February 19, 2014

Voltage Clamp
Refractory period
Intracellular Recording

Lab 2 final and Lab 3 Results
due tomorrow
Problems analyzing conductances underlying AP?
1. $I_{Cm}$ obscured $I_{Rm}$
2. Conductances changed during AP

Introduction to Voltage Clamp:
Current across membrane
Goal to understand the ionic conductances at each stage of the AP

\[ I_m = I_{Rm} + I_{Cm} \]
Ohm’s law for V-Clamp

\[ g_{ion} = \frac{I_{ion}}{E_{m} - E_{ion}} \]

- Recorded continuously...
- \( I_{ion} \) constant...clamped at a known level.
- \( E_{m} \) and \( E_{ion} \) constant...calculated from Nernst.
Ohm’s law for V-Clamp

How was this practically done?
Fast thermostat-like feedback
$g_{ion} = \frac{I_{ion}}{E_m - E_{ion}}$

- $E_m$: constant, clamped at a known level.
- $E_{ion}$: constant, calculated from Nernst.

**Ionic driving force**

**Diagram:**
- Inside: -
- Outside: +
- Electrical gradient (60 mV) from - to +
- Net gradient (15 mV) from inside to outside
- Concentration gradient (≈ 75 mV) from inside to outside
Ionic driving force

\[ g_{\text{ion}} = \frac{I_{\text{ion}}}{E_m - E_{\text{ion}}} \]

constant...clamped at a known level.

constant...calculated from Nernst.

INSIDE

\[ \text{electrical gradient (60mV)} \rightarrow \text{net gradient (110mV)} \rightarrow \text{concentration gradient (≈50mV)} \]

OUTSIDE
Current across membrane with hyperpolarizations

\[ I_m = I_{Rm} + I_{Cm} \]
Current across membrane with strong depolarizations

Current no longer ohmic!
I/V plot for current flow with larger depolarizations

Measure Im
Refractory periods: Why are the APs getting smaller and then fail?

Figure 5.C. Spike amplitude decrease. A Ca$^{2+}$ cell without spontaneous activity was stimulated with depolarizing current. The amplitude of APs after the first one decreases due to inactivation of voltage-gated Na$^+$ and/or Ca$^{2+}$ channels. Afterhyperpolarization amplitude decreases due to inactivation of the K$^+$ current responsible for repolarization. Spike failure (note the depolarization after the last AP) occurs when the membrane reaches AP threshold but there are too few active Na$^+$ channels to trigger a full AP.

Note changing AP threshold too.
Why doesn’t the AP go backwards?
2 reasons-

- Absolute refractory phase
- Relative refractory phase
Vr stable
Vth unstable
Vpeak could be stable but conductances are time and V sensitive
Refractory periods

D- $g_K \gg g_{Na}$
C- $g_{Na}$ recovering, but no AP
B- smaller AP
A- full AP

Notice threshold change
4910 recording of AP firing from a snail neuron
Intracellular potentials

Action Potentials

Synaptic Potentials

Receptor Potentials

MRO stretch in TTX
Motor Network Example

Extracellular

Intracellular

More detailed information from intracellular recording