

7.29 J 9.09

Cellular Neurobiology

Answers to 2010 Midterm Test

Question 1.

(a) **LHRH**. Peptide neurotransmitter co-released with acetylcholine by presynaptic terminals onto C cells in the bullfrog sympathetic ganglion. Diffuses, acts on postsynaptic receptors on B cells, responsible for late slow excitatory potential recorded postganglionically.

(b) **Xenopus**. African clawed toad (big ugly mother). Has a big oocyte that is used for testing of cloned genes for ion channels. Inject cDNA from the clone, patch-clamp the oocyte membrane and look for new conductance channels.

(c) The **Morris Water Maze** is used to assess hippocampus-dependent memory in rats and mice.

(d) **Donald O. Hebb**, Canadian psychologist, suggested the simplest properties for synapses that might mediate associative learning. He said that such a synapse (now called Hebbian) is strengthened when firing the presynaptic input contributes to the postsynaptic cell's activity. The shorthand catch-phrase is: "cells that wire together fire together."

Everybody for this part because of a statement I made during the review class that students were not responsible for names of researchers. got full credit

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

(f) **Calmodulin**. 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**.

(g) **CRE-containing oligonucleotides** were used to block long-term synaptic facilitation in co-cultured *Aplysia* sensory and motor neurons, demonstrating the probable involvement of cyclic-AMP-dependent gene induction(s) in this form of long-term memory.

(h) **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.

(i) Vagusstoff. Soluble messenger from Vagus nerve in Otto Loewi's frog-heart experiments. Eventually purified and identified as acetylcholine.

(j) Beta-propranolol. A blocker of adrenaline (epinephrine) at beta-adrenergic receptors. It attenuates the normal sympathetic response. Used (by me) to alleviate mild high blood pressure used (by musicians) to alleviate stage fright, used (by physiologists like Kuba) to block the postsynaptic component of the Orbeli effect (sympathetic potentiation of the neuromuscular junction).

Question 2

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 rats injected (intraventricularly) with APV or in mice with genetically engineered knockouts of the gene encoding an essential subunit of the NMDA receptor.

Question 3.

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.

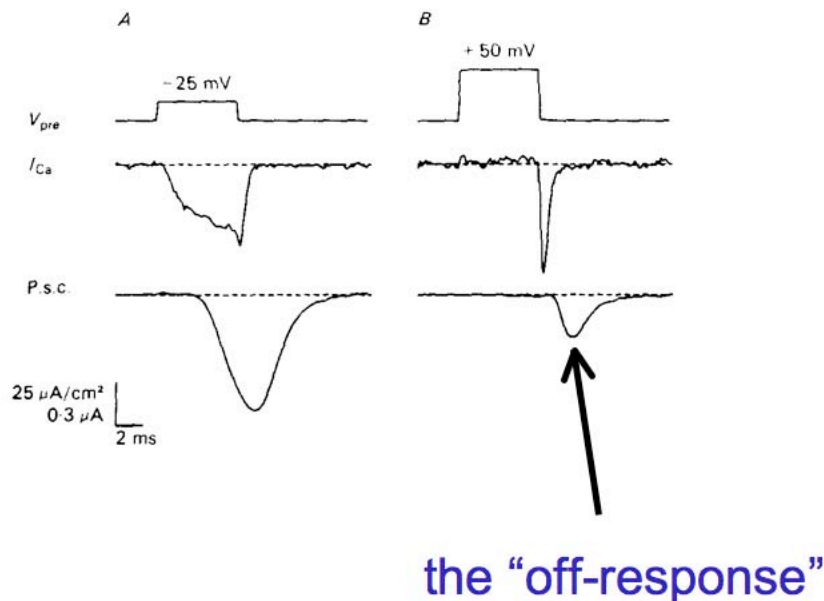
(a) Input leads are 1 and 2. They are connected to: (i) a wire running along the outside of the axon and (ii) a wire running through down the inside of the axon. (The outside lead actually has a box with a command voltage interposed in series, but you don't need to say this).

- (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 4.

- (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 so preventing 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 synaptic 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. Many variants are acceptable, most involving voltage)

Vesicle Fusion Can Occur in microseconds



the "off-response"

Question 5.

(a) Quantal analysis. Record intracellularly from the muscle, Stimulate the motor neuron, adjust the free calcium concentration in the bath to get few quanta. Compare normal and mutant neuromuscular junctions under same conditions. Measure (in each) mean quantal content m inferred from frequency of failures, P_0 .

Lower P_0 in the mutant means increased m in the mutant, *presynaptic* change in the mutant.

Similarly, record from normal and mutant muscles, *don't* stimulate, record size of spontaneous mini's v_1 . Change in mutant indicates *postsynaptic* alteration.

(b) The straightforward rationale is: Presynaptic effect. Fewer potassium channels in presynaptic nerve and terminal \Rightarrow delayed repolarization \Rightarrow broadening of action potential in motoneuron \Rightarrow increased influx of calcium ions into the presynaptic terminal \Rightarrow more vesicle fusion per action potential \Rightarrow increase in m , (decrease in P_0), increase in postsynaptic depolarization. A decrease in postsynaptic potassium conductance would increase depolarization but would prolong the synaptic potential –it gets some credit.

(c) The whole effect is analogous to the situation in Aplysia sensitization. Potassium S channel inactivation (by PKA) \Rightarrow spike broadening \Rightarrow synaptic facilitation.

Question 6.

a) Occlusion experiments. Record intracellularly from an Oblate cell. Apply sufficient GABA to obtain maximal hyperpolarization, then apply vasopressin. If the two neurotransmitters affect the same class of channel, there should be *no additional effect*. Then apply the two transmitters in the reverse order, and look again for no additional effect.

b) Put two intracellular electrodes in the Oblate cell -- one for recording voltage and one for passing pulses of current -- say 10 msec pulses of 100 nanoamps at 5 Herz. Then apply GABA (or vasopressin) and check the change in response to current pulses. Channel closure by the neurotransmitter induces an increased voltage excursion in response to the voltage pulses ($V = IR$, like the postsynaptic Orbeli effect studied by Kuba.) Another correct way to answer the question was to apply a voltage clamp to the cell, apply neurotransmitters to the synapse and look for an increase (channel opening) or decrease (channel closure) in the output current supplied by the amplifier..

c) Potassium or (less likely chloride are the only two possibilities for ions with Nernst equilibrium potentials below resting. "Potassium" or "potassium and chloride" get full credit.

d) Way cool, but any civil answer gets the 1 point.

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