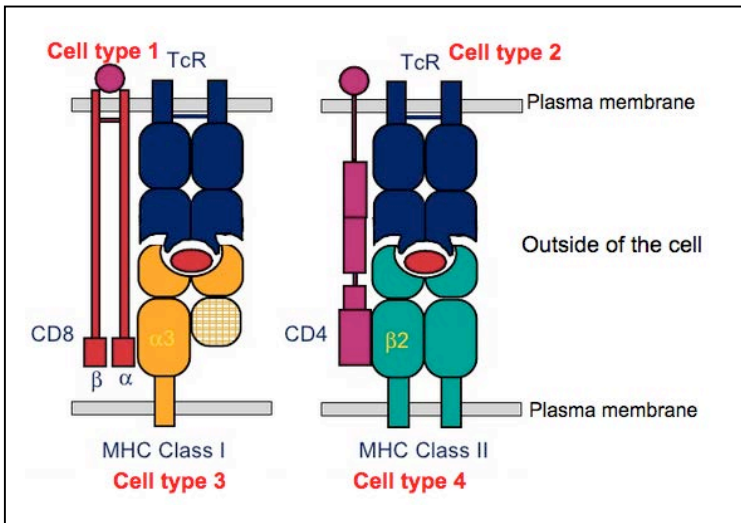


Solution key-7.013 Problem Set 7- 2018

Question 1 (4pts)

The schematic below shows the interaction between the T cell receptors (**TcR**), Major histocompatibility complex Type I (**MHCI**) and II (**MHCII**) and the accessory cell surface molecules (**CD4** and **CD8**) located on the surface of **cell types 1-4**.



a) Which cell type is likely a helper T cell (T_H): **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**? *CD4+ cell, type 2 (0.5pts, 0.25 each)*

b) Which cell type is likely a cytotoxic T cell (T_C): **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**? *CD8+, cell type 1(0.5pts, 0.25 each)*

c) Which cell type is most likely a professional antigen presenting cell (**APC**): **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**? *Cell type 4 are professional APC, MHCII expressing cell type 4(1pt, 0.5 each)*

d) Identify the cell types that interact with each other to trigger ...

i. B cell activation and humoral (Antibody mediated) immune response: **Cell type 1/2/ 3/ 4**

Type 2 and 4(0.5pts, 0.25 each)

ii. Cytotoxic T cell mediated killing of an infected cell: **Cell type 1/2/ 3/ 4**? *Type 1 and Type 3*

(0.5pts, 0.25 each)

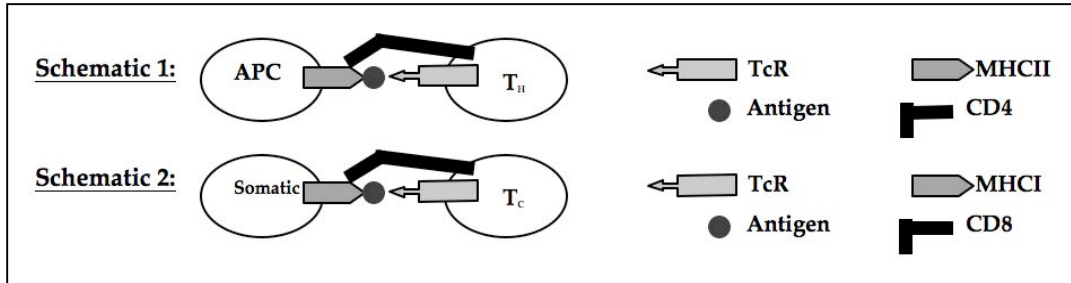
e) An individual who is heterozygous for two alleles of the Antibody (Ab)/ Immunoglobulin gene (Ig) expresses only ONE of the two alleles for this gene. Briefly **explain** why this is critical.

This is due to allelic exclusion, which is process that avoids dual specificity of T and B cells for antigens. A successful VDJ recombination sends a signal back to the nucleus to suppress recombination on other chromosomes/ homolog. If however, the first allele recombination fails, no suppression occurs and there is a VDJ recombination in the 2nd allele of the Immunoglobulin and/ or TcR gene. (1pt for the general idea)

Question 2 (6pts)

Gardasil is a preventive vaccine that was designed against the **surface proteins** of Human Papillomavirus (HPV), a **DNA virus** that causes cervical, head and neck cancer.

a) Which of the following schematics (1 or 2) represents the immune response that will be triggered following vaccination with Gardasil.

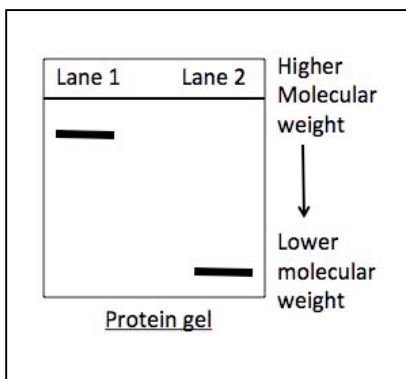


Schematic 1: Here you are using viral proteins, which can be engulfed by APC to trigger humoral immune response. (1pt)

b) Following vaccination with Gardasil, why the secondary immune response against HPV is **faster and more effective** than the primary immune response?

During the primary immune response, the memory B and T_H cells, against the specific antigen, are generated. During the secondary immune response, these can proliferate to produce plasma B cells that produce and secrete antibody, which has a higher affinity for the antigen than surface antibody. The secreted antibody molecules can bind to the antigen to neutralize its infecting ability. Furthermore, they also promote the phagocytosis of antibody bound antigen. (1pt)

c) During a primary and secondary humoral immune response to an antigen, the mature and memory B cells **produce surface antibodies**. Furthermore, the memory B cells, during secondary immune response can give rise to the **plasma B cells that produce secreted antibodies**. You isolate the antibodies that are specific to the HPV surface proteins and resolve them on a gel designed to separate proteins based on the difference in their molecular weight. The gel profile is shown below.



i. Which class of antibodies is present in **Lane 2** of the gel:
secreted IgG OR IgM?

Secreted IgG (1pt)

ii. From the gel, which B cell-type produces the antibody type shown in **Lane 2**?

Plasma B cells likely, they produce secreted antibody (IgG), which is monomeric as opposed to IgM of the memory B cells, which maybe pentameric. (1pt)

d) If you compare the structure of secreted and surface antibodies that are specific to the **same epitope** (antigenic determinant) of an antigen, would you expect these antibodies to have ...

i. The **same or different Variable** regions? **Why?**

There may be slight variations (if at all) since the antigen-binding site of secreted antibody has a higher affinity for the antigen as opposed to that of surface antibody. (0.5pt)

ii. The **same or different Constant** regions? **Why?**

There would be different since the secreted antibody will be lacking the transmembrane domain as opposed to surface antibody, which are usually on the surface of mature and memory B cells. (0.5pt)

Question 2 continued

e) Our immune system is capable of producing antibodies (IAb) and T- cell receptors (TcR), each of which has antigen-binding sites that are specific to a particular antigen. If our genome only has approximately 20,000 genes, **list three processes** by which our immune system can produce millions of Abs and TcRs.

*The immunoglobulin gene has approximately 100 variable (V) segments, 30 diversity (D) segments and 6 joining (J) segments, which can undergo **DNA recombination** in order to produce different combinations of V, D and J segments for heavy chains and V and J segments for light chains to produce millions of antibody molecules each of which is unique to a specific antigen. In addition, more variations at the DNA level can be generated through **somatic hypermutations**. Junctional diversity can be generated due to **terminal transferase activity**. **Class switching** and **affinity maturation** can further enhance the variation. (1pt)*

Question 3 (2pts)

Multiple sclerosis (MS) is an autoimmune disorder in which the immune system attacks and destroys the myelin sheath of a neuron.

a) What immune mechanism is disrupted in MS patients, resulting in the production of self-reactive antibodies? (0.25pts)

The self reactive T cell are usually eliminated in the thymus during the process of their development by negative selection or clonal deletion. If this goes wrong then autoimmune disorders such as MS arise.

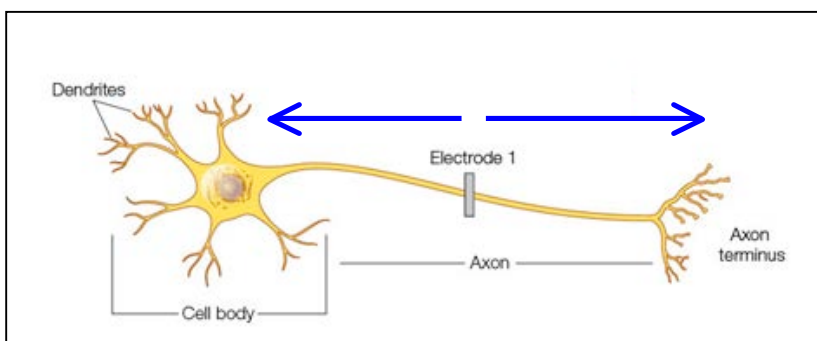
b) Immunosuppressive drugs are often prescribed to patients with autoimmune diseases. What would be the most common side effect of these drugs?

The patient will be highly prone to opportunistic infections since the patient's immune system will be compromised. (0.25pts)

c) What happens to the speed of propagation of action potentials in a neuron when the myelin sheath is destroyed: **slows down/ speeds up/ shows no change**? **Explain** why you selected this option.

The absence of the myelin sheath causes the ions to leak or diffuse instead of moving down the length of the axon. This slows down or abrogates the speed of propagation (conductance) of the action potentials along the length of the axon depending on the severity of MS. (0.5pt with 0.25pts for explain)

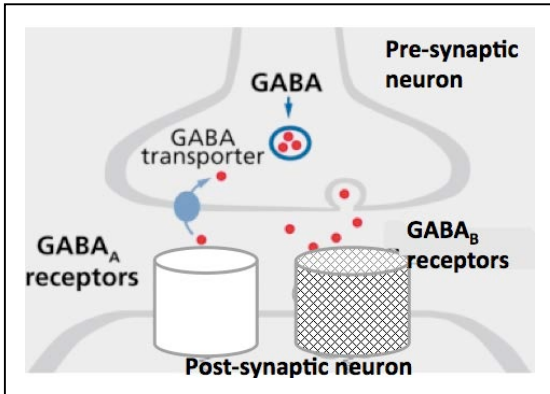
d) The schematic below a normal healthy neuron. Use an arrow(s) to show the propagation of the action potential (AP) after stimulating the neuron by passing current through electrode 1. **Explain** why it differs from the normal direction of propagation.



Since the signal begins in the middle of the axon, the VG Na⁺ channels are in the READY conformation on both sides of the stimulus. So the stimulus can therefore open these VG Na⁺ channels to yield an action potential signals that propagate in both directions. The action potential on either side will not revert back since the VG Na⁺ channels maintain a refractory phase once they close. (1pt with 0.5pts for explain)

Question 4 (4pts)

GABA is an inhibitory neurotransmitter in the central nervous system (CNS). It acts by binding to GABA-A receptors that are ligand gated chloride channels (Cl⁻) and GABA-B receptors that activate potassium channels (K⁺) via G proteins.



a) In response to GABA, would you expect both types of receptors to activate ion flow with the same time course? **Explain.**

No, GABA-A receptor will act fast since they are ligand gated ionotropic receptors. In comparison, GABA-B receptor are metabotropic (slow) receptors since they activate the K⁺ channels via G protein ie. they are not directly acting as receptors for GABA. (1pt, with 0.5 for explain)

b) Note that the K⁺ concentration is high inside the neuron, while the Ca²⁺, Na⁺ and Cl⁻ ion concentrations are high outside. In what direction would Cl⁻ ions flow when the GABA-A receptor is activated: **into OR out of the neuron**? How does this flow alter the likelihood of an action potential in the post-synaptic neuron? **Explain.**

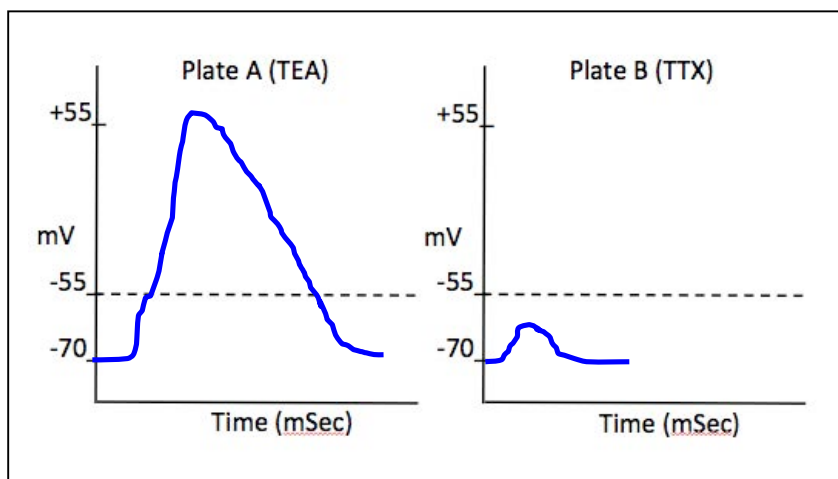
GABA is an inhibitory neurotransmitter. The chloride ions will diffuse down their concentration gradient from outside to inside thus hyperpolarizing the membrane and bringing the membrane potential further away from the threshold potential. So there won't be any action potential generated in the post-synaptic neuron. (1pt, with 0.5 for explain)

c) You culture a GABA producing neuron in the presence of the following neurotoxins in two separate cell culture plates (Plate A & Plate B). You then apply excitatory stimuli to both plates.

Plate A: Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K⁺ channels**.

Plate B: Neuron is treated with tetrodotoxin (TTX), which **inhibits voltage gated Na⁺ channels**.

Sketch the alteration in the action potential following the treatment of the GABA producing neuron with each neurotoxin. **Note:** If there is no change, please write **"NO CHANGE"** on the graph. The resting and threshold membrane potentials are -70mV and -55 mV respectively.

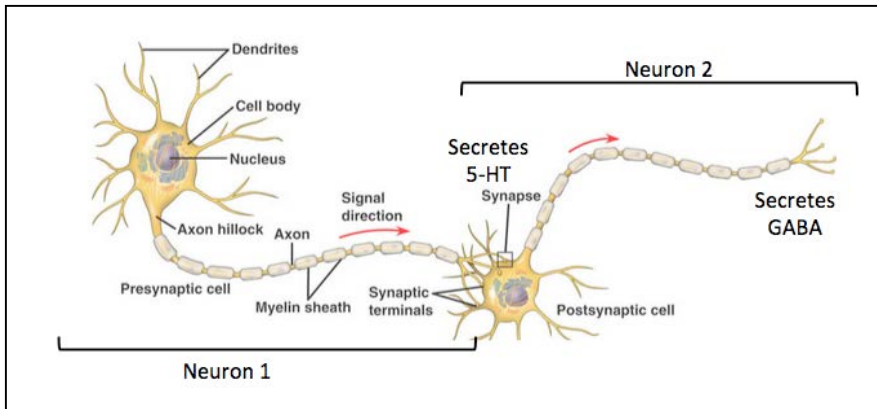


In the first graph, the slow repolarization is due to the open K⁺ channels. In the 2nd case the threshold level is never reached, so the voltage gated Na⁺ channels do not open to elicit the action potential.

(1pt, with 0.5 for each graph. Note: they can also draw small spikes for plate B)

Question 4 continued

d) Serotonin (5-HT) is an excitatory neurotransmitter. It acts by binding to 5-HT₃ (a ligand gated Na⁺ channel). In comparison, GABA is an inhibitory neurotransmitter, which acts by binding to the GABA-A receptors that act as Cl⁻ channels.



Neuron 1 secretes 5-HT and has GABA-A receptors

Neuron 2 secretes GABA and has 5-HT₃ receptors.

You stimulate neuron 1 in the presence of Prozac (an inhibitor of 5-HT reuptake from the synaptic cleft) and let it synapse with Neuron 2. Would neuron 2 secrete GABA at a **higher/ lower/ at the same rate** as the synapse that has not been treated with Prozac? **Explain.**

It will secrete GABA at a higher rate since it has 5-HT₃ receptors, which will respond to 5-HT that continues to stay in the synaptic cleft due to the action of Prozac. (0.5pt, with 0.25 for explain)

e) Over time, the postsynaptic neuron shows a decrease in responsiveness to 5-HT. Propose an **alteration** in the **post-synaptic neuron** that would account for decreased responsiveness.

- The post-synaptic neuron may express fewer 5-HT₃ receptors*
- The post-synaptic HT-3 receptors may have a decreased affinity to 5-HT*
- The 5-HT₃ receptors may be less reactive to 5-HT (0.5pt, Note: there can be other correct answers too)*

Question 5 (2pts)

Neuronal path finding is crucial for structured cellular organization and development of neural circuits. The elongation or retraction of the growth cone is dependent on guidance cues. You are looking at the response of the growth cone to the following guidance cues. **Note:** *In this example you may assume that both these guidance cues serve as **attractants**.*

- **Guidance cue 1: Fibronectin** protein that is a part of the extracellular matrix (ECM).
- **Guidance cue 2: Ephrins**, which diffuse to form a concentration gradient.

a) Which guidance cue, **Fibronectin OR ephrin** is short range and **why**?

Fibronectin. It does not form a gradient and directly binds to the receptors on the growth cone. (0.5pts)

b) You do a stripe assay to determine if fibronectin serves as an attractive guidance cue. Briefly **explain** how this assay works.

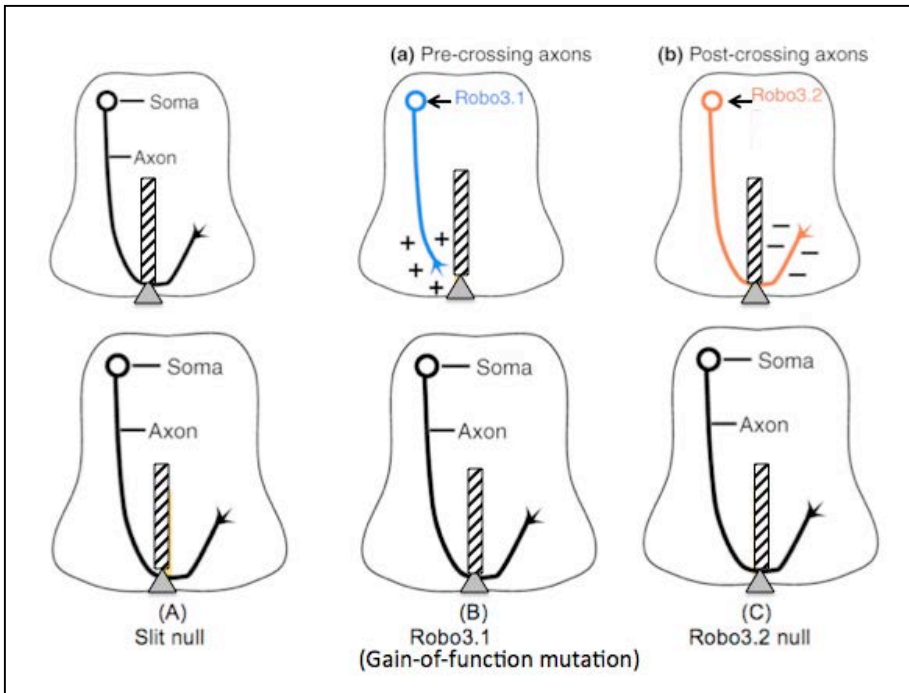
You coat the stripe with fibronectin, a short-range guidance cue. You place the neuron on one side. If this is an attractive guidance cue the growth cones of the neurons that have receptors specific for fibronectin will bind to it and will extend along the length of the stripe. If it is repulsive cue, the growth cone will collapse and will move away from the signal. (0.5pts)

Question 5 continued

As diagrammed below, in mice, spinal commissural axons (black) are attracted to grow towards the floor plate (shaded Δ) and then need to cross this to find their targets. The midline of the spinal cord expresses the **repulsive ligand Slit** (shown as striped rectangle).

- Before crossing the midline, the axons shown in blue express Robo3.1. Robo3.1 blocks the repulsive activity of Slit so the axons can cross.
- After midline crossing, the axons (shown in red) express Robo3.2, a second isoform of Robo3, in place of Robo3.1, which repels axons away from the midline so they do not grow back the wrong way. (*Ypsilanti et al, 2010*).

c) On the diagrams, draw in BLUE or RED or using a dashed line, how the axons would grow in the mutants indicated. **Note:** Normal axon growth is shown in each for reference.



-In Slit null mutant, the axon will grow towards the midline, but will likely not cross the midline since repulsive signal from slit is not available.

-Robo3.1 gain of function mutant, the axon would cross the midline but will likely not move away since Robo3.1 is always there.

-In, Robo3.2 null would cross the midline and go back and forth indiscriminately (No points)

d) Nocodazole is a chemical that disrupts the microtubules. What would be the effect of nocodazole treatment on the growth of commissural neurons?

Microtubule polymerization is involved in axon growth and migration. Interfering with this function could lead to aberrant axon extension or no growth at all. (0.5pts)

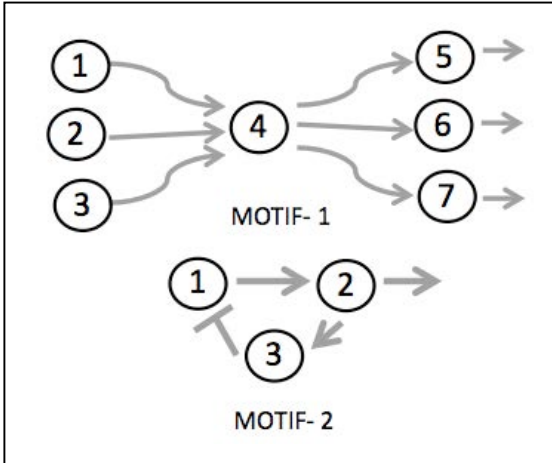
e) Slit is a secreted ligand acting at long-range. Would you be able to use a 'stripe assay' to monitor what regions of the spinal cord express Slit? **Why or why not?**

Most likely not since this is a long range guidance cue, which diffuses and forms a gradient. Also can say "Maybe". One could purify Slit ligand from spinal cord extracts and coat it as stripes, then perform a 'stripe assay' for neurons present at different regions of the spinal cord. Based on the patterns of response for each given area, one may infer the region in which the ligand is secreted endogenously. (0.5pts)

Question 6 (2pts)

C. elegans is a transparent nematode that has a total of 1031 cells of which 302 are neurons. All the neuronal connections in *C. elegans* have been mapped and many complex circuits have been defined.

The following are two motifs/ circuits in **C. elegans**. **Note:** The → represents activation and “T” represents inhibition. Each neuron is represented by a number.



a) Would you see an action potential generated in neurons 5, 6 and 7 of motif 1? **Why or why not?**

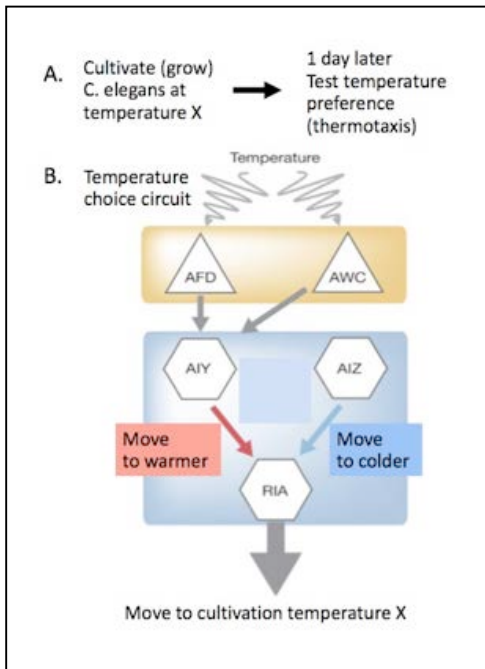
Most likely yes, since neuron 4 when activated releases an excitatory neurotransmitter (NT), which binds to specific receptors in the cell body or dendrites of neurons 5, 6 and 7 to generate an action potential. (0.5pts)

b) How would the activity of neuron 1 change if neuron 2 were removed, in Motif 2? **Explain your answer.**

It will never be inhibited. This circuitry is regulated by the feedback inhibitory signals that are provided by neuron 3. This motif is an example of feedback inhibitory loop. (0.5pts)

Thermotaxis is movement of an organism toward or away from a specific temperature. The worm *C. elegans* can be grown (cultivated) at a specific temperature and later will remember and choose to move towards that temperature through the process of thermotaxis. The specific neuronal circuitry involved is shown below.

You cultivate (grow) *C. elegans* at (temperature X for 1 day and then test their temperature preference. The temperature choice neuronal circuit for *C. elegans* is shown below (*adapted from Nishida et al*).



- Temperature is detected by AFD and AWC neurons.
- The activated AIY–RIA circuit promotes movement to a temperature **higher** than cultivation temperature.
- The activated AIZ–RIA circuit accelerates movement to a temperature **lower** than the cultivation temperature.
- The RIA neuron integrates and promotes temperature choice.

c) In the circuit to the left...

i. Is there a sensory neuron shown? **Explain** your choice.
AFD and AWC sense they detect/ sense the temperature (0.25pts)

ii. Is there an interneuron shown? **Explain** your choice.
AIY, since it gets the signals from the sensory neuron and relays it to motor neuron (0.25pts)

Question 6 continued

d) The circuit on Page 7 was defined by destroying (ablating) neurons and testing thermotaxis. Predict what would happen to the temperature memory from the following ablations. **Explain** your answers.

i. **AFD** ablated? *(No points)*

Move to colder temperature than cultivation temperature since there is no AIY-RIA circuitry.

ii. **AIZ** ablated? *(0.25pts)*

Move to warmer temperature than cultivation temperature, since there is no AIZ-RIA circuitry.

iii. **RIA** ablated? *(0.25pts)*

Confused since the RIA is not available to integrate and promote the temperature choices

e) Glutamate is an excitatory neurotransmitter normally produced by AFD. AIY produces a glutamate receptor. You engineer a mutant worm where both AWC and AFD produce glutamate. What would you predict would happen to thermotaxis? **Explain** your answer in terms of the action potential produced.

The worm will move to warmer temperature. Increased input will lead to increased number of action potentials from AIY. (No points)

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