

HST 131/Neuro 200  
19 September 2005

## Problem Set #2 Answer Key

### 1. Thresholds

We tend to think of threshold as a particular *voltage* at which the cell will fire an *action potential* if it is depolarized to or past that voltage.

The *action potential* is assumed to be an all-or-nothing phenomenon, though in the simulation, as in real life, we will manipulate certain physiological parameters to make it faster or broader. This should increase our appreciation for its ability to be modulated without diminishing an appreciation for its fundamental all-or-nothing character. Furthermore, as we will see, the precise *voltage* at which threshold is reached is also not fixed, but can be modified by a number of factors. However, within a relatively broad range of initial conditions, threshold varies little; thus, the concept of a specific threshold for the cell has taken root.

Run the APSIM program in active membrane with one pulse to explore threshold. It may also be helpful to open up all the plots (Membrane Currents, Membrane Conductances, Channel Gates), as well as all the parameters which might be modified (Maximal Conductances, Ionic Concentrations, Change Kinetics).

Using default values for all parameters, run the program. Since 20 uA stimulus for 0.5 millisecond is sufficient to elicit an AP, reduce the current until the AP goes away, while maintaining the duration of the current pulse constant. An AP is fired at 18.5 uA (there may be some variation in precise values depending on the version of the program used: PC/Mac, for instance). Using the measure tool, this is about -54.6 mV (for an empirical threshold). Instead of varying current, now hold current constant and vary pulse time required for an AP. I'll arbitrarily pick 10 uA. This requires 1ms current injection, with a measured threshold of -54.0 mV. (Pretty close!) So, now let's look at the total charge transfer, current injected multiplied by time (this could also be the integral of the current injected). This should correspond roughly to voltage change, since, given our capacitance equation,  $V=Q/C$ . Without knowing the capacitance (could be calculated) of the cell, I'll just use the arbitrary units of (uA)x(mseconds). In the first case,  $18.5 \times 0.5 = 9.25$ ; in the second  $10.0 \times 1.0 = 10$  (the program only allows 0.1 msec changes in duration). So, thus we might think of the cell as integrating the injected current to determine whether or not it reaches threshold and fires.

Why does the cell fire an AP at threshold? Because the sodium current entering the cell exceeds the potassium current tending to repolarize it. You can think of threshold as a balance in this competition. When sodium current is depolarizing the cell, this leads to increased depolarization. Voltage gated sodium channels (m gates) tend to open, allowing in more sodium. This positive feedback mechanism results in the fast rising phase of the AP. Remember that sodium channels are faster than potassium channels. At

the peak of the AP, two things happen to change: one, sodium currents inactivate (h gates), which reduces the sodium current; two, potassium current has had more time to activate, and can offset sodium current and repolarize the cell. This results in a rapid falling phase, and an afterhyperpolarization (AHP; this results in a membrane voltage below the resting potential due to extra delayed rectifier potassium conductance). Note that the peak of the AP cannot exceed  $E_{rev}$  for sodium nor can the AHP exceed the  $E_{rev}$  for potassium.

Of course, everything is not so simple! Imagine a very long current pulse of a very low magnitude current. What happens? One, leak currents tend to retard the depolarization (since low magnitude leak currents can better compete with low magnitude current injections). Second, as the cell depolarizes, sodium channels get more time to inactivate. Thus, by the time we reach threshold, so many sodium channels have inactivated that it is no longer possible to fire an AP! Set the time bar to 10ms and simulate this with a maximum duration pulse of 50ms. Inject  $2.5 \mu\text{A}$  current (depends on version – could be  $3.2 \mu\text{A}$ ). The cell voltage reaches a plateau less than 10ms into the current injection and then voltage goes down without reaching threshold, even as we persist in injecting current. Eventually, the cell reaches a new equilibrium resting voltage, when leak currents offset the injected current. Furthermore, looking at the channel gates plot, we see that our slow current injection did not change m or n gates, but we see that the largest magnitude change was the reduction of sodium current due to the closure of h gates (inactivation). (This mechanism of controlling excitability by variation in the steady state level of inactivation is seen, for example, in thalamic cells, and the change in resting membrane potential responsible may underlie the difference between firing modes in awake and unconscious states.)

Properties that might change threshold (to make cell more excitable):

-Conductances:

\*\*More sodium conductance

Less chloride conductance (simulates leak in this model; STUDENTS: please note that this is an artificiality of the model to allow us to manipulate leak current and delayed rectifier current independently. Potassium is also the charge carrier in physiological leak currents and generally sets the resting membrane potential.)

(Potassium delayed rectifier really have a small role, since they are shut at threshold.)

-Kinetics:

\*\*Faster sodium channel activation

\*Slower sodium channel inactivation

(Potassium delayed rectifier really have a small role, since they are shut at threshold.)

-Ionic Concentrations:

Sodium will vary this, since increasing the sodium driving force will mean less channels need to open to get enough current to reach threshold.

## 2. Refractory Period

*Refractory period* is the *time* period following an action potential during which the cell cannot fire a subsequent action potential. *Relative refractory period* is the period following an AP during which the same intensity stimulus that elicited the initial AP cannot evoke subsequent APs. *Absolute refractory period* is the period following an AP during which a stimulus of any intensity cannot evoke subsequent APs.

Run the APSIM program in active membrane with two pulses to explore refractory period. First, we will hold the stimulus intensity constant (current and duration) and vary only the interpulse interval. Set the time bar to 10ms. Cycle through pulses using the pulse number key. Pulse 1 and 2 are 20uA and 0.5 ms, a pulse near threshold. At interstimulus interval (ISI) less than 15.5ms, the second pulse only produces a depolarization with no AP. At 15.6 ms and longer ISI, APs occur. This is relative refractory period.

Now we'll look at absolute refractory period. Hold pulse one constant and determine a maximum amplitude size the the second pulse. For this example, I will select something physiological (1000 uA is probably too much), 150 uA for 2ms. Now we again vary only the ISI. Set the time bar to 4 for easy viewing. At 5ms ISI, the first AP is deep in its afterhyperpolarization, and even this large pulse can't cause an AP. A little later, though, between 5.6 ms and 6.0 ms, we see the AP slowly rise. (We will need to arbitrarily call anything above 0mV an AP). Thus, the absolute refractory period is about 5.8ms (5.7 in some versions).

Several effects to consider: First, after firing an AP, a second one is less likely due to sodium channel inactivation. Additionally, the cell is farther from threshold due to the lower voltage of the afterhyperpolarization. Lastly, and importantly, the open potassium delayed rectifier channels provide an additional conductance to the cell which means that the cell will depolarize less for any given current (or that a larger current is required for the same depolarization).

Properties that might change refractory period (to make the period shorter):

-Conductances:

More sodium conductance (only by making the cell more excitable)

\*Less potassium conductance (more important role; minimize degree of AHP).

-Kinetics:

Faster sodium channel activation (only by making the cell more excitable)

Slower sodium channel inactivation (only by making the cell more excitable)

\*\*Faster potassium channel activation (more important role, as the kinetics here determine length of AHP. Controls both ON/OFF of potassium channels, though a stronger pulse 1 may be required to test)

-Ionic Concentrations:

Sodium (only by making the cell more excitable)  
Potassium (by setting voltage of AHP)

### 3. Regulation of Peak and Repolarization

Which factors most affect the peak? This has been discussed in some detail above.

-Conductances:

\*Sodium conductance (a larger role in how rapidly the cell reaches peak, than on the value of the peak, which will be near but below  $E_{rev}$  for sodium).

Potassium conductance has a small role (since part of the peak is sodium inactivation)

-Kinetics

\*Faster sodium channel activation

\*Slower sodium channel inactivation

Potassium channel activation

-Ionic Concentrations:

\*\*Sodium (Most important in setting  $E_{rev}$  and thus maximum height of the peak).

Which factors most affect the repolarizing phase?

-Conductances:

Sodium conductance (only in terms of competing with potassium).

\*\*Potassium conductance (both how rapidly the falling phase occurs, and how far below  $V_{rest}$  the cell goes during AHP)

-Kinetics

Sodium channel inactivation (slower would provide a longer period of competition between Na/K currents)

\*\*Potassium channel activation (rapidity of falling phase), as well as the length of the AHP, which will be longer if the kinetics are slower.

-Ionic Concentrations:

\*\*Potassium (Most important in setting  $E_{rev}$  and thus level and degree of AHP).

### 4. Drugs and Toxins

Model drugs and toxins as reductions in the conductance of the various channel types. For example, TTX and lidocaine will block the sodium channel conductance, while TEA will block potassium channel conductance. (There are probably also some toxins that could affect the kinetics of the channel, but even ones that don't block the pore directly often prevent opening.)

Lidocaine is an anaesthetic. Blocking conduction of pain fibers, for instance, is one mechanism by which this drug might be used (thus reducing sodium conductance and

increasing threshold, though for practical purposes, even strong stimuli might not work). In other instances, for example an individual whose condition results in a reduction of the excitatory current available, we might wish to try to make cells more excitable (for example, by block with TEA). I'm not personally aware, however, of any cases in which this has been done clinically.

Regarding a difference between stationary and propagating action potentials: generally the sodium current and depolarization is suprathreshold for the subsequent node in an axon and this is not a concern. In the event of a change in the dendritic shape – for example, branching – the action potential may fail to sufficiently depolarize the region, and a phenomenon known as “branch point failure” can occur (and the AP fails to propagate).

## **5.Variability and Constancy among Cell Types**

The most open-ended of the questions. The model is designed for squid axon. Adjusting the concentrations to mammalian concentrations should work, though perhaps initial pulses will fail at default strength and duration, since they are just near threshold.

Parameter adjustments required:

\*All ion concentrations (to mammalian):

Na: 5/150

K: 140/5

Cl: 6/110

\*All kinetics (to account for the Q10 temperature dependence of mammalian temperatures versus squid):

Na activation: 2

Na inactivation: 2

K activation: 2

\*Current pulse (30uA, 1.0 ms)

(Other variations are possible)

Morphological Variation in Mammalian Neurons:

-Cell shape and size. Thus changes in capacitance. The cell will need more or less current to depolarize.

-Density of ion channels (max conductance). For example, the AP in mammalian muscle is always very robust, while that in other neurons may vary. There is, of course, a high concentration of ion channels in muscle.

-Example of spiral ganglion cells: those that must respond to high frequency stimulation (HF cells) have low resistance/high conductance to rapidly return to  $V_{rest}$  between stimuli; the opposite is the case for lower frequency (LF) cells (high resistance/low conductance).