Solution Key - 2010 7.012 Problem Set 7

Question 1
The MAP kinase cascade is activated by Epidermal Growth Factor (EGF) signaling. This pathway is used during development to induce various cell fates.

a) EGF can act as a morphogen. What is a morphogen?
A morphogen is a regulatory factor, either an inducer or a determinant, which regulates the fate of cells during development in a concentration dependent manner.

b) Assume that the cells below are producing and responding to EGF.

i. Which cells produce the EGF ligand? *Inducing cells*

ii. Which cells produce EGF receptor? *Responding cell*

iii. In which cells is the MAP kinase cascade activated? *Responding cell*

iv. In which cells will the activation of MAP kinase cause target gene expression? *Responding cell*

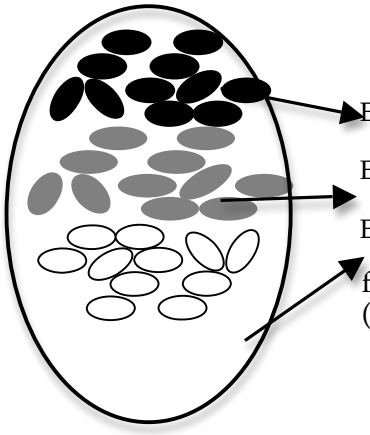
v. What type of morphogen (*inducer/determinant*) is EGF? **Explain** your choice.
EGF is a ligand secreted by the inducing cells. It induces the responding cells to acquire a specific cell fate by binding to specific receptors on their surface and activating the MAPK kinase cascade. So EGF is an inducer. Unlike an inducer, a determinant is a cell autonomous regulatory factor, which is never secreted.

c) Which class of genes (*regulatory/effector*) is expressed when an **uncommitted** cell becomes committed?

d) Which class of genes (*regulatory/effector*) is expressed when a **committed cell differentiates**?

e) What are the three classes of genes that make the combinatorial code of a differentiated cell?
Regulatory, effector and ubiquitously expressed genes in a specific cell make the combinatorial code of that cell.

A schematic of the embryonic spinal cord**, prior to any differentiation**, is shown below. Regulatory factors expressed in the cells of each region are indicated, as are the fates of the cells in these regions.



Express Pax7 and NKX2.2 proteins, future sensory neurons

Express NKX2.2 proteins, future inter neurons

Express FoxA2 and NKX2.2 proteins, future motor neurons

f) Based on the information in this schematic, could any of these genes (Pax7/NKX2.2/FoxA2)

- be an effector gene? *No since the schematic represents the embryonic spinal cord prior to differentiation.*
- be a ubiquitous gene? *Possibly NKX2.2, it is expressed in all three neurons. However, the NKX2.2 expression in non-spinal cells should be looked at to come to a conclusion.*

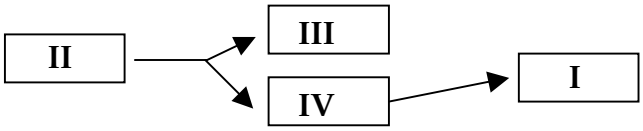
Question 2

A major goal of stem cell research is to repair diseased brains using neural stem cell lines. Four human embryonic cell lines, originally prepared from the **SAME embryo**, were tested for their potency **in vitro**, after exposure to retinoic acid, which promotes differentiation of all neuronal fates.

Cell line	Neuronal types differentiated in vitro	Potency (1=most potent)
I	motor	*4*
II	motor, sensory, lateral, hippocampal	*1*
III	sensory, lateral, hippocampal	*2*
IV	motor, sensory	*3*

a) Based on the data above, complete the table by ranking the neuronal potency of these cell lines.

b) Complete the lineage tree below using the information in the table.



c) Does each of these cell lines have the same DNA sequence? **Explain**.
They have the same DNA sequence since they are originating from the same embryo.

d) In a living organism (**in vivo**) what factors allow a cell type to acquire multiple cell fates?
The different regulatory factors that a cell receives from the surrounding cells or the surrounding environment i.e. ***niche*** *allow the cells to acquire multiple cell fates.*

e) The inducible pluripotent cells (iPS) hold great promise since they have the potential to differentiate into multiple cell types that make different organs.

i. Which cell types do you start with while making iPS cells? *Adult differentiated cells*

ii. How do you convert your starting cells to iPS cells?
In the adult differentiated cells, you introduce expression constructs for three –four transcription factors so that these transcription factors are expressed. Their expression converts some of these cells to inducible pluripotent cells (iPS) which are multi- potent i.e. can acquire multiple cell fates based on the inputs from their surrounding niche.

iii. What is the advantage of using iPS cells over embryonic cells in transplant experiments?
Since you make the iPS cells from the adult differentiated cells of the patient the iPS cells are autologous unlike the embryonic cells i.e. they will not be rejected by the immune system of the patient. The

Question 3

a) Is a functional neuron committed or differentiated? **Explain**.
A functional neuron is differentiated. It expresses a certain set of effector genes that it needs to perform its specialized functions.

b) Define a resting membrane potential and an action potential. Are these membrane potentials exclusively present in neurons? **Explain**.
All cells have an unequal distribution of charges across their plasma membrane and this results in the outside being more positive compared to inside. This unequal distribution of charges across the plasma membrane is called resting membrane potential. The resting membrane potential is an electrochemical potential of all cells, and can be exploited to do some forms of cellular work. The action potential is a transient change of the membrane potential in a tiny patch of the membrane. This transient change is propagated down the length of the neuronal axon or muscle fiber and can result in signals from one neuron to another. All the cells have a resting membrane potential. However, it is the neurons and muscle cells that fire an action potential.

Question 3, continued

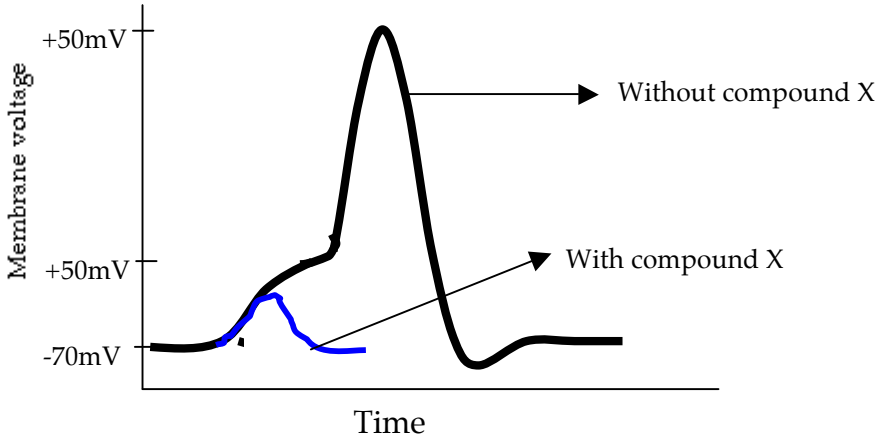
c) List the distinct protein complexes that are found in the plasma membrane of nerve cells and are essential in establishing and maintaining the **resting membrane potential**. For each protein complex you list, include what ion(s) move through that protein complex and in what direction that ion moves (into the cell or out of the cell) to maintain the resting membrane potential.

Protein complexes	Ion(s) moved	Net direction of ion movement
Open K+ channels	*K+*	*Out*
Na+K+ ATPase pump	*Na+ and K+*	*Na+ out , 2 K+ in*

d) Once the resting membrane potential has been established, which protein complex or complexes are essential to generate an **action potential** (*both the depolarization and repolarization phases*). For each protein complex you list, include what ion(s) move through that protein complex and in what direction that ion moves (into the cell or out of the cell) to generate an action potential.

Protein complexes	Ion(s) moved	Net direction of ion movement
Voltage gated Na+ channels	*Na+*	*In*
Voltage gated K+ channels	*K+*	*Out*

e) The compound X alters the action potential by interfering with one of the protein complexes listed above.



Which protein complex is most likely affected by this compound? **Explain** your reasoning.
Compound X prevents the membrane potential from reaching the threshold level and most likely inactivates the voltage gated Na+ channels.

f) Patients with episodic ataxia exhibit loss of coordinated movements during periods of mental or physical stress. This disorder is due to the absence of the neuronal **voltage gated K+ channels**. Would the deficit in the voltage gated K+ channel……

i. Make the resting potential of the neurons **more negative, less negative or not change it**, relative to normal neurons? **Explain**.

It will be not change. But it will take a longer time to reach the resting level since the repolarization in the absence of active voltage gated K+ channel, will be very slow and will occur only due to the open/resting K+ channels.

ii. Make the threshold potential of the neurons **more negative, less negative or not change it**, relative to normal neurons? **Explain**.

Threshold potential never changes.

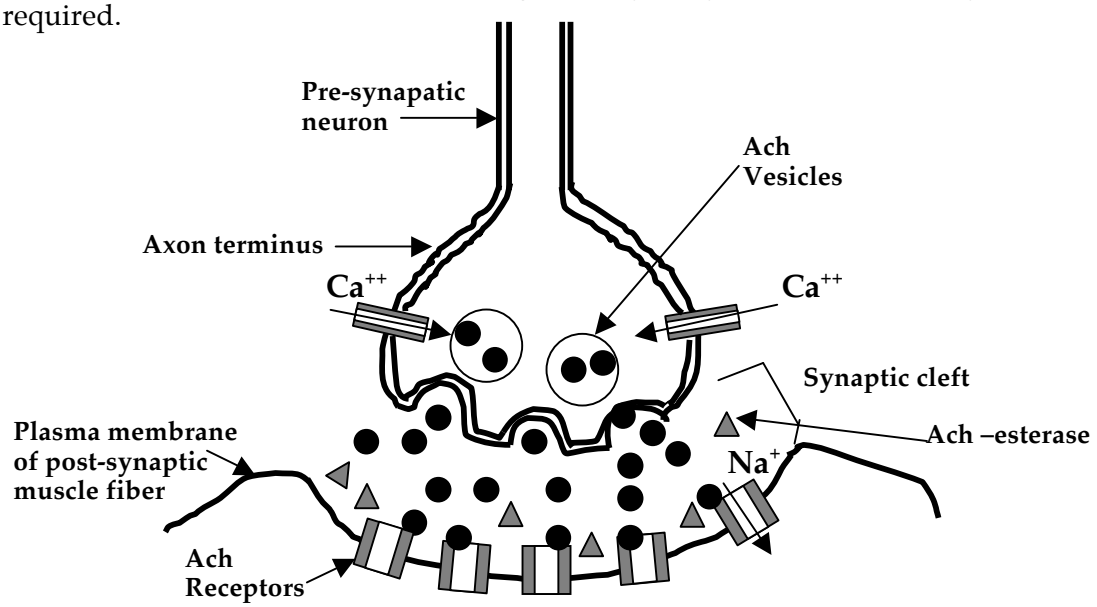
Question 3, continued

iii. **Increase/decrease/not change** the likelihood of the neuron to fire during periods of stress relative to normal neurons? **Explain**.

It will increase; since the repolarization in the absence of active voltage gated K+ channel, will be very slow and will occur only due to the open/resting K+ channels. Note: In fact, ataxia results from too frequent action potentials. Please look at the explanation in part (i).

Question 4

The following is a schematic of an excitatory neuromuscular junction. In this schematic, the axon is pre-synaptic and the muscle is post-synaptic. The excitatory neurotransmitter is acetylcholine (Ach), and it binds to nicotinic acetylcholine receptors (AchR), which are **ligand-gated Na+ channels**. This results in muscle contraction. The neurotransmitter is degraded by acetylcholinesterase enzyme when it is no longer required.



a) Once an action potential has been generated it travels down the axon of the pre-synaptic neuron. Outline the steps involved in how this action potential allows the cell to signal to a muscle cell and how the signal produces a depolarization of the muscle cell. Include any relevant channels, ions, and molecules specific for this process.

When the nerve impulse reaches the axon terminals, it causes a depolarization of the membrane that triggers the opening of voltage-gated Ca^{++} channels, Ca^{++} ions enter the nerve terminal through these channels. Ca^{++} interacts with and activates a Ca^{++} dependent kinase, which phosphorylates and inactivates a protein called synapsin and causes the release of vesicles containing neurotransmitters. This allows the fusion of the vesicles containing neurotransmitters (acetylcholine, Ach) with the membrane so that these neurotransmitters are release into the synaptic cleft. Ach diffuses across the synaptic cleft and binds to specific Ach receptors present in the muscle cell membrane. The Ach receptors act as a ligand-gated Na^{+}channel, and if enough acetylcholine is released, the muscle cell becomes temporarily more permeable to Na^{+} ions, which rush into the muscle cells thus generating an action potential. It should be noted that when an action potential is occurring the Ach gets broken down to acetic acid and choline by acetylcholineesterase enzyme AchE. Thus the muscle cell relaxes until stimulated by the next round of acetylcholine release.

b) In the autoimmune disease, myasthenia gravis, antibodies are produced against one's own acetylcholine (Ach) receptors in muscle. These antibodies either degrade the Ach receptors or impair their binding to Ach.

i. How would the frequency and amplitude of an action potential in the muscle of a myasthenia gravis patient differ from that in an unaffected individual? **Explain**.

Any loss of function *(i.e. blocking Ach receptor binding site, conformation change etc) of the Ach receptor can cause this disorder by preventing muscle from receiving the signal (from pre-synaptic neuron) so that it can contract. So there will be a decrease in the frequency of muscle contraction. However, if an action potential is elicited its amplitude will remain unchanged.*

Question 4, continued

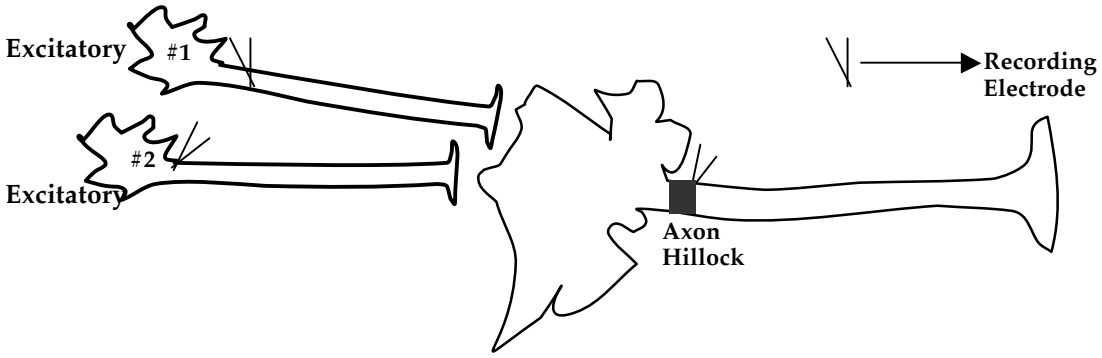
ii. Would an inhibitor of acetylcholinesterase be potentially useful in alleviating the symptoms of this autoimmune disease? **Explain** your answer.

Most likely yes since this will prevent the Acetylcholinesterase enzyme from degrading the Ach. Therefore the neurotransmitter can stay in the synapse for a prolonged time and will be available to bind to the fewer Ach receptor located on the surface of muscle cells in this patient to elicit the response.

c) Lambert-Eaton syndrome is associated with the production of antibodies that bind to and block pre-synaptic **voltage gated calcium channels**. **Explain** why such antibodies cause a decrease in muscle contraction in affected individuals relative to normal individuals.

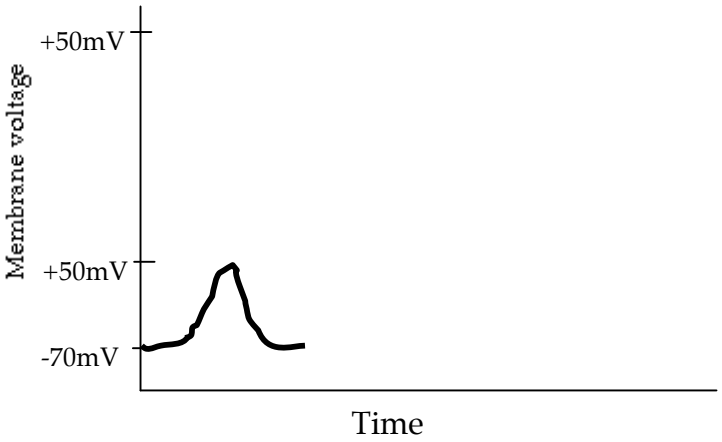
The antibodies will prevent calcium influx through voltage gated calcium channels that will cause ***the failure of vesicles to fuse with the plasma membrane and release the Ach in the synaptic cleft*** *leading to decreased or no muscle contraction.*

The following question refers to an experimental design depicted below. There are two excitatory pre-synaptic neurons that independently converge on a post-synaptic neuron. Electrodes placed into the two pre-synaptic neurons can be stimulated individually. In the absence of any stimulation, the recording electrode measures the electrode potential as -70mV.



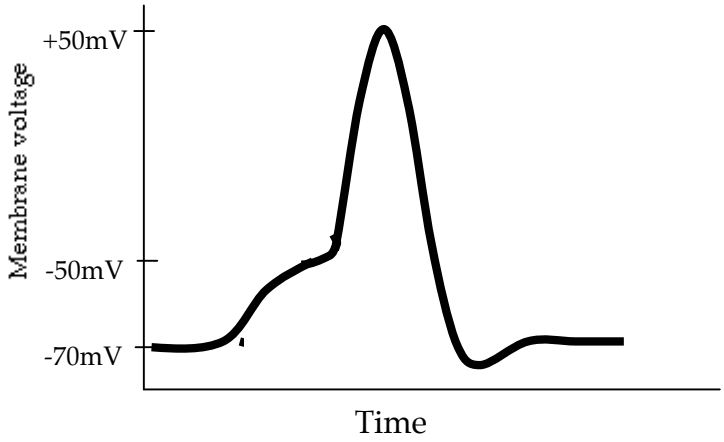
If **only one** excitatory pre-synaptic neuron is stimulated, you record a deviation from -70mV with the recording electrode in the post-synaptic neuron but you do not record an action potential. If **both** the excitatory pre-synaptic neurons are stimulated, you record an action potential in the post-synaptic neuron.

d) On the graph below sketch the changes in the post-synaptic neuronal membrane potential, as measured by the recording electrode, when **only one** excitatory pre-synaptic neuron is stimulated.



Question 4 continued

e) On the graph below sketch the changes in the post-synaptic neuron membrane potential when **both** the pre-synaptic neurons are stimulated.



Question 5

Aplysia californica, a sea slug, is a useful model in neurobiology to study neural transmission and neuronal circuits. It was Eric Kandel who studied the siphon mediated gill withdrawal reflex and was awarded the Nobel Prize in 2000.

a) You stimulate the siphon –mediated gill withdrawal reflex in aplysia in the following experiments.

- **Experiment 1**: You tap the siphon of this organism 30 times, once every 2 sec, at a stretch and observe a decrease in the intensity of the gill withdrawal reflex, which persists for a short duration.
- **Experiment 2**: You apply a strong electrical shock to the tail and simultaneously tap the siphon and measure a sharp and an intense increase in gill withdrawal reflex.
- **Experiment 3**: You repeatedly apply a strong electrical shock to the tail and simultaneously tap the siphon periodically for few days. You find that that intensity of the gill withdrawal reflex decreases over time. After many days you repeat the experiment and find that the decrease in the gill withdrawal reflex is persistent.

Based on what you have learned from Prof. Lander's neurobiology lecture series…

i. Which of the above experiments provide an example of habituation?

Both 1 and 3 are example of habituation. Please note that experiment 1 is an example of short-term habituation in comparison to experiment 3, which is an example of long-term habituation.

ii. Which of the above experiments is an example of sensitization?

Experiment 2

iii. Which of the above experiments is an example of learning and memory?

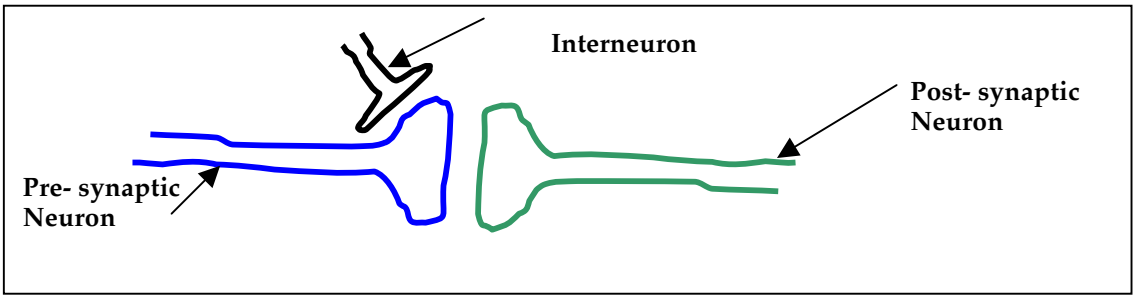
Experiment 3

b) Habituation is a result of the signaling events between a pre-synaptic neuron, that secretes an excitatory neurotransmitter 5-HT and synapses with a post-synaptic neuron of the gill. With habituation the pre-synaptic neuron show a progressive inactivation of **voltage gated calcium ion channels**. How may this explain the habituation shown by the post-synaptic neuron?

The inactivation of the voltage gated Ca^{++} channels results in a decreased influx of Ca^{++} ions into the axon terminus of the pre-synaptic neuron. Ca^{++} ions are essential for the fusion of the pre-synaptic vesicles with the membrane of the axon terminus and the release of 5-HT into the synaptic cleft. Therefore the inactivation of voltage gated Ca^{++} channels will slow the release of 5-HT. Thus less 5-HT will be available over time to bind to specific receptors on the surface of the post-synaptic neuron resulting in decreased response over time i.e. habituation to the applied stimulus.

Question 5 continued

In sensitization, an interneuron synapses with a pre-synaptic neuron which then synapses with a post-synaptic neuron as shown in the following schematic. Both the interneuron and the pre-synaptic neuron secrete 5-HT.



The interneuron, synapsing with the pre-synaptic neuron secretes 5-HT, which binds to its specific receptors located on the surface of the pre-synaptic terminal. This is followed by activation of an enzyme known as adenylyl cyclase, which in turn produces cyclic AMP from ATP. The cAMP then activates another enzyme, protein kinase A (PKA), which phosphorylates and inactivates voltage gated potassium ion (K+) channels.

c) How may the **inactivation of voltage gated K+ channels** in the pre-synaptic neuron explain the sensitization exhibited by the post-synaptic neuron?

This will result in a slow repolarization phase of the action potential. Hence the voltage gated Ca^{++} channels will remain open for a longer duration resulting in an increased influx of Ca^{++} for a longer period of time. This allows more vesicles fusion and an increase in the release of 5-HT in the synaptic cleft. Thus more 5-HT will be available for a longer time to bind to specific receptors on the surface of the post-synaptic neuron resulting in increased response.

d) Very briefly give one hypothesis to explain the results of experiment 3.

This experiment is an example of long-term memory that requires the activation of nuclear components. This may ultimately result in the alteration of synaptic connections or alterations in the type or amount of neurotransmitters or their corresponding receptors.