UNIVERSITY OF WATERLOO

BIOL 376: Cellular Neurophysiology

Review Notes

Fall 2021

Professor: Dr. David Spafford

Richard (Zhifei) Dong

Yolanda (Anqianyi) Tu

Ion Channels and Signalling

Fig. 4.3 Intracellular Recording of Channel Noise

- B: Application of ACh (from presynaptic terminal) causes an inward current of about -130 nA
    - Caused by entry of cation --> depolarize the membrane
- C: Fluctuation of current due to the random opening and closing of ACh-activated cation channels

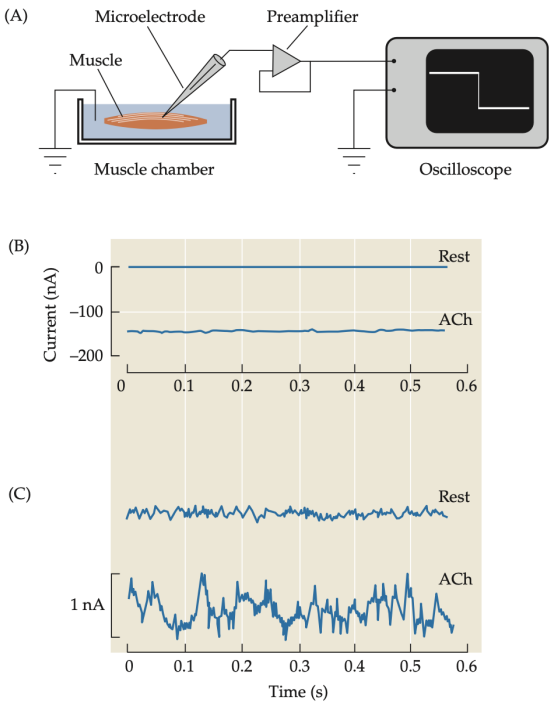


Fig. 4.5 Examples of Patch Clamp Recordings of Single Channels

- A: Glutamate-activated channel opens irregularly, at fixed amplitude and variable duration --> stochastic
- B: ACh-activated channel shows more than one (substate) current levels
    - Partly open/close (sub-conductance)
- C: Glycine-activated chloride channel show rapid closing and reopening (bursts)

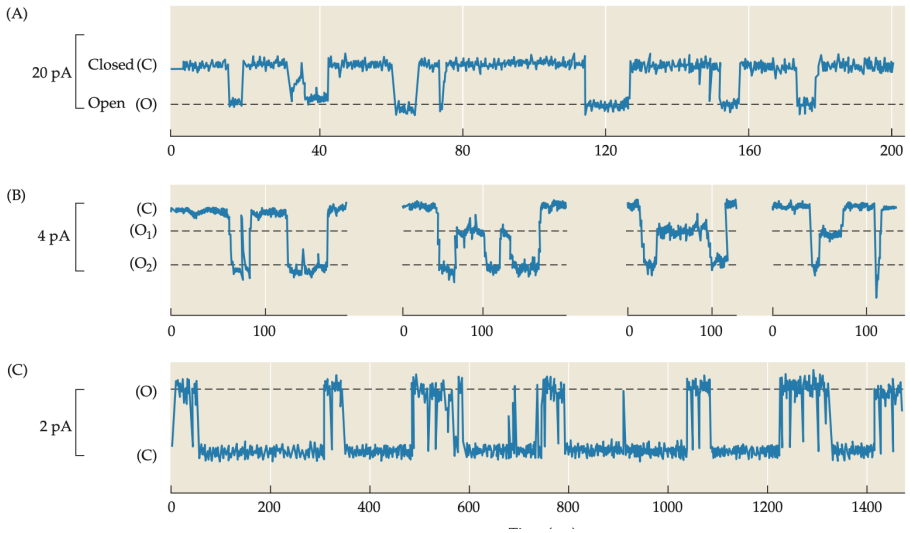


Fig. 4.6 Effect of Potential in Current on Potassium Channel

- A: Clamp voltage, measure current through (-ve = in, +ve = out); 150 mM [K+] for both in and out
- B: No potential is applied (0mV) --> No net movement of K+, no current measured
- C: Potential is applied (+20mV) --> outward current
- D: Potential is applied (-20mV) --> inward current
- E: Channel Current vs. Potential, where slope = conductance (V=IR=I/g)

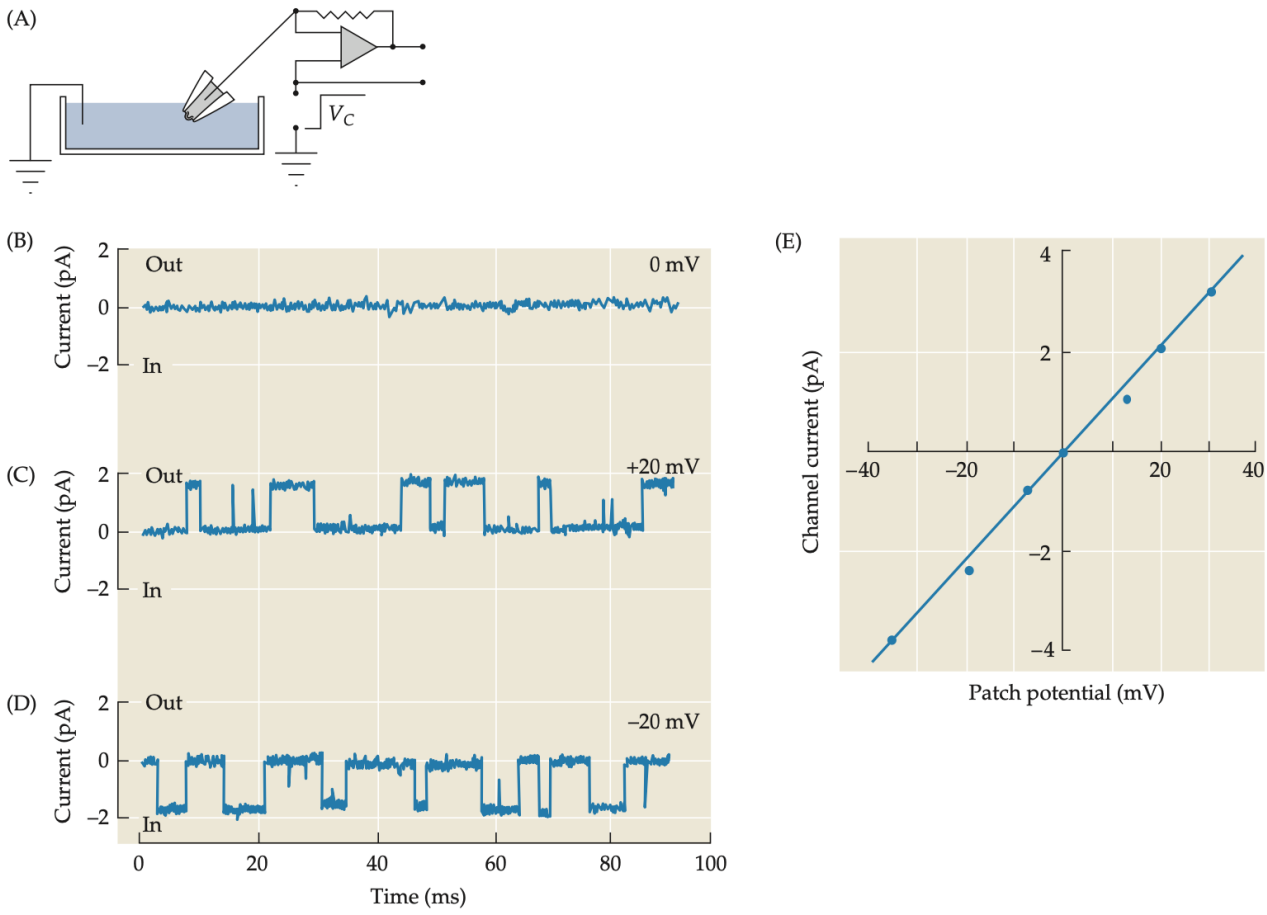
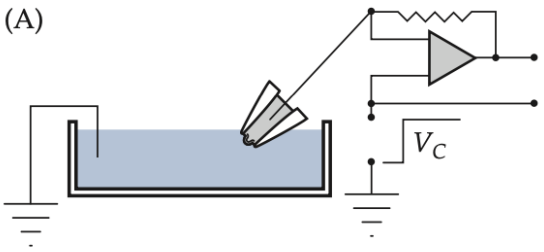
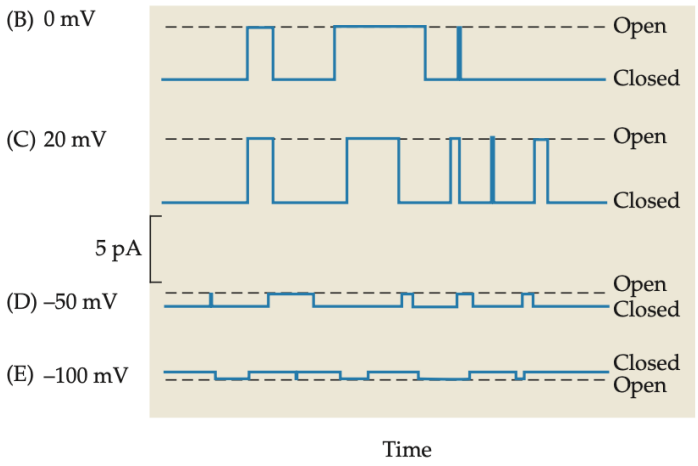


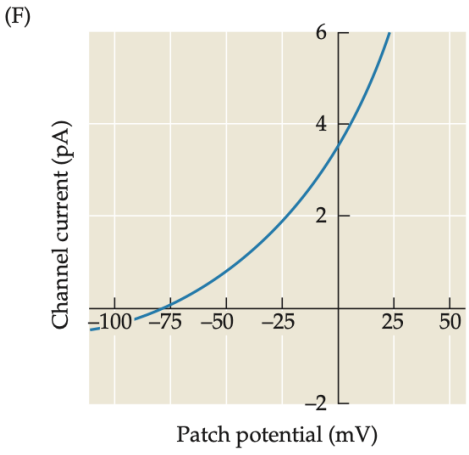
Fig. 4.7 Reversal Potential for Potassium Currents on Potassium Channel

- A: Clamp voltage, measure current through (-ve = in, +ve = out); 3mM in bath (extracellular) and 90mM in the electrode (intracellular)
- B: No potential applied, but still have current from the concentration gradient --> outward current
- C: Potential is applied (+20mV) --> larger outward current (outward rectification K+ Current --> current more easily to be passed outside)
    - outward rectifying -> majority of current vs. potential is outward current
    - inward rectifying -> majority of current vs. potential is inward current
- D: Potential is applied (-50mV) --> smaller outward current
- E: Potential is applied (-100mV) --> small inward current
- F: K+ Equilibrium potential (EK) at -85mV
- Nernst Equation for Equilibrium Potential

$$E_K = \frac{RT}{zF} \ln \frac{[K]_o}{[K]_i}$$







Box 4.1 Measuring Channel Conductance

- Single conductance value depends on concentration and linear relationship (cannot be rectified)
    - Otherwise, conductance changes constantly
- <u>Chord Conductance</u>
    - The slope between two points (red line)
- <u>Slope Conductance</u>
    - The instantaneous slope at one point

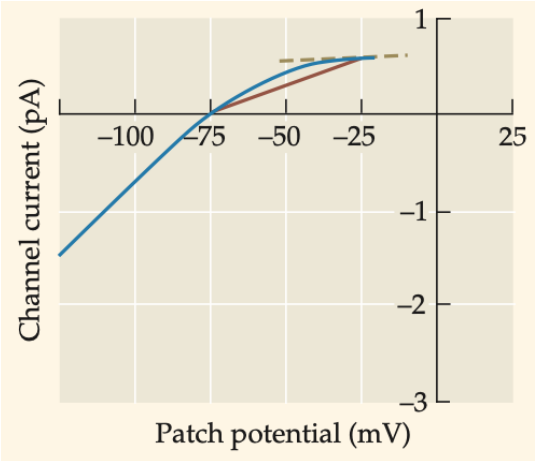


Fig 5.8 Structure of K+ KcsA Channel

- The four connections between the short helices and the inner helices form the <u>selectivity filter</u>
    - allows potassium, cesium, and rubidium to pass
    - excludes smaller cations: sodium and lithium
        - Negative charges of pore helix point --> dehydrate ions
        - Small dehydrated cations cannot perfectly fit the ion channel and fail to gain energy --> fail to pass through the filter

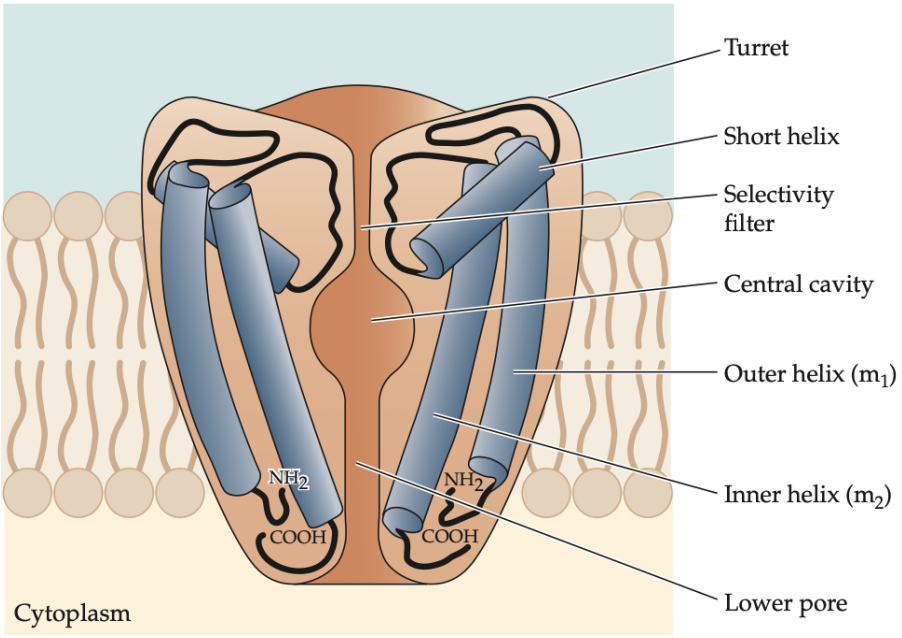


Fig. 9.1 Effects of Sodium Injection on Na/K ATPase

- To maintain viability after AP, we need to bring Na out and K+ in
    - Use Na/K ATPase
- B: Hyperpolarization caused by injection of Na+ but not Li+
    - 2K in and 3Na out --> net outward (-ve) current by ATPase
- C: Ouabain blocks Na+ Channel --> reduced hyperpolarization after Na+ injection
- D: Remove Extracellular K+ --> no hyperpolarization when Na+ is added. Hyperpolarization when extracellular K+ is restored
    - The pump requires both Na and K to work
- E: Measurements of membrane current; membrane potential clamped at -47 mV
    - Sodium injection results in an increase in intracellular sodium concentration, and an outward current across the cell membrane.
    - Sharp deflections on the sodium concentration record are artifacts from the injection system; the dashed line indicates the time course of the change in concentration

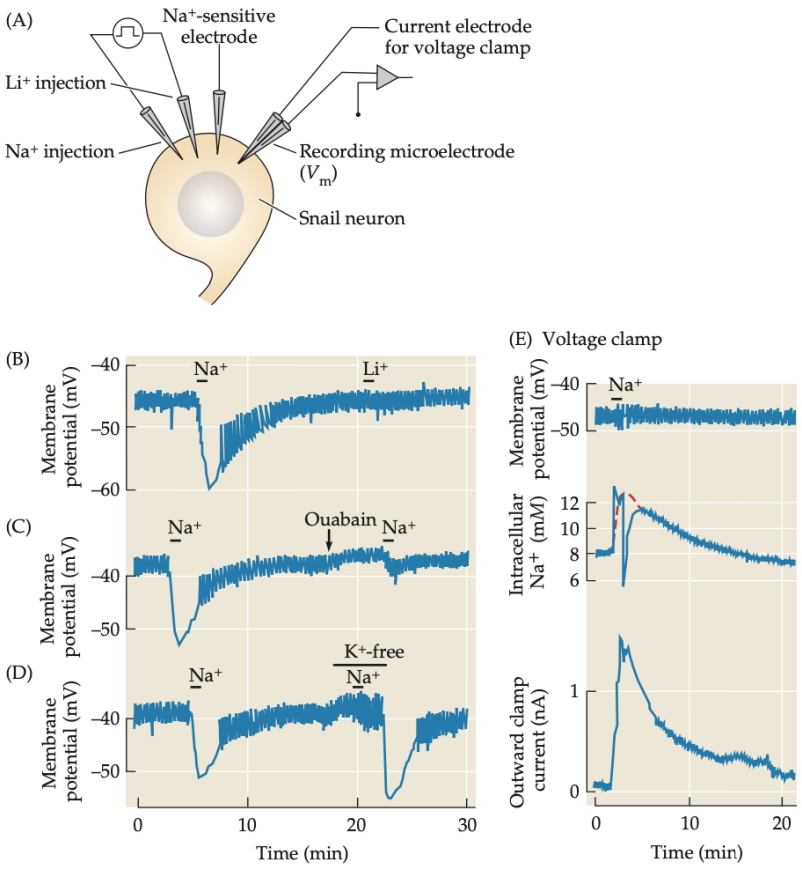
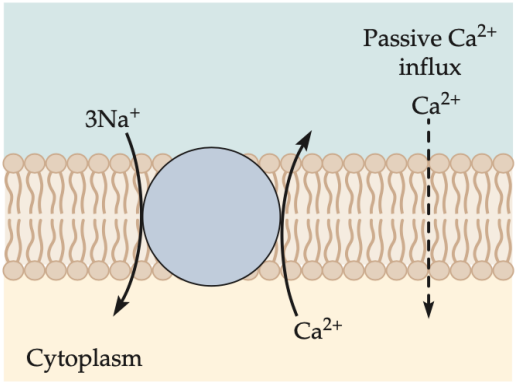


Fig 9.3 Transport of Calcium Ions

- A: Sodium/Calcium Exchange (NCX) System (no ATP --> down Na gradient)
  - 1 Ca2+ out and 3Na+ in to balance the natural influx of Ca leak --> <u>inward current</u>
  - Lower affinity for Ca than Ca ATPase --> due to less concentration
- B:
  - A lot of [Ca2+] out --> a lot of inward current due to increase of Ca2+ flow in
  - Reduce extracellular Na --> reduced gradient --> reduced outward calcium current --> more inner Ca2+ --> more current going in as the system trying to balance [Ca2+]in and [Ca2+]out
  - Replace Na with Li --> more Ca2+ inside --> more current in

(A)



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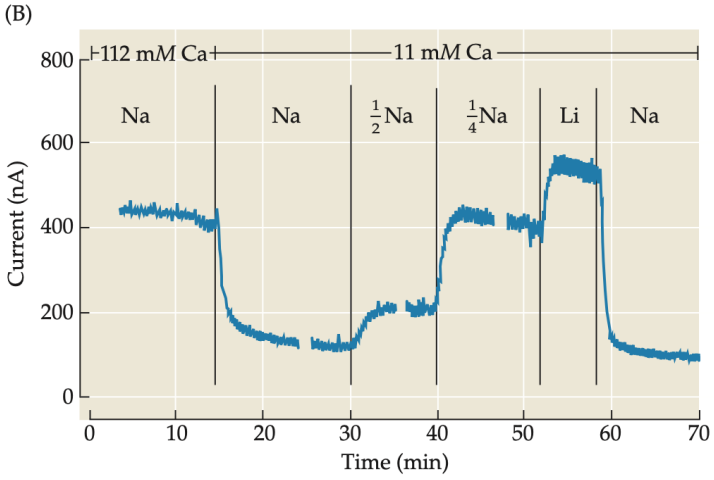


Action Potential

Fig. 7.1 Membrane Currents Produced by Depolarization

- A: Voltage clamped at -9mv -> 56 mV instan depolarization -> 3 currents
  - Measure current using I=V/R
- B: Capacitative Current and Leak Current
  - **Capacitative Current** (out)
    - due to change in potential results in the change of charge in membrane capacitance
    - Brief (about 20 micro sec)
  - **Leak current** (small and steady out)
    - caused by movement of K and Cl
    - often masked by other currents during AP
- C: **Early Current** (in)
  - due to Na entry
- D: **Late Current** (out)
  - due to K exit from opening of voltage-activated K Channels

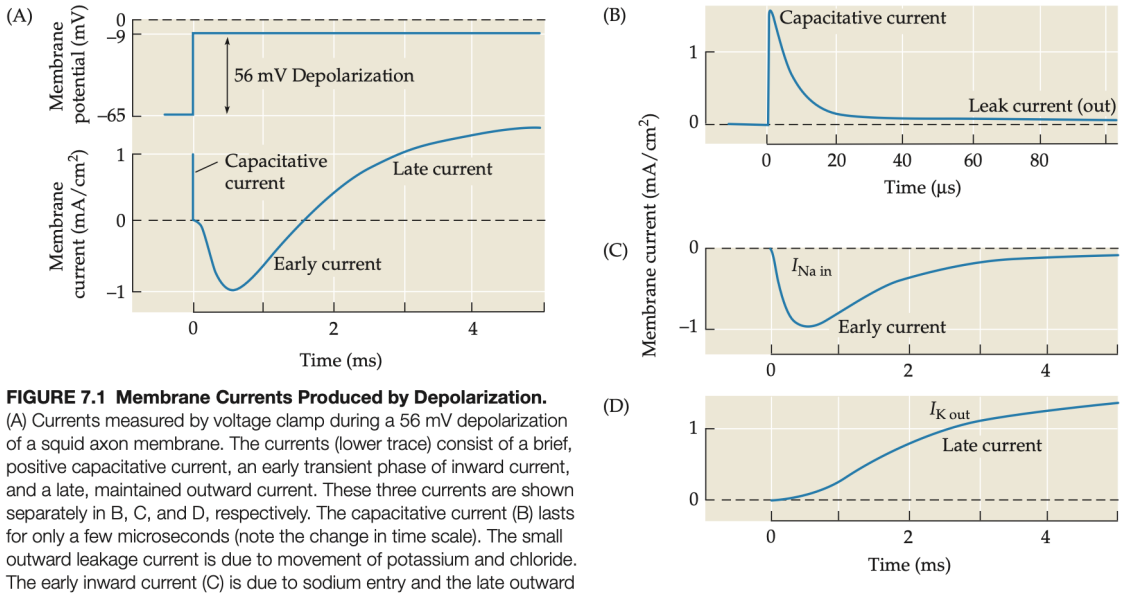


FIGURE 7.1 Membrane Currents Produced by Depolarization. (A) Currents measured by voltage clamp during a 56 mV depolarization of a squid axon membrane. The currents (lower trace) consist of a brief, positive capacitative current, an early transient phase of inward current, and a late, maintained outward current. These three currents are shown separately in B, C, and D, respectively. The capacitative current (B) lasts for only a few microseconds (note the change in time scale). The small outward leakage current is due to movement of potassium and chloride. The early inward current (C) is due to sodium entry and the late outward current (D) to potassium movement out of the fiber.

Fig. 7.2 Pharmacological Separation of Membrane Currents into Sodium and Potassium Components.

- B: TTX: blocks Na Channel --> Remove Na Early Current
- C: TEA: blocks K Channel --> Remove K Late Current

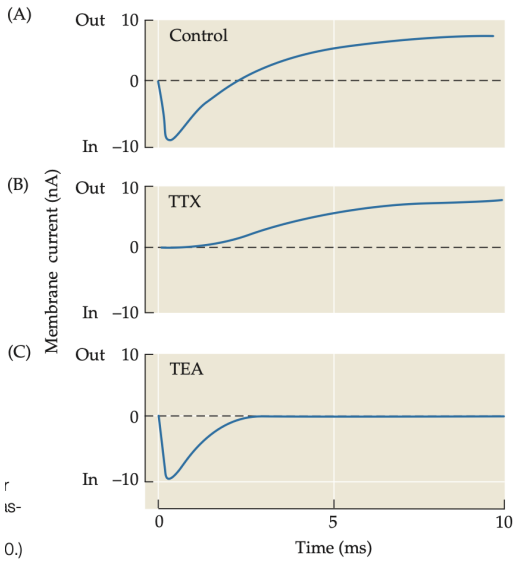


Fig. 7.3 Dependence of Early and Late Currents on Potential.

- A:
    - Hyperpolarization (at -85mv) only caused some leak current
    - Higher depolarization level, smaller early IN current
    - Early current disappears at +52mV (Na Equalibrium Potential), reverse OUT at +65mV
- B: Peak current vs. membrane potential
    - For **hyperpolarization**, no early/late current
        - Na and K channels remain closed
        - Current flows from high to low potential --> leak current balance out the more negative potential inside by flowing IN
    - **Early Current**
        - Na+ channels open --> current start to flow IN (since Na has ENa at +52mv)
        - **magnitude** of IN current start to <u>increase</u> (more Na opens), then <u>decrease</u> (decreased electromotive force), then hit 0 at +52mV (reveral potential), then becomes OUT (>reversal poential)

- **Late Current**
    - K+ channel open --> Current flow OUT due to EK at ~-77 mV
    - At you increase membrane potential, you increase the electromotive force --> more current OUT
- CURRENTx = g (Vm - Ex)
    - current = conductance x (membrane potential - reversal potential)

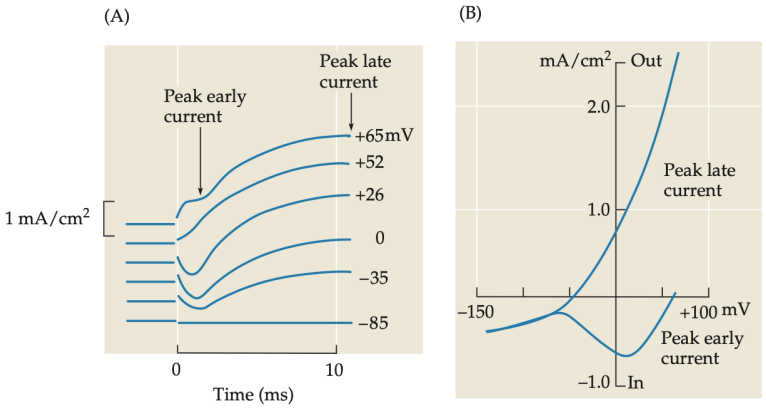
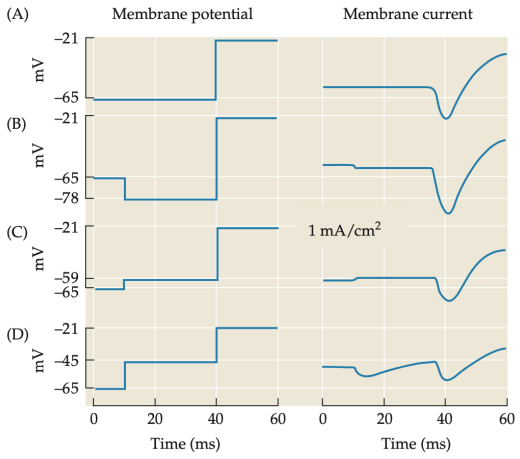


FIGURE 7.3 Dependence of Early and Late Currents on Potential. (A) Currents produced by voltage steps from a holding potential of –65 mV to a hyperpolarized level of –85 mV and then to successively increasing depolarized levels as indicated. The late potassium current increases as the depolarizing steps increase. The early sodium current first increases, then decreases with increasing depolarization, is absent at +52 mV and reversed in sign at +65 mV. (B) Peak currents plotted against the potential to which the membrane is stepped. Late outward current increases rapidly with depolarization. Early inward current first increases in magnitude, then decreases, reversing to outward current at about +52 mV, which is the sodium equilibrium potential. (After

Fig. 7.4 Effect of Membrane Potential on Sodium Currents.

- A: CONTROL -> depolarize to -21 means Na+ IN current followed by K+ OUT current
- B: Hyperpolarization + depolarization -> INCREASED size of Na+ IN
- C&D: Depo + depo -> REDUCED size of Na+ IN
- E: h --> 1 means fully activation of Na channel, 0 is no activation of Na channel
    - some degree of <u>Na+ channel inactivated</u> at resting membrane potential



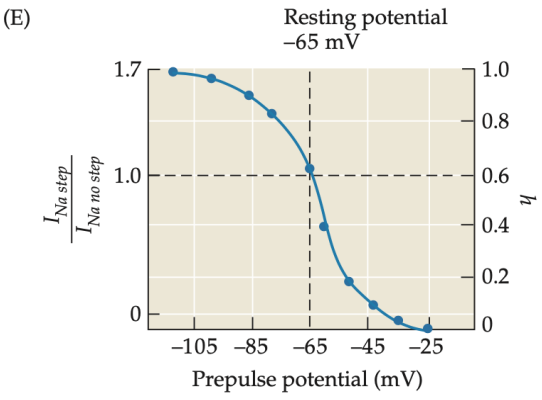


Fig. 7.5 Sodium and Potassium Conductance

- A: Increased depolarization = increased conductance
    - K+ opens for longer (NOT inactivated)
- B: K+ channels and Na+ channels have similar voltage sensitivity
    - the only difference is the opening time, NOT the sensitivity to voltage
- **Absolute Refractory Period**
    - No/very few Na+ channels are available for opening
- **Relative Refractory Period**
    - Large increase in gNa is required to override the gK to generate AP
    - During which the threshold gradually returns to normal as the potassium channels close and the sodium channels recover from inactivation.

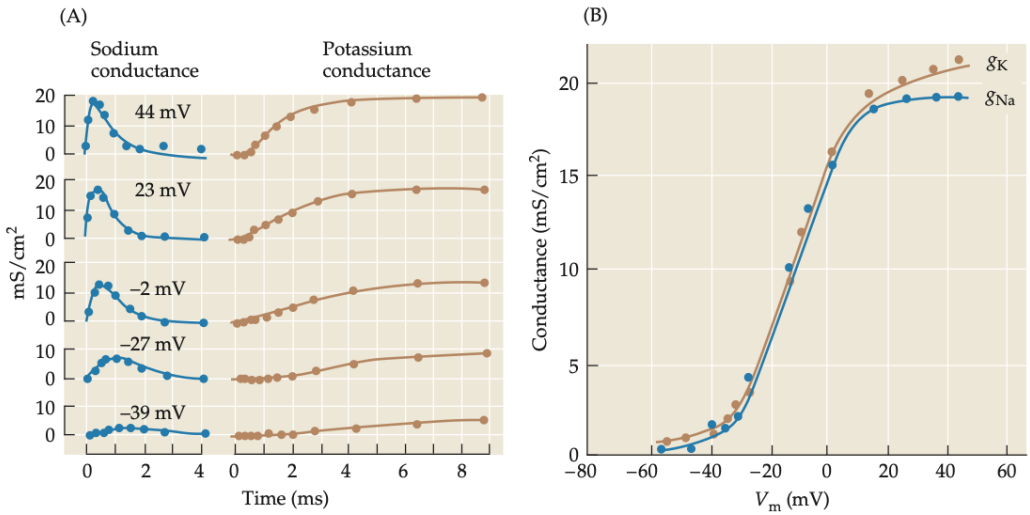


FIGURE 7.5 Sodium and Potassium Conductances. (A) Conductance changes produced by voltage steps from –65 mV to the indicated potentials. Peak sodium conductance and steady-state potassium conductance both increase with increasing depolarization. (B) Peak sodium conductance and steady-state potassium conductance plotted against the potential to which the membrane is stepped. Both increase steeply with depolarization between –20 and +10 mV. mS = millisiemens (After Hodgkin and Huxley, 1952a.)

Fig. 7.7 Sodium-Gating Current

- **Gating Current**
  - caused by the move of charged structure in the membrane
  - As the channel open, +ve charged core moved out to produced the gating current
- A:
  - Gating current is masked within the capacitive current during depolarization
  - Reversing the direction of potential (ie: hyperpolarization)
    - change the membrane the other way
    - capacitive current is IN, gating current is still out
  - Sum of a and b => gating current
- B:
  - A very small and fast gating current
- C:
  - No sodium current --> gating is more obvious

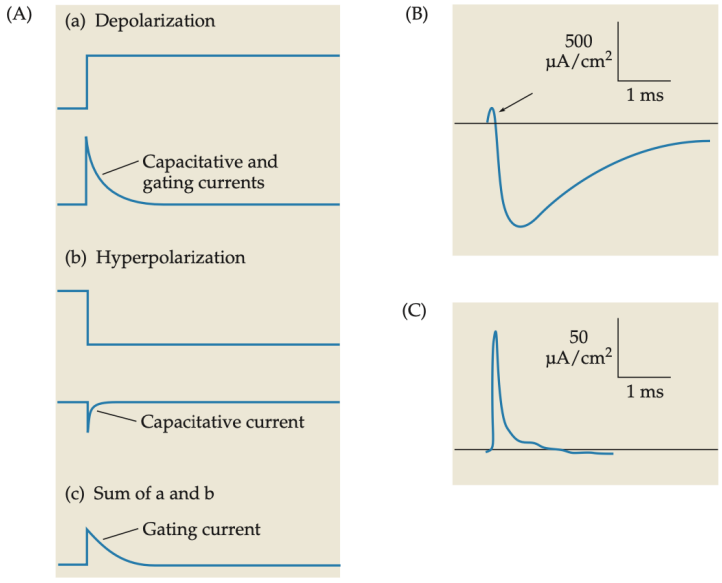


FIGURE 7.7 Sodium-Gating Current. (A) Method of separating gating current from capacitative current. Depolarizing pulse (a) produces a capacitative current in the membrane, plus a gating current. Hyperpolarizing pulse of the same amplitude (b) produces capacitative current only. When the responses to a hyperpolarizing and a depolarizing pulse are summed (c), capacitative currents cancel out and only the gating current remains. (B) Current record from a squid axon in response to depolarizing pulse, after cancellation of capacitative current. Inward sodium current is decreased by reducing extracellular sodium to 20% of normal. A small outward current (arrow) preceding the inward current is the sodium channel gating current. (C) Response to depolarization from same preparation after adding TTX to the bathing solution and recorded at higher amplification. Only gating current remains. (B and C after Armstrong and Bezanilla, 1977.)

Fig. 7.10 Sodium Channel Currents

- about 1/3 of the channels does not activate
    - multiple signals for those that do open, with most frequently near the onset of depolarization
- mean channel opening time is relatively short
- there is a probability such that the channel will open for a brief period of time

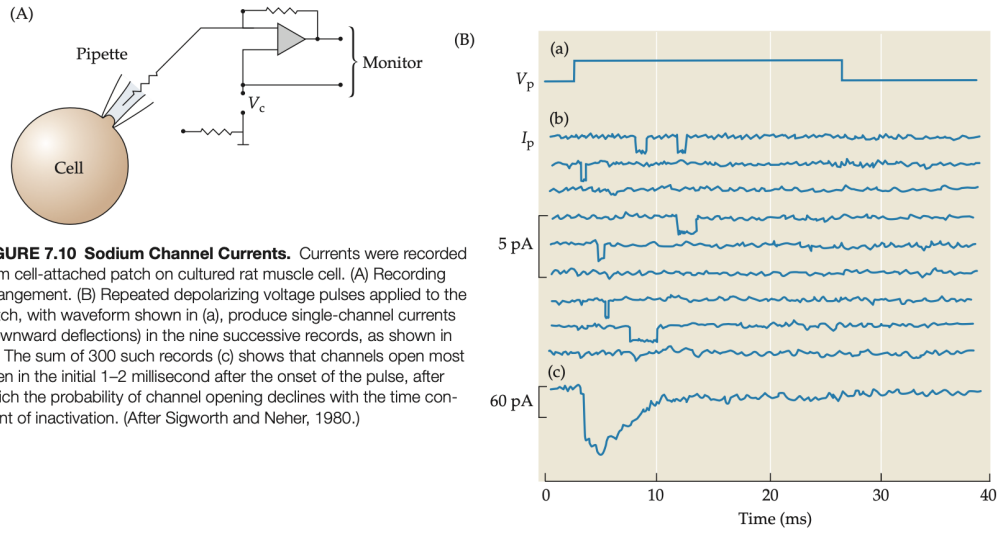
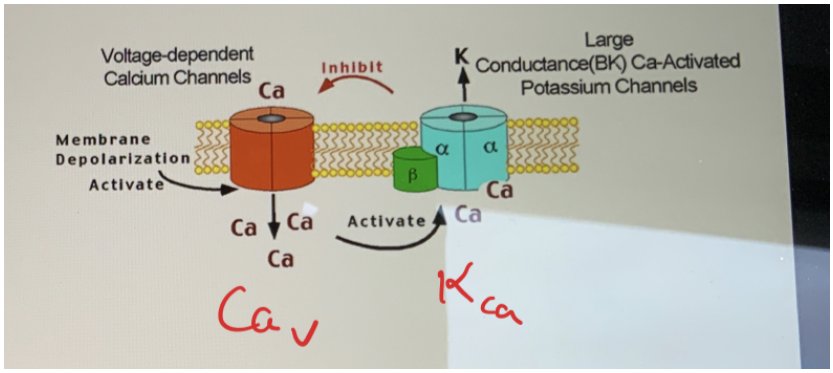


FIGURE 7.10 Sodium Channel Currents. Currents were recorded from cell-attached patch on cultured rat muscle cell. (A) Recording arrangement. (B) Repeated depolarizing voltage pulses applied to the patch, with waveform shown in (a), produce single-channel currents (downward deflections) in the nine successive records, as shown in (b). The sum of 300 such records (c) shows that channels open most often in the initial 1–2 millisecond after the onset of the pulse, after which the probability of channel opening declines with the time constant of inactivation. (After Sigworth and Neher, 1980.)

Fig. 7.11 Hyperpolarizing Afterpotential (AHP)

- Caused by delayed rectifier channels continue to open for a period that outlasts the action potential
- Fast Phase
    - Persistent activation of delayed rectifier channels (K)
    - allow a sustained K+ efflux to allow hyperpolarization
- Slow Phase
    - Potassium efflux through **calcium-activated potassium channels**
        - B: low Ca2+ out, <u>slow phase disappeared</u> -> slow phase depends on calcium concentration
        - Mediated by small-K (SK) channel and big-K (BK) channels
            - Ca2+ activates BK and pushes more K+ out



- C: accumulation of successive AP <u>increases</u> the size of slow phase
    - successive impulse increases intracellular [Ca2+] --> more K+ out
- D: massive AP (steady depolarization current) -> <u>frequency adaptation</u>
    - caused the depolarization falls (bc inc intracellular Ca2+ increases K conductance) below the threshold --> larger, slow AHP (longer in duration)
    - Spike Frequency Adaptation
- E: addition of cadmium blocks voltage-sensitive calcium channel, adaptation and slow AHP disappeared

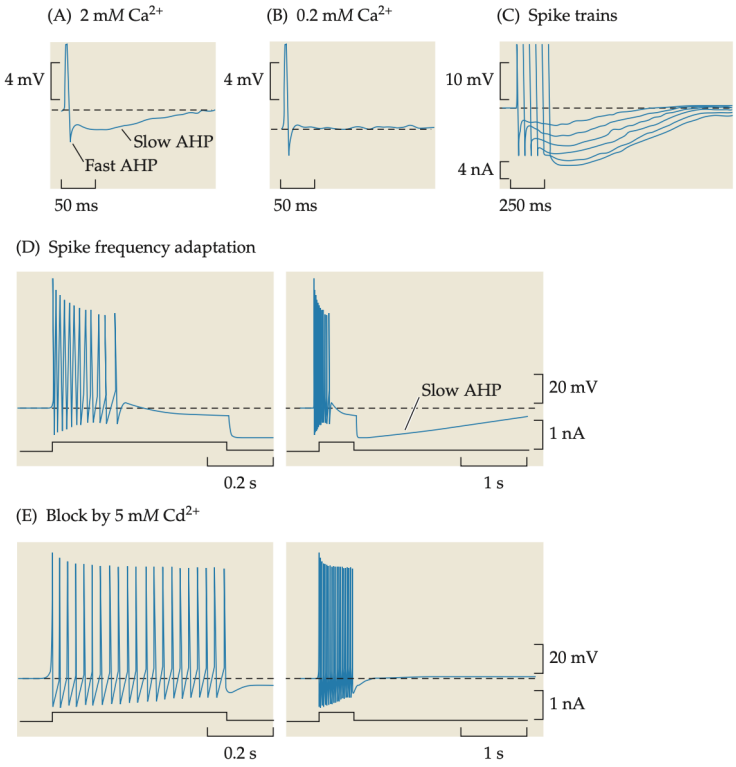


FIGURE 7.11 Hyperpolarizing Afterpotentials. (A) Action potential in a frog spinal motoneuron is followed by an afterhyperpolarization (AHP) with two phases: slow and fast. (B) After the preparation is soaked in low calcium bathing solution, the slow AHP disappears, suggesting that it depends on calcium influx during the action potential. (C) Superimposed records of trains of action potentials in a guinea pig vagal motoneuron. Size of the slow AHP increases as the number of successive action potentials in a train is increased from one to six. (D) A steady depolarizing current produces a train of action potentials that decreases in frequency (adapts) before dying out. Longer duration record reveals a large, slow AHP after the depolarizing current pulse is removed. (E) After block of voltage-sensitive calcium channels by the addition of cadmium to the bathing solution, the adaptation and slow AHP disappear. (A and B after Barrett and Barrett, 1976; B–E after Yarom, Sugimori, and Llinas, 1985.)

Fig. 7.12 Depolarizing Afterpotential (ADP)

- Prolonged depolarization
- activation of a **TTX-sensitive sodium current (*I*NaP)**
    - a low threshold of activation and very slow inactivation
- Decrease external Ca2+ --> reduce Ca2+ dependent K+ current --> larger ADP --> initiate second AP

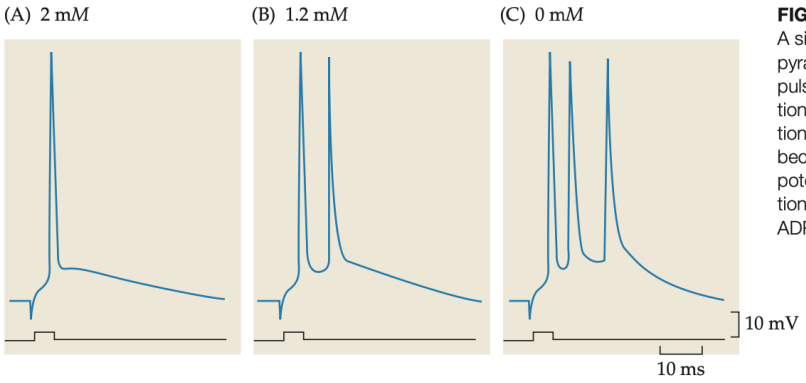
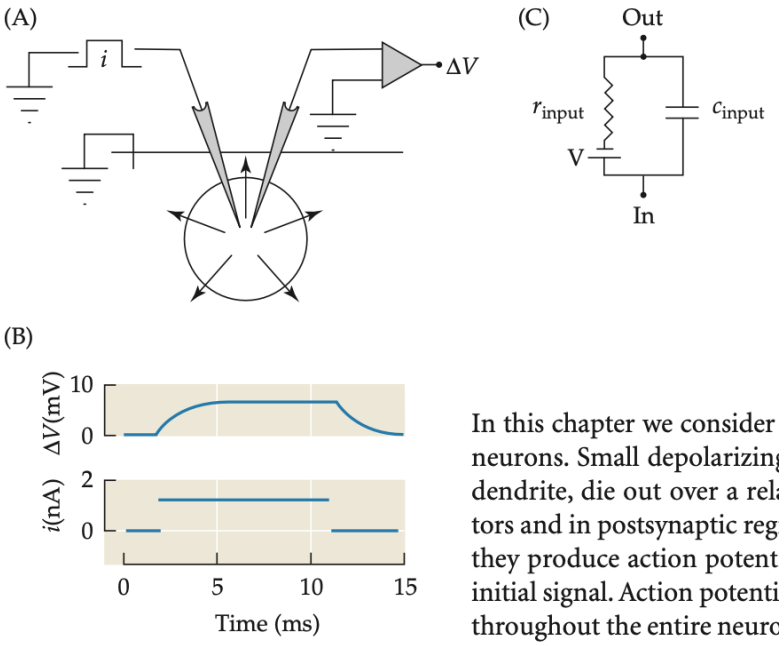


FIGURE 7.12 Depolarizing Afterpotentials. A single action potential in a rat hippocampal pyramidal cell, produced by a brief depolarizing pulse, is followed by a sustained afterdepolarization (ADP). When the external Ca^{2+} concentration is reduced from 2 m*M* to 1.2 m*M* the ADP becomes larger and initiates a second action potential. Reduction of external Ca^{2+} concentration to zero results in a further increase in the ADP. (After Golomb, Yue, and Yaari, 2006.)

Axon

Fig. 8.1 Response to Current Injection

- Positive current injected
- Voltage rise gradually
- when injection stopped -> decayed gradually
- The **input resistance** of the cell, ***r*input**, represents the pathway for current flow <u>across the cell membrane</u>
    - Input resistance is the inverse of the input conductance of the cell (*r*input = 1/*g*input)
    - Δ*V*max = *i* x *r*input
- **Specific capacitance** of the membrane (*C*m) is the capacitance of one square centimeter of membrane
- **Specific resistance** of the membrane (*R*m) is the resistance of one square centimeter of membrane.
- τm = *R*m*C*m



In this chapter we consider
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Fig. 8.2 Response to Current Injection

- B: The potential becomes smaller and rises more slowly as you move away from the place of the current injection
- C: Plot of response amplitude against distance from the current electrode. Decay of the response (ΔV) with distance is <u>exponential</u>.
  - $\Delta V = \Delta V_0 \, e^{-x/\lambda}$
    - λ is the **length constant**
- two factors, *r*input and λ, determine both the <u>size</u> of the response to current injection and <u>how far</u> the signal will spread along the fiber.
- **length constant** <u>increases</u> as <u>membrane resistance increases</u>, and <u>decreases</u> when the <u>internal longitudinal resistance increases</u>
- large diameter processes have <u>greater length constants</u> than small ones

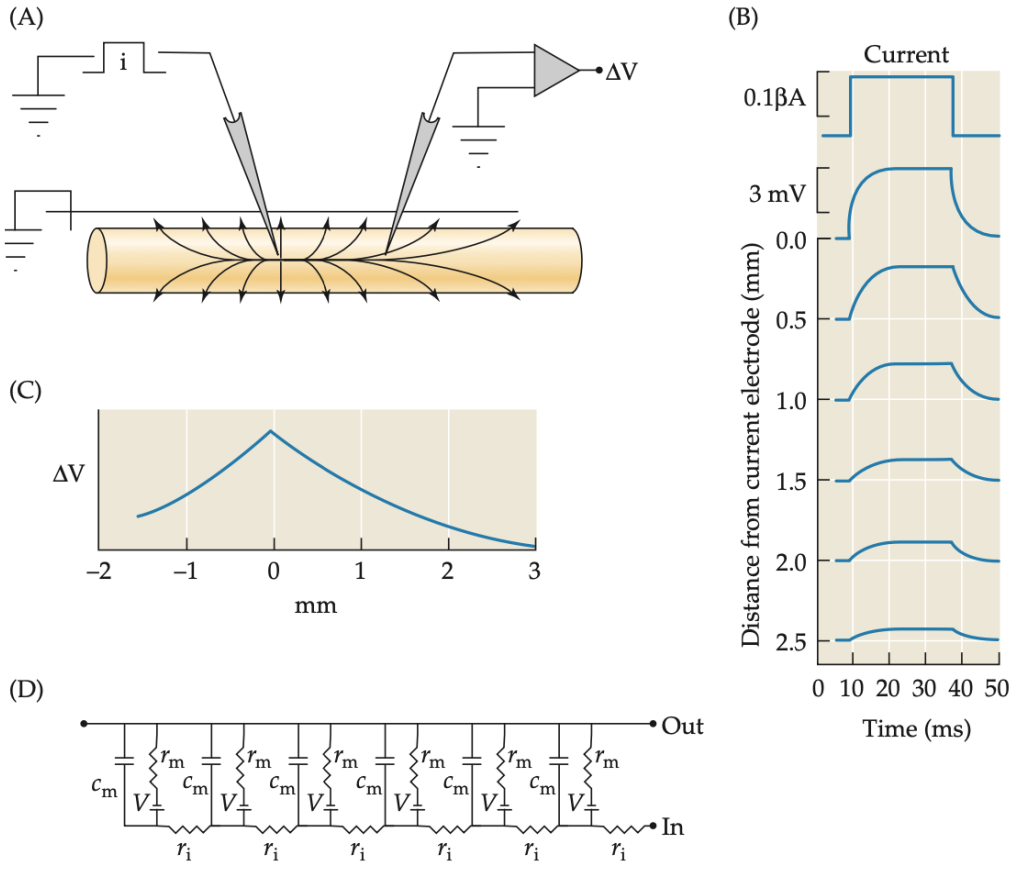
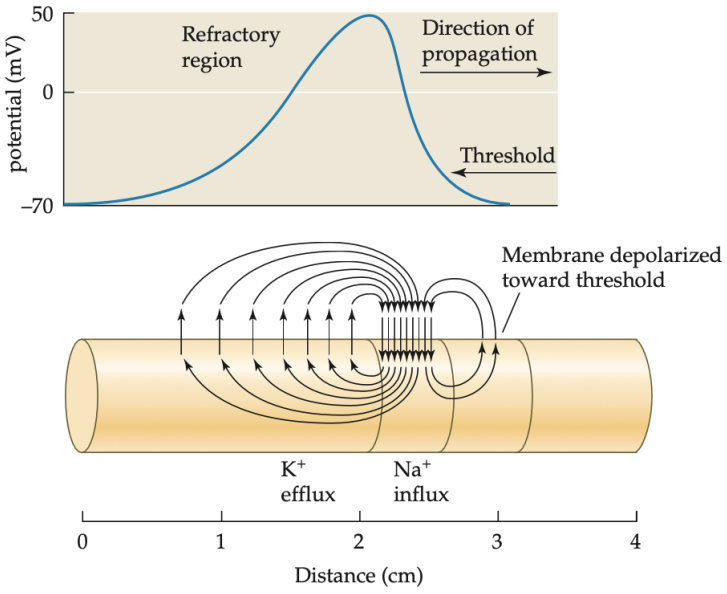


Fig. 8.3 Current Flow during an Action Potential

- Rapid depolarization on the rising phase of the action potential is due to an influx of positively charged sodium ions.
- The positive current spreads ahead of the impulse to depolarize the adjacent segment of membrane toward the threshold.
- Efflux of potassium ions behind the peak leads to repolarization.
    - An action potential cannot double back on itself, reversing its direction of propagation. This is because the peak depolarization is followed by a **refractory period** during which re-excitation cannot occur
- The rate of propagation of the action potential is influenced by both the space constant and time constant of the fiber.
    - If the time constant is small, the membrane will depolarize to threshold quickly and the conduction velocity will be relatively high.
    - If the space constant is large, the depolarization will spread a correspondingly large distance ahead of the active region, again speeding propagation of the signal.
        - large fibers conduct more rapidly than small fibers because of their larger space constants



Box 8.2 Classification of Vertebrate Nerve Fibers

- Group A fibers (group A refers to myelinated fibers in peripheral nerves) were further subdivided according to conduction velocity
    - α (80–120 m/s), β (30–80 m/s), and δ (5–30 m/s).
- γ fibers
    - motor nerves supplying muscle spindles, which have conduction velocities that span the β and lower part of the α range.
- Group B consists of myelinated fibers in the autonomic nervous system that have conduction velocities in the lower part of the A-fiber range.
- Group C refers to unmyelinated fibers, which conduct very slowly (less than 2 m/s).

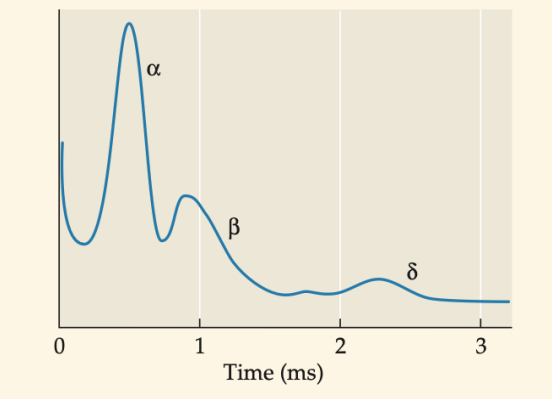


Fig. 8.5 Spread of Action Potentials in Dendrites.

- Propagation of impulse may fail when encounters an expanded area (ie: at axon branch)
    - Axon potential initiated in axon hillock, and propagates both outwards in the axon and back into the soma to the dendrite of the cell
- A: a cerebellar Purkinje cell (generate both Na AP in soma and Ca AP in dendrites), obtained by impaling the cell at the indicated locations and passing a depolarizing current through the electrode
    - (a) near end of dendrite: long duration calcium action potentials
    - (b,c) middle: depolarization produces calcium action potentials in the dendrite. Accompanying sodium action potentials generated in the soma spread passively into the dendritic tree and die out after a short distance
    - (d) cell soma: a steady depolarizing current produced high-frequency sodium action potentials, interrupted periodically by calcium action potentials
- B: Responses of a pyramidal cell to depolarization of the distal dendrite by activation of excitatory synapses

- (a) dendritic depolarization attenuates as it spreads passively toward the soma
    - Upon arrival, the depolarization produces a somatic action potential that then spreads back into the dendrite
- (b) direct activation of a dendritic calcium action potential that precedes the action potential generated in the soma

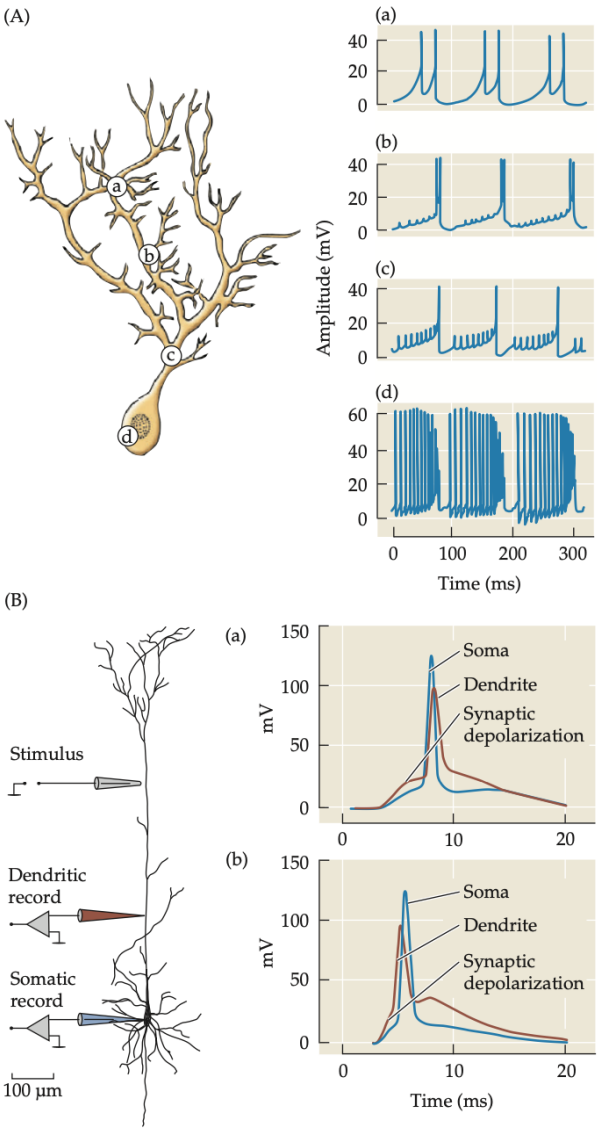


FIGURE 8.5 Spread of Action Potentials in Dendrites. (A) Records from a cerebellar Purkinje cell, obtained by impaling the cell at the indicated locations and passing a depolarizing current through the electrode. Near the end of the dendritic tree (a) depolarization produces long duration calcium action potentials. In the cell soma (d) a steady depolarizing current produced high-frequency sodium action potentials, interrupted periodically by calcium action potentials. At intermediate locations (b,c) depolarization produces calcium action potentials in the dendrite. Accompanying sodium action potentials generated in the soma spread passively into the dendritic tree and die out after a short distance. (B) Conduction in a cortical pyramidal cell. A cortical cell dendrite is depolarized by activating distal excitatory synapses. (a) Moderate depolarization of the dendrite spreads to the soma, where it initiates an action potential (blue record). In the dendrite, the initial depolarization is larger, and is followed by an action potential that spreads back from the soma (red record). (b) Larger depolarization produces a calcium action potential in the dendrite that precedes action potential initiation in the soma. (A after Llinás and Sugimori, 1980; B after Stuart, Schiller, and Sakmann, 1997.)

Direct Synaptic Transmission

Box 11.3 Action of Tubocurarine at Motor End Plate

- Tubocurarine acts as a reversible competitive antagonist of acetylcholine at the motor end plate
    - bind but not activating
- Thus, tubocurarine binds to the receptor about 100 times longer than acetylcholine;
    - so for equal concentrations, the competition is weighted in favour of tubocurarine
    - this would be equalized if the concentration of ACh were 100 times greater
- **DR - 1: Dose Ratio**
    - A_B/A
    - to get the same response to the agonist (Ach) in the presence of the antagonist as seen before adding the antagonist, the concentration of the agonist must be increased from A to AB such that $A_B / A = \{1 + [B] / K_B\}$.
    - More tubocurarine means you need to add more Ach to combate the effect

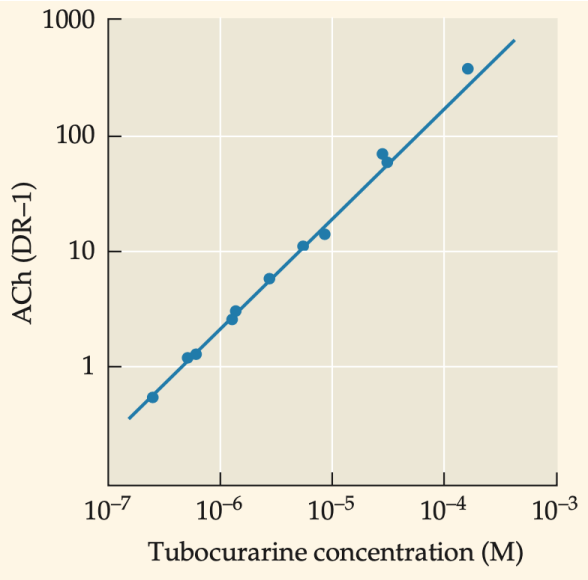


Fig. 11.3As they moved the recording microelectrode farther and farther away from the end plate, the end-plate potential amplitude became progressively smaller and its time to peak progressively longer.

Synaptic Potentials Recorded with an Intracellular Microelectrode

- Normally the amplitude of the end-plate potential in a skeletal muscle fiber is much greater than that needed to initiate an action potential.
- The amplitude can be reduced by adding curare, a blocker of the postsynaptic receptors, to the bathing solution
- With sufficient curare (about 1 μ*M*), the amplitude of the **end-plate potential (EPP)** is reduced to below threshold, so that it is no longer obscured by the action potential
- The curare concentration in the bathing solution was adjusted so that the amplitude of the synaptic potential (endplate potential) was near threshold and hence, on occasion evoked an action potential in the muscle fiber

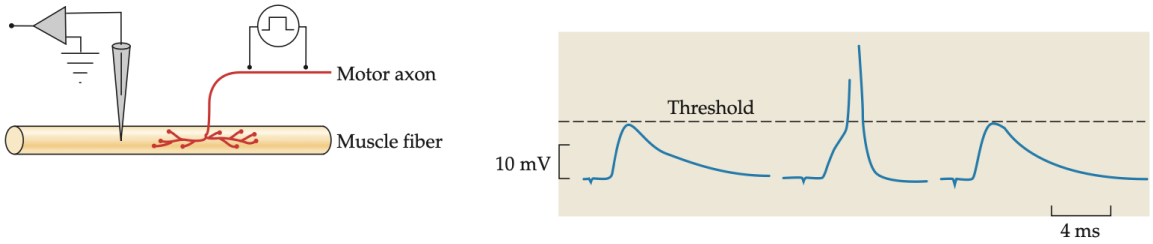


FIGURE 11.3 Synaptic Potentials Recorded with an Intracellular Microelectrode from a mammalian neuromuscular junction treated with curare. The curare concentration in the bathing solution was adjusted so that the amplitude of the synaptic potential was near threshold and hence, on occasion evoked an action potential in the muscle fiber. (From Boyd and Martin, 1956.)

Fig 11.4 Decay of Synaptic Potentials with Distance from the End-Plate Region of a Muscle Fiber

- As they moved the recording microelectrode farther away from the end plate, the end-plate potential amplitude became progressively smaller and its time to peak progressively longer.
- after reaching its peak, the end-plate potential decayed at a rate that was consistent with the time constant of the muscle fiber membrane

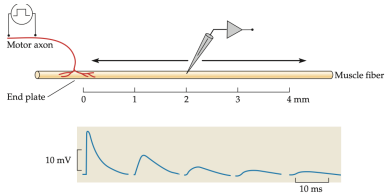


FIGURE 11.4 Decay of Synaptic Potentials with Distance from the End-Plate Region of a Muscle Fiber. As the distance from the end plate increases, synaptic potentials recorded by an intracellular electrode decrease in size and rise more slowly. (After Fatt and Katz, 1951.)

Fig 11.5 Mapping the Distribution of ACh Sensitivity by Ionophoresis at the Frog Neuromuscular Junction

- **Ionophoresis**: ejecting charged molecules (here are Ach ions) from the pipette.
- B: Responses to small ionophoretic pulses of ACh applied at different distances from the axon terminal
    - The amplitude and rate of rise of the response decrease rapidly as ACh is applied farther from the terminal --> **further = smaller EPP**

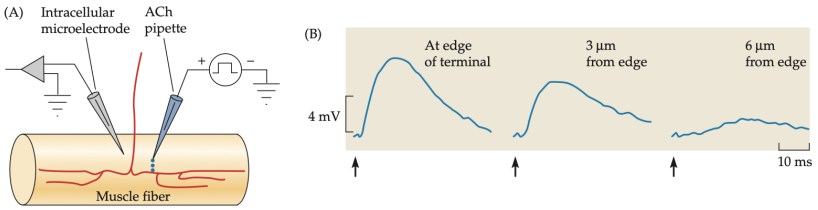


FIGURE 11.5 Mapping the Distribution of ACh Sensitivity by Ionophoresis at the Frog Neuromuscular Junction. (A) An ACh-filled pipette is placed close to the neuromuscular junction, and ACh is ejected from the tip by a brief, positive voltage pulse (ionophoresis). An intracellular microelectrode is used to record the response from the muscle fiber. (B) Responses to small ionophoretic pulses of ACh applied at different distances from the axon terminal (indicated by the blue dots in [A]). The amplitude and rate of rise of the response decrease rapidly as ACh is applied farther from the terminal. (After Peper and McMahan, 1972.)

Fig 11.6 Acetylcholine Receptor Distribution at the Skeletal Neuromuscular Junction of the Snake.

- A: The axon terminates in a cluster of **boutons**
- B: Synaptic vesicles mediate Ach release from nerve terminal with 50nm diameter
- D: At **synaptic region (crater) -->** uniformly high sensitivity to Ach; As we move away from the craters --> uniformly low sensitivity to ACh
- ACh receptors are highly concentrated in the region of the synapse.

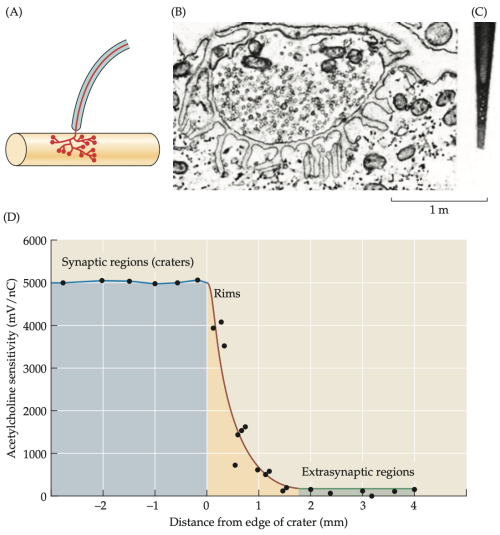


FIGURE 11.6 Acetylcholine Receptor Distribution at the Skeletal Neuromuscular Junction of the Snake. (A) An end plate on a skeletal muscle of a snake. The axon terminates in a cluster of boutons. (B) Electron micrograph of a cross section through a bouton. Synaptic vesicles, which mediate ACh release from the nerve terminal, are 50 nanometers (nm) in diameter. (C) Electron micrograph of the tip of a micropipette used for ionophoresis of ACh, shown at the same magnification as (B). The pipette has an outer diameter of 100 nm and an opening of about 50 nm. (D) ACh was applied by ionophoresis across the postsynaptic crater left after the nerve terminal was removed with collagenase. The craters have a uniformly high sensitivity to ACh (5000 mV/nC), with the sensitivity declining steeply at the rims of the craters. Extrasynaptic regions have a uniformly low ACh sensitivity (100 mV/nC). (After Kuffler and Yoshikami, 1975a.)

Fig 11.8 Reversal Potential for Synaptic Currents Measured by Voltage Clamp Recording

- A: Measuring reversal potential at motor end plate by clamping membrane voltage of muscle fibre and measuring current flow directions
- B: at -40 mV: inward current; at around 0: no current; at 22 mV: outward current
- C: peak amplitude of the end-plate current as a function of holding potential
    - **near 0, with some estimation to be around -15 mV**
- the effect of ACh was to produce a general increase in *cation* permeability (not Cl-)
    - The synaptic channel opened by Ach is electrically equivalent to two independent pathway for **Na and K**

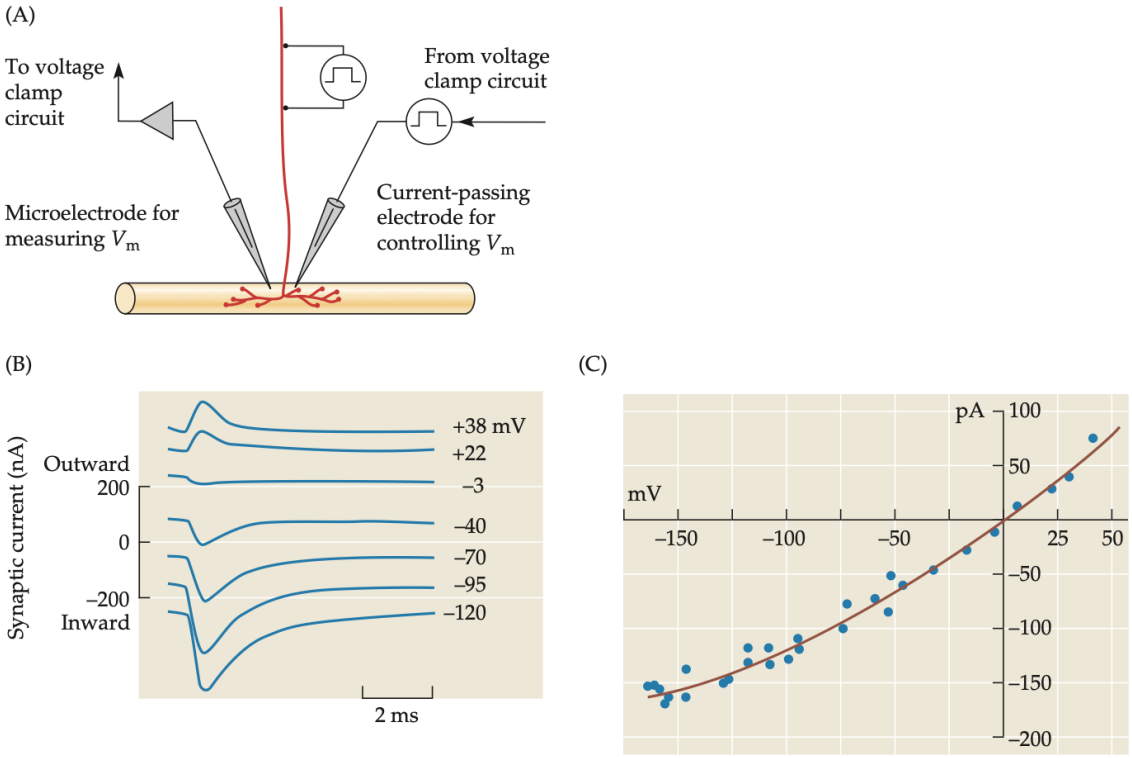


FIGURE 11.8 Reversal Potential for Synaptic Currents Measured by Voltage Clamp Recording. (A) Scheme for voltage clamp recording at the motor end plate. (B) Synaptic currents recorded at membrane potentials between –120 and +38 mV. When the muscle membrane potential is clamped below 0 mV, synaptic current flows into the muscle. Such inward current would depolarize the muscle if it were not voltage clamped. When the end-plate potential is clamped above 0 mV, synaptic current flows out of the cell. (C) Plot of peak end-plate current as a function of membrane potential. The relation is nearly linear, with the reversal potential close to 0 mV. (After Magleby and Stevens, 1972.)

Fig 11.10 Total End-Plate Current Is the Sum of Individual Channel Currents

- Current flow through six individual channels.
- Channels open instantaneously in response to ACh (added at the red marker point).
- ACh is rapidly hydrolyzed, so its concentration falls quickly (red marker), preventing any further channel openings. (Each channel APPEARS to be open only once)
- Channel open times are distributed exponentially.
- The individual channel currents sum to give the total end plate current (lower panel).
- The time constant of the decay of the total current is equal to the mean open time of the individual channels.

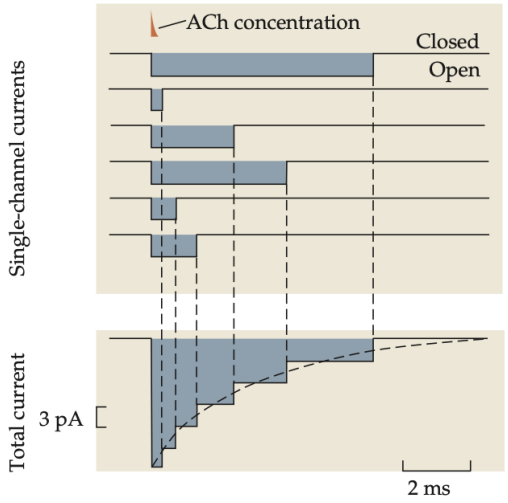


Fig 11.11 End-Plate Nicotinic Acetylcholine Receptors Open in Bursts

- Predicted **burst of three openings** (AR*) assuming the fastest possible binding rate (5 × 10^8 moles/s). The channel **flips briefly** to the ACh-bound but returns to the shut (AR) state twice before the acetylcholine dissociates and the channel reverts to the R state --> the original open channel lifetime is actually the mean burst length.
- High-resolution recording of an equivalent three-opening burst with two brief closures from a frog end-plate receptor activated by the nicotinic agonist suberyldicholine

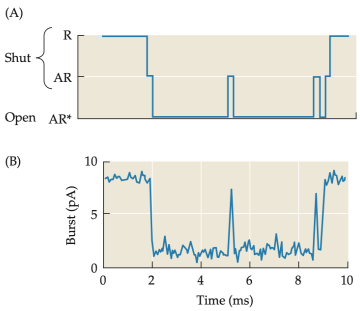


Fig 11.12 The Excitatory Neurotransmitter Glutamate Acts on Two Different Receptors to Produce Two Excitatory Postsynaptic Currents (EPSCs) in CNS

- The synaptic potentials were produced by stimulating the **glutamate-releasing afferent fibers** in the Schaeffer collaterals
- A: blocking AMPA receptor with CNQX (the competitive AMPA/kainate receptor antagonist) --> show activity of NMDA
    - **NMDA** is blocked by Mg at V_{rest}
    - voltage-dependent NMDA opens <u>slowly</u> due to high-frequency synaptic activity caused by high Ca2+ permeability
    - suppressed <u>all of the EPSC</u> at –80 mV but only the first part at +20 mV, leaving a large, slower component.
    - Key for memory formation
- B: blocking NMDA by APV --> show the activity of AMPA
    - **AMPA** receptors -> cannot be blocked by Mg2+
    - Responsible for the bulk of <u>fast</u> synaptic transmission due to low Ca2+ permeability --> AMPA receptor-mediated EPSC

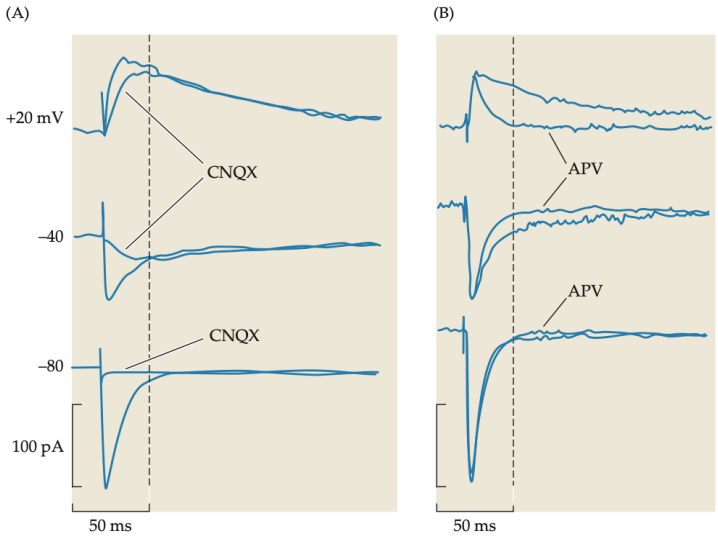


FIGURE 11.12 The Excitatory Neurotransmitter Glutamate Acts on Two Different Receptors to Produce Two Excitatory Postsynaptic Currents (EPSCs). EPSCs were recorded with a patch electrode from an interneuron in the CA1 region of a rat hippocampal slice preparation. The synaptic potentials were produced by stimulating the glutamate-releasing afferent fibers in the Schaeffer collaterals. The interneuron membrane potential was held at three different values, –80, –40, and +20 mV. (A) The traces show the effect of blocking the AMPA receptors with CNQX. This suppressed all of the EPSC at –80 mV but only the first part at +20 mV, leaving a large, slower component. The latter was due to the simultaneous activation of voltage-dependent NMDA receptors, since blocking the NMDA receptors with APV (B) left a pure, fast AMPA receptor-mediated EPSC. (C) Schematic of recording. PC = pyramidal cells; IN = interneuron; SC = Schaeffer collaterals; excitation (+); inhibition (–). (A and B after Sah et al., 1990).

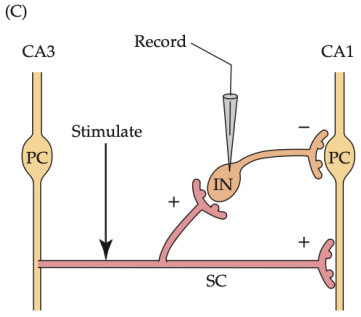
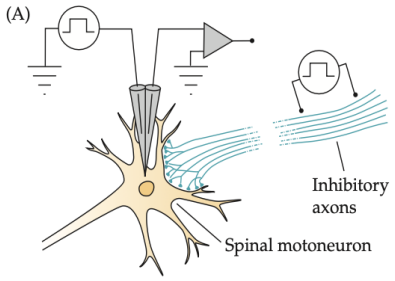


Fig 11.14 Direct Inhibitory Chemical Synaptic Transmission

- Inhibitory channels are permeable to anion --> here, Cl-
- at -75mV --> stimulation of the inhibitory inputs causes a slight <u>hyperpolarization</u> of the cell—the **inhibitory postsynaptic potential (IPSP)**
    - The flow of <u>Cl- INTO</u> the cell (influx) --> opposite of AP to hyperpolarize the membrane --> damping the excitation of AP
- **Reversal potential** between -74 mV and -82 mV (at around -80mV)



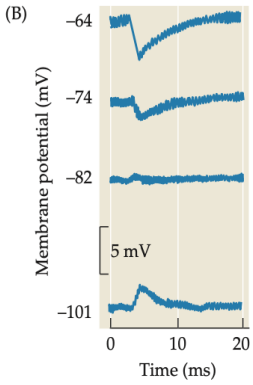


FIGURE 11.14 Direct Inhibitory Chemical Synaptic Transmission. (A) Scheme for intracellular recording from a cat spinal motoneuron and stimulation of inhibitory synaptic inputs. The membrane potential of the motoneuron is set to different levels by passing current through a second intracellular microelectrode. (B) Intracellular records of synaptic potentials evoked at membrane potentials between –64 and –101 mV. The reversal potential is between –74 and –82 mV. (After Coombs, Eccles, and Fatt, 1955b.)

Fig 11.15 Inhibitory Response to Glycine Depends on Chloride

- inhibitory synaptic transmission is mediated by glycine --> <u>glycine activates Cl channels</u>
- Glycine application resulted in a <u>slight hyperpolarization</u>, with a marked <u>reduction in input resistance</u> --> for the same current, less V change
- Removal of Cl- leads to no reduction in input resistance --> no Cl- through the channel
- The restoration of normal extracellular chloride concentration resulted in the restoration of the response
- Primary inhibitory transmitter: in PNS --> glycine; in CNS --> GABA

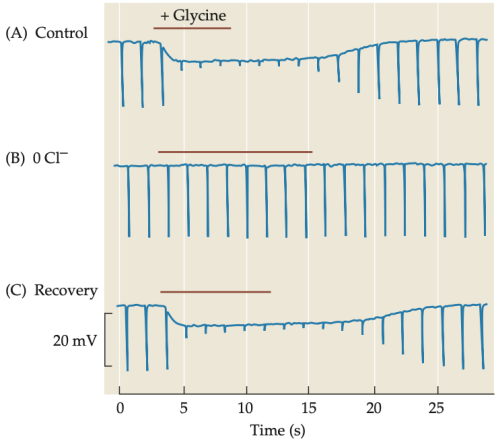


FIGURE 11.15 Inhibitory Response to Glycine Depends on Chloride. Intracellular microelectrode recordings from a neuron in the brainstem of the lamprey. (A) Resting membrane potential is –63 mV. Brief downward voltage deflections are produced by 10 nA current pulses from a second intracellular microelectrode; their amplitude indicates membrane resistance. On application of glycine (red bar), the cell is hyperpolarized by about 7 mV, and membrane resistance is reduced drastically. (B) After 20 minutes in chloride-free bathing solution, the response to glycine is abolished. (C) Five minutes after return to normal chloride solution the response has recovered. (From Gold and Martin, 1983.)

Fig 11.16 Presynaptic Inhibition in a Crustacean Muscle Fiber Innervated by One Excitatory and One Inhibitory Axon

- **Presynaptic** inhibition results in a reduction in the amount of transmitter released from excitatory nerve terminals
- A: Stimulation of the excitatory axon (E) produces a 2 mV EPSP;
- B: Stimulation of the inhibitory axon (I) produces a depolarizing IPSP of about 0.2 mV
- C: If the inhibitory stimulus follows the excitatory one by a short interval, there is no effect on the EPSP
- D: If the inhibitory stimulus precedes the excitatory one by a few milliseconds, the EPSP is almost abolished.

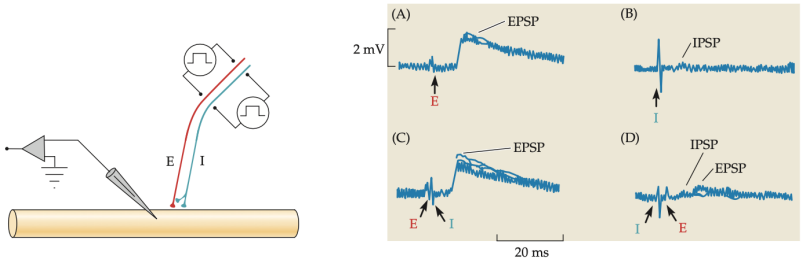


FIGURE 11.16 Presynaptic Inhibition in a Crustacean Muscle Fiber Innervated by One Excitatory and One Inhibitory Axon. (A) Stimulation of the excitatory axon (E) produces a 2 mV EPSP. (B) Stimulation of the inhibitory axon (I) produces a depolarizing IPSP of about 0.2 mV. (C) If the inhibitory stimulus follows the excitatory one by a short interval, there is no effect on the EPSP. (D) If the inhibitory stimulus precedes the excitatory one by a few milliseconds, the EPSP is almost abolished. The importance of precise timing indicates that the inhibitory nerve is having a presynaptic effect, reducing the amount of excitatory neurotransmitter that is released. (After Dudel and Kuffler, 1961.)

Fig 11.17 Presynaptic Auto-Inhibition at a GABA-Releasing Synapse in the Hippocampus

- **GABA-A Receptor** -> postsynaptic, chloride conducting; **GABA-B Receptor** -> presynaptic, metabotropic
- The IPSC evoked by the second stimulus is much smaller than that produced by the first stimulus (paired-pulse depression)
    - The second depression was largely reduced due to activation of the presynaptic $GABA_B$ receptors --> since the depression was reduced by blocking these receptors with $GABA_B$ antagonists (0.2mM 2-hydroxy-saclofen or 0.2 m*M* CGP 35348)
- GABA activates
    - GABA-A: on postsynaptic membrane to produce IPSC
    - GABA-B: inhibit Ca2+ channel and reduce transmitter release (slow activation due to GPCR)

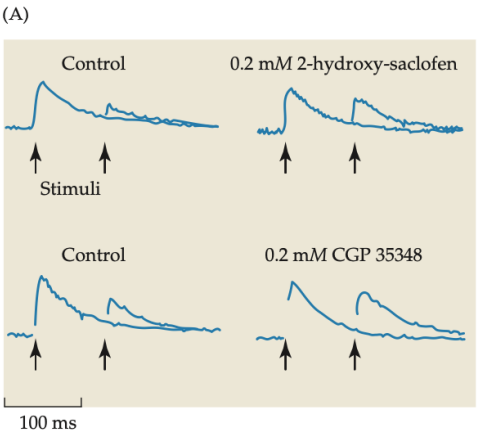


FIGURE 11.17 Presynaptic Auto-Inhibition at a GABA-Releasing Synapse in the Hippocampus. (A) Inhibitory synaptic currents (IPSCs) in a pyramidal neuron of a hippocampal slice preparation produced by two successive stimuli, delivered 100 ms apart, to inhibitory fibers in the adjacent stratum oriens. Excitatory currents were blocked with glutamate antagonists. The IPSC evoked by the second stimulus is much smaller than that produced by the first stimulus (paired pulse depression). This depression was largely due to activation of the presynaptic $GABA_B$ receptors, since it was reduced by blocking these receptors with $GABA_B$ antagonists (0.2 m*M* 2-hydroxy-saclofen or 0.2 m*M* CGP 35348; right-hand panels). (B) GABA released from the presynaptic terminal activates chloride-conducting $GABA_A$ receptors on the postsynaptic membrane to produce an inhibitory postsynaptic current (IPSC). It also activates metabotropic $GABA_B$ receptors on the presynaptic ending that inhibit calcium channels and reduce transmitter release. (A after Davies and Collingridge, 1993).

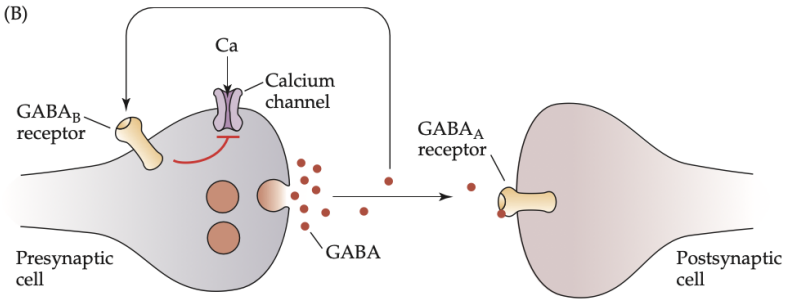
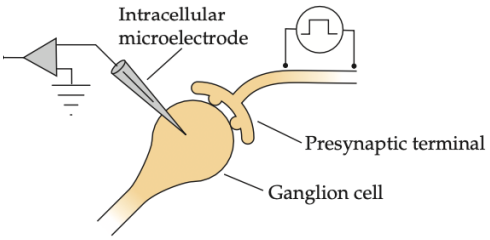
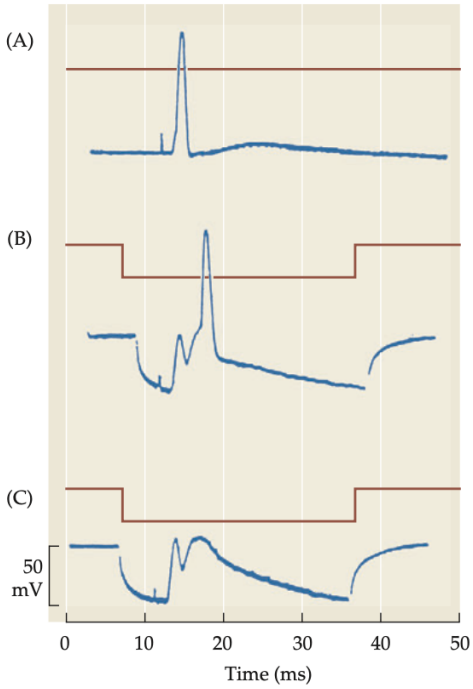


Fig 11.21 Electrical and Chemical Synaptic Transmission in a Chick Ciliary Ganglion Cell

- B: The ganglion cell is hyperpolarized by passing current through the recording electrode (red trace), the cell reaches threshold later, revealing an earlier, transient depolarization (blue trace).
    - This depolarization is an **electrical synaptic potential (coupling potential)**, caused by current flow into the ganglion cell from the presynaptic terminal
    - In A, the electrical synaptic potential depolarized the ganglion cell to threshold, initiating an action potential.
- C: Slightly greater hyperpolarization prevents the ganglion cell from reaching threshold, exposing a slower **chemical synaptic potential**.
    - The chemical synaptic potential follows the coupling potential with a **synaptic delay** of about 2 ms at room temperature
        - The delay is due to the time taken for the terminal to release the transmitter
    - No synaptic delay in electrical synapse


Intracellular
microelectrode
Presynaptic terminal
Ganglion cell


(A)
(B)
(C)
50
mV
0
10
20
30
40
50
Time (ms)

Indirect Synaptic Transmission

Use of 2nd messenger (mainly G-protein)

Fig 12.4 Acetylcholine (ACh) Opens Potassium Channels in Sinoatrial Cells of the Rabbit Heart

- A: brief ionophoretic ejection of ACh from a micropipette transiently hyperpolarizes the cell membrane and **inhibits** spontaneous action potentials for ~3 seconds
- B: When membrane current is recorded using voltage clamp, a similar ACh application produces an outward K+ current, IK, which starts after about 50 milliseconds (ms), and lasts about 1.5 seconds --> slow heart rate

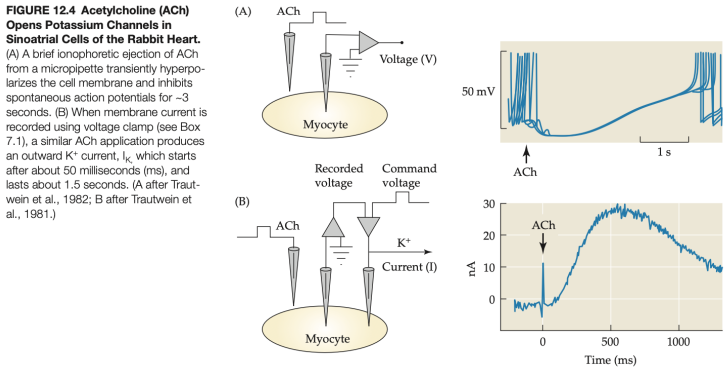


FIGURE 12.4 Acetylcholine (ACh) Opens Potassium Channels in Sinoatrial Cells of the Rabbit Heart. (A) A brief ionophoretic ejection of ACh from a micropipette transiently hyperpolarizes the cell membrane and inhibits spontaneous action potentials for ~3 seconds. (B) When membrane current is recorded using voltage clamp (see Box 7.1), a similar ACh application produces an outward K^+ current, I_K, which starts after about 50 milliseconds (ms), and lasts about 1.5 seconds. (A after Trautwein et al., 1982; B after Trautwein et al., 1981.)

Fig 12.5 Direct Modulation of Channel Function by G Protein

- inside-out clamp
- A: **Gβγ complex** to the **intracellular surface** (in bath) --> increase in potassium channel activity similar to that seen when acetylcholine (ACh) is added to the extracellular side of the patch (ACh in pipette, extracellular)
- B: Binding of ACh to muscarinic receptors (mAChR) activates a G protein
    - activated βγ-complex binds directly to and opens a potassium channel

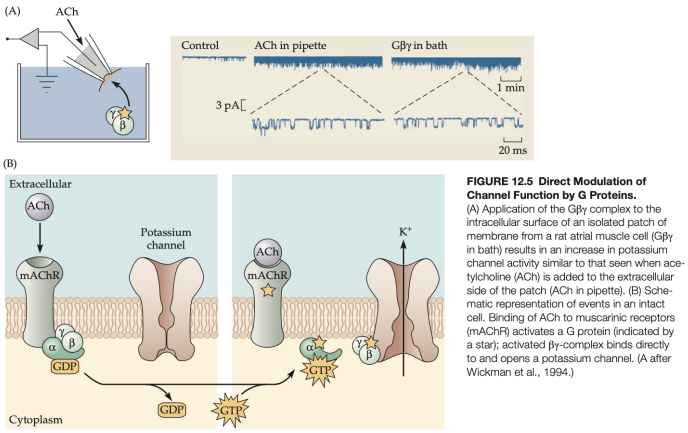


FIGURE 12.5 Direct Modulation of Channel Function by G Proteins. (A) Application of the Gβγ complex to the intracellular surface of an isolated patch of membrane from a rat atrial muscle cell (Gβγ in bath) results in an increase in potassium channel activity similar to that seen when acetylcholine (ACh) is added to the extracellular side of the patch (ACh in pipette). (B) Schematic representation of events in an intact cell. Binding of ACh to muscarinic receptors (mAChR) activates a G protein (indicated by a star); activated βγ-complex binds directly to and opens a potassium channel. (A after Wickman et al., 1994.)

Fig 12.6 Direct, or Membrane-Delimited, Effects of G Proteins Operate over Short Distances

- B: Recordings of single-channel currents before and during the addition of ACh.
    - Compared with the control, channel activity increased only when ACh was added to the patch pipette.
- it involves Gβγ complex --> membrane-bound, highly localized
    - if it involves alpha subunit, we wound have measured it

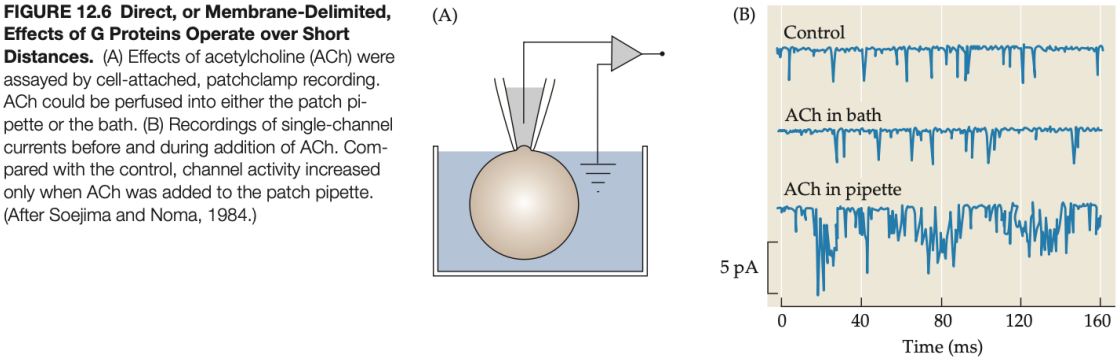


FIGURE 12.6 Direct, or Membrane-Delimited, Effects of G Proteins Operate over Short Distances. (A) Effects of acetylcholine (ACh) were assayed by cell-attached, patchclamp recording. ACh could be perfused into either the patch pipette or the bath. (B) Recordings of single-channel currents before and during addition of ACh. Compared with the control, channel activity increased only when ACh was added to the patch pipette. (After Soejima and Noma, 1984.)

Fig 12.7 Cholinergic Synaptic Inhibition in the Nervous System Results from the Opening of Potassium Channels

- A: Neurons in the 9th and 10th sympathetic ganglia are innervated by **descending cholinergic preganglionic** fibers in spinal nerves 7 and 8 and send their postganglionic axons out in the sciatic nerve
- B: Intracellular recordings from a small neuron in the 9th sympathetic ganglion.
    - A series of stimuli to the descending preganglionic fibers produces a slow hyperpolarization, which is imitated by electrophoretically applying Ach to the neuron through a micropipette & efflux of K+
    - This action results from stimulating **muscarinic (Ach) receptors** because the nicotinic receptors were blocked using tubocurarine
- C: Preganglionic stimulation also suppresses action potential discharges of the postganglionic neuron.
    - The neuron was made to fire repetitively by 4 minutes of preganglionic stimulation at 60 impulses per minute --> suppress by repetitive firing
    - This releases luteinizing hormone releasing hormone (LHRH), which depolarizes the cell because it produces a prolonged inhibition of the M-current

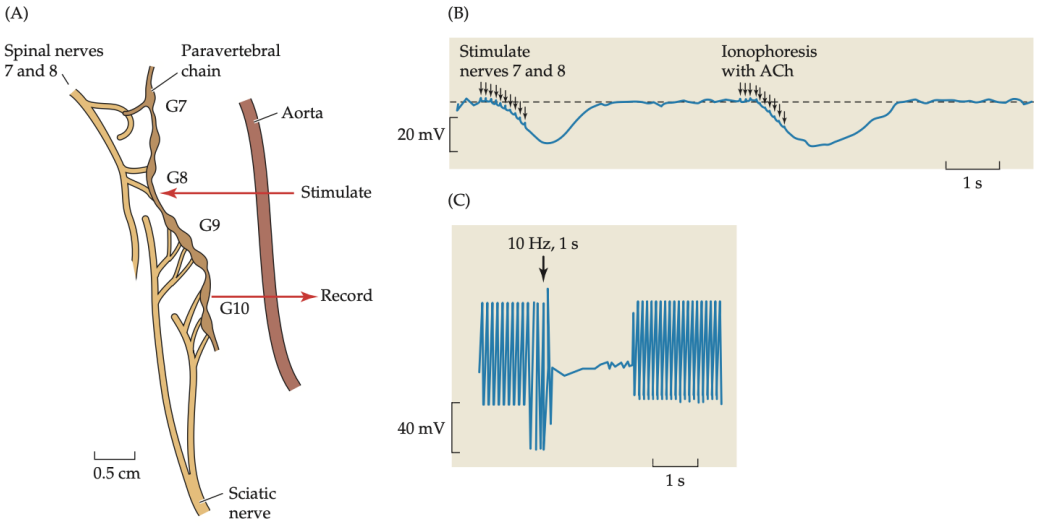
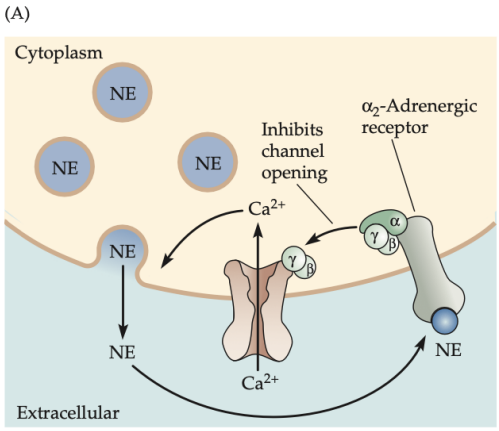


FIGURE 12.7 Cholinergic Synaptic Inhibition in the Nervous System Results from the Opening of Potassium Channels. (A) Drawing of the frog lumbar sympathetic nervous system. Neurons in the 9th and 10th sympathetic ganglia are innervated by descending cholinergic preganglionic fibers in spinal nerves 7 and 8 and send their postganglionic axons out in the sciatic nerve. (B) Intracellular recordings from a small neuron in the 9th sympathetic ganglion. A series of stimuli to the descending preganglionic fibers produces a slow hyperpolarization, which is imitated by electrophoretically applying acetylcholine to the neuron through a micropipette. This action results from stimulating muscarinic receptors because the nicotinic receptors were blocked using tubocurarine. (C) Preganglionic stimulation also suppresses action potential discharges of the postganglionic neuron. The neuron was made to fire repetitively by 4 minutes of preganglionic stimulation at 60 impulses per minute. This releases luteinizing hormone releasing hormone (LHRH; see Chapter 17), which depolarizes the cell because it produces a prolonged inhibition of the M-current. (A after Dodd and Horn, 1983a; B after Dodd and Horn, 1983b.)

Fig 12.8 Presynaptic Autoreceptors Reduce Transmitter Release

- A: **Norepinephrine (NE)** released from sympathetic neurons combines with **α2-adrenergic receptors** (called autoreceptors) in the terminal membrane, activating a G protein. The activated βγ-complex binds to calcium channels, **decreasing calcium influx** and so limiting further transmitter release
- B: Norepinephrine reduces the release of transmitter from sympathetic ganglia. Ganglia were loaded with radioactive norepinephrine and then enclosed in a perfusion chamber. Transmitter release was evoked by depolarization with a solution containing 50 m*M* potassium (green bars). Addition of 30 μ*M* unlabeled norepinephrine to the perfusion solution (red bar) **reduced the amount of radiolabeled transmitter released** in response to potassium-induced depolarization
    - NE inhibits Ca2+ --> inhibit further release of NE



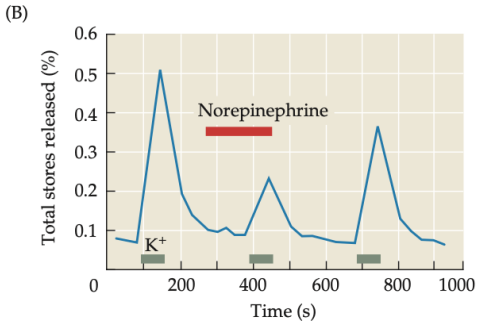


Fig 12.9 Norepinephrine Inhibits Calcium Channel Activity

- Single-channel currents were recorded in cell-attached patches
    - channels were activated with a depolarizing pulse (top trace)
    - When 30 μ*M* norepinephrine was included in the patch electrode, the unitary currents did not change in size (amplitude does not change), but channel openings were less frequent and of shorter duration

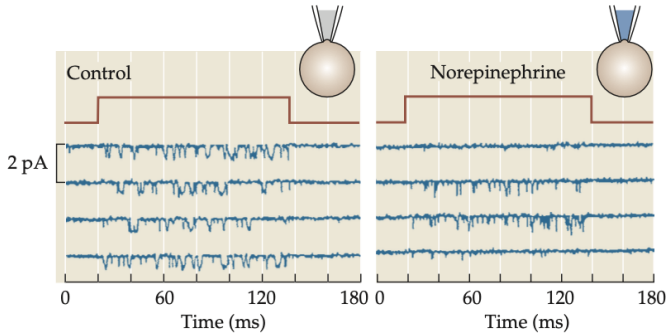


FIGURE 12.9 Norepinephrine Inhibits Calcium Channel Activity. Single-channel currents were recorded in cell-attached patches; channels were activated with a depolarizing pulse (top trace). When 30 μ*M* norepinephrine was included in the patch electrode, the unitary currents did not change in size, but channel openings were less frequent and of shorter duration. (After Lipscombe, Kongsamut, and Tsien, 1989.)

Fig 12.10 G Protein Regulation of Calcium Currents in a Sympathetic Neuron

- Records show N-type (CaV2.2) calcium currents recorded from a rat sympathetic neuron with a whole-cell patch pipette on stepping from –80 to +10 mV
- A: Norepinephrine (NE,10 μ*M*) inhibits the current and slows its activation (a).
    - This effect is imitated by adding 500 μ*M* of the non-hydrolyzable GTP-analog **GppNHp** (always activate GPCR) to the pipette solution (b), or when free G protein **βγ-subunits** were over-expressed by prior cDNA injection (c)
- B: The inhibitory actions of both norepinephrine (a) and Gβγ (b) are temporarily reversed by **strongly depolarizing the neuron** to +80 mV for 50 ms.
    - This is because the depolarization promotes the dissociation of the Gβγ-subunits from the calcium channel.
        - Voltage-dependent relief
        - Synaptic plasticity of CaV2.2 in presynaptic neuron by a train of AP and increase neurotransmitter release
            - Ca channel opens --> more NE is released --> more stimulation

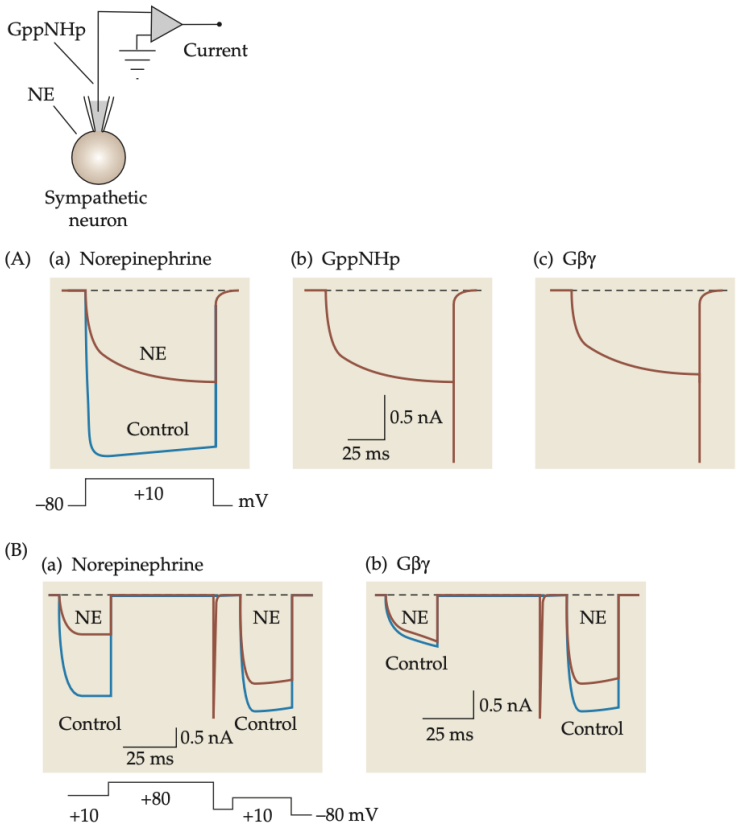


FIGURE 12.10 G Protein Regulation of Calcium Currents in a Sympathetic Neuron. Records show N-type ($Ca_V2.2$) calcium currents recorded from a rat sympathetic neuron with a whole-cell patch pipette on stepping from –80 to +10 mV. (A) Norepinephrine (NE, 10 μ*M*) inhibits the current and slows its activation (a). This effect is imitated by adding 500 μ*M* of the non-hydrolyzable GTP-analog GppNHp (see Box 12.2) to the pipette solution (b), or when free G protein βγ-subunits were over-expressed by prior cDNA injection (c). Calibration bars: 0.5 nA (vertical), 20 ms (horizontal). (B) The inhibitory actions of both norepinephrine (a) and Gβγ (b) are temporarily reversed by strongly depolarizing the neuron to +80 mV for 50 ms. This is because the depolarization promotes the dissociation of the Gβγ-subunits from the calcium channel. (After Ikeda, 1996.)

Fig 12.11 Activation of β-Adrenergic Receptors in Cardiac Muscle Increases Calcium Current

- A: The <u>increase in calcium current</u> produced by activation of **β-adrenergic receptors**, in this case by the addition of 10^{-6} *M* norepinephrine (NE), <u>increases action potential amplitude and duration</u> and the <u>tension</u> produced by cardiac muscle cells.
    - Ca2+ channel activity up --> lengthened plateau
- B: The current–voltage relationship of calcium current in a myocardial cell is measured under voltage clamp conditions in the <u>absence and presence</u> of 0.5 μ*M* epinephrine — a β-adrenergic receptor agonist (bind and activate)
    - No epinephrine --> low out current
    - With epinephrine --> high out current

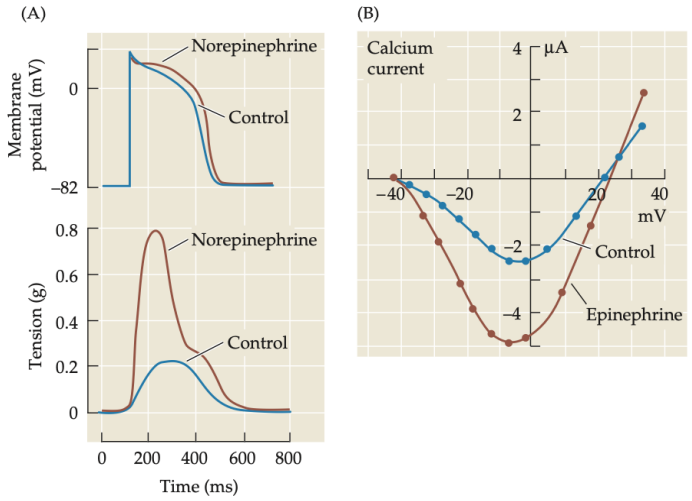


FIGURE 12.11 Activation of β-Adrenergic Receptors in Cardiac Muscle Increases Calcium Current. (A) The increase in calcium current produced by activation of β-adrenergic receptors, in this case by addition of 10^{-6} *M* norepinephrine, increases action potential amplitude and duration and the tension produced by cardiac muscle cells. (B) The current–voltage relationship of calcium current in a myocardial cell is measured under voltage clamp conditions in the absence and presence of 0.5 μ*M* epinephrine—a β-adrenergic receptor agonist. (A after Reuter et al., 1983; B after Reuter, 1974.)

Fig 12.12 β-Adrenergic Agonists Cause an Increase in Calcium Channel Activity

- Consecutive records of the activity of a patch containing two calcium chanels.
  - Addition of 14 μ*M* isoproterenol, a **β-adrenergic** agonist, to the <u>bath</u> causes an **increase in calcium channel activity** during depolarization

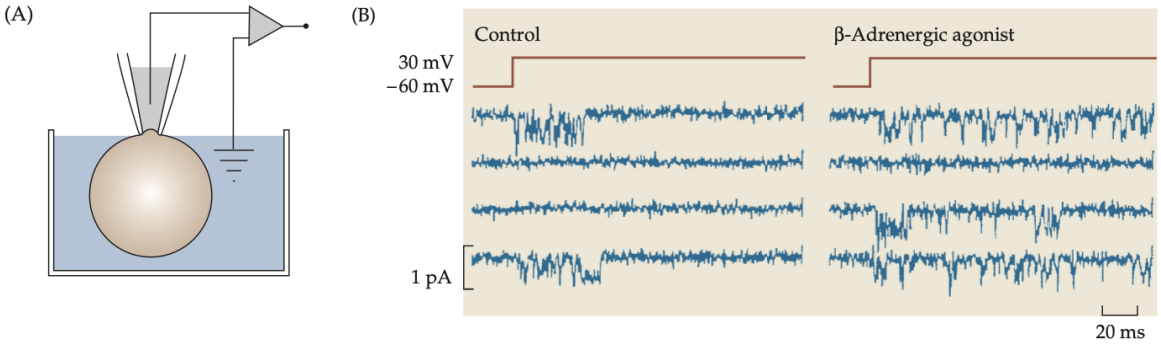


Fig 12.14 The Sinoatrial (SA) Node Heart Rate Is Regulated by β-Receptors through a Direct Effect of cAMP on the SA Pacemaker Current I_h

- The increase in **heart rate** produced by norepinephrine is due to a **direct** action of the cAMP on the channels responsible for the pacemaker current in the sinoatrial node
- (A). Recordings from an intact rabbit SA node fiber.
    - (a) The β-receptor agonist **isoprenaline** (Iso) increases the rate of spontaneous action potentials (red trace), whereas **acetylcholine** (ACh) reduces it (green trace).
    - (b). ACh and Iso have opposite effects on the inward pacemaker current Ih (activated by stepping to –85 mV).
        - an increase in the magnitude of the pacemaker current in Iso
        - inward current through cation-permanent, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (h-channels) that are opened by membrane hyperpolarization
        - HCN channels let Na+ in, depolarize the membrane and let heart beat faster
    - (c) ACh and Iso shift the activation curves for I_h in opposite directions.
        - Thus, h-channels open more readily with hyperpolarization in Iso solution, giving the faster-rising pacemaker current seen in (a), while ACh has the opposite effect.
        - the increased current is due to a shift in voltage sensitivity: in isoproterenol, less hyperpolarization is required to activate the current
- (B) Pacemaker h-current recorded in an excised inside-out SA node membrane patch with a 2-second step from –35 to –105 mV.
    - Current is increased by **cAMP** (a)
    - not by the catalytic subunit of **protein kinase A** (PKA) (b) --> No effect on HCN channels
        - PKA has no further effect after cAMP (c).
    - cAMP is needed to activate HCN channels

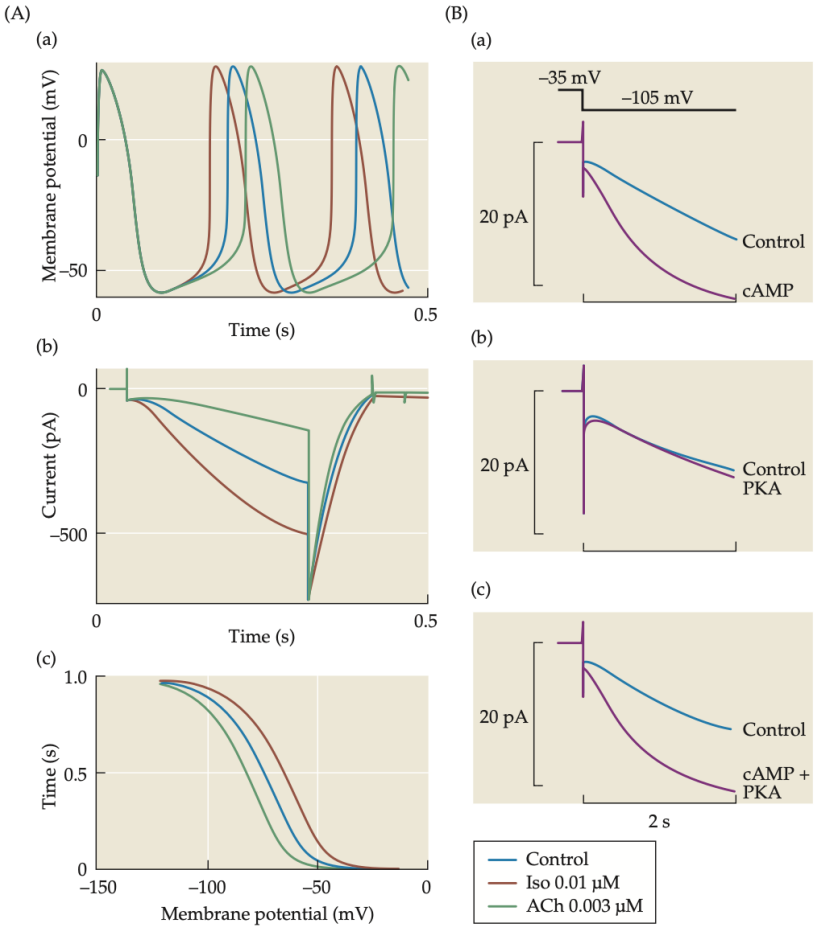


FIGURE 12.14 The Sinoatrial (SA) Node Heart Rate Is Regulated by β-Receptors through a Direct Effect of cAMP on the SA Pacemaker Current I_h. (A). Recordings from an intact rabbit SA node fiber. (a) The β-receptor agonist isoprenaline (Iso) increases the rate of spontaneous action potentials (red trace), whereas acetylcholine (ACh) reduces it (green trace). (b). ACh and Iso have opposite effects on the inward pacemaker current I_h (activated by stepping to –85 mV). (c) ACh and Iso shift the activation curves for I_h in opposite directions. Thus, h-channels open more readily with hyperpolarization in Iso solution, giving the faster-rising pacemaker current seen in (a), while ACh has the opposite effect. (B) Pacemaker h-current recorded in an excised inside-out SA node membrane patch with a 2-second step from –35 to –105 mV. Current is increased by cAMP (a) but not by the catalytic subunit of protein kinase A (PKA) (b), and PKA has no further effect after cAMP (c). (A after Accili et al., 2002; B after DiFrancesco and Tortora, 1991.)

Fig 12.16 Hydrolysis of Phosphatidylinositol-4,5-bisphosphate (PIP2)

- PIP2 accompanies <u>cholinergic inhibition of M-current</u> in an isolated rat sympathetic neuron.
- One such PIP2-regulated ion channel is the **M-channel**, which is a voltage-gated **K+ channel** that regulates the excitability of many central and peripheral neurons.
    - (A) The GFP-tagged PH-domain of phospholipase Cδ (**GFP- PLCδ-PH**) is used as a fluorescent probe to observe PIP2 hydrolysis.
        - At rest (a), the probe binds to PIP2 in the membrane.
        - The muscarinic (ACh) agonist **oxotremorine-M** (Oxo-M) (b) stimulates <u>PIP2 hydrolysis</u> and membrane PIP2 falls, so the probe leaves the membrane and goes into the cytoplasm -> binds to free **IP3**. On washing out the Oxo-M (c), PIP2 is resynthesized so the probe returns to the membrane.

- (B) The time course of GFP-PLCδ-PH movement, registered as the change of fluorescence in a region of the cytoplasm. Fluorescence is expressed as fractional increase over original baseline, ΔF/FO.
- (C) The time-course of M-current inhibition and recovery, recorded as the loss and recovery of outward K+ current at –20 mV.
    - The membrane was hyperpolarized to –50 mV for 2 seconds every 15 seconds to check the change of conductance (given by downward current deflections).
    - Note that the change of M-current closely follows the fluorescence change.
        - Cytoplasmic fluorescent = no PIP2 present -> inhibit
        - Add Oxo-M --> hydrolyze PIP2 -> drop in inhibition -> drop in K+ current

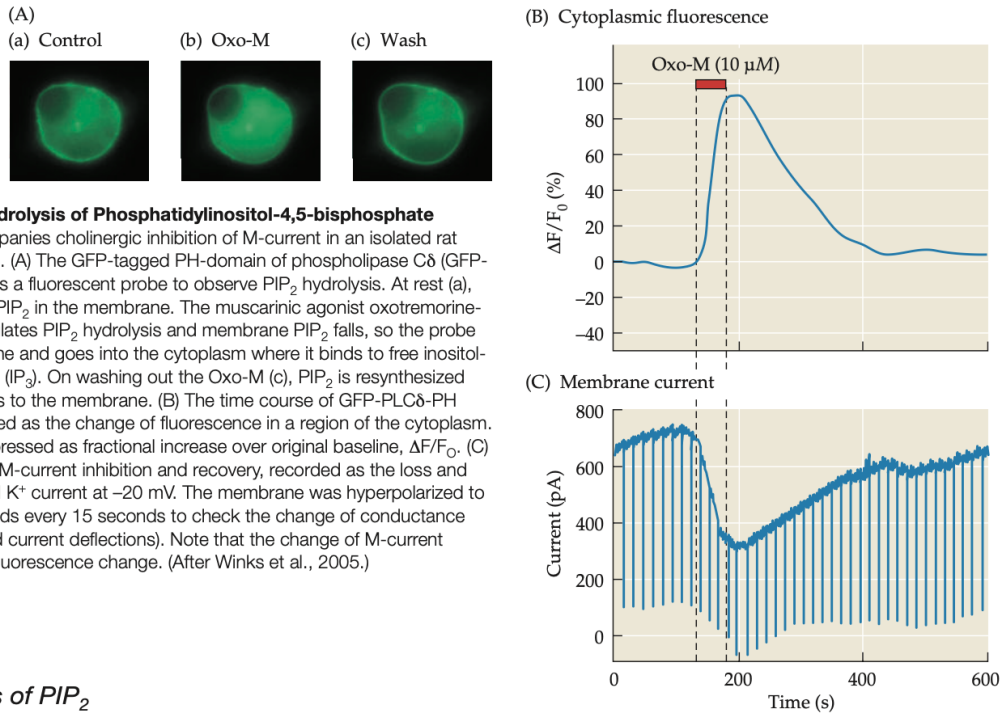


FIGURE 12.16 Hydrolysis of Phosphatidylinositol-4,5-bisphosphate (PIP_2). PIP_2 accompanies cholinergic inhibition of M-current in an isolated rat sympathetic neuron. (A) The GFP-tagged PH-domain of phospholipase Cδ (GFP-PLCδ-PH) is used as a fluorescent probe to observe PIP_2 hydrolysis. At rest (a), the probe binds to PIP_2 in the membrane. The muscarinic agonist oxotremorine-M (Oxo-M) (b) stimulates PIP_2 hydrolysis and membrane PIP_2 falls, so the probe leaves the membrane and goes into the cytoplasm where it binds to free inositol-1,4,5-trisphosphate (IP_3). On washing out the Oxo-M (c), PIP_2 is resynthesized so the probe returns to the membrane. (B) The time course of GFP-PLCδ-PH movement, registered as the change of fluorescence in a region of the cytoplasm. Fluorescence is expressed as fractional increase over original baseline, $\Delta F/F_O$. (C) The time-course of M-current inhibition and recovery, recorded as the loss and recovery of outward K^+ current at –20 mV. The membrane was hyperpolarized to –50 mV for 2 seconds every 15 seconds to check the change of conductance (given by downward current deflections). Note that the change of M-current closely follows the fluorescence change. (After Winks et al., 2005.)

Direct Actions of PIP_2

Fig 12.17 Phosphatidylinositol- 4,5-bisphosphate (PIP2) Is Necessary to Keep Kv7.2/7.3 (M-Type) Potassium Channels Open

- Kv7.2 and Kv7.3 mRNAs were co-expressed in a Chinese hamster ovary (CHO) cell and single-channel activity was recorded using a cell-attached pipette set to 0 mV membrane potential.
    - With the **pipette on-cell**, the channel shows a **modest open probability** (Po) of M potassium channels of 0.1–0.2.
    - When the membrane patch was excised into **inside-out mode** (inside surface facing the bath solution), <u>channel activity was lost</u>.
        - no secondary messenger (ie PIP2) outside
    - Addition of 50 µ*M* dioctanoyl-phosphatidylinositol- 4,5-bisphosphate (diC8-**PIP2**) to the **bath** solution <u>restored and increased activity.</u>
- (A) A continuous time-plot of open probability recorded in 3-second runs is shown, while (B) shows sample currents at a faster speed
    - <u>M-type potassium channels need PIP2 to open</u>

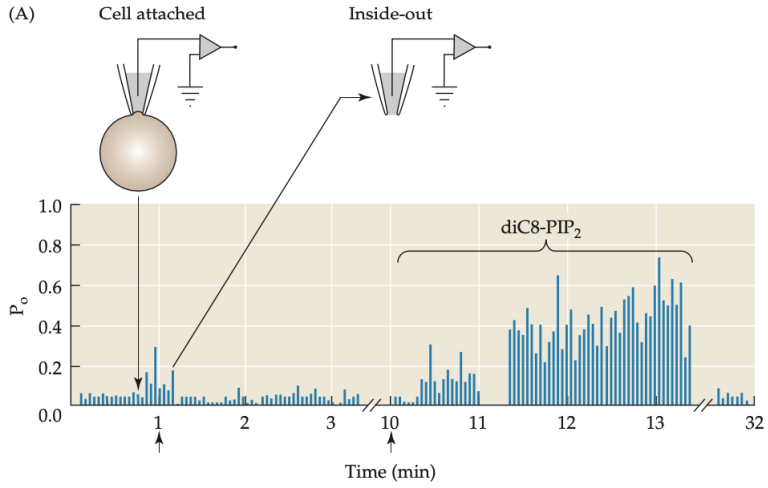


FIGURE 12.17 Phosphatidylinositol-4,5-bisphosphate (PIP_2) Is Necessary to Keep Kv7.2/7.3 (M-Type) Potassium Channels Open. Kv7.2 and Kv7.3 mRNAs were co-expressed in a Chinese hamster ovary (CHO) cell and single-channel activity recorded using a cell-attached pipette set to 0 mV membrane potential. With the pipette on-cell, the channel shows a modest open probability (P_o) of 0.1–0.2. When the membrane patch was excised into inside-out mode (inside surface facing the bath solution), channel activity was lost. Addition of 50 µ*M* dioctanoyl-phosphatidylinositol-4,5-bisphosphate (diC8-PIP_2) to the bath solution restored and increased activity. (A) A continuous time-plot of open probability recorded in 3-second runs is shown, while (B) shows sample currents at a faster speed. (After Li et al., 2005.)

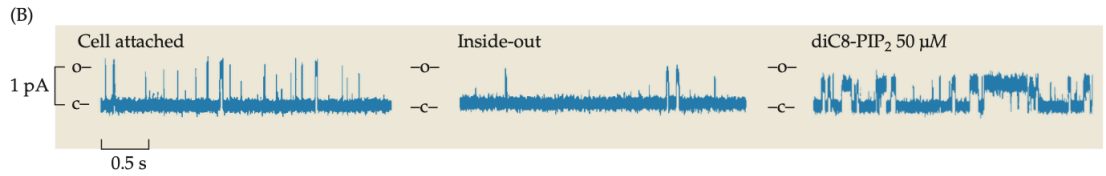
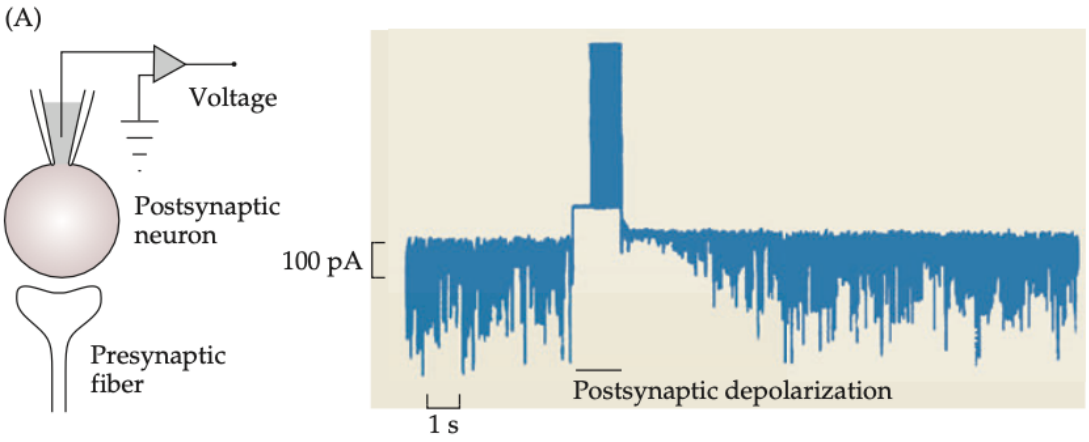


Fig 12.18 An Endocannabinoid Acts as a Retrograde Messenger for Depolarization-Induced Suppression of Inhibition (DSI)

- (A) Recording of spontaneous GABA-mediated inhibitory postsynaptic currents (IPSCs) in a hippocampal pyramidal neuron held at –80 mV.
    - The cell was depolarized for 1 second to induce a train of action potentials, which transiently **suppresses the IPSCs**.
        - depolarization-induced suppression of inhibition, or DSI.
- (B) Suggested explanation for hippocampal DSI.
    - Depolarization (or action potential activity) opens **Ca2+ channels** to produce an influx of Ca2+ ions.
        - activates phospholipase Cβ1 (**PLCβ1**) to hydrolyze phosphatidylinositol-4’5’-bisphosphate (**PIP2**), yielding diacylglycerol (**DAG**).
        - DAG is converted to 2-arachidonoyl glycerol (**2-AG**) by diacylglycerol lipase (**DAGL**).
        - 2-AG is then released into the interstitial space and activates the CB1 cannabinoid receptor (**CB1R**) on the presynaptic terminals.
        - The CB1 receptor activates **Gi/Go** and their **βγ-subunits** then inhibit Ca2+ entry through presynaptic Ca2+ channels; it may also inhibit synaptic vesicle fusion.
        - Metabotropic glutamate receptors (mGluR1 or mGluR5) or M1 or M3 muscarinic acetylcholine receptors can also independently activate phospholipase C via the G protein Gq, to generate **2-arachidonoyl glycerol** through the same biochemical pathway
- End result: inhibit the release of GABA to suppress inhibition


(A)
Voltage
Postsynaptic neuron
Presynaptic fiber
100 pA
Postsynaptic depolarization
1 s

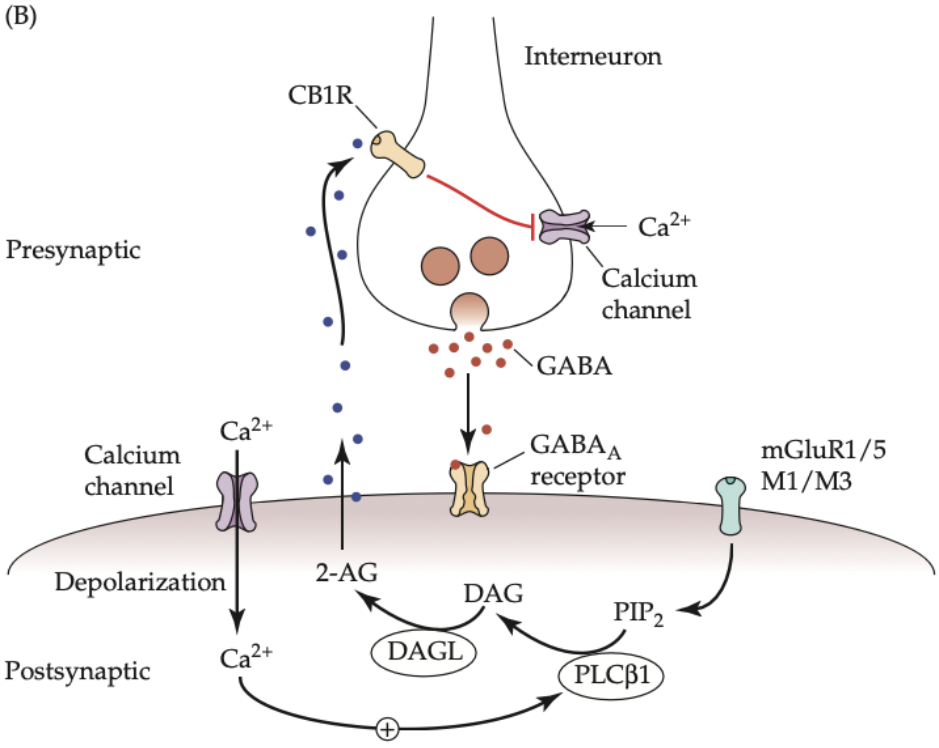

(B)
Interneuron
CB1R
Presynaptic
Ca^{2+}
Calcium channel
GABA
Ca^{2+}
Calcium channel
$GABA_A$ receptor
mGluR1/5 M1/M3
Depolarization
2-AG
DAG
PIP_2
DAGL
Postsynaptic
Ca^{2+}
PLCβ1
⊕

Fig 12.21 Inhibition by Ach-Activated Cation Channels

- (A) In chick cochlea hair cells, ACh binds to **nicotinic ionotropic receptors**
    - allow cations, including **calcium**, to flow into the cell.
    - Intracellular calcium causes **calcium-activated potassium channels** to open, leading to outward potassium current and hyperpolarization
- (B) In a whole-cell recording (inset), the application of ACh near the base of a hair cell produces a small, transient INWARD current (arrow, caused by Ca2+) followed by a large OUTWARD current (by K+).
    - In the intact cell, the outward current would be inhibitory.
- (C) If the calcium chelator (molecules that bind metal ions) **BAPTA** is added to the recording electrode, and hence to the cell cytoplasm, ACh application produces only inward current.
    - No outward current by K+ is seen because incoming calcium ions are chelated with BAPTA and so prevented from activating potassium channels.

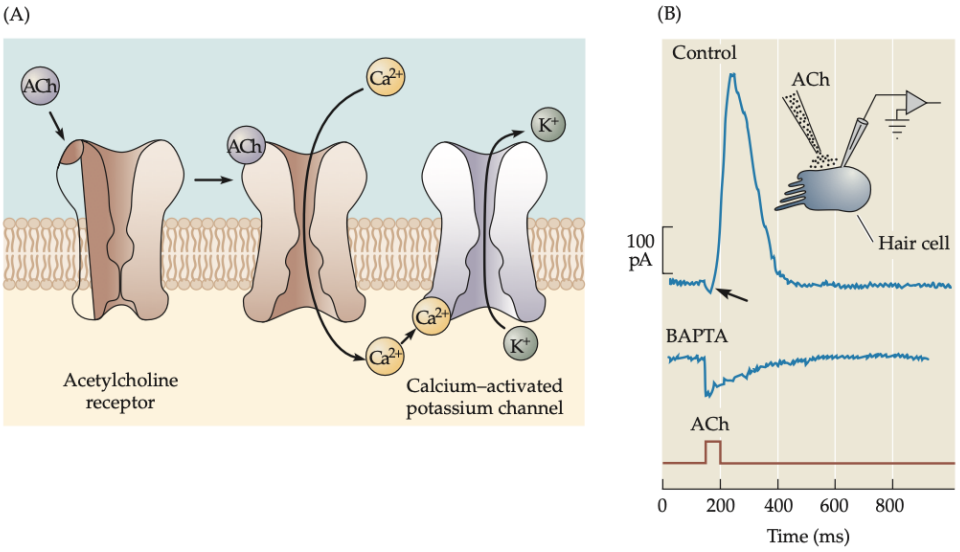


FIGURE 12.21 Inhibition by Ach-Activated Cation Channels. (A) In chick cochlea hair cells, ACh binds to nicotinic ionotropic receptors that allow cations, including calcium, to flow into the cell. Intracellular calcium causes calcium-activated potassium channels to open, leading to outward potassium current and hyperpolarization. (B) In a whole-cell recording (inset), application of ACh near the base of a hair cell produces a small, transient inward current (arrow) followed by a large outward current. In the intact cell, the outward current would be inhibitory. (C) If the calcium chelator BAPTA is added to the recording electrode, and hence to the cell cytoplasm, ACh application produces only inward current. No outward current is seen because incoming calcium ions are chelated and so prevented from activating potassium channels. (Records provided by P. A. Fuchs.)

Release of Transmitters

Fig. 13.1 Presynaptic Impulse and Postsynaptic Response

A. Stellate ganglion of squid, 2 large axons form chemical synapse

B. TTX block Na channels → reduced presynaptic action potential

   a. Lead to reduced postsynaptic action potential → disappears later when EPSP fails to reach threshold

C. Blue: presynaptic impulse as B

   Red: presynaptic terminal depolarizing current pulses after complete

   Threshold at about ~45 mV → after that, you get postsynaptic response

   - TTX (block of Na channel) has no effect on the sensitivity of the postsynaptic membrane to the transmitter, so the fall in synaptic potential amplitude indicates a reduction in the amount of transmitter released from the presynaptic terminal → only the amplitude of depolarization matters

- Fluxes of Na and K are responsible for action potential, but the release of transmitter only requires depolarization → **depolarization = transmitter release**

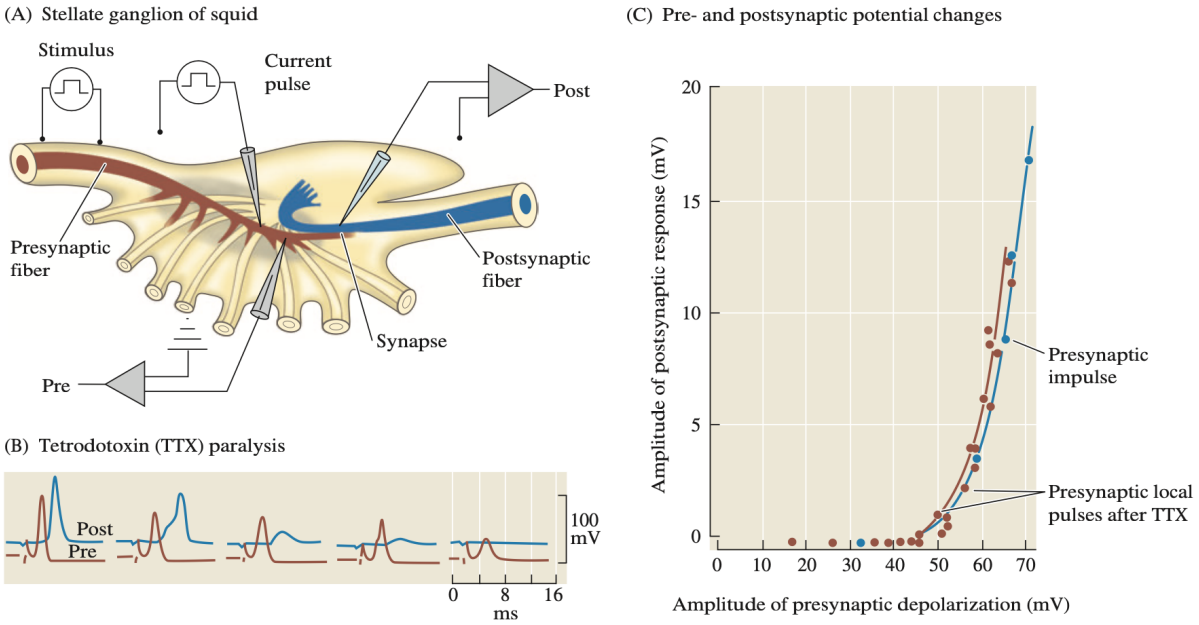


FIGURE 13.1 Presynaptic Impulse and Postsynaptic Response at a squid giant synapse. (A) Sketch of the stellate ganglion of the squid, illustrating the two large axons that form a chemical synapse. Pre- and postsynaptic axons are impaled with microelectrodes to record membrane potential, and an additional microelectrode is used to pass depolarizing current into the presynaptic terminal. (B) Simultaneous recordings from the presynaptic axons (red records) and postsynaptic axon (blue records) during the development of conduction block by tetrodotoxin (TTX). As the amplitude of the presynaptic action potential decreases, so does the size of the postsynaptic potential. Note that the two largest presynaptic action potentials evoke postsynaptic action potentials. (C) The relation between the amplitude of the presynaptic action potential and the postsynaptic potential. Blue circles represent results in B; red circles represent results obtained by applying depolarizing current pulses to the presynaptic terminals after complete TTX block. (A after Bullock and Hagiwara, 1957; B and C after Katz and Miledi, 1967b.)

Fig. 13.2 Synaptic Delay at a Chemical Synapse

- Synaptic delay → the time between AP in the nerve terminal and the beginning of end-plate current (EPC)
- B: There is a delay between AP and EPC. At 7 deg, delay is about 2ms; at 2.5 deg, delay is ~10ms
- C: as T increase, delay decrease
    - Delay is much more sensitive and longer to temperature than would be expected if it were due to diffusion across the synaptic cleft → delay is in the mechanism

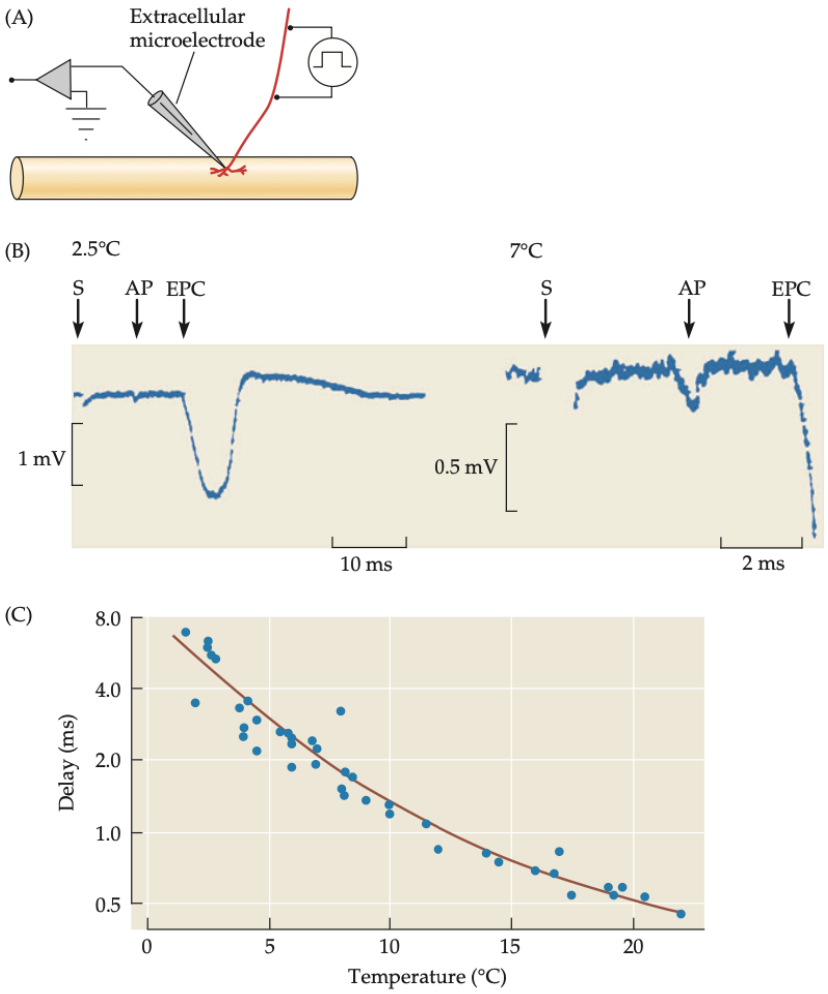


FIGURE 13.2 Synaptic Delay at a Chemical Synapse. (A) The motor nerve is stimulated while recording with an extracellular microelectrode at the frog neuromuscular junction. With this recording arrangement, current flowing into the nerve terminal or the muscle fiber is recorded as a negative potential. (B) Extracellular recordings of the stimulus artifact (S), the axon terminal action potential (AP), and the end-plate current (EPC) at 2.5°C and 7°C. The synaptic delay is the time between the action potential in the nerve terminal and the beginning of the end-plate current. (C) A plot of synaptic delay as a function of temperature , showing the decrease in synaptic delay with increasing temperature. (After Katz and Miledi, 1965.)

Fig. 13.3 Presynaptic Calcium and Transmitter Release at the Squid Giant Synapse

- TEA: blocks K+; TXX: blocks Na+ → Ca channel remained
- A:
    - slow Ca2+ entry in presynaptic cell with a small depolarization (to -18mV) → postsynaptic potential
    - no Ca2+ entry with a big depolarization to +60mV
        - <u>+60mV is the reversal potential for Ca</u> → no postsynaptic potential
    - Tail current → Dropping from +60 mV to -70 mV allows the entry of Ca2+ → driving force increase → postsynaptic potential
- B:
    - With pre-recorded AP-shape voltage clamp in presynaptic neuron
        - Ca current (black line) under AP clamp → lead to postsynaptic potential
- Synaptic delay → time required to <u>open calcium channels</u> and in part to the time <u>for calcium entry to trigger transmitter release</u>

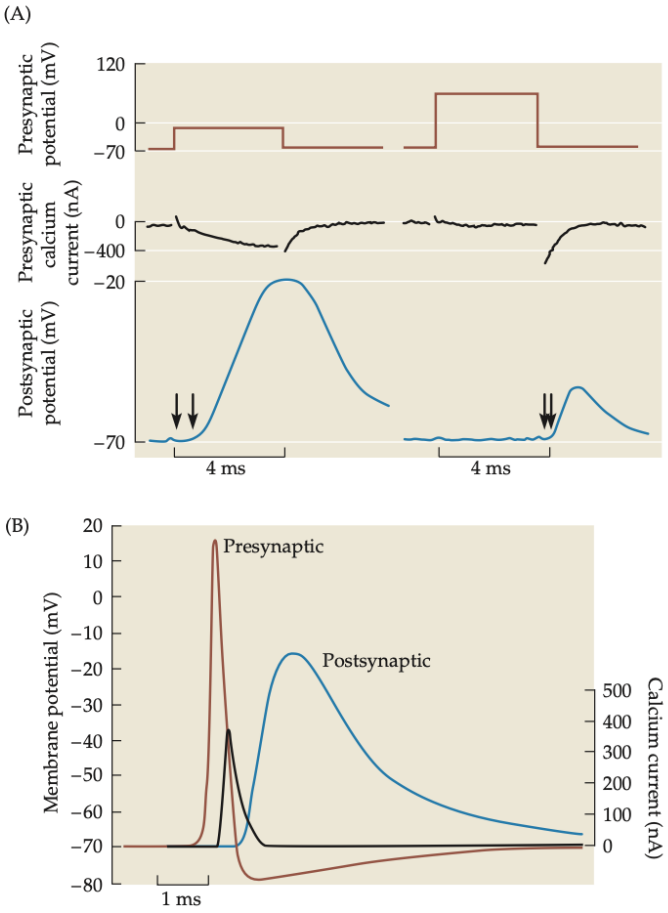


Fig. 13.4 Microdomains of Calcium within the Presynaptic Terminal at the Squid Giant Synapse

A) Distribution of calcium within the presynaptic axon terminal at rest, determined by intracellular injection of a calcium-sensitive dye

(B) A brief train of presynaptic action potentials results in the appearance of microdomains of high calcium concentration within the axon terminal → increase in concentration caused by Ca2+ entry

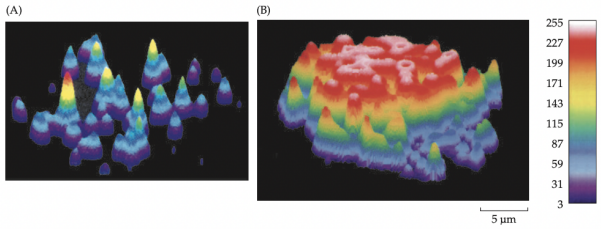


Fig. 13.5 Calcium Enters Near the Site of Transmitter Release

- BAPTA (chelator) binds to Ca2+ quickly; EGTA (chelator) binds to Ca2+ slowly
- A: 4 min injection of BAPTA (concentration) after presynaptic stimulus result in a more delayed and failed postsynaptic potential → Ca binds to BAPTA before it has time to reach Ca2+ sensor, which trigger transmitter release (B), without affecting presynaptic AP
- C: No Excitatory PSP (EPSP) is seen when adds EGTA
    - Ca2+ binds to sensor & release transmitter before EGTA can bind to Ca2+

Conclusion: Transmitter release process is close by (~100 nm) to Ca2+ channel

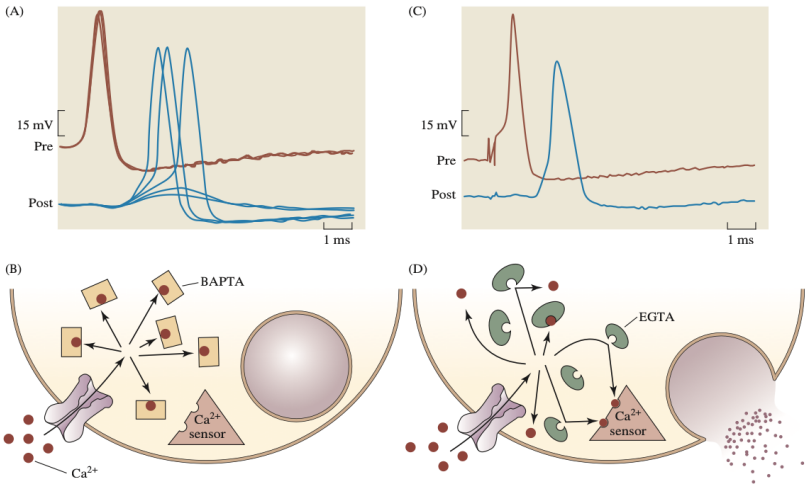


FIGURE 13.5 Calcium Enters Near the Site of Transmitter Release at the squid giant synapse. (A) Intracellular recordings from the presynaptic (Pre) and postsynaptic (Post) axons following injection of the fast calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA). Superimposed traces show the reduction in the EPSP during a 4-minute BAPTA injection. (B) Calcium is bound to BAPTA before it has time to reach the calcium sensor that triggers transmitter release. (C) Superimposed intracellular recordings during a 4-minute injection of ethylene glycol-bis[2-aminoethyl ether]-N,N,N′,N′-tetraacetic acid (EGTA), a chelator that binds calcium more slowly. No change in EPSP amplitude is seen. (D) Calcium reaches the sensor that triggers release faster than it becomes bound to EGTA, indicating that the site of calcium entry must be within 100 nm of the site at which calcium triggers transmitter release. (A and C after Adler et al., 1991.)

Fig. 13.6 An increased in intracellular Calcium is sufficient to trigger rapid transmitter release

- Nitrophen → caged Ca2+; release Ca2+ via UV light
- B: EPSP to postsynaptic potential has the same response as light on nitrophen
    - Downward blue line = UV response of nitrophen → sudden increase in Ca2+ intracellular concentration
    - An abrupt increase in intracellular Ca → increase transmitter release, with the same rate as that produced by presynaptic AP
    - Decay is slower due to addition of Ca2+ that brings the concentration to a higher level than normal

Conclusion: increase in [Ca2+] in could trigger postsynaptic potential just like EPSP

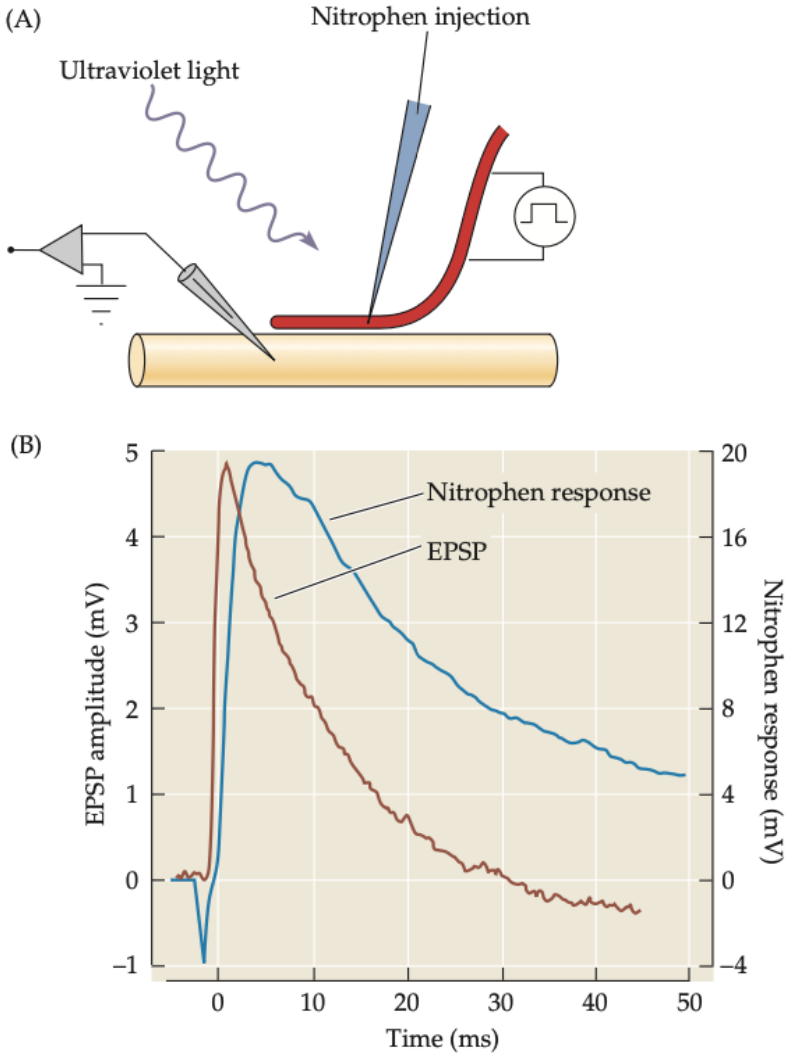


Fig. 13.7 Change in Presynaptic Calcium Concentration and EPSP current

- Upper: fluorescent signal indicating increase of presynaptic Ca2+ concentration
- Lower: postsynaptic currents produced by Ca2+ entry (solid) and presynaptic stimulus (dashed)
    - depolarization has no effect on transmitter release triggered by uncaged calcium
        - Ca2+ entry resulted in postsynp current response, not presynaptic depolarization

FIGURE 13.7 Change in Presynaptic Calcium Concentration and Excitatory Postsynaptic Currents at a synapse in the trapezoid body of the rat. The presynaptic calyx of Held was loaded with both caged calcium and a calcium indicator dye. Upper record: fluorescent signal indicating a transient increase in presynaptic calcium concentration, evoked by photolysis of caged calcium with a laser flash. Lower traces: postsynaptic currents produced by the calcium transient (solid curve) and by presynaptic stimulation (dashed curves). The postsynaptic currents are identical in time course. (Modified from Bollmann and Sakmann, 2005.)

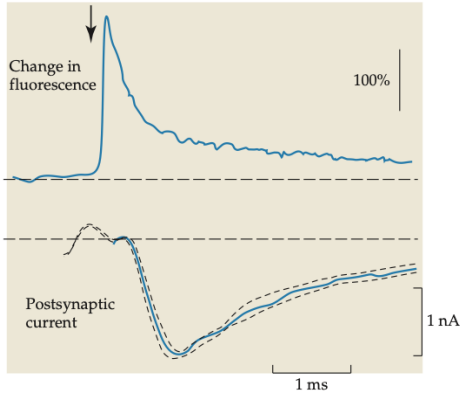


Fig. 13.8 Miniature End-Plate Potentials at the Frog Neuromuscular Junction

- B: miniature End-Plate Potentials (MEPPs) are about 1mV in amplitude and equal in size
- C: Adding **prostigmine** prevents ACh-esterase hydrolyzing activity
    - ACh stays longer → yet MEPP does not increase in frequency, but increase in duration + amplitude

Conclusion: MEPP is due to **quantal packet of ACh** not a single ACh molecule

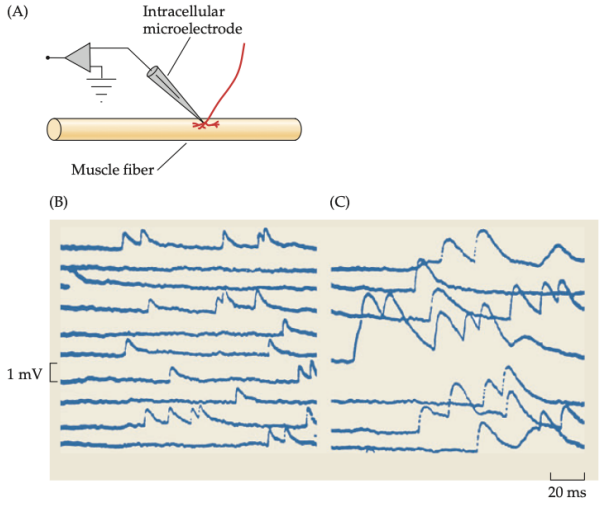


Fig. 13.9 The End-Plate Potential is composed of Quantal Units

- A: stepwise stimulus → EPP varies in a stepwise fashion

- Smallest change in response corresponds in amplitude in a MEPPs

- B: mean quantal content of EPP through Poisson distribution and EPP/MEPP
  - Linear relationship from both estimates

Conclusion: EPP is composed of quantal units that correspond to spontaneous MEPPs

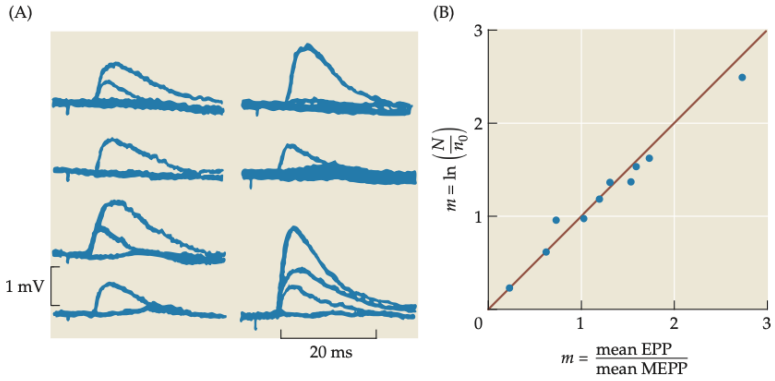


FIGURE 13.9 The End-Plate Potential Is Composed of Quantal Units that correspond to spontaneous miniature potentials. Presynaptic release of ACh at a frog neuromuscular junction was reduced by lowering the calcium concentration in the bathing solution. (A) Sets of intracellular records, each showing two to four superimposed responses to nerve stimulation. The amplitude of the end-plate potential (EPP) varies in a stepwise fashion; the smallest response corresponds in amplitude to a spontaneous miniature end-plate potential (MEPP). (B) Comparison of the mean quantal content (m) of the EPP, determined in two ways: by applying the Poisson distribution, $m = \ln(N/n_0)$ (ordinate), and by dividing the mean EPP amplitude by the mean MEPP amplitude (abscissa). Agreement of the two estimates supports the hypothesis that the EPP is composed of quantal units that correspond to spontaneous MEPPs. (A after Fatt and Katz, 1952; B after del Castillo and Katz, 1954a.)

Fig. 13.10 Amplitude distribution of End-plate potential

- Amplitude of spontaneous potentials (mean at 0.4 mV) is equal to 1 quanta
- Histogram shows # of EPPs at each amplitude
  - Peak at whole numbers (1,2,3,4) of spontaneous potentials
- Solid line is the theoretical distribution of Poisson if in quantal size

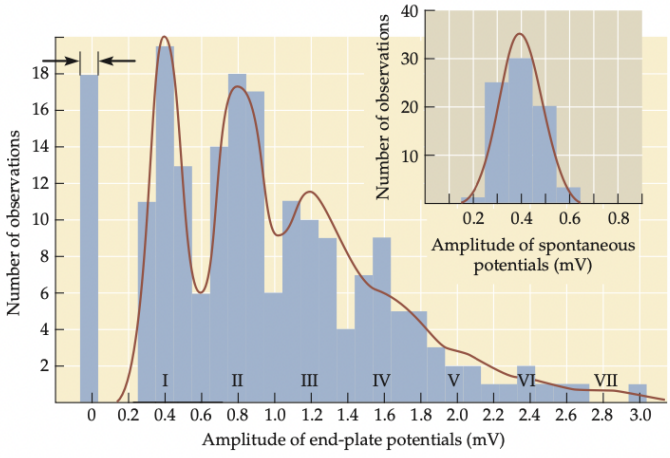


FIGURE 13.10 Amplitude Distribution of End-Plate Potentials at a mammalian neuromuscular junction in high (12.5 m*M*) magnesium solution. The histogram shows the number of end-plate potentials observed at each amplitude. The peaks of the histogram occur at 0 mV (failures) and at one, two, three, and four times the mean amplitude of the spontaneous MEPPs (inset), indicating responses comprising 1, 2, 3, and 4 quanta. The solid line represents the theoretical distribution of end-plate potential amplitudes calculated according to the Poisson equation and allowing for the spread in amplitude of the quantal size. The arrows indicate the predicted number of failures. (From Boyd and Martin, 1956.)

Fig. 13.11 The number of ACh Molecules in a Quantum

- A: iontophoretic application of ACh (-ve charged)

- B: Injection of ACh gives the similar response to postsynaptic membrane potential as MEPP
    - Yet the rate of rise ACh is slightly slower due to ACh is injected further from the postsynaptic membrane than the nerve terminal

Conclusion: By injecting a certain amount of Ach that produces the response of MEPP, we can measure the number of Ach in a quantum (7000 molecules)

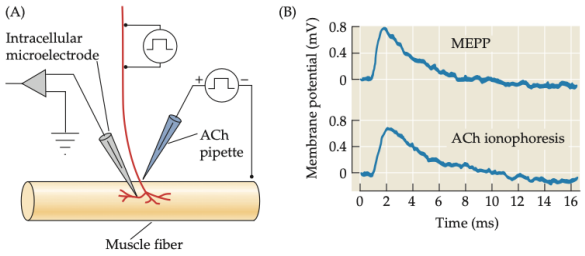


FIGURE 13.11 The Number of ACh Molecules in a Quantum, determined by mimicking an MEPP with an ionophoretic pulse of ACh. (A) An intracellular microelectrode records spontaneous MEPPs and the response to ionophoretic application of ACh. (B) An MEPP is mimicked almost exactly by an ionophoretic pulse of ACh. The rate of rise of the ionophoretic ACh pulse is slightly slower because the ACh pipette is further from the postsynaptic membrane than is the nerve terminal. (B after Kuffler and Yoshikami, 1975b.)

Fig. 13.17 Vesicle Exocytosis corresponds to Quantal Release

- B: when a large number of quanta is discharged → vesicle fusion occurs
- C: Block of K+ using 4-AP varies the amount of transmitter release → delay repolarization and extend stimulation to release more transmitter by have more vesicle opening
    - Same number of vesicle openings as number of quanta released → <u>each vesicle that opened released 1 quantum of transmitter</u>

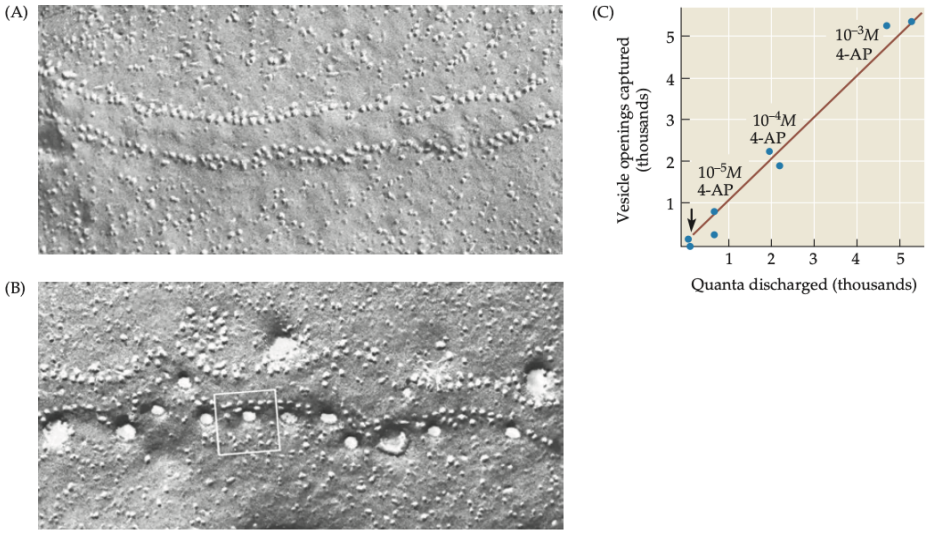
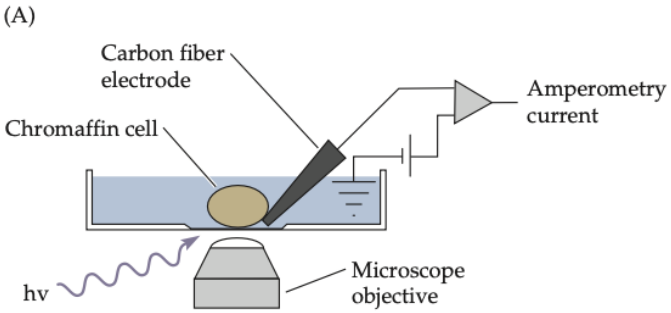


Fig. 13.18 Exocytosis Observed in Living Cells

- A: Evanescent-wave microscopy (detect fluorescence through TIRF); Measure release of catecholamines (a neurotransmitter) by amperometry
- B: Spot is catecholamines → disappears as the vesicle undergoes exocytosis and releases its fluorescent contents
- C: Extracellular K+ depolarizes the membrane
    - Amperometry current increase → catecholamine releases, and at the same time → Spot disappears
    - More events are observed by amperometry current thant by fluorescence
        - Exocytosis is over a large part of the cell, which cannot be all viewed by microscopy



layer of cytosol. Chromaffin granules within the cells were labeled

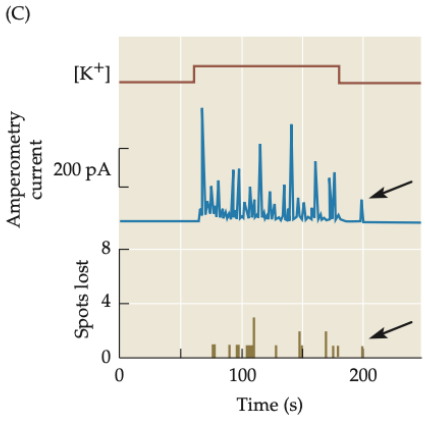


FIGURE 13.18 Exocytosis Observed in Living Cells. (A) Chromaffin cells growing on a glass cover slip in cell culture were labeled with a fluorescent dye, which becomes concentrated in chromaffin vesicles. Individual vesicles docked at the plasma membrane were visualized by evanescent-wave microscopy. At the same time, release of catecholamines was detected by amperometry. (B) High-power images of a single chromaffin vesicle at 2-second intervals after the cell was stimulated with high potassium. The spot disappears abruptly and permanently as the vesicle undergoes exocytosis and releases its fluorescent contents. (C) The time course of exocytosis in response to an increase in extracellular potassium concentration, as recorded by amperometric detection of catecholamine release and the disappearance of fluorescent spots. Note the coincidence of release and spot disappearance (the arrows mark one example). More events are recorded by amperometry than by fluorescence because the amperometric electrode detects exocytosis over a large part of the cell, while only a small portion of the cell surface is imaged by evanescent-wave microscopy. (After Steyer, Horstmann, and Almers, 1997; micrographs kindly provided by W. Almers.)

Fig. 13.19 Coincidence of Increases in membrane capacitance and release of catecholamines

- B:
  - Top: catecholamine release; bottom: capacitance
  - Catecholamine releases coincide with (small) increases in capacitance
    - Vesicle fuses with the membrane during exocytosis → increase in surface area → increase in capacitance (C=eA/d)

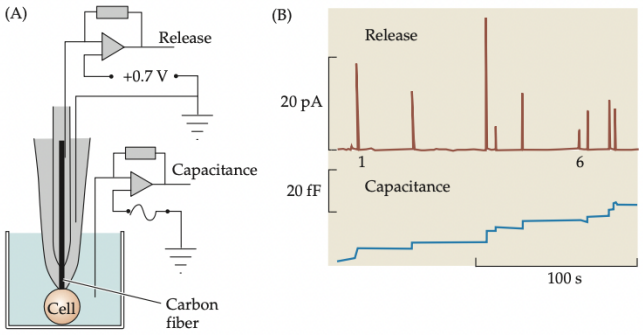


FIGURE 13.19 Coincident Increases in Membrane Capacitance and Release of Catecholamines from chromaffin cells. (A) A carbon fiber electrode inside the patch pipette measures catecholamine release by amperometry, while at the same time the electrode is used to measure capacitance within the patch. (B) Simultaneous recording of catecholamine release (top trace) and capacitance (bottom trace). All exocytotic events detected by catecholamine release coincide with increases in capacitance. Capacitance units are in femtofarads (1fF = 10^{-15} Farads). (After Albillos et al., 1997.)

Fig. 13.20 Release of Synaptic Vesicles from presynaptic nerve terminals (CNS)

- A: with stimulus events (circle) in presynaptic nerve, stepwise drops in fluorescence
  - Release of a single fluorescent signalling molecule
- B: a) Increase of membrane capacitance as vesicle is fused; b) shows a magnified record, showing <u>step-wise jumps in capacitance</u>

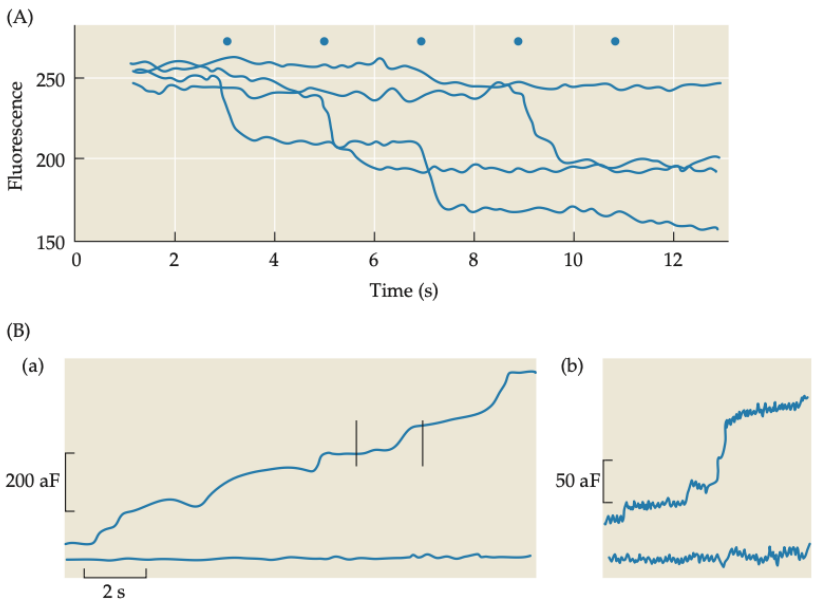
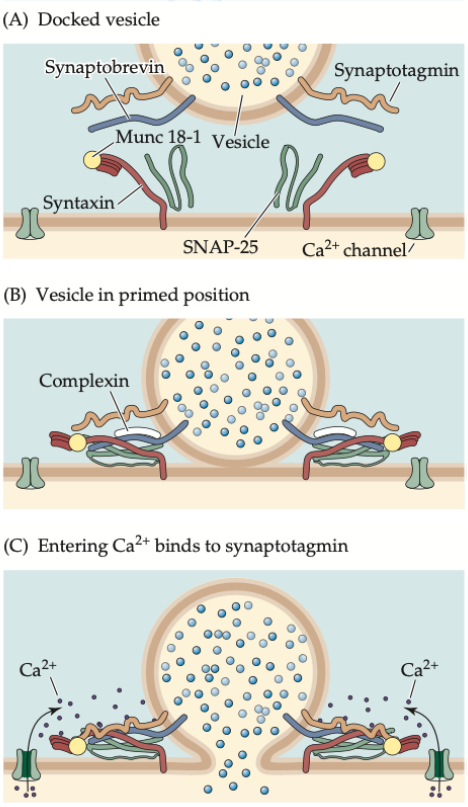


FIGURE 13.20 Release of Synaptic Vesicles from Presynaptic Nerve Terminals, monitored by loss of fluorescent dye (A) and increase in membrane capacitance (B). (A) Fluorescence records from four terminal boutons on a cultured hippocampal neuron. Vesicles in the bouton had been loaded previously with a fluorescent lipid marker. Stimulation of the presynaptic nerve (dots) caused step-wise drops in fluorescence, signaling single vesicle discharges. (B) Capacitance of a cell-attached membrane patch on the transmitter-releasing face of a calyx of Held. The patch was depolarized by perfusing the electrode with 25 m*M* KCl, starting at the beginning of the record. Membrane capacitance (upper trace) increased as vesicle membranes were incorporated into the patch (a). (b) is a magnified record of the segment between the two vertical lines in a, showing step-wise jumps. Capacitance calibration is in attofarads (1 aF = 10^{-18}F). (Records in A from Richards, 2009; in B from He et al., 2006.)

Fig. 13.21 Mechanism of Exocytosis



- A) Docked vesicle. The vesicle membrane contains the **SNARE protein synaptobrevin** and the **calcium sensor synaptotagmin**. Two additional SNARE proteins, **syntaxin** and **SNAP-25**, are anchored to the nerve terminal plasma membrane at the active zone. Syntaxin is held in a folded, inactive configuration by Munc18-1
- B) Vesicles in the primed position. **Syntaxin** has entered its open configuration and formed a **ternary SNARE complex** with synaptobrevin and the two arms of SNAP-25. The complex is stabilized by the presence of complexin
- C) Calcium binds to **synaptotagmin**, which in turn binds to the **SNARE complex**, displacing complexin and inducing pore formation

Fig. 13.25 Recycling of Synaptic Vesicle Membrane

- Stained with horseradish peroxidase (HRP)
- A) The nerve was stimulated for 1 minute in saline containing HRP; electron-dense reaction products can be seen in the extracellular space and in cisternae and **coated vesicles** → released vesicle product
- B) The nerve was stimulated for 15 minutes in HRP, then allowed to recover for 1 hour while the HRP was washed out of the muscle. Many synaptic vesicles contain HRP reaction products, indicating that they have been formed from membranes retrieved by endocytosis.
- (C) The axon terminal was loaded with HRP and allowed to rest, as in B, then stimulated a second time and allowed to recover an additional hour. Few vesicles are labeled (arrow), indicating that the previously recaptured membrane and enclosed HRP had been recycled into the vesicle population from which release occurs.

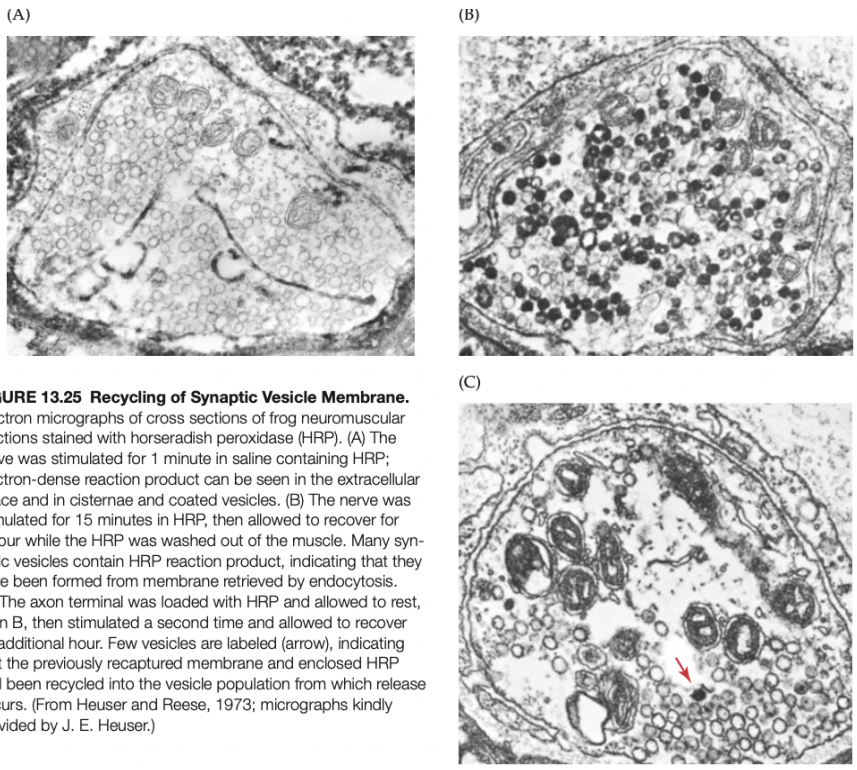


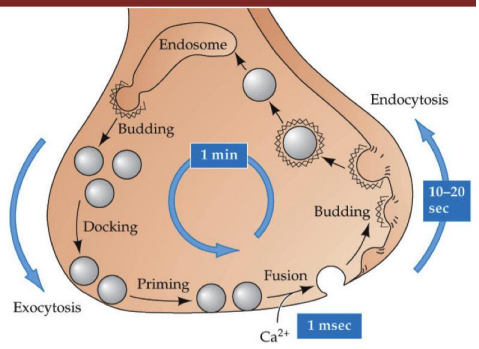
FIGURE 13.25 Recycling of Synaptic Vesicle Membrane. Electron micrographs of cross sections of frog neuromuscular junctions stained with horseradish peroxidase (HRP). (A) The nerve was stimulated for 1 minute in saline containing HRP; electron-dense reaction product can be seen in the extracellular space and in cisternae and coated vesicles. (B) The nerve was stimulated for 15 minutes in HRP, then allowed to recover for 1 hour while the HRP was washed out of the muscle. Many synaptic vesicles contain HRP reaction product, indicating that they have been formed from membrane retrieved by endocytosis. (C) The axon terminal was loaded with HRP and allowed to rest, as in B, then stimulated a second time and allowed to recover an additional hour. Few vesicles are labeled (arrow), indicating that the previously recaptured membrane and enclosed HRP had been recycled into the vesicle population from which release occurs. (From Heuser and Reese, 1973; micrographs kindly provided by J. E. Heuser.)

Synaptic vesicle cycle in presynaptic terminals

Heuser and Reese also showed that fused vesicle membrane is retrieved into the cytoplasm of the frog NMJ terminal (by endocytosis)

Horseradish peroxidase (HRP), enzyme produces a dense color reaction product visible under EM

Stimulation of endocytosis by presynaptic terminals cause HRP to be taken up into the presynaptic terminals via a pathway that includes (B) coated vesicles and (C) endosomes, and eventually (D) the HRP is found in the newly formed synaptic vesicles



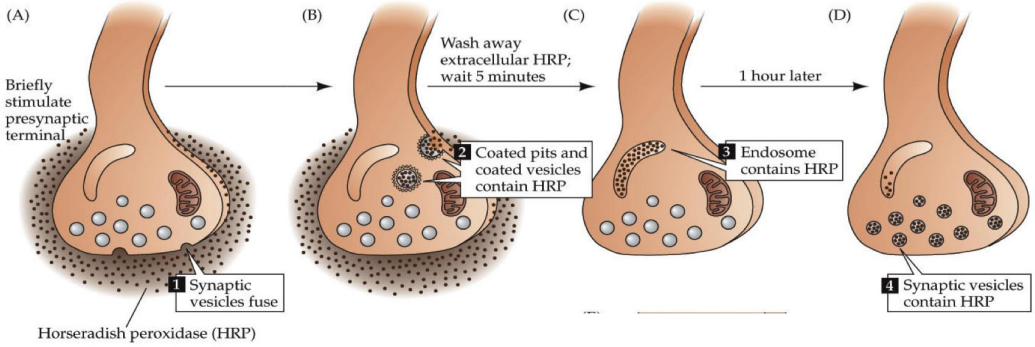


Fig. 13.28 Activity-Dependent Uptake and release of fluorescent dye by axon terminals at frog neuromuscular junction

- Fluorescence micrographs of axon terminals in a cutaneous pectoris muscle
- (A) Muscle was bathed for 5 minutes in fluorescent dye (2μ*M* FM1-43) and washed for 30 minutes. Only small amounts of dye remain associated with the terminal membrane
- (B) The same muscle was then bathed in dye for 5 minutes while the nerve was stimulated (10 Hz) and washed for 30 minutes. The fluorescent patches are **clusters of synaptic vesicles that were filled with dye** during recycling.
- (C) The same muscle was then stimulated at 10 Hz for 5 minutes and washed for 30 minutes. Stimulation released most of the dye.

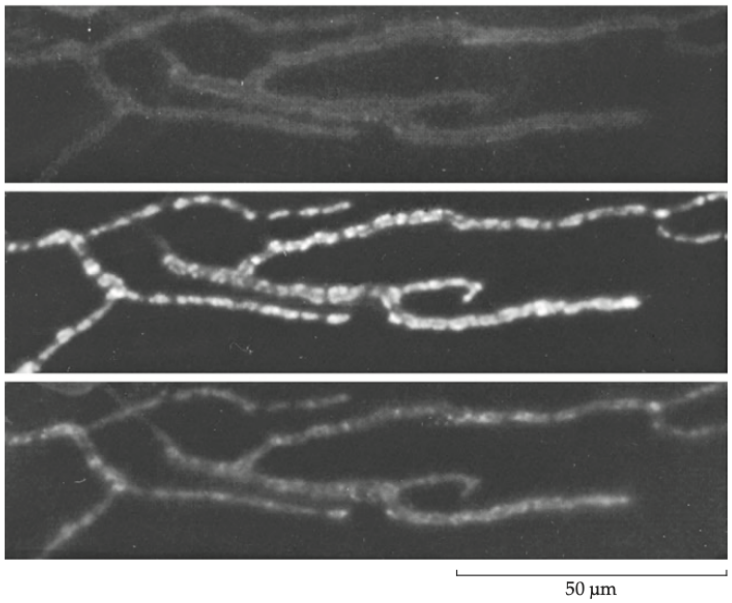


FIGURE 13.28 Activity-Dependent Uptake and Release of Fluorescent Dye by Axon Terminals at the Frog Neuromuscular Junction. Fluorescence micrographs of axon terminals in a cutaneous pectoris muscle. (A) Muscle was bathed for 5 minutes in fluorescent dye (2μ*M* FM1-43) and washed for 30 minutes. Only small amounts of dye remain associated with the terminal membrane. (B) The same muscle was then bathed in dye for 5 minutes while the nerve was stimulated (10 Hz) and washed for 30 minutes. The fluorescent patches are clusters of synaptic vesicles that were filled with dye during recycling. (C) The same muscle was then stimulated at 10 Hz for 5 minutes and washed for 30 minutes. Stimulation released most of the dye. (Micrographs kindly provided by W. J. Betz.)

Fig. 14.6 The GABAb-selective GABA analog Baclofen activates a potassium current in a Hippocampal Pyramidal neuron (CNS)

- Baclofen → activate postsynaptic GABAb receptor → GABAb agonist
- A: Baclofen increases the K+ conductance when being applied
  - Since same voltage change produces a larger current
  - When baclofen is removed, conductance of K+ dropped → current dropped
- B: Baclofen current changes as external [K+] changes
  - Inward rectifying current
  - The reversal potential shifted to the right as concentration for external [K+] increases → increased K+ driving force in so that it is more likely to move in → as expected for a K+ current

Conclusion: Baclofen increases postsynaptic K+ conductance

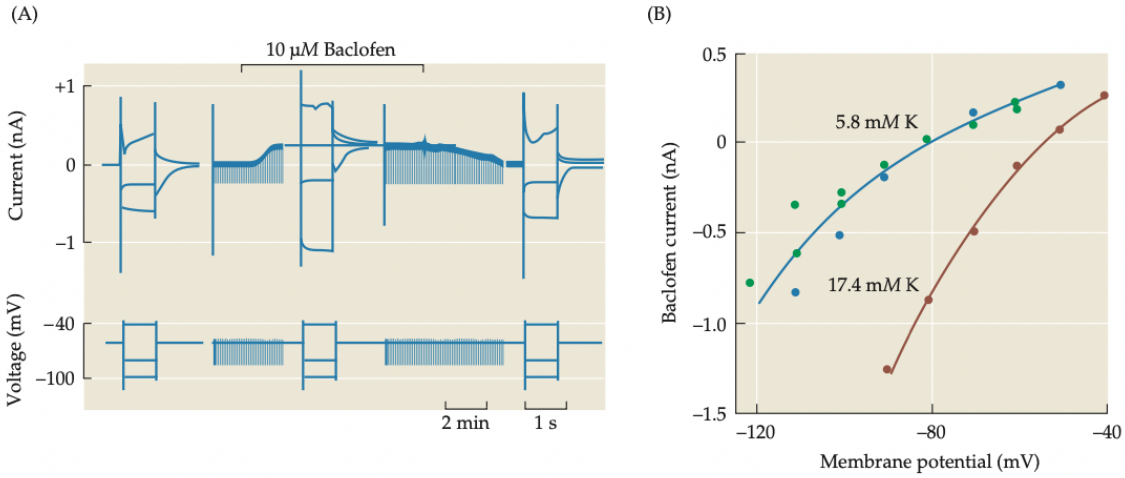


FIGURE 14.6 The $GABA_B$-Selective GABA Analog Baclofen Activates a Potassium Current in a Hippocampal Pyramidal Neuron. Records from an experiment on a slice of rat hippocampus cultured in vitro for 3 weeks. (A) A continuous record of membrane current recorded at –61 mV, with one-second steps to –81 mV applied every 5 seconds (downward deflections). Just before, during, and after application of baclofen, three voltage steps, to –41, –81 and –101 mV, were applied and the currents recorded at a faster speed. Baclofen produced an outward current at –61 mV and increased the membrane conductance as shown by the increased response to the voltage steps. (B) The extra current produced by baclofen (after subtracting the resting current) recorded from this cell in 5.8- and 17.4-m*M* external [K^+]. The currents were measured at the end of one-second steps from –61 mV to the voltages shown. The current reversal potential (that is, the potential at which the current–voltage curve crossed the zero-current line) shifted +26 mV on increasing the K^+ concentration threefold, which is near that (+29 mV) expected for a K^+ current (see Chapters 6 and 11). Note that the curve shows inward rectification—that is, the current is larger at potentials negative to the reversal potential (when the direction of net K^+ flow is into the cell) than at potentials more positive to the reversal potential, when it is outward. (After Gahwiler and Brown, 1985b.)

Fig. 14.7 Presynaptic GABAb receptors reduce transmitter release

- B: Baclofen activates presynaptic GABAb receptors
    - Presynaptic ICa current is reduced → reduced Ca channel activity
    - Postsynaptic EPSC is reduced → due to reduced ICa
- C: Same result can be obtained with decreased [Ca]
    - Baclofen does not alter the relation between ICa and transmitter release
        - All it does is to inhibit Ca current

Conclusion: activated presynaptic GABAb reduced Ca current and therefore reduced transmitter release

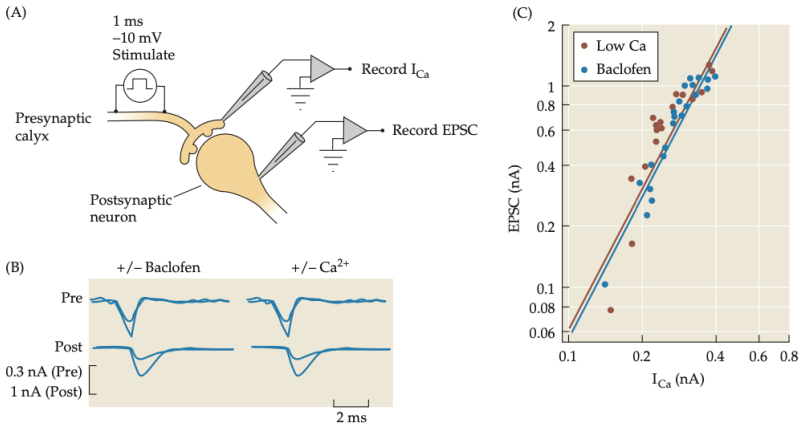


Fig. 14.9 Presynaptic Nicotinic receptors enhance excitatory transmission

- CNQX: block AMPA, a nicotinic receptor which releases **glutamate** when activated
- A: - with CNQX → no EPSC
    - with nicotine (that increases the release of glutamate by raising inward Ca2+ current & increase internal Ca2+) → increased EPSC
- B: Both nicotine and ACh binds to nicotinic receptors
    - It is not ACh that increase EPSC, but the activated nicotinic receptor to release glutamate and increase EPSC

Conclusion: Nicotine/Ach → activate Nicotinic receptor → glutatme release → enhance EPSC

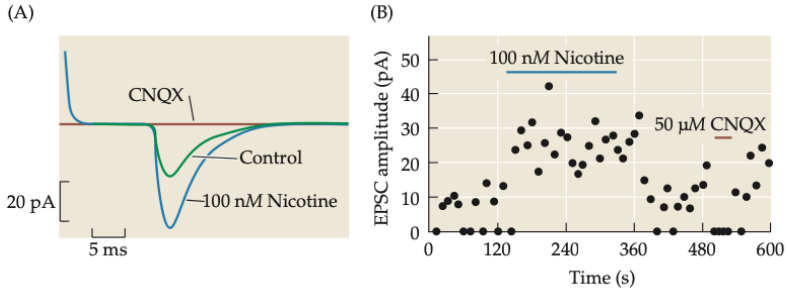


Fig. 14.10 Stimulation of Cholinergic Fibers from the Medial Septum Excites Hippocampal Pyramidal Cells

- Eserine: block ACh breakdown; Atropine: muscarinic receptor antagonist (blocker)
- B:
    - a: summed fast EPSP due to glutamate release (AMPA)
    - b: summed IPSP due to GABA released from interneurons
    - c: slow EPSP
        - With eserine: enhanced; with atropine: suppressed
- mAChR activation consists of a slow depolarization and enhanced AP discharge from inhibition of Ca-activated K channel (that normally generate a slow AHP) and M-type K channel current
    - Block M-type K channel = depolarization = lowers threshold for AP

Conclusion: Slow EPSP results from the activation of muscarinic AChRs that blocks Ca-activated K Channel and M-type K Channel

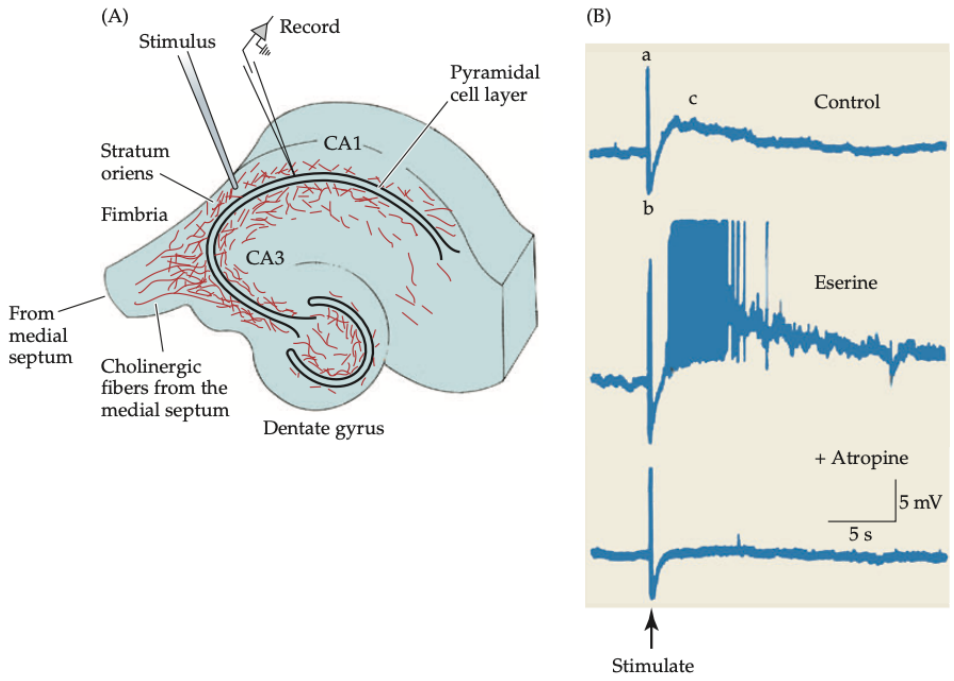


FIGURE 14.10 Stimulation of Cholinergic Fibers from the Medial Septum Excites Hippocampal Pyramidal Cells. (A) A drawing of a transverse hippocampal slice. Cholinergic fibers from the medial septum enter the slice through the fimbria and course through the stratum oriens. A stimulating electrode is placed in the stratum oriens and an intracellular pipette is used to record from a neuron in the pyramidal cell layer. (B) Stratum oriens stimulation for 5 seconds at 20 Hz produces: (a) summed fast excitatory postsynaptic potentials due to glutamate release (probably from the cholinergic fibers; see Chapter 15); (b) summed inhibitory synaptic potentials due to GABA release from interneurons; and (c) a slow excitatory postsynaptic potential. The latter is enhanced by the anticholinesterase eserine (physostigmine) and suppressed by the muscarinic receptor antagonist atropine. This effect of atropine shows that the slow excitatory postsynaptic potential results from activation of muscarinic acetylcholine receptors. (A after Nicoll, 1985; B after Cole and Nicoll, 1984.)

Fig. 14.11 Muscarinic Auto-Receptors Inhibit Acetylcholine Release from Cholinergic Forebrain Afferents

- A: Adding atropine resulted in the increased collection of ACh due to block of <u>presynaptic muscarinic ACh receptor</u>
- B: ACh released following each AP triggered <u>a burst of nicotinic channel opening (应该是muscarinic?)</u>
    - Using muscarine to bind to those channels result in reduced amount of ACh released
- M2 and M4 mAChR receptors activate G protein that inhibits Ca2+ current → inhibit ACh release

Conclusion: M2 and M4 mAChRs are auto-receptors that involves in the feedback activation/inhibition of the release of ACh presynaptically

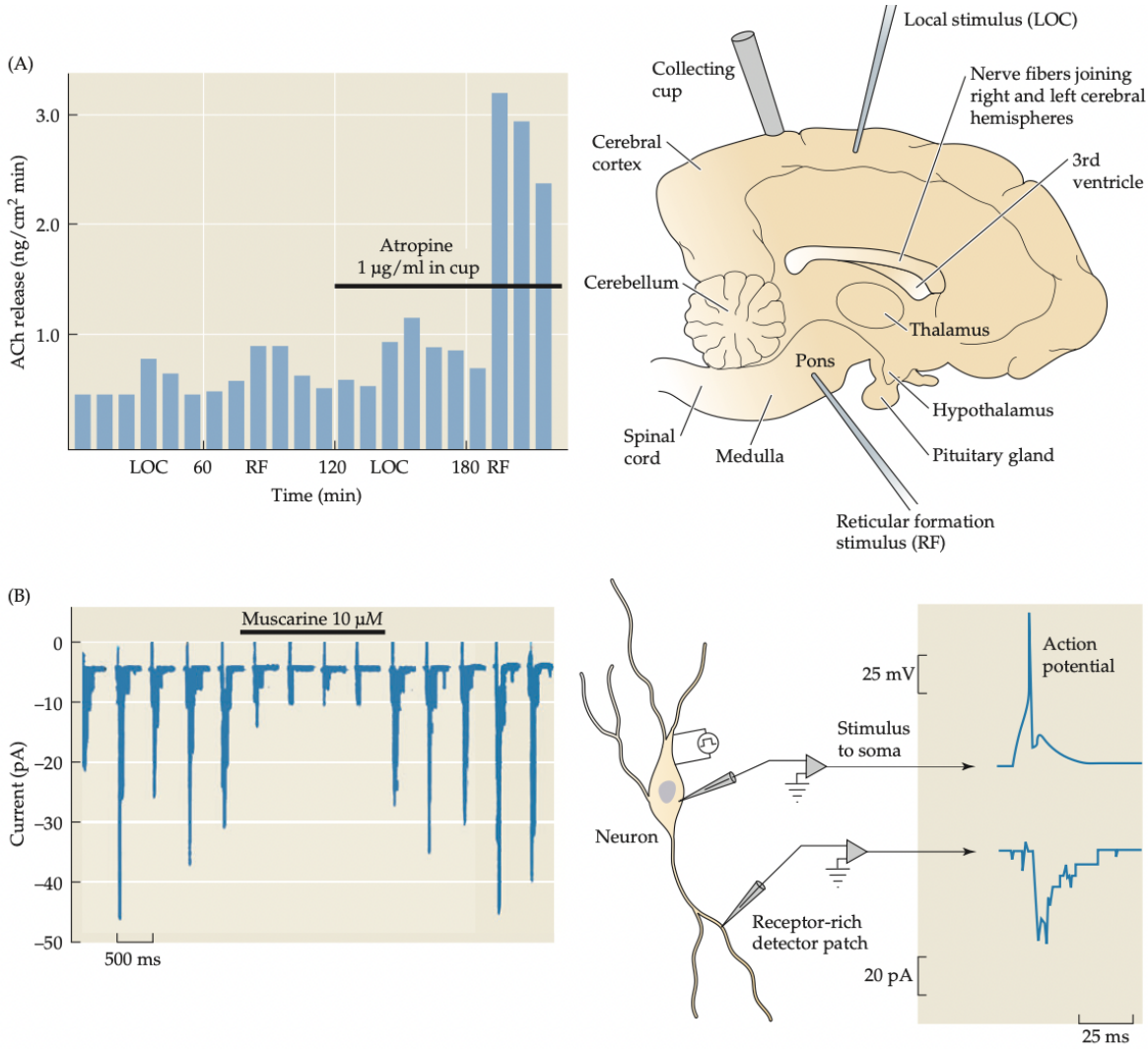


Fig. 14.13 Locus Coeruleus Stimulation Sharpens and Enhances Sensory Processing in the Cerebral Cortex

- Locus Coeruleus → The noradrenergic neurons that are concentrated in a nucleus in the dorsal part of the pons
    - Stimulus of LC is achieved by using NE
        - Inhibit calcium-activated K current through beta 2 receptor (increase sensory signal to noise ratio)
        - activating alpha 2 receptor → Hyperpolarizes the neuron (reducing background activity)
- Isoamyl Acetate → a stimulant
- A: No stimulation
    - The stimulant result in fewer AP
- B: with stimulation
    - The stimulant results in more AP → sharpen the response
    - Increase the reliability of the response (fewer blank horizontal in the upper plots)
    - Increase the clustering of the AP within the few hundred ms
    - Reducing the jitter in the delay to the first AP
- locus coeruleus can increase attention and facilitate learning and memory

Conclusion: NE is not responsible for the smell, but it enhances and sharpens it by using GPCR and inhibiting K+ current (enhance response to stimulation) + hyperpolarize to reduce noise → sharpens and enhances

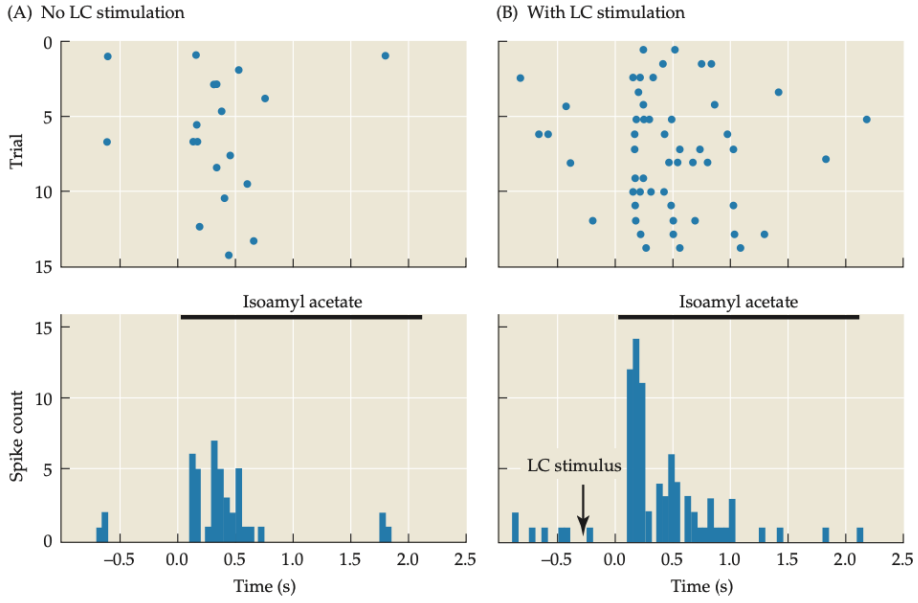


Fig. 14.17 Effects of Stimulating Three Different Histamine Receptors

- A: histamine activate postsynaptic H1 receptors → inhibit K current (including M-type) → caused depolarization and induce AP firing
    - Downward deflection: transient voltage response to brief hyperpolarizing current injection
    - Larger voltage deflection: reduced resting K conductance = more Na, less K, larger depolarization for the next AP
- B: histamine activate postsynaptic H2 receptor → inhibit calcium-activated K currents → no calcium-dependent AHP (human hippocampal pyramidal)
    - a. Eliminated long-lasting calcium-dependent K channel AHP after AP
    - b. Reduces accommodation during AP by increasing AP discharges
- C: histamine activate postsynaptic H3 auto-inhibitory receptors where they
    - reduce neuron natural firing rate and slows AP discharge
    - Block by antagonist thioperamide (-H3) and unblocked by omitting thioperamide (+H3)

Conclusion: H1 induce AP, H2 eliminate AHP, H3 reduce AP frequency

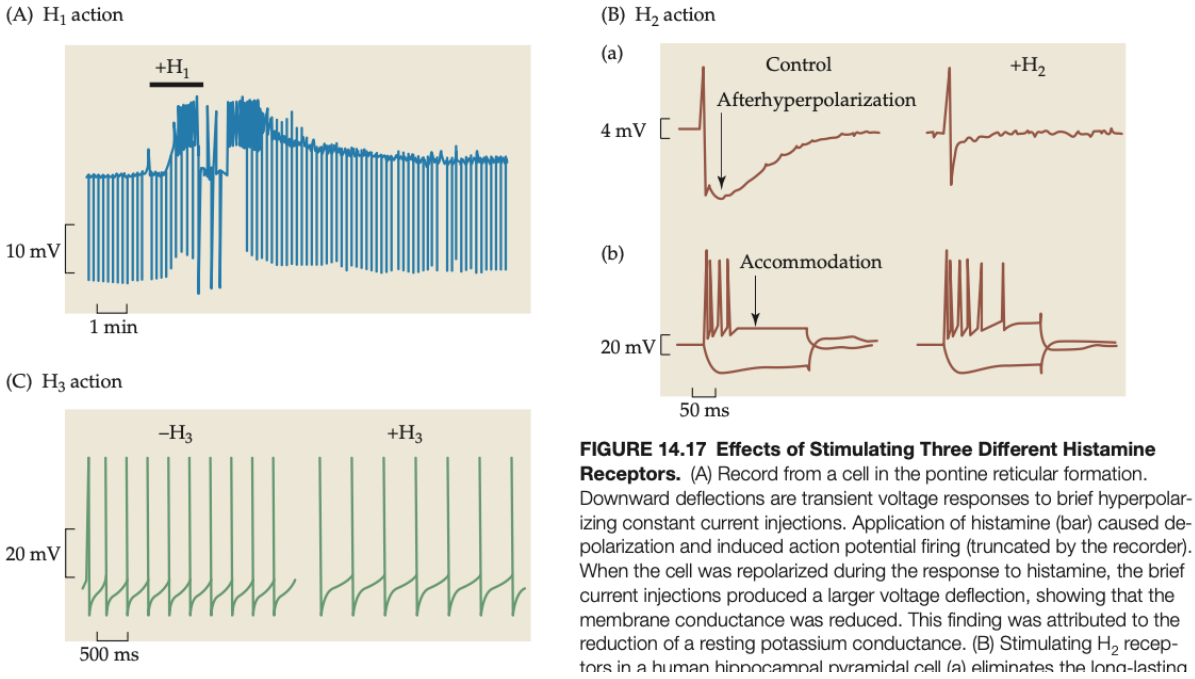


FIGURE 14.17 Effects of Stimulating Three Different Histamine Receptors. (A) Record from a cell in the pontine reticular formation. Downward deflections are transient voltage responses to brief hyperpolarizing constant current injections. Application of histamine (bar) caused depolarization and induced action potential firing (truncated by the recorder). When the cell was repolarized during the response to histamine, the brief current injections produced a larger voltage deflection, showing that the membrane conductance was reduced. This finding was attributed to the reduction of a resting potassium conductance. (B) Stimulating H_2 receptors in a human hippocampal pyramidal cell (a) eliminates the long-lasting

Fig. 14.18 Histaminergic Neuron Activity Is Related to the Awake State

- Only wake will generate action potential discharges

Conclusion: wakefulness is associated with histamine

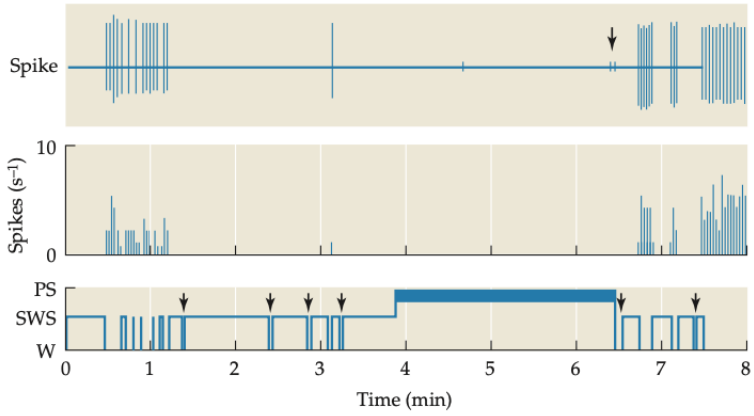


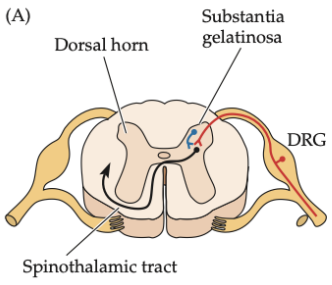
FIGURE 14.18 Histaminergic Neuron Activity Is Related to the Awake State. The top record shows action potential discharges (labeled "spike") from a single histaminergic neuron in the tuberomammillary nucleus of an unanaesthetized mouse with an extracellular recording electrode. The trace below is a ratemeter output of action potential frequency (spikes s^{-1}). The bottom diagram shows the awake-sleep state of the mouse, as judged from the electroencephalogram (EEG). W = awake; SWS = slow-wave sleep; PS = paradoxical sleep. The neuron only fired when the mouse was awake, and was completely silent when the mouse was asleep. It also stayed silent when the mouse woke up for just very short periods (arrowheads). (Adapted from Takahashi et al., 2006.)

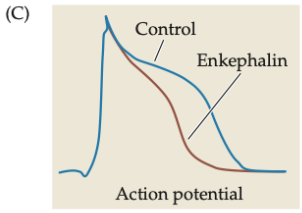
Fig. 14.20 Pathway for Transmission of Pain Sensation in the Spinal Cord

- A,B: Dorsal root ganglion (DRG) cells responding to noxious stimuli release **Substance P (SP) and Glutamate** at their synapses with interneurons in the dorsal horn of the spinal cord
    - Interneurons containing <u>enkephalin (ENK)</u> in the substantia gelatinosa of the dorsal horn <u>block transmission</u> partly by <u>inhibiting transmitter release</u> from terminals of the DRG cells
- C: Enkephalin (ENK) <u>reduces calcium current</u> → reduces duration of AP, and reduces transmitter release

Conclusion: ENK is a painkiller by blocking glutamate and Substance P

FIGURE 14.20 Pathway for Transmission of Pain Sensation in the Spinal Cord. (A,B) Dorsal root ganglion (DRG) cells responding to noxious stimuli release substance P (SP) and glutamate at their synapses with interneurons in the dorsal horn of the spinal cord. Interneurons containing enkephalin (ENK) in the substantia gelatinosa of the dorsal horn block transmission partly by inhibiting transmitter release from terminals of the DRG cells. (C) Intracellular recordings from the dorsal root ganglion cell demonstrate that enkephalin acts by causing a decrease in the duration of the action potential, reflecting reduced calcium current, which would reduce the amount of transmitter released from sensory nerve terminals. (C after Mudge, Leeman, and Fischbach, 1979.)





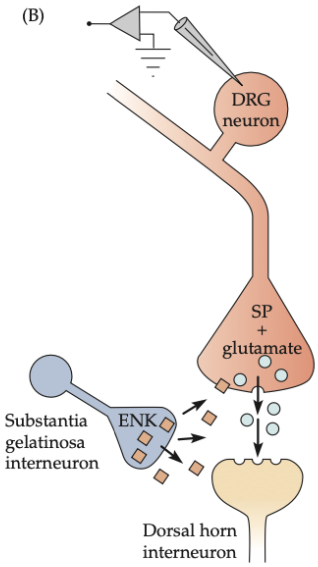


Fig. 14.23 Extracellular Glucose Concentration Regulates the Firing of Orexin- Containing Neurons

- A: increase glucose → hyperpolarized the Orexin-containing neuron and inhibit AP → less orexin released when high glucose → sleepy
- B: more glucose = more hyperpolarized membrane

Conclusion: glucose regulated orexin makes you feel not full and awake

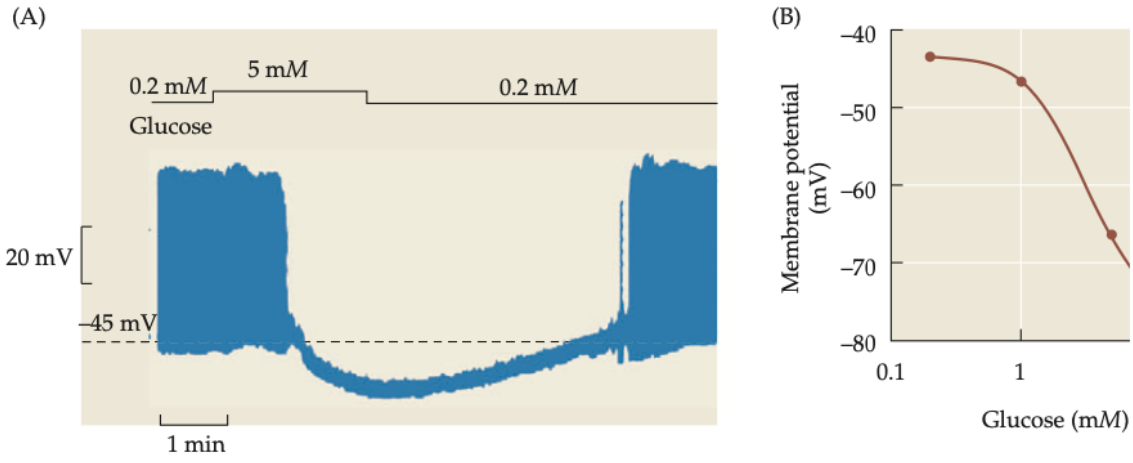


FIGURE 14.23 Extracellular Glucose Concentration Regulates the Firing of Orexin-Containing Neurons. (A) Spontaneous action potentials recorded from an orexin-expressing neuron in a slice of rat hypothalamus in vitro, recorded first in a solution containing 0.2 m*M* glucose and then on raising the glucose to 5 m*M*. Glucose hyperpolarized the neuron and inhibited action potentials. (B) Here, it is shown that the membrane potential is progressively increased as the glucose concentration is raised from 0.2 m*M* to 10 m*M*. (After Burdakov et al., 2005.)

Synaptic Plasticity

Focus:

- Short term vs. long term plasticity
- Hippocampal LTP / LTD / LTDP / input specificity and associativity of LTP

Short term changes

- A few minutes or less
- **Facilitation, augmentation, and potentiation** → causes persistent actions of calcium ions to enhance transmitter release
- Short term **depression** results from an activity dependent depletion of synaptic vesicles that are ready to undergo exocytosis

1. Synaptic (or paired-pulse) facilitation

- Increased synaptic strength → at least 2 AP invade the presynaptic terminal within few ms → stronger response in post
- Facilitation decreases exponentially as the second pulse follows the first pulse by tens of ms
    - Longer interval, less facilitation
- Caused by prolonged elevation of presynaptic calcium level
    - Build up of [Ca]i from closely arriving AP → allows for **greater transmitter release**

2. Synaptic depression

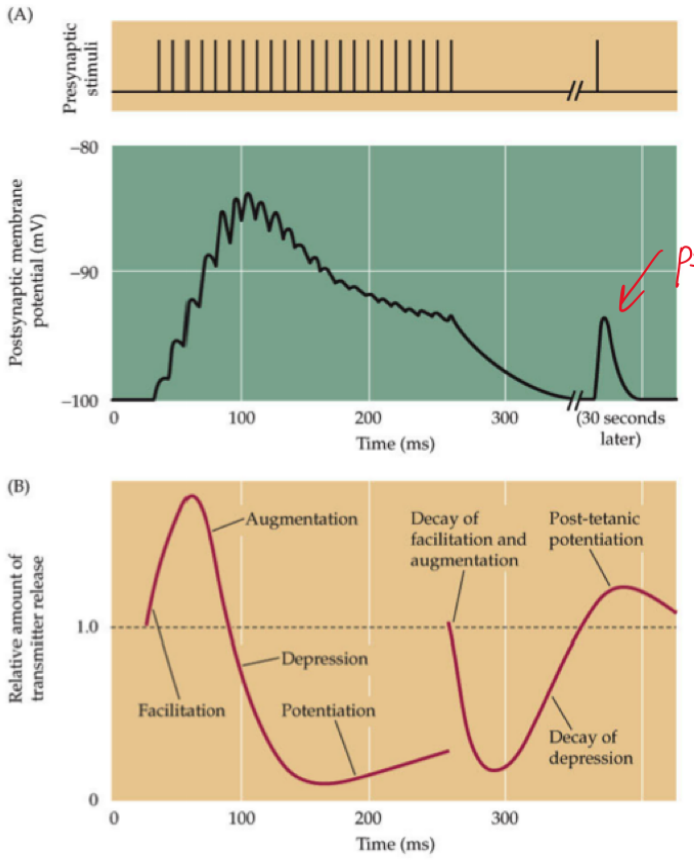
- Depression depends on the progressive depletion of the readily-releasable pool of synaptic vesicles in the presynaptic terminal (dubbed, “vesicle depletion hypothesis”)
    - When rates of release are high, readily-release pool of vesicles deplete rapidly causing depression, more rapidly than the mobilization and replenishment rate of vesicles from the reserve pool
- Lowering [Ca]o reduces the rate of depression → b/c lower total amount of transmitter released from presynaptic terminal
    - lower Ca = less Ca entry current = less transmitter released = more time to release transmitter = less rate of depression
- Mutant mice with impaired synapsin → enhanced synaptic depression
    - b/c synapsin is involved in inhibition of vesicle mobilization to the readily releasable pool

3. Synaptic augmentation and post-tetanic potentiation (PTP)

- **Augmentation (fast) and potentiation (slower)** → increase the amount of transmitter released from presynaptic terminals
- Augmentation rises and falls over a few seconds
    - Involves Ca enhancing the action of the protein **munc-13**
        - Involved in priming of docked, readily-releasable vesicles → increase the number of vesicles for release
- Post-tetanic potentiation (PTP) → greatly outlasts the timescale of the tetanus
    - Resulted from high frequency of stimulus → increase intracellular Ca
    - Acts over 10s to minutes
    - Involves Ca enhancing the actions of presynaptic protein kinases
        - Phosphorylate substrates eg. synapsin, regulate transmitter release

Short-term plasticity at the NMJ synapse

- High presynaptic stimulation → facilitation / augmentation for more transmitter release → depression: depletion due to lack of transmitter → PTP for more transmitter release

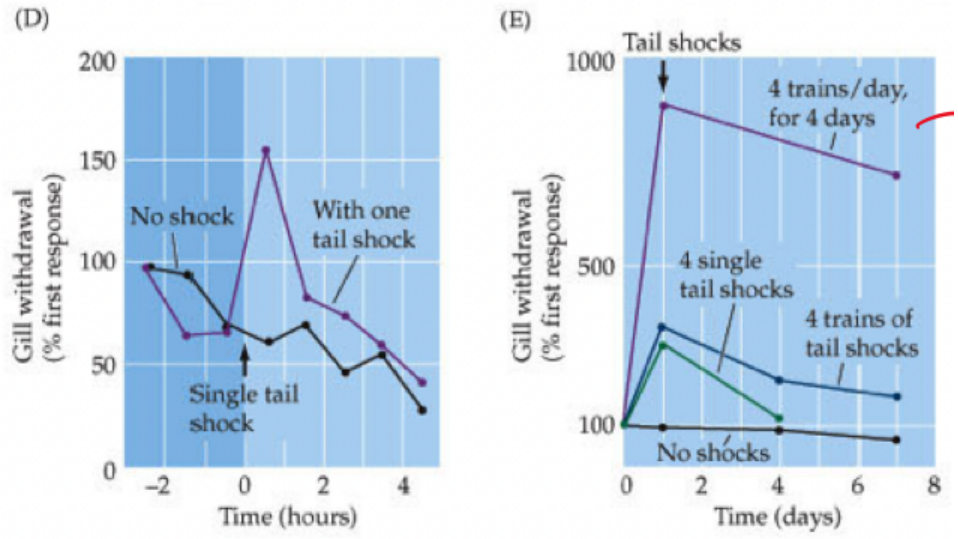


Long term changes

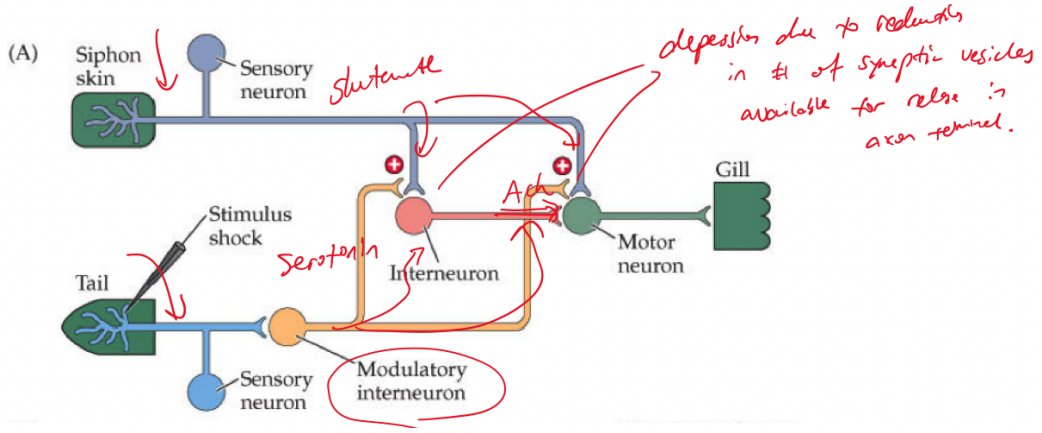
- **Long-term potentiation, long-term depression** lasts over 30 min to a life-time
- Two stages of laying down of long-term changes
    - A. post-translational modification of existing proteins, changing the trafficking of glutamate receptors
    - B. synaptic modification resulting from changes in gene expression: growth of synapses, and permanent modifications of brain function → learning and memory
- Aplysia as a model to study neuronal plasticity

Behavioral plasticity of Aplysia **gill withdrawal reflex**

1. **Habituation**
    - Less responsiveness to repeated occurrence of the same stimulus (eg tactile sensation on skin)
    - Habituation model: repeated light touch on siphon → causes gill withdrawal
2. **Sensitization (classical conditioning)**
    - A form of associative learning, popularized Ivan Pavlov dog
        - Pair conditioned stimulus (bellring&food) + unconditioned stimulus (food)
    - D: Pair siphon touch with electrical shock to the tail → restores a large and rapid gill contraction to siphon touch due to short-term sensitization
    - E: Repeat applications of tail shocks → prolonged sensitization of gill withdrawal response

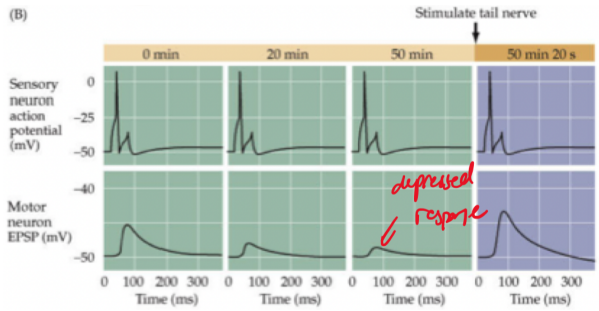


Neural circuitry

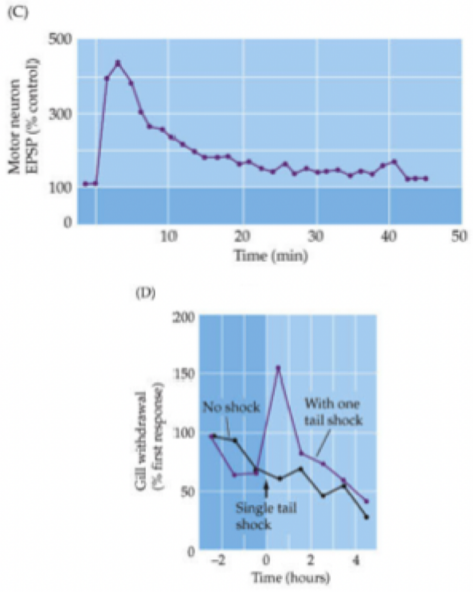


Changes in synaptic efficacy at the sensory-motor synapse

- **Habituation** - caused by **synaptic depression**
    - Caused by **presynaptic transmission** at the glutamatergic synapse between the <u>sensory and motor neurons</u>
        - Due to reduction in number of synaptic vesicles ready for release (B)



- **Sensitization** - modifies the function of circuit by <u>recruiting additional neurons</u>
    - Tail shock activates sensory neurons → excite modulatory interneurons → release **serotonin** onto the presynaptic terminal of sensory neurons in siphon
    - Serotonin enhances transmitter release → increase synaptic excitation of the motor neurons (C)
    - Time course of serotonin induces facilitation (C) ~= short term sensitization from single tail shock (D)

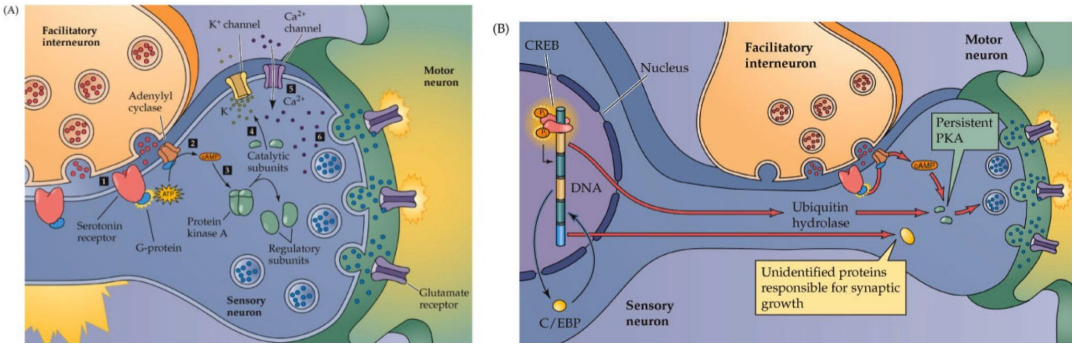


A: Molecular mechanism for <u>short term sensitization</u> involves enhanced <u>glutamate</u> release

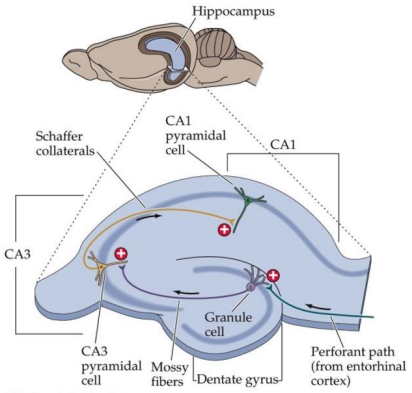
1. Serotonin from facilitatory interneurons binds to GPCR on the <u>presynaptic terminals</u> of the siphon sensory neurons
2. GPCRs stimulate the production of **cAMP**
3. cAMP binds to the regulatory subunits of **PKA**
4. cAMP binding liberates <u>catalytic subunits of PKA</u> → **phosphorylate K channels**
5. Phosphorylation lowers the K channel opening, <u>prolonging the presynaptic AP</u> and opening more <u>presynaptic Ca channels</u>
6. Greater [Ca]i increases the among of **glutamate released** onto motor neurons during sensory neurons AP

B: Molecular mechanism of <u>long term sensitization</u> is due to <u>increasing the production of building blocks</u> for synapses

- With repeat training, serotonin-activated **PKA phosphorylates** transcription factor **CREB**, which binds to **CREs** in regulatory regions of nuclear DNA to <u>increase the transcription</u> rate of downstream genes
- CREB also stimulates the synthesis of **ubiquitin hydroxylase** → stimulates the degradation of the regulatory subunit of PKA
    - Absence of the regulatory subunit causes a <u>persistent increase in the amount of free and active catalytic PKA subunit</u>, maintained without the requirement of serotonin
- CREB also stimulates **transcription factor C/EBP** → transcription of many genes required for building synaptic terminals, yielding a long-term increase in the <u>number of synapses</u> between sensory and motor neurons
- Long term synaptic changes (facilitation) involves cytoplasmic polyadenylation element binding protein **CPEB** → activates mRNA for controlling protein synthesis, and has self-sustaining properties like prion proteins



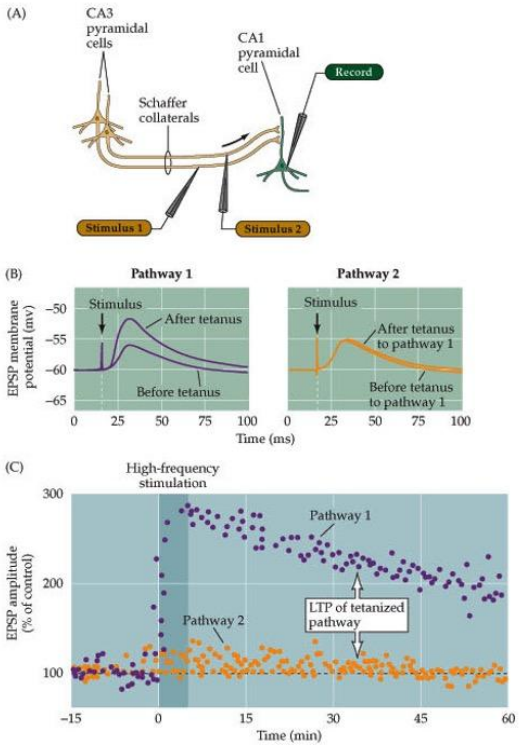
Synaptic plasticity in the mammalian hippocampus



- Long-term synaptic plasticity (potentiation: LTP/ depression: LTD)
- The hippocampus is essential for the formation and retrieval of memories
- Functional imaging shows the human <u>hippocampus is activated during memory tasks</u>
- Set of hippocampal neurons “place cells” encode spatial memories, and fire action potentials only when an animal is roaming in set locations
- Damage to the hippocampus results in an inability to form certain types of new memories and spatial navigation

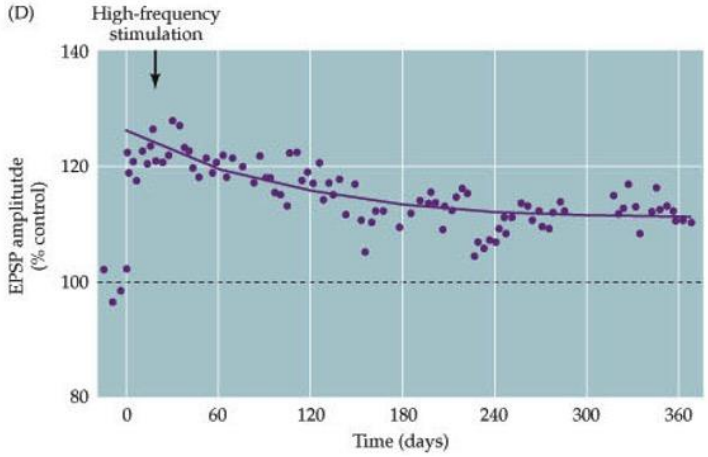
Hippocampal LTP in Schaffer collateral-CA1 synapse

- Electrical stimulation of the Schaffer collaterals generates EPSPs in the postsynaptic CA1 cells
- If Schaffer collaterals are stimulated <u>only 2-3 times/min</u>, the size of the evoked EPSP in the CA1 region <u>remains constant</u> (Pathway 2)
- A brief, <u>high-frequency</u> train of stimuli to other axons in the Schaffer collaterals causes **LTP → long-lasting increase in EPSP amplitude** (Pathway 1)



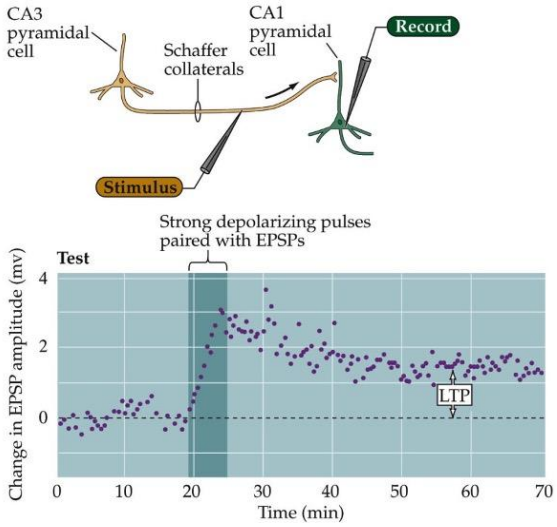
LTP is a mechanism for the long-lasting storage of information

- Recordings of EPSPs from the living hippocampus reveal that high-frequency stimulation can produce LTP that <u>lasts for greater than a year</u>
- Long lasting LTP has also been recorded in the cerebral cortex, amygdala and cerebellum



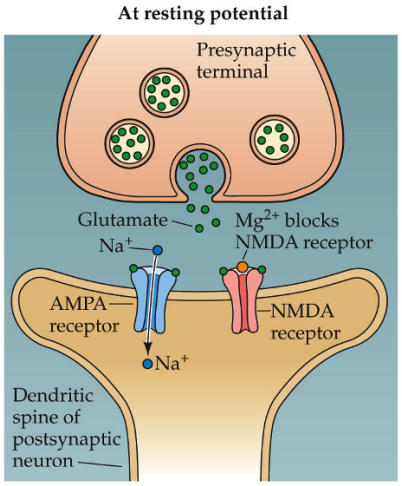
LTP is a Hebbian synapse: coincidence of presynaptic and postsynaptic activity causes LTP

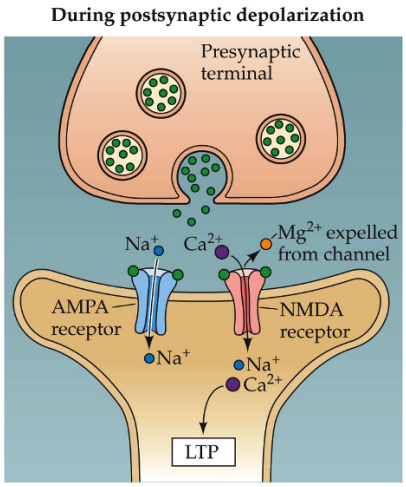
- Single stimulus (usually won't have LTP) to the Schaffer collaterals → if it is <u>paired with strong depolarization</u> of the postsynaptic CA1 cell, it will activated Schaffer collateral synapses **undergoes LTP**
- Strong <u>postsynaptic</u> depolarization must occur <u>within 100 ms</u> of <u>presynaptic</u> transmitter release
- Requirement for **coincident pre- and postsynaptic depolarization** was a central postulate of a theory of learning proposed by Donald Hebb in 1949



The NMDAR is a coincidence detector for generating LTP

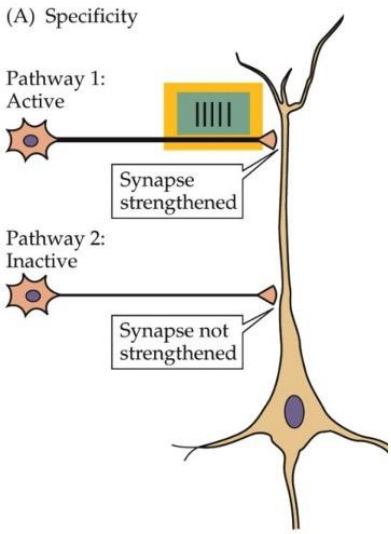
- Both AMPAR and NMDAR pass cations, but only NMDAR pass Ca^{2+} ions (second messenger for LTP)
- During low-frequency synaptic transmission, glutamate release by the Schaffer collaterals bind to both NMDA and AMPA GluR
    - NMDA receptor channel is blocked by Mg^{2+} when postsynaptic neuron is at normal resting mmbn potential → EPSP mediated entirely by AMPA receptors
- Mg^{2+} block of NMDAR is voltage-dependent, only open upon high frequency stimulation (summation of EPSPs) → prolonged depolarization of the postsynaptic membrane
    - Postsynaptic depolarization expels Mg^{2+} from the NMDA channel,Ca^{2+} flow through NMDAR
    - Increase in Ca^{2+} in the dendritic spines of the postsynaptic cell is the trigger for LTP
- The NMDAR behaves as a molecular coincidence detector, because it open to induce LTP only when there is simultaneous glutamate bound to the receptor and the postsynaptic cell is depolarized to relieve the Mg^{2+} block



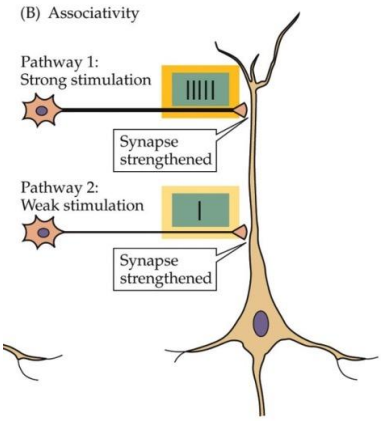


Input specificity of LTP

- LTP induced by activation of one synapse does not occur in other, inactive synapses that contact the same neuron
- LTP is restricted to activated synapses rather than to all of the synapses on a given cell
- LTP is confined to synaptic inputs that are active and releasing glutamate



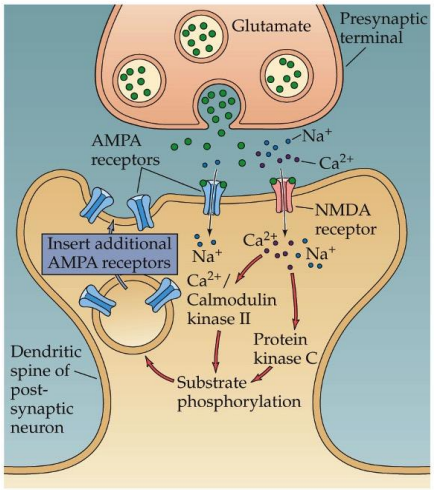
Associativity of LTP



- If a weak stimulus that does not normally itself trigger LTP is activated at the same time that a neighboring pathway undergoes LTP, <u>both synaptic pathways undergo LTP</u>
- Selective enhancement of conjointly activated sets of synaptic inputs is considered a cellular analog of associated learning or classical conditioning
- a weakly stimulated input, releases glutamate but cannot sufficiently depolarize the postsynaptic membrane to relieve the Mg^{2+} block, and requires the "<u>associative" depolarization of the strongly stimulating neighboring input</u> to relieve the Mg^{2+} block

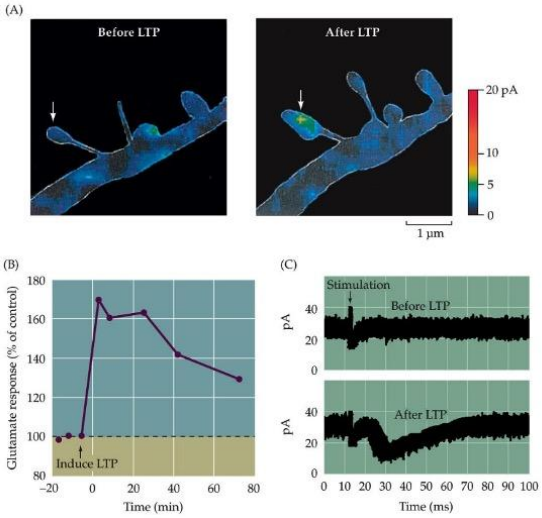
Calcium signaling underlying induction of LTP

- Injection of Ca^{2+} chelators blocks LTP induction, whereas elevation of Ca^{2+} levels in postsynaptic neurons potentiates synaptic transmission
- Ca entering through NMDAR activate **CaMKII and PKC**
- CaMKII is the most abundant postsynaptic protein at Schaffer collateral synapses
    - mutation of CaMKII prevents LTP
- <u>CaMKII is able to autophosphorylate and prolongs the duration of LTP</u>
    - Put AMPA receptor on cell membrane → more glutamate bind → prolong LTP
- Downstream targets of CaMKII is not fully known but apparently include AMPAR and other proteins



AMPAR insertion underlies the maintenance of LTP

- LTP is maintained from the kinase phosphorylation → increase membrane insertion of additional postsynaptic AMPAR
    - <u>increases the responsiveness of the postsynaptic dendritic spine to applied glutamate</u> at 120 minutes after LTP induction (voltage-sensitive dye, A)
- LTP can induce trafficking of AMPA receptors to previous silent synapses
- Induction of LTP (at time zero, B) increase glutamate sensitivity more than 60 minutes
    - Prior to inducing LTP, no EPSCs are elicited at -65 mV at a silent synapse (low AMPAR), but same stimulus produces EPSCs mediated by AMPA receptors after LTP induction (increase # of AMPAR) (C)



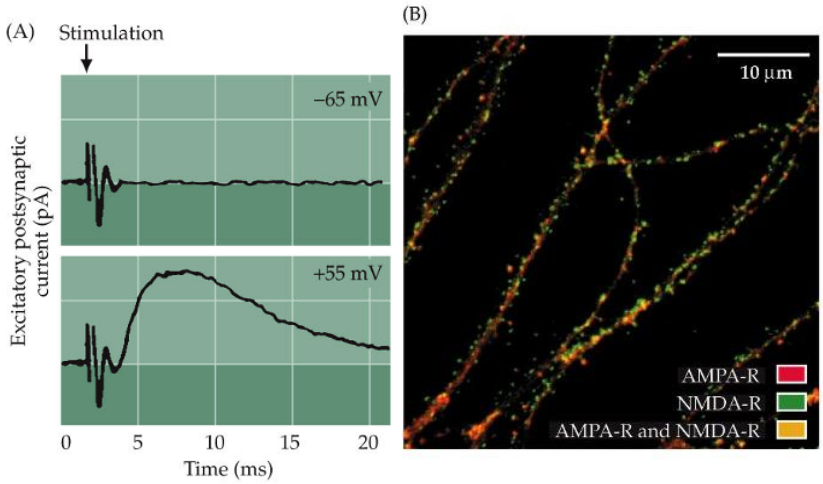
LTP can cause a increase in presynaptic glutamate release

- LTP is mainly a postsynaptic AMPAR mediated effect, but also involves a sustained increase in <u>presynaptic terminals</u> to release more glutamate
- Because LTP is triggered in the postsynaptic neuron, a **retrograde messenger** (nitrous oxide, NO) spreads from the postsynaptic spine back to presynaptic terminals → stimulate an enhanced glutamate release

Silent synapses in adults

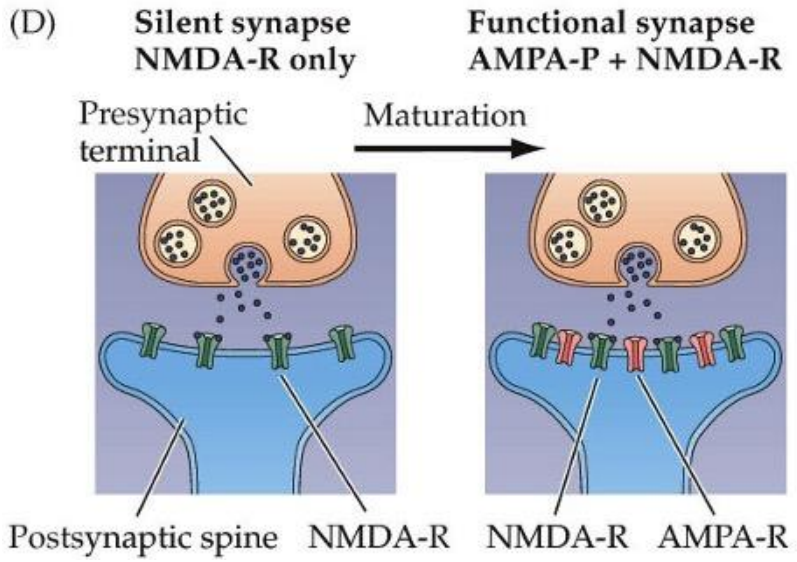
- “Silent synapses” are found in adult hippocampus, cerebral cortex and spinal cord
    - With NMDAR but no AMPAR
- Glutamate released at silent synapses <u>bind only to NMDAR</u> → but voltage-dependent block of NMDAR → no AP in post

- When the postsynaptic cell is held at -65 mV, the NMDAR are not responsive to presynaptic stimulation, but are <u>responsive to presynaptic stimulation at a +55 mV post-synaptic holding potential</u> (A)
    - +55 mV uncoupled Mg2
- Immunofluorescent localization indicates that silent synapses at dendritic spines lack AMPAR (red), but are positive for NMDAR only (green)



Silent synapses in development

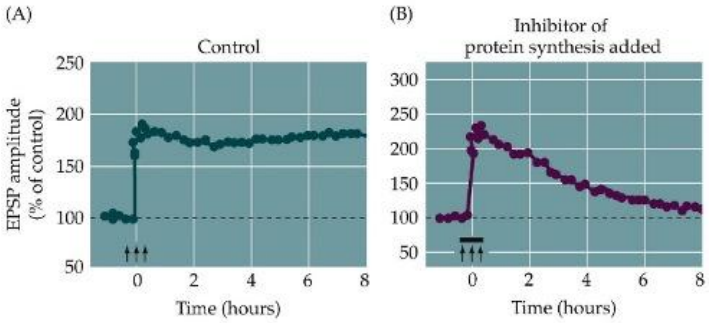
- Electron microscopy illustrates an absence of AMPA receptors in the CA1 region of the rodent hippocampus <u>from 10 day old</u> (juvenile) <u>but not 5 week old</u> (adult)
- **Maturation of silent synapses into functional ones involves insertion of AMPAR**



Long-term sustained LTP (> 2 hours) involves changes in gene and protein expression

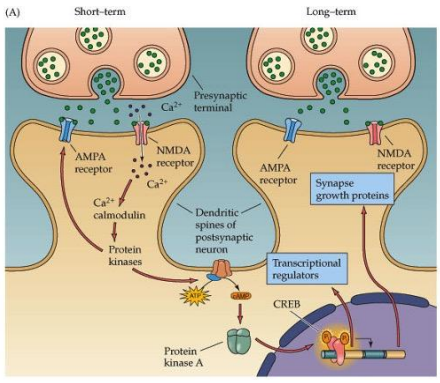
1) Requirements for protein expression

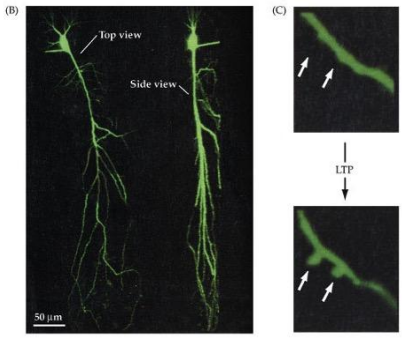
- High frequency stimulation induces LTP that persists for many hours (A)
- LTP is <u>not sustaining with protein synthesis inhibitor</u>, **anisomycin** (at bar, B)
    - LTP decays within a few hours after the high frequency stimulation



2) Requirements for gene expression

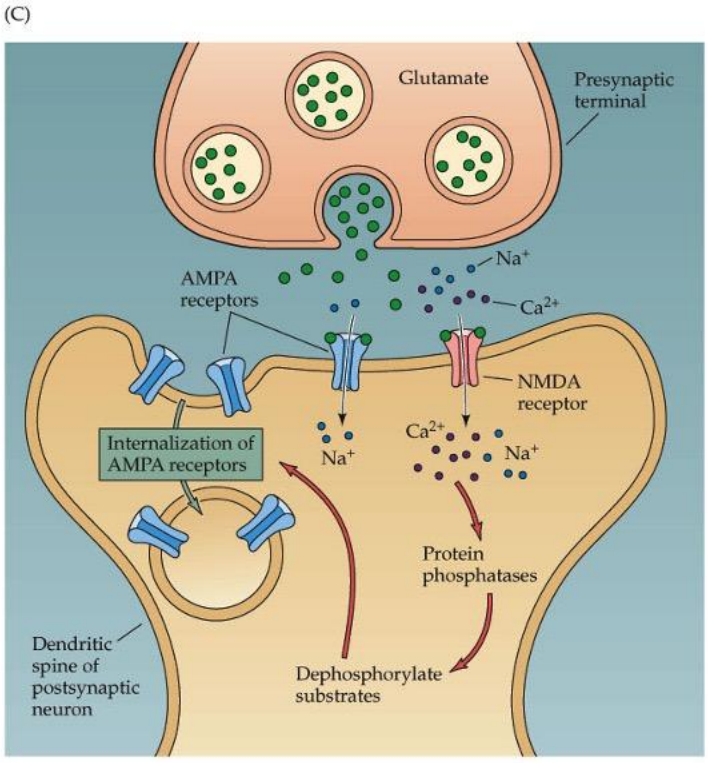
- Late phase of LTP is due to PKA → phosphorylates and activates CREB → turns on the gene transcription → produce long-lasting changes in PKA activity and synaptic structure (eg. AMPA receptor synthesis)
- <u>Number and size of synaptic contacts increases during LTP</u>, evident are new dendritic spines (C) after 1 hour post-LTP induction in CA1 pyramidal neuron filled with fluorescent dye (B)





Long-term synaptic depression in the hippocampus

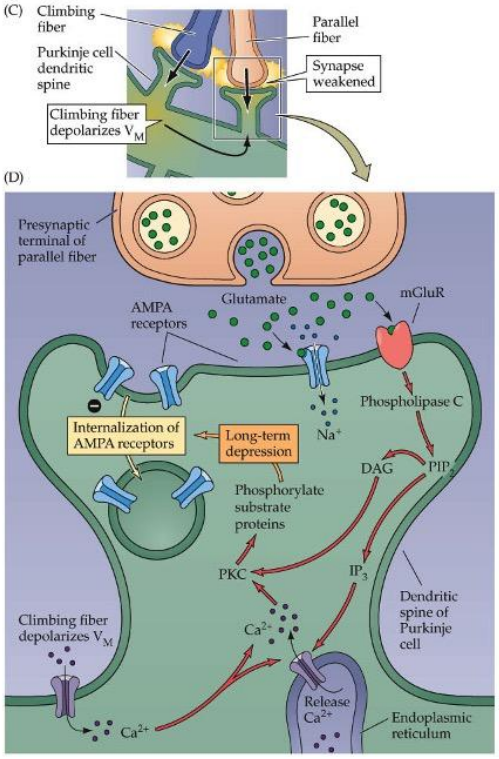
- While some synapses increase in strength as a result of LTP
    - other synapses are selectively weakened by LTD
- Low stimulation (1Hz) for long periods (10-15 minutes) induces long-lasting depression of synaptic transmission or LTD (A, B)
- LTP and LTD reversibly affect synaptic efficacy by acting at a common site
    - LTP can erase the decrease in EPSP size due to LTD
    - LTD can erase the increase in EPSP size due to LTP
- LTD, like LTP is input specific to the activated synapse, and is evoked by Ca entry into the postsynaptic cell through NMDAR
- Small and slow rises in Ca^{2+} trigger LTD
    - Large and fast increases in Ca^{2+} trigger LTP
- LTD involves activation of calcium-dependent phosphatases
    - While LTP involves activation of protein kinases
- Phosphatase (dephosphorylate) inhibitors prevent LTD but have no effect on LTP
- LTD is associated with the internalization of synaptic AMPAR, by a clathrin-dependent endocytosis mechanism
    - LTP put AMPAR to the mmbn



LTD in the cerebellum

1) Cerebellar LTD does not involve NMDAR

- LTD of parallel fiber (PF) and climbing fiber (CF) synaptic inputs onto cerebellar Purkinje cells
- LTD is implicated in the <u>motor learning</u> that mediates the coordination, acquisition and storage of complex movements in the cerebellum
- Glutamate released from PF activates purkinje cell AMPAR that results in membrane depolarization and activates **mGluR** that produces second messengers IP3 and DAG (C, D)

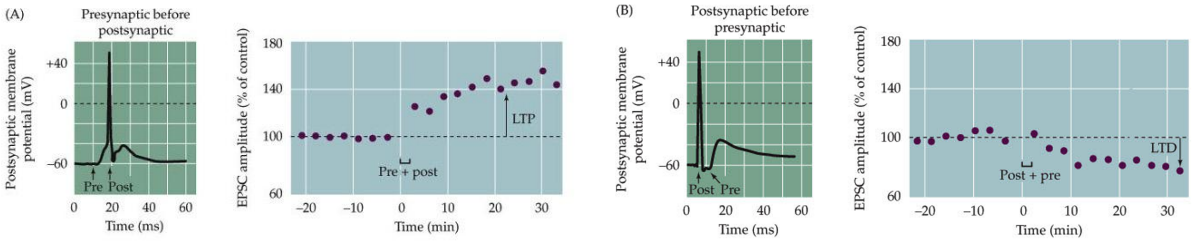


2) Cerebellar <u>LTD involves PKC</u> not a phosphatase

- CF activation causes an influx of Ca^{2+} through Vg-Ca channels and rises in [Ca]i
- Cerebellar LTD is **associative**
    - parallel fiber EPSP is reduced <u>only when PF and CF are co-activated</u>, synergistically activating the same second messenger activation cascade
- Rise in [Ca]i caused by coincident activation of Vg-Ca channels (CF) and IP3-stimulated Ca release from intracellular stores (PF)
- Synergistic rise in PKC by rise in [Ca]i (CF+PF) and DAG (PF)
- PKC activation leads to <u>sequestration of AMPA receptors</u> and <u>weakens the parallel fiber synapse</u>, and <u>long term stimulation of CREB</u>

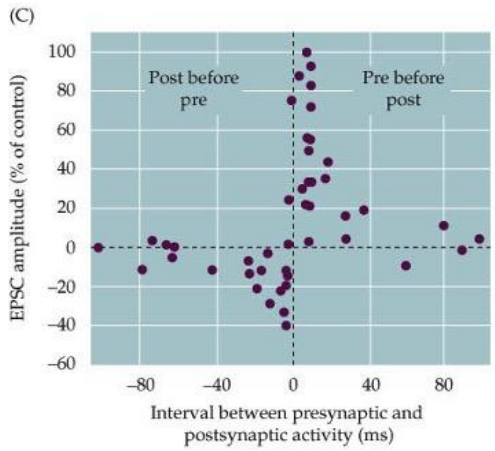
Spike timing-dependent synaptic plasticity (STDP)

- Different rates of synaptic activity will lead to LTP (high frequency activity) or LTD (low frequency activity)
- Also critical switch between LTP/LTD is the temporal relationship between activity in the presynaptic and postsynaptic cells (known as STDP)
- **Stimulation paradigm**:
    1. Stimulating a presynaptic neuron (Pre) causes an EPSP in the postsynaptic neuron (A,B left)
    2. Stimulus to the postsynaptic neuron (Post) causes an action potential that is superimposed on the EPSP (A, left)
- **Pre → Post causes LTP (A, right)**
- **Post → Pre causes LTD (B, right)**



STDP arises from timing differences in postsynaptic calcium signals

- If the postsynaptic neuron is activated after the presynaptic neuron (40 ms or less)
    - **LTP occurs**, because resulting depolarization relieve the Mg^{2+} block of NMDAR, causing a large Ca influx through NMDAR from presynaptic glutamate stimulation
- If the postsynaptic action potential is activated before the presynaptic action potential (40 ms or less)
    - **LTD is induced**, because the depolarization associated with the postsynaptic action potential will subside/reduce by the time an EPSP occurs, reducing the amount of Ca entry through NMDAR during presynaptic glutamate stimulation (LTD induction during STDP may involve other signals such as endocannabinoids)
- The timing of the presynaptic and postsynaptic stimulations of the synaptic change is highly precise
    - no LTD or LTP is observed if the presynaptic and postsynaptic activity are separated by 100 ms or longer

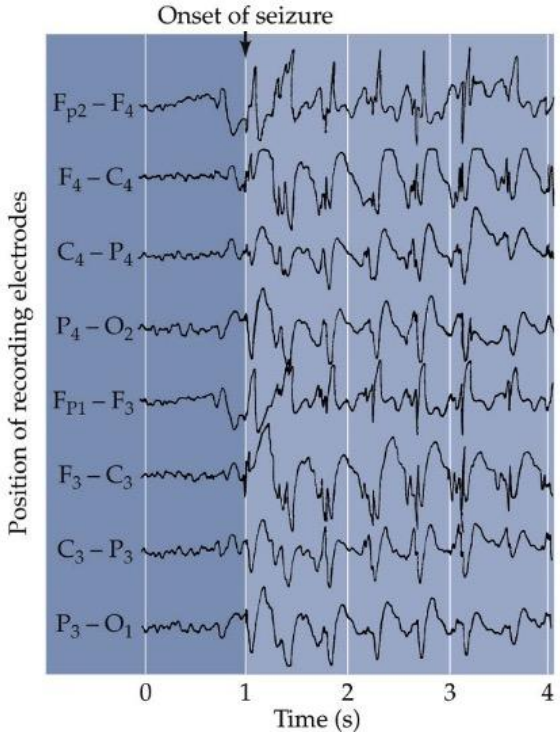


STDP provides a means to encode information about causality and input competition

- If a synapse generates a suprathreshold EPSP, the resulting postsynaptic action potential would rapidly follow presynaptic activity, producing LTP → postsynaptic action potential resulted from the activity of the synapse
- Stronger inputs would more likely produce suprathreshold EPSP and be reinforced by the resulting LTP, whereas weaker inputs would not generate postsynaptic action potentials that correlate with presynaptic activity

Epilepsy: The Effect of Pathological Activity on Neural Circuitry

- Epilepsy is a brain disorder characterized by periodic and unpredictable seizures mediated by persistent, synchronous, rhythmic firing of large groups of neurons (EEG in figure)
- Abnormal activity generates plasticity changes in the cortical circuitry that are critical to the progression of the disease
- Seizures are triggered in **foci** (small areas of the cerebral cortex) that spread to other synaptically connected regions
- Eg. seizure originating in the thumb area of the motor cortex will extend from the left thumb to proximal limb; whereas seizure in visual association area of the right hemisphere may be heralded by complex hallucinations in the left visual field
- Treatment includes enhancing function of GABA synapses or limited activity of Vg Na channels (using drugs such as carbamazepine, phenobarbital, phenytoin and valproic acid)
- Extreme cases physicians resort to cutting the corpus callosum to limit the spread of seizures between hemispheres



Kindling

- Animal model of seizure production resembling human epilepsy
- Induced by stimulating electrode implanted in the rodent brain (usually the amygdala)
- Weak electrical stimulation in the form of low-amplitude train of electrical pulses has no discernible effect on the animal's electrical activity patterns or behavior
    - Weak stimulation is repeated /day for several weeks, causes full blown seizures
    - Caused by STDP
- Kindling causes irreversible changes to the brain
    - One year later, same weak stimulus will again trigger a seizure

Fig. 17.2 Fast and Slow Synaptic Potentials in Sympathetic Ganglion Cells

- (A) Single stimulus to the preganglionic inputs evokes a large and fast EPSP and AP —> phasic response
- (B) Trains of stimuli (10 per second for 5 seconds) are required to elicit slow EPSP synaptic potentials —> tonic response
- (C) Cells in ganglia receive cholinergic and peptidergic inputs, which can be stimulated selectively
- (D) Slow excitatory potential evoked by stimulating peptidergic input (20 per second for 5 s). As in (B) the depolarization lasts several minutes
- (E) Application of GnRH (LHRH) to the same neuron by pressure from a micropipette
    - The peptide mimics the action of the naturally released transmitter

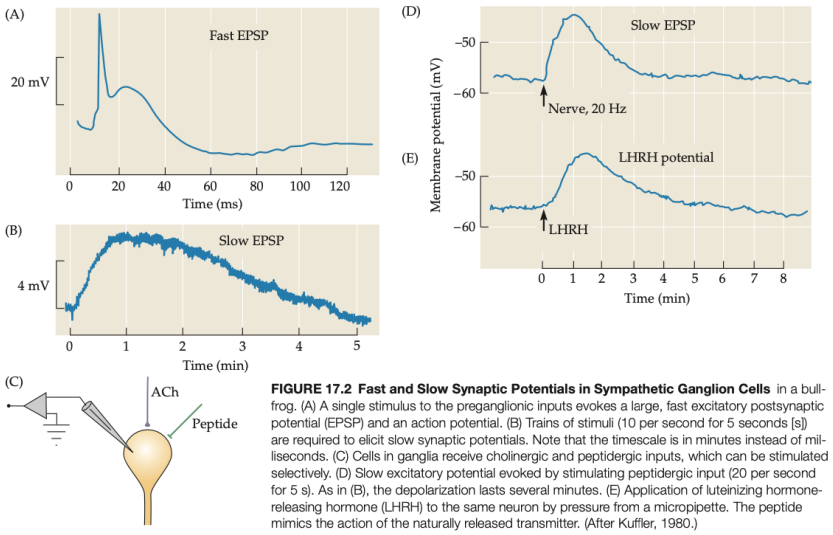


FIGURE 17.2 Fast and Slow Synaptic Potentials in Sympathetic Ganglion Cells in a bullfrog. (A) A single stimulus to the preganglionic inputs evokes a large, fast excitatory postsynaptic potential (EPSP) and an action potential. (B) Trains of stimuli (10 per second for 5 seconds [s]) are required to elicit slow synaptic potentials. Note that the timescale is in minutes instead of milliseconds. (C) Cells in ganglia receive cholinergic and peptidergic inputs, which can be stimulated selectively. (D) Slow excitatory potential evoked by stimulating peptidergic input (20 per second for 5 s). As in (B), the depolarization lasts several minutes. (E) Application of luteinizing hormone-releasing hormone (LHRH) to the same neuron by pressure from a micropipette. The peptide mimics the action of the naturally released transmitter. (After Kuffler, 1980.)

Fig. 17.3 Inhibition of Potassium Currents in sympathetic ganglion cells

- (A) Binding of ACh to muscarinic receptors (mAchR) and binding of GnRH (LHRH) to its receptor both **inhibit M-current potassium channels.**
    - Activation of muscarinic receptors causes M-channel to close
- (B) The effect of the decrease in the M-current during the slow synaptic potential is to increase the excitability of the ganglion cell —> AP is easier to fire
    - Depolarizing current pulses applied through the microelectrode (lower traces) before and after a slow synaptic potential produces a single action potential.
    - During the slow potential (where M current is inhibited), the same current pulse elicits a burst of action potentials → tonic-firing

- Depolarizing the ganglion cell (to the same extent as occurs during closure of M-channels) by injecting a maintained current has no effect on the responsiveness of the cell.
    - Because more current at same conductance = more voltage change —> just makes K+ more going out
- Initial depolarization opens M-channels -> M-channels generate an outward K current -> K current counteracts inward sodium current in initial phase of action potential -> prevents full action potential from developing
- M-current suppressed (M-channel closed): Neuron switches from phasic to tonic-firing

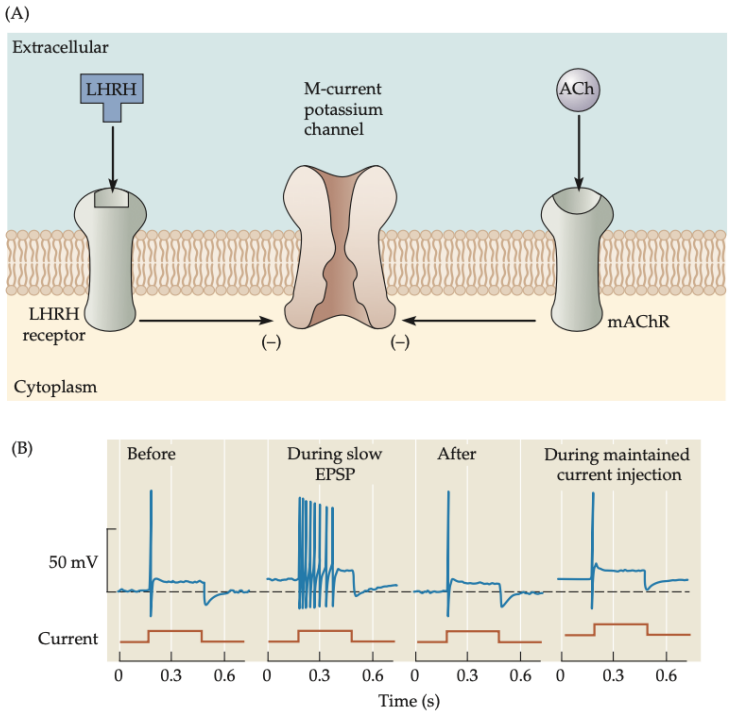


FIGURE 17.3 Inhibition of Potassium Currents in Sympathetic Ganglion Cells modulates responses to presynaptic stimulation. (A) Binding of ACh to muscarinic receptors (mAChR) and binding of LHRH to its receptor both inhibit M-current potassium channels. (B) The effect of the decrease in the M-current during the slow synaptic potential is to increase the excitability of the ganglion cell. Depolarizing current pulses applied through the microelectrode (lower traces) before and after a slow synaptic potential produce a single action potential. During the slow potential, the same current pulse elicits a burst of action potentials. Depolarizing the ganglion cell (to the same extent as occurs during closure of M-channels) by injecting a maintained current has no such effect on the responsiveness of the cell. (After Jones and Adams, 1987.)

Fig. 17.6 Firing of Carotid Sinus Stretch Receptors

- (A) Experimental arrangement for recording from sensory nerve fibers in the carotid sinus while it is distended by the circulation or, as in the diagram, artificially perfused
- (B) Relationship between blood pressure (lower trace) and the firing of a single afferent fiber from the carotid sinus at different levels of mean arterial pressure (from top down: 125, 80, and 42 mm of mercury measured with a manometer)
- (C) A classical record made in 1924 (with mercury manometer and smoked drum). The head of this animal was supplied with blood from a different animal so that blood pressure in the head arteries could be controlled separately by the experimenters (a)

Increased pressure in the head caused a fall in systemic blood pressure in the trunk of the animal. (b) Decreased pressure in the head caused an increase in systolic pressure.

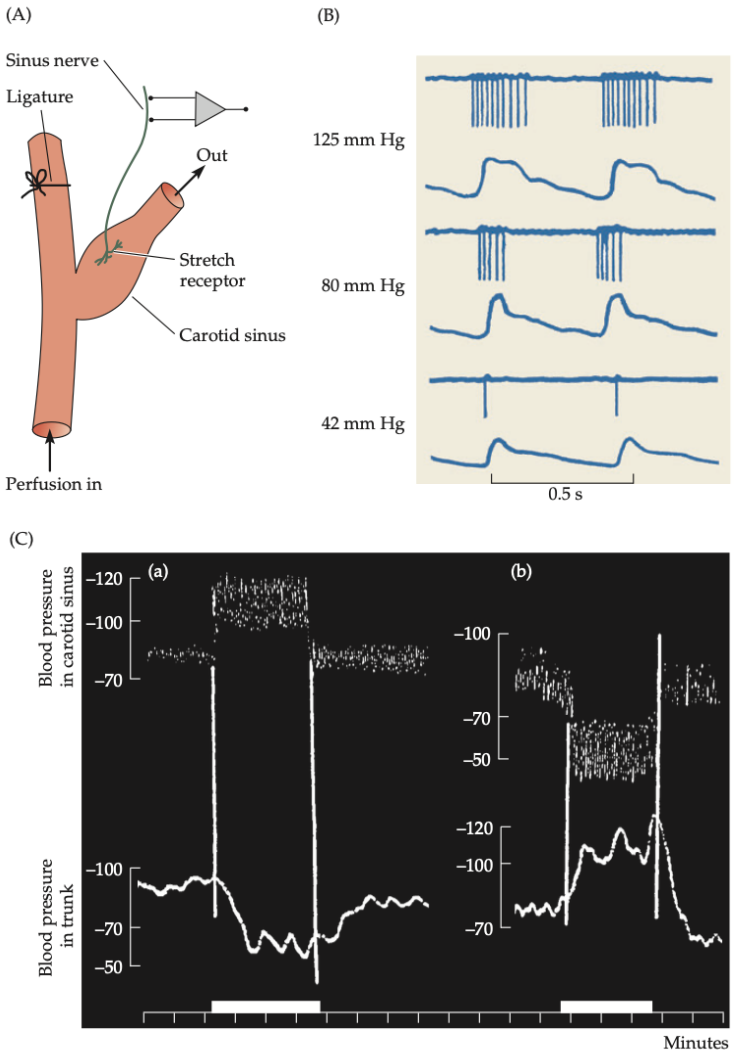


FIGURE 17.6 Firing of Carotid Sinus Stretch Receptors in response to raised blood pressure. (A) Experimental arrangement for recording from sensory nerve fibers in the carotid sinus while it is distended by the circulation or, as in the diagram, artificially perfused. (B) Relationship between blood pressure (lower trace) and the firing of a single afferent fiber from the carotid sinus at different levels of mean arterial pressure (from top down: 125, 80, and 42 mm of mercury, measured with a manometer). (C) A classic record made in 1924. The head of this animal was supplied with blood from a different animal so that blood pressure in the head arteries could be controlled separately by the experimenters. (a) Increased pressure in the head caused a fall in systemic blood pressure in the trunk of the animal. (b) Decreased pressure in the head caused an increase in systemic pressure. Such records were made before electrical recordings were possible; experimenters determined blood pressure using a mercury manometer and registered the movements with a fine pointer on a smoked drum. (B redrawn from Neil, 1954; C after Starling, 1941.)

Fig. 17.11 Circadian Rhythm of Slice of Rat Suprachiasmatic Nucleus maintained in culture

- GABA (causes Cl- current) was applied at different times while extracellular recordings were made from neurons. GABA gave rise to increases of action potential frequency in the daytime (A) and decreases at night (B)
- The recordings in C and D show that the effects of GABA were blocked by GABA antagonists (bicuculline and picrotoxin)
- The change from excitation to inhibition can be accounted for in terms of **changed intracellular chloride concentration**, which were assessed by whole-cell patch

recordings (not shown). The conductance change produced by GABA remains unchanged during the day-night cycle

- Regulated by NKCC1 and KCC2

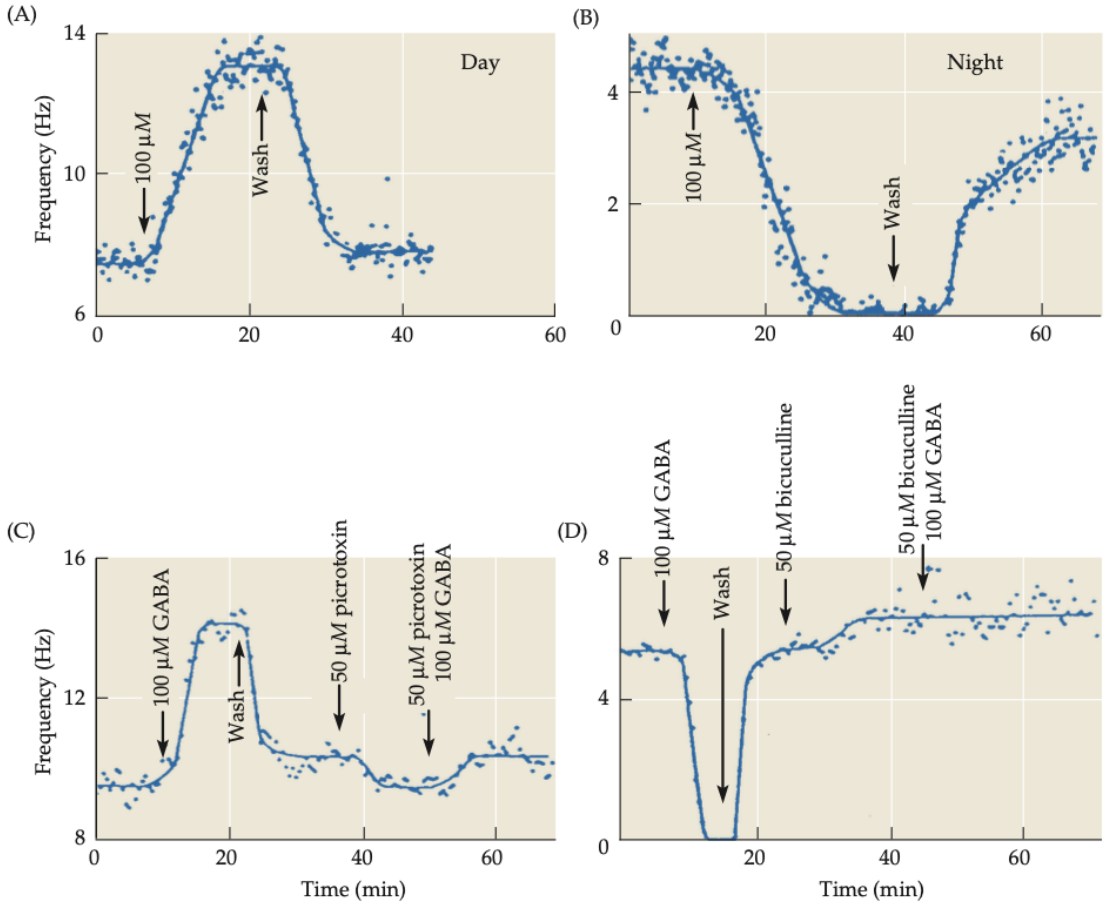


FIGURE 17.11 Circadian Rhythm of Slice of Rat Suprachiasmatic Nucleus maintained in culture. GABA was applied at different times while extracellular recordings were made from neurons. GABA gave rise to increases of action potential frequency in the daytime (A) and decreases at night (B). The recordings in C and D show that the effects of γ-aminobutyric acid (GABA) were blocked by GABA antagonists (bicuculline and picrotoxin). The change from excitation to inhibition can be accounted for in terms of changed intracellular chloride concentrations, which were assessed by whole-cell patch recordings (not shown). The conductance change produced by GABA remains unchanged during the day–night cycle. (After Wagner et al., 1997.)

Sensory Transduction

Fig. 19.2 Receptor potentials recorded extracellularly from a sensory nerve fiber supplying a muscle spindle

- The recording electrode is placed as close as possible to the receptor. Downward deflection of the voltage record (lower traces) indicates receptor depolarization
- (A) Stretching the muscle (upper trace) produces a **receptor potential**, upon which is superimposed a series of **action potentials** (lower trace)
- (B) Four stretches of increasing magnitude applied to the muscle after procaine (block Na channel) has been added to the bath solution. Action potentials are abolished by procaine, but the receptor potential remains
    - Stronger stretch, stronger response
- (C) Plot of receptor potential amplitude against increase in muscle length
    - More sensitive at lower range when compared to higher range
        - Adjust sensitivity of receptor so you are always in the lower range
- Weber-Fechner law = logarithmic relationship between stimulus and response

Conclusion: receptor potential is proportional to the muscle stretches in a log fashion

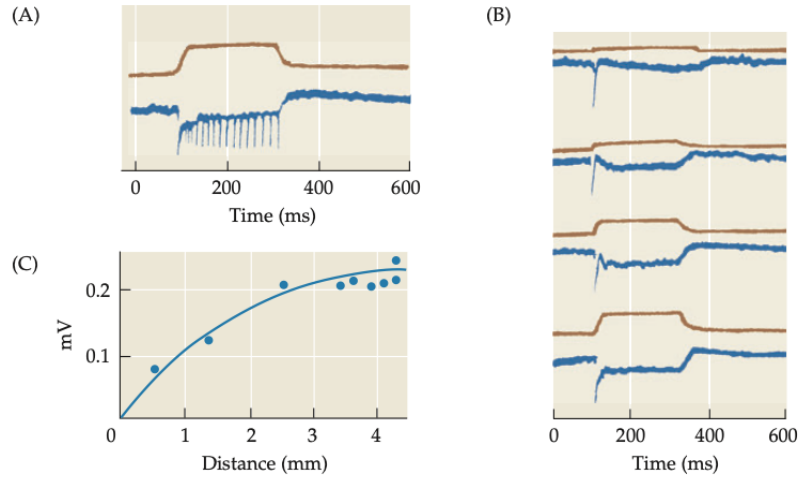


FIGURE 19.2 Receptor Potentials Recorded Extracellularly from a sensory nerve fiber supplying a muscle spindle. The recording electrode is placed as close as possible to the receptor. Downward deflection of the voltage record (lower traces) indicates receptor depolarization. (A) Stretching the muscle (upper trace) produces a receptor potential, upon which is superimposed a series of action potentials (lower trace). (B) Four stretches of increasing magnitude applied to the muscle after procaine has been added to the bathing solution. Action potentials (except for the first) are abolished by procaine, but the receptor potentials remain. (C) Plot of receptor potential amplitude against increase in muscle length. (After Katz, 1950.)

Fig. 19.3 Crustacean Stretch Receptor

- Crayfish stretch receptors lie in the periphery not in a ganglion (for vertebrates)
- (A) Superimposed picture of tubulin filaments in muscle (gray) and the receptor neurons (red)
- (B) Relation between stretch receptor neuron and muscle, indicating the method of intracellular recording. The excitatory fiber to the muscle produces contraction; the inhibitor fiber innervates the neuron

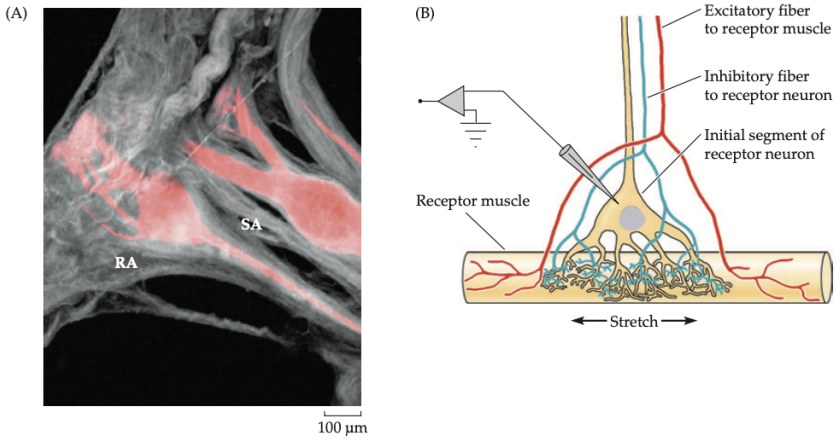


Fig. 19.4 Response to Stretch Receptor Neurons

- (A) In a slowly adapting receptor
  - a weak stretch (2s) produces a subthreshold receptor potential that persists throughout the stretch (upper record)
  - With a stronger stretch, a larger receptor potential sets up a series of action potentials (lower record) = indicates **period** of stretch or a "**static**" response (tonic response)
- (B) In a rapidly adapting receptor
  - In weak stimulus, receptor potential is not maintained (upper record),
  - During the large stretch, the action potential frequency declines due to opening of Ca2+ channel to open Ca activated K channels (lower record) = records the "**dynamic**" change (when on and when off) in stretch (phasic response)

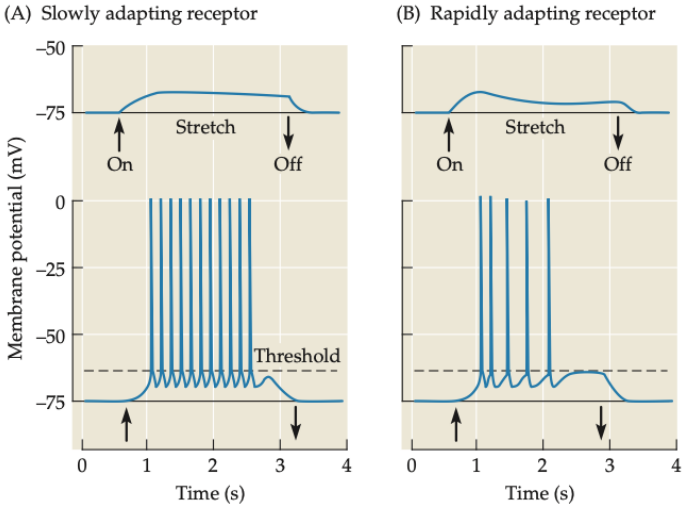


FIGURE 19.4 Responses of Stretch Receptor Neurons to increases in muscle length, recorded intracellularly as indicated in Figure 19.3B. (A) In a slowly adapting receptor, a weak stretch for about 2 seconds produces a subthreshold receptor potential that persists throughout the stretch (upper record). With a stronger stretch, a larger receptor potential sets up a series of action potentials (lower record). (B) In a rapidly adapting receptor, the receptor potential is not maintained (upper record), and during the large stretch, the action potential frequency declines (lower record). (After Eyzaguirre and Kuffler, 1955.)

Fig. 19.6 Specific Muscle Spindle Responses

- (A) Recordings of AP from single primary (group Ia, dynamic) and secondary (group II, static) sensory afferent fibers originating in a cat muscle spindle.
- The primary fiber
    - greatly increases its discharge rate as tension develops during the stretch;
    - during the maintained phase of the stretch, it quickly adapts to a lower rate —> dynamic due to opening of Ca activated K channels → phasic
- The secondary fiber
    - increases its firing rate more slowly as tension develops
    - and maintains its discharge during the steady stretch → tonic

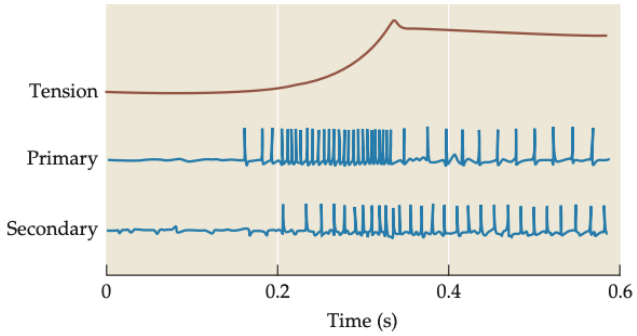
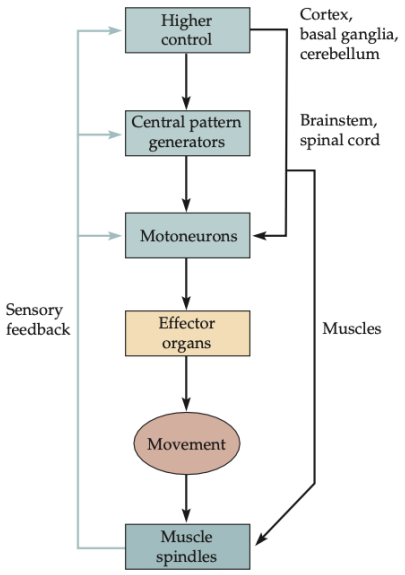


FIGURE 19.6 Specific Muscle Spindle Responses. Recordings of action potentials from single primary (group Ia) and secondary (group II) sensory afferent fibers originating in a cat muscle spindle. The primary fiber greatly increases its discharge rate as tension develops during the stretch; during the maintained phase of the stretch, it quickly adapts to a lower rate. The secondary fiber increases its firing rate more slowly as tension develops and maintains its discharge during the steady stretch. (After Jansen and Matthews, 1962.)

Circuits Controlling Reflexes, Respiration, and Coordinated Movements

Fig. 24.1 Scheme for Motor System Organization



- Limb muscles are controlled by **motor neurons** and **interneurons** of the spinal motor apparatus
- Interneurons within the spinal cord and brainstem make up **central pattern generators** that direct the motor apparatus
- Motor output is planned and refined by the motor cortex, basal ganglia and cerebellum. At every level of motor control, sensory input serves to initiate, inform and modulate output and is itself influenced by commands from higher levels

Fig. 24.2 Contacts between Stretch Receptor Afferents and Spinal Motor Neurons

- Average motor neurons receives many thousands of synaptic inputs
- Each **Ia afferent fiber** from a muscle sends an input to as many as 300 motor neurons
- (A) A single muscle spindle fiber (Ia) afferent sends branches to several motor neurons
- (B) A more detained view shows the afferent fiber passing over multiple dendritic branches, indicating many possible points of synaptic contact (red circles)
    - Innervation patterns observed by experimentally labeling afferent fibers and motor neurons with histological markers (eg. horseradish peroxidase, HRP)

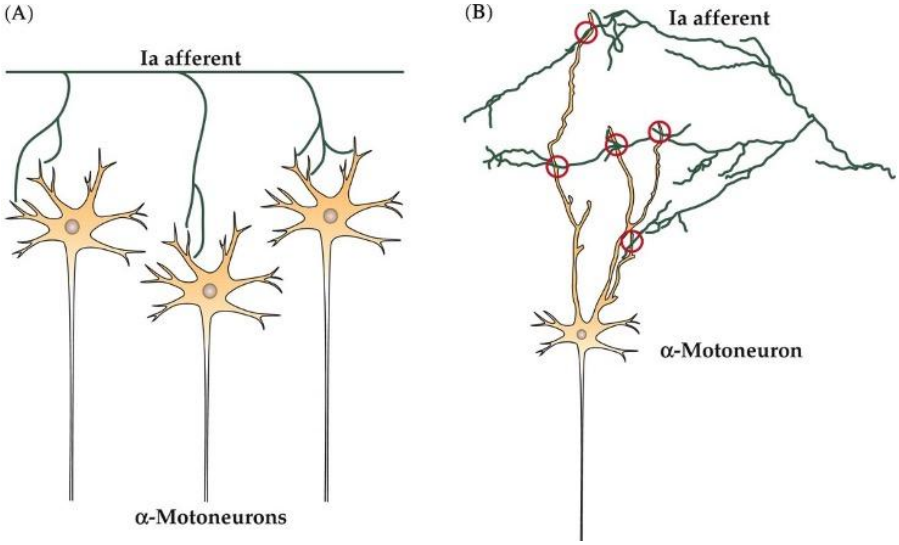


Fig. 24.3 Temporal and Spatial Summation

- (A) A single AP in a single Ia afferent produces a synaptic potential in a motor neuron that is only a fraction of a mV (blue). When the presynaptic fiber fires three AP rapidly in succession, the synaptic potentials (brown) ride on the falling phase of the previous one so they build up to a large depolarization **temporal** summation
- (B) A muscle, such as the soleus in a cat, may have as many as 50 muscle spindles, and an equivalent number of Ia afferent fibers
    - These all diverge to contact the majority of motor neurons in the motor pool. Thus, 50 Ia afferents converge onto each motor neuron. A strong stretch of the muscle can **activate all the Ia afferents**; the individual EPSPs add to depolarize the motoneuron by **spatial** summation
- The temporal summation is more effective, spatial summation is less effective → since it might be closer to reversal potential and decrease driving force

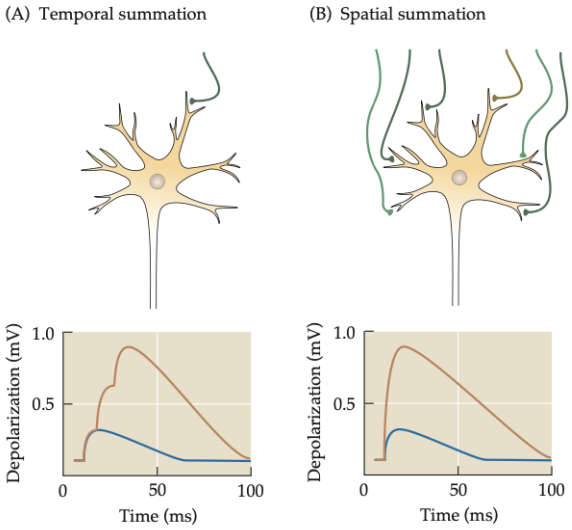


FIGURE 24.3 Temporal and Spatial Summation. (A) A single action potential in a single Ia afferent produces a synaptic potential in a motoneuron that is only a fraction of a millivolt (mV; blue trace). When the presynaptic fiber fires three action potentials rapidly in succession, the synaptic potentials (brown traces) ride on the falling phase of the previous one, so they build up to a larger depolarization—temporal summation. (B) A muscle, such as the soleus in a cat, may have as many as 50 muscle spindles, and an equivalent number of Ia afferent fibers. These all diverge to contact the majority of motoneurons in the motor pool. Thus, 50 Ia afferents converge onto each motoneuron. A strong stretch of the muscle can activate all the Ia afferents (only a few shown in the diagram): the individual excitatory postsynaptic potentials (EPSPs) add to depolarize the motoneuron by spatial summation.

Fig. 24.4 The size principle

- Current flow into a motor nerve cell produces a change in membrane potential that is proportional to the input resistance
- Input resistance is inversely proportional to the radius of the cell, so equivalent synaptic currents (Isyn) produce greater depolarization ($V_{syn} = I_{syn} \times r_{input}$) of smaller motor neurons
    - Smaller cell = higher resistance (due to reduced channel?)

- The small motor neuron on the left and the large one on the right, both receive the same input from Ia afferent fibers. The synaptic currents, which are the same in both cells (50 pA), produce a large depolarization in the smaller motoneuron
    - Small motor neuron will receive signal at weaker stimulus
- Also:
    - Larger dendritic tree = greater Cm = more current to charge = increased time constant

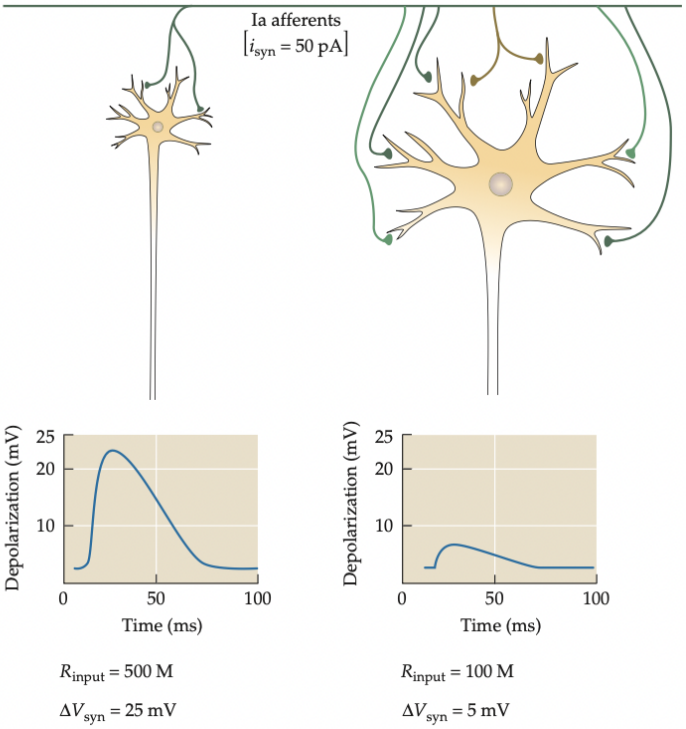


FIGURE 24.4 The Size Principle. Current flow into a motor nerve cell produces a change in membrane potential that is proportional to the input resistance (r_{input}). Input resistance is inversely proportional to the radius of the cell, so equivalent synaptic currents (i_{syn}) produce greater depolarization ($\Delta V_{syn} = i_{syn} r_{input}$) of smaller motoneurons (see Chapter 8). The small motor neuron on the left and the large one on the right, both receive the same input from Ia afferent fibers. The synaptic currents, which are the same in both cells (50 pA), produce a larger depolarization in the smaller motoneuron.

Fig. 24.5/9 Organization of Synaptic Connections for Spinal Reflexes & Flexor Reflex

- The spinal cord is shown in the transverse section, with inhibitory interneurons in blue
    - (A) In the myotatic reflex, stretch of the muscle spindle generates impulses that travel along group Ia afferent fibers to the spinal cord and produce monosynaptic excitation of alpha-motor neurons to that same muscle.
    - Impulses also excite **interneurons** → inhibit motor neurons supplying the antagonist muscles
- Figure 24.9: The flexor reflex is a limb-withdrawal reflex, produced in this example by stepping on a tack
    - Excitation of ADelta pain fibers results in elevation of the thigh (synaptic connections not shown) and flexing of the knee joint by polysynaptic excitation of flexor motor neurons and inhibition of extensors (blue interneurons are inhibitory).

Also not shown are contralateral connections that subserve extensions of the opposite leg for support

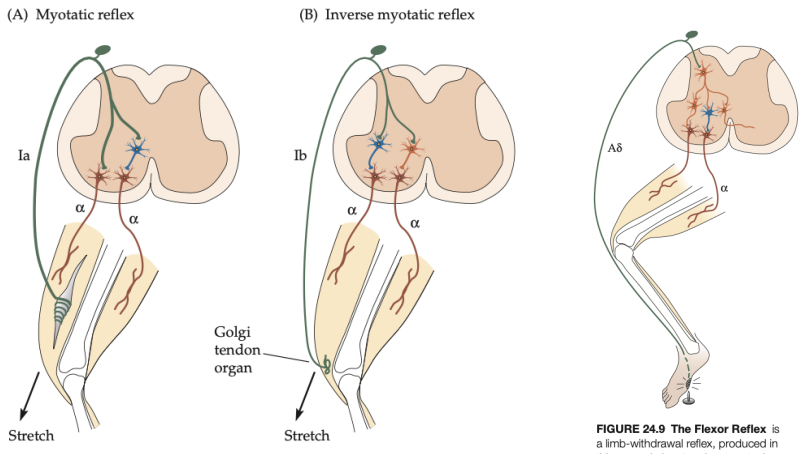


FIGURE 24.5 Organization of Synaptic Connections for Spinal Reflexes. The spinal cord is shown in transverse section, with inhibitory interneurons in blue. (A) In the myotatic reflex, stretch of the muscle spindle generates impulses that travel along group Ia afferent fibers to the spinal cord and produce monosynaptic excitation of α-motoneurons to that same muscle. Impulses also excite interneurons that, in turn, inhibit motoneurons supplying the antagonist muscles. (B) Stretch or contraction of the muscle pull on the tendon and generate impulses in the Golgi tendon organ's Ib afferent fiber. Ib fibers inhibit motoneurons that supply the same muscle.

FIGURE 24.9 The Flexor Reflex is a limb-withdrawal reflex, produced in this example by stepping on a tack. Excitation of Aδ pain fibers results in elevation of the thigh (synaptic connections not shown) and flexing of the knee joint by polysynaptic excitation of flexor motoneurons and inhibition of extensors (blue interneurons are inhibitory). Also not shown are contralateral connections that subserve extension of the opposite leg for support.

Fig. 24.6 Mammalian Muscle Spindle innervation

- The **spindle**, composed of <u>small intrafusal fibers</u>, is embedded in the bulk of the muscle
    - **Gamma motor** (or fusimotor) fibers supply the intrafusal muscle fibers
- The main muscle is made up of <u>large muscle fibers</u> supplied by <u>alpha-motor neurons</u>.
- <u>group I and group II afferent fibers</u> carry sensory signals from the muscle spindle to the spinal cord
- Note that the central region of the intrafusal muscle fiber is made up of nuclei and that the contractile material does not run from end to end. As a result, when the intrafusal fiber at each end of the spindle contracts, the sensory endings are stretched

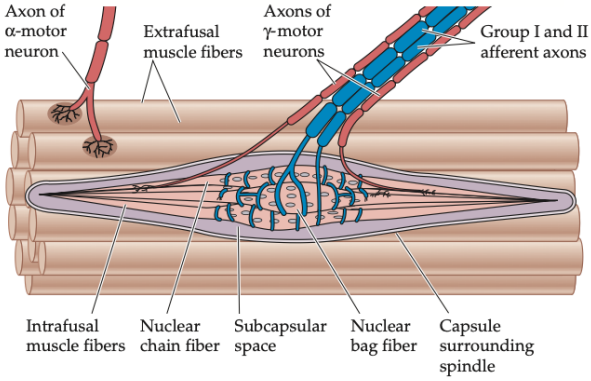


FIGURE 24.6 Mammalian Muscle Spindle. Scheme of mammalian muscle spindle innervation. The spindle, composed of small intrafusal fibers, is embedded in the bulk of the muscle. The main muscle is made up of large muscle fibers supplied by α-motoneurons. Gamma motor (or fusimotor) fibers supply the intrafusal muscle fibers, and group I and group II afferent fibers carry sensory signals from the muscle spindle to the spinal cord. Note that the central region of the intrafusal muscle fiber is made up of nuclei and that the contractile material does not run from end to end. As a result, when the intrafusal fiber at each end of the spindle contracts, the sensory endings are stretched (see also Figure 19.5).

Fig. 24.7 Efferent Regulation of Muscle Spindles

- Records of extrafusal muscle tension (red) and sensory discharge from muscle spindles (with intrafusal) (blue)
- (A) Stimulation of gamma-efferent fibers causes contraction of muscle spindles, producing discharge in the group 1 and 2 afferent fibers
- (B) Simulation of alpha-fibers supplying the main muscle causes it to contract, **reducing the stretch** on the intrafusal fibers, and the group 1 and 2 sensory fiber stops firing
- (C) When both alpha and gamma-motor fibers are stimulated tension on the muscle spindle remains unchanged and the sensory discharge is undisturbed → remain sensitive

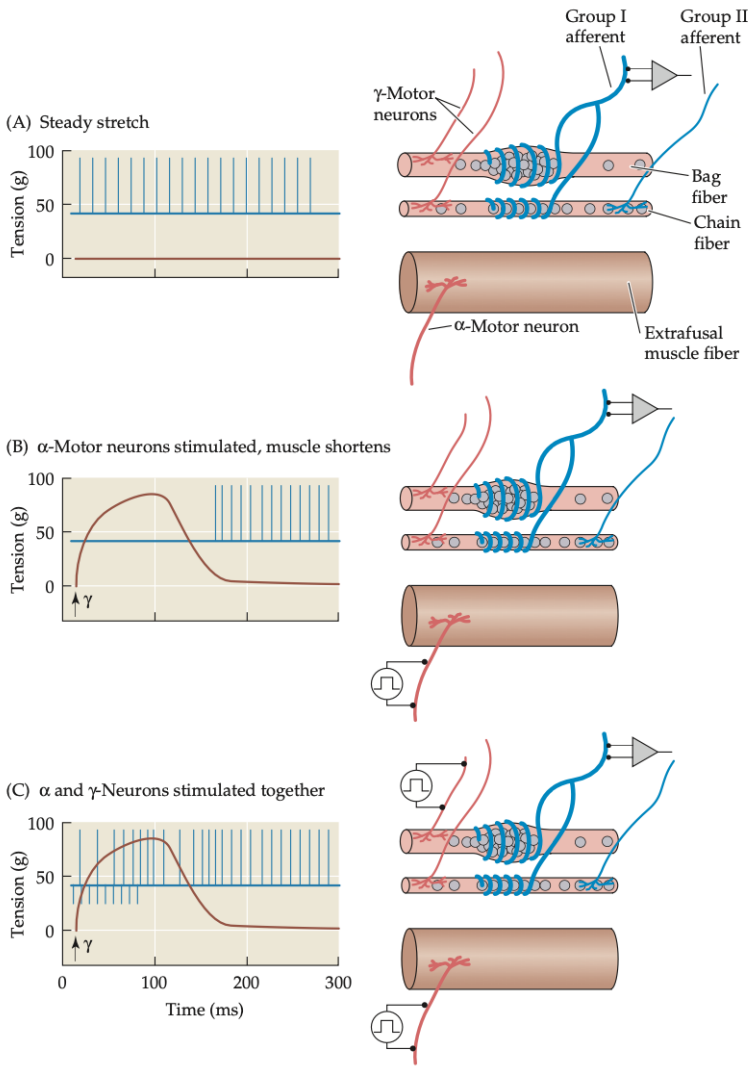


FIGURE 24.7 Efferent Regulation of Muscle Spindles. Records of extrafusal muscle tension (red) and sensory discharge from muscle spindles (blue). (A) Stimulation of γ-efferent fibers causes contraction of muscle spindles, producing discharge in the afferent fibers. (B) Stimulation of α-fibers supplying the main muscle causes it to contract, reducing the stretch on the intrafusal fibers, and the sensory fibers stop firing. (C) When both α and γ-motor fibers are stimulated tension on the muscle spindles remains unchanged and the sensory discharge is undisturbed.

Fig. 24.8 Coactivation of alpha and gamma-respiratory motor neurons

- (A) Extracellular recording of AP arising from a spindle in an inspiratory muscle during the respiratory cycle (red) → contraction of inspiratory muscle
    - sensory discharge from the inspiratory muscle spindle is highest during inspiration, while the muscles are shortening rather than being stretched
    - due to simultaneous activation of gamma-fusimotor fibers to the spindle
    - The spindle adjusts constantly to maintain a maximal response to the change in muscle tension in both expiration and inspiration
- (B) After the gamma fusimotor fibers are blocked by procaine (block Na+), the spindles behave passively → muscle become loose → no discharge during inspiration
    - The sensory discharge frequency increases during expiration, when the main mass of the muscle is stretched (not contracted)
    - sensory firing stops during inspiratory movements when the main mass of the muscle is shortened

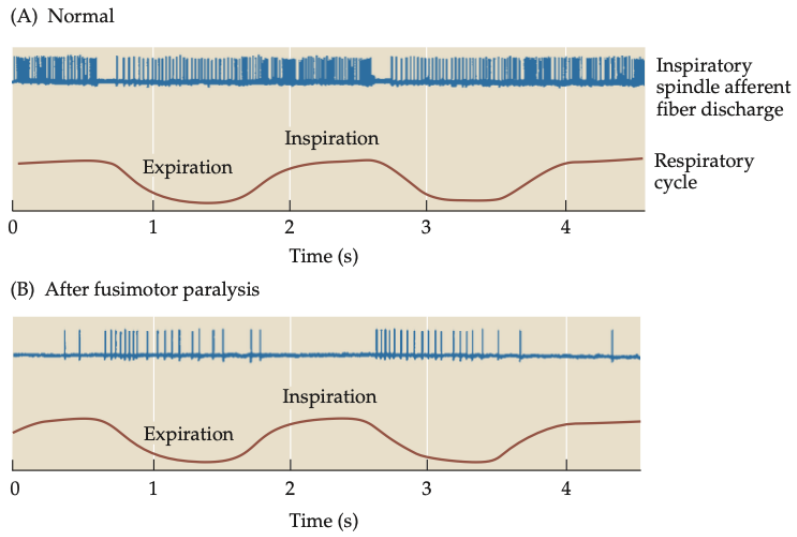


Fig. 24.10 Movements of Rib Cage and Respiratory Muscles during Inspiration and Expiration

- (A) **Inspiration**: diaphragm and **external** intercostal muscles contract (rib cage raised)
    - Volume of the chest is increased, the lung expands and air enters

    Expiration: relaxation of the diaphragm and contraction of the **internal** intercostal muscles
- (B) Activity of respiratory muscles in the cat recorded with needle electrodes. Discharges of the external and internal intercostal muscles are out of phase
    - Alternation of excitation and inhibition → cyclic output

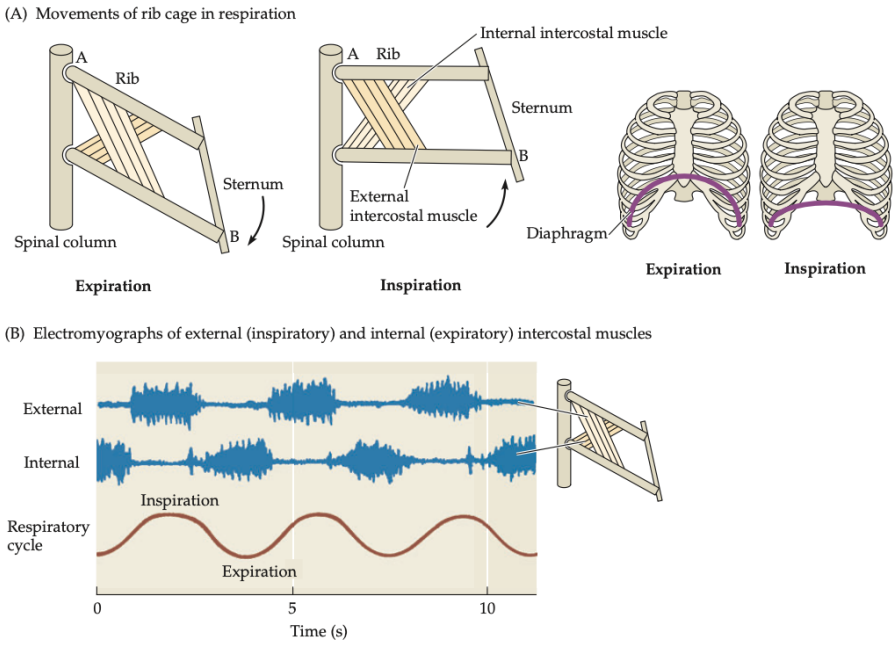


Fig. 24.12 Respiratory Rhythm Recorded from Brainstem Neurons in an isolated CNS from a neonatal opossum

- Extracellular record from a single neuron in the medulla, frequency of <u>short bursts</u> impulse is 0.5 Hz
- Simultaneous recording from a thoracic ventral root (efferent) that supplies the diaphragm shows the <u>corresponding rhythmic discharge</u> of respiratory motor neurons supplying inspiratory muscles → Within the pons and medulla, there are pools of neurons that fire during inspiration or expiration and produce excitation and inhibition of respiratory motor neurons → **central pattern generation**

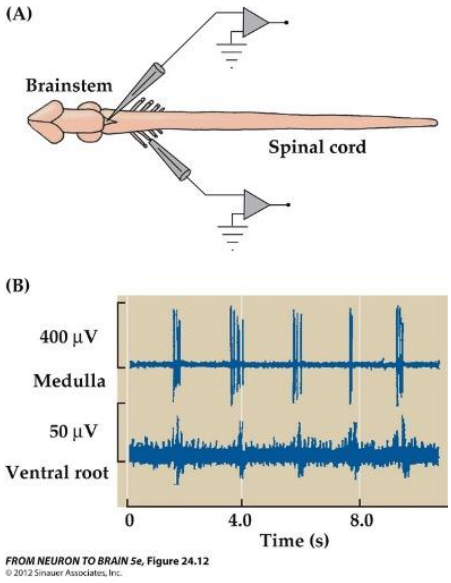


Fig. 24.16 Locomotion by a Cat on a treadmill

- A decerebrate cat can stand, but does not walk spontaneously
- Electrical stimulation of the mesencephalic locomotor region (MLR in the diagram) causes the animal to walk on the treadmill → start walking
- Electromyography associated with locomotor activity is recorded by electrodes in limb muscles
- The speed of walking or galloping depends on the rate of the treadmill → use muscle feedback and no involvement from the motor cortex
- As expected, cutting (sensory) dorsal (afferent) roots abolishes the response to different treadmill speeds but not the walking evoked by electrical stimulation
    - Afferent feedback from gamma afferent fibers (muscle spindles) modulates human gait at the spinal cord → central pattern generation
- Increasing the strength or the rate of stimulation increases the strength of limb movements but not their speed → the speed is purely based on the feedback

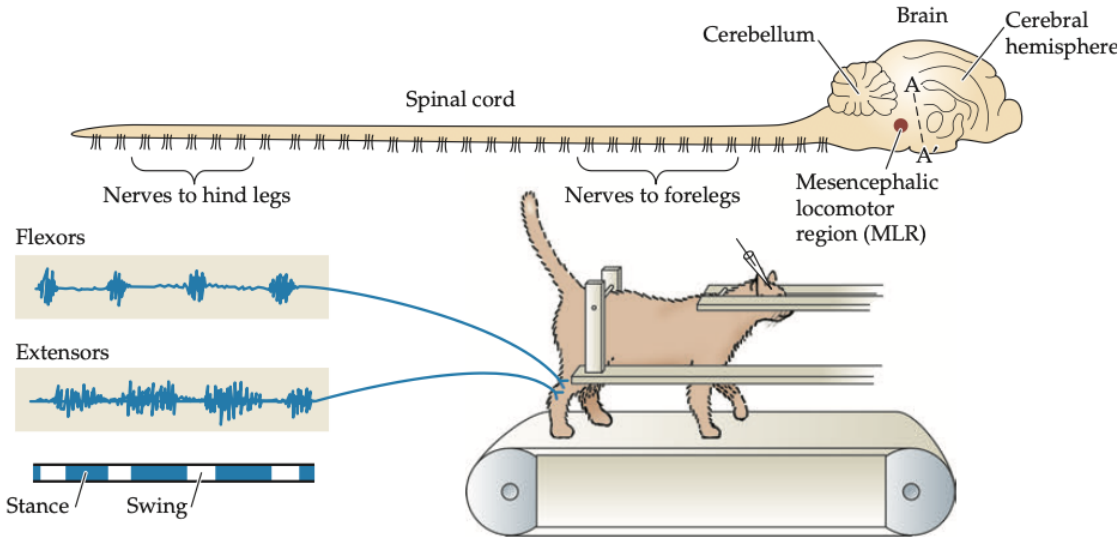


FIGURE 24.16 Locomotion by a Cat on a Treadmill after section of the brainstem (A–A′ in the diagram). Such an animal does not walk spontaneously. Electrical stimulation of the mesencephalic locomotor region (MLR in the diagram) causes the animal to walk on the treadmill. Electromyographs associated with locomotor activity are recorded by electrodes in limb muscles. The speed of walking or galloping depends on the rate of the treadmill. Increasing the strength or the rate of stimulation increases the strength of limb movements (as though the animal were walking uphill) but not their speed. (After Pearson, 1976.)

Fig. 25.6 The Vertebrate Hindbrain Develops as a Conspicuously Segmented Structure

- (A) Diagram of a 3-day chick embryo (lateral view), illustrating the segmental arrangement of **rhombomeres**(r1-r8) in the hindbrain
- (B) Pattern of cell organization in rhombomeres r1 to r7 of the 3-day chick embryonic hindbrain (dorsal view). Reticular neurons (green and black) and branchiomotor neurons (orange) occur in a segmentally repeating pattern. Motor neurons send their axons in cranial nerves V, VII, and IX
- (C) Segmental expression of genes in rhombomeres r1 to r8 of the vertebrate hindbrain (Gray bars indicate in which rhombomeres and how strongly individual genes are expressed → **different differentiation at different locations**
    - Black bars indicate a high level of expression. Early transcription factors, Eph family receptor tyrosine kinase, and Eph ligands establish the segmental pattern of rhombomeres. The Hox homeobox genes determine the fate of cells within each rhombomere in a segmentally specific way

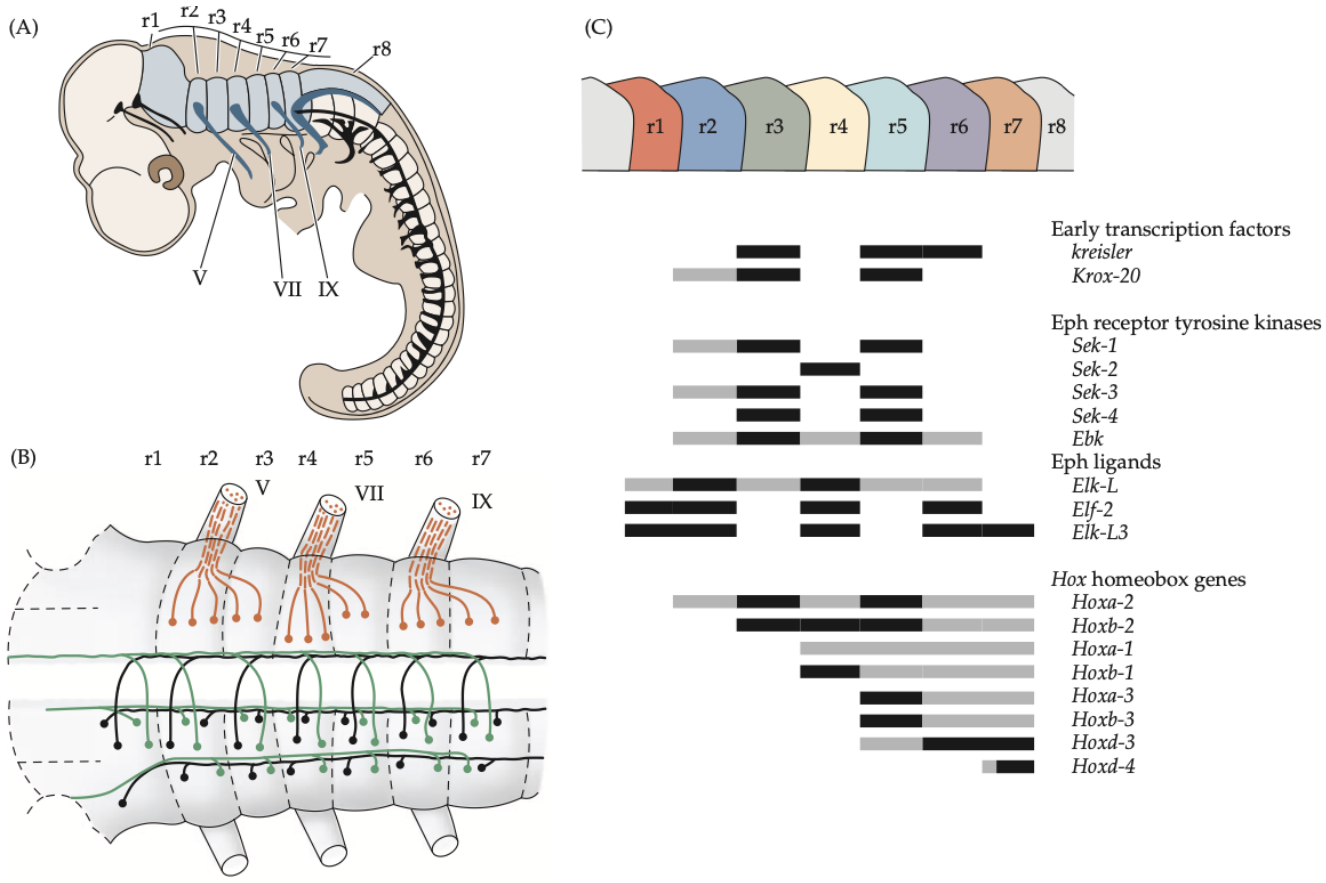


Fig. 25.13 Failure of Neurons to Divide in Adult Human Cerebral Cortex

- 14C radioactivity was measured in cell nuclei of neurons and non-neuronal cells in the human cortex in relation to the time of birth.
- (A) Neuronal (NeuN+, red) and nonneuronal (NeuN-, green) cell nuclei from the adult human cerebral neocortex were identified, separated and isolated by flow cytometry
- (B) In 1955-1963 the levels of 14C in the atmosphere increased as a result of nuclear weapons testing and subsequent decline (blue line). One can infer the time of birth of a cell population by first lelating the level of 14C in their DNA to that in the atmosphere (A to B) and then reading their age of the x-axis (vertical arrows). The age of the individual is given by the dashed line.
- The average age of all cells in the prefrontal cortex is less than the individual (black circle and arrow), indicating that they are younger, whereas neurons (red circle and arrow) are ~ as old as the individual. These results indicate that glial and endothelial cells continue to divide while cortical neurons do not.
- **Most neurons post birth stop dividing** (notable exception hippocampus dentate gyrus and olfactory bulb), decrease in 14C = younger cells = dividing cells

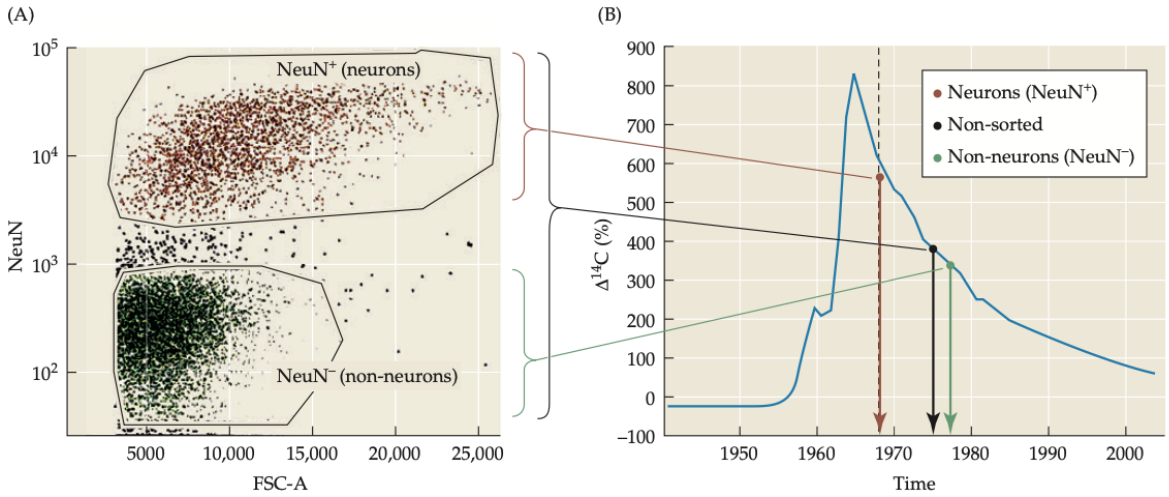


FIGURE 25.13 Failure of Neurons to Divide in Adult Human Cerebral Cortex. ^{14}C radioactivity was measured in cell nuclei of neurons and non-neuronal cells in human cortex in relation to the time of birth. (A) Neuronal (NeuN-positive, red) and nonneuronal (NeuN-negative, green) cell nuclei from the adult human cerebral necortex were identified, separated, and isolated by flow cytometry. (B) In 1955–1963 the levels of ^{14}C in the atmosphere increased dramatically as a result of nuclear weapons tests and subsequently declined (blue line). One can infer the time of birth of cell populations by first relating the level of ^{14}C in their DNA to that in the atmosphere (guidelines from A to B), and then reading their age off the *x* axis (vertical arrows). The age of the individual is given by the dashed line. The average age of all cells in the prefrontal cortex is less than the individual (black circle and arrow), indicating cell turnover. Dating of nonneuronal cells (green circle and arrow) indicates that they are younger, whereas neurons (red circle and arrow) are approximately as old as the individual. These results and others (see text) indicate that glial and endothelial cells continue to divide while cortical neurons did not. FSC-A = forward scatter channel-A. (After Bhardwaj et al., 2006.)

Fig. 25.17 GABA as a depolarizing transmitter during development

- Two days after birth, CA3 neurons in the rat hippocampus are depolarized by GABA and by inhibitory synaptic inputs
    - At early stages, the opening of chloride channels by GABA allows the negatively charged ion to exit
- By 12 days, the effect of GABA and inhibitory inputs have **reversed** and
    - In the adult, give rise to hyperpolarization (inward movement of Cl–) at the normal resting potential.
- The change to hyperpolarization is caused by a decrease in intracellular chloride concentration.
    - At 2 days, the concentration is high owing to the activity of an **inward chloride transporter (NKCC1)**, which virtually disappears by day 12

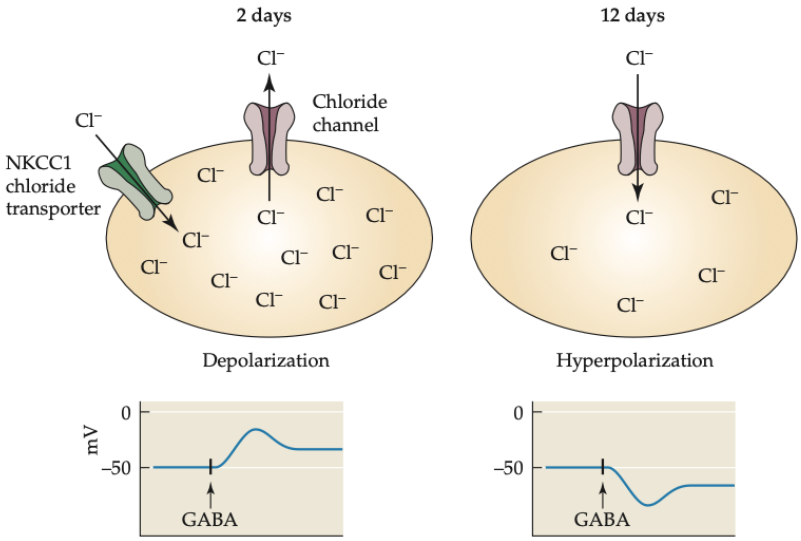


Fig. 25.20 Growth Cones of peripheral neurons rely on guidepost cells

- (B) In normal embryos, the axon of the Ti1 neuron encounters a series of **guide-post cells** on its route to the central nervous system: F1, F2, and two CT1 cells
    - Secreting gradient of growth cone cue
- (C) If the CT1 cells are killed early in development, the Ti1 neuron forms several axonal branches at the site of cell F2, with growth cones extending in abnormal directions

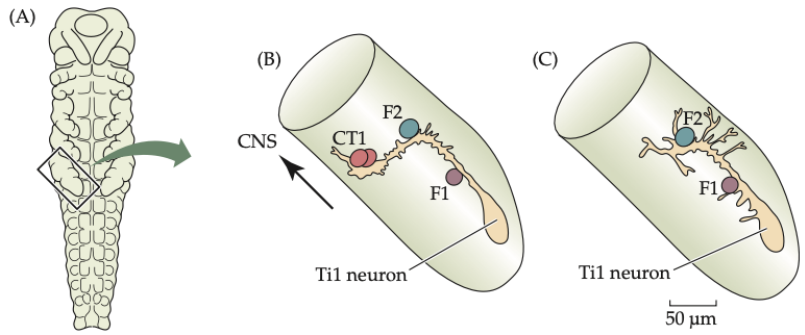


Fig. 25.21 Long- and Short-Range Chemoattraction and Chemorepulsion guide developing axons in the vertebrate spinal cord

- (A) **Netrin-1**, acting as a long-range chemoattractant, secreted by cells of the floor plate (blue), diffuses (black arrow), and binds to its receptor, **Deleted in Colorectal Carcinoma (DCC)** receptor on the growth cones of **commissural interneurons** (orange), attracting them (green arrow)
- (B) Transient axonal **glycoprotein-1 (TAG-1)** on commissural axon growth cones bind to **neuronal cell adhesion molecule (N-CAM)** on floor plate cells. This short-range chemoattraction (green arrow) facilitates extension of commissural axon growth cones across the floor plate
- (C) As they cross the midline, TAG-1 on commissural growth cones is replaced by **Roundabout (Robo)**, which binds to **slit** on floor plate cells.
    - This short-range chemorepulsion (red arrow) prevents the axons from re-crossing the floor plate —> repel
- (D) **Slit** and **netrin-1** diffuse from the floor plate (black arrow), interact with receptors on growth cones of **motor neurons** (blue), and repel them (red arrows). This long-range chemorepulsion helps direct the growth of motor axons away from the cord

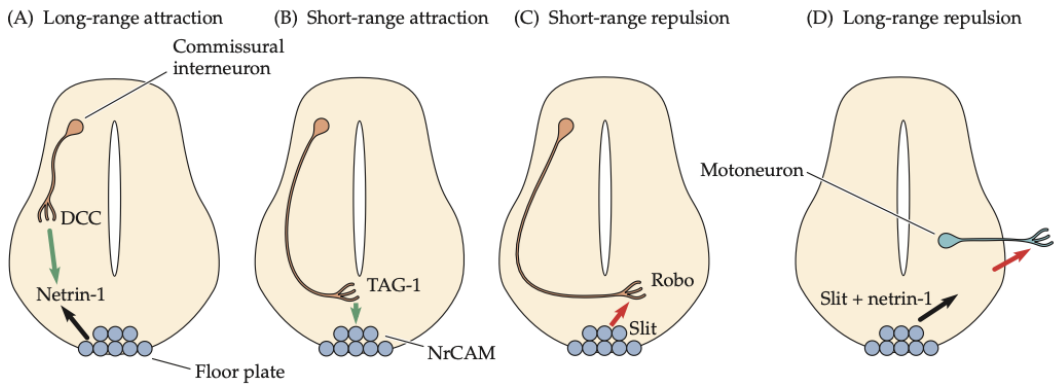


Fig. 25.22 Netrins and Netrin receptors

- Micrographs of pieces of dorsal (afferent) spinal cord from embryonic rats (top in each panel) cultured with a piece of floor plate tissue (left) —> floor plate attracts
- An aggregate of COS cells secreting recombinant netrin-1 (middle), or control COS cells (right) —> only netrin attracts
- The floor plate and netrin-1 both elicit the profuse and directed outgrowth of bundles of commissural axons from the dorsal spinal cord tissue

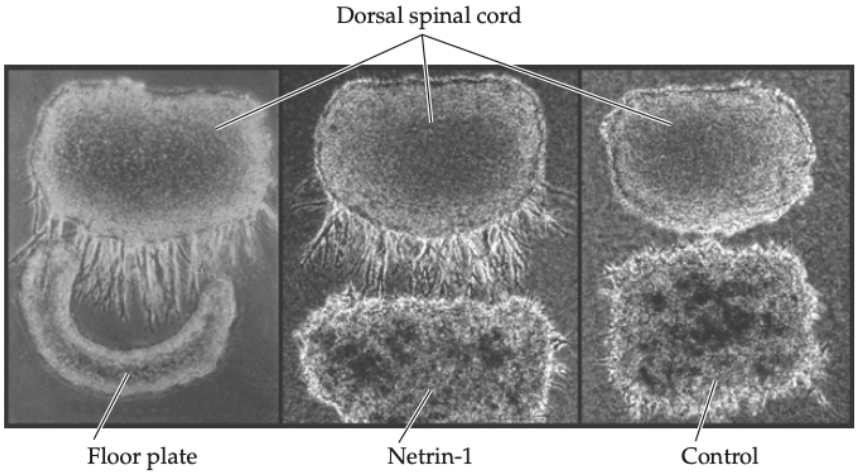


Fig. 25.23 Nerve Growth Factor (NGF) and Survival of Axon Branches

- (A) Neurons dissociated from neonatal sympathetic ganglia plated in the central compartment send neurites under a Teflon divider and into the adjacent compartments; all compartments contain NGF.
- (B) After initial outgrowth has occurred, removal of NGF from <u>compartment 1 for 20 days has no effect</u>, as neurons are maintained by NGF transported retrogradely from their terminals in the side compartments. Removal of NGF <u>from compartment 2 causes the neuritis entering it to degenerate</u>, while those in the compartment containing NGF remain → **receptor for NGF is at the terminal**

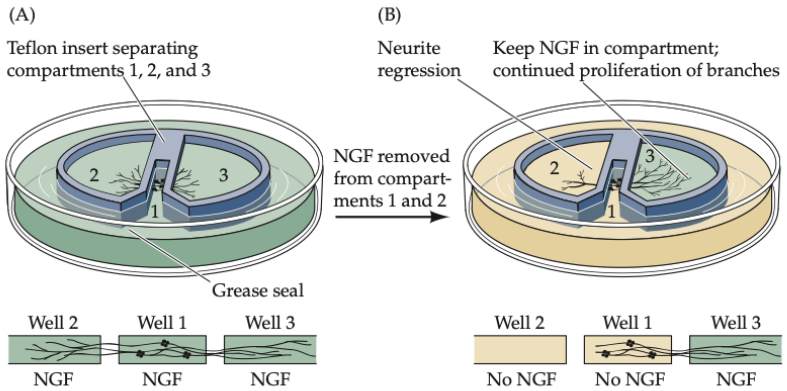
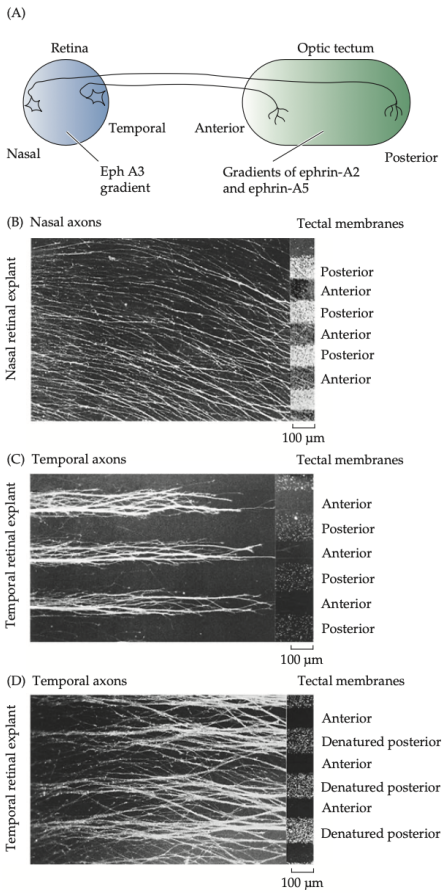


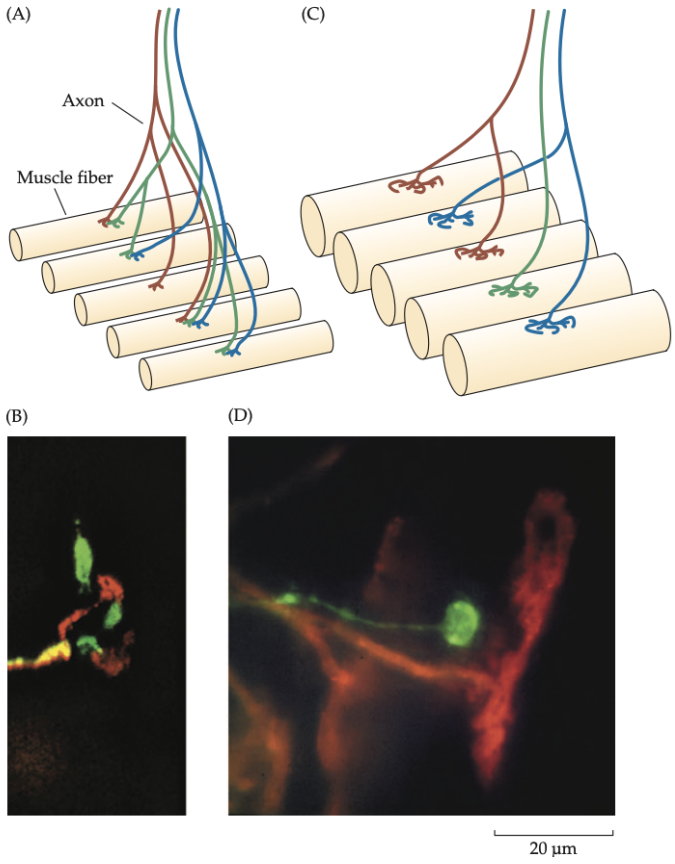
Fig. 25.25 The Role of Repulsive Interactions in Innervation of the optic tectum in the chick



- (A) Ganglion cells in the nasal retina innervate neurons in the posterior tectum中脑顶盖; ganglion cells in the temporal retina innervate neurons in the anterior tectum
    - **Nasotemporal gradient** of the **EphA3** receptor tyrosine kinase in retinal ganglion cells
    - **Anteroposterior gradients** of the **Eph** receptor ligands ephrin-A2 and ephrin-A5 in the tectum
- Axons of temporal ganglion cells are <u>prevented</u> from entering the posterior tectum by the <u>repulsive interaction of Eph receptors and ligands</u> → can only grow to anterior
- (B) In cell culture, axons from neurons in the <u>nasal</u> retina grow equally well on lanes coated with membranes isolated from anterior or posterior tectum
- (C) Axons from neurons in the <u>temporal</u> retina prefer to grow on <u>anterior</u> membranes → due to repulsive from posterior that contains Eph
- (D) Axons from the temporal retina grow <u>equally well</u> on <u>intact anterior membranes</u> and <u>denatured posterior membranes</u>, indicating that normally they are **repelled by heat-sensitive components** of the posterior membranes
    - Denature receptor → loss of replesion

Fig. 25.27 Polyneuronal Innervation and its Elimination

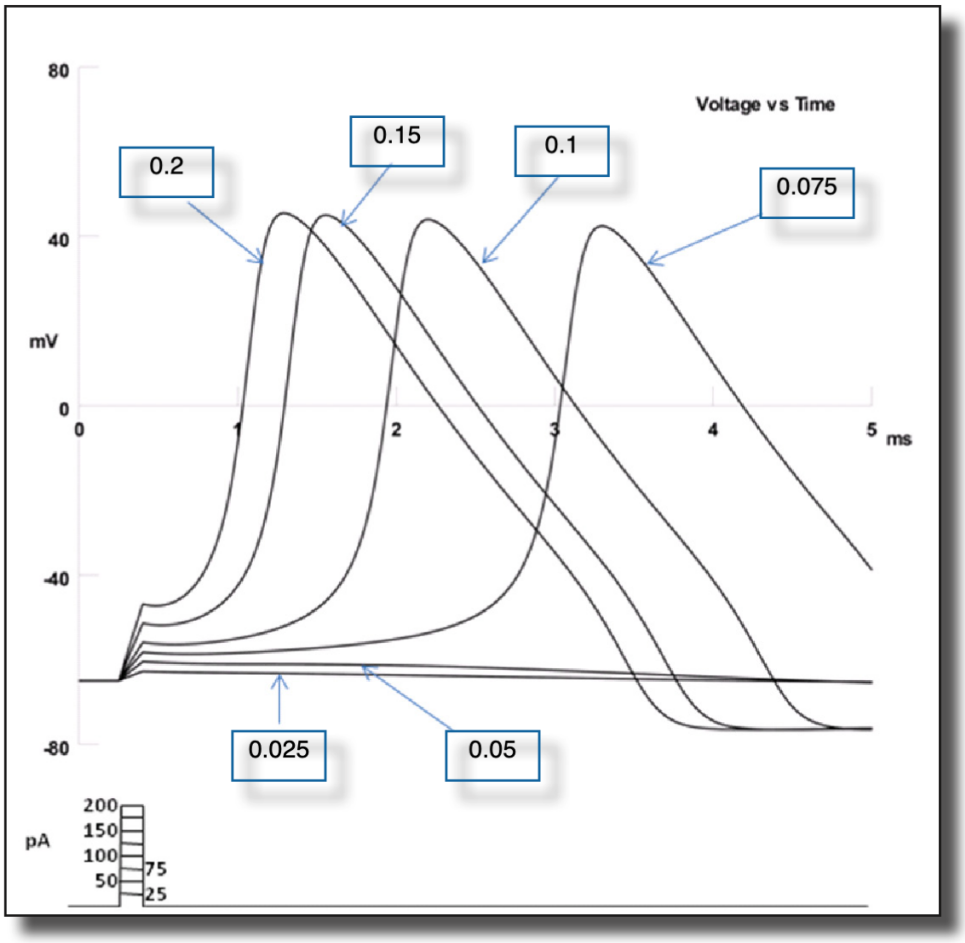
- (A) During embryonic development, motor axons branch to innervate many muscle fibers, and each muscle fiber is innervated by several motor axons
    - **polyneuronal innervation**
- (B) Fluorescence micrograph of a neuromuscular junction of an E18 mouse showing the distribution of terminals of two axons, each labeled with a lipophilic probe (dil in red, diA in green). During the period of polyneuronal innervation, the terminal arbors of all motor axons innervating a particular muscle fiber interdigitate at a single synaptic site.
- (C) After birth, weak inputs from polyneuronal innervation is eliminated as axon branches retract, leaving each muscle fiber innervated by a single motor axon.
- (D) Fluorescence micrograph of a mouse neuromuscular junction during the period of removal of polyneuronal innervation. Two axons innervating the junction were labeled as in B. All terminals of one axon (green) have been eliminated, and the axon is being withdrawn



Tutorial: Potentials

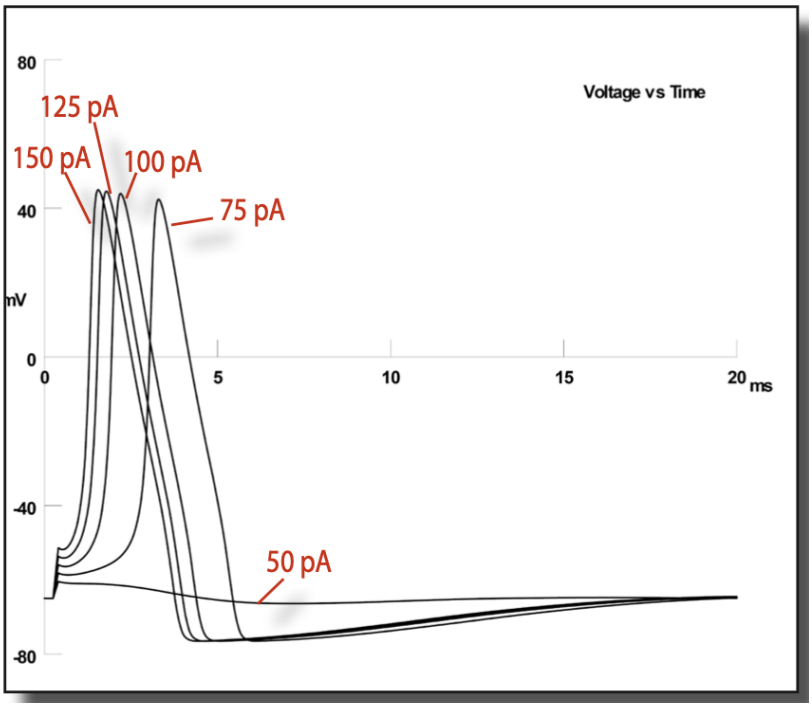
1.1 Current stimulus amplitude

- Ohm's law: V=I*R
    - Larger voltage = large current injected
- Conductance (g) = 1/R
    - No conductance = no ion flow
- There is a super-threshold for action potential generation
- The increasing amplitude of the current stimulus would cause
    - Upstroke to appear quicker
    - Peak AP to be higher (slight)
- The increasing amplitude of the current stimulus would not cause
    - Rate of AP upstroke to be quicker
    - Rate of repolarization to be quicker
    - Resting membrane potential to change



1.2 Observe the duration of the undershoot and the return of the voltage to rest

- An afterhyperpolarization or undershoot follows repolarization.
    - Neither the peak voltage nor time of recovery of the afterhyperpolarization varies with action potentials generated with different current stimulus intensities (75 pA to 150 pA).
- An **invariant refractory period** ensures a refractory period between subsequent action potentials.
- The afterhyperpolarization resets the Na channels which become inactivated or refractory during the extensive depolarization during an action potential
    - K+ current is responsible for both the repolarization and afterhyperpolarization

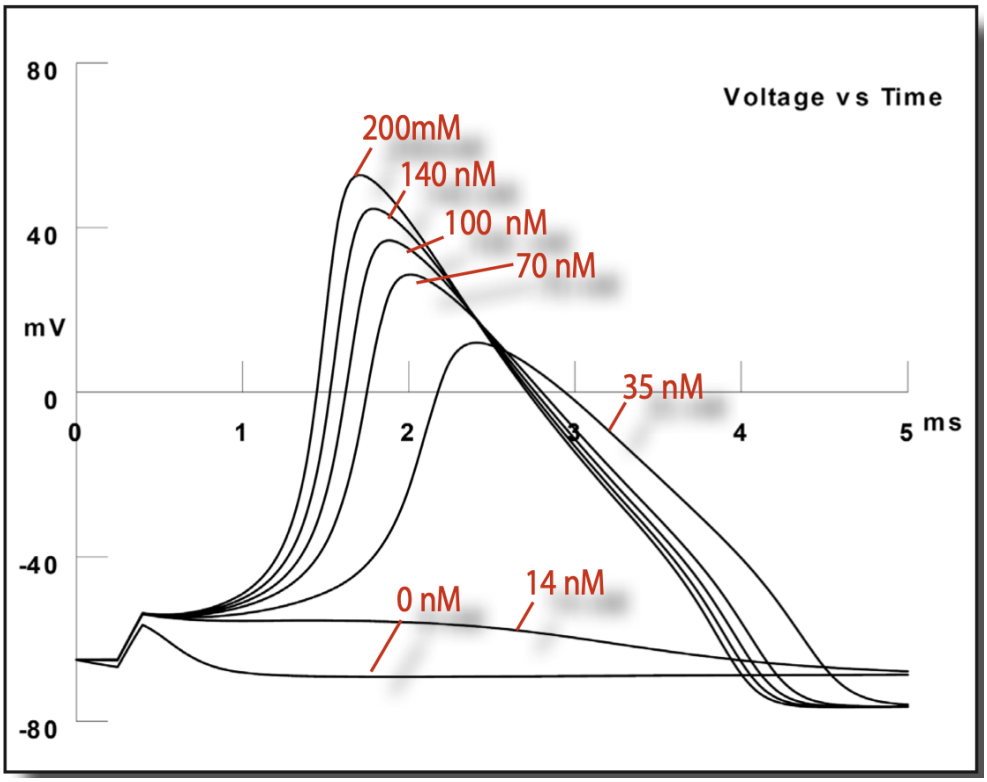


1.3 Effects of changing the extracellular sodium [Na]o on the action potential:

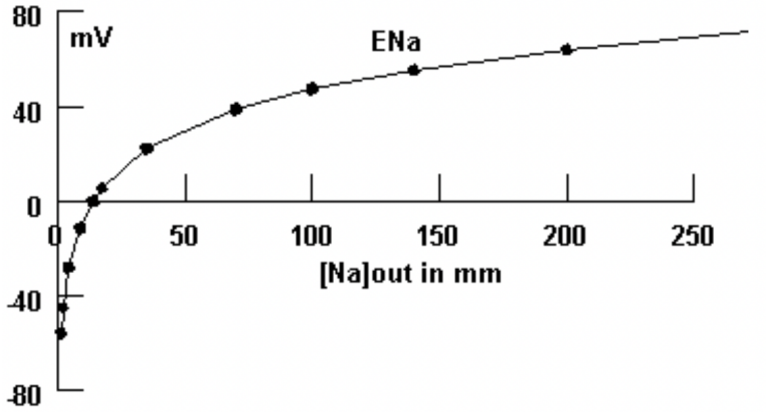
- As [Na]o is lowered, you observe that the <u>action potential peak decreases</u>. This reflects the <u>decrease in the equilibrium potential</u> for sodium channels
    - A strong current stimulus will depolarize the membrane above the threshold for opening sodium channels
    - <u>Na+ flows IN</u> by a strong electro-chemical gradient for sodium ions
        - The 10 fold membrane gradient from outside to inside [Na]o / [Na]I and an initial negative membrane potential (eg. -65 mV)
    - As Na+ flows, more positive inside → repulse more positive Na+ in
    - Eventually, a membrane potential is reached where current flow through the channel is zero → Sodium Equilibrium Potential (ENa)

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i}$$

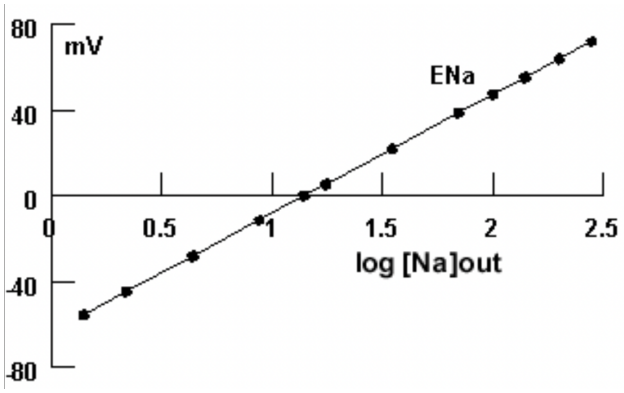
, RT/F = 58



- the ENa vs. [Na]o curve <u>rises exponentially</u> and that ENa equals zero when: [Na]o equals 14 mM, the concentration of [Na]i
    - At values of [Na]o less than 14 mM → Na+ OUT
    - More than 14mM → Na+ IN



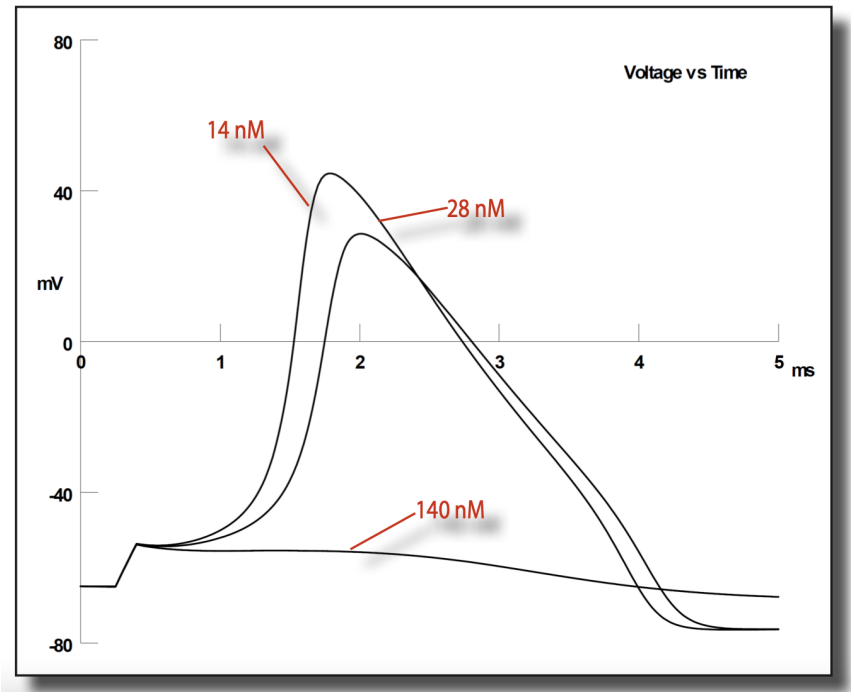
- ENa vs log [Na]o has the slope of 58 mV (RT/zF)



- action potential has a huge "**safety factor**"; that is, even if the [Na]o changes quite a bit, the action potential will still occur.

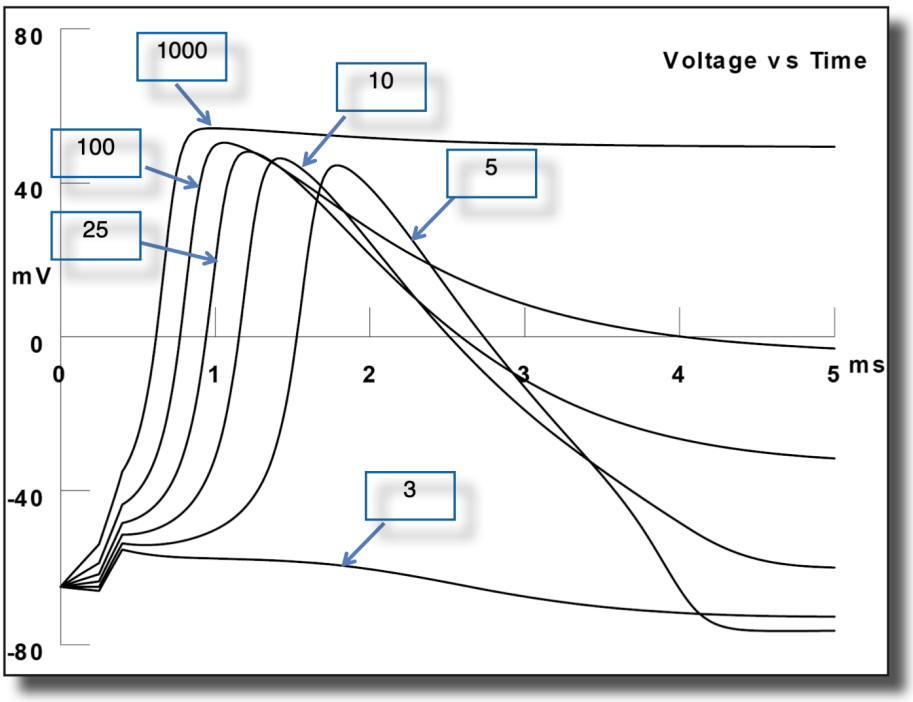
1.4 Effects of changing the intracellular sodium [Na]i on the action potential:

- raising the [Na]i retards the membrane excitability of neurons
    - It reduces the electro-chemical gradient



1.5 Effects of changing the extracellular potassium [K]o on the action potential:

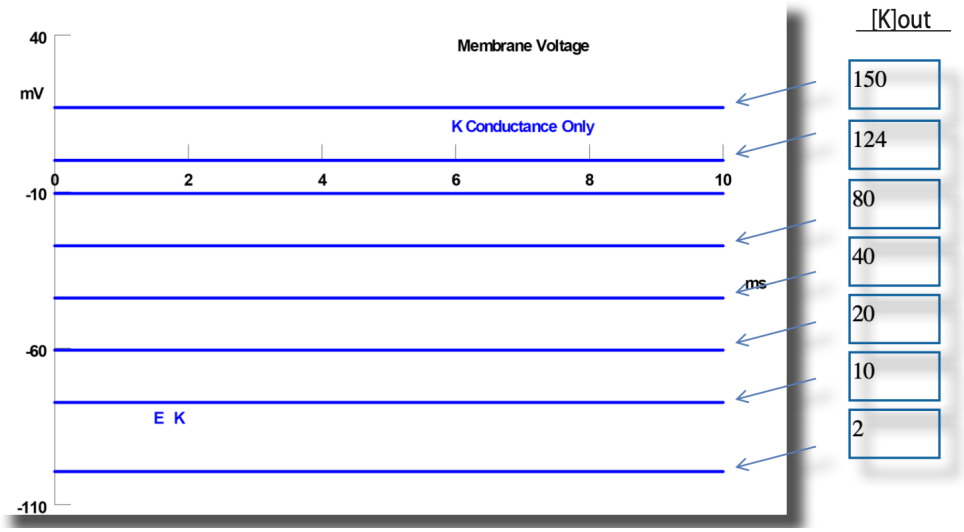
- Increasing [K]o from 5 mM to 1000 mM prevents repolarization of the action potential.
    - Blocking potassium channels with drugs like TEA (tetraethylammonium) and 4-AP (4-Aminopyridine) have the same effect
    - Raising [K]o = decreasing [K]o electrochemical gradient → fewer K+ out → prevention of repolarization
- Potassium ions are the most permeable ion at rest and thus are the most influential ions in establishing the resting membrane potential.
    - The equilibrium potential for potassium ions is close to the resting membrane potential
    - Raising [K]o makes the cell **hyperexcitable**, by elevating the resting membrane potential and depolarizing the cell membrane
        - Depolarization by Na+ happens earlier & repolarizes slower
- IV of KCl will raise [K]o → cause neurons to be hyperexcite



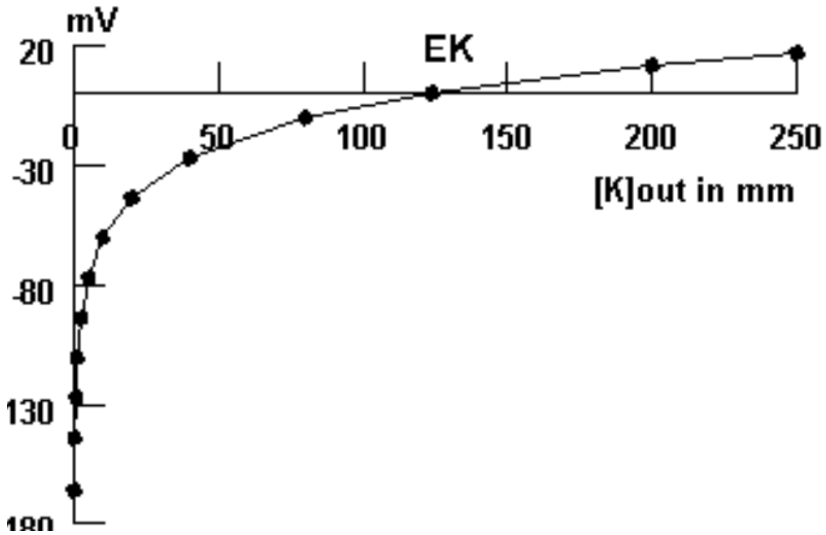
2.1 Experiment with a glial cell, which is solely permeable to K ions.

- Higher [K]out = higher membrane potential, which here is equal to EK (since only permeable to K)

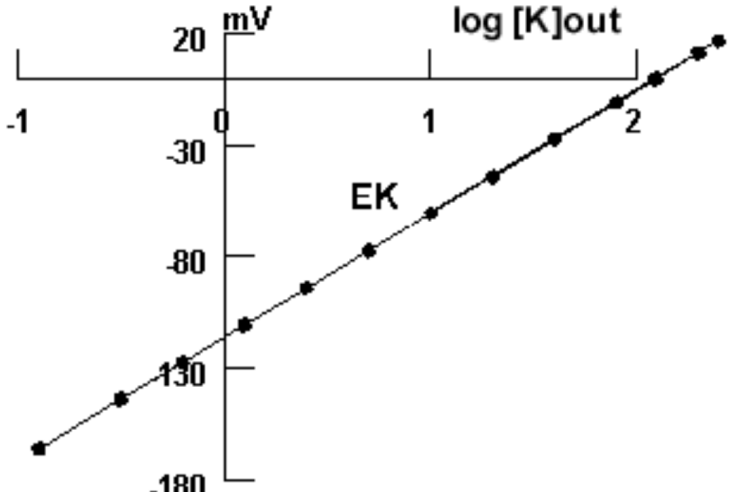
$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}$$



- **EK vs [K]o**
    - [K]o varied from its usual (mammalian) value of 5 mM.
    - The concentration of [K]i was 124 mM. Notice that the curve rises <u>exponentially</u> and that EK equals zero when [K]o equals 124 mM, the concentration of [K]i
        - At values of [K]o <u>less than 124 mM</u>, [K]i will be greater than the [K]o, the driving force on K will be outward

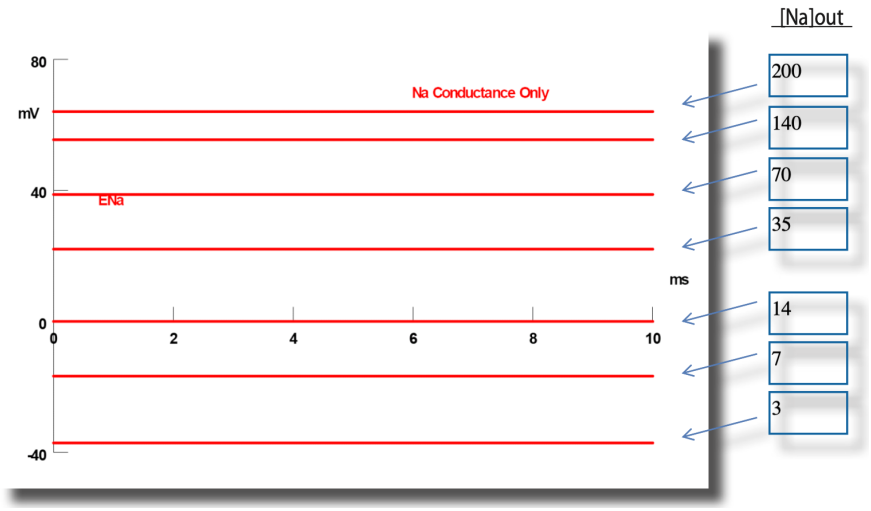


- **EK vs log ([K]o)**
    - EK is indeed proportional to the log of [K]o and has a slope of 58 mV (RT/zF).



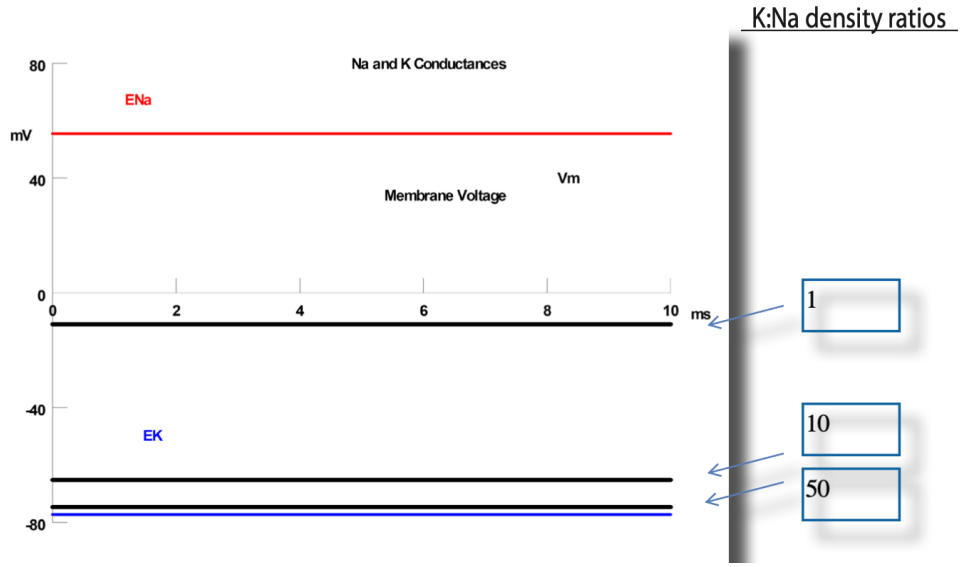
2.2 What would happen to Vm if the membrane were to become permeable only to Na ions?

- Higher [Na] out = higher ENa = higher membrane potential (since only permeable to Na)



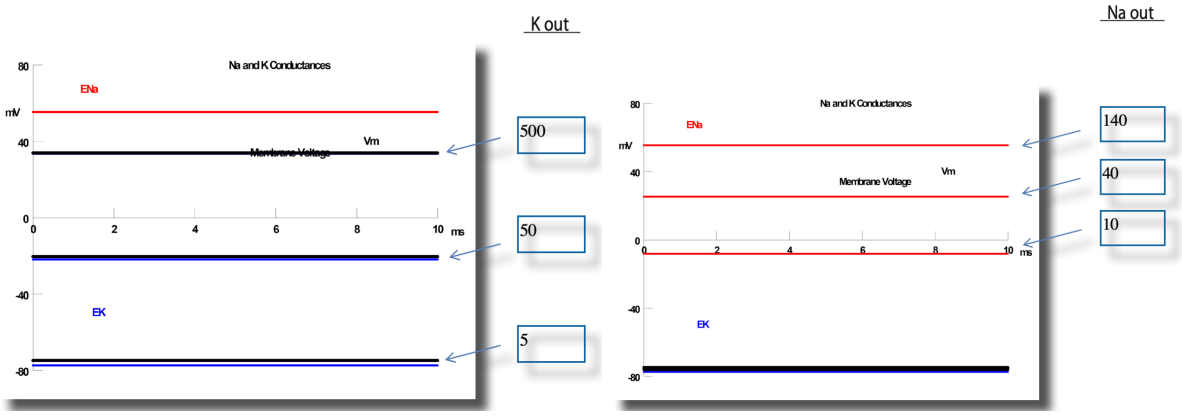
2.3 What determines the "resting potential" and how does it depend on ion concentrations?

- A typical neuron is permeable to both K and Na ions, although far less so to Na than to K
- The ratio of gK/gNa is typically 50:1 in neurons. Notice that at this ratio, V_m rests near E_K but is slightly depolarized to E_K's value.



2.4 Examination of the resting potential sensitivity to changes in K and Na ions in standard K: Na channel densities of 50:1 in neurons

- higher [K]o = higher membrane voltage
- higher [Na]o = higher membrane voltage
- Notice how Vm (= Vrest) tracks EK.
    - From this experiment you can see how a change in serum [K] would affect the Vrest of cells.
    - Because of the logarithmic relation in the Nernst equation, a change of 1 mM in the low values of [K]o has a much greater effect on Vm than a change of 1 mM at higher values.
- the resting potential so insensitive to the Na concentration when K is 50 times more permanent than Na
    - Na conductance is low + log nature of Ernest Equation

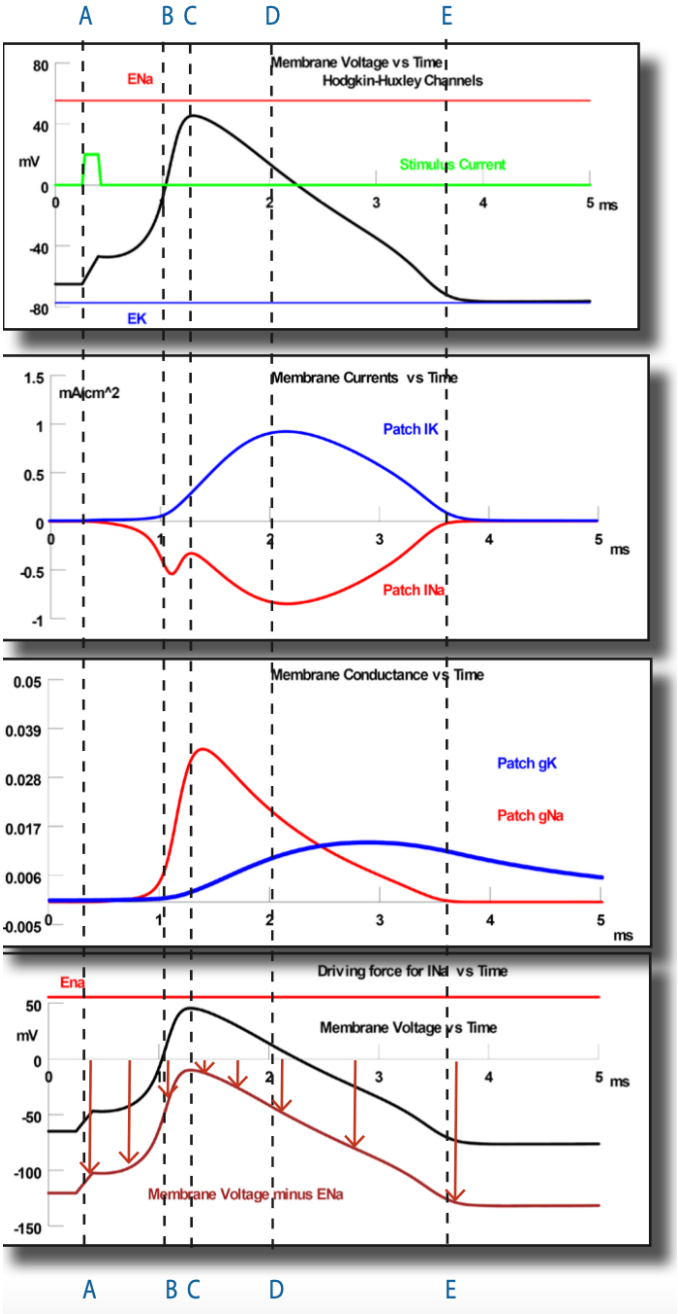


Generally

- EK = -77mV
- Erest = -65 mV
- ENa = +58 mV
- ECa = +135 mV

3.1 Action potentials and the shape of INa

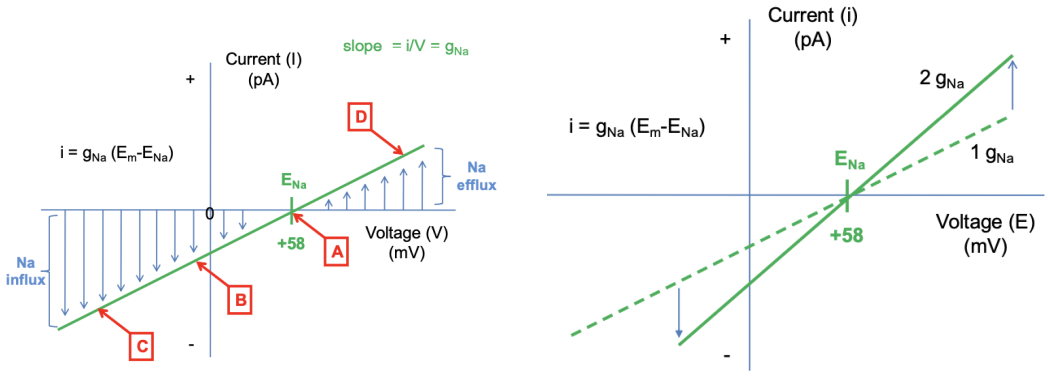
- $I_{ion} = g_{ion} * (V_m - E_{ion}) = g_{ion} *$ Driving force
- A:
  - high driving force for Na,
  - no conductance for Na
  - no Na current
- B: upstroke (dV/dt) at max
  - gNa increases
  - Driving force dropped to 44mV
  - gNa increases faster than decreasing driving force → iNa is increasing
- C:
  - gNa is max and stable
  - Driving force is lowest (14mV)
  - iNa is decreasing (df drops faster than gNa changes)
- D:
  - gNa drops
  - Driving force increases (30mV)
  - Driving force increases > loss of gNa → iNa is at max
- E:
  - gNa = 0
  - Driving force at max (>100mV)
  - No iNa



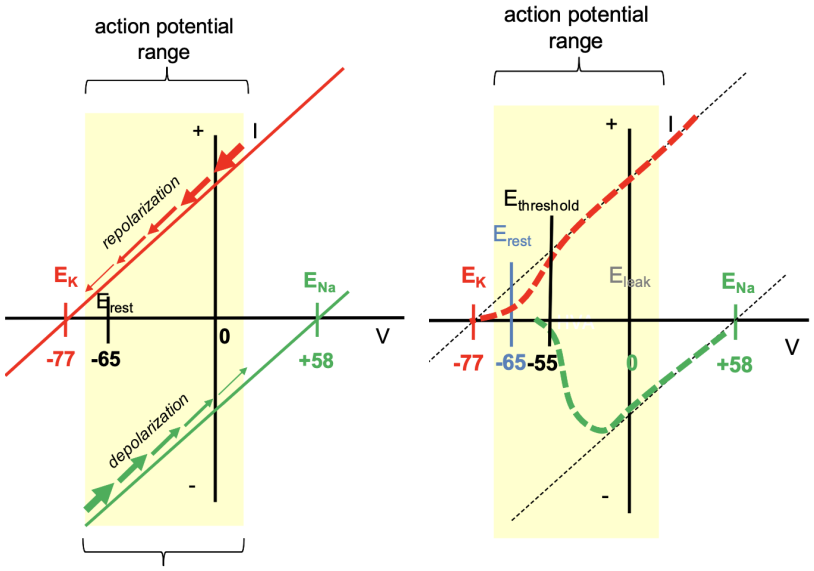
Note: ion membrane gradient is largely the same → consider them as constant

3.5 Electronic model of the plasma membrane

- As you move away from A, there is greater and greater Na efflux/influx
- Direction reverse at A
- Adding addition sodium channels in the membrane provides less resistance to current flow and additional routes for sodium influx
    - More ions passing through = more current at the same voltage

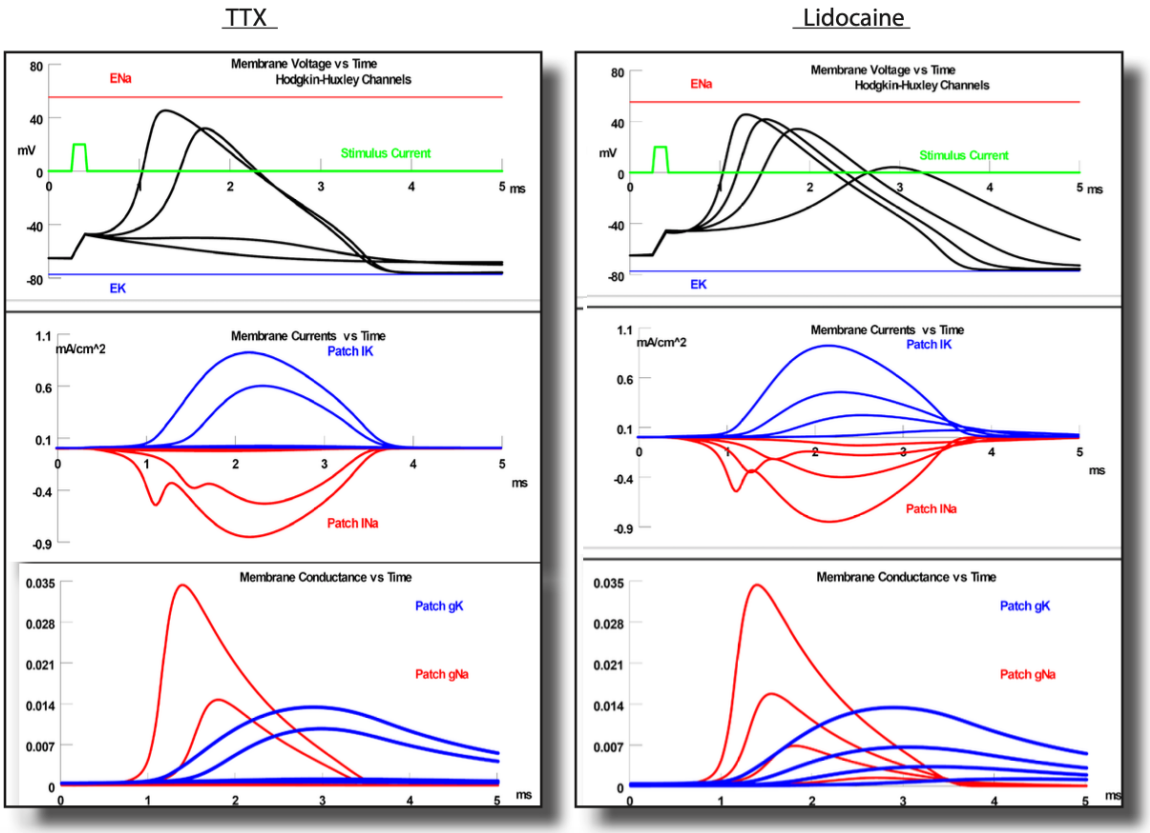


- During AP, ENa is always to the right of Em and EK is always to the left of Em
    - Na always flow IN (influx) to depolarize and K always flow OUT (efflux) to repolarize
- Ion channel gating prevent the major action potential generating Na and K channels to be conductive at rest
    - Below threshold, no current for both
    - At threshold, channel opens and quickly catches the supposed ion flow



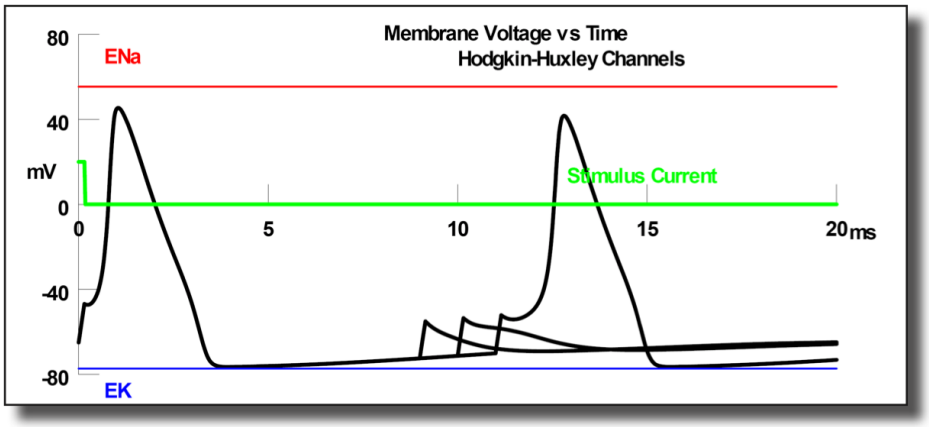
3.2 Bathe the axon in TTX or local anaesthetics

- **TTX** or **saxitoxin (STX)** is a highly specific blocker of Na channels and potent neurotoxin
- Local anesthetics like **procaine** and **lidocaine** reduce both the Na and K conductances by almost equal amounts
- Anaesthetics and TTX both slowing the rate of rise and peak of the action potential
    - TTX is more effective at blocking the action potential than an anaesthetic since it does not block K → allow for repolarization



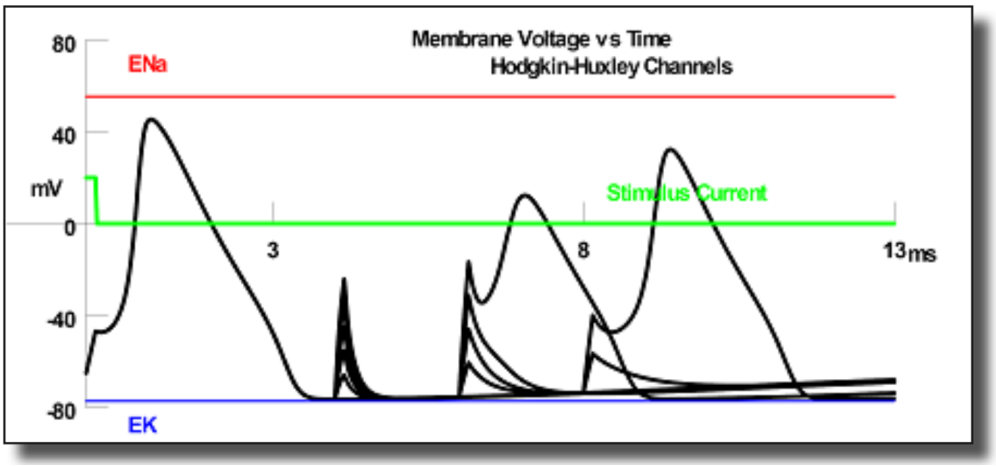
3.3 Refractory period of the action potential

- a period of reduced excitability following an action potential -> limits the frequency of impulses in a train of impulses
- During this period, most Na channels are completely inactivated → have to wait for a certain period of time before become available again → absolute refractory period



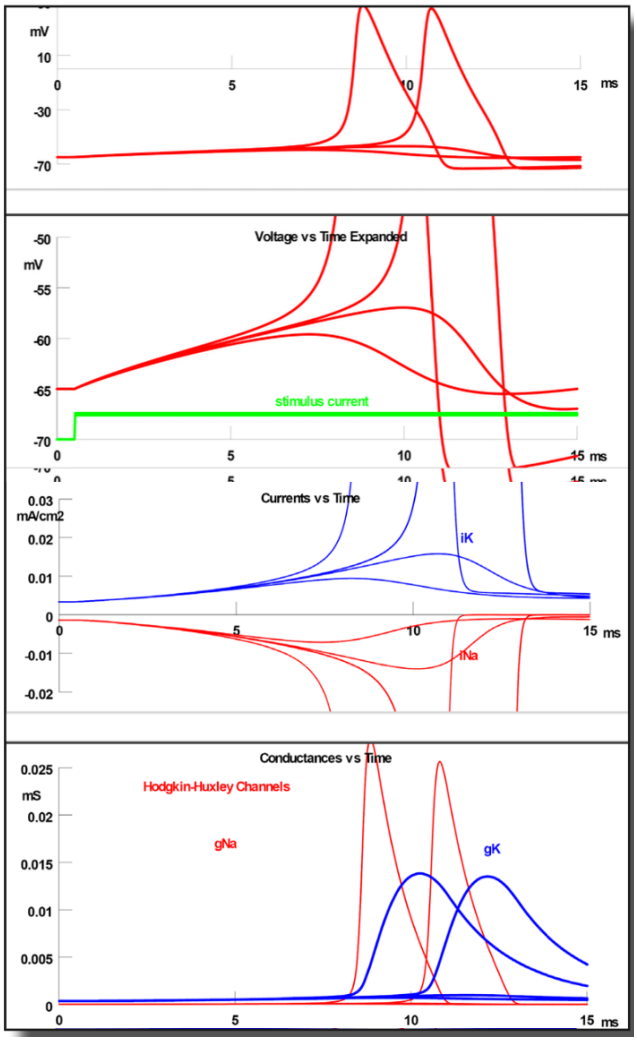
3.4 Relative and Absolute Refractory Period of the Action Potential

- When the second spike overlaps in the refractory period of the first spike, an action potential may be generated by increasing the size of the current pulse amplitude.
    - Shortening the delay further, the second spike runs into the **relative refractory period,** when the action potential is partially regenerative with a large current pulse
    - With a shorter delay still, the second spike falls in the **absolute refractory period**, where any sized spike will not be able to generate an action potential



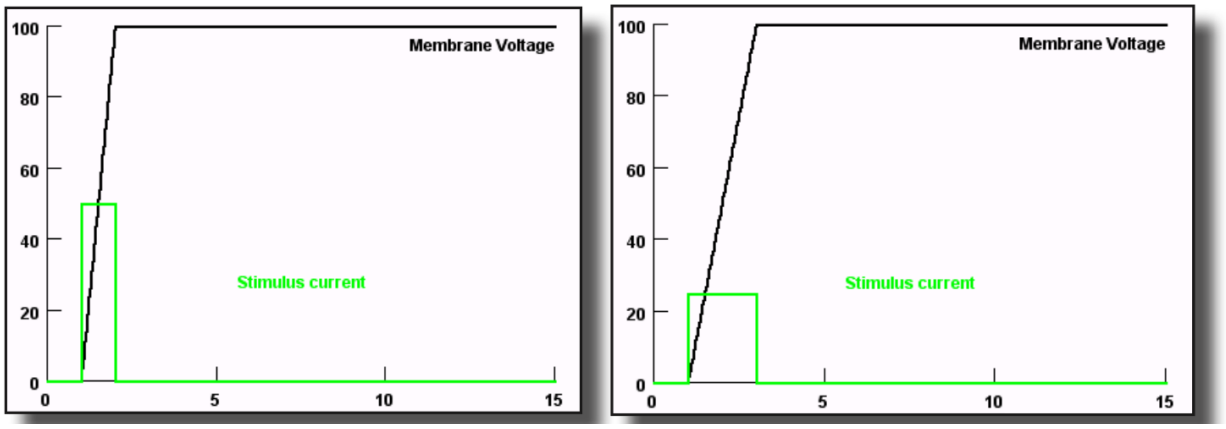
4.1 Is there a critical current or voltage threshold?

- In understanding whether a neuron will fire, one should think in terms of <u>whether there is more or less current depolarizing the membrane</u> rather than whether there is a voltage threshold.
    - As will be demonstrated in this tutorial, there is <u>no critical voltage threshold</u>.
    - subthreshold depolarization can continue to climb to a much larger depolarized level
    - **rate of change of the voltage (mV/ms)** is more important than the voltage alone (mV) for generating an action potential
    - Along a depolarizing ramp, both the Na and the K conductances increase. <u>The steeper the ramp, or rate of depolarization, the more the balance tips in favor of the Na conductance</u>, for it is by far the faster of the two.
    - At some critical rate of depolarization, the <u>Na current wins the battle and the depolarization becomes regenerative</u>.



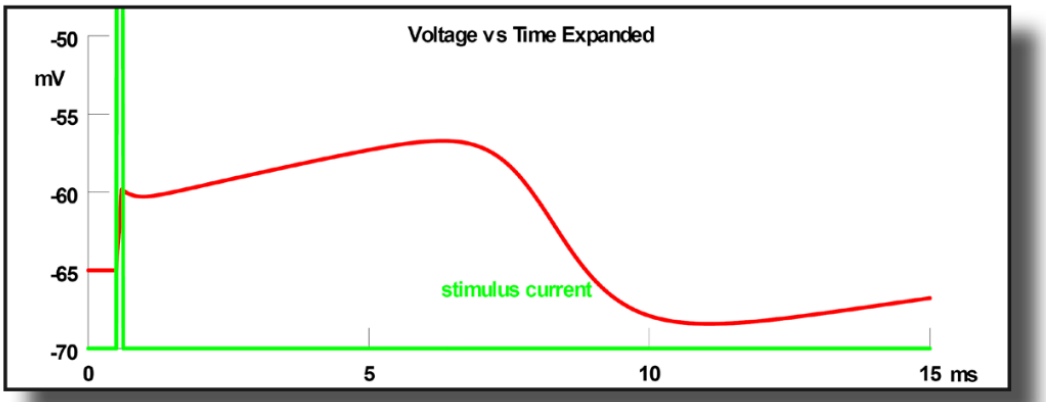
4.2 Treating myasthenia gravis

- autoimmune disease of antibody attacking ACh → less effective in engaging AP in muscle due to less current
- Treatment
    - if you prolong the action of a neurotransmitter at a synapse, resulting in a postsynaptic current for a longer time, you actually need less postsynaptic current to bring the muscle fiber (or neuron) to threshold.
        - inhibiting its breakdown using acetylcholinesterase inhibitors.
- In a short period of time, the stimulus current must be greater to generate the same voltage change (as shown below):
    - a brief, larger amplitude stimulus has the same effect as a longer, smaller amplitude stimulus on opening Na and K current because to be effective in spike generation, a brief stimulus must provide enough charge to quickly depolarize the membrane to the point where the Na conductance is great enough that the Na current can, by itself, depolarize the membrane faster than the K current can repolarize it.
    - For brief stimuli, threshold is achieved by delivering a certain depolarizing charge "all at once," so to speak, onto the membrane capacity.
    - Thus the current amplitude required is inversely proportional to its duration: the shorter the pulse duration, the more charge must be delivered.



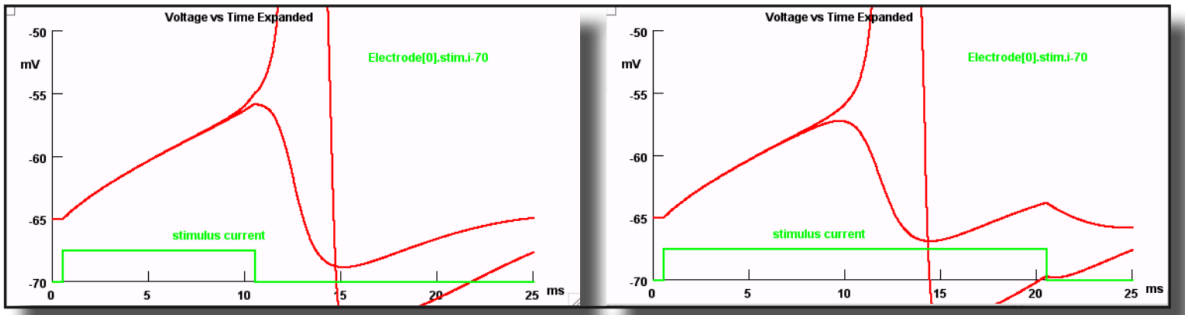
- there is a precise current threshold for synaptic potentials with a given duration
    - The amount of charge is what matters: charge equals pulse amplitude times its duration.

- Voltage change outlast stimulus in duration because after the stimulus, the Na and K channels are open and needs to reach equilibrium before closing
    - The membrane must exhausts all the charges

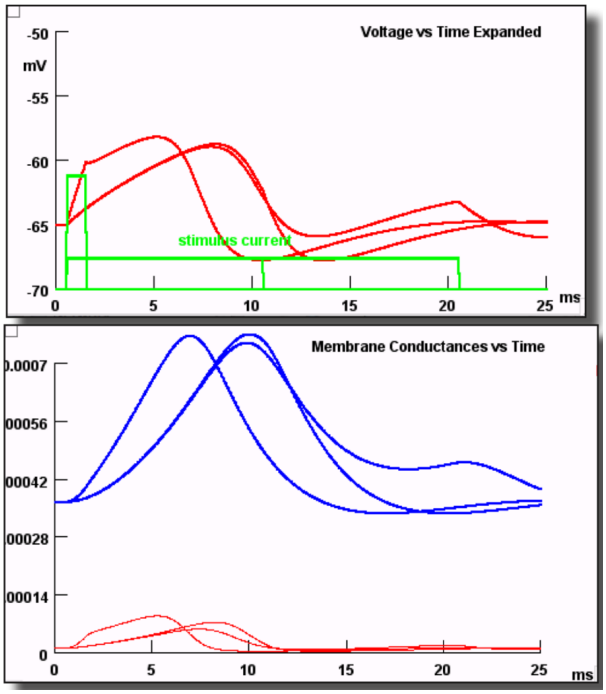


4.3 Longer Synaptic Potential

- Once the duration of the stimulus approaches **10 ms**, the amplitude of the stimulus current required to bring the patch to threshold reached a constant value.
    - Near identical voltage responses are observed for both a 10 ms (left) and a 20 ms current pulse



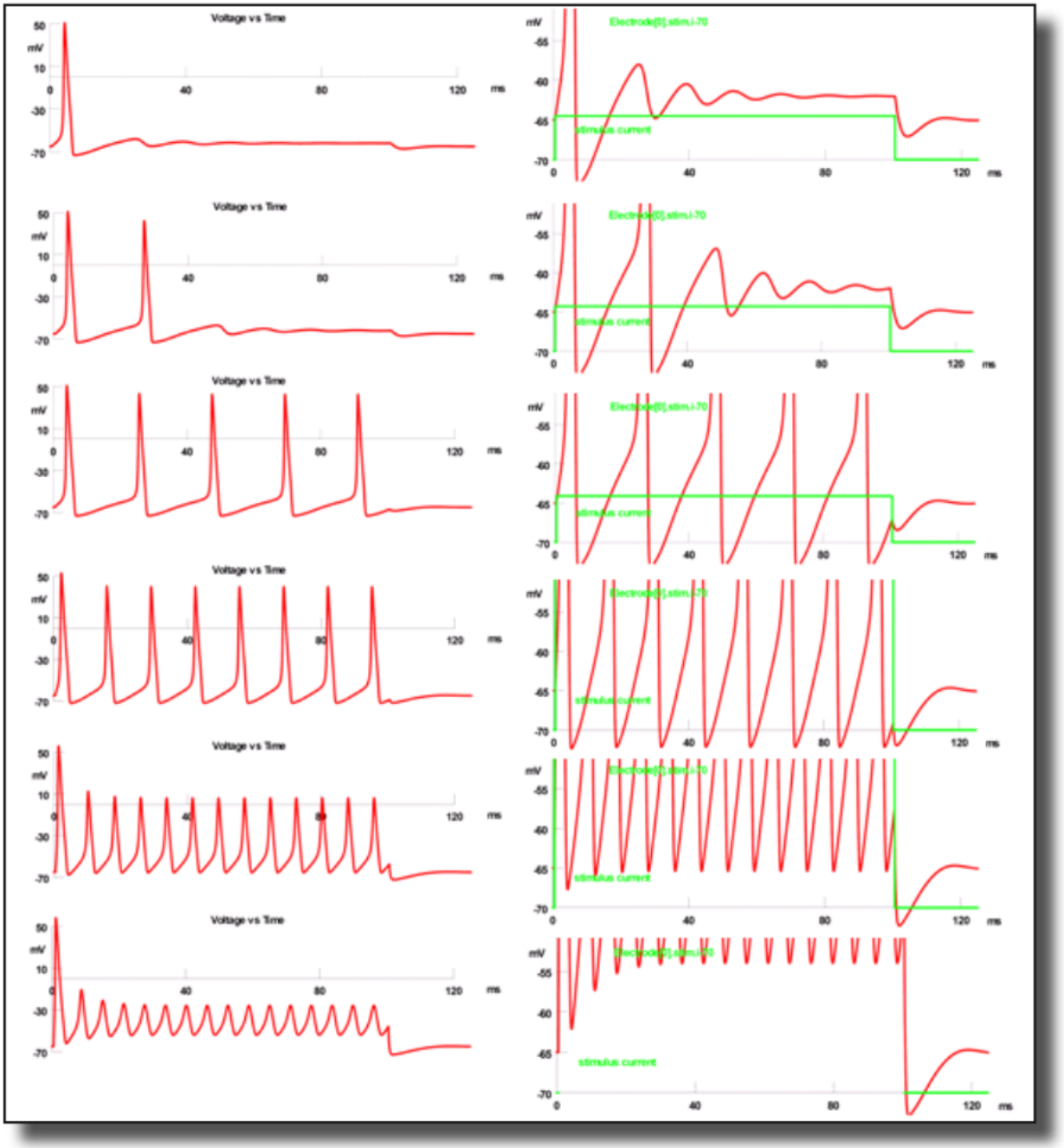
- For durations beyond 10 ms the stimulus duration exceeds the natural time courses of the Na and K conductances.
    - Consequently, further increases in duration are irrelevant to the interplay of the conductances that determines whether the membrane will reach threshold
    - Because all channels are opened → you just need enough current to bring it to AP threshold



4.4 Mechanosensory receptor potentials

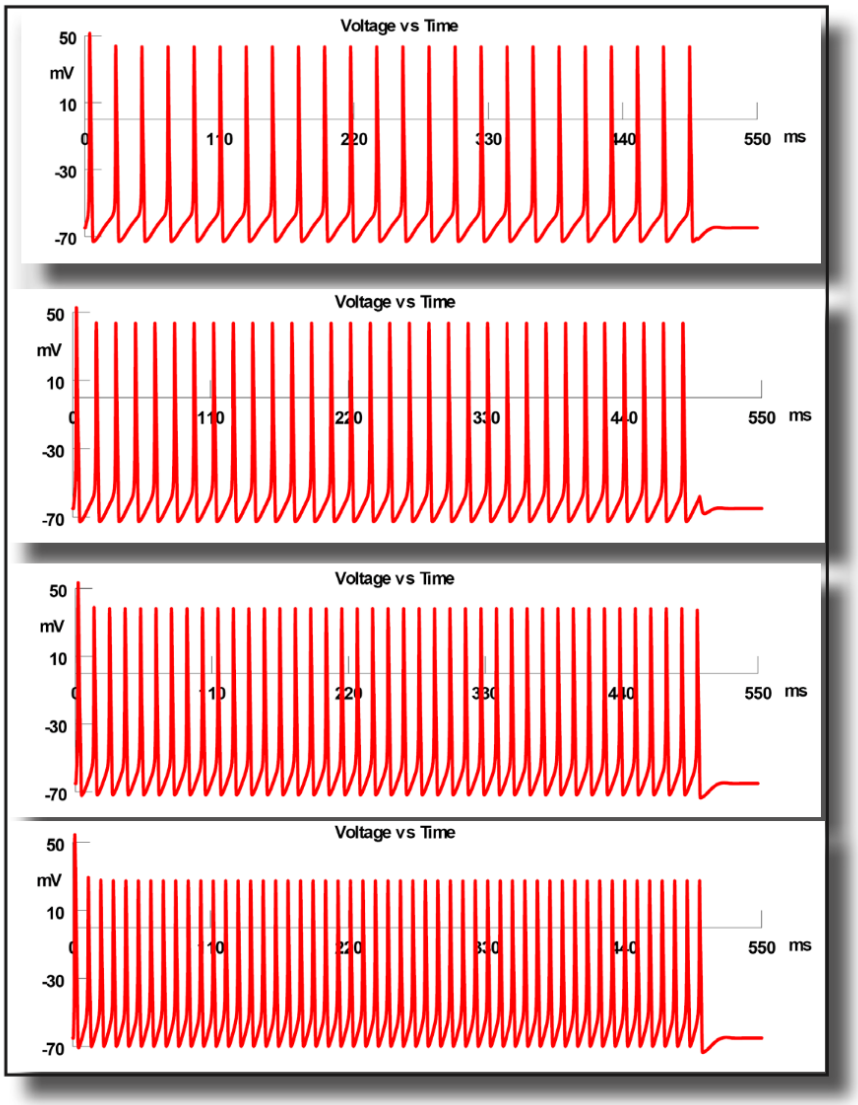
- Brief touch of 100ms
    - When you increase the amplitude of the stimulus current, you should observe damped oscillations and then a train of impulses as the amplitude is increased.
        - * first a single spike, followed by oscillations of decreasing amplitude,
        - * then oscillations increasing in amplitude and rate of rise,
        - * additional spikes generated by the oscillations,
        - * finally a sustained train of spikes.
    - If the stimulating current is persistent following the first spike in the train, it can cause a depolarizing voltage ramp and prevent the K conductance from returning to its resting value.
    - Although the membrane will be less excitable than at rest because the K conductance during the pulse is higher than it is at rest, the Na current will have an advantage over the K current (if the ramp has a steep enough slope) and a second spike will be generated.
    - Further increases in stimulus current give the Na current even more advantage, causing the second spike to be initiated earlier and subsequent spikes to be evoked.

- The ramp leading up to the first impulse is steeper than that leading up to the second impulse. This difference occurs because the higher K conductance during the sustained pulse slows the rise time of ramps subsequent to the first one.



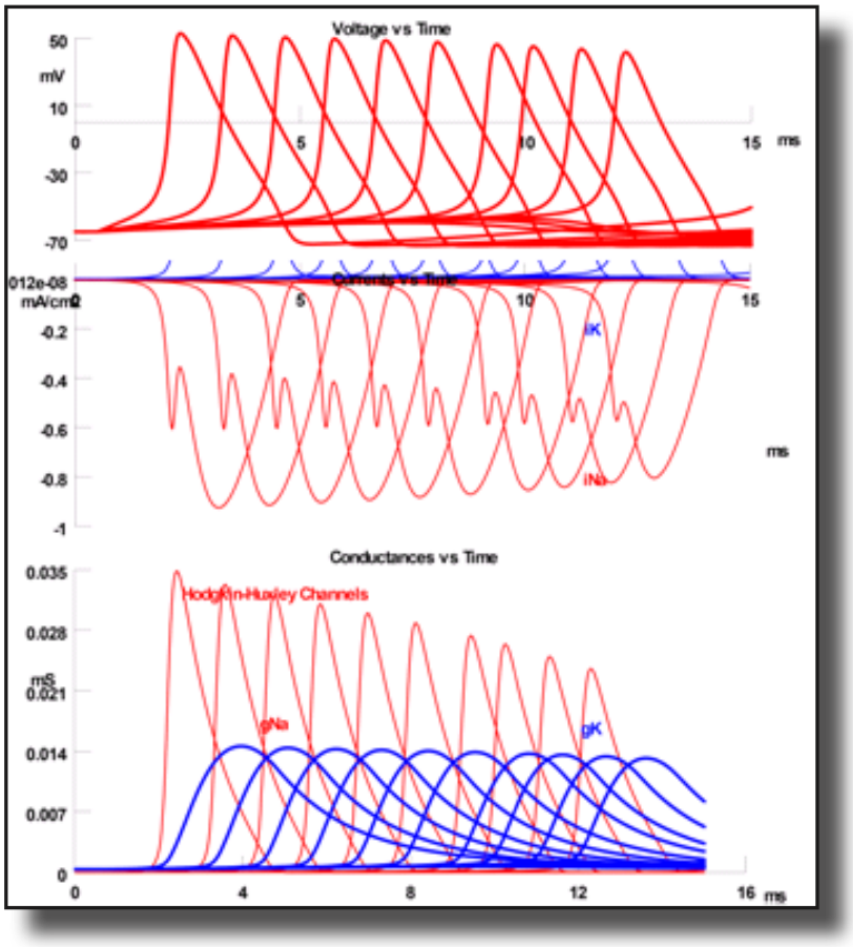
- Touch of 500ms

- ignoring adaptation in our sensory neuron: The rate of firing is essentially uniform throughout the stimulus
- the stronger the touch response, the greater the receptor activation and corresponding current stimulus amplitude
- Above a threshold amplitude, action potentials are uniform, except for their frequency.
    - Stronger stimulation, more frequent AP



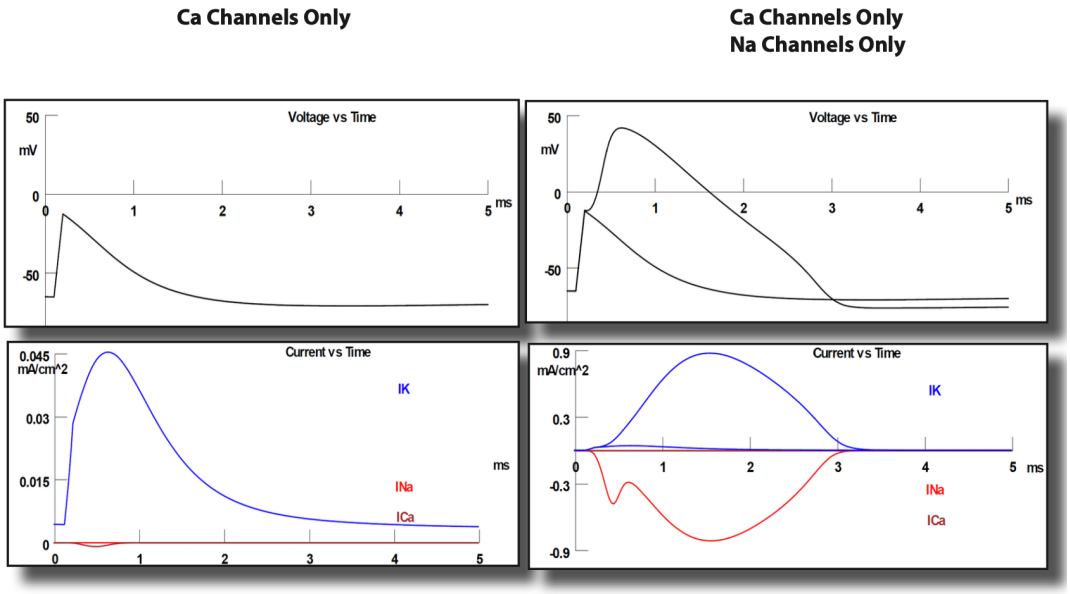
4.5 What happens to the peak amplitude of the action potential near threshold?

- The subthreshold response has the longest sustained depolarization but no action potential is generated.
- Just above threshold, the action potential is not generated until after nearly 10 ms of deliberation.
- As the stimulus increases above threshold, the action potential occurs progressively earlier and its amplitude grows larger.
- Small changes in the amplitude of the presynaptic action potential can have a large effect on the number of quanta released at the NMJ and thus on the postsynaptic response
- As we increases amplitude, Na current start to dominate & AP earlierarlier
    - For just above the threshold, K current will try to bring the membrane back

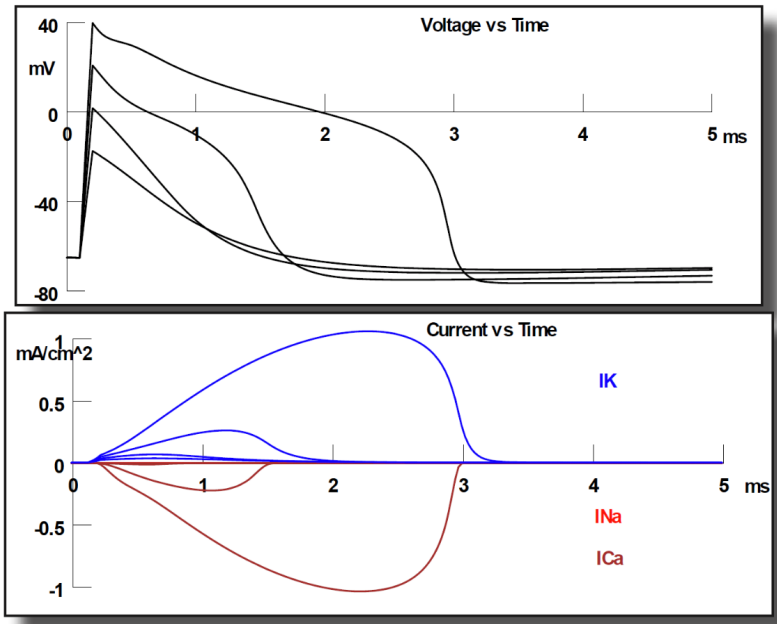


5.1 Compare Na-dependent and Ca-dependent action potentials and their underlying currents.

- At the offset of the pulse, the voltage of the Ca channel only current decays with a time course that appears to be exponential.
- The **L-type Ca channel** is "high-voltage-activated," and requires a depolarization to the level of **-30 mV for Ca channels** to open.
- In contrast, the Na channels begin to open with a small depolarization from a resting potential of -65 mV to about -60 mV
    - 0.55nA cannot depolarize enough activate Ca channel, only Na channel

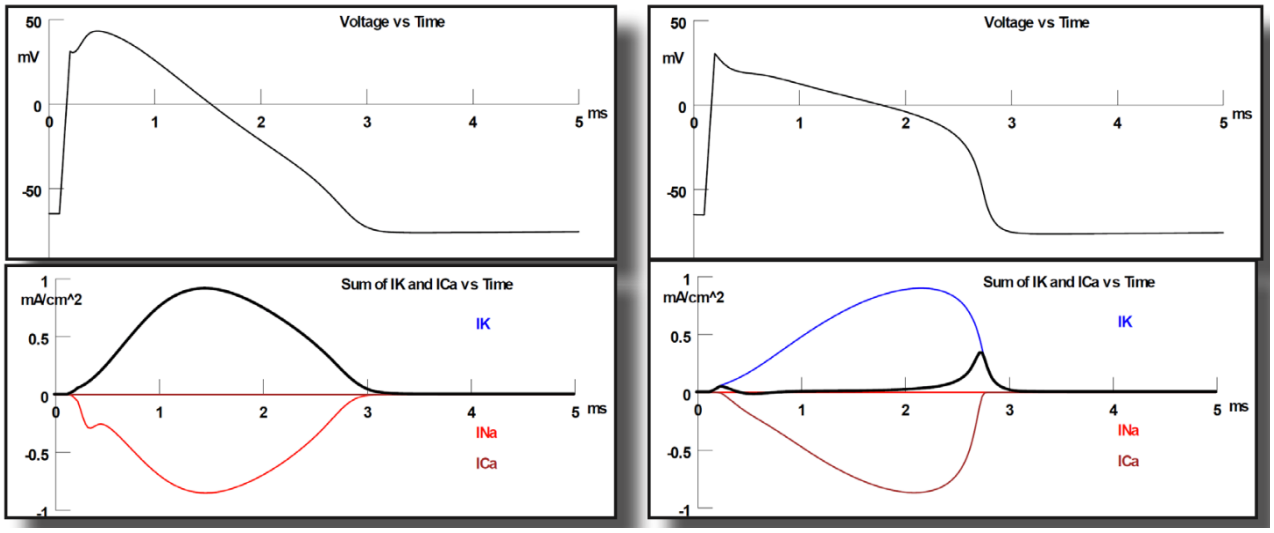


- increase the stimulus current but keep the duration the same (0.1 ms), the decline of the voltage after the pulse gradually looks like it is "hanging up," or developing a hump, or **plateau**, rather than declining exponentially.
- As the stimulus current amplitude, and the resulting depolarization, are increased, both the plateau and the undershoot increase
- When the stimulus is large enough to begin to open Ca channels, the resulting I**N Ca current** tends to drive the membrane potential towards ECa (+127.57 mV), a value even more positive than ENa (+50 mV).
- But this Ca current is fighting the opposing K current that also has been elicited by the depolarizing stimulus.

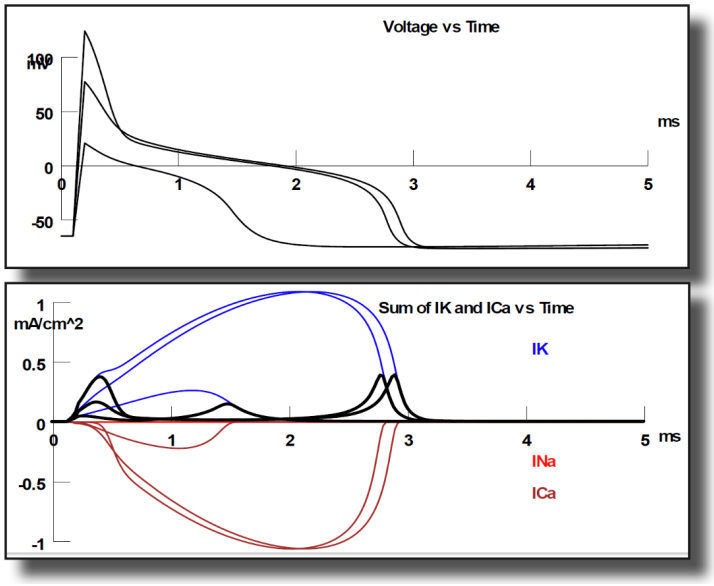


- Ca and K currents are fighting it out during the hump:
    - the sum of K and Ca currents evenutally favors K currents
    - K currents eventually win, but not before the Ca current has an effect on prolonging the falling phase of the voltage
    - Ca channels are slower to activate (open) and to become closed or refractory to opening (inactivated) than Na channels
        - It remain open during Na channel inactivation to prolong AP
    - The stronger the stimulus current, the more pronounced the undershoot, or hyperpolarization below the resting potential → due to more K+ efflux?
    - regenerative depolarization of the Na action potential can be preceded by a long “decision time” at threshold.
        - During this time, INa and IK are almost precisely balanced.
        - If INa gains the advantage over IK, more Na channels will open and more INa will enter, leading to further depolarization.
        - Because of the slight difference in the kinetics of the Na and K conductances, depolarization will open Na channels more quickly than K channels, more INa will enter, and the voltage will move explosively towards ENa.
    - iCa is also balanced by iK during repolarization → Ca closes, K repolarize?

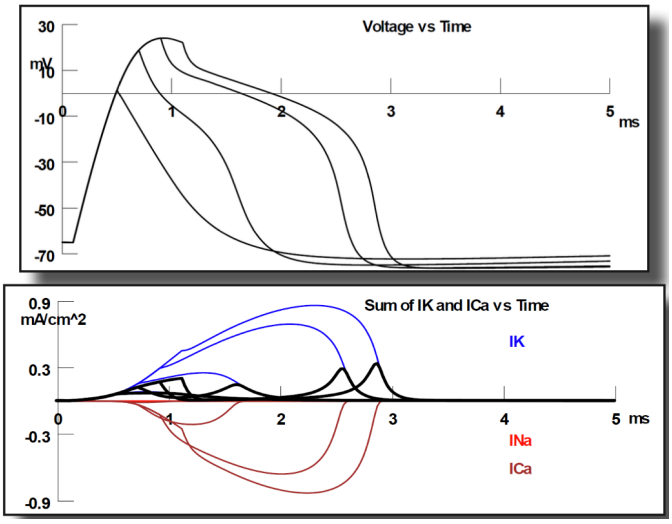
Na Channels Only **Ca Channels Only**



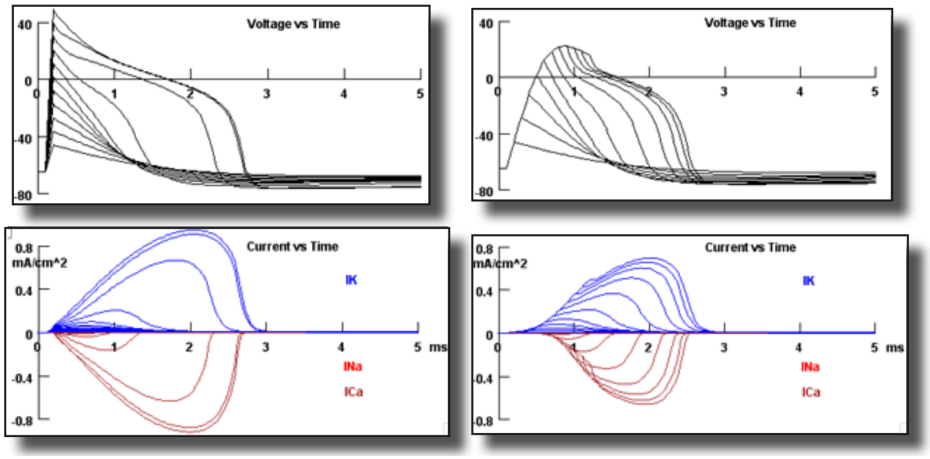
- consequence to Ca and K channel activity when the stimulus intensity reaches a maximum level:
    - the <u>size of the plateau</u> and the corresponding sizes of the ionic currents are nearly the <u>same</u> in response to current stimuli of amplitude 1.5 or 2.0 nA, while a current stimuli of 0.9 nA has smaller values
        - Due to similar current caused by maximal opening of Ca and K channels -> similar conductance between 1.5 nA and 2.0 nA



- **increasing the stimulus duration and current amp. on Ca and K channel activity**
    - In both cases the action potential gradually develops a prolonged plateau; at about 2.3-2.5 ms the action potential repolarizes and no further increase in either stimulus parameter can prolong it.
    - increasing in current duration (at submaximal current amplitude) compared to changes to current amplitude (to maximum)
        - rise time of the action potential is much slower, the maximal peak voltage attained is lower, and density of contributing Ca and K currents is much lower
        - Longer duration exceeds the natural conductance time range → conductance will not change as you increase time → you need more current stimulus for greater response

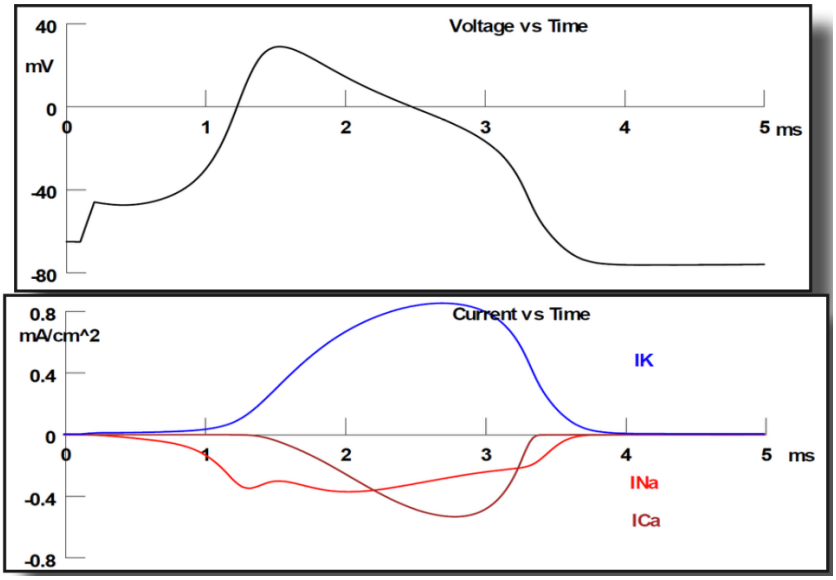


Vary stimulus amplitude **Vary stimulus duration**



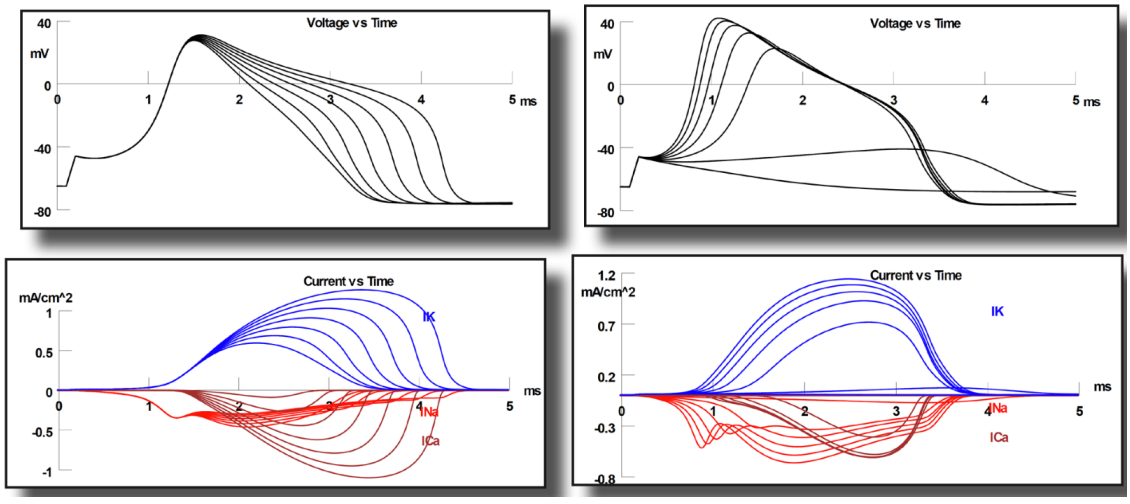
5.2 AP of Na and Ca current

- ICa being delayed compared to INa
    - Due to late opening of L-type Ca channels
- ICa turn off faster than INa
    - Because it closes faster since each single channel is more conducting



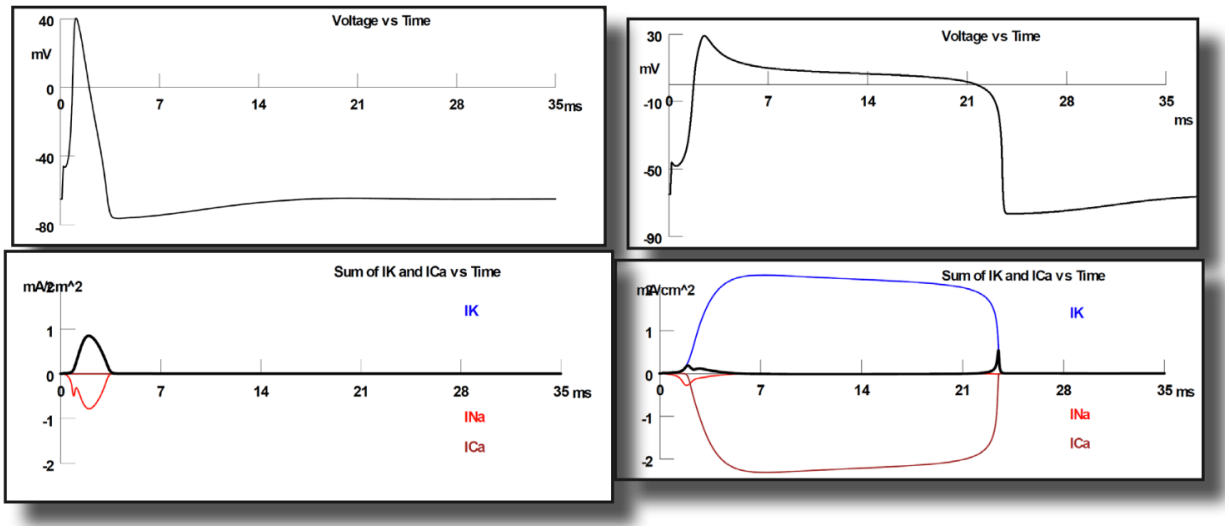
- effect of moderate changes in the ion channel density (gCa or gNa) on the action potential shape
    - Na channels → responsible for the steepness of rising AP
    - Ca channels → responsible for longer, sustaining plateau of AP

Increasing Ca Channel Density **Increasing Na Channel Density**



5.3 Cardiac AP

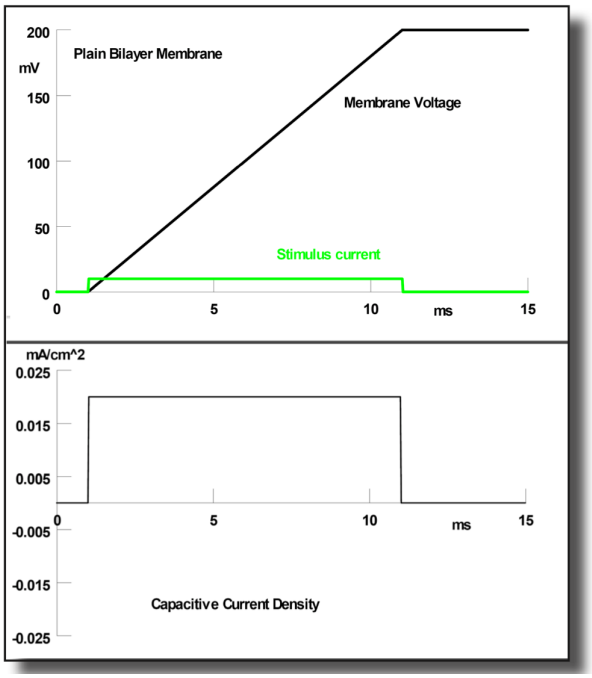
- The Ca channel density (0.39 S/cm2) is <u>much greater</u> than the Na channel density (0.04) in the cardiac action potential.
- The calcium influx is so much larger because the plateau triggers further release of Ca from intracellular stores, which supports muscular contraction of the heart ventricles.
- The long plateau also ensures a delay and refractoriness between contractions of the heart.



Tutorial: Axon

1.1 Charging the Plain Membrane

- Membrane capacitance (C) = capacity of the membrane to store charge / membrane potential
    - C = Q / Em
- Also: The membrane capacitance (C) = area of membrane x constant / thickness of membrane
    - C = A x ke / d
- Combine the 2 formula:
    - More surface area (ie at dendritic branch) = more current to charge
- The rate of change of the membrane voltage is proportional to this capacitive current.
- every change in membrane potential, there is a membrane capacitance change, and a capacitive current is generated to distribute charge across the membrane (i.e. the membrane capacitance)
    - Once the capacitive current stops, the <u>membrane capacitor holds the charge</u> across the membrane.
        - The membrane voltage rises and then remains high even after the stimulus current is no longer provided



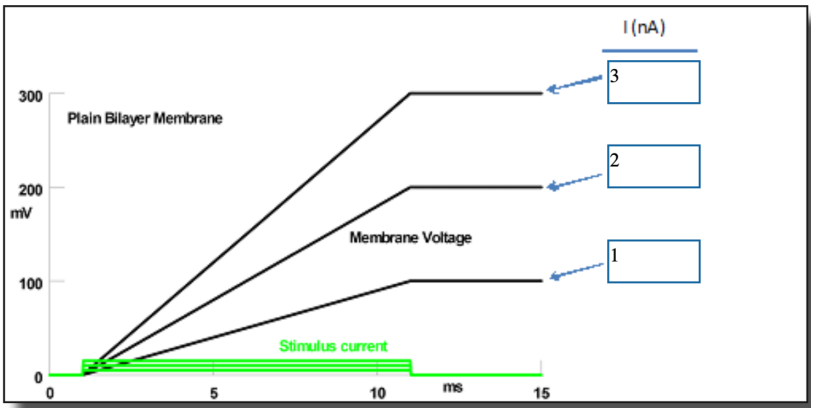
$$I_{cap} = C * [dV/dt]$$

or

$$dV/dt = I_{cap} / C$$

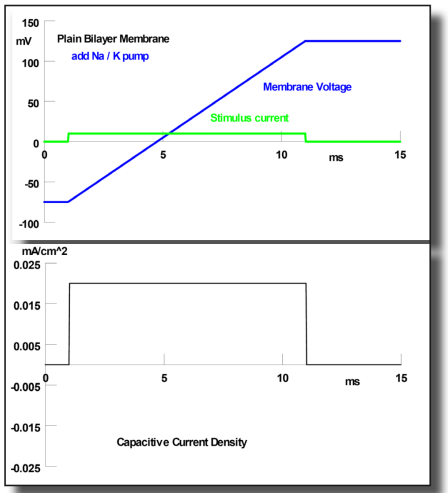
1.2 Change the stimulus amplitude of the current pulse

- With a stronger current stimulus,there is steeper rate of rise of the membrane voltage per time (dV/dt) and a maximal voltage stored on the capacitor per unit time
    - dV/dt = Icap/C → higher Icap = higher dV/dt



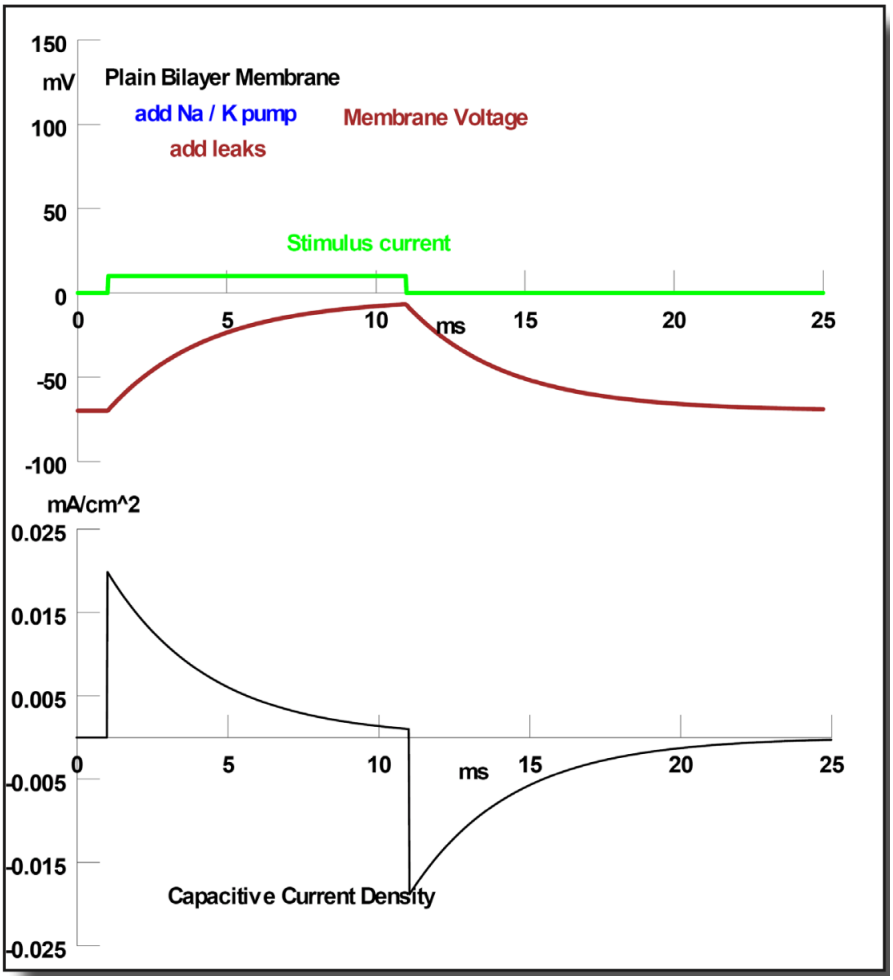
1.3 Establish a resting potential by adding the Na/K pump

- Na/K pump utilizes the energy from splitting ATP molecules to charge the battery that drives the action potential
    - 3Na out / 2K in
- restore the concentration gradients after the avalanche of ions that flow across the membrane during activity.
- The pump must move each of these ions against its concentration gradient
    - W/o pump, membrane voltage moves towards 0 mV
    - Resting membrane potential will not affect the slope of the voltage ramp in response to a current pulse → it is a passive process caused by ion flow



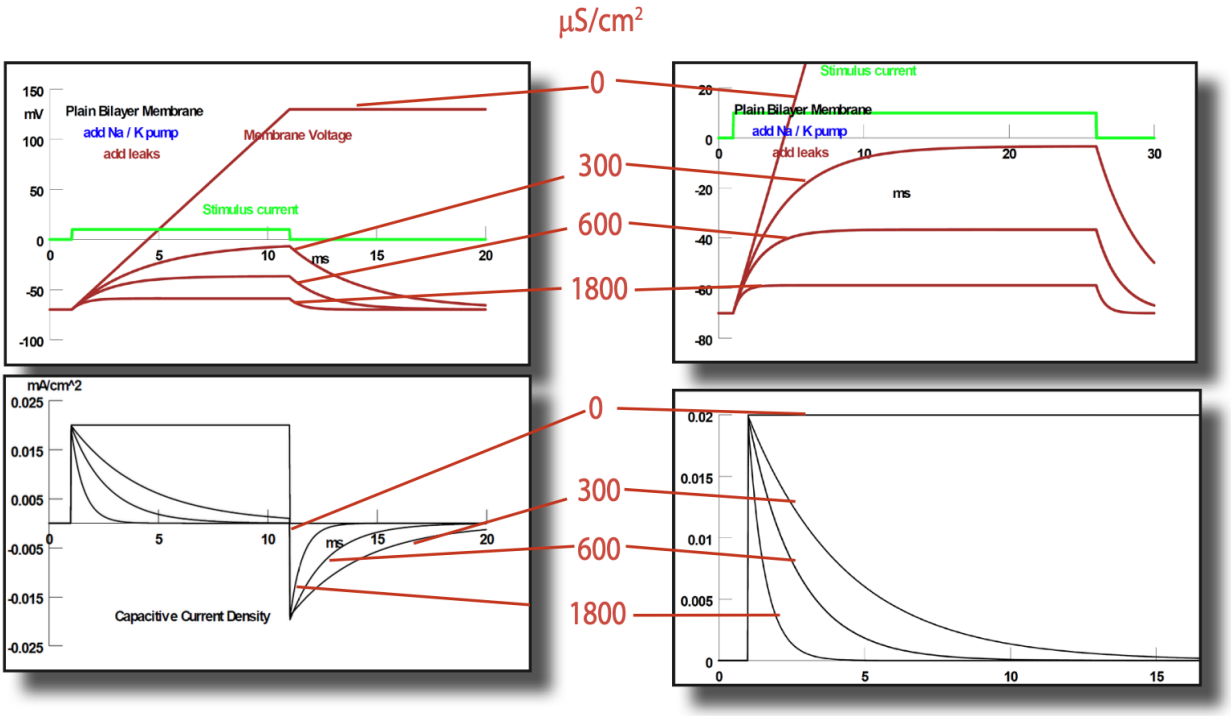
1.4 Leak Channels

- Adding a resistor (like a leak conductance) affects the charging and discharging of the lipid bilayer capacitor
- The membrane with leak channels and the capacitor is an RC circuit
    - The input current step is divided between the capacitive current (Icap) to charge the capacitor and the conductive current (Ileak) across the resistor (Rleak)
    - Icap jumps to the value of the step and then decays exponentially with time, while the Ileak, (through the resistance) starts from zero and rises exponentially as Icap decays.
    - Ileak always equals the value of the step minus Icap.
- **Tau (time constant)** is the time for the capacitive current to fall to within **1/e** of zero, which is **63.2%** (~2/3) of the way to zero
    - time for the leakage current to rise to **63.2 %** of its final value.



1.5 Measurement of time constants of responses to current pulses in experiments

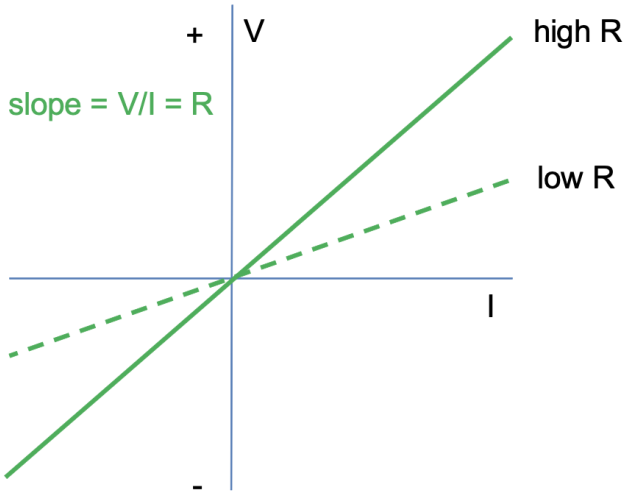
- as the number of channels are put in the membrane increases (leak conductance increases from 0 to 1.8 mS/cm2) the <u>total membrane voltage rise saturates</u> (brown traces) as more stimulus current is lost through the leak channels instead of charging the membrane capacitance
- As you increase leak conductance → **tau = $R_{leak}Cm = Cm/g_{leak}$** → tau becomes smaller
- <u>shorter taus</u> correspond to more <u>rapid charging / discharging</u> rate of the membrane capacitance
    - <u>shortest tau</u> values correspond to the <u>highest leak conductance</u> (gleak).



1.6 Input Resistance of neurons to a current stimulus

- **input resistance** = delivering a current step and measuring the resulting voltage change

- the steady-state level of the voltage divided by the current
- When the input resistance of the cell falls, tau also decreases since a membrane with greater conductance charges more rapidly
    - More Ri = less Ileak = less gm → same I gives you more V
    - Less Ri = more Ileak = more gm
- Increase in Ri can be caused by fewer leak channels → less place for current flow through, hence higher resistance

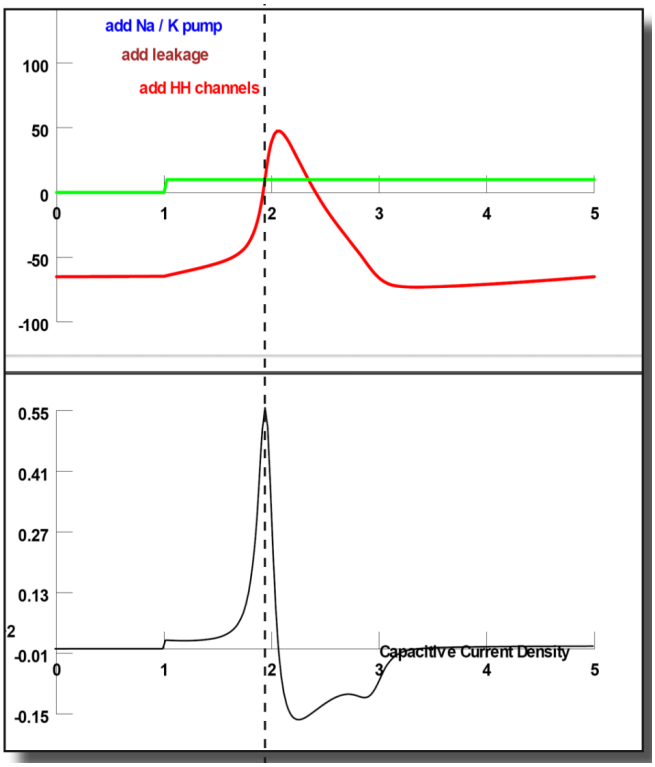


1.7 Calculating membrane surface are from the cell's capacitance measurement

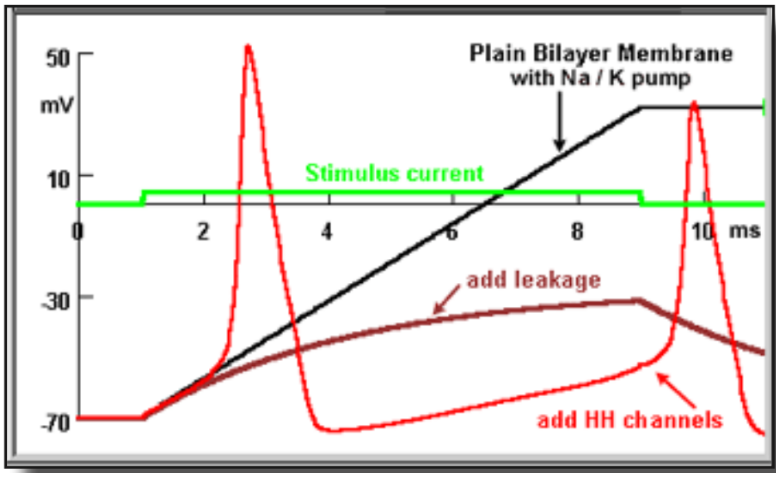
- C=Q/V
- Icap = C*dV/dt
    - Icap is non-existing at a steady state voltage level
- C=Er*A/d

1.8 Hodgkin-Huxley (HH) Voltage gated channels (Na and K Channels)

- capacitive current at its largest peak at the middle of the rising phase or depolarization of the action potential
    - Great change in dV/dt
- correspondingly smaller during the falling phase of repolarization
    - Reduction of dV/dt in magnitude
- Capacitive current cross 0 at the peak of AP



Summary

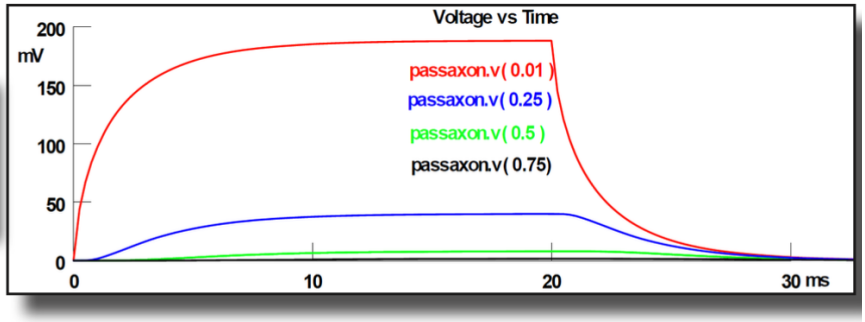


2.1 Voltage Spread

- voltage at the site of current injection rises very rapidly and without any delay.
- At the other locations farther along the axon, the voltage patterns show increasing delays before rising to maximal, steady-state amplitude
- At the cessation of current injection
    - the voltage at the location of the electrode falls immediately
    - but its return to rest is progressively delayed at locations farther from the source of current
- Tau determines the rate of rise of the voltage during the current stimulus and the following discharge rate at each electrode position along the axon after the current stimulus is over

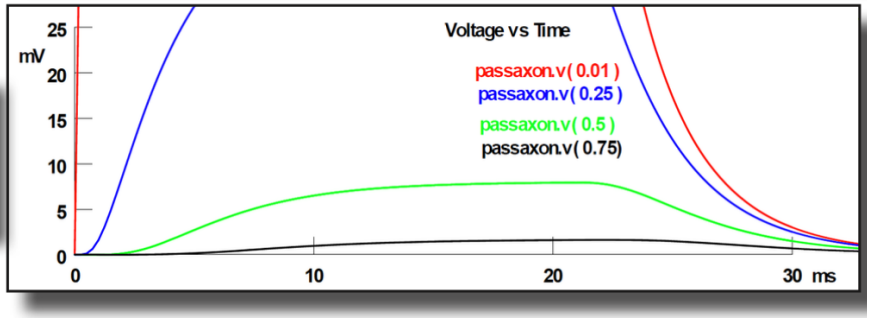
Graph 1:

V vs Time, 4 locations



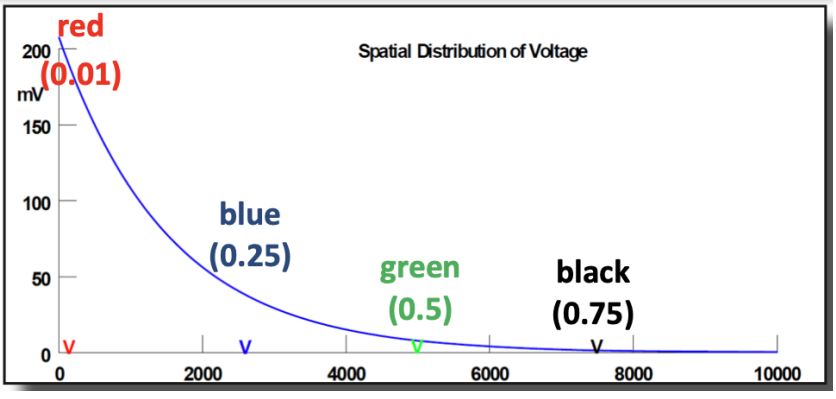
Graph 2:

V vs Time, Expanded



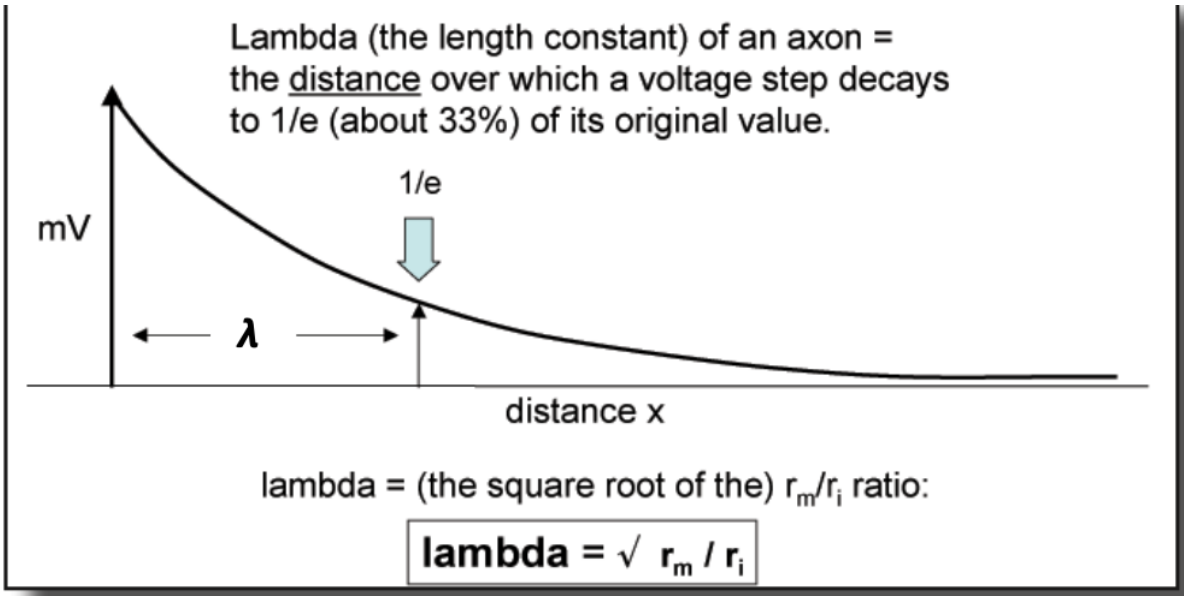
Graph 3:

Voltage vs Space



2.2 Length Constant

- **Length constant, lambda (λ)** is the distance over which the initial voltage response (Vo) decays to approximately one-third (⅓) of its initial value, or, more precisely, to **1/e** (0.368) of its initial value
- The longer a length constant is, the greater the spread of a passive signal

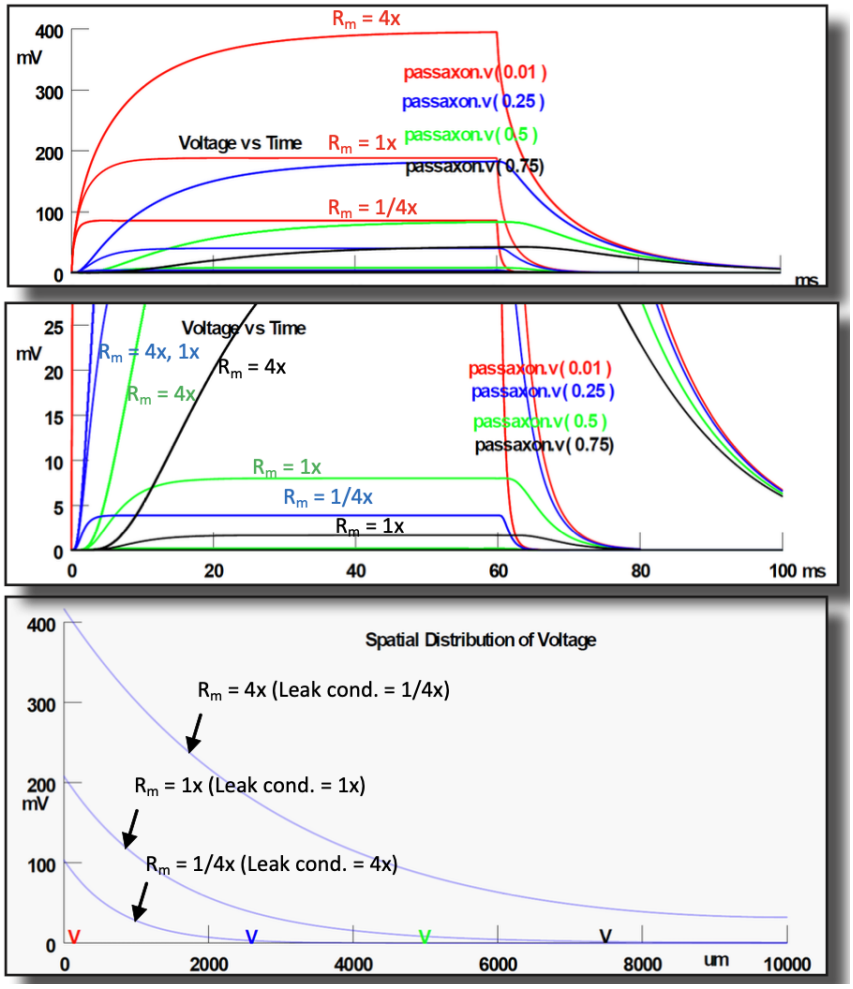


2.3 Membrane and axial resistance bot the length constant of an axon

- Voltage flow down the membrane is determined by
  - (1) how easily current flows across the membrane (**membrane current**) compared to → higher rm = lower membrane current
  - (2) how easily it flows down the axon (**axial current**) → higher ri = lower axial current
- Leakier axons have shorter length constants
  - Passive flow of current need high plasma membrane (rm) resistance and low axoplasma and extracellular medium resistance (ri)
- For an axon of a given diameter, the larger the membrane resistance, rm, the larger lambda
- the larger diameter axon (small ri) will have a larger lambda → easier to flow down due to small ri

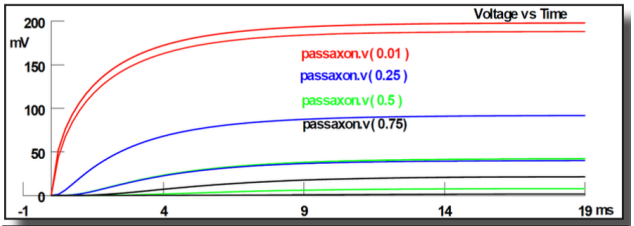
2.4 Length constant changes with membrane resistance

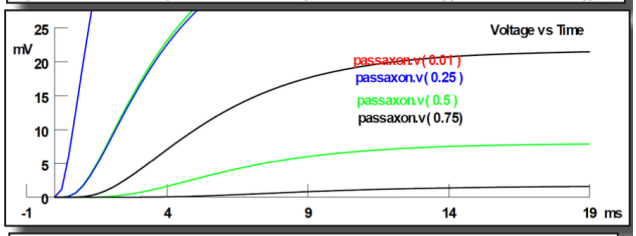
- Leak conductance = 1 / membrane resistance (Rm)
    - leakage conductance changes of 4x, 1x and 1/4x is equivalent to changes in Rm = 1/4x, 1x and 4x
    - 1/4x Rm = 1/2λ and 4x Rm = 2λ (since lambda = sqrt (rm/ri))
- if the membrane resistance is lower, more of the current will dissipate across the membrane, and accordingly reduces the current available to change the membrane potential, and thus the current pulse will not spread far down the axon.
    - With a leaky membrane, the length constant will be shorter

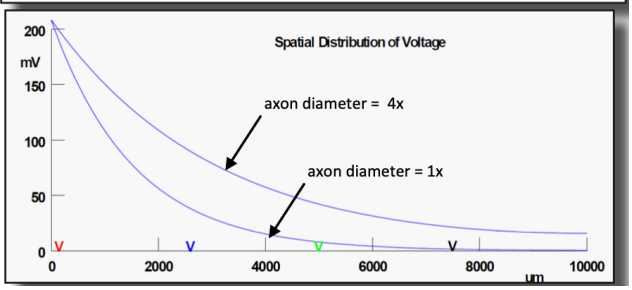


2.5 Lambda and Axon Diameter

- A 4x increase in axon diameter, **lowers** the axial resistance (ri) by 4x, and thus increases λ by 2x → increase voltage distribution

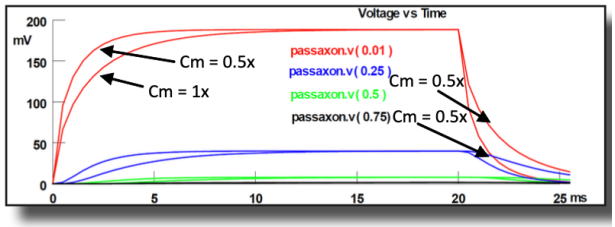


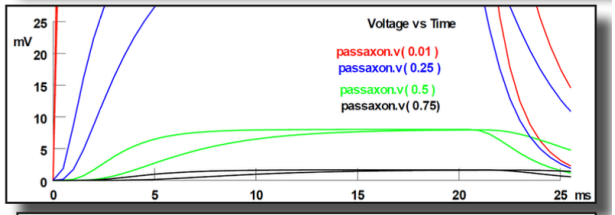


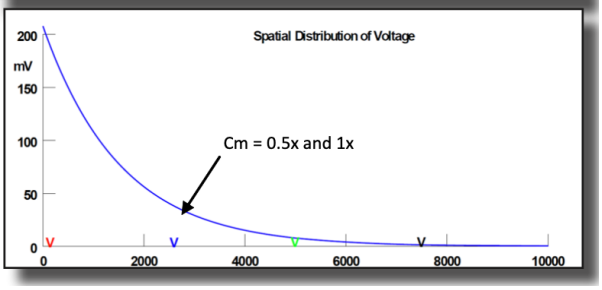


2.6 Lambda and Membrane Capacitance

- Membrane capacitance does not appear in the equation for the length constant
  - consequently you **should not** expect to see any change in this parameter with a change in capacitance.
- Cm affects tau
  - Lower Cm = lower tau
  - The lower the tau, the faster the voltage rise and decay

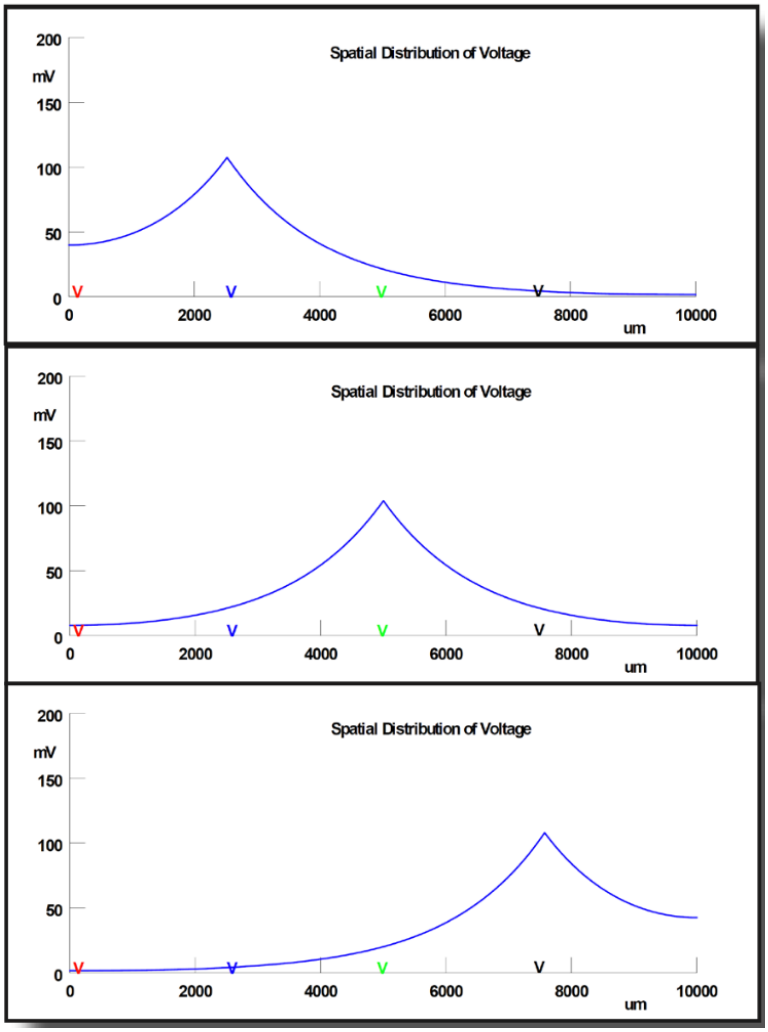






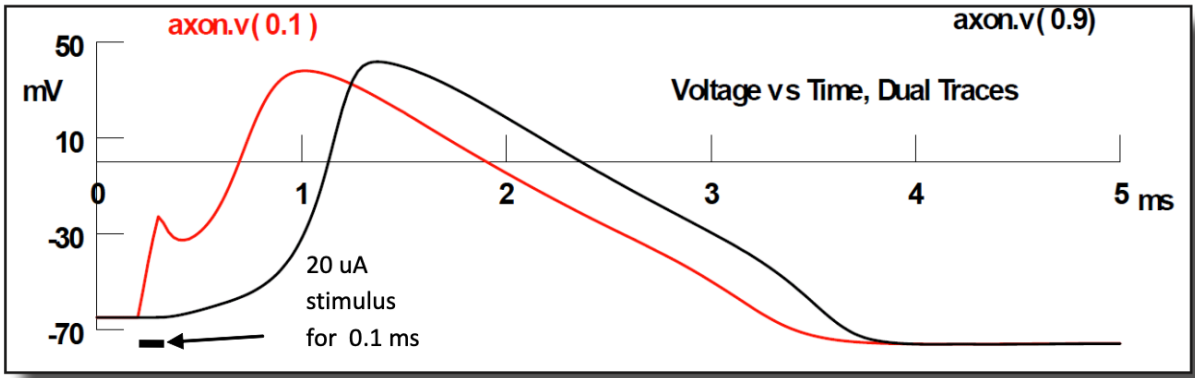
2.7 Spatial Distribution of Voltage at Different Points

- There is no decay of voltage at the very ends of the axon, when the axon is stimulated at an asymmetrical 1/4 or 3/4 points
    - The curves at the two ends become flat because there is **no current flow out of the sealed ends**, just like the ends of an axon which may be a presynaptic terminal or neuromuscular junction
    - V = IR → No current flow = no change in voltage



3.1 Velocity of Propagation of impulse

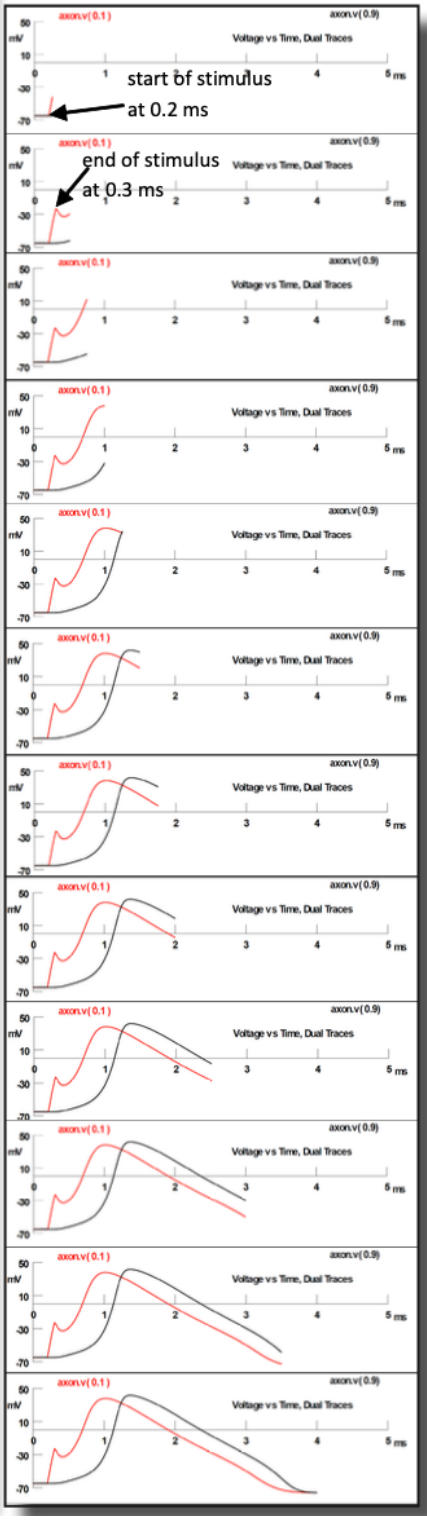
- Velocity = distance (0.9-0.1) / time for the peak to travel
- Stimulus artifact only visible at at 1mm since it is due to the injection of current → no injection of current at 0.9mm

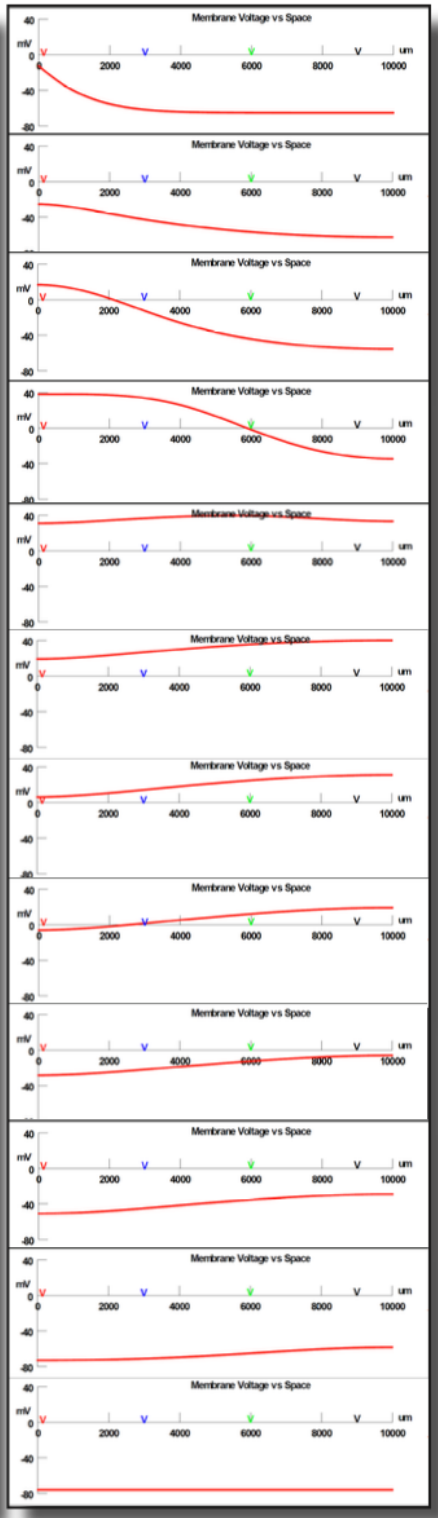


3.2 Action Potential Through Axon

- Depolarization opens Na channels locally and produces an action potential at point A of the axon (time t = 1).
    - The resulting inward current flows passively along the axon, depolarizing the adjacent region (point B) of the axon.
- At a later time (t=2), the depolarization of the adjacent membrane opens Na channels at point B, resulting in the initiation of the action potential at this site. and continues to spread to point C further along the axon.
- Note that as the action potential spreads, the membrane potential repolarizes due to K channel opening and Na channel inactivation, leaving a “wake” of refractoriness behind the action potential that prevents its backward propagation
- The local spread of depolarizing current in front of the action potential that leads to propagation of the action potential signal forward is carried by passive current flow.
    - This passive flow does not require the movement of Na ions along the axons.
    - The depolarization of the previous point causes a flow of current to the adjacent location and depolarizes that location

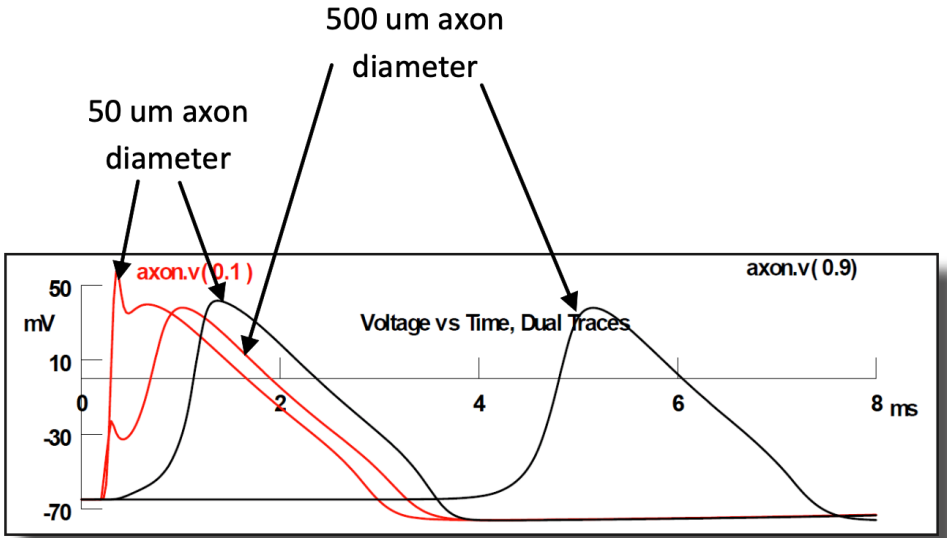
T (ms)	event
0.25	At E(0.1), V=-10 mV At E(0.9), V=-60 mV
0.5	At E(0.1), V=-20 mV At E(0.9), V=-65 mV
0.75	At E(0.1), V=+20 mV At E(0.9), V=-65 mV
1	At E(0.1), V=+20 mV At E(0.9), V=-35 mV
1.25	At E(0.1), V=+30 mV At E(0.9), V=+30 mV
1.5	At E(0.1), V=+20 mV At E(0.9), V=+40 mV
1.75	At E(0.1), V=+5 mV At E(0.9), V=+30 mV
2	At E(0.1), V=-5 mV At E(0.9), V=+20 mV
2.5	At E(0.1), V=-20 mV At E(0.9), V=-5 mV
3	At E(0.1), V=-50 mV At E(0.9), V=-30 mV
3.5	At E(0.1), V=-70 mV At E(0.9), V=-60 mV
4	At E(0.1), V=-75 mV At E(0.9), V=-75 mV





3.3 Diameter of Axon on Propagation Speed

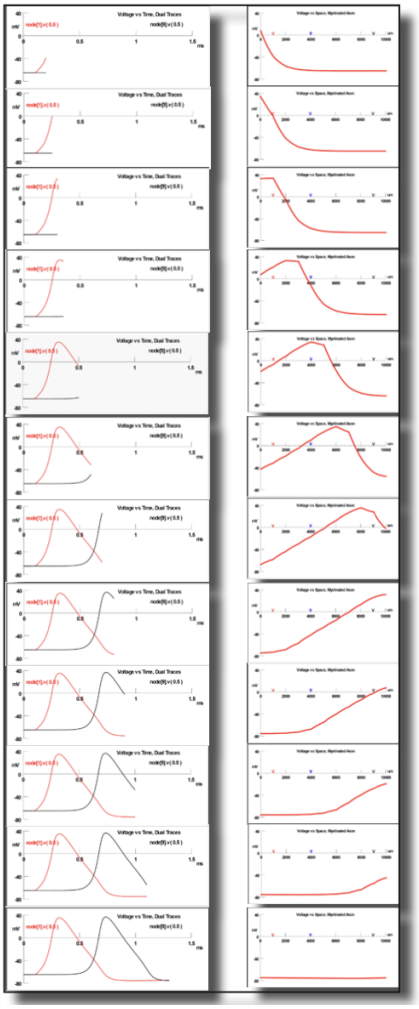
- Larger diameter = smaller time constant (due to reduced Cm) and larger length constant (due to reduced Ri)
    - Larger diameter axon propagates faster AP
- Artifact stimulus currents much larger for smaller diameter axon
    - Smaller Cm → same current can charge the membrane quicker and faster to reach AP
        - A brief stimulating current is more effective in stimulating the smaller axon because it charges the membrane faster and to a higher voltage.
- 图画错了：500红和50黑=500，50红和500黑=50



4.1 Propagation Through Myelinated Axon

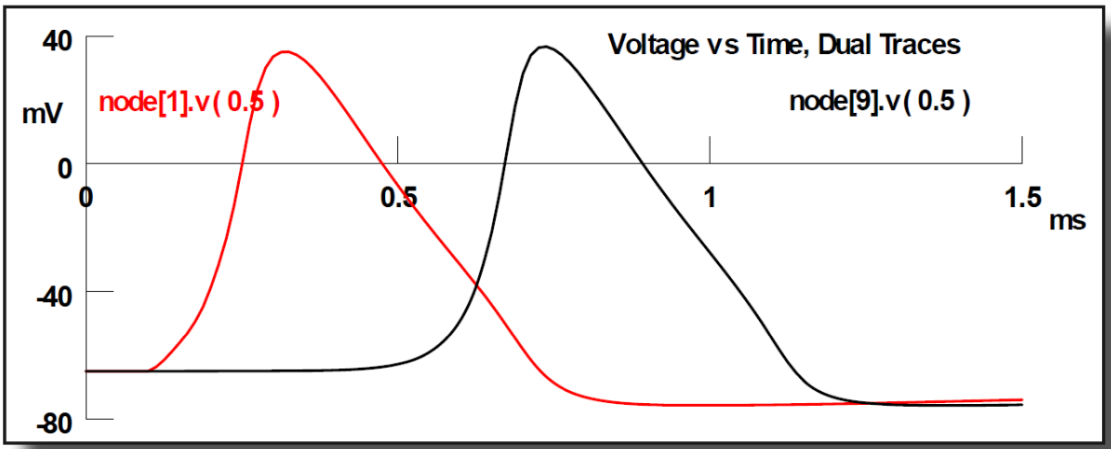
- High density of Na and K channels at Node of Ranvier (here 1000 um apart)
- Voltage cusps at the node:
    - On the rising phase of the action potential, these cusps are positive-going (Na is surging inward and tending to depolarize the node)
    - On the falling phase they are negative-going (K is flowing outward and tending to hyperpolarize the node)
    - Therefore, ion currents of Na and K causes cusps to form at the node
- Between the cusps / nodes
    - the voltage gradients are almost linear
    - This is because **voltage does not change** through the regions between nodes
        - Myelinated regions → AP jumps between nodes

T (ms)	event
0.2	At E(0.1), V= -40 mV At E(0.9), V= -65 mV
0.25	At E(0.1), V= 0 mV At E(0.9), V= -65 mV
0.3	At E(0.1), V= +36 mV At E(0.9), V= -65 mV
0.4	At E(0.1), V= +20 mV At E(0.9), V= -65 mV
0.5	At E(0.1), V= -10 mV At E(0.9), V= -55 mV
0.6	At E(0.1), V= -35 mV At E(0.9), V= -45 mV
0.7	At E(0.1), V= -60 mV At E(0.9), V= +20 mV
0.8	At E(0.1), V= -75 mV At E(0.9), V= +20 mV
0.9	At E(0.1), V=- 75 mV At E(0.9), V= 0 mV
1.0	At E(0.1), V=- 75 mV At E(0.9), V= -20 mV
1.1	At E(0.1), V=- 75 mV At E(0.9), V= -60 mV
1.3	At E(0.1), V= -75 mV At E(0.9), V= -75 mV



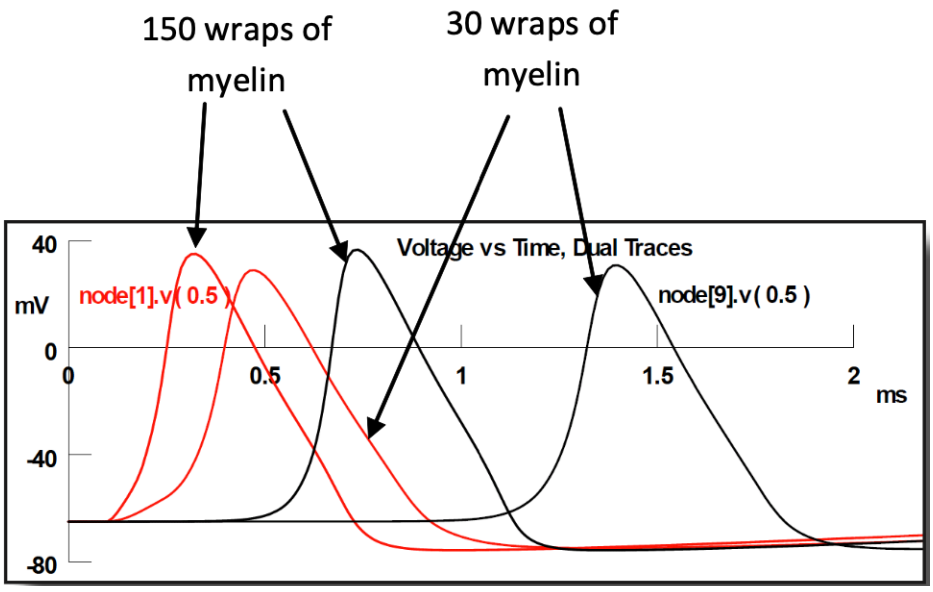
4.2 Degree of Myelination and Conduction Velocity

- Myelination speeds up the conduction velocity compared to unmyelinated axon



4.3 # of Myelin Wraps and Conductance and Capacitance

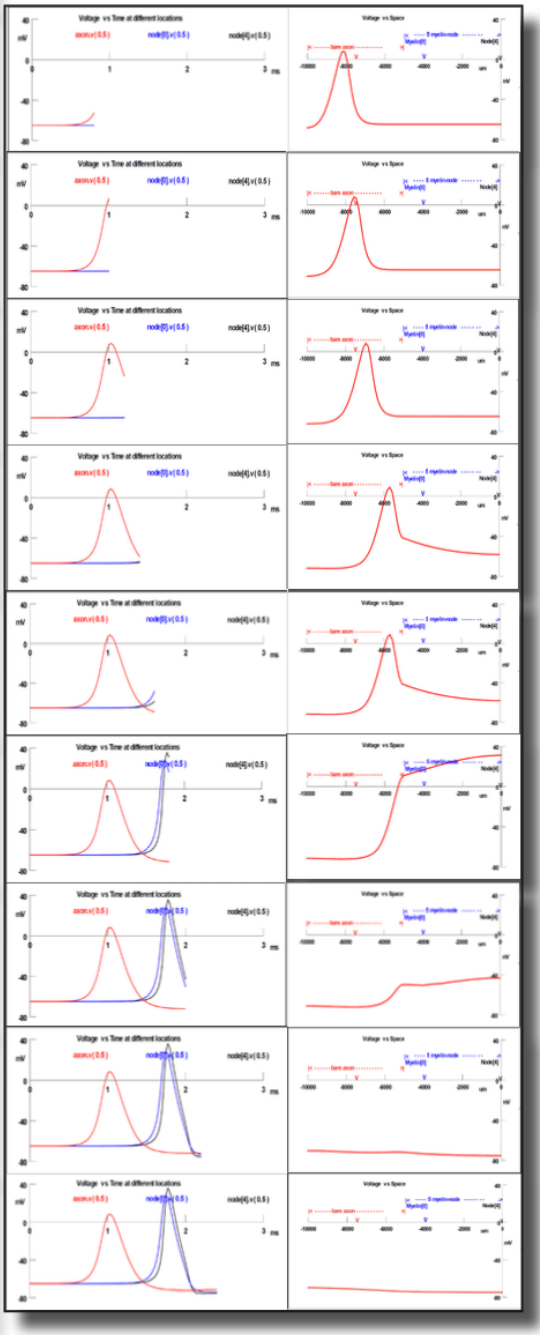
- More wraps = lower conductance (Cm) → <u>lower tau</u>, faster AP propagation
    - Cm = Cm with no wraps * 1/(# of wraps + 1)



5.1 From Demyelinated Axon to Myelinated Axon

- Because it is on the myelinated region → faster conduction velocity, faster rising time, and higher voltage amplitude,
    - Shorter time constant and longer length constant

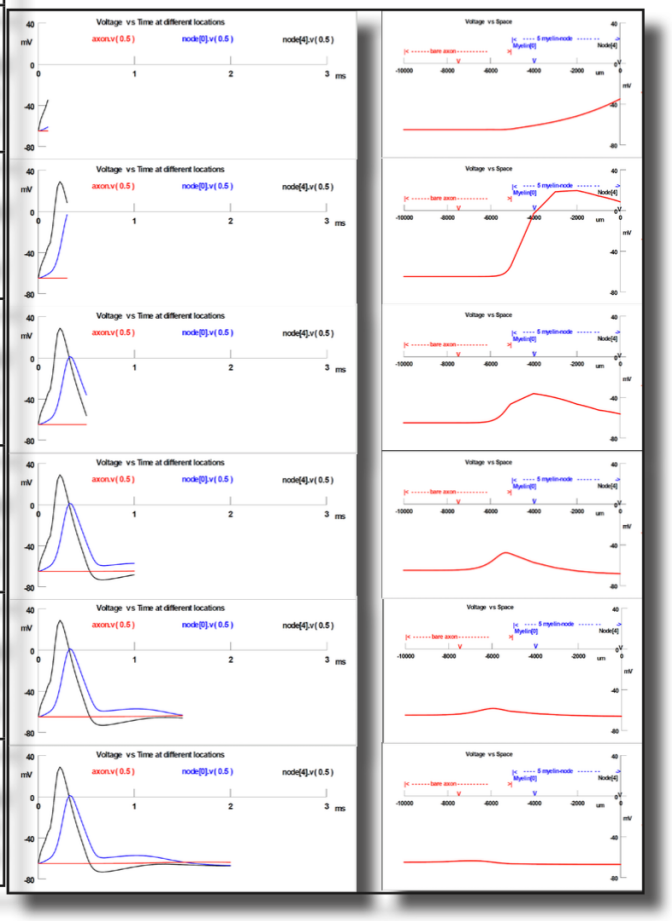
T (ms)	event
0.8	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
1	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
1.2	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
1.4	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
1.6	At bare axon: V= -65 mV At node[0]: V= -50 mV At node[4]: V= -55 mV
1.8	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
2	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
2.2	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV
2.4	At bare axon: V= -50 mV At node[0]: V= -65 mV At node[4]: V= -65 mV



5.2 From Myelinated to Demyelinated

- The impulse traveling in the myelinated region failed to invade the unmyelinated axon.
    - There is simply <u>not enough longitudinal current</u> spreading in front of the impulse in the myelinated axon to generate an impulse in the bare axon region
        - Bare axon has LARGER membrane capacitance → more current is used to charge the membrane → less is through ion channels to create AP
- Loss of impulse propagation due to demyelination is a characteristic feature of **Multiple Sclerosis** (MS). MS is an autoimmune condition, in which the body's own immune system attacks and damages the myelin

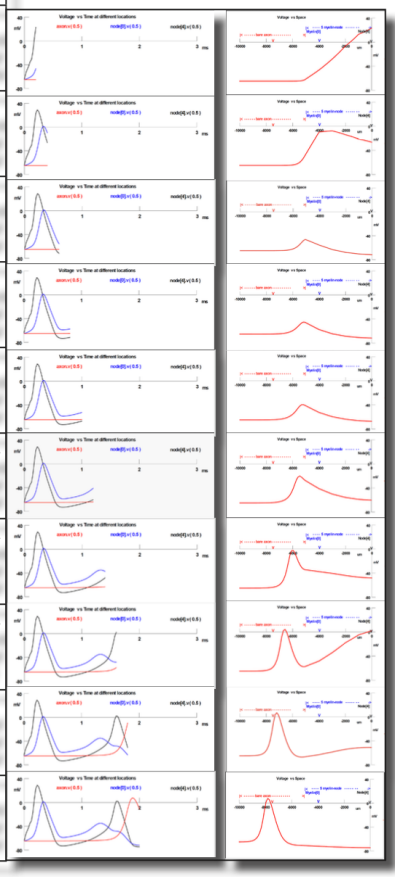
T (ms)	event
0.1	At bare axon: V= -65 mV At node[0]: V= -50 mV At node[4]: V= -38 mV
0.3	At bare axon: V= -65 mV At node[0]: V= - 5 mV At node[4]: V= +10 mV
0.5	At bare axon: V= -65 mV At node[0]: V= -35 mV At node[4]: V= -55 mV
1	At bare axon: V= -65 mV At node[0]: V= -50 mV At node[4]: V= -65 mV
1.5	At bare axon: V= -60 mV At node[0]: V= -60 mV At node[4]: V= -65 mV
2	At bare axon: V= -60 mV At node[0]: V= -65 mV At node[4]: V= -65 mV



5.3 Lowering the Temperature to Improve Conductance

- Changes in temperature affect the rates with which channels open, close, and inactivate and thus the speed with which the ionic conductances turn on and off
    - **Lowering Temperature** → SLOWING of the kinetics of ion channels, **INCREASING the time for ionic currents to deposit charges across the membrane** to drive membrane potential changes and generate action potentials.
- when the temperature drops by 0.1 oC, there is not only a forward propagating action potential through the unmyelinated region of the axon, but there is also back propagating action potential, known as a "**resurgence**" or "**reflection**" action potential back into the myelinated region from which it just came
    - abnormally long delay in the initiation of an impulse in the bare axon due to high Cm
    - a change in property in the Na channels in the long delay period that will permit the back propagating action potential.
        - increase in "recovery from inactivation" of sodium channels

T (ms)	event
0.2	At bare axon: V= -65 mV At node[0]: V= -40 mV At node[4]: V= +20 mV
0.4	At bare axon: V= -65 mV At node[0]: V= -10 mV At node[4]: V= -25 mV
0.6	At bare axon: V= -65 mV At node[0]: V= -50 mV At node[4]: V= -70 mV
0.8	At bare axon: V= -60 mV At node[0]: V= -55 mV At node[4]: V= -70 mV
1	At bare axon: V= -65 mV At node[0]: V= -55 mV At node[4]: V= -65 mV
1.2	At bare axon: V= -65 mV At node[0]: V= -50 mV At node[4]: V= -60 mV
1.4	At bare axon: V= -60 mV At node[0]: V= -40 mV At node[4]: V= -45 mV
1.6	At bare axon: V= -55 mV At node[0]: V= -45 mV At node[4]: V= 0 mV
1.8	At bare axon: V= 0 mV At node[0]: V= -60 mV At node[4]: V= -50 mV
2	At bare axon: V= -5 mV At node[0]: V= -70 mV At node[4]: V= -75 mV



5.4 Changes to the bare region on the effect of propagation from myelin to demyelin

- Treatment of MS: aminopyridine temporarily improved vision, coordination, and strength

- Problem: higher membrane capacitance of the bare axon due to leakage → larger tau & slower rise
- To lower the stimulus required for AP
    - Lower K Channel density by Using K Blocker → increase rate of depolarization
        - With backward propagation
    - Increase Na channel density by using Na agonist → increase rate of depolarization
        - With backward propagation
    - Decrease axon diameter → decrease Cm and increase input resistance → increase rate of rise and increase max voltage amplitude
        - Decrease diameter = slower conduction speed → less current is available to travel back for backward AP

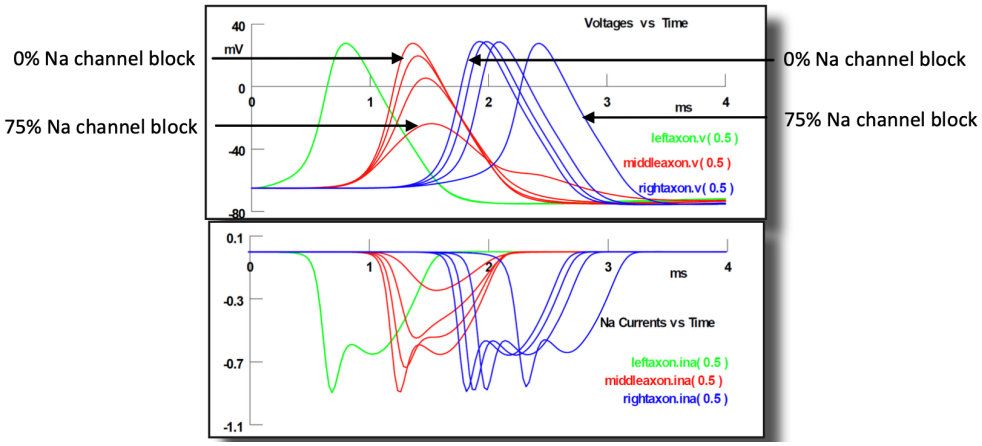
5.5 Changes to the myelinated region on the effect of propagation from myelin to demyelin → myelin [0] is the closest to the bare axon (ok with a smaller stimulus amplitude)

- **Length** → longest in myelin[0], shorter in others
    - DECREASES length has no effect on lambda
    - Yet, voltage decays over a shorter length of internode, which translates in a HIGHER voltage amplitude at the junction where the myelinated region meets the bare axon
    - A HIGHER voltage amplitude at the junction where the myelinated region meets the bare axon, translates into a LARGER longitudinal current available to generate an impulse in the bare axon
- **Capacitance** → lowest in myelin[0], no on other
    - DECREASES in the membrane capacitance (Cm) of the myelinated internodal region by ADDITION of more myelin wraps, translates into a SMALLER capacitive current (Icap) and a GREATER longitudinal current available through ionic Na and K channels to generate an impulse in the bare axon.
- **Diameter** → smallest in myelin[0], largest in meylin[2]
    - INCREASE diameter = increase length constant = slowing voltage decay rate = higher voltage amplitude at the junction = LARGER longitudinal current available to generate impulses in the bare axon

6.1 Block Na Channel Using TTX in the Middle Axon

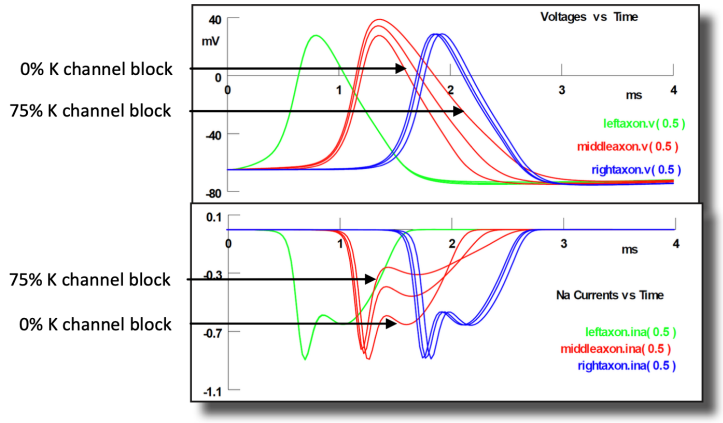
- There is a lot of resilience built into the impulse-generating mechanism.
- A large "**safety factor**" ensures transmission in the face of considerable difficulties.

- Whenever the struggling impulse in the middle segment manages to bring the right segment to threshold, the action potential there quickly resumes its normal shape and continues on



6.2 Block K Channel in the Middle Axon

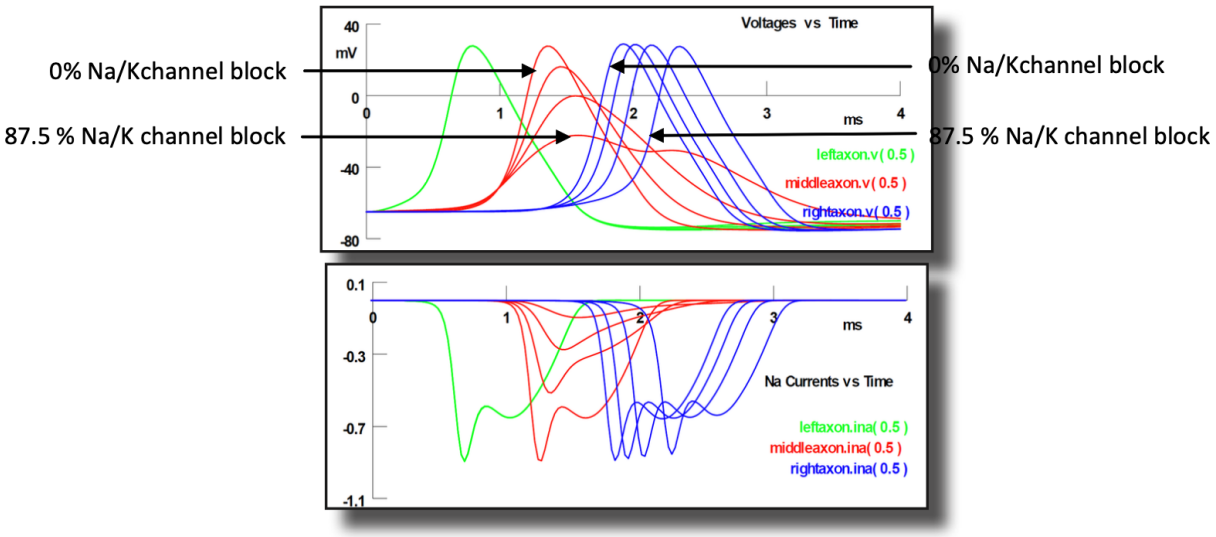
- Blocking the K conductance in the middle segment increases the speed of the depolarization and amplitude size of the action potential in the middle segment
  - The Na current is EXTENDED in time during repolarization though because the voltage decay during repolarization is SLOWER than normal
  - a higher and steeper action potential overshoot that is of a prolonged duration in the middle axon region
  - Conduction velocity is also faster with K blocker



6.3 Block both Na and K Channels Using Lidocaine

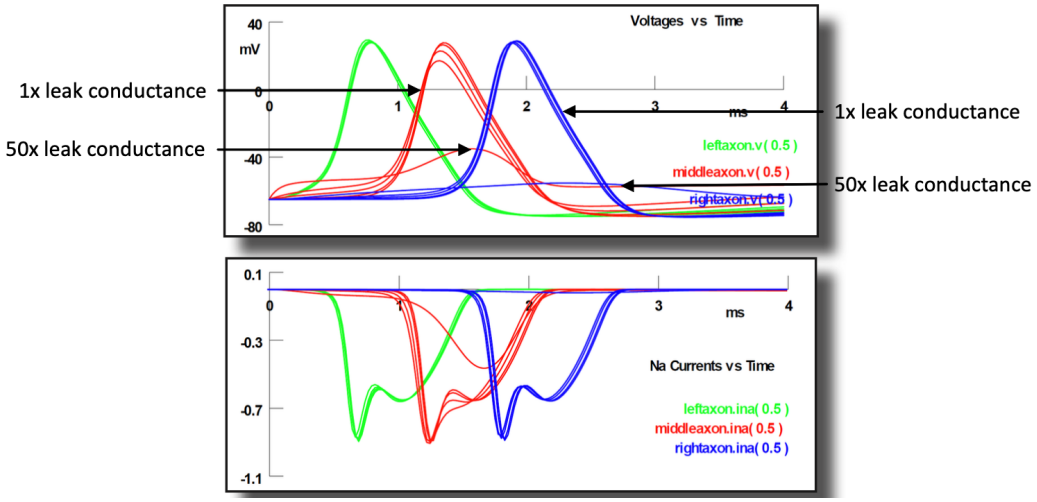
- Changes to sodium or potassium conductances WILL affect the conductances and currents for both sodium and potassium ions during an action potential.

- Both TTX and lidocaine both slow the rate of rise of the depolarization and reduces the action potential peak
- but TTX is more potent than lidocaine at the same dose, because TTX doesn't block K channel efflux, the counter ionic current to the sodium ion influx which mediates the action potential upstroke.
- All nerve fibres are sensitive to local anesthetics, but generally, those with a smaller diameter tend to be **more sensitive** than larger fibres
    - Pain sensory (nociceptive) neurons are carried in small myelinated axons, it means that pain sensation is blocked more readily than other sensory modalities.
    - Local anaesthetics effects small and unmyelinated due to its slower conduction speed (Larger Ri and smaller Rm) → easier to block than those with greater conduction speed



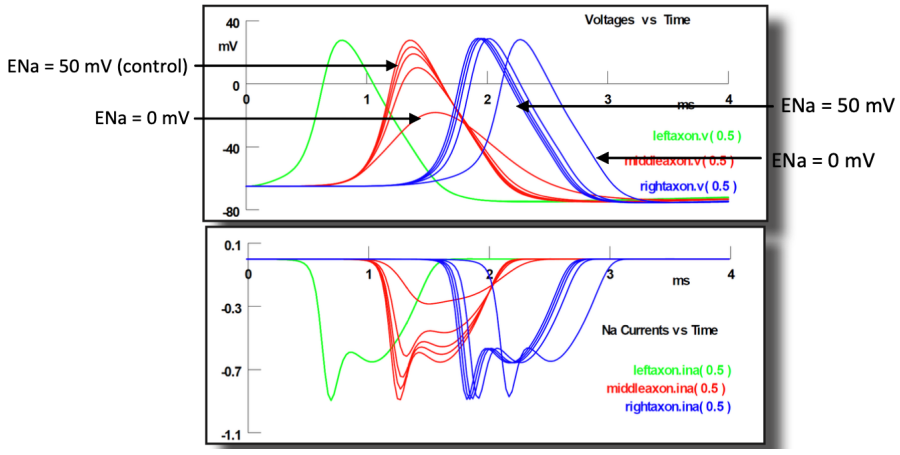
6.4 Increase of Leak Conductance in the Middle

- Small increases in leakiness in the middle segment does not interfere with transmission of action potentials
- A 50x increase in leak conductance in the middle axon segment will prevent impulse propagation



6.5 Damage of Na-K ATPase in the Middle

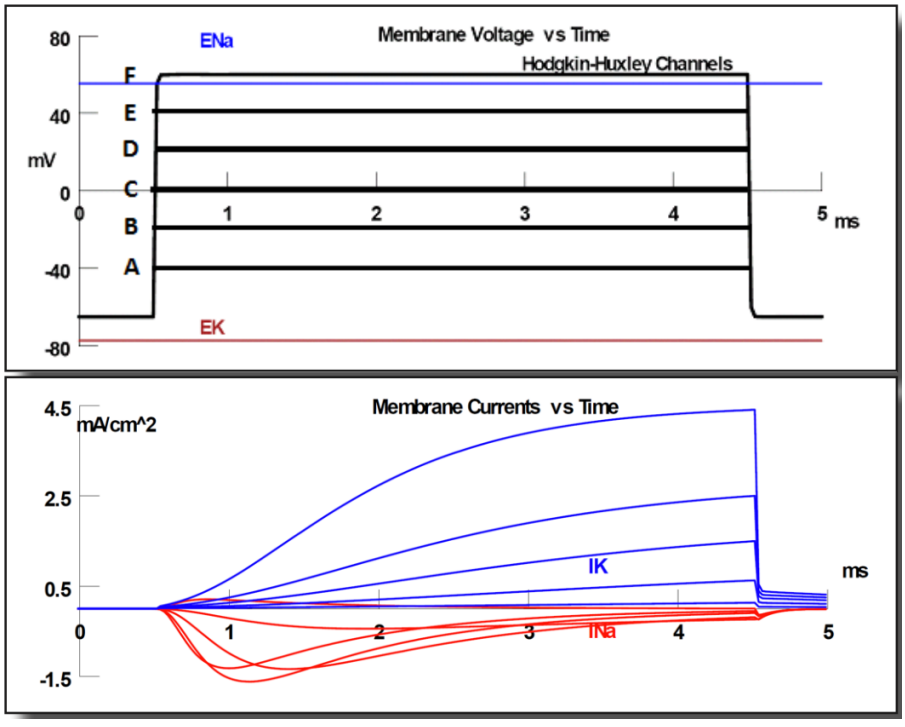
- Damage to Na-K ATPase → Lowered ENa
- there is a huge “safety factor” ensuring transmission of nerve signals, even in the face of a severe drop in Na channel density, or dramatically increased leakiness of axons (which occurs during brain trauma), or dramatic drop in reversal potential for Na ions (during periods of metabolic constraint when ATP levels are low)



Tutorial: PATCH

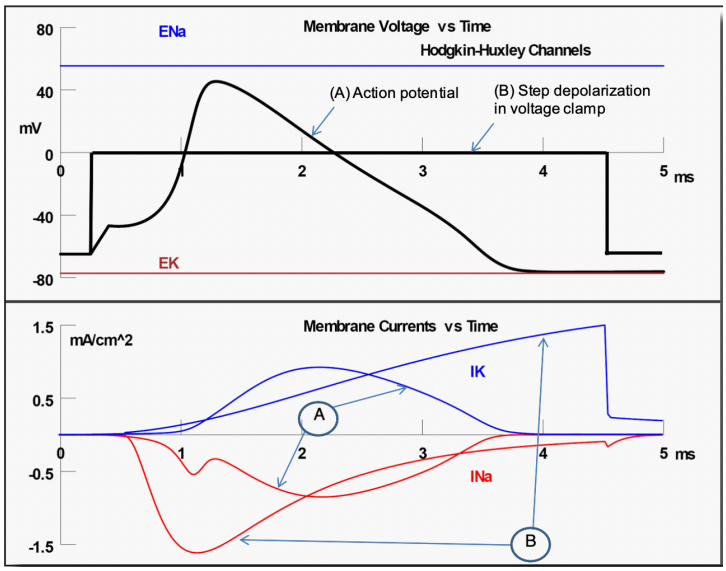
1.1 Observe the peak Na and K currents in response to step depolarizations:

- At -60mV → very low current for both Na and K → due to channels not yet opening
- For Na current:
    - AB: Keep decreasing as V increases
        - More and more inward current for Na+ due to channel opening
    - C: Reach minimum at ~ 0mV
        - Max opening and relatively greater driving force
    - DE: Keep increasing (toward 0) as V increase
        - Inward current decreases because of reduced driving force
    - Reach 0 at ~+60 mV
        - Reversal potential for Na+
    - F: Above 60 → +ve current (Na+ coming out)
- For K+ current:
    - <u>Always increasing</u> as V increases due to channel opening and moving away from reversal potential
- Na channels increase in conductance (i.e. Na channels open) at a lower (more hyperpolarized) voltage before the potassium channels increase in conductance (K channels open)



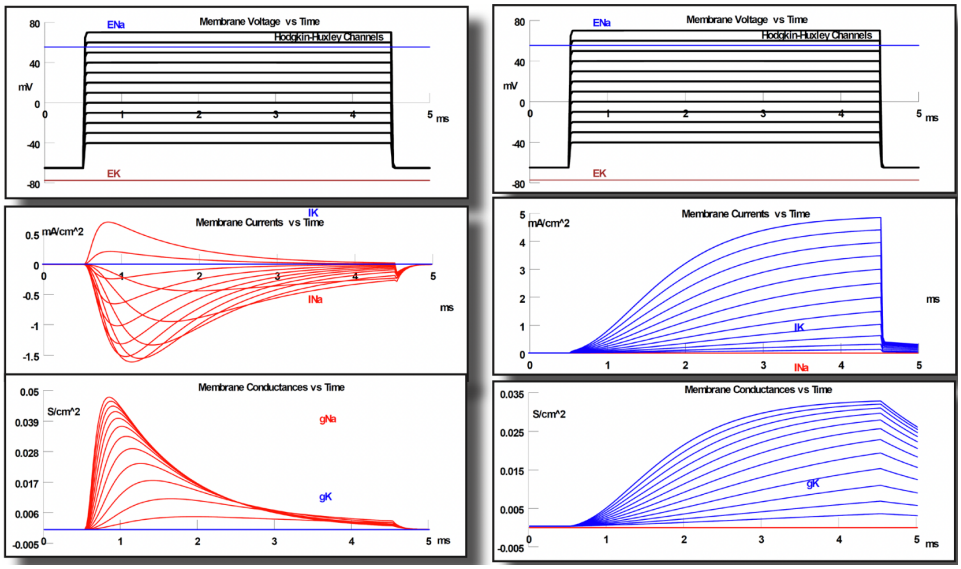
1.2 Compare the Na and K currents in response to a step depolarization or an action potential:

- The response observed for step depolarization is larger because the rate of change in voltage is larger



1.3 Analyse the family of Na and K currents in response to a series of step depolarizations to different voltages

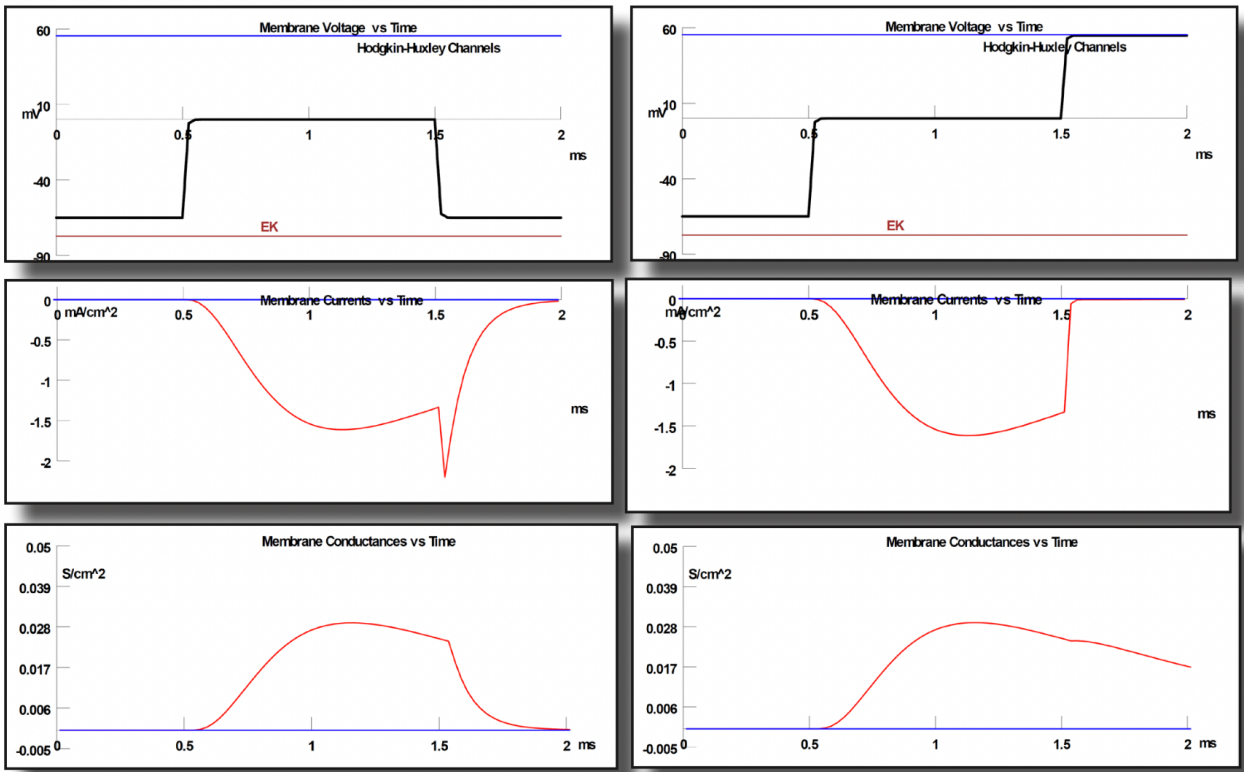
- K currents continue to grow in size with step depolarizations to potentials from rest to above 0 mv even though the maximum K conductance is beginning to saturate
    - Because we are getting away from the reversal potential → more DF



1.4 Observe and analyse the cause of “tail” currents

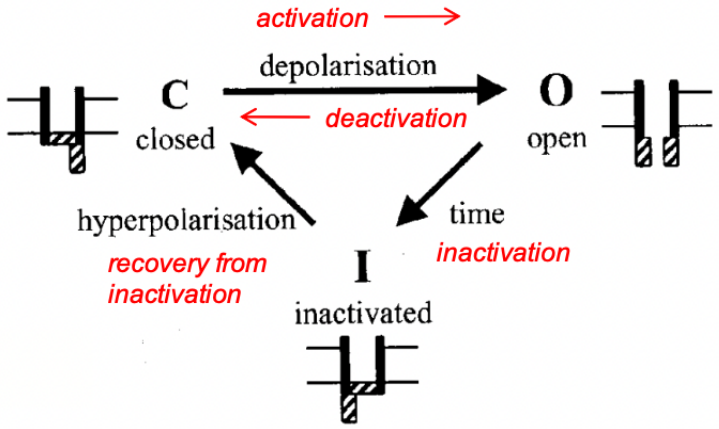
- Return level = -65mV
    - Sudden dip = sudden increase in driving force
    - Current magnitude decreases after due to decreased conductance
        - Na channels start to close when dropped to -65mV → it takes time to close
- Return level = + 55mV
    - Na current magnitude suddenly decrease due reduction in driving force
    - Na conductance slowly decreases at +55mV due to closing of Na channels

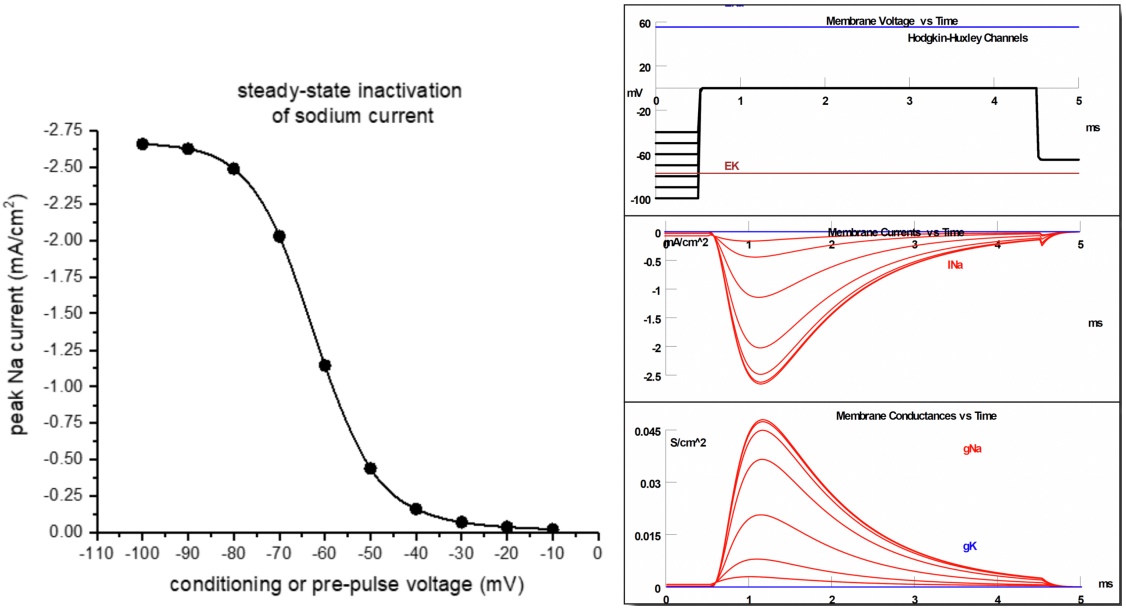
Return level = -65 mV (default) **Return level = +55.4 mV**



1.5 Demonstrate inactivation of the Na conductance

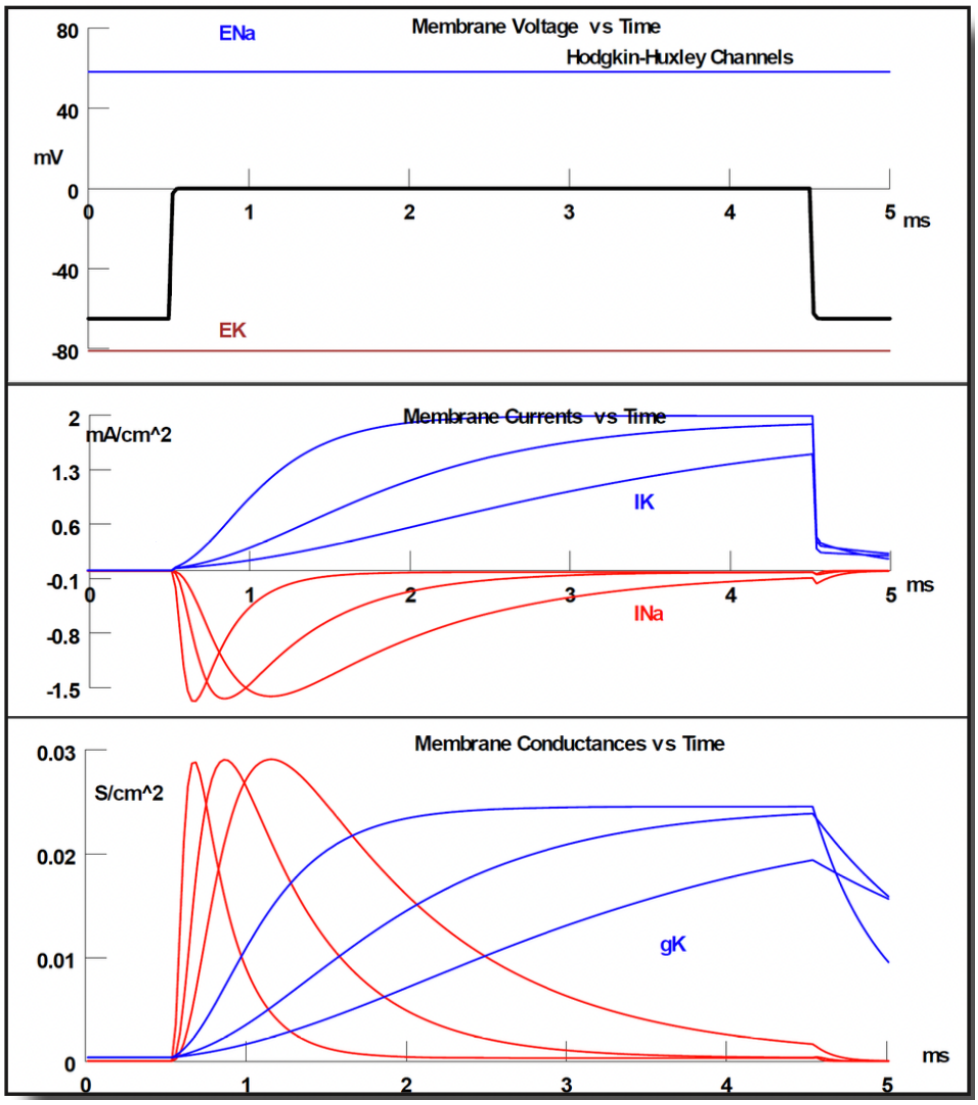
- Lower conditioning voltage = more peak Na current
    - More Na channels are <u>recovered from inactivated</u> state to <u>closed</u> state and be able to open up depolarization
    - With approximately 50% of Na channels inactivated at the resting membrane potential of -65 mV, a strong HYPERPOLARIZATION will relieve the inactivation of the 50% of the remaining Na channels.





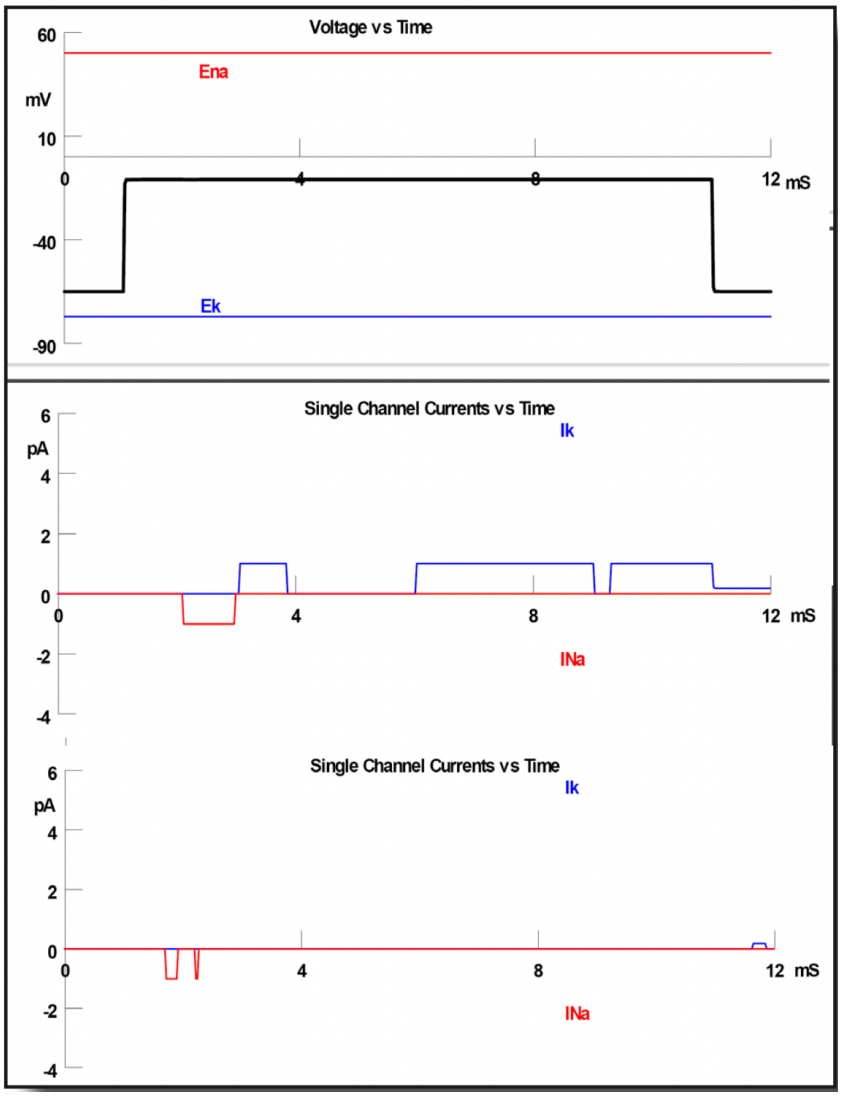
1.6 Observe the effect of temperature on the Na and K conductance kinetics

- Higher temperature = shorter time span of INa activity/duration
    - Na current lasts shorter



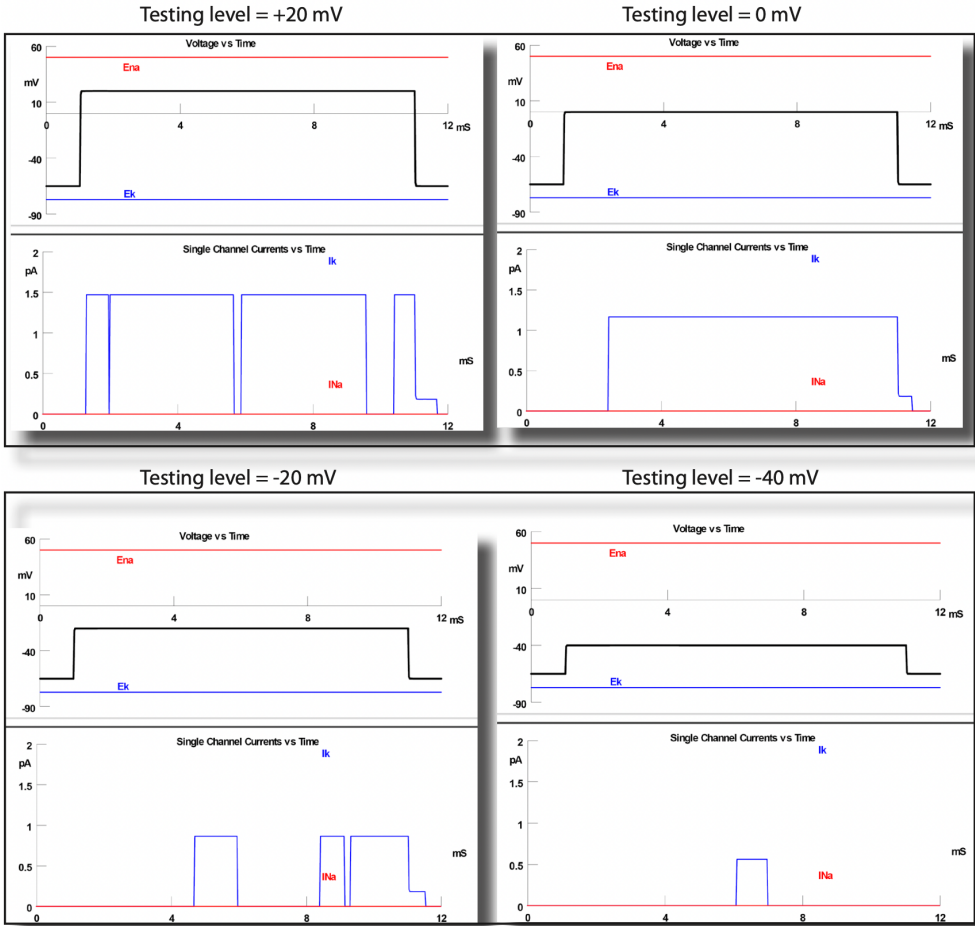
2.1 Observe Na and K channels in a patch in response to a depolarizing voltage step

- single sodium channel in the patch NOT ALWAYS opens and conducts an inward (negative) Na current in response to the strong membrane depolarization (-65 to 0 mV)
- When the Na current appearing during a sweep elicited by the -65 mV to 0 mV voltage step in the patch, the single Na channel NOT ALWAYS opens and passes Na current at the 1.638 ms average opening time point
- The Na channel after opening upon membrane depolarization, can close and reopen again, potentially many times before eventually succumbing to a state of inactivation (a state of refractoriness to channel opening)
- Openings of Na or K channels can occur at resting membrane potentials of -65 mV



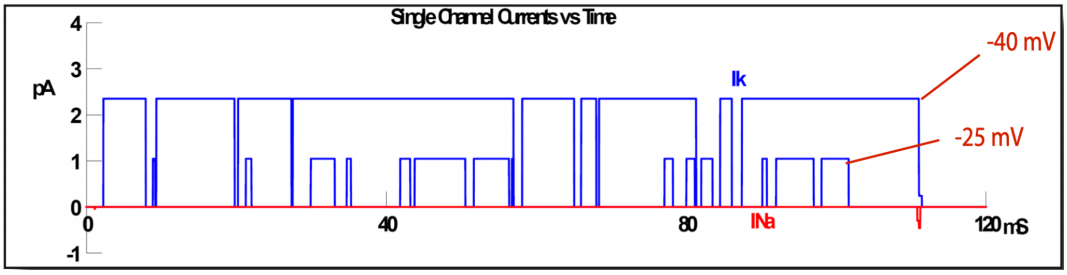
2.2 Observe single K channels in a patch in response to depolarizing voltage steps

- The observed behavior of single K channels indicate that K channels ARE NOT always, but USUALLY slower to open, and open for longer periods over the 110 ms duration of the depolarizing voltage step than single Na channels
- Greater depolarization = open early and longer (on average)
- Random occurrence



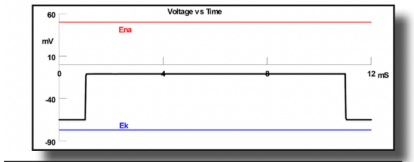
2.3 Observe the effect of membrane potential on the time that the channel spends in the open state.

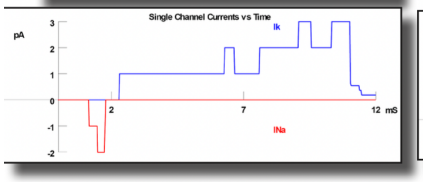
- The Na and K channel in the patch tends to open <u>more often, for longer mean open times and tends to open sooner</u> in the voltage step in response to the larger depolarization to +10 mV compared to a smaller depolarization to -35 mV



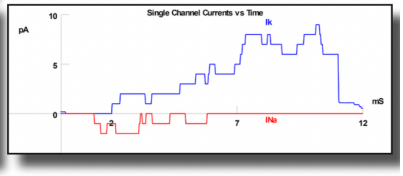
2.4 Change the number of channels in the patch.

- You can NEVER predict the behavior of a single ion channel (e.g. whether a single ion channel will open in response to a particular voltage stimulus in voltage-clamp), but the behavior of 10,000 ion channels will approach its expected probability and will result in the same approximate behavior observed in every sweep.
- Decay for Na starts at 2 ms due to inactivation of Na channel

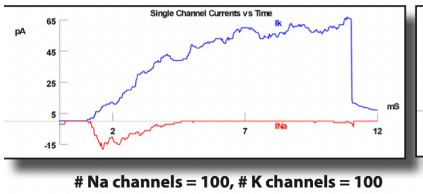




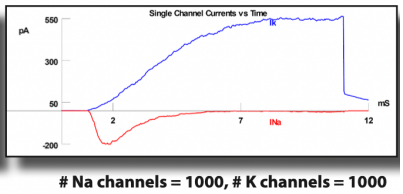
Na channels = 3, # K channels = 3



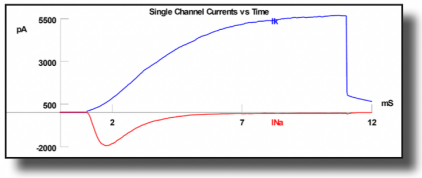
Na channels = 10, # K channels = 10



Na channels = 100, # K channels = 100



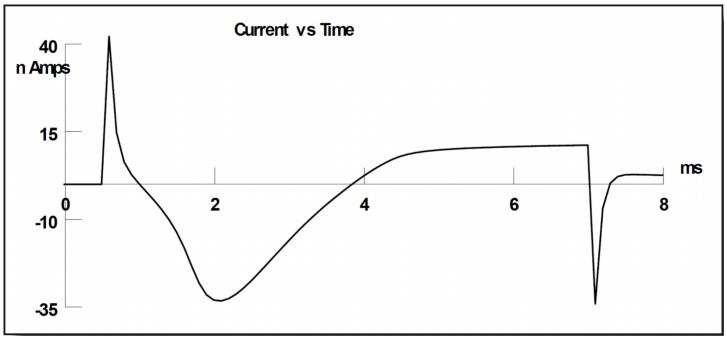
Na channels = 1000, # K channels = 1000



Na channels = 10,000, # K channels = 10,000

3.1 Clamp the soma with an ideal, low-resistance electrode and measure the resulting current

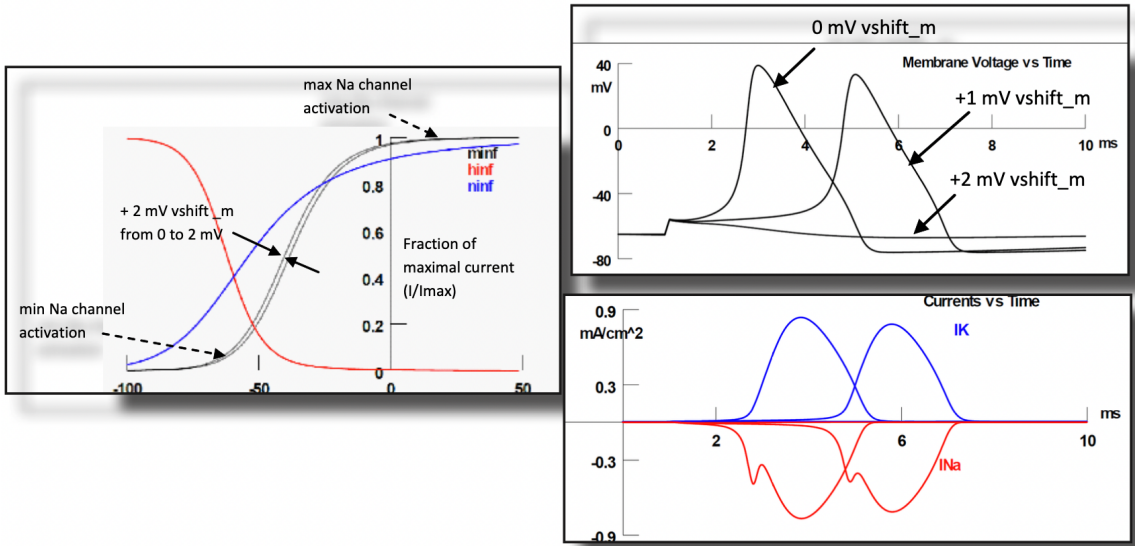
- Icap + Iion = Itot
- This current, Icap, charges and discharges the membrane capacitance. The lower the electrode resistance, the higher the current and the more quickly the capacitance is charged and discharged
- An inward Na current and then an outward K current follows the Icap



- Dendrites are slower to follow the command voltage than the cell soma under voltage clamp of neurons in “real” conditions, because of their much larger surface area of membrane.
- It is not possible to voltage clamp thin and long neuronal processes, such as axons, because of their HIGH internal resistance and SHORT length constant
- A high series resistance (due to the pipette and cellular access resistance in series) INCREASES the time constant (tau, τ), contributing to an “out of control” voltage clamp, just like how high resistant neuronal processes contribute to an “out of control” voltage clamp.

4.1 Shift the activation (m) curve for the Na channels on the voltage axis in the depolarizing direction

- The activation gate (or “m” gate) of Na channels has a steep-voltage dependence for opening, with a half-maximal activation at ~45 mV, maximal activation above 0 mV and initiation of channel opening above rest, near -60 mV.
- A small depolarizing shift in the activation curve of +1 mV, delays action potential generation, and action potential fails to develop with a greater shift in activation of +2 mV
    - The right shift allows for the additional opening time for K+, which hyperpolarizes the membrane and delay AP → decrease in excitability
    - When the activation curve is shifted to more depolarized potentials, fewer Na channels are recruited to conduct at each voltage level to generate an action potential.

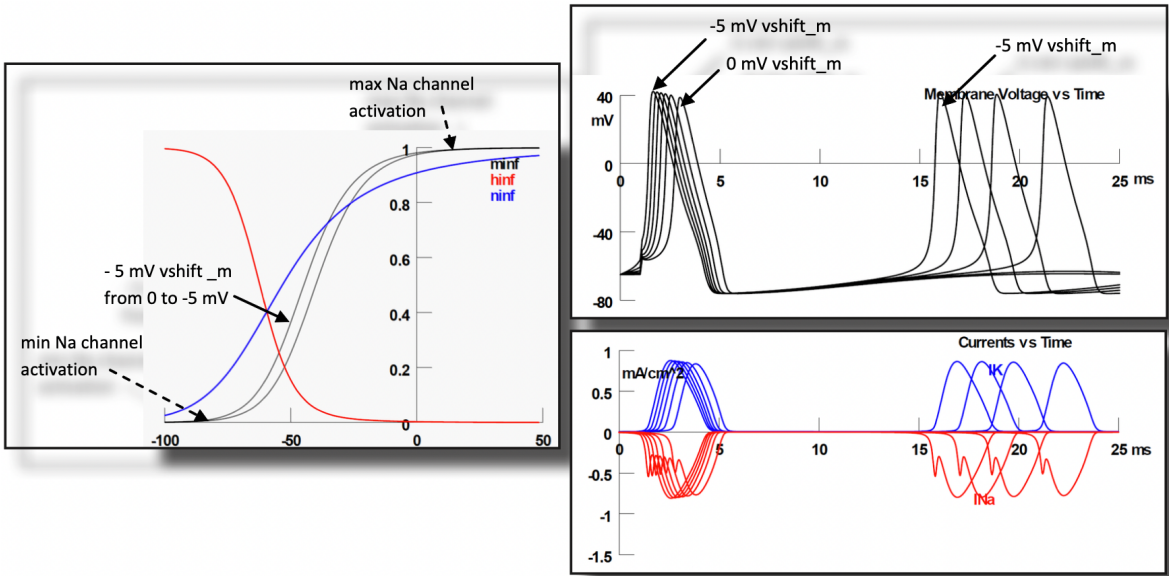


4.2 Examine how a strong depolarization can compensate for the decrease in excitability caused by a rightward shift the activation (m) curve for the Na channels:

- Larger stimulus brings the membrane potential higher (V=IR) → compensate for lower excitability → generate AP by reaching the opening threshold for Na quicker

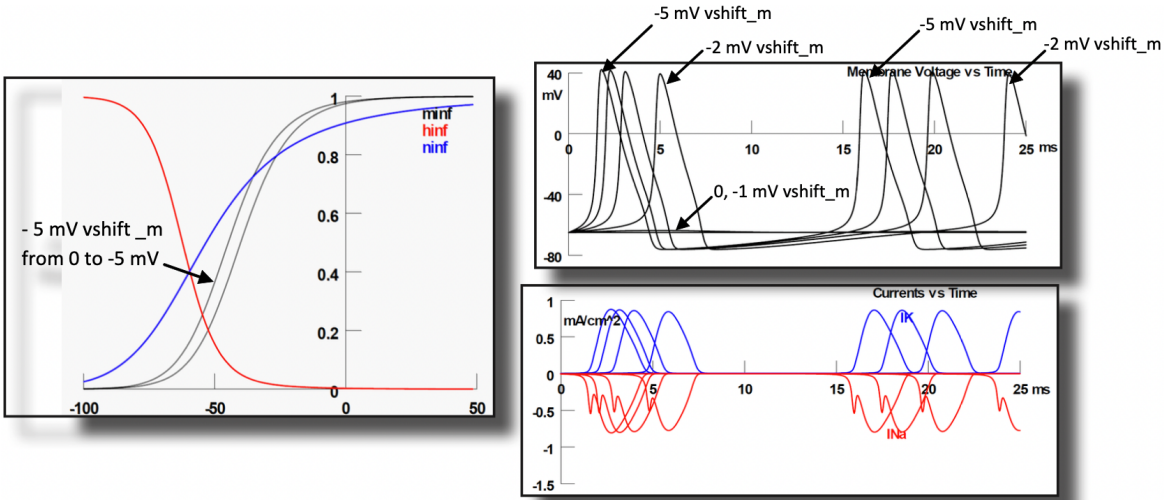
4.3 Shift the activation (m) curve for the Na channels on the voltage axis in the hyperpolarizing direction:

- We have removed the stimulus current, so when action potentials start to appear with hyperpolarizing, negative shifts in the “m gate” → <u>spontaneous, pace-making action potentials</u>, generated by “low threshold” Na channels
- A higher Na channel density is conducted at resting membrane potentials, generating a faster rate of rise in the action potential upstroke, after repolarization of the previous action potential, leading to a <u>higher frequency of action potentials</u>.



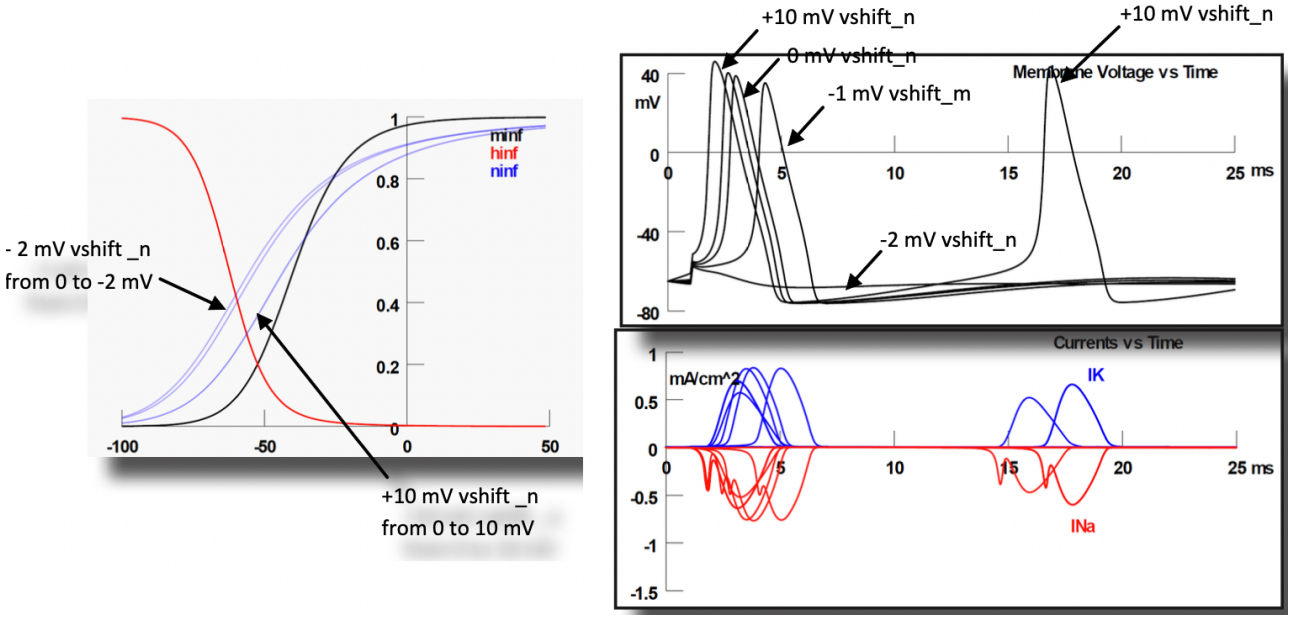
4.4 Show how the membrane will spontaneous firing action potentials in the absence of any stimulus with a shift in the m relation in the hyperpolarizing direction:

- observing spontaneous, pace-making action potentials, generated by “low threshold” Na channels. The more left-shifted the “m gate” of the activation curve, the greater the fraction of Na channels that are open and conducting at more negative potentials, such as the resting membrane potential
    - Ex: Cav3 T-type channels → open at resting membrane potential, peak current at -30mV → drive pace-making in the sinoatrial node of the heart
- The more negative shifting, the higher the frequency due to increased availability at resting membrane potential
- If the “m gate” is lowered so far, too many of the sodium channels are open at rest, elevating the resting membrane potential to -40 mV. At a membrane potential of -40 mV, too many of the sodium become inactivated after the action potential, but are unable to recovery from inactivation, because the membrane potential doesn’t fall below -40 mV



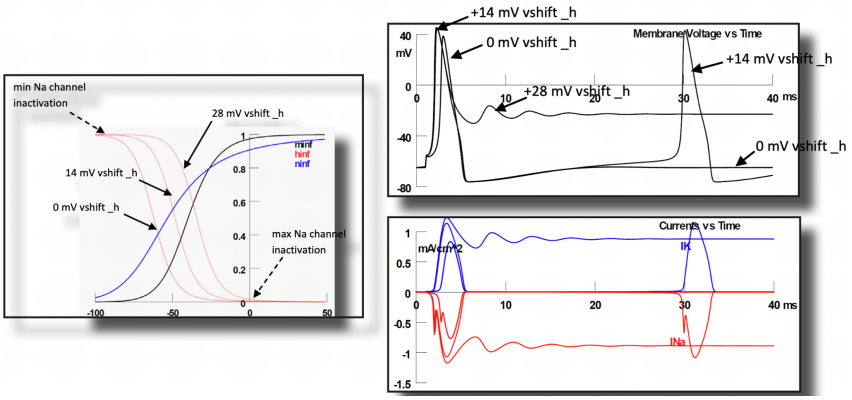
4.5 Examine how depolarizing or hyperpolarizing shifts in the activation relation for the K channels (n) influence the membrane excitability:

- Depolarizing (positive) shifts in K channel activation curves increase the rate of rise and the peak amplitude of action potentials and increase the frequency of firing of action potentials
    - as the Na current influx is increasingly less opposed by K efflux
    - At a very positive shift in the "n gate" activation curve, the K channels "n gate" is so positively-shifted, not enough K channels are recruited at depolarizing potentials to properly repolarize the membrane, sustaining an elevated membrane potential after a few action potentials are generated
- Hyperpolarizing shifts in K channel activation curves lowers the membrane excitability
- The differences in the time of the rise of the "**m (activation) gate**" of Na channels and **"n (activation) gate"** of K channels explains how Na influx can "win over" K efflux in generation of an action potential, even though the voltage-dependence of the Na and K channel conductances (activation curves) can be highly similar.



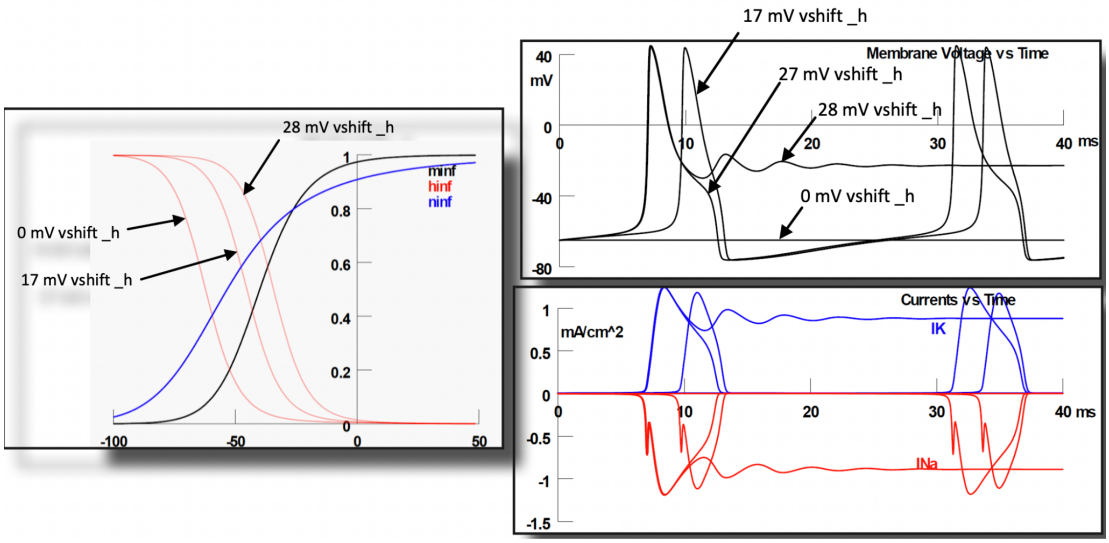
4.6 Experiment with rightward shifts in the inactivation curve (h) of the Na channel

- The h relation describes the fraction of channels that are probably refractory or in the inactivated state at a given voltage, and the remaining fraction are Na channels available for opening at a given voltage
    - At 1 → minimal Na channel inactivation
- The inactivation curve is **steeply voltage dependent**, so that at very hyperpolarized potentials (eg. -100 mV) essentially all of the channels are available for opening
    - As you move to more depolarized potentials (-70 mV), ~ 50% of the Na channels are available for opening and no channels are available at 0 mV.
- Right shifts in the Na channel inactivation curves +14 mV in the depolarization (positive) direction causes a decrease in action potential rise time, increase in amplitude and firing frequency.
- Even greater shifts of +28 mV prevents adequate repolarization of the action potential → too many Na channels are not inactivated and ready for opening
- h (inactivation) gate is time dependent and and voltage dependent
    - most (~93%) of the Na channels are refractory by the mid-point of repolarization → necessary refractory period for a recovery time from Na channel inactivation
    - The Na channels are “reset” from their refractoriness, and the h relation rapidly rises, during the hyperpolarization below resting membrane potentials (-75 mV to -65 mV)
- Only ~ 60% of Na channels are available to contribute to generating action potentials, because of the residual inactivation of Na channels at resting membrane potentials (-65 mV). The greater the DEPOLARIZATION of the membrane potential, the greater the percentage of Na channels become inactivated. The recovery rate from inactivation is driven by HYPERPOLARIZATION which removes inactivation



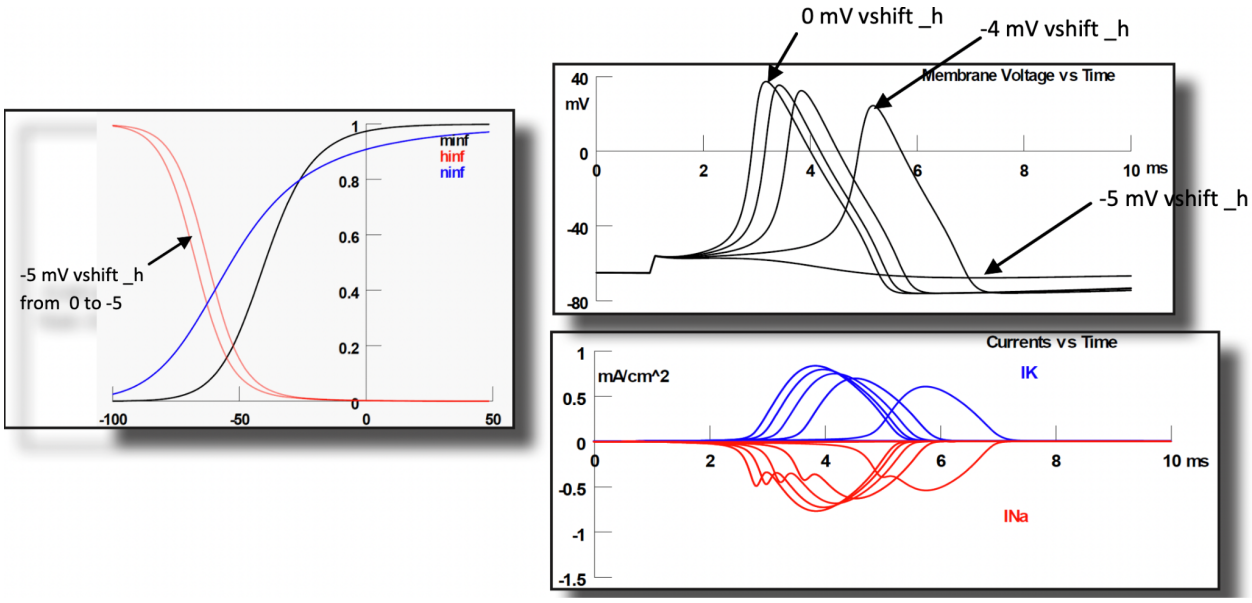
4.7 Examine how a rightward shift in the inactivation curve (h) of the Na channel will promote spontaneous action potential firing

- For same voltage, fewer channels are inactivated
- When Na channel inactivation curves are right shifted in the depolarization direction by +17 mV, the cell will spontaneously fire without any current stimulus. → more channels are available at resting membrane potential to fire AP since fewer are inactivated
- Further depolarizations to +27 mV and the <u>rate of action potential generation increases</u> and repolarization slows
- At a +28 mV depolarizing shift <u>repolarization is incomplete</u> after the first action potential is generated → too many channels are not inactivated, drives up membrane potential
- By shifting the “h gate” to <u>very positive potentials</u>, the Na channel eventually undergoes <u>very little inactivation</u>, because the inactivation state is so “high threshold” of a voltage to reach.
    - The membrane excitability for generating spontaneous, pacemaking action potentials is derived from the high probability of Na channel openings from the increasingly large pool of potentially conducting non-inactivated Na channels to meet the threshold of action potential generation.
    - Na channels are opening and closing, but are not significantly inactivating in response to large rightward voltage shifts for the “h” inactivation gate



4.8 Experiment with leftward shifts in the inactivation curve (h) of the Na channel.

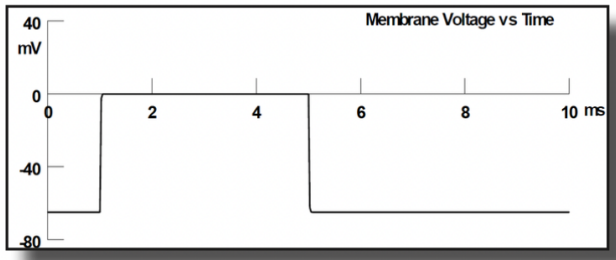
- With increasing hyperpolarizing (negative) shifts (h) in Na channel inactivation from 0 mV to -4 mV, the AP amplitude decreases and rise time increases.
    - more channels are inactivated and not able to conduct AP
- At a hyperpolarization shift of -5 mV, the action potential fails.
    - Hyperpolarization shifts in Na channel inactivation curves that retard AP can be overcome by **increasing the stimulus current amplitude**.
- When the inactivation curve is shifted in the negative direction (to the left), there are more Na channels that are inactivated at each membrane potential along the inactivation curve (i.e. the **h relation** is lower in value), including at resting membrane potentials (-65 mV). With fewer sodium channels available, not enough Na channels are recruited by the current stimulus to generate an action potential.
    - increase the amplitude of the current stimulus, to generate an action potential, by recruiting Na channels of the pool which are not inactivated.

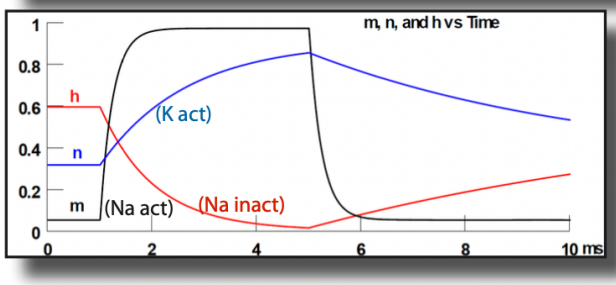


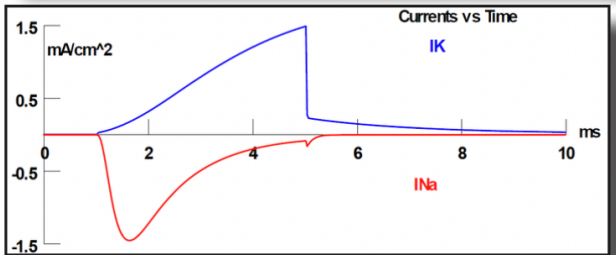
4.9 Observe changes in the Na and K currents with m,h,n curve shifts in voltage clamp.

4.10 Observe m and h during the action potential.

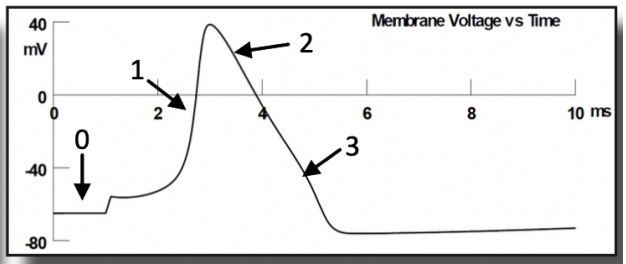
voltage step (-65 mV to 0 mV)

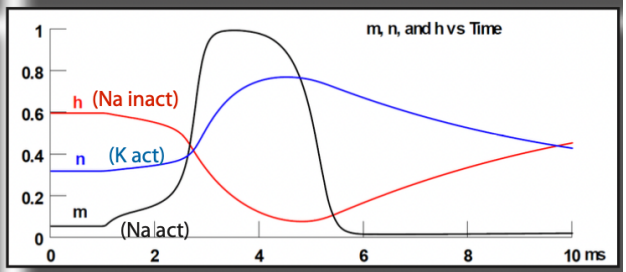


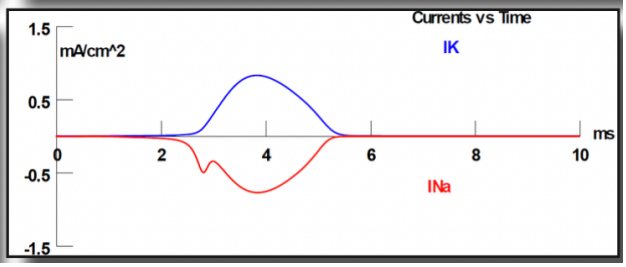




action potential

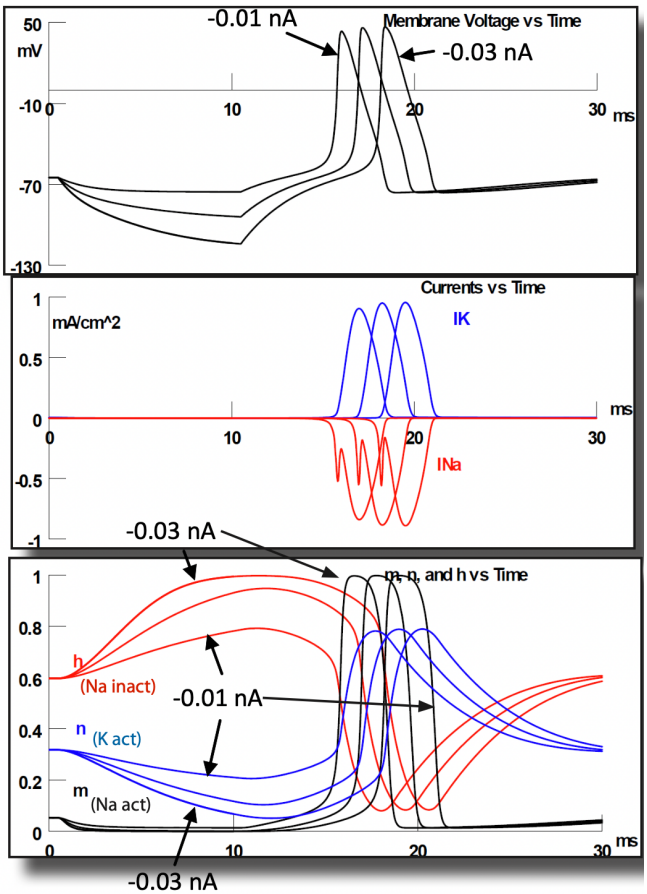






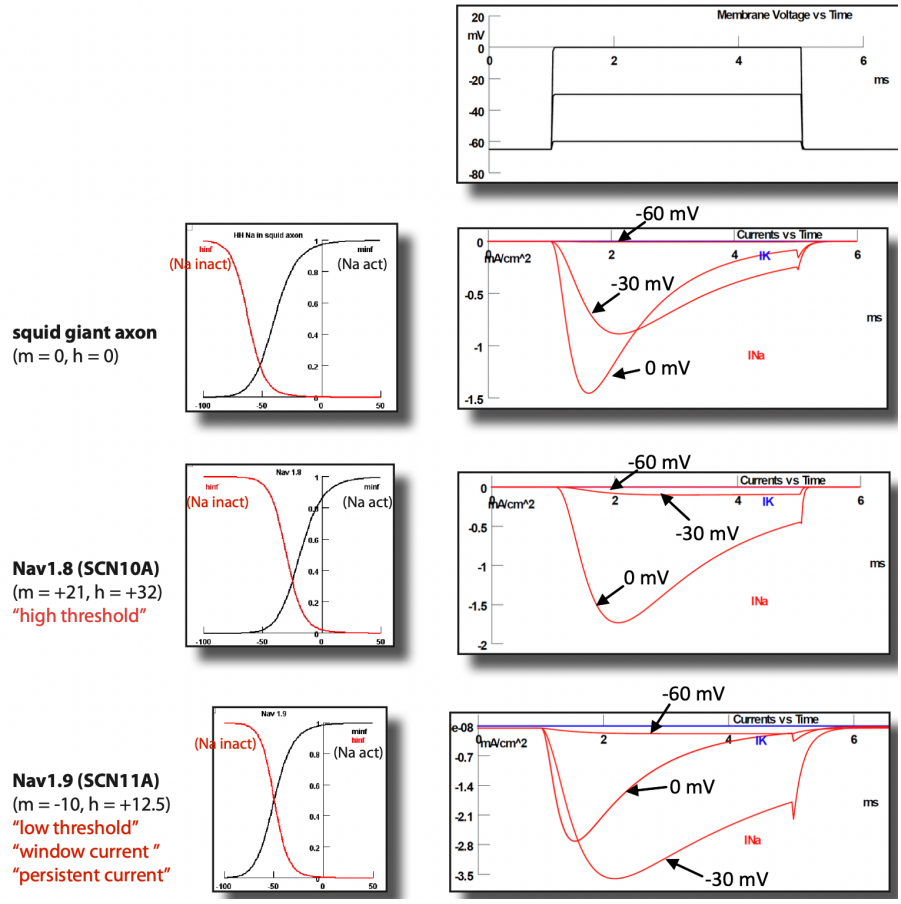
4.11 Understand what is occurring during anode break excitation

- Delivering a hyperpolarizing stimulus, relieves Na channel inactivation (h), and stimulates action potential generation after the hyperpolarizing stimulus has been interrupted
    - greater AP amplitude with greater hyperpolarizing stimulus
- Stronger hyperpolarizing pulses will relieve more Na channel inactivation (h). During the hyperpolarizing stimulus, Na channels (m gates) and K channels (n gates) are more reluctant to open (m and n values are lower). The delay to action potential firing is longer with stronger hyperpolarizing pulses
    - longer time to generate AP, but rise with faster rate
- In synapses, stronger hyperpolarizing pulses are equivalent to strong inhibitory presynaptic inputs, which would alter the timing of the postsynaptic action potential firing. This modulation of timing of the generation of action potential is crucial for integration of synaptic circuits. Anode break excitation is also known as an “off response” or “disinhibition” → release inactivated channels



4.12+13 Simulate different Na channel subtypes:

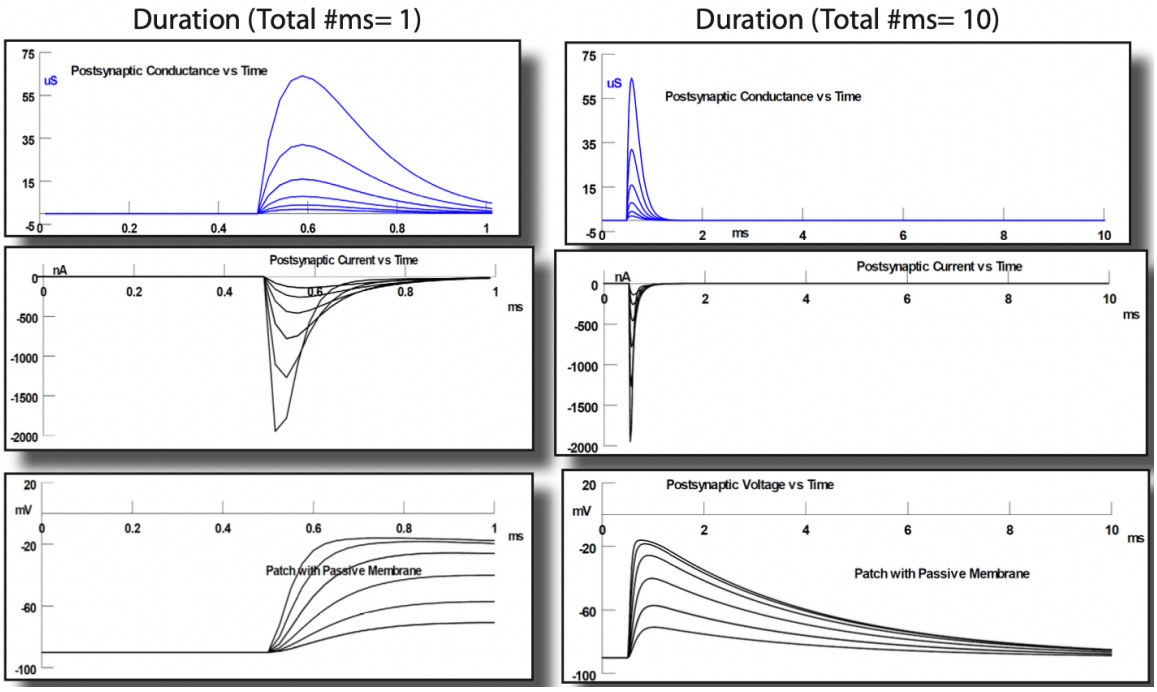
- Window Current
  - where m values are <u>above zero</u> (meaning a fraction of the Na channels are open) and h values are also above <u>zero too</u> (meaning that a fraction of the Na channels are not inactivated and available for opening).
  - The window current results in a small, but "**persistent Na current**" at rest.
  - At rest, Nav1.9 is depolarizing the membrane to first action potentials, especially if unimpeded by outward rectifying, repolarizing K channel current at rest. Nav1.9 destabilizes the resting state, so that action potentials are repeatedly firing in bursts of action potentials without intervals of rest in between.



Tutorial: Synapse

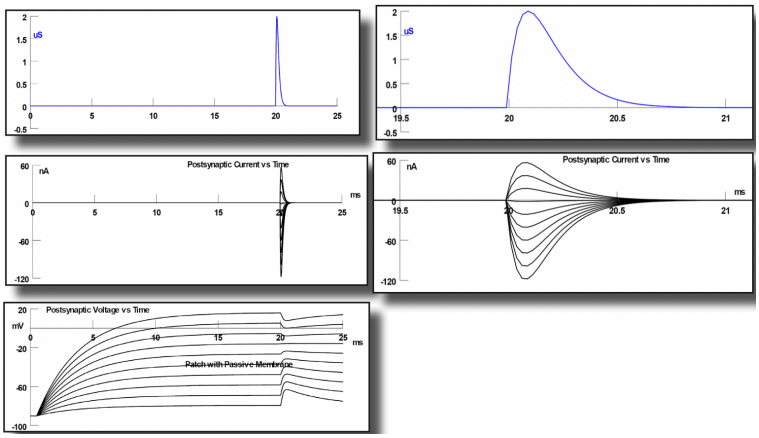
1.1 Observe the relation between synaptic strength (conductance) and EPP amplitude

- AchR do not allow Na+ and K+ ions to pass unless Ach is bound to it
    - the rise in synaptic conductance corresponds with Ach bound to AchRs and its decline after corresponds to the dissociation of Ach from AchRs
- The reversal potential of the AchR is -15 mV, which is approximately half-way between the reversal potentials for K ions and Na ions: EK = -80 mV and the ENa = 50 mV, since the AchR passes both Na+ and K+ ions in equal measure
    - Greater conductance = greater current
- Current duration is shorter than conductance because the opening will allow the membrane to reach reversal potential, which means no current
- The falling phase of the EPSP is determined by how quickly the membrane capacitance can discharge: that is, by how quickly charge can leak back out through the membrane → EPSP is longer than conductance opening
- At the end of synaptic current → membrane discharges capacitive and repolarizes the membrane



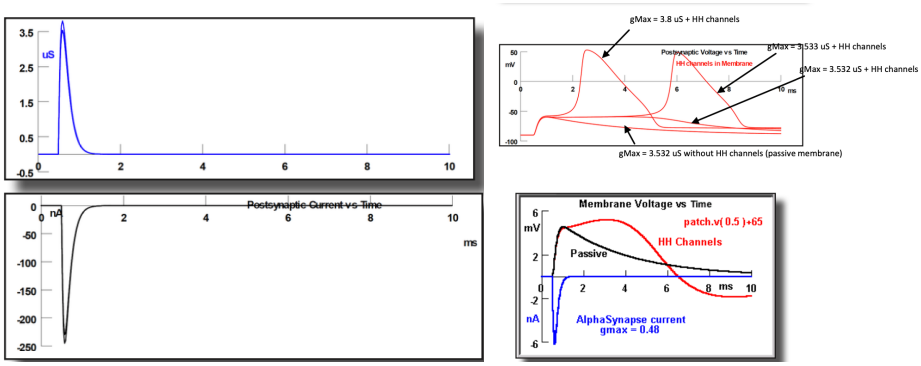
1.2 Determine the reversal potential of the ACh-gated EPP

- For all voltages between -80 and 50 (if there were ion flows) → Na in, K out
- Above reversal potential of AChR (-15mV) → net current out
    - We have more K current than Na current
- Below reversal potential of AChR → net current in
    - We have more Na current than K current
- At reversal potential of ACh → equal Na and K current = no net current

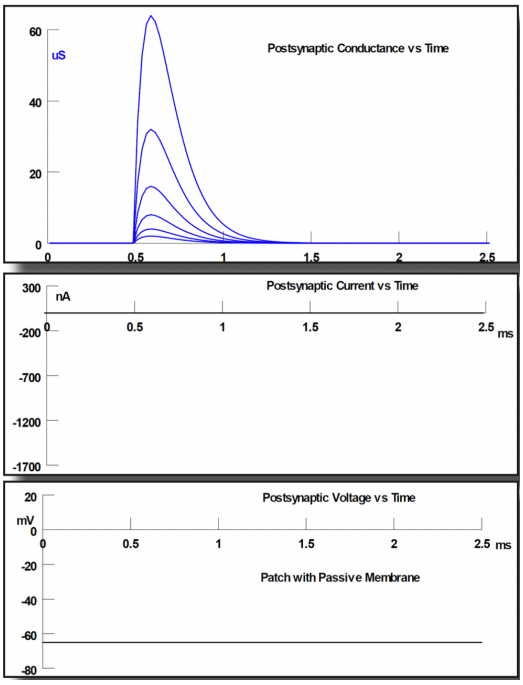


1.3 How do voltage-sensitive channels affect the shape of an EPSP?

- A nerve synapse may have a dendritic area containing thousands of synaptic terminals at tertiary, dendritic spines, <u>requiring summation of many coincident presynaptic inputs</u>, in order to ensure a total synaptic conductance (gmax, μs) in the dendrite above a threshold activation of Na channels to generate an overshooting action potential that conducts along nerve axons.
- HH Na channels maintain the subthreshold depolarization
- Enough synaptic current must be used to increase the membrane potential above the activation of HH Na channels for EPSP

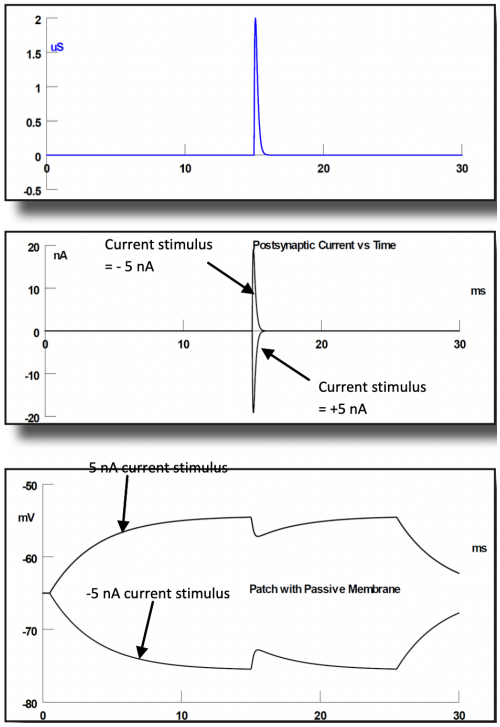


2.1 Observe the conductance, current, and voltage change (IPSP) in response to a pulse of an inhibitory transmitter.

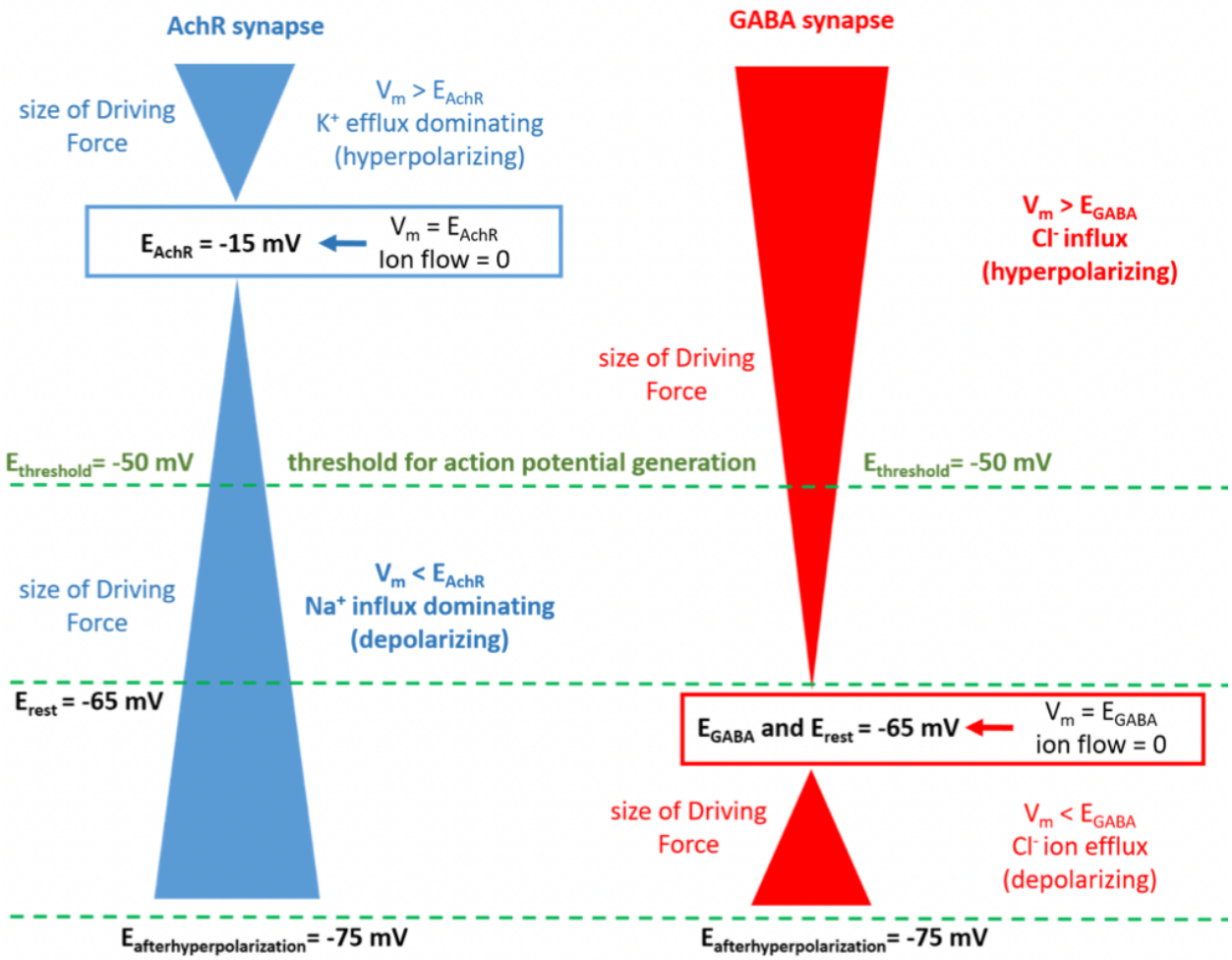


- IPSP: caused by increase in K and Cl conductance
    - GABA A and Glycine: increase Cl- influx (eq potential for Cl- = -65 mV)
    - GABA B and muscarinic ACh (M2M4): increase K+ efflux
- EGABAR = -65mV → No synaptic current or generation of IPSP around resting mbm
    - Above -65mV → GABA causes Cl- flows in and generate IPSP
    - Below -65mV → GABA causes Cl- flow out and generate EPSP
    - if the reversal potential is more positive than threshold, excitation results: inhibition occurs if the reversal potential is more negative than threshold.

2.2 Revealing the IPSP

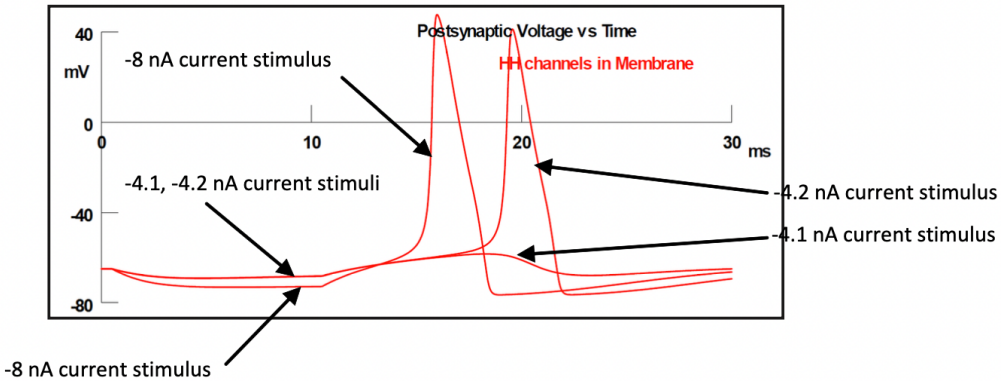


- GABA switches from being inhibitory to excitatory in synapses between mature versus immature neurons and in circadian clock neurons during the day versus night, because of the changing expression of chloride transporters in neuronal membranes
    - Reverse the gradient of Cl- changes the direction of Cl- flow
- AChR (-15mV) is above the threshold for AP → always excitatory (EPSP)



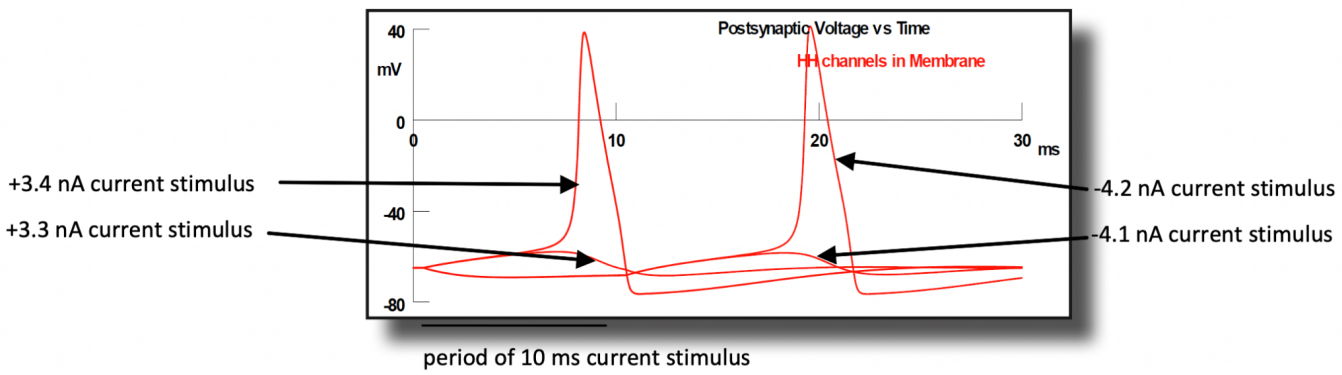
2.3 Excite a cell by disinhibition to generate an off-response action potential

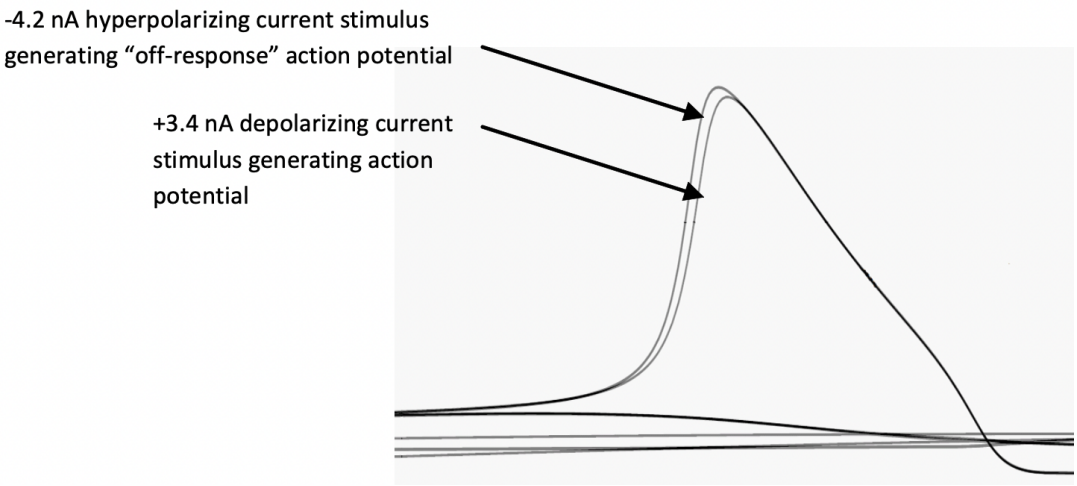
- Disinhibition = reactivation, "off response" = generate AP
- Trains of IPSPs or continuously released GABA will prolong inhibition
- At rest, a large fraction of Na channels are inactivated, but the recovery from inactivation is voltage-sensitive → <u>more hyperpolarization, more Na channels recover from inactivation are available</u> → more likely to generate a faster AP



2.4 Comparison of "off response" action potentials with action potentials elicited by depolarizing current pulses

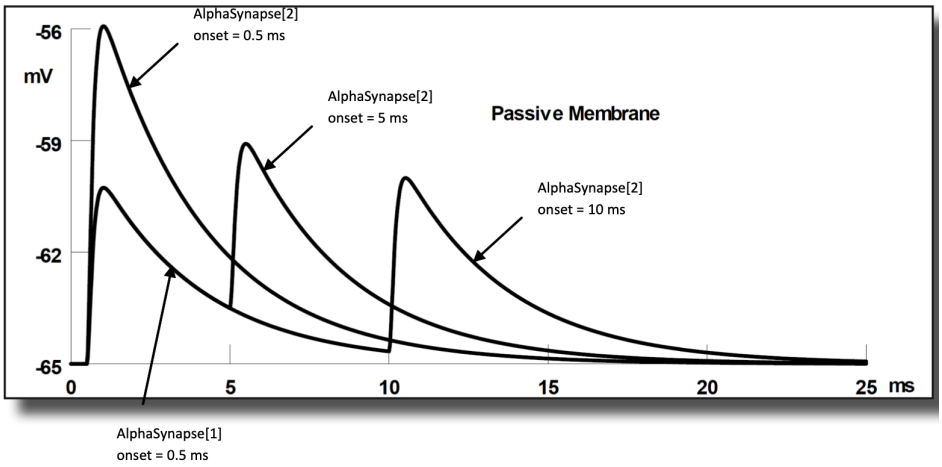
- A train of IPSPs generated from the synaptic conductance of inhibitory GABA receptors in the postsynaptic membrane can generate a faster rate of rise and higher peak voltage amplitude of action potentials than a train of EPSPs generated from the synaptic conductance of excitatory Ach receptors
    - Na channels are inactivated at rest, and the effect of a prolonged hyperpolarization is to remove Na channel inactivation, and provide a greater pool of available sodium channels for generating a faster and higher peak action potential than can be derived from a prolonged depolarization





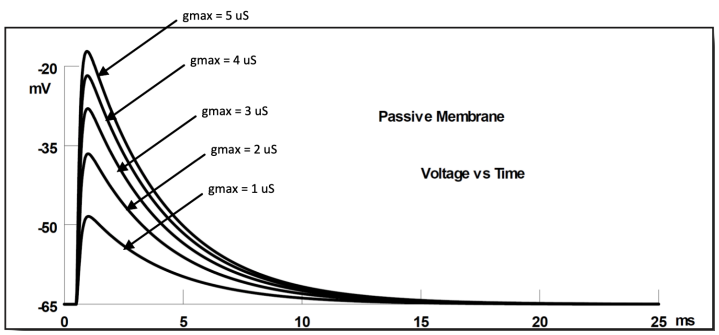
3.1 Summation of EPSPs in a passive postsynaptic membrane.

- EPSPs can sum to produce a larger EPSP
- Action potential would if the two EPSPs arrive simultaneously at the action potential
    - Above -56mV to activate Na channels → AP



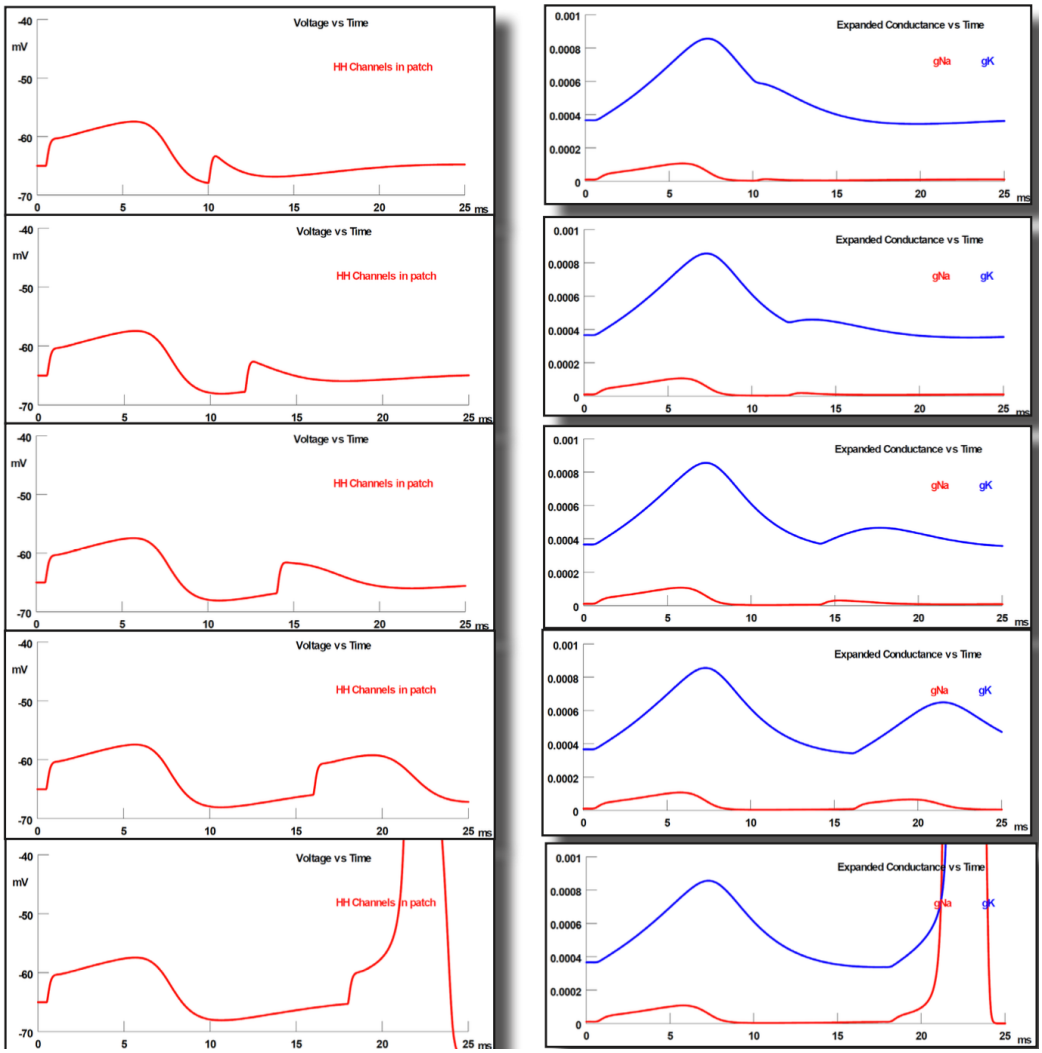
3.2 Change the conductance of each EPSP.

- When membrane voltage (V_m) rises to approach $E_{synapse}$, the current contributions at each synapse will drop, because of the <u>fall in driving force</u>, according to the equation $I = g_{max} (V_m - E_{synapse})$
- Thus when synaptic currents summate and drive membrane depolarization, each synapse contributes less and less current to facilitate voltage changes, as there is diminishing returns as the membrane potential rises and approaches the reversal potential of the synaptic receptor channel.
- Multiple excitatory synaptic inputs can summate and generate larger EPSP amplitudes, but the <u>contribution of synaptic currents are not additive</u>, and **diminish** as the <u>membrane potential approaches the reversal potential</u> for each synapse receptor channel



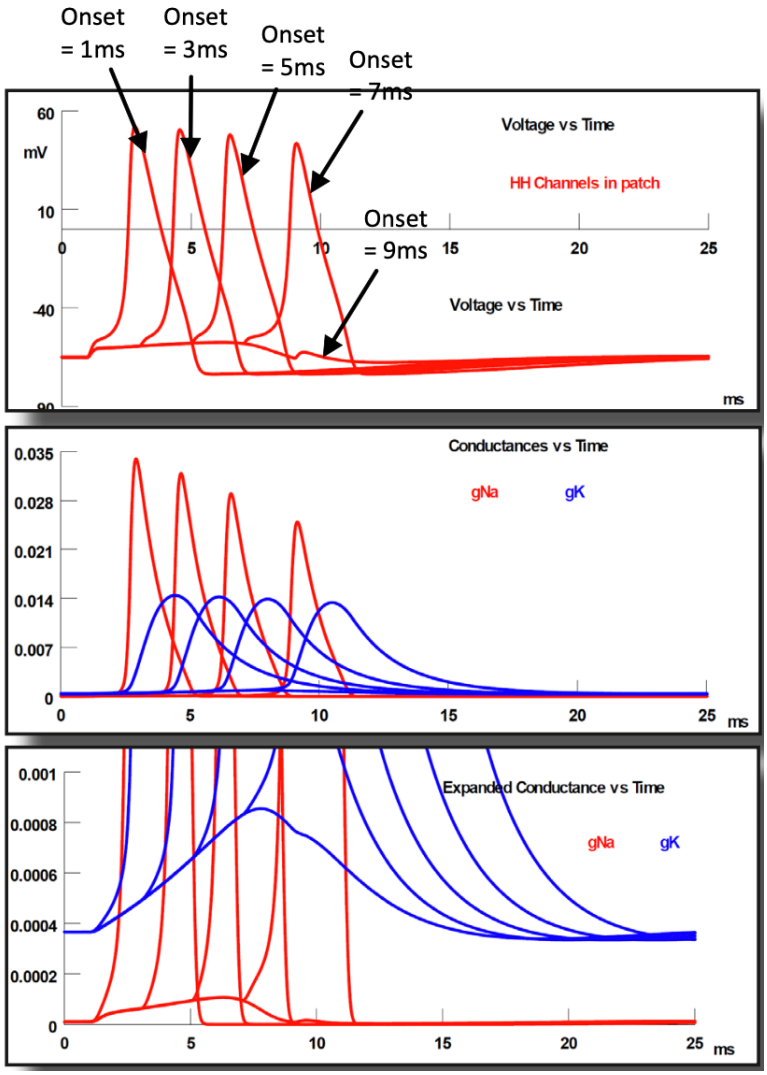
3.3 Summation of EPSPs in a postsynaptic membrane with voltage-gated Na and K channels: Time is of the essence!

- A first subthreshold EPSP will summate with a second subthreshold EPSP to generate an overshooting AP as long as it is <u>within the time period where the Na conductance is still rising</u> relative to the K conductance of the first threshold EPSP
- The summation of voltages of the two EPSPs is not linear for the conductance change in a passive membrane
    - Summation will be linear when the EPSP voltages are very small
- if a subthreshold EPSP does not reach threshold to generate an action potential, the subthreshold EPSP will INHIBIT a subsequent supra-threshold EPSP from reaching threshold to generate an action potential



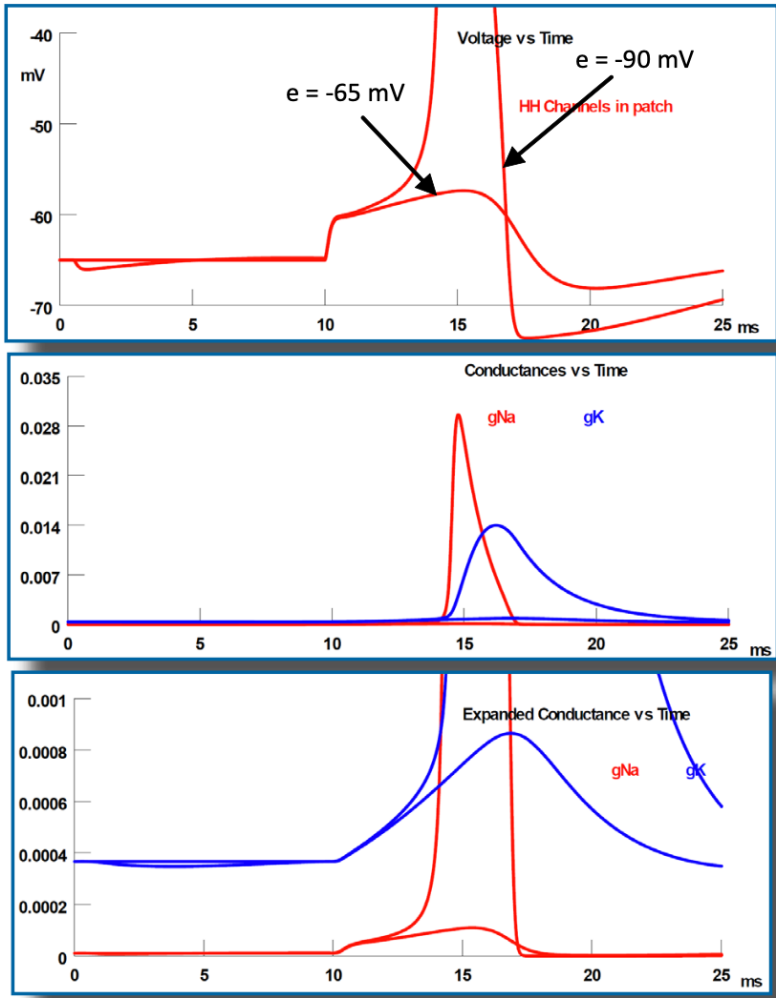
3.4 Combining two subthreshold EPSPs

- The first EPSP depolarizes the neuron for a progressively longer time before the onset of the second EPSP.
- However, the peak amplitude of the Na conductance generating the impulse (in response to the second EPSP) decreases markedly as the time interval between the two EPSPs increases
- If subthreshold EPSPs don't summate, and are spaced too long in time, the subthreshold EPSP creates a "**refractory period**" against subsequent action potential generation, until the voltage-gated Na and K channel conductances play out in time
- A subthreshold EPSPs can summate with another subthreshold EPSP and generate an action potential AND a subthreshold EPSP can prevent a supra-threshold EPSP from generating an action potential



3.5 What are the effects of an IPSP on membrane excitability?

- An IPSP can summate with a supra-threshold EPSP (if they overlap within a particular time window) and INHIBIT the generation of an action potential
- An IPSP can FACILITATE a subthreshold EPSP to generate an action potential, in what is termed an “off response” / “anode break excitation” after a time window for Na channels to recover from inactivation significantly in comparison to K channels
    - In addition, hyperpolarizing by IPSP also reduces voltage-gated K channels conductance to further promote AP

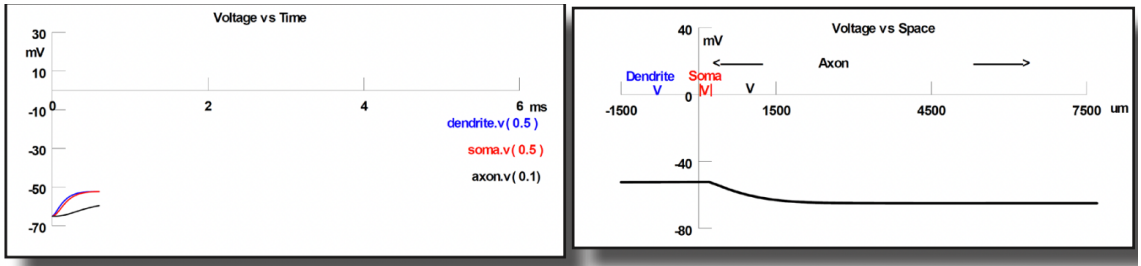


4.1 Where is the site of spike initiation? - Deliver a suprathreshold EPSP to the middle of the dendrite and study the movie and Voltage vs Time traces

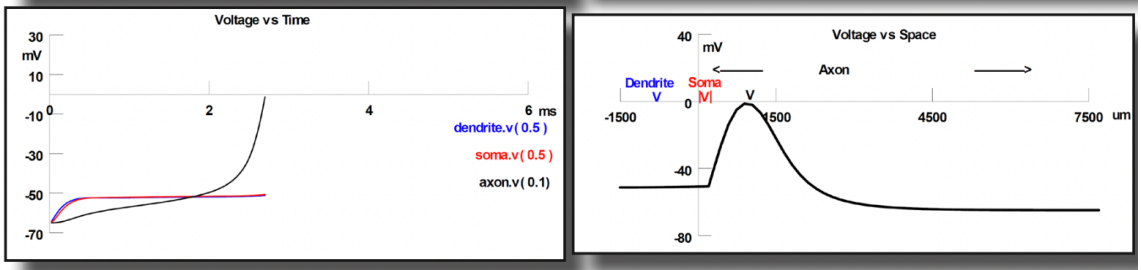
- The dendrites contain no HH ion channels compared to the soma. The dendrite depends on passive instead of active properties
    - Since the membrane contains no channels, there are no Na and K channels to help support an overshooting action potential in the dendrite
- The dendrite has a much larger membrane surface area compared to the soma
    - increase in surface area in dendrites = increased length of dendrites (1500 um) compared to the length of the soma (200 um)
        - the total diameter (200 um) is the same
    - The dendritic arbor has a high membrane capacitance (Cm) → a large current sink
- The action potential initiates first in the axon and then back propagates into soma and dendrites
- Both the soma and axon contain HH ion channels to support regenerative activity which could lead to generation of an action potential.
    - The reason why it is much easier to excite the axon to fire an action potential than the soma relates to the **small diameter of the axon** (15 um) compared to the soma (200 uM)
    - The end result is that the action potential initiates in the axon and begins propagation down its length while the soma is still struggling to generate its own action potential, dragged down by the dendritic arbor
- The action potential in the soma is of reduced amplitude compared to that in the axon. The reason for the reduced action potential amplitude relates to the slow rate of rise of the somatic action potential, because the soma has to struggle to supply current to the dendritic arbor
    - Slow rate of rise = more K+ activity to counter the current (?)
- Increasing the diameter size of the cell soma from 200 μm to 800 μm, facilitates an action potential to generate in the cell soma because there are now more Na and K channels

Conclusion: The tiny axon has much less membrane capacitance to be charged due to its small diameter and possess voltage-sensitive Na and K channels in its membrane to drive an action potential easily → reason why AP initiates in the axon

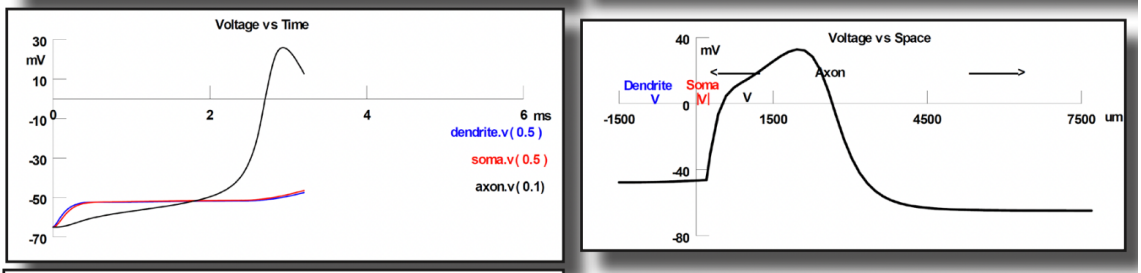
0.6 ms :



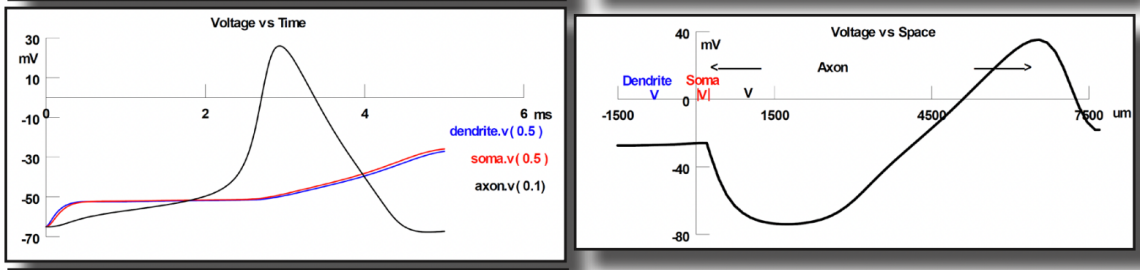
2.7 ms :



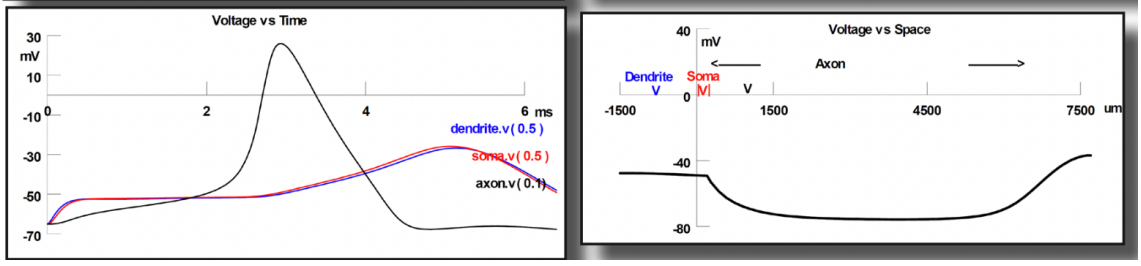
3.2 ms :



5.0 ms :



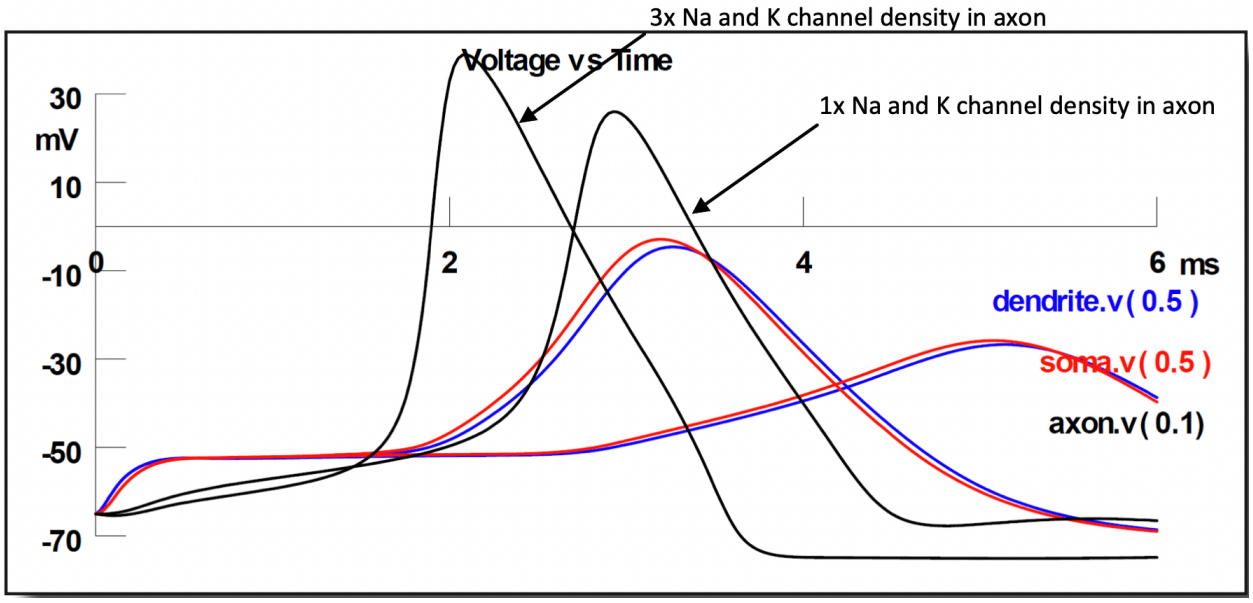
6.4 ms :



4.2 Change the ion channel densities.

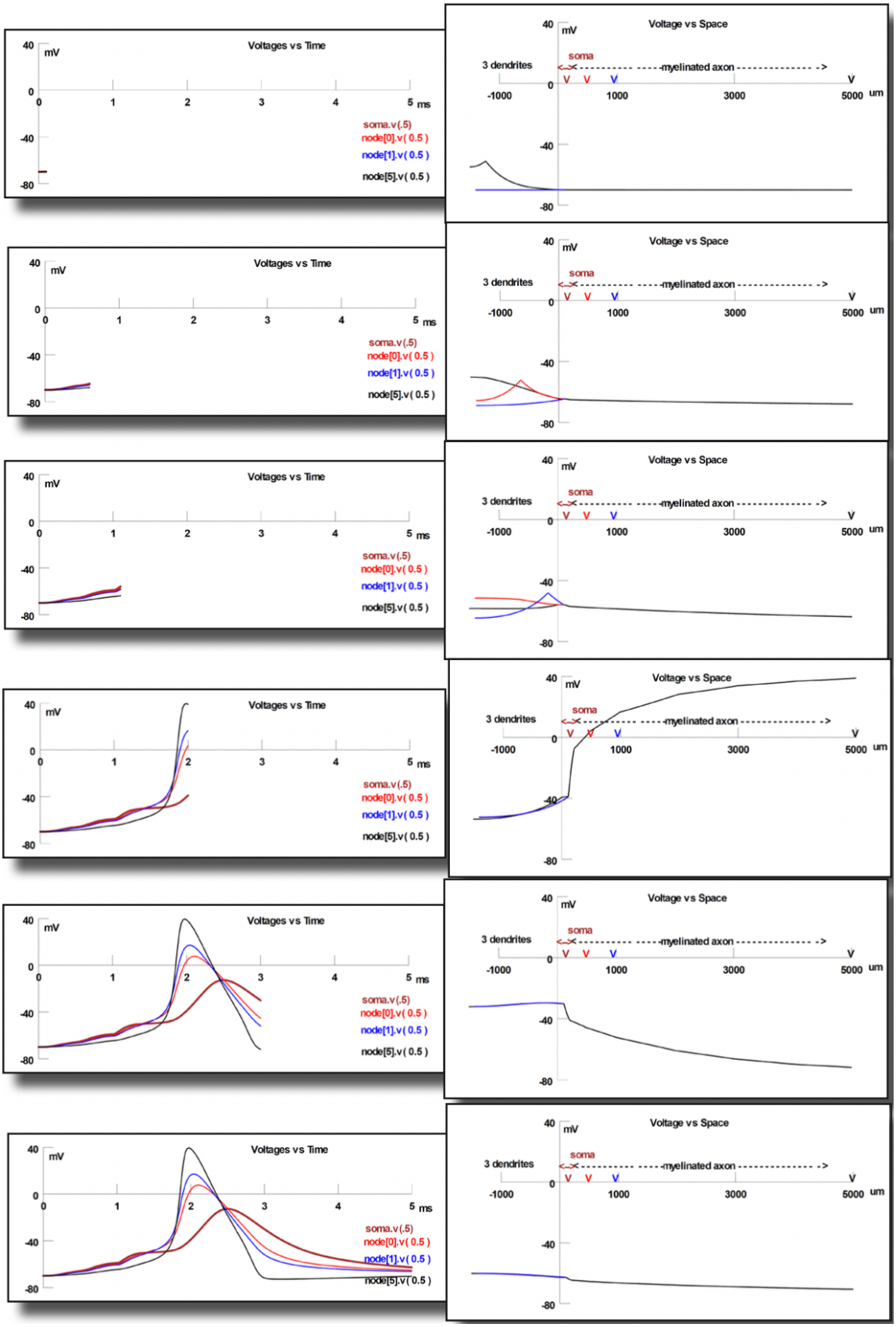
- You notice that increasing the Na and K channel densities causes an earlier action potential in the axon of higher voltage amplitude
    - More channels → less resistance → smaller tau and earlier onset
    - More powerful AP

A neuron's morphology per se causes the spike to initiate in the axon in a region close to the soma. Patch-clamp experiments on neocortical pyramidal cells in slices also localize the site of impulse initiation in the axon or initial segment. The probability that this region is the site of initiation is increased by an increased density of Na channels at this location



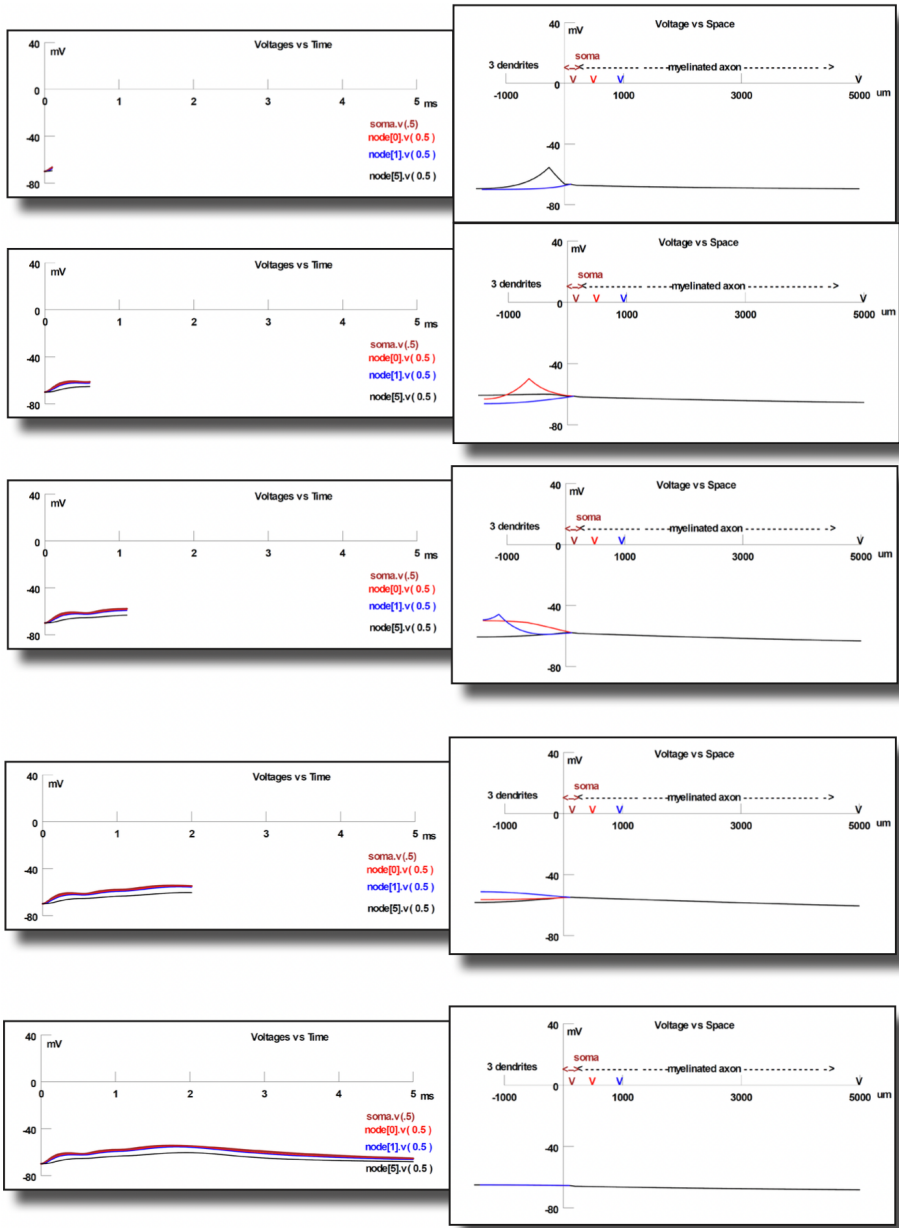
5.1 Generate EPSPs in the dendrites and observe where the spike initiates

- the action potential spike is initiated in more distal nodes of the myelinated axon, propagating back into the initial segment and from there into the soma
    - The action potential initiates in the axon initial segment because it possesses the smallest cell capacitance → shortest time constant (tau, τ) for voltage rises
    - The action potential initiates in the axon initial segment because it has a high input resistance, → voltage changes are greater with the current input from the cell soma
- The geometry of the neuron dictates how voltage changes across the neuron
    - The largest voltage drop occurs across the axon initial segment because of its small diameter (10 um) compared to the soma (100 um) while bearing the same length (100 um)
    - The longitudinal resistance is inversely proportional to the square of its diameter, and thus is a hundred-fold larger than that of the soma.
        - High longitudinal resistance in axon initial segment supports a large voltage drop due to short length constant
    - The soma is dragged down by the large surface area of the passive dendrite
        - Prevents depolarization due to high capacitance of the soma


Voltages vs Time
mV
ms
soma.v(.5)
node[0].v(0.5)
node[1].v(0.5)
node[5].v(0.5)
Voltage vs Space
3 dendrites
soma
myelinated axon
um

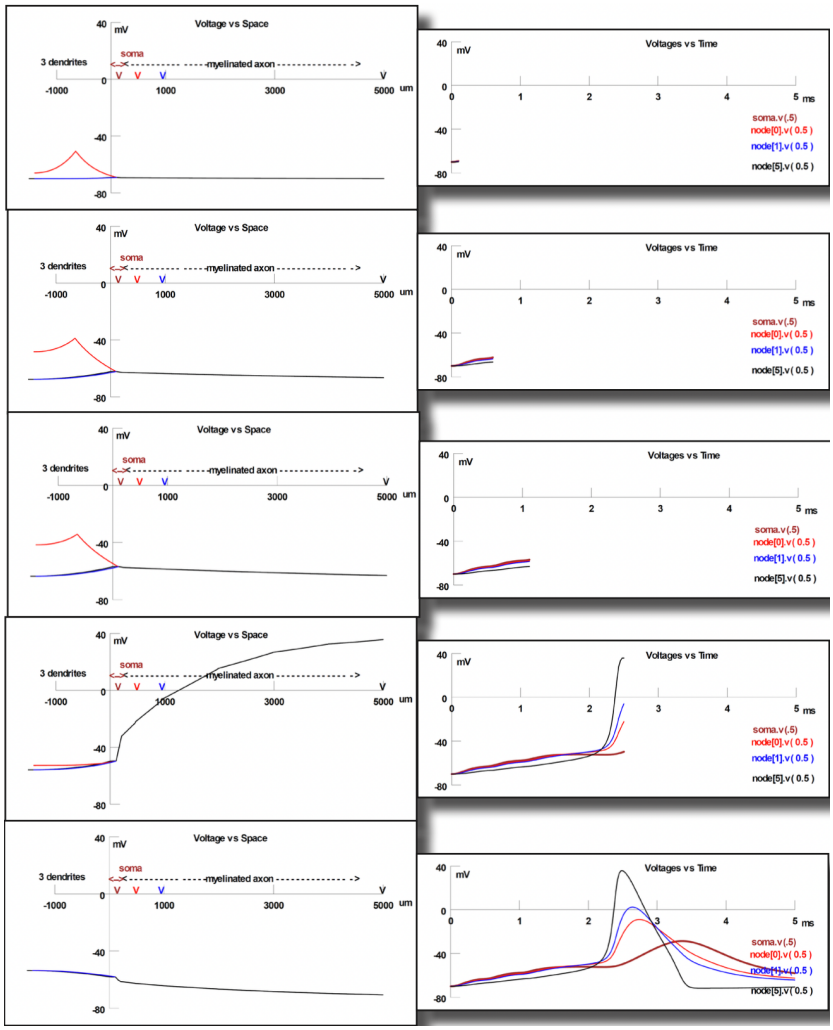
5.2 Reverse the timing or locations of the EPSPs so that the EPSP near the soma comes first (different location, different time)

- Deliver the first EPSP near the soma and the last near a dendritic
    - Action potential was not generated after this reversing of synaptic locations.
- When dendritic current is initiated first then soma current
    - Both currents can arrive at the same time and sum to generate AP
- When the soma current is initiated first then the dendritic current
    - Soma current arrives first, then the dendritic current (cannot sum) → failed to sum



5.3 Deliver a train of synaptic inputs at a single synaptic location.

- Same location at different times
- The identical conductance increases, but does not add linearly
    - Summation will only be linear when the EPSP voltages are so small that the change in voltage does not appreciably change the driving force on the synaptic current.
    - As the EPP became larger, the driving force (the difference between the voltage at the peak of the EPSP and the reversal potential of the EPP) decreased; this reduced the synaptic current as the EPP edged toward its reversal potential
        - Result: reduced driving force = less synaptic current

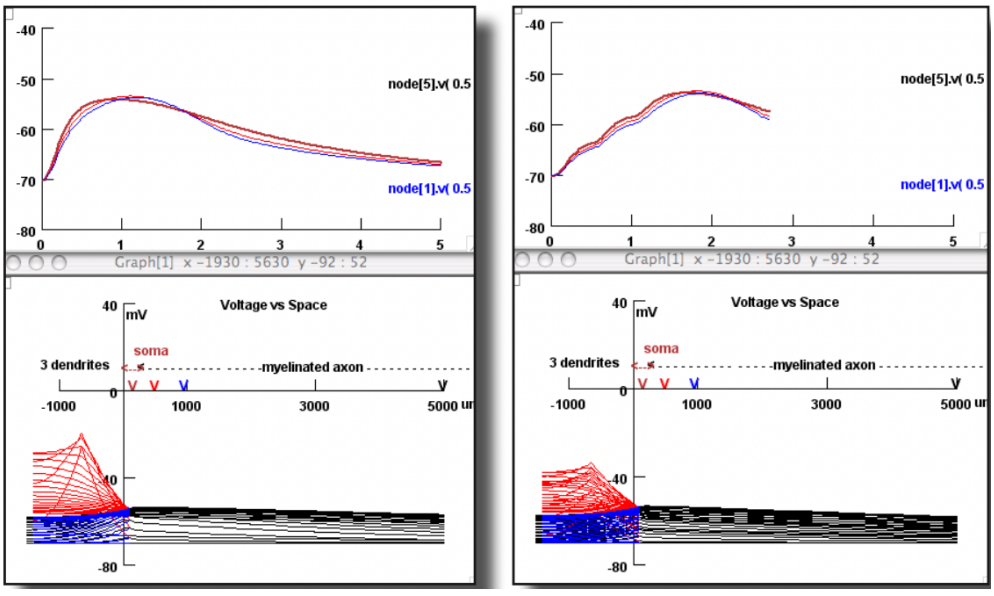


5.4 For bringing the neuron to threshold, are a train of EPSPs delivered from one dendrite equivalent to those same EPSPs occurring simultaneously from multiple dendrites?

- Synchronous EPSPs (same time same location) arriving at the same time lowers the driving force so dramatically, that a greater threshold synaptic conductance is required to generate an action potential
    - When the currents are simultaneous, the cause a large depolarization is close to the reversal potential (-15 mV)
        - Result: small driving force, small current
- compared to asynchronous EPSPs (different time same location) delivered in a train
    - Each EPSP delivered in a train raises the membrane potential in small increments (doesn't affect the driving force as much)
        - Result: smaller reduction in driving force, larger current
- Overall: synchronous EPSP will need a higher conductance for EPP generation
    - The synchronous synaptic currents add together, to their disadvantage;
    - The asynchronous synaptic currents do not add together.
    - With simultaneous inputs, less current flows into the dendrites and along the inside of the neuron to depolarize the spike generating region than when the inputs are asynchronous (?)

3 synchronous EPSPs on middle of dendrite
conductance 3.5 µS per EPSP

3 EPSPs in a train on middle of dendrite
conductance 2.4 µS per EPSP

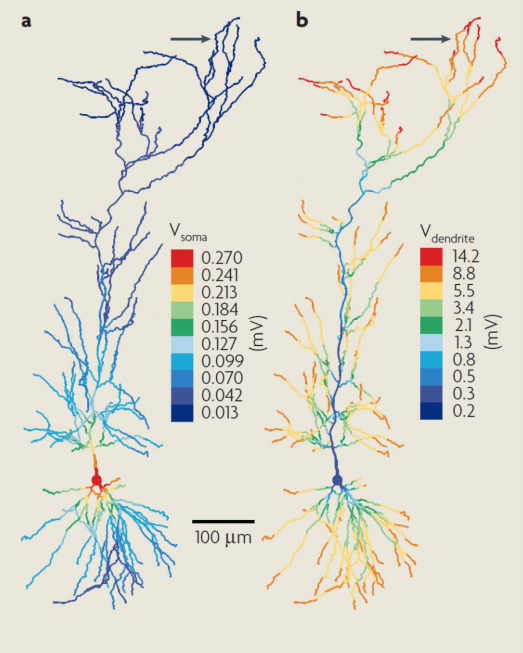


5.5 How does threshold change for the EPSPs in the train, and for those occurring simultaneously, as a function of synapse position on the dendrite?

The further from the cell soma, the greater threshold is required for EPP generation

5.6 The importance of the synaptic location on a dendrite:

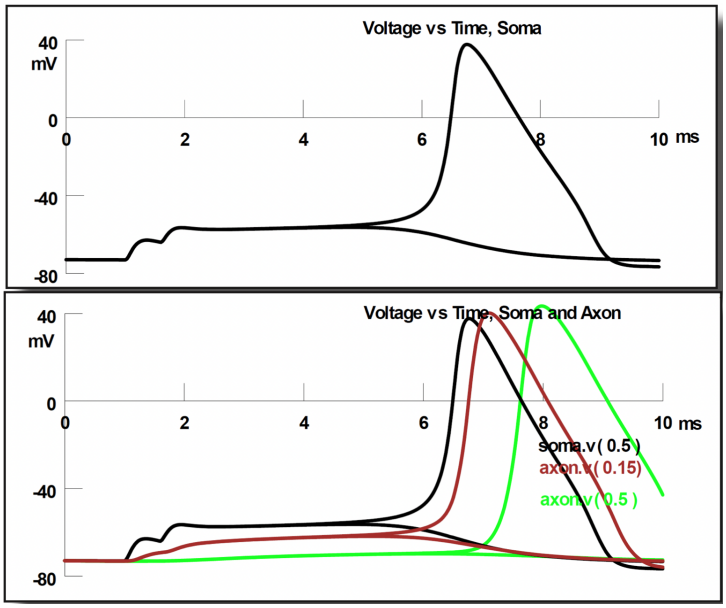
- As the distance from the soma increases, that part of the synaptic current flowing to the soma and axon is attenuated by two factors:
    - The increasing axial resistance.
    - The increasing capacitance (area) of the dendritic membrane that needs to be charged.
    - A location proximal to the soma is most advantageous for initiating a spike because both of these factors are minimized.
    - When synapse is moved to the distal tip of the dendrite, an enormous difference can be found between the conductances of simultaneous EPSPs (bring neuron to threshold) and EPSP in train
        - EPSP in train has lower conductance → does not sum to reduce driving force as simultaneous EPSPs
- High input resistances generate large EPSPs in distal dendritic synapses (increased length constant) compensating for the loss in voltage as the voltage spreads from the distal dendritic synapses to the cell soma
    - The more distal the dendrite, the smaller the dendrite



6.1 Find the duration of the CDW for a soma with two subthreshold excitatory inputs

- CDW: coincidence detection windows

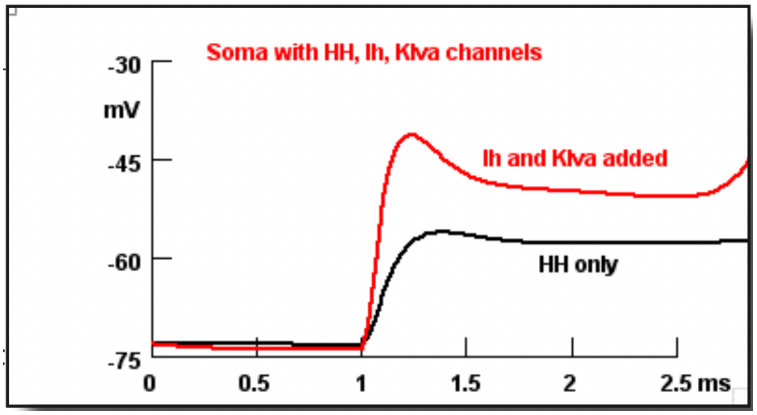
- 0.591ms difference is the duration of the CDW: the maximal duration of separation of the EPSPs that will generate an action potential

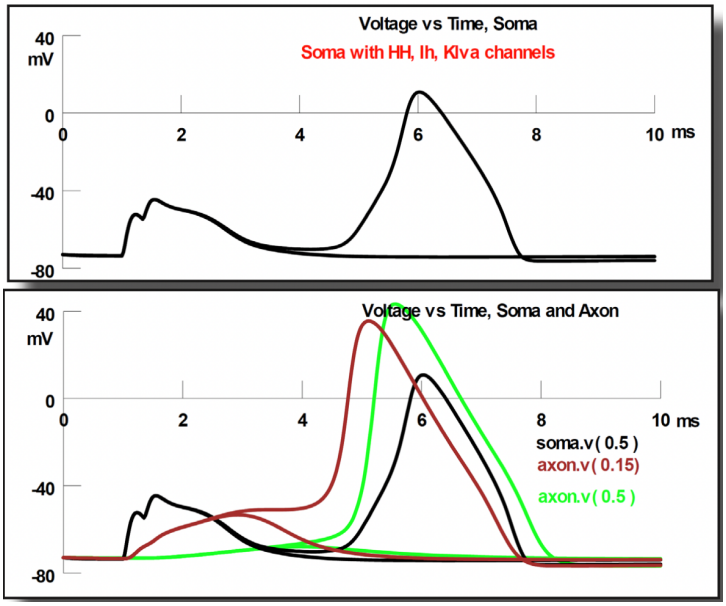


6.2 Simulate an auditory neuron by adding specialized channels to the soma.

- The more narrow the CDW, the greater the dynamic range that our brains can precisely identify the location of sounds
- **Ih (HCNO) and KIva**
  - These two opposing channel conductances operate to purposely lower the input resistance, and thus lowering the time constant (tau, τ) of these neurons
    - The new channels make the cell leakier (lowering the input resistance), demanding greater synaptic current to bring the membrane to threshold.
    - **Shorter time constant = narrower CDW**
    - With the low input resistance, a very short time constant results, about only several hundred microseconds, enabling the cells to have extremely short integration times. These properties enable the cells to fire in response to coincident inputs but discriminate against asynchronous inputs
  - Result: more current to depolarize (due to low input resistance) & the rate of rise is greater (due to more conductance)
- With channels: CDW is 0.342 ms (a two fold decrease)
- action potential now initiates in the axon when Ih and KIva channels are added to the soma

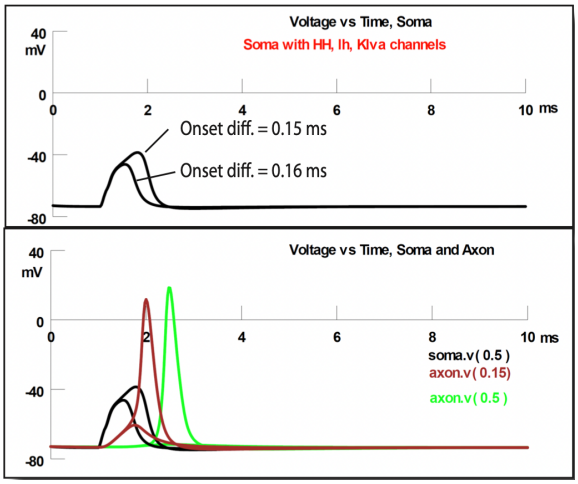
- higher resistance in axon
    - the current flowing into the axon can depolarize the small amount of axonal membrane more quickly than it can depolarize the large amount of leaky somatic membrane
    - The axon fires first and the action potential is then back-propagated into the soma.
- The addition of Ih and KIva channels to the cell soma, reduces the input resistance or increases the “leakiness” of the soma compared to the axon, enabling the axon to rise in voltage amplitude much quicker to the same inputted synaptic current as the soma

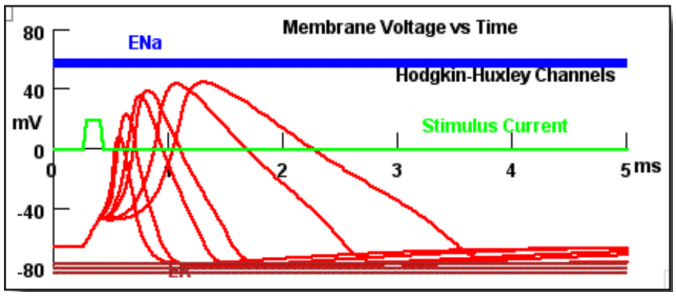




6.3 Would increasing the temperature narrow the CDW appreciably?

- A 10°C increase (6.3 to 16.3°C) causes the rate constants describing the channel kinetics to triple and reduces the spike amplitude to that seen in the fourth action potential from the right
    - As the temperature increases, channel kinetics become faster → less time to deposit charges and depolarize the membrane → reduced spike amplitude, but faster AP
- As the temperature becomes warmer, ENa becomes more positive and EK becomes more negative → reversal potential is higher at higher temperatures
- Raising temperature lowers threshold conductance and narrows CDW
    - Since the reversal potentials are higher at higher temperatures, we have a greater driving force
        - ion current flow is faster through Na channels and K channels, reaching the threshold for generation of action potentials, at a much reduced synaptic conductance required.
    - CDW narrows due to a faster AP → decreased time constant





6.4 Can you further narrow the CDW by decreasing the time to peak of the synaptic conductance?

- "Faster synapses" simulates one of the highly modified synapses in the mammalian auditory brainstem like the calyx of Held, which is designed specifically to ensure fast, reliable synaptic transmission, even at high input frequencies → reduce time to peak of EPSP
- Fast EPSP = lower tau = reduced CDW