The Unmyelinated Axon

Experiment with the Impulse Traveling in the Classic Giant Axon of the Squid

In previous tutorials we have simulated the stationary synaptic potentials that diffuse to the neighboring membrane units. Here we start to look at the propagating action potential. In this case, the stimuli elicits an action potential at one point. Then the depolarization potential diffuse to the neighboring membrane units and opens the ion channels in these units, eliciting additional action potentials. As the process goes on, the action potential is generated along the neuron, hence a propagation.

The squid axon is long and large in diameter. And it is unmyelinated. In mammals, however, most axons are myelinated to increase the propagation speed without having to increase the axon diameter. In mammals, these unmyelinated axons carry sensations that do not require action in the sub-second time.

In this tutorial, we are going to achieve the following goals:

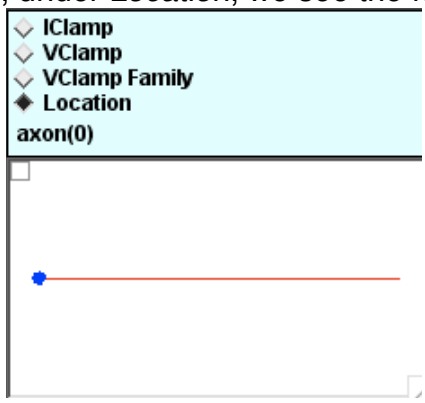
- To understand how the action potential is propagating along the axon.
- To relate the shape of the action potential as a function of time and as a function of space.
- To understand how axon diameter and temperature can affect the action potential and its propagation velocity.

Record the action potential as a function of time at various locations along the axon.

Propagating action potential at one point

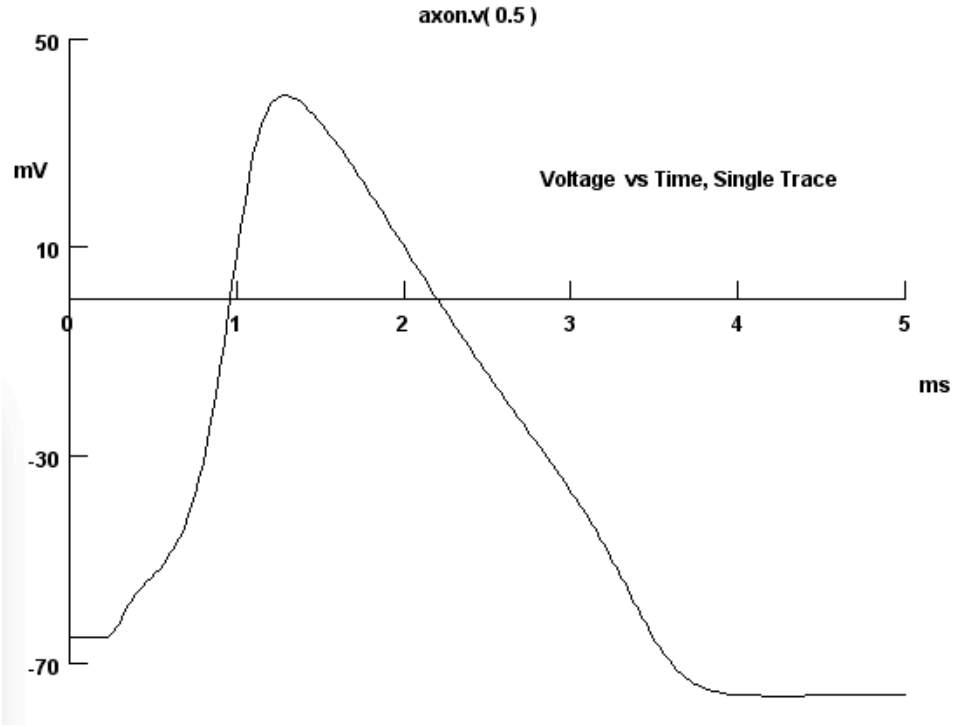
Start the simulation, one graph panel is brought up: *Voltage vs Time, Single Trace*.

In the *Stimulus Control* panel, under *Location*, we see the following image.



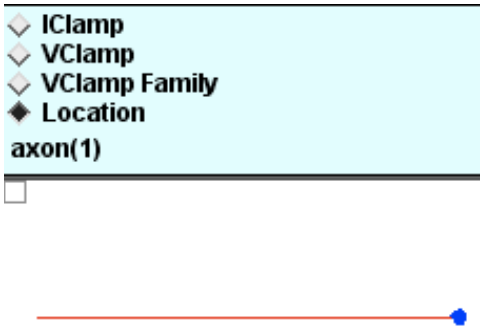
The red line represents the length of the axon, and the blue dot represents the place of the stimulus impulse. Here we have the impulse at the left end of the axon.

We may directly Reset & Run.

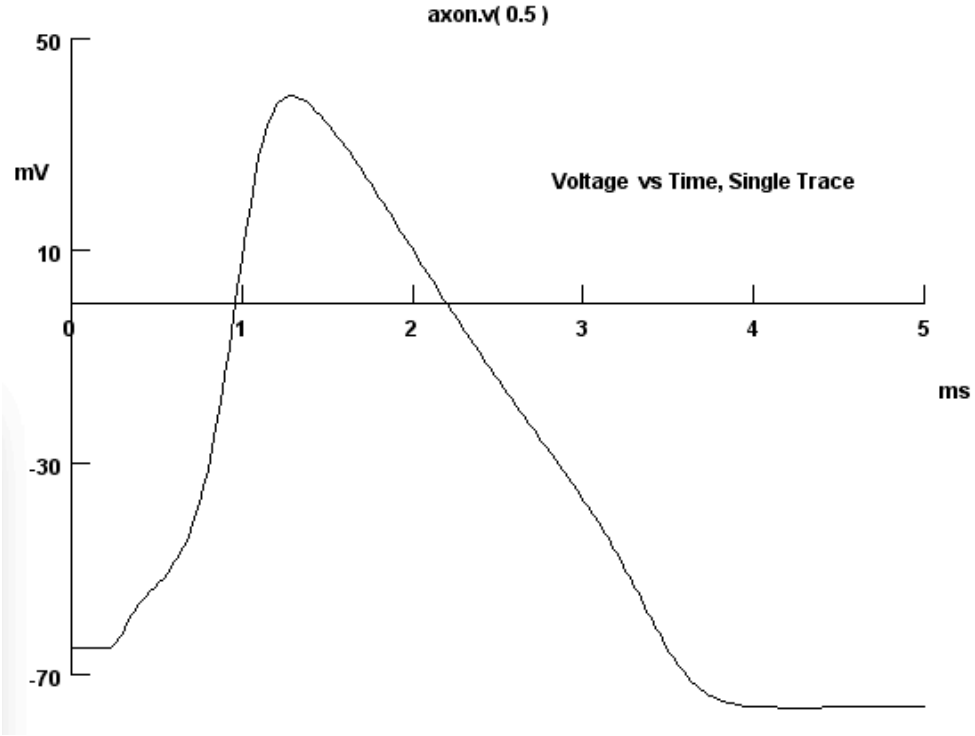


In the graph, *axon.v(x)* represents where the electrode is inserted to record the potential value. Here x=0.5 means the potential is recorded in the middle of the axon.

Move the stimuli to the right end of the axon,

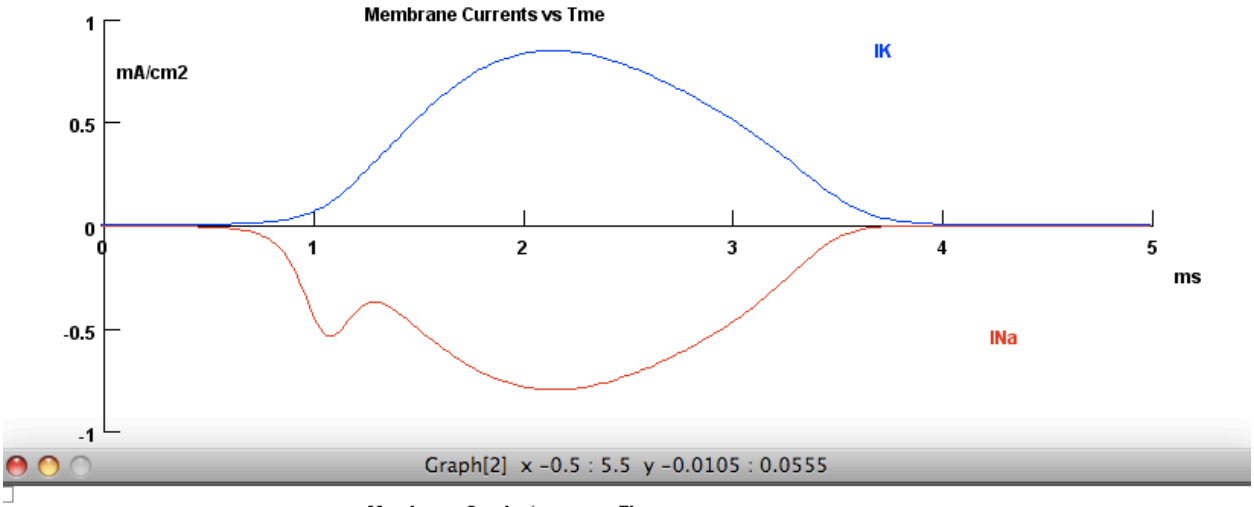


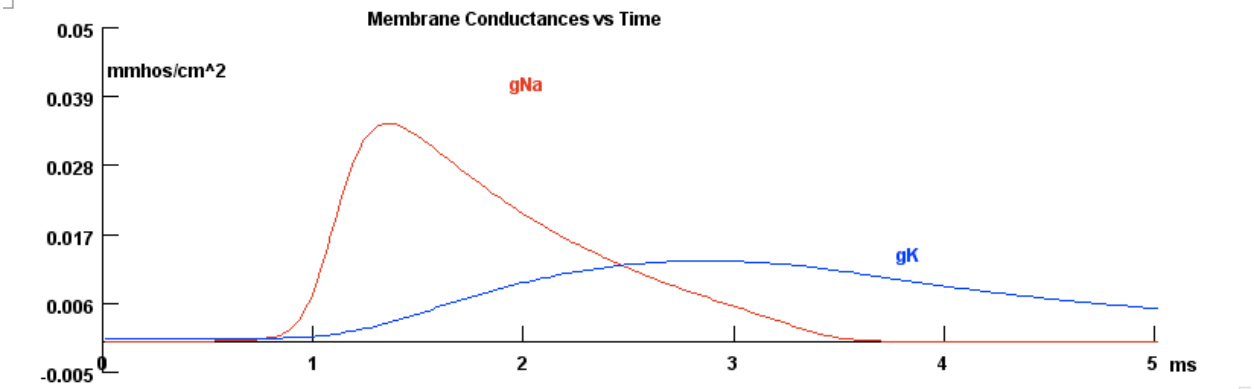
we have exactly the same shape of action potential at 0.5:



This result shows that the action potential is propagating both directions with the exactly same mechanism.

We observe the shape of the action potential is the same as what we saw in the stationary action potential in previous tutorials. Now we may bring up *Membrane Currents* and *Membrane Conductances* panels:

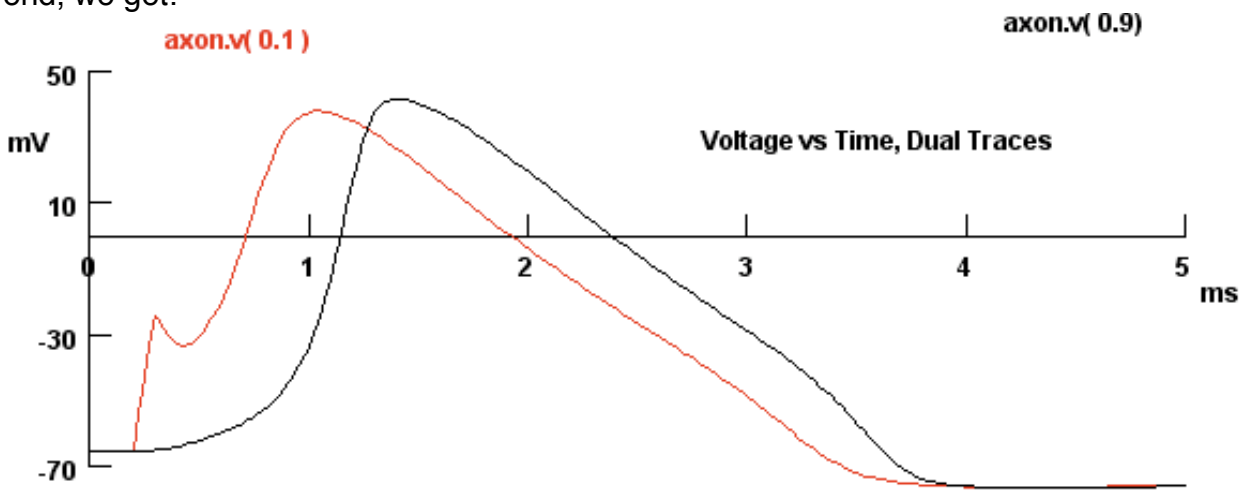




These figures are exactly the same as what we got for a stationary action potential. This result shows that at each local point, the propagating action potential works exactly the same way as if it is stationary.

Propagating action potential at two different points on axon

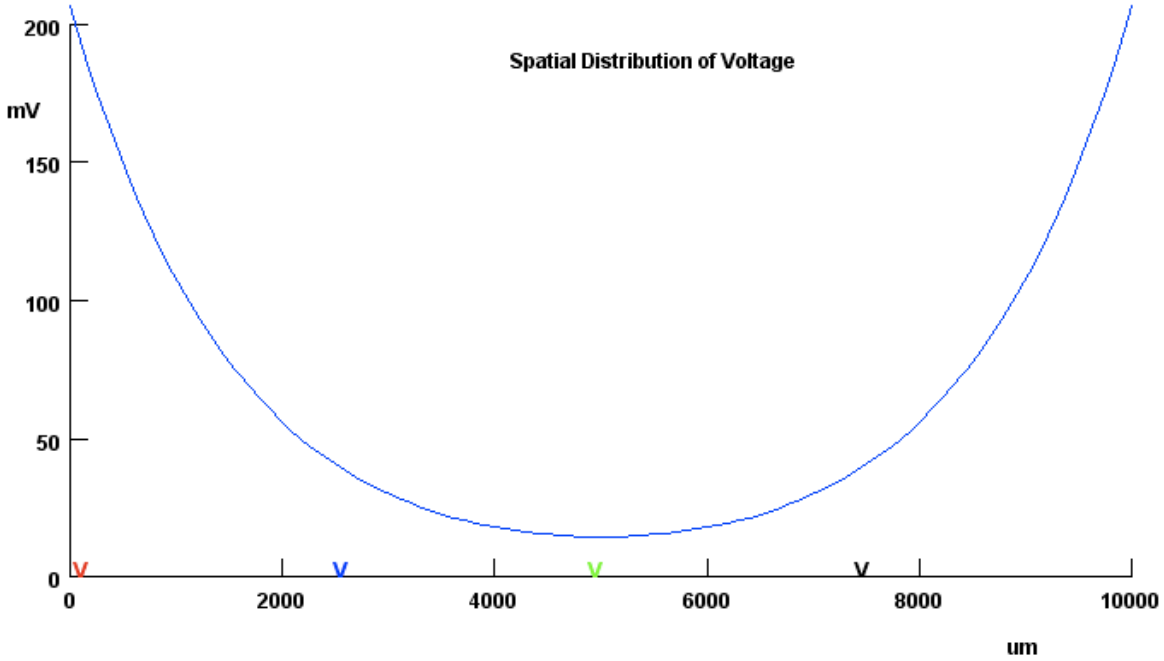
Now we pull up the *Voltage vs Time, Dual Traces* panel. Putting the stimuli at the left end, we get:



As before, *axon.v(0.1)* and *axon.v(0.9)* shows that the action potential are recorded at two points: 0.1 and 0.9. The red line represents the one at 0.1, and the black line represents the one at 0.9.

Here we have four observations:

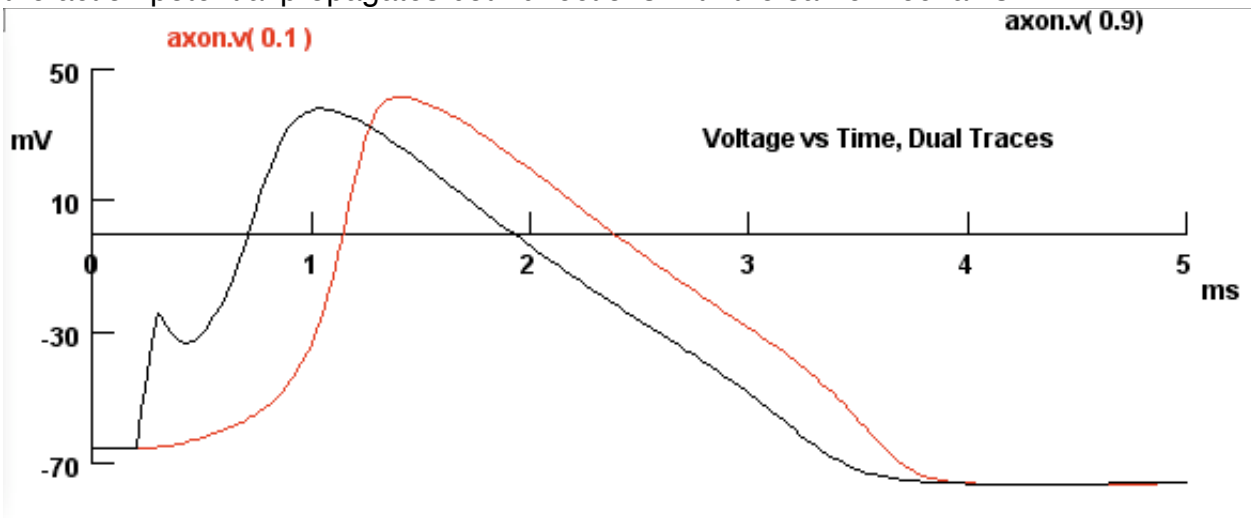
1. The red line proceeds the black line, meaning that the action potential occurs earlier at 0.1 position than at 0.9 position. This is one evidence of propagation.
2. In the depolarization period, there is a bump in the red line, which is due to the original stimuli. When the stimuli is removed, the potential drops a little and then continue to depolarize again. But there is no such bump in the black line, meaning that the effect of stimuli cannot reach 0.9 position in the axon. The action potential is solely evoked by the neighboring membrane depolarization diffusion.
3. The peak of the action potential is higher in the black line than in the red line. This is due to the close right end close to the 0.9 position. The boundary condition for action potentials with both open ends is the voltage being the resting potential. But when there is a closed end, the boundary condition changes. The action potential would act as if there is a symmetrical action potential on the other side of the closed end. Hence the boundary potential will be higher. This can be visualized by the following graph generated from *The Passive Axon* tutorial:



Here we put one stimuli each end of the axon. Instead of summing up, the two action potentials meet and connect with each other with even symmetry.

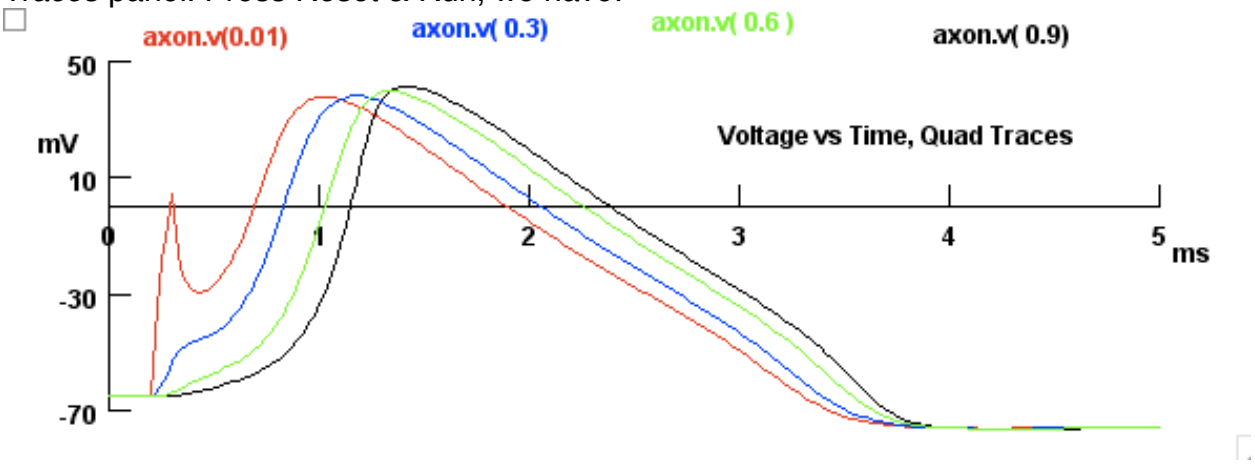
4. Approaching the end of the repolarization, the potential drops more abruptly in the black line than in the red line. This is also a result of the closed end.

By moving the stimuli to the right end of the axon, we observe exactly the same shape of the action potentials, with only the reverse of the position. This result confirms that the action potential propagates both directions with the same mechanism.



Propagating action potential at four different points

To further visualize the action potential propagation, pull up the *Voltage vs. Time, Quad Traces* panel. Press *Reset & Run*, we have:



In this graph, we can see more clearly about the four observations we made above:

1. The closer the recording electrode is to the stimulus electrode, the earlier the action potential is initiated, confirming the propagation of the action potential.
2. The closer the recording electrode is to the stimulus electrode, the more obvious the stimulus bump is in the beginning.
3. The peak values of the action potential in the first three positions are about the same, while the last one is slightly higher, as the effect of the closed end.
4. The closer the recording electrode is to the right end, the more obvious the bump near the end of repolarization, also as the effect of the closed end.

Display the impulse as it travels along the axon.

Bring up the *Voltage vs Time, Single Trace* panel and *Voltage vs Space* panel. Press *Reset & Run* to visualize the action potential propagating along the axon.

In the movie in the *Voltage vs Space* panel, we see that the stimuli at the left end of the axon elicits a depolarization initially before it falls a little. Then it continues to rise and move toward the right end.

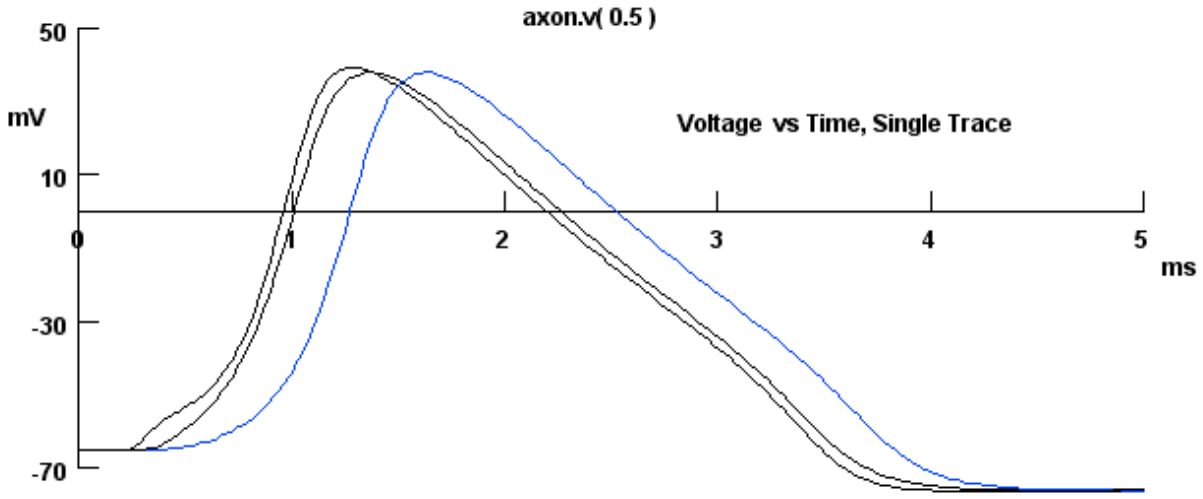
In particular, we may focus on the middle point in space and see how the potential rises and falls. This is exactly what is recorded in the *Voltage vs Time, Single Trace* panel.

Press *Reset* and then *Continue for* button to see the change in an advance-pause manner.

Observe the effect of changing the axon diameter on impulse propagation.

Select *Keep Lines* and press *Reset & Run*. This is the action potential for an axon with diameter of 500*μ*m.

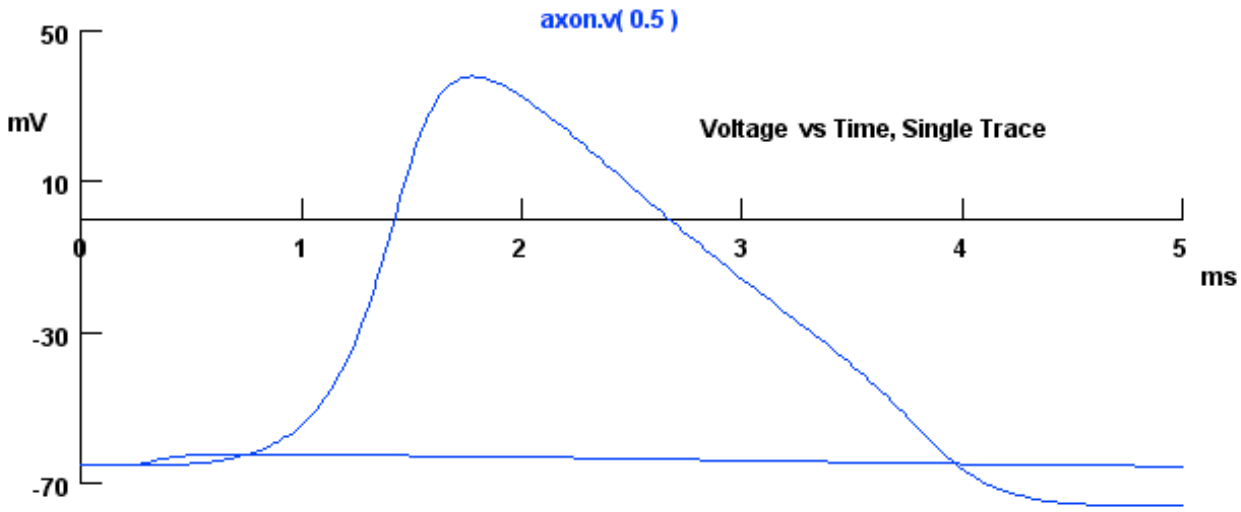
Press *Axon* Parameters and *c*hange the axon diameter to different values. Since the original value is large, we may try 100*μ*m and 50*μ*m. Also *Reset & Run*.



The left-most action potential is for 500μm; the one adjacent to its right is for 100μm; and the right-most action potential is for 50μm. We make the following three observations:

1. There is a right-shift trend with the diameter reduction. Given we have always been recording at the same place in the axon, the right-shift means that as the axon diameter gets smaller, the more delay there is for the action potential to propagate.
2. By looking at the initial depolarization, we notice the bump is getting smoother. For diameter of 50μm, the gradual and smooth curve looks the same as what we had in previous quad trace experiment at the point of 0.9. This is also an implication of the slower propagation.
3. The peak value of the action potential is getting smaller as the axon diameter getting smaller. This implies a loss of strength of action potential along the propagation.

For smaller axons, we should need weaker stimuli to evoke an action potential. We may select *Stimulus Control* panel, and change the *Amplitude* under *IClamp*, from 20000nA to 5000nA. Try axon diameter at 50μm and the default, 500μm. Keep lines, we have:



Indeed, the 5000nA stimulus current cannot evoke an action potential in an axon with diameter of 500μm, but can effectively evoke an action potential in an axon with diameter of 50μm. So smaller axons need not so large stimulus current to fire an action potential.

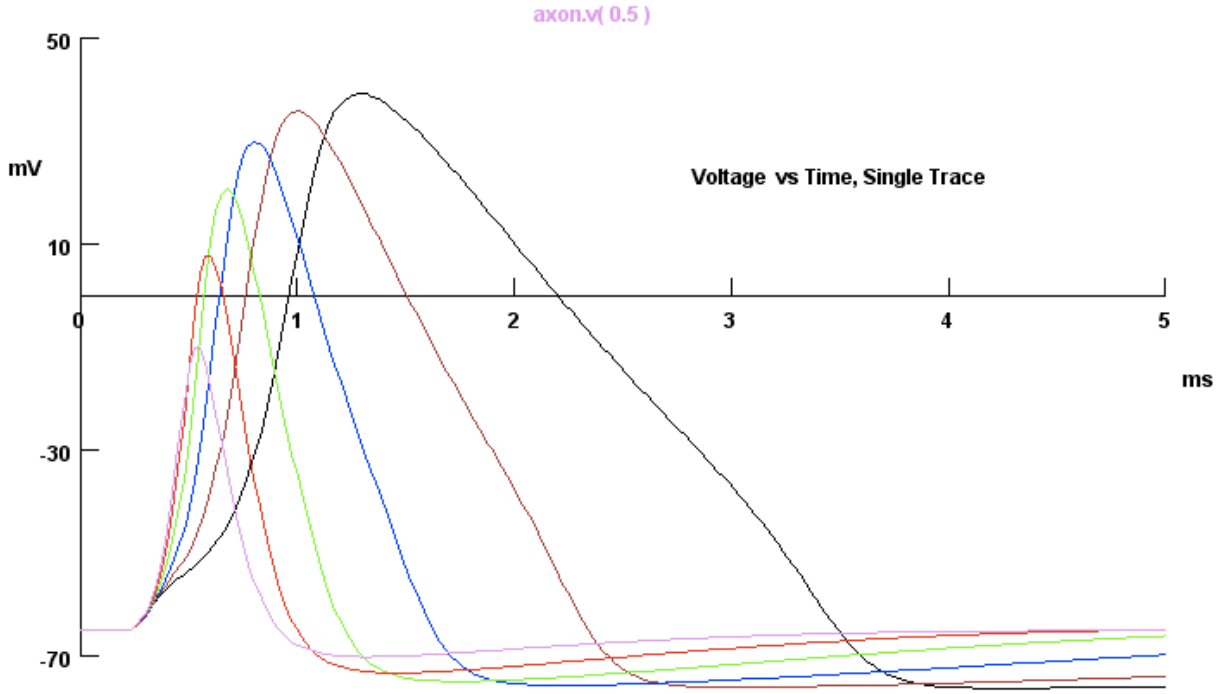
But why is it? The resting membrane has a fixed capacitance and conductance per unit area. Consider the Hodgkin & Huxley equation:

$$I = C_M \frac{dV}{dt} + \bar{g}_K n^4 (V - V_K) + \bar{g}_{Na} m^3 h (V - V_{Na}) + \bar{g}_l (V - V_l),$$

Total current | Capacitive current | Potasssium current | Sodium current | Leakage current

Here C_M=(Capacitance per unit area) X (Area),
and g_i=(Conductance per unit area) X (Area), i=K, Na, l.
So when the axon's diameter is decreased, its area per unit length is decreased. Given the same voltage-gated ion channels, to reach the threshold potential, we would need less current for smaller axons.

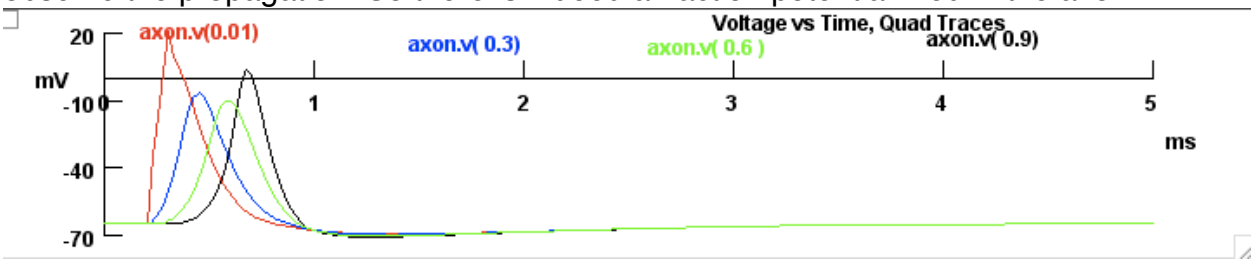
Observe the effect of changes in temperature on the propagation of the impulse.
Now we may reset everything back to default values.
In the *Run Control* panel, we may change the value of *Temp.* The default value is 6.3˚C. We may *Reset & Run* then select *Keep Lines*. We may increase the temperature by 5˚C from 6˚C and get the following graph:



And we summarize the data in the following table:

T (˚C)	6.3	11	16	21	26	31
t_peak (ms)	1.3	1	0.8	0.7	0.6	0.5
V_peak(mV)	39.3	35.7	29.7	20.7	7.7	-10.1

As a validity check, at 31˚C we use the *Voltage vs Time, Quad Traces* panel. We observe the propagation. So there is indeed an action potential fired in the axon.



From the table, we make the following three observations:

1. The time at which the action potential reaches its peak value is getting smaller. As we can also see from the graph above. This implies that the action potential propagation is getting faster with the temperature increase.
2. The peak value of the action potential gets smaller. Note that the depolarization is mainly contributed by Na, as opposed to K and other ions through the leak channel. This observation may be because when the action potential is

propagating too fast, although the Na channels can open up quickly sufficiently to form an action potential, they may not be fast enough to open up all Na channels available before the K channels are open sufficiently for repolarization.

3. The duration of the action potential decreases as the temperature goes up. This is another implication of briefer action potential.

Measure the velocity of propagation of the impulse.

To measure the velocity, we may insert two recording electrodes into the axon with known distance. Then we record the time when the action potential first passes zero at each site. And we may use the simple equation *velocity=distance/time* to solve for the propagation velocity.

Restore the default the values. To avoid the initial stimulus effect to propagation and the closed end effect, instead of using *Voltage vs Time, Dual Traces*, we use *Voltage vs Time, Quad Traces* and measure the time difference between 0.3 and 0.6 points. Note the distance between the two points is 3mm. You may also elongate the axon length to achieve a more accurate value. Now we may calculate:

$$v = \frac{\Delta s}{\Delta t} = \frac{3000\mu m}{1.03ms - 0.83ms} = 15.0m/s$$

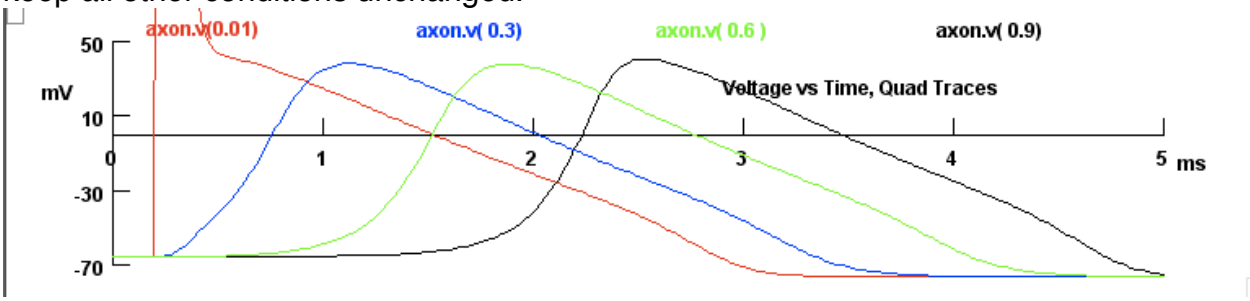
This is a very significant speed indeed.

Remember that we suggested changes in diameter and temperature affected the velocity. Now we are ready to tackle the problems by calculating them out.

<u>Velocity changes in response to axon diameter</u>

We postulated that the smaller axon is, the slower the action potential will be propagating.

Keep open the *Voltage vs Time, Quad Traces* panel. Reduce the diameter to 50*μ*m, and keep all other conditions unchanged.



And we may calculate

$$v = \frac{\Delta s}{\Delta t} = \frac{3000\mu m}{1.525ms - 0.765ms} = 3.95m/s$$

The velocity reduces about 83% as a result of axon diameter reduction.

How do we make sense of this difference? Recall the equation:

$$\tau \frac{\partial V}{\partial t} = \lambda^2 \frac{\partial^2 V}{\partial x^2} - I_{ion}$$

$\tau = r_m c_m$ is the time constant. r_m is the resistance across the membrane and c_m is the capacitance of the membrane. Recall that the resting membrane has a fixed capacitance and conductance per unit area. Conductance is just the reciprocal of resistance. So τ is independent of axon diameter.
λ is the length constant. r_i is the axial resistance along the axon.

$$I_{ion} = \sum_k g_k(V, \vec{W})(V - V_k)$$

Note that

$$\lambda = \sqrt{\frac{r_m}{r_i}} = \sqrt{\frac{R_m/\pi(d/2)}{R_i/\pi(d/2)^2}} = \sqrt{\frac{R_m}{2R_i}} \times \sqrt{d}$$

R_m is the membrane resistivity (Ω*cm^2), R_i is the intracellular resistivity (Ω*cm), d is the diameter of the axon. So λ is proportional to the square root of diameter.
Let

$$T = \frac{t}{\tau}$$

$$z = \frac{x}{\lambda}$$

then T and z are dimensionless variables. We may now define another variable

$$\theta = \frac{\Delta z}{\Delta T}.$$

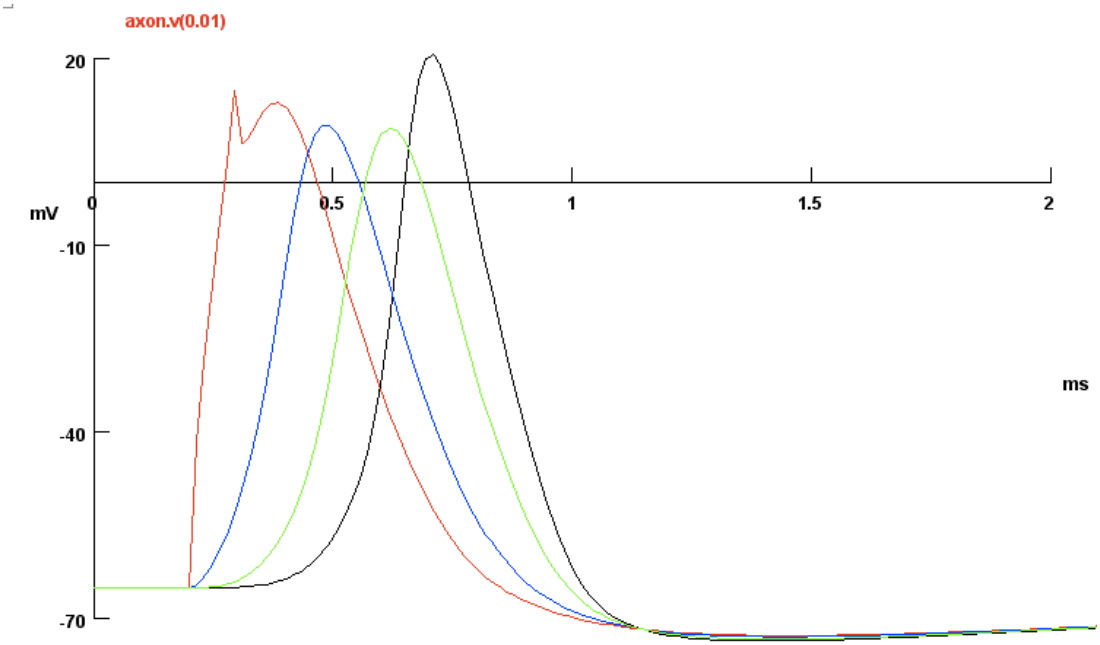
This "speed" variable can be used to relate velocity and the parameters:

$$\theta = \frac{\Delta x/\lambda}{\Delta t/\tau}$$

$$\frac{\Delta x}{\Delta t} = \theta \frac{\lambda}{\tau}$$

Note that since action potential is a traveling wave, from the above derivation, Θ can be regarded as wave numbers of the action potential. At the point of our measurement, we may consider the wave numbers in between are the same in the thick axon and the thin axon. So this equation is valid for us to estimate the velocity.
From the equation, the velocity is proportional to λ/τ, therefore square root of d. Since the axon diameter is reduced by 10 times, the velocity should be reduced by about 3.16 times, theoretically. In our empirical test, the velocity reduced by about 3.8 times, which is larger than what we expected.
This error can be reduced if we use longer axon for measurement. For example, if we use a very long axon of 100mm, then the ratio between the velocities of thick axon and thin axon is 3.32, much closer to 3.16.

<u>Velocity changes in response to temperature</u>
Keeping all other factors in their default value, we increase the temperature to 26.3˚C.



The velocity in this case is 22.22m/s, confirming our conjecture that the action potential propagates faster in higher temperature.

Indeed, in the Hodgkin & Huxley current balance equation, there are gating variables m, h, n coming into the equation. These variables are determined by

$$dm/dt = \varphi\,[m_\infty(V)-m]/\tau_m(V)$$
$$dh/dt = \varphi\,[h_\infty(V) - h]/\tau_h(V)$$
$$dn/dt = \varphi\,[n_\infty(V) - n]/\tau_n(V)$$

where ϕ is a temperature correction factor, $\phi=Q_{10}*[(temp-temp_{ref})/10]$, and in the HH equation, $Q_{10}=3$. So the higher the temperature is, the faster the gating variable is changing with time. This means the channel gates are responding more rapidly to depolarization. So we see the action potential travels through the axon in about 1ms.