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Protocol for chronic implantation of patch electrodes on the gastric muscle wall of the rat

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Electrical stimulation is a potential therapy for gastric disorders. Here we describe our surgical procedure for the implantation of patch electrodes on the muscle wall of the stomach of both male and female rats for use in chronic studies looking at the long-term effects of stimulation on physiology and behavior. The surgery is well tolerated by the subjects as rats return to pre-surgical body weight and normal solid chow intake within 7 to 10 days post-surgery. In our hands, rats implanted using the methods described below have been continuously run in feeding and motility experiments for 8 to 12 weeks with minimal to no loss of subjects. When failure does occur, it is typically a result of back mount failure at the site of attachment of the overhead tether to the back mount.

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Rats, Electrical Stimulation, Stomach, Electrodes, Chronic, Male, Female, Motility

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All procedures described were conducted in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* (8th ed., The National Academic Press, Washington, D.C.), and were approved by the Purdue University Animal Care and Use Committee.

Digital Storage Oscilloscope
Oscilloscope
Keysight **EDUX1002G**

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

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

MZ APO
Microscope
Leica **MZ APO**

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Skin Stapler
skin stapler
Fine Science Tools **12030-01**

LED Curing Light
LED Curing Light
Premium Plus **LED Curing Light**

Hot Bead Sterilizer
Sterilizer
Fine Science Tools **18000-45**

Vannas Spring Scissors
surgical scissors
Fine Science Tools **15000-08**

Delicate Suture Tying Forceps
forceps
Fine Science Tools **11063-07**

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Castroviejo Micro Needle Holders
needle holders
Fine Science Tools **12060-01**

Fine Scissors CeramaCut
Scissors
Fine Science Tools **14958-11**

Shaver w/ #50 Blade
shaver
Fine Science Tools **A5**

☒ Rat MD's Baytril (2 mg/tablet) **Bio-Serv Catalog #MD300-2**

☒ Rat MD's Baytril (2 mg/tablet) Rimadyl (2 mg/tablet) **Bio-Serv Catalog #F06801**

☒ Sterile Towel Drapes **Dynarex Catalog #4410**

☒ 2"x2" Sterile Gauze Pads **Honeywell Catalog #067522**

☒ sterile cotton swabs **Puritan Catalog #806-WC**

☒ Apexa Sterile Artificial Tears Ophthalmic Lubricant Ointment **Walmart**

☒ Chronic EMG Patch Electrode **Microprobes for Life Sciences**

☒ KWIK-SIL **World Precision Instruments Catalog #KWIK-SIL**

☒ Polyester Mesh **Surgicalmesh Catalog #PETKM14002**

☒ Sterile Saline **Nova-tech, Inc. Catalog #65207-807-60**

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Disposable Staples Cartridge **Fine Science Tools Catalog #12030-23**

3-0 Surgipro II Monofilament Polypropylene **Covidien Catalog #VP-760-X**

5-0 Surgipro II Monofilament Polypropylene **Covidien Catalog #VP-566-X**

Buprenorphine (Buprenex) **Midwest Veterinary SUpply Catalog # 191.26890.3**

Glycopyrrolate **Akorn Animal Health**

8-0 Nylon Black **Fine Science Tools Catalog #12051-08**

3-0 Silk LOOK **Midwest Veterinary SUpply**

Dust Cap **P1 Technologies Catalog #363DC**

6 Pin Pedestal **P1 Technologies Catalog #MS363**

Pedestal Backmount **P1 Technologies Catalog #E363/BM**

6 Channel Electrode Female Socket Pin **P1 Technologies Catalog #E363/0**

Fusio Self-adhesive Flowable Composite Pentron **Henry Schein Animal Health Catalog #A1**

Sprague-Dawley Rat **Envigo Catalog #RRID:RGD_70508**

Subjects

1 Male and/or female rats of the appropriate age and weight for the experimental design were used. Note: we have successfully implanted rats across a range of ages (3 to 6 months) and weights (250 to 550 grams).

2 Two days prior to surgery, start the rats on antibiotics (one 2 mg Baytril tablet per day).

Setup

3 Don a fresh pair of sterile latex powder-free gloves.

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4 Sterilize surgical instruments using a hot bead sterilizer and place on a sterile surgical drape.

5 Clean Surgisuite operating surface and heating pad with 70% ethanol and place a sterile drape over heating pad.

Anesthetic Induction and Preparation

6 Fast rat overnight with only access to water.

7 Induce anesthesia using 5% isoflurane (Somnosuite anesthesia system).

8 Place rat on sterile drape covered heating pad and lower isoflurane to 2%.

9 Apply sterile lubricant to both eyes to prevent drying (Artificial Tears Ophthalmic Lubricant Ointment).

10 Shave hair from back between shoulder blades and entire abdomen.

11 Clean shaved area with 70% ethanol followed by povidone-iodine solution and allow to dry.

12 Inject glycopyrrolate (0.2 mg/ml, s.c.) to minimize secretions.

13 Inject buprenorphine (Buprenex, 0.01 mg/kg, s.c.) for analgesia.

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14 Make a small incision using surgical scissors between the midline of the shoulder blades.

15 Change out sterile drape and don a fresh pair of sterile latex powder-free gloves.

16 Drape rat with opening in sterile drape to expose the abdomen.

Median Laparotomy

17 Apply pressure to the hindfoot of all four limbs to ensure there is no pain response.

18 Ensure that body temp and respiration rate are normal.

19 Make a 3-4 cm incision in the upper midline of the abdomen using surgical scissors, just below the xyphoid process as a landmark.

20 Drape perimeter of incision with sterile gauze pads and wet gauze with body temp sterile saline solution.

Mobilizing the Stomach

21 Using two wet sterile cotton swabs, gently lift the stomach out of the abdominal cavity and place on sterile wet gauze.

22 Drape additional sterile gauze over stomach leaving only the electrode placement site exposed.

23 Wet the gauze thoroughly with sterile, physiological saline warmed to body temperature.

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24 Note: continually wet the exposed region of the stomach throughout the surgical procedure using sterile saline warmed to body temperature ensuring that the muscle wall never dries out.

Surgical Attachment of Patch Electrode

25 Swing surgical scope into place.

26 Place chronic EMG patch electrode (MicroProbes) on the muscle wall of the stomach at appropriate location and ensure that suture points will not pierce the underlying vasculature. Note: prior to the start of the surgery check the impedance of the patch electrode with the electrode placed in physiological saline warmed to body temperature using an oscilloscope.

27 Using four stay sutures (8-0 nylon), one at each corner, attach the patch electrode to the serosal surface of the stomach muscle wall while being careful to avoid underlying blood vessels.

28 A fifth stay suture was routinely placed along the long edge of the patch electrode on the side opposite of the incoming lead wires.

29 Return the stomach to its natural position in the abdomen.

30 Using an oscilloscope, test patch the electrode again to ensure proper function.

Exteriorizing Patch Electrode Leads

31 Roll rat onto its side.

32 Starting at the incision site located between the shoulder blades, work a trocar (length 15 cm; I.D. 3 mm) between the skin and underlying muscle until roughly 2 cm past the rib cage and then puncture through muscle wall into abdominal cavity.

33 Bring patch electrode leads through the trocar.

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34 Remove trocar leaving just the pins of the leads exposed between the shoulder blades.

35 Return the rat to a supine position.

36 Ensure that the stomach along with intestines are in their natural position. If not, using wet sterile cotton swabs make the necessary adjustments to the organs.

37 Ensure that the patch electrode leads are positioned so that they do not create any unnecessary torque on the stomach.

38 Cover abdominal incision with wet sterile gauze.

39 Roll rat into a prone position.

Back Mount Construction and Implantation

40 Connect the exteriorized leads to a pedestal/pedestal back mount (P1 Technologies). Note: prior to start of surgery, a piece of polyester mesh (3 cm x 3 cm) should be attached to the pedestal back mount using 3-0 silk suture.

41 Place dummy pins in the remaining open slots in the pedestal/pedestal back mount.

42 While wearing appropriate eye protection, secure pins in place with Fusio self-adhesive flowable composite and LED curing light.

43 Using multiple layers of adhesive, slowly encapsulate the base of the pedestal back mount creating a watertight seal around the pins.

44 Screw dust cap (P1 Technologies) onto pedestal sealing it from exposure to liquids (e.g, blood and saline).

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45 Place the back mount in position between the shoulder blades.

46 Blunt dissect the skin away from underlying muscle as needed to make room for mesh.

47 Suture the mesh attached to the back mount to the underlying muscle using multiple stay sutures (Surgipro II, monofilament polypropylene, 5-0).

48 Return the skin to its natural lie and close around the pedestal of the back mount using interrupted sutures (Surgipro II, monofilament polypropylene, 3-0).

49 Roll rat onto back exposing abdominal opening.

Abdominal Closure

50 Ensure that organs and leads are in their appropriate positions.

51 Hydrate the rat by filling the abdominal cavity with 5-10 ml physiological sterile saline warmed to body temperature.

52 Close muscle with 5-0 polypropylene (Surgipro II) interrupted sutures.

53 Close the skin with 3-0 polypropylene (Surgipro II) interrupted sutures.

54 Reinforce skin sutures with staples. NOTE: staples are removed 10-14 post surgery.

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55 Using oscilloscope, check patency of patch electrode to ensure that no damage occurred during surgery.

56 Seal perimeter of dust cap using KWIK-SIL to ensure that the back mount is watertight during the healing process.

Postoperative Care

57 Rats are brought out of anesthesia slowly by reducing the flow of isoflurane by 0.5% every 15 min.

58 Once awake, rats are returned to their home cage and provided with a 5mg tablet of Baytril (2mg)/Rimadyl (2mg).

59 A 5 mg Baytril/Rimadyl tablet is then provided once each day for the following three days post-surgery followed by a 5 mg Baytril tablet once a day for the next four days.

60 Rats are housed individually to ensure proper healing of surgical sites.

61 Ad lib water is provided throughout the duration of post-surgical recovery.

62 Ad lib chow is provided starting one day post-surgery.

63 Remove skin staples post-operative day 10-14.

64 NOTE: Rats can be safely tethered immediately following surgery, however we have found that tethering the rats 14 days post-surgery results in optimal patency for long-term chronic use.

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