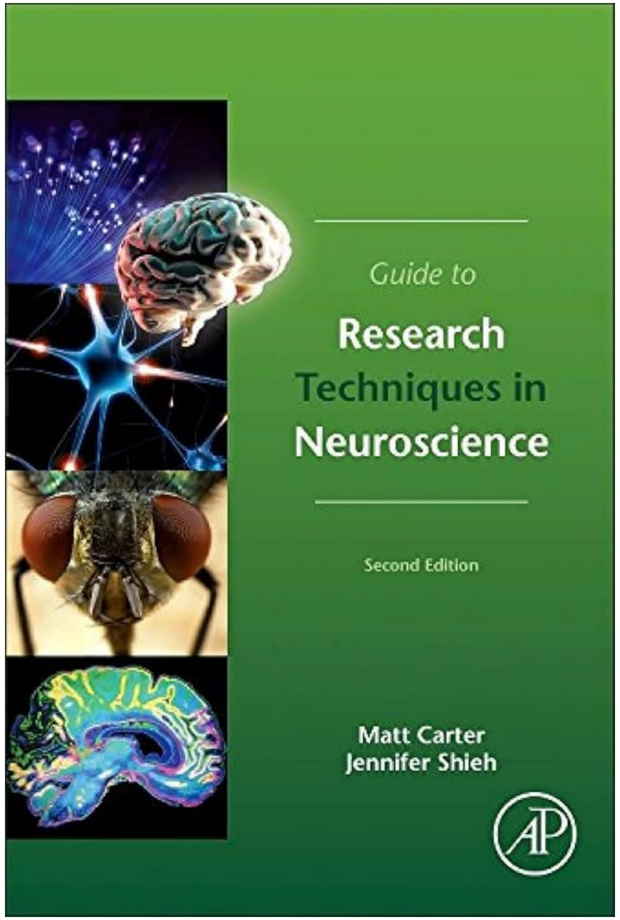
Electrophysiology Workshop

Misi Voroslakos
Postdoc
Buzsaki Lab

2025. 12. 11.

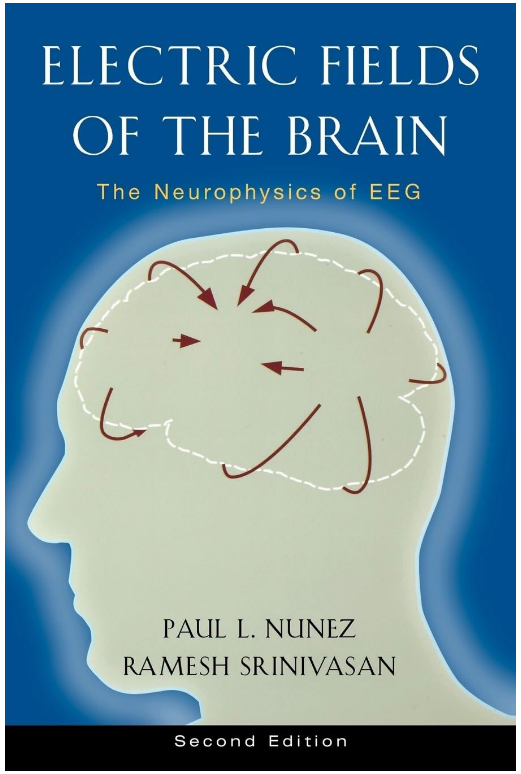
Postdoc Led- Electrophysiology Workshop

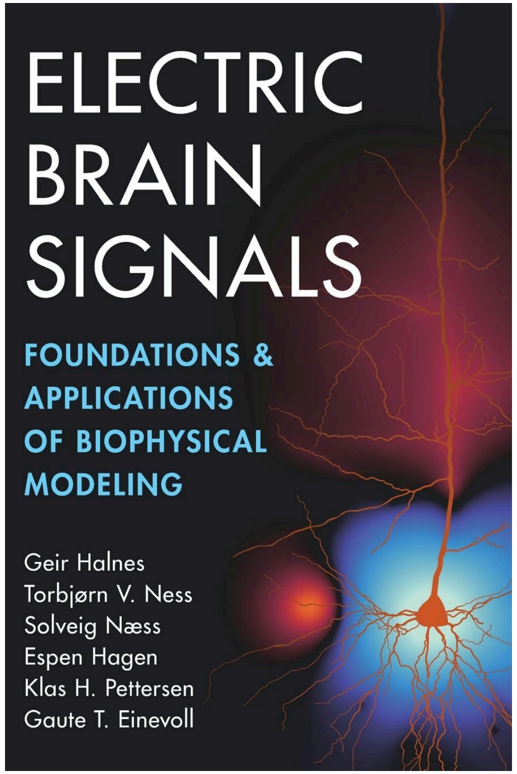
Background reading materials

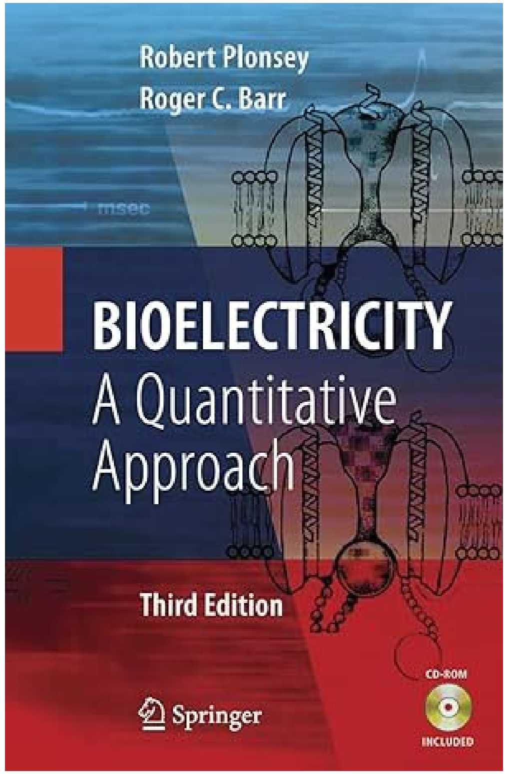


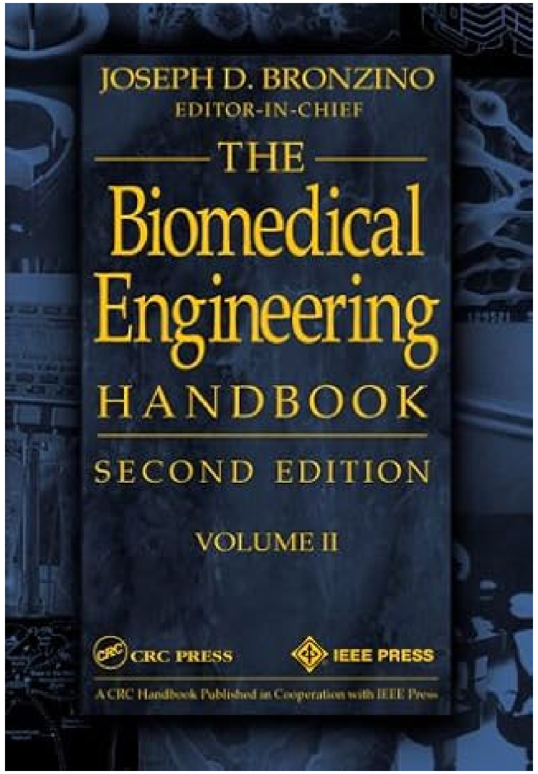
- **Chapter 3** - Stereotaxic Surgeries and In Vivo Techniques
- **Chapter 4** – Electrophysiology
- **Chapter 8** - Manipulating Neural Activity

Background reading materials









Section II
Section V
Chapter 70

Background reading materials

Microelectrodes, Microelectronics, and Implantable Neural Microsystems

Progress in development of tiny electrodes, cables, circuitry, signal processors and wireless interfaces promises to advance understanding of the human nervous system and its disorders.

By Kensall D. Wise, *Life Fellow IEEE*, Amir M. Sodagar, *Member IEEE*, Ying Yao, *Member IEEE*, Mayurachat Ning Gulari, Gayatri E. Perlin, and Khalil Najafi, *Fellow IEEE*

nature reviews methods primers https://doi.org/10.1038/s43586-025-00399-7

Primer Check for updates

In vivo microelectrode arrays for neuroscience

Nathaniel P. Williams[1,2], Mihaly Voroslakos[3], Delin Shi[1,2], May Yoon Pwint[1,2], Vittorino Lanzio[4], Hongwei Mao[2,5], Pavlo Zolotavin[6], Euisik Yoon[4,7,8,9], Thomas Stieglitz[10,11], Chong Xie[6,12,13], Timothy D. Harris[14,15], Andrew B. Schwartz[1,2,5] & Xinyan Tracy Cui[1,2]

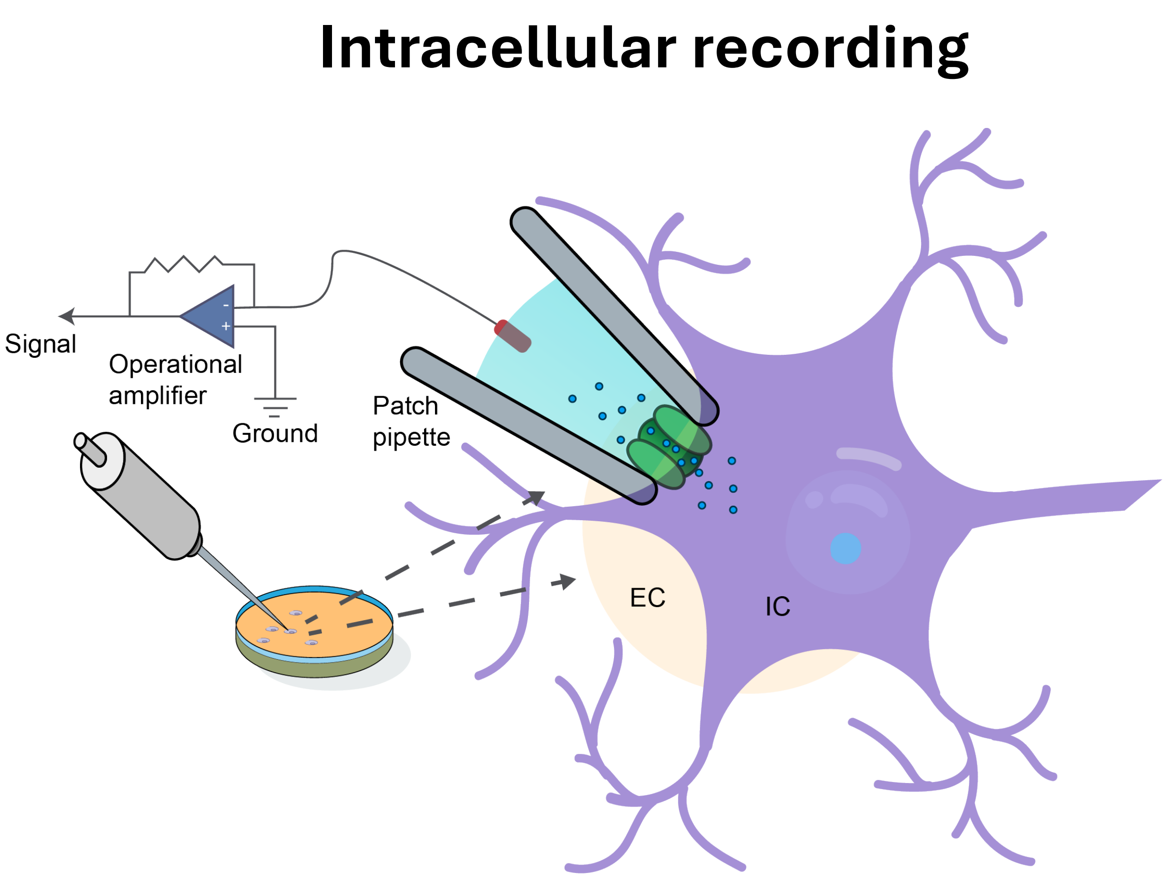
The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes

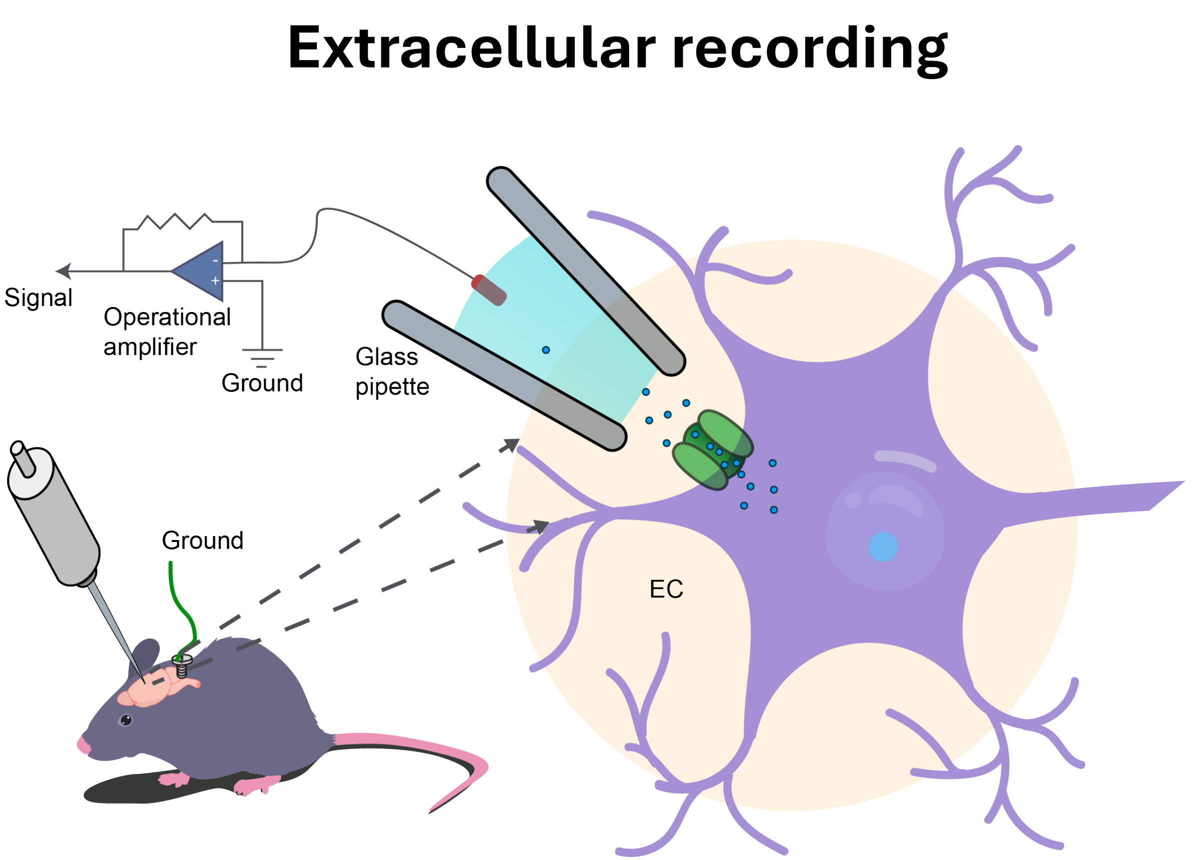
György Buzsáki[1,2,3], Costas A. Anastassiou[4] and Christof Koch[4,5]

REVIEW ARTICLE

State-of-the-art MEMS and microsystem tools for brain research

John P. Seymour[1], Fan Wu[2], Kensall D. Wise[1,3] and Euisik Yoon[1,3]





1930s–1950s: Glass micropipettes
“Intracellular revolution”

- Resting potential, AP shape, synaptic potentials
- Acute, mostly stationary preps

1953: Metal-filled glass micropipette

- Dowben & Rose, Science

1957: Tungsten microelectrode

- Hubel, Science
- reliable single units, chronic-ish in vivo

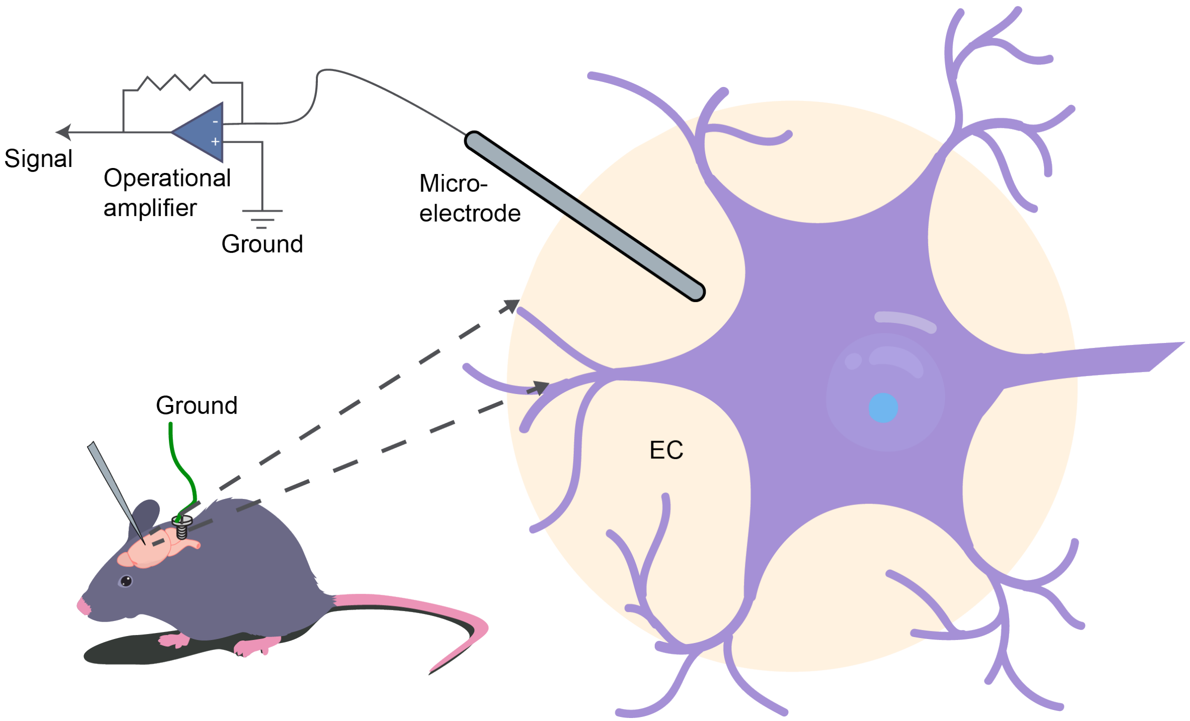
1958: Microwire bundles & chronic implants

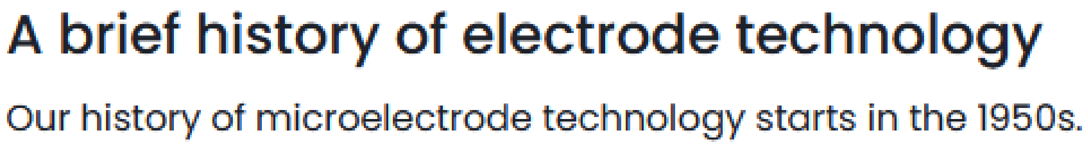
- Strumwasser (steel), Olds (nichrome)
- Long-term single units in unrestrained animals

Video worth watching

- Prof. Ken Wise
  - [The Rocky Road to Neurotechnology]
  - 1966 – 2016 overview of technology

Extracellular recording

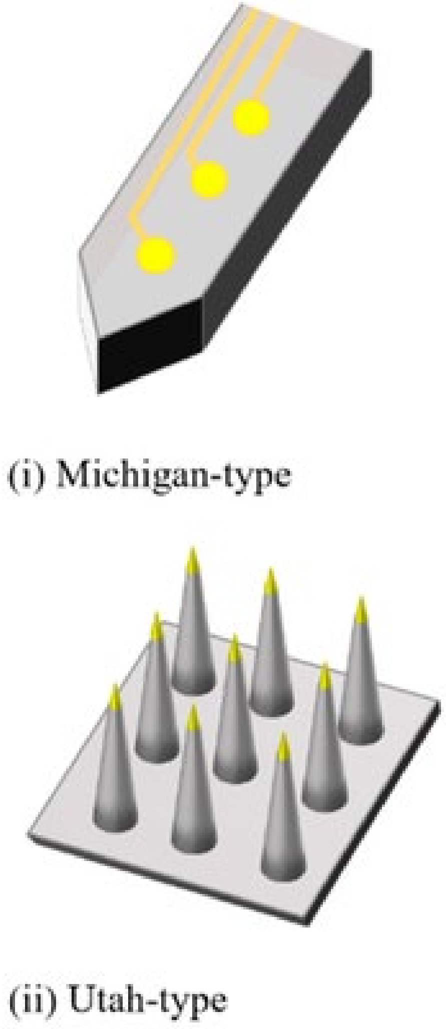




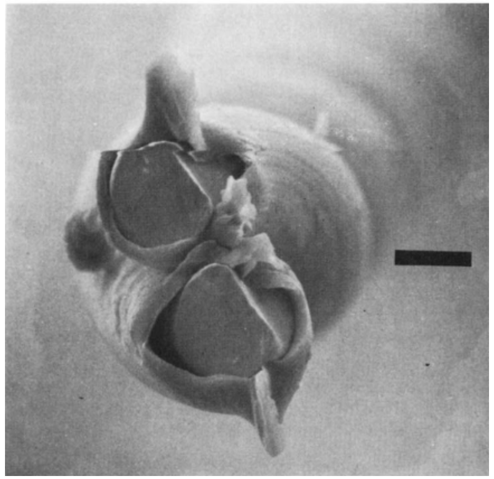
https://www.neuronexus.com/a-brief-history-of-electrode-technology/

The stereotrode: A new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records

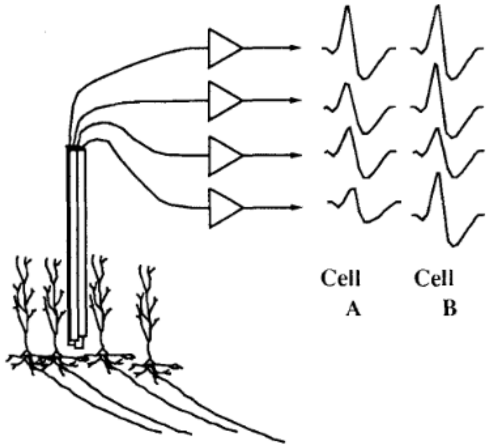
1990-



1983

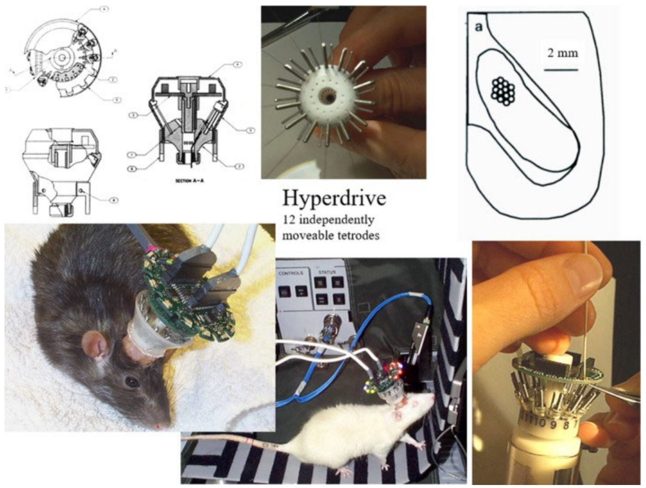


1993



Tetrode
Phase relationship between hippocampal place units and the EEG theta rhythm

1999

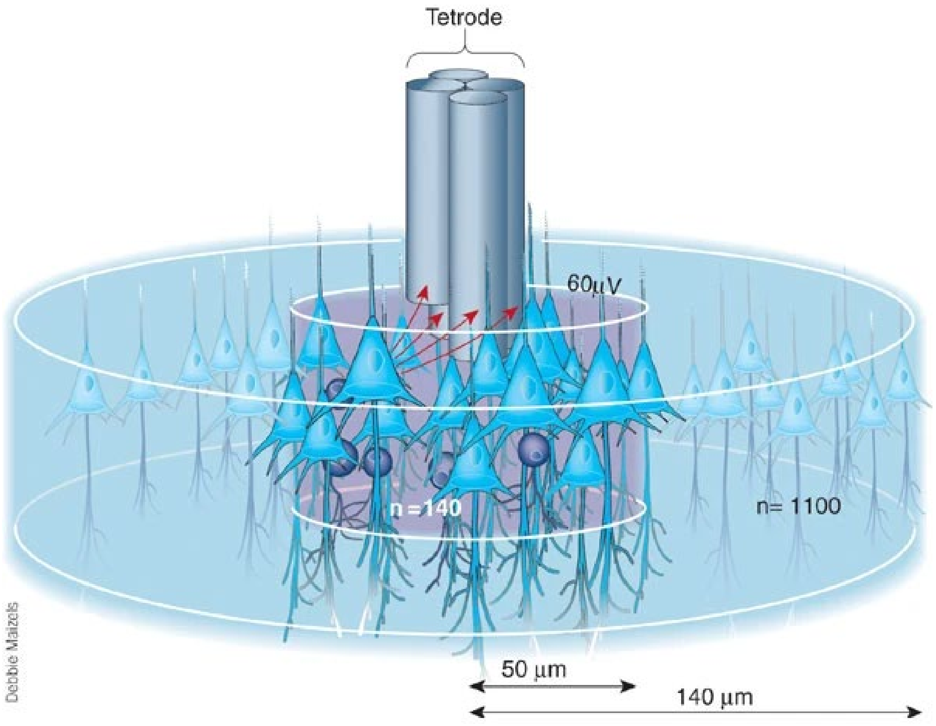


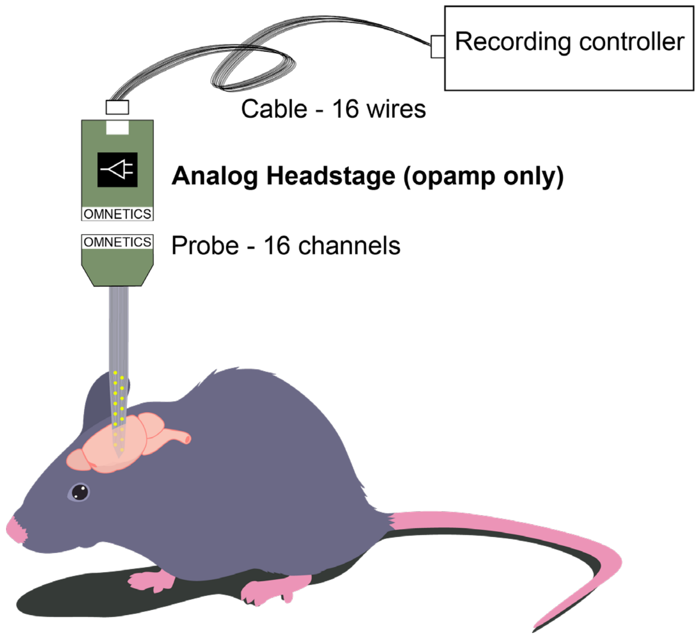
Hyperdrive (Bruce McNaughton)

Goals and limitations (for engineers)

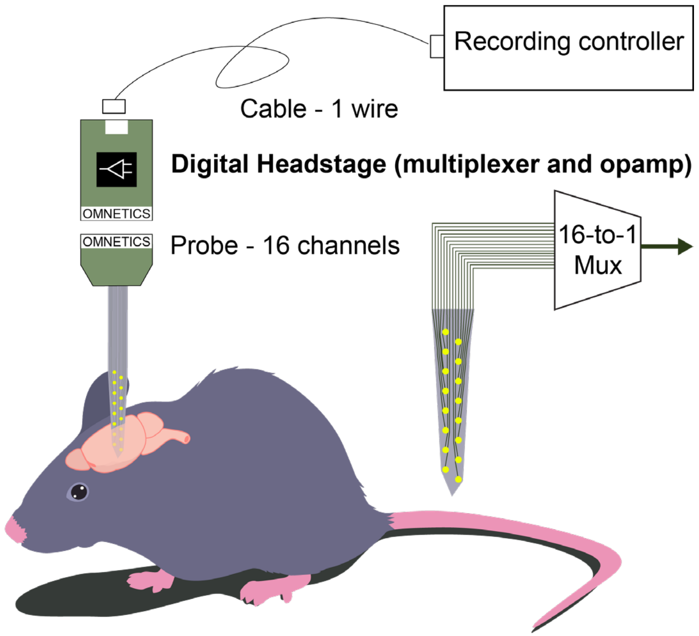
Channel count

As many channels as possible





Until 2010



Since 2010
Intan Technologies

Gyorgy Buzsaki, 2004

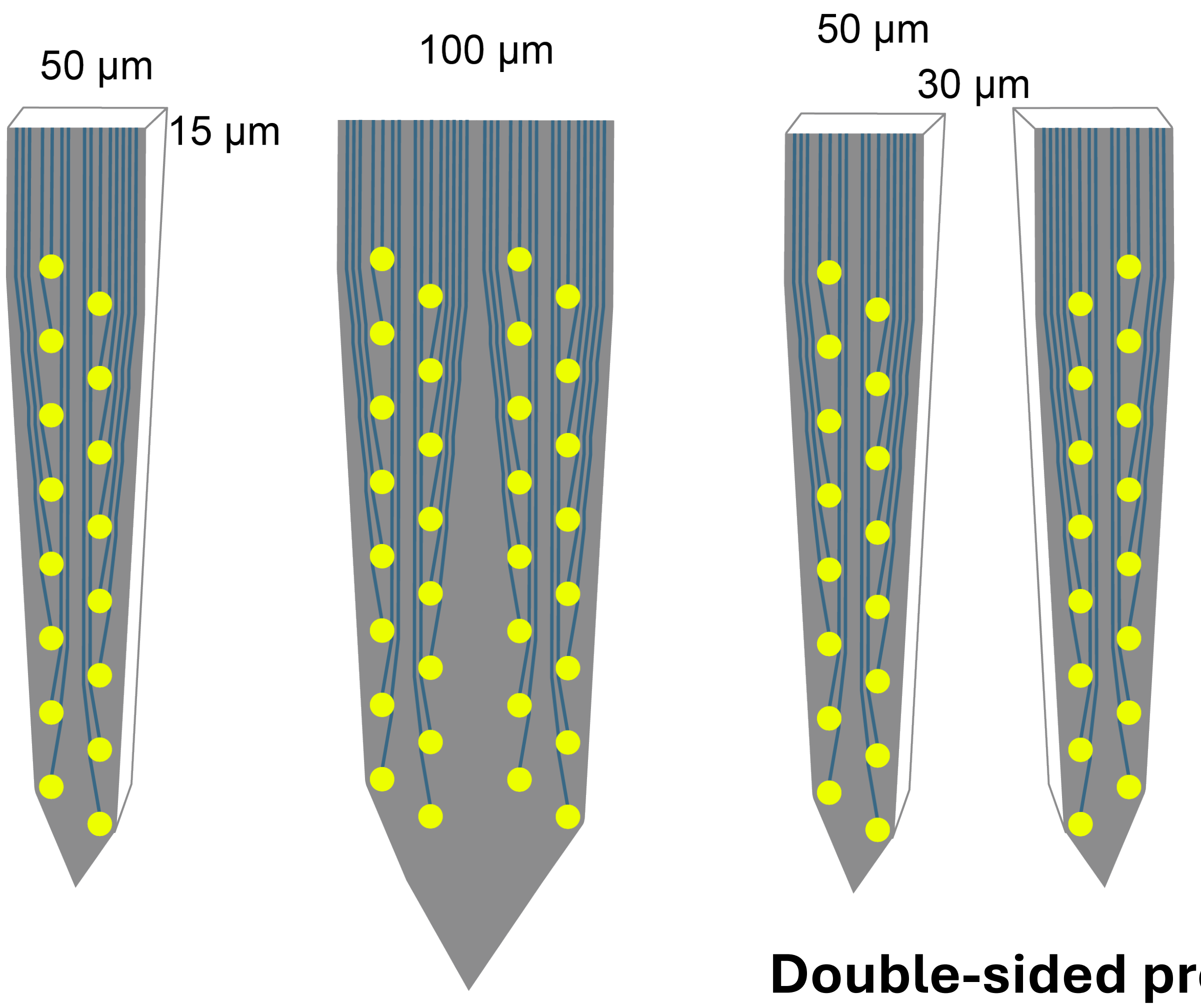
Goals and limitations (for engineers)

Channel count

As many channels as possible

Same time

Minimize tissue damage

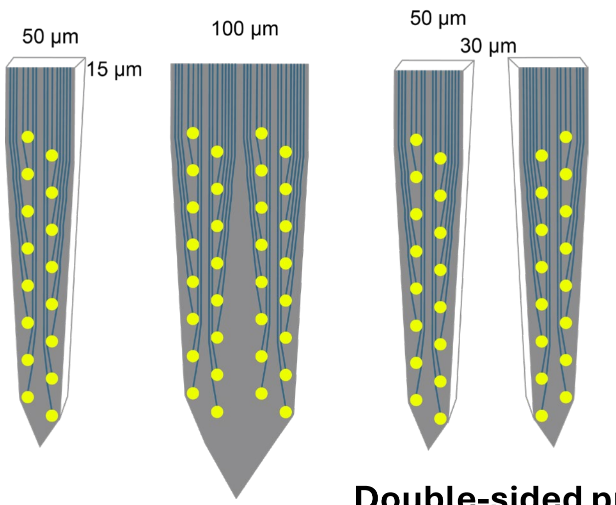


Double-sided probe

Goals and limitations (for engineers)

Channel count

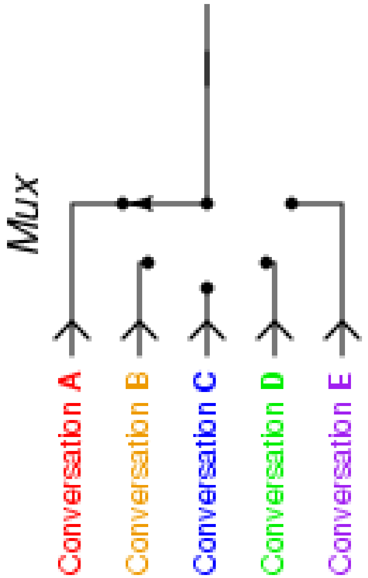
As many channels as possible



Double-sided probe

Number of metal lines (green) limit the number of channels

Multiplexing on the shank



Same time

Minimize tissue damage

CMOS probes
Neuropixels: 384 channels
SiNAPS: 1024 channels

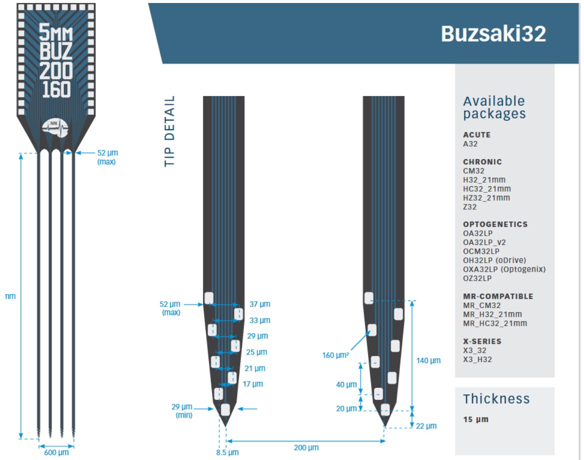
Active electronics on the probe shanks.

My electrophysiology journey

Martfű, Hungary (6000 people)

Szeged, Hungary → MD/PhD

2 x 32-channel probes (2013)
(anesthetized rats)



U of M, Ann Arbor

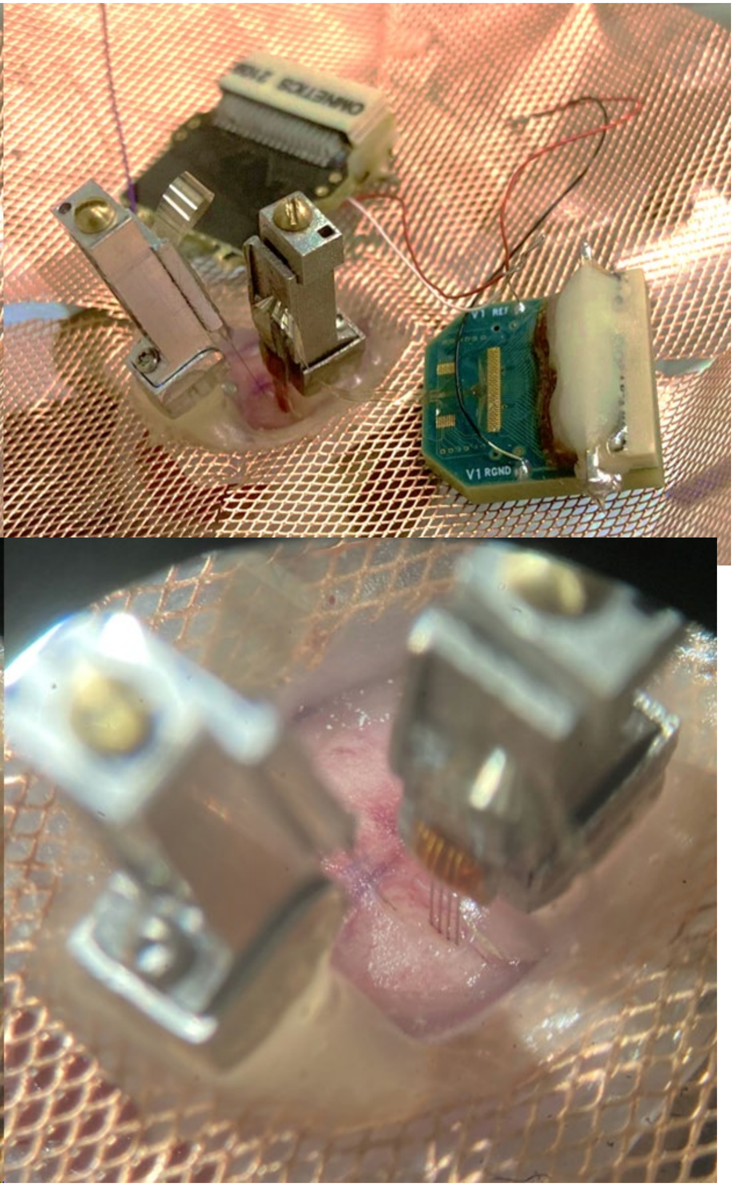
- Optogenetics
  - Optrode
  - Micro-LED probe
  - Waveguide probe
- Freely moving mice
- Head-fixed mice
- Freely moving rats

- Flexible probes
- Opto flexible probes

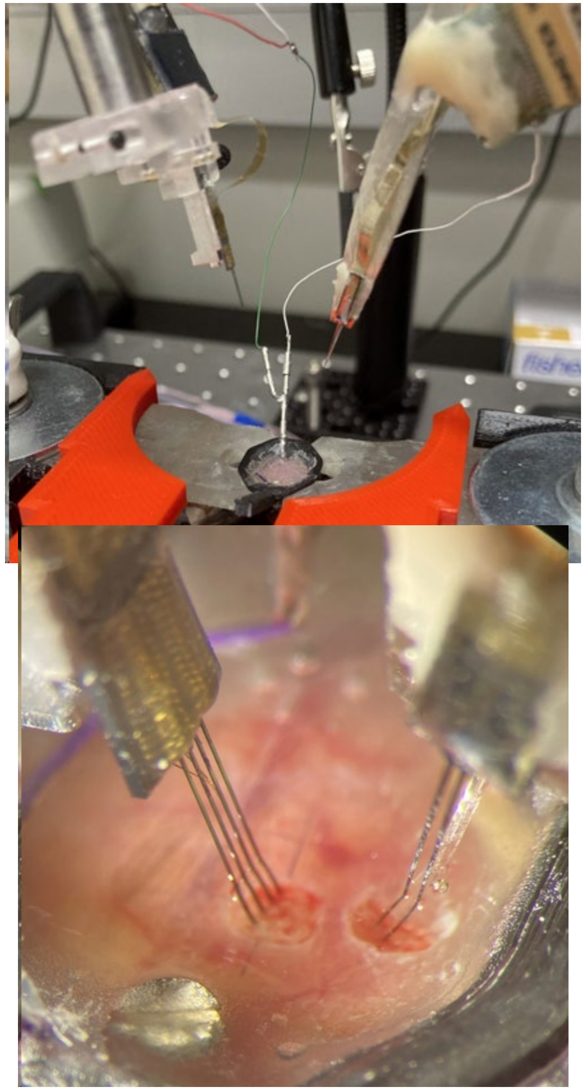
NYU NEUROSCIENCE

- Large-scale ephys
  - Neuropixels
  - SiNAPS
  - Hecto-STAR

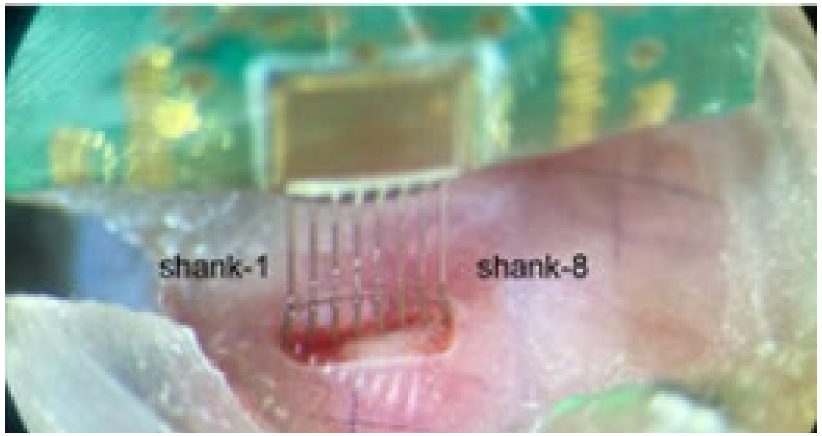
DBC-128 + micro-LED
(freely moving mouse)



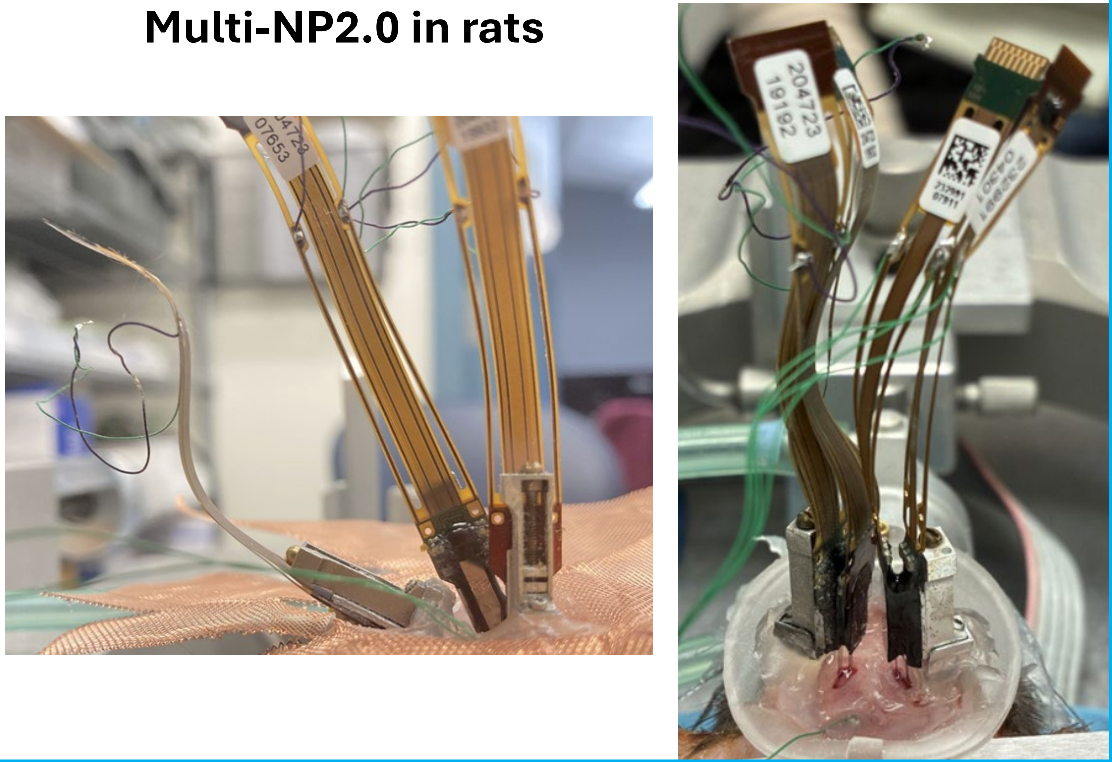
DBC-128 +
Cambridge optrode
(head-fixed mouse)



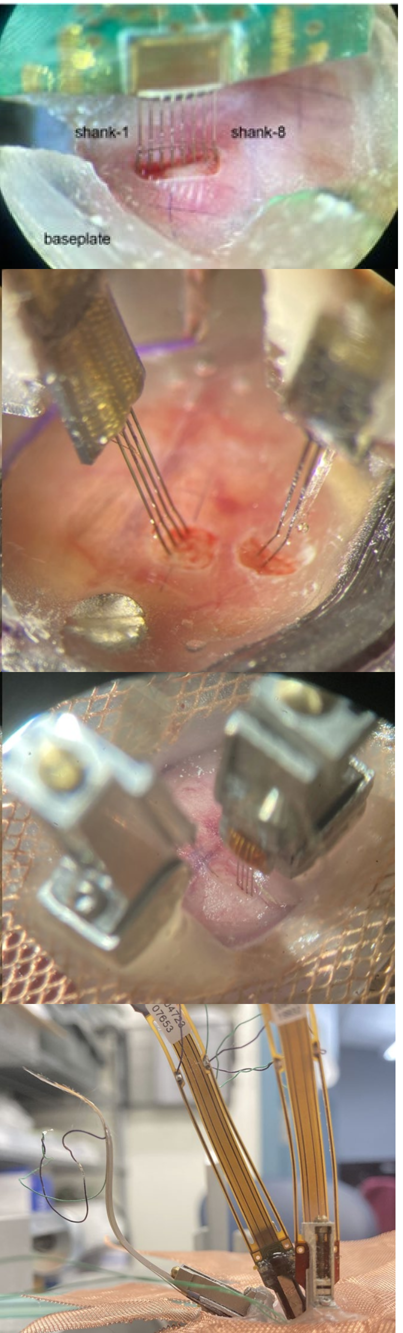
1024-channel SINAPS (freely moving mouse)



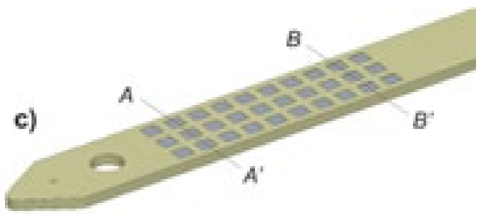
Multi-NP2.0 in rats



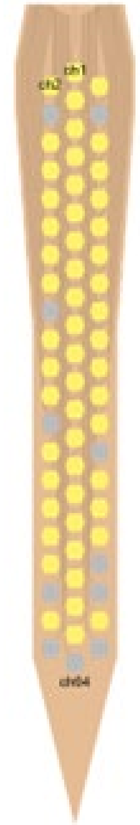
Experience with flexible probes



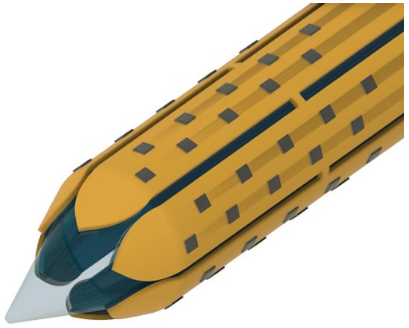
MANTA probe

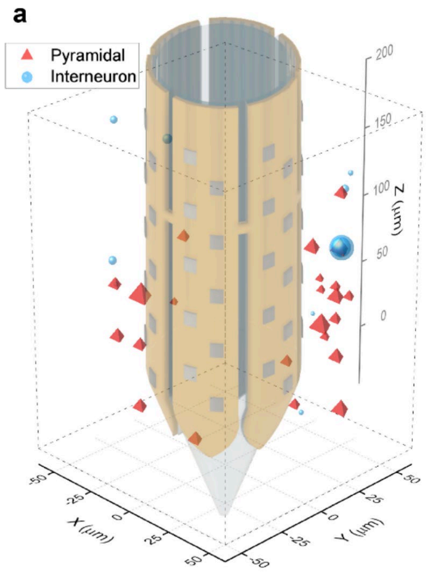


Polyimide probe
(4 µm thickness)

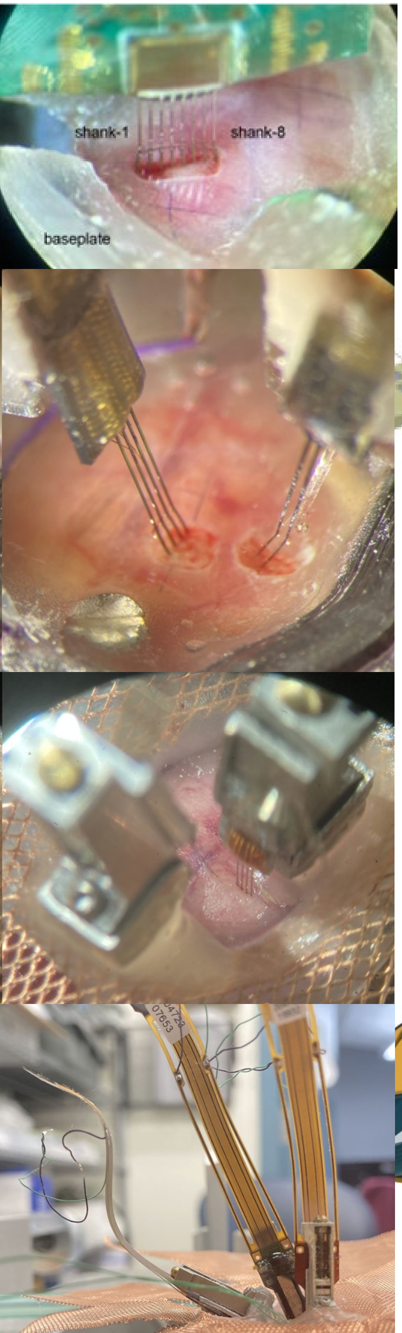


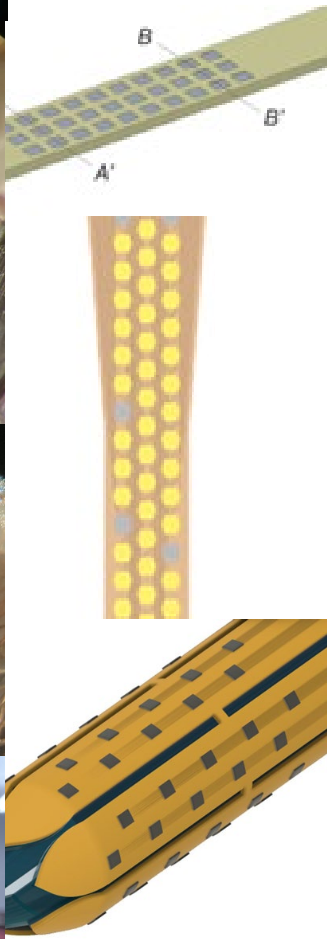
3D origami



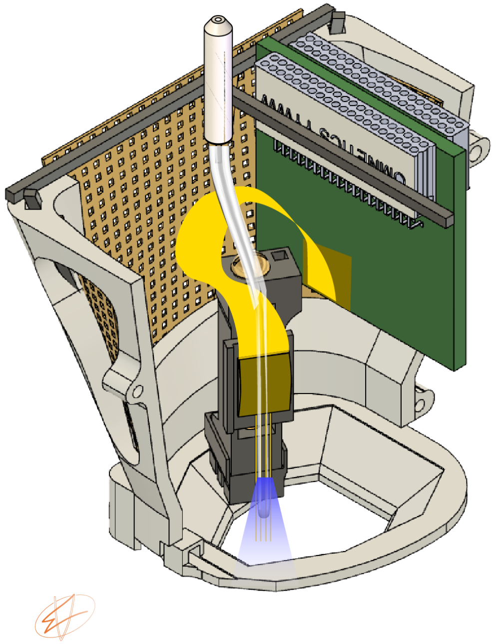


Experience with optogenetic probes





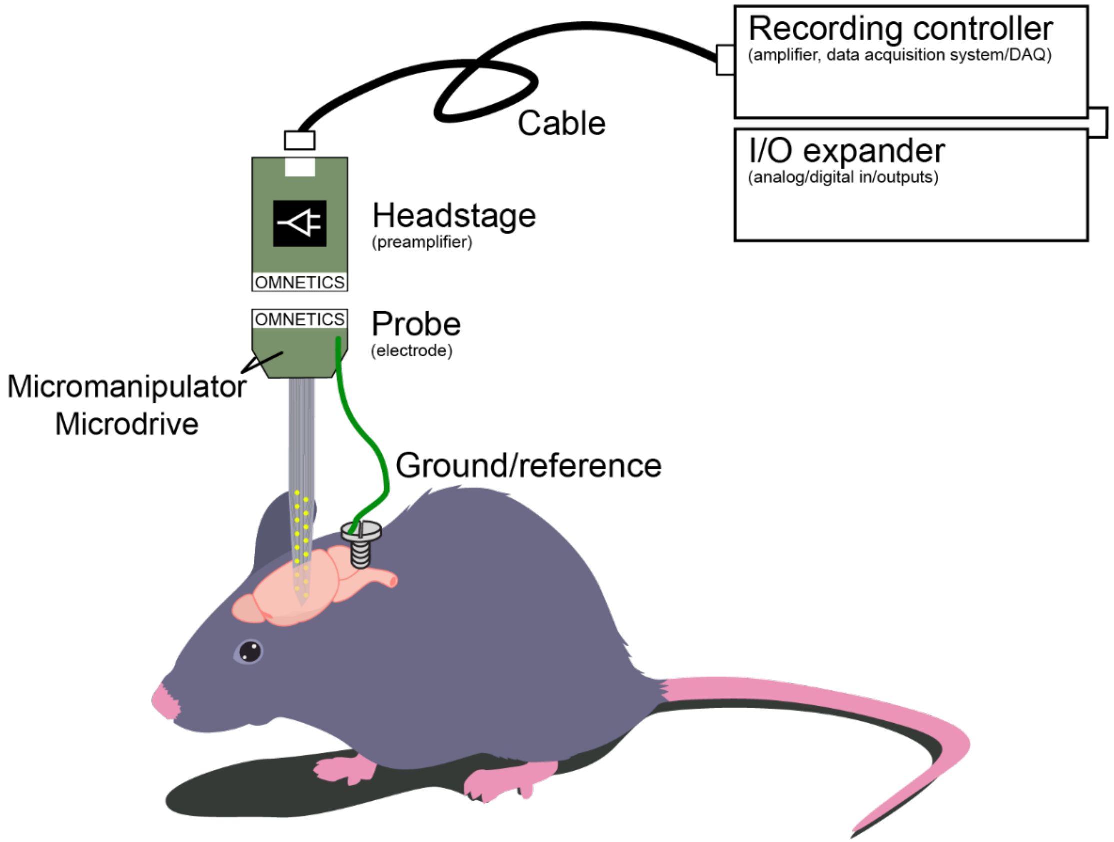
Optrode



Flexible Micro-LED

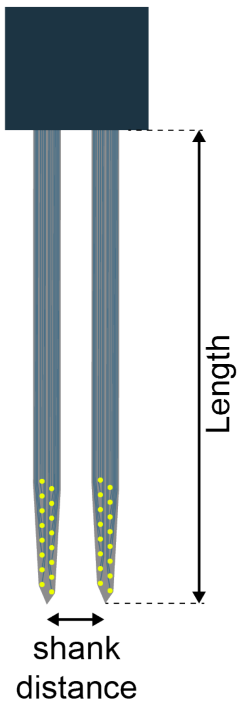
Micro-LED

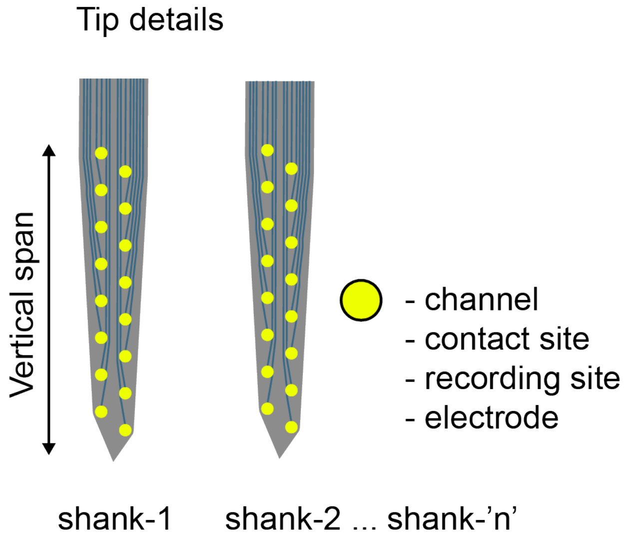
Parts of an extracellular ephys system

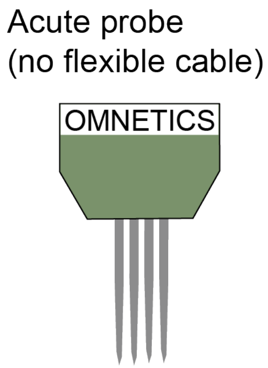


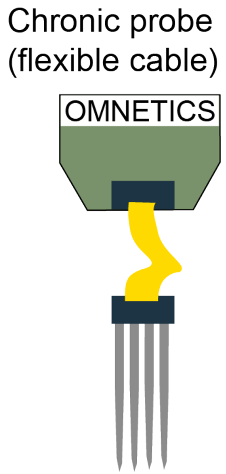
- Recording probe/electrode/device
- Microdrive/shuttle/drive/manipulator
- Headstage/preamp/amplifier
- Recording cable
- Recording device/DAQ
- I/O expander/analog_digital inputs
- Ground, reference

‘Anatomy’ of a probe









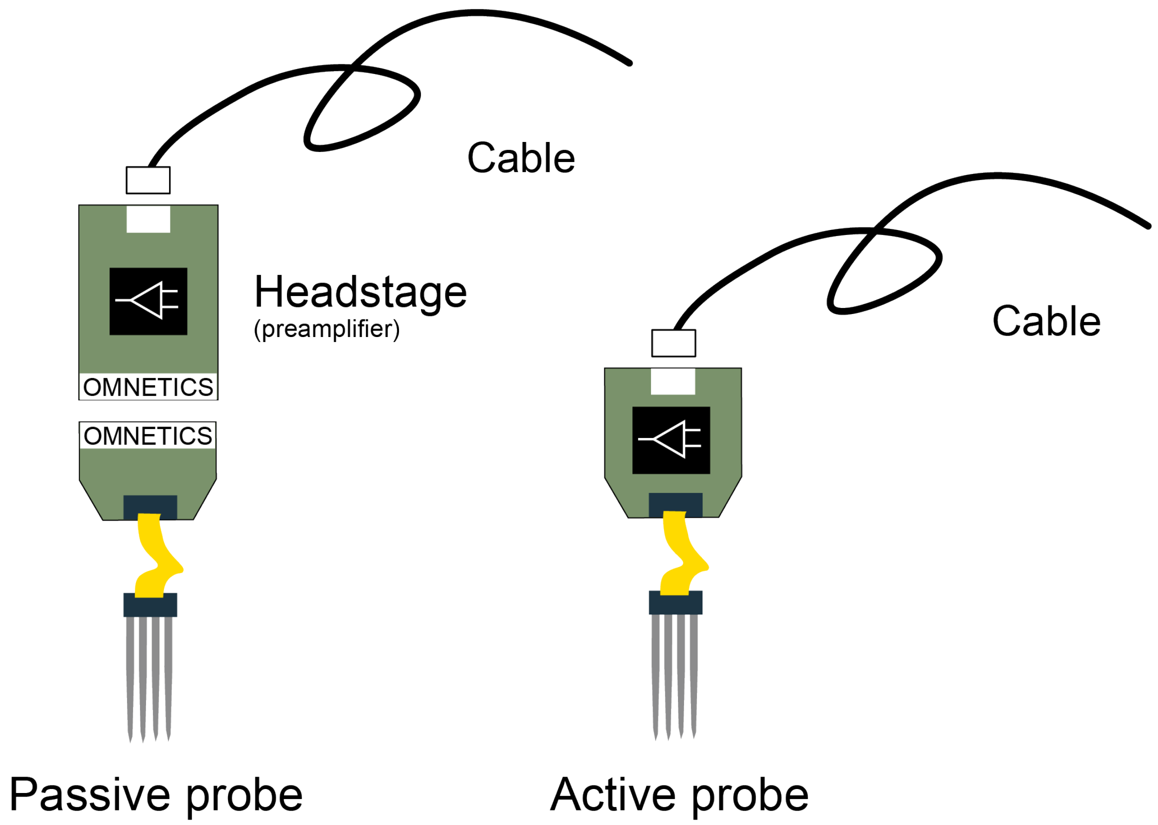
Acute probe

- No flex cable, very low noise
  - Parallel lines pick up EMI
- Head-fixed preparation
  - Awake, anesthesia

Chronic probe

- Flexible cable (yellow part) allows mechanical decoupling
- Attached to a Microdrive/shuttle
- Freely moving preparation
  - Can be used in head-fixed

‘Anatomy’ of a probe



Mouse weighs ~ 20-30 g

- We can place up to 20% of body weight

Passive probe

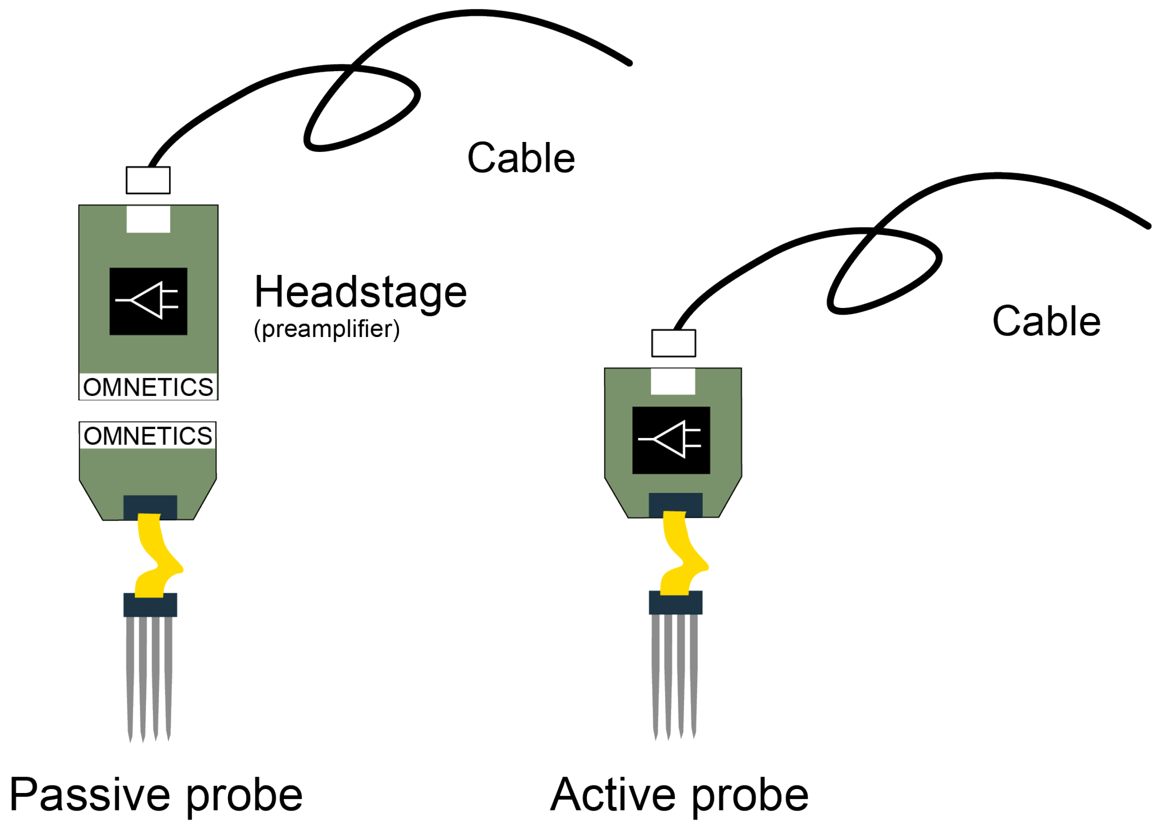
- Probe itself is **0.6 – 0.8 g**
- Headstage is **1.2 g**
    - **Animal must carry it during behavior!**

Active probe

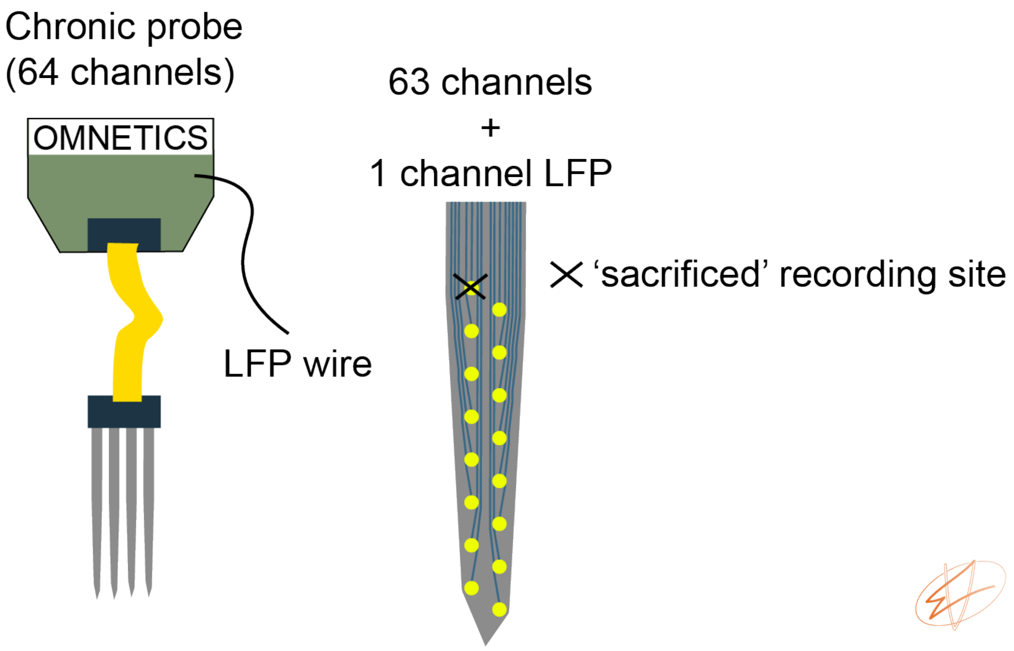
- Probe itself is **0.6 – 1.2 g**

MiniAmp (Cambridge)

‘Anatomy’ of a probe

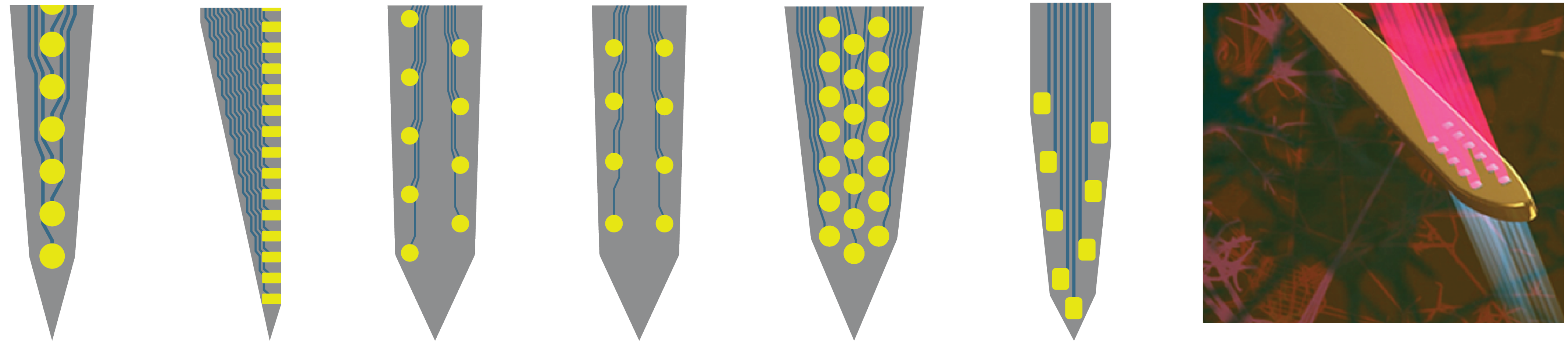


Multi-regional recordings with wires



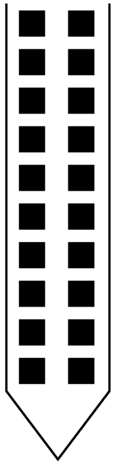
Silicon probes **16 / 32 / 64 / 128 / 256 channels** **5 or 10 mm**

Linear Edge Staggered Poly2 Poly3 Buzsaki Double-sided



CMOS probes

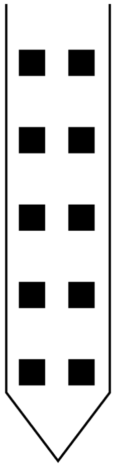
Neuropixels



384 out of 5120

10 mm

SiNAPS



1024 channels

5 mm

Cambridge Neurotech
IMEC
NeuroNexus
Plexon

(Alphabetical order)

‘Anatomy’ of a probe

Group-1

Freely moving mouse, targeting dorsal CA1. Your primary goal is to maximize the number of simultaneously recorded single units from the pyramidal cell layer.

Group-2

Mouse somatosensory cortex and study laminar information processing: how inputs and outputs differ across layers (L2/3, L4, L5, L6).

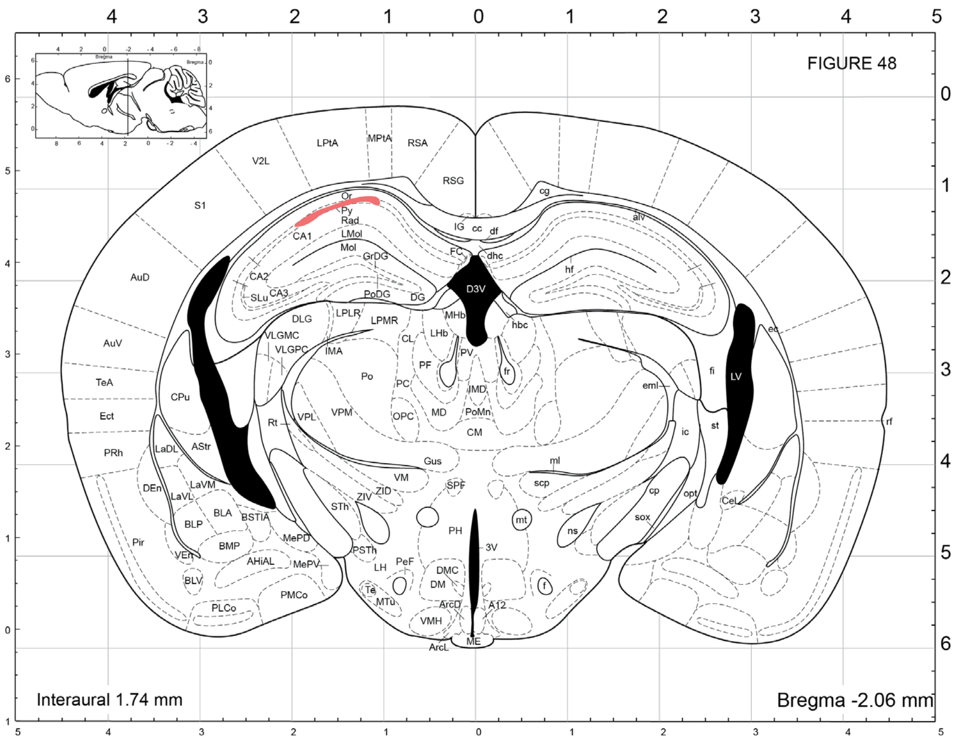
Group-3

Freely moving mouse, targeting CM nucleus of thalamus. Maximize single unit yield.

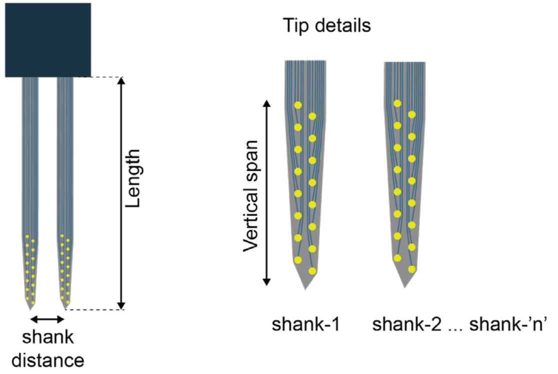
Group-4

Freely moving rat, targeting putamen and motor cortex. Maximize single unit yield in both regions.

Group-1



Select a probe that maximize the number of simultaneously recorded single units from the pyramidal cell layer



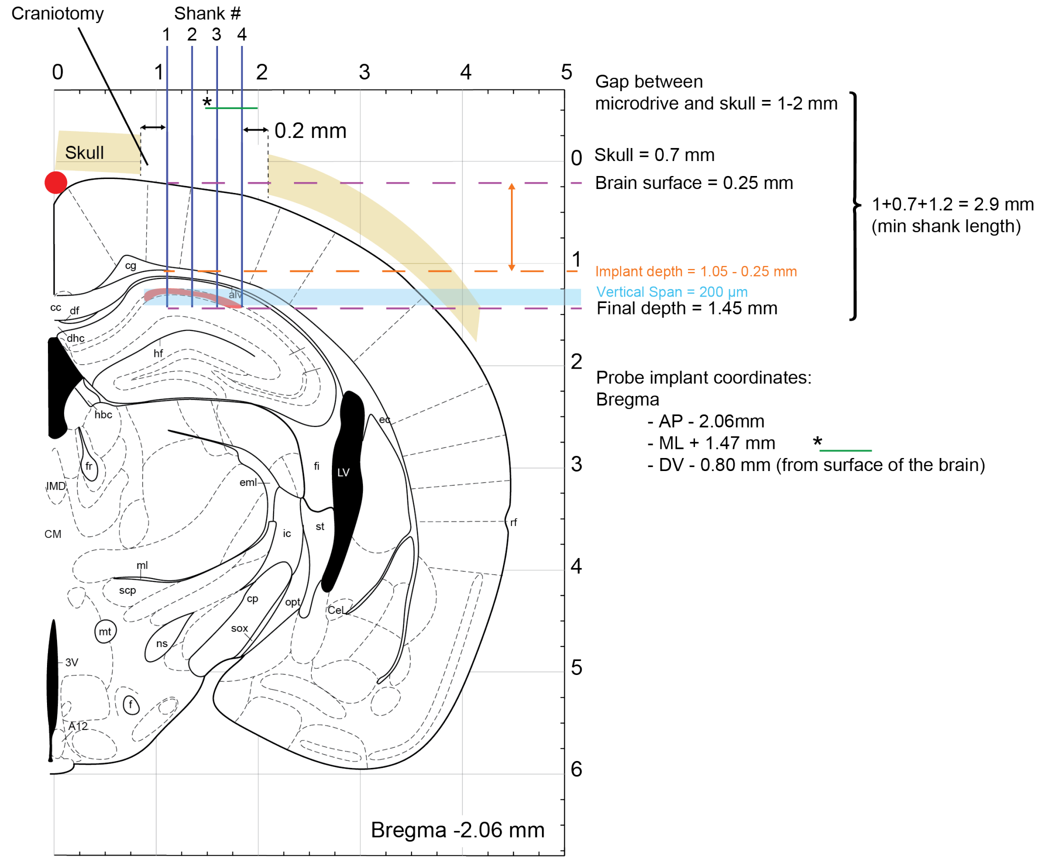
Length of shank:

Number of shank:

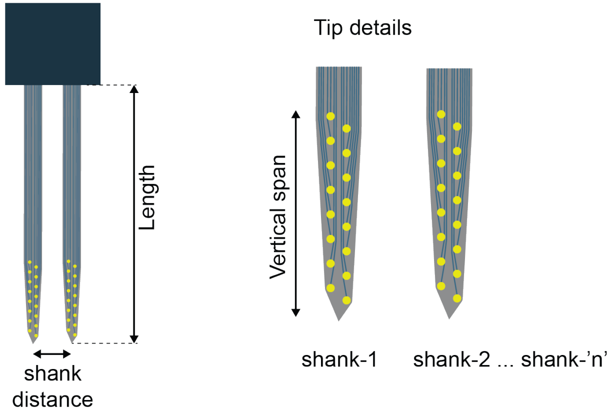
Number of channels:

Vertical span:

Channel layout:



Select a probe that maximize the number of simultaneously recorded single units from the pyramidal cell layer



Length of shank: 5 mm

Number of shank: 2-4

Number of channels: as many as possible

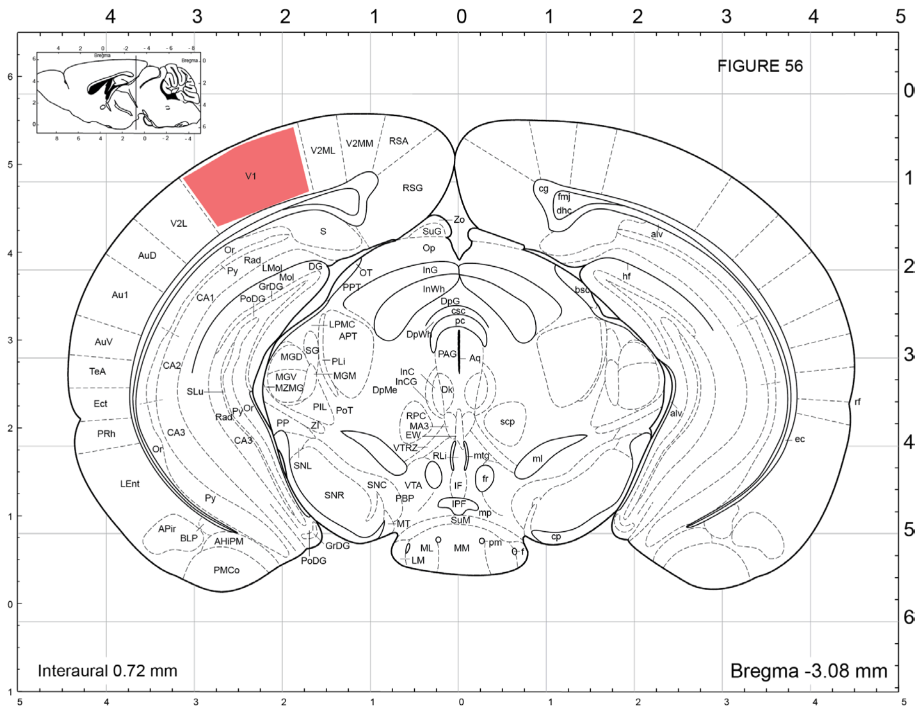
Vertical span: 2-300 µm

Channel layout: poly2 to higher density

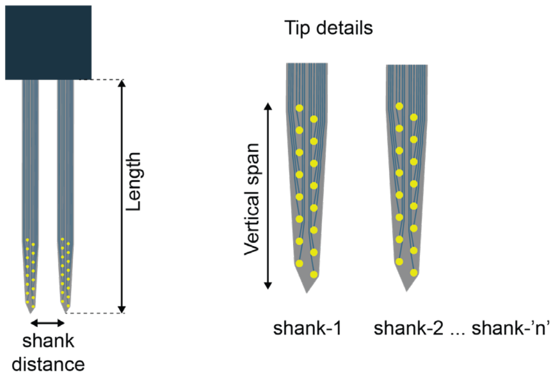
My choice:

- **double sided 128-channel**
- **poly3**
- **128-5 Cambridge Neurotech**

Group-2



You want to record from mouse primary visual cortex and study laminar information processing: how inputs and outputs differ across layers (L2/3, L4, L5, L6).



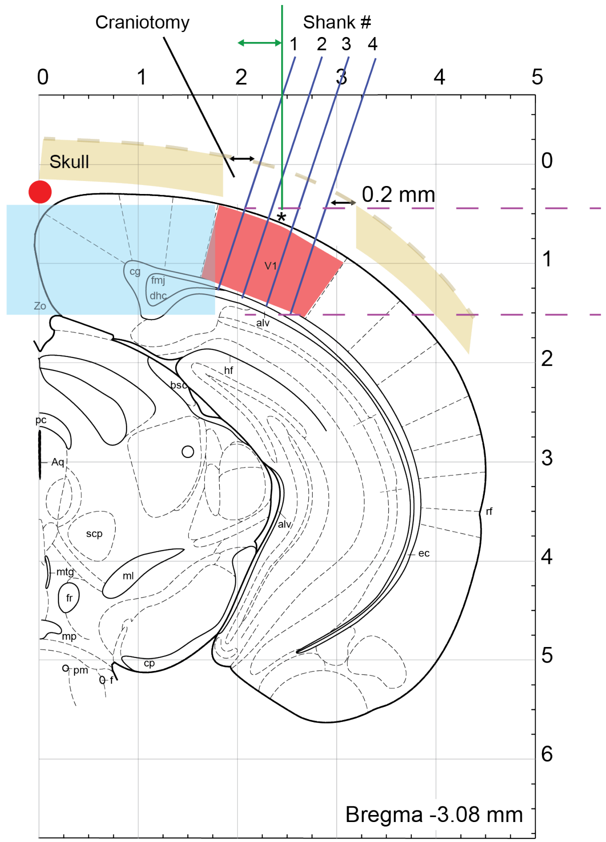
Length of shank:

Number of shank:

Number of channels:

Vertical span:

Channel layout:



Gap between microdrive and skull = 1-2 mm

Skull = 0.7 mm

Brain surface = 0.50 mm

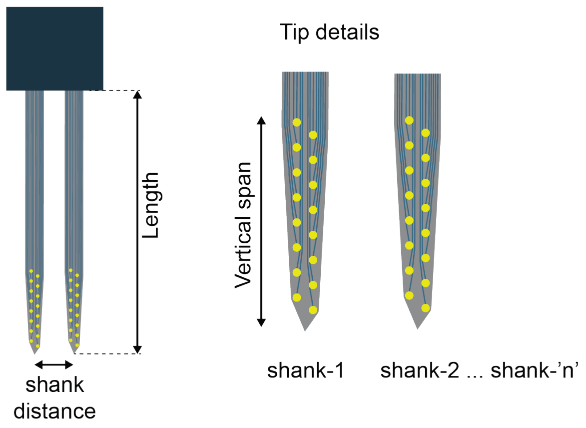
Vertical Span = 1100 µm

Final depth = 1.00 mm

1+0.7+1 = 2.7 mm (min shank length)

Probe implant coordinates:
Bregma
- AP - 3.08 mm
- ML + 2.45 mm *
- DV - 1.00 mm (from surface of the brain)
- 20 degrees from sagittal plane
  - straight down can work, but with this angle your channels will be in the same layers

You want to record from mouse primary visual cortex and study laminar information processing: how inputs and outputs differ across layers (L2/3, L4, L5, L6).



Length of shank: 5 mm

Number of shank: 4 or more

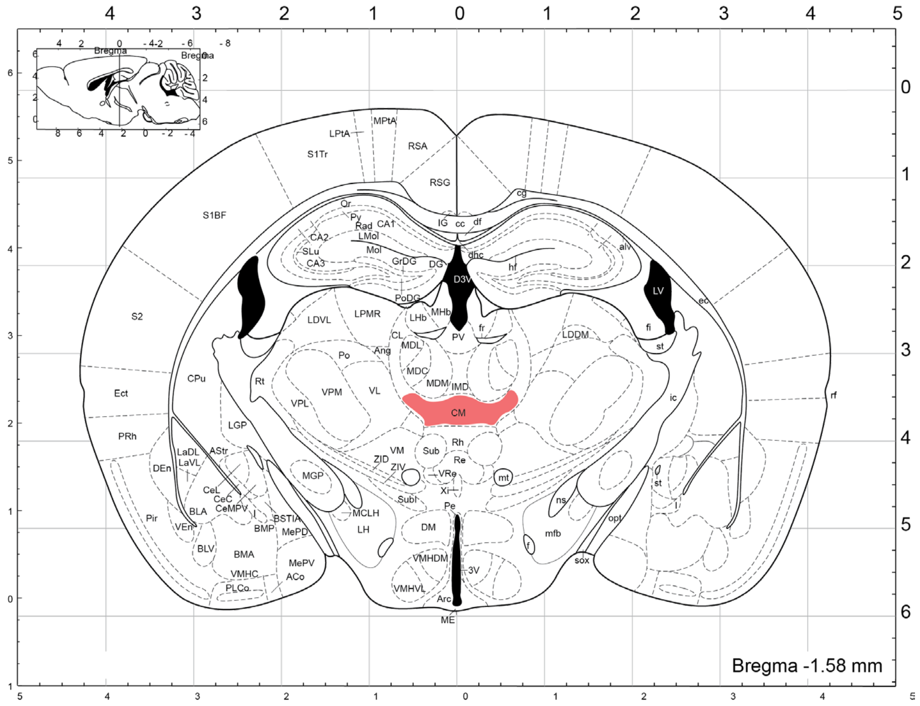
Number of channels: 128 or NP or SiNAPS

Vertical span: 900 µm

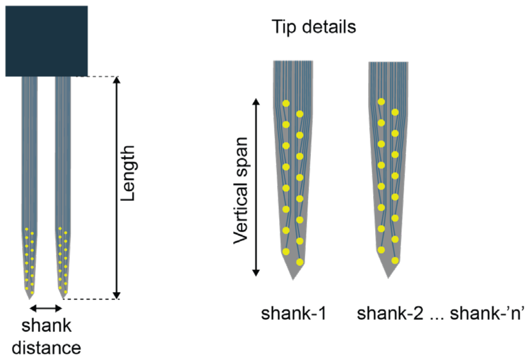
Channel layout: poly2

My choice:
- **NP 2.0**
  - **384 / 4 = 96 channels / shank**
  - **good spatial and unit yield**

Group-3



Select a probe that reaches thalamus and gives high density in the thalamic segment.
Explain how you would align the probe (angle, depth) to cover CM thalamus.



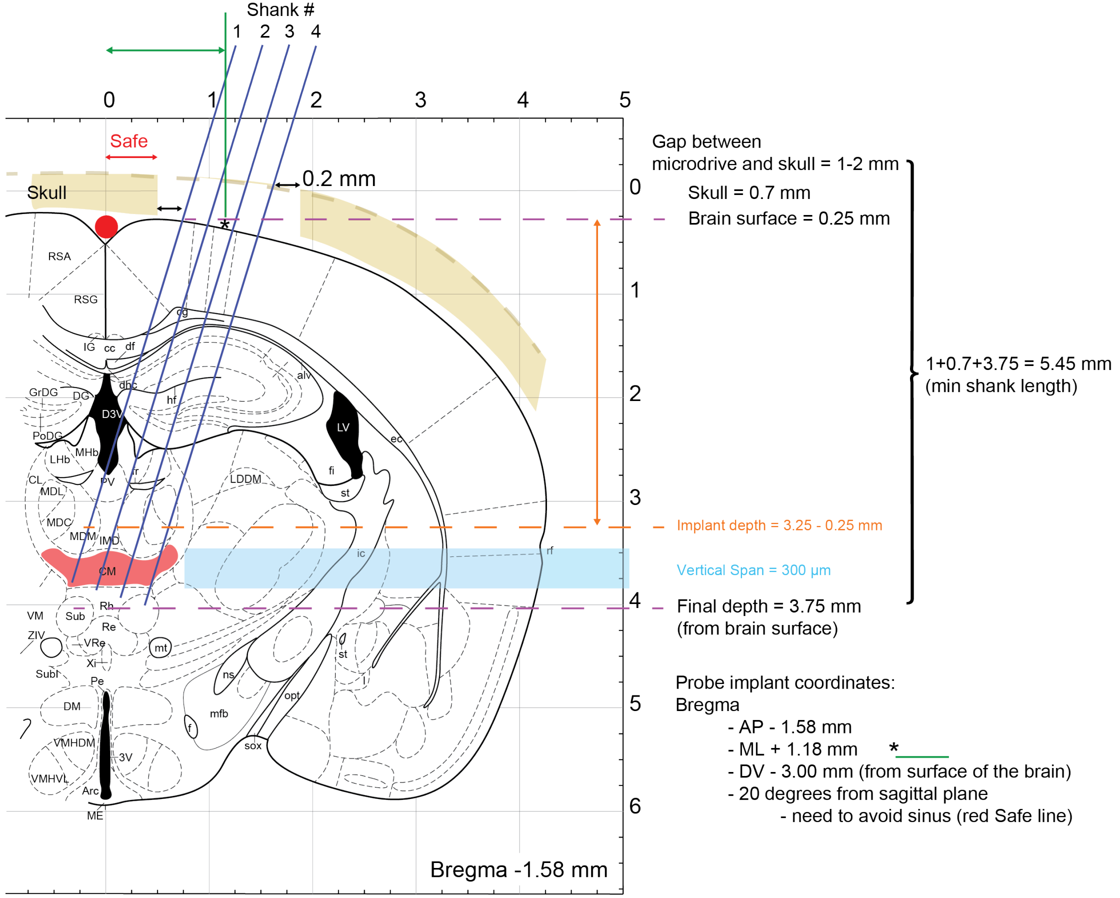
Length of shank:

Number of shank:

Number of channels:

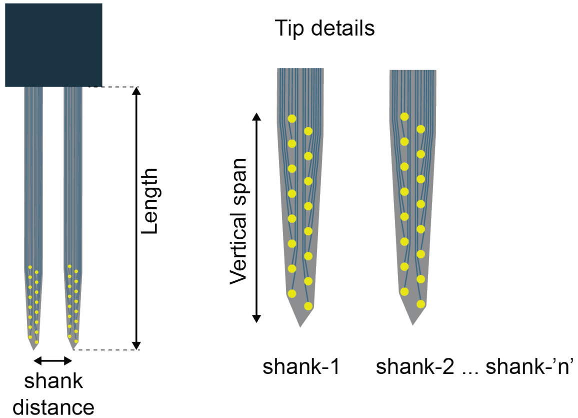
Vertical span:

Channel layout:



Probe implant coordinates:
Bregma
- AP - 1.58 mm
- ML + 1.18 mm *
- DV - 3.00 mm (from surface of the brain)
- 20 degrees from sagittal plane
  - need to avoid sinus (red Safe line)

Select a probe that reaches thalamus and gives high density in the thalamic segment.
Explain how you would align the probe (angle, depth) to cover CM thalamus.



Length of shank: 10 mm

Number of shank: 4

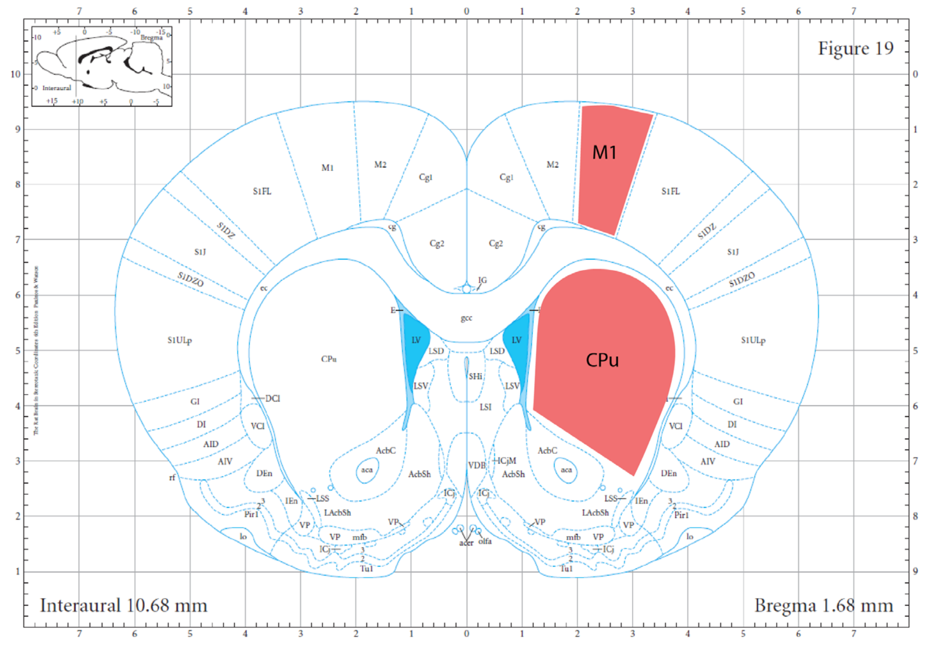
Number of channels: 128

Vertical span: 400 μm

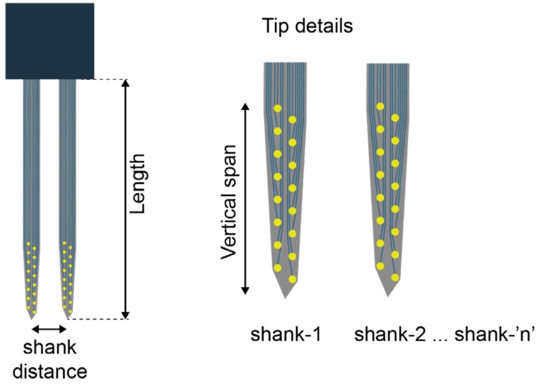
Channel layout: poly2

My choice:
- 128 channel poly 2

Group-4 (RAT)



Select a probe that records from CPu and M1.



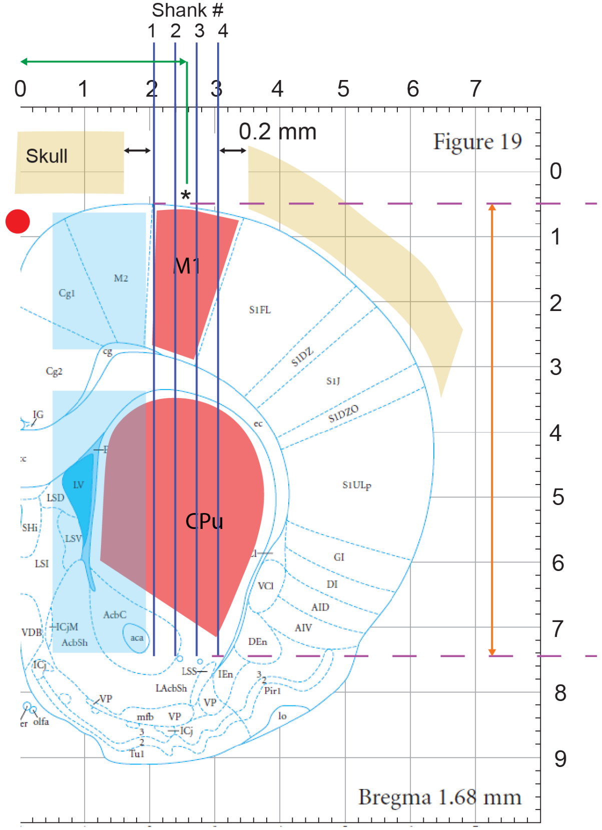
Length of shank:

Number of shank:

Number of channels:

Vertical span:

Channel layout:



Gap between microdrive and skull = 1-2 mm

Skull = 1.5 mm

Brain surface = 0.50 mm

Vertical Span = 2100 μm

1+1.5+6.90 = 9.40 mm (min shank length)

Implant depth = 6.90 mm

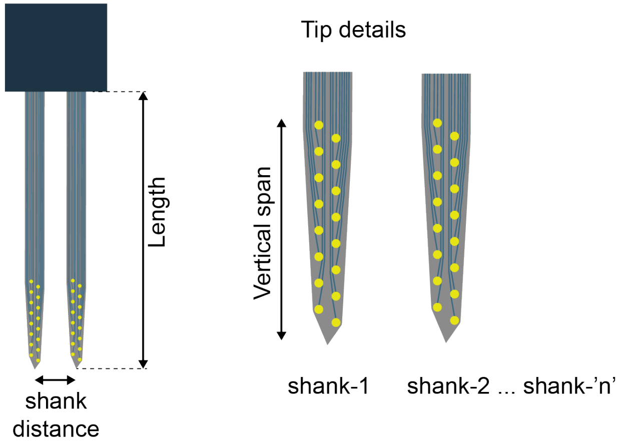
Note that Neuropixels is always implanted at final depth.

Vertical Span = 4000 μm

Final depth = 6.90 mm (from brain surface)

Probe implant coordinates:
Bregma
- AP + 1.68 mm
- ML + 2.60 mm
- DV - 6.90 mm (from surface of the brain)

Select a probe that records from CPu and M1.



Length of shank: 10

Number of shank: 4

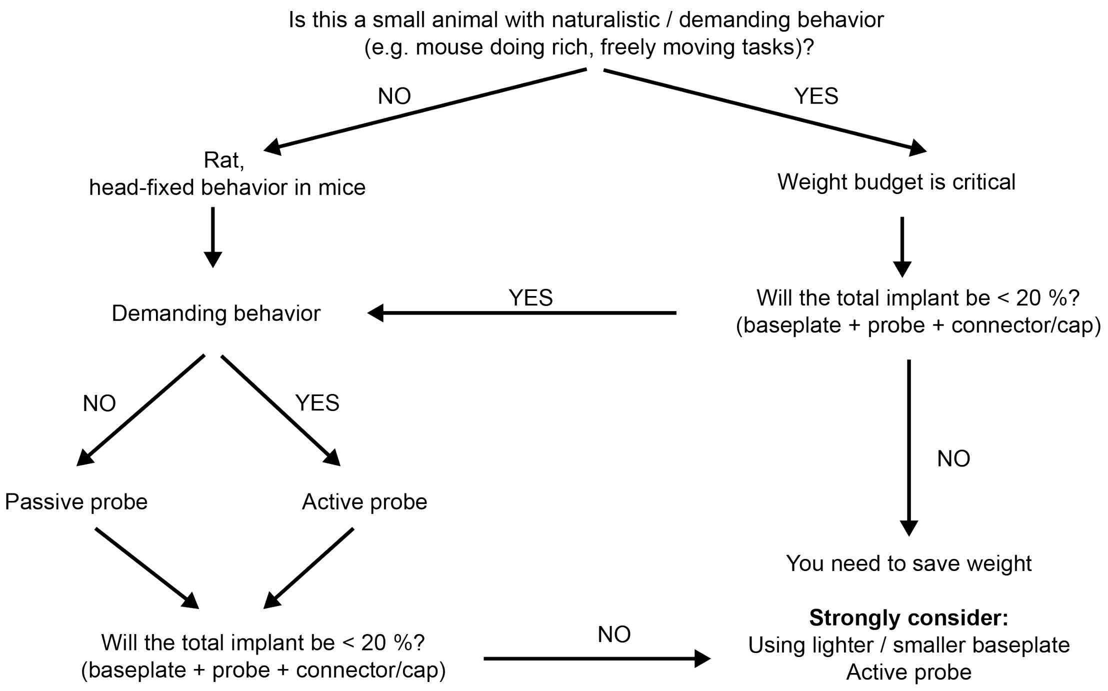
Number of channels: 384

Vertical span: 2100 and 4000

Channel layout: poly2 or linear

My choice:
- **Neuropixels 2.0**

Choosing a silicon probe for surgery



Passive probe setup

The headstage sits higher, center of mass is a bit further from the skull.
Cheaper and modular – probe failures don't destroy the entire active system
Easier to swap probes / reuse headstages

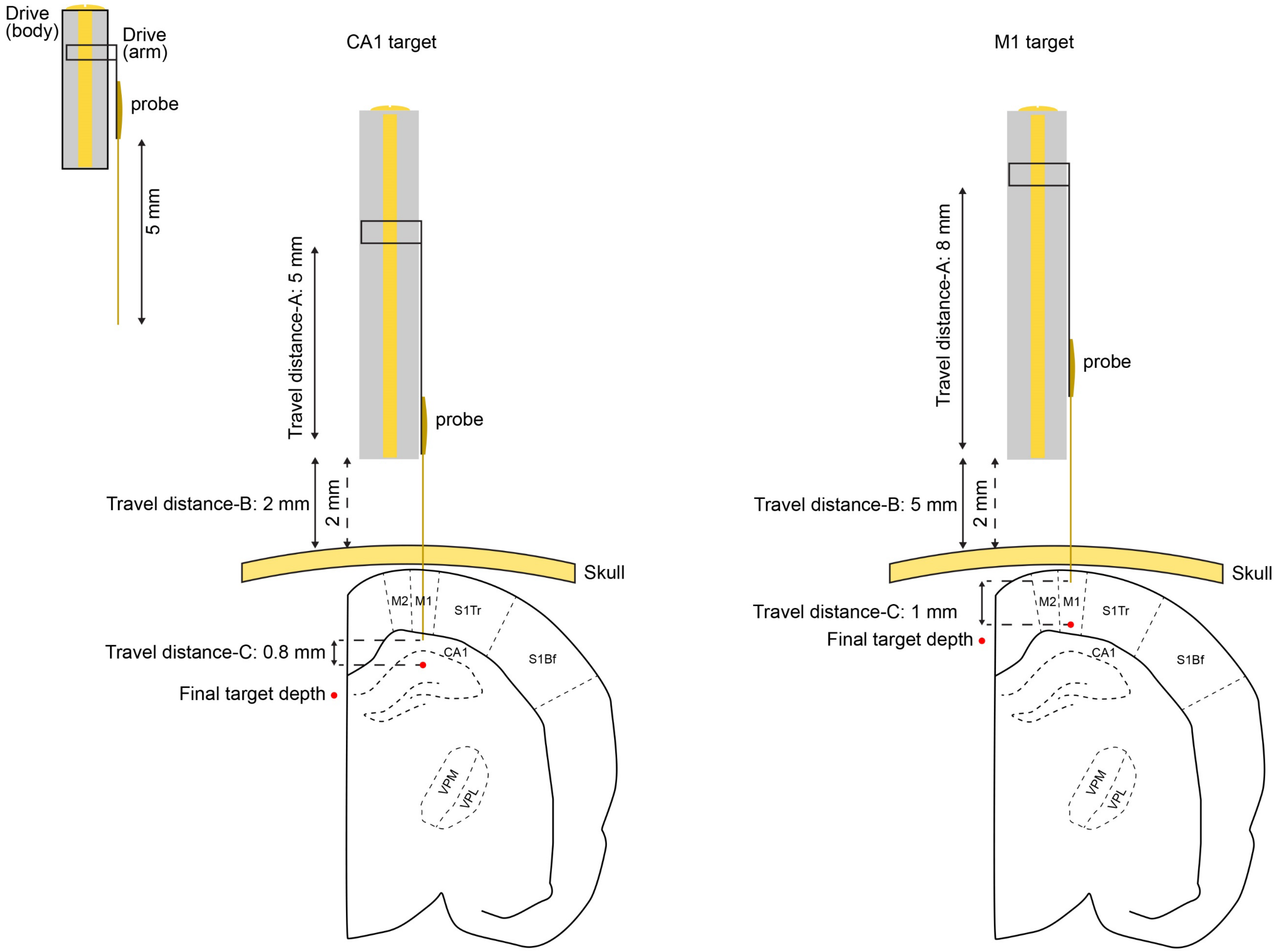
Works well when:
Animal is larger or behaves fine with a bit more weight / torque
You're less constrained by ultra-naturalistic behavior.

Active Probe

The headstage is shorter and the center of mass is closer to the skull.
This reduces torque on the neck and makes behavior more natural.

BUT

If the probe fails, you effectively lose the whole active assembly (probe + ASIC + PCB) – more expensive to sacrifice.
Good when you absolutely don't want implant weight to be the limiting factor for behavior.



Travel distance-A: total travel distance that the arm can move downwards inside the drive before hitting the bottom of the drive.

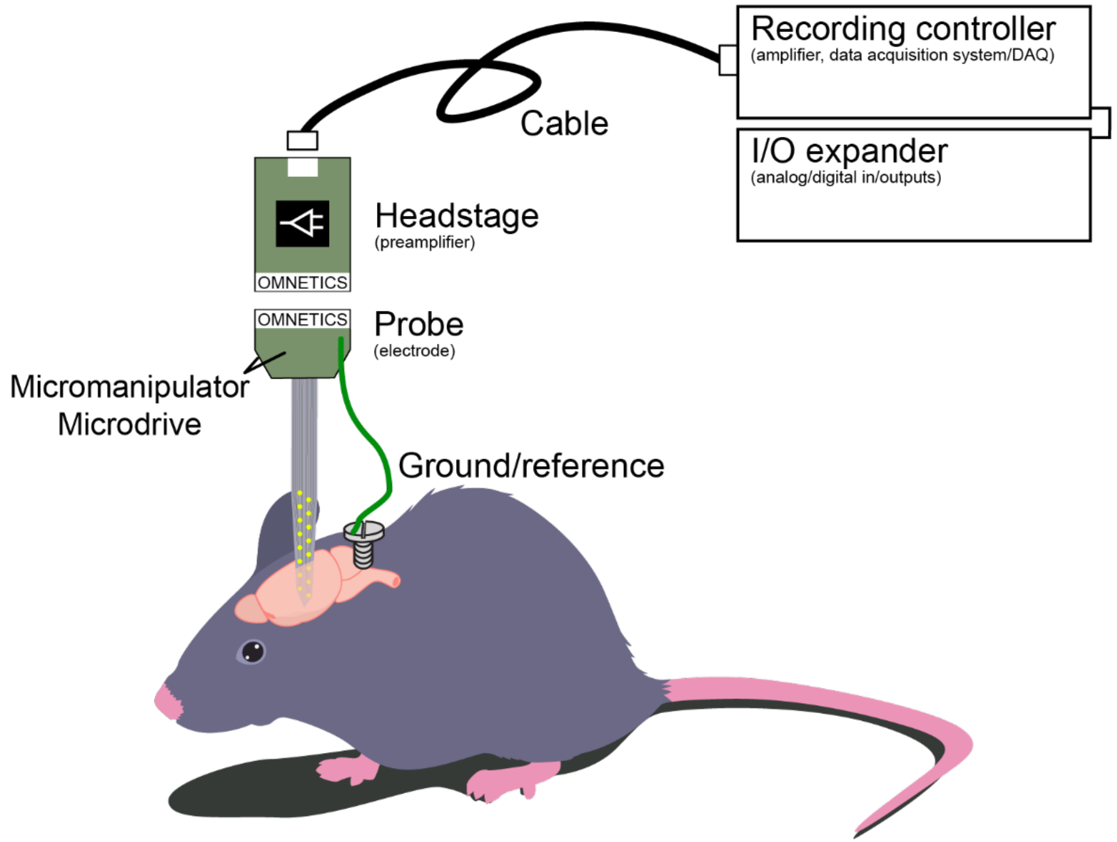
Travel distance-B: total travel distance that the arm can move downwards before hitting the skull.

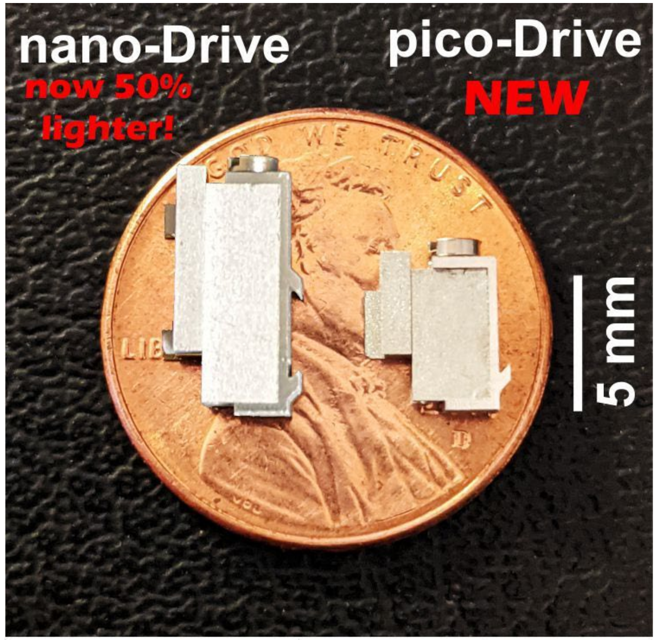
Travel distance-C: total travel distance that we want to move inside the brain (region dependent).

We always want to keep 1-2 mm gap between the skull and drive body. This is the space that we fill up with dental cement to secure the drive. If it is too close, it's difficult to fill it up. If it's too far, then we need ot put a lot of cement, making the whole structure less resistant to mechanical stress (the center of mass is moving upwards).

If we want to keep this distance constant we need to move the arm before surgery if we target different brain regions.

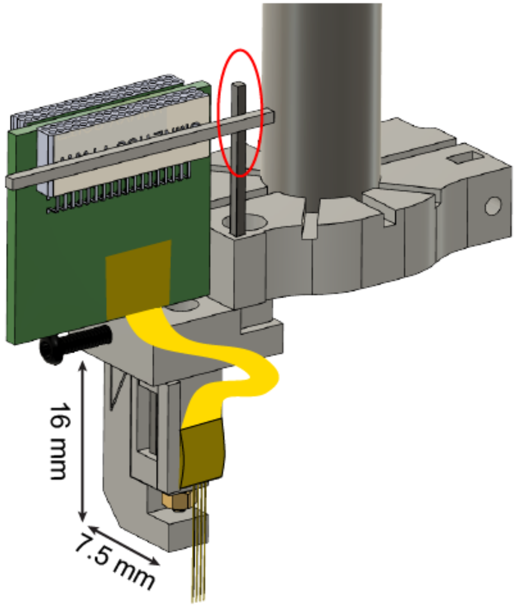
Microdrive, shuttle (just choose one:D)



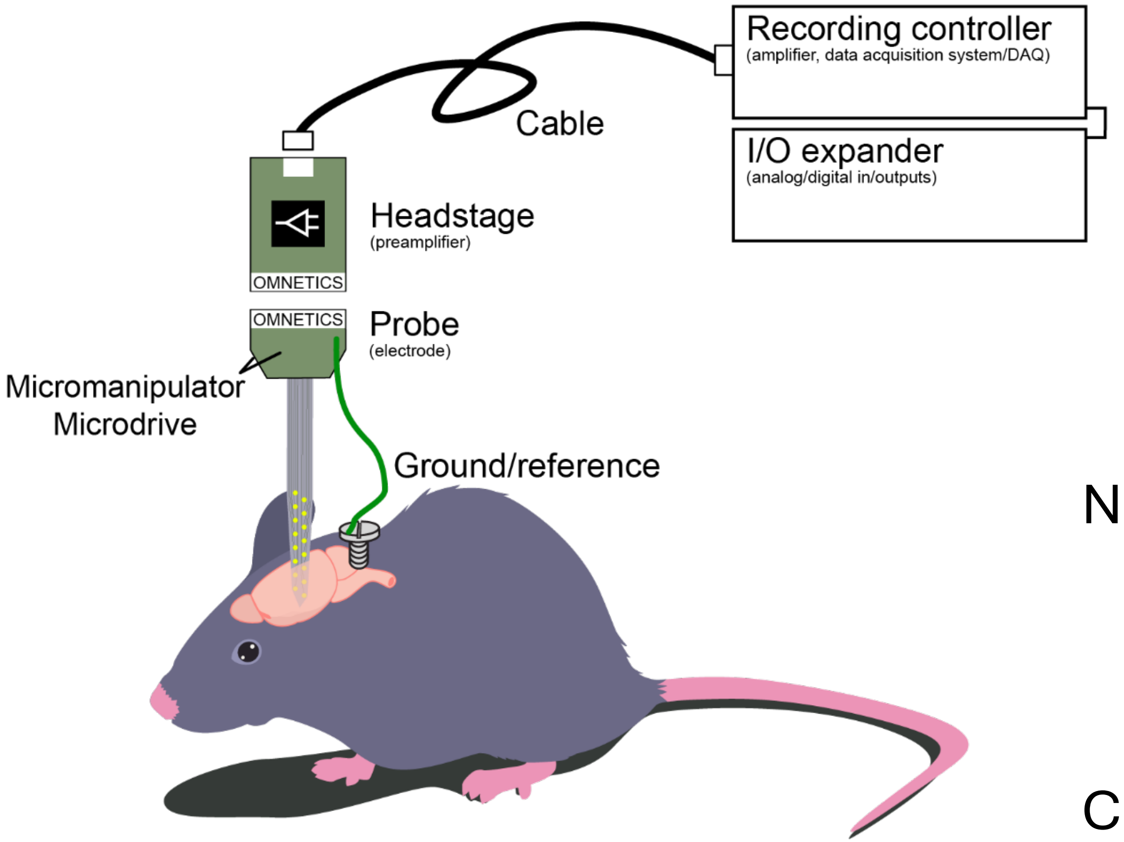


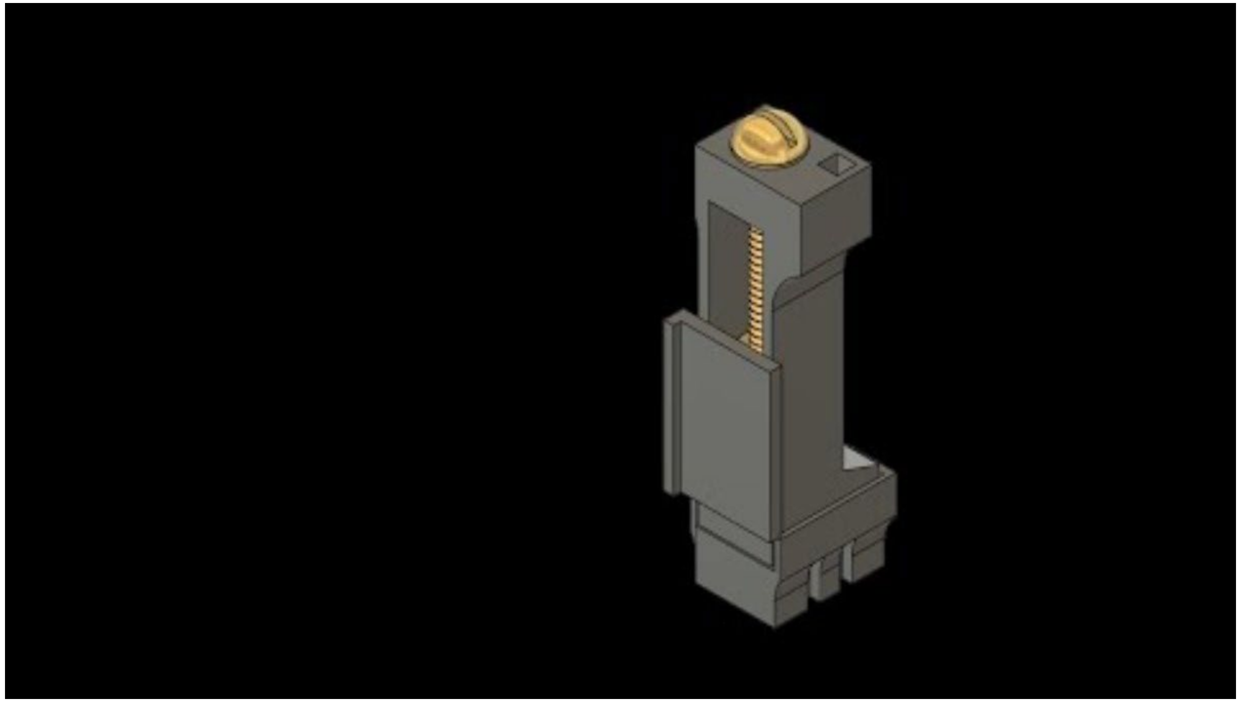
Feature	nano-Drive	pico-Drive
Size (mm)	2 x 5 x 11	2 x 5 x 7
Weight (g)	0.26	0.18
Travel (mm)	7.5	3.5
Resolution (um / turn)	250	250
Probe + Fiber Optic	✓	✓

https://www.cambridgeneurotech.com/nanodrives



Microdrive, shuttle





Play from 1:09 (it takes 1 hour to do what you see here for 7 seconds. Conclusion: try to understand and then adapt new methods.

None of the open-source will work

- Printer tolerance, not updated github, 'secrets' are not shown, etc.
- Contact first author if you have issues, try to visit the lab

Commercial ones are 10x more expensive

- There is quality control, they work as intended

Microdrive vs Neuropixels: unit yield vs spatial coverage (more details)

Microdrive (move probe physically)

- Implant ~2-300 μm above target; advance slowly (e.g., ¼–⅛ turns of ~280 μm/turn)
- Advance → **wait hours** → check signals (avoid tissue dimpling/buckling)
- **Pro:** entering into fresh tissue → **highest unit yield**, new cells every few days
- **Con:** slow, mechanical complexity, limited simultaneous spatial coverage

Neuropixels (move channels)

- Implant to span target; after recovery **map anatomy**, then pick channels along shank
- **Pro:** huge spatial coverage, multi-region simultaneity, great for circuit timing
- **Con: unit loss from day 1** is common; chronic yield usually < microdrive

Take-home: *unit yield vs spatial coverage — pick your poison.*

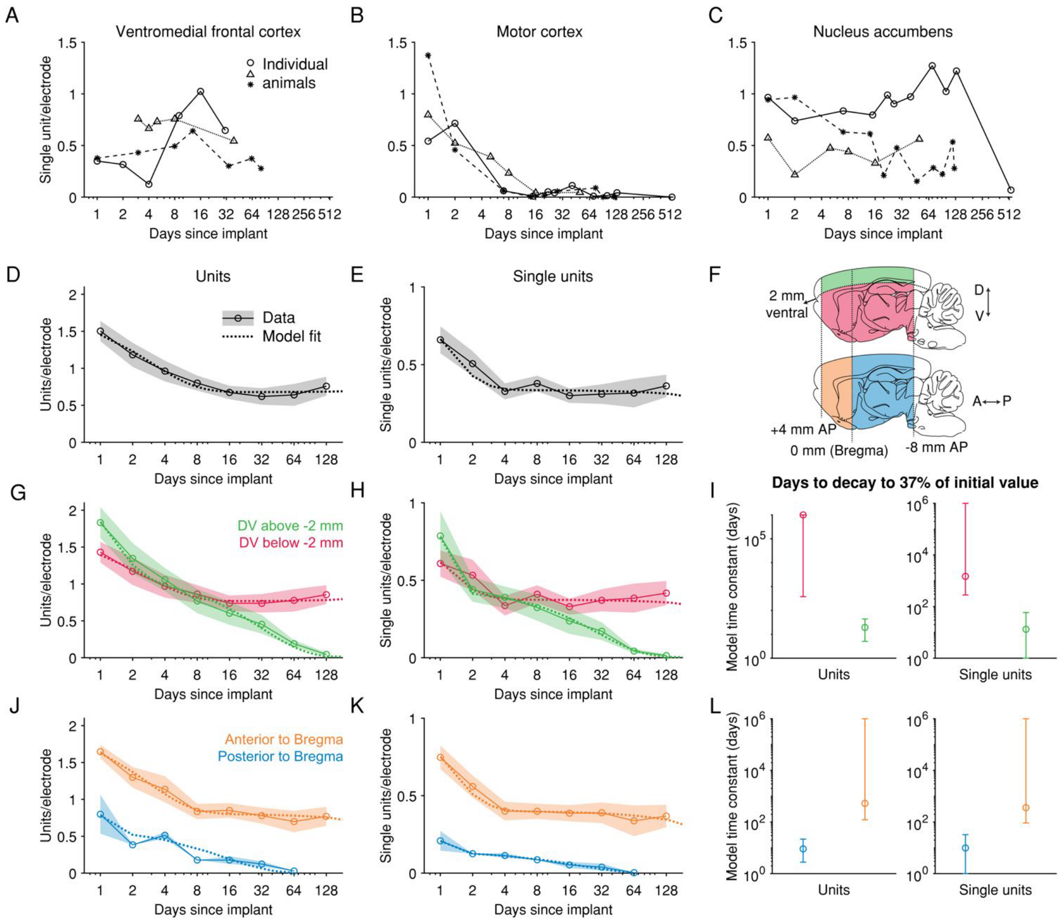
Microdrives

- https://elifesciences.org/articles/65859 (videos are available)
- https://bio-protocol.org/en/bpdetail?id=4137&type=0
- https://www.jove.com/v/3568/large-scale-recording-neurons-movable-silicon-probes-behaving
- https://www.nature.com/articles/s41598-017-03340-5
- https://www.sciencedirect.com/science/article/pii/S0165027006002883
- https://www.cambridgeneurotech.com/nanodrives
- https://www.3dneuro.com/products/r2drive/
- https://www.neuronexus.com/product_documentation/microdrive/

Neuropixels holders

- https://www.biorxiv.org/content/10.1101/2023.12.22.572441v2
- https://elifesciences.org/articles/98522
- https://www.nature.com/articles/s41596-021-00539-9
- https://www.protocols.io/view/chronic-recoverable-neuropixels-in-mice-e6nvwjo87lmk/v2
- https://elifesciences.org/articles/47188
- https://elifesciences.org/articles/59716

Losing cells over time with Neuropixels

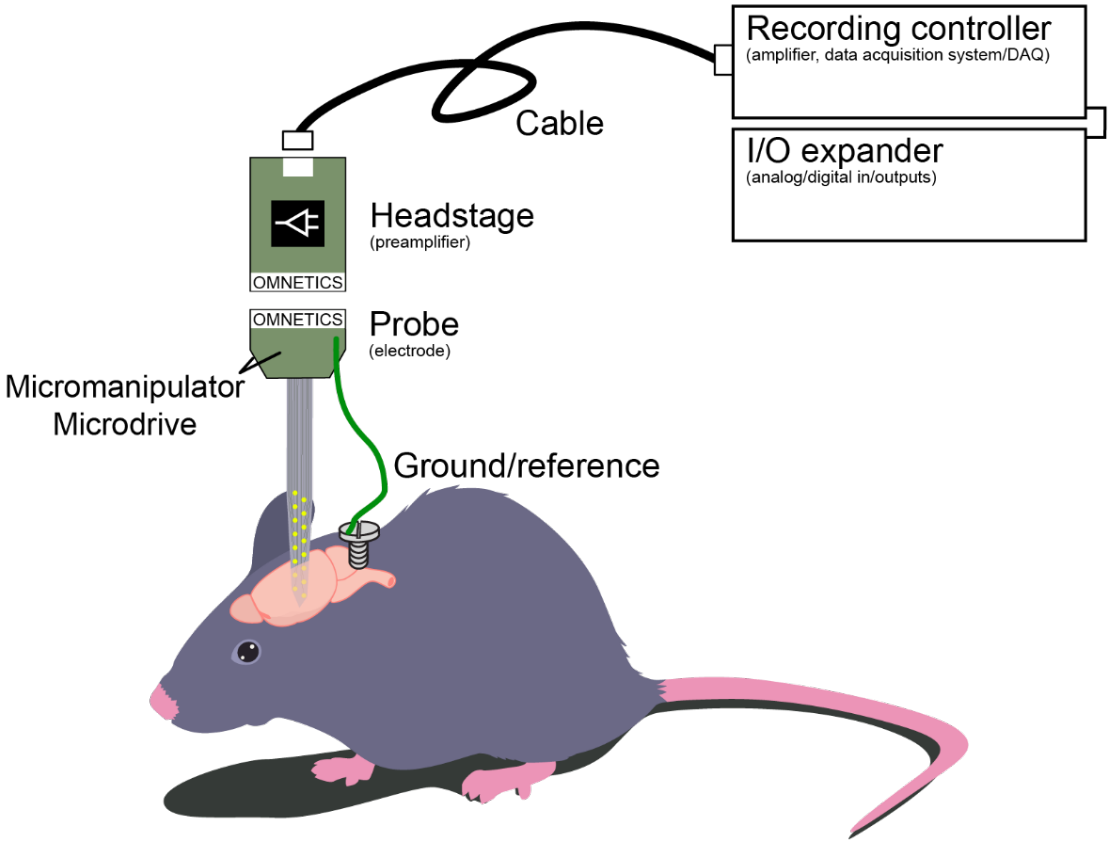


- https://elifesciences.org/articles/59716
- Note that single-unit yield is getting worse over days.
- This happens with all silicon probes if you don't move them, hence the Microdrive solution.

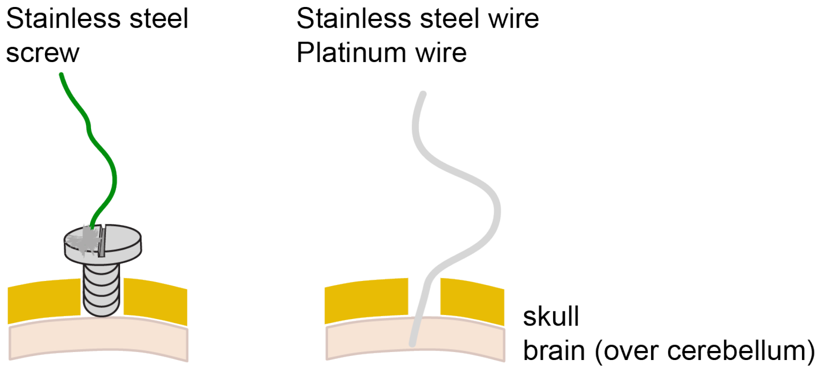
Ground and reference

Ask ten people and you'll get ten different answers.

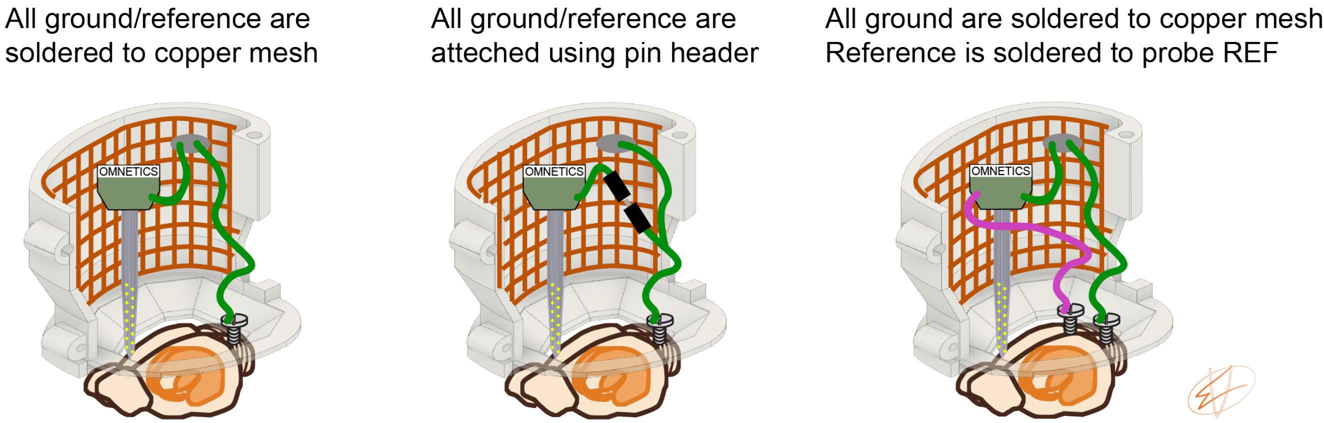
https://www.youtube.com/watch?v=_XFqGOXRdm4



Ground and reference choice



Ground and reference connection

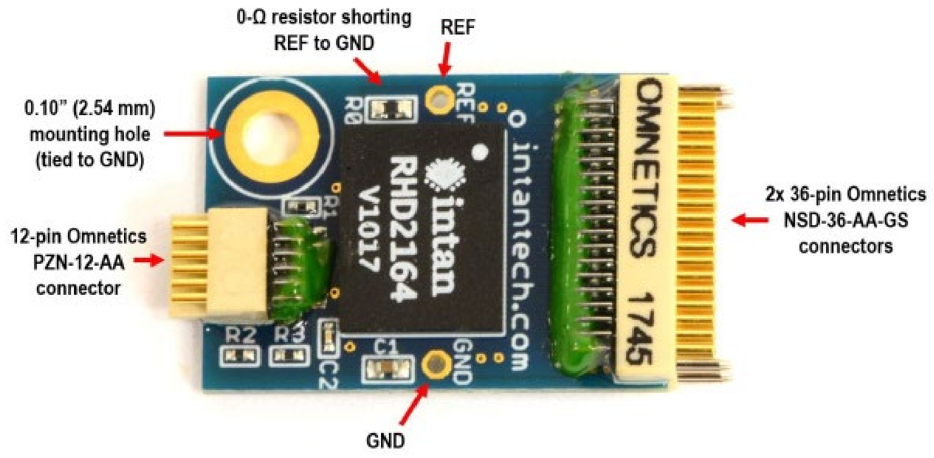


Ground and reference

“A ground connection is needed to establish a common DC voltage between the tissue and the amplifiers. In the old days where the amplifiers were across the room from the electrodes with long wires in between, a separate reference wire was useful because it would pick up (roughly) the same interference as the signal electrode wires on the long cable going to the amps, and then the differential amps would cancel this interference.

But now that we have tiny headstages with amp mounted a few mm away from the electrodes, interference isn't a big problem, so it works well just to tie the reference to ground.”

Reid Harrison, Ph.D.
Intan Technologies

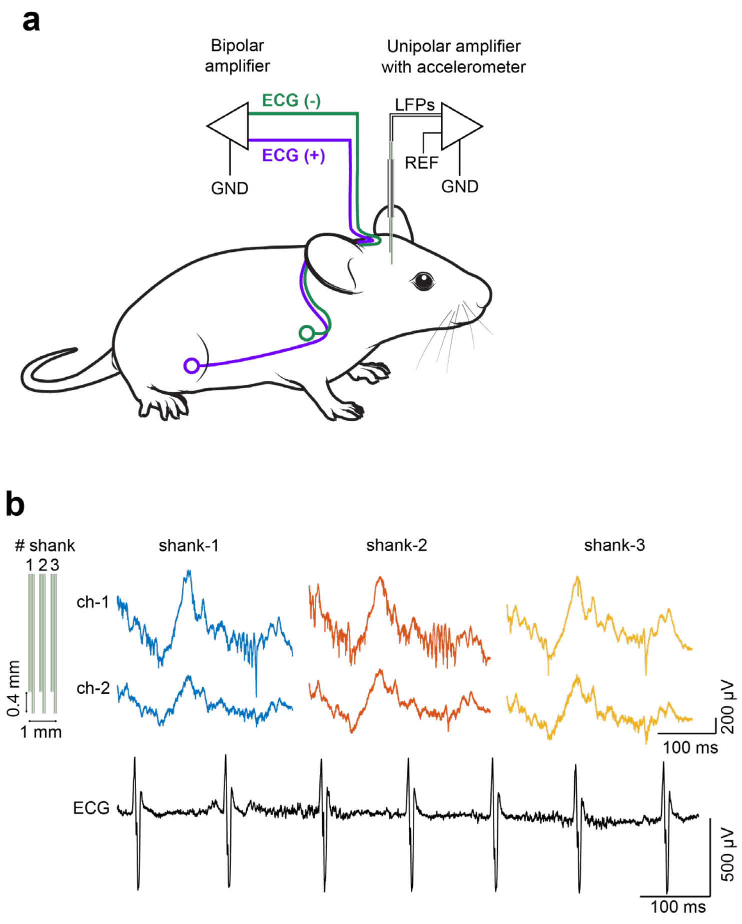


Read MORE on ground and reference

[Grounding and Referencing for Electrophysiology Recording Systems]

Ground and reference: Keep it separate if

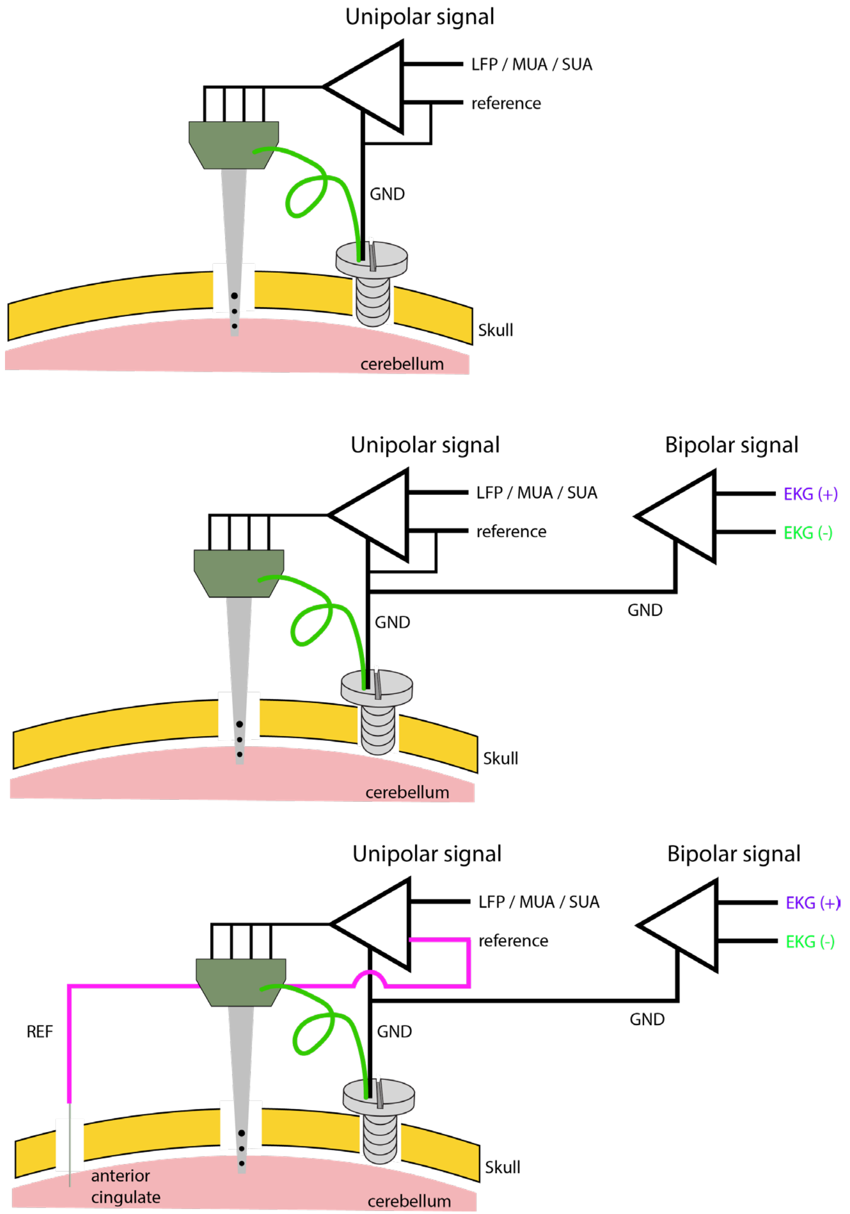
If you record other biopotentials
- EMG
- ECG



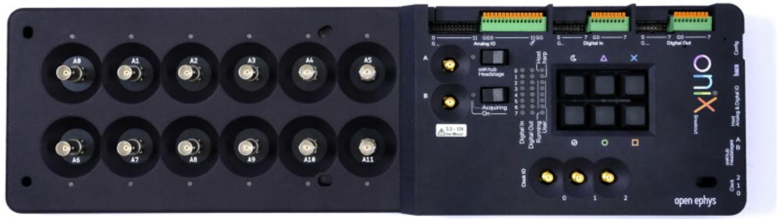
Recommended
Ephys only config

Shared Gnd/Ref will
introduce noise

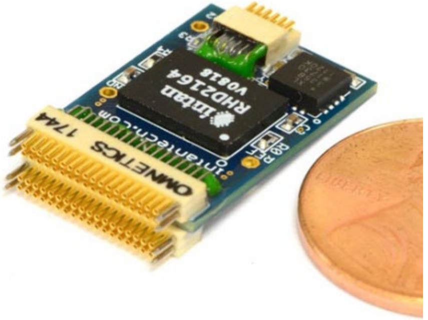
Separate Gnd/Ref



Headstage, cable, recording controller



OpenEphys



Intan Tech

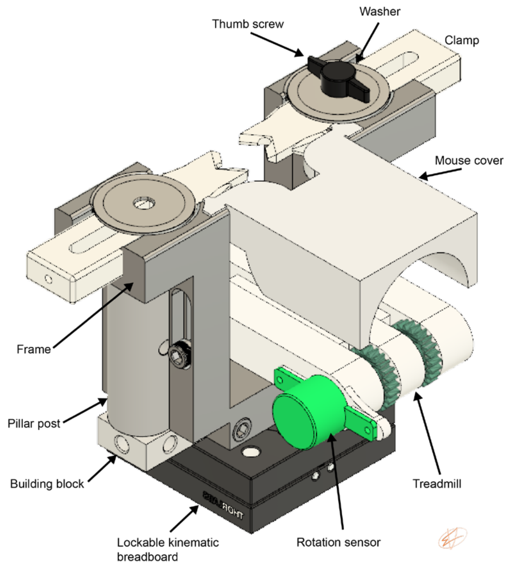
Headstage, cable, recording controller

OpenEphys	20k
IMEC for Neuropixels only	10k
Plexon	~50k
Tucker Davis	~50k
Spike Gadgets	~20k
Intan Tech	20k

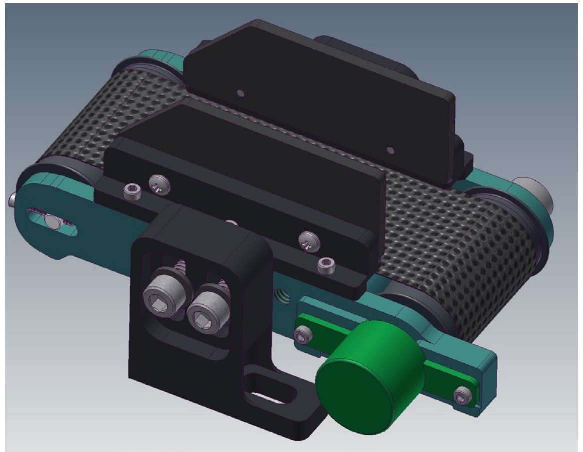
And some more, again just choose one

Head-fixed setup

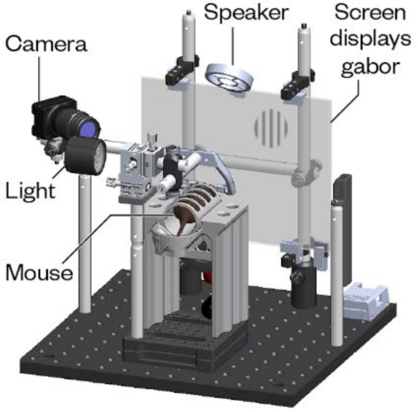
Rinberg's lab (NYU)



Janelia low-friction

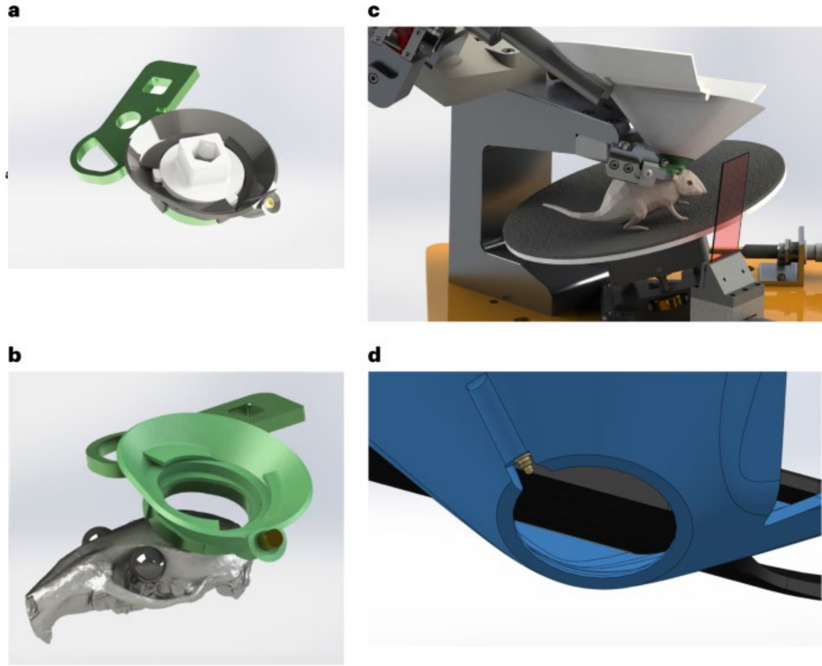


IBL



Allen Institute

Many more...



Advantages

High throughput
'Single setup' → can be months
Virtual reality
Every day a 'success'

Great for

Preliminary data collection
Circuit mapping
Pupil measurement
Controlled stimuli delivery
(odor, tactile, visual)

Disadvantages

No sleep (animals can be trained to 'sleep')
Limited time to record

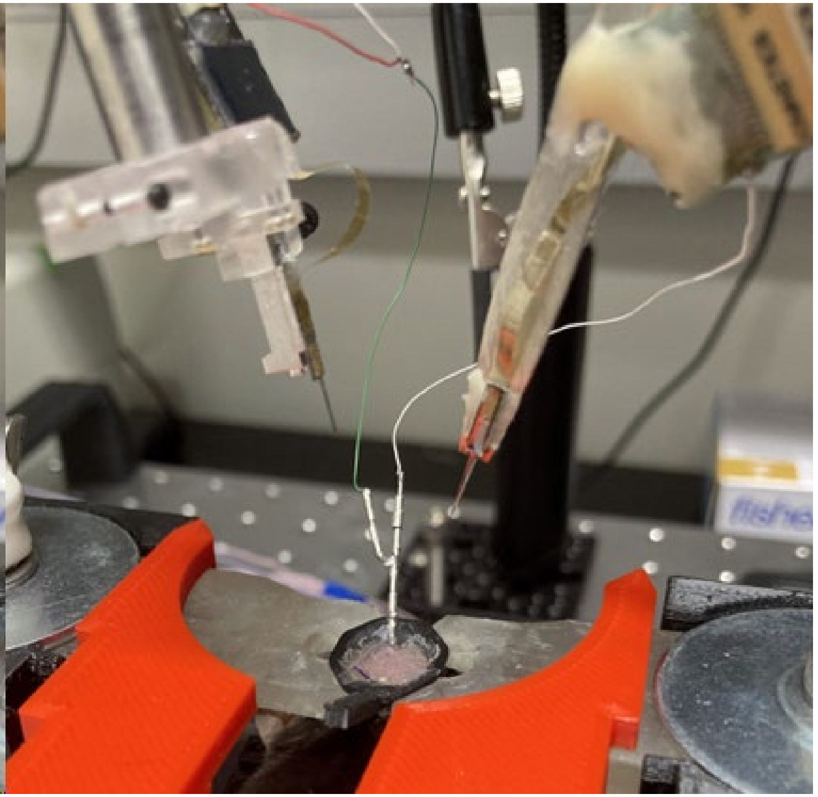
Head-fixed setups (most popular ones)

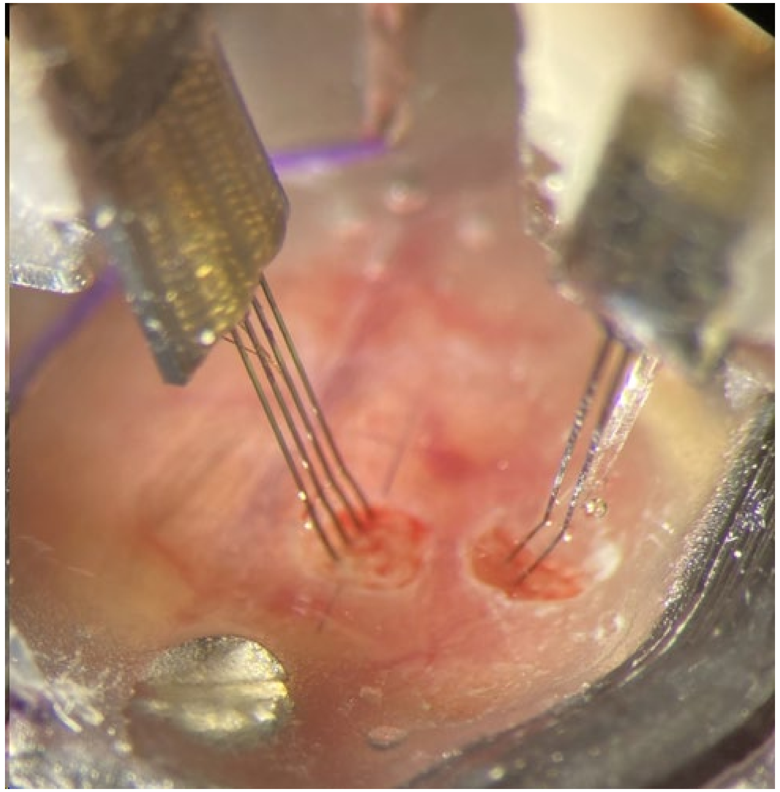
- https://www.janelia.org/open-science/low-friction-rodent-driven-belt-treadmill
- https://elifesciences.org/articles/63711
- https://github.com/misiVoroslakos/3D_printed_designs/tree/main/Treadmill_Rinberg
- https://www.sciencedirect.com/science/article/pii/S0165027020303459

- https://pmc.ncbi.nlm.nih.gov/articles/PMC7617528/

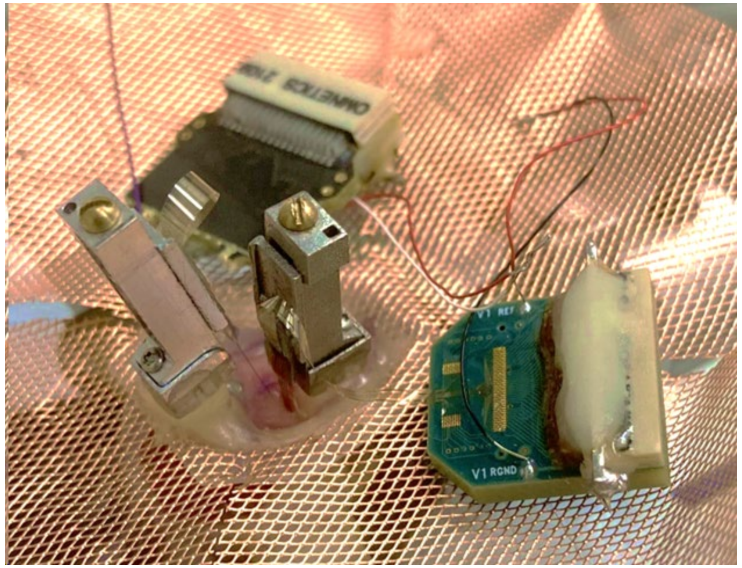
Head-fixed setup

Preliminary data
Setting up surgery coordinates

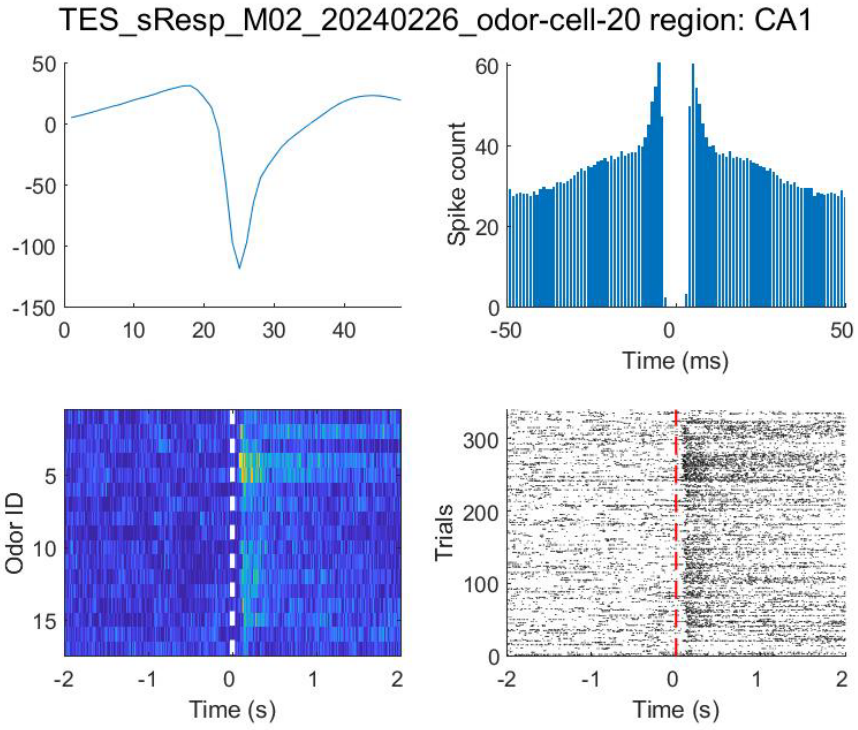




Chronic surgery based on
head-fixed results



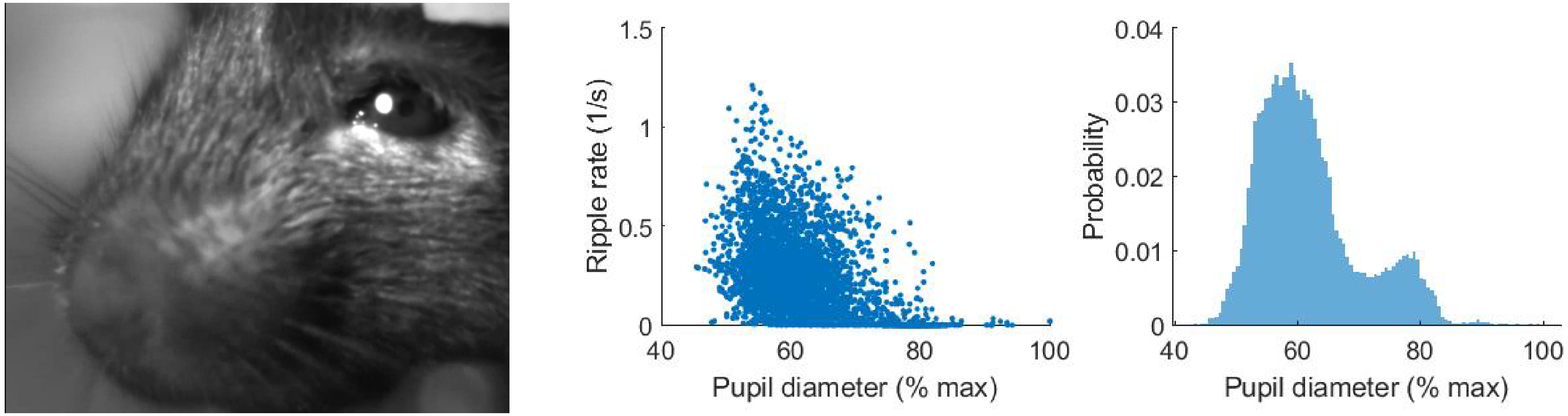
Head-fixed setup



Allows

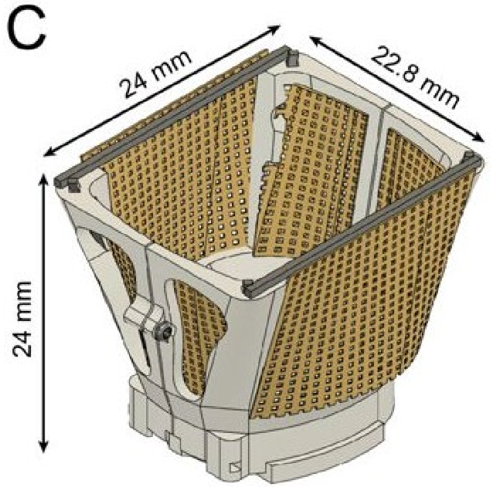
- Controlled delivery of stimuli
  - Whisker, odor, tactile

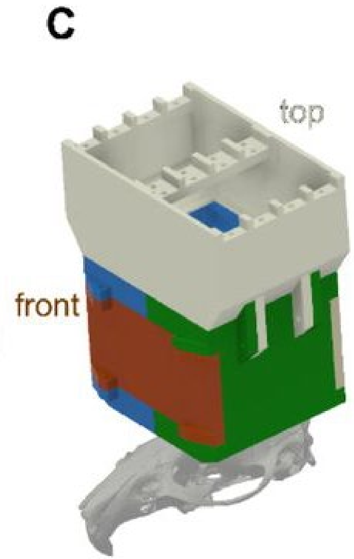
- Pupil measurement

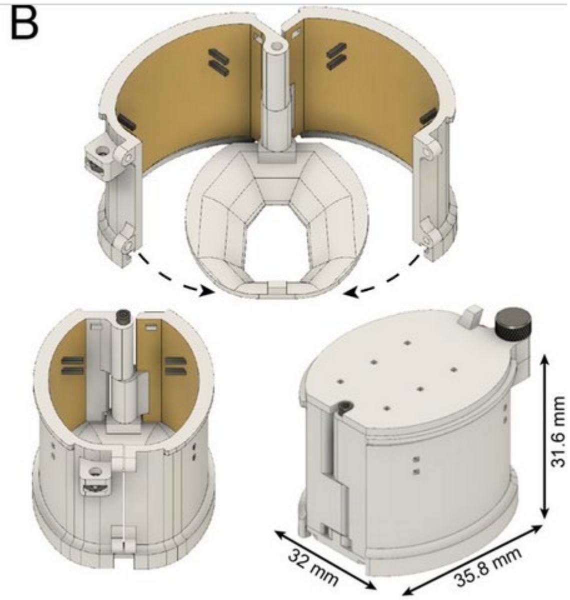


Freely moving experiments

Protection of implant

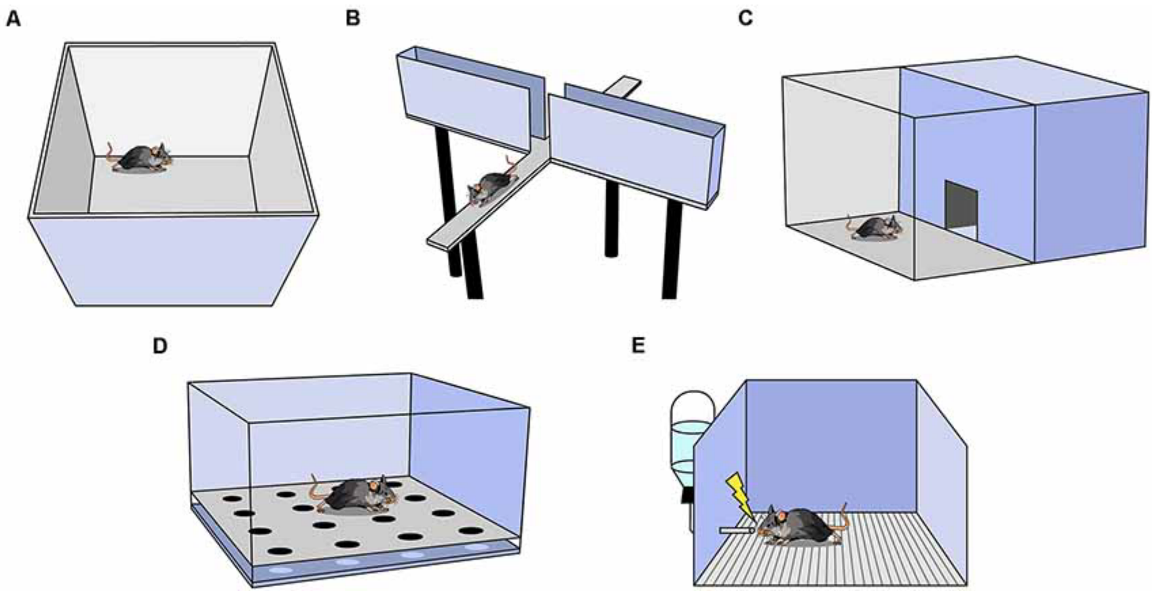


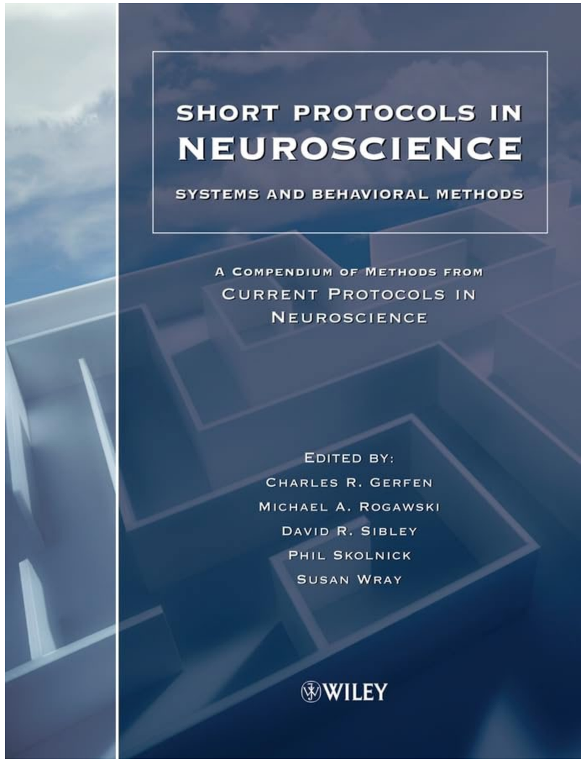




https://www.youtube.com/watch?v=SgM9TPhbf_Y
https://www.youtube.com/watch?v=_MYBLJf-178
https://www.youtube.com/watch?v=14ev8umGzIg

Freely moving experiments





https://neuroscience.stanford.edu/shared-resources/behavioral-functional-lab/services-and-rates/behavioral-models

https://www.frontiersin.org/journals/behavioral-neuroscience/articles/10.3389/fnbeh.2020.00145/full

Freely moving experiments

Again, ‘just’ choose one and adapt it…

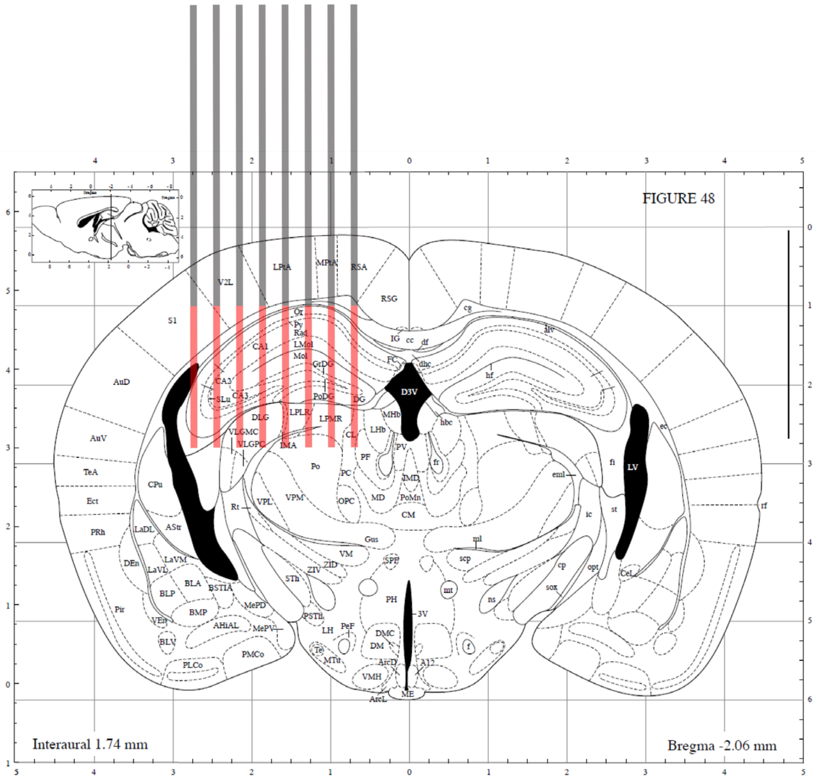
Practice on plastic skull

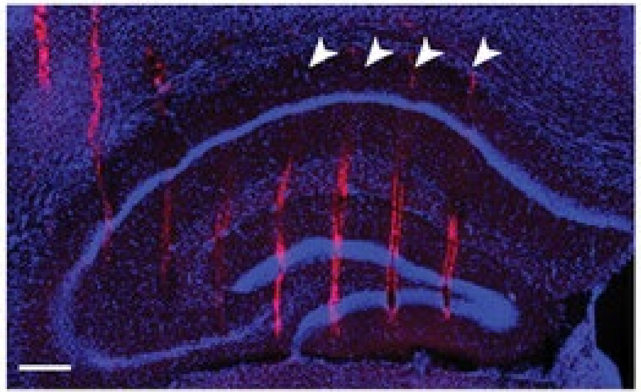
https://github.com/buzsakilab/3d_print_designs/tree/master/Rodent_models

Practice surgery

Plan surgery
Apply DiI on probe
Adjust if necessary

Bregma - 2 mm
first shank is ML+0.75 mm, center is 1.75mm
2.75 mm from brain surface





Neuroscience

An adaptable, reusable, and light implant for chronic Neuropixels probes

Célian Bimbard, Flóra Takács, Joana A Catarino, Julie MJ Fabre, Sukriti Gupta, Stephen C Lenzi, Maxwell D Melin, Nathanael O'Neill, Ivana Orsolic, Magdalena Robacha, James S Street, José Teixeira, Simon Townsend, Enny H van Beest, Arthur M Zhang, Anne K Churchland, Chunyu A Duan, Kenneth D Harris, Dimitri M Kullmann, Gabriele Lignani, Zachary F Mainen, Troy W Margrie, Nathalie L Rochefort, Andrew M Wikenheiser, Matteo Carandini, Philip Coen ... show less

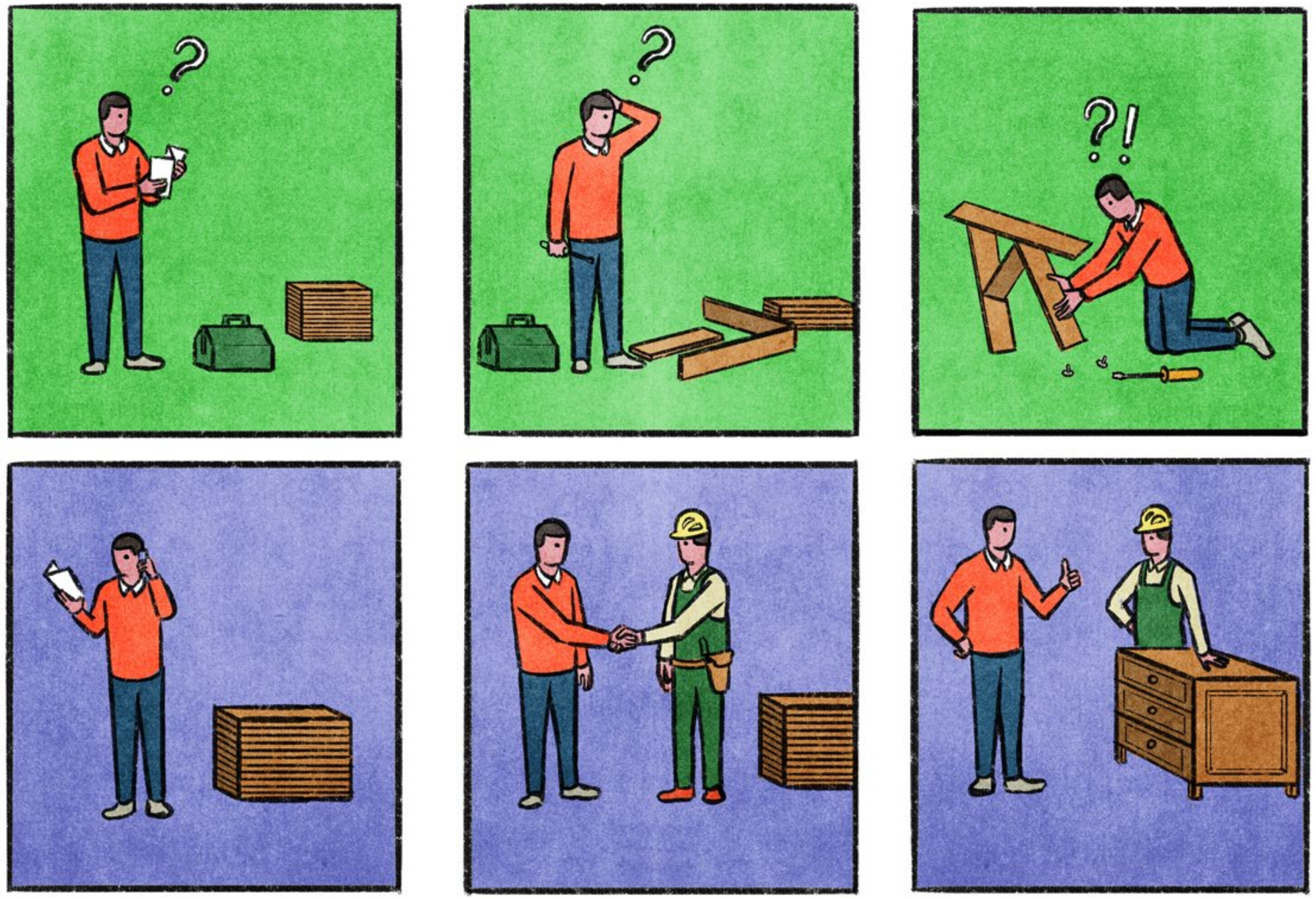
Lab	Probes (number & type)	Sharpening	Shank alignment	Grounding prep	Headstage
Carandini–Harris	1–2 × NP 2.0α, 4-shank	Yes (Narishige EG-45)	By eye	Ag wire shorting GND–REF, connected to headplate	Removable
Churchland	2 × NP 1.0	Yes (Narishige EG-45)	Checked in holder; Kapton on back; re-checked straightness	Ag wire shorting GND–REF, soldered to socket (bone-screw ground); separate screw socket for later detachment	Removable
Duan	1 × NP 1.0	Yes (Narishige EG-45)	By eye	Ag wire shorting GND–REF, soldered to grounding wire terminating in gold socket	"Permanent" epoxy block; recoverable via 3D-printed holder
Kullman/Lignani	1 × NP 1.0	Yes (Narishige EG-45)	By eye	Ag wire shorting GND–REF, soldered to additional Ag wire with male Mill-Max pin (to skull screw)	Removable
Mainen	1 × NP 1.0	Yes (Narishige EG-45)	Using graph paper	Ag wire shorting GND–REF	Removable
Margrie	1 × NP 2.0α or 2.0, 4-shank	Unsharpened	By eye	Ag wire shorting GND–REF	Removable
Rochefort	1 × NP 1.0	Unsharpened	By eye	Ag wire shorting GND–REF	Removable; docking surface cut at 44° for plate compatibility
Wikenheiser	1 × NP 1.0	Unsharpened	Using graph paper	Ag wire shorting GND–REF; additional Ag wire to male gold pin; matching skull-screw ground with female pin	Removable (SpikeGadgets); probe exit coated with silicone gel

An adaptable, reusable, and light implant for chronic Neuropixels probes

Célian Bimbard, Flóra Takács, Joana A Catarino, Julie MJ Fabre, Sukriti Gupta, Stephen C Lenzi, Maxwell D Melin, Nathanael O'Neill, Ivana Orsolic, Magdalena Robacha, James S Street, José Teixeira, Simon Townsend, Enny H van Beest, Arthur M Zhang, Anne K Churchland, Chunyu A Duan, Kenneth D Harris, Dimitri M Kullmann, Gabriele Lignani, Zachary F Mainen, Troy W Margrie, Nathalie L Rochefort, Andrew M Wikenheiser, Matteo Carandini, Philip Coen ... show less

Lab	Species / strain	Prep surgery timing	Skull / headplate prep (very high level)	Craniotomy (method, size)	Insertion speed & manipulator	Implant fixation	Analgesia / other treatment (summary)
Carandini–Harris	Adult M/F C57BL/6J mice	Separate day (headplate)	Ti headplate; skull cleared; VetBond + multiple layers of NOA81, then removed at implant; Super-Bond plate	Biopsy punch or drill; Dural-Gel cover (1–1.5 mm)	3–5 µm/s (Sensapex)	RelyX Unicem 2, then Super-Bond; Kwik-Sil in one case	Isoflurane; carprofen + dexamethasone peri-op; meloxicam/carprofen ×3 d
Churchland	Adult male C57BL/6J mice	Same session	Skull cleared; VetBond edges; headbar glued (Zap-a-gap); skull + headbar covered with Metabond	Drill; Dural-Gel cover	5 µm/s (Neurostar)	Kwik-Sil + UV cement (Wave A1), then Metabond	Isoflurane; meloxicam + enrofloxacin peri-op and ×3 d
Duan	Adult female C57BL/6J mice	Same session	Skull exposed, aligned; dental-cement well around craniotomy; skull covered with Super-Bond; cerebellar ground pin	Drill; Dural-Gel in ~1 mm well	10–20 µm/s for first 100–200 µm, then 3–5 µm/s (Scientifica S-IVM Mini)	RelyX Unicem 2; ground socket plugged + cured; implant wrapped in Micropore	Isoflurane; meloxicam peri-op and ×3 d
Kullman/Lignani	Adult male C57BL/6J mice	Same session	Skull cleared; contralateral skull screw with silver-wire ground; MedBond cyanoacrylate on skull/suture/skin edges	Biopsy punch + diamond bur; Dural-Gel cover (1.5 mm)	3–5 µm/s (Sensapex)	RelyX Unicem 2, then Super-Bond; ZIF protected with parafilm	Isoflurane; buprenorphine peri-op; meloxicam + amoxicillin post-op

Why (and how) we need to professionalize neuroscience



Over the past few decades, neuroscience has become so broad and technically sophisticated that individual researchers can no longer fully understand the technical foundations of their experiments.

The average systems neuroscience project, for example, requires in-depth knowledge of

- **animal surgery**
- **mechanical engineering**
- **optical engineering**
- **electrical engineering**
- **statistics and computer science**

Jakob Voigts
Group leader
Howard Hughes Medical Institute

Surgery, open-source data resources

Surgery planning software
PinPoint - https://virtualbrainlab.org/pinpoint/tutorials/tutorial_basics.html

Surgery video
Ask for a link from me at Voroslakos@gmail.com

Neuropixels training videos
https://www.ucl.ac.uk/neuropixels/training/2025-neuropixels-course
Neuropixels example dataset
https://www.steinmetzlab.net/shared/
Allen Institute Neuropixels dataset
https://allensdk.readthedocs.io/en/latest/visual_behavior_neuropixels.html

https://www.3dneuro.com/resources/ephys-behavior-resources/

Neuropixels is 'just' a fancy silicon probe.
80% of the knowledge can be applied to other silicon probes.

Synchronization, automation

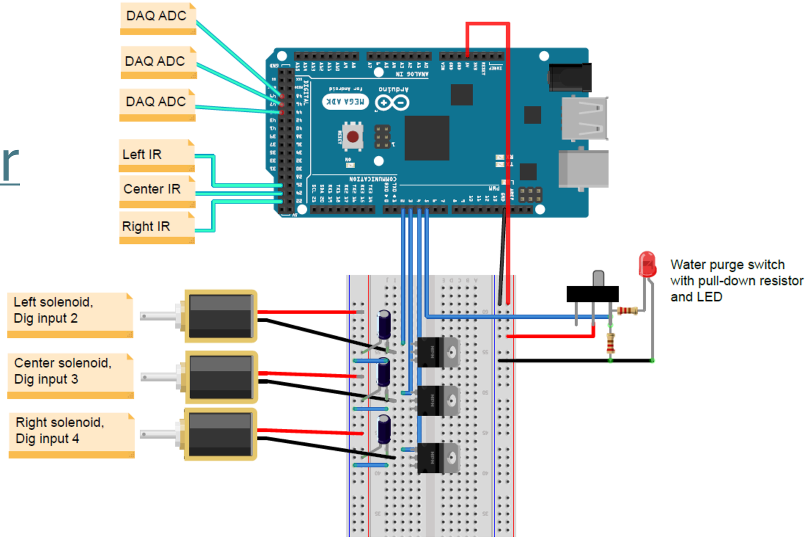
https://open-ephys.github.io/gui-docs/Tutorials/Data-Synchronization.html

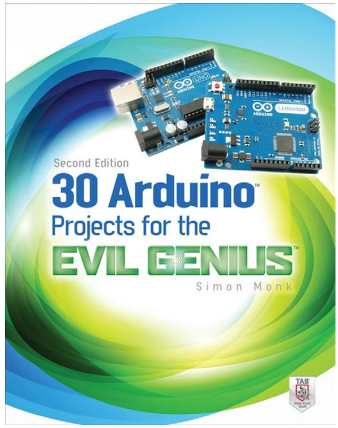
Bonsai
https://open-ephys.org/bonsai

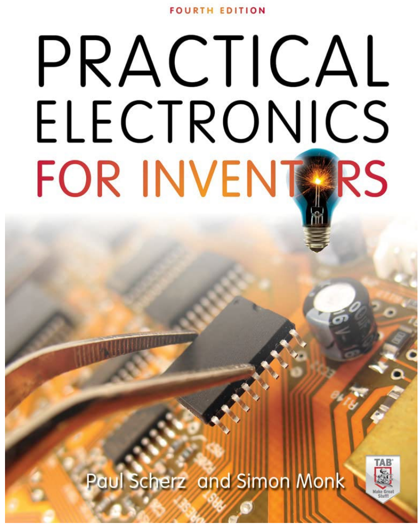
Arduino, raspberry PI
Infrared sensor, light sensor, vibration sensor

Sparkfun
https://www.sparkfun.com/

Adafruit
https://www.adafruit.com/







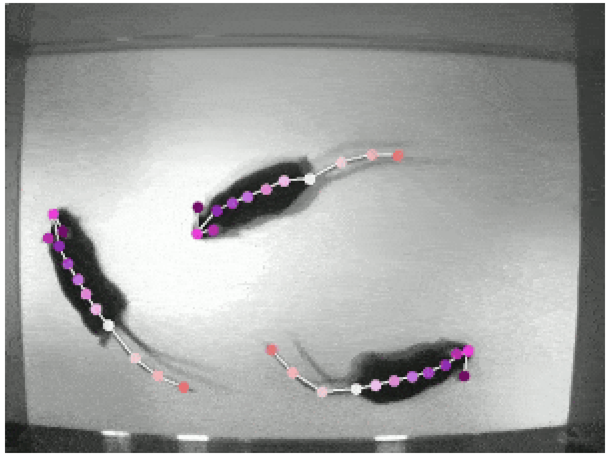
Behavior tracking

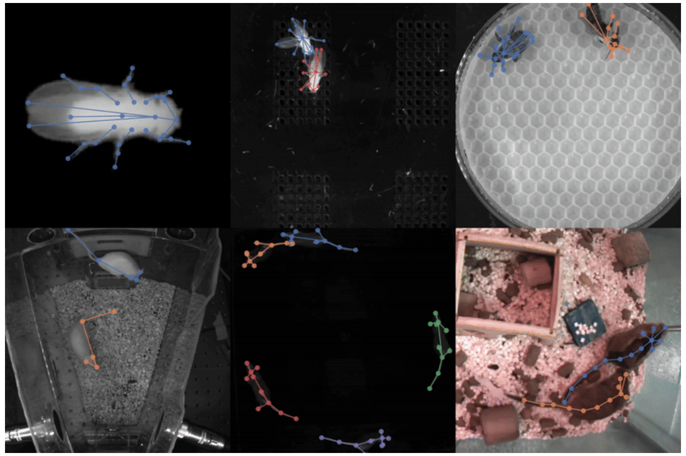
DeepLabCut
https://www.mackenziemathislab.org/deeplabcut

SLEAP
https://sleap.ai/

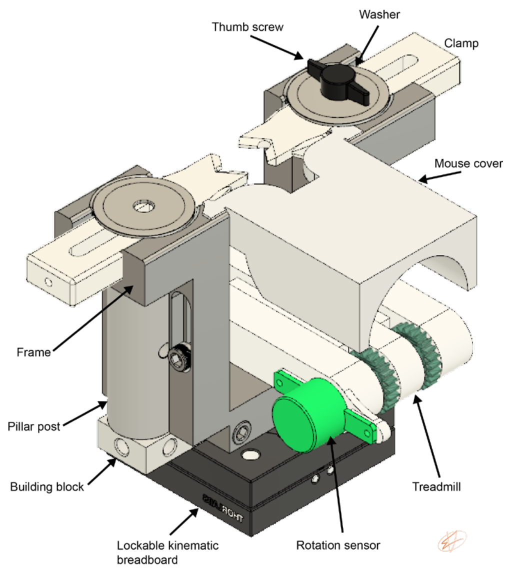
You need a good GPU (1-2 k price range, same one is needed for spike sorting).
Video analyzed in real time (30 min video takes 30 min to analyze).

Noldus
Ethovision (10k, plus yearly subscription)





CAD design



Autodesk Fusion
https://www.youtube.com/watch?v=A5bc9c3S12g

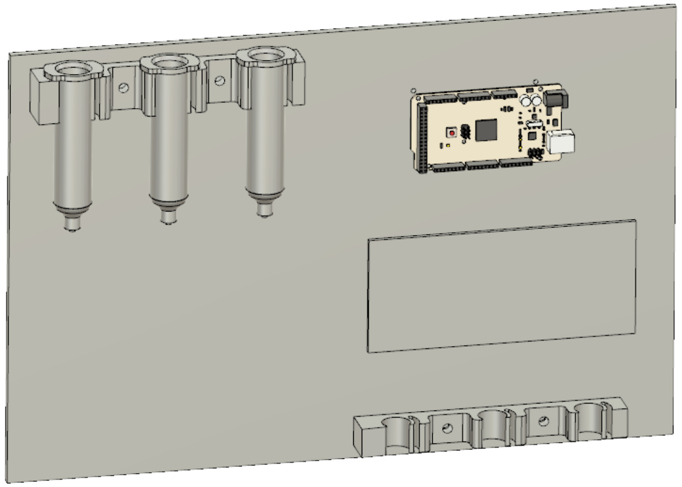
Autodesk Inventor, SolidWorks

If you have never used similar software before, it can take 1-2 months to get familiar with the concepts.
Is it worth your time to learn this?
Our designs are very simple, so once you know how to use the software and where each buttons are, designing can be fast and easy (you can create nice figures for workshops, tutorials:D).

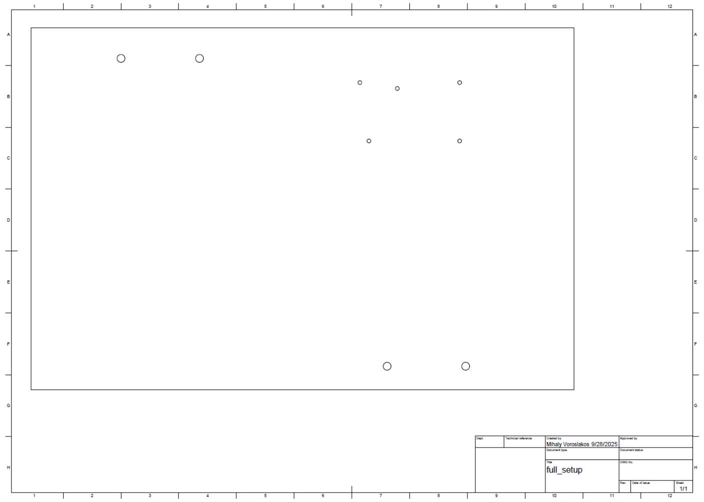
REMEMBER: most of the downloaded .stl files will not work. They are always adjusted to the printer's tolerance/specs. My experience, nothing will work when you just download. SHOW our GITHUB

CAD design

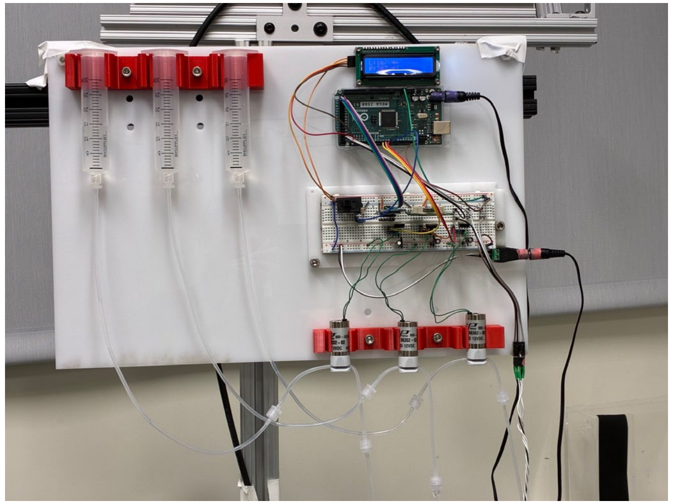
CAD design

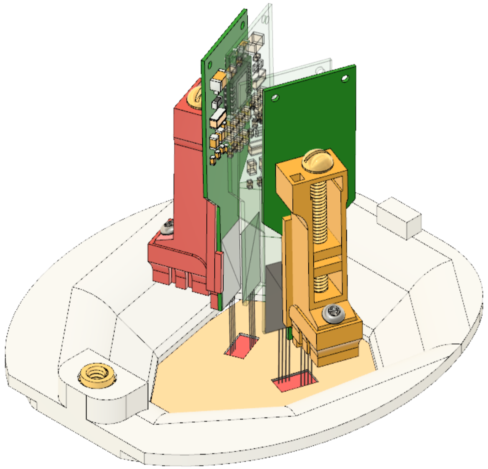


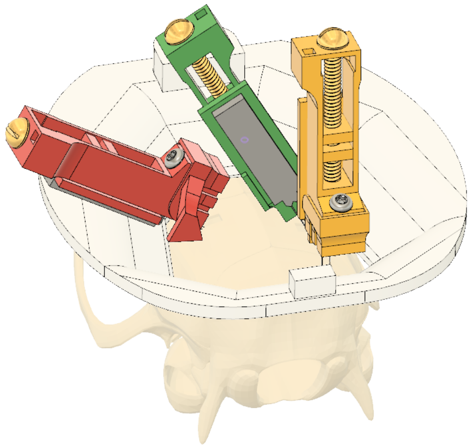
CAD drawing for laser cutter

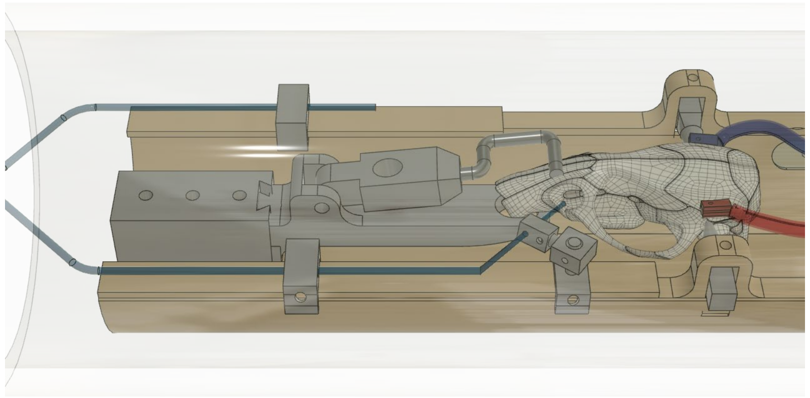


Assembled setup









Optogenetics

Optrode

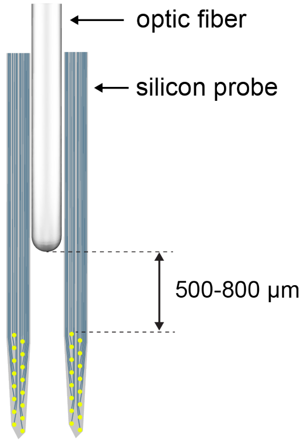
- Optic fiber attached to a silicon probe
- Companies offer this service
  - Cambridge NeuroTech - https://www.cambridgeneurotech.com/neural-probes/optogenetics
  - NeuroNexus - https://www.neuronexus.com/training_videos/how-to-order-optrode/

Light integrated on probe

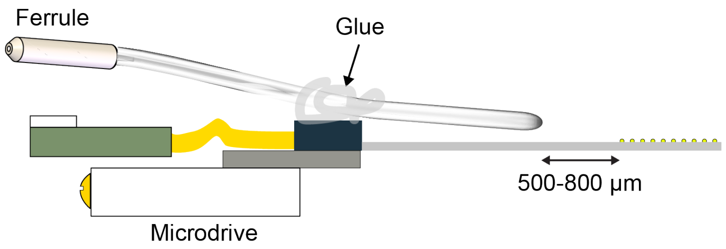
- microLED probe - https://plexon.com/products/neurolight-optoelectrode/
- Neuropixel opto - https://www.biorxiv.org/content/10.1101/2025.02.04.636286v1

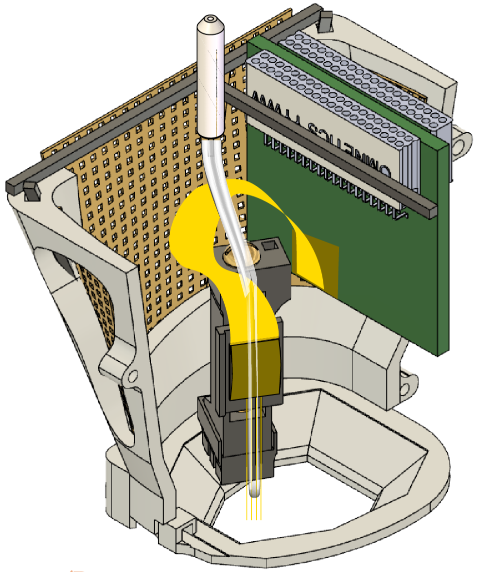
Optogenetics with optrode

FRONT VIEW



SIDE VIEW

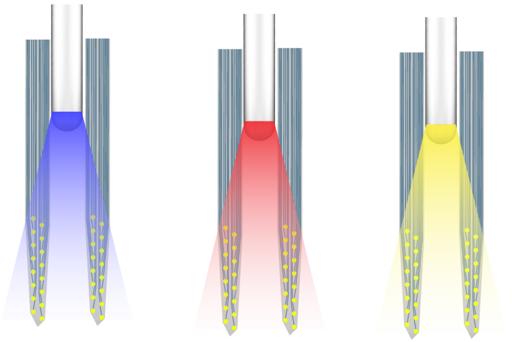


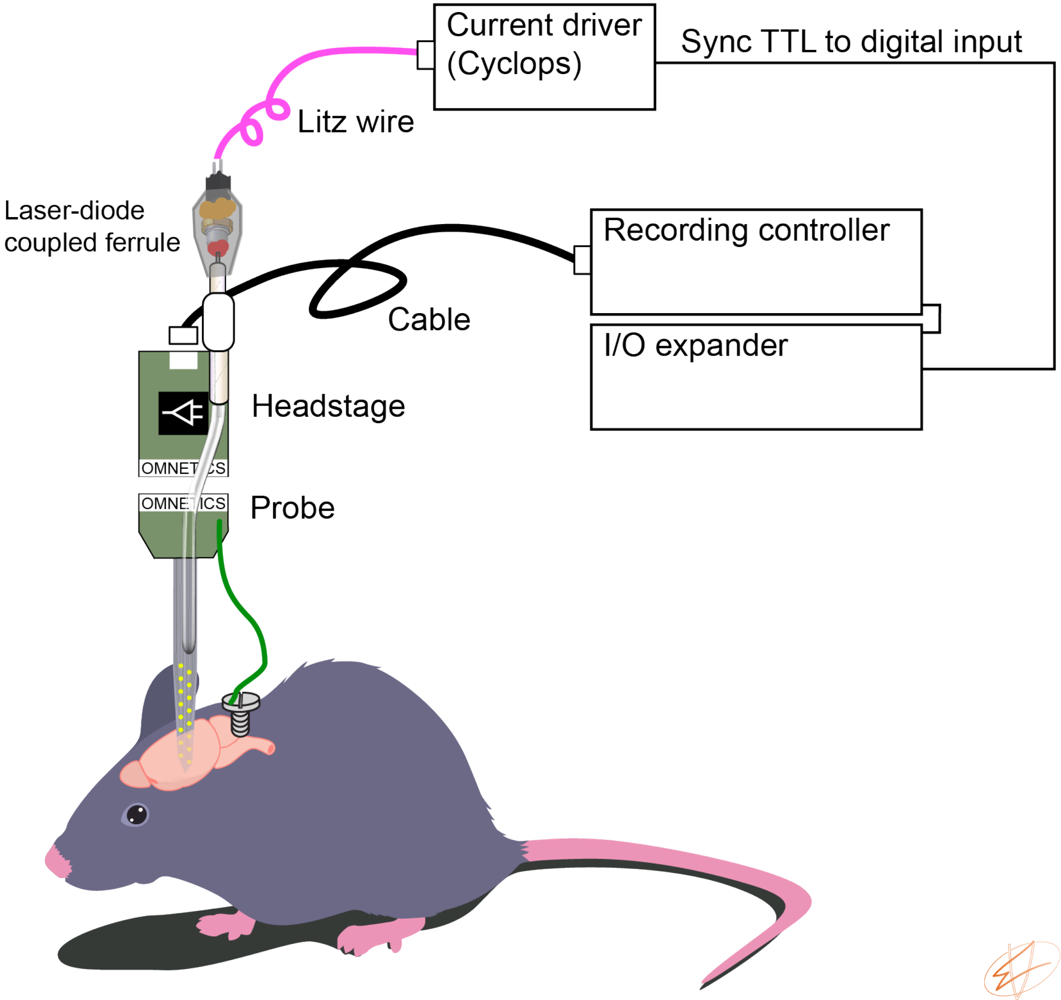


Ferrule is attached to copper mesh during surgery.

Laser-diode coupled ferrule is attached to animal to deliver light into the brain.

Different colors can be applied depending on the color of laser diode.





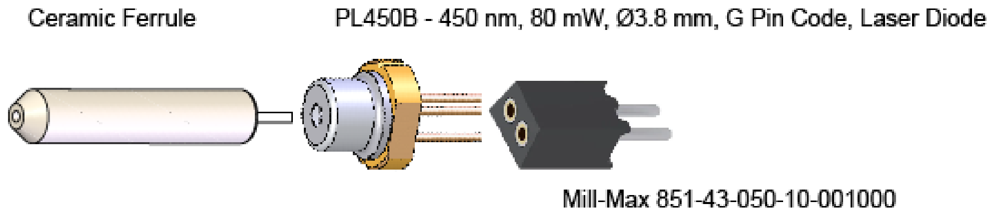
Optogenetics during behavior: light delivery

Fiber patch cable → ferrule

- Simple, but cable drag/torque can affect behavior
- A good commutator helps reduce twisting forces for fiber-based setups

On-head diode + ferrule

- deliver *current* via thin flexible wires → better mobility

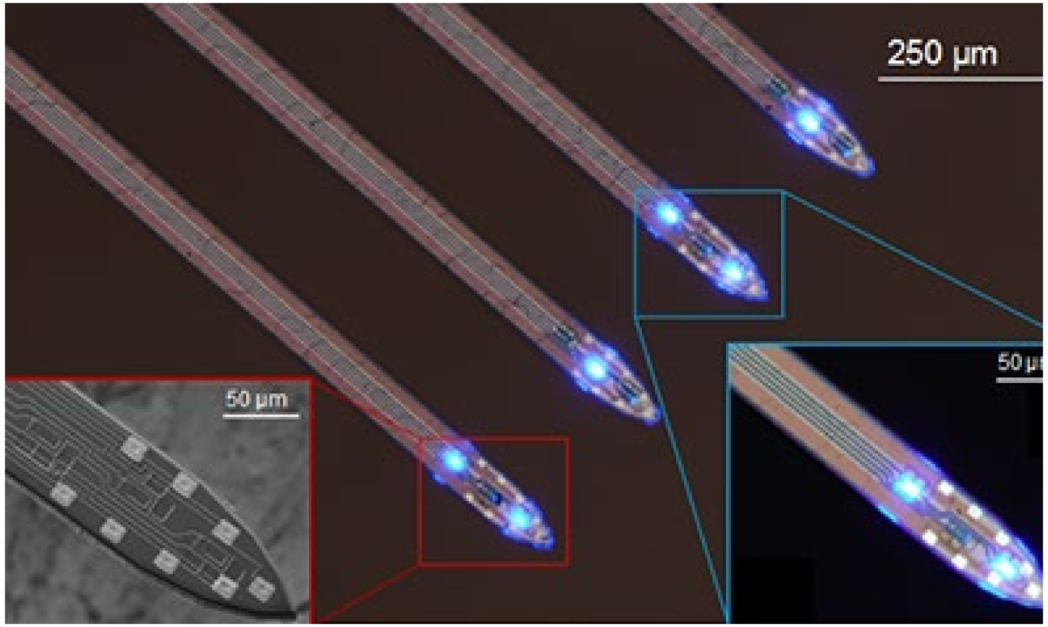


Fiber-to-silicon-probe attachment example: Stark et. al., 2012

Optogenetics with microLED probe

Good for

- Local light delivery
  - Effect is restricted to one shank
- Local circuit interrogation
- Few 10-100s of neurons are affected



Source: https://plexon.com/products/neurolight-optoelectrode/#1592930170634-2a8140fd-e25f

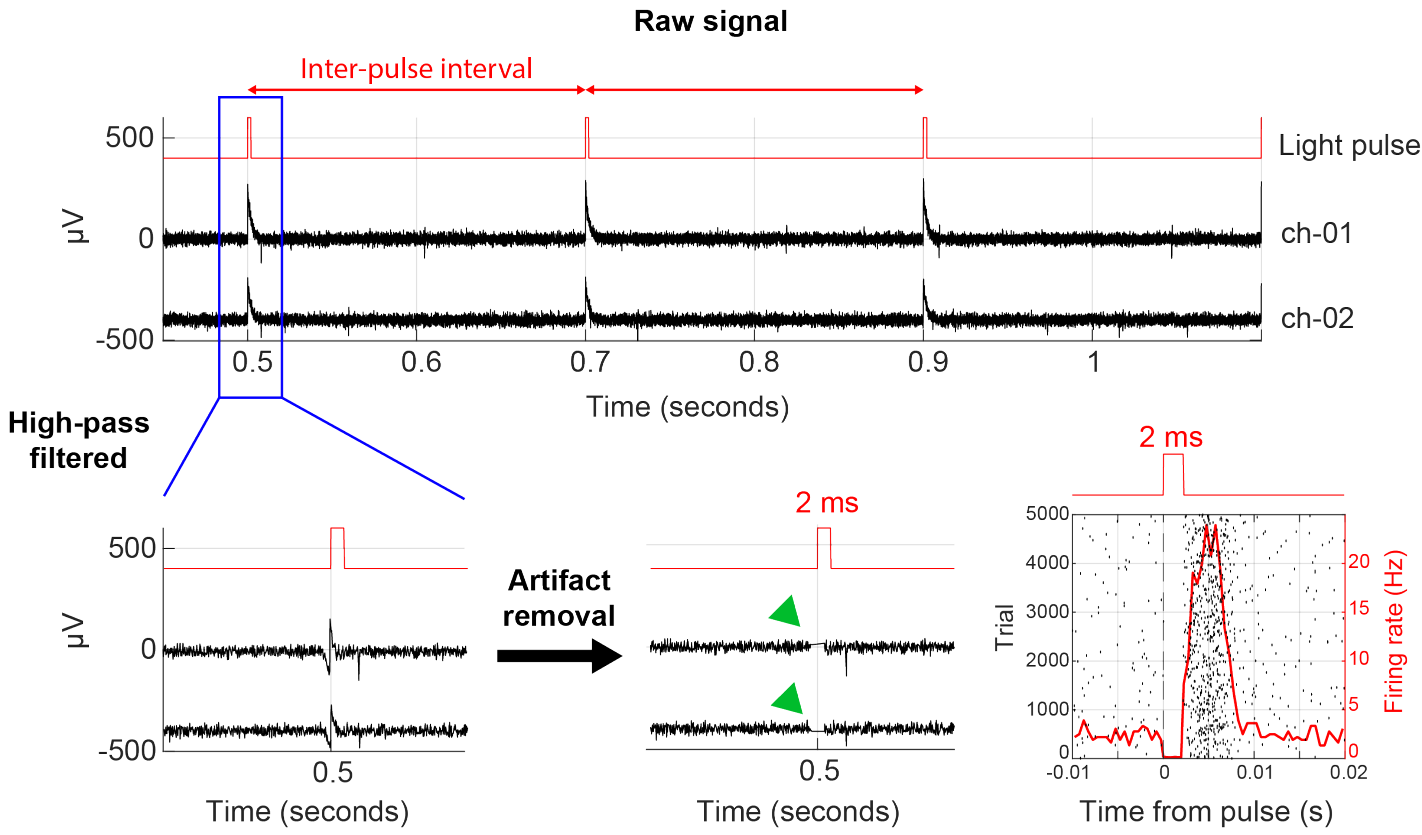
Optotagging experiments

Optotagging protocol

- Know opsin kinetics
- Pulse: <2 ms every 200 ms (5 Hz)
- Repetitions: ≥5000 (up to 10000)

- **Remove light artifacts** before spike sorting
- Expect increased spikes ≤8 ms
  - (longer may be polysynaptic)

- **Stats (SALT)**
  - [P, I] = SALT(SPT_BASELINE, SPT_TEST, DT, WN)
  - Compares spike-latency histograms (modified Jensen–Shannon divergence)



Green arrows show the cut-out data segment.

Flexible probes

Pros

- Potentially **less chronic inflammation / smaller long-term scar**, better for very long recordings (weeks→months).

Cons / reality check

- **Insertion is hard**: flexible shanks buckle, so you often need a temporary stiffener/shuttle (PEG-glued stiffener is common).
- The shuttle/stiffener can increase the acute footprint, so initial insertion damage may dominate if the delivery system is bulky.

Tech is improving (smaller-area shuttles exist), but it's still a "pick your poison" tradeoff for many labs.

Timeline intuition
Tissue encapsulation/scar around intracortical implants often becomes **dense around ~4–6 weeks** and then stabilizes—so early damage + early inflammation can set the ceiling for "forever" yield.

Flexible vs rigid https://www.nature.com/articles/s43586-025-00399-7

Pick flexible if:

- You need *very long* duration interfaces and can invest in (or already have) a good insertion/shuttle solution.
- Your lab can reliably execute delicate implant procedures and protect the implant mechanically.

Pick rigid probes if:

- You need **coverage now** (large tissue volumes, multi-region simultaneity).
- You want maximal practicality/reproducibility across animals with less surgical "heroics."

Flexible probes

- https://www.science.org/doi/10.1126/sciadv.1601966
- https://app.jove.com/t/59957/chronic-implantation-of-multiple-flexible-polymer-electrode-arrays
- https://advanced.onlinelibrary.wiley.com/doi/full/10.1002/advs.202207576
- https://www.nature.com/articles/s41593-024-01692-6
- https://www.nature.com/articles/s41467-024-49226-9

Flexible probes for human BCI

- https://neuralink.com/
- https://axoft.us/
- https://precisionneuro.io/

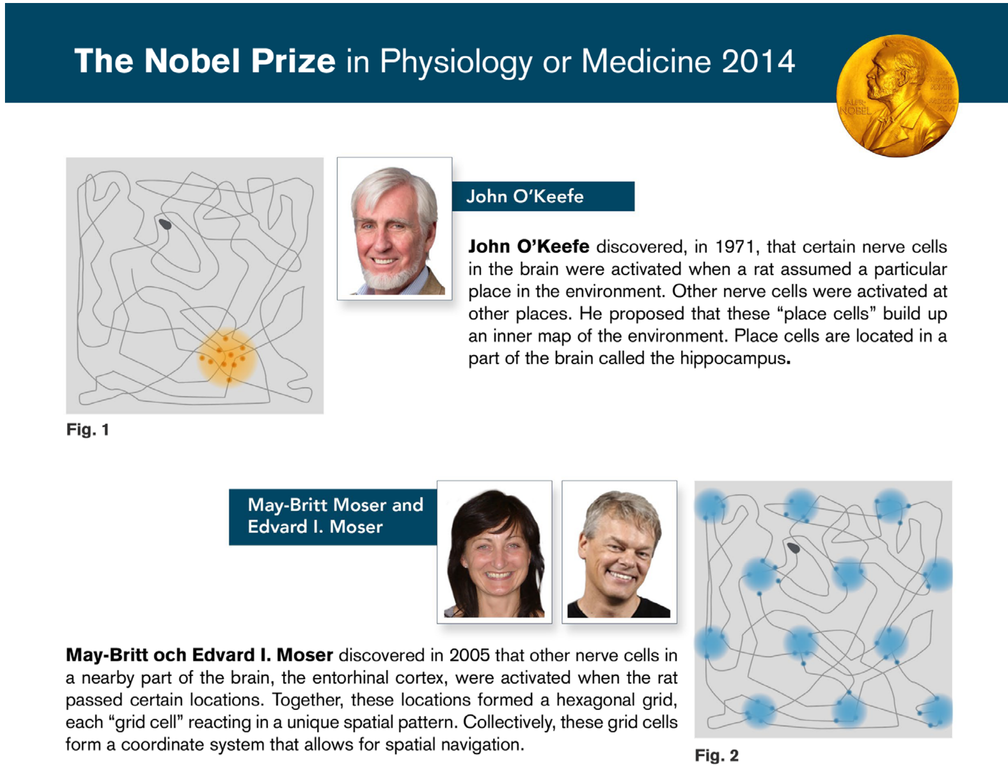
Flexible probe is preferred as it cannot break into the brain.

The field is moving toward:

- Recording more channels, more single units.
- Optogenetics in humans (Sahel et. al., 2021).
- Multi-color waveguide and uLED probes.
- Recording brain-body interactions.
  - Monitor heart-rate, temperature, blood sugar, blood pressure, etc.

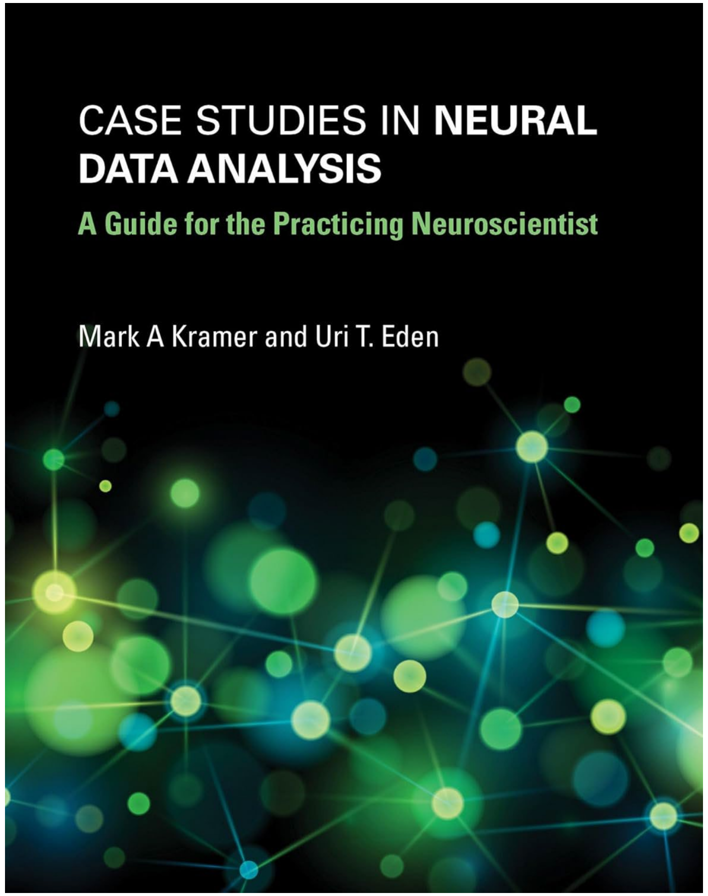
BUT

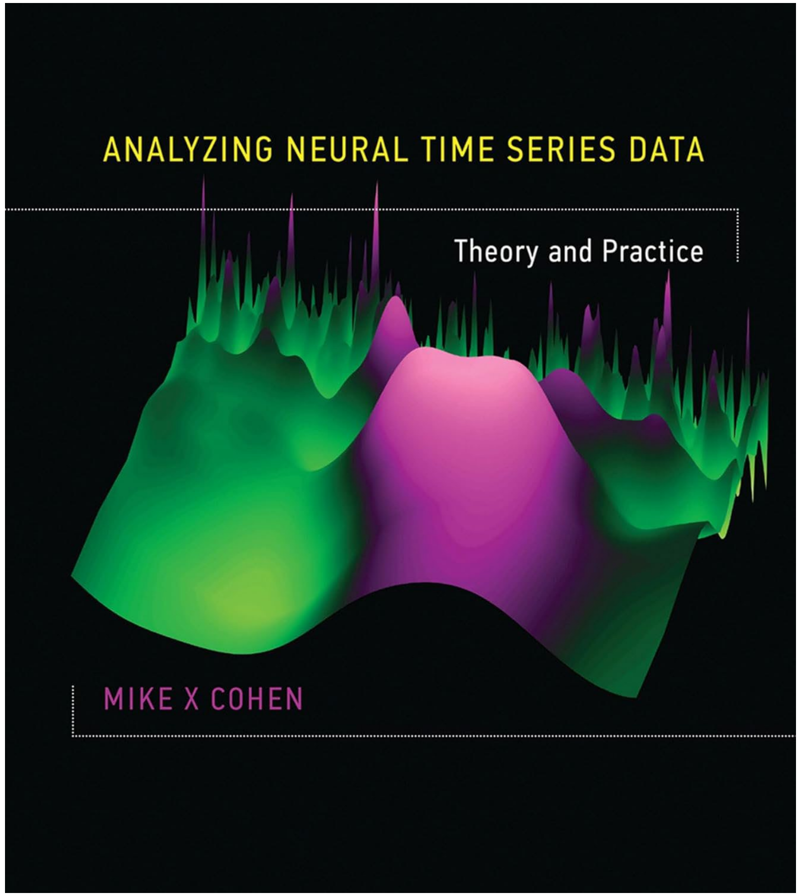
- The Nobel Prize discovery was made by single wires and a lot of thinking…



Computational resources

- [Python for the practicing neuroscientist: an online educational resource]()





Channel map creation (Headstage → Probe geometry)

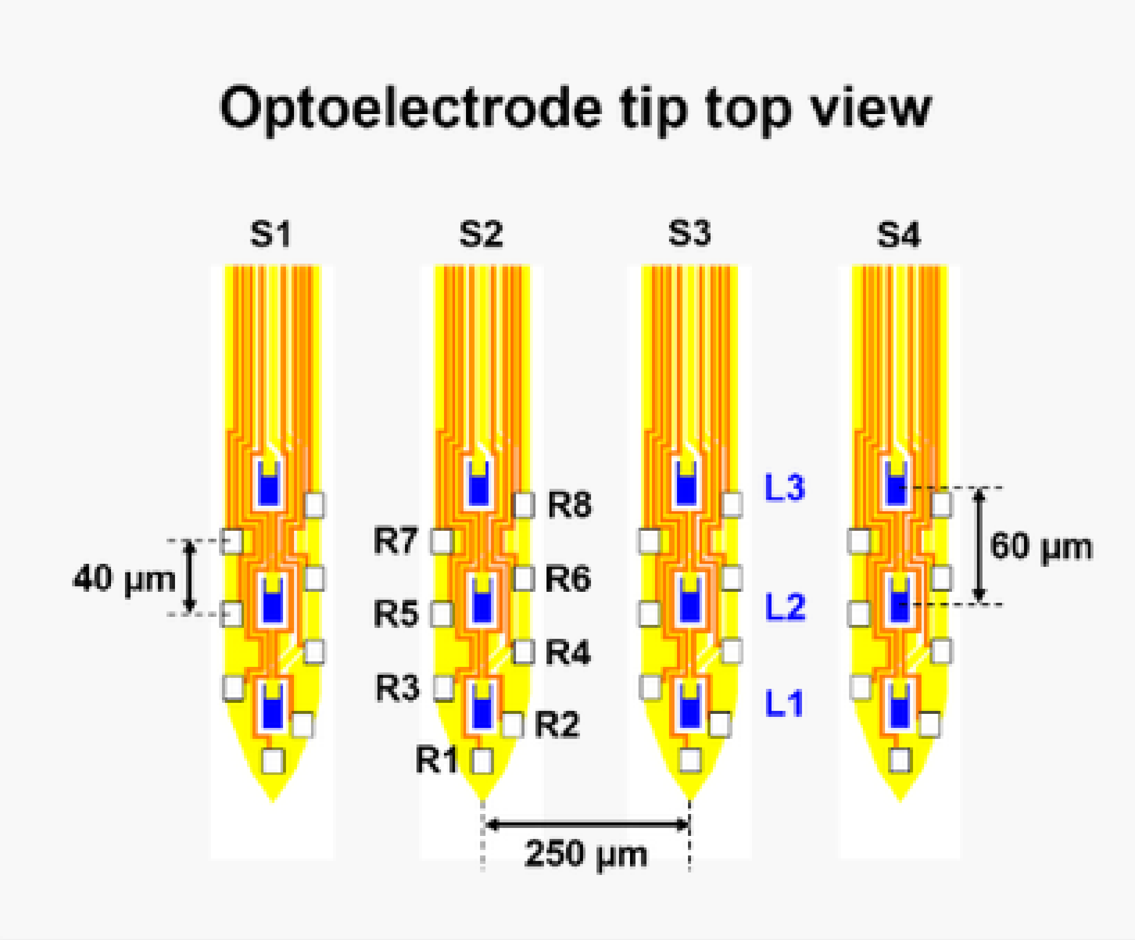
Goal

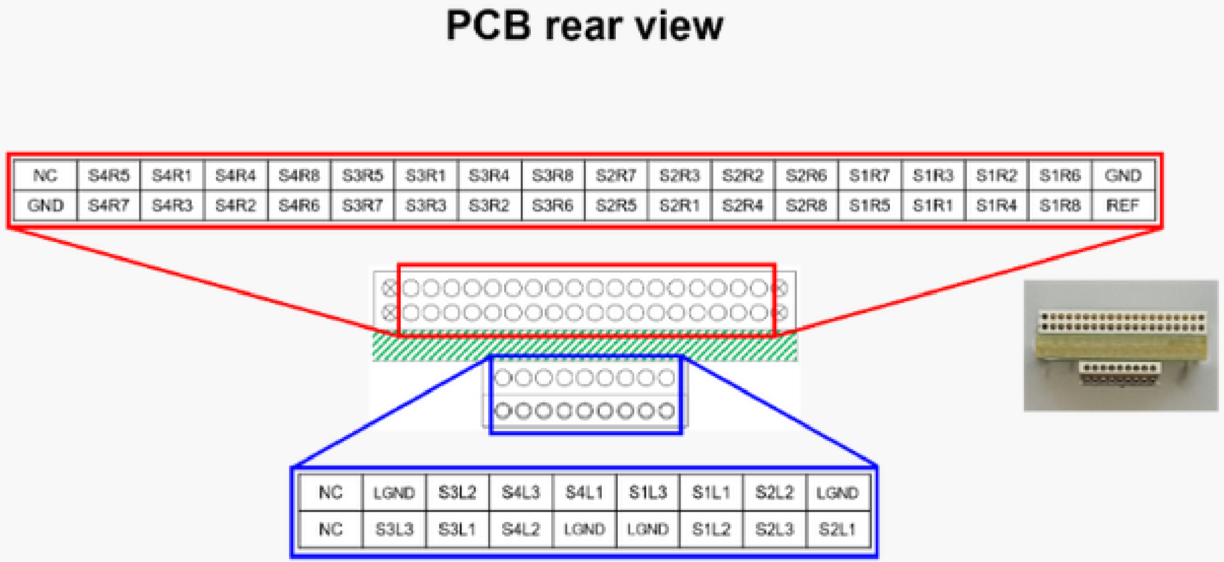
- Translate **recording software channel ID** (headstage channel number)
- into the **physical recording site** on the probe (**shank, x/y location**).

Inputs (3 different mappings)

- Probe recording channel layout (shank, x_um, y_um)
- Probe to connector pinout
- Connector to headstage pinout

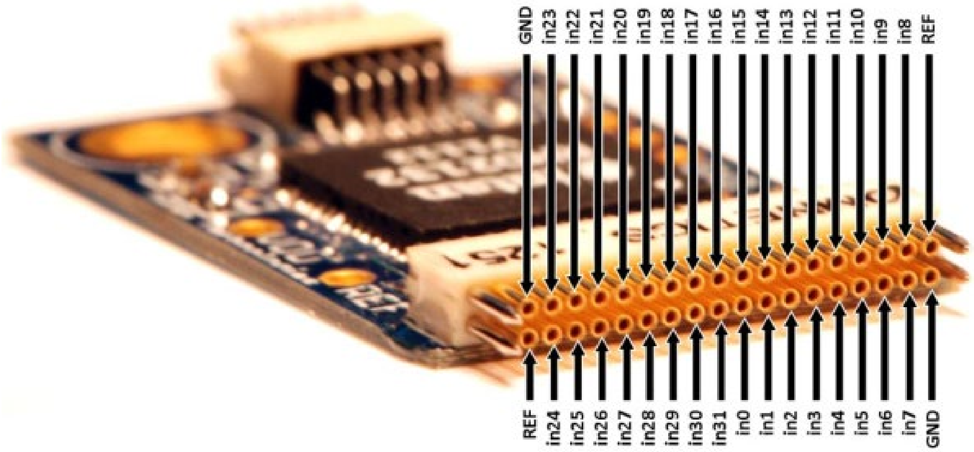
Creating your channel map





NC	S4R5	S4R1	S4R4	S4R8	S3R5	S3R1	S3R4	S3R8	S2R7	S2R3	S2R2	S2R6	S1R7	S1R3	S1R2	S1R6	GND
GND	S4R7	S4R3	S4R2	S4R6	S3R7	S3R3	S3R2	S3R6	S2R5	S2R1	S2R4	S2R8	S1R5	S1R1	S1R4	S1R8	REF

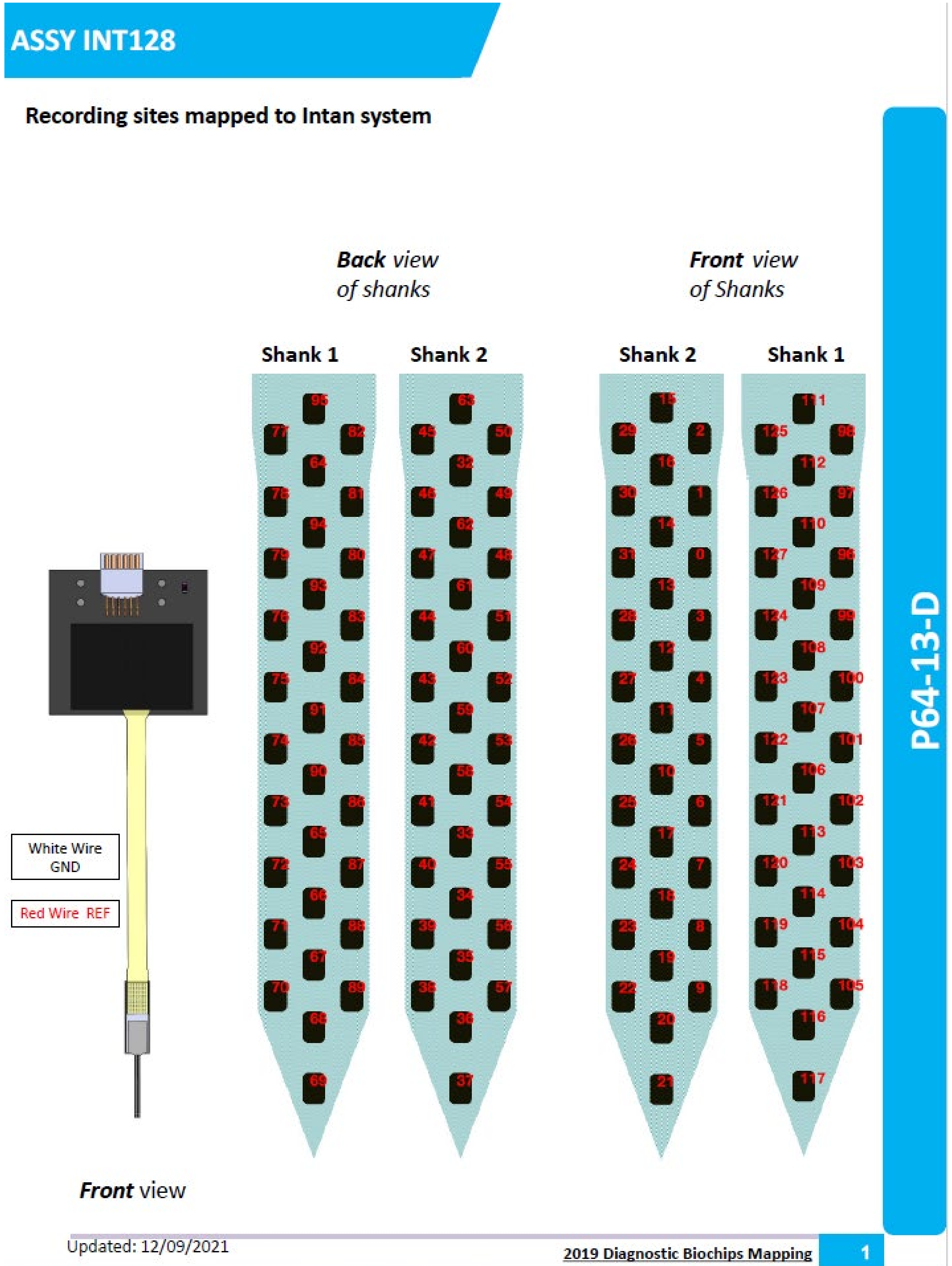
NC	LGND	S3L2	S4L3	S4L1	S1L3	S1L1	S2L2	LGND
NC	S3L3	S3L1	S4L2	LGND	LGND	S1L2	S2L3	S2L1



Creating your channel map

Inputs for active probes

Probe recording channel layout (shank, x_um, y_um)



Preprocessing data

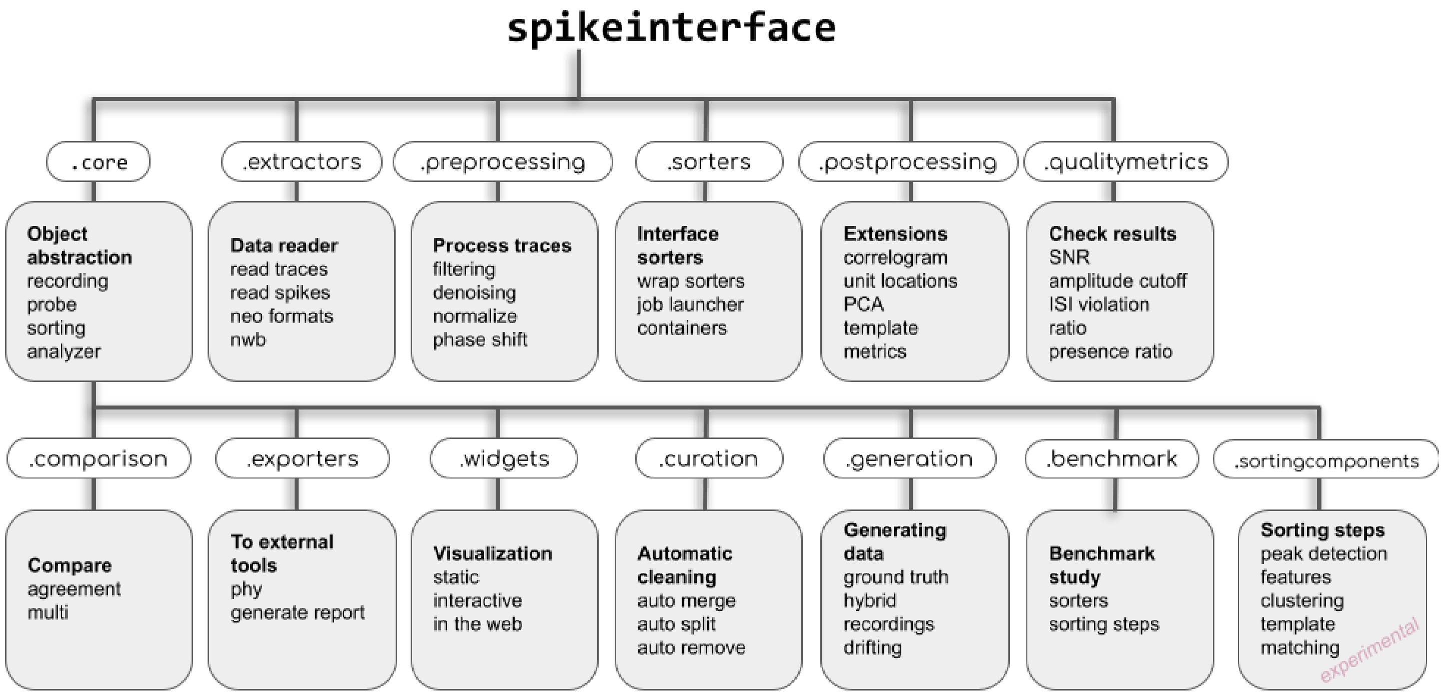
- Download Matlab functions BuzsakiLab github.

- bz_ConcatenateDats;
- bz_LFPfromDat(basepath);
- save([basepath filesep basename ‘.YOURmanipulation.manipulation.mat'],’YOURmanipulation’);
- RemoveArtefact_dat([basepath filesep basename '.dat'],artefact);
- removeNoiseFromDat(basepath,'method','substractMedian','ch', channels);
- SleepScoreMaster(basepath);
- TheStateEditor
- SpikeSort (Kilosort or similar)

Spikesorting

- Watch Neuropixels UCL training videos

- https://spikeinterface.readthedocs.io/en/stable/



If you have any questions:

Contact me at Voroslakos@gmail.com