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Protocol for the acute electrical stimulation of thoracolumbar and sacral nerve roots and colonic motility measurements in anesthetized pigs V.2

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We use this protocol and it's working

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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 on the sacral or thoracolumbar nerve roots in an acute anesthetized preparation. The effect of stimulation was quantified as motility index/area under the curve (AUC) assessments before, during and after stimulation, and the data was used to create a functional map of colonic motor response to spinal nerve roots stimulation.

Materials

Mikro-Cath™ Pressure Catheter, Millar Inc., Ref# 825-0101 https://millar.com/Clinical/MikroCath/#quicktabs-mikro_cath_diagnostic_pressure_c=2

PEC-10D Pressure cable, Millar Inc., Ref# 850-5090 **https://millar.com/Clinical/Control-Units-and-Accessories/**

PCU-2000, Dual Channel Pressure Control Unit, Millar Inc., Ref# 880-0129 **https://millar.com/Clinical/Control-Units-and-Accessories/**

Micro 1401, Data Acquisition Unit, Cambridge Electronic Design, Ltd, Ref# CED Micro1401 http://ced.co.uk/products/mic4in

Spike 2 7.10, Windows 7, Cambridge Electronic Design, Ltd, http://ced.co.uk/products/spkovin

Protocol materials

Yucatan minipig **S&S Farms** Step 1

EloxiJect (Meloxicam) Injection, 5mg/mL **Henry Schein Animal Health Catalog #**049755 Step 2

Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog #**059380 Step 2

Sodium Chloride Injection, USP, Preservative-Free, 0.9%, Baxter **Henry Schein Animal Health Catalog #**059382 Step 2

IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #**029405 Step 2

Heparin Sodium Injection, USP, 10,000 unit/mL **Henry Schein Animal Health Catalog #**067792 Step 2

Absorbable Hemostat Dressing Surgicel® Oxidized Regenerated Cellulose 1/2 X 2 Inch **McKesson Corporation Catalog #**188567 Step 2

Midazolam HCl Injection, 50mg/10mL (5mg/mL), C4 **Henry Schein Animal Health Catalog #**067695 Step 2

Ketamine HCl Injection, 200mg/20mL (10mg/mL), C3N **Henry Schein Animal Health Catalog #**068317 Step 2

Bone Wax 2.5 Gram **McKesson Corporation Catalog #**2045 Step 2

Euthasol Solution C3N **Henry Schein Animal Health Catalog #**009444 Step 2

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^2) 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 (8th 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)

EloxiJect (Meloxicam) Injection, 5mg/mL **Henry Schein Animal Health Catalog #**049755

injected intramuscularly.

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 first part of the surgical procedure, pigs were positioned on a heating pad (32°C) in supine position.

A femoral artery line was placed. When needed, the artery 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-CathTM 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.

Equipment

Mikro-Cath™	NAME
Pressure catheter	TYPE
Millar	BRAND
825-0101	SKU
https://millar.com/clinical/products/mikro-cath#quicktabs-mikro_cath_diagnostic_pressure_c=2	LINK

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. One sensor was also added in the anal canal (2 cm from the anal verge).

The abdomen was then closed and pigs placed on prone position, leaving access to the back.

The S1-S4 sacral spinal nerves or T12-L1 thoracolumbar spinal nerves were accessed via a laminectomy.(surgery time: 30 min-40 min).

Laminectomy is a common spine operation and refers to surgical removal of the vertebral lamina, thereby unroofing the vertebral canal. We followed similar processes as described in this **reference**.

Before surgery, we palpated the lumbar spinous processes along the midline and identified the vertebral levels of interest (T12-L1 or S1-S4). A midline incision using a scalpel/electrocautery was then performed and the subcutaneous tissue and fat dissected to gain access to the thoracolumbar or lumbosacral fascia, respectively. The fascia along the midline was cut to expose the supraspinous ligaments spanning between spinous processes. The supraspinous ligaments was open over a few millimiters. Then using a Freer elevator, the supraspinous ligaments was gently detached from spinous processes (subperiosteal dissection). The dissection was then extended to the interspinous ligaments up to the facet joints of the spinous processes along the area of interest. After locating the lamina, we opened the lamina using a surgical saw or a Kerrison rongeur to extract bone in a piece-wise fashion over the whole vertebral segment of interest in order to gain access to the ligamentum flavum, periosteum, epidural fat, and dura sac. The spinous processes corresponding to the region of interest were removed, allowing access to the meninges. The epidural fat was gently removed taking care not to damage the dura sac and allowing identification of the spinal roots.

During the surgical procedure and as the laminectomy was completed, hemostasis was performed with bipolar electrosurgery. Monopolar electrosurgery was not used because of the proximity of neural structures.

Bone wax was placed along sites of bleeding from exposed bone and absorbable hemostat dressing were used to obtain hemostasis near soft tissue. We also used cotton to wick serous fluid and blood away from the dissection where necessary.

Absorbable Hemostat Dressing Surgicel® Oxidized Regenerated Cellulose 1/2 X 2 Inch **McKesson Corporation** **Catalog #**188567

Bone Wax 2.5 Gram **McKesson Corporation** **Catalog #**2045

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, the left thoracolumbar (T12-L1) and sacral root nerves (S1, S2, S3 and S4) were identified and isolated using small pieces of cotton. Needle or cuff electrodes (NCX-2.5-3-500umPR-1-1-500SS-SUT, Microprobes for Life Science Inc., Gaithersburg, MD, USA) were placed on the roots.

Equipment

NCX-2.5-3-500umPR-1-1-500SS-SUT	NAME
nerve cuff electrode	TYPE
Microprobes for Life Science Inc.	BRAND
custom	SKU
X-wide contact nerve cuff electrode with 3 contacts made of 500 um platinum iridium ribbon, Cuff ID= 2.5 mm	SPECIFICATIONS

After positioning the stimulation electrodes, recording of the colonic motility using manometry began.

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

Equipment

Mikro-Cath™	NAME
Pressure catheter	TYPE
Millar	BRAND
825-0101	SKU
https://millar.com/clinical/products/mikro-cath#quicktabs-mikro_cath_diagnostic_pressure_c=2	LINK

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

Equipment

PEC-10D	NAME
Pressure cable	TYPE
Millar	BRAND
850-5090	SKU
https://millar.com/products/research/pressure/control-units/pcu-2000-dual-channel-pressure-control-unit-patient	LINK

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

Equipment

PCU-2000	NAME
Dual Channel Pressure Control Unit	TYPE
Millar	BRAND
880-0129	SKU
https://millar.com/products/research/pressure/control-units/pcu-2000-dual-channel-pressure-control-unit-patient	LINK

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

Equipment

Micro 1401	NAME
Data acquisition unit	TYPE
Cambridge Electronic Design, Ltd	BRAND
CED 1401	SKU
http://ced.co.uk/products/mic3in	LINK

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

Software	
Spike 2	NAME
Windows 7	OS
Cambridge Electronic Design, Ltd	DEVELOPER

A baseline was established for a period of at least 30 min, then the stimulation was initiated.

Thoracolumbar nerve electrical stimulation (TLNS) was performed on T12 and L1 roots, using a needle electrode unilaterally positioned on the left root targeting afferent (dorsal root) or both afferent/efferent fibers (dorsal and ventral roots past the DRG), without block. Two different stimulation protocols were performed 10 Hz, 0.3 ms, 0.5 mA, continuous (30 s on, 90s off) or 30 Hz, 0.3 ms, 0.5 mA, pulse-train (30 s on, 90 s off) for 10 min.

Sacral nerve electrical stimulation (SNS) was performed on S1, S2, S3 and S4 roots using a cuff electrode placed unilaterally on the left dorsal root or both ventral/dorsal root. Stimulation was done at 30 Hz, 0.3ms, 0.5 mA, pulse-train (30 s on, 90 s off) with or without afferent (AB) or efferent (EB) block (40 kHz, 0.1 ms, 2 mA) for 10 min.

Following stimulation, recording continued for at least another 30 minutes.

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 40 minutes immediately following the onset of stimulation (10 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.