Problem Sets: Questions and Answers

These problems are provided to aid in your understanding of basic neurobiological concepts and to guide your focus for in-depth study. These practice questions are optional and answers will not be graded, although they will be discussed during discussion sections. The topics in these problem sets parallel those covered in lecture.

Neurobiological concepts are complex and difficult, and oftentimes are not easily digested at first reading. We suggest that you review the course material several times by reading different texts/papers and talking through your difficulties with us and your fellow classmates. Stick with it — this material can be fully comprehended through persistent and focused effort.

Problems listed below are in order of topics covered in lecture. Problem set numbers do not always correspond to specific lectures or weeks of the term. There are 5 problem sets in this series.
**Problem Set 1 (IONS, MEMBRANES & EQUILIBRIA):**

1.1 The equilibrium potential for sodium ($E_{Na}$) = +58 mV if the concentration of Na on the outside of the cell is 10 times the concentration of Na on the inside of the cell. What happens to $E_{Na}$ if the extracellular concentration of sodium ([Na]o) is increased by a factor of 10? factor of 100? decreased by a factor of 10? Explain why $E_{Na}$ changes in the above examples in terms of the balance between chemical and electrical forces across the cell's plasma membrane.

Answer: The Nernst equation is the formula used to calculate the “equilibrium” or “reversal” potential for a particular ion given the relative internal and external concentrations of the ion. $E_{ion} = (58/z_{ion})* \log([ion_{outside}]/[ion_{inside}])$. Na has a plus one charge (a valence of +1) so $z$ is equal to 1. So, for normal Na concentrations inside and out, the Nernst equation gives $E_{Na} = 58*\log(10/1) = 58$ mV. When the extracellular concentration of Na is increased by a factor of ten the log term becomes $\log(100) = 2$, therefore the $E_{Na}$ doubles in magnitude to 116 mV. If the [Na]outside is increased again by a factor of ten the log term becomes $\log(1000) = 3$, therefore the $E_{Na}$ becomes $3 \times 58 = 174$ mV. When the [Na]outside is decreased by a factor of 10 from its original value the ratio out/in becomes 1. Since the $\log(1) = 0$ the $E_{Na}$ is now zero. This is intuitive. If the concentrations are equal on the inside and outside of the cell then no electrical gradient is required to balance the chemical gradient (because there is no chemical gradient). The Nernst equation answers the question: “what electrical potential is required inside the cell to equally oppose the chemical gradient for a particular ion?” It is called a “reversal potential” because at that potential there is no net movement of that ion across the membrane. At potentials above the reversal potential for an ion there will be a net flow of ions in one direction across the membrane, and at potentials below the reversal potential there will be net flow in the opposite direction. At the reversal potential the electrical and chemical gradients are equal and opposite. When the concentration of Na on the outside is increased there is a chemical gradient for Na directed inward. If the concentration gradient is to be maintained there needs to be an opposing electrical gradient to repel positively charged Na ions from the inside of the cell. This means that the reversal potential in the this case must be positive because positive charge repels positively charged ions.

1.2 Fill in the blank: the total conductance of a membrane for a particular ion ($g_{ion}$) is equal to the number of open channels for that ion x (multiplied by) ________________.

Answer: $g_{ion} = N_{ion} \times \gamma_{ion}$. This equation says that the conductance of a membrane for a particular ionic species is equal to the number of open channels for that ion multiplied by the **single channel conductance** ($\gamma_{ion}$).

1.3 Write an equation that describes the following statement in mathematical terms: The contribution to transmembrane potential ($V_m$) of each ionic battery is weighted in
proportion to the permeability of the membrane for each particular ion. Why is \( V_{\text{rest}} \) so much closer to \( E_K \) than it is to \( E_{Na} \)? Why is \( V_m \) at the peak of the action potential so much closer to \( E_{Na} \) than it is to \( E_K \)?

Answer: Unequal distribution of ions across the membrane creates what is called an ionic battery. There are two equations that describe how the different ionic batteries contribute to the membrane potential. They say the same thing in a different way. One is in terms of permeability and the other is in terms of conductance. The Goldman equation is in terms of permeability (\( P_{ion} \)).

\[
V_m = \frac{58 \times \log((P_K[K]^o + P_{Na}[Na]^o + P_{Cl}[Cl]^i))}{(P_K[K]^i + P_{Na}[Na]^i + P_{Cl}[Cl]^o))}
\]

This is the Nernst equation for each major ion combined into one formula with one additional term, permeability, which determines each ionic battery’s relative contribution to the membrane potential. Permeability is a measure of the relative ease with which an ion can pass through a membrane and is expressed as a value between 0 and 1. Looking at the Goldman equation you can see that the permeability factor gives different weights to each of the three ionic batteries in the equation. So, the **ionic battery corresponding to the ion that the membrane is most permeable to will contribute the most to the membrane potential.** Permeability and conductance vary in proportion to each other so the Goldman equation can be expressed in a different way when it is rearranged in terms of conductances and equilibrium potentials for each ion:

\[
V_m = \frac{(E_{Na}g_{Na} + E_Kg_{K} + E_{Cl}g_{Cl})}{(g_{Na} + g_{K} + g_{Cl})}
\]

This equation clearly says that the **contribution of each battery to the membrane potential is weighted based on the conductance for that ion.** If there is no conductance (\( g = 0 \)) for an ion then it contributes nothing to the membrane potential. \( V_{\text{rest}} (~-70 \text{ mV}) \) is much closer to \( E_K (~-80 \text{ mV}) \) than \( E_{Na} (+58 \text{ mV}) \) because, at rest, the conductance (and permeability) of the membrane to \( K \) is much greater than that of \( Na \). At the peak of the action potential waveform the membrane potential is positive (perhaps around +40 mV) because at this point in time the membrane is more permeable to \( Na \) due to the opening of voltage-gated \( Na \) channels.

1.4 What are the differences between permeability, conductance, and current? Explain each in terms of its relationship to ions and membrane properties.

Answer: Permeability, conductance, and current are terms that describe different aspects of the relationship between an ion and the cellular membrane. **Permeability is a proportionality (number between 0 and 1) that describes the ease with which an ion flows across a membrane, relative to the permeability of other ions.** **Permeability is proportional to the number of open channels for the particular ion.** A membrane can be highly permeable to an ion however if there are none of those ions present then there is no movement of those ions across the membrane. Therefore permeability does not require the movement of ions across the membrane but allows for it if the ions are present with a non-zero gradient. **Current is expressed as the movement of charge across the membrane in time (coulombs / second).** **Current is a measure of the rate of charge flow through the membrane.** Without the net movement of
charged ions across the membrane the current is zero. **Current (I\text{ion}) = g\text{ion} * (V_m-E_{ion})**. **The difference between the V_m and E_{ion} is called the “driving force” for a particular ion.** Remember that there is no net flow of a particular ion if V_m is equal to the equilibrium potential. So the existence of an ionic current relies on the existence of a driving force (V_m not equal to E_{ion}) and a conductance for that ion. **If either the driving force or conductance is zero then there is no current.** Conductance is an electrical term that is the reciprocal of resistance (G = 1/R) and has units of siemens. Conductance describes in quantifiable terms (different than permeability which is a term of proportionality) the amount of a particular charged ion that can flow across a membrane or single channel in a given amount of time at a particular membrane potential if the equilibrium potential is known. **g\text{ion} = I_{ion} / (V_m-E_{ion}).**

1.5 Propose 2 experiments to test the hypothesis that an increase in potassium conductance is responsible for the falling phase of the action potential in a neuron? (Hint: think about voltage clamping, the trough of the action potential, and that cesium and TEA both can be used to block some K currents.)

**Answer:** If potassium conductance (K ions moving outward, down their concentration gradient) is responsible for the falling phase of the action potential then adjusting the concentration gradient should affect the waveform of the falling phase of the action potential. The trough of the waveform is at an amplitude that is very close to the equilibrium potential for potassium because the inactivation of voltage-gated Na channels and the delayed opening of voltage-gated K channels makes the membrane very permeable to K, relative to other ions. For the experiment it is easiest to change the concentration gradient by changing the concentration of K on the outside of the cell. Decreasing the [K]_o will increase the concentration gradient directed outward. As a result the equilibrium potential for K will be a greater negative value. Now an action potential for this cell under the modified conditions should have a trough that is more negative. This is because when the cell becomes very permeable to K, ions will flow out of the cell until a sufficiently negative membrane potential is reached to counter act the flow of ions down the concentration gradient. This potential that the trough will drive toward is the equilibrium potential for K. So, increasing the [K]_o, a manipulation that decreases the concentration gradient and increases the equilibrium potential (less negative) will result in a action potential trough that is less negative.

In a voltage-clamp experiment you can change the membrane potential and measure the ionic currents that are turned on at that potential. During an action potential the membrane depolarizes due to the influx of Na ions. This depolarization turns on a voltage-gated outward current with a delay. The outward current is responsible for the repolarization of the cell back to the resting potential. **The cell can be held at a depolarized potential in a voltage-clamp experiment to simulate the depolarization due to the influx of Na.** The outward currents can then be measured at this depolarized membrane.
potential. If these outward currents are due to the movement of K ions then we should be able to eliminate these currents with chemicals that are known to block the flow of K ions. Cesium (Cs) and Tetra-ethyl-ammonium (TEA) are molecules that partially block K currents. Putting these molecules in the voltage-clamp recording electrode will cause them to be perfused into the cell. This manipulation should attenuate the outward current if K is the ion that carries it.

1.6 Explain why the opening of voltage-gated sodium channels proceeds in a positive feedback fashion during the generation of an action potential. Why isn’t the opening of voltage-gated potassium channels mediated via a similar positive feedback loop?

Answer: V-gated Na channels open upon depolarization from rest but they don’t all open at once because they have slightly different voltage sensitivities. The opening of V-gated Na channels is a positive feedback loop because Na influx will cause the cell to depolarize even more. When the cell depolarizes to a less negative value then more V-gated Na channels will open, resulting in further depolarization until all of the V-gated sodium channels have opened. This is positive feedback.

The depolarization due to influx of Na causes V-gated K channels to open with a delay. The efflux of K ions causes the cell to repolarize. The stimulus for V-gated channels to open is de-not re-polarization, so the flow of K ions is in the opposite direction than what would be required for positive feedback. It is negative feedback because the V-gated K channels are closed due to the repolarization that happens when current flows outward through the open V-gated K channel. This is negative feedback.

1.7 Explain the difference between the concepts of equilibrium and homeostasis (steady-state). (Hint: physical molecular forces and active cellular processes).

Answer: These two terms have a slightly different meaning in the context of neurophysiology. Let’s consider the idea of the resting membrane potential and the things that contribute to its existence. Equilibrium potentials are a description of the balance point between the two opposing, passive physical forces, namely the chemical and electrical gradients. E_{ion} is the potential at which the electrical gradient is equal and opposite to the chemical gradient. This balance of physical forces describes the equilibrium condition for a particular ion. There are active, energy requiring, cellular processes that contribute to keeping the neuron at a “steady-state” in which these equilibria are combined. The idea of a “steady-state” in which the system is stable (V_{rest}) is synonymous with the term homeostasis. The steady-state value of the membrane potential (V_{rest}) depends on the relative contribution of each ion’s equilibrium potential to the membrane potential. These relative contributions depend upon, for example, these two types of active cellular processes performed by the cell: 1) Production, assembly, and trafficking of the correct number and type of ion channels to the membrane. The cell must produce the correct number of channels for each ion if the steady-state is
to be maintained. This is an active process that contributes to balance of equilibria. This balance of equilibria is homeostasis. 2) Ionic pumps use energy to move ions across the membrane in order to establish concentration gradients. These active forces are in balance with the physical forces of equilibrium in order to establish the steady-state or homoestasis which is a balance of physical equilibria.
Problem Set 2 (ION CHANNELS):

In the answers for this problem set, membrane current ($I_m$) is drawn as resistive current ($I_R$) only. Capacitive currents ($I_C$) have been omitted for clarity.

2.1 Researchers have identified a gene that, they think, encodes a voltage-dependent potassium channel. How would you go about trying to prove that it does encode such a channel? Hint: frog oocytes can be used to express proteins of interest by injecting them with large amounts of RNA so that the product is of high concentration compared to other proteins in the cell. These cells can then be used for electrophysiology experiments.

Answer: After allowing the cells to express the protein you could perform the following experiments to determine the type of transmembrane ion channel encoded by the gene. In a voltage-clamp experiment we control the transmembrane potential of the cell while measuring the resulting ionic current at that voltage. The finely pulled glass electrode that is used to make the intracellular, whole-cell recording is filled with a saline that mimics the ionic content of the cytoplasm. The “intracellular” solution used in the recording electrode should have the ionic composition of a typical neuron because this solution will perfuse into the cell during the experiment. For the extracellular solution we should begin with the typical ionic concentrations required to establish the normal ionic concentration gradients across the membrane. Under these conditions the reversal potential for potassium will be approximately -80mV. Typically a voltage-gated channel will open upon significant depolarization from the resting potential. A voltage-gated potassium channel should therefore pass an outward current when the cell is depolarized from rest (-70mV) to a voltage at which channels are stimulated to open (eg –40mV).

Current amplitude is equal to the conductance of the membrane for that ion times the driving force: $I_{ion} = g_{ion} (V_m - E_{ion})$. The conductance is turned on when the cell is depolarized. **Experiment A** See figure. Now let’s depolarize the cell to successively higher voltages, for example, from rest to –40mV, then from rest to -30mV, -20mV, -10mV, etc. and think about what should happen to the magnitude of the voltage-gated current as we vary the stimulus. Since the driving force gets larger as the stimulus voltage moves away from $E_k$, the magnitude of the current measured should increase as we depolarize to successively higher voltages IF the gene encodes a potassium channel. If the result is as described above we have now shown that the gene encodes a voltage-gated channel that passes an ion with an equilibrium potential that is more negative than the voltage required to open the channel. But how can we show more conclusively that this ion channel is selective for potassium ions? We can do this by changing the concentration gradient for potassium so that different equilibrium potentials are produced. **Experiment B** See figure. Now if we do a series of experiments in which we depolarize the cells to the same potential but vary the concentration gradient you should be able to predict how the current magnitude will vary IF the voltage-gated channel is selective for potassium. **Experiment C** To further show that this
channel is a potassium channel we could add things that are known to block potassium channels to the intracellular solution. Cesium and TEA are potassium channel blockers that behave in a dose dependent and reversible fashion. See figure.
A) Measuring Whole-Cell Currents in Voltage-Clamp

Stimuli

$V_m (mV) \quad -70$

Responses

$I_m (nA) \quad 0$

B) Stimuli

$V_m (mV) \quad -70$

Responses

$I_m (nA)$

Same voltage stimulus at different $E_K$

- $E_K = -100 mV$
- $E_K = -80 mV$
- $E_K = -20 mV$
- $E_K = 10 mV$
- $E_K = 50 mV$
The term “whole-cell”, when put in front of the term “voltage-clamp”, refers to two things regarding the procedure of the electrophysiology experiment. First, it means that we are recording currents across the membrane of the whole cell, not currents from a small population of channels in a patch of membrane. Second, it means that the patch is ruptured and the inside of the cell becomes continuous with the inside of the recording electrode so that the contents of the electrode quickly equilibrate with the soluble contents of the cytoplasm. Soluble proteins are removed from the cell when this technique is used because the volume of the electrode is >> volume of cytoplasm. (This is in contrast to the “perforated-patch” technique in which electrical access through the patch is achieved via the action of enzymes in the recording electrode that create small holes in the patch of membrane. In this configuration whole-membrane currents are recorded but the endogenous contents of the cytoplasm are left inside because the holes in the membrane are only big enough to allow ions to pass in and out of the cell.)

Think about the shape of the whole-cell potassium currents. Can you explain the shape of these curves based on what you know about the gating characteristics of V-gated potassium channels? Think about the shape of whole-cell sodium
currents. How are they different from whole-cell potassium currents? Can you explain the shape of these curves based on what you know about the gating characteristics of V-gated sodium channels? Remember that a whole-cell current trace is the summation of thousands of individual channel currents.

2.2 You are performing a patch clamp experiment on a patch of membrane that has precisely 3 voltage-gated sodium channels.

A) Draw a current trace of all of the possible channel-opening events in response to a single depolarizing stimulus from rest (-70 mV) to above threshold (0 mV) and back to rest in a 1 sec period. (Hint: maximum of 3 events/ voltage step).

B) Explain why two single-channel events with different mean open times pass the same current and have the same conductance?

C) Explain why do 2 channels opening at once pass 2x the current of a single channel and have 2x the conductance of a single channel?

Measuring Channel Currents in Voltage-Clamp

Patch of membrane with 3 V-gated Na channels

- Stimulus
- Voltage (mV) -70
- Possible responses
- Current (pA)

Current = \frac{charge \text{ movement}}{time}  
Y axis: current  
X axis: time

Change = current \times time

Single channel current magnitude is constant but change carried by channel \text{ \&} time open
Answers: Voltage-gated sodium channels open upon depolarization and inactivate after a brief period. When the channel is inactivated it is unable to pass current. Inactivation of the channel can’t be removed until the cell is repolarized. One second is a long time in the world of a voltage-gated channel and plenty long enough to stimulate such a channel to open and close. Therefore each of the three channels will only open and close only once per depolarization. However the protein subunits that compose the channels’ voltage sensor and current gate may be caught in different “random” states of energetic activation at the onset of the depolarization. After the onset of a depolarization it takes different amounts of time (micro seconds) for a channel to open depending on the energetic state of the subunits. Therefore the single channel opening events could all happen discretely (one at a time), all at once, or something inbetween. See figure. Because of similar considerations of “random” energy states of the inactivation gate, channels may remain open for varying amounts of time. Current (coulombs/sec) in a measure of charge flow per second (amps). It is a rate. The same type of channel will have the same single-channel conductance ($\gamma_{\text{channel}}$) and therefore pass the same current at a given voltage regardless of how long it stays open. A channel staying open twice as long as another will therefore pass twice as much charge because it will pass the same current for twice as long. Two channels opening at once will, combined, pass twice the current, and therefore twice the charge, than would a single channel that is open for the same amount of time. The same can be said for patch membrane conductance ($g_{\text{ion}}$) since conductances, like currents, add in parallel and are both proportional to the number of open channels.

2.3 You are in the midst of a patch clamp experiment with a single voltage-activated Na channel. In response to a depolarizing voltage step, the channel current 1) turns on at the onset of depolarization; 2) remains on for a brief period; and 3) turns off (inactivate) before the end of the depolarizing stimulus. Draw the voltage step and the current trace from this experiment. Treat the patch with aconitine, repeat the same experimental protocol using the same voltage steps and draw the resultant current trace, explaining any differences. Rinse away the aconitine and repeat experiment using pronase.

Answer: Aconitine and pronase are two drugs that have different affects on voltage-gated Na channels. Aconitine is a lipid-soluble compound that will keep Na channels open to current flow once they have been stimulated to do so by a voltage stimulus. The shape of the channel is changed so that the inactivation gate and the voltage sensor no longer plug the hole. They are unable to inactivate and will not close, even when the cell is repolarized. Pronase is an enzyme that removes the inactivation gate and therefore removes the time-dependent inactivation of the channel. Channels pretreated with pronase will remain open for the entire duration of the depolarization. The voltage sensor will still close the channel at the end of the stimulus. See figure.
2.4 What properties of ions and their respective transmembrane channels make such channels selective for a specific ion? (Hint: the two main structural parts of channels are the pore and the selectivity filter).

Answer: The pore and the selectivity filter can make transmembrane channels selective for a particular ion or ions. Let’s consider sodium and potassium ions, and their respective channels. Forget about gating. These could be voltage-gated channels or channels that are always open, like the pores that pass the sodium and potassium leak current. In the case of voltage-gated and leak channels for sodium and potassium, all of these transmembrane channels need to be selective for sodium or potassium, not both. The pore is located at the mouth of the channel and works based on size exclusion. The size of the pore interacts with the size of the ion. Notice that the pore of a potassium channel is smaller than that of a sodium channel. This seems counter-intuitive because a potassium ion, one row down in the periodic table from sodium, is larger than a sodium ion. However since both ions have a charge of +1, this means that the smaller ion, sodium, has a greater charge density. A greater charge density attracts a larger shell of water molecules. An ion with a larger shell of hydration requires a larger pore to move through. Therefore sodium ions will be excluded from the small pore of a potassium channel whereas potassium ions, with a smaller water shell, will pass through. How then does a sodium channel remain selective for sodium given that its pore will not size-exclude a potassium ion? This is where the
hypothesis of a selectivity filter comes in. The selectivity filter is thought to work via the transient binding of the ion to specific charged amino acid residues on the inside of the channel. To fit through this part of the channel the ion must be stripped of some of its water molecules. However this process must be energetically favorable, meaning that the ion won’t give up its water molecule unless it is offered a stronger bond with an amino acid residue. In other words, to give up one or more of its water molecules and be moved through the filter, the ion’s positive charge must be stabilized by a negative charge on the inside of the channel. Then the ion has taken a step to overcome the energy barrier required to move through the channel (this is a simplified explanation of all of the thermodynamics involved in overcoming the energy barrier to cross the filter). The characteristics of the selectivity filter in the sodium channel make it selective for sodium ions whereas the diameter of the potassium ion is thought to be too large to be stabilized as effectively by the negatively charged amino acid residue. See figure.

2.5 Draw the current traces obtained from a patch clamp experiment on a single voltage-dependent Na channel exposed to a series of voltage steps (-110mV, -90mV, -65mV (subthreshold), 0mV, 20mV, 50mV, 70 mV) each starting from rest (-70mV) for a 100 msec duration. Repeat with a patch containing a single voltage-dependent K channel. (Hint: V=IR, so I=g(Vm-Ex where X= Na or K).
Answer: **single V-gated Na channel:**
*Subthreshold – no current because the channel doesn’t open
*Threshold – large inward current
*0mV – smaller inward current because closer to equilibrium potential
*20mV – even smaller inward current
*50mV – very small inward current because very close to equilibrium potential for Na (+58mV)
*70mV – small outward current because above $E_{Na}$
*90mV – no current because channel is gated by depolarization
*110 – no current because channel is gated by depolarization

**single V-gated K channel:**
*Subthreshold – no current because the channel doesn’t open
*Threshold – small outward current because close to equilibrium potential for K (-80mV)
*0mV – bigger outward current because moving away from equilibrium potential
*20mV – even larger outward current
*50mV – very large outward current
*70mV – still even larger outward current
*90mV – no current because channel is gated by depolarization
*110 – no current because channel is gated by depolarization
Problem Set 3 (CABLE THEORY):

3.1 Explain why an increase in the surface area of the plasma membrane of a neuron result in an increase of the cell’s time constant. (Hint: think about electrical properties of the cell that are affected by changes in the surface area of the plasma membrane.)

Answer: The time constant (tau) for a cell is equal to the product of the cell’s membrane capacitance and its membrane resistance: \( \tau = C_m R_m \). Consider the following situation. When a step change in current is applied to a cell in current clamp, the cell will respond with a change in voltage that reaches a new steady state value with a certain time course. The value of tau, typically in milliseconds, describes the time course with which the cell’s voltage will respond to an applied current. Specifically, it is the time required for the voltage response to reach \( [1-(1/e)] = 63\% \) of the way from the original to the new steady-state value. \( C_m \) and \( R_m \) are the determining parameters because they describe how much charge must flow to change the voltage across the membrane and what the resistance is to this charge flow, respectively. A change in either parameter will result in a change in tau. So, which parameter is affected by a change in a cell’s surface area? \( R_m \) is the specific resistance of the membrane per unit area so it is not changed simply by adding more membrane. Capacitance is proportional to the surface area of the plates of the capacitor, in this case the plasma membrane. It is also inversely proportional to the distance between the plates but since the distance between the two leaflets of the plasma membrane is a constant we only have to worry about changes in membrane surface area affecting capacitance. So, an increase in surface area increases the membrane capacitance, which increases tau because now that \( C_m \) is increased, more charge must be displaced from one side of the membrane to the other to achieve a change in voltage. Think about voltage as a measure of charge density. If you have a larger surface area for charge to flow onto, then it will take more charge movement to achieve the same change in charge density (voltage). If the resistance to charge flow stays the same (\( R_m \)) then it will take longer for the voltage to change…so tau is increased because it takes more time. Bigger tau means slower response.

3.2 What is the effect of an increase in input resistance on the length constant of a cell? Explain the reasons for your answer. What is the effect of an increase in axial resistance on the length constant of a cell? Explain. (Hint: consider current leaking across the membrane and current flowing down an axon.)

Answer: The length constant (lambda) for a cell is equal to the square root of the quotient of the internal (or axial) resistance into the membrane resistance: \( \lambda = \sqrt{R_m/R_a} \). Consider the following situation in which a hyperpolarizing current step is injected into an axon via a microelectrode at point 0. At the same time we will measure the voltage response at point 0 as well as at points 1, 2, 3, etc. that exist at increasing distances from point 0. The changes in voltage measured at those points will be called \( V_0, V_1, V_2, \) and \( V_3 \). During the current step, the largest
change in voltage will be measured at $V_0$. As we measure the voltage change at points distant from point 0 we notice that the voltage change gets smaller as the distance from point 0 increases. In fact, the change in voltage in response to the current step at any distance from point 0 is described by the following equation: $V_{\text{point at distance } x} = V_0(e^{-x/\lambda})$, where $x$ is the distance from the point of current injection to the point where the voltage change is measured and $\lambda$ is the length constant as described above. $X$ and $\lambda$ must be in the same units of length. Let’s look at that that equation. If we are at point 0 then $x = 0$, $e^0 = 1$, so $V_0 = V_0$. If we are at point 1 then $x = 1$, if we are at point 2 then $x = 2$, etc. As $x$ increases, the power term above $e$ decreases and is negative. An increasingly negative power term above $e$ means that $V_0$ will be multiplied by smaller and smaller numbers as $x$, the distance from point 0, increases. So, $V_x$ gets smaller as $x$ increases and $\lambda$ determines how rapidly the voltage response decreases as the signal travels down the axon. In fact, $\lambda$ is equal to the distance ($x$) at which the signal amplitude ($V_x$) is equal to $1/e = 37\%$ of $V_0$. Test this by substituting $\lambda$ for $x$ in the equation above: $e^{-\lambda/\lambda} = e^{-1} = 1/e = 37\%$. So, $V_\lambda = V_0(0.37)$. OK, so an increase in input resistance ($R_m$) gives us an increase in the length constant, $\lambda$, meaning that the signal will decrease more slowly as it travels down the axon because it travels further before reaching 37\% of the signal at point 0...but why? $R_m$ is a measure of how leaky the cell is with respect to charge carriers, specifically ions. As the voltage signal travels down the axon it is carried in the form of ions. One reason that the signal decreases with distance is because some of the ions leak out of the membrane. If less ions leak out, because of a greater $R_m$, then more ions travel down the axon to carry the signal further, thus a larger length constant ($\lambda$). Bigger $\lambda$ means bigger responses.

OK let’s think about axial resistance ($R_a$). Whereas a bigger membrane resistance increases $\lambda$, a bigger axial resistance rather decreases $\lambda$. Think about axial resistance of a cell’s axon as being analogous to the resistance of a metal conducting wire. Two conditions determine the resistance of that wire to charge flow: 1) the type of metal which determines the concentration of charge carriers (equivalent to the intrinsic resistive property of a neuron’s cytoplasm) and 2) the diameter of the wire, which determines the extent to which the charge carriers contact the surface of the wire, which impedes charge flow. The cellular analog of condition one is the ionic concentration of the cytoplasm, which is relatively constant from neuron to neuron. The important thing for this question is condition 2. The cellular analog of condition two is the diameter of the axon. Let’s compare two axons, one with a small and one with a large diameter. As the diameter, or radius, of a cylinder increases, so does the volume of the cytoplasm and therefore the volume of charge carriers. The surface area also increases. However, as we increase the radius, the volume of the cylinder increases faster than the surface area of the cylinder. This is because for a cylinder the volume increases with the square of the radius ($\text{Volume} = \text{length} \times \pi \times r^2$) whereas the surface area increases simply with the radius ($\text{SA} = \text{length} \times 2 \times \pi r$). Put in other words: as the radius of the axon increases,
the ratio of volume to surface area increases. As the volume to surface area ratio increases, less charge carriers come into contact with the membrane of the axon, which impedes charge flow or lets it leak out. Therefore, one way to increase axial resistance is to have an axon with a smaller diameter (smaller ratio of volume to surface area). An increase in axial resistance results in a decreased lambda because more internal resistance to charge flow down the length of the axon means that the signal will not travel as far before reaching 37% of the amplitude at point 0. Smaller lambda means smaller responses.

3.3 What is the effect of increasing the diameter of an axon on the speed of a propagated action potential? Explain the reasons for your answer. (Hint: consider the factors underlying the spread of charge in an axon).

Answer: One of the rate-limiting factors in the propagation of an action potential is the cable property of electrotonic conduction. To answer this question we must think about what an increase in diameter does to the parameters that affect the speed of electrotonic conduction, which is dependent on the rate of passive spread of voltage. Ions are charge carriers. Let’s compare an axon with a small radius (axon 1) to an axon with a large radius (axon 2). We notice that the increase in volume of ion-containing cytoplasm is greater than the increase of membrane surface area as we go from axon 1 to axon 2. This is explained by the geometric equations that relate changes in radius to changes in volume and surface area. Volume=length*pi*r². SA=length*2*pi*r. Increased volume is favorable to charge flow. Increased surface area is unfavorable. Since volume increase faster, and is favorable, axon 2 will have a lower internal resistance to charge flow. Therefore the increase in diameter results in a decreased axial resistance. Raxial α 1/radius. But what does this decrease in axial resistance do to the speed of electrotonic conduction? Consider two adjacent sections of membrane separated by a segment of axoplasm. Depolarization must spread from one segment to the other through the axoplasm in the form of capacitive current, thus changing the transmembrane potential. Ohm’s law tells us that if the resistance to charge flow is decreased then current through the loop is increased: I = V/R. More current means that more ions flow to change the charge on the adjacent area of membrane in a given amount of time: ΔV = ΔQ/Cm. So, less axial resistance means more charge flow (ΔQ), which results in a greater ΔV in a given amount of time and therefore a faster action potential. AP speed α 1/Raxial.

Now let’s think about the time constant, Larger diameter means more surface area, which means more capacitance, which means bigger time constant. So, the signal would travel slower if changes in speed were due to the change in this parameter alone. This is clear from the same equation we used above: ΔV = ΔQ/Cm. If Cm is larger then the change in voltage in a given amount of time will be smaller. AP speed α 1/Cm.
Speed of AP varies inversely with the product \((C_mR_{axial})\). Increasing the diameter of an axon will decrease \(R_{axial}\) and increase \(C_m\), so these two effects are at odds with each other. The question becomes: “which change has the predominant effect as we increase diameter?” If you do the math (outside the scope of this course) you will see that the change in axial resistance has the predominant effect.

Increasing the diameter of an axon means that an action potential will travel faster.

3.4 What is the effect of myelination of an axon on the time and length constants? Explain the reasons for your answers. (Hint: consider the effect of myelination).

Answer: In the previous problem we saw that one mechanism for increasing the speed of an action potential is to increase the diameter of the axon. This effect is due to the decrease in the product \(C_mR_{axial}\). In this problem we will see that myelination results in a proportionally larger decrease in \(C_mR_{axial}\). Therefore conduction in myelinated axons is typically much faster than in nonmyelinated axons of the same diameter. A myelinated axon results from the wrapping of glial cell membranes around an axon. This increases the effective thickness of the axonal membrane by as much as 100 times and therefore greatly reduces the capacitance of the membrane. These myelinated axon regions have low membrane capacitance, due to the thickening of the membrane, and high membrane resistance because of the low abundance of channels in these regions.

Length constant \((\lambda = \sqrt{R_m/R_a})\): Myelination \(\rightarrow \uparrow R_m \rightarrow \uparrow \lambda\). So when an action potential enters a region of myelination the signal is transferred down the axon with less decrease in amplitude than without myelin.

Time constant \((\tau = C_mR_m)\): Myelination \(\rightarrow \downarrow C_m \rightarrow \downarrow \tau\). So action potentials move very quickly through regions of myelinated axon. Here, the decrease in membrane capacitance is the predominant effect compared to the increase in membrane resistance.

An action potential is triggered at the axon hillock, a nonmyelinated region with a very high density of voltage-gated Na channels. The action potential then moves faster and with less decrement in amplitude in myelinated regions of axon but the signal still isn’t strong enough to make it all the way down an axon. This is because the low density of voltage-gated channels in myelinated regions means that very little inward current enters to boost the current flow from the trigger zone down the length of the axon. That is why regions of myelination are separated by spaces between the glial sheaths called the Nodes of Ranvier. These nodes have a high capacitance and therefore a larger time constant, so the action potential slows down at each node as voltage-gated current enters the axon to boost the signal into the next myelinated region and onward to
the next node. **These regularly spaced nodes prevent the action potential from dying out.**

3.5 Draw I-V curves for a rectifying and a non-rectifying electrical synapse. (Hint: consider drawing 4 curves total, 2 for each type of synapse: injecting depolarizing and hyperpolarizing current into pre-synaptic cell and measuring voltage in the post-synaptic cell (curves 1 & 2), and injecting depolarizing and hyperpolarizing current into post-synaptic cell and measuring voltage in pre-synaptic cell (curves 3 & 4)).

**Answer:** A non-rectifying electrical synapse will pass depolarizing or hyperpolarizing current in both directions, pre to post or post to pre. On the contrary, a rectifying electrical synapse will pass only depolarizing current forward (from pre to post) and only hyperpolarizing current backward (from post to pre). On the graphs below, the independent variable is injected current on the x-axis and the dependent variable is the measured voltage response of the other cell, plotted on the y-axis.

![Non-rectifying electrical synapse](image1)

![Rectifying electrical synapse](image2)
4.1 A) What is adaptation by sensory receptor cells? Describe 3 behavioral examples of adaptation (you have probably experienced dozens of them today unless you are dead).

B) Describe the different properties of slowly and rapidly adapting neurons. What different functions do they serve? (Hint: consider the different types of information contained in the physical stimulus).

Answers: A) Receptors encode a stimulus by giving a signal to the next cell in the sensory circuit. Many sensory receptors signal stimulus onset, rate of change, intensity, and duration by changing their action potential firing rate. When a stimulus lasts for a significant amount of time without a change in amplitude, perception of the stimulus intensity diminishes and is sometimes lost completely. Perceptual adaptation to sensory input is thought to occur in part because the response of a sensory cell adjusts to a persistent stimulus by decreasing its firing rate. The stimulus may then fade from consciousness. This type of sensory adaptation on a cellular level involves a change in the input-output function of the cell. Once adaptation occurs, the cell can now respond better to further changes in stimulus intensity, again, by changing its firing rate. If adaptation did not occur, the sensory receptor cell would be limited to encoding a much smaller range of stimulus intensity because there is an upper limit to firing frequency.

Behavioral examples of sensory adaptation are plentiful. Think of all the times when your perception of a stimulus diminishes even in the continuous presence of the stimulus. When you get into a hot shower it may, at first, feel very hot to your skin. However after a short period of adaptation, water of the same temperature no longer feels hot but warm because the temperature receptors in your skin have decreased their firing rates. Now if you turn up the heat again you can regain the same initial hot sensation but this time with a hotter temperature. Adaptation in this type of cell adjusts the stimulus range for which the cell is able to respond with a high degree of precision. Try to think of some other examples of perceptual adaptation. When you put a hat on your head the sensation is initially quite strong. Have you ever been looking for your hat only to find that it is right on top of your head all along?

B) To illustrate some of the differences between slowly and rapidly adapting neurons let’s consider some properties of the touch receptors in your skin, a.k.a. mechanoreceptors. Responses from both types of receptors are required to give you complete information about the stimulus because they respond to different aspects of the stimulus. Consider a probe stimulus on your skin that has an onset, is maintained constant for several seconds, and then ends. Slowly adapting receptors respond continuously throughout the duration of the stimulus by firing action potentials at a rate roughly proportional to the amplitude of skin indentation. The firing rate will become lower during the stimulus but firing will
not cease until the end, thus the term “slowly adapting.” They give us information about the intensity of a constant stimulus. On the other hand, rapidly adapting receptors respond only at the beginning and end of a stimulus, and when the stimulus intensity is changing. Whereas slowly adapting receptors respond to a constant stimulus with a proportionate firing rate, a rapidly adapting receptor encodes the rate of change of stimulus intensity by changing their firing rate proportionately. The faster the rate of change, the higher the frequency of firing for a rapidly adapting neuron. When the stimulus intensity stops changing, so does the firing, thus the term “rapidly adapting.”

In the figure below, the receptors’ voltage responses are shown above the corresponding mechanical probe stimulus. The vertical lines on the voltage trace are action potentials of the receptor cell in response to the touch on the skin.
4.2 Describe, in as much detail as possible, a G-protein-mediated receptor-second messenger cascade that results in a metabotropic response on a neuron’s ion channel. What functions are served by such a pathway?
The above figure is a simplified schematic of a G-protein mediated, metabotropic response to serotonin. Upon binding to its receptor, the neurotransmitter initiates a chain of activation of catalytic proteins. In the final step the active enzyme, protein kinase A, phosphorylates the channel using ATP as a cofactor. This closes the channel.

4.3 What is the difference between an ionotropic and a metabotropic receptor-mediated response? What is the utility of having metabotropic pathways when ionotropic pathways are faster and more direct?

Answer: An **ionotropic response is the direct gating of an ion channel** via the conformational change that happens in the transmembrane part of the channel in response to the binding of the receptor part of the channel to its neurotransmitter ligand. A **metabotropic response is the indirect gating of an ion channel by the action of a neurotransmitter as described above through a second messenger cascade, or the activation of any other downstream pathway.** Metabotropic responses may be beneficial for the reasons discussed in problem #2.

4.4 Describe two molecular mechanisms of sensory adaptation in sensory receptor cells. (Hint: consider changes in channel properties).

Answer: Inactivation of Na channels is one way to achieve short-term adaptation of a neurons response. Opening of V-gated Na channels depolarizes the cell and promotes the initiation of an action potential. However, remember that for a voltage-gated Na channel to be released from inactivation the cell must repolarize in between action potentials. If a cell is repeatedly stimulated such that the membrane potential is not allowed to return to rest for a long enough period to remove this inactivation, then the firing rate of the cell will decrease until the membrane voltage drops back to a depolarized level for a duration that is sufficient for removal of this inactivation.

Some types of sensory receptors have Ca-activated K channels. These potassium channels are opened by intracellular calcium. When a cell is excited (depolarized) upon sensory stimulation, Ca enters via V-gated channels and activates the release of neurotransmitter. If the cell is stimulated repeatedly, the concentration of Ca in the cytoplasm may increase to a level that activates Ca-activated K channels to an extent that the resulting efflux of K will counteract the influx of positive ions that causes the depolarization.

4.5 What is a receptive field? What type of neurons have receptive fields (be as specific as possible)? What is the effect of inhibition from lower order sensory cells on the receptive fields of higher order relay neurons?
A description of a neuron’s receptive field is a description of the particular range of environmental stimuli that excite the cell. Hair cells of the auditory system transduce mechanical energy in the form of sound waves into electrical energy. The receptive field for a particular cell is the range of sound frequency required to excite the cell. This process leads to synaptic transmission between the hair cell and the primary afferent neurons that lead to the brain. In vertebrates this bundle of axons and cell bodies, carrying information from the hair cells of the ear to the brain, is called the eighth cranial nerve. Neurons of the eighth cranial nerve have receptive fields corresponding to the receptive fields of the hair cells that they are individually connected to. Neurons in the various auditory nuclei in the brain may be tuned to a specific frequency range based on the receptive field of the cells they are connected to coming from the ear. When we consider a line of cells from the PNS to the brain, carrying information about a specific type of stimulus in the environment, we see that each cell in line has a receptive field. Receptive fields change in size as we go from more peripheral neurons to central neuronal nuclei. Sometimes receptive fields are more narrowly tuned as we head along a line of cells toward the brain, meaning the receptive fields are becoming smaller or more specific and therefore carrying more detailed information about the stimulus.

In other examples, receptive fields get larger along afferent pathways because receptive fields from multiple sensory cells converge onto higher order relay neurons. This is called convergent excitation. This can be achieved without loss of resolution. For example, a dorsal root ganglion cell that receives input from many touch receptors on the skin will have a larger receptive field than any one receptor alone. However the ganglion cell will still respond maximally to a touch stimulus that is centered in the patch of receptors that it is connected to. This happens because the ganglion cell is connected to inhibitory interneurons that are excited when receptors in the periphery of the ganglion cell’s receptive field are excited. This is called surround inhibition. Lateral inhibition works in a similar way via the asymmetric distribution of inhibitory interneurons functioning in a feed-forward manner. Lateral inhibition is very important for making distinctions between closely related stimulus features. Studying textbook diagrams may help you to better understand how lateral inhibition increases the perceptual resolution of a stimulus by endowing cells with a more clearly defined receptive field.

4.6 Describe the labeled line hypothesis of sensory perception. What is a sensory modality? A submodality?

Answer: Any particular neuron(s) respond maximally to a specific type of stimulus. This neuronal sensitivity to a particular type of stimuli is determined by the nature of the neurons that it is synaptically connected to, and ultimately to the sensory receptor neuron or other type of sensory cell that it is connected to. The labeled line hypothesis holds that cells in the CNS respond maximally to a particular type of stimulus originating in the outside
environment because of the line of cells that it is synaptically connected to leading out to the receptors in the PNS. The different general types of stimuli are called modalities. The main modalities are those of the 5 main senses: touch or somatosensory, smell or olfaction, sight or vision, hearing or audition, and taste or gustation. Afferent lines of synaptically connected cells originate in the PNS and lead to the brain. Cell bodies and axons from lines of cells of a particular modality associate spatially in the spinal chord and lead to brain regions which specialize in a certain modality. Within each sensory modality exist submodalities, thought to arise from further segregation of labeled lines of perception. The type of submodality perceived from an external stimulus depends upon the specific type or combination of receptor cells excited within the modality relevant for that stimulus. For example, the somatosensory modality has submodalities, some of which are the perceptions of hot, cold and pain. Submodalities for gustation are sweet, bitter, salty, and sour. Activation of various combinations of limited numbers of receptor cell subtypes at different intensities can lead to a virtually unlimited number of different perceptual submodalities. Take olfaction for example. Different types of receptor cells within a modality respond maximally to a particular quality of stimulus based on the physical properties of the receptor and the interaction between the environment and the organ the receptor is in. The specific range of physical stimuli for which a cell responds to is called the cell’s receptive field. For example, in the auditory system, the receptive field of a hair cell in the inner ear is the specific range of sound frequency vibrations that are capable of exciting the hair cell.

Problem Set 5 (CENTRAL PATTERN GENERATORS, LEARNING AND MEMORY):

5.1 Explain the concept of a central pattern generator (CPG). Name a human CPG and draw its cellular circuit with its specific neuronal connections (excitatory, inhibitory). What is the input? Output?

Answer: Coordinated motor behaviors are the result of the neuronal activity in a network of cells called a CPG. A central pattern generator is a circuit of neurons capable of producing a sustained rhythmic output in the absence of continuous sensory input from the PNS nor descending input from the brain. Some examples of the behavioral output produced by CPGs are walking, breathing, flying, chewing, and swimming. Sensory information from receptors in the PNS is used to modify the pattern of activity produced by the CPG. In this way the animal can concentrate on adjusting motor activity to suit the changing external environment while the CPG does the computations that keep the basic stereotyped pattern going. The properties of a CPG depend upon the properties of the cells in the network, the properties of the synapses that connect them, and the pattern of connectivity in the circuit.
The existence of CPG circuits for stereotyped behavior is very beneficial to an organism. First, it decreases the computational load on the brain by making patterned activity more or less automatic, once it is turned on. Secondly, they help to ensure coordinated movements for a group of muscles that may otherwise work against each other. This saves energy and is crucial to producing correct motor output.

The following figure is an example of a CPG circuit. This organization is called a **half-center circuit**. This type of network is used in walking, for example. When you move limbs there are opposing muscles at work called flexors and extensors which produce movements in opposite. **It is necessary when one is contracting that the other is relaxing.** This coordination between two opposing muscles is often guaranteed by inhibitory interneurons. Note the excitatory and inhibitory connections. When cell A fires, it excites the Flex MN and its muscle. At the same time, cell A excites an inhibitory interneuron that inhibits cell B. Inhibition of cell B ensures that the Extensor MN is not firing when the Flex MN is firing. **Each side of the circuit is called a half-center. Only one half, red or blue, is active at any given time.** This is one of the most simple CPGs. But why does this circuit produce a continuous, rhythmic output without the need for input once it is started? In many CPGs the answer is a phenomenon called post-inhibitory rebound. See problem #2.
5.2 Define the concept of post-inhibitory rebound (PIR) and its underlying molecular mechanisms. (Hint: consider the number of voltage-dependent Na channels that are inactivated at -70mV vs at -90mV). What is the function of PIR in a neuron of a CPG circuit?

Answer: Postinhibitory rebound is defined as the transient increase in excitability of a neuron after the termination of inhibitory input. If two neurons mutually inhibit each other and they both have the property of postinhibitory rebound, then their membrane potentials can oscillate in an alternating manner. In such an organization, the rhythmic pattern of output can be maintained without the need for additional input to the circuit once the pattern is started. How does post-inhibitory rebound work? For a cell to exhibit the property of post-inhibitory rebound it must have a fraction of its voltage-gated Na and Ca channels open at its resting potential. This fraction must be sufficiently large such that an action potential is triggered when they are opened all at once. This is explained in more detail in the next paragraph.

When a cell is held in a hyperpolarized state in response to inhibitory input, all of its voltage gated Na and Ca channels are released from inactivation because hyperpolarization and time are the required steps in removal of inactivation. This means that all of these voltage-gated channels are ready to open upon depolarization. When the inhibitory input is terminated, outward current turns off and the cell begins to repolarize back toward its resting potential. If the cell has the parameters that endow it with the property of post-inhibitory rebound, then a significant population of the voltage-gated channels will have voltage sensitivity in the range between hyperpolarized and rest. As the cell depolarizes toward rest, positive ions rush into the cell through voltage-gated channels. The driving force bringing these positive ions into the cell is very large around rest. This influx of positive ions gives the cell’s rising transmembrane potential the boost it needs to trigger the positive feedback loop of depolarization and opening of voltage-gated channels that produces an action potential. This is post-inhibitory rebound.

5.3 What is meant by LTP between a presynaptic and postsynaptic cell in the hippocampus? How is LTP induced in the hippocampus? What is the role of NMDA receptors in the postsynaptic cell in the induction of LTP? (Hint: the regulation of the NMDA receptor is complex) Postulate how LTP might be involved in learning and memory?

Answer: Long Term Potentiation is a type of facilitation between two neurons involving an activity-dependent increase in neuronal excitability and synaptic strength. Induction of LTP requires high frequency stimulation of the presynaptic cell, called tetanus, which causes a big depolarization postsynaptically. The resulting long-term enhancement of synaptic strength results in a potentiated synapse. Although many of the precise mechanisms are unknown, LTP is a phenomena thought to arise from both pre and postsynaptic modification. To describe induction of LTP let’s consider a
glutamatergic synapse with postsynaptic glutamate receptors of the AMPA and NMDA type. With **low frequency stimuli**, glutamate is released and binds to both types of receptor. **Na** flows in through the AMPA channels only. **Flow of cations into the cell through NMDA channels is blocked by Mg** which is bound to the inside of the pore. Under conditions of low frequency stimulation, the postsynaptic cell will only depolarize a little bit and return to rest before the next release of glutamate from the presynaptic cell.

**With high frequency stimuli**, the first release of glutamate would be followed closely by another release of glutamate into the synaptic cleft, before the postsynaptic cell returns to its resting potential. The flow of Na through AMPA channels now depolarizes the postsynaptic cell enough to **repel the positively charged Mg ion from the pore of the NMDA channel**. Glutamate bound to the NMDA receptor now opens the channel in the absence of a Mg block, and more positive ions flow in through the NMDA channels. **NMDA channels pass Ca into the cell** as well as Na. The resulting rise in intracellular calcium triggers **Ca-dependent kinases** to go into action. **This results in pre and postsynaptic modifications**. Postsynaptically, high Ca has the downstream effect of increasing the sensitivity to glutamate of non-NMDA receptors. Calcium is also though to cause the release of a retrograde messenger that travels to the presynaptic cell, increases activity of certain protein kinases, thereby enhancing neurotransmitter release.

**Activity-dependent changes in neuronal excitability and synaptic strength are thought to underlie memory encoding**. Thoughts, memories, and learned behaviors are the results of sequences of synaptic activity and patterns of neuronal activation. Any neuronal modification that modulates a sequence or pattern of activity changes the properties of the physiological circuits that underlie thoughts and memories. **Learning can result in enhancement of synaptic strength at a particular synapse**. If the synapse is excitatory, this means that the postsynaptic cell will have an increased probability of firing when the presynaptic cell receives excitatory input. This is how new associations are made. **After the learning (synaptic modification) has occurred, activity in the presynaptic cell is more likely to be associated with activity in the postsynaptic cell**. So, after the learning, the same input to the circuit (excitation of presynaptic cell) could result in new output of the circuit (excitation of the postsynaptic cell) that was not there before. **Such changes in synaptic physiology, termed synaptic plasticity, are correlated with the formation of new memories and learned behaviors**.

5.4 What is sensitization? Explain how sensitization occurs at the molecular level in a sensory neuron through the input from a facilitating interneuron. (Hint: gill withdrawal reflex in *Aplysia*.)

**Answer**: Sensitization is an increased response to a variety of stimuli after presentation of a harmful or noxious stimulus. It can be described on both a behavioral and a cellular level. When an animal is presented with a harmful stimulus it learns to respond more vigorously the next time that stimulus, or even...
a harmless stimulus, is presented. This is an important survival tactic because it involves an **enhancement of defensive reflexes**. In sensitization, a **stimulus applied to one pathway results in a change in the reflex strength in another pathway**. For an example of this we will look at the *Aplysia* gill withdrawal reflex. Sensitization is a **heterosynaptic process**.

A gill withdrawal can be elicited by touching the siphon because sensory neurons in the siphon are connected to motoneurons that innervate the gill. A light touch to the siphon results in a mild withdrawal of the gill. This circuit is also sensitive to stimuli at the tail of the animal. This is because sensory neurons of the tail are connected via facilitating interneurons to the terminals of the siphon sensory neurons that synapse with motoneurons innervating the gill. **When a painful or “sensitizing” stimulus is given to the tail, facilitating interneurons are excited by the tail sensory neurons. These facilitating interneurons release serotonin onto the terminal of the siphon sensory neuron via axoaxonal contacts. Serotonin evokes presynaptic modifications in the siphon sensory neuron terminal.** Two G-protein cascades become activated, resulting in decreased K conductance, increased Ca conductance, and mobilization of synaptic vesicles to the active zone. These three effects increase the excitability of the terminal and augment neurotransmitter release. Now, the same light touch to the siphon will result in a much bigger gill withdrawal reflex because the synapse, and therefore the behavior have been sensitized.

### 5.5 What is habituation to a physical stimulus? Dishabituation? Describe 2 behavioral examples of each and their functional significance to an organism’s survival.

**Answer:** **Habituation is a decreased response to a harmless or benign stimulus when the stimulus is presented repeatedly.** It can be described on both a behavioral and a cellular level. If an animal repeatedly encounters a harmless stimulus it learns to habituate to it. This is a form of learning in which an animal becomes comfortable with the properties of a harmless novel stimulus. One well-studied behavioral example is habituation of the gill withdrawal reflex in *Aplysia*. **If the same light touch is given to the siphon over and over again, the gill withdrawal reflex goes away.** This is an important energy saving mechanism for the animal. If the animal were to continue to waste energy by moving away from a harmless stimulus in the environment, the animal’s survival would be put at risk.

**A sensitizing stimulus can override the effects of habituation in a process known as dishabituation.** The habituated gill withdrawal reflex can be dishabituated by the sensitizing stimulus of a tail shock. Dishabituation serves to increase the defensive reflexes of an animal that has put its guard down. In the presence of danger, the energy saving function of habituation is no longer relevant to the survival of the organism.

### 5.6 Describe how second messengers modulate synaptic activity. Include pre- and post-synaptic effects. Give two examples of each.
Answer: For some presynaptic effects mediated by second-messengers let’s consider the example of sensitization of the gill withdrawal reflex by tail shock, as in question #4. Facilitating interneurons release serotonin onto the presynaptic terminal of the sensory neuron. The terminal of the sensory neuron is both pre- and postsynaptic, depending on which synapse we are referring to (see figure). In these examples the presynaptic modifications lead to increased excitatory synaptic activity between the sensory neuron (pre) and the motoneuron (post). In the answer to question #4 we saw that serotonin activates G-protein cascades that result in decreased K conductance, increased Ca conductance, and mobilization of synaptic vesicles. First, efflux of K repolarizes the nerve terminal after an action potential invades. If this K conductance is decreased then the depolarization of the terminal lasts longer, resulting in more Ca influx via voltage-gated channels and therefore more neurotransmitter release. Second, an increase in Ca conductance also has the effect of raising the intracellular calcium concentration, thus evoking more fusion of synaptic vesicles. Finally, mobilization of synaptic vesicles to the active zone means that more vesicles will be ready to fuse when calcium comes into the active zone to evoke fusion. All of these modifications converge on the downstream effect of more neurotransmitter release onto the motoneuron.

The modulatory effects on synaptic activity just described are also postsynaptic modifications from the point of view of the facilitating interneuron releasing the serotonin. These short-term modifications arise from the covalent modification of existing proteins in the pre and/or postsynaptic regions of the cell.

G-protein coupled second messenger cascades can also affect processes in the cell body that have modulatory influences on synaptic activity. These types of modifications result in much longer lasting effects on synaptic activity because they involve the synthesis of new proteins. Newly synthesized proteins are then directed toward the synapse where they may be involved in the growth of new synaptic connections.

5.7 What is the difference between declarative and non-declarative memory? Give two examples of each.

Answer: Declarative memory is also called “explicit” memory because it refers to the recalled knowledge about explicit facts and events. These memories are about specific details of people, places, events, and things or who, where, when, and what. An explicit memory is formed when we associate different bits of information into one. In this respect it is highly labile. Highly labile also means that it is highly vulnerable to extinction. For example, it is very easy to forget information about specific facts if you don’t practice recalling the knowledge. Explicit memory is recalled deliberately by conscious effort.

Non-declarative memories are also called “implicit” memories. Implicit memory refers to the stored information relating to how to perform a task or
type of action. Implicit memories are procedural. The ability to perform a certain skill or habit relies on non-declarative memory. Some examples of the types of behaviors that rely on the formation implicit memories are associative learning, nonassociative learning, and motor and perceptual reflex pathways. Implicit memory is recalled unconsciously. This type of memory is more permanent and rigid than explicit memory. Once you learn how to ride a bike you are unlikely to forget it, ever, even if you don’t practice.