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Trigeminal ganglion whole-cell patch clamp recording protocol for voltage-activated ion currents analysis followed by single cell RNA-Seq.

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We use this protocol and it's working

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Abstract

This protocol focuses on analyzing the currents of voltage-activated ion channels in trigeminal ganglion (TG) neurons that innervate the temporomandibular joint (TMJ) followed by single-cell RNA-Sequence. The goal is to classify these neurons into specific types based on both electrophysiology pattern and transcriptome. Each type of TG neuron displays a characteristic current and distinct molecular properties, including specific receptors, ion channels, and neuropeptides. The presence or absence of these components varies among TG neuron subtypes, contributing to their specialization in different sensory functions across the head and face.



Materials

- 1 liter of 1X Tyrode's Buffer (mM: 140NaCl, 4KCl, 2MgCl₂, 2CaCl₂, 10 glucose and 10 HEPES). Adjust to pH 7.4 with NaOH. Stored at -4°C for up to 1 month.
- 5ml of electrode solution (mM: 140 KCl, 1 CaCl₂, 10 EGTA, 10 HEPES, 2 MgCl₂). Adjust to pH 7.4 with KOH. Stored at -20°C for up to 3 months.
- 2 ml for each ganglia/animal of 2 mg/ml collagenase (Sigma, St. Louis, MO, Cat#: 10103578001) diluted in 1X Tyrode's buffer.
- 5 ml of 50ug/ml Poly-d-lysine diluted in sterile H₂O (Gibco, 0.1 mg/ml, Cat #A3890401).
- 7 units of 35×10mm dish, Nunclon™ Delta (Thermo Scientific, Cat #150318).
- Surgical tools (Micro Dissecting HP Tweezers #1625-0241 Oxford instruments; Vannas spring scissor, super fine, 8.5 cm, 7 mm tip, #500086).
- P-87 Flaming/Brown microelectrode puller (Sutter Instruments, Navato, CA).
- Glass capillary filament 1.5 MM x .86 MM, 4" (A-M Systems, Sequim, WA).
- Suprase-In RNase inhibitor (1U/μl, Invitrogen) in electrode solution. You can store the RNase inhibitor solution in aliquots at -20°C.
- 0.2 mL PCR strip tubes.

Trigeminal ganglion dissection

- 1 Fifteen days prior the experiments, anesthetize the animal using isoflurane inhalation (5% in O₂) and inject 15µl of red DiIC18 lipophilic tracer (2.5µg/ml; Molecular Probes) into both temporomandibular joints.
- 2 Prepare 1 liter of fresh 1X Tyrodes Buffer and adjust the pH to 7.4.
- 3 Prepare 2 ml of 2mg/ml collagenase in 1 X Tyrodes Buffer using a 15 ml tube.
- 4 Plate's coating: make 5 ml of 50ug/ml poly-d-lysine in ddH₂O. Place 1 ml of the poly-d-lysine solution on each petri dish and leave it in room temperature for at least one hour before seeding the cells.
- 5 For the dissection, prepare 2 petri dishes, one with 2ml of 5% antibiotic solution (Penicillin Streptomycin Solution, 100x) and one with 2ml of 1X Tyrode's buffer. Keep the solutions on ice until finishing the trigeminal ganglion dissection.
- 6 Sacrifice rat using isoflurane inhalation. After confirming the exposure was lethal, decapitate the animals to have a better access of the trigeminal ganglion.
- 7 Use forceps to open the upper part of the cranium. Gently extract the brain to expose the underlying structures. Make an incision at the bottom part of the trigeminal nerve. Using vanna scissors, cut around the trigeminal ganglion to remove the surrounding dura mater layer. Use tweezers to delicately remove any remaining dura mater around the trigeminal ganglion. Make small incisions at the top of the ophthalmic branch and in the maxillary branch of the trigeminal ganglion. Use tweezers for holding and removing the trigeminal ganglion from the cranial cavity.
- 8 Wash the ganglia in 5% antibiotic solution followed by Tyrode's buffer. Keep the ganglia in the Tyrode's buffer on ice during dissection of both TG ganglia.
- 9 After collecting the ganglia, spray alcohol on the closed petri dish and change gloves to avoid contamination. Place the ganglia into the collagenase solution and incubate the ganglia under agitation for 2 hours at 37°C.
- 10 Prepare the plate's coating with 1 ml of poly-d-lysine and incubate for at least 1 hour in room temperature. For each animal/ganglia, use 5 petri dishes.
- 11 After the incubation, gently triturate the ganglia with a 1000 µl plastic pipette.

- 12 Centrifuge the ganglia at 1000 rpm for 5min.
- 13 Gently remove the collagenase solution without disturbing the pellet. Add 10ml of fresh Tyrode's buffer and centrifugate at 1000 rpm for 5min.
- 14 Gently remove the supernatant without removing the pellet, and then resuspend with 1 ml of fresh Tyrode's buffer.
- 15 Remove the poly-d-lysine from the petri dishes before placing the cells. Do not wash the dishes before seeding the cells.
- 16 Split the cells among the 5 petri dishes by seeding 200ul into each one.
- 17 Add the necessary amount of Tyrode's buffer to complete 1 ml in each petri dish.
- 18 Leave the cells to adhere to the petri dish for 1 hour at room temperature prior to initiating the patch clamp recordings.

Whole-cell patch clamp recordings

- 19 During the experiments, the cells are superfused with Tyrode's buffer at 3.5mls per minute. Keep the bath's volume at approximately 2ml.
- 20 The temporomandibular joint(TMJ)-innervating neurons are identified by the expression of Dil using an inverted microscope equipped with fluorescence optics (Olympus IX70).
- 21 TMJ-innervating neurons labeled cells are whole cell patch clamped with 1.5mm glass electrodes filled with electrode buffer.
- 22 Data are collected using an Axopatch 200B amplifier, a Digidata 1200 analog to digital converter and PClamp8 software.
- 23 Voltage protocols for voltage gated sodium channels, voltage gated potassium channels and hyperpolarization-activated cyclic nucleotide-gated channels (HCN) are recorded as previously described by Petruska et al., 2000. Briefly, sodium channels were activated by hyperpolarizing the cell membrane to -100mV for 500ms from a holding current of

-60mV. The potential was then stepped in 10mV increments from -60mV to 10mV for 2ms. To activate voltage gated potassium channels the cells were hyperpolarized to -100mV for 500ms and then stepped from -60 to 40mV in 20 mV increments for 200ms. The HCN channels were activated by hyperpolarizing the cell membrane stepwise in 10mV increments from -60mV to -120mV for 500ms.

- 24 Following electrophysiology recording, the neurons are collected using the same glass pipette employed for the recordings. Negative pressure is applied, enabling the cells to adhere tightly to the pipette tips and be removed from the bath and placed into PCR strip tubes with 1U/ μ l RNase inhibitor solution. The cells are kept on ice until the end of the recordings, and then are transferred to -80°C until the RNA-Seq.

Single-Cell RNA-Seq

- 25 TG single cells are further processed with SMART-Seq Single Cell Plus Kit (Takara Bio, Cat. 634471) according to the manufacturer's instructions to construct the cDNA library. The purified cDNA library then is used for Illumina library construction using Nextera XT DNA Library Prep (Illumina, Cat. No. FC-131-1096) according to the user guide. Finally, the individual library is pooled at equal molar for sequencing on the Illumina NovaSeq X Plus platform. Specifically, the pool is sequenced on a 10B flow cell lane using the 2 $\times$ 150 cycles format (i.e., paired-end) to a depth of ~40-50 million reads per sample. The final library loading concentration is 130 pM with 5% PhiX spike-in control. The library construction is performed at Gene Expression Core, University of Florida (UF) (<https://biotech.ufl.edu/gene-expression-genotyping/>, RRID:SCR_019145). Sequencing is performed at the ICBR NextGen DNA Sequencing Core (<https://biotech.ufl.edu/next-gen-dna/>, RRID:SCR_019152).

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