to investigate any additional information coming from these areas with a negative predictive weight. In short, the pain network as defined in Chapter 5 might be incomplete.

GENERALIZING RESULTS TO THE POPULATION

Our patients either had a relatively short disease duration (Chapters 5 & Chapter 6), or had relatively intact cognitive functioning (chapter 3), both resulting in limited variance of cognitive outcome. The results from our studies can therefore not be extrapolated to the whole population of PD patients, but might only apply to those with relatively intact cognition (i.e. not to those with severe dementia). As a note, measuring pain is already a delicate matter in cognitively fit people, thus measuring pain in a cognitively severely impaired population is even more challenging, yet would be a clinically relevant next step.

THE DISADVANTAGE OF CROSS-SECTIONAL STUDIES AND SMALL SAMPLE SIZES

A major limitation inherent of cross-sectional studies is that no inferences can be made about causality: we can only conclude anything about co-occurrence at the time of measurement. Also, even though collectively more than 150 patients participated in the studies described in this thesis, the individual studies would have benefitted from larger sample sizes. This would have increased statistical power, allowing for adding covariates
such as the effects of age, education, or comorbidities. For chapter 4, this lack of power results in a less stable network of non-motor symptoms.

**STUDYING FUNCTIONAL NETWORKS WITH FMRI RESTING-STATE DATA**

Studying functional networks of the human resting-state is inextricably bound to methodological decisions, all of which influence results to some extent. First, we used the averaged timeseries from each atlas-region-of-interest (ROI) as nodes in subsequent network analyses. Several other methods have been proposed to extract ROI-values, such as using a peak voxel, median value, or a cluster of voxels around the ROI peak (Tong et al., 2016). Differences between measures are subtle, yet could have influenced our results (Tong et al., 2016). Furthermore, the adjacency matrix on which our connectivity analyses are based only contain information about strength between nodes, and not about direction of information flow, i.e. networks were weighted, but undirected. Adding information about direction can be achieved by calculating effective connectivity and could provide additional insights regarding both pain and cognition (Friston, 2011).

**COMMUNICABILITY OF THE NETWORK**

Correlation between pairs of nodes forms the basis for connectivity, i.e. assumed neural communication. The network measures that were calculated and used as estimates of network topology (i.e. global efficiency and betweenness centrality, Chapter 5) both make use of information on shortest paths between node-pairs. This means that we assume that neural communication between pairs of nodes generally only occurs through these shortest paths. However, indirect routes might hold additional information as well. Take the binary network in Figure 8.2, for example: node A and node D are connected with each other through their direct edge I. However, route III (A-B-D) and route II (A-C-B-D) might also be essential for proper communication within this module (Estrada et al., 2012; Rubinov and Sporns, 2010). Communicability, an estimate that includes all paths between two nodes and assigns higher weights to the shorter paths, could provide more specific routes of information spread (Estrada et al., 2012; Lella et al., 2018). In a model where spreading dynamics were investigated, shortest paths were only partially correlated with spread, indicating that alternative routes could be important for optimal
network functioning (Mišić et al., 2015). Taking communicability into account for weighted networks is more ambiguous but could be implemented as well.

![Figure 8.2. Four-node network with functional connections (undirected). For clarity of the figure, the network is kept in binary form. See text for details.](image)

**NEGATIVE EDGES**

The adjacency matrix, which is used for connectivity analyses, is calculated with absolute values of correlation coefficients. As a consequence, negative values become positive, and thus nodes that show a strong anti-correlation become strongly correlated in our analyses. In other words, we assume that strongly correlated signals have the same biological meaning (i.e. they are functionally connected) as strongly anti-correlated signals. However, negative edges have been implicated as having another biological implication than positive edges: the latter are more present between cortical areas, the former are more often observed between cortical and non-cortical areas (Goelman et al., 2014). Regarding all correlations as positive edges could potentially have affected the topology measures that we investigated: negative edges are strongly associated with shortest pathlength (Chen et al., 2011).
SUGGESTIONS FOR FUTURE STUDIES

1. CAREFUL SELECTION OF PATIENTS AND VARIABLES
For patients’ pain experience in clinical practice, it is imperative to take other symptoms and demographic factors (e.g. age, sex) into account. This seems like stating the obvious. Yet in addition, also the less obvious factors can be found and thus considered in the analyses by making use of a network approach. Many variables can be considered, but I would like to emphasize the central role that ‘mood’ (i.e. symptoms of anxiety and depression) plays in PD. Mood was found to be a central node and significant correlate to pain in our analyses, and also arises time and again as a major player in many other Parkinson studies (Ehrt et al., 2009; Fil et al., 2013). The choice of nodes is a crucial step when using networks to investigate symptoms, since this defines how the network looks (i.e. using individual questions or aggregates, choice of instruments, etc.). Nodes should thus be chosen carefully.

2. INVESTIGATING PAIN, COGNITION AND OTHER NON-MOTOR SYMPTOMS
The link between pain and cognition should be investigated further, because of its implications for patients with longer disease duration: when communicating in general becomes more troublesome because of cognitive problems, one might also not be able to communicate their pain properly and will thus risk pain undertreatment. Future studies could address this point using network science for symptom-interactions: longitudinal studies can provide insight into how symptom-networks change over time, and how the (indirect) relationship between pain and cognition changes over time, clearing the way for testing the causal hypothesis of symptom-networks by manipulation of symptoms and investigating its effects (Fried and Cramer, 2017). Future studies should also consider subgrouping the PD population. A first step could be to subgroup according to motor subtype, yet (in the light of this thesis’ subject) non-motor subgrouping would be clinically interesting as well.

3. FUTURE IMAGING STUDIES
The main suggestion for future imaging studies would be to further unravel the dynamics of functional connectivity, especially regarding cognitive functioning.
Subdividing existing resting-state networks such as the DMN and FPN to disentangle subtle effects of within- as well as between-network dynamics could also aid in this respect. In addition, one could investigate possible differential dynamic connectivity between the FPN with subnetworks, taking in mind the recently proposed diverse club, of which the FPN is one. With respect to the topology of networks and pain in PD, broadening the pain network by including the areas with negative predictive weight could potentially shed more light on the complex interactions within and between brain networks in pain. In addition, areas that do not fall in the traditional pain network might also be considered, especially because of the opposite associations that we found for HC and PD. One could also consider exploring the use of communicability as a measure of integration of the network.
NEDERLANDSE SAMENVATTING

Het hoofddoel van dit proefschrift was het bieden van inzicht in pijn en cognitie als non-motorische symptomen van de ziekte van Parkinson (ZvP). Dit werd bereikt door pijn en cognitief functioneren van patiënten met de ZvP te onderzoeken in een klinische setting (Hoofdstuk 2 & Hoofdstuk 3), door non-motorische symptoom interacties middels een netwerk analyse te onderzoeken (Hoofdstuk 4), en door mogelijke onderliggende neurale mechanismen van pijn en cognitie te onderzoeken (Hoofdstuk 5 & Hoofdstuk 6). In dit gedeelte volgt een korte samenvatting in het Nederlands.

DEEL I: KLINISCHE STUDIES

HOOFDSTUK 2: PIJN WORDT HERKENN ALS SYMPTOOM BIJ PARKINSON

De klinische beoordeling van pijn bij de ZvP werd behandeld in Hoofdstuk 2. Voor dit hoofdstuk waren wij vooral geïnteresseerd in hoe de beoordeling van pijn door een clinicus overeen zou komen met de beoordeling van pijn door de patiënt zelf. Daarnaast was het doel om de interbeoordelaarsbetrouwbaarheid van pijnevaluaties door neurologen te onderzoeken. Zevenenvijftig patiënten vulden vragenlijsten in waaronder pijn-gerelateerde vragen, en hun medische status werd gecontroleerd op pijn gerelateerde informatie. Daarnaast ondergingen 23 Parkinsonpatiënten met pijn een pijnevaluatie door drie onafhankelijke neurologen. Ten eerste vonden we geen significant niveau van overeenstemming op pijnbeoordeling tussen de patiënt en de clinicus. We zagen echter wel dat patiënten passende pijnstilling ontvingen, al namen mensen die pijnstilling naar de evaluatie van pijn volgens de clinicus, en niet die van patiënt zelf. Ten tweede vonden we dat de interbeoordelaarsbetrouwbaarheid tussen de drie neurologen het sterkste was voor het item over pijn gerelateerd aan respons-fluctuaties, overeenstemming op alle andere types pijn was lager.

HOOFDSTUK 3: ER IS GEEN DIRECTE ASSOCIATIE TUSSEN COGNITIE EN PIJN

In Hoofdstuk 3 onderzochten we een mogelijke associatie tussen cognitief functioneren en pijn, gebaseerd op de overlap in hersengebieden die bij beide functies betrokken zijn.
Het cognitief functioneren en de pijnbeleving van 48 patiënten met de ZvP en 57 gezonde controles werd getest en uitgevraagd. Zowel het affectieve als het sensorische aspect van pijn werd beoordeeld. De resultaten lieten zien dat de Parkinsonpatiënten meer pijn ervoeren dan de controlegroep, zeker wat betreft het affectieve aspect van pijn. Lineaire regressieanalyses lieten zien dat cognitief functioneren niet gerelateerd was aan pijn, maar dat pijn voornamelijk aan symptomen van angst en depressie was gerelateerd. Het verschil tussen het affectieve en het sensorisch aspect van pijn zou toe te schrijven kunnen zijn aan de neuropathologie van de ZvP, welke in het algemeen gezien het meest te vinden is in hersengebieden die het affectieve aspect van pijn verwerken (bijvoorbeeld de insula). Dit hoofdstuk onderstreept de noodzaak tot het betrekken van angst- en depressiesymptomen in de behandeling van en in het onderzoek naar pijn bij de ZvP.

HOOFDSTUK 4: NETWERK-ANALYSE VAN NON-MOTORISCHE SYMPTOMEN VERSCHAFT INZICHT IN SYMPTOOM-INTERACTIES

Bij onderzoek naar de impact van non-motorische symptomen (NMS) van de ZvP op het leven van patiënten, bekijken de meeste studies hoe individuele symptomen bijdragen aan de kwaliteit van leven. Symptomen kunnen echter ook invloed op elkaar uitoefenen. Deze potentiële interacties zouden over het hoofd kunnen worden gezien wanneer alleen naar die individuele contributie wordt gekeken. Het doel van Hoofdstuk 4 was ten eerste om te onderzoeken hoe NMS individueel verbonden zijn met kwaliteit van leven, en ten tweede om de symptoom-interacties te onderzoeken middels een netwerk-analyse. Netwerken bestaan uit nodes en de verbindingen ertussen (edges). Bij symptoom-netwerken zijn de symptomen de nodes, en de statistische associaties tussen de symptomen edges van het netwerk. Op deze manier kan een dimensioneel spectrum (zoals dat van NMS) worden onderzocht. Zeventig Parkinsonpatiënten vulden vragenlijsten aangaande NMS en kwaliteit van leven in. De NMS domeinen Stemming en Pijn waren significante voorspellers voor kwaliteit van leven. De netwerkanalyse liet zien dat zowel Stemming als Slaap een centrale rol speelden in het NMS-netwerk, en dat Stemming en Cognitie sterk met elkaar verbonden waren. Vanwege power-problemen is de generaliseerbaarheid van deze resultaten beperkt. Desalniettemin doet de complementaire informatie uit de netwerkanalyse vermoeden dat stemmingsproblemen
DEEL II: NEUROIMAGING STUDIES

HOOFDSTUK 5: PIJN BIJ PARKINSON IS GERELATEERD AAN DE ARCHITECTUUR VAN FUNCTIONELE NEURALE VERBINDINGEN

Onderzoek heeft aangetoond dat functionele connectiviteit tijdens rust is gecorreleerd met pijn bij de ZvP. Naast het onderzoeken van hoe sterk hersengebieden met elkaar verbonden zijn kan er ook naar de topologie (de structuur van functionele neurale verbindingen) van de hersenen worden gekeken. Functionele topologie is al vaker genoemd als een correlaat van menselijk gedrag. Het doel van Hoofdstuk 5 was ten eerste om te onderzoeken of er verschillen bestaan tussen Parkinsonpatiënten en controles wat betreft pijn en wat betreft topologie van hersennetwerken tijdens rust, en ten tweede om te onderzoeken of de topologie hersennetwerken tijdens rust geassocieerd kan worden met klinische pijn bij Parkinsonpatiënten en bij gezonde controles. Rust-activiteit van 24 Parkinsonpatiënten (waarbij zij éénmaal met en éénmaal zonder medicatie gescand werden, dus zowel ON als OFF) en 27 controles werd gemeten met fMRI. Pijn werd onderzocht middels de colored analogue scale en de McGill pijnvragenlijst. De volgende maten van functionele netwerk topologie zijn berekend: betweenness centrality (BC) diende als maat voor hubness van hersengebieden en global efficiency (GE) diende als maat voor integratie van het gehele netwerk. Deze twee maten werden berekend voor een specifiek pijnnetwerk en voor het default mode network (DMN). Voor het whole brain netwerk werd alleen GE berekend. De resultaten lieten zien dat Parkinsonpatiënten meer pijn ervoeren dan controles. GE (whole brain) was hoger bij Parkinsonpatiënten (zowel ON als OFF) vergeleken met controles. GE van het specifieke pijnnetwerk was ook hoger voor Parkinsonpatiënten vergeleken met controles, maar alleen tijdens ON. BC van het pijnnetwerk was lager in Parkinsonpatiënten (OFF). We vonden een positieve relatie tussen pijn en GE van het pijnnetwerk in Parkinsonpatiënten (OFF), en een negatieve relatie tussen pijn en zowel GE als BC van het pijnnetwerk in gezonde controles. Onze resultaten laten zien dat functionele netwerk topologie verschilt tussen Parkinsonpatiënten en gezonde controles en dat deze topologie gebruikt kan worden om onderliggende neurale mechanismen van pijnsymptomen in Parkinson bloot te leggen.
HOOFDSTUK 6: COGNITIE BIJ PARKINSON IS GERELATEERD AAN DE DYNAMICA VAN FUNCTIONELE NEURALE VERBINDINGEN

Naast stationaire, of statische, functionele connectiviteit, kan ook de variabiliteit van functionele connecties onderzocht worden. Uit de literatuur blijkt dat deze dynamische functionele connectiviteit (dFC) correleert met de prestatie op een aandachtstaak bij Parkinsonpatiënten, en met de ernst van hun motorische symptomen. Het doel van Hoofdstuk 6 was om dFC van zowel het DMN als het frontoparietal network (FPN) te onderzoeken als neurale correlaten van cognitief functioneren bij Parkinsonpatiënten. Daarnaast wilden we pijn en motorische problemen als symptomen van Parkinson relateren aan dFC. Hiervoor maakten we gebruik van dezelfde data als gebruikt in Hoofdstuk 5. Geheugen en executief functioneren werden getest met neuropsychologische tests. Pijn werd uitgevraagd middels de numeric rating scale, ernst van motorische problemen werd beoordeeld met de Unified Parkinson’s Disease Rating Scale. dFC van de activiteit tijdens rust werd bepaald door de variabiliteit van functionele connectiviteit over een aantal sliding windows te berekenen. dFC van zowel het DMN als het FPN met de rest van de hersenen werd berekend. Patiënten presteerden slechter op tests van het visuospatieel geheugen, het verbaal geheugen en het werkgeheugen. Er werd geen verschil gevonden tussen de groepen op dFC van het DMN, noch het FPN met de rest van de hersenen. Voor de groep patiënten werd een positieve correlatie gevonden tussen dFC van het DMN met de score op de test voor het visuospatieel geheugen. Onze resultaten laten zien dat hogere variabiliteit van connectiviteit van het DMN met de rest van de hersenen gunstig zou kunnen zijn voor visuospatieel geheugen. Daarnaast willen wij voorstellen dat dynamica van het DMN gelinkt kan worden aan cognitief functioneren in Parkinsonpatiënten, maar niet aan motorische of pijn-symptomen.

SUGGESTIES VOOR VERVOLGONDERZOEK

1. NAUWKERGRIGE PATIËNT- EN VARIABELE SELECTIE

Het is van groot belang om naast de pijnbeleving van Parkinsonpatiënten ook andere symptomen en demografische factoren (zoals geslacht en leeftijd) mee te nemen. Dit lijkt een open deur, maar door zoveel mogelijk symptomen in bijvoorbeeld een symptoomnetwerk analyse mee te nemen kunnen ook de minder voor de hand liggende symptomen
blikt gelegd worden. Hoewel er vele variabelen in ogenschouw genomen kunnen worden, zou ik hier graag de centrale rol willen benadrukken die ‘Stemming’ speelt in Parkinson (dat wil zeggen symptomen van angst- en depressie). Stemming was een centrale node in het symptoom netwerk van Hoofdstuk 4, een significant correlaat van pijn in onze analyses, en komt keer op keer boven als een belangrijke factor in verschillende Parkinsonstudies (Ehrt et al., 2009; Fil et al., 2013). De keuze van nodes in het netwerk is een cruciale stap bij het gebruik van netwerken om symptomen te onderzoeken, gezien deze mede bepalen hoe het netwerk eruitziet. Zo kan men bijvoorbeeld denken aan de keuze tussen ofwel een aggregaat van verschillende vragen als meer robuuste nodes (zoals verricht is in Hoofdstuk 4), ofwel specifieke sensitievere nodes in de vorm van losse vragen. Zorgvuldigheid is geboden.

2. ONDERZOEKEN VAN PIJN, COGNITIE EN ANDERE NON-MOTORISCHE SYMPTOMEN

De link tussen pijn en cognitie dient verder onderzocht te worden, temeer vanwege de consequenties voor patiënten die al in een vergevorderd stadium van de ziekte zitten: wanneer communicatie in het algemeen een probleem wordt door cognitieve achteruitgang, zal ook het communiceren van pijn lastig worden. Hierdoor loopt men kans op een niet adequate behandeling van pijn. Toekomstige studies zouden dit punt aan de orde kunnen stellen door symptoom-interacties te bekijken vanuit een netwerkperspectief: longitudinale studies kunnen inzicht bieden in hoe deze symptoomnetwerken veranderen over tijd en hoe de (indirecte) associatie tussen pijn en cognitie verandert over tijd, wat plaats maakt voor het testen van een causaal verband tussen symptomen door bijvoorbeeld bepaalde symptomen te manipuleren (behandelen) en de effecten hiervan te onderzoeken (Fried and Cramer, 2017). Toekomstige studies zouden ook subgroepen kunnen onderzoeken binnen de Parkinson populatie. Een eerste stap zou kunnen zijn om subgroepen te maken op basis van motor subtype, maar in het licht van dit proefschrift zou ook een onderverdeling naar aanleiding van non-motor symptomen een interessante keuze zijn.
3. TOEKOMSTIGE IMAGING STUDIES

In eerste instantie zouden toekomstige studies die gebruik maken van beeldvormende technieken de dynamica van hersennetten verder kunnen ontrafelen. Meer specifiek zou er gekeken kunnen worden hoe die dynamica onderliggend zijn aan cognitief functioneren en disfunctioneren in Parkinson. Het onderverdelen van bestaande rustnetwerken (zoals het DMN en het FPN) teneinde de meer subtiele nuances van *within* evenals *between* effecten van netwerk dynamica zou ook kunnen helpen. Daarnaast zou men ook kunnen onderzoeken of er verschillende dynamische connectiviteit bestaat tussen het FPN en verschillende subnetwerken, als men de recent voorgestelde *rich club* meeneemt, waar de FPN een voorbeeld van is. Aangaande de netwerk-topologie en pijn in Parkinson zou men het pijnnetwerk uit kunnen breiden door ook de negatief voorspellende gewichten mee te nemen om zo complexe interacties tussen en binnen hersennetten mee in ogenschouw te kunnen nemen. Daarnaast zouden ook gebieden die niet traditioneel in het pijnnetwerk vallen meegenomen kunnen worden, zeker gezien de tegenovergestelde associaties die wij hebben gevonden voor HC & PD. Men zou ook andere maten mee kunnen nemen, zoals het gebruik van *communicability* als maat van netwerk integratie.


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DANKWOORD

Het proefschrift is af. Voor bijna alles in het leven geldt dat je dingen nooit alleen doet, en zo ook zeker niet de voltooing van een proefschrift. Een these schrijven is als het maken van een EP’tje met een aantal nummers: zodra het af is en je boven ‘het werk’ hangt, zie je de samenhang beter, en kun je hopelijk het proces een beetje op waarde schatten. Ieder nummer, elk stuk is anders. Verschillende mensen hebben er op verschillende manieren aan bijgedragen, en iedere keer veranderden de arrangementen een beetje, waardoor elk stuk net iets anders klinkt. (Ook omdat het zo geschreven is: hier rubato, daar fortissimo!) Hoewel ik voor de totstandkoming van dit proefschrift veel meer mensen dank verschuldigd ben dan het aantal leden van een gemiddelde bigband, ga ik me toch wagen aan het voortzetten van deze analogie, met wellicht een kleine uitbreiding van de bigband-bezetting. Maar, lieve mensen, het is mijn dankwoord, dus deze twee kleine pagina’s gaat niemand redigeren.

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ABOUT THE AUTHOR

Gwenda was born on March 12, 1985. She finished her bachelor’s degree in psychology at Maastricht University, but not after she tried out theater studies, media & culture, and jazz-vocals at Maastricht Conservatorium. In 2009, she went to Amsterdam to get her (research)master’s degree in neurosciences at VU University. After finishing her master thesis under supervision of Prof. Erik Scherder, Gwenda continued with Prof. Scherder as a PhD-student at the department of Clinical Neuropsychology. In the beginning of 2019, she started as a post-doc at the University of Tilburg where she investigates associative memory in healthy aging. She is a neuroscientist at brain and a musician at heart. If you really want to know about Gwenda, give her a call!
LIST OF PUBLICATIONS

(CHRONOLOGICAL)


Dissease of the spectrum of Parkinson's and non-motor non-agonic movement.