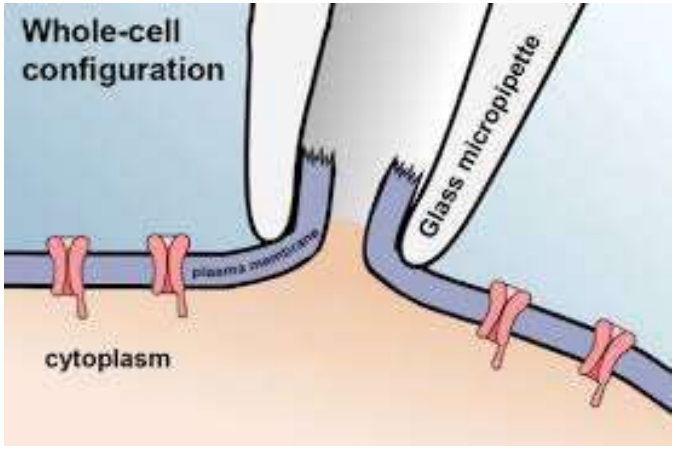
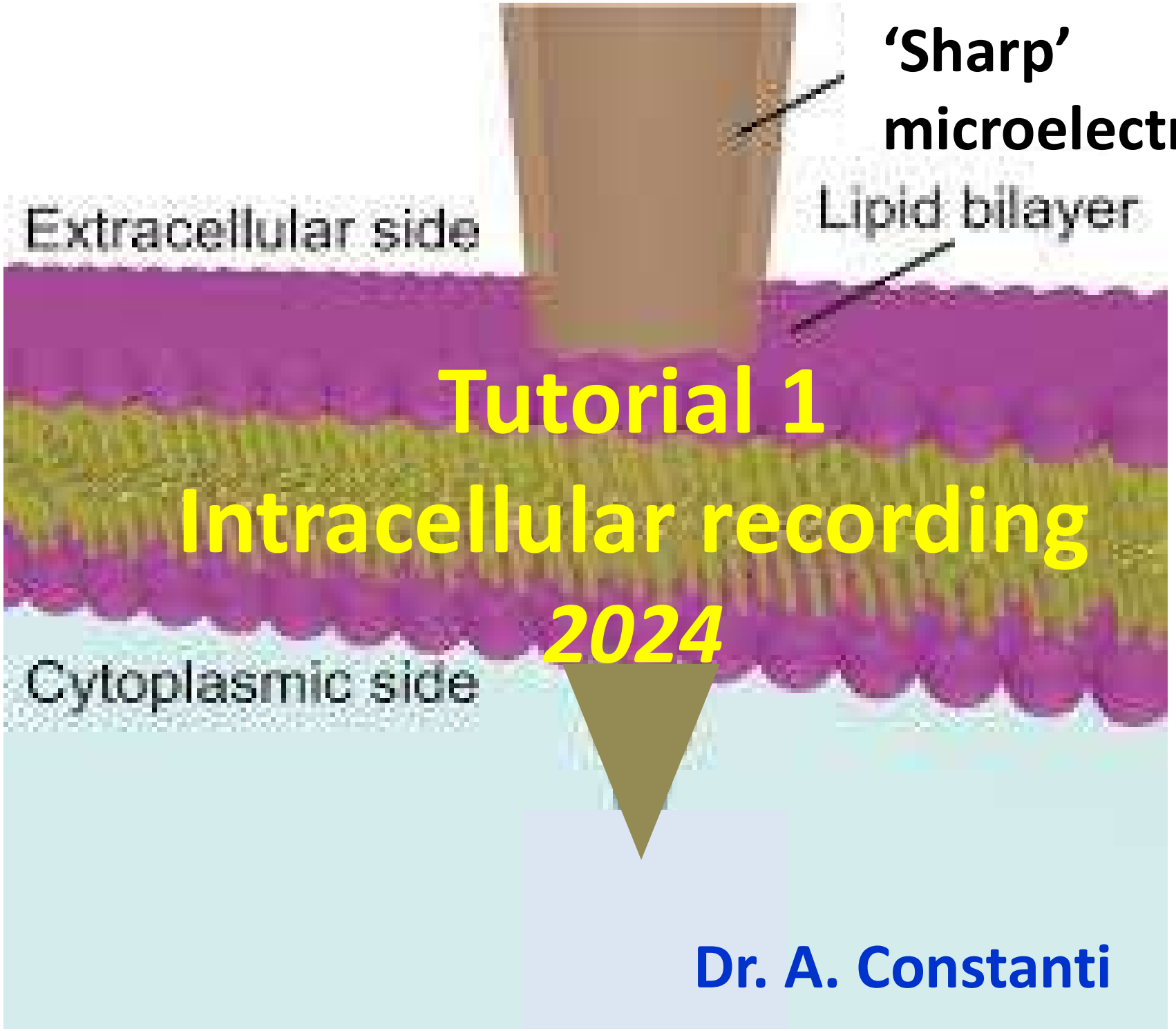
Interactive tutorials: Electrophysiological techniques in Pharmacology/Neuroscience

- 1. Intracellular recording
- 2. Voltage clamp
- 3. Patch clamp
- 4. Neuronal reconstruction
- 5. Advanced neuroscience methods





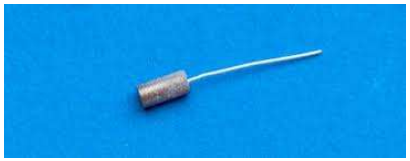
Tutorial 1
Intracellular recording
2024

Dr. A. Constanti

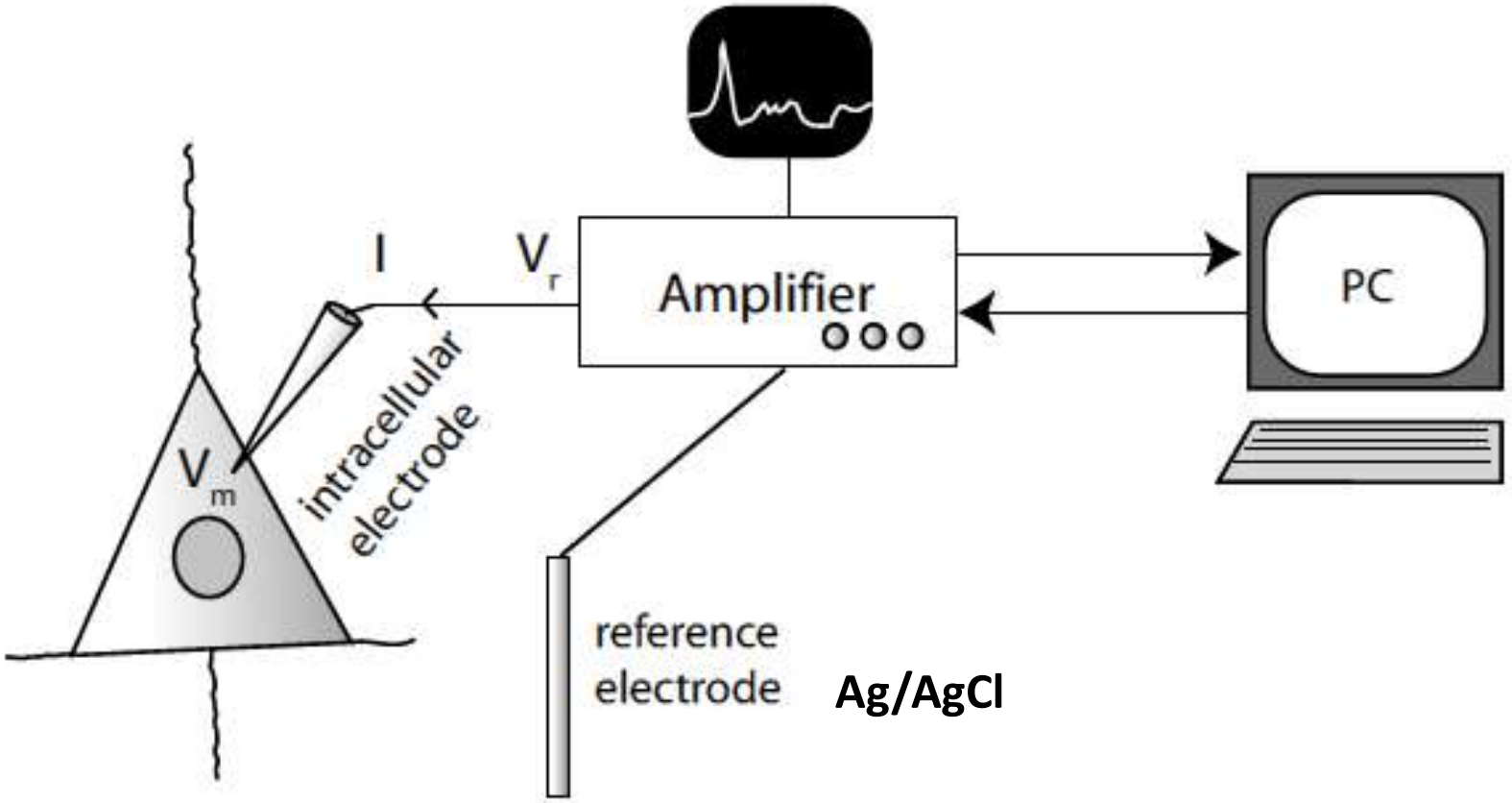
What is intracellular recording and how is it accomplished?

Intracellular recording

- **Intracellular recording** is the measurement of voltage or current across the lipid membrane of a cell. It typically involves the insertion of a “sharp” glass microelectrode filled with electrolyte into the cell interior and a reference (bath) electrode outside the cell.
- The electrode is connected to a *high input impedance* recording amplifier to measure the cell membrane potential relative to the bath reference electrode (usually a Ag/AgCl-coated wire/pellet).

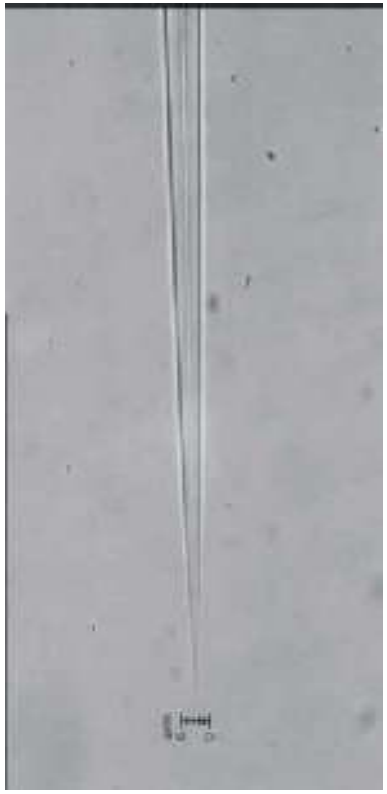


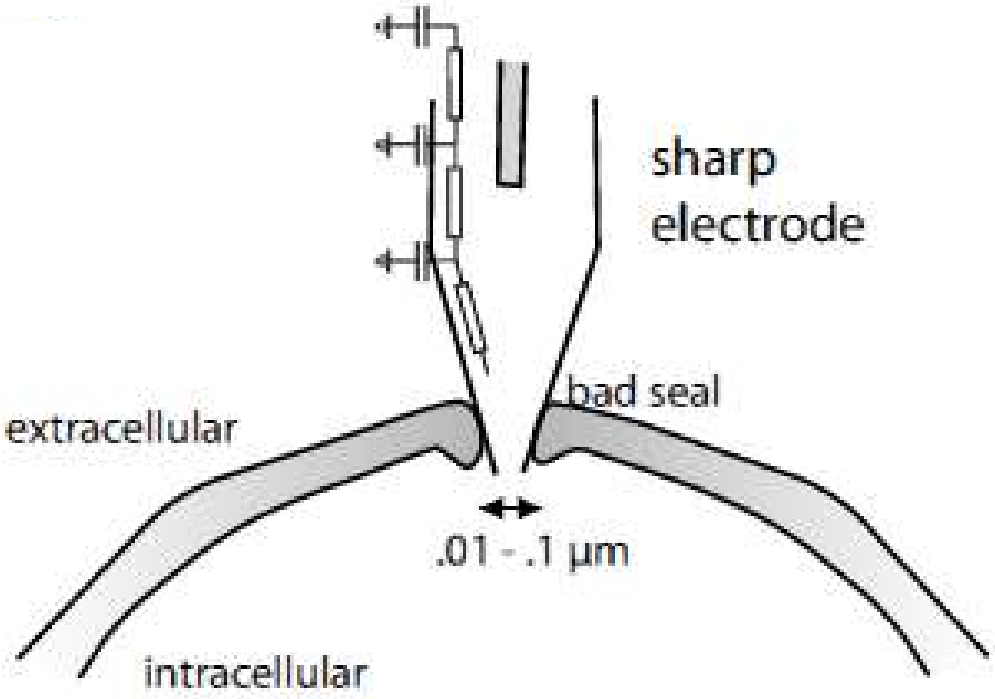
Intracellular recording

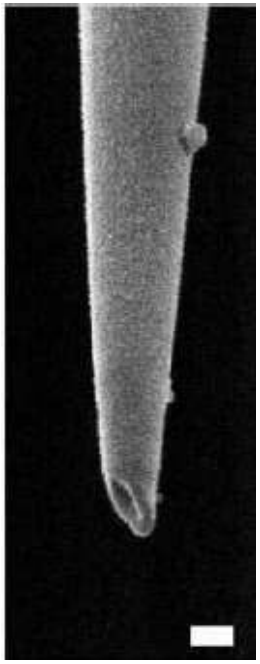


Basic intracellular experimental setup. An intracellular "sharp" microelectrode is impaled into the cell and connected to an amplifier, which records the intracellular membrane potential relative to that of a reference "bath" Ag/AgCl electrode.

Intracellular recording



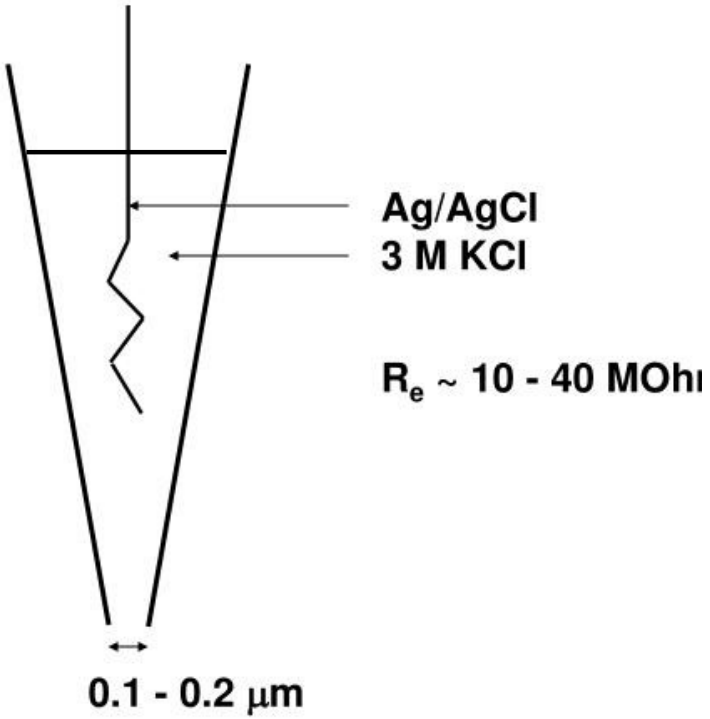




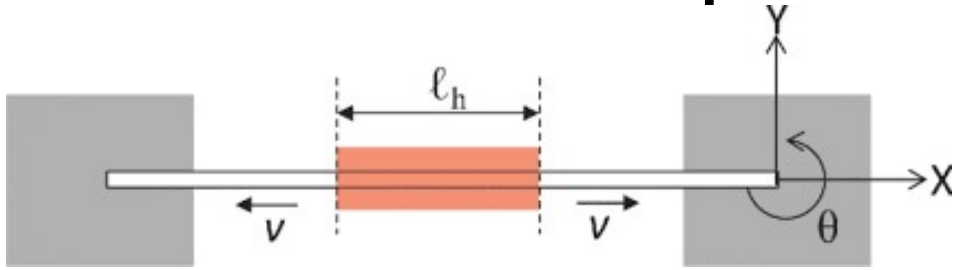
200 nm

"Sharp" microelectrodes typically have a small tip diameter (0.01-0.1 µm: equivalent electrical circuit superimposed on the left side of the electrode) and an input resistance when filled with electrolyte (*e.g*. 3M KCl), of around 10- 40 MΩ.

Intracellular recording

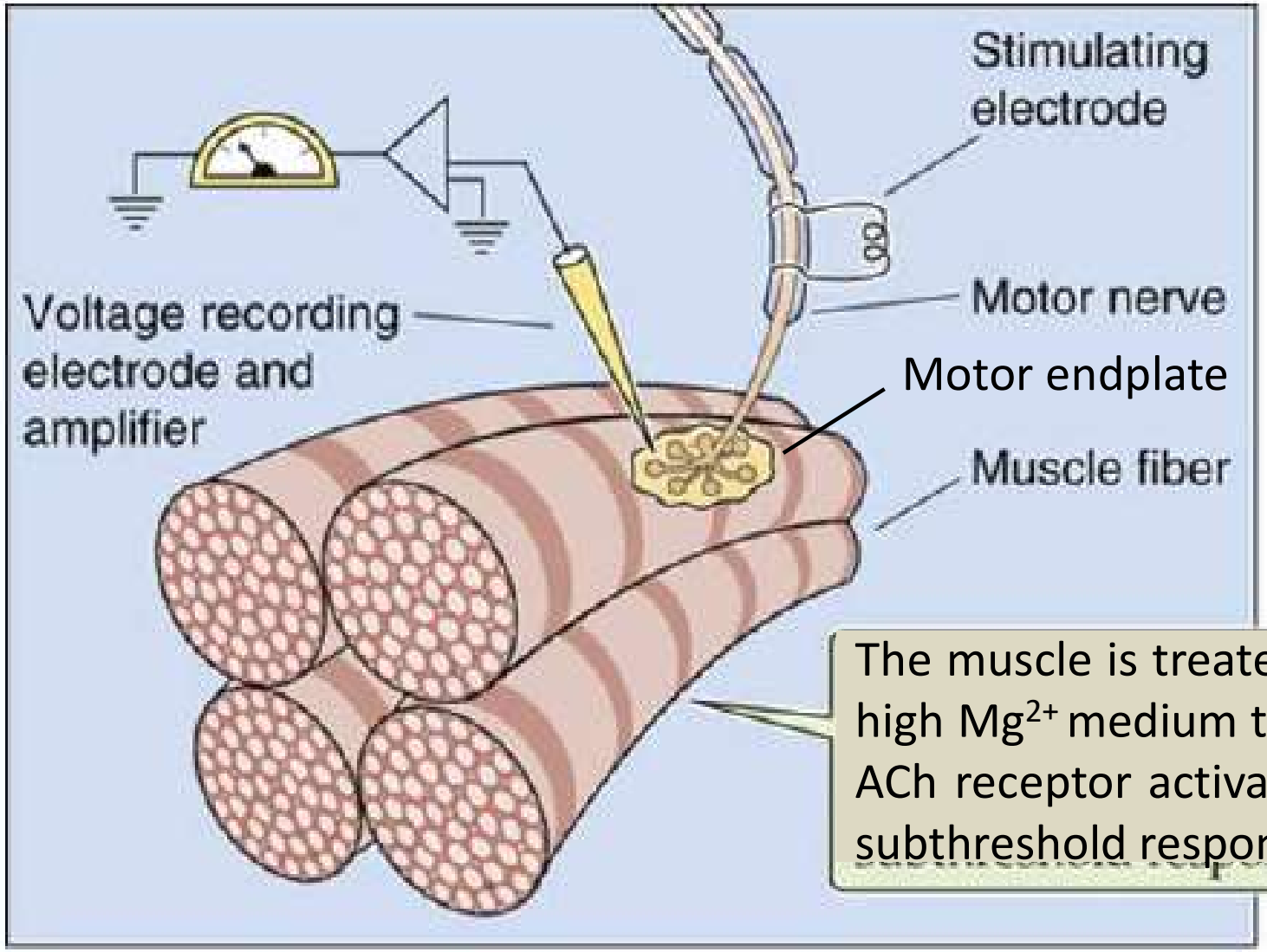


Sutter microelectrode puller



Intracellular recording

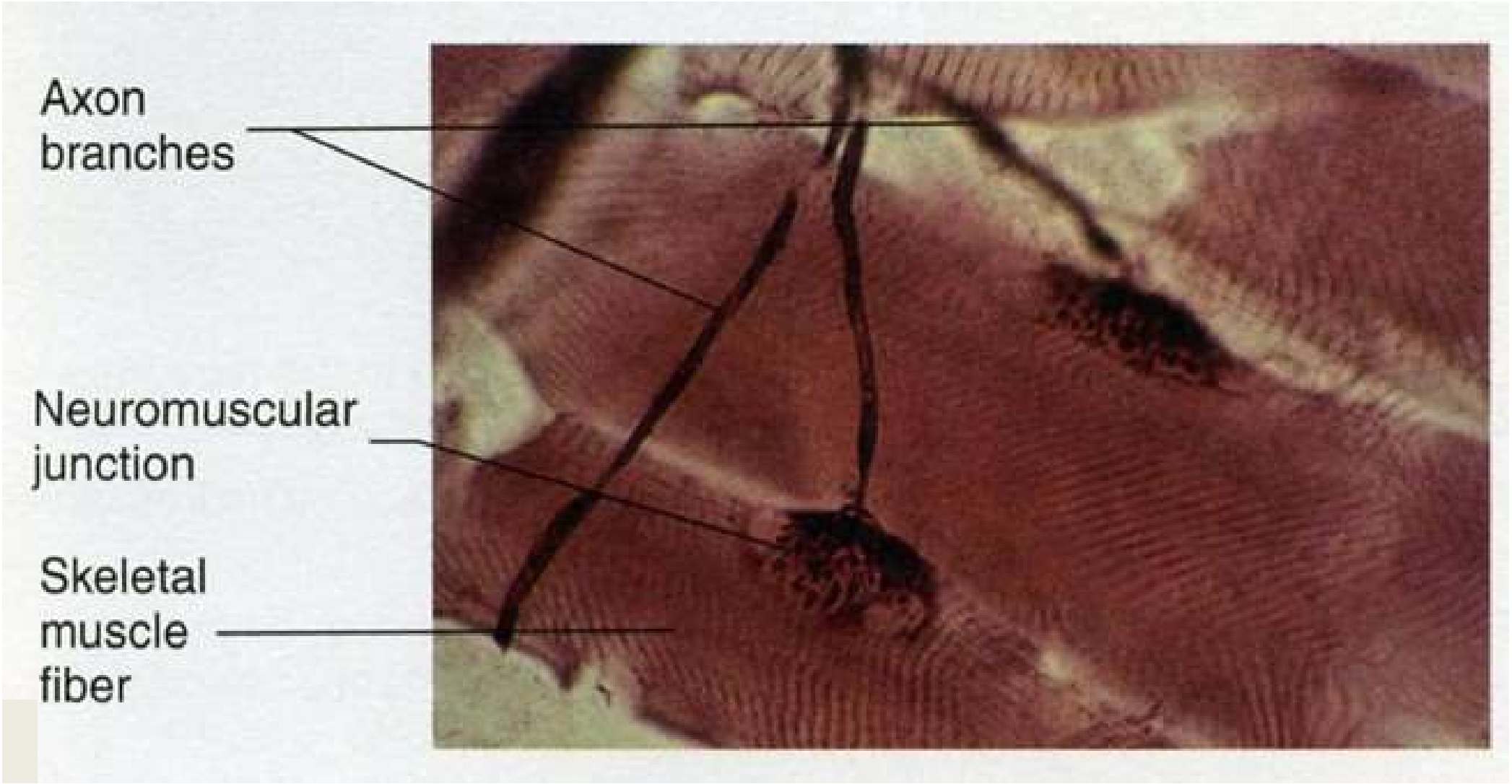
Neuromuscular junction



The muscle is treated with high Mg^{2+} medium to limit ACh receptor activation to subthreshold responses.

Intracellular recording

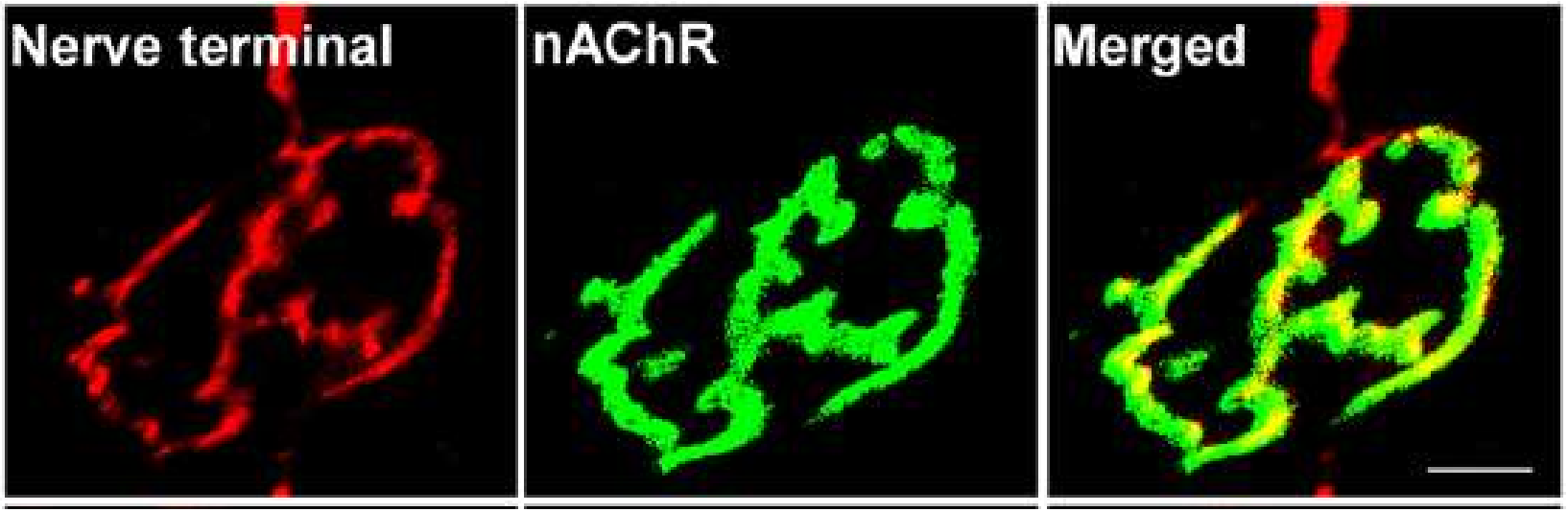
Neuromuscular junction



Gold chloride staining

Intracellular recording

Neuromuscular junction



Amplified photomicrographs for one NMJ located on mouse gastrocnemius muscle**. Synaptophysin** and **neurofilament 200** (red colour) and **α-bungarotoxin** (BTX; green colour) were used to label presynaptic nerve terminals and postsynaptic nAChR clusters respectively. From Tu et al., (2017). Front Physiol. 8:207.

Synaptophysin is a major synaptic vesicle protein;

Neurofilament 200 is involved in the maintenance and support of the neuronal cytoskeleton.

How is impalement of the microelectrode into the muscle cell achieved?

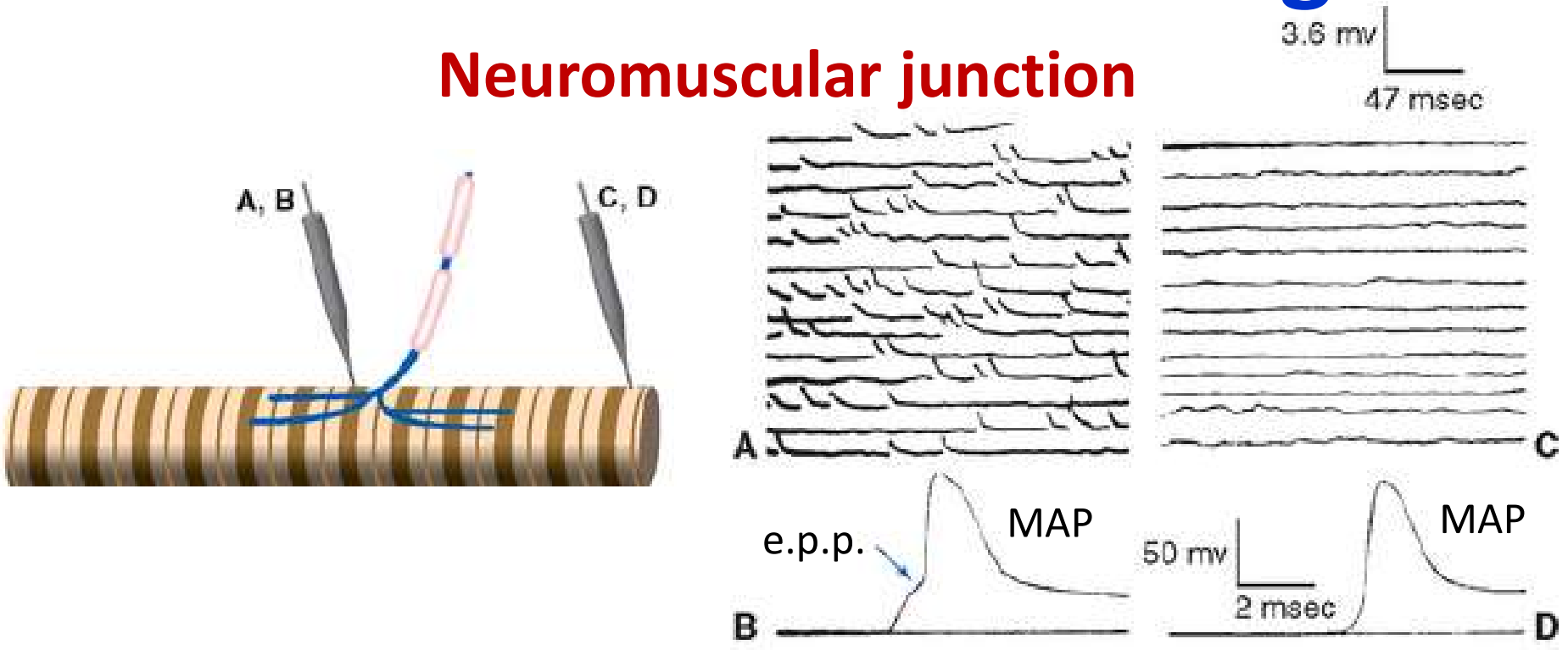
Intracellular recording

“Prior” micromanipulator

The intracellular microelectrode is mounted onto a micromanipulator that makes fine adjustments in position over the muscle cell preparation viewed under a stereo microscope, then manual penetration through the cell membrane is made using a fine control to register the membrane potential.

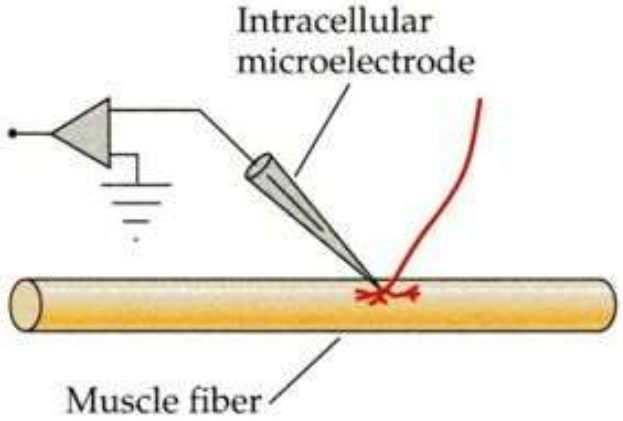
Intracellular recording

Neuromuscular junction

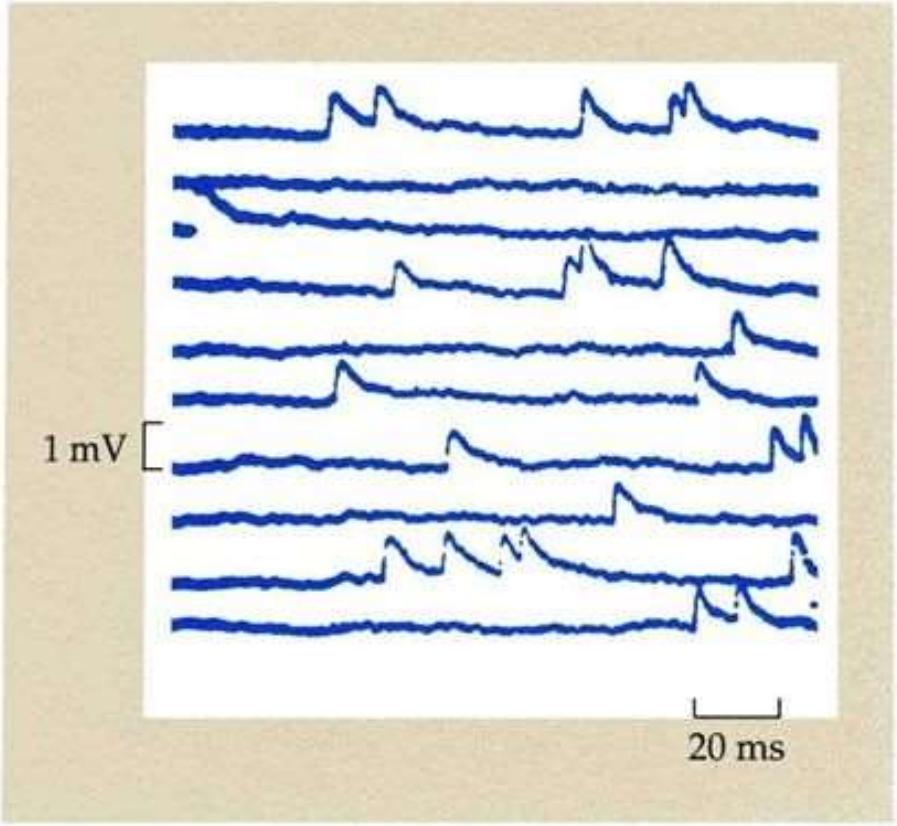


Intracellular recordings of spontaneous m.e.p.ps at the frog NMJ (A,B) and ~2 mm away from the end plate (C,D), demonstrating the localization of postjunctional potentials. Nerve stimulation elicits a large e.p.p. and MAP at the endplate (B), but 2 mm away (D), only the propagated MAP remains. From Fatt & Katz (1952) J Physiol 117:109.

The vesicles are “packets” of ACh

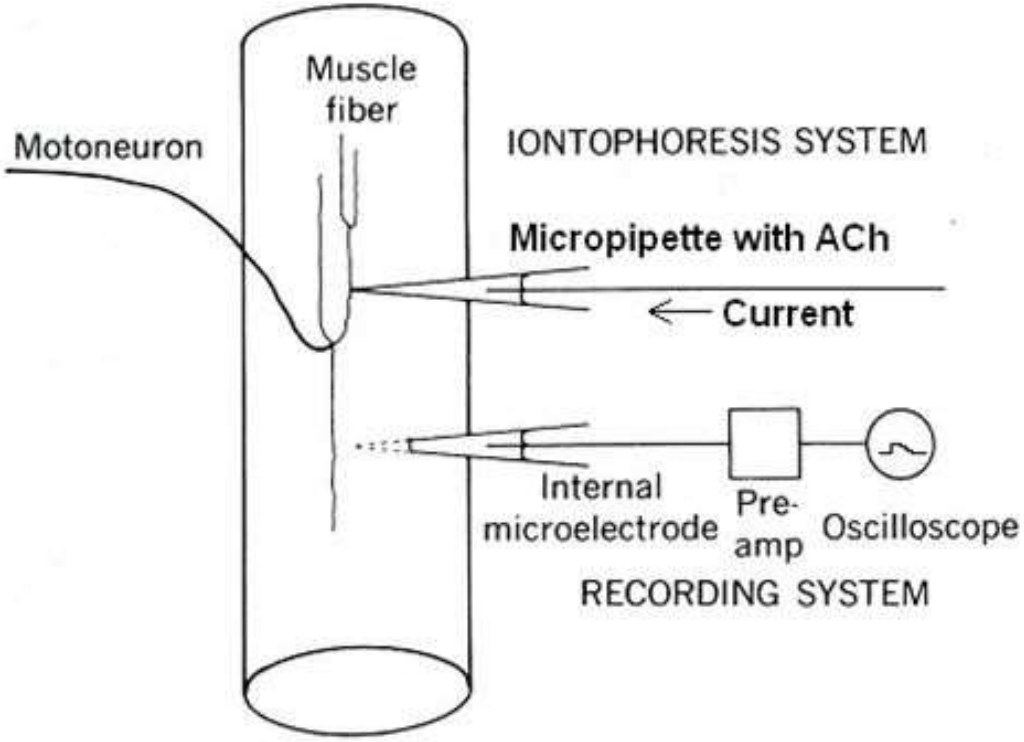


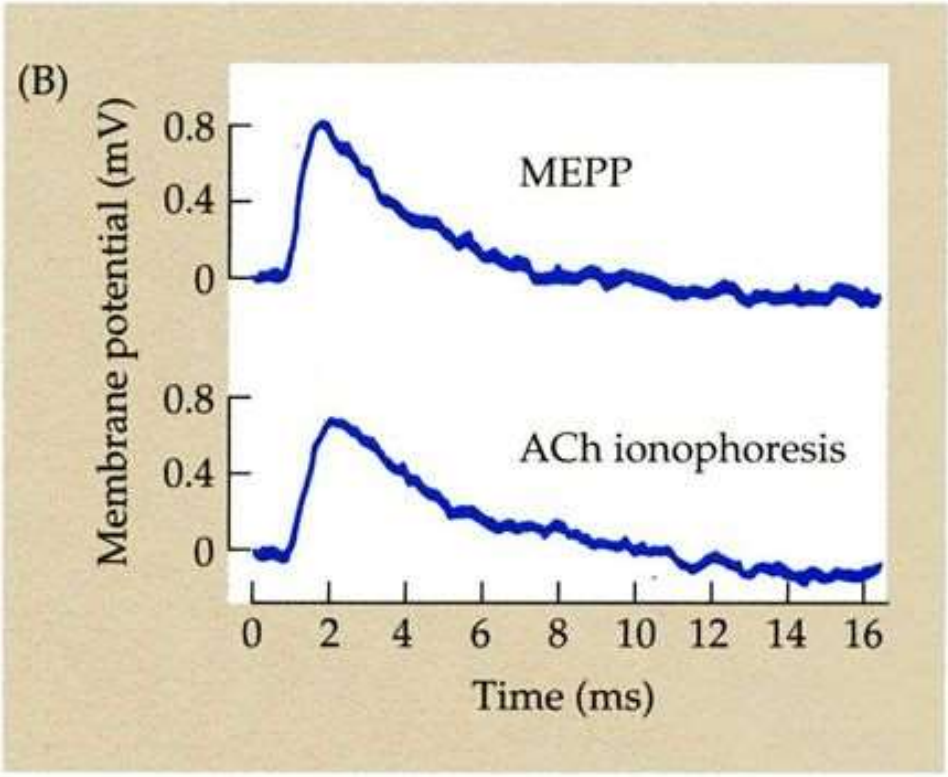
- Fatt & Katz (1952):
- Microelectrode recording at motor endplate



- Under these conditions we get MEPPs: spontaneous “miniature end plate potentials”
- How many ACh molecules are in a packet?

How many molecules in a packet of ACh?

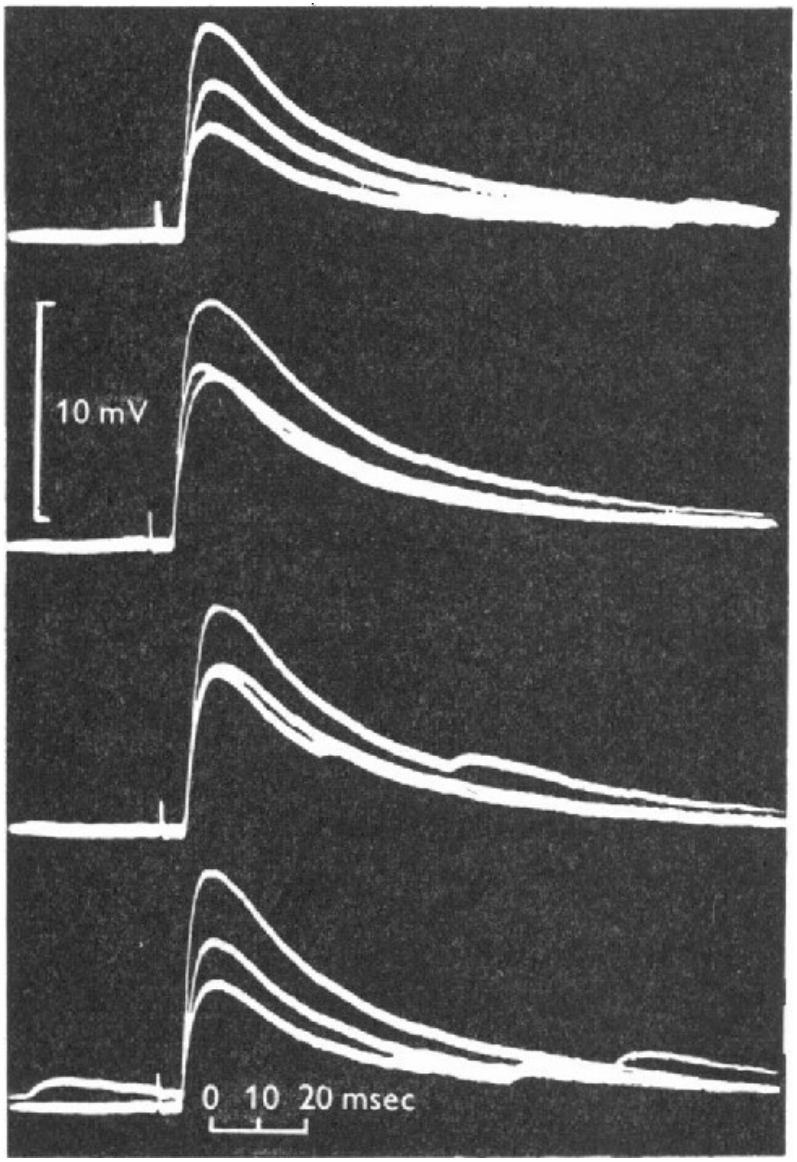




- MEPP can be reproduced exactly by iontophoresed ACh
- We can estimate exactly how many ACh molecules this needs because:
  - each ACh molecule has one positive charge
  - and we know how much current was used
- About 7000 ACh molecules are involved

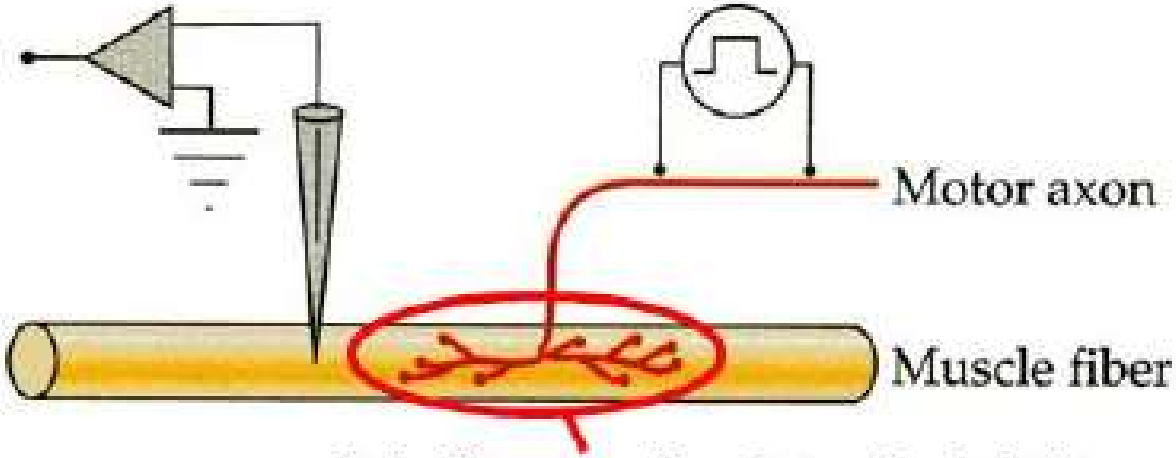
Intracellular recording

Neuromuscular junction



Fluctuation of evoked e.p.p. response at a single frog NMJ, treated with 10 mM-Mg^{2+} + 10^{-6}M prostigmine (anticholinesterase). In each record, three superimposed responses are seen. Note variation in amplitudes and scattered spontaneous miniature potentials. From Del Castillo & Katz (1954). Quantal components of the end-plate potential J. Physiol. 124(3):560-573.

EPP is made up of a sum of MEPPs

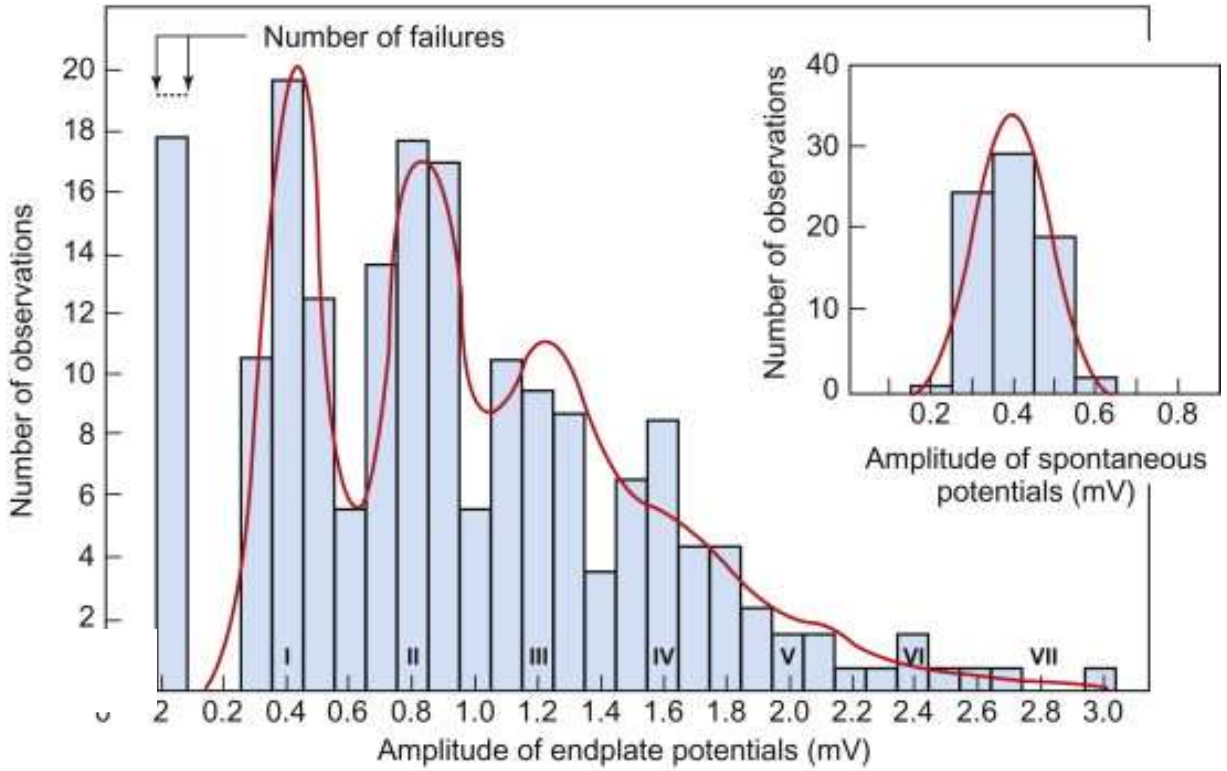


Mg^{2+} applied to inhibit transmitter release

- Because transmitter release is inhibited, each stimulus now gives only small EPPs and many failures...

Intracellular recording

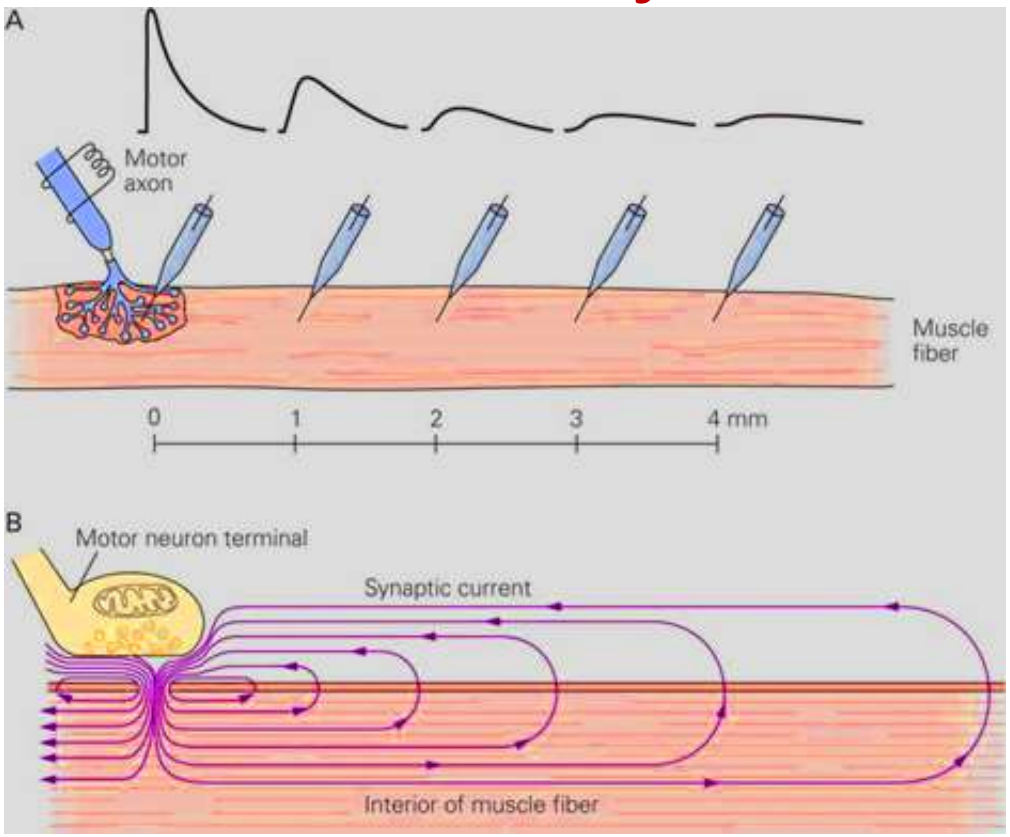
Neuromuscular junction



Distribution of spontaneous m.e.p.p and evoked e.p.p amplitudes recorded at the mammalian NMJ (+12.5 mM-Mg^{2+}). The e.p.p histograms show peaks at 1, 2, 3 and 4 times the mean amplitude of the m.e.p.p.s (0.4 mV) illustrating the quantal nature of transmission . From Boyd & Martin (1956). J.Physiol 132, 74-91.

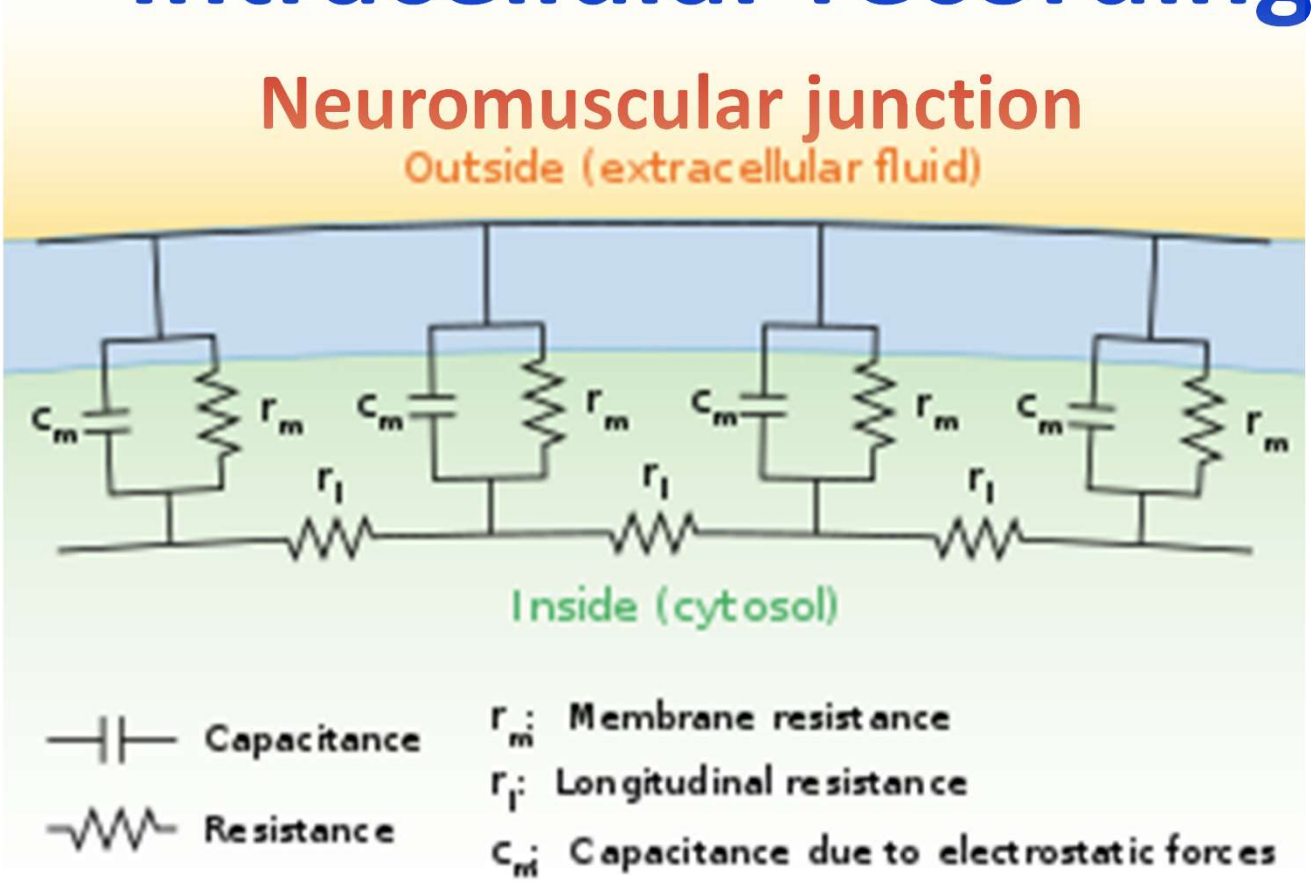
Intracellular recording

Neuromuscular junction



The amplitude of the e.p.p. decreases and the time course of the potential slows with distance from the site of initiation in the end-plate due to ionic “leakiness” of the muscle fibre membrane. Due to the long length of the fibre, it behaves like an “infinite” cable.

Intracellular recording



Cable theory's simplified electrical representation of a muscle fibre as an "infinite cable" .

$$\Delta V(x) = \Delta V(0)\,(e^{-x/\lambda})$$

length constant → $\lambda = \sqrt{\frac{r_m}{r_i}}$

r_m ← membrane resistance

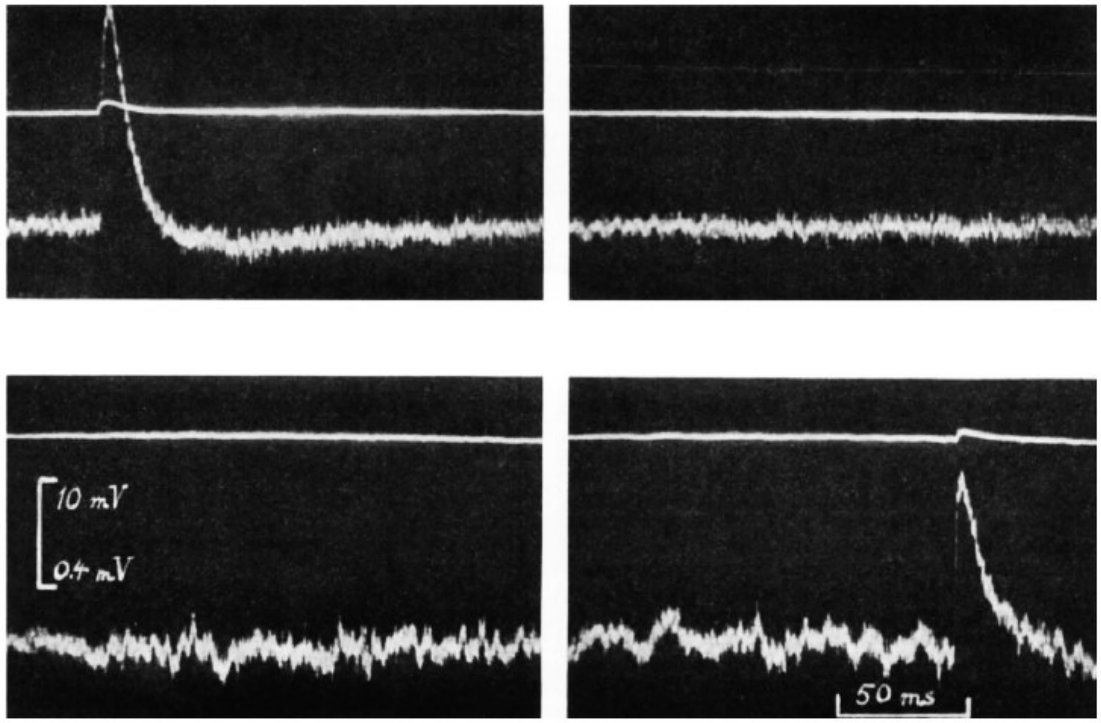
r_i ← internal resistance

Intracellular recording

Neuromuscular junction-membrane "noise"

Control baseline noise

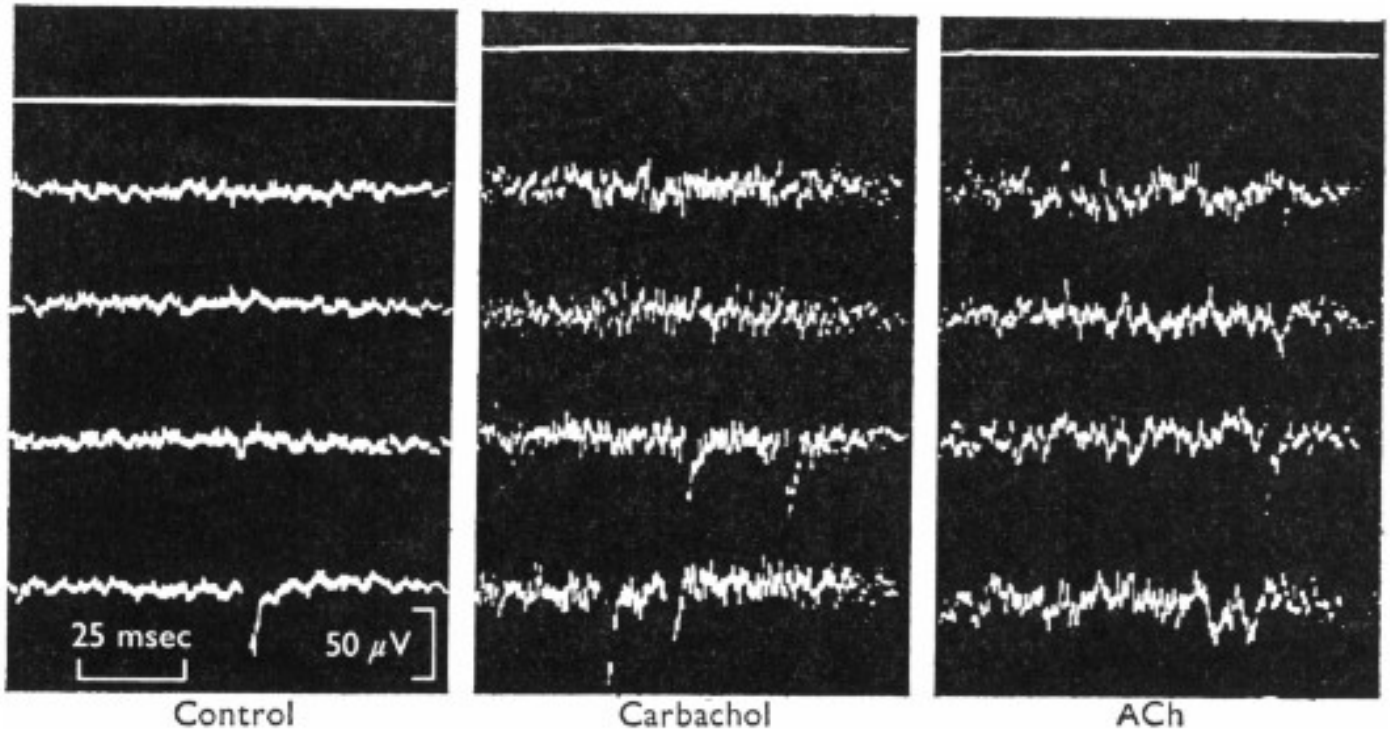
+ ACh



In 1970, Katz and Miledi noticed that there was an increase in baseline electrical "noise" accompanying the depolarization evoked by locally-applied ACh at the frog NMJ. They proposed that this reflected stochastic fluctuations in the numbers of open elementary ACh channels caused by random binding of ACh molecules to ACh receptors. From Katz & Miledi (1970). Nature, 226, 962-963.

Intracellular recording

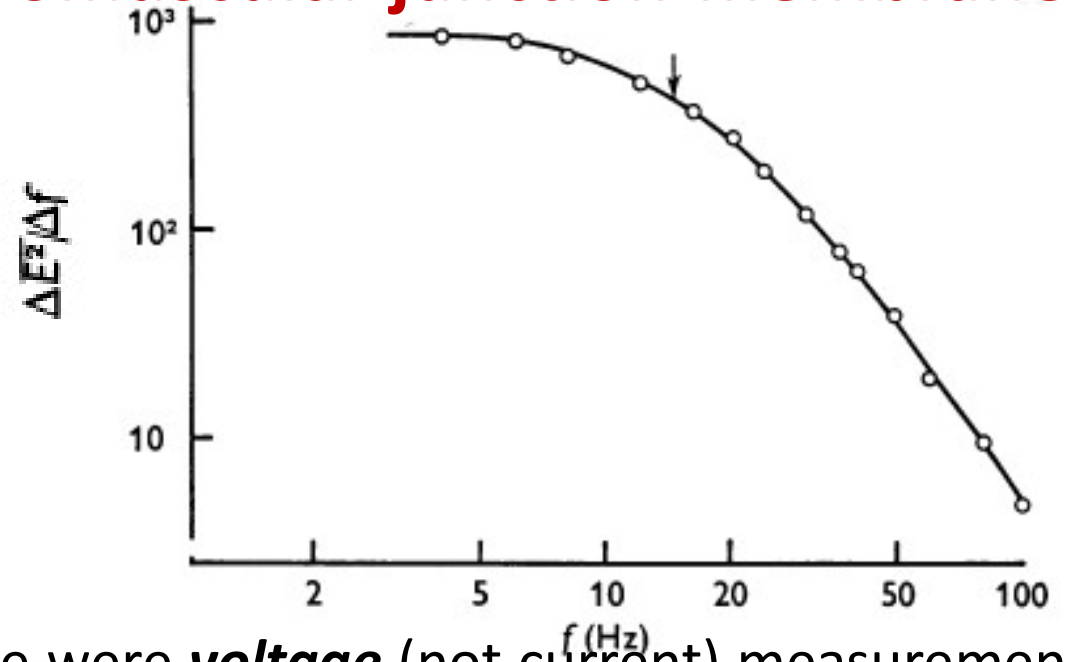
Neuromuscular junction-membrane "noise"



In a later paper, Katz and Miledi noticed that the noise produced by two different nicotinic agonists at the NMJ were different: thus, the noise produced by carbachol was "faster" than that produced by ACh, implying that the duration of the ion channel opened by carbachol was shorter than that activated by ACh. From Katz & Miledi (1972). J. Physiol. 224, 665-699.

Intracellular recording

Neuromuscular junction-membrane "noise"

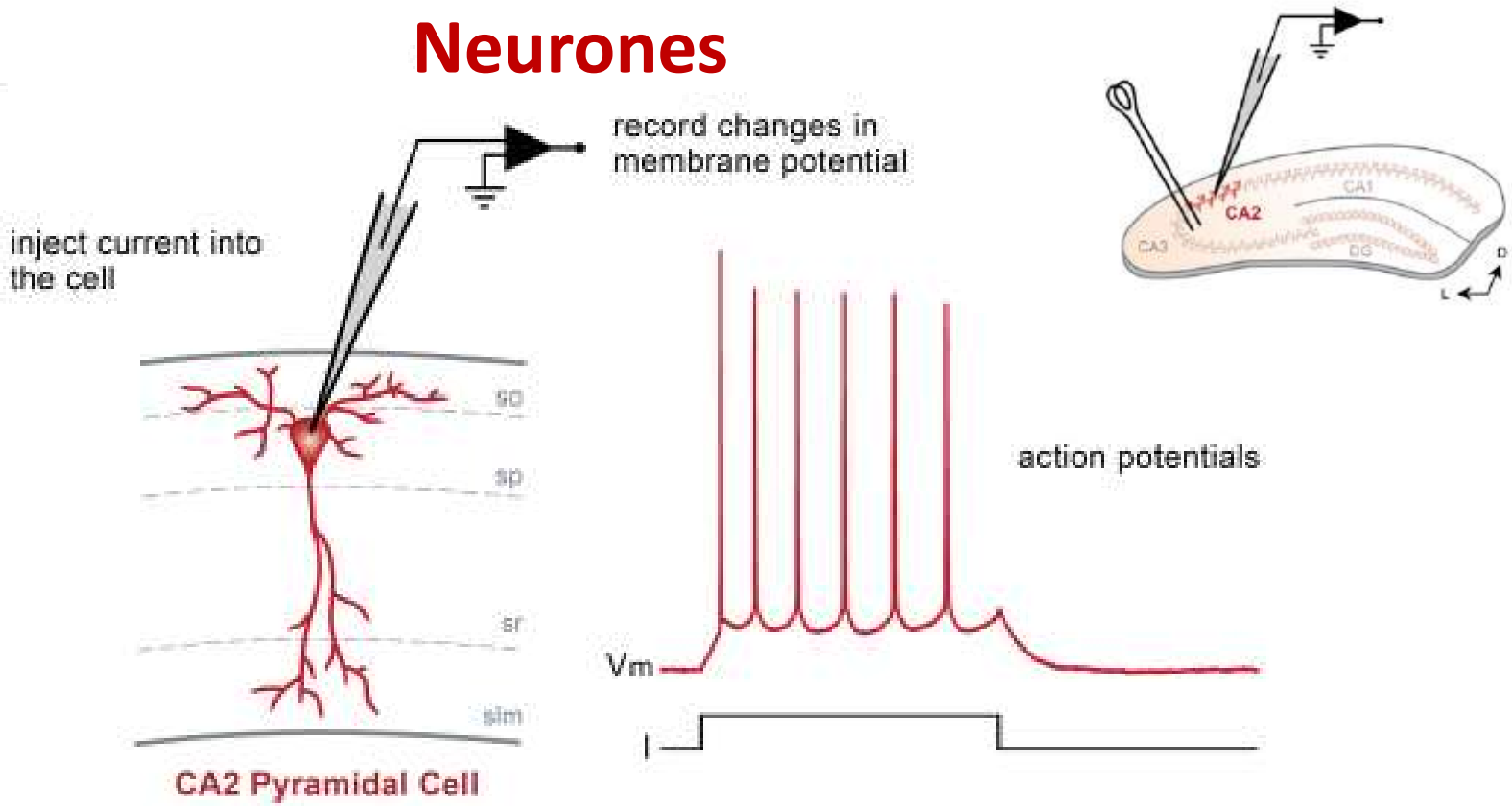


Note these were ***voltage*** (not current) measurements, the elementary "shot" effect produced by ACh (~0.3 µV, 1 ms) was ≡ a tiny m.e.p,p, with a time constant = time constant of the muscle fibre. Later voltage clamp recordings overcame this problem to give the **ACh single channel conductance and lifetime**. Analysis of the spectral distribution of noise variance produced a characteristic "power density spectrum" fitted by a Lorentzian function. From Katz & Miledi (1972). J. Physiol. 224, 665-699.

How do you record from neurones?

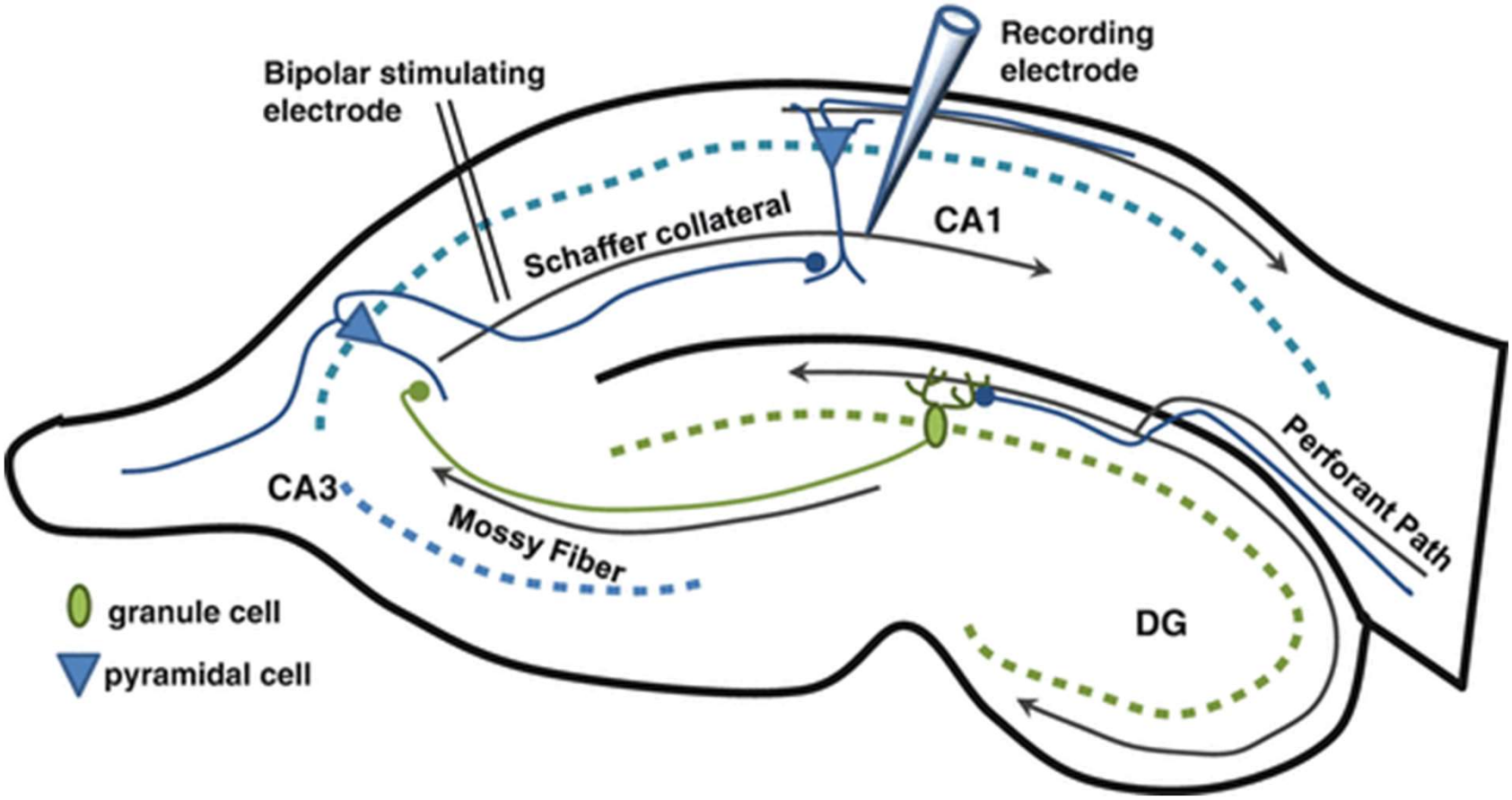
Intracellular recording

Neurones



Intracellular recordings using “sharp” microelectrodes can also be made from brain slice neurones *in vitro.* The electrode is impaled into the cell and connected to a “bridge”-type amplifier, which records membrane potential and passes intracellular current pulses into the cell to record intrinsic excitability.

Intracellular recording



Simplified representation of a transverse hippocampal brain slice preparation, widely used for intracellular microelectrode recording studies.

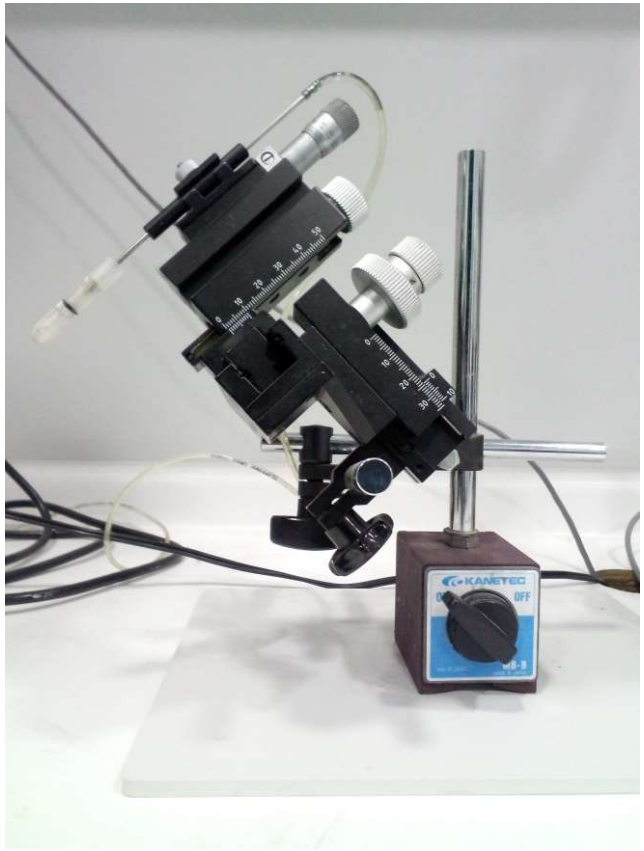
Intracellular recording

Leica VT1200 S : A vibrating blade “vibroslice” tissue cutter for preparing acute brain slices for recording.


Take a
Break!

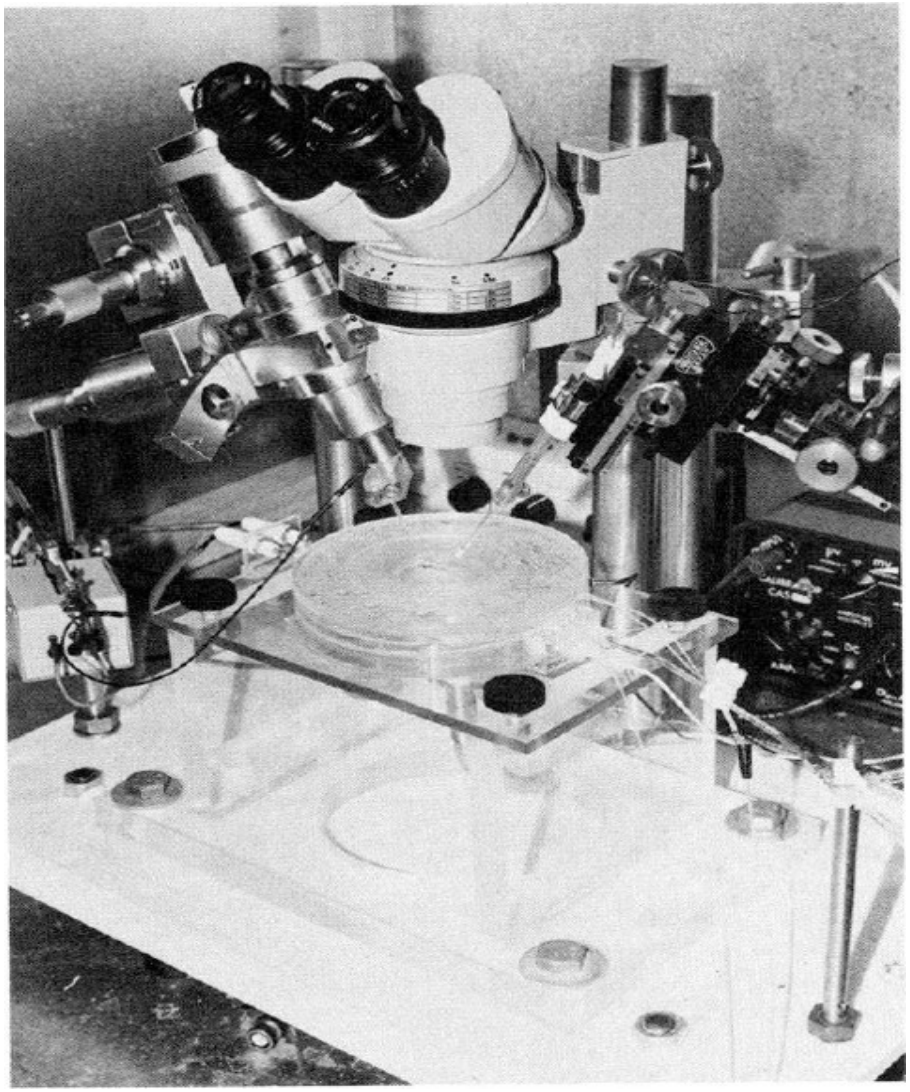
How is a neuronal intracellular recording made with the microelectrode?

Intracellular recording



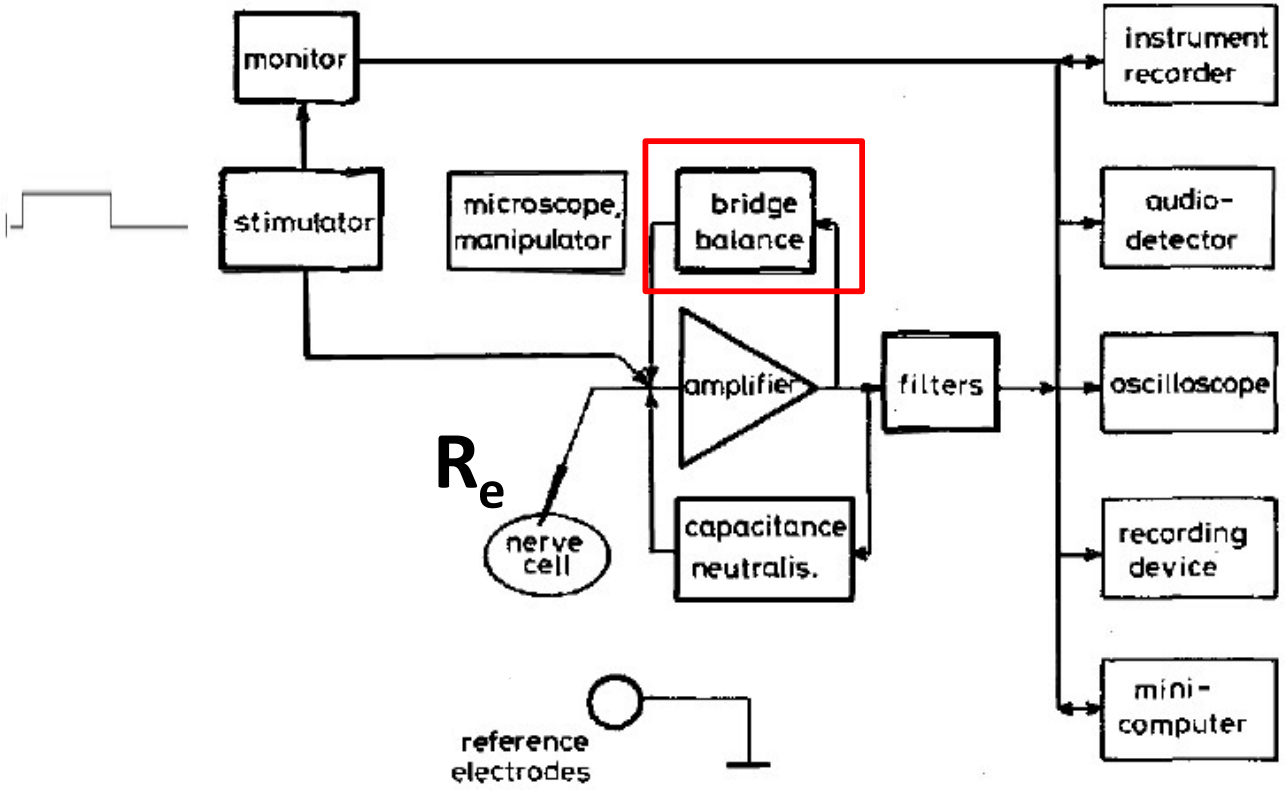
A more advance micromanipulator positioning system is usually preferred for making stable intracellular neuronal recordings, sometimes using a motorised or electromechanical (piezoelectric) control stage making fine step movements through the tissue.

Intracellular recording



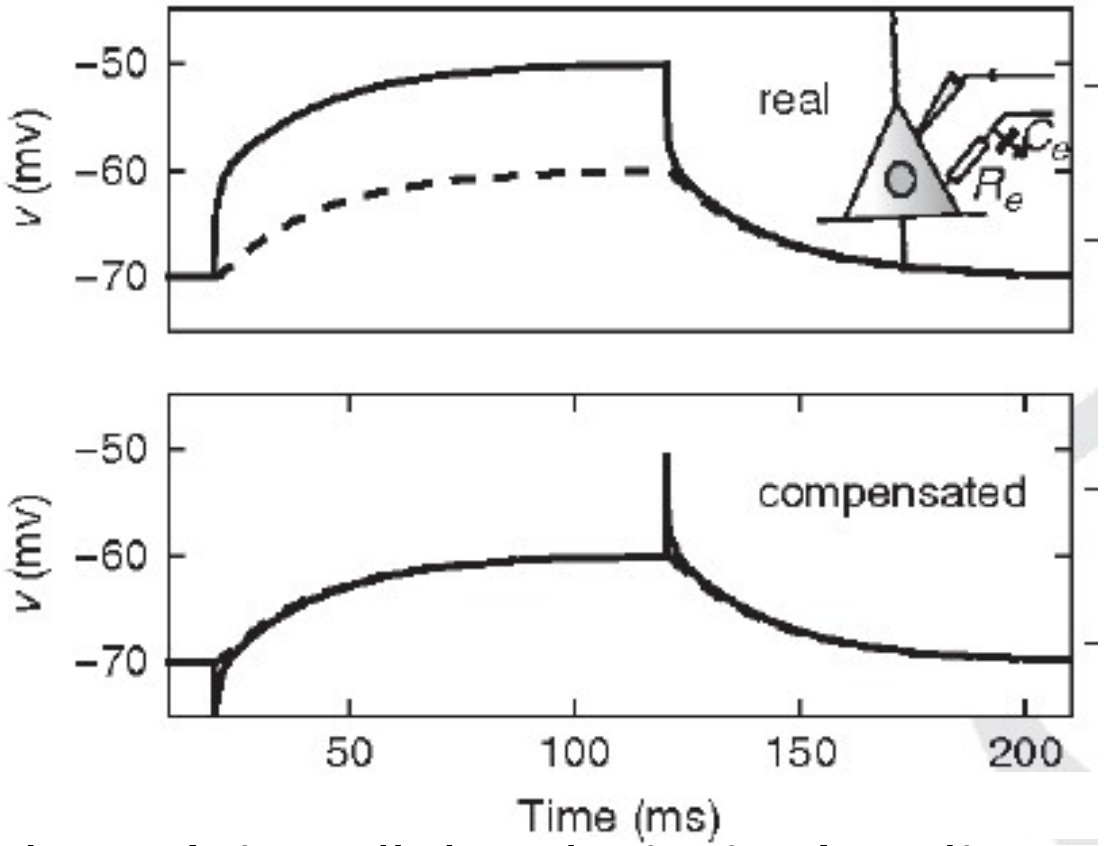
Photograph of a typical *in vitro* brain slice recording set-up.

Intracellular recording



An intracellular "bridge balance" amplifier allows for intracellular current pulses to be injected into the cell to assess membrane excitability and input resistance. Passing current through the microelectrode resistance $\mathbf{R_e}$ will however generate a voltage artefact that adds on to the real neuronal electrotonic response, therefore needs to be cancelled using a "bridge balance" circuit.

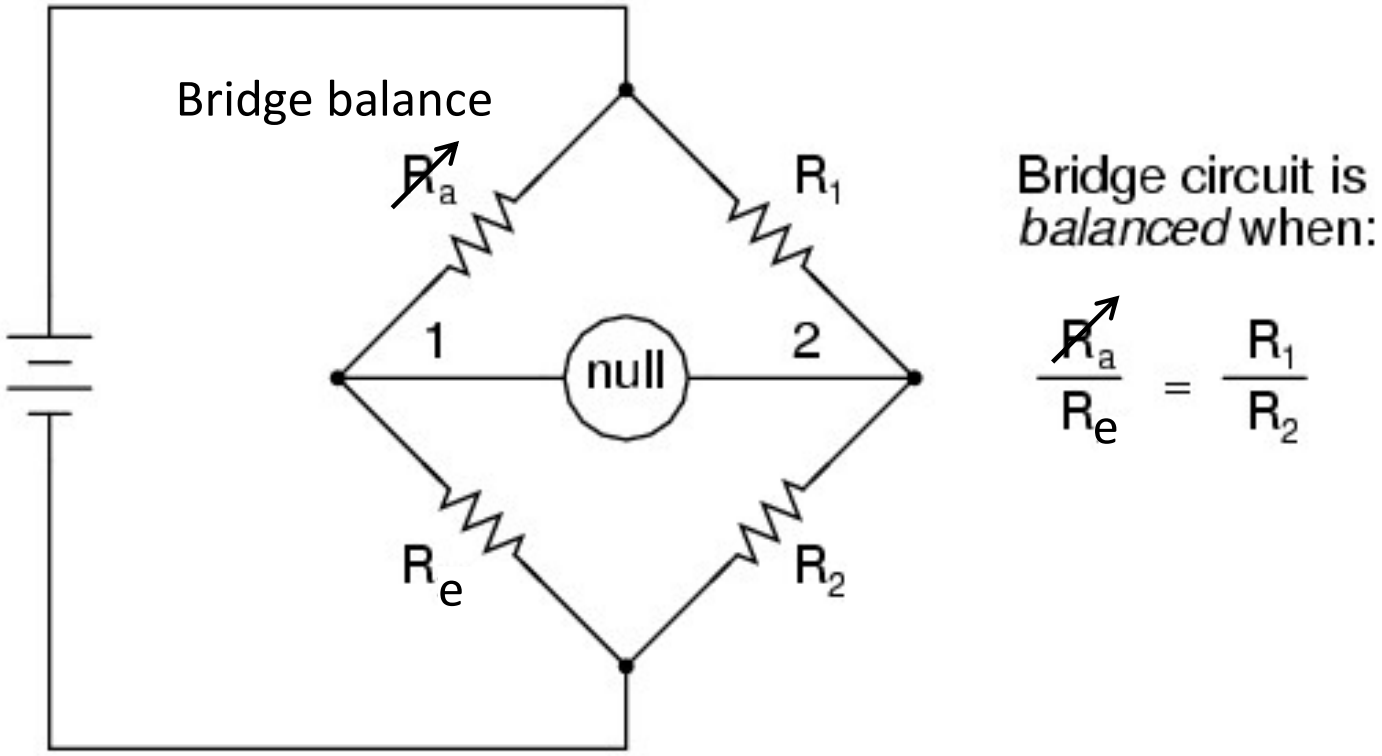
Intracellular recording



Upper trace shows intracellular electrotonic voltage response of a cell + microelectrode resistance artefact recorded following injection of a rectangular depolarizing current pulse. Lower trace shows the same response after bridge balance compensation for the microelectrode resistance R_e. This is **current clamp** recording.

Intracellular recording

Neurones



A standard Wheatstone Bridge circuit.

Intracellular recording

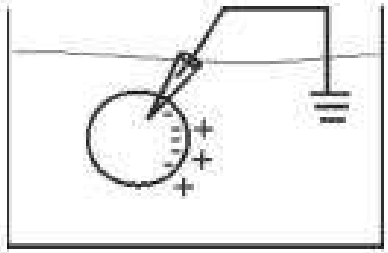
Axon Instruments Axioprobe 1A recording preamplifier:

Intracellular recording

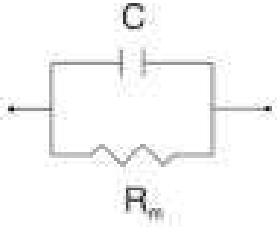
Current Clamp

Monitors the potential of the cell- therefore units will be in volts

By convention $V = E_{inside} - E_{outside}$



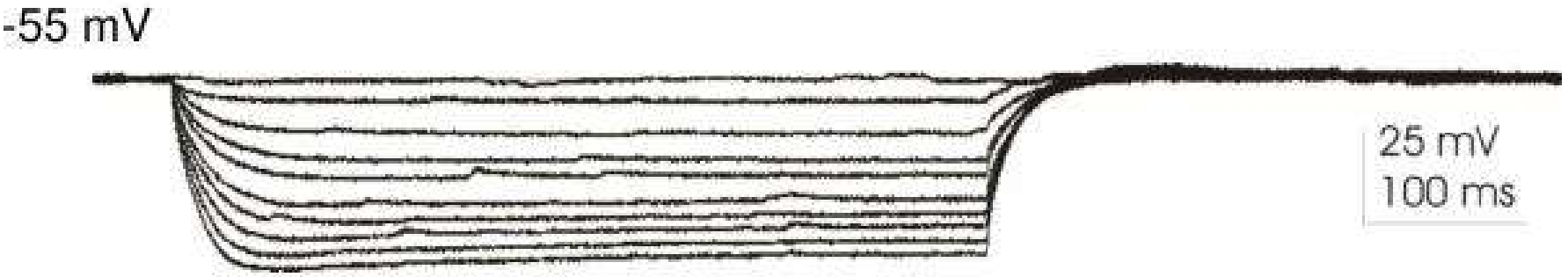
Upward deflections are depolarizing; downward are hyperpolarizing

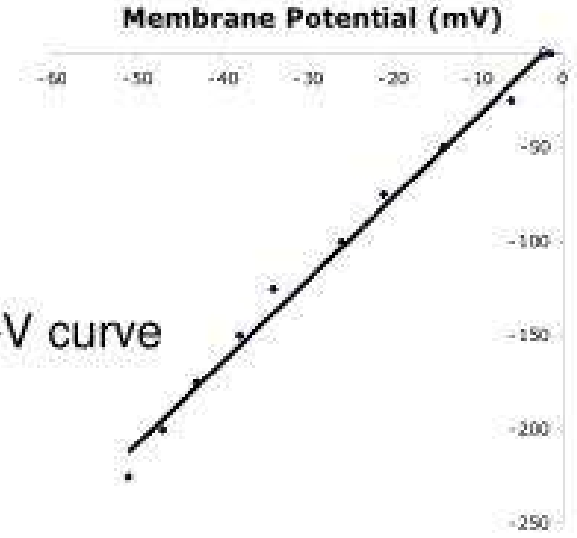


Membrane time constant = $R_m C_m$

Current Clamp

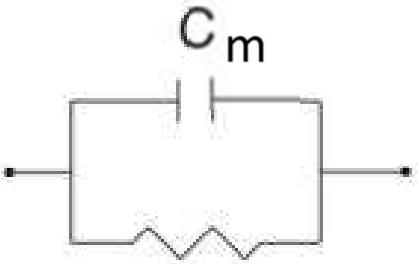
Current-Voltage Relationship & Measuring conductance (g):



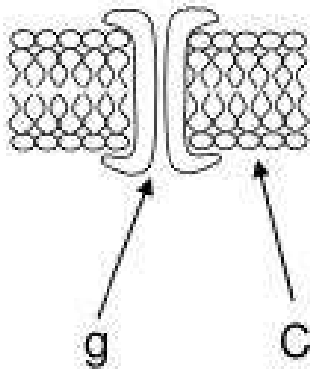


"Ohmic" I-V curve

I



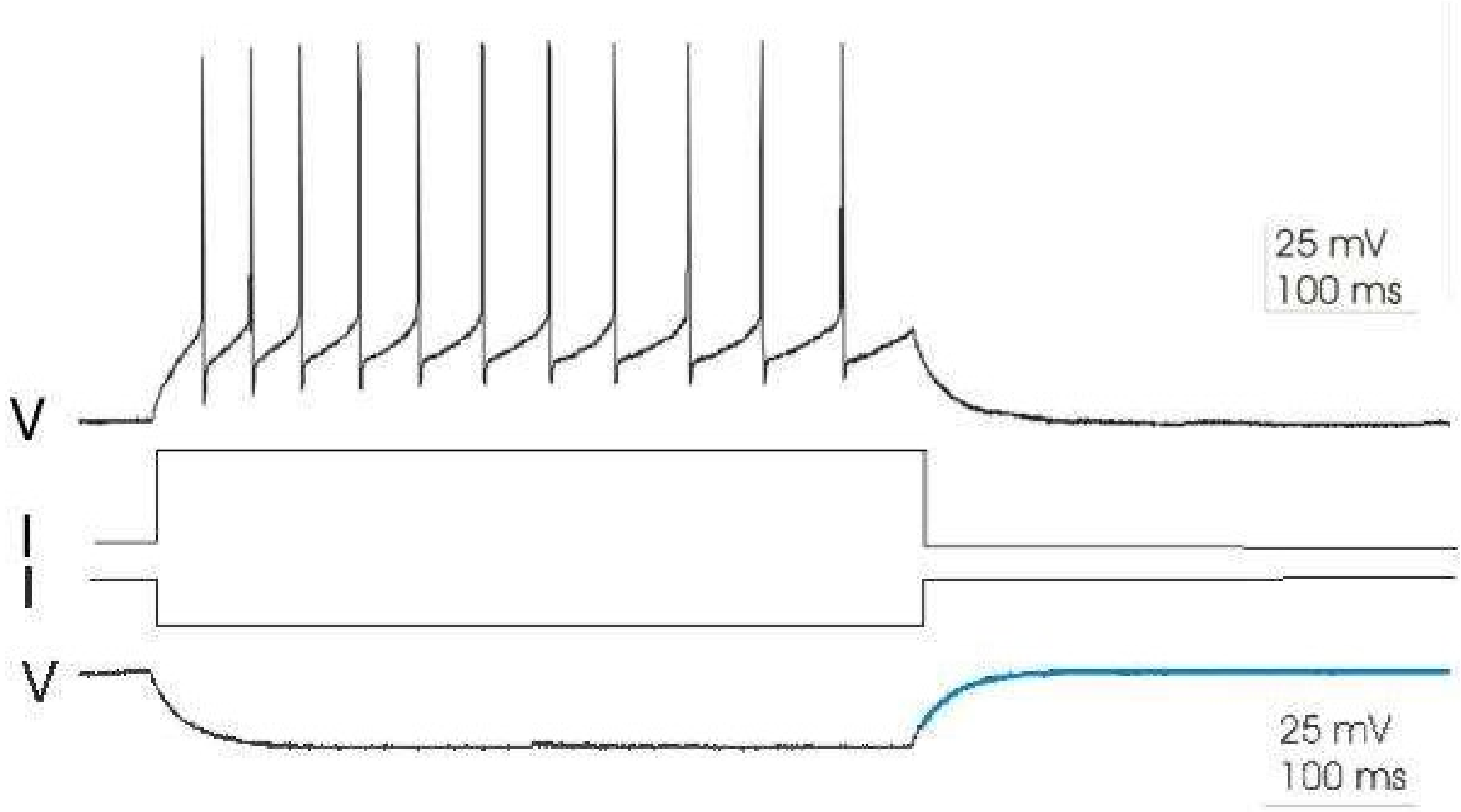
$R_m = 1/g$



Slope = $I/V_m = 1/R = g$ (conductance)

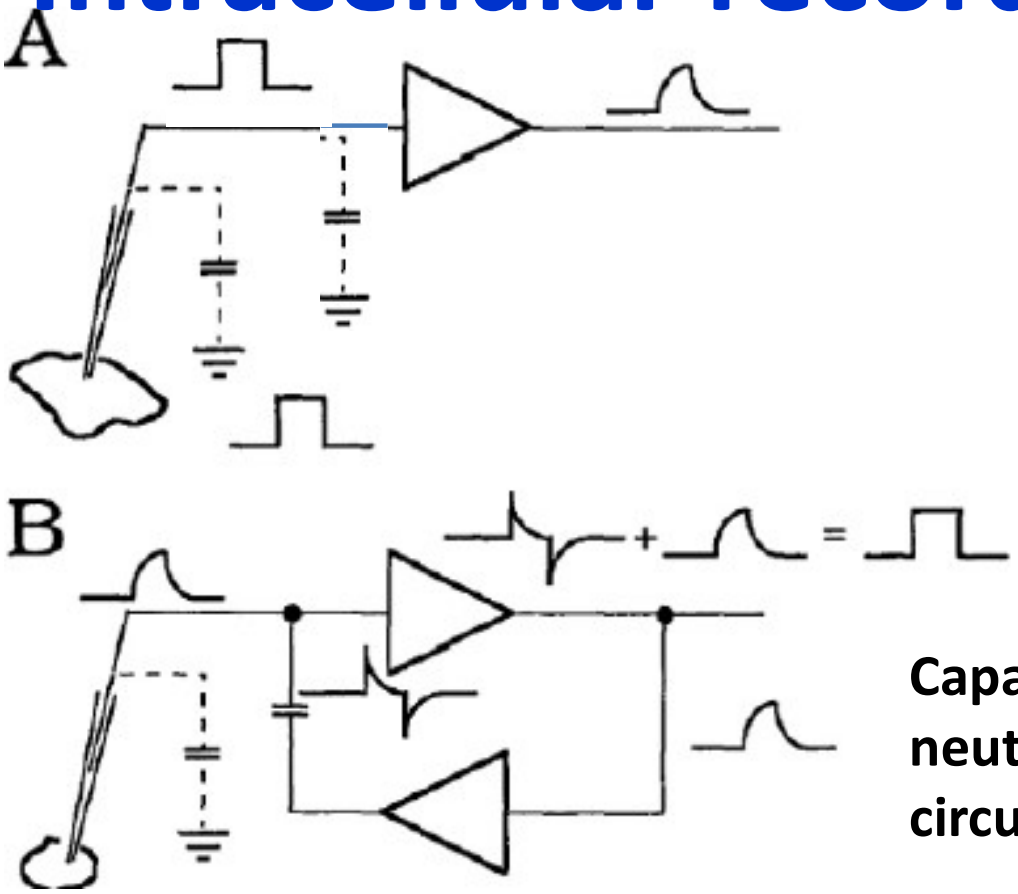
Current Clamp

Action potentials can be measured



How do you penetrate the neuronal cell membrane with the microelectrode?

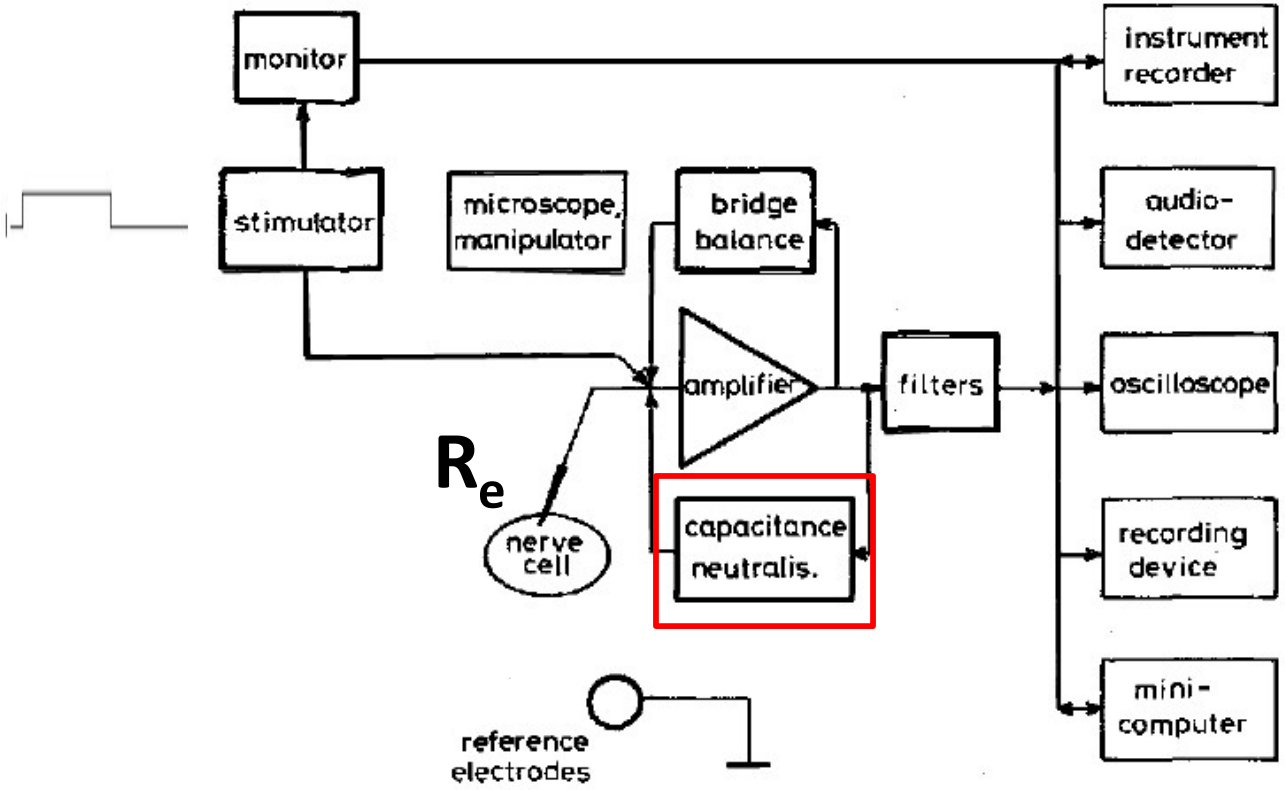
Intracellular recording



Capacitance neutralization circuit

(A) The input capacitance of the amplifier and the microelectrode capacitance combine with the electrode resistance to form a low-pass filter that attenuates the high-frequency components of the measured signal. (B) A capacitance neutralization feedback amplifier can restore the lost high-frequency components.

Intracellular recording



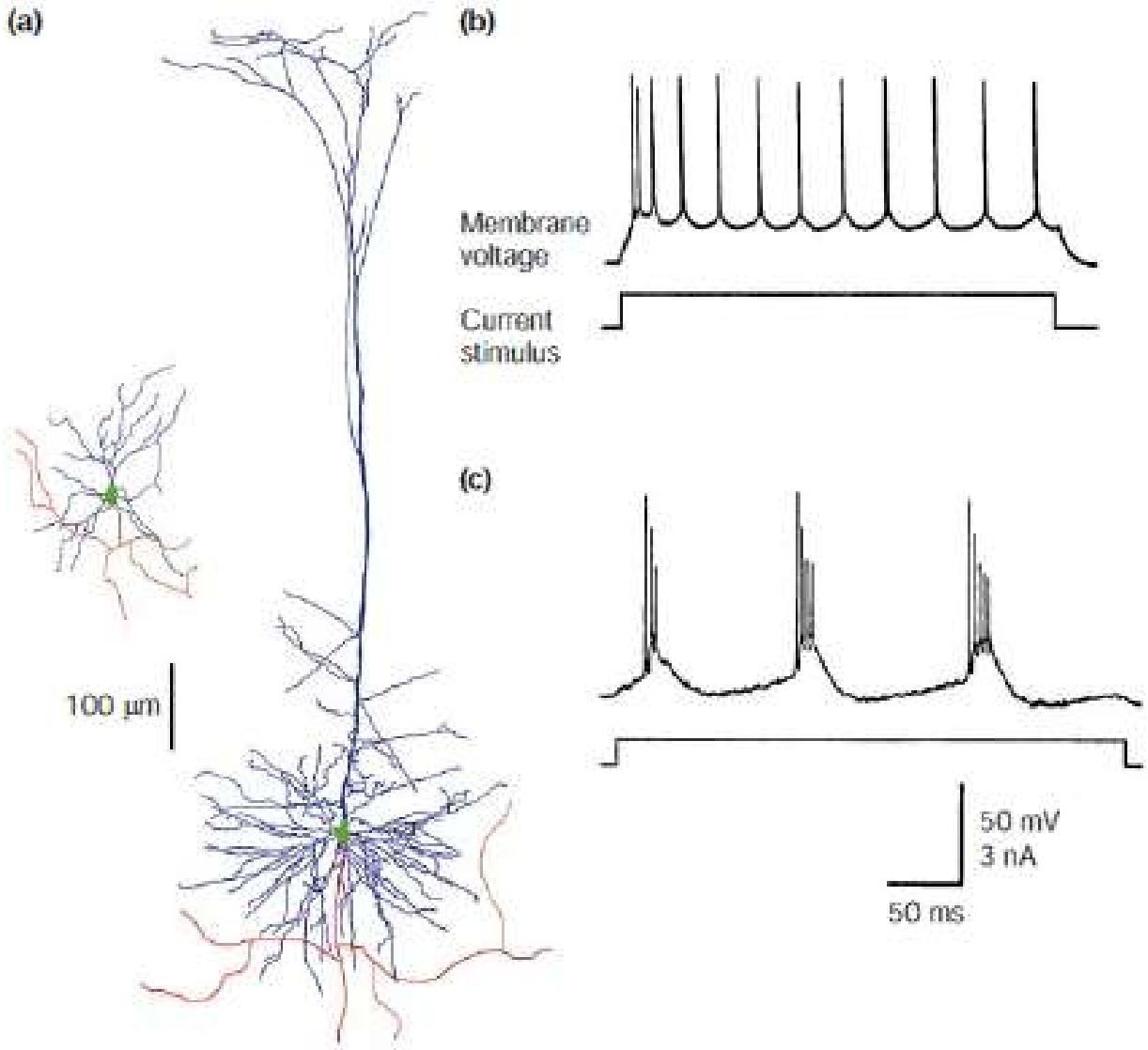
An intracellular "bridge balance" amplifier allows for intracellular current pulses to be injected into the cell to assess membrane excitability and input resistance. Passing current through the microelectrode resistance $\mathbf{R_e}$ will however generate a voltage artefact that adds on to the real neuronal electrotonic response, therefore needs to be cancelled using a "bridge balance" circuit.

Intracellular recording

Cell impalement can be achieved using a brief buzz or positive clear pulse.

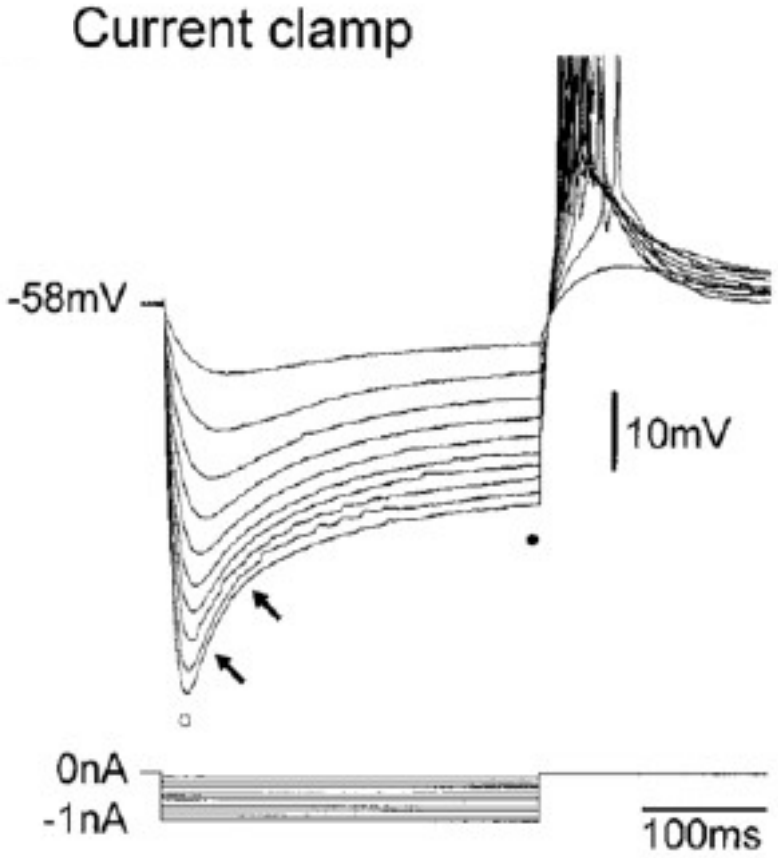
+ 15V

Intracellular recording



(a) Two mouse cortical neurones with very different morphologies: cell body (green), dendrites (blue), axon (red). Dendrites are input sites where the axons of other neurones form synapses; at excitatory synapses, ionic current flows across the membrane locally, generating e.p.s.ps that can propagate down the dendrites and into the soma. The axon is the output structure. **(b)** The firing of a 'regular-spiking' pyramidal neuron in response to an injected current pulse. **(c)** The response of an 'intrinsically bursting' pyramidal neurone. From Connors & Regehr (1996). Current Biology 6:1560–1562.

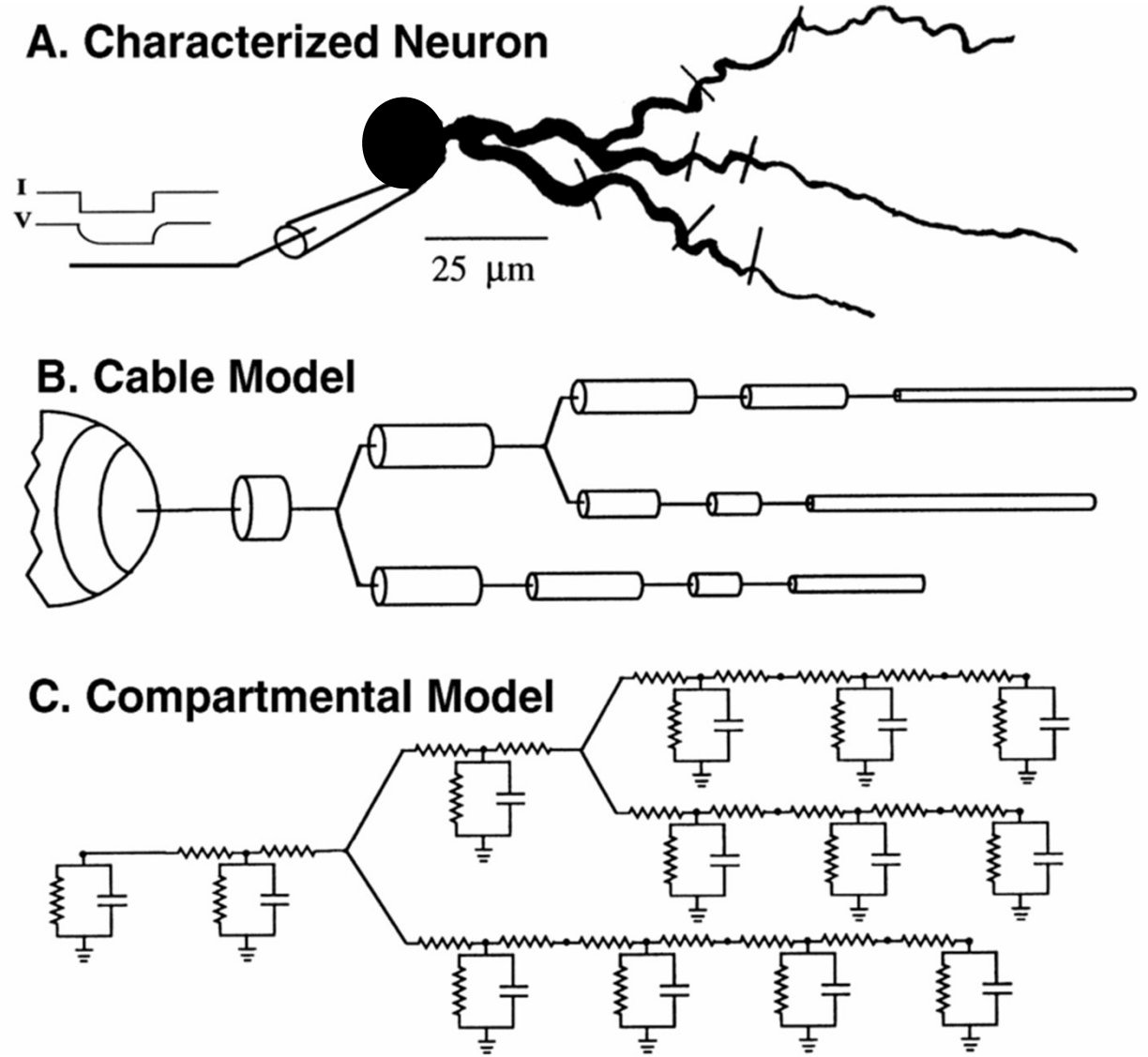
Intracellular recording



Entorhinal cortex neurone

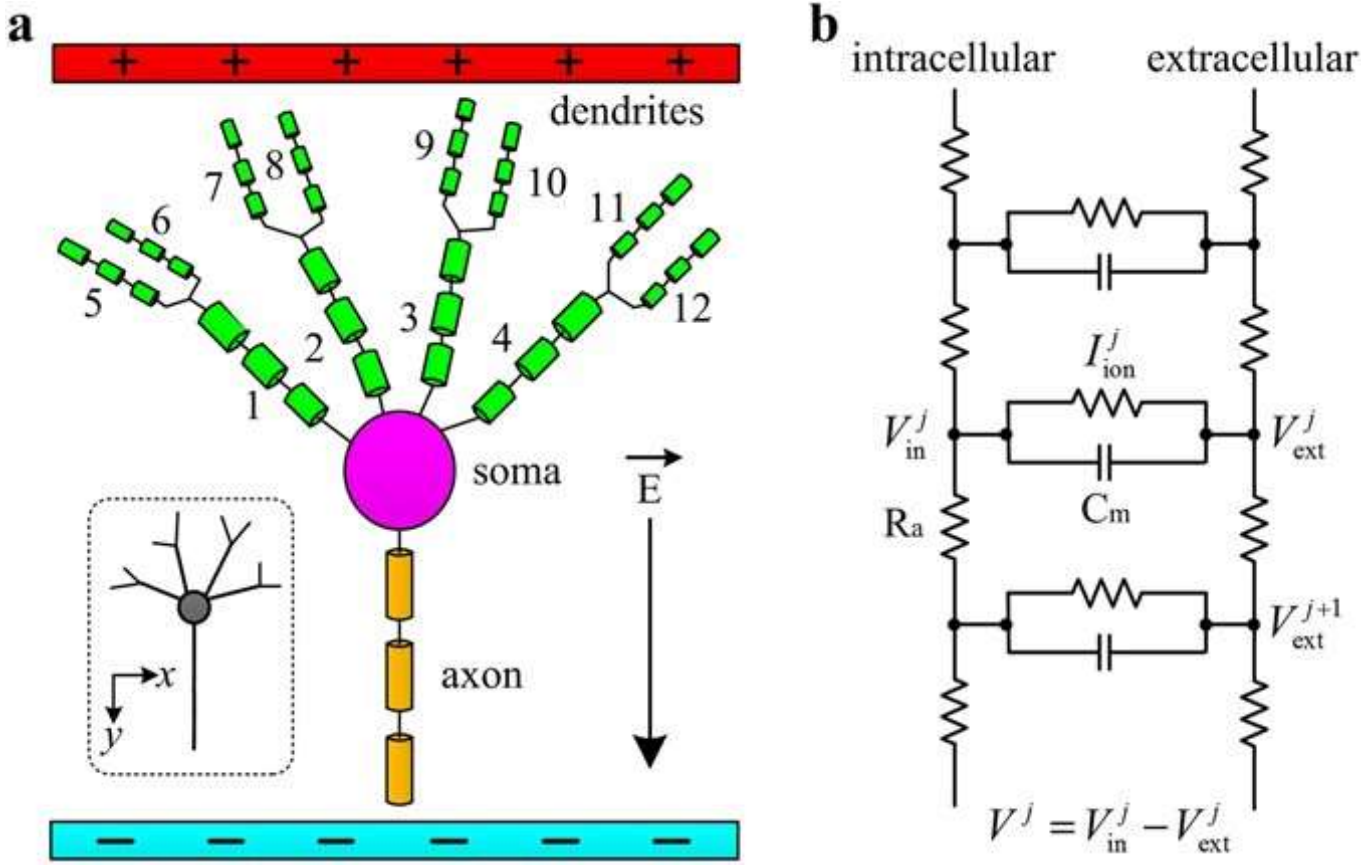
Intracellular recordings can also reveal interesting neuronal properties following injection of hyperpolarizing current pulses. Traces shows that in current-clamp conditions, hyperpolarizing pulses evoke voltage responses with a time-dependent depolarizing 'sag' due to activation of the I_h slow inward rectifier.

Intracellular recording



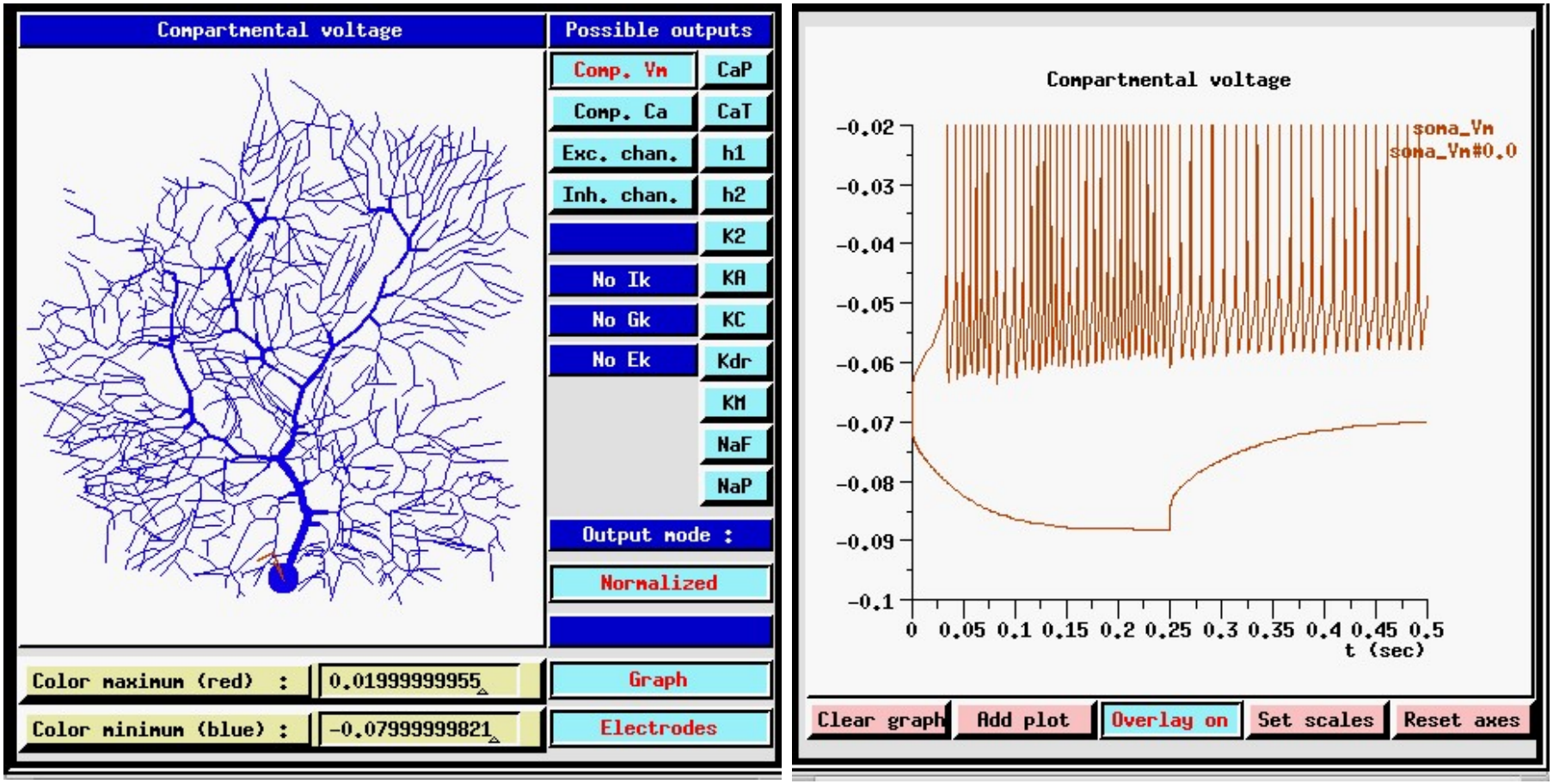
Cable or compartmental representation of a neurone

Intracellular recording



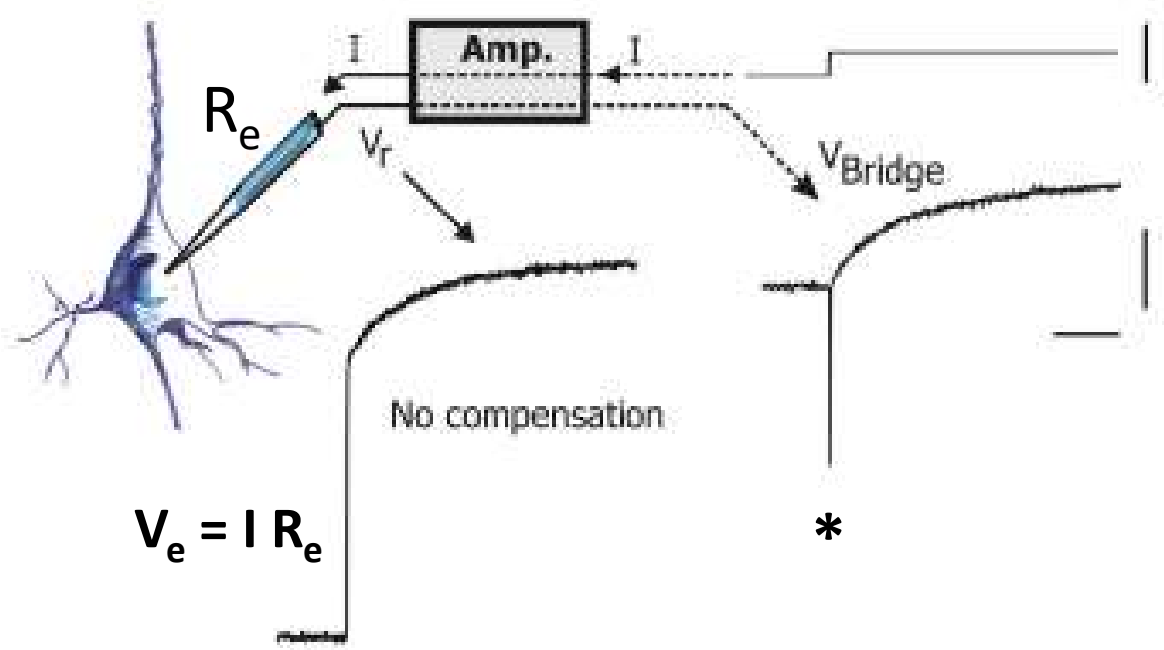
Artificial pyramidal neurone. (a) Schematic diagram of the multi-compartment artificial model simulated by the **NEURON** modelling program. (b) Circuit representation of the active compartments. From Yi et al., (2017) Scientific Reports, 7: 3210.

Intracellular recording



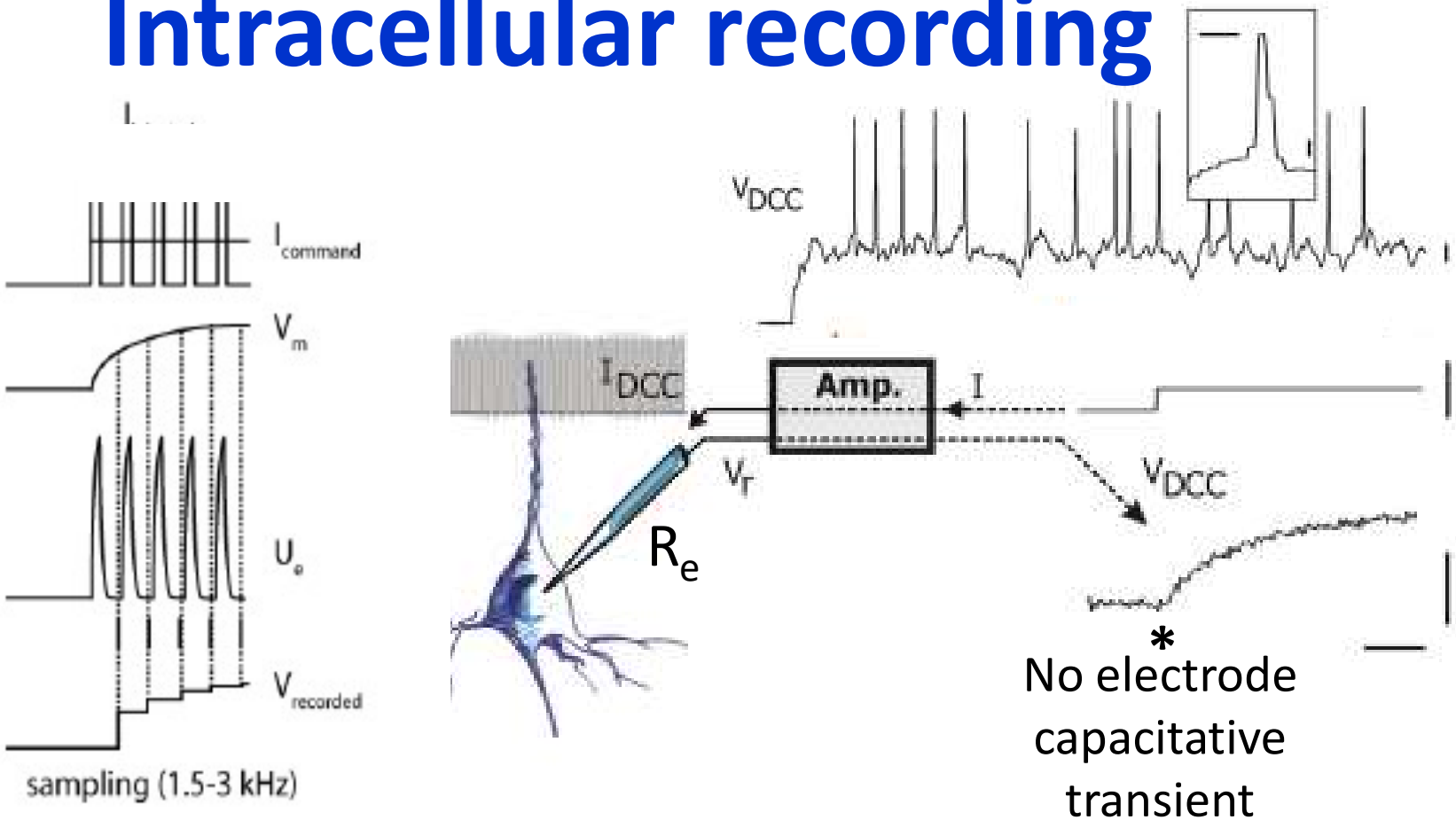
The Figure shows a cerebellar Purkinje cell model run on the **GENESIS** simulation program, with a current injection pulse applied to the soma. The plots show the superimposed results of ± 1 nA current pulses.

Intracellular recording



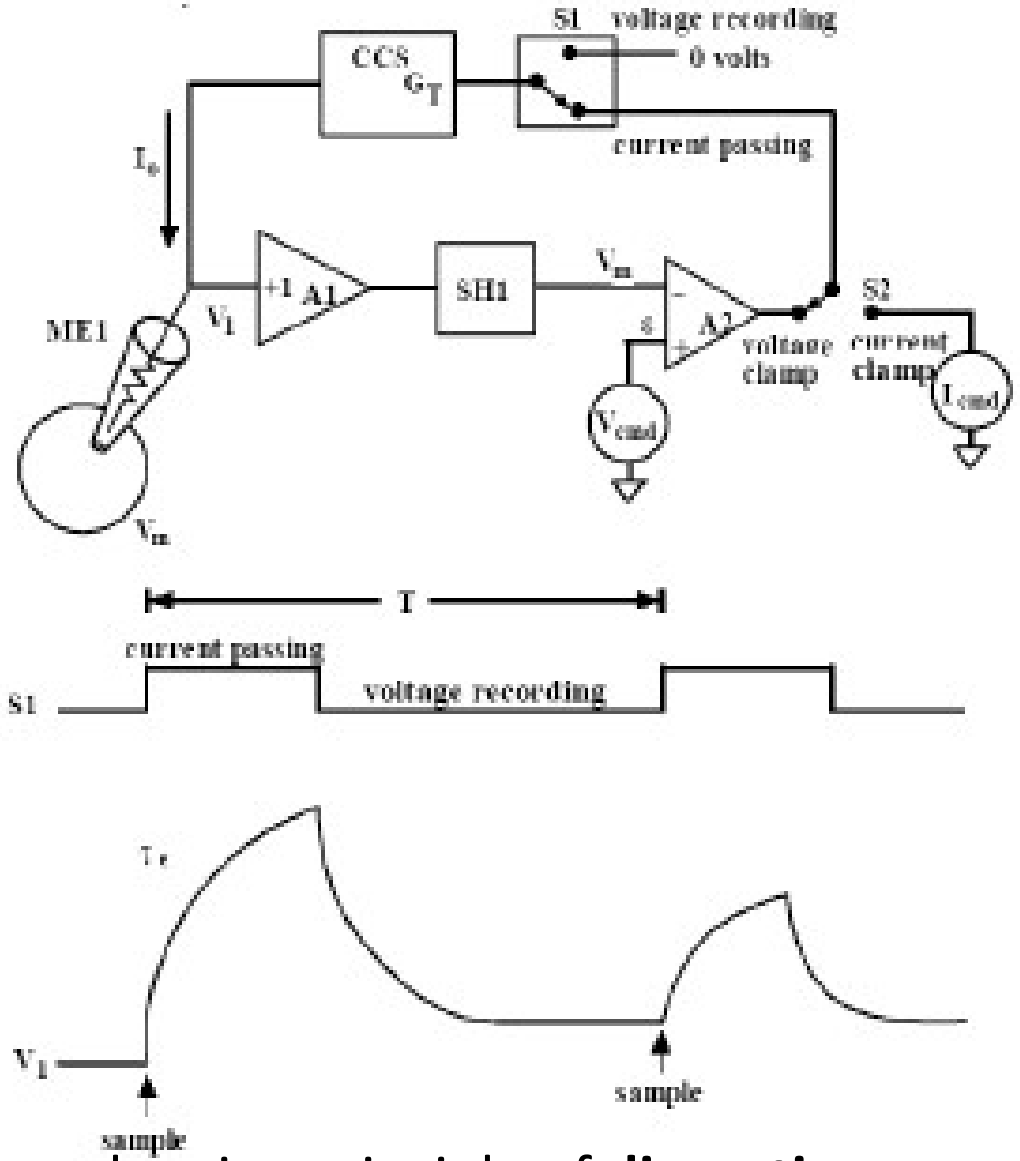
With standard bridge balance compensation, the accuracy of measurements can change if the electrode resistance $\mathbf{R_e}$ changes during the recording. Electrode capacitative transients (*) are also present at the make and break of the injected current pulse.

Intracellular recording



With **discontinuous current clamp**, current injection/voltage recording are rapidly alternated by a switching circuit such that voltage is sampled *after* the electrode artefact has subsided. The recording is therefore independent of $\mathbf{R_e}$, however the chopped sampling creates noisy records and fast action potentials cannot always be accurately resolved. Wilson & Goldner (1975). J. Neurobiology.6:411-422.

Intracellular recording



Circuit diagram showing principle of **discontinuous current clamp**, current injection/voltage recording preamplifier.

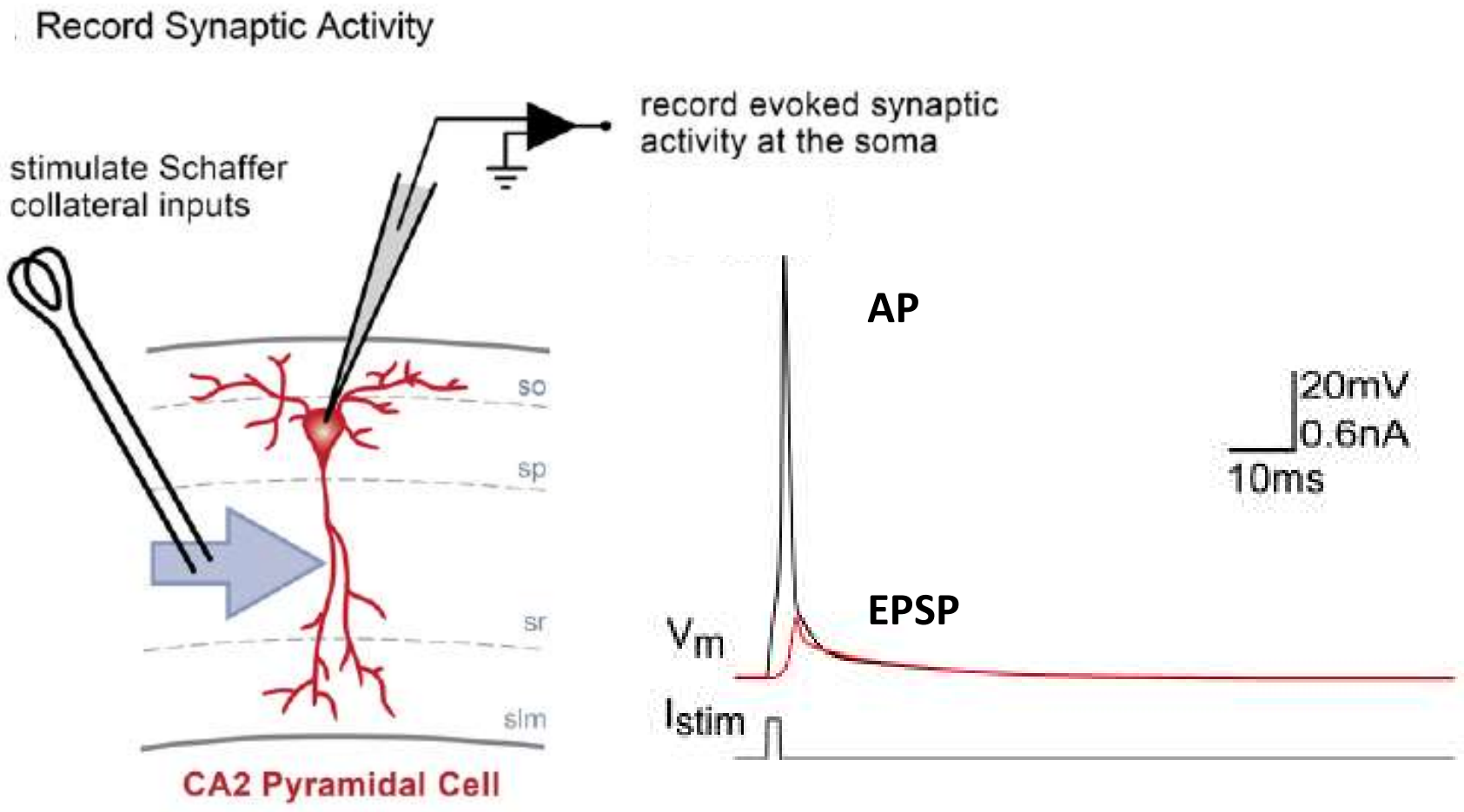
Intracellular recording

Axon Instruments Axoclamp 2B discontinuous current clamp/voltage clamp recording preamplifier:

Intracellular recording

The Axon Instruments MultiClamp™ 700B Microelectrode Amplifier is a more modern versatile, computer-controlled microelectrode amplifier designed for patch voltage-clamp or high-speed current clamp (sharp-electrode) recording within the same headstage.

Intracellular recording



Local electrical stimulation of intrinsic synaptic inputs allows recording of evoked synaptic activity recorded intracellularly at the soma.

Synaptically-evoked EPSP-fIPSP complex

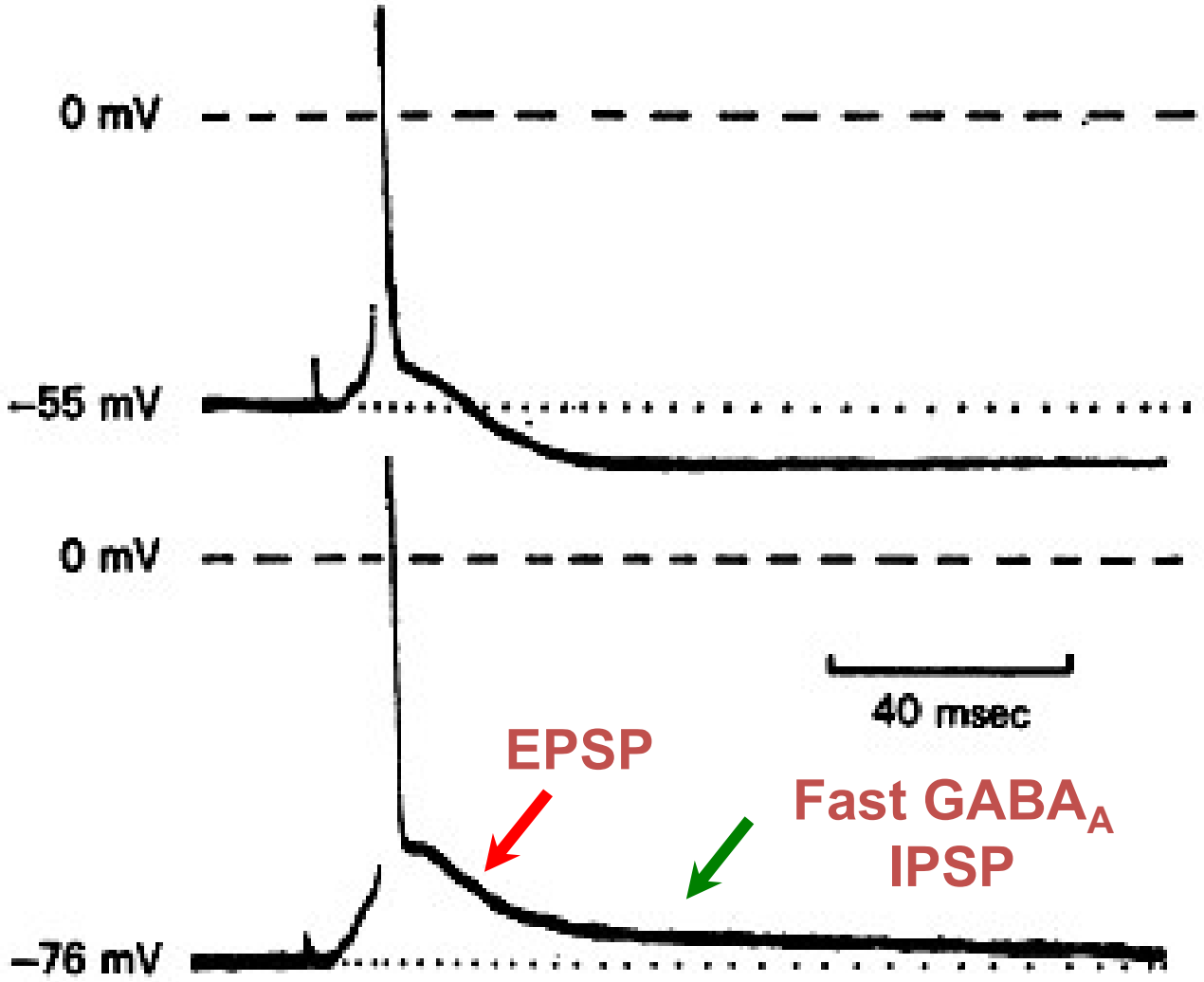


Fig. 1. An intracellular recording from a synaptically activated neurone in the olfactory cortex. The upper trace is a recording on stimulating the l.o.t. immediately after impalement when the membrane potential was — 55 mV.

Synaptically-evoked EPSP-fIPSP/sIPSP complex

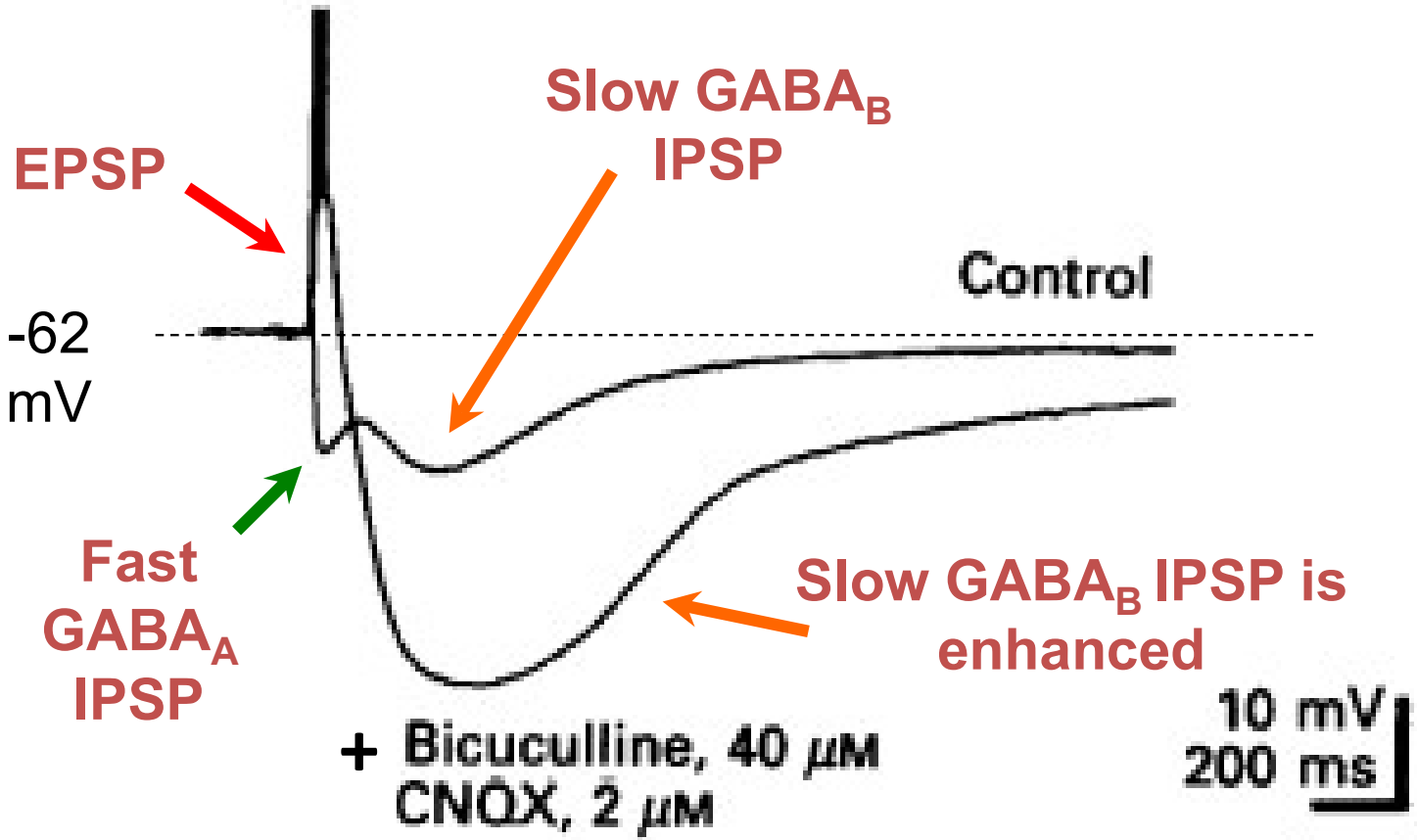
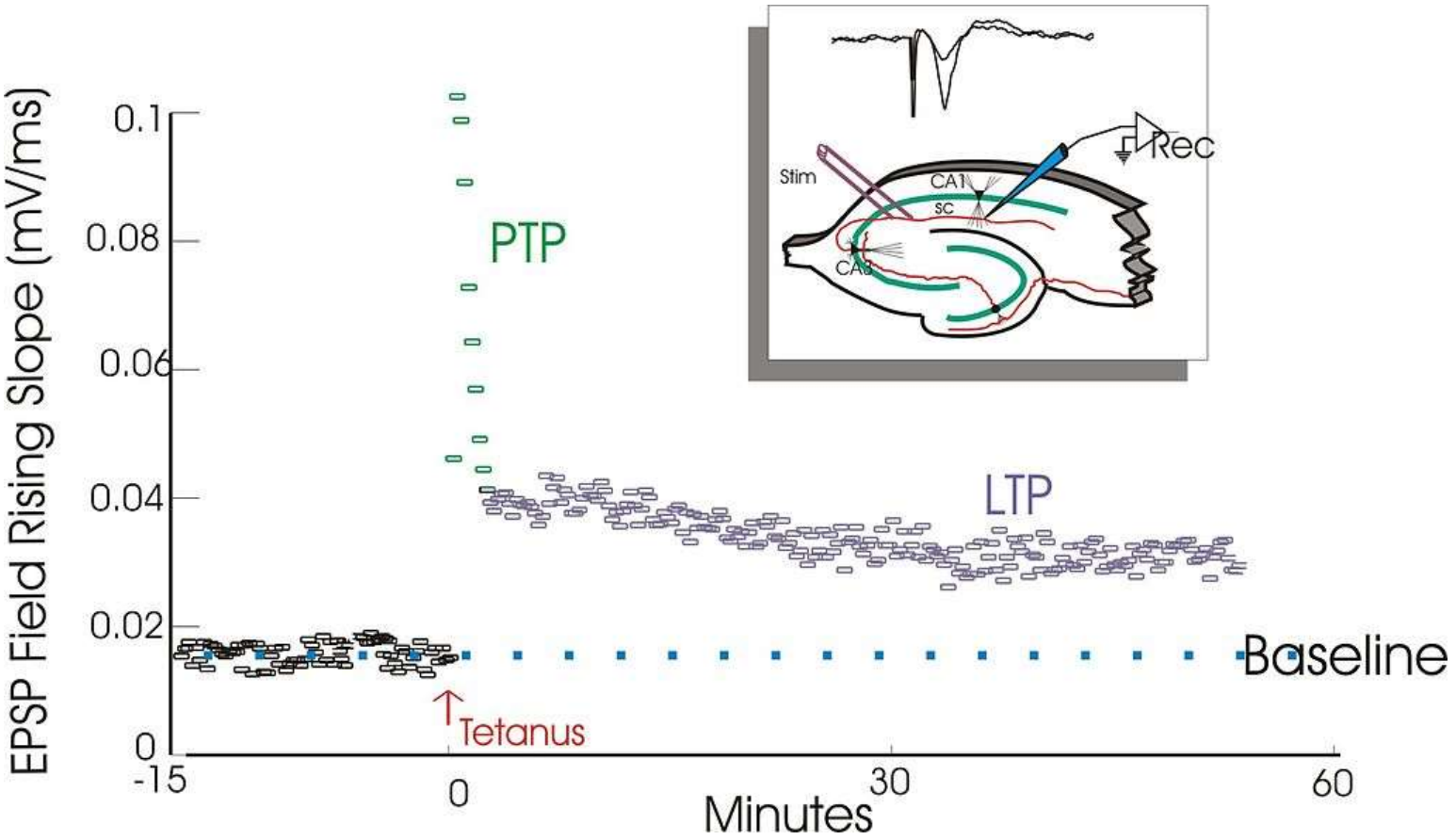


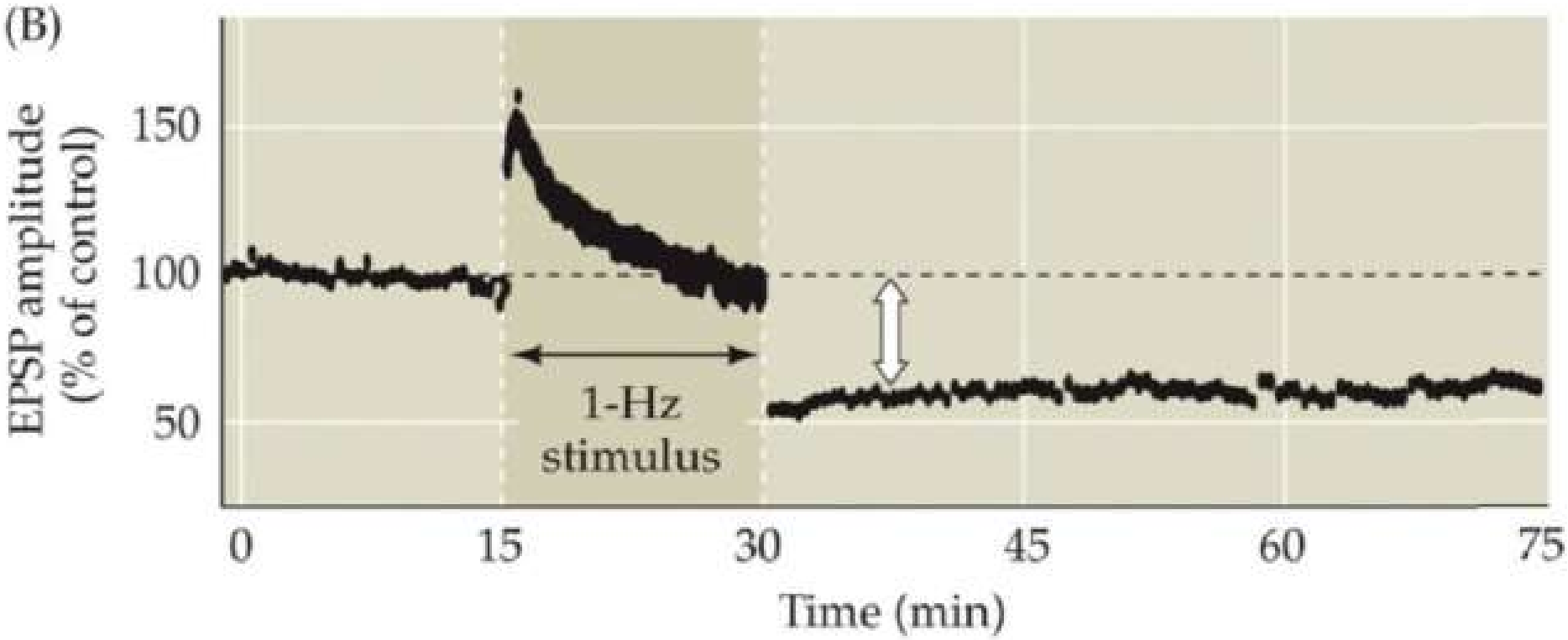
Fig. 3. *A*, the synaptic response of a CA3 cell in control conditions is superimposed on the response of the same cell in the presence of bicuculline (40 μM) and CNQX (2 μM). Note that in bicuculline and CNQX, the early component of the IPSP was blocked, revealing a large EPSP, while the late component of the IPSP was replaced by a paroxysmal inhibitory potential.

Long term potentiation



Long-term potentiation (LTP) is a persistent increase in synaptic strength following high-frequency (100 Hz, 1s) stimulation of a chemical synapse which is believed to underlie memory formation. LTP studies are commonly carried out in hippocampal brain slices.

Long term depression



Long-term depression (LTD) is a persistent activity-dependent *reduction* in synaptic efficacy following a long patterned stimulus. In opposing LTP, LTD is believed to selectively weaken specific synapses in order to facilitate synaptic strengthening caused by LTP. Both LTD and LTP are forms of synaptic plasticity.