Name: _____ **Student #: _____**

BIO 3305

Cellular Physiology

Prof: John Lewis

MIDTERM #2

November 4, 2019

- **4 Questions**
- **4 pages** total
- **40 marks** total

Please answer **ALL** questions.
Please write your name and student # on all pages.

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1. **(10 marks)** You are recording membrane potential from two neurons that are connected by a chemical synapse (i.e. transmitter binds to a ligand-gated ionotropic receptor). The resting potential of the presynaptic neuron is -70mV but it spontaneously fires an action potential (AP) once every two seconds. The resting potential of the postsynaptic neuron is -60mV and every AP in the presynaptic neuron results in a postsynaptic potential (PSP). In this context, determine whether each of the following statements is TRUE or FALSE. **Please circle the correct answer.**

(a) [TRUE] or FALSE | If the depolarization phase of the AP in the presynaptic neuron involves voltage-gated Na^+ channels (as in the squid axon), then blocking half of these channels (with a drug) should increase the threshold for AP firing.

(b) [TRUE] or FALSE | If the repolarization phase of the AP in the presynaptic neuron involves voltage-gated K^+ channels (as in the squid axon), then decreasing extracellular K^+ concentration should increase the refractory period of this neuron.

(c) [TRUE] or FALSE | If the ionotropic postsynaptic receptors are permeable to K^+ ions and the K^+ equilibrium potential (E_K) is -65mV, then the postsynaptic potentials should be hyperpolarizing.

(d) TRUE or [FALSE] | If the ionotropic postsynaptic receptors are permeable to K^+ ions and the K^+ equilibrium potential (E_K) is -65mV, then blocking voltage-gated Ca^{+2} channels in the presynaptic terminal will have no effect on the postsynaptic potentials.

(e) TRUE or [FALSE] | If the ionotropic postsynaptic receptors are permeable to Na^+ ions and the Na^+ equilibrium potential (E_{Na}) is +50mV, then increasing the extracellular concentration of K^+ could lead to an increased amplitude of the postsynaptic potentials.

2. **(4 marks)** Consider the factors that influence AP propagation along a single axon, and determine whether each of the following statements is TRUE or FALSE. **Please circle the correct answer.**

(a) TRUE or [FALSE] | Increasing the number of voltage-gated Na+ channels in an axon will increase its length constant.

(b) [TRUE] or FALSE | Applying a drug that increases an axon's membrane resistance should increase the velocity of AP propagation.

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3. (14 marks) Assume that you are recording from a neuron that has a resting potential of -70mV. It produces action potentials (APs) that appear similar to those of the "classic" squid axon, but you do not yet know their ionic basis. Interestingly, you find that an AP is produced following a brief (20ms) current stimulus that hyperpolarizes the membrane potential to -80mV.
Describe in detail **two distinct hypotheses** that could explain this observation. Then describe **one experimental manipulation** (along with **the associated predictions**) that would allow you to test **each** hypothesis (i.e. two manipulations in total, one for each hypothesis).
Feel free to use the back of this page if necessary.

There are a few possibilities here, two are summarized below. See also Q4b from the sample midterm 2 that I provided.

Hypothesis 1:
The hyperpolarizing stimulus would deactivate Na+ channels (decrease the number of activated channels) but also remove inactivation of Na+ channels. After the hyperpolarizing pulse, the membrane potential will recover towards the resting potential. But inactivation would recover more slowly than activation, so there will be more Na+ channels available (i.e. open) when the membrane potential reaches its original "resting level"; therefore the membrane will continue to depolarize. If the positive feedback cycle of Na+ activation can occur before Na+ inactivation "catches up", then an AP will be produced.

Testing this hypothesis could involve manipulation of:

- ***inactivation and/or activation time scales – if Na-channel inactivation is not sufficiently slow relative to activation time scale, then hyperpolarization will not result in an AP***
- ***voltage dependence of inactivation (i.e. inactivation curve) – shift to the left, so much less "removal of inactivation" for a given level of hyperpolarization… if removal of inactivation is not sufficient, then no AP.***

Hypothesis 2:
An ion channel (with E_{ion} sufficiently above Vrest) that is activated at hyperpolarized voltages. In other words, the activation curve for this channel is "flipped", resembling the classic Na inactivation curve. It would be opened (activated) by the hyperpolarizing stimulus and as long as it closes slowly enough, it would still be open when the membrane potential recovers to its original Vrest after the hyperpolarizing pulse, resulting in further depolarization and in principle an AP.

Test could involve manipulating the channel directly (block the channel with a drug), as we can assume it is not directly involved in AP production. Alternatively, manipulating the activation curve (e.g. shifting left should make hyperpolarization-induced AP more difficult) or the time scale of activation (e.g. slowing down channel closing should make it easier to produce an AP with the hyperpolarizing stimulus)

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4. (12 marks) You are studying motor neurons in a mutant population of mice for which action potential (AP) propagation is compromised compared to that in the wild-type population (such that many APs do not propagate the full length of the motor axon). As a first step towards characterizing this deficit, you decide to focus on **AP generation at one node of Ranvier**. Using a brief current pulse to generate a single AP, you find that the AP appears normal in the mutant population. Next, you deliver repeated (identical) current pulses at 30Hz for one second and find that in the wild-type, all stimuli produce identical APs. However, when you deliver this same set of pulses to the mutant motor neurons, you observe a peculiar pattern: (i) the first 10 pulses produce APs whose amplitude gradually decreases over time; (ii) the next 10 pulses do not produce any APs at all; and, (iii) the last 10 pulses produce APs with an amplitude that gradually increases back to the level seen for the first pulse.

Outline an hypothesis that could explain these observations. Then, describe a specific manipulation, along with the predicted outcome, that will allow a direct test of one aspect of your hypothesis. *Feel free to use the back of this page if necessary.*

This is pretty wide-open, so there are a number of possibilities here, some are summarized below. See also Q4a from the sample midterm 2 for a different take on similar concepts.

First of all, there are some important things to consider:

- ***i. the problem concerns AP production at a single point on the axon (i.e. a single node of ranvier)***
- ***ii. the voltage-dependence and time-dependence of the activation/inactivation for Na+ and K+, and how they can influence AP threshold and amplitude***
- ***iii. Any other combination of channels/channel properties that could change AP threshold and/or amplitude.***
- ***iv. The change in threshold (pulses 10-20 are below threshold; no AP) is accompanied by a progressive change in AP amplitude – the mechanisms could be linked or independent***

The proposed mechanism must be logical and also linked in some way to the progressive changes in the AP over the stimuli (the first and last third of the stimulus set, ~300ms each), as well as the lack of AP production by the middle stimulus pulses.

There are a number of things that are in general NOT appropriate, including (among others):

1. ***Axon geometry (radius) and/or length constant: these properties will be important for AP propagation, but the problem here focuses on AP production at a single node of ranvier.***
2. ***Na-K ATPase, unless it is assumed to be uncharacteristically fast: the changes to AP production occur over less than one second (i.e. 30 pulses at 30Hz). This pump could affect AP generation in a similar way, but typically over a much longer time scale. Similarly, changes in equilibrium potentials would also typically occur more slowly.***
3. ***"leak" channels: these are channels with no voltage-dependence that can influence membrane potential and membrane resistance. This influence is typically "static", and cannot alone explain the progression changes seen over this short time scale (unless a***

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mechanism is proposed for a dynamic conductance change, i.e. progressive increase/decrease in leak conductance).

Below are a few ideas that could form the basis for an appropriate hypothesis (this is not meant to be a comprehensive list):

1. *The gradual decrease in AP amplitude and eventual absence of APs, could involve a gradual build-up of Na+ inactivation that could occur if inactivation were too slow to fully recover between successive stimuli. The result would be a progressive increase in the number of channels that remain inactivated (i.e. fewer Na+ channels each stimulus), leading to a decreased AP amplitude, increased AP threshold, and eventual failure of AP generation. This failure would allow time to recover so that the pulse is once again above threshold. The gradual increase in amplitude (such that the original decay in amplitude is gradually offset) could arise from a gradual activation (for example via a build up of Ca+2 or Na+) of a voltage dependent K-channel (Kv3.1) that would speed recovery of Vm during the AP and eliminate the gradual inactivation seen in the first 10 pulses.*

2. *Another set of approaches could involve the activation and/or inactivation curves themselves:*
    a. *Phase 1 (decrease AP amplitude, increase threshold):*
        i. *a gradual left-shift of the Na+ inactivation curve would result in a similar affect as in #1.*
        ii. *a gradual left-shift in the K+ activation curve would lead to a gradual increase in threshold, longer AHP, and decrease in AP amplitude.*
        iii. *a gradual right-shift of Na+ activation curve, leading to an increase in threshold and lower AP amplitude because inactivation would have relatively more influence (similar to (i)).*
    b. *Phase 2 (AP recovery, increasing amplitude), slower time scale compared to phase 1. Can consider opposite shifts compared to those discussed for Phase 2. E.g. a gradual left shift of the Na+ activation curve on a slower time-scale.*

    This type of hypothesis should also include some mention of what signal caused the shift in activaton/inactivation curve. For example, a build-up of Na+ (or Ca+2 or ??) could lead to modulation of the channel gating properties.

3. *Another set of approaches, could involve a gradual change in time scales of inactivation/activation. This is a more complicated version of #1 and would also have to include a signalling mechanism underlying the modulation, as in #2.*

Testing the hypotheses should involve direct manipulation of the key parameter in your mechanism (for example, time scale of Na+ inactivation, the "build-up" of Ca+2, or a "special" ion channel). The goal being to "rescue" consistent AP production over the 30 pulses, or at least over the first or second phase, depending on the specifics of the hypothesis.