# Foundations I Fall 2016
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## IONIC BASIS OF THE ACTION POTENTIAL

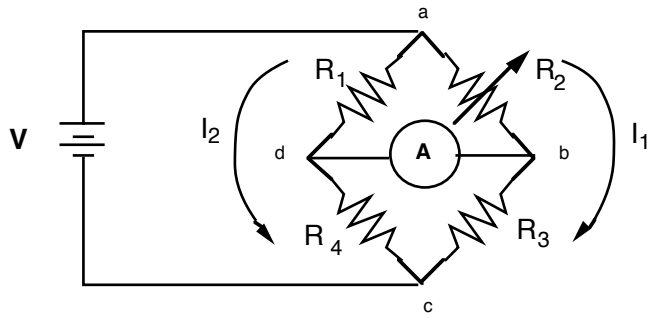
### I. Action Potentials and Active Properties

**A. Bernstein Hypothesis** - The action potential was originally thought to be due to "shorting out" of the membrane potential due to a breakdown of selective permeability to $K^+$ (J. Bernstein, c. 1912).

**B. Role of axoplasm** -There was a great debate about the nature of the action potential and the role of the axoplasm vs. the axonal membrane, lasting well into the first quarter of this century. Hermann (1872, 1905) was the first to recognize that the potential changes associated with an excited region of axon would send small currents (Strömchen) down the axoplasm, which would electrotonically, excite adjacent regions and thus account for self-perpetuating action potential.

**C. Wheatstone Bridge**
First applied to neurophysiology around 1920:

Wheatstone Bridge




1. Instrument for determining the value of an unknown resistor
2. The (known) variable resistor is adjusted until there is no current flow between points d and b detected by the ammeter (A).
3. At this point the bridge is said to be *balanced*, and the value of the unknown resistor ($R_1$) can be calculated by $R_1=R_2R_4/R_3$.

How and Why?

According to *Kirchoff's Law,* the algebraic sum of the potential rises and drops around a closed loop is zero. Since V=IR for all paths in the circuit, if there is no current flowing between d and b, there must be no potential difference between d and b. In other words, $V_d=V_b$.

Thus $$V_d=I_2R_1=I_1R_2 = V_b=I_2R_4=I_1R_3$$

Solve for $I_2$:

$$I_2R_1=I_1R_2$$

$$I_2=I_1R_2/R_1$$
and
$$I_2=I_1R_3/R_4$$

now solve for the unknown, $R_1$

$$I_1R_2/R_1=I_1R_3/R_4$$
$$R_2/R_1=R_3/R_4$$

$$R_1=R_2R_4/R_3$$

[Note that this is not precisely what the bridge balance control on the front of a modern intracellular preamplifier does. Today we use "bridge" amplifiers that simply subtract a voltage from the amplifier output that is equal to $IR_{electrode}$ (when balanced), but the net result is the same.]

The Wheatstone bridge made it possible to measure, for the first time, the resistance of the cell membrane by discounting or "balancing out" the series resistance of the recording microelectrode.

## C. First Intracellular Recordings

1. Curtis and Cole (1939) put a squid axon into a Wheatstone bridge. The total resistance of the intracellular pipette and the membrane resistance in series formed the unknown resistor. By passing a sine wave through the intracellular electrode while the bridge was balanced, and then depolarizing the axon with an electrical stimulus, they showed for the first time that the action potential was associated with an all or none transient reduction in membrane resistance. Conductance increased some 40 times during the peak of the action potential with only a 2% change in capacitance. Thus, for the first time, there was direct experimental evidence support for at least part of the Bernstein hypothesis, i.e., that the resistance of the membrane decreased during the action potential.

2. Soon thereafter, Hodgkin and Huxley (1939) and Curtis and Cole (1940) showed that action potential overshot 0 potential by several tens of mV and inside of membrane went positive. Furthermore, for a short time immediately after the peak of the action potential, the membrane *hyperpolarized* beyond the resting membrane potential. Furthermore, subthreshold depolarizations caused graded increases in membrane conductance. Thus, Bernstein's hypothesis couldn't be correct. [Actually, Bernstein himself noticed the overshoot in nerve before the turn of the century, but failed to observe it in muscle and apparently discounted the overshoot as some sort of artifact.]

3. WWII intervened and cost several years but Hodgkin and Katz correctly inferred that overshoot could be accounted for if membrane transiently became very permeable to $Na^+$ during the action potential. They verified this after the war in 1949 using standard intracellular recording techniques from the squid giant axon.

At this point it was known that the action potential was an *"all-or none"* event under normal conditions, that it depended on the presence of extracellular sodium, and that it was associated with a large increase in membrane conductance. A difficulty in trying to

understand the mechanisms of the action potential arises since the depolarization of the membrane is dependent on membrane conductance, which in turn is dependent on membrane potential and time. In other words, membrane depolarization increased the membrane permeability to Na+, which in turn, further increased depolarization etc. Thus, there is a kind of vicious cycle of positive feedback wherein depolarization increases $P_{Na}$which increases depolarization which increases $P_{Na}$ etc. In order to figure out what is really going on, it is necessary to break the cycle by controlling membrane permeability or membrane potential.

## D. Voltage Clamp and HH Kinetics

**1. In typical intracellular recording experiment in *current clamp mode*, the experimenter varies a stimulus current and measures the membrane voltage changes that result.** *Voltage clamp* recording reverses this procedure, and measures transmembrane currents by holding the membrane potential constant by passing varying amounts of current through the membrane that are equal in magnitude and time course and opposite in sign to the currents generated by the activity of the membrane itself.

a. The original voltage clamp designed independently by Marmount, Cole and Hodgkin, Huxley and Katz in the late 1940s consisted of 2 intracellular electrodes - one for recording membrane potential and one for "clamping" membrane potential by passing current.

b. 2 amplifiers - both differential - one is like a conventional amplifier that amplifies the difference between the recording electrode and extracellular reference electrode. The other amplifies the difference between the output of the 1st amplifier ($V_m$) and a *command voltage* input, and outputs the difference to the current passing electrode.

c. If the command voltage equals the membrane potential, the 2nd amplifier sees no difference and no current is passed. If there is a difference, it is amplified, inverted and the current passing electrode is charged to a voltage just sufficient to pass enough current in the opposite direction to balance the difference. The current passed by this electrode is continuously monitored, and is the output of the voltage clamp. Note also that in the case of the squid axon voltage clamp it was possible to insert a large wire down the center of the axon for current passing. This enabled H&H to also achieve a *space clamp* as well as a voltage clamp, since they were able to maintain a large area of the axon at the same potential simultaneously.

> [Most modern voltage clamping in the CNS is done with a single intracellular microelectrode coupled to a circuit that switches back and forth between current measuring and current passing duties. In practice, it is difficult to achieve a good space clamp, i.e., to maintain all regions of the cell at the same potential, this way, and this can lead to serious errors in the voltage clamp measurements.]

**2. There are two oppositely directed currents during an action potential**

a. Early *transient* inward current - depolarizes the membrane

b. Later *sustained* outward current - hyperpolarizes the membrane

[The first current seen is actually a very brief outward current due to the discharge of the membrane capacitance as the membrane is stepped to a depolarized value and is termed a capacitative artifact. A similar artifact (opposite in sign is also seen upon release of the clamp. What follow the capacitative artifact are the "real" *ionic* currents.]

**3. When $Na^+$ was eliminated from the extracellular medium, the transient inward current was abolished.** The current reversed in sign and became outward when the command voltage exceeded the $Na^+$ equilibrium potential. Thus, the early inward current is due to $Na^+$ influx and the late current is due to potassium. [In the absence of specific channel blockers, how could they know that the late outward current was due to $K^+$?]

**4. Ionic conductances**

Hodgkin and Huxley introduced the concept of ionic conductances to study the instantaneous current-voltage relations as follows:

(recall that conductance g=1/R and that R=V/I. Thus, g=I/V)

$$g_{Na}=I_{Na}/(E_m-E_{Na})$$
and
$$g_K=I_K/(E_m-E_K)$$

By plotting values for $g_{Na}$ and $g_K$ obtained from measurements of their respective currents, they established the time course of the conductance changes. Note that the "V" in this case of g=I/V is *not* the membrane potential, but the difference between the membrane potential and the equilibrium potential. This difference is called the *driving force* on the ion.

Note that the Na conductance activates then inactivates within a few ms, whereas the K conductance, once activated, stays activated (as long as the membrane potential is clamped at a depolarized level). Note also that the inactivation of the Na channel is much slower than its activation, and that once inactivated, reactivation (de-inactivation) requires re-polarization for a period of time, and is thus both voltage- and time-dependent.

**5. Time dependence of HH kinetics**

a. $g_K$ - HH assumed some type of molecular event underlay the changes in membrane permeability. The time course of the onset of the K current is S-shaped, whereas the decay (after the depolarizing step is removed) is exponential. A phenomenon like this could obtain (and so HH hypothesized) if one or more membrane bound elements were acting independently to control or "gate" the permeability, and that for the current to activate, all such particles would have to be in the same permissive state simultaneously. Because $I_K$ is voltage dependent, the particles must be charged, i.e. dipoles. If the probability that one particle is in the "permissive" state is n, then the probability that x such independent particles are in that state is $n^x$. Empirically, it turns out that the time course of $I_K$ activation is best fit by assuming that x=4. [Since n is a fraction between 0 and 1, $n^x$ is smaller than n, which causes $n^x$ to rise more slowly than n.] Note that the time course of $I_K$ decay is much faster, as if only one of the 4 particles had to flip back to its non-permissive state to turn the current off. Thus:

$$I_K=n^4G_K\,(E_m-E_K)$$

[where G is maximum conductance]

The voltage and time dependent changes of n are given by the first order reaction:

$$1\text{-}n \underset{\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} n$$

where the n particles make transitions between the permissive and non-permissive forms and where $a_n$ and $b_n$ are the *voltage dependent rate constants* in the first order reaction in which the gating particles move from one state to the other:

If the initial value of the probability, n, is known, then other values can be calculated by solving:

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

An alternative description created by HH defined the voltage dependent time constant, $\tau_n$ and the steady -state value of n ($n_\infty$) as:

$$\tau_n = \frac{1}{\alpha_n + \beta_n} \quad \text{and} \quad n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}$$

where $t_n$ is the time constant for the transition of n and $n_\infty$is the steady state voltage-dependent value of n. Values of n can be calculated by solving the differential equation:

$$\frac{dn}{dt} = \frac{n_\infty - n}{\tau_n}$$

*[These equations represent the most common methods for mathematical simulations of conductances in neuronal compartments, i.e., this is how different conductances are instantiated in neuronal models.]*

b. $I_{Na}$ was handled similarly, but was a more complex case because of its time-dependent inactivation. HH proposed two different gating particles, one for activation (m) and one for inactivation (h [h is actually the probability that the Na channel is *not* inactivated]). Thus:

$$I_{Na} = m^3 h G_{Na}(E_m - E_{Na})$$

[where G is maximum conductance]

c. In this way, the total ionic current across the membrane at any time is given by:

$$I_i = (I_K + I_{Na} + I_l) = n^4 G_K (E_m - E_K) + m^3 h G_{Na}(E_m - E_{Na}) + G_l(E_m - E_l)$$

where $G_l$ is a fixed background leak current
(unlike the gated channels, the leak channels are always open, and are not voltage sensitive)

6. The movement of these "hypothetical" particles was first measured in the mid 1970's and gives rise to *gating currents*. The key experiment (Armstrong and Bezanilla, 1974) was to take advantage of the asymmetry of the capacitative responses to depolarizing and hyperpolarizing current pulses of equal amplitude under voltage clamp conditions. Responses to hyperpolarizing and depolarizing voltage steps of equal magnitude are recorded in a preparation in which the Na and K currents are minimized or eliminated by replacement of Na and K ions or by pharmacological blockade. Since the Na channel opens under depolarization but not hyperpolarization, there should be a gating current in response to the former but not the latter, but the capacitative currents should be equal. By subtracting the averaged responses to hyperpolarizing steps from that of depolarizing steps of equal magnitude, the gating current can be seen.

7. Note that HH kinetics were deduced from empirical measurements. They *do not* represent a model based on a priori theoretical considerations. In the most severe and conservative approach, one could say that they merely represent curve fitting. However, one should also note that they accurately predict the time course and voltage dependence of action potentials, as well as action potential propagation and refractory periods. And despite all the criticisms of the model, most modern neuron simulating software that make compartmental models of neurons take as their input equations describing the various conductances in basically the same form as that first described by H&H.

**a. Absolute refractory period** - For a short time immediately after a neuron fires a spike the cell cannot be made to fire another action potential no matter how strong the stimulus. This is due to residual sodium channel inactivation and can be seen in the HH equations by the time dependence of inactivation and the recovery from inactivation ($\tau_h$.)

**b. Relative refractory period** - For a short period of time after the absolute refractory period the neuron's threshold for action potential initiation is increased. It can be made to fire a spike, but requires a stronger than normal stimulus. This is due to an afterhyperpolarization resulting from residual $I_K$, as well as to some number of sodium channels still being in the inactivated state.

**c. Threshold** - As the membrane is depolarized, $g_{Na}$ increases, but so do $g_K$ and $g_l$. Although the individual channels have no thresholds - the *membrane* threshold derives from the statistical nature of the proportion of m, h and n gates in one state or another and the resulting probabilities (proportions) of channels begin opened. Threshold is the point at which the depolarization increases $g_{Na}$ to the point that the inward current just overbalances the net outward currents of $g_K$ and $g_l$. At this point the inward current becomes regenerative and there is a rapid influx of sodium as all the remaining Na channels open.

**d. Accommodation** - Normally, neurons have a relatively stable threshold. However, if a ramp of depolarizing current is injected with a sufficiently low slope, $g_K$ and sodium inactivation increase in parallel with $g_{Na}$. This serves to raise the voltage at which the net inward current exceeds the outward current, and the threshold is raised. With a slow enough ramp, it is possible to depolarize a neuron past the point at which it can fire a spike by inactivating enough Na channels. A related phenomenon is the increase in interspike interval between spikes in a train generated, for example, by a long intracellular current injection. This is termed *spike-frequency adaptation*. Although spike-frequency

adaptation is a complex phenomenon with several different components, one of them is residual sodium channel inactivation.

## E. Action Potential Propagation

**1. Mechanism of Propagation** - Hermann was essentially correct. The passive electrotonic propagation of depolarization arising from the action potential at a given site spreads along the axon, thereby depolarizing adjacent regions to threshold. Note that this indicates that if an action potential is initiated in the middle of an axon, it will simultaneously propagate in both directions away from the site of initiation (see below). There is no polarization to the direction of action potential propagation.

**2. Speed of conduction** - The rate at which action potentials propagate is directly affected by the electrotonic properties of the axon.

a. The time it takes for current to spread axially through the axoplasm varies with the product $t=r_a c_m$. Since the axial resistance is inversely proportional to axon diameter, t is smaller in larger axons and thus adjacent regions of axoplasm will depolarize faster. Note that this is not the same membrane time constant that we discussed earlier. In that case we were concerned with $r_m$, not $r_a$. What can you conclude about decreases in $r_m$ versus $r_a$ and speed of conduction?

b. The membrane length constant also plays a role here. Recall that:

$$\lambda=\sqrt{r_m/r_a}$$

Thus when $r_m$ increases or $r_a$ decreases, the length constant increases and a depolarization will spread further.

Myelination increases conduction velocity by both of these mechanisms. The myelin greatly increases $r_m$ and forces the action potential to jump from node to node, a phenomenon known as saltatory conduction). At the same time, myelination also decreases $c_m$ by increasing the distance between the extracellular space and the axoplasm. This decreases $\tau=r_a c_m$. and thus speeds the rate at which adjacent sections of the membrane depolarize passively. In these ways, conduction velocity is greatly enhanced by myelination.

**3. Antidromic Action Potentials** - Axons can be stimulated to threshold at any site along their trajectory including at their terminal endings. When this occurs, the action potential propagates backwards, towards the cell body. This direction is called *antidromic* (as opposed to *orthodromic*, or forward-going) and resulting action potentials are termed antidromic action potentials. Antidromic stimulation is a powerful tool for demonstrating "live and on-line" that a neuron one is recording from is a projection neuron, and the site to which it projects. Antidromically elicited action potentials can be discriminated from orthodromically elicited action potentials by several means.

**a. Constant Latency** - because there are no synapses involved, the latency of an antidromic action potential in a neuron from a given stimulating site is constant to within a few tens or hundreds of microseconds.

**b. High Frequency Following** - again, because there are no synapses, and no possibility of synaptic fatigue, antidromic responses can follow faithfully high frequency stimulation (usually given in the form of double pulse stimuli) up to 100 Hz or more.

**c. One Response Per Stimulus** - orthodromic responses often consist of multiple spikes whereas antidromic responses consist of one action potential per stimulus.

**d. Collision Test** - All of the above criteria (which are often used) are valuable, but subject to error. Very tight monosynaptic responses can fulfill all of them. The collision test is the best, and most rigorous test of the antidromic nature of an action potential. Antidromic responses will be extinguished by collision with a spontaneous action potential that occurs within a period of time before or after the stimulus onset equal to the conduction latency plus the refractory period. This occurs because the two spikes encounter one another somewhere along the axon between the initial segment and the stimulating site and encounter each other's absolute refractory period, thereby stopping the propagation of both spikes.

**4. Super and Subnormal Periods -** Action potential (both orthodromic and antidromic) conduction velocity and axonal excitability can vary depending on the immediate prior history of activity of the axon. Immediately after the relative refractory, there is a *subnormal period* wherein conduction velocity is slightly slowed and excitability is depressed due to residual sodium channel inactivation. Immediately following the subnormal period a *supernormal period* may occur, in which conduction velocity and excitability are both increased due to a build up of extracellular $K^+$ ions.

## F. Neuronal Action Potentials

A. In most of the preceding discussion concerning the active properties of neuronal membranes and action potential initiation and conduction, we used the axon as a model. We also implicitly assumed that all excitable membrane was roughly equivalent, something that is obviously incorrect (recall the differing densities of sodium and potassium channels along most axons compared to those at nodes of ranvier in myelinated axons). We also implicitly assumed that neurons fire action potentials, since they travel along axons and the axons originate from neuron. What does it mean to say that a neuron fires an action potential, and where on the neuron does it originate? For the sake of convenience, we can divide a neuron up into three distinct functional areas, the initial segment, the cell body and the dendritic arborization.

**1. The initial segment** - Axons emerge from different points on different neurons. In some cases, they originate directly from the cell body. In others, they issue as processes from primary or even secondary dendrites. In all these cases, the point initial region of the axon is referred to morphologically as the axon hillock, and functionally as the initial segment (IS). Regardless of whether a neuron is excited to threshold by antidromic stimulation, orthodromic stimulation, or by direct intracellular depolarization, in almost all cases, the initial segment fires first, i.e., it has the lowest threshold for spiking. This is (presumably) because the highest density of sodium channels on the cell are in this region. Sometimes there is a considerable delay between the firing of the IS region

and the rest of the cell, known as the IS-SD (soma-dendritic) delay. Under certain conditions, a stimulus can elicit spikes in the IS region without triggering an action potential in the rest of the cell.

**2. The cell body.** Is the soma electrically excitable? Before we say "obviously yes", consider the current sources and sinks that could account for the waveform of an extracellularly recorded action potential in which the IS spike and the SD spike both have the same positive polarity. If the IS spike is initiated at a distance from the cell body (as seems to be the case from intracellular recordings where the IS spike is only a fraction of the amplitude of the SD spike due to electrotonic degradation), then the currents responsible for the observed spike must have their source at the IS and their sink at the soma. Similarly, the same holds for the SD spike, which has the same polarity as the IS spike. If the soma itself spikes, we would expect a polarity reversal just after the IS spike when the soma switches from sink to source. By this argument, the cell body of the neuron does not fire a spike (Grace and Bunney, 1983). (Note that this argument involves assumptions about the nature of the IS spike and the analysis of extracellular field potentials that could be incorrect. To wit, see Hausser et al., 1995, showing directly the presence of $Na^+$ and $K^+$ channels in both the dendrites and cell body of the dopamine neuron, the same ones that Grace and Bunney claimed do not fire somatic spikes. The point is, it is not so easy to analyze these events as one might imagine.)

**3. The dendrites** - In many different cells, the dendrites have been shown to fire dendritic spikes. In some cases, the dendritic spikes have been shown to be primarily $Ca^{++}$ - dependent, not $Na^+$ dependent.

**a. Purkinje Cell** - The principal cell of the cerebellar cortex has a massive and extensively branched dendritic arbor. The dendrites of these cells possess voltage dependent $Ca^{++}$ conductances that underlie regenerative $Ca^{++}$ action potentials that may last for hundreds of ms. Interestingly, the somatic $Na^+$ spikes do not propagate very well into the proximal and middle dendrites, and not at all into the distal dendrites, whereas the dendritic $Ca^{++}$ spikes do affect burst firing of $Na^+$ spikes at the cell body level via a $K_{Ca}$ conductance.

**4. Conclusion** - Thus, in a typical neuron, we can conceive of the sequence of events in the "normal" orthodromic initiation of an action potential as follows:

1) Dendritic depolarization via excitatory synaptic input spreads passively up the dendrites to the initial segment

2) $Na^+$ action potential initiated at initial segment propagates actively along axon and

3) electrotonically back through slightly depolarized cell body to trigger a Na+ spike in the soma (maybe?) and

4) electrotonically (or actively?) through soma to dendrites to trigger a $Ca^{++}$ spike (maybe in some cases a $Na^+$ spike)