

## Introduction to ion-channels & electrophysiology

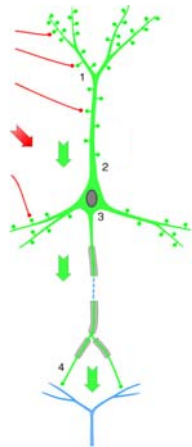
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## Lecture Outline

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

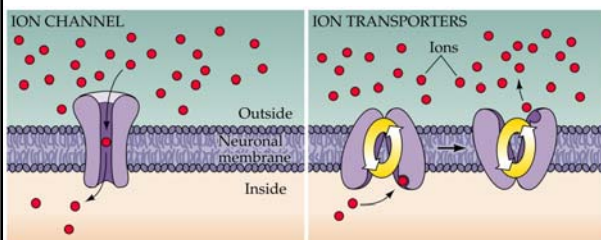


- neuron receives synaptic input
- if membrane potential reaches threshold, cell fires action potential
- action potential propagates along axon
- AP triggers release of neurotransmitter at synapse

## 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

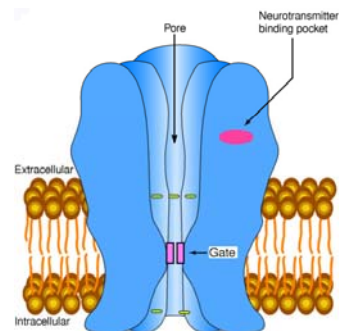
## Difference between ion channels and transporters (pumps)



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

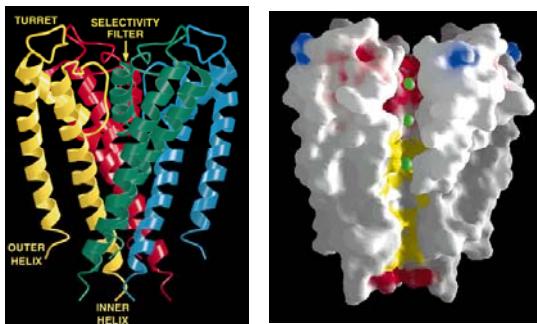
- voltage gated
  - ligand gated
  - "leak"
- |                           |                   |                                   |
|---------------------------|-------------------|-----------------------------------|
| Na <sup>+</sup> channels  | nACh              | 2P domain K <sup>+</sup> channels |
| K <sup>+</sup> channels   | GABA <sub>A</sub> | Na <sup>+</sup> channels          |
| Ca <sup>2+</sup> channels | glycine           | Cl <sup>-</sup> channels          |
|                           | glutamate         |                                   |

### Ion channels are selective

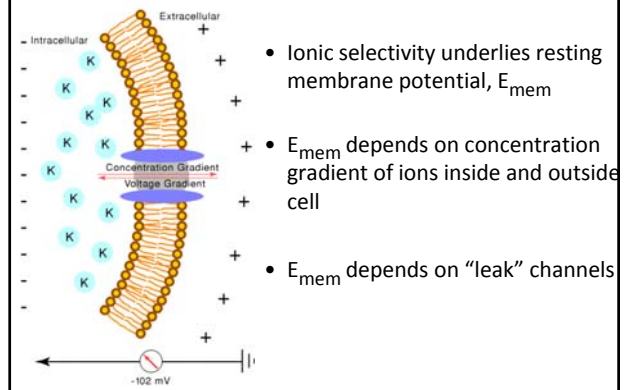
- Channels are selective for certain ions
- May allow only one ion to pass (e.g. Na<sup>+</sup>, K<sup>+</sup>)
- May select for cations or anions
- May select for divalent or monovalent cations

### X-ray crystallographic structure of a K<sup>+</sup> channel

Doyle *et al* 1998 Science



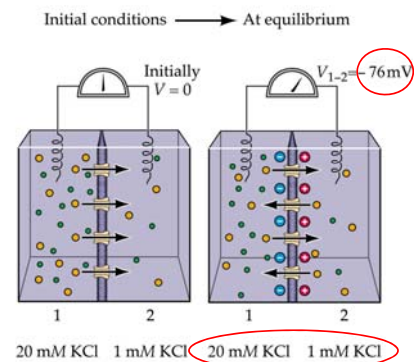
### The Excitable Membrane at Rest



### 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<sup>+</sup> 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 ( $\sim 10^{-12}$  moles/cm<sup>2</sup>) so doesn't change the concentration gradient

The Nernst equation relates equilibrium membrane potential to ion concentration

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

at 20 °C

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

### Ion concentration (mM)

squid giant axon      mammalian cell

Ion	External	Internal	External	Internal
K <sup>+</sup>	20	400	4	155
Na <sup>+</sup>	440	50	145	12
Cl <sup>-</sup>	560	40	123	4

### Nernst potentials for the squid axon at 20°C

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

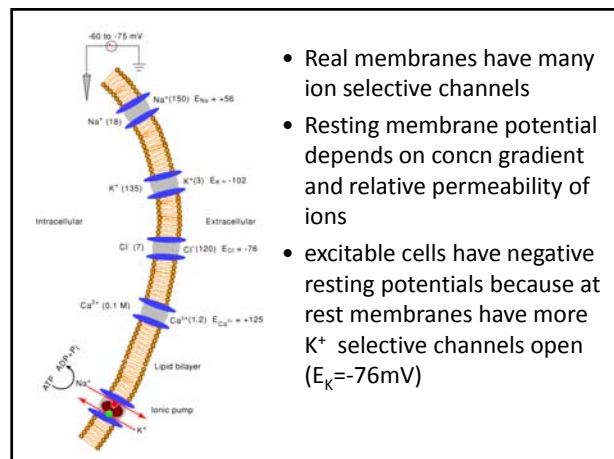
$$= -76 \text{ mV}$$

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

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

### Significance of Nernst potential?

- If membrane channels are permeable to only one ion then  $E_{\text{mem}} = E_{\text{Nernst}}$
- If  $E_{\text{mem}}$  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<sup>+</sup> selective channels open ( $E_K = -76\text{mV}$ )

## Action Potentials

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

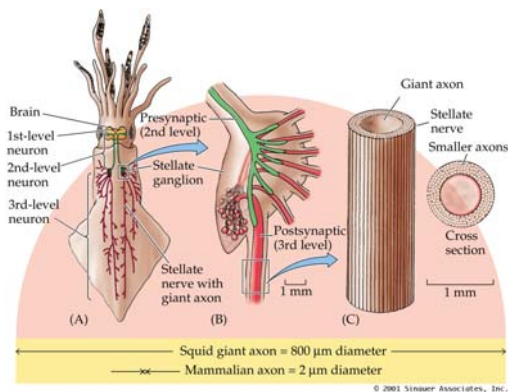
J. Physiol. (1952) 117, 500-544

A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE  
By A. L. HODGKIN AND A. F. HUXLEY  
From the Physiological Laboratory, University of Cambridge  
(Received 10 March 1952)

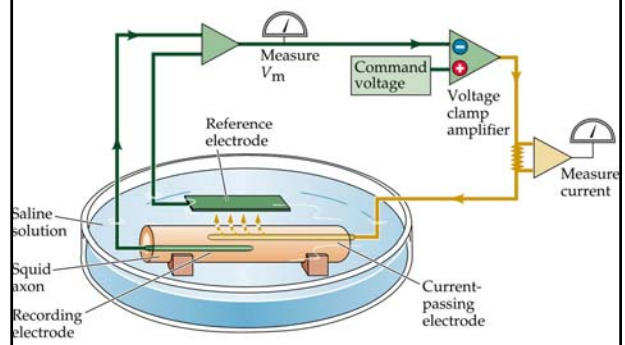


A squid of the species *Loligo*

## Squid giant axon

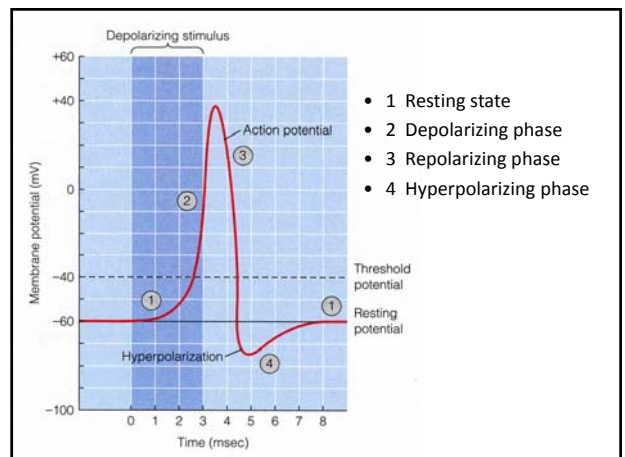
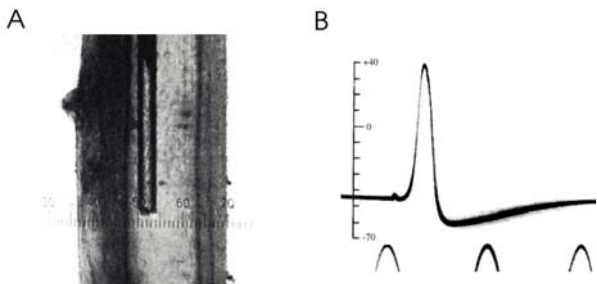


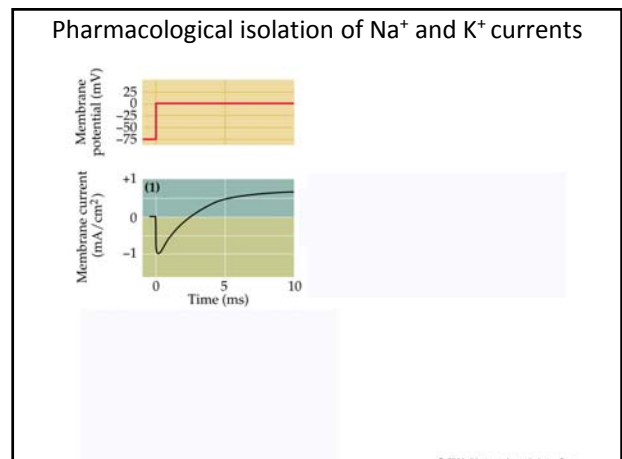
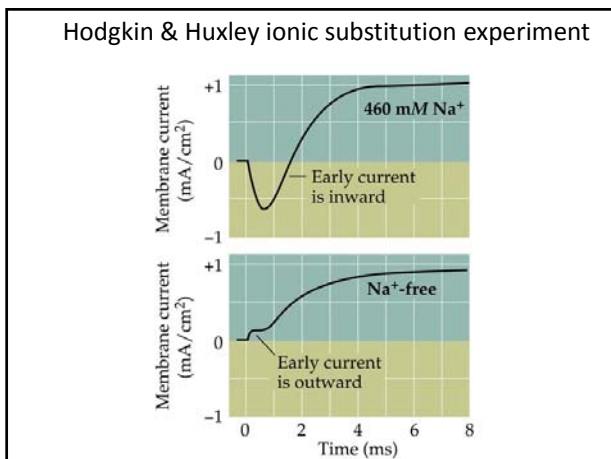
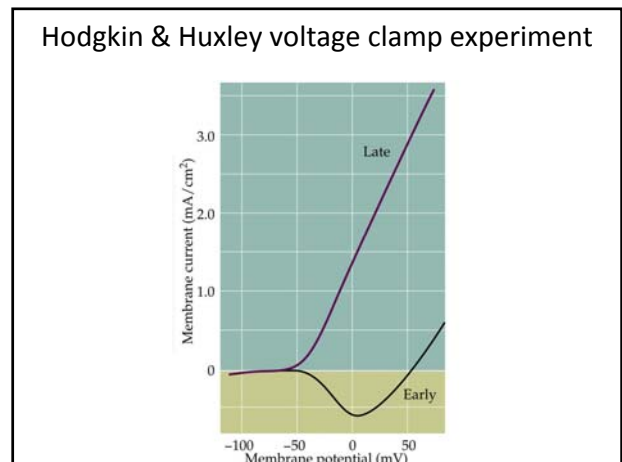
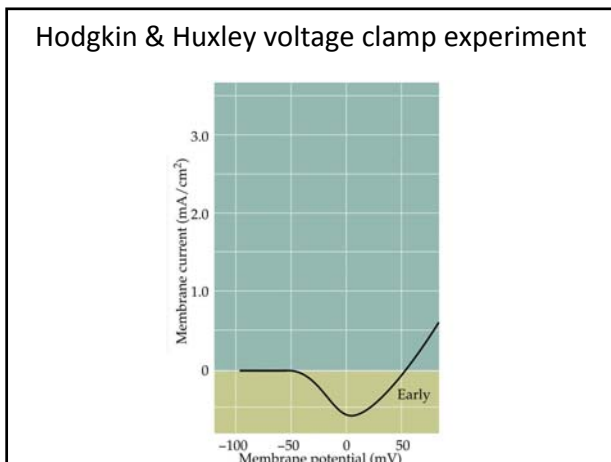
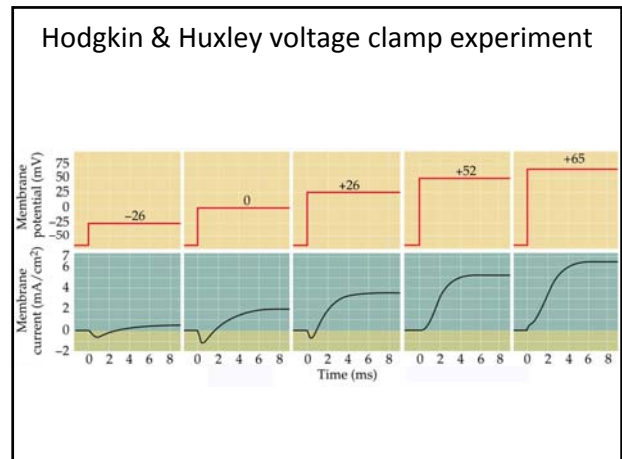
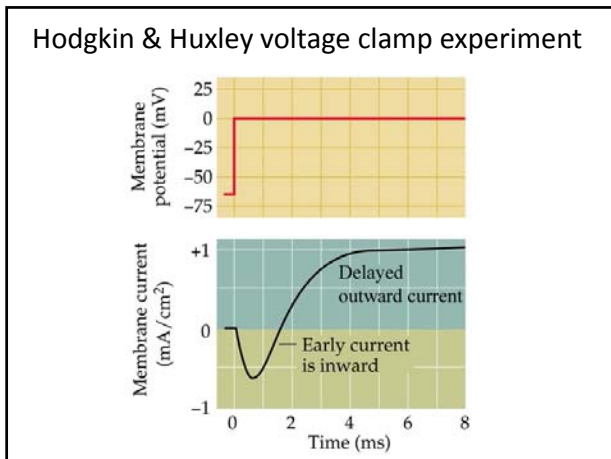
## Hodgkin & Huxley's Experiment

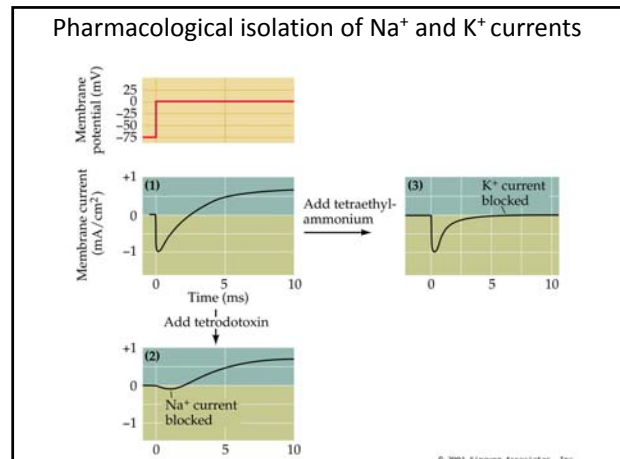
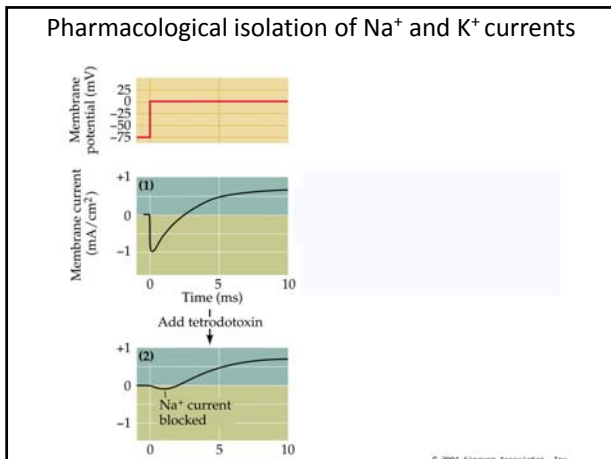


## Axon potential propagation in the squid giant axon

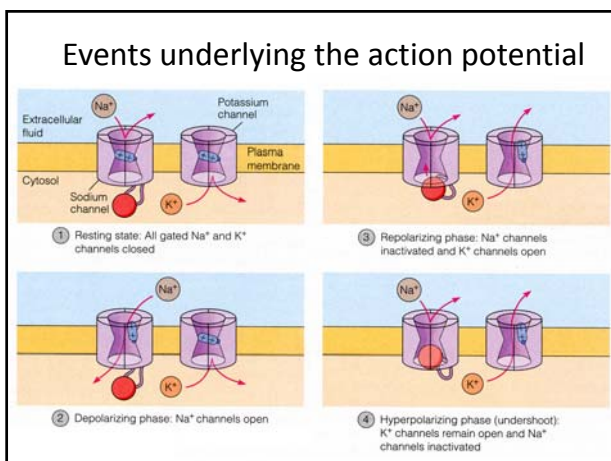
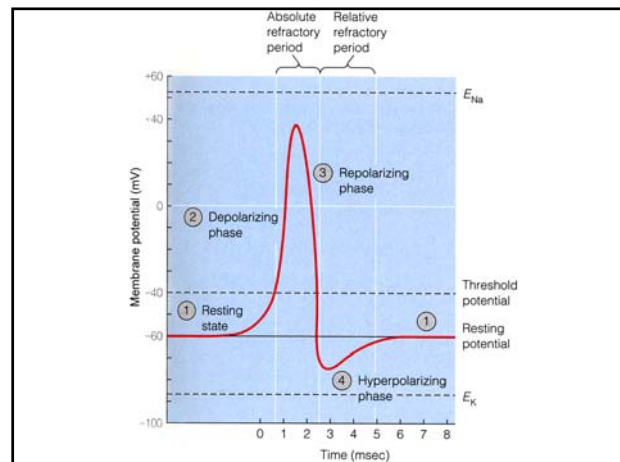
Hodgkin & Huxley c1950



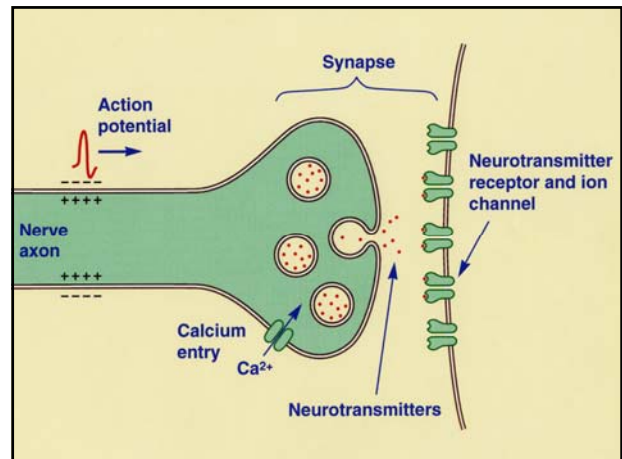
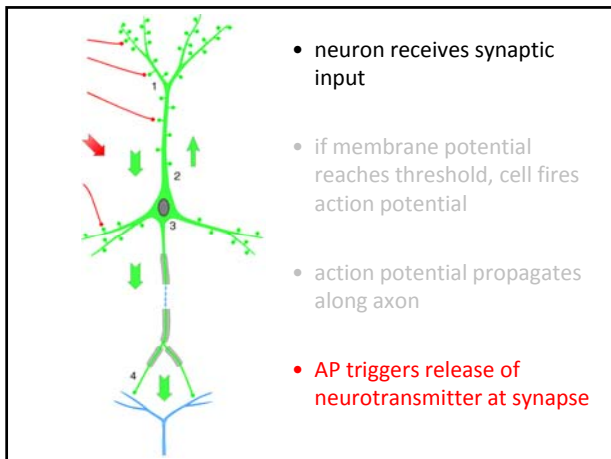




- ### Voltage dependent Na<sup>+</sup> and K<sup>+</sup> channels underlie the action potential
- Rapid activation of Na<sup>+</sup> channels
  - Inactivation of Na<sup>+</sup> channels
  - Slower activation of K<sup>+</sup> channels



- ### Key stages in Action Potential generation
- membrane is depolarised past threshold
  - voltage-gated Na<sup>+</sup> channels open - Na<sup>+</sup> enters cell causing further depolarisation - further activates voltage-gated Na<sup>+</sup> channels –rapid depolarisation (positive feedback)
  - Na<sup>+</sup> channels start to inactivate
  - voltage-gated K<sup>+</sup> channels open - K<sup>+</sup> flows out of cell - membrane starts to repolarise (negative feedback)
  - K<sup>+</sup> channels remain open (Na<sup>+</sup> channels closed) hyperpolarising phase
  - K<sup>+</sup> channels close slowly in response to repolarisation of membrane - return to resting potential



### Ways in which general anaesthetics could interfere with synaptic transmission

- Block action potential propagation?
  - voltage gated Na<sup>+</sup> and K<sup>+</sup> channels insensitive to g.a.
- Affect release of neurotransmitter?
  - voltage gated Ca<sup>2+</sup> 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

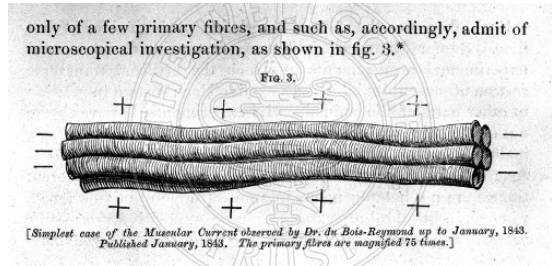
### First demonstration of bioelectricity c.1780



### Emil Heinrich Dubois-Reymond (1818-1896)



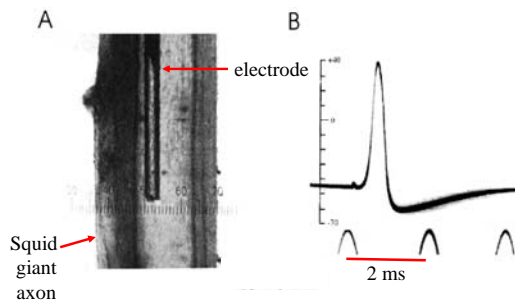
“Action Current” measured by Dubois Reymond in muscle fibres



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)



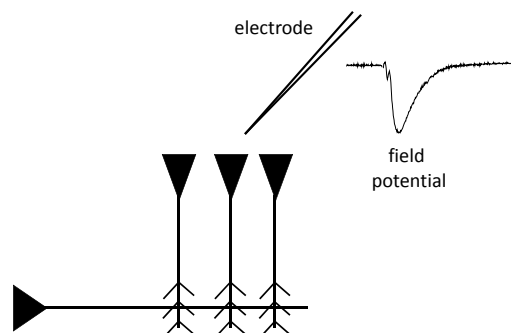
Electrophysiological techniques used today

- 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

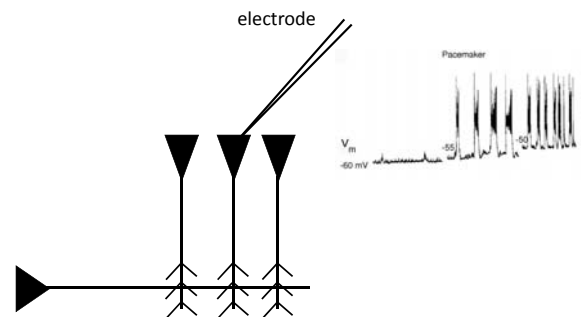
### Limitations of extracellular recording

- extracellular potentials are small ( $\sim 10 - 100 \mu\text{V}$ )
- 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

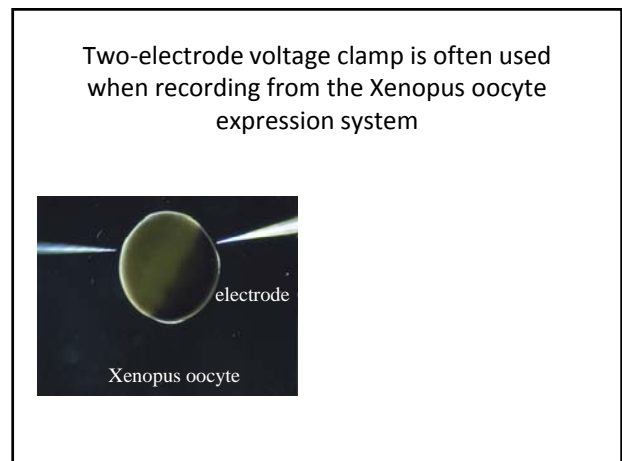
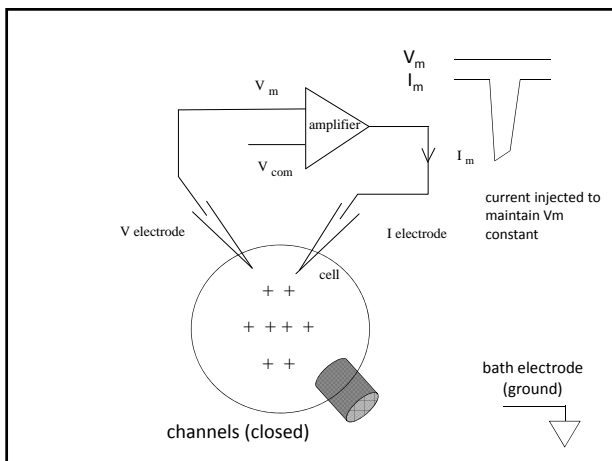
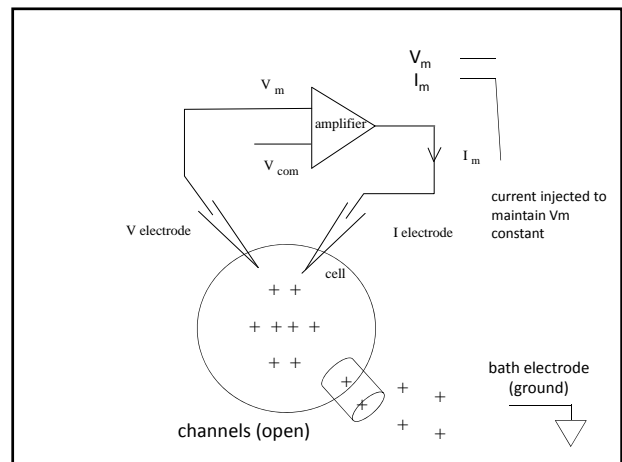
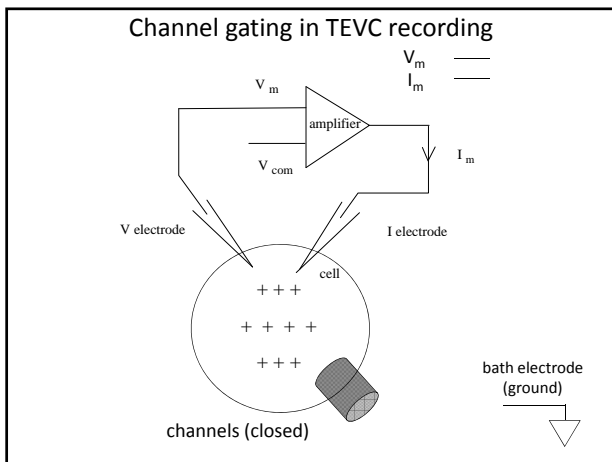
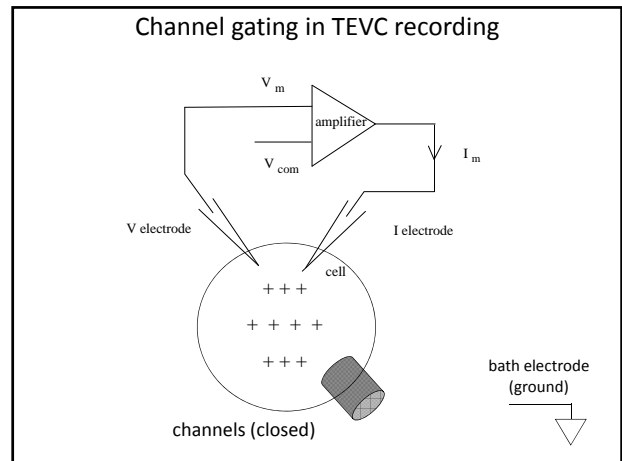
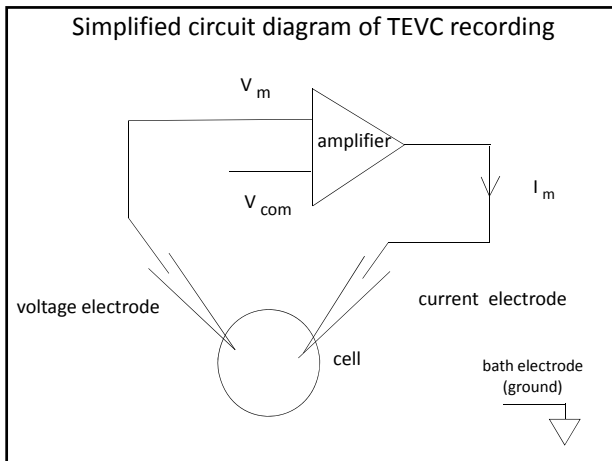


### Why control membrane potential?

- 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



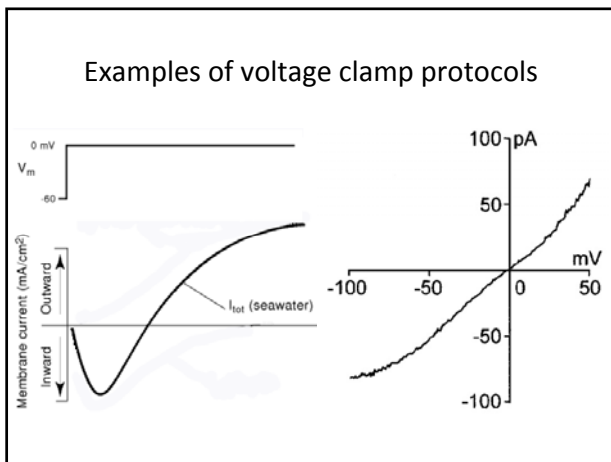
### The capsaicin receptor: a heat-activated ion channel in the pain pathway

Michael J. Caterina\*, Mark A. Schumacher<sup>†</sup>, Makoto Tominaga<sup>‡</sup>, Tobias A. Rosen<sup>§</sup>, Jon D. Levine<sup>¶</sup> & David Julius\*

The figure illustrates the study of the capsaicin receptor. On the left, a Xenopus oocyte is shown with an electrode inserted. On the right, several capsaicin receptor variants are depicted: Capsaicin, Habanero, Thai green, Wax, and Pubesno verde. Each variant is associated with a characteristic inward current trace (IV plot) and a corresponding image of the plant source. A bar graph at the bottom right shows the relative response of these variants, with Capsaicin (C) having the highest response (100%), followed by Habanero (H), Thai green (T), Wax (W), and Pubesno verde (P).

### 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

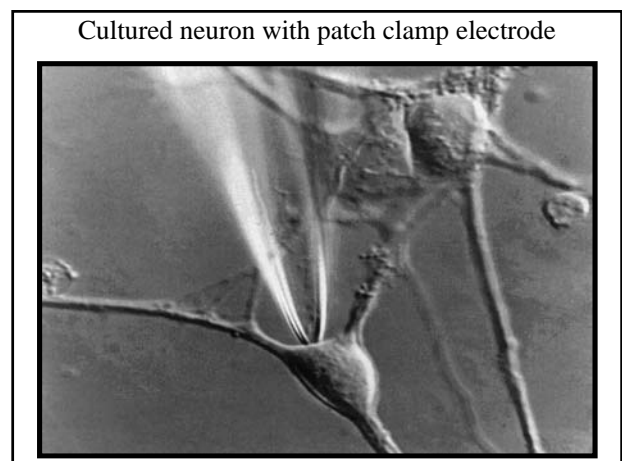
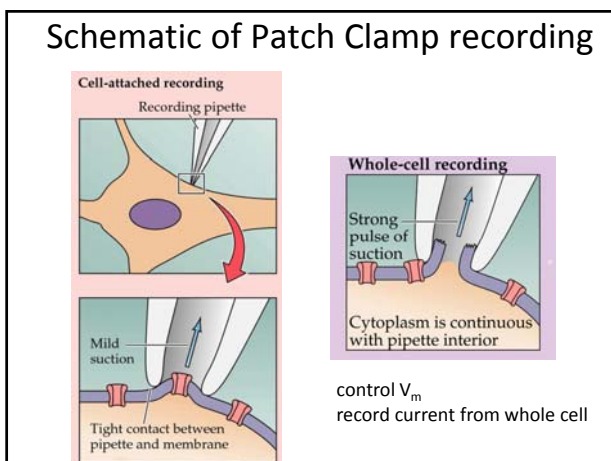


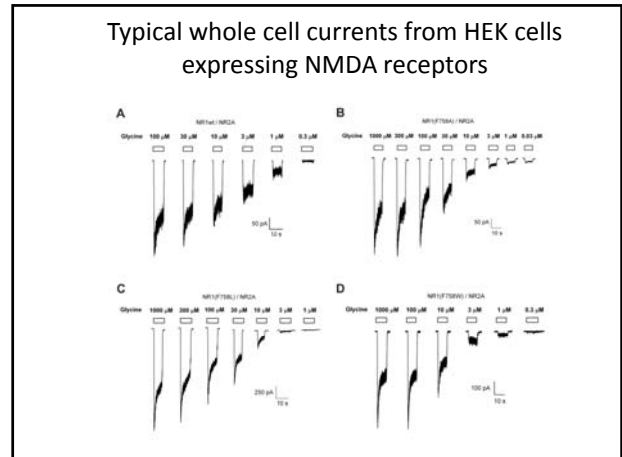
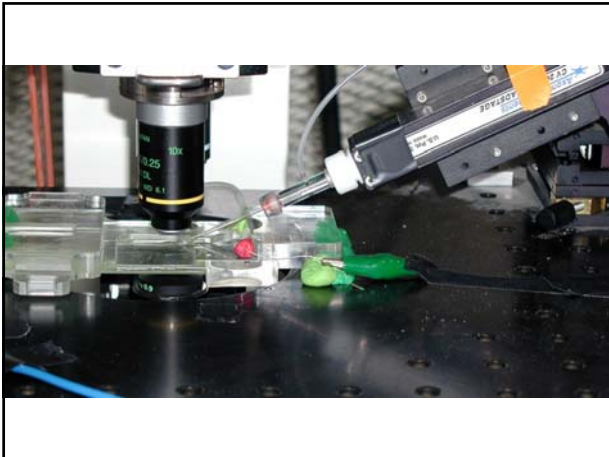
### Patch clamp technique

- 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*.

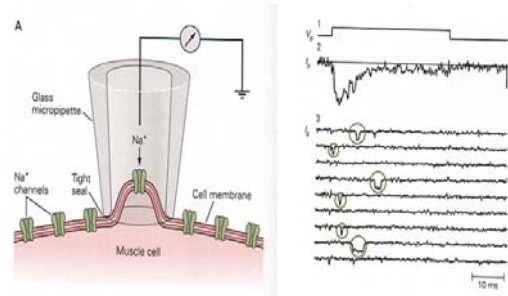




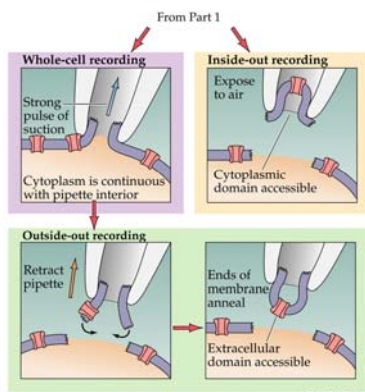
### Single channel recording using patch clamp technique

- Sakmann & Neher originally interested in measuring the activity of single ion-channels
- single channel current  $\sim$ pA so need ultra low noise recording
  - hence need high resistance “seal” between pipette and cell membrane.
- seal resistance  $\sim$ giga-ohms ( $10^9$  ohms)
  - so called “giga-seal”

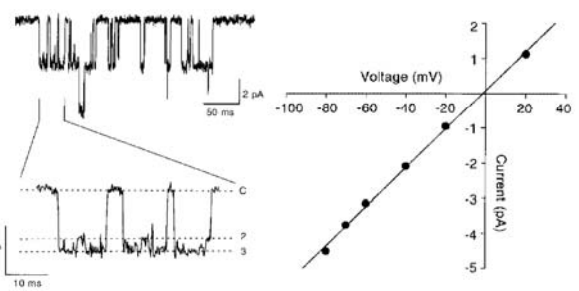
### Cell-attached patch recording



### 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. 2<sup>nd</sup> 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](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?

