Answers To Midterm 2011

Question 1.

a) **Isoproterenol.** Used to dissect presynaptic and postsynaptic components of sympathetic modulation of neuromuscular junction (Orbelli effect). Specifically activates postsynaptic component (increase in mean quantal size).

b) **Vagusstoff.** This is the diffusible substance which slowed the frog hearts in Loewi's early experiments (first identification of neurotransmitter). It turned out to be acetylcholine.

c) Neostigmine, a cholinesterase inhibitor, is used to potentiate the action of acetylcholine at neuromuscular junctions of patients with the autoimmune disease Myasthenia Gravis,

d) **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

e) **Potassium S channel**. The channel closed by the action of protein kinase A in *Aplysia* sensory neurons.

f) **CNQX.** a specific antagonist for the AMPA (non-NMDA) class of glutamate receptors on the CA1 pyramidal cells of the hippocampus. Used in pharmacological dissection of normal synaptic transmission and LTP on those synapses

g) **Freeze fracture.** Electron microscopic technique used in fast-freezing experiments by Heuser et al. on neuromuscular junction to verify vesicle fusion with presynaptic terminal membrane

h) **Dopamine antagonist.** Those mentioned in class are antipsychotic drugs, with pharmacological efficacy correlated with their binding affinity (at dopamine D2 receptors).

i) **Somatotopic.** Refers to continuous, two-dimensional a point-to point from the body surface to somatosensory or motor areas on the cerebral cortex (homunculi and whatnot).

j) **Membrane action potential.** This is an action potential evoked in a space-clamped axon, in which the whole membrane fires at once. It is in contrast to a propagated action potential.

Question 2

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.
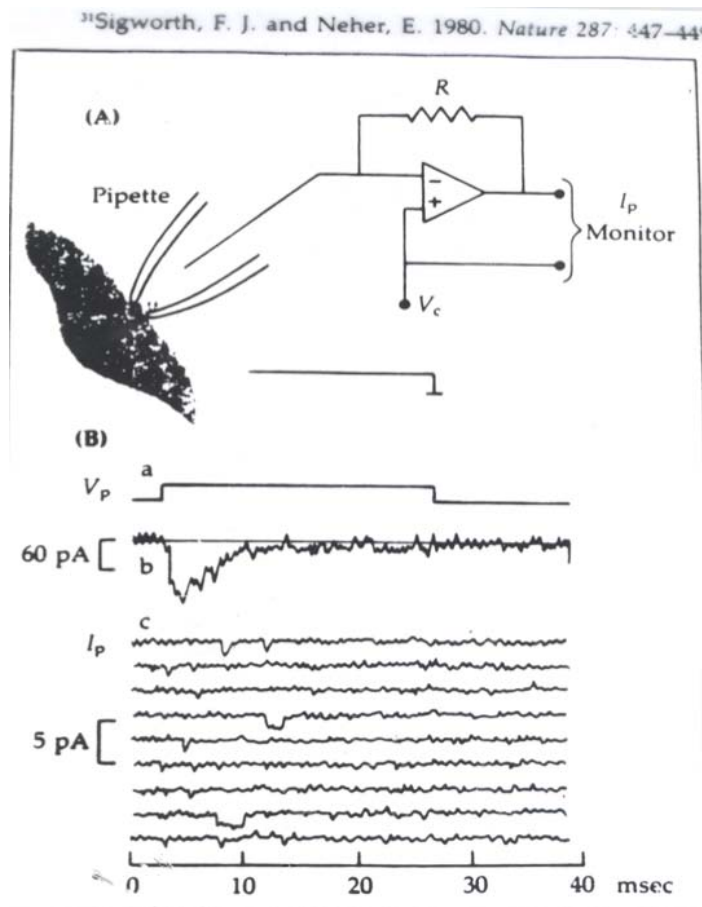
Question 3

   a) . The receptors are the nicotinic and muscarinic acetylcholine receptors.

   b) The evidence comes with specific agonists and antagonists. The nicotinic antagonist curare blocks only the fast EPSP (excitatory postsynaptic potential) after preganglionic stimulation; the muscarinic antagonist atropine blocks only the slow EPSP after such stimulation. Iontophoretically applied nicotine potentiates only the fast EPSP, applied muscarine elicits only the slow EPSP.

   c) After nicotinic receptor binding -- opening of excitatory ion channel (mixed sodium and potassium current. After muscarinic receptor binding –- closing of potassium M channel (via unknown second-messenger system).

   d) Occlusion: Apply muscarine to give maximal slow EPSP, then apply LHRH – no additional excitatory effect. (The occlusion experiment works when the order of applied agonists is reversed.) AND – do patch-clamping experiment to show that applied muscarine and applied LHRH inactivate the same potassium channel (same single-channel conductance).

   e) LHRH-like immunoreactivity is found only in presynaptic endings onto the C (small cells), but LHRH elicits physiological effects only when applied to B (big) cells.

Question 4

   a) Both setups use a voltage clamp; ie, a differential amplifier in negative feedback mode with a voltage command box on one lead. Both have input (voltage-sensing) leads connected with the fluid on the cytoplasmic and the external face

of the membrane. Both have the output (current-passing) lead running back to the fluid on one side (inside the electrode for the patch clamp; inside the axon for the squid-axon voltage clamp.) The voltage-clamp amplifier for a patch clamp can be much smaller and has to be much quicker.

b) The squid axon voltage clamp gives a **smooth curve** showing transient inward current (the delayed, steady outward current is blocked by TEA). The patch clamp gives a series of very small, square-wave looking **step increases in inward current**, as the individual channels open and close. Probabilistically the strep current profiles tend to occur soon after membrane depolarizations by the voltage clamp.

c) You would average the steppity current profiles from many runs of the patch-clamp setup to give a smooth curve of current vs time that matches the (many-channel) squid axon profile. In other words, total current from one channel depolarized many times = total current from many channels depolarized once.

[1]Sigworth, F. J. and Neher, E. 1980. *Nature* 287: 447–449.

(figure from lecture).

Question 5

Most of 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 magnitude of 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 with the presynaptic cell membrane -- hotcha!

Question 6

a) For the answer to this question we take a turn around the predictive   cycles used by Hodgkin and Huxley, going from conductance to   current to dV/dt. One then multiplies dV/dt, the rate of voltage change, by the time interval to

get the total change in the interval $(t_2 - t_1)$ and adds it to the old voltage to get the new value

First:

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

$I = 5 \times 10^{-3} \, S/cm^2 (-40 \times 10^{-3} \, V - 55 \times 10^{-3} \, V)$

$\qquad\qquad\qquad\qquad + 10^{-3} \, S/cm^2 (-40 \times 10^{-3} \, V - [-80 \times 10^{-3} \, V])$

$I = -425 \times 10^{-6} A + 40 \times 10^{-6} A$

$I = -435$ microamps$/cm^2$ (inward--sodium dominated)

Second:

$I = -C \cdot dV/dt$

$dV/dt = -I/C = 435$ microamps$/cm^2 \times 1/1$microfarad

$dV/dt = -(435 \text{ Volts/sec}) = 385$ mV/msec

Third:

$V_1 = V_o + dV/dt \cdot \Delta t$

$V_1 = -40$ mV $+ 435$mV/msec $\cdot 01$ msec

$V_1 = -35.65$ mV (-36mVis good enough)

b) You need to know g_{Na}, g_K from the new voltage, +36 mV, which you can look up in tables from Hodgkin-Huxley experiments (you actually look up the n, m and h values). You can calculate another round, as above, using same predictive cycle and equations.

7.29J / 9.09J Cellular Neurobiology

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