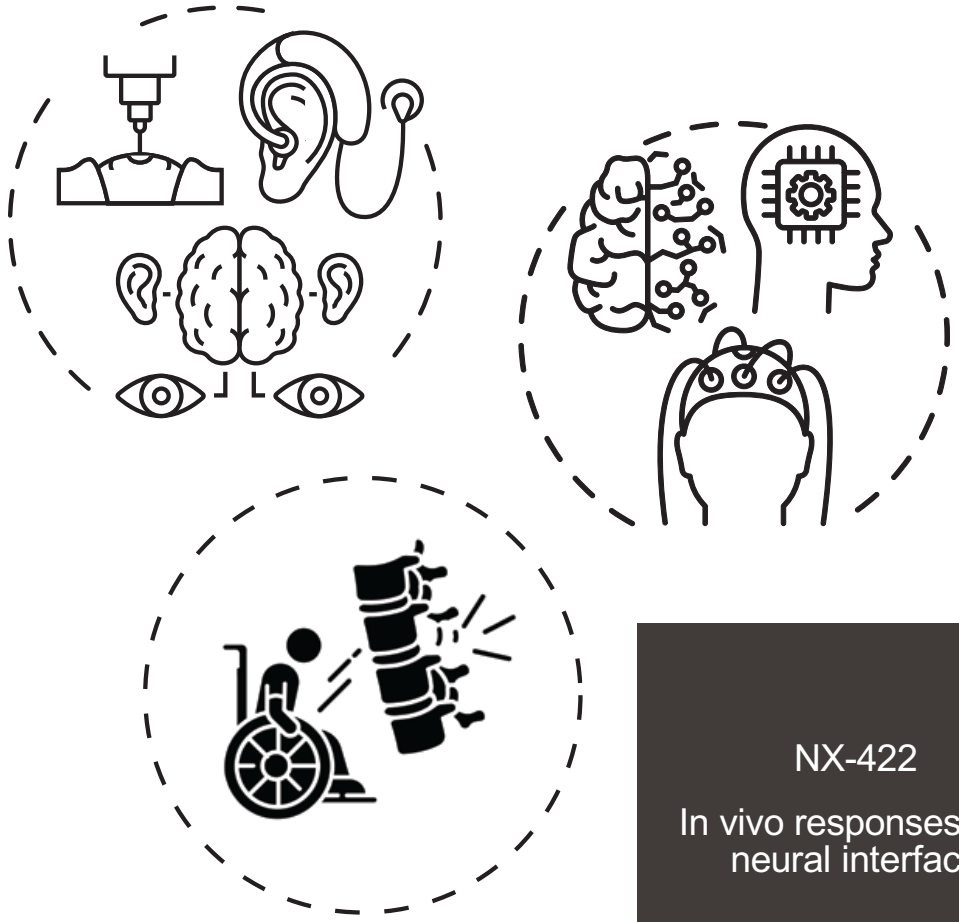


Neural Interfaces

NX-422

In vivo responses to a neural interface

Stéphanie P. Lacour

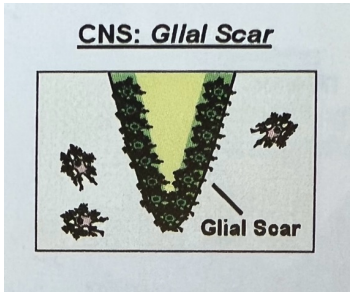
Neuro-X Institute

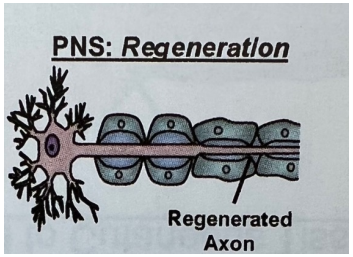
Topics

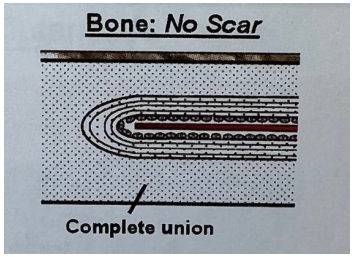
- Foreign body reaction (FBR)
- Performance of current implantable neural interfaces
- Investigation methods of FBR in vivo
- Strategies to modulate FBR and implant failure

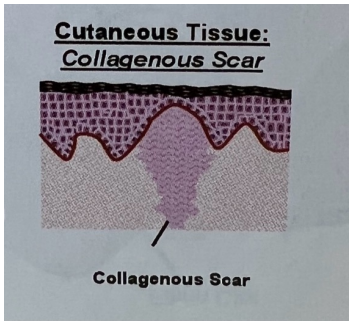
Foreign Body Reaction (FBR)

- 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.

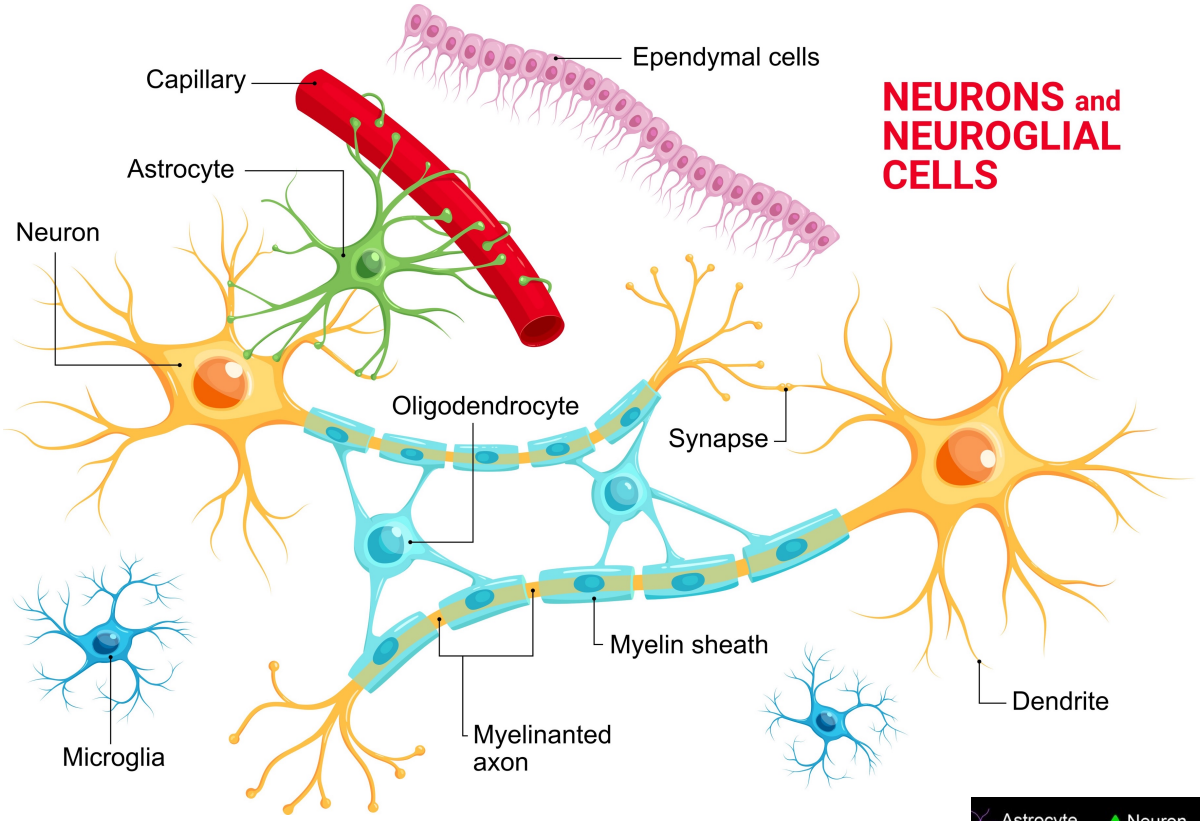


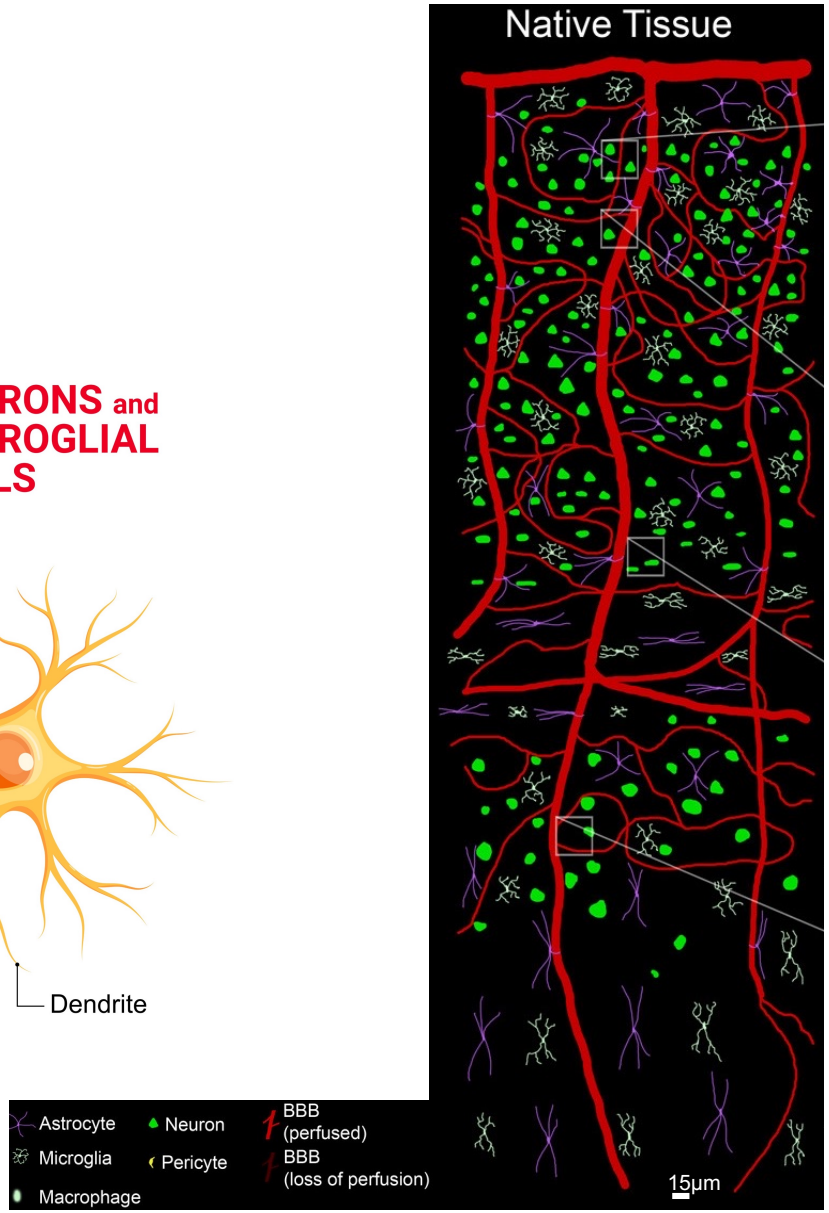






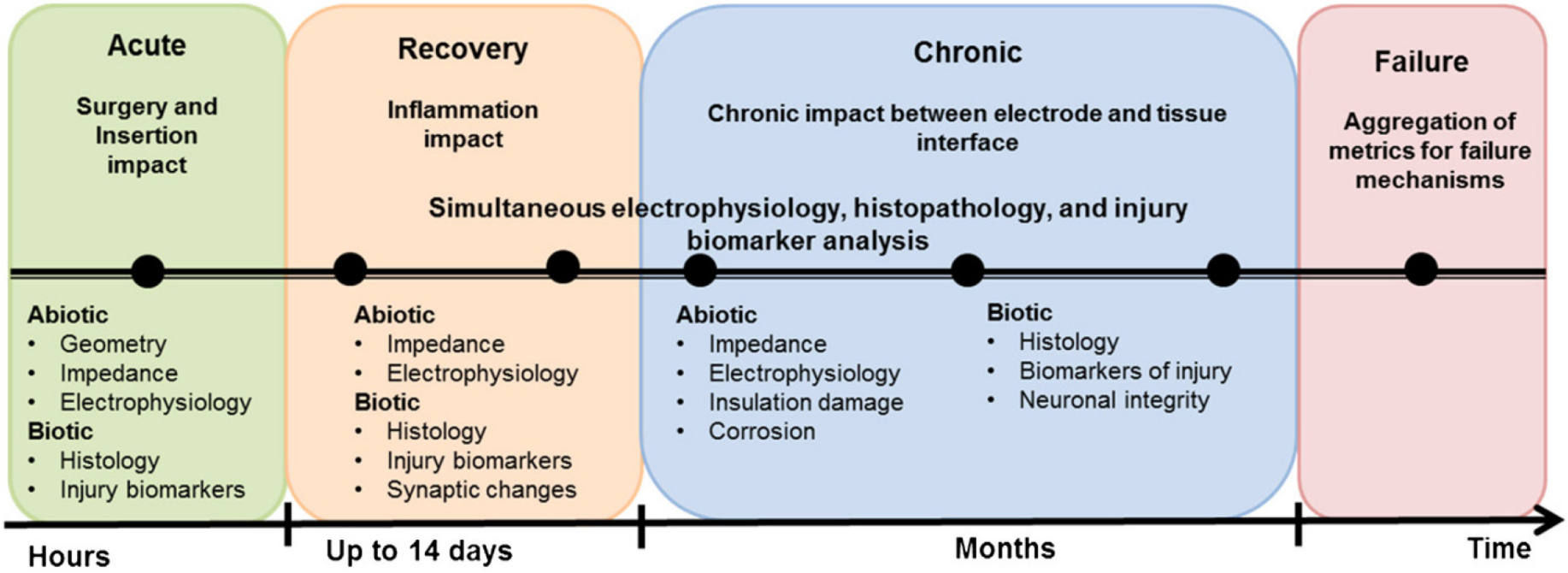
Participants in the CNS





ACS Chem. Neurosci. 2015, 6, 1, 48–67

Timeline of major events following chronic implantation *in vivo*



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

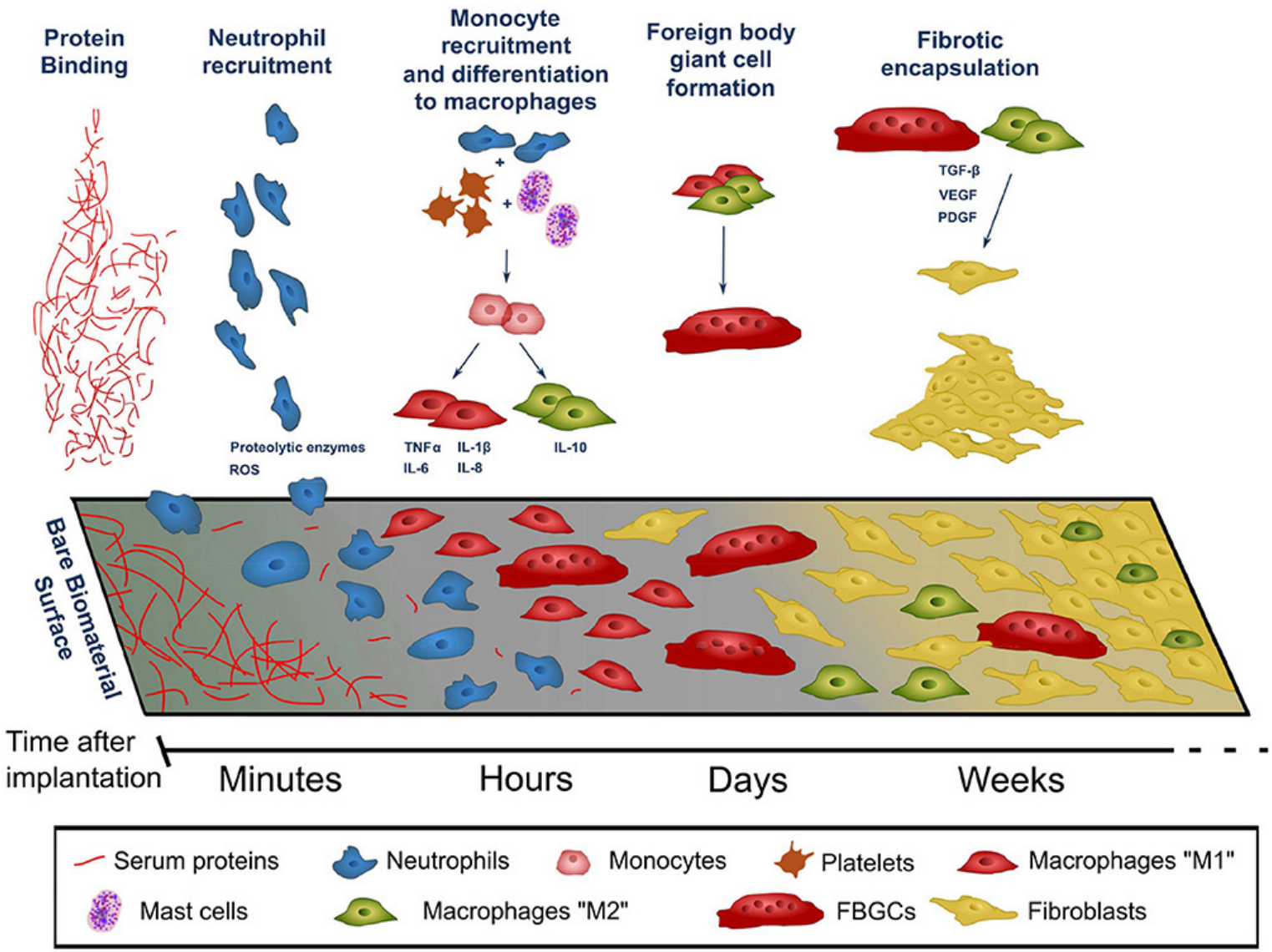
NX-422 ©LSBI

Timeline

Acute (inflammation)

Then

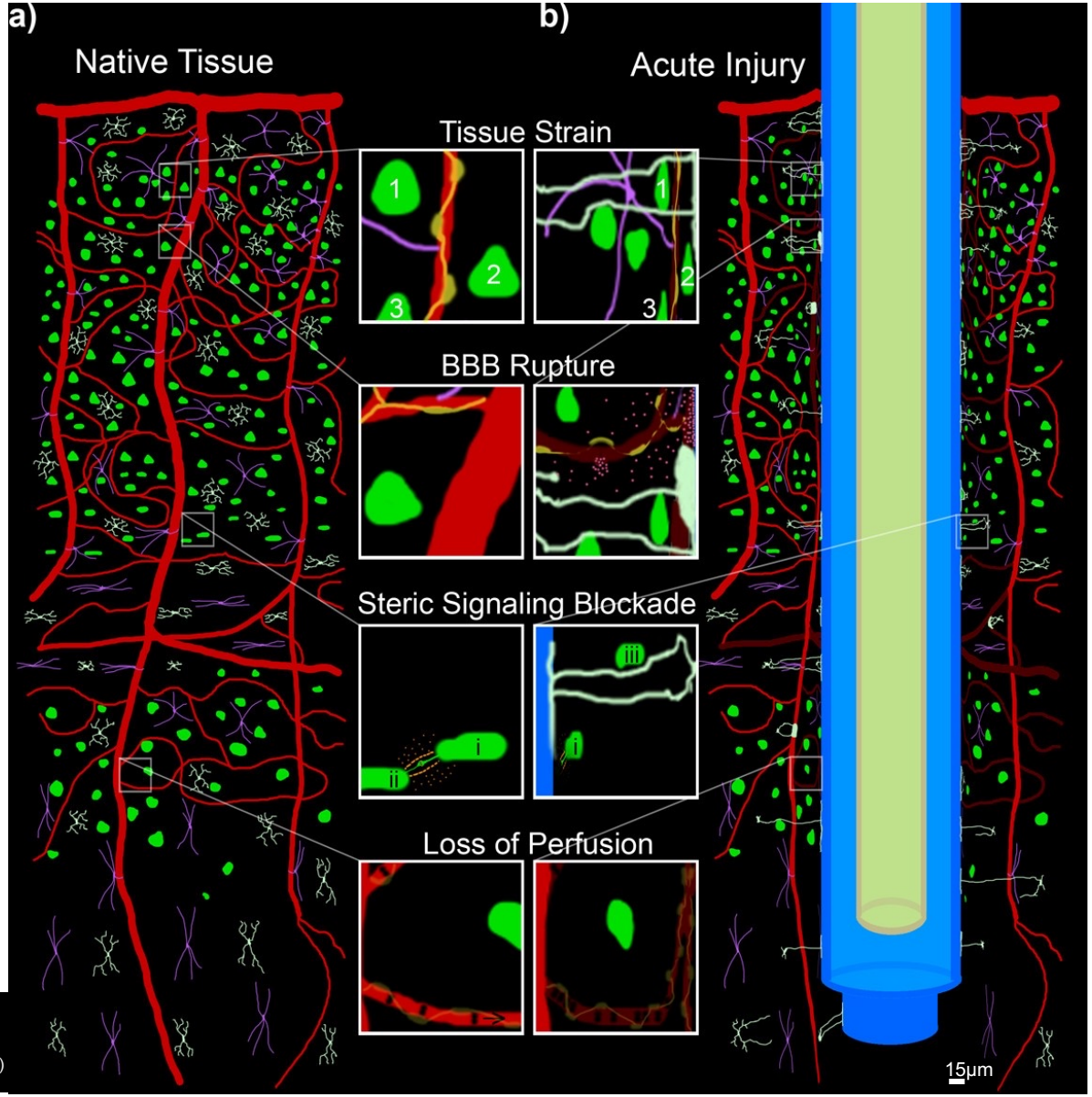
Chronic (fibrosis)



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

Insertion of an implant

- Acute phase



ACS Chem. Neurosci. 2015, 6, 1, 48–67

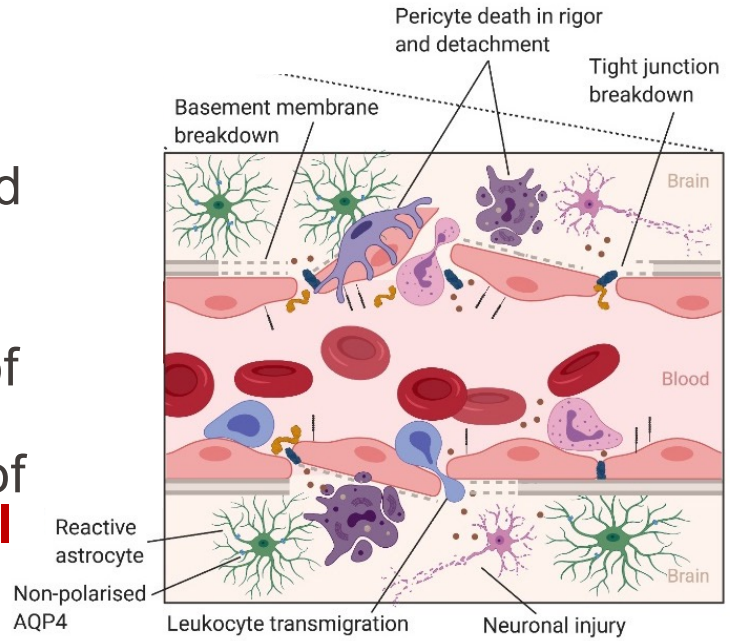
Perfusion and blood flow

- 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 strains

BBB rupture

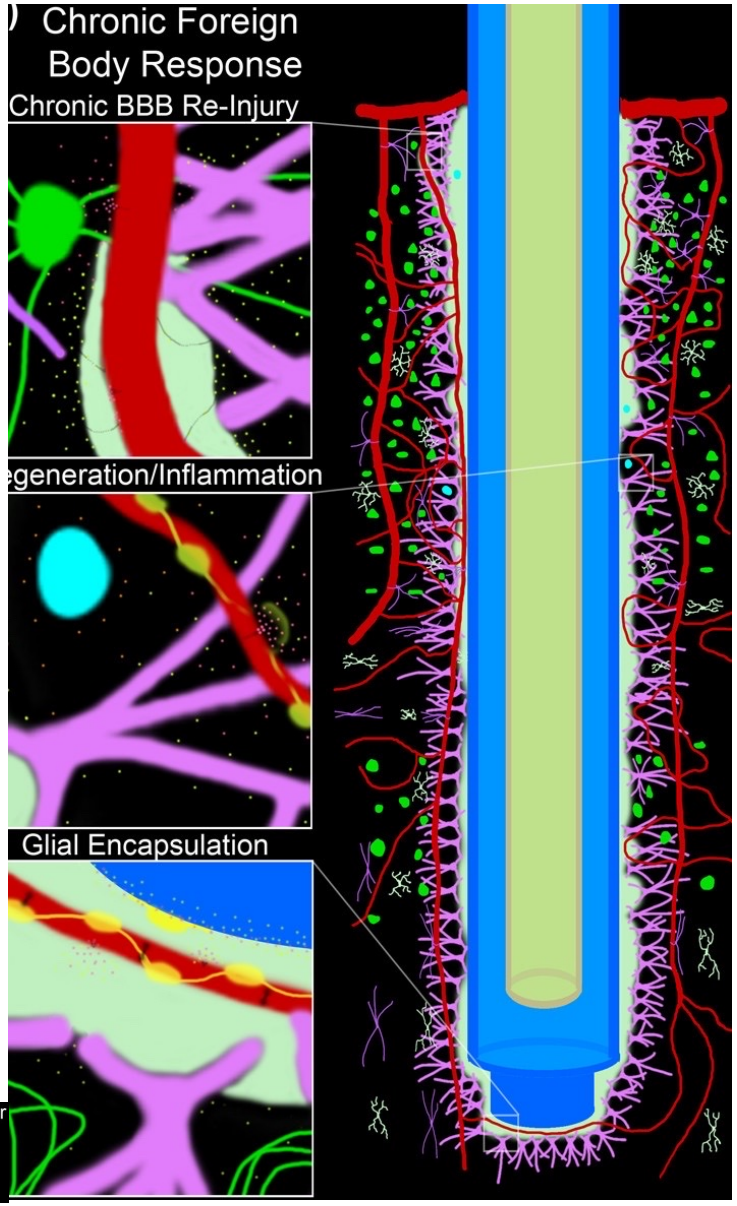
1. Deposition of plasma proteins foreign to the CNS and red blood cells
2. Increase of hemoglobin leads to increases in reactive oxygen species and reactive nitrogen species (oxidative stresses) → **inflammation**
3. Activation of macrophages → cleaning of debris but also production of pro-inflammatory 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

- Starts after a week
- Increased cell proliferation, capillary budding and synthesis of extracellular matrix (ECM)
- In the brain, lost axons are not replaced
- Glial scar is formed by reactive astrocytes and inhibits axon regeneration

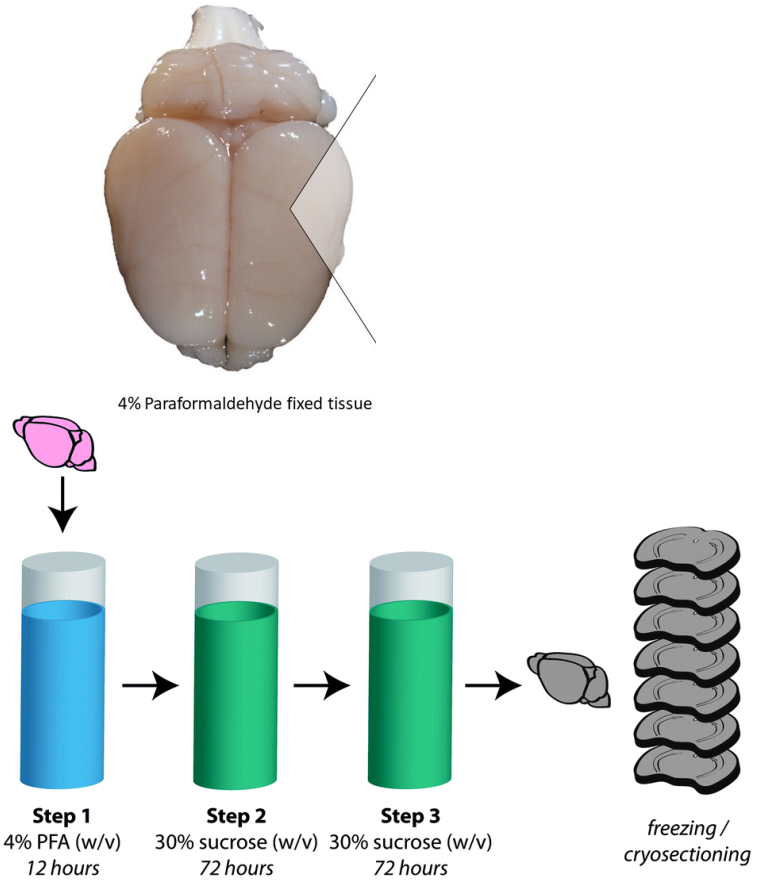


ACS Chem. Neurosci. 2015, 6, 1, 48–67

22 ©LSBI

Imaging

- **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

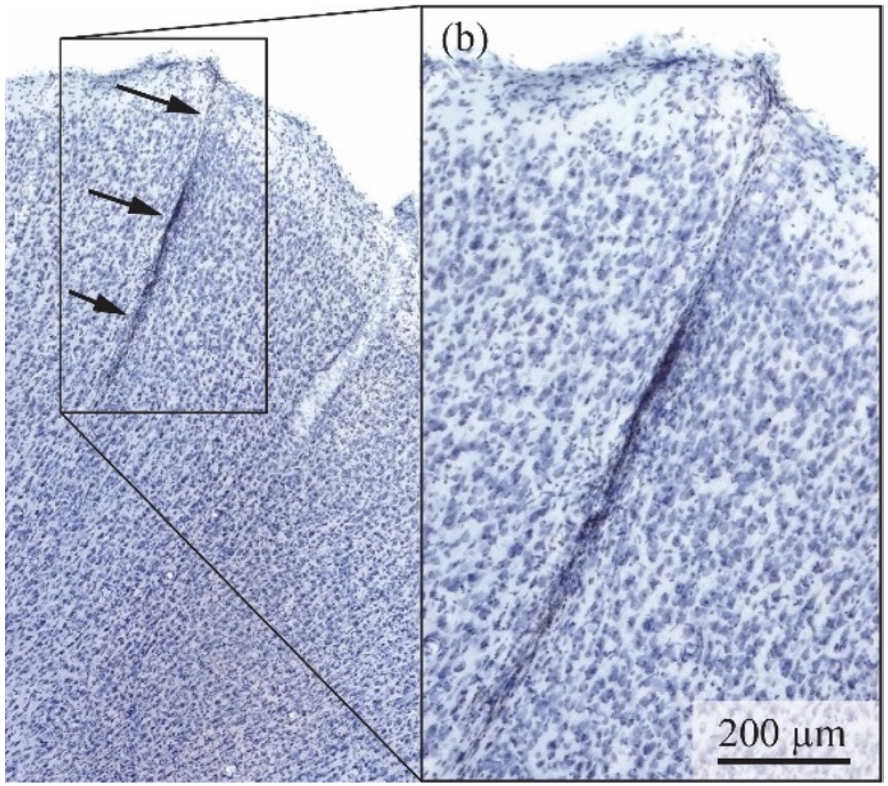


J. Anal. At. Spectrom., 2014,**29**, 565-570

Cell 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

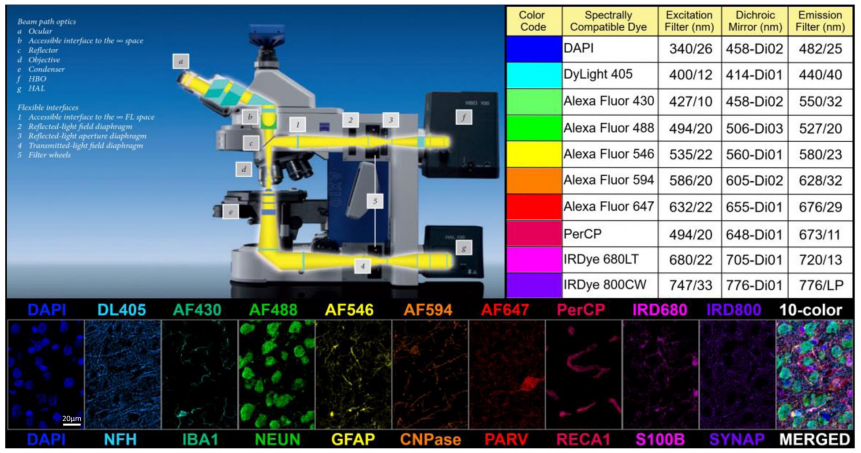


J. Neural Eng. 14 (2017) 036010

Histochemistry

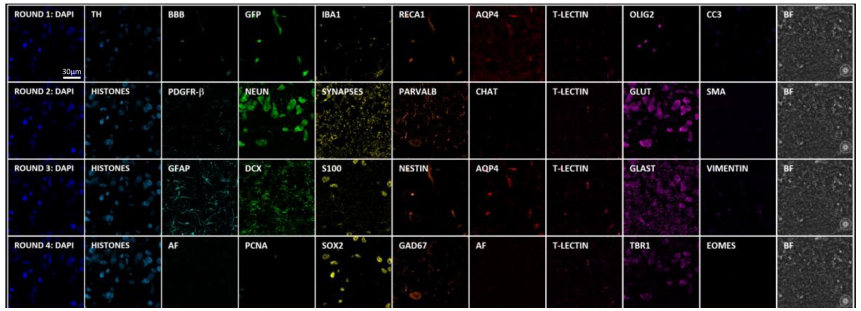
- selectively stain particular cell types, axonal fascicles, glial processes or blood vessels
- Expression of fluorescent proteins to visualise different neurons
- from ultraviolet to near-infrared (350–800 nm)
- Fluorescence microscopy (2D), confocal microscopy (3D)

a



Color Code	Spectrally Compatible Dye	Excitation Filter (nm)	Dichroic Mirror (nm)	Emission Filter (nm)
	DAPI	340/26	458-Di02	482/25
	DyLight 405	400/12	414-Di01	440/40
	Alexa Fluor 430	427/10	458-Di02	550/32
	Alexa Fluor 488	494/20	506-Di03	527/20
	Alexa Fluor 546	535/22	560-Di01	580/23
	Alexa Fluor 594	586/20	605-Di02	628/32
	Alexa Fluor 647	632/22	655-Di01	676/29
	PerCP	494/20	648-Di01	673/11
	IRDye 680LT	680/22	705-Di01	720/13
	IRDye 800CW	747/33	776-Di01	776/LP

b

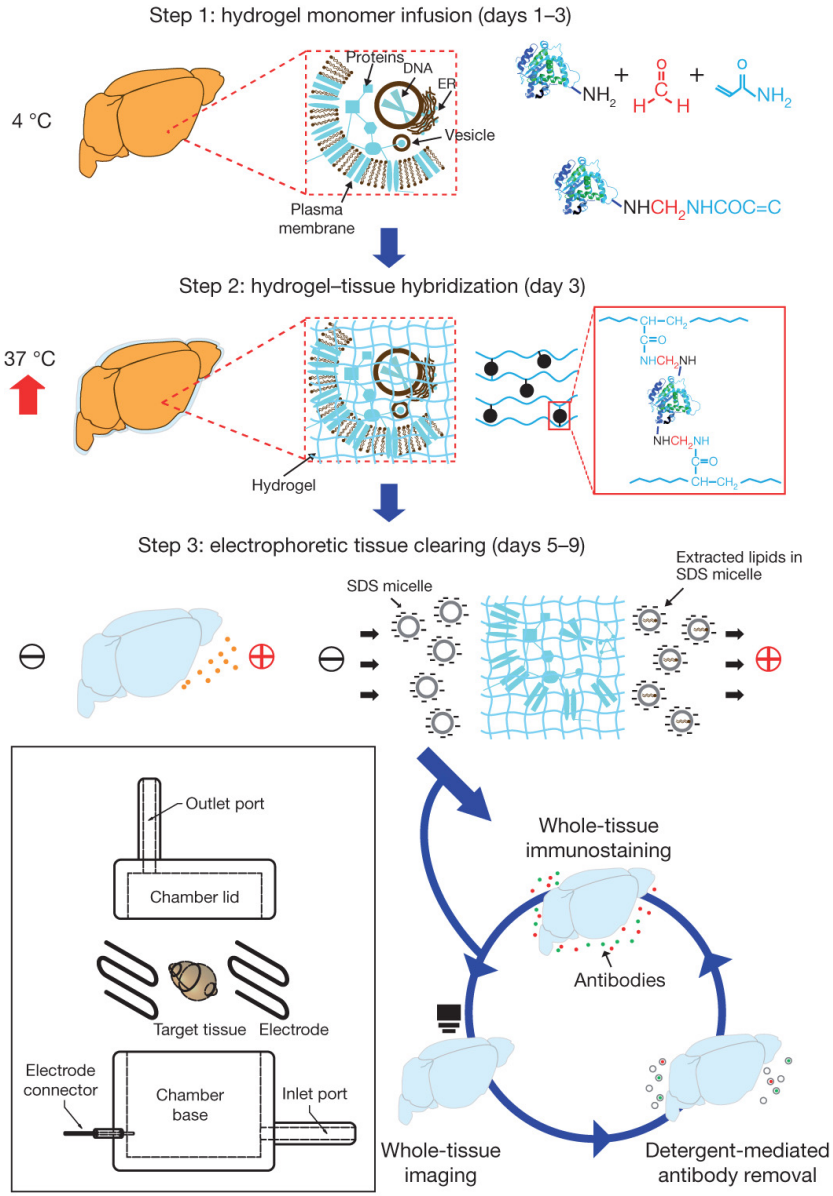


Nature Communications volume 12, Article number: 1550 (2021)

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)

Light sheet microscopy

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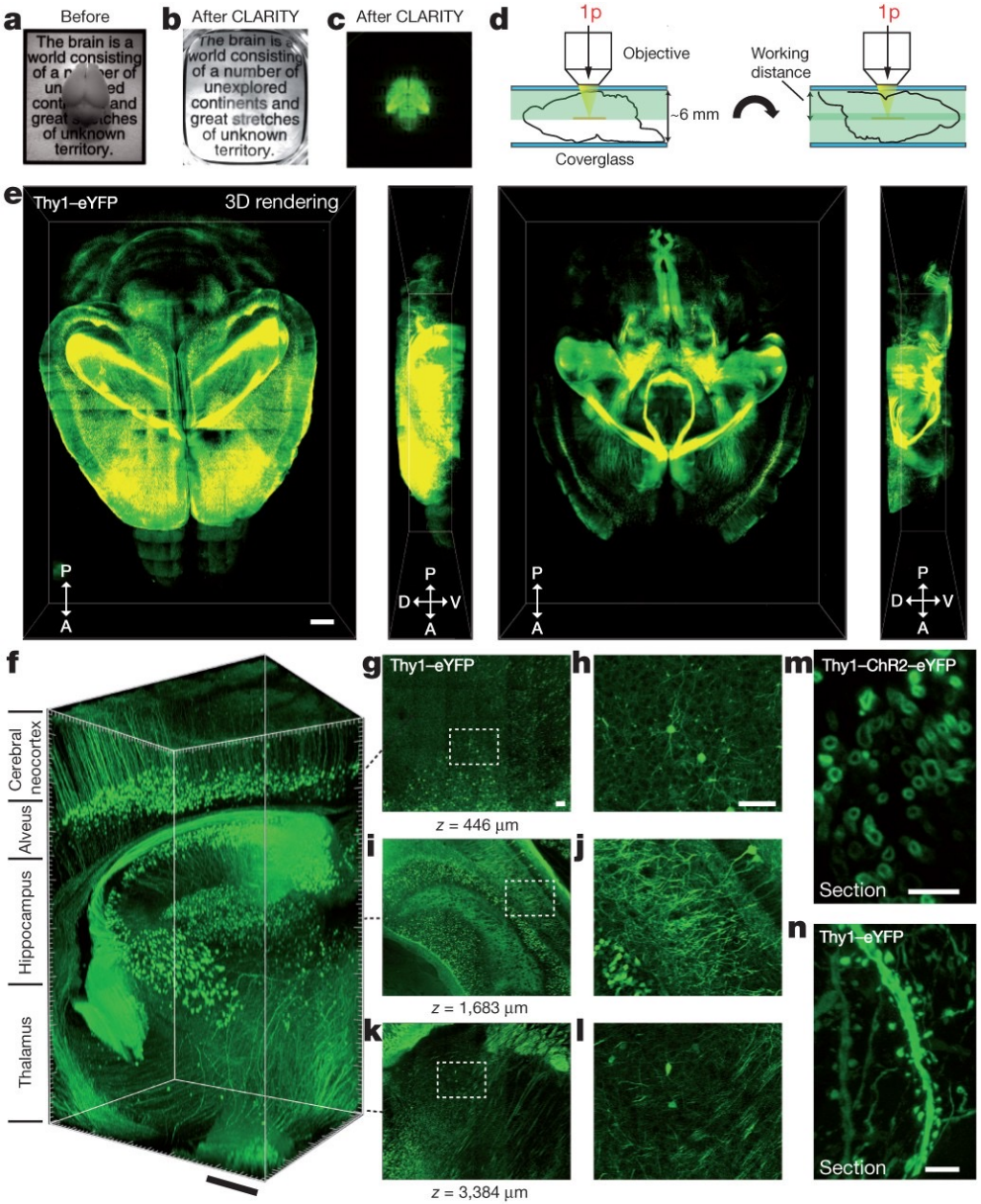
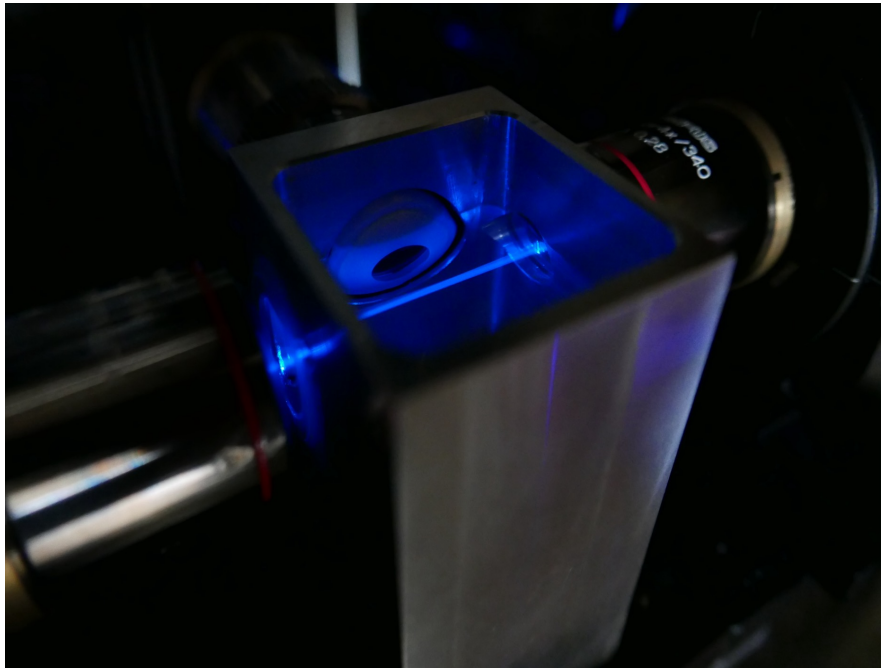
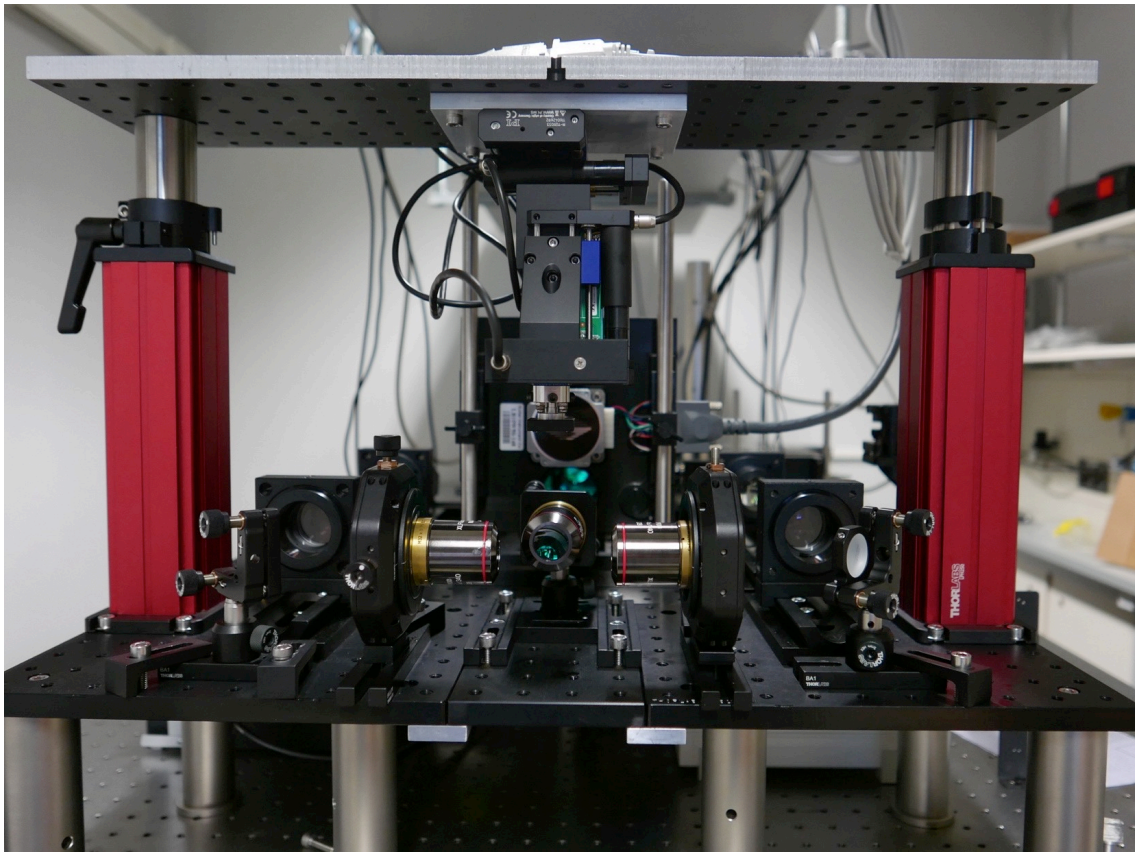


Nature volume **497**, pages 332–337 (2013)

Microscope





Photos par Q. Barraud, G. Courtine, EPFL

Cerebrospinal tract

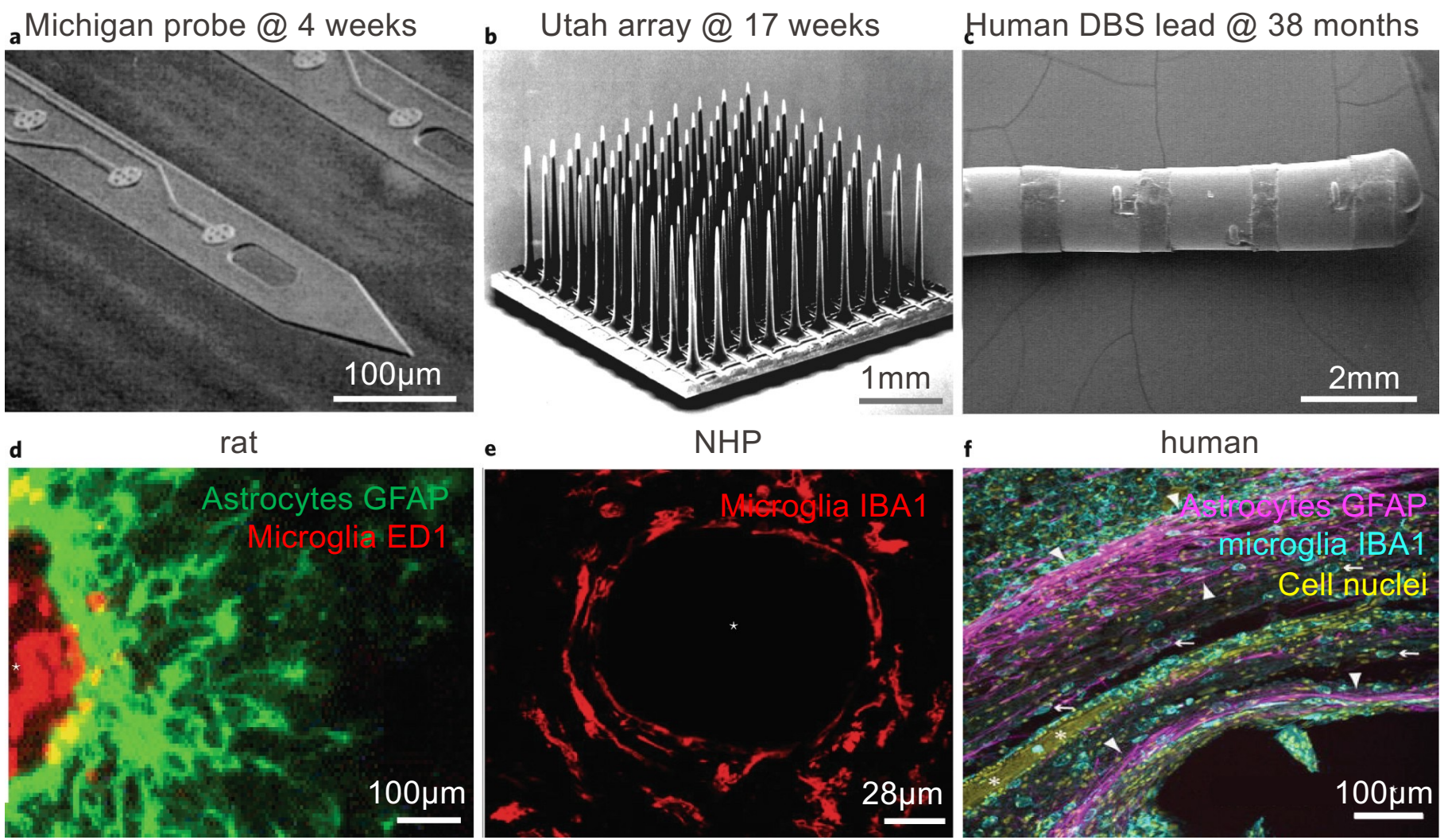
Vsx2-expressing neurons

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

Faisceau corticospinal
Faisceau reticulospinal

Videos par Q. Barraud, G. Courtine, EPFL

Gliosis



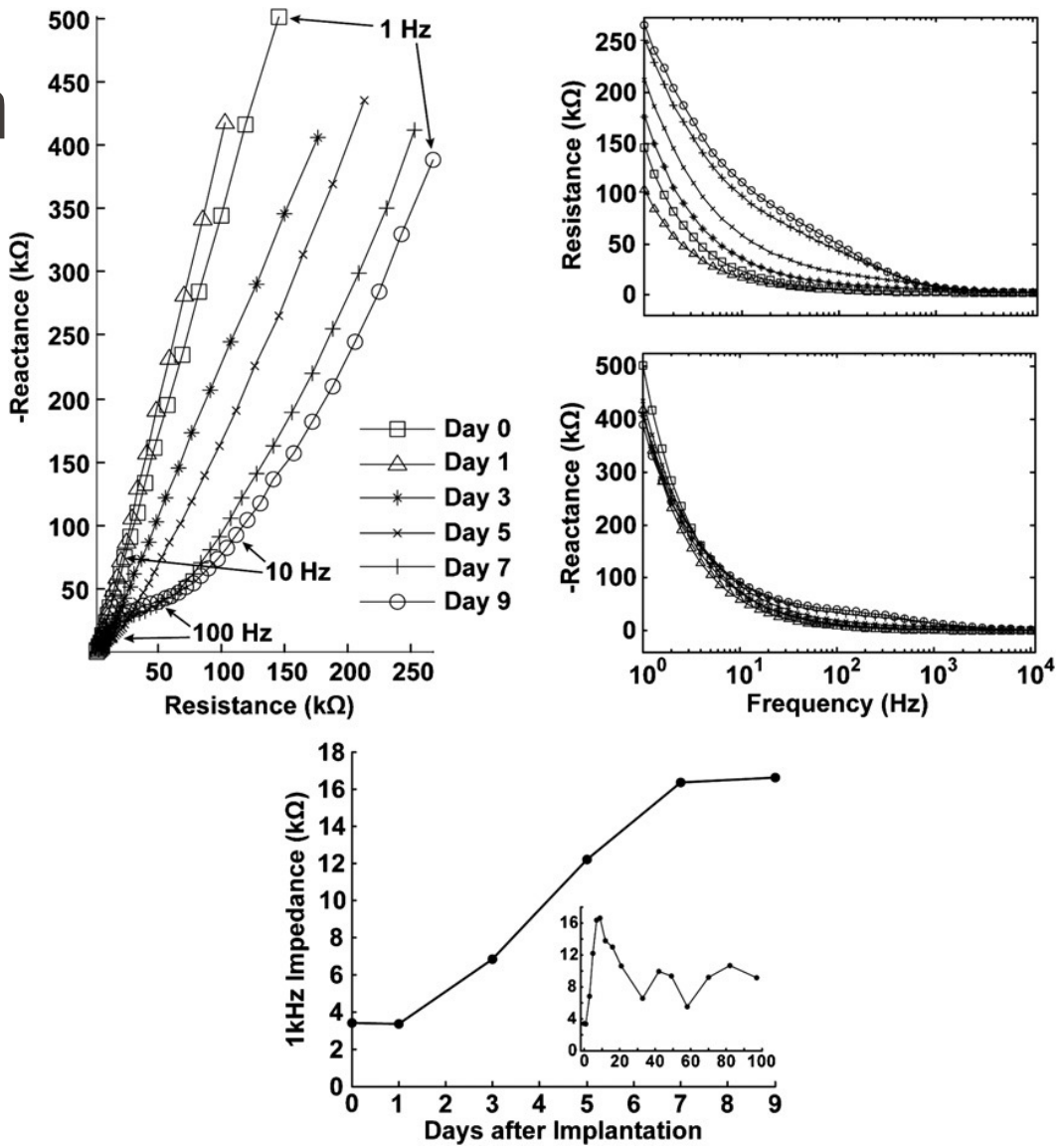
Nature Biomedical Engineering **volume 1**, pages 862–877 (2017)

NX-422 ©LSBI

Electrode impedance and glial consolidation

DBS electrode impedance after implantation

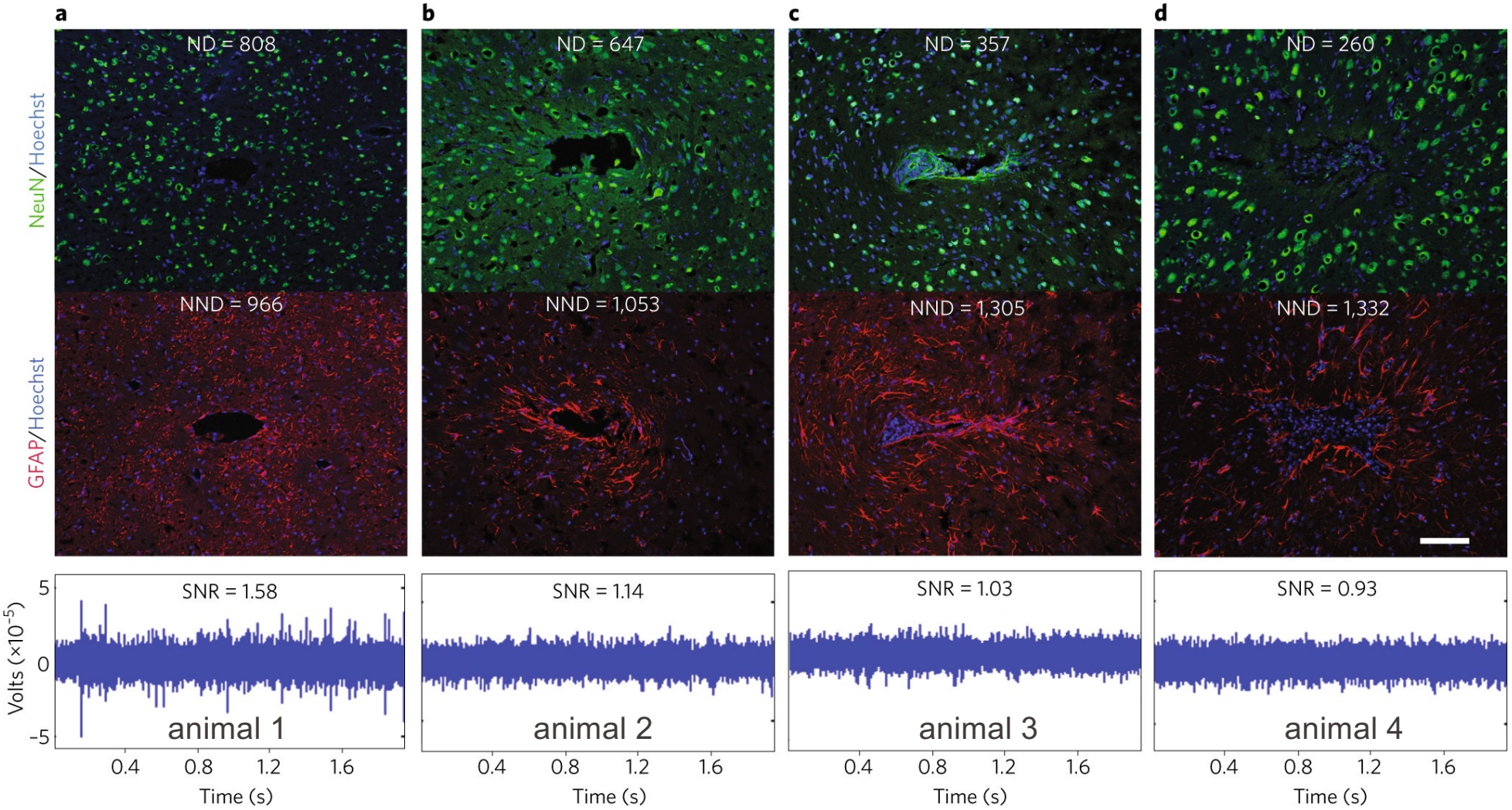
Under constant-voltage stimulation, alteration of the current effectively reaching neural tissue



J. Neural Eng. 6 (2009) 046001

Negative impact on recording quality

ND neuronal density
NND non neuronal density
28 day post implantation



NX-422 ©LSBI

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

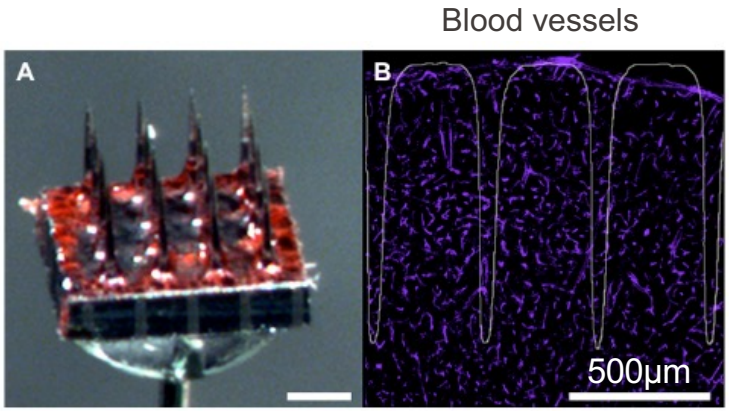
Biomaterials 53 (2015) 753-762

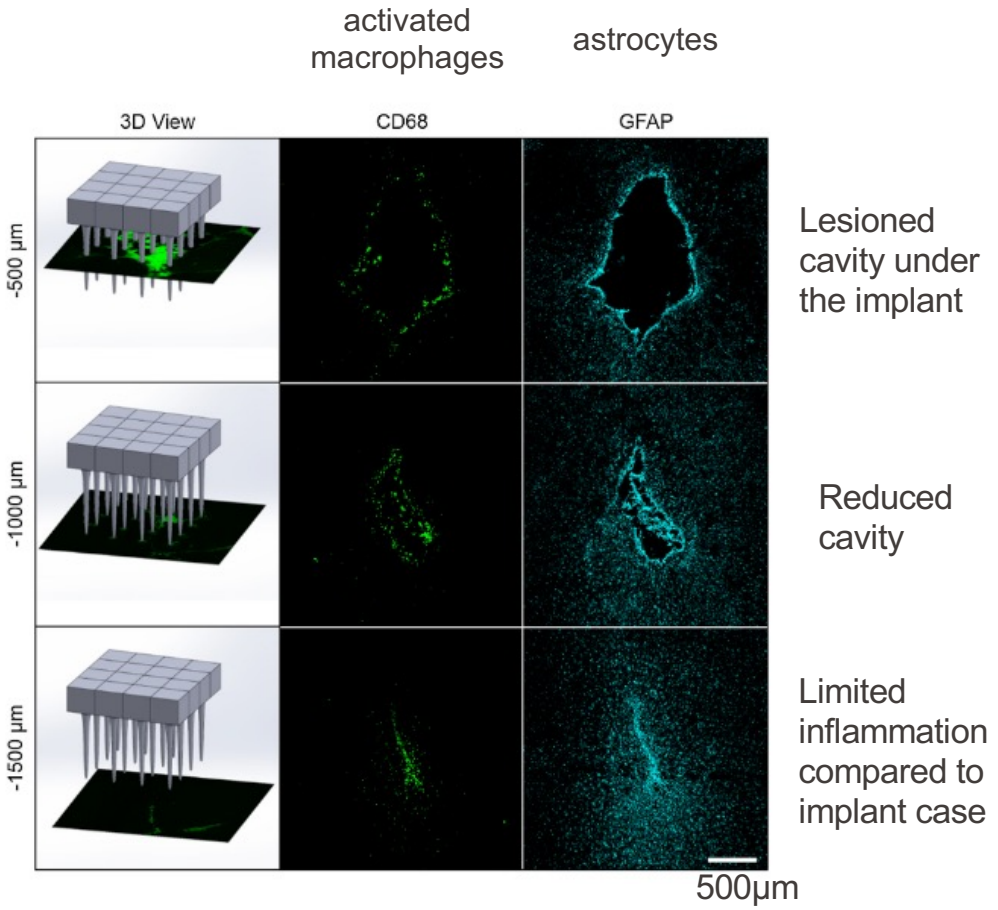
FBR and stab injury

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

Stab wound:
2min in then out

Histology at week 4





Lesioned cavity under the implant

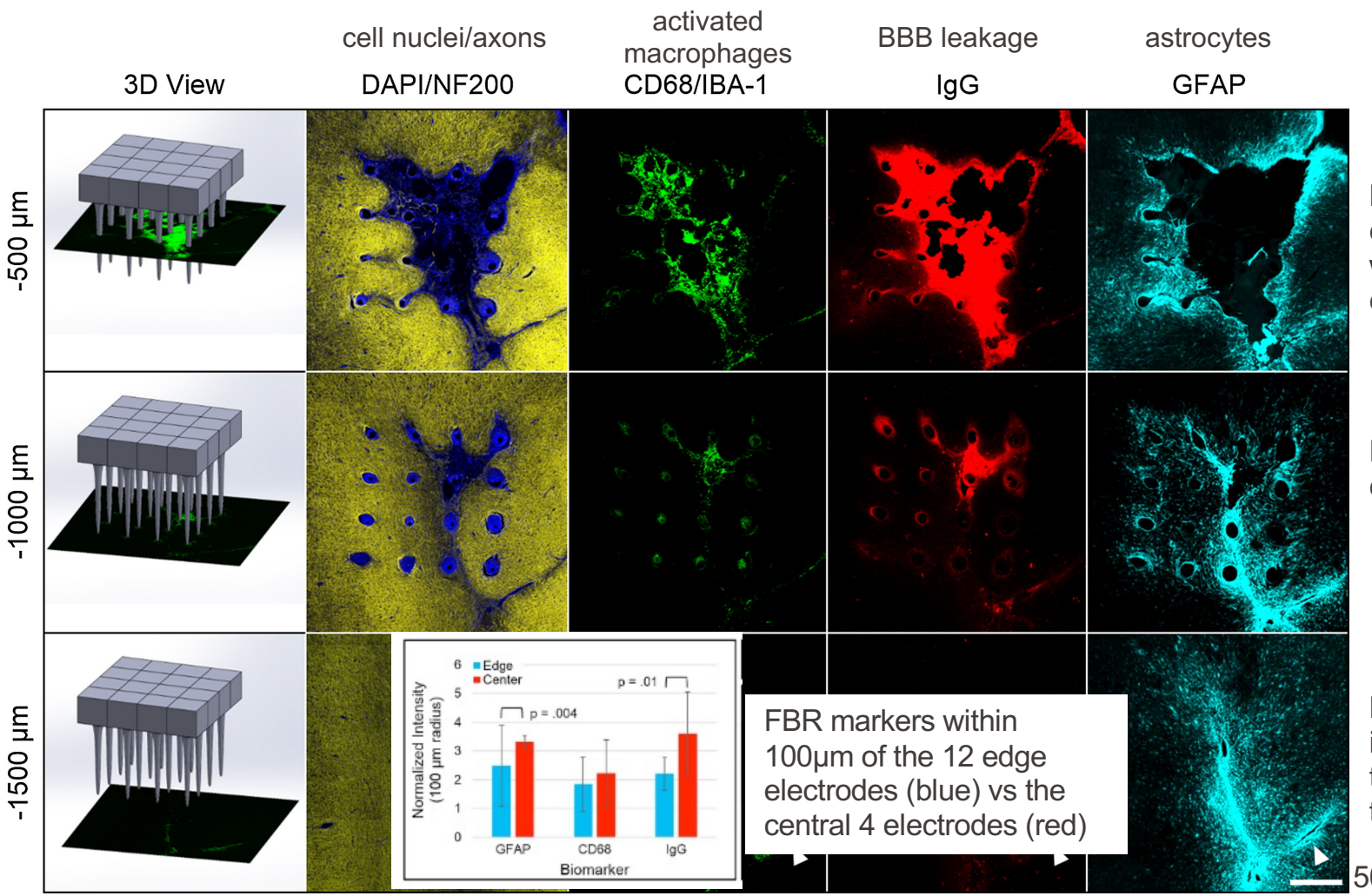
Reduced cavity

Limited inflammation compared to implant case

FBR and shank density

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

Timepoint: 4w

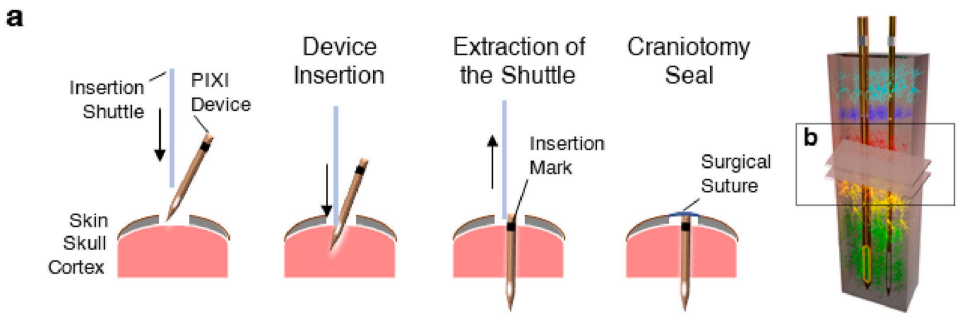


Biomaterials 53 (2015) 753-762

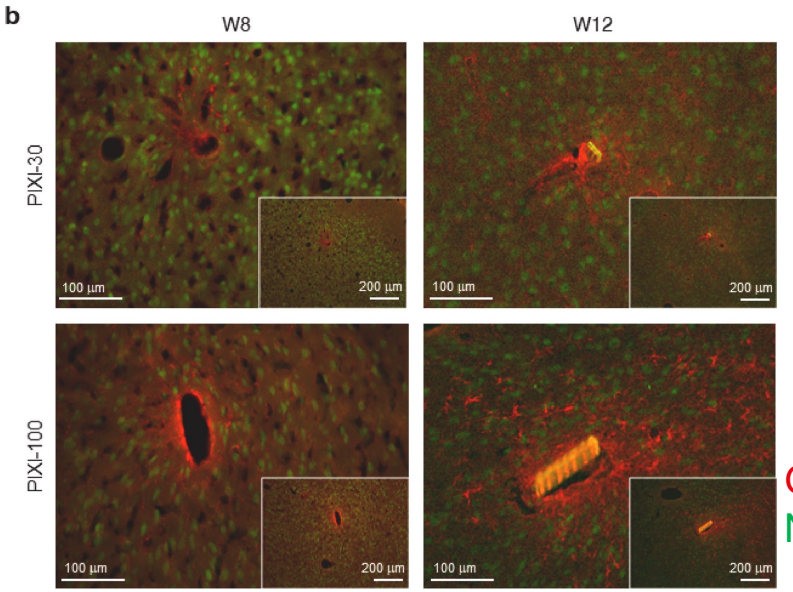
NX-422 ©LSBI

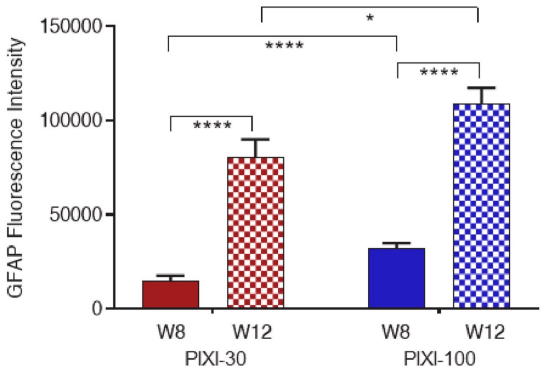
Effect of implant size on FBR

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



Sections in layer IV of the cortex



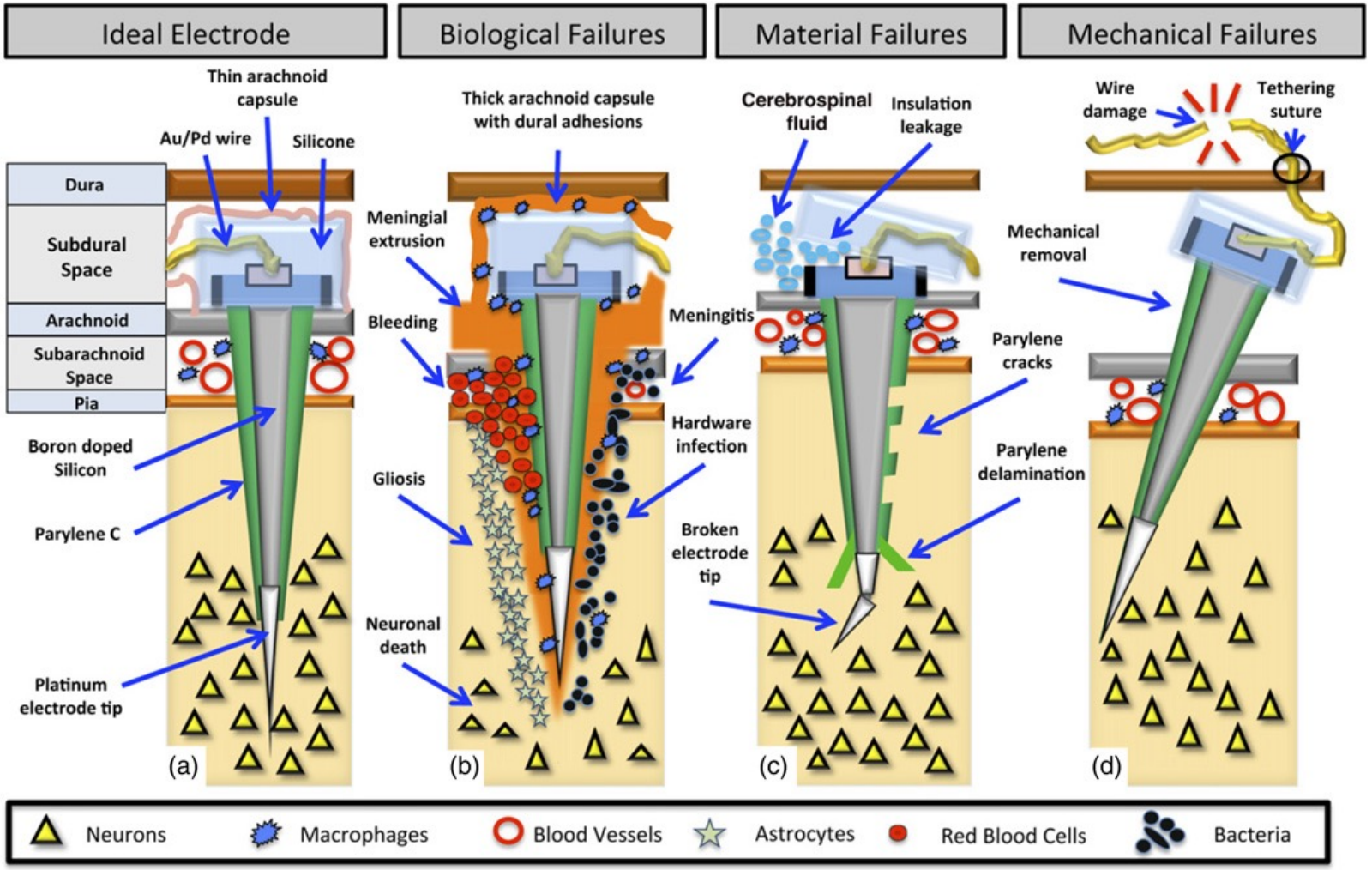


Footprint of scar depends on size of the implant

Biomaterials Volume 281, February 2022, 121372

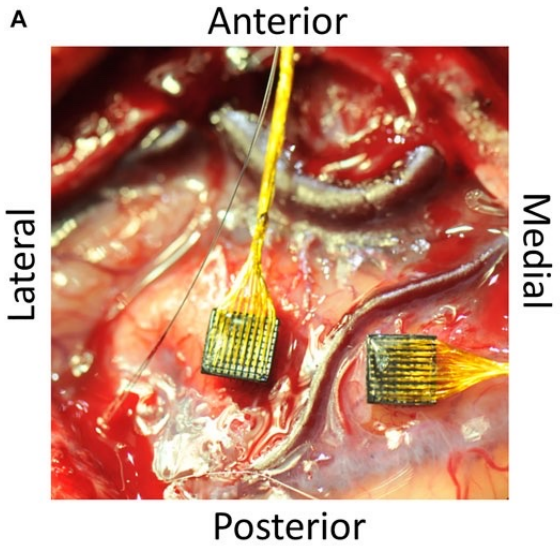
doi: 10.1088/1741-2560/10/6/066014

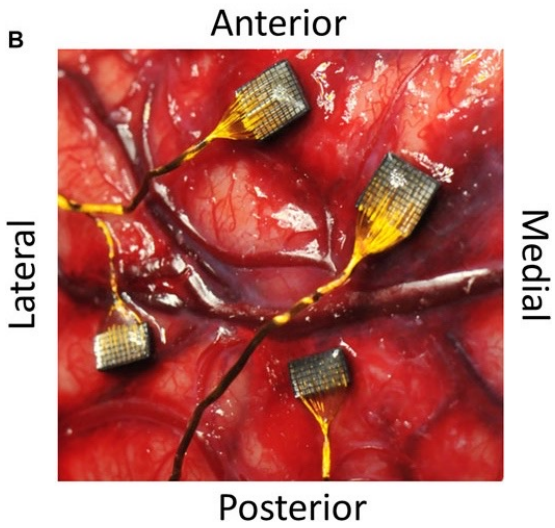
Implant damage



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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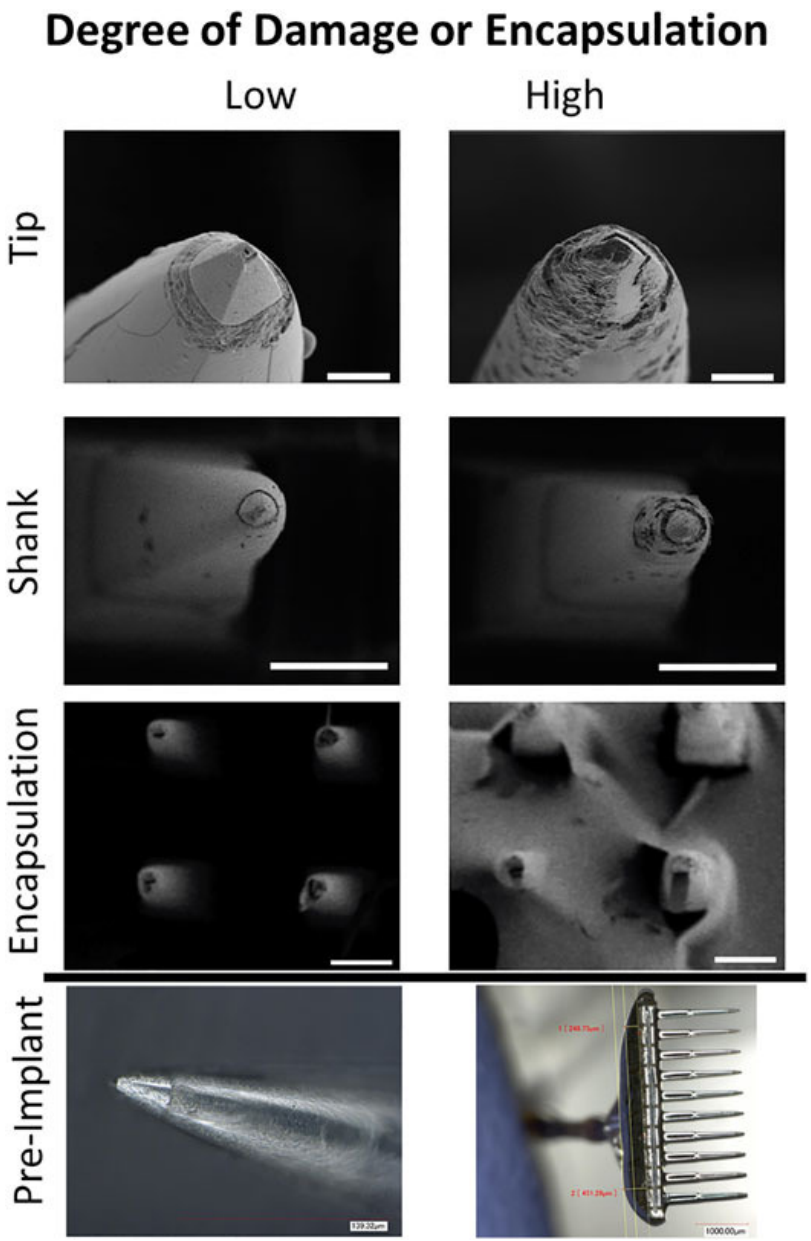
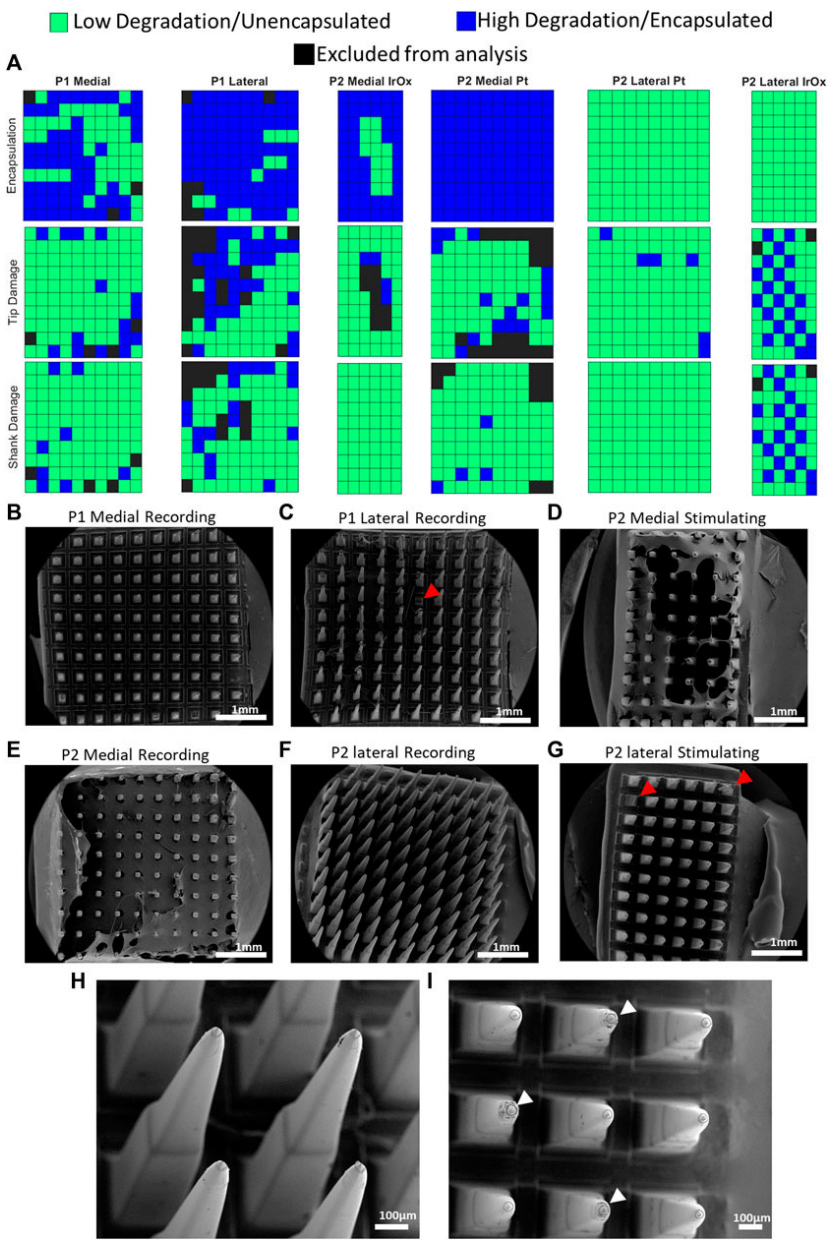


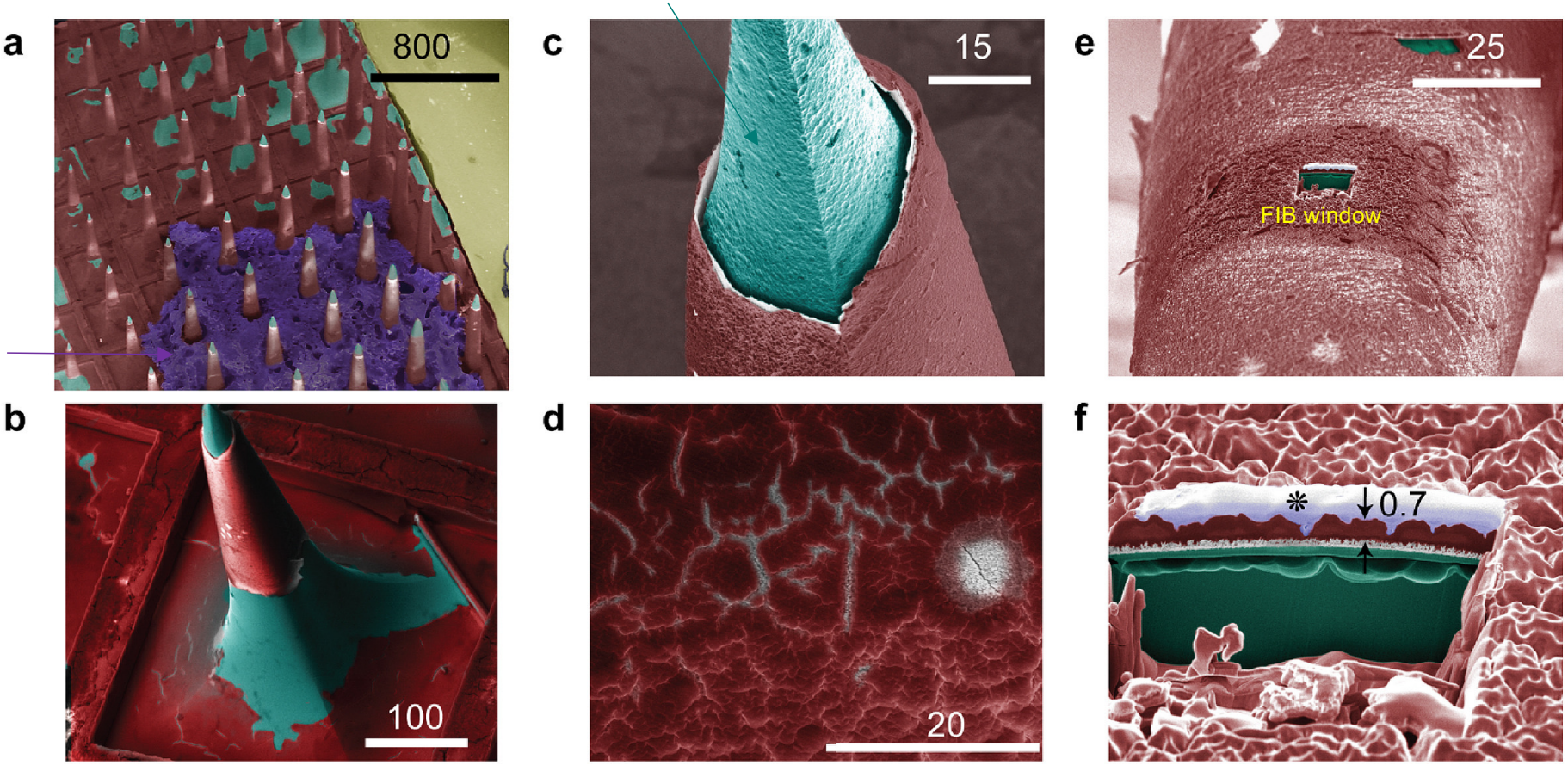


NX-422 ©LSBI

Parylene damage in vivo

Metal tip damage
Parylene degradation

residual tissue



Explanted after 3.25 years in cat sciatic nerve

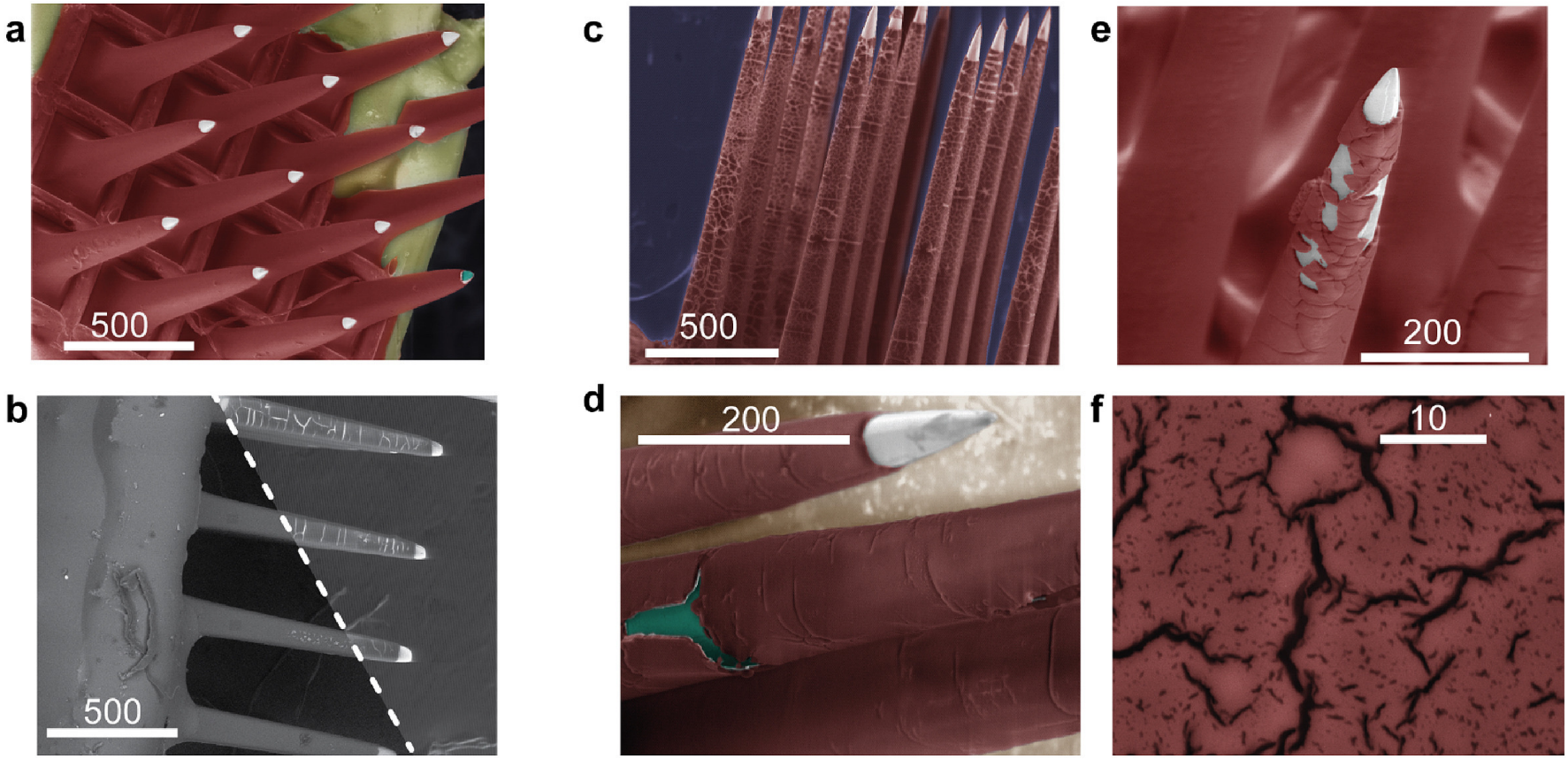
Biomaterials Volume 232, February 2020, 119731

NX-422 ©LSBI

Accelerated aging in reactive environment

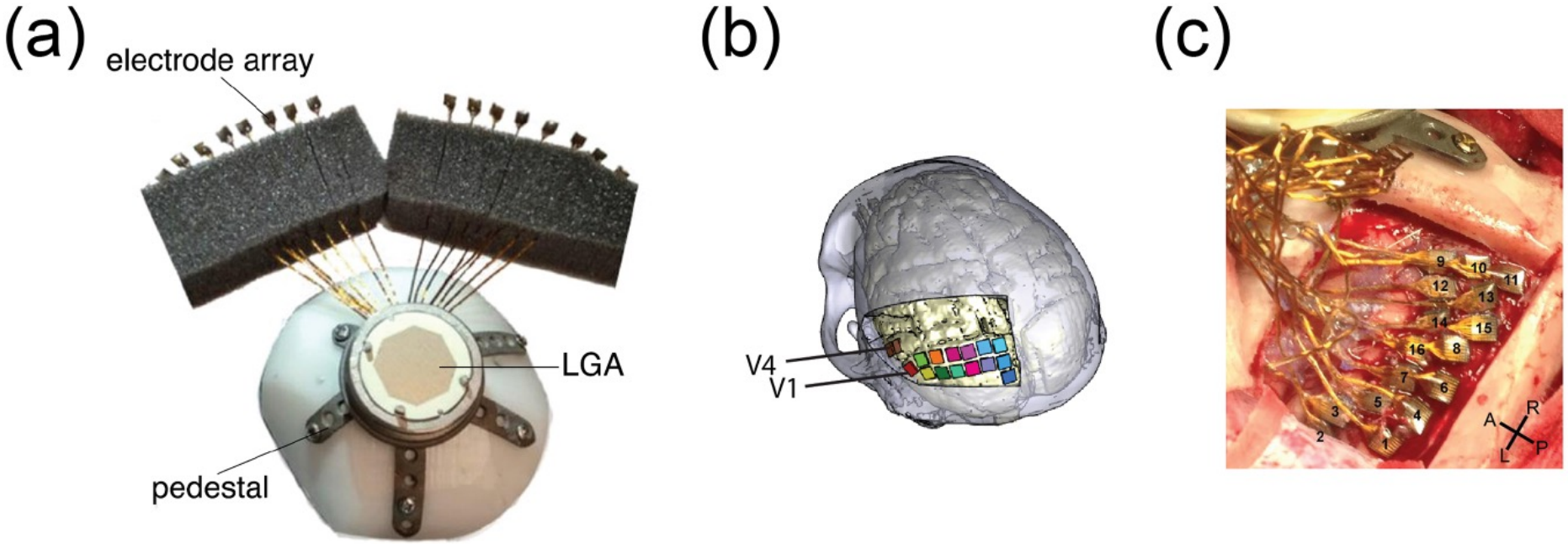
devices aged in PBS at 37°C

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



Biomaterials Volume 232, February 2020, 119731

Chronic stability with multiple adjacent Utah arrays

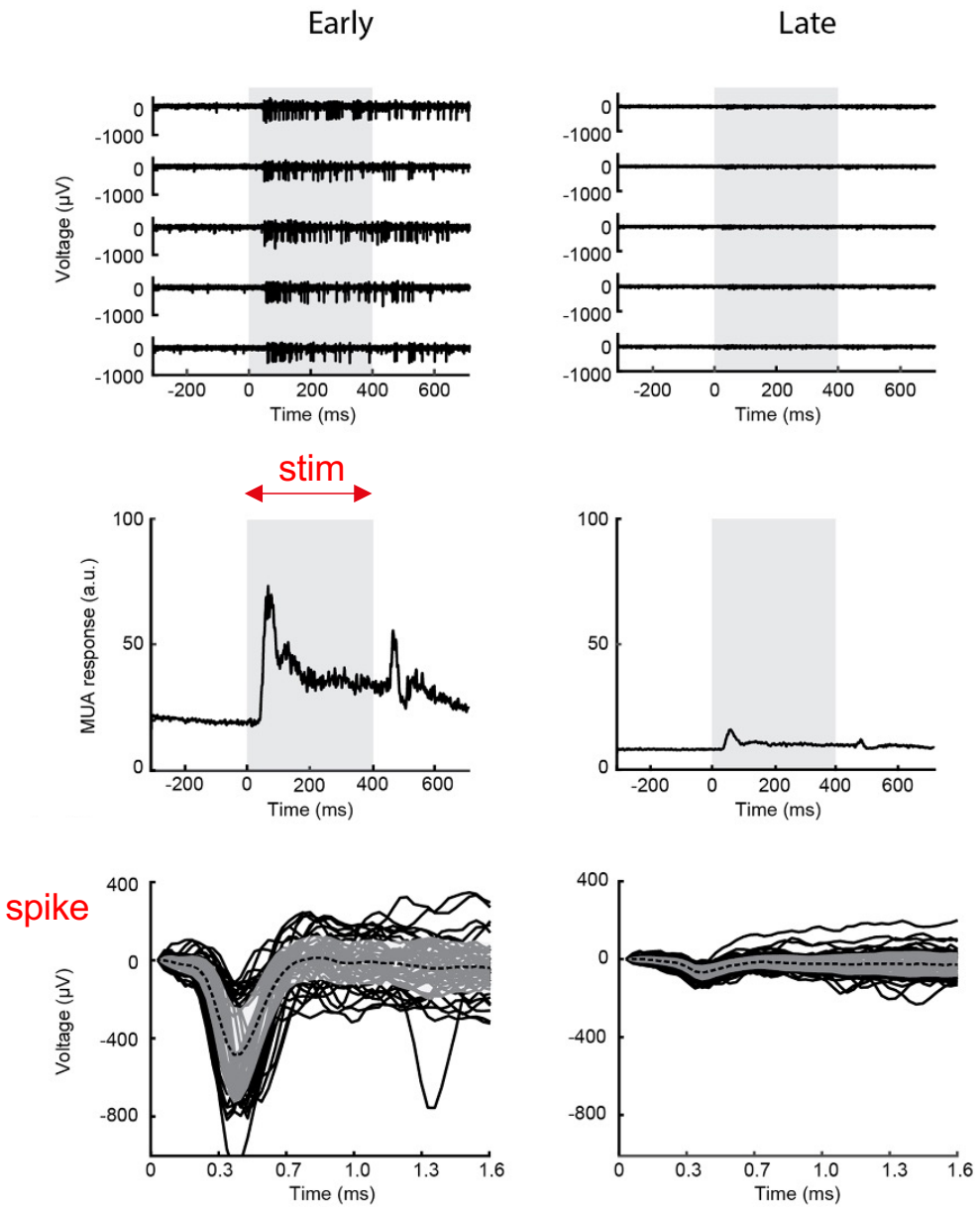


Journal of Neural Eng.2023 **20** 036039

Representative recordings

Early: 91 days
Late: 279 days

MUA: multiunit activity



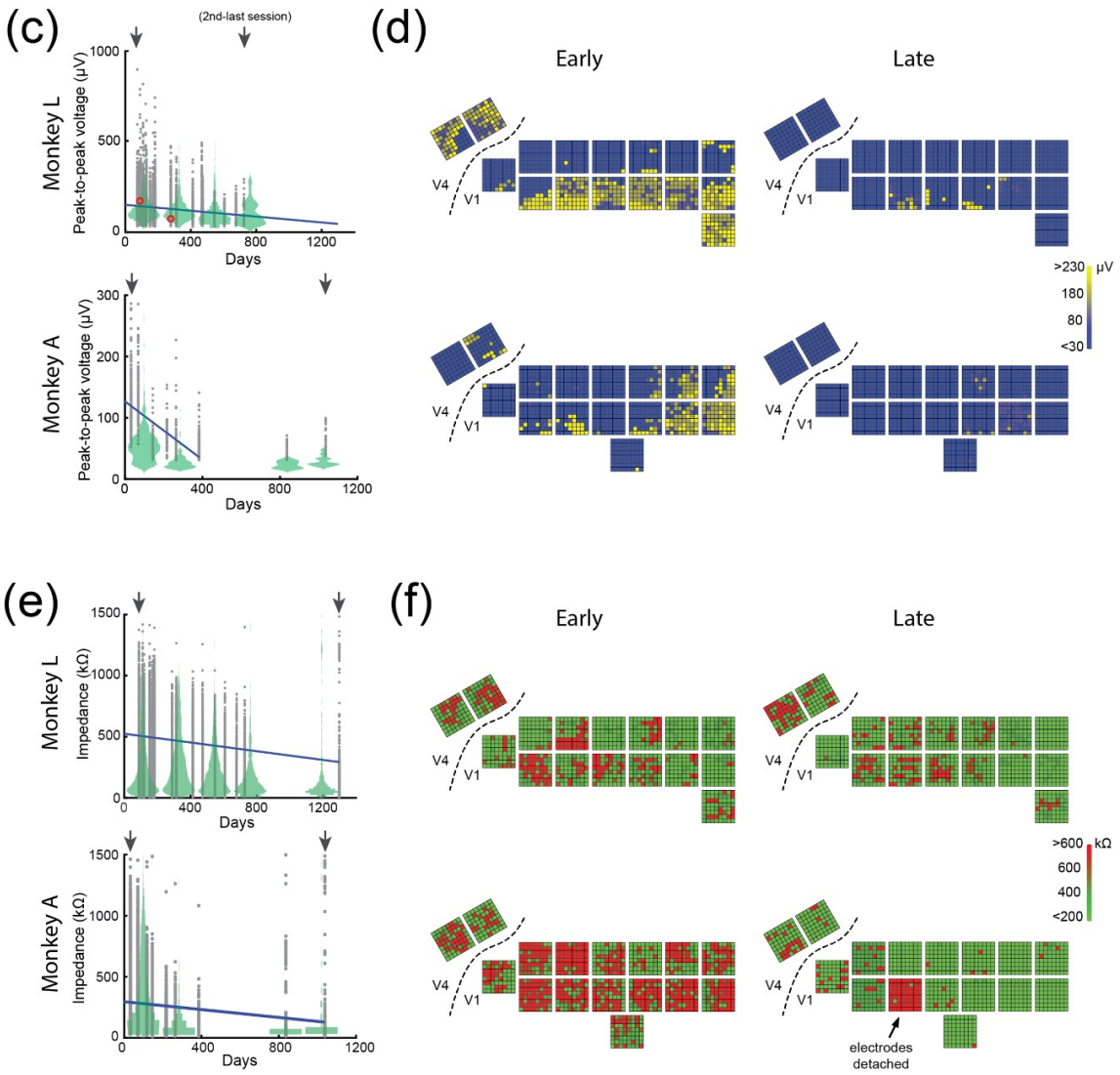
Journal of Neural Eng.2023 **20** 036039

NX-422 ©LSBI

Representative recordings across all arrays

Early: 91 days
Late: 279 days

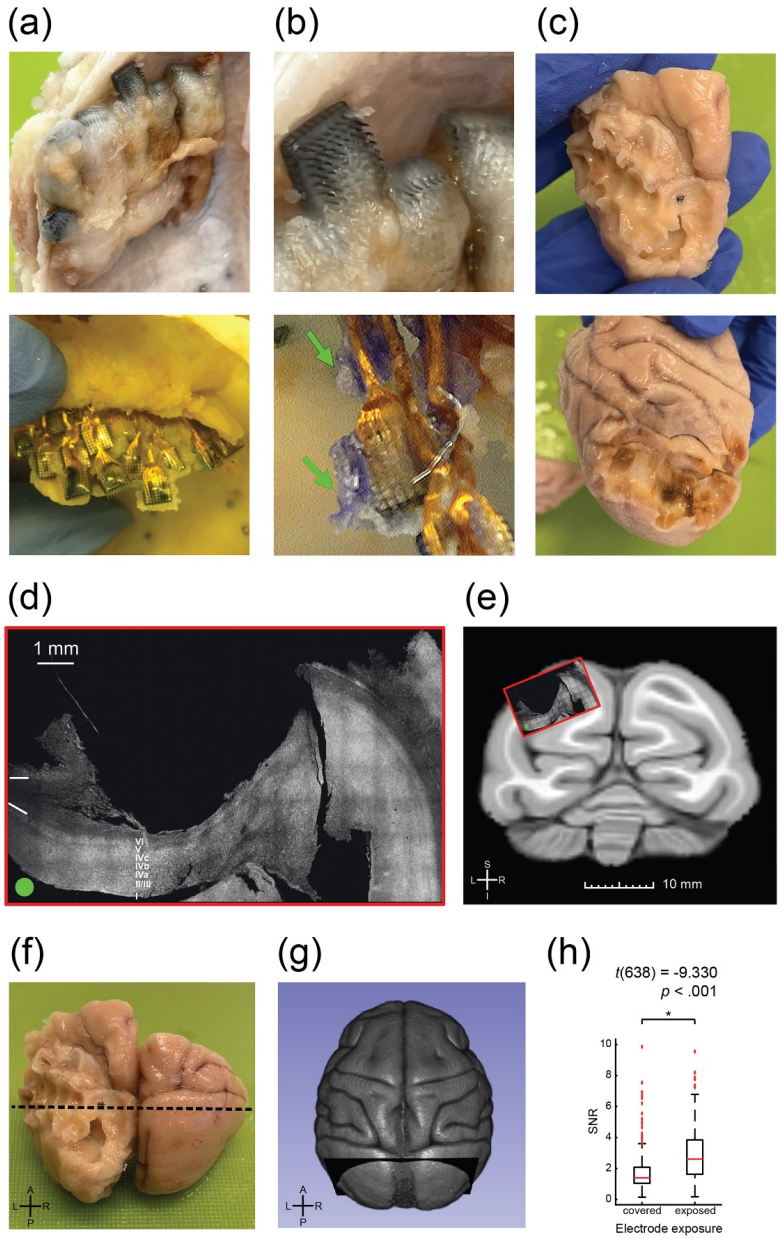
MUA: multiunit activity



Journal of Neural Eng.2023 20 036039

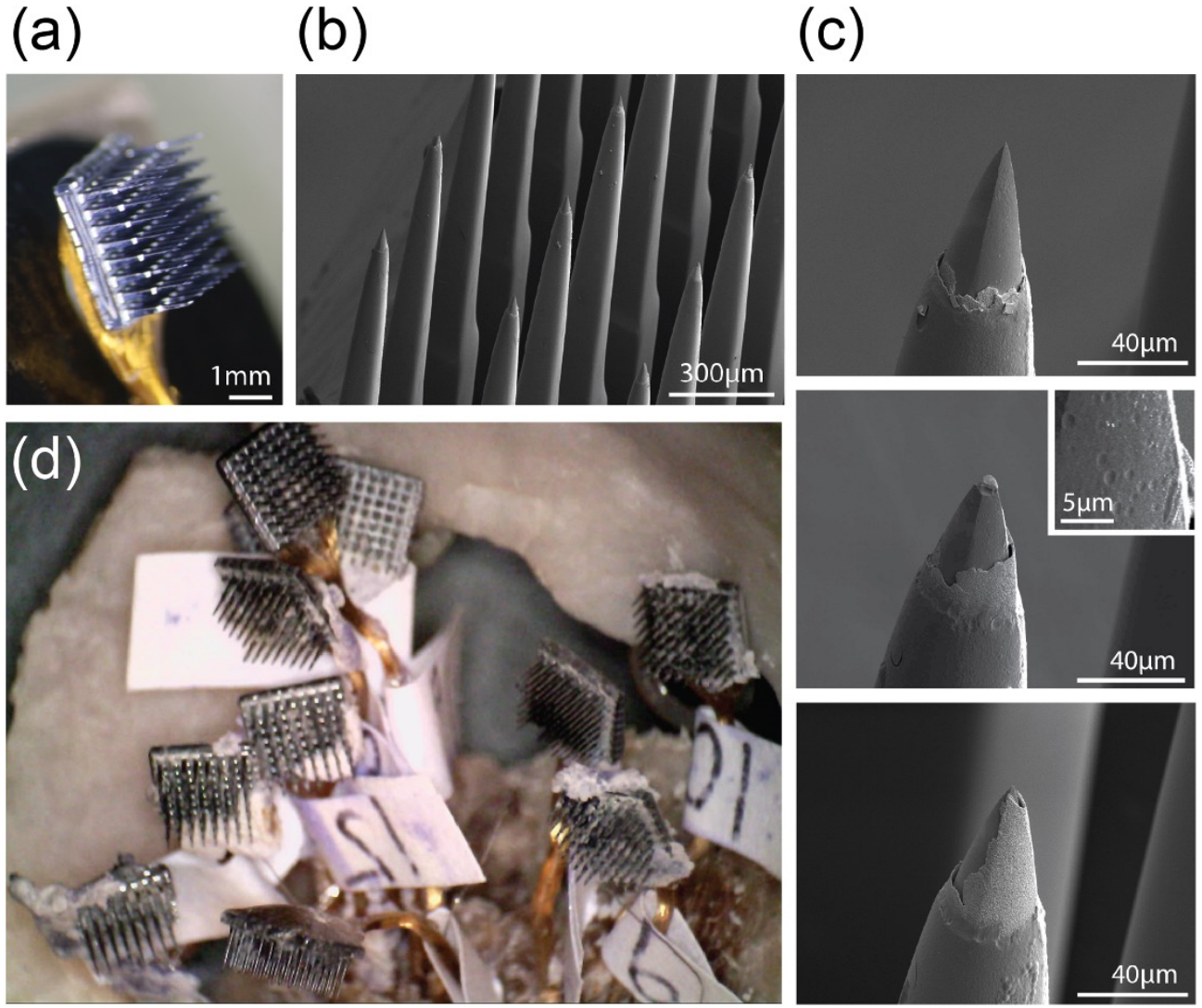
Tissue response and histology

Day 638



Journal of Neural Eng.2023 20 036039

Explanted arrays



Day 638

damaged IrOx

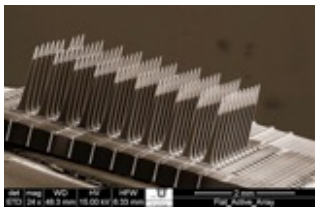
broken Si tip

pitting Si surface

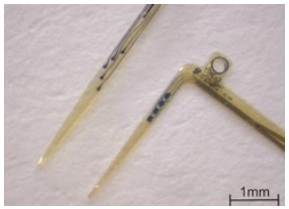
Journal of Neural Eng.2023 **20** 036039

Intracortical probes

INTERFACES



‘1991’ intracortical
Si microneedle implant



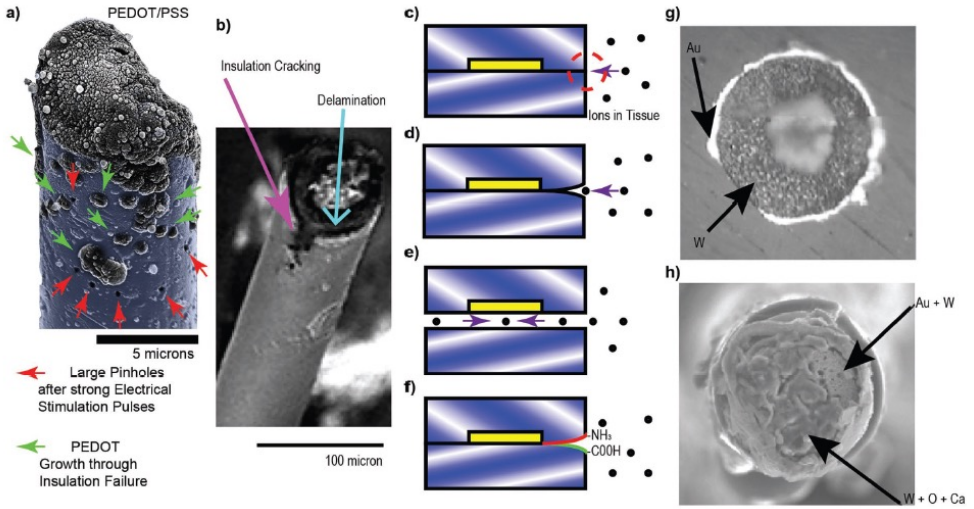
‘2000’ intrafascicular
polyimide implant

E

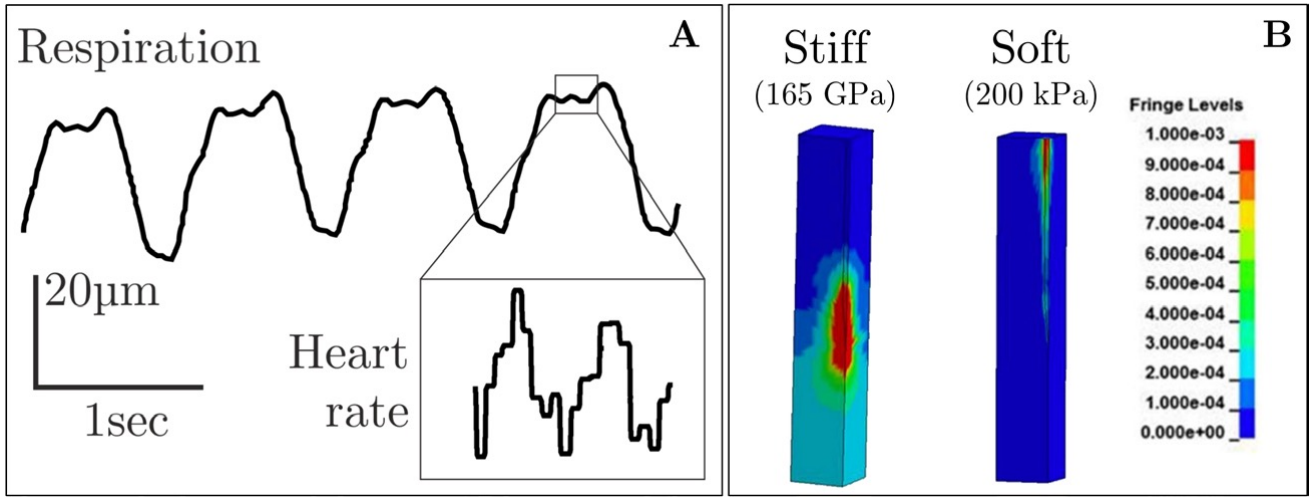
100GPa 1GPa 1MPa 1kPa

stiff interfaces

static interfaces

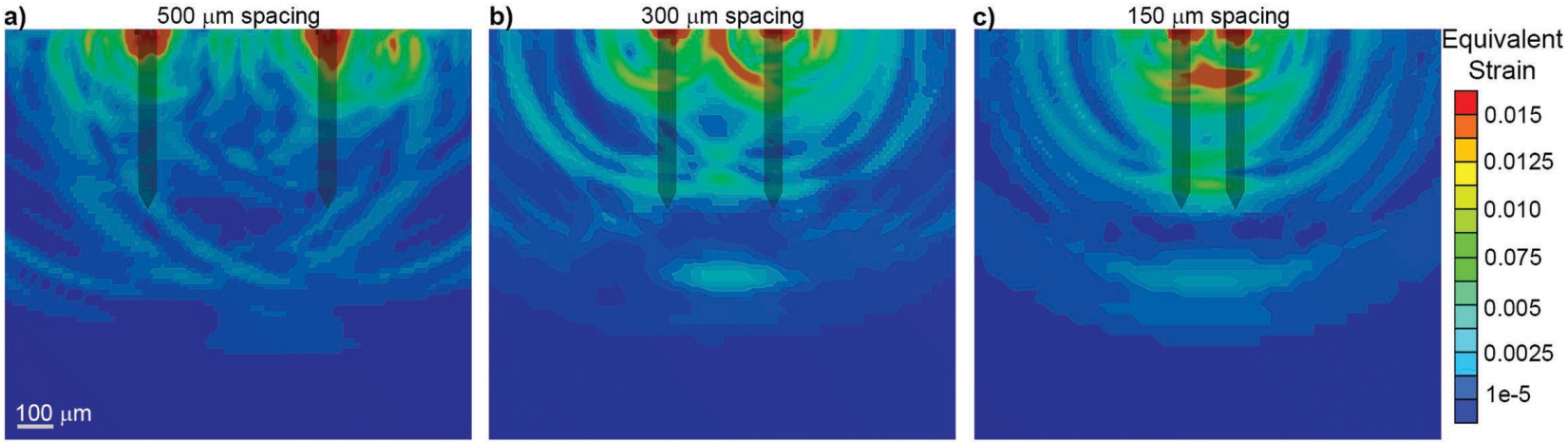


Probe mechanics



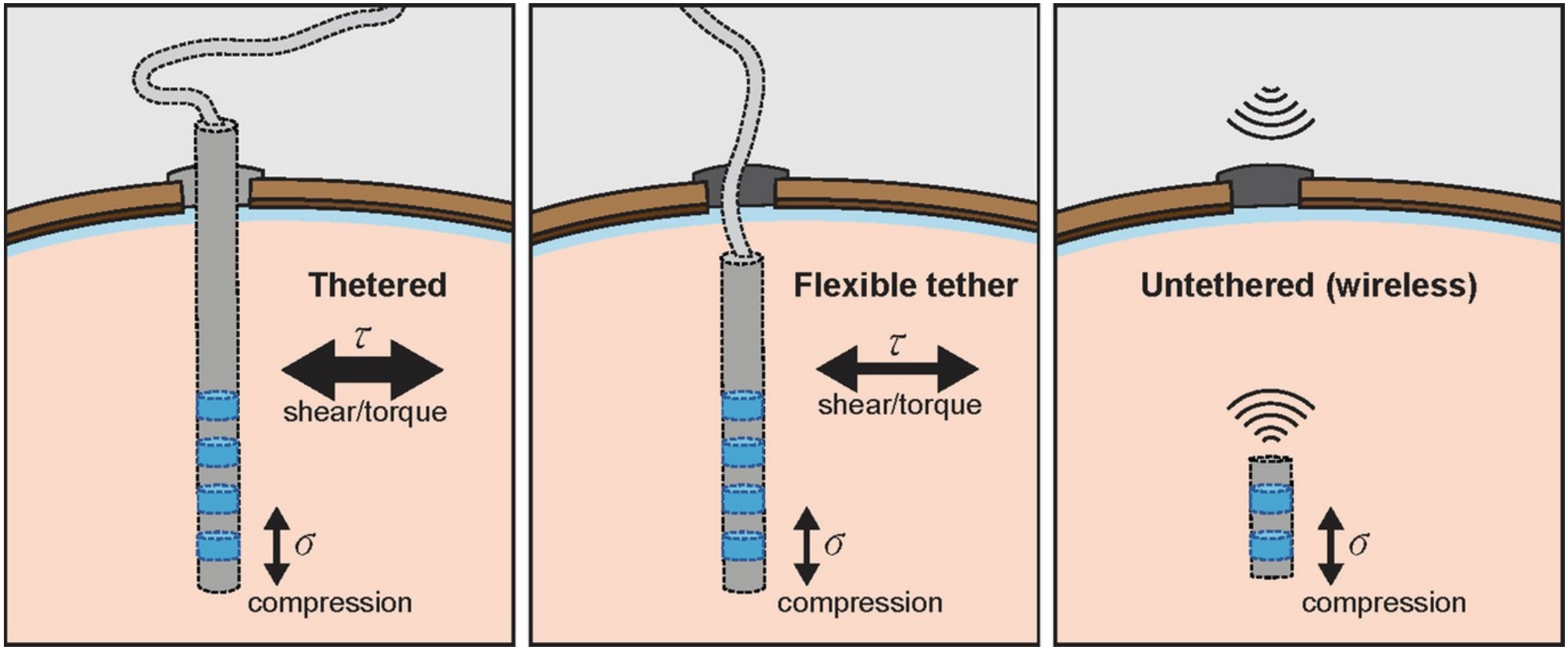
Modelled strain distribution at the probe/tissue interface using a micromotion displacement of 4 µm

J. Neural Eng. 15 (2018) 031001



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

Probe tethering

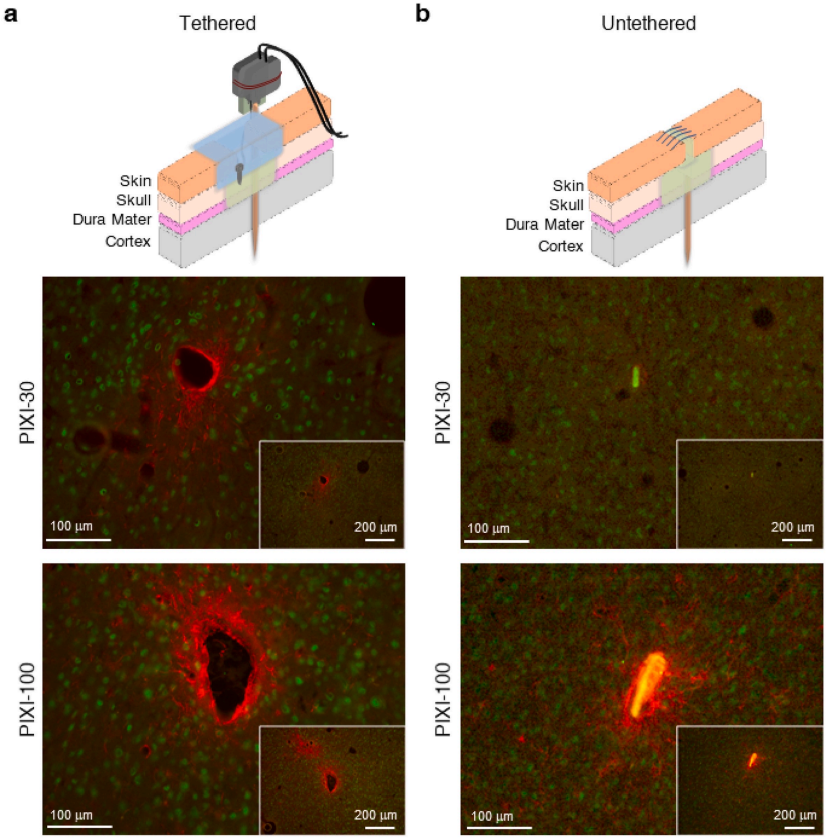


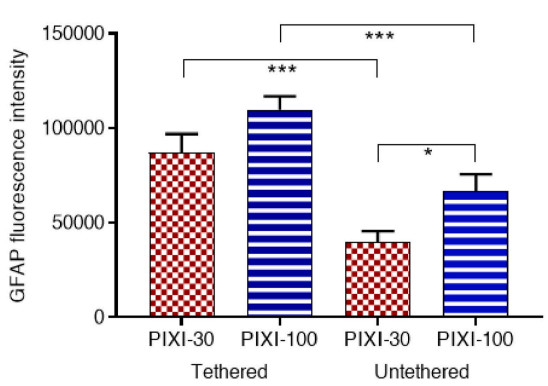
Neural Interface Engineering pp 123–172

Effect of tethering on FBR

12w post-implantation

GFAP
NeuN



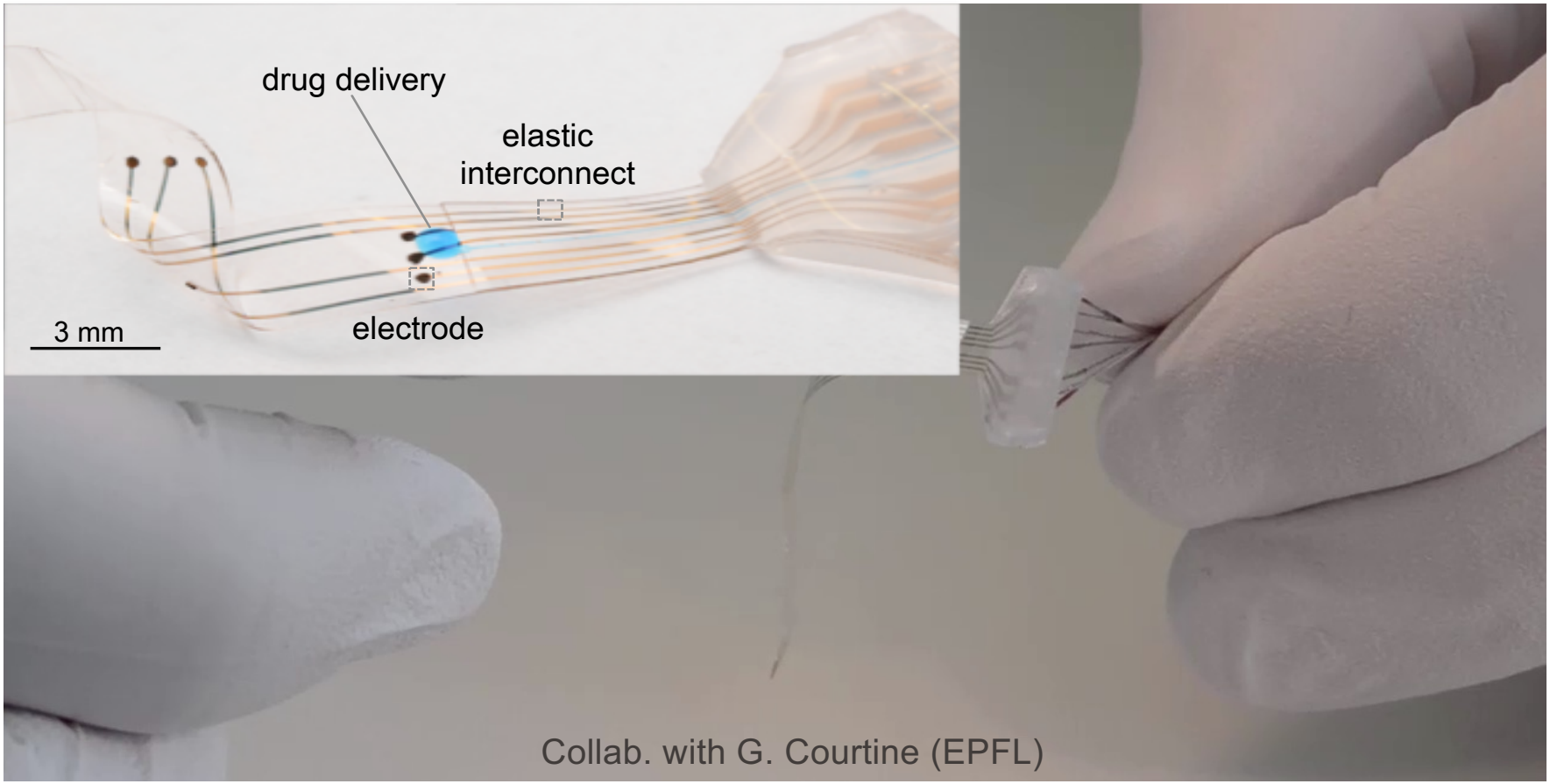


"smaller and untethered devices are the least harmful for the brain"

Biomaterials Volume 281, February 2022, 121372

NX-422 ©LSBI

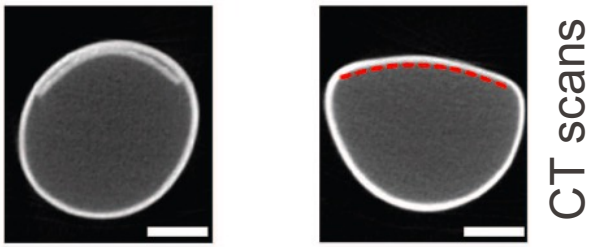
Matching mechanics



NX-422 ©LSBI

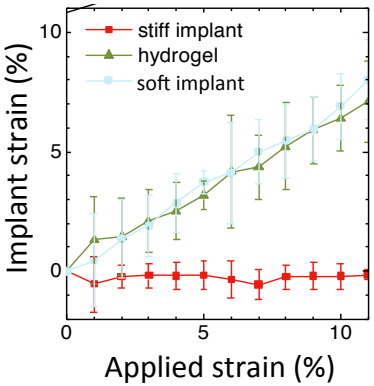
Does softness matter?

Bending stiffness ⇨ macroscopic distortion



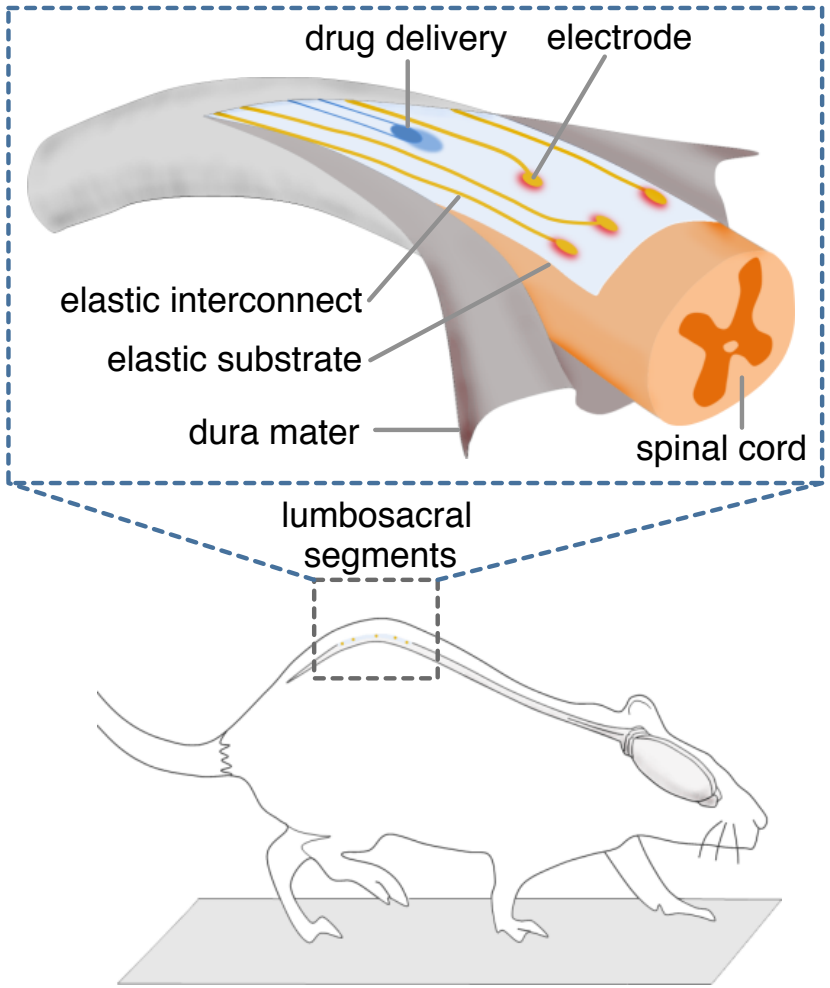
soft implant stiff implant

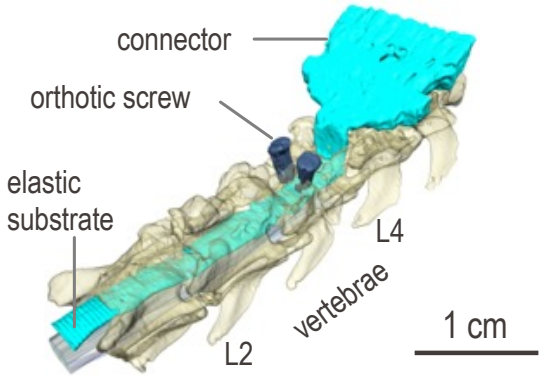
Elastic stiffness ⇨ motion restriction



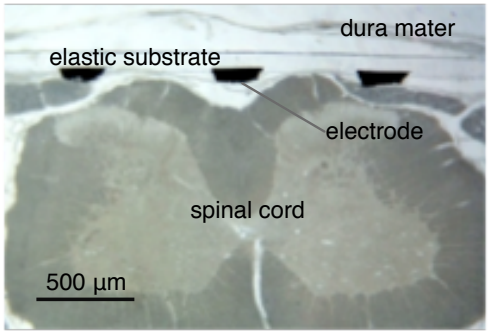
Minev*, Musienko » et al, **Science** 2015

Electronic dura mater



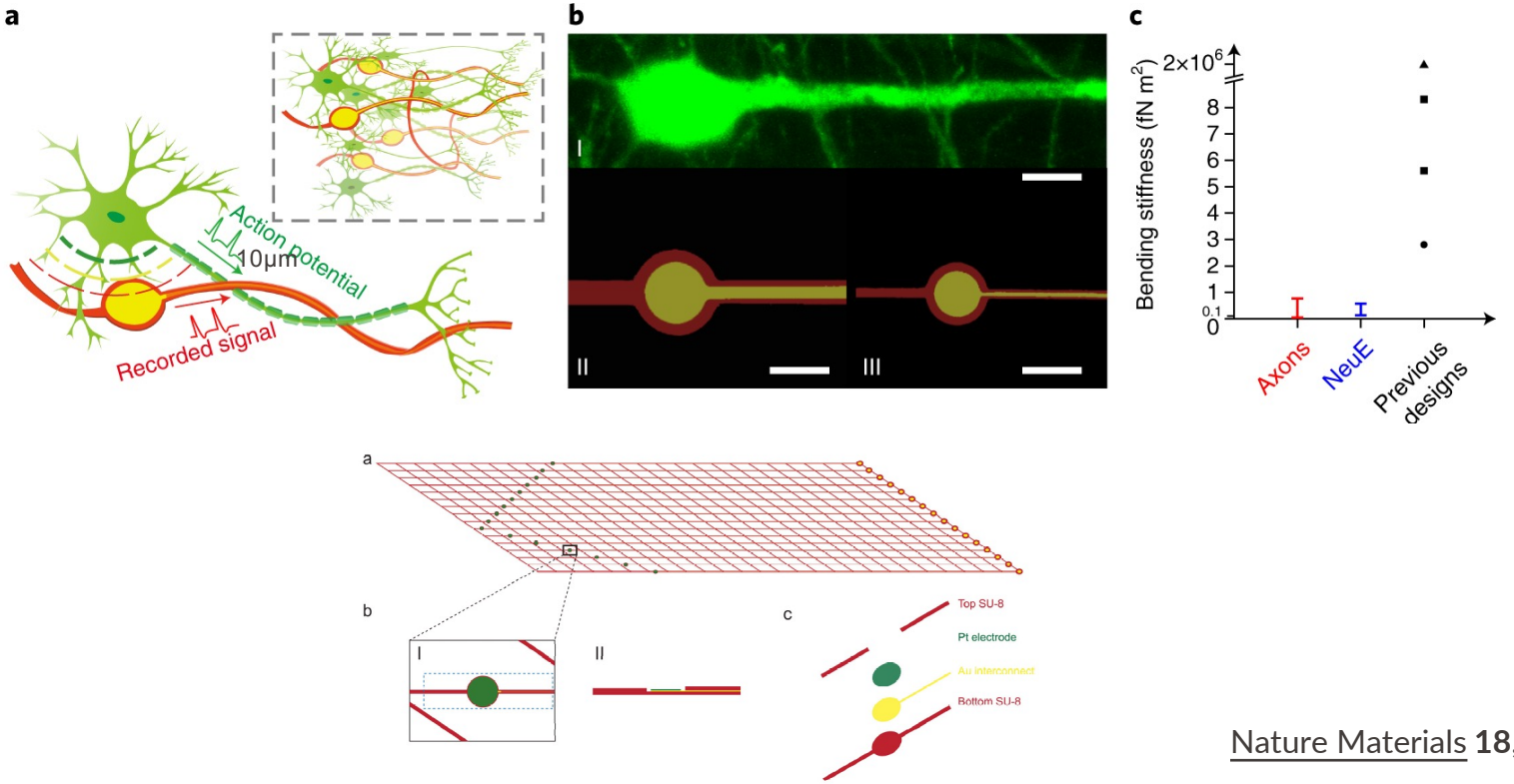


biointegration
in the spinal subdural space
@ 2 month post implantation



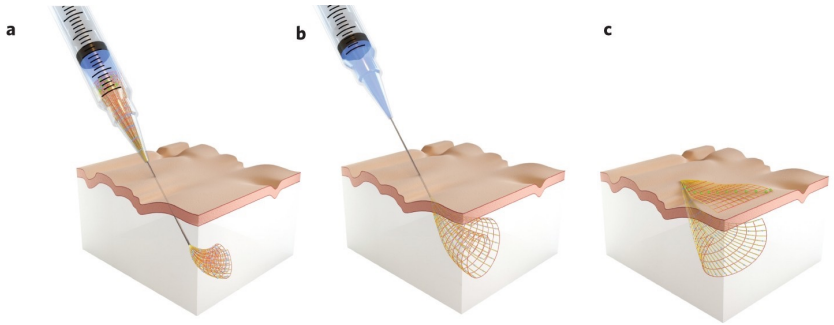
Minev*, Musienko » et al, **Science** 2015

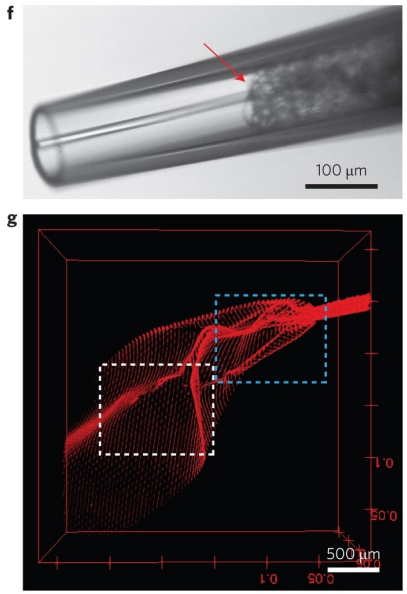
Nanomesh design

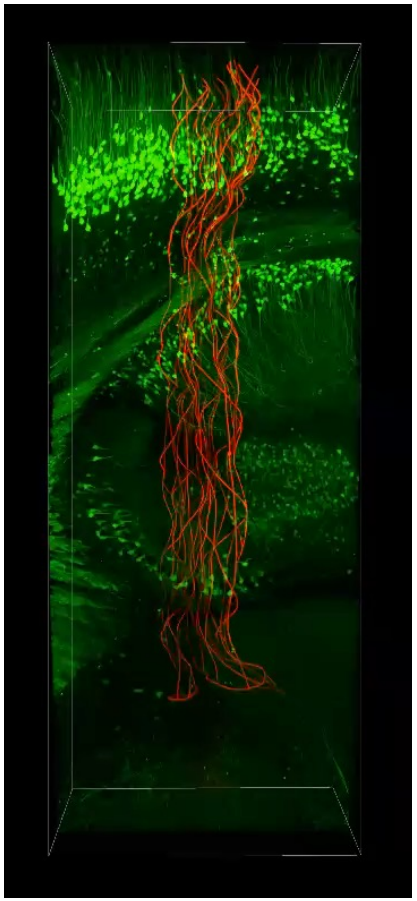


Nature Materials **18**, 510–517 (2019)

Nanomesh electrodes in vivo



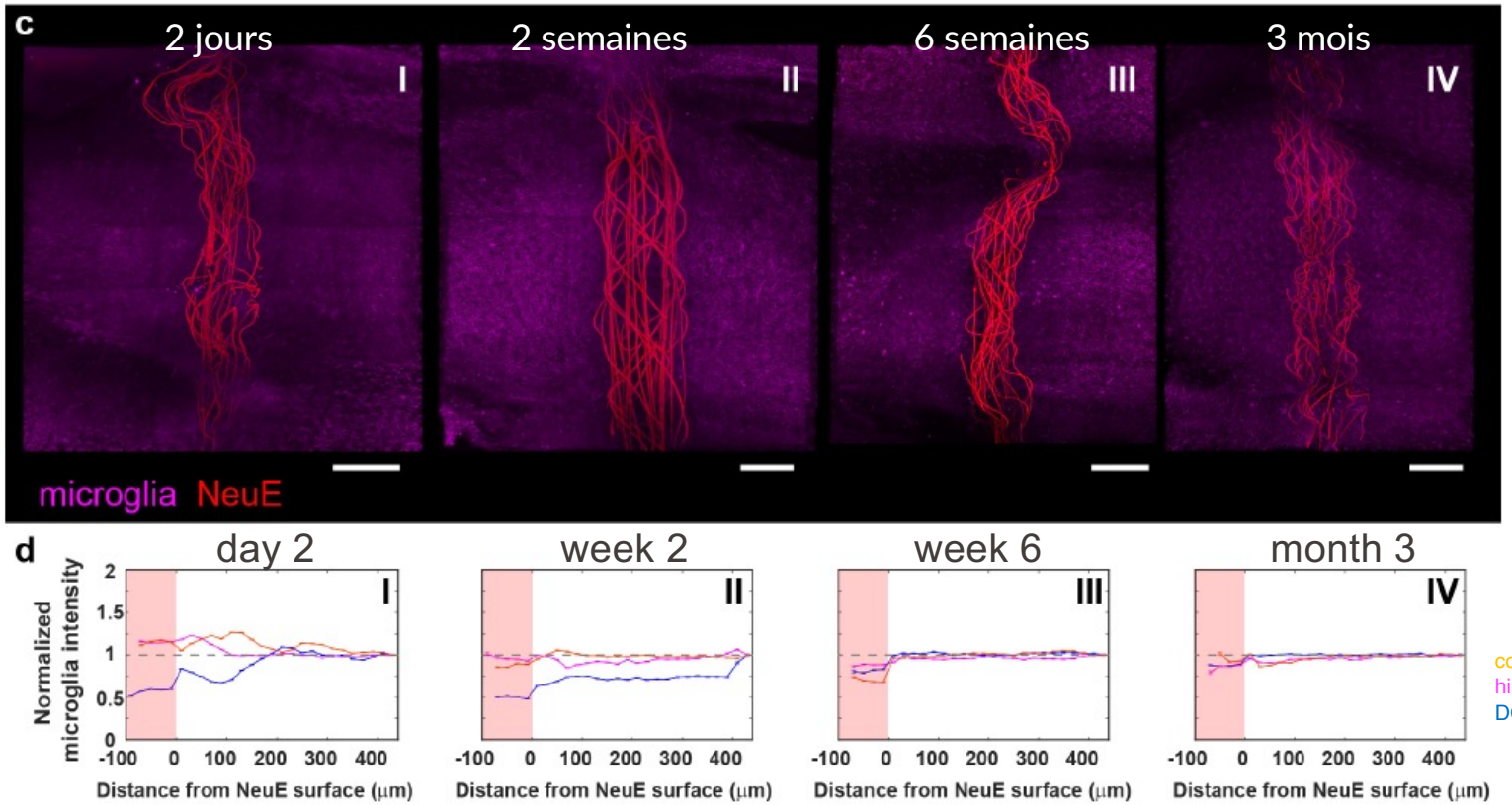




Suspension dans une solution saline et injection dans le cortex (95µm dia.)

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

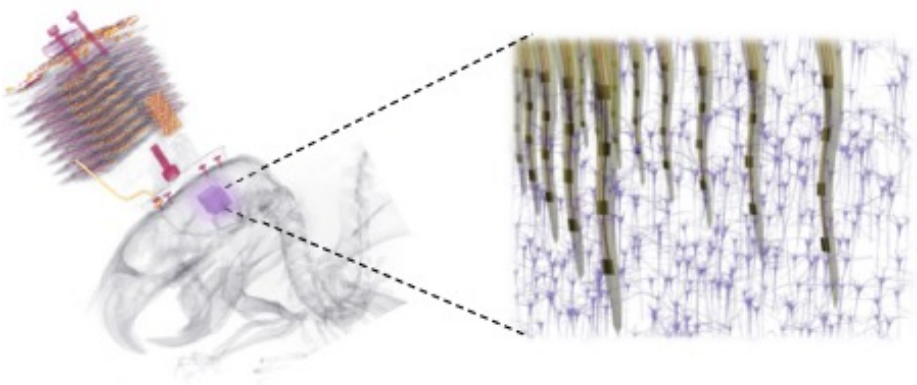
Glial cells and electrodes over time

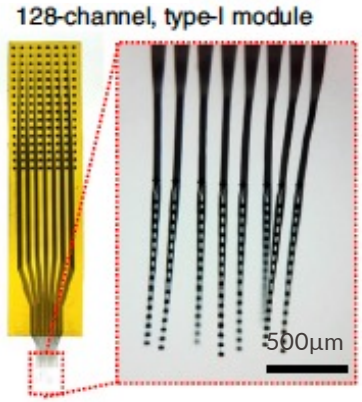


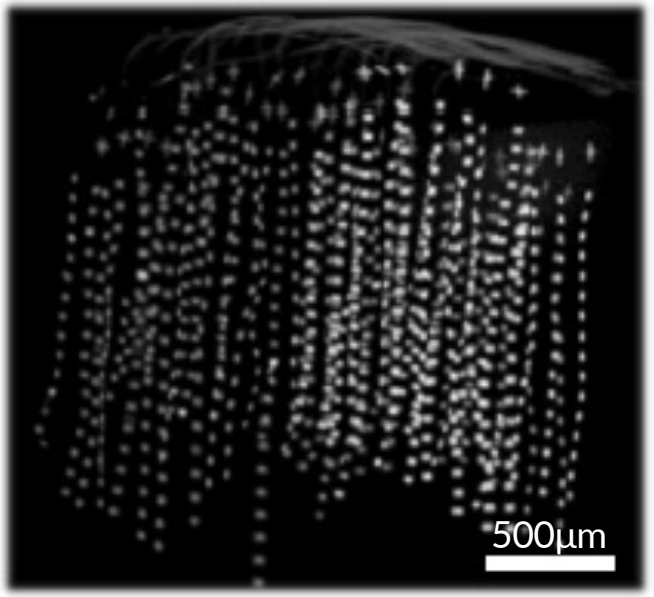
cortex (orange),
hippocampal CA1 (magenta)
DG (blue)

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

Ultra-miniaturised implant: NET electrodes







NET: ultraflexible NanoElectronic Thread electrodes

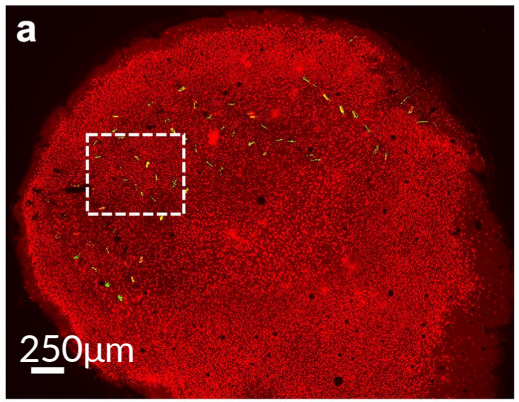
section ~ soma
SU-8 / Pt ou Au

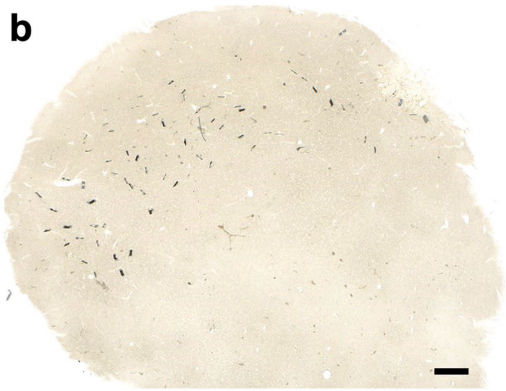
1 x 1 x 1 mm^3
(8x8x16) 1'024 électrodes

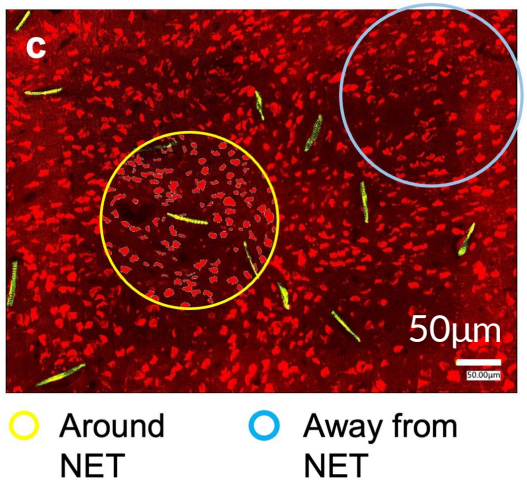
Nature BME **7**, 520-532 (2022)

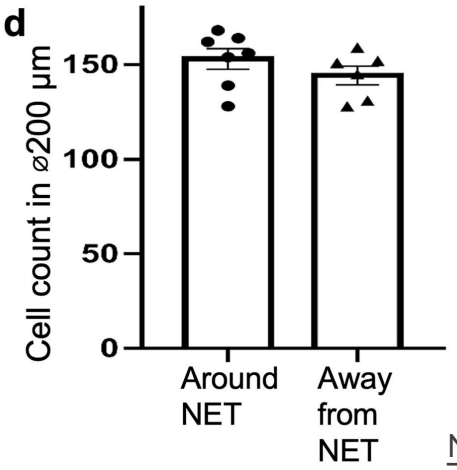
Biointegration

Implantation (>1 month)
visual cortex
mouse









number of neurons

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

Summary

- Foreign Body Reaction is inevitable with current neural interface designs
- An acute phase and a chronic phase
  - Activation of glial cells
- FBR has biological consequences on the host tissue
- FBR challenges the integrity and stability of neural interfaces