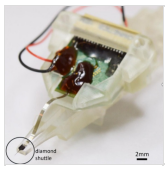

Jan 10, 2021

High-Density Penetrating Microelectrode Recordings from Anesthetized Feline Dorsal Root Ganglia

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1 Works for me dx.doi.org/10.17504/protocols.io.w5nfg5e

SPARC
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ABSTRACT

Dorsal root ganglia (DRG) are components of spinal roots containing sensory cell bodies, and hold significant promise as neural recording sites for sensory neuroprostheses.This protocol details the fabrication and use of a high-density flexible polyimide electrode array into the sacral DRG of an anesthetized feline for neural recordings.

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Na, K., Sperry, Z. J., Lu, J., Voeroeslakos, M., Parizi, S. S., Bruns, T. M., Yoon, E., Seymour, J. P. (2018). Novel diamond shuttle to deliver flexible bioelectronics with reduced tissue compression. BioRxiv, 435800. https://doi.org/10.1101/435800

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KEYWORDS

microelectrode, electrophysiology, dorsal root ganglia, cat

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All animal subjects research was approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC; protocol PRO00007296).

ABSTRACT

Dorsal root ganglia (DRG) are components of spinal roots containing sensory cell bodies, and hold significant promise as neural recording sites for sensory neuroprostheses.This protocol details the fabrication and use of a high-density flexible polyimide electrode array into the sacral DRG of an anesthetized feline for neural recordings.

Electrode Array Fabrication

1 Spin lower 2.2 µm thick layer of polyimide onto 4" silicon wafer.

Polyimide (PI-2610) **HD Microsystems Catalog #PI-2610**

2 Apply thin titanium dioxide adhesion layer.

3 Lift-off pattern gold/platiunum interconnect lines (2.5 µm wide).

4 Spin on upper 2.2 µm thick layer of polyimide.

Polyimide (PI-2610) **HD Microsystems Catalog #PI-2610**

5 Apply thin titanium dioxide adhesion layer.

6 Pattern outer structure of arrays and contact openings with hard mask and O_2 plasma etching.

7 Use liftoff patterning to deposit stack of titanium-platinum-iridium contact sites.

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8 Release arrays from silicon wafer.

Diamond Shuttle Fabrication

9 Pattern tapered trenches onto silicon wafer.

10 Fill trenches with moderately conformal ultrananocrystalline diamond (UNCD) using hot filament chemical vapor deposition (CVD) (proprietary method).

11 Etch UNCD shuttle profile.

12 Release shuttle from silicon wafer.

Recording Apparatus Assembly

13 Bond array interconnects to circuit board for connection to Omnetics adaptor.

Nano Dual Row Horizontal Surface Mount Tail (36 pin connector)
Adaptor

Omnetics **NPD-36-AA**

14 3D print recording apparatus **shuttle jig.stl**, **circuit board jacket.stl**, and **motor adaptor.stl**.

Form 2
3D Printer

Formlabs **Form 2**

Clear
Resin **Formlabs Catalog #RS-F2-GPCL-04**

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15 Tap 1/4"-20 thread into top hole in motor adaptor (opposite the D-hole). Tap 6-32 thread into side hole of motor adaptor. Tap 4-40 thread into all side holes in shuttle jig.

16 Glue 1" 1/4"-20 threaded rod into top of motor adaptor (opposite D-hole). Screw in partially (but do not glue) 1/4" 4-40 set screws into side holes of shuttle jig.

1/4" 4-40 Cup-Point Set Screw
Set Screw
McMaster-Carr **92311A106**

1" 1/4"-20 Threaded Rod
Threaded Rod
McMaster-Carr **90322A645**

Threaded rods and set screws can be ordered in shorter or longer sizes as desired.

17 Glue 2-56 nuts into circuit board holder (hexagonal cutouts).

Helpful to screw in 2-56 screws into opposite side to align nuts and pull into circuit board holder for glue curing. These screws will provide holding force to keep circuit board in circuit board holder.

2-56 Nut
Nut
McMaster-Carr **91841A003**

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2-56 Torx Screw
Screw

McMaster-Carr 90910A682

18 Use UV-curable glue to glue UNCD shuttle to tip of shuttle jig, aligning with guides at the tip of the jig.

Use microscope for good alignment. Try not to get glue anywhere on UNCD shuttle except for rectangular mounting section. This step and those following in this section require good manual dexterity and a lot of practice.

If only acute recording is desired, may skip to step 21 and then secure array to shuttle using UV curable glue. Array will not release from shuttle during surgery, but may be more secure in DRG.

19 Prepare [M] **40 Mass Percent** PEG (8000MW) in DI water and heat in glass dish on hotplate (**90 °C**) until dissolved.

Depth of mixture should be about 1 cm to accomodate dip coating, about 3g PEG to 4.5g water in a small dish. Keep PEG on hotplate except when dipping.

Polyethylene Glycol (PEG) - 8,000MW **Sigma Aldrich Catalog #202452**

20 Remove 40% PEG8000 mixture from heat and dip UNCD shuttle in PEG mixture once to coat. Allow to dry (a few seconds).

Hand dipping is acceptable, as long as shuttle jig tip is not coated. Mounting to a micromanipulator and visualizing with a camera may provide more repeatability. Final thickness of PEG mixture should be about 2 µm and smooth.

21 Insert circuit board with mounted array into circuit board holder, insert slider of circuit board holder into shuttle jig track, and secure circuit board holder in track using threaded rods in side of shuttle jig so that array just hangs over the shuttle jig tip onto the UNCD shuttle.

May need to back screws out of shuttle jig side holes for free movement of circuit board holder. Predefined marks on shuttle jig correspond to forward edge of circuit board holder ("15.5mm" for 20mm array, "31.5mm" for 40mm

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array)

22 Prepare [M]**60 Mass Percent** PEG in DI water and heat in glass dish on hotplate (**90 °C**) until dissolved.

Polyethylene Glycol (PEG) - 4,000MW **Sigma Aldrich Catalog #81292**

23 Align array to UNCD tip under microscope. Use a drop of 60% PEG4000 mixture to secure array ribbon to rectangular UNCD shuttle backing (do not wet tip).

Array only needs to be roughly aligned, to about 10 micron precision. Final alignment will occur during following dip step.

24 Add relief to array ribbon by loosening shuttle jig side screws, sliding circuit board holder forward, and re-tightening screws.

25 Remove 40% PEG8000 mixture from heat and dip shuttle+array into 40% PEG8000 mixture. Check alignment of array and shuttle.

Surgical DRG Exposure

26 Anesthetize animal with a ketamine (6.6mg kg^{-1})-butorphanol (0.66 mg kg^{-1})-dexmedetomidine (0.033 mg kg^{-1}) intramuscular (IM) dose, intubate, then maintain on isoflurane anesthesia [M]**3 % volume range: 2-4%** during surgical procedures. Insert intravenous (IV) line into one or both cephalic veins for drug and fluid infusions. Infuse IV fluids (1:1 ratio of lactated Ringers solution and 5% dextrose) at a rate of 5–10 ml/kg/h and increase up to 30 ml/kg/h during surgery as needed. Monitor respiratory rate, heart rate, end-tidal CO_2, and O_2perfusion and adjust anesthesia/temperature support as needed to maintain a deep plane. Follow other procedures per an IACUC approved surgical protocol.

Surgivet Advisor
Vitals Sign Monitor
Smiths Medical **V9203**

27 Insert a urethral catheter and allow bladder to drain into a collection receptacle.

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28 With the subject prone, use a scalpel to create a medial incision over the lower lumbar and sacral spine.

29 Following a medial incision over the lower lumbar and sacral spine, use rongeurs to perform a laminectomy exposing the lumbosacral spinal cord and DRG (typically L7-S2). Keep nerves moist during surgery.

30 Use scalpel to expose bilateral posterior superior iliac crest. Use a Dremel to create a hole in the bone, and secure self-tapping bone screws (size of hole and screws may vary).

Insertion and Stabilization Apparatus Assembly

31 Move subject to support stand with overhead rack and ability to lower legs below back legs below thorax.

We built a custom stand out of materials from 80/20.

32 Wrap stainless steel wire around pelvic bone screws and use to suspend cat pelvis, lowering leg supports so legs are extended.

Ensure that cat spine is parallel to the surgical table to ensure proper lung inflation. Monitor vitals carefully following suspension and adjust as necessary to keep excessive weight off diaphragm.

33 Place an optical stabilization board below the stand and assemble a support structure for a micromanipulator over the surgical site and a USB microscope camera viewing the surface of the target DRG.

We assembled our support structure using materials from Thorlabs and custom machined/3D printed a system for mounting the micromanipulator, which was taken from a WPI stereotaxic frame. Support structure should allow for very little movement. It is helpful to secure the optical stabilization board to the surgical table using C-clamps.

USB Microscope
Microscope

Teslong **MS100**

Mount the linear actuator to the micromanipulator for precise positioning of the array. Connect to a computer.

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34

High-Resolution Linear Actuator with DC Motor
Linear actuator
Physik Instrumente **M-235.DD**

35 Use a thread adaptor to secure the motor adaptor to the linear actuator.

Adapter with Internal M4 x 0.7 Threads and External 1/4"-20 Threaded Stud
Thread Adaptor
Thorlabs **AS4M25E**

Array Insertion

36 Mount a recording apparatus (shuttle jig+circuit board holder+circuit board+array) to the motor adaptor. Secure with a 6-32 set screw.

3/8" 6-3218-8 Stainless Steel Cup-Point Set Screw
Set screw
McMaster-Carr **92311A146**

37 Connect 2 Ripple nano headstages to the circuit board, connect the headstages to the NIP and start Trellis software.

Neural Interface Processor
Neural Interface Processor
Ripple **NIP**

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nano2 Recording Headstages
Electrophysiology Headstage

Ripple Neuroscience **nano2**

Trellis 11.1
Microsoft Windows 10
source by Ripple Neuroscience

Connect Ripple nano headstages to the circuit board, connect the headstages to the NIP and start Trellis software.

Neural Interface Processor
Neural Interface Processor

Ripple **NIP**

nano2 Recording Headstages
Electrophysiology Headstage

Ripple Neuroscience **nano2**

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Neural Interface Processor
Neural Interface Processor

Ripple NIP

nano2 Recording Headstages
Electrophysiology Headstage

Ripple Neuroscience nano2

Trellis 11.1
Microsoft Windows 10
source by Ripple Neuroscience

Original study used discontinued nano rather than nano2 headstages, which are slightly wider and require a thicker circuit board (or other solution) in order to fit together. 2 32 channel headstages are required.

38 Use micromanipulator to align shuttle tip over desired DRG

39 Use the micromanipulator and linear actuator software controller (included with actuator) to lower the shuttle tip about 5 mm above the surface of the target DRG, using the USB microscope for visualization

40 Suspend animal breathing to reduce movement (do not suspend for more than 1 minute). Use the linear actuator software to advance the shuttle into the DRG at 1-2 mm/s. Immediately resume breathing.

Record during insertion to immediately see neural firing. Firing will increase gradually over 1-2 hours after initial

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insertion shock.

The following steps are required only if shuttle removal is desired. Recording is possible with the shuttle in place, but it will break and not be reusable.

41 Rinse array backing with saline. Watch for dissolution of hardened PEG.

42 Once PEG has dissolved, use fine forcep tips or a bristle from a broom to gently release the array ribbon from the shuttle jig and shuttle.

Do not jostle any part of the setup including the shuttle jig, as this will result in breakage of the delicate shuttle.

43 Use the linear actuator software to slowly retract the shuttle, watching to ensure array is not moving with shuttle. Jiggle back and forth as needed.

Sensory Testing

44 Activate sensory neurons by performing bladder cystometry, dermatome brushing, or nerve cuff electrical stimulation (pudendal nerve and pelvic nerve are lower urinary tract nerves in the sacral DRG). Details of these procedures are not included as part of this protocol and will depend on experimental goals. The Ripple system and Trellis software are used to achieve neural recording

Data Analysis

45 Analyze raw neural data using IronClust software. Details of using this software are available at the repository. IronClust requires MATLABb 2016 or later.

IronClust 4.3.3
Microsoft Windows 10
source by James Jun

MATLAB 2017a
Microsoft Windows 10
source by Mathworks

As of 6/21/2020, IronClust is still being supported and updated for new versions of MATLAB. Use the latest available versions of both software packages and contact the developer with issues using the Github repository.

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