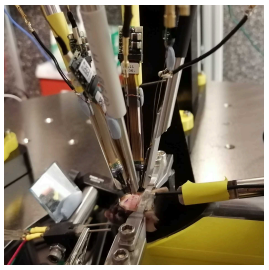
May 20, 2021

Recordings with multiple Neuropixels probes in head-restrained mice



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

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Abstract

Step-by-step instructions for electrophysiological recordings using multiple Neuropixels probes in head-fixed mice

Materials

IMEC user guidelines:
https://e2f49b05-3010-45e2-a7bf-b8f67811425a.filesusr.com/ugd/832f20_93fb9fa4481c4b3eb0975a6410a38722.pdf

Sensapex micromanipulator:
https://www.sensapex.com/products/ump-micromanipulation-system/

Software SpikeGLX:
http://billkarsh.github.io/SpikeGLX/

Cortex buffer:
125mM NaCl
5mM KCl
10mM Glucose
10mM HEPES
2mM CaCl2
2mM MgSO4
pH 7.4

Probe cleaning solution option 1:
Freshly prepared 1% Tergazyme solution

Probe cleaning solution option 2:
10mM NaCl
0.5% SDS
50 mM Tris Base (pH ~8)

Kwik-Cast Sealant:
https://www.wpiinc.com/kwik-cast-kwik-cast-sealant

Sugi sponge:
https://www.sugisponge.com/material-properties/absorption/

IPA (2-propanol)
https://www.sigmaaldrich.com/catalog/product/sigma/i9516?lang=en®ion=US

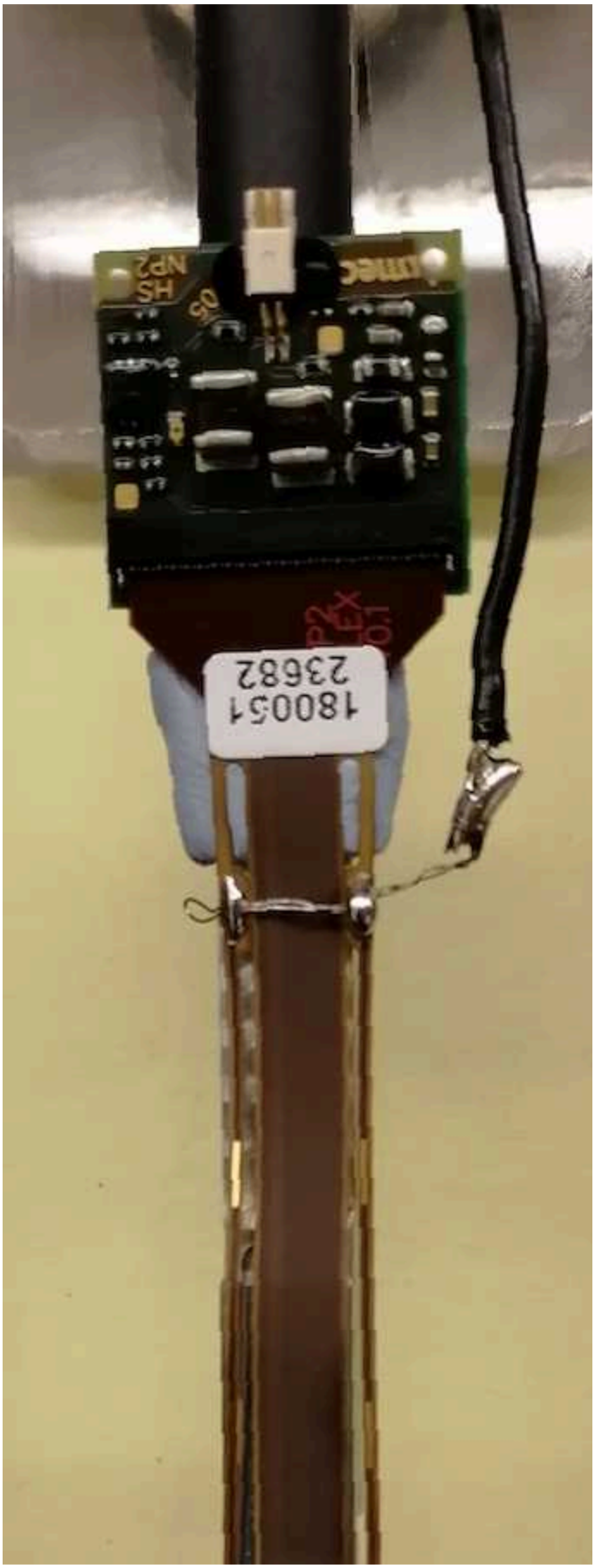
Tergazyme
https://www.sigmaaldrich.com/catalog/product/aldrich/z273287?lang=en®ion=US

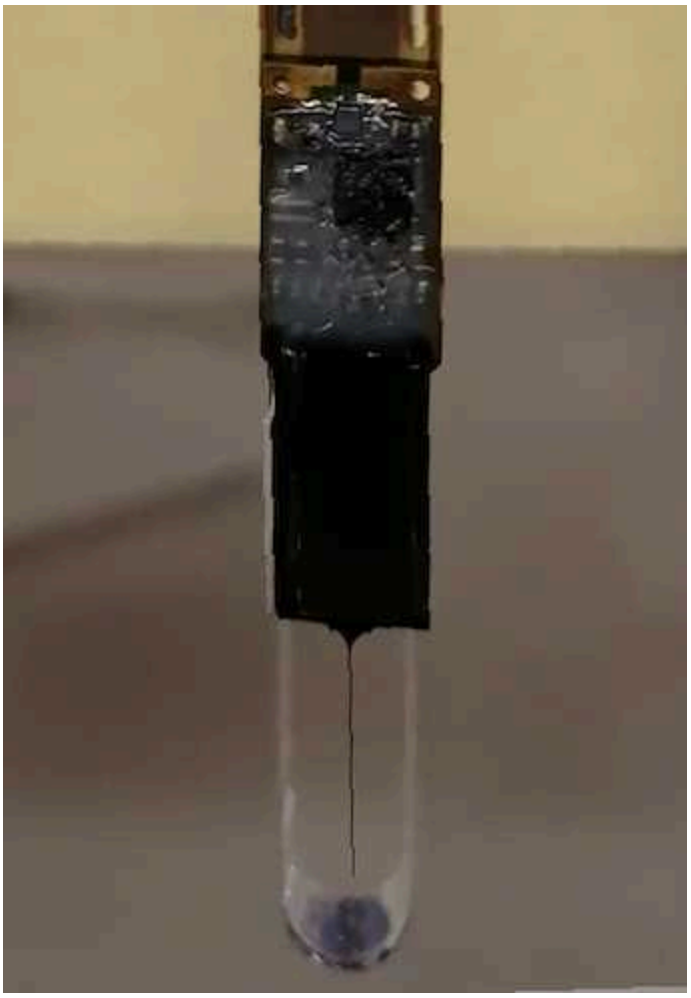
Troubleshooting

1 Based on target brain regions, set up manipulator azimuth and pitch angles before each recording session. Manipulators should be positioned to be able to reach target areas while not colliding with each other.

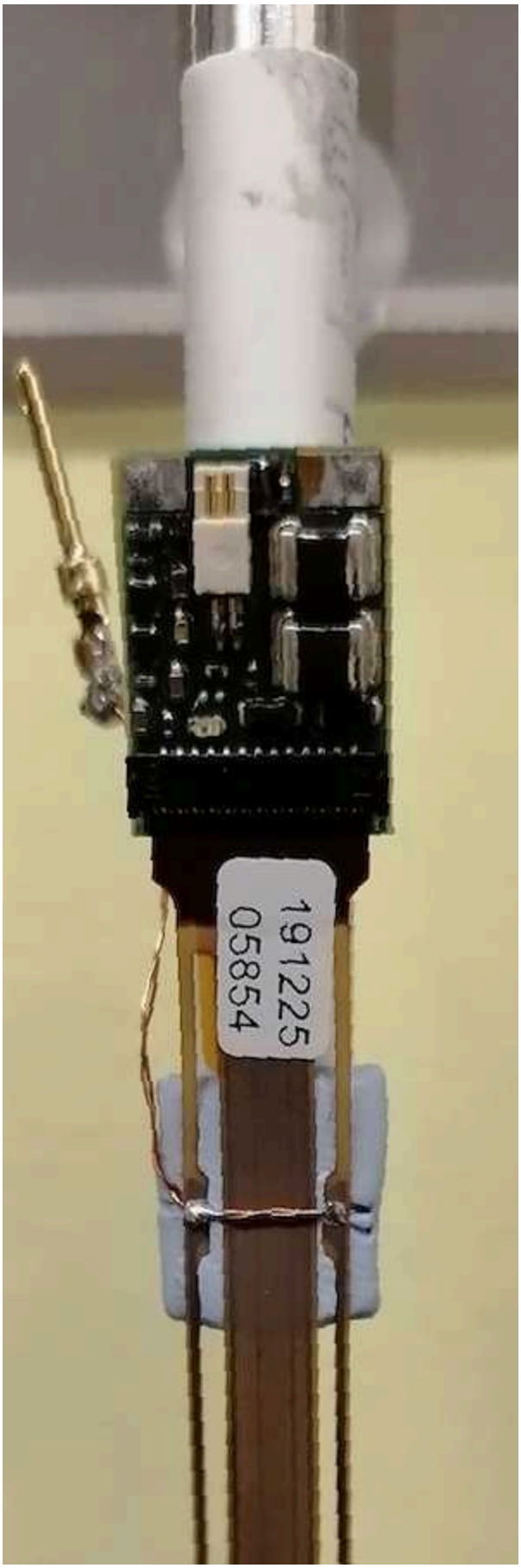
2 Paint individual electrodes with CM-DiI (https://www.protocols.io/view/painting-neuropixels-probes-and-other-silicon-prob-wxqffmw).

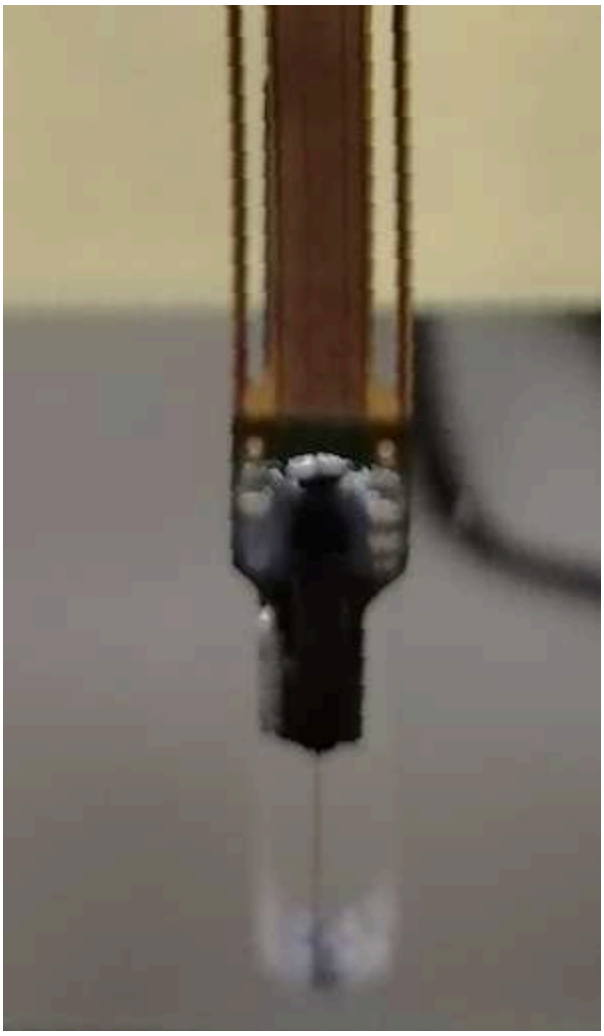
3 Mount and fix individual probes onto their manipulators. Connect Neuropixels probe cables to headstages. Connect individual reference cables to pins soldered onto each probe. Ground and external reference pads are shorted together on the flex.


180051
23682



Example of shorted external reference (i.e. left column pad on the flex) and ground (i.e. right column pad on the flex) on a Neuropixels 1.0 probe.

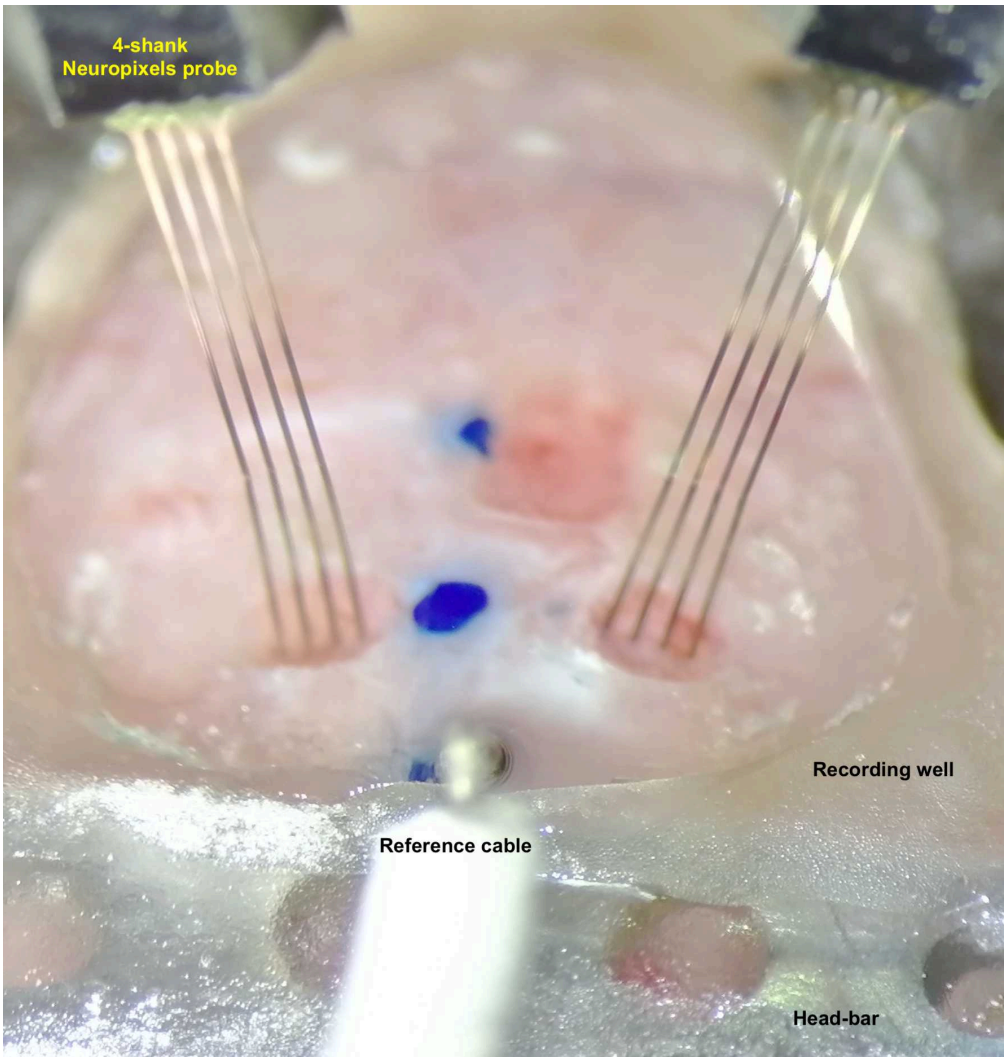

191225
05854



Example of shorted external reference (i.e. left column pad on the flex) and ground (i.e. right column pad on the flex) on a Neuropixels 2.0 probe.

All electrode reference cables should connect to a common reference electrode. Start software SpikeGLX (**https://billkarsh.github.io/SpikeGLX/**) to make sure all probes can be successfully detected. Set up recording configuration including Imec Readout Table (imRo), trigger mode, where to save files, etc in SpikeGLX (https://github.com/billkarsh/SpikeGLX/blob/gh-pages/Support/Metadata_30.md).

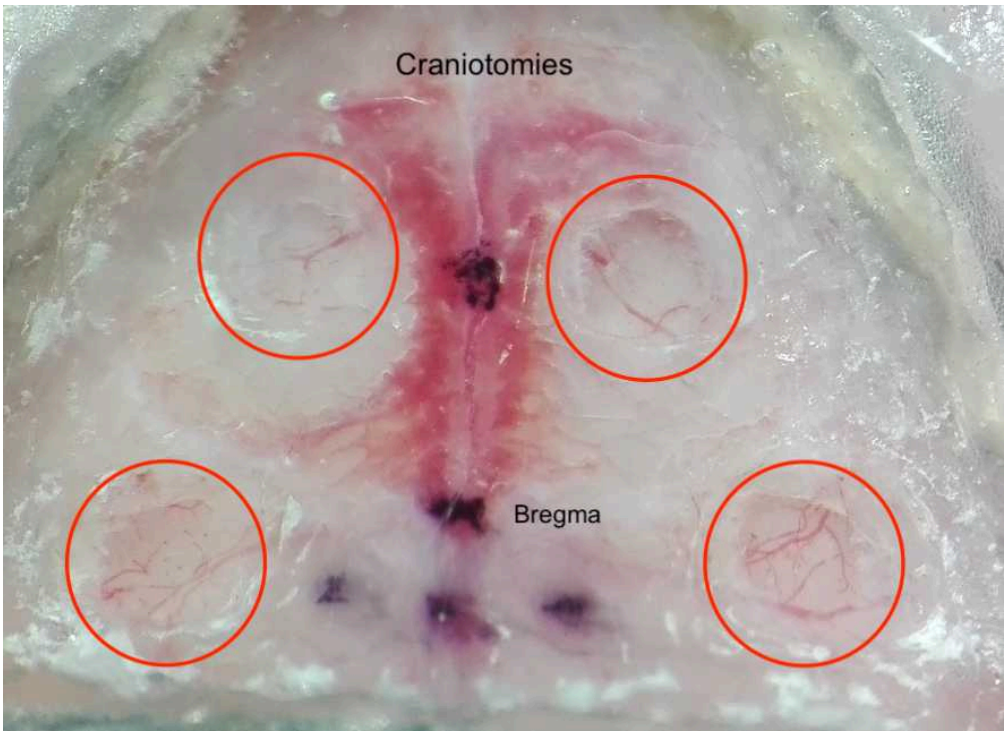
4 Load animal into recording apparatus and immobilize the head. Remove dura seal (i.e. Kwik-cast sealant) and apply ~0.1 ml of cortex buffer to fill the recording well (i.e. built with layers of dental cement prior to acute recording https://www.protocols.io/view/recording-well-preparation-9a8h2hw). Place common reference electrode (cable) into cortex buffer bath (see white cable in the example shown below), and keep it far from any potential insertion craniotomy.



Example of two Neuropixels 2.0 multi-shank probes inserted into brain with reference cable in position (white cable). Recording well is filled with cortex butter to keep the brain from drying out.

Rinse the well and reference electrode multiple times with cortex buffer to remove floating debris. Fill up the well with cortex buffer.

5 Move individual probes above corresponding target craniotomies. Identify clean entry point for each insertion; avoid surface blood vessel; record the entry point of each recording day/session within individual craniotomies. The electrode location with respect to Bregma is noted at the level of each craniotomy (https://www.protocols.io/view/recording-well-and-craniotomy-preparation-9a8h2hw). The entry points from separate sessions should be at least 200 µm apart if using the same angle of penetration. This results in probe tracks that are easy to distinguish in histology.



Example of four craniotomies performed on a mouse for four simultaneous Neuropixels probes recording. Red circles highlight craniotomies.

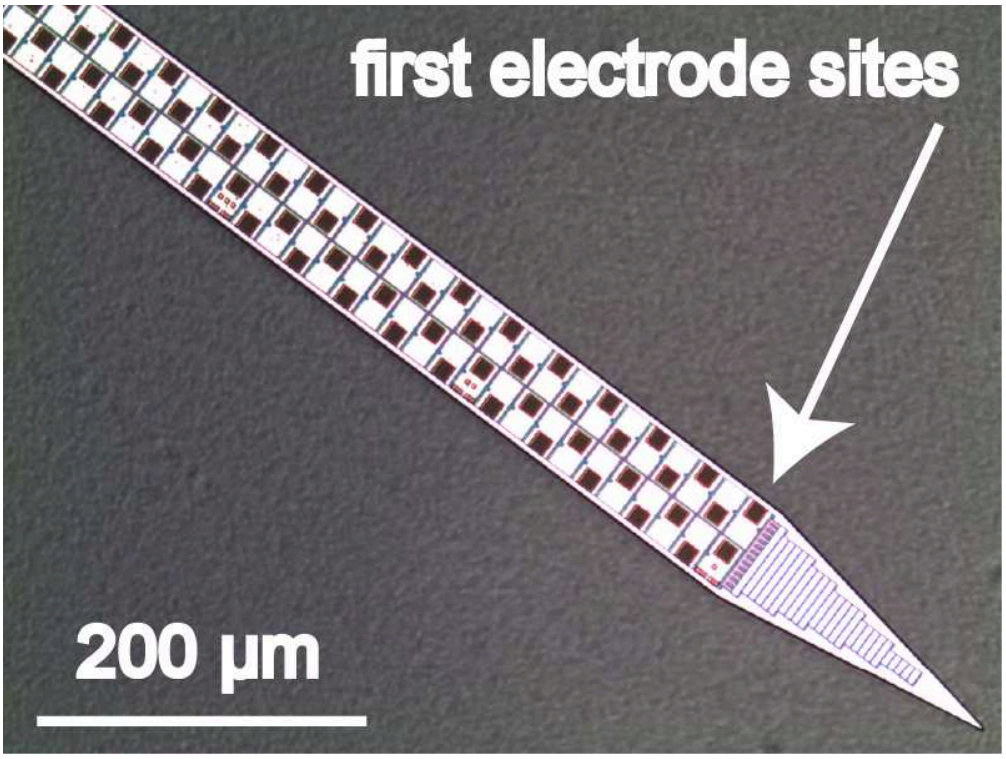


Example of recording log of entry points in four craniotomies from a different brain. Numbers correspond to session dates and their locations record respective entry points. It is also helpful to draw the pattern of blood vessels within each craniotomy to help determine different entry points over consecutive days.

Fine-adjust entry point using micro-manipulator. Once entry point is determined, move probe along penetration axis to assist dura penetration. It is highly recommended to sharpen all probes before usage. This is to minimize compression of the brain while

penetrating through dura (https://vimeo.com/359133527). During insertion, the recommended bending of Neuropixels probe from base to tip is < 100 µm (page 11 https://e2f49b05-3010-45e2-a7bf-b8f67811425a.filesusr.com/ugd/832f20_93fb9fa4481c4b3eb0975a6410a38722.pdf).

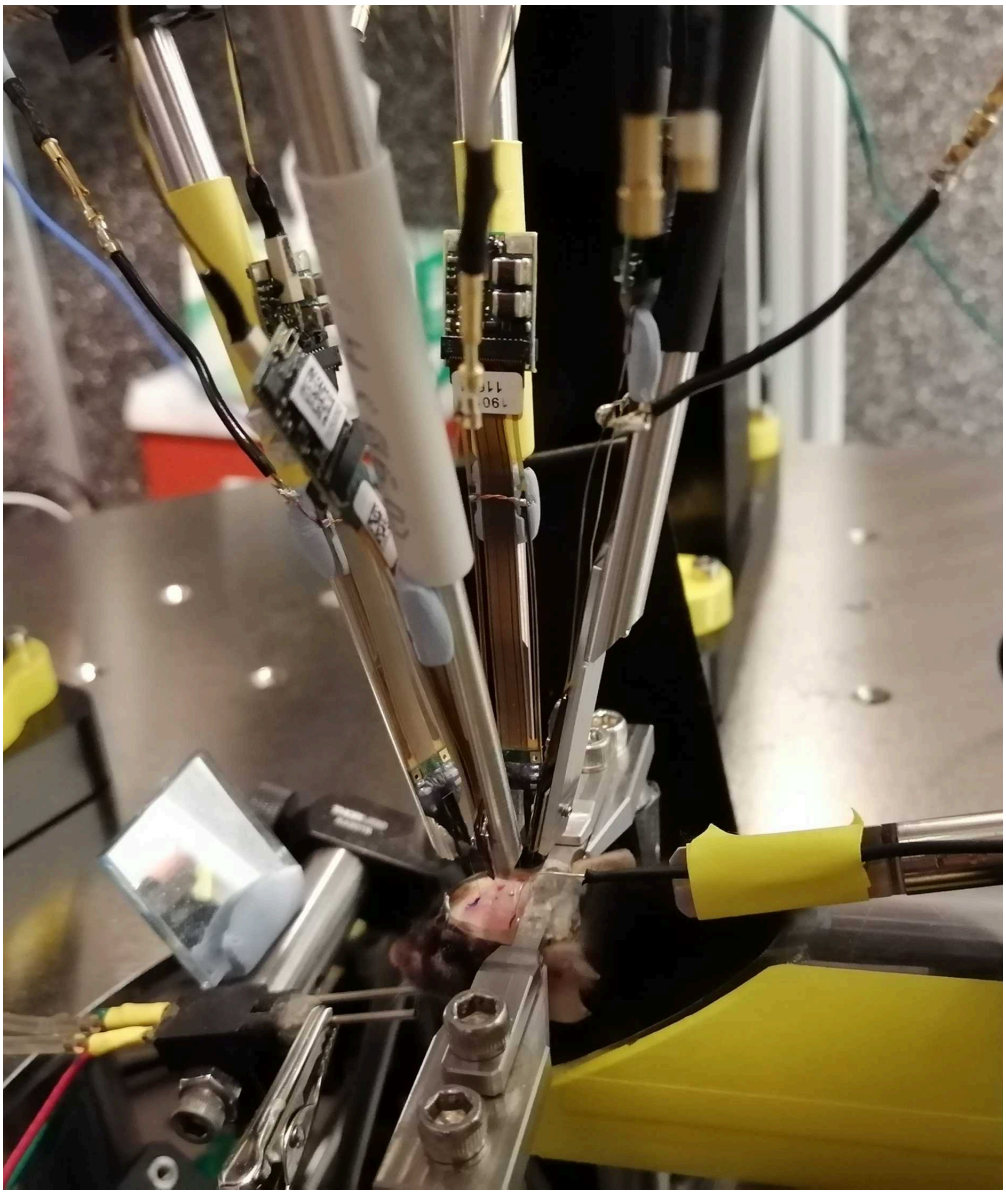
When encountering difficulties during dura penetration (e.g. dura thickens up over consecutive days), first bend the probe for less than 200 µm (distance read from manipulator), and then use the tip of a fine syringe (gauge 28) to gently tap the convex side of the probe until it penetrates through dura. Probe should straighten when penetration is achieved. Move probe slowly in and out until the first row of recording sites is at the brain surface (by visual inspection through a high-power stereo microscope). This depth should be recorded as the surface of the brain. It is good practice to zero the manipulator depth reading at the surface of the brain.



For each insertion, make sure the tip of the shank (triangle region with no recording site) is completely inside the brain before zero the manipulator.

Penetrate probes through dura one at a time. Once penetration and zeroing is done for all probes, probes can be driven to targeted depths by automatic stepping, all at the same time, with a speed of approximately 6~8 µm/s (e.g. 3~4 µm/step, 1 step = 0.5 sec). When the desired depths are reached, record these depths as the distance from the surface of the brain.

6 Once all probes have been fully inserted, wait 00:10:00 for brain to settle before recording. Fill the recording well with at least 0.1 ml of cortex buffer to avoid drying of the brain surface over a session that lasts for 1~2 hours.



Example of four inserted Neuropixels probes targeting multiple brain regions.

7 After the recording is finished, retract probes using manipulators, one at a time manually, or simultaneously using automated program. Disconnect probes and reference cables. Dismount probes from manipulators and immerse probes (only shank part but not base) in cleaning solution at RT. Recommended soaking time on shelf is 12:00:00 . 12h

Remove common reference electrode from recording well. Rinse well with cortex buffer

multiple times. Absorb all cortex buffer with sugi sponge before sealing the well. Make sure the brain surface is moist but without excessive liquid, and apply Kwik-cast sealant to cover all craniotomies. Remove animal from rig after Kwik-cast has solidified (~ 3 min).

8 Before the next day's experiment all probes should have been washed in distilled water for 02:00:00 followed by a quick dip in IPA (to remove DiI from previous penetration). Cleaned probes can be left dry in air before the next use. For cleaning guidelines see IMEC user manual (page 41 https://e2f49b05-3010-45e2-a7bf-b8f67811425a.filesusr.com/ugd/832f20_93fb9fa4481c4b3eb0975a6410a38722.pdf)