7.29 J 9.09
Cellular Neurobiology
Answers to Midterm Test

Question 1.

a) M Channel. Potassium channel that is closed (indirectly by second-messenger system) consequent to binding of acetylcholine to muscarinic receptor or LHRH to LHRH receptor on sympathetic ganglion cells. Closing is responsible for slow EPSP and late-slow EPSP.

b)Xenopus. African clawed toad. Has a big oocyte that is used for testing of cloned genes for ion channels. Inject cDNA from the clone, patch-clamp the oocyte membrane and look for new conductance channels.

c) Yeast mutants. Yeast sec mutants (involved in Golgi vesicle trafficking) used to clone sec genes. Sequence of these genes corresponds to many vesicle proteins involved in calcium-evoked neurotransmitter release.

d) Freeze Fracture. A technique for visualization of biological material by electron microscopy. Fracture lines go between layers of biological membranes. Used by Heuser et al. to visualize synaptic vesicle fusion at neuromuscular junction.

e) Saltatory. Describes "jumping" conduction of action potential from node to node of Ranvier in meyelinated axons.

f) Cyclic AMP dependent kinase. Closes S-type potassium channel in *Aplysia*. Involved in synaptic facilitation, sensitization, and differential conditioning of the gill, mantle, and siphon withdrawal reflex(es) in that animal.

g) Schaeffer Collateral. An a xon to presynaptic terminals of synapses in CA1 region of hippocampus. These synapses are studied for long-term potentiation (LTP).

h) Tetraethylammonium. A blocker of potassium channels in squid axon. Used in pharmacological dissection of sodium and potassium currents in action potential (and ultimate resolution of g_K and g_{Na} kinetics).

i) Vagusstoff. The perfusible factor found by Loewi in studies of vagus nerve action on heart muscle. Turned out to be acetylcholine.

j) Atropine. The active principle in Belladonna (plant). An antagonist (receptor blocker) at muscarinic cholinergic synapses.

Question 2.

a) They changed the $[Na^+]_o$, the sodium concentration in the (seawater-like) solution bathing the axon and observed a diminished height of the action potential. (Importantly, they noted that the effect was $[Na^+]_o$-dependent and reversible.)

b) At the top of the overshoot, total ionic current = 0, so sodium and potassium currents are equal (and opposite).

$g_K(V_m - E_K) + g_{Na}(V_m - E_{Na}) = 0.$

g_K (50mV-[-80mV]) + g_{Na} (50 mV-55mV) = 0

g_K (130mV)+ g_{Na} (-5mV) = 0

$g_K/g_{Na} = 5/130 = 1/26.$

c) The major approximating assumtion is that $I=I_K+I_{Na}$, and that other currents (Leakage current, chloride current, etc) are negligible.

d) At the top of the overshoot, dV/dt = 0; so capacitative current, C dV/dt = 0.

Question 3.

a) $\lambda = [R_m/(R_i + R_o)]^{1/2}$.

b) Running wires in parallel to resistances will *minimize* the resistances = make them virtually zero. If you want to maximize the expression in a, you want to insert wires in parallel to the resistances in the denominator. How about running a wire down the center of the axon (parallel to R_i) and another wire parallel to this just outside the axon (parallel to R_o). This will cause both terms in the denominator to approximate zero and cause:

c)the expression for λ to approximate infinity. Maximized enough for you?

d) Velocity of axon propagation is proportional to λ, so velocity,Θ, is approximately infinite.

e) The space clamp of Hodgkin and Huxley. Note that infinite Θ corresponds to no spatial variation in V_m.

Question 4.

a) The synapse is inhibitory, because its reversal potential is below threshold.

b) If you stimulate the cell at rest, nothing happens. You have to stimulate while you fire an action potential, stimulate another (depolarizing or hyperpolarizing) synapse, (better)inject current into the cell or (best) voltage clamp the cell above resting, stimulate the synapse, and observe an increase inthe voltage-clamps current output (=change in the ionic current across the cell membrane).

c) To do this: (i) Voltage-clamp the cell (*to a voltage different from resting*), stimulate the synapse, and measure the total synaptic current, I_T. (ii) Patch-clamp a portion of the postsynaptic membrane (outside-out) to the same voltage as in (i) above, add gaba, and measure a single-channel evoked current, I_S. Divide I_T by I_S and, voila! channel number. (There are other ways to patch clamp but you have to make sure the outside of the receptor is accessible to gaba) similarly, you can clamp to different voltages, but then you need to specify more arithmetic.)

Question 5.

a) The synapse is excitatory, because if you have other synaptic input to raise the membrane potential near threshold, then you fire this synapse, you depolarize the cell more. The direct analogy is the postsynaptic side of the Orbelli effect--the closing of leakage channels in the muscle to increase the voltage deflection consequent to a given synaptic current. A less exact analogy is the phenomenon of slow and late slow epsp's in sympathetic ganglion cells.

b) Same answer as the answer to question 4(b), except that the direction of the observed effect would be opposite. In particular, if you voltage clamped above resting and stimulated, you would observe a *decrease* in the voltage-clamp output.

c) The synapse has a reversal potential. It is different from the usual synaptic reversal potential (that results from opening ion conductance channels) because if you depolarize above the reversal potential and stimulate the synapse you get *more* depolarization and if you hyperpolarize below the reversal potential you get *more hyperpolarization*.

Question 6

a) The sympathetic ganglion (slow and late-slow epsp's). Stimulate the preganglionic nerve and record intracellularly from postganglionic **B** cells.

b) Occlusion is the blocking of the effect of one physiological stimulus bu the overlapping application of anotherstimulus. Thus, if you iontophoretically apply sufficient muscarine to obtain a maximal depolarization and then apply LHRH (overlapping the muscarine application), you will see no additional depolarization. Conversely, if you apply LHRH to get a maximal depolarization and then apply muscarine, you will again see no additional effect.

c) They concluded that the pathways downstream from the two transmitter receptors converged at some point before the observable output (closure of M channels) so that maximal closure by one transmitter woukd occlude any effect from the other transmitter.

d) You could apply a cell-attached patch clamp to the B cell, separately iontophorese both muscarine and LHRH, and note that they inactivated conductance channels that had the same single-channel conductance, and reversal potential (and mean open time).