7.29 J 9.09
Cellular Neurobiology
Answers to 2004 Midterm Test

Question 1.

a) 2-amino-5-phosphonovaleric acid (APV). A specific blocker for the NMDA subtype of glutamate receptor. When applied to hippocampal CA1 cells, it prevents LTP without interfering much with normal synaptic transmission.
b) Adenylyl Cyclase The enzyme that synthesizes cyclic AMP from ATP. A crucial second-messenger enzyme in facilitation of the *Aplysia* sensory neuron-motoneuron synapses implicated in behavioral sensitization.
c) **Sarin**. Nerve gas (used in Tokyo subway attacks). An inhibitor of the enzyme acetylcholinesterase.
d) Belladonna ("Beautiful woman") Source of Atropine, an antagonist of acetylcholine at muscarinic receptors.
e) Norepinephrine (= Noradrenaline). Acts on alpha-adrenergic receptors on presynaptic nerve terminals to potentiate neuromuscular transmission (Part of Orbelli effect). Also a CNS neurotransmitter made in locus coeruleus cells -- but not in material for this test.
f) Tetraethylammonium (TEA) Blocker of potassium channels in squid giant axon. Used in pharmacological dissection of sodium and potassium currents and conductances during action potential there.
g) Differential Amplifier. The principal component in a voltage clamp. An electronic circuit which senses the voltage (difference in electrical potential) between two input wires and sends a proportional current from its output wire.
h) Schaeffer Collateral. A fiber in the mammalian hippocampus which terminates as a presynaptic input onto the pyramidal cells in region CA1. Important in LTP studies.
i) Tetanus toxin. A bacterial protein -- a serine protease which specifically cleaves the vesicle protein synaptobrevin and so blocks transmitter release from the presynaptic terminal.
j) Succinylcholine. A reversible antagonist of acetylcholine at nicotinic cholinergic receptors such as those at the vertebrate neuromuscular junction. Used as a "muscle relaxant" by, like, surgeons.

Question 2.

A. $5 \times 1/3 = 1\ 2/3 =$ **1.67** (quanta per evoked release).

B. $P_{(0)}$ from binomial distribution = $(2/3)^5 = 32/243 =$ **0.13.**

C. $P_{(0)}$ from Poisson distribution = $e^{-1.67}$ = **0.189**. This is a pretty large discrepancy. The principal difference is that the Poisson distribution assumes a very large (actually an infinite) number of vesicles per synapse rather than the five we have here.

Question 3.

A. Quantal analysis. Bathe synapse in low Ca^{++} solution. Look for decrease in frequencu of failures (indicates presynaptic action) or increase in spontaneous mini's (indicates postsynaptic action.

B. Locally iontophorese (or pressure-extrude) SCP locally onto the sensory neuron or the motoneuron and look for a facilitatory effect of one or the other application.

C. Occlusion experiments. Iontophorese or bath-apply serotonin on the preparation until you get a maximal effect, then (overlapping in time) apply SCP. (You can do this in either order.)Occlusion (lack of additional effect) indicates shared pathway elements.

D. You know that the final molecular effect of serotonin is closure of potassium S channels. Apply SCP to cells, use cell-attached patch, and look for closure of same channels.

Question 4.

A. The fancy electrical properties of the axon, including the ability to generate action potentials, result from the cell membrane, not the cytoplasm (except for the salts.

B. They measured internal salt concentrations, $[K^+]_i$ and $[Na^+]_i$, (by doing flame photometry on the extruded cytoplasm.

C. Re-inflate the axon with potassium that includes a radioactive isotope at a known concentration. Tie off or plug up the ends. Fire one, or (better) n action potentials. Measure the concentration of radioactive potassium in the bath solution multiply by the isotope dilution factor, divide by n and, bingo, you've got the number of moles of

potassium efflux per action potentioa. Multiply by Avogadro's number if you want the number of molecules.

D. From capacitance and the voltage excursion during an action potential. I =-CdV/dt. Q= -CΔV. ΔV during an action potential is about -110 mV (for the potassium phase. Capacitance is about 1 microfarad per square centimeter. Calculate the area of the membrane cylinder and you get the charge in Coulombs -- moles of monovalent ions.

Question 5

Most of the the experiments are by Katz and collaborators. One was by Heuser et al.

A. In the first of the experiments they set up a frog (sartorius muscle) neuromuscular junction, stimulated the presynaptic nerve, recorded intracellularly from a muscle fiber,. They varied the concentration of calcium ions in the external bath and found that the magnitudeof the excitatory post-synaptic potential (or excitatory junctional potential), measured in millivolts above resting, varied with the fourth power of free calcium concentration $[Ca^{++}]_0$.

B. In the second such experiment, the setup was the same except that they removed all free calcium from the bath solution and actually added cobalt (chloride) to block any residual calcium leaking from damaged tissue. In this case they applied calcium locally and acutely via a calcium electrophoretic pipette. They found that they had to apply calcium ti the immediate vicinity of the presynaptic terminal.

C. Setup exactly the same as above. With temporal control of calcium release from the electrophoretic pipette, they found they had to release calcium immediately before or at the time that the action potential invaded the nerve terminal. Iin a refinement, theyfound that they could block the action potential with tetrodotoxin and substitute straight depolarization of the nerve terminasl by applied current to the nerve. All this indicates that free calcium ions have to be immediately outside the presynaptic terminal at the time it is depolarized.

D. Heuser et all placed a frog (cutaneus pectoris) neuromuscular junction, stimulated it as it was falling through the air toward a cold steel plate, and froze it just as the action

potential entered the presynaptic terminal. When they subjected the tissue to (freeze-fracture or conventional transmission) electron microscopy, they saw vesicles fused wuth the presynaptic cell membrane -- hotcha!

Question 6.

A.

Area conductance of membrane:

$= 5x10^{-12}$ Siemens/channel x $5x10^{10}$ channels/cm^2

$= 0.25$ Siemens/ cm^2

Nernst potential for sodium:

$Ena=58\ mV \log [Na^+]_0/[Na^+]_i$

$=58mV \log \{400mM]/40mM]$

$= 58\ mV$

Current density through membrane:

$I_m = g_{Na} (V_m-E_{Na})$

$=0.25\ S/cm^2\ [-22mV -(=58mV)]$

$= 0.25\ S/cm^2 \times (-0.08\ V)$

$= -0.2\ A\ cm^2.$

Rate of change of voltage:

$I=-CdV/dt$

$dV/dt=-I/C$

$=-(-0.2A/cm^2)/10^{-6}F/\ cm^2.$

$=2x10^5V/sec$

$=2x10^4mV/msec$

B. Doubling the diameter of the membrane would not affect the rate of depolarization. This is because doubling the area of the membrane would double the number of channels in the membrane and hence double the current , but it would also double the capacitance, which is proportional to area. Thus dV/dt=-I/C would not be affected.

C. This calculated value is way too fast because of an exponent error of mine. The estimate is also a little too fast because (a) not all the sodium chammels are likely to be open at once and (b) there is an opposing (repolarizing) current from potassium current -- potassium conductance is never negligible and is coming on, albeit slowly, during the action potential.