Influence of Commensal Microbiota on the Enteric Nervous System and Its Role in Neurodegenerative Diseases

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Keywords

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Abstract

When thinking about neurodegenerative diseases, the first symptoms that come to mind are loss of memory and learning capabilities, which all resemble hallmarks of manifestation of such diseases in the central nervous system (CNS). However, the gut comprises the largest nervous system outside the CNS that is autonomously active and in close interplay with its microbiota. Therefore, the enteric nervous system (ENS) might serve as an indicator of degenerative pathomechanisms that also affect the CNS. On the other hand, it might offer an entry point for devastating influences from the microbial community or – conversely – for therapeutic approaches via gut commensals. Within the last years, the ENS and gut microbiota therefore have sparked the interest of researchers of CNS diseases and we here report on recent findings and open questions, especially with regard to Alzheimer and Parkinson diseases.

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Introduction

The gastrointestinal (GI) tract harbors a huge amount of commensal bacteria that easily varies depending on the diet, pharmacological intervention, and diseases. Since it is known that the microbiome impacts the development of the central nervous system (CNS) [1] and even might modulate it throughout the life span [2], it is wise to have a closer look at the particular part of the nervous system that is closest to the microbiome, i.e., the enteric nervous system (ENS). Comparison of the ENS with other parts of the nervous system clearly reveals that the ENS is both the largest and the most complex part of the peripheral nervous system. In contrast to the CNS, the ENS is not structured in a compact mass of neurons and glial cells but it is instead organized in distinct networks within the gut wall, where individual, relatively small ganglia are interconnected by dense fiber bundles. Two major ganglionated networks are located in the gut, i.e., the myenteric and the submucous plexuses (Fig. 1). The morphology and ganglionic size vary enormously between both individual segments and species.

This work was conducted at both institutes.

Fig. 1. Brain-gut-axis communication and structure of the enteric nervous system. The brain and the gut interact via different routes and biochemical signatures. A neuronal structure that is in quasi direct contact with the gastrointestinal tract microbiota and contributes to this mutual interplay is the enteric nervous system, which is shown on the right (all depicted microscopic pictures are of murine origin). Gut commensal microbiota can be found in the lumen of the gut (*) but also attached to the villi in close contact with the epithelial cells. Enteric neurons form mesh-like structures

with ganglionic swellings and are located in the myenteric plexus (MP, also Auerbach plexus) between the circular and longitudinal muscular layers or within the submucous plexus (SMP; Meissner plexus) underneath the circular muscle, within the submucous region. The plexus depicted in the scheme are so-called whole-mount preparations, displaying the 3-dimensional character of the networks. Between the plexus pictures a cross-section of a gut villus with the enteric glial cells (EGC) attached to the enterocyte lining is shown.

Moreover, there is a variety of individual neuronal subtypes that comes close to that found in the CNS. Next to cholinergic and nitrergic neurons, there is a high abundance of calretinin- or neuropeptide-expressing [3, 4], as well as catecholaminergic and inhibitory GABA [5] neurons. Interestingly, there are specific bacterial strains that produce, e.g., GABA [6] and short-chain fatty acids [7] or might influence the serotonin production of colonic enterochromaffin cells [8]. All of these metabolites or signaling molecules can affect gut motility, and the corresponding receptors for both GABA and serotonin are spread all over the gut [9]. Interestingly, the majority of the body serotonin (~95%) is produced in the GI tract and can signal via at least 14 different receptors [10].

The neuronal networks positioned in the gut wall form complex neuronal circuits, similar to the CNS, that allow easy communication among the gut lumen, the ENS, and muscle as well as immune cells, and also via the vagus

nerve, with the brain. The ENS not only comprises a broad range of individual neuronal subtypes but it also harbors glial cells. Enteric glial cells differ significantly from the Schwann cells seen in the peripheral nervous system in that they do not form basal laminae [11]. While the enteric glia were for a long time seen as a rather uniform entity, today it is known that they show many similarities to CNS glia, both in morphology and in function. Already in the 80s it could be shown that enteric glial cells express GFAP and thus exhibit astrocyte properties [12]. Moreover, enteric glia respond to inflammation in the same way as CNS-reactive glia, i.e., with an increase in GFAP and the neurotrophic factor GDNF [13]. Enteric glial cells play a central role in maintenance of the mucosal barrier [14] and as such bear another similarity to astrocytes, which play a central role in formation of the blood-brain-barrier.

Enteric neurons express Toll-like receptors (TLR) [15] strongly suggestive of a direct impact of bacterial metab-

olites on the ENS. Interestingly, in TLR4 knockout mice, a delay in GI transit was seen that was comparable to that observed in germ-free (GF) animal models which were studied in parallel [16]. These animals also showed a decrease in nitrergic innervation and, correlated to this finding, a decreased colonic relaxation in response to electrical field stimulation. TLR4 is also expressed in enteric neural stem cells and might be involved in maintenance of the neural stem cell niche within the ENS [17]. Moreover, there is an intense interplay between bacterial lipopolysaccharides (LPS) and the host's Wnt signaling, a pathway that is involved in enteric stem cell regulation [18]. So there is strong evidence that the cross talk between neural and enteric stem cells, and thus the maintenance of the GI epithelial barrier depends on microbial metabolites.

Since it is well known that the microbiome has a strong impact on brain development and homeostasis, this impact might be mediated by the "brain-gut-axis," the interconnection between the gastrointestinal tract and the CNS. Although the term "axis" suggests a paved road between the brain and the gut, there are several potential alternatives. There is one "hard-wire" connection between the brain and the gut, i.e., the vagus nerve (Fig. 1), which connects the midbrain with multiple organs and muscles in the periphery, among them the esophagus, the stomach, and the intestines. Additionally, immune routes are observed that are mediated by cytokines, hormonal pathways represented by the neuroendocrine-hypothalamic-pituitary-adrenal axis, but also neurotransmitters, which are produced by different microbial strains. Thus, the brain-gut-axis is not a mere transportation route but also a potent immune modulator. Moreover, there are interactions between the hard-wired vagus nerve and the immune system [19]. It is now quite obvious that the vagus nerve regulates inflammation in the gut and other peripheral tissues and might thus maintain or restore the local immune homeostasis [20]. Over the last decade, evidence has accumulated that the vagus nerve might even be the transporting element not only for prions but also for pathological peptides that are generated within the gut.

So the ENS works as a relay station between the brain, the immune system, and the microbiome. It can easily be affected by microbiome alterations and lead to gastrointestinal disorders but likewise be involved in neurodegenerative diseases. These strong interactions make it probable that neurodegeneration might be influenced by diet and the microbiome.

Interaction of Microbiota and the ENS

The gastrointestinal mucosal barrier consists of a single epithelial layer, i.e., the enterocyte lining, which protects the human body from the inner/outer milieu of the microbiome, thus avoiding severe diseases. Nevertheless, there are always single microbes translocating the mucosal barrier without generating severe trouble, due to their immediate contact with the intrinsic mucosal immune system. Since the mucosal barrier disintegrates with age [e.g., 21] or disease [e.g., 22], the amount of bacteria crossing the epithelium can increase. The quality of the mucosal barrier is maintained by the protective effect of the ENS, and especially the enteric glia, which are in close contact with the enterocytes. Defects of the ENS, based on developmental (Hirschsprung disease, see below) or, e.g., neurotrophic factor deficits may lead to increased translocation of bacteria [23].

Besides pathological effects due to barrier dysfunction, there are also physiological effects of the microbiome or individual bacterial strains on gut function. Stress-induced gut-motility alterations can be attenuated, for example, by application of a specific *Lactobacillus* strain (JB-1) [24].

A convincing explanation for the impact of commensal bacteria on gut motility was delivered by Muller et al. [25]: based on the finding that GF mice present gastrointestinal disturbances, mice were treated with antibiotics and properties of the gut walls were analyzed. In correlation with a significant dysmotility, lower numbers of muscular macrophages, and decreased levels of bone morphogenetic protein 2 (BMP2) and colony-stimulatory factor 1 (CSF1) were found. Since muscular macrophages modulate enteric neurons via BMP2 signaling and, vice versa, enteric neurons muscular macrophages via CSF1, the authors could elegantly demonstrate an LPS-dependent cross talk between the ENS and the muscular macrophages. A second mechanism for a direct bacterial impact on gut motility is provided by the induction of 5-HT synthesis in colonic enterocytes, which can systemically be liberated and immediately affect the submucous nerve endings [8]. Additional modes of action by bacteria that produce short-chain fatty acids can be hypothesized.

Independently of their functional impact on gastrointestinal motility, bacteria do evenly influence ENS development and homeostasis. In GF mice, the ENS is compromised, especially in those areas where bacteria are normally found. While Anitha et al. [16] demonstrated a significant nitrergic deficit in the colon of adult GF mice,

Collins et al. [26] showed a general reduction in myenteric plexus density in both the jejunum and the ileum but not in the duodenum of postnatal (p3) GF mice. Interestingly, the relative number of nitregic neurons was higher in the GF mice than in conventionally raised controls. The commensal microbiome supports the postnatal establishment of the ENS and takes part in its homeostasis in adult life. Kabouridis et al. [27] demonstrated that an enteric glial subpopulation in the submucous microenvironment is highly dependent on stimulation by commensal bacteria. Mucosal enteric glial cells were continuously renewed and their homeostasis was triggered by the gut microbiome. Also the numbers and quality of neural stem cells within the gut wall are variable in different gut segments. While specific nestin-positive neurons were mainly found in the colon, within a potent microbiotic environment, neuron-glial intermediate cells were preferentially encountered in the proximal intestine [28].

Increasing evidence shows that neurodegenerative diseases, such as Parkinson disease (PD), might even originate from the gut [29] and spread via the vagus nerve to the brain. Therefore, the possible correlation between the disabled function of enteric neurons, microbiota, and CNS diseases needs to be analyzed in depth in the future.

Neurological Disorders of the Gut

Since the gut harbors a “second brain,” i.e., the ENS, it is likely and quite obvious that this “brain” may be similarly affected whenever the CNS is subject to neurodegeneration or other systemic diseases. The gastrointestinal tract and the ENS therefore need to be included in our general perspective of neurological diseases. Neurological disorders of the gastrointestinal system can: (1) manifest as defects of gut innervation itself, (2) be correlated with general neurological disorders, or (3) present as neurological features occurring in certain gut disorders along with other symptoms.

Defects in Gut Innervation

The CNS is a very delicate part of the body that is prone to trauma and degeneration and susceptible to systemic diseases throughout life. The risks are even higher during embryogenesis, so developmental failures can occur. This happens not only to the CNS but also to the ENS, where a very complex migration route and procedure leads to well-developed and interconnected networks within the gut wall. The lack of migration or differentiation may result in hypo- or aganglionic innervation of the GI tract,

which can manifest in diseases such as achalasia or Hirschsprung disease.

Achalasia is characterized by lack of peristalsis in the esophageal body and failure of relaxation of the lower esophageal sphincter. It has been discussed whether the problem of innervation rests in the dorsal motor vagal nucleus, the vagus nerve itself, or the intrinsic innervation of the esophagus. Esophageal biopsy or resection shows an almost complete loss of ganglion cells and inflammatory reaction [30].

Hirschsprung disease is a developmental disorder which presents at, or soon after, birth, is more prominent in males (4:1 compared to females), and occurs as a rare disease with a live birth ratio of 1/5,000 [31, 32]. Typical features of Hirschsprung disease are constipation and gaseous abdominal distension. Morphologically, a narrowed aganglionic distal segment of bowel with a length varying between ultra-short and total aganglionosis (Zülzer-Wilson syndrome) [33] is found. The defects are a result of incomplete migration of neural crest-derived stem cells along the gut axis. The underlying mechanism is multigenic and many different genes that promote the survival, proliferation, differentiation, and migration of enteric neural crest cells are involved [for a concise review, see 34].

Hirschsprung disease patients are prone to increased bacterial translocation and thus present a high risk of developing a severe life-threatening enterocolitis (Hirschsprung-associated enterocolitis). First studies have shown that Hirschsprung-associated enterocolitis is closely related to disturbance of the intestinal microbiota [35].

Gastrointestinal Disorders Caused by Neurological or Systemic Diseases

Gastrointestinal function can also be compromised in relation to traumatic alteration of the brain (stroke) or by neuronal deficits induced by metabolic diseases such as diabetes. Neurogenic dysphagia may arise from defects in either motor or sensory components and involve cortical areas concerned with swallowing, the respective efferent pathway, the brain stem motor or sensory nuclei, the lower cranial nerves in their distal course, their neuromuscular junctions, or muscular components of the swallowing pathway. A major cause of dysphagia is stroke, with nearly 50% of patients being affected. However, a wide variety of other neurological diseases, such as PD, multiple sclerosis, Guillain-Barré syndrome, amyotrophic lateral sclerosis, and myasthenia gravis, also lead to dysphagia. Next to these neurological causes, dysphagia also can result from muscular dysfunction, which will be not addressed in this review.

The mechanism underlying abnormal gut peristalsis is dysfunction of the autonomic innervation of the gut. One example is gastroparesis in diabetes mellitus, where the continuous metabolic syndrome leads to neuronal cell death and decreased numbers of interstitial cells of Cajal [36].

Primary Gut Disorders Associated with Neurological Disease

Malabsorption is one of the main reasons why gut diseases may lead to neurological symptoms. Examples are vitamin B₁₂, nicotinamide, and thiamin deficiencies. Endemic pellagra – a disease no longer seen in developed countries – is linked to dietary nicotinamide deficiency and associated with dementia as the neurological symptom. Coeliac disease, which is characterized by malabsorption, abnormal small bowel mucosa, and intolerance to gluten, leads to neurological manifestations in up to 36% of adult patients [37]. Affected structures of the CNS include, e.g., the thalamus, the caudate nucleus, the putamen, the amygdala, the anterior hypothalamic nuclei, and the substantia nigra, as well as various cranial nerve nuclei. Ulcerative colitis and Crohn disease, both inflammatory bowel diseases, may lead to thromboembolic complications and thereby evoke neurological consequences. Moreover, peripheral neuropathy in the form of an acute or chronic inflammatory demyelinating polyneuropathy has been found in both diseases [38].

These examples clearly indicate that the nervous system of the gut is vulnerable to neurological disorders but also that gut function may contribute to neurological malfunction inside and outside of the gut. An obstacle to understanding cause and consequence relates to the fact that pathological constitution is determined not only by the host tissue itself but also by the huge number and variability of gut commensals, i.e., the microbiota. Profound knowledge of the underlying pathomechanisms, microbial metabolites, or the host's response to them is still lacking, although the research landscape has widened over the last decades and now also the typical CNS neurodegenerative diseases such as PD are being considered as having a potential influence on the gut.

Impact of Gut Microbiota on Neurodegenerative Diseases

The term “neurodegenerative diseases” covers – in our contemporary understanding – a range of conditions which primarily affect neurons in the human brain. As de-

Fig. 2. Current number of publications on Alzheimer/ Parkinson disease and microbiota. Data are based on a PubMed search (<https://www.ncbi.nlm.nih.gov/pubmed>) performed on September 28, 2017, using the terms “Alzheimer’s disease” and “microbiome” or “microbiota” or “gut bacteria”; the same applies for Parkinson disease. Irrelevant data sets are those that, e.g., contain Parkinson or Alzheimer in the author list but do not match the content. Observed vs. expected rates were calculated assuming 10% irrelevant data sets and the mean proportion of reviews for the search criteria “Alzheimer” or “Parkinson” (19 and 17%). $p < 0.0001$.

scribed above, however, also gut enteric neurons may suffer from degenerative processes and pathological conditions may affect both the brain and the gut. Therefore, it is intriguing to investigate whether the gut and its commensals can serve as indicators for degenerative processes and/or might contribute to the pathogenesis of brain diseases. Although a wide variety of CNS neurodegenerative disorders is known, the focus of this review will be on 2 of the most common disorders: PD and Alzheimer disease (AD).

Our first step consisted of screening the literature published so far on AD and PD and microbiomes. The results of our screening revealed a steep increase in the number of publications but, surprisingly, also a tremendous misbalance between the available data and reviews on the scarce facts; as such, review papers outnumbered original papers by a factor of 2 (Fig. 2). Although some relevant investigations lacking the respective key words might not have been included, we feel that there is a pressing need for facts before it can unambiguously be decided whether our “gut feeling,” i.e., that commensals might indicate, prevent, or contribute to neurological diseases of the brain, has a real basis. Nevertheless, we here present the data available so far for both diseases.

Parkinson Disease

PD affects approximately 1% of individuals older than 60 years and is characterized by 2 major neuropathological findings: a loss of pigmented dopaminergic neurons of the substantia nigra pars compacta and the presence of Lewy bodies and Lewy neurites with abnormal α -synuclein filaments. For many years, PD has been considered a movement disorder; however, in recent years a wide range of nonmotor features have increasingly been addressed. For example, the so-called PD dementia may occur in proportions as high as 70% [39]. In addition, disturbances of gut function such as constipation have been described in patients [reviewed in 29]. Using the Rome III questionnaire, Mertsalmi et al. [40] investigated the incidence of inflammatory bowel syndrome-like symptoms in 74 PD patients and 75 healthy controls. These symptoms were found to be elevated in 24.3% of the PD patients versus 5.3% of controls. Another example of a potential interplay between the gut and PD relates to the reduced risk of colorectal cancer as reported by 2 studies with high numbers of patients and matched controls [41, 42]. The assumption that PD correlates also with intestinal dysbiosis was recently tested in a mouse model of the disease: mice that overexpressed α -synuclein displayed decreased pathophysiological signs upon antibiotics treatment [43]. This could be reverted by recolonialization. Interestingly, α -synuclein-overexpressing mice that were raised GF even showed enhanced physical impairment upon being recolonized by fecal microbiota obtained from PD patients as compared to those recolonized with a “healthy” microbiome. As far as changes observed in human PD patients are concerned, the results are conflicting, which might be due to differences in sample collection, preparation, and analysis. One example where those discrepancies are obvious is the family Prevotellaceae: 2 reports describe a decreased number in fecal samples of PD patients [41, 44], whereas 2 other studies report no significant deviation from healthy controls [45, 46] and 1 investigation describes even elevated numbers of this family in PD patients [47]; however, samples here were taken from the oral cavity. An example where the literature seems to be more consistent is the family Lactobacillaceae, with at least 3 reports showing elevated numbers [45–47]. This is of interest because enhanced stress-coping has been attributed to Lactobacillaceae in humans and animal models of psychiatric diseases [e.g. reviewed in 48]. Why the changes in the microbiome occur in PD and what the consequences for the host are remain highly enigmatic; already in 2008 a lowered colonic motility was shown in a PD mouse model [49], which

might be a direct consequence of overexpression of α -synuclein in the myenteric plexus by the Thy-1-driven transgene [50]. Characteristic deposition of α -synuclein in human PD patients [51] and altered patterns of phospho- α -synuclein aggregates [52] as well as elevated total intestinal permeability [53] have been reported. Together with the fact that SNP within peptidoglycan-recognizing protein genes are associated with PD [54] and that systemic application of LPS leads to an immediate and progressive increase in α -synuclein expression in the large intestine of mice [55], the gut itself might have the potential to contribute to disease progression in PD rather than merely reflecting pathology in a non-CNS organ.

Alzheimer Disease

AD is a progressive neurodegenerative disorder that affects about 30% of all humans aged over 80 years. Hallmarks of the disease are intracellular neurofibrillary tangles built of hyperphosphorylated Tau protein and extracellular deposits of amyloid- β peptides. In comparison to PD there is no or little systematic information about gastrointestinal symptoms in AD. However, weight loss, even before a clinical diagnosis, has been found in patients in several studies [e.g., 56]. Additionally, inflammatory bowel syndrome has been shown to be associated with a higher risk of dementia in patients aged over 50 years [57], even if this was not specific for AD. This might already hint at dysfunctions of the gastrointestinal system and dysbiosis in AD. Very early on – already in 1989 – the group of Dennis Selkoe recognized amyloid- β -positive staining in gut sections from an AD patient’s biopsy [58]. The presence of neurotoxic peptides has been confirmed in different mouse models of the disease [59, 60], which led to speculation about the presence of pathological abnormalities within the gut. We recently identified an early dysbiosis in the 5xFAD mouse model, in which an increased Firmicutes/Bacteroidetes ratio was observed along with a body weight gain reduction and a trypsin deficiency [59]. Even more interestingly, an investigation on amyloid-positive and amyloid-negative AD patients in comparison to healthy controls revealed that there is a possible association of certain taxa with a peripheral inflammatory state in patients with cognitive impairment and brain amyloidosis [61]: while the proinflammatory taxon *Escherichia/Shigella* was elevated in such patients, the anti-inflammatory *Escherichia rectale* was found to be reduced in fecal samples.

In a small cohort of AD patients ($n = 14$ AD cases and $n = 12$ controls [62]) a general increase in the bacterial population and a specific increase in *Propionibacterium*

acnes was described in frozen and formaldehyde-fixed brain tissue samples. *P. acnes* is known to nonspecifically stimulate the innate immune system and thereby might impact the AD pathological condition. In this regard, it is of interest that LPS has been observed in the brain of AD patients (e.g., 3-fold increase in the hippocampus in comparison to healthy controls [63]) and that serum IgG levels to common periodontal microbiota such as *Actinomyces naeslundii* have been associated with the risk of developing AD [64]. A recent in silico analysis of human proteins that might act autoimmunogenically by mimicking gut-bacterial peptides revealed AD as one of the major diseases represented in the data set [65]. Interestingly, GF AD model mice revealed a reduced amyloid- β pathology and reconstitution of these mice with especially microbiota from AD mice aggravated pathological aggregates in comparison to reconstitution with material derived from wild-type mice [66]. Moreover, transfer of an aged microbiome (from 17-month-old mice) in young GF mice promoted inflammation in the small intestine and thereby potentially increased vulnerability against neurodegenerative conditions [67]. This, together with the knowledge that the gut endothelial barrier and the blood-brain-barrier show decreased integrity with aging, might hint at the gastrointestinal microbial community being one of the drivers of sporadic AD in the elderly [67].

Microbiota as a Therapeutic Target in Neurodegenerative Diseases of the Brain

Fecal microbiota transplantation has been successfully used for the treatment of *Clostridium difficile* infection, Crohn disease, and ulcerative colitis. Therefore, it is tempting to speculate that the transfer of “healthy” microbiota (whatever this might mean) might also be a route for treating neurodegenerative diseases of the brain. However, there are many hindrances and there is a vast lack of fundamental data: selection of donors, standardized preparation of the transplantation material, and potential duration, as well as onset of medication, have to be considered and only single case reports for certain neuropsychiatric diseases are available [e.g., reviewed in 68]. To our knowledge, no single description of a controlled investigation regarding PD has been published and the same holds true for AD. Single attempts have been made to analyze how the gut microbiota might be involved in therapeutic approaches per se. For example, pomegranate juice and extracts showed neuroprotective effects against AD in several animal studies. The ellagitannins contained in these

plant products are rapidly converted by gut microbiota and do not reach the circulation in an intact chemical structure. Yuan et al. [69] reported that urolithins derived by microbial conversion of ellagitannins might be a bioactive compound that is capable of preventing A- β oligomerization in vitro and of protecting *Caenorhabditis elegans* from amyloid- β -induced toxicity. More of such relations will be surely found in future studies and microbial organisms contributing to such conversion then might offer assistance in therapeutic approaches.

Conclusions

Microbes and their metabolites are not only important for shaping of the CNS; they also interfere with the part of the nervous system that is closest to them, i.e., the ENS. Changing the microbial conditions in the gut might thus not only lead to metabolic alterations but it will also affect the ENS and the brain-gut axis, and as such it is crucial not only for the well-being of the patient but also for finding the cause and course of and the appropriate treatment for neurodegenerative diseases. Therefore, there is an urgent need for much more reliable facts that elucidate the microbial impact on the CNS, the ENS, and the brain-gut axis based on data that have been gathered following a consensus in the field, e.g., on sample collection, storage and analyzed species and models.

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