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Review

Animal Models of Gastrointestinal Problems in Parkinson's Disease

James G. Greene*
Department of Neurology, Emory University, Whitehead Biomedical Research Bldg, Atlanta, GA, USA

Abstract. Gastrointestinal (GI) symptoms are being increasingly recognized as early, common, and severe features of Parkinson's disease (PD), and they are important determinants of quality of life and disability throughout the disease course. In addition, the concept that GI symptoms could represent "pre-motor" PD has been hotly debated and has recently become a driving force for investigations into the pathophysiology and neuropathology of PD. Clinical data has been supported by many pathological studies demonstrating α-synuclein neuritic pathology and neuronal loss in central and peripheral areas relevant to GI function. As understanding has matured concerning the impact of GI dysmotility on patients and its potential relationship to incipient PD, behavioral and neuropathological examination of the GI tract has become a critical aspect of evaluation and validation of PD animal models. This review will briefly summarize GI symptoms and neuropathology in PD important to model in animals, behavioral and neuropathological examination of the GI tract in animals, and the current state of modeling parkinsonian GI dysfunction in animal model systems.

Keywords: Parkinson's, gastrointestinal, enteric, constipation, gut, gastric emptying, animal models, MPTP, rotenone, synuclein

Non-motor symptoms are being increasingly recognized as early, common, and severe features of Parkinson's disease (PD), and they are important determinants of quality of life and disability throughout the disease course. In addition, the concept that certain constellations of non-motor symptoms could represent "pre-motor" PD has been hotly debated and has recently become a driving force for investigations into the pathophysiology and neuropathology of PD.

Gastrointestinal (GI) dysfunction occurs in almost every PD patient at some point. Most PD-related GI symptoms, such as constipation, bloating, and dysphagia, are results of abnormal motility of the GI tract [1–4]. GI symptoms have a highly negative impact on quality of life and frequently precede the onset of motor abnormalities [1, 5–8]. For example, there is an association between frequency of bowel movements early in life and incidence of PD such that patients with long-term constipation have 2–5 times higher risk of being diagnosed with PD later in life [6, 8].

Clinical data has been supported by many pathological studies demonstrating 'parkinsonian' neuropathology in central and peripheral areas relevant to GI function. Since the first demonstration of Lewy bodies in the enteric nervous system (ENS) in 1984, Lewy pathology has been confirmed in the GI tract in nearly every case examined pathologically [9–15]. The advent of α-synuclein immunostaining as a marker for Lewy pathology has provided a very sensitive tool to explore the neuropathological effect of PD on GI function. Not only is there extensive α-synuclein neuritic

*Correspondence to: James G. Greene, MD, PhD, Department of Neurology, Emory University, 505 H Whitehead Biomedical Research Bldg, 615 Michael St, Atlanta, GA 30322, USA. Tel.: +1 404 727 9579; Fax: +1 404 727 3728; E-mail: james.greene@emory.edu.

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pathology in the ENS, but autonomic control areas extrinsic to the GI tract, such as sympathetic ganglia and the dorsal motor nucleus of the vagus (DMV) are also dramatically affected [16–24]. In addition, neuronal loss in sympathetic ganglia and the DMV is a prominent feature of PD neuropathology that likely significantly impacts GI function [19, 25–36].

As understanding has matured concerning the impact of GI dysmotility on patients and its potential relationship to incipient PD, behavioral and neuropathological examination of the GI tract has become a critical aspect of evaluation and validation of PD animal models. In addition to the hope that animal models will provide insight into the pathogenesis of the human disease, the main goal for developing animal models of GI dysfunction in PD is to develop platforms for testing symptomatic and disease-modifying therapeutics.

MODELING GI DYSFUNCTION IN PD – WHAT TO MIMIC?

Before embarking on a discussion of animal models of GI dysfunction in PD, it is important to address characteristics of an ideal model system. While practical issues such as cost, ease of use, and reliability are all relevant, the most critical aspect is accuracy.

With that in mind, it is clear that an accurate understanding of human symptoms and pathology is required to provide the basis for preliminary validation of any proposed animal models.

Symptom and behavior modeling

As alluded to above, the majority of GI symptoms problematic in PD are related to slow motility in the GI tract. Symptoms can affect every segment from the mouth to the anus. For example, dysphagia (trouble swallowing) and sialorrhea (drooling) are well-known proximal GI tract complications of PD that have been shown to occur in up to half of PD patients [1, 5, 37, 38]. Aside from the more obvious serious complications related to them, like aspiration pneumonia, they can also contribute to decreased appetite and food intake, weight loss, and malnutrition [39–42].

Delayed and uncoordinated emptying of stomach contents is also frequent in PD occurring in 43–88% of patients studied using radiographic methods [43–48]. In addition to bloating, early satiation (feeling very full after a meal), and poor nutritional absorption, it can contribute to fluctuating motor symptoms by interfering with timely medication absorption from the duodenum [49–53].

Constipation (slowed colon transit) and defecatory dysfunction (increased straining to stool) are common in early PD (40–50% of patients) and increase in frequency (70–80%) as the disease advances [5, 54–58].

Two points concerning clinical symptoms of GI dysfunction in PD are particularly relevant to animal models. First, subjective GI symptoms are notoriously underreported by PD patients; even patients who specifically deny GI symptoms when asked usually display objective evidence of GI dysmotility when evaluated using radiographic or electrophysiological tests [43, 48, 59]. This means that the problem is significantly more prominent than suggested by surveys of clinical symptoms and that objective measures of GI function are particularly important to evaluate – not a problem for animal systems since that is all that is available. Second, different symptoms occur with different frequencies at different stages of the disease and progress at different rates, suggesting that not all GI symptoms are caused by the same underlying pathology. For example, sialorrhea is almost exclusively a complication of late stage PD, while constipation and gastric dysfunction are seen at all disease stages [1, 5]. The potential sites for neuropathology contributing to individual symptoms are varied and include the enteric nervous system (ENS), the parasympathetic and sympathetic branches of the autonomic nervous system (ANS), higher central nervous system (CNS) autonomic integrative areas, and pyramidal and extra pyramidal somatic motor areas.

Neuropathological modeling

One of the factors especially limiting the development of animal models of parkinsonian GI disease has been inadequate understanding of the causative pathological factors resulting in GI symptoms. For example, constipation can be caused by a variety of things including decreased mobility, decreased water and fiber intake, medication side effects, or specific and non-specific pathology at any level from the GI mucosa to the brain.

Several factors have led authors to hypothesize that GI symptoms are directly related to the PD disease process and not an epiphenomenon of advancing age and neurodegenerative disease [4, 60–62]. For instance, GI disturbances such as constipation and defecatory dysfunction may be sentinel events in the manifestation of PD occurring not merely early in the course of motor symptoms but heralding their appearance. This has been reported anecdotally for years, and recent data from a large scale prospective study suggests

that constipation decades earlier is associated with an increased risk of future PD [6, 8]. In addition, parkinsonian neuropathology in GI-related areas has been reported in nearly every post-mortem case in which it has been sought, and recent reports indicate it is also consistently detectable in biopsy samples from living patients [11, 17]. The remainder of this review will be based on the presumption that GI dysfunction in PD is part of the specific disease process and, as such, most likely localized to neural elements involved in controlling the GI tract.

Neuropathology has been demonstrated in several neural elements in PD, and while complete characterization of pathology in PD patients is proceeding, several points appear critical enough that animal models should recapitulate them.

The first critical pathological finding is α-synuclein neuritic pathology. As mentioned above, Lewy pathology has been described in the GI tract in over 95% of patients in which it has been specifically sought since its original description in 1984 [9, 10, 12, 14]. Initially, methods were based on standard histological stains, but recently, detection sensitivity has been somewhat improved by the use of α-synuclein immunostaining (Fig. 1). A recent paper by Beach et al detected abnormal α-synuclein in the GI tract in over 93% of cases [17]. We have recently detected pathology in 12/13 stomach samples by examining only a single six micron-thick paraffin section from 1–2 cm length tissue samples. Contrary to an early report implying increased vulnerability of VIP (vasoactive intestinal peptide) enteric neurons, Lewy pathology has not been localized to any specific neural subtype in the GI tract [11, 12]. Interestingly, synuclein pathology is not randomly distributed throughout the GI tract, but is much more abundant in proximal segments [13, 17]. In particular, we have recently described a distribution that correlates nearly perfectly with the expected extrinsic innervation of the GI tract by the vagus nerve, a finding that has previously been suggested by other groups (Greene et al., unpublished results) [13, 17]. This finding is also consistent with the known predilection of the DMV to development of α-synuclein neuritic pathology [16, 24]. It has been hypothesized that synuclein pathology occurs in the gut at least as early as in any area of the brain, but primary impact on peripheral systems has yet to be proven [15, 63, 64].

In addition to the DMV, sacral parasympathetic nuclei and sympathetic ganglia are known to accumulate abnormal α-synuclein, as are CNS centers important for the integration of autonomic inflow and outflow [20, 21, 34, 35, 65]. In the heart, elegant

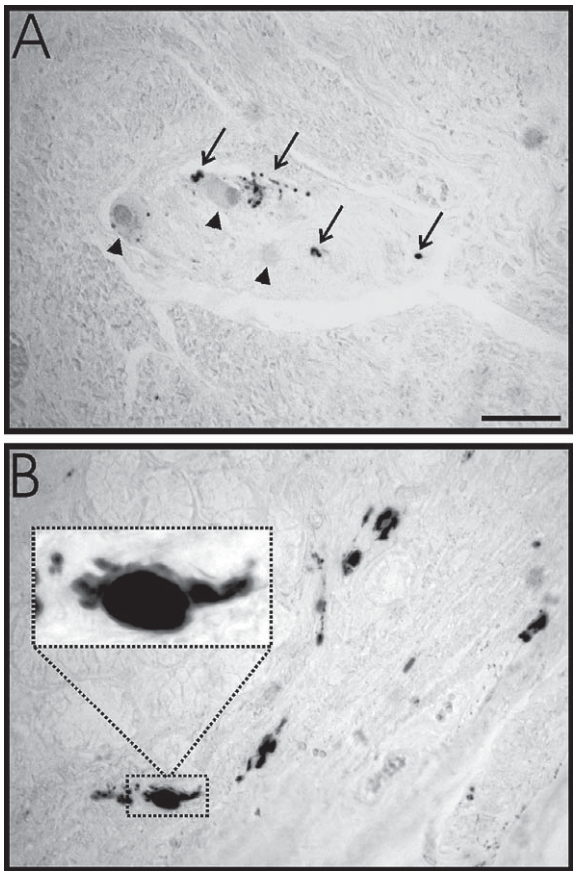

Fig. 1. Examples of synuclein pathology in the GI tract. **A**. α-Synuclein neuritic pathology (arrows) in a gastric myenteric ganglion from a patient with PD. The ganglion is centered and surrounded by intestinal smooth muscle. Counterstained myenteric neurons are denoted by arrowheads. Scale bar = 50 μm **B**. α-Synuclein neuritic pathology in the gastric submucosa from a patient with PD. Inset shows a Lewy body at higher magnification.

pathological studies have suggested that synuclein accumulates first in sympathetic terminals, causing their neurodegeneration and subsequent accumulation of abnormal synuclein in the cell body, but the same sequence of events has not yet been sought in the GI tract [33, 36, 66].

The second critical pathological finding is neuronal loss in areas referable to GI function. The DMV has long been known to degenerate in PD, in many instances showing neuronal loss to a degree similar to that seen in the substantia nigra [19, 27, 28, 30]. Loss of pre- and post-ganglionic sympathetic neurons has also been described, but whether or not those are GI-projecting cells is not known [18, 21, 22, 29, 32, 34, 62, 67]. Enteric neuron loss has not been convincingly described and is only now beginning to be sought to any

significant degree. One recent study has suggested that there may be a small loss of submucosal neurons in PD [11]. We have recently generated convincing data indicating that myenteric neuron numbers are no different between PD and control cases whether examined globally or for specific neurochemical phenotypes (Greene et al., unpublished results).

One critical aspect heretofore almost completely uninvestigated is clinicopathological correlation of GI symptoms and pathology in PD [11, 68]. Only one study to date has roughly correlated α-synuclein neuritic pathology with PD symptoms [11]. No investigation of correlation between symptoms and neuronal loss has yet been undertaken, although one might expect neuronal loss to be a later feature of the disease, and thus particularly relevant to more advanced symptoms. Although such studies would provide correlative not causative proof, a more complete understanding of the relationship between symptoms and pathology would provide needed direction to both human and animal model investigations.

With that limitation in mind, at present, an accurate animal model of GI problems in PD would encompass objective evidence of slowed GI motility as well as α-synuclein neuritic pathology in the GI tract and autonomic nuclei and associated neuronal dysfunction and loss in appropriate areas as discussed above.

BEHAVIORAL EVALUATION OF THE GI TRACT IN ANIMAL MODELS OF PD

The next two sections will address behavioral and pathological methods for validation of animal models of GI problems in PD. The focus will be on evaluation of rodents, since that encompasses the majority of work performed to date.

From a behavioral standpoint, several aspects of GI motility can be evaluated in rodents. In particular, total gut transit time, liquid and solid gastric emptying, stool frequency, and distal colon motility are most commonly and easily measured. Total transit time has sometimes been measured using heat-stable spores from *Bacillus stearothermophilus*, but is now more easily evaluated using non-absorbable fluorescent dyes [69–71]. For either reagent, animals are gavaged at time zero and stools are collected over time. The amount of indicator in stool (either colonies grown or fluorescence intensity) is plotted as a function of time (Fig. 2). Results are expressed in units of time, such as time to first appearance, time to maximum intensity, time to elimination, or mean transit time based on calculations of geometric mean. Total gut transit time can give estimates about overall GI motility, but is fairly insensitive and does not provide information about specific aspects of GI motility which may have more relevance to human disease.

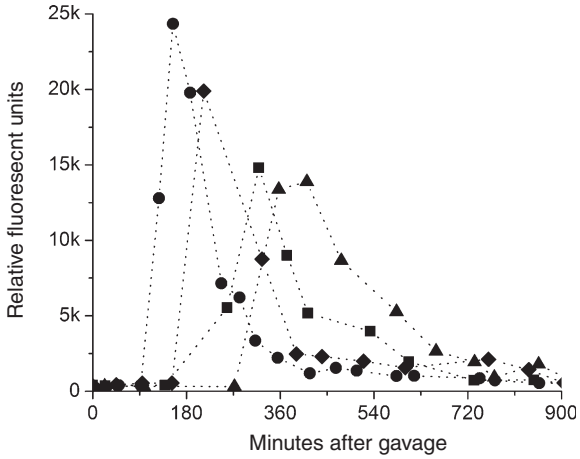

Fig. 2. Total gut transit time example. Example plots derived from 5 individual mice gavaged at time 0 with 100 μl microliters of 5 μm CW800 carboxylate infrared flouroescent dye (Li-Cor Biosciences). Stools were collected serially and scanned at the end of the experiment on an Odyssey Infrared Imaging System (Li-Cor). As expected, the peak signal was highest for the shortest transit time (circle) and lowest for the longest transit time (triangle).

Gastric emptying is typically measured in rodents by measuring residual stomach contents after administration of a known quantity of solid or liquid food. For example, in our lab, after an overnight fast, animals are given free access to rodent chow for a defined period (20–60 min), and food is weighed before and after the access period. Animals are killed 1–2 hours after food removal, and stomach contents are weighed, dried, and weighed again. Percent gastric emptying is calculated using the formula: 100 – (100 * dry stomach contents ÷ food eaten) [72, 73]. Similar methods are used for liquid gastric emptying, although since analysis is based on spectrophotometric measurement of stomach concentration of gavaged dye, a full time course must usually be performed and compared to animals killed immediately after dye administration [74].

One clear drawback to these methods is the inability to perform repetitive or longitudinal analyses due to the terminal nature of the assays. Traditional nuclear medicine measurements similar to those employed in humans have been used in rodents, but they are expensive, highly-specialized assays that require substantial infrastructure and experience to perform and analyze properly [75–77]. While still complex, ^{13}C-octanoic acid breath analysis has proven more accessible to the

research community. Liquid or solid meals containing a known quantity of ^{13}C-octanoic acid are administered, and animals are placed in individual sealed chambers with continuous airflow. Breath samples are analyzed for $^{13}CO_2$ content, and gastric half emptying times ($t_{1/2}$) are calculated from the resulting $^{13}CO_2$ excretion curves [78–80].

Measurement of colon motility is typically performed using two complementary methods. First, one-hour stool frequency is a good indicator of motility of the entire colon. Stool frequency directly correlates with stool water content providing a good assessment of water absorption, which is the main function of the proximal colon [69, 70, 72, 74]. Distal colon motility can be more specifically measured by the amount of time required to expel a bead inserted 2 cm past the anal verge; this is also an assay of defecation [69, 70].

There are a number of caveats to the interpretation of behavioral tests of GI motility in rodents. For example, differences in food and water intake may artificially alter GI motility indices or conversely may be a symptom of dysmotility. For example, decreased food intake will result in decreased fecal output, not necessarily from slowed motility, but from decreased substrate. From another viewpoint, animals with poor gastric motility tend to change their food intake. For instance, after vagotomy, rats tend to eat less, with smaller meals over a longer period of time, since their stomachs do not empty efficiently [81]. As discussed below, we have encountered this interpretive difficulty examining the effect of rotenone intoxication on GI motility in rats. The rats have decreased stool output that correlates with decreased gastric emptying, but deciding which effect is primary is nearly impossible [72].

Especially relevant to PD model systems, decreased motor function and activity level can result in alterations in transit time through the entire GI tract as well as through specific segments. Time of day is another important variable to be noted, since food and water intake and activity level are dramatically affected by circadian rhythm. Age and weight should also be matched as closely as possible for comparison groups.

Of particular note, stress and anxiety level can significantly impact GI motility [82–84]. For example, when animals are placed in the novel environment, such as a clean empty cage, there is an initial rapid defecation (Fig. 3). It is especially important to take this time course into account when evaluating the impact of drugs on colon motility and adjust assay conditions to allow for absorption of drug prior to testing. Furthermore, interpretation of results must be cautious if the therpeutic agent of interest has anxiolytic or anxiogenic properties. To make matters more complicated, gastric emptying testing is typically affected in the opposite direction; stress causes a delay in gastric emptying [83]. When performing that assay we and others perform 'dry runs' of fasting and food exposure in the 7–10 days prior to the test to minimize the effect. Even so, interpretation of results is complicated. As discussed below, mice with very low levels of VMAT2 replicate several motor and non-motor symptoms of PD. From a GI standpoint, they show delayed gastric emptying and accelerated colon motility. While this could be due to direct effects of abnormal catecholaminergic neurotransmission on GI function, the accentuated anxiety phenotype discernable in these mice may secondarily result in the observed GI effects [85].

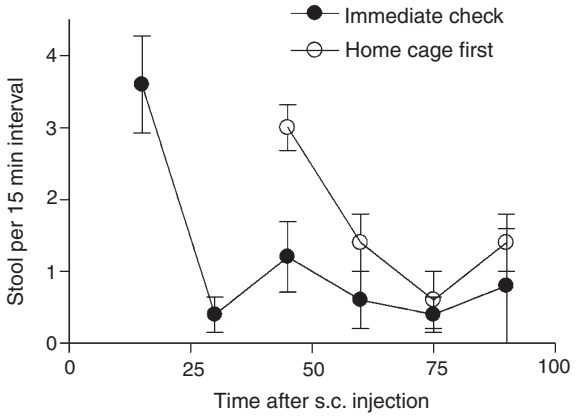

Fig. 3. Time course of defecation in a novel environment. Mice were injected subcutaneously with saline at time 0 and placed in a clean cage either immediately (filled) or 30 min (open) after injection. Stools were collected in 15 min epochs. Note the rapid defecation associated with entering the new environment.

NEUROPATHOLOGICAL EVALUATION OF GI DYSFUNCTION IN ANIMAL MODELS OF PD

This section will not discuss specific methods, but instead will describe the neural systems, both intrinsic and extrinsic to the gut, that modulate GI motility (Fig. 4). Neuropathological features to be examined in animal models should include α-synuclein aggregation and neuronal loss (as described in the *Neuropathological modeling* section).

The central autonomic network receives viscero-ceptive, humoral, and environmental information and contributes to autonomic, endocrine, behavioral motor, emotional, attentional, and anti-nociceptive responses

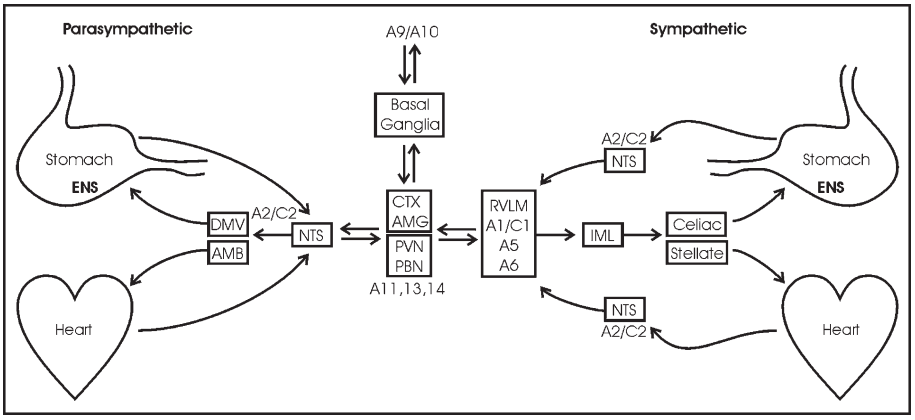

Fig. 4. Schematized autonomic circuitry including catecholaminergic cell groups. Scheme is highly simplified and designed to highlight the interaction between central and peripheral neural circuits. ENS, enteric nervous system; DMV, dorsal motor nucleus of the vagus; AMB, nucleus ambiguus; NTS, nucleus tractus solitarius; CTX, cortex (primarily anterior cingulate, insula, and orbital); AMG, amygdala; PVN, paraventricular nucleus; PBN, parabrachial nucleus; RVLM, rostral ventrolateral medulla; IML, intermediolateral cell column. **(A)** indicates noradrenergic (1, 2, 5, 6) or dopaminergic (9–14) cell group. **(C)** indicates adrenergic cell group. Post-ganglionic sympathetic neurons (celiac and stellate ganglia, among others) are noradrenergic.

[86, 87]. It is anatomically and functionally composed of scattered interconnected circuitries that are involved in the sensory and motor components of autonomic function [88]. The structures forming the central autonomic network are distributed at all levels of the nervous system, including the cerebral cortex, basal forebrain, basal ganglia, midbrain, thalamus, hypothalamus, pons, and medulla [89]. From the brainstem, information is carried to and from the organs via the sympathetic and parasympathetic autonomic pathways (Fig. 4).

The insula, cingulate, and medial prefrontal cortices are involved at the highest level of integration of autonomic responses [86, 89–91]. The prefrontal cortex, and its medial part especially, projects to the ventromedial part of the caudate nucleus [92], the globus pallidus [93], and the substantia nigra [94], the insula projects to the ventral part of the caudate nucleus [95]. These areas of cortex also project to the parafasicular nucleus of the thalamus [96] which receives projections from the striatum [97, 98] and the substantia nigra [99]. In addition, they project to the nucleus basalis [93, 100], the extended amygdale [93, 101], the hypothalamus [102, 103], and the brainstem [96] where they interface with the peripheral autonomic system.

The relationship between basal ganglia and autonomic dysfunction has been examined in a few previous studies. In rats, salivary response is dependent upon the activity of dopamine receptors in the striatum and decreased by striatal lesion [104–106]. Baroreflex regulation of blood pressure has been reported to be dependent on the nigrostriatal pathway in rodents [107–111]. In addition, gastrointestinal motility can be modulated by basal ganglia activity, especially in the caudate [85, 112, 113].

Signals from higher centers (cortex, amygdala, hypothalamus, etc.) are integrated with sensory input in the brainstem to modulate central autonomic outflow through the sympathetic and parasympathetic segments of the ANS. Cell groups in the rostral ventrolateral medulla (RVLM) receive central input from higher centers and peripheral input from the nucleus of the tractus solitarius (NTS) and project to preganglionic sympathetic neurons in the spinal cord. These neurons in turn project to peripheral sympathetic ganglia which send postganglionic, primarily noradrenergic, fibers to the organs. Gastrointestinal sympathetic innervation is primarily to the myenteric plexus and vasculature [87].

Peripheral parasympathetic drive is carried by the cranial nerves or those originating from sacral spinal segments. Almost all of the parasympathetic innervation of the heart and GI tract is contained in the vagus nerve. Fibers projecting to the heart originate in the nucleus ambiguus and fibers projecting to the GI tract the dorsal motor nucleus of the vagus (DMV). Preganglionic parasympathetic neurons relay onto the enteric nervous system (ENS).

The ENS is semi-autonomous network of neurons that lines nearly the entire longitudinal extent of the GI tract that coordinates precise temporal coordination of inhibition and excitation of GI smooth muscle leading

to effective peristalsis. It consists of a deep myenteric and more superficial submucosal plexus [114]. The myenteric plexus is thought to be the more important of the two in terms of controlling motility, but they work in concert. Though neurons producing virtually every neurotransmitter seen in the central nervous system have been identified within the ENS, acetylcholine serves as a primary excitatory neurotransmitter, and nitric oxide and vasoactive intestinal peptide (VIP) play primarily, but not exclusively, inhibitory roles [114].

Multiple GI reflexes are mediated by the ENS and modified by central influences. For example, fatty contents in the duodenum stimulate vagal afferent fibers to excite inhibitory neurons in the NTS. Activation of those NTS neurons inhibits outflow from the DMV, decreasing gastric motility and delaying gastric emptying [115]. Modulation of GI reflexes is iterative in nature, with multiple anatomical and functional loops at nearly every level of the nervous system.

As discussed above, in human PD, cortical areas, basal ganglia, brainstem nuclei (including the DMV), sympathetic ganglia, and the ENS all develop abnormal accumulations of α-synuclein. Several areas, including the DMV and autonomic ganglia lose neurons. Thus, this extensive enteric and autonomic circuitry needs to be considered when evaluating pathology related to the GI tract in PD model systems.

ANIMAL MODELS OF GI PROBLEMS IN PD

In this section, we will summarize data reported from several common PD model systems referable to the GI tract. Nearly all of the published data to date is from rodent model systems, so those will be the focus; data from non-human primate experiments will be included when available. We will first discuss toxic and genetic models that primarily target dopaminergic neurons and follow that up with a discussion of more generalized PD models, specifically mice with abnormalities in α-synuclein expression.

Models based on selective catecholaminergic dysfunction

As reviewed above, the pathological lesion(s) causing GI dysfunction in PD are unknown. It is possible that since dopamine depletion produces most of the motor symptoms that it contributes to GI symptoms as well. A single article in 1995 suggested that there was dopamine depletion in the colon from PD patients [68]. Using an anti-dopamine antibody, it was noted a majority of PD patients had lower numbers of positive neurons; in one depicted case, the loss was nearly complete. However, in the same samples, there was no difference in numbers of tyrosine hydroxylase-positive neurons which is usually considered a more accurate marker for dopamine neurons. As such, the reported drop in colonic dopamine neurons should be interpreted cautiously pending replication.

The most common animal model of PD employs systemic injection of the selective dopamine neurotoxin MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine). Selective vulnerability of central dopamine neurons has been well-described in mice after a variety of doses and time courses of MPTP injection [116–122]. In monkeys, the model is even more convincing, reliably reproducing not only graded dopamine neuron loss, but also most of the motor signs and symptoms associated with parkinsonism in humans [123–125].

The effects of MPTP administration on GI function have been reported several times over the past five years, and all studies are in agreement [74, 126, 127]. We reported a 40% decrease in myenteric dopamine neurons after MPTP intoxication in mice that was associated with electrophysiological and behavioral changes consistent with enteric dopamine depletion; unpublished data from our lab also indicates an associated depletion of gastrointestinal dopamine as measured by HPLC [74]. Decreased catecholaminergic innervation of the GI tract in mice after systemic MPTP has been confirmed by two separate laboratories using RT-PCR, western blot, and immunostaining [126, 127]. In addition, one report suggests a similar loss of myenteric catecholaminergic neurons in the colon occurs in MPTP-treated monkeys [128].

From a behavioral standpoint, we have described a transient *increase* in colon motility of about 40% after MPTP administration. Obviously, this is the opposite of what is observed in PD; however, it is the expected physiological response to enteric dopamine depletion. For example, dopamine antagonists, such as metoclopromide and domperidone, have been used for years as effective pro-motility agents [129–132]. Conversely, dopamine replacement therapy is known to slow GI motility in the stomach and colon. In mice, knockout of the D2 dopamine receptor causes a profound increase in GI motility and decreased transit time similar to that observed after MPTP intoxication [70]. In addition, mice deficient in VMAT2 (vesicular monoamine transporter 2), and thus profoundly hypocatecholaminergic, also display accelerated colon transit; similar to MPTP,

this GI symptomatology occurs despite the fact that VMAT2 mice mimic many of the other motor and non-motor symptoms of parkinsonism [85]. Taken together, these results indicate that enteric dopamine depletion *does not* cause GI symptoms in PD regardless of its presence (or lack thereof) in patients.

A small amount of preliminary evidence suggests that central dopamine depletion might cause different results. Bilateral 6-hydroxydopamine (6-OHDA) injection into the rat midbrain, which causes severe selective loss of midbrain dopamine neurons, has been suggested to cause an *increase* in tyrosine hydroxylase and dopamine transporter protein levels in the stomach, duodenum, and colon [127]. From a behavioral standpoint, Blandini et al. [133] have implied that central dopamine lesions caused by 6-OHDA injection may cause decreased stool output and slowed colon motility [133]. The data in that report was somewhat confounded by decreased water intake in the treated animals, but both reports provide intriguing evidence that central dopamine systems may modulate the neurochemistry and motility of the GI tract. The mechanism for such modulation is entirely speculative, but could involve interaction between the central autonomic network and the enteric nervous system. There is precedent for such an effect in that lesion of the noradrenergic locus ceruleus in the pons (also severely affected in human PD) causes abnormal myoelectric rhythms in the GI tract [134–137].

Rotenone

Rotenone is a naturally-occurring mitochondrial poison that is used agriculturally as an organic pesticide and pissicide. Systemic administration of the compound to rats has been used to mimic behavioral and neuropathological findings of human PD, including bradykinesia, selective nigral dopamine neuron loss, and α-synuclein aggregation with varying degrees of consistency and success [138–144]. We have shown that rotenone causes delayed gastric emptying in rats, even in the absence of motor symptoms and frank neurodegeneration in the CNS or ENS [72]. This finding has been replicated and expanded to include suggestion of α-synuclein aggregation and some myenteric neuronal loss in the ileum 6 months after initiation of rotenone treatment [71].

A study has recently been published describing progressive α-synuclein aggregation and nigral dopamine neuron loss after gastric administration of rotenone over a period of months [145]. The α-synuclein aggregation was reported in the enteric nervous system and with progression to involve the spinal cord, brainstem (specifically the DMV), and ultimately the substantia nigra. This is the exact propagation scheme of α-synuclein neuritic pathology proposed by Braak et al. [16] in human PD and would tend to support the hypothesis that exposure to an environmental agent could play a role in this progression [16, 146]. The results are very provocative, but the PD research community maintains legitimate skepticism pending replication.

α-Synuclein transgenic mice

Mutation in the gene for α-synuclein was described in 1997 as a rare cause of familial autosomal dominant parkinsonism. Shortly thereafter Lewy bodies, were found to contain large amounts of aggregated α-synuclein in both familial and sporadic cases [147, 148]. As detailed above, in addition to being found in multiple brain regions, synuclein pathology has been described consistently in peripheral nervous tissue, including the ENS [11, 15, 17, 62].

Generation of α-synuclein transgenic mice has had mixed success in replicating pathological and behavioral features of parkinsonism in mice [149–153]. This section will focus on a few recent studies in which α-synuclein transgenic mice have been found to have GI abnormalities [69, 154].

Mice expressing wild-type human α-synuclein under control of the Thy1 promoter were shown to have variable abnormalities in colon motility [154]. These alterations included an increased retention of stool pellets in the colon two hours after a meal, decreased stool production in a familiar environment, and increased bead expulsion time. Conversely, there was an increase in stool production in response to a novel environment. As mentioned, these changes were highly variable. They were not correlated with any pathological evaluation of tissue as relates to neuronal damage or α-synuclein accumulation.

Another recent series of experiments investigated aspects of GI function and pathology in mice expressing mutant human α-synuclein (A53T or A30P) on a P1 artificial chromosome (PAC) in the absence of mouse synuclein [69]. Mice expressing both types of mutant synuclein expressed high levels in brain and colon. Mice displayed lengthened whole gut transit time and increased distal colon transit time as measured by fluorescent dye transit and bead expulsion, respectively. The A53T mice were more severely affected, and in all cases male mice were affected to a much more significant degree. Human synuclein immunore-

activity was demonstrated in enteric neurons in both the submucosal and myenteric plexuses; there was some coincident staining with tyrosine hydroxylase and nitric oxide synthase. The majority of enteric α-synuclein was localized to neural processes and synaptic varicosities.

This report is very interesting for several reasons, including expression of synuclein in the context of presumed upstream regulatory elements, expression in and around the ENS especially in synaptic elements, and associated measurable GI dysfunction. As in all synuclein transgenic models, there are several cautionary caveats. First, these mice displayed a massive over-expression of synuclein in the colon, which is not a part of human PD. Second, although there is a male predilection toward development of PD itself, the same cannot be said for development of GI symptoms in PD patients making the meaning of the dramatic male-female difference in the mice unclear. Third, no pathological changes aside from α-synuclein overexpression were demonstrated [69]. Nevertheless, this provides an intriguing model system for further study, and more detailed exploration of it is clearly warranted.

FUTURE DIRECTIONS

Exploration of GI problems in PD animal models is in the early stages. The goal of these types of experiments is not only to replicate PD symptoms to provide a platform for testing symptomatic and disease-modifying therapeutics, but also to provide leads for return investigation in patients concerning the pathophysiology of PD.

In general, the 're-discovery' of non-motor symptoms as an integral part of PD pathophysiology has redirected the goal of animal modeling from an essentially exclusive focus on dopamine neurons to replicating PD as a whole. This understanding has called into question not only the accuracy of current models, but more critically, their relevance and validity. As a result, investigating GI symptoms in current PD models and developing new models based on current theories of PD pathophysiology involving the GI tract is critical to mimicking human PD in the laboratory setting. The ideal laboratory model of PD will encompass all symptoms and neuropathology encountered in the human disease and will dramatically improve not only our understanding of the disease process, but our chances of finding a cure.

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