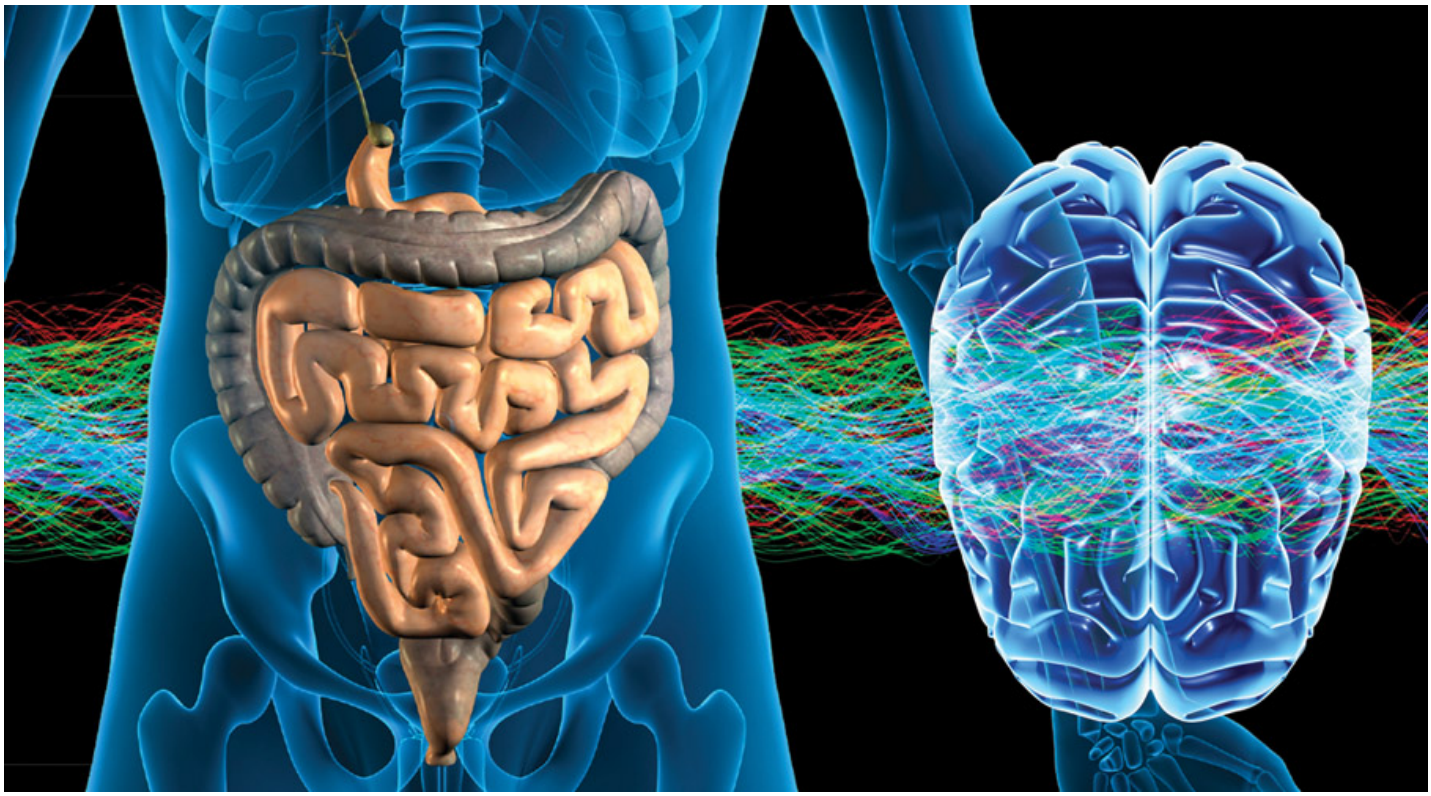
The role of the gastro-intestinal microbiota on the onset and progress of Parkinson's disease



Bachelor thesis
Date: 03-11-2017
Faculty of Sience and Engineering
Author: Bine Schoenmaker; S2756404
Supervisor: Prof. Dr. Ody Sibon

The picture on the front is from *[37]*.

Summary

Parkinson's disease (PD) is the second most common neurodegenerative disease in de world. By 2030, it is estimated that around 10 million people will suffer from PD. PD is characterized by the accumulation of Lewy bodies in the central nervous system (CNS), especially in the dopaminergic neurons of the substantia nigra. The main component of Lewy bodies is aggregated α-synuclein. Patients suffering from PD show several motor symptoms such as tremor, rigidity, bradykinesia and PIGD (postural instability and gait difficulty). Until now, there is no effective treatment available to cure PD. Besides that, PD is often diagnosed when the first motor symptoms occur. Braak et al., proposed in a time line for PD that before motor symptoms occur, already an abnormal α-synuclein aggregate deposition takes place in the gastric myenteric plexus in the enteric nervous system (ENS). Subsequently they suggest that this α-synuclein aggregates are transported to the CNS by retrograde axonal transport via the vagal nerves. Moreover, research has shown intestinal dysbiosis of the microbiota in the intestine before onset of PD. Therefore, a way to earlier diagnose PD would be a helpful approach in terms of being able to administer treatment to patients before the onset of PD motor symptoms. To decipher this, it is required to know how, why, and by what factors the α-synuclein aggregates develop in the ENS. Recently, research has shown that dysbiosis of the intestine microbiome might be important for the onset of PD. Therefore, the question of this thesis is 'What is the role of the gastro-intestinal microbiome in the onset and progress of Parkinson's disease?'. Literature research shows that the answer on this question is a complex interplay between several factors. For example, as mentioned, dysbiosis of the intestinal microbiota, as well as intestinal permeability and a pro-inflammatory environment.

Index

Introduction

For the past years, there has been a large amount of research concerning neurodegenerative diseases to decipher the pathological features and find an effective therapy to decrease their progression, but still for most neurodegenerative diseases both the pathophysiology and effective therapies are largely unknown. Examples of neurodegenerative diseases are Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease and amyotrophic lateral sclerosis (ALS). They all have in common that they are characterized by the accumulation of incorrectly folded proteins in the brain and that their prevalence becomes higher with increasing age *[1]*.

It is estimated that by 2030, there will be around 10 million people suffering from Parkinson's disease. PD is therefore the second most common neurodegenerative disease after Alzheimer's disease *[2] [3]*. It is predicted that PD affects 1% of the individuals over the age of 65 years and that the age-adjusted prevalence is around 50-175 per 100.000 persons *[4]*.

External symptoms shown by PD patients are mainly motor symptoms such as tremor (continuous shaking movement of a body part), rigidity (stiffness), bradykinesia (slow movements) and PIGD (postural instability and gait difficulty). Motor symptoms are mainly caused by the loss of dopaminergic neurons in the substantia nigra (see figure 1). Though, there are more regions involved in the neuropathological changes of PD. For example, the autonomic nervous system, olfactory structures, the lower brain stem and the cerebral cortex are also involved in PD pathology *[5]*.

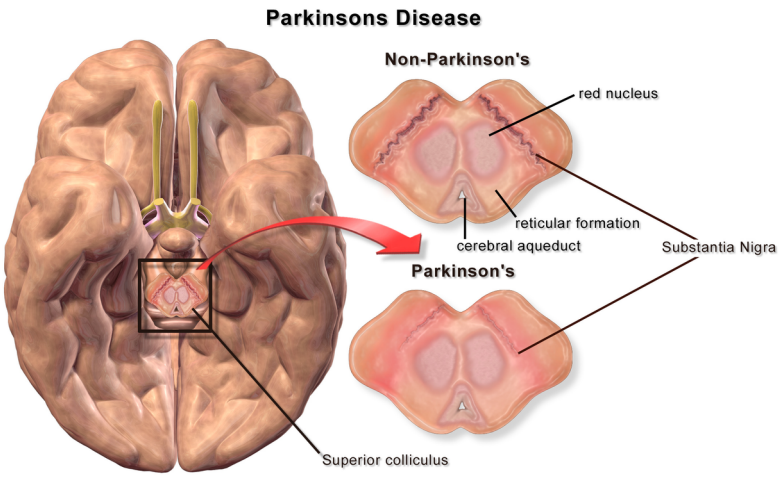


Figure 1 [36]

Symptoms of PD pathology as seen within the body are mainly the Lewy bodies. The Lewy bodies' main component is aggregation and phosphorylation of the **α**-synuclein protein. **α**-synuclein aggregates are thought to be the first step in the onset of the neuronal loss observed in PD. This neuronal loss is caused mainly by the loss of dopaminergic neurons in the substantia nigra and is being held responsible for the neurological symptoms in PD *[6] [7]*.

Braak et al. proposed six stages for the progression of PD. They proposed that in stage 1 there is abnormal **α**-synuclein deposition in the gastric myenteric plexus, part of the enteric nervous system (ENS). Stage 2 is characterized by the involvement of the coeruleus/subcoeruleus complex and from stage 3, typical PD features are seen such as tremor (continuous shake movement caused by involuntary muscle contractions), rigidity (stiffness) and bradykinesia (slow movements). Stage 4, 5 and 6 are primarily characterized with severe and widespread cortical damage. By proposing this stages for PD progression, Braak and his colleagues propose that PD pathology finds its onset in the ENS and then spreads to the CNS *[8]*.

For the origin of PD, several genetic and environmental factors that contribute to the onset of the disease have been identified but still it remains to be deciphered how PD exactly originates *[9] [10]*. Examples of environmental factors are certain pesticides and fungicides, for instance paraquat, maneb and rotenone. Also heavy metals such as cadmium, lead, mercury, arsenic, manganese and iron are identified as environmental factors that are able to induce PD *[11]*.

Another example of an environmental factor that plays a role in PD is the GMB (gut microbiota). Microbiota is defined as the human symbiotic microorganisms that colonize the body, especialy bacteria, but also viruses, protozoa, archaea and fungi *[12]*. The intestinal microbiota is found to have multiple functions in the body. The GMB play a role in the absorption of nutrients, vitamins, medications, toxic compounds and also influence the immune system *[13] [14] [15] [16] [17] [18]*. The GMB as well has a function in maintaining human health, such as anti-microbial protection, immunomodulation and the regulations of the host-epigenetic machinery *[Marizzoni et al. 6,7,8,9,]*. Moreover, the GMB is found to influence brain activity, behaviour and levels of neurotransmitter receptors and neurotrophic factors *[19] [20] [21] [22]*.

In most cases of PD, the patients are diagnosed and receive treatment after neurological symptoms have started. The treatment used for decreasing PD progress is mostly with levodopa (Ldopa). Ldopa is a pharmacological treatment and currently the most effective one for PD. Although it is the most effective treatment known, using Ldopa has some side effects such as involuntary movements (dyskinesias) and nausea *[23]*. Although levodopa is an effective treatment, its efficacy decreases with PD getting in a more advanced stage. Moreover, every patient with PD eventualy develops the most severe form of PD so Ldopa is not a cure for PD. Therefore, a way to diagnose PD and start treatment before the onset of the neurological symptoms that indicate neuronal loss and an important neuronal dysfunction and motor symptoms, would be a helpful approach in diagnosing PD *[24]*. The GMB might be helpful for an earlier diagnosis of PD since gastrointestinal disturbances are a common symptom in PD and often antedate earlier, up to several years, than the motor symptoms *[25]*.

Recently, evidence was presented that the gastro-intestinal microbiome may contribute to the onset of neurodegenerative diseases such as PD. It was shown that the intestinal microbiome has an effect on the brain via the gut and microbiota. *[26].* Also, often there is a gastro-intestinal dysregulation seen few years before the onset of PD *[27].* For example, **α**-synuclein appearance in the ENS (enteric nervous system) seems to be a frequent and plausible premotor sign of PD *[27] [28]*.

The ENS is the neuronal network of the digestion system. The ENS is organized into two plexuses, the myenteric plexus and the submucosal plexus. The myenteric plexus is involved in controlling smooth muscle activity, while the submucosal plexus plays a role in the regulation of the secretion and the microvasculature *[29] [30] [31]*.

α-synuclein lesions in the ENS seem to be appearing early in PD, since they have been observed in cases with advanced PD, but also in cases with non-symptomatic PD. Also, it is unlikely that the lesions in both de ENS and CNS develop independent from each other. It is more likely that a joint pathological event takes place which triggers the misfolding and aggregation.

Furthermore, an anatomical assay strengthens this thought by showing that the ENS and the CNS are interconnected with each other via long-axonal nerve cell types. This demonstrates that there is a route present for neurotropic pathogens to enter the gastric epithelial lining, induce misfolding and aggregation of **α**-synuclein and then affect the ENS and the CNS via projection neurons with long axons (Figure 2) *[27].*

In addition, in the CNS, early **α**-synuclein aggregates are mostly found in the structures needed for parasympathical innervation to the gut, which again suggests a clear link between PD and the gut. Moreover, gut microbiota are found to influence the activity of the ENS neurons which might also possibly affect cellular alpha-synuclein secretion. All this results enforce the thought that the vagal nerve is a possible route for the spread of **α**-synuclein neuropathology form the ENS to CNS. *[32] [33] [34] [35]*.

Given this information, an interesting link is suggested between the onset and progress of PD and de gastro-intestinal microbiome. Therefore, the main question of this thesis is: 'What is the role of the gastro-intestinal microbiome in the onset and progress of Parkinson's disease?'.

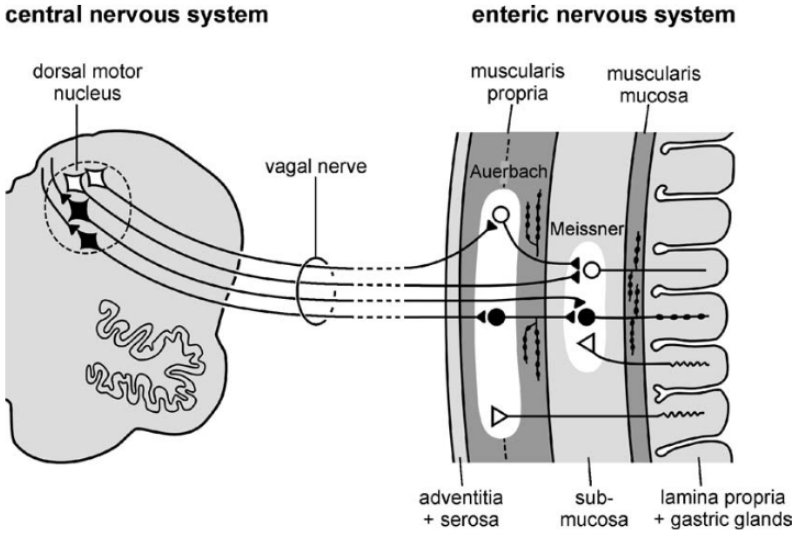


Figure 2 Figure which shows the proposed connection between the ENS and the CNS. Also, Meissner's and Auerbach's plexus are shown here [27].

Results from literature

There are several research papers that show a link between the gut microbiome and PD. One of the first researchers proposing this link was Heiko Braak. Braak and his colleagues proposed that the Lewy pathology seen in PD, primes the ENS and subsequently spreads to the brain. They suggested an active retrograde transport of α-synuclein via the vagal nerve. Braak et al. also investigated that Lewy pathology appears in the neurons of the ENS long before the Lewy pathology is present in the dopaminergic neurons of the midbrain and typical PD motor symptoms such as tremor and rigidity show up. This gap between stage 1, which is characterized by abnormal α-synuclein deposition in the gastric myenteric plexus in the ENS, and stage 3, which is characterized by the emerge of typical PD features like tremor, rigidity and bradykinesia, can take many years *[6] [38]*.

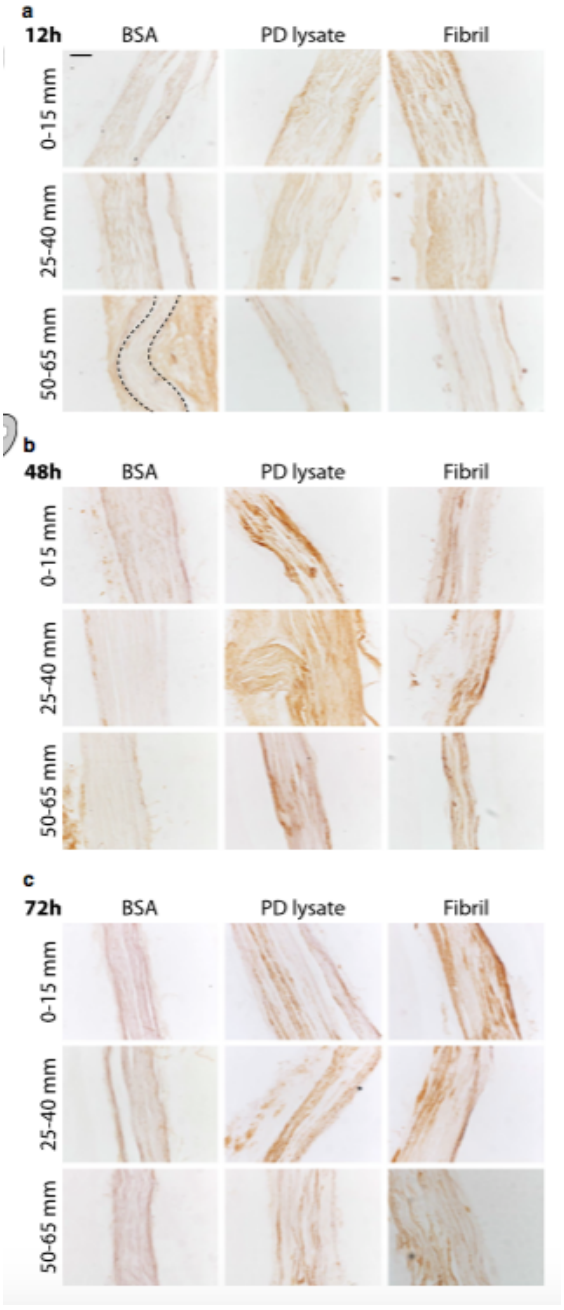


Figure 3. The time-dependend distribution of α-synuclein trough the vagal nerve. On the left: vagal nerve segments of the BSA injected rats, in the middel: vagal nerve segments of the PD lysate injected rats, on the right: vagal nerve segments of α-synuclein injected rats. An immunostaining for human α -synuclein was used [38]..

Another example is the research done by Holmqvist et al. They experimentally tested the hypothesis proposed by Braak et al. In the research done by Holmqvist et al., human PD brain lysate was injected into the intestine wall of rats. This lysate contained different forms of α-synuclein and was obtained from the substantia nigra from neuropathologically confirmed PD patients. Immuno-histological analysis was used to confirm the presence of α-synuclein positive Lewy bodies and the Lewy-positive ones were used for the brain lysate preparation. This lysate was injected into the intestine of wildtype adult rats. They used 2 other groups of rats, one injected with α-synuclein fibrils and one injected with BSA (bovine serum albumin) as a control. Holmqvist et al. hypothesized that this α-synuclein from the brain lysate reaches the dorsal motor nucleus of de vagus in the brainstem in a time-dependent manner after injection into the intestine wall. They found in this research that different α-synuclein forms can be distributed from the gut to the brain over a long distance via the vagal nerves in a time-dependent manner. This can be seen in figure 1. The darker the

staining, the more α-synnuclein. The result of their research contributes to the hypothesis of Braak et al. that PD may originate from the periphery and spreads to the brain where it causes the typical PD symptoms. *[38]*.

Since the discovery that Lewy bodies are found in the intestinal enteric nerves, Forsyth et al. hypothesized that the intestine might be an early place for the start of PD in response to environmental factors such as toxins or pathogens that might be secreted by the gut microbiota. They investigated this by examining the intestinal permeability of patients recently diagnosed with PD compared to healthy people. Also, Forsyth and his co-workers made intestinal biopsies from both groups to be able to apply immunohistochemistry. By using immunohistochemistry, the bacterial translocation, oxidative stress and α-synuclein could be determined and it could also be seen if the increased permeability of the intestine was associated with an increased translocation of intestinal bacterial products. Also, serum markers of endotoxin exposure, for example LPS binding protein (LPB) could be measured. They found that PD patients show a significant higher intestinal permeability compared to healthy controls *[figure 2]*. They also found this intestinal hyper permeability to be correlated significantly with increased intestinal mucosa staining for E. coli bacteria, oxidative stress and α-synuclein and decreased serum LPB levels in PD patients *[figure 3]*. In samples from PD patients, there was found a significantly more intense staining of E. coli in the *lamina propria* zone compared to controls. The increased intestinal permeability is correlated with intestinal endotoxin (LPS) exposure, which is indicated by the increased intestinal mucosal staining for *E. coli* and the decreased level of serum LPS.

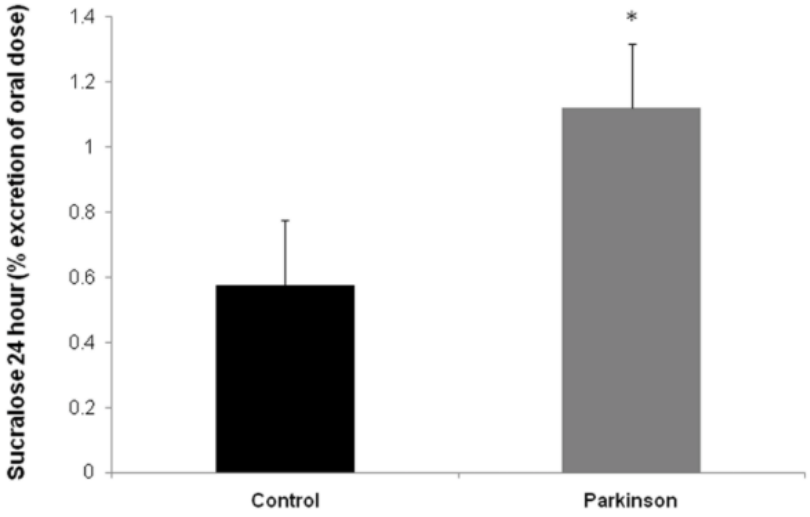


Figure 2. Urinary sucralose is used for measuring the intestinal permeability. PD patientes show higher urinary sucralose levels, suggesting a greater intestinal permeability [39].

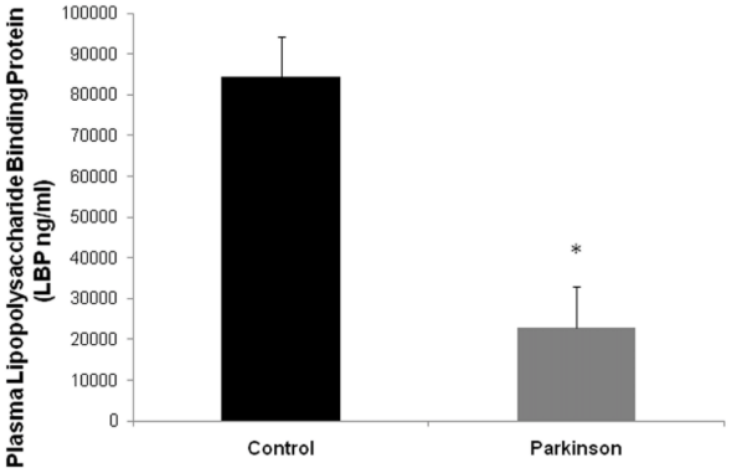


Figure 3. In this figure is shown that plasma LPB is significantly lower in PD patients. The level of LPB is an indirect measure of systemic endotoxin exposure and is used as an indicator of intestinal permeability. Lower levels op plasma LPB are associated with increased exposure to gram negative bacteria, which is an indicator of a greater intestinal permeability [39].

This suggests higher translocation of intestinal bacterial products. Also, a significant correlation between the urinary sucralose, which is used to measure intestinal permeability, and the intensity of the E. coli staining was found. The greater intestinal permeability might be the cause for the translocation of bacterial products such as LPS (known as endotoxin). The researches also investigated whether the increased intestinal permeability to inflammatory bacterial products measured in PD subjects was correlated with the intestinal markers for PD and oxidative stress. They found that the PD subjects displayed significantly more staining for E. coli, α-synuclein and nitrotyrosine (oxidative stress staining) compared to the stained controls. The researches as well found that the increased intestinal permeability and E. coli staining correlated significantly with the α-synuclein staining in PD samples, but not in the control samples. They suggested that a pro-inflammatory environment is created by this greater intestinal permeability. To summarize, the researchers suggested that this greater intestinal permeability they found in PD, in combination with a genetic sensibility for PD might be a crucial early step for the promotion of the pro-inflammatory environment, which contributes to the start and progression of PD. An important consequence of the increased intestinal permeability is the translocation of bacteria (E. coli) and bacterial products such as LPS (endotoxin). Both contribute to the development of a pro-inflammatory environment. This results also enforce the hypothesis that the GI tract can act as a 'gate' for PD pathogen, which than triggers pathological changes in the submucosal/myenteric neurons of the ENS, and spreads via the vagus nerve to the medulla oblongata, as proposed by Braak et al. It also suggests that the increased permeability of the intestine in PD contributes to the spread of α-synuclein from the gut to the CNS. *[39]*.

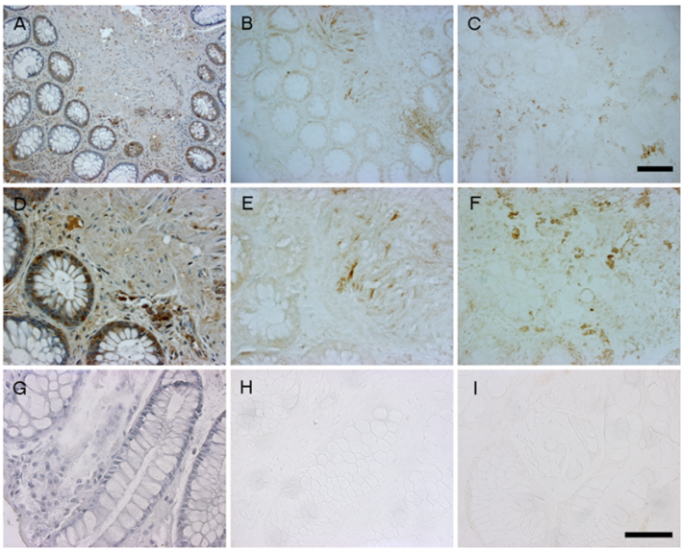


Figure 4. This figure shows the immunohistological staining of intestinal biopsies. A,B,C,D,E,F are biopsies from PD, G,H and I are controls. A,D and G are stained for E.coli. B, E and H are stained for α-synuclein. C, F and I are stained for nitrotyrosine. PD biopsies are found te be stained significantly more for all three stainings [39]

Another research paper is by Scheperjans et al. Given that, recently, research has shown that intestinal microbiome interacts with both the autonomic and central nervous system, by, among other things the enteric nervous system (ENS) and the vagal nerve, they investigated the microbiome in PD compared to the microbiome of healthy subjects. The researchers hypothesized that the faecal microbiome of PD patients differs from the faecal microbiome of control subjects as to bacterial diversity or taxonomic composition. They compared the faecal microbiomes of 72 PD patients with 72 control subjects. The outcome of this research was that

the mean abundance of *prevotellaceae* in the faeces of PD patients was reduced by 77,6% compared to controls. Also, a positive connection between the amount of *enterobacteriaceae* and PIGD (postural instability and gait difficulty) symptoms was found. PIGD is a typical symptom for PD. The researchers found this decreased abundance of *prevotellaceae* to be in line with some characteristics such as increased intestinal permeability. Low *prevotellaceae* levels have been found as an indication for decreased mucin synthesis, which is linked to an increased intestinal permeability. Also, high abundance of *prevotellaceae* is found to be associated with a high capacity for the biosynthesis of thiamine and folate (both vitamins). In PD, low levels of these vitamins are found. Therefore, low abundance of *prevotellaceae* is in line with this finding *[32] [40] [41] [42] [43] [44]*. This research suggests a different GMB composition in PD compared to healthy subjects, which might indicate some kind of GMB dysbiosis taking place in PD.

Moreover, there has been more research that suggested a GMB dysbiosis to be a trigger for PD. Dysbiosis means that there are unfavourable changes in the composition of the microbiota. Results suggesting this come from a study in humans that showed an increased abundance of butyrate producing bacteria. Larger quantity of butyrate producing bacteria are thought of as anti-inflammatory, such as *Blautia, Coprococus* and *Roseburia*, and more abundant in faecal samples from healthy subjects compared to PD patients. Also, in PD, a higher amount of *Ralstonia* is found in the mucosa. *Ralstonia* is considered as pro-inflammatory *[45]*. This results suggests that GMB dysbiosis is taking place in PD patients that is among other things causing a pro-inflammatory environment.

There has been more research showing the connection between PD and the GMB. For example, there has been a study with an α-synuclein overexpressing mouse model. Antibiotic treatment of this mouse model improved motor defects, microglia activation and α-synuclein aggregation while the administration of microbial metabolites made these features getting worse. The researchers found this to be prove for the key role of the GMB in PD, since the amount of α-synuclein aggregates improved with the use of anti-biotics which ‘turns of’ the GMB *[46]*.

Conclusions and discussion

To come back to the research question of this thesis: 'What is the role of the gastro-intestinal microbiome on the onset and progress of Parkinson's disease?', it is possible to conclude that in PD the intestine and its microbiota definitely play an important role in the onset and progress of the disease. As proposed by Braak et al. in 2005 and researched by Holmqvist and his colleagues, it seems that α-synuclein is retrogradely transported from the ENS, via the vagal nerve, to area's in the CNS. This was proven by the research done by Holmqvist et al., in which they showed that different α-synuclein forms can be distributed from the intestine to the CNS over a long distance via the vagal nerves. Also, as shown by Forsyth et al., there is a higher intestinal permeability in PD. They found this to be linked to a higher staining for *e. coli,* oxidative stress, α-synuclein and serum LPB (LPS binding protein,) and also found this to correlate with markers of increased exposure to endotoxin and an oxidative stress marker. This implicates that the greater intestinal permeability might be the cause for greater translocation of bacterial products and LPS (endotoxin) which contribute to a pro-inflammatory environment. This pro-inflammatory environment then contributes then again to a more permeable intestine, which all together contribute to the onset and progression of PD. Furthermore, this implicates that the exposure to endotoxin and oxidative stress might be the cause for the pathological accumulation of α-synuclein in neurons of the ENS.

A group of researchers that investigated the possible pathophysiological route of gut microbiota in PD is the group of Marizzoni et al (see figure 5). With the help of other research showing different compositions of the PD microbiome compared to the healthy microbiome, they suggest that GMB dysbiosis is a possible factor playing a role in causing a pro-inflammatory environment in the intestine and a greater intestine permeability. Subsequently, this will lead to α-synuclein aggregate deposition in the ENS and subsequently α-synuclein will possibly be transported via the vagus nerve to the CNS *[47]*. How exactly the pro-inflammatory state leads to the aggregation and deposition of α-synuclein remains to be deciphered, but as mentioned research has shown that the GMB

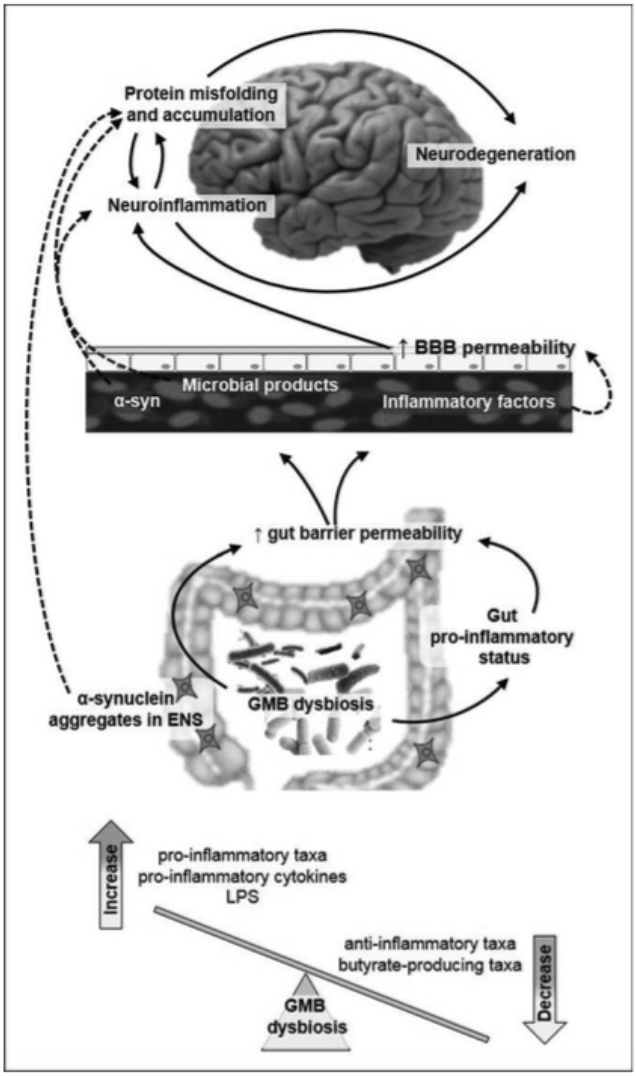


Figure 5. Proposed pathofysiological route of the onset of PD from the intestine, as proposed by Marizzoni et al. [47]

has an influence in this process since antibiotic treatment improved PD symptoms, microglia activation (indicator for a pro-inflammatory state) and α-synuclein aggregation. This transportation of α-synuclein from the ENS to the CNS is made possible by both an inflammatory gut status and a greater intestinal permeability, caused by GMB dysbiosis. Because of the inflammation and greater intestinal permeability, α-synuclein is then able to be transported from the ENS to the CNS. How exactly these α-synuclein aggregates develop in this conditions, remains to be deciphered *[47]*.

Also, an interesting question is whether the increased intestinal permeability is causal to the pro-inflammatory status in the gut, or if the pro-inflammatory status is causal to the higher intestinal permeability. It has been suggested that a neurotoxin absorbed by the body is able to permeate the gastrointestinal mucosa and reaches the ENS were it can stimulate the aggregation of α-synuclein and subsequently reach the vagal nerves to and be transported to the CNS. In this case, this neurotoxin would be the first pathological event leading to the PD development. Alternatively, it has been discovered that the severity and frequency of gastrointestinal symptoms increased with further progression of the disease, which suggests that the involvement of the gastrointestinal symptoms might be dynamic over the progression of the disease *[48]*. The truth about the gastrointestinal symptoms as a primary pathological event is probably found in the middle of both.

Concluding about the influence of the GMB on the onset and progress of PD, it is possible to say that it is a complex interplay between different factors such as gastrointestinal permeability, a pro-inflammatory environment, GMB dysbiosis and a genetic aptitude which probably all in some way induce α-synuclein aggregation and therefore influence the progress and onset of PD. Subsequently α-synulcein aggregates are transported to the CNS via the vagal nerves with retrograde axonal transport. The function of the GMB in this process is inducing the pro-inflammatory state and the intestinal permeability which both lead to α-synuclein aggregate deposition.

Moreover, GMB symbiosis is mentioned in some of the articles as an early biomarker for the onset of PD. Indeed, in some studies, for example the study from Scheperjans and his colleagues discussed in the result section, show a different composition in faecal samples from PD compared to healthy subjects. However, the reduction in *prevotellaceae* and the increase in *enterobacteriaceae* found in among other things the research of Scheprjans et al. *[40]* and some other studies done in de E.U., was not found in studies done in the U.S. A possible explanation for this might be differences in dietary habits between the U.S. and the E.U. Therefore, it is difficult to indicate one specific microbiota species that suggests the onset of PD. It might however be possible to indicate the individual GMB composition and from there monitor if any dysbiosis takes place.

Also, not every kind of GMB dysbiosis seems to be specific for PD. For example, low abundance of *prevotellaea* was also found in studies about the GMB linked to autism and diabetes type 1 *[49] [50].* Although the GMB dysbiosis might not be specific, it does suggest the relevance of the bacteria in the context of disorders in de CNS.

Although decreased abundance of *prevotellaea* do not seem to be specific for PD, decreased *prevotellaea* has been associated with decreased levels of ghrelin. Ghrelin is a gut hormone that induces appetite. Also, ghrelin regulates nigrostriatal dopamine function and might restrict neurodegeneration in PD. In PD, an impaired ghrelin secretion has been reported. Therefore, ghrelin secretion might be an interesting therapeutical target for the reduction of the progress in PD *[51] [52] [53].*

For the future, more research should be done to investigate how exactly α-synuclein aggregates develop under the influence of a pro-inflammatory environment in the ENS and under the influence of a greater intestinal permeability. If it is known how these aggregates develop in the early stage of PD, before the typical PD symptoms are visible, this might be a possible interesting therapeutic target. If it is possible to delay the progress of PD in an early stage, it might be possible to postpone or reduce the typical motor and neurological symptoms of PD for a longer period of time. It would at least be interesting to see if there is any possibility for a treatment in the early stage of the disease that takes place in the ENS and the intestine, and also to see if any more biomarkers can be found to make an earlier diagnosis possible since this is of course a condition for a possible treatment in an earlier stage of the disease.

Literature

1. *Bagynszky E et al. 2017.* Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis. *J Neurol Sci 367:242-254*
2. *Lees AJ et al. 2009.* Parkinson's disease. *Lancet 373: 2055-2066*
3. *Dorsey ER et al. 2007.* Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology 68: 384-386*
4. *Muangpaisan W et al. 2011.* A systematic review of the worldwide prevalence and incidence of Parkinson's disease. *J. Med. Assoc. Thai. 94(6), 749-755*
5. *Braak H et al. 2003.* Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of ageing. 24:197-21*
6. *Braak H et al. 2008.* Nervous system pathology in sporadic Parkinson disease. *Neurology 70: 1916:1925*
7. *Dickson DW et al. 2009. Neuropathology of non-motor features of parkinson's disease. Parkinsonism Relat Disord. 15: S1-S5*
8. Hawkes CH et al. 2010. A time line for Parkinson's disease. Parkinsonism Related Disorders. *Parkinsonims and related disorders 16(2): 79-84.*
9. *Gasser T et al. 2011.* Milestones in PD genetics. *Mov. Disord, 26(6), 1042-1048*
10. *Elbaz A et al. 2007.* Epidemiologic studies of environmental exposures in Parkinson's disease. *J. Neurol. Sci. 262 (1-2), 37-44*
11. *Ghaisas S et al. 2016.* Gut microbiome in health and disease: linking the microbiome-brain-axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacol Ther. 158: 52-62.*
12. Eckburg PD et al. 2005. Diversity of the human intestinal microbial flora. *Science* 308:1635-1638.
13. Li M et al. 2008. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci USA* 464(7285):2117-2122.
14. Sommer F et al. 2013. The gut microbiota: masters of host development and physiology. *Nat Rev Microbiol* 11:227-238.
15. *Goldsmith* JR et al. 2014. The role of diet on intestinal microbiota metabolism: Downstream impacts on host immune function and health, and therapeutic implications. *J Gastroenterol* 49:785-798.
16. Abt MC et al. 2009. The intestinal microbiota in health and disease: the influence of microbial products on immune cell homeostasis. *Curr Opin Gastroenterol*. 25:496-502.
17. Nicholson JK et al. 2012. Host-gut microbiota metabolic interactions. *Science* 336:1262-1267.
18. Holmes E et al. 2012. Gut microbiota composition and activity in relation to host metabolic phenotype and disease risk. *Cell Metab* 16:559-564.
19. Ubeda C et al. 2017. Roles of the intestinal microbiota in pathogen protection. *Clin Transl Immunol* 6:e128.
20. Ivanov II et al. 2012. Intestinal commensal microbes as immune modulators. *Cell Host Microbe* 12:496-508.
21. Krautkramer KA et al. 2016. Diet-microbiota interactions mediate global epigenetic programming in multiple host tissues. *Mol Cell* 64:982-992.
22. Kumar H et al. 2014. Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. *MBio* 5:pii:e02113-14.
23. Chou KL et al. 2008. Adverse events from the treatment of Parkinson's disease. *Neurol Clin* 26:S65-S83.

24. Christopher B Forsyth et al. 2011. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early parkinson's disease. *PLoS ONE* 6(12): e28032
25. Postuma RB et al. 2012. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord* 27:617-626.
26. *Collins SM et al. 2012.* The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol *10(11): 735-42*
27. Braak et al. 2005. Gastric alfa-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neuroscience letters* 396:67-72.
28. Shannon KM et al 2012. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. *Mov Disord* 27:716-719.
29. Benarroch EE et al. 2007. Enteric nervous system: functional organization and neurologic implications. *Neurology* 69:1953-1957.
30. Neunlist M et al. 2003. Changes in chemical coding of myenteric neurones in ulcerative colitis. *Gut* 52: 84-90.
31. Schemann M et al. 2004. *The human enteric nervous system.* Neurogastroenterol. Motil. 16: 55-59.
32. Cryan JF et al. 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 13: 701-712.
33. Forsythe P et al. 2013. Voices from within: gut microbes and the CNS. *Cell Mol Life Sci* 70:55-69.
34. Kunze WA et al. 2009. Lacobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. J Cell Mol Med 13(8B):2261-2270.
35. Paillusson S et al. 2013. Activity-dependent secretion of alpha-synuclein by enteric neurons. *J Neuroc*hem 125:1051-1059.
36. *Blausen.com* staff *(2014). ''Medical gallery of Blaussen medical 2014''. Wikijournal of medicine 1(2). Doi: 10.15347/wjm/2014.010. ISSN 2002-4436.*
37. Harvey B (2017-10-17). Brain Gut connection – enteric, sympathic and parasympathic nervous system. Retreived from: https://justinhealth.com/brain-gut-connection-nervous-system-podcast-39/
38. Holmqvist S et al. 2014. Direct evidence of Parkinson pathology spread from the gastrointestinal tract tot he brain in rats. *Acta neuropathol* 128(6):805-820.
39. Forsyth CB et al. 2011. Increased Intestinal Permeability Correlates with Sigmoid Mucosa alpha-Synuclein Staining and Endotoxin Exposure Marker in Early Parkinson's Disease. *PLoS ONE* 6(12): e28032.
40. Scherjans F et al. 2014. Gut Microbiota Are Related to Parkinson's Disease and Clinical Phenotype. *Movement Disorders* 3(30):350-358.
41. Arumugam M et al. 2011. Enterotypes of the human gut microbiome. *Nature* 473:174-180.
42. Ou J et al. 2013. Diet, microbiota, and microbial matabolites in colon cancer risk in rural africans and african americans. *Am J Clin Nutr* 98:111-120.
43. Dos Santos EF et al. 2009. Evidence that folic acid deficiency is a major determinant of hyperhomocysteinemia in Parkinson's disease. *Metab Brain Dis* 24:257-269.
44. Luong KV et al. 2013. The beneficial role of thiamine in parkinson disease. *CNS Neuerosci Ther* 19:461-468.
45. Keshavarzian A et al. 2015. Colonic bacterial composition in Parkinson's disease. *Mov Disord* 30:1351-1360.
46. Sampson TR et al. 2016. Gut microbiota regulate motor deficts and neuroinflammation in a model of Parkinson's disease. *Cell* 167:1469-1480.

47. Marizzoni M et al. 2017. Microbiota and neurodegeneratieve diseases. *Curr Opin Neurol* 30(6):630-638.
48. Salat-Foix D et al. 2012. Increased Intestinal Permeability and Parkinson Disease Patients: Chicken or Egg? *The Canadian Journal Of Neurological Sciences* 39:185-188.
49. Kang DW et al. 2013. Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic childeren. *PLoS One* 8:e76993.
50. Brown CT et al. 2011. Gut microbiome metagenomics analysis suggests a functional model fort he development of autoimmunity for type 1-diabetes. *PLoS One* 6:e25792.
51. Queipo-Ortuno MI et al. 2013. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One* 8:e65465.
52. Bayliss J et al. 2011. Gut hormones restrict neurodegeneration in Parkinson's disease. *Advanced Understanding of Neurodegenerative Diseases* 269-284.
53. Unger MM et al. 2011. Postprandial ghrelin response is reduced in patients with parkinson's disease and idiopathic REM sleep behaviour disorder: a peripheral biomarker for early Parkinson's disease? *J Neurol* 4:e7227.

Epilogue

For the past three weeks, I have been working hard on the writing of this thesis and I have learned a lot. Not only about the topic of this thesis, but also about writing a thesis in English, the construction of a thesis and how to separate the information into important and less important for my thesis. Since I had only a short period to write this thesis, it was especially difficult to stop searching for more information to add. I hope that all the persons reading this thesis, enjoy it and, although it might not be their specific interest, find it interesting to read.

Moreover, there are a few persons I would like to thank in this epilogue. Firstly, I would like to thank Prof. Dr. Ody Sibon for being my supervisor for this thesis. I am very happy that she gave me the chance, despite being the only bachelor student writing a thesis in October, to write my thesis and to be my supervisor. Without her willing to do this, I would not have been able to do so.

Next, I would like to thank Yu Yi, who helped me to find this topic for my thesis and supervised me with my bachelor researched. I learned a lot from him, also with relevance for this thesis, such as reading articles more efficiently and writing abstracts.

Also, I would like to thank the study advisor Wouter van Egmond. After my concussion he helped me to find a place where I could write my thesis and arranged this with the exam committee. Without his help, I would also not have been able to write my thesis.