Molecular, structural, and behavioral differences between tremor dominant and non-tremor Parkinson's disease

Annelies van Nuland

DONDERS SERIES

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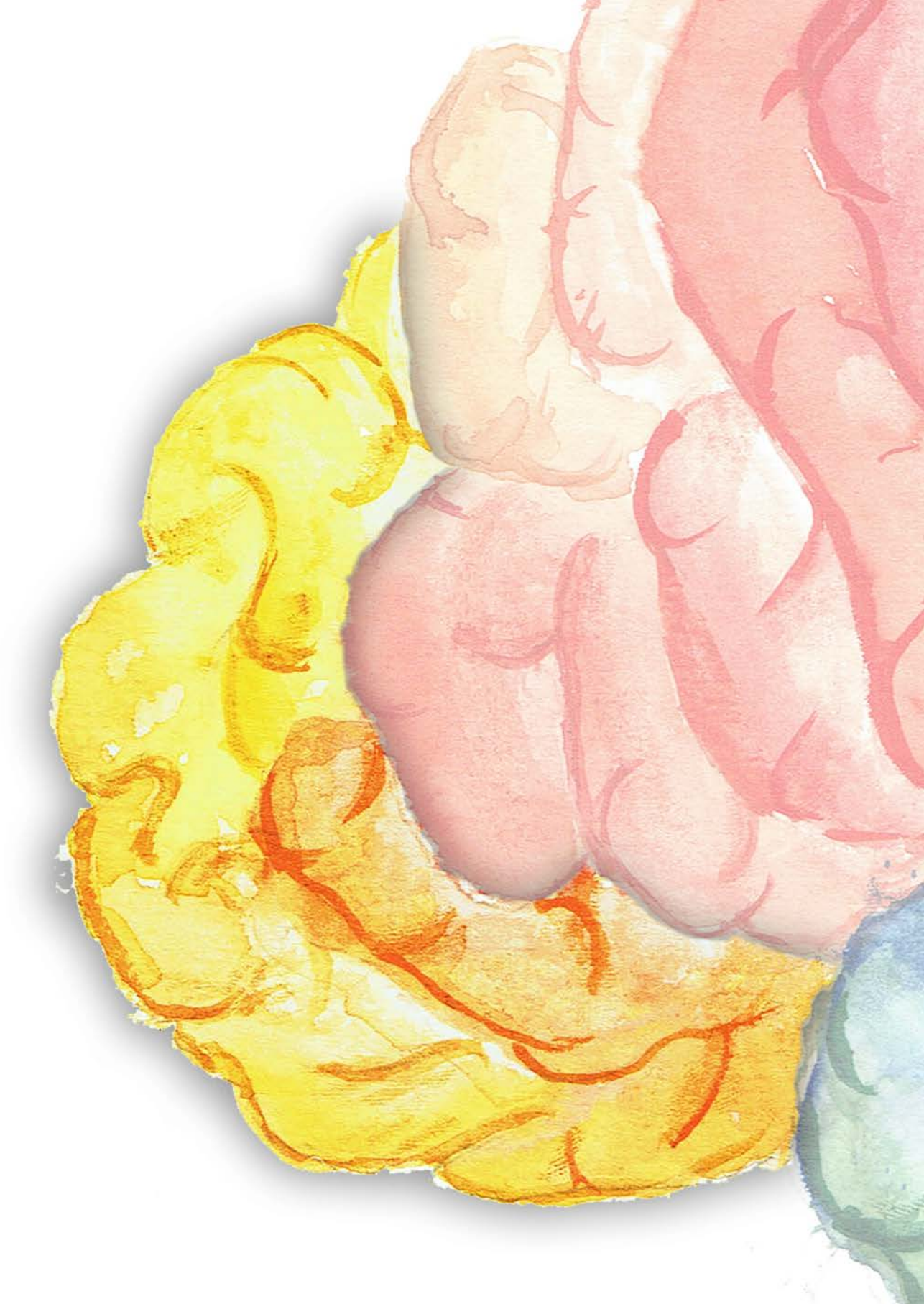

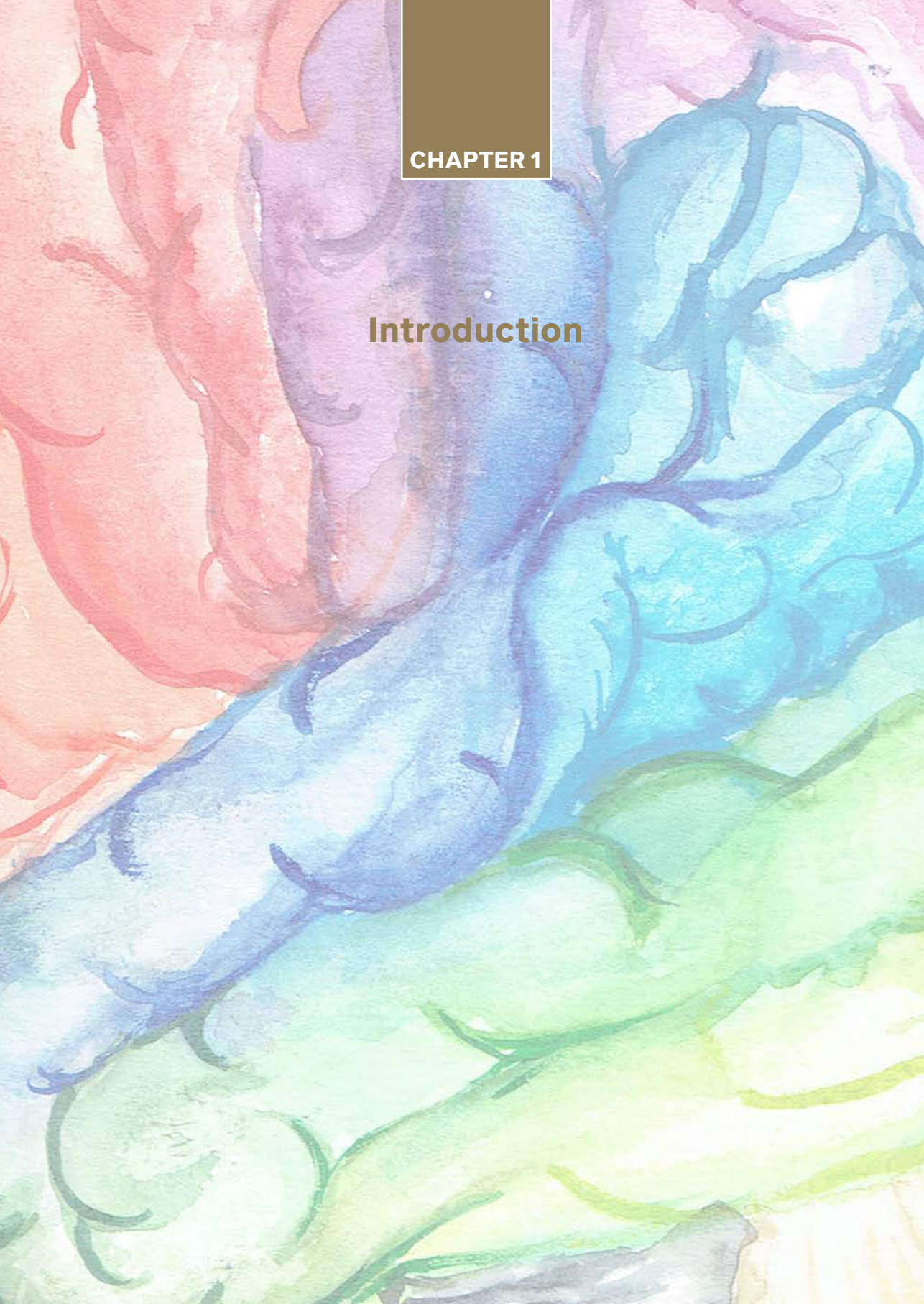

CHAPTER 1

Introduction

1.1 | Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disorder (after Alzheimer's disease), with a prevalence around 1% of the population in the population above 60 (Nussbaum and Ellis 2003). It is a movement disorder marked by bradykinesia (slowed movement), rigidity (increased muscle tone), and a typical 'pill rolling' tremor (resembling an action of trying to roll a pill or another small object between the thumb and index finger). Most early symptoms are thought to depend on progressive neurodegeneration originating in the brainstem, most prominently involving dopaminergic neurons. In general, motor symptoms are attributed to a loss of dopaminergic neurons in the substantia nigra (SN) pars compacta, which causes subsequent striatal dopamine depletion (Kish, Shannak *et al*. 1988). However, in late stages of Parkinson's disease, neurodegeneration becomes more widespread, resulting in cortical Lewy body pathology that is associated with non-motor symptoms such as dementia (Spillantini, Crowther *et al*. 1998). In general, apart from the dominant involvement of motor impairment, Parkinson's disease has substantially more widespread impact, with a variety of non-motor symptoms based on dysfunction throughout the nervous system. Very early symptoms (often preceding motor symptoms by many years) may include impaired olfaction, disordered sleep, and constipation (Abbott, Petrovitch *et al*. 2001, Khoo, Yarnall *et al*. 2013). Many patients also show cognitive and motivational dysfunction, which can cause symptoms like depression, anxiety, hallucinations and in later stages, dementia(Chaudhuri, Healy *et al*. 2006).

1.2 | Tremor-dominant and non-tremor subtypes

Parkinson's disease is a heterogeneous neurodegenerative disorder, with a diverse level of expression of its dominant symptoms. A core example is tremor: Some patients suffer from a prominent and disabling tremor, while others never develop this symptom (Hoehn and Yahr 1998). This observation has led to a subdivision of patients into two clinical phenotypes: a tremor-dominant phenotype and an a-kinetic/rigid (non-tremor) phenotype (Zetusky, Jankovic *et al*. 1985, Jankovic, McDermott *et al*. 1990, Rajput, Pahwa *et al*. 1993, Lewis, Foltynie *et al*. 2005, Burn, Rowan *et al*. 2006). Based on several clinical parameters such as symptom severity, disease onset and clinical progression, cluster analyses confirm the 'intuitive' separation into tremor and non-tremor phenotypes (Lewis, Foltynie *et al*. 2005). Clinically, tremor-dominant patients and non-tremor patients differ in a number of key aspects. Tremor-dominant patients show a slower overall disease progression, with lower annual increases in symptom severity (Selikhova, Williams *et al*. 2009), and a slower progression into the higher (>4) Hoehn and Yahr scores (Rajput, Pahwa *et al*. 1993). Tremor-dominant patients also outperform non-tremor patients on cognitive tests (Burn, Rowan *et al*. 2006,

Wu, Le *et al*. 2011) and were found to have a reduced likelihood to develop Parkinson associated dementia (Aarsland, Andersen *et al*. 2003, Williams-Gray, Foltynie *et al*. 2007). Behaviorally, we see differences in motor impulsivity between these two motor subtypes, with non-tremor patients showing higher susceptibility to motor impulses compared with tremor patients (Wylie, van den Wildenberg *et al*. 2012), and increased levels of anxiety (Dissanayaka, Sellbach *et al*. 2010). Such a dichotomy in cognitive impairment within Parkinson's disease patient subgroups could potentially relate to a different distinction made based on cognitive heterogeneity. In a 5-year follow-up cohort, (Williams-Gray, Evans *et al*. 2009) showed two distinct genetic factors in the development of cognitive dysfunction in Parkinson's disease, namely the genes for catechol-O-methyltransferase (COMT Val158Met) and microtubule-associated protein tau (MAPT) H1/H2. Both of these genes could substantially predict increases in cognitive impairment. The MAPT H1/H1 genotype was an independent predictor of dementia risk, while the COMT genotype had no effect on dementia, but a significant impact on Tower of London performance, an executive function based task thought to involve the fronto-striatal system. The same study also reported that the non-tremor phenotype (in contrast to the tremor dominant subtype) represented a notable risk factor towards increased cognitive decline. However, still unreported remained whether these two methods of patient separation might in fact be related, although suggestions in this direction were made in a later literature overview (Robbins and Cools 2014).

From a neuro-anatomical perspective, post-mortem studies show differences between tremor-dominant and non-tremor patients in the pattern of dopaminergic cell loss in the midbrain. Specifically, non-tremor patients had higher substantia nigra pars compacta (SNc) degeneration (Jellinger and Paulus 1992). The reverse was found in the dopaminergic retro-rubral area (RRA), where tremor-dominant patients had more neurodegeneration than non-tremor patients (Hirsch, Mouatt *et al*. 1992). These findings are also consistent with work in non-human primates, were injection of a dopamine specific neurotoxin caused differential patterns of neuronal damage in rhesus and vervet monkeys. While rhesus monkeys showed primarily damage to the SN, accompanied by an akinetic/rigid (non-tremor) phenotype, vervet monkeys presented with primarily damage to the RRA and a tremor dominant phenotype (Bergman, Raz *et al*. 1998, Rivlin-Etzion, Elias *et al*. 2010). In humans, there is recent work in Parkinson's disease patients comparing tremor-dominant patients to a postural instability gait difficulty (PIGD)-subgroup using a neuromelanin sensitive MRI protocol. The PIGD subtype has slightly different classification criteria compared to our non-tremor patients. However, in general it still holds a large overlap with non-tremor Parkinson's disease patients (Stebbins, Goetz *et al*. 2013). PIGD patients are not exclusively defined as a non-tremor group; however, they have predominant balance and gait symptoms, as compared to other symptoms. Clinically, these patients usually have little tremor and relatively severe bradykinesia and rigidity. They

found that PIGD patients showed a more severe decline in neuromelanin-containing neurons in the SNc than the tremor-dominant subgroup (Xiang, Gong *et al*. 2017). Neuromelanin is a waste product of the oxidative metabolism of catecholamines and is used to quantify the number of catecholaminergic neurons (Nahimi, Kinnerup *et al*. 2018). These findings match results from metabolic imaging data, where PET data shows reduced dopamine receptor density in the striatum in the tremor-dominant subtype (Spiegel *et al*., 2007; Rossi *et al*., 2010; Helmich *et al*., 2011). Overall, the evidence suggests a milder nigral pathology in tremor-dominant patients, possibly with a different spatial distribution within dopaminergic regions.

Apart from dopaminergic differences, there are signs that tremor dominant patients show lower levels of thalamic serotonin transporters than non-tremor patients (Caretti, Stoffers *et al*. 2008, Qamhawi, Towey *et al*. 2015), with serotonin levels relating to tremor severity. Furthermore, there is post-mortem evidence that non-tremor patients have more locus coeruleus degeneration than tremor-dominant Parkinson's disease (Paulus and Jellinger 1991). Together, it suggests that tremor-dominant patients show a broader neurochemical decline than their non-tremor counterparts.

1.2.1 | Parkinson's disease tremor

Tremor in Parkinson's disease has a different pathophysiology compared with most other motor symptoms. Tremor intensity does not progress at the same rate as rigidity and bradykinesia (Louis, Tang *et al*. 1999), and tremor severity does not correlate with other motor symptoms (Louis, Levy *et al*. 2001). Most prominently, tremor activity has been associated with a cerebral circuit including the motor cortex, thalamus, and cerebellum, known as the cerebello-thalamo-cortical circuit. In combined fMRI electromyography (EMG) work, we see that BOLD activity in these regions correlates with fluctuations in tremor amplitude (Helmich, Hallett *et al*. 2012, Dirkx, den Ouden *et al*. 2016, Dirkx, den Ouden *et al*. 2017). PET studies comparing cerebral blood flow in Parkinson's disease patients ON and OFF DBS also report that this cerebral network is involved in tremor (Fukuda, Barnes *et al*. 2004).

Outside of the cerebello-thalamo-cortical circuit, there is a clear role for the pallidum in tremor generation. DBS into the GPi is very effective in reducing tremor (Kumar, Lang *et al*. 2000). Electrophysiological studies have identified cells firing at tremor frequency in the pallidum (Raz, Vaadia *et al*. 2000) along with the VIM and subthalamic nucleus (STN) (Lenz, Kwan *et al*. 1994, Levy, Hutchison *et al*. 2000, Magnin, Morel *et al*. 2000). Furthermore, using functional MRI, Helmich *et al*. have shown that the GPi drives tremulous activity (Helmich, Janssen *et al*. 2011). Based on these data it was suggested that tremor is regulated through the dimmer switch model (Helmich, Hallett *et al*. 2012), in which the cerebello-thalamo-cortical circuit influences resting

tremor amplitude “analogous to a light dimmer”, while the pallidum functions as the “switch”, responsible for triggering tremor activity.

Unlike for the motor symptoms, bradykinesia and rigidity, the relationship between tremor and dopamine is unclear. The response of tremor to dopaminergic medication varies greatly between patients, with some patients showing little to no tremor reduction following dopaminergic treatment (Koller, Busenbark *et al*. 1994, Fishman 2008). Moreover, unlike other motor symptoms, tremor does not correlate with the degree of striatal dopamine depletion (Pirker 2003). However, PET/SPECT receptor binding measures have shown a link between tremor and pallidal dopamine depletion (Mounayar, Boulet *et al*. 2007, Helmich, Janssen *et al*. 2011). Indeed, dopaminergic medication was found to reduce tremor onset-related activity in the globus pallidus (Dirkx, den Ouden *et al*. 2017). Network connectivity modelling of fMRI data showed that dopaminergic medication directly (rather than indirectly) increased self-inhibition of the ventral intermediate nucleus of the thalamus, with the magnitude of thalamic self-inhibition predicting the clinical dopamine response of tremor (Dirkx, den Ouden *et al*. 2017). This suggests that Parkinson’s disease tremor results from reduced thalamic inhibition, especially dopamine-resistant tremor (Helmich 2018). In contrast, non-dopaminergic mechanisms may contribute to dopamine-resistant tremor. This hypothesis has recently been confirmed by a direct comparison of Parkinson’s disease patients with dopamine-responsive versus dopamine-resistant tremor, where both groups were measured ON and OFF dopaminergic medication. Patients with dopamine-resistant tremor had more tremor-related activity in the cerebellum (across both medication sessions) and they had less inhibition of thalamic activity (ON versus OFF dopaminergic medication) (Dirkx, Zach *et al*. 2019).

BOX 1 | CLASSIC PATHOPHYSIOLOGICAL MODEL OF THE BASAL GANGLIA

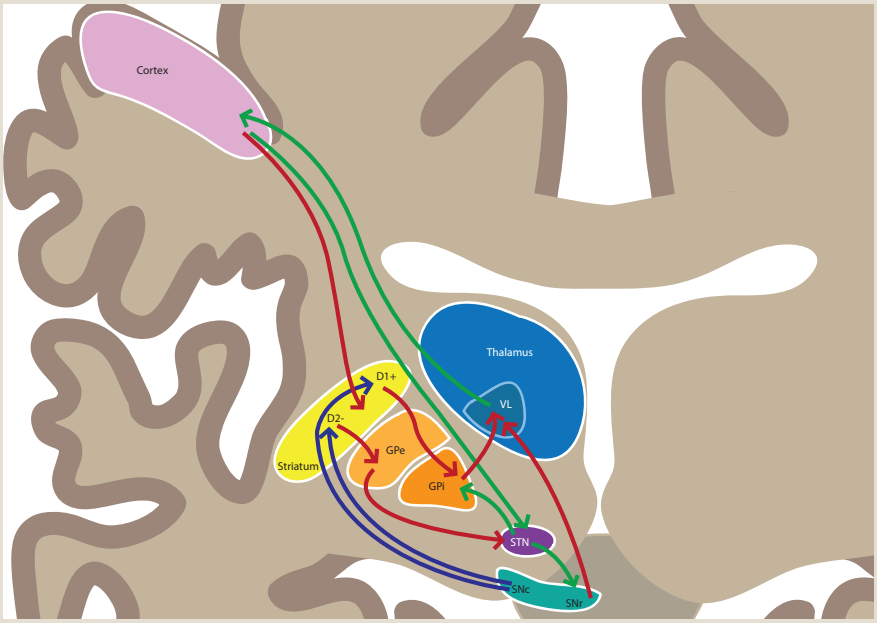

Figure 1 | A visual representation of the classic model of the basal ganglia: dopaminergic connections are depicted in blue, GABAergic (inhibiting) input is represented as a red line while glutamatergic (stimulating) input is in green.

In this model, dopamine from the SNc acts on dopaminergic receptors of the direct and indirect pathway in the striatum. This increases activity of the direct pathway through stimulation of D1 receptors, while stimulation of the D2 receptors of the indirect pathway reduces this pathway's activity. Dopamine therefore changes the balance between of activity between both circuits. Activity of the direct pathway facilitates cortical firing. This occurs to a (relatively) simple interaction through the internal globus pallidus (GPi), and VL of the thalamus. Activity of the direct pathway inhibits the GPi, thereby reducing the GPi's GABAergic inhibition upon the VL nucleus. Now the VL nucleus is free from inhibition it can facilitate and enhance the information that is originating from the (motor) cortex.

The indirect pathway follows a more complicated process of double inhibition: D2 associated GABAergic striatal neurons inhibit activity of the external globus pallidus (GPe). This reduces the baseline inhibition the GPe exerts on the sub thalamic nucleus (STN). When active, the STN stimulates both activity of the GPi and SNr, both inhibiting activity of the VL of the thalamus, and thereby blocking cortical activity. Dopamine, by reducing activity of the D2 associated striatal neurons, now allows the GPe to inhibit the STN, which releases the negative load upon the thalamus and the VL nucleus, facilitating movement. In later versions, the hyper-direct pathway was added, which forms a direct stimulation from the cortex on the STN, with similar subsequent effect.

1.3 | Dopamine depletion & the basal ganglia

Dopaminergic input from the SN is critical to normal functioning of the basal ganglia. The basal ganglia form an important central hub responsible for behavioral control, motor control and action selection. It consists of the striatum (caudate nucleus and putamen), globus pallidus - internal (GPi) and external (GPe), substantia nigra (SN) and subthalamic nucleus (STN). These nuclei control behavior through intricate multi-inhibitory pathways. Most nuclei largely consist of GABAergic projecting neurons in addition to a small proportion of GABAergic and cholinergic interneurons. The exception to this is the STN, whose output neurons are glutamatergic. The striatum functions as the main input nucleus of the basal ganglia. It receives glutamatergic cortical inputs from the many sub-divisions of the neocortex and is thought to filter out uncorrelated synaptic cortical inputs (Hammond, Bergman *et al*. 2007). According to the 'classic' basal ganglia 'rate' model (Albin, Young *et al*. 1989), information is delivered through two separate pathways; the *direct* pathway (which facilitates responding) and the *indirect* pathway (which suppresses responding) [see box 1 for a descriptive breakdown of these pathways]. Dopamine has a central role in controlling the relative activity of both pathways. Increased dopaminergic input will effectively upregulate activity of the *direct* pathway through excitatory D1 receptors, while downregulating activity of the *indirect* pathway through inhibitory D2 receptors. In addition to receiving input from the *indirect* pathway, the STN receives direct projections from the cortex (originating from somato-motor cortical areas) forming what is known as the hyper-direct pathway.

The dopamine deficiency in Parkinson's disease effectively disrupts the balance between these pathways. It is thought to cause a maladaptive upregulation of the indirect pathway relative to the direct pathway, which increases GABAergic inhibition onto the thalamus. This disrupts thalamo-cortical communication and suppresses motor output. Classically, this disruption is explained mainly through an abnormal increase in GABAergic thalamic inhibition. However, some argue that it is not so much the quantity of GABAergic inhibition, but the frequency and synchrony of firing that is affected (Hutchison, Dostrovsky *et al*. 2004). In favor of this argument, studies have shown that there is a relationship between β oscillatory activity in the STN and the parkinsonian rigidity and akinesia (Guo, Zhuang *et al*. 2012). Additionally, animal models of Parkinson's disease show excessive synchrony of basal ganglia regions such as the STN, GPe and GPi (Hammond, Bergman *et al*. 2007).

The disruption caused by dopamine depletion is not exclusive to the motor system. As a central hub in cortical information processing, the basal ganglia serve a variety of functions including motor, cognitive, motivation and limbic function. Broadly speaking, the basal ganglia-thalamocortical circuits are organized in a parallel manner (Alexander and Crutcher 1990, Alexander, Crutcher *et al*. 1991). The motor circuit

consists of the posterior substantia nigra connecting to the putamen, influencing the ventral lateral anterior (VLa) nucleus of the thalamus to the motor cortex and SMA. The anterior substantia nigra connecting to the caudate nucleus, ventral anterior thalamus and prefrontal cortex, and the ventral tegmental area (VTA) connecting to the nucleus accumbens (ventral striatum) connecting to the anterior cingulate cortex (ACC) and orbitofrontal cortex through the mid dorsal nucleus of the thalamus [see box 2 for an illustration]. Dopamine neuronal degeneration in Parkinson's disease is most severe in the posterior SN, thereby strongly affecting the putamen and associated motor circuits (Fearnley and Lees 1991, Vaillancourt, Spraker *et al*. 2009). Nevertheless, degeneration of anterior SN and VTA is still substantial (Fearnley and Lees 1991), leading to the well documented cognitive and motivational challenges of the Parkinson's disease phenotype.

BOX 2 | VENTROMEDIAL TO DORSOLATERAL DIRECTION OF INFORMATION FLOW

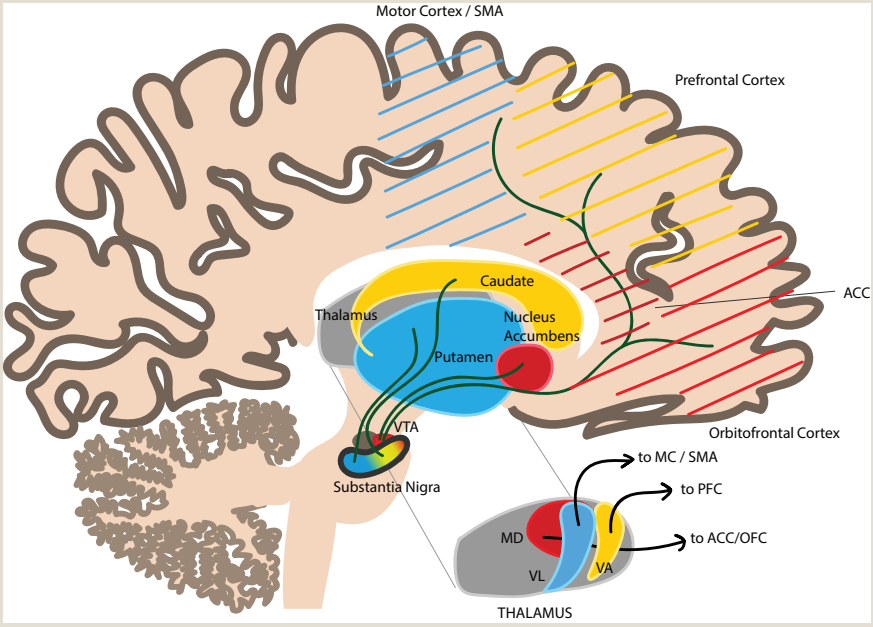

Figure 2 | Visualisation of the frontostriatal loops involved in motivational control (red), cognitive control (yellow), and motor control (blue). N. Acc = nucleus accumbens (ventromedial striatum); caudate= caudate nucleus (dorsomedial striatum); Put = putamen (dorsolateral striatum); OFC = orbitofrontal cortex; ACC = anterior cingulate cortex; MC = motor cortex, MD = medial dorsal nucleus of the thalamus, PFC = prefrontal cortex; PMC = premotor cortex, VA = ventral anterior nucleus of the thalamus, VL = ventral lateral nucleus of the thalamus, VTA = ventral tegmental area.

1.4 | Effects of dopaminergic changes on motivated behavior

To maximize chances of survival, an organism needs to be able to select behavior that leads to the best possible outcome. This includes incorporating information on rewarding and punishing experiences. In this process, dopamine is a key neuromodulator involved in decision-making and drives several different behavioral selection and learning processes.

Prominent work has revealed an important role for dopamine in orchestrating reward processing. Schultz *et al* showed that in vivo dopamine recordings mirrored reward prediction theory, that is, the unexpected delivery of reward was associated with positive peaks in dopamine firing forming a positive reward prediction error (Schultz, Dayan *et al*. 1997).When the expected reward was omitted, dopaminergic firing would decrease from the norm, forming a negative reward prediction error. This formed the basis of subsequent research into the relationship between dopamine and reward learning.

Dopaminergic neurons innervate large parts of the brain, including the striatum. This region has often been implicated to be involved in motivation reward-based learning (Robbins and Everitt 1992, Cools and Robbins 2004, Frank 2005, Pessiglione, Seymour *et al*. 2006, Clatworthy, Lewis *et al*. 2009). Reward-based learning is thought to depend strongly on the interaction between the D1 and D2 receptors of the direct and indirect pathways (Frank 2005). Approach is mediated by the reward cue induced dopamine release, which activates the direct pathway through striatal D1 receptor stimulation. Avoidance occurs after a dip in DA, which disinhibits the indirect pathway due to reduction of D2 receptor stimulation, leading to action inhibition (Cox, Frank *et al*. 2015). Together this can facilitate learning from both positive and negative outcomes (Frank 2005, Cox, Frank *et al*. 2015).

1.4.1 | Motivation-action coupling

In theory, using reward and punishment based instrumental learning we should be able to make near optimal decisions maximizing the reward outcome. However, we are often bad decision makers, so why is this the case? and One prominent idea is that we depend on fast and efficient motivational biases to cut down decision time costs, which comes at the risk of being less correct. A prominent example of such a motivational bias is the coupling between action and valence. In this deeply ingrained bias (also known as the Pavlovian bias), we tend to perform an action to gain a reward and hold back to avoid a punishment. Dopamine is suggested to have an important role in the driving these motivational biases (Boureau and Dayan 2011,

Cools, Nakamura *et al*. 2011, Guitart-Masip, Chowdhury *et al*. 2012, Guitart-Masip, Huys *et al*. 2012, Swart, Froböse *et al*. 2017). Dopamine is essential to both establish active motivated behavior, as well as to support instrumental learning. As dopamine holds an important role in increasing both behavioral vigor and reward learning it was suggested to also subsequently influence the coupling between action and valence. Indeed, some early work into dopamine and motivational biases shows that dopamine can potentiate appetitive motivational approach biases in experimental animals (Parkinson, Olmstead *et al*. 1999, Dickinson, Smith *et al*. 2000, Lex and Hauber 2008). Interestingly, a recent study suggested that dopamine enhancement may also affect motivational bias in the opposite direction, reducing the effective bias (Guitart-Masip, Economides *et al*. 2014). Potentially, this occurs through facilitation of prefrontal behavioral control.

1.4.2 | Parkinson's disease and behavior

As Parkinson's disease patients suffer from dopaminergic neurodegeneration, this has an effect on behavior related to the previously mentioned functions of dopamine. Classically, we see a reduction in action invigoration. Parkinson's disease is characterized by stiffness and the inability to initiate movement. Related to reward sensitivity, one striking and often replicated observation is that Parkinson's disease patients off their dopaminergic medication showed a decreased ability to learn from rewarding outcomes, yet increased ability to learn from aversive outcomes. This effect is reversed by dopaminergic medication, leading to enhanced learning from rewards (Frank, Seeberger *et al*. 2004, Cools 2006). Clinically, there are related neuropsychiatric side effects of dopaminergic treatments such as impulse control disorders and behavioral addictions such as gambling (Beaulieu-Boire and Lang 2015, Weintraub, David *et al*. 2015). Additionally, there is a wealth of evidence showing significant alterations in cognitive control, which is strongly dependent on prefrontal dopamine levels (Cools 2006, Cools and D'Esposito 2011). As mentioned earlier, dopamine depletion is relatively restricted to the dorsal striatum (i.e. putamen and dorsal caudate nucleus) in early stages of the disease, with progression to limbic and cortical structures (nucleus accumbens and prefrontal cortex) in later stages of the disease. During early stages, ventral striatal dysfunction could also be attributed to a local dopamine *overdose*. The overdose hypothesis states that administration of dopaminergic medication to Parkinson's disease patients may replenish dopamine depleted circuits (including the dorsal striatum), but 'overdose' relatively intact circuits (including the ventral striatum) (Gotham, Brown *et al*. 1988, Swainson, Rogers *et al*. 2000, Cools, Barker *et al*. 2001). Such overdosing could cause patients to perform poorly on related behavioral tasks such as probabilistic reversal learning and other cognitive tasks, associated with intact dopamine-dependent brain regions (Cools, Barker *et al*. 2001, Cools 2006, Kwak, Müller *et al*. 2009). Evidence from recent studies with patients with Parkinson's disease have revealed that effects of

dopaminergic medication on reinforcement learning tasks can be attributed, at least in part, to modulation of choice (Shiner *et al*., 2012; Smittenaar *et al*., 2012). However, those studies do not exclude that medication alters both learning and choice, as these could not be assessed simultaneously (Collins and Frank, 2014).

1.5 | Outline and key questions

The aim of this thesis is to investigate the pathophysiological basis of Parkinson's disease. Specifically, I will focus on cerebral and neuropsychological differences between tremor-dominant and non-tremor patients, focusing particularly dopaminergic nuclei and their subcortical projection targets, and behavioral consequences of dopamine depletion and administration. I acknowledge the complexity of the disease by approaching this topic from three different but related angles looking at the molecular, structural, and behavioral differences between tremor dominant and non-tremor Parkinson's disease. Together these approaches represent important factors that underlie the pathophysiology of Parkinson's disease.

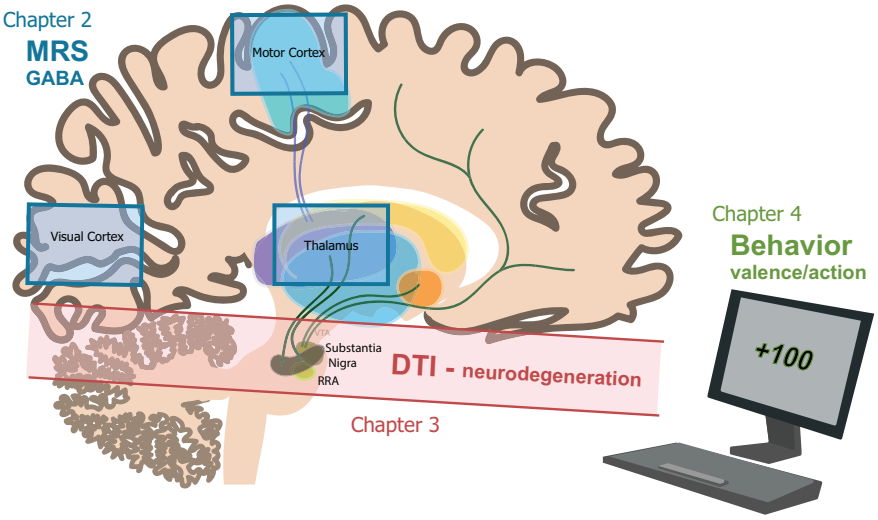

Figure 3 | Overview of each of the three chapters in this thesis, each of these chapters covers a different link in the neurological systems involved in Parkinson's disease.

Parkinson's disease is associated with dopaminergic cell loss in the midbrain, particularly in the substantia nigra. As discussed in section 1.2, there are many hints that neurodegeneration in dopaminergic regions differ between tremor and non-tremor patients. In **chapter 3**, I will investigate structural decline using diffusion tensor imaging (DTI) in two relevant dopaminergic nuclei: the substantia nigra (SN) and retro rubral area (RRA). I will investigate whether there is a difference in structural integrity

of the substantia nigra and the retro-rubral area between tremor dominant and non-tremor Parkinson's disease patients, and whether these measures of degeneration relate to symptom expression. Specifically, I will test the hypothesis that tremor-dominant patients have reduced cell loss in the substantia nigra (SN) but increased cell loss in the retro-rubral area (RRA). As these regions have been associated with bradykinesia and tremor severity respectively, I further tested whether increased free water levels in the SN and RRA corresponded to increases in the symptom severity of bradykinesia and tremor.

As discussed in section 1.3, the 'classic' basal ganglia (rate) model suggests that many physiological consequences of dopaminergic decline arise from an imbalance in GABAergic signaling and a subsequent increase in GABAergic inhibition from the striatum on the thalamo-cortical circuit. This system is thought to play a vital source in motor (and motivational) dysfunction seen in Parkinson's disease. In **chapter 2**, I use magnetic resonance spectroscopy (MRS, see box 3) to study the molecular GABAergic input on the thalamus and motor cortex, with the visual cortex as a control region. I investigate the hypothesis that GABA in the thalamo-cortical motor circuit is increased in Parkinson's disease compared to healthy controls. Furthermore, I will test the hypothesis that there is a difference in GABA concentrations between tremor-dominant and non-tremor Parkinson's disease patients in the motor circuit, along with dopamine-responsive and dopamine-resistant Parkinson's disease patients with tremor. In addition, as the increase in GABAergic inhibition was suggested to originate from the decline in dopaminergic input from the SN, we will further test the hypothesis that GABA levels change (decrease) as a function of dopaminergic drugs. Moreover, as GABA levels are thought to increase in relation to symptom progression, we follow up to see whether GABA levels correlate to disease severity.

As discussed in section 1.4 and 1.5, dopamine deficiency is known to affect both motor function and influence motivational reasoning, showing effects on reinforcement learning. We see substantial signs of motivational impairment in Parkinson's disease thought to be related to their dopaminergic decline. Therefore, in addition to studying specific anatomical and neurochemical changes in Parkinson's disease, I will also study the effect on behavior in **chapter 4**.

As discussed in section 1.2, tremor and non-tremor phenotypes were found to differ in their cognitive abilities, with non-tremor patients showing an overall faster cognitive decline. Compounding evidence suggests that non-tremor patients suffer from greater dopamine depletion, with more degeneration in the SN and lower dopamine activity in the striatum as measured by metabolic imaging. Based on these differences in cognitive and dopamine dysfunction, I hypothesized that well-established effects of dopaminergic medication on motivational learning may differ between tremor-dominant and non-tremor patients. Moreover, as discussed in section 1.5, medication

might alter both reinforcement learning as choice bias in Parkinson's disease. Here, I investigate the differences in dopamine-sensitive motivated behavior between tremor-dominant patients and non-tremor patients, using a behavioral task designed to assess motivated learning and choice. I use computational modelling to disentangle the influence of hardwired, 'Pavlovian' biases from motivated learning and increase our understanding of the computational mechanisms driving dopaminergic-influenced changes in behavior.

Together I provide a multifaceted take on a complex disorder, with a high regard for patient-to-patient variation. Taken together, this thesis presents a state-of-the-art multidisciplinary approach, combining a causal pharmacological intervention, with sophisticated behavioral paradigms, computational modelling, multi-modal neuroimaging, and clinical assessment, to further our understanding of the pathophysiological basis and neuro-computational mechanisms involved in Parkinson's disease. In **chapter 5**, I provide a summary of the main findings, discuss and integrate the most relevant findings of this thesis, and highlight future directions.

This work is part of a larger study which includes the works of Heidemarie Zach and Michiel F.M. Dirkx. The overall study investigates several factors including i) differences between tremor and non-tremor Parkinson's disease, and ii) within tremor patients, the differences between patients whose tremor symptoms are responsive to DA medication, and those who are not. Finally, iii) it adds an investigation into the physiological properties of and cause behind tremor in Parkinson's disease. The total study consisted of three Parkinson subgroups (tremor DA responsive, tremor DA resistant and non-tremor), with an aim of 20 participants in each group.

BOX 3 | MAGNETIC RESONANCE SPECTROSCOPY

Magnetic resonance spectroscopy (MRS) allows us to obtain biochemical information on tissues of the human body in a non-invasive way. Like the more common variants of magnetic resonance imaging (MRI), it utilizes the interaction between magnetic field strength and resonance frequency. The resonance frequency of a certain nuclei is always relative to the magnetic field strength times this nucleus specific constant. While this relationship is commonly mainly used to determine localization of the measured signal, MRS utilizes small changes in frequency shift to determine the type (and abundance) of a metabolite that is emitting the signal. Like standard MRI, signal is typically acquired from protons, although other endogenous nuclei such as those of carbon, nitrogen, and phosphorus can also be used. The measure relies on the fact that each metabolite consists of a specific configuration of atoms that form a small metabolite specific 'micro-environment'. This micro-environment slightly

influences the effective magnetic field that the groups of protons experience. Due to the relationship between magnetic field and resonance frequency, this means these protons in this micro-environment will also resonate a frequency slightly higher (or lower) than the overall frequency that is based on the baseline magnetic field applied by the MRI system. Each group of protons (e.g. all protons surrounding a single 'C' or 'O' atom) in this metabolite has a similar micro-environment, and a similar frequency shift. Together, this means that each metabolite will have its own specific fingerprint of frequency specific signal peaks, that can help us to identify the metabolite in question. The signal intensity of these frequency peaks relates to the presence of the metabolite in the tissue and allows us to estimate its relative concentration. Since there are many other factors that can influence the intensity of MRI signal, this does not offer us an absolute quantity. However, as these factors generally affect all products/metabolites measured equally it does allow us to quantify the product in relation to others. Most commonly, quantification is done in relation to either water (which generally requires a separate scan), or to metabolites that are considered stable, and/or otherwise relevant, such as Creatine (which can be used as a proxy of cell count), and NAA (a proxy for neuron count).

To be able to reliably measure any metabolite using MRS, the availability needs to be sufficient such that the signal peaks this product creates are bigger than the noise that is present in the signal itself. Achieving large enough signal requires several factors: 1) A voxel size big enough to encompass a large enough quantity of metabolite to contribute to the signal, while at the same reduce the relative influence of local noise sources as noise has spatial fluctuations while metabolite's profile is stable. 2) A relatively long scanning time, to acquire multiple measures averaging over time, to increase overall signal to noise ratio as noise has temporal fluctuations while the metabolite is stable. 3) Minimization of voxel field inhomogeneity (shimming) to reduce fluctuation of the frequency orientating from the metabolite of interest, resulting in an improved (smaller) linewidth of the signal peaks. Because of the stringent signal to noise requirements, the number of neurotransmitters that can be successfully quantified by MRS is quite small. In general, only GABA and the combined measure of Glutamate and Glutamine (these two products have almost identical 'frequency fingerprints' and estimation is therefore combined) are abundant enough to be detected. Abundance of other metabolites such as dopamine is too low to detect using standard 1H-MRS

1.6 | References

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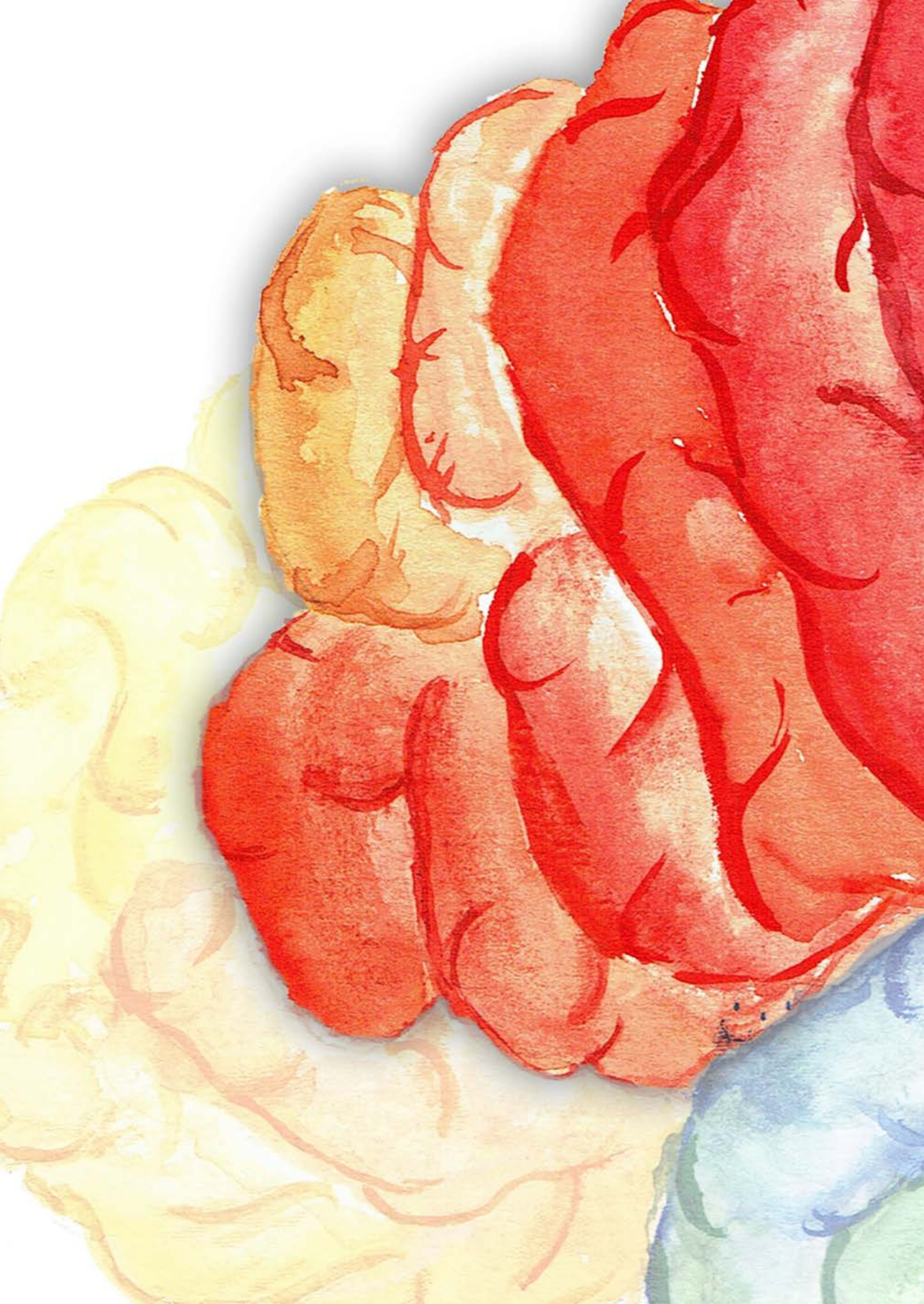

CHAPTER 2

GABA-ergic changes in the thalamo-cortical circuit in Parkinson's disease

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2.1 | Abstract

Parkinson's disease is characterized by bradykinesia, rigidity, and tremor. These symptoms have been related to an increased GABAergic inhibitory drive from globus pallidus onto the thalamus. However, in vivo empirical evidence for the role of GABA in Parkinson's disease is limited. Some discrepancies in the literature may be explained by the presence or absence of tremor. Specifically, recent fMRI findings suggest that Parkinson's tremor is associated with reduced, dopamine-dependent thalamic inhibition. Here we tested the hypothesis that GABA in the thalamo-cortical motor circuit is increased in Parkinson's disease, and we explored differences between clinical phenotypes. We included 60 Parkinson patients with dopamine-resistant tremor (n=17), dopamine-responsive tremor (n=23), or no tremor (n=20), and healthy controls (n=22). Using magnetic resonance spectroscopy, we measured GABA-to-total-Creatine ratio in motor cortex, thalamus, and a control region (visual cortex) on two separate days (ON and OFF dopaminergic medication). GABA levels were unaltered by Parkinson's disease, clinical phenotype, or medication. However, motor cortex GABA levels were inversely correlated with disease severity, particularly rigidity and tremor, both ON and OFF medication. We conclude that cortical GABA plays a beneficial rather than a detrimental role in Parkinson's disease, and that GABA depletion may contribute to increased motor symptom expression.

2.2 | Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized by bradykinesia, rigidity, and resting tremor. It is generally thought that the loss of dopaminergic neurons in the substantia nigra pars compacta, and subsequent striatal dopamine depletion, underlies bradykinesia (Kish, Shannak *et al*. 1988). According to the classical 'rate' model of basal ganglia function, striatal dopamine depletion produces an imbalance between the direct (facilitatory) and the indirect (inhibitory) pathways through the basal ganglia (Albin, Young *et al*. 1989). More specifically, dopamine depletion is thought to elicit a release of indirect pathway activity, resulting in an abnormally increased GABA-ergic, inhibitory drive from the internal globus pallidus (GPi) onto the ventral lateral (VL) nucleus of the thalamus. The VL nucleus in turn facilitates cortical activity in the motor cortex. According to this model, dopamine depletion in Parkinson's disease should therefore cause both increased concentrations of GABA in the thalamus and reduced facilitation of the motor cortex. This model has been very influential in explaining the role of dopamine in motor control and it has contributed to important breakthroughs such as the development of stereotactic surgery for treating Parkinson's disease (Hamani, Dostrovsky *et al*. 2006).

However, several predictions of this model have yielded opposing results (Ellens and Leventhal 2013, Nelson and Kreitzer 2014). Animal and patient studies report only small increases in GPi firing rates in the parkinsonian state (Hutchison, Lozano *et al*. 1994, Levy, Dostrovsky *et al*. 2001), and no suppression of thalamic firing (Ellens and Leventhal 2013, Nelson and Kreitzer 2014). Furthermore, there is limited (and contradictory) experimental evidence for altered GABA levels in the thalamus in Parkinson's disease. For instance, microdialysis in patients with Parkinson's disease with deep brain stimulation (DBS) showed that both levodopa administration and DBS of the subthalamic nucleus (STN) *decreased* GABA levels in the VL nucleus of the thalamus accompanied with motor improvement (Stefani 2011, Stefani, Fedele *et al*. 2011). Using MR spectroscopy, others found *increased* GABA levels in Parkinson's disease compared with controls in a small voxel contained within the thalamus (Dharmadhikari, Ma *et al*. 2015). However, covering the entire basal ganglia and part of the thalamus, others found a *reduction* of GABA levels in Parkinson's disease, and even lower GABA levels for tremulous Parkinson's disease compared with non-tremor Parkinson's disease (Gong, Xiang *et al*. 2017). Finally, a post-mortem study found a 36% *reduction* in thalamic GABA concentrations in patients with Parkinson's disease compared with controls (Gerlach, Gsell *et al*. 1996). Accordingly, it has been suggested that not intensity of inhibition, but the pattern and synchrony of neural firing is most affected in Parkinson's disease (Hutchison, Dostrovsky *et al*. 2004, Uhlhaas and Singer 2006). More specifically, these authors suggest that enhanced synchronization, particularly in the beta-frequency range, is responsible for bradykinesia in Parkinson's disease (Brittain, Sharott *et al*. 2014).

Adding to the disparity, it is clear that the classic box-and-arrow model does not easily explain Parkinson's disease rigidity and tremor, which are both associated with excessive rather than reduced motor activity (Rodriguez-Oroz, Jahanshahi *et al*. 2009). Rigidity has been linked to enhanced excitability of the motor cortex, as evidenced by increased motor evoked potentials and a reduced cortical silent period in patients versus controls (Cantello, Gianelli *et al*. 1991). Furthermore, higher rigidity scores were associated with increased motor cortex activity during voluntary movements (Yu, Sternad *et al*. 2007). Resting tremor has been hypothesized to result from increased thalamic inhibition (thalamic hyperpolarization hypothesis (Llinás 1988)), however, subsequent findings have cast doubt on this idea. The proposed low-threshold calcium-dependent spiking behaviour was not found in the thalamic region associated with resting tremor, i.e. the posterior portion of the ventrolateral thalamus (Magnin, Morel *et al*. 2000). Further, a recent functional MRI study using dynamic causal modelling, showed that dopaminergic medication reduced tremor severity by increasing, rather than decreasing, thalamic self-inhibition (Dirkx, den Ouden *et al*. 2017). This effect was related to the clinical response of tremor to dopamine, i.e. patients with dopamine-responsive tremor showed enhanced thalamic inhibition. This suggests that tremor, especially dopamine-resistant tremor, may result from reduced, rather than increased, thalamic inhibition (Helmich 2018).

Thus, there is evidence for increased as well as reduced GABA concentrations in the thalamus and motor cortex of Parkinson's disease patients, and these discrepancies may depend on differences in clinical phenotype. Using MRS, here we aimed to clarify this issue by testing the effect of dopaminergic medication in carefully selected clinical phenotypes in Parkinson's disease, on GABA levels in the primary motor cortex, thalamus, and a control region in the visual cortex. Following the classical basal ganglia model, we expected higher thalamic GABA levels in patients than controls, which should be (partly) remediated by dopaminergic medication, and which should increase with bradykinesia severity. As MRS is sensitive to extracellular, unbound GABA (Rae 2014, Stagg 2014, Dyke, Pépés *et al*. 2017) – which is involved in tonic rather than phasic inhibition – this method is appropriate to detect the predicted increase in tonic GABAergic tone in the thalamus in Parkinson's disease (Redgrave, Rodriguez *et al*. 2010). The classical model does not offer such clear predictions on changes in the motor cortex: although increased thalamic inhibition may lead to increased inhibition of the motor cortex, other transcortical influences likely also play a role. Furthermore, based on our previous data (Dirkx, den Ouden *et al*. 2017), we hypothesized an opposite relationship between thalamic GABA and resting tremor. More specifically, we expected reduced GABA concentrations in tremor-dominant patients compared with non-tremor patients, and more so in patients with a dopamine-responsive tremor than a dopamine-resistant tremor.

2.3 | Methods

2.3.1 | Subjects

The study was conducted according to the standards of the 1964 Declaration of Helsinki and was approved by the local ethics committee. Before inclusion, all participants provided their informed written consent.

This project included three groups of patients with Parkinson's disease (patients with dopamine *resistant* tremor [n=17, 4 Female (F), 13 Male (M)], patients with dopamine *responsive* tremor [n=23, 14F, 9M], non-tremor patients [n=20, 9F, 11M]), as well as one group of age matched healthy controls [n=22, 10F, 12M]. Clinical details are presented in *Table 2*. For patients, inclusion criteria were: idiopathic Parkinson's disease, and one of these three possible clinical phenotypes. Exclusion criteria were cognitive dysfunction defined as Mini-Mental State Examination (MMSE)<26 (Cockrell and Folstein 2002) and frontal assessment battery (FAB)<13 (Lima, Meireles *et al*. 2008), neurological or a severe psychiatric comorbidity (such as personality disorder, clinically defined major depression, psychosis), severe head-tremor, known allergy against levodopa-benserazide or domperidone and severe dyskinesia's. Patients with mild psychiatric symptoms were not excluded.

Tremor-dominant Parkinson's disease was defined as a history of tremor and a resting tremor score of 1 point or more in at least one arm on item 17 of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS part III) (Dirkx, den Ouden *et al*. 2017). These tremor patients were divided into dopamine-responsive and dopamine-resistant phenotypes. Specifically, in a separate session before MRI examinations, 83 tremor-dominant Parkinson's disease patients (as defined above) were pre-screened to measure their dopamine responsiveness. During this session, patients' MDS-UDPRS motor scores were measured twice on one day, both before (OFF medication) and after a levodopa challenge (200/50 mg levodopa-benserazide plus domperidone 10 mg). Dopamine-resistant tremor was defined as a clinical dopamine response of ≤20% on tremor UPDRS (items 15-18); while dopamine-responsive tremor was defined as a clinical dopamine response of ≥70% on tremor UPDRS. All selected patients had a dopamine response for bradykinesia / rigidity of at least 20%, to exclude trivial causes for dopamine-resistance (such as gastro-intestinal problems). This resulted in 17 Parkinson's disease patients with dopamine *resistant* tremor and 23 Parkinson's disease patients with dopamine responsive tremor, who were included in this study. Non-tremor Parkinson's disease (n=20) was defined as the absence of resting tremor in all limbs (UPDRS resting tremor score of 0 (Helmich, Janssen *et al*. 2011). Action tremor was allowed given that this tremor has a different pathophysiology (Dirkx, Zach *et al*. 2018). In our sample 52 patients took levodopa, 16 patients took dopamine agonists,

4 patients took a catechol-O-methyltransferase (COMT) inhibitor and 2 patients took tricyclic antidepressants.

From all 60 Parkinson's disease patients, 4 patients dropped out on the second day due to claustrophobia (2 in the responsive, 1 in the resistant and 1 in the non-tremor group). In the dopamine *responsive* tremor group, 1 MRS-session could not be completed due to technical problems with the MRI-scanner on day 1. Out of 22 controls, 3 participants did not return for session 2. We further excluded patients whose MRS spectra did not pass quality control requirements (*see 1H-MRS post-processing below*), which resulted in an average of 2-3 rejected spectra per group in the thalamus, 0-1 in the motor cortex, and 0-4 in the visual cortex (see *Supplementary Figure 1* for a precise description of rejected spectra).

2.3.2 | Dopaminergic intervention

Both patients and healthy participants were measured on two separate occasions, always in the morning. Parkinson's disease patients were measured in pseudorandomized order with respect to the dopaminergic intervention. On both sessions, patients came in an OFF state, i.e. >12 hours after their last dose of levodopa, > 48-72 hours after their last dose of dopamine-agonist (>3 times the drug half-life). On both sessions, all patients received a dose of domperidone 10 mg 1 hour before drug/placebo intake, to increase gastro-intestinal absorption and to reduce side effects. During one session, patients received a standardized dose of 200/50mg of dispersible levodopa-benserazide (ON state), dispersed in water. Levodopa dose was on average 70% higher than the patients' own morning dose. During the other session, patients received a placebo (cellulose dispersed in water, which matches the dispersible levodopa both visually and in terms of taste). All healthy subjects were measured on two separate sessions to control for repetition effects.

2.3.3 | Measurements

Clinical Ratings: Full tremor scores consisted of all 18 items of the MDS-UPDRS part III; sub-scores were used to calculate the dopamine response for each of the three motor symptoms separately: *resting tremor and re-emergent tremor* (non-kinetic tremor: items 15 and 17), *bradykinesia*: (items 4-8 & 14) and *rigidity* (item 3). The clinical rater was blinded to the medication administration of the patients.

MRI: All participants underwent a similar counterbalanced MRI scanning protocol using a 3 Tesla MRI Siemens PRISMA system (Siemens Healthcare, Erlangen, Germany), equipped with a 32-channel head coil. A whole brain high resolution T1-weighted anatomical scan was acquired using a three-dimensional magnetization prepared rapid gradient echo acquisition sequence (MP2RAGE (repetition time (TR)

= 5000ms; echo time (TE) = 2.96ms TI1=700ms, TI2=2500ms; matrix 350x263x350 mm, voxels 1.0 mm^3 isotropic).

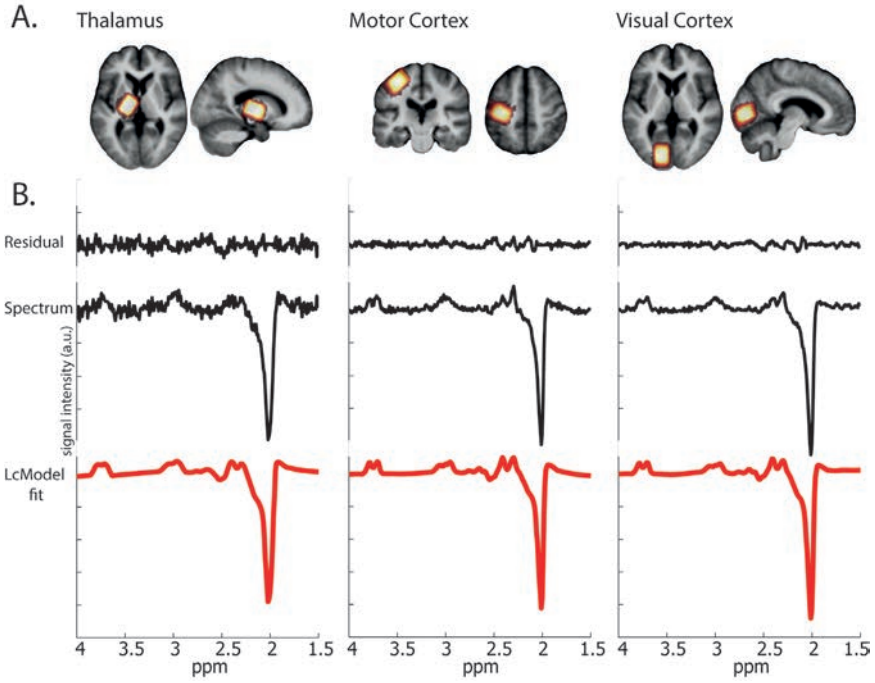

Figure 1 | Anatomical location of MRS voxels. Panel **A** shows a heat plot of the MRS voxels in the thalamus, motor cortex and visual cortex (in red-yellow), which was created by normalizing all anatomical T1 scans to standard stereotactic space, keeping associated region of interests in line, and calculating the overlap of regions of interest between subjects. They are shown superimposed on the averaged anatomical scan of all patients. Voxels were placed on the side contralateral to the most affected side; this resulted in 30 participants being scanned on the left, and the other 30 on the right side, controls were scanned on the right and left side equally in random order (11 were scanned on the left and 11 on the right). Only the left side is shown as an example. **B**. Representative MR-spectra as recorded in a Parkinson's disease patient, measured in each of the three voxels, decomposed into the LCModel fit, the original spectrum, and difference between original and fitted spectra (residual) from bottom to top, respectively. a.u. = arbitrary units; MRS = Magnetic Resonance Spectroscopy

1H-MRS: MRS data were acquired after the T1 acquisition, using a standard Siemens MEGA-PRESS acquisition protocol for GABA detection (Mescher, Merkle *et al*. 1998), with a TR = 1500ms, TE = 68ms, acquisition bandwidth = 1200Hz and water suppression at 4.7 ppm (Mullins, McGonigle *et al*. 2014), using the CHESS water suppression method (Ogg, Kingsley *et al*. 1994). On the odd-numbered acquisitions a frequency selective refocusing pulse was applied at 1.9 parts per million (ppm). Subtracting odd from even acquisitions reveals the GABA resonance at around 3.0 ppm. (See *supplementary materials* for a more extended description of the MRS

sequence). We focused on three different brain regions: the thalamus, motor cortex, and visual cortex. Our main hypothesis of altered GABA concentrations in Parkinson's disease concerned the thalamus and motor cortex; the visual cortex was added as a non-motor control region. In patients, these voxels were placed in the hemisphere contralateral to the body side with most prominent motor symptoms. In the healthy controls, the voxels were placed equally on either the left or right side of the brain, randomized across control participants. Voxel dimensions (18x24x18mm=7.78ml) were optimized for the slightly elongated shape of the thalamus. Number of averages was optimized for signal to noise drop off towards the centre of the brain. This resulted in 96 averages in cortical regions (in pairs of scans), and 128 in the thalamus. Voxels were placed manually on the first session, and voxel coordinates relative to the individual anatomical MRI were saved using the vendor-provided Auto Align function. This guaranteed that the voxel was placed in the same anatomical locations across the two sessions for each individual subject. During session 2, this automatic voxel placement was always visually checked using a new anatomical T1 scan. The thalamus voxel was placed to avoid neighbouring nuclei such as globus pallidus and caudate nucleus, and to minimize inclusion of cerebrospinal fluid (CSF). The voxel was oriented along the transversal axis and rotated along the white matter tract posterior and lateral from the thalamus tissue. Its size was restricted from the start of the globus pallidus on the anterior side, to the CSF on posterior and medial side. The motor cortex voxel was placed to cover the motor hand area, or 'hand knob', see also (Caulo, Briganti *et al*. 2007), and oriented along the central sulcus, and was aligned with the skull in the coronal plane. The visual cortex voxel was aligned with the calcarine sulcus and rotated with a slight (+/- 10 degree) angle from the anterior-posterior line, to avoid distortion originating from the sagittal sinus and jugular veins. See *Figure 1* for a representation of the average location of the three voxels.

2.3.4 | 1H-MRS post-processing

Metabolite quantification was performed with LCModel software (Provencher 2001). LCModel performs fitting, frequency alignment, phase adjustment, eddy current correction and baseline correction. Relative concentrations were estimated by fitting the measured signal with a simulated basis set for both the difference and the original spectrum. Most prominently, spectra of gamma-aminobutyric acid (GABA), N-Acetyl Aspartate (NAA), N-Acetyl-Aspartyl Glutamate (NAAG), Glutamate (Glu), Glutamine (Gln) and total (Phospho-) Creatine (tCre) were simulated using the MEGA-PRESS editing and non-editing sequence, including pulse shapes and pulse timings. A full overview of the metabolites included in the simulated basis sets of both spectra can be found in the *supplementary materials*. Quality control of the spectra was based on the Cramer-Rao lower bounds (given as % Standard Deviation (%SD)-value by the LCModel program), FWHM and further visual inspection (see *Table 1* for the average CRLB and FWHM values per subgroup). Especially in spectra with resonances

with low signal intensity, such as GABA, a low cut-off Cramer-Rao lower bound as quality control introduces a systematic bias towards higher levels of that particular resonance (Kreis, 2016). Therefore, we chose to use Spectra with a %SD value < 50 as the threshold for acceptance of the GABA fit (Marjańska, Lehéricy *et al*. 2013), see *Table 1* for average %SD values. Our main outcome measure was the region-specific GABA amount, which was calculated as the ratio between the GABA and the tCre signal, were the GABA signal was taken from the edited experiment and the tCre signal from the non-edited spectrum. This was done to normalize the GABA levels to a stable counterpart without the issues of calculating absolute concentrations.

Table 1 | Comparison of spectral quality between groups. The mean (standard deviation) of linewidth (FWHM, based on the NAA peak), and Cramer Rao Lower Bounds (%SD) of the quality-controlled GABA fits for GABA-edited spectra from thalamus, motor cortex and visual cortex are shown. sig. = significance level (t-test) FWHM= full width half maximum

	Thalamus			Motor Cortex			Visual Cortex		
	Patient	Control	sig.	Patient	Control	sig.	Patient	Control	sig.
FWHM:	9.2 (4.4)	9.6 (4.5)	0.347	7.3 (2.1)	7.4 (2.8)	0.188	7.4 (3.0)	7.7 (3.2)	0.466
CRLB:	24.1 (13.9)	22.6 (13.4)	0.154	15.3 (5.5)	14.5 (5.8)	0.171	17.9 (10.0)	16.8 (11.0)	0.226

2.3.5 | Statistical analysis

Statistical analyses were performed with Statistical Package for Social Science Software (SPSS, version 2, Chicago, IL, USA) for Windows. A p-value of <0.05 was considered significant. In addition to classical statistics we supplement these analyses with Bayesian statistics using JASP for Bayesian analysis (JASP Team (2017, Version 0.8.2)). Bayesian statistics are added to provide insight into the validity of a null response. Bayes factors quantify the ratio of accumulated evidence for the null hypothesis and the alternative hypothesis. We report both Bayes Factors showing evidence towards the alternative hypothesis (BF_{10}) and Bayes Factors showing evidence towards the null hypothesis (BF_{01}). The reported Bayes Factors (BF_{10} or BF_{01}) are interpreted according to the guidelines provided in JASP (Wagenmakers, Love *et al*. 2017) in which a BF between 1-3 was interpreted as anecdotal effect, a BF between 3-7 as a moderate effect (BF=3 can be roughly seen as the 'equivalent' denotation of p<0.05) and a BF >7 as a strong effect. Our main analyses can be divided into three main parts:

– First, we tested whether GABA levels differed between patients and controls, and whether these effects were region-specific. For this analysis, we combined the three patient groups into one patient group, and we averaged across the two medication sessions. We used a [2x3] repeated measures analysis of variance (ANOVA) in SPSS, with group (patients versus controls) as between-subject factor and region

(thalamus, motor cortex, visual cortex) as a within-subject factor. We also assessed the direction of these effects using [2x3] Bayesian repeated measured ANOVA in JASP, to test for evidence in favour or against the null hypothesis.

– Second, in patients, we tested whether GABA levels were influenced by disease phenotype (Parkinson's disease with dopamine-resistant tremor, dopamine-responsive tremor, or no tremor) and by dopaminergic medication (OFF versus ON). We used a [3x2] repeated measures ANOVA in SPSS with factors region and medication as within-subject factors and group as between subject factor. Again, we assessed the direction of effects using the [3x2] Bayesian repeated measured ANOVA in JASP.

– Third, we tested whether GABA levels were correlated with disease severity (total MDS-UPDRS motor score). First, to investigate the overall effect of disease severity and to test whether these correlations were significantly different between regions, we used a repeated measures-analysis of variance (ANOVA) in SPSS, with region (thalamus, motor cortex, visual cortex) as a within-subject factor and mean UPDRS scores as a covariate. Next, in accordance with a-prior expectations, we separately performed Multiple Linear Regression analyses on the GABA/tCre and MDS-UPDRS values in each of the three regions (averaged over sessions). We also assessed Bayesian scores of each correlation using Bayesian Correlation Pairs in JASP on the mean values for each participant, to see whether evidence supports the presence or absence of a correlation. These previous results were inspected using an adjusted p-value of $p<0.007$, based on a Bonferroni correction to account for our 7 tests of interest: patient vs controls, patient-group, medication and correlation with UPDRS including each of the three regions.

We performed several post-hoc exploratory analyses to elaborate on our previous results. First, we ran the three multiple linear regression analyses (UPDRS by GABA in the three regions) separately for ON and OFF sessions. In addition, we investigated whether each of the three main symptoms of Parkinson's disease (rigidity, tremor and bradykinesia) were correlated with GABA levels in the motor cortex, using Multiple Linear Regression analyses both ON and OFF medication and Bayesian Correlation Pairs over the mean scores. Given that we collected unilateral GABA measurements, we used symptom severity ratings for the contralateral (most-affected) side. We compared all possible correlations between the three symptoms (separately ON and OFF medication) using the cocor toolbox, developed by (Diedenhofen and Musch 2015).

2.4 | Results

2.4.1 | Clinical differences between groups

There were no differences between patient groups and controls in gender balance, age, FAB and MMSE scores. There were also no differences between Parkinson phenotypes in terms of age, FAB and MMSE, Levodopa Equivalent Daily Dose (LEDD), and MDS-UPDRS motor scores of the following subsets: axial symptoms, bradykinesia, rigidity and the total non-tremor score (*see Table 2*). There was no difference in non-tremor MDS-UPDRS response (difference between OFF and ON). However, there was a trend-level difference in gender balance between the three Parkinson's disease phenotypes [$\chi 2(2)=5.51$, $p=0.064$]. Furthermore, the disease duration of the dopamine resistant group was shorter than that of other groups [resistant/responsive: $t(39)=-2.78$, $p=0.020$, resistant/non-tremor: $t(36)=-2.43$, $p=0.046$]. Tremor scores differed between patient groups, reflecting our inclusion procedure: MDS-UPDRS scores differed significantly between the two tremor groups ON medication [$t(39)=3.52$, $p=0.002$], but not OFF medication [$t(39)=1.00$, $p=0.579$]. The non-tremor patient group showed significantly different tremor scores from the tremor patients, both ON [resistant/non-tremor: $t(36)=8.97$, $p<0.001$, responsive/non-tremor: $t(42)=6.00$, $p<0.001$], and OFF dopamine [resistant/non-tremor: $t(36)=9.30$, $p<0.001$, responsive/non-tremor: $t(42)=8.99$, $p<0.001$].

2.4.2 | Effects of Parkinson's disease and brain region on GABA

GABA/tCre ratios differed per brain region, such that the thalamus had the highest GABA/tCre ratio, followed by the motor cortex and visual cortex [factor REGION: $F(2,142)=10.012$, $\eta^2=0.121$, $p<0.001$]. There were no significant differences between patients and controls [factor GROUP: $F(3,71)=0.03$, $\eta^2<0.001$, $p=0.859$], and no interaction between group and region [$F(6,71)=0.149$, $\eta^2=0.002$, $p=0.858$], see also *Figure 2*. Bayesian analyses comparing GABA levels between controls and patients revealed moderate evidence towards the null hypothesis [$BF_{01}=4.72$]. This provides statistical evidence in favour of no effect between groups, suggesting that the lack of a statistical difference in our conventional analysis was not driven by a lack of statistical power.

Table 2 | Disease characteristics of participants. Disease severity as measured by MDS-UPDRS part III (maximum score is 108). Bradykinesia refers to the sum of UPDRS items 8-17,23; Rigidity to the sum of UPDRS items 3-7,18; Axial to the sum of UPDRS items 1,2,18-23), and Tremor refers to MDS-UPDRS item 24-33. The Frontal Assessment Battery (FAB, maximum is 18) and Mini Mental State Examination (MMSE, maximum is 30) were used as a measure of cognitive function. To compare between controls and patients we used a series of two-tailed T-tests for our continuous variables, and the $\chi 2$ test for the categorical variable 'gender'. The three patient groups were compared using a one-way multivariate ANOVA (or MANOVA) for the continuous variables. MDS-UPDRS subscores were checked separately for ON and OFF medication, all resulting significant scores (p values are in ***bold***), were post-hoc compared between each of the three groups with two- sample t-test, two-tailed. If the individual t-test comparison is significantly different from both other groups, the mean score with standard deviation is depicted in bold as well. Brady+rigid = combination of bradykinesia and rigidity scores; F = female, FAB = frontal assessment battery, LEDD = levodopa equivalent dose; M = male; MMSE = Mini-Mental State Examination; sig. = significance level.

	Resist	Respond	NoTremor	sig.		Patients	Controls	sig.
sample size	17	23	20			60	22	
gender	F:4 M:13	F:14 M:9	F:9 M:11	p = 0.064		F:27 M:33	F:10 M:12	p = 0.971
age	60.9 (9.6)	61.3 (12.8)	60.2 (9.2)	p = 0.886		60.8(10.7)	63.1(10.5)	p = 0.455
FAB	17.5 (0.6)	17.0 (1.1)	16.5 (4.4)	p = 0.109		17.0 (1.4)	17.5 (0.7)	p = 0.166
MMSE	29.1 (1.3)	29.2 (1.4)	29.2 (7.0)	p = 0.969		29.2 (1.3)	29.4 (0.9)	p = 0.503
LEDD	381 (329)	518 (289)	645 (503)	p = 0.219				
duration	***2.6 (1.8)***	5.7 (5.4)	5.1 (2.1)	***p = 0.019***				
	ON				**OFF**			
sub-UPDRS:	Resist	Respond	NoTremor	sig.	Resist	Respond	NoTremor	sig.
Axial	5.2 (4.3)	5.7 (3.3)	5.5 (4.3)	p = 0.947	6.4(4.8)	8.1 (4.3)	7.0 (4.2)	p = 0.492
Bradykinesia	13.1 (9.0)	13.3 (5.8)	12.5 (5.9)	p = 0.906	16.4 (9.2)	18.7 (5.8)	18.6 (8.7)	p = 0.619
Rigidity	4.8 (4.3)	4.7 (3.2)	5.3 (3.2)	p = 0.888	5.0 (5.8)	6.1 (3.8)	7.5 (3.8)	p = 0.249
Rest tremor	***10.4 (2.9)***	***6.2 (4.5)***	***0.0 (0.0)***	***p < 0.001***	10.4 (3.2)	9.3 (4.3)	***0.0 (0.0)***	***p < 0.001***
Tremor	***13.9(5.0)***	***8.9 (5.0)***	***0.7 (0.7)***	***p < 0.001***	14.4 (5.1)	13.0 (5.2)	***1.3 (1.2)***	***p < 0.001***
Brady+rigid	17.8(12.5)	18.0 (7.8)	17.8 (8.4)	p = 0.990	21.4(13.9)	24.8(8.9)	26.1(11.7)	p = 0.461

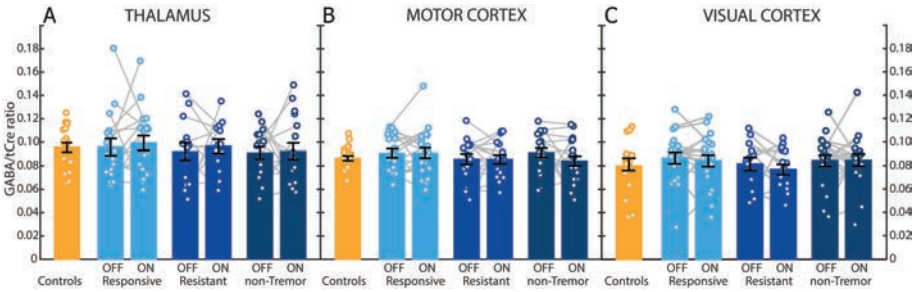

Figure 2 | GABA-ergic changes in Parkinson's disease. GABA-to-total-creatine (GABA/tCre) values measured in controls (n=22) and in the three Parkinson phenotypes (dopamine-responsive tremor (n=23), dopamine-resistant tremor (n=17), non-tremor (n=20)), during both ON and OFF medication, are shown for the thalamus (panel A), the motor cortex (panel B) and the visual cortex (panel C). Histograms indicate mean and standard error of the mean (in black). Against our expectations, we found no evidence for effects of disease, phenotype, or dopaminergic medication on GABA levels. MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale; tCre = total Creatine

2.4.3 | Effect of dopaminergic medication and Parkinson phenotype on GABA

There was no effect of medication [$F(1,32)=0.02$, $\eta^2<0.001$, $BF_{01}=6.64$, $p=0.894$], or medication by region [$F(2,64)=0.35$, $\eta^2=0.011$, $p=0.708$; $BF_{01}=10.11$], on GABA/tCre. In addition, there was no effect of patient phenotype [$F(2,32)=1.13$, $\eta^2=0.066$, $p=0.336$, $BF_{01}=3.96$], region by patient phenotype interaction [$F(4,64)=0.87$, $\eta^2=0.052$, $p=0.486$; $BF_{01}=6.77$], or medication by patient phenotype interaction [$F(2,32)=0.62$, $\eta^2=0.038$; $p=0.542$; $BF_{01}=3.84$]. As outlined above, the Bayesian analyses provided positive though not strong statistical evidence in favour of no effect (BF>3).

2.4.4 | Correlations between total disease severity and GABA/tCre ratio

There was a significant correlation between overall GABA concentration and total UPDRS motor scores [$F(1,38)=6.33$, $p=0.015$, $\eta^2=0.108$], which was not significantly different between regions [region x UPDRS: $F(2,)=0.13$, $\eta^2=0.003$, $p=0.876$]. In line with our a-priori prediction that disease severity as specifically related to GABA concentrations in the thalamus and motor cortex, we inspected the individual correlations between each of the three regions of interest. The effect was strongly significant in the motor cortex, with Bayesian statistics showing very strong evidence towards the H1 [$T(57)=-3.55$, $r^2=0.18$, $p<0.001$; $BF_{10}=40.57$], holding up to a strict Bonferroni-adjusted p-value of $p<0.007$. However, this effect was not significant in the thalamus [$T(54)=-3.09$, $r^2=0.04$, $p=0.084$; $BF_{01}=1.40$] or visual cortex [$T(57)=-1.53$, $r^2=0.02$, $p=0.132$; $BF_{01}=2.03$], both with anecdotal evidence in favour of the null

hypothesis. As a follow up we looked at consistency over sessions and found that the correlation in the motor cortex was significant in both sessions, see *Figure 3*; ON[T(55)=-2.54, r^2=0.11, p=0.014] and OFF medication [T(52)=-2.56, r^2=0.11, p=0.013]. In contrast, there were no significant correlations for the thalamus (ON: [T(48)=-1.43, r2=0.04, p=0.159] and OFF: [T(45)=-0.83, r^2=0.02, p=0.412]) or the visual cortex (ON: [T(51)=-1.20, r^2=0.03, p=0.236] and OFF: [T(50)=-0.78, r^2=0.01, p=0.438]). These effects did not differ substantially when correcting for age grey matter ratio, and Grey to White matter ratios (see *supplementary materials*). Furthermore, we found that there was no relationship between head motion (estimated using fMRI scans collected in the same session) and variables of interest (e.g. MDS-UPDRS, GABA/tCre). Additionally, correcting for head motion did not change the results (see *supplementary materials*). The negative correlation with UPDRS was specific for GABA, and not present for Glx/tCre (Glx is glutamate+glutamine) (see *supplementary materials*). Tremor-dominant patients (but not non-tremor patients) also showed a significant negative correlation between disease severity and GABA levels in the thalamus (see *supplementary materials*).

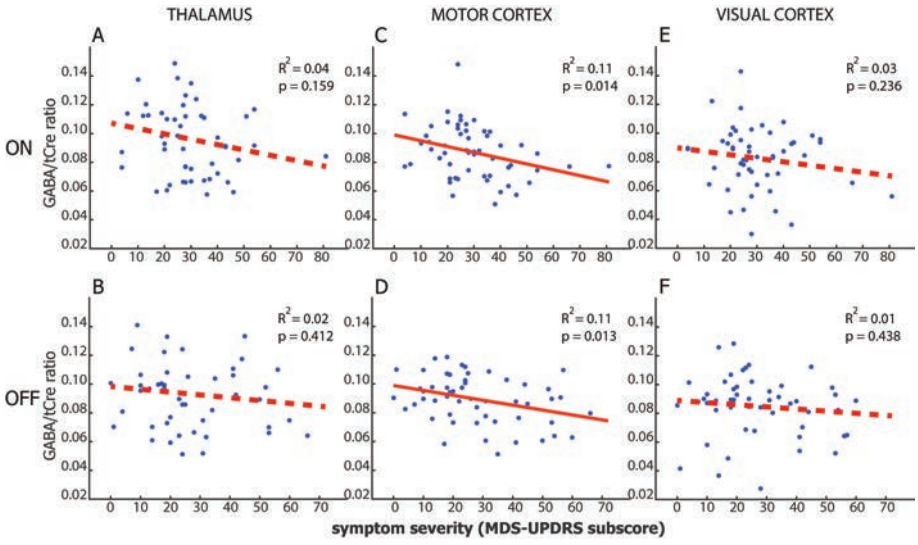

Figure 3 | Relationship between GABA and disease severity. Scatterplots of the relation between GABA/tCre and total MDS-UPDRS scores across the three Parkinson phenotypes, both ON medication (panels A, C, and E) and OFF medication (panels B, D, and F) for each of the three regions (first, second, and third column). Significant correlations are marked with a continuous red line. We find a consistent significant correlation between MDS-UPDRS scores and GABA levels in the motor cortex, both ON (panel C) and OFF medication (panel D), but no significant correlations for the thalamus (panel A, B) and visual cortex (panel E, F).
MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale; tCre = total Creatine.

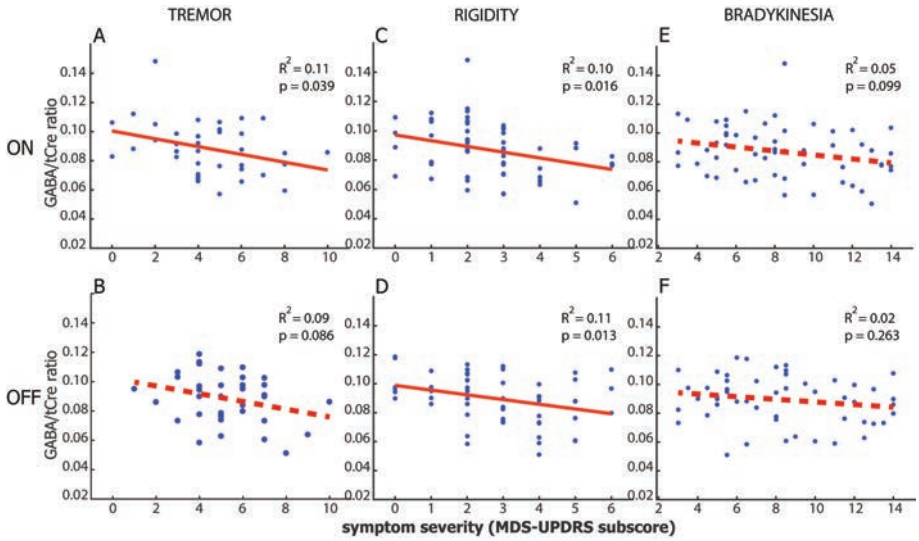

Figure 4 | Relationship between motor cortex GABA and motor symptom severity. This figure shows scatterplots of the relationship between GABA/tCre in the motor cortex and MDS-UPDRS sub-scores on the most affected side, during ON medication (upper row) and OFF medication (bottom row). The MDS-UPDRS sub-scores concern tremor (non-kinetic tremor: items 15 and 17), (panels A and B), rigidity (item 3) (panels C and D), and bradykinesia (items 4-8 & 14) (panels E and F). For definition of sub-scores, see Table 2. Significant correlations are marked with a continuous red line. We find the most consistent correlation for rigidity both ON and OFF medication, followed by tremor with a significant correlation for tremor ON medication, and at trend level OFF medication. Bradykinesia showed a marginal trend-level correlation ON medication, but no effect OFF medication, showing that rigidity scores match the results best, but the relationship between motor cortex GABA and symptom severity does not differ markedly between symptom type. MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale; tCre = total Creatine.

2.4.5 | Correlations between motor symptom severity and GABA/tCre ratio

As an exploratory analysis we tested post-hoc whether the correlation between motor cortex GABA and total UPDRS was driven by one of the three motor symptoms (bradykinesia, rigidity, and tremor). We found a significant correlation in the same direction for rigidity, both ON medication, *see Figure 4*, [T(55)=-2.49, r^2=0.10, p=0.016] and OFF medication [T(52)=-2.56, r^2=0.11, p=0.013]. In addition, we found a significant correlation for tremor ON medication: [T(36)=-2.14, r^2=0.11, p=0.039; BF_{10}=3.34] and a trend level correlation OFF medication: [T(33)=-1.18, r^2=0.09, p=0.086]. Finally, for bradykinesia we found a marginal trend-level correlation ON medication: [T(55)=-1.68, r^2=0.05, p=0.099] and no effect OFF medication: [T(52)=-1.131, r^2=0.02, p=0.263]. Looking at the overall evidence for each motor symptom, we found that the evidence was strongest for rigidity [BF_{10}=7.55], intermediate for tremor [BF_{10}=3.62],

and weakest for bradykinesia [BF_{10}=1.16]. There were no significant differences in the strength of the GABA-severity correlation between the three motor symptoms (p>0.5 for all possible comparisons). Taken together, these findings suggest that the relationship between motor cortex GABA and symptom severity does not differ markedly between symptom type, suggesting that higher motor cortex GABA levels are associated with a more benign symptom expression.

2.5 | Discussion

We investigated whether GABA levels in the thalamus and primary motor cortex, as well as a non-motor control region (visual cortex), differed between Parkinson's disease patients and controls, and whether patient phenotype and dopaminergic medication influenced GABA levels. In line with the classical model of basal ganglia dysfunction (Albin, Young *et al*. 1989), we expected patients to have higher GABA levels in the thalamus than controls. We further expected GABA levels to positively predict motor symptoms (specifically bradykinesia), and to normalize with dopaminergic medication. In addition, we investigated whether discrepancies between previous findings might be related to patient phenotype. This relationship was explored by including patients with a tremor-dominant Parkinson's disease phenotype (dopamine-responsive versus dopamine-resistant tremor) and patients with a non-tremor phenotype. Contrary to our predictions, the present study did not provide positive evidence for effects of disease, phenotype, or dopaminergic medication on GABA levels. In fact, there is a strong and consistent *negative* relationship between GABA in the motor cortex and symptom severity. This suggests that GABA plays a modulatory role in the pathophysiology of Parkinson's disease, which is independent of dopaminergic medication.

2.5.1 | Parkinson's disease is not associated with increased thalamic GABA

We did not confirm our hypothesis of increased GABA concentrations in the thalamus of Parkinson's disease. Thus, our findings are different from those by Dharmadhikari *et al*., who showed increased GABA levels in the thalamus of Parkinson's disease patients (Dharmadhikari, Ma *et al*. 2015), or those by Gong *et al*., who found a reduction of GABA levels in patients in a larger thalamus/basal ganglia voxel (Gong, Xiang *et al*. 2017). We considered several possible explanations for this null finding. First, a lack of power due to small sample size is unlikely, since our patient sample (N=60) was considerably larger than previous samples (n= 22 (Gong, Xiang *et al*. 2017), n=19 (Dharmadhikari, Ma *et al*. 2015). Second, methodological differences may have played a role: previous studies used a GABA-to-water ratio, whereas here we used a GABA-to-Creatine ratio. There is some evidence that in Parkinson's disease, Creatine (and

NAA) are also reduced in the thalamus and basal ganglia (Kickler, Krack *et al*. 2007, Gong, Xiang *et al*. 2017). However, reduced Creatine levels in Parkinson's disease would bias us towards confirming, rather than rejecting, our a priori hypothesis of an increased thalamic GABA-to-Creatine ratio in Parkinson's disease. Also, our findings were unchanged when using the GABA-to-Glx ratio. Third, although the voxel we used covers a large portion of the thalamus, by comparing tremor-dominant and non-tremor Parkinson's disease patients, we could partly rule out the possibility that opposing GABA-ergic changes in different thalamic nuclei may have balanced each other out. More specifically, increased GABA in the anterior ventrolateral nucleus that is involved in bradykinesia and rigidity, and reduced GABA in the posterior ventrolateral nucleus that is involved in tremor (Brodkey, Tasker *et al*. 2004, Helmich, Janssen *et al*. 2011). If this were the case, then only the non-tremor patient group would have shown increased thalamic GABA levels, while in fact there were no differences between clinical phenotypes. Taken together, our findings do not support the idea that increased thalamic GABA levels are associated with Parkinson's disease symptoms, as predicted by the classical basal ganglia model. This is consistent with other pathophysiological models that focus more on abnormal oscillations (Brittain, Sharott *et al*. 2014), or that explain Parkinson symptoms by increased thalamic rebound firing rather than enhanced thalamic inhibition (Kim, Kim *et al*. 2017).

2.5.2 | There is an inverse relationship between disease severity and GABA levels in the motor cortex

There are no previous reports of changes in GABA levels in the motor cortex of Parkinson's disease patients. However, our finding that lower GABA levels were associated with higher disease severity is in line with previous work using other modalities: fMRI results showed increased motor cortex BOLD activity in Parkinson's disease during thumb pressing movements, and this effect correlated with higher rigidity scores (Yu, Sternad *et al*. 2007). The positive relationship between motor cortex activity (fMRI) and disease severity may reflect the same mechanism as the inverse relationship between motor cortex inhibition (GABA) and disease severity found here: several studies in healthy subjects showed that reduced GABA levels were associated with increased task-related BOLD responses in the cortex with examples from the motor cortex (Stagg, Bachtiar *et al*. 2011), the anterior cingulate cortex (Northoff, Walter *et al*. 2007), and visual cortex (Donahue, Near *et al*. 2010).

Others have looked at cortical excitability in Parkinson's disease using transcranial magnetic stimulation (TMS). Cortical excitability is thought to represent the relative influence of intracortical inhibitory and facilitatory networks (Bunse, Wobrock *et al*. 2014). Many studies have reported reduced short interval intracortical inhibition (SICI) in Parkinson's disease (MacKinnon, Gilley *et al*. 2005, Rothwell, Day *et al*. 2009, Carrillo, Palomar *et al*. 2013) and a progressive reduction of SICI with disease

progression (Kojovic, Kassavetis *et al*. 2015). Pharmacological studies suggest that SICI is predominantly dependent on (inhibitory) GABAA receptor activity (Ziemann 2013). As cortical excitability represents the relative influence of intracortical inhibitory and facilitatory networks, it is not clear whether SICI necessarily involves impaired inhibition or is driven by increased intracortical facilitation (MacKinnon, Gilley *et al*. 2005). Using MRS, we verified that disease severity was not explained by variations in glutamate: Glx (glutamate+glutamine) concentrations did not correlate with disease severity, while the GABA/Glx ratio followed the same negative correlation as reported for the GABA/tCre ratio (see *supplementary materials*). This suggests that, within our dataset, it would be a change in cortical inhibition, not facilitation, that explains inter-individual variations in disease severity. The functional role of the shifted cortical inhibition/facilitation balance towards enhanced cortical excitation in Parkinson's disease remains unclear. On the one hand, enhanced motor cortex excitability could represent a compensatory downregulation to overcome the excessive inhibitory influence from the basal ganglia (John C. Rothwell 2013). On the other hand, enhanced motor cortex excitability could interfere with processing of inputs from upstream areas, thereby disrupting the encoding of motor parameters – resulting in bradykinesia (Kumar *et al*., 2010). The latter interpretation would fit best with our findings.

2.5.3 | Is cerebral GABA beneficial for Parkinson's disease?

The negative correlation between GABA and disease severity we report may relate to converging ideas suggesting that GABA acts as a neuroprotective agent in neurodegenerative disorders. This idea was put forward in the GABA-collapse hypothesis (Hurley, Brandon *et al*. 2013, Błaszczyk 2016), which proposes that GABAergic input protects neurons from calcium-based neurotoxicity. This notably affects dopamine neurons, because of high energy requirements and dependence on regular slow calcium-based pacemaker activity (Hurley, Brandon *et al*. 2013). Evidence for the contribution of calcium-based neurotoxicity in Parkinson's disease comes from post-mortem studies showing a brain-wide imbalance of calcium channels (Hurley, Brandon *et al*. 2013). In rats, GABA-producing transplants increases survival rates of implanted dopaminergic neurons (Winkler, Bentlage *et al*. 1999). In multiple human intervention studies in late stage Parkinson's disease, upregulating the GABA-producing glutamic acid decarboxylase (GAD) protein with gene therapy improved motor symptoms (LeWitt, Rezai *et al*. 2011), and less invasive alternatives in the form of GABA receptor modulators, such as benzodiazepines or zolpidem, have also been reported to help normalize Parkinson's disease related symptoms (Pourcher, Bonnet *et al*. 1989, Hall, Prokic *et al*. 2014) – although opinions differ with respect to the use of these drugs (Lavoisy and Marsac 1997). Outside of the motor system; a study by Firbank *et al*. showed that visual hallucinations in Parkinson's disease were associated with reduced GABA in the visual cortex, while GABA levels of non-affected

patients showed no difference from control subjects (Firbank, Parikh *et al*. 2018). The role of GABA in neurodegeneration is likely not specific to Parkinson's disease, given the role of altered GABA levels in other neurodegenerative diseases such as dystonia (Levy and Hallett 2002, Marjańska, Lehéricy *et al*. 2013). Taken together, this study provides evidence for an inverse association between motor cortex inhibition (GABA) and disease severity in Parkinson's disease, which may be related to a protective role of GABAergic inhibition. Intervention studies are necessary to test whether a potentiation of GABA-ergic mechanisms worsens or improves Parkinson's disease symptoms, to establish whether GABA plays a compensatory or pathophysiological role in Parkinson's disease.

2.5.4 | Reliability & Limitations

This study consists of a large number of Parkinson's disease subjects (n=60), with respectable group sizes (n=23/n=20/n=17). Since we measured GABA and UPDRS on two independent days (ON and OFF dopaminergic medication), this also provides a measure of replicability. The reported GABA correlations with disease severity were not only strongly significant, but consistent over sessions: scores on both days yielded similar, significant negative correlations. Furthermore, we were able to rule out that the correlation between GABA and disease severity was driven by age or by grey matter atrophy (see *supplementary materials*), and GABA/tCre values were comparable to similar studies, especially when considering participants within this age range (Geramita, van der Veen *et al*. 2011, Gao, Edden *et al*. 2013). We also found that the relative GABA/tCre levels between regions (thalamus>motor cortex>visual cortex) were consistent with other studies (Levy and Hallett 2002). This study relied on the ratio of GABA to creatine, which may have had mixed consequences; while we do not anticipate problems in our cortical voxels, there are reports of reduced thalamic creatine, which suggest that this may have led to reduced sensitivity for patient GABA reduction in our thalamic region of interest (as discussed above). The sequence in this study was not optimized to eliminate macromolecules from our spectra (which are known to be co-edited with the GABA signal at 3ppm) - thus a macromolecule estimation was included in the LCModel fit. So far, literature on spectroscopy in PD does not provide evidence for either elevated or reduced macromolecule levels (Emir, Tuite *et al*. 2012). However, as potential macromolecule signal was not fully eliminated, we cannot exclude the possibility that our results are affected by its presence. Finally, considering the reduced power and sensitivity of MRS in subcortical structures (Bottomley 1987), it is possible that this approach is not suitable to detect subtle changes in thalamic GABA, or is only sensitive to GABA in specific compartments. According to recent consensus, MRS is most sensitive to extracellular unbound GABA, which is involved in tonic inhibition (Rae 2014, Stagg 2014, Dyke, Pépés *et al*. 2017). Specifically, extracellular GABA depends on accumulation of GABA spill-over from synaptic transmission (Glykys and Mody 2007), in addition to a small percentage

of GABA released by local glia cells (Lee, Yoon *et al*. 2010). Intracellularly, GABA is converted from glutamate only when there is a demand in the axon terminal itself, and subsequently contributes only minimally to the MRS signal (Martin and Rimvall 1993, Buddhala, Hsu *et al*. 2009). In Parkinson's disease, the predicted increase in tonic inhibition of the thalamus (Redgrave, Rodriguez *et al*. 2010) should therefore lead to increased extracellular GABA, which MRS can measure. Taken together, it is unlikely that our method (MRS) was blind to the thalamic changes predicted by the classical basal ganglia model, and this explains our negative finding.

2.5.5 | Conclusion

Our findings show that GABA concentrations in the primary motor cortex are inversely correlated with disease severity, independent of dopaminergic medication (i.e. present across OFF and ON dopaminergic medication sessions), and independent of the type of motor symptom. This suggests that GABA may play a modulatory role in the pathophysiology of Parkinson's disease, independent of dopaminergic denervation. We speculate that cerebral GABA might have a protective role, either at the neuronal level (e.g. by preventing calcium-based neurotoxicity) or at the circuit level (e.g. by preventing dysfunctional motor hyperactivity). If proven to be correct in further studies (using other modalities, e.g. flumazenil PET), a potential neuroprotective role of GABA-ergic mechanisms could have important implications for the treatment of patients with Parkinson's disease.

2.6 | References

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2.7 | Supplementary materials

2.7.1 | Full description of siemens MRS protocol (svs_edit_859)

The PRESS 90° pulses were Optimised-90 pulses, with a duration of 2.6ms with a bandwidth of 2400 Hz, the 180° refocusing pulses consisted of a HyperSecant pulse with a duration of 6.0 ms, covering a bandwidth of 3200 Hz. The edited pulses were a Gauss pulse, with a duration of 17.2 ms and a bandwidth of 44 Hz (not double banded). Shimming was performed according to the provided automated Siemens shimming procedure, GRESHIM: A fieldmap was acquired as a double gradient echo sequence and the shim coil currents were computed by fitting 2nd order spherical harmonics in the spectroscopy volume of interest. This procedure was repeated twice, using the NAA line-width as part of the quality assessment.

2.7.2 | LCModel stimulated basis sets

We performed LCModel fits using simulated spectra for both the difference spectrum (provided by Siemens) and non-edited spectrum as these contained our metabolites of interest (GABA and creatine).

The simulated spectra contained the following metabolites:
Difference: GABA, NAA, NAAG, Glu, Gln.
Non-edited: NAA, NAAG, Cr, PCr, GABA, Gln, Glu, 2HG, Lac, Glc, Cho, GPC, PC, PE, mI, Tau, Act, Asp, Ala, GSH, bHb, Gly, Scyllo, Gua, Pyr, Suc.

Additionally, LCModel estimates the contributions of MM17 and MM20 (macro molecular components at 1.7 and 2.0 ppm) in the difference spectrum, and the contributions of Lip13a, Lip09 (lipid components at 1.3 and 0.9 ppm), MM09, MM12, MM14 and MM17 (macro molecular components at 0.9, 1.2, 1.4 and 1.7 ppm) in the original spectrum

2.7.3 | Nuisance variables

Movement correction: Movement is an important confound in any MRI measurement, especially so for MRS datasets. Whereas fMRI offers an easy way of estimating (and correcting) the level of movement, MRS does not. Here, we have addressed this problem in two different ways: First, we have tried to minimize movement by adding head pads and fixing the head as much as possible (as much as patient comfort would allow). Second, we minimized the effect of signal intensity by using the ratio of GABA to creatine – this means that any general loss of signal should be evenly represented in both the signal intensity of GABA, as in the signal intensity of creatine.

To address this issue further, we have further control analysis to estimate subjects' head movements, taking advantage of fMRI data that were collected in the same session in the same participants:

Aside from MRS, patients also underwent a 10-minute fMRI resting state scan (Dirkx *et al*., in press in Brain), just before the anatomical scan and subsequent spectroscopy scans. As fMRI does provide a method to estimate movement (spatial realignment), we included this measure to serve as an indicator for the level of movement of the subject lying in the scanner. To this end, we used the scan-by-scan realignment parameters calculated during fMRI preprocessing. Specifically, we calculated the Euclidean distance traveled by the head from the first to the last scan. Next, we investigated whether these measures correlated with MSD-UPDRS scores and GABA/tCre ratio's in the motor cortex, and whether including these measures influenced the reported relationship between MSD-UPDRS and GABA/tCre ratio's in the motor cortex. We found that there was no relationship between head movements and either MDS-UPDRS scores [$T(55)=1.01$, $p=0.319$, $r^2=0.02$] or GABA/tCre ratios in the motor cortex [$T(55)=-0.27$, $p=0.787$, $r^2<0.01$]. Additionally, we found that the relationship between MDS-UPDRS scores or GABA/tCre ratios was unaffected by including head movements as a confound (original correlation [$T(57)=-3.55$, $r^2=0.18$, $p<0.001$] and correlation after correction [$T(54)=-3.40$, $r^2=0.18$, $p=0.001$]).

Grey matter ratio (GMR): The correlation of GMR to GABA/tCre values was tested using a repeated measures ANOVA of GABA/tCre values with region as a fixed between subject variable, medication as a within subject variable and GMR as a covariate as GMR values differ for each region. We found no overall effect of GMR [$F(1,168)=0.09$, $\eta^2<0.001$, $p=0.769$], but a significant interaction between region and GMR [$F(2,168)=3.43$, $\eta^2=0.039$, $p=0.039$], suggesting that this interaction is regionally specific. We indeed found a significant effect of negative GMR correlation with GABA/tCre in the thalamus (Thalamus: $T(35) =-2.05$, $p=0.045$, $r^2=0.07$], no effect in the motor cortex [$T(57)=0.03$, $p=0.977$, $r2<0.01$], and no effect in the visual cortex [$T(57)=1.53$, $p=0.181$, $r^2=0.03$]).

Age: The correlation of age to GABA/tCre values was tested using a repeated measures ANOVA of GABA/tCre values with region and medication as a within subject variable and age as a covariate. We find no overall effect of age [$F(1,33)=0.68$, $\eta^2=0.020$, $p=0.415$], and no interaction between region and age [$F(2,66)=0.767$, $\eta^2=0.023$, $p=0.469$].

One of the main questions that arises is whether age and/or GMR would affect the results we report above. Here we show the correlation of GABA/tCre with UPDRS scores in the motor cortex corrected for age and GMR, we show the correlation for mean-UPDRS and UPDRS ON and OFF separately using Multiple Linear Regression

analyses. We found a strongly significant correlation for GABA/tCre with mean UPDRS after correction [T(48)=-2.86, p=0.006], with nuisance variables age: [T(48)=-1.53, p=0.132], and GMR: [T(48)=-0.687, p=0.495], a significant correlation with GABA/tCre and UPDRS OFF medication: [T(50)=-2.32, p=0.025], with nuisance variables age: [T(50)=-1.22, p=0.227], and GMR: [T(50)=-1.16, p=0.250]. The correlation with GABA/tCre and UPDRS ON medication is still at trend level, [T(53)=-1.93, p=0.059], with both nuisance variables showing a non-significant interaction; age: [T(50)=-1.01, p=0.316], and GMR: [T(50)=0.07, p=0.943].

Grey/white matter ratio: There was no significant difference between GM/WM ratios between groups, in any of the three MRS voxels [Thalamus: F(3,75)=0.43, p=0.732. Motor Cortex F(3,75)=0.60, p=0.616, Visual Cortex: F(3,75)=2.06, p=0.112], as determined by multivariate ANOVA. For a visual illustration, see *Supplementary Figure 2* included below. In general, the proportion of CSF was very low, causing the GM ratio and the GM/WM ratio to be highly correlated [Thalamus: T(77)=20.7, p< 2.9E-33, r^2=0.85], Motor Cortex: T(77)=19.9, p< 3.9E-32, r^2=0.84], visual Cortex T(77)=16.1, p< 2.3E-26, r^2=0.77]). As we expect the GABA to originate mainly from grey matter, we focused solely on grey matter in our analyses; however, considering the very high correlation between the two values we would not expect major differences in our results using either method.

2.7.4 | Cortical excitability: Glx and GABA/Glx ratio

When discussing cortical excitability, it is relevant to report the inhibitory/excitatory balance; or in this case, the balance between GABA and Glutamate. Here we report the correlation of mean Glx with mean UPDRS symptoms (averaged over both sessions) and the inhibitory balance, or the mean GABA/Glx ratio to mean UPDRS scores using Multiple Linear Regression analyses.

Glx: We observed no correlation in the motor cortex [T(57)=-1.12, p=0.268, r^2=0.02, BF_{10}=0.19, BF_{01}=5.22], the thalamus [T(57)=0.562, p=0.562, r^2<0.01, BF_{10}=0.30, BF_{01}=3.38], or the visual cortex [T(58)=-0.50, p=0.634, r^2<0.01, BF_{10}=0.18, BF_{01}=5.56]. This suggests that the concentration of excitatory neurotransmitters does not explain disease severity.

GABA/Glx: We saw a significant correlation in the motor cortex [T(57)=-2.26, p=0.028, r^2=0.08, BF_{10}=0.50, BF_{01}=1.99], but not a significant correlation in thalamus [T(53)=-1.52, p=0.134, r^2=0.04, BF_{10}=1.71, BF_{01}=0.58], or visual cortex [T(57)=-0.80, p=0.426, r^2=0.01, BF_{10}=0.22, BF_{01}=4.52]. Taken together, this shows that there is a specific negative correlation between disease severity and motor cortex levels of GABA, but not Glx.

2.7.5 | Disease Severity: tremor/non-tremor group

We tested whether the correlation between GABA and disease severity was present for each Parkinson's disease phenotype independently. In patients with tremor-dominant Parkinson's disease (both dopamine-responsive and dopamine-resistant), we found a similar correlation between GABA/tCre concentration and total UPDRS scores as for the entire patients group [$F(1,33)=6.47$, $p=0.016$, $\eta^2=0.164$]. The correlation with UPDRS was not significantly different between regions [region x UPDRS: $F(2,66)=0.44$, $p=0.635$, $\eta^2=0.013$]. As for the entire sample, the effect was most pronounced in the in the motor cortex (see *Supplementary Figure 1*), where the correlation was strongly significant [$T(37)=-3.38$, $p=0.002$, $r^2=0.24$]. We also found a significant negative correlation in the thalamus [$T(35)=-2.28$, $p=0.029$, $r^2=0.13$], but not the visual cortex [$T(37)=-1.48$, $p=0.146$, $r^2=0.06$]. When looking at the ON and OFF sessions separately, we found that the correlation between motor cortex GABA and disease severity (total UPDRS) was significant for each of the two sessions, while the correlation in the thalamus was trend-level significant, and the correlation in the visual cortex was non-significant on both days.

Motor Cortex ON: [$T(36)=-2.84$, $p=0.007$, $r^2=0.18$] and OFF: [$T(33)=-2.12$, $p=0.042$, $r^2=0.12$],
Thalamus, ON: [$T(31)=-1.70$, $p=0.099$, $r^2=0.09$] and OFF: [$T(29)=-0.83$, $p=0.106$, $r^2=0.09$].
Visual cortex, ON: [$T(33)=-1.06$, $p=0.296$, $r^2=0.03$] and OFF: [$T(31)=-1.00$, $p=0.326$, $r^2=0.03$].

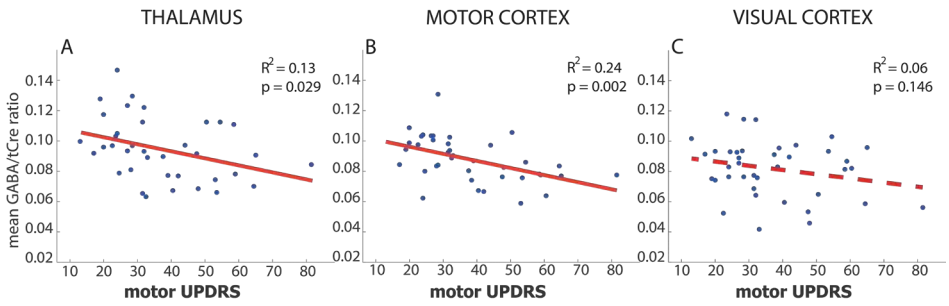
Using Bayesian statistics, we found strong evidence towards the H1 in the motor cortex [$BF_{10}=22.91$], indicating a strong correlation between UPDRS scores and GABA/tCre values. Furthermore, we found anecdotal evidence towards H1, in the thalamus [$BF_{10}=2.05$], and moderate evidence toward a null effect for the visual cortex [$BF_{10}=0.26$, or $BF_{01}=3.89$].

In non-tremor group, (which is much smaller and possibly underpowered), we found no correlation between disease severity (total UPDRS) and GABA levels in the motor cortex [$T(18)=-1.56$, $p=0.136$, $r^2=0.12$, $BF_{10}=0.79$, or $BF_{01}=3.52$], the thalamus [$T(17)=0.07$, $p=0.943$, $r^2=0.00$, $BF_{10}=0.28$, or $BF_{01}=1.27$], or the visual cortex [$T(37)=-1.48$, $p=0.146$, $r^2=0.06$, $BF_{10}=0.30$, or $BF_{01}=3.39$]. Taken together, this analysis suggests that there is a negative correlation between disease severity and thalamic GABA in tremor-dominant Parkinson's disease, while the lack of an effect in non-tremor Parkinson's disease may be explained by a (much) smaller group.

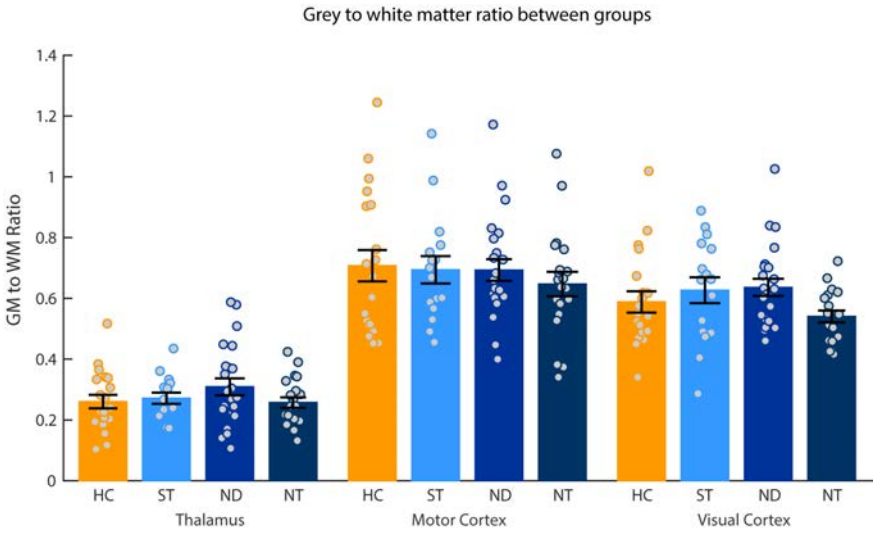
2.7.6 | Supplementary Figures

Supplementary Table 1 | Total patients and Controls in each group of whom we have datasets available (total) on both days. For each region is depicted which spectra could be estimated reliably with an %SD<50, and the number of spectra did not meet these requirements; (accepted %SD<50|rejected).

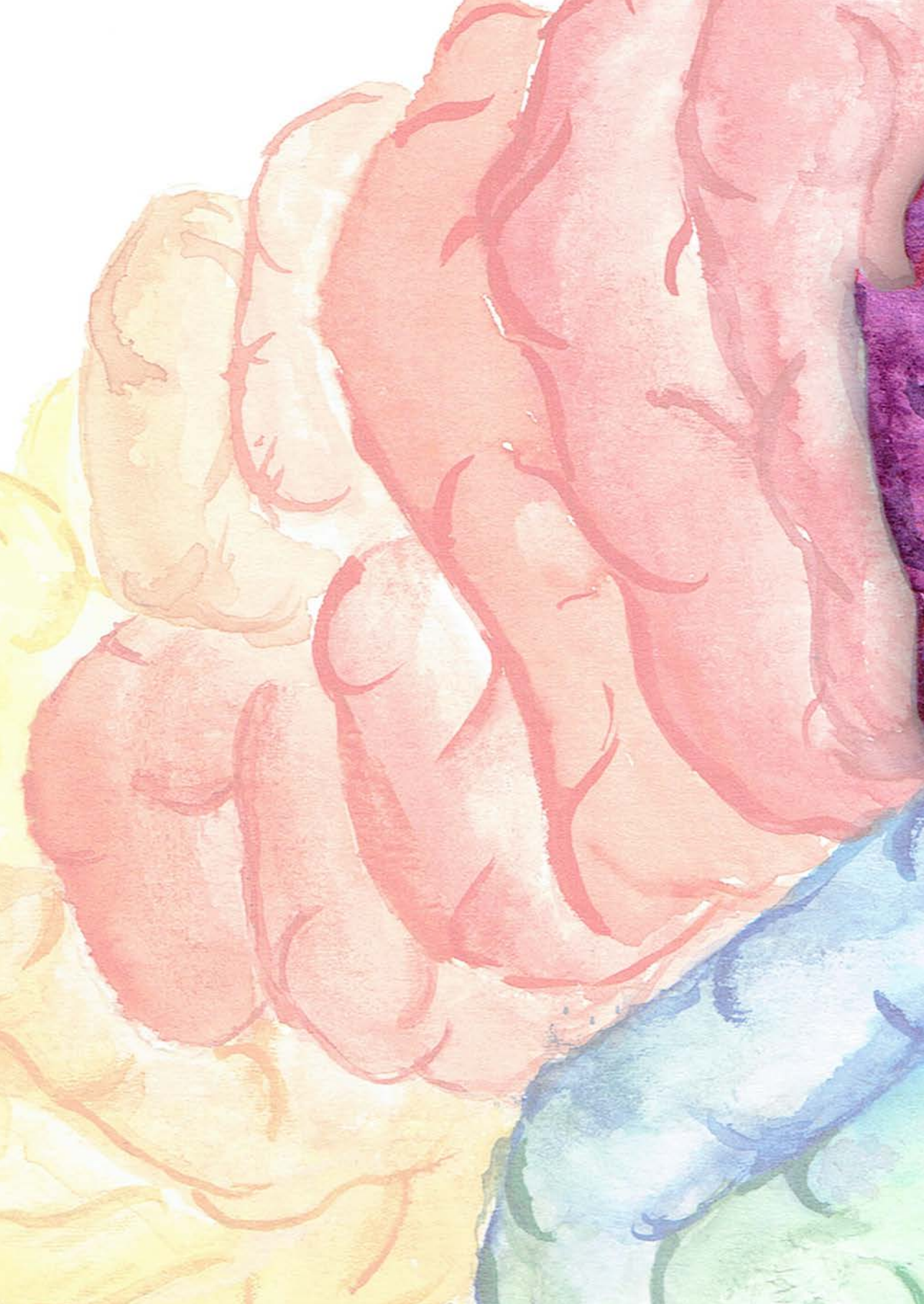
Group		Total	Thalamus	Motor cortex	Visual cortex
resistant	day 1	17	14|3	17|0	15|2
	day 2	16	13|3	16|0	12|4
responsive	day 1	22	20|2	21|1	22|0
	day 2	20	17|3	19|1	19|1
noTremor	day 1	20	17|3	20|0	19|1
	day 2	19	16|3	18|1	18|1
control	day 1	22	19|3	21|1	22|0
	day 2	19	17|2	18|1	16|3



Supplementary Figure 1 | Tremor patients only; ccorrelation of total UPDRS scores (averaged over both scanning days) and GABA/total creatine ratio. **(A)** We find a significant decrease in GABA for higher UPDRS scores in the Thalamus, **(B)** and a strongly significant decrease in the motor cortex. **(C)** We find no correlation in the visual cortex



Supplementary Figure 2 | Grey Matter (GM) to White Matter (WM) ratios (GM/WM) measured in all subgroups in the three regions of interest (thalamus, motor cortex, visual cortex). Yellow depicts the Healthy Controls (n=22) with the three Parkinson Disease (PD) phenotypes in blue (PD resiSTant to dopamine (n=17), PD respoNDing to dopamine (n=23) and PD Non-Tremor (n=20). Histograms indicate mean and standard error of the mean (in black). There is no significant difference between subgroups for each of the three regions of interest.



CHAPTER 3

Midbrain neurodegeneration in tremor-dominant and non-tremor Parkinson's disease Evidence from free water imaging

Contributing Authors
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3.1 | Abstract

Parkinson's disease is characterized by tremor, rigidity, and bradykinesia, and is associated with dopaminergic cell loss in the midbrain. An important clinical factor to distinguish Parkinson subtypes is the absence or presence of resting tremor. Post-mortem studies indicate that tremor and non-tremor subtypes are associated with different patterns of neurodegeneration in the midbrain. Specifically, tremor-dominant patients have reduced cell loss in the substantia nigra (SN), but increased cell loss in the retro-rubral area (RRA). However, in vivo evidence is lacking. Here we investigated the degree of neurodegeneration in the SN and RRA between patients with tremor-dominant (n=37) and non-tremor (n=19) Parkinson's disease, along with 23 healthy controls. In all subjects, we used diffusion tensor MRI to quantify free water, a marker of neurodegeneration, in manually defined regions of interest in the SN and the RRA. We further tested whether free water intensity in these regions correlated with bradykinesia and tremor severity. We found increased free water signal for non-tremor compared with tremor-dominant patients with Parkinson's disease in the posterior SN, but no differences in the RRA. However, we found a strong positive correlation between clinical resting tremor severity and free water signal in the RRA. We conclude that different patterns of neurodegeneration in the midbrain are associated with tremor (severity) and non-tremor motor symptoms. Motor subtype is a relevant factor that should be considered in future structural imaging studies.

3.2 | Introduction

Parkinson's disease is a neurodegenerative disorder characterized by bradykinesia, rigidity, and tremor. These symptoms are thought to be caused by a progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta, and subsequent striatal dopamine depletion (Kish, Shannak *et al*. 1988). However, the expression of these motor symptoms varies markedly between patients. On the basis of their predominant motor symptoms, Parkinson patients have been divided into clinical subtypes. One of the most commonly used distinctions is between tremor-dominant patients (who have a clear tremor in addition to akinetic-rigid symptoms) and non-tremor patients (who only have akinetic-rigid symptoms) (Helmich 2018).

Tremor-dominant and non-tremor patients also differ in other aspects than merely the presence or absence of the tremor itself. Clinically, tremor is thought to be a marker of benign Parkinson's disease: tremor-dominant patients show a slower overall disease progression (Selikhova, Williams *et al*. 2009), a slower cognitive decline (Wu, Le *et al*. 2011), and reduced likelihood to develop Parkinson-associated dementia (Aarsland, Andersen *et al*. 2003, Williams-Gray, Foltynie *et al*. 2007). Post-mortem studies report differences in the pattern of dopaminergic cell loss in the midbrain. Specifically, non-tremor patients show higher substantia nigra pars compacta (SNc) degeneration (Jellinger and Paulus 1992). The reverse was found in the dopaminergic retro-rubral area (RRA), where tremor-dominant patients had more neurodegeneration than non-tremor patients (Hirsch, Mouatt *et al*. 1992). These cerebral differences may thus account for some of the clinical variation: increased SNc degradation suggests reduced (striatal) dopaminergic function, which could be a substrate for the faster decline and emergence of some cognitive symptoms. Indeed, PET imaging studies show lower dopamine transporter binding in the striatum for non-tremor patients (Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). On the other hand, increased RRA degeneration in tremor-dominant patients might play a role in tremor symptoms (Helmich, Hallett *et al*. 2012). Experiments involving non-human primates have shown that animals with predominant RRA damage most resembled the tremor phenotype (Deutch, Elsworth *et al*. 1986, Bergman, Raz *et al*. 1998), while primarily SNc affected animals appeared more akinetic. Using functional MRI, it has been shown that dopamine reduces Parkinson's tremor by acting on the globus pallidus and on the ventrolateral thalamus (Dirkx, den Ouden *et al*. 2017). Both of these regions receive dopaminergic input from the RRA (Jan, François *et al*. 2000, Sánchez-González, García-Cabezas *et al*. 2005). This specific pattern of dopamine depletion may cause abnormal pallidal activity that triggers tremor oscillations in the basal ganglia, which are then transmitted to the cerebello-thalamo-cortical circuit that maintains and amplifies the tremor (dimmer-switch hypothesis; (Helmich 2018)). Here we test whether differences in the pattern of dopaminergic cell loss between tremor-dominant and non-tremor patients, which so far have only been demonstrated

in post-mortem studies, can be shown in vivo. MR imaging allows us to consider a summary metric of tissue degeneration (rather than focusing on dopaminergic neurons). Furthermore, using an in-vivo approach enables us to assess a larger proportion of patients at earlier stages of the disease.

Imaging studies can offer insight in anatomical changes during the course of the disease. Work with diffusion tensor imaging (DTI) has shown a significant decrease in fractional anisotropy (FA, a proxy for cell degeneration) in the posterior SN of patients compared to healthy controls (Vaillancourt, Spraker *et al*. 2009, Péran, Cherubini *et al*. 2010, Rolheiser, Fulton *et al*. 2011, Lehéricy, Sharman *et al*. 2012, Zhan, Kang *et al*. 2012), although other studies have failed to replicate these FA findings (Menke, Jbabdi *et al*. 2010, Focke, Helms *et al*. 2011, Schwarz, Abaei *et al*. 2013). Furthermore, an improved diffusion-based method using a two-compartment model is a promising proxy of neurodegeneration (Pasternak, Sochen *et al*. 2009). This two-compartment model separately estimates two different modalities, a free water compartment characterized by an isotropic tensor with diffusivity of free water, and a tissue compartment modelled by a diffusion tensor (Pasternak, Sochen *et al*. 2009). The estimated free water component within a voxel fully covering grey (or white) matter areas is theorised to be a *direct* consequence of atrophy-based neurodegeneration. Using this method, a consistent pattern of free water increase for Parkinson's disease versus controls was found in several single-site and multi-site studies (Ofori, Pasternak *et al*. 2015, Planetta, Ofori *et al*. 2015, Burciu, Ofori *et al*. 2017, Ofori, Krismer *et al*. 2017, Guttuso, Bergsland *et al*. 2018, Yang, Archer *et al*. 2019). Moreover, further studies show consistent increases in free water signal over several years, mirroring the progression of the disease (Ofori, Pasternak *et al*. 2015, Burciu, Ofori *et al*. 2017, Guttuso, Bergsland *et al*. 2018). Together, this suggests that free water intensity in the midbrain is sensitive to the pattern of neurodegeneration seen in Parkinson's disease, and that it may also be sensitive to differences between motor phenotypes.

Here we aimed to revisit, in vivo, previous post-mortem results showing different levels of neuronal degeneration in the SN and RRA for Parkinson's disease patients with a tremor-dominant versus a non-tremor phenotype (Hirsch, Mouatt *et al*. 1992, Jellinger and Paulus 1992), in addition to baseline differences in free water levels between patients and controls. We tested the hypothesis that there is increased (posterior) SN free water in non-tremor versus tremor-dominant Parkinson's disease, while there should be higher free water intensity in the RRA in tremor-dominant versus non-tremor Parkinson's disease. We also tested whether free water intensity in the SN and RRA correlated with bradykinesia and tremor severity, respectively.

3.3 | Methods

3.3.1 | Subjects

The study was conducted according to the standards of the 1964 Declaration of Helsinki and was approved by the local ethics committee (reference: CMO 2014/014). Before inclusion, all participants provided their informed written consent.

This project included two groups of patients with Parkinson's disease: tremor-dominant patients [n=37, 16 Female (F), 21 Male (M)] and non-tremor patients [n=19, 8F, 11M]), as well as one group of age matched healthy controls [n=23, 11F, 12M]. Clinical details are presented in *Table 1*. Patients were included if they fitted either of the two clinical groups of interest. Tremor-dominant Parkinson's disease was defined as a resting tremor score of 1 point or more in at least one arm on item 17 of the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS part III) (Dirkx, den Ouden *et al*. 2017), and a clear history of tremor. Non-tremor Parkinson's disease was defined as the absence of resting tremor in all limbs (MDS-UPDRS resting tremor score of 0) (Helmich, Janssen *et al*. 2011). Kinetic tremor, however, was allowed given that this tremor has a different pathophysiology (Dirkx, Zach *et al*. 2018). Exclusion criteria were cognitive dysfunction defined as Mini-Mental State Examination (MMSE)<26 (Cockrell and Folstein, 2002) and frontal assessment battery (FAB)<13 (Lima *et al*., 2008), neurological or psychiatric comorbidity, severe head-tremor, known allergy against levodopa-benserazide or domperidone and severe dyskinesias.

3.3.2 | Measurements

Clinical Ratings: Full tremor scores consisted of all 18 items of the MDS-UPDRS part III; sub-scores were used to calculate the dopamine response for each of the three motor symptoms separately: resting tremor and re-emergent tremor (non-kinetic tremor: items 15 and 17), bradykinesia: (items 4-8 & 14) and rigidity (items 3 and 9).

MRI: DTI were acquired on a 3 Tesla MRI Siemens PRISMA system (Siemens Healthcare, Erlangen, Germany), equipped with a 32-channel head coil, using the DTI RESOLVE sequence (Cohen-Adad, 2012). The sequence consisted of the following parameters (repetition time (TR) = 2200ms; echo time (TE) = 69ms; b values = 0-1000 s/mm2; diffusion gradient directions = 34; matrix = 220x220, slice thickness = 1.8 mm, slice number = 14). AutoAlign Head LS was used to place the DTI slab in the same orientation for all participants. The top slice was placed approximately beneath the splenum and rostrum part of the corpus callosum. On the same day, participants had further (MR) measurements taken, which will be reported on in other manuscripts.

3.3.3 | Longitudinal free-water mapping analysis

Data preprocessing was performed with the FMRIB Software Library (FSL; Oxford, UK) and custom UNIX shell scripts. After acquisition, each diffusion scan underwent the following preprocessing steps; motion correction, eddy current correction, b-vec rotation and skull stripping to remove non-brain tissue from the diffusion volumes. Motion was extracted from the affine motion and eddy current correction, and quantified by the root mean square deviation averaged over all volumes. The root mean square deviation is the average difference between the center of volume of the b0 and each diffusion volume in millimetres. The gradient directions were rotated to match the eddy current corrections.

Free-water maps and free-water corrected diffusion tensor maps were calculated from the motion and eddy current corrected volumes using a custom written MATLAB R2013a (The Mathworks) code provided by their developers (Pasternak, Sochen *et al*. 2009, Pasternak, Westin *et al*. 2012). The process includes a minimization procedure that fits a two-compartment model, to quantify the fraction of free-water volume in each voxel generating a free water map. The two-compartment model predicts the attenuation of the signal by the free water and is built up as the sum of two compartments: one that models free water, and a second tissue compartment that models water molecules in the vicinity of tissue membranes. This process is described in detail in (Pasternak, Sochen *et al*. 2009).

3.3.4 | Regions of interest

For each subject, regions of interest were manually placed on the b0 image in MNI space. They were hand-drawn by two trained independent raters (AN and DA), blinded to the free-water map and blinded to the group category. After setting the regions of interest, free-water was quantified within each region of interest. Our regions of interest consisted of the left and right posterior SN, and the center of the RRA. Each region of interest was 2x2 mm, spanning two slices, which were placed separately to guarantee optimal placement in each slice. The SN was visible as a dark region on the B0 and the ROIs were placed in the most posterior part of visible SN – at least one voxel removed from the border of the pons and the surrounding ventricle. The RRA was placed based on anatomical landmarks as described in (Damier, Hirsch *et al*. 1999) in the same slices as the SN, one voxel more lateral to the center of the red nucleus, at least two voxels posterior to the posterior border of the red nucleus.

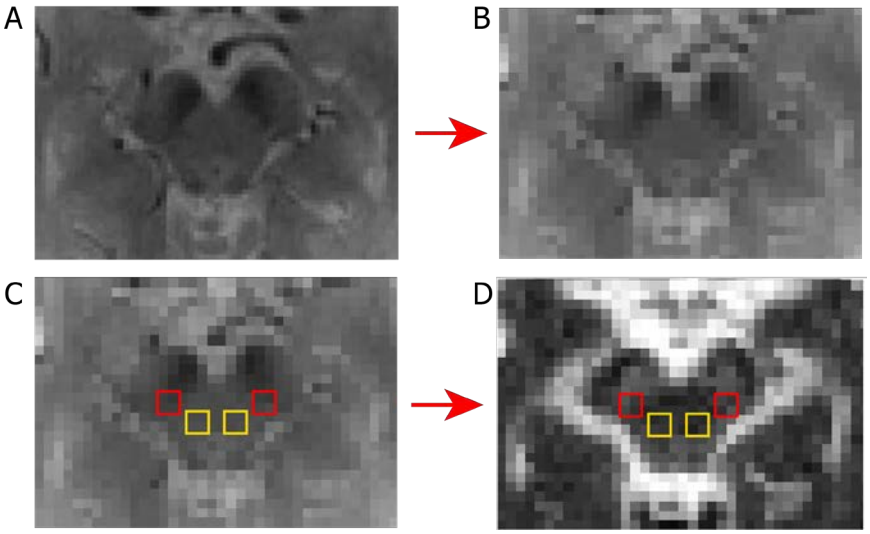

Figure 1 | ROI placement. The original B0 image **(A)** is down sampled **(B)** to a standard 2x2x2 millimeter space. **(C)** ROI regions (size: 2x2 voxels, spanning 2 slices) are placed based on the B0 image: the posterior SN (right and left) is visible in red, the RRA (right and left) is visible in yellow. **(D)** Free-water map of the same subject showing the regions of interest that are used to extract free water values. RRA = retro-rubral area; ROI = region of interest; SN = substantia nigra.

3.3.5 | Statistical analysis

Statistical analyses were performed with SPSS (version 2, Chicago, IL, USA) for Windows. Inter-rater reliability of manual region of interest delineation was examined using intraclass correlation coefficients (ICC) between the two raters using a 2- way random model with absolute agreement. When the ICC proved sufficient, the average of both results was used for further analyses.

Outliers were defined as 1.5 times the Interquartile range (standard SPSS outlier detection), and removed before further analyses. Results without outlier removal are provided in *supplementary materials*. Each group was tested for normal distribution using the Shapiro-Wilk test for normality, and the Levene test for equality of variance. When one (or more groups) of the tested groups did not pass this requirement, statistical testing was done using a non-parametric analysis. We found no such deviation in the control and tremor-dominant group; however, the non-tremor group did not pass these requirements in both the posterior SN (non-normal distribution), and the RRA (unequal distribution). To this effect, we compared differences between tremor-dominant and non-tremor patients in both regions using a Mann-Whitney U test. To detect differences between Parkinson's disease patients and healthy controls we used a univariate ANCOVA with subgroup as between-subject factor and gender

and age as covariates for the two regions. As we had specific hypotheses concerning the direction of the effect in both regions, and between patients and controls, we used a 1-tailed analysis in these cases. The results were inspected using an adjusted p-value of $p<0.017$, based on a Bonferroni correction to account for our three main tests of interest. As a post-hoc analysis, we further compared differences between the control group and the two patient subgroups in the posterior SN using a similar non-parametric procedure (Mann-Withney U test).

Furthermore, we investigated if there was a relationship between free water intensity and symptom severity. We tested the correlation between MDS-UPDRS bradykinesia subscores and posterior SN free water levels (across all Parkinson patients), and the correlation between MDS-UPDRS tremor subscores and RRA free water levels (only in the tremor-dominant group), using linear regression. Finally, to test whether these correlations were symptom-specific and region-specific, we statistically compared the correlation coefficients of two correlations using the cocor toolbox (Diedenhofen and Musch 2015). For each significant result, we contrasted both symptom (bradykinesia versus tremor) for the associated region, and compared regions (SN, RRA) for the specified symptom.

Table 1 | Subject characteristics. Disease severity as measured by MDS-UPDRS part III (maximum score is 108). Bradykinesia refers to the sum of MDS-UPDRS items 4-8, 14; Rigidity to the sum of MDS-UPDRS items 3, 9; and RestingTremor refers to MDS-UPDRS item 17-18, Tremor refers to MDS-UPDRS item 15-18. The Frontal Assessment Battery (FAB, maximum is 18) and Mini Mental State Examination (MMSE, maximum is 30) were used as a measure of cognitive function. To compare between controls vs patients, and tremor-dominant vs non-tremor patients we used a series of two-tailed T-tests for our continuous variables, and the $\chi 2$ test for the categorical variable 'gender'. F = female, FAB = frontal assessment battery, M = male; MMSE = Mini-Mental State Examination; sig. = significance level; MSD-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale.

	Tremor	NonTremor	sig.	Patients	Controls	sig.
sample size	37	19		56	23	
gender	F:16 M:21	F:8 M:11	p = 0.935	F:24 M:32	F:11 M:12	p = 0.686
age	61.2 (10.5)	60.1 (9.4)	p = 0.713	60.8 (10.1)	62.0 (9.8)	p = 0.618
FAB	17.2 (1.0)	16.6 (2.1)	p = 0.114	17.0 (1.5)	17.6 (0.7)	p = 0.096
MMSE	29.2 (1.4)	29.2 (1.3)	p = 0.954	29.2 (1.3)	29.4 (0.9)	p = 0.563
UPDRS OFF:	40.6 (16.9)	33.2 (14.7)	p = 0.110			
Total non-tremor	27.6 (13.7)	31.8 (14.0)	p = 0.283			
Bradykinesia	17.2 (7.6)	18.9 (8.8)	p = 0.448			
Rigidity	5.3 (4.3)	3.6 (1.4)	*p = 0.036*			
RestTremor	9.4 (3.9)	0.0 (0.0)	$p<1.5E^{-16}$			
Tremor	13.0 (5.2)	1.4 (1.2)	$p<2.3E^{-13}$			

3.4 | Results

3.4.1 | Clinical differences between groups

There were no differences between patient groups and controls in gender balance, age, FAB, and MMSE scores. Patient phenotypes did not differ in total non-tremor MDS-UPDRS scores or bradykinesia subscores (see *Table 1*). There was, however, a significant difference in rigidity between the two Parkinson's disease phenotypes [t(54)=-2.15, p=0.036]. Tremor scores significantly differed between patient groups (tremor: [t(54)=9.66, $p<2.3E^{-13}$], resting tremor: [t(54)=10.30, $p<1.5E^{-16}$]), reflecting our inclusion criteria.

3.4.2 | Free Water differences between patients and controls

Given our a priori hypotheses, we tested for differences between tremor-dominant and non-tremor patients in the posterior SN and the RRA. In line with our hypothesis, we found higher free water levels in the posterior SN in the non-tremor group compared to the tremor-dominant group [z(53)=-2.01, p=0.016, 95%=0.014-0.018], as seen in *Figure 1A*. However, in the RRA, we found no differences between tremor-dominant and non-tremor patients [z(55)=-0.08, p=0.465, 95%=0.455-0.475]. Previous research has shown increased free water values in Parkinson's disease patients compared to healthy controls, and we tested for similar effects. However, there were no significant differences in free water levels between Parkinson patients and controls in either the posterior SN [F(1,75)< 0.01, η^2=0.050, p=0.954] or the RRA [F(1,75)=0.01, η^2=0.051, p=0.922]. These results were inspected using an adjusted p-value of p<0.017, based on a Bonferroni correction to account for these three questions of interest.

As we found significantly higher free water levels for non-tremor patients compared to tremor-dominant patients in the posterior SN, but no difference between patients and controls overall, we added a post-hoc analysis to assess how each patient subgroup differed from controls. However, we found no significant difference between healthy controls and non-tremor patients [p=0.259, 95%=0.251-0.268], nor between healthy controls and tremor dominant patients [p=0.463, 95%=0.454-0.473].

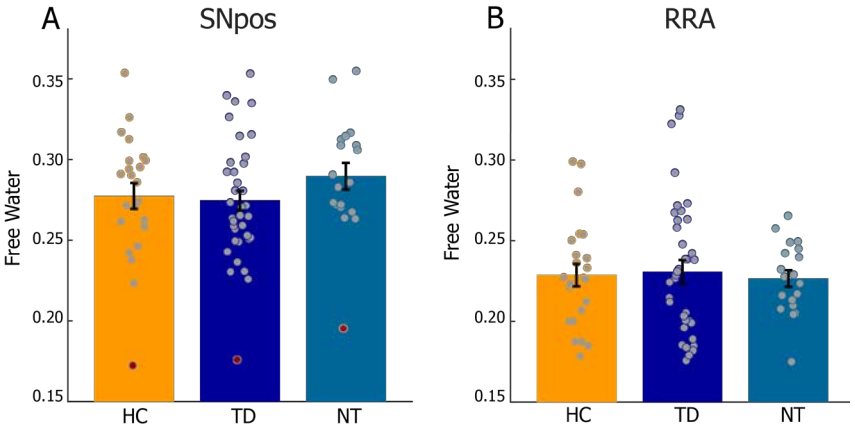

Figure 2 | Difference in free water levels between subgroups. Free-water levels in healthy controls (yellow), tremor-dominant Parkinson's disease (dark blue) and non-tremor Parkinson's disease (light blue) in the posterior SN **(A)** and the RRA **(B)**. We found no overall difference between groups, but a significant difference between tremor-dominant and non-tremor patients. Histograms indicate mean and standard error of the mean (in black).
HC = healthy controls; NT = non-tremor (Parkinson's disease); PD = Parkinson's disease; RRA = retro-rubral area; SNpos = posterior substantia nigra; TD = tremor-dominant (Parkinson's disease).

3.4.3 | Correlation with disease severity

Across all Parkinson patients, we found no significant correlation between SN free water levels and bradykinesia [$t(35)= 0.24$, $R^2=0.001$, $p=0.814$]. In the tremor-dominant group, resting tremor severity was significantly correlated with free water values in the RRA [$t(34)= 3.47$, $R^2=0.256$, $p=0.001$], as shown in *Figure 2B*. In contrast, bradykinesia severity did not correlate with free water levels in the RRA [$t(54)= 0.73$, $R^2=0.010$, $p=0.470$]. A post-hoc comparison of correlation coefficients showed that free water values in the RRA were significantly stronger correlated to resting tremor than to bradykinesia [$z(34)= 2.31$, $p=0.011$]. Furthermore, resting tremor severity was significantly more correlated to free water values in the RRA than in the posterior SN [$z(34)= 2.22$, $p=0.013$]. This suggests that the association between tremor severity and free water concentration in the RRA is both regionally and symptom specific.

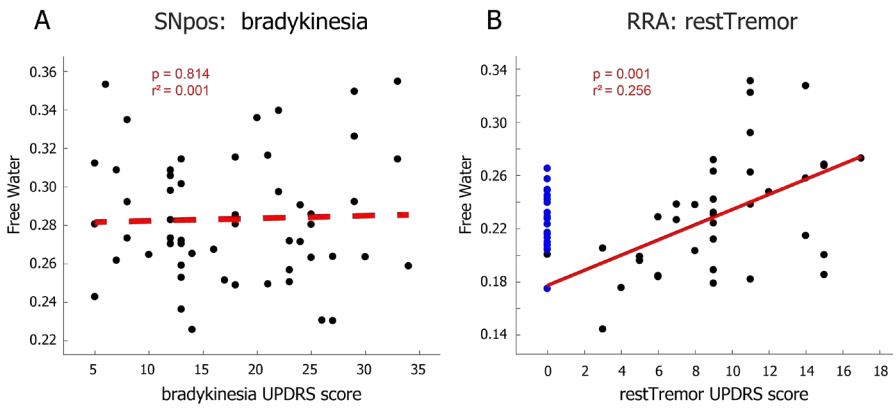

Figure 3 | Relationship between free water levels and disease severity. Scatterplots of the relation between free water scores (a.u.) and MSD-UPDRS sub-scores relevant to the region. Significant correlations are marked with a continuous red line; otherwise, the estimate is shown as a dashed line. **(A)** The relationship between MSD-UPDRS bradykinesia and free water levels in the posterior SN. There was no significant correlation between bradykinesia and posterior SN free water levels. **(B)** The relationship between MSD-UPDRS tremor scores and free water levels in the RRA. In figure 2B, blue dots represent non-tremor patients (shown for comparison), while black dots represent tremor-dominant patients. As non-tremor patients inherently show a resting tremor score of zero, they were omitted from this specific analysis. As a result, we found a significant relationship between tremor and RRA free water levels in accordance to our previous hypotheses. RRA = retro-rubral area; SNpos = posterior substantia nigra; MSD-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale.

3.5 | Discussion

In this study, we compared levels of neuronal degeneration in the SN and RRA between patients with tremor-dominant and non-tremor Parkinson's disease, indexed by diffusion based free water imaging. In line with previous post-mortem findings, we expected increased (posterior) SN free water in non-tremor versus tremor-dominant patients, but increased RRA free water in tremor-dominant versus non-tremor Parkinson's disease. Furthermore, we expected SN free water to correlate with bradykinesia, but RRA free water to correlate with resting tremor. Contrary to our predictions, we did not find evidence for differences between patients and controls in the posterior SN and the RRA, nor did posterior SN free water levels correlate with bradykinesia severity. However, we report increased free water in the posterior SN in non-tremor patients compared to tremor patients. Furthermore, we report a significant correlation between resting tremor severity and free water in the RRA levels. Both of these findings are in line with our predictions.

3.5.1 | Structural brain differences between tremor-dominant and non-tremor Parkinson's disease

Our findings fit with post-mortem studies reporting differences in the pattern of dopaminergic cell loss in the midbrain: non-tremor patients showed more degeneration of the SNc than tremor-dominant patients (Jellinger and Paulus 1992), while the reverse was found in the retro-rubral area (RRA) (Hirsch, Mouatt *et al*. 1992). These findings are also consistent with work in non-human primates, were MPTP injection caused differential patterns of dopamine-based neuronal damage in rhesus and vervet monkeys. While rhesus monkeys showed primarily damage to the SN, accompanied by an akinetic/rigid (non-tremor) phenotype, vervet monkeys presented with primarily damage to the RRA and a tremor dominant phenotype (Bergman, Raz *et al*. 1998, Rivlin-Etzion, Elias *et al*. 2010). In humans, there is recent work in Parkinson's disease patients comparing tremor-dominant patients to a postural instability gait difficulty (PIGD) subgroup using a neuromelanin sensitive MRI protocol. Although not fully the same, a PIGD subgroup has a large overlap with non-tremor Parkinson's disease patients (Helmich, Hallett *et al*. 2012). PIGD patients are not exclusively defined as a non-tremor group; however, they have predominant balance and gait symptoms, as compared to other symptoms. Clinically, these patients usually have little tremor and relatively severe bradykinesia and rigidity. They found that PIGD patients showed a more severe neuromelanin decline in the SNc than the tremor-dominant subgroup (Xiang, Gong *et al*. 2017). These results are put in broader perspective by imaging studies showing further differences in striatal signaling. PET imaging studies show lower dopamine transporter binding in the striatum of non-tremor patients than tremor-dominant patients (Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). Additionally, voxel-based morphometry (VBM) results show lower globus pallidus grey matter volumes for PIGD patients compared to tremor-dominant Parkinson's disease (Rosenberg-Katz, Herman *et al*. 2016). Taken together, these results paint a picture of disparity between motor phentoypes in dopaminergic degeneration and subsequent striatal functioning, which may culminate in the observed differences in disease expression.

There are also indications that non-tremor patients have more severe structural brain abnormalities outside the dopaminergic system. DTI studies have shown widespread FA reductions in PIGD (but not tremor-dominant) patients involving the superior longitudinal fasciculi and corpus callosum, suggesting a more widespread microstructural decline (Vervoort, Leunissen *et al*. 2016). Other work shows increased grey matter atrophy in the PIGD group in several brain areas including motor as well as cognitive, associative, and limbic regions (Rosenberg-Katz, Herman *et al*. 2013) with the level of grey matter atrophy corresponding to increased severity of motor symptoms and reduced cognitive performance. In general, non-tremor patients seem to encompass a more severe form of Parkinson's disease

3.5.2 | The involvement of the RRA in resting tremor

Although we found no significant differences between subgroups in RRA free water levels, the specific correlation between clinical tremor severity (measured off dopaminergic medication) and RRA free water intensity suggests that (dopaminergic) cell loss in this region may have a role in tremor expression. Specifically, the strength of the correlation suggests that RRA degeneration was able to explain 25.6% of the measured tremor intensity. This is in line with previous SPECT data showing a correlation for dopamine depletion in the pallidum, which receives dopaminergic projections from the RRA, but not in the striatum. The striatum receives dopaminergic projections from the (posterior) SN) (Helmich, Janssen *et al*. 2011). As outlined above, these findings also concur with results from a post-mortem study, where non-tremor patients showed higher RRA degeneration (Hirsch, Mouatt *et al*. 1992). However, as there was no between-group differences here, other mechanisms must be at play as well. For instance, imaging studies indicate that abnormalities in the noradrenergic (Isaias, Marzegan *et al*. 2012) and serotonergic systems (Doder, Rabiner *et al*. 2003, Caretti, Stoffers *et al*. 2008, Qamhawi, Towey *et al*. 2015) may be associated with Parkinson's tremor. Specifically, these studies have found an association between reduced midbrain raphe 5-HT1A binding and increased tremor severity (Doder, Rabiner *et al*. 2003), and reduced levels of thalamic serotonin transporter levels in tremor-dominant Parkinson's disease compared to non-tremor patients (Caretti, Stoffers *et al*. 2008). Furthermore, there is post-mortem evidence that non-tremor patients have more locus coeruleus degeneration than tremor-dominant Parkinson's disease (Paulus and Jellinger 1991). Serotonergic and noradrenergic factors might have independent contributions to tremor, or affect dopaminergic firing rates (Lategan, Marien *et al*. 1992, Grenhoff and Svensson 1993).

3.5.3 | Reliability & Limitations

Our findings differ from previous studies that all showed increased free water signal in the SN of Parkinson's disease patients compared to controls (Ofori, Pasternak *et al*. 2015, Ofori, Pasternak *et al*. 2015, Burciu, Ofori *et al*. 2017, Ofori, Krismer *et al*. 2017, Guttuso, Bergsland *et al*. 2018, Yang, Archer *et al*. 2019). This warrants caution when interpreting the free water differences between Parkinson's disease phenotypes that we report. Below we discuss possible reasons for this null finding, as well as relevant issues to be considered in future studies.

First, we considered differences in the diffusion imaging sequence that was used (see *supplementary materials* for an overview). Compared to previous studies, the diffusion imaging sequence used here had a lower TR, a higher spatial resolution, and a relatively small field of view. This was purposely done to optimize spatial resolution, given our goal to assess Parkinson subtype-specific anatomical patterns

of (dopaminergic) cell loss in the midbrain. More specifically, in our study, the TR was 2200 ms compared to much higher values (range of 7748-8800) in previous work (Ofori, Pasternak *et al*. 2015, Ofori, Pasternak *et al*. 2015, Planetta, Ofori *et al*. 2015, Burciu, Ofori *et al*. 2017, Ofori, Krismer *et al*. 2017, Guttuso, Bergsland *et al*. 2018, Yang, Archer *et al*. 2019). For details, see *Supplementary Table 1*. In addition, we worked with a higher spatial resolution of 1.0x1.0x1.8 mm, which is different from the 2 mm isotropic resolution used previously. Both measures contribute to a reduced signal to noise ratio, making it more difficult to detect group differences. The relatively small field of view ('slab' of 25.2 mm in thickness) was used to obtain a high scanning resolution. While this was sufficient to fully encompass the SN and RRA, a narrow field of view makes it more difficult to align its placement between patients (e.g. angle through the mesencephalon). On the other hand, we took several measures to minimize this issue: during data acquisition, we used a predetermined orientation that was applied to all participants using an automated alignment procedure. Furthermore, localization of ROIs was done individually, for each participant, in accordance to strict placement guidelines. Finally, to check for any remaining variability in the location of the posterior SN between participants, we checked the relative position of the ROI based on the full span of the SN tissue and showed that all ROIs where located in the 20-30% most posterior section of the SN. This suggests that it is unlikely that localization errors have contributed to our null finding.

Second, we considered clinical differences between the patients included in our study versus previous ones. Given that our goal was to investigate differences between Parkinson subtypes, our sample contained a relatively large proportion of tremor-dominant patients (66.1%), and this subgroup had a relatively severe resting tremor (average resting tremor (MDS-UPDRS-III item *17-18*) of 9.4 points, and an average tremor score (item *15-18*) of 13.0). It is not clear to what extent our sample differs from previous studies, given that the relative severity of individual motor symptoms is generally not reported. A noteworthy exception are two studies (Ofori, Pasternak *et al*. 2015, Yang, Archer *et al*. 2019). In both studies, tremor scores were substantially lower than in the current work, especially when taking the severity of non-tremor symptoms into account. It is conceivable that other studies also included patients with relatively little tremor, as tremor introduces potential problems in MRI studies (such as movement artefacts, or motor-related activity when fMRI is also measured). This could introduce a slight measurement bias between both patient groups. The stronger tendency towards an increased SN free water signal in the non-tremor group, compared to the tremor group, suggests that it is important for prospective studies to report the clinical (motor) phenotype of patients in more detail.

3.5.4 | Conclusion

We found in vivo evidence for differential patterns of cell loss in the mesencephalon between tremor-dominant and non-tremor phenotypes of Parkinson's disease. Specifically, we report increased free water signal for non-tremor Parkinson patients compared to tremor-dominant patients in the posterior SN, which is consistent with previous post-mortem results. In addition, we found a strong correlation between clinical resting tremor severity and free water signal in the RRA, which suggests that dopaminergic cell loss in this region may contribute to tremor expression. However, we did not find reduced free water signal in posterior SN of Parkinson patients versus healthy controls, which may relate to methodological and clinical differences between this study and previous work. These results illustrate that motor phenotype is a relevant factor that should be considered (and at least reported) in future structural imaging studies.

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3.7 | Supplementary materials

3.7.1 | Results without outlier removal

In the posterior SN, three outliers were removed before analyses. The resulting analyses without outlier removal are as follows:

There were no significant differences in free water levels between Parkinson's disease patients and controls in either the posterior SN [F(75)= 0.06, η^2=0.001, p=0.808]. We found no group difference in free water levels between the three subgroups overall (Healthy Control, Tremor-Dominant, Non-Tremor) [p=0.205, 95%=0.197-0.213], see *Figure 1B*. Using the full data set (including outliers) we only find trend level difference in free water levels in the posterior SN in the non-tremor group than in the tremor-dominant group [p=0.068, 95%=0.063-0.073]. Similar to the corrected results, we did not find a significant difference between healthy controls and non-tremor patients [p=0.300, 95%=0.291-0.309], or between healthy controls and tremor dominant patients [p=0.558, 95%=0.548-0.568].

3.7.2 | Comparing study parameters

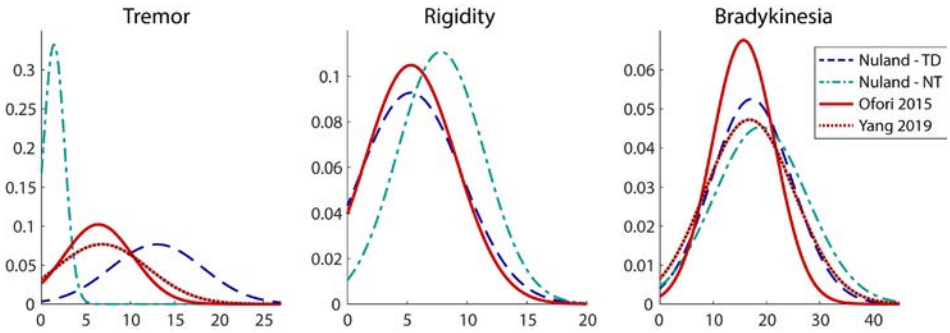
The current number of studies reporting (SN) Free Water differences between Parkinson's disease patients and controls is still somewhat limited. We have made an overview of recently published papers on this topic and their scanning/patient parameters. We find that most notably our resolution and TR diverge from the list provided here, in addition to a potential difference in tremor symptom/intensity.

Supplementary Table 1 | Overview of study/scanning parameters of DTI studies using Free Water values successfully showing differences between Parkinson patients and Healthy Control subjects. We observed specific parameter differences in a few factors. We find that we have lower resolution and TR. RU = Radboud University, UF= University of Florida, PPMI = Parkinson's Progressive Marker Initiative, UB = University of Buffalo.

Study	Cohort	Controls	PD	MRI vendor	Directions	B-values	resolution	TR (ms)
van Nuland 2019	*RU*	*20*	60	3T Siemens	34	1000	**1.0*1.0*1.8**	**2200**
Yang 2019	*Michigan*	75	129	3T Philips	15	800	1.75*1.75*1.75	8044
Ofori 2015 Brain	*UF*	*19*	25	3T Philips	64	1000	2.0*2.0*2.0	7748
Planetta 2016	*UF*	18	18	3T Philips	64	1000	2.0*2.0*2.0	7748
Ofori 2015 NbOfAg.	*UF*	20	28	3T Philips	64	1000	2.0*2.0*2.0	7748
	PPMI	56	78	3T Siemens	64	1000	1.98*1.98*2.0	8400-8800
Burciu 2017	*PPMI*	49	103,46,30	3T Siemens	64	1000	1.98*1.98*2.0	?
Ofori 2017 Mov Dis	*Austria*	41	85	3T Siemens	20	1000	2.0*2.0*3.0	8200
Guttuso 2018	*UB*	19	19	3T GE Signa	25	1000	2.0*2.0*4.0	?

Supplementary Table 2 | Comparison of patient parameters between studies and their cohorts. We find that comparatively, our patients score between comparable- to on the high end of most disease symptoms. This suggests that the patients in our cohorts would show a measure of degeneration that is at least similar to previous papers showing positive results. There is only one paper reporting tremor sub scores (Ofori 2015, Brain), which allows us to loosely compare specific differences between the populations. This points out a potential difference in tremor rates, showing relatively high tremor scores for our patient population, especially our tremor group overall. This suggests that their population might either consist of a smaller number of tremor patients, or contain patients with lower tremor intensity. This table is further visualized in Supplementary Figure 1. H&Y = Hoehn and Yahr, Nb of Ag. = Neurobiology of Aging, PPMI = Parkinson's Progressive Marker Initiative, UF= University of Florida, UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale, yr = year.

Study	Subgroup	Tremor	Rigidity	Bradykinesia	H&Y	Duration (yr)	UPDRS
van Nuland 2019	*Tremor*	13.0 (5.2)	5.3 (4.3)	17.2 (7.6)	2.2 (0.5)	3.9 (4.5)	40.6 (16.8)
	No-Tremor	1.4 (1.2)	7.8 (3.6)	18.9 (8.8)	2.0 (0.6)	4.8 (1.8)	33.2 (19.0)
	combined PD	9.1 (7.0)	6.1 (4.3)	17.8 (8.0)	2.1 (0.5)	4.2 (3.8)	38.1 (16.4)
Yang 2019	*PD*	6.8 (5.2)	N.A.	16.8 (8.4)	2.4 (0.6)	6.0 (4.2)	32.6 (14.3)
Ofori 2015 Brain	*Baseline*	6.4 (3.9)	5.8 (4.0)	12.2 (4.7)	1.9 (0.4)	3.0 (1.5)	28.5 (10.2)
	Year 2	6.4 (3.9)	5.3 (3.8)	15.7 (5.9)	2.0 (0.6)	4.2 (1.5)	31.0 (10.5)
Planetta 2016	*PD*	N.A.	N.A.	N.A.	N.A.	N.A.	37.3 (6.6)
Ofori 2015 Nb of Ag.	*UF*	N.A.	N.A.	N.A.	N.A.	3.4 (1.8)	29.8 (9.2)
	PPMI	N.A.	N.A.	N.A.	N.A.	0.7 (0.7)	22.9 (8.7)
Burciu 2017	*Cohort 1*	N.A.	N.A.	N.A.	N.A.	0.6 (0.6)	20.0 (8.8)
	Cohort 2	N.A.	N.A.	N.A.	N.A.	0.6 (0.6)	21.3 (8.7)
	Cohort 3	N.A.	N.A.	N.A.	N.A.	0.6 (0.6)	22.0 (7.4)
Ofori 2017 Mov Dis	*PD*	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Guttuso 2018	*Baseline*	N.A.	N.A.	N.A.	1.9	7.1 (5.1)	18.8 (7.3)
	Follow-up	N.A.	N.A.	N.A.	2.4	10.4 (5.3)	22.1 (9.2)



Supplementary Figure 1 | Visualization of the comparison of MDRS-UPDRS subscores between studies and their cohorts. The mean and standard deviation of these variables were plotted as a normal distribution, further visualizing the difference/similarity between the cohorts. A few groups were specifically highlighted here; this includes our two subgroups (tremor/non-tremor patients – blue dotted lines), and the 1 year Ofori 2015 cohort, along with the Yang 2019 cohort in comparison. This points out a potential difference in tremor rates, showing relatively high tremor scores for our patient population, especially our tremor group overall. Nuland TD, Nuland NT = referring to the present thesis chapter Tremor Dominant and Non Tremor groups, H&Y = Hoehn and Yahr, MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale.

CHAPTER 4

Effects of dopamine on reinforcement learning in Parkinson's disease depend on motor phenotype

Contributing Authors

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4.1 | Abstract

Parkinson's disease is clinically defined by bradykinesia, along with rigidity and tremor symptoms. However, the severity of these motor symptoms is greatly variable between individuals, particularly the presence of tremor. This variability in motor symptoms has been established to relate to variation in cognitive/motivational impairment as well as neurodegeneration, including dopamine depletion. Here we focus on reinforcement learning, a cognitive function particularly strongly implicated in Parkinson's disease due to its pervasive association with dopamine. Specifically, we hypothesized that effects of dopaminergic medication on reinforcement learning differ between tremor-dominant and non-tremor patients. 40 tremor-dominant and 20 non-tremor patients with Parkinson's disease were tested both OFF and ON dopaminergic medication (200/50 mg levodopa-benserazide), while 20 age-matched controls were tested twice OFF medication. Participants performed a reinforcement learning task designed to dissociate effects on learning rate from effects on motivational choice bias. As predicted, the effect of dopaminergic medication depended on Parkinson tremor phenotype: In non-tremor patients, dopaminergic medication improved reward-based choice, replicating previous studies (Frank, Seeberger *et al*. 2004, Cools 2006). In contrast, in tremor dominant patients dopaminergic medication improved learning from punishment. Computational modelling showed that these two effects were captured by effects on motivational choice bias and learning rate, respectively. This divergent effect of medication as a function of motor phenotype is especially relevant in light of established clinical cognitive/motivational differences between tremor and non-tremor patients. Importantly, our findings may have brought to light a structural selection bias against tremor patients in earlier studies, and strongly underline the importance of increased awareness of interpatient diversity in future studies.

4.2 | Introduction

Parkinson's disease is clinically defined by bradykinesia, rigidity, and tremor. However, the severity of motor symptoms differs considerably between patients. Arguably, the main clinical characterization is between patients with a tremor-dominant phenotype and those with a non-tremor phenotype (Helmich, Hallett *et al*. 2012). Compared with tremor-dominant patients with Parkinson's disease, non-tremor patients suffer more from gait and balance problems. However, non-tremor patients also suffer from more severe cognitive decline and earlier dementia (Williams-Gray, Foltynie *et al*. 2007, Williams-Gray, Evans *et al*. 2009, Wu, Le *et al*. 2011), increased motivational dysfunction indicative of impaired impulse control (Wylie, van den Wildenberg *et al*. 2012), and increased levels of anxiety (Dissanayaka, Sellbach *et al*. 2010). These clinical differences are mirrored by a variety of neurochemical alterations, predominantly in the dopaminergic system. Specifically, dopamine cell loss has been demonstrated, in both post-mortem and nuclear imaging studies, to be more severe in non-tremor patients than in tremor-dominant patients (Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). Furthermore, non-tremor patients have more extensive substantia nigra (SN) degeneration (Jellinger and Paulus 1992), which in non-human primates contains 76% of mesencephalic dopaminergic neurons (Francois, Yelnik *et al*. 1999). In contrast, tremor-dominant patients have more extensive retro-rubral area (RRA) degeneration (Hirsch, Mouatt *et al*. 1992), which in non-human primates, contains only 10% of mesencephalic dopaminergic neurons (and the remaining 14% in the ventral tegmental area) (Francois, Yelnik *et al*. 1999).

Brain dopamine has long been implicated not only in motor behaviour but also in a wide range of cognitive functions. Most pervasive is the role of dopamine in reinforcement learning. Yet, given the well- established importance of dopamine in the neural implementation of reinforcement learning (Schultz, Dayan *et al*. 1997, Holroyd and Coles 2002, Fiorillo, Tobler *et al*. 2003, Steinberg, Keiflin *et al*. 2013), there is a puzzlingly large variability in the effects of dopaminergic medication on reinforcement learning in Parkinson disease (Frank, Seeberger *et al*. 2004, Cools 2006, Grogan, Tsivos *et al*. 2017, Timmer, Sescousse *et al*. 2017). Here we exploit clinically relevant variance in Parkinson phenotypes, namely the presence or absence of tremor, to characterize this large variability in dopaminergic drug effects on reinforcement learning.

Multiple controlled medication withdrawal studies in Parkinson's disease have demonstrated that dopaminergic medication enhances learning from reward, while impairing learning from punishment (Frank, Seeberger *et al*. 2004, Cools 2006, Frank, Samanta *et al*. 2007, Moustafa, Cohen *et al*. 2008, Bódi, Kéri *et al*. 2009, Palminteri, Lebreton *et al*. 2009). This medication-related shift away from punishment towards reward learning is grounded in neural network modelling work (Frank 2005). According

to this work, medication potentiates reward prediction error-related phasic dopamine bursts, while blocking punishment prediction error-related dopamine dips. These effects are compelling, both theoretically and empirically, but several recent studies have failed to replicate them (Grogan, Tsivos *et al*. 2017, Timmer, Sescousse *et al*. 2017). While at first puzzling, this variability in the effects of dopaminergic medication is not so surprising: It concurs with extensive evidence from pharmacological work demonstrating great variability in dopaminergic drug effects, for example as a function of variation in baseline dopamine levels (Cools and D'Esposito 2011). Based on this literature, and the different dopaminergic phenotype of tremor-dominant and non-tremor Parkinson patients, we hypothesized that the variability in dopaminergic medication effects on reinforcement learning reflects differential effects depending on motor phenotype, with non-tremor patients exhibiting greater medication-related increases in reward versus punishment learning than tremor patients.

A second key open issue about dopamine's effects on reinforcement learning is the degree to which these effects reflect modulation of learning or, rather, of motivational choice biases (Berridge 2007). According to the learning hypothesis, dopamine prediction errors drive reward and punishment learning through selective modulation (long term potentiation and depression) of direct 'Go' and indirect 'NoGo' pathway activity (Frank 2005). According to the alternative motivational choice biasing hypothesis, dopamine alters only the expression of learning on choice, invigorating action in the face of reward (Berridge 2007, Robbins and Everitt 2007) and suppressing action in the face of punishment (Guitart-Masip, Economides *et al*. 2014, Lloyd and Dayan 2016). Disentangling these hypotheses has been difficult because correctly learned performance on most learning tasks requires responses that are congruent with motivational biases (go-for-reward or nogo-to-avoid-punishment) (Frank, Seeberger *et al*. 2004, Cools, Altamirano *et al*. 2006, Bódi, Kéri *et al*. 2009). Thus, some effects that have been attributed to modulation of learning might in fact reflect biasing of motivational choice (Guitart-Masip, Chowdhury *et al*. 2012, de Boer, Axelsson *et al*. 2019). In keeping with this hypothesis, evidence from recent studies with patients with Parkinson's disease have revealed that effects of dopaminergic medication on reinforcement learning tasks can be attributed, at least in part, to modulation of choice (Shiner, Seymour *et al*. 2012, Smittenaar, Chase *et al*. 2012). However, those studies do not exclude that medication alters both learning and choice, as these could not be assessed simultaneously (Collins and Frank 2014). Here we address this issue by combining computational reinforcement learning modelling with the use of a reinforcement learning task where go/nogo response requirements and motivational valence were manipulated independently (Guitart-Masip, Fuentemilla *et al*. 2011). The task capitalizes on the fact that rewards and punishments elicit differential action biases of activation and inhibition of behaviour respectively. Effects on learning would affect learning across action domains, leading to changes in accuracy as a function of valence, but not of the go/nogo response

requirement. Conversely effects on motivational choice bias would drive choice accuracy in opposite directions depending on whether the required action was congruent (i.e. go-for-reward) or incongruent (i.e. nogo-for-reward) with the valence of the cue. We used this design to compare the effect of dopaminergic medication on reinforcement learning between two carefully selected Parkinson subtypes, i.e. tremor-dominant and non-tremor patients, as well as healthy controls.

4.3 | Methods

4.3.1 | Subjects

We tested 63 patients with Parkinson's disease, in addition to 22 healthy controls. This work is part of a larger study investigating i) differences between tremor and non-tremor Parkinson's disease, and ii) within tremor patients, the differences between patients whose tremor symptoms are responsive to DA medication, and those who are not. The total study therefore consisted of three Parkinson subgroups (tremor DA responsive, tremor DA resistant and non-tremor), with an aim of 20 participants in each group. It is important to note that the DA responsiveness criterion was tremor-specific; all patients were responsive to DA with respect to their other symptoms. There was no theoretical basis to expect a difference between DA responsive and non-responsive tremor patients in the current task, and therefore Parkinson patients were grouped solely based on the presence or absence of tremor (see supplemental Results). This resulted in 43 tremor dominant patients [18F], and 20 non-tremor patients [9F]. For patients, inclusion criteria were idiopathic Parkinson's disease, and fitting into either clinical phenotype (see below). Exclusion criteria were cognitive dysfunction defined as a Mini-Mental State Examination (MMSE) score <26 (Cockrell and Folstein 2002), a frontal assessment battery (FAB) score <13 (Lima, Meireles *et al*. 2008), severe dyskinesias, neurological or psychiatric comorbidity, severe head-tremor, known allergy against levodopa-benserazide or domperidone. Complete clinical and demographic information is presented in *Table 1*.

Tremor-dominant Parkinson's disease was defined as a history of tremor and a resting tremor score of 1 point or more in at least one arm on item 17 of the Movement Disorders Society Unified Parkinson's disease Rating Scale (MDS-UPDRS part III). Non-tremor Parkinson's disease was defined as the absence of resting tremor in all limbs (UPDRS resting tremor score of 0). These definitions were previously used in (Helmich, Janssen *et al*. 2011). Action tremor was not an exclusion criterion for this group, given that action tremor has a different pathophysiology (Dirkx, Zach *et al*. 2018).

In the tremor dominant group, one patient dropped out on day 2 due to claustrophobia. Due to a technical error, behavioural data of one patient on day 1, and one patient on day 2 was incomplete/unusable; this resulted in 40 full datasets (42 on day 1, 41 on day 2). In the non-tremor group, one patient dropped out on day 2 due to claustrophobia, and one patient did not complete the behavioural task resulting in 18 complete datasets (20 on day 1, 18 on day 2).

4.3.2 | Setup and medication regime

Both patients and healthy participants were measured on two separate occasions, always in the morning.

On both sessions, patients came in an OFF state, defined as abstinence from medication for >3 times the drug half-life, i.e. >12 hours after their last dose of levodopa, > 48-72 hours after their last dose of dopamine-agonist. All healthy participants were measured on two separate sessions to test and control for task repetition effects. Parkinson patients were measured in pseudorandomized order with respect to the dopaminergic intervention (*see medication regime below*).

Testing procedure: For patients only, each day started with a measurement of their motor symptoms (using the UPDRS motor scale), followed by administration of medication. Next all participants moved to the MRI scanner to undergo a combination of fMRI and anatomical scans that lasted for approximately 2 hours (results reported elsewhere (Dirkx, Zach *et al*. 2019, van Nuland, Archer *et al*. Submitted, van Nuland, den Ouden *et al*. Submitted)). After a short break, participants performed the behavioural task (outside the scanner). Finally, patients repeated the measurement of their motor symptoms, to get a measurement of symptom severity 'ON' medication (or placebo). Cognitive assessment (FAD/MMSE) was done on the second day, either in between UPDRS/MRI or behavioural sessions (as participants were often faster with more experience), or at the end of the behavioural session if necessary. This choice was optimized to match the overall timing of the first and second day.

Medication regime: On both sessions, all patients received a dose of domperidone 10 mg 1 hour before drug/placebo intake, to increase gastro-intestinal absorption and to reduce side effects. During one session, patients received a standardized dose of 200/50mg of dispersible levodopa-benserazide (ON state), dispersed in water. Levodopa dose was on average 70% higher than the patients' own morning dose (cf. *Table 1* for average levodopa equivalent daily dose per patient group). During the other session, patients received a placebo (cellulose dispersed in water, which matches the dispersible levodopa both visually and in terms of taste).

4.3.3 | Motivational Go/Nogo Task Design

Participants performed a reinforcement learning task with 4 different task conditions to disentangle the separate but interacting axes of motor response requirement (Go/NoGo) and motivational valence (win/avoid). Each trial started with the presentation of a cue (*Figure 2A*). During cue presentation (3 seconds) participants could decide to press a button (Go response) or abstain from responding (NoGo response). 100 milliseconds after cue offset, participants received feedback based on their response. Valence of the cues was signalled by a coloured edge. Cues with a green edge (Win cues) could be followed by reward (100 points) or neutral feedback (0 points). Cues with a red edge (Avoid cues) could be followed by neutral feedback (0 points) or punishment (-100 points). Subjects were informed about these contingencies, and instructed to try to maximize the number of points won while minimizing the total points lost.

For each cue, there was one correct response (Go or NoGo; *Figure 1*), which participants had to learn by trial and error. Feedback validity was 80%, that is, correct responses were followed by the desirable outcome 80% of the time. There were four cues in total (*Figure 2B*). The order of cue presentation was pseudorandom, with a maximum cue repetition of 2. Each cue was presented 45 times. The task lasted approximately 30 min, including instructions and two self-paced breaks split evenly between trials. Prior to the task, instructions were presented on screen, in which participants were informed about the probabilistic nature of the feedback and that each cue had one optimal response. At the end of the task the total number of points won or lost was displayed on screen. Punishment consisted of a red text with '-100', reward of a green text with '+100', and neutral feedback was a grey text with '000'. All cues were uniquely shaped, with colours that were well distinguishable from the red and green edge. On each testing day, a unique stimulus set was used, the order of which was counter- balanced across participants and drug conditions.

This task allows us separately investigate effects of motor response requirement (go vs nogo) and of motivational valence (reward vs punishment). In addition, it indexes the motivational bias that couples these two systems. In general, we expect subjects to perform better on trials in accordance with this motivational bias - e.g. go response for reward-associated cues (go2win), and avoidance behaviour for cues that are associated with punishment (nogo2avoid), while performing worse on trials incongruent with this innate motivational bias (go2avoid, nogo2win).

4.3.4 | Statistical analysis

4.3.4.1 | *Data quality checks*

Before statistical analyses, we performed the following data quality checks: First, any responses with a response time<200ms were removed from the dataset, assuming that these were spurious responses in which the participant could not have processed the stimulus value. Participants made such spurious fast responses very rarely. Across all trials in the study, patients reacted too fast on average on 0.26% of trials), with a maximum of 8 out of 180 trials (4.4%) in a single patient. Controls responded too fast on average on 0.13% of trials, with a maximum of 2 out of 180 trials (1.1%) in a single individual. Next, we checked whether the distribution of our key measure of interest, accuracy (see below for definition), deviated significantly from normal using Kolmogorov–Smirnov test, which showed no significant deviation from normal distribution. Next, we quantified test-retest differences on the main task measures in both healthy controls and patients, to assess validity of using a within-participant design (for details see below). Finally, we assessed whether overall, participants understood the task / learnt to make the correct responses. We differentiated between congruent stimuli (expected to start high) and incongruent stimuli (expected to start at low performance, but improve over time). For the incongruent stimuli, we compared whether performance in the first and last block showed significant improvement using a paired samples t-test. As we expect above-chance performance from the start for the congruent stimuli, for these stimuli we looked whether performance in the first block was significantly above chance (>0.5) using a one sample t-test against a test value of 0.5.

4.3.4.2 | *Task effects*

Our main measure of interest was the ability to learn to select the response that most often led to the desired outcome. This accuracy score was quantified as the proportion correct responses computed for each of the four conditions. The basic analysis comprised a 2x2 repeated-measures analysis of variance (ANOVA) with accuracy score as dependent variable and required action (go/nogo), valence (win/avoid loss) as factors. This ANOVA allows us to quantify the following effects: the intercept describes overall performance in the task (>0.5 is above chance), The main effect of valence effect describes the ability to learn from reward relative to punishment. The main effect of required action effects show whether people are better at learning to make a go rather than nogo response. Finally, the interaction between Required action and Valence describes the degree to which learning motivation and action learning are coupled: The so-called motivational (or Pavlovian) bias is a well-replicated phenomenon, with an increased performance on go-to-win and nogo-to-avoid stimuli, as well as decreasing performance for go-to-avoid and

nogo-to-win trials (Guitart-Masip, Fuentemilla *et al*. 2011). This task is particularly relevant in Parkinson's disease as it taps into multiple factors that are specifically implicated in this disease: i) motivation ii) motor learning and execution ii) their interaction (motivational bias).

This basic 2x2 ANOVA was extended to assess the following factors: 1) test-retest effects, as part of our quality checks, 2) medication and patient group effects, as our main hypothesis-testing, 3) robustness of findings, considering nuisance variables. We will describe these below.

4.3.4.3 | *Test-retest reliability*

Learning dependent behavioural tasks are inherently vulnerable to test-retest differences as performance often increases at second task exposure. It is therefore important to check whether there are consistent test-retest biases that affect our main analyses or factors of interest (e.g. interaction with valence, action, or valence x action). We performed a 2x2x2 ANOVA [Valence x Action x Testing day] on accuracy, with participant status [control/patient] as a between participant factor. For patients, we collapsed over medication status. There was a significant interaction of Testing day and Valence, such that people learnt better from reward than punishment on day 1, but vice versa on day 2 (for details see Results). Importantly this effect was present in and not significantly different between healthy controls and patients (where medication was a potential confound). We therefore decided to take testing order into account in our main analysis. Specifically, in the patient group a testing day effect would show up as a testing order x medication interaction. Thus, in case of the latter, we limit our analyses to session 1. This would keep equal distribution of medication status between each patient group (given our counterbalanced design), but include the effect of medication as a between participant factor (as opposed to a within participant factor). Exclusion of day 2 data resulted in a final sample of N=42 (23/19 ON/OFF medication) tremor patients, and N=20 (10/10 ON/OFFs medication) non-tremor patients, and N=22 healthy controls.

4.3.4.4 | *Medication and patient group effects*

Our two main questions centred on i) effects of dopaminergic medication on valence learning in the context of different required actions, and ii) potential differences of these dopaminergic effects between tremor and non-tremor patients. Given the test-retest differences above described, we restricted this analysis to day one. We extended the basic [2x2] repeated-measures ANOVA with accuracy as dependent variable and Required action (go/nogo) and Valence (win/avoid loss) as within participant factors, with medication status (On/Off) and patient group (Tremor/NonTremor) as between participant factors. We followed up any patient group effects

with a comparison to healthy controls to assess whether medication 'normalizes' altered behaviour, or disturbs normal behaviour: For each patient group (tremor/ non-tremor) we performed two separate t-test comparing behaviour ON and OFF medication to healthy controls.

4.3.4.5 | *Control analyses*

Group assignment: To quantify relevant clinical or demographic differences between groups (controls vs. patients, and tremor vs. non-tremor patients) we used a series of two-tailed T-tests for our continuous variables – see *Table 1*.

Confounds: When a difference between groups was detected, we followed this up with extra control analyses dedicated to this particular variable. Specifically, we observed a difference in the delay between medication intake and task performance for the different patient groups. To assess whether this difference could explain our patient group results, we reanalysed the behavioural data using a smaller subset that was matched with respect to the confounding variable to establish the robustness of the findings. We further compare behaviour between the matched and non-matching subsets, to see whether their behaviour is significantly different. Finally, we repeat our main analyses with medication delay as a potential nuisance variable.

Nuisance variables: In addition to test-retest effects, we assessed age and gender. We further inspected LEDD and drug-delay (time between drug administration and performance on the behavioural task), to control for relative medication levels between patients. Both affect variability in effective dopamine levels and could alter the subsequent effects of dopaminergic medication. These nuisance variables were added to our main ANOVA, adding age, drug-delay and LEDD as a covariate and gender as between participant factor.

4.3.5 | Computational modelling

As described in previous literature, changes in reward and punishment-based behaviour can result from both altered motivational learning (from reward versus punishment) (Frank, Seeberger *et al*. 2004), but also from changes in motivational choice bias, i.e. an increased (or decreased) tendency to invigorate responding in the context of a reward cue, and inhibit responding in the context of a punishment cue (Guitart-Masip, Chowdhury *et al*. 2012). Increased (or decreased) performance in our task could in principle arise from both mechanisms (Frank, Seeberger *et al*. 2004, Guitart-Masip, Huys *et al*. 2012, Swart, Froböse *et al*. 2017, Swart, Frank *et al*. 2018). To assess which of these mechanisms gave rise to the observed effects of dopaminergic medication, we fitted computational models that allowed us to independently

quantify these processes. We fitted the following six models to choice behaviour (Go/Nogo) using hierarchical Bayesian parameter estimation implemented in RStan.

All models started with a simple Rescorla-Wagner reinforcement learning model: action weights (w) are estimated for each response option (a) for all trials (t) per cue (s). Choice probabilities are computed using a softmax function based on these action weights:

$$p(a_t|s_t) = \left[\frac{\exp(w(a_t,s_t))}{\sum_{a'} \exp(w(a',s_t))} \right] \quad (1)$$

In the simplest, Rescorla-Wagner, model (M1) the action weights are fully determined by the learned action values (Q-values). These action values are learned through a standard delta-rule learning with two free parameters: a learning rate (ε) which scales the update term, and feedback sensitivity (ρ) scaling the outcome value (comparable to the softmax temperature):

$$Q_t(a_t,s_t) = Q_{t-1}(a_t,s_t) + \varepsilon(\rho r_t - Q_{t-1}(a_t,s_t)) \quad (2)$$

Outcomes are reflected by r, which incorporates negative, neutral and positive outcomes: $r \in (-1,0,1)$. As cue valence is instructed in our paradigm (using green and red cue edges), initial Q-values (Q_0) are set to $\rho * 0.5$ for Win cues and $\rho * -0.5$ for Avoid cues.

In M2 we add a go bias parameter (b) to allow for a differential 'base rate' of Go responding, independent of valence.

$$w(a_t,s_t) = \begin{cases} Q(a_t,s_t) + b & \text{if } a = Go \\ Q(a_t,s_t) & \text{else} \end{cases} \quad (3)$$

In the next models, we implemented various mechanisms through which motivational valence could affect responding. First, we model valence effects on choice bias. In M3 a motivational bias is added that modulates Go responding according to cue valence. This (Pavlovian) cue valence (V) contributes to the action weights by increasing the weight of Go responses for positive and decreasing them for negative cues:

$$w(a_t,s_t) = \begin{cases} Q(a_t,s_t) + \pi V_{(s)} + b & \text{if } a = Go \\ Q(a_t,s_t) & \text{else} \end{cases} \quad (4)$$

$V_{(S)} = 0.5 \quad \text{if } s = \text{win cue}$
$V_{(S)} = -0.5 \quad \text{if } s = \text{avoid cue}$

V is fixed because cue valence is instructed. The impact of Pavlovian valence is determined by the parameter π.

Next, we extend M3 to allow either outcome or cue valence to differentially impact learning. In model M4 and M5 we explore whether there is evidence for differential learning based on cue valence (M5, see e.g. (Swart, Froböse *et al*. 2017)), or as a function of outcome valence (M4 (Frank, Seeberger *et al*. 2004, Cools, Altamirano *et al*. 2006)).

In model M4, the two learning rates correspond to the *sign of the prediction error*: meaning that any *outcome that is better than expected* results in a positive learning rate ε_{win} (i.e. a neutral outcome after a punishment cue or a win after a reward), while impact of outcomes that are worse than expected, will be governed by for ε_{loss}. Note that M4 allows us to test for previously observed effects of DA medication on reward versus punishment learning (Frank, Seeberger *et al*. 2004, Cools, Altamirano *et al*. 2006), yet this model cannot capture the biased motivation-action coupling.

$$\varepsilon = \begin{cases} \varepsilon_{win}: \ if\ r = 1\ \&\ s = win\ cue \quad \text{OR } if\ r = 0\ \&\ s = avoid\ cue \\ \varepsilon_{loss}: \ if\ r = -1\ \&\ s = avoid\ cue \quad \text{OR } if\ r = 0\ \&\ s = win\ cue \end{cases} \tag{5}$$

In model M5, the two learning rates are based on cue valence, so that patients may learn differently from a Win cue than an Avoid cue:

$$\varepsilon = \begin{cases} \varepsilon_{win}: \ if\ s = win\ cue \\ \varepsilon_{avoid}: \ if\ s = avoid\ cue \end{cases} \tag{6}$$

To estimate model parameters and model fit, we used a sampling- based method for hierarchical Bayesian estimation of group-level and participant-level parameters. Here, group-level parameters (X) serve as priors for the individual-level parameters (x), such that $x \sim N(X,\sigma)$. The hyperpriors for s are specified by a half-Cauchy (Gelman 2006) with a scale of 2. The hyperpriors for X are centered around 0 (with the exception of (X_{ρ}) and weakly informative: $X\rho \sim NN(2,3)$, $X\varepsilon \sim NN(0,2)$, $Xb,\pi \sim NN(0,3)$.

Parameters b,π are unconstrained, ρ was constrained to be positive through and exponential transform, learning rates ε were constrained to [0 1] through an inverse logit transform.

Model estimation procedure was identical to (Swart, Froböse *et al*. 2017), using Stan software in R (RStan) (Stan-Development-Team 2016). Stan provides full Bayesian inference with Markov chain Monte Carlo (MCMC) sampling methods (Metropolis, Rosenbluth *et al*. 1953). The number of Markov chains was set at 4, with 200 burn-in iterations and 1000 post burn-in iterations per chains (4000 total). Model convergence was considered when the potential scale reduction factor R^ < 1.1 for all parameters (Gelman and Rubin 1992). Model comparison was evaluated using the Watanabe-Akaike Information Criteria (WAIC) (Watanabe 2010). WAIC is an estimate of the likelihood of the data given the model parameters, penalized for the effective number of parameters to adjust for overfitting. Lower (i.e. more negative) WAIC

values indicate better model fit. As WAIC is reported on the deviance scale (Gelman, Hwang *et al*. 2014), a difference in WAIC value of 2–6 is considered positive evidence, 6–10 strong evidence, and >10 very strong evidence (Kass and Raftery 1995).

4.4 | Results

4.4.1 | Participants exhibit learning and motivational choice biases

Overall, participants exhibited a bias towards making a Go response (Action: [F(1,76)=106.3, η^2=0.58, p<0.001]), i.e. better performance on Go vs. NoGo cues. They showed no overall differential performance for Win vs. Avoid cues (Valence: [F(1,76)=0.6, η^2=0.007, p=0.5]). Subjects were more likely to make a Go response to Win cues and NoGo response to Avoid cues, thus leading to better performance for bias-congruent Go2Win and NoGo2Avoid cues relative to bias-incongruent 'inNoGo2Win and Go2Avoid cues (Action x Valence F(1,76)=171. 7, η^2=0.69, p<0.001) (*Figure 1C-E*).

Furthermore, participants successfully learned the task in all conditions (*Figure 1C,D*), indexed particularly by performance changes across time on 'incongruent' conditions (Go2Avoid, NoGo2Win), indexed by a significantly larger number of correct responses in the 3rd versus 1st block of the task. In these incongruent conditions, participants have to learn to make a response that goes against their motivation-action coupling tendency. Accuracy in block 3 was significantly higher than in block1, for both Go2Avoid [Δ(pCorrect), Mean=0.13, SD=0.27, t(61)=3.9, p<.001] and NoGo2Win [Δ(pCorrect), Mean=0.15, SD=0.19 t(61)=3.5, p<0.001]. For the congruent scores, accuracy was above chance from the start of the experiment (Block 1 only, Go2Win: mean = 0.85, SD = 0.19, accuracy>0.5: t(61) = 14.8, p <.001; NoGo2Avoid: mean = 0.70, SD = 0.21, accuracy>0.5: t(61) = 7.7, p <.001) Here good performance reflects a combination of learning and baseline motivational bias that drives responses in the 'correct' direction. Taken together, this indicates that participants are able to learn the task in both congruent and incongruent trials. Finally, as described in the Methods, there was an unexpected test-retest effect on task performance across patients and controls (described in more detail further below), which led us to restrict analysis of medication effects to day 1 only (see test-retest effects, *supplementary materials*).

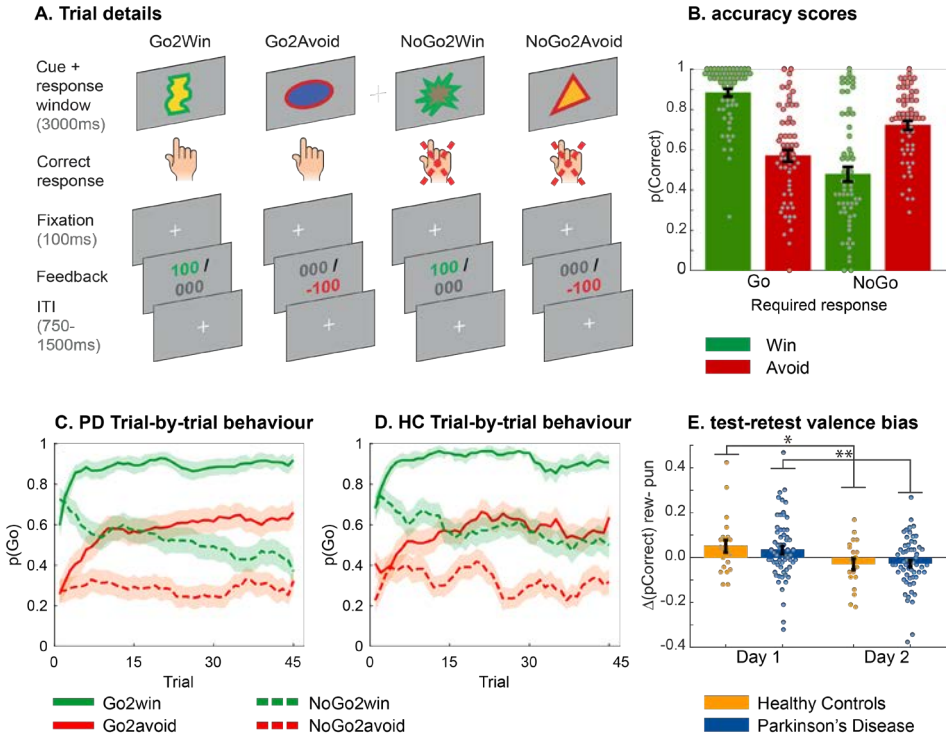

Figure 1 | Motivational Go/NoGo learning task and performance. **(A)** Each trial starts with either a Win or an Avoid cue; signaled by the green or red edge of the cue. For each cue, the participant needs to learn to correct response – either press the spacebar ('Go') or not ('NoGo'). Participants can respond while the cue is on the screen. Outcomes are presented 100 ms after cue offset. In total, 4 cues are presented, reflecting the 2x2 factorial design of response requirement (Go/Nogo) and cue valence (Win/Avoid), such that for each valence there is one cue where 'Go' is correct, and one cue where 'NoGo' is correct. Feedback is probabilistic: correct responses are followed by reward (Win cues) or a neutral outcome (Avoid cues) in 80% of the time, and by a neutral outcome (Win cues) or punishment (Avoid cues) otherwise. For incorrect responses, these probabilities are reversed. **(B)** Average accuracy of patients' responses during the whole experiment for each cue type – performance of the cues congruent with the automatic motivational bias (Go2Win, NoGo2Avoid) is higher than for the incongruent trials (NoGo2Win, Go2Avoid). **(C/D)** Trial-by-trial proportion of Go responses (±SEM), displayed using a within subject 5-trial average sliding window, for both Parkinson patients **(C)** and healthy controls **(D)**. From the first trial onwards, a clear motivational bias is apparent as participants start by making more Go responses for Win cues, and more NoGo responses for Avoid cues. However, during the course of the experiment both participant groups learn to adjust responses towards the correct contingencies. **(E)** Test-retest effects: Both Healthy Controls (orange) and Parkinson patients (blue) show better learning for Win cues on day 1, which switches to better learning for Avoid cues on day 2.

4.4.2 | Levodopa affects performance as a function of valence and Parkinson motor phenotype

We did not replicate previous reports that levodopa medication improved performance on the Win versus the Avoid trials [F(1,58)= 0.95, η^2=0.016, p=0.3]. Instead, we found that the interaction of medication and valence was strongly modulated by patient-group [Tremor Group x Medication x Valence: F(1,58)= 15.2, η^2=0.21, p<0.001]. Both groups showed a significant modulation by levodopa of performance on Win versus Avoid cues [Medication x Valence: tremor - F(1,40)= 7.43, η^2=0.160, p=0.010; non-tremor - F(1,18)= 5.96, η^2=0.210, p=0.026], but in opposite directions. The non-tremor group largely replicated the previous literature: Those ON levodopa exhibited higher accuracy on Win versus Avoid trials than those OFF levodopa, with a simple main effect of Valence in the ON group [F(1,9)= 8.90, η^2=0.331, p=0.008], but not the OFF group [F(1,9)= 0.34, η^2=0.019, p=0.564]. In contrast, in the tremor group, those ON levodopa exhibited increased accuracy on the Avoid versus the Win trials than those OFF levodopa, with no simple main effect of valence ON levodopa [F(1,22)= 0.59, η^2=0.024, p=0.471], but, surprisingly, lower accuracy on Avoid than Win trials OFF levodopa [F(1,18)= 10.23, η^2=0.362, p=0.005].

When comparing each patient group ON and OFF levodopa with healthy controls (*Figure 2*), there were no significant differences. There were trend level differences between the behaviour of healthy controls and tremor dominant patients ON medication [HC vs. TD-ON, F(1,43)= 3.7, η^2=0.08, *p=0.06*], and healthy controls and non-tremor OFF medication [HC vs. NT-OFF, F(1,30)= 2.9, η^2=0.09, *p=0.10*]. The other comparisons were not significant [HC vs. TD-OFF, F(1,39)= 0.4, η^2=0.01, p=0.5], [HC vs. NT-ON, F(1,30)= 2.5, η^2=0.08, p=0.13]. As reported in the supplement, healthy controls performed better on Win than Avoid trials. This was also true for non-tremor patients when they were ON medication and for tremor patients when they were OFF levodopa.

4.4.3 | Motivational Bias is affected by levodopa

Across patient populations, the motivational choice bias was *weaker* ON levodopa than OFF (Action x Valence x Medication [F(1,58)= 4.2, η^2=0.068, p=0.04], see *Figure 2B*). This effect, however, was not as strong as the Valence x Medication x Patient Group effect reported above, and showed no further interaction with relevant variables of interest. There was no interaction with Parkinson motor phenotype (Action x Valence x Medication x Group: [F(1,58)= 2.74, η^2=0.045, p=0.103]. Finally, there was no significant group difference in terms of motivational bias [Valence x Action x Patient Group: F(1,58)= 1.7, η^2=0.03, p=0.2], nor did medication affect differential learning to Go or NoGo (Medication x Action: [F(1,58)= 0.2, η^2=0.004, p=0.6], nor as a function of motor phenotype (Action x Medication x Group: [F(1,58)= 0.8, η^2=0.01, p=0.4]).

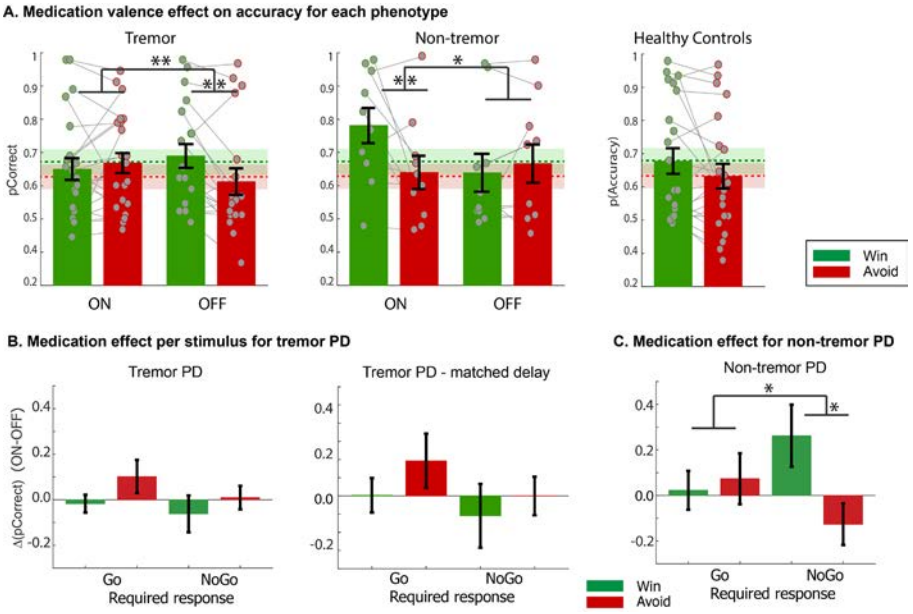

Figure 2 | Performance in response to medication. Error bars represent standard error of the mean (SEM). A) Average accuracy scores over the whole experiment for reward and avoid cues – subdivided by group. For both Parkinson motor phenotypes, we find a significant medication by valence bias. In Tremor patients (left) this is explained by a decrease in performance for avoid trials relative to win trials OFF levodopa. While in the non-tremor group (middle) we find a reverse effect: a relative increase in reward learning ON levodopa. Healthy controls are plotted to the right. To provide a better comparison, the average performance of the healthy controls is plotted as green (win) and red (avoid cues) dotted lines (±SEM) in the background of the two patient groups. B) Performance in relation to dopaminergic medication (ON-OFF, positive score relates to an increase in performance ON levodopa) for each cue type (go2win, go2avoid, nogo2win, nogo2avoid) for tremor and for the subgroup of tremor patients matched with the non-tremor group in medication-delay. C) Relative performance per cue type as a response to medication (ON-OFF) as found in non-tremor patients.

4.4.4 | Computational modelling

We used computational modelling to differentiate between a number of algorithms that could account for behaviour, and to assess which latent variables, i.e. computational mechanisms, may mediate the effects of medication on performance in the two patient groups. Specifically, we aimed to assess the relative contribution of motivational choice bias and reinforcement learning, and whether these mechanisms differed between groups. We started by considering a Rescorla-Wagner model (M1). Stepwise addition of a go bias, motivational choice bias, and separate learning rates for Win and Avoid cues improved WAIC model evidence (*Table 1*). Crucially, model M5 with separate learning rates for Win cues vs. Avoid cues significantly outperformed Model 4 with separate learning rates for 'positive' prediction errors (upon a win or not a punishment) vs. 'negative' prediction errors (upon a punishment or not a win).

Given that the effects of medication depended on tremor phenotype, we next assessed how parameters differed as a function of patient group and medication status, analysing parameter estimates from the winning model (M5). We focused on both the motivational bias parameter and the cue valence dependent learning rates (Win/Avoid), for each group, because modulation of only these parameters can account for the observed valence-based performance differences. In the non-tremor group, there was a significant *reduction* in motivational bias [$F(1,18)= 4.7$, $\eta^2=0.206$, $p=0.04$], but no changes in learning rates [ε-win: $F(1,18)= 0.9$, $\eta^2=0.05$, $p=0.4$], [ε-avoid: $F(1,18)<0.01$, $\eta^2<0.001$, $p=0.97$]. In contrast, in the tremor group, the Avoid learning rate was higher in patients ON than those OFF levodopa [$F(1,40)= 4.4$, $\eta^2=0.099$, $p=0.04$], but no changes in reward learning rate [$F(1,40)= 0.12$, $\eta^2=0.003$, $p=0.7$], or Motivational Bias [$F(1,40)= 0.1$, $\eta^2=0.001$, $p=0.8$]. This change in punishment-learning rate can easily explain the raw performance effects, i.e. relatively better performance for Avoid cues in tremor patients ON versus OFF medication.

For the non-tremor group, the change in the motivational bias parameter is puzzling at first sight, because in this group, the main effect of interest was an increase in performance on Win cues. The current observation suggests that the increased performance does not originate from an increase in reward learning (as is often assumed), but rather from reducing the automatic influence of reward cues on action invigoration, thereby allowing for a relatively greater impact of adaptively learnt instrumental values on the final choice, surfacing primarily on nogo2win trials.

Given this observation of reduced motivational bias in non-tremor patients ON medication, we performed a post-hoc ANOVA to assess a change in motivational bias as a function of medication in the raw choice data, specifically for the non-tremor patients. Here we observe a significant interaction between Action x Valence x Medication [$F(1,18)= 4.5$, $\eta^2=0.20$, $p=0.048$], c.f. *Figure 2C*), due to a disproportionate levodopa-related increase in accuracy on nogo2win trials. For completeness, this interaction was not present in the tremor group: tremor: [$F(1,40)= 0.13$, $\eta^2=0.003$, $p=0.7$). While this result should be interpreted cautiously given the absence of a significant 4-way interaction (Action x Valence x Medication x Group: [$F(1,58)= 2.74$, $\eta^2=0.045$, $p=0.103$], it illustrates why the effect of medication on performance for non-tremor patients is captured by the parameter indexing the motivational (choice) bias, rather than a (differential) effect of valence learning.

Table 1 | Base models. Median [25–75 percentile] of subject-level parameter estimates in model space. WAIC is as the estimate of model evidence, as well as WAIC relative to the winning model.

	M1	M2	M3	M4	M5 - WINNING	M5b
WAIC	-12860	-12059	-10311	-10274	-10020	-10017
ΔWAIC	-2840	-2039	-291	-254	0	-3
ρ	2.5 [1.2 6.5]	2.2 [0.7 12.0]	2.1 [0.9 7.5]	3.6 [2.5 6.3]	3.9 [1.3 13.5]	3.9 [1.3 13.9]
ε_0	0.04 [0.01 0.29]	0.03 [0.02 0.06]	0.06 [0.03 0.23]			
b		0.37 [0.13 0.59]	0.45 [0.13 0.73]	0.22 [0.05 0.46]	0.61 [0.35 0.85]	
π			1.7 [0.99 2.35]	0.96 [0.66 1.55]	2.04 [1.07 2.65]	
ε_{win}				0.22 [0.11 0.63]	0.05 [0.04 0.09]	0.05 [0.03 0.09]
ε_{loss}				0.02 [0.01 0.04]	0.02 [0.01 0.04]	0.02 [0.01 0.04]
π_{win}						3.54 [1.78 4.21]
π_{los}						0.69 [-0.05 1.53]

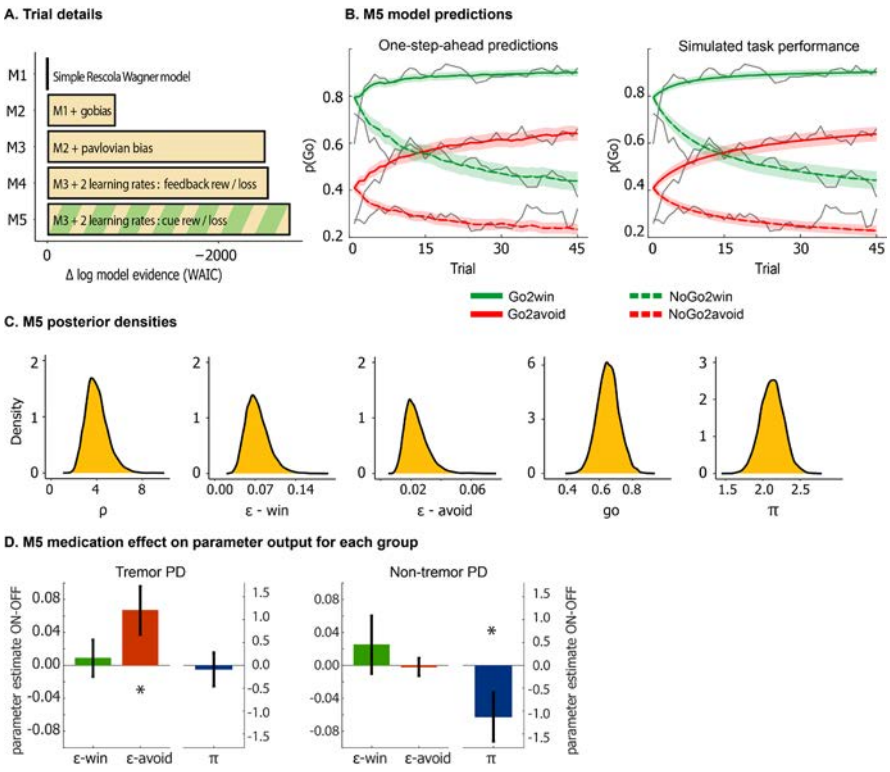

Figure 3 | Model and parameter inference. **(A)** Model evidence, relative to simplest model M1, this clearly favours M5. The simplest model M1 contains a feedback sensitivity (r) and learning rate (e) parameter. Stepwise addition of the go-bias (b) (M2), motivational (Pavlovian) bias (π) (M3), and valence based learning rates, i.e. the valence of the prediction error (M4) and valence of the cue (M5) improve model fit. The final model (M5) which models valence dependent learning rates by cue valence, performs best - as quantified by WAIC (estimated log model evidence). Lower (i.e. more negative) WAIC indicates better model fit. **(B)** One-step-ahead predictions and posterior predictive model simulations of winning base model M5, this shows how the winning model captures the behavioural data (grey lines). Both methods use the fitted model parameters to compute the choice probabilities: The one-step-ahead predictions compute probabilities based on the history of each participant's actual choices and outcomes, whereas the simulation method generates new choices and outcomes based on the response probabilities. **(C)** Posterior densities of the winning base model M5. **(D)** Effect of levodopa on parameter estimates generated from M5: (Difference ON-OFF levodopa in each Parkinson motor phenotype); we find that the tremor dominant group shows a significant increase in punishment learning, while the non-tremor groups shows a significant decrease in motivational bias.

4.4.5 | Clinical differences between groups

There were no differences between patient groups and controls in gender balance, age, FAB and MMSE scores. There were also no differences between Parkinson phenotypes in terms of age, FAB and MMSE and MDS-UPDRS non-tremor score motor scores (see *Table 1*). Tremor scores significantly differed between patient groups $t(54)=9.79$, $p<1.5E-16$, reflecting our inclusion procedure. There was a difference in average "task-delay" (representing the delay between medicine administration and the onset of the behavioural task) of approximately 30 minutes, reflecting the finding that non-tremor patients were structurally faster throughout the experimental procedure preceding the behavioural go/nogo task. This is a potential confound for group comparison, as longer time between drug intake and task onset could affect the efficacy of the drug during the task. To assess this confound, we reanalysed the behavioural data using a subset of the tremor patient group (which was considerably larger than the non-tremor group), to match the drug-delay of non-tremor patients ("drug-selection", *Table 1*). We found no notable difference in behaviour of this 'low-delay' subgroup to the main tremor group (see also *Figure 2B (left)* and *FIgure 2C*). In addition, we found no difference in the direction and outcome of the main analyses when it was limited to non-tremor patients and the low delay subgroup compared to the analysis including the full list of patients (see *supplementary materials*). Finally, the factor 'drug-delay' and 'LEDD' were added as a potential confounding variable in the overall analysis.

Table 2 | Disease characteristics of participants. Reporting mean (standard deviation). For disease scores, min-max possible scores are reported. Patients were successfully matched for gender, age, cognitive function (Frontal Assessment Battery and Mini Mental State Examination (MMSE)) and non-tremor disease severity (MDS-UPDRS part III, items 1-23), although there was a trend level difference in levodopa equivalent daily dose (LEDD), with higher medication for non-tremor patients. Subjects were successfully differentially included based on resting tremor scores (MDS-UPDRS items 28-33). Two-tailed T-tests were used for continuous variables, and $\chi 2$ test for the categorical variable 'gender'. F = female.

	range	Tremor	NoTremor	p-value	Patients	Controls	p-value
sample size		43	20		60	23	
gender		F:18 M:25	F:9 M:11	p = 0.935	F:27 M:36	F:9 M:13	p = 0.874
Age (yr)		61.4 (11.1)	60.2 (9.2)	p = 0.652	61.0 (10.5)	64.3 (9.1)	p = 0.194
FAB	0 – 18	17.3 (0.9)	16.5 (2.1)	p = 0.118	17.0 (1.4)	17.5 (0.9)	p = 0.774
MMSE	0 – 30	29.2 (1.3)	29.2 (1.3)	p = 0.997	29.2 (1.3)	29.3 (0.9)	p = 0.159
LEDD	0 – 2255	449 (302)	645 (503)	p = 0.052			
UPDRS non-tremor	0 – 108	49.7 (24.2)	53.6 (23.1)	p = 0.553			
UPDRSrestTremor	0 – 16	9.4 (3.9)	0.0 (0.0)	p<1.9E-18			
drug-delay (min.)		164 (23)	134 (16)	P < 0.001			
delay-selection (min.)		139 (17)	134 (16)	p = 0.409			

4.4.6 | Confound variable analysis: Age, Gender, LEDD and drug-delay

Accuracy decreased with increasing age [$F(1,74)=19.6$, $\eta^2=0.21$, $p<0.001$]. There was no significant effect of gender [$F(1,74)=0.7$, $\eta^2=0.01$, $p=0.4$], or drug-delay [$F(1,56)=0.1$, $\eta^2=0.002$, $p=0.8$]. We also assessed whether patients' usual dopaminergic medication dose (LEDD) mediated the effects of medication. Given that LEDD may reflect baseline hypodopaminergic state, it could affect the impact of a fixed dose of dopaminergic medication. Finally, we analysed whether LEDD predicted performance independent of medication, testing the hypothesis that variability in LEDD would have a similar effect to medication administration. However, there was no significant effects of LEDD as a main effect, or on relevant effects of interest (LEDD: [$F(1,56)=0.03$, $\eta^2=0.001$, $p=0.863$], valence x LEDD: [$F(1,56)=1.85$, $\eta^2=0.032$, $p=0.179$].

As age was found to predict accuracy, all analyses were repeated including age as a covariate. Age did not interact with any of the effects of interest, nor did inclusion affect the significance of these findings (see *supplementary materials*).

4.5 | Discussion

In this study, we aimed to understand whether and how differences in the cognitive effects of dopaminergic medication relate to a fundamental clinical variation in the Parkinson's disease phenotype, i.e. the presence or absence of tremor. Building on known differences in clinical and dopaminergic phenotypes of tremor-dominant and non-tremor Parkinson patients, we investigated whether those two Parkinson groups have different dopamine-dependent reinforcement learning deficits. We tested this hypothesis using a reinforcement learning task that allowed us to disentangle effects of dopaminergic medication on motivational choice from effects on learning rate. The main finding provides evidence for different computational effects of dopamine-enhancing medication in tremor-dominant and non-tremor Parkinson patients. We confirm that dopaminergic medication enhances performance when cues signal a potential win (Frank, Seeberger *et al*. 2004, Cools 2006, Frank, Samanta *et al*. 2007, Bódi, Kéri *et al*. 2009, Palminteri, Lebreton *et al*. 2009), and we add a consequential qualification. The dopaminergic effect on motivational choice bias is bound to non-tremor Parkinson patients. In contrast, tremor-dominant patients under levodopa learned faster during trials when punishment needed to be avoided.

4.5.1 | Different cognitive effects of dopamine in different motor Parkinson's disease phenotypes

It has been argued that dopaminergic modulations of valence-dependent learning might, in fact, reflect biasing of motivational choice (Guitart-Masip, Chowdhury *et al*. 2012, de Boer, Axelsson *et al*. 2019). The current findings add to that debate by showing that patients with different motor phenotypes selectively change learning- and choice-related computations when receiving levodopa. The findings indicate that, in non-tremor patients, levodopa decreases motivational choice bias during reward trials. This effect matches the decrease in motivational bias evoked by levodopa in healthy participants performing the same task (Guitart-Masip, Economides *et al*. 2014). In tremor-dominant patients, dopaminergic medication modifies a different computational mechanism of learning: levodopa increases learning rate towards Avoid cues. This effect is opposite to previous reports showing dopamine improves learning from reward (Frank, Seeberger *et al*. 2004, Cools, Altamirano *et al*. 2006). On the other hand, the effect of dopamine found in tremor-dominant patients fits with the stimulus-locked dopaminergic surge observed during go-to-avoid trials (Gentry, Lee *et al*. 2016). This could be an instance of the suggested role of dopamine in "safety learning", i.e. the active avoidance of an unpleasant stimulus (Mowrer 1947, Mowrer 1956, Gentry, Lee *et al*. 2016, Lloyd and Dayan 2018).

Taken together, the current findings provide evidence for the notion that dopamine can modulate different computations contributing to value-based choice. There are a number of neural accounts of the differential effects in the two Parkinsonian motor phenotypes. One possibility is that they reflect distinct functional anatomical alterations in tremor-dominant and non-tremor Parkinson patients, such as the different spatial distribution of dopaminergic degeneration in the midbrain of those two Parkinson phenotypes (Hirsch, Mouatt *et al*. 1992, Jellinger 2012). A second possibility is that the severity of dopaminergic depletion, besides its spatial distribution, plays a role, although this is less likely given the absence of effects of levodopa equivalent daily dose (LEDD), an indirect marker of dopamine depletion. There might also be a role for any or more of the other monoamines, given that resting tremor in Parkinson's disease has been associated with abnormalities in the noradrenergic and serotonergic system (Isaias, Marzegan *et al*. 2012, Qamhawi, Towey *et al*. 2015, Pasquini, Ceravolo *et al*. 2018). Indeed, dopaminergic medication in Parkinson's disease has been shown to alter serotonin transmission (Mayeux, Stern *et al*. 1984, Reader and Dewar 1999, Kerenyi, Ricaurte *et al*. 2003, Miguelez, Navailles *et al*. 2016), and serotonin is well known to be implicated in punishment learning (Soubrié 1986, Deakin and Graeff 1991, Chamberlain, Müller *et al*. 2006, Dayan and Huys 2008, Crockett, Clark *et al*. 2009).

Our findings have important clinical implications. Enhanced reward-based learning in Parkinson's disease has previously been demonstrated to be exacerbated in patients with impulse control disorders such as pathological gambling (Voon, Pessiglione *et al*. 2010). Although we did not quantify susceptibility to impulse control disorders here, our findings suggest that non-tremor patients may be more susceptible to developing these symptoms after dopaminergic medication than tremor-dominant patients. In line with this idea, it has been shown that non-tremor patients showed more impulsive motor behaviour during a speeded reaction task (Wylie, van den Wildenberg *et al*. 2012), and that frequent fallers (a motor sign associated with a non-tremor phenotype) score higher on the Barratt Impulsiveness Scale than non-fallers (Smulders, Esselink *et al*. 2014), but see (Hurt, Alkufri *et al*. 2014).

4.5.2 | Interpretational issues

This study involved a relatively large number of Parkinson patients (n=63), all of whom were measured both ON and OFF dopaminergic medication, and it was designed to assess behaviour in each group under both medication conditions. Unfortunately, we found test-retest differences in task performance across patients and healthy controls. Therefore, we had to limit analyses to day one and shift towards a between-subject group design. Nonetheless, the novel finding of this study emerged from a sizeable sample (20 On-dopamine and 23 Off-dopamine tremor-dominant patients) given the large effect size (η^2=0.182).

We used a standardized levodopa dose instead of the patients' own dopaminergic medication, to avoid heterogeneity in the effects of (different) dopamine agonists and different regimes of levodopa. The dose used here (200/50 mg levodopa-benserazide) was higher than the normal dose for most patients, as quantified using their LEDD. The difference in LEDD between patient groups was close to the statistical threshold, raising the possibility that tremor-dominant patients were overdosed relatively to non-tremor patients. However, control analyses indicate that LEDD did not predict performance, and the findings did not change when including LEDD as a covariate.

Finally, it might be argued that other clinical subdivisions of Parkinson's disease could have been considered, e.g. related to the age at onset or rate of progression (Sauerbier, Jenner *et al*. 2016, Fereshtehnejad and Postuma 2017). On the other hand, the observed clinical differences between tremor-dominant and non-tremor Parkinson's disease are firmly grounded in work showing subtype-specific differences in the structural and functional integrity of the dopaminergic system – which was the focus of the current study (Hirsch, Mouatt *et al*. 1992, Helmich, Hallett *et al*. 2012, Jellinger 2012).

4.5.3 | Conclusion

Our key finding is that often-replicated effects of dopaminergic medication in Parkinson's disease hold only for a subgroup of patients, namely patients without tremor (Hughes, Daniel *et al*. 1993, Helmich, Hallett *et al*. 2012). In line with the previous literature, non-tremor patients ON dopaminergic medication showed better performance in the reward domain than did patients OFF medication. In line with recent work in healthy subjects (Guitart-Masip, Economides *et al*. 2014), this effect reflected a decrease in motivational choice bias. In stark opposition to non-tremor patients, patients with tremor symptoms ON medication showed better go-to-avoid learning in the punishment domain than did patients OFF medication. This improvement might reflect dopamine-related changes in safety learning. These divergent effects of dopaminergic medication during reinforcement learning as a function of motor phenotype are especially relevant in light of established clinical cognitive/motivational differences between these patient groups (Dissanayaka, Sellbach *et al*. 2010, Wu, Le *et al*. 2011, Wylie, van den Wildenberg *et al*. 2012), and associated differences in degeneration of dopaminergic nuclei such as substantia nigra and retro-rubral area. Our findings suggest the relevance of considering motor phenotype in future reinforcement learning studies in Parkinson's disease.

4.6 | References

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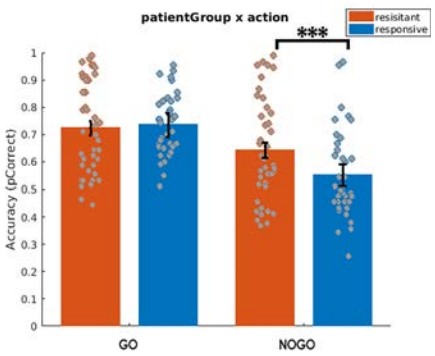
4.7 | Supplemental Results

4.7.1 | Dopamine resistant versus responsive Tremor patients.

There was no theoretical basis to expect a difference between DA responsive and non-responsive tremor patients in terms of valence processing in the current task, and therefore Parkinson patients were grouped solely based on the presence or absence of tremor. Here, we verify this assumption in a supplemental ANOVA on tremor patients only, with factors Action x Valence x Medication x Medication Response Group.

There was no effect of either Valence or Valence x Action (i.e. motivational bias) as a function of medication responsiveness, nor did this interaction with medication administration. We did find a significant interaction between patient group and action (F=10.8, p=0.002), such that the resistant group was less accurate on NoGo trials (see *Supplemental figure 1*), in other words, that they make fewer 'Go' responses on this task overall. However, this effect is irrelevant for the main findings reported in this manuscript as it does not interact with Valence nor with Medication, as the key variable of interest. We therefore do not elaborate on this finding in the main manuscript.

Contrast	Day 1 and 2		Day 1 only	
	F (1,38)	p value	F(1,38)	p value
Medication Response Group	0.8	.4	0.35	.6
Action x Medication Response Group	**10.8**	**.002****	**6.4**	**.015 ***
Valence x Medication Response Group	0.2	.7	0.01	.9
Action x Valence x Medication Response Group	0.5	.5	.01	.9
Medication Response Group x Medication	1.3	.3	3.2	.08
Action x Medication Response Group x Medication	0.1	.8	3.4	.07
Valence x Medication Response Group x Medication	1.3	.3	0.06	.8
Action x Valence x Medication Response Group x Medication	0.5	.0.5	2.5	.12



Supplemental Figure 1 | illustration of patient group (responsive vs non responsive) x action interaction on performance for Day 1 only.

4.7.2 | Test-retest effect

The degree to which participants were able to learn from reward versus punishment feedback changed over testing days (valence x day: [F(1,76)=14.41, η^2=0.159, p<0.001], see *Figure 1F*), such that participants learnt better for Win cues on day 1 (Day 1: Win vs. Avoid, [F(1,76)=8.06, η^2=0.096, p=0.006] and better for Avoid cues on day 2 (Day 2: Win vs. Avoid, [F(1,76)=4.272, η^2=0.053, p=0.042]. This was true for both patients (Valence x Day; [F(1,57)=9.24, η^2=0.139, p=0.004] and controls (Valence x Day; F(1,18)=5.12, η^2=0.222, p=0.036]. Because of this significant difference between performance on day 1 and 2 in terms of valence effects, we added 'testing order' as a factor in the within-participant analysis of patient data to assess whether potential test-retest effects interacted with effects of medication. There was a medication x valence x testorder interaction [F(1,54)= 8.9, η^2=0.14, p=0.004], in addition to a medication x valence x action x testorder interaction [F(1,54)= 5.33, η^2=0.090, p=0.025]. Here it should be noted that a Medication x TestOrder interaction is mathematically identical to a main effect of testing day. Given the presence of such an effect of testing day in the healthy controls, it is not possible to meaningfully interpret the Medication x Testorder interaction. Therefore, we restricted our analysis to data from day 1, considering Medication as a between-participants factor instead of a within-participants factor.

4.7.3 | Nuisance correction: Low delay group

In our control analysis, we found that the tremor group on average had a longer delay between medication admission and the task onset. In this section, we test whether restricting ourselves to a low delay tremor-group that matches the delay of the non-tremor group changes the main results. We find that the results do not change, and our main analysis all give comparable results. Similar to earlier results, we do not replicate previous reports that levodopa medication improved performance on the Win versus the Avoid trials [F(1,31)= 0.05, η^2=0.001, p=0.8]. We still find that the interaction of medication and valence was strongly modulated by patient-group [Tremor Group x Medication x Valence: F(1,31)= 12.0, η^2=0.28, p=0.002]. Both groups showed a significant modulation by levodopa of performance on Win versus Avoid cues [Medication x Valence: tremor - F(1,13)= 8.05, η^2=0.382, p=0.014; non-tremor - F(1,18)= 6.37, η^2=0.261, p=0.021], but in opposite directions.

4.7.4 | Nuisance correction: Age

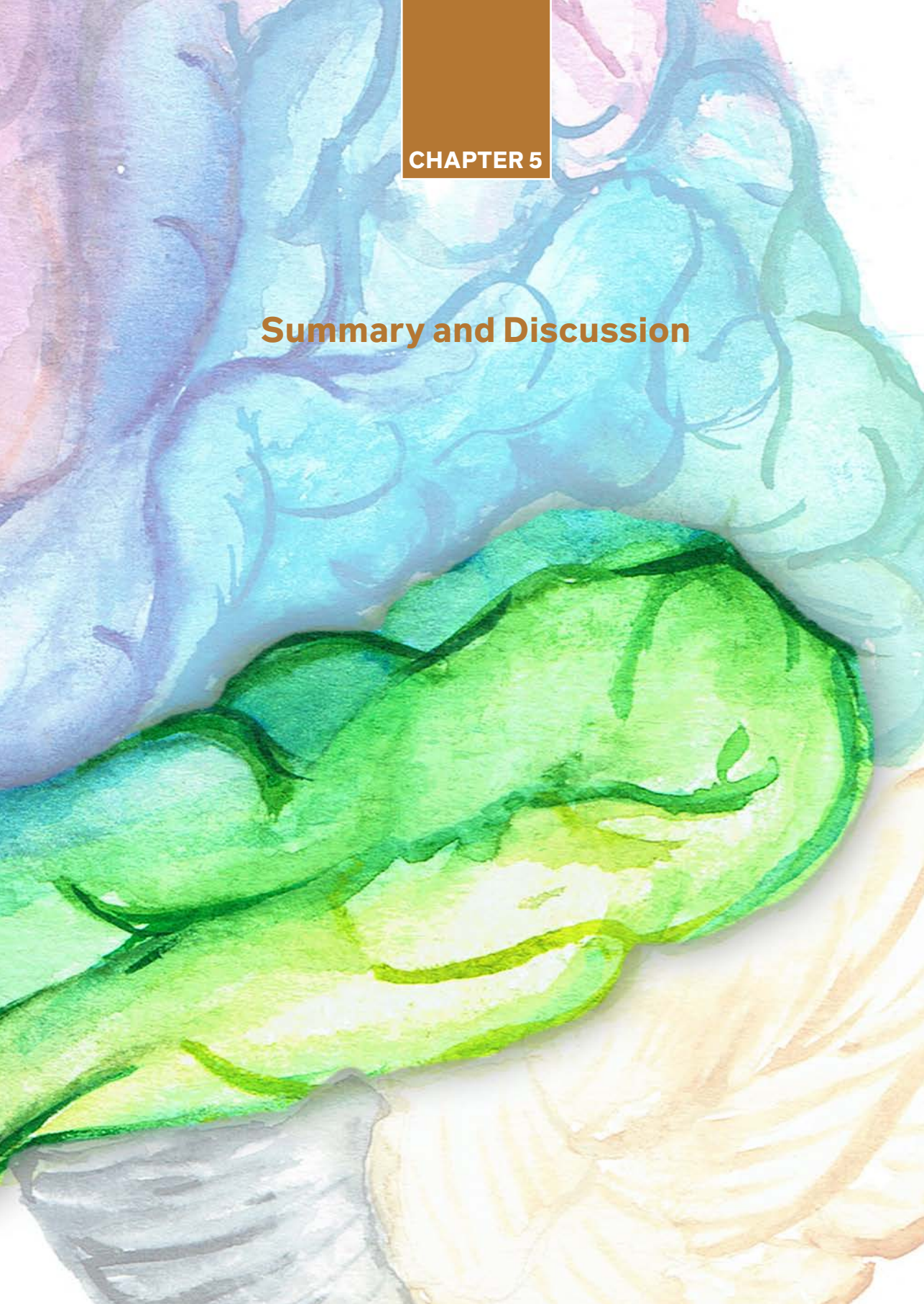
We found that although age significantly influences overall performance (participants perform better when they are younger), age does not correlate with any of the effects of interest:

Contrast	F (1,38)	p value
Age	**13.4**	**<.001**
Valence x Age	0.3	.6
Valence x Medication x Age	0.2	.5
Valence x Medication Response Group x Age	0.0	.9
Valence x Medication x Medication Response Group x Age	0.1	.9

We find that the results do not change, and our main analysis all give comparable results. Similar to earlier results, we do not replicate previous reports that levodopa medication improved performance on the Win versus the Avoid trials [F(1,57)= 0.95, η^2=0.016, p=0.33]. We still find that the interaction of medication and valence was strongly modulated by patient-group [Tremor Group x Medication x Valence: F(1,57)= 15.2, η^2=0.21, p<0.001]. Both groups showed a significant modulation by levodopa of performance on Win versus Avoid cues [Medication x Valence: tremor - F(1,39)= 7.13, η^2=0.160, p=0.010; non-tremor - F(1,17)= 5.96, η^2=0.260, p=0.026], but in opposite directions.

CHAPTER 5

Summary and Discussion



In this thesis I aimed to unravel some of the fundamental differences between tremor and non-tremor subtypes of Parkinson's disease. Although the differentiation between tremor and non-tremor patients was originally inspired by clinical observations, it was shown to hold wider pathophysiological relevance. In this work I addressed three (neural) cause and (behavioral) effects of Parkinson's disease focusing specifically on this relevant clinical subdivision. I studies these underlying neural systems by assessing both the degree of focal nigrostriatal cell loss and the differences GABA levels in motor circuit in tremor-dominant and non-tremor phenotypes. Furthermore, I assessed the effects of those clinical phenotypes on motivational behavior. In this final chapter, I will summarize and discuss the findings of this thesis. I will end by sketching future perspectives on patient research and clinical relevance.

Summary

In **chapter 2**, I investigated the hypothesis that GABA in the thalamo-cortical motor circuit is increased in Parkinson's disease compared to healthy controls. Moreover, I explored whether there are differences in baseline GABA levels between non-tremor and tremor patients, including tremor patients whose tremor symptoms are resistant, and patients with tremor responsive to DA medication, as a function of dopaminergic medication. I measured GABA-to-total-Creatine ratio in motor cortex, thalamus, and a control region (visual cortex) on two separate days, to get separate measures ON and OFF dopaminergic medication. I found that GABA levels were unaltered by Parkinson's disease, clinical phenotype, or medication. Our findings show that GABA concentrations in the primary motor cortex are inversely correlated with disease severity (total MSD-UPDRS score), independent of dopaminergic medication (i.e. present across OFF and ON dopaminergic medication sessions), and independent of the type of symptom-subscore (tremor, bradykinesia, rigidity). This suggests that GABA may play a modulatory role in the pathophysiology of Parkinson's disease that is independent of dopaminergic denervation. I speculate that GABA in the motor cortex might have a protective role, either at the neuronal level (e.g. by preventing calcium-based neurotoxicity) or at the circuit level (e.g. by preventing dysfunctional motor hyperactivity), and that GABA depletion may contribute to increased motor symptom expression.

In **chapter 3**, I focused on differences in the degree of neurodegeneration in the SN and RRA between patients with tremor-dominant and non-tremor Parkinson's disease. This was based on post-mortem studies indicating altered patterns of neurodegeneration between clinical subtypes in these two dopaminergic nuclei. Specifically, this work had shown that tremor-dominant patients show reduced cell loss in the substantia nigra (SN) but increased cell loss in the retro-rubral area (RRA). In this chapter, I tested whether these results could be replicated in vivo. In all subjects, I used diffusion tensor MRI to quantify free water concentration, a marker of neurodegeneration, in the

relevant regions of interest. In line with previous results showing correlations between bradykinesia and SN degeneration, and the theoretical dimmer-switch model which suggests a relation between RRA degeneration and tremor, I further tested whether free water intensity in the SN and RRA correlated with bradykinesia and tremor severity, respectively. Free water signal was increased for non-tremor compared to tremor-dominant Parkinson patients in the posterior SN, but there were no differences for the RRA. However, there was a strong positive correlation between clinical resting tremor severity and free water signal in the RRA. Nevertheless, this study failed to replicate previously reports of SN free water differences between Parkinson patients and healthy controls. I conclude that different patterns of neurodegeneration in the midbrain are associated with tremor (severity) and non-tremor motor symptoms.

In **chapter 4**, I investigated the effects of dopaminergic medication on motivated behavior in tremor-dominant and non-tremor patients and healthy controls. In a wealth of previous studies, Parkinson's disease patients have been shown to differ from healthy controls on reinforcement learning tasks. However, there were signs that this process might be differentially affected in tremor and non-tremor patients. Research has shown that non-tremor patients present with a faster cognitive decline, along with reduced DAT binding in the striatum (Helmich, Janssen *et al*. 2011) as well as higher substantia nigra (SN) degradation (Jellinger and Paulus 1992). Based on these differences in cognitive and dopamine dysfunction, I hypothesized that well-established effects of dopaminergic medication on reinforcement learning may differ between tremor-dominant and non-tremor patients. Participants performed a reinforcement-learning task exploring the effect of dopaminergic medication on both motor response and motivational valence. In this chapter, I show that the effect of medication on reinforcement learning was indeed predicted by Parkinson motor phenotype. Specifically, I found that results from non-tremor patients replicated the previous literature, as patients showed increased learning from rewards relative to punishments on dopaminergic medication. The behavior of tremor dominant patients however, was in direct opposition to the previous literature. While in the previous literature punishment learning was relatively worse on dopaminergic medication, in the current study tremor-dominant patients showed a relative improvement in learning from punishment after medication. This change in behavior was sub served by different computational mechanisms, with dopaminergic medication affecting motivational choice bias and learning rate in non-tremor versus tremor-dominant patients, respectively. My findings suggest that effects of dopaminergic medication on reinforcement learning are modulated by the Parkinson motor phenotype. I speculate that this may relate to differences in the pattern of dopaminergic cell loss in the mesencephalon. A further important implication of these findings is that I may have uncovered a consistent selection bias in behavioral studies in Parkinson's disease, with a bias towards (more easily testable) non-tremor patients, suggesting that previous literature is perhaps not generalizable to the entire population of Parkinson's disease patients.

Discussion

5.1 | What have my findings contributed to our understanding of the pathophysiology of tremor?

This work made two specific contributions concerning the pathophysiology of tremor. First, in chapter 3, I investigated the association between tremor and neurodegeneration in the RRA. Second, in chapter 2, I assessed the relationship between thalamic GABA levels and the occurrence of tremor (comparing tremor-dominant and non-tremor patients) as well as the clinical severity of tremor. The RRA and the thalamus play an important role in the dimmer-switch hypothesis (Helmich, Janssen *et al*. 2011, Helmich, Hallett *et al*. 2012). In this model of tremor in Parkinson's disease, dopaminergic cell death in the RRA causes dopamine depletion in the pallidum and thalamus. Pallidal dopamine depletion then leads to emergence of abnormal pallidal activity that triggers tremor oscillations in the basal ganglia, which are then transmitted to the cerebello-thalamo-cortical circuit that maintains and amplifies the tremor. The pathological activity in the striatal-pallidal circuit triggers tremor episodes (analogous to a light switch), while the cerebello-thalamo-cortical circuit produces the tremor and controls its amplitude (analogous to a light dimmer).

5.1.1 | *Support for role of RRA in tremor*

Prior to this thesis, there were a few (indirect) pieces of evidence supporting the role of the RRA in this tremor network: Post-mortem work shows higher degeneration in the dopaminergic retro-rubral area (RRA) of tremor-dominant patients compared to non-tremor patients (Hirsch, Mouatt *et al*. 1992). In contrast, non-tremor patients show higher substantia nigra pars compacta (SNc) degeneration (Jellinger and Paulus 1992). Additionally, experiments involving non-human primates exposed to a neurotoxin specific to dopaminergic neurons have shown that animals with predominant RRA damage most resembled the tremor phenotype (Deutch, Elsworth *et al*. 1986, Bergman, Raz *et al*. 1998), while primarily SNc affected animals were more akinetic. Using functional MRI, it had been shown that dopamine reduces Parkinson's disease tremor by acting on the globus pallidus and on the ventrolateral thalamus (Dirkx, den Ouden *et al*. 2017). Both of these regions receive dopaminergic input from the RRA (Jan, François *et al*. 2000, Sánchez-González, García-Cabezas *et al*. 2005). Finally, SPECT data showed a correlation between tremor severity and dopamine depletion in the pallidum, which receives dopaminergic projections from the RRA, but not in the striatum, which receives dopaminergic projections from the (posterior) SN) (Helmich, Janssen *et al*. 2011).

In chapter 3, I show for the first time, an association (in vivo) between clinical tremor severity and RRA neurodegeneration (as indexed by free water level). This measure

of RRA degeneration explained 26% of the measured tremor intensity. In addition, this correlation was specific for the tremor symptoms. There was no correlation between free water levels in the RRA and measures of bradykinesia. It was also regionally specific, as there was no correlation between tremor severity and SN neurodegeneration. This fits with the dimmer-switch model, showing that the RRA indeed holds an important relation to the emergence of tremor. I did not find between-group differences in RRA degeneration between tremor and non-tremor patients, as reported in an earlier post-mortem study (Hirsch, Mouatt *et al*. 1992). This null finding might be due to differences between patient populations. The patients included in this study were still relatively mildly afflicted, with a maximum H&Y score of three, and an average of only four year since initial diagnosis. However, the disparity between the strong correlation between RRA free water levels and the lack of between-group difference does suggest that the RRA could not be the sole origin behind the tremor symptoms, and that there are other mechanisms at play as well. Potential candidates may involve the noradrenergic and serotonergic system, which have both been implicated to play a role in the occurrence and severity of tremor (Doder, Rabiner *et al*. 2003, Caretti, Stoffers *et al*. 2008, Isaias, Marzegan *et al*. 2012, Qamhawi, Towey *et al*. 2015), and have been known to also be affected in Parkinson's disease.

5.1.2 | *No evidence for role of thalamic hyperpolarization in tremor*

The hyperpolarization theory was one of the major theories offering an explanation of the differences between tremor and non-tremor patients. This theory states that parkinsonian tremor could be caused by strongly increased GABAergic input from the pallidum into the thalamus, causing local hyperpolarization of thalamic neurons. This idea was based on intrinsic biophysical properties of thalamic neurons, which under laboratory conditions oscillate at 6 Hz while hyperpolarized (Llinás 1988) coinciding with the frequency of Parkinson's disease tremor. Hyperpolarization theory predicts that thalamic GABA levels are increased in tremor patients compared with non-tremor patients causing GABAergic tone to increase beyond a certain barrier necessary to instigate local hyperpolarization. Potentially, this could be related to the increased pallidal dysfunction seen in the tremor group.

However, subsequent findings from related studies have cast doubt on the validity of this theory. The proposed low-threshold calcium-dependent spiking behavior was not present in the specific thalamic region associated with resting tremor, i.e. the posterior portion of the ventrolateral thalamus (Magnin, Morel *et al*. 2000). Further, a recent functional MRI study using dynamic causal modelling showed that dopaminergic medication reduced tremor severity by increasing, rather than decreasing, thalamic self-inhibition (Dirkx, den Ouden *et al*. 2017). In the work presented in chapter 2, I also find no evidence for increased GABAergic concentration in tremor-dominant compared to non-tremor patients, nor do I find a change in

GABAergic tone in response to dopaminergic medication. This further cements the idea that tremor is not caused by thalamic hyperpolarization, but rather due to different processes involving the cerebello-thalamic-cortical circuit.

5.2 | Cerebral differences between motor phenotypes in Parkinson's disease

Although there was no difference in GABA levels between the two motor phenotypes in either the thalamus or the motor cortex, I did find prominent differences between the two subtypes in both substantia nigra free water levels as well as in motivated behavior. As hypothesized, I found signs of stronger neurodegeneration in the posterior SN of non-tremor patients in chapter 3, and different patterns of dopamine related reward and punishment learning between the two Parkinson's disease subtypes in chapter 4. These differences between motor phenotypes do not stand on their own. There has been a growing number of studies showing variations of disease expression between Parkinson's disease motor subtypes – either contrasting tremor to non-tremor patients, or patients differing in postural instability gait difficulty (PIGD). The PIGD subtype has different selection criteria compared to our non-tremor patients (i.e. emphasis on the presence of gait and balance problems rather than the absence of tremor). However, given that tremor is usually relatively mild (or absent) in Parkinson's disease patients with predominant gait and balance problems, in practice the PIGD and non-tremor phenotypes largely overlap (Stebbins, Goetz *et al*. 2013). Differences span a broad variety of subjects; non-tremor and tremor patients show altered brain wide degeneration patterns (Rosenberg-Katz, Herman *et al*. 2013, Vervoort, Leunissen *et al*. 2016), distinct dopamine-linked degeneration (Hirsch, Mouatt *et al*. 1992, Jellinger and Paulus 1992), and behavioral and clinical differences (Wu, Le *et al*. 2011, Helmich, Hallett *et al*. 2012, Wylie, van den Wildenberg *et al*. 2012). Examples of brain-wide structural differences include widespread DTI fractional anisotropy reductions for PIGD (but not tremor-dominant) patients involving the superior longitudinal fasciculi and corpus callosum, suggesting stronger widespread microstructural decline (Vervoort, Leunissen *et al*. 2016). Other work shows increased grey matter atrophy in the PIGD group in several brain areas including motor as well as cognitive, associative, and limbic regions (Rosenberg-Katz, Herman *et al*. 2013). Combined, we see that non-tremor patients show higher levels of brain-wide neurodegeneration.

This notion seems to be mostly mirrored in dopamine specific degeneration patterns. In line with our free water results in chapter 3, post-mortem work shows differences in the pattern of dopaminergic cell loss in the midbrain. Non-tremor patients show higher substantia nigra pars compacta (SNc) degeneration (Jellinger and Paulus 1992), while the reverse was found in the dopaminergic retro-rubral area (RRA), where tremor-dominant patients had more neurodegeneration than non-tremor

patients (Hirsch, Mouatt *et al*. 1992). In vivo, recent work comparing tremor patients to a PIGD-subgroup using a neuromelanin sensitive MRI protocol, showed more severe neuromelanin decline in the SNc of the PIGD group (Xiang, Gong *et al*. 2017). In addition to midbrain degeneration, research has found subsequent functional differences in striatal signaling and integrity: PET imaging studies show lower dopamine transporter binding in the striatum for non-tremor patients (Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). Additionally, VBM results show lower globus pallidus grey matter volumes for PIGD patients compared to tremor dominant Parkinson's disease (Rosenberg-Katz, Herman *et al*. 2016).

Behaviorally and clinically, we see a similar pattern. Non-tremor patients show a faster overall disease progression (Selikhova, Williams *et al*. 2009), with a more rapid cognitive decline (Wu, Le *et al*. 2011), and increased likelihood to develop Parkinson-associated dementia (Aarsland, Andersen *et al*. 2003, Williams-Gray, Foltynie *et al*. 2007). In general, non-tremor patients seem to encompass a more severe form of Parkinson's disease

Our behavioral results however, show qualitative rather than quantitative differences between tremor-dominant and non-tremor Parkinson's disease patients. Specifically, instead of simply showing a less and more severe phenotype, the non-tremor and tremor-dominant patients show distinctly different behavioral patterns in response to dopaminergic medication. In line with earlier results, the non-tremor patients responded to dopaminergic medication by showing increased performance for cues signaling a potential win ON levodopa. In contrast, upon receiving levodopa, tremor-dominant patients performed better for cues where punishment needed to be avoided, in direct opposition of previous findings. Some of the differences in behavior might be explained by differences in SN degeneration; (Chowdhury, Guitart-Masip *et al*. 2013) showed a relationship between degree of neurodegeneration in the SN (estimated using magnetization transfer (MT) imaging) and the ability to suppress maladaptive motivationally driven invigoration. They showed that more *damage* to the SN related to better performance on trials where subject had to suppress making an action to gain a reward (go-to-win). Therefore, it is possible that the distinct reward-associated response driven by the go-to-win cues in the non-tremor patients is related to this particular finding. Indeed, we show higher SN degeneration in non-tremor patients compared to tremor patients in chapter 3. However, it is difficult to explain these behavioral differences between Parkinson's disease motor phenotypes on dopaminergic changes alone. This especially true considering the tremor-dominant patients' unorthodox response to dopaminergic medication, showing an increase in punishment associated learning.

A plausible secondary source of altered behavioral response in the tremor-dominant group could be due to influences from either serotonergic (and/or) noradrenergic

systems. Serotonin has been implicated in punishment processing (Crockett, Clark *et al*. 2009, Watson, Ghodasra *et al*. 2009), while non-adrenergic system seems to relate to attention, arousal (Bouret 2019) and behavioral flexibility (Jahn, Gilardeau *et al*. 2018). In support of this idea there are at least two studies showing that tremor dominant patients have lower levels of thalamic serotonin transporters compared to non-tremor patients (Caretti, Stoffers *et al*. 2008, Qamhawi, Towey *et al*. 2015), with serotonin levels relating to tremor severity. With respect to noradrenaline, noradrenergic mechanisms were found to be increased in tremor-dominant patients relative to non-tremor patients (Isaias, Marzegan *et al*. 2012).

Combined, the literature and these present results paint a picture of differential dopaminergic mechanisms in tremor-dominant and non-tremor Parkinson's disease patients. In this thesis, I offer an in-vivo conceptual replication of the findings showing increased degeneration in the substantia nigra for non-tremor patients in chapter 3. Moreover, I further these results by showing altered dopamine dependent motivated behavior in chapter 4. These results implicate that the dopaminergic response on motivated behavior in Parkinson's disease is more complicated that generally assumed, and strongly relates to patient motor-phenotype.

5.3 | Clinical implications

Parkinson's disease is a highly heterogeneous disorder, that often requires a variable treatment regime depending on the individual patient. Finding each patient's optimal treatment can be an arduous and time-consuming process, requiring a lot of trial and error. By furthering our understanding of the mechanisms, pathology and behavioral consequences associated with variability in symptoms, we hope that these results may eventually help to expedite this process, or potentially provide new angles for treatment.

In each empirical chapter of my thesis I provides a separate insight in the pathology of Parkinson's disease, which I will discuss below. In **chapter 2** I have shown that thalamic GABA did not significantly differ between non-tremor and tremor (DA resistant and responsive) phenotypes. However, I found a strongly significant correlation between motor cortex GABA levels and tremor severity. This strong correlation between GABA in the motor cortex and disease severity could suggest that GABA plays a modulatory role in the pathophysiology of Parkinson's disease, independent of dopaminergic denervation. I speculate that cerebral GABA has a protective role, either at the neuronal level (e.g. by preventing calcium-based neurotoxicity) or at the circuit level (e.g. by preventing dysfunctional motor hyperactivity). If such a neuroprotective role of GABA indeed holds this could have important implications for the treatment of patients with Parkinson's disease. After sufficient verification in subsequent studies using other modalities (e.g. flumazenil PET, which measures GABAA receptors

binding), treatments using GABAergic medication could be considered to slow disease progression, or to potentially reduce patients' symptom severity.

In **chapter 3**, I am the first to show *in vivo* evidence towards increased neurodegeneration in (posterior) substantia nigra tissue for non-tremor patients compared to their tremor-dominant counterparts using Free water DTI. This confirms results from post-mortem (Jellinger and Paulus 1992) and animal studies (Deutch, Elsworth *et al*. 1986, Bergman, Raz *et al*. 1998). This greater neurodegeneration is accompanied by changes in striatal dopaminergic activity, with PET imaging studies show lower dopamine transporter binding in the striatum for non-tremor patients (Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). The discriminatory power of the differences between the two subgroups was relatively poor in this study, as previous studies showed larger differences between patients and controls. This is likely due to signal to noise issues. However, there are clear signs that power levels could be improved with proper optimization of the DTI sequence and the subsequent analysis procedure (see chapter 3 for a more extensive review on this topic). With sufficient signal to noise, and subsequent discriminatory power, Free Water levels could provide a non-invasive biomarker of dopaminergic decline, and potentially predict aspects of patients' subsequent disease trajectory – in line with the clear differences in disease expression between non-tremor and tremor dominant Parkinson's disease patients.

In **chapter 4**, I find notable differences in motivational behavior between the two motor-phenotype based Parkinson's disease subgroups. This is expressed as improved reward learning caused by a *decrease* in motivation-action coupling in response to dopaminergic medication for non-tremor patients, and faster punishment learning for tremor-dominant patients. Changes in motivated behavior could be associated with a variety of clinical behavioral symptoms. Relevant examples include impulse control disorder, anxiety, apathy and depression, as they are often associated with Parkinson's disease. Indeed, a recent study shows reduced action-specific motivational biases in depressed patients relative to healthy controls in the context of a Pavlovian-instrumental transfer task (Huys, Gölzer *et al*. 2016). Additionally, (Mkrtchian, Aylward *et al*. 2017) showed that mood and anxiety disorders were associated with increased reliance on an avoidance bias (to withhold responding in the face of punishments) during reinforcement learning. This shows that such biases are indeed altered in relation to mental health. An increase in avoidance bias might therefore prime patients towards higher anxiety, or related mental health problems such as depressive symptoms. However, due to the design and purpose of this study, there is insufficient evidence to definitively link cognitive/motivational processing to clinical cognitive/motivational symptoms.

Overall, I find remarkable differences between tremor and non-tremor patients both in dopamine related motivated behavior, as in markers for degeneration of dopamine associated neuronal tissue. Combined, this paints a message of disparity in dopaminergic decline between the two tremor groups, with non-tremor patients showing a larger dopaminergic deficit. These results could therefore inform likely medication targets for improved treatment specificity in tremor and non-tremor patients.

5.4 | Future directions

5.4.1 | *Tremor subtype is a relevant factor to consider in future related studies.*

In two separate modalities, I showed a notable difference in pathophysiology between the tremor and non-tremor disease phenotype. Specifically, in line with our hypotheses I show a significant difference in substantia nigra free water levels (as a proxy of neurodegeneration). In addition, I found a remarkable difference in the interaction between disease subtype, dopaminergic medication and motivational behavior. In both instances, analyses across these two phenotypes reduced the ability to detect disease-specific differences between patients and controls. Thus, separation into these two clinical phenotypes reduced the intra-group variation and uncovered group specific differences.

These results also highlight the importance of avoiding selection bias in patient recruitment, and underline the importance of increased awareness and representation of inter-patient diversity. I would therefore strongly advice to include tremor phenotype as a factor of interest in future work – or, at the very least, report UPDRS subscores of the included Parkinson patient groups so that these differences can be assessed.

5.4.2 | *Re-utilizing previous data*

There is great merit in replicating each of the findings presented in this thesis, as all three finding represent relatively novel research topics. Replication is costly and time intensive, limiting the number of replication studies performed. However, in this case, replication studies could likely be achieved through re-analyzing old datasets. In many studies of Parkinson's disease patients, MSD-UPDRS symptoms were recorded yet never used as a potential method to detect clinical patient subsets. Reexamining old datasets based on their UPDRS sub-scores, would allow us to confirm a potential bias towards non-tremor patients in previous studies. Moreover, it would allow us to revisit and potentially confirm the results put forth in this study. For example, revisiting free water results on previously measured Parkinson's disease patients (e.g. such as in the research line of Efori *et al.*) would allow us to follow up on the results from chapter 3. Separating these existing patients into motor subgroups, could confirm differences

in substantia nigra degeneration tremor-dominant and non-tremor patients. A more thorough effort, which includes revisiting raw B0 and free water images of these studies and manually locating the RRA as a region of interest in the subjects, could confirm the relationship between RRA degradation and tremor severity.

In addition, revisiting motivational studies (Frank, Seeberger *et al*. 2004, Cools 2006, Bódi, Kéri *et al*. 2009), in terms of tremor vs. non-tremor patient subgroups, could be very informative. This would show whether classic results showing a reversal between preferential reward and punishment learning as a function of medication hold for both subgroups. A Alternatively – in the unfortunate event that the patient selection bias proves to be so substantial that studies included very few tremor patients, this will put those previous results in perspective.

5.4.3 | *Revisiting GABA results with methodological improvements*

When aiming to identify the differences in GABA levels in the thalamus and motor cortex in chapter 2, the main limitation proved to be the limited signal to noise that could be achieved in the thalamic region. In this study, I report a null finding showing no differences in GABA levels in Parkinson's disease patients compared to healthy controls. However, this study was limited in regards to signal to noise in this region. Furthermore, we find that recent papers on the same topic have shown contradicting results. Therefore, a replication with optimized GABAergic measurements could help to further cement these findings, and definitively show whether GABA levels differ or indeed remain unchanged in Parkinson's disease patients and patient-subgroups. MRS studies in the thalamus are inherently limited in coverage, as the shape and size of the thalamus limits the use of larger voxel sizes. Further optimization might be gained by longer scanning times, secure head fixation to limit movement and further technical advancements. There are promising results showing increased reliability using water referencing (Bogner, Gruber *et al*. 2010, Mullins, McGonigle *et al*. 2014), macromolecule suppression (Henry, Dautry *et al*. 2001) and the introduction of higher field strengths (Terpstra, Ugurbil *et al*. 2002).

Apart from MRS, there are other options to detect GABA activity in the brain such as Flumazenil PET. Measurements in GABAergic changes using Flumazenil PET in related disorders such as dystonia and essential tremor, has already shown promising results. For example, it has revealed a reduced GABA-A receptor binding in the cerebellum and sensorimotor cortex of dystonia patients, while showing increased GABA-A receptor binding in the cerebellum (Gallea, Herath *et al*. 2018), ventrolateral thalamus and lateral premotor cortex of essential tremor patients (Boecker, Weindl *et al*. 2010).

5.4.4 | *Tremor subtypes relating to cognitive heterogeneity*

In this study, I found a clear dichotomy in motivational behavior within Parkinson's disease motor subgroups. This was in line with earlier studies showing higher cognitive decline in non-tremor patients (Aarsland, Andersen *et al*. 2003, Burn, Rowan *et al*. 2006, Williams-Gray, Foltynie *et al*. 2007, Wu, Le *et al*. 2011), as well as reduced dopaminergic levels in the striatum for this same subgroup (Jellinger and Paulus 1992, Spiegel, Hellwig *et al*. 2007, Rossi, Frosini *et al*. 2010, Helmich, Janssen *et al*. 2011). A related literature on cognitive heterogeneity within Parkinson's disease patients (Williams-Gray, Evans *et al*. 2009), reported a different distinction between patients. They found two distinct genetic factors that could substantially predict whether patients showed increases in cognitive impairment. One of these genes was an independent predictor of dementia risk, while the other had no effect on dementia, but a significant impact on Tower of London performance. This same study also reported that the non-tremor phenotype (in opposition to a tremor dominant subtype) represented a notable risk factor towards increased cognitive decline. The same work however failed to report whether these genetic factors might further be able to predict development towards either the tremor or non-tremor phenotypes. Seeing that both non-tremor phenotype as these genetic factors are a reliable predictor of cognitive decline, a further investigation of their relation might provide a further understanding of the development of the tremor and non-tremor phenotypes and their relation to genetic diversity and behavioral or motivational dysfunction.

5.5 | Concluding remarks

The aim of this thesis was to investigate the pathophysiological basis of Parkinson's disease, while focusing on cerebral and neuropsychological differences between tremor-dominant and non-tremor patients, using multifaceted approach. This has resulted in several key findings:

First, in chapter 2, I found that GABA concentrations in the primary motor cortex were inversely correlated with disease severity, independent of dopaminergic medication. I speculate that this suggests that GABA may play a modulatory role in the pathophysiology of Parkinson's disease. Second, in chapter 3, I was the first to show in-vivo evidence in Parkinson's disease patients for a relationship between RRA neurodegeneration and tremor severity, confirming a previous post-mortem study. Furthermore, in this same chapter, I showed that increased free water signal for non-tremor Parkinson patients compared to tremor-dominant patients in the posterior SN. Finally, in chapter 4, I found a clear and distinct difference in motivational behavior between the two motor-phenotype (tremor) based Parkinson's disease subgroups. I revealed that established ideas on dopaminergic influence on value learning only held for the non-tremor subgroup.

Together this thesis paints a picture of disparity in dopaminergic decline between the two Parkinson phenotypes. Overall, I find evidence in the direction of non-tremor patients showing a larger dopaminergic deficit. They show signs of more neurodegeneration in the posterior SN, and showed a higher divergence from healthy controls in motivated behavior OFF medication. This tendency is in line with ideas brought forth in previous literature, suggesting that non-tremor patients encompass a more severe form of Parkinson's disease. In addition, in my anatomical work, I show a specific relationship between RRA denegation and tremor.

Overall, I found that merging the two symptom groups reduced our ability to detect disease specific differences between patients and controls. The separation into these two clinical phenotypes reduced the intra-group variation and helped me identify group specific behavior. This highlights the importance of avoiding selection bias in the patient recruitment, and underlining the importance of increased awareness and representation of inter-patient diversity. I hope that my observations will promote incorporation of detailed descriptions of individual symptom severity, and an increased attention towards differences between these two motor phenotypes.

5.6 | References

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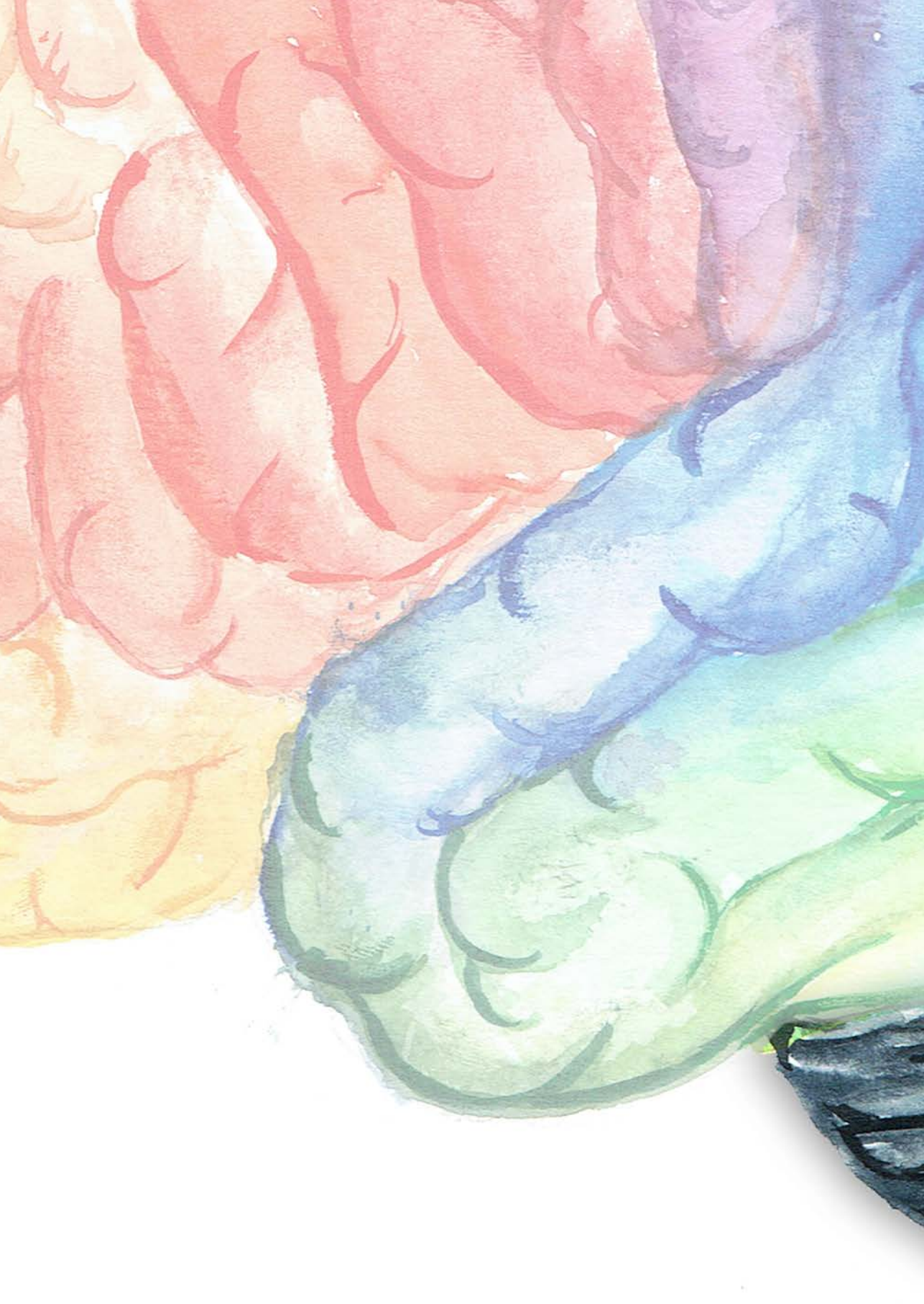


CHAPTER 6

Appendices

Nederlandse samenvatting

Inleiding

In deze thesis wordt een belangrijk symptoom van de ziekte van Parkinson vanuit drie verschillende invalshoeken onderzocht. Hiermee richt ik me op dopamine-producerende cellen in de substantia nigra, en op de invloed hiervan op de basale ganglia. Dit zijn belangrijke hersenonderdelen voor beweging en motivatie. Specifiek kijk ik naar wat in deze gebieden verschillen zijn tussen twee (en soms drie) verschillende patiëntgroepen.

Wat is de ziekte van Parkinson?

De ziekte van Parkinson is een degeneratieve ziekte. Dat betekent dat de symptomen van de ziekte steeds sterker worden. Patiënten krijgen moeite met bewegen: lopen met schuifelende pasjes, een voorovergebogen rug, en soms ook trillende handen. In de hersenen van deze patiënten sterven de dopamine-producerende cellen langzaam af. Dopamine is een neurotransmitter die belangrijk is bij het starten van bewegingen. Er zijn een paar kleine hersengebieden waar dopamine wordt aangemaakt en rondgestuurd; de grootste is de substantia nigra.

Wat doen de basale ganglia?

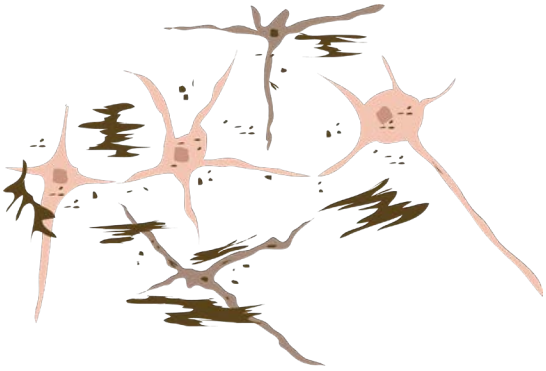
Om te begrijpen wat de substantia nigra doet moet je eigenlijk begrijpen wat de basale ganglia doen. De basale ganglia zijn een groep hersengebieden die de doorschakeling van allerlei informatie regelen. Ze regelen ook het doorgeven van hersensignalen bij het maken van bewegingen. In de communicatie in je hersenen bestaat veel ruis. De taak van de basale ganglia is onder andere om nuttige hersensignalen te scheiden van die ruis. Als je basale ganglia te veel informatie zouden doorlaten, zou je de hele tijd ongecontroleerde bewegingen maken, of er van alles ineens uitschreeuwen (dit is er mis bij het syndroom van Tourette). Maar de controlerende werking van de basale ganglia mag ook niet te sterk zijn. Als je echt een beweging wilt maken, moeten de basale ganglia dat niet tegenhouden.

Wat doet de substantia nigra?

Het reguleren van de basale ganglia is de taak van de substantia nigra. De substantia nigra controleert de basale ganglia met behulp van de neurotransmitter dopamine (neuro = hersenen, transmitter = doorgever).

Als je substantia nigra niet goed werkt worden je bewegingsimpulsen te hard gefilterd door de basale ganglia. Het signaal voor het beginnen van een beweging moet dan

heel sterk zijn om door de basale ganglia heen te komen. Dit veroorzaakt de traagheid en het moeilijke bewegen bij Parkinson. Het is bij Parkinson niet zo dat de spieren niet kunnen bewegen – de hersenen hebben het moeilijk. Dit komt doordat de dopamine-makende neuronen in de substantia nigra steeds verder afsterven.



Stervende dopamine neuronen omgeven door te veel eiwit-afval

Waarom de dopamine-cellen?

Helemaal precies weten we het niet wat er bij Parkinson misgaat in de substantia nigra, maar we weten wel dat een aantal specifieke eiwitten ermee te maken hebben. Één van deze eiwitten wordt alpha synucleïne genoemd.

U kunt zich een hersencel voorstellen als een grote eiwitfabriek met een lange lopende band waar van alles gebeurt. In een bepaalde productiestap gaat er iets mis in de fabriek en wordt er heel wat afval geproduceerd. In het geval van Parkinson zijn dat bijvoorbeeld te veel verkeerd gevouwen alpha synucleïne-eiwitten. Als de fabriek te weinig wordt schoongemaakt, of als de machine in de fabriek meer afval dan normaal produceert, dan heeft de hersencel een afvalprobleem. De verkeerd gevouwen alpha synucleïne-eiwitten hopen zich op, klonteren samen en vormen hele bouwwerken binnen en buiten de cellen. Op een gegeven moment stort de cel in.

Waarom gaat het juist bij dopamine cellen in de substantia nigra fout? Alpha synucleïne wordt meer aangemaakt in de dopamine-producerende cellen, waardoor er in de cellen van de substantia nigra als eerste een probleem ontstaat. We zien dat uiteindelijk de rest de hersenen ook schade begint op te lopen. Eigenlijk gaat het allemaal nog best lang wél goed. De hersenen zijn best sterk en houden het lang uit. Pas als ongeveer 70% van de cellen in de substantia nigra doodgegaan is beginnen we het te merken - en dan noemen we het Parkinson. Zo veel afgestorven cellen kun je niet zomaar reparen want hersencellen groeien niet zomaar uit zichzelf weer aan. Daarom is Parkinson niet te genezen.

Tremor en non-tremor patiënten

Als we een Parkinson's patiënt voor ogen hebben denken we vaak aan een schuifelende persoon met trillende handen en benen. Dit trillen, ook wel de tremor genoemd, komt maar bij ongeveer 75% van de patiënten voor.. Het opmerkelijke is ten eerste dat veel patiënten een symptoom hebben dat andere patiënten totaal niet vertonen. Maar vooral dat we tussen de trillende en niet-trillen patiënten (tremor en non-tremor) bredere verschillen zien dan alleen deze tremoren. Zo hebben niet-trillende patiënten vaak een sneller ziekteverloop en zien we in deze groep ook eerder mentale klachten verschijnen. Zijn deze patiënten misschien net iets anders aangedaan? Gaan er misschien andere hersendelen eerder achteruit bij groep één dan groep twee? Is er iets dat de tremor veroorzaakt dat beschermd tegen andere achteruitgang? Om hier meer duidelijkheid in te krijgen heb ik in deze thesis drie hele specifieke vragen gesteld.

VRAAG 1 | ***Zijn de problemen in de Basale Ganglia terug te zien in de neurotransmitter-spiegel van twee belangrijke bewegingsgebieden?***

(En zien we hier verschillen tussen de patiëntgroepen? Is de ene groep bijvoorbeeld meer aangedaan dan de andere?)

Veel van de werking van de hersenen is gebaseerd op een balans van stimulering en remming. Wat belangrijk is wordt gestimuleerd, bijzaken worden geremd. Dit gebeurt via twee belangrijke neurotransmitters: Glutamaat, de stimulant; en GABA, de remmer. Door middel van een principe genaamd MRS (Magnetic Resonance Spectroscopy) zijn we in staat van een aantal stoffen in de hersenen de hoeveelheid te meten. Dit is echter niet makkelijk: de concentratie van deze stoffen moet hoog genoeg zijn om een goede meting te maken. Daarom is deze methode met de meeste neurotransmitters geen optie. Omdat Glutamaat en GABA zo belangrijk zijn in de hersenen wordt het veel gebruikt en geproduceerd. Er is in de hersenen dus veel meer Glutamaat en GABA aanwezig dan de meeste andere neurotransmitters. Genoeg zelfs om te kunnen detecteren met MRS. Dit komt goed uit, want GABA is heel belangrijk in de werking van de Basale Ganglia, één van de grote spelers bij Parkinson.

Een groot deel van de controlerende werking van de basale ganglia gebeurt door een complex systeem van remmingen. Dit wordt dus voornamelijk met GABA geregeld. Dopamine kan de balans tussen meer of minder remming normaal goed controleren. Één van de belangrijke hypotheses bij Parkinson is dat door het gebrek aan dopamine de GABA afhankelijke remming niet meer goed gecontroleerd kan worden. In de Basale Ganglia dient de thalamus normaal als een doorgeefluik en

selectiemechanisme van bewegingsinstructies van de motor cortex (de 'bedenker' van beweging) naar de spieren (de 'uitvoerders'). Een overdosis van GABA in dit kleine gebied zorgt dat het doorgeefluik op slot gaat. Er komt bijna niets meer doorheen, en bewegen gaat dus maar moeilijk.

De vraag hier was - kunnen we ook zien dat de hoogte van GABA in de thalamus veranderd is, en verschilt dit dan tussen de patiëntengroepen? Daarvoor kijken we naar concentraties in de thalamus, de motor cortex (motorisch/bewegings gebied) en de visuele cortex (het visuele gebied). We namen hier het visuele gebied alleen mee als controle, we wilden immers zeker weten dat de verschillen die we in de bewegingsgebieden zagen wel specifiek waren voor dit gebied.

Helaas bleek het erg moeilijk om in de thalamus - die diep in de hersenen ligt - goede metingen te krijgen. Door de diepe ligging is er een grotere afstand tot de signaalontvangers, wat voor veel extra ruis zorgt. We zagen bij onze metingen in de thalamus geen verschil tussen patiëntgroepen of tussen patiënten en gezonde controles. Het is echter in dit gebied moeilijk om zeker te weten dat dit verschil er ook echt niet was. Misschien verdween het verschil in de ruis, en was het verschil kleiner dan we goed konden meten. Gelukkig bleek dit onderzoek niet voor niets, want we hebben wel wat anders interessants ontdekt. We zagen dat de concentratie van GABA in de motor cortex, samenhangt met de ernst van de symptomen. In dit gebied lijkt GABA juist beschermend te werken. Meer GABA hangt hier samen met mildere symptomen. Een prettiger ziekteverloop dus.

Dit is interessant omdat er vanuit de neurobiologie al signalen kwamen dat GABA misschien ook een beschermende werking zou kunnen hebben. Remmen is namelijk soms ook best nuttig. Het zorgt dat de hersenen af en toe als het ware 'uit kunnen rusten'. Meer GABA in kwetsbare gebieden zou dus wel eens goed zou kunnen zijn voor Parkinson patiënten. Het zou kunnen zijn dat als de hersenen meer rust krijgen, er ook meer tijd is om op te ruimen en te herstellen. Dit kan celsterfte bij patiënten tegen gaan. We hopen dat hier in de toekomst nog beter naar gekeken wordt.

VRAAG 2 | *Zien we een ander patroon van degeneratie (=celsterfte) in de dopamine-kernen?*

Één van de mogelijke redenen dat patiënten verschillen in symptomen laten zien is dat de patiëntengroepen ook verschillen in de precieze gebieden waar de meeste celsterfte plaatsvindt. De ene groep heeft bijvoorbeeld meer celsterfte in gebied 1, maar minder in gebied 2, terwijl dit bij de andere groep juist andersom is.

Dopamine wordt voornamelijk gemaakt in de substantia nigra, medeverantwoordelijk voor het reguleren van beweging en belangrijk voor het ontstaan van Parkinson.

Maar ons brein heeft meerdere dopamine-kernen, met elk een ander hoofddoel. Er is bijvoorbeeld de retrorubral area (RRA). Dit gebied was voor ons onderzoek naar trillen in Parkinson erg interessant omdat bij apen bleek dat beschadiging van dit gebied kon leiden tot symptomen van trillen. Een ander bekende dopamine-kern is de zogenoemde VTA (Ventral Tegmental Area), belangrijk voor beloning en motivatie.

Voor vraag 2 hebben we specifiek gekeken naar de Substantia Nigra, die belangrijk was bij het ontstaan van bewegingsproblemen in Parkinson. Daarnaast hebben we gefocust op de RRA, die al eerder geassocieerd was met trillen. We maakten hier gebruik van MRI technieken (de DTI of diffusion tensor imaging) die ons een plaatje kan geven van de dichtheid en structuur van de hersenen. We gebruikten hiervoor Free Water values - of 'vrij water' metingen. We verwachten dat als er veel cellen doodgaan de structuur van de hersenen minder 'hecht' wordt. Er vallen kleine gaten tussen de overblijvende weefsels dat opgevuld wordt met water of vocht. Hoe meer 'vrij water' we meten, hoe meer weefselafbraak er heeft plaatsgevonden.

We zagen dat patiënten die trilden minder 'vrij water' in de substantia nigra hadden. Hun substantia nigra was dus nog gezonder. Dit klopt met wat we zien in de symptomen. We weten namelijk dat trillende patiënten een langzamer en rustiger ziekteverloop hebben, en nog langer redelijk goed kunnen bewegen. In de RRA zagen we juist dat hoe meer een patiënt trilde, hoe meer vrij water er in dit gebied aanwezig was. Dit is in lijn met het eerdere onderzoek bij apen, en helpt ons beter begrijpen waar de tremor bij Parkinson's patiënten vandaan komt.

Bij elkaar suggeren deze resultaten dat het ontwikkelen van verschillende symptomen bij verschillende patiëntengroepen met Parkinson inderdaad samenhangt met een ander patroon van degeneratie of celsterfte.

VRAAG 3 | *Zien we een ander patroon in gedrag en beloningsgevoeligheid tussen patiënten?*

Dopamine is niet alleen belangrijk voor initiëren van beweging, maar ook voor het initiëren van gedrag. Dopamine helpt hier om ons gemotiveerd te houden. We zien bijvoorbeeld een kleine toename in dopamine als reactie op beloning, en een verlaging als reactie op straf. Als je eenmaal hebt geleerd dat iets belonend is dan krijg je meteen al een dopaminepiek als je weet dat die beloning eraan kan komen. Het alleen maar zien van een roze geglazuurde donut kan dus al heel wat bewerkstelligen. Je bent nu meer gemotiveerd om even van de bank op te staan om jouw mooie prijs te bemachtigen. Omdat de ziekte van Parkinson de dopamineproductie aantast heeft dit ook effecten op onze beloningsgevoeligheid. Eerdere experimenten lieten zien dat Parkinson's patiënten met hun lage dopaminespiegel minder goed leerden van beloningen, en juist meer van straf. Als we deze patiënten dan medicatie gaven die ze

weer veel dopamine gaf zagen we het omgekeerde. Met de extra dopamine leerden de patiënten juist goed van beloning, en minder goed van straf.

We zien bij patiënten die trillen en patiënten die niet trillen een aantal belangrijke verschillen: Ten eerste zien we dat trillende patiënten een langzamer ziekteverloop hebben - hun bewegingsklachten worden dus langzamer erger. Dit geldt ook voor de mentale effecten. Patiënten die trillen hebben minder cognitieve achteruitgang. Wij dachten dat dit misschien te maken heeft met dopamine-specifiek leren.

Om dit te testen hebben we een hele specifieke gedragstaak gebruikt die het leren van beloning en straf los kan meten van het maken van een beweging of niet. We werken hier immers met patiënten die juist ook problemen hebben met bewegen. Mensen moesten leren dat of het slim was om wél of níet op een knop te drukken, en hier konden ze óf een beloning mee verdienen, óf een straf proberen te vermijden. Alle opties waren mogelijk. Het kon dus zijn dat je bij één symbool juist wel moest drukken voor je prijs, en bij een ander symbool juist niet. Hetzelfde gold voor straf, bij één symbool moest je drukken om de straf te voorkomen bij het andere symbool juist niet. Uiteindelijk konden we dus precies zien of mensen goed konden leren voor beloning en straf, onafhankelijk van of ze goed konden leren om te drukken of niet. Ook konden we kijken naar de verbanden tussen drukken (of niet drukken) en beloning en straf. Dit maakt het een hele precieze, maar ook best moeilijke test.

Uiteindelijk zagen we bij de niet-trillende patiënten het traditionele idee over beloning en Parkinson's patiënten terug: zonder dopamine leerden ze relatief slecht van beloning. Toen deze patiënten dopamine hadden gekregen leerden ze juist extra goed van beloning. Maar wanneer we naar onze trillende patiënten keken zagen we dit effect helemaal niet. Sterker nog, de patiënten leerden met dopamine juist best goed van straf, en niet zozeer van beloning.

Na het vergelijken met oude experimenten, en het praten met eerdere onderzoekers geeft dit een verontrustend beeld. Het lijkt erop dat zij voornamelijk niet-trillende patiënten hebben gemeten. Ergens wel logisch, want trillen is natuurlijk maar onhandig bij het drukken op toetsen tijdens zo'n gedragstest. Maar nu blijkt het dat trillende en niet-trillende patiënten toch heel ander gedrag vertonen. Het is dus heel belangrijk om tussen deze patiëntgroepen onderscheid te maken in ons gedragsonderzoek. Zeker omdat de niet-trillende patiënten maar 25% van de totale patiëntengroep beslaan. We hopen dat in de toekomst meer duidelijk wordt over de het verschil in gedrag tussen de twee patiëntgroepen, en waar dat precies door veroorzaakt wordt. Ook hopen we dat toekomstige onderzoekers genoeg trillende patiënten gaan meenemen in hun onderzoek.

Samenvattend

Uit het onderzoek hebben we een aantal belangrijke lessen geleerd:

Ten eerste lijkt het interessant om verder onderzoek te doen naar GABA en Parkinson, omdat GABA in onze resultaten een beschermende werking lijkt te hebben.

Ten tweede laten we zien dat trillende en niet-trillende Parkinson's patiënten best wel van elkaar verschillen. We zien dat ze andere patronen van celsterfte hebben, en ook andere effecten op gedrag en beloningsgevoeligheid. Het is dus belangrijk om bewust te zijn van de mogelijke verschillen tussen deze patiënten in toekomstig onderzoek naar de ziekte van Parkinson.

Uiteindelijk zijn we ook verder gekomen in het onderzoek naar het trillen zelf. We tonen hier aan dat de afbraak van de RRA samenhangt met de ernst van het trillen.

Onderzoek staat nooit op zichzelf; om zeker te weten dat onze resultaten kloppen is het belangrijk dat dit soort experimenten door anderen herhaald worden. Ook levert dit werk weer nieuwe vragen op: Als GABA beschermd lijkt te werken, werkt GABA medicatie dan ook? Hoe komt het precies dat het gedrag van trillende en niet trillende patiënten verschilt? Bij elkaar heeft dit werk belangrijke toevoegingen gegeven op het gebied van tremor in Parkinson, van het belang van GABA en in de effecten van patiëntgroepen op gedrag.

List of publications

Published articles in this thesis

1 **van Nuland AJM**, den Ouden HEM, Zach H, Dirkx MF, van Asten JJ, Scheenen TW, Toni I, Cools R, Helmich RC. GABAergic changes in the thalamocortical circuit in Parkinson's disease. *Human brain mapping. 2019 Nov 13*.

2 **van Nuland AJM**, Archer DB, Zach H, Dirkx MF, Toni I, Vaillancourt DE, *et al*. Midbrain neurodegeneration in tremor-dominant and non-tremor Parkinson's disease – evidence from free water imaging. *Submitted*.

3 **van Nuland AJM**, Helmich RC, Dirkx MF, Zach H, Toni I, Cools R, *et al*. Effects of dopamine on reinforcement learning in Parkinson's disease depend on motor phenotype. BRAIN. *Submitted*.

Other Articles

4 Dirkx MF, Zach H, **van Nuland AJM**, Bloem BR, Toni I, Helmich RC. Cerebral differences between dopamine-resistant and dopamine-responsive tremor patients. *Brain.* 2019.

5 Dirkx MF, Zach H, **van Nuland AJM**, Bloem BR, Toni I, Helmich RC. Cognitive load amplifies Parkinson's tremor through excitatory network influences onto the thalamus. *Brain*. 2020.

Curriculum Vitae

Annelies van Nuland was born on November 3rd 1991 in Tiel, The Netherlands. After completing pre-university education at the Lek & Linge College in Culemborg in 2009, she started studying Biology at the Radboud University in Nijmegen. During this bachelor she specialised in a minor Medical Biology, and performed a research internship at the Department of Anatomy of the Radboud University Nijmegen Medical Centre. Her bachelor thesis "*the effect of long chain polyunsaturated fatty acids on neuronal development*" was done under the supervision of Dr. Amanda Kiliaan. She completed her bachelor's degree in Medical Biology (Cum Laude) in 2012. She then enrolled in the Master's programme in Cognitive Neuroscience at the Radboud University, following the plasticity and memory track, graduating (Cum Laude) in 2014. Her master thesis "*Striatal GABAergic control of human reward anticipation*" was supervised by Dr. Hanneke den Ouden and Prof. Roshan Cools. In 2014, she was awarded the TOP TALENT personal PhD grant to study "*the cerebral mechanisms of Parkinson's disease subtypes*" under the supervision of Dr. Rick Helmich, Dr. Hanneke den Ouden, Prof. Ivan Toni and Prof. Roshan Cools. This thesis is a result of that work. In October 2019, Annelies has started in a position as Data Scientist at Vantage AI.

Research data management

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. The medical and ethical review board Committee on Research Involving Human Subjects Region Arnhem Nijmegen, Nijmegen, the Netherlands has given approval to conduct these studies.

During this PhD project data was collected at the Clinical Neurophysiology department of the Radboudumc (electromyography and accelerometry recordings for tremor patient selection) and at the Donders Centre for Cognitive Neuroimaging (electromyography, accelerometry, MRI, pupil diameter, heart rate, breathing, behavioral data). Data is stored at local servers at the Donders (/project/3017044.01/raw/) and will be backed up at the Donders repository (https://www.ru.nl/donders/research/research-data-management/).

The paper data collected is stored at the DCCN. Paper data was also anonymously digitized in excel files (stored at the local department's hard drive) where privacy of the participants is warranted by use of unique individual subject codes. A separate encrypted, password-protected excel file was used for decoding.

The data will be saved for 15 years after termination of the study (November 30th, 2018).

PhD Portfolio

Name PhD candidate	Annelies Jopie Maria van Nuland
Research School	Radboud Unviersity Nijmegen Donders Institute for Brain Cognition and Behaviour
Department	Donders Centre for Cognitive Neuroimaging
Promotors	Prof. dr. R. Cools, Prof. dr. I. Toni
Copromotors	Dr. R.C.G. Helmich, Dr. H.E.M. den Ouden
Research period	September 2014 – September 2019

PhD Training

Subject	Location	Year
General courses		
Management voor Promovendi	Radboud University Nijmegen	2016
Programming Skills: Python	Radboud University Nijmegen	2016
The Art of Presenting science	Radboud University Nijmegen	2017
Solliciteren en Netwerken	Radboud University Nijmegen	2018
Conferences		
Society for Neuroscience Congress	Chicago	2014
Movement Disorders Congress	Berlin	2015
Nederlandse Vereniging voor Psychonomie (NVP) Congress	Egmond	2017
4th international symposium on MRS of GABA	Leuven	2017
Society for Neuroscience Congress	San Diego	2019
Teaching		
MR spectroscopy and neuroscience for the Tool-kit on advanced MR Techniques	Nijmegen	2016
Other activities		
Writer, Editor and Website maintenance for the Donders Wonders Blog	Nijmegen	2014-2019

Acknowledgements

As much as I learned about science, about movement, about motivation. I learned that no thoughts are born in isolation and all work is covered with many prints. It's the people you meet and the minds you share that makes it all worthwhile in the end.

I count myself extremely privileged to have grown, learned and worked with so many wonderful people. In this section (which is secretly the most important part of the whole book – because we all read it first), I get to thank all the people that have been my rock, my joy and my inspiration throughout the writing of this thesis. Buckle up, this is about to take a while.

First, I'd like to thank my supervisors who've helped keep this complicated project on track, and who've been the source of many discussions and helped spark new and interesting ideas.

Rick Helmich | I can still remember a quick meeting (after a talk about an RA position) in which I asked whether this "GABA in Parkinson" idea might lend itself to an interesting extension and could be fleshed out to a full-fledged PhD project proposal. Now roughly six years later I'd say that has certainly been successful. I know few people who are as driven, resourceful, with such vision, *true* interest and passion for their topic as you. Although I sometimes had to disappoint you in feeling that some interesting new angle *really did not fit into our time frame*, I think this project has become immensely richer with all your ideas, creativity and guidance. You've helped me stay on track, focus and rekindle my spark of excitement about our project on days that I was stuck. I hope you will be able to make the scientific progress you hope for, and I surely know our Parkinson research is in good hands.

Hanneke den Ouden | It's been quite a long time coming – what started as an ambitious master thesis was followed by a whole PhD (and two lovely mini-Hannekes). There've been grants, major success stories (two of which I mentioned just now) but also hard times and struggles to handle all the ridiculousness science can demand sometimes. I'm glad we've made it through with experience gained, with projects completed and bright futures ahead. I wish you all the best, and hope you'll make a badass professor someday! (Otherwise I honestly don't know what's wrong with them). Thank you for the care and help given. Best wishes.

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and start writing down. I also want to thank you for your excellent taste in PhDs and Postdocs, because we had a wonderful set of people in our lab!

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My lovely paranymphs

Jessica Määtä | Dear Jessica: You are utterly wonderful, and I'm still so very sorry I didn't get to team up with you on a project. Having you as an orchestrator would have been amazing, and without a doubt have saved me many months of panicking on forms, calls and complicated procedures. Having you as a friend however, was worth more than all that combined. We went through hell together, and I only hope we will keep coming out stronger every time. There need to be many more sauna trips to plan, many more dinners and tea and excellent Swedish cookies. I love you dearly, and am forever grateful to have you as a friend.

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Laurens van Dijk | Dear amazing source of wicked plans, intricate dinners and my own private rowing coach! As prime representative of our glorious #RU and longtime friend, I'm glad to count you as one of my paranymphs. I hope there will be many more trips to Germany, hikes, Port-nights and all. Thank you for the private DJ lessons, for all the fun during the good times and the support during sad times. Over all those years you've always ALWAYS had my back <3. Know that I'll always have yours. Also, I will definitely require more pictures of golden retriever pups.

My master student

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The scanning team

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My fellow PhDs and Postdocs

The blessing (and oftentimes curse) of science is the coming and going of students, PhDs and PostDocs (due to temporary contracts of varying duration). I've met many many wonderful colleagues over the years and although I fear I will undoubtedly forget an embarrassing number of names, I will take a shot at thanking you all.

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Joost | Het is zo fijn samen <3

“It always seems impossible until it’s done.”

Nelson Mandela

Max Planck Institute
for Psycholinguistics

Radboud University

Radboudumc