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Cellular Neurobiology

Answers to 2009 Midterm Test

Question 1.

(a) Gene for **NMDA receptor subunit knocked out selectively in hippocampus** – No LTP in hippocampal region CA1, no water-maze learning by mouse.

(b) Yeast **secretory mutants** – identify gene products involved in vesicle trafficking in Golgi – some are homologous to synaptic vesicle proteins.

(c) **Belladonna** – Plant from which atropine (muscarinic cholinergic receptor antagonist) is isolated. (Means "beautiful lady," because renaissance Italian women used it to dilate their pupils to make 'em look interested.)

(d) **Eels** (and rays) can be strongly electric fish, whose electric organs (degenerate muscles – almost entirely synapses) are rich sources of the proteins for Acetylcholine Receptor and for the voltage-gated Sodium Channel – also for the mRNA's that encode these proteins. Used to clone the genes for both these proteins.

(e) **Snakes** – *bungarus multicinctus,* the Taiwanese sea snake (a Krait), makes alpha-bungarotoxin, a high-affinity ligand for the alpha subunits of the acetylcholine receptor. Used to clone this, the first channel protein.

(f) **Frog hearts** were used in the Otto Loewi experiment to identify vagusstoffe and purify it to acetylcholine, the first neurotransmitter.

(g) A **Bacterial Potassium channel** was the first channel protein to be crystallized and structurally analyzed by X-ray diffraction studies. Gave hard information about the mechanisms of ion selectivity and voltage gating, got Rod McKinnon the Nobel prize.

(h) An **Incompetent organic chemist** who undertook a shortcut synthesis of Heroin wound up with a sample contaminated with the compound MPTP, which induced rapid onset Parkinson's disease symptoms in himself and his clients. Resulted in animal models of Parkinson's, understanding in environmental contributions to the disease.

(i,j) **Puffer Fish (and some Newts)** make Tetrodotoxin. Used (i) as a high-affinity ligand in the purification and cloning of the sodium channel (ii) in the pharmacological separation of sodium and potassium currents in squid-axon voltage-clamp experiments and (iii) in experiments by Miledi and Katz to show that calcium entered the presynaptic terminal through voltage-gated channels to

effect transmitter release. Any two of these, clearly identified, will get you full credit

Question 2

(a) The Orbelli effect is potentiation of neuromuscular transmission after sympathetic stimulation of the animal. Part of the sympathetic fight-or-flight response, it helped your forbears pursue and eat cute little deer and escape from fierce saber-tooth tigers.

(b) Set up spinal (headless) frog, with accessible sartorius or cutaneus pectoris neuromuscular junction. Put (extracellular) stimulating electrode on motor nerve, record intracellularly from muscle fiber. Curarize animal to partially block neuromuscular transmission so you don't get action potentials (low calcium works less well but is an acceptable substitute). Stimulate motor nerve, increase stimulus until the motor neuron axon to your muscle fiber fires; record excitatory postsynaptic potential (EPSP) in millivolts.
Stimulate animal's sympathetic nervous system by electrically stimulating spine or directly stimulating preganglionic nerves. Then fire motorneuron axon again and record change (increase in EPSP).

(c) The presynaptic mechanism proceeds from norepinephrine released from local sympathetic nerve terminals, and results in an increase in quantal content (therefore decrease in failures). Pure presynaptic facilitation can be obtained by treating preparation with norepinephrine or by running the experiment in (b) in the presence of an alpha-adrenergic receptor antagonist such as clonidine. The postsynaptic mechanism proceeds from circulating epinephrine (adrenaline) from the adrenal gland), and results in an increase in quantal size (most conveniently measured with spontaneous minis). A pure postsynaptic response can be obtained with s specific beta agonist such as isoproterenol or by running (b) above in the presence of a beta-adrenergic receptor blocker such as beta-propanolol.

(d) The postsynaptic mechanism goes through beta-adrenergic receptors and has its effect in the closure of (non-ion-specific) conductance channels on the whole muscle fiber, resulting in an increase in the cell's leakage resistance to any applied current. This results in an increase in quantal size in response to a given synaptic current; V=I**R**, and can be measured by putting a second micropipette into the muscle fiber, injecting varying measured amounts of current through it, and plotting the consequent voltage excursions as a function of current. One observes the increased resistance as an increase in the slope $R = dV/dI$.
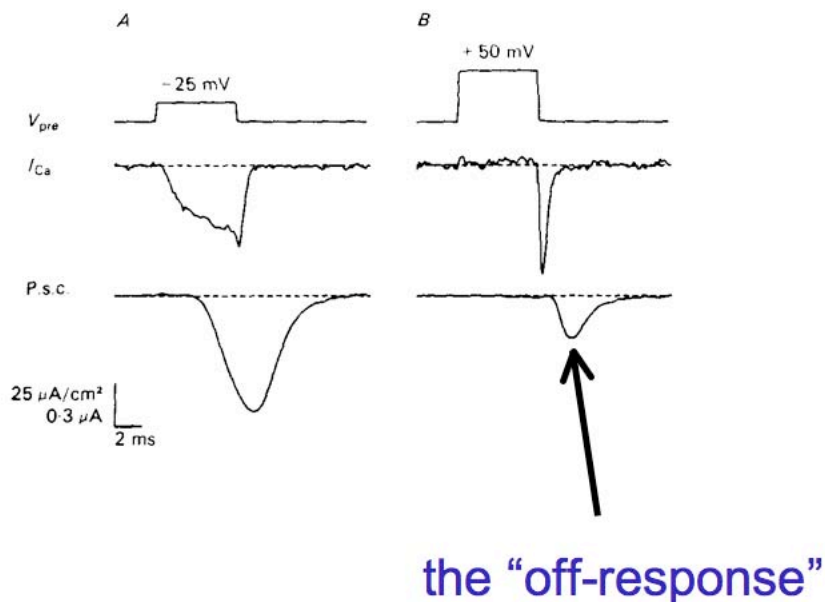
Question 3.

(a) The giant synapse of the squid.

(b) Insert a current-passing electrode and voltage-recording electrode into the presynaptic terminal (this is just about the only synapse with a presynaptic terminal large enough to do this – it can be done with one electrode if you are good at electronics). Insert a voltage-recording electrode into the postsynaptic neuron.

(c) The experimenters depolarized the presynaptic terminal above to +50 mV – more positive than the reversal potential of calcium – and in doing so prevented net calcium current from entering the cells. They then allowed the circuitry quickly to return to resting with a **very brief** interval during which the voltage-gated calcium channels remain open and the calcium can enter the cell. They then measured the postsynaptic response (they actually measured the synaptic current but you can measure the voltage), which started within microseconds of the onset of the presynaptic calcium influx.

(d) This is the slide from the lecture . The relevant figure is B, and the important point is that postsynaptic electrical activity (current in this case) begins within microseconds after the onset of presynaptic calcium current. (Variants are acceptable, most involving voltage).

Vesicle Fusion Can Occur in microseconds

the "off-response"

Question 4.

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 assumption 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 5

(a) First let's find the equilibrium potential (reversal potential) of the channel opened by GABA. Because this is neither excitatory nor inhibitory, it should correspond to threshold. For this we use the weighted average equation (Ohm's law for membranes solved for the equilibrium case of no net current). Plugging in also : $g_K = 9g_{Na}$,

$V_m = [g_K E_K + g_{Na}E_{Na}]/[g_K+g_{Na}]$
$V_m = [9g_{Na}(-80mV) + g_{Na}(+50mV)]/[9g_{Na} + g_{Na}]$
$V_m = [-720mV + 50mV]/10$
$V_m = -67\ mV$ at threshold.

Resting potential is 8mV more hyperpolarized than threshold $= -67mV - 8mV$
$= -75mV$ (surprise!)

(b) For the conductances at resting we use the same ol' weighted-average equation, now with the voltage known:
$V_m = [g_K E_K + g_{Na}E_{Na}]/[g_K+g_{Na}]$
$-75mV = [g_K(-80mV) + (100\mu S/cm^2)(+50mV)]/[g_K + 100\mu S/cm^2]$

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Clearing the denominator:

$$g_K \, (\text{-75mV}) \, + 7500\mu S.mV/cm^2 = g_K \, (\text{-80mV}) - 5000\mu S.mV/cm^2$$
$$g_K \, (\text{+5 mV}) = 12500\mu S.mV/cm^2$$
$$g_K = 2500\mu S/cm^2 = 2.5 \text{ milliSiemens}/cm^2$$

(There is a quicker intuitive way to do this, knowing that the conductance ratio is going to be the inverse of the ratio of the driving forces, $(V_m - E_{ion})$.

Question 6.

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 (or speed of light). 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.

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