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Neuropathy Phentotyping Protocols - Animal Perfusion/Tissue Fixation

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy

In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:



Neuropathy



Materials

Reagents:

♦ Fixatives

- 4% paraformaldehyde
- 2% paraformaldehyde
- Trumps 4% para and 0.5% glutaraldehyde
- Zamboni's
- Bouin's

♦ Buffers Phosphate buffer pH 7.2, 0.1 M

- Phosphate buffered saline same as above with 150 mM NaCl
- Tris pH 7.2-7.6, 0.1 M
- Cacodylate buffer 0.15 M

Supplies:

- 21 X 3/4 butterfly needle (rat)
- 25 X 3/4 butterfly needle (mouse)
- 2 liter aspirator bottle
- tubing
- clamps
- sample jars/vials
- Dissecting tools scissors, large and small forceps, large and small hemostats

Troubleshooting

1 Intracardiac perfusion:

For intracardiac perfusion, the fixative may be administered by peristaltic pump or by gravity. The Morphology Core uses gravity.

1. Fix bottles are placed at a height of 1 meter above the animal.
2. The animal is placed on an open metal grid over a large tray to catch the fixative. **NO FIXATIVE MAY GO DOWN THE DRAIN!!!**
3. The animal should be deeply anesthetized, not responding to very firm toe pinch.
4. Rinse the chest and abdomen with 70% ethanol to reduce static and make the hair easier to cut.
5. Make an incision in the upper abdomen and cut laterally.
6. Find the xyphoid process, lift it up and cut through the diaphragm.
7. Cut the ribs laterally then cranially, avoiding the mammary arteries do not puncture the lungs.
8. Free the heart from the pericardium.
9. Insert the fixative needle into the left ventricle, start the flow of fixative, clip the right atrium.
10. Allow fix to flow until the animal is quite stiff or at least 300 ml (rat) or 30 ml (mouse) of fix has gone through.
11. Following perfusion, dissect areas of interest and immerse in the same fixative for several hours or overnight in a mix of 50% fixative and 50% buffer solution (phosphate buffer, cacodylate buffer or Tris).

2 Immersion:

Small animals, i.e., embryos or dissected tissue (pieces should be small, fix will not penetrate more than 3 mm) of larger animals.

1. Place tissue in fixative for 6-8 hours or overnight, change fixative after 3 hours, may fix longer in fixative and buffer if the first immersion isn't adequate.



2. Stirring or rocking increase mixing and result in better fixation. When stirring, place tissue in autopsy basket to protect it from stir bar. 5% DMSO may be added to aid in penetration of the fixative; however this also disrupts plasma membranes, and shouldn't be used for EM unless the EM protocol specifies its use.