Cellular Biophysics – Electrophysiological Measurements

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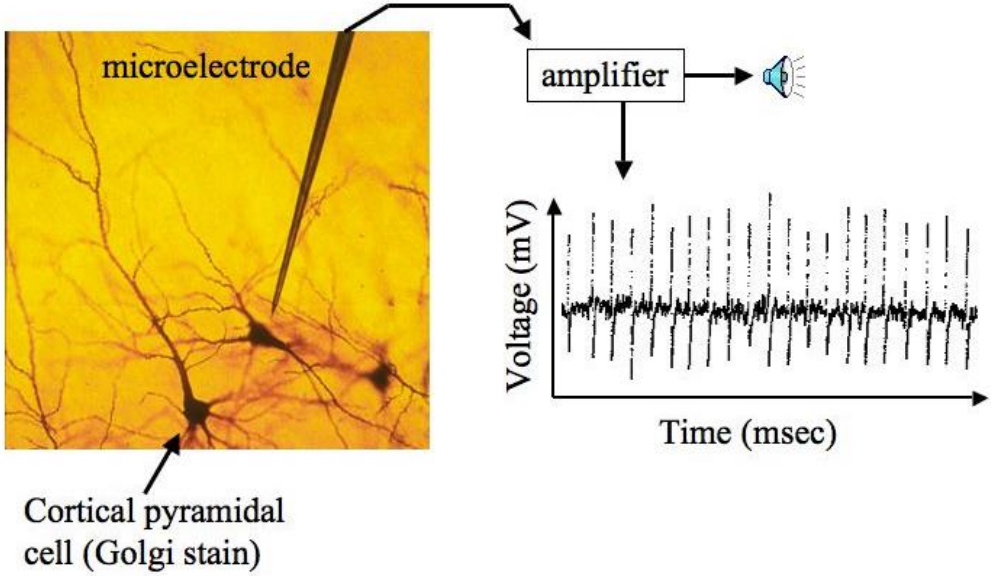
Contents of the lecture

- Principles of electrophysiological measurements
- Extracellular recordings
- Intracellular recordings
- Patch clamp technique
- Calcium imaging

Electrophysiological measurements

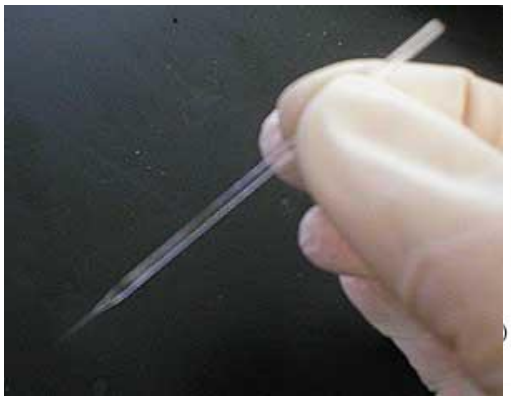
- Utilize recording techniques that enable the measurement of ion flow in biological tissues
- **Electrodes** are placed into various preparations of biological tissue and signal needs to be **amplified**
- Charge carriers in tissues and cells:
  - Ions (Na^+ , Cl^- , K^+ , Ca^{2+} …)

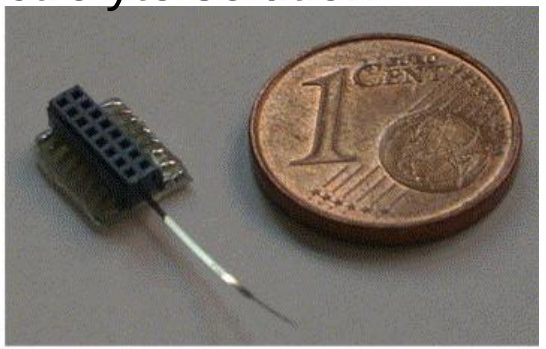


Electrodes

- An electrical conductor used to make contact with a nonmetallic part of a circuit
- Typical electrodes:

- Electrode pads (use conductive gel in contac, e.g. EEG, ECG)
- Simple solid conductors, e.g. needles/wires, disks, pellets (can be singles or arrays, often insulated except for the tip); common materials: Ag, AgCl, Au, Pt, TiO, IrO
- Tracings on printed circuit boards, insulated except for the tip
- Hollow tubes filled with an electrolyte, such as glass pipettes filled with potassium chloride solution or another electrolyte solution.





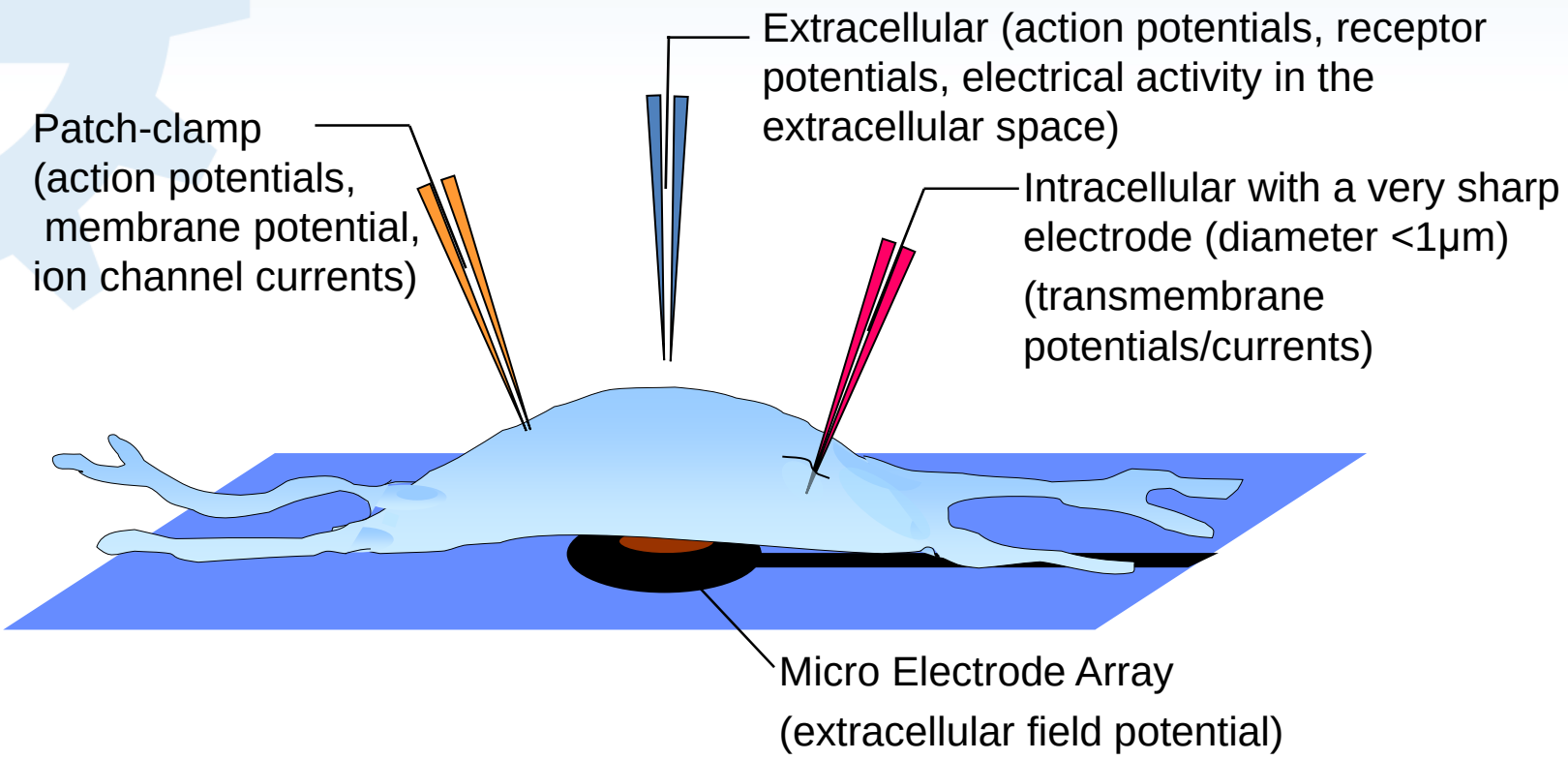
Typical tissues/samples

- The principal tissue preparations
  - Living organisms (*in vivo* measurements, e.g. EEG)
  - Excised tissue (acute or cultured)
  - Dissociated cells from excised tissue (acute or cultured)
  - Artificially grown cells or tissues
  - Hybrids of the above

in vitro

- *In vitro* conditions mimic natural *in vivo* conditions as well as possible
  - Perfusion
  - Medium composition
  - Temperature
  - Electrical environment

Intracellular and extracellular recordings

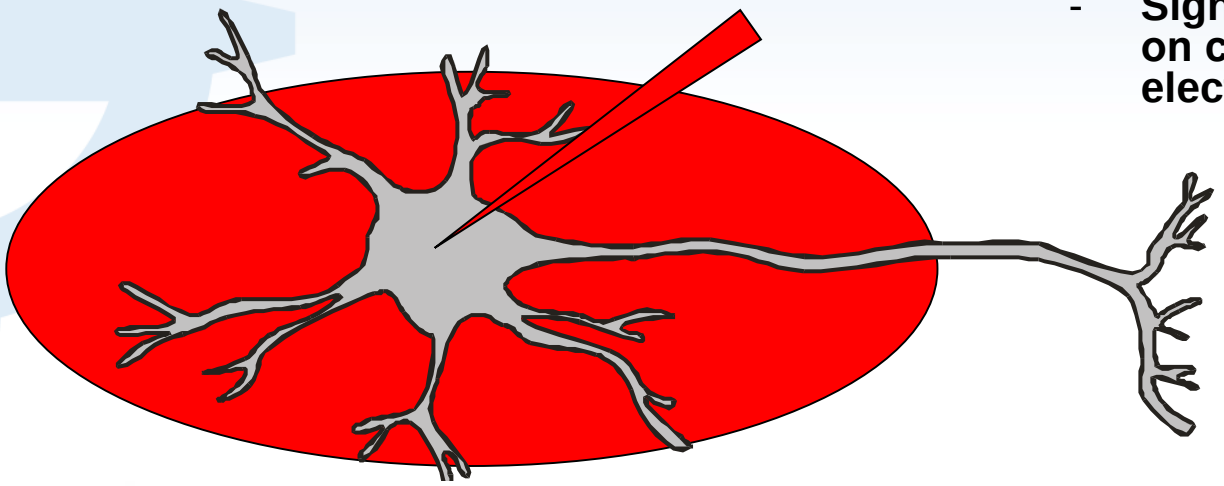


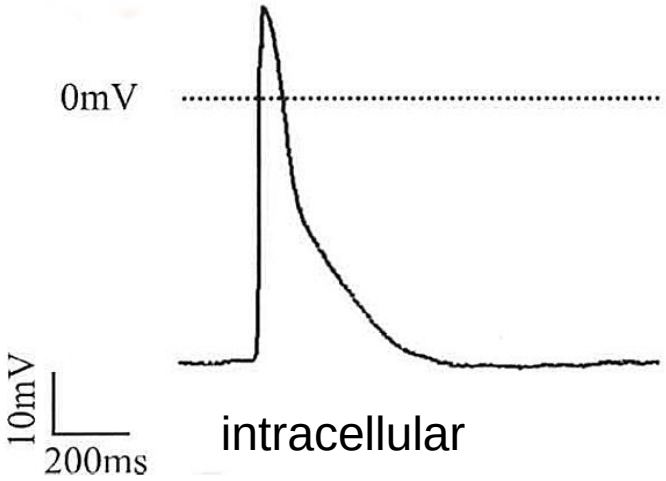
Modified from Lohmann Research Equipment, http://www.lohres.de/products/05_qt-screen.htm

Intracellular and extracellular recordings

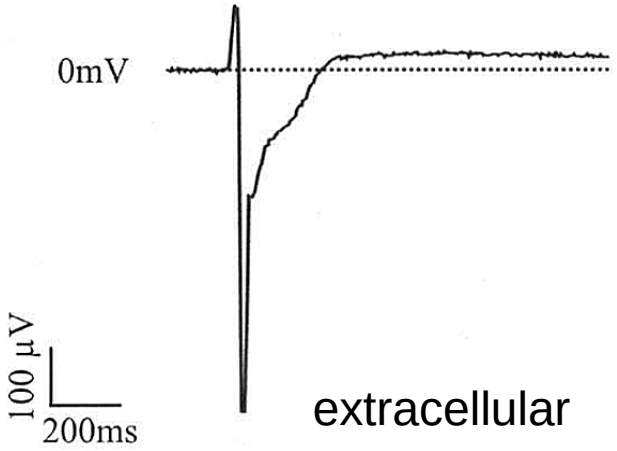
Extracellular recordings:

- **Composite signals**
- **Signal amplitude depends on cell's distance to the electrode**





intracellular



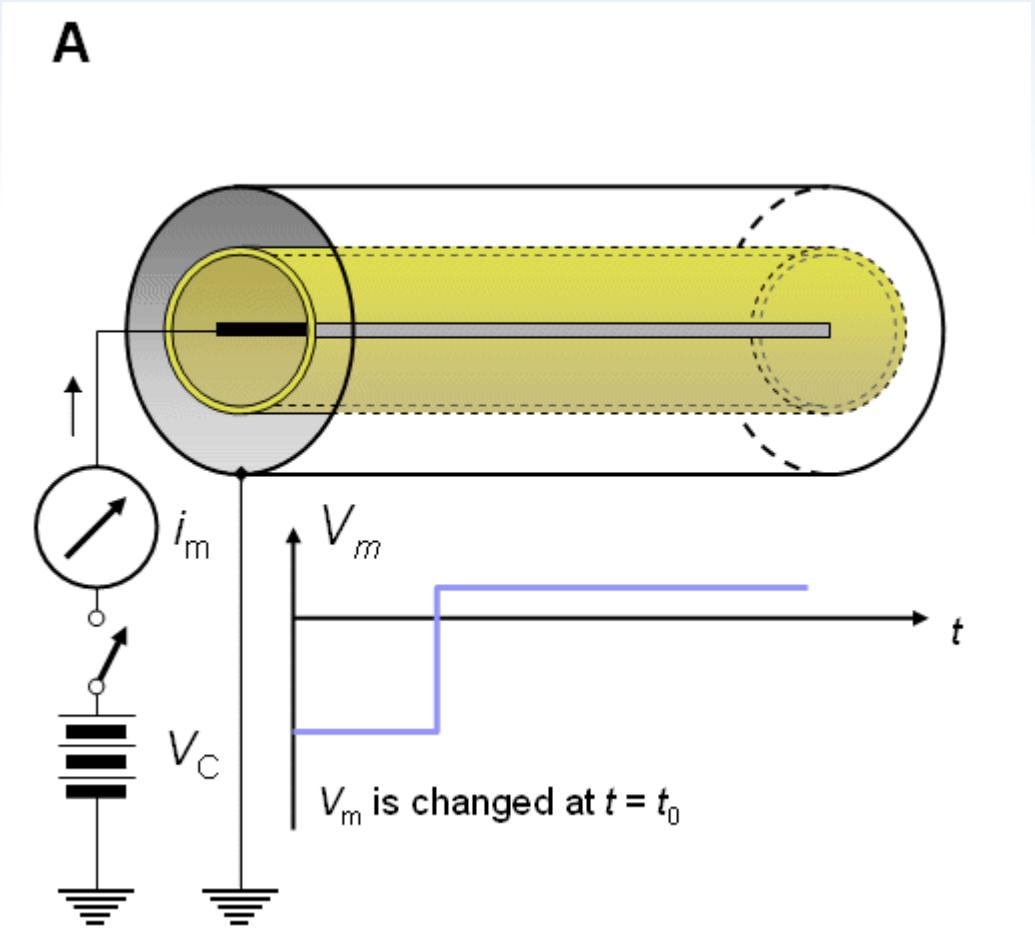
extracellular

Pros and cons of different techniques

	Advantage	Disadvantage
Intracellular Patch clamp	✱ high resolution information from the tissue ✱ measurements of single cells ✱ measurements of single ion channels	✱ invasive: causes irreversible damage to the tissue/cells ✱ dialyze of intracellular compartments ✱ needs considerable skills for quick and precise micromanipulation ✱ extremely difficult to keep stable and reliable recording conditions
Extracellular recordings	✱ noninvasive ✱ long term study possible ✱ network information ✱ measurements more robust and easier to carry out	✱ Composite signals (signal source not always known) ✱ Signal amplitude depends on cell's distance to the electrode

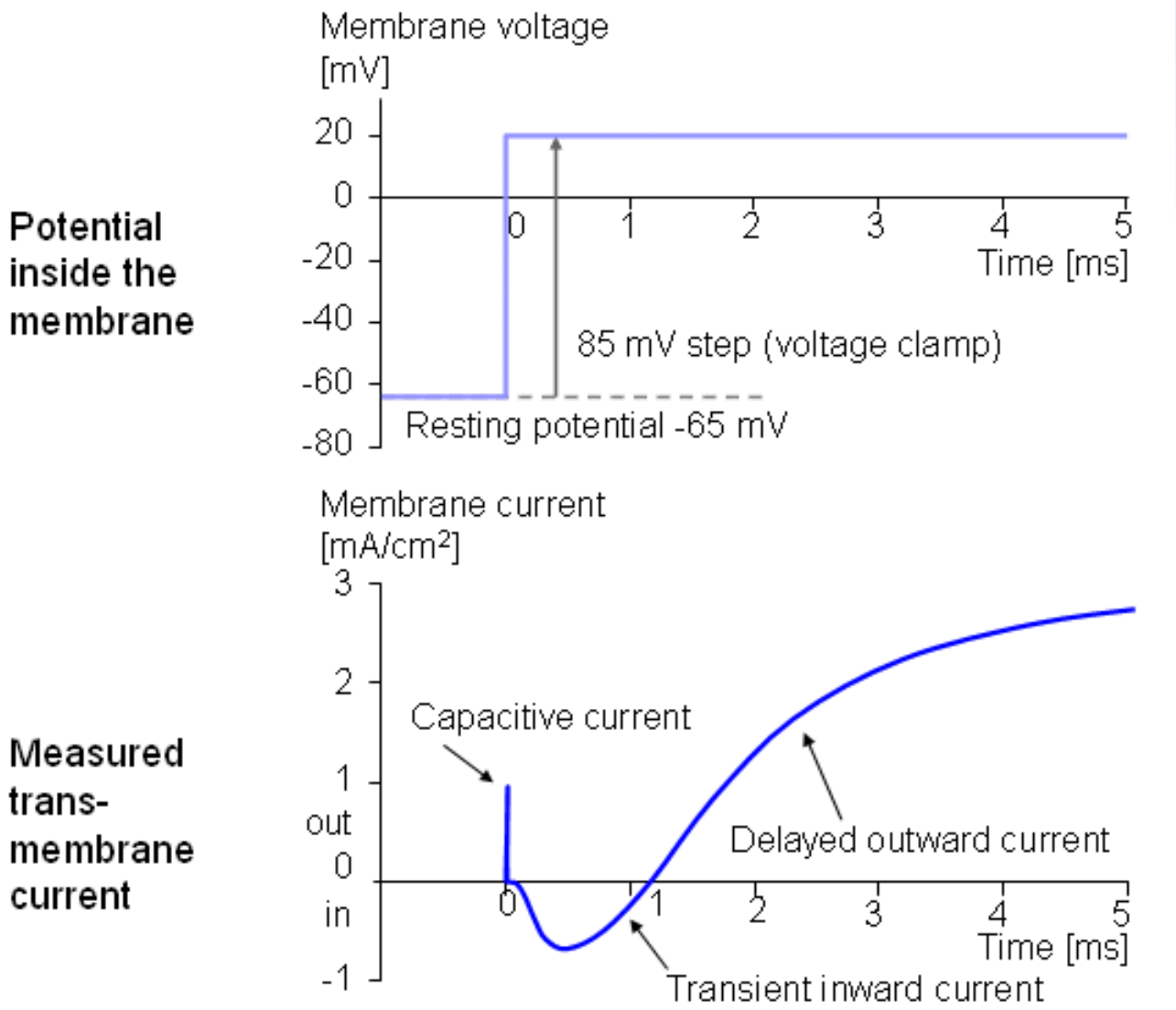
Intracellular recordings – voltage clamp

- **Voltage is clamped** to desired value
- **Current is measured**
- Typically:
  - voltage step is applied between the electrodes inside and outside the cell
  - the current flowing between these electrodes (i.e., the transmembrane current) is measured



http://www.bem.fi/book/ Fig 4.3

Voltage clamp



http://www.bem.fi/book/ Fig 4.5

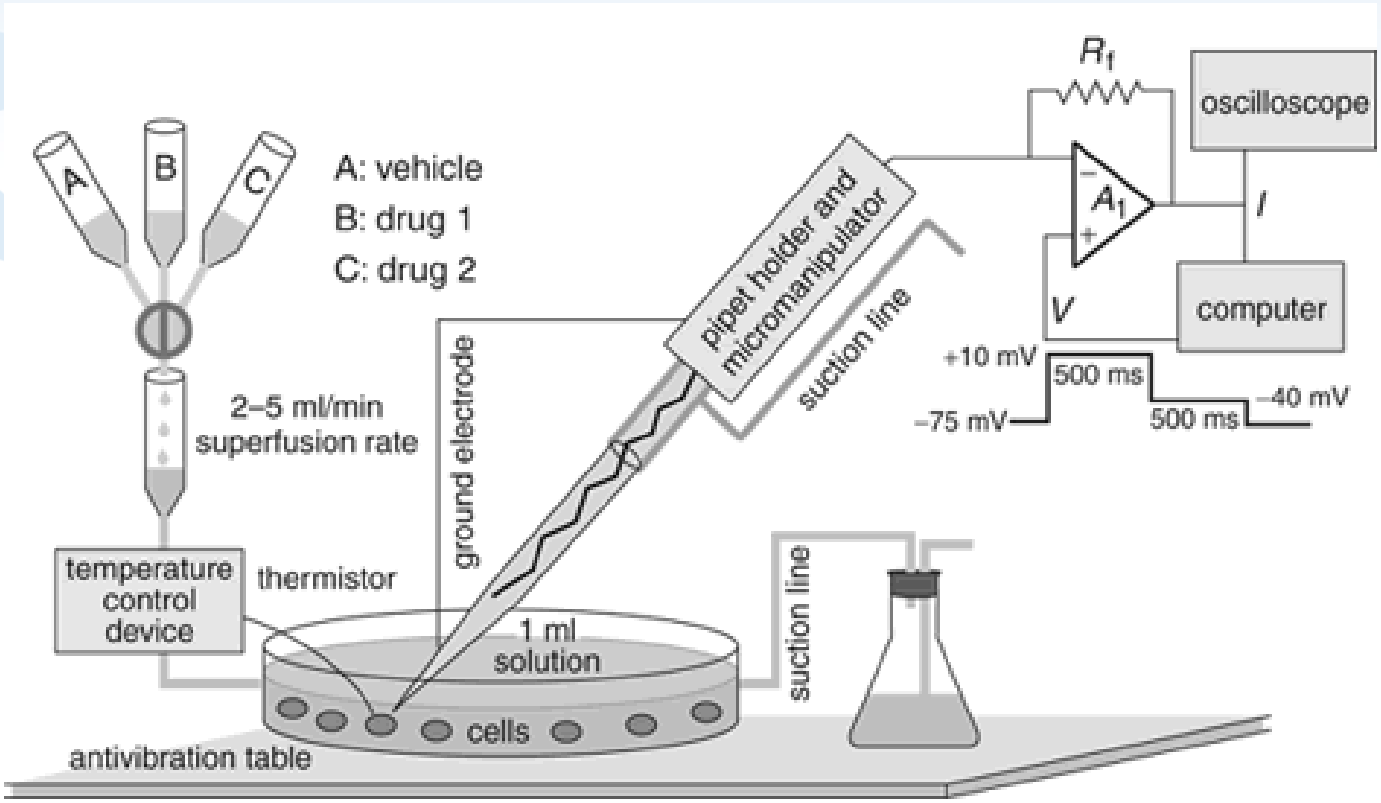
Intracellular recordings – current clamp

- **Current is injected** into a cell and **membrane potential is recorded**

- Main difference between voltage clamp and current clamp:
  - In **voltage clamp** mode the membrane potential is held at a level determined by the experimenter and the transmembrane current is recorded
  - In **current clamp** mode the current is held at a level determined by the experimenter and the membrane potential is free to vary. The amplifier records the voltage that the cell generates on its own or as a result of stimulation.

http://www.bem.fi/book/ Fig 4.3

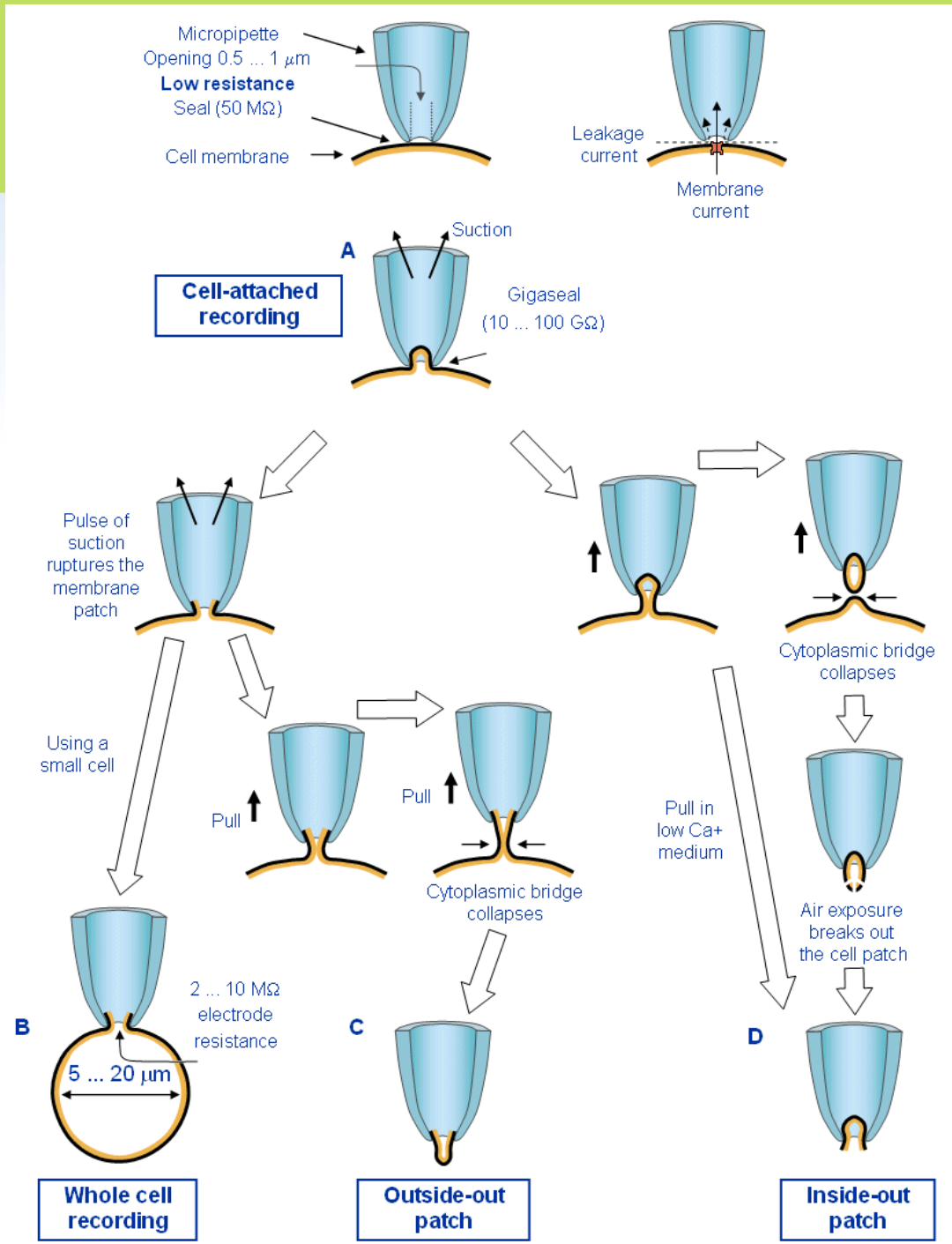
Patch clamp technique



Typical patch clamp setup

Patch clamp

http://sites.sinauer.com/neuroscience5e/animations04.01.html

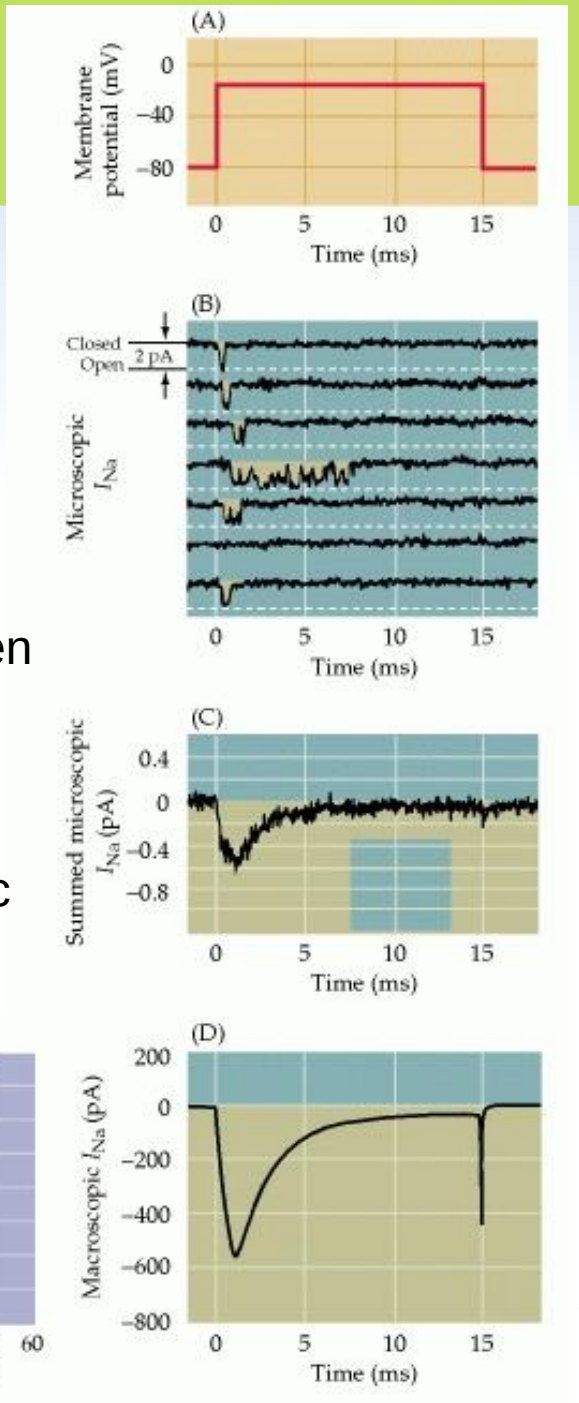


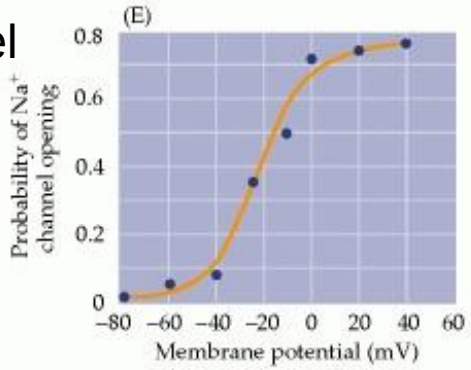
http://www.bem.fi/book/ Fig 4.27

Patch clamp measurements, Na+

- Measurements of ionic currents through single Na+ channel in a squid giant axon.
- Voltage-gated K+ channels blocked by Cs+.
- Depolarizing voltage pulses (A) applied to a patch of membrane containing a single Na+ channel
- Recorded currents (B, downward deflections).
- (C) Sum of many current recordings. Most channels open rapidly, after which the probability of channel openings diminishes (channel inactivation).
- (D) Macroscopic current measured from another axon. Close correlation between microscopic and macroscopic Na+ currents.

- (E) The probability of an Na+ channel opening depends on the membrane potential, increasing as the membrane is depolarized.

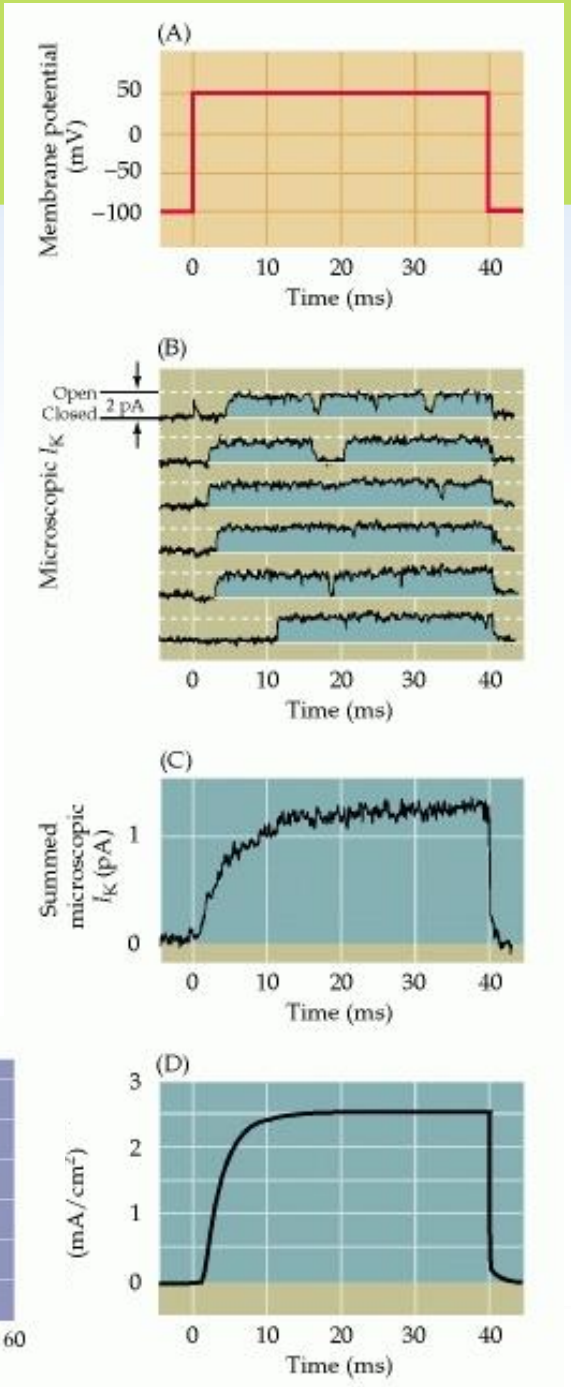




Purves et al. (2008) Neuroscience, Fig. 4.1

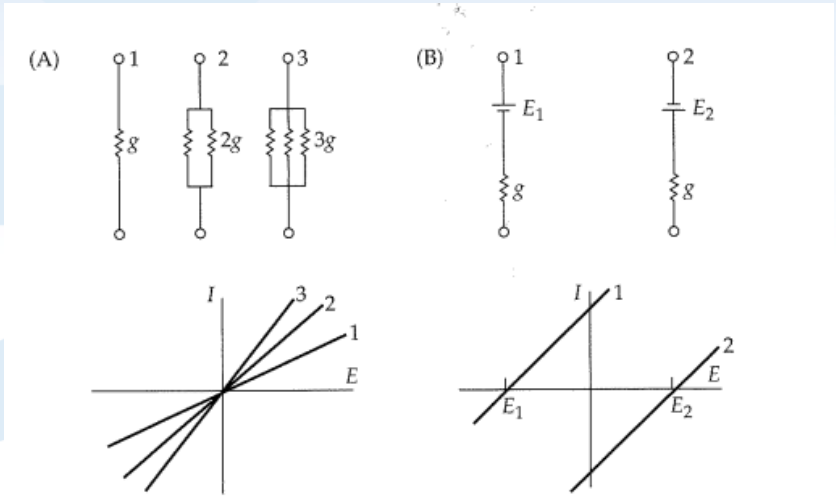
Patch clamp measurements, K+

- Patch clamp measurements of ionic currents through single K+ channels in a squid giant axon. Tetrodotoxin is applied to block voltage-gated Na+ channels.
- Depolarizing voltage pulses (A) applied to a patch of membrane containing a single K+ channel
- Recorded currents (B, upward deflections).
- (C) Sum of such current recordings shows that most channels open with a delay, but remain open for the duration of the depolarization.
- (D) A macroscopic current shows the correlation in time courses of microscopic and macroscopic currents.
- (E) The probability of a K+ channel opening depends on the membrane potential, increasing as the membrane is depolarized.



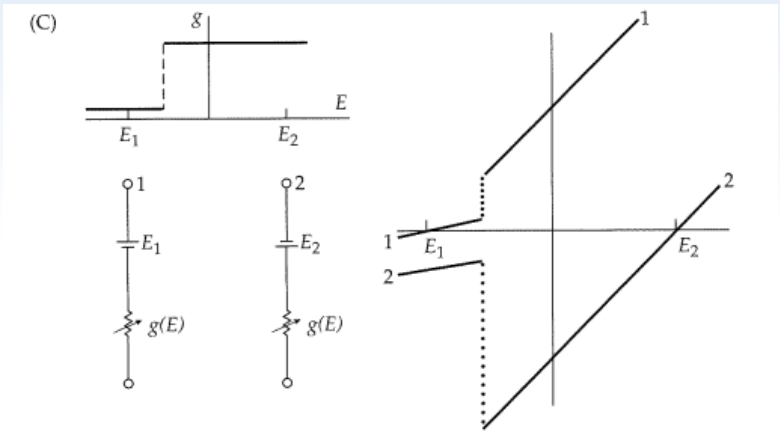
Purves et al. (2008) Neuroscience, Fig 4.2

Current-voltage reltions of membranes

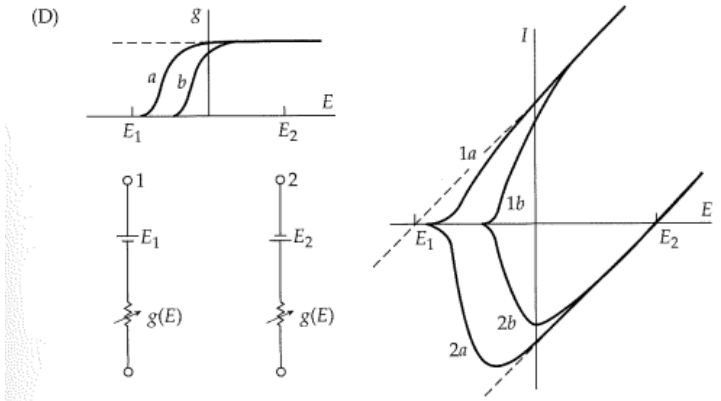


A) Membranes with 1, 2 and 3 pores open

B) Pores with negative (E_1) or positive (E_2) electromotive forces (i.e. equilibrium potentials)



C) Pores with an abrupt change from low conductance state to high conductance state

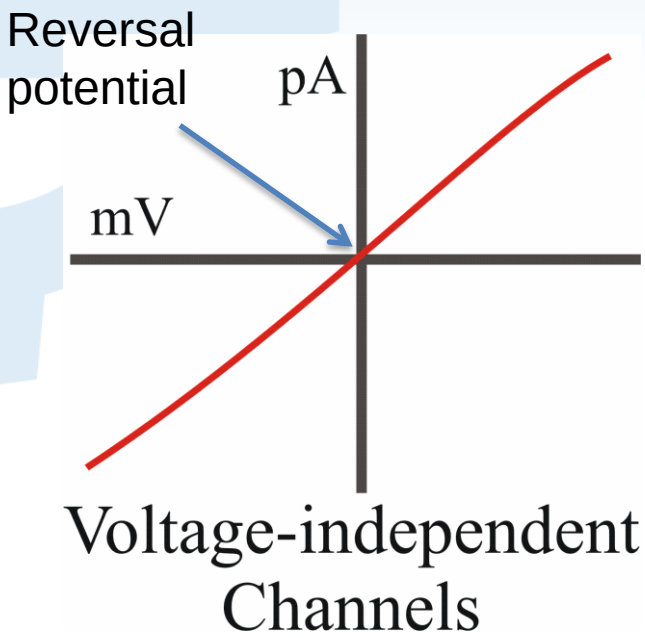


D) Pores with smoothly voltage-dependent probability of being open

Note: These curves are called I-V curves; Here V is denoted as E

B. Hille: Ion Channels of Excitable Membranes, 3rd Ed., Sinauer

Current-voltage curve (I-V curve)



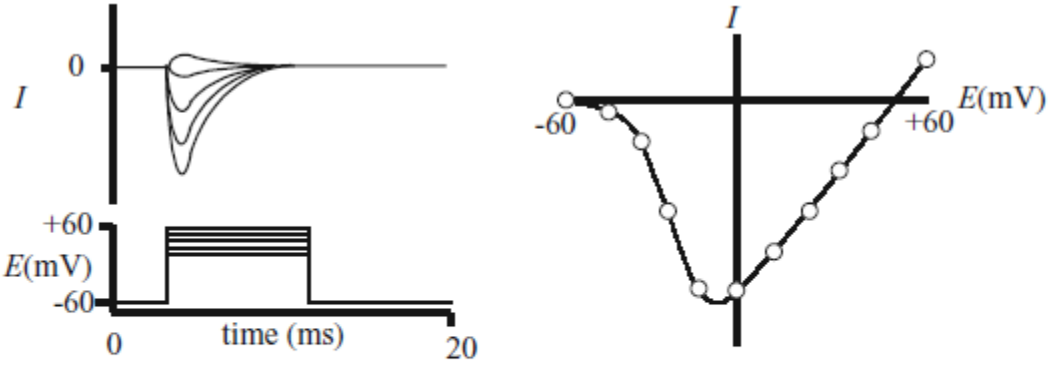


Figure 5.10 Results of a step protocol in the study of an inward current. Depolarising voltage steps with increments of 10 mV are applied from −60 to + 60 mV. In the left-hand panel only raw data from the last five steps are shown for clarity. The right-hand panel shows the relation between peak-evoked current and step potential. The maximum peak-evoked current can be of the order of several nano-amperes in large neurones

Voltage dependent channels

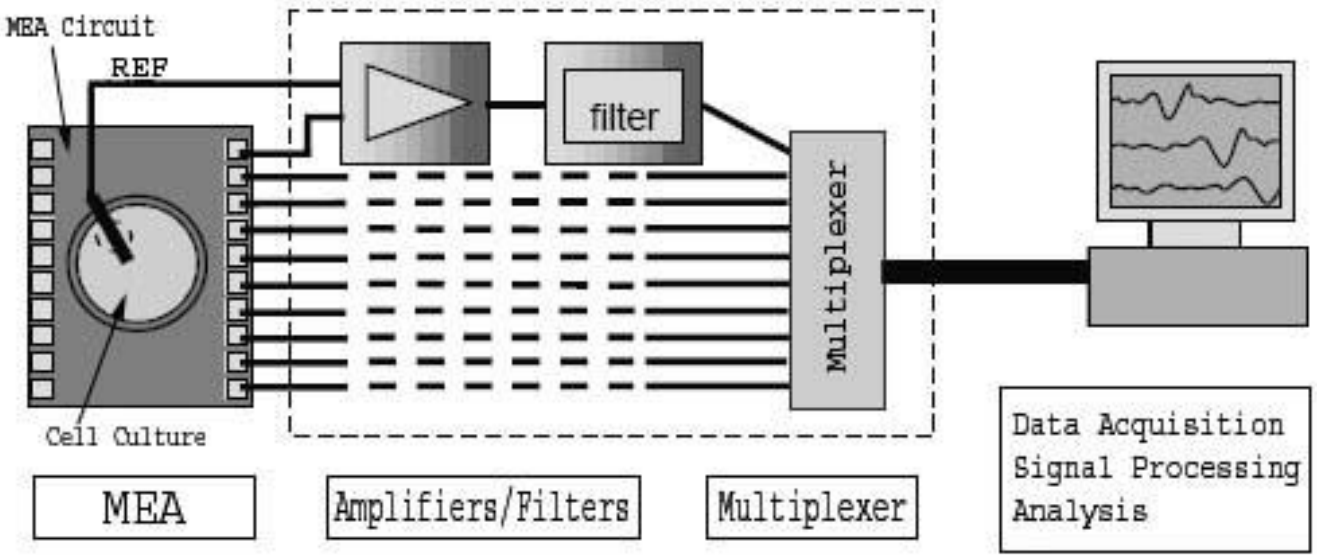
Molleman: Patch clamping (2003)

With patch clamp technique you can…

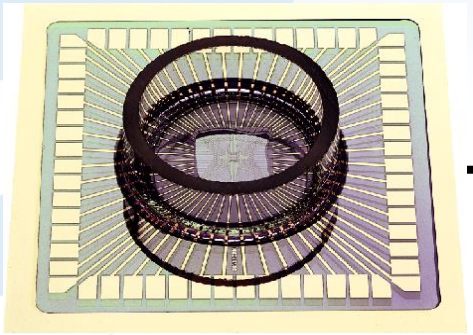
- …characterize ionic currents through single ion channels
- …characterize ionic currents through ion channels in a whole cell
- …determine specific properties of ion channels such as voltage dependency or ligand dependency
- …measure reversal potentials for ions
- …investigate the functioning of the cell (e.g. firing of action potentials, their duration, amplitude etc.)
- …measure the resting membrane potential of the cell
- …investigate the effects of different drugs on single ion channels or whole cells

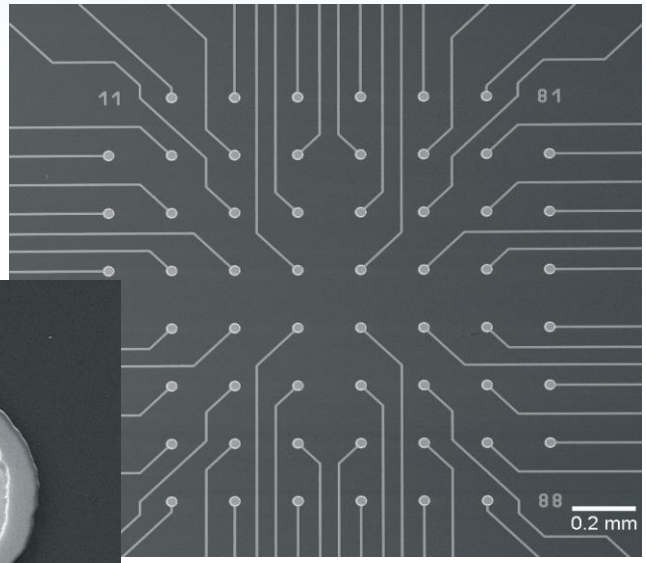
Field potential recordings - MEA

- Microelectrode array technique (MEA) is a good way to record field potentials from excitable cells
  - Neural tissue / cell cultures
  - Cardiac tissue / cell cultures

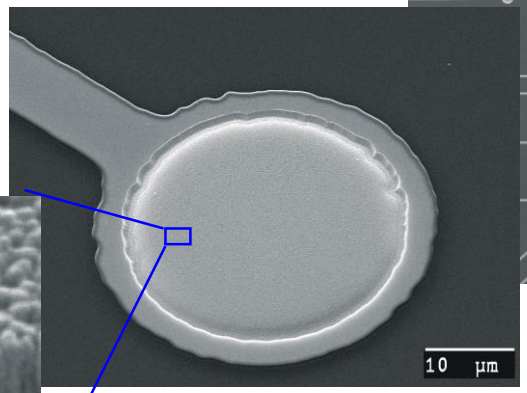


MEA electrode plates

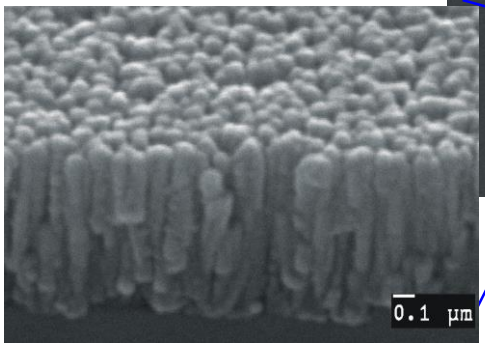




100 or 200 µm grid



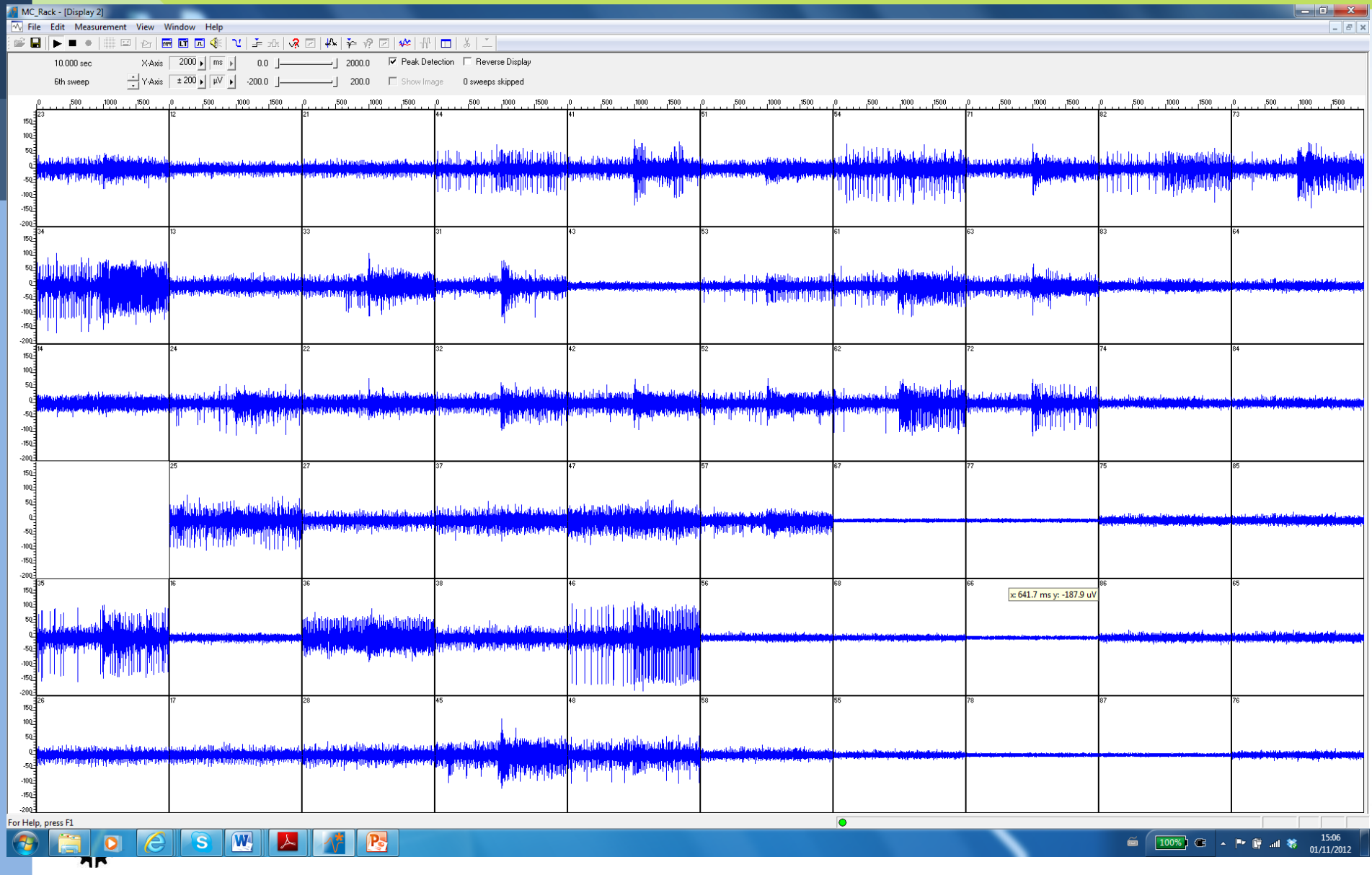
10-30 µm electrode diameter



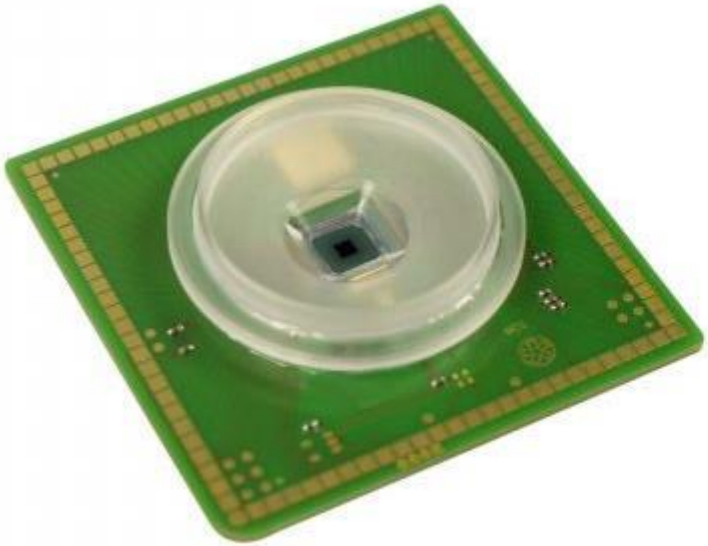
impedance: 30-300 kΩ

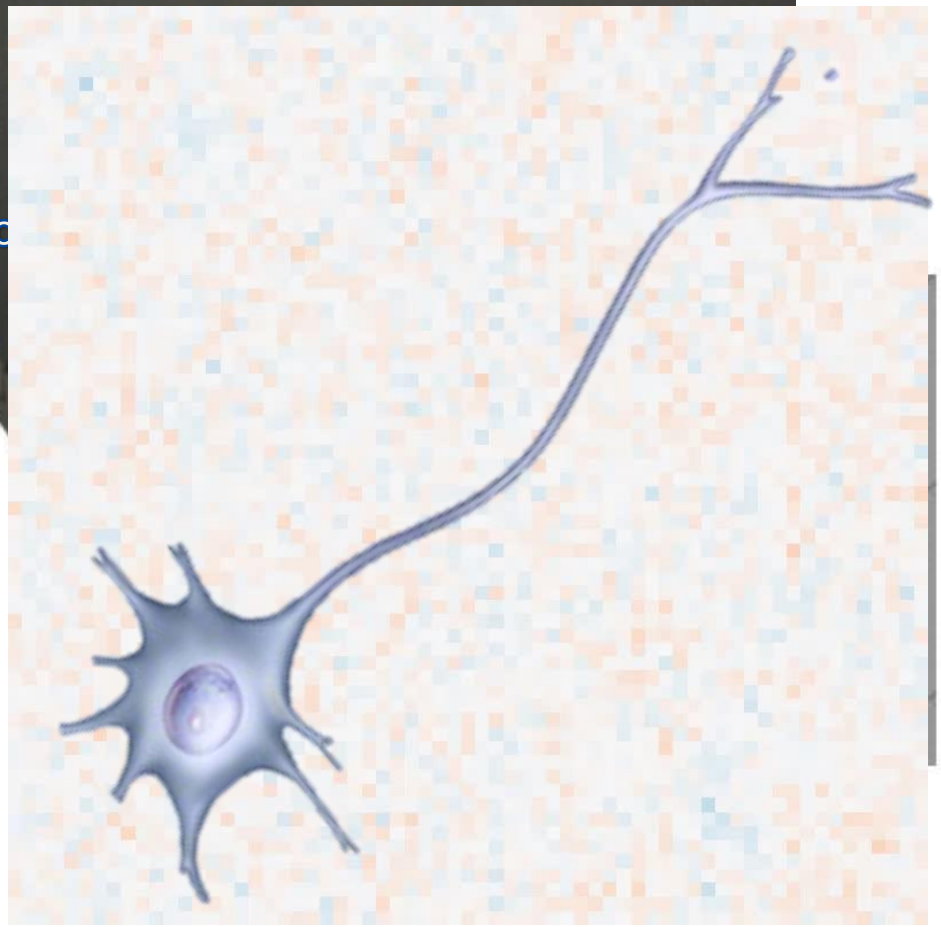
Egert et al. 1998

MEA recordings – retinal action potentials



New CMOS MEA





Calcium signalling

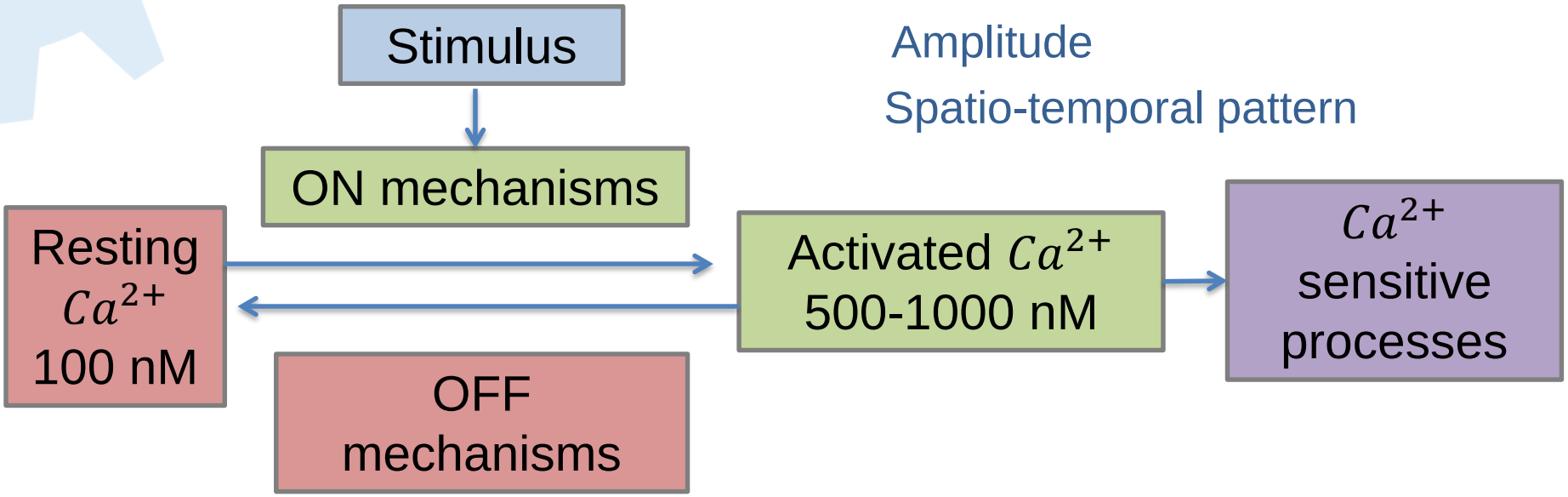
- Many cellular processes involve changes in calcium concentration => Ca^{2+} is extremely important in cell signalling

Important concepts:

Speed

Amplitude

Spatio-temporal pattern

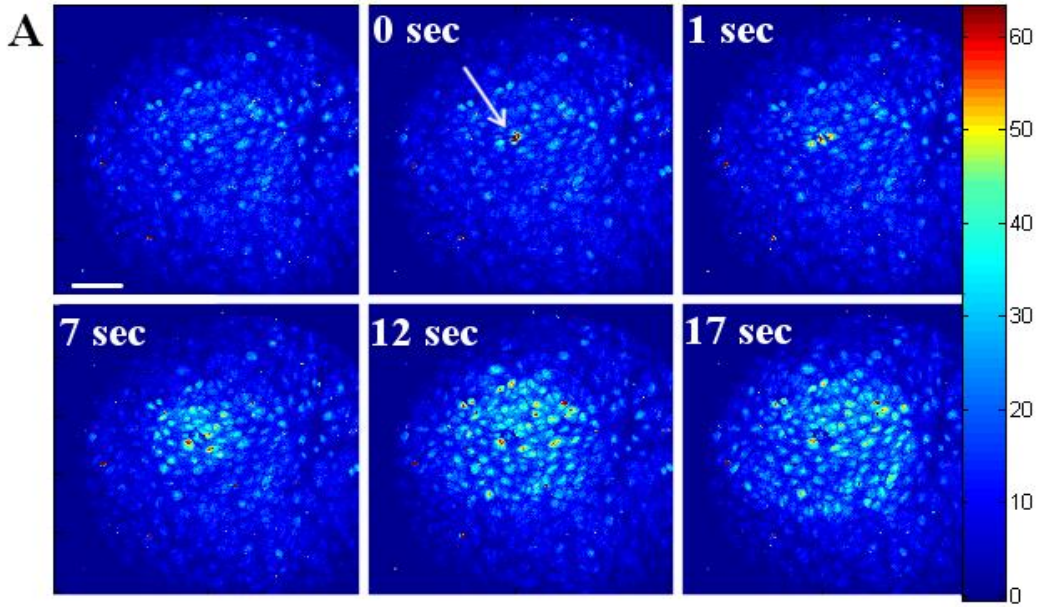


Nature Rev. Mol. Cell Biol. 1. 11-21 (2000)

Calcium imaging

- Calcium imaging shows the Ca^{2+} status of a tissue or medium.
- It uses calcium indicators, molecules that can respond to the binding of Ca^{2+} ions by changing their spectral properties.
- Two main classes of calcium indicators: chemical indicators and genetically encoded indicators.

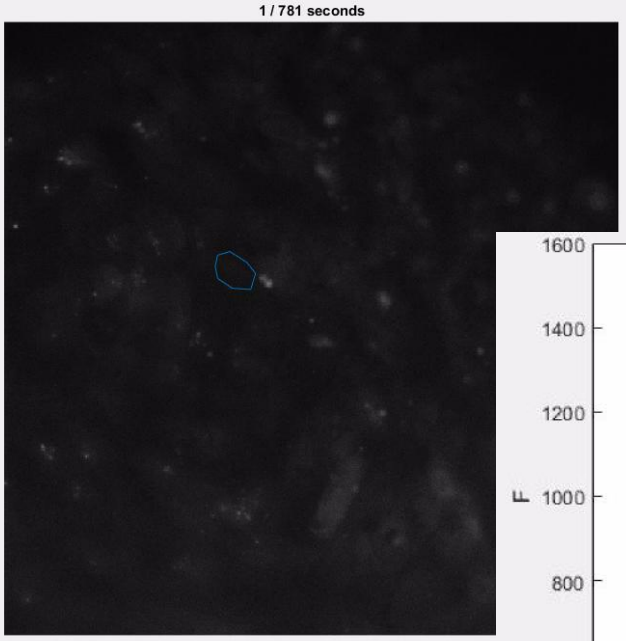
Calcium wave
in ARPE-19 cells

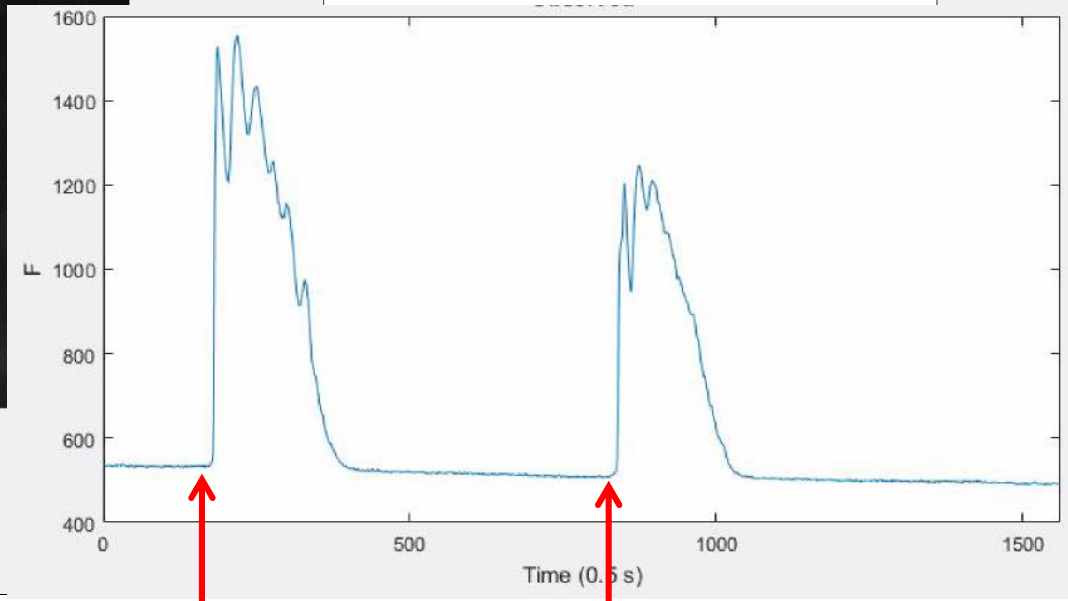


AbuKhamidakh et al., 2013

Response of RPE to ATP stimulation

Calcium imaging using Fluo-4-am



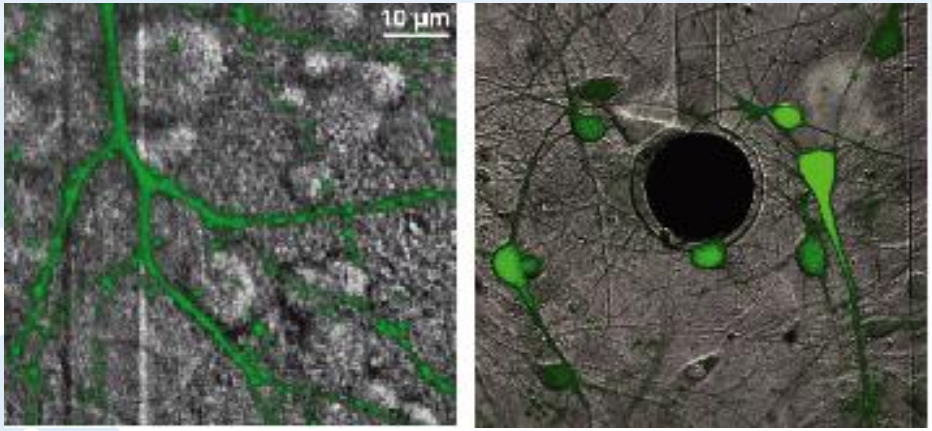


100 µM ATP for 30s

Calcium imaging

- Chemical indicators:
  - Small molecules that can chelate (=bind) calcium ions.
  - Need to be loaded into the cells
  - Examples: fura-2, indo-1, fluo-3, fluo-4, Calcium Green-1.
- Genetically encoded indicators
  - Fluorescent proteins derived from green fluorescent protein (GFP) or its variants, fused with calmodulin (CaM).
  - Do not need to be loaded into the cells
  - Genes encoding for these proteins can be transfected to cell lines.
  - Transgenic animals expressing the dye in all cells or selectively in certain cellular subtypes, can be created.

Combining techniques



Kindly provided by A. Minerbi and N. Ziv, Technion Faculty of Medicine, Haifa, Israel

- Fluorescence microscopy
- Calcium imaging
- Patch clamp
- Single electrode extracellular recording
- MEA

Electrophysiological measurements in general

- Electrophysiological measurements:
  - Record electrical activity (=ion movements) in biological tissues and cells
  - Are carried out in conditions that mimic natural environment
  - Involve electrodes
- More in
  - http://www.bem.fi/book/
  - Chapter 4
- Note!

  Laboratory visit next week in Arvo-building of the University of Tampere, Arvo Ylpön katu 34.

  This replaces the lecture