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.

1
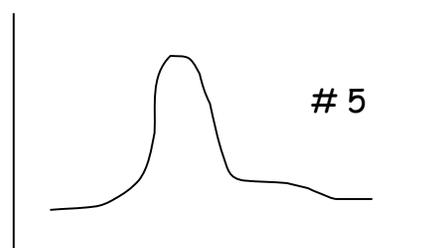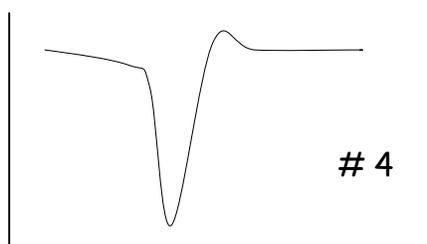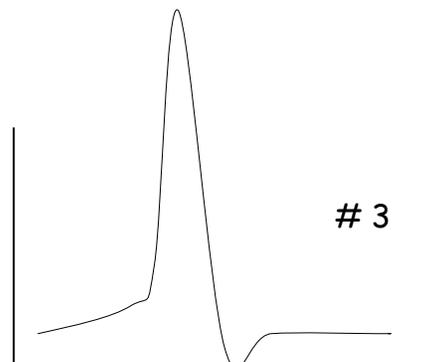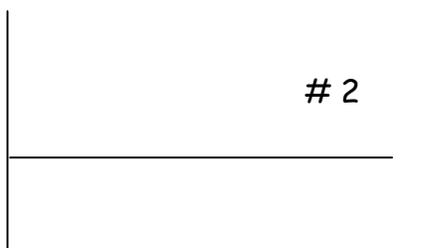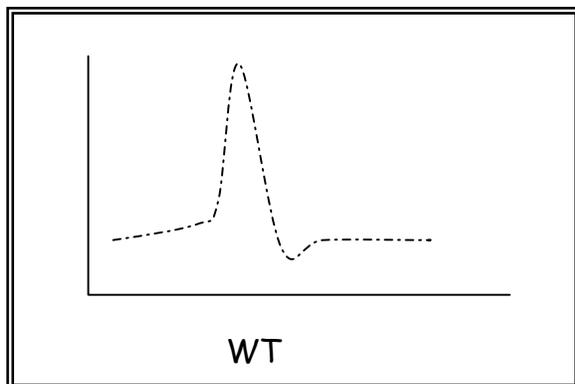
b) Acetylcholinesterase is an enzyme that degrades acetylcholine in normal individuals. Which of the following is true?

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 Ca2+ 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.)

2
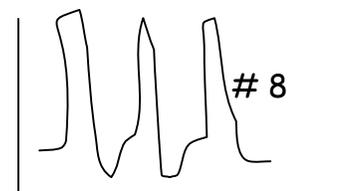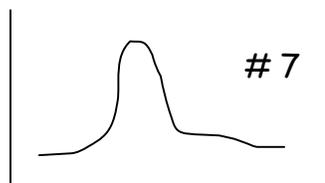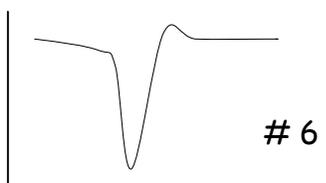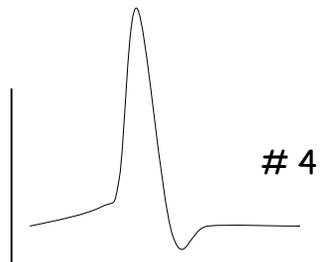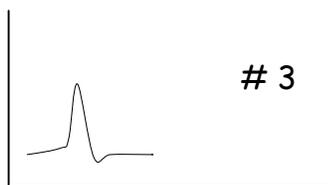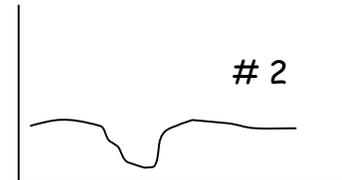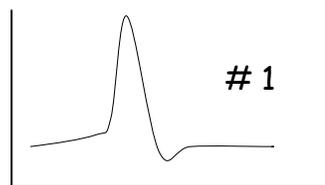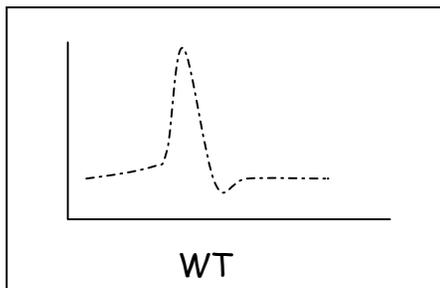
e) If you were to make a neuromuscular synapse between a Lambert-Eaton presynaptic neuron and a myasthenia gravis postsynaptic muscle, which of the following would you expect?

   i) Normal frequency of muscle action potentials

   ii) Decreased frequency of muscle action potentials

   iii) Increased frequency of muscle action potentials

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g) What is an ionotropic receptor?

h) What takes place at the axon hillock?

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?

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

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

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

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":

ionic flow in post synaptic cell

stimulus

before LTP ———

after LTP - - - - - -

Time in milliseconds
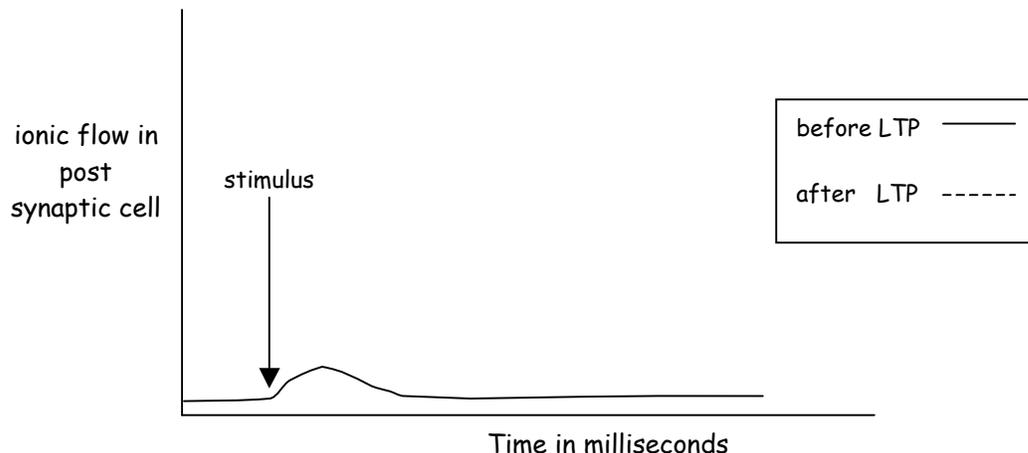
Question 3

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⁻ 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?

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

- 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?

- 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?

c) You decide to perform a different type of Ames test. This time you use the *lac Z* reporter gene, which encodes the ß -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 ß-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 ß -galactosidase non-functional.
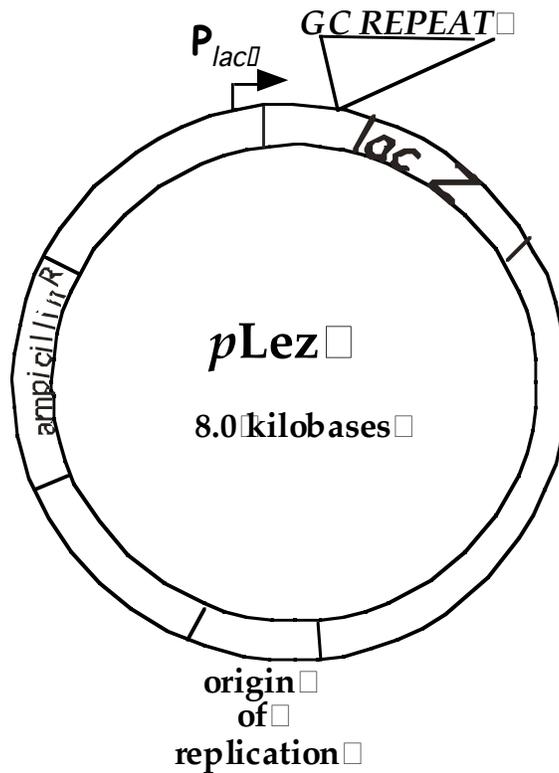
a) How does expansion or contraction of the dinucleotide repeat result in a nonfunctional ß-galactosidase enzyme?

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?

| X-gal medium | X-gal medium plus DIOXIN | X-gal medium plus Nitrous Acid | X-gal medium plus Charles River Water |
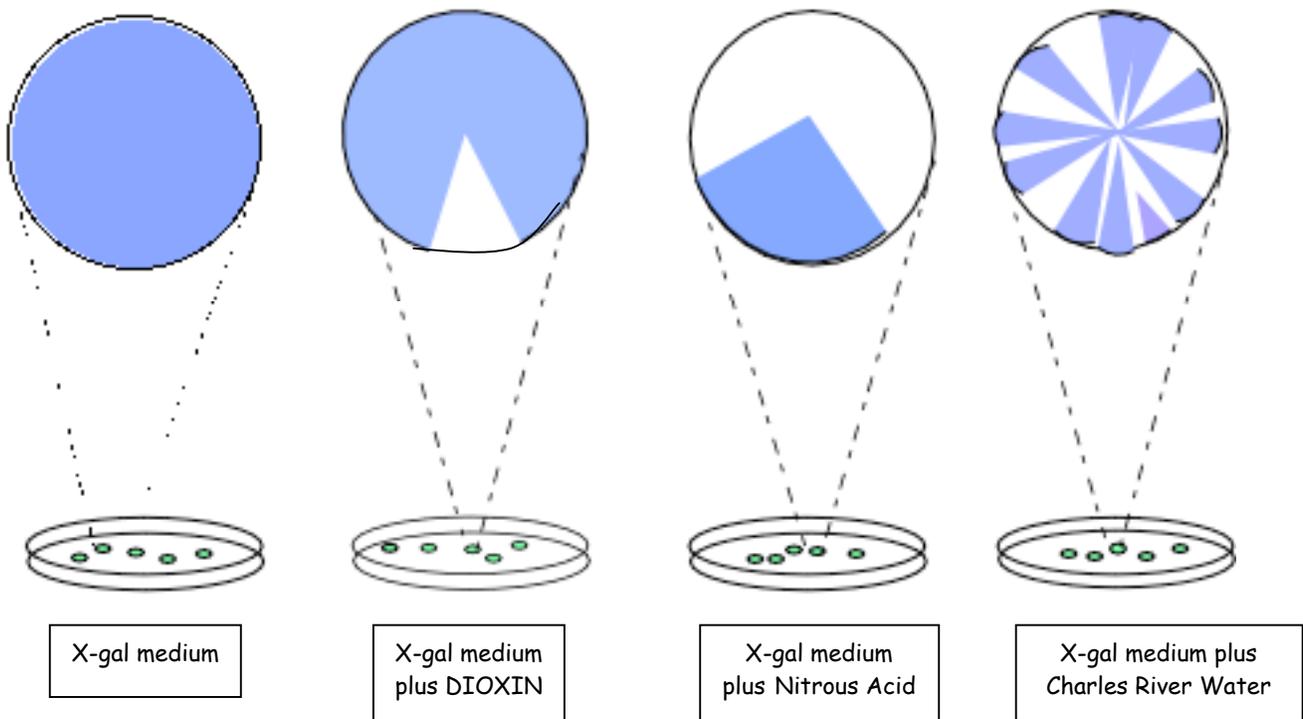
c) How did you determine b)?

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