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Retrograde labeling of brown adipose tissue (BAT)-projecting sympathetic neurons with cholera toxin B (CTB)

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We use this protocol and it's working

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Abstract

This protocol describes how to retrogradely label the Brown Adipose Tissue (BAT) - projecting sympathetic neruons in stellate ganglia (SG) using Cholera toxin subunit B (CTB) conjugated with Alexa Flour 555.

Guidelines

Do not repeat freeze and thaw cycle with the CTB stock bottles.

Materials

MATERIALS

Cholera Toxin Subunit B (Recombinant) Alexa Fluor™ 555 Conjugate **Thermo Fisher Scientific Catalog #**C34776

iSpacer **Catalog #**IS010

VECTASHIELD® Hardset™ Antifade Mounting Medium **Catalog #**H-1400

Materials and tools needed for the survival surgery

1. Sterilized surgery tools (Scissors, forceps, suture scissors)
2. Anesthesics (ex. Isoflurance)
3. Heat pad
4. Analgesics (ex. Buprenorephine)
5. Trimmer (for hair removal)
6. Povidone-Iodine scrub
7. Alcohol prep
8. Sterile Q-tips
9. Sterile draping
10. Ophthalmic ointment
11. Suture
12. CTB solution
13. Sterile gloves
14. Syringes (BD 30G ultra-fine)

Materials and tools needed for the dissection

1. Dissection tools (Scissors, forceps, #5 forceps)
2. Anesthesics (ex. Avertin)
3. Slide glass
4. iSpacer
5. Vectashield mounting media
6. Coverslips

Troubleshooting

Before start

Aliquot the diluted CTB (in Saline) in amber tubes and store at -20ºC.

CTB preparation

1 Dilute CTB in saline to make 1% stock solution: 500 ug CTB powder in 50 ul saline

CTB preparation

2 Aliquot 4 ul of 1% CTB solution to each bottle and store it at -20ºC

3 When ready to use, take out the frozen CTB solution and adjust with more saline to final volume desired (ex. add 16 ul of saline each bottle to get 20 ul of total solution, which will be injected 10 ul to each depot)

Survival surgery for CTB injection into BAT

4 Anesthetize the animal with isoflurane, and keep it anesthetized for the whole procedure

5 Once animal is unconscious (check with toe pinch), place it on heating pad and lubricate the eyes with ophthalamic ointment

6 Inject buprenorphine (100 µl/ 10 µg) on the surgical site for analgesia

7 Remove the hair of interscapular region

8 Scrub the surgical area using povidone-iodine and 70% isopropyl alcohol, 3 times each

9 Open the sterilized pouch with tools, put the suture on the sterile space (save the plastic cover for CTB drops), uncover the sterile draping, place on the sterile space

10 Prepare CTB in syringe (10 ul for each depot), put on sterile gloves
*Alternatively, 1-2 ul of less volume can be injected using Hamilton syringe or mouth pipette with a pulled glass needle

11 Cut the sterile draping to make a hole that has a size of the operating site

12 Make a shallow incision at the center of the shaved area (1 cm below the head) and extend about 0.5 cm bilaterally

13 Grasp the adipose tissue of one side, locate BAT inside and inject the CTB solution

14 Repeat the same injection on the opposite side to complete the bilateral injection

15 Gently put back the fat pad and close the wound with a 4-0 PS-4 suture

16 Wipe the closed surgical wound with Povidone-iodine and alcohol prep

17 Inject buprenorphine or appropriate analgesics every 4-12 hours after thesurgery for 48 hours

Stellate Ganglia collection

18 Sacrifice the animals 3 days after the injection, dissect out stellate ganglia (SG) on a slide with cold PBS

19 Fix the dissected SG in 4% PFA in 0.1M PB for 1 hour and wash with PBS overnight at 4ºC

20 Attach an iSpacer on a slide glass, carefully locate the SG inside the spacer (best to stretch SG horizontally for imaging) and mount with Vectashield (~110 ul) and a cover slip

Imaging

21 SG was imaged with Zeiss confocal 710 using tile scan and z-stack options.