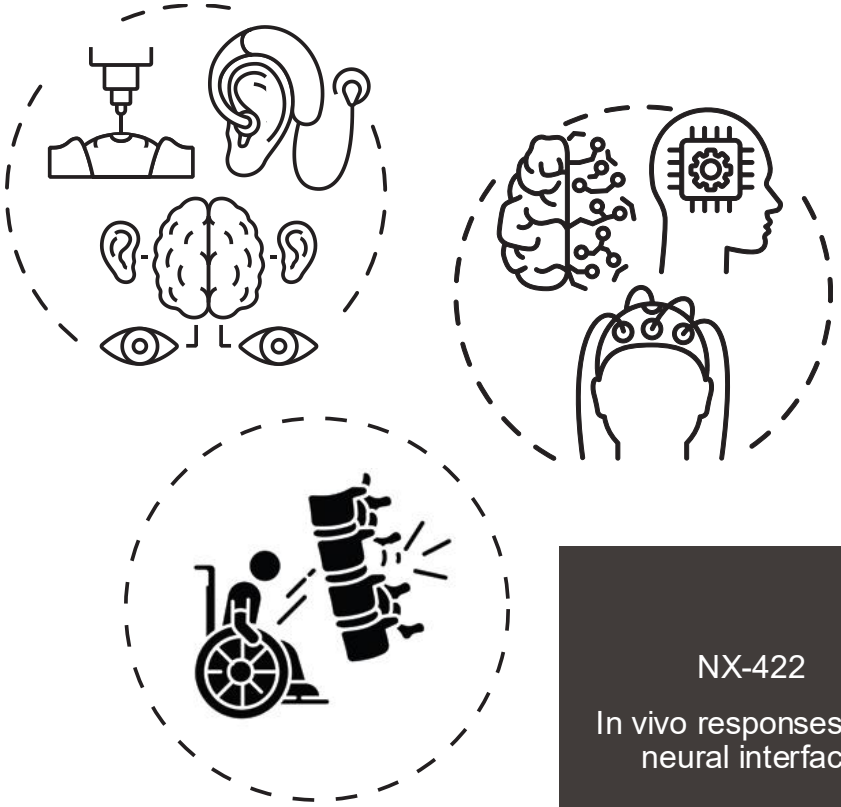


Neural Interfaces

NX-422

In vivo responses to a neural interface

Giuseppe Schiavone

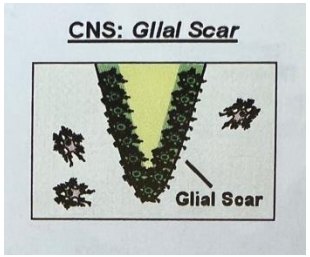
Neuro-X Institute

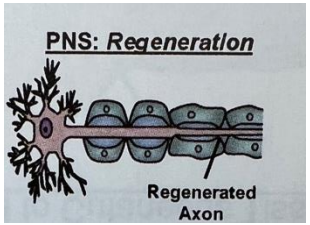
Topics

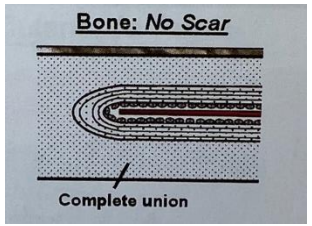
- Foreign body reaction (FBR)
- Investigation methods of FBR in vivo
- Performance of current implantable neural interfaces
- Strategies to modulate FBR and implant failure

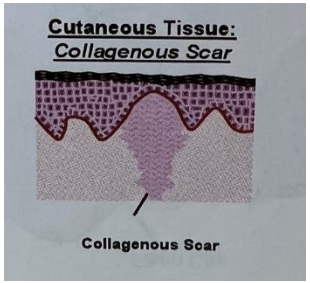
Foreign Body Reaction (FBR)

- An inevitable response to any implant in a biological tissue
  - hemostasis → inflammation → repair → remodelling
  - Nature of the **encapsulation tissue** and **cellular participants** in the immune reaction depend on the site of implantation, the type of tissue and the implantation procedure.



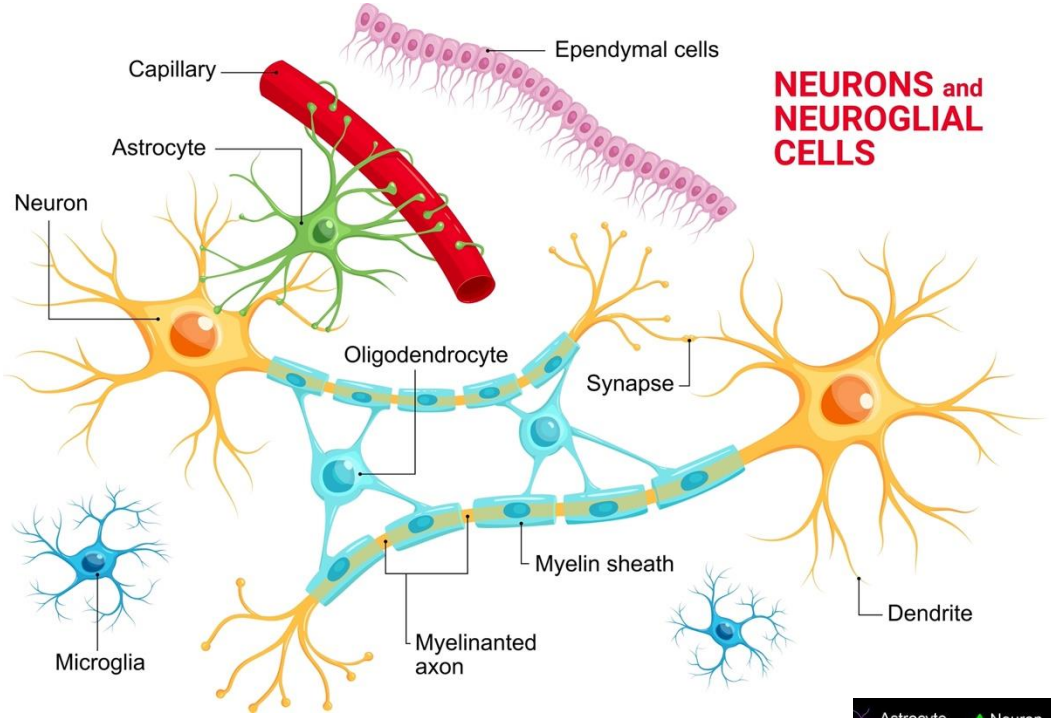


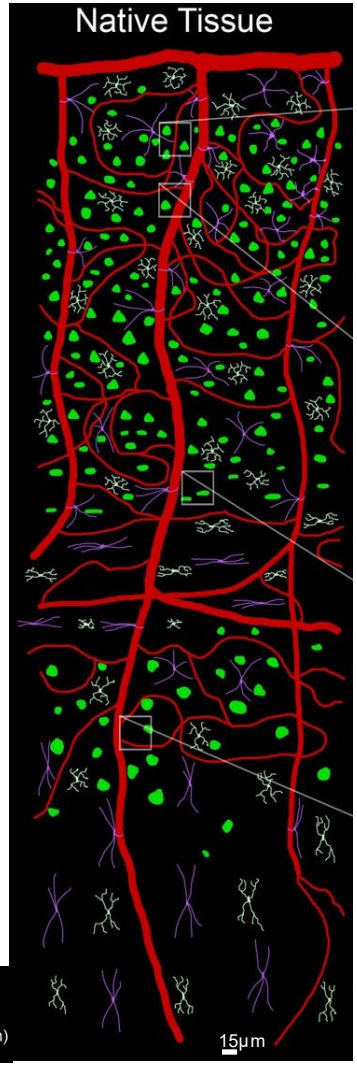




Indwelling Neural implants. Ed. by WM Reichert CRC Press 2008

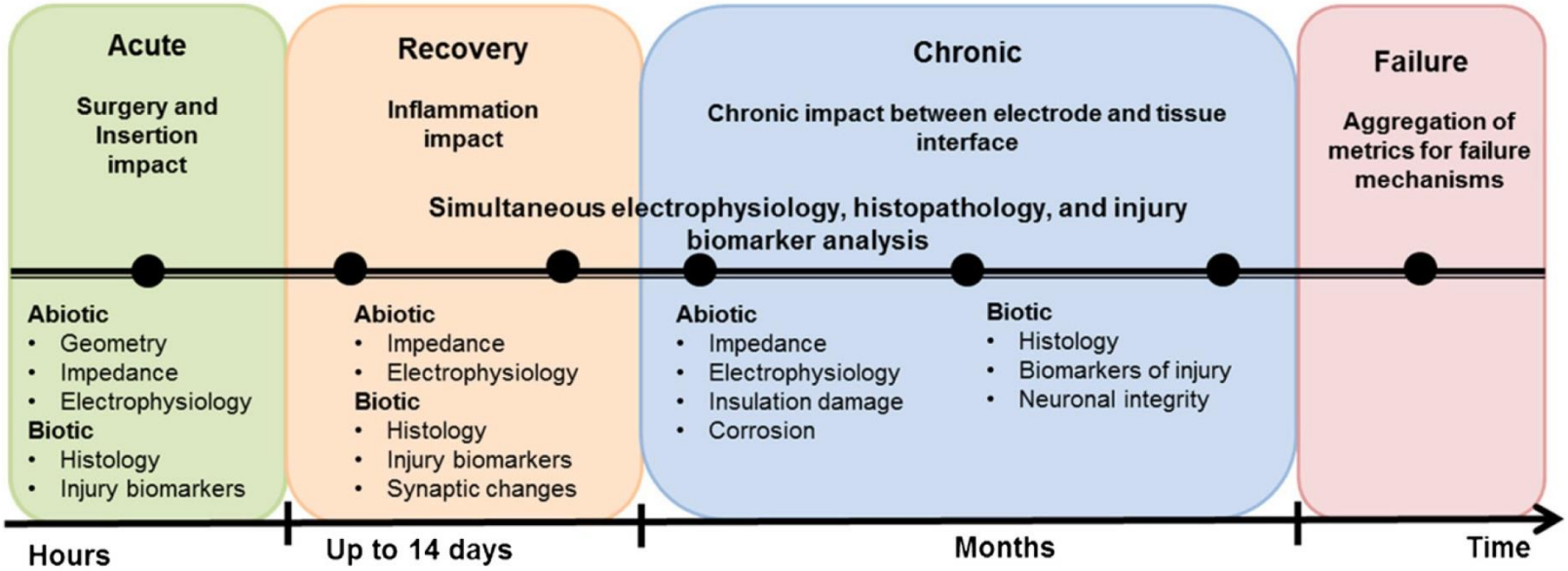
Participants in the CNS





ACS Chem. Neurosci. 2015, 6, 1, 48–67

Timeline of major events following chronic implantation *in vivo*



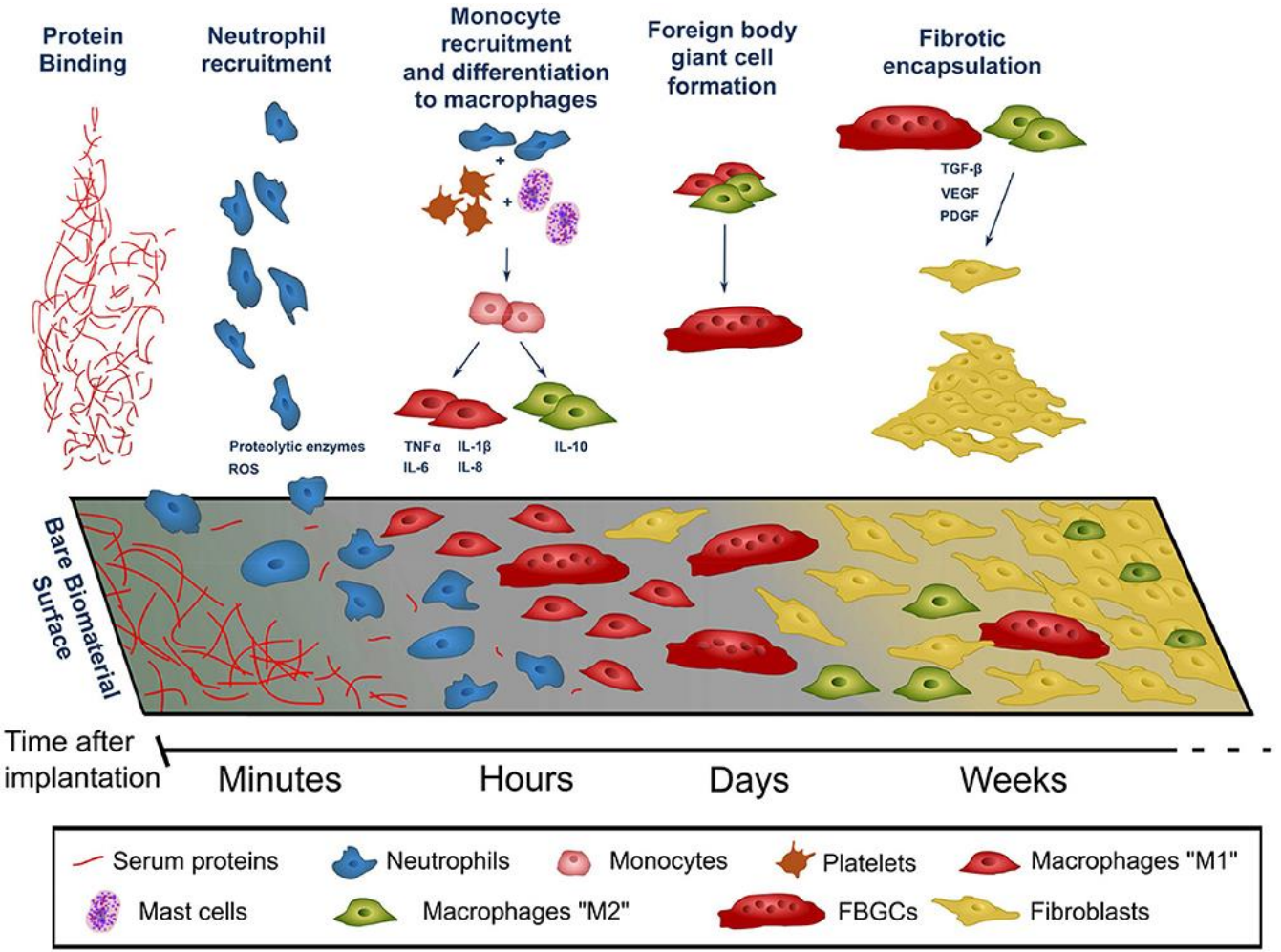
2018 *J. Neural Eng.* **15** 041004

Timeline

Acute (inflammation)

Then

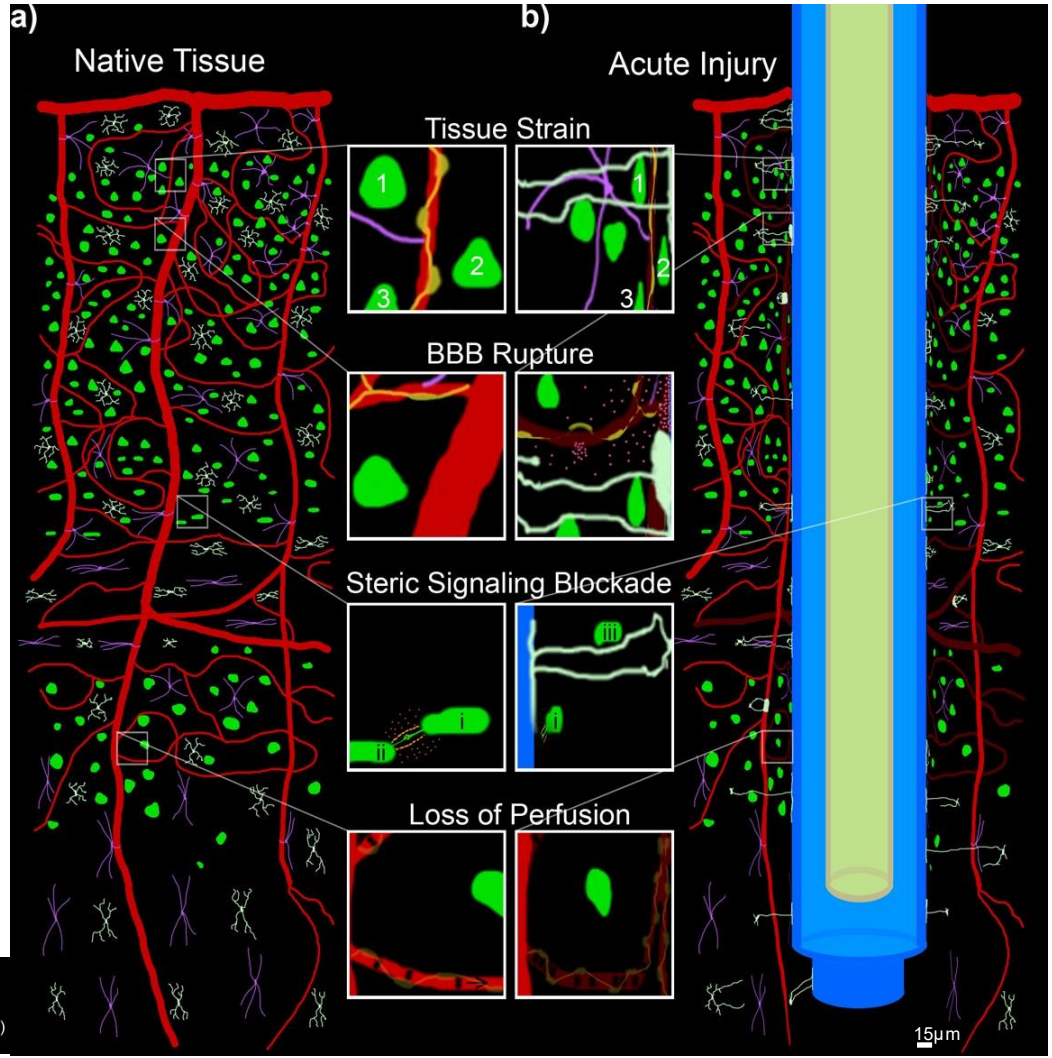
Chronic (fibrosis)



https://doi.org/10.3389/fbioe.2021.622524

Insertion of an implant

- Acute phase



ACS Chem. Neurosci. 2015, 6, 1, 48–67

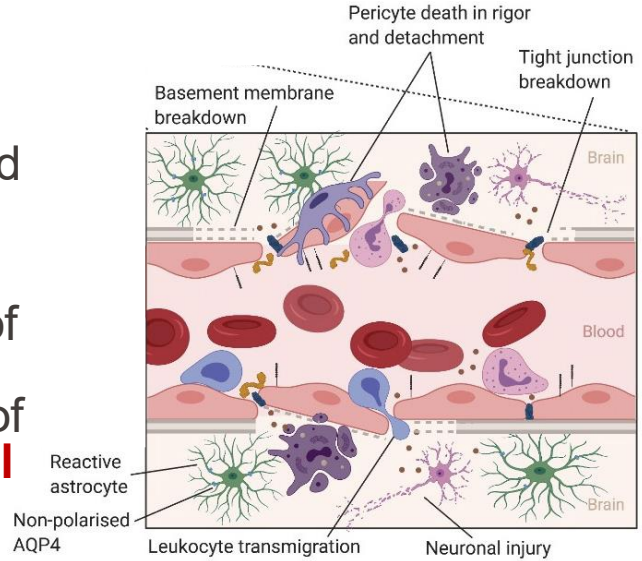
Perfusion and blood flow

- Insertion of an implant → diminution of local blood flow and loss of perfusion: **local ischemia**
- **Lower oxygen and nutrient delivery**
- Impaired removal of neurotoxic waste → promote inflammation

- **Implant volume and stiffness**
  - Interspacing in the vasculature: **50-60 µm** (rat cortex)
  - Device volume also leads to differential tissue **strains** at time of implantation (compression) and chronically (shear)
  - The stiffer the implant relative to the brain the higher the tissue strains (think of a system of springs where each absorbs some stress)

BBB rupture

1. Deposition of plasma proteins foreign to the CNS and red blood cells
2. Increase of hemoglobin leads to increases in reactive oxygen species and reactive nitrogen species (oxidative stresses) → **inflammation**
3. Activation of macrophages → cleaning of debris but also production of pro-inflammatory cytokines for the duration of the implantation → progression of a **glial sheath** (reduce signal quality)
4. Reactive **astrocytes** (within hours)
   - Mediate inflammation

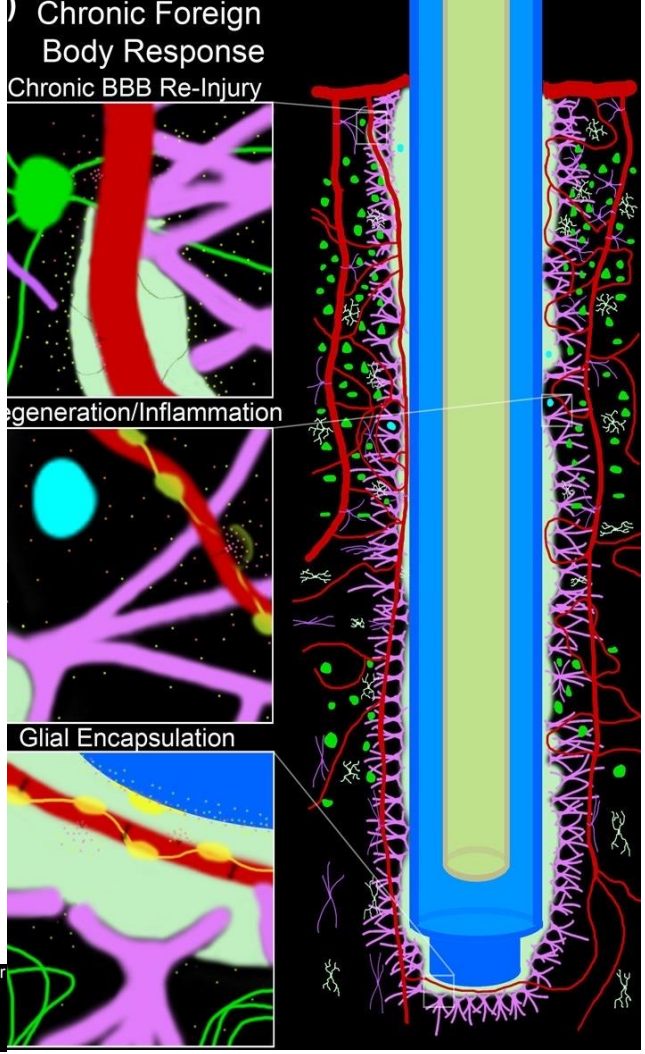


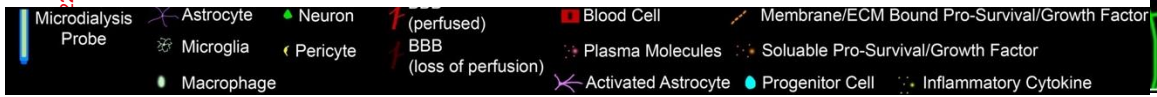
Int. J. Mol. Sci. 2021 22, 4280

Repair (scarring)

- Starts after a week

- Increased cell proliferation, capillary budding and synthesis of extracellular matrix (ECM)
- Glial scar is formed by reactive astrocytes and inhibits axon regeneration
- The glial sheath is made of multiple layers of activated microglia, macrophage, and astrocytes that form an ionic barrier through tight junctions with neighboring cells.
- While tissue regeneration can occur, the glial scar prevents neuronal cell bodies and neural filament from reoccupying regions within the glial scar. = In the brain, lost axons are not replaced

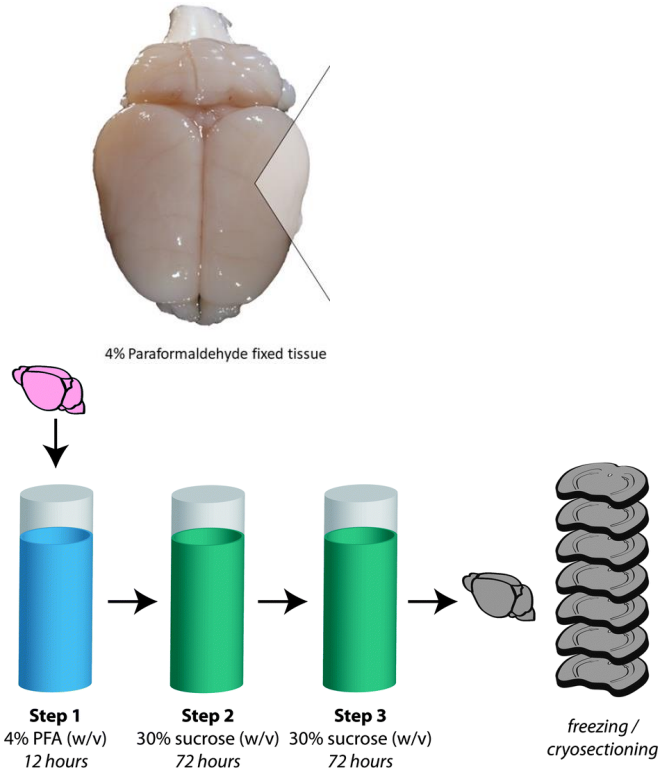




ACS Chem. Neurosci. 2015, 6, 1, 48–67

Imaging – fixation and slicing

- **Explanted tissue**
  - Under deep anaesthesia, transcardial perfusion with heparin infused phosphate-buffered saline (PBS) pH 7.4 followed by 4% paraformaldehyde (PFA) in PBS
  - Explantation
  - Post-fixation in PFA/PBS at 4°C for 12-24h and cryoprotection in sucrose
  - Embedding in resin, freezing
  - Slicing in ~10 µm thick slices

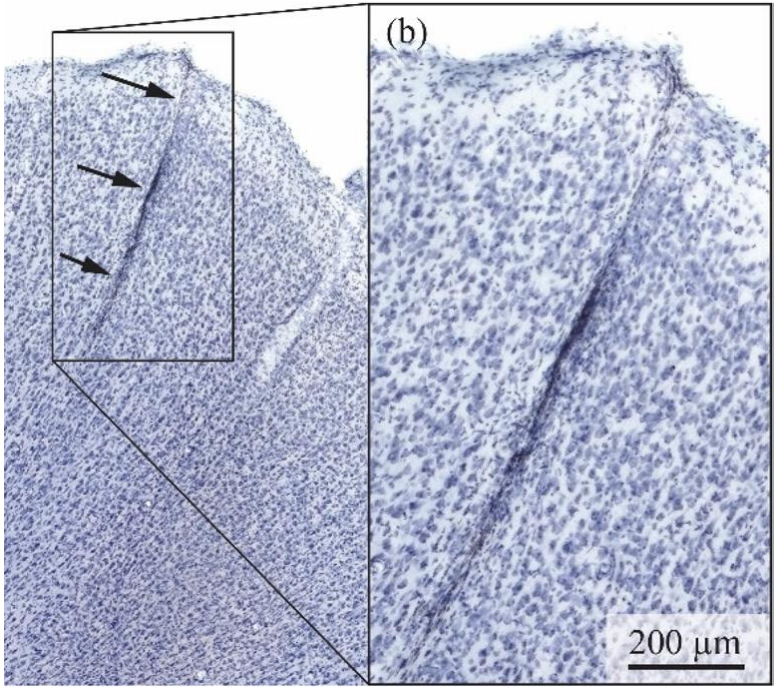


J. Anal. At. Spectrom., 2014,29, 565-570

Tissue staining

- Visualisation technique to enhance contrast of particular features

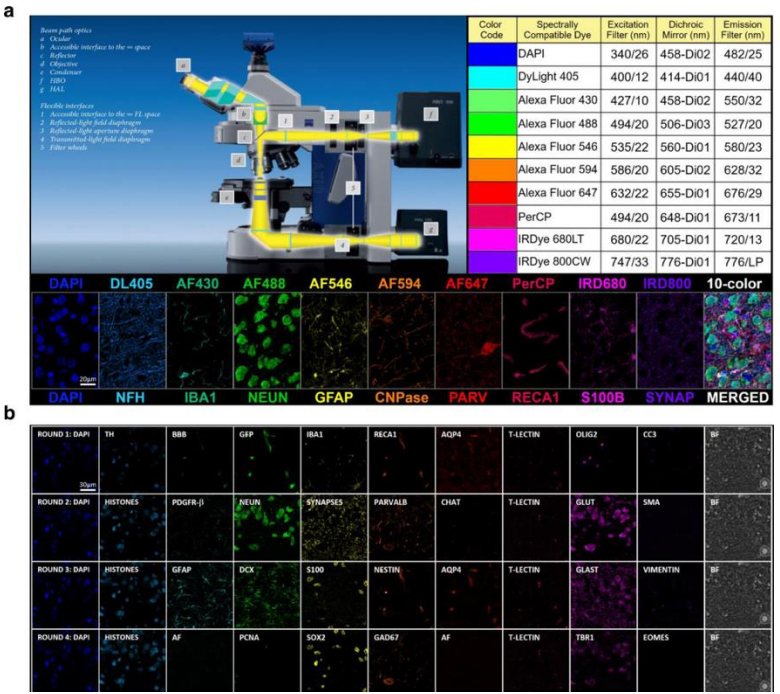
- **Nissl staining**: method to study morphology and pathology of neural tissue
  - aniline basic dye e.g. toluidine blue or methylene blue, that stains the nucleic acid content of cells esp. in the rough endoplasmic reticulum and ribosomes in the cytoplasm, which is abundant in neurons



J. Neural Eng. 14 (2017) 036010

Histochemistry

- selectively stain particular cell types, axonal fascicles, glial processes or blood vessels
- Expression of fluorescent proteins to visualise different neurons
- from ultraviolet to near-infrared (350–800 nm)
- Fluorescence microscopy (2D), confocal microscopy (3D)

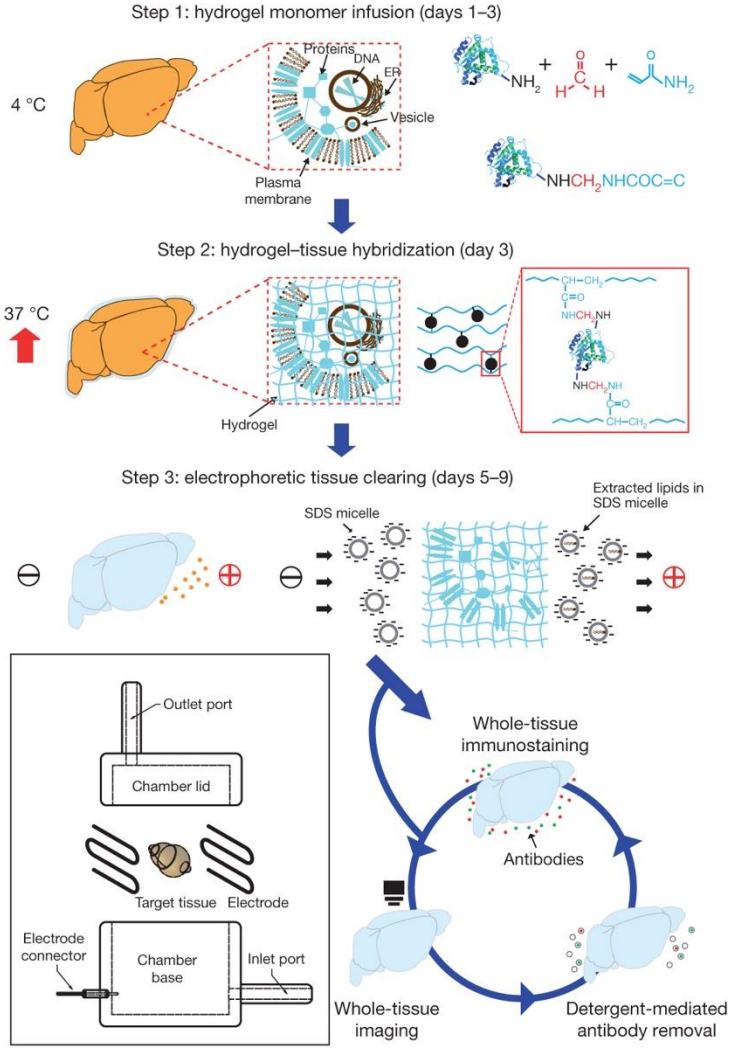


Nature Communications volume 12, Article number: 1550 (2021)

Light sheet microscopy

1. Tissue clearing
   - Lipid bilayers are replaced with a more rigid and porous hydrogel-based infrastructure

2. Enabling 3D molecular and optical interrogation of large assembled biological systems



Nature volume 497, pages 332–337 (2013)

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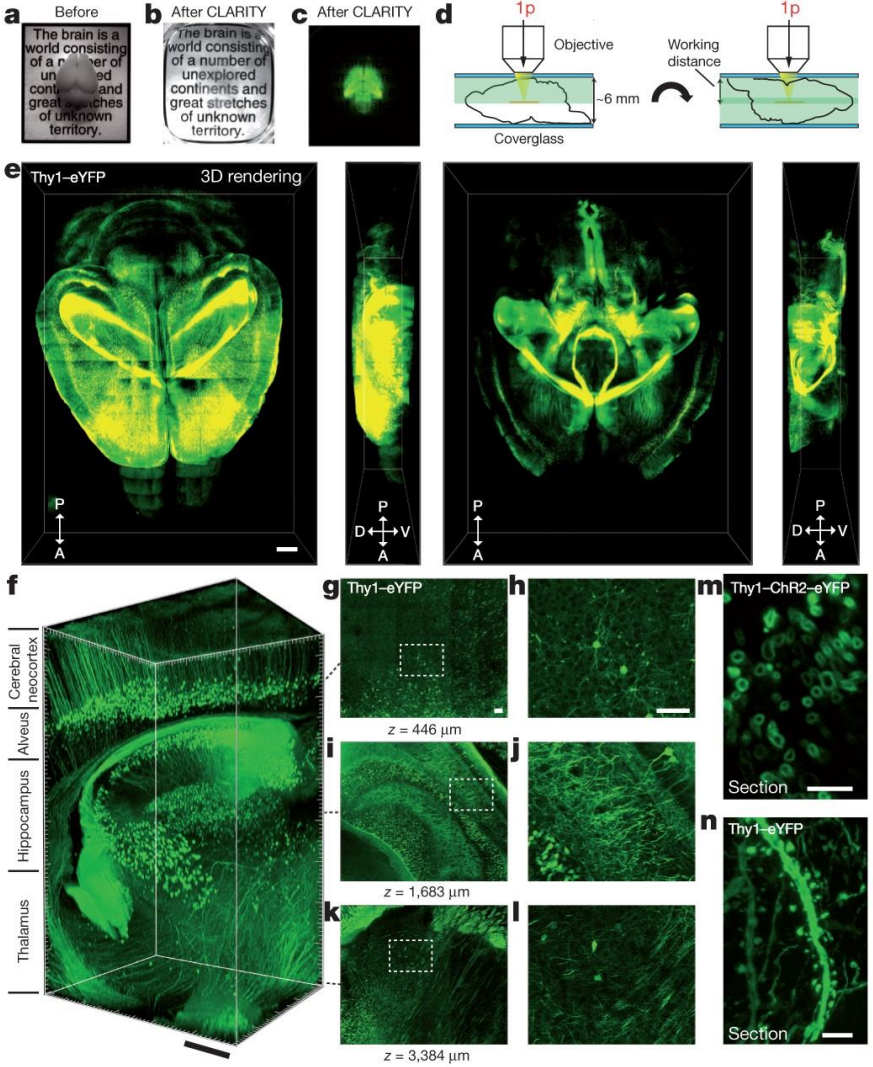
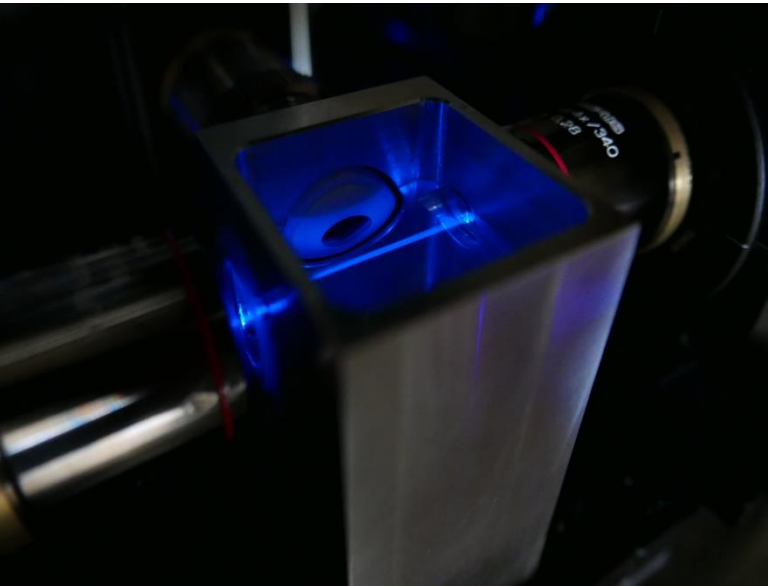
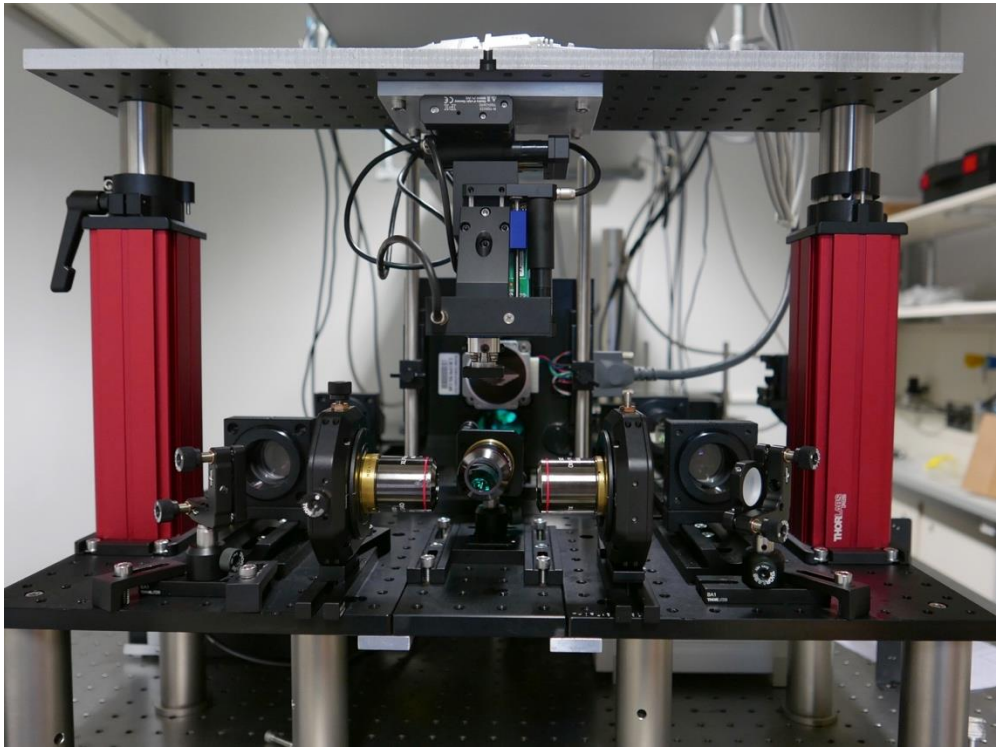


Nature volume 497, pages 332–337 (2013)

Microscope

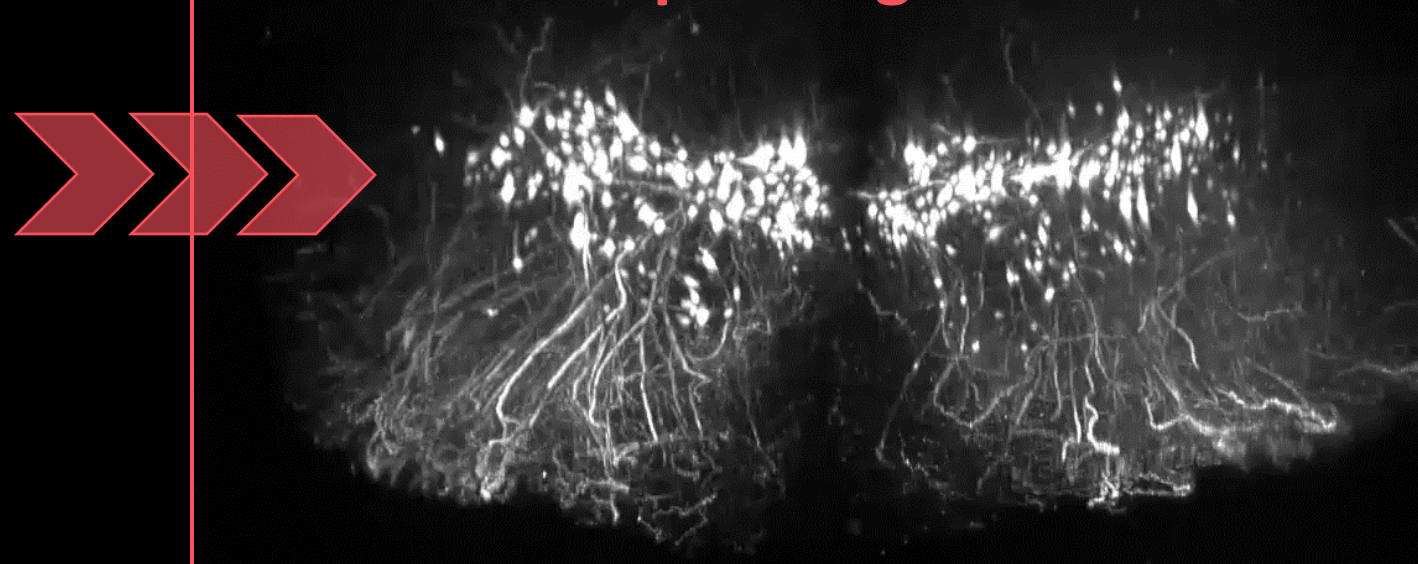




Photos par Q. Barraud, G. Courtine, EPFL

Cerebrospinal tract

Vsx2-expressing neurons



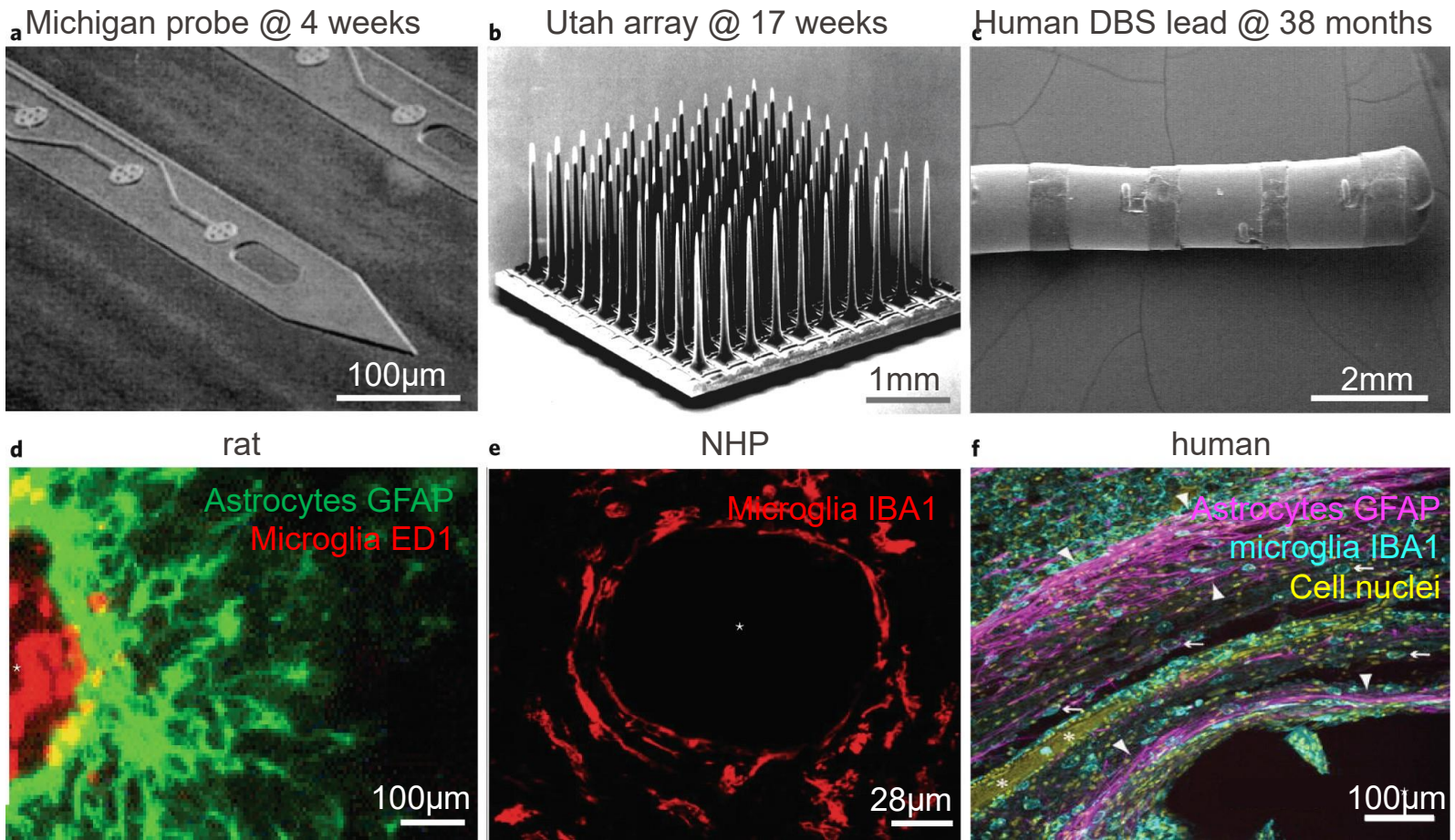
KATHE*, SKINIDER*, HUTDSON* ET AL. ¦ NATURE ¦ 2022

Faisceau corticospinal
Faisceau reticulospinal

4000 um

Videos par Q. Barraud, G. Courtine, EPFL

Gliosis

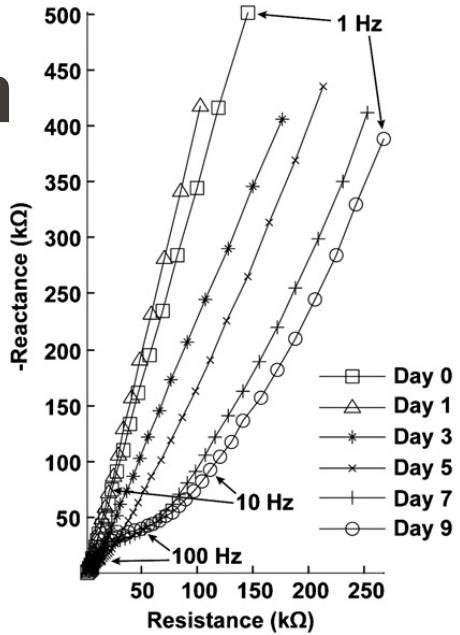


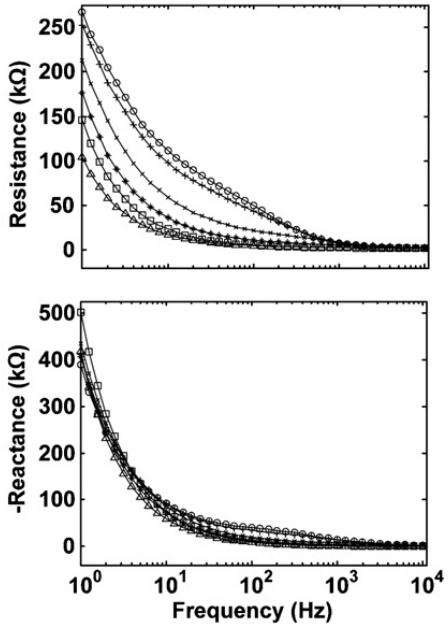
Nature Biomedical Engineering **volume 1**, pages 862–877 (2017)

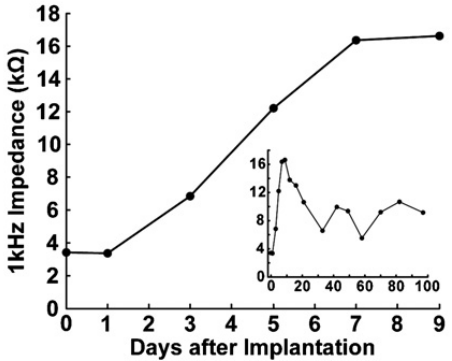
Electrode impedance and glial consolidation

DBS electrode impedance after implantation

Under constant-voltage stimulation, alteration of the current effectively reaching neural tissue



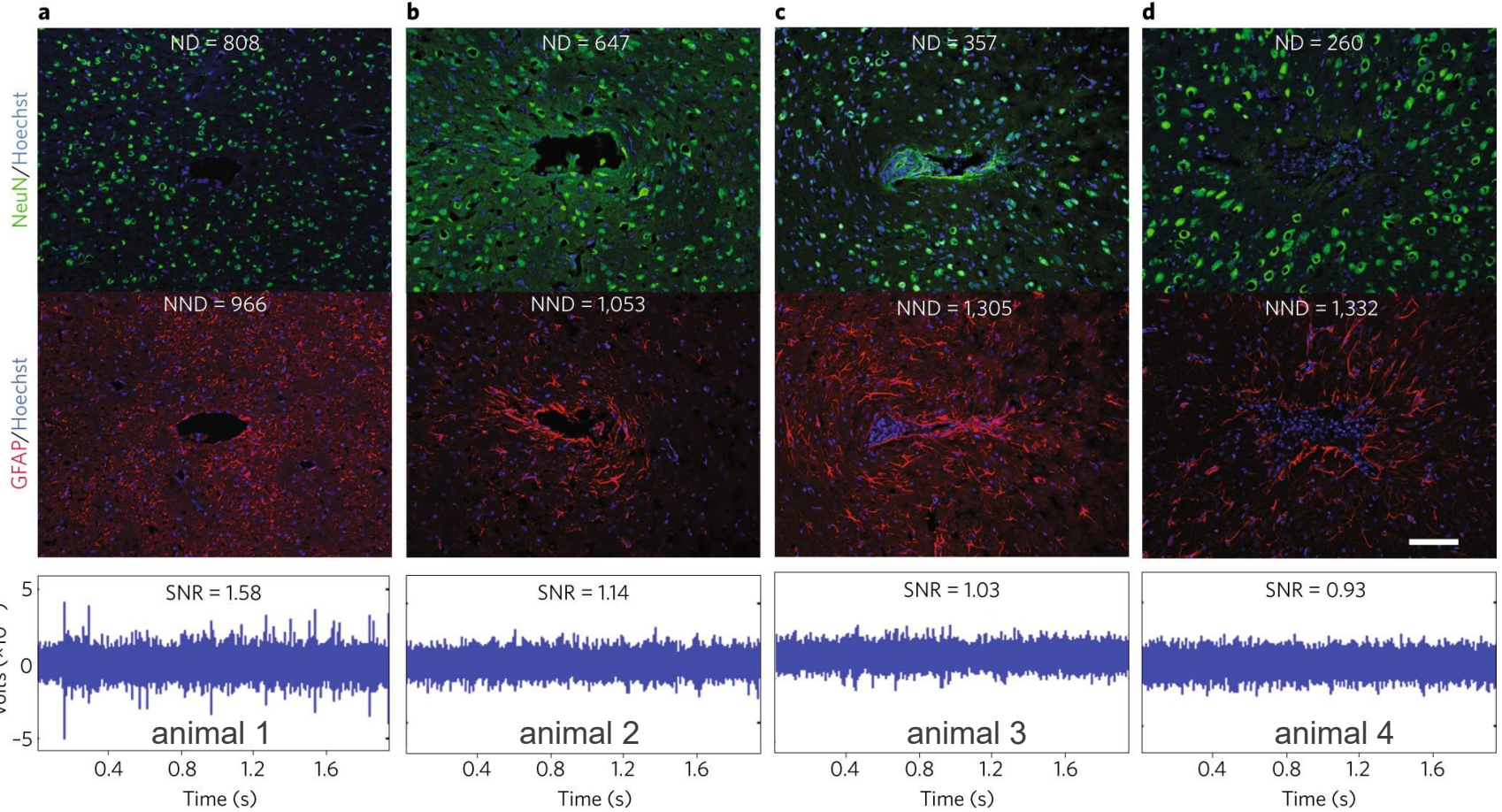




J. Neural Eng. 6 (2009) 046001

Negative impact on recording quality

ND neuronal density
NND non neuronal density
28 day post implantation



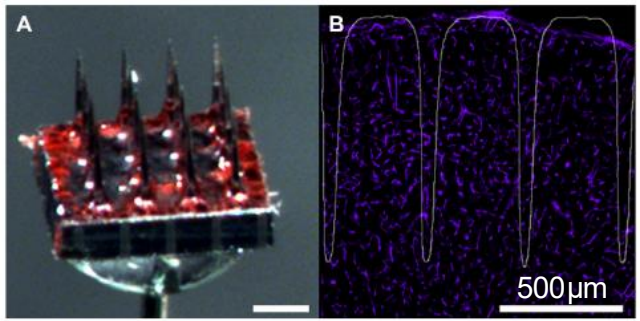
Nature Biomedical Engineering **volume 1**, pages 862–877 (2017)

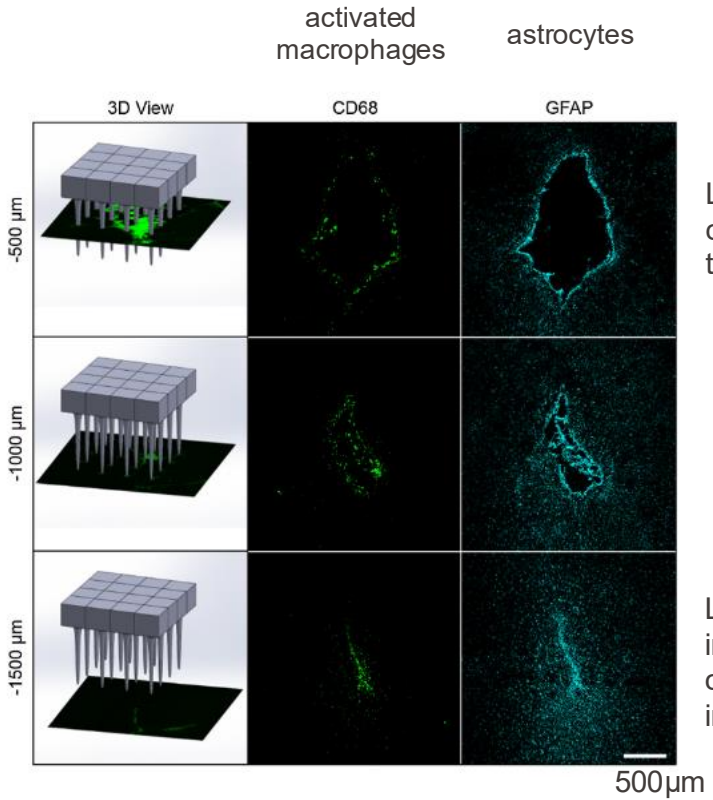
FBR and stab injury

4x4 Si shanks,
1mm long,
400µm apart
1.2x1.2 mm^2

Stab wound:
2min in then out

Histology at week 4





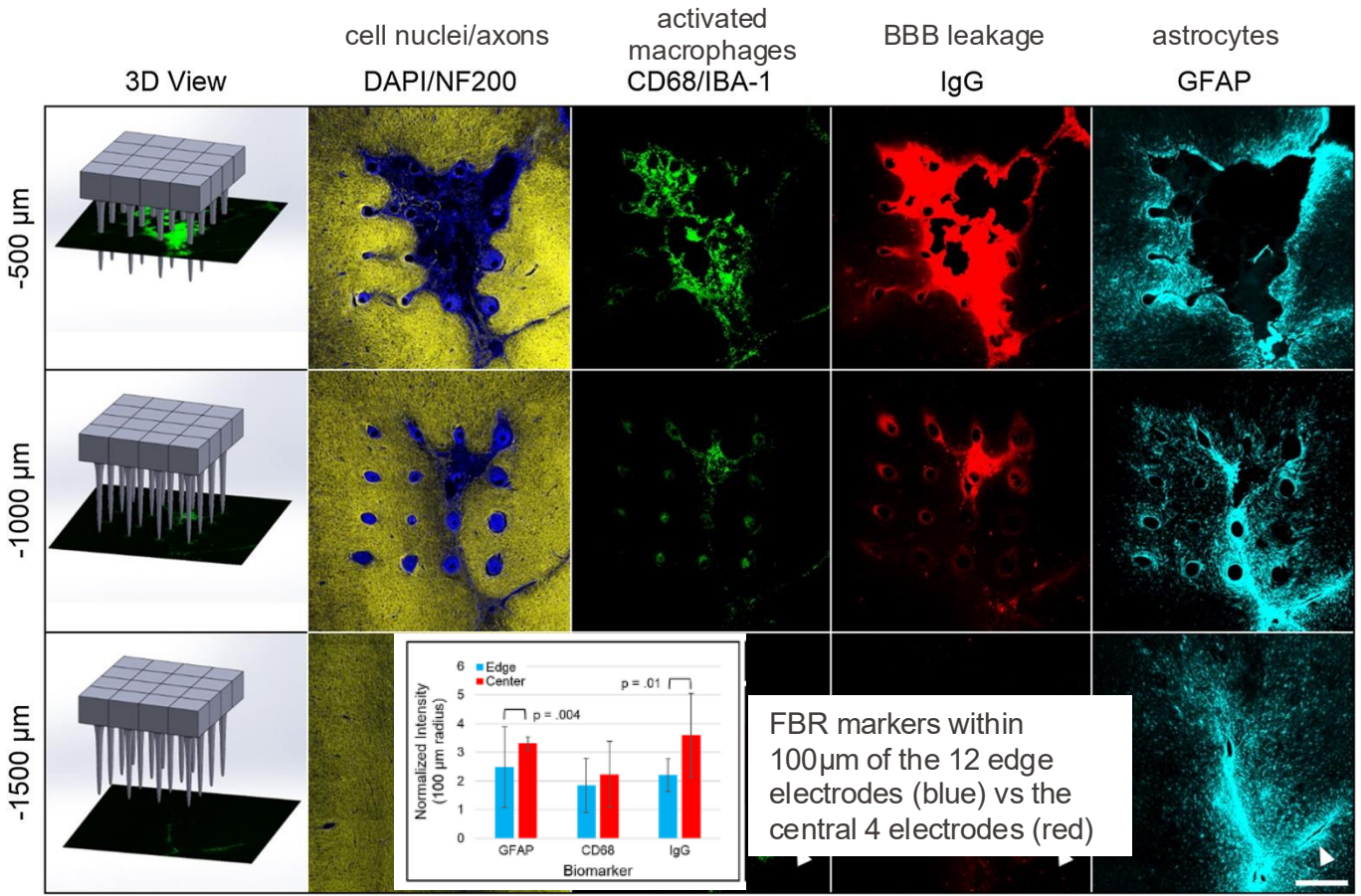
Biomaterials 53 (2015) 753-762

FBR and shank density

Biomaterials 53 (2015) 753-762

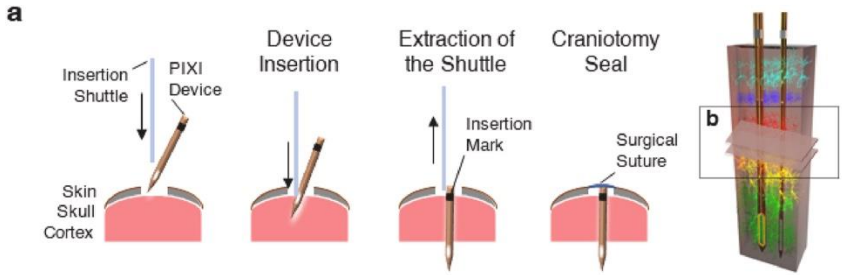
4x4 Si shanks,
1mm long,
400µm apart
1.2x1.2 mm^2

Timepoint: 4w

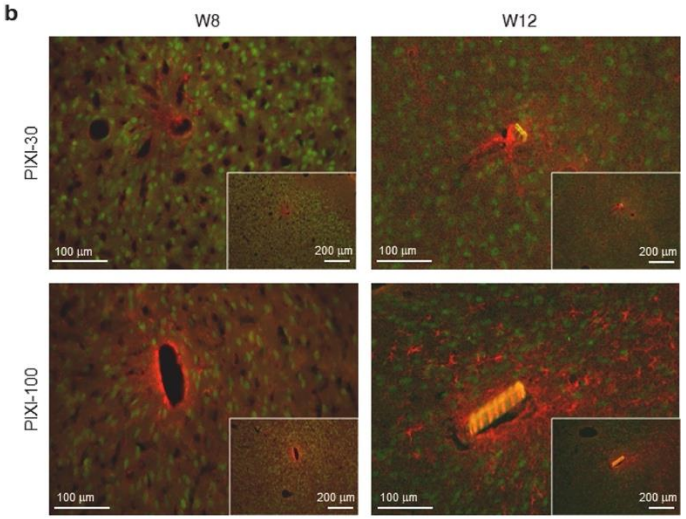


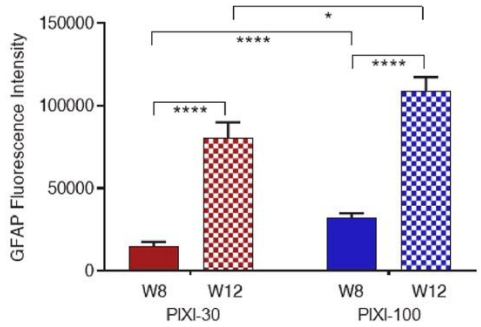
Effect of implant size on FBR

PIXI (PolyImide-based fleXible Intracortical) devices
30 or 100 µm in width
12 µm thick



Sections in layer IV of the cortex

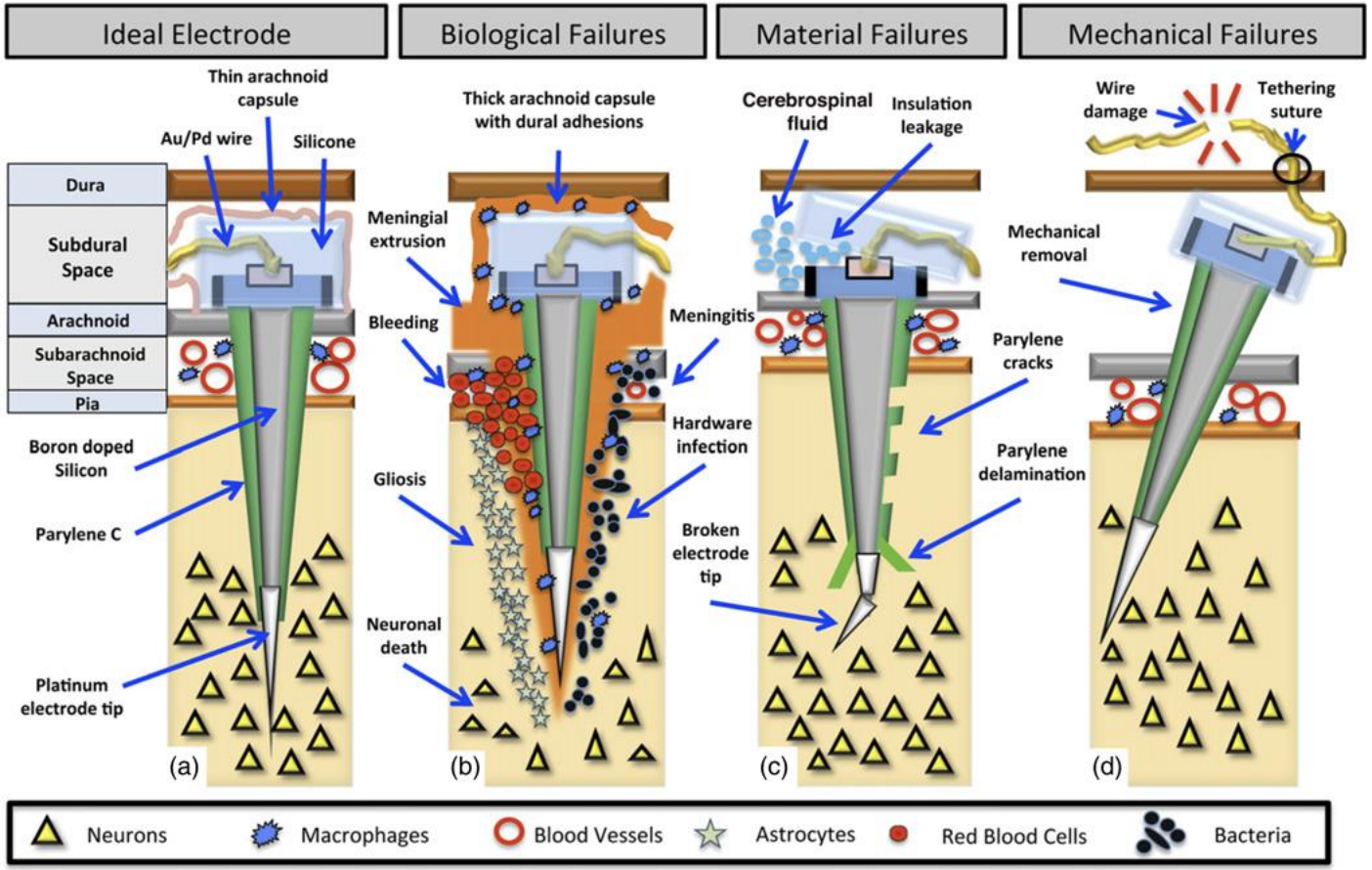




Footprint of scar depends on size of the implant

Biomaterials Volume 281, February 2022, 121372

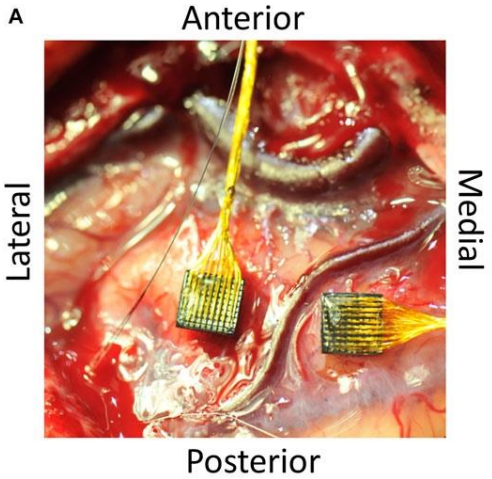
Implant damage

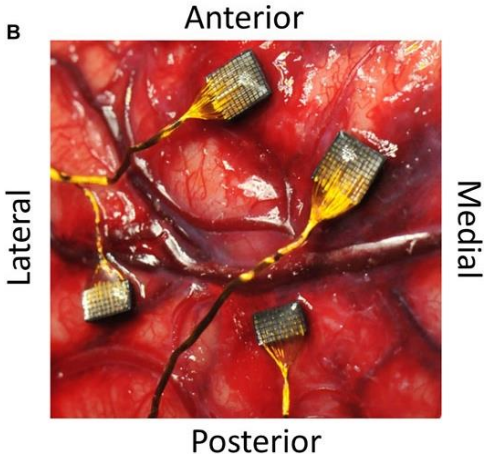


doi: 10.1088/1741-2560/10/6/066014

Materials failure
Study case.

- Two participants were implanted with 6 Utah arrays in the brain
  - P1: 2 platinum electrode recording in motor cortex; 980 days
  - P2: 2 platinum electrode recording in somatosensory cortex, 2 IrOx electrode stimulating in parietal cortex; 180 days





https://doi.org/10.3389/fbioe.2021.759711

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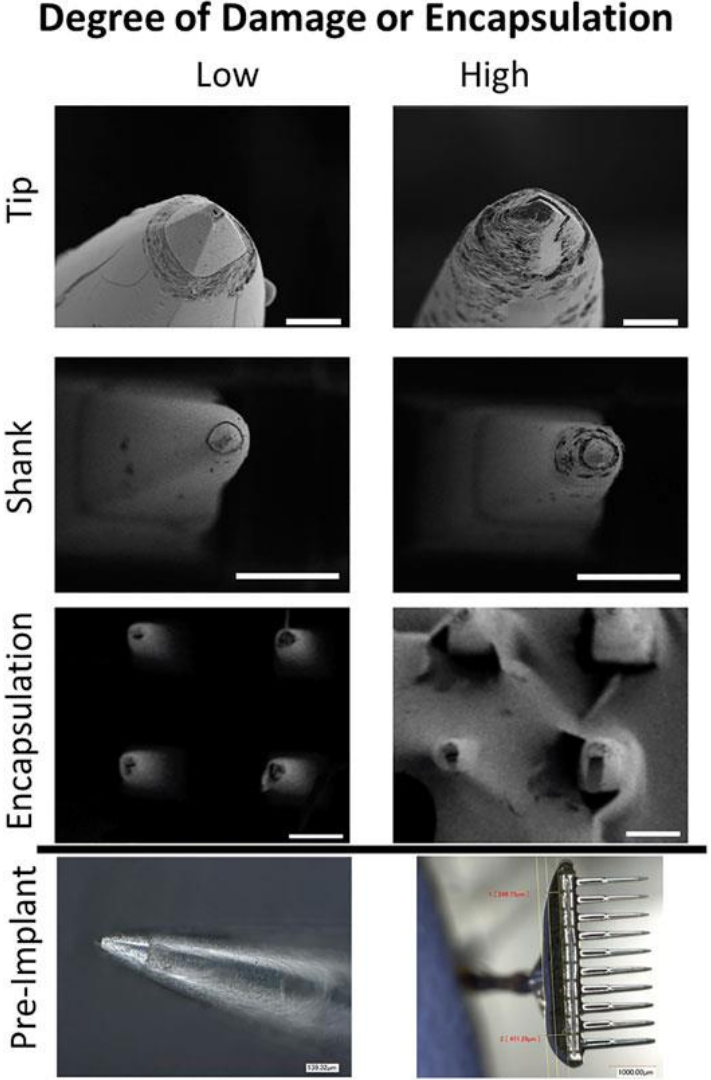
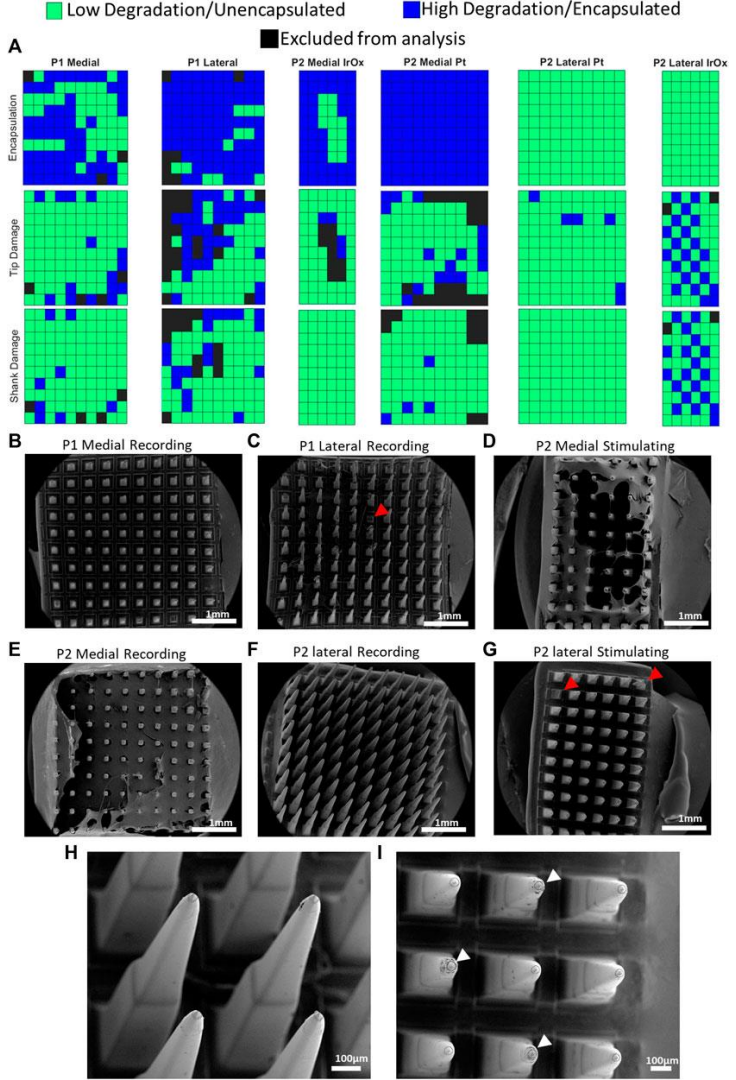
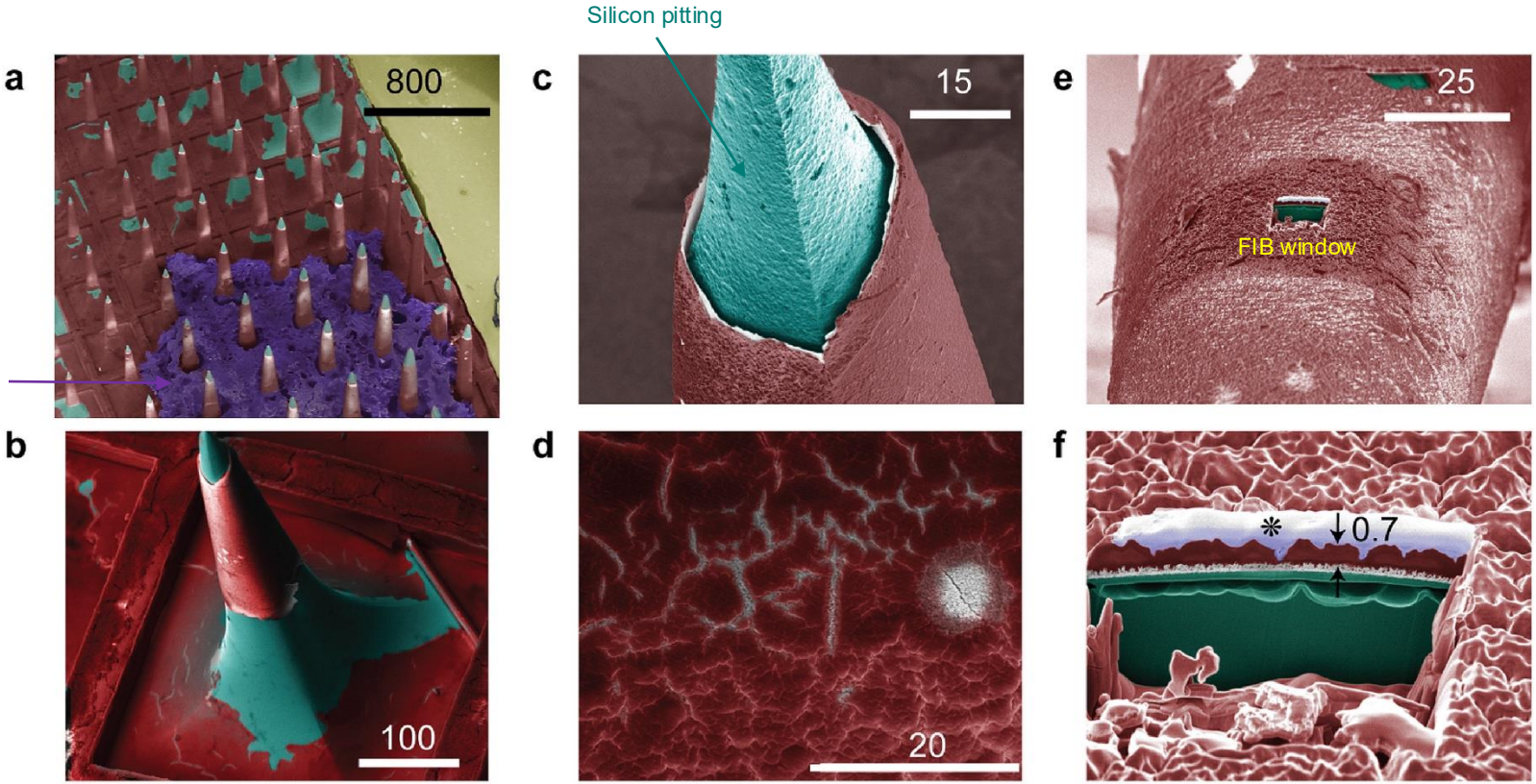




Parylene damage in vivo

Metal tip damage
Parylene degradation

residual tissue



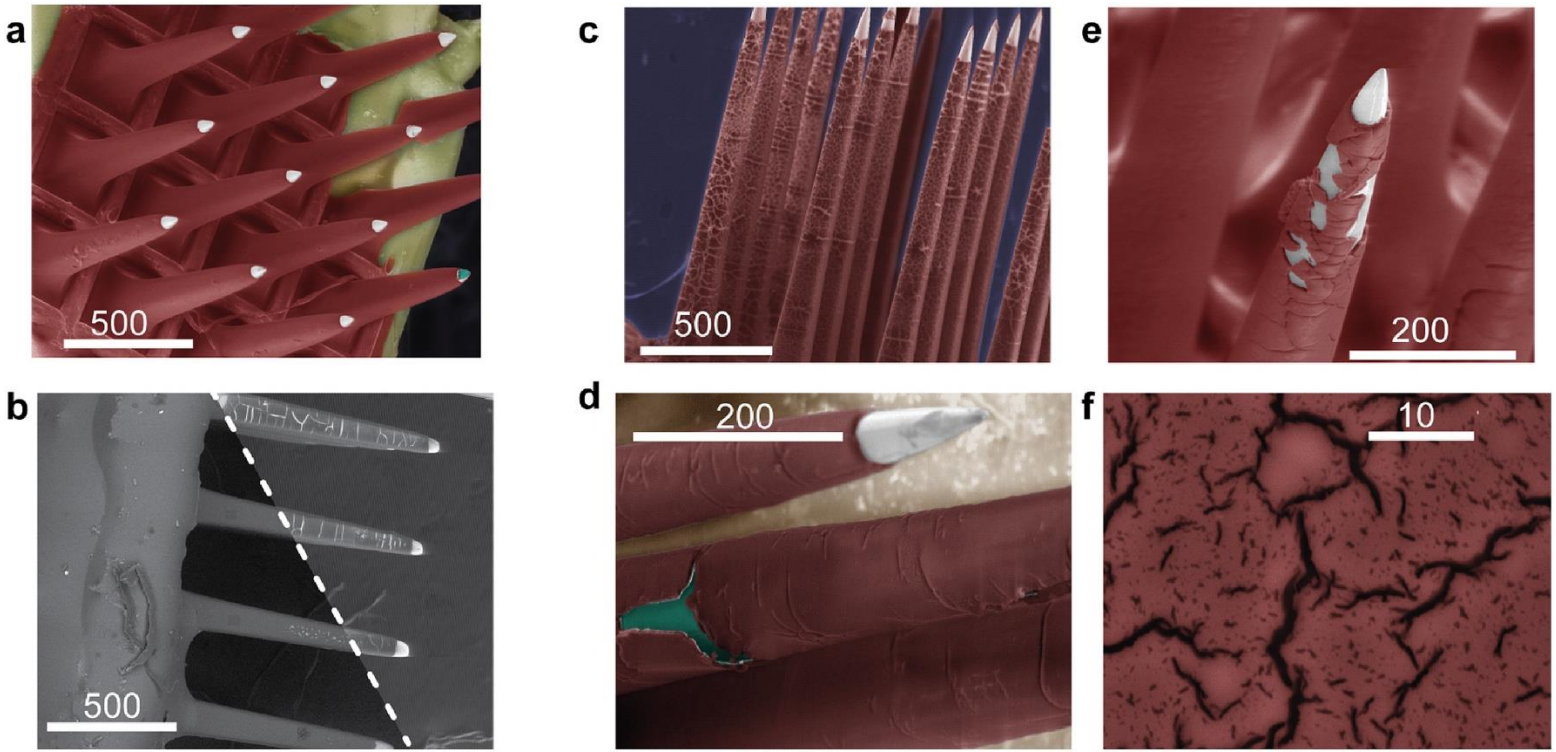
Explanted after 3.25 years in cat sciatic nerve

Biomaterials Volume 232, February 2020, 119731

Accelerated aging in reactive environment

devices aged in PBS at 37°C

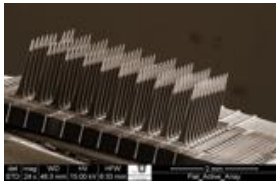
devices aged using RAA (PBS + 20 mM H_2O_2) at 67 °C



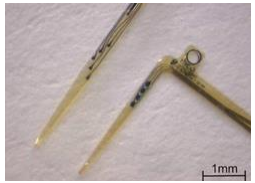
Biomaterials Volume 232, February 2020, 119731

Intracortical probes

INTERFACES



‘1991’ intracortical
Si microneedle implant



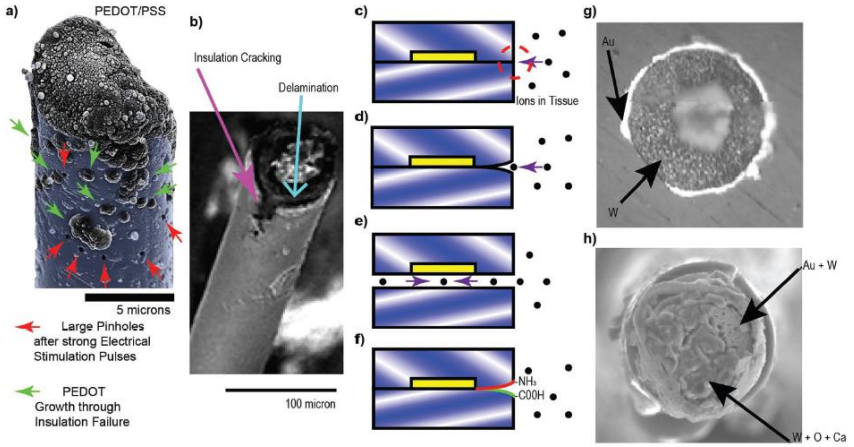
‘2000’ intrafascicular
polyimide implant

E

100GPa | 1GPa | 1MPa | 1kPa

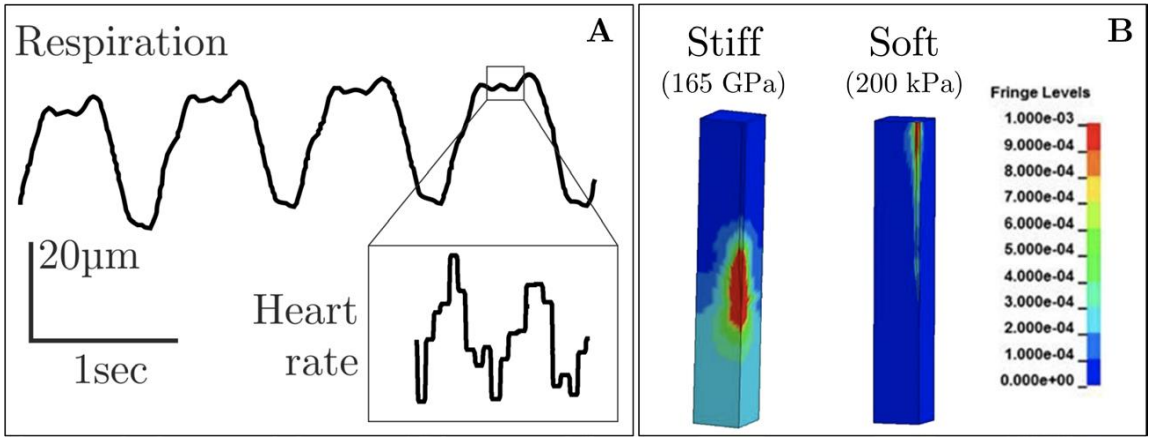
stiff interfaces

static interfaces



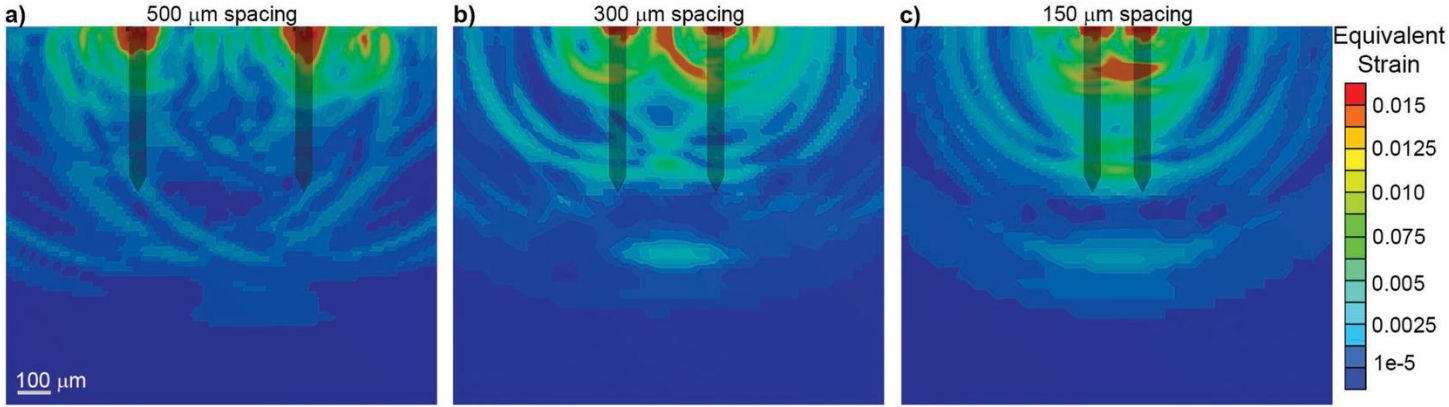
SM Wellman et al, Adv. Funct. Mat. | 2017

Probe mechanics – dynamic behaviour



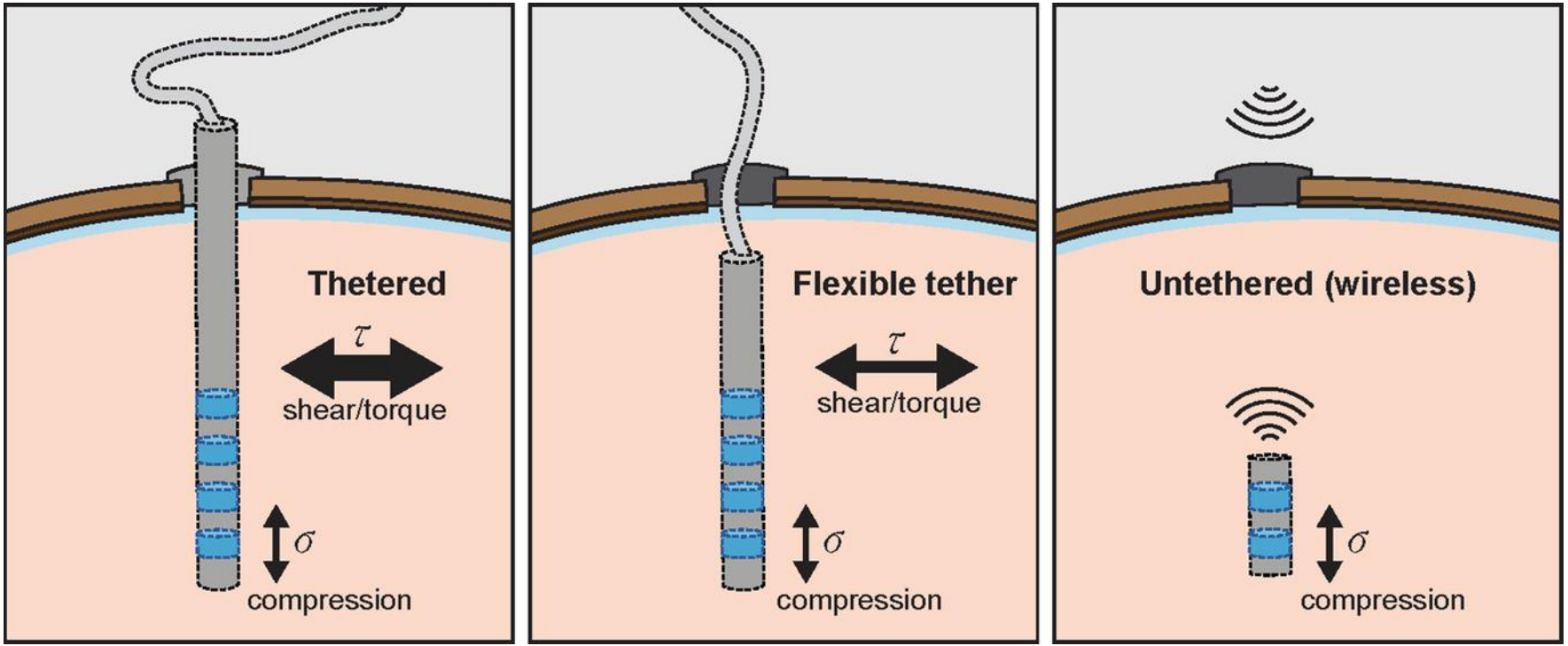
Modelled strain distribution at the probe/tissue interface using a micromotion displacement of 4 µm

J. Neural Eng. 15 (2018) 031001



Closely packed stiff shanks lead to high strain zone in the surrounding tissue

Probe tethering

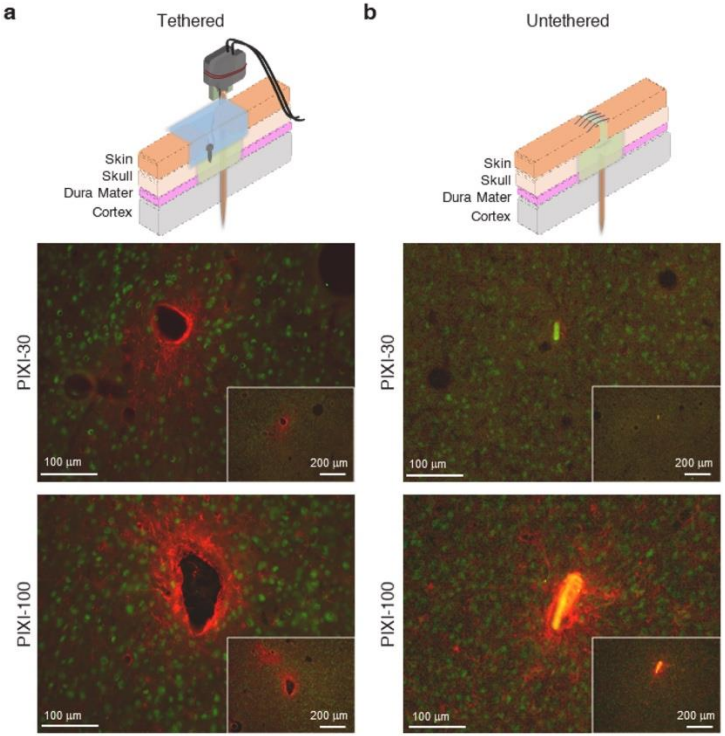


Neural Interface Engineering, pp 123–172

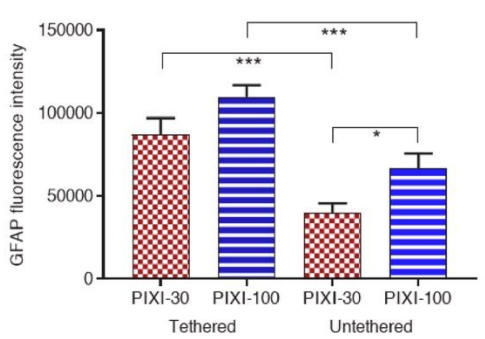
Effect of tethering on FBR

Biomaterials Volume 281, February 2022, 121372

12w post-implantation

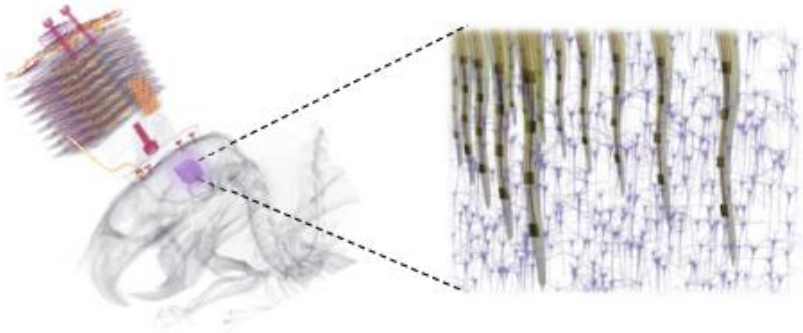


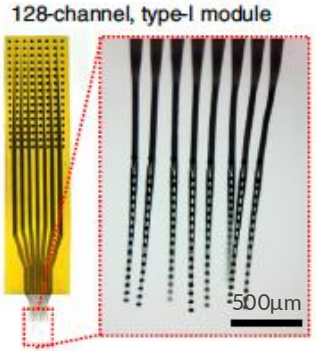
GFAP
NeuN

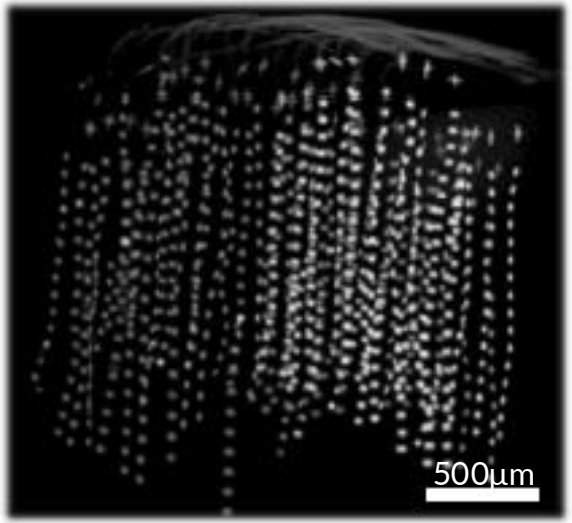


"smaller and untethered devices are the least harmful for the brain"

Ultra-miniaturised implant: NET electrodes







NET: ultraflexible NanoElectronic Thread electrodes

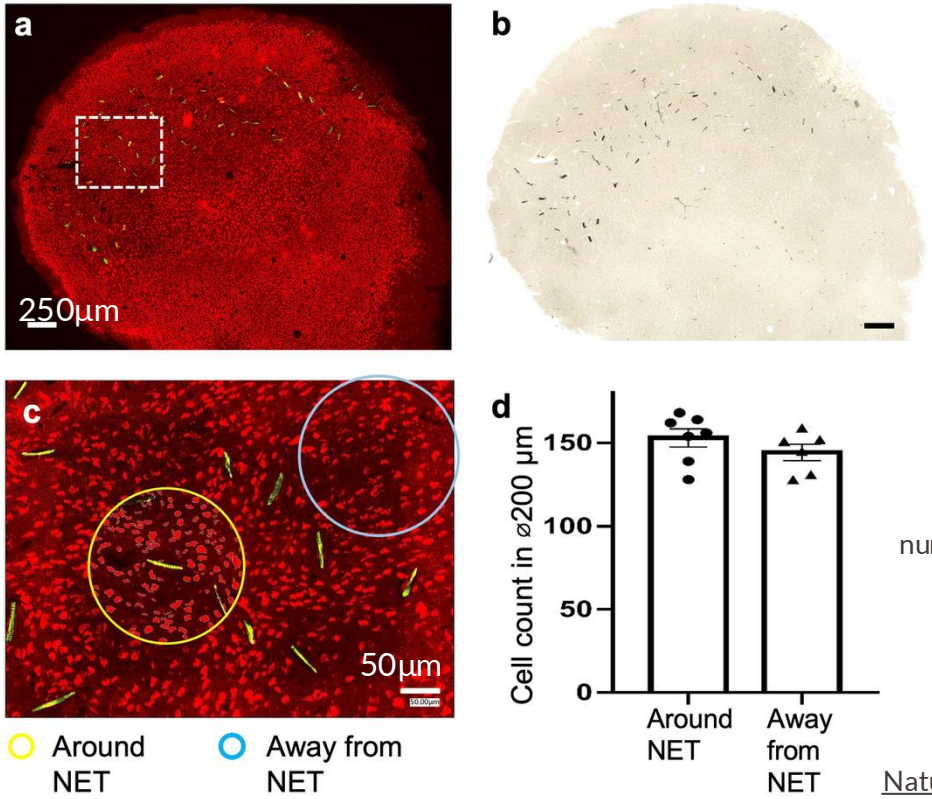
section ~ soma
SU-8 / Pt ou Au

1 x 1 x 1 mm^3
(8x8x16) 1'024 électrodes

Nature BME **7**, 520-532 (2022)

Biointegration

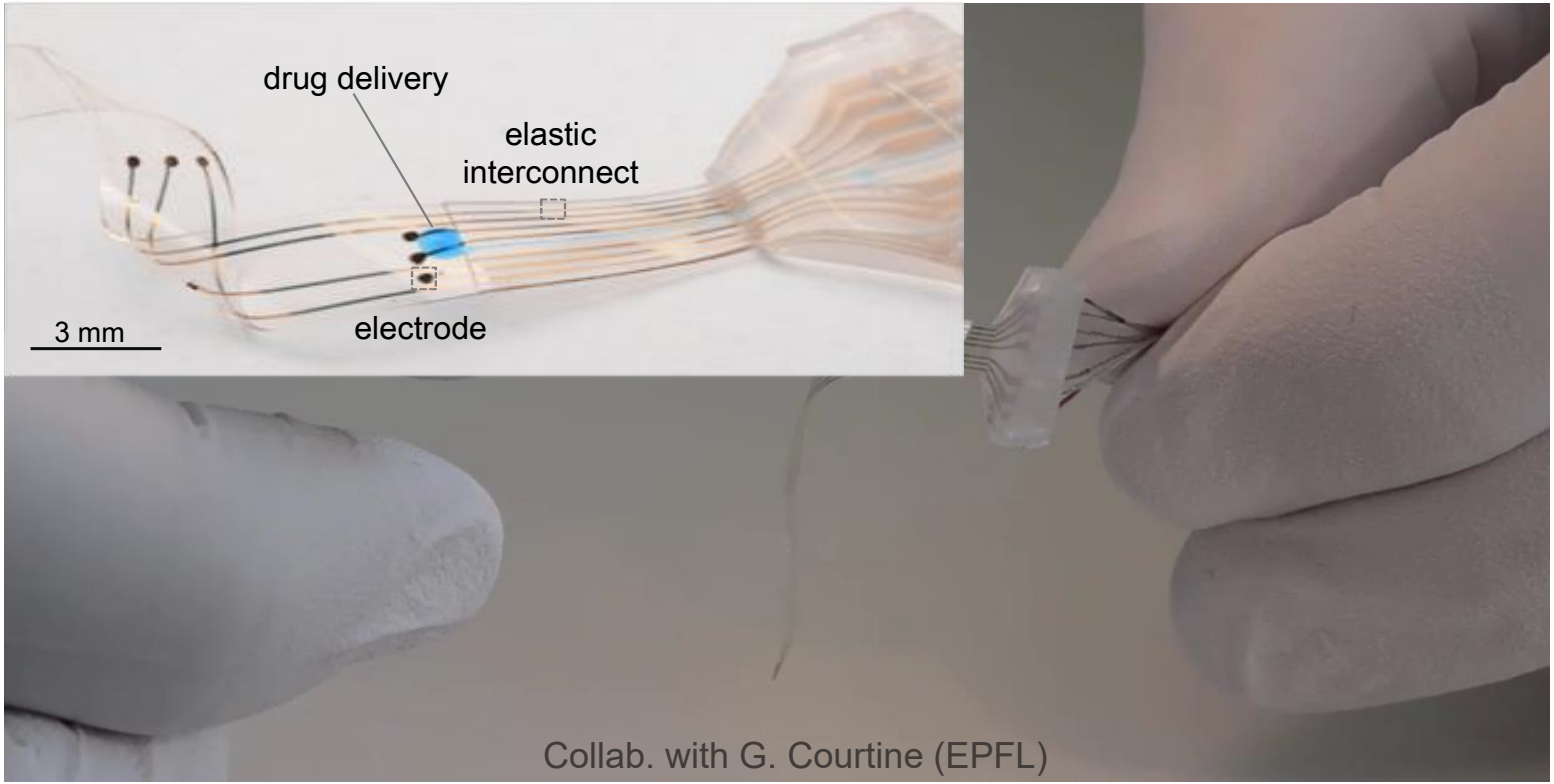
Implantation (>1 month)
visual cortex
mouse



number of neurons

Nature BME **7**, 520-532 (2022)

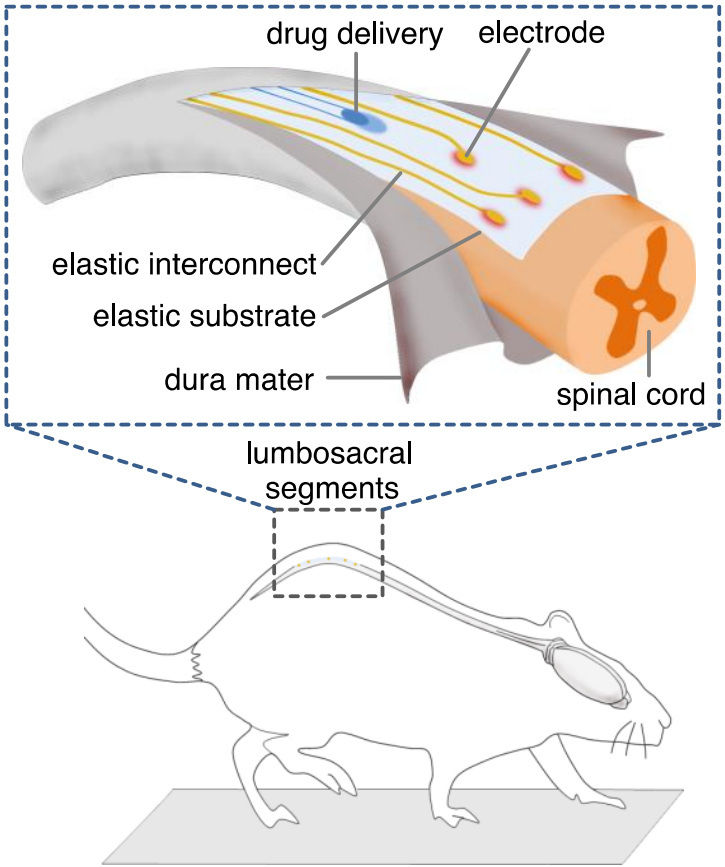
Matching mechanics

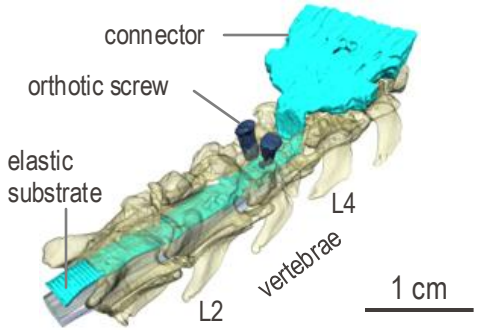


Collab. with G. Courtine (EPFL)

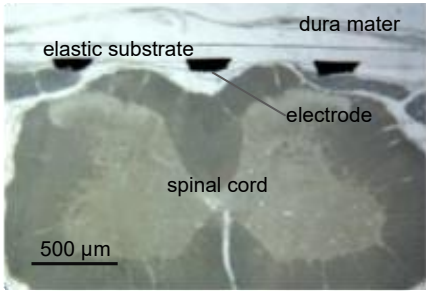
I. Minev*, P. Musienko* et al.| Science |2015

Electronic dura mater





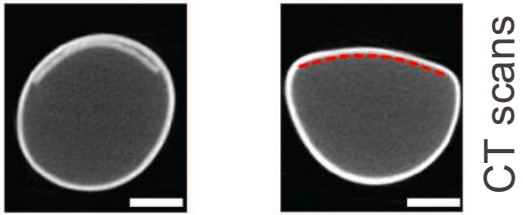
biointegration
in the spinal subdural space
@ 2 month post implantation



Minev*, Musienko » et al, **Science** 2015

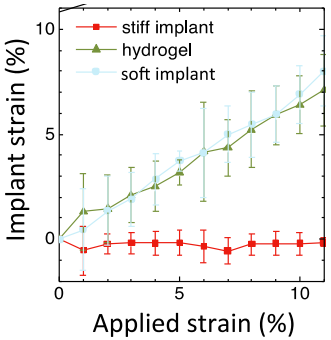
Does softness matter?

Bending stiffness ⇨ macroscopic distortion



soft implant stiff implant

Elastic stiffness ⇨ motion restriction

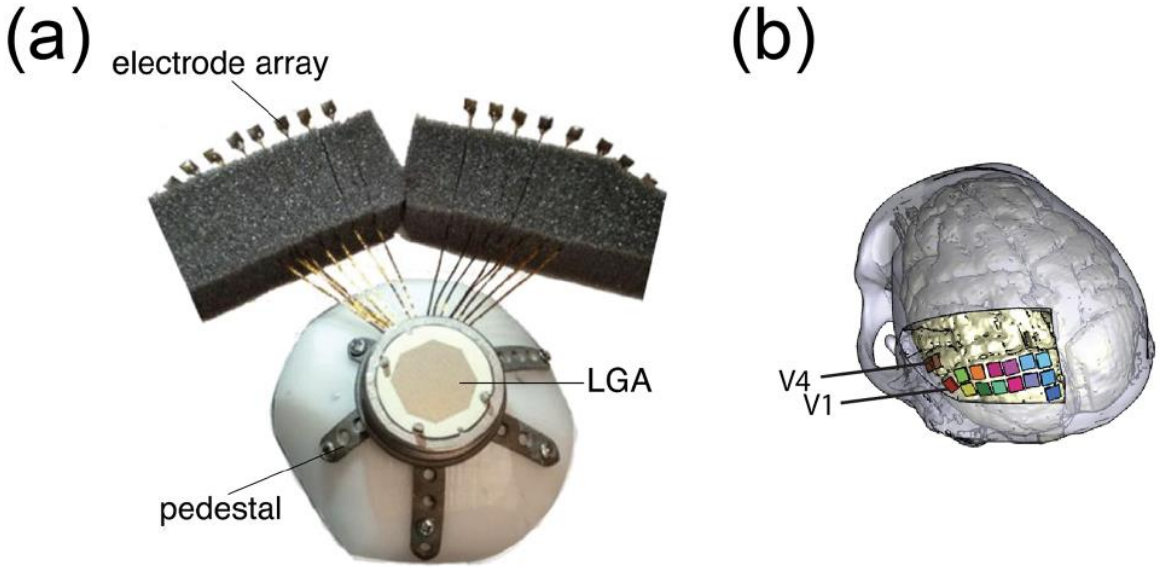


Minev*, Musienko » et al, **Science** 2015

Take-home messages

- Foreign Body Reaction is inevitable with current neural interface designs
- An acute phase and a chronic phase
  - Activation of glial cells – implant “encapsulation” (another meaning of the term)
- FBR has biological consequences on the host tissue
- FBR challenges the integrity and stability of neural interfaces
- Design features can modulate FBR: mechanics, geometry, surface chemistry, tethering, etc.

Chronic stability with multiple adjacent Utah arrays

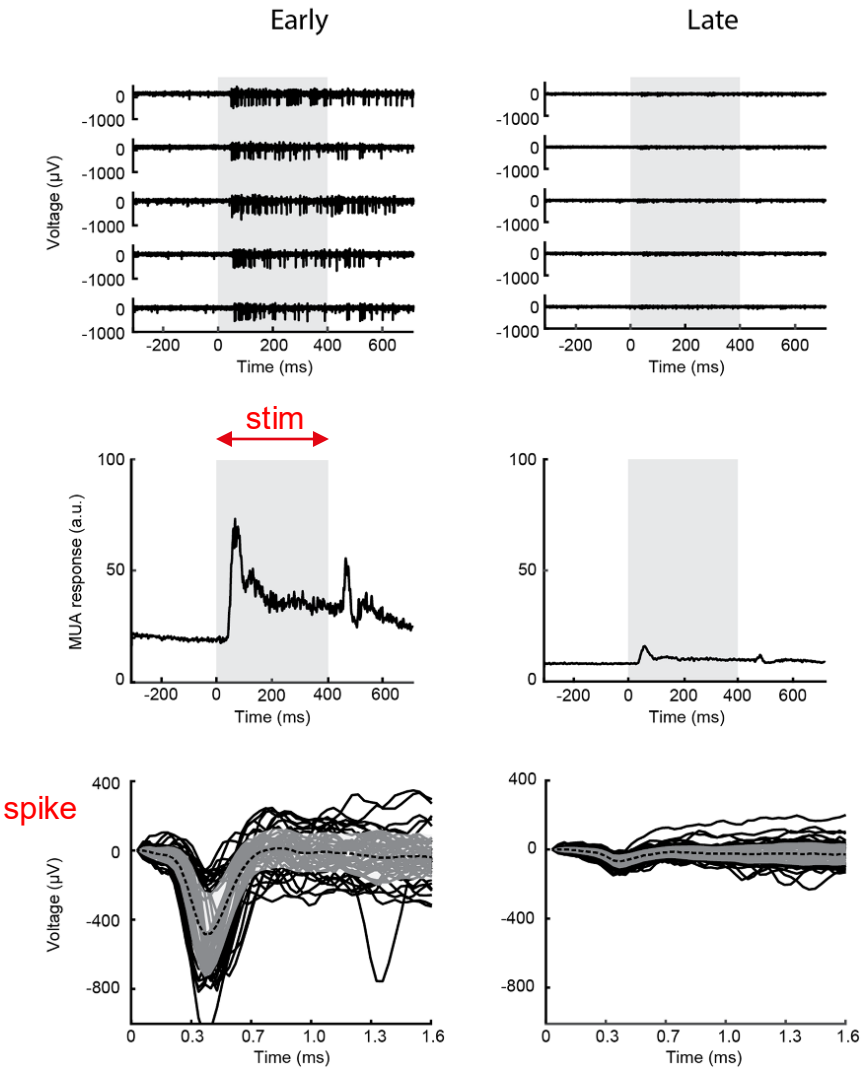


Journal of Neural Eng. 2023 **20** 036039

Representative recordings

Early: 91 days
Late: 279 days

MUA: multiunit activity

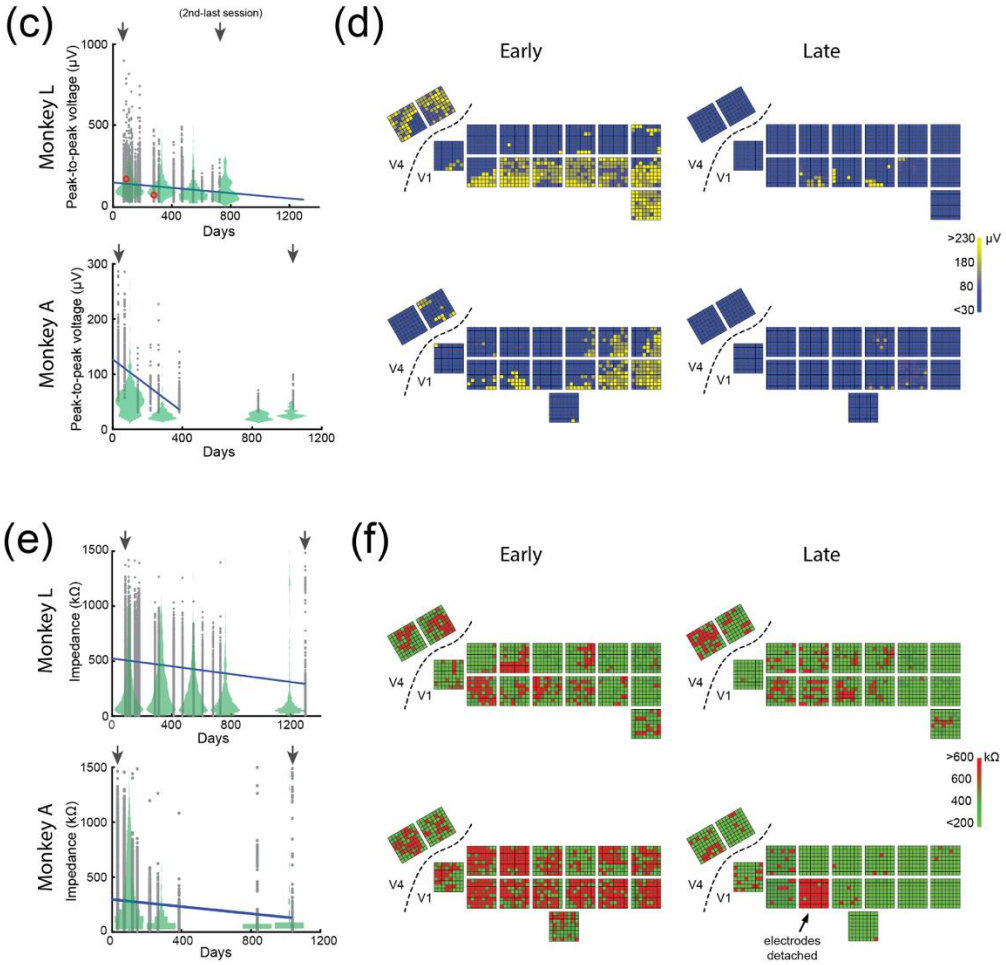


Journal of Neural Eng. 2023 20 036039

Representative recordings across all arrays

Early: 91 days
Late: 279 days

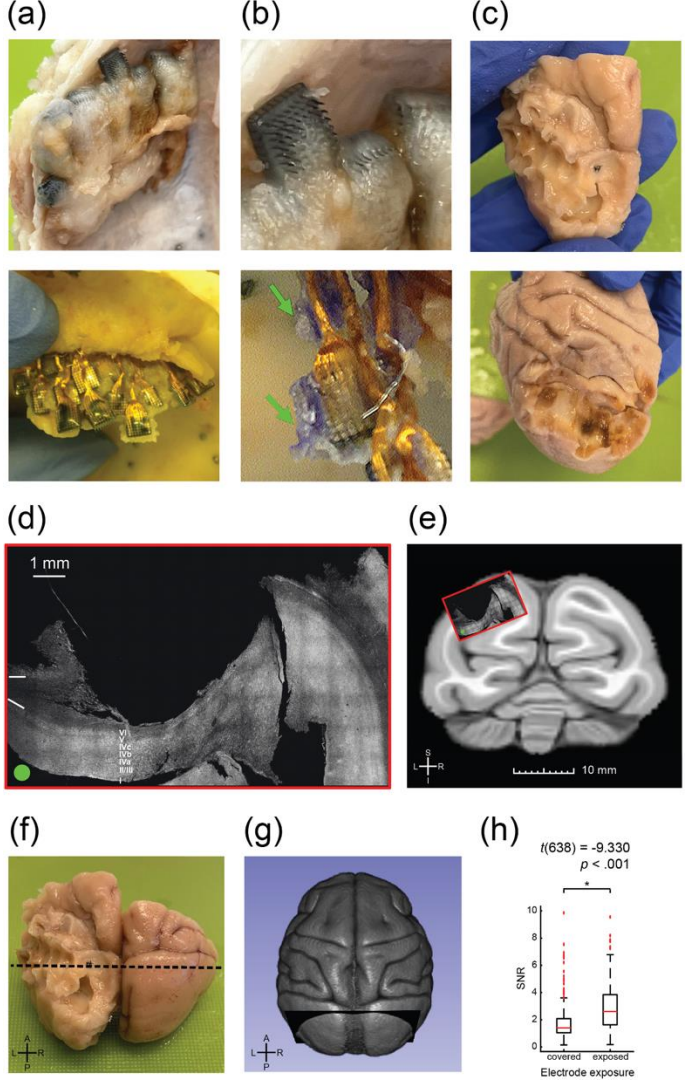
MUA: multiunit activity



Journal of Neural Eng. 2023 20 036039

Tissue response and histology

Day 638



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Explanted arrays

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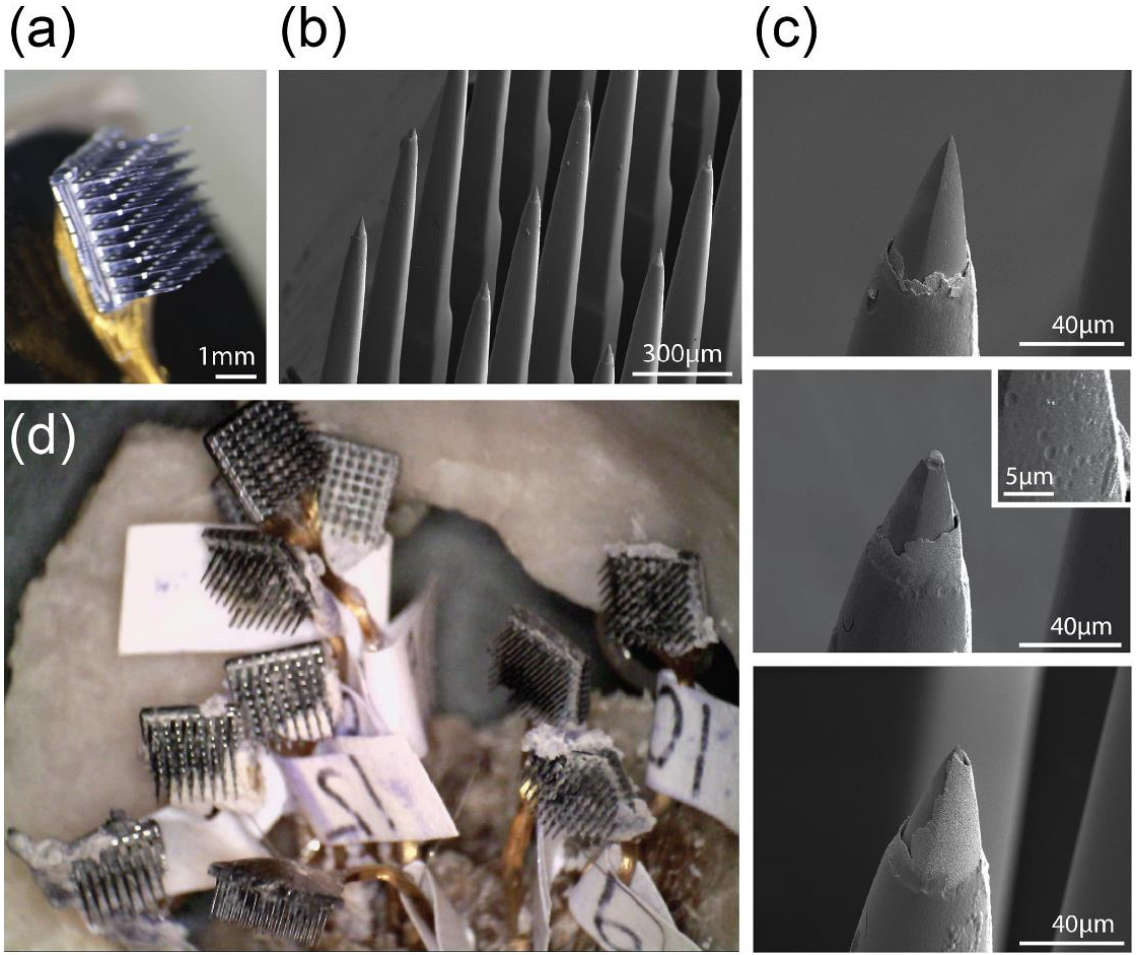
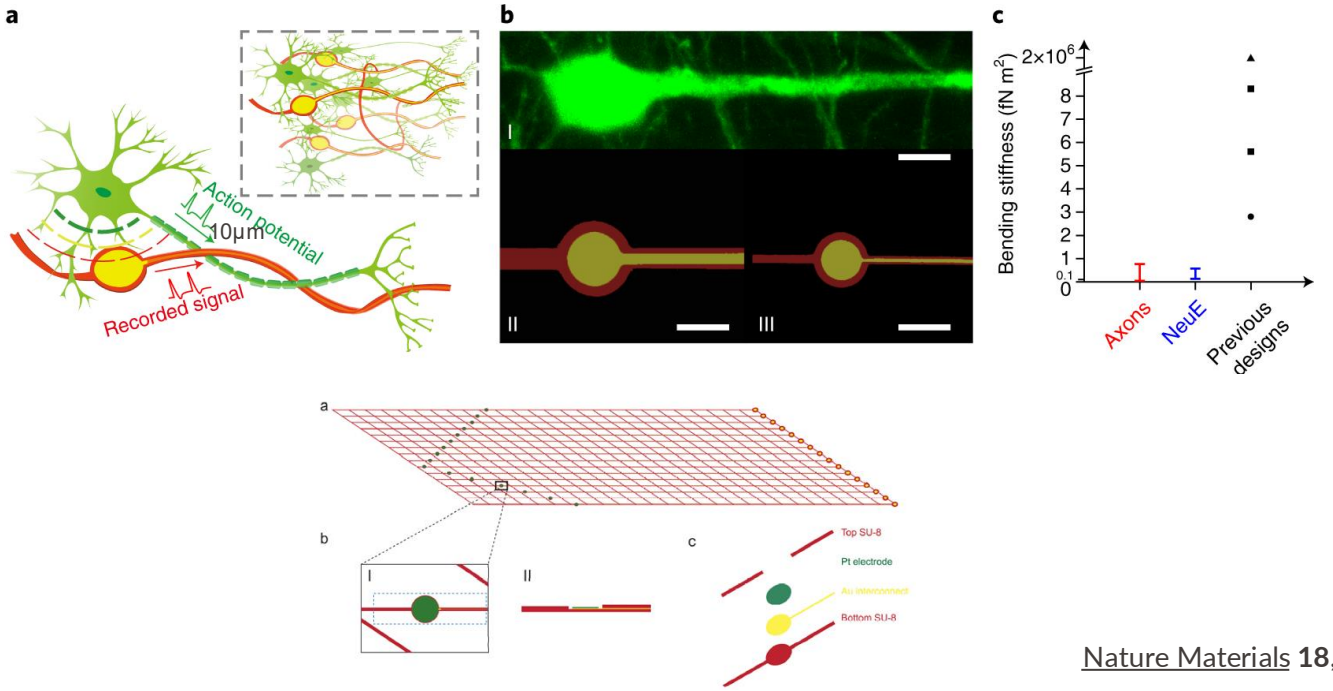


damaged IrOx

broken Si tip

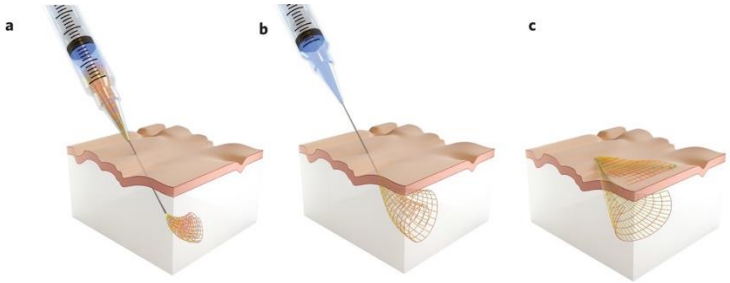
pitting Si surface

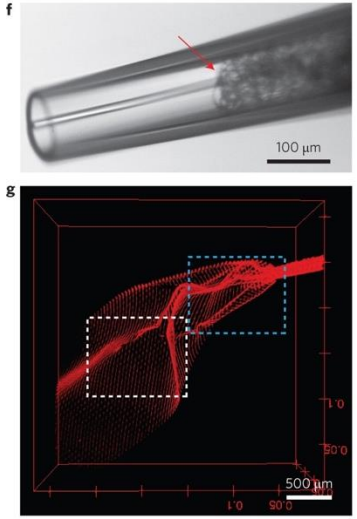
Nanomesh design

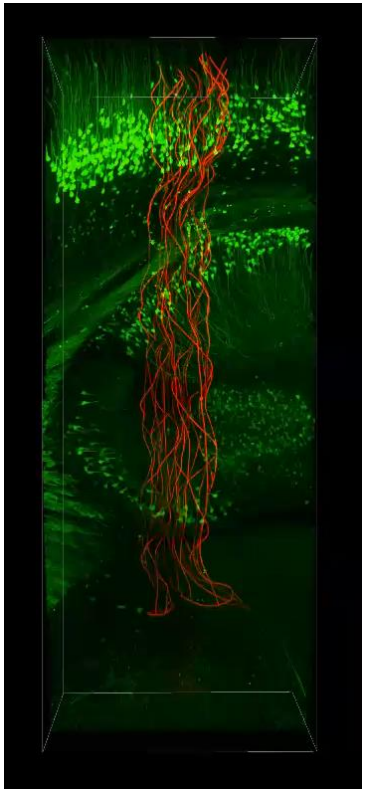


Nature Materials **18**, 510–517 (2019)

Nanomesh electrodes in vivo



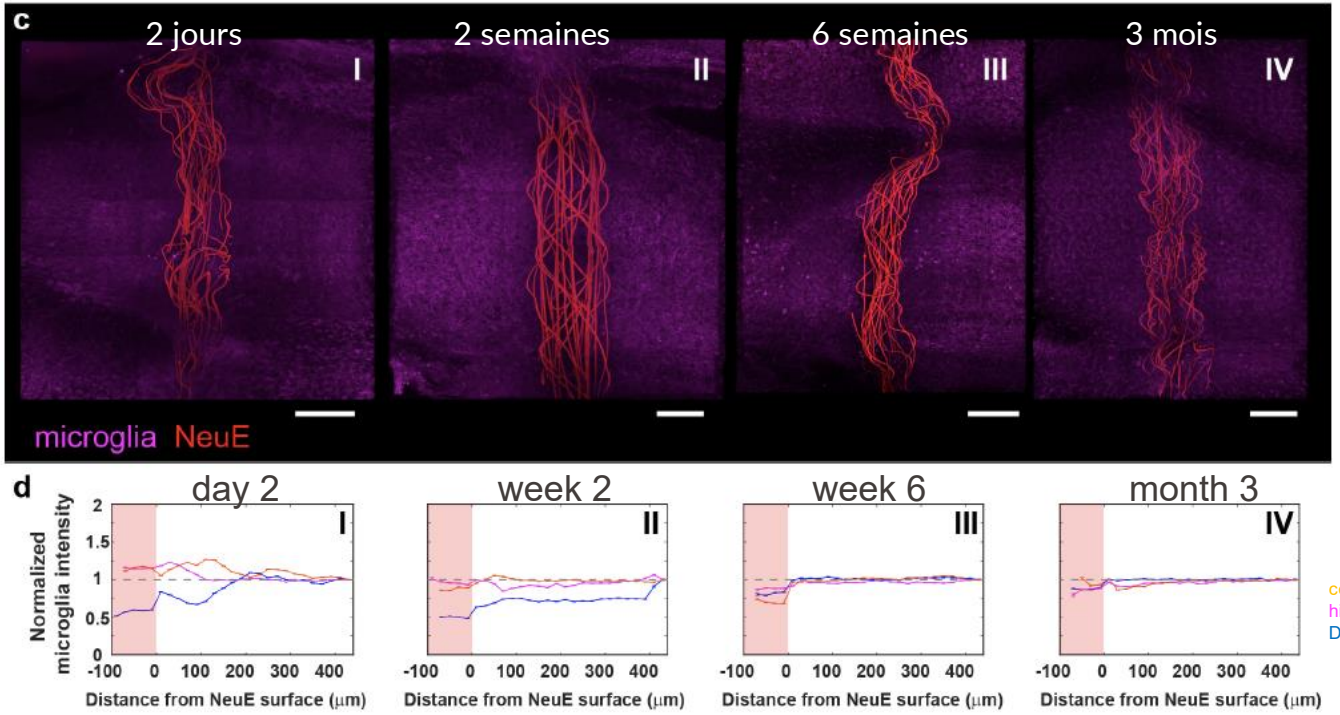




Suspension dans une solution saline et injection dans le cortex (95µm dia.)

Nature Nanotech **10**, 629-636 (2015)
Nature Materials **18**, 510–517 (2019)

Glial cells and electrodes over time



cortex (orange),
hippocampal CA1 (magenta)
DG (blue)

Nature Nanotech **10**, 629-636 (2015)
Nature Materials **18**, 510–517 (2019)