1. [20 points] A single shock to a presynaptic nerve evokes an EPSP in a postsynaptic cell following a delay of 1 ms. The excitatory postsynaptic current through the AMPA receptors that generate the EPSP lasts for only 1 ms. EPSP time-constant (τ) = 10 ms. EPSP amplitude = 10 mV. Answer following and explain your reasoning:

a. [6 points] What happens during the delay between nerve stimulation and the generation of the EPSP?

The AP conducts from the site of stimulation to the presynaptic nerve terminal [not required as part of answer, but correct]

Voltage-gated Ca++ channels open during the presynaptic AP letting in Ca++. The Ca++ binds to the synaptic vesicle protein synaptotagmin. This induces fusion of the vesicle membrane with the plasma membrane and release of the transmitter into the extracellular space. The transmitter diffuses across the synaptic cleft and binds to and opens the transmitter-gated receptor/channels letting Na+ enter the cell and generate the EPSP.

b. [7 points] What happens to EPSP amplitude when external Ca++ is reduced from the normal level of 1.5 mM to 0.5 mM.

The probability of release drops because less Ca++ enters during the AP and so synaptotagmin is activated less. As a result, fewer vesicles fuse and the EPSP amplitude gets smaller. It could become possible to see single fusion events (one vesicle or quantum per stimulus) and some failures of release. [not required, but correct]

c. [7 points] What happens to EPSP amplitude if you return to normal external Ca++ and stimulate a burst of 5 presynaptic APs at 20 Hz (interval between APs = 50 ms)?

This interval is too long for one EPSP to summate with the next postsynaptically, but is short enough so that there remains residual Ca++ in the presynaptic terminal from one AP when the next one arrives. This residual Ca++ activates CaMKII, which phosphorylates synapsin, a vesicle protein that links reserve pool vesicles to actin and immobilizes them. The phosphorylation breaks the link to the actin permitting the vesicle to migrate to the docking site, leading to a rise in the number of docked vesicles and thus to the number of vesicles that are released. This is facilitation, which generates larger EPSPs.

During the train of 5 stimuli there will be progressive facilitation from AP2-AP5. [True but not necessary]
2. **[10 points]** You are Bob Zucker. In a flash of inspiration you invent a new kind of microscopy that enables you to visualize synaptic vesicles in the presynaptic nerve terminal. You load the presynaptic terminal with a “caged Ca++” (Ca++ inside a light-sensitive chelator). The vesicles are immobile. The postsynaptic cell is at resting potential. A small flash makes the vesicles dance around for 200 ms, but produces no postsynaptic change. A larger flash does the same thing, but also evokes a bunch of EPSPs in the postsynaptic cell. Explain.

The small flash elevates Ca++ high enough to activate CaMKII to phosphorylate synapsin and release vesicles from actin. The bigger flash raises Ca++ to level that binds to lower Ca++ affinity synaptotagmin and trigger fusion.

3. **[10 points]** A shock stimulates enough presynaptic axons to fire an action potential in a postsynaptic cell. The same shock given 5ms after the first does not fire the cell. Why? How does the neuron recover from this less excitable state? How do Ca++-activated K+ channels influence this recovery?

The second shock arrives during the relative refractory period when the delayed rectifier K+ channels are still open from the previous AP. During this time the membrane is hyperpolarized so that it is further from the AP threshold. In addition, the membrane resistance is lower because in addition to the usual leak channels which are always open the delayed rectifier channels are open, so that a larger postsynaptic current would be needed to produce the same size EPSP as in the first stimulus (this is as in $V = IR$, with a smaller $R$ the same $I$ will give a smaller $V$, a smaller EPSP). Thus to fire the cell will require a much bigger synaptic current.

Ca++-activated K+ channels remain open much longer after the AP than do delayed rectifier channels, so this effect persists for hundreds of ms.
4. **[10 points total]** A neuron is bathed in physiological solution (150 mM Na+, 1.5 mM K+, 2 mM Mg++, 1.5 mM Ca++, and 156.5 mM Cl-), has only leak K+ channels open, and has the usual ionic gradients (E_K = -120 mV, E_Cl = -70 mV, E_Na = +60 mV). The cell has AMPARs. It also has a muscarinic G-protein coupled receptor (mAChR), which activates K+ channels. Explain what happens to the membrane potential in the following situations and over what timescale these changes occur:

a. **[2.5 points]** You stimulate a presynaptic input that releases acetylcholine.

The membrane potential is already at E_K, since only leak K+ channels are open. Opening more K+ channels by activating mAChRs will not change the voltage.

b. **[2.5 points]** You stimulate a presynaptic input that releases glutamate.

[2 points] The membrane is depolarized in an EPSP because at the negative resting potential more Na+ enters than K+ leaves, with the voltage going toward V_{rev} = -30 mV, midway between E_Na and E_K.

[0.5 points] that rises for ~1 ms and decays in ~10 ms.

c. **[5 points]** You stimulate the glutamate and acetylcholine inputs at the same time.

A fast depolarizing EPSP (triggered after an ~1 ms delay and lasting for ~10 ms) is followed by a slow hyperpolarizing IPSP [name not needed]. The IPSP has a longer delay because of the second messenger steps that require diffusion (GPCR to Gbg to channel) and lasts longer, because it takes a while for the GTPase activity of the G-protein to turn the activation off.

5. **[10 points]** The Action Potential (AP) is initiated at the spike initiation zone (SIZ, in the initial segment of the axon) by membrane depolarization. It is then conducted down the axon. Explain why conduction is faster if the axon is myelinated.

Myelination is a glial wrap that adds many layers of tightly apposed membrane. It acts as an insulating (hydrophobic/greasy) wrap around the axon, which makes it harder for ions that exit from leak channels in the axon to reach the bulk extracellular space (thus increasing R_m) and it increases the distance between the polar head groups of the inner leaflet of the axon membrane and the outer leaflet facing the bulk, which is from the outermost glial membrane (thus decreasing C_m). As a result fewer ions leak out under the myelin (higher R_m) and fewer are needed to charge the membrane to the full depolarization of the AP (higher C_m), so that most of the charge travels axially leading to faster conduction.

[changes in constants w/ related equations, drawings of an axon, or equivalent circuits that illustrate these concepts are acceptable too]
6) [15 points] Explain why a 5 Hz burst of presynaptic action potentials produces long-term depression (LTD), while a 100 Hz burst produces long-term potentiation (LTP).

Both forms of plasticity require Ca++ to influx through NMDARs. Since NMDARs are blocked by external Mg++ for this to occur depolarization must be induced by activation of AMPARs. The 5Hz burst has intervals of 200 ms between EPSPs, too long a time for the EPSPs to summate and not much chance for postsynaptic internal Ca++ to accumulate. As a result at 5Hz only a little Ca++ enter through NMDARs and this is only enough to activate the high Ca++ affinity phosphatase calcineurin. At 100 Hz the EPSPs summate leading to more Mg++ unblock and postsynaptic Ca++ accumulates more resulting in enough Ca++ to activate the lower affinity kinase CaMKII.

When Ca++ is high both CaMKII and calcineurin are activated, but CaMKII “wins” because it is present in much larger amounts. CaMKII phosphorylates the same proteins as calcineurin dephosphorylates, resulting in opposite effects.

There are two target proteins to the phosp/dephosph:
   i) AMPARs: phosph increases their opening leading to bigger EPSPs
   ii) proteins that control the delivery and removal of AMPARs from the cells surface: phosph, increases the # on the surface, leading to bigger EPSPs

7) [10 points] A prolonged exposure of AMPA receptor to glutamate results in a transient current, that turns on and then turns off (a process called desensitization), even when glutamate is still there. Describe a molecular model for how glutamate binding opens the channel and why it desensitizes.

Each GluR subunit has a clamshell ligand binding domain. They are paired in a back-to-back orientation. When glu binds the clamshell closes. Because the backs of the clamshell are attached (“velcroed”) to each other the top “jaw” cannot move down to close so the lower one moves up. This pulls on a linker attached to the gate of the channel and pulls the gate open. This is a high tension situation. The tension is relieved when the back-to-back velcro attachment breaks. Even with the glu bound and the clamshell closed this break allows the clamshell to rotate “down” toward the membrane to relieve the tension on the linker and allow the gate to close. This is desensitization.
8) [15 points] Describe two mechanisms for ion selectivity, one for how channels select between cations (+) and anions (−). The second how K⁺ channels are so good at letting K⁺ through, but not Na⁺.

Selectivity for cations vs anions is achieved by placing counter-charges (amino acids of the opposite charge of the permeant ion) at the internal and external mouths of the pore. For example, a cation selective channel will have negatively charged glutamate or aspartate at the mouth of channel to repel anions and attract cations.

Selectivity for K⁺ over Na⁺ depends not on charge but on size. The selectivity filter contains a series of oxygens (backbone carbonyls) 4 from each of the 4 subunits in a row. The spacing between them perfectly mimics the geometry of oxygens in the first layer of waters in solution, so that K⁺ sits in a low energy (stable) state between them. Na⁺ is too small to satisfactorily interact with the Os from all 4 subunits and so cannot be stabilized in its dehydrated state. But if it tries to hold on to one of its waters of hydration it is too big to fit in the selectivity filter. Thus the bigger ion is preferred over the smaller one.