

7.29 J 9.09

Cellular Neurobiology Answers to 2012 Midterm Test

[Material in brackets is non-essential information]

Question 1

- a) **A voltage-gated potassium channel from bacteria** was purified, crystallized, and analyzed by X-ray diffraction [by Rod McKinnon's group]. The resulting structure yielded the structure and selectivity mechanism of the ion-selective pore and yielded clues about the mechanism of voltage-dependent gating.
- b) **Saltatory**. Describes "jumping" conduction of action potential from node to node of Ranvier in myelinated axons.
- c) **Tetanus** kills people by blocking neurotransmission [at inhibitory synapses in the spinal cord]. The toxin, when purified, was found to be a {serine} protease that specifically cleaved synaptobrevin/VAMP, implicating this (otherwise boring-looking) protein in vesicle exocytosis.
- d) **Nicotine** [the addictive ingredient in tobacco] is a specific agonist [actually a potentiator] at nicotinic cholinergic receptor (ionotropic receptors). The compound was used in pharmacological dissection of cholinergic action in the bullfrog sympathetic ganglion. Nicotinic receptors mediate the fast EPSP, [muscarinic receptors mediate the slow EPSP].
- e) An **antibody to LHRH** localized immunoreactivity to presynaptic endings onto C cells (small cells) in the bullfrog sympathetic ganglion. But LHRH only acted on postsynaptic receptors on the B cells, constraining an interpretation of limited diffusion from one presynaptic terminal to another postsynaptic cell some distance away {acting somewhere between a classical neurotransmitter and a circulating hormone}.
- f) Atropine is a specific antagonist of acetylcholine at muscarinic receptors – used in pharmacological dissection of cholinergic action in the bullfrog sympathetic ganglion. Atropine blocks the slow EPSP.
- g,h) **Eserine or neostigmine** (acetylcholinesterase inhibitors) potentiate and prolong the action of acetylcholine, for our purposes at the neuromuscular junction. They can be used to alleviate the symptoms of myasthenia gravis, to correlate pharmacological effects of iontophoretically applied acetylcholine with evoked stimulation by the nerve, or to demonstrate the cholinergic nature of spontaneous mini's.
- i,j) Tetrodotoxin [from newts or puffer fish] is a high-affinity blocker of conductance at voltage-gated sodium channels. It was used as a high-affinity binding molecule to tag and purify the sodium-channel protein [from electric-organ preparations] and so to clone the gene, and also in the pharmacological dissection of sodium and potassium currents in squid-axon voltage-clamp studies.

Question 2

A.

$$V(x) = V(0) e^{-x/\lambda} \text{ and } \lambda = [R_m / (R_i + R_o)]^{1/2}$$

B. Note: The question mistakenly said ‘spread’ instead of ‘speed’, making the problem somewhat ill-defined. Because of this, any answers to this section which showed some factual relevance were given generous partial credit.

The action potential is propagated by local current loops, spreading depolarizing current (coming in as sodium conductance increases at the rising phase of the action potential) to regions ahead of the action potential and depolarizing them to threshold. The larger the space constant, the farther ahead the depolarizing voltage is transmitted (for a specified decrement) and the faster the excitation will travel. The proportionality turns out to be pretty exact.

C. Squids want to avoid sperm whales and Captain Nemo, so have evolved relatively rapidly-conducting axons in their escape circuits – giant axons. Here, by increasing their axon diameter (say, doubling it) they increase the cross-sectional diameter of the R_i unit resistor (they would quadruple it) and thus decrease R_i fourfold. Is in the denominator of and so they would increase λ , ideally by a factor of 2. Things don't quite work out quite this well, as R_m gets halved when you double the axon diameter, but the net effect is still an increase in λ (by $2^{1/2}$), and a proportional increase in action-potential speed.

Vertebrates (who want to run fast to avoid saber-tooth tigers and also think fast to do well on tests) have more efficiently increased λ by increasing its numerator, R_m . This they do by myelination –sheathing the axon membrane with multiple wrappings by glial cells. Then to get around the problem of blockade of all conductance channels, they interrupt the myelin sheathing at intervals (nodes of Ranvier) and concentrating all the voltage-gated sodium and potassium channels there, resulting in **saltatory conduction**.

D. The refractory period prevents backward propagation of an action potential. This is predominantly specified by the property of sodium inactivation, due to closing of the inactivation gate (h-gate) of the sodium channel protein. The h-gate is a cytoplasmic loop of the sodium channel protein that plugs up the channel after (relatively) prolonged depolarization.

Question 3

A. The patch had to be **inside-out** so that the **added** PKA catalytic subunit could act on the **cytoplasmic** face of the membrane.

B. The electronics is a little **voltage clamp**. Keeps a constant voltage across the membrane, measure current through membrane.

- C. **Current across the membrane** (in picoamps), Conductance is acceptable.)
- D. **Because of the opening and closing of individual potassium S conductance channels**
- E. **The catalytic subunit of cyclic AMP dependent protein kinase (PKA) was added to the solution outside the patch-clamp electrode.**
- F. **PKA inactivates a species of potassium conductance channels in Aplysia sensory neurons.**
- G. **It provides a defined mechanism whereby activation of the cyclic AMP second messenger system leads, via potassium channel inactivation, spike broadening, enhanced calcium entry and enhanced vesicle exocytosis, to potentiation of synaptic transmission following sensitization in this reflex circuit.**

Question 4

Conductances, voltages and current should bring to mind Ohm's law for membranes:

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

The individual sections of the questions ask about individual sodium and potassium terms.

A. For sodium

$$I_{Na} = 1\text{mS/cm}^2 (0 - 40\text{mV}) = \mathbf{-40\mu\text{A/cm}^2 \text{ (inward)}}$$

B. For potassium

$$I_K = 9\text{mS/cm}^2 (0 - (-80\text{mV})) = \mathbf{720 \mu\text{A/cm}^2 \text{ (outward)}}$$

C. Total membrane current = $I_{Na} + I_K = 680\mu\text{A/cm}^2$ (outward) The amplifier must be providing an equal and opposite current to maintain zero charge flow, ie., $680 \mu\text{A/cm}^2$

$$\begin{aligned} &= 680\mu \text{ A/cm}^2 \cdot \text{sec} \times (\text{area of membrane}) \\ &= 680\mu \text{ A/cm}^2 \times (3.14 \times 64 \text{ mm}^2) \\ &= 680\mu \text{ A/cm}^2 \times (201 \text{ mm}^2) \\ &= 680\mu \text{ A/cm}^2 \times (2.01 \text{ cm}^2) \\ &= \mathbf{1360\mu \text{ A} \text{ -- positive ions to the inside of the axon.}} \end{aligned}$$

D. Hyperpolarizing.

E. $I = -C dV/dt$

(You can do it in current and capacitance per axon, but it's easier in current and capacitance per square centimeter.)

$$dV/dt = -I/C = (-680 \times 10^{-6} \text{ A/cm}^2) / (10^{-6} \text{ Farad/cm}^2)$$

$$dV/dt = (-680 \times 10^{-6} [\text{coulomb/sec}/\text{cm}^2]) / (10^{-6} [\text{coulomb/volt}/\text{cm}^2]) \\ = -680 \text{ volts/sec}$$

$$dV/dt = \mathbf{-680 \text{ millivolts per millisecond.}}$$

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: $g_K = 9g_{Na}$,

$$V_m = [g_K E_K + g_{Na} E_{Na}] / [g_K + g_{Na}]$$

$$V_m = [9g_{Na} (-80\text{mV}) + g_{Na} (+50\text{mV})] / [9g_{Na} + g_{Na}]$$

$$V_m = [-720\text{mV} + 50\text{mV}] / 10$$

$$V_m = -67 \text{ mV at threshold.}$$

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

(b) for the conductances at resting we use the same old weighted-average equation, now with the voltage known:

$$V_m = [g_K E_K + g_{Na} E_{Na}] / [g_K + g_{Na}]$$

$$-75\text{mV} = [g_K (-80\text{mV}) + (100\mu\text{S}/\text{cm}^2)(+50\text{mV})] / [g_K + 100\mu\text{S}/\text{cm}^2]$$

Clearing the denominator:

$$g_K (-75\text{mV}) + 7500\mu\text{S} \cdot \text{mV}/\text{cm}^2 = g_K (-80\text{mV}) - 5000\mu\text{S} \cdot \text{mV}/\text{cm}^2$$

$$g_K (+5 \text{ mV}) = 12500\mu\text{S} \cdot \text{mV}/\text{cm}^2$$

$$g_K = 2500\mu\text{S}/\text{cm}^2 = 2.5 \text{ milliSiemens}/\text{cm}^2$$

(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

First experiment: [Heuser, et al.]

Frog neuromuscular junction [cutaneous pectoris – flat muscle] on end of falling piston. Nerve is stimulated so action potential invades presynaptic terminal just as preparation hits very cold steel plate (cooled with liquid nitrogen) and is instantly frozen. Preparation is prepared and shadowed for freeze-fracture electron microscopy – shows dark circles where freeze-fracture plane has cut across exocytosis hole – but not for non-stimulated preparations and shows partial recovery shortly after stimulation.. [Also, later prepared for transmission EM. In that case, one could visualize omega membrane figures from stimulated but not non-stimulated synapses].

Second experiment: [Fernandez, Neher, Gomperts]

Mast cells (involved in inflammatory response) are whole-cell clamped (electrode sealed to membrane, big hole opened), then Alternating-current signal is passed across membrane. At proper AC frequency, current transmitted is proportional to capacitance. Membrane capacitance is proportional to membrane area. What was observed was step-shaped increases in membrane capacitance after stimulation of neurosecretion from the Mast cells. This increased capacitance plausibly comes from increased membrane area as exocytosed vesicle membrane is made continuous with the cell membrane. {In later experiments the stepwise increases are temporally correlated with measured spikes in secreted neurotransmitter.

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Spring 2012

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