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Measuring the Visceromotor Response in Rodents

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We use this protocol and it's working

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Abstract

Protocol for measuring the visceromotor response (VMR) to colorectal distension in mice to investigate visceral nociception.

Guidelines

Follow all institutional requirements for vertebrate animal work

Materials

Surgery:
Standard small animal surgical instruments
Betadine (or similar)
Fur clipper
Heating Pad
Sterile sutures with small cutting needle (6-0, non-dissolvable)
Surgical staples (optional)
Analgesics
Sterile Saline for fluid resuscitation
27G needles

Wire Implantation
FE6350 (LVM) Wire (0.175mm thickness, quadruple PTFE insulated)
Sterile cannula (for threading wire)

Wireless Implantation
Data Systems International (DSI) ETA-F10 transmitter

Wired Recordings
NeuroLog
- Band-Pass Filter NL125/6
- Pressure Amplifier NL108A
- Extracellular Recording Amplifier NL104A
- Compact System Case NL905
- Headstage NL100AK
- Disposable Physiological Pressure Transducer NL108T2

CED Data Acquisition Unit Micro1401-3
Spike2 software

Wireless Recordings
Data Systems International Ponemah
- MX2 Matrix 2.0
- Signal Interface
- RPC-1 Receiver

Distensions
Surgilube (or similar)
Disposable gavage needle
1 ml syringe
Water
Interlog Distender Series IIR Barostat

Broomenator style restrainer
Balloon catheter with 2cm or shorter balloon

Safety warnings

Standard precautions for animal work

Before start

Ensure all equipment is functional and reagents available prior to start

EMG Wire Implantation

1 Prepare surgical area in accordance with institutional requirements.
- Clean and sterilize instruments
- Warm heating pad
- Check all equipment prior to starting

2 Prepare the mouse
- Anesthetize with 1.5% inhaled Isoflurane
- Shave neck and right abdomen
- Clean exposed skin with Betadine

3 Surgical incisions
- Make a small (< 1 cm) skin incision in right lower abdomen above the inguinal region to expose the abdominal muscle
- Turn over mouse and make small incision on nape of neck

4 Wire Placement
- Tunnel sterile cannula subcutaneously from neck incision along the side of the mouse and out the abdominal incision taking care to not injure/enter the abdominal compartment
- Thread two sterilized EMG wires (~20cm length) through cannula. Holding the wires from the abdominal side, withdraw the cannula through the neck incision leaving the wires in place
- Remove 2-3mm of insulation from the abdominal end of the wires and tunnel into the abdominal muscle utilizing a 27g needle one at a time, ensuring the wires are close to each but do not touch
- Secure wires using 6-0 non-dissolvable sutures
- Close skin incisions with interrupted sutures
- Coil the wires extending from the next into a flat disc and suture to nape of neck to prevent disruption

EMG Wireless Transmitter Implantation

5 - Prepare surgical area in accordance with institutional requirements.
- Clean and sterilize instruments
- Warm heating pad
- Check all equipment prior to starting

6 Prepare the mouse
- Anesthetize with 1.5% inhaled Isoflurane
- Shave right abdomen
- Clean exposed skin with Betadine

7 Surgical incision
- Make a ~ 1 cm skin incision in right lower abdomen above the inguinal region to expose the abdominal muscle sufficient to admit passage of the transmitter body

8 Transmitter Implantation
- Use blunt dissection to create a pocket in the right flank sufficient to house the transmitter. Take care to avoid making the pocket too large, which could result in seroma formation.
- Remove 2mm of insulation from each wire. Set aside insulation for later use.
- Using a 27 G needle, tunnel each wire individually through abdominal muscle. Wires should be placed closely together but should not touch
- Place insulation back on the exposed ends of each wire as they emerge from the muscle tissue and secure in place with 6-0 non-dissolvable sutures. Note: the insulation can be stretched with fine forceps to aid placement back into the wire.
- Suture the wires proximally to muscle entry to both secure the wires in place and to prevent moisture seepage into the transmitter.
- Close skin with either interrupted sutures or surgical staples

Postoperative Care

9 - Provide analgesia per institutional surgical protocol
- Administer 1ml of warm, sterile saline subcutaneously to mitigate dehydration
- Animals should be singly housed to prevent surgical site and EMG wire disruption prior to VMR experiment

Preparation for VMR

10 - Lightly anesthetize mouse with Isoflurane
- If using wired VMR, take down coil by carefully cutting sutures and unspooling wire. Take care not to cut the wire
- Lubricate disposable gavage needle with Surgilube or similar and administer 100 uL water enema to remove fecal pellets
- Insert balloon catheter to depth of 2 cm and secure in place by taping tubing to animal tail
- Place mouse into broomenator style restrainer and allow to wake up

Colorectal Distension

11 - Follow manufactures instructions for recording EMG and pressure tracings
- We use a NeuroLog NL100AK headstage, Band-Pass Filter (NL125/6), Pressure Amp (NL108A), and Extracellular Recording Amplifier (NL104A) feeding into Spike2 for our

wired recordings and the Data Sciences International Ponemah system for our wireless recordings
- Program barostat (Distended Series IIR) to deliver sequential balloon inflations of 20, 40, 50, 60, 70, and 80 mmHg for 20 s each with a 2 m rest period in between distensions