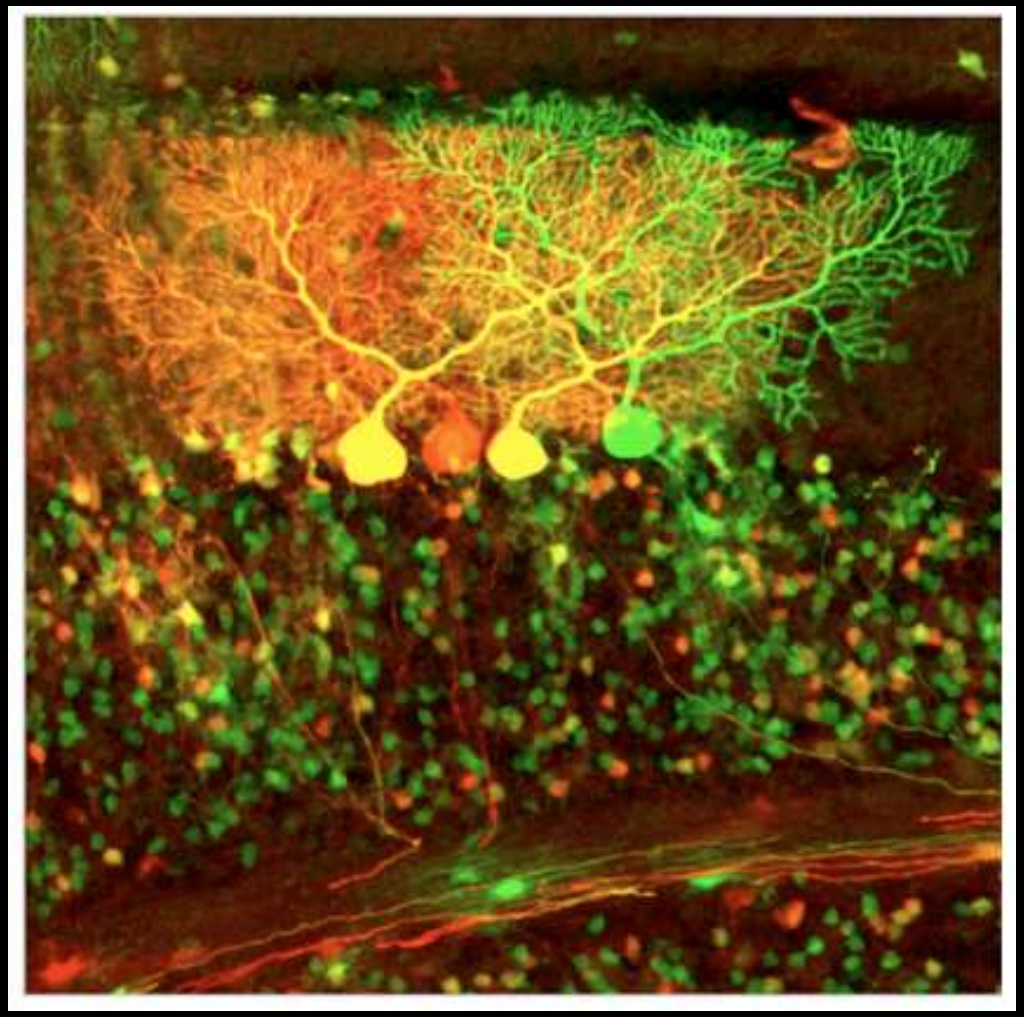
Techniques in Probing Neural Circuits



Dayu Lin (Dayu.lin@nyumc.org)

Basic strategies in studying Neural circuits

- **Visualize cells**
- **Monitor cell activity**
- **Manipulate cell and gene activity**

Visualize cells

- Any cells
- Cells with certain genetic features
- Cells with certain developmental lineage
- Connected cells

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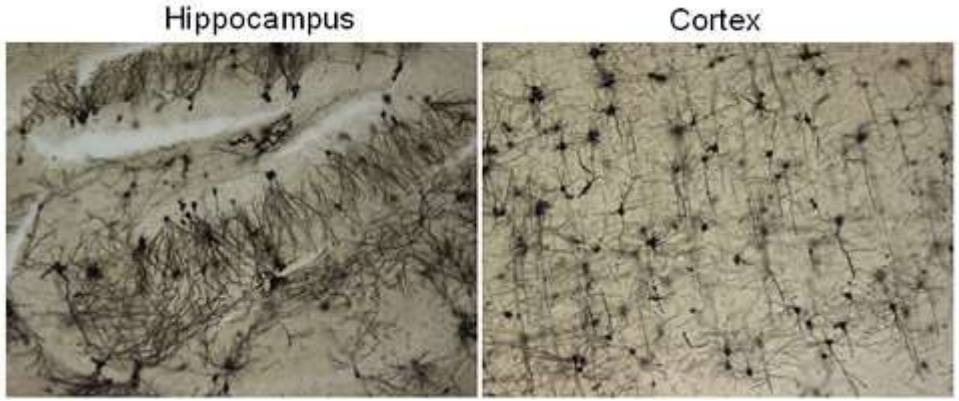
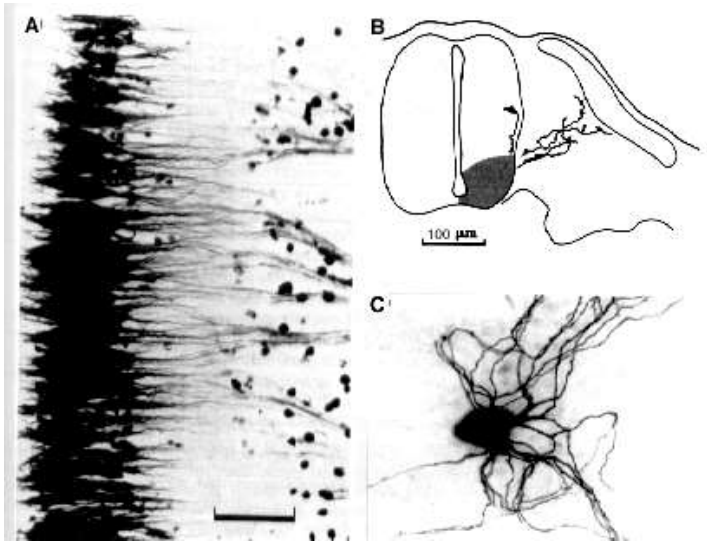
Methods to simply label cells

Golgi staining (Silver chromate, $Ag_2CrO_{4)}$)



horseradish peroxidase (HRP)



Other tracers

subunit B of cholera toxin (CTB)

Phaseolus vulgaris leucoagglutinin (PHA-L)—a plant lectin

Biotinylated dextran amine (BDA)

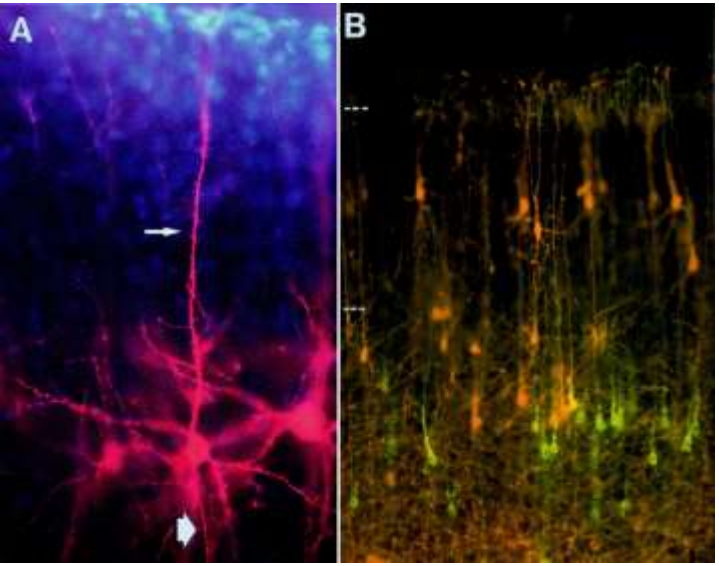
Biocytin and neurobiotin (a small molecule)

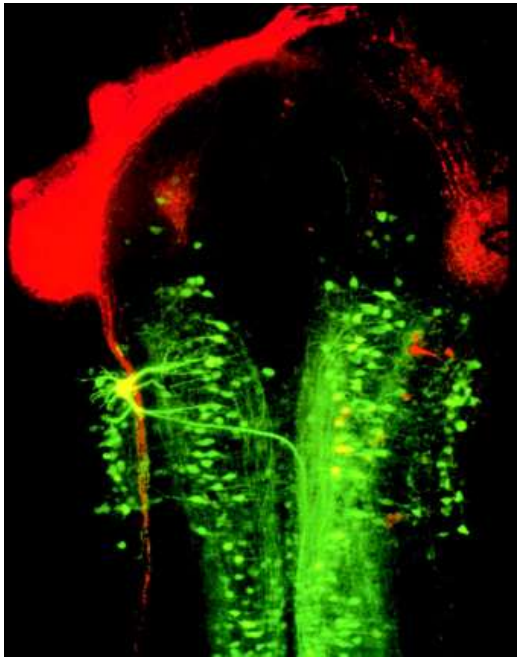
Conventional methods for neuroanatomy-Fluorescent dyes

Lipophilic dyes

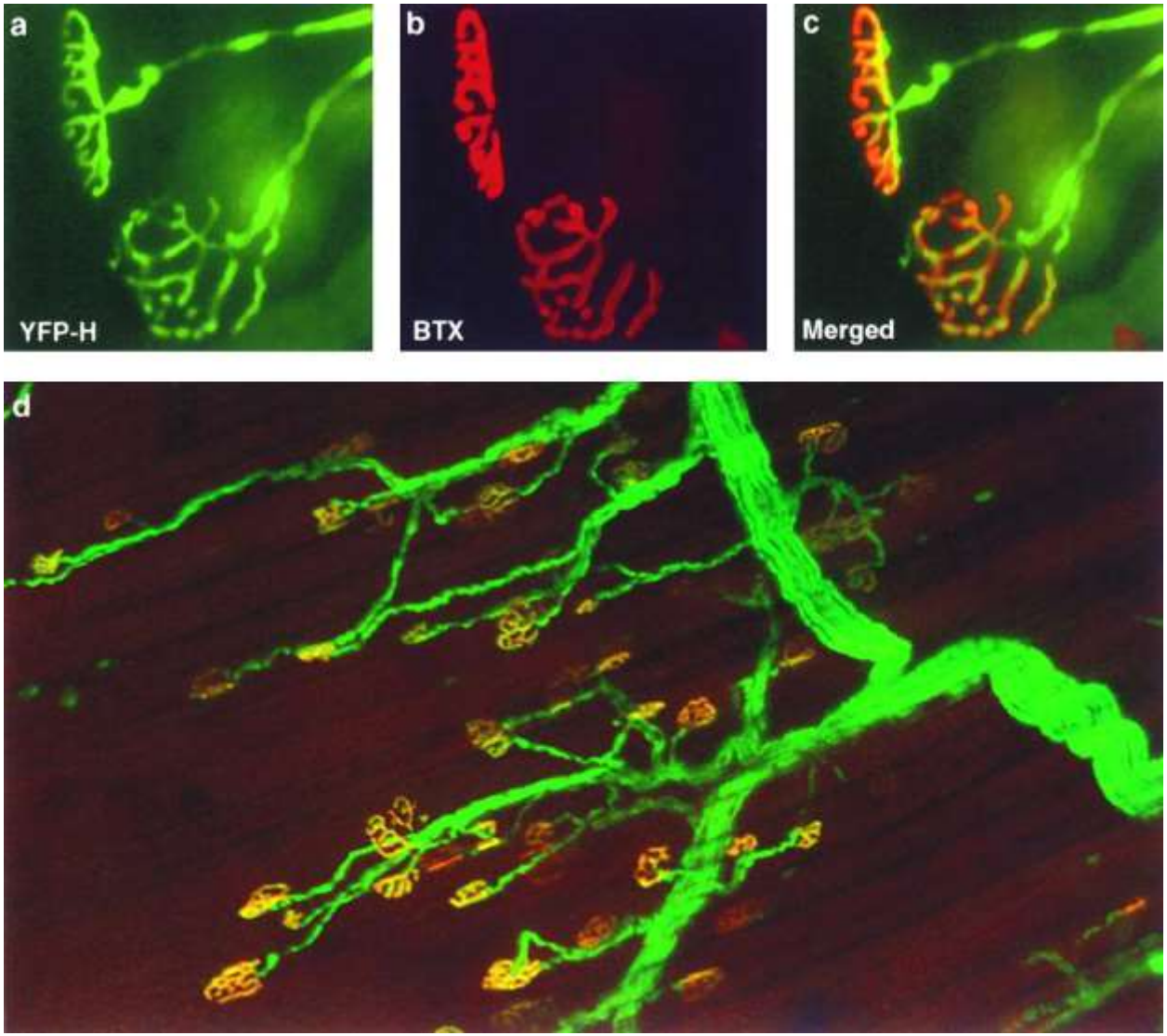
Fluorescent dextrans
(Lucifer Yellow, Texas Red, fluorescein)

fluorescent microspheres



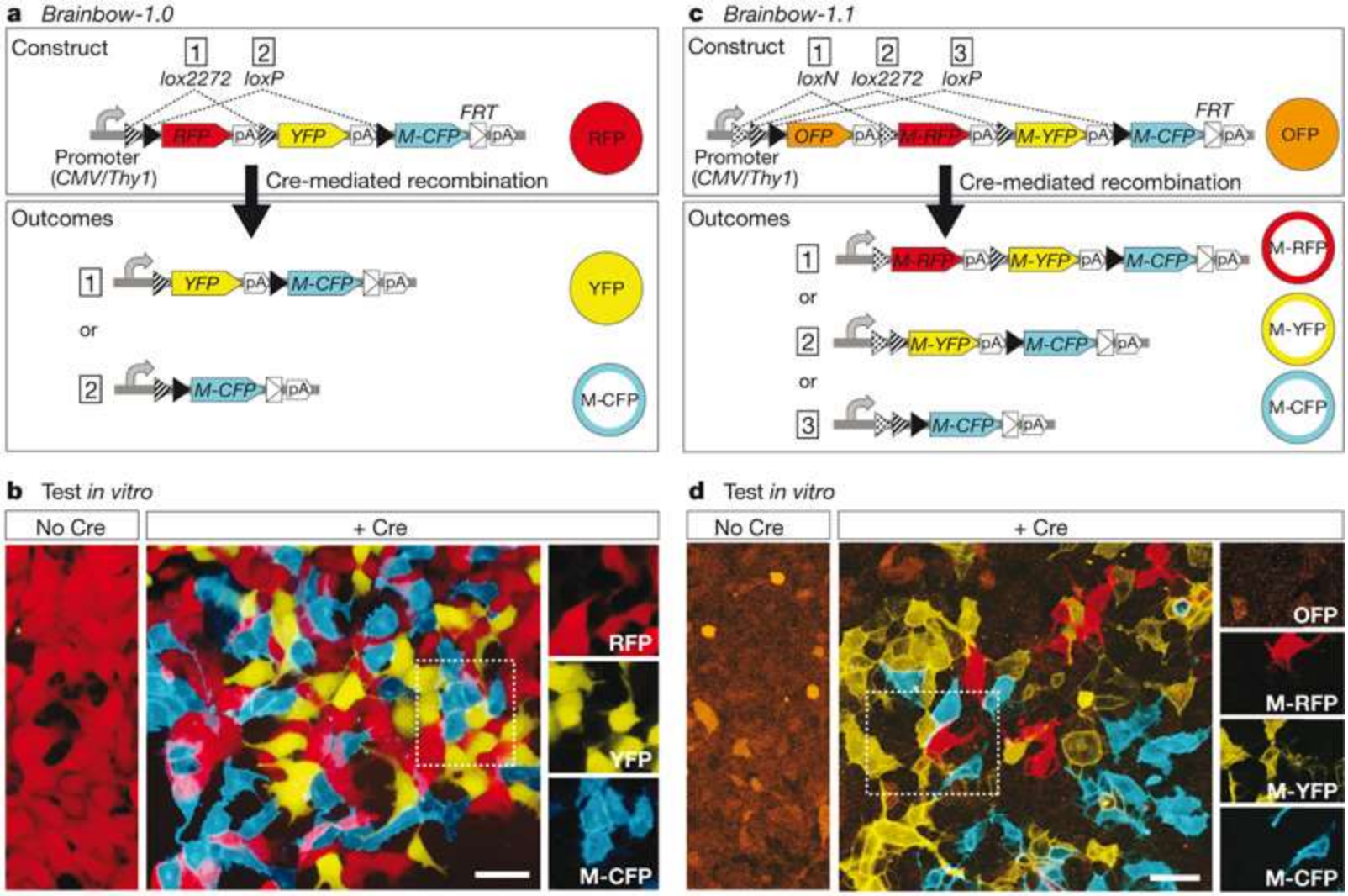


Label cells reproducibly--Thy1-GFP mice



Feng et al, 2000, Neuron,
Cite >1600

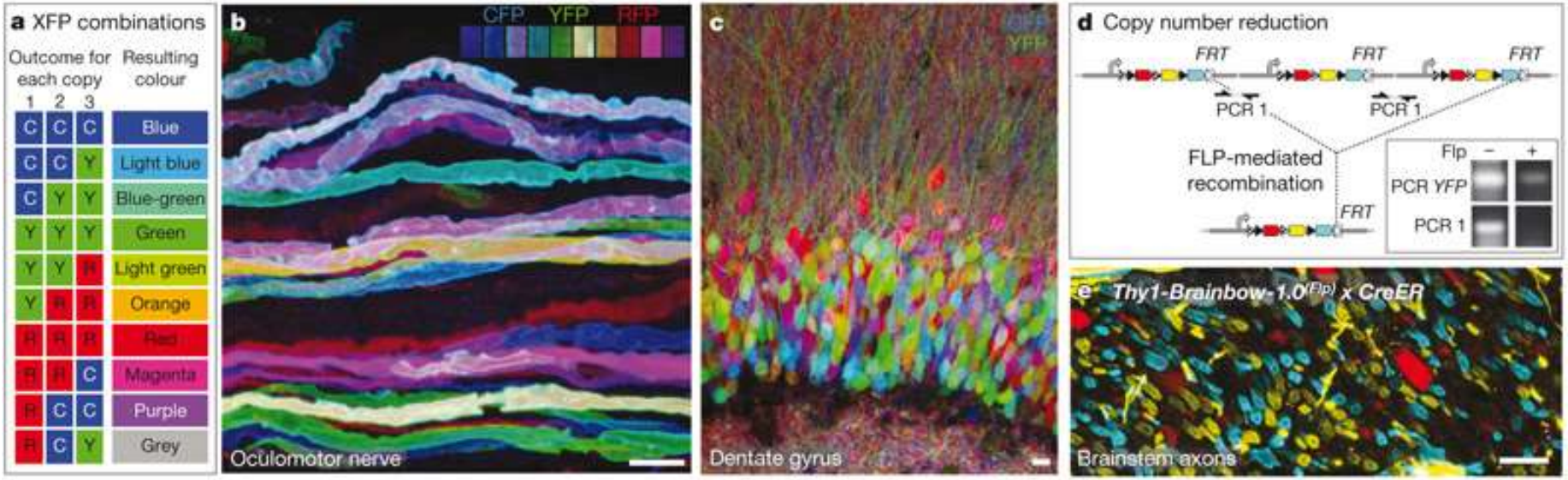
Label many cells but differently—Brainbow mice



Livet et al, Nature, 2007, Cite ~800

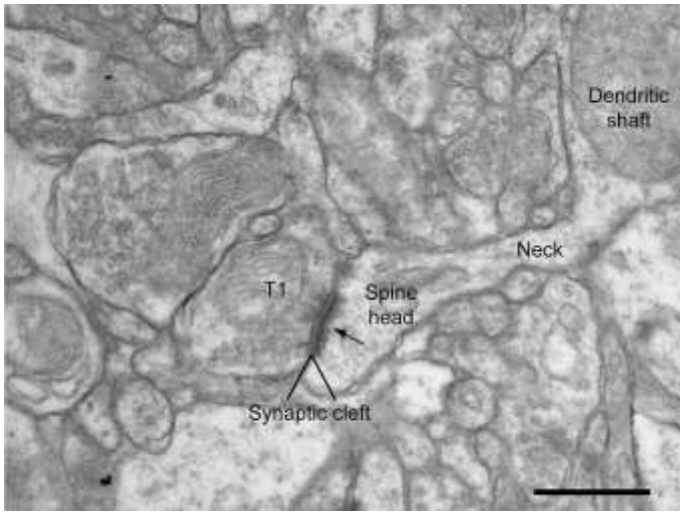
Jeff W. Lichtman and Joshua R. Sanes

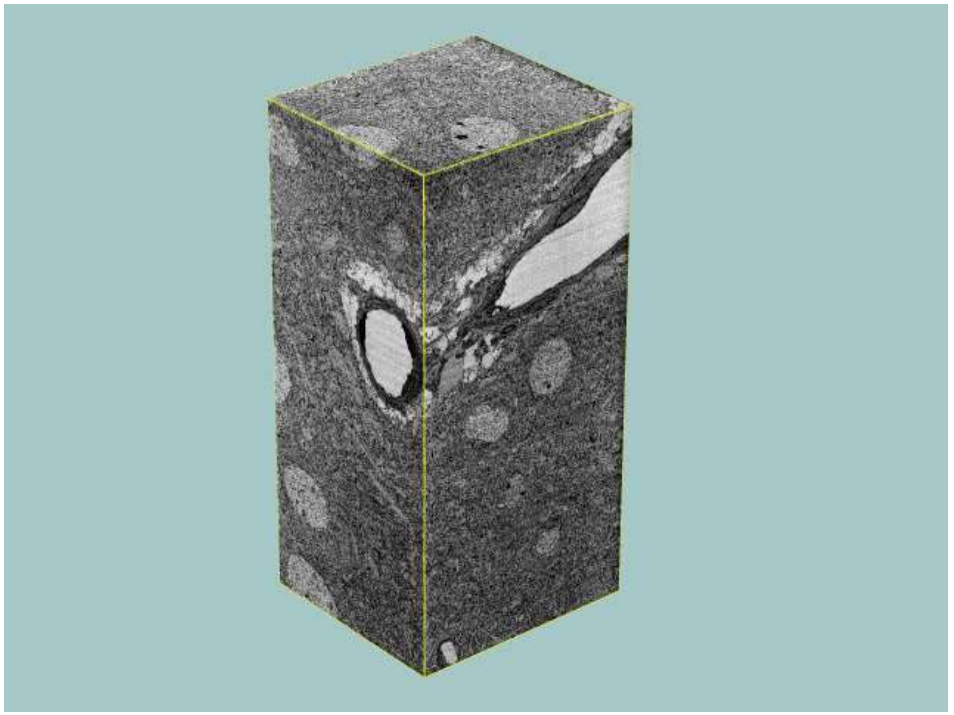
Membrane bound GFP facilitated neuroanatomy—Brainbow mice



Livet et al, 2007

Electron microscope (EM)--Connectome





http://www.cgl.ucsf.edu/chimera/animations/ratbrain/ratbrain.html

Visualize cells

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- Cells with certain genetic features
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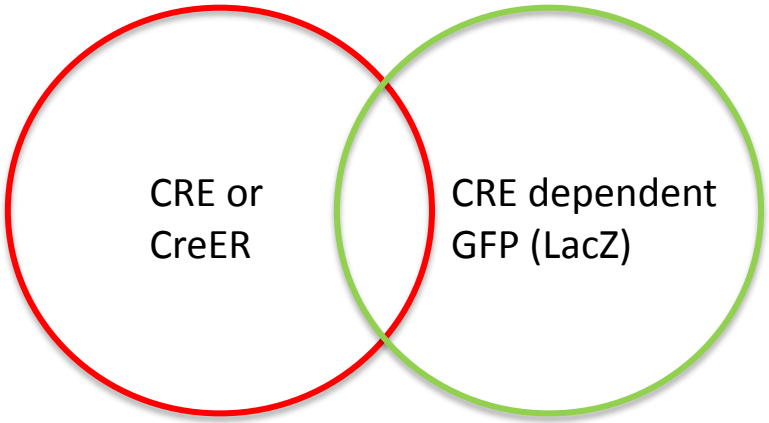
- Connected cells

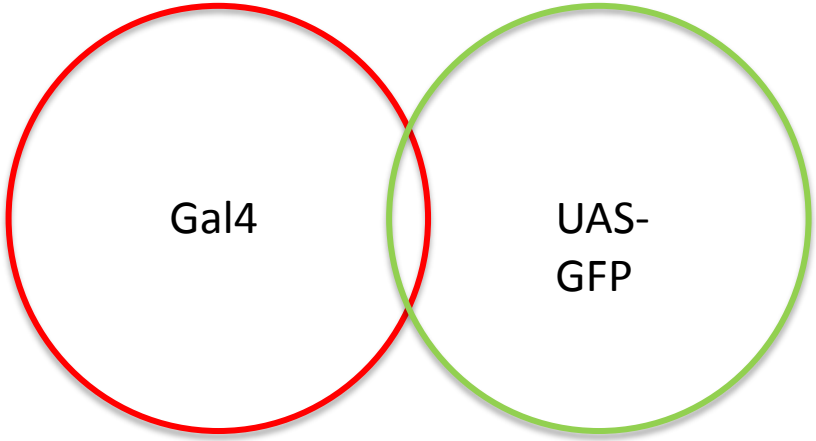
Label genetically defined cell type

mice



http://cre.jax.org/index.html
http://www.gensat.org/cre.jsp
http://connectivity.brain-map.org/transgenic

fly



http://flystocks.bio.indiana.edu/

Visualize cells

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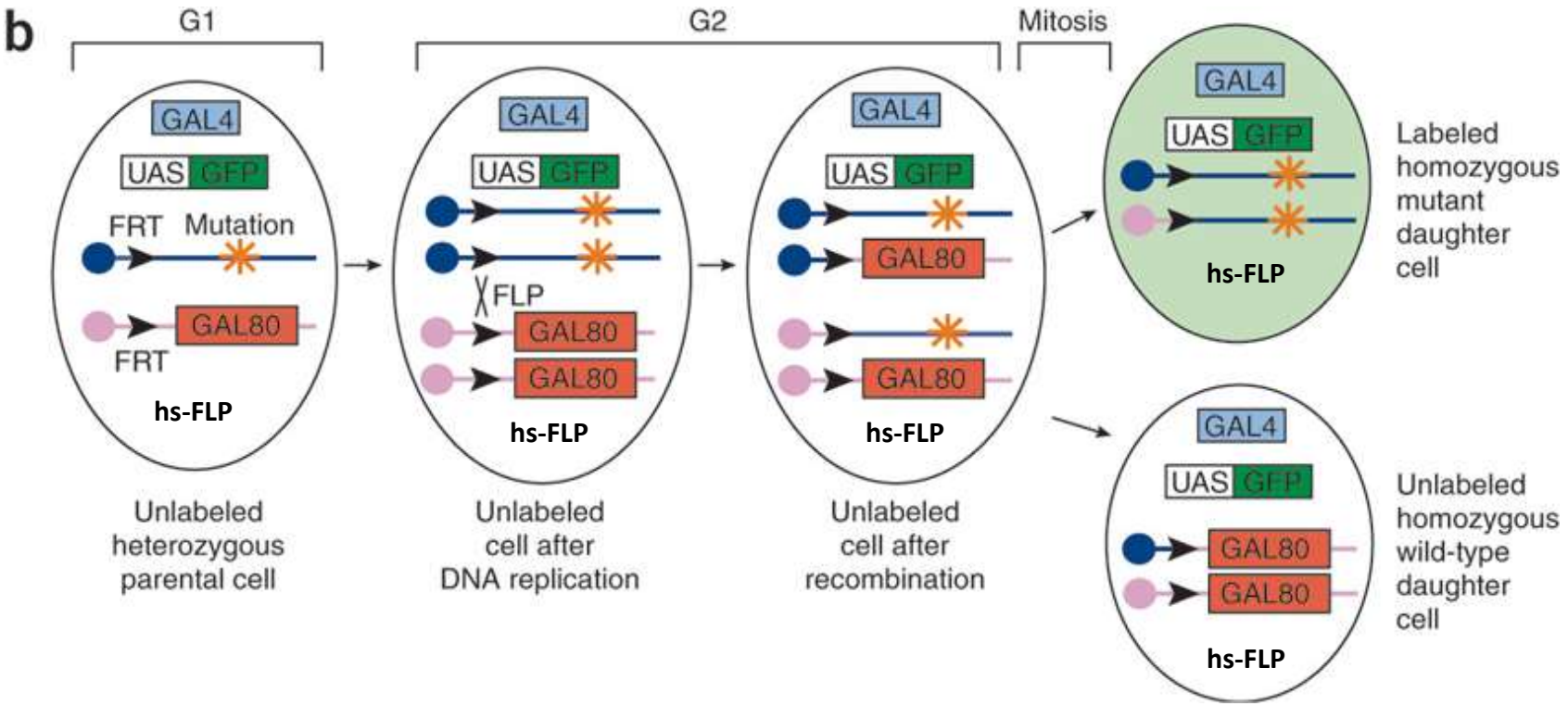
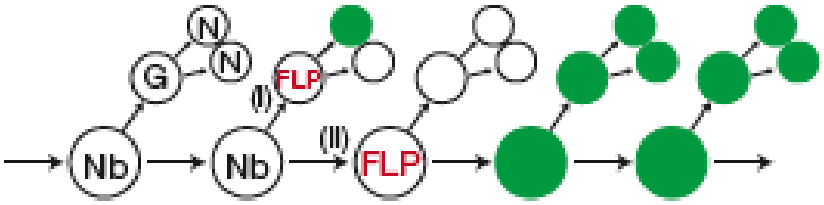
MARCM

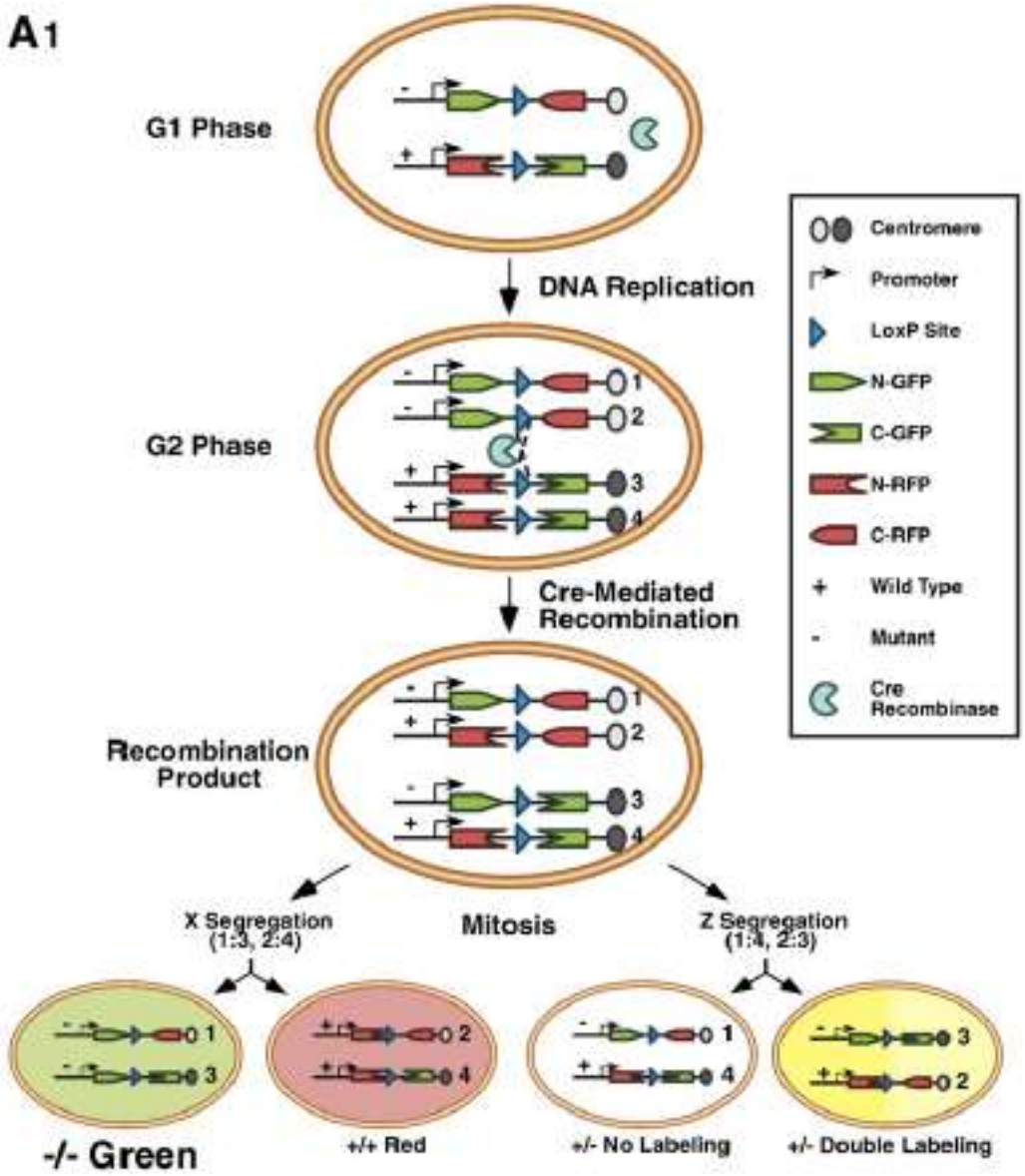
Mosaic Analysis with a Repressible Cell Marker– Label one or set of mutant cell with GFP

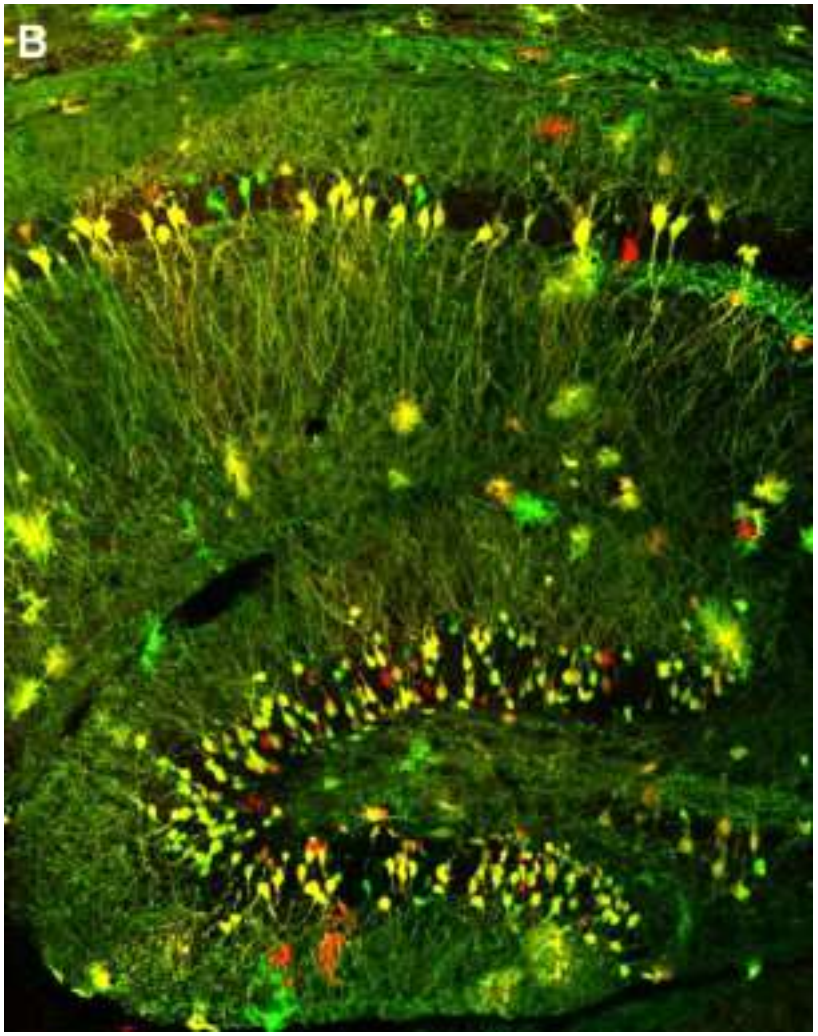




T Lee, Trends Neuroscience, 2001
Cite>500

MADM: Mosaic Analysis with Double Markers





Zong, 2005, Cell, Cite>200

Visualize cells

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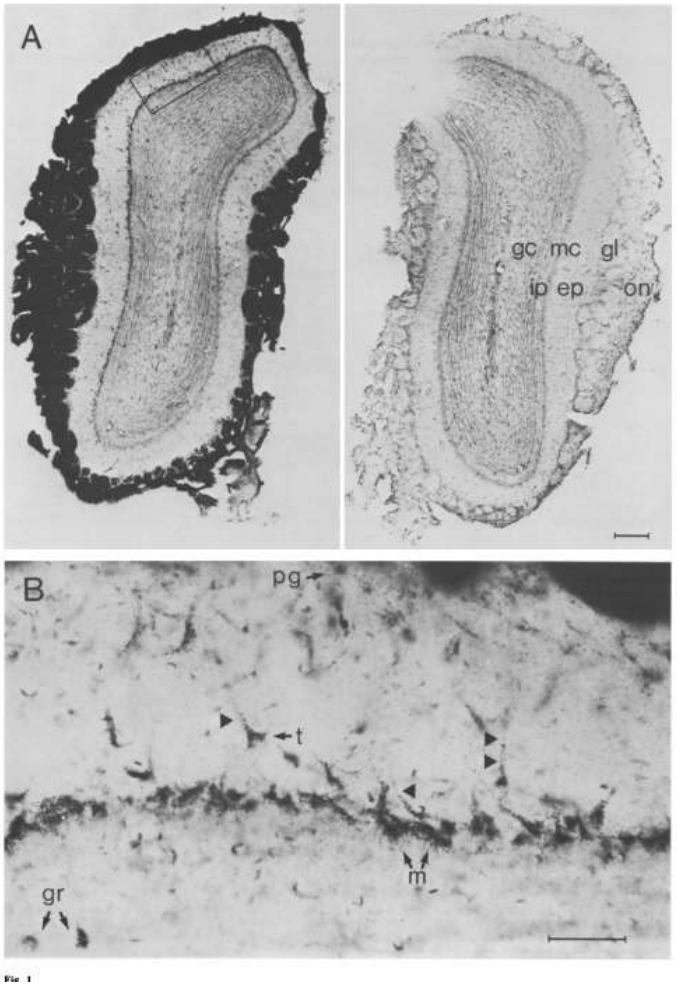
Label cells transneuronally

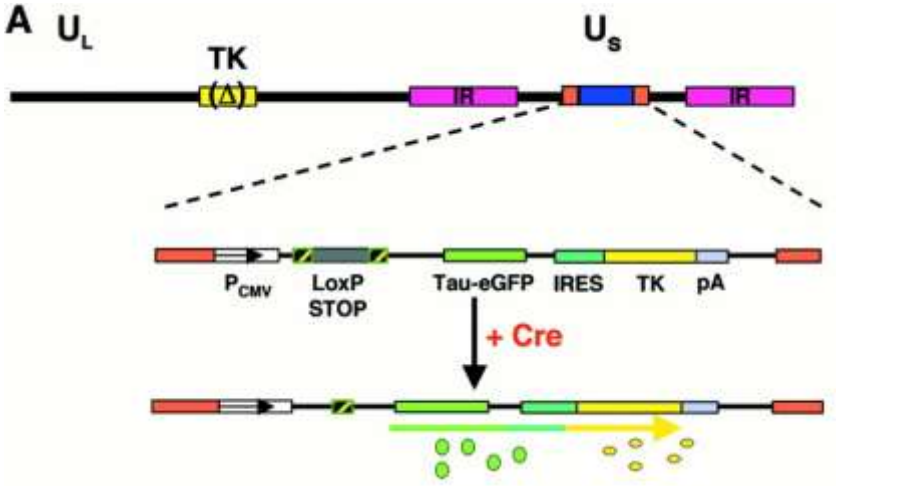
WGA: wheat germ agglutinin (an antegrade and retrograde transneuronal tracer)

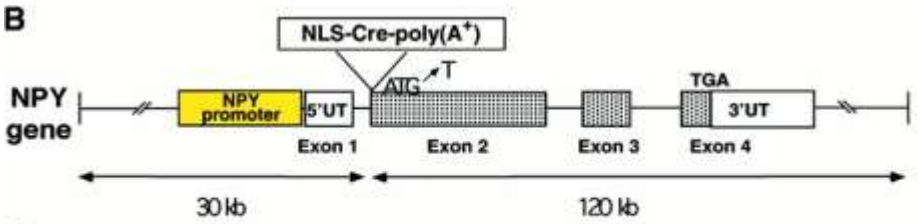


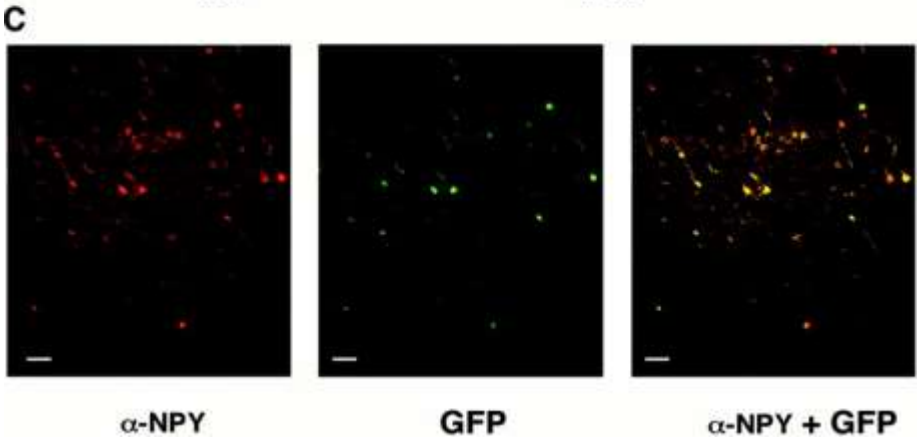
C.I. de Zeeuw et al. Brain Research, 447(1988)269

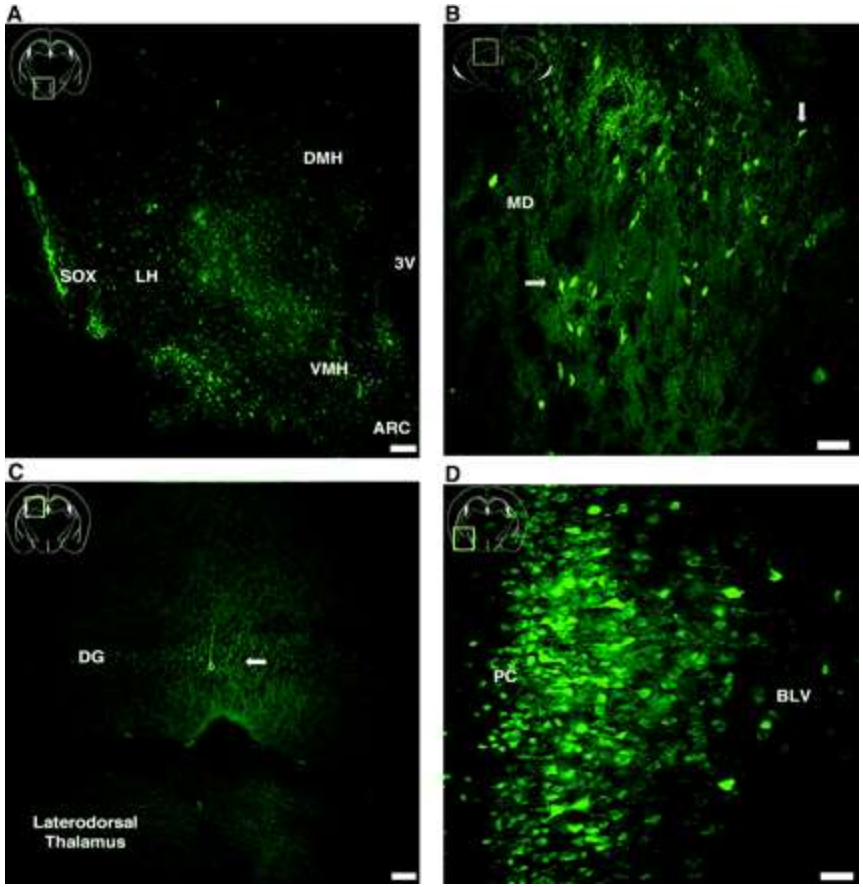
Transneuronal viral tracing
Anterograde: Herpes simplex virus (HSV)
Retrograde: Pseudorabies Virus (PRV)

TK: thymidine kinase





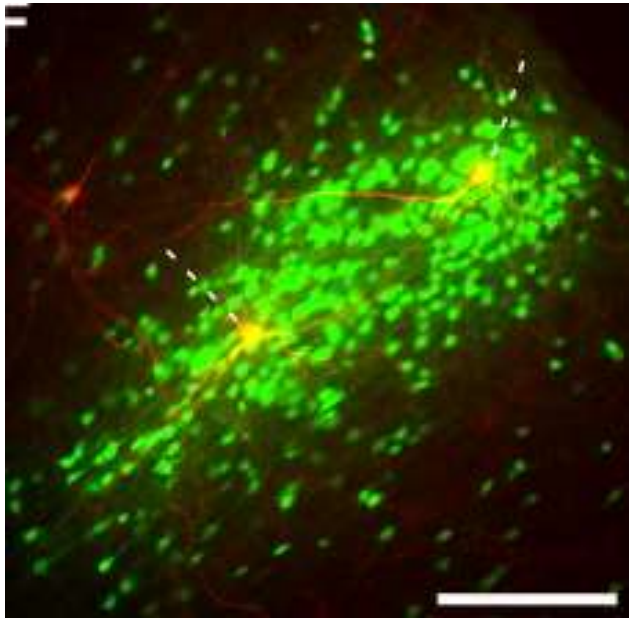


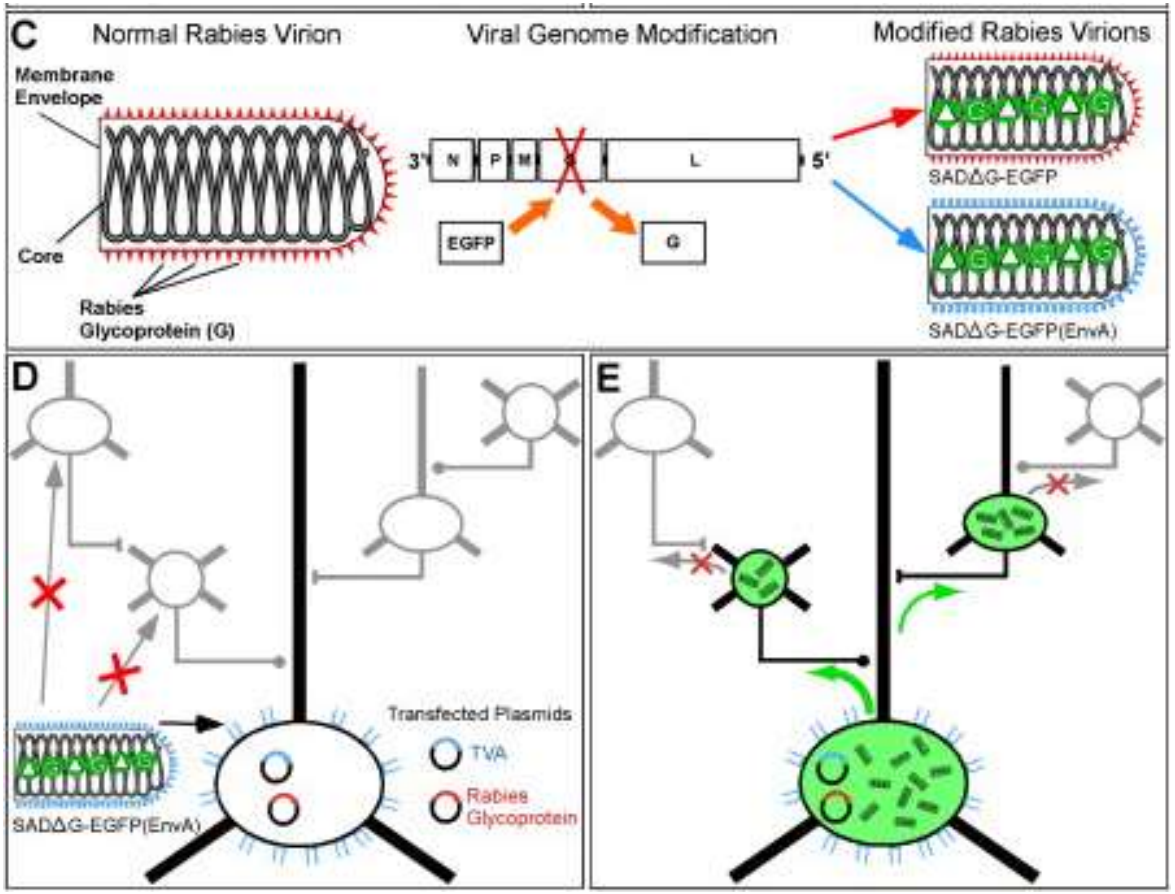


DeFalco … Jeffery Friedman. 2001, Science
~ 250 citation

Monosynaptic trans-neuronal tracing

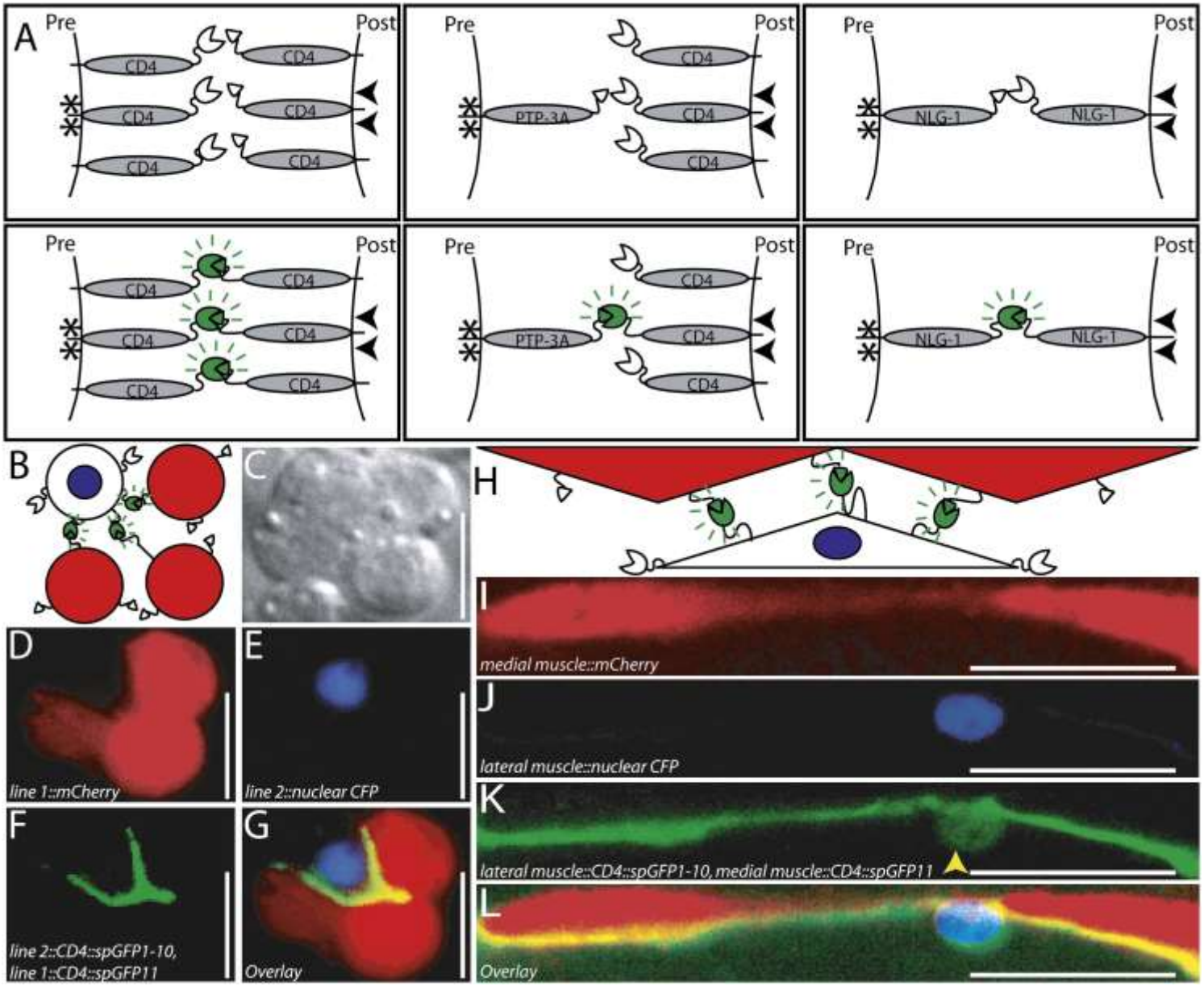
G protein: essential for viral particle amplification
EnvA: make virus only infect TVA expressing cells





Wickersham et al, 2007 Citation: ~390

GFP Reconstitution Across Synaptic Partners (GRASP) Defines Cell Contacts and Synapses

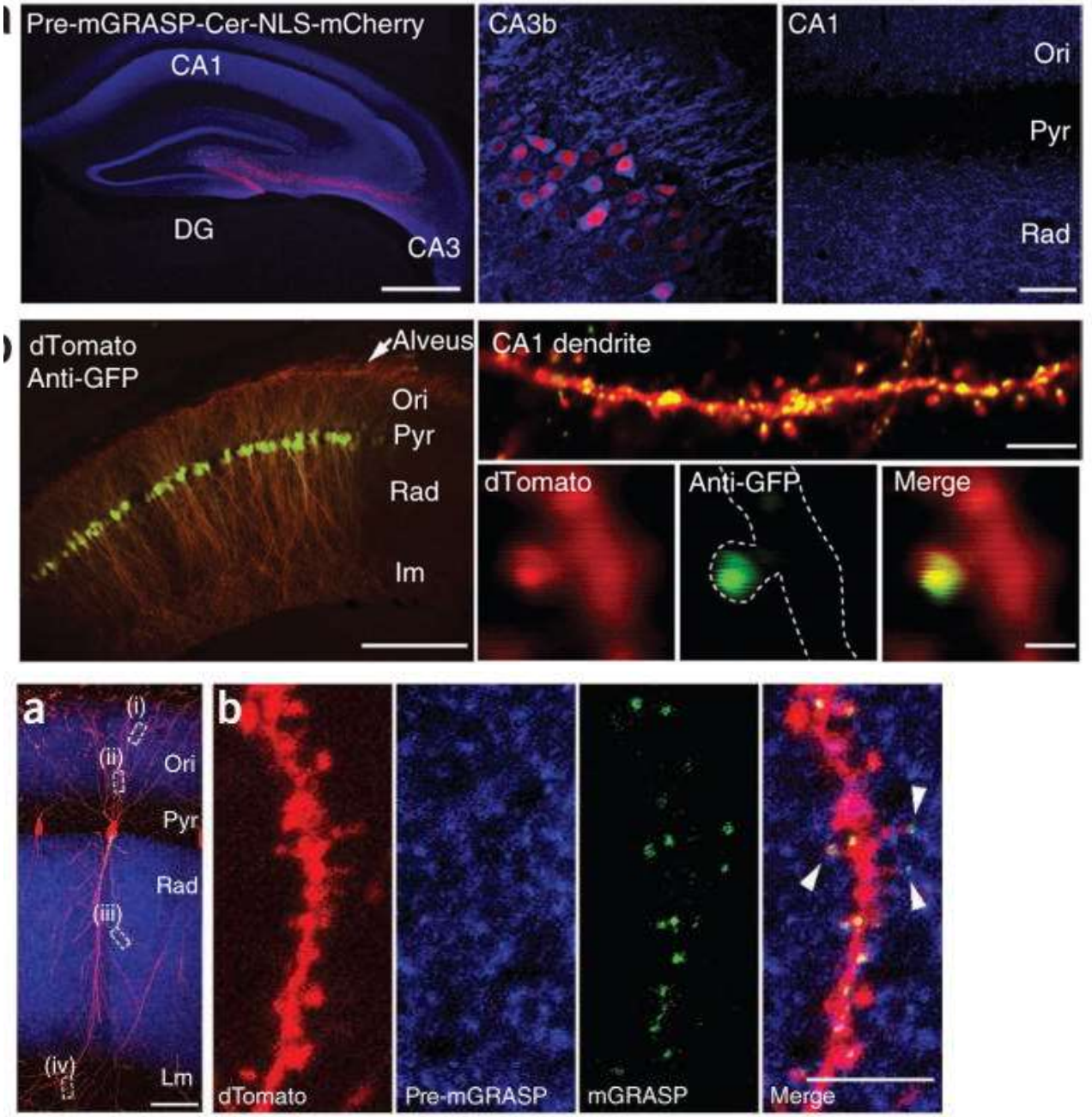


Feinberg EH, et al. Neuron, 2008, citation ~230

Mammaliam GRASP

CA3:
pre-mGRASP-mCerulean
NLS-mCherry

CA1:
post-mGRASP
(recognized with GFP antibody)
dTomato



Kim J, et al. 2011, Nature methods, cite ~70

Basic strategies in studying Neural circuits

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- Manipulate cell activity

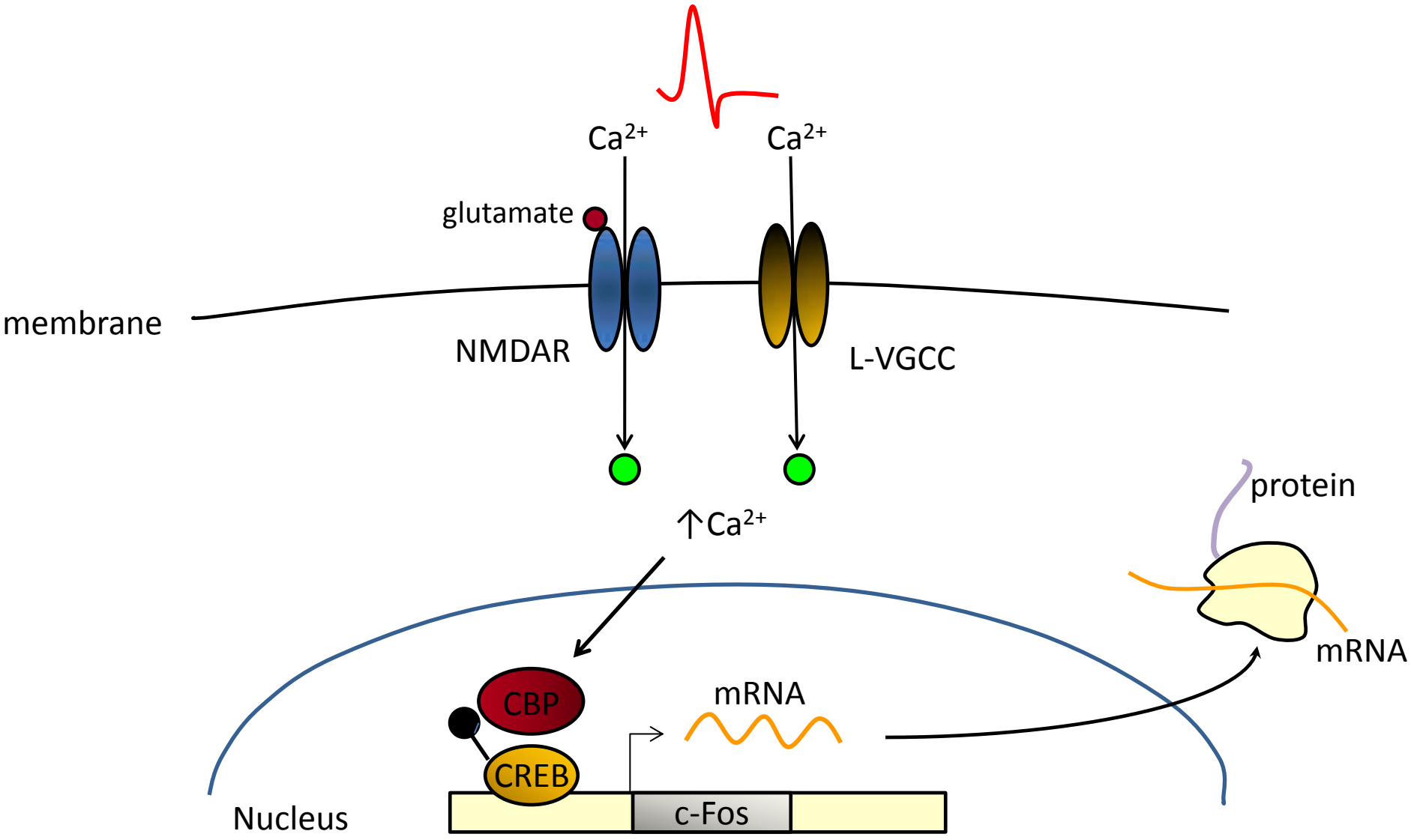
Monitor Cell activity

Immediate early gene mapping

Electrophysiology

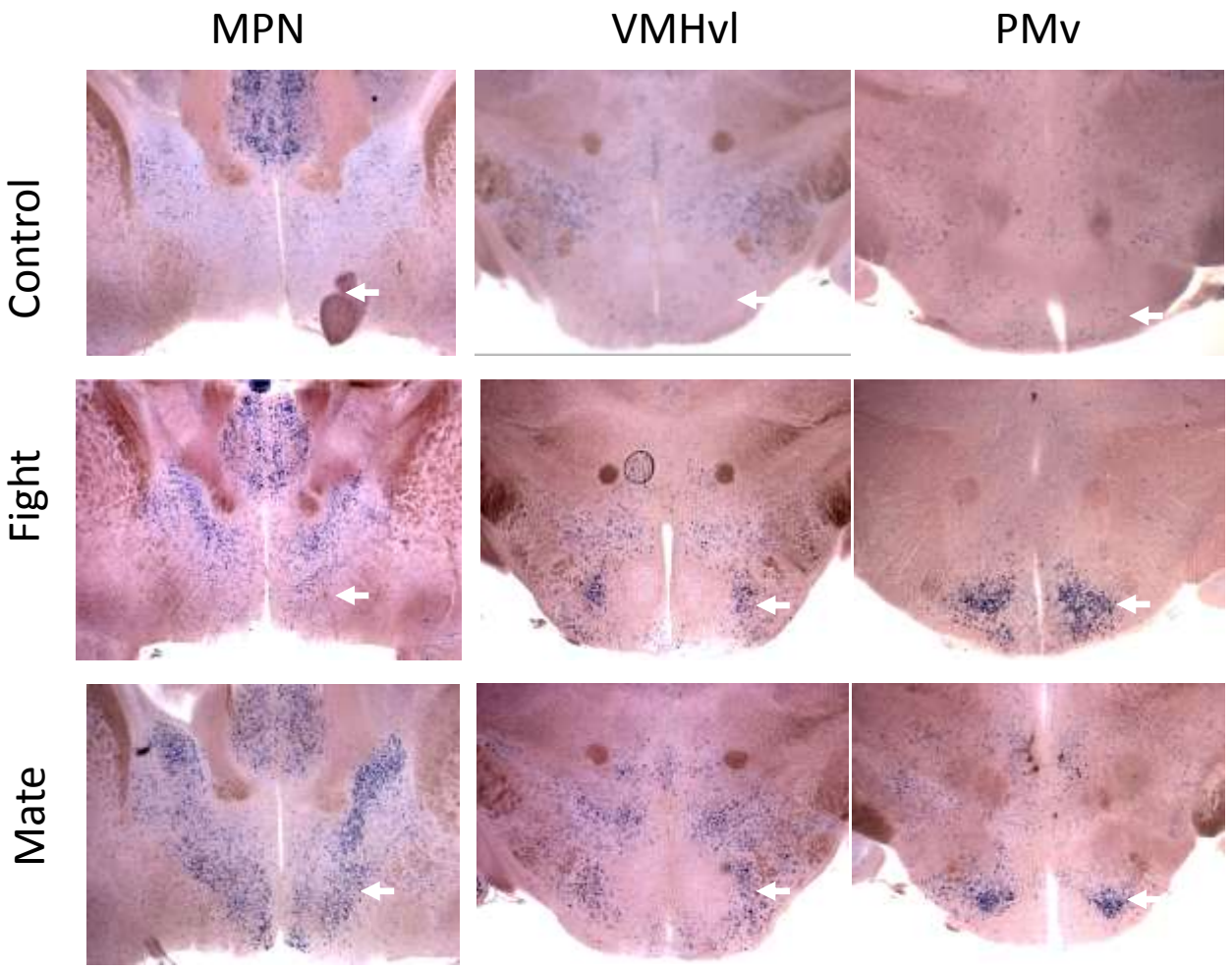
Functional Imaging

Immediate early gene response



Modified from Nature Reviews Neuroscience 3:921-31

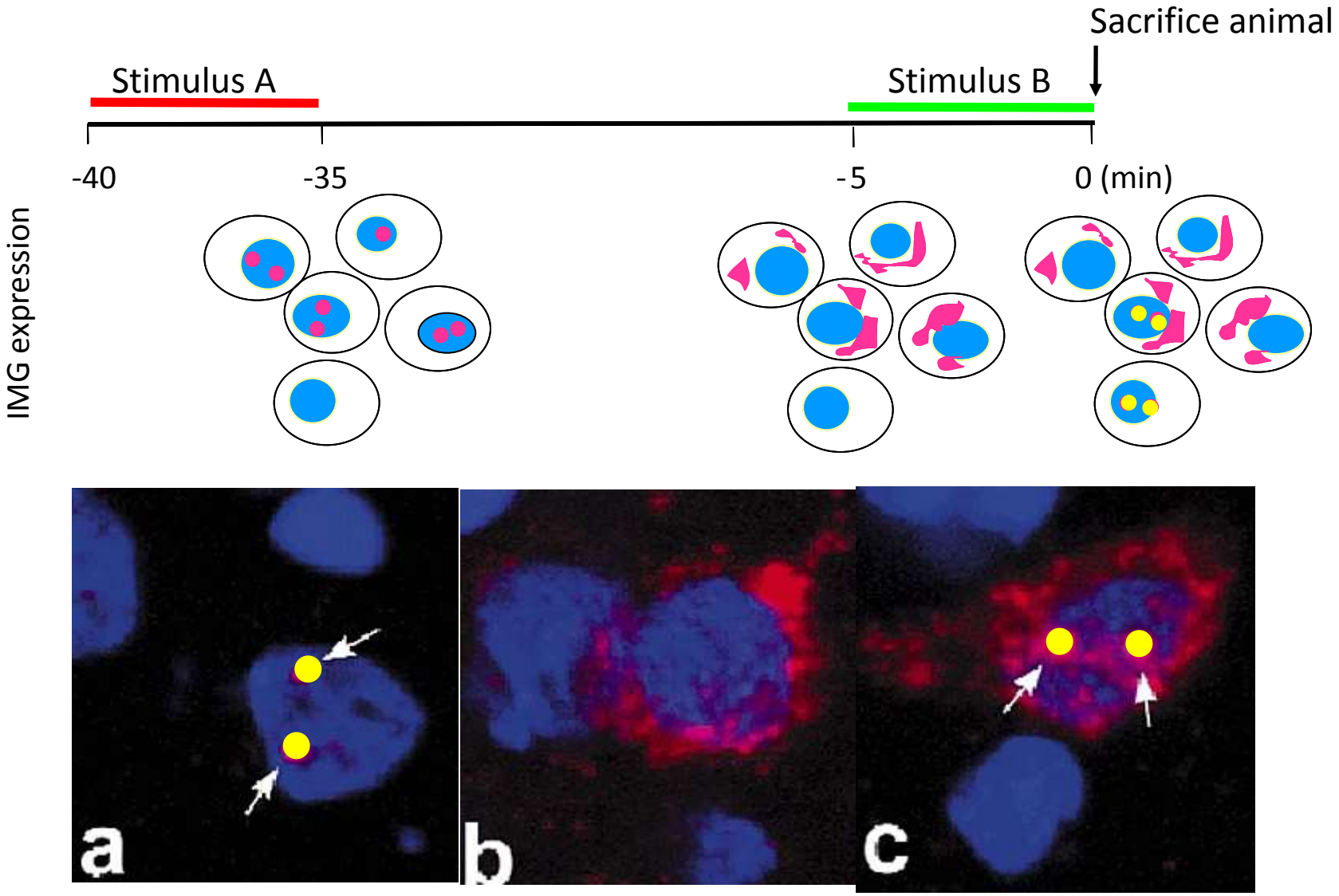
Fos expression patterns after fighting and mating



PMv: premammillary nucleus
MPN: Medial preoptic nucleus
VMH: Ventromedial hypothalamic nucleus

Immediate early gene (IMG) CatFISH —

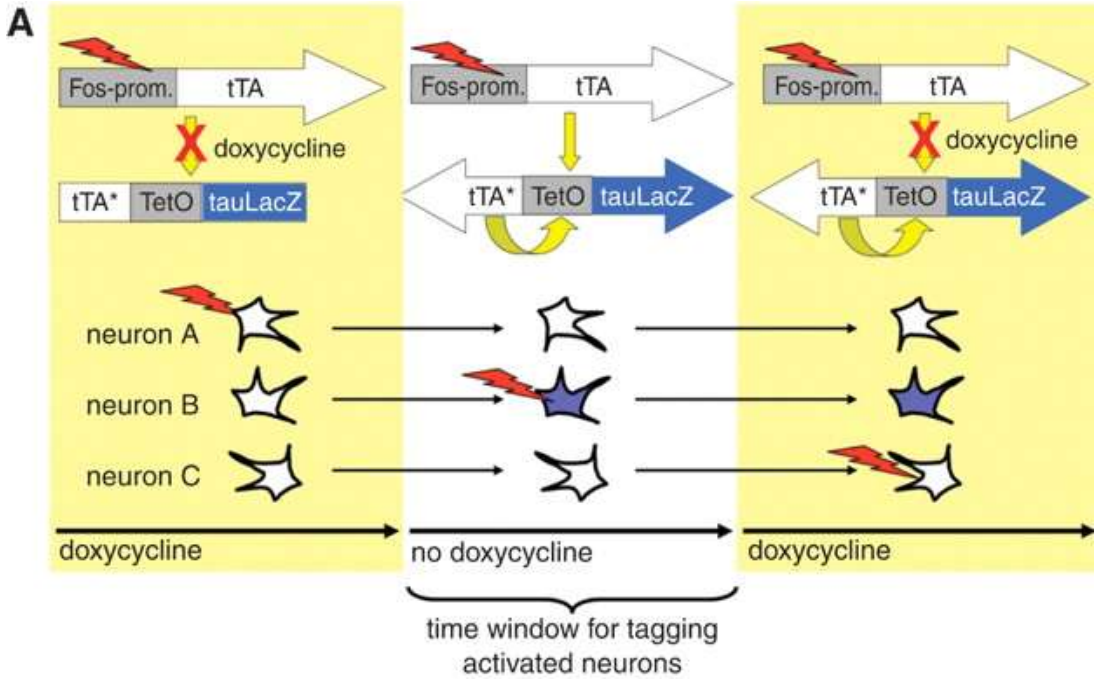
cellular compartment analysis of temporal activity by fluorescence in situ hybridization

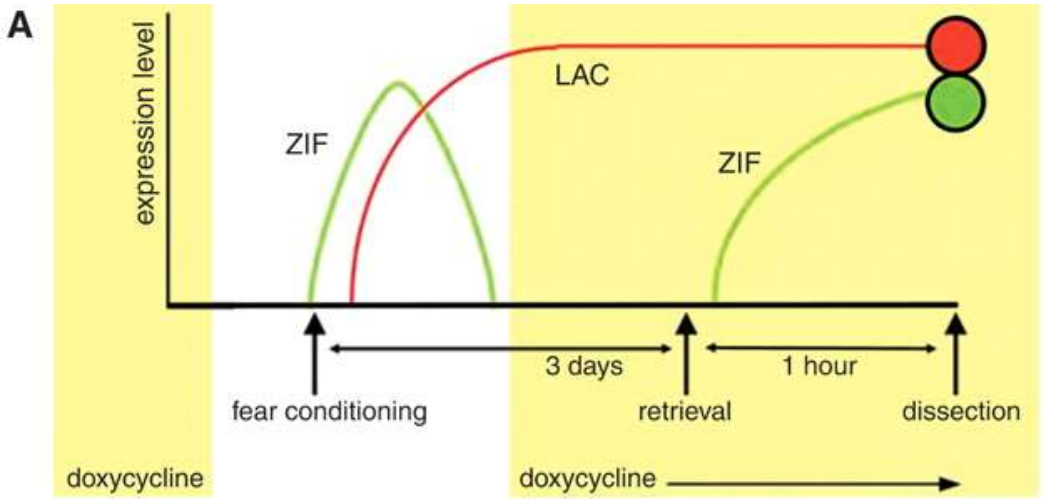


Guzowski JF et al. Nature Neuroscience1999.
Cite ~550

Long term tagging activated cells

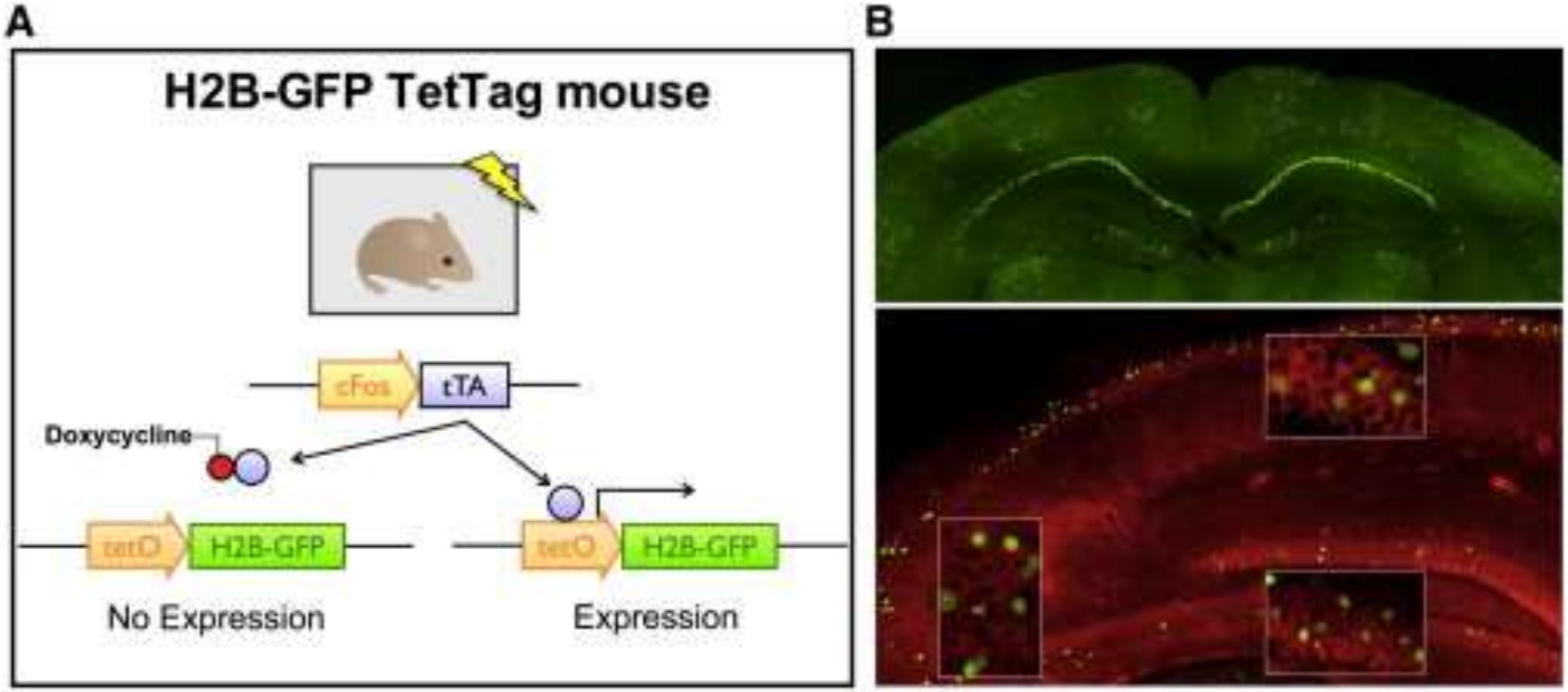
tTA: tetracycline transactivator
tetO: tTA binding sites





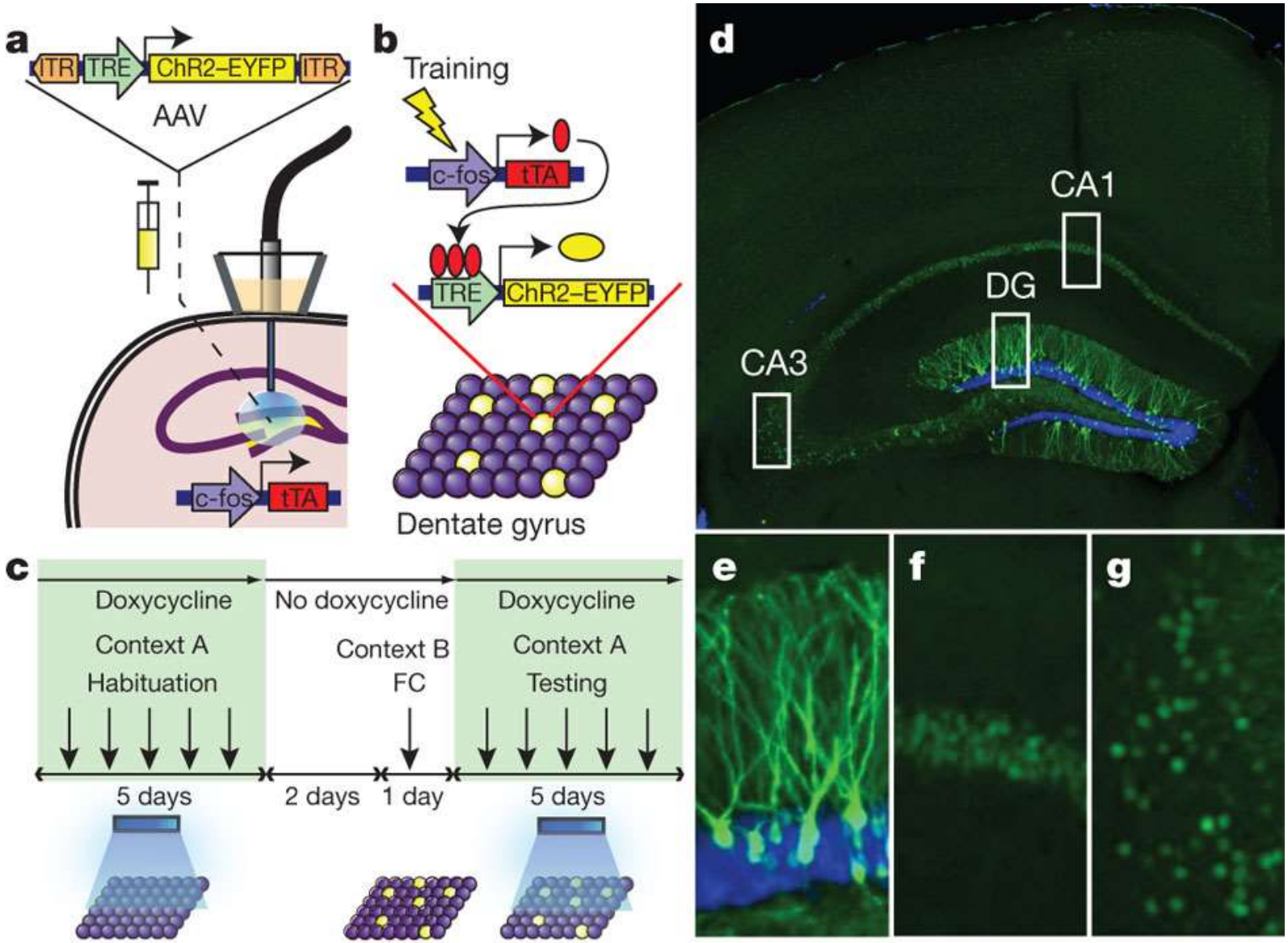
Reijmers et al, Science, 2007, cite ~240

TetTag mouse with long lasting GFP



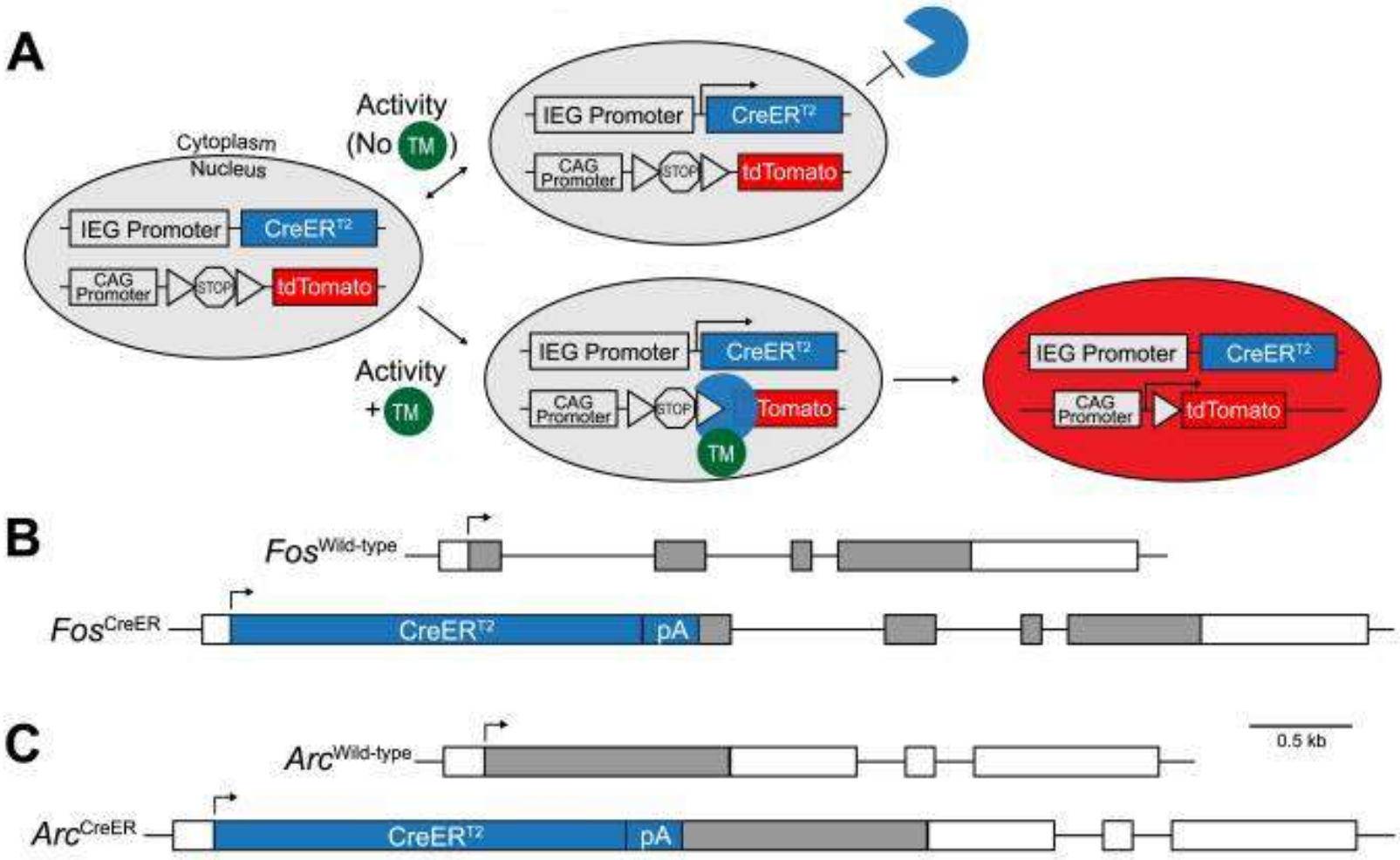
Tayler K et al. Current Biology, 2013

TetTag system to label active cells with ChR2



Xu Liu… Susumu Tonegawa. Nature, 2012, cite ~330

Labeling of transiently activated neurons using TRAP



Guenthner C… Liqun Luo, Neuron 2013

Electrophysiology

Methods:

Intracellular recording (Patch clamp or sharp electrode)

Cell attach recording

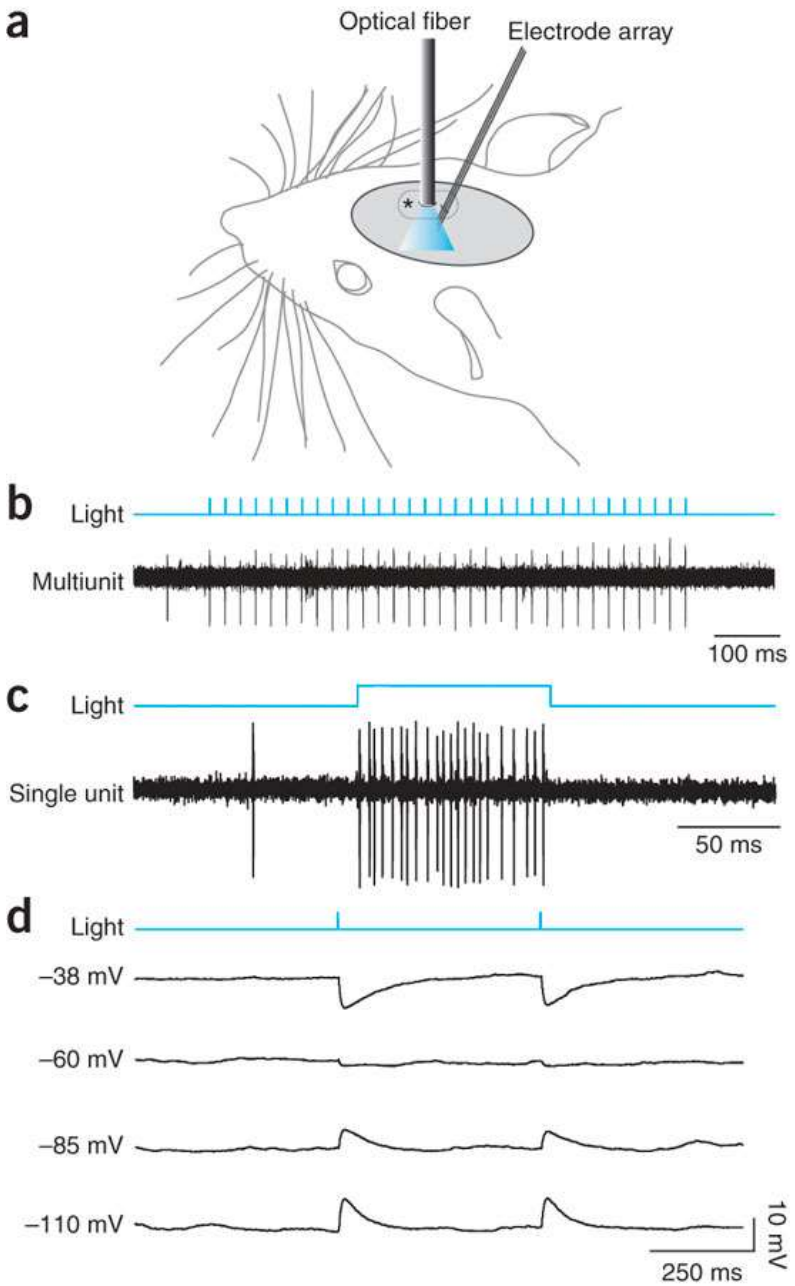
Extracellular recording (single or multiple electrodes)

Preparation:

In vitro (slice or cells)

In vivo (anaesthetized animals, head fixed awake animal, free moving animals)

Combining ChR2 and electrophysiology



Functional imaging --detecting the activity of neurons by optical imaging

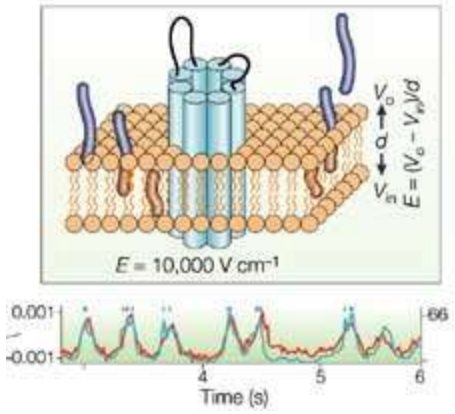
What kind of changes will occur when a brain area (or cell) becomes active?

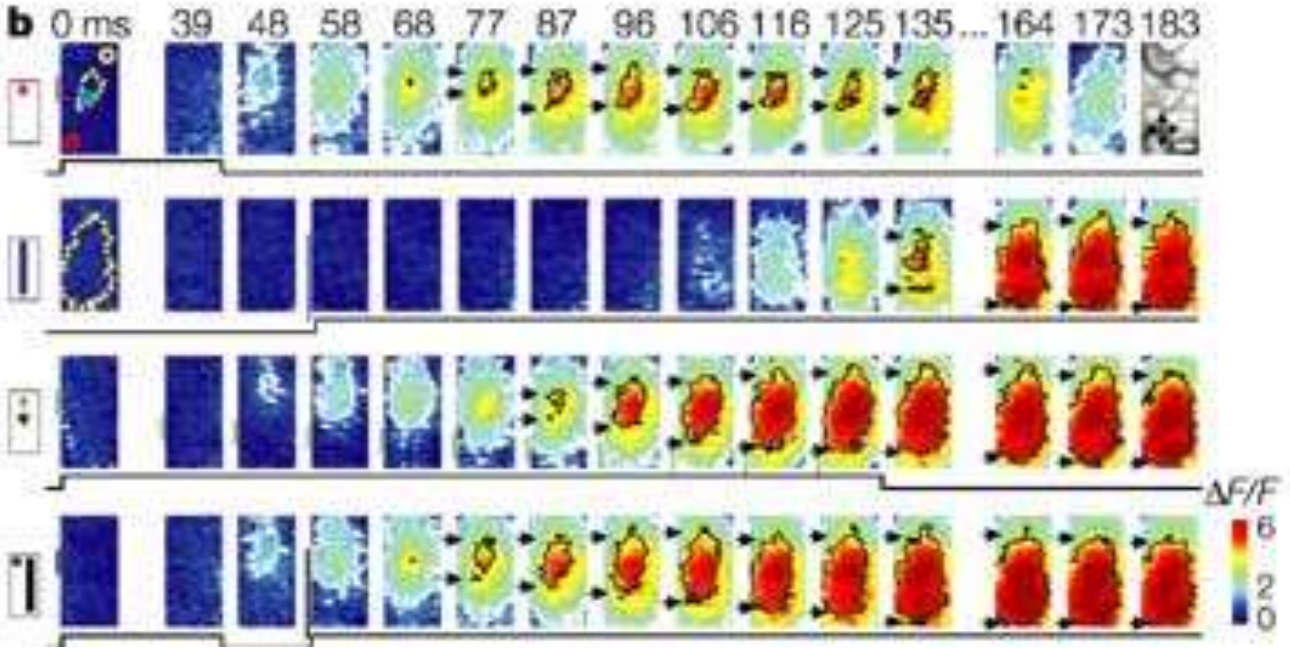
Membrane potential
Calcium concentration
Chloride concentration
Sodium concentration
Potassium concentration
PH level (synapse)
Oxygen level

How to convert non-optical changes to optical changes?

Voltage sensitive dye
Calcium sensitive dye
Synapto-pHluorin
Camgaroo- ratiometric GECl

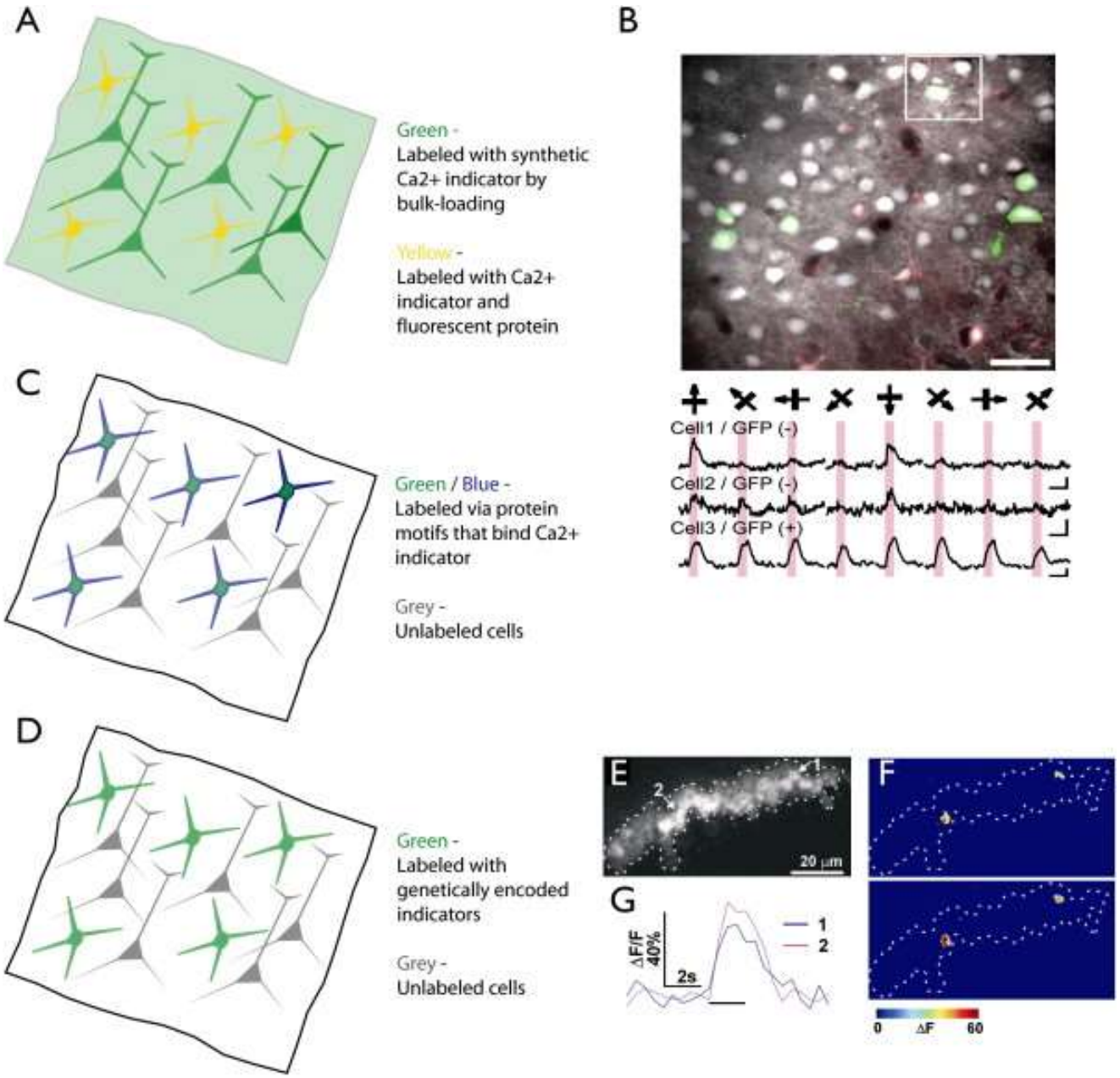
Functional imaging--Voltage sensitive dye



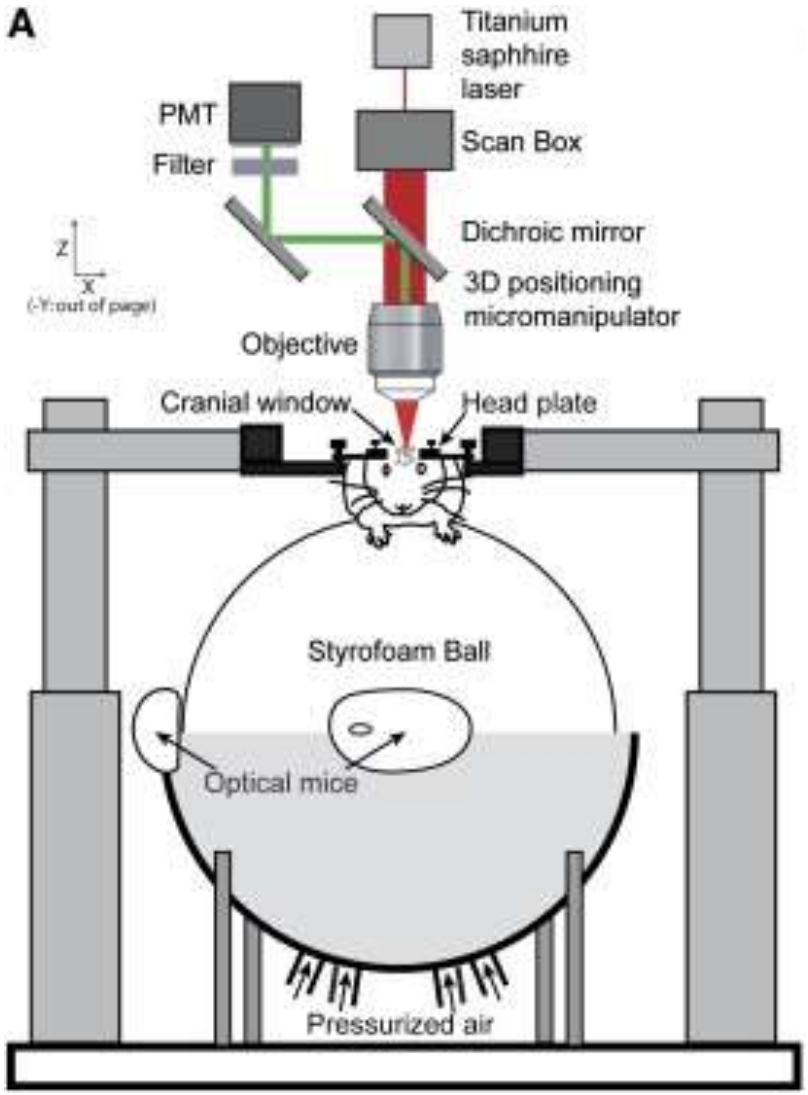


Jancke, 2004, Nature

Functional Imaging –Calcium imaging

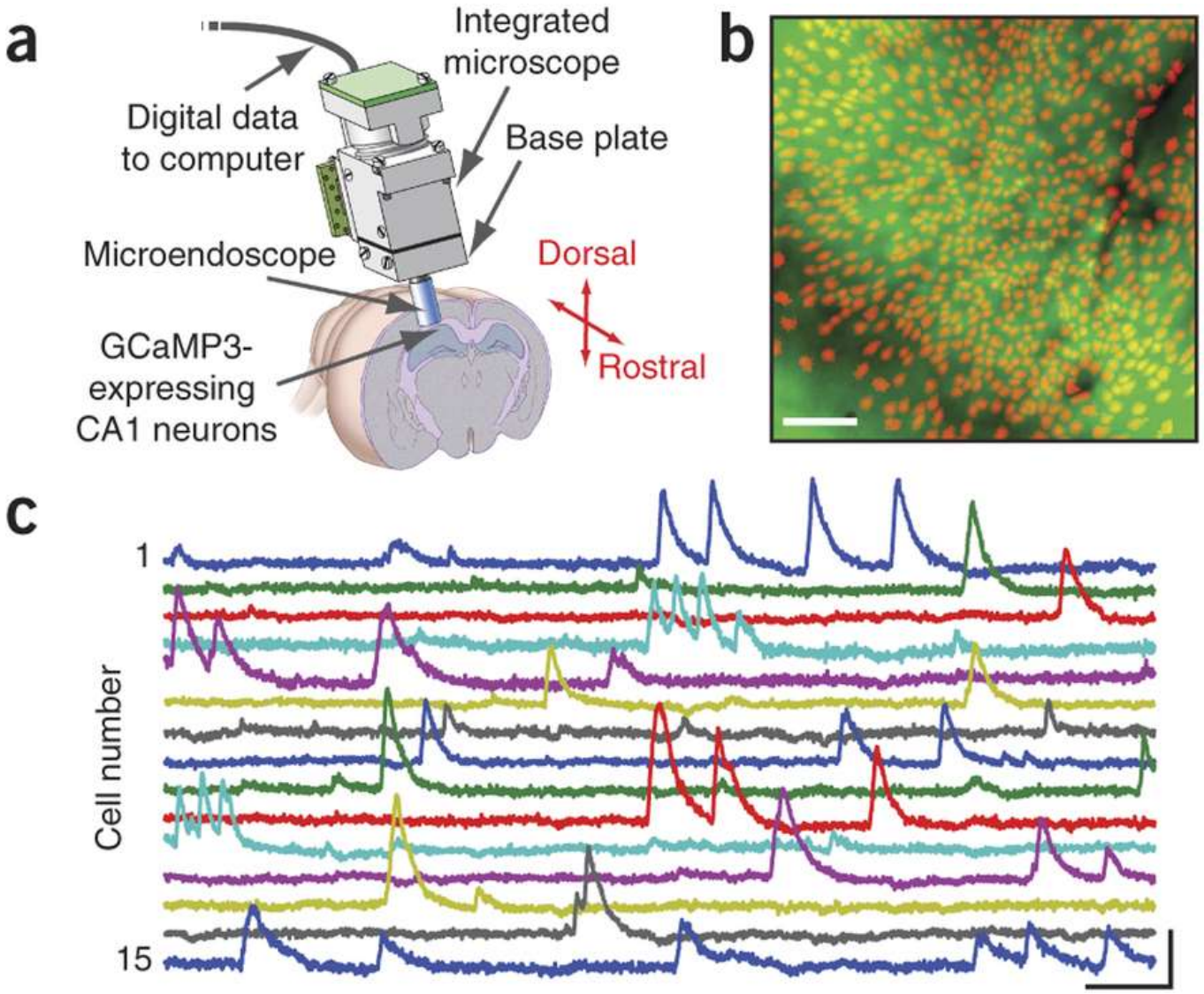


Head Fixed 2-photon calcium imaging



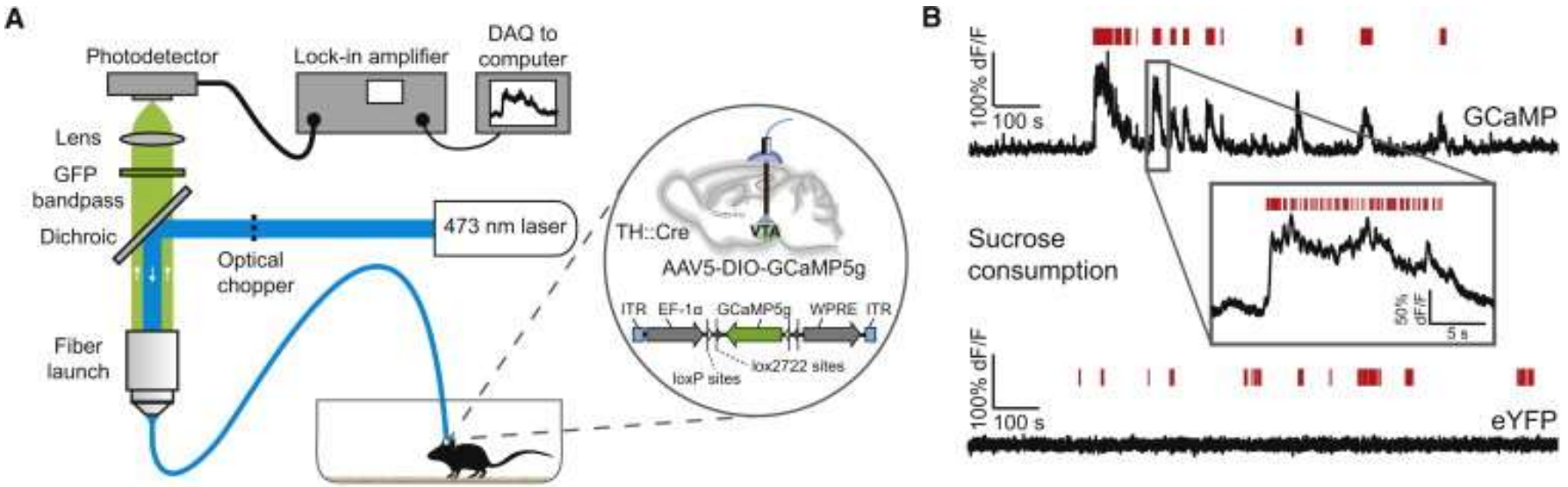
Daniel A. Dombeck… David Tank
Nature Neuroscience, 2010, Cite 230

1-photon Calcium imaging in awake behaving animals



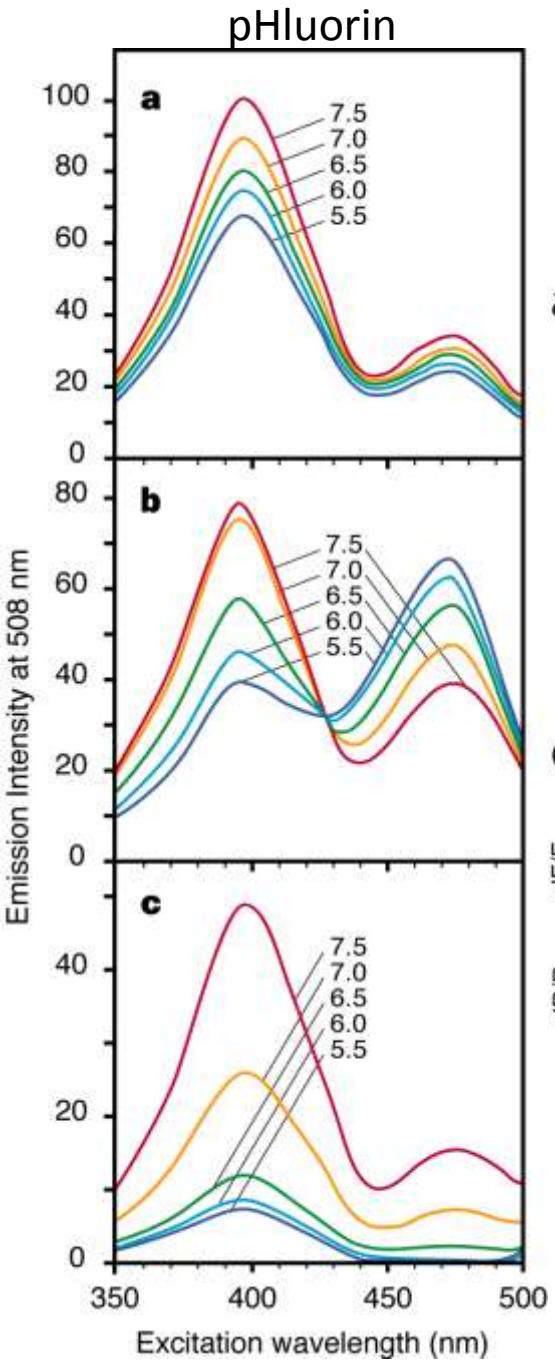
Yaniv Ziv … Mark Schnitzer 2013, Nature Neuroscience, cite ~140

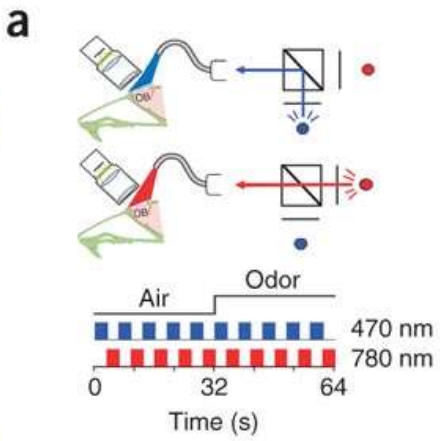
Population calcium imaging in awake behaving animals –fiber photometry

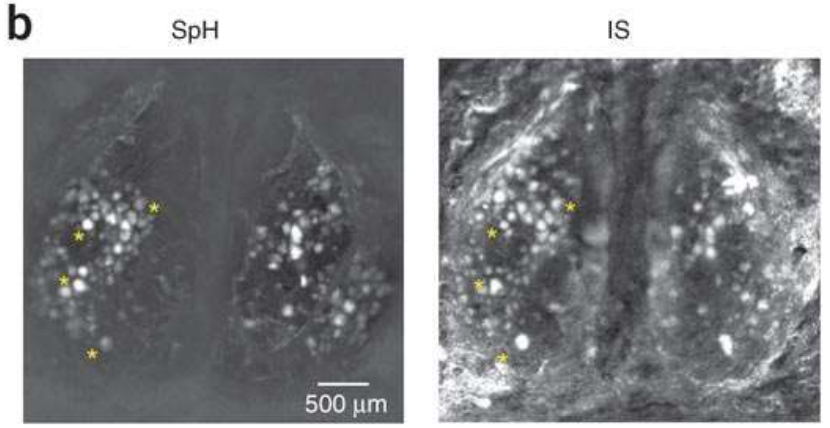


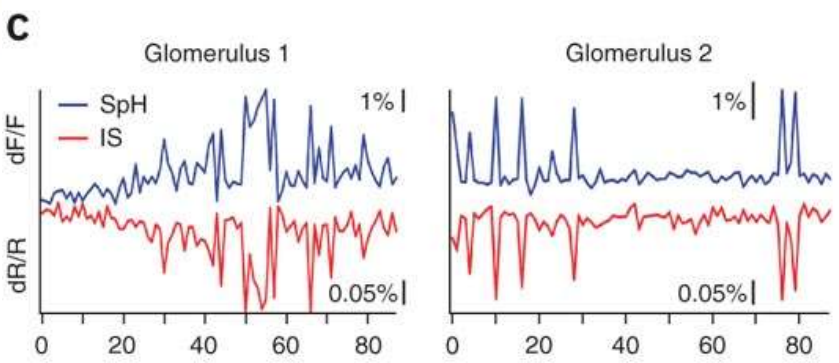
Lisa A. Gunaydin… Karl Deisseroth. Cell, 2014

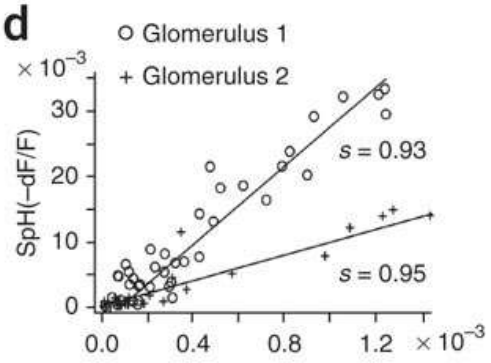
Synapto-pHluorin and Intrinsic signal imaging





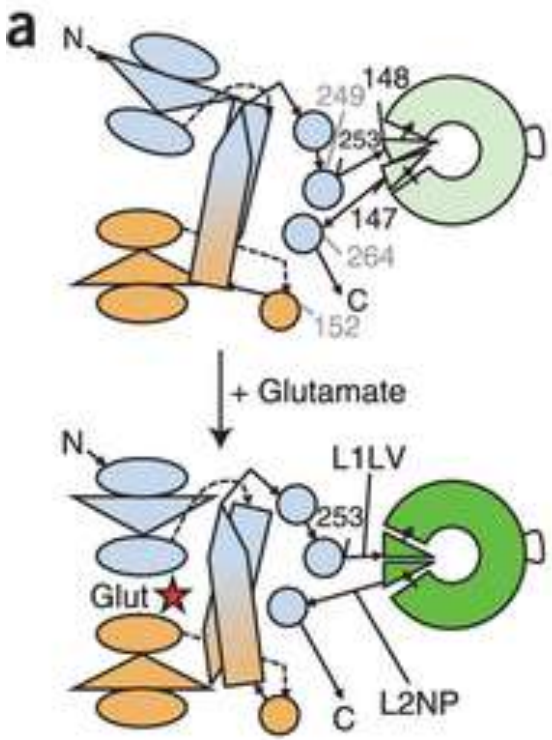


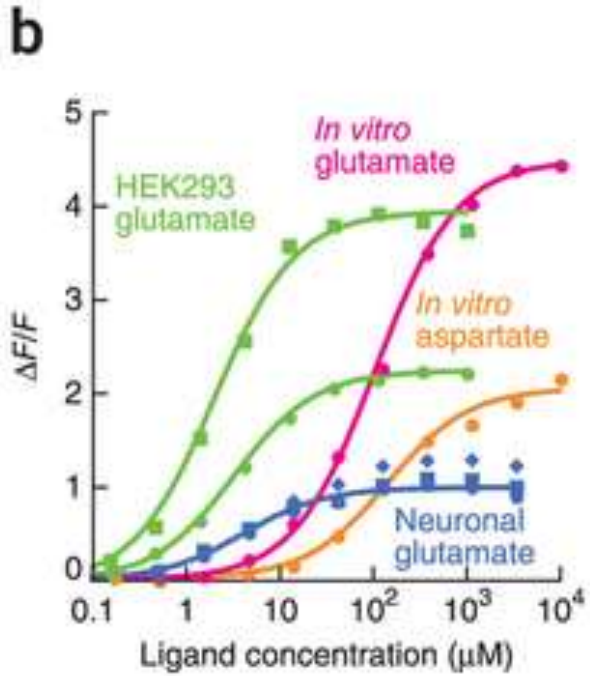


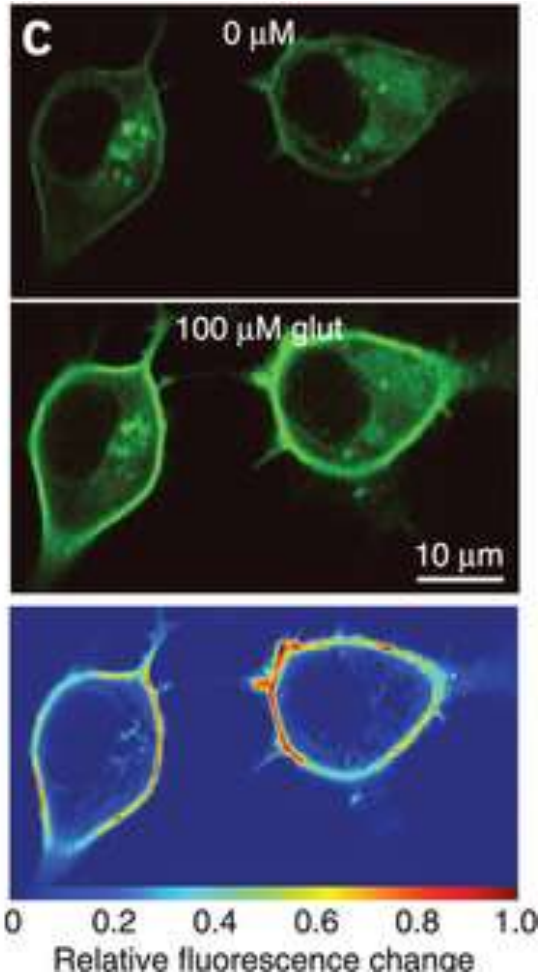


iGluSnFR as an glutamate sensor

Bacterial periplasmic binding proteins (PBPs)







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- **Visualize cells**
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- **Manipulate cell/gene activity**

Manipulate cell/gene activity

Region

1. Electrical Lesion
2. Pharmacological inactivation
3. Electrical stimulation
4. Pharmacological activation

Cells

1. Inactivate
2. Activate
3. Killing

Genes

1. Traditional gene targeting
2. Transgenic
3. CRISPR/Cas9

Receptors

1. Antagonist
2. Agonist
3. Ligand uncaging

Manipulate cell/gene activity

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2. Pharmacological inactivation
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Genes

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1. Antagonist
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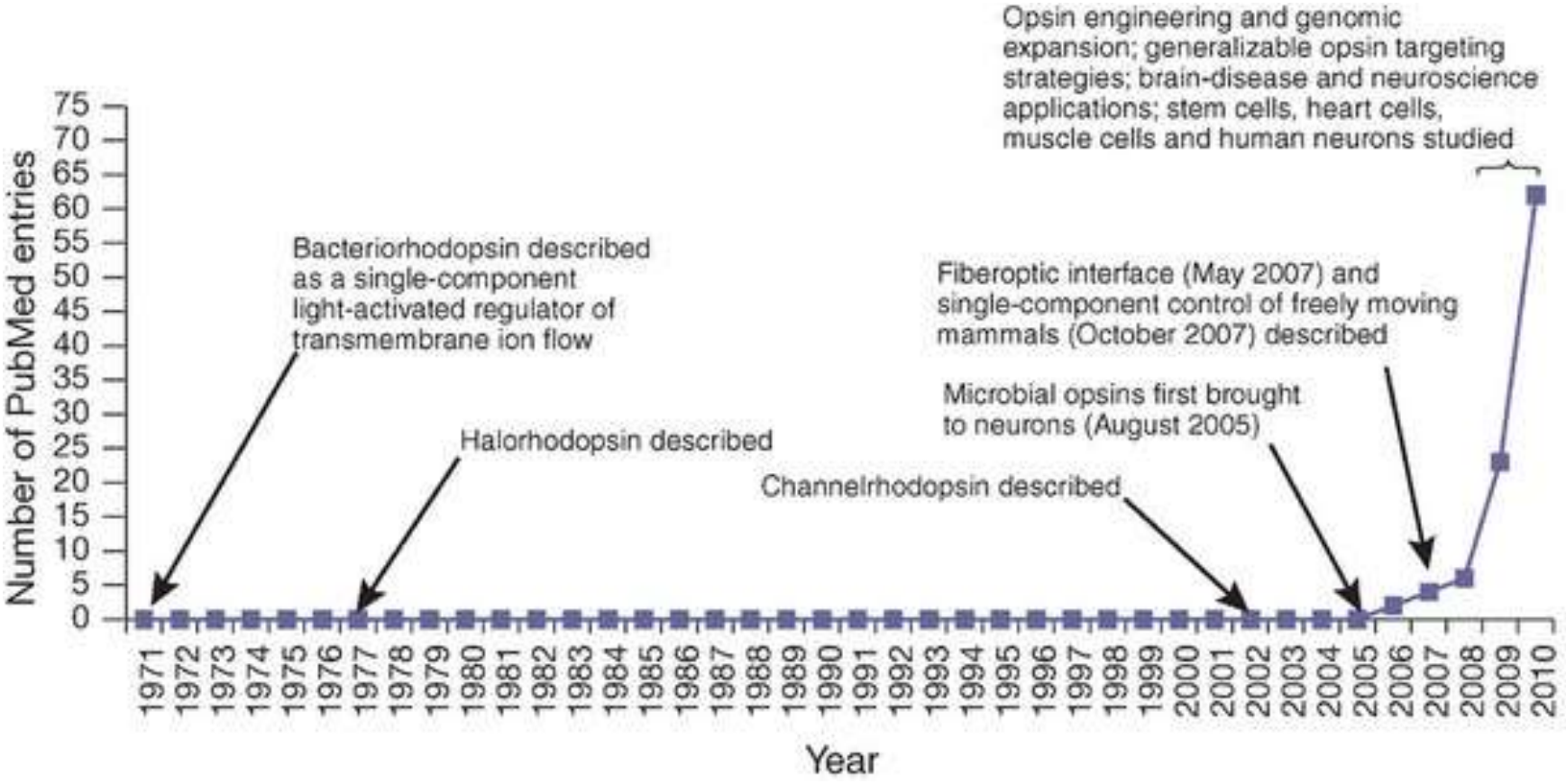
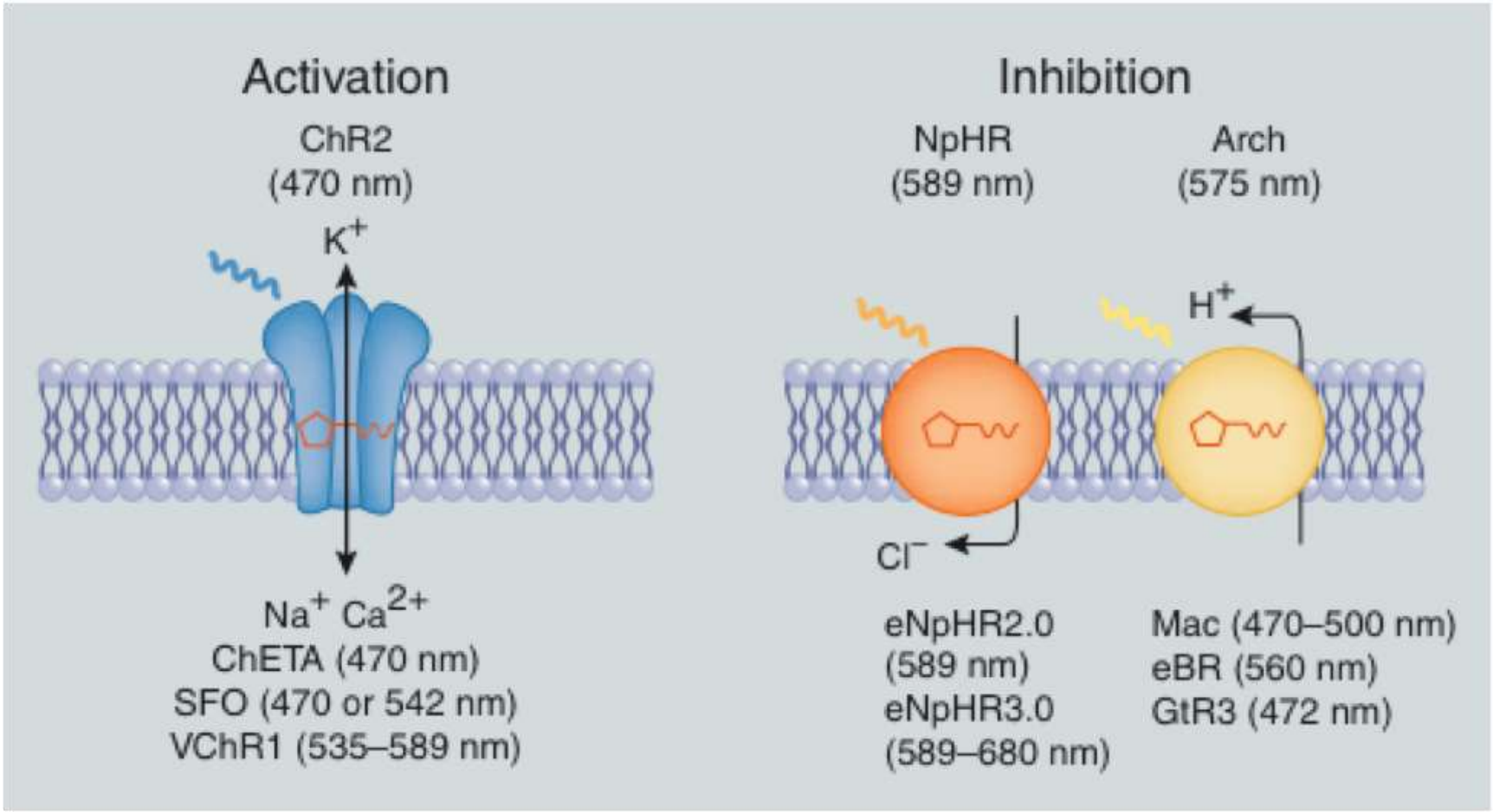
Light mediated cell control -- optogenetics



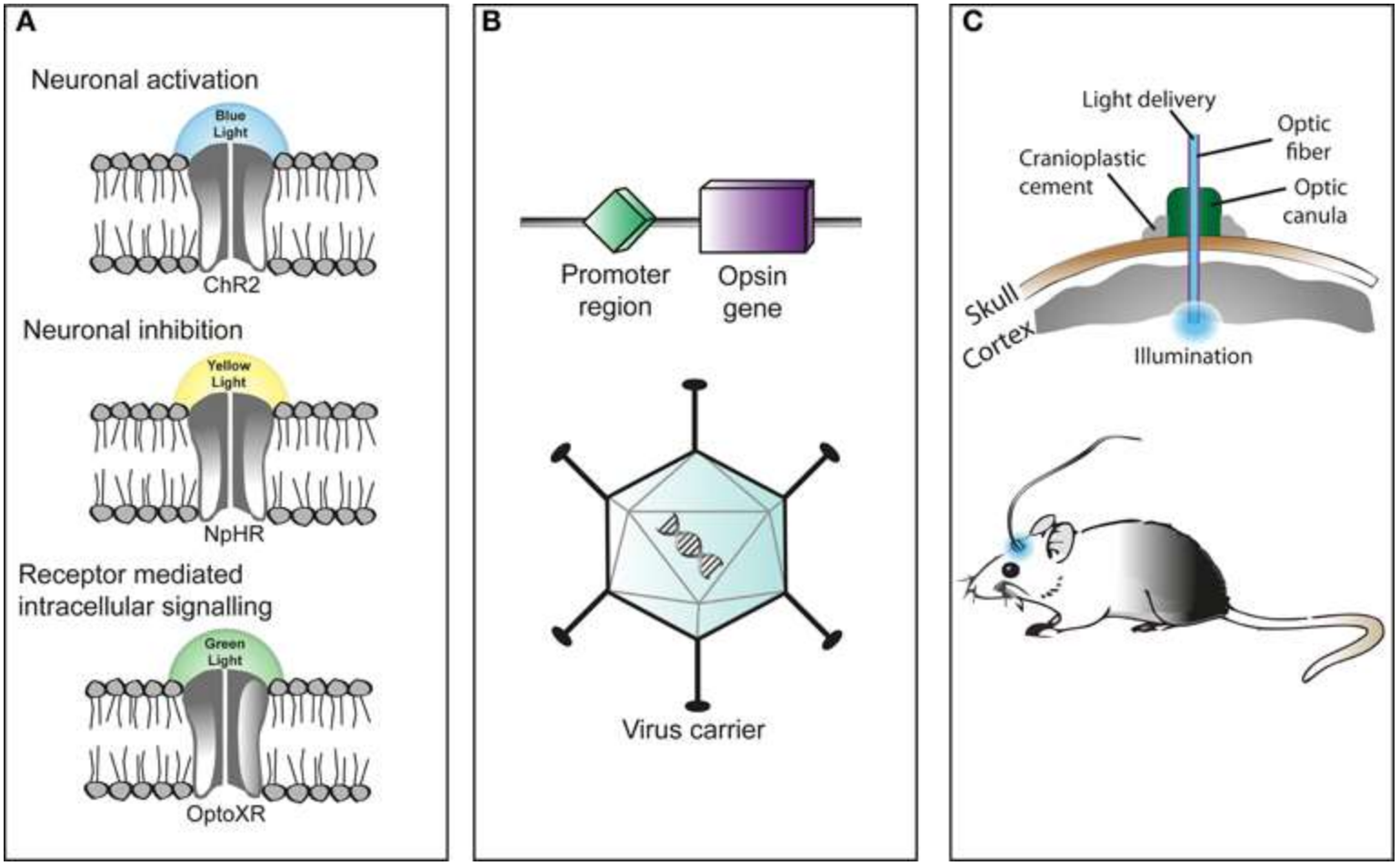
Karl Deisseroth and Ed Boyden
2005 Nature ~2000 citations

Optogenetics



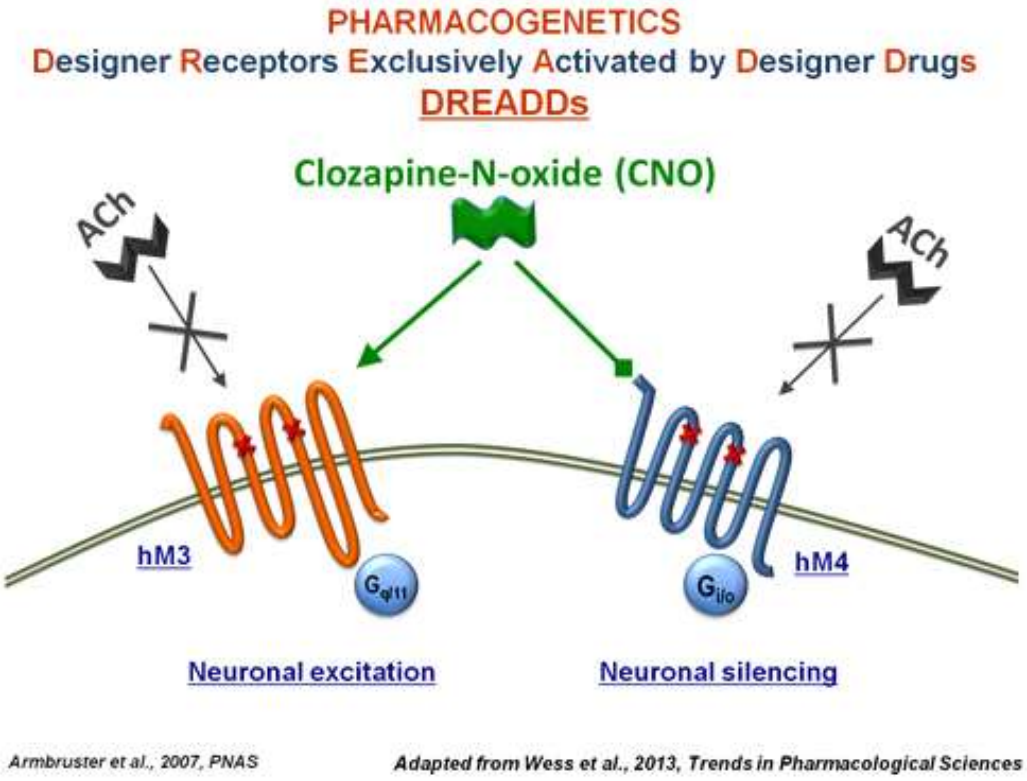
Karl Deisseroth and Ed Boyden
2005 Nature ~2000 citations

In vivo optogenetic control of cell activity



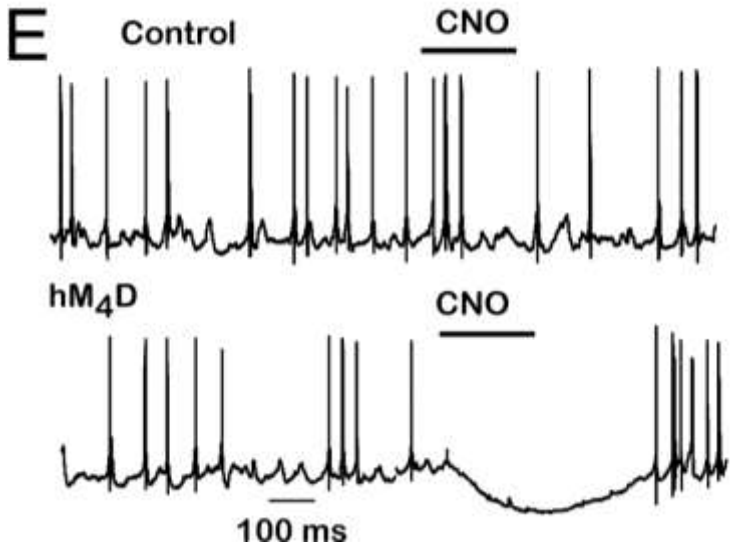
Ligand mediated cell control --phamacogenetics

DEARDD



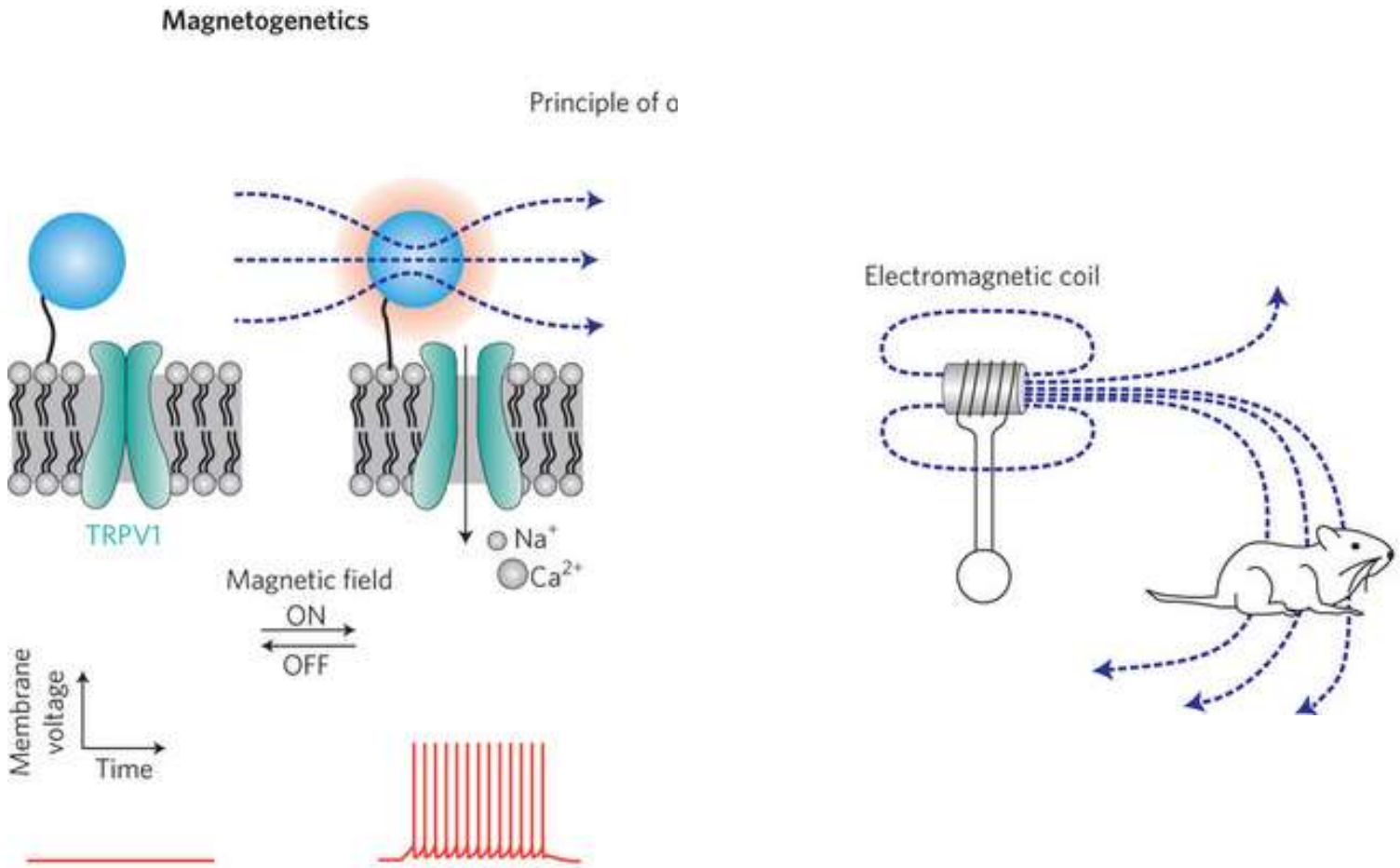
Armbruster et al., 2007, PNAS

Adapted from Wess et al., 2013, Trends in Pharmacological Sciences



2007 PNAS Armbruster BN et al.
Cite ~390

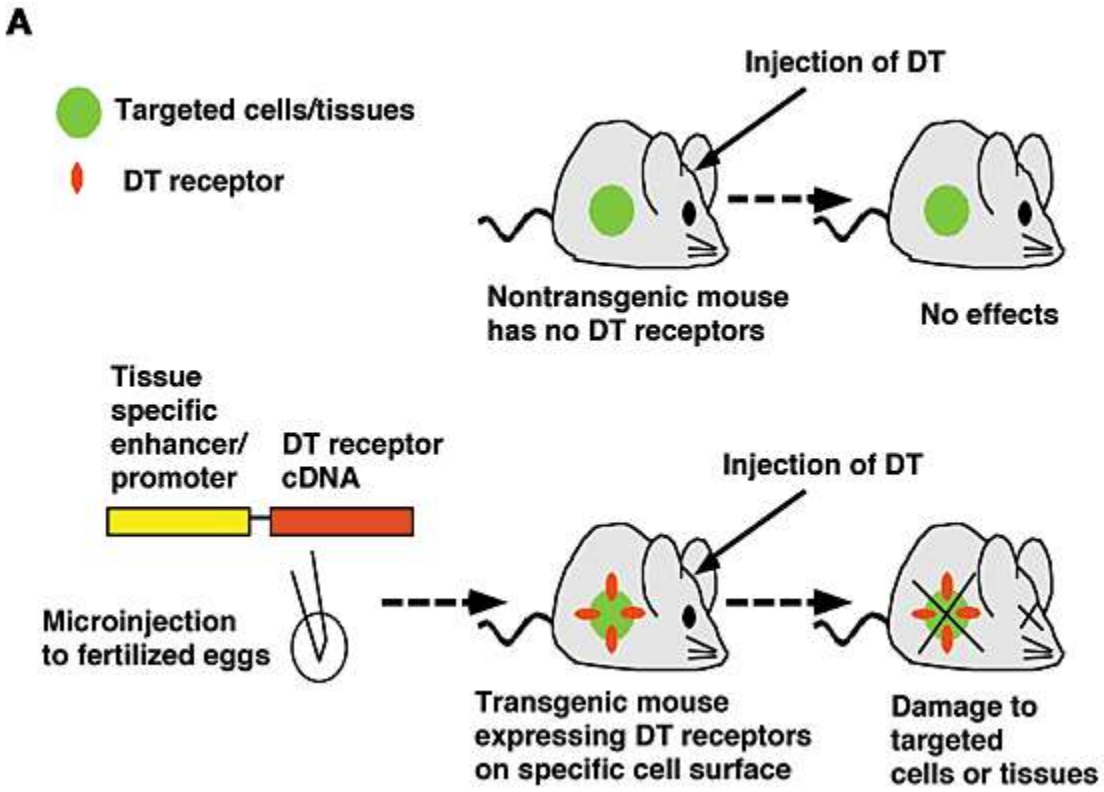
Magnetic field mediated cell control -- Meganogenetic



Heng Huang et al. Nature Nanotechnology, 2010
Cite ~220

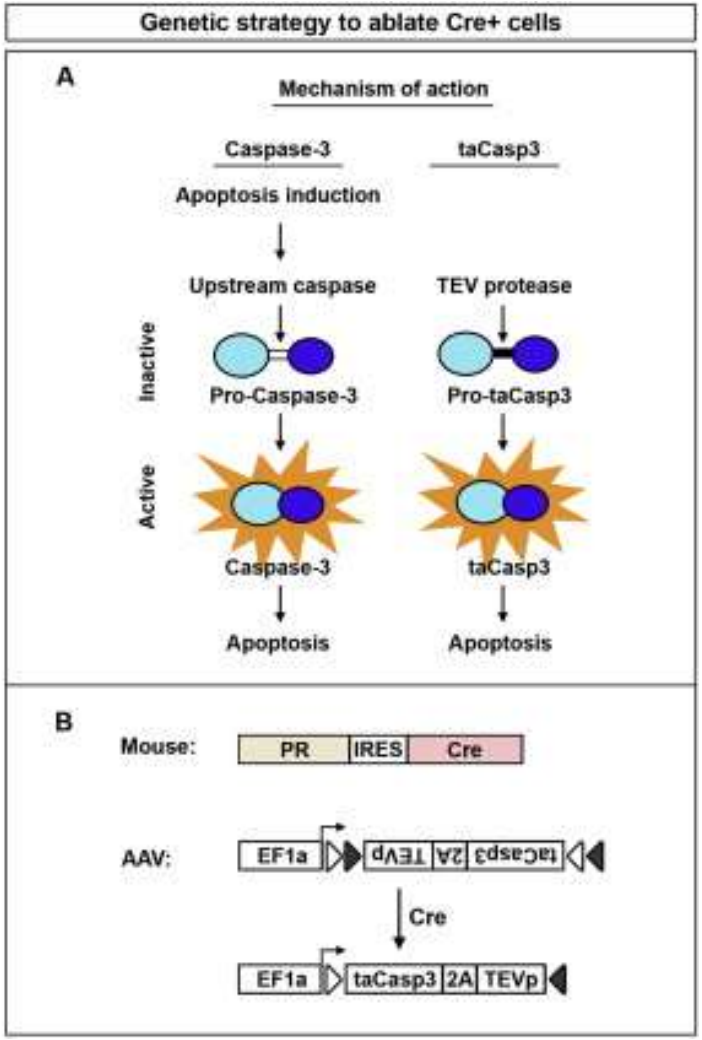
Diphtheria toxin receptor (DTR) mediated cell killing

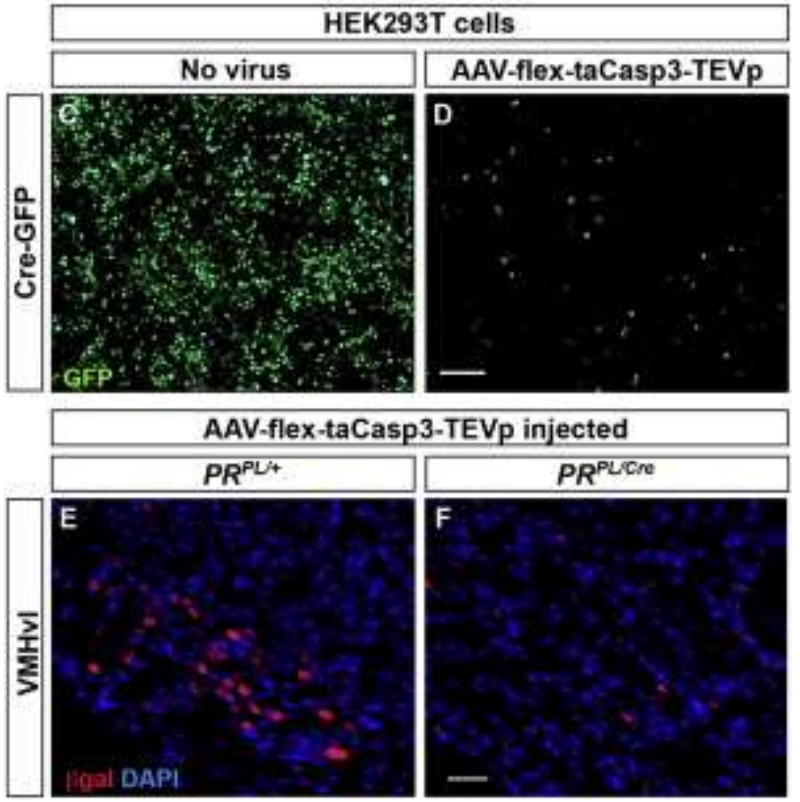
One molecular of DT can kill a cell – Cell, 1978, Yamaizumi, M.



Michiko Saito et al. 2001, Nature Biotechnology
Cite ~250

Caspase 3 mediated cell killing





Cindy F. Yang et al. Cell, 2013, ~60

Manipulate cell/gene activity

Region

1. Electrical Lesion
2. Pharmacological inactivation
3. Electrical stimulation
4. Pharmacological activation

Cells

1. Inactivate
2. Activate
3. Killing

Genes

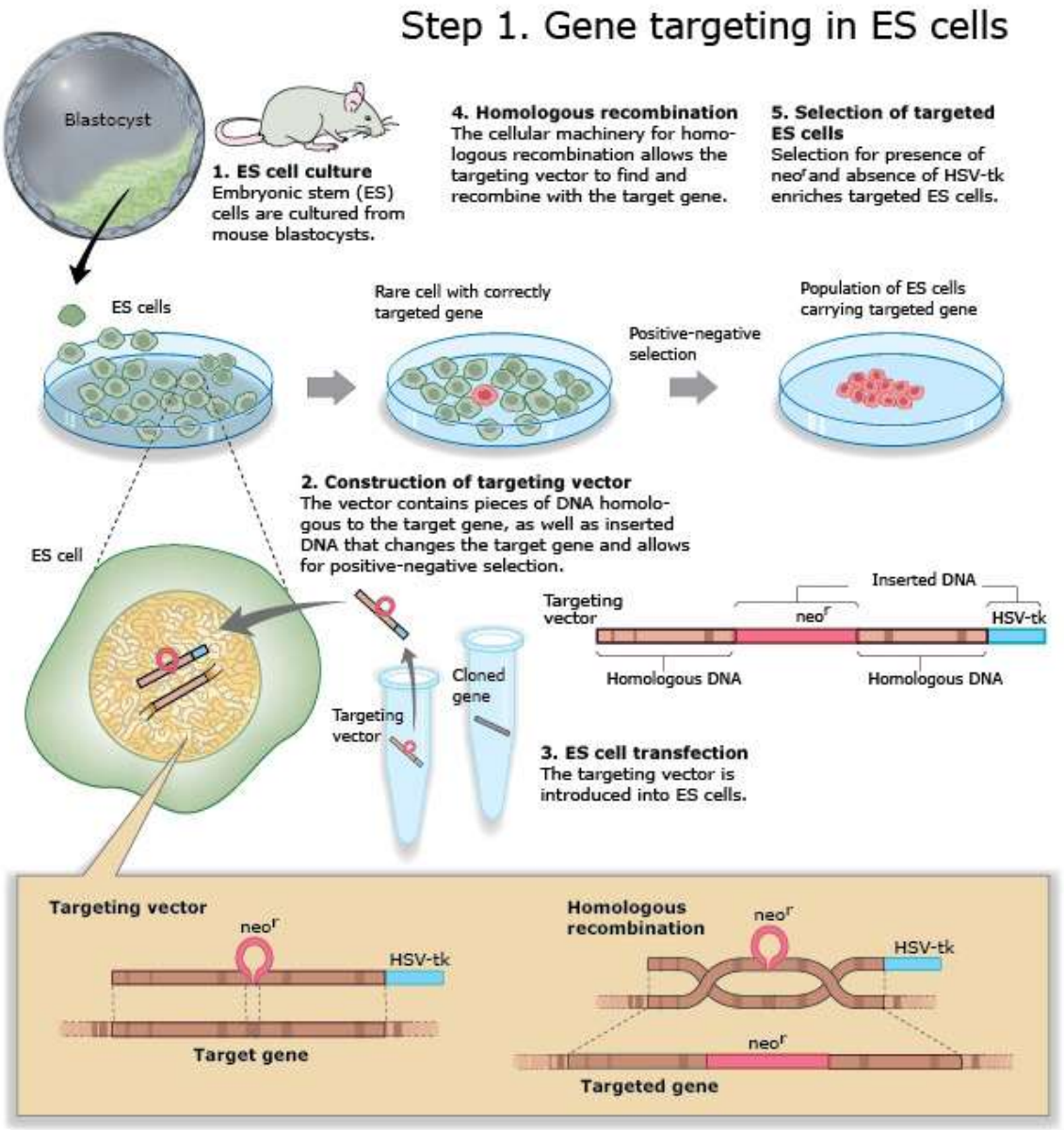
1. Traditional gene targeting
2. Transgenic
3. CRISPR/Cas9

Receptors

1. Antagonist
2. Agonist
3. Ligand uncaging

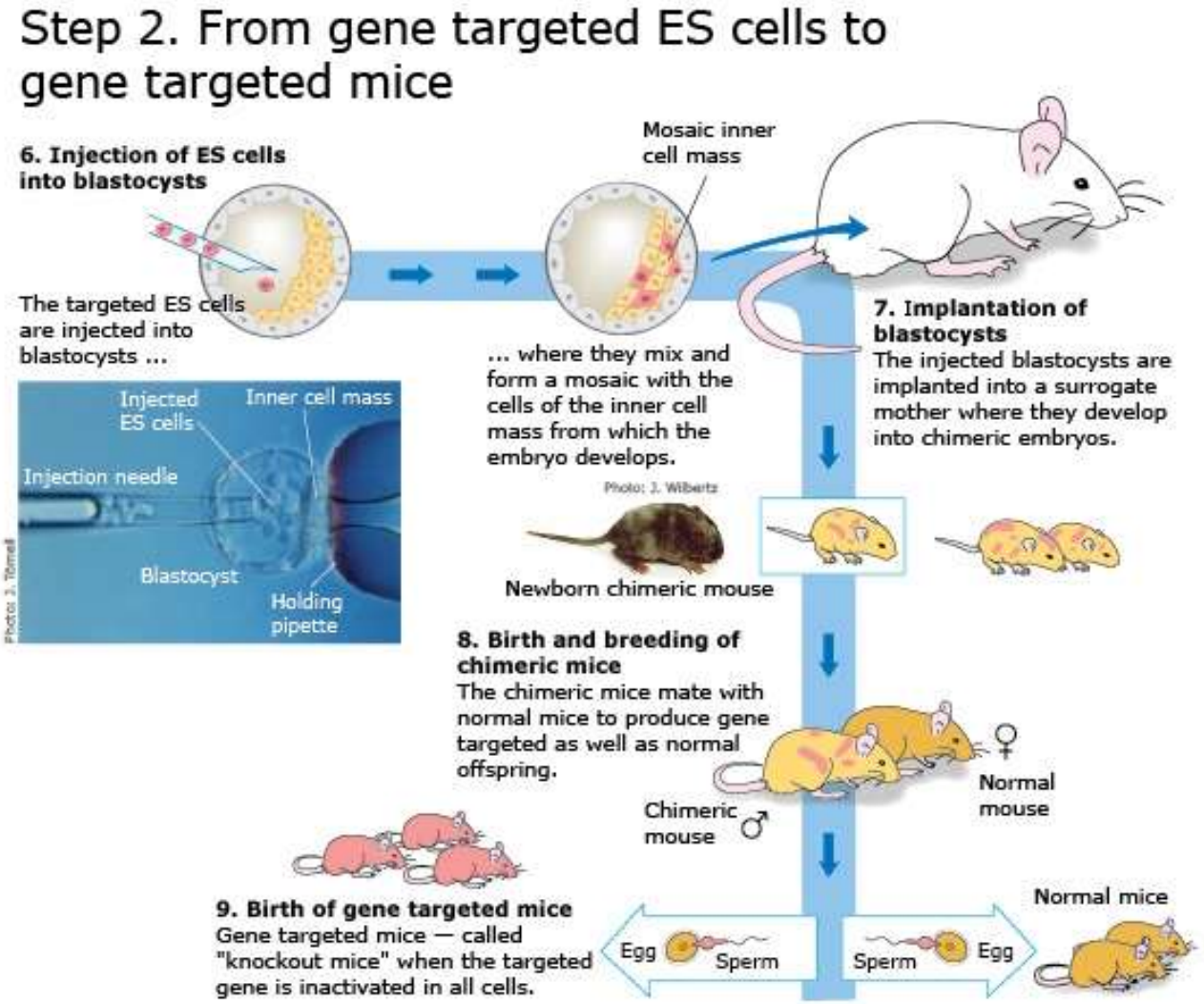
Traditional gene targeting

Rely on rare homologous recombination in ER cells

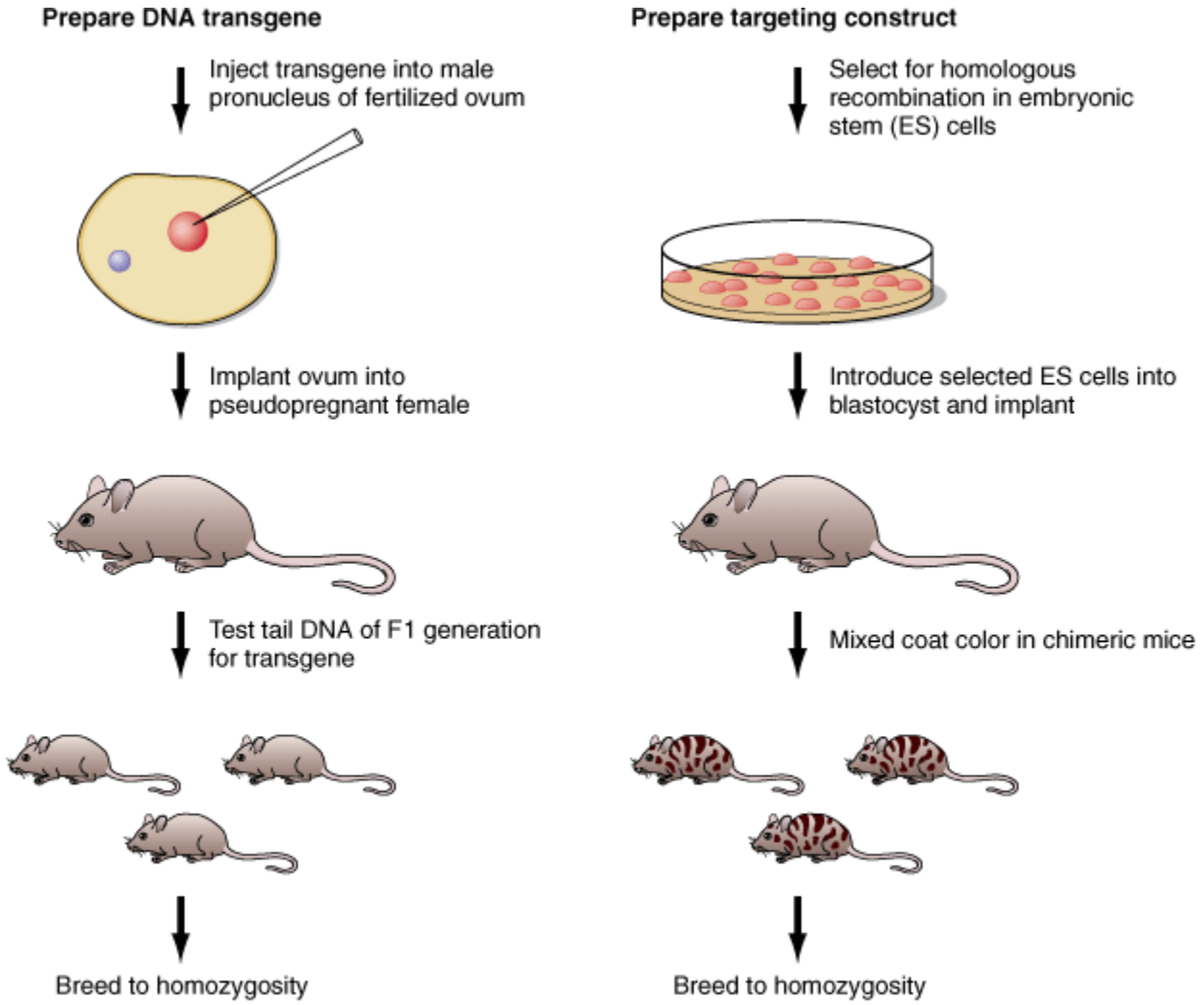


Mario R. Capecchi,
Martin J. Evans and
Oliver Smithies
Nobel Prize 2007

Traditional gene targeting

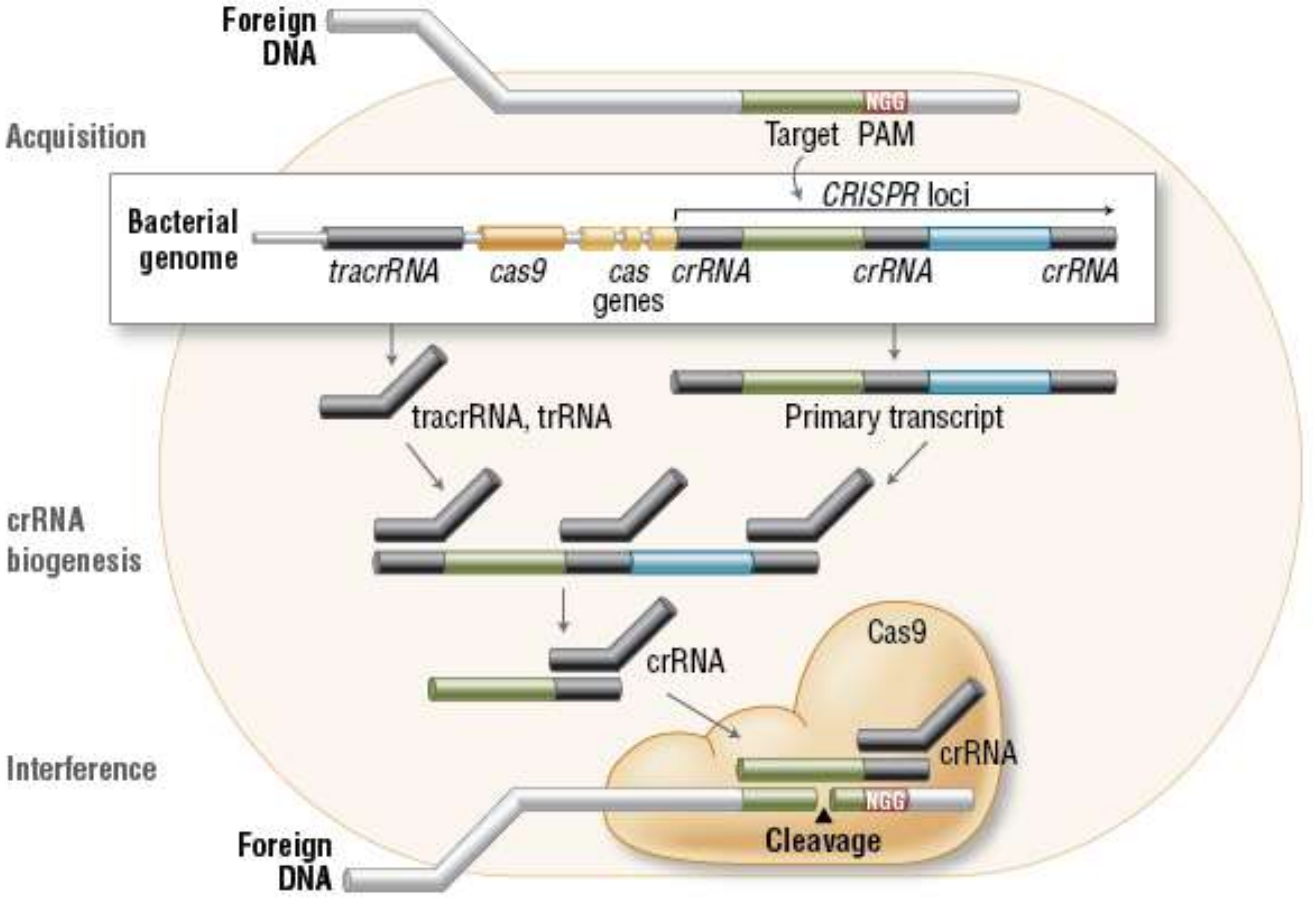


Transgenic vs. gene targeting



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

CRISPR/Cas9:Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR Associated (Cas) system-- bacterial self-defense mechanism



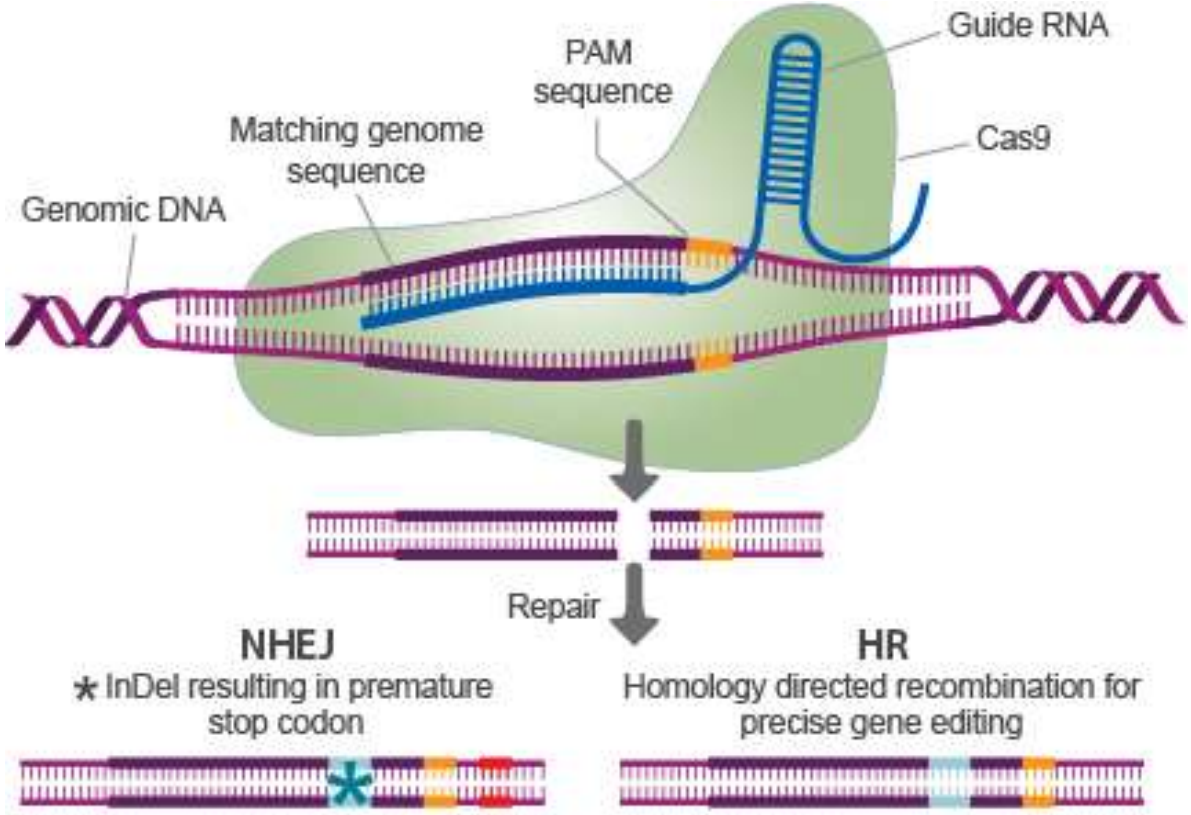
Ishino, Y., et al. (1987) *J. Bacteriol.* 169, 5429–5433.

PAM: protospacer-associated motif

crRNA: CRISPR targeting RNA

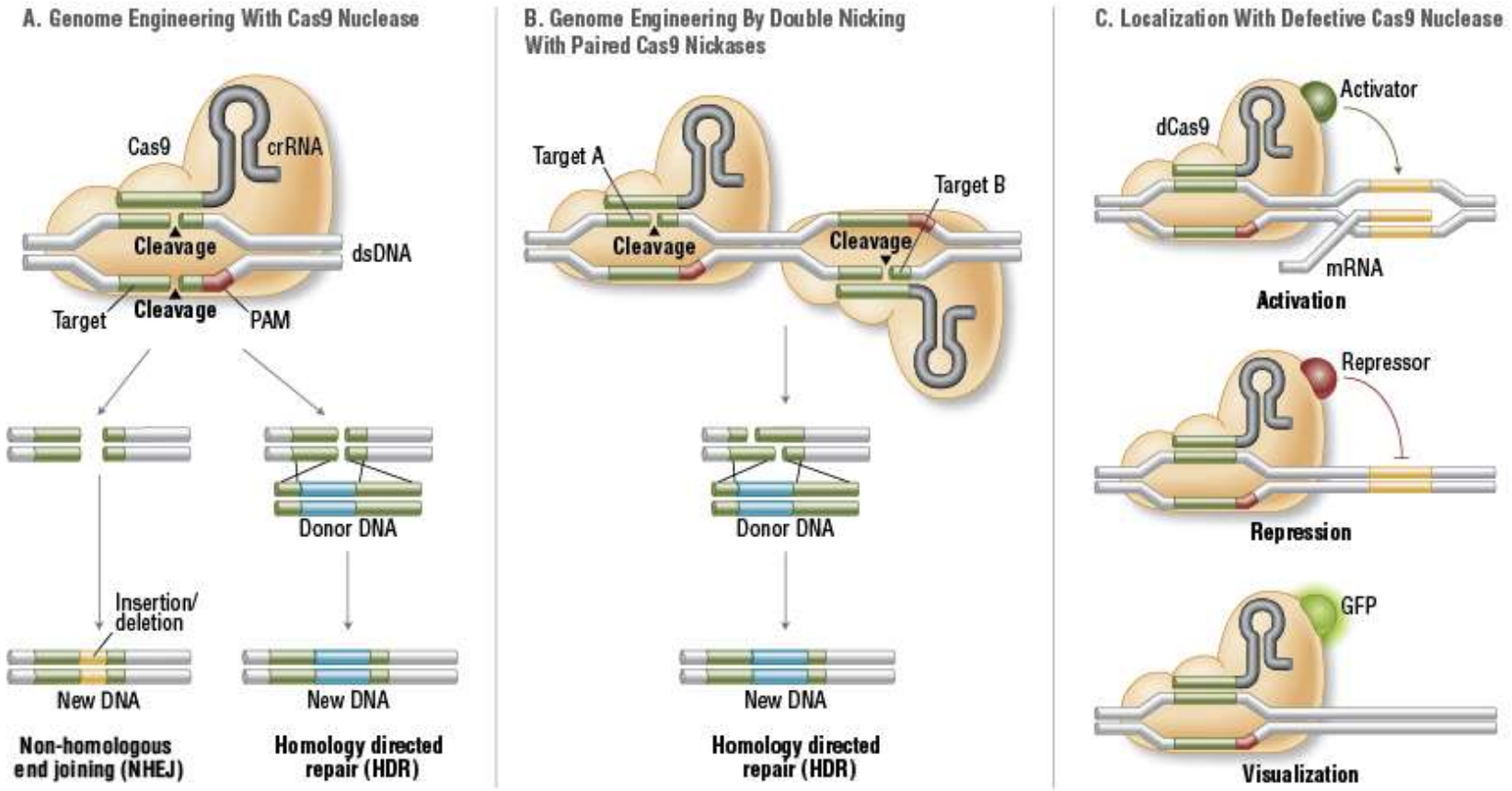
Required for the Cas(to recognized the sequence 2-5 conserved bp

CRISPR/Cas9 mediated gene targeting -- a much more efficient method

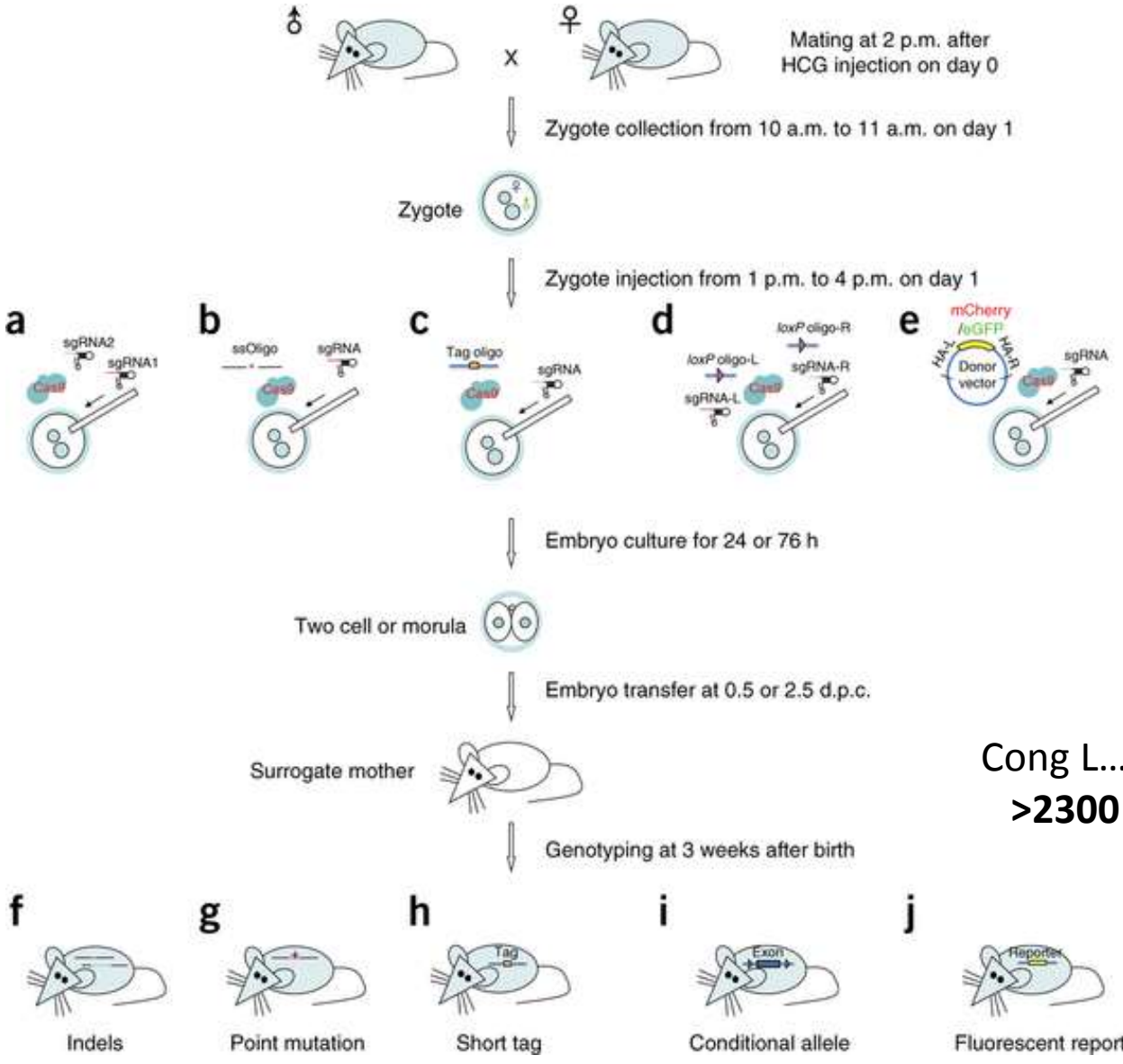


Deltcheva, E., et al. (2011) *Nature,* 471, 602–607. Cite ~410

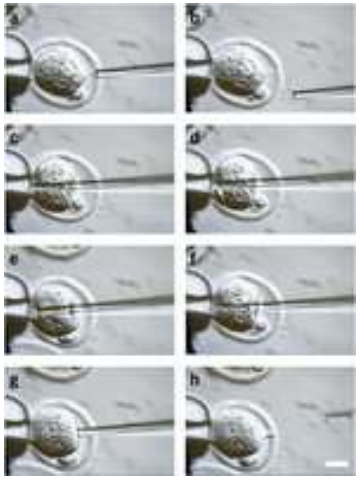
Other variations of CRISPR/Cas9



CRISPR/Cas9 mediated gene targeting
Time: 1-3 months



Injection of zygotes



Cong L… Zheng F. (2013) *Science*
>2300 Citations!