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# Tache\_Mulugeta\_OT20D024899\_Colon tissue electrical stimulation and colonic motility measurements

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## ABSTRACT

This protocol describes a process for the measurement of acute electrical stimulation-induced effects on colonic motility in anesthetized young adult Yucatan minipigs. Signals recorded from manometry probes inserted into the proximal, transverse and distal colonic regions were used to measure the effect of stimulation by serosally placed electrodes opposed to the colonic tissue at multiple sites in an acute anesthetized preparation. The effect of stimulation was quantified as motility index assessments before, during and after stimulation, and the data was used to create a functional map of colonic motor response to localized colon stimulation.

## THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Larauche M, Wang Y, Wang PM, Dubrovsky G, Lo YK, Hsiang EL, Dunn JCY, Taché Y, Liu W, Million M. The effect of colonic tissue electrical stimulation and celiac branch of the abdominal vagus nerve neuromodulation on colonic motility in anesthetized pigs. *Neurogastroenterol Motil.* 2020 Nov;32(11):e13925. doi: 10.1111/nmo.13925. Epub 2020 Jun 23. PMID: 32578346; PMCID: PMC7606494.

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## KEYWORDS

colon, direct tissue electrical stimulation, motility, pig

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This protocol describes a process for the measurement of acute electrical stimulation-induced effects on colonic motility in anesthetized young adult Yucatan minipigs. Signals recorded from manometry probes inserted into the proximal, transverse and distal colonic regions were used to measure the effect of stimulation by serosally placed electrodes opposed to the colonic tissue at multiple sites in an acute anesthetized preparation. The effect of stimulation was quantified as motility index assessments before, during and after stimulation, and the data was used to create a functional map of colonic motor response to localized colon stimulation.

## Animals

## 1

Six-to-seven months old (25-36 kg) male Yucatan minipigs (S&S farms, Ramona, CA), castrated at 7 days of age, were group housed in pens (either bedding or grate floor depending on housing availabilities - 2 pigs/pen, 42ft<sup>2</sup>) in an environmentally controlled room (lights on/off 6AM/6PM, 61-81°F) under SPF conditions.

[Yucatan minipig S&S Farms](#)

All pigs were offered ad libitum access to diet (5p94 Prolab mini pig diet, PMI nutrition) and filtered tap water.

All husbandry practices and procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals (8<sup>th</sup> edition) and were reviewed and approved by the UCLA Animal Research Committee (Institutional Animal Care and Use Committee). All efforts were made to minimize any suffering as well as the number of animals used.

## Surgical procedures

## 2 Pigs were fasted for at least 12h prior to surgery with free access to water.

For surgical level anesthesia, pigs were premedicated with midazolam (1 mg/kg),

[Midazolam HCl Injection, 50mg/10mL \(5mg/mL\), C4 Henry Schein Animal](#)

**Health Catalog #067695**

ketamine (15 mg/kg)

[Ketamine HCl Injection, 200mg/20mL \(10mg/mL\), C3N Henry Schein Animal](#)

**Health Catalog #068317**

and meloxicam (0.3 mg/kg) injected intramuscularly.

[EloxiJect \(Meloxicam\) Injection, 5mg/mL Henry Schein Animal](#)

**Health Catalog #049755**

They were then intubated, connected to a respirator for ventilation (breathing rate maintained between 13-16 breaths/min), and maintained under general anesthesia with 1-3% inhaled isoflurane.

[IsoThesia \(Isoflurane\) Solution Henry Schein Animal](#)

**Health Catalog #029405**

Maintenance fluids (lactated ringers ) were administered at 10 ml/kg/h.

[Lactated Ringers Injection, USP, Preservative-Free, Baxter Henry Schein Animal](#)

**Health Catalog #059380**

During the surgical procedure, pigs were positioned on a heating pad (32°C) in supine position. A femoral heart line was placed. When needed, the heart line was flushed with sterile saline containing 0.5% heparin.

[☒ Sodium Chloride Injection, USP, Preservative-Free, 0.9%, Baxter Henry Schein Animal](#)

**Health Catalog #059382**

[☒ Heparin Sodium Injection, USP, 10,000 unit/mL Henry Schein Animal](#)

**Health Catalog #067792**

A midline abdominal incision was performed to gain access the peritoneum. Three colonic regions of interest - proximal/ascending, transverse, distal/descending - were identified and externalized.

Still-manometry probes (Mikro-Cath™ diagnostic pressure catheter, #825-0101, Millar, Houston, TX) were inserted into the colon via a small incision and maintained in position with a loophole silk ligature.

Mikro-Cath™  
Pressure catheter

Millar      825-0101      [↗](#)

For the proximal colon, 4 manometry probes were inserted about 10 cm below the ceco-colic junction, at 10, 13, 16 and 19 cm from the point of entry. For the transverse colon, 4 manometry probes were inserted at the end of the proximal colon, at 10, 13, 16 and 19 cm from the point of entry. Distal probes were inserted in the distal colon through the anus with sensors at 10, 13, 16 and 19 cm proximal to the anal verge.

Planar electrode arrays (made in-house) were placed onto the serosal surface of the proximal, transverse and distal colon corresponding to the luminal pressures sensors position of the intestine for stimulation and recording.

The pigs were euthanized at the end of the experiment with an intravenous injection of pentobarbital (100 mg/kg).

[☒ Euthasol Solution C3N Henry Schein Animal](#)

**Health Catalog #009444**

## Stimulation experiment

- 3 After surgery, recording of the colonic motility using manometry and bioimpedance began.

Manometry measurements were made by connecting the still-manometry probes (Mikro-Cath™ diagnostic pressure catheter, #825-0101, Millar, Houston, TX)

Mikro-Cath™  
Pressure catheter

Millar      825-0101      [↗](#)

via pressure cables (PEC-10D, #850-5090, Millar, Houston, TX)

PEC-10D  
Pressure cable

Millar      850-5090      [↗](#)

to a transducer (PCU-2000, Millar, Houston, TX)

PCU-2000  
Dual Channel Pressure Control Unit

Millar      880-0129      [↗](#)

itself connected to a data acquisition system (Micro 1401, CED, Cambridge, UK)

Micro 1401  
Data acquisition unit

Cambridge Electronic Design, Ltd      CED 1401      [↗](#)

and raw data were collected using the Spike 2 software (CED, Cambridge, UK).

**Spike 2 7.10** [↗](#)

Windows 7  
by Cambridge Electronic Design, Ltd

Bioimpedance measurements were recorded using the OmniPlex data acquisition system (Plexon, Dallas, TX).

OmniPlex® D  
Data acquisition system

Plexon      OmniPlex® D      [↗](#)

Data were then extracted and analyzed using Matlab (Mathworks, Natick, MA, USA).

**Matlab R2016b** [↗](#)

MacOS 10.14.5  
by Mathworks

A baseline was established for a period of 30 min, the stimulation was initiated using customized stimulation device (Lo et al, 2015).

Lo, Y., Wagner, J., Chang, C., Rouch, J. D., Dunn, J., & Liu, W. (2015). Single-Electrode Colon Stimulation and Impedance Monitoring in an Intestinal Aganglionosis Model.. *Gastroenterology*.  
[http://doi:10.1016/s0016-5085\(15\)30422-4](http://doi:10.1016/s0016-5085(15)30422-4)

Stimulation was applied alternatively via the two planar electrode arrays placed about 2.5 cm apart.

The stimulation parameters were as follows: pulse-train, I = 15 mA, pw = 2 ms, 10 Hz, 30s-on-60s-off, 10 one-minute cycles.

Following stimulation, recording continued for another 30 minutes.

#### Motility data analysis

- 4 Recording of motility data began after surgery was complete and continued for at least 30 min for stabilization of baseline motility, and then for at least another 30 min following completion of the stimulation experiment.

The data in the accompanying dataset was obtained by analyzing the following two subsets of that entire recording: (1) 30 min prior to stimulation; and (2) the 45 minutes immediately following the onset of stimulation (15 min during stimulation, together with another 30 min of recording post stimulation).

The raw manometry data were filtered and rectified using a lab-written Spike 2 code. The same code provided the motility index (MI) defined as the the area under the curve before, during and after stimulation per each minute.

The raw bioimpedance data were processed and analyzed using a lab-written Matlab code. The same code provided the average impedance value before and after stimulation per each second.