ELEC ENG 3BB3 – CELLULAR BIOELECTRICITY (2013)

Solutions to Hodgkin-Huxley Simulator Lab Assignment

2. Threshold current and potential

a. Open the `Stimuli` window by checking the checkbox in the HHsim main window.

b. In the main window, try applying `Stimulus 1` with various (positive) current amplitudes (and the default pulse duration of 1 ms).

What is the threshold *current* for a 1-ms long depolarizing pulse?

I_{th} is 8.8 nA for a 1-ms long depolarizing pulse.

What is the threshold *potential* (in mV) for this model and stimulus set-up?

V_{th} is just above ≈ -55.3 mV.

Describe your methodology for determining the threshold *potential*.

It is not possible to determine the threshold potential from the action potential (AP) waveform itself. It is necessary to find the *maximum subthreshold* stimulus current level (here 8.7 nA) and from that determine the *maximum subthreshold* membrane potential (≈ -55.3 mV). The threshold potential must be slightly above this potential.

What determines the threshold *potential* for an excitable membrane?

The threshold potential is determined primarily by the sodium activation versus membrane potential behaviour, i.e., m_∞ vs V_m. If the membrane is depolarized enough (i.e., to the threshold potential) to start opening up substantially greater numbers of sodium channels, this will cause a positive feedback loop (i.e., opening sodium channels depolarizes the membrane further which opens even more sodium channels and so on), generating an AP.

c. Now try applying `Stimulus 2`.

Adjust the amplitude (keeping it negative) and duration of `Stimulus 2` so that it generates an action potential. Report one set of values that you find can generate an AP.

The default values of -10 nA and 2 ms do not produce an AP. One set of stimulus parameters that does generate an AP is an amplitude of -10 nA and a duration of 4 ms. Another is -16 nA and 2 ms.

Describe briefly how a hyperpolarizing current can generate an AP in terms of membrane potential and gating particle behaviour.

"Anode break excitation" is generated at the offset of the pulse if the membrane has been hyperpolarized by a sufficient amount and for long enough such that there is substantial sodium deinactivation (i.e., opening of *h* particles) and potassium deactivation (closing of *n* particles). Sodium deactivation (closing of *m* particles) also occurs with the hyperpolarization, but because of the faster dynamics of the *m* particles, when the membrane potential starts returning to rest after the end of the stimulus pulse, sodium activation is ahead of sodium inactivation and potassium activation in returning to its

resting value, so the sodium current is larger than its resting magnitude and the potassium current is smaller. Consequently, the membrane potential overshoots the resting potential and may trigger an action potential if it overshoots it by enough.

3. Threshold current versus stimulus duration

a. In the `Stimuli` window, reset the values for the stimuli by pushing the `Reset` button.

Find the (positive) threshold *current* for at least 5 different current durations between 0.1 ms and 40 ms. (Try to pick durations that best show where the threshold current is changing as a function of duration.)

Some example durations and corresponding threshold currents are:

Duration (ms)	0.1	0.5	1.0	2.0	5.0
I_{th} (nA)	74.3	13.5	8.8	5.8	5.1

What produces the observed changes in threshold current as a function of current pulse duration?

The major factor is how much the membrane is depolarized by the time the current pulse is turned off. Consequently, shorter pulses need a larger current amplitude to produce sufficient depolarization, and thus sodium channel activation, while the pulse is still on. The rates of ion channel activation and inactivation are secondary factors.

4. Refractory effects

a. In the `Stimuli` window, reset the values for the stimuli by pushing the `Reset` button. Now set the amplitude of `Pulse 1` for `Stimulus 1` to the threshold current for a 1-ms pulse, as found in part 2b above. Set the amplitude of `Pulse 2` for `Stimulus 1` to the maximum allowable value of 100.

Find the *absolute refractory period* by adjusting the `Inter-pulse interval` of `Stimulus 1`.

How many milliseconds **long is the <u>absolute</u> refractory period, as measured from the *bottom of the falling phase of the first action potential* to the *onset* of `Pulse 2`?**

The minimum inter-pulse interval to produce a pair of action potentials (with a first-pulse amplitude of 8.8 nA and a second-pulse amplitude of 100 nA, both of duration 1 ms) is ~3.9 ms. Thus, the absolute refractory period measured from the bottom of the falling phase of the first action potential to the onset of `Pulse 2` is ~0.7 ms.

How does the value for the absolute refractory period measured in this way compare to the *minimum inter-spike interval* that you can observe?

The minimum inter-spike interval for this pair of pulses is ~3.1 ms (measured from the peak of the first AP to the peak of the second AP). Thus, the absolute refractory period measured from the bottom of the falling phase of the first action potential to the onset of `Pulse 2` is *much shorter* than the minimum inter-spike interval. This is because this way of measuring the absolute refractory period does not include the falling phase of the first AP and the rising phase of the second AP. Therefore, care should be taken to *not* interpret the absolute refractory period measured in this way as the fastest time between spikes.

Explain the source of the absolute refractory period in terms of ion channel dynamics.

The absolute refractory period is caused by the substantial sodium and potassium currents through the voltage-gated ion channels that cause the rising and falling phases, respectively, of the first action potential. During the period while these ionic currents are so large, the effects of the stimulating current on the membrane's capacitive current are negligible, such that a second action potential cannot be generated by the second pulse. In addition, even while the ionic currents are returning to normal, sodium *inactivation* can prevent a regrowth of the sodium current that would otherwise produce an action potential.

b. Find the *relative refractory period* by appropriately adjusting the `Height` of `Pulse 2` and the `Inter-pulse interval` for `Stimulus 1`.

How many milliseconds long is the <u>relative</u> refractory period, as measured from the *bottom of the falling phase of the first action potential* to the *onset* of `Pulse 2`?

The minimum inter-pulse interval to produce a pair of action potentials with both pulses having an amplitude of 8.8 nA and a of duration 1 ms is ~9.0 ms. The relative refractory period measured from the bottom of the falling phase of the first action potential to the onset of `Pulse 2` is ~5.8 ms.

Explain the source of the relative refractory period in terms of ion channel dynamics.

The relative refractory period is caused by the time that it takes for the transmembrane potential and the conductances of the voltage-gated ion channels to return to their resting values following generation of an action potential. During the period while the ionic conductances are larger than their resting values, the effects of the stimulating current on the membrane potential is reduced, such that a larger stimulating current is required to reach threshold and produce a second action potential. In addition, if there is a hyperpolarized after-potential, i.e., the membrane has overshot the resting potential in the repolarizing phase, then this will contribute to a larger stimulating current being required to reach threshold, and thus plays a part in the refractory period.