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Ex vivo Skin-Nerve-Ganglion-Spinal cord preparation to study pain-related plasticity in sensory afferents.

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Emily Tran¹, LaTasha K Crawford¹

¹University of Wisconsin-Madison



Emily Tran

University of Wisconsin Madison

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We use this protocol and it's working

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Keywords: ex vivo, spinal cord, dorsal root ganglia, skin, sciatic nerve, intact, electrophysiology , electrical stimulation of drg neuron, dorsal root ganglia, drg neuron, dorsal root, stimulation of the innervated tissue, nerve, ex vivo drg dissection, methods for ex vivo drg dissection, sciatic nerve, spinal cord preparation, relevant stimulation during neurophysiological recording, plasticity in sensory afferent, innervated tissue, sensory afferent, neuron cell body, spinal cord, ganglion, sensory, neurophysiological recording, mechanisms within drg, electrical stimulation, paw skin, stimulation, naturalistic sensory input, signaling mechanism, relevant stimulation, drg

Disclaimer

This preparation has been tested to record extracellular activity of lumbar DRG in response to skin stimulation. Stimulation of skin included sharp forceps pinch, light indentation, thermal, and pin prick.

Abstract

Sensory signaling within dorsal root ganglia (DRG) involves a host of interactions between neuron cell bodies, satellite glia, and other non-neuronal cells. A better understanding cell signaling mechanisms within DRG is attainable when the entire DRG microenvironment is intact. While chemical or electrical stimulation of DRG neurons can produce important insight, they lack the naturalistic sensory input that results from stimulation of the innervated tissue. This protocol describes methods for *ex vivo* DRG dissection where the spinal cord, dorsal root, DRG, sciatic nerve and innervated paw skin remain intact, enabling physiologically relevant stimulation during neurophysiological recordings of DRG neurons.

Materials

- *Dissection microscope*
- *Electric razor*
- *Ice bucket*
- *Ice box*
- *Black Bone Cutting Scissors (Fine Science Tools, 91604-09)*
- *Fine Forceps (Fine Science Tools, 91110-10)*
- *Large & small scissors (VWR 82027-592 and -582)*
- *Razor blade*
- *Transfer pipette*
- *Set of sharp forceps (for handling DRG and nerves later)*
- *Dissection chamber (Sylgard bottom for stabilizing tissue)*
- *Insect pins (to stabilize tissue)*

Modular electrophysiology equipment including Axon Devices Multiclamp 700A microelectrode amplifier with CV-7B headstage and PClamp 11 software, Axon Devices Digidata 1550B digitizer with Humsilencer, antivibration Air Table, Scientifica PatchStar low drift, 3-axis micromanipulator with manual control cube and dual control cards expandable to an additional device, Scientifica post and platforms with magnetic base, and Linlab II software.

Dissecting Microscope with 3.5 to 90X magnification and boom stand

Troubleshooting

Problem

DRG is viable but not nerve or skin

Solution

Keeping the skin and connected nerves alive is the most challenging part of this preparation. During the skin dissection, only manipulate or maneuver the skin by holding on the the edges, and try to void maneuvering the nerves directly at all costs. It is also helpful to be mindful of the skin while dissecting the rest of the preparation (spinal cord and DRG). Make sure the nerves and skin remain submerged in the acsfd (creating an icy slurry of acsfd to keep the skin and nerve covered is what I have the most success with.



Safety warnings

- ⚠ There are differences between mouse strains in lower lumbar DRG - some have L5 *and* L6, some only have L5 before S1 appears. This protocols utilizes mice with L5 and L6 DRG. It will be important to check which vertebral levels your mice have before proceeding.

Ethics statement

All animal use has been approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee (IACUC) (Protocol # : V006184-R01-A02).

Before start

This preparation is intended to preserve sural/lateral dermatomes of hind paw skin through the sciatic nerve. Many nerve-skin preparations utilize the saphenous nerve. Careful considerations for dissection of nerve terminals in the skin that innervate the sural region should be noted prior to dissection.

Reagent Recipes

- 1 Dissections were done in homemade sucrose-based artificial cerebrospinal fluid buffer for dissection (aCSFd) made up of Sucrose (0.234M), KCl (0.0025M), NaH_2PO_4 (0.0013M), NaHCO_3 (0.026M), glucose (0.011M), MgSO_4 (0.01M), and CaCl_2 (0.0005M, added day-of recording). At least one hour prior to dissections buffer was placed in the freezer to make a semi-frozen slurry for easier preservation of whole tissue.

Extracellular artificial cerebrospinal fluid for recording buffer (aCSFr) was made according to Hachisuka et al (2016) using NaCl (0.177M), KCl (0.0036M), MgCl_2 (0.0012M), NaH_2PO_4 (0.0012M), NaHCO_3 (0.025M), Glucose (0.011M), and CaCl_2 (0.002M, added day-of recording). Intracellular buffer was made in reference to Kitai and Park (1990), using 0.05M Tris hydrochloride buffer and 0.5M KCl, adjusting to a pH 7.6 using HCl in a drop-wise fashion. Buffers were kept at room temp for recording.

Items

- 2
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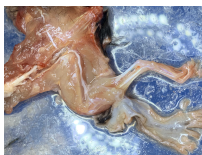
Steps

- 3 Euthanize animals via intraperitoneal injection of 70% ethanol (Worthington et al., 2015) in sterile saline and shave on dorsal and ventral side of the animal from mid-dorsum to paw.
- 4 Acutely dissect mice for their spinal column (head detached), keeping left hind limb attached, and dorsal and hindlimb skin intact submerged in aerated aCSF for dissection (aCSFd, see Recipes). Dissections should be completed on ice.

- 4.1 Replace aerated aCSF at least three times with fresh aCSF throughout the dissection, with the preparation constantly submerged.
- 5 Pin the muscle around the spinal column as rostral as possible so the prep lays flat – paw closest to you (note: always grab at areas of skin NOT being recorded from to avoid crushing of target nerves).
- 6 Begin dissection dorsal-side up and pin the skin between fourth and fifth digit of the left hind paw so the glabrous/plantar side is face-up.
- 7 Cut along dorsum of leg down to the tip of the third digit along its lateral edge.
- 8 At the tip of the digit, cut width-wise all the way around its circumference (just below nailbed), and repeat for second-first digit.
- 9 Free up the skin along each digit, gently using blunt forceps to pull up and apart from the muscle. Loosen skin around sides of the leg by gently pulling away from muscle, careful to avoid severing the skin from important nerves that run just below the skin.
- 10 Once glabrous/plantar skin is loosened, flip the prep and re-pin so body is ventral-side up and hairy paw up. Pin again between fourth and fifth digit.
- 10.1 **This dissection is intended to keep the **lateral/sural** region of the paw intact.
- 11 Continue cutting along the tip of the digits and down the sides of each digit, freeing up the skin and nerve (cutting should reach bone so muscle will be collected too).
- 12 Once the digits are free, cut laterally across the digit tips and pull tissue, carefully freeing skin to the heel/ankle (look for the nerve that may drag behind, often right next to a blood vessel).
- 12.1 While lifting the skin, continue to cut laterally on both sides toward the thigh.
- 12.2 At the knee, the nerve and blood vessel dive toward the muscle, continue to free up the tissue and dissect the rest of the skin away from the muscle.
- 12.3 On either side of the nerve, while lifting up the skin, cut the muscle tissue along its length and carefully free up the nerve from the blood vessel (free up this side of the

nerve/muscle first as it is the most challenging), moving from caudal to rostral, until the paw skin and nerve are free-floating in the aCSF.

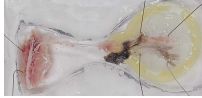
- 13 Flip the prep again so it is dorsum-side up and re-pin, keeping free-floating skin-nerve submerged but out of the way (do not pin down). Pin down what is remaining of the paw tissue to hold the prep steady. Trim the muscle along the dorsum from rostral to caudal.
- 14 Insert one end of fine bone-cutting scissors into cross-section of spinal column where the head was detached (avoid poking and harming the spinal cord) from the rostral end and cut lengthwise toward the caudal end. Make the same cut on the other side of the cross-section of the spinal column.
- 15 Continue length-wise along the dorsum half of the spinal column to expose the spinal cord and connected roots until you reach the sacrum. At the llium, cut through the bone by inserting one side of bone-cutting scissors under the bone and one above to avoid severing nerves underneath.
- 15.1 Cut away bone and muscle from this upper thigh/dorsum region to expose the sciatic nerve.
- 15.2 Follow the sciatic nerve back to the connected dorsal roots at L4 and L5.
- 16 Expose L4, L5, L6, and L3 DRG by cutting away bone and muscle (careful not to sever connections from L6-L5 and L3-L4).
- 17 Continue to free up DRG, connected nerves, and sciatic nerve from surrounding muscle and bone, eventually working rostral to caudal.
- 18 Where the sciatic nerve dives into the muscle towards the lower thigh and into the bend of the knee, carefully free up the nerve and cut away muscle.
- 18.1 Once most of the thigh muscle is cut away from the nerve, begin to free up the nerve and its branches from the remaining muscle and bone of the tibia and fibula (eventually cutting through the bone near the ankle).
- 18.2 Example dissection of (top) spinal cord and DRG with connected sciatic nerve and (bottom) connected paw skin.



(Out of solution) Lower lumbar spinal cord and DRG dissection with connected sciatic nerve and branches in paw skin.

19 Clean up the rest of the extra tissue or skin from the dorsum. Replenish with fresh ice-cold aCSFr in the recording chamber, remove the prep from the ice-cold aCSFd and immediately place in aCSFr for recording (aCSFr, see Recipes).

19.1 Example whole preparation in customized recording chamber with (top) muscle around spinal cord pinned and (bottom) edges of paw skin (leaving lateral/sural region untouched) pinned.



20 Let rest and bring to room temp

Extracellular electrophysiology and prep validation

21 Recordings were completed in pCLAMPTM Software Suite utilizing micromanipulator hardware from Scientifica. To capture inward currents over a duration of time, recording was set to Gap-free mode. Prior to recording, electrodes were filled with intracellular buffer (see Recipes section) and positioned adjacent to L3, L4, or L5 DRG.

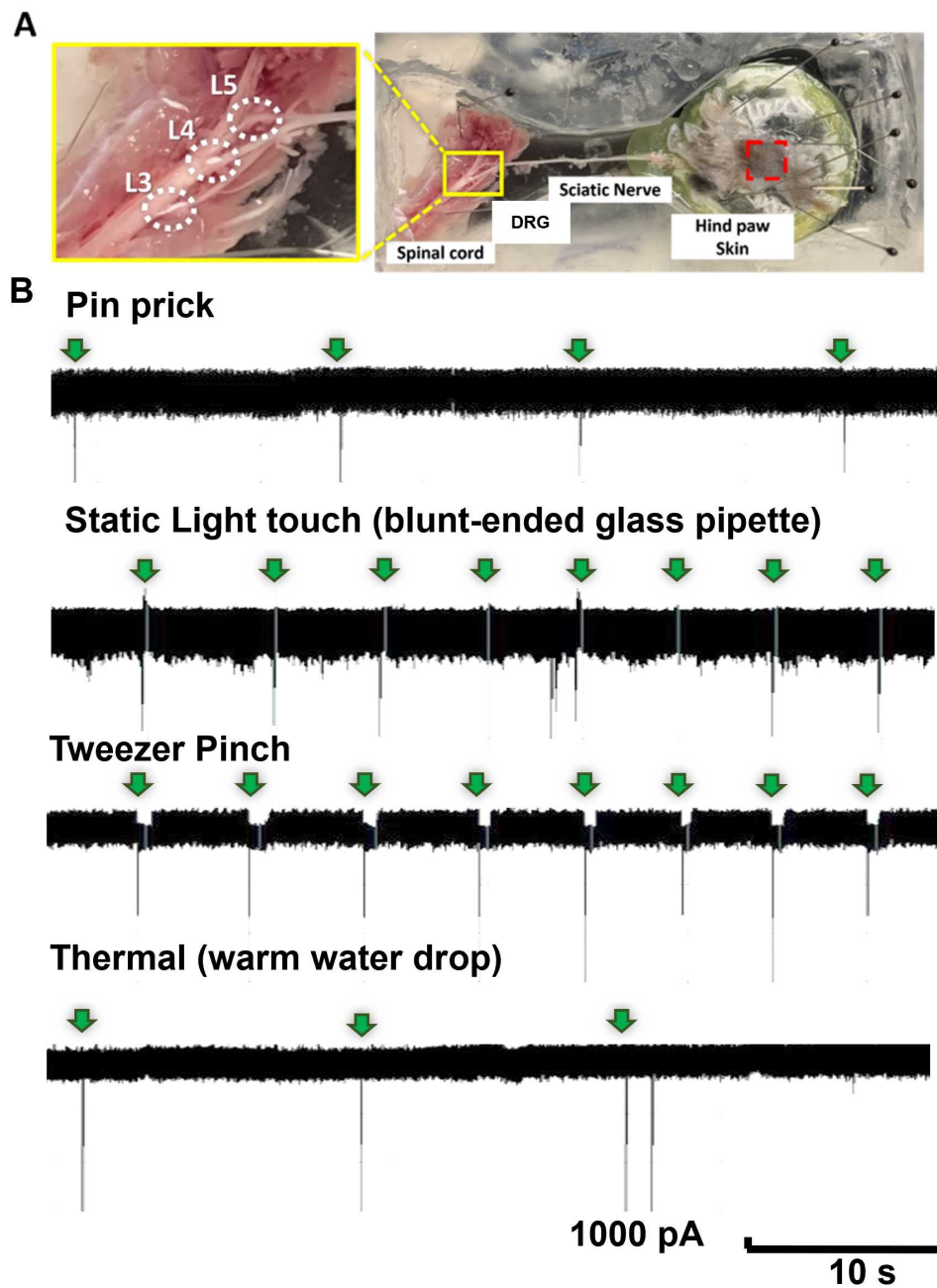
Initial viability testing of each prep was conducted by light pin-prick of the sural region of the hind paw skin followed by light indentation, recording between L3-L4 DRG. If these resulted in clear inward currents, thorough testing of viability was completed. If none, or only some of these stimulations evoked currents, tactile stimulation of branching nerves or the sciatic nerve by pinprick or pinching with forceps was conducted to determine viability of DRG and nerves. If tactile nerve stimulation resulted in inward currents, it was concluded that hind paw skin connections, or their nerve terminals, were no longer viable but DRG alone were. These were noted, and the prep was discarded.

Full range viability testing was conducted using a stopwatch to ensure inward current intervals matched real-time second marks. All stimulation of skin was conducted on the sural region (in the prep, should be in the middle region with visible hair and glabrous sections). Pin prick of skin was repeated around 10 second intervals using a 20-gauge needle. After cessation of stimulation, recordings were continued for a short duration to ensure the absence of unevoked currents, after which the next set of stimulation was conducted. Testing of light-touch was completed using a blunt-end glass probe, pressed lightly on the skin to produce a small indentation, then immediately removed, over an



interval of roughly 5 seconds, again followed by a period of no stimulation. Thermal sensitivity was assessed utilizing hot DIH₂O, warmed in the microwave and pipetted drop-wise at 10 second intervals. DIH₂O was heated to about 32-37 degrees Celsius (90-100 degrees Fahrenheit). After ensuring the absence of unevoked currents, sharp forceps pinch was conducted at 5 second intervals to test pain sensitivity.

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Viability testing of an ex vivo skin-nerve-ganglion-cord preparation responding to multiple types of skin stimulation. **A.** Diagram of the preparation and corresponding dorsal root ganglion (insert). **(B)** Extracellular recordings of ganglion response to (i) pin prick, (ii) sharp forceps pinch, (iii) light touch, and (iv) thermal stimulation of hind paw skin.

Protocol references

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