7.29 J 9.09
Cellular Neurobiology
Answers to Midterm Test
20 March, 2002

Question 1. This question asks you to go around one of Hodgkin and Huxley's predictive cycles. Half the problem is tightly analogous to a homework problem.

(a) First step: Given conductances and voltage forces, calculate the resultant current. Use Ohm's law for membranes.

$I = g_{Na}(V_m - E_{Na}) + g_K(V_m - E_K)$
$I = (2\ mS/cm^2)\ [-50mV - (+50mV)] + 5mS/cm^2\ [-50mV - (-80mV)]$
$I = (2 \times 10^{-3}\ S/cm^2)\ (-100 \times 10^{-3}\ V) + (5\ 10^{-3}\ S/cm^2)\ (30 \times 10^{-3}\ V)$
$I = (-2 \times 10^{--4}\ Amps/cm^2) + (1.5 \times 10^{--4}\ Amps/cm^2)$
$I = -5 \times 10^{--5}\ Amps/cm^2$

Note that the sodium (negative) current is the larger term; therefore the net current is negative = inward = *depolarizing*.

Second step: Use the differentiated definition of capacitance to get the voltage slope.

$I = C\ dV/dt$
$dV/dt = I/C$
$dV/dt = (-5 \times 10^{--5}\ Amps/cm^2)/(10^{-6}\ Farad/cm^2)$
$dV/dt = (-5 \times 10^{--5}\ Coulombs/sec/cm^2)/(10^{-6}\ Farad/cm^2)$
$dV/dt = -50\ Volts/sec$
$= 50$ millivolts/millisec in depolarizing direction.

$V' = V + \Delta V$
$\Delta V = (50\ millivolts/millisec) \times (1\ millisec)$
$V' = -50mV + 50\ mV$
$V' = 0$ mV

(b) Three principal approximations:

(i) Top of overshoot approximares E_{Na}
(ii) Bottom of undershoot approximates E_K
(iii) the conductances g_{Na}, g_K and the voltage driving forces don't change much over one millisecond. This is the most questionable of the three approximations.

Question 2. The triangle represents a **differential amplifier**. In Hodgkin and Huxley's day it was a system of tubes and resistors. Nowadays it's transistors and integrated circuits in a box that you buy.

(a) Input leads are 1 and 2. They are connected to: (i) a wire running along the outside of the axon and (ii) a wire running through down the inside of the axon. (The outside lead actually has a box with a command voltage interposed in series, but you don't need to say this).

(b) The output lead, 3, goes back to a wire running down the inside of the axon.

(c) The output **current** is proportional to the input **voltage** – the potential difference between leads 1 and 2.

(d) The output current of the amplifier is exactly equal to the (ionic) current across the axon membrane -- because they are in the same circuit loop.

(e) If you switched leads 1 and 2 you would put the amplifier circuit into **positive feedback mode**. This would cause the amplifier to send all the current it could produce across the axon membrane, thereby frying the membrane.

Question 3.

(a) The first sodium channel cloned was from the electric organ **of electric eels or rays** (actually a ray). The sodium channel was purified from tissue extracts using tritium-labeled **tetrodotoxin**, which comes from **newts or puffer fish .** The purified protein was partially sequenced (using the Edman reaction) and the sequence information used to generate oligonucleotides with some of the gene's sequence. These were used to probe cDNA and genomic libraries made from electric organ tissue. The functionality if the cDNA was confirmed by expression in **Xenopus oocytes** (not required).

(b) Salient sequence features of the coding region:

(i) fourfold internal homology – the gene codes for four similar large subdomains.

(ii) each of these subdomains has six large membrane-spanning alpha-helices. These stretches of amino acids are identified because they have lots of hydrophobic amino acids, no prolines.

(iii) In ne of the six alpha helices in each subdomain , every third amino acid is positively charged (either a lysine or an arginine). This helix apparently forms part or all of the voltage activation gate.

(iv) a positively charged cytoplasmic domain which forms the inactivation gate (as shown by in vitro mutagenesis studies.

(v) [In text}] An extracellular domaun which apparently re-enters the membrane to form part or all of the ion selectivity filter.

(c) The first potassium channel was cloned by positionally cloning the **Drosophila shaker gene,** which encodes such a channel. "Drosophila" and "mutant" gets full credit, unless you identified another mutant.

Question 4.

(a) In the **abdominal ganglion**, between (presynaptic) **sensory neurons** with receptive fields in the mantle shelf ("gill" is OK.) and postsynaptic **motoneurons** which drive gill-contracting muscles The presynaptic neuron is most responsible for the change.

(b) (i) **tail shock** (the UCS or reinforcing stimulus) and (ii) **touch to a specific locus on the mantle shelf** ("on the gill" is OK.)

(c) **serotonin** secreted in vicinity of sensory neuron presynaptic terminals *or* serotonin bound to receptors on presynaptic terminals *or* **activated G_S** protein (any one of these is OK

(d) **Ca^{++} influx** into the sensory neuron consequent to action potentials.

(e) Calcium-calmodulin-dependent **adenylyl cyclase.** The consequence is greatly increased production of cyclic AMP.

Question 5.

(a) 5 x 0.5 = **2.5** (quanta per evoked release).

(b) $P_{(0)}$ from binomial distribution = $(0.5)^5$ = **0.031.**

(c) $P_{(0)}$ from Poisson distribution = $e^{-2.5}$ = **0.82**. 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.

(d) **Botulinum toxin, tetanus toxin, cobalt**, (veratridine, tetrodotoxin).

(e) Awful.

Question 6.

(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) **Muscaria**—spotted, lawn-ornament mushroom, produces muscarine, selective agonist at muscarinic acetylcholine receptors.

(d) **Fyodor Dostoievsky** (Or V. van Gogh or T. Jones) exemplifies beehavioral traits associated with temporal-lobe epilepsy.

(e) **H. M.** His operation for intractable epilepsy produced severe anterogtade amnesia for declarative memories, elucidating the role of hippocampus and other medial-temporal structures in declarative memory.

(f) Rubidium ions **are more permeant trrough most "potassium" channels than are potassium ions**. This fact helps in understanding mechanism of ion-selectivity.

(g) In expriments by Miledi and Katz using this agent, **strong passive depolarization was able to substitute for action potential in producing evoked release**. Shows that action potential can be replaced simply by depolarization of presynaptic terminal.

(h) Injection of these oligonucleotides into the nuclei of an ***Aplysia*** **sensory neuron blocks long-term (24-hour) synaptic facilitation** of this neuron's synapses onto a co-cultured motor neuron. This finding **suggests a role for CREB-dependent gene transcription in long-term memory**.

(i) Xenopus **oocytes** are used as an **expression system to verify function** of cloned genes for conductance channels – inject the gene's transcript and look for new conductance channels with patch clamps of the oocyte membrane.

(j) **Any patch-clamp experiment.**

(k) You can find many examples in the text, but the one mentioned in class was the **localization of LHRH peptide to presynaptic terminals** (onto C cells) **in** bullfrog **sympathetic ganglia**.