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Loss of enteric dopaminergic neurons and associated changes in colon motility in an MPTP mouse model of Parkinson's disease

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Abstract

Gastrointestinal (GI) dysfunction is the most common non-motor symptom of Parkinson's disease (PD). Symptoms of GI dysmotility include early satiety and nausea from delayed gastric emptying, bloating from poor small bowel coordination, and constipation and defecatory dysfunction from impaired colonic transit. Understanding the pathophysiology and treatment of these symptoms in PD patients has been hampered by the lack of investigation into GI symptoms and pathology in PD animal models. We report that the prototypical parkinsonian neurotoxin, MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), is a selective dopamine neuron toxin in the enteric nervous system (ENS). When examined 10 days after treatment, there was a 40% reduction of dopamine neurons in the ENS of C57Bl/6 mice administered MPTP (60 mg/kg). There were no differences in the density of cholinergic or nitric oxide neurons. Electrophysiological recording of neural-mediated muscle contraction in isolated colon from MPTP-treated animals confirmed a relaxation defect associated with dopaminergic degeneration. Behaviorally, MPTP induced a transient increase in colon motility, but no changes in gastric emptying or small intestine transit. These results provide the first comprehensive assessment of gastrointestinal pathophysiology in an animal model of PD. They provide insight into the impact of dopaminergic dysfunction on gastrointestinal motility and a benchmark for assessment of other PD model systems.

Keywords

Parkinson; gastrointestinal; dopamine; colon; gastric; MPTP; constipation; enteric; gut; myenteric

Introduction

Gastrointestinal dysfunction is a prominent non motor feature of Parkinson's disease (PD). PD patients experience symptoms that span the entire alimentary tract including abnormal salivation, dysphagia, delayed gastric emptying, constipation, and defecatory dysfunction (Pfeiffer, 2003, Pfeiffer and Quigley, 1999). Dysfunctional motility is the pathophysiological mechanism underlying many of these symptoms. This dysmotility contributes directly to the morbidity of PD and complicates the disease's clinical management. For example, in the

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stomach, delayed emptying leads to nausea, contributes to weight loss because of decreased food intake, and adds to fluctuations in motor impairment from variable absorption of medication (Djaldetti, et al., 1996, Goetze, et al., 2006, Goetze, et al., 2005, Kurlan, et al., 1988). In the colon, longer transit time due to poor motility causes harder stools and constipation by increasing the absorption of water (Ashraf, et al., 1995, Edwards, et al., 1992, Ueki and Otsuka, 2004). The exact mechanism of motility dysfunction in PD is poorly understood. Lack of understanding of the changes in the gastrointestinal tract in PD has led to limited success in the treatment of gastrointestinal dysfunction in PD.

Control of gastrointestinal motility arises from both local and central locations. Local control is directed by the intrinsic enteric nervous system (ENS), a semiautonomous network of nerves that consists of a deep myenteric and more superficial submucosal plexus (Johnson, 2001). The myenteric plexus is the more important of the two in terms of controlling motility. Circuitry in this plexus controls the contraction and relaxation of the circular and longitudinal smooth muscle that line the length of the gastrointestinal tract. Though neurons producing virtually every neurotransmitter seen in the central nervous system have been identified within the ENS, acetylcholine serves as the primary excitatory neurotransmitter, while nitric oxide and vasoactive intestinal peptide are the prominent inhibitory transmitters (Johnson, 2001). Effective peristalsis depends on precise temporal coordination of inhibition and excitation of the two layers of smooth muscle. Central control arises from the autonomic inputs imposed on the local enteric nervous system. Parasympathetic, cholinergic innervation originates primarily in the dorsal motor nucleus of the vagus (DMV) in the medulla and generally promotes increased motility. Sympathetic input originates in paravertebral and sacral ganglia, and generally serves to inhibit gastrointestinal motility.

Although PD has traditionally been considered a disease of dopaminergic neurons in the substantia nigra, pathological analyses of brain and gastrointestinal samples from PD patients have suggested neuronal loss in other areas. Lewy bodies have been described in both the myenteric and submucosal plexuses of the ENS (Braak, et al., 2006, Kupsky, et al., 1987, Singaram, et al., 1995, Wakabayashi, et al., 1990, Wakabayashi, et al., 1988, Wakabayashi, et al., 1989), and other research has found lower levels of enteric dopaminergic neurons in ENS of PD patients (Singaram, et al., 1995). Central areas associated with gastrointestinal motility have also been implicated. For example, Braak et. al. (2003) suggest that the DMV is perhaps the earliest central site affected in PD because α -synuclein pathology in the DMV was found in brains without damage to the substantia nigra (Braak, et al., 2003).

One factor that has made research into the etiology of gastrointestinal dysfunction and evaluation of effective treatments difficult has been the absence of an animal model for this aspect of PD. The aim of the present research was to evaluate gastrointestinal dysfunction in a well-described animal model of PD using the selective dopamine neurotoxin MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) (Jackson-Lewis, et al., 1995). Animals treated with MPTP were behaviorally evaluated for signs of dysmotility in the stomach, small intestine, and colon. In addition, samples were examined for evidence of neuronal dysfunction and loss in the ENS. The results suggest that MPTP is a selective dopaminergic neurotoxin in the ENS and causes changes in colonic motility.

Materials and Methods

Animals and MPTP treatment

All experimental procedures were in accordance with the NIH Guide for the Care and Use of Experimental Animals and approved by the Emory University Institutional Animal Care and Use Committee. Male C57Bl/6 mice (6-8 months old) were administered MPTP in saline vehicle at a total dose of 60 mg/kg of MPTP given in four intraperitoneal injections 2 hours

apart on a single day (Teismann, et al., 2003). Controls received saline using the same protocol. Following behavioral analysis, mice were anesthetized with isoflurane and killed by decapitation. Brain, stomach, small intestine and colon were removed from the animals for electrophysiological and histopathological analyses.

Measurement of colon motility by one-hour stool collection

Each animal was placed in a clear plastic cage for one hour. Stools were collected immediately after expulsion and placed in sealed tubes. The total stools were weighed to provide a wet weight, then dried overnight at 65°C and weighed again to provide a dry weight (Li, et al., 2006).

Liquid gastric emptying

Following a 12-hour fast, each animal was administered 0.1 ml of methylene blue labeled 10% glucose by oral gavage. Following sacrifice (0-60 minutes after gavage) the animals' stomachs were clamped with a string tied both at the lower esophageal sphincter and the pylorus to prevent dye leakage. Stomachs were weighed and frozen at -70°C. To assess the amount of methylene blue that remained in the stomachs, they were ground and emulsified in 15 ml of 0.1 N NaOH for 30 seconds. An additional 5 ml 0.1 N NaOH was added, and the sample was allowed to settle at room temperature for 1 hour. Five ml of the supernatant was centrifuged at 1,250 g for 20 minutes at 4°C. Methylene blue absorbance of the resulting supernatant was measured at 570 nm (Anitha, et al., 2006). Results were normalized to the absorbance at time zero.

Solid gastric emptying

Following a 12-hour fast, mice were allowed free access to food for one hour. Two hours later, animals were killed and the stomach contents were weighed. Food was weighed before and after the feeding period to determine the amount consumed (Whited, et al., 2006).

Small intestine transit

The small intestine was removed along with the stomach as described for liquid gastric emptying. Small intestine transit was assessed by measuring the distance from the pylorus the dye front had traveled (Anitha, et al., 2006).

Isometric muscular force recording

Segments of proximal colon were collected for isometric muscle recordings using electrical field stimulation (EFS) according to previously published methods (Anitha, et al., 2006, Anitha, et al., 2006). Samples of longitudinal or circular muscle along with their enteric innervation were attached to two hooks placed between platinum electrodes suspended in Krebs buffer either perpendicular to normal colonic flow (circular) or in parallel (longitudinal). Each sample was tightened to a force of 3 mN and allowed to equilibrate for at least 1 hour. Amplitude and frequency of contractions were assessed following this normalization period. Contraction of circular muscle by EFS (24V, 20Hz, 5 msec pulse width, 30 sec) was assessed in the presence of L-NAME (100 µM). Magnitude of relaxation induced by EFS (24V, 8Hz, 5 msec pulse width, 30 sec) was assessed in longitudinal muscle after incubation with atropine (1 µM). For quantification, the average force from the 30 second period of EFS was compared to the average force from the 30 second period immediately prior to EFS. Results are expressed as percentage contraction or relaxation as compared to baseline force.

Immunocytochemistry

Samples of ileum immediately adjacent to the cecum were prepared for staining according to previously published methods (Anitha, et al., 2006). Ileum was excised from the mouse, and the contents were washed out with phosphate buffered saline (PBS). Each section was then cut along the mesentery, stretched flat on a silicone coated plate, and fixed in 4% formaldehyde at room temperature for two hours. After fixing, longitudinal muscle and the underlying myenteric plexus were peeled from the submucosa using a dissecting microscope. Sections were then stained for tyrosine hydroxylase (1:100; rabbit anti-TH; Chemicon) or choline acetyltransferase (1:100; goat anti-ChAT; Chemicon). On the first day, sections were treated with 0.3% hydrogen peroxide in de-ionized water, washed in 1% tris-buffered saline (TBS), blocked with NGS or NHS for 30 minutes, and then incubated with primary antibody solution overnight at 4 °C. The following day, sections were washed with TBS and incubated with secondary antibody for 1 hour (1:500; biotinylated goat anti-rabbit or donkey anti-goat; Jackson). Sections were then exposed to ABC solution (Vectastain) for 1 hour, washed, and incubated with DAB in de-ionized water for ten minutes. After the final wash, sections were mounted on glass slides, dehydrated, and coverslipped.

NADPH diaphorase staining

Each section of peeled tissue was washed in PBS and incubated in diaphorase solution (NADPH, 1 mg/ml; nitroblue tetrazolium, 0.1 mg/ml; and 0.3% Triton-X 100 in PBS) for 1 hour at 37 °C. After washing, sections were mounted on glass slides, dehydrated, and coverslipped (9).

Neuron counting

Neuron counting was performed by a reader blinded to treatment group. All TH-positive neurons in a section were counted along with all visible ganglia; results are presented as positive neurons per ganglion. ChAT- and NADPH-positive neurons were counted using a 1 mm² grid, and the total number of positive neurons in each of five randomly chosen grids was recorded (Anitha, et al., 2006).

Tyrosine hydroxylase immunoblotting

Tissue samples were homogenized in Tissue Protein Extraction Reagent, TPER (Pierce) supplemented with 700 U/ml DNase and 1% β-mercaptoethanol (Sigma) and centrifuged at 10,000×g for 10 min. Supernatant was collected, and protein content was assayed using Bio-Rad protein assay (BioRad) according to manufacturer's protocol. Protein samples were run on 12% polyacrylamide gels, transferred to PVDF membranes (Immobilon, Millipore) and probed for tyrosine hydroxylase (mouse polyclonal 1:1000, Chemicon). Rabbit polyclonal anti-MAPK (1:1000, Cell Signaling Tech, Beverly, MA) was used to confirm equal loading of wells. AlexaFluor 680 donkey anti-mouse (1:5,000; Molecular Probes, Eugene, OR) and IRDye 800 goat anti-rabbit (1:5,000; Rockland, Gilbertsville, PA) secondary antibodies were used. Blots were dried, scanned, and quantified with an Odyssey Infrared Imaging System (Li-Cor Biosciences) (Betarbet, et al., 2006).

Statistics

Results are expressed as means. Data were compared using t-tests, and a p-value of less than 0.05 was considered significant.

Results

MPTP selectively damages catecholaminergic neurons in the ENS

When examined after 10 days, MPTP-treated animals had a greater than 40% reduction in the density of TH-positive neurons in the ENS (Fig 1A). Due to the relatively low number of TH-positive enteric neurons, results are expressed as TH-positive neurons per ganglion to normalize for section area (Fig 1B). Quantitative assessment of fiber density was not performed, but there were clearly TH-positive fibers remaining in the myenteric plexus. There were no significant differences in NADPH (nitric oxide) or ChAT (cholinergic) neuron density between saline- and MPTP-treated animals (Fig 2). As expected for this model, immunoblot evaluation of the brain revealed significant decreases in TH-immunoreactivity in the substantia nigra and striatum of 57% and 52%, respectively ($p < 0.05$ v. saline) in MPTP-treated animals (not shown).

MPTP-treated mice exhibit a physiological defect of inhibitory neurons in the ENS

Circular muscle from MPTP-treated animals (10 days) showed significantly greater neural-mediated contraction after EFS than circular muscle from saline-treated animals (Fig 3A, B). In the presence of L-NAME, EFS induced contraction in both saline and MPTP samples; rebound contraction following cessation of EFS was intermittently observed and tended to be more robust in MPTP-treated samples (Fig 3A). Isometric relaxation of longitudinal muscle was dramatically impaired following MPTP (Fig 3C, D). In the presence of atropine, EFS induced a quick relaxation of longitudinal muscle in control samples that could easily be distinguished from the baseline rhythmic oscillation of longitudinal muscle. Relaxation was much less prominent in samples from MPTP-treated mice (Fig 3C). Taken together, these results indicate dysfunction of an inhibitory subpopulation of ENS neurons.

MPTP-treated mice exhibit a transient increase in colon motility

MPTP-treated animals had a significantly higher one-hour stool frequency than saline-treated animals when assayed 2-3 days after treatment (Fig 4A). By 8-10 days after treatment, stool frequency was not different between the groups. It remained similar for at least 21 days (not shown). There was a robust correlation between percent solid matter and stool frequency ($R = -0.69$) in both saline- and MPTP-treated animals (Fig 4B), supporting the validity of one-hour stool collection as a measure of colon motility.

MPTP treatment does not affect gastric emptying or small intestine transit

There was no difference in either liquid or solid gastric emptying between saline- and MPTP-treated mice (Fig 5). Both groups of animals consumed the same amount of food and water during the ad libitum phase of the solid gastric emptying studies (not shown). Small intestine transit, as measured by movement of methylene blue dye over time after ingestion, was identical between the two groups (Fig 6). Gastric emptying and small intestine transit were measured in separate sets of animals both 3 and 10 days after MPTP administration. There were no differences at either time point.

Discussion

This is the first comprehensive description of gastrointestinal dysmotility in an animal model of Parkinson's disease. Parenteral administration of MPTP using a dosing paradigm that consistently causes dopaminergic neurodegeneration in the substantia nigra of mice concomitantly induces dopaminergic neurodegeneration in the enteric nervous system (ENS) that is associated with behavioral and electrophysiological consequences. The accelerated colon motility and colonic muscle relaxation defect observed after MPTP intoxication are

consistent with the inhibitory nature of dopamine neurons in the ENS (Li, et al., 2006, Walker, et al., 2000).

Immunostaining results provide direct evidence for dopaminergic damage in the ENS. Tyrosine hydroxylase is a marker of catecholaminergic neurons, but adrenergic and noradrenergic inputs to the GI tract are largely extrinsic. Thus, the majority of TH-positive neurons with cell bodies in the myenteric plexus can be considered dopaminergic (Li, et al., 2004). Furthermore, ten days after the insult, the absence of TH-positive cell bodies most likely represents a loss of cells, rather than a mere downregulation of tyrosine hydroxylase (Jackson-Lewis, et al., 1995). The finding of reduced TH-positive neurons in the myenteric ganglia in the absence of any effect on cholinergic or nitric oxide neurons confirms that MPTP is selectively toxic to dopamine neurons in the ENS, much as it is in the brain (Bove, et al., 2005, Heikkila, et al., 1984, Jackson-Lewis, et al., 1995). Our results suggest that nigral and enteric dopamine neurons exhibit grossly similar levels of sensitivity to parenteral MPTP, but the relative vulnerability between gut and brain dopamine neurons is difficult to assess. Given the graded levels of susceptibility to MPTP of central dopamine neurons (e.g., substantia nigra > ventral tegmental area > hypothalamus), investigation of lower or more chronic doses of MPTP may provide provocative information about where enteric dopamine neurons fit into that continuum (Braak, et al., 2003, German, et al., 1992, Varastet, et al., 1994).

Our electrophysiological recording results provide confirmatory evidence for a dopaminergic defect in the ENS of MPTP-treated mice. Colon muscle from MPTP-treated mice displayed augmented contraction and impaired relaxation in response to electric field stimulation of enteric neurons. These results are complimentary and indicate impaired function of an inhibitory subpopulation of enteric neurons, in this case dopamine neurons. This is in agreement with previous functional evaluations of the effect of dopamine on enteric neural-mediated muscle contraction (Walker, et al., 2000). Exogenous dopamine has been shown to antagonize colon muscle contractility in a receptor-dependent manner. Endogenous, physiologically released dopamine has a similar effect mediated predominantly by D1 and D2 dopamine receptors (Li, et al., 2006, Walker, et al., 2000).

Given our neuropathological and electrophysiological results, we hypothesize dopamine neuron dysfunction and death to be the cause of the transient increase in colon motility seen after MPTP intoxication. Decreased dopaminergic inhibitory tone results in more rapid colonic transit due to a relative abundance of stimulatory neuronal input (Li, et al., 2006, 9). The mechanisms by which motility is restored following its initial perturbation by MPTP are unknown. It is possible that surviving dopamine neurons become more active to compensate for dopamine neuron loss, but that is unlikely given the persistent electrophysiological deficit. It is more likely that alteration in other ENS transmitters offsets the dopamine dysfunction. In order to favor contraction or relaxation, our physiologic testing of ENS function was done in the presence of L-NAME or atropine, respectively. By removing competing inhibitory (nitric oxide) or excitatory (cholinergic) inputs, evaluation of less prominent systems involved in contraction and relaxation, such as dopamine, is more straightforward. However, this strategy makes it difficult to evaluate the effects of competing compensatory influences that may result in behavioral normalization of colon function. Further pharmacological analysis of enteric neuron function after MPTP may tease out the involvement of other neurotransmitter systems in the resumption of normal colon motility.

Despite several different animal groups, experimental paradigms, and assessment time points, we were unable to detect any differences in gastric emptying (liquid or solid) or small intestine transit following MPTP intoxication. This is interesting, given the recently described distribution of dopamine neurons in the murine ENS, which suggests that dopamine neurons are more numerous in the proximal GI tract than the distal (Li, et al., 2004). It is possible that

dopamine denervation has a more profound effect on the physiology and function of the lower GI tract than the upper precisely due to that proximal to distal gradient; greater reserve capacity might make upper GI function more resistant than lower GI function to similar degrees of dopaminergic denervation. It may also be that since central influences play a larger role in upper GI motility, particularly gastric emptying, the effects of enteric dopamine damage are less pronounced. It is less likely that our methods for detection of upper GI behavioral abnormalities are insufficiently sensitive to detect a small change (Travagli, et al., 2006, Whited, et al., 2006). Finally, although unlikely, there is the possibility that enteric neurons in the lower GI tract are more sensitive to MPTP toxicity. Further investigation will be required to address these possibilities, but there is an interesting parallel with human PD, in which lower GI dysmotility and constipation may be one of the earliest detectable symptoms (Abbott, et al., 2001, Ueki and Otsuka, 2004).

Since colon motility normalizes in the presence of persistent dopamine neuron damage, it is possible that colonic dysmotility after MPTP is unrelated to the dopaminergic dysfunction. It is conceivable that MPTP leads to a temporary loss of inhibitory sympathetic tone in the GI tract, which would be supported by previous research suggesting that MPTP leads to transient sympathetomy in the mouse heart (Fuller, et al., 1988). Since sympathetic input tends to inhibit motility, the result of GI sympathetomy might be an overall increase in GI output due to increased forward flow (Umezawa, et al., 2003). Our isometric muscle contraction data argue against transient sympathetomy as the sole mechanism of enhanced colon motility, since EFS is primarily an assay of ENS (not PNS) function (Ouyang, et al., 1996), and the electrophysiological abnormalities persisted despite resolution of the behavioral deficit. Alternatively, MPTP has been shown to induce transient gastric and duodenal erosions in rodents, raising the possibility that mucosal injury in the stomach and duodenum may cause a temporary increase in intestinal transit velocity by altering the composition of intestinal contents (Sikiric, et al., 1999, Szabo, et al., 1985).

Loss of enteric dopamine neurons has been reported in PD, but the typical gastrointestinal symptom profile exhibited by PD patients includes delayed gastric emptying and constipation, which MPTP-treated mice do not develop (Pfeiffer, 2003, Pfeiffer and Quigley, 1999, Singaram, et al., 1995). In fact, dopaminergic damage transiently causes colonic hypermotility in mice, which is similar to results previously reported in D2 dopamine receptor knockout mice (Li, et al., 2006). This difference may be due to intrinsic differences in the role of dopamine between murine and human enteric nervous systems. In addition, the correlation between electrophysiological and behavioral findings may be different between mice and humans. For example, compared with the severe central dopamine deficiency observed, the motor deficits displayed by MPTP-treated mice are relatively mild (Hunot, et al., 2004, Sundstrom, et al., 1990). A similar situation may occur in the enteric nervous system. Furthermore, in humans, hyper-contractility sometimes causes paradoxically slowed intestinal transit due to muscle spasm. In such a situation, an enteric dopaminergic deficit may be sufficient to induce constipation in PD patients. Another perhaps more likely possibility, as yet essentially unexplored, is dysfunction or death of non-dopaminergic enteric neurons in PD.

Comparison of these results from MPTP-treated mice to other toxic and genetic animal models of PD will help to tease out the role of various ENS transmitters in the genesis of PD-related GI symptoms. With that background, detailed neurochemical coding of ENS damage from PD patients should provide a foundation for rationally designed GI therapies based on the specific neuropathological pattern of ENS damage in PD. Furthermore, given the effects noted in the lower GI tract, colorectal biopsy of PD patients may provide the means to routinely neuropathologically diagnose and monitor PD in living patients (Singaram, et al., 1995). Finally, animal models of PD that exhibit ENS pathology may provide a tool to confirm or

refute hypotheses related to pathogenesis and selective vulnerability in parkinsonism (Braak, et al., 2006).

In summary, as in the brain, MPTP is a selective dopaminergic neurotoxin in the mouse enteric nervous system. ENS dopaminergic denervation produces a physiological impairment in ENS inhibitory function causing behavioral symptoms of colon hypermotility. These experiments are a necessary first step in beginning to explore the pathophysiological underpinnings of GI dysmotility in PD and in the development of an animal model in which to investigate the debilitating GI symptoms associated with PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. MPTP causes dopamine neuron loss in the ENS

A. Bar graph indicating the number of TH-positive neurons per ganglion in the myenteric plexus of the distal ileum in mice ten days after treatment with saline (N=8) or MPTP (60 mg/kg; N=7). * $p < 0.05$. B. Photograph of the myenteric plexus from mouse ileum immunostained for TH. The arrow denotes a TH-positive neuron shown at higher magnification in the inset. The dashed lines outline two ganglia.

Figure 2. MPTP does not affect numbers of nitric oxide or cholinergic neurons in the ENS

A. Bar graph indicating the number of NADPH-diaphorase-positive neurons per mm² in the myenteric plexus of the distal ileum in mice ten days after treatment with saline (N=7) or MPTP (N=7). B. Bar graph indicating the number of ChAT-positive neurons per mm² in the myenteric plexus of the distal ileum in mice ten days after treatment with saline (N=4) or MPTP (N=7). C. Photograph of the myenteric plexus from mouse ileum immunostained for ChAT (brown) and histochemically stained for NADPH-diaphorase (blue). Note the relative abundance of both neuron types as compared to TH-positive cells. Inset shows a higher magnification view of one ChAT-positive and one NADPH-positive neuron.

Figure 3. Dopamine neuron loss causes impaired neural-mediated relaxation of proximal colon

A. Representative tracing of EFS-induced muscle contraction in circular muscle preparations from a saline- and an MPTP-treated mouse. B. Compiled data from 4 experiments like that shown in A quantifying enhanced contraction in MPTP-treated mice. C. Representative tracing of EFS-induced muscle relaxation in longitudinal muscle preparations from a saline- and an MPTP-treated mouse. D. Compiled data from 4 experiments like that shown in C quantifying impaired relaxation in MPTP-treated mice. * $p < 0.05$.

Figure 4. Transiently increased colon motility after MPTP treatment

A. Stool frequency was dramatically higher in MPTP-treated animals 2-3 days after treatment, but was similar to saline-treated controls by 8-10 days after MPTP. (N=8 per group). * $p < 0.05$. B. Solid matter in stool correlates with stool frequency ($R = -0.69$). Since the colon functions to remove water, this confirms the utility of one-hour stool frequency as a measure of colon transit time. Data are from multiple one-hour collection periods from saline- (N=14) and MPTP-treated (N=12) animals across the entire time course of the experiment (from prior to injection through 10 days after).

Figure 5. Gastric emptying is unaffected by MPTP

A. Time course of gastric dye retention in saline- and MPTP-treated animals ten days after treatment. Amount of dye remaining in the stomach was normalized between the zero and 1 hour time points. (N=3 per group per time point). B. The amount of solid food remaining in the stomach after 2 hours was no different between groups (N=13 per group).

Figure 6. Small intestinal transit is unaffected by MPTP

Distance from the pylorus to the dye front is plotted against time. (N=3 per group per time point).