Apr 27, 2021

Sympathetic chain ganglia dissection_Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.baagiabw

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ABSTRACT

The study goal is to identify the gene expression profile of interscapular brown fat (iBAT)-related ganglia (SG/T1 & T3) and inguinal white fat (Iwat)-related ganglia (T13/L1 & L2).

DOI

dx.doi.org/10.17504/protocols.io.baagiabw

PROTOCOL CITATION

Rui Zhang, Heike Muenzberg 2021. Sympathetic chain ganglia dissection_Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.baagiabw

CREATED

Dec 09, 2019

LAST MODIFIED

Apr 27, 2021

PROTOCOL INTEGER ID

30760

MATERIALS TEXT

- CO_2 machine for euthanization
- Scale for measuring mice body weights
- Liquid nitrogen
- Dry ice
- Regular ice
- Sterile petri dish
- Sterile RNase-free 1.5 mL micro-centrifuge tubes with anti-freezing labels
- Cold sterile saline
- Two 60 ml syringes
- 23 Gauge needle
- Vaculet
- 70% ethanol spray bottle
- Scissors (1 big/medium size for opening the peritoneum, and 1 small size for cutting the diaphragm and heart)
- Mosquito forceps/hemostat
- Forceps (1 pair of big/medium size for holding the heart, and a pair of small size for ganglia collection)
- Waste container for the mixture of perfusion run off (blood and saline)

BEFORE STARTING

Day before tissue collection:
Label the sterile, 1.5mL centrifuge tubes
Chill a sterile saline bag in 4C fridge overnight
Autoclave dissection tools

1 Weigh mouse, then euthanize via CO2 inhalation method, and record start time

2 **Perfusion** - mice will be perfused with cold, sterile saline to remove the blood from the animal tissues.

2.1 Prepare the 50 ml syringe for perfusion by filling up with at least 30 ml ice-cold sterile saline, and connecting it with the vaculet by attaching the tubing to the tip of the syringe. Once attached, depress the plunger on the syringe to expel any air bubbles in the tubing. You should clearly see a jet of saline shoot from the tip of the butterfly needle.

2.2 Spray the mouse belly with 70% ethanol.
Make an incision using the medium scissors by pulling up part of the mouse's skin above the abdomen. Be careful to not cut too deep; only cut deep enough to open up the mouse's organ space (peritoneum, you can see the mouse's intact intestines if done properly).

2.3 Continue to open the peritoneum by sliding the scissors along and cutting up the sides of the mouse until you reach and can clearly see its entire rib cage.

2.4 Pierce the diaphragm using small scissors. Be careful not to poke/pierce the heart as you do this!

2.5 Cut each side of the rib cage to completely open up the chest cavity and clamp the sternum with a mosquito forceps/hemostat to retract it up and away from the heart. The heart should be completely exposed and clearly visible.

2.6 With the other mosquito forceps/hemostat gently grasp the heart to position the tip of the left ventricle so that it just barely peeks out from the edge of the forceps/hemostat. (It is important to not clamp too much of the heart as this will impede flow and reduce the effectiveness of the perfusion.)

2.7 Using small scissors, snip the mouse's right atrium

2.8 With your free hand, insert the butterfly needle into the tip of the left ventricle. Insert needle as shallow as possible as to not stab through the heart and destroy the integrity of the left ventricle/atrium.

2.9 Slowly depress the plunger of the 50mL syringe to perfuse at least 30mL of ice-cold saline. Monitor the liver until it becomes completely clear to ensure proper perfusion. It should slowly change from deep red to a grey/beige color.

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2.10 Once you have witnessed the color change in the liver, remove the needle from the left ventricle. Remove the set of mosquito forceps/hemostat clamped to the sternum.

2.11 Dispose of the perfusion run off (blood, and saline) mixture that has collected in the appropriate waste container.

3 Tissue dissection and collection

3.1 Remove all the inner organs below diaphragm, except the kidneys. The kidneys will act as a landmark to help identify relevant structures while dissecting. If you wish to keep the organs, store in designated tube and drop into liquid nitrogen tank.

3.2 Remove the heart and lungs. If you wish to keep the organs, store in designated tube and drop into liquid nitrogen tank.

3.3 Remove the surrounding tissues that block the view of the sympathetic chain ganglia that innervate iBAT.

3.4 Ganglia are collected bilaterally and each ganglia pair is collected separately. Surrounding ganglia fat is pooled from several thoracic level and used to control for possible contamination of collected ganglia.

3.5 Place collected samples in designated tubes and store on dry ice throughout remainder of procedure.

3.6 Record the end of the dissection time for each mouse.

3.7 Clean tools with 70% ethanol in between dissection of each animal.

4 Clean up

4.1 Transfer all samples to the -80 freezer (including samples in liquid nitrogen).

4.2 Clean the working area with 70% ethanol, wash tools with soap and warm water. Once tools are dry, make necessary preparations so they can be autoclaved.

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4.3 Update the dissection information for records (e.g. body weight, dissection time, storage info).

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