

NAME _____ TA _____

7.013 Spring 2005 Problem Set 4

FRIDAY April 8th, 2005

Problem sets will NOT be accepted late.

Question 1

a) A friend knows you are taking 7.013 and therefore an expert on cloning, so he asks you to clone a cat for him. He suggests several cell types from which you could take the nucleus for the experiment. Match the cell types with your reactions and write a brief explanation of why you reacted this way. Use each letter once.

A	early stage embryonic cell
B	B cell (see Figs 18. 8 and 18.19.a, p.382-383)
C	Sperm
D	Neuron
E	Red Blood Cell

i) "Forget it. I could do the transfer but we'd never get a viable clone." _____ C _____
haploid.

ii) "Okay. Not a bad place to start." _____ D _____

iii) "Terrific! That will maximize our chance of success!" _____ A _____

iv) "Are you kidding? I couldn't even do the experiment with that." _____ E _____
No nucleus.

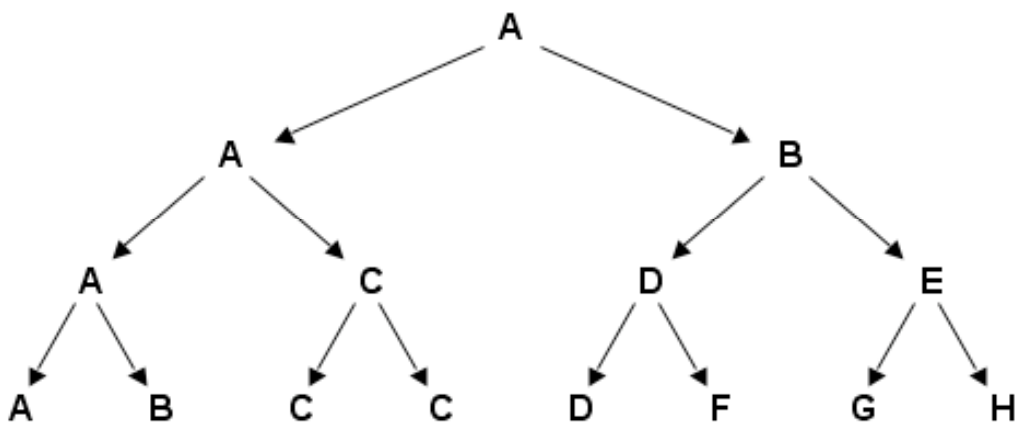
v) "Hm.... I can try, but we'll probably end up with one sick cat." _____ B _____
Missing Variability of antibody types.

b) What will you transfer the nucleus into?
enucleated egg

c) What are the two key characteristics of a stem cell?

1) Self-renewing.
2) Can differentiate into other cell types.

d) Which cells in the diagram below are stem cells? _____ A, D _____



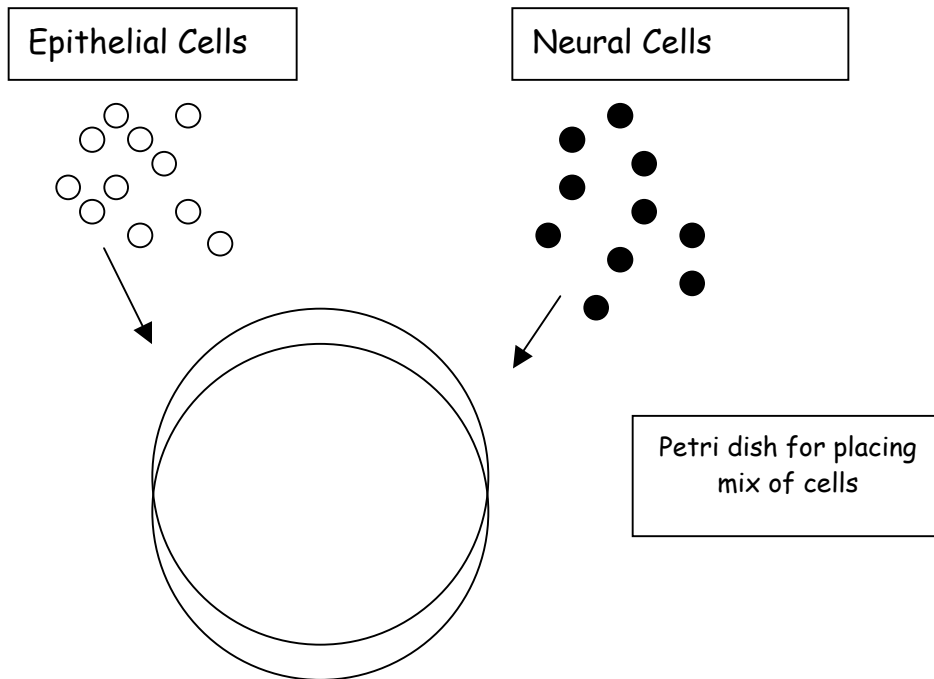
e) Which cell in the diagram above would you most want to use to obtain a nucleus for the cloning experiment above? Explain briefly. *A-most pluripotent*

f) What is the difference between embryonic stem cells and stem cells from adults? *Embryonic stem cells are more pluripotent.*

g) The Massachusetts legislature is currently debating a bill that would make it much easier to do somatic cell nuclear transfer and stem cell research in the state. Do you personally think such a bill should pass or do you have concerns about it? Explain in three or four sentences.

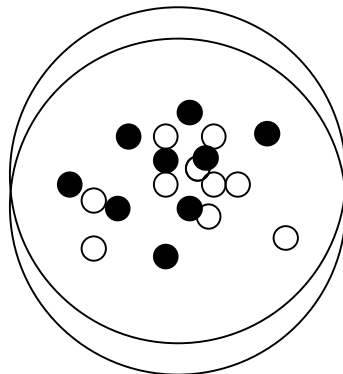
Question 2

A. To examine the role of cell adhesion in tissue formation, you remove epithelial cells and neural cells from a *Drosophila* embryo, dissociate them so that any existing connections are gone and each cell is independent. You then label each type of cell with a different color dye and mix the two cell populations together on a plate.

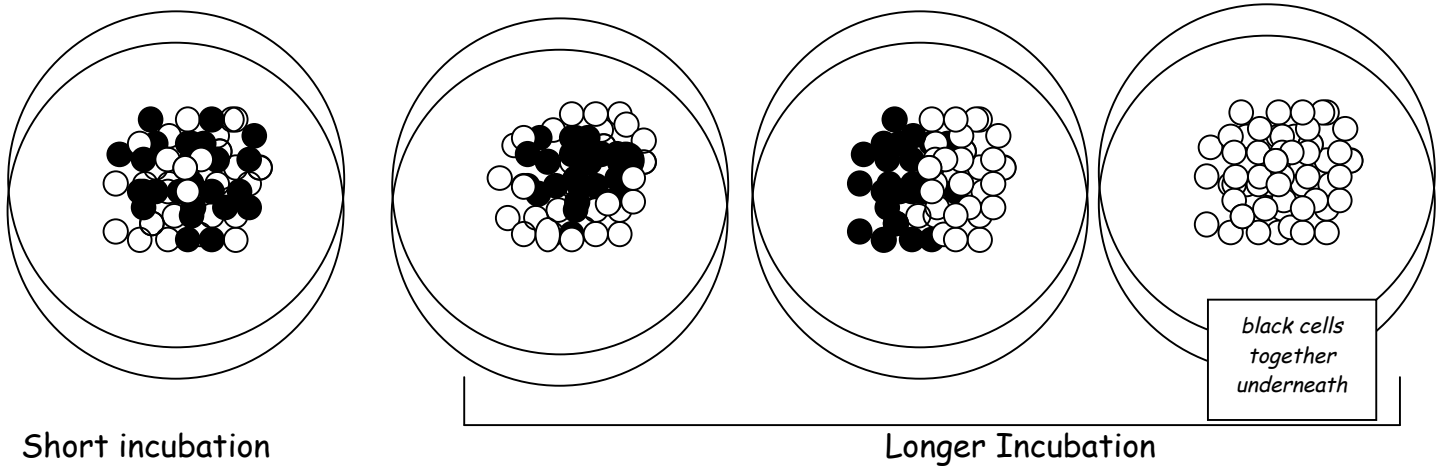


For parts a through c use empty circles to represent epithelial cells and filled circles to represent neural cells (like above).

a) What would the cells look like immediately after mixing them? Draw what you would expect to see in the petri dish below.

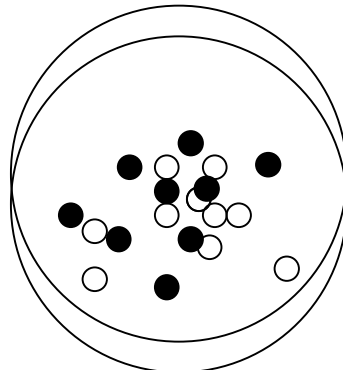


b) What would you expect to see if you looked at the cells at later time points after mixing? Assume for simplicity that mitosis has not occurred. Draw what you expect to see after a short incubation and then after a longer incubation. in the petri dishes below.



c) EDTA is a calcium chelator that sequesters calcium making it useless. How would your results in b) change if you added EDTA to the cells before mixing them?

i) Draw the expected results in the petri dish below.



ii) What aspect of cell behavior are we disrupting by the addition of EDTA above and what molecules are involved?

Cell adhesion / Cadherins

iii) Design an experiment that would allow identification of the molecules involved in this behavior.

Create mutants that lack adhesion ability. Or create mutants that are resistant to EDTA. Construct a wild-type cDNA library transform into these mutant lines and look for cells that regain the wild type ability to adhere (for the adherent defective strain) or look for the susceptibility to EDTA (for the EDTA resistant strain).

B. During your summer research internship in the Amazon, you stumble upon a never before seen spectacular creature (shown leaping below), which you give it the genus and species name *Nihplod reggit*.

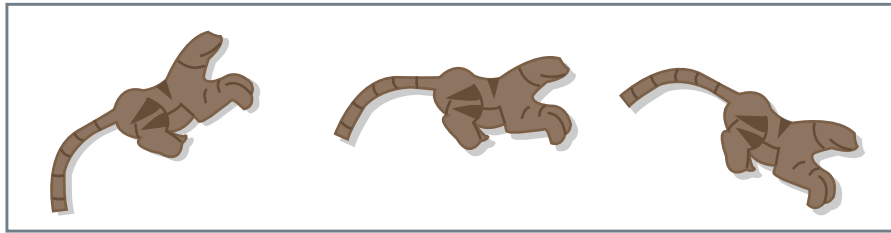


Figure by MIT OCW.

You decide to study the early development of *Nihplod reggit* to see how if you can find similarities to known organisms. You discover that the *Nihplod reggit* has a unique organ that acts as a combination heart and lung. This organ forms from a series of primitive tubes that develop from a specific region of the mesoderm.

a) What type of experiment could you use to determine which cells of the mesoderm give rise to this organ? *Fate mapping*

b) Describe one of the three possible ways that these tubes can form. What type of cells are involved in each process?

- 1) *ballooning -Epithelial sheets*
- 2) *cavitation-Epithelial sheets*
- 3) *condensation-Mesenchymal Cells*

Development of the mature heart lung organ requires elongation and thinning (changing layer thickness and number) of the primitive tubes.

c) What types of cell movements could be leading to these changes? Are these changes likely occurring in sheets of cells or in mesenchyme?

Changes in layer number through processes such as delamination and radial intercolation. Changes in length through intercolation, cobnvergent extension. Most likely occurring in sheets.

d) These shape changes are triggered by the production of heart growth factor (HGF) in neighboring cells. How could a growth factor induce changes in cell shape and movement?

Through transcriptional changes and signal transduction resulting in actin polymerization and depolymerization.

e) What role does actin play in cell movement?

It polymerizes at one end of a cell into projectiles that extend out of the plasma membrane and depolymerizes at the opposite end of the cell allowing the cell to move forward.

f) What effect would addition of a drug that prevented actin polymerization or depolymerization have on cell movement?

Since both polymerization and depolymerization of actin is required for cell movement and shape change, the cells would no longer be able to move.

Question 3

Circle the correct answers.

a) With respect to the outside, the inside of a neuron at rest is:

i) **More negatively charged**

ii) More positively charged

iii) The same charge

b) Na^+ and Cl^- are:

i) More concentrated inside the neuron

ii) **More concentrated outside the neuron**

iii) Are found in equal concentrations inside and outside the neuron

c) K^+ is:

i) **More concentrated inside the neuron**

ii) More concentrated outside the neuron

iii) Is found in equal concentrations inside and outside the neuron

d) The resting membrane potential of a neuron is determined by:

i) **Ions which can travel freely through channels in the resting neuron**

ii) Ions which cannot cross the resting neuron membrane

iii) **All ions (okay)**

iv) Ions do not determine the resting membrane potential

e) Action potentials are generated by the passage of ions through

i) Resting ion channels

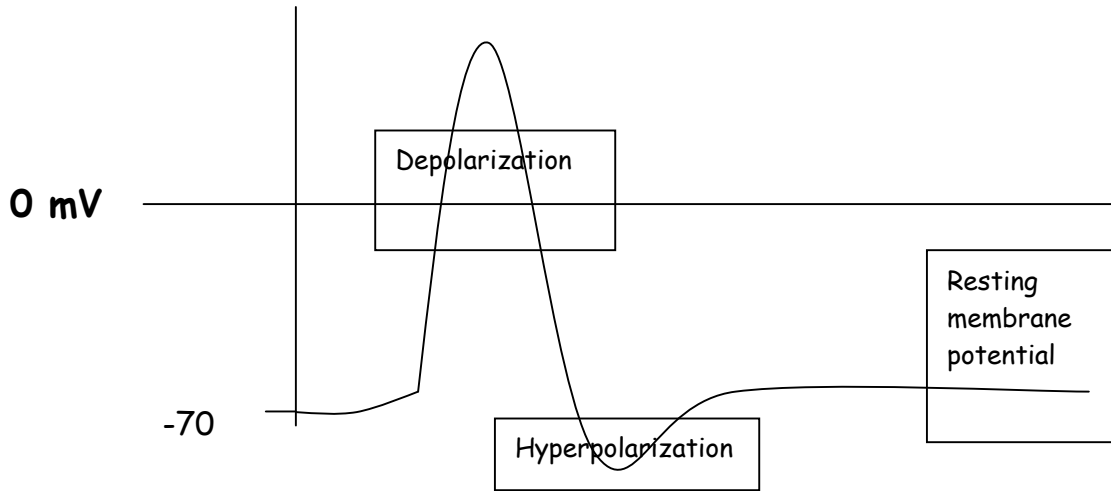
ii) **Voltage-gated ion channels**

iii) G-protein coupled receptors

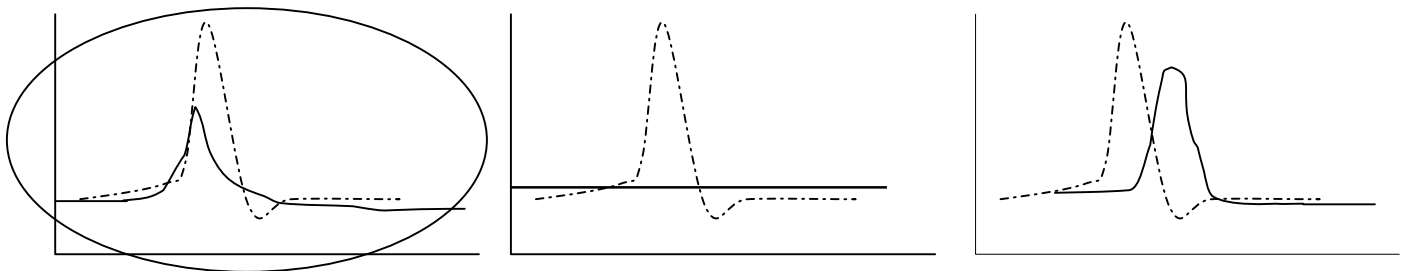
iv) Receptor tyrosine kinases

f) In the action potential depicted below, label the following:

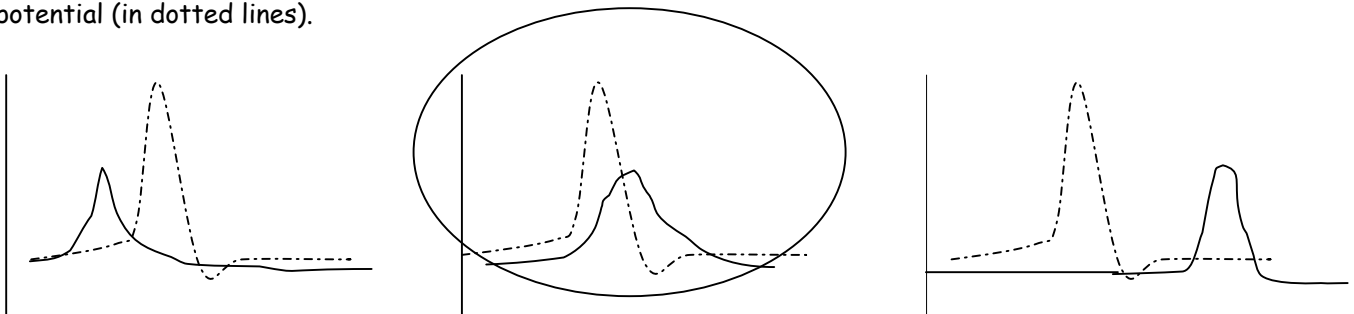
Resting membrane potential
 Depolarization
 Hyperpolarization



g) Which of the following correctly depicts Na⁺ conductance (in solid lines) over the course of the action potential (in dotted lines). Conductance refers to the permeability of sodium through the neuronal membrane.



h) Which of the following correctly depicts K⁺ conductance (in solid lines) over the course of the action potential (in dotted lines).



i) When voltage-gated potassium channels in the neuronal membrane open, the flow of potassium will be:

- a) **inside to outside**
- b) outside to inside
- c) no flow

j) When voltage-gated sodium channels in the neuronal membrane open, the flow of sodium will be:

- a) inside to outside
- b) **outside to inside**
- c) no flow

Question 4

a) What is the normal "resting potential" of a neuron? How does it manage to stay at this potential?

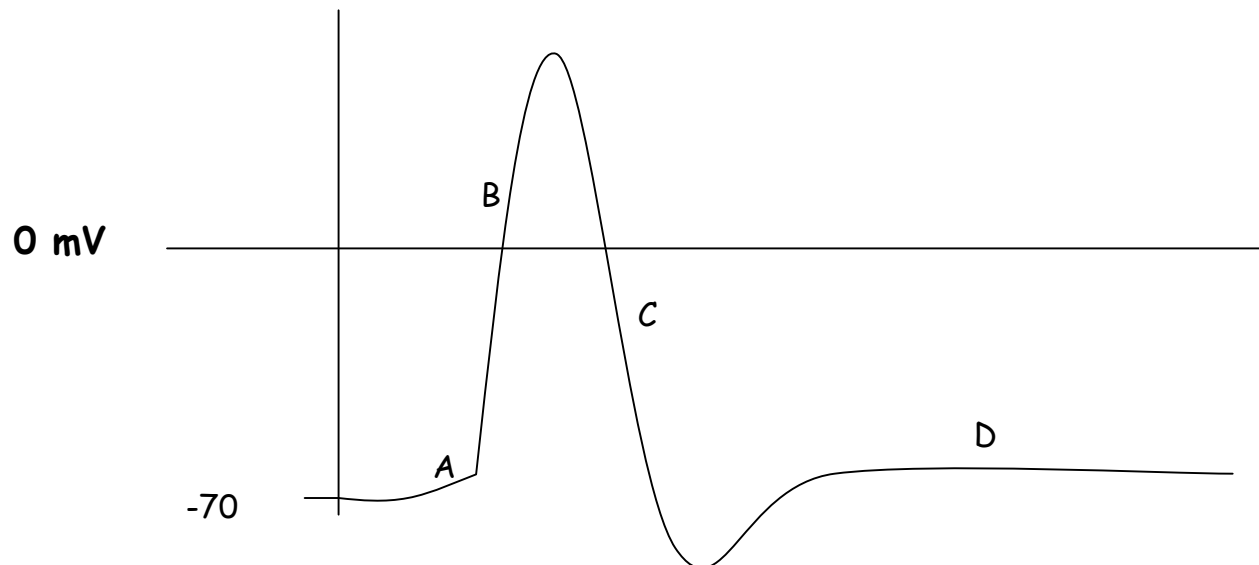
The size of the resting potential in neurons is about -70 millivolts (mv).

The resting potential arises and is maintained by 2 channels...

1) *The sodium/potassium ATPase pump. This pump pushes exchanges potassium ions (K⁺) into the cell for sodium ions (Na⁺) it pumps out of the cell.*

2) *The leak potassium channels in the plasma membrane are "leaky" allowing a slow facilitated diffusion of K⁺ out of the cell. The movement of ions across the cell membrane depend both on concentration and electrostatic gradients.*

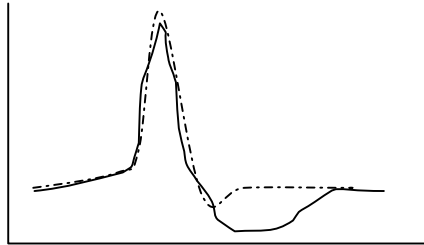
b) Match each letter on the diagram below to the appropriate states of the **voltage-gated Na⁺ and K⁺ channels** in the axonal membrane. **Not every statement will have a matching letter.**



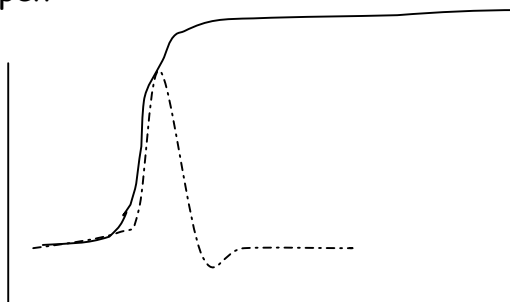
	All Na ⁺ channels are reactivated but closed: most K ⁺ channels are open
B	All Na ⁺ channels are open; all K ⁺ are closed
	All Na ⁺ channels are inactivated; all K ⁺ channels are closed
A	Some Na ⁺ channels are open; all K ⁺ are closed
C	All Na ⁺ channels are inactivated; All K ⁺ channels are open
	All Na ⁺ channels are closed; some K ⁺ are open
	Some Na ⁺ channels are open; some K ⁺ are open
D	All Na ⁺ channels are reactivated but closed: All K ⁺ channels are closed
	All Na ⁺ channels are inactivated; all K ⁺ channels are closed

c) For each of the following cases where neurons are stimulated just to the normal threshold level, draw on the axes below how an action potential would look relative to a normal action potential (drawn with dotted line).

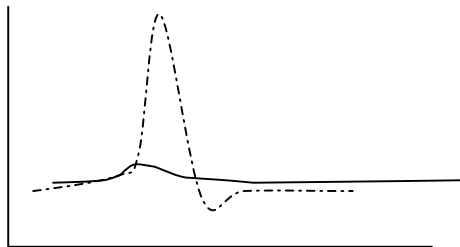
i) Voltage-gated K^+ channels take longer to close



ii) Voltage-gated K^+ channels never open



iii) Voltage-gated Na^+ channels never open



iv) Mutant Voltage-gated Na^+ channels have a higher threshold

