MCB 160 - MIDTERM EXAM #1
MONDAY MARCH 3, 2008
ANSWER KEY

Name ______________
ID#______________

Instructions:
-Only tests written in pen will be regarded
-Please submit a written request indicating where and why you deserve more points
-Original exams must be submitted with written requests
-Please submit regrade requests by end of class, Wednesday, March 19.

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1. [10 points] An action potential (AP) triggers transmitter release from the presynaptic nerve terminal (bouton) and generates an EPSP. Release take place over a period of about 1 ms. What is the mechanism of AP-triggered release and why is it limited to such a short period of time?

AP in nerve terminal opens voltage-gated Ca++ channels, yielding Ca++ influx.
Near internal mouth of Ca++ channel Ca++ concentration becomes very high. Ca++ concentration drops below levels that trigger release as soon as channel closes.
Ca++ becomes high enough to bind to low affinity C2 domains of Synaptotagmin, but only on synaptic vesicles that are docked near Ca++ channels in the active zone.
Ca++-bound synaptotagmin triggers fusion.

2. [10 points] A second AP that arrives 100 ms after the first evokes a larger EPSP. Why is this so? Design an experiment that explains the role of calcium for this scenario.

Ca++ levels away from Ca++ channel (in bulk) rise, but to a much lesser extent than near channel.
High enough, though, to bind to calmodulin, and have Ca++-CaM bind to and activate CaMIIK
CamIIK phosphorylates Synapsin, which links reserve pool vesicles to actin. Phospho-synapsin lets go of actin, allowing vesicles to migrate to active zone and dock. Increased in number of docked vesicles increases release.
Facilitation can be seen with flash photolysis of caged Ca++ to level too low to trigger fusion if AP is evoked.

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3. [10 points] A single presynaptic AP evokes a fast EPSP. A burst of 20 APs at 50 Hz (pulses per second) evokes 20 fast EPSPs that start of very big and then get smaller and smaller. These fast EPSPs are followed by a delayed long lasting slow EPSP. Explain these observations and the role of the different receptors involved.

The fast EPSPs are evoked by a classical excitatory transmitter (e.g. glutamate or acetylcholine) activating ionotropic receptors (receptors that are themselves ion channels). Release declines because of depletion of available vesicles.

The accumulated release of the classical transmitter during the burst (or the release of a peptide transmitter that only takes place if there is a high frequency burst) activates GPCRs that are on the edge of the postsynaptic area. This produces a delayed and protracted response due to the time course of the second messenger cascade (slow to turn on / slow to turn off) (time it takes for the receptor to activate G-protein, for the G-protein to then activate its effector, e..g PLC and then IP3, Ca++, and finally hit an ion channel).

4. [15 points total] 10 inputs to a cell release excitatory transmitter within 5 ms of each other, but the cell does not fire. 100 inputs to the cell release excitatory transmitter within 5 ms and the cell fires an action potential.

a) [5 points] Where in the cell is the action potential initiated? Why there?

Spike Initiation Zone / Axon Hillock / Initial Segment
Site of highest VG Na+ channel density, therefore at given depolarization larger number of channels will open per unit area: thus lowest threshold.

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b) [5 points] The action potential is conducted down to the nerve terminal. Why does it not "reflect" from there and get conducted back up the axon?

VG Na+ channels in the conducting axon inactivate after the AP passes. At the end of the axon the VG Na+ channels in the immediately preceding segment are still inactivated (in the absolute refractory period), preventing reflection.

c) [5 points] Why do the inputs have to release transmitter so close to one another in time to fire the postsynaptic cell?

EPSPs must summate and will only do so if they occur close in time (temporal summation)

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5. [10 points total]. During early development GABA receptors are excitatory, while later and in adult they become inhibitory! This is because there is a switch from one Cl- pump to another. What can this difference be? How does the GABA receptor select for Cl- ions and exclude Na+?

Early pump leaves more Cl- in cell (OK to say is weaker pump or pumps Cl- inward) so that E_{Cl-} is more positive than resting potential of -70 mV so that when GABA channels open Cl- exits cell and depolarizes voltage.
Ring of positive charges at internal and external mouths of channel repel cations and attract Cl-.

6. [20 points total] Axons from three presynaptic neurons synapse onto the same dendrite, close to the cell body ("near input"), far out on the dendrite ("far input"), and in the middle ("middle input"). The three have an equal probability of fusion of vesicles. "Far input" and "near input" release glutamate, while "middle input" releases GABA. Respond to following questions and explain your answers.

a. [10 points] Compare the size of the voltage response recorded in the postsynaptic cell body in response to a single action potential in the "near" versus the "far" glutamate input? Explain and illustrate.

Near bigger than far because it has shorter distance to conduct decrementally (passively).
Either:

Lamda=sqrt(Rm/Ra) with explanation
Or equiv circuit
Or cartoon of dendrite showing leak through membrane and axial conduction.

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b. [10 points] How will the size and shape (decay rate) of the postsynaptic voltage response differ if "far" and "middle" fire together? Draw the responses and explain your logic.

GABAR will decrease Rm

Therefore EPSP due to AMPAR will be short-circuited:

V=IR (smaller EPSP size due to reduced Rm)
τ=RC (shorter lasting EPSP due to reduced Rm)

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7. [15 points] Depolarization opens Na+ channels after a short delay. Soon after opening they stop conducting. What happens during the delay before opening? Why do they stop conducting even though the membrane is still depolarized and what sets the duration of opening before conductance stops? Support your explanation with an illustration of the channel. Label the relevant parts and explain how they work. Describe an experiment that demonstrates the mechanism that stops conduction after a short time.

Voltage sensing and S6 gate

Each subunit has an S4 segment which has a positively charged arginine at every third position (+XX+XX+XX…), where Xs are hydrophobic. Since S4 sits in membrane span arginines feel force of membrane voltage and are pulled inward to resting state at negative voltage and are pushed outward at depolarized voltage. Once all of the channels 4 S4s have been activated then S6 gate can open at the internal mouth of the channel. The S4 movements occur during the delay before opening.

Inactivation ball

Once the S6 gate has opened the inactivation ball (in III-IV linker of Na+ channel, N terminus of K+ channel) can bind and block. The effective concentration of the ball near the mouth of the channel (i.e. the length of the "chain") determines how long the ball randomly wanders around before entering the mouth and blocking the channel.

Experiment

Intracellular protease (pronase or trypsin) or deletion of ball-encoding segment of gene yield non-inactivating channels. Addition of synthetic peptide copied from ball region reintroduces inactivation to ball-less channel when applied to internal face of membrane.

If mechanism is clear in illustration not necessary to repeat in words.

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8. [10 points] You mix together vesicles containing the one V-SNARE and a green dye with vesicles containing the two T-SNAREs and a red dye. What happens? Explain and name the various parts of the SNARE complex. How is this affected when you put on BOTOX, a protease that cleaves VAMP? How is it affected if you add Ca++ (in the absence of BOTOX)?

The V-SNARE VAMP in one vesicle type will form a snare complex with the T-SNAREs syntaxin and SNAP-25 on the other vesicle type and “glue” (“dock”) the vesicles to each other. At a slow rate the vesicles will fuse (yielding yellow dye mixture).

BOTOX will block both steps.

Ca++ will have no effect, unless synaptotagmin is present, in which case fusion and dye mixing will be greatly accelerated.