Bi 250b Systems Neuroscience
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Application of molecular and genetic techniques to the study of systems neuroscience
We wish to use genetic/molecular tools to:
- activate neurons or genes
- silence neurons or genes
- trace circuitry/define connections
- detect neuronal activity

What assay?
- behavior
- recordings from/imaging single neurons
- recordings from/imaging populations of neurons
Expression systems:
Flies: (GAL4/UAS, split GAL4, LexA/VP16)
Worms: promoter fusions
Fish: T7, promoter fusions
Mice: Cre/Lox
Rats/other mammals: viral injection of promoter fusion (target non-dividing cells)
Neuronal Activation

ChannelRhodopsin2:
- evoke single APs with light (~400nm)
- Very precise spike timing (<5ms jitter) and reproducible firing in long trains
- requires high light intensity, must use fiber optic cables and lasers (not 2PE) to target neurons in vivo
- must add retinal for insect preps
- Using light to stimulate can interfere with imaging neuronal activity
- Ability to activate neuron depends on level of expression of ChR2

In Drosophila larvae
**LiGluR (light-activated glutamate receptor)**

![Diagram of LiGluR mechanism]

*Szobota et al. Neuron 2007*

- **A** illustrates the receptor structure and how it responds to light.
- **B** shows the voltage response at different wavelengths.
- **C** demonstrates the effect of depolarization on the response.

Amplitude of response depends on depolarization wavelength.

Effective current clamp with light. No desensitization. Stays on while light is on.
Silencing Neurons

(a) Blue light activates ChR2, causing a influx of K⁺ and efflux of Na⁺.

(b) Graph showing wavelength vs. activation for ChR2 and NpHR.

(c) Graph showing neuronal activity with blue light stimulation.
Tracing Circuitry / Defining Connections

rabies virus - moves retrogradely across the synapse:
- may not label all connections
- may inappropriately target non-synaptically connected neurons

Wickersham et al. Neuron 2007
Brainbow

lox variants that allow recombination with only identical lox sequences

Livet et al. Nature 2007
Genetically-Encoded (GE) Sensors:
• why use GE sensors?
• what does the sensor do to the cell?
• what is being imaged?

Advantages of genetically encoded sensors:
• No injection
• Similar expression levels across animals
• Can control expression levels with copy number or by choosing different promoters and enhancers
• Express in the same pattern of cells across animals
• Target neurons of interest reliably

Imaging Ca++ changes:
• FRET-based sensors
• Circularly Permutated GFP
Currently, the best GE calcium sensor is GCaMP1.6:
- high signal to noise ratio, although kinetics are slow
- problems with photobleaching
- probe does not fold properly at 37°C
- affects normal calmodulin function? (can use troponin C instead)
- local calcium concentration may be altered
- nonlinear relationship between Ca++ binding and AP
- only detects neuronal activity at ~20Hz or higher
Projection Neuron-GAL4; UAS-GCaMP

Wang et al. 2003 Cell
Other options

voltage sensors

synapto-pHfluorin
Tuning to odors is narrow at the level of the receptor neurons and broad at the level of the second-order projection neurons.

Hypothesis: There are excitatory lateral connections between projection neurons. HOW TO TEST THIS HYPOTHESIS?
What is the total impact of all lateral synaptic input to a PN?
Does each ORN type provide indirect input to many glomeruli? 2 methods for selective stimulation of only one ORN type.