MCB 160 – MIDTERM EXAM 1 KEY
Wednesday, February 22, 2012

Name: _____

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Instructions:

- Write in pen. (No regrades if written in pencil.)
- Write name on top of each page.
- Clearly label any illustrations.

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1. [20 points total] A single shock to a motor axon evokes an excitatory postsynaptic current (EPSC) measured in a voltage clamped muscle cell. The EPSC, which is generated by nicotinic receptors, is carried mainly by Na^+ and K^+. Muscle V_{rest} = -80mV, E_K = -100mV, E_{Na} = +60mV. Answer following and explain your reasoning:

a. [2 points] What neurotransmitter is released by the motor axon?

Acetylcholine

b. [12 points] You vary the holding potential (the voltage of the membrane) by voltage clamping the muscle cell and then stimulate the axon at each of these potentials. What happens to the EPSC amplitude as the holding potential is changed from -80mV to -40mV to 0mV to +40mV? (No numbers required. Answer in relative terms and explain.)

-80mV: normal inward EPSC, negative current, dominated by Na+ due to its large driving force
-40mV: smaller EPSC due to a larger driving force on K+ and a smaller driving force on Na+
0mV: EPSC now outward (pos. current) because the K+ driving force is now larger than the Na+ driving force, same amplitude as the current at -40mV because the two holding potentials are the same distance from Vrev (-20mV)
+40mV: larger positive EPSC, same amplitude as at -80mV, dominated by the large driving force on K+

Longer Explanation: The reversal potential for current flowing through this channel is -20mV, the midpoint between the equilibrium potentials for K+ and Na+. At -80mV the driving force for Na+ is large ($Vm - E_{Na}$ = -140mV) and the driving force for K+ is small ($Vm - E_K$ = 20mV) so the current will be dominated by Na+ flowing into the cell resulting in a large negative EPSC. At -40mV the driving force for Na+ (-100mV) is still larger than that for K+ (60mV) so the net current will still be inward (negative), but since the relative driving forces are more equal, the amplitude of the EPSC will be smaller but still negative. At 0mV (Na+ driving force is +60mV and K+ is driving force -100mV) the current flows outward (dominated by K+). At +40mV (Na+ driving force +20mV and K+ driving force -140mV) the current is now even more heavily dominated by K+ so again the current is positive (outward). Now comparing -40mV and 0mV, the holding potentials are the same relative distances from the reversal potential (-20mV), and therefore the EPSC amplitude at 0mV will be the same as at -40mV although opposite directions. Similarly, the EPSC amplitude at +40mV will be the same as at -80mV but again they have opposite signs.

c. [6 points] You switch your setup to measure voltage, what is the relationship between the evoked EPSP and the spontaneous miniature EPSP?

The evoked EPSP amplitude is a multiple of the mini amplitude. The miniature EPSP amplitude reflects the postsynaptic cell's response to a single vesicle (quantum) and the evoked EPSP will reflect the response to multiple vesicles.

2. [10 points] In a flash of inspiration you invent a new kind of fluorescent dye that gets brighter when it binds Ca^{2+}. You then find a mutation in the voltage gated Ca^{2+} channel that breaks its interaction with Piccolo (a protein in the presynaptic transmitter release site). This mutation eliminates action potential-evoked transmitter release, even though your dye shows you that the mutation does not affect Ca^{2+} flow into the cell. Explain how this might happen.

By eliminating the interaction with Piccolo but not affecting calcium influx you could be changing the localization of the calcium channels if Piccolo is some protein responsible for clustering calcium channels near the release site. Alternatively, Piccolo could be involved in docking or anchoring vesicles to the release site thus preventing vesicles from being in close proximity to the calcium channels. In either case the high local concentration of calcium (nanodomain) at the calcium channels will not be appropriately positioned to interact with the low affinity calcium sensor synaptotagmin and thus no release will occur.

Longer Explanation: Since Ca2+ influx occurs normally, the defect in transmitter release must occur downstream of the Ca2+ signal—either a problem with docking/fusion/release of vesicles or with sensing the incoming Ca2+ itself. Synaptotagmin, the low affinity Ca2+ binding protein that is required for initiating vesicle release, must be located within the nanodomain at the mouth of the voltage gated Ca2+ channels in order for the local effective concentration of Ca2+ to reach threshold levels for binding and transmitter release. Away from the mouth of the Ca2+ channel, the concentration of Ca2+ diffuses too quickly into the bulk volume of the nerve terminal to ever reach these threshold values. By breaking the link between the Ca2+ channel and a protein that is at the presynaptic release site (Piccolo), the Ca2+ channel may no longer anchored to the complex of proteins that includes the docked vesicles and synaptotagmin on the vesicles. Without the anchor, the channel diffuses laterally in the membrane and incoming Ca2+will already have dropped to low levels by the time it nears synaptotagmin. In a similar way Piccolo could provide a link between the Ca2+ channel and the release machinery such that the SNARE proteins and vesicles are also not properly localized to the active zone. Alternatively, Piccolo could also be a low affinity Ca2+ sensitive protein involved in the release process and breaking its link with the Ca2+ channel would, like synaptotagmin in the previous example, prevent it from being localized next to where there is a high local concentration of Ca2+.

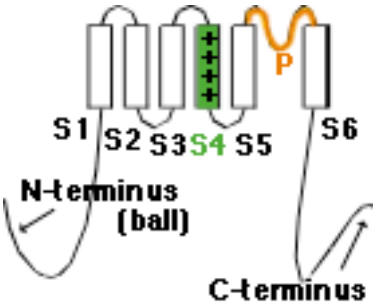
3. [10 points] A spike in a presynaptic neuron (axon #1) generates an EPSP in the postsynaptic cell that is 25mV in amplitude, decays with a time-constant (τ) of 5ms, and elicits an action potential (AP). A spike one minute later in a different presynaptic neuron (axon #2) generates a similar postsynaptic EPSP and AP. However, when the spike in axon #2 follows the spike in axon #1 by only 15ms the second EPSP is much smaller (only 15mV), decays more quickly (τ = 2ms), and it does not fire the postsynaptic cell. Note: voltage gated Na^+ channels recover from inactivation faster than 15ms. Explain what happens in the postsynaptic cell to account for this and describe how the cell recovers.

The postsynaptic cell is in the relative refractory period. During this time the delayed rectifier potassium channels remain open leading to an afterhyperpolarization (AHP) and a decrease in the membrane resistance. The AHP will mean that the second EPSP is farther from threshold so no action potential is fired. The lower membrane resistance will lead to a smaller EPSP (V=IR). The lower membrane resistance will also reduce the time constant (τ=RC). The cell recovers by allowing the delayed rectifying potassium channels to close.

Longer Explanation: When the EPSPs that generate action potentials are given sufficient time in between EPSPs, the responses in the postsynaptic cell are the same because there is no influence from the absolute or relative refractory periods. We can infer that in one minute, the postsynaptic cell will have recovered to its prior state (i.e. exited either refractory period) and therefore these two inputs must then also be the same. When the two inputs are separated by only 15ms and we see a different response to identical inputs, we can infer that the postsynaptic cell

must not be in the same initial state. Since the postsynaptic cell fired an AP in response to the first input, it is likely that the cell is in the relative refractory period as the voltage gated Na+ channels will have recovered in this time window. During the relative refractory period, voltage gated K+ channels (delayed rectifiers) are still open, even though the cell's Vm has dropped below their threshold voltage for opening/closing. Due to the prolonged conductance of K+, the Vm drops below the resting membrane potential (-70mV) towards E_K (afterhyperpolarization or AHP). During this time, three key factors dictate the EPSP of the input from axon #2: The decrease in membrane resistance (due to the large number of K+ channels still open) will result in a smaller ΔV (EPSP) in response to the inward current caused by transmitter released from axon #2 (Ohm's Law: V=IR). Similarly, the decrease in Rm will shorten the time constant τ (τ =RC) leading to a shorter duration EPSP and a smaller chance of eliciting a second action potential. Lastly, due to the afterhyperpolarization following the action potential, the Vm will be farther from the threshold required to provoke a new AP and would require a larger depolarization to fire an AP. The cell will recovers by allowing the delayed rectifying K+ channels to close. This will increase the Rm and increase the Vm. Both changes will increase the size and duration of EPSPs and at the same time bring them closer to the AP threshold.

4. [15 points] Depolarizing voltage steps (in voltage clamp) activate a K^+ channel, but it inactivates soon after opening. Describe the molecular mechanisms by which the channel senses voltage, opens, and inactivates. Draw a cartoon to illustrate your explanation.



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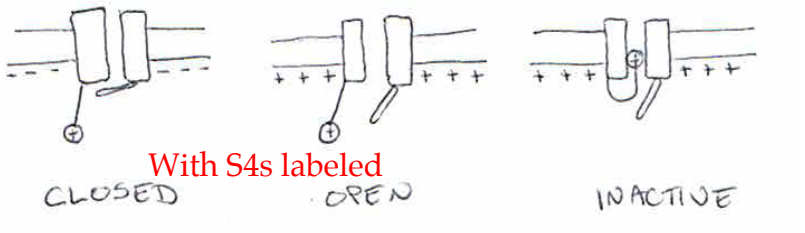

Voltage sensitivity is implemented by the S4 transmembrane domain. This domain has positively charged amino acids (arginines) at every third position (RXXRXXRXXR) and these residues sense the voltage of the membrane when they are sitting between the two leaflets of the membrane. At negative potentials, like the Vrest, the S4 domain is pulled inward and when the membrane depolarizes due to an EPSP or experimental current injection, there is an outward force on S4 moving it out of the membrane. When S4 moves out (as pictured), interactions (Van der Waals) with the intracellular side of S6 cause a conformational change in S6 (movement around the glycine hinge) that allows the gate to open. Ions flow through the pore of the channel for approximately 1ms, until the inactivation ball swings in to block the channel. The inactivation ball is comprised of 20 amino acid residues on the N-terminal end of the protein. These residues only can block the pore of the channel after the gate has already opened.

5. [10 points] A presynaptic neuron fires an action potential and releases glutamate into the synaptic cleft. The glutamate activates both ionotropic and metabotropic receptors in the postsynaptic cell. The ionotropic receptors generate depolarization and the metabotropic receptors generate hyperpolarization. What is the relative timing of these two postsynaptic responses? Explain.

Since the ionotropic receptor IS the effector, ligand binding and channel gating happen within the same macromolecule. Only one step is required to go from ligand-binding to ion flux = fast.

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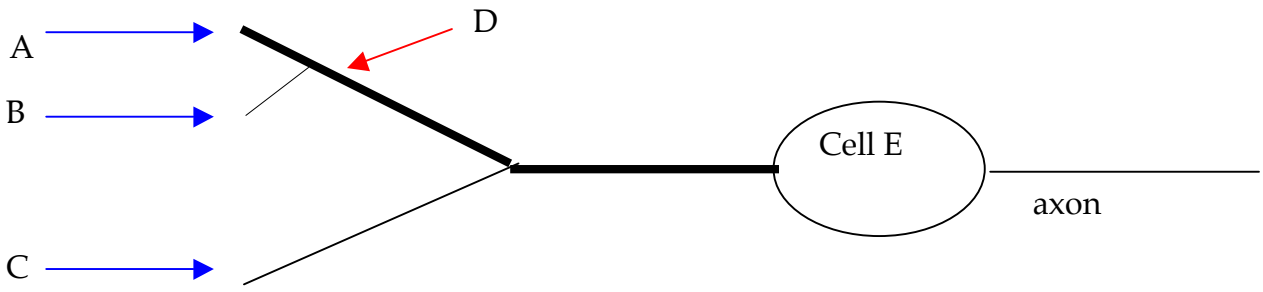
Ligand binding a metabotropic receptor activates a G-protein which needs to diffuse and interact with downstream effectors in a cascade of reactions involving second messenger molecules, which eventually regulate channel function and ion flux. Each step takes time = slower.

Longer Explanation: Postsynaptic responses due to the action of glutamate on ionotropic glutamate receptors (iGluRs: AMPA or Kainate receptors primarily, also NMDA receptors) would lead to fast depolarizations because all iGluRs are relatively nonselective cation channels. Hyperpolarizations due to glutamate binding to metabotropic glutamate receptors (mGluRs) would be much slower. Because mGluRs are GPCRs they must act via second messengers to have any effects in the postsynaptic cell. Thus activation of the GPCR will lead to activation of the heterotrimeric G-protein. In some cases the G protien can directly gate ion channels with active Gβγ (ex. GIRKs). GIRKs as K+ channels will lead to hyperpolarization of the membrane on slower time scales due to the need for activation of the G-protein. In some cases GPCR and G-protein activation will lead to generation of second messengers (ex. cAMP, DAG, IP3, Ca2+) that can then either directly act on ion channels (ex. Ca2+ sensitive channels or CNG channels). For example activation of a Ca2+ sensitive Cl- channel will lead to hyperpolarization in most cells (not true for olfactory neurons). Second messengers can activate other proteins (PKA, PKC, CaM->CaMKII) that affect the function of ion channels. For example phosphorylation of potassium channels might cause them to open, which would lead to hyperpolarization. Phosphorylation and closing of more sodium channels could also have a similar hyperpolarization (though less likely). In any of the cases the hyperpolarization due to the mGluRs would be slower than the iGluR depolarization.

6. [10 points total] Four presynaptic inputs converge onto cell E, as shown. Inputs A, B, and C release glutamate in equal amounts. The dendritic spines (not shown) responding to A, B, and C all have the same number of AMPARs (ionotropic glutamate receptors). Input D releases GABA onto a spine with ionotropic GABAa receptors. The thickness of the dendrites is as shown. You record from the cell body of E. Answer questions below and explain your answers.



a. [5 points] Compare the postsynaptic potentials in E when you stimulate A *versus* B. Explain.

The postsynaptic potential from synaptic input A will be slightly larger than B. The larger diameter of the dendrite that A travels through has a slightly longer length constant than the dendrite B travels. The length constant $\lambda_m = \sqrt{R_m/R_a}$ increases because the axial resistance decreases faster than the membrane resistance with increasing diameter. This means the EPSP from A will be larger because it will decay less over the same distance.

Longer Explanation: The postsynaptic potential from synaptic input A will be slightly larger and longer lasting than B. This is due to the relative diameter (radius) of the dendrites between input A and input B. With increasing diameter of the dendrite, the length constant increases ($\lambda_m=\sqrt{R_m/R_a}$) due to a larger decrease in your axial resistance compared to your membrane resistance. Increasing the length constant decreases the amount of decay the EPSP from A will experience by the time it reaches E. B has to travel through a smaller dendrite and that dendrite has a shorter length constant so it will decay more rapidly over the length of the dendrite. Also the EPSP from input A will travel slightly faster so it will reach the cell body slightly before B.

b. [5 points] Compare the postsynaptic potentials in E when you stimulate A alone, or D alone, or A + D together. Explain.

A: normal EPSP
D: GABA opens Cl- conducting channels that will either hyperpolarize the cell (IPSP) or have no effect depending on if there is a difference between Vrest and E_{Cl}.
A+D: The EPSP from A will be smaller and shorter. Influx of Cl- will counteract the depolarization from A and make the EPSP smaller. The decreased membrane resistance (more channels open) will decrease the ΔV from the same magnitude current input at A ($V=IR$). This decreased Rm will also reduce the time constant and the EPSP will be shorter ($\tau=RC$).

Longer Explanation: When stimulating D alone, because it is GABAergic, postsynaptic GABA receptors will allow Cl- to flow through them. This Cl- current will either hyperpolarize the cell body (IPSP) or there will be no change in the membrane potential if the cell is at or near the E_{Cl-}. Stimulating A alone will produce a large depolarizing EPSP. If A and D are stimulated together the EPSP from A will be smaller and decay faster. This is due to two factors. Opening GABA receptor channels will decrease the Rm, which decreases τ ($\tau=RC$) thus causing the EPSP decay faster. Inward Cl- currents will also act to hyperpolarize the cell so this will decrease the amplitude of the depolarizing EPSP. The lower Rm caused by opening channels at D will also reduce the size of the voltage change caused by the inward current at A ($V=IR$).

7. [10 points total] Consider the organization of the auditory system and how it encodes information about a sound stimulus.

a. [6 points] How is information about the frequency of a sound stimulus encoded in the cochlea? Which parts of the cochlea are sensitive to high pitched versus low pitched sounds and why? Use a diagram to illustrate your answer.

High pitched or high frequency sounds are encoded at the base of the basilar membrane and the low pitch or low frequency sounds are encoded at the apex. This tonotopic map corresponds to decreasing stiffness of the basilar membrane as you go from the base to the apex.

Longer Explanation: A tonotopic map along the basilar membrane encodes frequency in the cochlea with high frequencies being primarily detected at the base of the cochlea (near the oval/round windows) while low frequency is detected at the apex of the cochlea. This is primarily due to a gradient of stiffness along the basilar membrane which allows it to vibrate to different frequencies with high frequencies producing a maximum deflection at the base where the basilar membrane is stiffer and low frequencies at the apex where the basilar membrane is more floppy and thus is deflected better by low frequency sound. Also the local tuning properties of the inner hair cells are optimized for the detection of certain frequencies.

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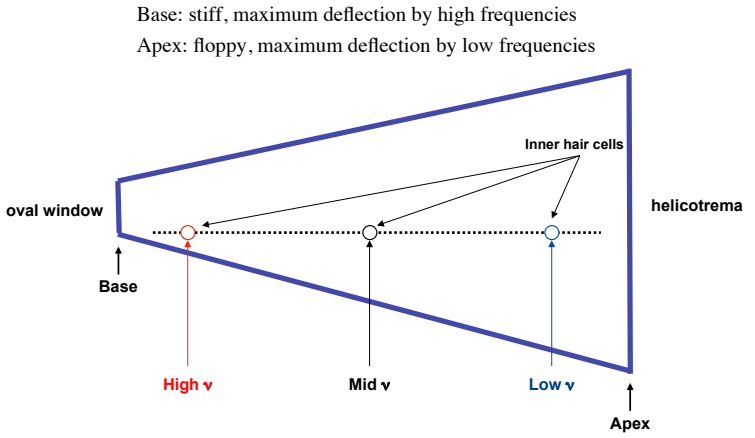

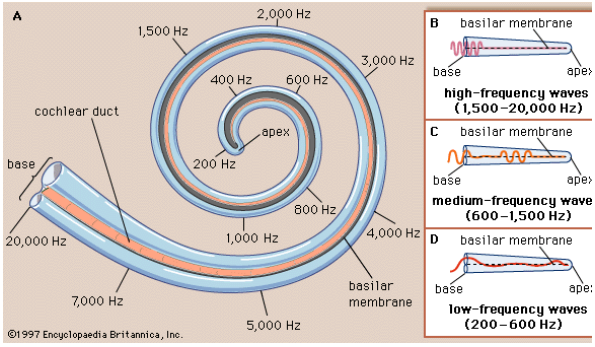

Hair cell's frequency tuning is mainly determined by its position along basilar membrane

b. [4 points] How are the frequency tuning and magnitude of the response of the sensory inner hair cells modified by the activity of the outer hair cells?

Outer hair cells receive efferent input from the brain, which causes them to move thus displacing the basilar membrane. This displacement allows for control of the local stiffness of the basilar membrane. This allows that portion of the basilar membrane to respond slightly differently to vibrations. This manipulation of the local stiffness then allows for both amplification and sharpening of the tuning curve of the inner hair cell response in a process called the "active response"

8. [5 points] You are working late in the lab recording responses of isolated olfactory sensory neurons to odors. You find that an individual neuron can respond to multiple odors. But wait: you know that each olfactory neuron expresses just a single type of odorant receptor. How do you reconcile these two observations?

Olfactory neurons express one and only one receptor however each olfactory receptor can detect multiple odorants. The identity of a particular odorant is thus identified based on the relative activation of several olfactory receptors, a combinatorial coding strategy.

9. [10 points total] You are working in the lab analyzing taste perception in a newly identified species of mammal, *Oskiebear domesticus*. You discover that *O. domesticus* can detect sour, sweet, salty, and bitter compounds via its taste system, but does not respond at all to "savory" compounds such as mono-sodium glutamate (MSG). You sequence the genome and discover a single inactivating mutation in a gene that could account for this.

a. [2 points] What protein do you think this gene encodes?

T1R1

b. [6 points] Why does this mutation affect the recognition of savory stimuli but not the recognition of sweet or bitter stimuli in these animals?

Sweet is detected by heterodimers of T1R2 and T1R3 so a mutation in T1R1 would not affect recognition of sweet. Bitter is detected by many different T2Rs so a mutation in T1R1 would not

affect bitter cells. Because taste modalities are encoded by a labeled line strategy lack of umami (savory) responses would not be expected to affect sweet or bitter taste cells or signaling to the brain.

c. [2 points] Why does this mutation not affect the recognition of sour or salty taste stimuli?

Sour taste stimuli are recognized by PKD21 and salty stimuli are recognized by ENaC. Because of the labeled line coding strategy both of the receptors for these taste modalities are in different cells and thus not affected by the umami taste modality mutation.