The Neuromuscular Junction

Classic Model of Synaptic Excitation
Ach binds to nicotinic receptors, causing channels to open, causing a postsynaptic EPC that leads to a postsynaptic EPP.
Simulation Panels

Description of the Panels and Windows Customized for this Tutorial

1. **NEURON's representation of a postsynaptic potential**
   
   The conductance change caused by ACh has the kinetic form shown to the right. Since this form is often represented mathematically by a function called the alpha function, in NEURON it is referred to as the AlphaSynapse.

   The AlphaSynapse panel controls the parameters of the synaptic input as follows:
   - **onset**: the time of onset of the synaptic potential in ms
   - **Tpeak**: the time to peak of the synaptic conductance change in ms
   - **gmax**: the maximum synaptic conductance in µS
   - **e**: the reversal potential, set by default at −15 mV for the ACh-gated channels

2. **The graphs**
   - A Postsynaptic Conductance-vs-Time graph shows the conductance change caused by the ACh.
   - A Postsynaptic Current-vs-Time graph plots the postsynaptic currents through the ACh-gated channels.
   - A Postsynaptic-Voltage-vs-Time graph plots the membrane potential, Vm. Its time course and amplitude will depend on:
     - The time course and amplitude of the current
     - The capacitance and resistance of the postsynaptic membrane

3. **The Patch Parameters panel**
   Notice that the tutorial begins with the Na and K channel densities set to zero and only a leak conductance in the membrane.

4. **Stimulus Control**
   When launched during the tutorial, this panel "inserts" a stimulating electrode and controls a current pulse delivered to the postsynaptic membrane patch. When this panel is launched, there are then two ways of injecting current into the patch: (1) through the postsynaptic ACh-gated channels, controlled by the AlphaSynapse panel, and (2) through the electrode, controlled by the Stimulus Control panel.
Presynaptic Input

Experiments and Observations

Observe the relation between synaptic strength (conductance) and EPP amplitude.

1. **Stimulate the presynaptic input.**
   - When you click R&R you will release a small amount of ACh from a virtual presynaptic terminal; in response you will see a change in the synaptic conductance, the resulting synaptic current (EPSC), and a small change in the postsynaptic voltage (EPP). The patch is passive; the Hodgkin-Huxley (HH) Na and K conductances are set to zero by default. (Notice in the Reset field that the membrane potential is −90 mV, the typical resting potential of a muscle fiber.)

2. **Double the synaptic conductance (gmax) several times.**
   - Double the conductance, choose Keep Lines in each plotting window, and re-run the simulation. Keep doubling the conductance up to 64 µS (or above). You will see that there is no further significant increase in the amplitude of the voltage even though the synaptic conductance and current continue to increase.

When John (“Jack”) Eccles, Bernard Katz, and Steve Kuffler observed the EPP in frog muscle fibers in 1941, they had to include curare in the bathing medium to reduce the EPP's amplitude below threshold for eliciting an action potential in the muscle. You need not worry about using curare because the HH channels in your patch have zero conductance for now.

3. **Explain the results of this experiment.**
   - The time courses of the synaptic currents can have considerably shorter durations and different shapes than those of the conductance changes. **Why?**
   - The time course of the voltage change, the EPP, is much slower than either that of the synaptic conductance change or of the synaptic current. Its peak is reached when the current returns to zero! What shapes the **time course** of the EPP?
   - Clearly, as the conductance increases, the EPP approaches an asymptotic value. What is the **significance** of this value?
Neuromuscular Junction

Experiments...
- Increase EPP amplitude
- Observe reversal potential
- Add voltage-gated channels

Reference

What is... Interactive Equivalents of Original Papers
PDFs of Original Papers

Help with...
Presynaptic Input (2)

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No further significant increase in amplitude of postsynaptic voltage.
Results

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**The time course of the synaptic conductance**

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**The time course of the current**

The time course of the current, on the other hand, depends on the driving force as well as the conductance. At any time point, the synaptic current equals the synaptic conductance multiplied by the driving force on the current. Since the driving force is the difference between the equilibrium potential for the transmitter-gated conductance (-15 mV for ACh) and Vm, it will change with Vm. In particular, as Vm approaches the synaptic reversal potential, the driving force approaches zero and the current amplitude also approaches zero even though the conductance may still be high.
Results (2)

The time course of the voltage change, the EPP, is much slower than either that of the synaptic conductance change or of the synaptic current. Its peak is reached when the current returns to zero! What shapes the **time course** of the EPP?

**The rising phase**

The rising phase of the EPP (or EPSP) is determined by the synaptic current depolarizing the capacitance of the postsynaptic membrane. The equation for capacitance holds: $I_{cap}$ (synaptic current, in this case) is equal to $C$ (essentially the membrane area) times $dV/dt$, the rate of change of $V_m$.

**The falling phase**

The falling phase of the EPP is determined by how quickly the membrane capacitance can discharge: that is, by how quickly charge can leak back out through the membrane.
Results (3)

Clearly, as the conductance increases, the EPP approaches an asymptotic value. What is the significance of this value?

The EPP amplitude approaches a maximum asymptotically as the conductance change increases. Indeed the amplitude approaches a particular value of $V_m$, the "equilibrium potential" for the transmitter-gated conductance; that is, since ACh gates a channel that is equally permeable to Na and K, the "equilibrium potential" for that channel will be a value halfway between $E_{Na}$ and $E_{K}$, in this case -15 mV.

This "ACh equilibrium potential" is also the potential at which both the voltage change and the current reverse in sign, and so it is also called the "reversal potential." In the case of ACh, there is an equal increase in permeability to both Na and K, and so the equilibrium potential is at -15 mV, a value halfway between $E_{K}$ and $E_{Na}$. 
4. Compare your observations with a figure from a classic paper. If you re-run your simulation with Total # (ms) set to 30, you can compare your voltage changes to the uppermost trace in Figure 5 of the classic study of the EPP by Paul Fatt and Bernard Katz in 1951. In their case, the muscle is curarized and the EPP is subthreshold. Consult this link for a simulation of how an EPP would spread along the muscle fiber, as in the Fatt and Katz experiments.

5. Return gmax to its default value of 2 µS. Erase the traces from your previous experiment.
Reversal Potential

Determine the reversal potential of the ACh-gated EPP.

1. Time the EPP to arrive during a pulse of injected current.
   To observe reversal of the EPP you will have to change Vm to different values with a prolonged current pulse. The EPP can then be timed to occur after the pulse has reached a steady state.
   - Set the Total # (ms) to 25.
   - Insert a current-passing electrode into the patch.
     Bring up the Stimulus Control panel. Check the pulse parameters in IClamp. The duration of your depolarizing current pulse has been set at 25 ms (that is, it terminates just off the graph) and its amplitude initially is set at 5 nA.
   - Time the EPP.
     In the AlphaSynapse panel, change the onset of the EPP to 20 ms so that it will occur after the depolarized voltage response has reached a steady state.

2. Run the experiment.
   Using Keep Lines in all of the plotting windows, deliver a set of depolarizing current pulses to the patch, increasing the amplitude of the current pulse with each R&R in 5 nA steps to 50 nA from its initial value of 5 nA. What is the value of the reversal potential?
   - Use View = plot to expand the conductance and current trace amplitudes.
     The amplitudes of the conductance and current traces will be small. Expand them and examine them. What determines the amplitude of each current?
   - Expand the time base of a selected region.
     You can expand the region around the reversal potential on any graph by choosing New View on the pop-up menu (right mouse button). Use the left mouse button to box the interesting area. Resize the box; pull it out horizontally to see the events on a faster time base, or vertically for more y-axis resolution.
Reversal Potential (2)
Reversal Potential (3)
Adding HH Na and K channels

How do voltage-sensitive channels affect the shape of an EPP or an EPSP?
The next experiments will use a patch containing HH Na and K channels rather than a passive patch. In the muscle fiber, depolarization of the postsynaptic end-plate region must gate the Na and K channels in the surrounding membrane to elicit an action potential and cause contraction.

1. **Set the Total # (ms) to 10 and reset the default parameters.**
   You may have to bring up new windows to replace those in which you employed View = plot. The Patch Parameters panel should be open.

2. **Click the "Add HH channels" button (in the P&G manager).**
   Clicking this button will reset three parameters as follows:
   - The densities of the Na and K channels will be changed to the standard HH values.
   - The stimulus current will be set to zero.
   - The synaptic conductance (gmax) will be changed to 3.532 µS, just below the threshold level of 3.533 µS for generating an impulse.

3. **Run the simulation.**
   Click R&R to see the waveform of an EPP in an active patch membrane. If you "increase the number of transmitter quanta" by increasing gmax the slightest bit, to 3.533 µS, this EPP will (after prolonged deliberation) generate an action potential.

   A very small difference in the transmitter-gated current can determine whether or not the postsynaptic membrane fires. You may remember this result from the Threshold tutorial. Remember, also, that at threshold the Na and K currents can be equal and opposite for quite some time, as you see here, before one of them gains the advantage. Note that many milliseconds passed between the increase in ACh-gated conductance (and the resulting increase in current) and the postsynaptic action potential it triggered.

4. **Question:**
   Can you explain the shape of your subthreshold EPP in this active membrane? If you are puzzled about EPP or EPSP shapes observed experimentally when recording from intact muscle fibers or neurons (rather than a patch), a detailed explanation is available in this link.

5. **Compare EPPs in a passive and active patch.**
   If you wish to compare this EPP in an active patch (containing the voltage-gated Na and K channels) with that in a passive patch, bring up the Parameters Panel to reset the conductances of the Na and K channels to zero.
A very small difference in the transmitter-gated current can determine whether or not the postsynaptic membrane fires.
Can you explain the shape of your subthreshold EPP?

*Compare the voltage change in passive and active membrane.*

Shown to the right are the shapes of the *synaptically induced current*, the resulting *voltage change in a passive muscle fiber* and the *voltage change with HH channels* in the fiber.

The traces are plotted from zero so that voltage and current may be plotted together since the point of interest is response shape, not reversal potential.

*The difference comes primarily after the voltage change has reached a peak.*

Notice that the sub-threshold, inward synaptic current drives a quick voltage rise in both the passive and the active membranes to approximately the same value. At the cessation of the synaptic current, however, the voltage across the active membrane is temporarily maintained by activation of the Na conductance instead of returning exponentially to the resting level (zero here) as it does for the passive membrane. After a few milliseconds, the voltage in the active membrane is pulled down so rapidly by K channel activation that it undershoots the resting level before settling to zero.
In a patch of membrane containing Hodgkin-Huxley (HH) channels, the response to a subthreshold synaptic input is partially regenerative. In contrast, the shapes of end plate potentials (EPPs) at neuromuscular junctions and of excitatory post-synaptic potentials (EPSPs) in nerve cells may look much more like the response of a passive membrane despite the presence of active channels in the membrane. There may be several reasons for these differences, including the value of the resting potential and the "electrical load" faced by the synaptic input, as will be detailed below.

What determines the shape of the postsynaptic response?

The shape of the potential change induced by a postsynaptic conductance change in response to a squirt of transmitter depends on several factors:

- The nature of the transmitter-gated event: its magnitude, duration, and reversal potential
- The "electrical load" of the postsynaptic membrane. This load represents the total resistance and capacitance through which the postsynaptic current flows.
- The presence or absence of voltage-gated channels
- The postsynaptic voltage range over which the EPP or EPSP occurs. Vertebrate skeletal muscle fibers can have a resting potential as large as -90 mV; depolarizations from this level may have to be tens of millivolts in amplitude in order to activate voltage-sensitive channels. Neurons, on the other hand, rest more depolarized, typically at about -65 mV, where much smaller depolarizations can activate the HH channels.
EPP/EPSP Shape (3)

What is the ”electrical load” of the postsynaptic membrane?

The ”electrical load” of a patch of membrane, or of an intact neuron or muscle fiber, refers to how much current is required to change its voltage.

For a patch or an isopotential soma:
A patch has a light electrical load because it has no neighboring compartments. It neither receives current from neighboring segments nor sends current to them. All of the transmitter-gated current is applied directly to the process of charging the relatively small capacitance of the patch of membrane. Consequently, the electrical load of a patch is considerably less than that of an axon or muscle fiber, where current spreads longitudinally and must charge more capacitance.

Thus, when synaptic currents are injected into patches, they easily depolarize the patch. If the patch contains HH channels, even a small synaptic current can activate a fraction of the channels, producing partially regenerative currents. These currents distort the voltage response expected from a passive membrane, as shown in this figure. (Here the resting potential of the patch is -65 mV but is set at zero so that voltage can be plotted in the upper portion of the figure and current in the lower portion.)
The shape of the end plate's response in the intact muscle fiber depends on the amplitude of the EPP.

The simulations here compare the EPP of a passive end plate membrane (black trace) to three EPPs (red traces) generated in an active end plate membrane (containing HH channels) by squirts of ACh of increasing concentration. All of the EPPs are recorded at the end plate.

The three EPPs in active membrane are prolonged and of complex waveform compared to the EPP in passive membrane. All three have an initial "bump" at about 1.8 ms. This first bump is the attempt of the end plate membrane itself to generate an action potential, shorted out by the huge conductance caused by the ACh.

As the ACh concentration increases, the EPPs are prolonged (due to more ACh) and a second "bump" appears, an action potential generated in a neighboring region and spreading back to the end plate region. The highest concentration of ACh caused a full-blown spike to occur within a millimeter of the junction (not shown in this figure) that is recorded in the figure as a shunted spike at the end plate.