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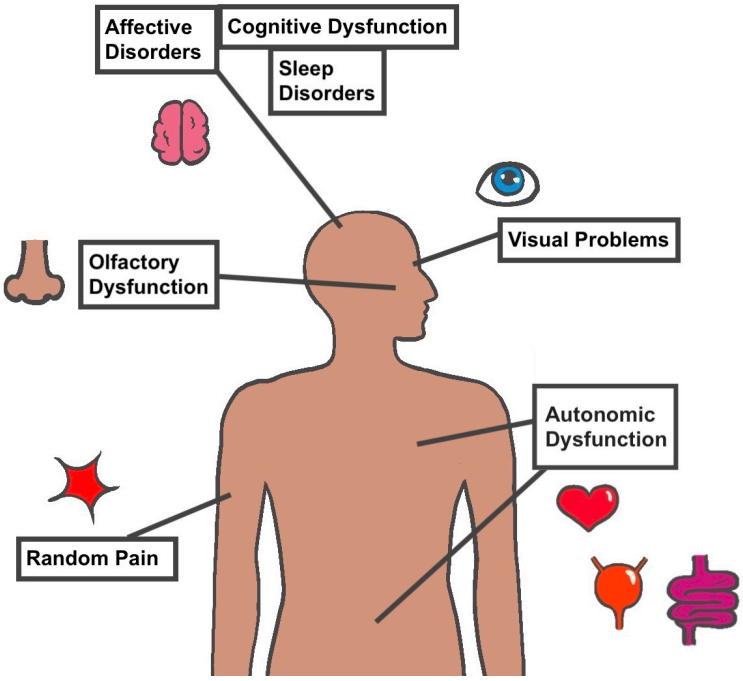
Non-motor symptoms and their use as markers for prodromal and early Parkinson's Disease



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Abbreviations

BDI	Beck depression inventory-II
BPI	Brief pain inventory
B-SIT	Brief smell identification test
CANTAB	Cambridge neuropsychological test automated battery
CFT	Category fluency test
DSM-IV	Diagnostic and statistical manual of mental disorders version IV
D1R	Dopamine D1 receptor
D2R	Dopamine D2 receptor
dmX	Dorsal motor nucleus of the vagus nerve
ECG	Electrocardiogram
ERP	Event related potentials
EDS	Excessive daytime sleepiness
FAB	Frontal assessment battery
GPe	Globus pallidus external segment
GPi	Globus pallidus internal segment
LANSS	Leeds assessment of neuropathic symptoms and signs
LB	Lewy body
LN	Lewy neurite
MCI	Mild cognitive impairment
MMSE	Mini-mental state examination
MDS-UPDRS	Movement disorder society sponsored UPDRS revision
NPI	Neuropsychiatric inventory
NMSQuest	Non-motor symptom questionnaire
NMS	Non-motor symptoms
OCD	Obsessive compulsive disorder

OCT	Optical coherence tomography
PD	Parkinson's disease
PDQ-8	Parkinson's disease questionnaire with eight dimensions
PDSS	Parkinson's disease sleeping scale
PSG	Polysomnography
QOL	Quality of life
RBD	REM-sleep behavior disorder
RAVLT	Rey auditory verbal learning task
SCOPA-AUT	Scales for outcomes in Parkinson's autonomic dysfunction
SPECT	Single-photon emission tomography
SNpc	Substantia nigra *pars compacta*
STN	Subthalamic nucleus
SNCA	Synuclein-alpha gene
TMT-A	Trail making test A
UPDRS	Unified Parkinson's disease rating scale
UPSIT	University of Pennsylvania smell identification test
VA/VL complex	Ventral anterior/Ventral lateral nuclei complex of the thalamus
VEP	Visually evoked potentials
α-SN	α-Synuclein

** Due to the ongoing pandemic, this degree project was conducted by comparative analysis of current literature paralleled by statistical analysis of data obtained by Serra/Mackenzie in a previous experimental study*

1. Abstract

Parkinson's Disease (PD) is the second most common neurodegenerative disorder. It is a disease with a broad spectrum of symptoms, both motor and non-motor, but is often only diagnosed when the motor symptoms begin to appear. By this time however, a large amount of the dopaminergic neurons of the substantia nigra *pars compacta* have already deteriorated. It is therefore of great interest to be able to diagnose the disease earlier on in its progression and perhaps slow down or halt its course. Recent literature has supported the idea that non-motor symptoms begin to appear years, perhaps even decades, before the motor symptoms are visible. This makes them a prime candidate for diagnosing PD earlier on. With the aim of assessing the prevalence of different NMS in prodromal and early Parkinson's, 19 studies addressing different NMS were analyzed. It was found that NMS are prevalent in both prodromal and early PD. The strongest prodromal predictors for PD were found to be olfactory dysfunction and REM-sleep behavior disorder (RBD).

2. Introduction

2.1 Background

PD was first described in 1817 as a 'shaking palsy' by James Parkinson. Today, it affects an estimated 3% of the population over age 65, remaining considerably rare before the age of 50 (except for in the case of hereditary PD, roughly 5-10% of all PD cases). Being the second most common neurodegenerative disorder, PD is the focus of several studies and research efforts (Poewe 2017).

Presently, clinical PD diagnosis relies almost entirely upon the presence of motor symptoms such as bradykinesia, resting tremor, and akinesia. In the last two decades, however, a multitude of articles have been published addressing the non-motor symptoms (NMS) of PD and their importance in not only aiding diagnosis, but also in impacting a patient's quality of life (Jellinger 2011, Poewe 2017).

Despite the numerous publications, the use of NMS to help diagnose PD is still lacking and NMS are widely underreported and untreated as a result (Chaudhuri & Schapira 2009). Perhaps the most compelling reason to include NMS in the clinical diagnosis is that the motor symptoms of PD only begin to manifest after the disease has progressed considerably, with an estimated 50% of the dopaminergic neurons in the substantia nigra *pars compacta* (SNpc) already being degenerated (Cova & Priori 2018, Jellinger 2017). If NMS could be used to recognize PD before the motor symptoms begin to show, the disease could be caught and treated much earlier on in its progression, and maybe the deterioration of dopaminergic cells could be slowed, if not halted entirely.

2.2 Aims

With the interest of encouraging the inclusion of NMS in the clinical picture of PD, this essay aims to answer two questions via meta-analysis:

1. Is there evidence that NMS exist before the classical motor symptoms in PD?

2. If we knew how to identify and diagnose these NMS when they first appeared, would it help to improve treatment as the disease progresses?

2.3 The Basal Ganglia and its normal function

The basal ganglia (BG) is a bundle of nuclei at the base of the forebrain that consists of the striatum (the caudate nucleus & the putamen), the pallidum (the globus pallidus internal (GPi) & external segments (GPe)), the subthalamic nucleus (STN) and the SNpc (Parent & Hazrati 1995).

The BG is believed to be involved in many pathways controlling motor, associative, cognitive, motivational & affective functions. The basic pathway mechanism for each circuit is as follows: the parietal, temporal, occipital and frontal lobes all send massive amounts of projections to the striatum, where the projections are organized in a topographic manner. Signals are then sent further to the pallidum, and from there to the thalamus before being sent back to the associated cortical area (fig. 1) (Buot & Yelnik 2012, Poewe 2017). The BG circuits can be divided into three parallel but somewhat segregated loops: the limbic loop, the sensorimotor loop and the associative loop (Parent & Hazrati 1995).

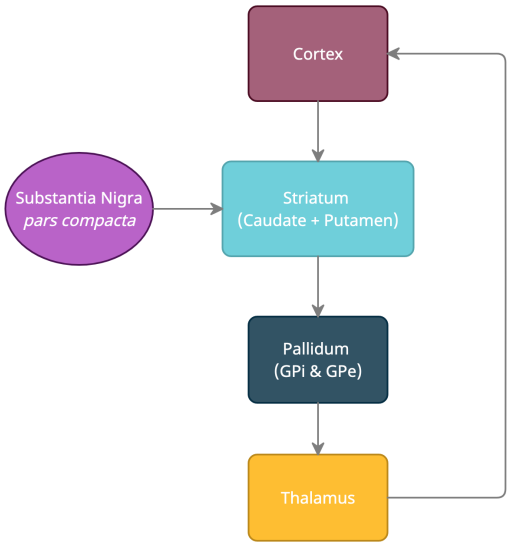

Figure 1: A simplified diagram of a thalamocortical BG circuit.

An alternative view of the BG pathways has been provided by Herrero, Barcia & Navarro (2002). In this view, the BG is separated into dorsal & ventral components. The dorsal pathway is where the striatum

receives information from the associative areas of the neo- & sensorimotor cortices. This is the motor component of the BG. The ventral pathway is where the nucleus accumbens (the ventral caudate nucleus and putamen, a part of the striatum) receives information from the orbitofrontal cortex, limbic cortical areas and from the hippocampus and amygdala. This is the limbic component of the BG.

The exact structure and organization of the BG's circuits and especially that of the limbic system is still widely discussed (Buot & Yelnik 2012). Regardless, it is known that these circuits are involved in a multitude of functions such as regulation of voluntary and involuntary movements, regulation of behavior and learning via positive/negative reinforcement, planning for future events, attention and spatial working memory. It is therefore easy to understand that diseases of the BG, such as PD entail not only motor dysfunction, but also a slew of cognitive, affective, autonomic and other impairments (Herrero, Barcia & Navarro 2002).

2.4 Parkinson's Disease - symptoms & pathology

The symptoms of PD are somewhat variable. For example, certain patients can present all of the classical motor symptoms (bradykinesia, tremor, etc.) and perhaps one or two non-motor symptoms (olfactory dysfunction, insomnia, etc.), while others can present some of the motor symptoms and a plethora of non-motor symptoms. This is due to the variable and far-reaching pathology of the disease (Sauerbier et al. 2016).

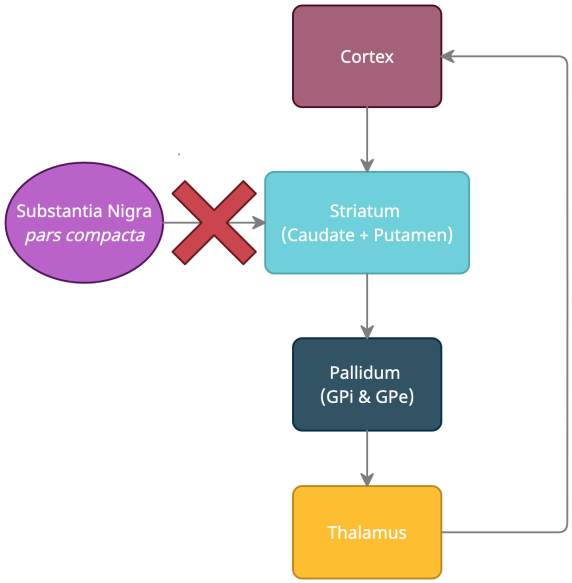

Figure 2: A simplified diagram of a thalamocortical BG circuit, this time showing the pathway that is deteriorated in PD.

The hallmark of PD pathology is the deterioration of dopaminergic projections that travel between the SNpc & the striatum (fig. 2). The SNpc plays a pivotal role in facilitating movement and other functions carried out by the BG. It does this by strengthening or dampening signals that come from the different cortices and

converge on the striatum. Degeneration of the dopaminergic cells leads to improper regulation of the BG circuits, and ultimately the motor and non-motor symptoms of PD (Onla-or & Winstein 2001, Poewe 2017).

Interestingly, different degrees of neuronal degeneration have been shown to occur not only in the striatum but also in the brainstem, vagus nerve & other structures (Sauerbier et al. 2016). Deficits are therefore found in several neuronal systems: cholinergic, dopaminergic, noradrenergic, and serotonergic among others.

As stated before, the pathological changes of PD have been shown to start long before the motor symptoms are detectable (Braak et al. 2003). By the time motor symptoms appear, approximately 30% of the dopaminergic neurons and 50% of all dopaminergic neuron axon terminals in the SNpc have already been degraded (Cova & Priori 2018, Jellinger 2017).

The aetiology and progression of PD is still unknown and requires further research.

2.4.1 Other pathologies

Neurotoxic protein aggregates known as Lewy bodies (LB) and Lewy neurites (LN) can be distributed across several different brain structures. LBs and LNs consist of abnormally phosphorylated, nitrated & oxidized α-synuclein (α-SN) proteins and proteolytic stress proteins such as ubiquitin (Del Tredici et al. 2002, Ferrer 2011).

α-SN proteins are encoded by the synuclein-alpha gene (SNCA) and each is roughly 140 amino acids long. The role of α-SN in the brain is not fully understood, but it is believed to play a role in the mechanisms of synaptic vesicles, molecular chaperoning, mitochondrial functions & intracellular trafficking. It has been found to occur in cytosol and is believed to also occur in the mitochondria and nucleus of cells. α-SN becomes a neurotoxin when the individual proteins begin to aggregate and form α-SN fibrils, which can further group together and become the aforementioned LBs and LNs (Poewe 2017).

LB and LN aggregation only compounds the issues brought about by dopaminergic cell degradation, adding to the breakdown of motor loop functions of the BG. The mesolimbic, mesocortical, and tuberoinfundibular systems are also affected by this (Dal Ben et al. 2019, Kingsbury 2010).

According to Braak et al. (2003) the spread of LB & LN follows a caudal to rostral progression. The manifestation begins in the dorsal motor nucleus of the vagus nerve (dmX) and reticular zone, spreads onward to the raphe nuclei, reticular formation & SNpc and then progresses to the prosencephalon, temporal mesocortex & allocortex. Lastly, LB & LNs spread to the neo- & prefrontal cortices, premotor areas, and primary sensory areas. While Braak et al. (2003) have attempted to describe the severity of PD in a progressive and structured manner, others such as Kingsbury et al. (2010) have suggested that, from onset in the dmX, progression of LB & LN to other parts of the brain is so rapid that actual disease progression is better measured by the level of deterioration/α-SN aggregation in the individual affected areas.

Mitochondrial dysfunction, neuroimmune responses, oxidative stress and point mutations are also part of PD pathology. These can be better understood by studying hereditary PD, where the disease is often diagnosed early on and at a younger age (Poewe 2017).

From hereditary PD we have learned that disturbances of certain pathways cause symptoms exactly like those found in sporadic PD. For example, point mutations in or duplications of the SNCA gene can cause misfolding or overproduction of α-SN. This leads to disruptions in the α-SN pathway and can result in protein aggregation, forming LBs and LNs (Cacabelos 2017, Poewe 2017).

A consequence of LBs and LNs forming in neurons is mitochondrial dysfunction. It has been theorized that low levels of α-SN are normally present in mitochondria, but aggregations of it cause oxidative stress & mitochondrial dysfunction. Oxidative stress in the mitochondria can lead to oxidation of RNA, which compromises the nucleic acid's stability. This can lead to increased mutation rates and therefore protein aggregation or dissociation, creating a kind of feedback loop between α-SN aggregation and mitochondrial instability. It is still debated whether oxidative stress occurs early on or later in a neuron's degradation, due to the feedback loop created (Poewe 2017).

It is very important to note that dopaminergic neurons in the SNpc are particularly sensitive to oxidative stress breakdown in the mitochondria because they have long, unmyelinated axons with several synapses and require a lot of energy to function (Poewe 2017).

Another insight gleaned from studying hereditary PD are the effects of neuroinflammation. This has shown to be a feature of PD via autopsies and brain imaging. Some have theorized that prodromal inflammation of the olfactory system or gut could be a trigger of α-SN aggregation that perhaps later spread to the brain. On the other hand, α-SN aggregation in PD has been shown to trigger immune responses such as neuroinflammation. Overall, neuroinflammation aids α-SN aggregation, and α-SN triggers neuroinflammation, resulting in yet another feedback loop (Poewe 2017).

2.4.2 The motor symptoms of PD

PD has been and still is clinically recognized primarily by its motor symptoms (Poewe 2017). These motor symptoms are due to the disruption of the motor circuit of the BG, which is critical for switching from one movement to another smoothly & without hesitation. There are three different motor pathways, each with a different role:

1. The Direct pathway, which informs cortical areas of upcoming intended movement (Onla-or & Winstein 2001).
2. The Indirect pathway, which inhibits competing motor commands while intended movement is in progress (Onla-or & Winstein 2001).
3. The Hyperdirect pathway, which increases regulation of the direct pathway and stops premature and/or unwanted movements.

Dopaminergic projections from the SNpc to the striatum balance the indirect & direct pathways by facilitating movement in the direct pathway via D1R projections and by strengthening the inhibitory actions of the indirect pathway via D2R projections (Poewe 2017).

In the hyperdirect pathway, excitatory projections from cortical areas are sent directly to the subthalamic nucleus (STN). The STN, when triggered by the cortical areas, will send excitatory signals to the GPi. The GPi's inhibitory signals to the VA/VL complex of the thalamus are then strengthened, thus suppressing unwanted movements (Poewe 2017).

In PD, loss of the dopaminergic projections from the SNpc leads to hyperactivity in the GPi, SNpr and STN. Hyperactivity in these areas leads to reinforcement of the indirect pathway, while the direct pathway is weakened following the loss of dopamine input from the SNpc. More simply put, the indirect pathway is strengthened while the direct pathway is weakened. This ultimately results in the classic PD motor symptoms such as difficulty switching between movements (halting or staggering, aka akinesia), slowed movements (bradykinesia), resting tremor, and difficulty initiating movements (Herrero, Barcia & Navarro 2002, Onla-or & Winstein 2001).

2.4.3 The non-motor symptoms of PD

Anatomical studies have revealed that the BG has ties to the limbic system. The limbic system is a complex system lacking fixed boundaries and is involved in a multitude of functions such as memory, perception, emotion and motivation. The central grey nuclei of the BG have been shown to connect to cortical areas that are involved in limbic processing such as the prefrontal and insular cortices. The medial parts of the caudate + putamen receive projections from the lateral orbitofrontal & dorsolateral prefrontal cortices, comprising the associative territory of the striatum. On the other hand, the ventral caudate + putamen receive projections from the medial orbitofrontal, anterior cingulate & insular cortices and comprise the limbic territory. The lateral Habenula, which receives a large number of projections from limbic areas, projects onto the dopaminergic neurons of the SNpc, providing a pathway for limbic information to affect the activity of the BG. This anatomical data supports the idea of the BG's role in cognitive and emotional processing (Buot & Yelnik 2012).

In further support of the BG's role in more than motor processing, it has been shown that stimulation of BG can trigger impulsivity and hypomania, as well as relieve tics in Tourette's syndrome and compulsions in obsessive compulsive disorder (OCD) (Buot & Yelnik 2012).

Below, each non-motor symptom that will be addressed in this essay as well as what is known about the pathology behind it is described in detail. It is worth noting that there exist more NMS than are taken up in this essay. Due to time restraints, only a select amount were studied.

2.4.3.1 Olfactory dysfunction

Although most often individuals with PD are unaware of changes in olfaction, olfactory dysfunction (such as hyposmia or anosmia) are symptoms that occur in up to 90% of PD patients (Doty 2012, Pfeiffer 2016). There is even some evidence that increasing severity of hyposmia is related to increasing risk of PD patients developing cognitive symptoms such as dementia (Cavaco et al. 2015).

The pathology behind olfactory dysfunction starts when α-SN aggregates begin to form in the olfactory bulb and associated areas in the brain such as the amygdala and perirhinal-primary olfactory cortex, affecting neurons there (Ferrer 2011, Jellinger 2017). The amount of LBs that have aggregated in the olfactory bulb is directly related to the amount of cell loss in the anterior olfactory nucleus and the progression of PD in a patient (Doty 2012). Although α-SN aggregation does cause a fair share of problems in the olfactory bulb, the fact that - in the majority of cases - the relative amount of aggregates there is low suggests that there are disruptions in other molecular pathways that are contributing to olfactory dysfunction. Also, aging in general increases oxidative stress and damage in the olfactory bulb, and hyposmia is therefore a normal part of aging, making it difficult to determine what is due to age and what could possibly be PD (Bohnen et al. 2010, Ferrer 2011). However, biopsies of the olfactory bulb can be used to determine abnormal α-SN deposition and possibly distinguish olfactory dysfunction that is due to PD (Jellinger 2017).

2.4.3.2 Visual problems

In a study carried out by Nowacka et al. (2014), it was found that PD patients had reduced contrast sensitivity & color discrimination, as well as increased frequency of problems affecting the eyes or structures around the eyes such as glaucoma, dry eye syndrome, cataracts, meibomian gland disease and seborrheic blepharitis. PD patients usually have worsened red/green color discrimination, which can be compared to subjects whose vision has worsened due to aging, where the problem is primarily blue/yellow discrimination (Hasanov 2018).

Hasanov and colleagues (2018) have shown that structural and functional changes occur in the retina & optic nerves of patients with PD. The pathology behind these changes is varied. Firstly, it has been proven that α-SN aggregates form in the retinal layers of a PD patient's eyes. This can contribute to foveal pit deformation and retinal thinning, which cause visual dysfunction (Bodis-Wollner et al. 2014). Secondly, dopamine is a neurotransmitter in amacrine cells and in several other neurons involved in visual processes, so the loss of dopaminergic transmission that occurs due to PD affects these cells as well, causing problems with vision. Lastly, oxidative stress, which occurs as part of PD pathology, has a positive effect on cataract development, decreasing visual ability (Hasanov 2018).

2.4.3.3 Random pain

About 76% of individuals with PD report pain as a NMS, with musculoskeletal pain being the most frequent type of pain reported (Pfeiffer 2016).

The pathology behind random pain is multifactorial. Depletion of dopaminergic transmission to the striatum affects the projections that run between the striatum and the lateral and medial thalamus, aka the higher centers for pain control. This causes misfiring pain signals, leading to random pain. On top of this, disruption of serotonergic pathways due to PD can cause problems with pain modulation. Yet another factor can be cutaneous denervation due to α-SN aggregation (Jellinger 2017).

2.4.3.4 Cognitive dysfunction

Dementia & MCI: Dementia is a symptom that happens eventually to about 80% of all PD patients who survive for 20 years or more after clinical PD diagnosis. Mild cognitive impairment (MCI) can be present earlier in PD as an early sign of cognitive decline, leading ultimately to dementia (Pfeiffer 2016). Mild cognitive impairment includes problems with memory, attention & language.

Dopamine deficiency in the striatum leads to problems in the circuit between the BG and the prefrontal and parietal cortices. As a consequence, the patient experiences cognitive impairments. Another cause of cognitive impairment is the aggregation of LBs in the brainstem, limbic loop & neocortex (Jellinger 2017).

2.4.3.5 Autonomic dysfunction

Orthostatic hypotension: This is also known as a sudden drop in blood pressure. This is the most widely recognized autonomic dysfunction of PD, present in roughly 60% of all patients. It is difficult to diagnose because the symptoms are not always visible, for example not all patients experience lightheadedness upon standing but instead could experience blurred vision or lethargy (Pfeiffer 2016).
Gastrointestinal issues: Autonomic dysfunction can cause gastrointestinal issues such as constipation, incomplete bowel emptying, difficulties swallowing (dysphagia) and gastroparesis. Dysphagia can result in other symptoms such as excessive drooling (sialorrhea) and aspiration. Gastroparesis can result in bloating, nausea, early feelings of satiety and reduced appetite. It can also lead to reduced effectiveness of levodopa, a medication commonly used to substitute dopamine loss in PD patients, because levodopa must reach the small intestine in order to be absorbed (Pfeiffer 2016).
Urinary problems: This category of NMS is experienced by roughly 25-50% of PD patients. The most frequent issue is overactivity of the detrusor muscle in the walls of the bladder. This can lead to nocturia, urinary urgency, frequent urination or incontinence (Pfeiffer 2016).

The pathology of PD in the autonomic nervous system is still being researched. LBs have been shown to be present in the autonomous nervous system in PD patients (sympathetic, parasympathetic & enteric

systems). They are found in the hypothalamus, in the intermediodorsal nucleus of the thoracic part of the spinal cord and sympathetic ganglia as well as in the dorsal vagal & sacral parasympathetic nuclei and peripheral parasympathetic ganglia and in the enteric plexus. In the medullary autonomic areas, the number of raphe nuclei neurons are significantly decreased (Ferrer 2011). LBs have also been shown to aggregate in distal axons of the cardiovascular autonomic system before moving inward toward the paravertebral sympathetic ganglia (Ferrer 2011).

LBs presence in the autonomic system correlates with dysfunction of the different systems there but does not prove direct causation. The problems arising in the autonomic system due to PD likely have a multifactorial origin and require more research to fully understand (Ferrer 2011).

2.4.3.6 Sleep problems

Sleep disturbances: Problems with sleep are very common in PD, estimated to be experienced by about 90% of PD patients. The most common sleep disturbance is a type of insomnia called sleep fragmentation, wherein the patient wakes several times during the night. There are several factors that contribute to sleep fragmentation such as muscle rigidity and bradykinesia making it difficult to roll or turn during sleep, nocturia, side effects of medication, and more. Other NMS such as fatigue and excessive daytime sleepiness could possibly be a result of sleep disturbances (Pfeiffer 2016).
RBD: REM-sleep behavior disorder (RBD) is a disorder in which the patient is not paralyzed during REM sleep and is therefore able to flail and act out their dreams. It is strongly implicated as a risk factor for the development of PD. The frequency of RBD in the PD population is estimated to be about 25-50% (Pfeiffer 2016).
Fatigue: Fatigue is defined by exhaustion that is not due to medications or psychiatric disorders. This can be an incredibly disabling symptom and is experienced by 33-58% of all PD patients (Siciliano et al. 2018).
EDS: Excessive daytime sleepiness (EDS) has a prevalence of roughly 35% of all PD patients. Male gender, MCI, high doses of levodopa and autonomic dysfunction are all risk factors for EDS (Al-Qassabi, Fereshtehnejad & Postuma 2017).

There are several pathways involving sleep that are disturbed because of PD. To start, post-mortem studies of PD patients have shown degeneration of serotonergic neurons as well as LBs in the median raphe nucleus. Serotonergic neurons are active during states of wakefulness, and less active during sleep, so it is believed that disruption of these pathways can cause daytime sleepiness and possibly fatigue. Second, it has been shown that in advanced PD there is a loss of hypocretin neurons in the posterolateral hypothalamus. Hypocretin has important effects on wakefulness, so loss of these neurons can lead to sleepiness and fatigue. Third, cholinergic neurons fire during REM sleep and wakefulness and are believed to play a role in REM sleep atonia. Loss of these could possibly be the cause of RBD. Fourth, dopaminergic neurons are active during wakefulness and REM sleep. Degeneration of these has been theorized to lead to EDS, fatigue, and RBD. Lastly, glutaminergic neurons in the locus coeruleus are active during wakefulness and REM sleep. Degeneration of these neurons due to PD could possibly be the cause of RBD (Al-Qassabi, Fereshtehnejad & Postuma 2017).

2.4.3.7 Affective disorders

Depression: Depressed PD patients have been shown to have worsened motor symptoms, more cognitive symptoms, and a lower quality of life. Depression in PD can manifest as minor depressive disorder (22% of patients), major depressive disorder (17% of patients), or chronic depression (dysthymia, 13% of patients) (Reijnders et al. 2007). Depression is often attributed to serotonin deficiency (Pfeiffer 2016).
Anxiety: Anxiety in patients with PD varies between 25-50%. It can manifest as generalized anxiety disorder, panic attacks, OCD, social phobia, phobic disorder, or agoraphobia (Simuni & Fernandez 2012).
Apathy: Apathy appears in about 40% of PD patients. Apathy appears often alongside depression, and it can be difficult to diagnose as a separate symptom. There are connections between apathy and difficulty with initiation (executive dysfunction) in PD (Pfeiffer 2016).

Problems (such as lesions or malfunctions) with the orbitofronto-thalamic and amygdalo-thalamic circuits have been shown to cause affective disorders. In PD these circuits are degenerated, and this could be the cause of the affective disorders experienced by PD patients (Borgonovo et al. 2017).

Aside from being involved in the motor circuit, the SNpc is also involved in producing reward feelings & motivation. In PD, the neurons of the SNpc are degenerated, and this could be a cause for apathy and/or depression (Borgonovo et al. 2017).

It is difficult to pinpoint the exact cause of affective disorders in PD after clinical diagnosis because they can be due to the pathology of the disease and/or the psychological stress of being diagnosed with PD (Aarsland et al. 2009).

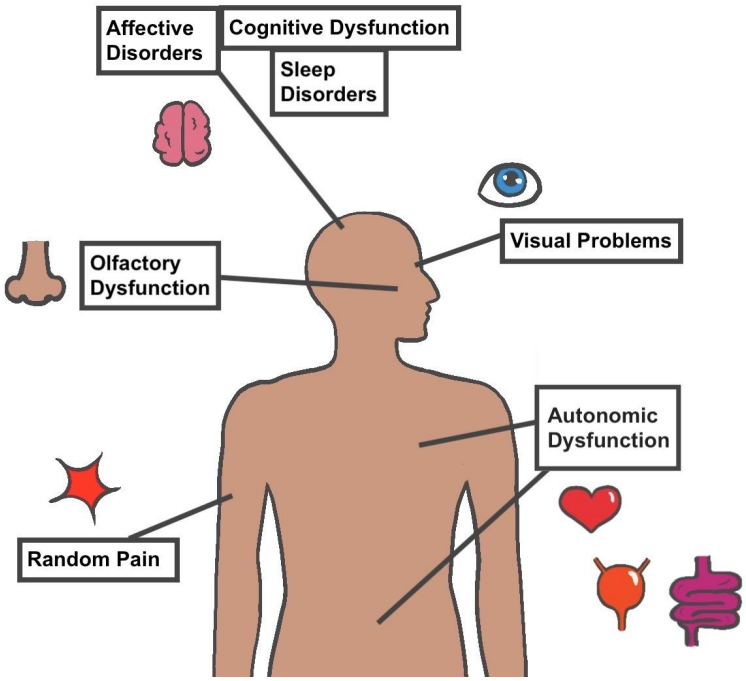

Figure 4: A diagram of the human body showing the different NMS and the parts of the body they affect.

It is important to note that several of what are considered NMS occur also as a normal part of aging, and this can make it difficult to distinguish what is due to PD and what isn't. PD patients tend to have a larger amount of NMS though, whereas individuals not affected by a neurodegenerative disease in the same age group tend to express only one or two NMS (Pfeiffer 2016).

2.5 Methods of evaluating NMS in PD

There are multiple methods to evaluate a patient's NMS, but a couple are used more often than others and cover multiple NMS.

In the 1980's the Unified Parkinson's Disease Rating Scale (UPDRS) was created, and in 2001 the Movement Disorder Society (MDS) sponsored a revision of this questionnaire and the so-called MDS-sponsored UPDRS revision (MDS-UPDRS) was created. This is a comprehensive questionnaire including both motor and non-motor symptoms. It uses a number scale for rating the severity of symptoms (Goetz et al. 2008).

One of the most widely used is the non-motor symptoms questionnaire (NMSQuest). This is an internationally accepted and comprehensive questionnaire specifically designed to make evaluation of NMS easier and more common. It uses 'yes' or 'no' options to determine the presence of NMS and a number scale for the rating of severity and frequency (Klingelhoefer, Jitkritsadakul & Bhidayasiri 2017).

Outside of these, there are a plethora of other tests focusing on multiple NMS or one specific NMS.

3. Methods

Initially, two separate searches were carried out via pubmed according to the following criteria:

- *Search 1*: ((Early) AND (Parkinson's)) AND ((nonmotor) OR (non-motor) OR (non motor))
- *Search 2*: (Parkinson) AND (prodromal) AND ((non motor) OR (non-motor) OR (NMS))

The filters clinical trial, meta-analysis, and randomized controlled trial were applied in each search. These searches provided 80 results together. From these, 77 essays were excluded based on their titles. Title criterion was the mention of prodromal or early PD and/or the mention of NMS. The final 3 essays were assessed based on their abstracts and 1 essay was excluded. Articles were excluded if their abstract didn't address NMS and prodromal or early PD.

Thereafter, the reference lists of the essays used in the introduction and background were combed through and 60 essays were selected based on their titles. 12 essays were found to be relevant after reading through the abstracts, and one more essay was excluded after a read through.

Lastly, three more searches were carried out via pubmed according to the following criteria:

- *Search 3*: (parkinson) AND ((prodromal) OR (early) OR (pre-clinical) OR (pre-motor)) AND (olfaction)
- *Search 4*: (parkinson) AND ((prodromal) OR (early) OR (pre-clinical) OR (pre-motor)) AND ((visual*) OR (optic*))
- *Search 5*: (parkinson) AND ((prodromal) OR (early) OR (pre-clinical) OR (pre-motor)) AND (sleep)

The filters clinical trial, meta-analysis, and randomized controlled trial were applied in each search. These searches provided a total of 105 results. From these, 96 essays were excluded based on their titles (using the same title criterion as before). The final 9 essays were then assessed based on their abstracts and a further 3 essays were excluded (using the same abstract criterion as before).

In total, 19 essays were selected to be analyzed (fig.5).

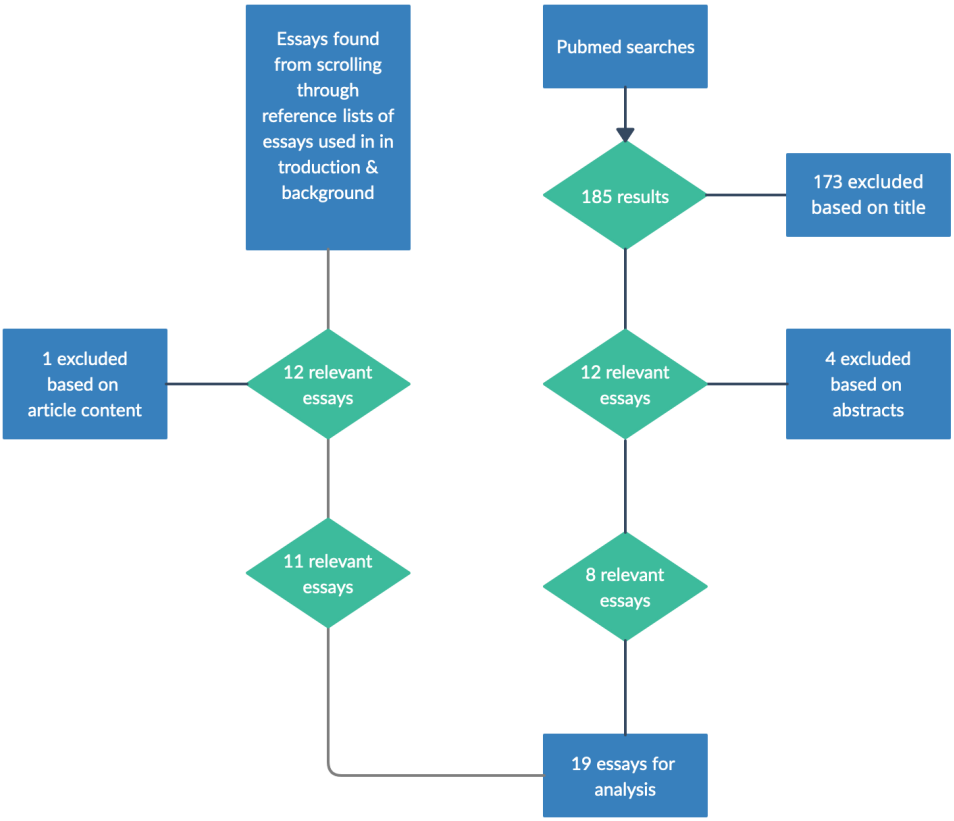

Figure 5: A flowchart illustrating how articles were excluded and collected for analysis in this essay.

4. Results

4.1 Study characteristics

Of 19 studies, 4 included multiple categories of NMS and 15 focused on only one category (table 1).

2 studies were meta-analyses, 7 were clinical trials, and 10 were randomized controlled trials with a group of controls being compared to a test group (table 1).

12 essays addressed NMS of early PD, whereof 7 defined early as within the first five years after clinical diagnosis, 3 defined early as Hoehn & Yahr stages 1-2.5, and 2 used both definitions. The remaining 7 essays addressed prodromal PD (table 3).

7 studies addressed olfactory dysfunction, 3 studies addressed visual problems, 3 addressed random pain and 6 studies addressed cognitive dysfunction. 12 studies tested a symptom in the category of autonomic dysfunction (6 orthostatic hypotension, 3 gastrointestinal issues, and 3 urinary problems). 12 studies addressed a symptom within the category of sleep problems (4 sleep disturbances, 2 fatigue, 2 EDS, and 4 RBD). 11 studies tested a symptom within the category of affective disorders (4 depression, 4 anxiety, and 3 apathy).

1 of the 4 focusing on multiple NMS included 2 NMS categories (Aarsland et al. 2009), whereas the other 3 contained 6 or all NMS categories (Pont-Sunyer et al. 2014, Diedrich et al. 2010 & Khoo et al. 2013).

Table 1:
▲ = meta-analysis
◉ = clinical trial
★ = randomized controlled trial

Author/s & Study Type	Characteristics of Report		
	NMS category focused on	Study Type (▲,◉,★) and parameters	Tests Used
Sui et al. 2019	Olfactory Dysfunction	▲ 7 essays	–
Ponsen et al. 2004	Olfactory Dysfunction	◉ 78 normosmic & hyposmic first-degree relatives of PD patients	SPECT scans Odor detection & discrimination tasks

Ross et al. 2008	Olfactory Dysfunction	◉ 2267 men	B-SIT
Valkovic et al. 2015	Random Pain	◉ 47 early stage PD patients 53 late stage PD patients	BPI LANSS pain scale PDQ-8 BDI
Pont-Sunyer et al. 2014	Olfactory Dysfunction Random Pain Cognitive Dysfunction Autonomic Dysfunction (OH, GI, UP) Sleep Problems (SD, Fat., EDS, RBD) Affective Disorders (Anx., Apat.)	★ 109 early PD patients 107 controls	Custom-made questionnaire
Awerbuch & Sandyk 1994	Autonomic Dysfunction (OH)	★ 20 early PD patients 20 healthy controls	ECG Ewing Cardiovascular Test
Shiba et al. 2000	Affective Disorders (Dep., Anx.)	★ 196 PD patients 196 controls	DSM-IV
Hasanov et al. 2018	Visual Problems	★ 19 early PD patients 19 healthy controls	Contrast sensitivity with MetroVision Ishihara pseudoisochromatic plates Humphrey Field analyzer II VEP testing with MetroVision OCT
Sommer et al. 2004	Olfactory Dysfunction	◉ 30 patients with idiopathic hyposmia	SPECT scans Sniffin' Sticks
Stefanova et al. 2015	Cognitive Dysfunction	★ 111 early PD patients 105 controls	RAVLT CANTAB Stroop Test Digital Ordering Test TMT-A Boston Naming Task CFT Clock Drawing Test Hooper Visual Organization Test MDS

Antal et al. 2002	Cognitive Dysfunction	★ 16 early PD patients 20 controls	Recorded ERPs
Dupouy et al. 2017	Cognitive Dysfunction	★ 70 early PD patients 70 controls	Custom Test
Goldstein 2006	Autonomic Dysfunction (OH)	◉ 35 patients with PD + OH	Timing of OH diagnosis as compared to PD diagnosis
Bonucelli et al. 2003	Autonomic Dysfunction (OH)	◉ 51 early PD patients	Ewing Cardiovascular Test
Diedrich et al. 2010	Olfactory Dysfunction Visual Problems Cognitive Dysfunction Autonomic Dysfunction (OH, GI, UP) Sleep Problems (SD, RBD) Affective Disorders (Dep.)	★ 30 early PD patients 30 healthy controls	FM 100 hue Test Pelli-Robinson Test Vistech Test UPSIT PDSS PSG NMSQuest SCOPA-AUT FAB TMT A BDI
Postuma et al. 2008	Sleep Problems (SD)	◉ 93 patients with idiopathic RBD	UPDRS MMSE
Siciliano et al. 2018	Sleep Problems (Fat.)	▲ 44 essays	–
Aarsland et al. 2009	Sleep Problems (SD) Affective Disorders (Dep., Anx., Apat.)	★ 175 early PD patients 166 controls	NPI
Khoo et al. 2013	Olfactory Dysfunction Visual Problems Random Pain Cognitive Dysfunction Autonomic Dysfunction (OH, GI, UP) Sleep Problems (SD, EDS, RBD) Affective Disorders (Dep., Anx., Apat.)	★ 159 early PD patients 99 controls	NMSQuest

4.2 Study Results

All of the following results can be found summarized in tables 2, 3 and 4 below.

4.2.1 Olfactory Dysfunction

Out of the 7 studies addressing olfactory dysfunction, 3 were studies that focused on multiple NMS and 4 were studies that solely addressed olfactory dysfunction.

5 of 7 studies focused on prodromal olfactory dysfunction. In all 5 of these, the presence of this NMS before clinical diagnosis of PD was confirmed (Pont-Sunyer et al. 2014, Sui et al. 2019, Ponsen et al. 2004, Ross et al. 2008 & Sommer et al. 2004).

The remaining 2 of 7 studies focused on the presence of olfactory dysfunction in early PD, and both found evidence for this NMS in early PD (Diedrich et al. 2010 & Khoo et al. 2013).

4.2.2 Visual Problems

2 of the 3 studies addressing visual problems addressed multiple NMS and 1 addressed solely visual problems.

All 3 of the studies tested early PD patients. In all 3 studies (Khoo et al. 2013, Hasanov et al. 2018 & Diedrich et al. 2010) the presence of visual problems in early PD patients was confirmed. It is worth noting that one of the results (Khoo et al. 2013) was on the borderline to being insignificant ($p = 0.048$, where $p<0.05$ was significant).

4.2.3 Random Pain

Out of 19 studies, 3 tested for the symptom of random pain. 2 of these studies addressed multiple NMS and 1 addressed solely random pain.

1 of these studies tested for and confirmed the presence of random pain as a prodromal NMS (Pont-Sunyer et al. 2014). The other 2 studies tested for random pain in early PD and both were able to confirm it (Valkovic et al. 2015 & Khoo et al. 2013).

4.2.4 Cognitive Dysfunction

A total of 6 studies addressed cognitive dysfunction. 3 studies also addressed other NMS, and the other 3 addressed solely cognitive dysfunction.

1 study tested for cognitive dysfunction as a prodromal NMS and confirmed its presence (Pont-Sunyer et al. 2014). 5 studies tested for cognitive dysfunction as an early NMS. 4 of these studies were able to confirm (Stefanova et al. 2015, Antal et al. 2002, Dupouy et al. 2017 & Khoo et al. 2013), while 1 study did not find significant results for cognitive dysfunction as an early NMS (Diedrich et al. 2010). As a note, the study that did not find significant results for cognitive dysfunction in early PD had results that were on the borderline of significance ($p = 0.06$ & $p = 0.05$ for $p<0.05$ significance).

4.2.5 Autonomic Dysfunction

There were a total of 12 studies addressing an NMS within the category of autonomic dysfunction.

6 studies focused on orthostatic hypotension. Of these 6 studies, 3 addressed multiple NMS and 3 focused solely on orthostatic hypotension. 4 of the 6 studies focused on early PD. All 4 of these were able to confirm orthostatic hypotension as an NMS in early PD (Awerbuch & Sandyk 1994, Bonucelli et al. 2003, Diedrich et al. 2010 & Khoo et al. 2013). 1 study focused on orthostatic hypotension as an NMS in prodromal as well as early PD and was able to confirm its presence in both cases (Goldstein 2006). The remaining study tested for orthostatic hypotension as a prodromal NMS and was not able to confirm it as a symptom (Pont-Sunyer et al. 2014).

3 of the total 12 studies focused on gastrointestinal issues. All 3 of these studies addressed multiple NMS. 1 study focused on prodromal symptoms of PD and was able to confirm gastrointestinal issues as a prodromal NMS (Pont-Sunyer et al. 2014). 2 studies focused on early PD symptoms, and both were able to confirm gastrointestinal issues as an early NMS (Diedrich et al. 2010 & Khoo et al. 2013).

The remaining 3 of 12 studies focused on urinary problems, all 3 of these addressed multiple NMS. 1 study tested for urinary problems as a prodromal NMS but was not able to confirm its presence (Pont-Sunyer et al. 2014). 2 studies tested for urinary problems as an early NMS, and both found significant results (Diedrich et al. 2010 & Khoo et al. 2013).

4.2.6 Sleep Problems

There were 12 studies that addressed an NMS in the category of sleep problems.

Out of these, 4 focused on sleep disturbances and all 4 also tested multiple categories of NMS. 1 of 4 tested sleep disturbances in prodromal PD and did not find evidence of them (Pont-Sunyer et al. 2014).

The remaining 3 focused on sleep disturbances in early PD and 2 of 3 confirmed (Diedrich et al. 2010 & Aarsland et al. 2009) while 1 found no significant results (Khoo et al. 2013).

2 of 14 focused on fatigue, 1 testing for multiple NMS and 1 for solely fatigue. 1 tested for fatigue as an early NMS (Siciliano et al. 2018) and 1 tested for fatigue as a prodromal NMS (Pont-Sunyer et al. 2014). All studies testing for fatigue found significant results.

EDS as an NMS was tested for by 2 studies, both testing for multiple NMS. 1 study tested for EDS in prodromal PD and found significant results (Pont-Sunyer et al. 2014) and 1 study tested for EDS in early PD and also found significant results (Khoo et al. 2013).

The remaining 4 of 14 tested for RBD, 3 of which focused on multiple NMS and 1 of which focused solely on RBD. 2 tested for RBD in prodromal PD (Pont-Sunyer et al. 2014 & Postuma et al. 2008) and 2 tested for RBD in early PD (Diedrich et al. 2010 & Khoo et al. 2013). All studies testing for RBD had significant results.

4.2.7 Affective Disorders

11 studies addressed an NMS in the category of affective disorders.

4 studies tested for depression. 2 focused on multiple NMS categories and 1 focused solely on affective disorders. 1 study addressed prodromal PD (Shiba et al. 2000) and the other 3 addressed early PD (Aarsland et al. 2009, Diedrich et al. 2010 & Khoo et al. 2013). All studies testing for depression had significant results.

Out of the 11 studies addressing affective disorders, 4 addressed anxiety as an NMS. 3 studied multiple NMS and 1 studied solely affective disorders. 2 of 4 studies addressing anxiety focused on prodromal PD. Of these 2, 1 found significant results (Shiba et al. 2000) and 1 did not (Pont-Sunyer et al. 2014). The other 2 of 4 studies focused on early PD, and both found significant results (Aarsland et al. 2009 & Khoo et al. 2013).

The remaining 3 of 11 studies addressed apathy. All 3 studied multiple NMS. 1 study focused on prodromal PD, confirming the presence of apathy as a prodromal NMS (Pont-Sunyer et al. 2014). The other 2 studies tested for apathy as an early NMS in PD and both had significant results (Aarsland et al. 2009 & Khoo et al. 2013).

Table 2: a table summarizing the findings of each study.
P *= Tested for prodromal stages of PD;* ***E*** *= Tested for early PD (first 5 years after diagnosis or Hoehn & Yahr stages 1-2);* ***B*** *= Tested for both prodromal & early PD;* ***X*** *= Did not test for this NMS;* ******* *= Results were on the borderline of significance;* ***Olfac. Dys.*** *= Olfactory Dysfunction;* ***Visual Prob.*** *= Visual Problems;* ***Ran. Pain*** *= Random Pain;* ***Cog. Dys.*** *= Cognitive Dysfunction;* ***OH*** *= Orthostatic Hypotension;* ***GI*** *= Gastrointestinal Issues;* ***Urin. Prob.*** *= Urinary Problems;* ***Sleep Dist.*** *= Sleep Disturbances;* ***Fat.*** *= Fatigue;* ***EDS*** *= Excessive Daytime Sleepiness;* ***RBD*** *= REM-sleep Behavior Disorder;* ***Dep.*** *= Depression;* ***Anx.*** *= Anxiety;* ***Apat.*** *= Apathy*

	Non-motor Symptom (Yes/No/–)													
Author/s	Olfac. Dys.	Visual Prob.	Ran. Pain	Cog. Dys.	OH	GI	Urin. Prob.	Sleep Dist.	Fat.	EDS	RBD	Dep.	Anx.	Apat.
Sui et al. 2019	Yes^{P}	–	–	–	–	–	–	–	–	–	–	–	–	–
Ponsen et al. 2004	Yes^{P}	–	–	–	–	–	–	–	–	–	–	–	–	–
Ross et al. 2008	Yes^{P}	–	–	–	–	–	–	–	–	–	–	–	–	–
Valkovic et al. 2015	–	–	Yes^{E}	–	–	–	–	–	–	–	–	–	–	–
Pont-Sunyer et al. 2014	Yes^{P}	–	Yes^{P}	Yes^{P}	No^{P}	Yes^{P}	No^{P}	No^{P}	Yes^{P}	Yes^{P}	Yes^{P}	–	No^{P}	Yes^{P}
Awerbuch & Sandyk 1994	–	–	–	–	Yes^{E}	–	–	–	–	–	–	–	–	–
Shiba et al. 2000	–	–	–	–	–	–	–	–	–	–	–	Yes^{P}	Yes^{P}	–
Hasanov et al. 2018	–	Yes^{E}	–	–	–	–	–	–	–	–	–	–	–	–
Sommer et al. 2004	Yes^{P}	–	–	–	–	–	–	–	–	–	–	–	–	–

Stefanova et al. 2015	–	–	–	Yes[E]	–	–	–	–	–	–	–	–	–	–
Antal et al. 2002	–	–	–	Yes[E]	–	–	–	–	–	–	–	–	–	–
Dupouy et al. 2017	–	–	–	Yes[E]	–	–	–	–	–	–	–	–	–	–
Goldstein 2006	–	–	–	–	Yes[B]	–	–	–	–	–	–	–	–	–
Bonucelli et al. 2003	–	–	–	–	Yes[E]	–	–	–	–	–	–	–	–	–
Diedrich et al. 2010	Yes[E]	Yes[E]	–	No[E]*	Yes[E]	Yes[E]	Yes[E]	Yes[E]	–	–	Yes[E]	Yes[E]	–	–
Postuma et al. 2008	–	–	–	–	–	–	–	–	–	–	Yes[P]	–	–	–
Siciliano et al. 2018	–	–	–	–	–	–	–	–	Yes[E]	–	–	–	–	–
Aarsland et al. 2009	–	–	–	–	–	–	–	Yes[E]	–	–	–	Yes[E]	Yes[E]	Yes[E]
Khoo et al. 2013	Yes[E]	Yes[E]*	Yes[E]	Yes[E]	Yes[E]	Yes[E]	Yes[E]	No[E]	–	Yes[E]	Yes[E]	Yes[E]	Yes[E]	Yes[E]

Table 3: A table showing which studies support each NMS, prodromal and early.
* = *within first 5 years after PD diagnosis*
** = *H&Y stages 1-2*

Non-motor symptom	Gives evidence of prodromal manifestation:	Gives evidence of early manifestation:	Earliest prodromal detection (time before clinical diagnosis)
Olfactory Dysfunction	Sui et al. 2019 Ponsen et al. 2004 Sommer et al. 2004 Ross et al. 2008 Pont-Sunyer et al. 2014	Diedrich et al. 2010* Khoo et al. 2013*	4 years (Ross et al. 2008)
Visual Problems	–	Hasanov et al. 2018** Diedrich et al. 2010* Khoo et al. 2013*	–
Random Pain	Pont-Sunyer et al. 2014	Valkovic et al. 2015** Khoo et al. 2013*	2-10 years (Pont-Sunyer et al. 2014)
Cognitive Dysfunction	Pont-Sunyer et al. 2014	Stefanova et al. 2015** Antal et al. 2002* Dupouy et al. 2017* Khoo et al. 2013*	2 years (Pont-Sunyer et al. 2014)
Orthostatic Hypotension	Goldstein 2006	Goldstein 2006* Awerbuch & Sandyk 1994* Bonucelli et al. 2003*/ **	–

		Diedrich et al. 2010* Khoo et al. 2013*	
Gastrointestinal Issues	Pont-Sunyer et al. 2014	Diedrich et al. 2010* Khoo et al. 2013*	10 years (Pont-Sunyer et al. 2014)
Urinary Problems	–	Diedrich et al. 2010* Khoo et al. 2013*	–
Sleep Disturbances	–	Diedrich et al. 2010* Aarsland et al. 2009*	–
Fatigue	Pont-Sunyer et al. 2014	Siciliano et al. 2018*/**	2-10 years (Pont-Sunyer et al. 2014)
EDS	Pont-Sunyer et al. 2014	Khoo et al. 2013*	10 years (Pont-Sunyer et al. 2014)
RBD	Pont-Sunyer et al. 2014 Postuma et al. 2008	Diedrich et al. 2010*	12 years (Postuma et al. 2008)
Depression	Shiba et al. 2000	Diedrich et al. 2010* Aarsland et al. 2009* Khoo et al. 2013*	5 years (Shiba et al. 2000)
Anxiety	Shiba et al. 2000	Aarsland et al. 2009* Khoo et al. 2013*	20 years (Shiba et al. 2000)
Apathy	Pont-Sunyer et al. 2014	Khoo et al. 2013* Aarsland et al. 2009*	2 years (Pont-Sunyer et al. 2014)

Table 4: A table summarizing the evidence found supporting each NMS's presence in prodromal or early PD. Results are separated into prodromal and early categories, with the total representing the amount of studies that tested that NMS in that specific stage. A (–) symbol means that there were no studies testing for that NMS in that stage.

	Non-motor Symptom													
	Olfac. Dys.	Visual Prob.	Ran. Pain	Cog. Dys.	OH	GI	Urin. Prob.	Sleep Dist.	Fat.	EDS	RBD	Dep.	Anx.	Apat.
Prodr-omal	5/5	–	1/1	1/1	1/2	1/1	0/1	0/1	1/1	1/1	2/2	1/1	1/2	1/1
Early	2/2	3/3	2/2	4/5	5/5	2/2	2/2	2/3	1/1	1/1	2/2	3/3	2/2	2/2

5. Discussion

Prodromal findings indicate olfactory dysfunction & RBD as the strongest predictors of developing PD. All 5 studies focusing on prodromal olfactory dysfunction found significant results and the 2 studies focusing on prodromal RBD found significant results as well. Furthermore, olfactory dysfunction could appear up to 4 years before a clinical diagnosis of PD was made (Ross et al. 2008) and RBD could appear up to 12 years before a clinical diagnosis was made (Postuma et al. 2008).

Other possible prodromal signs of PD were gastrointestinal issues, random pain, cognitive impairment (MCI), fatigue, EDS, depression, and apathy. There were less studies focusing on these as there were on olfactory dysfunction and RBD, however.

EDS and gastrointestinal issues were found to predate PD by up to 10 years. Fatigue and random pain were found to appear anywhere from 2-10 years before PD (Pont-Sunyer et al. 2014). Depression could predate PD by up to 5 years (Shiba et al. 2000). Apathy and cognitive dysfunction were found to predate PD by up to 2 years (Pont-Sunyer et al. 2014).

Prodromal anxiety and orthostatic hypotension were each tested by 2 studies. Both had one study that found significant results and one study that did not. Therefore, further research is suggested before drawing a conclusion about these two NMS. In one study, anxiety was found to predate PD by up to 20

years, so if it could be a confirmed prodromal NMS, it could be the earliest marker of prodromal PD (Shiba et al. 2000).

Only two prodromal NMS had no significant results: urinary problems and sleep disturbances (such as insomnia, sleep fragmentation, etc.) (Pont-Sunyer et al 2014). Based on this, it can be concluded that these two perhaps are not the best markers for possibly developing PD. However, to really be able to exclude these NMS, more studies need to be collected and analyzed.

Based on the prodromal findings, different combinations of NMS appearing (without reasonable explanation) in a patient can be suggested as a type of 'red flag' for possible early PD development. RBD and olfactory dysfunction together would be a very strong reason to suspect PD, but other combinations, for example, gastrointestinal issues and EDS, could also be reason to suspect PD. Both RBD and olfactory dysfunction were recorded appearing roughly 10 years ahead of PD diagnosis. Perhaps one of these on its own wouldn't give cause to suspect PD, but both appearing in the same decade would be a definite cause for concern.

Overall, appearance of any combination of the confirmed prodromal NMS should be considered a possible sign of developing PD and warrant therefore appropriate screening. Furthermore, the appearance of idiopathic RBD or olfactory dysfunction should be considered possible signs of developing PD, even if unaccompanied by other NMS.

Early findings indicate several NMS as markers of early PD. Almost every single study testing for an NMS in early PD found significant results. These findings are of interest because signs that an NMS is present already in early PD could mean that it is already present in prodromal PD, but that it went undiagnosed/unnoticed.

Pont-Sunyer et al. (2014), Diedrich et al. (2010) and Khoo et al. (2013) all studied NMS in almost every category. The latter two of these three focused on NMS in early PD, while the first focused on prodromal PD. Despite studying two different PD stages, they had very similar results, with the differences appearing in the NMS categories of sleep disturbances, urinary problems and orthostatic hypotension. This supports the idea that early NMS are already present in the prodromal stages of PD. In order to really confirm this idea though, more prodromal studies would need to be carried out and analyzed.

Some of the limitations of this study were that only a few of the analyzed studies covered all NMS, meaning that most NMS were only covered by a few or even one study. Another limitation was that not all NMS reported were covered/considered. Further, many of the control groups also showed some (albeit much less) amount of NMS, and it is possible that some of the age-matched healthy controls could have had prodromal PD, still in the stages where it goes unnoticed. This plus the natural occurrence of some NMS due to aging, makes it hard to be 100% sure that the reported NMS were due to PD or not.

The accuracy of clinical PD diagnosis has not much improved over the last few decades. Currently, it sits at about 75%, with 25% of PD patients being initially misdiagnosed. The accuracy of diagnosis increases as the disease progresses, but is lowest in the early stages of PD. On top of this, a study done by O'Sullivan et al. (2008) showed that PD patients presenting with NMS instead of motor symptoms were

more likely to be misdiagnosed. Aside from prolonging the time until the patient receives adequate care, this also makes it hard to gather subjects for early PD and NMS clinical trials (Rizzo et al. 2016).

NMS often go unnoticed and untreated. In a study by Chaudhuri et al. (2010), it was found that all NMS experienced by PD patients go somewhat unreported, with a considerable amount being unreported by 50% or more of the patients studied. The most reported NMS was diplopia, with only 33% going unreported. The least reported NMS was delusions, with a staggering 65% going unreported. On average, each PD patient had about 4 NMS that had gone non-declared.

NMS have a significant effect on quality of life (QOL) of PD patients. In a study by Martinez-Martin et al. (2011), they found that as NMS screening scores increased, QOL scores decreased. In particular, cognitive impairment and depression have been shown to have very strong correlations with decreased QOL (Schrag 2000). The combination of NMS being underreported and their diminishing of QOL means that considerable amounts of PD patients are not getting optimal treatment for the disease. This only adds to the fact that NMS need to be a prominent part of the clinical PD picture.

A suggestion for better incorporation of NMS in early clinical PD diagnosis and treatment is the required administration of relatively simple NMS questionnaires such as the NMSQuest or NMSS as soon as a patient is diagnosed with PD and several more times throughout treatment.

In the case of detecting prodromal PD with NMS, specific questionnaires addressing idiopathic NMS or NMS clusters common to PD need to be researched and developed. These questionnaires could then be administered somewhat regularly by clinicians once a certain age has been reached, or used as a first step when a patient presents to their clinician with NMS. If the criteria of the questionnaire are then filled, the patient can be recommended for further screening (for example SPECT screening) to see if PD is present or not. Even if the patient doesn't fill the criteria at the time, the possibility of developing PD has been acknowledged and a closer eye can be kept on any developing symptoms by both patient and caregiver, perhaps allowing for earlier detection.

Some have complained that screening for NMS is too time consuming or too expensive, but some examples of easy screenings include:

- Detection of α-SN deposits in cutaneous nerves via peripheral tissue skin biopsies (Jellinger 2017).
- The trigeminal system (detects spiciness, freshness, etc.) is usually damaged in olfactory dysfunction. However, in olfactory dysfunction caused by PD, the trigeminal system is intact. This can be used to detect whether OD is caused by PD or other factors and possibly give earlier detection of PD (Tremblay, Durand Martel & Frasnelli 2017).
- Noninvasive transcranial sonography as a way to identify patients at risk for developing PD (Sommer 2004).

There are several other suggestions and methods for easier NMS & PD screening, these are only a few.

Better diagnosis of NMS would allow for better treatment of symptoms and heightened quality of life. Currently, there are several treatments available for NMS, including both dopaminergic and non-dopaminergic options, so the only barrier for their treatment is recognition and diagnosis (Chaudhuri & Schapira 2009). Using NMS for earlier diagnosis of PD will help us to better understand the pathology and aetiology of the disease, improve patients QOL, and improve treatment for PD overall. Therefore, more research on NMS and better incorporation into the clinical picture of PD would provide benefits for both patients and researchers.

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6. References

Aarsland, D., Bronnick, K., Alves, G., Tysnes, O. B., Pedersen, K. F., Ehrt, U., & Larsen, J. P. (2009). The spectrum of neuropsychiatric symptoms in patients with early untreated parkinson's disease. Journal of Neurology, Neurosurgery & Psychiatry, 80(8), 928-930. doi:10.1136/jnnp.2008.166959

Al-Qassabi, A., Fereshtehnejad, S.-M., & Postuma, R. B. (2017). Sleep Disturbances in the Prodromal Stage of Parkinson Disease. Current Treatment Options in Neurology, 19(6). https://doi.org/10.1007/s11940-017-0458-1

Antal, A., Kéri, S., Dibó, G., Benedek, G., Janka, Z., Vécsei, L., & Bodis-Wollner, I. (2002). Electrophysiological correlates of visual categorization: evidence for cognitive dysfunctions in early Parkinson’s disease. Cognitive Brain Research, 13(2), 153–158. https://doi.org/10.1016/s0926-6410(01)00090-8

Awerbuch, G. I., & Sandyk, R. (1994). Autonomic functions in the early stages of Parkinson's disease. International Journal of Neuroscience, 74(1-4), 9-16. doi:10.3109/00207459408987224

Bodis-Wollner, I., Kozlowski, P. B., Glazman, S., & Miri, S. (2014). A-Synuclein in the inner retina in parkinson disease. Annals of Neurology, 75(6), 964-966. doi:10.1002/ana.24182

Bohnen, N. I., Muller, M. L., Kotagal, V., Koeppe, R. A., Kilbourn, M. A., Albin, R. L., & Frey, K. A. (2010). Olfactory dysfunction, central cholinergic integrity and cognitive impairment in parkinson's disease. Brain, 133(6), 1747-1754. doi:10.1093/brain/awq079

Bonuccelli, U., Lucetti, C., Del Dotto, P., Ceravolo, R., Gambaccini, G., Bernardini, S., Rossi, G., Piaggesi, A. (2003). Orthostatic hypotension in de NOVO Parkinson Disease. Archives of Neurology, 60(10), 1400. doi:10.1001/archneur.60.10.1400

Borgonovo, J., Allende-Castro, C., Laliena, A., Guerrero, N., Silva, H., & Concha, M. L. (2017). Changes in neural circuitry associated with depression at pre-clinical, pre-motor and early motor phases of Parkinson's disease. Parkinsonism & Related Disorders, 35, 17–24. https://doi.org/10.1016/j.parkreldis.2016.11.009

Braak, H., Tredici, K. D., Rüb, U., De Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic parkinson’s disease. Neurobiology of Aging, 24(2), 197-211. doi:10.1016/s0197-4580(02)00065-9

Buot, A., & Yelnik, J. (2012). Functional anatomy of the basal ganglia: Limbic aspects. Revue Neurologique, 168(8-9), 569–575. https://doi.org/10.1016/j.neurol.2012.06.015

Cacabelos, R. (2017). Parkinson’s Disease: From Pathogenesis to Pharmacogenomics. International Journal of Molecular Sciences, 18(3), 551. https://doi.org/10.3390/ijms18030551

Cavaco, S., Gonçalves, A., Mendes, A., Vila-Chã, N., Moreira, I., Fernandes, J., Damásio, J., Teixeira-Pinto, A., Bastos Lima, A. (2015). Abnormal olfaction in parkinson's disease is related to faster disease progression. Behavioural Neurology, 2015, 1-5. doi:10.1155/2015/976589

Chaudhuri, K. R., & Schapira, A. H. V. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. The Lancet Neurology, 8(5), 464–474. https://doi.org/10.1016/s1474-4422(09)70068-7

Cova, I., & Priori, A. (2018). Diagnostic biomarkers for parkinson's disease at a glance: Where are we? Journal of Neural Transmission, 125(10), 1417-1432. doi:10.1007/s00702-018-1910-4

Dal Ben, M., Bongiovanni, R., Tuniz, S., Fioriti, E., Tiribelli, C., Moretti, R., & Gazzin, S. (2019). Earliest mechanisms of dopaminergic neurons sufferance in a novel slow progressing ex vivo model of parkinson disease in rat organotypic cultures of substantia nigra. International Journal of Molecular Sciences, 20(9), 2224. doi:10.3390/ijms20092224

Del Tredici, K., Rüb, U., De Vos, R. A., Bohl, J. R., & Braak, H. (2002). Where Does Parkinson Disease Pathology Begin in the Brain? Journal of Neuropathology & Experimental Neurology, 61(5), 413-426. doi:10.1093/jnen/61.5.413

Diederich, N. J., Pieri, V., Hipp, G., Rufra, O., Blyth, S., & Vaillant, M. (2010). Discriminative power of Different Nonmotor signs in early Parkinson's Disease. a case-control study. Movement Disorders, 25(7), 882-887. doi:10.1002/mds.22963

Doty, R. L. (2012). Olfaction in parkinson's disease and related disorders. Neurobiology of Disease, 46(3), 527-552. doi:10.1016/j.nbd.2011.10.026

Dupouy, J., Ory-Magne, F., Mekies, C., Rousseau, V., Puel, M., Rerat, K., Pariente, J., Brefel-Courbon, C. (2017). Cognitive complaint in early Parkinson's disease: A pilot study. Acta Neurologica Scandinavica, 137(1), 59–66. https://doi.org/10.1111/ane.12808

Ferrer, I. (2011). Neuropathology and Neurochemistry of Nonmotor Symptoms in Parkinson's Disease. Parkinson's Disease, 2011, 1–13. https://doi.org/10.4061/2011/708404

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., Poewe, W., Sampaio, C., Stern, M. B., Dodel, R., Dubois, B., Holloway, R., Jankovic, J., Kulisevsky, J., Lang, A. E., Lees, A., Leurgans, S., LeWitt, P. A., Nyenhuis, D., Olanow, C.W., Rascol, O., Schrag, A., Teresi, J.A., van Hilten, J.J & LaPelle, N. (2008). Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Movement Disorders, 23(15), 2129–2170. https://doi.org/10.1002/mds.22340

Goldstein, D. S. (2006). Orthostatic hypotension as an early finding in parkinson's disease. Clinical Autonomic Research, 16(1), 46-54. doi:10.1007/s10286-006-0317-8

Hasanov, S., Demirkilinc Biler, E., Acarer, A., Akkın, C., Colakoglu, Z., & Uretmen, O. (2018). Functional and morphological assessment of ocular structures and follow-up of patients with early-stage

Parkinson’s disease. International Ophthalmology, 39(6), 1255–1262. https://doi.org/10.1007/s10792-018-0934-y

Herrero, M., Barcia, C., & Navarro, J. M. (2002). Functional anatomy of thalamus and basal ganglia. Child's Nervous System, 18(8), 386-404. doi:10.1007/s00381-002-0604-1

Jellinger, K. A. (2011). Neuropathology of sporadic Parkinson's disease: Evaluation and changes of concepts. Movement Disorders, 27(1), 8–30. https://doi.org/10.1002/mds.23795

Jellinger, K. A. (2017). Neuropathology of Nonmotor symptoms of Parkinson's disease. International Review of Neurobiology, 133, 13-62. doi:10.1016/bs.irn.2017.05.005

Khoo, T. K., Yarnall, A. J., Duncan, G. W., Coleman, S., O'Brien, J. T., Brooks, D. J., Barker, R. A., Burn, D. J. (2013). The spectrum of nonmotor symptoms in early parkinson disease. Neurology, 80(3), 276-281. doi:10.1212/wnl.0b013e31827deb74

Kingsbury, A. E., Bandopadhyay, R., Silveira-Moriyama, L., Ayling, H., Kallis, C., Sterlacci, W., Maeir, H., Poewe, W. & Lees, A. J. (2010). Brain stem pathology in parkinson's Disease: An evaluation of the Braak staging model. Movement Disorders, 25(15), 2508-2515. doi:10.1002/mds.23305

Klingelhoefer, L., Jitkritsadakul, O., & Bhidayasiri, R. (2017). Objective Measurement and Monitoring of Nonmotor Symptoms in Parkinson's Disease. International Review of Neurobiology, 347–387. https://doi.org/10.1016/bs.irn.2017.04.004

Martinez-Martin, P., Schapira, A. H., Stocchi, F., Sethi, K., Odin, P., MacPhee, G., Brown, R. G., Naidu Y., Clayton L., Abe K., Tsuboi, Y., MacMahon D., Barone, P., Rabey, M., Bonuccelli, U., Forbes, A., Breen, K., Tluk, S., Olanow, C.W., Thomas, S., Rye, D., Hand, A., Williams, A. J., Ondo, W. & Chaudhuri, K. R. (2007). Prevalence of nonmotor symptoms in Parkinson's disease in an international setting; study using nonmotor Symptoms questionnaire in 545 patients. Movement Disorders, 22(11), 1623-1629. doi:10.1002/mds.21586

Martinez-Martin, P., Rodriguez-Blazquez, C., Kurtis, M. M., & Chaudhuri, K. R. (2011). The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. *Movement Disorders*, *26*(3), 399–406. https://doi.org/10.1002/mds.23462

Nowacka, B., Lubiński, W., Honczarenko, K., Potemkowski, A., & Safranow, K. (2014). Ophthalmological features of parkinson disease. Medical Science Monitor, 20, 2243-2249. doi:10.12659/msm.890861

Onla-or, S., & Winstein, C. J. (2001). Function of the ‘direct’ and ‘indirect’ pathways of the basal ganglia motor loop: Evidence from reciprocal aiming movements in parkinson’s disease. Cognitive Brain Research, 10(3), 329-332. doi:10.1016/s0926-6410(00)00046-x

O'Sullivan, S. S., Williams, D. R., Gallagher, D. A., Massey, L. A., Silveira-Moriyama, L., & Lees, A. J. (2008). Nonmotor symptoms as presenting complaints in Parkinson's disease: A clinicopathological study. *Movement Disorders*, *23*(1), 101–106. https://doi.org/10.1002/mds.21813

Parkinson, J. (1969). AN ESSAY ON THE SHAKING PALSY. Archives of Neurology, 20(4), 441–445. https://doi.org/10.1001/archneur.1969.00480100117017

Parent, A., & Hazrati, L. (1995). Functional anatomy of the basal ganglia. i. the cortico-basal ganglia-thalamo-cortical loop. Brain Research Reviews, 20(1), 91-127. doi:10.1016/0165-0173(94)00007-c

Pfeiffer, R. F. (2016). Non-motor symptoms in parkinson's disease. Parkinsonism & Related Disorders, 22, 119-122. doi:10.1016/j.parkreldis.2015.09.004

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A. E., Lang, A. E. (2017). Parkinson disease. Nature Reviews Disease Primers, 3(1). doi:10.1038/nrdp.2017.13

Ponsen, M. M., Stoffers, D., Booij, J., van Eck-Smit, B. L., Wolters, E. C., & Berendse, H. W. (2004). Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Annals of Neurology, 56(2), 173–181. https://doi.org/10.1002/ana.20160

Pont-Sunyer, C., Hotter, A., Gaig, C., Seppi, K., Compta, Y., Katzenschlager, R., Mas, N., Hofeneder, D., Brücke, T., Bayés, A., Wenzel, K., Infante, J., Zach, H., Pirker, W., Posada, I., J., Álvarez, R., Ispierto, L., Fàbregues, O. D., Callén, A., Palasí, A., Aguilar, M., Martí, M. J., Valldeoriola, F., Salamero, M, Poewe, W. & Tolosa, E. (2014). The onset of nonmotor symptoms in parkinson's disease (the onset pdstudy). Movement Disorders, 30(2), 229-237. doi:10.1002/mds.26077

Postuma, R. B., Gagnon, J. F., Vendette, M., Fantini, M. L., Massicotte-Marquez, J., & Montplaisir, J. (2008). Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. Neurology, 72(15), 1296–1300. https://doi.org/10.1212/01.wnl.0000340980.19702.6e

Reijnders, J. S., Ehrt, U., Weber, W. E., Aarsland, D., & Leentjens, A. F. (2007). A systematic review of prevalence studies of depression in parkinson's disease. Movement Disorders, 23(2), 183-189. doi:10.1002/mds.21803

Rizzo, G., Copetti, M., Arcuti, S., Martino, D., Fontana, A., & Logroscino, G. (2016). Accuracy of clinical diagnosis of Parkinson disease. Neurology, 86(6), 566–576. https://doi.org/10.1212/wnl.0000000000002350

Ross, G. W., Petrovitch, H., Abbott, R. D., Tanner, C. M., Popper, J., Masaki, K., Launer, R., White, L. R. (2008). Association of olfactory dysfunction with risk for future Parkinson's disease. Annals of Neurology, 63(2), 167–173. https://doi.org/10.1002/ana.21291

Sauerbier, A., Jenner, P., Todorova, A., & Chaudhuri, K. R. (2016). Non motor subtypes and Parkinson's disease. Parkinsonism & Related Disorders, 22(Supplement 1), p.41-46. 10.1016/j.parkreldis.2015.09.027

Schrag, A. (2000). What contributes to quality of life in patients with Parkinson's disease? *Journal of Neurology, Neurosurgery & Psychiatry*, *69*(3), 308–312. https://doi.org/10.1136/jnnp.69.3.308

Shiba, M., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., Schaid, D. J., Rocca, W. A. (2000). Anxiety disorders and depressive disorders preceding parkinson's disease: A case-control study. Movement Disorders, 15(4), 669-677. doi:10.1002/1531-8257(200007)15:43.0.co;2-5

Siciliano, M., Trojano, L., Santangelo, G., De Micco, R., Tedeschi, G., & Tessitore, A. (2018). Fatigue in Parkinson's disease: A systematic review and meta-analysis. Movement Disorders, 33(11), 1712–1723. https://doi.org/10.1002/mds.27461

Simuni, T., & Fernandez, H. H. (2012). Anxiety in parkinson's disease. Parkinson's Disease and Nonmotor Dysfunction, 17-29. doi:10.1007/978-1-60761-429-6_2

Sommer, U., Hummel, T., Cormann, K., Mueller, A., Frasnelli, J., Kropp, J., & Reichmann, H. (2004). Detection of presymptomatic Parkinson's disease: Combining smell tests, transcranial sonography, and SPECT. Movement Disorders, 19(10), 1196–1202. https://doi.org/10.1002/mds.20141

Stefanova, E., Žiropadja, L., Stojković, T., Stanković, I., Tomić, A., Ječmenica-Lukić, M., Petrović, I., & Kostić, V. (2015). Mild Cognitive Impairment in Early Parkinson's Disease Using the Movement Disorder Society Task Force Criteria: Cross-Sectional Study in Hoehn and Yahr Stage 1. Dementia and Geriatric Cognitive Disorders, 40(3-4), 199–209. https://doi.org/10.1159/000433421

Sui, X., Zhou, C., Li, J., Chen, L., Yang, X., & Li, F. (2019). Hyposmia as a Predictive Marker of Parkinson's Disease: A Systematic Review and Meta-Analysis. BioMed Research International, 2019, 1–9. https://doi.org/10.1155/2019/3753786

Tremblay, C., Durand Martel, P., & Frasnelli, J. (2017). Trigeminal system in Parkinson's disease: A potential avenue to detect Parkinson-specific olfactory dysfunction. *Parkinsonism & Related Disorders*, *44*, 85–90. https://doi.org/10.1016/j.parkreldis.2017.09.010

Valkovic, P., Minar, M., Singliarova, H., Harsany, J., Hanakova, M., Martinkova, J., & Benetin, J. (2015). Pain in Parkinson´s Disease: A Cross-Sectional Study of Its Prevalence, Types, and Relationship to Depression and Quality of Life. PLOS ONE, 10(8). https://doi.org/10.1371/journal.pone.0136541

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7. Supplementary Data Analysis

Background: The subthalamic nucleus (STN) is vital for performing intended movements. It is a key player in the indirect pathway, the pathway that suppresses unwanted movements. STN dysfunction is known to occur in neurodegenerative diseases, such as Parkinson's disease.

Aim of the essay: To test the impact of the STN on motor functions such as locomotion, coordination and balance.

Aim of data analysis: To gain practical experience with data analysis and interpretation.

Method: Data from a study by Guillaumin et al. (2021) was used to perform a data analysis. AA2V virus containing a DNA vector that encoded for a light sensitive protein was injected into the experimental group of mice's (ChR2) STN. This way the ChR2 group's STN could be activated by light and the reaction of the mice when the STN was known to be active could be studied.

Results: During activation of the STN via light, the ChR2 group of mice showed a significantly longer grooming duration than the control group (Ctrl). There was also a significantly longer duration of grooming in the ChR2 group when the STN was activated as compared to when it was not. There was no significant difference between the ON and OFF states on the Ctrl group

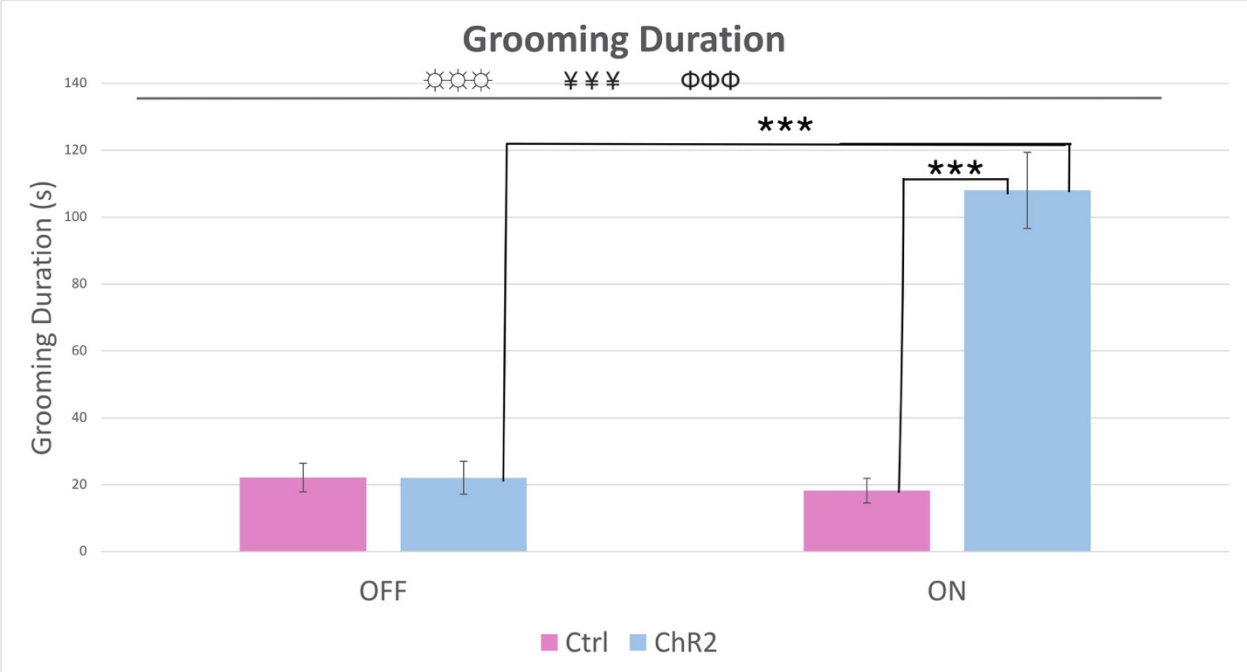

Figure 6: A diagram showing the differences between the Ctrl (pink) and ChR2 (blue) groups during the ON and OFF states. Significant results are shown with symbols (*, ☼, ¥, Φ) and have the following values: 1 symbol = $p<0.05$; 2 symbols = $p<0.01$; 3 symbols = $p<0.001$. * = two-sided t-test comparison; ☼ = stimulation effect; ¥ = treatment effect; Φ = interaktion effect.

Reference:

Guillaumin, A., Serra, G. P., Georges, F., & Wallén-Mackenzie, Å. (2021). Experimental investigation into the role of the subthalamic nucleus (STN) in motor control using optogenetics in mice. Brain Research, 1755, 147226. https://doi.org/10.1016/j.brainres.2020.147226