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③ Vagotomy and Tamoxifen treatment

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We use this protocol and it's working

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Abstract

This protocol provides the methodology of vagotomy procedure and tamoxifen injections in a murine model, investigating the pathological role of alpha-synuclein in the gastrointestinal (GI) system. The mouse model includes features of exclusive expression of alpha-synuclein in cholecystokinin (CCK) cells within the gut. The protocol intricately details the surgical procedure, including a dissection of the vagus node to ensure the mice not only survive but thrive post-surgery. Subsequent tamoxifen injections, utilizing a tamoxifen-inducible promoter, aim to induce the expression of alpha-synuclein.

The significance of this protocol lies in presenting a unified and standardized procedure for both vagotomy and tamoxifen injections. This streamlined approach is crucial for investigating the role of alpha-synuclein in the GI system.

Attachments



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19KB



Materials

Materials

- Syringe
- Needles
- Scalpels
- Ketamine
- Xylazine
- Bupivacaine
- Buprenorphine hydrochloride
- Tamoxifen
- Corn oil
- Stainless steel wound clips (MikRon Precision Inc)

Troubleshooting



Vagotomy and Tamoxifen treatment



- 1 Perform surgical subdiaphragmatic vagotomy in 1-month-old male and female SNCAbow;Vil-CreERT2 mice.
- Prior to surgery, sterilize all surgical instruments. Use syringe, needles, sutures, and scalpels from sterile packs, open it at the time of the operation.
- 3 Sterilize dissecting instruments before using on the next mouse.
- Surgeon washed their hands thoroughly for 00:05:00 before donning sterile gloves.

5m

- 5 Surgeon maintained a sterile operating field during the operation.
- 6 Apply lubricant eye ointment to mouse eyes to prevent corneal drying.
- Anesthetize mice with ketamine/xylazine at a dose of 87/13 mg/kg by intraperitoneal injection. Determine adequate anesthesia by lack of movement, lack of response to tail pinch, and lack of whisker twitching.
- 8 Administer buprenorphine \perp 0.05 mg/kg subcutaneously before the surgery.
- After initial anesthesia, place the mouse on top of a heating pad lined with a silicone pad within the stereotactic apparatus field.

Note

Note: Maintain heating pad at a temperature of \$\mathbb{\mathbb{E}}\$ 37 °C .

- Make a mid-line incision with scissors to expose the abdominal contents.
- 11 Immediately below the diaphragm, identify the vagus nerve and isolate it from surrounding connective tissue and vessels.

- 12 Excise 2 mm section of the vagus nerve.
- Monitor mice for intra-operatively signs of arousal. Continuously monitor mouse breathing throughout the surgery by observing chest wall movement.
- 14 Close the laparotomy in two layers with suturing and surgical clips. Suture the inner layer of skin with a continuous suture pattern using 5-0 monofilament absorbable suture and close the outer skin with 9 mm stainless steel wound clips (MikRon Precision Inc).
- Apply analgesic bupivacaine (1-2 drops) to the incision site after suturing and allow the animals to awaken.
- Following surgery, assess mice every 00:30:00 until they return to baseline level of activity with no signs of breathing complications or lasting motor deficits.

30m

- 17 Record the well-being of the mice in the post-surgery monitoring log. Postoperatively, give mice analgesics.
- Administer mice analgesics (buprenorphine hydrochloride at a dose of and observe daily for 5 days for any signs of infection, distress, or changes in behavior.
- 19 Give mice free access to food and water.
- In sham-operated animals, perform abdominal laparotomy, and expose the vagus nerve but do not excise it.
- Weight loss of ~15% was noted in mice undergoing vagotomy compared to sham surgery.
- One week after surgery, treat mice with tamoxifen dissolved in corn oil (\$\to\$ 50 mg/kg) or vehicle administer by intraperitoneal injection daily for five days.