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„Lewy Pathology in Parkinson´s disease: Perspectives on the gut-origin hypothesis, role of the gut microbiota and α-Synuclein induced neurodegeneration in the brain"

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I. Abstract

A key pathological hallmark of Parkinson's disease (PD) is the presence of Lewy pathology (LP), which refers to intraneuronal inclusion bodies, termed Lewy bodies and Lewy neurites, that are primarily composed of the protein α-Synuclein.

With respect to LP, this work addresses three main issues: 1) What are the arguments for and against the hypothesis that the gut is the onset site for LP before brain involvement? 2) Does the gut microbiome have an impact on the PD pathogenesis? 3) How does Lewy pathology leads to neurodegeneration in the brain?

Although many studies support the gut-origin hypothesis, it is still considered controversial, as there are too little cases of "gut-only" LP. Other researchers attempt to explain this controversy by proposing that there are multiple phenotypes: body-first and brain-first PD.

In regard to the gut microbiome, it has been clearly shown that PD patients have differences in β-diversity compared to controls, particularly a reduction in SCFA-producing bacteria, which in turn is associated to the increased permeability in the large intestines observed in PD patients. Such a „leaky gut" may play a causative role in PD, as the translocation of bacterial derived pro-inflammatory products, such as LPS, could induce α-Syn aggregation. These α-Syn aggregates exert their dopaminergic neurotoxicity through compromising various intracellular mechanisms and contribute significantly to the uncontrolled neuroinflammation in PD.

II. Zusammenfassung

Ein wesentliches pathologisches Merkmal der Parkinson-Krankheit (PD) ist das Vorkommen der Lewy-Pathologie (LP). Diese beschreibt intraneuronale Einschlusskörper, sogenannte Lewy-Körperchen und Lewy-Neuriten, welche primär aus dem Protein α-Synuclein bestehen. Im Hinblick auf die LP befasst sich diese Arbeit mit drei Hauptfragen: 1) Welche Argumente sprechen für und gegen die Hypothese, dass LP im Darm beginnt, bevor das Gehirn davon betroffen ist? 2) Hat das Darmmikrobiom einen Einfluss auf die Pathogenese von Parkinson? 3) Wie führt die Lewy-Pathologie zur Neurodegeneration im Gehirn?

Obwohl viele Studien die Darm-Ursprungs Hypothese stützen, gilt sie immer noch als umstritten, da es nur eine beschränkte Anzahl von "gut-only"-LP Fällen gibt. Diese Kontroversität erklären sich andere Forscher über die Existenz mehrerer Phänotypen: "Body-first"- und "Brain-first"-PD.

In Bezug auf das Darmmikrobiom wurde eindeutig nachgewiesen, dass Parkinson Patienten im Vergleich zu Kontrollpersonen Unterschiede in der β-Diversität aufweisen, insbesondere eine Verringerung der SCFA-produzierenden Bakterien, was wiederum mit der bestätigten erhöhten Permeabilität im Dickdarm bei Morbus-Parkinson-Patienten assoziiert wird. Ein solcher "leaky gut" könnte eine ursächliche Rolle bei Morbus Parkinson spielen, da die Translokation von bakteriellen pro-inflammatorischen Produkten wie LPS die α-Syn Aggregation auslösen könnte.

Diese α-Syn Aggregate üben ihre dopaminerge Neurotoxizität durch die Beeinträchtigung verschiedener intrazellulärer Mechanismen aus und tragen wesentlich zur unkontrollierten Neuroinflammation bei Parkinson bei.

III. Acknowledgements

I would like to express my sincere thanks to my supervisor, Dr. Alexander Haslberger, who supported and advised me in my work. Thank you for the spontaneous acceptance to supervise my master thesis, but also for the given freedom in structuring my work and the flexible and uncomplicated support.

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V. Material and Methods

The aim of this work was to provide a comprehensive review of the scientific literature concerning the issues addressed in this work.

Since the topic is controversial, a particular focus was the integration of in-vitro and in-vivo studies, which should support or invalidate the respective hypothesis.

I have tried not only to include the most recent studies, but to provide an overview of all studies conducted, regardless of the year of publication.

For the literature search, I used the Pubmed[a] database and the following search terms: Parkinson´s disease, Lewy pathology, Parkinson´s disease and the gut-origin hypothesis, brain-first vs. body-first PD, microbiota-gut-brain axis, altered microbiota in PD, SCFA´s, Leaky gut and PD, endotoxin-hypothesis, α-Syn induced neurodegeneration, uncontrolled neuroinflammation in PD, microglia mediated dopaminergic neurotoxicity and several others.

[a] https://pubmed.ncbi.nlm.nih.gov

VI. Abbreviations

α-Syn Alpha-Synuclein
ALP Autophagy-lysosomal pathway
ATP Adenosintriphosphat
CNS Central nervous system
CSF Cerebrospinal fluid
DAMPs Damage-associated molecular patterns
DAT Dopamine transporter
DMV Dorsal motor nucleus of the vagus
ENS Enteric nervous system
ER Endoplasmic reticulum
GF Germ-free
GIT Gastrointestinal tract
GM Gut microbiota
HC Healthy control
HLA Human leucocyte antigen
IHC Immunohistochemistry
ILBD Incidental Lewy body disease
iRBD isolated RBD
JAM Junctional adhesion molecules
LBD Lewy body dementia
LBP LPS binding protein
LP Lewy pathology
LPS Lipopolysaccharide
MCNSCs Mouse cortical neuronal stem cells
MGBA Microbiota-gut-brain axis
MHC Major histocompatibility
MO Microorganism
MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA Multiple system atrophy
NMS Non-motor symptoms
PD Parkinson´s disease
PFF Preformed fibrils
PNS Peripheral nervous system
RBD Rapid eye movement sleep behaviour disorder
REM Rapid eye movement
ROS Reactive oxygen species
SCFA Short-chain fatty acids
SIBO Small intestinal microbial overgrowth
SNARE Soluble N-ethylmaleimide-sensitive-factor attachment receptor
SNc Substantia nigra pars compacta
SPF Specific pathogen-free
TJ Tight junction
TOM20 Translocase of outer mitochondrial membrane 20
TV Truncal vagotomy
UPR Unfold protein response
UPS Ubiquitin-proteasome system
WT Wild type
XBP1 X-box binding protein 1
ZO Zonula occludens

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1. Introduction

1.1 Parkinson´s disease

Affecting more than 10 million people worldwide, Parkinson's disease (PD) is one of the most common neurodegenerative disorders. PD is an age-associated disease, with the highest prevalence observed in the population over the age of 80 years.[1]
Although most cases are idiopathic, genomic mutations account for 10% of subjects, particulary found within young people.[2]

Pathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), the primary center in the brain responsible for coordination of body movements. As a result, the levels of the neurotransmitter dopamine are reduced, which eventually leads to motor disorders.[1]

1.2 Motor and non-motor symptoms

Impaired motor functions are characteristic for Parkinson's disease. It is estimated that these first begin to manifest when approximately 50-60% of the dopaminergic neurons have been lost.[3] The main features are bradykinesia, resting tremor, rigidity, and postural instability, collectively referred to as parkinsonism or Parkinson's syndrome.[1] Other features include speech and swallowing disorders, micrographia and a mask-like facial expression.[4]
Since a pathological confirmation of PD is only possible by autopsy examination, the diagnosis of PD relies on these motor disorders.[5]
However, it is important to note that PD is also associated with a broad spectrum of non-motor symptoms (NMS), which can vary between patients and occur at different stages of the disease. Not only do they expand the scope of the clinical picture of PD, but some of these symptoms, such as hyposmia, sleep behavior disorder, constipation, and depression, can precede the onset of motor features by several years.[3] NMS appear across all PD patients, including individuals with genetic causes, and may in the future be a useful biomarker for a pre-motor stage of the disease.[3]

1.3 Lewy Pathology

In addition to the loss of dopaminergic neurons and motor symptoms, another key hallmark of Parkinson's disease is the presence of Lewy pathology (LP) in the affected brain regions. LP refers to the deposition of abnormal protein aggregates in neurons, defined as Lewy bodies (spherical) and Lewy neurites (spindle-like).[5]
These consist of a large number of molecules, but mostly of α-Synuclein (α-Syn).[6]
Together with other neurodegenerative diseases in which LP is also found, such as Lewy body dementia and multiple system atrophy (MSA), PD belongs to a subgroup termed synucleinopathies.[5]

1.3.1 Alpha-Synuclein (α-Syn)

α-Syn is a protein of 140 amino acids that is abundantly expressed in neurons.
Its physiological function has not been fully understood, but it appears that its main function is to regulate the release of neurotransmitters by modulating the SNARE complex.[7]
α-Syn first attracted interest in 1997 when Polymeropoulos et al. reported a gene mutation that was associated with early-onset PD in Italian and Greek families. The mutation affects the SCNA gene, which is encoding for the protein α-Syn.[7]
After the development of antibodies against α-Syn, it was found that α-Syn is strongly expressed in Lewy bodies[7], which were first described in 1912.[6] Since then α-Syn immunohistochemistry has been the gold standard for neuropathological confirmation of PD.[7]

1.3.2 Loss of function or gain of toxicity?

> *"In order to understand the role of α-syn in mediating the pathogenesis of PD, the researchers studied α-syn knockout mice and found that although they showed some defects in the nigrostriatal DA pathway, they could survive and give birth without any abnormalities or characteristics of PD. This study suggests that PD is caused by the acquisition of toxic function of α-syn, not by the loss of its normal function. The gain of toxic function of α-syn is related to the accumulation of α-syn, which leads to its aggregation."*
> *(S. He et al. 2021)[8]*

1.3.3 α-Syn aggregation

α-Syn is a natively unstructered and monomeric protein that can adopt a helical state when it is bound to highly curved membranes, such as synaptic vesicles. (Fig. 1)
Under pathological conditions, α-Syn monomers gradually aggregate into complex structures that eventually deposit as Lewy bodies.[9] This process is a crucial step for α-Syn to acquire its toxic properties[9] and many mechanisms have been reported to promote this formation.[6]

Experiments with test tubes, whose medium resembles the cytosol of a neuron, showed that higher levels of α-Syn enhances kinetics of fibrillization.[10] Other studies confirmed that overexpressed α-Syn in cell cultures and animal models resulted in the formation of aggregates.[6] Such concentration-dependent phenomenon is also supported by the fact that gene mutations causing duplication and triplication of the SCNA gene are associated with familial PD.[10]

Second, α-Syn forms insoluble aggregates when exposed to H_2O_2 and ferrous iron, indicating that oxidation processes may be a possible cause for α-Syn aggregation.[6] Interestingly, there is evidence for excessive oxidation in the brains of PD patients.[6]

Finally, posttranslational modifications to α-Syn, particularly phosphorylation of α-Syn at Ser^{129}, have been linked to the pathogenesis of PD. Phosphorylated α-Syn has been shown both in-vivo and in-vitro to increase α-Syn toxicity, presumably by accelerating the fibrillization of α-Syn.[11] In fact, phosphorylated α-Syn account for over 90% of aggregated α-Syn within Lewy bodies.[12]

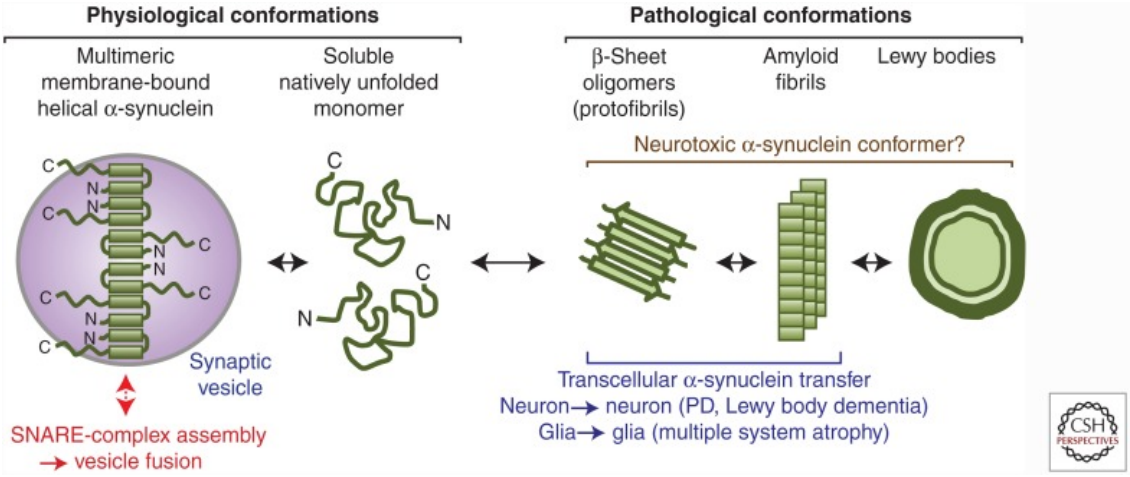

Figure 1: Conformational states of α-Synuclein. Edited after Burre et al. (2018)[9]

2. The gut-origin hypothesis

2.1 Introduction

After initially being described in the brain[6], autopsy studies have indicated that the topographical distribution of Lewy bodies and neurites is much greater than previously assumed.[13] Besides the brain, Lewy pathology is prevalent throughout the spinal cord and peripheral nervous system (PNS) of PD patients. This raises the question of where the aggregation of α-Syn starts.[13]

Traditionally, the nigrostriatal system is considered the first region affected by α-Syn pathology.[14] However, since GI symptoms are related to dysfunction of PNS[13] and often precede motor disorders by up to a decade in PD patients[15], researchers have become interested by the GI-origin hypothesis, by which the nervous system of the intestine, known as the enteric nervous system (ENS) may be the onset site of α-Syn aggregation, preceding CNS involvement.[14]

Savica et al. (2009) conducted a case-control study investigating the hypothesis that constipation may be a premotor manifestation of PD. The authors had access to a medical record system that made it possible to study the time period between appearance of constipation and onset of motor disorders. A total of 196 PD subjects were included in the study. Each case was matched by age and sex to controls without PD.

The study results showed that constipation is associated with an increased risk of PD. This finding remained significant after adjustment for smoking and alcohol consumption and after exclusion of possible medication induced constipation. Notably, in 34 cases, constipation preceded the onset of PD by more than 20 years.[16]

The results are consistent with findings from a prospective cohort study by Abbott et al. (2001), which reported that subjects with <1 bowel movement/day had a 4.1-fold higher risk for PD than subjects with >2/day.[17]

2.2 Enteric nervous system (ENS)

The enteric nervous system is the largest division of PNS, innervating the GI tract and regulating many GI functions. It consists of neurons and glia cells, which are organized in two main plexuses, the myenteric (Auerbach's) plexus and submucosal (Meissner's) plexus. (Fig. 2) The myenteric plexus primarily controls the relaxation and contraction of the intestinal wall, whereas the submucosal plexus is responsible for sensing the luminal environment and regulating GI blood flow and epithelial fluid secretion. In view of this, it seems plausibel that the degeneration of enteric neurons could be responsible for GI symptoms commonly observed in PD patients.[18]

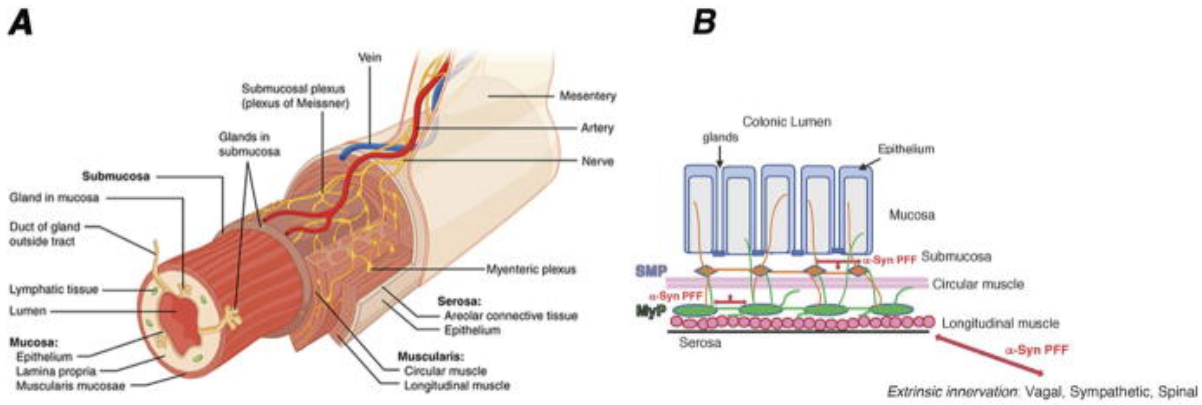

Figure 2: Organization of the ENS. Edited after A. Chalazonitis & M. Rao (2018)[18]

The presence of Lewy bodies in ENS was for the first time described by Qualman et al. in 1984.[5] The authors identified intracytoplasmic inclusions, characteristically found in the brainstem of PD, in the esophagus of one PD patient and in the colon of another.[19]
Since then, numerous autopsy studies provided further evidence that Lewy Pathology is widely distributed in Auerbach´s and Meissner`s plexus affecting almost the entire GIT.[13, 20, 21]

Along those findings, Lewy pathology has been observed to follow a rostro-caudal gradient within the gastrointestinal tract.[5] A study by Beach et al. (2010) revealed that the submandibular gland and the lower esophagus were the most affected by phosporylated α-Syn, whereas the colon and the rectum showed the lowest involvement.[13]

2.3 Braak´s hypothesis

> *„The first descriptions, in the 1980s, of Lewy bodies (LBs) and neurites, the pathological hallmarks of Parkinson's disease (PD), in the gastrointestinal tract of PD subjects were quickly recognized to have clinical implications but otherwise found relatively little echo among the movement disorders community. It took 20 years and the publications by Braak and coworkers for this topic to ignite." (Lionnet et al. 2017)*[22]

Braak et al. raised the question of whether α-Syn pathology evolves simultaneously in all areas of the brain or wether it follows a specific pattern. Therefore, in 2003, the authors conducted an autopsy study that included 110 brain obtained from individuals who were either diagnosed with Parkinson's disease or classified as having incidental Lewy body disease (ILBD), which refers to individuals who did not exhibit PD symptoms but whose brains showed the presence of Lewy pathology. Based on the study results, Braak et al. concluded that α-Syn pathology follows a caudo-rostral gradient in the brain, with the dorsal motor nucleus of the vagus (DMV) being an inevitable point of passage.[22]

Considering that the DMV extensively innervates the neurons of the ENS and previous studies have confirmed Lewy pathology in the intestinal wall in some PD patients, Braak et al. suggested that the onset of the pathological process may be in the enteric neurons, which then reach the CNS via the vagus nerve.[22]

To support his hypothesis, Braak et al. did a postmortem examination on five individuals with different severity of LP in the brain. All five cases were found to have α-Syn immunoreactive inclusions in the myenteric and submucosal plexus as well as in the DMV. Among these subjects, three were diagnosed with PD and two met the criteria for ILBD.
The α-Syn pathology was present in the substantia nigra in all three PD patients, whereas only one of the ILBD cases was positive in the SN.
Braak et al. concluded that ENS involvement manifests early and is not confined to the end stage of the disease, as lesions in the ENS were observed in both clinically diagnosed PD patients and non-symptomatic individuals.[22]

2.4 The prion-like spreading of α-Syn

Prion proteins are infectious agents that have the ability to propagate from an infected cell to a healthy one and trigger native cellular proteins to fold abnormally.[23]
The suggestion that α-Syn spreads in a prion-like manner stems from autopsy studies of PD patients in which Lewy pathology was observed in embryonic grafted dopamine neurons that had been transplanted into the putamen 10-22 years earlier.[24]

In 2009, Desplats et al. transplanted mouse cortical neuronal stem cells (MCNSCs) into the hippocampus of transgenic mice expressing human α-Syn. After 4 weeks, 15% of the grafted cells were tested positive for human α-Syn and some developed inclusion bodies, whereas the MCNSCs in nontransgenic mice were negative.[25]
Similar findings were also observed in other animal studies, providing strong evidence for neuron-to-neuron propagation of α-Syn pathology.[23]

While α-Syn is predominantly localized in the cytosol, it has been also found in different biofluids, including cerebrospinal fluid (CSF), plasma and saliva, supporting the idea that α-Syn can be secreted to the extracellular space.
Several in-vitro studies reported different possibilities for α-Syn transmission (Fig. 3), but the exact mechanism for each α-Syn species remains unclear and requires further studies.[23]

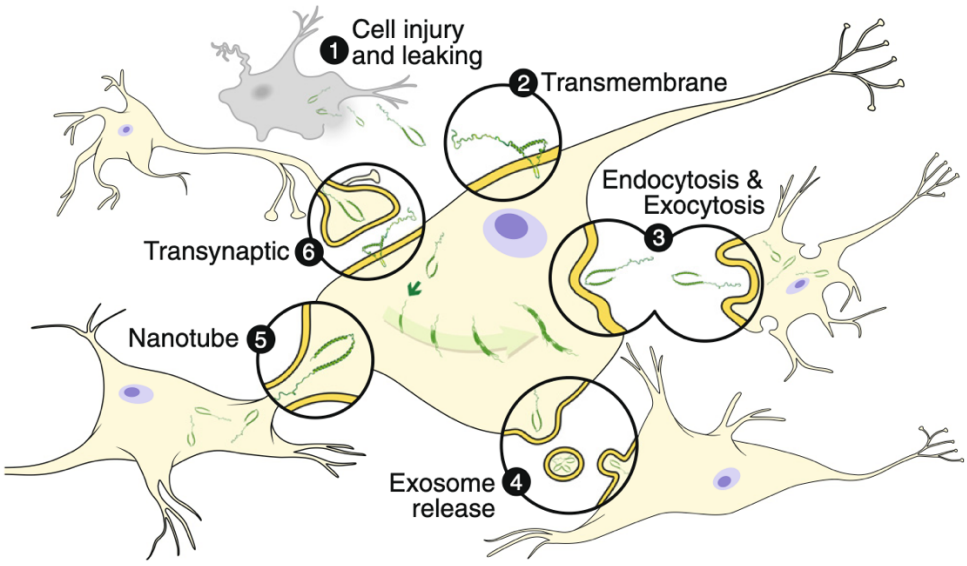

Figure 3: Potential mechanism for neuron-to-neuron propagation of α-Syn. Edited after Visanji et al. (2013)[24]

2.5 α-Syn propagation via vagus nerve

In the gut-to-brain hypothesis, the vagus nerve plays a central role by conveying the α-Syn pathology from the ENS to the CNS.[22] (Fig. 4)

To test this postulate, Kim et al. (2019) conducted an animal study. The authors first injected preformed fibrils (PFF) of α-Syn into the muscle layers of the pylorus and duodenum of mice, both of which are intensively innvervated by the vagus nerve.

The mice were then divided in two groups, one that underwent a truncal vagotomy (TV), and the other remained as before (WT). After 7 months, Kim et al. observed phosphorylated α-Syn in the substantia nigra only in WT-mice, whereas the TV-mice were spared from α-Syn pathology in the brain.[26]

> *"PFF injection causes a significant loss of DA neurons, which is completely prevented by TV […]" (Kim et al. 2019)[26]*

In addition to preventing the spread of α-Syn pathology and associated neurodegeneration, vagotomy also prevented olfactory dysfunction, cognitive deficits and behavioral changes induced by α-Syn PFF injection.

These findings support Braak´s hypothesis that α-Syn pathology can propagate in a stereotyped fashion from the GIT to the brain via the vagus nerve.[26]

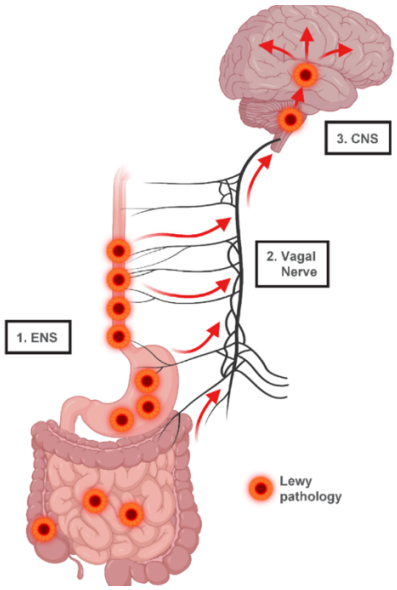

Figure 4: Gut-to-brain α-Syn pathology transmission via vagus nerve. Edited after Chen & Mor (2023)[27]

Regarding the role of the vagus nerve, Svensson et al. (2015) investigated the risk of PD in subjects who underwent vagotomy between 1977 and 1995. This surgical procedure was formerly a common treatment for gastric ulcer and there were 2 types: full truncal vagotomy and superselective vagotomy.

While in the first one, both vagus trunks are severed, the second method involves only the nerves that supply the fundus and the body of the stomach.

The authors hypothesized that truncal vagotomy should have a protective effect on PD pathogenesis, whereas superselective vagotomy has little effect.

The study results showed that patients who did a full truncal vagotomy, 5.339 subjects, had a significant lower incidence rate compared to their matched controls. This effect was especially observed when the follow-up period was extended to more than 20 years.

On the other hand, the incidence rate in the superselective vagotomy cohort, 5.870 subjects, was similar with the rate of their comparison cohort. In conclusion, the results suggest that an intact vagus nerve increases the risk for PD.[28]

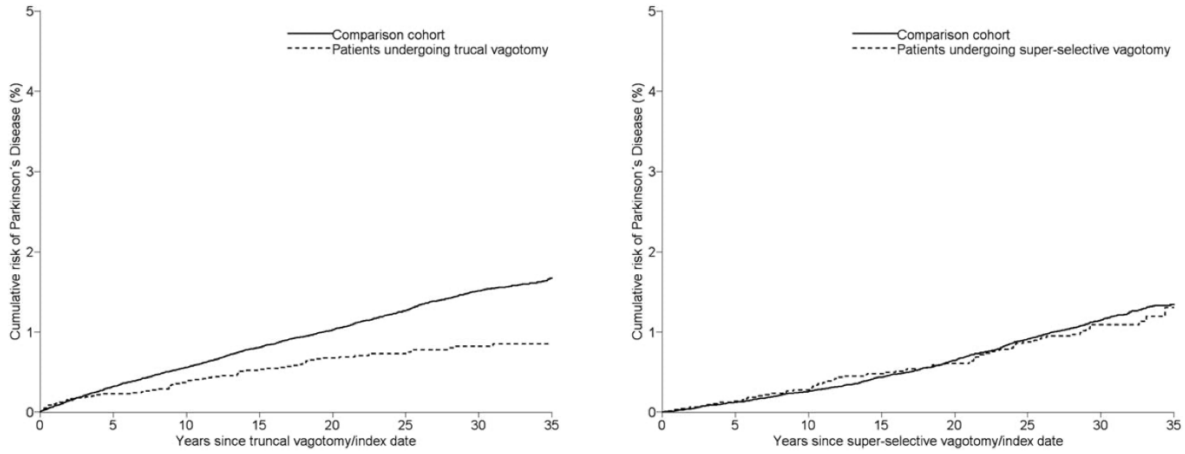

Figure 5: Cumulative PD incidence curve for patients who underwent either truncal or superselective vagotomy compared to their matched controls. Edited after Svensson et al. (2015)[28]

2.6 Histological evidence for GI-origin hypothesis

Now that we know that anatomical structures and the prion-like properties of α-Syn theoretically allow for transmission of its pathology from the ENS to the CNS, the question remains as to what evidence supports that initial α-Syn aggregates originates in the gut rather than the brain.

A major limitation of Braak´s hypothesis is that it is based on static postmortem pathology. However, since Lewy pathology is present in the gut, it is possible to track it via biopsies in living individuals. This has prompted researchers to screen gastrointestinal tissue collected during endoscopy for α-Syn pathology.[22]

The study of Shannon et al. (2012) included three PD patients who underwent a colonoscopy with tissue removal 2-5 year before the onset of the first motor symptoms. The tissue obtained included one mucosal biopsy and two benign polyps.

All patients displayed intense staining for α-Syn in their tissue samples, whereas this was not the case in colonic biopsies from 23 healthy control subjects.[29]

Another study by Stokholm et al. (2016) identified archived tissue samples from 57 PD patients (98 samples) and 90 control subjects (98 samples). These were collected from the period 1992-2013 and some individuals contributed > 1 tissue sample. Among the 57 PD subject, 39 had tissue removed before they were diagnosed with PD. The authors applied two different immunohistochemistry (IHC) techniques, one staining for aggregated α-Syn and one for phosporylated α-Syn. The study results are summarized in Figure 6.[30]

Region	Prodromal PD			PD			CTR		
	No.	a-α-syn Positive (%)	p-α-syn Positive (%)	No.	a-α-syn Positive (%)	p-α-syn Positive (%)	No.	a-α-syn Positive (%)	p-α-syn Positive (%)
Nasal region	2	0	0	1	1 (100)	1 (100)	3	0	1 (33)
Oral region	5	3 (60)	1 (20)	3	0	0	8	1 (13)	2 (25)
Salivary gland	3	2 (67)	3 (100)	1	1 (100)	0	4	2 (50)	1 (25)
Esophagus	16	3 (19)	2 (13)	7	2 (29)	4 (57)	23	5 (22)	2 (9)
Stomach	21	10 (48)	9 (43)	10	7 (70)	4 (40)	31	18 (58)	5 (16)
Small intestine	7	6 (86)	6 (86)	1	1 (100)	1 (100)	8	7 (88)	3 (38)
Appendix	5	5 (100)	5 (100)	3	3 (100)	2 (67)	8	8 (100)	6 (75)
Colon	8	6 (75)	4 (50)	5	4 (80)	3 (60)	13	5 (38)	5 (38)
Total	67	35 (52)	30 (45)	31	19 (61)	15 (48)	98	46 (47)	25 (26)

Figure 6: α-Syn immunochemistry results from the study by Stokholm et al. (2016)[30]

The greatest immunoreactivity was observed in the myenteric and submucosal ganglia structures, however, some subjects had also intense staining in the mucosa layers.
Taken all PD patients together they had significantly more positive tissues compared to the control group, but only when applying IHC against phosphorylated α-Syn. Thus, the IHC method used to detect α-Syn pathology is critical to the frequency of positive tissue samples. On average, tissue samples from prodromal PD patients were removed 7 years before the diagnosis (3,8 months to 20,2 years). This study showed that 22 of 39 (56%) of these prodromal PD patients were tested positive for p-α-Syn up to 20 years prior the diagnosis. This finding supports the hypothesis that PD is a multisystem disease with an extended prodromal phase. Moreover, the authors detected p-α-Syn in 23 of 90 control subjects, which can be interpreted as ILBD cases.[30]

Finally, Hilton et al. (2013) observed accumulation of aggregated α-Syn in tissue samples of 3 PD patients, which were taken 8, 8 and 7 years prior the diagnosis. These samples included gastric and colonic biopsies.[31]

2.7 Criticism to Braak´s hypothesis

Although Braak's hypothesis is supported by in vitro, in vivo and clinical studies, it is still considered controversial, as it´s proposed staging schema doesn´t apply for all PD patients.[36] Kalaitzakis et al. (2008) examined 71 cases of PD and assessed the topographical distribution of α-Syn pathology in different brain regions. The substantia nigra (SN) was affected in all cases, however, the authors found that 47% of cases did not follow a caudo-rostral spread of α-Syn pathology through the PD brain and 7% of cases were lacking pathology in the DMV. This finding suggest that the DMV is not an obligatory point of passage for the pathological process.[32]
The other main concern is that the conclusions from Braak´s group were based on the findings of only 5 cases. In a study by Beach et al. that included 417 autopsy cases, not a single subject was found with α-Syn histopathology in the PNS in the absence of CNS involvement. Autopsy studies from other researcher groups found only two cases out of several hundred, that were positive in the PNS but not in the CNS.[22]

2.8 Body-first versus brain-first PD

The scientific discussion regarding the onset site of PD pathogenesis often takes a one-sided course. Evidence is examined to support that the pathology always originates in the PNS, or alternatively, in the CNS, but rarely has it been considered that both cases could apply.[33]
In 2019, Borghammer and Van Den Bergen first hypothesized that PD can be divided in two phenotypes: body-first and brain-first PD[33] (Fig. 7)

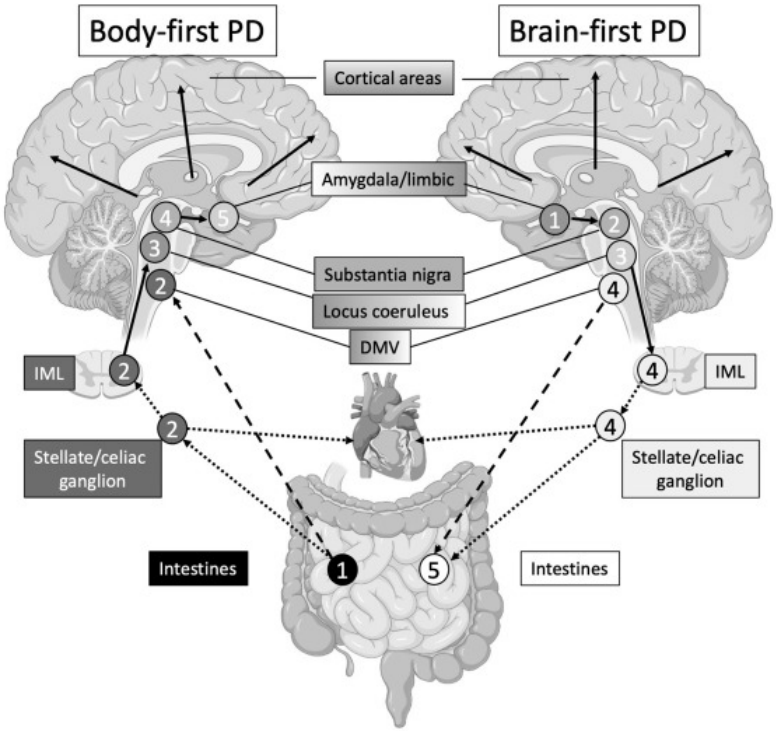

Figure 7: Schematic illustration of α-Syn pathology spreading patterns in both PD phenotypes. Edited after Horsager et al. (2022)[34]

A central component of this hypothesis is that the two subtypes are distinguished by different clinical symptoms early in the disease course, which become similar at later stages of the disease due to greater dissemination of α-Syn pathology.[34]
Rapid eye movement sleep behavior disorder (RBD) is a form of parasomnia characterized by loss of REM sleep atonia, causing dream-enacting behaviour.[33] Studies have found that isolated RBD (iRBD) is a strong predictor for prodromal phase of Lewy body disorders, such as PD or Lewy body dementia (LBD).[34]

In a study conducted by Iranzo et al (2013), a cohort of iRBD patients recruited between 1991 and 2003 was followed up until 2012 to assess possible neurodegenerative syndromes. The cohort included 44 subjects and at the assessment in 2012, 16 were diagnosed with PD, 14 with LBD, one with multiple system atrophy and five had mild cognitive impairment. Of the remaining 8 subjects, 4 died or were not followed up, and 4 were disease-free but had decreased uptake by striatal dopamine transporter (DAT), which is a risk marker for developing PD or LBD.[35]

Since studies have shown that almost all iRBD patients have marked cardiac sympathetic denervation, while at the same time most have a fully or nearly intact nigrostriatal system, iRBD is considered a marker for body-first PD.

On the other hand, many patients in the early stages of PD, which are RBD-negative, have normal cardiac sympathetic innervation. This changes in later stages of PD, as they then exhibit severely reduced cardiac innervation. Thus, such cases may represent a brain-first PD.[33]

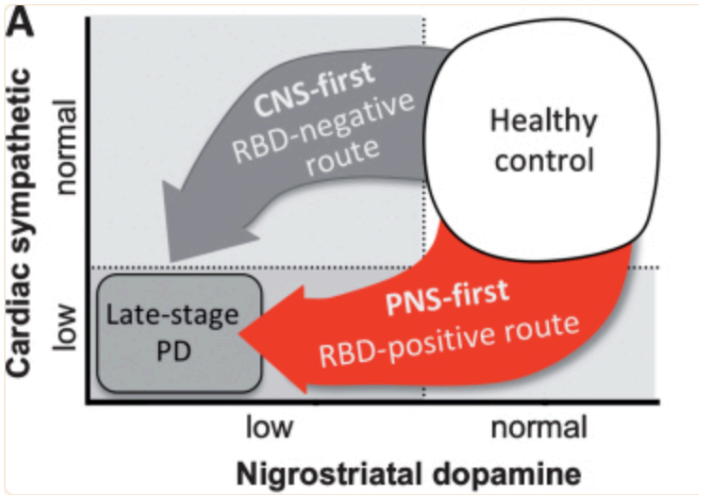

Figure 8: CNS (RBD-negative) vs PNS (RBD-positive) route. Edited after Borghammer and Van Den Berge (2019)[33]

In a review, Horsager et al. (2022) pointed out that other clinical markers besides RBD could be relevant indicators of one or the other phenotype.

Autonomic symptoms, in particular constipation and orthostatic hypotension, are more prevalent in body-first patients. In addition, they have reduced colonic parasympathetic and cardiac sympathetic innervation and have a higher detection rate of intestinal and skin α-Syn. In contrast, the brain-first patients display a more asymmetric nigrostriatal neurodegeneration consistent with an asymmetrical onset of motor symptoms.[34]

3. Gut microbiota and Parkinson´s disease

3.1 Introduction

The human microbiota is the collective term for trillions[40] of microorganisms (MO), such as bacteria, archaea, fungi and viruses, that inhabit almost every enviromentally exposed body surface[37] and exist symbiotically with the human host.[38]

As one of the largest interfaces between enviroment and the host[62], the gastrointestinal tract hosts the greatest density and absolute abundance of MO in the human body, known as the gut microbiota (GM).[37] Instead of being just passive passengers, the gut microbiota regulates a range of physiological functions (Figure 1) in our bodies.[37]

Functions of gut microbiota
Maintain metabolic homeostasis
Inhibit pathogen overgrowth
Involve in development of enteric protection
Biosynthesis of vitamins (B12, folate)
Immunological and xenobiotic effects
Aid in drug metabolism
Produce short-chain fatty acids: butyrate, acetate, propionate
Produce bile acids, choline, amino acids and phenolic derivatives, and polysaccharide A
Produce nicotinic acid, aminoethylsulfonic acid, and retinoic acid

Figure 9: Functions of gut microbiota. Edited after Metta et al. (2021).[39]

Since a balanced microbiota is important for maintaing host health[37], it is not suprising that a GM imbalance is associated with the pathogenesis of several inflammatory diseases and infections.[62]

GM is a dynamic entity and responsive to host and enviromental factors. Its composition as well as activity is being shaped by age, genetics, diet, drugs, exercise, air pollution and geography.[37]

3.2 Microbiota-Gut-Brain Axis

In the past decades, the gut microbiota has emerged as one of the key regulators of gut-brain function, leading to the appreciation of a distinct microbiota-gut-brain axis (MGBA).[40]
MGBA refers to the network of multiple biological systems that allows a bidirectional communication between the brain and gut microbiota, which takes place via several pathways including chemical transmitters, neuronal pathways and the immune system.[37]

Research using germ-free (GF) mice has provided the strongest evidence for an impact of the gut microbiota on the development and function of the CNS. GF-mice are raised in germ-free conditions and are therefore a useful tool to study the MGBA.[41]
Figure 2 illustrates how the brain is affected by the absence of gut microbiota.

Further evidence for the influence of the gut microbiota on the CNS has been provided by studies investigating behavioral changes in mice in response to various GM interventions.[41]
Goehler et al. (2008) indicated that mice treated with Campylobacter jejuni, a pathogenic bacterium, exhibited increased anxiety-like behaviour.[63]
Similarly, a study by Bercik et al. (2011) found that administration of oral antimicrobials to specific-pathogen-free (SPF)-mice reduces anxiety-like behaviour. At the same time, the behaviour of GF-mice was not affected by the administered antimicrobials.[64]

Other studies showed altered behaviour in animals when they were given specific strains of bacteria.[40] Savignac et al. (2014) found that two strains of bifidobacteria, B. breve and B. longum 1714, reduce stress-related behaviours in mice.[65]
The psychobiotic effects of B. longum 1714 were further investigated in a study with healthy volunteers by Allen et al. (2016), which demonstrated an association between B. longum consumption and stress reduction and memory improvement.[66]

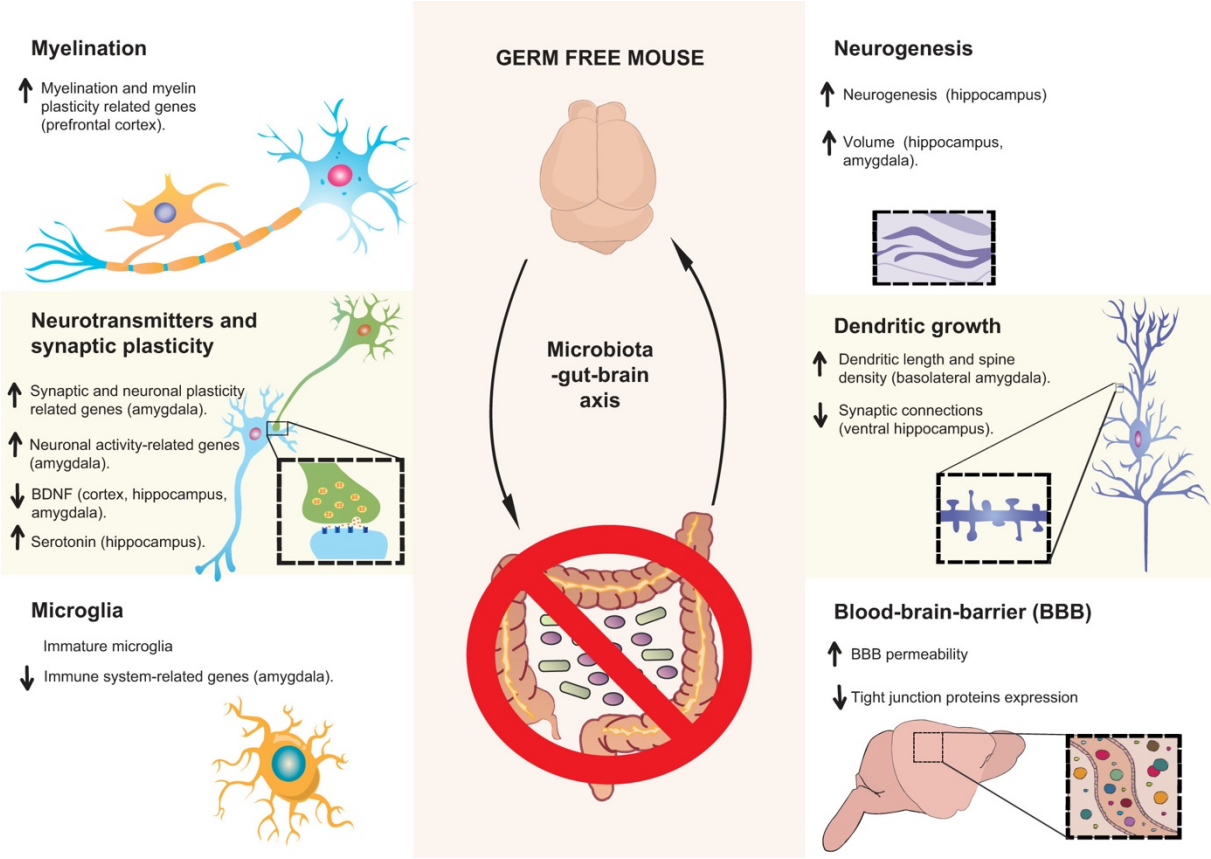

Figure 10: CNS changes in GF-mice. Edited after Cryan et al. (2019).[40]

3.3 Altered gut microbiota in PD

Based on the evidence that the gut microbiota has an impact on the CNS function, its potential role in PD pathogenesis has received increasing attention.[38]

In fact, several studies (Table 2) reported the existence of an alternation in gut microbiota composition, called dysbiosis.[38]

Since bacteria account for the largest part of GM and their role in Parkinson´s disease has been most studied[42], this review focuses solely on the changes of the bacterial taxa, even though the term “gut microbiota” includes all microorganisms colonizing the gut.

3.3.1 α- diversity

α-diversity is an indicator of the species diversity of the gut microbiome within an individual subject.[49] Its decrease is considered by many experts as a potential cause of gastrointestinal, immune and metabolic disorders and is therefore an important marker of intestinal health.[50]

However, this led to the question if α-diversity could also be a promising predictor for neurological diseases.[50]

A meta-analyses study, conducted by Plassais et al. (2021), investigated the potential link between α-diversity and Parkinson´s disease and aimed to determine if α-diversity from stool samples could be used as a marker to diagnose PD.
The meta-analyses included 7 studies with a total of 1067 samples (631 PD, 436 HC).
The authors concluded that PD is not associated with a loss of bacterial diversity and suggested that unlike the striking difference in α-diversity observed in GI diseases, PD exhibit a more subtle dysbiosis with only a limited number of bacteria involved.[50]

3.3.2 β-diversity

While it was shown that α-diversity is similar between PD patients and healthy controls[50], many studies (Table 2) revealed differences in the composition of bacterial taxa (β-diversity) between both groups.[49]
The results in terms of differences in specific taxa are heterogenous (Table 2), for which several factors may play a role.[51]
One is due to technical aspects, such as differences in study design, sample size and lack of standardization in sample collection and sequencing techniques.[51]
Second, due to differences in participant characteristics, such as genetic and geographical background, lifestyle, dietary habits, co-morbidities and use of medication.[57]
Finally, the heterogenous nature of PD also contributes to such variability.[51]

Nevertheless, meta-analyses revealed discernible patterns. Bacteria of the genera Blautia, Coprococcus, Lachnospira, Roseburia and Faecalibacterium are reduced in PD patients compared to healthy controls. In contrast, bacteria of the genera Bifidobacterium, Akkermansia, and Lactobacillus are increased. In addition, oppurtunistic pathogens and proinflammatory bacteria of the genera Escherichia, Corynebacterium, and Desulfovibrio are reported to be enriched in PD patients.[57]

Study number	Study	Participants	Sample used	Increased in PD	Decreased in PD
1	Hasegawa et al. (2015)[43]	PD: 52 Control: 36	Stool	Lactobacillus (G)	Clostridium coccoides (Sp), Bacteroides fragilis (Sp)
2	Scheper-jans et al. (2015)[44]	PD: 72 Control: 72	Stool	Lactobacillaceae (F), Verrucomicrobiaceae (F), Bradyrhizobiaceae (F), Clostridiales (F)	Prevotellaceae (F)
3	Unger et al. (2016)[45]	PD: 34 Control: 34	Stool	Enterobacteriaceae (F), Bifidobacterium (G)	Bacteroidetes (P), Prevotellaceae (F), Lactobacillaceae (F), Enterococcaceae (F), Faecalibacterium prausnitzii (Sp)
4	Petrov et al. (2017)[46]	PD: 89 Control: 66	Stool	Christensenella (G), Catabacter (G), Lactobacillus (G), Oscillospira (G), Bifidobacterium (G)	Dorea (G), Bacteroides (G), Prevotella (G), Faecalibacterium (G)
5	Kesha-varzian et al. (2015)[47]	PD: 38 Control: 34	Sigmoid-mucosal biopsies and stool	Bacteroidetes (P), Proteobacteria (P), Verrucomicrobia (P), Clostridiaceae (F), Oscillospira (G), Akkermansia (G)	Firmicutes (P), Lachnospiraceae (F), Coprobacillaceae (F), Blautia (G), Coprococcus (G), Dorea (G), Roseburia (G)
6	Bedarf et al. (2017)[48]	PD: 31 Control: 28	Stool	Verrucomicrobiaceae (F), Firmicutes (P), Akkermansia (G)	Prevotellaceae (F), Erysipeli-trichaceae (F)

Table 1: Altered gut microbiota composition in PD patients.
P: Phylum, F: Family, G: Genus, Sp: Species

3.3.3 Helicobacter pylori

H. pylori is a gram-negative bacterium and is one of the most prevalent pathogens, infecting an estimated 50% of the world´s population.[53]

While it was initially found to cause peptic ulcer and gastritis[52], various studies have suggested a role of H. pylori infections in PD pathogenesis.[54]

A meta-analysis by Shen et al. (2017), which included 33,125 participants, concluded that H. pylori positive patients have a 59% higher risk of developing Parkinson's disease than patients without such infection, suggesting that H. pylori infection may be a risk factor for PD.[55]

3.3.4 Small intestinal microbial overgrowth (SIBO)

> *"Small intestinal bacterial overgrowth (SIBO) is a gut dysbiosis in which the small intestine is excessively colonized by bacteria that are typically found in the large intestine. […]*
>
> *SIBO is relatively common in people with PD, including those with recent onset of motor symptoms, around half of the patients with PD testing positive for SIBO, compared with only up to a quarter in the general population. Notably, however, the prevalence may be as low as 14%, or as high as 67%, depending on the demographic and clinical characteristic of the population included in the study, […]*
>
> *The relation between SIBO and specific characteristics of sporadic PD is not straightforward, but it is biologically plausible that SIBO might influence the etiopathogenesis, clinical phenotype and progression of sporadic PD, […]" (Danau et al. 2021)[56]*

3.4 Altered gut microbial metabolites

The gut microbiota communicates with its host through microbial secreted metabolites and toxins[57], whose production is altered in GM dysbiosis.[38]
Numerous studies have found differences between PD patients and control subjects regarding levels of microbial metabolites in various samples, including feces, plasma, and cerebrospinal fluid. Some of them correlate with the progression and severity of PD, suggesting that they play a role in disease pathogenesis and may serve as disease biomarkers.[57]

3.4.1 Short-chain fatty acids (SCFA)

SCFAs are metabolites derived from the microbial fermentation of nondigestable carbohydrates. Different SCFA-producing bacteria produce different SCFAs[49], but the most abundant in the human colon are acetate, propionate and butyrate.[57]
PD patients have shown (Table 1) a decrease of the families Prevotellaceae and Lachnospiraceae as well as a decrease of the genera Blautia, Roseburia and Faecalibacterium, all of which comprise SCFA-producing species.[49]

In addition to their role as an energy source for colonocytes, SCFAs are considered beneficial for promoting intestinal barrier integrity and their anti-inflammatory effects.[57]
Their ability to inhibit the enzyme histone deacetylase in colonocytes and immune cells results in an increased expression of anti-inflammatory cytokines, such as IL-37 and IL-10, as well as antimicrobial cathelicidin-derived peptide. At the same time, its inhibition is also associated with the downregulation of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-8, and the stimulation of colonic regulatory T-cell differentiation to control the gut inflammation.[49]

Several studies observed reduced fecal levels of SCFAs in PD patients compared to healthy controls. A decrease of SCFAs was found to correlate with loss of tight junctions proteins in the instestines, causing increased intestinal permeability and inflammation.[109]
Moreover, Tan et al. (2021) indicated in their study, including 104 PD patients, that low levels of fecal SCFAs are associated with worse cognitive and motor symptoms.[110]

3.4.2 Hydrogen sulfide (H_2S)

Many studies revealed a decrease of Prevotellaceae in PD patients.
In addition to SCFAs, Prevotella is a important producer of hydrogen sulfide, a gasotransmitter that exerts neuroprotective effects.[58]
GF-mice exhibit significantly reduced free H_2S levels in their plasma and colon compared to SPF-mice, highlighting the contribution of gut microbiota to free H_2S levels.[58]
Kida et al. (2011) examined the effects of inhaled H_2S in mice with PD-like symptoms, induced by the neurotoxin MPTP. The study showed that the inhalation of H_2S prevented microglia activation and dopaminergic neurodegeneration as well as restored the MPTP-induced movement disorder.[59]

3.4.3 Ghrelin secretion

Accumulating evidence indicate that the gut microbiota have influence on the secretion of GI peptides, such as the orexigenic hormone ghrelin.[105]
Studies found that a decrease in Prevotella and an increase in Lactobacillus and Bifidobacterium is associated with a reduced ghrelin secretion in the gut.
Since these bacterial changes are observed in PD patients (Table 1), their ghrelin levels should be decreased.[60]
Song et al. (2017) addressed this issue by investigating plasma ghrelin levels in early-stage PD patients. The results showed that both total and active plasma ghrelin levels were decreased in PD patients compared to the control group. In particular, the levels of active ghrelin were nearly halved.[106]
In a study, conducted by Cheng et al. (2016), ghrelin was shown to upregulate the expression levels of tight junction proteins, such as ZO-1 and claudin-5. At the same time ghrelin downregulated the ICAM-1 expression, a protein that contributes to intestinal barrier dysfunction.[107]
Besides the positive effects on the gut barrier integrity, ghrelin has been also found to have neuroprotective effects in dopaminergic neurons of the substantia nigra in a mouse model.[60]

3.4.4 Mucin degrading activity

Another consisting finding in fecal samples of PD patients was a increase of Akkermansia muciniphila (Table 1), a bacterium species of the phylum Verrucomicrobia.[108]
It is known as a mucin-degrading bacterium, as it is utilizing mucus as a sole carbon, nitrogen and energy source.[108] The breakdown of the mucus layer makes the intestinal wall more susceptible to pathogens, which leads to inflammation.[60]

3.4.5 Other bacterial metabolites

Bacterial-derived product	**Bacterial taxa**	**Increased or decreased in PD patients vs controls**	**Physiological effect**	**Source**
Gasotransmitter: Hydrogen (H_2)	Blautia coccoides (Sp), Clostridium leptum (Sp)	Decreased	Neutralize toxic radical, downregulation of pro-inflammatory factors, neuroprotective	58
Lipopolysaccharide (LPS)	Escherichia (G)	Increased	Pro-inflammatory	57
Indole-3-acetic acid (IAA)	Clostridium (G)	Decreased	Downregulation of pro-inflammatory factors, attenuating inflammation	57, 58
p-Cresol sulfate	Bacteroidaceae (F), Clostridiaceae (F)	Increased	Pro-inflammatory	58, 61
Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin)	Prevotella (G), Bacteroides (G)	Decreased	Neuroprotective	60

Table 2: Altered bacterial metabolites in PD patients.

4. “Leaky gut” and Parkinson´s disease

4.1 Introduction

The GI epithelial barrier consists of a monolayer of epithelial cells that are covered by a thick layer of mucus and are connected by intercellular junctions. The barrier and its integrity are critical for preventing translocation of luminal bacteria and their metabolites.[67]
Since the gut microbiota and its products play an important role in maintaining the epithelial barrier, it is not surprising that dysbiosis of the GM, as observed in PD patients, is more often associated to disrupted barrier function[67], termed “leaky gut”.[76]

4.2 What is the evidence for a “leaky gut” in PD?

4.2.1 In vivo permeability tests

A non-invasive in-vivo technique for assessing intestinal permeability is to measure urinary excretion of orally ingested non-metabolizable sugars.[68]
Such sugar solutions are most commonly combinations of lactulose, a disaccharide, and mannitol, a monosaccharide. While mannitol, a relatively small molecule, can easily pass through the intestinal barrier, the larger lactulose is usually absorbed to a lesser extent. Hence, an increased L/M ratio is an indicator of increased intestinal permeability.[68]

As early as 1996, Davies et al. conducted a study to assess the intestinal permeability in 15 PD patients. The study found that the L/M ratio was significantly higher in the patient group than in the matched control group, however, urinary mannitol was significantly decreased in the PD group, whereas urinary lactulose was similar in the two groups.[69]
Thus, the L/M ratio difference between both groups must be interpreted cautiously.[68]

In the study by Salat-Foix et al (2012) it was shown that the L/M ratio was increased in only 3 of 12 PD subjects.[70]
Two more studies published by Forsyth et al. (2011) and Perez-Pardo et al. (2019) found no difference in the average L/M ratio of 9 and 6 PD patients compared to healthy controls.[71, 72]

Notably, the L/M ratio only reflects small intestine permeability. In order to assess the permeability in the large intestine, studies used sucralose, an artificial disaccharide, which, unlike lactulose and mannitol, doesn´t get metabolized by colonic bacteria.[68]
Forsyth et al. (2011) and Perez-Pardo et al. (2019) both demonstrated significantly higher urinary sucralose in PD subjects compared to controls. (Figure 3)[71, 72]

"Together, the existing in vivo data on gut permeability in PD suggest that the colon, but not the small intestine of parkinsonian patients is hyperpermeant." (van IJzendoorn SCD & Derkinderen P., 2019)[68]

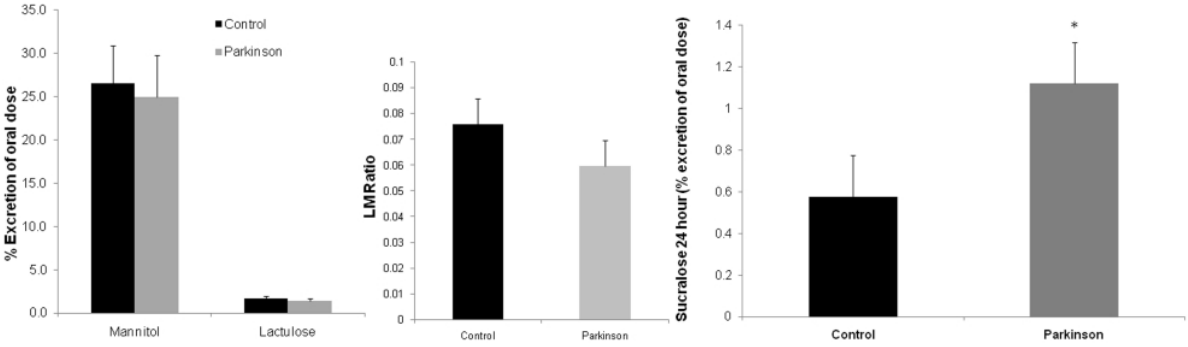

Figure 11: Intestinal permeability in PD subjects. Edited after Forsyth et al. (2011).[71]

Alternatively, measurement of alpha-1-antitrypsin and zonulin in the feces of PD patients is also a possible approach to assess the gut barrier function in vivo, as for both, elevated fecal levels are an indirect marker of a disrupted gut barrier.[68]
Schwiertz et al. (2018) conducted a study, which applied this approach to 36 PD patients and 28 control subjects. The results demonstrated that significantly more PD patients had increased levels of both markers compared to the control group.
Moreover, the authors found significantly increased levels of calprotectin, a marker for intestinal inflammation, in the feces of PD. However, none of the reported markers are specific for PD, nor do they correlate with disease severity.[104]

4.2.3 Structural changes

Tight junctions (TJ), also known as zona occludens, are intercellular junctions, which are crucial for the intestinal barrier function. Its loss of integrity results in an increased paracellular permeability.[68]

TJ consists of transmembrane proteins, including occludin, claudin and junctional adhesion molecules (JAM) that interact in the paracellular space with their kind of proteins from neighboring cells, ensuring that the apical intercellular space is completely closed.
Other proteins, such as zonula occludens (ZO) -1, -2, and -3, provide a link between the TJ complex and the actin cytoskeleton, whose interplay maintains TJs function.[73]

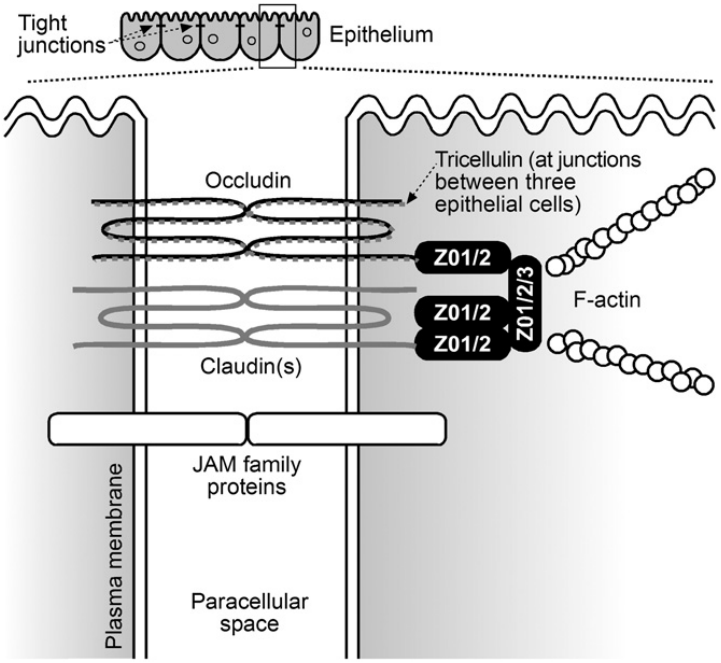

Figure 12: Structure of tight junctions. Edited after Ulluwishewa (2011).[74]

Clairembault et al. (2015) investigated wether PD patients exhibit structural changes in the intestinal epithelial barrier. Using western blot, they analyzed the expression levels of occludin and ZO-1 in lysates of sigmoid/descending colon biopsy samples of 31 PD patients and 11 healthy subjects. The study revealed that the expression of occludin is reduced in PD patients by 50% compared with healthy controls. At the same time, no change in ZO-1 levels was observed.
In addition, immunofluorescence experiments performed on colon biopsies by Chairembault et al. showed significant differences in the cellular distribution of ZO-1 and occludin in PD, which may also indicate for an impaired TJ integrity.[75]
Another immunofluorescence staining analyses, conducted by Perez-Pardo et al. (2019), showed a significant reduction of ZO-1 expression in sigmoid colon biopsies from 6 PD subjects compared to controls (n=6).[72]

4.3 How might a "leaky gut" contribute to PD pathogenesis?

Many literatures speculated that the increased intestinal permeability in PD patients allows the translocation of bacterial derived pro-inflammatory products, such as lipopolysaccharide (LPS). This may then promotes local inflammation and oxidative stress, which in turn induces α-Syn overexpression and pathological aggregation in enteric neurons.[71, 68, 76, 77] (Figure 7)

This scenario is supported by an animal study, conducted by Kelly et al. (2014), which showed a progressive increase in α-Syn expression when mice were given systemic low-dose LPS for 5 months.

Figure 5 illustrate that the average number of severe α-Syn-immunoreactive myenteric neurons per ganglia increased in LPS-treated mice over time, implying for an α-Syn overexpression in the enteric nervous system. However, this increase is only observed in the large intestine, whereas the average number of myenteric neurons per ganglia in the small intestine was not affected by LPS in any category.

Moreover, mice treated with LPS exhibited phosphorylated α-Syn in a subset of colon myenteric neurons at month 4 and 5.[78]

This finding suggest that the large intestine is preferentially susceptible not only to hyperpermeabilty but also to endotoxin exposure compared with the small intestine.[78]

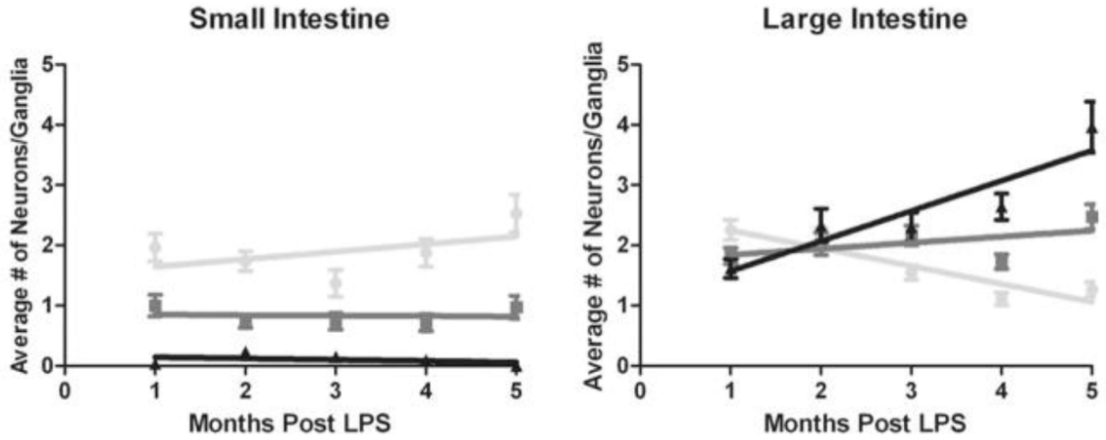

Figure 13: Effects of systemic LPS on α-Syn expression in mice. Mild (light grey), moderate (dark grey), severe (black) α-Syn-immunoreactive myenteric neurons.
Edited after by Kelly et al. (2014)[78]

Forsyth et al. (2011) investigated wether the increased intestinal permeability in PD patients, is accompanied with increased translocation of bacterial-derived products.
The study found that both epithelial and lamina propria zones of sigmoid mucosal samples of PD subjects had significantly more intense staining of E. coli, a gram-negative bacterium, compared to controls.
As an alternative approach, the authors measured plasma LPS binding protein (LBP), a protein which can neutralize endotoxin and remove LPS from the blood.
The mean level of LBP was significantly lower in PD subjects compared to controls, indicating a greater exposure to gram-negative bacteria. However, serum endotoxin was not significant increased in PD patients.[71]

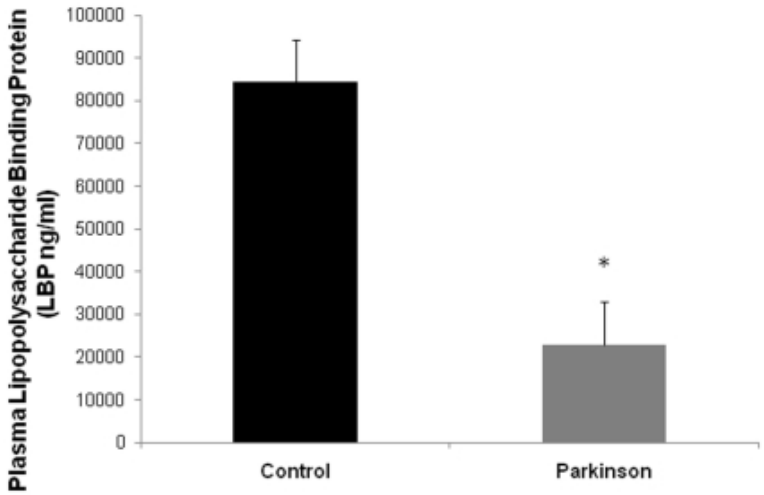

Figure 14: Plasma LBP PD vs. healthy subjects. Edited after Forsyth et al. (2011)[71]

Quantification of LPS in blood samples from PD patients has been conducted in many studies, with different results. This is due to the heterogeneity of Parkinson's disease, but also because of the difficulties of measuring LPS in blood samples.
Not only are the concentrations of LPS in blood very low and can therefore be below the detection range in many commercially available assays, but their half-life is also short.
Another significant issue is a potential contamination during blood sampling and sample preparation. Also plasma should be preferred to serum, since blood coagulation leads to an additional loss of endotoxin activity.
Because of these difficulties, measurement of an indirect marker such as LBP may be more reliable and is commonly used to determine endotoxin exposure.[77]

Further aim of the study by Forsyth et al. (2011) was to examine if increased intestinal permeability is also associated with the generation of oxidative stress in the mucosa and the abnormal accumulation of α-Syn in the enteric neurons.
Intestinal biopsies from PD subjects showed a significantly more intense staining for α-Syn and 3-nitrotyrosine, a biomarker for oxidative stress, when compared to controls.[71]

The authors noted that the study results do not establish a causal relationship between increased intestinal permeability and PD pathogenesis, but rather support a new model of PD pathogenesis that involves increased intestinal permeability to bacteria and their pro-inflammatory products. More studies are needed to determine if a "leaky gut" plays a causative role in PD or is a consequence of PD.[71]

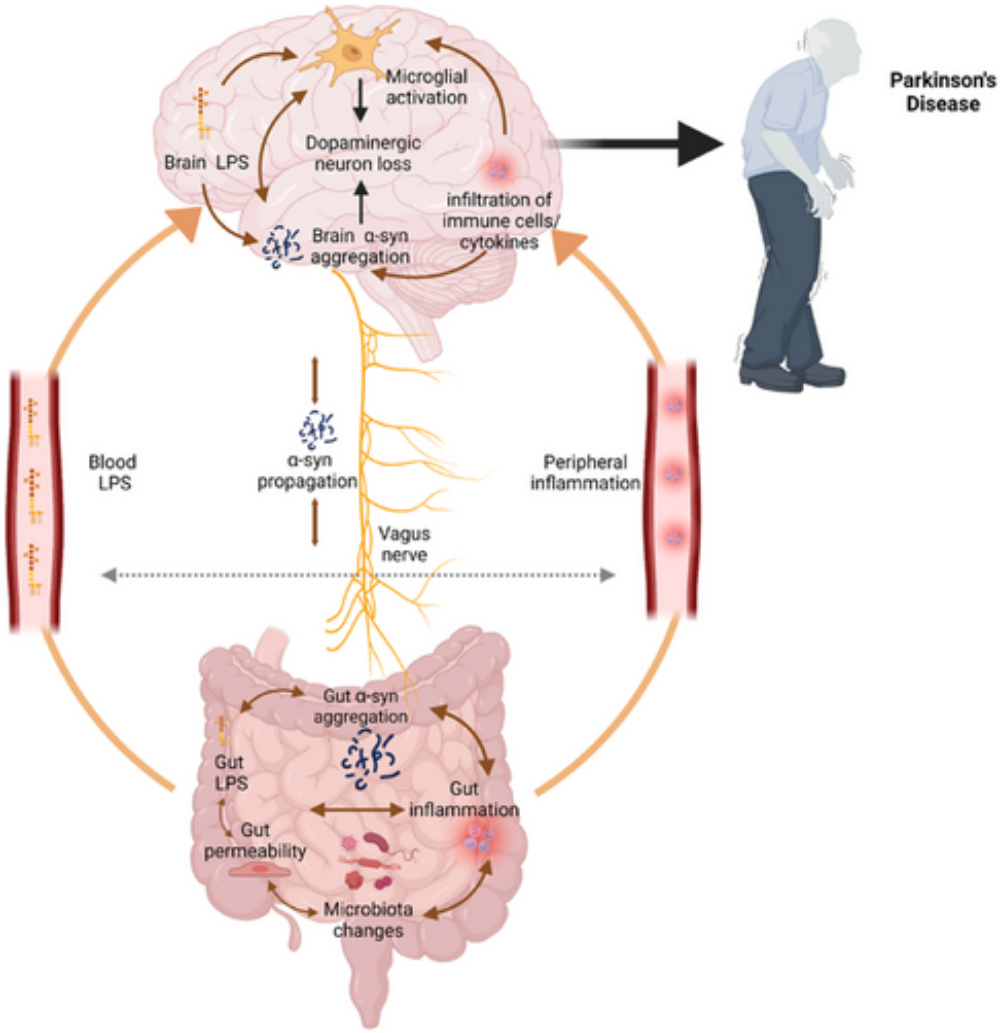

Figure 15: The endotoxin hypothesis in PD. Edited after Brown et al. (2023)[77]

5. α-Syn induced neurodegeneration

5.1 Lewy pathology: "innocent bystander"?

Although Lewy pathology is associated with the clinical symptoms of Parkinson's disease, its role in the neurodegenerative process remains questioned. Instead, α-Syn oligomers and fibrils are considered to be the toxic species.[79]

Several lines of evidence support the idea that Lewy bodies/neurites may be "innocent bystanders" in PD pathogenesis.[79]

First, in two different reviews examining PD patients with genetic cause, Poulopoulos et al. (2012) and Cookson et al. (2008) showed that some gene mutations are associated with PD phenotypes in which neuronal degeneration is present but in the absence of Lewy pathology.[80, 81]

Second, Lewy bodies/neurites have been found regularly post-mortem in eldery individuals without neurological impairment.[79]

Finally, unlike in Alzheimer´s disease, the burden of Lewy pathology in PD patients has not been shown to correlate with the severity of PD symptoms.[79]

One could argue, that Lewy body/neurite formation provides a protective mechanism by isolating the toxic species from the cytoplasma.[79] This is consistent with the observation that Lewy body-positive dopaminergic neurons appear to be healthier than neighboring inclusion-free neurons.[82]

However, correlation does not equal causality and if they are indeed protective entities, they can only prolong the survival time of the affected neurons, but not ultimately rescue them.[79]

5.2 Role of α-Syn oligomers and fibrils in PD pathogenesis

Studies have shown that contrary to previous assumptions where α-Syn fibrils were believed to be the toxic species responsible for PD pathogenesis, α-Syn oligomers are the most potent neurotoxic α-Syn species.[83]
Karpinar et al. (2009) investigated the effects of α-Syn mutants in-vivo, which are designed to increase oligomer formation, but reduce their propensity for fibrillization.
When exposed to mammalian neurons or organisms, like C. elegans and D. melanogaster, the study showed a strong correlation between α-Syn oligomers with neuronal toxicity and behavioural defects.[85]

To evaluate the toxicity of both species Winner et al. (2011) developed α-Syn mutants that promote either for oligomer or fibril formation.
In-vivo tests with rats revealed that α-Syn oligomer-forming variants caused the most severe dopaminergic loss compared to the α-Syn fibril-forming variants, which showed no significant toxic effect.[84]
The lack of toxicity of α-Syn fibrils is further supported by various studies with cell-culture models.[83]

Although α-Syn fibrils exert less neurotoxic effects than the oligomers, it has been reported that α-Syn fibrils, on the other hand, play a crucial role in the spread of α-Syn pathology. In vitro studies have shown that the aggregation of endogenous monomeric alpha-synuclein can be triggered by exogenously introduced alpha-synuclein fibrils. These fibrils act as seeds that can propagate from one cell to another, thus driving the progression of Parkinson's disease.[83]

5.3 Mechanisms of α-Syn oligomer toxicity

5.3.1 Compromised cell membrane

In a study, conducted by Danzer et al. (2007), it was shown that α-Syn oligomers can induce a disruption of cellular ion homeostasis by creating pores in the cell membrane, leading to an influx of extracellular calcium and subsequently to cell death.[86]
Interestingly, another study found that a depletion of calcium in the extracellular space appears to reduce oligomer-induced cell death.[87]
Moreover, α-Syn oligomers can decrease neuronal excitability by altering membrane properties, such as input resistance.[87]

5.3.2 Mitochondrial dysfunction

Mitochondrial dysfunction have been strongly implicated in PD pathogenesis and it has appeared to be related to α-Syn accumulation.[96]
Di Maio et al. (2016) reported that α-Syn oligomers bind with high-affinity to the TOM20 receptor, a subunit of the mitochondrial protein import machinery, preventing its interaction with TOM22. As a consequence of the resulting inhibition of mitochondrial protein import, mitochondrial respiration is reduced, ROS production is increased, and mitochondrial membrane potential is decreased.
As opposed to α-Syn oligomers, α-Syn fibrils had no impact on protein import impairment. Furthermore, Di maio et al. also examined post-mortem brain tissue from PD patients and controls. It has been found that the nigrostriatal dopamine neurons from PD cases exhibit an abberant α-Syn:TOM20 interaction, which is associated with loss of imported mitochondrial protein, thus, confirming the relevance of their findings from in-vitro experiments.[96]

In addition to impairment of protein import, α- Syn oligomer could induce mitochondrial dysfunction by inhibiting mitochondrial complex I, an enzym of the respiratory chain, leading to oxidation of ATP synthase and mitchondrial lipid peroxidation. This event promotes the formation of osmotic transition pore, which result in mitochondrial swelling and eventually cell death.[88]

5.3.3 Synaptic Impairment

Choi et al. (2013) investigated wether α-Syn oligomers can cause synaptic dysfunction. The authors found that α-Syn oligomers are able to bind to the N-terminal domain of synaptobrevin-2, a membrane protein of synaptic vesicles, which is relevant for preventing vesicles from docking with each other and for the formation of the SNARE complex.[91]

Giving the fact that α-Syn oligomers have mutliple binding sites for synaptobrevin-2, α-Syn oligomers can either sequester most of the synaptobrevin-2 of a single vesicle, or bind synaptobrevin-2 from mulitple vesicles, forming a vesicle cluster. Both pathways are ultimately leading to an inihibition of the formation of the SNARE complex, a fusion maschinery relevant for the fusion of vesicles with the neuronal plasma membrane. This has been further confirmed by the finding that an insertion of α-Syn oligomers directly into the cytoplasm of PC12 cells significantly reduced the exocytosis in these cells.[91]

"In vivo, the result would be decreased neurotransmitter release and the possible ensuing loss of connectivity may lead to neuronal dysfunction and death."

(Bengoa-Vergniory et al. 2017)[87]

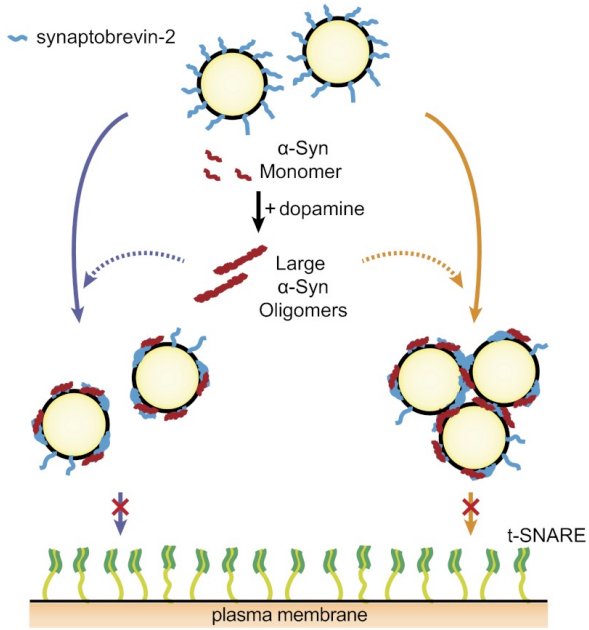

Figure 16: Inhibitory effects of dopamin-induced α-Syn oligomers on exocytosis. Edited after Choi et al. (2013)[91]

5.3.4 Oxidative stress

Deas et al. (2016) found in their study that both α-Syn oligomers and fibrils are capable of generating ROS in neuronal cells, however, this response was significantly higher, when the cells were exposed to α-Syn oligomers than to α-Syn fibrils.

Further investigations showed that the α-Syn oligomer-induced ROS production is independent of known cellular ROS production mechanisms, such as mitochondria, cytosolic NADPH oxidase activity and catecholamine metabolism, but rather through interacting with metal ions. This is supported by the finding that chelation of free iron and copper can prevent the α-Syn oligomer-induced ROS generation.

To assess wether α-Syn oligomer-induced ROS generation is able to induce oxidative stress, the authors measured the level of glutathion, the major antioxidant in the brain.

The results indicated that α-Syn oligomers significantly reduced endogenous gluathion levels in neuronal cultures, confirming their ability to induce oxidative stress. Although α-Syn fibrils were able to induce ROS production, the generating ROS level was insufficient to reduce the levels of glutathion.

Finally, Deas et al. found that α-Syn oligomers-induced oxidative stress can trigger the activation of caspase-3 activation, a major enzyme involved in the cascade of cell apoptosis, which concludes that α-Syn oligomers can induce cell death through generating ROS.[90]

5.3.5 ER stress

Endoplasmic reticulum stress occurs when misfolded proteins accumulate in a cell. As a protective cellular response to this, unfold protein response (UPR) become triggered, which should counteract the accumulation.[92]

Castillo-Carranza et al. (2012) showed in their study that α-Syn oligomers are strong inducers of XBP1 (X-box binding protein 1) activation, a key component of UPR, indicating that α-Syn oligomers induce ER stress.

As opposed to α-Syn oligomers, α-Syn fibrils had a much weaker effect on XBP1 activation, although the change was significant with respect to monomeric α-Syn and untreated cells.[93]

Another study, conducted by Colla et al. (2012), indicated that α-Syn oligomers accumulation within the ER resulted in chronic ER stress, which in turn contributed to neurodegeneration through activation of ER-associated caspases.[92]

5.3.6 Loss of intracellular degradation

The main pathways responsible for degradation of overexpressed α-Syn and its aggregates are the ubiquitin-proteasome system (UPS) and autophagy-lysosomal pathway (ALP).[88]
Giving the fact that Lewy bodies are highly ubiquitinated, a mechanism which tags unwanted proteins for degradation by the proteasome, implicate that the UPS is impaired.[94]
In fact, post-mortem studies with PD patients have revealed a reduced expression of proteasomal subunits and a compromised catalytic activity of proteasome.[95]
Lindersson et al. (2004) demonstrated in their study that both α-Syn oligomers and fibrils can inhibit the function of proteasome.[94]
Similarly, studies on cellular and animal models showed that α-Syn aggregates also induce ALP dysfunction.[95]
In summary, both degradation pathways can be downregulated by α-Syn aggregates, leading to a vicious cycle in which the aggregates accumulate and in turn further enhance the inhibition of the degradation process.[88]

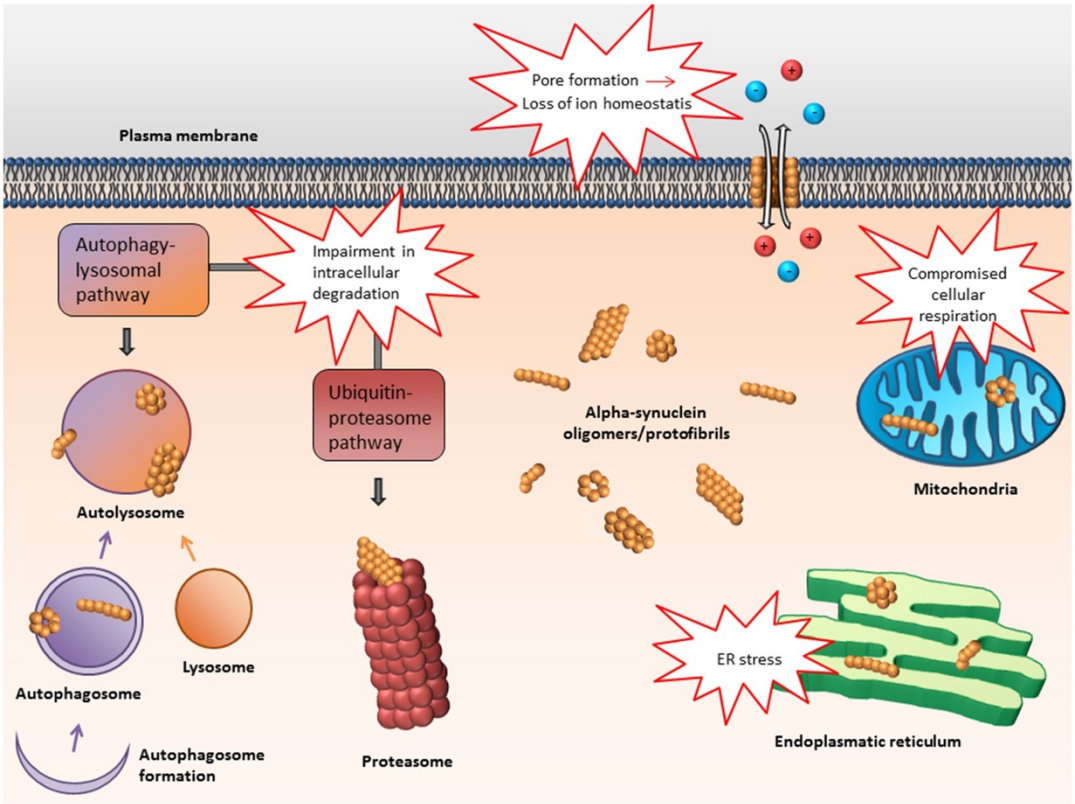

Figure 17: Intracellular targets of α-Syn oligomers. Edited after M. Ingelsson (2016)[89]

5.3 α-Syn mediated neuroinflammation

5.4.1 Introduction

Apart from the direct cytotoxic effects, α-Syn also contributes to PD pathogenesis by inducing neuroinflammation.[88] As a protective response of the CNS, neuroinflammation is important for the removal of harmful stimuli, including pathogenic protein aggregates, and for initiating the healing process.
Its key regulators are glia cells, such as astrocytes and microglia.[97]

The role of neuroinflammation in neurodegenerative diseases has become more evident and is now considered as a double-edged sword, as it exerts both beneficial and detrimental effects.[97]
As for PD, the activation of astrocytes and microglia by α-Syn aggregates on the one hand promotes the clearing process from such misfolded proteins, on the other hand it enhances neurotoxicity due to the release of pro-inflammatory mediators.[87]

5.4.2 Microglia mediated dopaminergic neurotoxicity

Post-mortem studies on PD patients revealed increased staining for HLA-DR, a MHC class II cell surface receptor, in the substantia nigra, confirming the presence of activated microglia and suggesting that they may play a pathological role in PD.[98]
Several other studies support the causal role of microglia on dopaminergic neuron damage.[99]

In-vitro and in-vivo studies have shown not only that the activation of microglia by LPS result in loss of dopaminergic neurons, but also that LPS is toxic to neurons only in the presence of microglia. Interestingly, dopaminergic neurons seem to be particularly sensitive to microglia activation, being the first cell type to be affected.[99]

Similarly, many other enviromental triggers, such as rotenone, paraquat, maneb and diesel exhaust particles, cause neurotoxicity by directly activating microglia.[99]
Another study using mutant mice, which are deficient in pro-inflammatory factors, showed significantly reduced toxicity when exposed to MPTP, indicating that inflammation is a crucial contributor to neuron damage.[99]

Zhang et al. (2005) conducted a study to assess the influence of glia cells on α-Syn induced dopaminergic neurotoxicity. The authors performed three different experiments. (Figure ?)

The study results indicated that increased percentage of astroglia in neuron-enriched cultures, treated with 250nM α-Synuclein for 10 days, was associated with preservation of dopaminergic neurons. Notably, the α-Syn treatment consisted mainly of α-Syn oligomers. In contrast, under the same conditions, an increasing percentage of microglia showed a progressive increase in dopaminergic neurotoxicity.
A third experiment demonstrated that α-Syn dopaminergic neurotoxicity was significantly prevented when microglia were depleted in a neuron-glia mixed culture (< 1% of total cells), further confirming that microglia accelerate α-Syn dopaminergic neurotoxicity.[100]

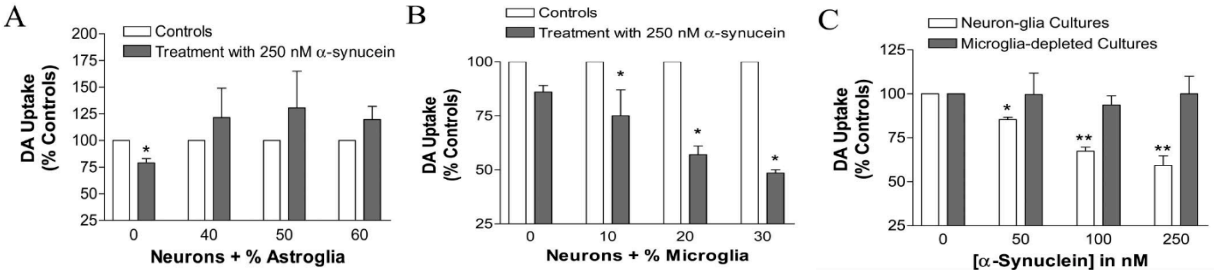

Figure 18: Influence of glia cells on α-Syn mediated neurotoxicity. Neurotoxicity was assessed by uptake of [^{3}H] dopamin. ∗P < 0,05 / ∗∗P < 0,01.
Edited after Zhang et al. (2005)[100]

5.4.3 Phenotypes of microglia

To understand the role of microglia in neurodegeneration, it is important to know their phenotypes.
Under physiological conditions the microglia is in a homeostatic state, where they constantly survey and monitor pathological changes in their enviroment. Once a harmful stimuli compromising the neurological system, microglia cells switch to a temporarly reactive state.[102]

The M1 microglia is reffered to the inflammatory phenotype, as it releases pro-inflammatory cytokines and reactive oxygen species. This response is important to remove pathologic material, however, it needs to be eventually downregulated in order to repair the damage. Therefore, a properly regulated immune response results in the transition from the M1 to the M2 phenotype. By releasing anti-inflammatory mediators, M2 microglia inhibits the initial inflammatory response, promotes tissue repairing and restore the homeostasis.[101]

5.4.4 Uncontrolled inflammation and "reactive microgliosis"

"In contrast to the beneficial housekeeping duties of resting and moderately activated microglia, over-activation of microglia resulting in excess production of inflammatory mediators is in fact neurotoxic […]"

(Lull ME & Block ML 2010)[98]

As the insult, in particular α-Syn pathology, is persistent in PD[97], continuous pathological stimulation increases the number of M1 microglia[103], leading to a failed reparative M2 response.[101] The release of a large number of pro-inflammatory mediators without anti-inflammatory mediators as counterparts results in local tissue damage. In response to neuronal injury, damage-associated molecular patterns (DAMPs) are released, which further increase inflammation and microglia activation[101], forming a vicious inflammatory cycle that is referred to as "reactive microgliosis".[99]

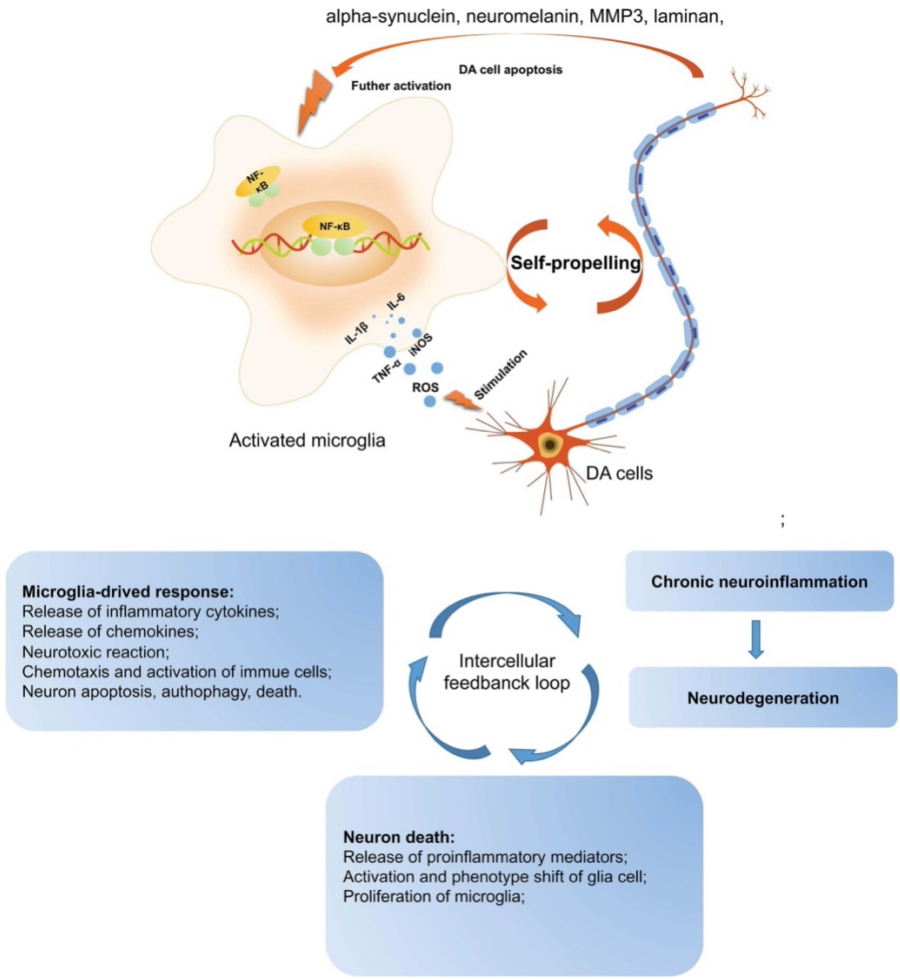

Figure 19: Self-propelled degeneration cycle in PD. Edited after Yao et al. (2021).[103]

6. Conclusio

Reviewing the current literature it can be concluded that the gut-origin hypothesis is supported by much evidence. First, gastrointestinal disorders, in particular obstipation, often precede motor symptoms by up to a decade in PD patients. Second, several animal studies showed that the α-Syn pathology is capable to spread from the ENS to the CNS via the vagus nerve and that vagotomy reduces the risk for PD.

Finally, Stokholm et al. tested tissue samples positive for phosphorylated α-Syn from 22 of 39 subjects taken an average of 7 years before PD diagnosis.

However, a lack of "gut-only" cases in other autopsy studies is considered as a strong argument against the hypothesis. This in turn may be due to a number of reasons.

Most cases in such post-mortem analyses had some degree of α-Syn pathology in the brain and are therefore not eligible for investigation of "gut-only" pathology. Next, one can argue that α-Syn pathology in the ENS is highly localized, especially in the initial phase. Considering the large surface area of the GIT, it would require a large amount of tissue samples from different areas of the GIT to rule out gut pathology with certainty. It is also possible that in some cases, the time window for α-Syn pathology to spread to the CNS is relatively short to identify it as "gut-only".

Therefore, further studies are necessary, which include several different tissue samples taken from patients before the PD diagnosis. Furthermore, the methodology, starting from the localization of the tissue removal to the staining technique, needs to be standardized to provide comparable results.

It is conceivable that not all PD patients follow the same pathway, but that multiple phenotypes exist: body-first and brain-first PD. This is reinforced by the different expression of clinical symptoms in the prodromal or early phase of PD. PD patients with RBD and a higher burden of autonomic dysfunction, such as constipation, orthostatic hypotension, urinary, sexual, and olfactory dysfunction before the onset of motor symptoms are considered to belong to the body-first phenotype. In contrast, the brain-first phenotype displays an asymmetrical onset of motor symptoms.

Regarding the gut microbiome, we are still in the early stages of understanding what impact relative shifts in the microbiome have functionally. In PD patients, a reduction in SCFA-producing species has been observed, which is associated with increased intestinal permeability and inflammation. In vivo data showed that PD patients tend to have a hyperpermeable colon. Forsyth et al. found that sigmoid mucosal samples from PD patients had significantly more intense staining for E. coli, 3-nitrotyrosine, and α-Syn compared with control subjects. In addition, the level of LBP in the plasma of PD patients was significantly lower than that of control subjects, indicating a higher exposure of LPS in the blood. Although these results support the endotoxin hypothesis, it remains unclear whether "leaky gut" plays a causative role in PD or is a consequence of the disease.

While many arguments suggest that Lewy bodies/neurites are only "innocent bystanders" and are even considered as a protective mechanism, the toxicity of α-Syn oligomers has been confirmed in numerous studies. α-Syn oligomers compromise many different intracellular processes and can induce cell death. α-Syn fibrils, on the other hand, have a less toxic effect, but play a major role in the propagation of α-Syn pathology by acting as seeds that trigger the aggreagtion of endogenous monomeric α-Syn.
Another crucial factor in the neurodegenerative process is the α-Syn mediated neuroinflammation. A persistent insult, such as α-Syn aggregates in PD, leads to continuous pathological stimulation and increases the number of pro-inflammatory M1 microglia, which is a key regulator of neuroinflammation. Under physiological conditions, the insult would eventually be removed and the reparative response of M2 microglia could be initiated. Since this is not the case here, the counter mechanism fails, which result in neuronal injury that triggers the release of DAMPs. These further amplify inflammation and activation of M1 microglia, eventually leading to a self-propelling degenerative cycle.
From this I conclude that neurodegeneration in PD, but also in other neurodegenerative diseases, is the consequence of a pro-inflammatory vicious circle from which the body can not escape on its own. Future therapies should therefore aim to interrupt this cycle, either by eliminating the α-Syn aggregates or by delivery of exogenous anti-inflammatory mediators that will induce a transition from M1 to M2 microglia.

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