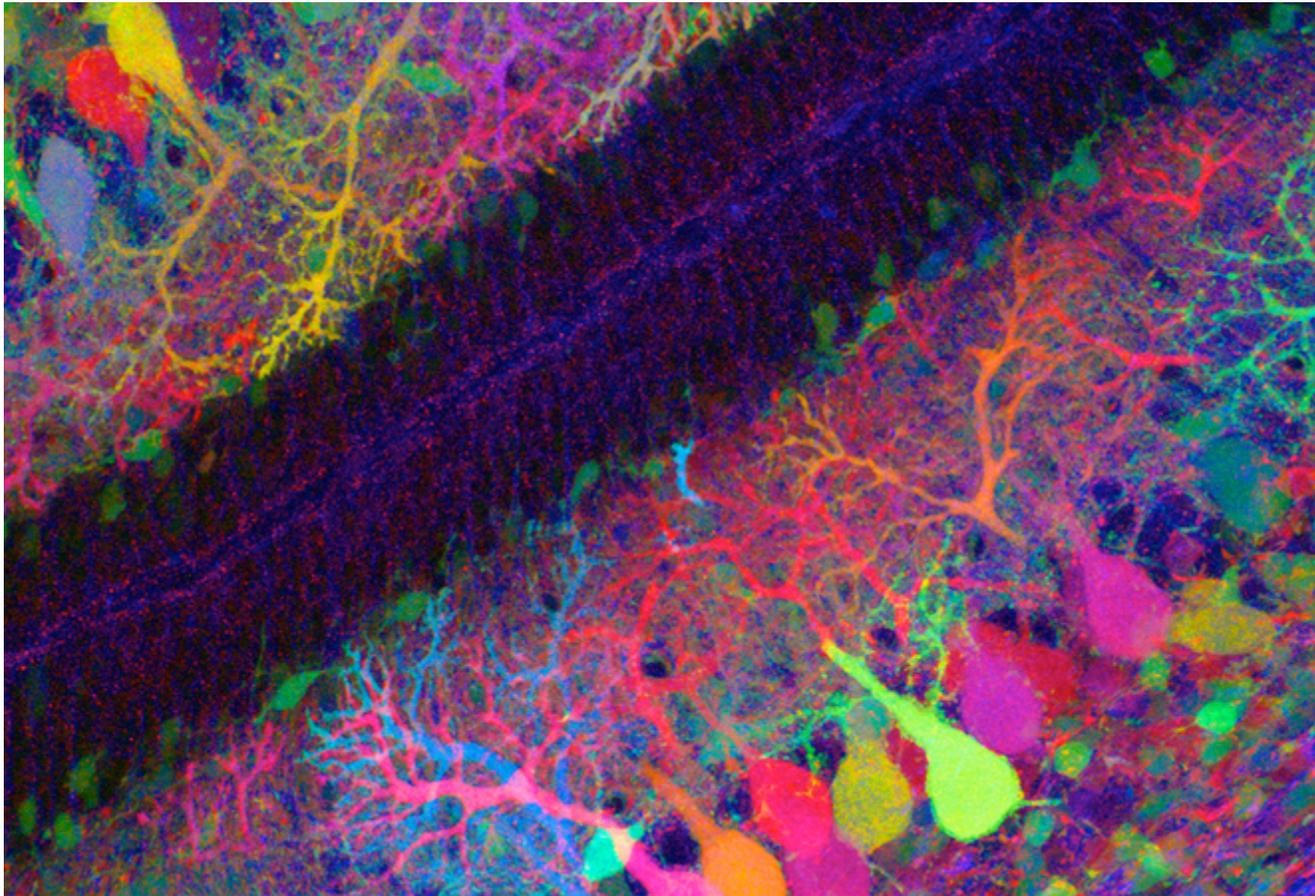


Optogenetics Toolbox

Developmental Neurobiology
9/11/13



Family Weissman, Harvard University

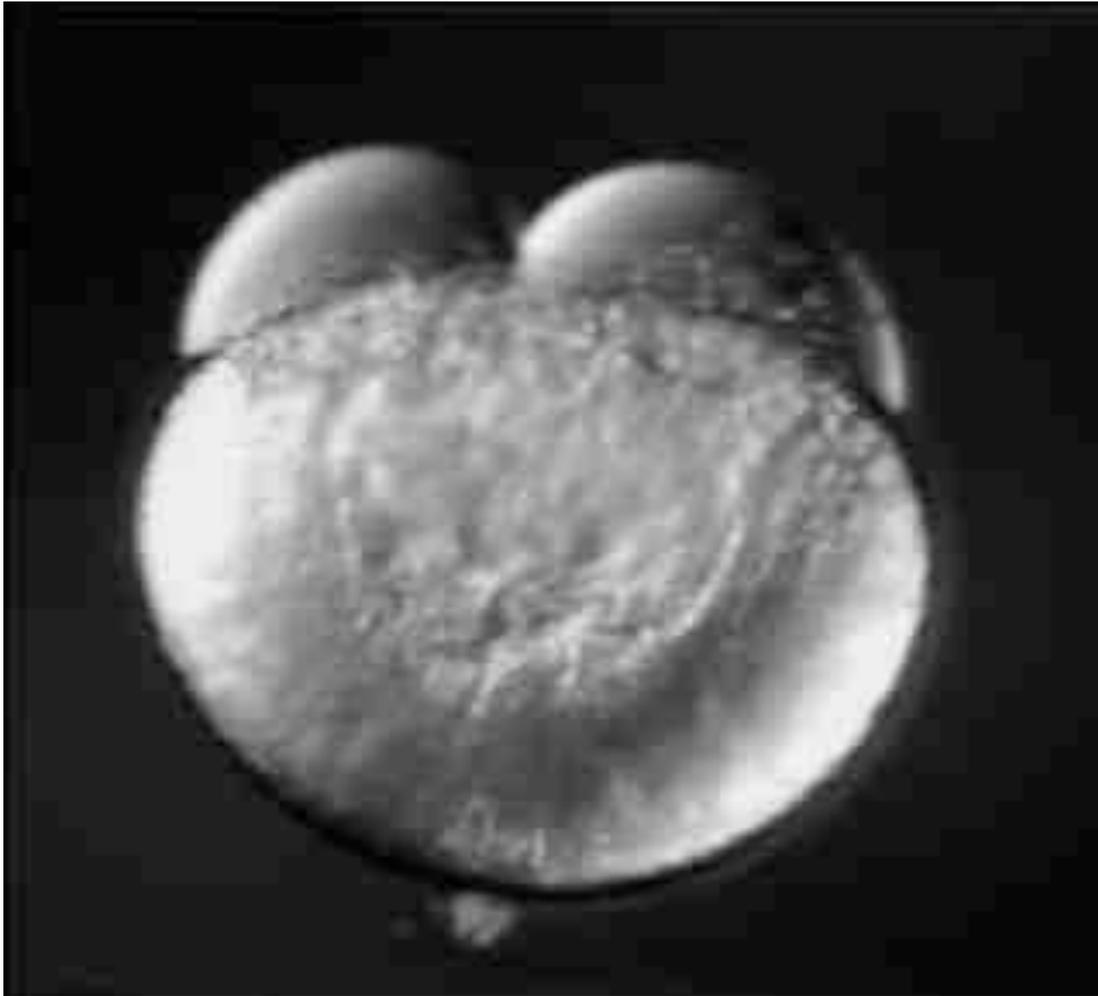
“The scientific mind does not so much provide the right answers as ask the right questions.” - Claude Levi-Strauss, anthropologist

Goals for today:

- Introduce optogenetics
- Choosing the right tool for the right job
- There’s always a catch



Let's just take a look ...



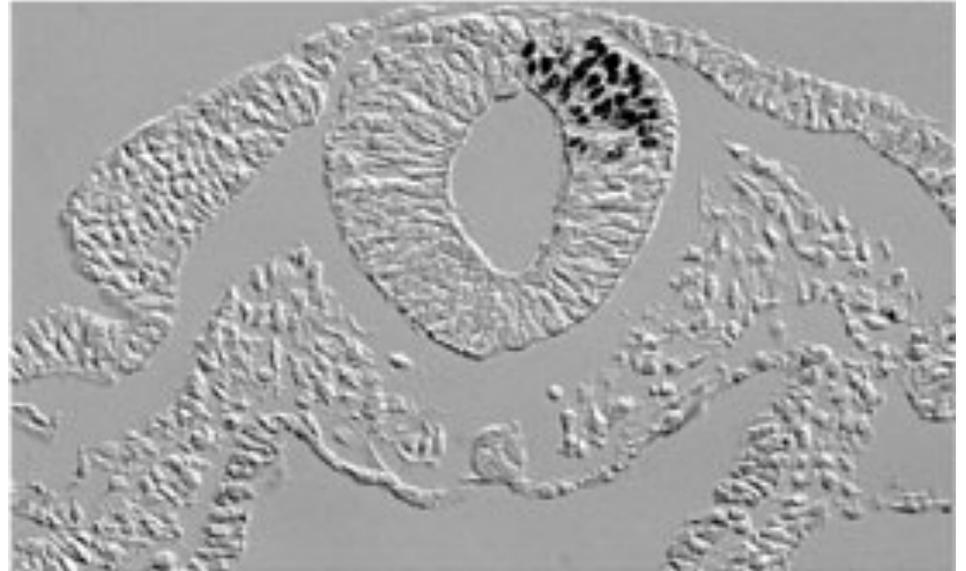
Pretty impressive, but also pretty limited.

Source: Joe Fetcho

“Paint It” Revisited

Topic: Cell Fate

Q: Where do cells originally located at site X end up in the developing embryo?

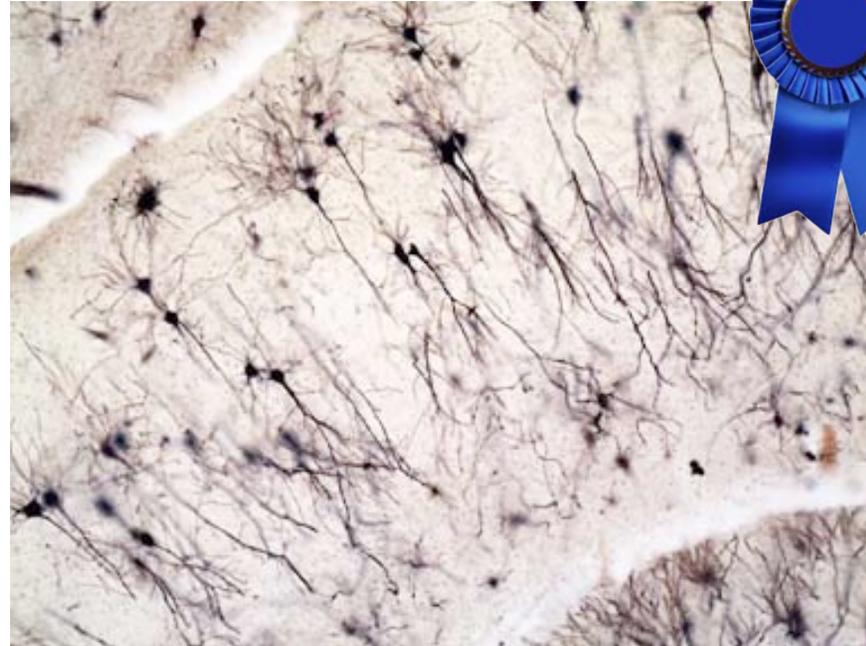


Schoenwolf GC (2001) *Nature Reviews Neuroscience*

“Paint It” Revisited

Topic: Cell Morphology

Q: Do neurons in area X share particular morphological features?

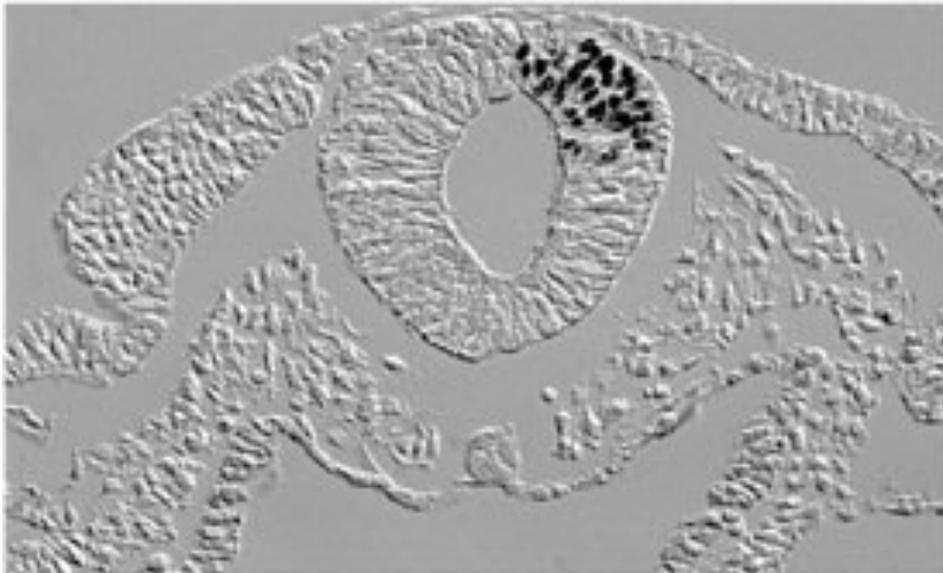


Source: Neurodigitech

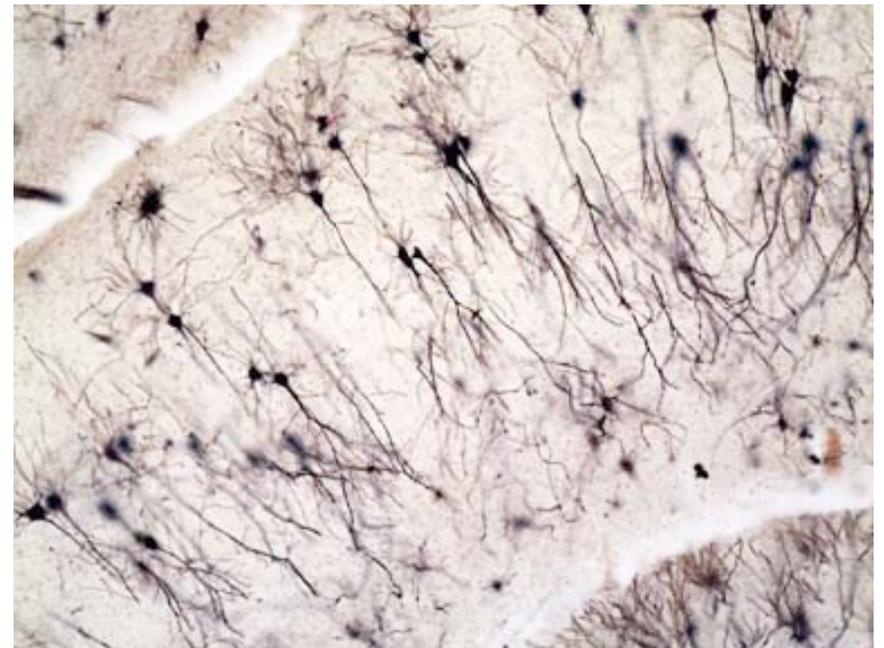
“Paint It” Revisited

Revolutionary discoveries ... but what's the catch?

Staining requires serendipity and a static specimen.



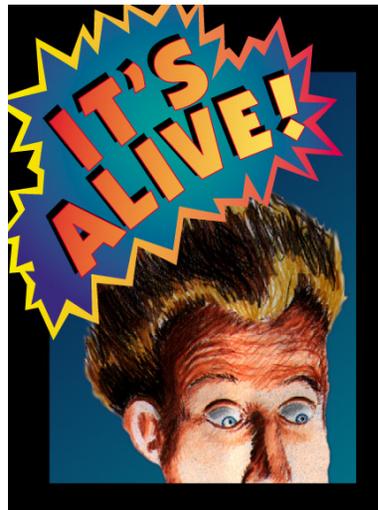
Schoenwolf GC (2001) *Nature Reviews Neuroscience*



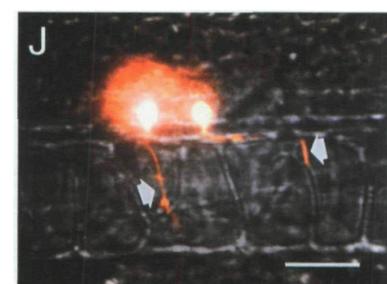
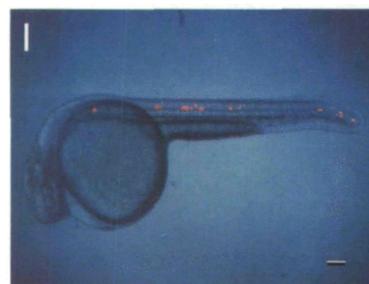
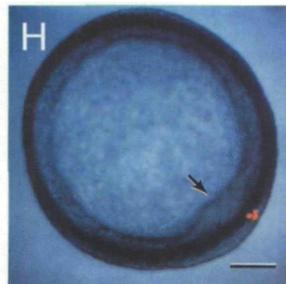
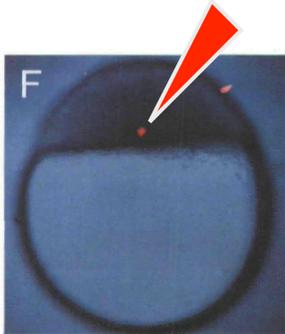
Source: Neurodigitech

“Paint It” Revisited

Fluorescent dyes - what’s the big deal?



Fluorescein



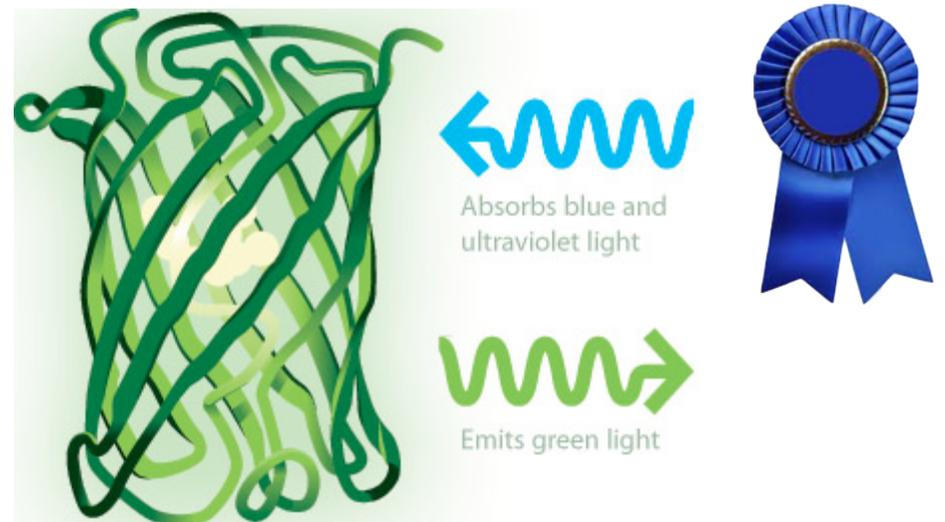
Kimmel CB et al. (1990) *Development*

“Paint It” Revisited

Green fluorescent protein,
discovered in jellyfish

Other fluorescent dyes
(ex: fluorescein) are
synthetic

What’s the big deal?

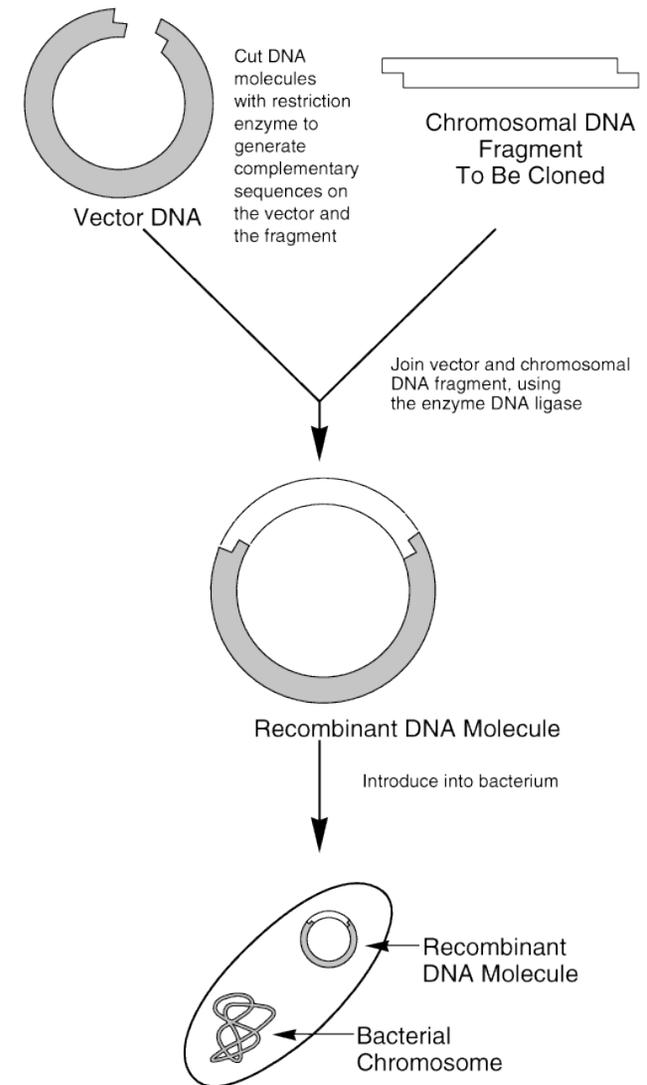


Source: www.nobelprize.org

Meanwhile, back at the ranch ...

Recombinant DNA technology has advanced, to allow for the creation of transgenic organisms

DNA sequencing has advanced, allowing us to identify and clone promoter regions for specific genes

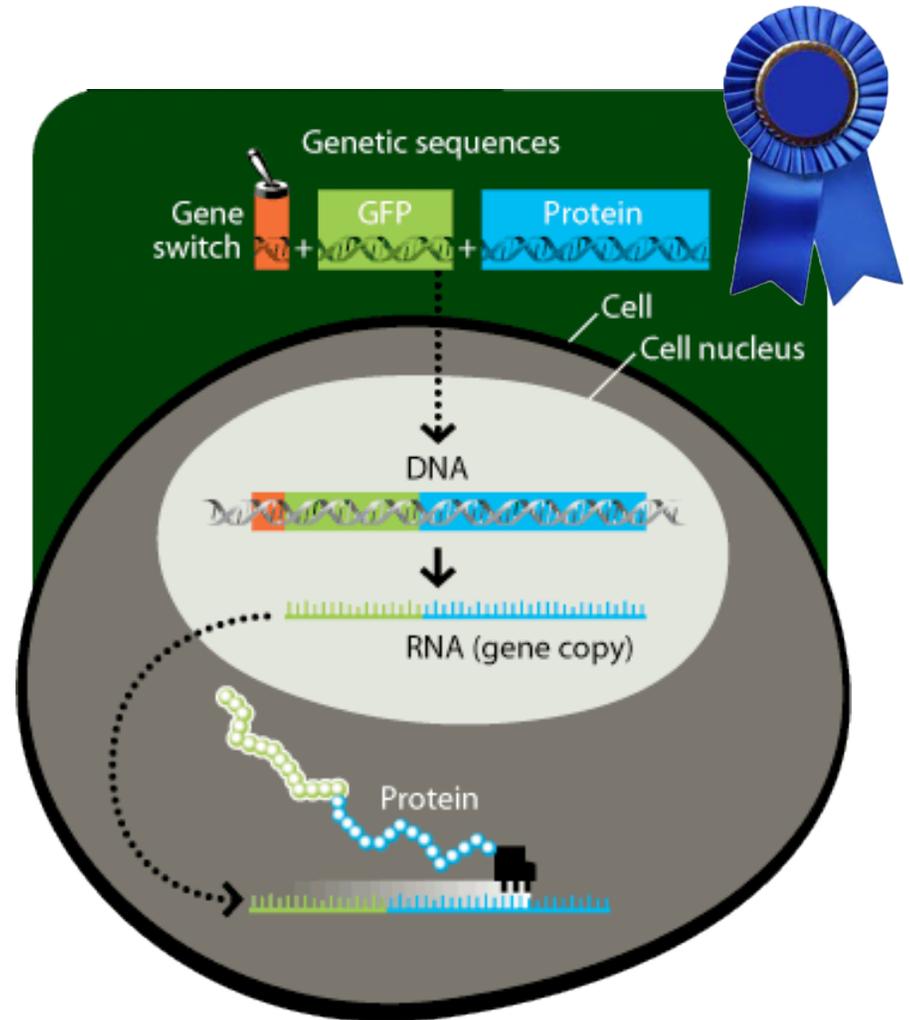


Source: <http://library.thinkquest.org>

GFP Revolution

Fluorescent protein that we can express in transgenic animals

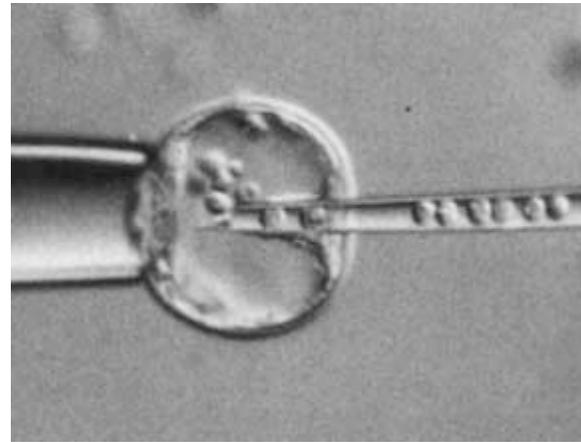
We can use our understanding of cell-specific gene expression to target



Optogenetics

Light-
Activated

Genetically
Encoded



Genetic techniques allow you to control when and where a protein of interest is expressed, and optical techniques allow you to control when and where this protein is activated ...

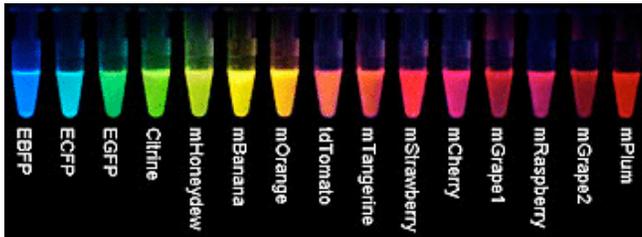


... so what do you want to express?

Sensors & Effectors



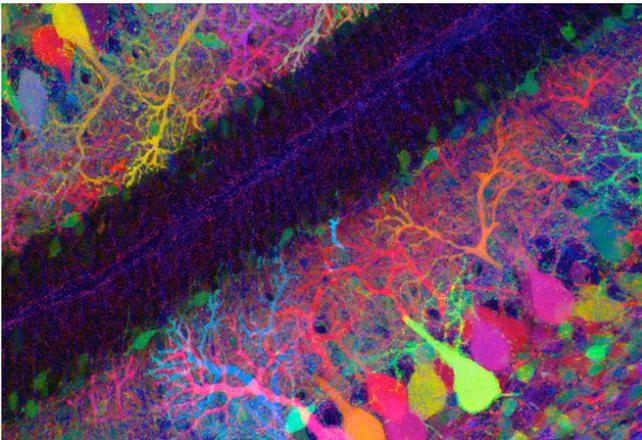
Sensors: Fluorescent Proteins



Source: <http://www.conncoll.edu/ccacad/zimmer/GFP-ww/tsien.html>



Rasse TM, et al. (2005) *Nature Neuroscience*

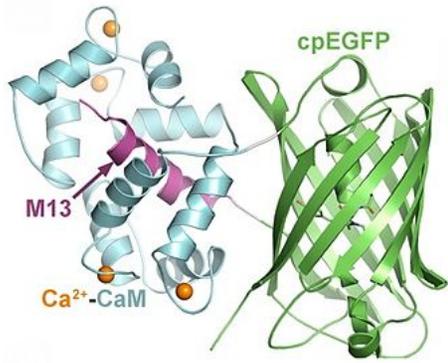


Family Weissman, Harvard University



Source: Joe Fetcho

Sensors: GECIs



Akerboom J, et al. (2009)
J Biol Chem

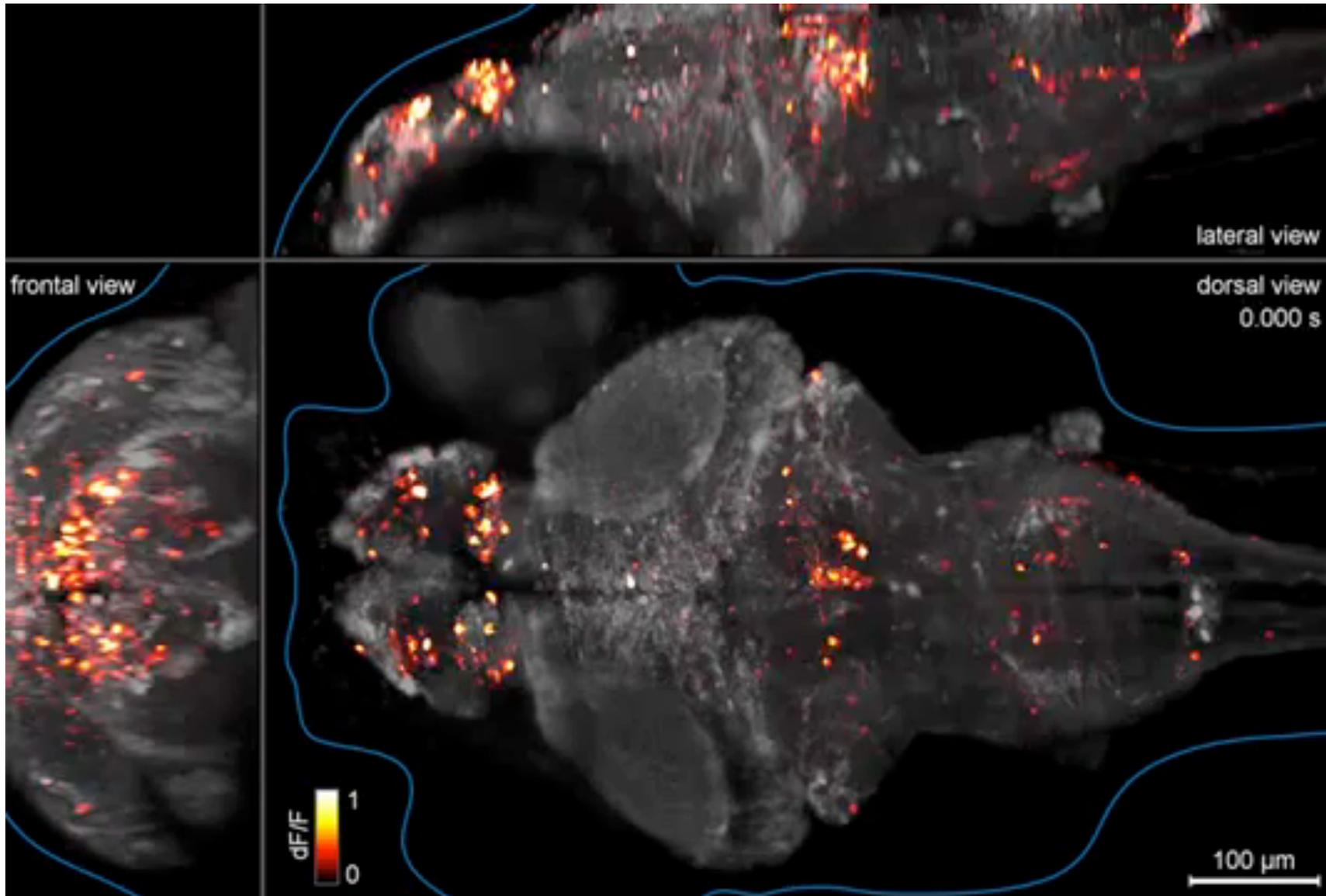
Electrophysiology
Calcium imaging

20%
0.5mV
1s

10 μ m

Chen T-W, et al. (2013) *Nature*

Sensors: GECIs

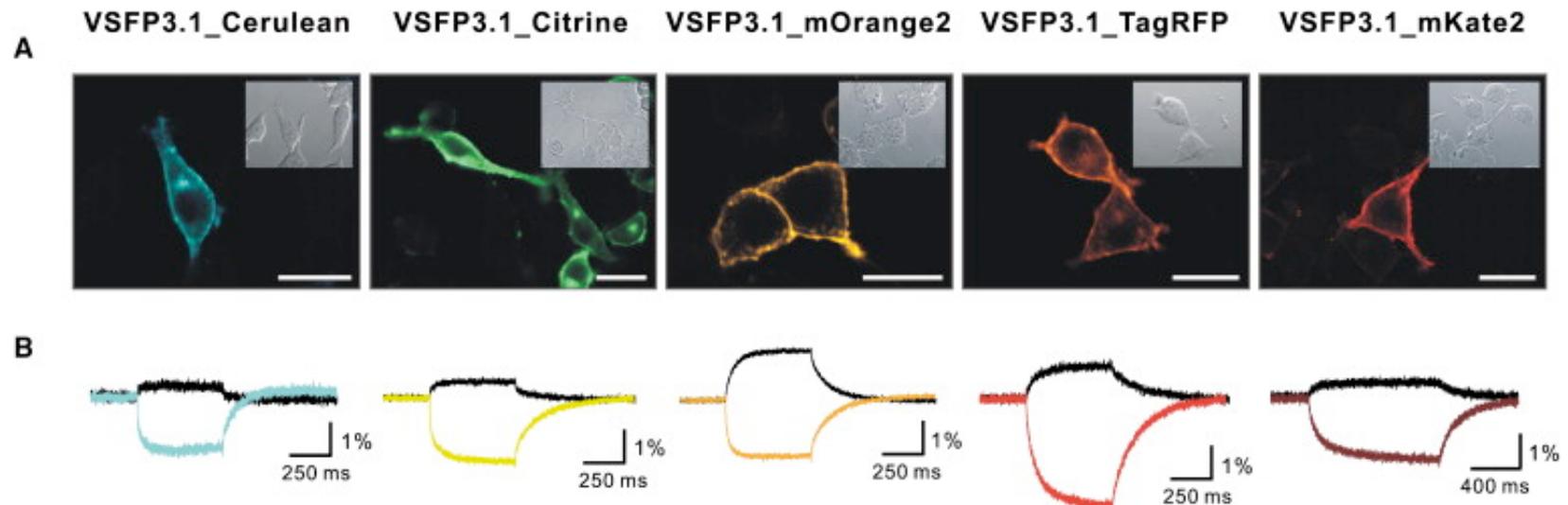


Ahrens MB, et al. (2013) *Nature Methods*

Sensors: GEVIs

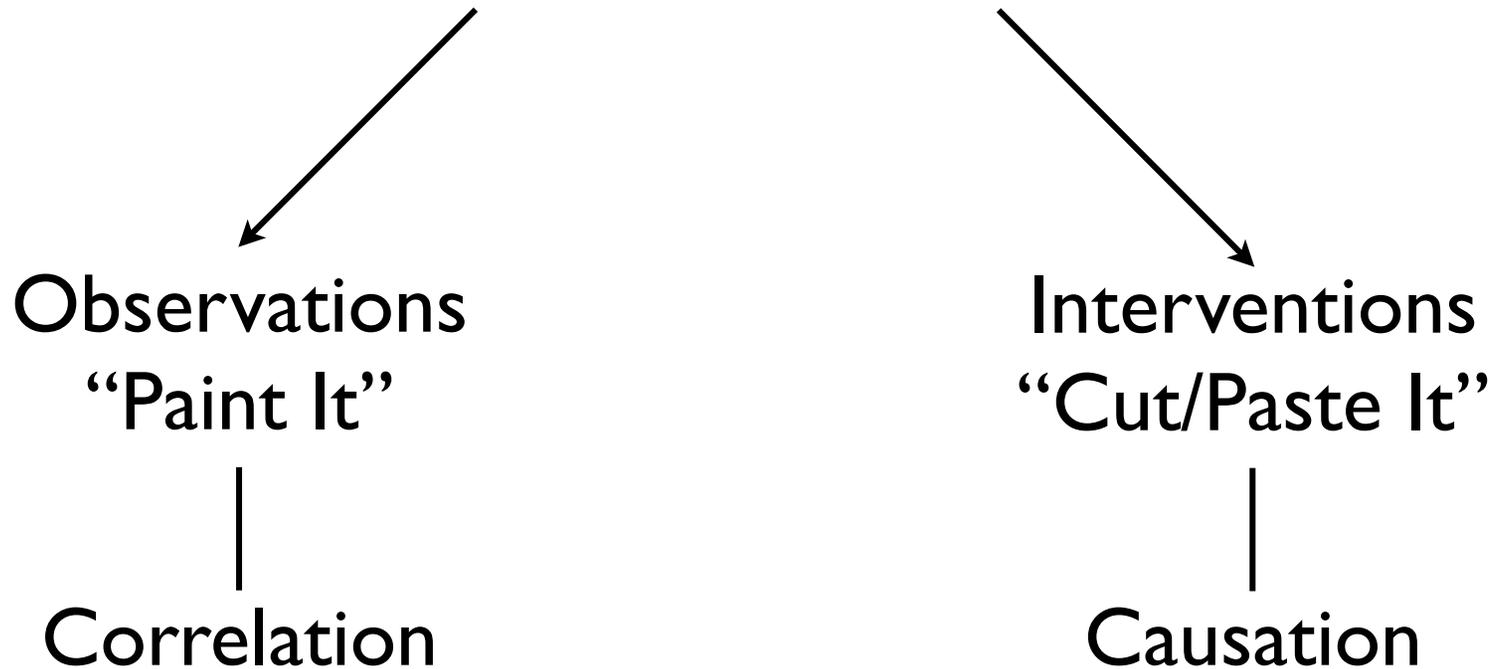
What are the limitations of calcium imaging?

What else could we sense instead of calcium?



Perron A, et al. (2009) *Chemistry & Biology*

Sensors & Effectors



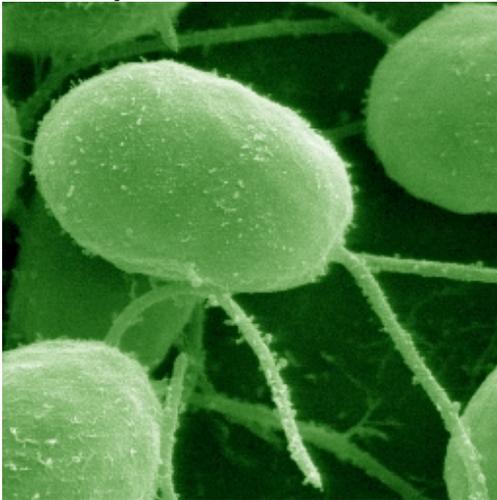
One of the next requirements ... is to be able to turn the firing of one or more types of neuron on and off in the alert animal in a rapid manner. The ideal signal would be light ... This seems rather far-fetched but it is conceivable that molecular biologists could engineer a particular cell type to be sensitive to light in this way.

- Francis Crick (1999)

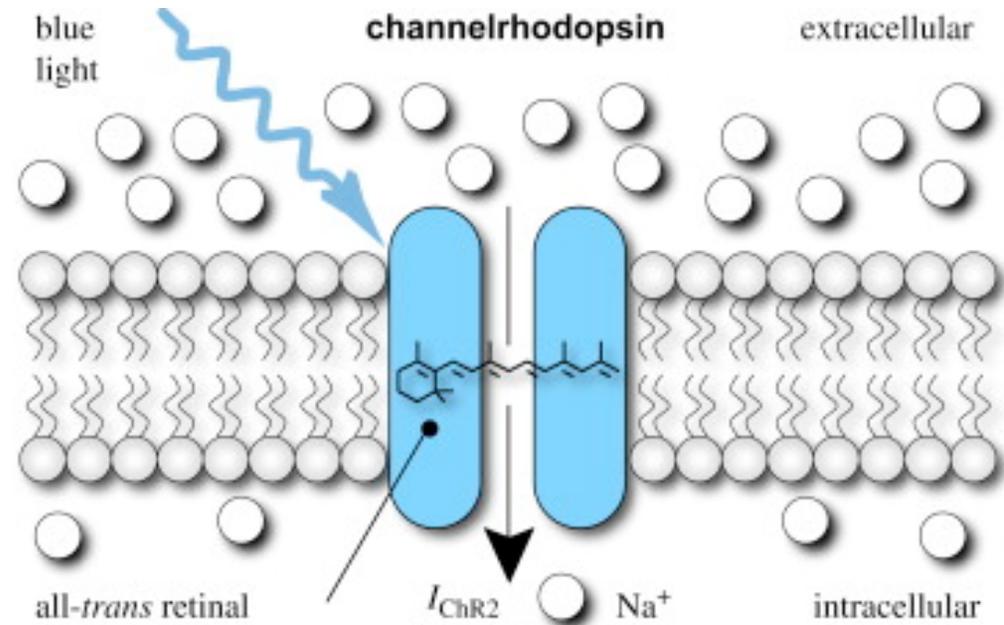
Green Algae

Opsins: single-component, light-mediated ion channels involved in phototaxis

Chlamydomonas reinhardtii



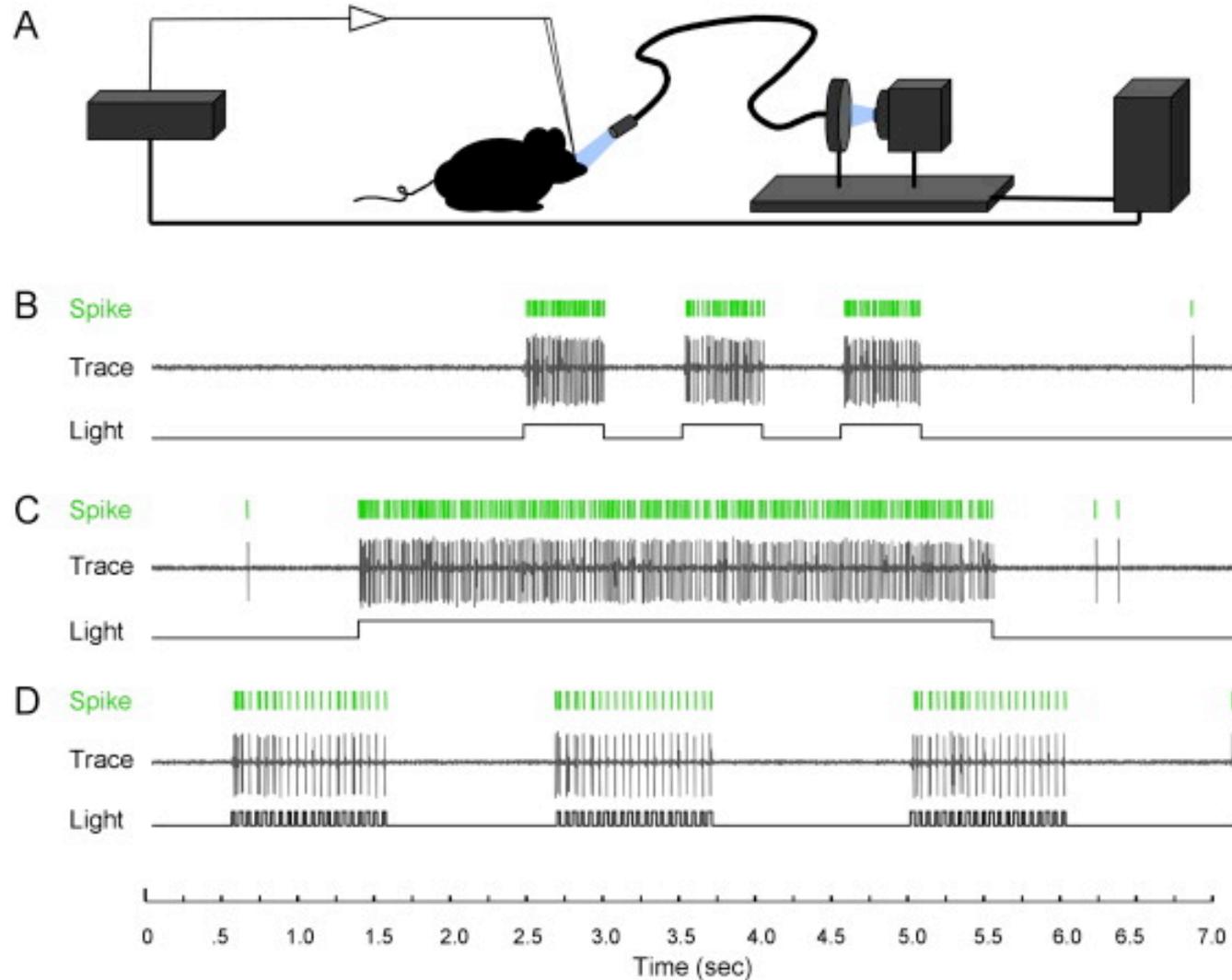
Louisa Howard,
Dartmouth University



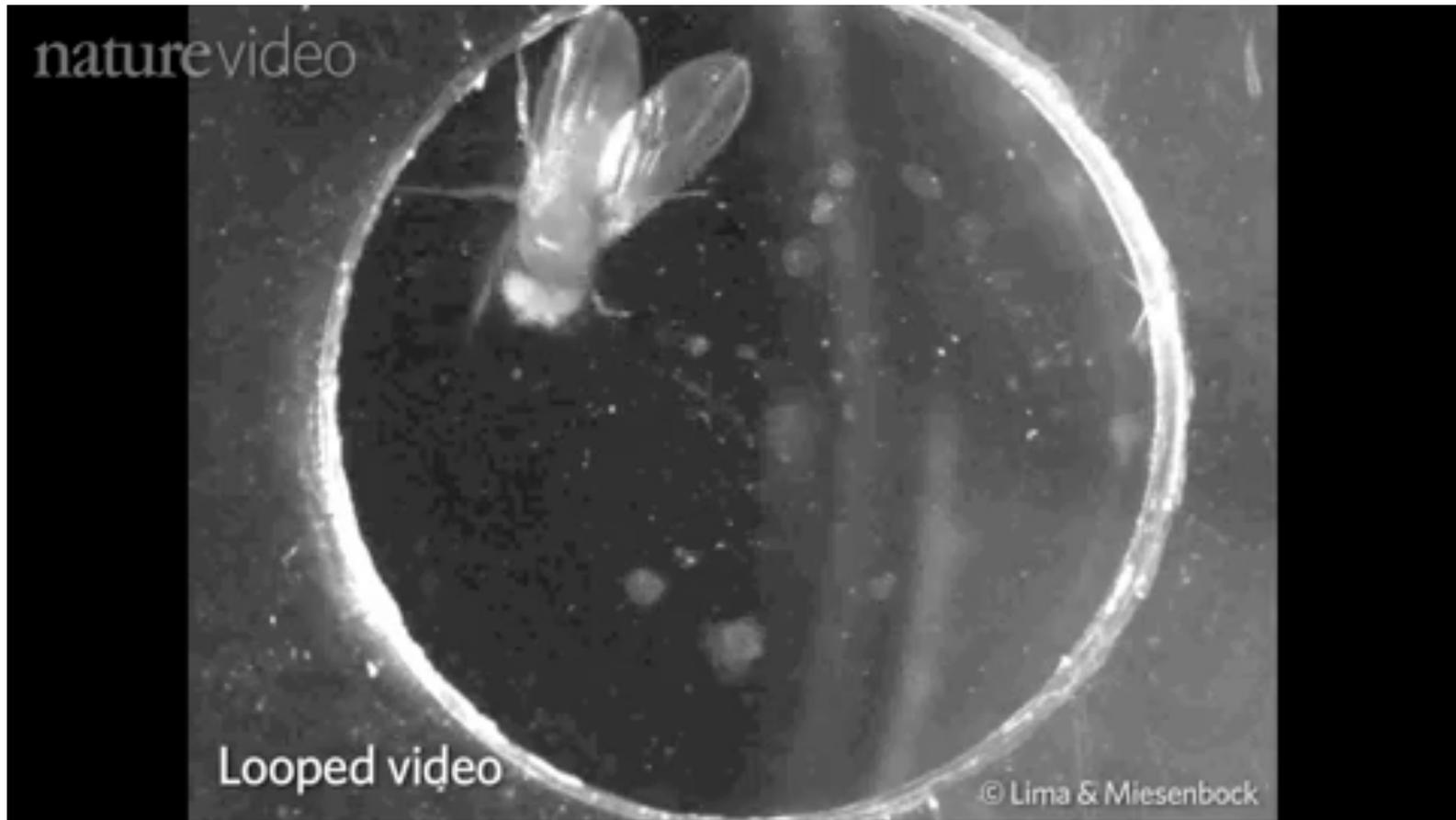
Wong J, et al. (2012) *Journal of Mechanics and Physics of Solids*

What's the big deal?

Effectors: Channelrhodopsin

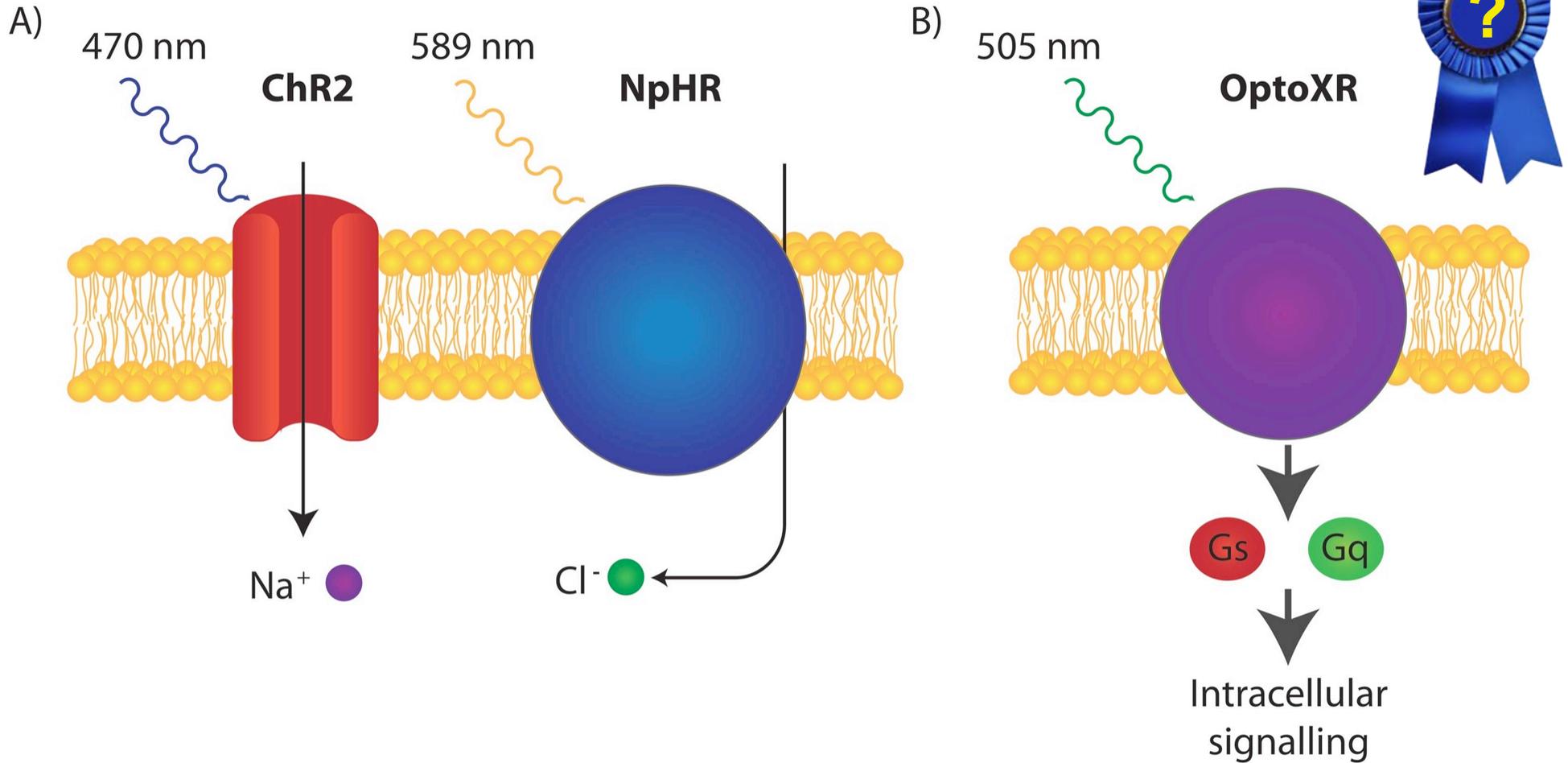


Effectors: Channelrhodopsins



Nature Method of the Year (2010)

Effectors: Channelrhodopsins +

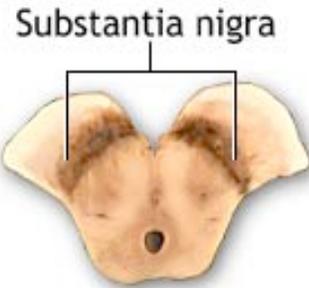


Adapted from Zhang F, et al. (2010) *Nature Protocols*
Source: www.mn.uio.no

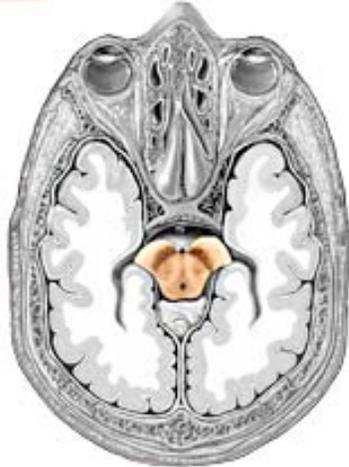
Clinical Applications



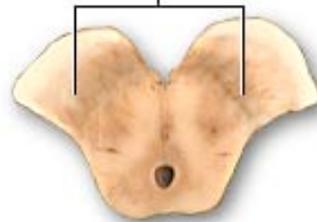
Cut section of the midbrain where a portion of the substantia nigra is visible



Substantia nigra

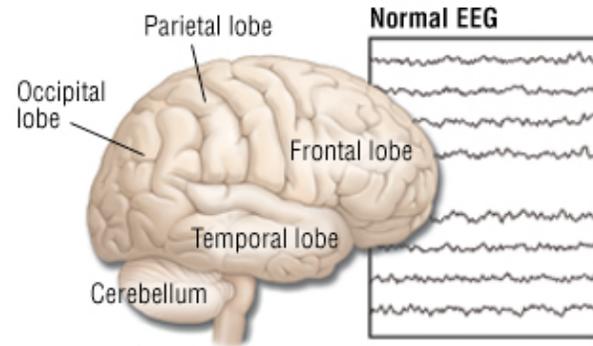


Diminished substantia nigra as seen in Parkinson's disease

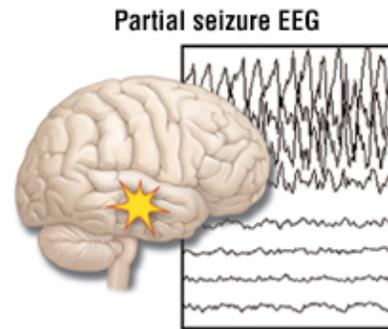


ADAM

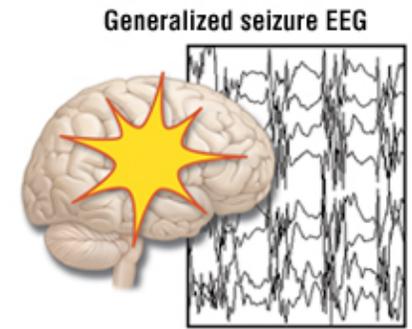
Source: health.rush.edu



Normal EEG



Partial seizure EEG



Generalized seizure EEG

Source: Krames StayWell