Introduction to ion-channels & electrophysiology

Dr Robert Dickinson
Dept. of Anaesthetics, Pain Medicine & Intensive Care
Imperial College
Biophysics Group
Blackett Laboratory
South Kensington Campus

r.dickinson@imperial.ac.uk

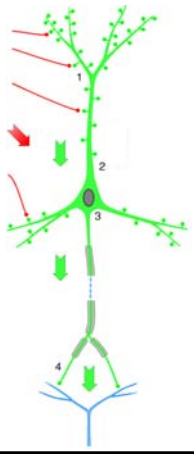
Lecture Outline

ion channels: voltage gated, ligand gated, "leak" channels

membrane potential: – Nernst equation

Hodgkin Huxley Experiment – action potentials

Electrophysiological techniques- Two electrode voltage clamp; Patch clamp

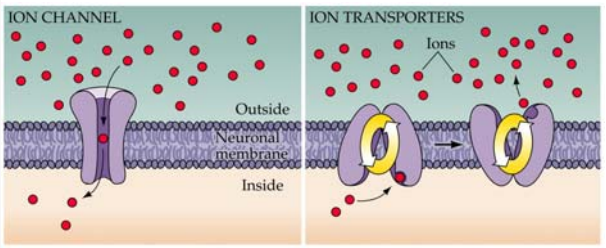


- neuron receives synaptic input
- if membrane potential reaches threshold, cell fires action potential
- action potential propagates along axon
- AP triggers release of neurotransmitter at synapse

Ion Channels

- Integral membrane proteins
- Contain aqueous pore which allows passive flow of ions across membrane
- Background or "leak" channels: always open
- Gated: open or shut

Difference between ion channels and transporters (pumps)



Passive (no energy required)

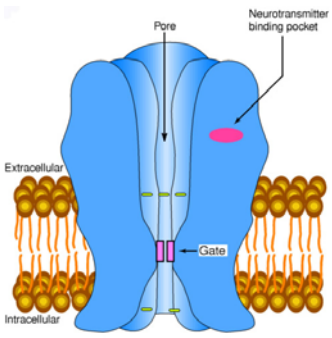
Ions flow down concn. gradient

Active (requires energy *e.g.* ATP)

Move ions against concn. gradient

Maintain concn. gradient

Schematic based on nACh receptor



Classification of ion channels

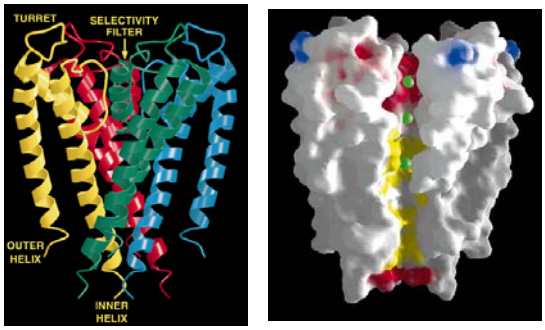
•voltage gated	•ligand gated	• "leak"
Na^+ channels	nACh	2P domain K^+ channels
K^+ channels	$GABA_A$	Na^+ channels
Ca^{2+} channels	glycine	Cl^- channels
	glutamate	

Ion channels are selective

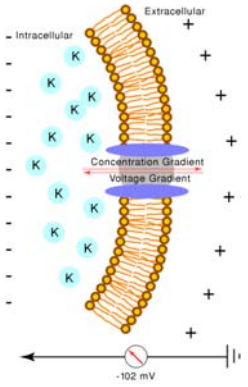
- Channels are selective for certain ions
- May allow only one ion to pass (*e.g.* Na^+, K^+)
- May select for cations or anions
- May select for divalent or monovalent cations

X-ray crystallographic structure of a K^+channel

Doyle *et al* 1998 Science



The Excitable Membrane at Rest

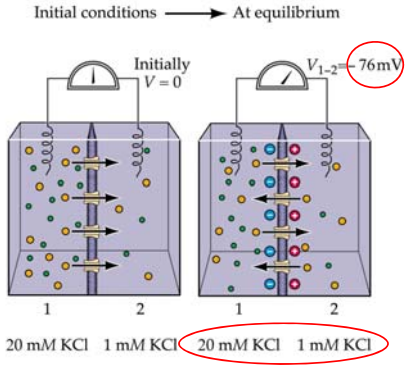


- Ionic selectivity underlies resting membrane potential, E_{mem}
- E_{mem} depends on concentration gradient of ions inside and outside cell
- E_{mem} depends on "leak" channels

All systems like to reach equilibrium

- ions want to move down their concentration gradient
- movement of charge sets up an electric field counteracting movement

Membrane permeable only to K^+ ions



Selectively permeable membrane

- at equilibrium electromotive force balances effect of concentration gradient
- the value of membrane potential depends on the size of the concentration gradient
- number of ions that flow across membrane to establish the membrane potential is small (~10^{-12} moles/cm^2) so doesn't change the concentration gradient

The Nernst equation relates equilibrium membrane potential to ion concentration

$$E_X = \frac{RT}{zF} \ln \frac{[X]_{external}}{[X]_{internal}}$$

at 20 °C

$$E_X \text{ (mV)} = \frac{25.26}{z} \ln \frac{[X]_{external}}{[X]_{internal}}$$

Ion concentration (mM)

Ion	squid giant axon External	squid giant axon Internal	mammalian cell External	mammalian cell Internal
K^+	20	400	4	155
Na^+	440	50	145	12
Cl^-	560	40	123	4

Nernst potentials for the squid axon at 20°C

$$E_K = 25.26 \ln \frac{[20]_{external}}{[400]_{internal}} \text{ (mV)}$$

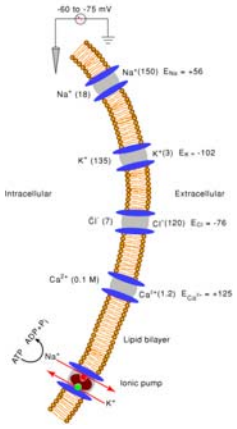
$$= -76 \text{ mV}$$

$$E_{Na} = +55 \text{ mV}$$

$$E_{Cl} = -66 \text{ mV}$$

Significance of Nernst potential?

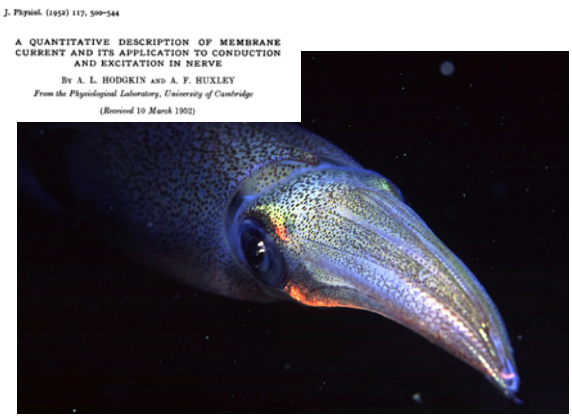
- If membrane channels are permeable to only one ion then E_{mem} = Nernst potential
- If E_{mem} is held at Nernst potential for a given ion there will be no net flux of ion through channels selective for that ion



- Real membranes have many ion selective channels
- Resting membrane potential depends on concn gradient and relative permeability of ions
- excitable cells have negative resting potentials because at rest membranes have more K^+ selective channels open (E_K=-76mV)

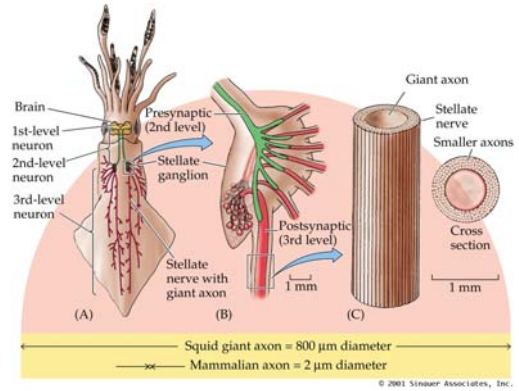
Action Potentials

- involve voltage-gated ion channels
- voltage gated Na^+ channels
- voltage gated K^+ channels
- ionic permeability of membrane depends on membrane potential (and time)

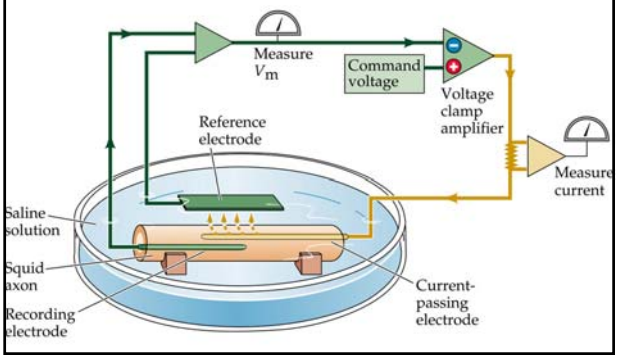


A squid of the species *Loligo*

Squid giant axon

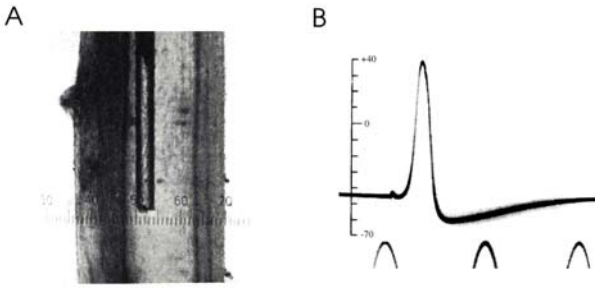


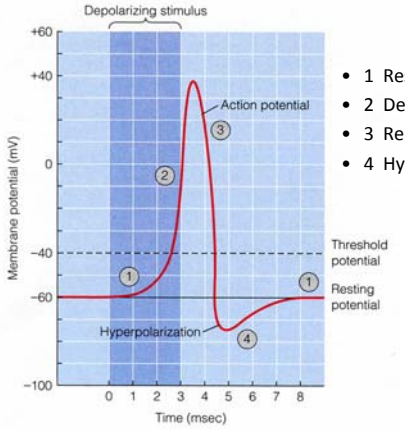
Hodgkin & Huxley's Experiment



Axon potential propagation in the squid giant axon

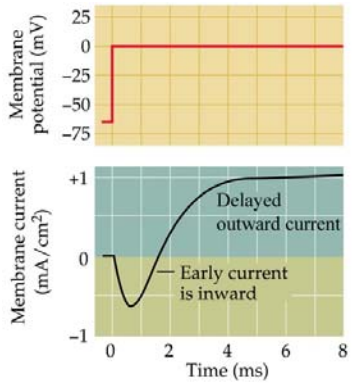
Hodgkin & Huxley *c*1950



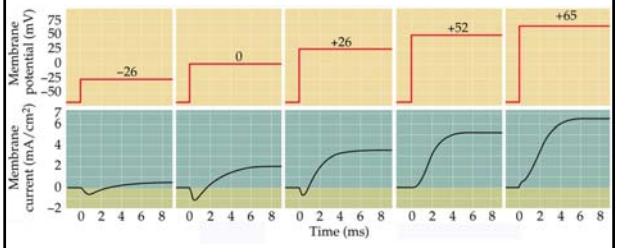


- 1 Resting state
- 2 Depolarizing phase
- 3 Repolarizing phase
- 4 Hyperpolarizing phase

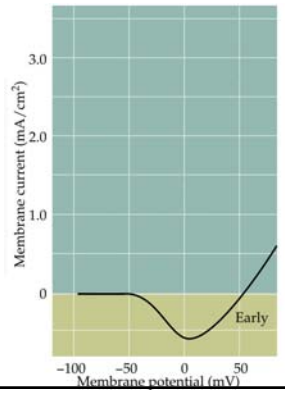
Hodgkin & Huxley voltage clamp experiment



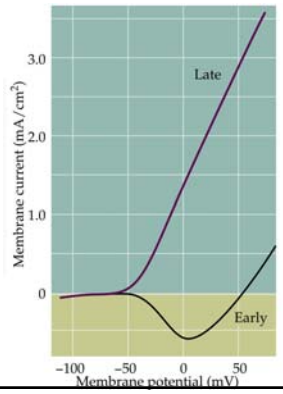
Hodgkin & Huxley voltage clamp experiment



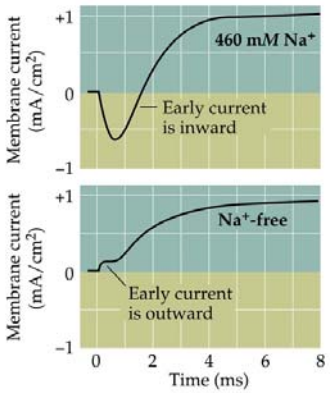
Hodgkin & Huxley voltage clamp experiment



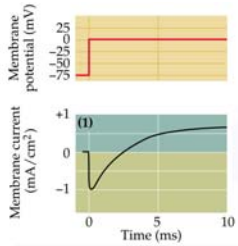
Hodgkin & Huxley voltage clamp experiment



Hodgkin & Huxley ionic substitution experiment



Pharmacological isolation of Na^+ and K^+ currents



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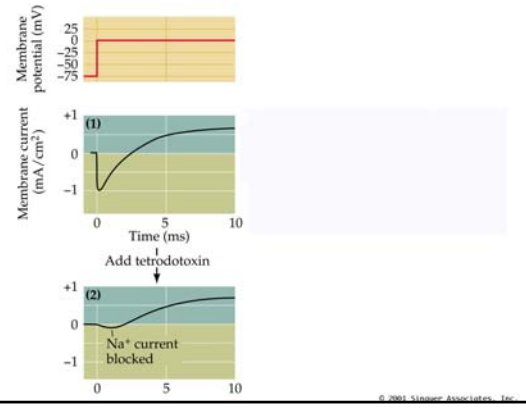

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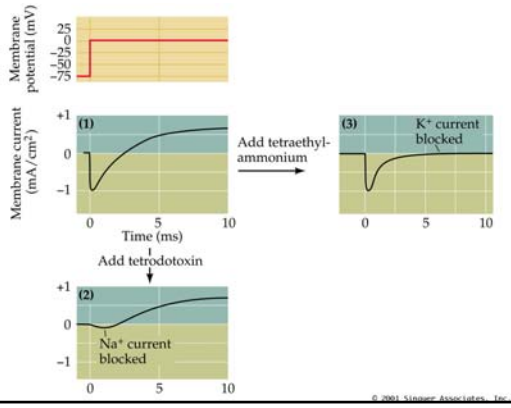
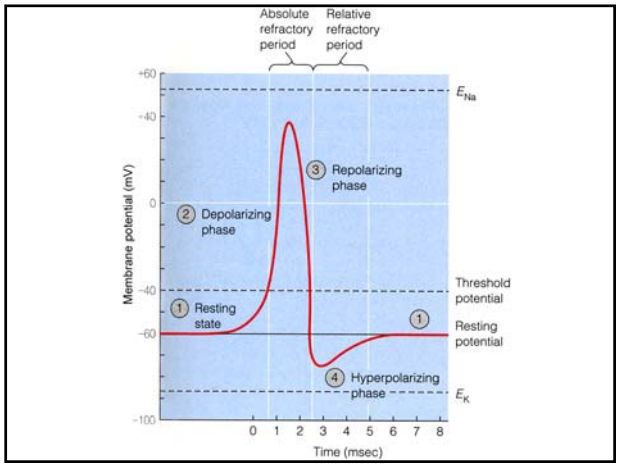
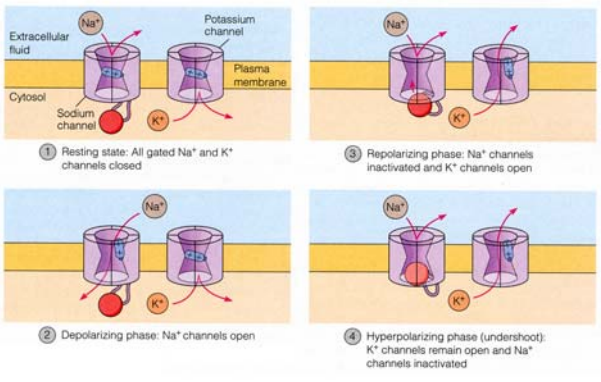


Voltage dependent Na^+ and K^+ channels underlie the action potential

- Rapid activation of Na^+ channels
- Inactivation of Na^+ channels
- Slower activation of K^+ channels

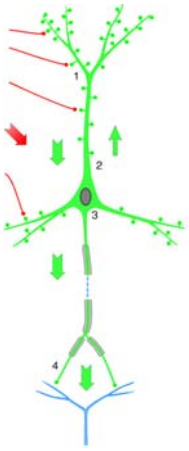


Events underlying the action potential

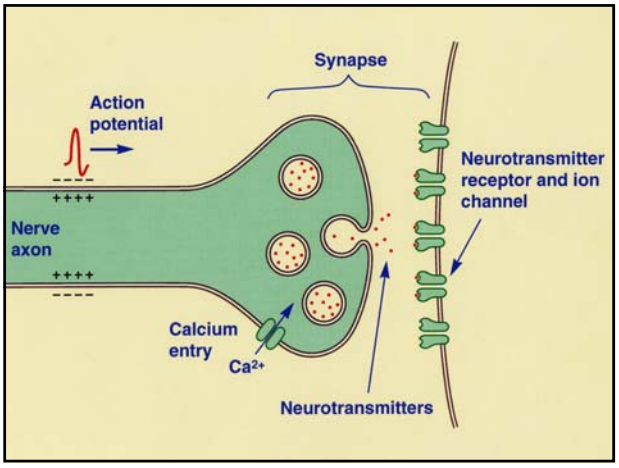


Key stages in Action Potential generation

- membrane is depolarised past threshold
- voltage-gated Na^+ channels open - Na^+ enters cell causing further depolarisation - further activates voltage-gated Na^+ channels –rapid depolarisation (postive feedback)
- Na^+ channels start to inactivate
- voltage-gated K^+ channels open - K^+ flows out of cell - membrane starts to repolarise (negative feedback)
- K^+ channels remain open (Na^+ channels closed) hyperpolarising phase
- K^+ channels close slowly in response to repolarisation of membrane - return to resting potential



- neuron receives synaptic input
- if membrane potential reaches threshold, cell fires action potential
- action potential propagates along axon
- AP triggers release of neurotransmitter at synapse



Ways in which general anaesthetics could interfere with synaptic transmission

- Block action potential propagation?
  - voltage gated Na^+ and K^+ channels insensitive to g.a.
- Affect release of neurotransmitter?
  - voltage gated Ca^{2+} channels insensitive to g.a.
- Reducing excitability of cell such that no action potential is generated?
  - affect "leak" channel to change resting membrane potential
- Modulating function of postsynaptic receptors?
  - potentiate or inhibit response of receptors to neurotransmitter

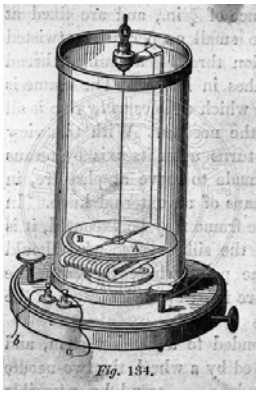
Electrophysiological Techniques

Why be interested in electrophysiology?

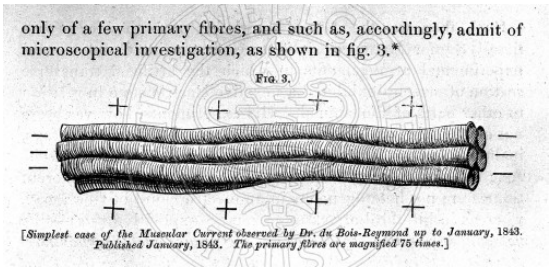
- widely used technique in neuroscience research
- understand molecular mechanisms of drug action
- lead to novel treatments or new drugs
- e.g. xenon

First demonstration of bioelectricity c.1780

Emil Heinrich Dubois-Reymond (1818-1896)



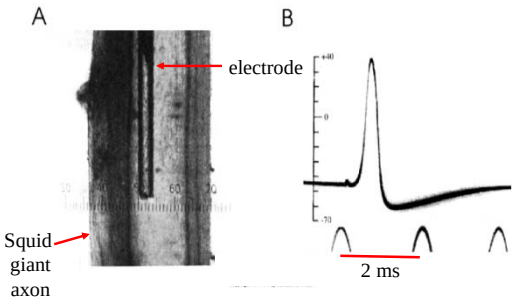
"Action Current" measured by Dubois Reymond in muscle fibres



Limitations of 19th Century Measurement Techniques

- signals are very small (microvolts)
  - no amplifiers or electronics!
- signals are very fast (milliseconds)
  - galvanometers have slow response
  - can't accurately resolve timecourse

Axon potential propagation in the squid giant axon (Hodgkin & Huxley, c 1950)



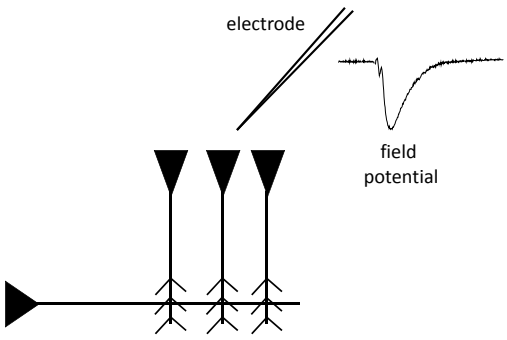
Electrophysiological techniques used today

- Extracellular recording
- Intracellular recording
  - Two Electrode Voltage Clamp
  - Patch Clamp

Extracellular recording

- simplest technique: single electrode and amplifier
- record extracellular potentials arising from a single cell or from a population of cells
- can be used *in vivo* and with *in vitro* brain slice preparations

A schematic view of extracellular recording.



Extracellular Recording Electrodes

- insulated metal (platinum, tungsten)
- insulated carbon fibre
- glass microelectrode filled with conducting solution

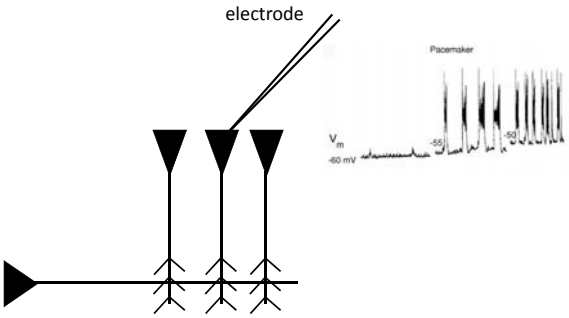
Limitations of extracellular recording

- extracellular potentials are small (~10 – 100 μV)
- difficult to separate activity of single cell from its neighbours
- can't control the membrane potential of the cell being studied

Intracellular Recording

- Two Electrode Voltage Clamp
- Patch Clamp

A schematic view of intracellular recording

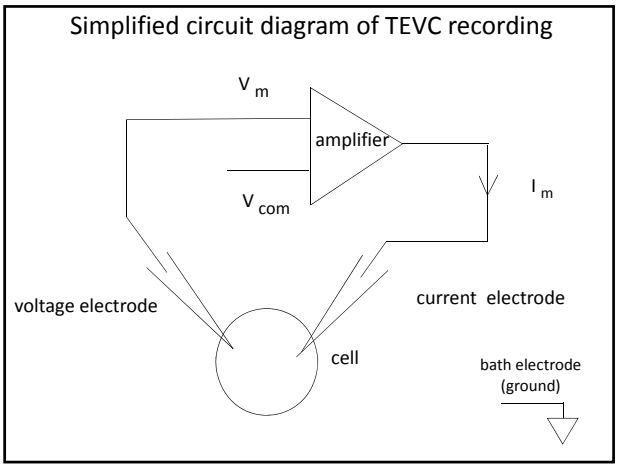


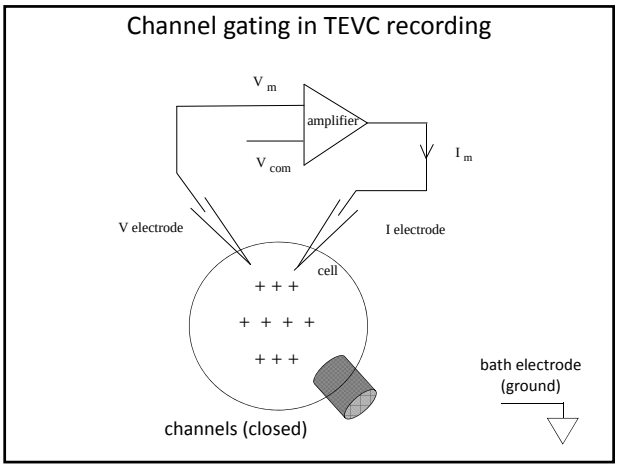
Why control membrane potential?

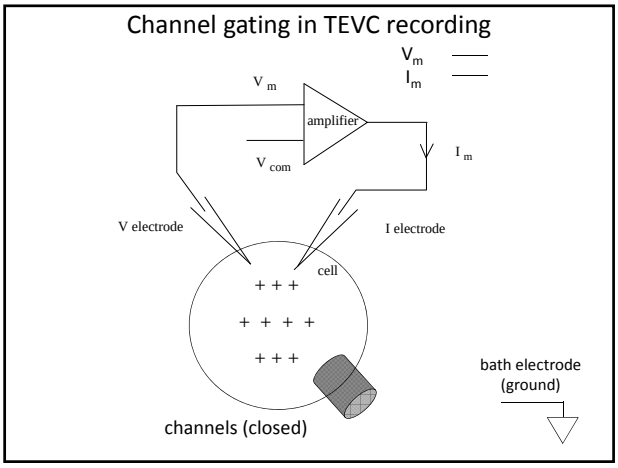
- activity of cell may depend on V_m
- allows separation of capacitive and ionic (channel) current
- gating of channels often influenced by V_m
  - easier to study channel properties

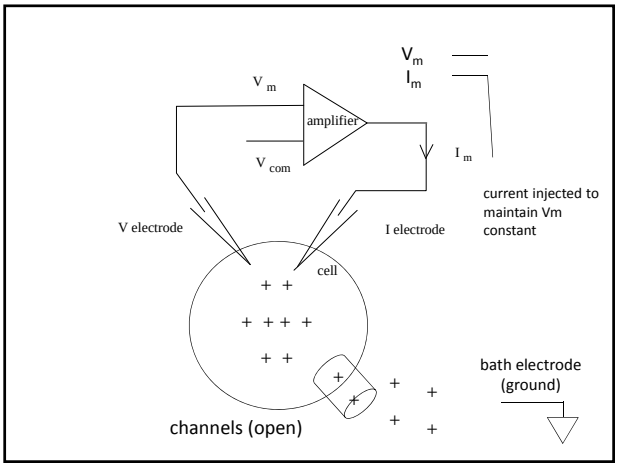
Two electrode voltage clamp

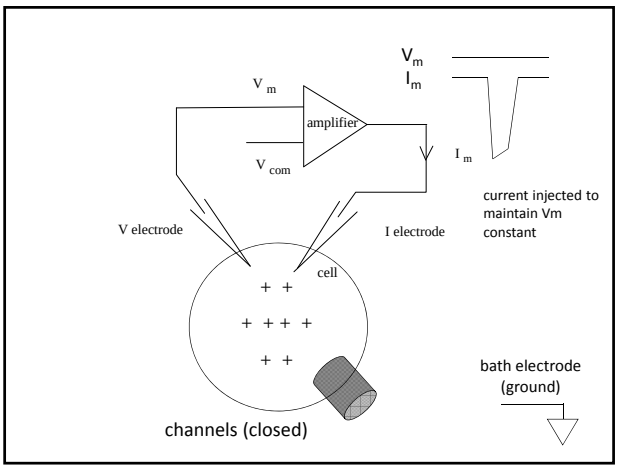
- developed by Cole (1949) and Hodgkin & Huxley (1952)
- one electrode measures the membrane potential (V_m)
- another electrode injects current into cell
- a feedback amplifier determines the amount of current to inject to maintain V_m at a set value


Simplified circuit diagram of TEVC recording
V m
amplifier
V com
I m
voltage electrode
current electrode
cell
bath electrode
(ground)


Channel gating in TEVC recording
V m
amplifier
V com
I m
V electrode
I electrode
cell
+ + +
+ + + +
+ + +
bath electrode
(ground)
channels (closed)


Channel gating in TEVC recording
Vm
Im
V m
amplifier
V com
I m
V electrode
I electrode
cell
+ + +
+ + + +
+ + +
bath electrode
(ground)
channels (closed)


Vm
Im
V m
amplifier
V com
I m
current injected to
maintain Vm
constant
V electrode
I electrode
cell
+ +
+ + + +
+ +
bath electrode
(ground)
channels (open)


Vm
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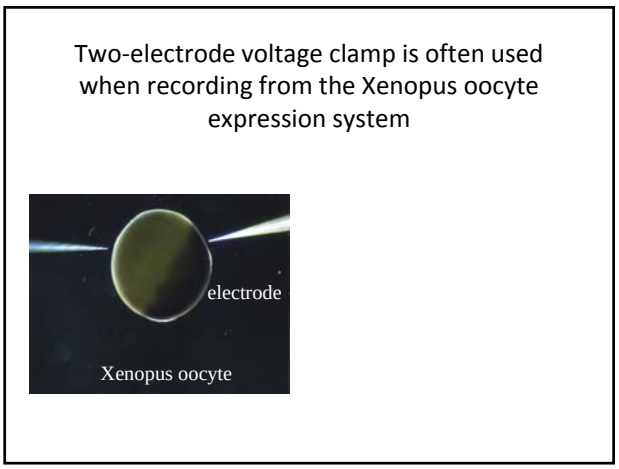
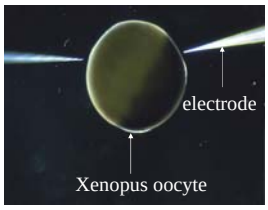
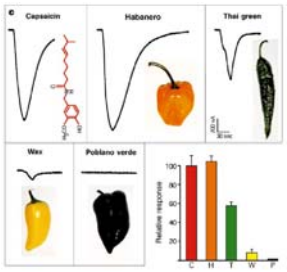
+ +
+ + + +
+ +
bath electrode
(ground)
channels (closed)


Two-electrode voltage clamp is often used
when recording from the Xenopus oocyte
expression system
electrode
Xenopus oocyte

The capsaicin receptor: a heat-activated ion channel in the pain pathway

Michael J. Caterina*, Mark A. Schumacher†‖, Makoto Tominaga*‖, Tobias A. Rosen*, Jon D. Levine‡ & David Julius*

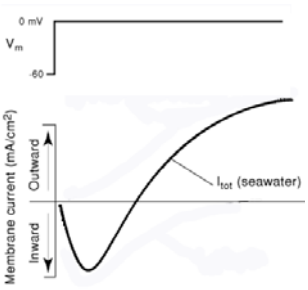


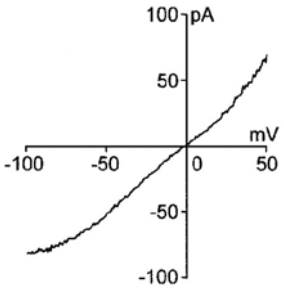


Voltage clamp protocols

- V_m constant – ligand gated channels
- voltage step – voltage gated channels
- voltage ramp – identify channel from reversal potential and shape of IV plot

Examples of voltage clamp protocols





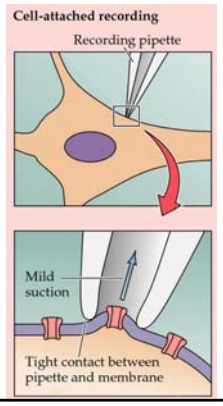
Patch clamp technique

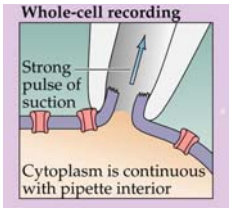
- Smaller cells cannot accept two microelectrodes without killing cell, in this case we need another technique:

Single electrode voltage-clamp or Patch Clamp

- Sakmann and Neher received the 1991 Nobel Prize for Physiology or Medicine for developing the technique.
- Relies on very high resistance seal between cell membrane and electrode
- Used with mammalian neurons, HEK cells, in vitro brain slices, even intracellular recordings *in vivo*.

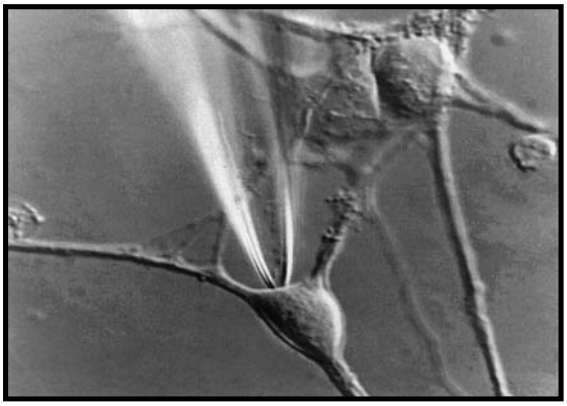
Schematic of Patch Clamp recording

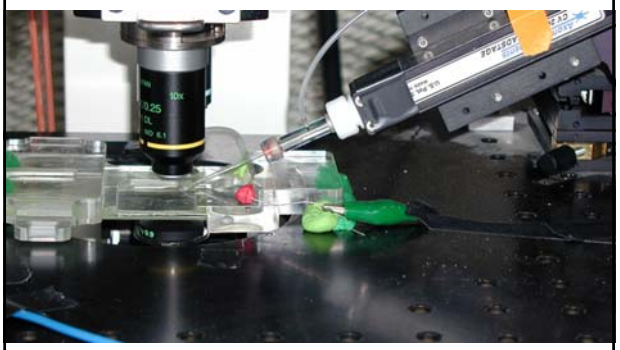




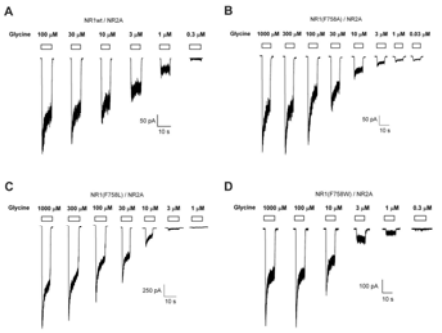
control V_m
record current from whole cell

Cultured neuron with patch clamp electrode





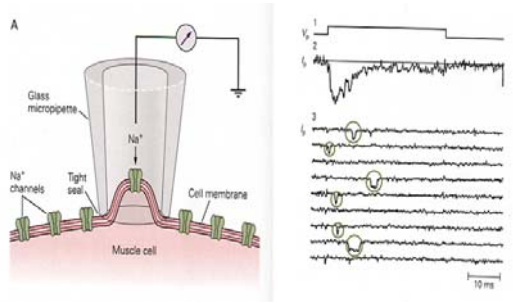
Typical whole cell currents from HEK cells expressing NMDA receptors



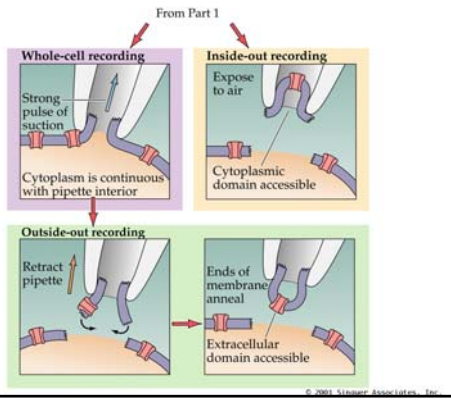
Single channel recording using patch clamp technique

- Sakmann & Nehr originally interested in measuring the activity of single ion-channels
- single channel current ~pA so need ultra low noise recording
  - hence need high resistance "seal" between pipette and cell membrane.
- seal resistance ~giga-ohms (10^6 ohms)
  - so called "giga-seal"

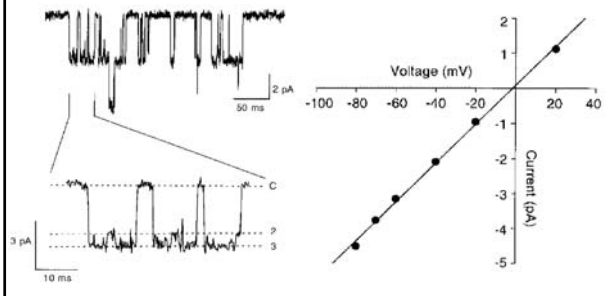
Cell-attached patch recording



Excision of membrane patch allows greater control



Single channel properties allow identification of channel and permeant ion



Misra *et al* (2000) J. Physiol. 524, p147

Further reading

Ion Channels

Principals of Neural Science (2000).
Kandel, E., Schwartz, J. & Jessel, T. Chapters 7,8,10

Ion channels of excitable membranes (1992).
Hille, B. Chapters 1 & 2

Electrophysiology

Microelectrode techniques: The Plymouth workshop handbook. 2nd Ed. Ogden, D (editor)

Single channel recording, Sackmann, B. & Neher, E

The Axon Guide for Electrophysiology and Biophysics
online: http://stg.rutgers.edu/stg_lab/protocols/The%20axon%20Guide.pdf

Exercise

- After visiting the research labs, can you identify the equipment in the following pictures?