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Protocol status: Working
We use this protocol and it's working

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Isolation and co-culture of nodose ganglion neurons

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ABSTRACT

Protocol useful for co-cultured intestinal organoids from SNCA^{A53T} mice with nodose ganglia neurons from Snca$^{-/-}$ mice that lack any α-synuclein expression. Though enteric ganglia might natively express low levels of α-synuclein protein, culturing the neurons from the Snca$^{-/-}$ mice and utilizing a monoclonal anti-human α-synuclein antibody previously validated in the Snca$^{-/-}$
mice ensures reliability for detection of authentic α-synuclein spread from the intestinal organoids to the co-cultured nerves.

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Isolation of Nodose Ganglion for Culture

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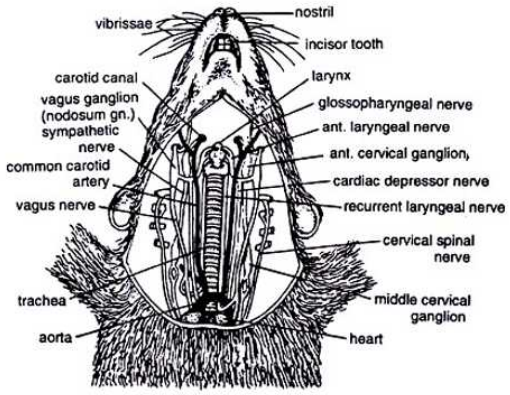
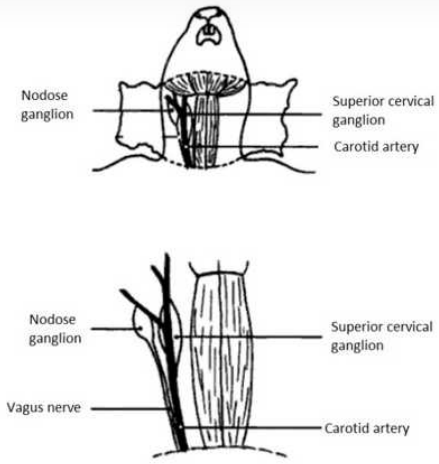


Fig. 19.9 : Rattus sp. Dissection of the neck region. Ventral view



Isolation of Nodose Ganglion for Culture

1.1 Coat the wells of an 8 well chamber slide with 10ul growth factor reduced matrigel (Corning, #354230) and place into a 37C incubator to polymerize.

1.2 Follow carotid artery down, removing musculature and fascia. Remove nodose ganglia from the left and right side of two-three mice. * I use 2-3 mice per slide. Place into 300ul ice cold mouse intesticult media (Stem Cell Technologies: #06005) with 25ng/ml (final concentration) of nerve growth factor-2 (Sigma, Cat # N6009). Keep on ice while collecting.

1.3 Add 20ul of 2.5mg/ml Liberase (Liberase TL, Roche, #05401020001). Heat at 37C for 30min, gently tapping tube every 5min to knock nodose from bottom.

1.4 Allow nodose to settle. Remove liberase media. Add 500ul intesticult media + NGF and titurate using a 200ul pipette until ganglion are broken up.

1.5 Filter through 70 micron mesh. Wash tube with an extra 500ul intesticult media + NGF and filter into same tube.

1.6 Centrifuge and 800rcf for 4min.

1.7 Remove media, leaving visible pellet behind. Add half the amount of needed media + NGF to tube to resuspend pellet (in this case 40ul). Place on ice.

1.8 Add the other half needed (in this case 40ul) of GFR matrigel (with organoids) and mix well.

1.9 Spread 10ul of ganglion mixture onto prepared slide. Place into incubator and incubate to polymerize 30min at 37C.

1.10 Add 300ul warm (@37C) intesticult media + NGF. Incubate to grow.

Co-culture of Organoids and Nodose Ganglion

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2.1 Isolate organoids (via splitting protocol; organoids need to be about 3-4 weeks old) and re-suspend in growth factor reduced matrigel. Add to isolated nodose ganglion (Isolation of Nodose ganglion for culture). Gently pipette.

2.2 Spread 10-14ul of organoids/nodose/matrigel onto prepared slide (Treat each well of eight well chamber slide with 10ul of growth factor reduced matrigel spread on bottom. Allow to polymerize at 37C for 30min before adding organoids/nodose).

2.3 Allow organoids/nodose/matrigel to polymerize for 20-30min at 37C. Add 300ul of Intesticult Media (Stemcell Technologies, Cat # 06005) with 25ng/ml (final concentration) nerve growth factor (Sigma, Cat # N6009). *Make sure to pre-warm to 37C.

2.4 Co-culture for AT LEAST 7 days. If matrigel is OK, try and co-culture for 10 days to see transfer of alpha synuclein onto nerves.

Whole Mount Staining

3

3.1 Remove media from co-culture or organoids.

3.2 Fix for 20min at room temperature using 300ul 4%PFA/1XPBS (pre-warmed at 37C, otherwise matrigel will depolymerize).

3.3 Remove PFA and add 300ul 0.5% Triton X100/1XPBS (pre-warmed at 37C). Incubate for 25 minutes at room temperature.

3.4 Wash 3x20min 100mM Glycine.

3.5 Block with 5%BSA/5%Donkey Serum/0.25% Triton X 100/1XPBS for 2 hours at room temp.

3.6 Add primary antibody (in block buffer above). Incubate at 4C overnight in a humidified chamber (Do NOT use the cover of the chamber slide on it's top, this will lead the antibody to leak out the top- I know, weird- but just leave it off. Just use humidified chamber and slide).

3.7 Wash 3x20min with 300ul/well of IF Buffer (IF Buffer: 0.1%BSA, 0.2% Triton X-100, 0.05% Tween 20).

3.8 Add secondary antibody (in same block buffer) for 1 hour at room temperature, in the dark.

3.9 Wash 3x20min with 300ul/well IF Buffer.

3.10 Apply Dapi for 5 min at room temperature. Wash 1x5min in 1XPBS.

3.11 Remove wells (keep PBS on wells during this time). Gently run razor blade along bottom of wells/slide junction. Use chamber remover and carefully pull off wells. Tip off excess PBS. Add 1 drop pro-long gold/well and lay coverslip on top. Let dry for 10 min (in dark) at room temperature. Seal with nail-polish and allow to dry again (in dark). Store at 4C. Image at confocal.