# NEU 501a Problem Set 2

Due: Saturday, October 10th, 2020 @ 11:59 am

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1. The answers to this problem set are to be submitted by canvas. Please copy this google doc, enter your answers and **submit as a pdf.**
2. **Please do not add your name to document or file name so we can grade anonymously**
3. If there are papers pertinent to the question, we linked to them in the question.
4. If you are using any literature to guide your hypotheses, please cite it (name, year & link)
5. Some of these questions are open ended - we will be evaluating how you support your claim. Please keep answers as succinct as possible.
6. You may work with your classmates on the problem set, but your answers must represent your own understanding of the solutions.
7. Problem sets that are turned in late will receive a 10 point deduction per late day.

[**NEU 501a Problem Set 2**](#_wctbx6j9ep38) **1**

[**Question 1 [10]**](#_423znl5cdzd3) **2**

[**Question 2 [5]**](#_ydnz3w9ce350) **3**

[**Question 3 [15]**](#_6ba5cn3sflkk) **4**

[**Question 4 [5]**](#_rqd0dsez6z9g) **6**

[**Question 5 [15]**](#_m1m9srhwny8e) **7**

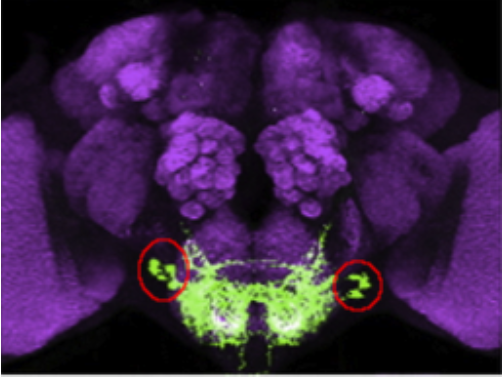
[**Question 6 [10]**](#_qq2b3q9wrtc4) **8**

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### Question 1 [10]

You want to determine if there is a CRM (cis-regulatory motif) upstream of genes involved in GABAergic neuron differentiation in *Drosophila melanogaster* (also a genetic model system like *C. elegans*). You decide to look into the following genes: GAD, VGAT, and GAT. All three genes are known to be expressed in GABAergic neurons - there are 100 GABAergic neurons in the *Drosophila* brain, but you focus on 4 interneurons that are all involved in feeding behaviors (see picture below of the fly brain (magenta) with 4 GABAergic interneurons per hemisphere labeled with GFP (green), cell bodies circled in red).



1. Sketch the reporter construct (using the gene for GFP, green fluorescent protein) you would design to test candidate CRM DNA sequences? [2]
2. Briefly, how would you confirm the existence of a common CRM using reporter constructs?[2]
3. One of your constructs expresses GFP in all of the neurons shown above in the picture but in none of the GABAergic neurons in the olfactory pathway (different neurons from the feeding neurons). Provide an explanation.[2]
4. Now you want to determine which transcription factor binds to the CRM you identified for the GABAergic feeding neurons. You do this by expressing your reporter construct in various mutant backgrounds. You find that your reporter construct in flies that are mutant for the gene *Pointed* results in no expression. List two experiments that would show that Pointed is **necessary or sufficient** to specify feeding GABAergic neuron cell fate (indicate whether the experiment tests necessity or sufficiency and also explain any caveats that might affect the interpretation of each experiment). [4]

### Question 2 [5]

Based on the Yogev and Shen review article, list 5 steps of axon development, starting with the release of an extrinsic cue and ending with the elongation and maturation of the axon. Please include the following words in your answer: microtubule, plus end, minus end, actin waves, actin rings, dendrite, axon, neurite, amplification, kinesin, dynein, symmetry, and stable. Please highlight each of the words above in your answer.[5 points, ¼ off for each incorrect usage]

### Question 3 [15]

In a frog, the normal mapping of retina to optic tectum looks like this:

A-->a

B-->b

C-->c

D-->d

E-->e

… and a to e then map to internal brain representations of the visual world.

**Part I**

Consider an experiment in which the optic nerve is cut, and then the eye is rotated 180 degrees and reimplanted. In frogs, regeneration then occurs. In a situation in which each of the following mechanisms was dominant, what mapping would you expect? In addition to identifying the mapping, explain why this mapping would occur.

1. Guidance by a secreted chemical that attracts axon ingrowth generally. Further, what is such a chemical called? [2]
2. Guidance by five different chemicals secreted by a, b, c, d, and e. [1]

**Part II**

**\*\* Note for all questions below, if you base your claim off of previous literature, please provide citations. \*\***

1. In wild type frogs (no eye rotation like above, and A → a, B → b ...etc), do you expect you’d find ocular dominance columns? Why or why not? [3]
2. Suppose you have access to frogs where the typical retina-tectum mapping has been changed to the following (don't worry about how this change was accomplished):

A→ a

B→ a

C→ b

D → b

E → b

All else being equal, would you expect to see any broad changes in the wiring organization of the frog's optic tectum over time compared to wildtype animals? (Ignore the trivial fact that retinal cells project to fewer target zones, but do assume that the size of each target is the same as in part A). Explain why you would or would not expect a change. [3]

1. Given the mapping outlined above, if you removed the eyes shortly after birth, would your answer in **e)** change? Why or why not? If you base your claim off of previous literature, please provide citations. [3]
2. You design VR (virtual reality) goggles for the frogs described in **d)** such that both eyes receive identical visual patterns. Would you expect to see differences in the wiring organization of the tectum compared to **d)** ? Why or why not? You may ignore any possible contribution from critical periods or general changes in plasticity over time. [3]

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### Question 4 [5]

Glucocorticoid receptors (GRs) shut down acute HPA stress response through negative feedback mechanisms. In clinical depression, and in animal models of chronic stress, GRs are decreased in the hippocampus. What effect would this have on acute stress response?

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### Question 5 [15]

**Part I**

Consider an outside-out patch with a single active channel permeable to potassium. The conductance of this channel is 200 picosiemens. (Siemens is the unit of conductance. 1S = 1-1 ). Recall, . These facts can be used to determine the scale of your current axis when sketching. Draw a trace of current versus time to illustrate the flow of current in the following scenarios:

1. 100mM potassium in both the electrode and the bathing solution with no voltage applied to the electrode.[1.5]
2. Application of +5mV to the electrode.[1.5]
3. Application +20mV to the electrode.[1.5]
4. Application of -5mV to the electrode. [1.5]
5. Application of -40mV to the electrode.[1.5]

**Part II**

In the same experimental set up, you set the electrode concentration of potassium to 100mM and the concentration of the bathing solution to 4mM of potassium.

1. Do you observe inward or outward currents?[1.5]
2. What happens to the amplitude of the current when you apply a positive potential to the electrode? [1.5]
3. What is the equilibrium potential for this potassium channel? Please include your calculations & round to the hundredths place [3]
4. What happens when you apply the equilibrium potential to the electrode? [1.5]

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### Question 6 [10]

You measure various properties of a neuron, observing ,,,. From the literature, you find the typical conductances for this type of neuron , , . For these questions, assume there are sodium, potassium, and chloride channels, but no sodium-potassium transporter, so you will use the equations below. Only the potassium equations for Nernst and voltage are listed below, but you should also use the sodium and chloride equations when appropriate.

1. What are the values of , , and ?[2]
2. What is the extracellular sodium concentration if the intracellular sodium concentration is 30mM?[1.5]
3. If you inactivate chloride channels, so that , what is ? [1.5]
4. If you inactivate chloride channels, what extracellular concentration of sodium is necessary to make(Assume intracellular sodium concentration is 30mM, as in part b)?[2]
5. Instead of inactivating chloride channels, you now increase the temperature of the environment by 10%. What does this make ? (Hint: You should also think about how , , and will change.) If the intracellular concentration of potassium is 50mM, what extracellular concentration of potassium is necessary to maintain ? (You can assume the temperature , however you might notice that you can solve the problem without using a specific temperature.) [3]