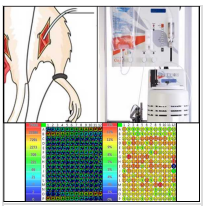

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Quantification of the effect of gastric electrical stimulation location on circulating blood hormone levels

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SPARC
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ABSTRACT

This protocol describes a process for the quantification of electrical stimulation-induced effects on circulating blood hormone levels in young adult Sprague-Dawley rats. Blood samples were collected via the left femoral artery using a Culex sampling system both before and after a discrete stimulation period. Stimulation occurred via patch electrodes implanted at multiple sites across the rat stomach in an acute anesthetized preparation.

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KEYWORDS

rat, stomach, gastric electrical stimulation, blood, hormone

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Animals

1 Two-to four-month-old ☒Sprague-Dawley **Envigo** male and female 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 18 h with free access to water. Rats were then anesthetized with ☒Isoflurane **Akorn Animal Health Catalog #NDC: 59399-106-01** (5%) in an induction box and transferred to a

Somnosuite Low-flow Anesthesia System
Anesthesia system
Kent Scientific **SomnoSuite**

on a surgery platform

SurgiSuite Multifunction Surgery Platform
Surgery platform
Kent Scientific **SurgiSuite**

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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. Custom-made stimulation patch electrodes

Custom patch electrodes
Electrodes
Microprobes for Life Science N/A

were sutured on the serosal surface of stomach, either one or two electrodes.

4 A custom-made strain gauge (4x3.5mm, Clunbury Scientific LLC, Bloomfield Hills, MI) constructed from two strain gauge elements

EA-06-031CE-350
Strain gauge
Micromeasurements EA-06-031CE-350

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).

The original goal of these experiments was to correlate hormone release and duodenal motility; however, it was determined that the blood draw itself affected the motility results, hence, while the strain gauge is implanted so that the state of the animal is comparable to that in the motility experiments, the strain gauge data is not usable.

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

6 With the rat on a supine position, a 1.0-1.5cm incision was made on the angle of the left hind

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leg and the left femoral artery was then exposed and separated from the connective tissue and femoral vein by blunt dissection. A catheter (CX-80002S, www.BASinc.com) was inserted into the femoral artery (2-3 cm) via a pre-cut 45 degree angle incision with micro-dissecting scissors in the femoral wall. The catheter was pre-filled with heparined (Heparin: Meitheal Pharmaceuticals Inc.) saline (10units/ml). The catheter was tied in position with sutures and connected to the Culex automated blood sample system.

7 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.

Blood draw set up

8 See **steps 1-5** of the following protocol:

SPARC - Preparation of Plasma Samples from Rats
by J Paul Robinson
PREVIEW RUN

Stimulation and blood draw

9 About one hour after the end of surgery, the animal was considered stable enough to begin the blood draw/stimulation experiment. This was typically confirmed by assessment of the duodenal motility signal (see Protocol "Measurement of duodenal motility using implanted strain gauges"), but this process is not necessary.

Blood draw timings were defined relative to the start of the 5 min period of stimulation. Blood samples (0.15ml each) were withdrawn at the following times: -30 min, -5 min, 5 min, 15 min, 30 min.

Stimulation was provided by a PlexStim electrical stimulator

PlexStim Electical Stimulator
Stimulator
Plexstor **PlexStim**

Stimulation parameters were as follows: biphasic, I = 0.3mA, pw = 0.2ms, 10 Hz, 20s-on-40s-off, 5 one-minute cycles.

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Blood processing

10 See **steps 6-21** of the following protocol:

SPARC - Preparation of Plasma Samples from Rats
by J Paul Robinson
PREVIEW RUN

The rest of the hormone analysis process is captured in four other protocols:

- SPARC - Millipore Metabolic Rat Multiplex Bead Assay for Flow Cytometry
- SPARC - Attune NxT Set-up for Milli-metabolic bead assay Acquisition
- SPARC - Setting up the BEADS for the Millipore Metabolic Rat Milliplex Assay
- SPARC - Analysis of multiplexed bead data using MPLEX software

Perfusion

11 Once the blood sampling was complete, the animals were given a lethal dose of
Ketamine **Patterson**
Veterinary Catalog #07-803-6637
and
Xylazine **Akorn Animal**
Health Catalog #NDC: 59399-110-20
(i.p. 275mg/kg ketamine and 27.5 mg/kg xylazine).

The locations of 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/100g of rat weight) 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

The ventral half stomach was placed in PBS in a dissecting dish under a stereomicroscope,

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12 with the inner surface facing up, and the locations of the electrodes were marked using pins to clearly show each end of the electrode, 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 off 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.

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