BIOL 455 – Midterm exam #1 (2017) **NAME:**

Important Equations:

Nernst equation (at 20°C) : $E_{ion} = \frac{58}{z} \log \frac{[ion]_{out}}{[ion]_{in}}$

Goldman equation (at 20°C): $V_m = 58 \log \frac{(P_{K+}[K^+]_{out} + P_{Na+}[Na^+]_{out} + P_{Cl-}[Cl^-]_{in})}{(P_{K+}[K^+]_{in} + P_{Na+}[Na^+]_{in} + P_{Cl-}[Cl^-]_{out})}$

Please make sure you have all 6 pages. There are 9 questions and 25 total points.

Answer all of the following questions. You can write in full sentences or in point form, but whatever you write must consist of coherent thoughts. Calculators are permitted. There should be enough room to answer each question (ie try to be succinct), but if you absolutely need to, you may write on the back of the pages; however, you must make it absolutely clear which question any extra writing is answering.

Questions:

1. You are studying some proprioceptive neurons in centipedes to figure out how they keep track of all those legs. You find that they have somewhat unusual characteristics. You measure the V_m and ion concentrations inside and outside the neurons as follows:

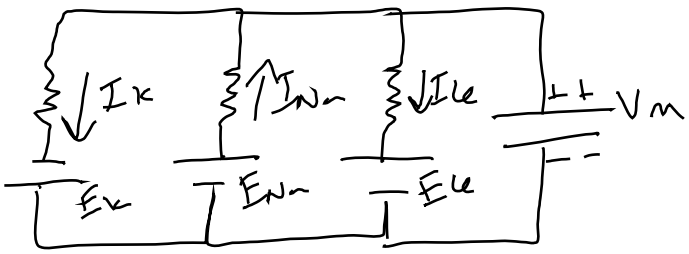
V_m = -70 mV

ion	[inside]	[outside]
K^+	30 mM	300 mM
Na^+	400 mM	4 mM
Cl^-	10 mM	100 mM

Important: for this question, assume that the neuron is only permeable to these three ions.

a) Draw the neuron equivalent circuit for this neuron at rest (no ion pumps or injected current), making sure to label each component. **(3 points)**

first, some calculations: E_K = 58 mV, E_{Na} = -116 mV, E_{Cl} = -58 mV



-1 for each mistake (aside from battery directions); -0.5 for 1 wrong battery, -1 for all 3 batteries wrong. Maximum of -2 for getting all 3 currents wrong.

b) You dunk the caterpillar in a drug that completely eliminates Na^+ conductance for your neuron ($g_{Na} = 0$). With the information you have, what can you say about how the Cl^- current (I_{Cl}) will change after addition of the drug? Explain your answer **(2 points)**.

Without any Na current, the Vm will have to sit somewhere between E_K and E_{Cl} (i.e. between -58 mV and + 58 mV. This means that we will go from Vm < Ecl before the drug, to Vm > Ecl after the drug. So we know that the Cl current will reverse direction, going from inward before the drug to outward after the drug. We can't say how the magnitude will compare, without more information.

If you said that there would be less inward current because Vm moves towards Ecl, then you get 1 mark. Additional note: You can see on your circuit diagram that INa is the only outward current. If INa becomes 0, you're left in a situation where both your remaining currents were inward. This can't be the case (net current has to =0) so you know that one of them has to switch directions (and based on the Eions, you can figure out that it has to be Cl that switches).

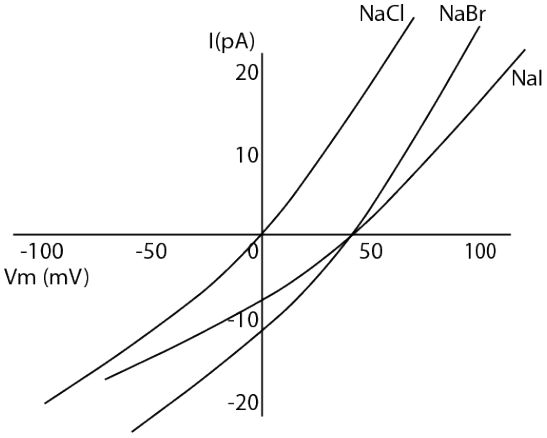
c) At the moment you add the drug in part (b), you would expect any capacitive current? If so, would I_{cap} be positive or negative? **(1 point)**

Yes, you would see a very brief positive I_{cap} since V_m changes in a positive direction.

2. You idolize David Julius, whose lab discovered the capsaicin receptor, VR1. So you set out to characterize the receptor for another very important culinary ligand, called caprisun. You discover the caprisun receptor, which you call CSR1. In order to determine the ion permeability for CSR1, you express it in HEK 293 cells (just like the Julius lab did for VR1), and perform whole-cell patch clamp with 140 mM NaCl in your pipette. You have previously determined that CSR1 is only permeable to anions, so you make IV curves with the following extracellular solutions:

1) 140 mM NaCl
2) 140 mM NaBr (sodium bromide)
3) 140 mM NaI (sodium iodide)

Your IV curves look like this:



a) Using <, >, or + signs, describe the relative permeability of CSR1 to Cl^-, Br^- and I^-. Briefly explain your answer. (**2 marks**).

Pcl > PBr = Pi.

The easiest part of the answer is that PBr = Pi. This is because the Erev for both curves is the same. (1 mark)

The harder part of the answer is that Pcl > PBr and Pi (1 mark). Many people got this reversed, which would be the case if these were cations. However, with anions, the Erevs are going to be opposite, and therefore you end up with the opposite conclusion. There are basically three ways you can look at this and still get the right answer:

1) "Math way" #1: Using the NaBr curve as an example. Because Cl is high on the inside and nothing on the outside, and it's an anion, ECl has to be very positive. Because Br is high on the outside, EBr has to be negative. Seeing that Erev is positive, you know that it is towards ECl, so you can conclude that ECl is more permeable.

2) Math way #2: From the Goldman equation, using the NaBr curve as an example: Erev = 58 log ((PBr[Br]in + PCl[Cl]in)/(PBr[Br]out + PCl[Cl]out)) note that [ion]out are in the denominator rather than the numerator, because they are anions
So, Erev = 58 log((0 + PCl(140)/PBr(140) + 0)) = 58 log (PCl(140)/PBr(140))
Because Erev > 0, PCl must be greater than PBr (to make log term positive)

3) Non-math way. Again using NaBr as an example. Cl is on the inside, Br on the outside. If they are equally permeable, then when Vm = 0 you'd see equal currents in both directions (so Erev would be 0). However, we see equal currents in both directions (Erev) when Vm >0. This means that you need to build up positive charge on the inside in order to equalize the currents. Positive charge on the inside will tend to increase the flow of Br in, and decrease the flow of Cl out. Thus, you can conclude that Cl must be more permeable (because Br needs the "help" of a positive membrane potential to equalize its current to Cl).

b) Imagine that CSR1 is expressed in a neuron with the following characteristics:
Vm = -65 mV ; threshold = -50 mV
E_K = -80 mV ; E_{Na} = +55 mV ; E_{Cl} = -55 mV ; E_{Br} = -53 mV ; E_I = -58 mV.

Would binding of caprisun to CSR1 be inhibitory or excitatory? Explain (**2 points**)

Based on the Eions for all the anions, Erev for CSR1 has to be somewhere between -58 and -53 mV. Since threshold is -50 mV, Erev is below threshold, which would make CSR1 inhibitory.

3. You want to test the involvement of octopaminergic (another monoamine neurotransmitter) neurons in fruit fly learning. To do this, you generate the following two types (genotypes) of flies to test:

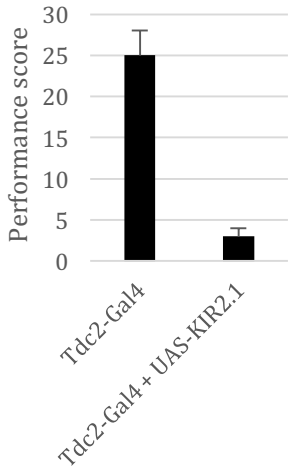
i) flies carrying only Tdc2-Gal4
ii) flies carrying Tdc2-Gal4 and UAS-KIR2.1

Tdc2-Gal4 drives Gal4 in octopaminergic neurons.
KIR2.1 is an inward rectifying potassium channel.

a) What do you expect to be the effect of KIR2.1 expression on membrane potential and action potential firing? Explain. **(2 points)**

We expect an inward rectifier to be open at polarized and hyperpolarized potentials (negative potentials). Therefore, expression of an inward rectifier K channel will hyperpolarize the neuron (make Vm more negative) because the increased K conductance will push Vm towards E_K and E_K is generally more negative than resting potential. This will cause inhibition of action potential firing. An important point here: As we discussed in class, just because a channel is inward rectifying **doesn't** mean it will always pass inward current. The direction of the current is **always** determined by the driving force. The inward rectifier is simply open at negative potentials and closed at positive potentials, which means that *in general*, inward current will pass more easily than outward current (because negative potentials are more likely to result in inward current due to the driving force).

b) You then expose each genotype to odour A in the presence of sugar and then to odour B alone (you repeat this 3 times). Then you test them for preference of A versus B in a T-maze. You get the results shown to the right. State the conclusion of your experiment in one sentence, **using the terminology we've discussed in class**. (**1 point**)



Octopaminergic neurons are necessary for appetitive learning.

Will also accept "octopamine is necessary for appetitive learning".
If you said that "expression of Kir2.1 in octopaminergic neurons is sufficient to block appetitive learning" you got 0.5. This statement is technically correct, but not the major biological conclusion here, because our experiment is designed to test the function of octopaminergic neurons in learning, not the function of KIR2.1 on octopaminergic neurons. Remember that when we expressed Shi in dopaminergic neurons (an analogous experiment) the conclusion was that dopaminergic neurons are necessary for aversive learning.

4. What ionic current is largely responsible for the undershoot portion of the action potential? **(1 point)**

K current

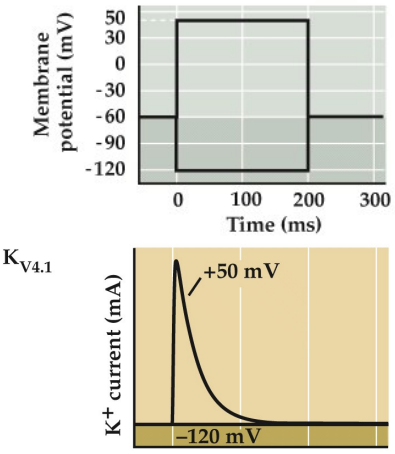
5. Ketamine is known to impair memory. Explain why this may be the case. **(2 points)**

Ketamine sits in the pore of the NMDA receptor and blocks current from flowing (1 point). NMDA receptor function plays an important role in memory (1 point); therefore, interfering with its function would be expected to interfere with memory.

6. One of the main ingredients of "Icy Hot" is menthol. What part of the Icy Hot experience do you think menthol contributes to, and how does it do this? (**2 points**)

Menthol produces the sensation of cold (1 point). It does this by activating the TrpM8 receptor (1 point)

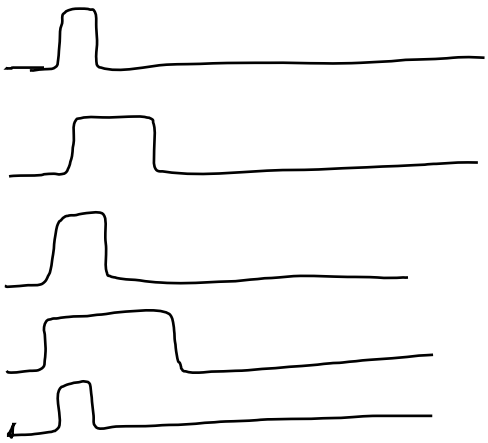
7. The image to the right shows the currents from the $K_{v4.1}$ channel during the two illustrated voltage steps.



a) State two <u>gating</u> properties of the $K_{v4.1}$ channel that account for this current shape. (**2 points**)

1) opens on depolarization
2) rapidly inactivates

b) Draw what you would expect **5** single channel recordings of $K_{v4.1}$ might look like during the + 50 mV voltage step. Assume the conditions are the same as the experiment illustrated, and try to make your 5 representative of the whole population. (**1 point**)



Something like these → most short-lived, but very slightly in duration.

8. In class, we discussed that serotonin has been implicated in regulating mood.

a) What fact, presented in class, could be taken as evidence that serotonin is sufficient to increase mood? (**1 point**)

SSRIs elevated serotonin levels, and have a positive effect on mood. (sufficient)

b) In 1-2 sentences, propose one "right time and place" type of experiment that would connect serotonin with mood regulation. *note: your experiment does not have to demonstrate both time and place. One of those would be fine. (**1 point**)

You could do some sort of recording of serotonergic neurons in a model (monkeys, rats) who were made to be depressed or happy.

You could show that serotonergic neurons innervate areas of the brain implicated in mood.

9. In class we discussed a paper identifying the capsaicin receptor.

a) Which of the three major research aims we discussed (necessity, sufficiency, right time and place) was *not* demonstrated in the paper. (**1 point**)

necessity

b) **Briefly** describe a subsequent follow-up experiment/result from the same group that satisfies the missing aim from above. (**1 point**).

They made a knockout mutant for VR1 in mice. They then showed that these mice had lowered response to capsaicin – examples of the experiments include: showing that DRG neurons lost capsaicin-induced currents; showing that the mice no longer licked their paws as much when capsaicin was applied; showing that the mice were no longer deterred from drinking water with capsaicin in it.