7.29 J 9.09
Cellular Neurobiology
Answers to 2005 Midterm Test

Question 1.

a) Bacterial Toxin. These are **tetanus toxin** and **botulinum toxin**, various components of which bind to identified synaptic proteins (e.g. synaptobrevin, syntaxin) and verify their involvement in synaptic docking and exocytosis.

b) Calmodukin. A small (5000 Dalton) protein which binds four **calcium** ions and changes shape acting as an adaptor for the second-messenger calcium in molecules such as **CaM Kinase II**.

c) Node of Ranvier. One of many periodic gaps in the **meyelination** of meyelinated axons. This is the locus of sodium and potassium conduction in **saltatory conduction of the action potential.**

d) Orbelli effect. Sympathetic stimulation of an animal **potentiates transmission** at its neuromuscular junctions (synapses).
f) Yeast mutants. Yeast sec mutants, involved in **vesicle fusion in Golgi trafficking**, are **homologous** in sequence to some **synaptic vesicle proteins**, arguing for their functional role in vesicle docking and release.

g) DDT. Insecticide (*The Silent Spring*) specifically **blocks inactivation** of the voltage-dependent **sodium conductance channel.**

h) Cobalt. Blocks calcium entry through voltage gated calcium channels, **interferes with synaptic transmission** in Miledi and Katz's iontophoresis experiments.

i) B Cell. Big cell in sympathetic ganglion; responds to LHRH but indirectly,: .

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

Question 2.

A. The patch had to be i**nside-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 3

This question had an error by me and was logically inconsistent (made little sense) -- see part B below. Consequently, nearly everybody tried to answer it. Those who pointed out the inconsistency instantly got full or nearly full credit. Others were graded pretty generously because of the situation I put them in.

A. With a **voltage clamp on the cel**l or (better) a cell-attached patch clamp. Dial the electronics to various membrane voltages around -58 mV, apply puffs of norepinephrine and note any changes in the membrane current. New current should be outwardat voltages below (more hyperpolarized than) -58 mV and inwardat voltages above -58 mV (the opposite of the usual situation, because the neurotrasnsmitter closes the channel). There should be no new current at -58 mV. --- An alternative and acceptable experiment **is a cell-attached patch clamp that detects the closure of a defined channel species with applied neurotransmitter**, and voltage -clamp- varying experiments to show that the current through this channel disappears and reverses sign at -58 mV. The patch has to be cell-attached or the second messenger system won-t work.

B Plug into the Nernst equation**. E_K = +58mV; E_{Cl} = +58mV.** In making up this question I switched the I and o subscripts for the molar Ion concentrations (Both $[K^+]$ and $[Cl^-]$) Consequently, these values are inconsistent with the reversal potential given, and with common sense for neurons.

C. **Insufficient information**. Decide by experimental cetup in (A) above, **changing $[K^+]_o$ and then $[Cl^-]_o$, and noting any change in reversal potential.** The ion whose change affects the reversal potential is the ion conducted by the channel.

D. **Inhibitory**. It **closes** a channel whose reversal potential is way above threshold.

Question 4.

a) **Glutamate**
b) **NMDA** (N-methyl-D-aspartate)
c) **APV** (2-amino-5-phosphonovaleric acid)
d) Na^+ and Ca^{++}
e) The NMDA receptor is a ligand-gated channel. To conduct sodium and calcium ions it needs to bind **glutamate, released from the presynaptic terminal**. – But for efficient conduction it also requires **depolarization of the postsynaptic membrane, to avoid** (by charge repulsion**) magnesium blockade**.
f) The NMDA receptor's function was described on the membrane of **pyramidal cells in hippocampal region CA1, on the postsynaptic side of synapses made by Schaeffer collaterals** onto these cells.
g) Its conduction requirements (see e above) make it a logical and gate – a Hebbian molecule. **Experiments with APV show that it mediates only a small fraction of normal synaptic transmission but is necessary for all synaptic plasticity –LTP** in this lecture.
h) NMDA receptor function is required for normal hippocampal-dependent learning as assayed in the Morris Water Maze hidden-platform task. This is shown by the disruption of such learning in ratsinjected (intraventricularly) with APV or in mice withgenetically engineered knockouts of the gene encoding an essential subunit of the NMDA receptor.

Question 5

First, in a wild (non-clamped axon, replacing the outside seawater with seawater containing (e.g) 30% sodium (the rest replaced by the impermeant ion choline) the height (overshoot) of the action potential was diminished (whereas the resting potential was essentially unaffected. Second, in a voltage-clamped axon, the reversal potential for the early inward current matched E_{Na} (and was similarly sensitive to changes in $[Na^+]_o$.

B. Voltage-clamp the axon, find the reversal potential for the early current, change $[Na^+]_o$, and note that the of the reversal potential does not change 58 mV for every tenfold change in $[Na^+]_o$.

C. Ca^{++}, because Calcium ions are known to carry some of the inward current in some neurons, e.g. Aplysia sensory neurons.

D. Voltage-clamp the axon, find the reversal potential for the early current, change $[Ca^{++}]_o$, and note any change inthe reversal potential (see B above).

E. From the relative slopes of the change in the reversal potentials with changes in the outside molar ion concentrations, $[Na^+]_o$ and $[Ca^{++}]_o$.

Question 6.

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

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

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

A. For sodium

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

B. For potassium

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

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

$= 680\mu\ A/cm^2 \cdot sec$ x (area of membrane)

$= 680\mu\ A/cm^2$ x ($3.14 \times 64\ mm^2$)

$= 680\mu\ A/cm^2$ x ($201\ mm^2$)

$= 680\mu\ A/cm^2$ x ($2.01\ cm^2$)

=1360µ A -- positive ions to the inside of the axon.

D. **Hyperpolarizing.**

E. $I = - CdV/dt$

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

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

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

$= -680$ volts/sec

dV/dt = -680millivolts per millisecond.