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The substantia nigra modulates proximal colon tone and motility in a vagally-dependent manner in the rat

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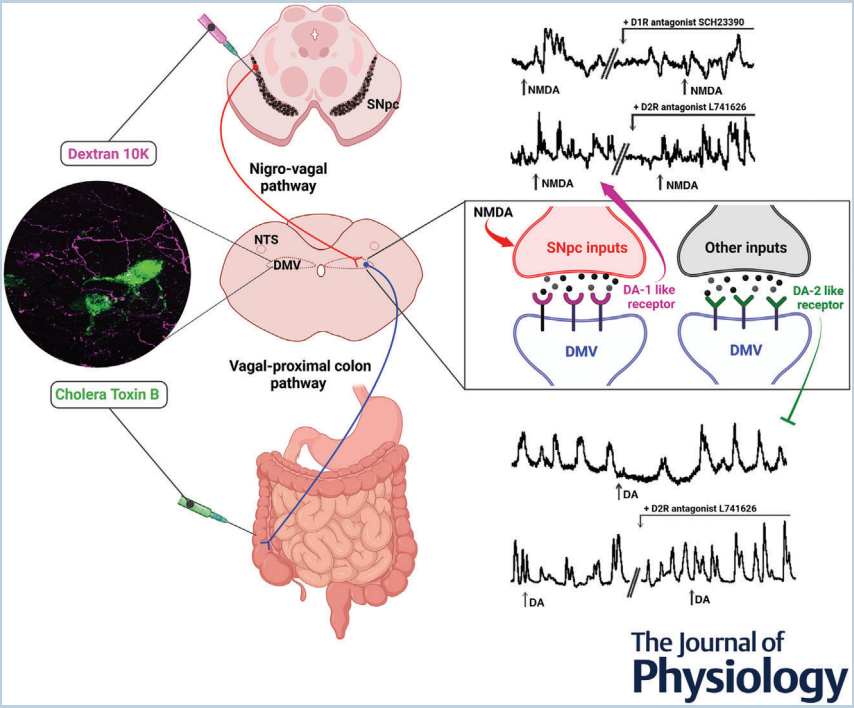

Abstract A monosynaptic pathway connects the substantia nigra pars compacta (SNpc) to neurons of the dorsal motor nucleus of the vagus (DMV). This monosynaptic pathway modulates the vagal control of gastric motility. It is not known, however, whether this nigro-vagal pathway

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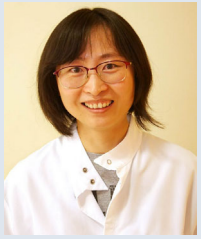

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also modulates the tone and motility of the proximal colon. In rats, microinjection of retrograde tracers in the proximal colon and of anterograde tracers in SNpc showed that bilaterally labelled colonic-projecting neurons in the DMV received inputs from SNpc neurons. Microinjections of the ionotropic glutamate receptor agonist, NMDA, in the SNpc increased proximal colonic motility and tone, as measured via a strain gauge aligned with the colonic circular smooth muscle; the motility increase was inhibited by acute subdiaphragmatic vagotomy. Upon transfection of SNpc with pAAV-hSyn-hM3D(Gq)-mCherry, chemogenetic activation of nigro-vagal nerve terminals by brainstem application of clozapine-*N*-oxide increased the firing rate of DMV neurons and proximal colon motility; both responses were abolished by brainstem pretreatment with the dopaminergic D1-like antagonist SCH23390. Chemogenetic inhibition of nigro-vagal nerve terminals following SNpc transfection with pAAV-hSyn-hM4D(Gi)-mCherry decreased the firing rate of DMV neurons and inhibited proximal colon motility. These data suggest that a nigro-vagal pathway modulates activity of the proximal colon motility tonically via a discrete dopaminergic synapse in a manner dependent on vagal efferent nerve activity. Impairment of this nigro-vagal pathway may contribute to the severely reduced colonic transit and prominent constipation observed in both patients and animal models of parkinsonism.

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Abstract figure legend The dorsal vagal complex (DVC) receives inputs from the substantia nigra pars compacta (SNpc) via a nigro-vagal pathway. The present study examined whether this nigro-vagal pathway extends its influence to the proximal colon. In rats, colonic-projecting neurons in the dorsal motor nucleus of the vagus (DMV) receive inputs from SNpc neurons, as demonstrated by simultaneous anterograde (SNpc) and retrograde (proximal colon) neuronal tracing. Activation of SNpc neurons via the ionotropic glutamate selective agonist, NMDA, increases proximal colonic motility and tone in a manner that was reduced by brainstem pretreatment with the dopaminergic D1-like antagonist SCH23390, but not by the D2-like antagonist L741646. These studies suggest that the nigro-vagal pathway tonically modulates the tone and motility of the proximal colon and excites vagal efferent motoneurons via activation of dopaminergic D1 receptors.

Key points

- Substantia nigra pars compacta (SNpc) neurons are connected to the dorsal motor nucleus of the vagus (DMV) neurons via a presumed direct pathway.
- Brainstem neurons in the lateral DMV innervate the proximal colon. Colonic-projecting DMV neurons receive inputs from neurons of the SNpc.
- The nigro-vagal pathway modulates tone and motility of the proximal colon via D1-like receptors in the DMV.
- The present study provides the mechanistic basis for explaining how SNpc alterations may lead to a high rate of constipation in patients with Parkinson's Disease.

Introduction

The gastrointestinal (GI) tract from the lower third of the oesophagus to the transverse colon undergoes robust modulation by vagal inputs (Browning & Travagli, 2014; Travagli & Anselmi, 2016). The vagal motor inputs to the GI tract arise from preganglionic cholinergic parasympathetic motoneurons of the dorsal motor nucleus of the vagus (DMV), which project to postganglionic myenteric neurons of the enteric nervous system (ENS) that ultimately modulate, motility amongst other responses (Browning & Travagli, 2014). One of the remarkable, and fundamental, properties of GI-projecting DMV motoneurons is their spontaneous, slow pacemaker-like activity, the rate of which is modulated by synaptic inputs (Browning & Travagli, 2014). One immediate implication is that the activity of the GI tract, even at rest, is modulated by DMV neurons and vagal efferent outflow, the activity of which is continuously sculpted by a large array of synaptic

inputs from brainstem, midbrain and higher central nervous system (CNS) centres (Browning & Travagli, 2014). Among the several physiologically relevant inputs to DMV neurons, we have shown that a nigro-vagal pathway, originating from dopaminergic neurons of the substantia nigra pars compacta (SNpc), exerts a tonic modulation over gastric motor activity (Anselmi et al., 2017a).

Degeneration of dopaminergic neurons in the SNpc induces uncontrolled tremors at rest, bradykinesia, postural instability and rigidity, which are the movement disorders commonly associated with Parkinson's disease (PD) (Del Rey et al., 2018; Dickson, 2018; Goedert & Compston, 2018; Goedert et al., 2013; Johnson et al., 2019). There are, however, several non-motor symptoms, including autonomic dysfunctions, that increase the overall morbidity of parkinsonian patients significantly (Cersosimo & Benarroch, 2012; Coon et al., 2018; Goldstein, 2014; Postuma & Berg, 2016; Schapira et al., 2017). GI dysfunctions, including delayed gastric emptying and severe constipation, are amongst the most prominent non-motor manifestations of PD (Travagli et al., 2020); these GI issues can precede the onset of motor symptoms by several decades and reduce significantly the patients' quality of life. Specifically, the delayed colonic transit observed in PD patients is a major complication that may evolve in chronic constipation unresponsive to first-line treatments (Giancola et al., 2017; Travagli et al., 2020).

Among various hypotheses for the aetiology of PD (Johnson et al., 2019; Liddle, 2018; Marras et al., 2019; Surmeier et al., 2017; Wichmann, 2019), a notable hypothesis postulates that the infiltration and ENS absorption of ingested toxicants and the spread of the resulting synucleinopathy via retrograde transport through vagal pathways to the DMV and ultimately to the SNpc, basal ganglia and cerebral cortex (Anselmi et al., 2018; Braak, Rub et al., 2003; Goedert et al., 2013) induces neuronal loss.

The functions of the colon include the absorption of water and electrolytes, as well as peristalsis to move intestinal contents aborally (Furness, 2012). Vagal efferent innervation of the proximal colon, although more sparse than that projecting to the stomach and upper small intestine, may represent another area of the GI tract that is implicated in the infiltration of soluble ingested toxicants, their retrograde transport to the CNS and ultimately to the SNpc, basal ganglia and cerebral cortex. The synucleinopathy observed in the proximal colon (Anselmi et al., 2018) may impair motility, although little is known about the extent to which or the mechanism of action of vagal inputs from the SNpc modulate the function of the proximal colon. Indeed, the anatomical evidence and the physiological significance of a potential link between the SNpc and the proximal colon has not been reported.

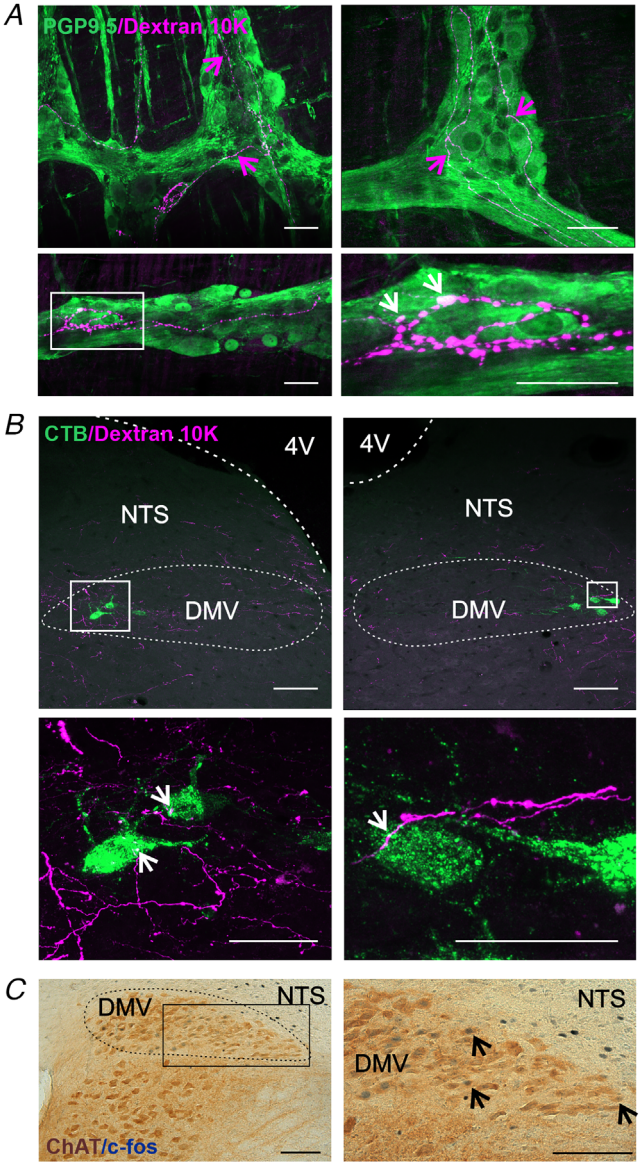

Figure 1. SNpc provides an excitatory input to neurons of the dorsal motor nucleus of the vagus that innervate the proximal colon
A, representative micrograph of vagal efferent fibers apposing myenteric neurons of the proximal colon. Following microinjection of an anterograde tracer (magenta) in the dorsal vagal complex (DVC; $n = 4$), labelled fibers are observed in PGP9.5-IR myenteric neurons of the proximal colon (magenta arrows). Some PGP9.5-IR neurons appeared to receive close contacts from vagal efferent fibers and were encircled with dextran labelled fibers (white arrows). *B*, representative micrograph of neurons in the DMV. Following microinjection of an anterograde tracer in the SNpc, labelled fibers (white arrows) can be seen apposing DMV neurons labelled following injections of the retrograde tracer CTB in the proximal colon ($n = 5$). *C*, representative micrographs of ChAT-IR neurons (brown) and c-fos (blue–black) within the within the DMV. Black arrows indicate c-fos-IR neurons within the lateral DMV following NMDA microinjection into the DMV. Scale bars = 100 μm. DMV, dorsal motor nucleus of the vagus; NTS, nucleus tractus solitarius; 4V, fourth ventricle.

The present study aimed (1) to demonstrate the existence of a nigro-vagal-proximal colon pathway; (2) to characterize this connection; and (3) to assess whether this pathway regulates the tone and motility of the proximal colon.

Preliminary accounts of the present work have been presented at the 2020 Digestive Disease Week (Nanni & Travagli, 2020).

Methods

Ethical approval

Male Sprague–Dawley rats (n = 87) (Charles River, Wilmington, MA, USA; weighing 150–200 g at the beginning of the experiments) were housed in an AAALAC accredited animal care facility at 24°C under a 12:12 h light/dark photocycle with food and water available *ad libitum*. Surgical procedures were performed using aseptic techniques and were conducted in accordance with NIH guidelines, with the approval of the Penn State University College of Medicine Institutional Animal Care and Use Committee (protocol #47 048), and in accordance with the ARRIVE guidelines for animal care and use.

Although we acknowledge the potential limitations in restricting the present study to male rats only, recent studies have shown that oestrogens act both peripherally (Balasuriya et al., 2021; Li et al., 2016; Liu et al., 2019) and centrally (Jiang et al., 2019; Meister et al., 2019) to modulate GI functions, rendering data interpretation problematic unless experiments are conducted in an oestrogen-controlled environment, which was beyond the scope of the present study.

Neuronal tracing

Rats (n = 7) were anaesthetized with a solution of ketamine/xyalzine/acepromazine (80/1.6/5 mg mL^{-1}kg^{-1} I.P.). The anaesthesia level was monitored continuously throughout the experiment and the core temperature was maintained at 37°C via a heating pad. Once a deep plane of anaesthesia was achieved (i.e. absence of palpebral reflex), an abdominal laparotomy was performed to expose the colon. Five to ten microinjections of the retrograde tracer cholera toxin-B (CTB; 5–10 μL (microliters) each injection, 0.5% w/v; List Biological Laboratories, Campbell, CA, USA) were made into the proximal colon ∼2–3 cm from the ileocecal valve; the wound was then closed with 5:0 Vicryl suture (Ethicon Inc., Bridgewater, NJ, USA). The rats were then placed on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA), the head was shaved, the skin retracted and the skull was exposed. Two to three millimetre wide holes were drilled bilaterally to allow microinjection of the anterograde tracers biotinylated or fluorescein dextran 10,000 MW (5% of 6.5% w/v, respectively, dissolved in sterile saline; Life Technologies, Grand Island, NY, USA) into the SNpc. Four microinjections of tracer (600 nL for each location) were made bilaterally into the SNpc at sites: rostrocaudal, −5.0 mm from bregma, mediolateral, ±2.4 mm from midline, dorsoventral, −7.6 mm from

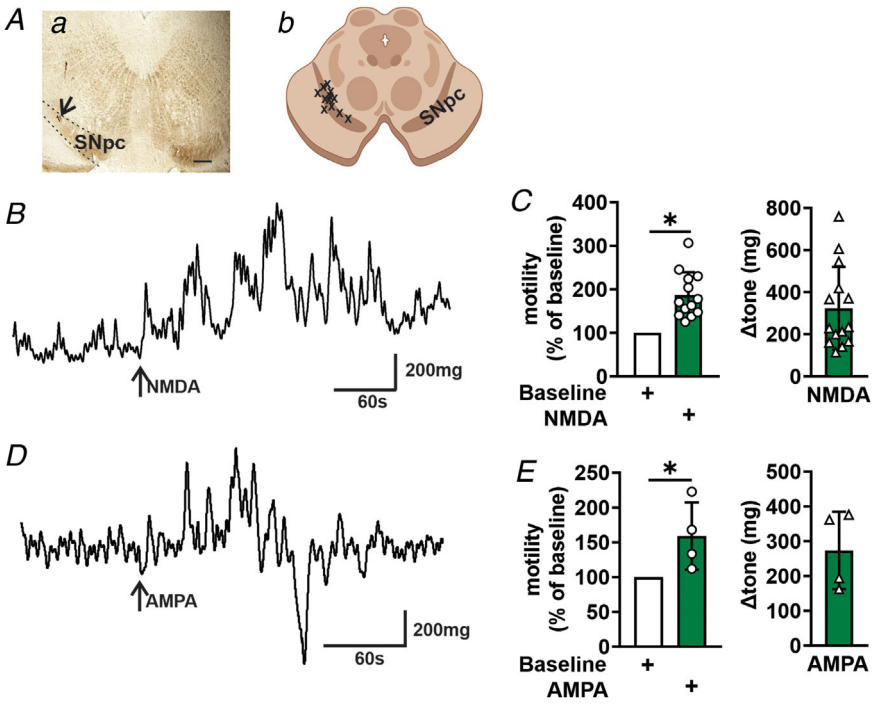

Figure 2. NMDA microinjection in the SNpc increases the tone and motility of the proximal colon
A, representative micrograph showing the location of a NMDA injection (arrow) in SNpc (*Aa*, scale bar = 500 μm). Schematic image summarizing the location of the injections in SNpc (*Ab*, n = 21). *B*, representative trace showing that the NMDA (5 nmol/210 nL) microinjection in the left SNpc (arrow) increases both tone and motility of the proximal colon. *C*, summary graphic showing the increase in motility (n = 21, $P < 0.0001$, one-tailed paired t test) and tone (n = 21) of the proximal colon upon NMDA microinjection in the left SNpc. *D*, representative trace showing that the AMPA (5 nmol/210 nL) microinjection in the left SNpc (arrow) increases both tone and motility of the proximal colon. *E*, summary graphic showing the increase in motility (n = 4, P = 0.0463, one-tailed paired t test) and tone (n = 4) of the proximal colon upon AMPA microinjection in the left SNpc.

the surface of the dura mater, as well as rostrocaudal, −5.6 mm, mediolateral, ±1.6 mm and dorsoventral, −7.8 mm. The scalp was sutured (5/0 Vicryl) and rats were allowed to recover. Rats received carprofen (5 mg kg^{-1} s.c.) perioperatively and at 24 h intervals postoperatively for analgesia, and Baytril (enrofloxacin; Elanco, Indianapolis, IN, USA) (5 mg kg^{-1} s.c.) for 5 days as a prophylactic antibiotic. After 10–15 days, rats were anaesthetized with Inactin (sodium thiobutabarbitol; Sigma, St Louis, MO, USA; 100–150 mg kg^{-1} I.P.) and killed via administration of a bilateral pneumothorax (laparotomy and diaphragm penetration), before being perfused transcardially with 0.9% saline followed by 4% paraformaldehyde (PFA).

In another set of neuronal tracing experiments ($n = 4$), dextran 10,000 MW (in either the biotinylated (5% w/v) or the fluorescein (7.5% w/v) formulation; Life Technologies, Carlsbad, CA, USA) was injected in the dorsal vagal complex (DVC) (rostrocaudal, 0.0–0.6 mm from calamus scriptorius; mediolateral, ±0.5–0.7 mm from midline; dorsroventral, 0.55–0.7 mm from the brainstem surface); after 15–30 days of recovery, rats were anaesthetized with Inactin (100–150 mg kg^{-1} I.P.; abolition of the foot pinch withdrawal reflex) and killed via administration of a bilateral pneumothorax (laparotomy and diaphragm penetration), before being perfused transcardially with 0.9% saline followed by 4% PFA.

Tissue processing

After transcardial perfusion, brains were removed and post-fixed for 4 days at 4°C with 4% PFA containing 20% sucrose before being transferred to a solution containing phosphate-buffered saline (PBS) and 20% sucrose for at least 1 day. Transverse slices (50 μm) were made throughout the entire rostrocaudal extent of SNpc as well as the DVC using a freezing sledge microtome. Slices were cut into sets of four and preserved in long-term storage buffer (PBS, 0.1 M, sucrose 30%, ethylene glycol 30%) at −20°C.

In the rats that received microinjection of anterograde tracer in the DVC, the proximal colon was extracted prior to PFA perfusion and immersed in PBS. The colon was opened along the mesenteric border, washed and pinned under tension to the bottom of silicon-coated dishes. Specimens were fixed for 1–2 days in 4% PFA at 4°C, washed in PBS and stored in PBS + sodium azide 0.05% until dissection within 2–5 days. Specimens were then dissected under magnification to produce longitudinal muscle-myenteric plexus whole mount preparations by peeling away the mucosa, submucosa and circular muscle.

Immunohistochemical analyses

All immunohistochemistry steps were performed at room temperature on a shaker. After being washed in Tris-PBS containing 0.03% Triton-X (TPBS) followed by incubation in 1% normal donkey serum (NDS), specimens were incubated in primary antibodies diluted in TPBS containing 10% NDS for 3 days. After washing in PBS, the sections were incubated overnight in secondary antibodies diluted in TPBS containing 1% NDS. After several washes, tissues were mounted on gelatin-subbed slides and coverslipped using Fluoromount-G (Southern Biotechnology Associated, Birmingham, AL, USA). The primary antibodies were goat α-CTB (dilution 1:100 000; List Biological Labs, Campbell, CA, USA); rabbit-α-PGP 9.5 (dilution 1:500; Millipore, Billerica, MA, USA); rabbit-α-choline acetyltransferase (dilution 1:1000; Sigma); mouse α-tyrosine hydroxylase (dilution 1:1000; ImmunoStar, Hudson, WI, USA); and rabbit anti-c-fos (dilution 1:500; EnCor Biotechnology Inc,., Gainesville, FL, USA) and their dilutions were determined by titration in tissue fixed and processed in the same ways as the experimental tissue. The secondary antibodies used were donkey anti-mouse/rabbit/goat Alexa Fluor 488, 568 or 647; all fluorescent secondary antibodies were purchased from Life Technologies (Grand Island, NY, USA) and were used at 1:500 dilution.

Immunofluorescence images were captured with an AXIO Observer Z1 confocal laser scanning microscope (Zeiss, Oberkochen, Germany). In rats ($n = 4$) that received microinjections of anterograde tracers in the DVC, a minimum of 200 proximal colon PGP9.5-immunoreactive (-IR) myenteric neurons were analysed for their close association with labelled efferent vagal fibres. Data are reported as percentage of PGP9.5-IR neurons that received close contacts from vagal fibres. In a different group of rats ($n = 5$) that received microinjections of anterograde tracers in the SNpc and retrograde tracers in the proximal colon, every fourth DVC slice was analysed to assess the percentage of labelled DMV neurons receiving close contacts from SNpc-labelled fibres.

Motility and tone studies

Rats ($n = 69$) were fasted overnight (water *ad libitum*) and anaesthetized with Inactin (100–150 mg kg^{-1} I.P.). The anaesthesia level was monitored continuously throughout the experiment, and the core temperature was maintained at 37°C via a heating pad. Once a deep plane of anaesthesia was achieved (absence of palpebral reflex), rats were intubated with a tracheal catheter, a midline laparotomy was performed to expose the proximal colonic wall and a miniature strain gauge (MSR Neurobiology, Roaring Spring, PA, USA) was sutured to the serosal surface of the circular smooth muscle of the proximal colon ∼2–3 cm from the ileocecal valve, and the leads were exteriorized prior to abdominal closure. Rats were then placed on

a stereotaxic frame, neck muscles were blunt dissected and the brainstem was exposed following removal of meningeal membranes above the fourth ventricle. The skull was then exposed and 2–3 mm wide holes were drilled bilaterally at: rostrocaudal, 5.6 mm from bregma and mediolateral, ±1.6 mm from bregma to allow subsequent microinjection of drugs into the SNpc. Injections into the DVC were made at rostrocaudal, 0.0–0.6 mm from calamus scriptorius, mediolateral, ±0.5–0.7 mm from midline and dorsoventral, 0.55–0.7 mm from the brainstem surface. Rats were allowed to stabilize for at least 45 min before beginning the tone/motility experiment and supplemented with 5 mL of pre-warmed saline (s.c.) prior to beginning the experiment. The depth of anaesthesia was assessed every 15–30 min throughout the duration of the experiment.

NMDA (5 nmol/210 nL; $n = 21$) or AMPA (5 nmol/210 nL; $n = 4$) was microinjected into the SNpc over the course of 2 min and the resulting change in colonic tone and motility noted. After 30 min of recovery, 2 μL of PBS solution containing the D1- or the D2-like receptor antagonists SCH23390 or L741626, respectively (45 nmol each) was applied to the surface of the fourth ventricle using an 5 μL Hamilton syringe, taking care to not touch the floor of the fourth ventricle or the adjacent area postrema, followed 2–5 min later by a second NMDA microinjection. All drugs were dissolved in isotonic PBS. In the experiments in which the effects of antagonist application were assessed, their effects on motility and tone were monitored for ∼5 min.

In different group of rats ($n = 5$), the subdiaphragmatic right vagus was sectioned prior to strain gauge apposition and the left cervical vagus was exposed and loosely ligated with a thread that was exteriorized for easy access later in the experiment. NMDA was microinjected in the left SNpc. Following a recovery period of at least 30 min, the left cervical vagus was severed by pulling the exteriorized thread, thus attaining a complete vagotomy. After a 45 min stabilization period, the NMDA microinjection into the left SNpc was repeated.

At the conclusion of the experiments, rats were killed via administration of a bilateral pneumothorax (laparotomy and diaphragm penetration) and perfused transcardially with saline (0.9%) followed by 4% PFA. The brain was post-fixed as above for subsequent immunohistochemical localization of the microinjection sites.

Colonic tone was measured as absolute tone variation (in mg) from baseline. Colonic motility was calculated using the following formula, as described previously (Anselmi et al., 2017b):

$$\text{Motility index} = (N_1 * 1 + N_2 * 2 + N_3 * 4 + N_4 * 8)/t \times 100\%$$

where N equals the number of peaks in a particular force range (N_1 = 25–50 mg, N_2 = 51–100 mg, N_3 = 101–200 mg, $N_4 > 201$ mg) and t equals the time interval over which the colonic motility was measured. The effect of drugs on colonic motility was measured relative to the averaged value (expressed as arbitrary units, a.u.) of motility before microinjection (baseline = 100%).

Chemogenetic studies

Following induction of anaesthesia with ketamine/xyalzine/acepromazine (80/1.6/5 mg mL^{-1} kg^{-1} I.P.), and once a deep plane of anaesthesia was obtained (abolition of the foot pinch withdrawal reflex), rats ($n = 27$) were laid on a homeothermic heating pad that maintained their core body temperature at 37°C. pAAV-hSyn-hM3D(Gq)-mCherry ['hM3D(Gq)'] (2 × 10^{12} vg mL^{-1}; Plasmid #50474; Addgene, Watertown, MA, USA) or pAAV-hSyn-DIO-hM4D(Gi)-mCherry ['hM4D(Gq)'] (2 ×10^{12} vg mL^{-1}; Plasmid #44362; Addgene) or rAAV2/hsyn-EYFP ('empty vector') (3.4 × 10^{12} vg mL^{-1}; Gene Therapy Center, UNC Vector Core, Chapel Hill, NC, USA) were injected bilaterally into the SNpc at sites: rostrocaudal, −5.0 mm from bregma, mediolateral, ±2.4 mm from midline and dorsoventral, −7.6 mm from the surface of the dura mater, and rostrocaudal, −5.6 mm, mediolateral, ±1.6 mm and dorsoventral, −7.8 mm. The scalp was sutured (5/0 Vicryl) and rats were allowed to recover; rats received carprofen (5 mg kg^{-1} s.c.) perioperatively and at 24 h intervals postoperatively for analgesia, and Baytril (5 mg kg^{-1} s.c.) for 5 days as a prophylactic antibiotic. After allowing sufficient time for recovery from surgery and expression of the microinjected viral vectors (3–4 weeks), a group of rats ($n = 18$) was then anaesthetized with Inactin (100–150 mg kg^{-1} I.P.). The anaesthesia level was monitored continuously throughout the experiment, and the core temperature was maintained at 37°C via a heating pad. Once a deep plane of anaesthesia was achieved (absence of the palpebral reflex), rats were instrumented for *in vivo* colonic recordings as described above, and chemostimulation of the DVC was performed using clozapine-*N*-oxide (CNO) (Sigma; 2 μL/200 nmol) applied to the floor of the fourth ventricle. The depth of anaesthesia was assessed every 15–30 min throughout the duration of the experiment. At the conclusion of the experiments, rats were killed via administration of a bilateral pneumothorax (laparotomy and diaphragm penetration) and perfused transcardially with saline (0.9%) following by 4% paraformaldehyde. The brain was post-fixed as above for subsequent immunohistochemical localization of the microinjection sites.

A subgroup of virally-transfected rats ($n = 9$) was used for whole cell patch clamp recordings from DMV neurons

in thin brainstem slices. Rats were anaesthetized with isoflurane (5% in air) until a deep place of anaesthesia was induced (abolition foot pinch withdrawal reflex), before administration of a bilateral pneumothorax (laparotomy and diaphragm penetration). The brainstem was excised quickly, submerged in cold (4°C) oxygenated Krebs' solution and cut into 300 μm-thick sections, which were placed in warm (30°C) oxygenated Krebs' solution for 90 min before recording.

A brainstem slice was placed in a perfusion chamber (volume 500 μL; MSR Neurobiology) fitted on the stage of a E600FN microscope (Nikon, Tokyo, Japan) and perfused with warmed (32°C) Krebs' solution (in mM: 126 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4 and 10 D-glucose, kept at pH 7.4 by bubbling with 95% O_2/5% CO_2). Electrophysiological recordings of DMV neurons were made using 2–4 MΩ patch pipettes filled with a potassium gluconate (in mM: 128 K gluconate, 10 KCl, 0.3 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 1 EGTA, 1 NaATP and 0.25 NaGTP adjusted to pH 7.35) and a single electrode voltage clamp amplifier (Axopatch 200B; Molecular Devices, Union City, CA, USA). Only one cell per brainstem slice was used to avoid potential confounding results from multiple drug applications. Data were filtered at 2 kHz digitized via a Digidata 1440 Interface and stored and analysed on a PC with pClamp 10 software (Molecular Devices). Recordings with a series resistance of >20 MΩ were eliminated from the study. DMV neurons were current clamped at a membrane potential that allowed spontaneous action potential firing of ∼1 event s^{-1}. Freshly prepared CNO (10 μM diluted in Krebs' solution) was perfused until a stable response was observed or for 5 min if no response was observed. Each neuron served as its own control, with responses assessed before and after CNO application. A neuron was considered responsive if CNO altered its firing rate by >25% relative to baseline.

Data collection and analysis

Immunofluorescence images were captured with an AXIO Observer Z1 confocal laser scanning microscope (Zeiss) with Zen 3.3 (blue edition) software.

Motility and/or tone data were collected from all animals; six rats in which the motility and tone response were assessed following NMDA injection into the SNpc were excluded from the final analysis. Of these six rats, four were excluded because they did not show a measurable change in tone or motility following microinjection; subsequent *post hoc* verification confirmed the injection site was off-target. The remaining two rats were excluded from analysis because of failure of the strain gauge during the recording. Colonic tone and motility traces were analysed with Axoscope software (Molecular Devices). All data were analysed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, or a *t* test with Prism, version 9 (GraphPad Software Inc., La Jolla, CA, USA). Data are expressed as the mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Anatomical characterization of the nigro-vagal-proximal colon connection

To confirm that brainstem vagal neurons innervate the proximal colon, vagal efferent neurons were labelled with microinjections of an anterograde tracer in the DMV ($n = 4$). Subsequent immunohistochemical analyses of the proximal colon showed that out of 220 ± 14.6 PGP 9.5-IR myenteric neurons, 105 ± 5.5 (i.e. 47.9 ± 2.81%) received close appositions from, and were occasionally encircled by vagal efferent fibres (Fig. 1*A*).

To investigate whether colon-projecting vagal efferent motoneurons receive projections from the SNpc, the retrograde tracer, CTB, was injected in the proximal colon and the anterograde tracer, dextran 10,000 MW was microinjected into the SNpc ($n = 7$). Subsequent analysis of every fourth vagal brainstem slice showed 61 CTB-positive DMV neurons in the lateral tips of the DMV throughout the rostrocaudal extent of the DVC; these neurons were located in the rostral (bregma −13.3 mm; $n = 22$), intermediate (bregma −13.9 mm; $n = 32$) and caudal (bregma −14.3 mm; $n = 7$) DMV. Forty-two (i.e. 68.8%) of these retrogradely labelled neurons received close contacts from dextran labelled SNpc fibre projections (17 of 22 neurons in the rostral, 19 of 32 neurons in the intermediate and six of seven neurons in the caudal DMV, respectively) (Fig. 1*B*).

To confirm that the nigro-vagal pathway modulates the activity of DMV neurons, brainstem slices from an additional four rats that had undergone NMDA microinjection into the SNpc (see below) were assessed for the co-localization of c-fos and ChAT-IR. SNpc stimulation induced c-fos expression in DMV neurons throughout the rostrocaudal extent of the DVC, including in the lateral third of the DMV that contains presumed colon-projecting neurons (Fig. 1*C*). These neurons were located in the rostral (bregma −13.3 mm; $n = 12$ laterally located of a total of 21 DMV c-fos-IR neurons), intermediate (bregma −13.9 mm, $n = 14$ of 60 neurons) and caudal (bregma −14.2 mm, $n = 6$ of 16 neurons).

These data provide anatomical proof of a discrete nigro-vagal pathway through which SNpc neurons are presumed to innervate proximal colon-projecting DMV neurons.

The nigro-vagal pathway modulates tone and motility of the proximal colon

To investigate whether SNpc neurons modulate proximal colon tone and motility, NMDA was microinjected into the left SNpc (injection locations shown in Fig. 2*A* and *B*) when colon tone and motility were assessed ($n = 21$). Microinjection of NMDA increased both the motility 157 ± 117.5 to 255 ± 130.3 a.u. (i.e. 190 ± 98.9% of baseline; $t_{20} = 4.189$, $P < 0.0001$ *vs.* baseline, one-tailed paired *t* test) and the tone (399 ± 330.4 mg) of the proximal colon (Fig. 2*B* and *C*). Because the baseline motility was similar for all the experiments, the results of the experiments described below are expressed as a percentage of baseline.

To confirm that the response to NMDA microinjection was due to physiological rather than excitotoxic activation of SNpc neurons, microinjections of AMPA were made into the SNpc of four rats, ∼60 min after recovery from NMDA injection. In these rats, AMPA increased both the motility (158 ± 48.4% of baseline; $t_3 = 2.438$, $P = 0.0463$ *vs.* baseline, one-tailed paired *t* test) and tone (273 ± 111.1 mg) of the proximal colon.

To investigate whether the increase in proximal colon tone and motility were vagally-dependent, NMDA was microinjected in the left SNpc after posterior subdiaphragmatic vagotomy. NMDA microinjection increased tone by 558 ± 264.9 mg and motility to 207 ± 63.7% of baseline ($P = 0.0097$ *vs.* baseline; $n = 5$; one-tailed paired *t* test). In the same rats, after complete subdiaphragmatic vagotomy, subsequent NMDA microinjection had no effect on tone (9 ± 30 mg; $n = 5$) or motility (79.33 ± 41.35% of baseline; $n = 5$; $t_4 = 1.118$, $P = 0.3263$, two-tailed paired *t* test) of the proximal colon (Fig. 3).

These data indicate that the SNpc modulates the tone and motility of the proximal colon in a vagally-dependent manner.

Chemogenetic stimulation of DVC confirms SNpc inputs modulate DMV neuronal activity

To confirm that the nigro-vagal pathway exerts modulatory control over proximal colon activity, the effects of chemogenetic modulation of nigro-vagal terminals on the activity of DMV neurons and proximal colon tone and motility were assessed. *Post hoc* verification of transfection location and efficacy in hM3D(Gq) ($n = 7$), hM4D(Gi) ($n = 5$) or empty (control) vector ($n = 6$) rats in the SNpc (Fig. 4*A*) showed co-localization of vector in tyrosine hydroxylase-IR SNpc neurons (tyrosine hydroxylase-IR, blue) (Fig. 4*B*, upper) and mCherry and YFP labelled fibres scattered bilaterally throughout the DMV (Fig. 4*B*, lower).

Fourth ventricular application of CNO (2 μL/200 nmol) had no effect on either proximal colon motility (93 ± 23.9% of baseline; $n = 6$; $t_5 = 0.7066$, $P = 0.5114$, two-tailed paired *t* test) or tone (8 ± 41.2 mg) in any of the six rats that were transfected with the empty vector Fig. 4*C* and *D*) . By contrast, CNO application to hM3D(Gq) transfected rats increased motility (192 ± 95.3% of baseline; $n = 7$; $t_6 = 2.552$, $P = 0.0434$, two-tailed paired *t* test) and tone (137 ± 136 mg, $n = 7$) of the proximal colon (Fig. 4*E* and *F*), whereas CNO application to hM4D(Gi) transfected rats decreased tone (−71 ± 25.6 mg, $n = 6$) and motility (50 ± 20.1% of baseline; $n = 5$; $t_4 = 5.564$, $P = 0.0051$, two-tailed paired *t* test) of the proximal colon (Fig. 4*G* and *H*).

In a subgroup of rats in which the SNpc was transfected with hM3D(Gq), hM4D(Gi) or empty (control) vector in the SNpc, whole cell patch clamp recordings were made from DMV neurons and the effects of CNO

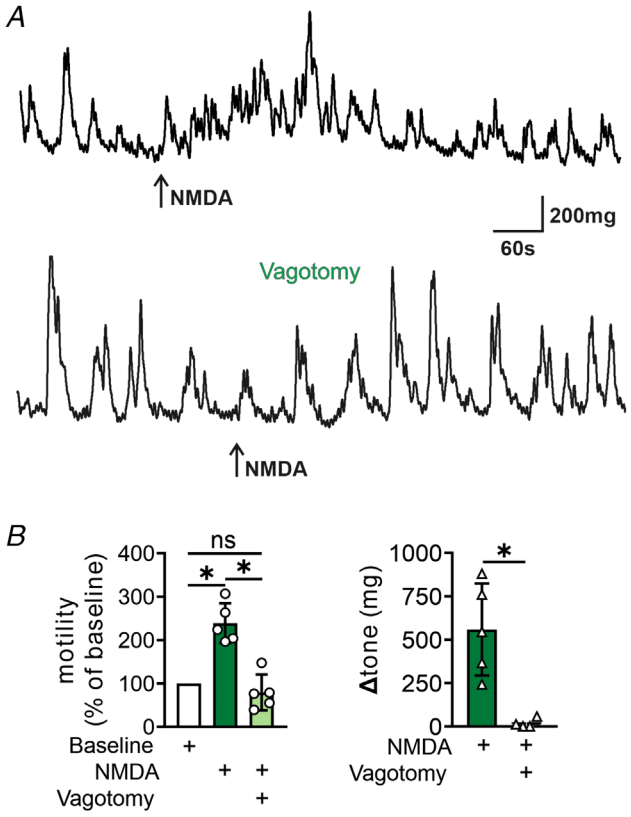

Figure 3. Vagotomy prevents the increase in tone and motility of the proximal colon following SNpc microinjection of NMDA microinjection
A, representative traces showing that the increase in tone and motility of the proximal colon induced by NMDA (5 nmol/210 nL, arrow) microinjection in the left SNpc (upper) is prevented by vagotomy (lower). *B*, summary graphic showing the increase in motility and tone observed in response to NMDA before ($n = 5$; $P = 0.0097$ NMDA *vs.* baseline, one-tailed paired *t* test) and after vagotomy (ns: $P = 0.3263$ Vagotomy + NMDA *vs.* baseline, two-tailed paired t test; tone: $P = 0.0097$ NMDA *vs.* Vagotomy + NMDA, one-tailed paired *t* test). ns, not significant.

(10 μM) to modulate action potential firing rate were assessed. In seven of eight neurons from three hM3D(Gq) rats, CNO increased the firing rate of DMV neurons from 1.2 ± 0.26 to 1.9 ± 0.55 events s^{-1}. This increase in firing rate was prevented by pretreatment with the D1-like antagonist SCH23390 [1.07 ± 0.21 events s^{-1} with SCH23390+CNO; P = 0.0255; F(DFn, DFd = 0.9080, 4994) = 10.17, Geissner–Greenhouse epsilon = 0.4540; one-way ANOVA, mixed effects model] (Fig. 5*C* and *D*). Conversely, in seven out of eight neurons from three hM4D(Gi) rats, CNO decreased the firing rate of DMV neurons from 1.1 ± 0.33 to 0.6 ± 0.52 events s^{-1} (t_6 = 2.949, P = 0.0257; two-tailed paired t test) (Fig. 5*E* and *F*). Perfusion with CNO had no effect on the firing rate in seven out of eight DMV neurons from three empty vector rats (1.2 ± 0.35 and 1.1 ± 0.57 events s^{-1} at baseline and after CNO perfusion, respectively (t_7 = 0.6608, P = 0.5300; two-tailed paired t test) (Fig. 5*A* and *B*).

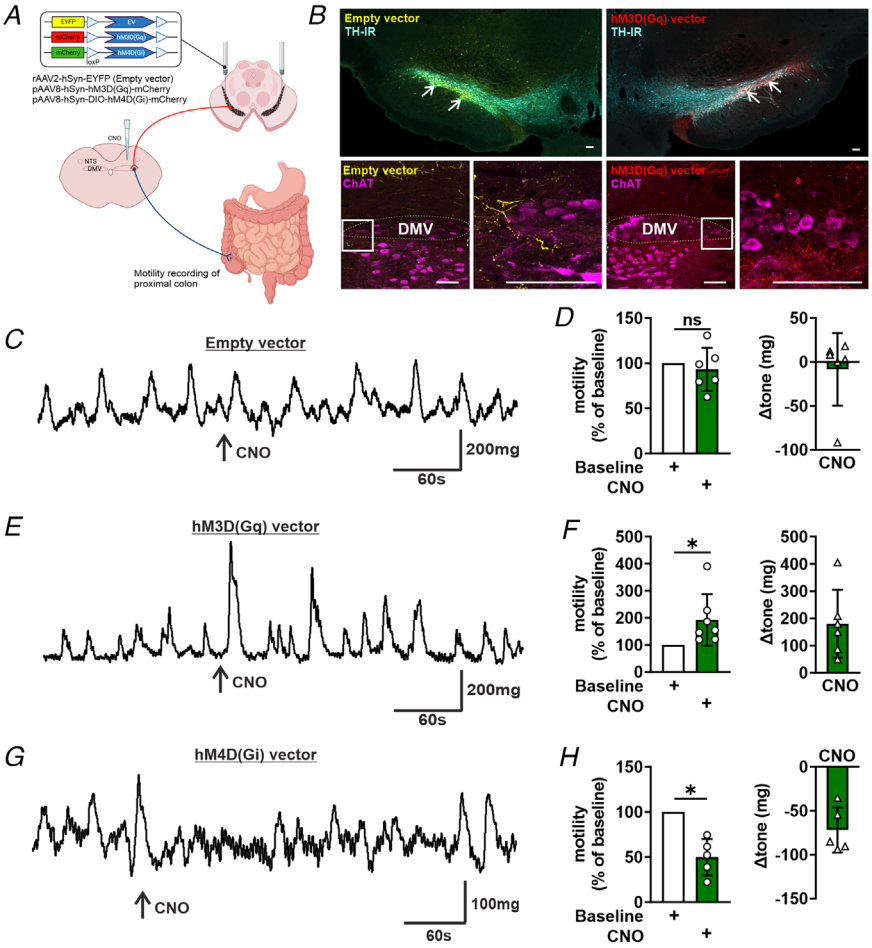

Figure 4. Chemogenetic activation of nigro-vagal projections increases the tone and motility of the proximal colon
A, schematic diagram illustrating the experimental protocol used for chemogenetic manipulation of the nigro-vagal pathway. *B*, representative micrograph showing the location of hM3D(Gq) (upper left; red) and empty vector (upper right, yellow) microinjection. SNpc neurons are labelled with tyrosine hydroxylase. Representative micrograph showing the location of the mCherry and EYFP labelled fibers in the DMV following SNpc transfection, and apposing choline acetyl transferase-IR neurons (lower). *C*, recording of proximal colon function in an empty vector transfected rat following application of CNO to the fourth ventricle. *D*, graphical summary of the change in proximal colon motility (left, n = 6; P = 0.5114, two-tailed paired t test) and tone (right) in empty vector transfected rats in response to brainstem application of CNO. *E*, recording of proximal colon function in an hM3D(Gq) transfected rat following application of CNO to the fourth ventricle. *F*, graphical summary of the change in proximal colon motility (left, n = 7; P = 0.0434, two-tailed paired t test) and tone (right) in hM3D(Gq) transfected rats following brainstem application of CNO. *G*, recording of proximal colon function in an hM4D(Gi) transfected rat following application of CNO to the fourth ventricle. *H*, graphical summary of the change in proximal colon motility (left, n = 5; P = 0.0051, two-tailed paired t test) and tone (right) in hM4D(gi) transfected rats following brainstem application of CNO. Scale bars = 100 μm. ns, not significant.

These data indicate that the nigro-vagal pathway exerts a tonically active and physiological relevant modulatory control over DMV neuronal activity as well as tone and motility of the proximal colon.

The nigro-vagal pathway modulates tone and motility of the proximal colon via D1-like receptors in the DMV

To investigate the neurotransmitter(s) used by nigro-vagal pathway to increase the tone and motility of the proximal colon, the effects of NMDA stimulation of the SNpc were carried out before and after application of the D1-like antagonist SCH23390 or the D2-like antagonist L741646 (both at 45 nmol/2 μL) applied to the floor of the fourth ventricle.

In a subgroup of rats (n = 5), fourth ventricular application of SCH23390 had no effect on motility (108 ± 10.8% of baseline; $t_4 = 1.784$, $P = 0.1491$ *vs.* baseline, two-tailed paired t test) or tone (−3 ± 10.9 mg) (Fig. 6*B*) of the proximal colon. In the presence of SCH23390, the NMDA-induced increase in tone was reduced from 692 ± 565.4 mg to 327 ± 286.1 mg (n = 5; $t_4 = 2.716$, $P = 0.0266$ *vs.* NMDA alone, one-tailed paired t test). Similarly, NMDA microinjection in the SNpc increased the motility of the proximal colon to 219 ± 68.7% of baseline (n = 4; $t_3 = 3.456$, $P = 0.0204$, one-tailed paired t test); this increase was reduced significantly to 94 ± 13.1% of baseline in the presence of SCH23390 (n = 4; $t_3 = 3.085$, $P = 0.0270$, one-tailed paired t test) (Fig. 6*A* and *B*).

In a subgroup of rats (n = 5), fourth ventricular application of the D2-like antagonist L741646 increased both the motility (190 ± 51.9% of baseline; $t_4 = 3.898$, $P = 0.0088$ *vs.* baseline, one-tailed paired t test) and the tone (325 ± 108.2 mg) (Fig. 6*D*). In the presence of L741646, however, the increase in tone or the motility of the proximal colon following microinjection of NMDA into the SNpc was unaltered (tone: NMDA 221 ± 130.8 mg *vs.* L741646 + NMDA 212 ± 90.3 mg; n = 5; $t_4 = 0.2636$, $P = 0.8051$, two-tailed paired t test; motility: 199 ± 36.2% *vs.* 227 ± 57.9% of baseline in NMDA *vs.* L741646 + NMDA, respectively; n = 5; $t_4 = 0.9419$, $P = 0.3996$, two-tailed paired t test) (Fig. 6*C* and *D*).

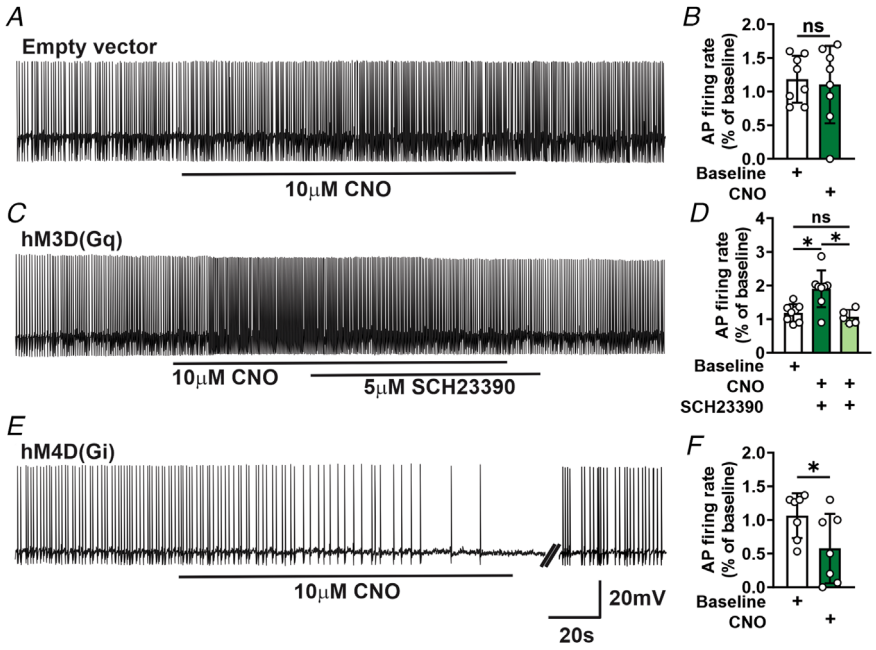

Figure 5. Chemogenetic activation of nigro-vagal projections modulates DMV neuronal activity
Whole cell patch clamp recordings from a DMV neuron current clamped at a potential to allow spontaneous firing at ∼1 event s^{-1}. *A*, in an empty vector transfected rat, superfusion with CNO had no effect on action potential firing rate. *B*, graphical summary of the effects of CNO on action potential firing rate in DMV neurons from empty vector transfected rats (n = 7, P = 0.5300; two-tailed paired t test). *C*, in an hM3D(Gq) transfected rat, superfusion with CNO increased action potential firing in a manner that was reversed following superfusion with the D1-like receptor antagonist, SCH23390. *D*, graphical summary of the effects of CNO to alter action potential firing rate in DMV neurons from hM3D(Gq) transfected rats (n = 7; P = 0.0454 baseline *vs.* CNO, P = 0.0112 CNO *vs.* SCH, P = 0.4204 baseline *vs.* SCH, one-way ANOVA, mixed effects model). *E*, in an hM4D(Gi) transfected rat, superfusion with CNO decreased action potential firing in a reversible manner. *F*, graphical summary of the effects of CNO to alter action potential firing rate in DMV neurons from hM4D(Gq) transfected rats (n = 7; P = 0.0257; two-tailed paired t test). Bars indicate a 2 min interval. ns, not significant.

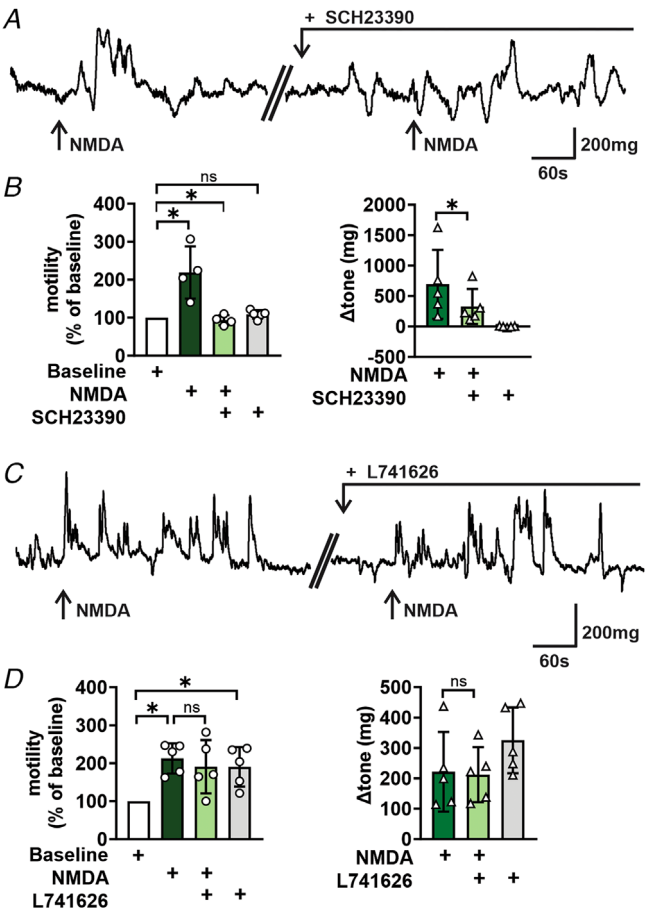

Figure 6. Pretreatment with the dopamine D1-like antagonist SCH23390 prevents the increase in tone and motility of the proximal colon following NMDA microinjection in the left SNpc

A, representative traces showing that the increase in tone and motility of the proximal colon induced by NMDA (5 nmol/210 nL, arrow) microinjection in the left SNpc (left) is prevented by fourth ventricular application of the dopamine D1-like antagonist SCH23,390 (45 nmol/2 μL) (right). *B*, summary graphic showing that the increase in motility (n = 4; P = 0.0204, one-tailed paired t test) and tone (n = 5) of the proximal colon observed in response to SNpc microinjection of NMDA is prevented by pretreatment with SCH23390 in the fourth ventricle (motility: P = 0.3974 *vs*. baseline, one-tailed paired t test; tone: P = 0.0266, one-tailed paired t test). Fourth ventricular application of SCH23390 had no effect on motility (n = 5; P = 0.1491 *vs*. baseline, two-tailed paired t test) or tone of the proximal colon. *C*, representative traces showing that the increase in tone and motility of the proximal colon induced by NMDA (5 nmol/210 nL, arrow) microinjection in the left SNpc (left) is not affected by pretreatment with the dopamine D2-like antagonist L741646 (45 nmol/2 μL) on the floor of the fourth ventricle (right). *D*, summary graphic showing that the NMDA-induced increase in motility (n = 5; P = 0.0018, one-tailed paired t test) and tone (n = 5) of the proximal colon is not altered by pretreatment with L741646 (motility: P = 0.3996 NMDA *vs*. L741646 + NMDA, two-tailed paired t test; tone P = 0.8051, two-tailed paired t test). Fourth ventricular application of the D2-like antagonist L741646 increased both the motility (n = 5; P = 0.0088 *vs*. baseline, one-tailed paired t test) and the tone. Bars indicate a 40 min interval. ns, not significant.

Overall, these data indicate that excitation of the nigro-vagal pathway increases tone and motility of the proximal colon via activation of DMV D1-like receptors, but that this nigro-vagal pathway is distinct from that which results in tonic activation of D2-like receptors to inhibit activity of the proximal colon.

To confirm the distinct and different effects of DA to modulate vagal control of the proximal colon, dopamine (DA; 100 nmol/60 nL) was microinjected in the left DMV when proximal colon tone and motility were recorded. By contrast to the D1-like receptor-dependent excitation observed following SNpc stimulation, microinjection of DA decreased both the tone (-89 ± 41.7 mg, $n = 10$) and motility ($46 \pm 23.7\%$ of baseline; $n = 10$; $t_9 = 7.163$, $P < 0.0001$, one-tailed paired t test) of the proximal colon (Fig. 7*B* and *C*). These inhibitory effects were attenuated significantly by application of the D2-like antagonist L741646; indeed, the response to exogenous application of DA became excitatory (tone: -99.5 ± 58.9 mg *vs*. 99 ± 100.1 mg; $n = 4$; $t_3 = 2.567$, $P = 0.0414$, one-tailed paired t test; motility: $62 \pm 16.7\%$ and $137 \pm 40.9\%$ of baseline in the absence and presence of L741646 respectively; $n = 4$; $t_3 = 2.725$, $P = 0.0.0361$, one-tailed paired t test) (Fig. 7*D* and *E*)

These data indicate that the SNpc modulates the tone and motility of the proximal colon via a nigro-vagal neurocircuit which activates D1 receptors on DMV neurons. They also suggest that DMV neurons responding to SNpc stimulation form a discrete neurocircuit that can be differentiated from the other DMV neurons receiving different dopaminergic inputs.

Discussion

In the present study, we have demonstrated: (1) the existence of an anatomically defined pathway that connects the SNpc to brainstem vagal motoneurons that innervate the proximal colon; (2) that nigral fibres form close anatomically connections with a discrete subpopulation of vagal proximal-colon projecting motoneurons and modulate proximal colon tone and motility; and (3) that this nigro-vagal pathway is dopaminergic and excitatory. These novel observations suggest the possibility that the impairment of this nigro-vagal dopaminergic pathway may be involved in the severely reduced colonic transit and prominent constipation observed in parkinsonism regardless of the etiology (central or peripheral) of PD.

Traditionally, the vagal innervation of the distal intestine was considered extremely sparse, despite tracing studies having revealed vagal projections that extend to the transverse colon (Berthoud et al., 1991; Powley, 2021). Furthermore, mapping of the peripheral projections of brainstem vagal motoneurons identified clusters of

intestinal-projecting neurons in the lateral tips of the DMV (Altschuler et al., 1993; Browning et al., 1999; Fox & Powley, 1985; Norgren & Smith, 1988), in the area that we report here as receiving projections from SNpc fibres. In the present study, we used a combination of anterograde tracing from the SNpc and retrograde tracing from the proximal colon to confirm the location of colonic-projecting DMV neurons, and also to demonstrate that these vagal motoneurons receive close anatomical connections from the SNpc. Indeed, neither the neuronal tracers (dextran or CTB), nor the viral constructs [hM3D(Gq) or hM4D(Gi)] used in the present study cross synapses, and both the anatomical and functional studies described suggest that the nigro-vagal pathway may be monosynaptic.

The physiological relevance of the nigro-vagal pathway and its modulation of proximal colonic function is provided by the observation that both pharmacological and chemogenetic stimulation of this pathway increase the tone and motility of the proximal colon in a vagally-dependent manner. As reported previously relative to the control of gastric tone and motility (Anselmi et al., 2017a), the nigro-vagal pathway described in the present study also appears to use dopamine (DA) as the main neurotransmitter that activates proximal colon-projecting DMV neurons. Indeed, the excitatory effects of SNpc stimulation were attenuated significantly by brainstem application of the D1-like antagonist SCH 23 390, but not by the D2-like antagonist L741646, implying that the nigrovagal pathway excites vagal efferent motoneurons via activation of dopaminergic D1 receptors.

Interestingly, the excitatory D1 receptor mediated effects on proximal colon function following nigro-vagal stimulation is in sharp contrast to the inhibitory effects observed upon exogenous application of DA to the DVC. It should be noted, however, that this inhibitory effect was reversed by the application of a D2 receptor antagonist, implying that the physiological response to brainstem application of DA is a balance between excitatory D1 and inhibitory D2 receptor activation. Notably, the observation that pharmacological and chemogenetic stimulations of the nigro-vagal pathway elicit only excitatory effects on proximal colon function provides

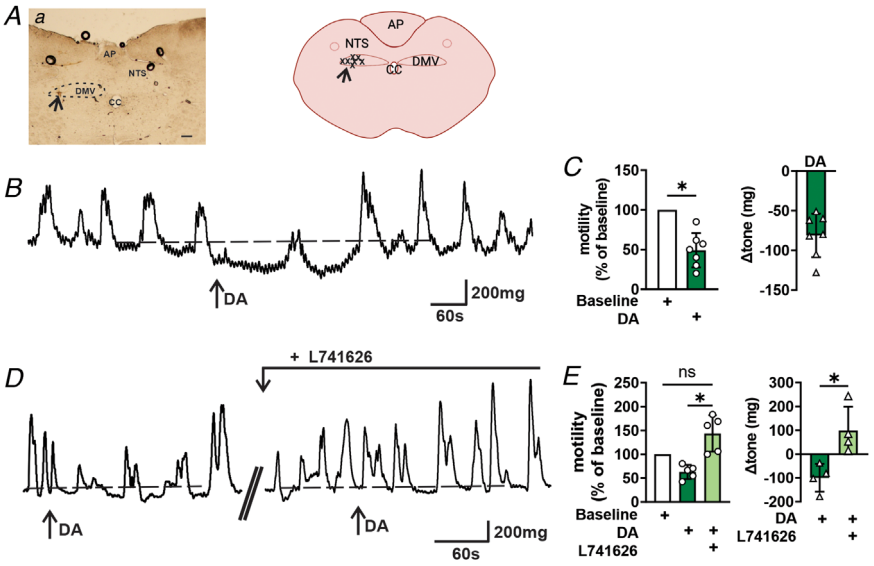

Figure 7. Pretreatment with the dopamine D2-like antagonist L741646 attenuates the decrease in motility of the proximal colon following DA microinjection in the DVC
A, representative micrograph showing the location of a dopamine (DA) injection (arrow) in the DMV (*Aa*, scale bar = 100 μm). Schematic image summarizing the location of the injection of DA in the DMV (*Ab*, n = 5, other microinjection locations were omitted for clarity). DMV, dorsal motor nucleus of the vagus; NTS, nucleus tractus solitarius; cc, central canal; AP, area postrema *B*, representative trace showing that DVC microinjection of DA (100 nmol/60 nL, arrow) decreases both the tone and motility of the proximal colon. *C*, summary graphic showing the decrease in motility (left; n = 10; $P < 0.0001$, one-tailed paired t test) and tone (right; n = 10) of the proximal colon upon DA microinjection in the left DMV. *D*, representative traces showing that the decrease in motility and tone of the proximal colon induced by DVC microinjection of DA (100 nmol/60 nL, left trace) attenuated by fourth ventricular application of the dopamine D2-like antagonist L741646 (45 nmol/2 μL, right trace). Bars indicate a 40 min interval. *E*, summary graphic showing that the decrease in proximal colon motility (n = 5; P = 0.01, one-tailed paired t test) and tone (n = 4) observed after DVC microinjection of DA is attenuated by fourth ventricle application of L741646 (motility: P = 0.2386 baseline *vs.* L741626+DA, two-tailed paired t test, P = 0.0361 DA *vs.* L741626+DA one-tailed paired t test; tone: P = 0.0414 DA *vs.* L741626 + DA, one-tailed paired t test). Bars indicate a 40 min interval. ns, not significant.

further support for the selectivity and specificity of brain-stem vagal neurocircuit organization, as put forward previously (Anselmi et al., 2017b; Babic et al., 2011; Browning et al., 1999, 2005; Browning & Travagli, 2014; Davis et al., 2004; Evans et al., 2003; Gao et al., 2009; Grabauskas & Moises, 2003),

The ENS comprises a large number of neurons whose density increases gradually from the proximal toward the distal portions of the GI tract (Furness, 2012; Grundy et al., 2006; Wood, 1987). Myenteric neurons of the ENS play a fundamental role in the control of small and large intestine functions, including the organization of appropriate behaviour patterns that direct effective secretion, absorption and transit. The present study provides the first experimental evidence, both anatomical and physiological, indicating that a nigro-vagal neurocircuit regulates proximal colonic functions in a vagally-dependent manner. Furthermore, the anatomical association between proximal colon, vagal efferent motor neurons within the DMV and SNpc delineates a neural pathway by which colonic dysfunction may be explained in both 'top down' (i.e. PD pathology is initiated centrally in the SNpc) and 'bottom up' (i.e. PD pathology is initiated in the gut and travels centrally as per Braak's hypothesis Braak & Del Tredici, 2017; Braak, Del et al., 2003) forms of parkinsonism.

Although the aetiology of PD is still open to debate, with evidence for both central and peripheral disease origins (Johnson et al., 2019; Liddle, 2018; Marras et al., 2019; Surmeier et al., 2017; Wichmann, 2019), it is also clear that the majority of parkinsonian patients experience prodromal GI issues that comprise, among other pathologies, severe constipation (Cersosimo et al., 2013; De Pablo-Fernandez et al., 2019; Edwards et al., 1992; Fasano et al., 2015; Giancola et al., 2017; Knudsen et al., 2017; Liddle, 2018; Pfeiffer, 1998; Ramprasad et al., 2018; Travagli et al., 2020). Indeed, misfolded α-synuclein, the histological hallmark of PD (Spillantini et al., 1997), has been described in both enteric and DMV neurons (Goedert, 2015; Goedert et al., 2013), which led to the hypothesis that, in some instances, PD may originate in the GI tract with the misfolding of α-synuclein originating in myenteric neurons of the GI tract before being transported retrogradely, initially to vagal motoneurons of the DMV and then onto higher centres such as the SNpc (Braak & Del Tredici, 2017; Braak, Del et al., 2003; Goedert et al., 2013; Hawkes et al., 2010). The initial involvement of the GI tract may not only account for the prodromal GI-related motor dysfunctions experienced by Parkinsonian patients, but also highlight the relevance and importance of vagal neurocircuitry in disease pathology and pathogenesis. Indeed, analysis of parkinsonian patients has shown that their gastric myoelectric activity is altered (Lu et al., 2004; Naftali et al., 2005) and is similar to that recorded in patients who received a recent vagotomy (Soykan et al., 1999). Furthermore, a large Scandinavian retrospective study suggested that truncal vagotomy reduces the incidence of PD (Kalaitzakis et al., 2008; Svensson et al., 2015). Notably, our previous studies using an environmental model of parkinsonism demonstrated that motor deficits were preceded by gastric dysmotility, that vagotomy prevented the development of parkinsonian motor dysfunctions, as well as the loss of SNpc neurons, and that vagotomy constrained the location of α-synuclein aggregates to the enteric nervous system (Anselmi et al., 2018).

The present study, which demonstrates that proximal colon motility is under similar tonic modulation by a nigro-vagal pathway, provides further support for a gut–brain etiology of PD, as well as a functional and anatomical support for Braak's staging hypothesis Braak & Del Tredici, 2017; Braak, Rub et al., 2003. It is important to note, however, that although Braak's hypothesis may explain the pathology of some instances of PD, it does not provide explanations for all instances of idiopathic PD. The present study, by demonstrating that the nigro-vagal pathway we described previously (Anselmi et al., 2017a) modulates not only gastric tone and motility, but also extends its influence distally to the proximal colon, delineates a neural pathway by which GI dysfunctions in PD may be explained independently of central or peripheral aetiology.

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Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Author contributions

R.A.T. and K.N.B. conceived and designed the research. T.X., G.N. and C.R.B. performed experiments and collected data. All authors analysed data, interpreted results of experiments, prepared figures, edited and revised manuscript, and approved the final version of manuscript submitted for publication. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved;

all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

brain stem, dorsal motor nucleus of the vagus, proximal colon motility, substantia nigra pars compacta

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document
Peer Review History

Translational perspective

The present study provides the first anatomical and physiological experimental evidence that a nigro-vagal neurocircuit regulates proximal colonic functions in a vagally-dependent manner. Furthermore, the close anatomical association between substantia nigra pars compacta (SNpc) neuronal projections and proximal colon-projecting vagal efferent motor neurons within the dorsal motor nucleus of the vagus provides a synaptically-connected conduit that might be responsible for the prodromal gastrointestinal dysfunctions, including severe constipation, observed in most Parkinson's disease (PD) patients. Future studies will define whether this nigro-vagal-proximal colon pathway represents a direct route by which environmental toxins or synucleinopathies can travel retrogradely to central nervous system centres and promote the degeneration of dopaminergic neurons of the SNpc, which would then trigger the prodromal severe constipation observed in most PD patients.