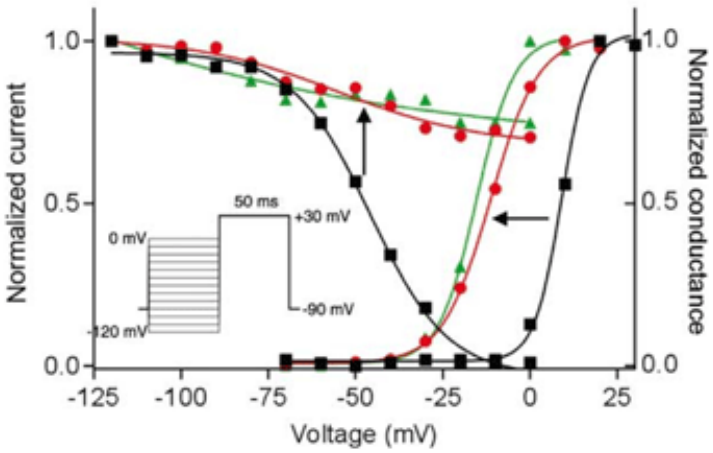
Lab R

With Emphasis on Effective Communication

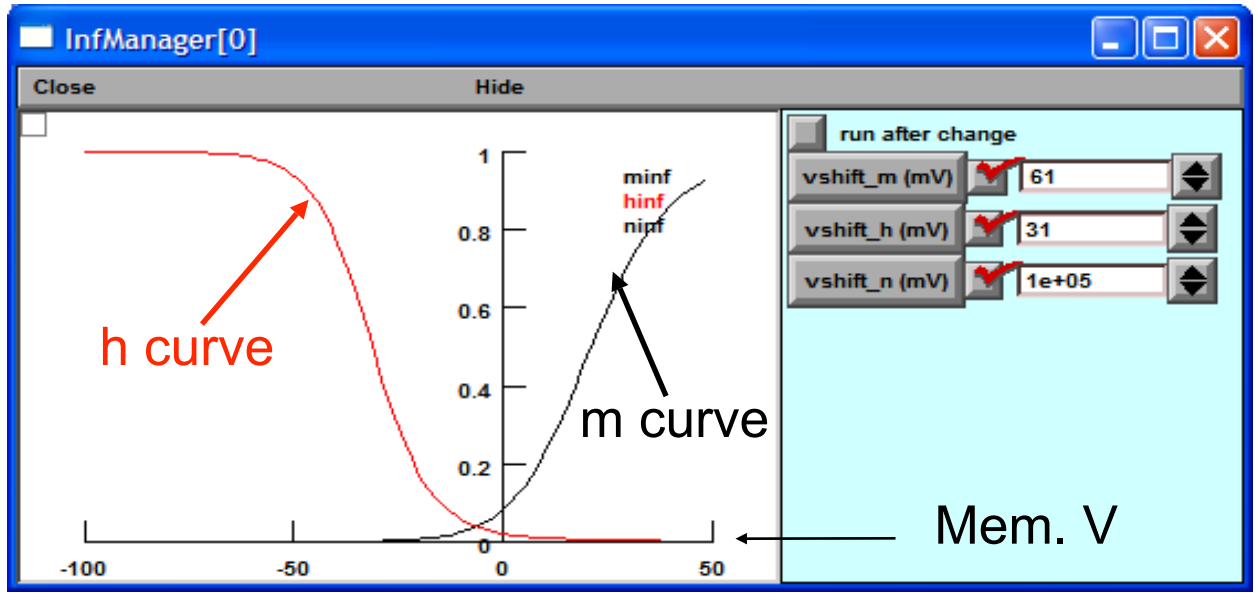
Biological Data Showing a BTX Block (Bosmans et al.)

Control m curve is on the right and h is on the left both in black. The curves with the two green curves represent the m and h (left and flat) after 10 um of BTX treatment.

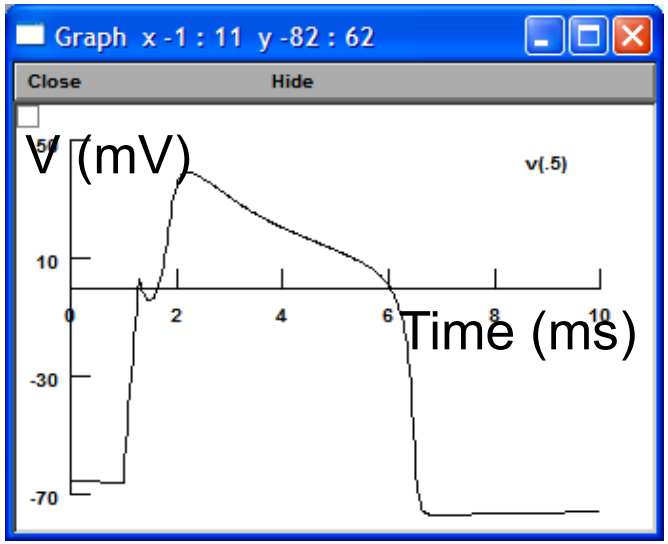


$Na_v1.8$ channel, before BTX treatment

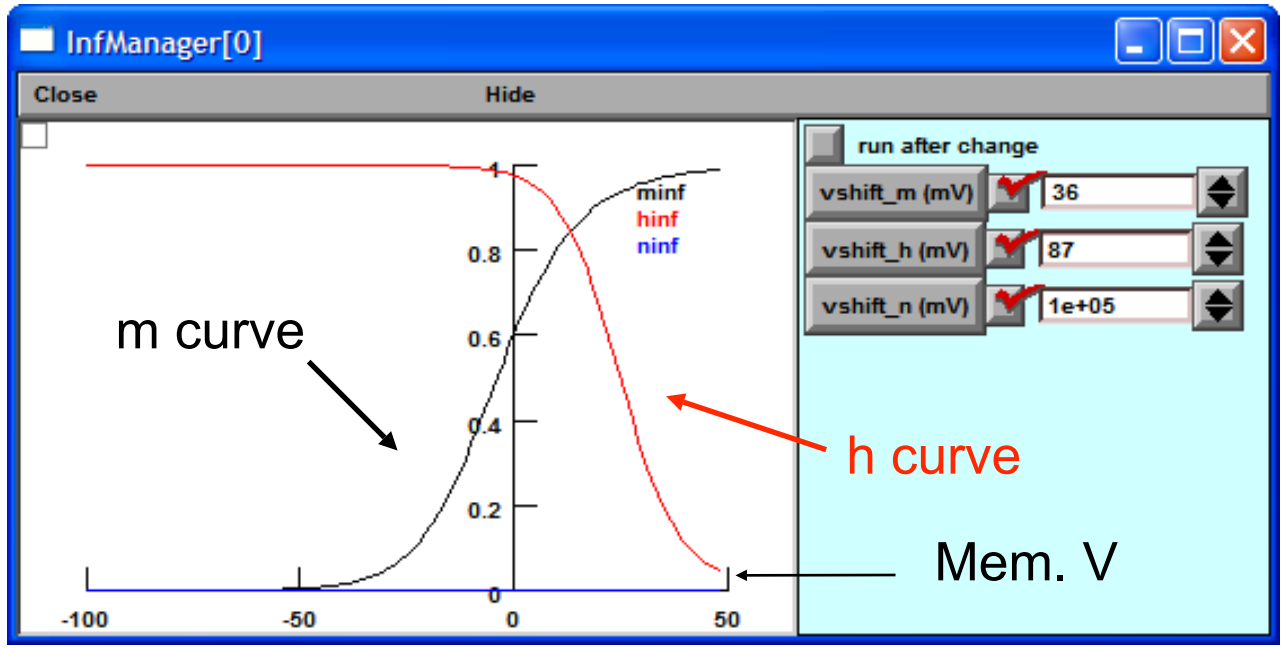
Below the biological results are reproduced with the computational model. (Only the 10 um block was reproduced. Also, the resulting action potential from the block is shown at the bottom left of the slide.



Resulting Action Potential from 10 µm BTX Block



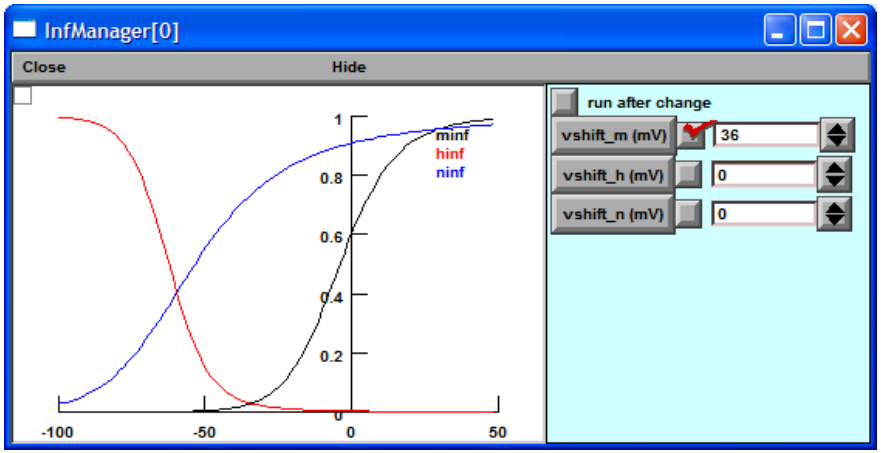
$Na_v1.8$ channel, after BTX treatment (10µm)

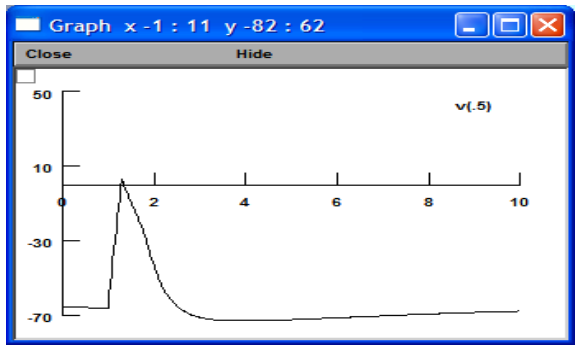


What do you think is the more effective action of this toxin in disrupting normal excitable membrane function—the blocking of inactivation or the shifting of the activation curve, m, to more negative voltages?

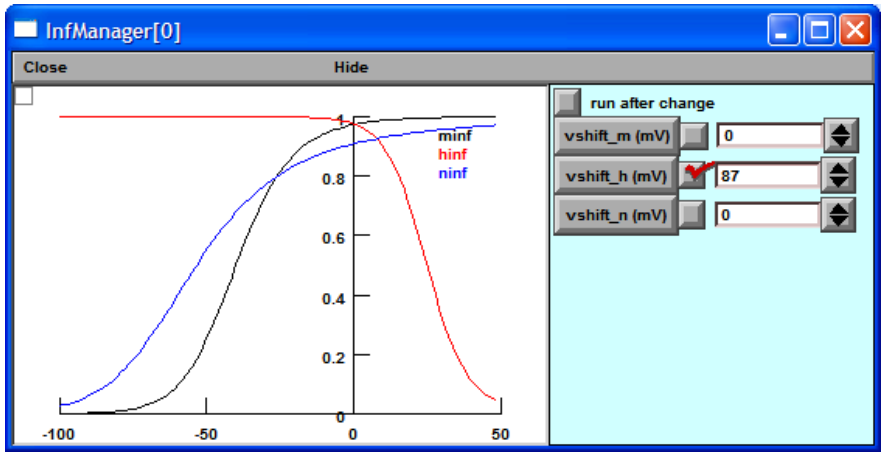
To test this we create two altered BTX affects: one that shifts the m curve exactly like that seen on the previous slide while leaving h untouched; and a second that shifts h while leaving m untouched. Then we can compare the resulting action potentials and make a conclusion.

BTX only having an affect on m

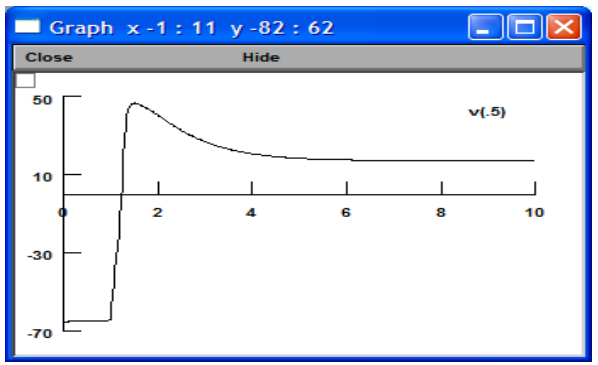




BTX only having an affect on h



The graphs have the same axes as the corresponding graphs on the previous slide. On the m and h plot is also the plot of ninf in blue.



The results are not so clear cut. However, in my opinion, because the affect on m still leaves the neuron with sum semblance of an action potential (although very small in amplitude), I think BTX's affect on h is more harmful. It completely abolishes any ability for a neuron to fire a train of action potentials and leaves the neuron at high voltage for a prolonged time—something that can cause cell death.

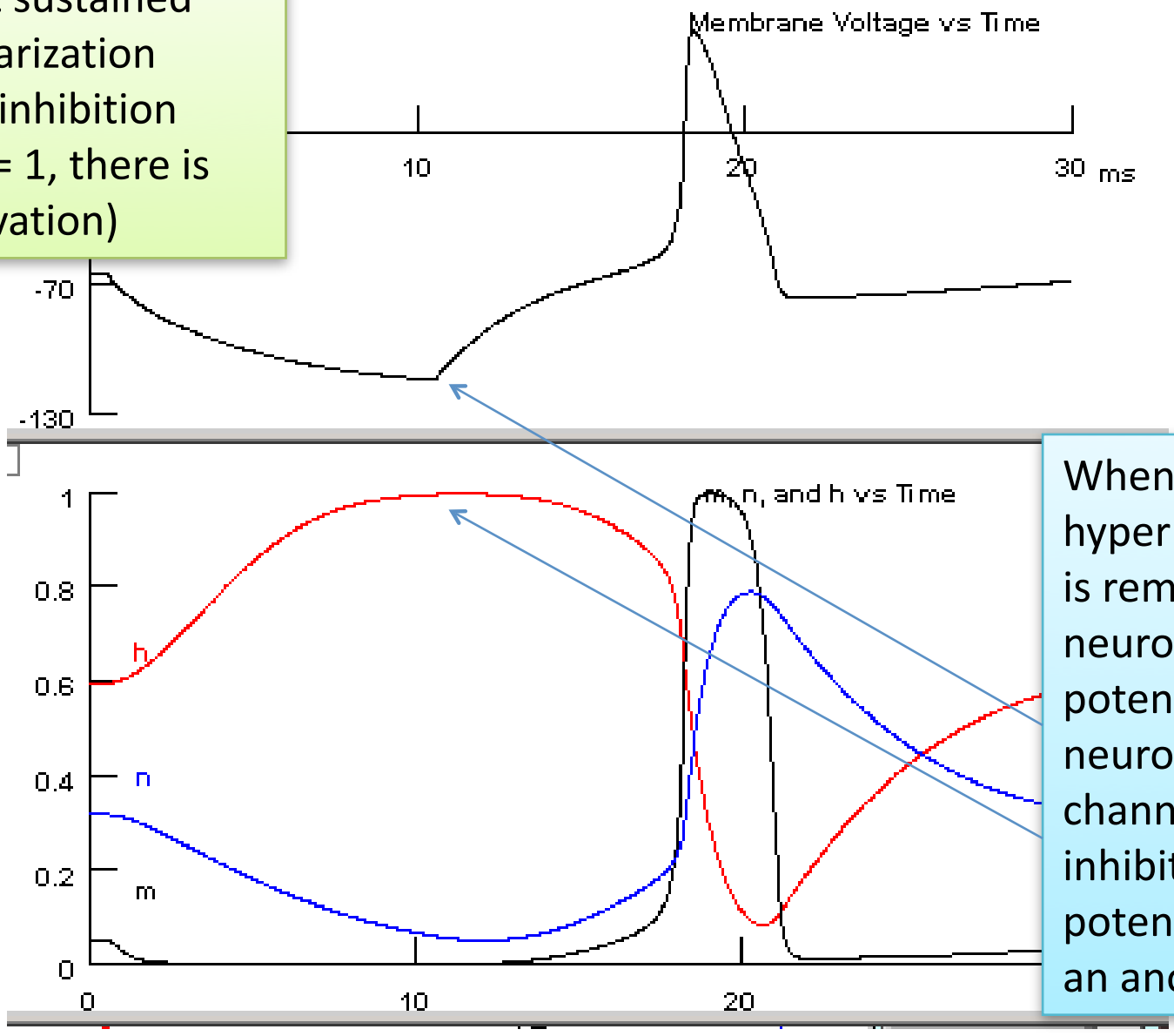
Explanation of Figures and BTX Block

BTX is a neurotoxin that comes from poison dart frogs. The LD_{50} is 2 table salt sized grains of the poison. It changes both the permeability and selectivity of the sodium ion channels. My guess is that the figure produced from biological data was created by applying BTX to a neuronal culture, then doing an electrophysiological work up to obtain the m and h curves in terms of the HH definitions. BTX is known to increase Na permeability and cause the cell to fire action potentials at resting potential. This corresponds to a leftward shift of the m curve—that is, an individual Na channel is more likely to open at more polarized voltages (near resting potential). Also, it seems from the biological data there is also an affect on h, the inactivation variable. With BTX applied, inactivation activity is increased over the range of all voltages, which translates to a sustained action potential that plateaus (no down phase). Notice that the computational results support this.

To mimic the affect of BTX in the computational model, m and n curves were shifted to match with the biological data. This is rather crude and does not provide much insight into BTX mechanism, or how the shift in the curves actually arises. However, it does provide an easy way to see the affect of BTX on the action potential (something we did not have biological data for). The results were a sustained action potential, which again is supported by the biological knowledge that BTX increases permeability while increasing Na channel inactivation.

Anode Break – an action potential from hyperpolarization

Sufficient sustained hyperpolarization removes inhibition (when h = 1, there is no inactivation)

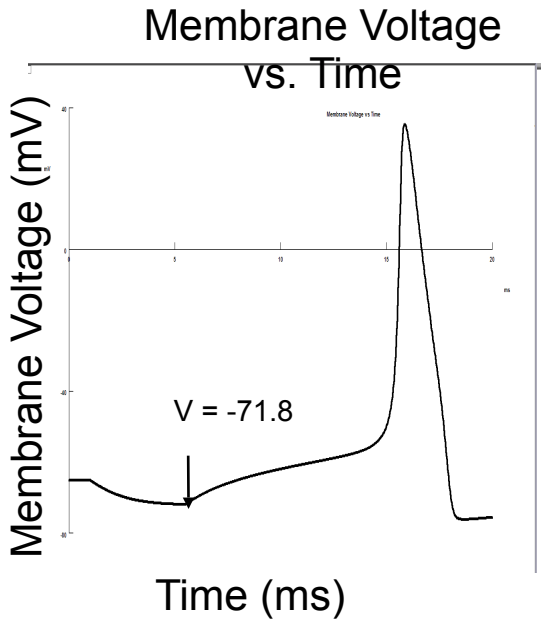


When the hyperpolarizing stimulus is removed and the neuron returns to resting potential (t = 10), the neuron's inactivation channels are still inhibited and an action potential occurs, called an anode break

The Anode Break

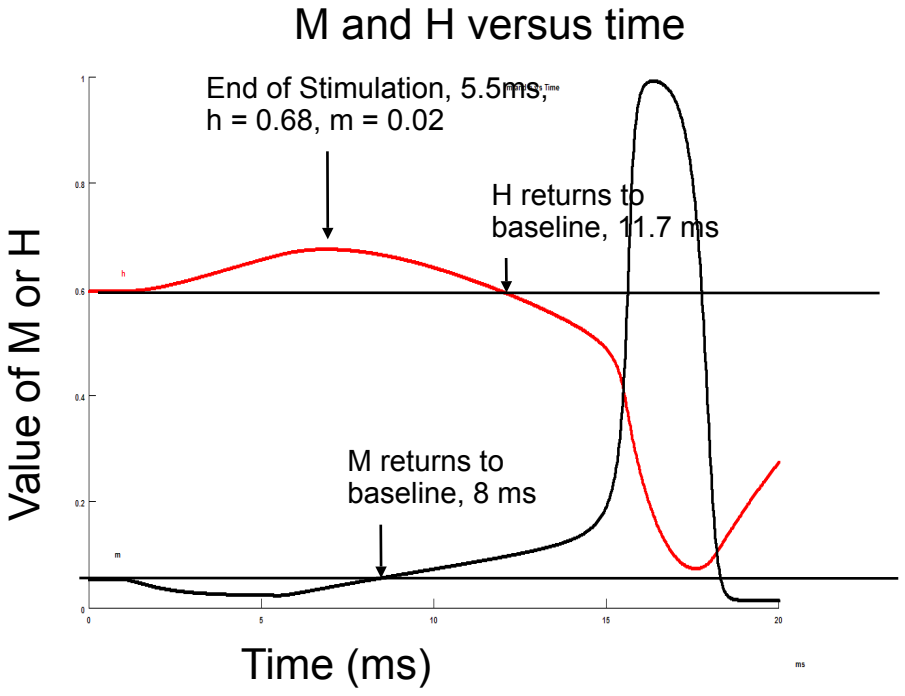
In the Hodgkin-Huxley model and interesting scenario occurs where briefly hyperpolarizing a cell and then releasing that stimulus can lead to an action potential. This occurs because the sodium channel is governed by both the kinetic variable m, the probability that an available channel will open, and h, the percent of channels that are not currently inactive.

When a small -0.005nA, 4.5ms stimulus is applied, the cell hyperpolarizes to -71.8mV. During this period an increasing number of the total channels become available for opening. Simultaneously, the more negative voltage reduces the probability that any given sodium channel will open.

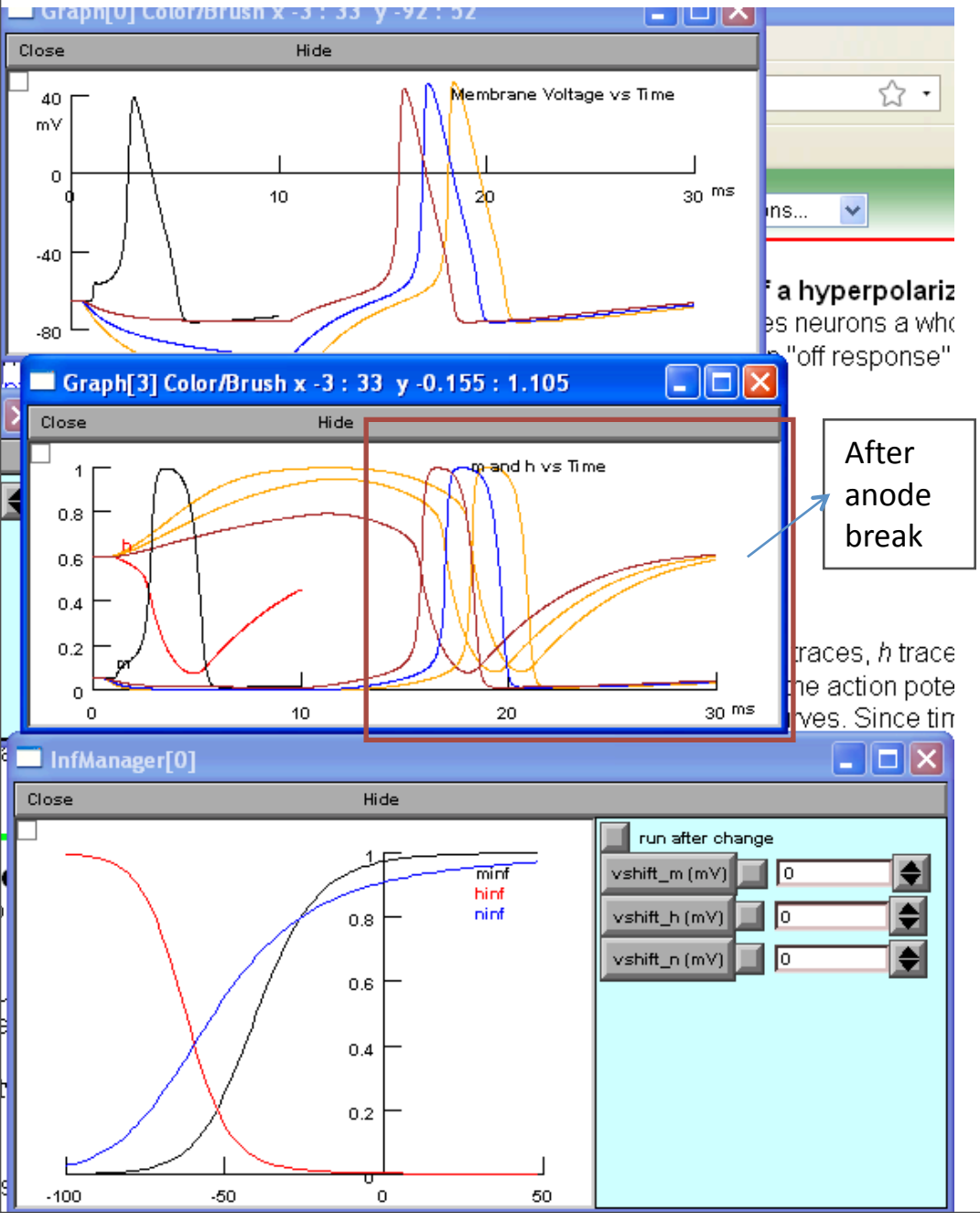


However, a significant difference exists in the rates at which these changes can occur. It appears from the graphs that the chance that a channel will open at a given voltage varies almost directly with changes in membrane potential. In fact, at the end of the stimulus, m is only slightly behind the value it would take the cell were permanently voltage clamped to -71.8mV. H, on the other hand, relies of much slower cellular processes. As a result, at the end of the stimulus h only rises to 0.68, far from the 0.8 that would be expected if the cell were held at -71.8mV.

It is this difference in rates that causes an action potential to occur. Once the stimulus ends, the chance that a channel will open quickly returns to baseline (8ms). However, since more channels are currently active in the synapse, a greater influx of sodium ions occurs, driving the cell past this point and into an action potential.



Exercise 4



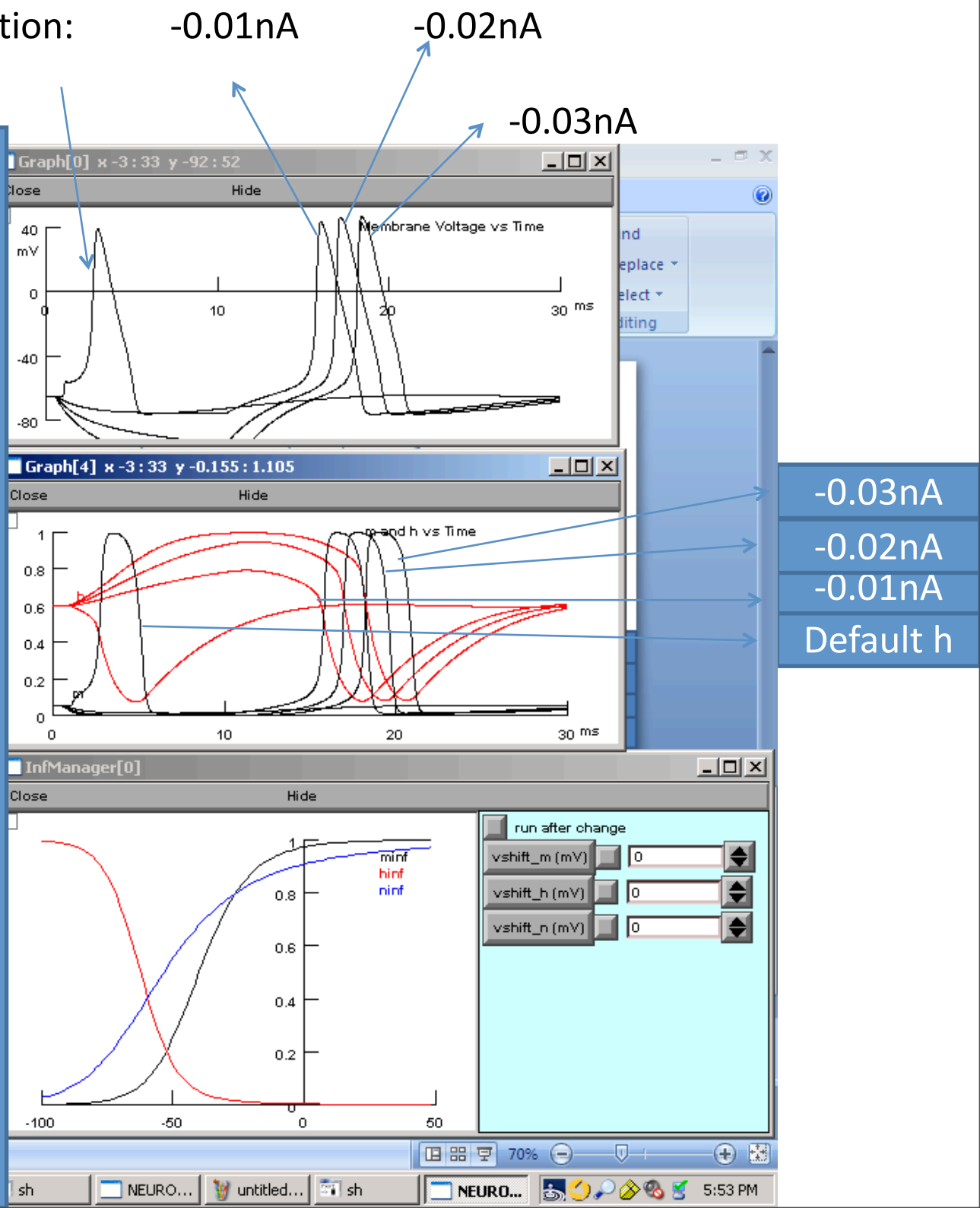
The graph shows m and h during action potential at three different IClamp amplitudes (-0.03,-0.02,-0.01nA-orange,blue, red respectively). The m surge to 1 when action potential is already descending. For the h, at a membrane potential of -76 mV, many of the Na channels would be ready to open. It is surprising that 60% of Na channels are actually de-inactivated before the action potential is actually taking place. The time course of h lags behind the action potential. Also, when the amplitude changes to 0.03,-0.02,-0.01nA, anode break (the offset of a hyperpolarizing pulse used to evoke an action potential in a neuron) happens. This is because the 10ms hyperpolarization gives time for the probability h to change to 1 so that all of inactivation is removed.

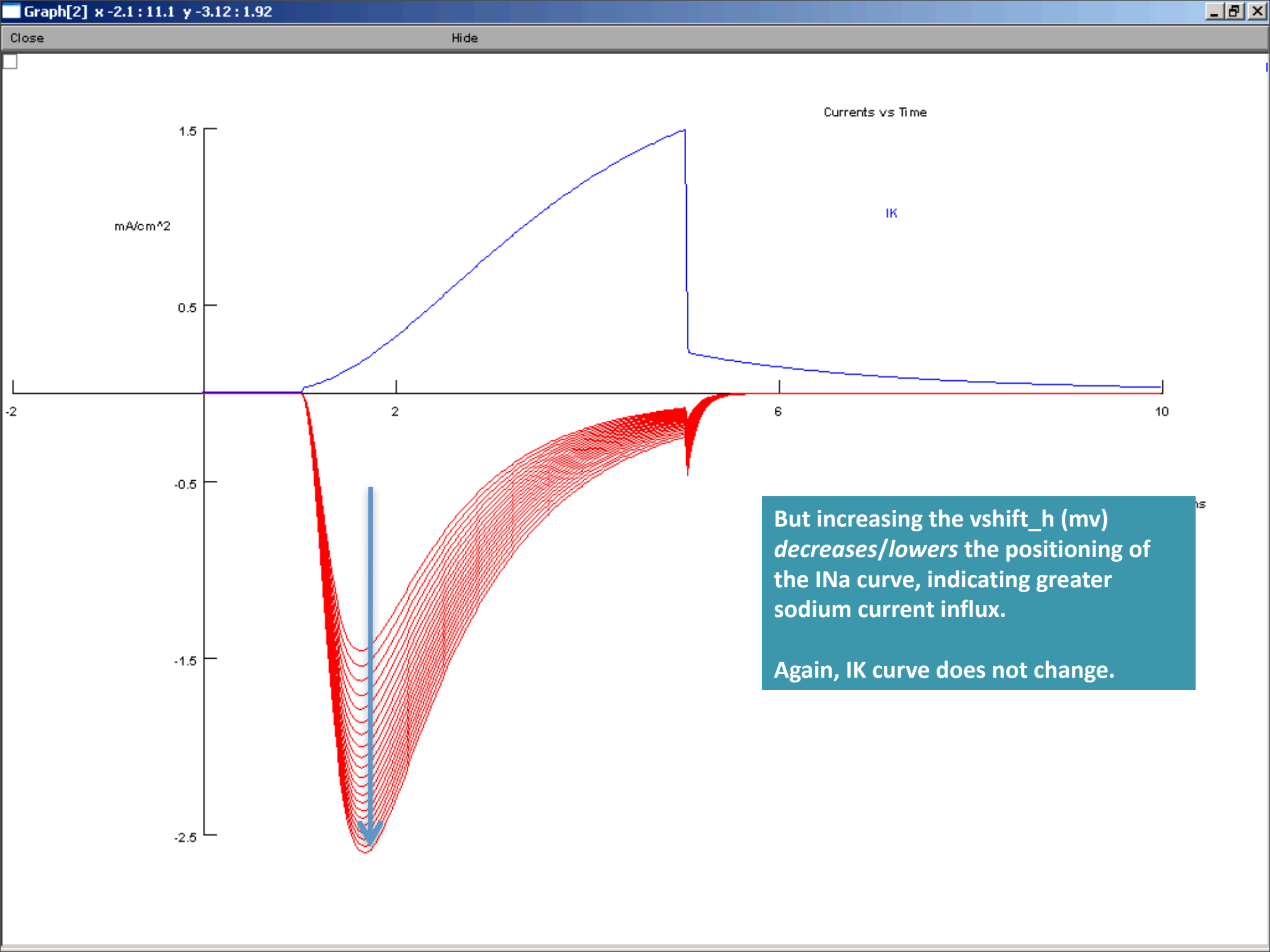
Anode Break

The term "anode break" refers to the offset of a hyperpolarizing pulse used to evoke an action potential in a neuron. If the pulse is of great enough amplitude and duration, it can remove enough inactivation from Na channels so that at the end of the pulse, when the voltage is suddenly returned to its resting value, the depolarization opens these channels and leads to an action potential.

Removing inactivation occurs by delivering a hyperpolarizing stimulus, which leads neurons to generate an action potential. It is often referred to as “off response" or "disinhibition.”

“Anode break" action potential occurs when all of inactivation is removed from the Na channels, triggering an action potential.




Graph[2] x -2.1 : 11.1 y -3.12 : 1.92
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Currents vs Time
IK
mA/cm^2
1.5
0.5
-0.5
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But increasing the vshift_h (mv) *decreases/lowers* the positioning of the INa curve, indicating greater sodium current influx.
Again, IK curve does not change.