

**1** We will now discuss ionic conductances in more detail and show how changes in ionic conductances are responsible for the **action potential**. We say that the sodium current, for example, can be expressed as  $I_{Na} = g_{Na}(V - E_{Na})$  and we have discussed how  $(V - E_{Na})$  is the driving force but what precisely is  $g_{Na}$ ? Conductance occurs through open ion channels. Total conductance is the sum over all channels.

We know that ions cross the membrane through channels. Channels are proteins in the lipid bilayer. If all channels were always open, life would be pretty dull if it would exist at all! It turns out that many channels can make the transition between open and closed states by changes in the conformation of the protein that makes up the channel.

We can view the channel as having a gate and the channel can switch between states stochastically (with some probability). For example, here is a channel with one open state and one closed state. If the  $C \rightarrow O$  rate equals the  $O \rightarrow C$  rate then the channel will spend equal times in the open and closed states. If the  $C \rightarrow O$  rate (here  $k_1$ ) is greater than the  $O \rightarrow C$  rate, then the channel will spend more time in the open state than in the closed state. Note that how long a channel spends in the open state is a function of its closing rate  $k_2$  and how long it spends in the open state depends on the opening rate  $k_1$ . These rates are in units of  $ms^{-1}$ , so  $1/k_2$  would give the average open time, although the actual open time in any one case would be random and drawn from an exponential distribution.

**2** This figure shows the current for a single open channel at given voltages. When the voltage is changed, the amplitude of the current changes? Why? The amplitude would change because of the change in the driving force. If the voltage were set to  $E_{ion}$  then we would not see a channel current at all. The conductance of the single channel is not changed by voltage. We can see this by plotting single channel current vs. voltage.

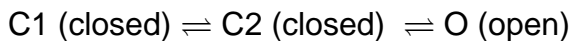
**3** Now the membrane has many channels and the current for a particular ion at a particular voltage depends on how many of these channels are open. For example, here we see the current for one channel, 3 channels, dozens of channels and on a different scale the whole cell current.

For a given driving force, the current will depend on  $g_{Na}$  which is determined by the number of channels present times the probability the channel is open times the conductance of a single open channel.

$g_{Na} = N_{Na} P_{o,Na} \gamma_{Na}$  where  $N$  is the number of channels,  $P_o$  is the probability a channel is open, and  $\gamma$  is the single channel conductance. To increase  $g_{Na}$  one could increase 1) the number of channels. However this takes time and over short periods  $N$  is constant. 2) increase  $P_o$ . It turns out that this is what happens to generate the action potential. 3) Increase the single channel conductance. Generally this is fixed.

What is the maximum Na conductance? The maximum occurs when  $P_0 = 1$  and equals  $N\gamma$ . The notation we use for maximum conductance is  $\bar{g}_{Na}$  with a bar over the  $g_{Na}$ . Then the actual sodium conductance is the maximum conductance multiplied by the open probability of a single channel.  $\bar{g}_{Na} = g_{Na}P_0$ . We will discuss this more later.

**4** Now some channels have multiple closed states. For example



This can lead to interesting behaviors of channel openings. For example if the rates between C1 and C2 are slow and the rates between C2 and O are fast, we can observe “bursty” behavior with some long closed times (where the channel is trapped in the C1 state for long periods) and short closed times where the channel makes transitions between C2 and O.

The reason we are talking about single channels and transitions among various open and closed states is that:

1) we know that Na and K channels responsible for the action potential can exist in multiple closed states, and

2) the transitions among states are not necessarily constant.

Na and K channel transitions, in particular, are altered by changes in voltage. We also know that transition rates for some ion channels may also be affected by calcium concentration (2 lectures from now), or by phosphorylation (later in the course).

Changes in the transition rates can lead to different distributions of open times, different probabilities of a channel being open ( $P_o$ ), and consequently different current flows across the membrane. (In some cases single channel conductance may be affected, but for now we will consider single channel conductance to be constant).

**5** For “voltage-dependent ion channels” the transition rates are functions of voltage. Let’s consider potassium. As voltage changes, charged “gating particles” in the channel move (see Fig 7.6). With sufficient movement the channel becomes open.

The transition to the open state still occurs stochastically. However, for potassium, the probability of the channel being open increases with depolarization as shown here. If we add up the open times and closed times of a single channel at a given voltage, we can construct a plot of the open probability of a channel as a function of voltage as shown in the bottom of this overhead.

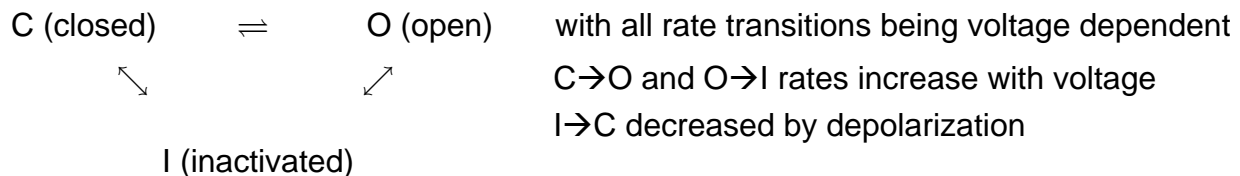
I should mention here that in the literature you will see curves of this nature described as activation curves. It is common to fit activation curves to the function:

$$\frac{1}{1 + \exp\left(\frac{V - V_{1/2}}{-k}\right)}$$

where  $V_{1/2}$  is the voltage where the probability of a channel being open is 0.5 and  $k$  determines the slope of the curve. In models the activation curve for each conductance is given a letter and for the K conductance that repolarizes the action potential, the letter

is  $n$ . What this activation curve tells us is that if we were able to change the voltage to a particular value, the permeability would change over time to a new steady-state value that can be read from this graph.

**6** For sodium the situation is a bit different. It turns out that the Na channel has multiple closed states, one of which we call the inactivated state. In the simplest model of state transitions for a Na channel we have



$\text{C} \rightarrow \text{O}$  is fast,  $\text{O} \rightarrow \text{I}$  is somewhat slower and  $\text{I} \rightarrow \text{C}$  is slow. Think of Na as having two gates, an activation gate and an inactivation gate. What does Na conductance and current look like for these sequential transitions?

**7** It is possible to construct an activation curve for Na analogous to the activation curve shown for K above. The activation curve for Na as a function of voltage is given the letter  $m$ . However here there is also an inactivation process. We can construct an inactivation curve as a function of voltage and to this we assign the letter  $h$ . These are shown here and in Fig. 7-11 in the book.

How can we interpret these curves? When thinking about these curves, it is best to think of the Na channel as having an activation gate and an inactivation gate as shown here. At hyperpolarized potentials the activation gate is closed and  $m$  is approximately 0. This means that the probability of the Na channel being open is quite small. At depolarized channels,  $m$  is approximately 1, which means that the channels have been activated. However  $h$  here is approximately 0 so the probability of the channel being open is again quite small. So how does the channel ever open?

To think of this consider

$m = 0$  means the  $m$  gate is closed,  $m = 1$  means the  $m$  gate is open

$h = 0$  means the  $h$  gate is closed,  $h = 1$  means the  $h$  gate is open

For the Na channel to be open both the  $m$  and  $h$  gates must be open. How can we ever get the Na channel to open? Fortunately the **timing** of the processes is what matters. At rest the  $m$  gate is closed and the  $h$  gate is open. When the voltage is stepped to a depolarizing level, the  $m$  gate opens quickly. At some time later the  $h$  gate closes and the permeability drops back to near zero.

$m$  closed,  $h$  open  $\rightarrow$  (fast)  $m$  open,  $h$  open  $\rightarrow$  (slower)  $m$  open,  $h$  closed. Later,  $m$  closed,  $h$  closed and then back to  $m$  closed,  $h$  open.

**8** Now let's consider what happens during an action potential during a transient suprathreshold depolarization.

First, Na channels open (m increases, Na permeability increases, Na C→O rate increases)

1) If the permeability for Na is increased 500-fold, then the new  $V_m$  (calculated with the GHK equation) would be +30 to +40 mV. Biophysically this increased permeability reflects the fact that the probability of Na channels being in the open state has increased drastically. Because the permeability change happens so quickly (100  $\mu$ s) and is regenerative (see next point), the voltage does indeed rise to these levels.

2) The probability of Na channel opening (and hence an increased permeability) is dependent on voltage and increases with depolarization. The process is regenerative. A sufficiently large initial depolarization increases the Na channel openings which increases Na current, which induces further depolarization, which induces more Na channel openings, more current, more depolarization, etc.

Second, K channels open (n increases, K permeability increases, K C→O rate increases).

1) The opening probability of the delayed rectifier K channel increases with voltage. We call this channel the “delayed rectifier” because the channel opens more slowly than Na channels and may be considered to be delayed with respect to Na channel opening.

2) Even with large increases in K permeability, we still need a means to shut off the Na current to bring the voltage back to rest and Na inactivation provides this.

Third, Na channels inactivate (h decreases, Na permeability decreases, Na O→I rate increases).

So as voltage increases, the open probability (activation) increases quickly, but at the same time, but at a much slower rate, the open probability decreases because of inactivation. See Fig. 5-7. The combination of Na inactivation and K activation cause termination of the action potential and the voltage hyperpolarizes. In fact the voltage hyperpolarizes below rest usually.

**9** Fourth, K channels deactivate (n decreases, K permeability decreases). As the voltage hyperpolarizes, K channels deactivate (reverse of activation). Note that K channels do not inactivate like Na channels. At 0 mV K activation will remain constant. The voltage that at this time is hyperpolarized to rest (we say there is an afterhyperpolarization) and now starts to return to rest. Note, the very few Na channels that were not inactivated are deactivated in this period.

Fifth, Na channels deinactivate (h increases). The inactivated Na channels return to the closed state and become ready to be activated again. The afterhyperpolarization allows inactivation to be relieved (see the h inactivation curve).

**10 THRESHOLD.** Let's talk about threshold. I have talked about threshold as if it were some magical fixed value. While it is clear that a threshold exists, it is not necessarily fixed and does not need to be mysterious. What makes up this threshold?

**SMALL DEPOLARIZATIONS.** At rest there is a relatively large background K conductance (note the relative permeabilities mentioned earlier) in addition to the delayed rectifier K conductance. When voltage is slightly depolarized what happens?

The potassium current increases. Why? Because even if conductance is constant, the driving force increases since  $I_K = g_K(V - E_K)$ . Plus any increase in delayed rectifier conductance will increase K current further. The increase in  $I_K$  is at least linear.

What about Na? Na conductance increases, as we know, but what about the Na driving force ( $V - E_{Na}$ )? The driving force decreases. For small depolarizations, the reduced Na driving force offsets any gain in  $g_{Na}$  from the depolarization. The result is that the increase in  $I_{Na}$  is sublinear. Thus  $I_{Na}$  increases less than  $I_K$  and the net current is outward (hyperpolarizing). The voltage due to the small depolarization is brought back to rest and the regenerative Na process is blocked.

**LARGER DEPOLARIZATIONS.** With larger depolarizations you can get the induced  $I_{Na}$  to be greater than linear. Net current will be inward (depolarizing) and the regenerative Na process takes off. It is this interaction of two processes with different voltage dependencies (background K and regenerative Na) that creates the threshold.

**11** This is illustrated in this overhead. Here the current injection causes a small depolarization. Because of the driving force effect you see an initial outward current, countered a bit (but not enough) by the increased Na current and later the delayed rectifier conductance increase kicks in making the voltage hyperpolarize back to rest.

If the depolarization is a little larger, you still see the initial outward current increase because of the driving force, but the Na conductance slowly overruns this and is able to give an action potential.

Note: If a depolarization is just barely suprathreshold, the action potential may be delayed considerably. You should try changing the current amplitude in the computer simulations to see this delay.

**12 REFRACTORY PERIOD** After the action potential there is a period of time called the refractory period in which it is either impossible (absolute refractory period, 0.5 – 1.0

ms generally) or possible only with severe effort (relative refractory period beyond 1 ms) to generate an action potential.

What causes the absolute refractory period?

Immediately after an action potential, almost all Na channels are inactivated (as opposed to being in the open or closed states). In addition K channels are still activated because they deactivate (close) slowly. To get an action potential Na channels need to move from the C state to the O state. However if most channels are in the I state, there will not be enough channels making the C → O transition during a subsequent depolarization to cause an action potential. Furthermore, a depolarization will activate more K channels and the increased Na current caused by the few Na channels that can activate is overwhelmed by the remaining K current and the newly generated K current.

In time we reach a relative refractory period. Eventually the afterhyperpolarization reduces Na inactivation and deactivates K. With more Na channels in the C state as opposed to the I state, a depolarization now, if large enough, could make enough Na channels become activated to start an action potential.

This depolarization needs to be much larger than normal for two reasons. 1) Because the proportion of inactivated Na channels is still significant, more depolarization is needed to activate a larger proportion of the remaining Na channels. 2) A larger depolarization has the effect of reducing the Na driving force and increasing the K driving force. We may need to depolarize the cell twice as much to get the same increase in  $g_{Na}$  as before, but the  $I_{Na}$  would be less because of the reduced driving force.

**13** Finally, Na inactivation can cause an interesting phenomenon. A stimulus in the relative refractory period may cause an action potential with a long delay. However if this stimulus is delivered a short time later, the action potential is generated sooner. The delay allows the inactivation gate to open a little more as shown here (so there is slightly less inactivation). You might see if you can see this phenomenon in the computer lab.

Finally (again), I should note that although action potentials increase concentrations of Na inside and K outside, the concentration changes are quite small, in the same ballpark as the numbers we calculated earlier, in the  $\mu\text{M}$  range for Na for example. Pumps quickly restore the resting concentrations.

## PROPAGATION OF THE ACTION POTENTIAL

**14** Once the action potential is generated at the initiation site (axon hillock, first node, spike generating zone), there is passive spread of depolarization to the next patch of membrane and when this patch exceeds threshold, a new action potential is developed.

The old analogy of a gunpowder fuse holds here. Heat from the presently burning portion brings the adjacent portion above ignition temperature and the burning self-propagates.

Because of the absolute refractory period, the action potential can only go in one direction (i.e., cannot turn around). Why? By analogy with the fuse, the ash trail cannot burn again! Once a patch of axon enters the absolute refractory period, passive spread of depolarization from a neighboring axon patch cannot depolarize the refractory patch.

However, if you inject current into the middle of an axon, what happens? Action potentials spread away from the point of injection in both directions. Alternatively, if action potentials are generated at two points in an axon and the action potentials travel towards each other, what happens when they meet? Do they pass through each other? No, they are both extinguished since the action potentials cannot pass through each other's absolute refractory period. You will do this in computer lab.

So what determines propagation velocity in an unmyelinated axon? Earlier I mentioned that conduction velocity is proportional to the square root of the diameter. If you want to see where this comes from, consider that velocity is distance divided by time. Using our cable parameters velocity should be proportional to the space constant divided by the time constant or  $\lambda/\tau$ .

**15** If we plug in our previous definitions of  $\lambda$  and  $\tau$  we get  $\frac{\lambda}{\tau} = \frac{\sqrt{\frac{R_m d}{4R_a}}}{R_m C_m} \propto \frac{\sqrt{d}}{C_m \sqrt{R_m R_a}}$  so

we see that conduction velocity increases as  $d$  is increased and decreases as  $R_m$ ,  $R_a$ , and  $C_m$  are increased.

For an action potential in an unmyelinated axon, the important thing is to depolarize the next patch of membrane above threshold. Suppose we approximate the action potential by a 100 mV depolarization. We have already learned that the steady-state voltage along an infinite cable will decay according to  $V=V_0 \exp(-x/\lambda)$  and for larger diameter,  $\lambda$  will be larger and the decay will be less as shown here.

However, action potentials are not steady-states and we need to see the time course of this voltage at given distances. This is shown in the plot on the right. Here we see the voltage profile over distance at times  $t=0, 0.5, 1.0,$  and  $1.5$  for diameters equal to 1 and 4. (Note if diameter is increased, input resistance decreases and we need a larger current to get a given voltage. In the plot shown here current was 1 nA for the diameter=1 cable and 8 nA for the diameter = 4 cable. With this adjustment, the action potential at the site of injection (the origin of the cable) was identical in time in the two cases)

What we see here is that when the diameter is increased, the decay with distance at each time point is less (voltage is higher). Thus, voltage will cross threshold sooner for earlier time points in the cable with the larger diameter.

**16** I don't want to dwell on this point much longer, but here are some plots illustrating action potentials at 0, 500 and 1000  $\mu\text{m}$  along an axon where the diameter is either 1 or 4. The stimulus was calibrated (as noted above) to get the action potential at  $x=0$  to have the same time course with both diameters. We clearly see that the action potential travels faster in the larger diameter axon.

In the lower part of this overhead we have voltage plotted as a function of distance along the axon every 0.5 ms. The early part of these plots was shown in the previous overhead. What may seem strange about these plots is that they show that the voltage is highly depolarized over a large distance in the axon. For example, at 3 ms voltage is above 0 mV for about 200-900  $\mu\text{m}$  in the diameter =1 axon and over 500-1900  $\mu\text{m}$  in the diameter =4 axon.

**17** So for unmyelinated neurons, the way conduction velocity can be increased is through increasing diameter. Invertebrates use this mechanism and this is why the squid giant axon has such a large diameter. Conduction velocity is very important for escaping predators. Increasing diameter does have a cost however. First what happens to input resistance when diameter is increased? It goes down. This means that it takes more input to initiate the action potential. Apparently the fear of your personal demise elicits sufficient input! A second cost is that a larger diameter with more Na and K channels will cause, in absolute numbers, more Na and K ions to cross the membrane. This means that more ATP has to be used to restore the resting concentrations.

Vertebrates have evolved another means to increase conduction velocity, and that is to surround the axon with myelin (oligodendrocytes or Schwann cells). Since the myelin is membrane, a reasonable approximation is that  $n$  layers of myelin will reduce  $C_m$  by  $n$  and will increase  $R_m$  by  $n$ . This is the situation that you simulated in computer lab when you divided  $C_m$  by 10 and multiplied  $R_m$  by 10 although larger numbers could be used. With a smaller capacitance, there is no need to charge up much capacitance; with a larger  $R_m$  the current cannot escape through the membrane as easily. The result is that the current goes down through the axial resistance to the next node. The action potential thus leaps from one node to the next where it is regenerated. The conduction velocity thus depends on the distance between nodes.

Anatomically, the internode distance seems to be proportional to the diameter, so the conduction velocity here is actually proportional to diameter instead of the square root of diameter as in unmyelinated fibers. Advantages of myelination are that since input resistance of the axon is larger ( $R_m$  larger), less input is needed to send the action potential down the axon. Since Na and K channels only have to be at nodes the metabolic cost is less for the axon. However, there is a cost of developing and maintaining the myelin sheaths and the risk of demyelinating diseases such as multiple sclerosis.