Student number: _____

Module I: Action potential and cable properties (25 pts.)
Please choose 3 out of 5. Keep your answers brief.

1) You are recording from two different neurons, each with one electrode in the soma and another in the distal dendrite. You notice that, at low firing rates, neuron A exhibits reliable retrograde conduction of action potentials (AP's) whereas you rarely record retrograde AP's in the dendrites of neuron B. List 2 possible contributing factors to this difference in retrograde AP conduction, and describe at least 1 experiment to test each possibility.

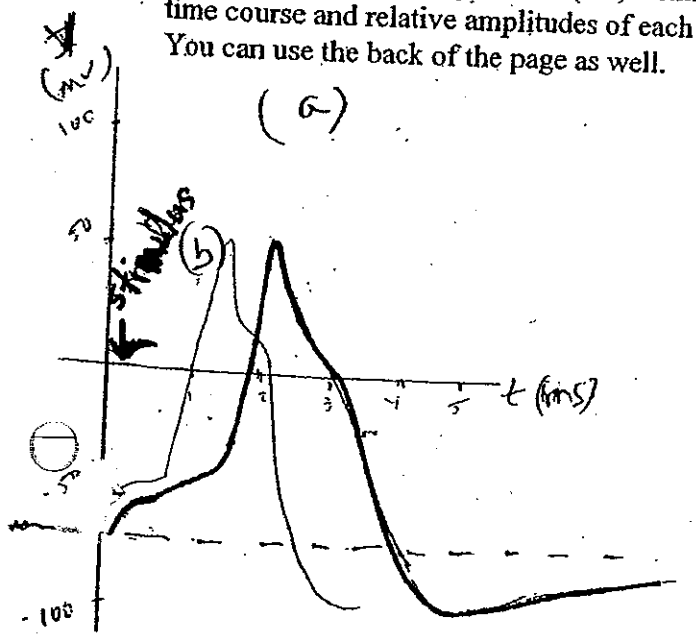
B: longer refractory period
- slower stim

conductance dependant on λ...
- λ dependant on r_i and r_m → leak conductance } measure V_m at diff dist
  ↓ diameter of dendrite

- ↑ slow inactivating Na^+ channels? → paired-pulse to see inactivation
  ↑ A-type K^+ → block w/ 4-AP

2) You have an electrode in the cell body of a neuron in a brain slice, recording under unclamped conditions in order to follow the changes in membrane potential in response to incoming stimuli. You stimulate presynpatic afferents to generate an EPSP that results in an action potential. Draw the action potential under the following conditions:
   a) Normal recording conditions (room temperature). – drawn for you, to get you started
   b) At increased temperature (~37° C) vs. low temperature (~10° C).
   c) In the presence of a specific inhibitor of I_A (fast-inactivating K channel) current.
   d) In the presence of a specific inhibitor of small conductance Ca-sensitive K channel.
   e) In the presence of a specific inhibitor of delayed rectifier K channels.
   f) In a neuron expressing a mutant form of Na^+ channel that shows slow inactivation. V-indep

The starting membrane potential (i.e., resting potential prior to the EPSP) is –70 mV. Indicate the time course and relative amplitudes of each AP by drawing them on axes (Y=mV; X=milliseconds). You can use the back of the page as well.



(c) rise quicker, steeper
(d) no AHP, slower repol?
(e) delayed repolarize
(f) " "

Student number: _____

3) You are recording as described in question #2, using an extracellular solution that is similar to the composition of normal cerebrospinal fluid, and an intracellular solution with ~140 KCl, 1 $MgCl_2$, Ca^{2+} chelators and usual buffering, ATP, etc.

a) Describe two experimental approaches you could use to determine the contribution of voltage-gated Ca channels to the AP recorded from the cell soma.

b) How would you determine which class(es) of Ca^{2+} channel (i.e., N, P/Q, or L) are involved? (Give two approaches).

c) Draw the AP in a neuron overexpressing L-type Ca^{2+} channels.

a) block Ca^{2+} + trigger ap, other?...
of cadmium

b) block Na^+/K^+ → current?

a - remove Ca^{++} from outside
- voltage-clamp (?)

↑ or ap peak
quick fall?
↓
Chelators
↓
2 responses

c. L-type - slow inactivation
P/Q - slow inactivation
T - repetitive spikes

- specific blockers;
- based on kinetics of ap?
- inactivation rate
- inactivation membrane potential
- current waveform

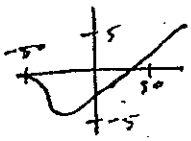
4) Choose one type of voltage-gated channel (i.e., Na^+, K^+ or Ca^{2+}) and:

a) Draw its I-V curve

b) Briefly describe its salient structural features (membrane topology, subunits/domains, etc.)

c) Describe the mechanism for voltage sensing/gating or inactivation, and two types of experiments you could use to help determine this information.

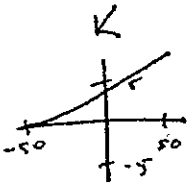
Na



6 Tm/ domain
4 domains,
S4 V-sensor
P segment, S5, S6 → pore

S4 - V sensor
helical tail inactivation
IFM inactivation gate

K



6TM
tetramer
N-term "ball"
S4 V-sensor
P segment, S5, S6 → pore

S4 - V sensor
N-term "ball" inactivation
substitute S4 w/ S4 from diff ion channel
mutate S4 residues
intracellular trypsin

5) You are recording from 2 different neurons in a brain slice. Neuron A has a high density of leakage-type K channels, whereas neuron B has a relatively low density. Assume strictly passive conduction of EPSP from dendrites to soma, and that each neuron receives an equivalent stimulus to dendritic synapses (including amplitude of EPSP generated, and distance from the soma).

a) Draw the EPSP recorded in the soma of neuron A vs. neuron B, showing any differences in time course and/or amplitude.

b) Give a rough sketch of the firing patterns of these 2 neurons to a series of EPSP's given a few milliseconds apart.

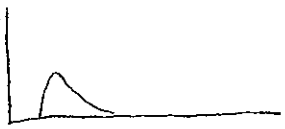
c) How would overexpression of an inwardly rectifying-type K^+ channel affect the properties (name at least one property affected) and firing pattern of these neurons?

what does this do?

↓firing rate

A

a.

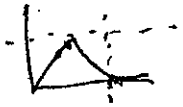


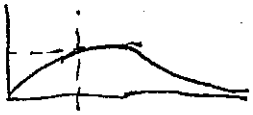
B

?

b.

c. (activated when hyperpolarized – below G_K?)
inactive when depolarized
- ~~lower~~ raises resting potential
- ~~decreased~~ faster firing rate (req. more current to reach threshold
- shorter AP (?)
faster





Module 2 answer 3 of 5 questions.

Question 1. Student #:_____

Outline 10 steps from the substance P neuropeptide gene to the peptide actually being released from a distant synaptic terminal. (give rough locations of the steps, mitochondria, terminal etc., and the form of the neuropeptide DNA, RNA other?)

1. transcription of gene in nucleus to mRNA (pre-mRNA → mRNA)
2. RNA – neuropeptide protein or per in cytoplasm
3. ER → golgi transport (protein)
4. synaptic vesicle formation in trans-golgi network (TGN)
5. budding of vesicle from endosome
6. transport of vesicle down microtubules by motor proteins to terminal
7. docking on vesicle to presy release site of plasma membrane
8. AP invades terminal → opening of V-gated Ca channels, Ca entry.
9. SNARE zippering (Complex)
10. fusion of vesicle w/ membrane, release of peptide into cleft

1. nucleus : gene transcription to mRNA
2. signal peptide targeting to rER (cytoplasmic proteins → free ribosomes)
3. signal recognition peptide dissociates, protein translation
4. translocation to golgi (protein)
5. sorting into vesicles in trans golgi network (protein → maturation) (modifications)
6. budding of vesicles from golgi
7. mature protein in vesicle transports through cytosol towards synaptic terminal (microtubule)
8. near membrane, transfer to actin transport
9. anchoring of vesicle to reserve/readily releasable pool
10. receptor activation (G-protein or Ca^{++}), SNARE complex zippering, fusion of vesicle, release of mature neuropeptide to synaptic cleft

Question 3. Student #:_____

Describe the functional significance of postsynaptic anchoring proteins such as PSD95; **a) tell why they are important, b) how does their structure facilitate this role, c) draw a cartoon of this concept.**

a) -

Student #:_____

Question 4.

It is not uncommon for a single nerve terminal to exhibit sustained transmitter release at frequencies of up to 20 Hz (20 times a sec.) for several seconds.

a) Describe strategies nature uses to make chemical synaptic transmission fast and to permit it to follow these frequencies.

- increased RRP
- more docked, fully assembled fusion complexes
- more synaptic enzymes
- more mitochondria
- localized Ca receptors

b) After 10 sec of 20 Hz stimulation you find that synaptic transmission has stopped, give 2 reasons for why.

- depletion of vesicles
- depletion of NT
- run out of Ca
- post syn receptor desens

1.) _____

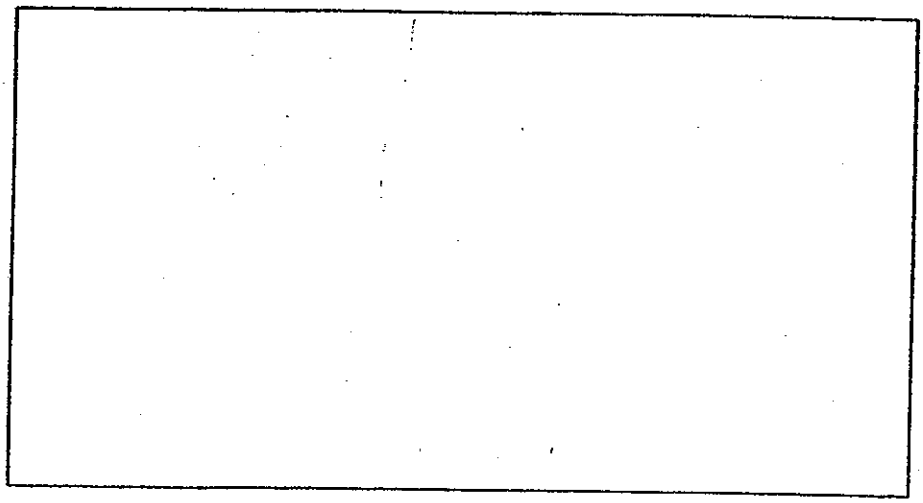
_____

2.)_____

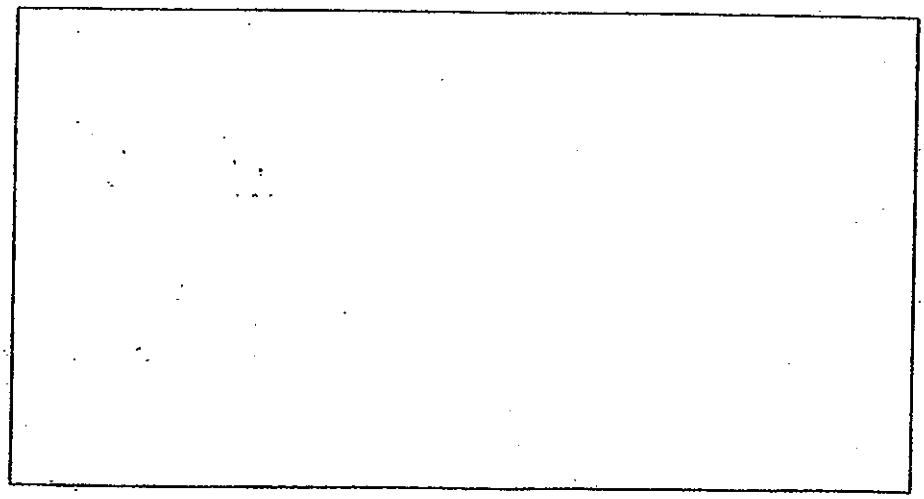
_____

Question 5 Student #:_____

Draw an example of what a single **a.) subthreshold** and **b.) suprathreshold (for spike)** EPSP would look like, give approximate time scales and voltages. The voltage you plot includes the influence of both voltage and transmitter gated conductances. c.) Show an example of desensitization.



Plot subthreshold and suprathreshold EPSP above



Plot synaptic EPSP desensitization above.