Primary culture of nodose ganglion neurons from mouse

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Abstract

The present protocol explains how to prepare a primary culture of isolated neurons from the mouse nodose ganglion.

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Protocol

Anaesthesia and ganglia extraction

Step 1.

Deeply anaesthesise the mouse using a CO_2 chamber or isoflurane overdose.

NOTES
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Mice are SWISS CD1 strain

30-60 days old

Male and females.

12-h light : 12-h dark cycle

Fed ab libitum

Anaesthesia and ganglia extraction

Step 2.

Check that the animal is anaesthesised assesing *foot* pad pinch. If the animal responds to this stimulation, apply further anaesthetic.

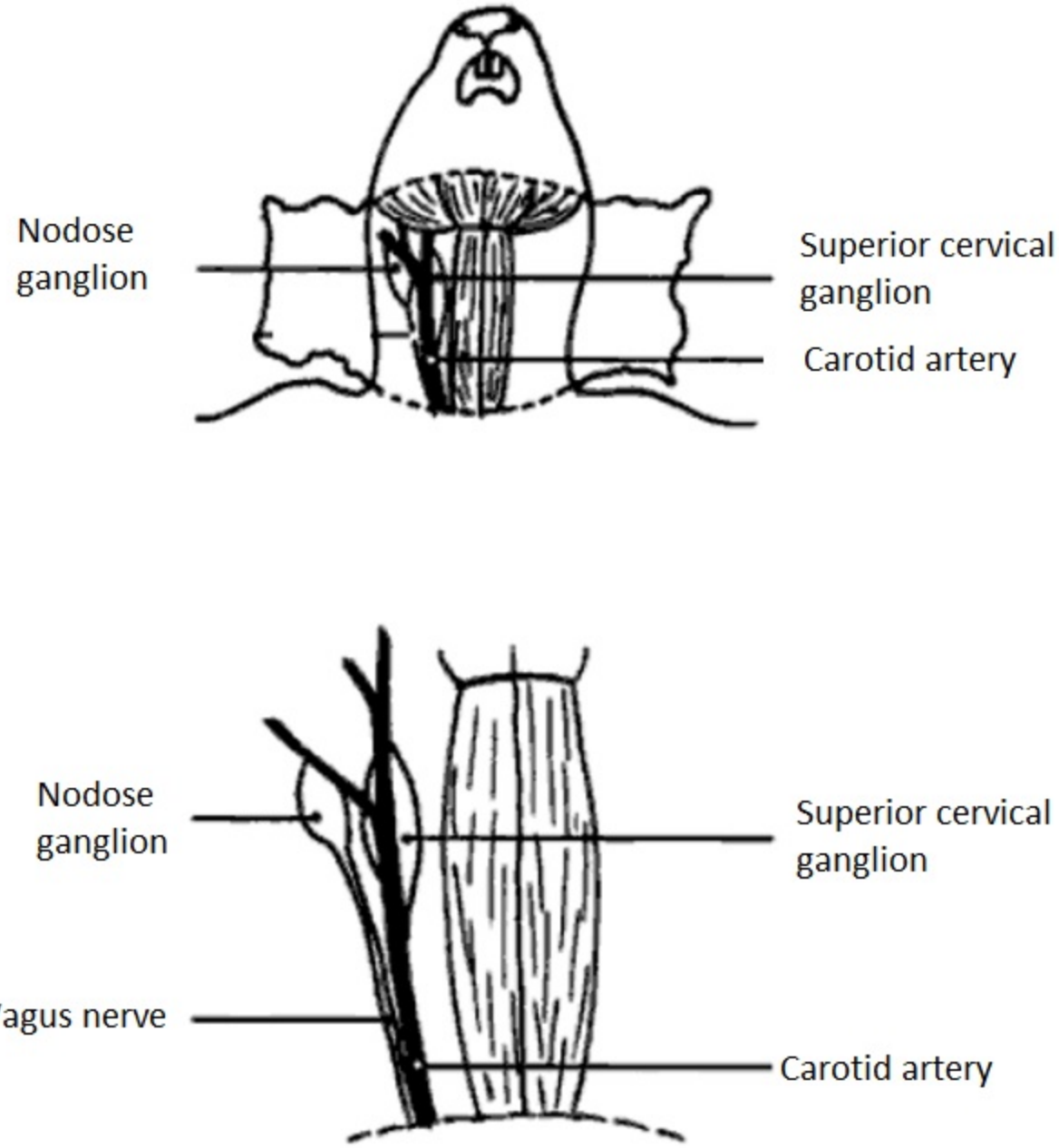
Anaesthesia and ganglia extraction

Step 3.

While anaesthesised, quickly sacrifice the animal by decapitation.

Position the backwards and pin. Cut medial from the base of the neck to the mouth. Pin the traquea to

see the nodose ganglion as an enlargement of the vagus nerve, which goes parallel to the carotid artery (see figure).



NOTES

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Use autoclaved surgery material during all extraction procedures. Material should be kept in 70º alcohol while it is not being used during the procedure. Before use, it has to be wash in destilate water and saline (NaCl 0.9 %) for removal of ganglia.

Anaesthesia and ganglia extraction

Step 4.

Quickly isolate the ganglion by cutting it free from the vagus nerve. Do the same procedure with the

second, billateral ganglion.

Anaesthesia and ganglia extraction

Step 5.

Transfer both ganglia to a 35 mm well filled with 1 mL of cold medium (L-15).

AMOUNT

1 ml Additional info: L-15 per well

REAGENTS

Leibovitzs L-15 Medium L4386 by Sigma Aldrich

Anaesthesia and ganglia extraction

Step 6.

Under magnification, clean both ganglia from rests of other tissue, specially blood.

Enzymatic treatment

Step 7.

The following material must be sterilized and kept in a *laminar flow* cabinet:

- Corning wells 35 mm.
- Tubes 10 mL.
- Two pasteur glass pipettes (one with a thin polished mouth and another with a broader mouth).
- L-15 medium without L-glutamine.
- *Hanks' Balanced Salt Solution* (HBSS) + HEPES. HEPES 1 M is added to HBSS to get a final concentration of 10 mM HEPES.
- Culture medium: 50 mL L-15 supplemented with supplemented with 10% foetal calf serum, 24 mM $NaHCO_3$, 38 mM D-glucose, 100 UI/ml penicillin-100 µg/ml streptomicyn, 2 mM L-glutamine and 50 ng/ml nerve growth factor. Keep at 4º C before use.
- Trypsin: 1 mg/ml in HBSS + 6 mg/mL albumin.
- Colagenase: 2.5 mg/ml in HBSS + 6 mg/ml albúmin.
- Laminin: prepare 3 wells with 200 µL of laminin each (10 mg/ml solved in *Earle's Balanced Salts Solution* (EBSS)). Before harvesting, drop of laminin must be incubated for at least two hours in the incubator (37 ºC, 95% O_2/5% CO_2).

Enzymatic treatment

Step 8.

After cleaning for extra tissue, In the laminar flow cabinet ganglia must be washed 3 times with 2 ml of HBSS + HEPES.

Enzymatic treatment

Step 9.

Add 2 mL of colagenase solution and incubate during 15 minutes (37 ºC, 95% O_2/5% CO_2).

Enzymatic treatment

Step 10.

Wash again two times with HBSS + HEPES to remove colagenase.

Add 2 mL of trypsin solution and incubate during 30 minutes (37 ºC, 95% O_2/5% CO_2).

Tissue disaggregation and seeding

Step 11.

Take both ganglia with the smallest possible amount of trypsin and place them in a 10 mL tube containing 2 mL medium (FCS in the medium will stop trypsinization).

Using the small tip pasteur pipette, gently dissociate the ganglia until they are no longer visible.

Tissue disaggregation and seeding

Step 12.

Transfer the 3 mL cell suspension to another 10 mL tube containing 3 mL L-15.

Centrifugate this tube.

Tissue disaggregation and seeding

Step 13.

During centrifugation, take the laminin coated wells and remove the laminin. Wash the place were the laminin drop was at least 2 times with L-15. Then remove the L-15 and leave the well empty.

Tissue disaggregation and seeding

Step 14.

After centrifugation, carefully poor the supernatant out of the tube.

Add culture medium to the remaining pellet of neurons (visible as a white spot at the bottom of the tube).

AMOUNT

600 µl Additional info: cultured medium to the tube with the pellet

Tissue disaggregation and seeding

Step 15.

Seed 200 mL of medium + cells in suspension in the centre of each of the three wells (where the laminin drop was previously adhered).

Incubation

Step 16.

Place the wells with cells in the incubator.

TEMPERATURE

37 °C Additional info: 95% O2/5% CO2

Incubation

Step 17.

Carefully, add 2 mL of culture medium to each well. Make sure that the medium is not cold.

Keep cells in the incubator during at least 24 hours before the electrophysiological experiments.

TEMPERATURE

37 °C Additional info: 95% O2/5% CO2

Warnings

Maintain and handle animals in accordance with the experimental procedures approved by the regional Research Council and Scientific Committee responsible in your area and institution.