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The bowel and beyond: the enteric nervous system in neurological disorders

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Abstract

The enteric nervous system (ENS) is large, complex and uniquely able to orchestrate gastrointestinal behaviour independently of the central nervous system (CNS). An intact ENS is essential for life and ENS dysfunction is often linked to digestive disorders. The part the ENS plays in neurological disorders, as a portal or participant, has also become increasingly evident. ENS structure and neurochemistry resemble that of the CNS, therefore pathogenic mechanisms that give rise to CNS disorders might also lead to ENS dysfunction, and nerves that interconnect the ENS and CNS can be conduits for disease spread. We review evidence for ENS dysfunction in the aetiopathogenesis of autism spectrum disorder, amyotrophic lateral sclerosis, transmissible spongiform encephalopathies, Parkinson disease and Alzheimer disease. Animal models suggest that common pathophysiological mechanisms account for the frequency of gastrointestinal comorbidity in these conditions. Moreover, the neurotropic pathogen, varicella zoster virus (VZV), unexpectedly establishes latency in enteric and other autonomic neurons that do not innervate skin. VZV reactivation in these neurons produces no rash and is therefore a clandestine cause of gastrointestinal disease, meningitis and strokes. The gut–brain alliance has raised consciousness as a contributor to health, but a gut–brain axis that contributes to disease merits equal attention.

The gut is a complicated organ[1]. A byzantine array of events is required for digestion and absorption to be successful. Muscular sphincters compartmentalize the bowel, dividing it into functionally distinct regions with radically different luminal environments. Neuronal monitoring of luminal contents permits ingested material to be transported aborally at a rate that allows each compartment to accomplish its task[2]. Contractions by smooth muscle (along the entire gastrointestinal tract) and skeletal muscle (in the oesophagus and anus) are thus choreographed into activity patterns, such as churning (in the stomach), segmentation (in the small intestine), or haustration (in the colon) that grind, mix, or temporarily hold luminal contents, in addition to providing aboral power propulsion and retropulsion[2]. Secretory

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Author contributions

The authors contributed equally to the review.

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mechanisms maintain a regionally appropriate pH as well as tightly regulated concentrations of electrolytes, enzymes and mucus. A thin semipermeable epithelial barrier, which is continuously regenerated from gastrointestinal stem cells[3], separates the lumen from the body's internal milieu. This barrier facilitates absorption, but also prevents the leakage of essential molecules into the intestinal lumen as well as the translocation of digestive enzymes, toxins and gut microbiota into the body from the lumen[4]. A scaffold of loose connective tissue, which contains the body's largest array of immune effector cells, provides mechanical and defensive support for the barrier[5]. All of these functions — secretion, motility, mucosal maintenance and immunological defence — require an exquisite degree of regulation and coordination, which the enteric nervous system (ENS) provides (FIG. 1).

The ENS is one of three divisions of the autonomic nervous system, defined as sympathetic, parasympathetic and enteric by the British physiologist John Newport Langley[6]. The human ENS contains more than 100 million neurons, which dwarf the number of efferent fibres that reach the gut in the vagus nerves[7].The complexity of managing the behaviour of the bowel is sufficiently great that, in contrast to the remainder of the peripheral nervous system (PNS), evolution has endowed the ENS with the ability to manifest integrative neuronal activity (that is, uniting complex inputs into a coherent and purposeful behavioural output) and the ability to control gastrointestinal behaviour independently of input from brain or spinal cord[1,2,8]. The ENS has more neurons than the aggregate of all other peripheral ganglia, containing at least as many as the spinal cord. Uniquely for the PNS, the ENS is organized in microcircuits, with interneurons and intrinsic primary afferent neurons (IPANs), which are able to initiate reflexes (FIGS 2,3). Enteric neuronal phenotypic diversity is extensive and virtually every class of neurotransmitter found in the CNS has also been detected in the ENS[2]. Although the ENS can function without input from the CNS, it does not normally do so; the CNS influences enteric behaviour and the gut also sends information to the brain. In fact, 90% of vagal fibres between the gut and brain are afferent, suggesting that the brain is more of a receiver than a transmitter with respect to brain–gut communication[2,9]. Some of the signals that the brain receives initiate vagovagal reflexes in which neurons within the CNS respond to enteric stimuli to regulate motility patterns in the oesophagus or stomach[2,9]. Additionally, gut-to-brain signalling transmits sensations of nausea, bloating, or satiety. Much of the information sent from the bowel to the CNS, however, is 'homeostatic' and might not reach consciousness, yet could be a determinant of mood. Vagus nerve stimulation, which mimics afferent signalling from the bowel, has been used successfully to treat depression and has been demonstrated to improve learning and memory in animals and humans[10,11]. Furthermore, the vagus nerve is also a potential conduit of signals from the luminal microbiota that affect mood, behaviour and brain development[9,12–14].

Key points

- The enteric nervous system (ENS) is the largest component of the autonomic nervous system and is uniquely equipped with intrinsic microcircuits that enable it to orchestrate gastrointestinal function independently of central nervous system (CNS) input

- Because many neurotransmitters, signalling pathways and anatomical properties are common to the ENS and CNS, pathophysiological processes that underlie CNS disease often have enteric manifestations
- Neuronal connections and the immune system might provide conduits that allow diseases acquired in the gut to spread to the brain
- Transmissible spongiform encephalopathies, autistic spectrum disorders, Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis, and varicella zoster virus (VZV) infection are examples of disorders with both gastrointestinal and neurological consequences
- VZV reactivations from latency in enteric and other autonomic neurons that lack cutaneous projections are occult causes of zoster without rash that lead to gastrointestinal disease, meningitis and strokes
- Research on the gut–brain axis of disease is reasonably new, concepts are changing rapidly, and further investigation is much needed

The importance of efferent signal traffic from the CNS is greatest in the most proximal and distal bowel[2]. Mastication and swallowing are CNS-dependent; moreover, the CNS directly innervates oesophageal striated muscle, which responds to vagovagal reflexes and pattern generators within the brain. Vagovagal reflexes also control gastric motility and compliance, which enables the stomach to expand without increasing intraluminal pressure and thus to store and grind down ingested contents until they can be moved to the small intestine[2]. By contrast, the details of the movements of the small and large intestines are controlled and safeguarded by the ENS, and in fact, if all connections to the CNS are severed, essential motility in these regions of the bowel is unimpaired. Conversely, a lethal pseudo-obstruction occurs if even a small segment of the ENS is missing, which might happen congenitally (in Hirschsprung disease)[15–17] or as a acquired illness (in Chagas disease)[18]. The ENS is therefore essential for life.

Primary disorders of the CNS

Given the importance, size and complexity of the ENS, it is not surprising that it contributes to the pathophysiology of gastrointestinal disorders. There is also no reason to believe that pathophysiological mechanisms underlying CNS disorders should not also affect the ENS. Many neurotransmitters are common to the CNS and ENS[2] and similar mechanisms govern development of both systems[19]. The pathophysiology that gives rise to CNS disorders therefore might also be operative in the ENS. ENS deficits are now reported to accompany an increasing number of CNS disorders (FIG. 4), from neurodevelopmental to neurodegenerative, and dysfunctional gastrointestinal manifestations might occur even before CNS symptoms become evident[20].

Transmissible spongiform encephalopathies

Because afferent and efferent nerves each link the CNS to the gut, it is plausible that these nerves act as conduits, enabling the spread of disorders from one to the other. Transmissible

spongiform encephalopathies (TSEs), which include kuru, variant Creutzfeldt–Jakob disease (vCJD), scrapie, chronic wasting disease and bovine spongiform encephalopathy, are progressive and uniformly fatal diseases that provide a paradigm of CNS disorders that might originate or enter the body through the gut[21]. The pathogenic agents that cause TSEs are abnormally folded forms of normal cellular prion glycoproteins: the normal protein is called PrP^{C} (prion protein cellular) and the abnormal protein derived from it is called PrP^{Sc} (prion protein scrapie, no matter in which TSE it occurs)[22]. PrP^{Sc} is a transmissible agent that acts as a template that causes replacement of alpha-helix with beta-sheet conformations in normal cellular PrP^{C}, which then undergoes abnormal folding. Misfolded PrP aggregates into oligomers and becomes PrP^{Sc} (REF. 23), which spreads inexorably to and through the CNS. The neurodegeneration that underlies the signs and symptoms of TSEs follows in the wake of spreading PrP^{Sc} (REFS 22,24). In addition to these neural pathways, the immune system provides a potential gut-to-brain conduit for ingested PrP^{Sc} (REF. 25). M cells might translocate PrP^{Sc} to lymphoid cells, and receptors for PrP^{Sc} could also be present on the brush border of enterocytes[26,27]. Furthermore, enteric glia[28] and neurons[29] express PrP^{C} and might thus be vulnerable to ingested PrP^{Sc} early in the disease course because of their proximity to the gut lumen[26].

Autism spectrum disorder

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder that is diagnosed in childhood on the basis of evidence of social withdrawal, impaired communication and repetitive behaviours[30]. Gastrointestinal symptoms are 3–4 times more common in children with ASD than in unaffected children[31] but are often overlooked or considered components of comorbidities. ASD is diagnosed on the basis of behaviour, which directs focus to the brain, and no anatomical or biochemical diagnostic marker is currently available. Moreover, many different behaviourally defined conditions have now been combined under the umbrella term ASD in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders[32], which means that the phenotypes of individuals with ASD are necessarily varied. The genetic causes of ASD are similarly diverse, with at least 100 and as many as 800 genes or genomic regions associated with ASD to date[33–37].

Because of the diversity of ASD manifestations and abundance of genes associated with the condition, it has been difficult to link underlying molecular mechanisms to subtypes of ASD. One way to bring order to a diverse condition defined only by symptoms is to first identify a genetic abnormality and then to define the phenotype that arises from it. This classification of a condition can be achieved by ‘reverse phenotyping’, a process in which genetic marker data is used to refine phenotypic descriptions. This approach is the reverse of the traditional method by which signs and symptoms are first used to define a disease and a search is subsequently made for the underlying genotype. Reverse phenotyping is a potentially valuable method of determining molecular mechanisms that cause disease[38]. An example of the power of this approach is the analysis of the syndrome associated with a defect in the gene encoding chromodomain-helicase-DNA-binding protein 8 (CHD8), a transcriptional repressor that negatively regulates the Wnt signalling pathway. Mutations in the *CHD8* gene have been associated with a genetically defined ASD subtype, typified by macrocephaly, characteristic facial features and gastrointestinal symptoms due to slow-

transit constipation[39]. Expression of the abnormal human gene encoding CHD8 in zebrafish caused a deficiency in enteric neurogenesis and slow gastrointestinal transit[39]. These observations suggest that slow gastrointestinal transit is not just a comorbidity but as much a part of the *CHD8* phenotype as the behavioural symptoms of ASD. Similarly, Pitt–Hopkins syndrome is a subtype of ASD caused by haploinsufficiency of transcription factor 4 (TCF4). Constipation and gastro-oesophageal reflux are common in patients with Pitt–Hopkins syndrome[40]. Consistent with these human manifestations, mice with TCF4 haploinsufficiency have impaired upper gastrointestinal transit and rectal motility[41].

A study published in 2016 examining patients with ASD indicated that lower gastrointestinal symptoms, including abdominal pain, stool retention and large bowel movements, correlated with the level of serotonin (5-HT) in the blood[42]. Furthermore, an elevated level of blood 5-HT has been found in about one-third of patients with ASD[43]. Blood 5-HT is almost entirely derived from the gut. Although platelets carry 5-HT in blood they do not synthesize it[44], and instead utilize the sodium-dependent 5-HT transporter (SERT, also known as SLC6A4) to take up this molecule[45] as they circulate through the bowel. An elevation of blood 5-HT levels is, therefore, itself suggestive of gastrointestinal dysfunction. Indeed, gain-of-function mutations in the *SLC6A4* gene that encodes SERT are over-transmitted in patients with ASD[46]. In mice, expression of SERT Ala56 — a mutated form of the protein in which alanine is substituted for glycine 56 — results in ASD-like behaviour and a gastrointestinal phenotype characterized by ENS hypoplasia and slow gastrointestinal transit[47]. This observation is consistent with the idea that an ENS abnormality might define yet another subset of ASD.

The observations discussed in the previous paragraphs suggest that genetic defects affecting CNS function in a neurodevelopmental disorder, such as ASD, might also affect ENS function, leading to the high prevalence of gastrointestinal symptoms. Data from animal models of ASD suggest that agents causing congenitally acquired insults to the CNS could similarly provoke an ENS defect. Large epidemiological studies have shown a strong link between prenatal exposure to the anti-epileptic drug valproic acid and increased risk of autism[48,49]. Rats and mice exposed to valproic acid during fetal development exhibit altered social interaction, increased anxiety and repetitive behaviours, mimicking deficits in children with autism[50]. Male mice exposed to valproic acid *in utero* also exhibit intestinal inflammation, which is associated with decreased intestinal 5-HT levels[51], suggesting that toxic exposures that affect the CNS also affect the gut. Although it is not clear whether the decrease of intestinal 5-HT levels is due to a direct effect of valproic acid or inflammation resulting from valproic acid, mucosal 5-HT is strongly pro-inflammatory whereas enteric neuronal 5-HT is anti-inflammatory[52–56]. These data are thus consistent with the possibility that *in utero* exposure to valproic acid predisposes children to intestinal inflammation through altered enteric 5-HT signalling.

Interest in the possibility that the gastrointestinal tract is not just an innocent bystander in ASD, but that it might actually play a part in pathogenesis, is long-standing. The ‘leaky gut’ hypothesis of ASD pathophysiology is based on the idea that defects in intestinal epithelial barrier permeability lead to inappropriate signalling by luminal components including bacteria, environmental toxins and even dietary macromolecules[57]. Although this hypothesis

remains popular and has driven many parents to place children with autism on special diets, the data to support an intestinal permeability deficit in ASD are limited and conflicting, given that both increased intestinal permeability[58] and normal intestinal permeability[59] in ASD have been reported. One study reported that the permeability of the intestine to lactulose and mannitol was abnormal in 36.7% of children with ASD, in 21.2% of their first-degree relatives and in 4.8% of healthy adults as controls, but was always normal in children without ASD[60]. Mouse models provide some support for the 'leaky gut' hypothesis and suggest that host gut microbiota are important determinants. In the maternal immune activation (MIA) model of ASD, pregnant mice are treated with immune-stimulatory molecules that mimic viral infection. MIA pups exhibit features of ASD including decreased ultrasonic vocalizations, impaired social interactions and repetitive behaviours[61]. MIA pups also have increased intestinal barrier permeability[62]. Additionally, treating MIA mice with the bacteria *Bacteroides fragilis* restores the integrity of the intestinal barrier, and is associated with improvement in some of the ASD-like behaviours, such as open field exploration, impaired sensorimotor gating and repetitive marble burying[62]. This exciting study shows that CNS impairments that result in an ASD-like behaviour, such as reduced expressive communication, are not necessarily irreversible and could be ameliorated by altering the intestinal microbiota and barrier permeability. Enteric neurons and glia both have important roles in maintaining the integrity of the intestinal epithelial barrier[63]. Further study is therefore needed to determine whether ENS dysfunction underlies barrier defects in ASD, and whether modulating ENS function might offer an opportunity for treatment of behavioural and/or gastrointestinal symptoms in patients with ASD.

Parkinson disease

Progressive neurodegenerative disorders such as Parkinson disease (PD), Alzheimer disease (AD) and amyotrophic lateral sclerosis (ALS) are defined by CNS dysfunction in specific regions characteristic to each disease. In PD, for example, degeneration of nigrostriatal dopaminergic neurons is associated with a movement disorder characterized by rigidity, resting tremor, postural instability and bradykinesia[64]. The identification of gastrointestinal abnormalities in patients with PD over 30 years ago first raised the possibility that the ENS could be involved in PD[65–67]. Dopaminergic neurons are found in the ENS[68,69] and are essential for normal gastrointestinal motility[70]. Both chemical injury and genetically derived animal models commonly used to study the pathogenesis of PD suggest that the ENS is vulnerable to degeneration in PD. One such animal model involves systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes nigrostriatal damage and PD-like symptoms in mice, primates and humans[71]. MPTP damages the ENS and the CNS, with up to 40% loss of enteric dopaminergic neurons occurring within 10 days of MPTP administration in mice, which suggests that toxic insults to the CNS associated with PD can exert similar effects on the ENS[72]. Mouse genetic models of PD pathogenesis also exhibit varying degrees of enteric neuronal dysfunction. α-synuclein is a member of a presynaptic family of synuclein proteins that are expressed widely in the nervous system[73–75], including the ENS[20,76–78]. Synucleins can function as chaperone proteins and are necessary for neuronal survival[73]. Monomeric synucleins also function in promoting the curvature of cell membranes[74], and regulate the kinetics of endocytosis of synaptic vesicle proteins[75]. In PD, α-synuclein forms protease-resistant

aggregates that are the primary molecular constituent of Lewy bodies, the pathognomonic lesion that remains the gold standard for definitive neuropathological diagnosis of PD[20,78,79]. Transgenic mice engineered to overexpress normal human α-synuclein in neurons exhibit colonic dysmotility associated with insoluble α-synuclein aggregates within enteric neurons, which precedes onset of subcortical changes[80–82]. Autosomal dominant familial forms of PD have been linked to two missense mutations in α-synuclein, encoding Ala30Pro and Ala53Thr substitutions[83,84]. Expression of these mutant forms of human α-synuclein in mice lacking endogenous α-synuclein leads to proteinase-K-resistant inclusion bodies within enteric neurons that display α-synuclein-immunoreactivity[85]. Ala30Pro and Ala53Thr mice have a statistically significantly greater total gastrointestinal transit time and slower colonic motility than wild-type or α-synuclein-null mice, even in the absence of CNS pathology[85]. Mouse models of PD therefore suggest that both toxic and genetic insults linked to PD are associated with ENS deficits, which could precede the onset of CNS symptoms or pathology. One limitation of the genetic models remains that they all involve overexpression of either wild-type or mutant α-synuclein proteins, which may or may not reflect the pathophysiology of human disease. Regardless of this limitation, the observations support the exciting possibility that the ENS offers an accessible site for the diagnosis and monitoring of treatment in PD[78].

Routine biopsy samples obtained by colonoscopy can sample up to 150 submucosal neurons and many more mucosal nerve fibres[86], making it plausible that ENS pathology could be used to diagnose and/or monitor PD. Since the initial report of Lewy bodies in the human ENS[65], investigations have sought to determine whether enteric Lewy bodies are specific for PD, whether the extent of Lewy pathology in the ENS correlates with disease severity, and whether PD causes enteric neuronal degeneration. One study examined the ENS in seven patients with PD who had undergone autopsy and in age-matched controls without PD in whom brain sections revealed no Lewy bodies, and detected Lewy bodies in both enteric plexuses of all patients with PD examined, but only eight of 24 controls[67]. Lewy bodies were also more numerous in patients with PD than in control patients. Another autopsy study compared enteric α-synuclein expression in ten patients with PD, eight patients with AD and 77 controls who were negative for markers of PD or AD. Enteric α-synuclein expression was detectable in at least half of the controls and patients with AD, but was found universally in patients with PD; furthermore, levels of staining were also much higher in the bowel of PD patients than in other patients[87]. Additional studies have also focused on the prognostic value of endoscopic biopsies, which sample mostly mucosa and some submucosa but are routinely performed in the USA and Germany for colon cancer screening in adults over the age of 50, according to guidelines of the US Preventative Services Task Force[88]. Some of these studies have suggested that α-synuclein^{+} Lewy neurites detected in the submucosa of colonic biopsy samples are specific for PD[86,89,90], whereas others have shown that aggregated α-synuclein in the mucosa and submucosa is a common finding that is unrelated to PD[91]. The use of multiple antibodies and careful examination of the morphologies of the resulting patterns of immunostaining might improve the prognostic value of immunocytochemical detection of α-synucleinopathies[78]. The discrepancies in reported observations of Lewy bodies in the ENS will have to be resolved before intestinal biopsy samples can be used to diagnose PD. Diagnostic use of Lewy pathology in the ENS,

perhaps in association with another biomarker, ought to be beneficial because evidence is good that prodromal lesions appear in the bowel in a substantial fraction of patients with PD up to 20 years before the onset of CNS manifestations[92].

Lewy pathology can affect the gut, however, it remains unresolved to what degree PD causes neurodegeneration in the ENS and, if it does, whether dopaminergic neurons are more vulnerable than other subpopulations. One group of investigators compared colonic surgical specimens from 11 patients with advanced PD with specimens from patients who underwent colonic resection for either adenocarcinoma or chronic constipation[93]. They reported that the proportion of myenteric dopaminergic neurons was selectively decreased by 10-fold in patients with PD; moreover, the PD-related decrease in enteric neurons was associated with the presence of Lewy bodies in both dopaminergic and non-dopaminergic myenteric neurons[93]. By contrast, another group reported that, compared with age-matched controls, there was no difference in either the density of total myenteric neurons or the density of subpopulations of myenteric neurons throughout the gastrointestinal tract in postmortem samples from 13 patients with advanced PD[94]. Several challenges unique to studying the ENS have made it particularly difficult to assess the extent of neurodegeneration. Enteric neurons are irregularly distributed in two interconnected plexuses that are integrated into 7.5 m (~25 ft) of bowel in a human adult. Histopathological studies using paraffin sections from autopsy or surgical specimens to quantify neuronal density are therefore highly prone to sampling error. The sections pass randomly through the enteric plexuses and relatively few neurons are present in any one section. The underlying logic of ENS organization, furthermore, is not fully understood. This aspect means that enteric neurodegeneration in PD might be restricted to functionally specific regions or ganglia, much as it is in the CNS, and be easily missed. Finally, tyrosine hydroxylase immunoreactivity is often used to identify dopaminergic neurons both in the bowel[69] and the brain[95]. Tyrosine hydroxylase, however, is not actually a dopaminergic neuronal marker because it is also found in noradrenergic neurons[96]. In the gut, moreover, the vast majority of tyrosine hydroxylase-immunoreactive fibres are sympathetic axons, which are noradrenergic projections from the extrinsic prevertebral ganglia to the bowel[69]. Greater understanding of ENS biology and more effective strategies to quantify the densities of total and dopaminergic neurons in human bowel are therefore needed.

In sum, data from human and mouse studies suggest that α-synuclein has a role in ENS function in the normal bowel and that protease-resistant aggregates of α-synuclein can be seen in enteric ganglia in the presence and absence of CNS pathology. Studies of transgenic mice engineered to express human mutant α-synuclein, as well as studies of the pathology of human specimens, suggest that the myenteric plexus might be more affected by Lewy body pathology than the submucosal plexus. Additionally, these studies indicate that pathological findings in the ENS are not restricted to dopaminergic neurons. Although the myenteric plexus is not sampled by routine endoscopic biopsies, it remains much more accessible than the CNS. Further studies are needed to determine whether full-thickness biopsies of the bowel wall that include the myenteric plexus will be helpful in the diagnosis or monitoring of PD.

Although the data on the diagnostic utility of Lewy pathology in the ENS remains equivocal, evidence exists that the gastrointestinal tract might play a part in PD pathogenesis. Braak and colleagues characterized brainstem pathology in patients with PD and found, unexpectedly, that α-synuclein^{+} inclusion bodies could be identified within very specific subpopulations of neurons in some brainstem nuclei, including visceromotor projection neurons in the dorsal motor nucleus of the vagus (DMV)[97,98]. This finding led Braak to propose that PD might be caused by a neurotropic pathogen, which first crosses the mucosal barrier to enter enteric neurons and then moves by retrograde transport along efferent vagal fibres to the DMV to gain access to the cerebral cortex[99]. Consistent with Braak's hypothesis, Lewy pathology is detectable in the peripheral vagus, as well as the enteric plexuses in the stomach and oesophagus of patients with PD[100]; moreover, rat vagal efferent axons are α-synuclein-immunoreactive, whereas vagal afferent axons are not[101]. Studies in rodents have now provided the proof of principle that gastrointestinal exposure to a toxic insult can trigger CNS pathology in PD[95,96]. Pesticide exposure has been linked to PD in epidemiological studies[102] and the administration of the pesticide rotenone has been explored in a mouse model of PD[71]. Chronic intragastric administration of low doses of rotenone leads to progressive accumulation of α-synuclein^{+} inclusion bodies — first in enteric neurons, then the DMV, and finally in the substantia nigra — in the absence of detectable rotenone in the CNS or systemic circulation[103,104]. Other groups have shown that injection of human α-synuclein or brain lysate from a patient with PD into the intestinal walls of rats leads, over time, to progressive α-synuclein immunoreactivity along vagal fibres and eventually in the DMV[105,106]. These data demonstrate that retrograde transport of α-synuclein in vagal efferent axons from the DMV is possible, and that insults to the gastrointestinal tract can trigger ascending pathology along the gut–brain axis.

Although causality is difficult to assess in humans, intriguing evidence supporting Braak's hypothesis is provided by one epidemiological study, which proposed that if vagal projections to the gastrointestinal tract are indeed important in the pathogenesis of PD, then patients who undergo a vagotomy would be protected from developing PD[107]. To test this hypothesis, the researchers assembled a cohort of over 14,000 patients who underwent a vagotomy over an 18-year period in Denmark, and a control cohort of matched individuals from the general population. Patients typically underwent either truncal vagotomy, in which both vagal trunks were severed below the level of the diaphragm, or superselective vagotomy, in which only the vagal projections to the gastric fundus and body were ligated. Truncal vagotomy eliminates vagal projections to the gastric antrum, pylorus, liver, biliary tree, pancreas, small intestine and large intestine, whereas superselective vagotomy leaves these projections intact. Patients who underwent truncal vagotomy and had at least 20 years of postsurgical follow-up were found to have an adjusted hazard ratio of 0.53 (95% CI 0.28–0.99) for PD, compared with the general population cohort[107]. This protective effect was not observed with superselective vagotomy[107], supporting the idea that vagal innervation to the gastrointestinal tract is important for PD pathogenesis but that the area of vulnerability includes more than the stomach. Subsequent analysis by another group of this same data set expanded to include a larger time frame and using a different type of statistical analysis, however, has questioned the strength of these findings[108]; therefore, further study is needed.

Research in animal models shows that the gut-to-brain transmission of PD is plausible, but it remains unclear to what extent the gastrointestinal tract plays a part in the overall incidence of human PD. Histopathological data from patients suggests that the ascending pathology proposed by Braak is unlikely to be the only route of disease progression[109–111]. Postmortem examination of the brainstem in patients with PD has found that at least 7% do not have α-synuclein^{+} inclusions in the DMV despite extensive nigrostriatal pathology, and that the severity of DMV pathology does not always correlate with extent of pathology in higher brain regions[109–111]. The combination of animal and human studies nevertheless presents a strong case for gastrointestinal involvement in the pathogenesis and/or pathophysiology of at least some cases of PD, and makes it imperative to continue to investigate the role of the ENS in PD.

Alzheimer disease

In contrast with PD, studies investigating the enteric manifestations of AD remain limited. In the CNS, AD is associated with the progressive accumulation of extracellular plaques containing amyloid beta (Aβ), intracellular accumulations of neurofibrillary tangles containing hyperphosphorylated tau protein[112], and dysfunction of cholinergic neurons of the basal forebrain[113]. These pathological changes trigger neurodegeneration and synapse loss, leading to impaired working memory and progressive dementia. Amyloid precursor protein (APP), the molecule from which Aβ is derived, is normally expressed in the ENS[114] and is essential for normal gastrointestinal motility, immunity and secretion[115]. Over 70% of enteric neurons are cholinergic[7], further supporting the potential effects of AD pathophysiology in the ENS. Transgenic mice that express three mutant forms of APP (Lys670Asn, Met671Leu, and Val717Phe)[116] or APP with a double mutation (Lys670Asn and Met671Leu) plus mutant presenilin1 (PS1-dE9), all of which are associated with familial AD[117], develop progressive accumulation of Aβ within enteric neurons. This Aβ accumulation is associated with a decrease in numbers of enteric neurons, dysmotility and increased vulnerability to intestinal inflammation, depending on the transgene and promoter used[116,117]. These observations suggest that enteric neurons are vulnerable to APP abnormalities, and imply that ENS dysfunction could occur in AD. Studies of the human ENS in AD, however, have been sparse. Aβ-immunoreactive plaques were reported in the intestinal submucosa of two patients with AD[118], but there have been no follow-up studies. Human enteric neurons express microtubule-associated tau protein[119,120] and hyperphosphorylated tau aggregates have also been detected in the myenteric plexus of ageing rats[121]. Only one study has compared the ENS in patients with AD with that of healthy individuals and those with other forms of dementia, in which no enteric neuronal loss or tau pathology specific to AD was found[122]. Given the increasing prevalence of AD and its severe financial burden, further exploration of a potential link between human AD and ENS pathology is clearly needed.

Amyotrophic lateral sclerosis

ALS and frontotemporal dementia (FTD) are neurodegenerative disorders that overlap in their underlying genetic bases and pathology; both involve nuclear–cytoplasmic transport dysfunction at the cellular level[123]. As new genetic mutations have been identified in ALS and FTD, specifically in *TARDBP* (encoding TAR DNA-binding protein 43; TDP-43), *FUS*

and *C9orf72*, new links have also been revealed between CNS disorders and previously unsuspected ENS dysfunction. TDP-43 is a multi-functional RNA-binding protein that acts primarily in the nucleus as a transcriptional repressor and can also move to the cytosol to modulate mRNA transport and stability[124]. Mutations in *TARDBP* are responsible for 2–5% of familial ALS[125–127]; furthermore, hyperphosphorylated, ubiquitinated aggregates of TDP-43 are found within inclusion bodies in the brain and spinal cord of many patients with FTD and/or ALS[128]. A number of TDP-43 transgenic mouse models have been generated to study the pathogenesis of FTD and ALS. One model, in which the mouse prion promoter drives expression of the Ala315Thr mutant form of TDP-43 (Prp-TDP43^{Ala315Thr})[129], causes symptoms of intestinal obstruction followed by sudden death[130,131]. Enteric neurons and glia normally express TDP-43 and the mouse prion promoter is active in both cell types[132]. A progressive, age-related loss of neurons and glia occurs in the myenteric plexus in Prp-TDP43^{Ala315Thr} mice, which is associated with delayed total gastrointestinal transit time secondary to slow colonic motility, even in *ex vivo* preparations that lack CNS input[132,133]. These data suggest that the ENS is vulnerable to genetic insults associated with ALS and FTD. Enteric manifestations of these and other TDP-43 associated neurodegenerative disorders require further study in which the concept of reverse phenotyping (as discussed earlier) might prove useful.

Interestingly, a gastrointestinal phenotype also occurs in transgenic mice that express superoxide dismutase 1 (SOD1) Gly93Ala, the most studied of the *SOD1* mutations[134]. In contrast to Prp-TDP43^{Ala315Thr} mice, however, the defect consists of abnormal tight junctions between enterocytes and increased mucosal permeability[135]. The SOD1^{Gly93Ala} mucosa, moreover, has increased numbers of Paneth cells, but decreased expression of the antibacterial peptide defensin-5, which Paneth cells produce. Perhaps because of the diminished antimicrobial ability in these animals, the microbiota of SOD1^{Gly93Ala} mice are highly abnormal. The ENS is an important regulator of the proliferation and differentiation of the mucosal epithelium; serotonergic neurons in the myenteric plexus activate submucosal cholinergic neurons that innervate intestinal crypts to stimulate proliferation[136]. Conceivably, a defect could be present in enteric neurons of SOD1^{Gly93Ala} mice, as in Prp-TDP43^{Ala315Thr} animals, which causes the mucosal barrier abnormalities in these animals; however, investigations into the ENS in SOD1^{Gly93Ala} mice are lacking, despite the popularity of these mice as a model of ALS.

The link between the CNS and the ENS is particularly relevant in neurodegenerative disorders because of the emerging hypothesis that the misfolded proteins that occur in these disorders share common cell-to-cell transmission mechanisms[137,138], analogous to those responsible for the spread of PrP^{Sc} (REF. 22). Either the ENS or the CNS could, in theory, be a point of origin for the pathophysiology. Regardless of where the pathology begins, the misfolded proteins form aggregates, which are displayed on plasma membranes of affected cells and are secreted or cleaved to reach the extracellular space. These proteins are glycated (subjected to non-enzymatic glycosylation) to form advanced glycation end-products (AGEs) that bind to AGE receptors (RAGEs) on neighbouring cells and are internalized. The proteins might be cleared as a result of autophagy but, if not, they are neurotoxic[139]. These features are common for PrP^{Sc}, α-synuclein in PD, as well as Aβ and tau in AD. AGEs have also been postulated to have a role in the abnormal folding of mutated forms of SOD1 and

TDP-43. Findings from experiments in mice expressing a series of mutations in *FUS* (a gene closely related to TDP-43), which are associated with highly aggressive and juvenile forms of ALS[140], suggest that the mutant FUS protein causes degeneration of motor neurons through a gain-of-function mechanism that does not involve a loss of the normal activity of FUS protein[141]. Studies of FUS mutations as well as repeat expansions in *C9orf72,* another gain-of-function mutation linked to familial ALS[142], highlight the importance of protein misfolding in ALS pathogenesis. No work has yet been done on FUS or C9orf72 in the ENS of humans with ALS or animal models of ALS.

Unsuspected viral infection of the ENS

Studies of autism and neurodegenerative disorders have shown that the ENS can be both an innocent bystander as well as an active player in the pathophysiology of CNS disease. The emergence of a well-known neurological infection as a clandestine cause of potentially serious gastrointestinal disturbance highlights the importance of the ENS and its connections to the rest of the nervous system. Varicella zoster virus (VZV) is the aetiological agent of varicella (chickenpox) and zoster (shingles)[143]. VZV is highly infectious but its primary disseminated infection, varicella, is usually a childhood disease of mild–moderate severity that provides VZV with access to neurons in which the virus establishes lifelong latency[143]. Zoster, in its classic manifestation, is a localized secondary infection caused by reactivation of VZV from latency, which causes pain, cutaneous hyperalgesia and allodynia. VZV has long been known to establish latency in neurons of dorsal root ganglia and cranial nerve ganglia[143]; however, it is now recognized also to establish latency in enteric neurons of virtually all patients who have experienced varicella or been vaccinated against it[143–145]. If an immunodeficiency exists, varicella causes lytic infection, which destroys enteric neurons and leads to ileus[146].

A vesicular rash due to epidermal infection is the characteristic feature that suggests a diagnosis of varicella or zoster[143]. In varicella, the rash is due to VZV that has been transported to the epidermis within skin-homing T cells from the portal of virus entry in the upper respiratory tract[147]. In zoster, anterograde transport within cutaneous projections of the dorsal root ganglia and cranial nerve ganglia neurons containing the reactivated VZV delivers the virus to the epidermis. When VZV reactivates in neurons of the ENS (FIG. 5), which do not project to the skin, enteric zoster occurs, causing gastrointestinal dysfunction without a rash[144]. Because there is no rash, enteric zoster is rarely suspected or diagnosed before surgery or autopsy, when a viral screen can reveal VZV to be the cause of intestinal pseudo-obstruction[148]. VZV reactivation in autonomic ganglia is similarly a clandestine cause of CNS disease and can cause strokes or meningitis without manifesting a telltale rash[143]. Reactivation of VZV leads to lytic infection and death of enteric neurons[146]; therefore, the intestinal pseudo-obstruction in enteric zoster is the result of an acquired aganglionosis, similar to that of Chagas disease[18]. Pseudo-obstruction, however, is not the only manifestation of enteric zoster, which can also cause perforating gastric ulcers and/or otherwise unexplained abdominal pain[144]. The examination of saliva in the context of abdominal pain for which no cause has been found after a gastroenterological workup provides a non-invasive diagnostic test for enteric zoster[144]. DNA encoding VZV genes appears in saliva when there is a productive VZV infection somewhere in the body, but

cannot be detected in control individuals that lack such an infection[144,149]. The appearance of VZV DNA in the saliva of patients with abdominal pain therefore suggests enteric zoster. In a small clinical trial of patients in whom salivary VZV DNA caused enteric zoster to be suspected, treatment of three of three patients with the antiviral drug valaciclovir was followed by the rapid disappearance of abdominal pain[144]. Because zoster is associated with a decrease in cellular immunity[150], population ageing and the increasingly common use of immunosuppressive therapies have caused the incidence of zoster in the USA to rise by about threefold since studies carried out between 1945 and 1959; similar rises have been reported in other developed countries[151]. The use of salivary VZV DNA for the diagnosis of zoster has already revealed that enteric zoster is not rare[144], and once it begins to be widely suspected, this pathology is likely to be encountered with increasing frequency. The possibility that enteric zoster contributes to the initiation of idiopathic gastrointestinal conditions, such as IBS, gastroparesis and IBD, merits future investigation.

Conclusions

The major site of integrative neuronal activity is the brain; however, the functions of the bowel are so complex that evolution has provided the gut with an intrinsic nervous system that can uniquely provide the necessary regulation of enteric behaviour and integration of multiple sensory inputs independently from the CNS. Details of the control of enteric behaviour have been delegated to the ENS. This ‘peripheralization’ has freed the brain from providing space for the hundreds of millions of neurons that are housed in the bowel, and eliminated a need for vast numbers of nerve fibres to connect these neurons to the gut. Still, despite the potential independence of the ENS, the brain and gut are connected and, for better or worse, able to influence one another (FIG. 4). In health, this complex mutual inter-relationship works well, but disorders of the CNS might have enteric manifestations and could even be acquired from the gut. Many developmental mechanisms that operate in CNS formation are also operative in ENS ontogeny, and the two nervous systems share a considerable degree of cellular and molecular identity. Several conditions that are commonly thought to be CNS disorders, such as TSE, ASD, PD, AD and ALS have enteric consequences. Additionally, many gastrointestinal disorders, such as IBS, chronic intestinal pseudo-obstruction and gastroparesis, are idiopathic. One fairly common cause of occult gastrointestinal disease is infection with VZV, which has been diagnosed infrequently due to lack of previous consideration. Other such conditions probably exist. Further exploration of brain–gut disease with molecular tools and the application of genetic approaches, including reverse phenotyping, will probably shrink the current idiopathic category of gastrointestinal disease and enlarge that of disorders common to the CNS and the ENS.

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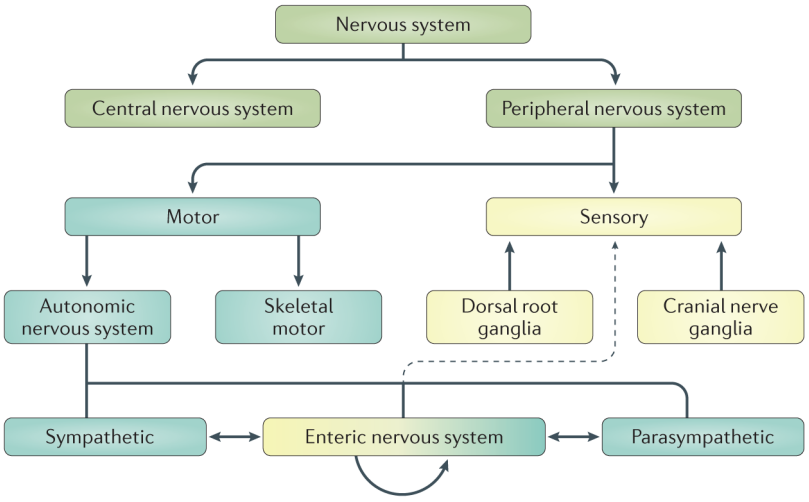

Figure 1. Relationship between the ENS and components of the peripheral nervous system

The enteric nervous system (ENS) is a large division of the peripheral nervous system (PNS) that can control gastrointestinal behaviour independently of central nervous system (CNS) input. Mammalian neurons are located in either the CNS (brain and spinal cord) or PNS (cells with soma outside the brain and spinal cord). Afferent information from the periphery to the CNS is conveyed by neurons located in dorsal root or cranial nerve ganglia, which constitute the 'sensory' division of the PNS (yellow). Afferent information integrated by the CNS leads to efferent output through the 'motor' division of the PNS (blue). Efferent projections from the CNS target either skeletal muscle (skeletal motor) or the autonomic nervous system, which is divided into three parts: sympathetic, parasympathetic and enteric[6]. In contrast to the neurons in sympathetic or parasympathetic ganglia, most enteric neurons receive no direct innervation from the CNS. Enteric neurons are organized in microcircuits that contain intrinsic primary afferent neurons that can respond intrinsically to local stimuli to integrate information and coordinate motor output. The ENS is therefore unique in having both sensory and motor properties (dotted line). Thus, ENS can mediate behaviour independently of the CNS; nevertheless, a two-way communication normally occurs between the bowel and the CNS, which influence one another.

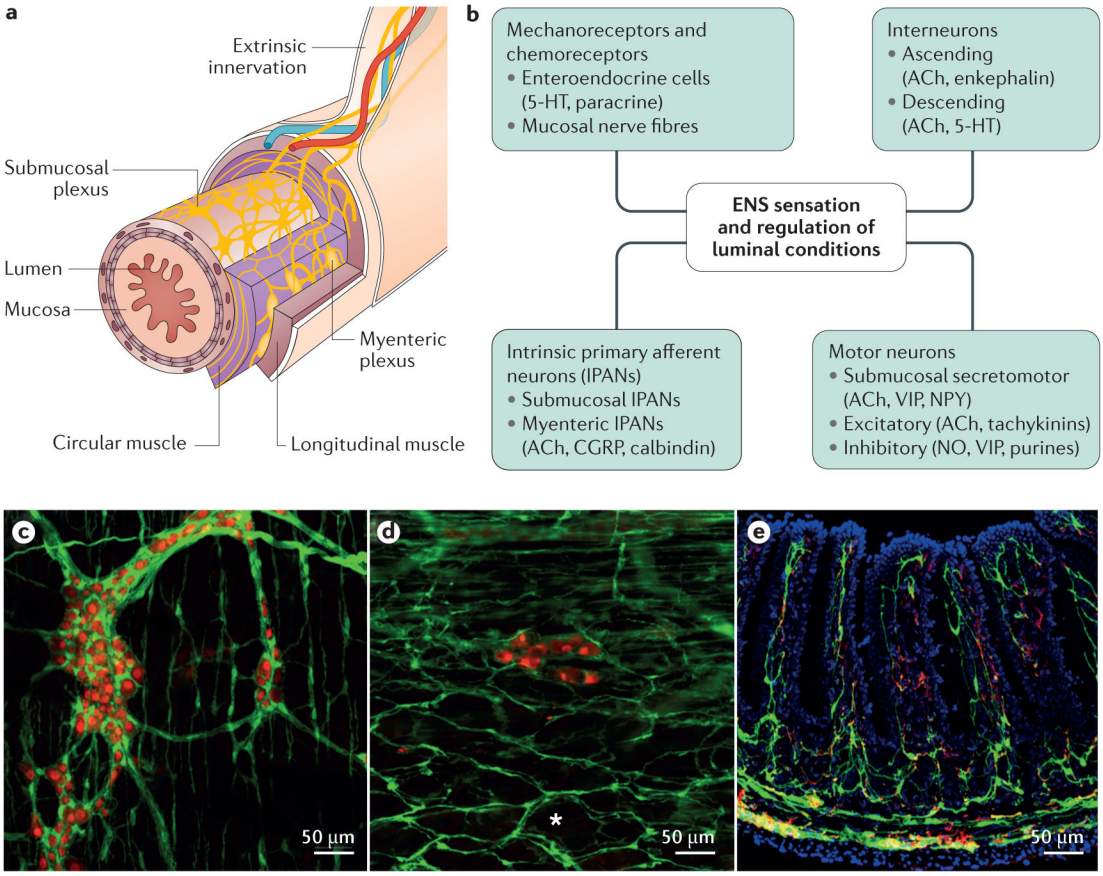

Figure 2. The ENS can regulate intestinal behaviours in the absence of CNS input

The neurons and glia of the ENS form an extensive network that extends through the layers of the small and large intestine. **a** | Schematic of the small intestine illustrating the organization of the ENS in its location within the intestinal wall. The myenteric plexus is located between the longitudinal and circular layers of smooth muscle whereas the smaller submucosal plexus is located in the dense connective tissue of the submucosa, just underneath the mucosa. Note that no nerve fibres actually enter the enteric lumen or its epithelial lining. The extrinsic innervation reaches the bowel through the mesentery along with the vasculature. **b** | The major components of the gastrointestinal tract that allow the bowel to sense and respond to luminal conditions are listed. **c** | Organization of myenteric ganglia. A colonic segment from an adult PLP1-eGFP mouse immunostained with the neuronal cell body marker, ANNA-1 (red) and the PLP1-eGFP glial reporter (green). Scale bar = 50μm. **d** | Organization of the submucosal ganglia. A colonic segment from an adult PLP1-eGFP mouse immunostained with the neuronal cell body marker, ANNA-1 (red) and the PLP1-eGFP glial reporter (green). The asterisk indicates a non-immunostained crypt base encircled by mucosal glia (green). Scale bar = 50μm. **e** | A cross-section of ileum from a PLP1-eGFP mouse immunostained with the neuronal marker, PGP9.5, which identifies neurites as well as cell bodies (red). The extensive innervation of the intestine, as well as the presence of enteric glia (green) throughout the lamina propria of the mucosa, is illustrated. DAPI (blue) was used to stain cell nuclei. The image was obtained from a maximum intensity projection of a 20 μm confocal stack. Scale bar = 50 μm. 5-HT, 5-

hydroxytryptamine or serotonin; ACh, acetylcholine; CGRP, calcitonin gene-related peptide; CNS, central nervous system; ENS, enteric nervous system; NO, nitric oxide; NPY, neuropeptide Y; VIP, vasoactive intestinal peptide.

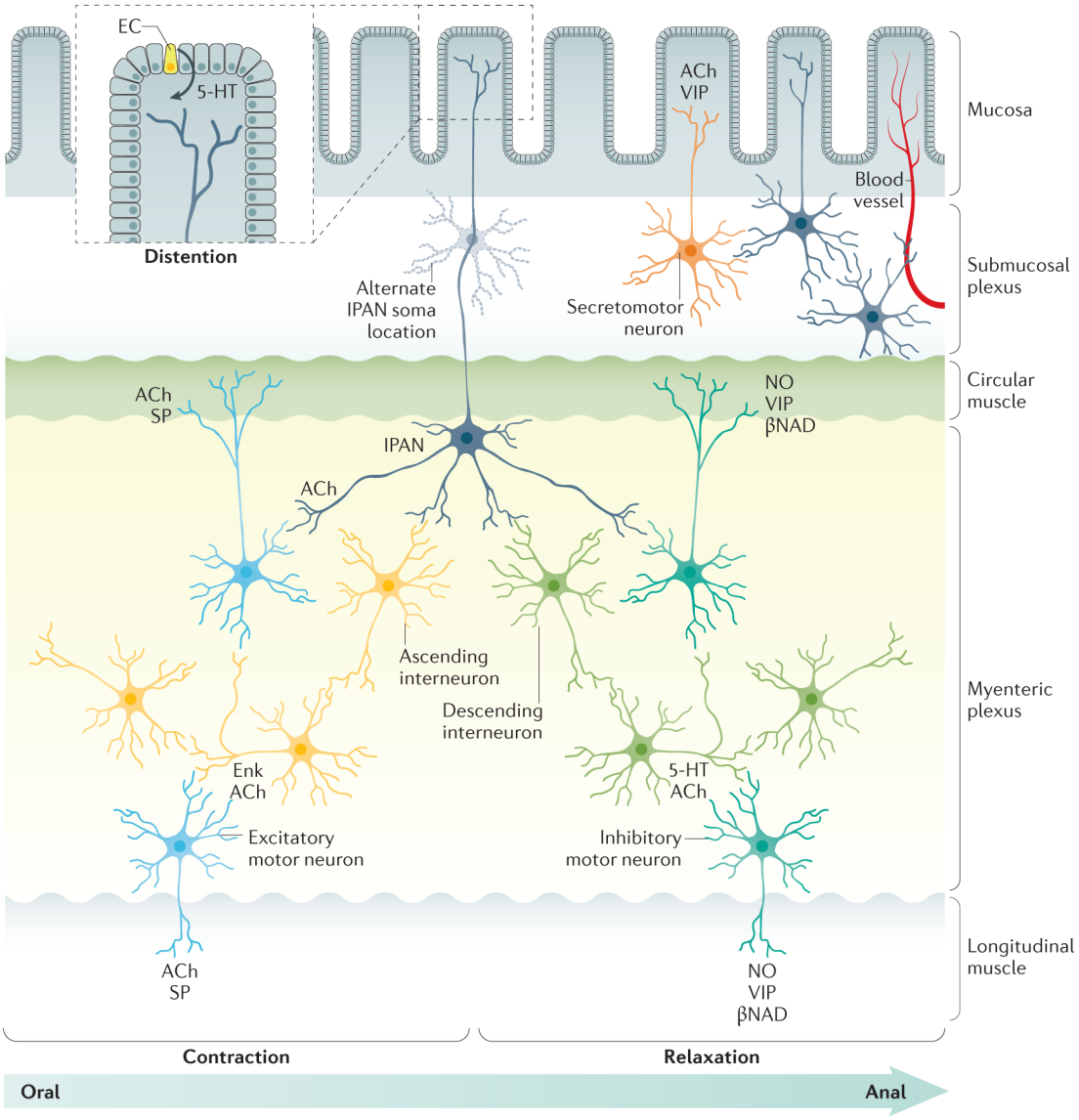

Figure 3. Schematic of the peristaltic reflex microcircuit required for aboral propulsion of luminal contents

Luminal distention or distortion triggers direct activation of mechanoreceptive endings of intrinsic primary afferent neurons (IPANs), as well as indirect activation of IPANs upon serotonin (5-HT) release by enterochromaffin cells (ECs) in the epithelium. IPANs activate ascending and descending interneurons, which stimulate excitatory and inhibitory motor neurons, respectively. Motor neuron activity leads to oral contraction and anal relaxation of intestinal smooth muscle, which propels luminal contents in the proximal–distal direction. ACh refers to neurons that contain acetylcholine. SP refers to neurons that contain substance P. Enk refers to encephalin-expressing ascending interneurons. NO and VIP indicate inhibitory motor neurons secreting nitric oxide and vasoactive intestinal peptide. βNAD refers to inhibitory motor neurons secreting the purine, β-nicotinamide adenine dinucleotide. Secretomotor and vasomotor neurons of the submucosal plexus secrete ACh or VIP.

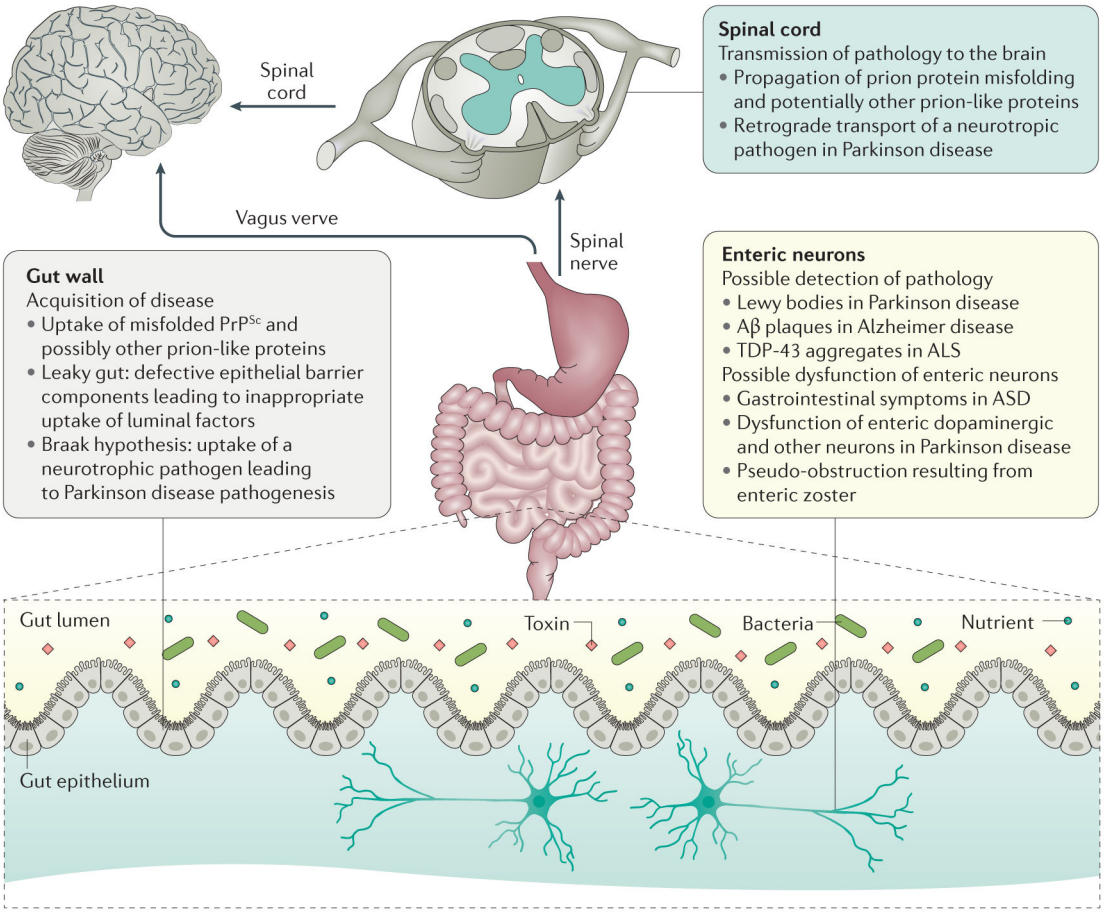

Figure 4. Summary of primary disease interactions between the gut and brain

Aβ, amyloid-beta; ALS, amyotrophic lateral sclerosis; ASD, autism spectrum disorder; PrP^{Sc}, prion protein scrapie.

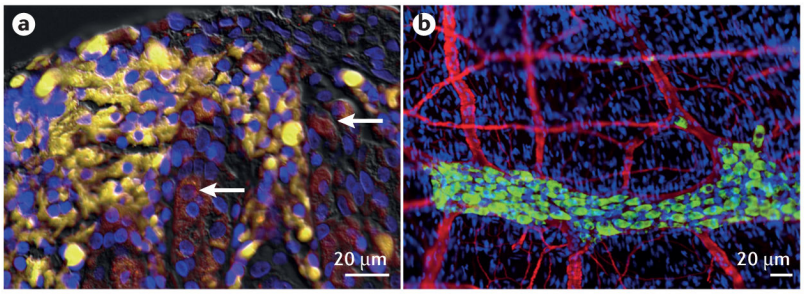

Figure 5. Enteric manifestations of lytic VZV infection of the mucosa and latent VZV infection of the ENS

a | Mucosal biopsy sample from a patient with a perforated gastric ulcer. The patient's saliva and stomach contained DNA encoding varicella zoster virus (VZV) gene products. The gastric epithelium has been infected and shows the immunoreactivity of gE (ORF68p; green) and ORF63p (red). DNA has been stained with bisbenzimide (blue). There is a slight superimposition of white light in interference contrast to allow all components of the tissue to be visualized. Newly infected cells (arrows) where the virus is spreading are mostly red fluorescent because ORF63 is an immediate early gene and gE is a late gene. The highly infected cells contain both gene products and are yellow. Scale bar = 20 μm. **b** | Ileum of a guinea pig injected with VZV-infected lymphocytes. The VZV expresses green fluorescent protein (GFP) under the control of the VZV promoter for ORF66. The red fluorescence is the immunoreactivity of β3-tubulin, a neuronal marker. Virtually all enteric neurons contain latent VZV. Note that the VZV is confined to nerve cell bodies and is not seen in neurites within the interganglionic connectives (red). Thus, the neurons are not actually producing viral particles. The restriction of ORF66-GFP to cell bodies is therefore an indicator of viral latency. DNA has been stained with bisbenzimide (blue). Scale bar = 20 μm.