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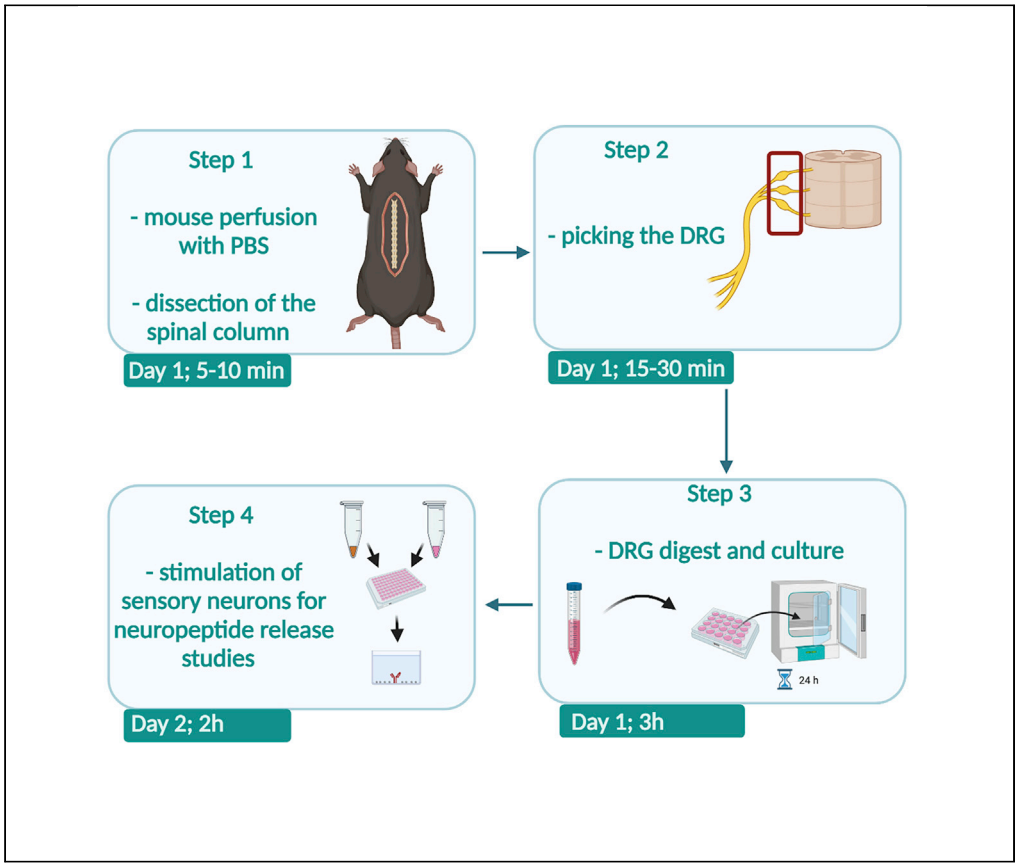
Protocol for dissection and culture of murine dorsal root ganglia neurons to study neuropeptide release



In this protocol, we provide step-by-step instructions for dissection and culture of primary murine dorsal root ganglia (DRG), which provide an opportunity to study the functional properties of peripheral sensory neurons *in vitro*. Further, we describe the analysis of neuropeptide release by ELISA as a possible downstream application. In addition, isolated DRGs can be used directly for immunofluorescence, flow cytometry, RNA sequencing or proteomic approaches, electrophysiology, and calcium imaging.

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HIGHLIGHTS

Localization, dissection, and removal of murine dorsal root ganglia (DRG)

DRGs are processed into single-cell suspensions for sensory neuron culture

Applicable for assays including *in vitro* activation and neuropeptide release

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Protocol for dissection and culture of murine dorsal root ganglia neurons to study neuropeptide release

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SUMMARY

In this protocol, we provide step-by-step instructions for dissection and culture of primary murine dorsal root ganglia (DRG), which provide an opportunity to study the functional properties of peripheral sensory neurons *in vitro*. Further, we describe the analysis of neuropeptide release by ELISA as a possible downstream application. In addition, isolated DRGs can be used directly for immunofluorescence, flow cytometry, RNA sequencing or proteomic approaches, electrophysiology, and calcium imaging.

For complete details on the use and execution of this protocol, please refer to Perner et al. (2020).

BEFORE YOU BEGIN

Preparation of reagents

Timing: 2–3 h, prepare before starting the procedure

1. Media and Buffers (see detailed recipes in the Materials and equipment section):
   a. Supplemented DMEM
      500 mL bottle with L-Glutamine + 10% (50 mL) of heat inactivated FBS + 1% (5 mL) Penicillin/Streptomycin (stored at 4°C), use within 3 months.

   Note: You can store the media in aliquots for longer term at −20°C.
   b. Supplemented Neurobasal media
      i. Neurobasal-A media 500 mL (protect from light) + 1% (5 mL) Penicillin/Streptomycin + 1% (5 mL) GlutaMax 100× + 10 mL B-27 supplement (stored at 4°C for up to 3 months).
   c. 500 mL HBSS + 1% (5 mL) GlutaMax + 1% (5 mL) Penicillin/Streptomycin (stored at 4°C for up to 3 months)
   d. 1× PBS (stored at 4°C for up to 3 months).

2. Enzymes/Reagents:
   a. Collagenase A/Dispase II mix (see also the Materials and equipment section)
      i. First, make Collagenase A stock solution and Dispase II stock solution at 100 mg/mL by dissolving Dispase II and Collagenase A lyophilized powder in PBS (e.g., dissolve 100 mg Collagenase A in 1 mL PBS, and 1 g Dispase II in 10 mL PBS).

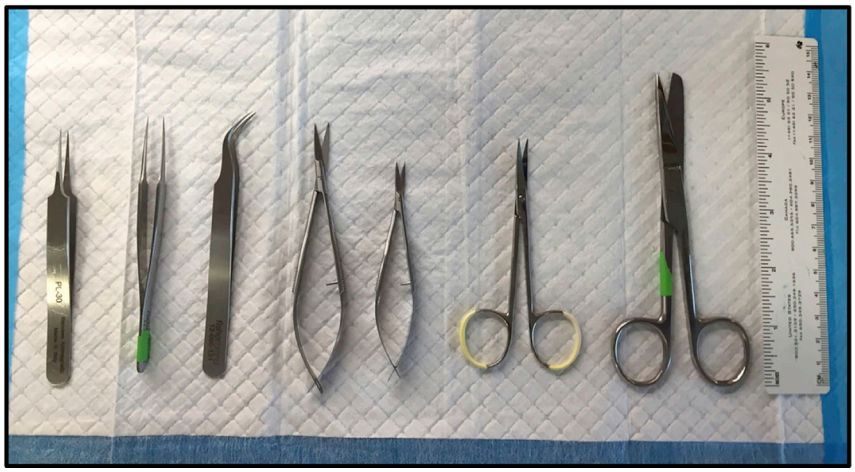

Figure 1. Tools for dissection

From left to right: 2 Micro Dissecting Tweezers Dumont Pattern #5SF, super fine tips RS-4955, ROBOZ; 1 Micro Dissection Tweezer Dumont Pattern #7, RS-5047, ROBOZ; 1 Micro Dissecting Spring Scissor, Micro Dissecting RS-5680, ROBOZ; 1 Micro Dissecting Spring Scissor, McPherson-Vannas, RS-5602 ROBOZ; 1 Micro Dissecting Scissors, RS-5882, ROBOZ; 1 Operating scissors Veterinary, RS-6940, ROBOZ.

ii. Add 1,000 μL of Dispase II solution (100 mg/mL or 80 U/mL) and 500 μL Collagenase A solution (100 mg/mL or 10 U/mL) to 40 mL 1× PBS for the combined stock solution.
iii. The combined 1.25 mg/mL collagenase A + 2.5 mg/mL Dispase II stock solution, prepared in 2aii, should be stored at −20°C for up to 6 months in 3 mL aliquots and put into 37°C water bath shortly before digestion of DRG.

b. Laminin: prepare 10 μg/mL solution (1:100 dilution from Stock)
i. Aliquot 50 μL of Laminin stock solution (1 mg/mL Laminin; Sigma-Aldrich; Cat#: L2020). Store stock solution in working aliquots at −20°C (long term storage).
ii. Dilute 50 μL of 1 mg/mL Laminin stock solution in 5 mL PBS (1:100) for the working concentration of 10 μg/mL.

c. Prepare murine NGF 2.5S (Nerve Growth Factor) 100 μg/mL in 20 μL aliquots, store at −20°C (long term storage). (It is supplied at a concentration of 100 μg/mL in 10 mM sodium acetate (pH 5))

d. Prepare rat GDNF (Glial cell line-Derived Neurotrophic Factor) 2 μg/mL in 50 μL aliquots, store at −20°C (long term storage). (Stock solutions of 100 μg/mL can be prepared in the vial by adding sterile phosphate buffered saline containing at least 0.1% human serum albumin or bovine serum albumin)

e. Prepare Ara-C (Arabinosylcytosine) by dissolving it in water to a concentration of 10 mM. Divide into 100 μL aliquots, store at −20°C for up to 1 year.

3. Tools for dissection (see Key resources table and Figure 1)

Laminin coating of the tissue culture plate

Timing: 10–20 min

4. Laminin coating of tissue culture plate: directly before starting the DRG dissection protocol, place a 30 μL droplet of 10 μg/mL Laminin solution (1:100 from stock) onto the center of each well of a 24 well plate, or in the lower corner of each well of a 96 well plate. Put in the 37°C incubator until your cells are ready for plating. Adding water or PBS between wells will prevent evaporation of the laminin droplet.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Substance P ELISA Kit	Cayman Chemical Company	Cat #: 583751
CGRP (rat) EIA Kit	Cayman Chemical Company	Cat #: 589001
Experimental models: organisms and strains		
Mouse: C57BL/6 (6–8 weeks of age, male or female)	Charles River Laboratory	Strain No.: 027
Chemicals, peptides, and recombinant proteins		
Collagenase A	Sigma Aldrich	Cat#: 10103578001
Dispase II	Sigma-Aldrich	Cat #: D4693-1G
Cytosine beta-D-arabinofuranoside (Ara-C)	Sigma-Aldrich	Cat#: C6645
B-27 Supplement (50×)	Thermo Fisher Scientific	Cat#: 10889038
GlutaMAX	Gibco	Cat#: 35050061
Laminin	Sigma-Aldrich	Cat#: L2020
GDNF	Sigma-Aldrich	Cat#: G1401
Nerve growth factor (NGF) 2.5S	Invitrogen	Cat#: 13257019
Papain	Sigma-Aldrich	Cat: 5125
PBS	Corning	Ref #: 21-031-CV
DMEM	Corning	10-014-CV
HBSS	Sigma-Aldrich	Cat#: H6648
Neurobasal-A medium	Thermo Fisher Scientific	Cat #: 10888022
Penicillin/streptomycin	Thermo Fisher Scientific	Cat#: 15070063
Fetal bovine serum	Sigma-Aldrich	Cat #: F4135-500ML
Software and algorithms		
Prism v 7.0c	GraphPad	https://www.graphpad.com/demos
Softmax Pro	Molecular Devices	https://www.moleculardevices.com/systems/microplate-readers/softmax-pro-7-software
Other		
Steritop Quick Release0.22 µm filter system	Millipore Sigma	Cat #: S2GPT05RE
Falcon conical tubes, 50 mL	STEMCELL Technologies	Cat # 100-0090
Falcon conical tubes, 15 mL	STEMCELL Technologies	Cat # 100-0092
KONTES Dounce tissue grinders, Kimble Chase	VWR	KT885300-0002
Corning 96 well TC-treated microplates	Millipore SiGMa	Cat #: CLS3595
Corning Costar TC-treated multiple well plates	Millipore SiGMa	Cat #: CLS3527
10 mL syringe	BD	Cat #: 309604
1 mL syringe	BD	Cat #: 309659
18G needle	BD	Cat #: 305196
21G needle	BD	Cat #: 305167
26G needle	BD	Cat #: 305110
Corning tissue-culture treated culture dishes	Corning	Cat #: CLS430166
Benchmark Incu-Shaker mini shaking incubator with non-slip rubber mat	TEquipment	Cat #: H1001-M
Micro dissecting tweezers Dumont pattern #5SF, super fine tips	ROBOZ	Cat #: RS-4955
Micro dissection tweezer Dumont pattern #7	ROBOZ	Cat #: RS-5047
Micro dissecting spring scissor, micro dissecting	ROBOZ	Cat #: RS-5680
Micro dissecting spring scissor, McPherson-Vannas	ROBOZ	Cat #: RS-5602
Micro dissecting scissors	ROBOZ	Cat #: RS-5882
Operating scissors veterinary	ROBOZ	Cat #: RS-6940
20× and 40× trinocular stereo microscope on single arm boom stand	AmScope	Cat #: SW-3T24

MATERIALS AND EQUIPMENT

Supplemented DMEM	Final concentration	Amount
DMEM	1×	500 mL
FBS heat inactivated	10%	50 mL
Penicillin/streptomycin	10 U per mL/10 μg per mL	5 mL
Total	**N/A**	**555 mL**

Note: This penicillin/streptomycin has a concentration of 5,000 U/mL (penicillin) / 5,000 μg/mL (streptomycin). Filter supplemented DMEM through a 0.22 μm filter system (Steritop Quick Release; Millipore Sigma; Catalog #: S2GPT05RE), store at 4°C for up to 3 months.

Supplemented Neurobasal media	Final concentration	Amount
Neurobasal A medium	1×	500 mL
Penicillin/streptomycin	10 U per mL/10 μg per mL	5 mL
GlutaMax 100×	1×	5 mL
B-27 supplement	1×	10 mL
Total	**N/A**	**520 mL**

Note: This penicillin/streptomycin has a concentration of 5,000 U/mL (penicillin) / 5,000 μg/mL (streptomycin). Filter supplemented Neurobasal media through a 0.22 μm filter system (Steritop Quick Release; Millipore Sigma; Catalog #: S2GPT05RE), store at 4°C for up to 3 months.

Collagenase/Dispase mix	Final concentration	Amount
Sterile PBS	1×	40 mL
Dispase II (100 mg/mL)	2.5 mg/mL (0.2 U/mL)	1,000 μL
Collagenase A (100 mg/mL)	1.25 mg/mL (0.125 U/mL)	500 μL
Total	**N/A**	**41.5 mL**

Note: First, dissolve Dispase II and Collagenase A lyophilized powder in PBS to 100 mg/mL solutions (e.g., dissolve 100 mg Collagenase A in 1 mL PBS and 1 g Dispase II in 10 mL PBS).

Culture media (make fresh every time) pre warm 37°C	Final concentration	Amount
Supplemented neurobasal medium	N/A	30 mL
NGF 2.5S	50 ng/mL	15 μL
GDNF	2 ng/mL	30 μL
Ara-C	10 μM	30 μL
Total	**N/A**	**30.045 mL**

Supplemented HBSS (storage: 3 months at 4°C)	Final concentration	Amount
HBSS	1×	500 mL
Penicillin/streptomycin	10 U per mL/10 μg per mL	5 mL
GlutaMax 100×	1×	5 mL
Total	**N/A**	**510 mL**

⚠ CRITICAL: Ara-C (arabinosylcytosine) is a harmful substance; follow the safety data sheet when handling. Ara-C is optional in this protocol. It reduces glia cell proliferation, which can be important especially in longer term experiments where glial cell proliferation may affect the results of your experiment. We have not found a significant effect of Ara-C in calcium imaging or in short term neuropeptide/neurotransmitter release assay.

STEP-BY-STEP METHOD DETAILS

Day 1: Dissection of vertebral column

Timing: 5–10 min per mouse

Dorsal Root Ganglia (DRG) are located in the intervertebral foramen adjacent to the spinal cord. It is therefore necessary to dissect the vertebral column before isolating the DRG. To decrease blood contamination, animals can be perfused with PBS prior to the dissection of the vertebral column.

1. Prepare the perfusion syringe by adapting an 18G needle to a 10 mL syringe and aspirate 1 × PBS. You can use one syringe for several mice.
2. Prepare 1 × 50 mL Falcon tube for the collection of the vertebral column.
   a. Fill the 50 mL Falcon with 20 mL of supplemented HBSS solution.
3. Sacrifice mouse
   a. Sacrifice mice using CO_2 inhalation, confirming the exposure was lethal.

⚠ CRITICAL: Do not perform cervical dislocation! This would disrupt the spinal cord and pulls on the spinal nerves that are connected to the DRG!

4. After the mouse is sacrificed, lay the mouse on the dissection board in a supine position. Pin feet down with a needle for stabilization. Spray 70% ethanol on the fur of the mouse. Open the chest and expose the heart for perfusion. (Methods Video S1)

   a. Use the forceps to pick up the skin around the bottom of the sternum (the highest point of the mouse chest when lying in a supine position). Make an incision using a pair of scissors.
   b. Lift the bottom of the sternum with the forceps and cut along the diaphragm using the scissors. To avoid damage of the heart or lung, hold the forceps high and keep the tip of the scissor tip pointed ventrally, just under the sternum and the ribs.
   c. Lift the rib cage and expose the heart. You can cut out a little window of the sternum and the ribs to have a better view on the right atrium of the heart.
   d. Make an incision on the right atrium (deep red color compared to light red color of the ventricles) so the blood and perfusion buffer (PBS) can escape.
   e. Use forceps to carefully hold the heart, then pierce the heart at the left apex into the left ventricle using the perfusion syringe. Perfuse with 2–4 mL room temperature PBS.

5. Dissection of the vertebral column (Methods Video S2)

   a. Turn the mouse to the ventral side and pin it to the dissection board again. Lift the skin at the base of the tail and make an incision over the whole back to the neck. Pin or cut away the skin to the sides so you have good access to the back of the mouse.
   b. Make a cut with your scissors at the lumbosacral joint and separate the sacrum from the lumbar spine.
   c. Lift the spine with a forceps and cut through the muscles and ribs on the left and right side in cranial direction until the base of the skull.
   d. Make a cut through the spine at the atlanto-occipital joint.

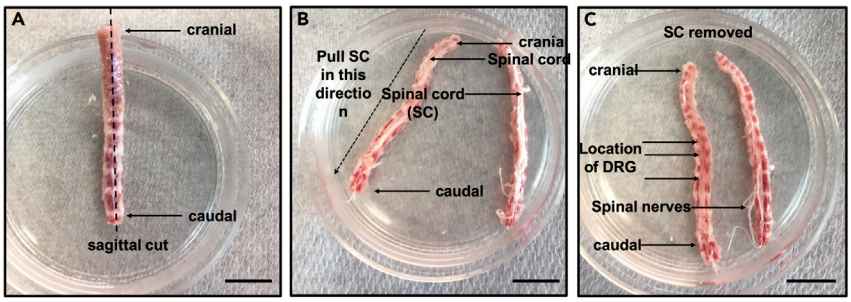

Figure 2. Cutting the vertebral column sagittal in half and removal of the spinal cord

(A) Dorsal view of vertebral column cleaned from all soft tissue and attached ribs. Vertebral proccessi spinosi and vertebral arcs are visible. The cut must be made exactly through processi spinosi in a sagittal approach from caudal to cranial.

(B) Vertebral column split in half, view into the spinal canal with the remaining spinal cord on both sides.

(C) View into the spinal canal after removal of the spinal cord. Intervertebral foramina and DRG are visible. Scale bar: 1 cm.

e. Remove any organs that might be attached to the tissue surrounding the spine.

f. Place the spine in a 50 mL Falcon tube filled with supplemented HBSS and put it on ice.

6. Cleaning the spine from muscle and fatty tissues (Methods Video S3)

a. To make the vertebral column better accessible, using fine bladed scissors carefully cut away all surrounding muscle, fat, and other soft tissue. Be careful to not damage the vertebral column. Also cut away the ribs from the thoracic part of the vertebral column.

DRG dissection

Timing: 15–30 min per mouse

The DRG dissection is the trickiest part of this protocol and it needs some practice. For this step you need a stereomicroscope.

7. Open the vertebral column to remove the spinal cord and gain access to the DRG

a. Once the vertebral column is cleared from the surrounding soft tissue and ribs, take a small micro-dissection scissor and put one scissor tip into the caudal spinal canal. Now carefully cut in the sagittal plane one vertebra at a time in cranial direction until the vertebral column is half opened. The easiest way is to hold the vertebral column with your fingers, so you have the best control over where you cut. Make sure the cut is in the middle of the vertebrae, as this will allow the best access to the DRG. (Figure 2)

b. Now turn the vertebral column and cut through the opposite side again from caudal to cranial, one processus spinosus vertebrae at a time in a sagittal direction.

c. Now the vertebral column is split in half along the sagittal plane. Take a petri dish filled with supplemented HBSS. Lay down both half pieces so the spinal cord is visible. Take one tweezer to hold the vertebral column down and one tweezer to grasp the cranial aspect of the spinal cord and pull it out from cranial to caudal. Do this with both half pieces. Be careful not to pull too hard on the spinal roots, so the DRGs do not get pulled out. Put the petri dish with the two half pieces of the vertebral column on ice.

Note: These steps can be performed with or without using the stereomicroscope. This is personal preference. (Methods Video S4)

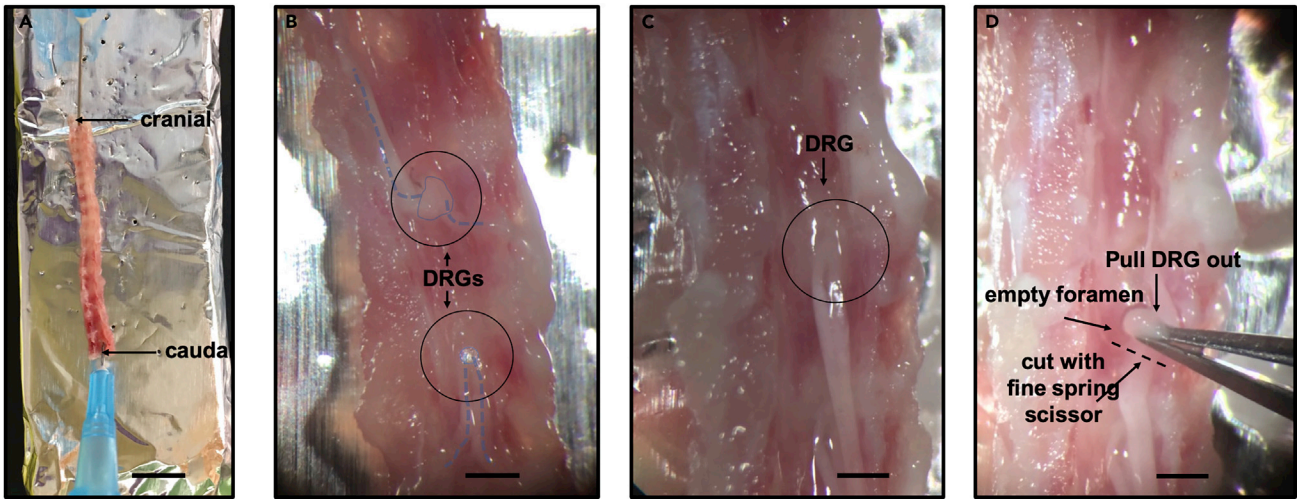

Figure 3. Picking DRG out of the intervertebral foramina and cut away the attached roots and spinal nerves

(A) Vertebral column pinned onto a Styr pore pin pad for the DRG dissection.

(B) View through a stereo microscope. DRGs are circled. Blue dotted line indicates ventral and dorsal roots. Attached are the dorsal and ventral root that can be used as an orientation.

(C) DRG is circled.

(D) DRG is tightly grasped by the fine tweezer and pulled out of the intervertebral foramen. Use a spring scissor to cut away the attached axon roots as depicted by the dotted line. Scale bars: (A) 1 cm, (B) 2 mm, (C) 2 mm, (D) 1.5 mm.

8. Picking the DRG

Note: These steps should be performed using a stereomicroscope.

a. The DRG are located in the intervertebral foramina connected to the spinal cord through the dorsal root but separated through the meninges.

b. Prepare a 15 mL Falcon containing 15 mL supplemented DMEM media and keep it on ice, open the lid so you can easily put the DRG in the media.

c. Take one half of the vertebral column from the Petri dish and leave the other in the HBSS on ice while you dissect the DRG from the first half.

d. To adequately visualize the DRG, pin down one half of the vertebral column (e.g., using a Styr pore pin pad) so that the spinal canal is facing up. Now you are able to see the DRG in the intervertebral foramen beneath a shiny layer of meninges. Sometimes it is not easy to see them shine through. Especially in the beginning it is also helpful to use your tweezers to feel the openings between bony vertebrae where the foramina are and in which the DRG are located.

e. Take a fine tweezer in one hand and a fine spring scissor in the other hand. Now feel that there are the bony vertebral arcs, and between them there is a soft hole, that is the intervertebral foramen. Take the tweezer and push with slightly opened ends into the foramen and take the DRG between the tweezer endings.

f. Tightly grasp the dorsal and ventral axon roots close to the DRG using your tweezers. Carefully pull it up a few millimeters, maintaining a tight grasp with your tweezers. (Figure 3)

g. Now take the spring scissor and cut away the spinal roots and spinal nerves that are attached to the DRG. The DRG is a slightly darker/yellow/beige in color then the spinal roots and nerves (appear white).

⚠ CRITICAL: This step will help you reduce myelin debris and glial cell contamination, which is important for downstream analysis like protein/RNA profiling.

h. Now put the DRG into the 15 mL Falcon with supplemented DMEM. If it is a DRG, it will sink down.

i. Continue collecting each DRG from all the intervertebral foramen.

j. Discard the now DRG-free vertebral column and repeat with the other half.

Troubleshooting 2 If you cannot easily see or access the intervertebral foramina it can help to spin the pin pad 180° and start from the other side. It does not matter if the DRGs are collected from cranial to caudal or vice versa.

Optional: If you want to use the DRG for downstream RNA profiling, you can directly collect them into 350 µL of RLT buffer into a 1.5 mL Eppendorf tube (we recommend using the RNeasy plus micro kit from QIAGEN for RNA isolation).

Note: Before starting the RNA isolation protocol, it is best to homogenize the DRG in RLT buffer using a Dounce-homogenizer.

Optional: If you want to use the DRG for microscopy, you can directly collect them into 4% Paraformaldehyde (PFA) into a 1.5 mL Eppendorf tube. Leave the DRG in 4% paraformaldehyde for 6–18 h at 22°C for fixation. Take the DRG out of the PFA using a tweezer and put them into 15% sucrose solution (15 g sucrose in 100 mL PBS) into a new Eppendorf tube for 24 h at 4°C. After that, take the DRG out and put them into 30% sucrose solution (30 g sucrose in 100 mL PBS) and store at 4°C for at least 24 h or for longer time periods until you want to perform cryostat cutting. Prior to cryostat sectioning, first place the DRG in OCT and keep for at least 18 h or longer time periods at −80°C. OCT frozen DRGs are now ready for cryostat cutting and staining (Hill et al., 2018; Kalinski et al., 2020).

Note: Perfusion of the mouse with 4% paraformaldehyde instead of PBS, prior to dissection, improves the downstream immune fluorescence and can be performed if no DRGs are collected for live cell applications.

DRG digest and plating for culture

Timing: 3 h

Note: These steps should be performed in a tissue culture hood.

9. Place the Collagenase A/Dispase II Mix in a 37°C water bath.
10. Spin down the DRG in a 15 mL Falcon at 161 × *g* (1,000 rpm) for 5 min.
11. Remove DMEM carefully by using a serological pipette. The DRG do not form a firm pellet, so there is a significant risk of losing them by removing the media using a vacuum.
12. Add 3 mL of 37°C Collagenase A/Dispase II mix to the DRG.
13. Leave the DRG in the Collagenase A/Dispase II mix in a 37°C incubator shaking (around 150 rpm) for 15 min.
14. Spin down at 161 × *g* for 5 min. Remove the Collagenase A/Dispase II solution carefully by using a serological pipette.
15. Carefully wash the pellet by adding 10 mL DMEM. Do not resuspend! Spin down 161 × *g* for 5 min.
16. Remove DMEM carefully by using a serological pipette.
17. Resuspend in culture media (supplemented Neurobasal media containing cytokines). For the amount of media to use in this step, calculate the number of wells you want to culture + 2 × 30 µL (1 mL max). Whether you use a 24 well plate or 96 well plate you will add the same amount of neurons in media to each well (e.g., for 20 wells of either a 24 well plate or a 96 well plate, plan for the culture of sensory neurons from 1 mouse → (20 + 2) × 30 µL = resuspend in 660 µL culture media).

18. Take a 1 mL syringe and triturate the DRG solution by aspirating and ejecting the DRGs in solution 7 times up and down for each needle size starting with an 18 gauge needle, followed by a 23 gauge, and 28 gauge needle.

⚠ CRITICAL: Count cells using a hemocytometer to plate equal cell numbers if you have different mouse lines/conditions that you are comparing and for inter-experimental reproducibility. There will be significant debris visible in the hemocytometer, just count the round cell bodies that are approximately 10 μm in diameter. You should get >40,000–60,000 per one adult mouse. Plate neurons with a density of approximately 15,000 neurons per cm^2, which equals 2,500–3,000 neurons per 30 μL droplet.

19. Remove the Laminin droplet from 4 wells using vacuum so there is thin liquid layer left and immediately plate 30 μL of the cell suspension on the area that is laminin coated. Do not let the laminin evaporate completely before you add the cell suspension. Keep the time between removing the droplet of laminin and the plating of the cell suspension droplet to less than 1 min.
20. Continue with the next 4 wells by removing the laminin and plating 30 μL of cell suspension.
21. Incubate the plate or wells for 1 h at 37°C.
22. Add 200 μL culture media per well (96 well plate), 500 μL culture media per well (24 well plate), or 2 mL culture media/well for 2.5 cm petri dish single wells (e.g., for calcium imaging).
23. Incubate 18–24 h at 37°C.
24. The cells are ready for experiments.

Note: If you want to plate more than 20 wells (by pooling the DRG of several mice), you can resuspend in more than 1 mL in step 17, depending on the number of wells and mice you use. It is critical therefore to take a 5 mL syringe in the following step 18.

Day 2: Sensory neuron *in vitro* stimulation with papain to study Substance P and CGRP release

Timing: 2 h

Note: The description is for a set of 8 wells with cultured sensory neurons in a 96 well plate. 4 wells will be left unstimulated with only the vehicle control and 4 wells will be stimulated with 25 μg/mL Papain (750 USP units per mL).

Note: Prepare the 10 mg/mL papain solution fresh by dissolving 100 mg papain in 10 mL PBS. Keep solution on ice.

25. Prepare 2 Eppendorf tubes with 1 mL supplemented Neurobasal media without cytokines.
26. Add 2.5 μL media only in the unstimulated control tube.
27. Add 2.5 μL of papain dissolved in PBS (10 mg/mL) to the papain tube to get a 25 μg/mL papain concentration.
28. Vortex both tubes for 5–10 s.
29. Carefully remove the media of 4 wells at a time by bringing your pipette to the edge of the plate where no DRG neurons are attached and slowly aspirate.
30. Add 200 μL of either the unstimulated media (4 wells) or the papain containing media (the other 4 wells) to each well by slowly dripping it down the wall of the well.
31. Incubate for 1 h at 37°C.
32. Carefully remove the supernatant by placing your pipette on the edge of the plate where no DRG neurons are attached and slowly aspirate.
33. Pipette the supernatant into a fresh 96 well plate.
34. You can either snap freeze the supernatant on dry ice and then store at −80°C until you proceed with the Substance P and CGRP ELISA, or you can put it on ice and use it directly.
35. For the ELISA see the manufactures instructions:

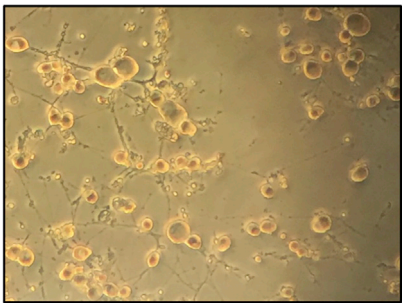

Figure 4. DRG culture 24 h after plating

Large luminous oval cell bodies of sensory neurons attached to the well. Sensory neurons start forming new axons. Smaller cells surrounding the sensory neuron cell bodies are glia cells. 200× magnification.

a. Substance P ELISA Kit: https://www.caymanchem.com/pdfs/583751.pdf
b. CGRP (rat) EIA Kit: https://www.caymanchem.com/pdfs/589001.pdf

EXPECTED OUTCOMES

From one adult mouse it is expected to get around 40,000 to 60,000 sensory neurons if you collect around 30 DRG. Therefore, we recommend plating no more than 20 wells per mouse (2,500–3,000 sensory neurons per well). Under the tissue culture microscope you will be able to see the round sensory neuron cell bodies that appear like a luminous oval. You will see some glial and axon debris as well, but this will not attach to the bottom of the well and instead will float in or on top of the media. By changing the media on day 2, much of the debris will be removed with the media. (Figure 4)

The ELISA results are expected to show an increase in Substance P release, and a decrease in CGRP release after stimulation with 25 μg/mL papain compared to the unstimulated control. (Figure 5)

LIMITATIONS

The protocol describes the dissection of DRGs from the whole accessible spine of a mouse. It does not describe the dissection of the DRG from certain spinal segments that might be needed for studies on peripheral nerves (e.g., sciatic nerve) (Kalinski et al., 2020; Swett et al., 1991). Also, there is no description of the dissection of the trigeminal ganglion provided (Katzenell et al., 2017), which contains the cell bodies of the sensory neurons that innervate the head and face. Furthermore, we provide the description to culture murine sensory neurons that are not sorted from glial cells and the few immune cells that are part of the DRG. Adding Ara-C to the neurobasal culture medium will decrease glial cell proliferation.

TROUBLESHOOTING

Problem 1

The process of cutting the vertebral column sagittal in half requires concentration and fine handling. By cutting too much to one side of the vertebral arc, it is possible to cut too close to the DRG which makes it very hard or impossible to pick them afterwards (step 7).

Potential solution

Make sure you cut right in the middle of the processus spinosus of the vertebrae. Try different scissors. A fine micro dissection scissor or a fine spring scissor (a bit larger than the spring scissor you need for the step where you pick the DRG). Alternatively, you can cut the column in 3 pieces, place the column piece by piece dorsal side facing up and secure it with thick forceps. Now use a raiser plate to cut the column sagittal in half (Sleigh et al., 2016).

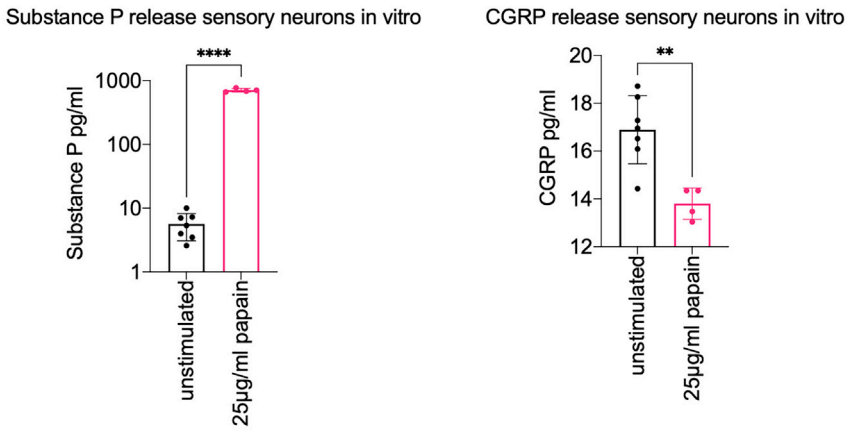

Figure 5. ELISA results for Substance P and CGRP release from *in vitro* stimulated sensory neurons

Substance P release is significantly increased after *in vitro* stimulation of sensory neurons, while CGRP release is significantly decreased. Both ELISAs were performed using the supernatants from the same experiment, so the dissection, culture, and stimulation conditions were equal.

Problem 2

It is often the case that after carefully removing the spinal cord, there will be some residuals of the spinal cord and of the ventral and dorsal roots that can make it harder to see and locate the DRG (step 8).

Potential solution

The ventral and dorsal roots as well as the spinal cord are whiter in color, which helps to distinguish them from DRGs and allows to remove them carefully if they are hiding the view on the intervertebral foramina. To find the DRG within the tissue and the handling of the dissection tools requires some training if not done frequently. That should be considered before planning the experiment.

Problem 3

Digestion of DRG must be balanced between separating the sensory neurons from the surrounding tissue without harming the neurons through the digestion itself (step 13).

Potential solution

The 70 min we propose in this protocol is on the longer time edge on what we would recommend for the digestion and a time frame that works in our lab. Nevertheless, we have found that this time varies in different labs working with DRG culture between 15–30 min up to 90 min. This is probably depending on the enzymatic activity and concentration of the Collagenase A/Dispase II mix used.

If you find yourself having troubles with the viability of your cultured DRG neurons we recommend to titrate the time down.

Problem 4

Counting sensory neurons before plating may be necessary if you have different mouse lines or conditions to compare. Because of the rough digestion process and the trituration, you will find a lot of debris in your cytometer field of view so it can be hard to see the actual sensory neuron cell bodies (step 18).

Potential solution

To make sure that the counting is reliably working, we recommend taking a cytometer (e.g., Neubauer chamber) undiluted, or with a maximum dilution of 1:1 with Trypan blue. This should allow you to

find enough sensory neuron cell bodies in the four quadrants of the Neubauer chamber. You will see a lot of small round cells and debris as well as worm like structures. These are not the neuron cell bodies. The neuron cell bodies are large round cells, approximately 5× the size of a leucocyte. Only count these cells.

Problem 5

During the process of changing the media, it is possible that some sensory neurons might float off, leading to the loss of sensory neurons, which can affect your experiment (step 29).

Potential solution

If you have plated your cells in the center of a 24 well plate well, remove the media very slowly and carefully by tipping the plate and putting the pipette tip in the bottom rim of the well.

If you have plated your cells in the lower edge of 96 well plate wells, remove the media very slowly and carefully by tipping the plate and putting the pipette tip in the upper rim opposite of the neurons.

Carefully fill the wells with new media by slowly rinsing out down the wall of the well.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Caroline L. Sokol, MD, PhD (clsokol@mgh.harvard.edu).

Materials availability

There are no special mouse lines necessary for this protocol. All materials are listed in the Key resources table including the catalog numbers.

Data and code availability

This study did not generate or analyze datasets/code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100333.

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AUTHOR CONTRIBUTIONS

C.P. and C.L.S. designed the study and wrote the manuscript.

DECLARATION OF INTERESTS

C.L.S. is a paid consultant for Bayer and Merck.

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