

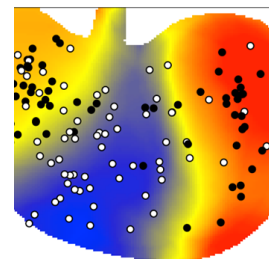
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Version 1

Measurement of duodenal motility using implanted strain gauges V.1

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Terry Powley¹, Zhenjun Tan¹, Matthew Ward¹

¹Purdue University

SPARC

Tech. support email: info@neuinfo.org



Deborah Jaffey

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes a process for the measurement of electrical stimulation-induced effects on duodenal motility in young adult Sprague-Dawley rats. Signals recorded from strain gauges attached to the proximal duodenal surface were used to measure the effect of stimulation by patch electrodes implanted at multiple sites across the rat stomach in an acute anesthetized preparation. The effect of stimulation was quantified as the ratio of various motility assessments during and after stimulation vs. before stimulation, and the data was used to create a functional map of duodenal motor response to localized gastric stimulation.

Materials

STEP MATERIALS

-  Vetbond **3M corporation Catalog #1469SB**
-  Sprague-Dawley **Envigo**
-  2018 Teklad global 18% protein rodent diet **Envigo**
-  Isoflurane **Akorn Inc Catalog #NDC: 59399-106-01**
-  Ketamine **Patterson Veterinary Catalog #07-803-6637**
-  Xylazine **Akorn Inc Catalog #NDC: 59399-110-20**



Protocol materials

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Troubleshooting

Animals

- 1 Two-to four-month-old male

 Sprague-Dawley **Envigo**

rats were housed in vented rack cages in an Association for Assessment and Accreditation of Laboratory Animal Care-approved temperature (22–24 °C) and humidity (40–60%) controlled colony room. The room was maintained on a 12-hour light–dark schedule. Pelleted chow

 2018 Teklad global 18% protein rodent diet **Envigo**

and filtered tap water were provided ad libitum. All husbandry practices conformed to the NIH *Guide for the Care and Use of Laboratory Animals* (8th edition) and were reviewed and approved by the Purdue University Animal Care and Use Committee. All efforts were made to minimize any suffering as well as the number of animals used.

Surgical Procedures

- 2 Animals were transferred to wire hanging cages the day before surgery and then fasted for overnight with free access to water. Rats were then anesthetized with

 Isoflurane **Akorn Inc Catalog #NDC: 59399-106-01**

(5%) in an induction box and transferred to a

Equipment

Somnosuite Low-flow Anesthesia System

NAME

Anesthesia system

TYPE

Kent Scientific

BRAND

SomnoSuite

SKU

<https://www.kentscientific.com/products/somnosuite/>^{LINK}

on a surgery platform

Equipment

SurgiSuite Multifunction Surgery Platform NAME

Surgery platform TYPE

Kent Scientific BRAND

SurgiSuite SKU

<https://www.kentscientific.com/products/surgisuite/> LINK

A servo-controlled homoeothermic heating blanket, equipped with a rectal thermometer, was used to maintain body temperature at 36 °C. The level of anesthesia was reduced to 2.5% isoflurane for the surgical procedure.

- 3 After midline laparotomy, the stomach and 3-4cm of proximal duodenum were exteriorized onto saline-soaked gauze pads. One to three custom-made stimulation patch electrodes (each consisting of two Pt/Ir foil plates, each about 1mm x 2mm, spaced about 4mm apart (center to center), and mounted to a silicone pad).

Equipment

Custom patch electrodes NAME

Electrodes TYPE

Microprobes for Life Science BRAND

N/A SKU

<https://microprobes.com/products/peripheral-electrodes/emg-patch> LINK

were sutured on the serosal surface of stomach. Electrodes were typically aligned with or at right angles to the angle of the local greater curvature.

- 4 A custom-made strain gauge (4×3.5mm, Clunbury Scientific LLC, Bloomfield Hills, MI) constructed from two strain gauge elements

Equipment

EA-06-031CE-350

NAME

Strain gauge

TYPE

Micromeasurements

BRAND

EA-06-031CE-350

SKU

was then glued to the serosal surface of the proximal duodenum (5-15mm distal to pyloric sphincter) using

 Vetbond **3M corporation Catalog #1469SB**

The strain gauge was oriented parallel to the longitudinal or circular muscle.

The fine wire leads attached to the stain gauge and patch electrodes were exteriorized and connected to a DC bridge amplifier and stimulator respectively (see below).

- 5 The animal was kept in a supine position with the abdominal area covered by saline-soaked gauze pads. Normal saline (2.0ml/hr) was injected continuously i.p. using a syringe pump

Equipment

GenieTouch syringe pump

NAME

syringe pump

TYPE

Kent Scientific

BRAND

GenieTouch™ Syringe Pump

SKU

<https://www.kentscientific.com/products/genietouch-syringe-pump/>^{LINK}

The animal was then covered with a blanket to help maintain body temperature, and anesthesia was reduced to 1.5% isoflurane and maintained at that level for the reminder of the experiment.

Stimulation experiment

- 6 After surgery, recording of duodenal motility began. Strain gauge measurements were made using a DC bridge amplifier system purchased from MDE gmbh

Equipment

DC bridge amplifier (4 channel)

NAME

Amplifier

TYPE

MDE

BRAND

EXP-SG-4

SKU

<http://mdegmbh.eu/en/simple-amplifiers/dc-bridge-amplifier.html#available-models>

LINK

Once the strain gauge signal reached a stable baseline and had maintained that stable baseline for at least 20 min, stimulation was initiated. Stimulation was provided by a PlexStim electrical stimulator

Equipment

PlexStim Electrical Stimulator

NAME

Stimulator

TYPE

Plexstor

BRAND

PlexStim

SKU

<https://plexon.com/products/plexstim-electrical-stimulator-2-system/>

LINK

Stimulation parameters were as follows: biphasic, $I = 0.3\text{mA}$, $p_w = 0.2\text{ms}$, 10 Hz, 20s-on-40s-off, 5 one-minute cycles.

Following stimulation, recording continued for about another hour.

Perfusion

- 7 Once the experiment was complete, the animals were given a lethal dose of

 Ketamine **Patterson Veterinary Catalog #07-803-6637**

and

 Xylazine **Akorn Inc Catalog #NDC: 59399-110-20**

(i.p. 275mg/kg ketamine and 27.5 mg/kg xylazine).

The locations of the plates of the electrodes used in the experiment were marked with blue suture thread before the electrodes were removed.

To ensure that the stomach was normally distended at the time of fixation, the organ was inspected for normal distension or accommodation, and, as required, physiological saline (3.3ml/100gwt) that had been warmed to body temperature was slowly infused into the stomach by gavage catheter. With the stomach normally dilated, the animal was first transcardially perfused through the vasculature with physiological saline and then with 4% paraformaldehyde in 0.1 mol/liter PBS; pH 7.4). After perfusion, the distal esophagus and the proximal duodenum were transected, and the stomach was freed and removed. The organ was then opened with a longitudinal cut along the greater curvature. Next, the ventral and dorsal stomach walls were separated by an incision along the lesser curvature, thus yielding two whole mounts per animal.

Electrode location measurement

- 8 The ventral half stomach was placed in PBS in a dissecting dish under a stereomicroscope, with the inner surface facing up, and the locations of two plates on each the electrodes were clearly marked with pins, and a photograph of the stomach capturing the entire surface was then taken.

The image of the stomach at a consistent magnification for each stomach to be measured was printed, and x and y locations of the midpoint of the electrode measured from the image, together with the size of the stomach itself so that electrode location could be reported as percentage measurements relative to the pylorus end of the stomach contour (x) and relative to the bottom edge of the stomach at the greater curvature (y). In addition, the orientation of the electrode relative to a line from the top of the limiting ridge (near the LES) to the bottom point near the greater curvature where the limiting ridge changes direction was measured.

Motility data analysis

- 9 Recording of motility data began after surgery was complete and continued for at least an hour for stabilization of baseline motility, and then for at least another hour following

completion of the stimulation experiment. Data analysis typically used two subsets of that entire recording: (1) 15 min immediately prior to stimulation; and (2) the 15 min immediately following the onset of stimulation (5 min during stimulation together with another 10 min of recording). The raw strain gauge data was filtered and rectified using lab-written MATLAB code. The same code provided three quantitative assessments of the ratio of motility during and after stimulation to motility before stimulation. These three assessments are: (1) average amplitude ratio; (2) average frequency ratio (defined as number of excursions exceeding 10% of maximum, per unit time); and (3) motility index (MI) defined as the ratio of the area under the curve during and after stimulation to the area under the curve before stimulation, per unit time. Results obtained from the various stimulation locations were mapped in a variety of ways including contour maps and maps of locations where MI exceeded 1.0 vs. locations where MI was less than 1.0.