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Review

A Critical Analysis of Intestinal Enteric Neuron Loss and Constipation in Parkinson's Disease

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Abstract. Constipation afflicts many patients with Parkinson's disease (PD) and significantly impacts on patient quality of life. PD-related constipation is caused by intestinal dysfunction, but the etiology of this dysfunction in patients is unknown. One possible cause is neuron loss within the enteric nervous system (ENS) of the intestine. This review aims to 1) Critically evaluate the evidence for and against intestinal enteric neuron loss in PD patients, 2) Justify why PD-related constipation must be objectively measured, 3) Explore the potential link between loss of enteric neurons in the intestine and constipation in PD, 4) Provide potential explanations for disparities in the literature, and 5) Outline data and study design considerations to improve future research. Before the connection between intestinal enteric neuron loss and PD-related constipation can be confidently described, future research must use sufficiently large samples representative of the patient population (majority diagnosed with idiopathic PD for at least 5 years), implement a consistent neuronal quantification method and study design, including standardized patient recruitment criteria, objectively quantify intestinal dysfunctions, publish with a high degree of data transparency and account for potential PD heterogeneity. Further investigation into other potential influencers of PD-related constipation is also required, including changes in the function, connectivity, mitochondria and/or α -synuclein proteins of enteric neurons and their extrinsic innervation. The connection between enteric neuron loss and other PD-related gastrointestinal (GI) issues, including gastroparesis and dysphagia, as well as changes in nutrient absorption and the microbiome, should be explored in future research.

Keywords: Parkinson's disease, α -synuclein, constipation, enteric neuron, degeneration, dysfunction, gastrointestinal, microbiome, mitochondria, neurodegeneration

INTRODUCTION

Parkinson's disease (PD) is a progressive and currently incurable neurodegenerative disease [1]. PD is clinically characterized by motor abnormalities, including slowness of voluntary movement (bradykinesia), involuntary movement (resting tremor) and/or

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increased muscle tone (rigidity) [2, 3], which are commonly accompanied by psychiatric and autonomic dysfunction [3].

The motor dysfunctions in PD primarily manifest due to an impaired ability of the basal ganglia to regulate movement, stemming from dopamine depletion in the striatum [2, 4, 5]. The level of striatal dopamine significantly diminishes once a proportion of substantia nigra pars compacta (SNpc) dopaminergic neurons, and an even greater proportion of their projections, degenerate [6]. The causes and mechanisms underlying this degeneration remain the subject of ongoing research [7, 8], such that it cannot be delayed or prevented by existing clinical interventions [9]. Fortunately, the gold-standard PD medication l-3,4-dihydroxyphenylalanine (levodopa) [10], which is believed to increase the amount of dopamine within the striatum [11], provides substantial and superior relief from PD motor symptoms [9, 12].

In addition to the characteristic movement issues, PD patients commonly suffer from sleep, mood, cognitive and gastrointestinal (GI) dysfunction (collectively termed 'non-motor symptoms'), none of which are relieved by levodopa or other therapeutics effective at alleviating motor symptoms [13, 14]. These findings, paired with the appearance of hyposmia, depression, constipation and sleep disturbances sometimes years before motor symptom onset, suggest that depletion of striatal dopamine is not what gives rise to non-motor symptoms in PD [13, 15].

In terms of PD-related GI symptoms, which include dysphagia (swallowing difficulty), gastroparesis (slowed stomach emptying), and constipation [16], it has been suggested that dysfunction of the enteric nervous system (ENS) may contribute to their occurrence [17]. The ENS is an extensive neural network embedded within the gut wall, from the esophagus to the internal anal sphincter, and it plays important roles in the control of GI activities [18]. There are connections and bi-directional communication between the central nervous system (CNS) and the ENS, which allow these systems to influence one another and collectively modulate gut function [18]. However, the CNS primarily regulates esophageal movement and stomach contractions [19], while intestinal motility is dominantly controlled by the ENS [20] with input from the vagus nerve, pelvic nerves, and sympathetic prevertebral ganglia [18]. Prolonged colonic transit time (CTT), typically due to decreased colonic motility [21, 22], is thought to be a major contributor to PD-related constipation [23].

Although the prevalence of constipation among PD patients varies between studies [24, 25], constipation is more prevalent in the PD population than in the general population aged 60 years and over [26, 27], can precede the onset of PD motor dysfunction by more than 10 years [28, 29] and significantly impacts on patient quality of life [30]. Current therapies aimed at managing constipation tend to have limited efficacy in PD patients [31, 32]. As loss of SNpc neurons and their striatal projections in the CNS is associated with development of PD motor symptoms, it is hypothesized that neuronal damage and death in the intestinal ENS may underlie the initiation of PD-related constipation.

This review will critically evaluate the evidence for and against intestinal enteric neuron loss in PD patients, justify why PD-related constipation must be objectively measured and explore the potential link between loss of enteric neurons in the intestine and constipation in PD. Importantly, this review will highlight discrepancies in the literature on intestinal enteric neuron loss in PD patients, present potential explanations for these disparities and suggest solutions that could be implemented by future studies.

THE ENTERIC NERVOUS SYSTEM CONTROL OF INTESTINAL CONTENT MOVEMENT

Within the small and large intestine, most enteric neurons are organized into two ganglionated plexuses—the myenteric plexus and the submucosal plexus [18, 33]. Neurons of the myenteric plexus originate between the longitudinal and circular muscle layers of the intestine, while neurons of the submucosal plexus stem from the region between the circular muscle and the intestinal mucosa [18, 33] (Fig. 1).

Studies of the guinea pig intestine demonstrate that neurons in each plexus interact and influence one another (Fig. 2). For example, submucosal intrinsic sensory neurons (ISNs), which indirectly detect and respond to mechanical stimulation of the intestinal mucosa [35, 36], project to the myenteric plexus [36–38] where the majority of neurons innervating the circular and longitudinal muscle of the intestine reside [18]. Myenteric ISNs, which can directly detect stretching of the intestinal wall [39] or chemical stimulation of the mucosa [40, 41], also provide excitatory input to interneurons and motor neurons in the myenteric plexus [40]. Secretomotor neurons in

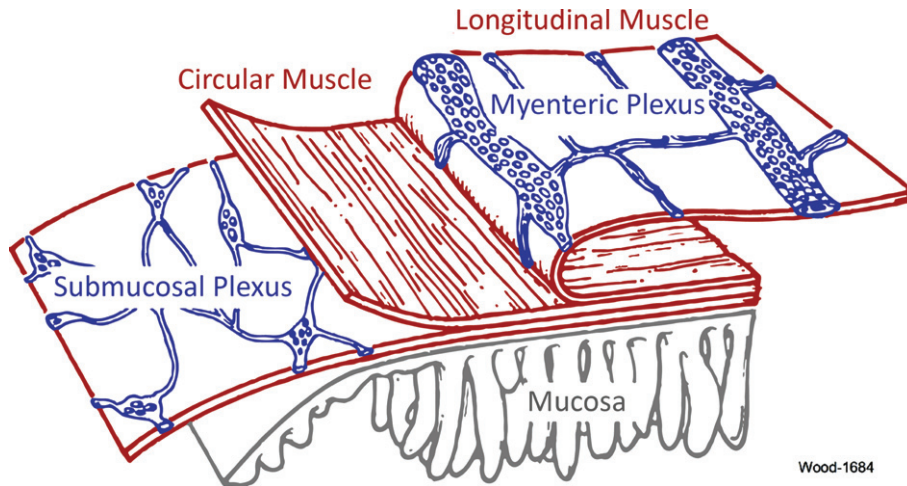


Fig. 1. Structure of the intestinal ENS. This simplified representation depicts how the myenteric and submucosal plexuses are arranged within the walls of the small intestine, relative to the longitudinal and circular muscle. The ENS within the large intestine exhibits a near-identical organization [20], but the mucosa is not organized into villi [34]. Reproduced by permission from Morgan & Claypool Publishers and Prof Jackie D. Wood from *Enteric Nervous System: The Brain-In-The-Gut* by Wood © 2011 Morgan & Claypool Life Sciences [33].

the submucosal plexus receive excitatory input from certain myenteric neurons [42], as well as from submucosal ISNs [35], and in turn stimulate mucosal epithelial cells to secrete chloride, which facilitates secretion of water and sodium into the intestinal lumen [35].

Collectively, the complex interplay between various types of myenteric and submucosal neurons underpin secretory and motility reflexes of the intestine [18, 35, 42]. These interrelated reflexes allow intestinal contents to be easily transported (orally to anally) at a speed appropriate for optimal nutrient and water absorption. When intestinal transit is slowed and liquidity of the intestinal contents lowered due to suppression of enteric neuronal activity, increased absorption and inhibited secretion of water and electrolytes results in drier-harder stools and associated constipation [44]. Therefore, if enteric neurons are lost from either plexus in PD patients, this could similarly compromise the timely execution and coordination of reflexes governing movement of intestinal contents, and this may give rise to PD-related constipation. Constipation in patients with severe gut dysmotility correlates with loss of both myenteric and submucosal neurons in the small intestine [45], and neurons in both the myenteric and submucosal plexus are significantly reduced in the colon of patients with slow-transit constipation [46]. Several other conditions involving substantially impaired GI motility are also associated with enteric neuron loss, including Chagas' disease [47, 48], achalasia and chronic intestinal pseudo-obstruction [31].

INTESTINAL ENTERIC NEURON LOSS IN PARKINSON'S DISEASE

A summary of key results from studies assessing intestinal enteric neuron loss in PD patients is provided in Table 1. This table is intended to provide a general overview of relevant study findings, but the supplementary tables contain the level of detail required to accurately compare and critique these studies.

Small intestine

The small intestine has three divisions: the duodenum, the jejunum, and the ileum [34]. In addition to different anatomical positions and subtle structural differences, each division includes a unique combination of glands, lymphoid follicles and neurovasculature [34, 55, 56]. In light of this, enteric neuron loss in each region of the small intestine should be considered separately.

Duodenum

No enteric neuron loss has so far been discovered in the myenteric plexus [51] nor the submucosal plexus [53] of the duodenum in PD patients relative to controls (Fig. 3A; Supplementary Table 1, D and G). However, over 50% of patients recruited into the study by Desmet and colleagues [53], which investigated PD-related submucosal neuron loss in the duodenum, had early-onset PD (onset before age

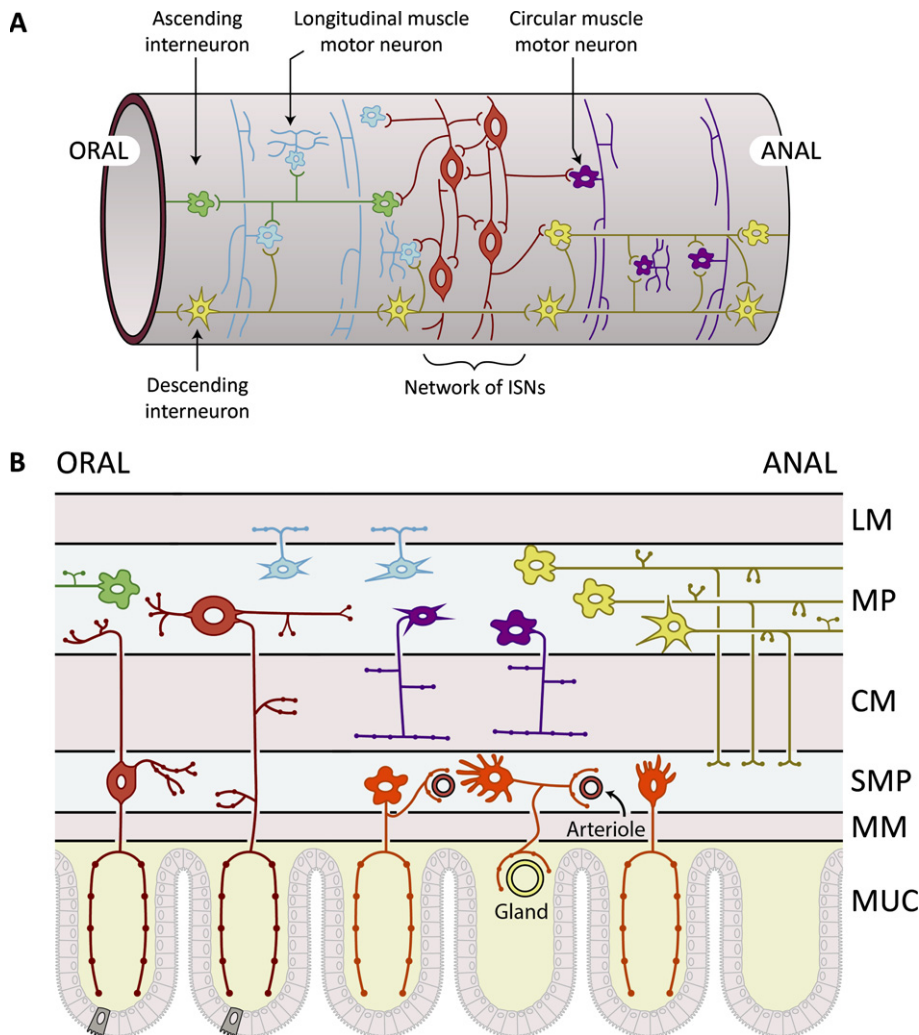


Fig. 2. Interactions between intestinal enteric neurons. **A**) Simplified representation of enteric neuronal circuits directly governing intestinal motility, which involve both myenteric and submucosal neurons. **B**) Simplified representation of the enteric neuronal interactions directly and indirectly facilitating movement of intestinal contents (neuron types colored as in A, with secretomotor neurons additionally depicted in orange). LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; SMP, submucosal plexus; MM, muscularis mucosa; MUC, mucosa (of the small intestine — Mucosa of the large intestine is not organized into villi [34]). Adapted by permission from Springer Nature: Springer, *The Enteric Nervous System and Gastrointestinal Innervation: Integrated Local and Central Control* by Furness et al. © Springer New York 2014 [18] and from *Progress in Neurobiology*, 72, Furness et al., *Intrinsic primary afferent neurons and nerve circuits within the intestine*, 143-164 © 2003 Elsevier Ltd [43], with permission from Elsevier Ltd.

50 [57–60]) (Supplementary Table 2). Early-onset PD accounts for approximately 10–20% of all PD cases [59] and is commonly associated with mutations in specific genes, including DJ-1 (PARK7), PINK1 (PARK6), and Parkin (PARK2) [61, 62]. Thus, although it is not synonymous with familial PD, early-onset PD is often familial in nature. Therefore, Desmet et al. [53] genetically analyzed the Parkin (PARK2) gene of 5/6 patients with disease onset at or before age 45, but did not discover any mutations. However, this study did not addition-

ally screen for familial PD-linked mutations in DJ-1, PINK1 or other genes—these mutations may have been present in the early-onset patients examined, and perhaps contributed to a profile of symptoms and intestinal neuropathology different from idiopathic PD [53, 59]. Only patients with idiopathic PD were analyzed in the study estimating constipation to precede clinical diagnosis by over 17 years [29], and early-onset cases do not appear to have been analyzed. It is also unclear whether the prior systematic review and meta-analysis of constipation

Table 1
Summary of key results from studies assessing intestinal enteric neuron loss in PD patients

Study reference	Region of the intestine examined ^A	Subjects assessed ^B	PD-related enteric neuron loss ^C in the intestinal region?	Correlation between intestinal enteric neuron loss and constipation ^D ?
Lebouvier et al. [49]	Colon (ascending)	5 PD 3 CC 5 control	SMP: No MP: Not assessed	No correlations performed
Lebouvier et al. [50]	Colon (ascending, descending)	29 PD 10 control	SMP: Yes MP: Not assessed	No
Annerino et al. [51]	Duodenum, colon (transverse)	13 PD 12 control	SMP: Not assessed MP: No	Constipation not assessed
Barrenschée et al. [52]	Upper dorsal rectal wall	12 PD 11* control	SMP: No MP: Not assessed	No relevant correlation performed
Desmet et al. [53]	Second part of the duodenum	15 PD 15 [^] control	SMP: No MP: Not assessed	Constipation not specifically assessed
Giancola et al. [54]	Colon (descending)	29 [#] PD 10 CC 20 control	SMP: No MP: Not assessed	No relevant correlation performed

PD, PD patients; CC, constipated controls; control, healthy and/or non-constipated controls; SMP, submucosal plexus; MP, myenteric plexus. *10/[^]14/[#]15 included in neuronal quantity analysis. ^AFor information on tissue collection and tissue processing in each study, refer to Supplementary Table 1. ^BFor detailed demographics of the PD patients evaluated in each study, refer to Supplementary Table 2. ^CFor detailed information on how enteric neuron loss was assessed in each study, refer to Supplementary Table 1. Correlations between PD patient demographics and intestinal enteric neuron quantities in each study are presented in Supplementary Table 4. ^DFor detailed information on how constipation presence and/or severity was assessed in the relevant studies, refer to Supplementary Table 3. Correlations between PD patient demographics and constipation severity score in the relevant studies are presented in Supplementary Table 5. Differences in the descriptive statistics, *p*-values and individual participant data provided by each study, in addition to the types of statistical tests performed, are detailed in Supplementary Table 6.

preceding PD motor symptoms [28] is applicable to early-onset and/or familial PD. To our knowledge, the incidence of constipation specifically among patients with familial and/or early-onset PD has not been explored. Therefore, the lack of submucosal neuron loss in patients studied by Desmet et al. [52] may not be applicable to the constipated PD population (Table 1).

Jejunum

Ohlsson and Englund [63] examined samples of jejunum and colon from PD patients and controls. The pilot study reported the presence of atrophic/pyknotic neurons in 15/20 PD patients, either within the submucosal plexus (10/15), myenteric plexus (13/15) or both (9/15), and found no evidence of degenerating neurons in controls [63]. However, it is unclear from this report whether the neuronal degeneration observed in PD patient tissue was identified in samples of jejunum, colon or both. Furthermore, there was no quantitation of intestinal enteric neurons in this study, and thereby no comparison of enteric neuron quantities between PD patients and controls. Consequently, whether or not there is appreciable loss of submucosal or myenteric neurons within the jejunum in PD remains an open question (Supplementary Table 1, I).

Large intestine

The large intestine includes the caecum, appendix, colon, rectum and anal canal [34], and the colon has four subdivisions: the ascending colon, the transverse colon, the descending colon and the sigmoid colon [34]. Each colonic region has a distinct amalgam of arterial blood supply, venous blood drainage, innervation and lymphatics, in addition to specific anatomical positions and unique relations to other abdominal structures [64]. Accordingly, it is best to consider enteric neuron loss in each subdivision of the colon separately, where possible.

Colon and rectum

Several colonic regions have been examined for enteric neuron loss in PD patients [49–51, 54] (Fig. 3B–D; Supplementary Table 1, B–D and H). One study found a significant 14% decrease in the mean number of colonic submucosal neurons per ganglion, averaged from one ascending colon sample and one descending colon sample per subject, in PD patients compared to controls [50] (Fig. 3C; Supplementary Table 1, C). A subsequent study found no change in the mean percentage of total colonic neurons that were positive for tyrosine hydroxylase (TH), a marker of catecholaminergic (dopaminergic and

noradrenergic) neurons, in the submucosal plexus of PD patients relative to controls [65] (Supplementary Table 1, E). It is important to consider that if catecholaminergic and other neuronal subtypes within the submucosal plexus were lost in equal amounts, the relative proportion of submucosal catecholaminergic neurons to total submucosal neurons would remain the same. Therefore, the results of these two studies [50, 65] together indicate that there may be proportional loss of catecholaminergic neurons and other types of enteric neurons within the human colon, the majority of which are cholinergic [54, 66], from the colonic submucosa in PD.

However, another study compared the mean number of submucosal neurons per ganglion in the descending colon of constipated PD patients, constipated individuals without PD (constipated controls) and control subjects without constipation (healthy controls), and did not find any significant difference between these groups [54] (Fig. 3D; Supplementary Table 1, H). The study by Annerino and colleagues [51] also failed to find myenteric neuron loss in the transverse colon of PD patients relative to controls (Supplementary Table 1, D). Nonetheless, neither of these studies [51, 54] examined enteric neurons within the ascending colon—the study which discovered enteric neuron loss in the colon of PD patients [50] collectively evaluated submucosal neuron quantities across the ascending and descending colon. Additionally, Giancola et al. [54] derived mean submucosal neurons per ganglion from a high proportion of patients with early PD (diagnosed with PD for less than 5 years [67, 68]) relative to Lebouvier et al. [50] (Supplementary Table 2). As it is challenging to distinguish between PD and related conditions within the first 5 years of PD-like symptoms [67, 69, 70], a considerable proportion of patients in the study that did not discover colonic neuron loss [54] may have had a condition other than PD. Indeed, the diagnostic criteria for clinically established PD [3, 68], approximate to earlier definitions of definite PD [71] and probable PD [72], are very difficult for early PD patients to satisfy [68, 71, 72] (Supplementary Table 2). Therefore, it may be the case that patients with clinically established PD experience neuron loss in the colonic submucosal plexus. This loss may be isolated to the ascending and descending subdivisions, although the study which investigated PD-related neuron loss in the transverse colon [51] exclusively examined the myenteric plexus. Interestingly, a pilot study which only included patients diagnosed with PD for over 5 years [49] did not

find submucosal neuron loss in the ascending colon of subjectively constipated PD patients relative to both healthy and constipated controls, respectively (Fig. 3B; Supplementary Table 1, B). However, it has been suggested that these results may lack validity due to the very small number of subjects examined [50]. A study of the rectum [52] failed to find submucosal neuron loss in PD patients relative to controls (Fig. 3E; Supplementary Table 1, F).

Before continuing, it is worth noting that Singaram et al. [73] examined neurons of the submucosal and myenteric plexuses within the ascending colon of severely constipated PD patients, intractably constipated individuals without PD (constipated controls) and control subjects without constipation (healthy controls), and reported no significant difference between groups in the percentage of neurons positive for vasoactive intestinal peptide (VIP) nor TH in either plexus (Supplementary Table 1, A). Although this study evaluated the total number of submucosal and myenteric neurons in the ascending colon of each subject via staining for protein gene product 9.5 (PGP 9.5) [73], whether or not there was a significant difference in the total number of these neurons between any of the groups assessed was not reported. Additionally, as is the case with Corbillé et al. [65], a lack of significant change in the proportion of TH-positive neurons does not necessarily reflect a lack of significant change in the number of TH-positive nor total neurons, as catecholaminergic and other neuronal subtypes (including VIP-positive neurons) may have been lost in proportionate amounts.

Singaram and colleagues [73] also concluded that their severely constipated PD patients had a reduced proportion of myenteric dopaminergic neurons within the ascending colon relative to constipated and healthy controls. This conclusion was based on the findings of immunohistochemical staining for dopamine [73], which is not a validated method for the *in situ* identification of dopaminergic neurons [51, 65]. The results of TH immunohistochemistry, a technique which is commonly used to identify noradrenergic and dopaminergic neurons *in situ* [51, 65], did not indicate a significant reduction in the percentage of myenteric catecholaminergic neurons within the ascending colon of PD patients [73]. Therefore, others have suggested that the reduced proportion of dopaminergic neurons in the myenteric plexus of these patients was artifactual [51, 65]. Artifacts are artificial structures or tissue alterations that result from extraneous factors, including tissue sampling and processing procedures [74],

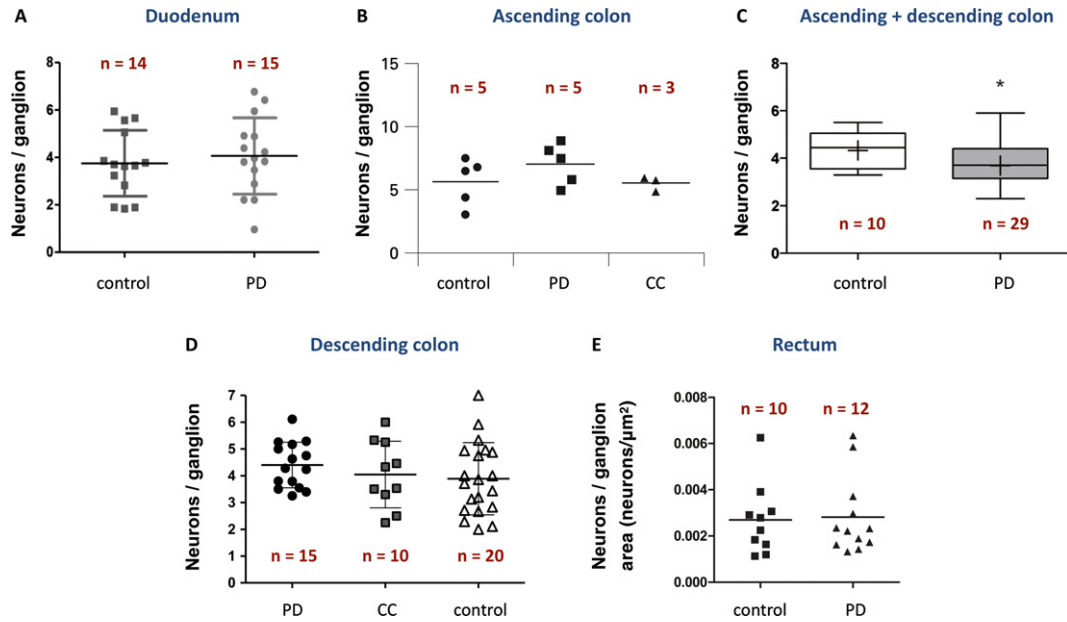


Fig. 3. Comparisons of mean intestinal enteric neuron quantities between PD and control groups. A) Enteric neurons in the submucosal plexus of the duodenum were detected via immunostaining for HuC/HuD (HuC/D) and neurofilament (NF). Modified from [46] Copyright Desmet et al., eLife article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>). B) Enteric neurons in the submucosal plexus of the ascending colon were detected via immunostaining for HuC/D. Adapted by permission from BMJ Publishing Group Limited. Pathological lesions in colonic biopsies during Parkinson's disease, Lebouvier et al., Gut 57:1741-1743 © 2008 [49]. C) Enteric neurons in the submucosal plexus of the ascending + descending colon were detected via immunostaining for NF. Modified from [50] © 2010 Lebouvier et al., PLoS ONE article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>). D) Enteric neurons in the submucosal plexus of the descending colon were detected via immunostaining for HuC/D. Adapted by permission from John Wiley and Sons: Neurogastroenterology & Motility, 29, Giancola et al., Downregulation of neuronal vasoactive intestinal polypeptide in Parkinson's disease and chronic constipation, e12995 © 2016 John Wiley & Sons Ltd [54]. E) Enteric neurons in the submucosal plexus of the rectum were detected via immunostaining for protein gene product 9.5 (PGP 9.5). Modified from [52] © 2017 Barrenschee et al., Acta Neuropathologica Communications article distributed under the terms of the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0>). Annerino et al. [51] detected enteric neuron density (neurons/mm²) between PD patients and controls (not shown). PD, PD patients; CC, constipated control subjects; control, healthy and/or non-constipated control subjects. * $p < 0.05$ as compared with control.

and complicate the histomorphological evaluation of intestinal tissues. As some PD patients in Singaram et al. [73], but no control subjects, had colonic tissue sampled at autopsy (Supplementary Table 1), postmortem artefacts [75] may have been observed exclusively in the PD patient tissue. However, it is also worth noting that the duration and severity of PD in the patients examined [73] far surpassed that of patients in other studies aimed at investigating PD-related intestinal enteric neuron loss (Supplementary Table 2).

Clinically established PD-related neuron loss in the colonic submucosal plexus

Overall, the results of the studies explored above may together be consistent with proportional loss of neuronal subtypes (catecholaminergic, VIPergic,

cholinergic and potentially others) in the colonic submucosal plexus of patients with clinically established PD (Supplementary Tables 1 and 2). Whether there is enteric neuron loss within the rectal myenteric plexus, the ileum, caecum, appendix, anal canal, sigmoid colon, myenteric plexus of the descending colon or submucosal plexus of the transverse colon in PD has yet to be investigated.

CONSTIPATION IN PARKINSON'S DISEASE

Intestinal dysfunction (colonic and/or anorectal dysfunction) gives rise to PD-related constipation

There are three types of constipation commonly encountered in PD patients—slow transit constipa-

tion caused by prolonged CTT [16, 23, 76], outlet obstruction, sometimes termed 'defecatory dysfunction', due to uncoordinated (dyssynergic) contraction or insufficient relaxation of the puborectalis muscle and anal sphincters during defecation [16, 23, 76] and, most commonly, a combination of both [23]. Objective measures of colonic and anorectal dysfunction are able to distinguish between these subtypes—CTT can be measured via retention of radiopaque markers (ROM) or a wireless motility capsule in the colon [23, 25, 76], while contractility of the anal sphincters, rectal sensory function and recto-anal reflexes can be evaluated using anorectal manometry (ARM) [23, 54, 77]. The state of the puborectalis muscle during defecation attempts can also be discerned via magnetic resonance (MR) imaging (MR defecography) [23, 77]. However, constipation criteria and related questionnaires are more widely utilized by researchers to evaluate PD-related constipation than these objective measures [25]. This is mainly because it has been presumed that less than three bowel movements per week, a common constipation criterion, is reflective of slowed CTT [16, 76] and that anorectal symptoms, such as straining during defecation and sensation of incomplete bowel emptying after defecation (other common constipation criteria), are due to outlet obstruction [16, 76]. For example, the Rome III Criteria for Functional Constipation [78] has frequently been used to diagnose constipation in studies involving PD patients [30, 50, 54, 79–81], and many studies involving PD patients have used the sum of positive responses to constipation items on a related questionnaire, the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders [82], as a semi-quantitative assessment of constipation severity [50, 54, 79–81, 83].

Constipation items on PD questionnaires do not appropriately evaluate intestinal dysfunction

Summing the positive responses to Rome III constipation items is not a validated method of scoring constipation severity [50, 81], nor has the Rome III constipation criteria and related questionnaire themselves been appropriately validated for use in PD research [25]. Nonetheless, it is understandable why several studies involving PD patients utilized either 6 or 7 Rome III constipation items to derive a constipation severity score—all the non-motor symptom questionnaires validated for use in PD patients

[84–87] include no more than 1 or 2 items relevant to constipation (Fig. 4), and these do not evaluate colonic and anorectal dysfunction in the same detail as the relevant Rome III items [25]. For example, the Non-Motor Symptoms Scale (NMSS) [84] defines constipation as less than three bowel movements per week (Fig. 4A), which, although thought to reflect prolonged CTT, fails to account for the anorectal symptoms of constipation prominent in PD. Furthermore, definitions of constipation based solely on bowel movement frequency are known to be unreliable [81, 88] and, if reliant on subjects remembering their weekly number of bowel movements, are particularly prone to recall bias [89]. The Non-Motor Symptoms Questionnaire (NMSQuest) [85] and SCOPA-AUT (Scales for Outcomes in Parkinson's disease—AUTonomic dysfunction) [87] both enquire about the need to strain during defecation, in addition to whether a patient experiences less than three bowel movements per week [85, 87] (Fig. 4B, C). However, only the latter is again utilized as the constipation definition, whereas straining is distinguished from constipation—the reason for this is unclear. Although, the NMSQuest employs a single question to determine if a patient has experienced constipation or straining within the past month [85], which seems to undermine its exclusion of straining from the constipation definition. The NMSQuest additionally appraises sensation of incomplete bowel emptying following defecation (Question 7) [85], but the SCOPA-AUT does not include an equivalent question. Finally, the constipation item on the Unified Parkinson's Disease Rating Scale (MDS-UPDRS (I), item 11) [86] appears to initially define constipation as bowel movement difficulty, but the descriptions of each constipation severity include inconsistent details pertaining to level of defecation effort, discomfort, activity disturbance, and need for physical help (Fig. 4D)—this may lead to confusion among patients attempting to define their constipation severity. In contrast to constipation items on the SCOPA-AUT, NMSQuest, NMSS, and MDS-UPDRS (I), the Rome III constipation items [78, 90] examine frequency, and thus severity, of the inability to relax the anorectum or the sensation of anorectal blockage, the need for manual maneuvers to facilitate defecation and the occurrence of hard or lumpy stools, in addition to abnormally reduced bowel movements, straining during defecation and sensation of incomplete bowel emptying following defecation [78, 90].

A **NMSS constipation item**

Symptoms assessed over the last month. Each symptom scored with respect to:
Severity: 0 = None, 1 = Mild: symptoms present but causes little distress or disturbance to patient;
2 = Moderate: some distress or disturbance to patient; 3 = Severe: major source of distress or disturbance to patient.

Frequency: 1 = Rarely (<1/wk); 2 = Often (1/wk); 3 = Frequent (several times per week);
4 = Very Frequent (daily or all the time).

Domains will be weighed differentially. Yes/ No answers are not included in final frequency x severity calculation.
(Bracketed text in questions within the scale is included as an explanatory aid).

Domain 6: Gastrointestinal tract	Severity	Frequency	Frequency x Severity
21. Does the patient suffer from constipation? (Bowel action less than three times weekly)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

B **NMSQuest constipation items**

Have you experienced any of the following in the last month?

5. Constipation (less than 3 bowel movements a week) or having to strain to pass a stool (faeces) Yes No

7. Feeling that your bowel emptying is incomplete after having been to the toilet Yes No

C **SCOPA-AUT constipation items**

5. Constipation is a blockage of the bowel, a condition in which someone has a bowel movement twice a week or less.
In the past month, have you had problems with constipation?

never sometimes regularly often

6. In the past month, did you have to strain hard to pass stools?

never sometimes regularly often

D **MDS-UPDRS constipation item**

1.11 CONSTIPATION PROBLEMS	SCORE
Over the past week have you had constipation troubles that cause you difficulty moving your bowels?	
0: Normal: No constipation.	
1: Slight: I have been constipated. I use extra effort to move my bowels. However, this problem does not disturb my activities or my being comfortable.	<input type="checkbox"/>
2: Mild: Constipation causes me to have some troubles doing things or being comfortable.	
3: Moderate: Constipation causes me to have a lot of trouble doing things or being comfortable. However, it does not stop me from doing anything.	
4: Severe: I usually need physical help from someone else to empty my bowels.	

Fig. 4. Constipation assessment items on each PD non-motor symptom questionnaire. A) The question assessing constipation on the Non-Motor Symptoms Scale (NMSS) for PD patients. This figure incorporates the NMSS, which is owned and licensed by the International Parkinson and Movement Disorder Society (MDS). Permission to reproduce the NMSS in this figure was granted by MDS, the copyright holder. Copyright © 2007 International Parkinson and Movement Disorder Society (MDS). All Rights Reserved. B) The question/s assessing constipation on the Non-Motor Symptoms Questionnaire (NMSQuest/NMSQ) for PD patients. This figure incorporates the NMSQ, which is owned and licensed by the International Parkinson and Movement Disorder Society (MDS). Permission to reproduce the NMSQ in this figure was granted by MDS, the copyright holder. Copyright © 2006 International Parkinson and Movement Disorder Society (MDS). All Rights Reserved. C) The question/s assessing constipation on the SCOPA-AUT (SCales for Outcomes in Parkinson's disease — AUTonomic dysfunction). This figure incorporates the SCOPA-AUT, which is owned and licensed by the International Parkinson and Movement Disorder Society (MDS). Permission to reproduce the SCOPA-AUT in this figure was granted by MDS, the copyright holder. Copyright © 2019 International Parkinson and Movement Disorder Society (MDS). All Rights Reserved. D) The item assessing constipation on the Unified Parkinson's Disease Rating Scale (MDS-UPDRS). This figure incorporates the MDS-UPDRS, which is owned and licensed by the International Parkinson and Movement Disorder Society (MDS). Permission to reproduce the MDS-UPDRS in this figure was granted by MDS, the copyright holder. Copyright © 2008 International Parkinson and Movement Disorder Society (MDS). All Rights Reserved.

Constipation Scoring System survey results correlate with colonic and anorectal dysfunction in PD

Similar to the Rome III constipation items, the Constipation Scoring System (CSS) questionnaire provides a comprehensive assessment of constipation [88], and has occasionally been utilized in studies involving PD patients [52, 91, 92]. This survey consists of 8 questions (scored on a scale of 0–4, bar one) associated with different aspects of constipation, including bowel movement frequency, painful defecation effort, and sensation of incomplete bowel emptying after defecation [88]. The resulting CSS score has been shown to correlate well with objective measures of colonic and anorectal dysfunction in constipated patients [88], including the results of ARM, CTT, and cinedefectography, a predecessor of

MR defecography [93]. Although the CSS score is not validated for use in PD research [81], one study found that objective indicators of PD-related constipation were only completely corroborated by the CSS score, relative to the sum of positive responses to Rome III constipation items and both NMSQuest Question 5 (<3 bowel movements per week or straining during defecation) and 7 (sensation of incomplete bowel emptying following defecation) [81]. Specifically, PD patients had a significantly higher total number of retained ROM in the colon, indicative of prolonged CTT, and a significantly larger total colonic volume, predominantly due to transverse colon and rectosigmoid region volume increases (suggestive of co-occurring outlet obstruction) [81]. When the different constipation assessments were compared with these objective measures, all three (NMSQuest Questions 5 and 7, the sum of positive responses to Rome

III constipation items and the CSS score) correlated with total colonic volume, but only the CSS score correlated significantly with total colonic ROM (CTT) [81].

Correlations between any constipation items/criteria and intestinal dysfunction are weak in PD

However, the significant correlation between CSS score and total colonic ROM was weak ($R^2 = 0.19$), as was the significant correlation between total colonic volume and both the CSS score ($R^2 = 0.34$) and the sum of positive responses to Rome III constipation items ($R^2 = 0.33$) [81]. Although the significant correlations between Questions 5 and 7 of the NMSQuest and total colonic volume were moderate ($r = 0.504$ and $r = 0.538$, respectively) [81], the strength of these correlations appear to be due to an outlier (Supplementary Figure 1). This lack of strong correlations between quantitative indicators of PD-related constipation and constipation as defined by various questionnaires, validated for use in PD research or otherwise, reflects an overarching issue: objectively evaluated colonic abnormalities and criteria-defined constipation are weakly related. The prevalence of constipation among PD patients is known to vary substantially between studies [24, 25], but this was thought to be due mainly to variable definitions of constipation being utilized across studies [25, 94]. However, the comparative results of two recent studies demonstrate that, in PD patients with the same prevalence of prolonged CTT (79% vs. 78%), the prevalence of constipation diagnosed by identical criteria still varies greatly (32% vs. 100%) [54, 81].

Intestinal dysfunction occurs in the majority of PD patients and must be objectively evaluated

In contrast to the apparent inconsistency of criteria-based constipation assessments, CTT is significantly prolonged in PD patients relative to controls [81, 95–98] or normal values [99] in most cases. Anorectal dysfunction results among PD patients appear more variable [95, 98, 100, 101], but delayed colonic transit plus impaired anorectal function seems to be the most common finding in PD patients who match constipation criteria or believe they do (60–65%), relative to anorectal dysfunction only (13–26.7%), isolated slow transit (6.7–13%) or no transit and anorectal impairment (6.7–9%) [54, 77]. More broadly, intesti-

nal dysfunction seems to occur in the majority of PD patients, both when the majority match constipation criteria or believe they do [54, 77, 98, 99] and when the majority do not [81, 97]. Therefore, it is clear that constipation criteria and related questionnaire items do not reliably assess characteristics of slow transit constipation nor outlet obstruction, and thus cannot reliably identify the types of constipation encountered in PD patients.

RELATIONSHIP BETWEEN COLONIC ENTERIC NEURON LOSS AND PD-RELATED CONSTIPATION

Each of the studies that specifically investigated submucosal neuron loss in the colon or rectum of PD patients [49, 50, 52, 54] also assessed which participants exhibited constipation [50, 52], or exclusively recruited patients who were constipated to compare with constipated and healthy (non-constipated) controls [49, 54] (Supplementary Table 3).

Both Lebouvier et al. [50] and Giancola et al. [54] used the Rome III Criteria for Functional Constipation [78] to investigate constipation in their studies—the former study diagnosed constipation in 23/29 PD patients and 1/10 control subjects according to these criteria [50], while the latter utilized fulfilment of the criteria to guide recruitment of constipated individuals with and without PD, as well as non-constipated controls [54]. Lebouvier and colleagues [50] additionally employed the number of positive responses to 6 constipation items from the Rome III Diagnostic Questionnaire for Adult Functional Gastrointestinal Disorders [82] as a semi-quantitative assessment of constipation severity. Giancola and colleagues [54] included a correlation involving the severity of Rome III-criteria defined constipation in PD (on a scale of 0–6) in their supporting results (Supplementary Figure 2). However, as explored earlier, summing the positive responses to constipation items on the relevant Rome III questionnaire has not been validated as a means of scoring constipation severity [50, 81]. More importantly, a constipation diagnosis based on subjective criteria or related questionnaire items does not reliably correspond with prolonged CTT nor anorectal abnormalities, which objectively define the types of constipation encountered in PD patients [16, 23, 76]. The results of both studies [50, 54] must be considered in light of these limitations.

Lebouvier et al. [50] found no correlation between the number of neurons per ganglion in the colon and constipation severity [50] (Supplementary Table 3, B). However, this does not exclude the possibility that the colonic submucosal neuron loss discovered by this study contributed to colonic dysfunction in the PD patients examined, as colonic function was not objectively evaluated. Giancola et al. [54] did not report a relationship between the mean number of submucosal neurons per ganglion within the descending colon and the presence of Rome III criteria-defined constipation, likely because there was no significant difference in this mean between their PD patients, constipated controls and healthy controls [54] (Supplementary Table 3, C). However, this result does not rule out a connection between the mean number of submucosal neurons per ganglion in the descending colon and colonic function. Fortunately, Giancola et al. [54] objectively evaluated colonic function in PD patients via CTT assessment, in addition to examining their anal and rectal function using ARM [54]. In the subset of PD patients who underwent both ARM and CTT assessment, 78% were found to experience colonic dysfunction (prolonged CTT) and 65% additionally experienced anorectal dysfunction (ARM abnormalities) [54]. However, this study did not seek to correlate the mean number of submucosal neurons per ganglion in the descending colon with the mean number of residual intracolonic ROM within the PD group, likely because these means were derived from different, though partially overlapping, subsets of PD patients [54, 102].

Giancola and colleagues [54] also reported no significant difference in the mean number of residual intracolonic ROM between the 23 PD patients and the 9 constipated controls who completed both CTT evaluation and ARM [54]. However, 55% of the patients with known disease duration included in this analysis (12/22) had been diagnosed with PD for less than 5 years (Supplementary Table 2). Therefore, the appreciable misdiagnosis rate among early PD patients [67, 69, 70] may have contributed to this finding, such that it may not be applicable to the PD population. Assuming the patients with early PD were accurately diagnosed, the lack of significant difference in mean CTT between PD patients and constipated controls [54] still may not be generalizable to patients with clinically established PD [3, 68] (similar to probable PD [72] or definite PD [71] in earlier diagnostic criteria), who constitute the majority of patients examined in other studies of PD-related intestinal enteric neu-

ron loss (Supplementary Table 2). Indeed, the amount of residual intracolonic ROM was positively correlated with PD duration (spanning 1–10 years) in patients who had their CTT evaluated by Giancola et al. [54]—this could reflect an increase in the severity of PD-related constipation with years since PD diagnosis, or with increasing diagnostic accuracy, but this correlation should be interpreted with caution (Supplementary Figure 2).

The study that examined the ascending colon of subjectively constipated PD patients, all of whom had been diagnosed with PD for over 5 years, did not report a relationship between the mean number of submucosal neurons per ganglion and the presence of constipation [49] (Supplementary Table 3, A). However, the PD patients assessed in this study were not formally diagnosed with constipation [49], and the intractably constipated controls may have been constipated due to isolated outlet obstruction or other issues not directly related to colonic enteric neuropathy. Others have also suggested that the very small number of participants examined in this pilot study limit the validity of its findings [50].

One study examined both rectal submucosal neuron density and constipation severity of PD patients and control subjects [52]. Although no correlation between these variables was reported, neither mean neuron density within the submucosal plexus of the rectum nor mean constipation severity score (CSS score) differed significantly between the PD and control groups [52] (Supplementary Table 3, D). However, it is unclear whether any participant was 'constipated' according to their CSS score, and constipation was not objectively evaluated in any PD patient nor control subject [52]. Furthermore, the control group may have consisted of both constipated and non-constipated individuals, as rectal tissue from some control subjects was obtained during colonoscopy for GI symptoms [52], whereas the presence of existing GI issues in the PD patients examined was not described. Regardless, constipation in any participant assessed may have been related to enteric neuropathy within the colon, rather than enteric neuron loss in the rectum.

LIMITATIONS, DISPARITIES AND SUGGESTIONS FOR FUTURE STUDIES

The main limitations of the reviewed studies and relevant recommendations for future research are summarized in Table 2.

Table 2
Main limitations of the reviewed studies and suggestions to improve future research

Limitations of reviewed studies	Suggestions for future research
Small sample sizes impede applicability of intestinal enteric neuron loss results to the PD population	Sufficiently large patient and control samples should be utilized.
Recruitment/over-representation of early and early-onset PD patients may have confounded studies' results	Recruitment criteria should ensure patient samples are representative of the PD population (majority diagnosed with idiopathic PD for at least 5 years)
Differences in intestinal enteric neuron loss results between studies may be influenced by PD heterogeneity	The factors currently believed to contribute to PD heterogeneity, including particular genetic variants, RNA expression patterns, pesticide exposures and sleep disturbances, should be additionally assessed.
Incomplete reporting and different outcome definitions limit ability to perform a meta-analysis/compare study results and draw accurate conclusions	Individual participant results and/or relevant descriptive statistics, <i>p</i> -values and details of statistical tests should be provided. Study outcomes must be defined and analysed in the same manner across studies. Specifically: <ol style="list-style-type: none"> 1) A consistent neuronal quantification method and study design (including standardized statistical analyses) should be implemented. 2) Constipation should be assessed via objective measures of colonic transit and anorectal function. Furthermore, complete data and methodological transparency would enhance the validity of any future retrospective analysis.

Small sample sizes impede applicability of intestinal enteric neuron loss results to the PD population

The majority of studies that compared intestinal enteric neuron quantities between PD patients and controls derived these quantities from 15 or fewer participants per group (Supplementary Table 1), which is typically considered too few for findings to be generalizable [103, 104]. The small sample sizes utilized by the reviewed studies therefore limited their ability to detect any real PD-related change in intestinal enteric neuron quantities, and any relationship between constipation and intestinal enteric neuron loss [103]. This also limited the power of any correlation analyses performed (Supplementary Tables 1 and 3), including correlations run between PD patient demographics and intestinal enteric neuron quantities (Supplementary Table 4) or constipation severity scores (Supplementary Table 5). Although the only study to report a significant PD-related reduction in total intestinal enteric neurons evaluated 29 patients [50], their results were compared against only 10 controls [103, 104]. Future studies must therefore utilize larger sample sizes if their PD-related intestinal enteric neuron loss results are to be confidently generalized to the patient population. Although the exact sample sizes required should be estimated via *a priori* power analyses [104], PD and control groups of at least $n = 30$ could be an ideal place to start.

Incomplete reporting and different outcome definitions limit ability to perform a meta-analysis

When several studies with small samples seek to answer the same question, it is not unusual for researchers to perform a meta-analysis on the quantitative findings from each study to derive conclusions with higher power [105–107]. Unfortunately, individual participant results and/or relevant descriptive statistics, *p*-values and details of statistical tests were not consistently reported across the studies that sought to assess intestinal enteric neuron loss in PD (Supplementary Table 6), limiting the ability to perform a meta-analysis [108]. It is also difficult to perform a meta-analysis when the same outcome is treated or analyzed differently across studies [108] – as well as examining different regions of the intestine (Table 1), mean neurons per ganglion was quantified differently in each reviewed study and neuronal density was instead quantified in some studies (Supplementary Table 1). Additionally, the constipation assessments utilized were not consistent across relevant studies and thus the same outcome (constipation) was defined differently (Supplementary Table 3). Future studies should implement a consistent neuronal quantification method and study design (including standardized statistical analyses, where possible) and diagnose constipation via objective measures of colonic transit and anorectal function

[16, 23, 76] rather than using constipation criteria or related questionnaire items.

Recruitment/over-representation of early and early-onset PD patients may have confounded studies' results

The studies that sought to evaluate intestinal enteric neuron loss in PD utilized different recruitment criteria for both patients and control subjects (Supplementary Tables 1 and 3). Furthermore, the proportion of recruited patients with early PD (diagnosed with PD for less than 5 years [67, 68]), early-onset PD (onset before age 50 [57–60]), and severe PD (Hoehn and Yahr Stage 4 or greater [109, 110], and perhaps also indicated by a dopa-responsiveness $\leq 30\%$ [3, 50], UPDRS-III score ≥ 45 [111]/MDS-UPDRS (III) score ≥ 59 [86, 112] or a UPDRS-III axial score ≥ 21 [50, 111]) varied between studies (Supplementary Table 2), and over 50% of patients in some studies had early PD [54] or early-onset PD [53] (Supplementary Table 2). PD onset age, duration and severity may independently or jointly influence intestinal dysfunction and neuropathology [113–116]. Furthermore, given early-onset PD accounts for only 10–20% of PD cases [59] and might not be associated with constipation, while patients with early PD have appreciable rates of misdiagnosis [67, 69, 70], these patient groups should not be over-represented. Recruitment criteria utilized in future investigations should ensure patient samples are representative of the PD population (majority diagnosed with idiopathic PD for at least 5 years).

Discrepancies between colonic enteric neuron loss results may instead be due to PD heterogeneity

Only one of the three studies that specifically investigated enteric neuron quantities in the colon discovered PD-related submucosal neuron loss [50] (Supplementary Table 1, B, C and H). The validity of preliminary ascending colon results from Lebourvier et al. [49] has been questioned in their follow-up study [50], while the lack of descending colon submucosal neuron loss in Giancola et al. [54] may be explained by the disproportionate inclusion of potentially misdiagnosed (early PD) patients [67, 69, 70]. However, assuming the majority of early PD patients evaluated by Giancola and colleagues [54] were not misdiagnosed, and recognizing that constipation can predate

PD diagnosis by over a decade [28, 29], it could be argued that significant enteric neuron loss should be present in the colon of these patients, if such loss were a main contributor to PD-related constipation. In this case, the absence of PD-related submucosal neuron loss found [54] would contradict the significant submucosal neuron loss in PD patients observed by Lebourvier and colleagues [50], if the latter were due to substantial neuron reductions in the descending (or both ascending and descending) colon (Table 1). Rather than being an outright inconsistency, however, this potential disparity in the literature may be attributable to PD heterogeneity.

For several years, it has been recognized that PD is highly diverse in terms of onset, symptoms and impairments, disease progression and response to medication [14, 117–122]. The combination of clinical symptoms experienced and the biological processes underpinning these manifestations are potentially unique to each PD patient, such that PD may be resistant to useful subtyping [123]. Individual patient variation is thought to be due to the complex interplay between a variety of factors [123], including numerous genetic variants [124–128] and altered RNA expression [129]. Medical co-morbidities such as diabetes, anemia and cancer can additionally influence the pathogenesis and progression of PD [130]. Heterogeneity in the sleep disturbances experienced by PD patients, which include REM sleep behavior disorder (RBD) and insomnia, may be related to the heterogeneity in genetics, RNA expression and motor symptoms [131], and may exacerbate motor symptoms and cognitive issues to variable degrees [132]. Other factors such as pesticide exposures [133–136], age and sex [137] likely contribute to the individual-level heterogeneity, with PD sex differences themselves seemingly influenced by the population of origin (Western vs. Asian) and the age at disease onset [138]. Concussions have been shown to significantly increase the risk of PD development [139], while cerebral microbleeds have been posited to contribute to heterogeneous PD pathogenesis [140].

In light of the heterogeneity prevalent in PD, intestinal enteric neuron loss might occur in some patients but not in others, or in distinct amounts (and perhaps involving different neuronal subtypes) in each patient. Such heterogeneous loss could contribute to the diversity of intestinal dysfunctions underlying PD-related constipation [54, 77]. Future studies in this area should account for potential heterogeneity by including assessment of the

phenomena currently believed to interact and contribute to individual patient variation.

Data and methodological transparency would enhance the validity of any future retrospective analysis

Each future study of intestinal enteric neuron loss and/or constipation in PD should make all methodological considerations and data acquired available online along with the published research article, including:

- Enteric neuron quantities in exact amounts/numbers, rather than in proportions/percentages, and all parameters (neuronal, ganglionic, tissue or otherwise) relevant to the neuronal quantification method implemented. The total number of neurons and neurons per ganglion/neuronal density, for example, in each plexus and in each intestinal region examined should be presented separately. How an enteric ganglion was defined, how many ganglia were examined in each sample and/or how intestinal tissue stretch was accounted for should also be specified.
- Clarification/explanation regarding which participants underwent objective assessments of intestinal function (CTT evaluation and ARM, for example) and which patients and controls were included in neuronal quantification and correlation analyzes. A scatterplot with regression line, if applicable, and correlation coefficient for each correlation performed should also be presented.
- Detailed, de-identified information on every individual examined, including age, sex, ethnicity and other demographics, specific medications, co-morbidities and history of particular medical conditions, cerebral injuries or pesticide exposures, the presence of particular genetic variants, RNA expression patterns or sleep disturbances, and the disease duration, severity and age at onset for each PD patient.

Ensuring the information listed above is freely accessible would allow for precise and complete comparisons between relevant future studies, and enable incorporation of future study data into large-scale retrospective analyzes capable of determining the variables that may influence intestinal enteric neuron loss and/or constipation in PD patients.

OTHER CONSIDERATIONS

Challenges in obtaining objective constipation measures and myenteric plexus samples from PD patients

It can be difficult to consistently evaluate intestinal dysfunction among PD patients. For example, while a few patients refuse CTT evaluation and/or ARM in some studies [54], only a small subgroup of patients consent to combined assessment of colonic transit and anorectal function in other studies [77]. Patients may also refuse the endoscopy required for intestinal biopsy collection [54], without which numbers and properties of intestinal enteric neurons cannot be evaluated, but endoscopy refusal does not occur in most studies [50, 53, 65]. Even when objective assessments of both colonic transit and anorectal function are performed in patients from whom intestinal biopsies are also obtained, these biopsies often only contain the mucosa and submucosa—sampling the myenteric plexus via routine endoscopic biopsies presents a bleeding risk, and so cannot be done safely [53]. Full-thickness intestinal wall samples include the myenteric plexus [16], but these can only be obtained from living PD patients if part of their colon is resected [73]. Moreover, the need for colectomy entails that the patient likely suffers from an intestinal dysfunction unrepresentative of most PD patients. For example, two large-scale (though CSS score-based) studies suggest that severe constipation, one reason for colonic resection [73], is not common among PD patients [91, 92]. Other potential reasons for colectomy such as cancer [73] or a bowel disease, in addition to the resection itself, could contribute to any dysfunction recorded and thus confound results. Samples of intestinal myenteric plexus are easier to obtain from PD patients postmortem [51, 63] but, for obvious reasons, any myenteric neuron loss found in these samples cannot be subsequently compared with functional colonic and anorectal assessments.

The link between enteric neuron loss and intestinal dysfunction in preclinical studies is unclear

Due to the challenges associated with intestinal myenteric plexus sampling from live PD patients, combined with the difficulties in obtaining measures of intestinal function that are not influenced by issues other than PD, some researchers have resorted to PD animal model studies to elucidate the connection

between intestinal myenteric neurons and PD-related constipation. Unfortunately, when myenteric neuronal parameters within the intestine and intestinal function of several PD mouse models were compared in the same study, the onset and severity of both myenteric neuropathy and intestinal dysfunction were heavily influenced by mouse age, sex and species, as well as neurotoxin doses, duration of neurotoxin treatment and timing of post-treatment assessments [141]. Because of these limitations, the extent to which intestinal dysfunction is correlated with myenteric degeneration in various mouse models of PD remains uncertain.

Intestinal tissue artifacts could impact on neuronal quantification

Post-mortem artifacts can occur in intestinal tissue sampled at autopsy [75] and operation-associated artifacts may be present in surgical specimens [74, 142]. Such artifacts impact on histomorphological evaluation of intestinal tissues, including quantification of enteric neurons. None of the studies that quantified intestinal enteric neuron loss compared PD and control tissue sampled via different methods (Supplementary Table 1) and future studies should continue to only compare intestinal tissues collected via the same method (at autopsy, via biopsy or via resection, for example) in an attempt to partially control for sampling artifacts.

Enteric neuron dysfunction and extrinsic innervation may contribute to PD-related constipation

Aside from enteric neuron loss, changes in the function, connectivity, mitochondria and/or α -synuclein proteins of enteric neurons, especially VIPergic and cholinergic subpopulations [54, 66], may underlie or contribute to intestinal dysfunctions in PD patients. However, to our knowledge, only one study has evaluated the function of intestinal enteric neurons and their mitochondria in PD patients, and discovered no neuronal nor mitochondrial dysfunction relative to controls [53]. The potential causative role of altered enteric neurotransmission in PD-related constipation, whether due to loss of neuronal subtypes or neurotransmitter expression, is contentious [50, 51, 54, 65, 143] and studies of intestinal α -synuclein alterations in PD patients have produced diverse results deserving of a discussion beyond the scope of this review. Since constipation precedes the

onset of PD motor dysfunction and diagnosis by 10–20 years in some cases [28, 29], a large-scale prospective study of constipated individuals, wherein their intestinal function and enteric neuron function, connectivity, mitochondria and/or α -synuclein protein parameters are periodically assessed over the long-term, could be an ideal way to elucidate the role of enteric neuronal changes in PD development and symptoms. A large multicenter study of a similar style provided valuable information on the conversion rate of RBD to parkinsonisms and dementias, and determined the predictive value of various prodromal markers [144].

Extrinsic innervation to the ENS is also important for gastrointestinal function and thus extrinsic fibers, including vagal nerves, may play a role in PD-related constipation. Further research is required to better understand the role of both sympathetic and parasympathetic extrinsic visceral sensory afferent and visceral motor efferent fibers in the intestinal dysfunctions and disease pathology of PD patients.

The potential impact of enteric neuron loss on other GI functions requires investigation in PD patients

Although patients suffer from a range of GI issues, including gastroparesis and dysphagia [16], constipation is the only GI dysfunction that has been specifically evaluated and discussed in studies of PD-related enteric neuron loss. Additionally, the potential impact of enteric neuron loss on small intestinal functions, such as nutrient absorption, and the microbiome [145–147] is yet to be investigated in PD patients. Future clinical studies should explore the connection between loss of enteric neurons and microbiome alterations, malabsorption and other PD-related GI dysfunctions.

CONCLUSIONS

The studies that sought to investigate PD-related intestinal enteric neuron loss were limited by small sample sizes and over-representation of minority PD patients (early-onset PD patients) or patients potentially misdiagnosed with PD (early PD patients). However, when these studies are considered relative to one another in terms of sample size and the proportion of early PD patients included, results suggest neuron loss may occur in the colonic submucosal plexus of patients with clinically established PD. The studies which specifically investigated PD-related

submucosal neuron loss in the colon or rectum additionally explored constipation, but the criteria-based assessments these studies utilized do not reliably evaluate the intestinal dysfunctions of PD patients. One study did objectively measure intestinal function, but over 50% of the examined patients had early PD. In preclinical investigations, enteric neuropathy and intestinal function findings are heavily influenced by animal, treatment, and assessment parameters, such that the extent to which intestinal dysfunction is correlated with enteric degeneration in PD mouse models remains uncertain.

Before the connection between intestinal enteric neuron loss and PD-related constipation can be confidently described, future research must objectively quantify intestinal dysfunctions in PD patients and use sufficiently large samples representative of the patient population (majority diagnosed with idiopathic PD for at least 5 years). Future investigations should also implement a consistent neuronal quantification method and study design, including standardized patient recruitment criteria, publish with a high degree of data transparency and account for potential PD heterogeneity.

Further investigation into other potential influencers of PD-related constipation is required, including changes in the function, connectivity, mitochondria and/or α -synuclein proteins of enteric neurons and their extrinsic innervation. The connection between enteric neuron loss and other PD-related GI issues, including gastroparesis and dysphagia, as well as changes in nutrient absorption and the microbiome, should be explored in future research.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report. The funder had no role in the design nor writing of this review.

DATA AVAILABILITY

Most data utilized and analyzed during the writing of this review are available within published articles. Unpublished datasets (supplied by Prof. Roberto De Giorgio and Prof. Fiorella Giancola) were also analyzed and can be obtained from these authors upon reasonable request.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <https://dx.doi.org/10.3233/JPD-223262>.

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