ZOO 332H1S
Lecture 5,6
Jan. 2003 (AJE)

Channels, resting and action potentials: ***Experimental evidence***

Hodgkin (1949) and Huxley (1974)

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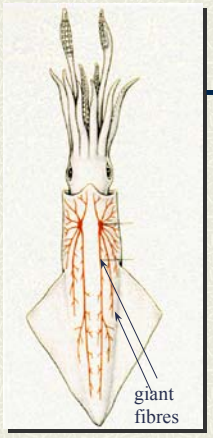
Major advances occur because:

- Discover a good preparation
- Find or develop appropriate technology
- Ask the right questions
- Work hard and think clearly

2



Loligo, the squid

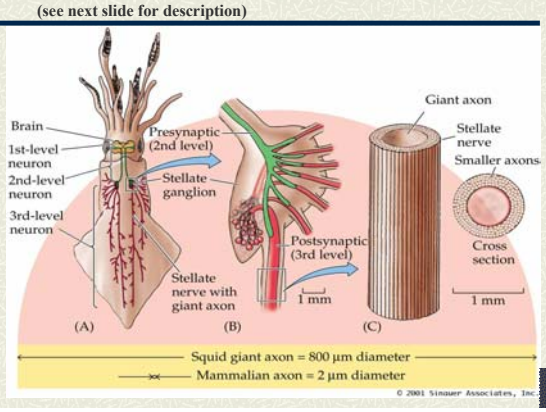
"It is arguable that the introduction of the squid giant nerve fibre by J.Z. Young in 1936 did more for axonology than any other single advance during the last forty years. Indeed a distinguished neurophysiologist remarked recently at a congress dinner (not, I thought, with the utmost tact), 'It's the squid that really ought to be given the Nobel Prize.' "

A.L. Hodgkin, 1973

3

Giant Nerve Cells of the Squid

(see next slide for description)



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Cont...Giant Nerve Cells of the Squid

After Purves et al (2001):

Diagram of squid, showing location of its giant nerve cells. Different colours indicate the neuronal components of the escape circuitry. The first- and second-level neurons originate in the brain, while the third-level neurons are in the stellate ganglion and innervate muscle cells of the mantle. (B) Giant synapses within the stellate ganglion. The second-level neuron forms a series of fingerlike processes, each of which makes an extraordinarily large synapse with a single third-level neuron. (C) Structure of a giant axon of a third-level neuron lying within its nerve. The difference in the diameters of a squid giant axon and a mammalian axon are shown below. However, note that some mammalian motor neurons are as large as 20 µm in diameter.

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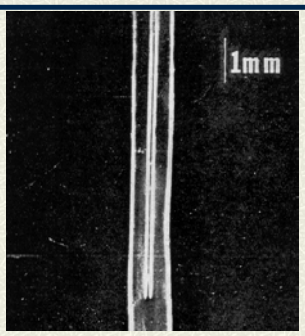
Using "microelectrodes"



Hodgkin, Huxley, and others pioneered recording intra-cellularly.
They used glass capillaries inserted longitudinally into the squid giant axon.

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Baker, Hodgkin, and Shaw (1962)(revisited)

- What's essential for an AP?
  - Squid giant axon
  - Cannulate and extrude axoplasm
  - Refill with appropriate salt solution
  - Stimulate

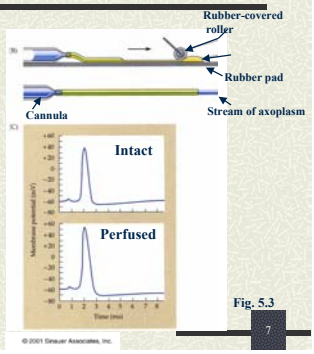


Fig. 5.3

Effect of reducing $[Na^+]_{out}$ on AP

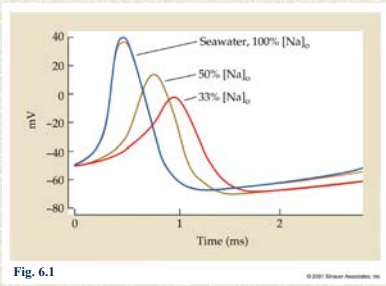


Fig. 6.1

Hodgkin & Katz, 1949

Effects of increasing conductance's (g_{Na+} and g_{K+})

(A) Positive feedback of Na^+ entry on depolarization

(B) K^+ efflux leads to repolarization

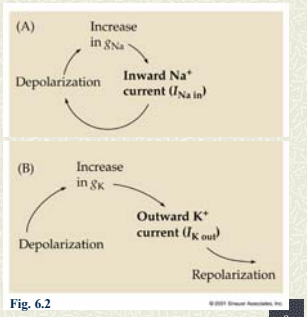
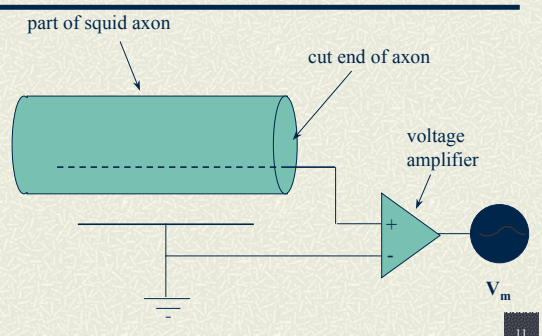


Fig. 6.2

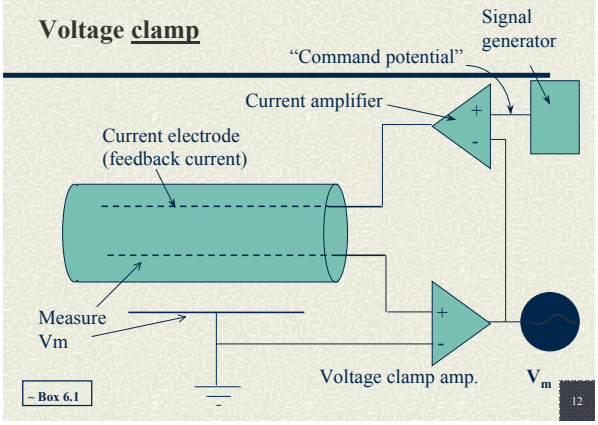
To figure out ionic currents and changes in conductance

- Problem: during AP, V_m, I_m, and G_m are all changing
- Solution: if we hold one variable steady, measure a second, we can calculate the third
- The "voltage clamp" was developed to do this by Cole, Hodgkin, Huxley, Katz and others around 1949

Intracellular voltage recording (squid giant axon)



Voltage clamp



How voltage clamp works...

- Voltage electrode records V_m
- Signal generator outputs command potential, *i.e.*, desired voltage
- Current amplifier sends out current if command potential different from V_m
- This current causes V_m to move to the command potential

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...*Voltage clamp continued*

- The size of the current needed to "clamp" V_m, *i.e.*, to counteract ΔV_m, depends on membrane conductance (Ohm's Law)
- Therefore the size of the current gives a measure of the *actual current flowing* at this voltage, and of the *underlying conductance changes*, and their *time course*

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Cont... Voltage clamp

(A) membrane (command) potential (top) and total membrane current (bottom)

NOTE: what is happening to membrane potential? What is causing the current flow?

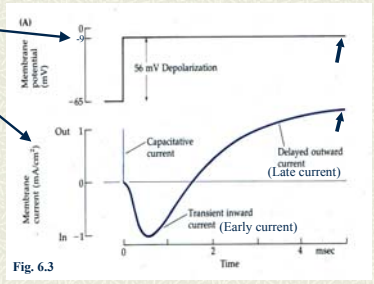

Fig. 6.3

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...cont. Voltage clamp

Capacitive Current → membrane, Na^+ ch gates

(B) Capacitive and leakage current

- caused by ___; direction ___; time course ___**μsec** for I_{cap};

(C) Transient inward current

- caused by ___; direction ___; time course ___msec;

(D) Delayed (late) outward current - (same);

note some overlap

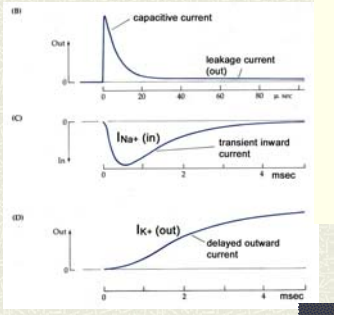

Fig. 6.3

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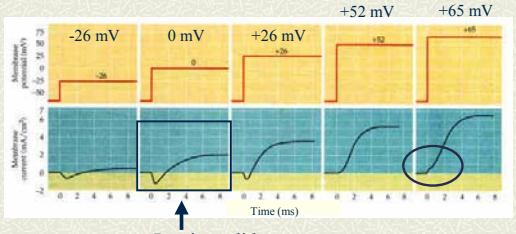
Voltage clamp - "family" of command potentials to depolarizing levels



~ Previous slide

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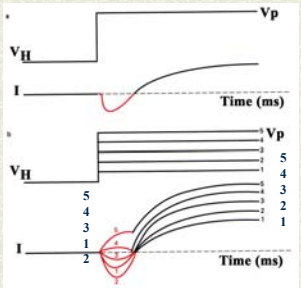
….*Cont.* V/C

"Stacked" version of whole cell voltage clamp family of clamp steps (same as previous slide)



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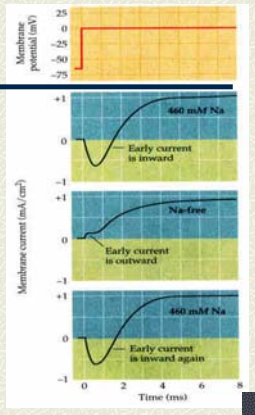
Voltage clamp of squid giant axon (H&H, 1952) - the components

- V/C to 0 mV
- normal external Na^+
- Na^+-free (**choline chloride**)
- back to normal $[Na^+]_o$



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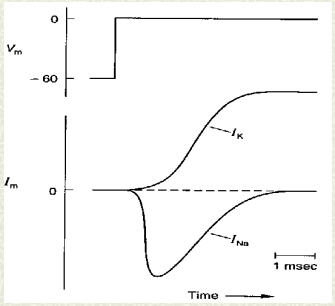
Separation of currents – note some overlap



Reduction of external $[Na^+]$ to same concn. as inside abolishes the inward Na^+ current, leaving the late outward K^+ current I_K. Subtraction of this from the overall current reveals I_{Na}.

These currents are also revealed using TTX and TEA (Na^+ and K^+ channel blockers) – pharmacology not available to H & H.

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Discovery of neuroactive drugs, examples…

- *Tetrodotoxin* (TTX) from puffer fish selectively blocks voltage-gated Na^+ channels (other sources available as well)
- *Tetraethylammonium* (TEA) ions block voltage-gated K^+ channels

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cont…Discovery of neuroactive drugs has led to powerful tools

- **powerful tools with which to investigate channels**
  - **Kinetics**
  - **Operation**
  - **Pharmacology**
  - ***Etc.***

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Pharmacological separation of membrane currents

- Voltage clamp to 0 mV
- (A) is normal bath
- 300 nM TTX
- Low mM TEA
- Time-course of individual conductance changes during an A.P. can be calculated from v/c records

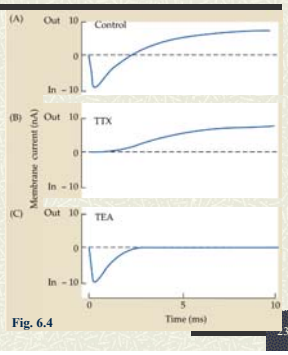

Fig. 6.4

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Dependence of early and late currents on potential

- From H & H, 1952
- Holding potential of –65mV
- Depolarize to various levels
- Complex curve breaks into two components
- Measure peak currents, plot against potential

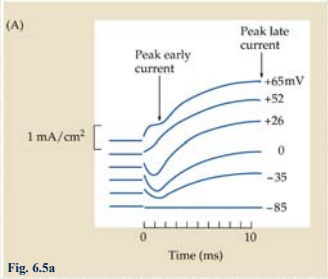

Fig. 6.5a

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cont…Dependence of early and late currents on potential

- Plot peak current *vs*. potential to which membrane is stepped
- Peak late current, <-65mV is linear (passive resistor); depolarization involves v-activated K^+ conductance, additional current thru membrane
- Early current complex: with depolarization dealing with decreasing driving force on Na^+, increasing conductance of v-activated Na^+ channels, (inactivation)
- Negative slope conductance

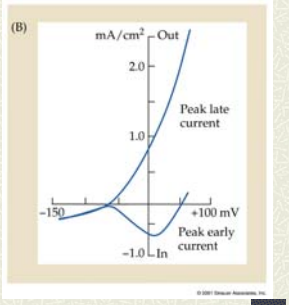


Fig. 6.5b

Complex interaction between channels and membrane potential

- Clearly have (at least) two sets of channels operating during AP
- Operate independently
- Dependence on voltage
- ? Does the starting point of V_m influence subsequent inward current recorded?
- ? What about immediate 'prehistory' of the neuron?

Effect of membrane potential on I_{Na+}

Feature of v-activated Na^+ channels, inactivation, first elucidated by H & H.

(A) control - no prepulse

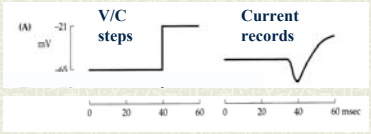
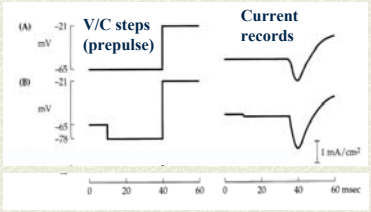


Fig. 6.6a

cont…Effect of membrane potential on I_{Na+}

(A) control - no prepulse

(B) hyperpol'ing prepulse

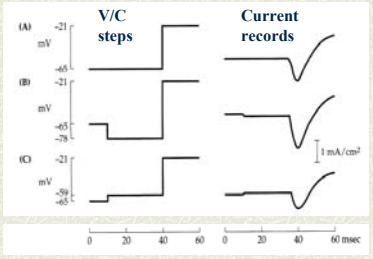


cont… Effect of membrane potential on I_{Na+}

(A) control - no prepulse

(B) hyperpol'ing prepulse

(C) small depol'ing prepulse



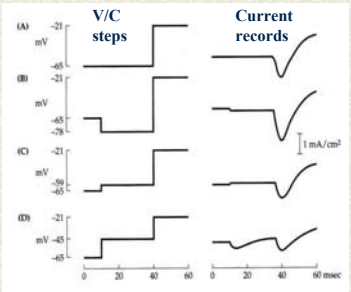
cont…Effect of membrane potential on I_{Na+}

(A) control - no prepulse

(B) hyperpol'ing prepulse

(C) small depol'ing prepulse

(D) large depol'ing prepulse



Peak Na^+ current *vs.* prepulse potential - amount of activation in the "system"

- $I_{Na\ no\ step}$ *constant*
- If current **increases** after a step then $I_{Na^+\ step} / I_{Na^+\ no\ step}$ is > 1.0
- If current **decreases** after a step then $I_{Na^+\ step} / I_{Na^+\ no\ step}$ is < 1.0
- intersection of **RP** and **no change (1.0)** shows amount of activation normally in system at rest
- ***h*** - represents amount of activation

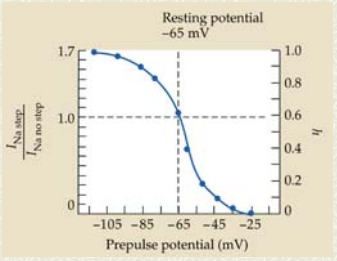


Fig. 6.6e

A further note on gNa^+ and gK^+

- Examples of data for Na^+ and K^+ conductances
- Voltage steps to indicated potentials
- Peak g_{Na+} and g_{K+} both increase with depol'n

$$g_{Na+} = \frac{I_{Na+}}{(V_m - E_{Na})};$$

And similarly for g_{K+}

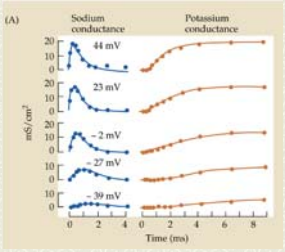


Fig. 6.7

cont…A further note on gNa^+ and gK^+

- both increase progressively as neuron is depol'd
- note the similar voltage dependence for each "g"
- both sigmoidal functions of membrane potential
- quite small at -ve potentials
- maximal at +ve potentials
- exquisitely dependent on Vm at intermediate potentials
- deduction about mechanism of g-change senses V across membrane

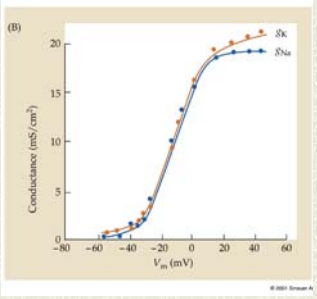


Fig. 6.7

Threshold and refractory period

- **Threshold**
  - Generally about 15mV depolarization required to reach (several factors influence exact value)
  - Point at which gNa^+ > outward gK^+ , initiates +ve feedback cycle of Na^+ entry
- **Refractory Period (absolute and relative)**
  - Na^+ - inactivation
  - high g_{K+}

Hodgkin, Huxley, and Katz (1952)

Hodgkin & Huxley (and Katz) reconstructed the conductance changes underlying the AP from voltage clamp data.

- Apply change in potential (ΔV) across the membrane (V/C);
- Measure current flow (I);
- Calculate conductance (G) (remember: V=IR (R=1/G))

Bottom lines: point by point reconstruction; data generated experimentally essentially same as during an AP. Mathematical model accurate, AP is a predictable phenomenon.

Also, What does this tell you about the voltage clamp technique?

H & H finally compared their calculated APs with the observed APs

Reconstruction of the AP (mathematically)

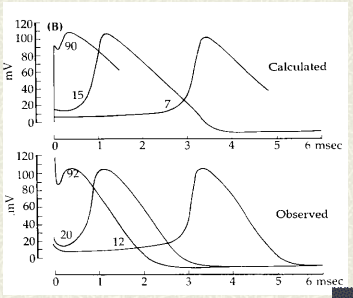


Fig. 6.8

Timing of conductance changes with respect to the action potential

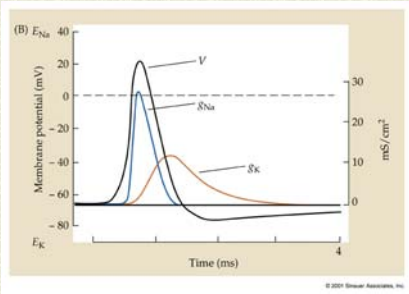


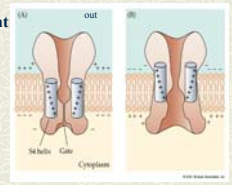
Fig. 6.8

Hodgkin and Huxley's prediction *re.* Na^+ channel "gating currents" (1952)

- suggested Na^+ channel **activation** >> **movement** of **charged particles** ("structure") within the membrane

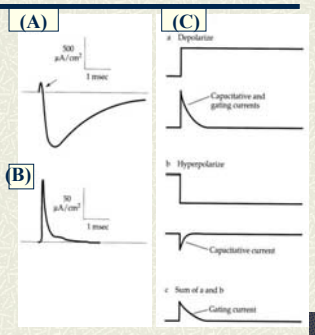
- appear as very small current in response to voltage step (**asymmetrical**), *i.e.*, doesn't occur when hyperpolarize

- block Na^+ channels with TTX and cancel symmetrical capacitive current due to voltage step; remainder = gating current (Armstrong and Bezanilla, **1974**)

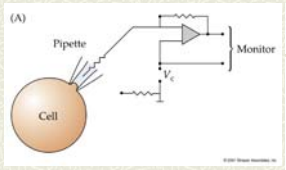


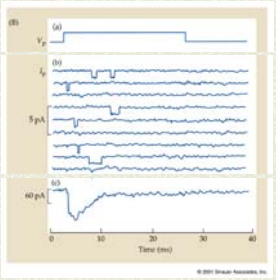
cont. Na^+ channels - gating currents

- **(C)** method of separating gating current from capacitative current
  - for depol'g and hyperpol'g V/C steps, I_{cap} are equal and opposite
    - electronically subtract out I_{cap} and I_{gating} remains
- (A) I_{gating} and I_{Na} (when reduced $[Na]_o$)
- (B) I_{gating} alone (TTX)



P/C recording from Na^+ channels





- V_p (a) - voltage clamp Ip
- (b) - current records (channels opening and closing)
- (c) - many current records "piled" up

Fig. 6.10

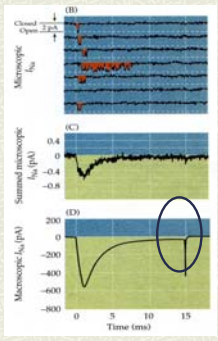
Altogether - from macro to micro

V/C →

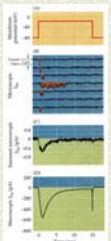
Microscopic I_{Na+} →

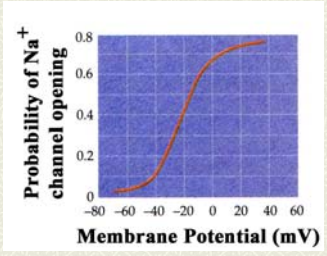
Summed microscopic I_{Na+} →

Macroscopic I_{Na+} →



cont... From macro to micro - Probability of Na^+ channel opening





More on Na^+ currents - what's in the "*system*" ?

Remember...

- probabilistic events - Na^+ channel opening and closing

- dependence on voltage

- Na^+ channel activation and inactivation appear to be "separate" events

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What experimental evidence suggested:

- **inactivation** was a distinct phenomenon, **separable** from the **activation** process (by H & H, 1952 !)
- **pronase** experiments supported this (pronase - what? where? Not effective externally)(1973, 1977)
- further support - **veratridine (lily) and batrachotoxin (BTX)(frog skin)**, both eliminate inactivation; but also alter V-dependence of activation (Na^+ channels open at rest)(1980)(*i.e.*, not perfectly specific)
- **antibody** directed at cytoplasmic domain of Na^+ channel - greatly prolonged single channel Na^+ currents (1989)
- **site-directed mutagenesis** - alter or delete specific a.a. in cytoplasmic domain (3 specific aa's in loop that acts to "plug" the channel), inactivation not occur (1997)

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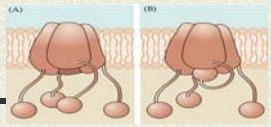
Notes on other channels



- Delayed rectifier (*a.k.a.* voltage gated K^+ channel) does not inactivate
- Many species of K^+ channels, different properties; for *eg.*
  - A-channels: inactivate with maintained depolarization; ball and chain model similar to Na^+ channels (NMWF 107, 109); inactivate rapidly and usually inactivated at rest (require hyperpolarization to activate)
  - M-channels: muscarinic AChR; 2nd messenger involved; activate gK^+ (note mistake in text pg 109)
  - S-channels: open at rest; 5-HT via 2nd messenger, closes K^+ channel
  - Ca^{2+}-activated K^+ channels

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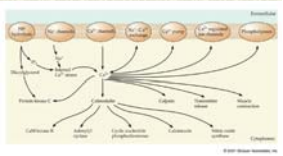
Role of calcium in excitation

- Voltage-gated Ca^{2+} channels
- Distribution
- Importance
  - excitability (threshold – complex interaction with local charges)
  - release of neurotransmitter
  - contraction of muscle fibres
  - *etc.*



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NEXT…..

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