Name _____

(write your name on every sheet)

HST 131/Neuro 200
Exam I, Sept 29, 2004

There are 23 questions.
Point values for each are given.
86 points total.

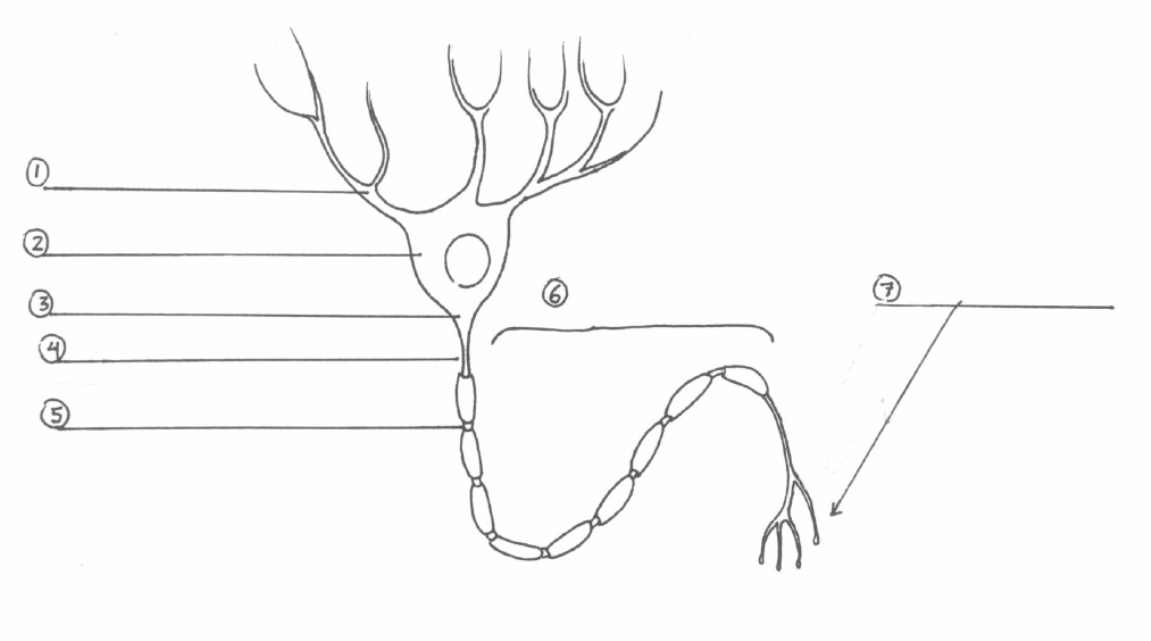
KEY KEY KEY KEY KEY KEY KEY KEY KEY KEY KEY KEY KEY KEY

1. (5 pts) It is important to have a sense for the relative orders of magnitude of cellular components. Circle the answer which is closest to correct for each physical parameter for a CNS synapse. **(Correct Answers *)**

vesicle diameter	0.5 nm	5 nm	**50 nm***	500 nm
synapse width (length of active zone)	5 nm	50 nm	**500 nm***	5000 nm
Lipid bilayer thickness	.05 nm	.5 nm	**5 nm***	50 nm
Vesicles released per active zone per action potential	**1***	10	100	1000
Synaptic delay (pre AP to post AP)	0.1 ms	**1 ms***	10 ms	100 ms
Synaptic cleft width	0.2 nm	2 nm	**20 nm***	200 nm
glutamate molecules/ vesicle	50	500	**5,000***	50,000
resting [Ca^{2+}] in terminal	**0.1 µM***	1 µM	10 µM	100 µM
[Ca^{2+}] near vesicle for release	0.5 µM	5 µM	**50 µM***	500 µM
AMPA receptor protein diameter	1 nm	**10 nm***	100 nm	1000 nm

2. (2.5 pts) Indicate which of the following are true. **ANSWERS: A, C, E**
   a. Microtubules possess great tensile strength that enables axons to withstand mechanical stress.
   b. The initial segment and nodes of Ranvier are enormously enriched in delayed rectifier potassium channels.
   c. The speed of slow axonal transport is only one order of magnitude faster than simple diffusion.
   d. Presynaptic proteins are often synthesized in the axonal terminal, while dendritic proteins are exclusively made in the soma.
   e. Dendritic spines are generally thought to constitute the site of long-term, stable memory in CNS neurons.

3.a. (3.5 pts) Label the following diagram:



b. (2.5 pts) Which one of the above structures is *best* known to
i. trigger action potentials? **AXON HILLOCK (3)**
ii. contain microtubules that are mostly oriented in the same direction? **AXON (6)**
iii. contain microtubules that are of mixed orientation? **DENDRITE (1)**
iv. contain Nissl substance? **CELL BODY (2)**
v. be a site of protein synthesis? **CELL BODY (2)**

4. (3 pts) Myelin... (circle all that apply) **ANSWERS: B only**
a. is made by oligodendrocytes cells in peripheral sensory nerves
b. decreases the effective capacitance of an axon
c. decreases the length constant of an axon
d. decreases the effective resistance of an axonal membrane
e. is lost in the disease myaesthenia gravis
f. increases conduction velocity to 20-100 mm/s

5. (2.5 pts) A typical mammalian CNS synapse differs from the neuromuscular junction (NMJ) in the following ways (circle all that apply): **ANSWERS: A, D, E**
a. A CNS presynaptic terminal typically releases 1 vesicle per action potential while a NMJ terminal releases hundreds.
b. Acetylcholine is hydrolyzed by acetylcholinesterase in the cleft at the NMJ, while glutamate is cleaved by glutamate hydrolase in the cleft in CNS synapses.
c. NMJ synapses have dense-core vesicles while CNS vesicles are usually clear.
d. CNS, but not NMJ presynaptic terminals can be postsynaptic to inhibitory neurons.
e. A muscle cell is innervated by a single motor neuron while a CNS neuron can be postsynaptic to many neurons.

6. (2 pts) Which processes contribute importantly to the resting membrane potential?
ANSWERS: A, C

a. Na-K ATPase pump
b. K+ going through voltage-gated K+ channels
c. K+ going through inward-rectifying K+ channels
d. Small negatively charged molecules inside the cell

7. (2.5 pts) For channels in the open conformation, we frequently approximate I(V) relationships as Ohmic, but we know that they are not strictly linear. Which of the following contribute to nonlinearities in open-channel I(V) relationships?
ANSWERS: C, D

a. Voltage-dependent gating
b. Membrane capacitance
c. Voltage-dependent block of pores by intracellular or extracellular ions.
d. Differences in the concentration of charge carriers across the membrane.
e. Voltage-dependent changes in permeability of the lipid bilayer

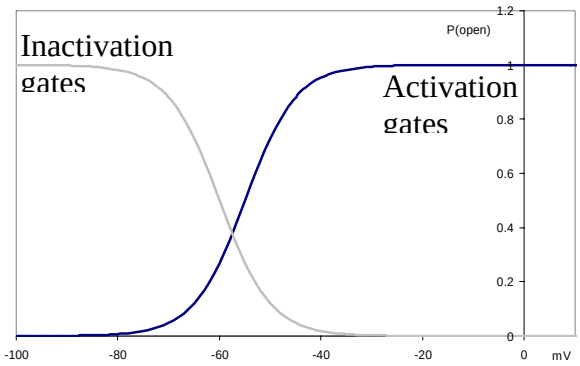
8. (2 pts) The resting conductance of an ordinary neuron is due to a K+ channel that is always open at the resting potential. If you double the extracellular K+ concentration, from 5 to 10 mM, what happens to the membrane time constant?
ANSWERS: E

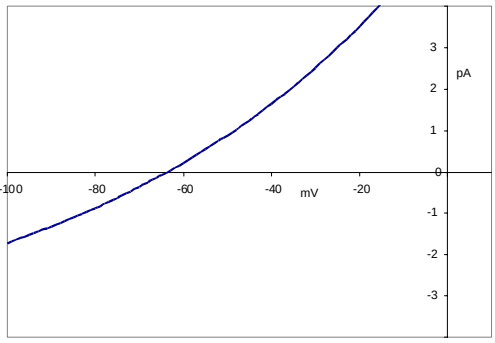
a. nearly doubles
b. increases slightly
c. no effect on time constant
d. decreases slightly
e. nearly halves

9. (3 pts) In a thermal vent deep in the Pacific, you discover a new bacterium, and decide to characterize its channels. One of them tends to flicker open and closed, so it's easy to measure its reversal potential. Under the following ionic conditions (given in mM), the current reverses at about +29 mV. What ion is the channel permeable to? **Mg++**
ALLOW SOME PARTIAL CREDIT FOR WORK SHOWN

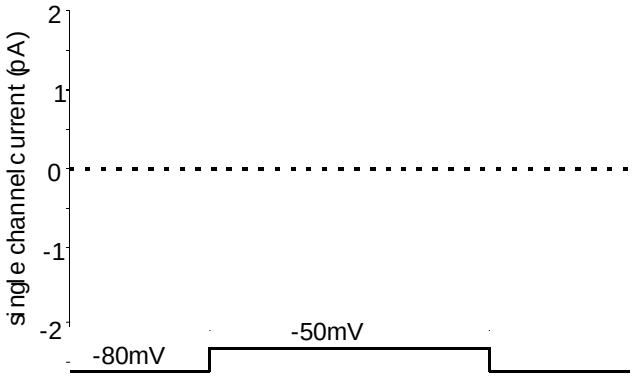
Ion	*OUT*	*IN*
Mg^{2+}	50	5
Sr^{2+}	5	50
Cl^{-}	260	65
I^{-}	5	50
La^{3+}	50	5
Dextrose	0	195

10. One channel type that we did not dwell on is the A-type potassium channel, which activates much like other delayed rectifier channels but which inactivates fairly rapidly. Suppose an A-channel in a particular cell has the following voltage dependencies of activation and inactivation (left) and the following open-channel I(V) curve for a single channel (right). Suppose also that at –50mV, the A-current activates with a τ of 0.5ms and inactivates with a τ of 15ms.

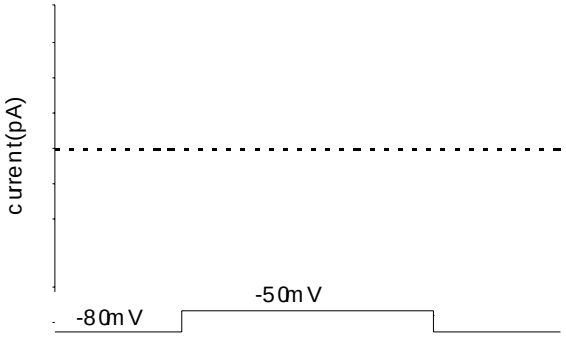




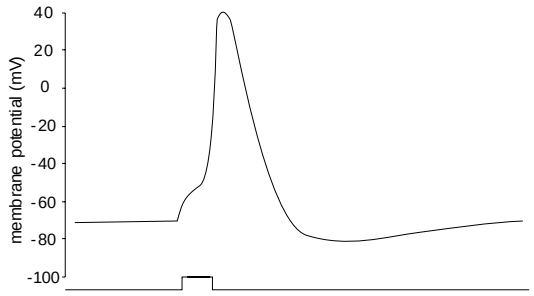
a. (4 pts) With a patch clamp, you record from a SINGLE A-type potassium channel, holding at –80mV and stepping to –50mV for 10ms. Draw a typical record for the current through a single channel. **Amplitude from IV: about +1pA; Flickers open fairly fast (tau opening 0.5ms); may not inactivate during 10ms pulse since inactivation takes 15ms (tau). Opening/closing will be square wave shape. If open, may see "tail" current until it shuts rapidly (?)**



10b. (4 pts) After blocking all other channels, you record from the WHOLE-CELL, holding at –80mV and pulsing to –50mV for 10ms. Assuming your cell has ~1000 A-type K+ channels, draw the expected current, indicating an appropriate current scale.
Amplitude rises fast (0.5ms tau). Peaks about +700pA (assuming ideal clamp and 0.7 Popen at -50mV). Inactivation in 10ms is about 1/e^10/15, or about 51% current remaining.
Step to -80mV, immediately shift to -2pA*1000*Popen=0.7*51% = -700pA tail current, which rapidly shuts.



c. (2 pts) To see the effect of the A-type channels on the cell's action potential, you pass current (not in voltage clamp) to stimulate an action potential. If the action potential shown below is in the absence of the A channels, draw what you would expect to happen when they are present. **Since they open pretty fast (0.5ms), may slow the rising phase, reduce amplitude, and speed the falling phase. Probably won't affect AHP since they will deactivate fast. Inactivation (with 15ms tau) won't be much.**



11. (4 pts) For a standard voltage-gated potassium channel that has inactivation, which part(s) of the protein correspond to each of these functions?

a. the voltage sensor_____**S4**_____

b. the selectivity filter_____**the loop between S5 and S6 with the GYG sequence**_

c. the activation gate_____**the S5 and S6 proximity; S6 moving out**_____

d. fast inactivation_____**the N-terminal (ball)**_____

12. You are working with the depicted neuron whose dendritic arborizations are several electrotonic length constants long. You are able to measure Ca^{2+} concentrations in the dendrites using calcium-sensitive fluorescent dyes. Your adviser tells you that Ca^{2+} entry into dendrites is only mediated by voltage-gated calcium channels.

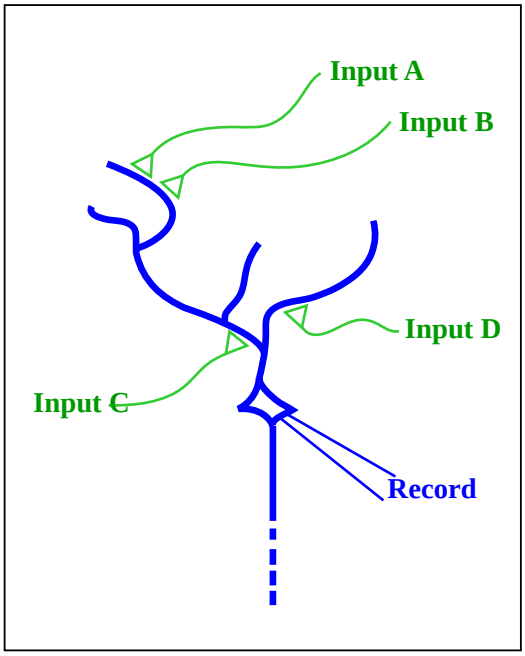


Figure courtesy of MIT OCW.

a. (1 pts) Knowing that voltage-gated calcium channels are closed at –80 mV, you voltage clamp the soma at –80 mV and stimulate input A. To your surprise, you see robust Ca^{2+} entry in the distal dendrite! You conclude that the calcium could not have entered through voltage-gated calcium channels, but your adviser disagrees. Why?

may not be voltage clamped out there

b. (1 pts) You insist, however, that voltage-gated Ca2+ channels are not the only possible explanation for increased cytosolic Ca2+. How else might a glutamatergic synaptic input increase intracellular calcium?

AMPA receptors that are Ca^{2+} permeable
Or Ca++ release from internal stores possible

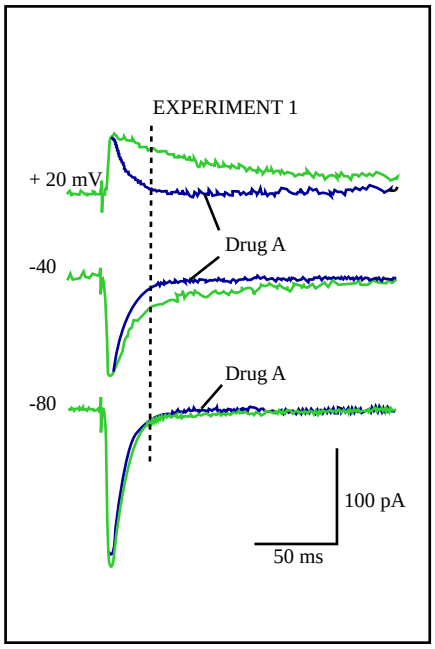
c. (3.5 pts) You now switch to voltage recording (current clamp). You see that a single stimulus at input A or at input B produces a very small EPSP in the soma. However, when you stimulate them simultaneously, you see an EPSP which is greater than the sum of the two. Which of the following are likely to explain this phenomenon? (choose all that apply) **ANSWERS: A, E**

a. activation of voltage-gated Ca^{2+} channels in the distal dendrites
b. activation of $GABA_A$ channels in the distal dendrites
c. facilitation
d. an increase in the electrochemical driving force for Ca^{2+}
e. presence of NMDA receptors
f. metabotropic mGluRs opening dendritic K^+ channels
g. activation of a postsynaptic Ca^{2+} ATPase

12. d. (3 pts) Assume all inputs have ionotropic glutamate receptors except for input C, which is a GABAergic synapse dominated by $GABA_A$ receptors. You study the effect of input C on inputs B and D. Although input D is electrotonically closer to input C, you find that input C has a greater inhibitory effect on input B. Why?

We're looking for the concept of shunting inhibition which preferentially effects distal inputs. $GABA_A$ channels are primarily Cl- permeable. When opened, they result in a shunting inhibition that does not pass significant current at rest. Inputs which are distal to the Cl- conductance (such as inputs A and B) and which must pass along that membrane are shunted. Input D can spread to the soma without directly crossing the membrane at input C, and is therefore less effected by the shunting effect.

13. You voltage clamp a hippocampal pyramidal cell at various holding potentials, stimulate its glutamatergic input fibers and observe the following EPSCs in response (drawn in black.) You are surprised that the time course is quite different at positive and negative holding potentials. When you add Drug A, the time course becomes much more consistent (drawn in gray).



a. (2 pts) What is the likely target of Drug A?
Drug acts by blocking NMDARs. The remaining current is carried by AMPARs.

b. (2 pts) Why does Drug A have little or no effect at a holding potential of –80 mV?
NMDARs are not open (blocked by Mg++) at –80 mV.

c. (1 pts) You decide to do the experiment one more time (just to be sure), but realize right before that you have run out of external solution for your experiment. You hurriedly make up a new batch. This time, however, you're surprised to see that at –80 mV the EPSC now has a long, slow time course similar to that seen at +20 mV. Furthermore, addition of Drug A makes the EPSC at –80 mV quick again, as in Experiment 1. What ion did you forget to add to your solution?

Mg++, thus eliminating the voltage-dependent blockade of the NMDAR. But drug A can still inhibit the NMDAR current.

14. (2.5 pts) Which of the following is true about transmission of EPSPs along dendrites. **ANSWERS: B, D, E**

a. EPSPs become smaller in amplitude and shorter in duration as they move along passive dendrites

b. Potentials travel more readily away from the soma than towards it

c. Inhibitory inputs shunt EPSPs more readily when then are near the cell body than when they are in distal dendrites **(Exclude because antecedent of "they" could be either the inhibitory or excitatory inputs.)**

d. At dendritic branch points the length constant can either increase, decrease or stay the same

e. EPSPs from distal dendrites are often larger than expected from passive propagation both because synapses are stronger at distal locations and because they can stimulate action potentials in the dendrites

15. (2.5 pts) Which of the following are true about the propagation of the action potential down an axon (circle all that apply). **ANSWERS: A, C**

a. increasing axon diameter speeds up propagation

b. increase the number of K+ leak channels speeds up propagation

c. increasing the number of voltage-gated Na+ channels speeds up propagation

d. the myelin sheath primarily increases propagation velocity by decreasing R_m

e. because conduction between nodes is saltatory, eliminating a single node of Ranvier would abolish propagation along the axon

16. (2 pts) There is potassium channel made of KCNQ2 and KCNQ3 subunits, which is partly open at rest, and slowly opens more when depolarized. This channel is closed by acetylcholine or muscarine, and so current through this channel is called the M-current. Which are likely to be true about the M-current? **ANSWERS: A, B, C**

a. acetylcholine probably acts on the current through a second messenger

b. application of acetylcholine to dendrites containing KCNQ2/3 will increase the length constant of the dendrite

c. cells with KCNQ2/3 will have a harder time firing a burst of action potentials than a single action potential

d. application of acetylcholine to a presynaptic terminal containing KCNQ2/3 will decrease vesicle release

17. (3 pts) How do tetanus toxin and botulinum toxin interfere with synaptic transmission (explain in molecular detail)? How does one produce a rigid paralysis, while the other causes flaccid paralysis?

Toxin's heavy and light chain required to enter cell. Light chain then freed and acts as metalloprotease to enzymatically cleave different proteins of the SNARE complex (required for synaptic vesicle fusion).

Tetanus tends to hit inhibitory synapses (thus rigid, due to disinhibition of motoneuron), and botulinum toxin hits NMJs and others. (Tropism of toxin.)

18. (2.5 pts) To impress your parents and show them that their tuition loan was worth it all, you begin to tell them all you learned about the sites of action of cannabinoids in the brain. Your recollection is a little foggy, however, as you still have a buzz from that very fine stuff you smoked last night. What are some of the things you are trying to recall? (circle all that apply) **ANSWERS: B, D, E**

a. tetrahydrocannabinol (also the active ingredient in marijuana) is normally synthesized by medium spiny neurons of the striatum
b. anandamide is a membrane-permeant cannabinoid which can diffuse from a postsynaptic neuron to a presynaptic terminal as a form of retrograde transmission
c. binding of anandamide to CB1 receptors potentiates voltage-gated Ca^{2+} channels, leading to more neurotransmitter release
d. cannabinoids inhibit GABA release by neurons of the nucleus accumbens, stimulating dopaminergic neurons of the ventral tegmental area
e. morphine inhibits GABA release by neurons of the nucleus accumbens, stimulating dopaminergic neurons of the ventral tegmental area

19. (2.5 pts) Long term potentiation in the hippocampus, at CA3 to CA1 synapses, has the following characteristics (circle all that apply) **ANSWERS: A, C, D, E**

a. it lasts minutes to hours
b. it requires the influx of Ca^{2+} primarily through AMPA receptors
c. it requires insertion of new AMPA receptors in the postsynaptic membrane
d. it can be a mechanism for associating signals from two neurons, as depolarization evoked by one neuron can potentiate the synapse from another neuron
e. it differs from LTP at mossy fiber synapses (dendate gyrus to CA3), which is primarily presynaptic

20. (2 pts) You have spent years to identify the gene for a rare, dominantly inherited paralytic disease. The disease is characterized by a temporary inability to generate action potentials in the muscle after heavy exercise. The gene you finally identify encodes an inwardly rectifying potassium channel, and the disease is correlated with a single amino acid change in its selectivity filter. Experiments on mutant channels expressed in cultured cells indicate that the channel becomes less selective for K^+ when lactic acid builds up and the residue is protonated. What is the single most likely etiology for the disease? **ANSWERS: A**

a. Na^+ influx depolarizes the muscle to a region where voltage-gated Na^+ channels are largely inactivated.
b. Because the channel is less K^+ -selective, K^+ leaks into the muscle, shifting the K^+ Nernst potential more negative and hyperpolarizing the cell
c. Ca^{2+} influx stimulates continuous neurotransmitter release, and desensitization of nACh receptors
d. K^+ accumulation in the T-tubules depolarizes the muscle cells

21. (6 pts) Execution by lethal injection involves administration of the following drugs, in order: sodium thiopental (a barbiturate), tubocurarine chloride (a.k.a. curare), and potassium chloride. For each, indicate the important molecular target, and the effect on the inmate.

sodium thiopental
target: **____Agonist of $GABA_A$_____**

effect: **anesthesia**

tubocurarine chloride
target: **_____Antagonist of nAChR at NMJ esp diaphragm_____**

effect: **paralysis, stops breathing**

potassium chloride
target: **___Na^+ channel esp heart**

effect: **cardiac arrest, brain meltdown**
(cause arrhythmia by depolarization)

22. (3 pts) What are three widely used treatments for epilepsy?

drugs (dilantin, phenobarbitol, phenytoin, tegretol, etc.)
ketogenic diet
surgery to remove focus
vagal nerve stimulator
(Any 3 of 4)

23. (4 pts) Bipolar disorder is often treated with drugs such as Prozac, Celexa, Zoloft, and imipramine. While they apparently target synapses, an unusual feature of them is that they don't have much effect for the first 2-3 weeks.

a. What synaptic process or molecule is thought to be their target?

They are SSRIs (selective serotonin reuptake inhibitors) and target SERT (Serotonin Reuptake Transporter).

b. What new hypothesis for their action would explain the delay in efficacy?

It is thought that they may stimulate neurogenesis through stem cell differentiation