April 16, 2014

What did they do in the first paper??

1) Showed that Na\(^+\) is the inward charge carrier for the AP.
2) Separated \(I_{Na}\) and \(I_k\).
3) Expressed the currents in terms of conductance.
4) Applied this to calculations of the time course of \(g_{Na}\) and \(g_k\).
5) Suggested the importance of these currents for the AP.

Voltage clamp of squid giant axon

This paper was concerned with:

1) Providing some quantitative evidence about the influence of membrane potential on the process responsible for inactivation.
2) Quantifying the rate at which repolarization restores the ability of the membrane to respond with its characteristic increase in Na\(^+\) permeability.
Changing conditioning pulse time—depolarizing duration

Pre-pulse > 20 ms, 40% reduction of $I_{\text{in}}$

Changing conditioning pulse time—hyperpolarizing duration

$I_{\text{in}}$ inactivation at rest!

Inactivation and recovery exponential

Reached steady state

Faster at larger $V$ steps

Changing conditioning pulse amplitude

Time is constant, pre-step is changed
h = ability of membrane to undergo a change in Na permeability

40% inactivation at rest!

h infinity curve

depolarize hyperpolarize

0 = -55 mV

Fig. 5. Influence of membrane potential on "inactivation" in the steady state. A: brisk phase: displacement of membrane potential from its resting value during conditioning step. B: steady state, transient current during test step relative to transient current in unconditioned test step (left-hand side) or during to membrane depolarization (right-hand side). The inward transient current is plotted in all panels. Inactivation was measured at 11°C. All panels represent the experiments as shown in the legend (inset). The graph is based on the results obtained in Fig. 4. Different conditions were depicted in the manner shown in Fig. 5.

What is the state of the Na channel?

3 states of Na channel: closed open inactivated

Twin pulse recovery experiments

Explains the refractory period!!

Description of I_{Na}

Modeling I_{Na}

\[ g_{Na} = m^3 h g_{Na} \]
2 states of K channel:
- closed
- open

First high profile computational work

These experiments explain:
1) the shorter latency of the AP with a large depolarizing stimulus.
2) Refractory period
3) Accommodation.

These experiments set the stage for the major paradigms we still use today:
- Identifying ionic currents by:
  - ion substitution
  - reversal potential
  - description of I/V curves
  - activation and inactivation parameters
- Modelling

Real neurons have many different currents distinguished by:
- Conducting ions
- Time course (activation/inactivation)
- Sensitivity to membrane potential
- Sensitivity to neuroactive compounds
Patch clamp recording

H&H predicted separate channels for each ion, with no hard evidence

Early evidence for single channels

Biophysicists found antibiotic channels in black lipid membranes

Lipid bilayer currents flowed in steps

Scale of biological currents

The Nobel Prize in Physiology or Medicine 1991

"for their discoveries concerning the function of single ion channels in cells"
The Gohm seal is possible:
1) the membrane lipid bilayer is attracted to the surface of electrode glass.
2) the membrane is fluid, allowing it to be pulled into the electrode tip.

Early discoveries
Openings fast (10 µS), measuring speed of protein conformational changes!!!
Early discoveries

Channels follow Ohm’s law

Wide range of channel conductances

Defining channel kinetics for opening and closing

Single channel currents matched macro currents

Na⁺ channels
K⁺ channels
Also staining, adding experimental compounds: channel blockers, second messengers

Hamill et al 1981

Channel States defined

Twin pulse experiments:

Hodgkin and Huxley
3 states of Na channel:
closed  open  inactivated

Na channels don’t need to open to inactivate

H&H
3 states of Na channel:
closed  open  inactivated

No Presynapse

Prepulse

No Openings

Positive Openings