NAME_____TA_____ SEC____

7.012 Problem Set 6 FRIDAY November 19, 2004

Answers to this problem set must be inserted into the box outside

Problem sets will NOT be accepted late. Solutions will be posted on the web.

Question 1

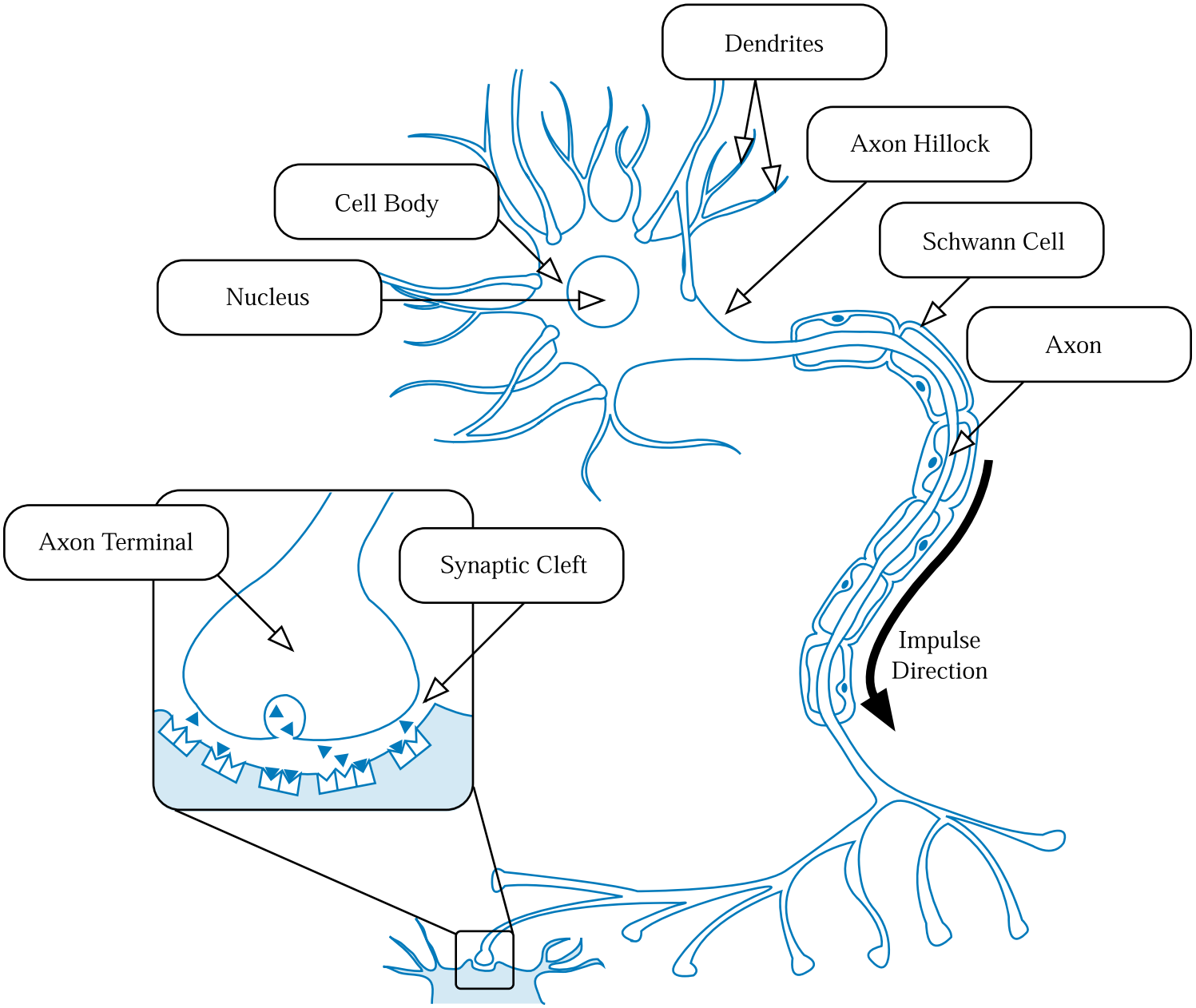


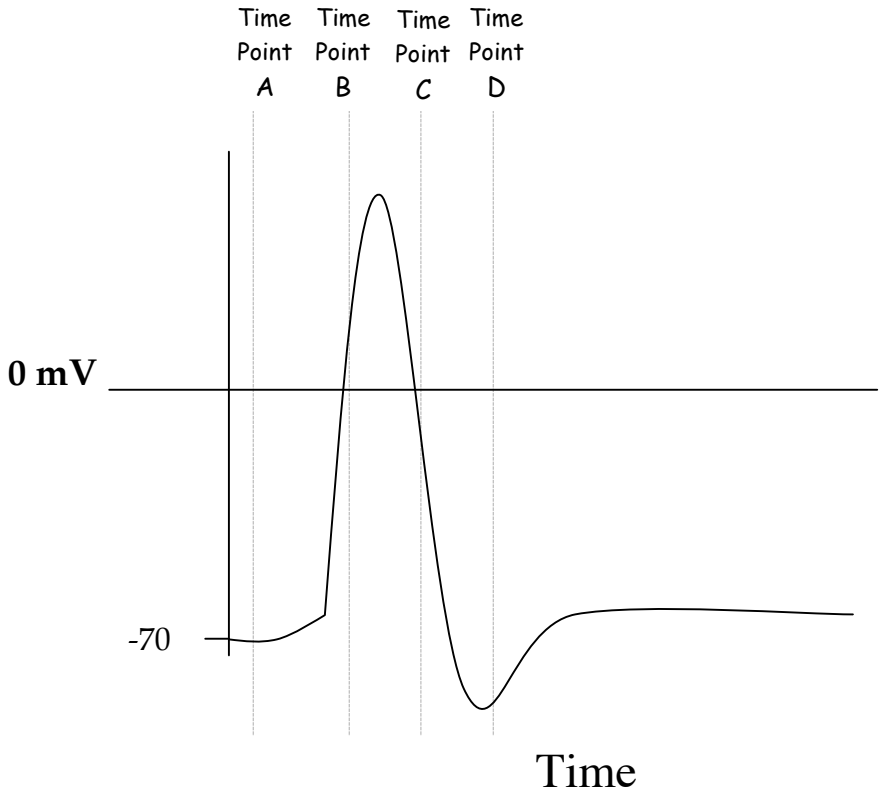
Figure by MIT OCW.

Question 1 continued

b) In a neuron, where do the signals from the excitatory and inhibitory synapses get summed up in the decision to fire an action potential? (Circle the correct one.)

Cell Body ***Axon Hillock*** Myelin Sheath Nerve Terminal Synaptic Vesicle Synaptic Cleft

Shown below is a plot of the membrane potential at a single point on an axon as a function of time as an action potential travels down that axon.



c) For each of the four time points indicated (A-D), fill in the following: the stage of the action potential, the status of the sodium and potassium channels, and the ion flux through each channel at the point along the axon where the potential is being measured.

(For the potassium channels give the answers for the voltage-gated type.)

Please use the terms provided.

	Time Point			
	A	B	C	D
Stage of action potential				
Voltage-gated Na^+ Channel status (**Open or Closed/Inactive**)				
Voltage-gated K^+ Channel status (**Closed, Open**)				
Na^+ Flow through Na^+ Channel (**Into axon, Zero, Out of axon**)				
K^+ Flow through K^+ Channel (**Into axon, Zero, Out of axon**)				

	A	B	C	D
Stage of action potential	***Resting potential***	***Depolarization***	***Repolarization***	***Hyperpolarization***
Voltage-gated Na^+ Channel status (Open or Closed/Inactive)	***Closed/inactive***	***Open***	***Closed/inactive***	***Closed/inactive***
Voltage-gated K^+ Channel status (Closed, Open)	***Closed***	***Closed***	***Open***	***Open***
Na^+ Flow through Na^+ Channel (Into axon, Zero, Out of axon)	***Zero***	***Into Axon***	***Zero***	***Zero***
K^+ Flow through K^+ Channel (Into axon, Zero, Out of axon)	***Zero***	***Zero***	***Out of Axon***	***Zero***

d) What role do the Na^+/K^+ ATP-driven pumps (present in neuronal cell membranes) play in maintaining the resting potential?

The sodium/potassium pump maintains the sodium and potassium ion concentration gradients across the plasma membrane.

e) Consider an experimental injection of a number of positive ions into the axonal cytoplasm of a neuron. Explain how the voltage-sensitive sodium (Na^+) channels initiate an action potential in response to this injection.

If the injection results in a depolarization above threshold, many sodium channels open, allowing a local influx of sodium ions--which causes a depolarization of the axon sufficient to initiate an action potential.

f) In many cells there are two types of potassium (K^+) channels present in the axonal membrane. What is the role of each type of K^+ channel, with regard to the membrane potential?

The resting K^+ channel (which is always open) results in a basal leak of potassium ions out of the cell, creating a negative membrane potential (the resting potential). The voltage-sensitive K^+ channel opens in the latter stage of the action potential, accelerating repolarization across the membrane.

g) The voltage-sensitive sodium channel has an inactivated state, in which the channel is not only closed, but also unable to open in response to depolarization. When does the channel shift into this conformation? What effect does this have on the direction of propagation of the action potential?

The sodium channels spontaneously inactivate after being open briefly; allowing subsequent repolarization of the membrane to occur. Since the refractory period of the sodium channels prevents the action potential from moving in the direction from which it came, it can progress in only one direction down the axon.

Question 2

A. You have some drugs that affect neurotransmission, and you wish to determine what steps they are likely to affect.

In a Petri dish culture of neurons, you depolarize a pre-synaptic cell with an electrode and you observe a resulting action potential in that pre-synaptic cell. This step is the same regardless of whether drugs are added. However, the effect on the post-synaptic cell varies.

Additions	**What you see in post-synaptic cell...**
No drug	You see a slight depolarization in the post-synaptic cell followed by no action potential in the post-synaptic cell.
Drug 1	You see a large depolarization in the post-synaptic cell followed by an action potential in the post-synaptic cell
Drug 2	You see no depolarization in the post-synaptic cell and no action potential in the post-synaptic cell

c) For the following, complete the statement with the letter(s) from the list below.

i) The effect of Drug 1 could be that the…_____A D

ii) The effect of Drug 2 could be that the…_____B C E

A.	*Neurotransmitter is inhibited from degradation.*
B.	*Neurotransmitter is degraded.*
C.	*Neurotransmitter's reuptake is accelerated.*
D.	*Neurotransmitter's reuptake is inhibited.*
E.	*Neurotransmitter's receptor is blocked.*

Question 2, Part B

You are working in lab that studies the adrenaline response. Many athletes come to your lab looking for a drug that will give them an extra kick-just like adrenaline. You know that adrenaline (a.k.a. epinephrine) works in the "flight or fight" response and that it can trigger action potentials in cardiac muscle, but you need to learn more.

a) You find out that epinephrine results in an overall increase in calcium ion levels within the cytoplasm of muscle cells. Given this fact, what do you think is required to restore the cardiac muscle cell back to its resting state?

In addition to the removal of epinephrine from synapse,
Calcium pumps in the membrane to lower cytoplasmic calcium ion levels

b) What are three ways that neurotransmitter can be removed from the synapse?

Diffusion, uptake by nerve terminal, and degradation

c) You find out that epinephrine is removed from the synapse by uptake by the axon. With this knowledge, how could you design a drug to give the athletes the extra kick they are looking for? How would it work?

You could design a drug that prevents the uptake of epinephrine by the axon so it remains in the synapse to signal longer.

d) What possible side effects would this drug cause?

Excessive and forceful muscle spasms. (Epinephrine overdose includes symptoms of an increased and irregular heartbeat, a more forceful heartbeat, a severe, throbbing headache, and stroke.)

Question 3

a) You receive a letter in the mail from ACDC (Americans for Cloning Dead Celebrities), asking for a $10 donation to support the cloning of a famous movie star, Goldie Starlet, who died in a plane crash in 1994 and no remains of her were found. Your friend, who is a huge fan of Goldie, sees the letter and promptly whips out $10 to send to ACDC. Do you think your friend made a wise decision? Explain your answer.

No. A nucleus would have to be obtained from one of Goldie's cells in order to clone her.

b) A few months later, you watch a documentary on organ transplants, where a woman named Rosie claims to have received Goldie's left kidney during a kidney transplant in 1993. Briefly outline the steps by which Goldie could be cloned with the help of Rosie.

Take the nucleus from one of the cells of the donated kidney. Inject the nucleus into an enucleated oocyte. Activate the egg and implant the blastocyst in the uterus of a surrogate mother.

c) ACDC clones Goldie with some assistance from Rosie. However, the clone has a short stature (typical of a known mitochondrially inherited disorder), whereas Goldie was much taller. Is Rosie a fraud or could there be another explanation?

The mitochondria of the oocyte donor probably carried a defect that gave rise to the clone's short stature.

d) The ACDC finds out that Goldie had some of her eggs frozen in a fertilization clinic. Could the nucleus of one of these unfertilized eggs be used to clone Goldie Starlet? Why or why not?

No the nucleus of an unfertilized egg cannot be used to clone because it is haploid.

e) Your friend thinks that ACDC should give Rosie a second chance. What steps could one take so that this time the clone has the same stature as Goldie's?

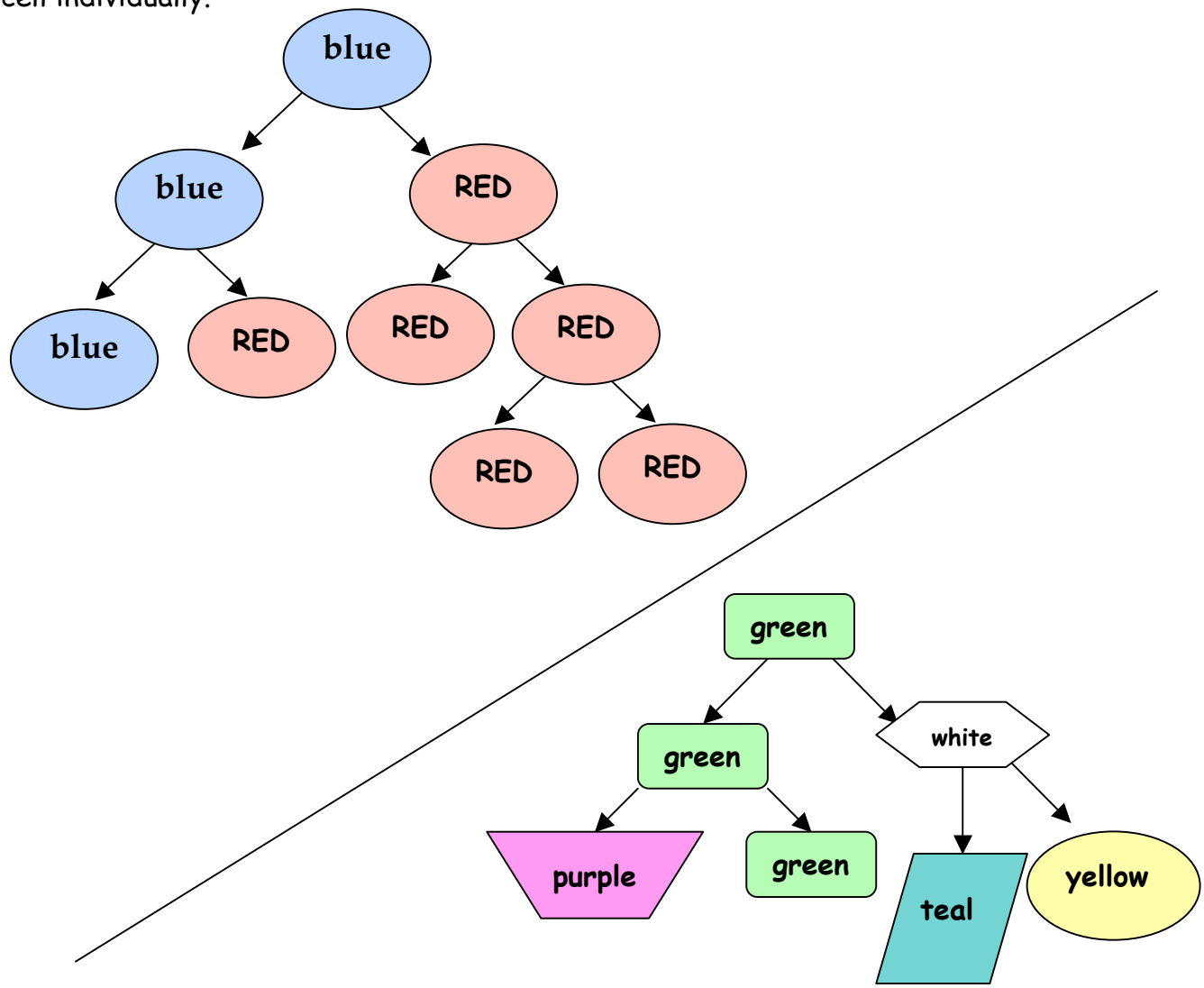
Use one of the oocytes that Goldie had frozen in a fertilization clinic. Alternatively, use oocytes from one of her family members—mother, sister or daughter, if available.

f) After including your suggestions from part d), can you feel confident that the clone will be 100% identical to Goldie in every way. Explain your answer.

No. There are epigenetic differences, so different sets of genes may be active or inactive in the parent and clone.

Question 4

Divya finds a new brightly colored mammalian species, which he names *Magnificus colores*. She isolates blue and green cells from different parts of this organism, and cultures each cell individually.



a) Are the blue cells stem cells? Why or why not?

Yes, because they can self-renew and give rise to other cell types.

b) Given that the green cell is a stem cell, can Divya tell whether it is pluripotent or unipotent? Explain your answer.

It is multipotent because it can give rise to more than one cell type.

c) Divya finds out that development in *M. colores* is similar to human development. She wants to obtain a single cell that can give rise to an individual *M. colores*. Do you have any suggestions? What is the property that this cell possesses?

A fertilized egg could be used. A cell that can give rise to an entire organism is "totipotent".

d) She now wants to isolate embryonic stem cells from an *M. colores* embryo. Where exactly in a developing embryo can she find such cells?

The inner cell mass of the blastocyst.

e) *M. colores* have coats with multi-colored spots, which are produced by the *speckle* gene. One functional copy of *speckle* is sufficient to give multi-colored spots. While studying different embryos, Divya detects that one 8-cell stage embryo has both copies of *speckle* mutated. Suggest a strategy using stem cells by which Divya could try to rescue this defect in the embryo.

Grow the embryo to the blastocyst stage. Obtain ES cells from the embryo. Insert a normal copy of the speckle gene in these cells and inject them into the blastocyst again and let the embryo develop.

f) One of the *M. colores* has a weak heart that has only 10% of the normal functioning capacity. If technology were advanced enough, how could ES cells from this animal potentially be used to save it from heart failure? Why would this be preferred over a heart transplant from another individual of this species?

The ES cells (previously frozen down) are pluripotent. If they could be induced to differentiate into cardiac tissue, this tissue could be used to replace the existing one. Such a technique would be preferred over a heart transplant from another individual because one would not have to deal with issues of immune rejection.