NEU501
Fall 2009
Homework #2 - Due Thursday, Oct. 22nd
Please turn in to Sam Wang's mailbox in Schultz Lab

1. PREDICTIONS OF CEREBELLAR CIRCUIT TRACING. The lab for October 12-16th involves injection of pseudorabies (PRV)-152 Bartha into the granule cell layer of Crus II in the right cerebellar hemisphere. Answer the following questions using primary scientific literature on cerebellar connectivity and what you have learned about the virus. Please cite all references used.

a) **List the major regions** one might expect to be labeled if the virus were only transported retrogradely from granule cells to mossy fibers and regions upstream of mossy fibers. Be as specific as possible regarding the relative density of cells labeled and the time course of labeling. What types of behavior might these connections support?

b) **List the primary regions** one might expect to be labeled if the virus were only transported via climbing fibers. Give two paths the virus can go by to enter climbing fibers even if the injection is localized to the granule cell layer.

c) Labeling in several presynaptic regions could arise by retrograde transport of the virus through both the mossy fiber and climbing fiber pathway. **Design an experiment** that would rule out afferent labeling through the climbing fiber pathway.

2. SPECIFICITY OF TRANSSYNAPTIC LABELING. Various strains of PRV have found broad use for both retrograde and anterograde tracing of synaptic pathways.

a) **Critically evaluate** the extent to which you expect the labeling mechanism to be synapse-specific. Are all retrogradely expressing neurons synaptically connected to the site of initial infection?

b) **Design a control experiment** to test the synapse-specificity of PRV by adapting the GRASP strategy for use in mammalian cells.

Feinberg EH, Vanhoven MK, Bendesky A, Wang G, Fetter RD, Shen K, Bargmann CI (2008) GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. *Neuron,* 57:353-363. http://www.ncbi.nlm.nih.gov/pubmed/18255029

3. GENETICALLY ENCODABLE CALCIUM INDICATORS (GECIs). No GECI has yet matched the brightness and speed of synthetic indicators such as Oregon Green BAPTA-1. But some GECIs may still be well enough matched to the properties of some neurons (and other cell types) to provide information on temporal activity patterns.

For the following questions assume that a GECI-expressing neuron has a resting calcium concentration of 70 nM and, in response to an action potential, generates a calcium transient:

- in the cell body that is 3 nM in amplitude with a $t_{1/2}$ of 1 s, and
- in the dendrites that is 30 nM in amplitude with a $t_{1/2}$ of 0.1 s.

Assume that action potentials in rapid succession still generate equal influxes of calcium.

a) **Plot the somatic and dendritic calcium signals** that would arise from:

i) a neuron that is known to fire one AP at a time at intervals of >1 s.

ii) a neuron that fires bursts of 2-5 APs at ~100 Hz, with ~1 s between bursts (similar to hippocampal place cells).

iii) a neuron that fires between 10-50 Hz with rate modulations on a time scale of 0.1-1 s (similar to many interneurons).

b) **Plot the expected fluorescence signals** in the neuron types described in (a) using **G-CaMP2** (a circularly permuted GFP-based GECI), **YC3.60** (FRET/CaM-based), and **TN-XL** (FRET/troponin-based). Assume that each protein has the signaling properties found in presynaptic motoneuron boutons as described in Hendel et al. (2008). In each case make an argument for which indicator would be optimal for extracting activity information, and the quality of the information extracted.

The following references should be useful:

Hendel T, Mank M, Schnell B, Griesbeck O, Borst A, Reiff DF (2008) Fluorescence changes of genetic calcium indicators and OGB-1 correlated with neural activity and calcium in vivo and in vitro. *Journal of Neuroscience,* 28:7399-7411. http://www.ncbi.nlm.nih.gov/pubmed/18632944

Hires SA, Tian L, Looger LL (2008) Reporting neural activity with genetically encoded calcium indicators. *Brain Cell Biology*, 36:69-86. http://www.ncbi.nlm.nih.gov/pubmed/18941901

c) **Describe the expected distortions** in the signal if expression is so high that the GECIs bind to a large fraction of entering calcium ions.