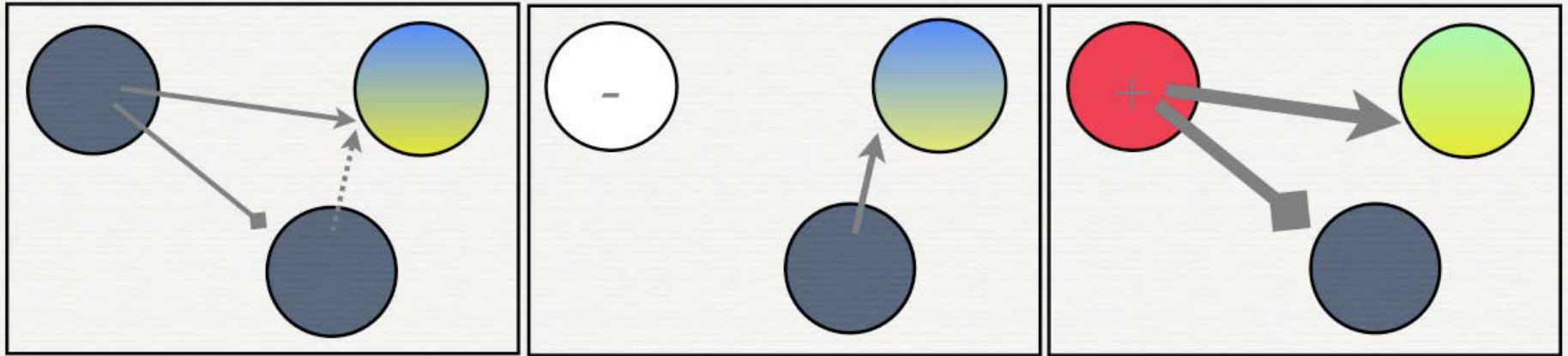


Bi 250b Systems Neuroscience
Mala Murthy, PhD
Laurent Lab

*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:

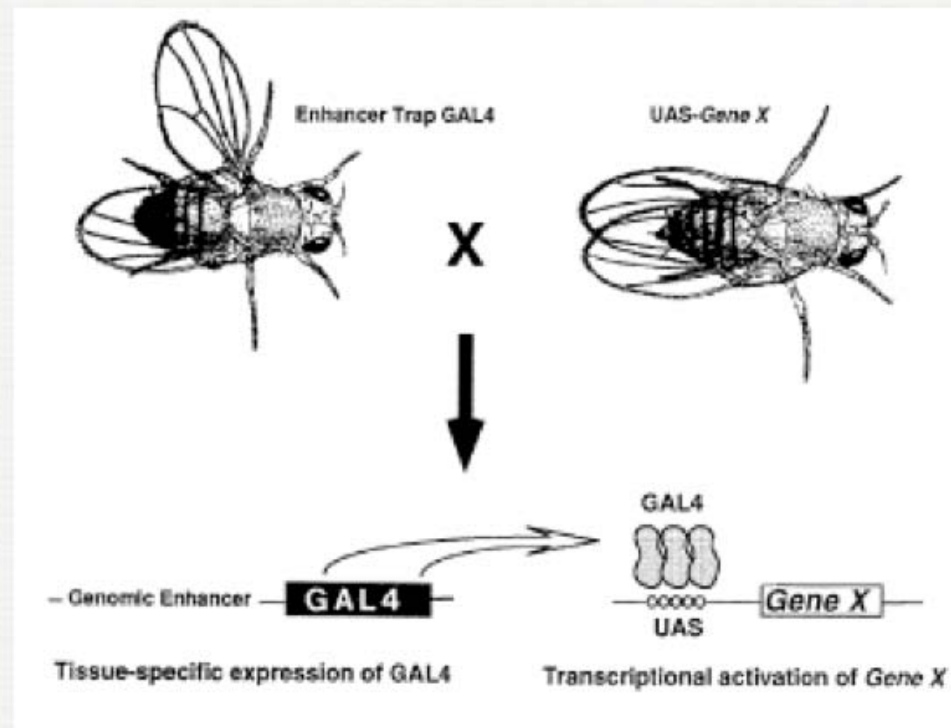
Flies: (GAL4/UAS, split GAL4, LexA/VP16)

Worms: promoter fusions

Fish: T7, promoter fusions

Mice: Cre/Lox

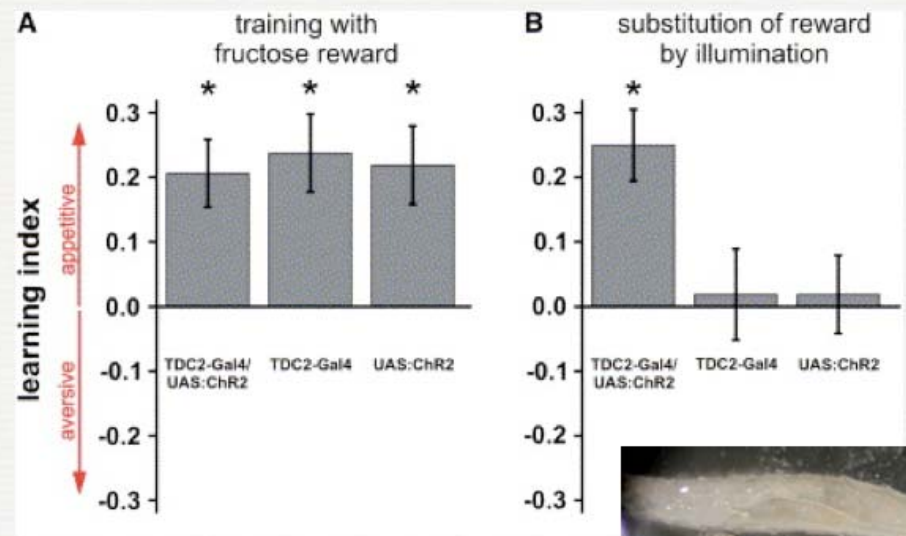
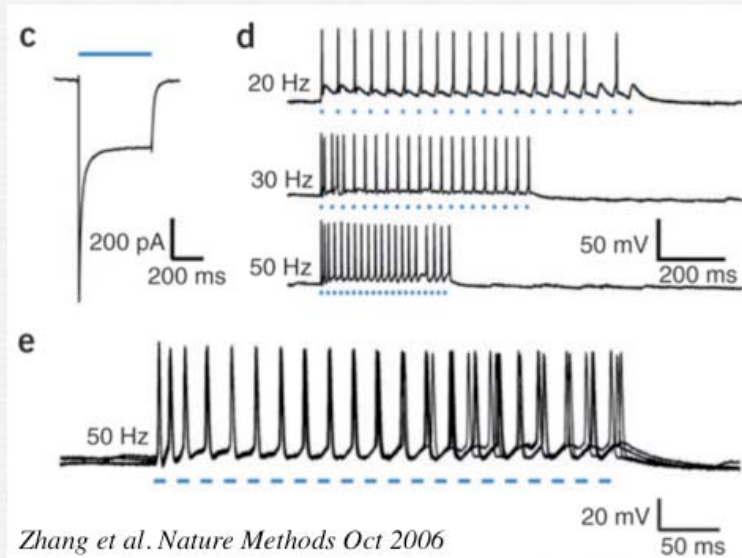
Rats/other mammals: viral injection of promoter fusion (target non-dividing cells)



Neuronal Activation

ChannelRhodopsin2:

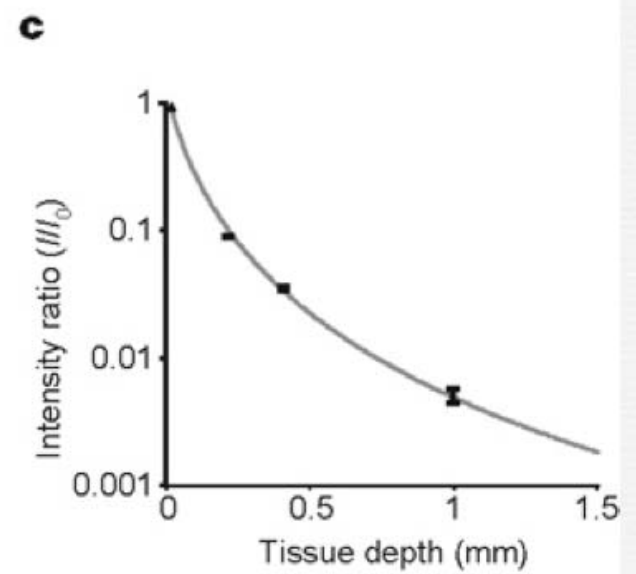
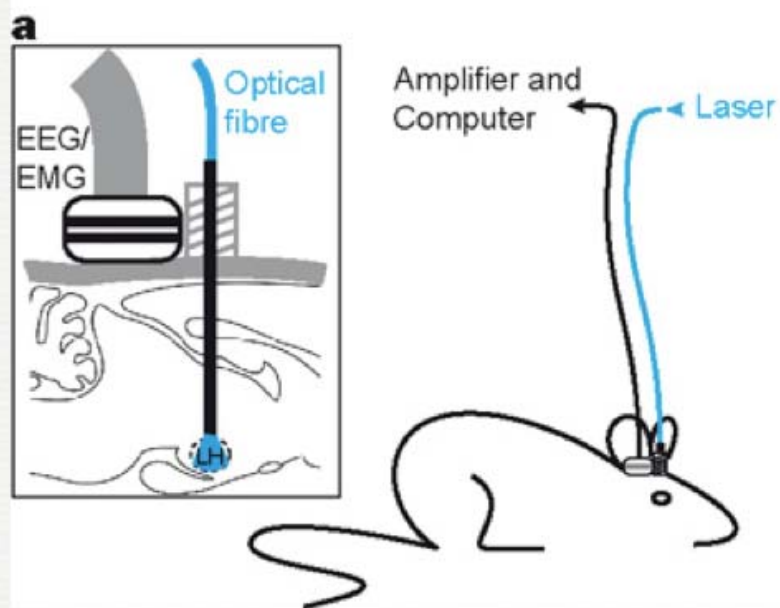
- o evoke single APs with light (~400nm)
- o Very precise spike timing (<5ms jitter) and reproducible firing in long trains
- o requires high light intensity, must use fiber optic cables and lasers (not 2PE) to target neurons in vivo
- o must add retinal for insect preps
- o Using light to stimulate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



In *Drosophila* larvae

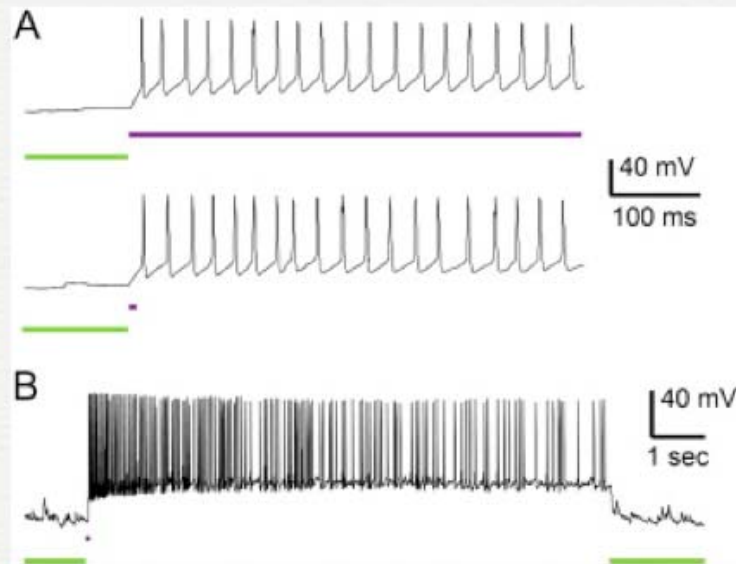
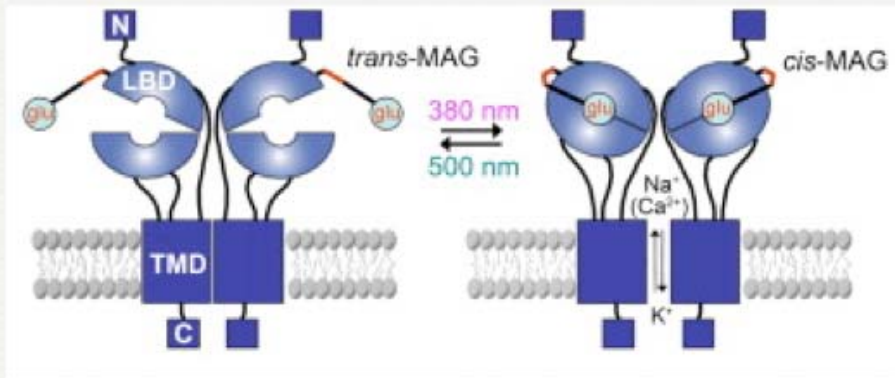
Schroll et al. Curr Biol 2007



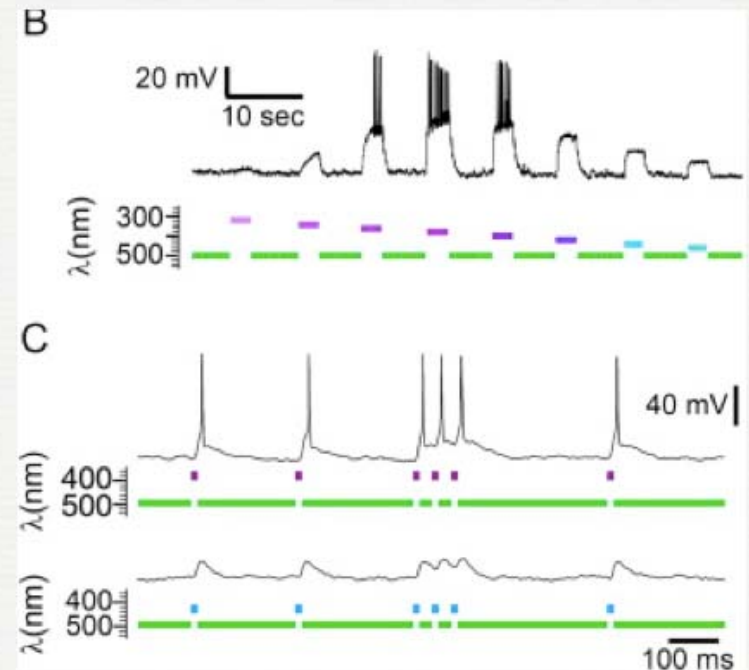


Adamantidis et al. Nature 2007

LiGluR (light-activated glutamate receptor)



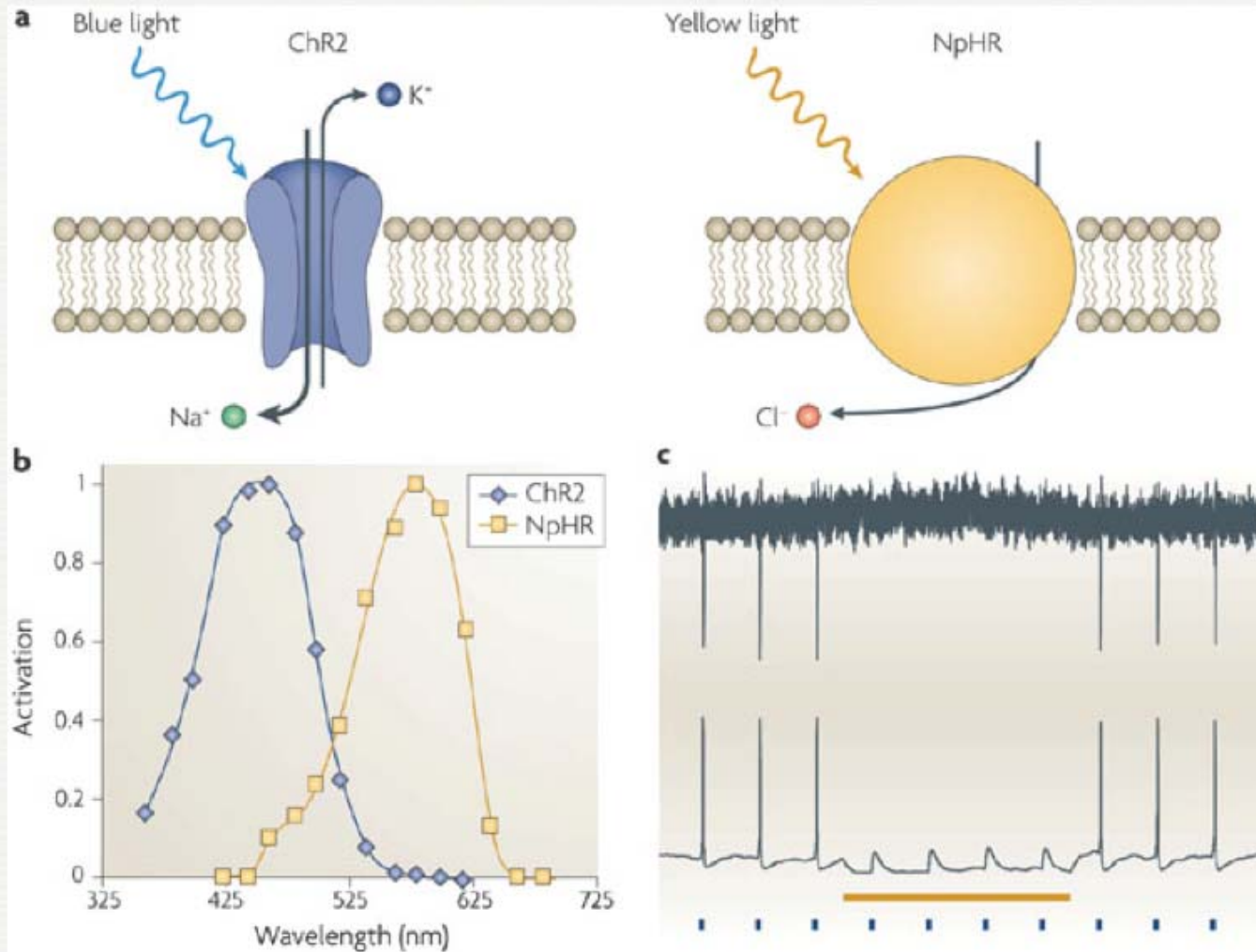
Szobota et al. Neuron 2007



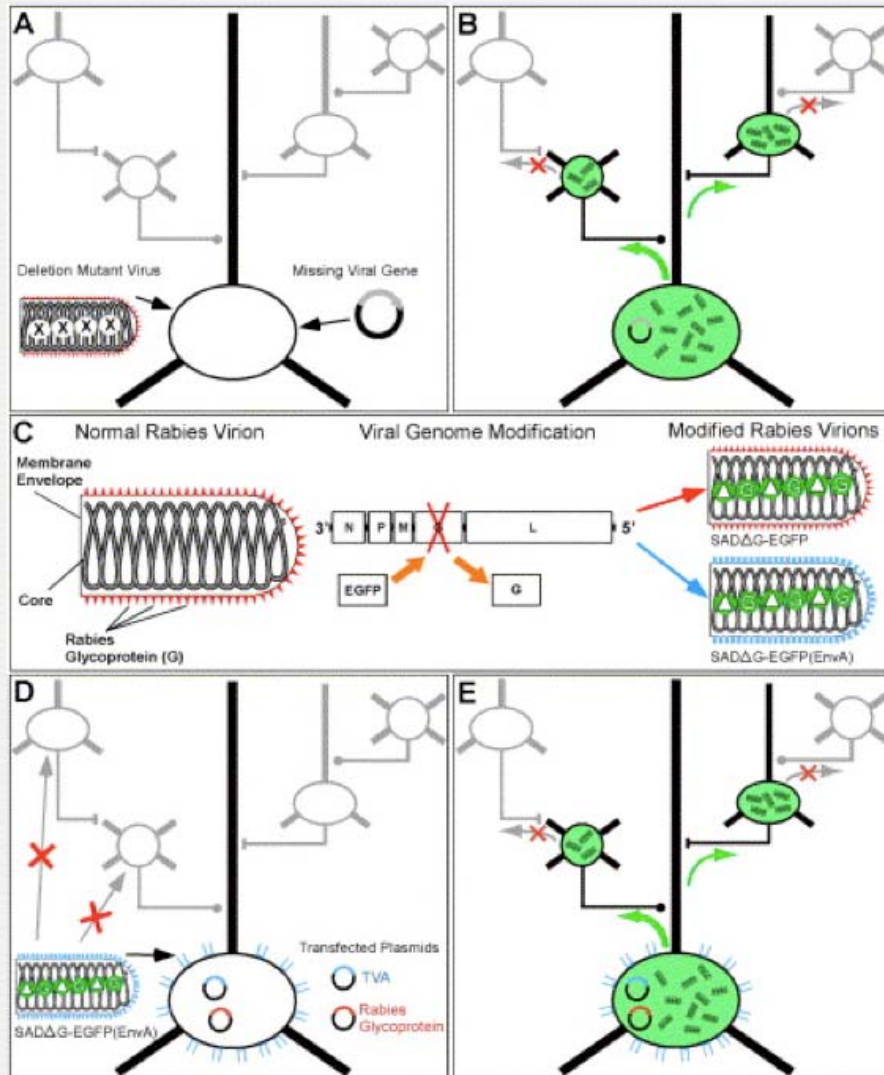
amplitude of response depends on
depolarization wavelength

effective current clamp with light.
no desensitization.
stays on while light is on.

Silencing Neurons



Tracing Circuitry / Defining Connections



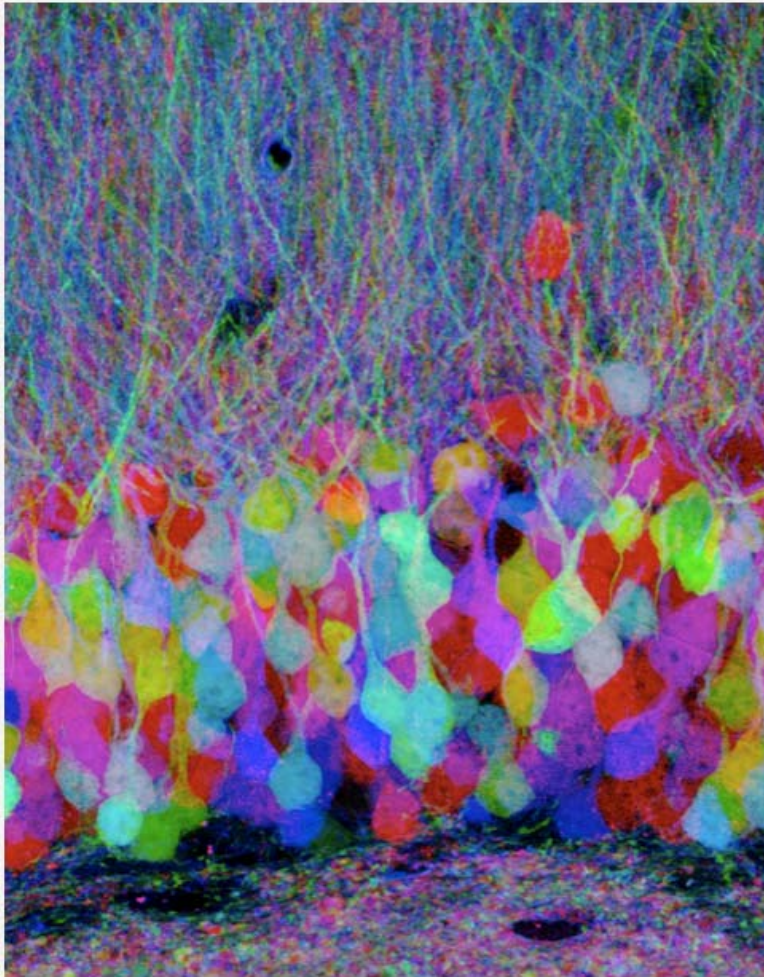
rabies virus - moves retrogradely across the synapse:

- may not label all connections
- may inappropriately target non-synaptically connected neurons

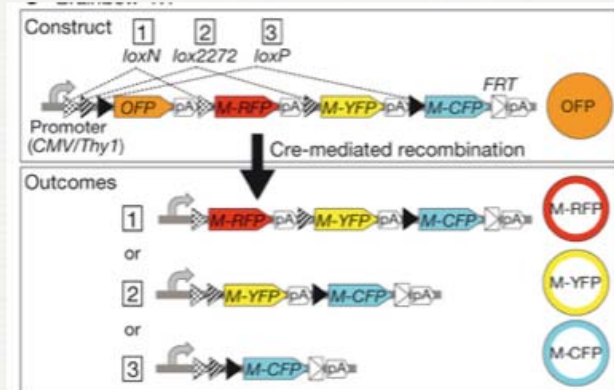
Wickersham et al. Neuron 2007

Brainbow

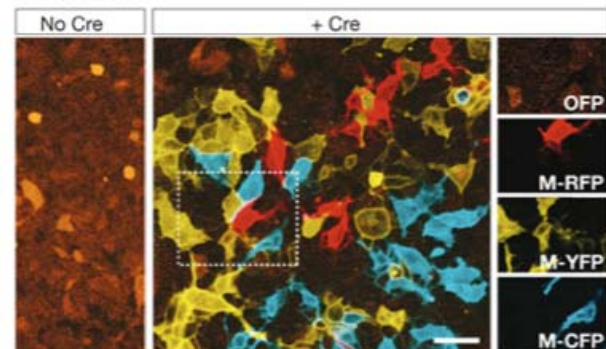
lox variants that allow recombination with only identical lox sequences



Livet et al. Nature 2007



d Test in vitro

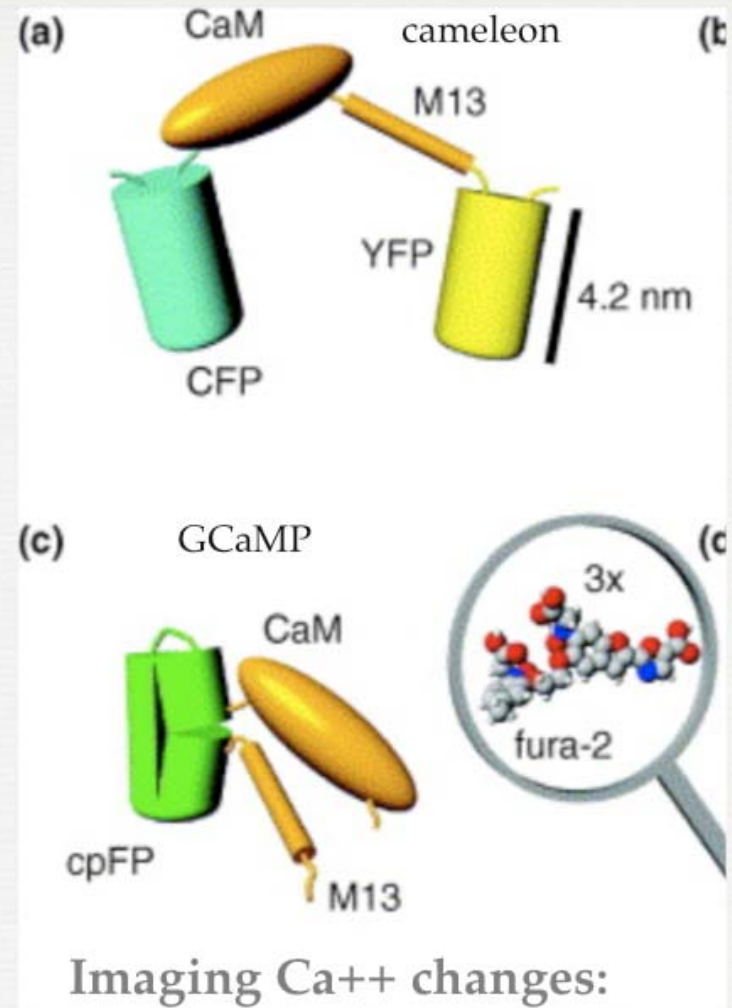


Genetically-Encoded (GE) Sensors:

- 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*

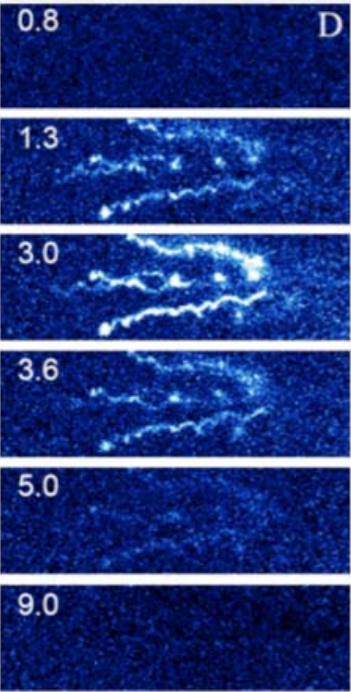


Imaging Ca^{++} changes:

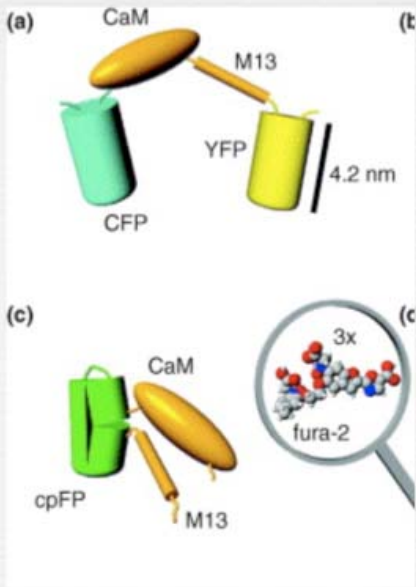
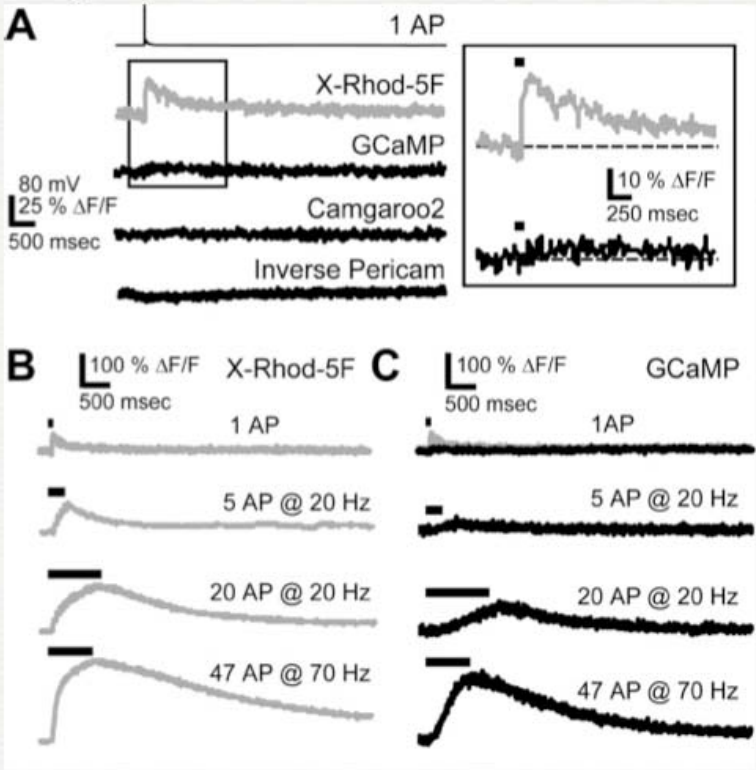
- FRET-based sensors
- Circularly Permutated GFP

Currently, the best GE calcium sensor is GCaMP1.6:

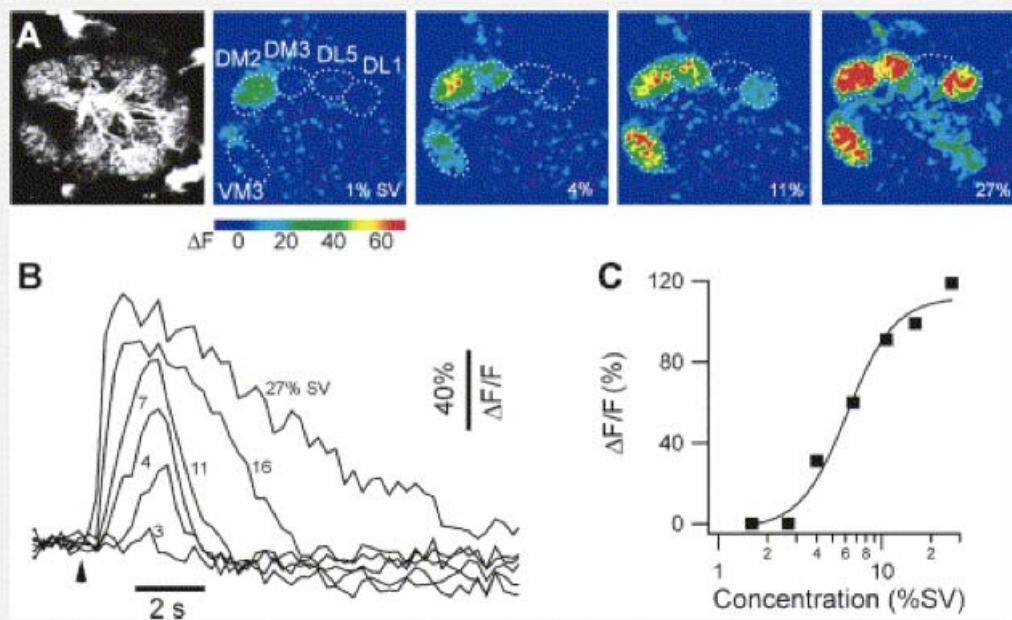
- high signal to noise ratio, although kinetics are slow
- problems with photobleaching
- probe does not fold properly at 37C
- affects normal calmodulin function? (can use troponin C instead)
- local calcium concentration may be altered
- nonlinear relationship between Ca^{++} binding and AP
- only detects neuronal activity at ~20Hz or higher



Drosophila NMJ

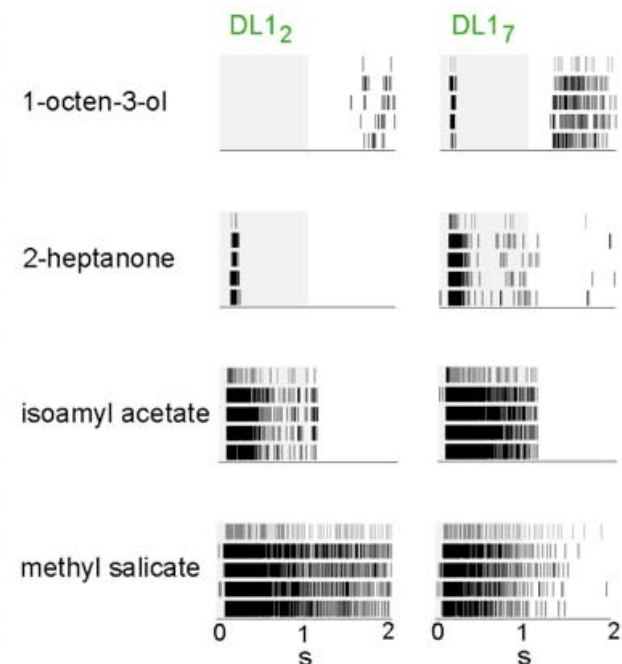


transfected cortical slices



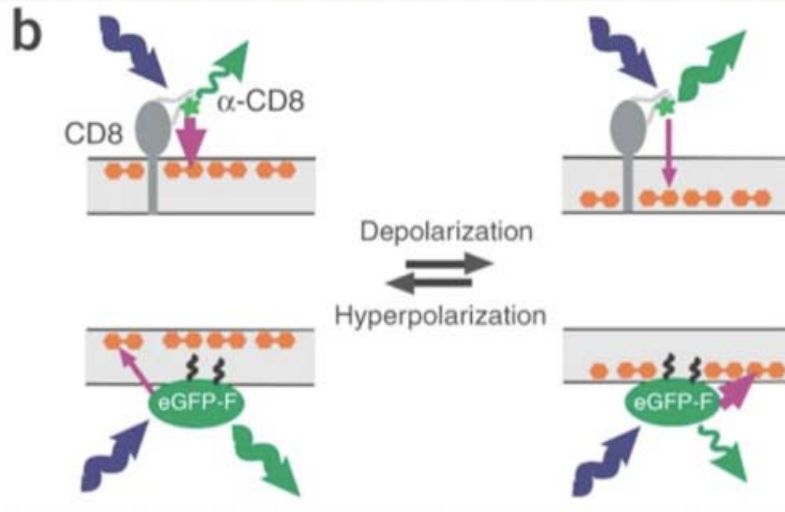
Projection Neuron-GAL4; UAS-GCaMP

Wang et al. 2003 Cell

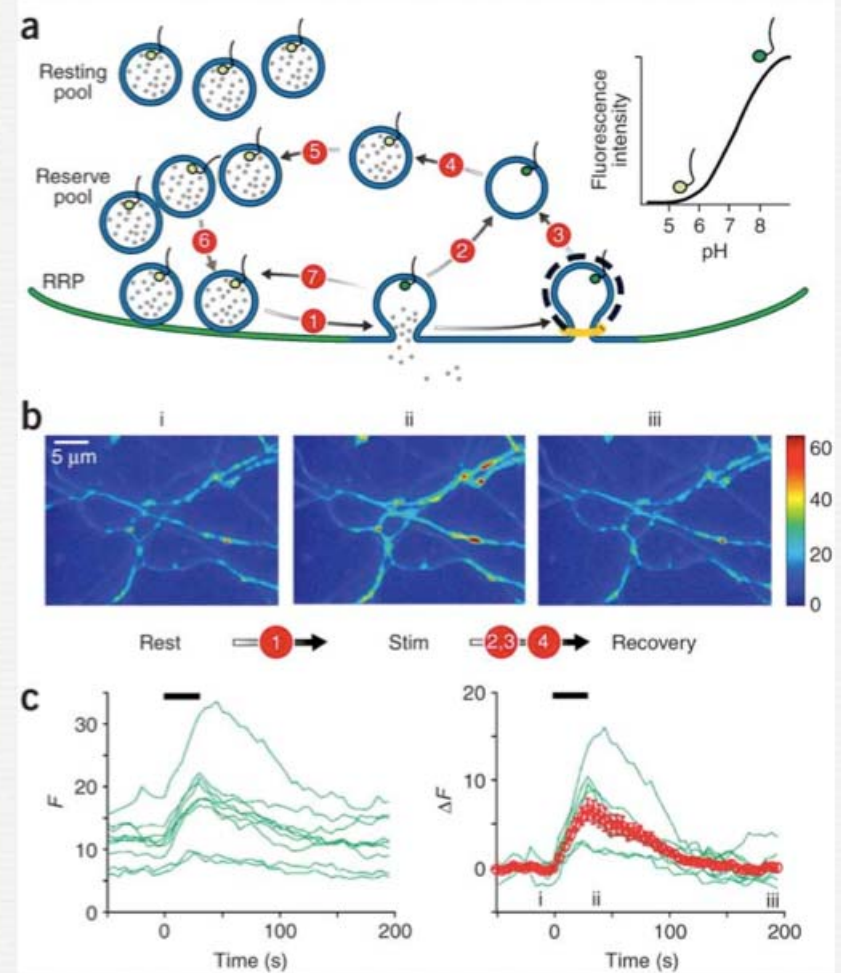


Other options

voltage sensors



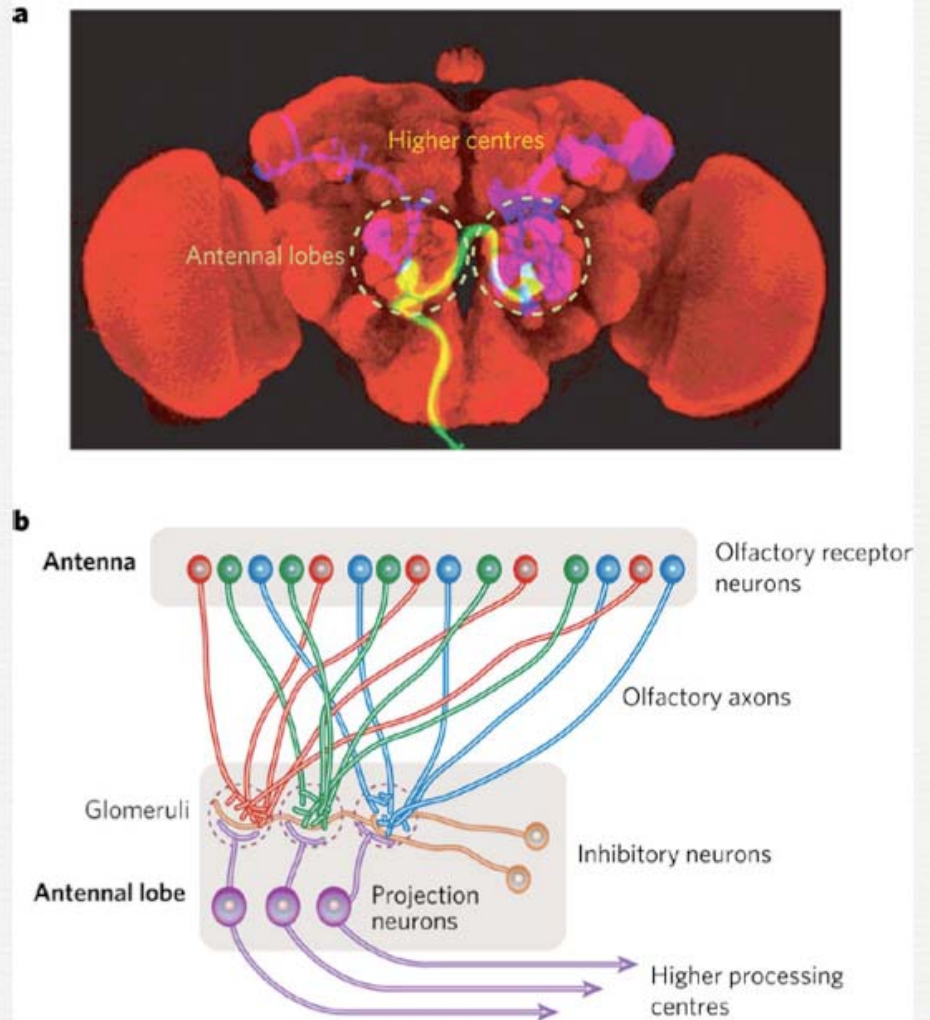
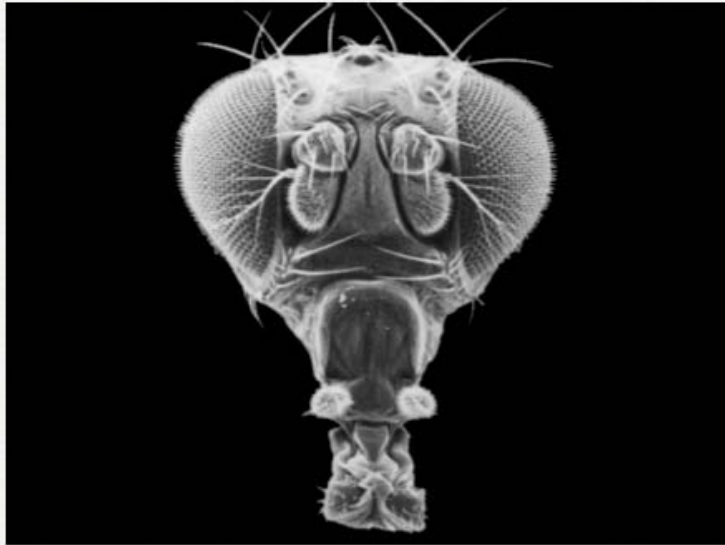
synapto-pHluorin

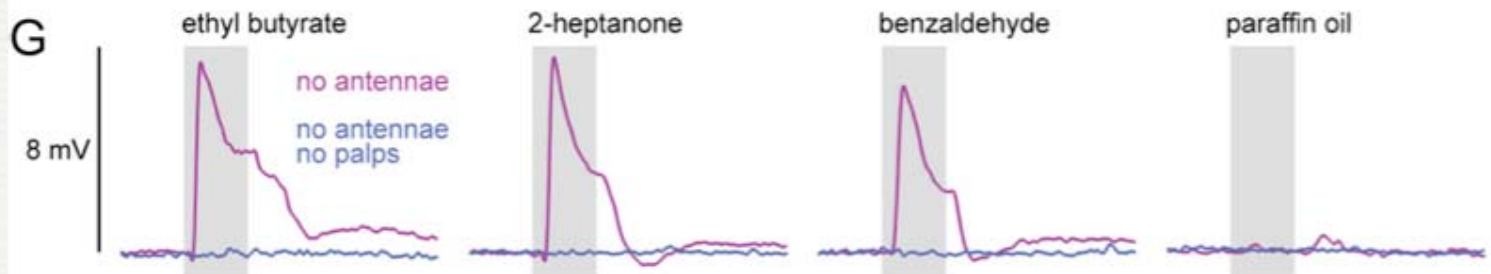
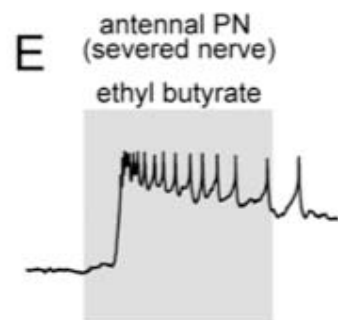
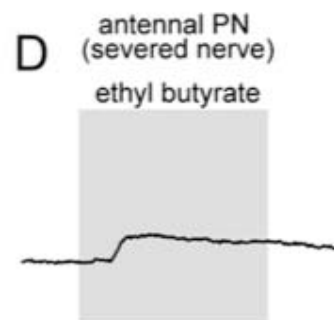
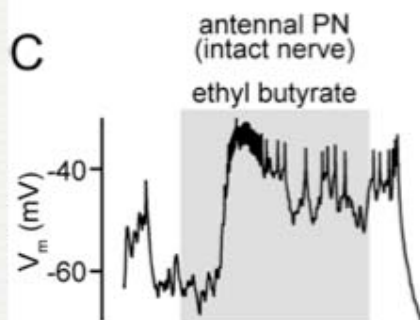
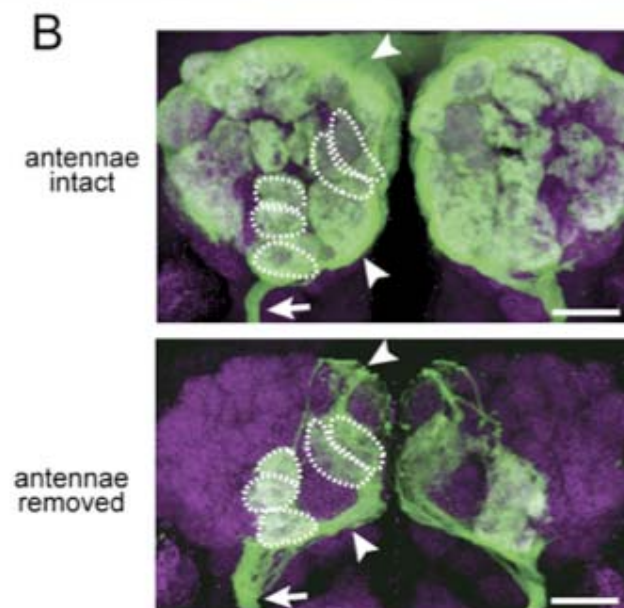
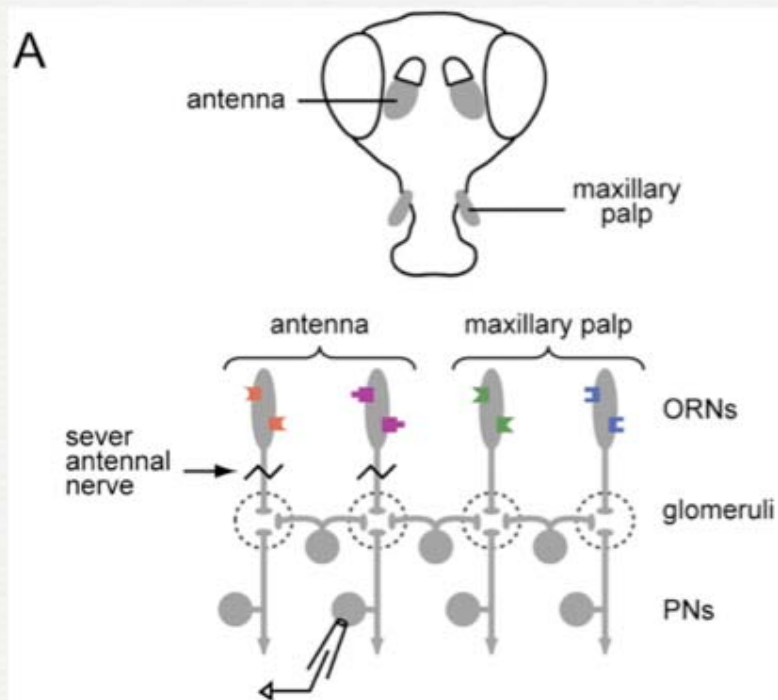


Olsen et al.: Excitatory Interactions between Olfactory Processing Channels in the Drosophila Antennal Lobe

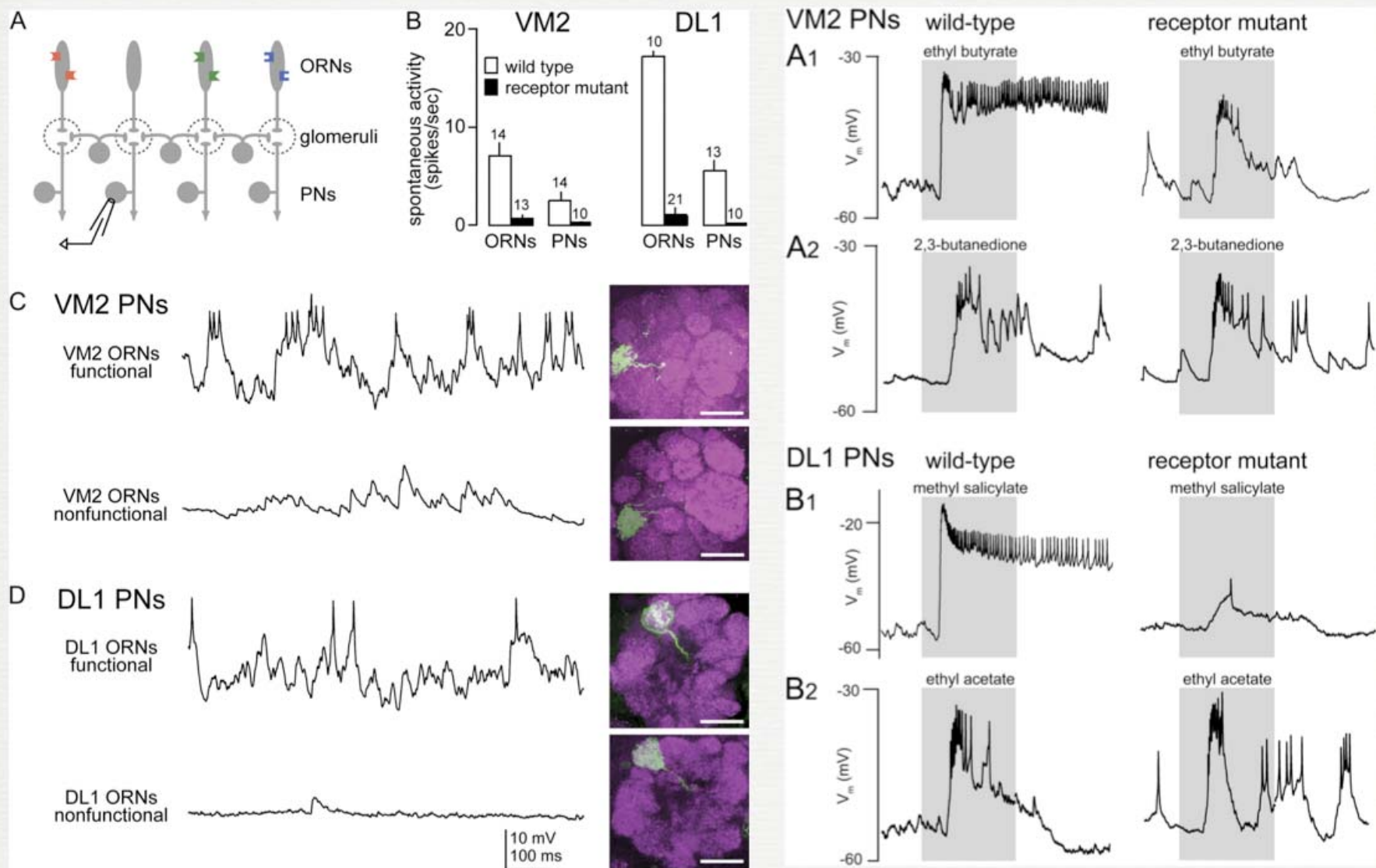
Tuning to odors is narrow at the level of the receptor neurons and broad at the level of the second-order projection neurons.

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.

