

NAME \_\_\_\_\_ TA \_\_\_\_\_

## 7.013 Spring 2005 Problem Set 5

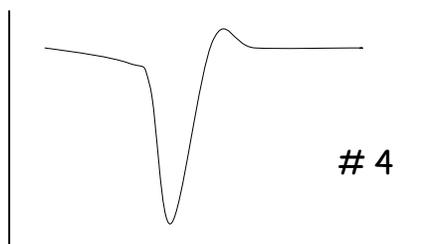
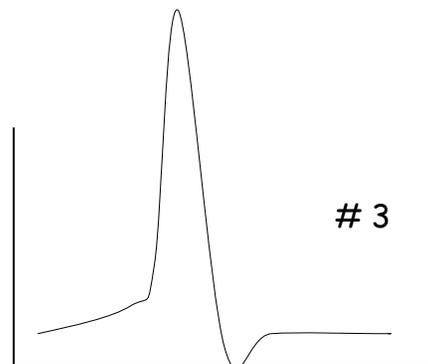
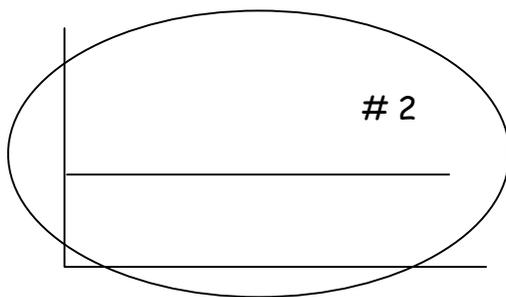
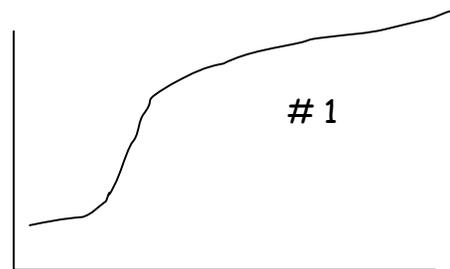
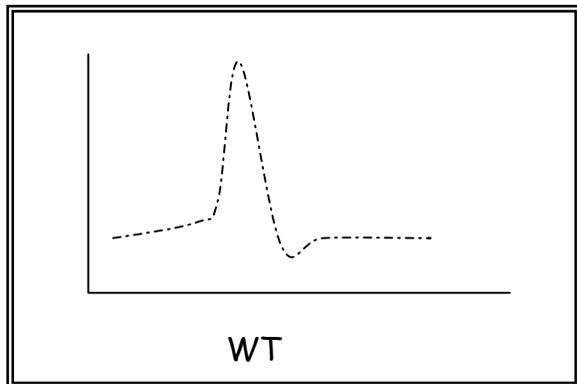
FRIDAY April 15th, 2005

**Problem sets will NOT be accepted late.**

### Question 1

In the autoimmune disease, myasthenia gravis, antibodies are produced against one's own acetylcholine (ACh) receptors in muscle. The result of this antibody production can either lead to the degradation of the ACh receptors, or to impaired binding of acetylcholine.

a) A post-synaptic muscle cell experiences action potentials similar to a neuron. See the wildtype (WT) diagram below. Compared with the WT muscle action potential, which of the following muscle action potentials (#1-5) would you expect to see from a person with advanced myasthenia gravis.



b) Acetylcholinesterase is an enzyme that degrades acetylcholine in normal individuals. Which of the following is true?

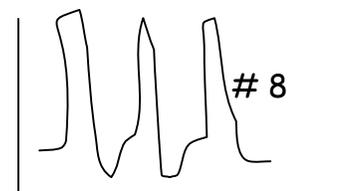
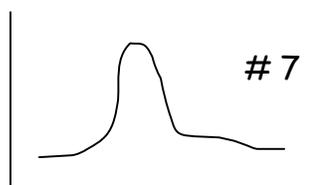
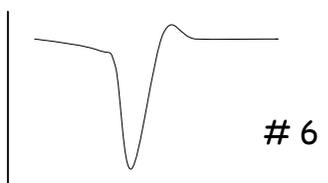
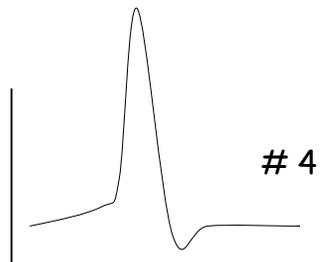
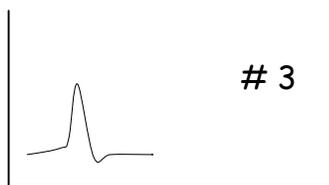
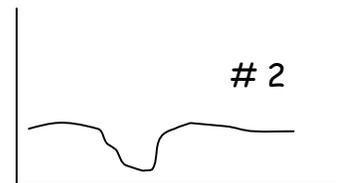
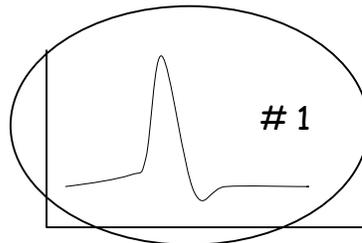
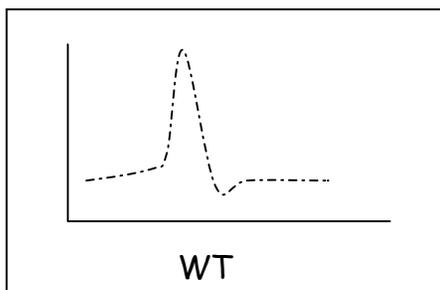
- i) Drugs that stimulate acetylcholinesterase would improve myasthenia gravis.
- ii) Drugs that inhibit acetylcholinesterase would improve myasthenia gravis.**
- iii) Drugs modulating acetylcholinesterase would have no effect on myasthenia gravis.

Two other diseases affecting the neuromuscular junction are Lambert-Eaton syndrome and human botulism. In Lambert-Eaton syndrome, an autoimmune disease, antibodies are produced against the presynaptic  $Ca^{2+}$  channels at the neuromuscular junction. In botulism, a bacterial toxin prevents fusion of neurotransmitter-containing vesicles with the presynaptic plasma membrane.

c) Calcium is important in synapses because...

- i) Calcium is physically required for binding of Ach to its receptor.
- ii) Calcium influx in the axon is required for fusion of presynaptic vesicles with the membrane.**
- iii) Calcium prevents neurotransmitter from being degraded.

d) If you isolated **neurons** from a person affected by L-E syndrome or botulism and stimulated it, which of the following action potentials would you likely see? (Normal action potential-shown by dotted line; affected action potential-shown by solid line.)



e) If you were to make a neuromuscular synapse between a Lambert-Eaton presynaptic neuron and a myasthenia gravis postsynaptic muscle, which of the following would you expect?

i) Normal frequency of muscle action potentials

**ii) Decreased frequency of muscle action potentials**

iii) Increased frequency of muscle action potentials

f) If you were to make a neuromuscular synapse between a myasthenia gravis presynaptic neuron and a Lambert-Eaton postsynaptic muscle, which of the following would you expect

**i) Normal frequency of muscle action potentials**

ii) Decreased frequency of muscle action potentials

iii) Increased frequency of muscle action potentials

g) What is an ionotropic receptor?

A receptor that is also a channel for ions.

h) What takes place at the axon hillock?

Charges are summed up and if threshold is reached an action potential is fired.

## Question 2

As discussed in lecture and in Purves, pages 898-9, Long term potentiation, (LTP), is thought by many scientists to underlie learning and memory. In the questions below, briefly describe each of the following events that are involved in LTP.

a) How does an action potential result in the release of neurotransmitter into the synapse?

*Action potential depolarizes membrane at axon terminal. Opening of voltage-gated  $Ca^{++}$  channel cause a rise in intracellular calcium. Calcium promotes fusion of vesicles with membrane and release of contents into the synapse.*

b) What is the general name of molecules released from vesicles the presynaptic neuron into the synapse? What is the molecule released in LTP?

*Neurotransmitter (Specifically-Glutamate in LTP)*

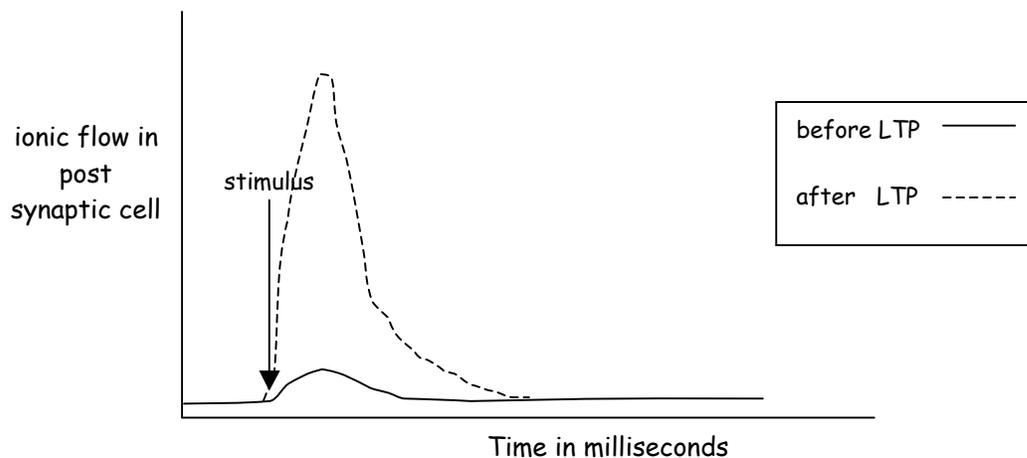
c) To what do these molecules bind on the surface of the post-synaptic neuron?

*RECEPTORS. (Specifically, Glutamate receptors, which are also ligand-gated ion channels (NMDA and AMPA receptors). Allosteric changes that result from glutamate binding cause these channels to open.)*

d) This results in an excitory interaction. What kind of ions flow into the cell as a result?

**POSITIVE. Sodium, for example.**

d) After repeated cycles of LTP process, surface receptors on the post-synaptic cell ore up-regulated. (More appear.) How does this change affect the response of the post-synaptic cell? Draw your answer on the graph below relative to the curve labeled "before LTP":



### Question 3

a) Which of the following assumptions is the "Ames Test" based on? Circle all that apply.

i) **Mutagens are likely to be carcinogens**

ii) Histidine is a carcinogen

iii) Histidine is a mutagen

iv) Bacteria cause cancer

v) Many genes are carcinogens

b)

- You spread His<sup>-</sup> bacteria on a plate with medium containing Chemical X and lacking any histidine. No colonies are able to grow on this plate. Is Chemical X a mutagen?

**NO**

- You grow His<sup>-</sup> bacteria on a plate lacking histidine with Chemical Y. You see many colonies. Is Chemical Y a mutagen?

**YES**

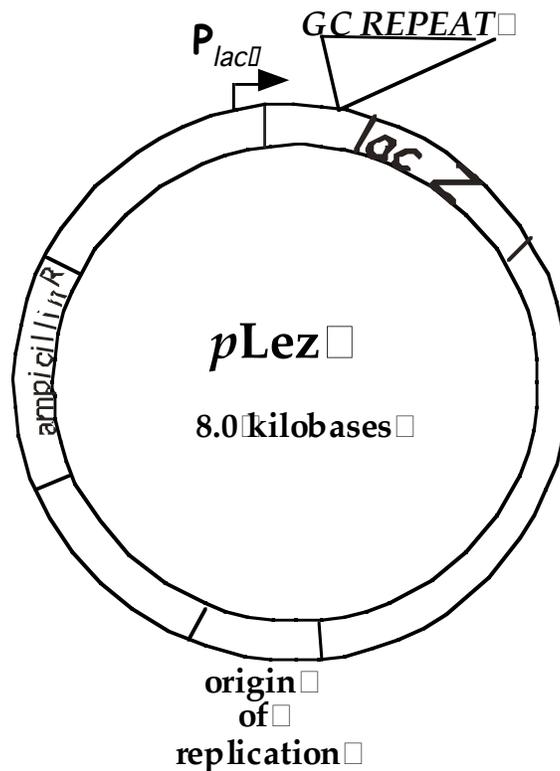
- You repeat the Ames test with Chemical X, but include this time rat liver enzymes. You now see many colonies. Why is this result different from the earlier test?

**Liver enzymes modified Chemical X and it now MUTAGENIC.**

- You repeat the Ames test with Chemical Y, but include rat liver enzymes this now. Now you see no colonies. Why is this result different from the earlier test?

**Liver enzymes modified Chemical X and it now DETOXIFIED.**

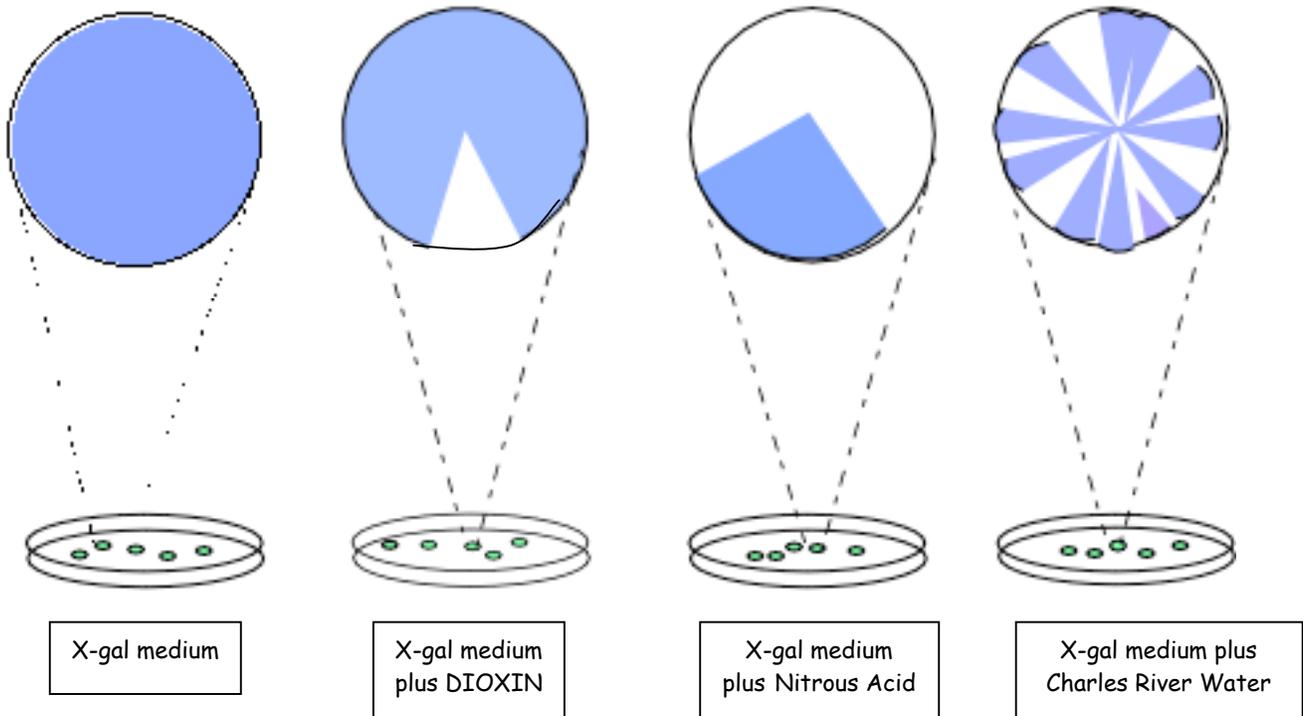
c) You decide to perform a different type of Ames test. This time you use the *lac Z* reporter gene, which encodes the  $\beta$ -galactosidase enzyme that converts X-gal into a blue product. You use a modified *lac Z* gene that contains an internal repeat with the sequence *GCGCGCGCGCGC*. This sequence does not impair  $\beta$ -galactosidase activity. You generate a plasmid containing this modified *lac Z* (below), transform it into *E. coli* bacteria. You will use this as your strain in the Ames test to screen for white colonies on X-gal medium. These white colonies result from expansion or contraction of the *GC* repeat that renders the  $\beta$ -galactosidase non-functional.



a) How does expansion or contraction of the dinucleotide repeat result in a nonfunctional  $\beta$ -galactosidase enzyme?

***By creating a frameshift in the gene***

b) Below are Petri plates spread and incubated with the bacterial strain constructed on the previous page. Above each Petri plate an aerial and close-up view of one typical colony found on that plate is portrayed, each exposed to a different substance. Which substance appears to be most mutagenic?



***Charles River Water is most mutagenic.***

c) How did you determine b)? ***More mutations → more sectors. One early mutation during the second division might give a pattern observed in the third plate. or if the mutation occurred after 3 divisions it would look like the second plate. In both the second and third plate there was only one mutational event. The last plate is the result of many many mutations.***

d) You observe that even when treated with the strongest mutagens that you can find, colonies have blue sectors. Why is this the case?

***If you add 3 copies of the dinucleotide, you get functional enzyme back. Likewise for contraction***

