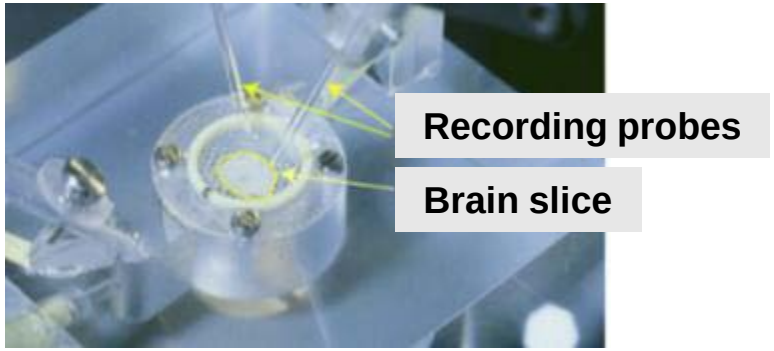
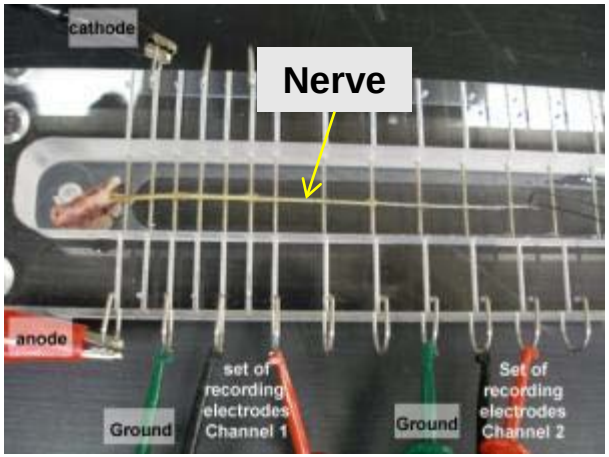
3-4. Bioelectric Phenomena

Recording methods

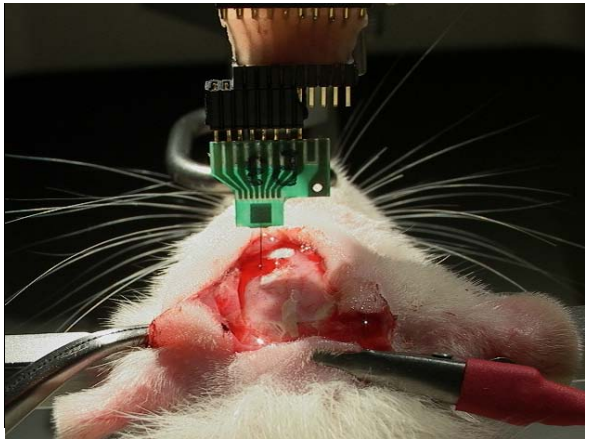
Method of recording APs

- In vitro recording



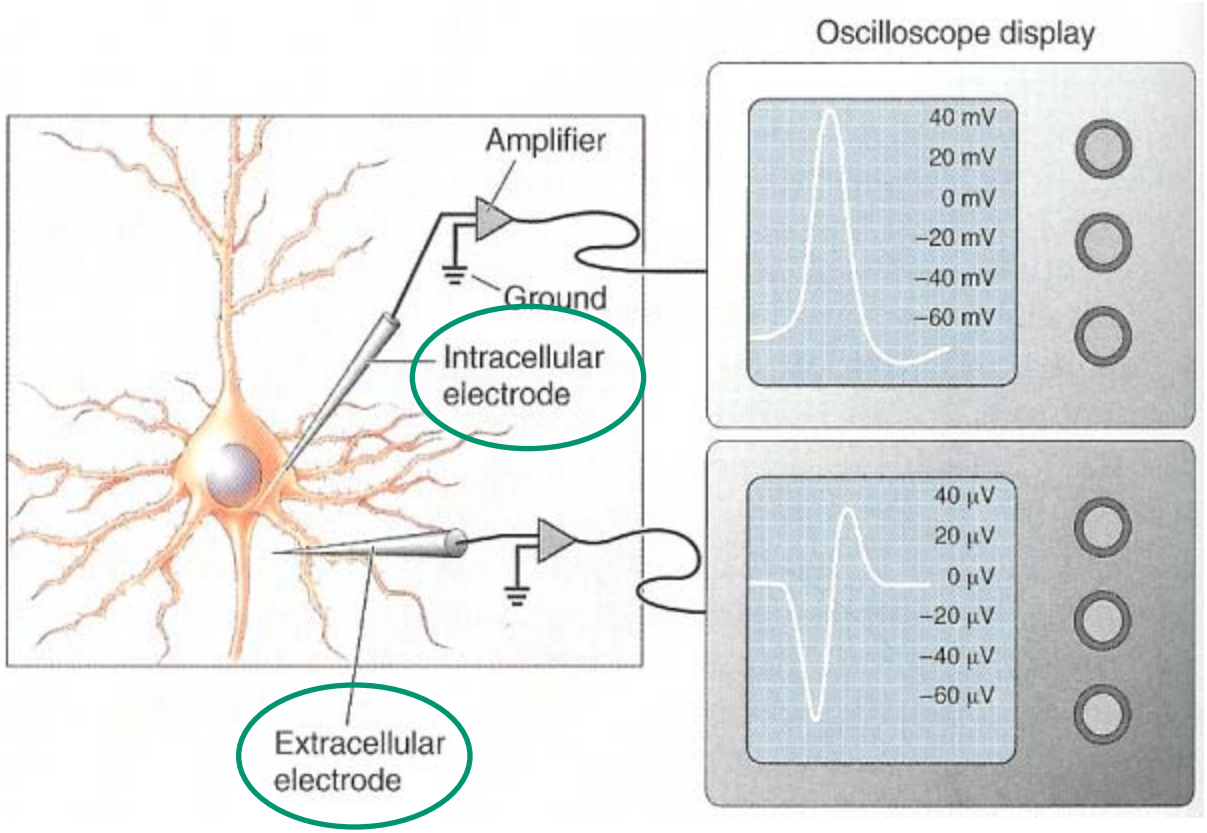


- In vivo recording



Method of recording APs

- Intracellular & extracellular recording



Intracellular recording

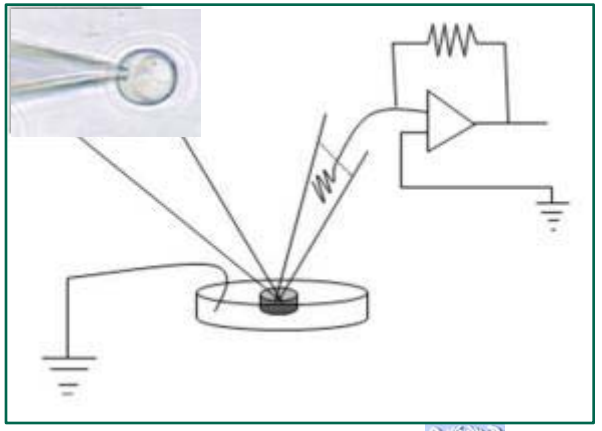
Patch-clamp

Intracellular recording: patch-clamp

- Measuring voltage and/or current across the membrane of a cell.
- Inserted inside the cell by glass micropipette electrode.
  - Filled with a solution
  - Tip diameter < 1 μm
  - Resistance: several MΩ
  - Seal Resistance: Giga Ohms (called Giga-seal)
  - Trade-off between size & resistance
    - Size: small enough to penetrate a single cell w/o damage
    - Resistance: low enough so that small neuronal signals can be discerned from thermal noise in the electrode tip

What is patch-clamp?

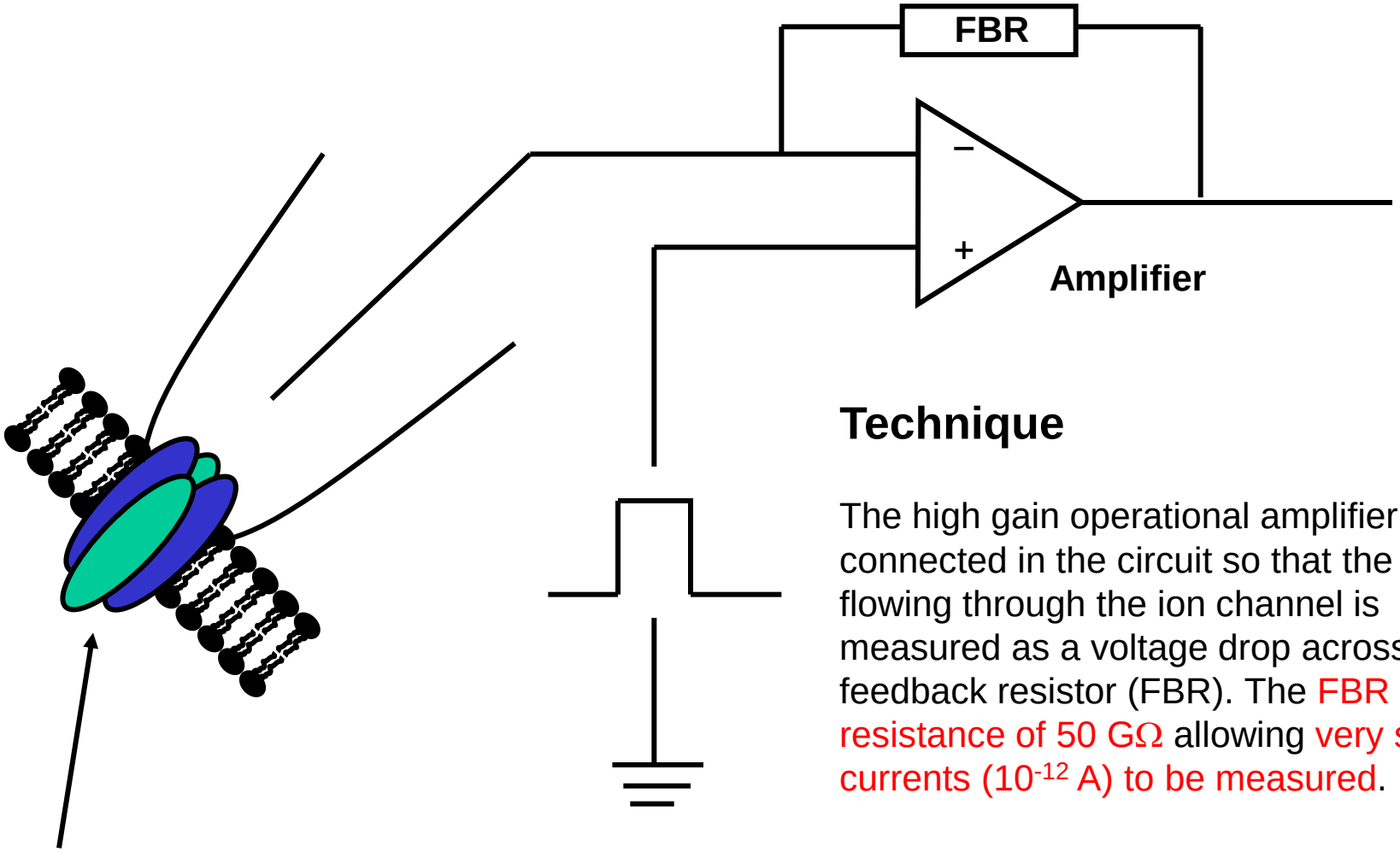
- An electrophysiological technique in which we are able to clamp the voltage of an isolated piece of cell membrane or whole-cell.
- By clamping the voltage we are able to observe currents that flow through ion channels.
- Possible to measure very small currents (10^{-12} A).



Patch clampers win Nobel Prize

- Sakmann, Neher et al revolutionized the field of electrophysiology in 1981 with their paper.
  - "Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches" (*Pflügers Arch*. **391**, 85-100).
- With patch-clamp recording, the movement of single molecules can be observed in real-time.
- In 1991, Neher and Sakmann were rewarded for their pioneering efforts in patch-clamp recording when they jointly won *the Nobel Prize in Physiology or Medicine*.

Patch-clamp circuit

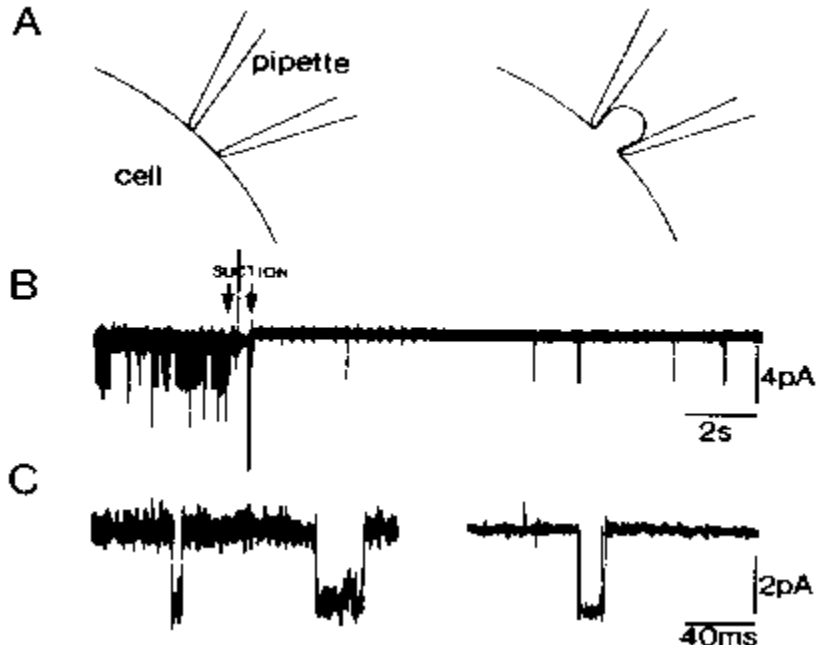


Technique

The high gain operational amplifier is connected in the circuit so that the current flowing through the ion channel is measured as a voltage drop across the feedback resistor (FBR). The FBR has a resistance of 50 GΩ allowing very small currents (10^{-12} A) to be measured.

Patch of cell membrane with ion channel

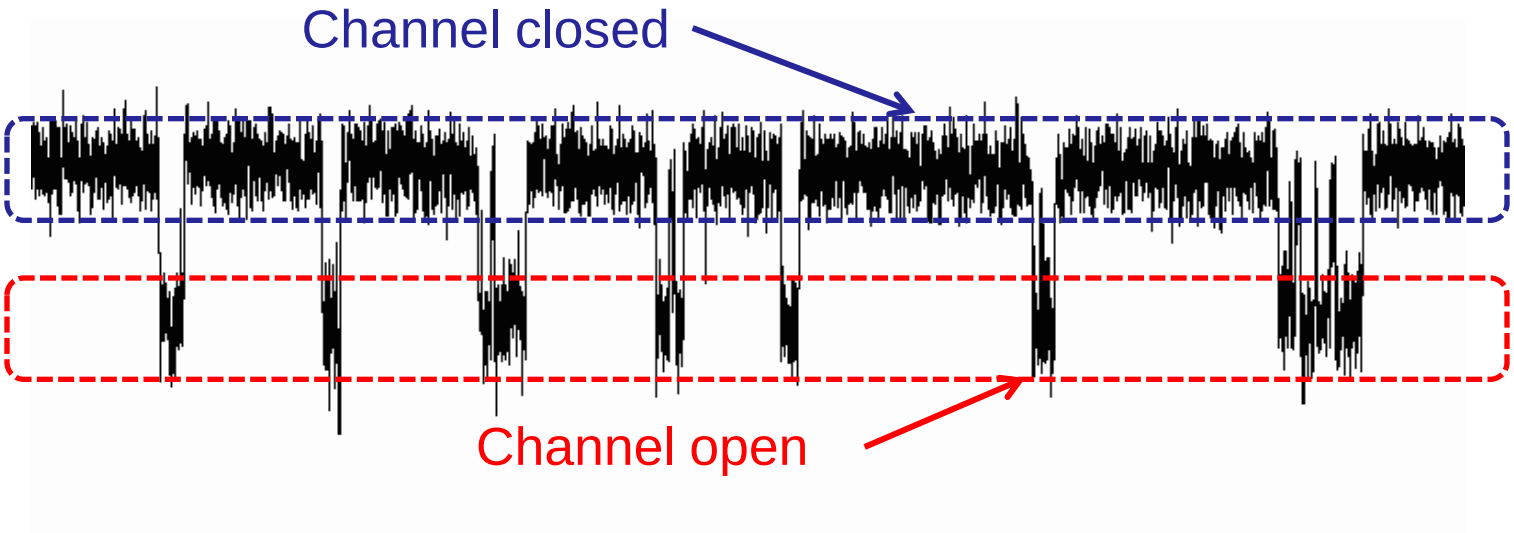
Patch-clamp example



From Hamill *et al* 1981

By increasing the seal resistance we reduce the noise level and increase temporal resolution.

Single channel currents

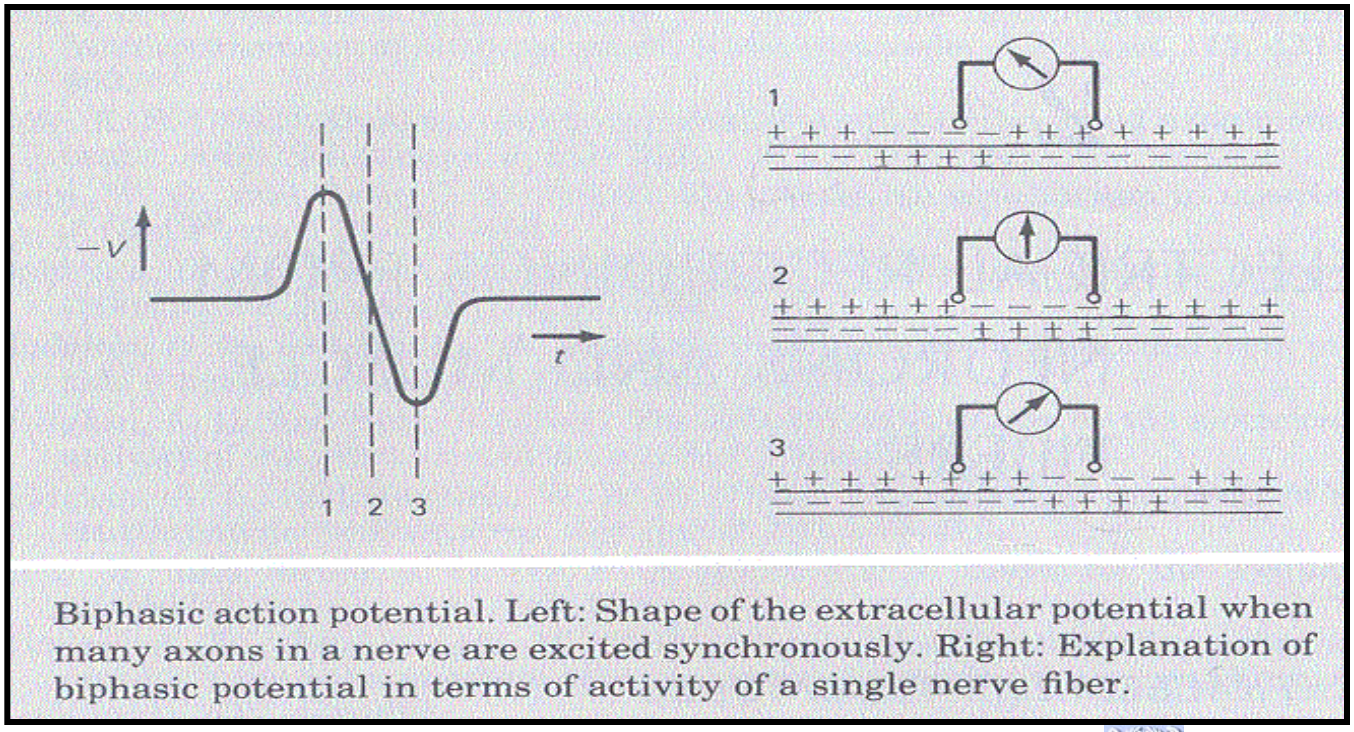


- A patch clamp recording reveals transitions between two conductance states of a single ion channel: closed (at top) and open (at bottom).
- Diffusion through a single channel is "all-or-none”.

Extracellular recording

Biphasic action potential

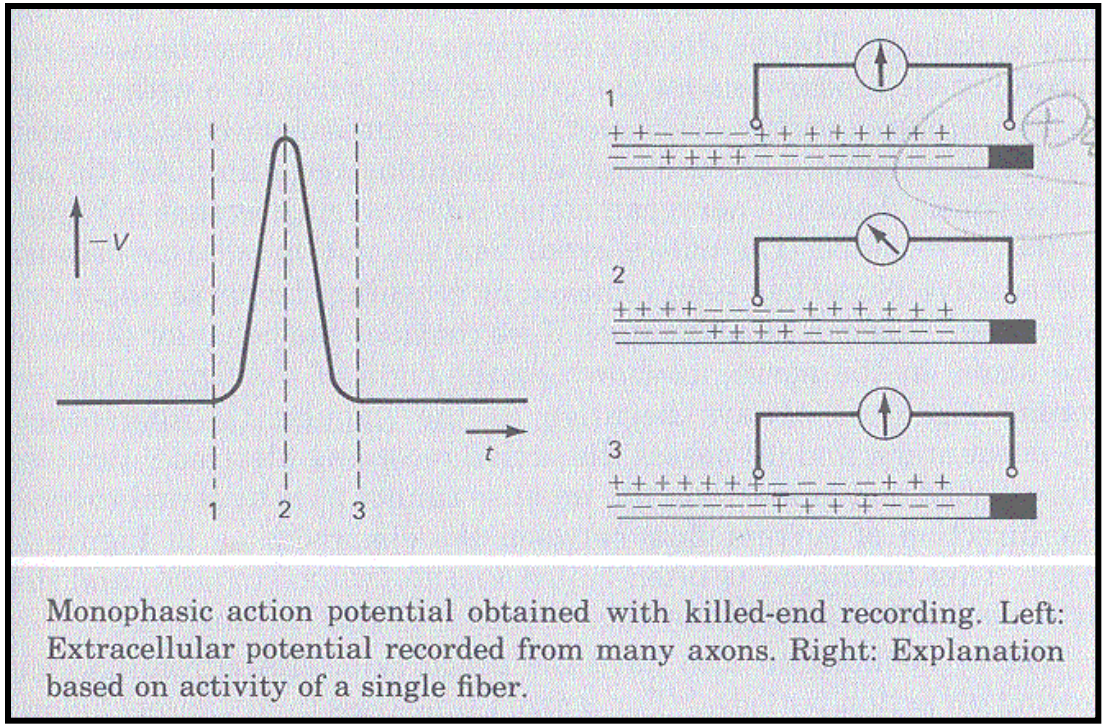
- Extracellular recording: Biphasic AP using bipolar electrodes.



Biphasic action potential. Left: Shape of the extracellular potential when many axons in a nerve are excited synchronously. Right: Explanation of biphasic potential in terms of activity of a single nerve fiber.

Monophasic action potential

- Extracellular recording: Monophasic AP recorded using killed-end recording.



Monophasic action potential obtained with killed-end recording. Left: Extracellular potential recorded from many axons. Right: Explanation based on activity of a single fiber.

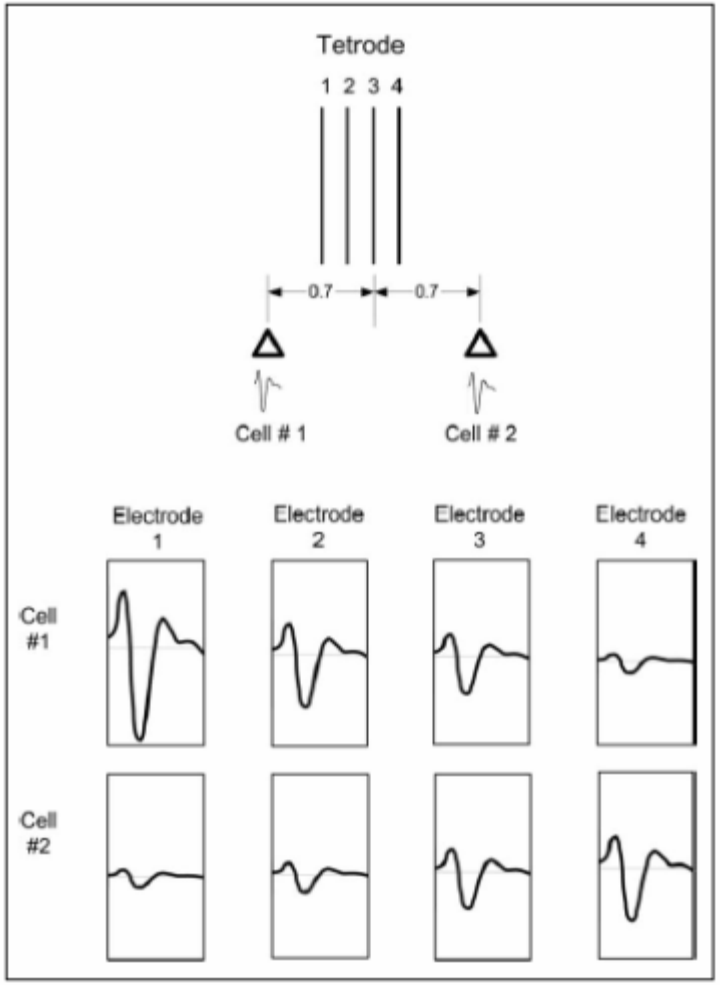
Single unit vs. multi-unit recording

- Single-unit recording: If the tip is small enough, such a configuration may allow indirect observation and recording of the electrical activity of a single cell.

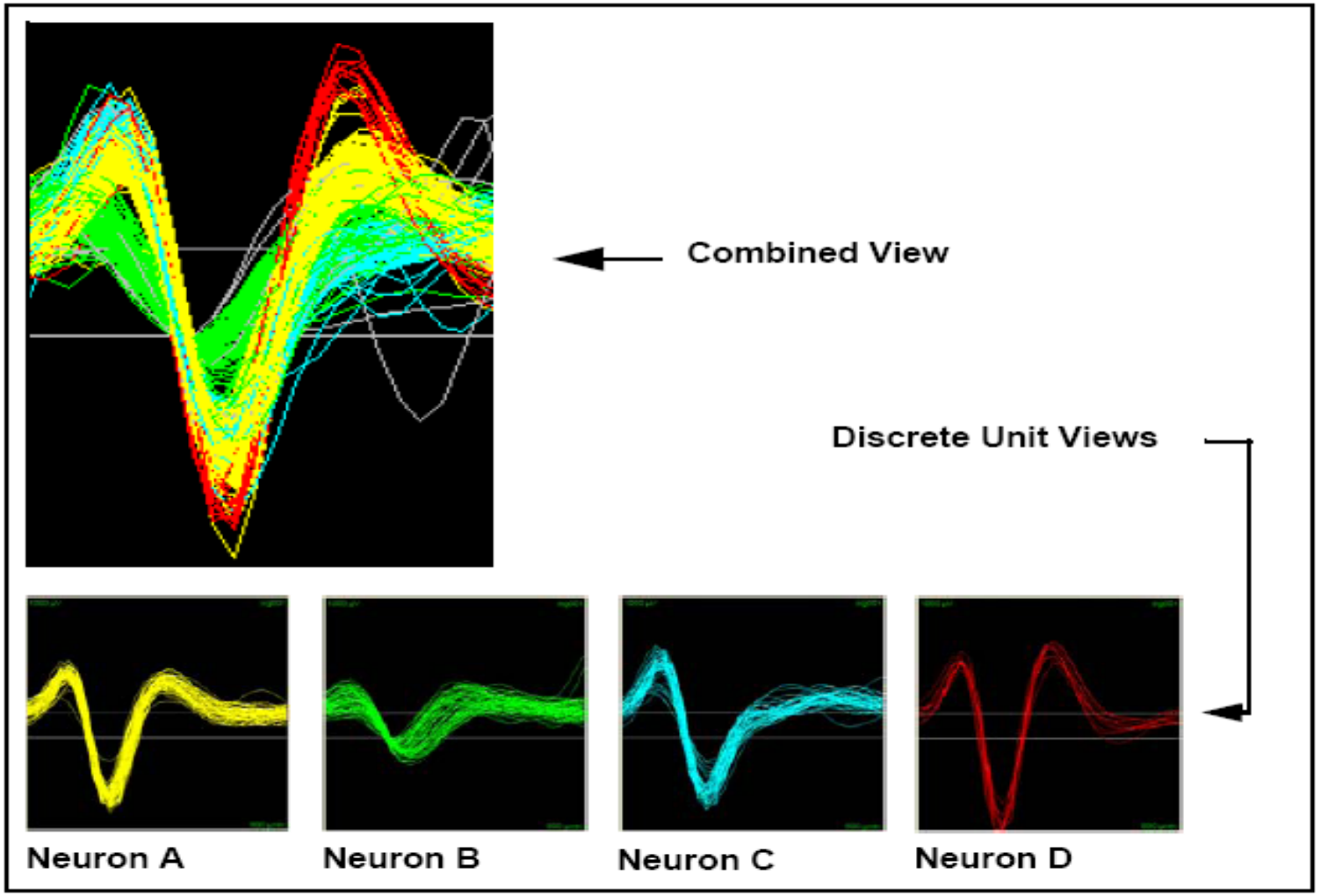
- Multi-unit recording: Depending on the electrode size and placement, an extracellular configuration may pick up the activity of several nearby cells simultaneously.

Principles of spike sorting

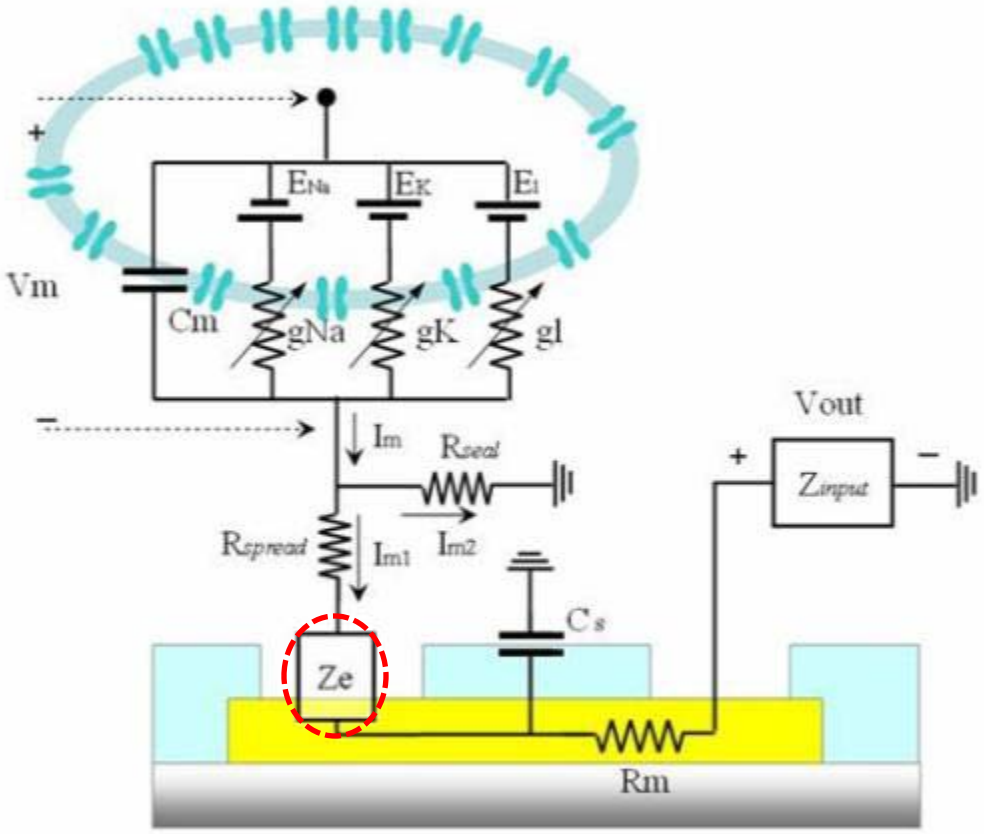
- Multi-unit recording needs spike sorting to separate the signals.
- Spike sorting depends on the shape of waveform.
  (distance between neuron & electrode)
- If cells have similar morphology and they are at the same distance from an electrode, their waveform shapes can be indistinguishable.



Spike sorting



Extracellular recording circuit



Schematic diagram of extracellular recording

※ Impedance of recording site (Z_e ~ few hundred kilo-ohm)

※ keep the electrode surface clean for small neural signal recording. If not, I_{m2} (leakage current) is relatively increased because of the capacitance factor at the electrode surface.

Trade-off between sensitivity & selectivity

- *For high sensitivity :*

  –Needs relatively small Z_e (site impedance) compared with R_{seal} .

  → but, large site area reduces the impedance.

- *For high selectivity* :

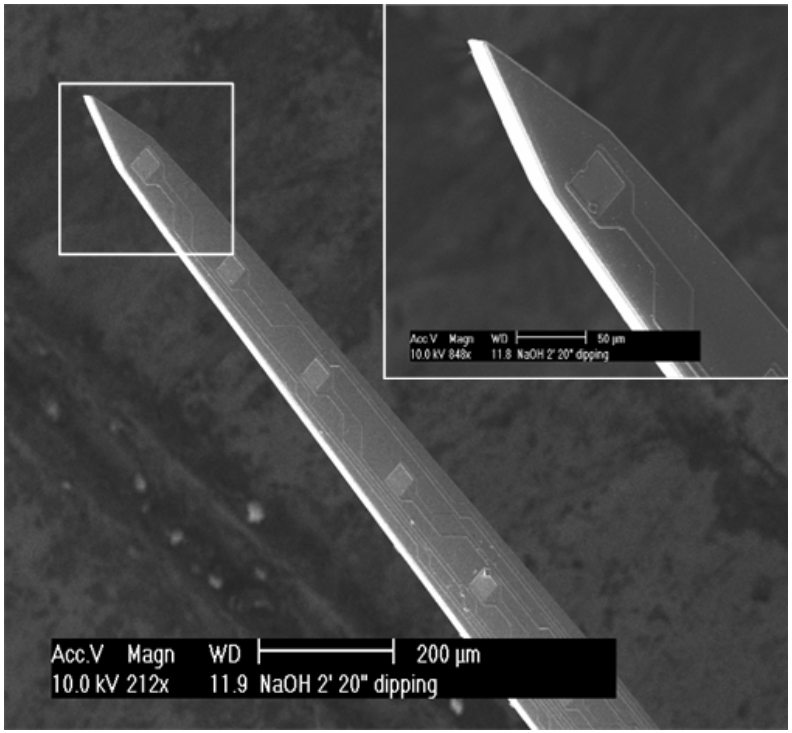
  –Needs relatively small site area to recording single unit signal.

  → but, small site area increases the impedance.

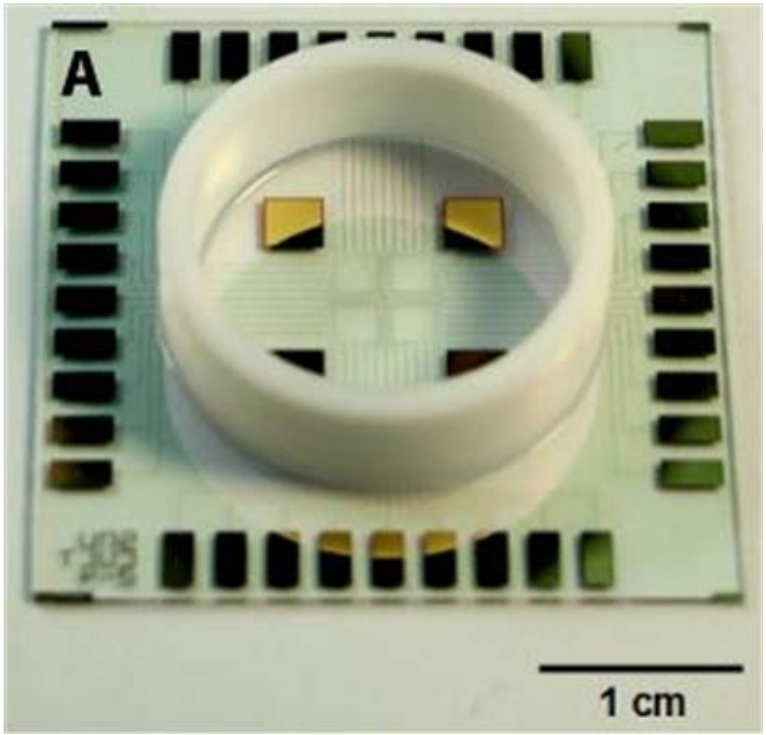
Electrodes & applications

MEA recording technique

Neural Recording Devices: SNU electrodes

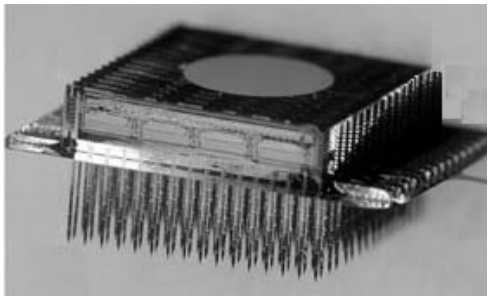


SNU depth-type
Silicon Electrode

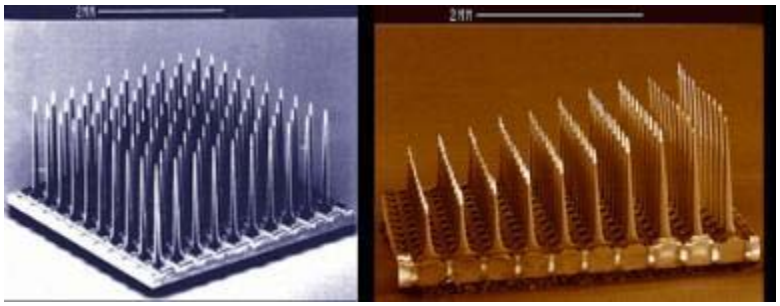


SNU planar-type MEA
(microelectrode array)

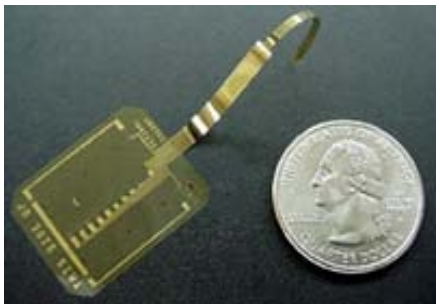
Other Electrodes



Michigan Probe

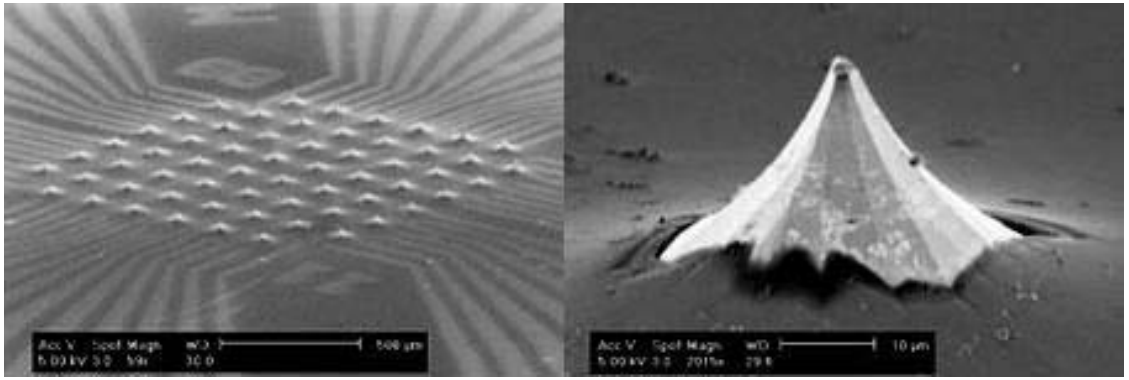


Utah Probe



Flexible Probe

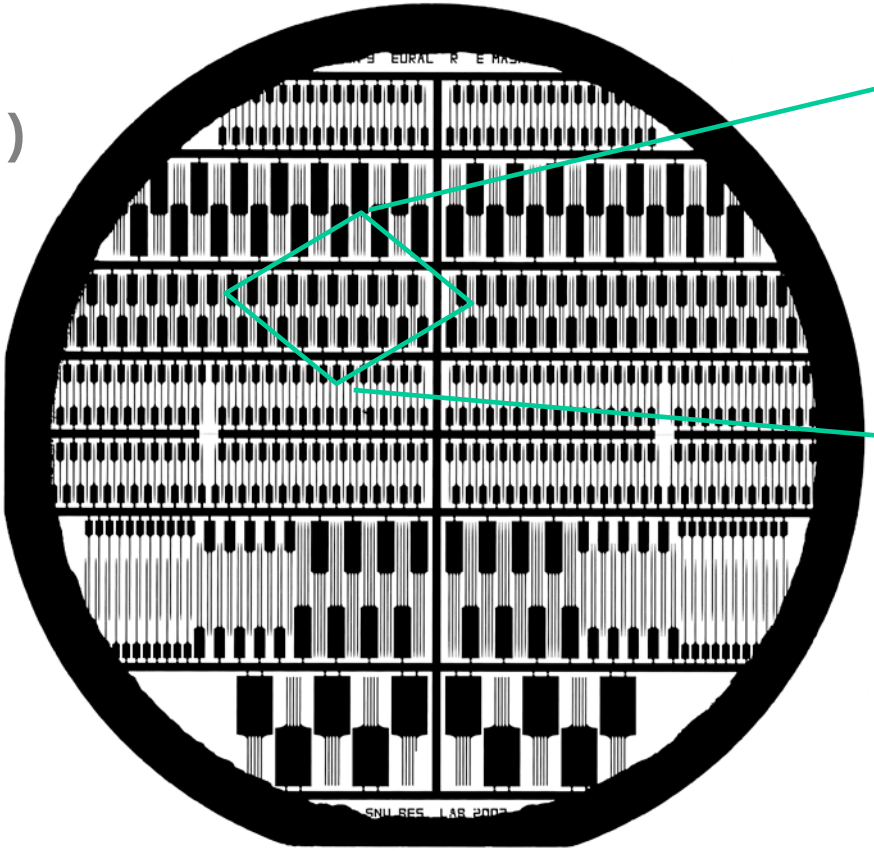
Multichannel systems
60-channel MEA



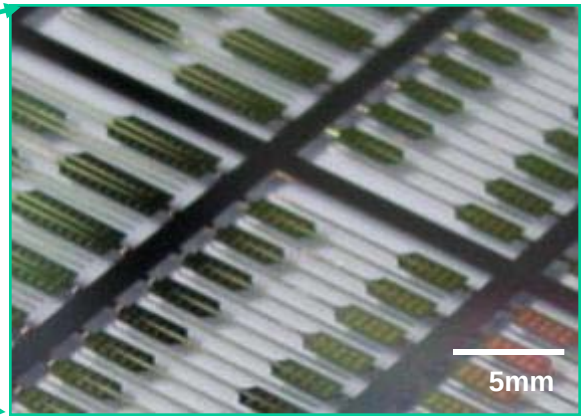
Multichannel systems
- 3D Electrode

Depth- type silicone neural probe

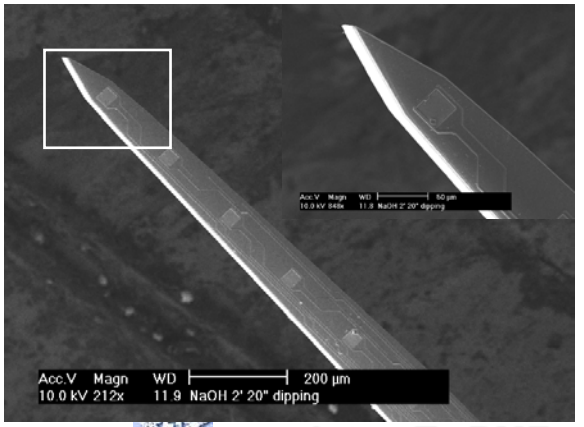
(A)



(B)



(C)

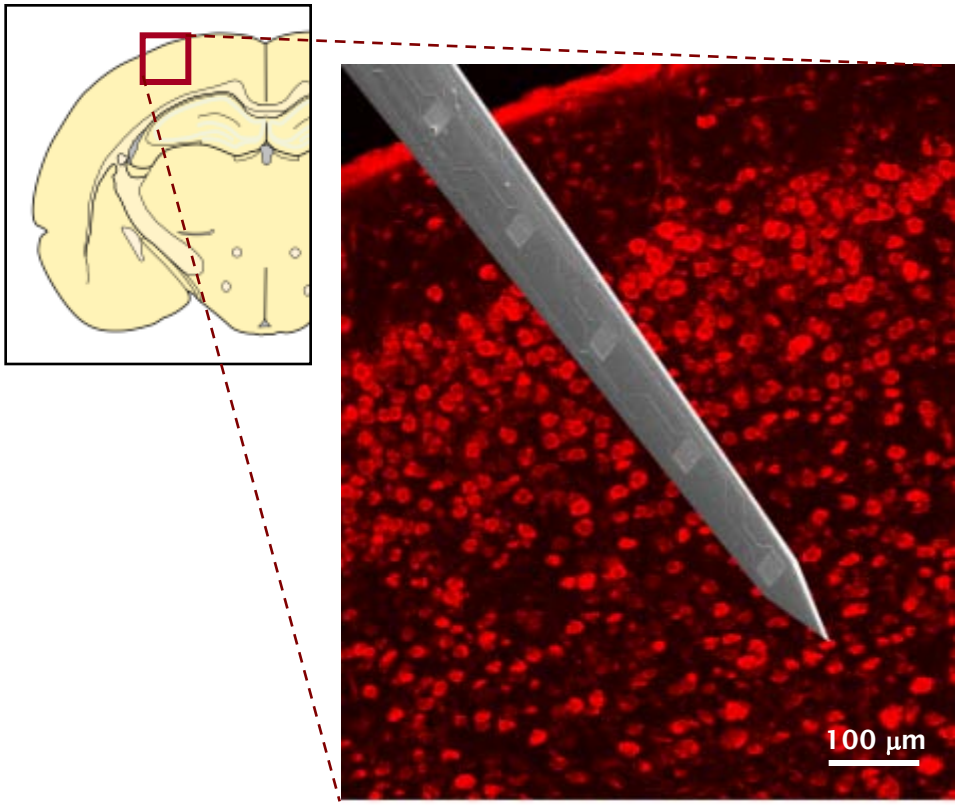


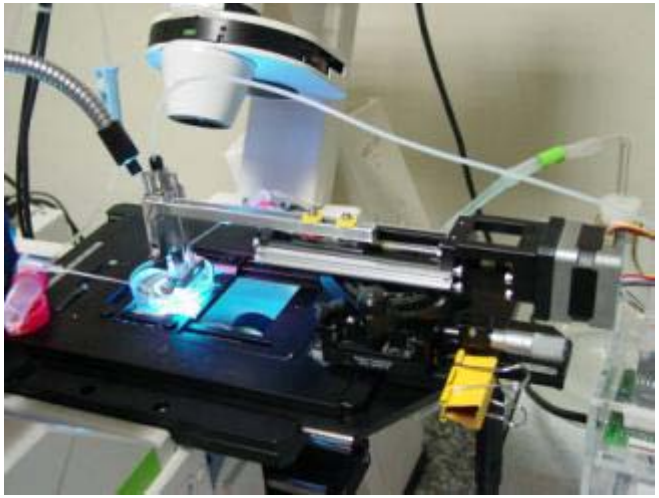
A) Released whole wafer image,

B) Fabricated 1 shank neural probe

C) SEM Image - electrode sites and probe tip

Image recording of tissue damage

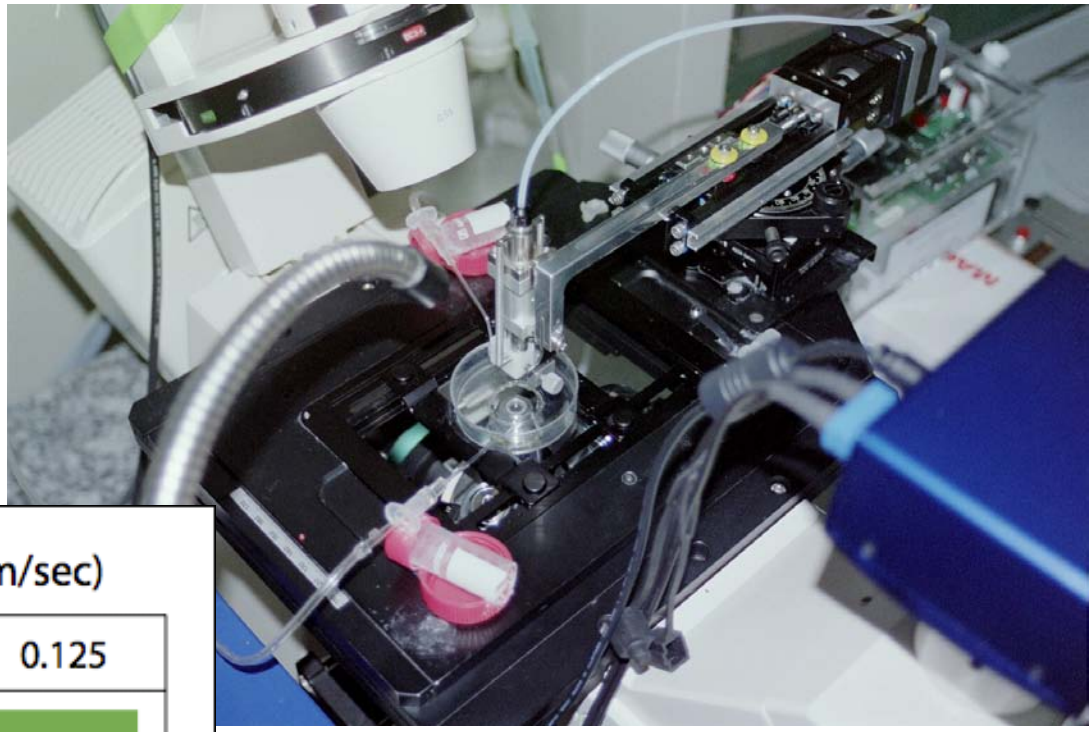




Tissue damage in forms of compression and distortion of tissue, Severing, Dragging, Rupturing of Blood Vessels.

Ex vivo insertion experimental design

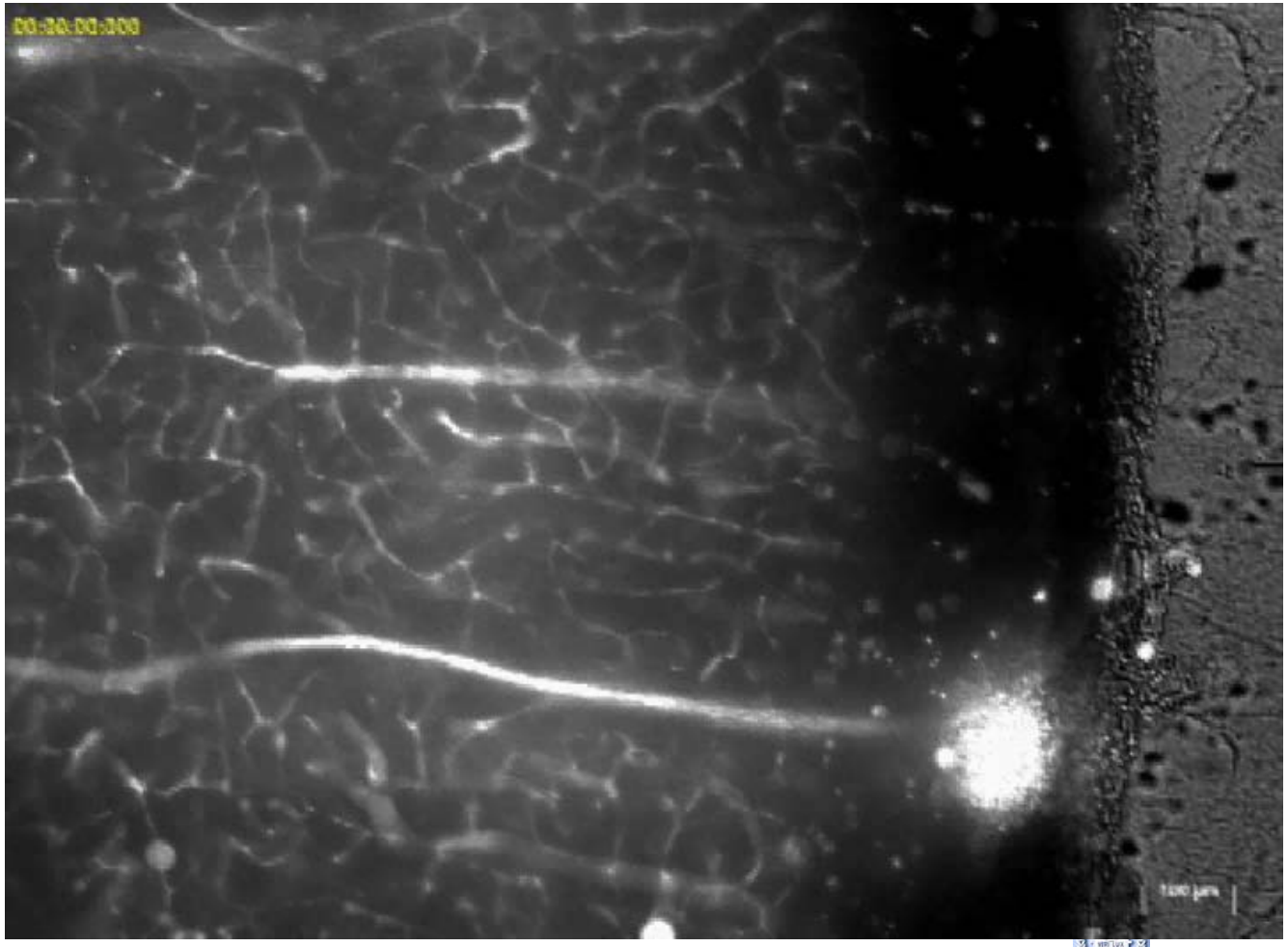
To present an *Ex Vivo* system that permits qualitative observations and the quantitative analysis of tissue damage in real time during probe insertion.



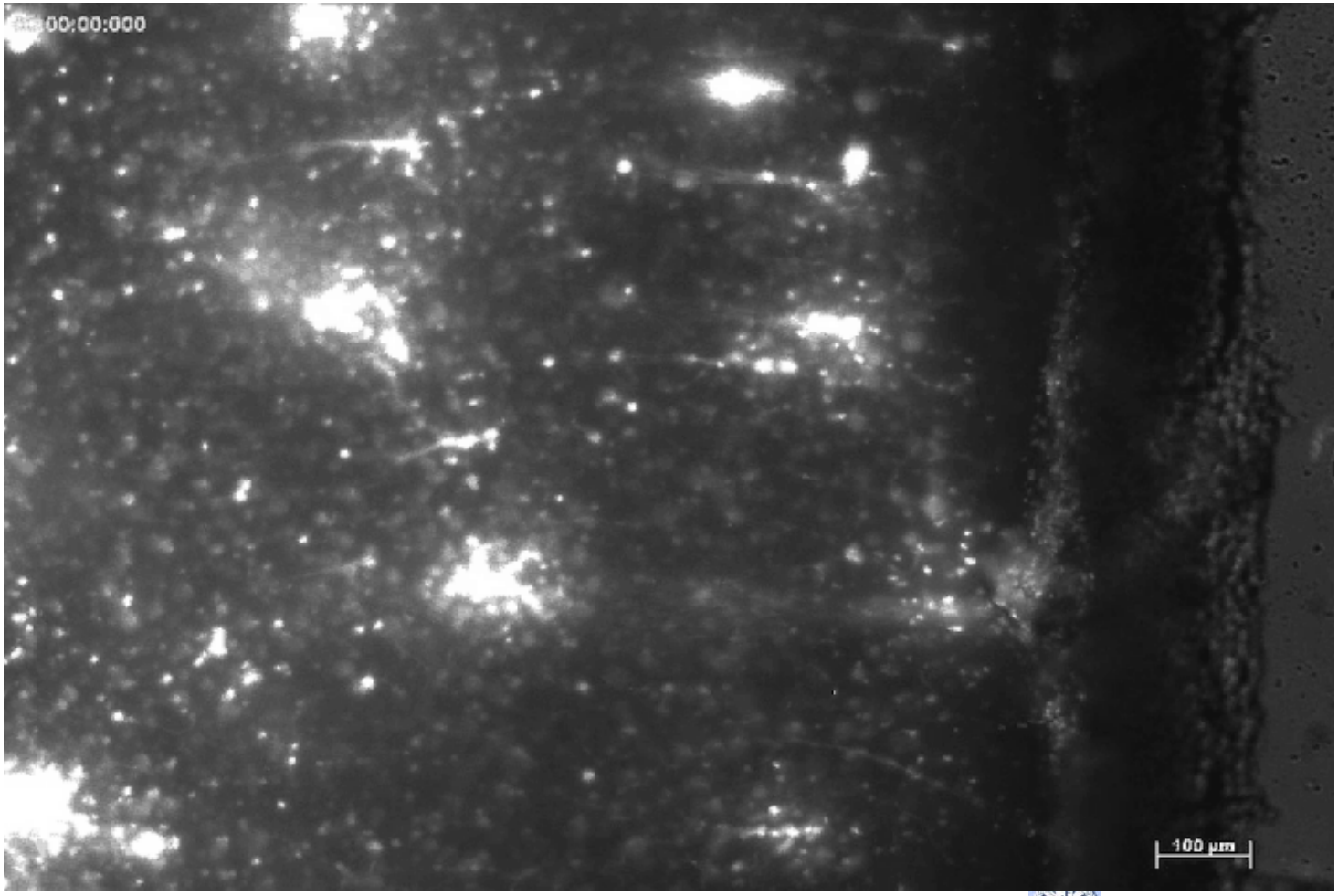
Ex vivo imaging system

Device tip shape	Insertion Speed (mm/sec)		
	2.000	0.500	0.125
tip angle = 150°	3	6	8
tip angle = 90°	3	5	7
tip angle = 5°	8	11	8

Ex vivo device insertion - near best condition



Ex vivo device insertion - position on pial surface

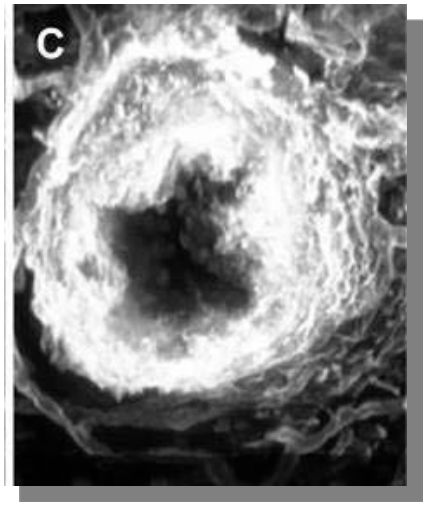


Ex vivo device insertion - maximum effective strain

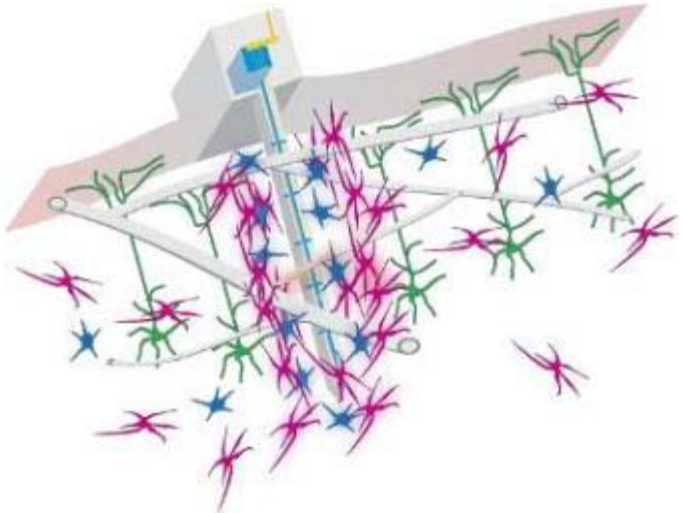
- **Speed**
  - **Penetration into Pia is easier with Faster insertion, causing less compression and distortion.**
  - **Faster insertion seems to cause less vessel damage except it tends to sever vessels.**
- **Sharpness**
  - **Sharp ones seems to cause less damage but not as relevant as the speed.**

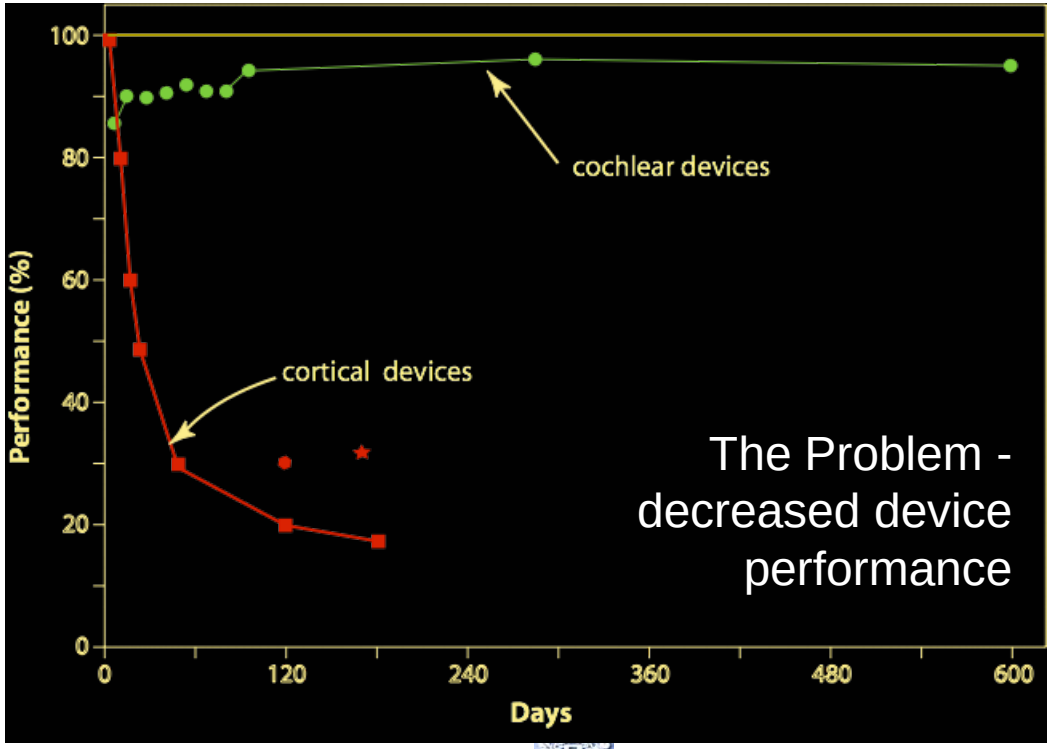
Device tip shape	Insertion Speed (mm/sec) 2.000	0.500	0.125	mean
tip angle = 150°	0.25 (0.13)	0.22 (0.05)	0.55 (0.25)	0.34
tip angle = 90°	0.15 (0.01)	0.20 (0.08)	0.46 (0.22)	0.27
tip angle = 5°	0.25 (0.12)	0.24 (0.11)	0.28 (0.16)	0.25
mean	0.21	0.22	0.43	

Glial Encapsulation



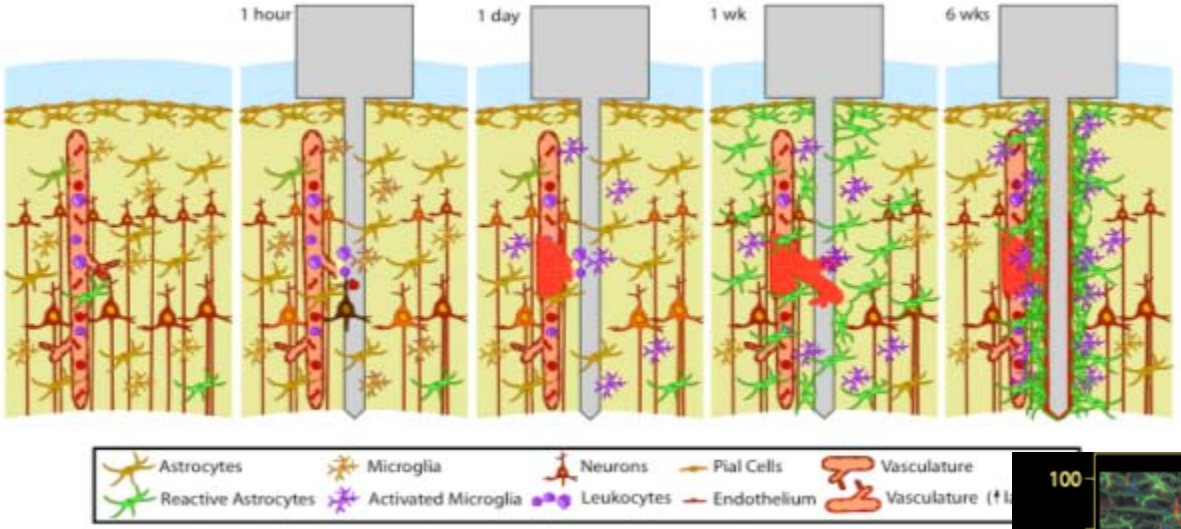
Chronic implant is limited due to glial encapsulation that electrically isolates devices from neuronal networks.

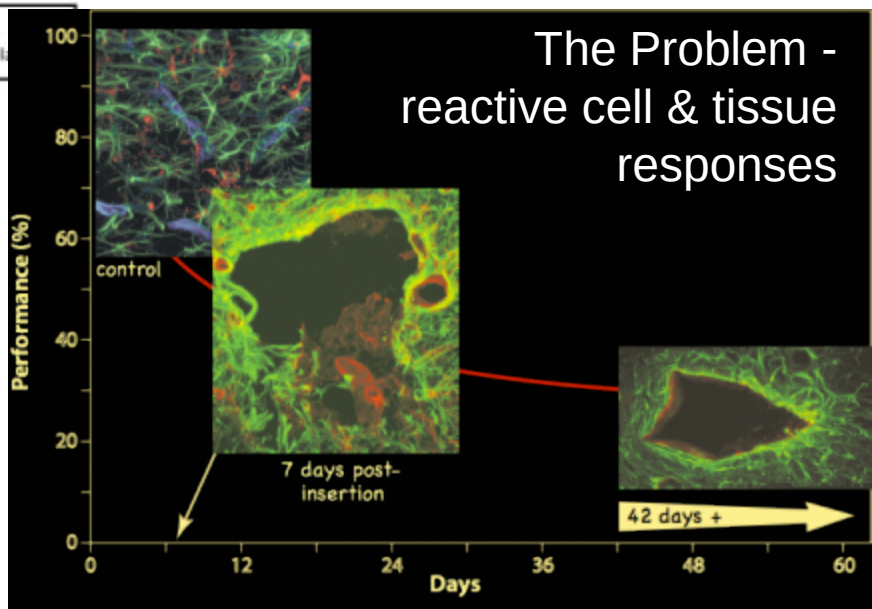




Biocompatibility of Neural Probe

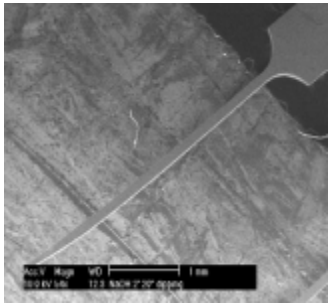
Control **<--** **Early Responses** **-->** **Sustained**



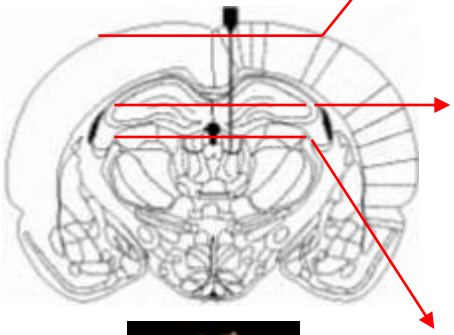


Time Course and Regional Difference of Reactive Responses

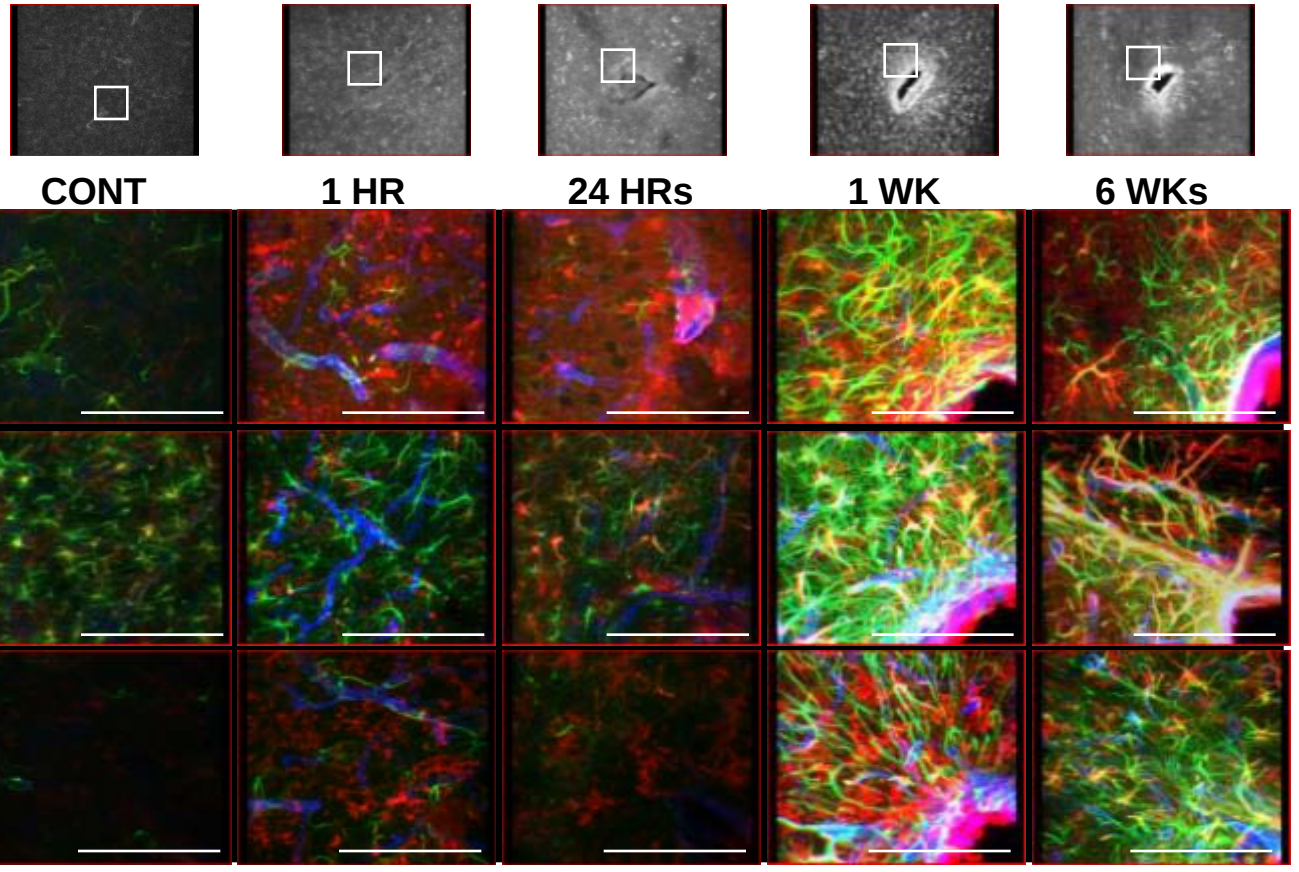
Single-shank silicon probe



Stereotaxic Coodinate



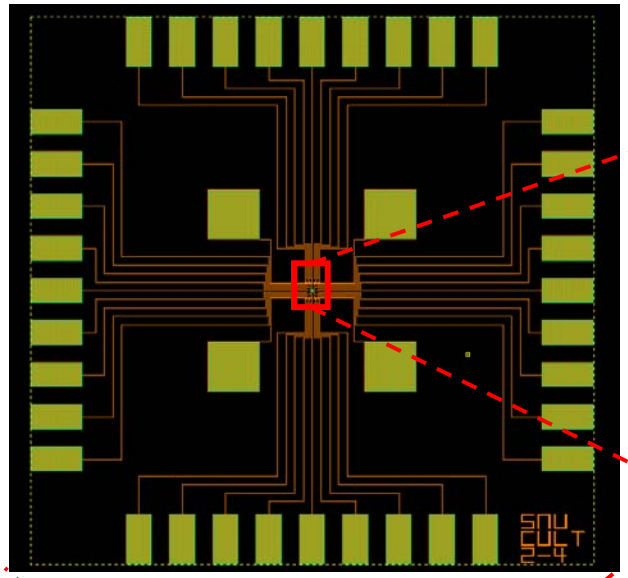
Time Post Probe Insertion

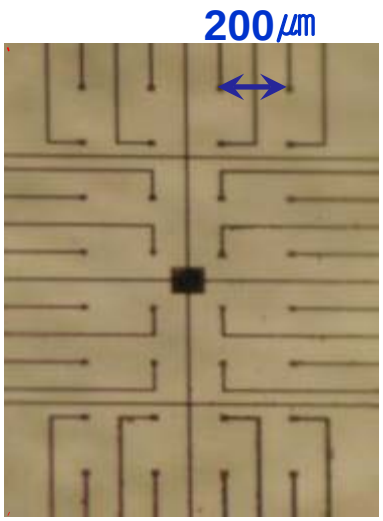


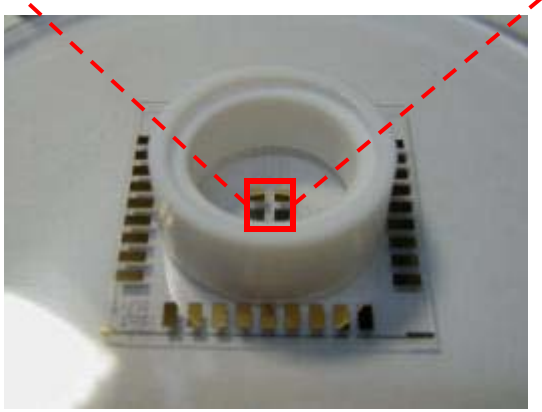
Regional Difference/Time response

- Reactivity of astrocytes (GFAP) was greatest in the Hippocampus. (>neocortex > thalamus)
- Microglia(CD11b) was comparable in neocortex and hippocampus.
- Blood vessel (Laminin) in all regions extended considerable distances from insertion sites at one hour and decreased at later times.
- Microglia(CD11b) is peaked at 1 week, while astrocyte(GFAP) is at 6 weeks.

Planar-Type MEA design







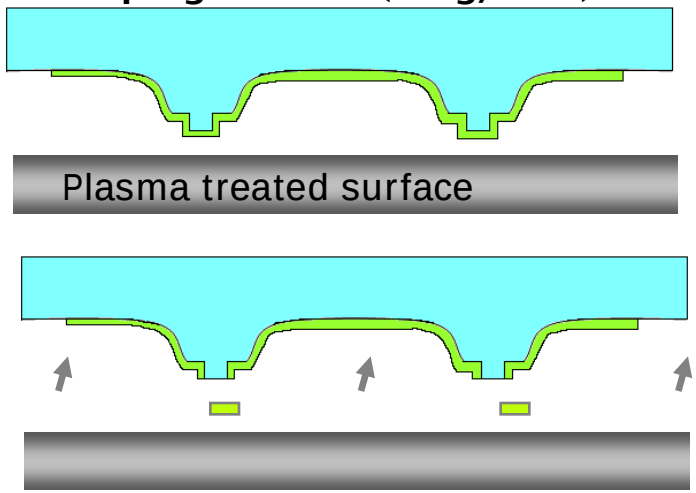
- 8 × 4 array
- Each electrode size : 10×10μm^2
- Interelectrode Spacing : 200 μm

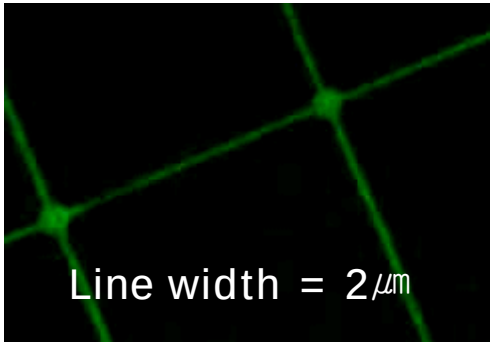
Microstamping of Poly-L-lysine

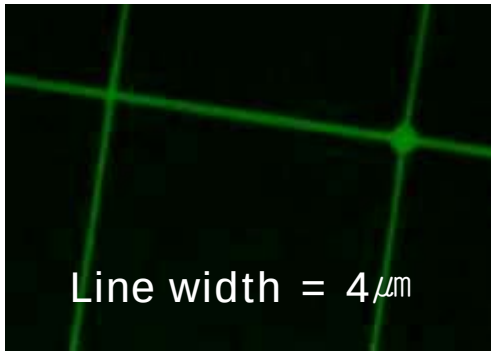
PDMS stamp molding with stampmater

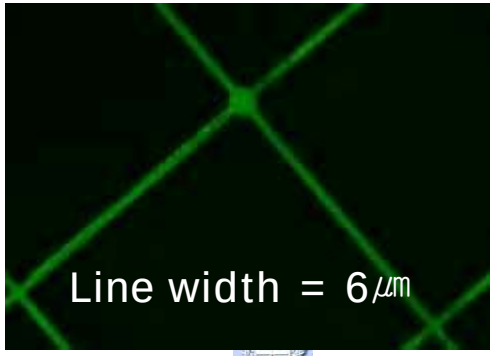
Poly- L- lysine (diluted in BBS) Inking for 2 hr

Stamping for 1 hr (50 g/cm^2)

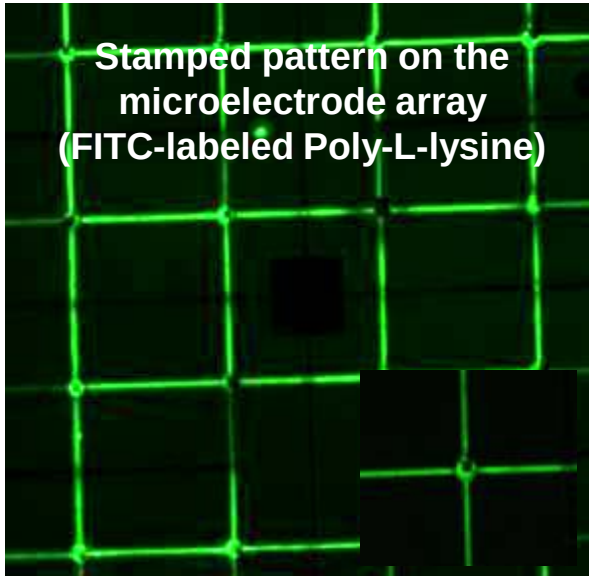


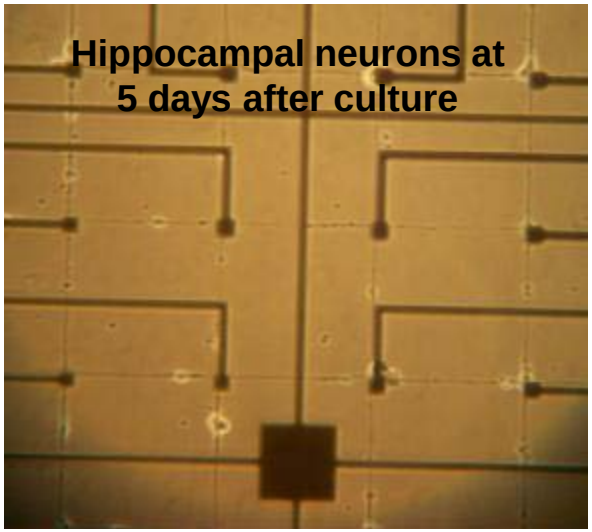






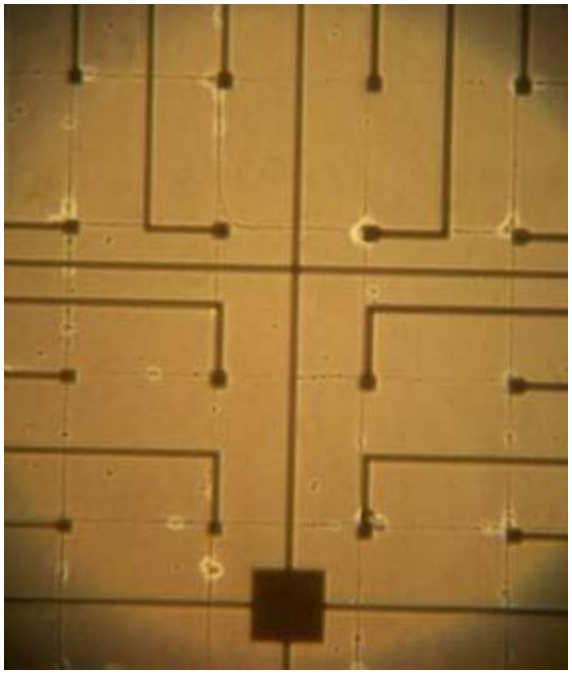
Stamped protein pattern on MEA





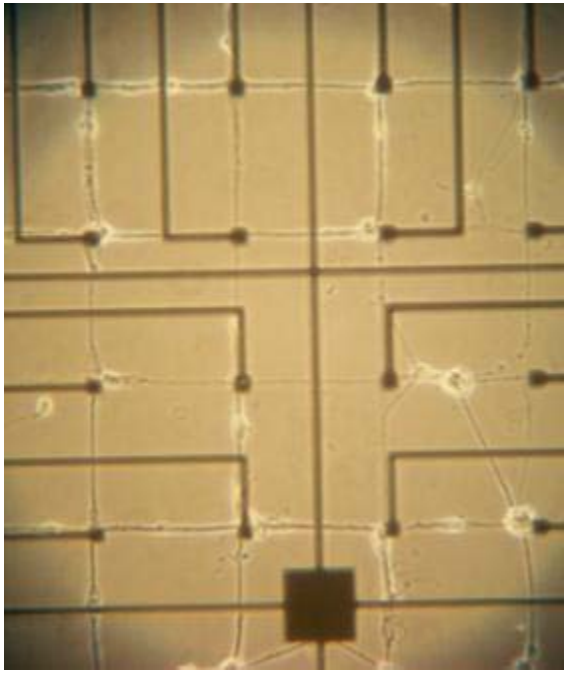
Optimizing cell density

100 cells/㎟



Too small number of cells are attached to record signals especially to see the signal penetration.

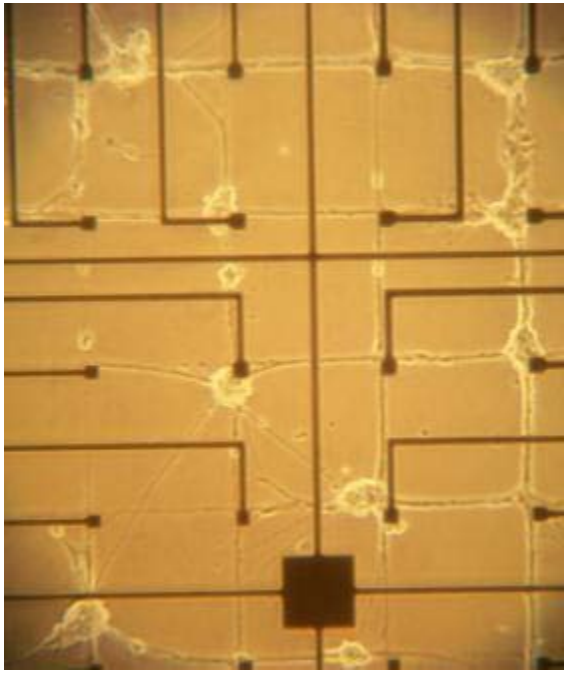
200 cells/㎟



Activities from single cell could be recorded.

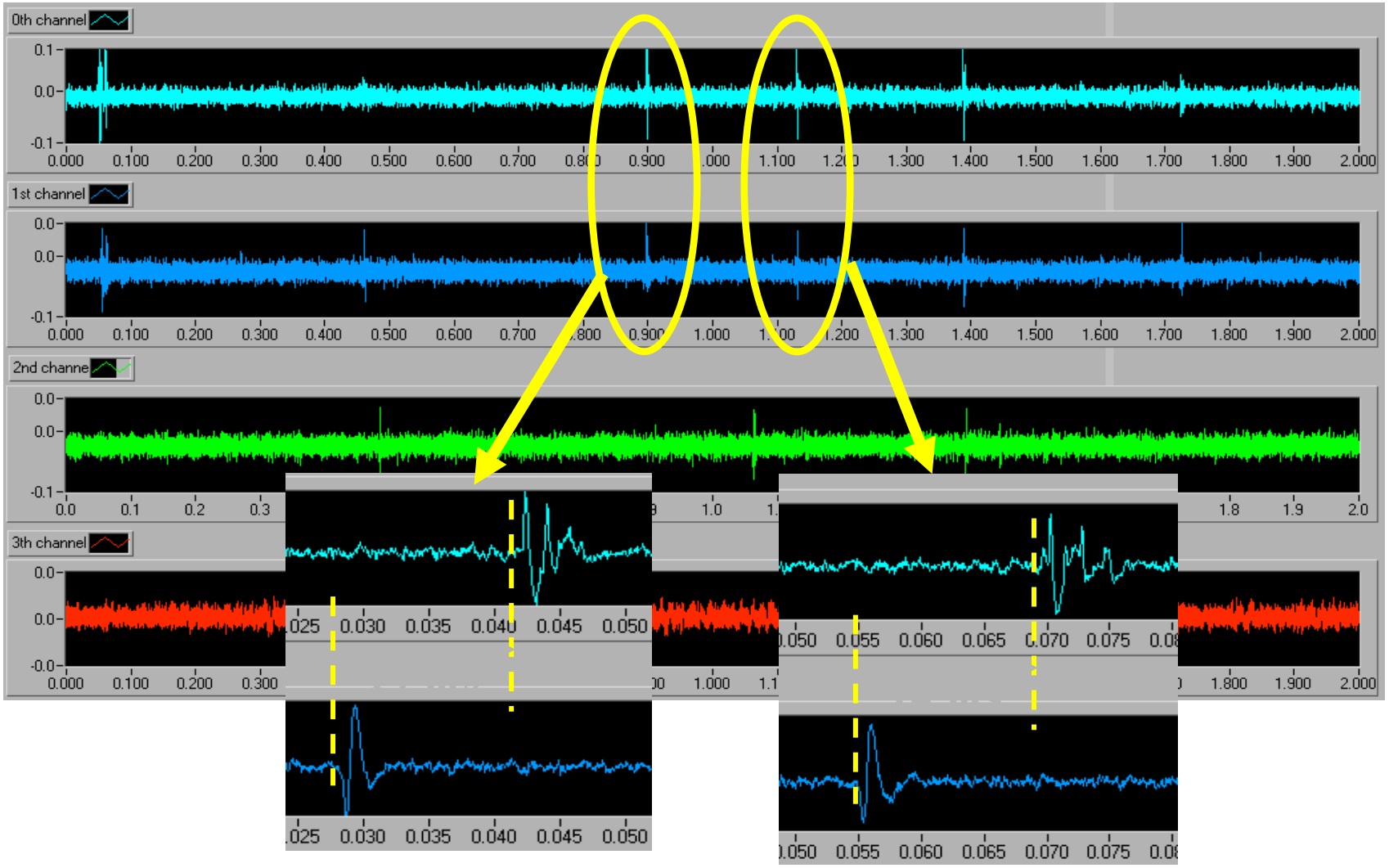
The penetration of signals could also monitored.

400 cells/㎟

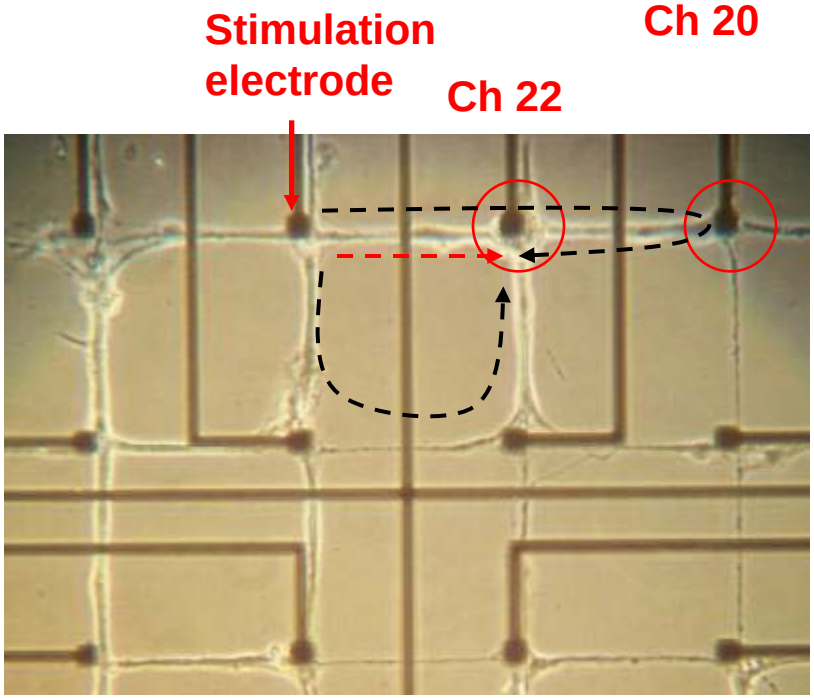


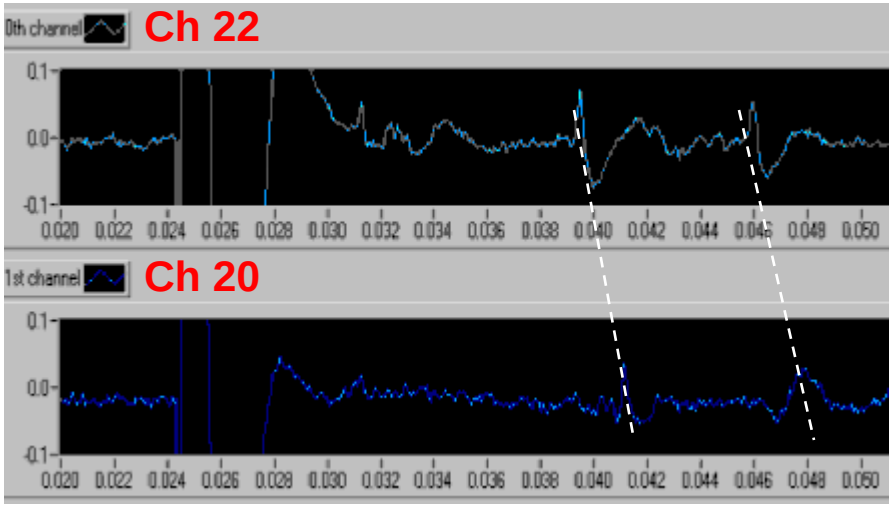
Too many cells aggregate to record single cell activities.

Electrical connectivity in spontaneous activities



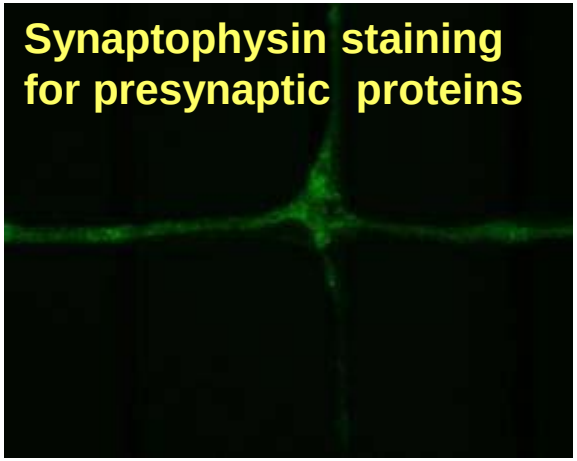
Electrical stimulation-evoked activities

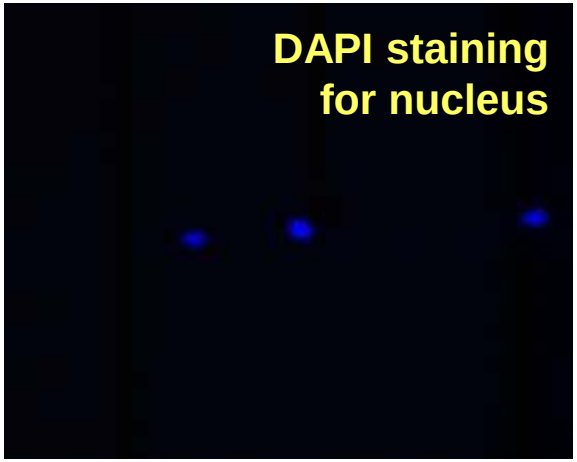


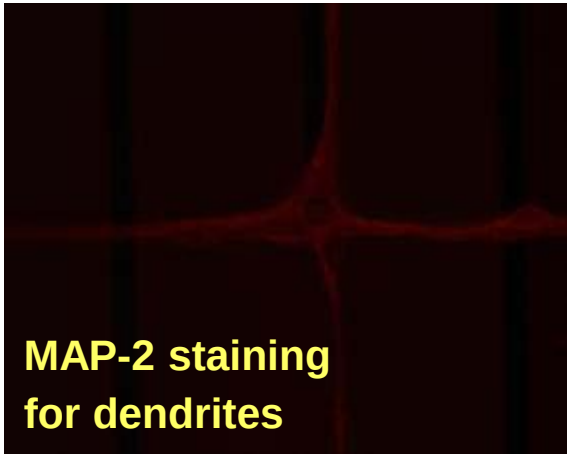


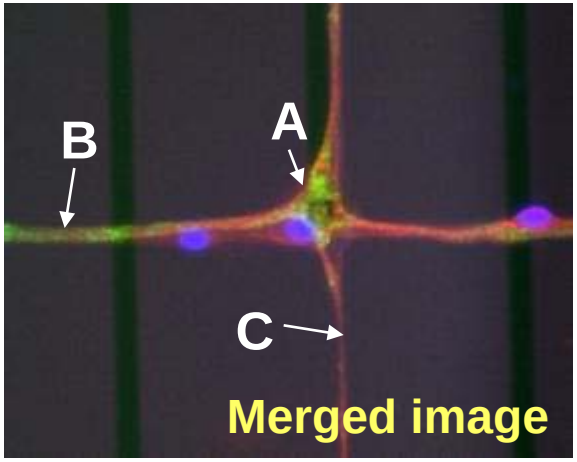
The responses with similar shape show that there are several conduction pathway from the stimulation point to the recording site

Immunostaining image









A. A cell body is located near the electrode site.

B. At the bundle of neurites, the synapses are developed along the bundle. The synapses seem to be formed between axon and dendrites going through the bundle.

C. At the single process, no synapse is seen

Scanning Electron Microscopy (SEM) image of neurons