Problem Set  #4 Answers

1. A neuron’s intracellular calcium level can increase by:
   - flow of calcium into the cell through:
     o NMDA receptors
     o AMPA receptors that do not contain GluR2
     o Voltage-gated calcium channels
   - release of calcium from intracellular stores (initiated by activation of metabotropic glutamate receptors)

2. CaMKII cannot interact with calmodulin that is not bound to calcium. CaMKII is a good candidate molecular mechanism for learning and memory because it can remain active even after the event that initiated its activity has ended. More concretely, CaMKII can remain active through autophosphorylation (at Thr286) even when calcium-calmodulin, which originally activated CaMKII, is no longer bound to it. The ring structure of CaMKII may serve to facilitate the response of the molecule; once one CaMKII is phosphorylated, it’s neighboring CaMKIIs (within the same ring) are more readily phosphorylated.

3. CaMKII has two activation features—the activation due to the binding of calcium-calmodulin and the long-lasting activation due to the phosphorylation at Thr286. If you knockout CaMKII, you therefore knock out activity that is due to both of these features, and if you then observe an alteration of the physiology or behavior, you wouldn’t know which form of activation was the one underlying the change that you observe. In other words, the observed change could be due to the absence of calcium-calmodulin activation, the absence of Thr286-dependent activation, or both. An alternative to knocking out CaMKII is to create a Thr286A mutation. This mutation significantly reduces the level of LTP that can be induced (observed in a Thr286A mutant mouse).

4. To find out if a protein is phosphorylated, you can make an antibody that is specific to the phosphorylated form of that particular protein or you can load a cell with radioactive phosphate and test whether the protein becomes radioactive (indicating it was phosphorylated). To figure out the site of phosphorylation, you can first start with the observation that phosphorylation occurs at only three amino acids, only two of which are acted upon by CaMKII (Ser and Thr). You can then look for consensus sites on different proteins that are phosphorylated by CaMKII in order to determine candidate phosphorylation sites. You can section the GluR1 protein and find which section is phosphorylated by CaMKII, and then find the phosphorylation site in the phosphorylated section by creating mutations of the candidate sites and testing which site(s) is necessary for phosphorylation to occur. The CaMKII phosphorylation site on GluR1 was found to be S831.