Methods in Systems Neuroscience

NS201C

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Lecture Outline

- Methods for in vivo monitoring of neural activity with single-cell resolution

- Methods for in vivo perturbation of brain function

- Methods for in vivo manipulation of neural signaling with single cell resolution

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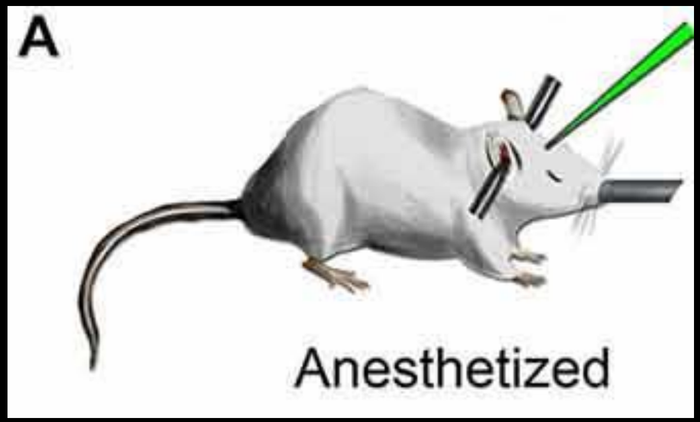
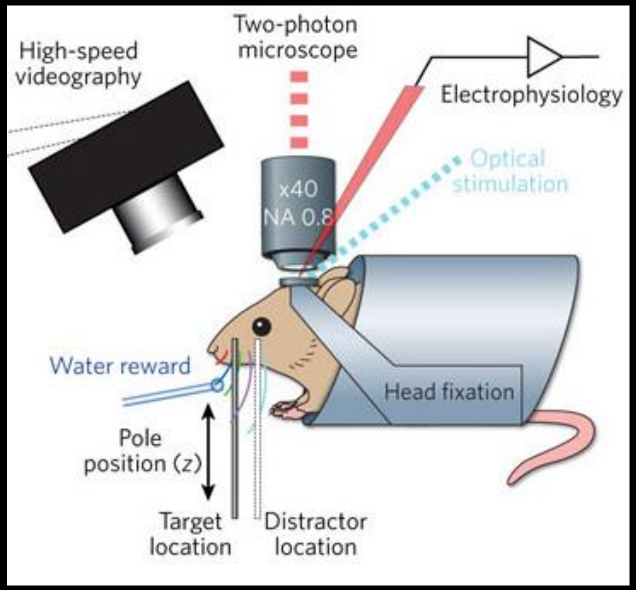
Experimental Preparations for In Vivo Recording

- Anesthetized, head-fixed animals
- Awake, head-fixed animals
- Awake, freely-moving animals

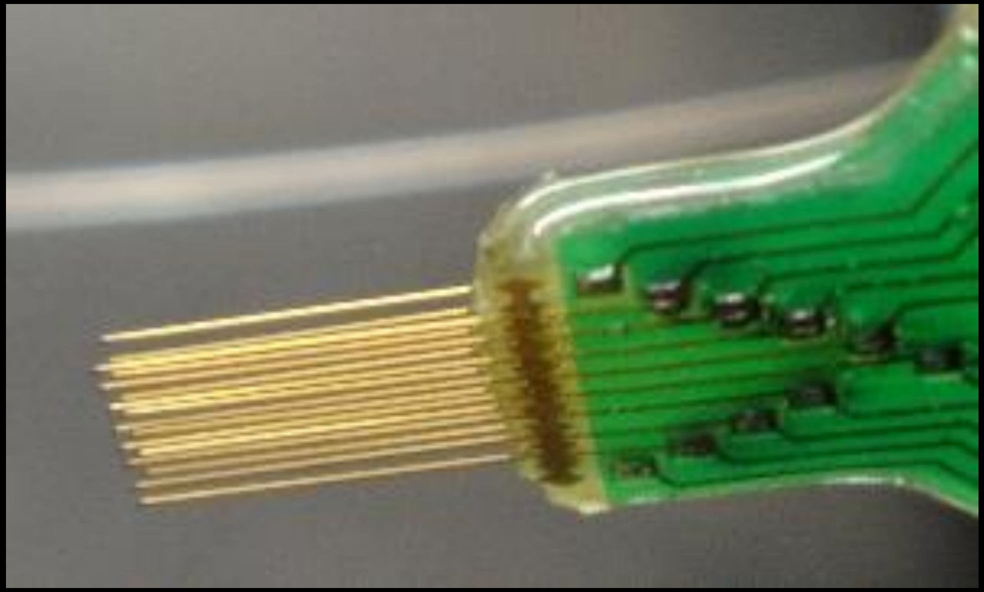




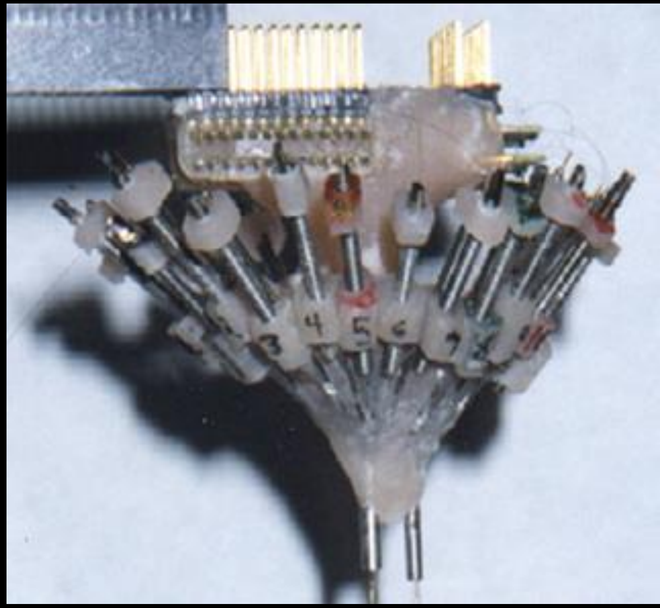
Monitoring Neural Activity with Single Cell Resolution

- Extracellular single-unit recording: microwire arrays, silicon probes
- Intracellular recording: sharp electrodes, patch-clamp
- Achieving cell-type specificity with genetic/viral methods and genetically-encoded indicators: photo-tagging, GECIs, voltage sensors

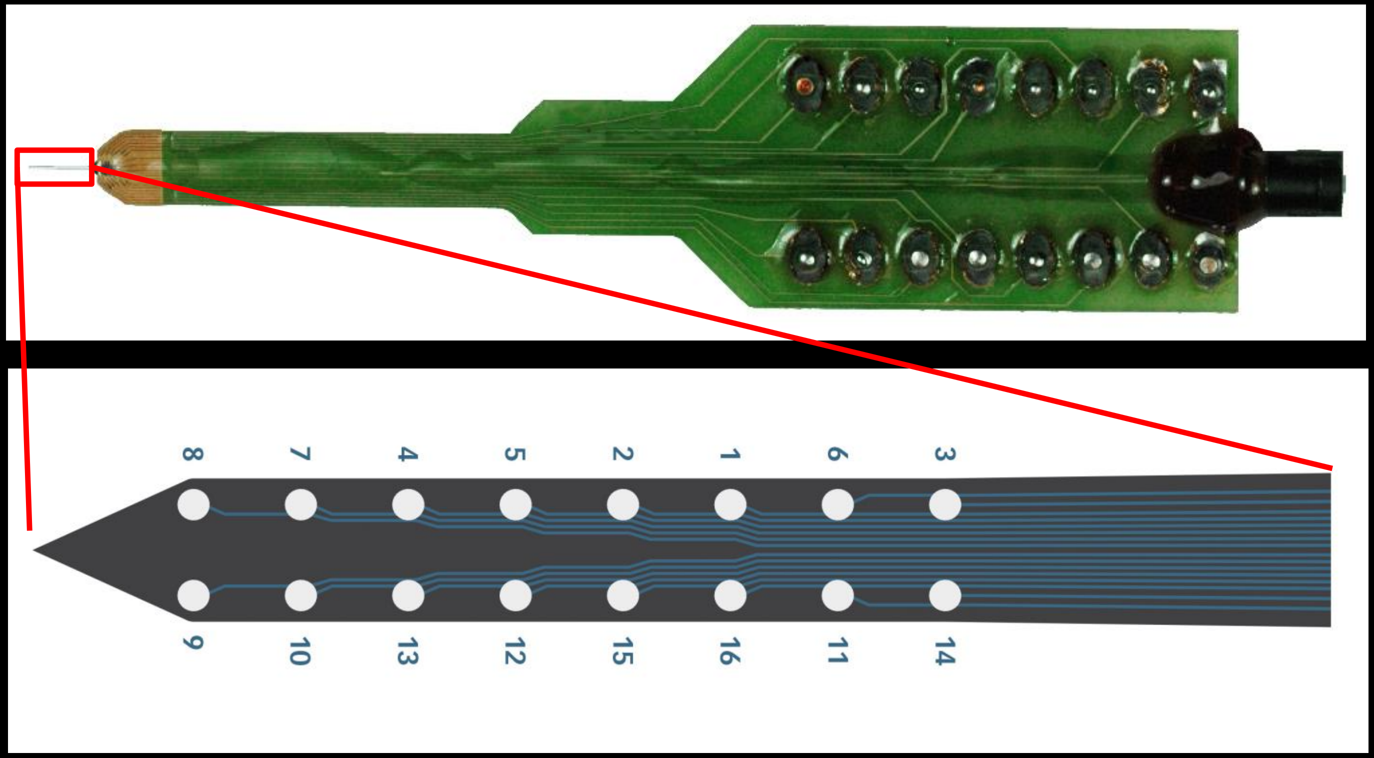
Microwire, Tetrode, and Silicon Probes for Extracellular Multi-Unit Recording



fixed microwire array

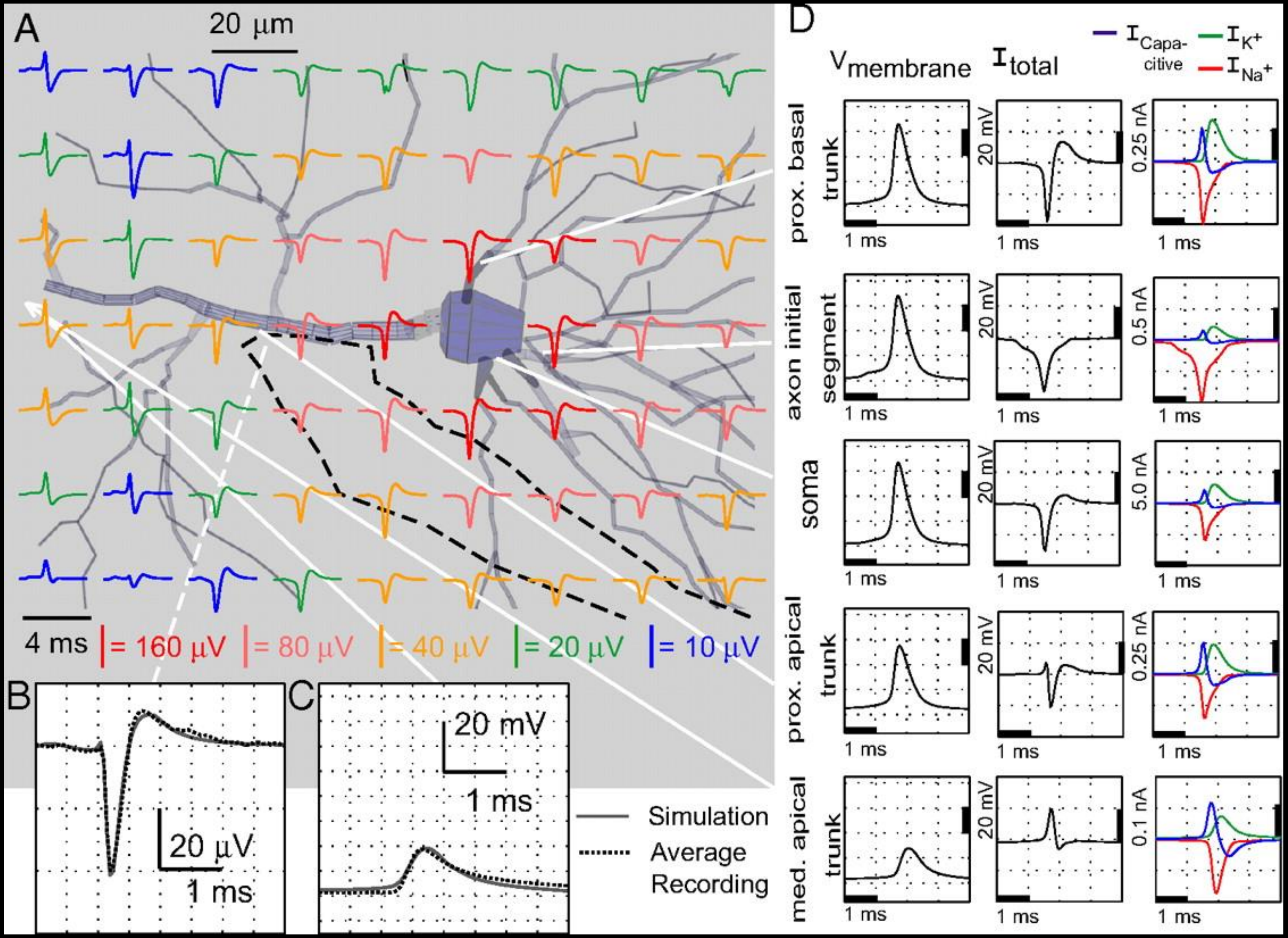


drivable tetrode array



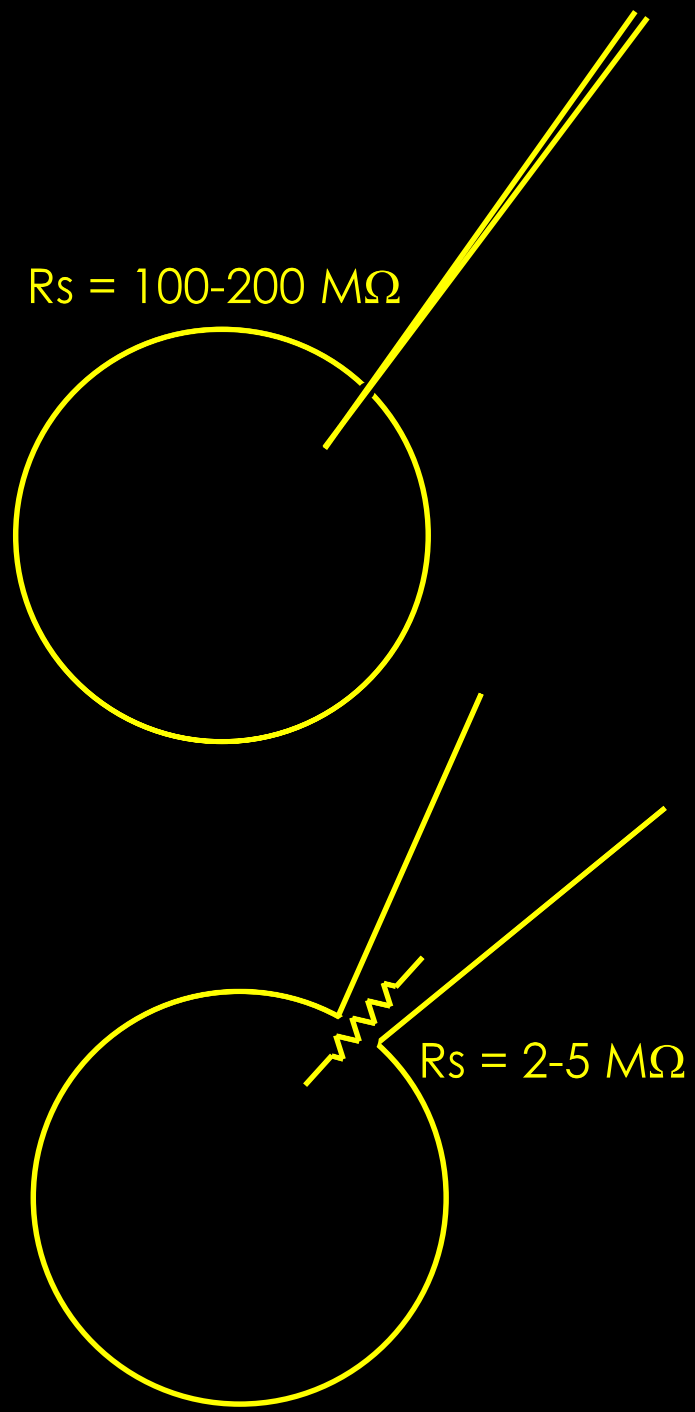
silicon probe

Origins of Extracellular Waveform



Recording and Simulation D151. *A*: extracellular action potentials (EAPs) in the transverse section containing the soma and the tip of the electrode track (dotted line). *B*: enlargement of the EAP at the estimated electrode position, and comparison to the recording (strongest channel of the tetrode). EAP is made up of 3 distinct phases: *1*) a brief, positive peak; *2*) a much larger negative peak; and *3*) a positive period of longer duration and slowly decaying amplitude. *C*: comparison of the average intracellular recording with the simulated spike in the proximal apical trunk. Lack of pronounced afterhyperpolarization (AHP) suggests the intracellular electrode was not at the soma. *D*: details of the simulation in the indicated compartments. Shape of the EAP waveform is given by the shape of the net membrane current across the membrane at the soma and proximal dendrites (*2nd column*). *Third column*: makeup of the membrane current in terms of Na^+, K^+, and mixed-ion capacitive current. All 3 currents are simultaneously active throughout the action potential (AP); the 3 phases of the EAP correspond to the current that is dominant at that time: Brief positive peak at the start of the waveform is attributed to the positive capacitive current; the main negative peak is attributed to the influx of Na^+ current driving the action potential; the final positive phase results from repolarizing K^+ current flowing out of the cell.

Intracellular Recording: Sharp or Patch Electrodes



Advantages:

1. Subthreshold activity
2. Measure inhibition vs excitation
3. Can be targeted to specific cell types

Disadvantages:

1. Low Stability (1-10 min)
2. Leak current (sharps)
3. Requires head-fixed preparation

BUT…How Can We Record from Specific Cell Types In Vivo?

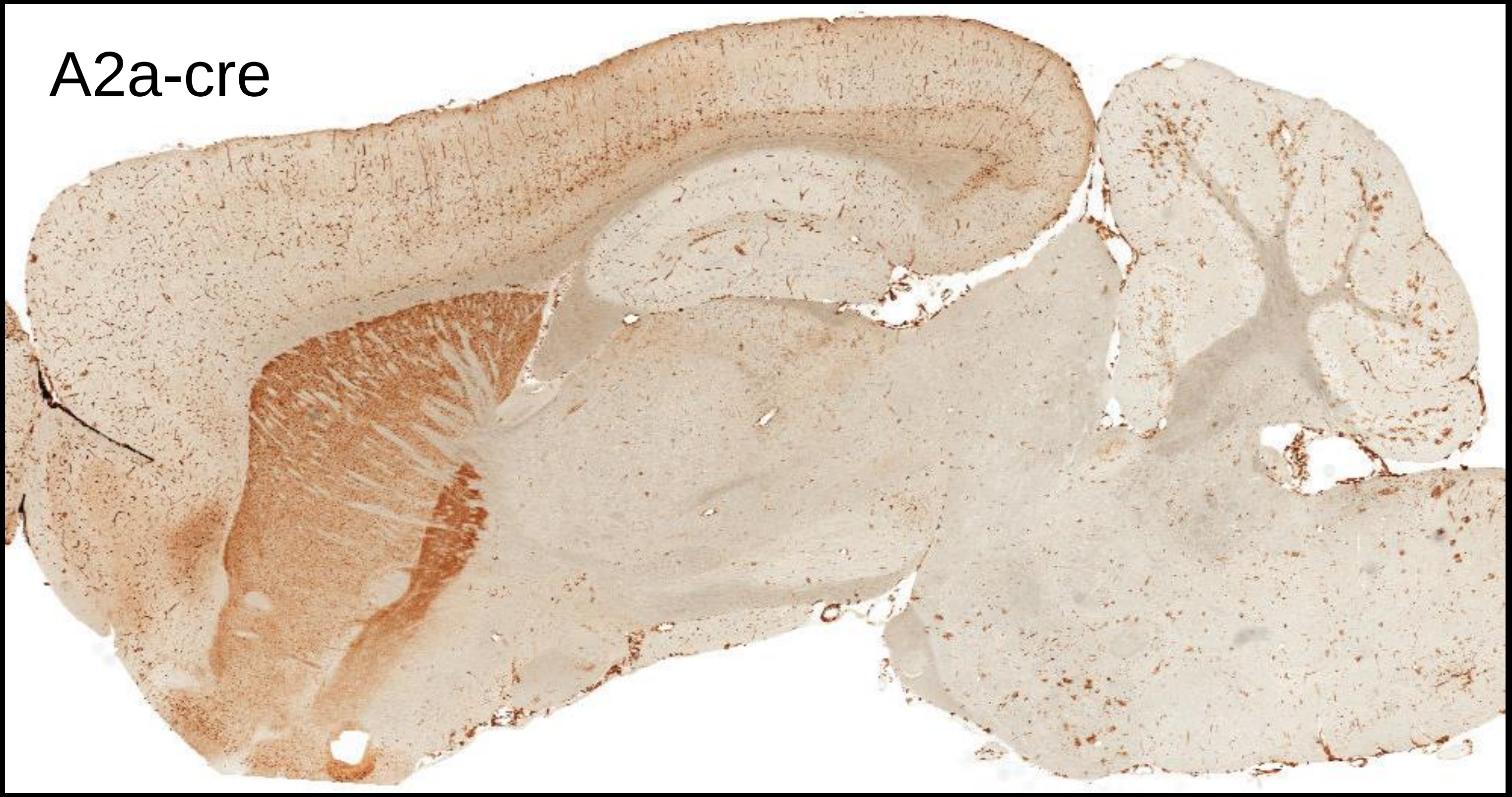
1. Cell-Type-Specific Expression of Proteins
   - Transgenic driver lines (Cre, Flp, Dre) + contingent viruses
   - Viruses with cell-type-specific promoters
   - Transynaptic or projection targeting

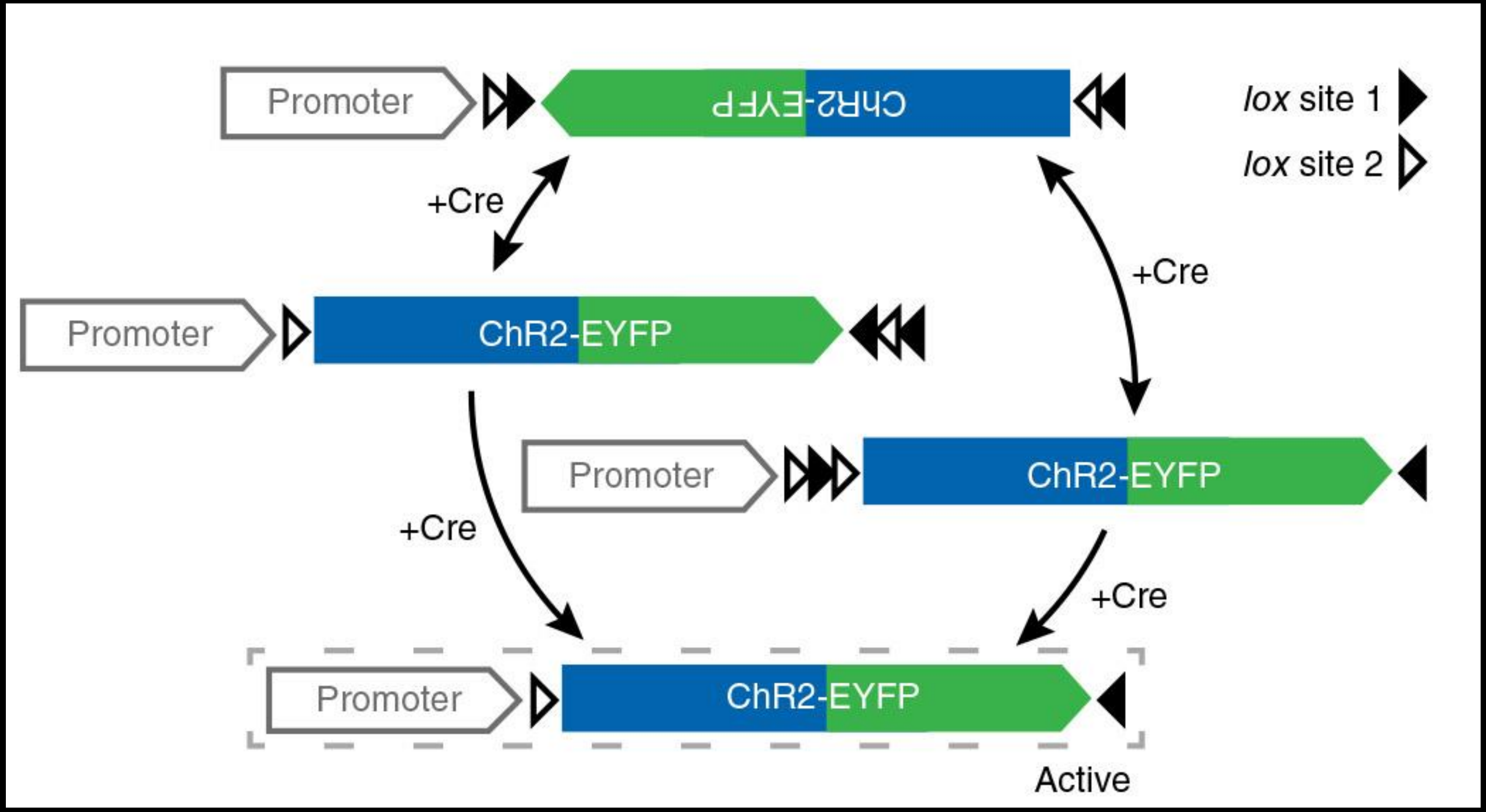
2. Phototagging neurons during electrophysiological recording

3. GECI imaging

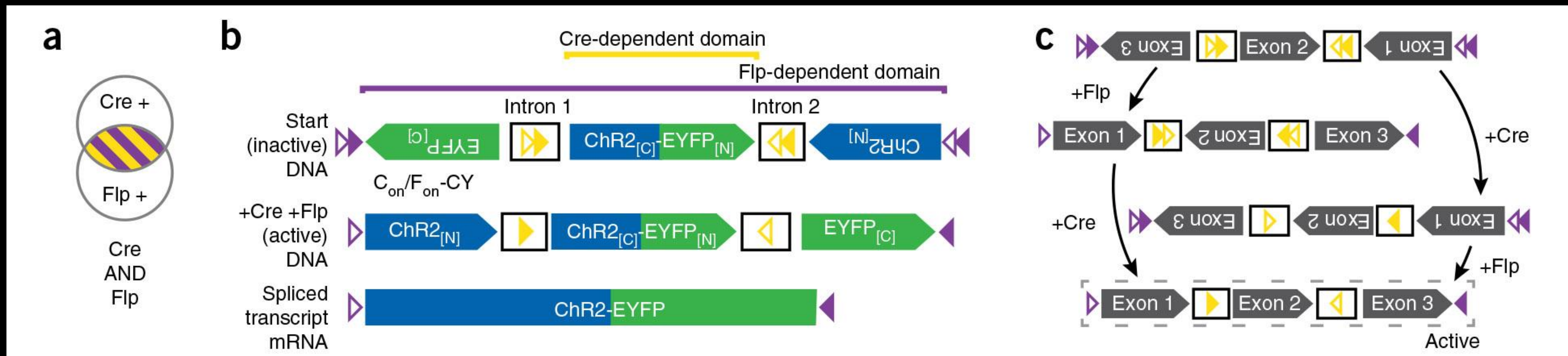
4. Voltage imaging

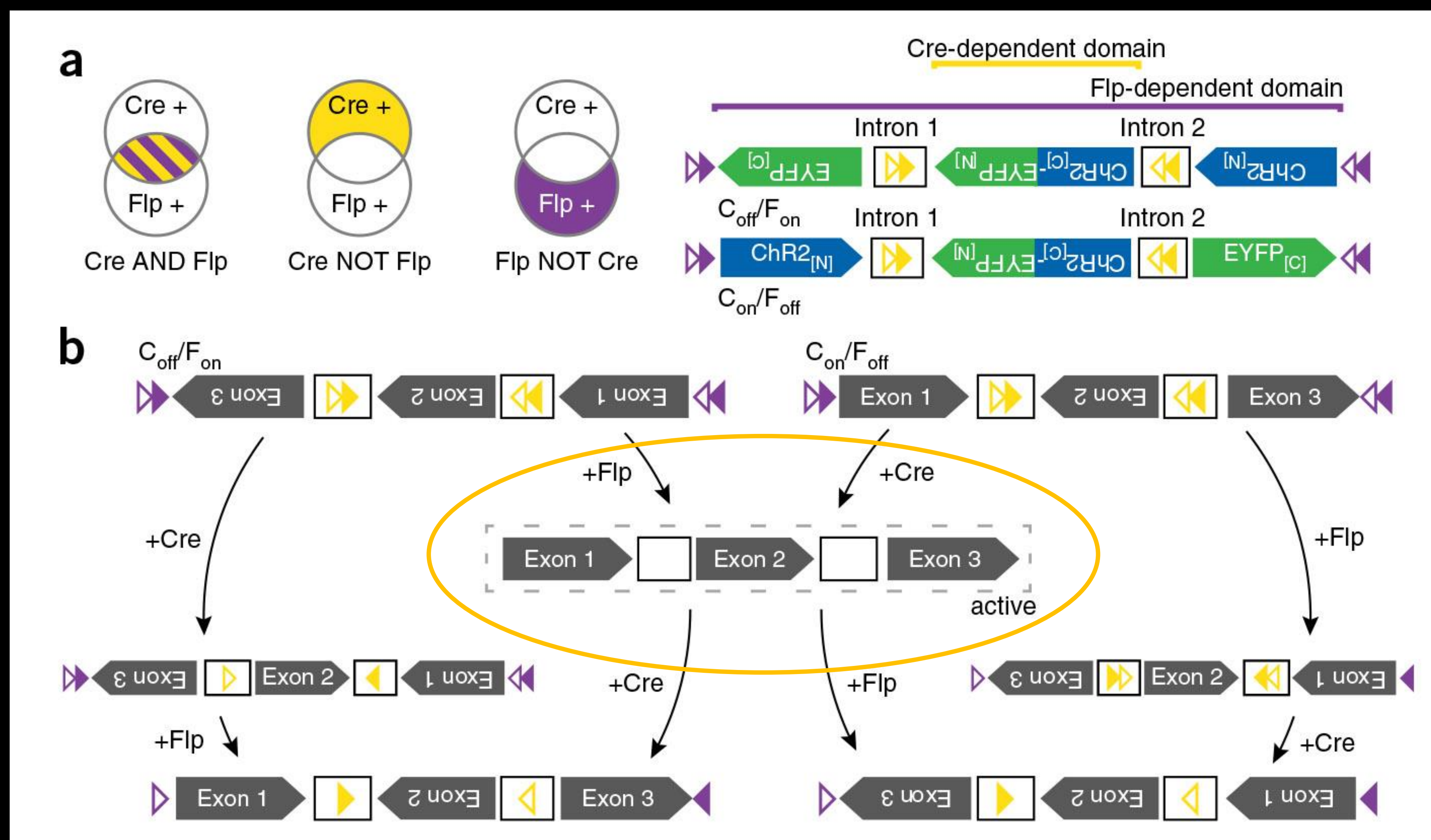
Strategies for Targeting Cell Types





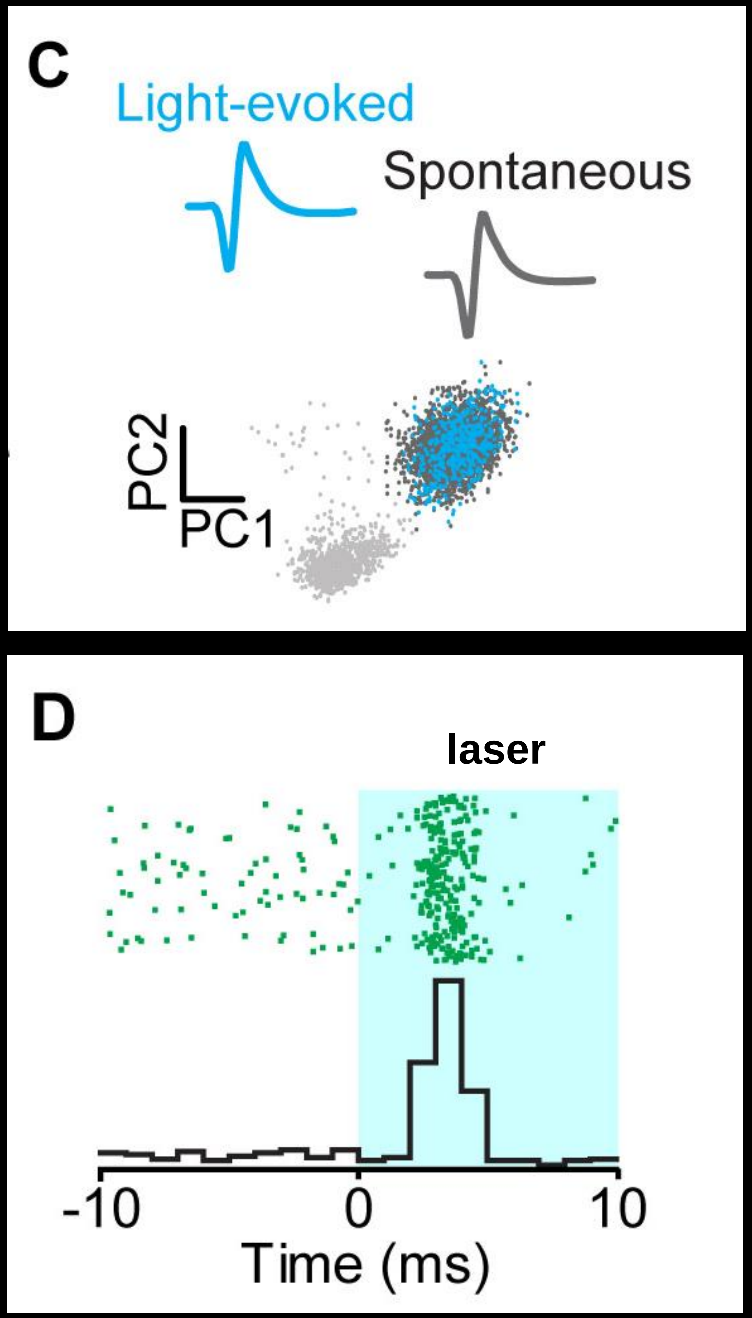
Strategies for Targeting Cell Types





Fenno et al, Nat Methods 2014

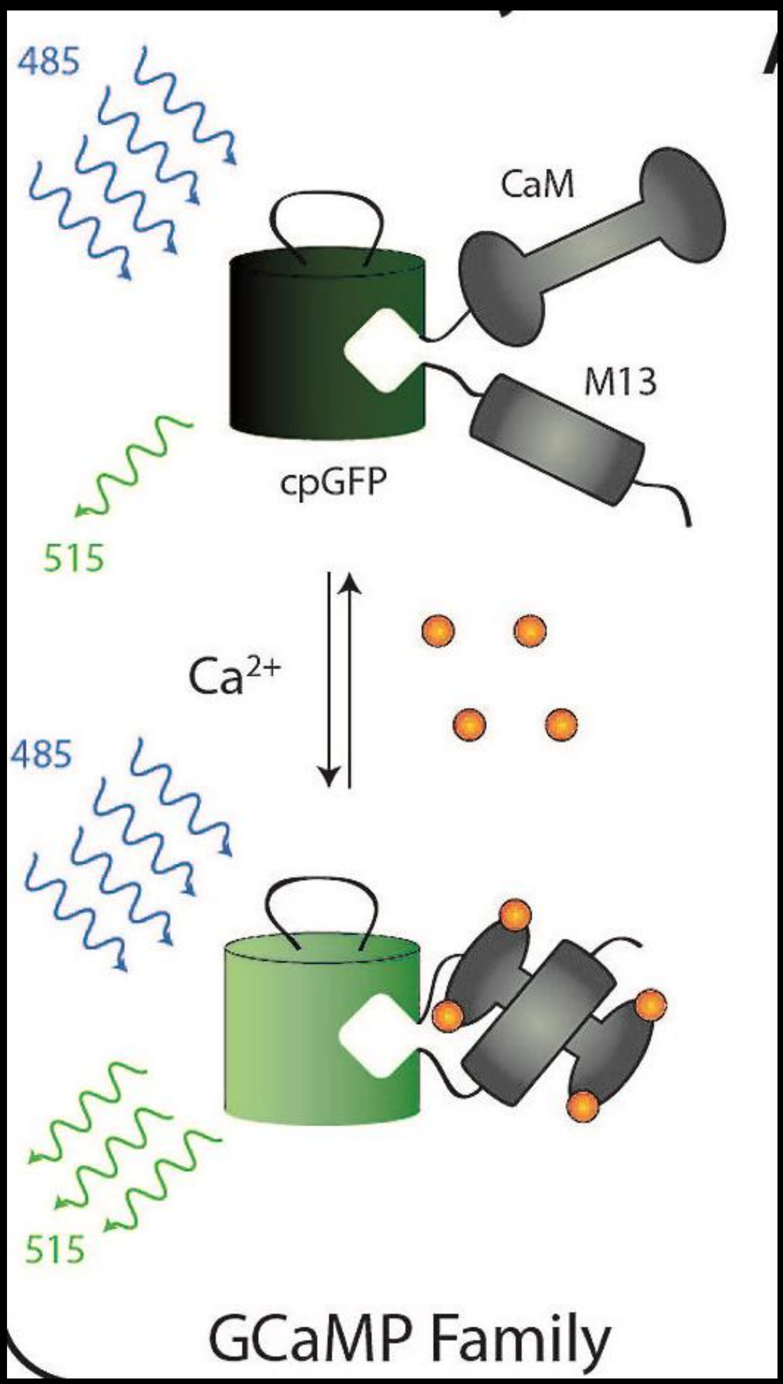
Phototagging with ChR2



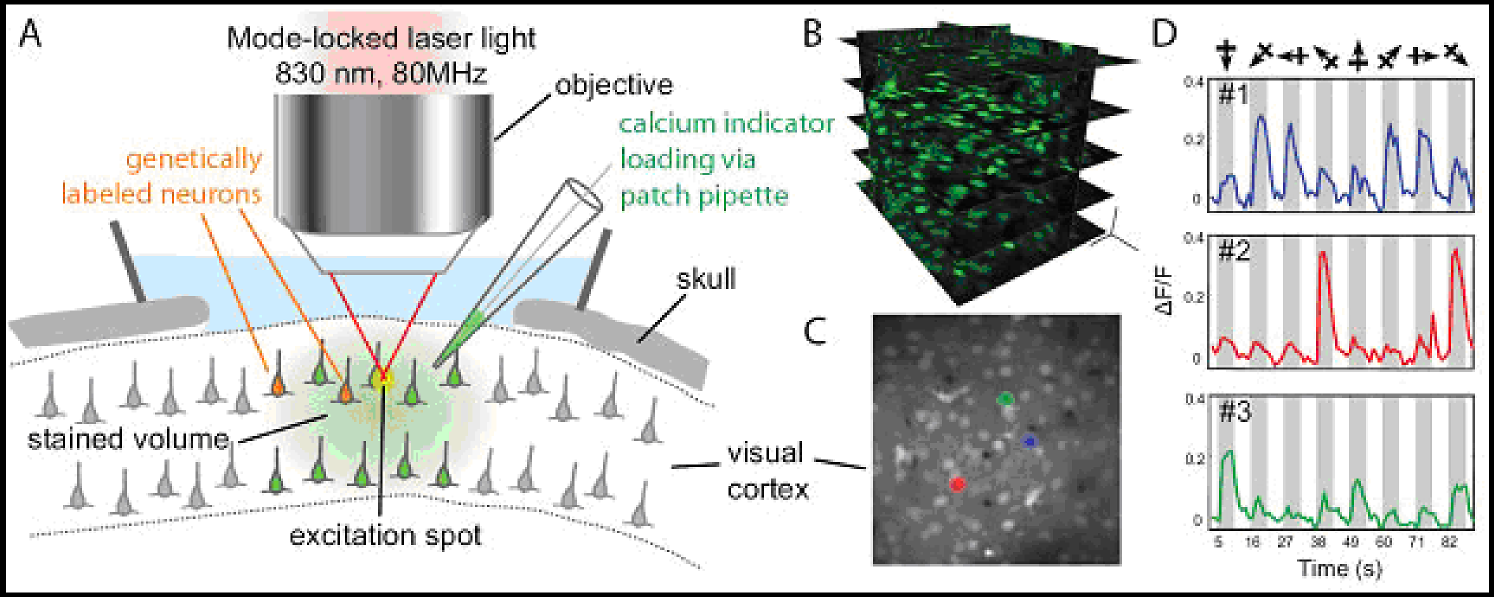
Roseberry et al, Cell 2016

See also:
Cardin et al 2009
Lima et al 2009
Zhao et al 2011
Cohen et al 2012
Royer et al 2012
Kravitz et al 2013

Imaging Neural Activity with GECIs



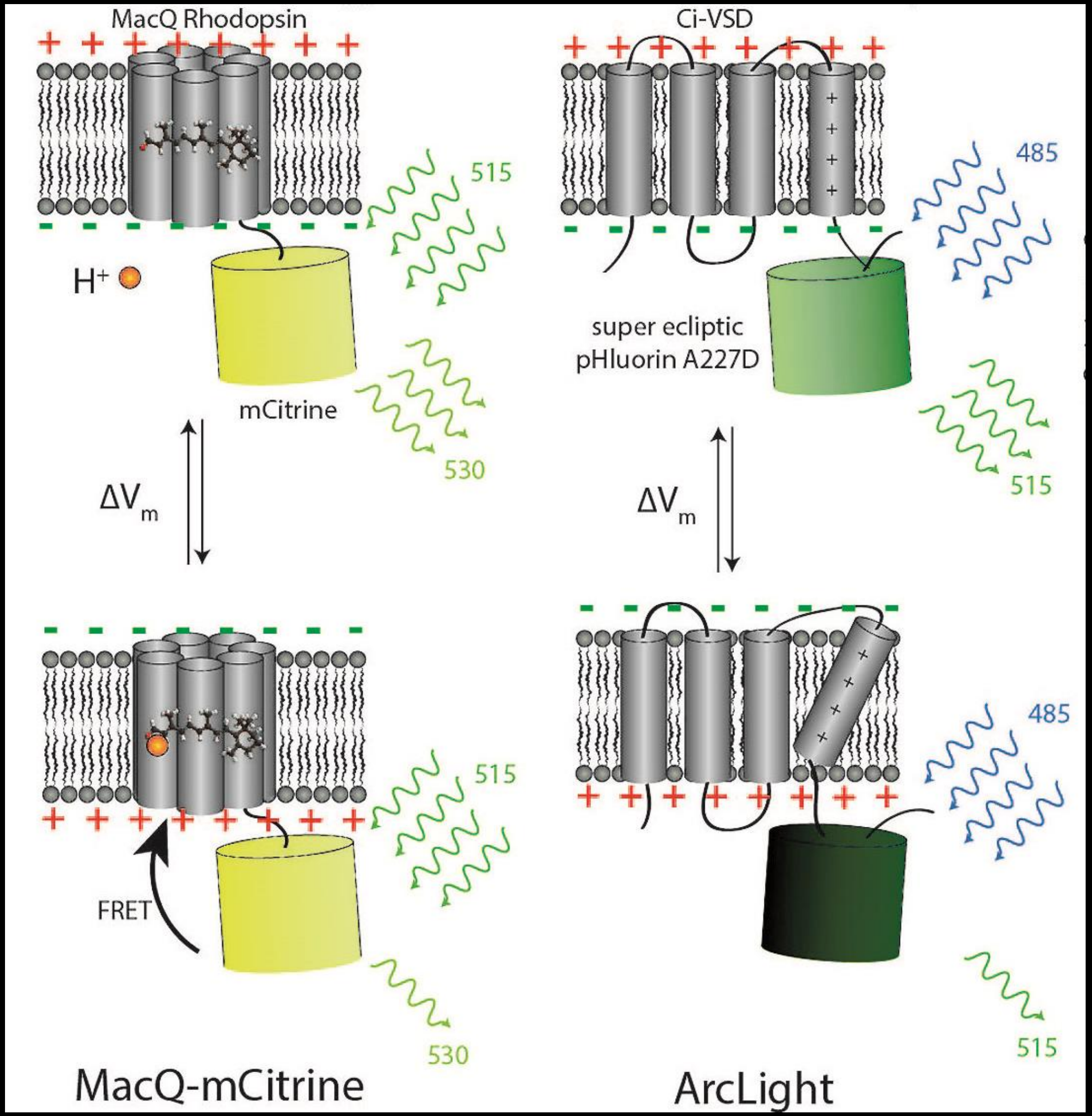
GECI	$\Delta F/F$	K_d (nM)
R-GECO1	8.7 ± 0.7	223 ± 95
R-CaMP1.07	14.4 ± 1.4	192 ± 4
R-CaMP2	4.8 ± 0.6	69 ± 8
R-GECO2L	4.1 ± 0.3	26 ± 3
GCaMP3	8.4 ± 0.2	365 ± 8
GCaMP5G	18.2 ± 1.0	371 ± 13
GCaMP6f	22.1 ± 3.0	296 ± 8
GCaMP6s	30.8 ± 3.0	152 ± 8

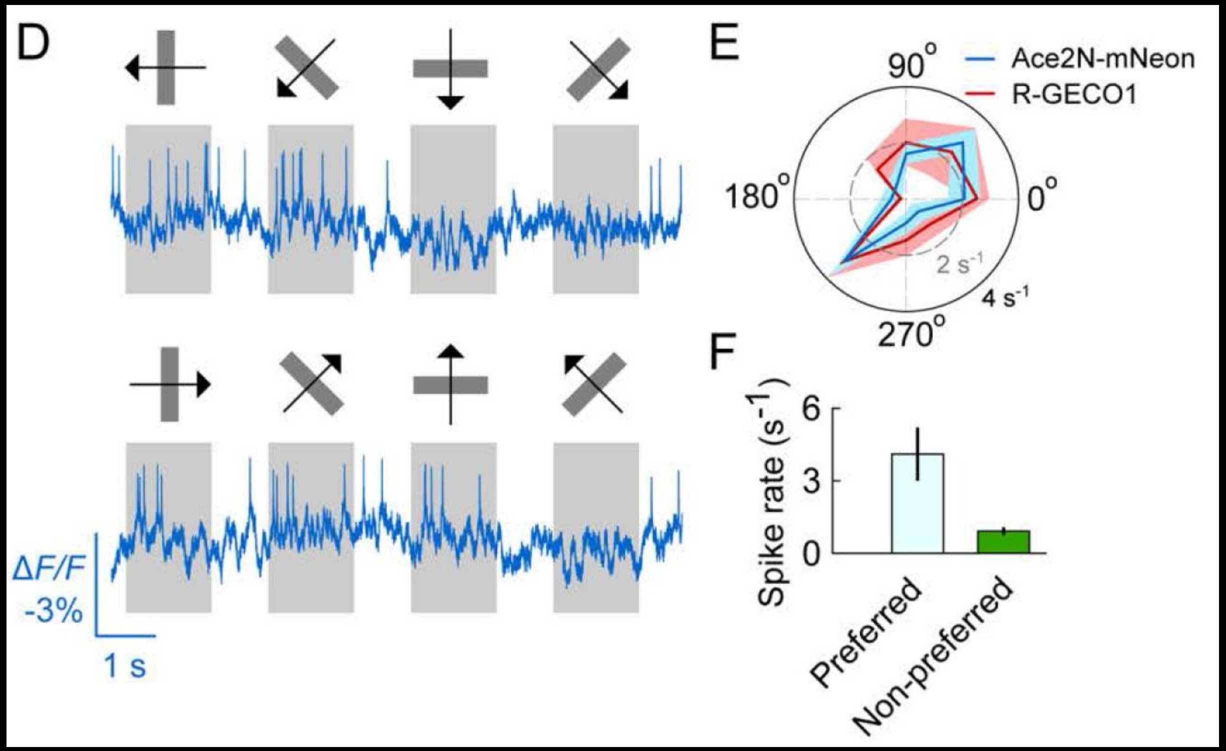


Calcium Imaging: Caveats

- Calcium influx is not the same as spiking! Calcium influx can occur without spikes, and spikes can occur without calcium influx
- Different cell types will translate spikes into calcium influx differently. Generally, calcium transients will reflect bursts and not single spikes
- Calcium indicators can saturate, particularly in neurons with high firing rates and high densities of calcium channels
- Calcium indicators bind calcium, and therefore have the potential (at high concentrations) to buffer intracellular calcium signaling and alter neuronal properties

Imaging Neural Activity with GEVIs





Voltage Imaging: Caveats

Development of GEVIs has lagged behind GECIs because:

1. Speed (need kinetics to report APs that are ~1ms in duration)

2. High sensitivity (need to report subthreshold changes in voltage ~5mV)

3. Restricted imaging volume (membrane bound)

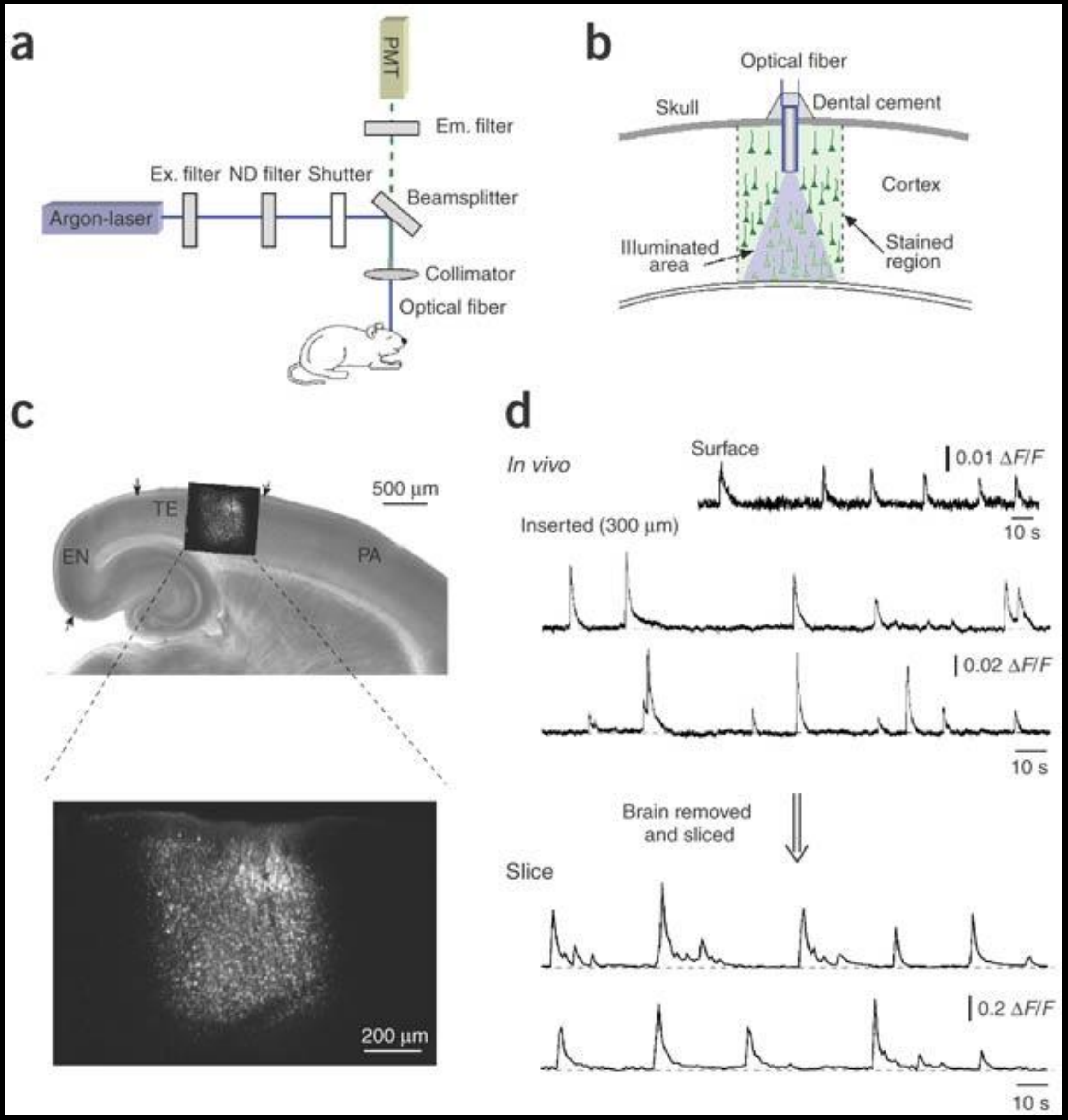
Optical Strategies for Monitoring Neural Activity In Vivo

1. Fiber Photometry (freely moving)

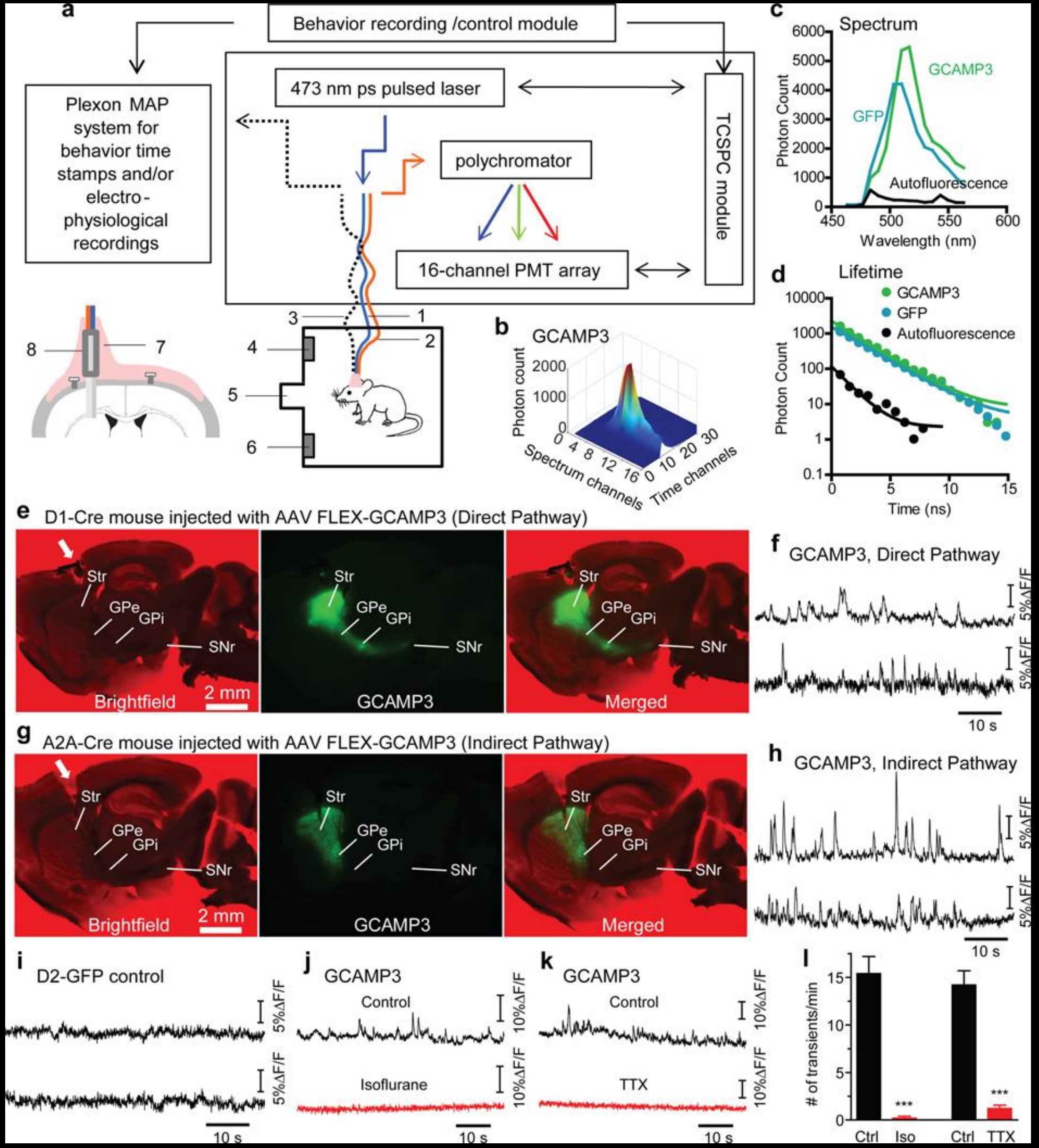
2. Microendoscopy (e.g. Inscopix cameras) (freely moving)

3. 2-photon microscopy (head fixed)

Fiber Photometry

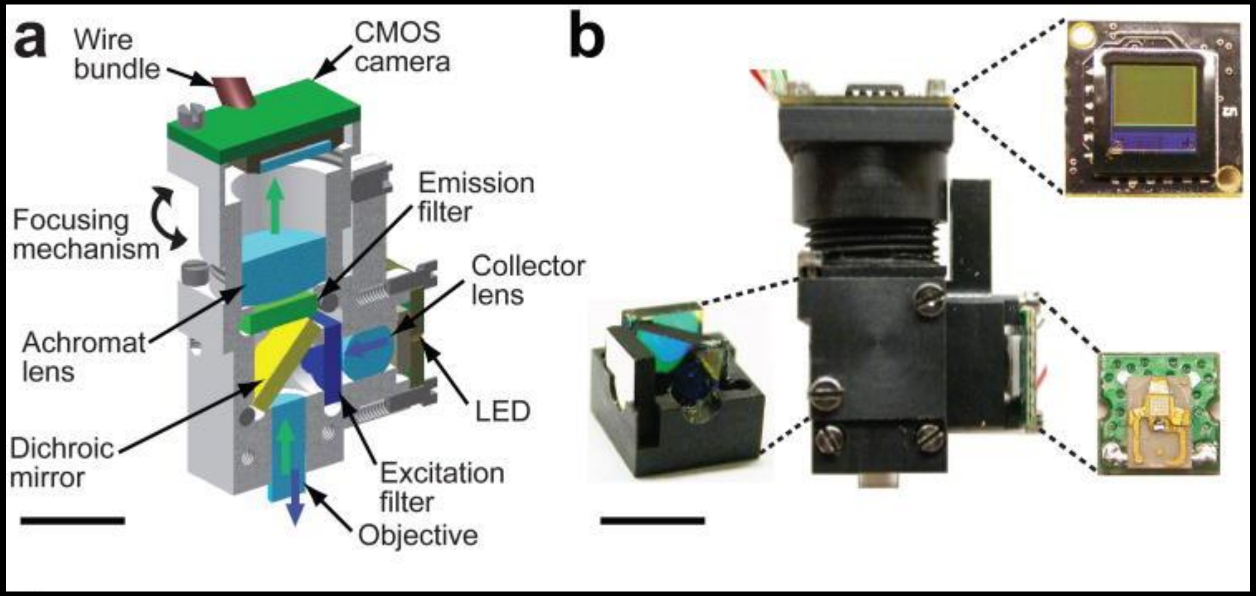


Adelsberger et al, Nat Neurosci 2005



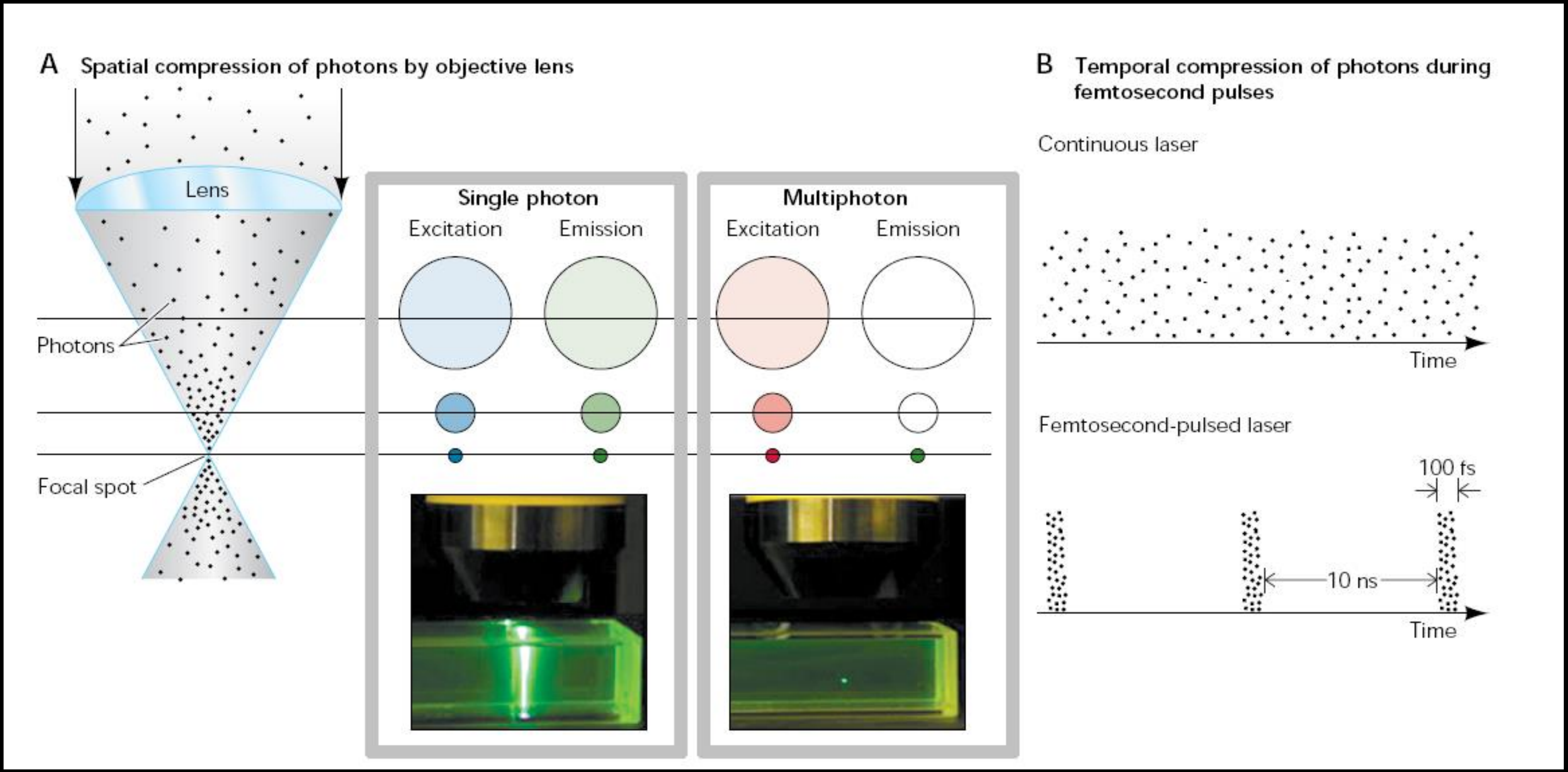
Cui et al, Nature 2013

Microendoscopy



Ghosh et al, Nat Meth 2011

2-Photon Imaging



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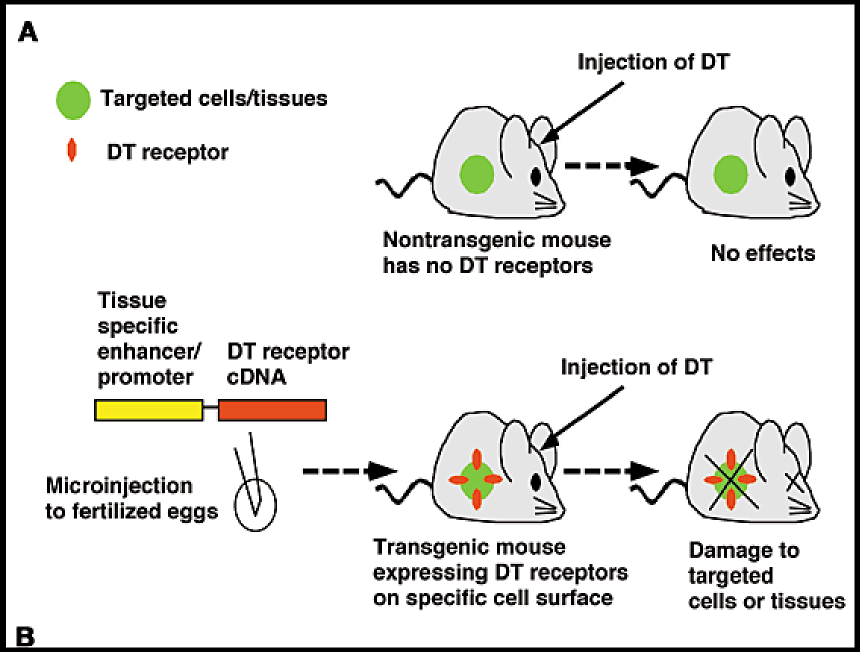
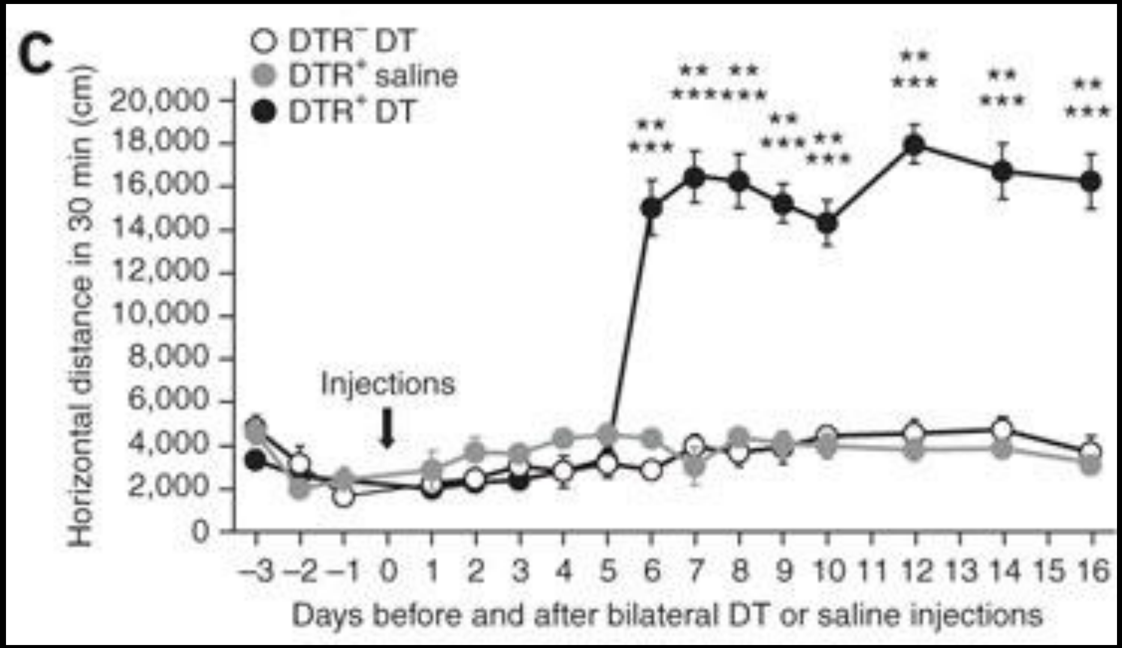
Lesions: Irreversible and Non-Specific

- Mechanical lesions: aspiration of brain tissue (removes everything from large area)
- Electrolytic lesions: local heating and coagulation (targets smaller regions)
- Chemical lesions: ibotenic acid, kainic acid (spares fibers of passage)

Toxins: Irreversible and Specific

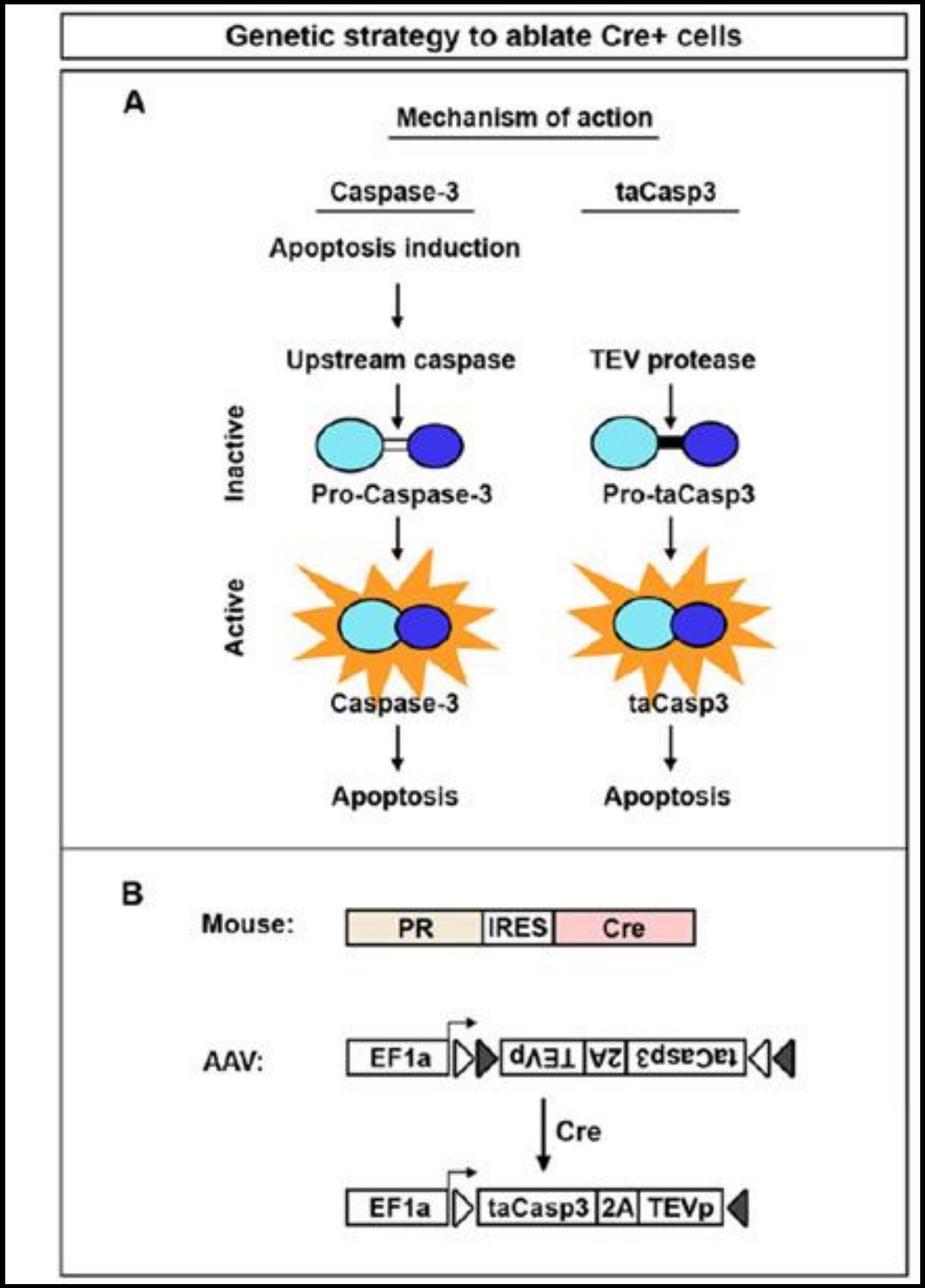
- Diphtheria Toxin: binds human, but not murine membrane-bound HB-EGF. Thus, express human receptor in mice using cell-type-specific targeting strategies, administer toxin systemically (Saito et al, 2001)





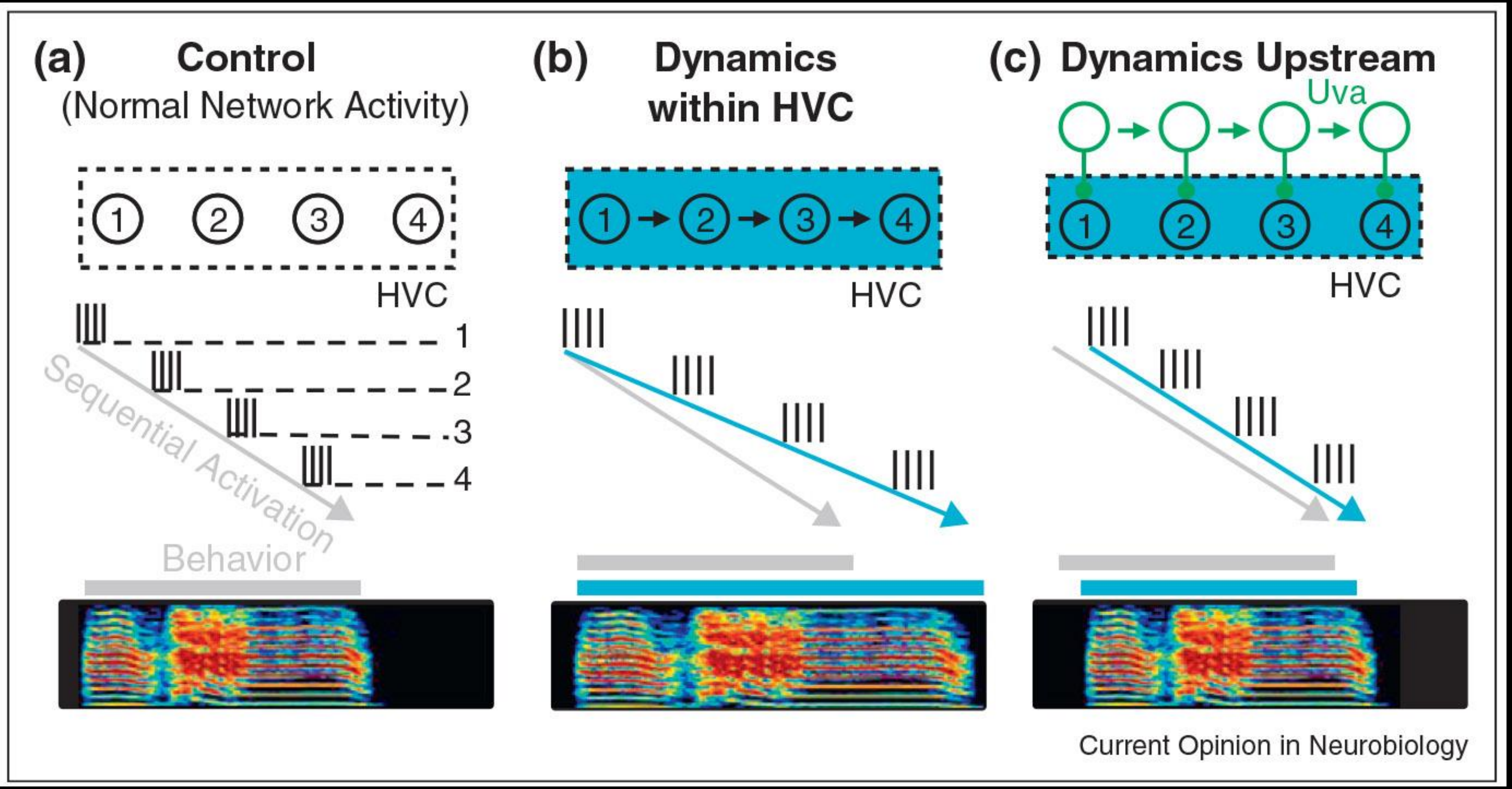
Toxins: Irreversible and Specific

- taCasp3:



(Yang et al, Cell 2013)

Cooling: Reversible and Non-Specific



Long and Fee, 2011

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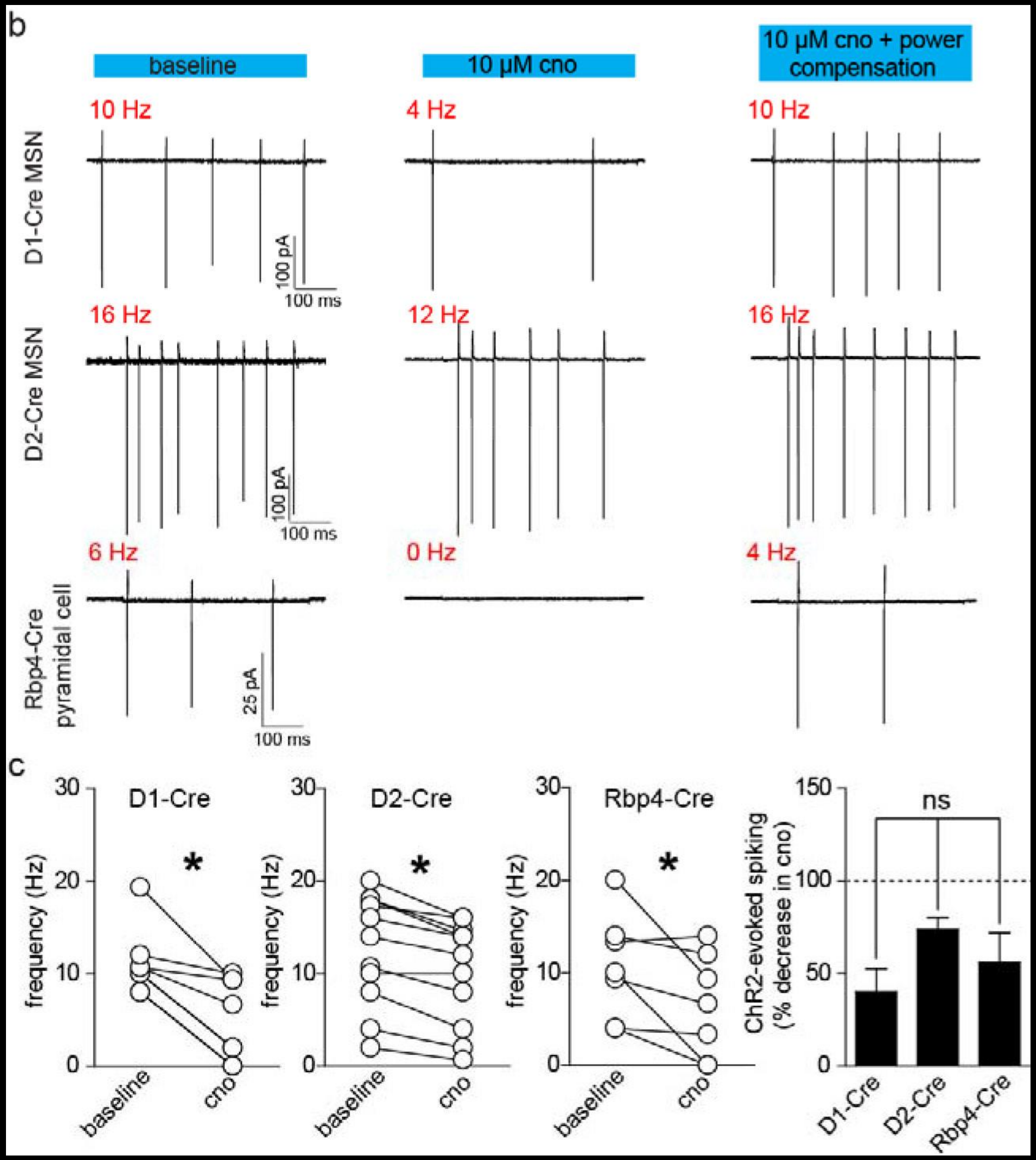
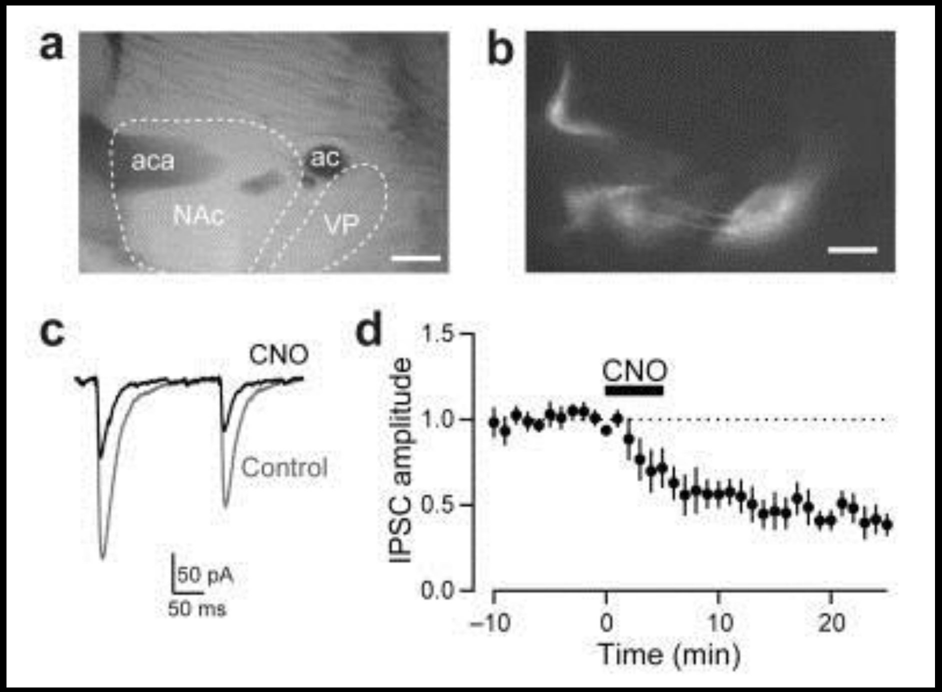
Chemogenetics

- Engineered GPCRs: Strader et al, 1991; Coward et al 1998, DREADDs (Armbruster, Li, Herlitze, Roth, PNAS 2007) – low constitutive activity, insensitivity to native ligand, nanomolar binding to inert orally-active ligand (CNO).
- Non-mammalian GPCRs: allatostatin receptor (Lechner…Callaway, J Neurosci 2002; Tan et al, Neuron 2006)
- Non-mammalian ion channels: c. elegans GluCl (opened by ivermectin) (Slimko et al, J Neurosci 2002; Lerchner et al, Neuron 2007)
- Mammalian ion channels (TRPV1, GABA γ2 subunit)
- Engineered mammalian ion channels (PSAM, PSEM) (Magnus…Sternson, Science 2011)

2 Modes of DREADD action: Somatic vs Axonal Inhibition



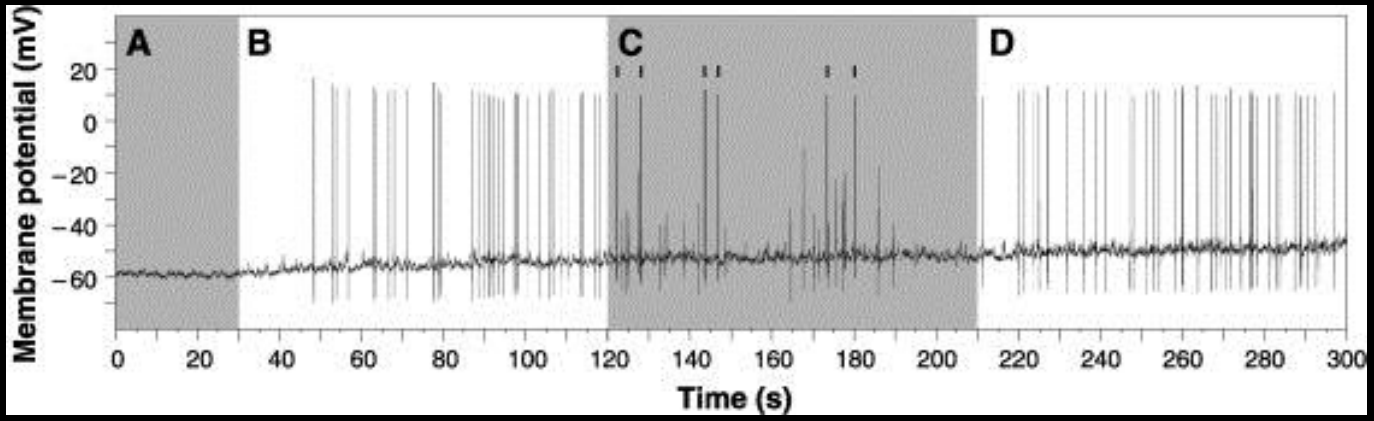
Kozorovitskiy et al, Nature 2012



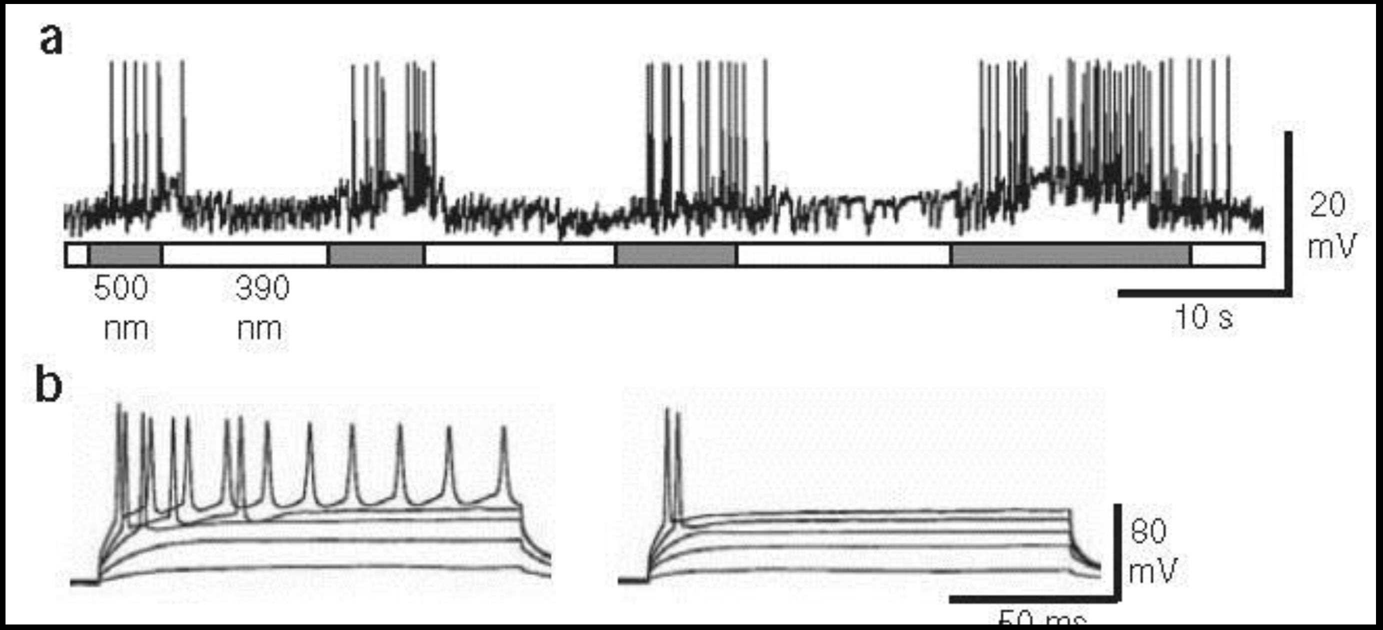
Bock et al, Nature Neurosci 2013

Early Optogenetics

Early attempts required multiple components and lacked temporal precision

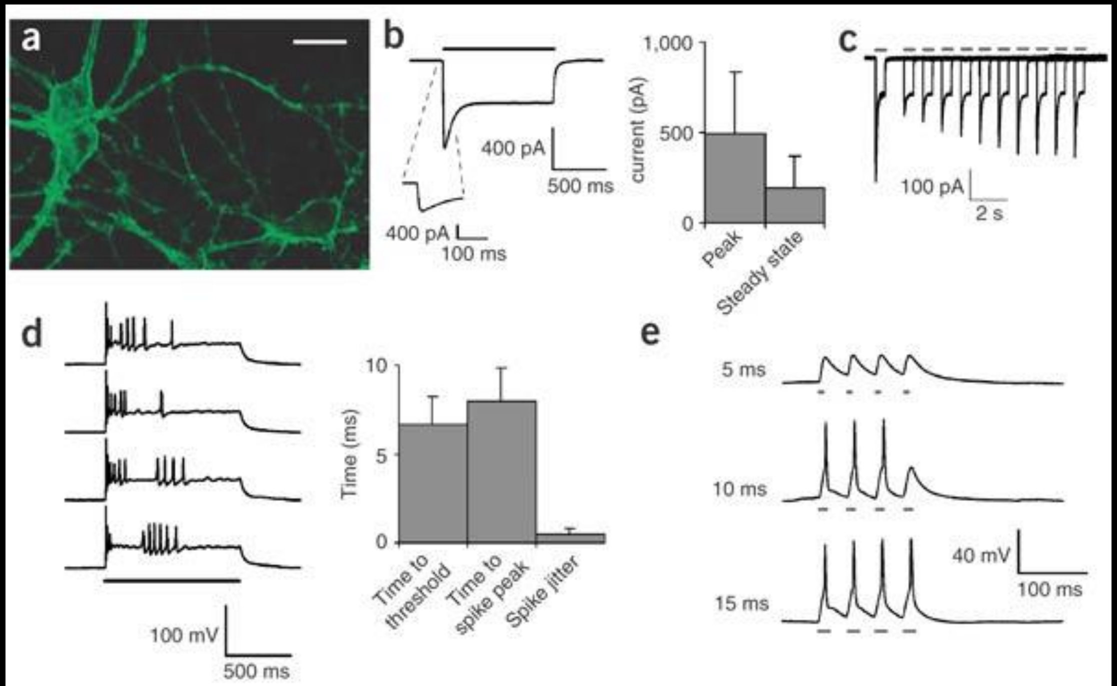


Zemelman…Miesenbock, Neuron 2002

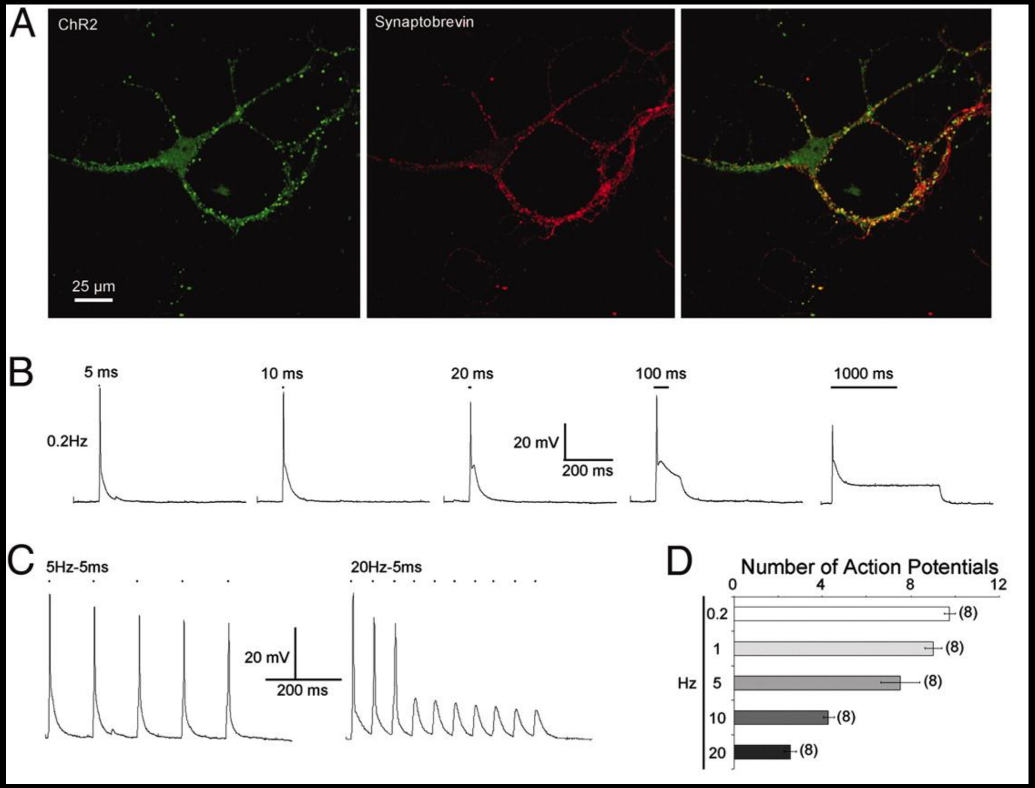


Banghart…Kramer, Nat Neurosci 2004

Single Component Optogenetics: ChR2

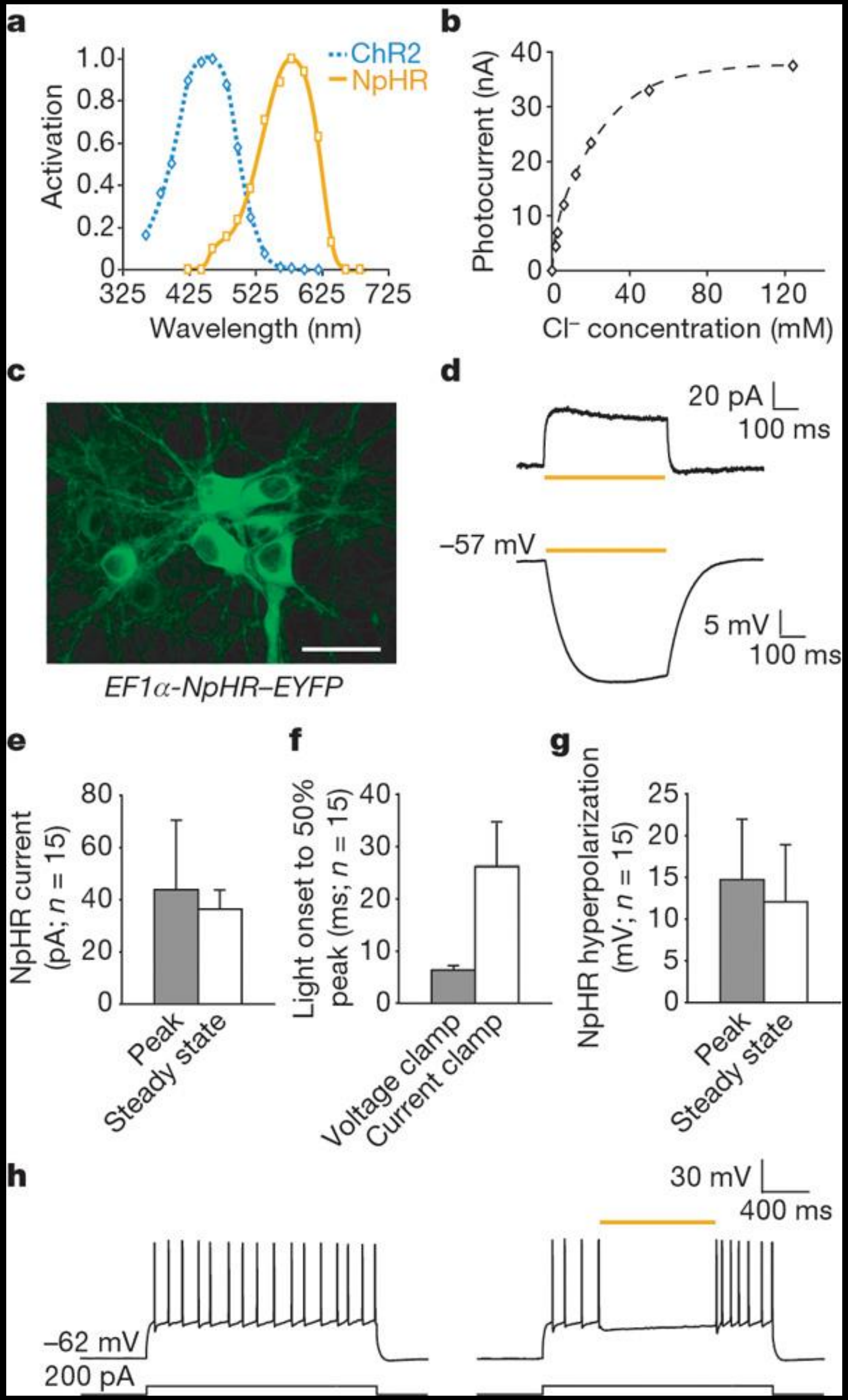


Boyden…Deisseroth, Nat Neurosci Sept 2005

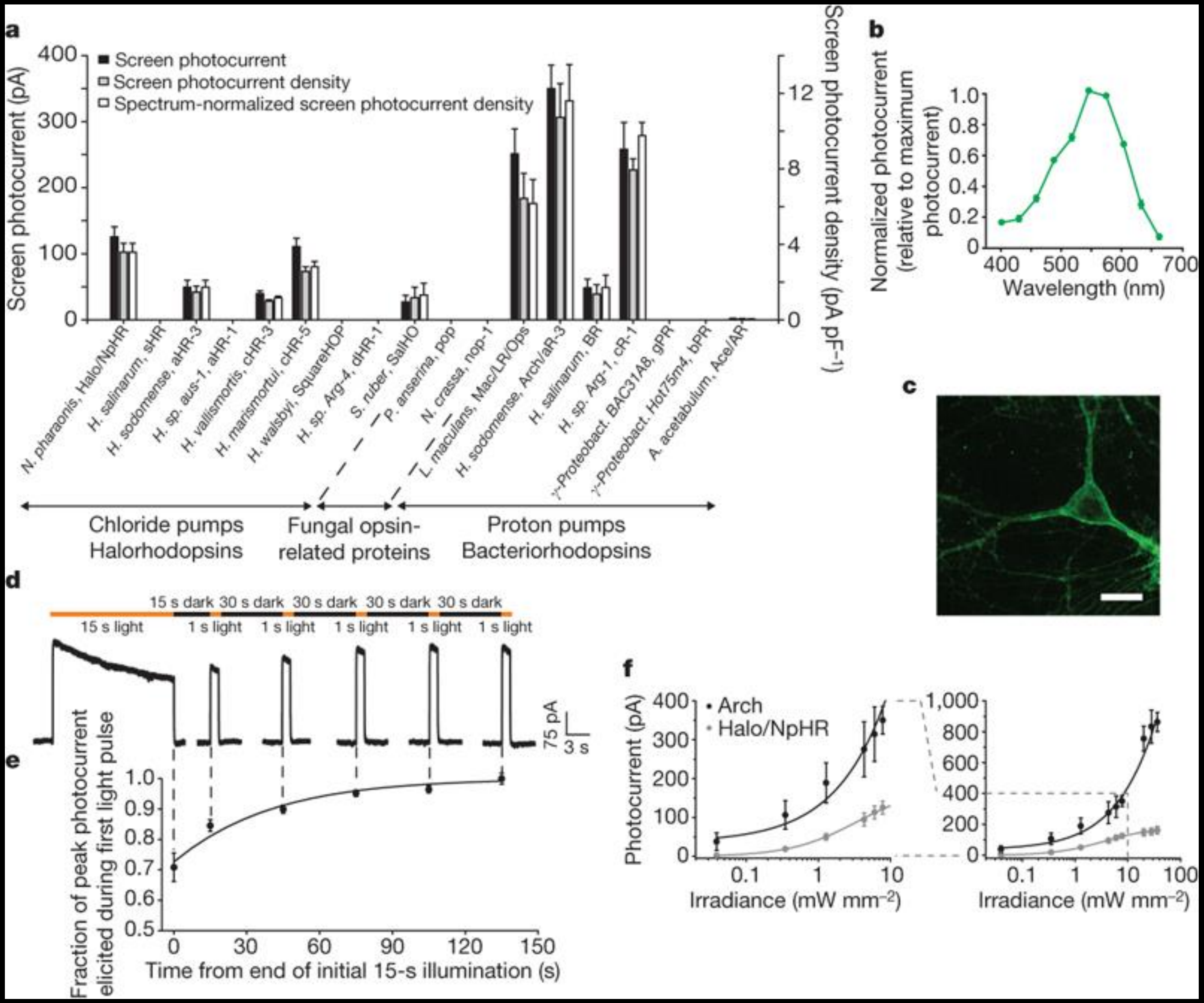


Li…Herlitze, PNAS Dec 2005

Optogenetic Inhibition: eNpHR and Arch



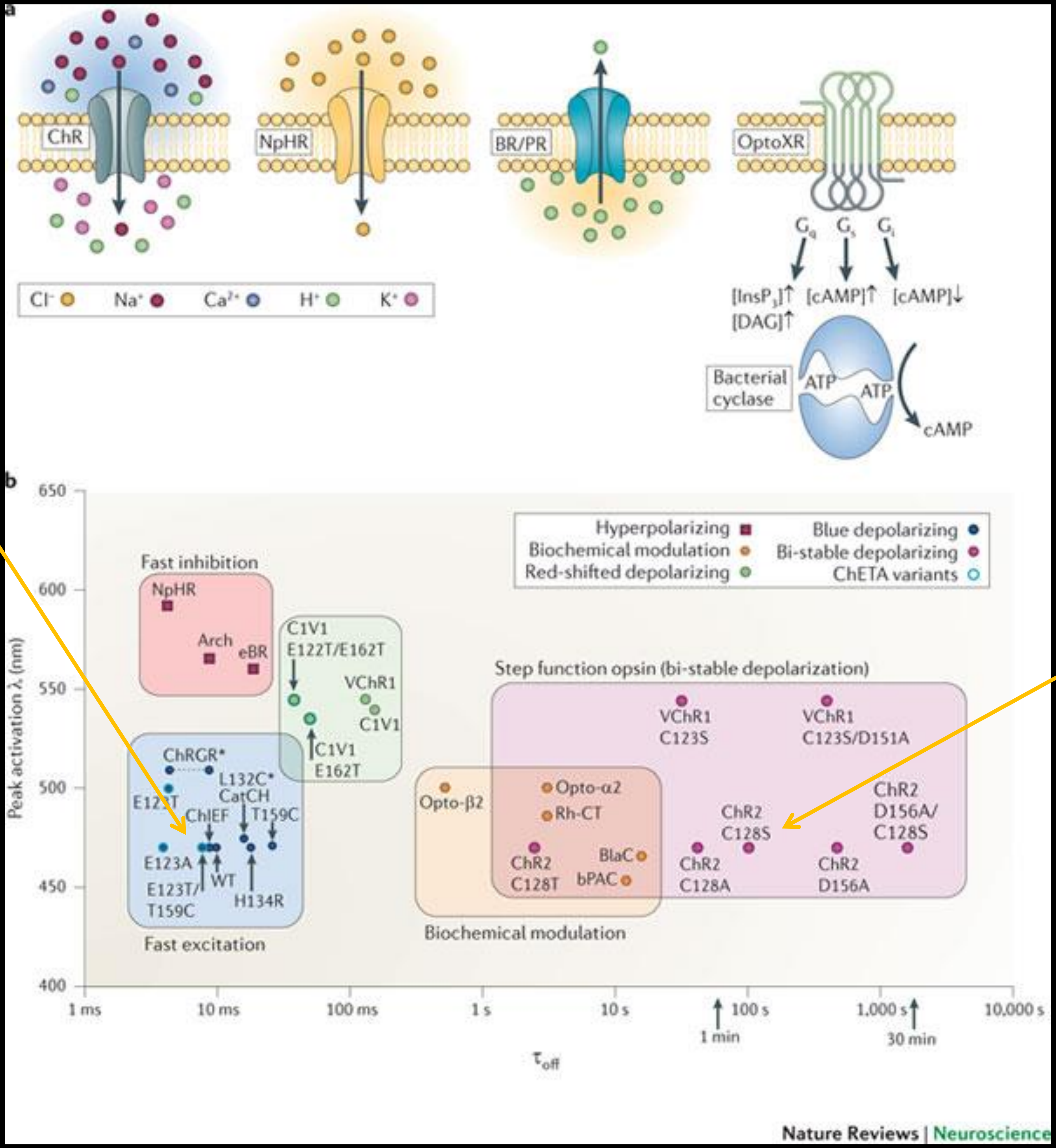
Zhang…Deisseroth, Nature 2007



Chow…Boyden, Nature 2010

Optogenetic Tool Summary

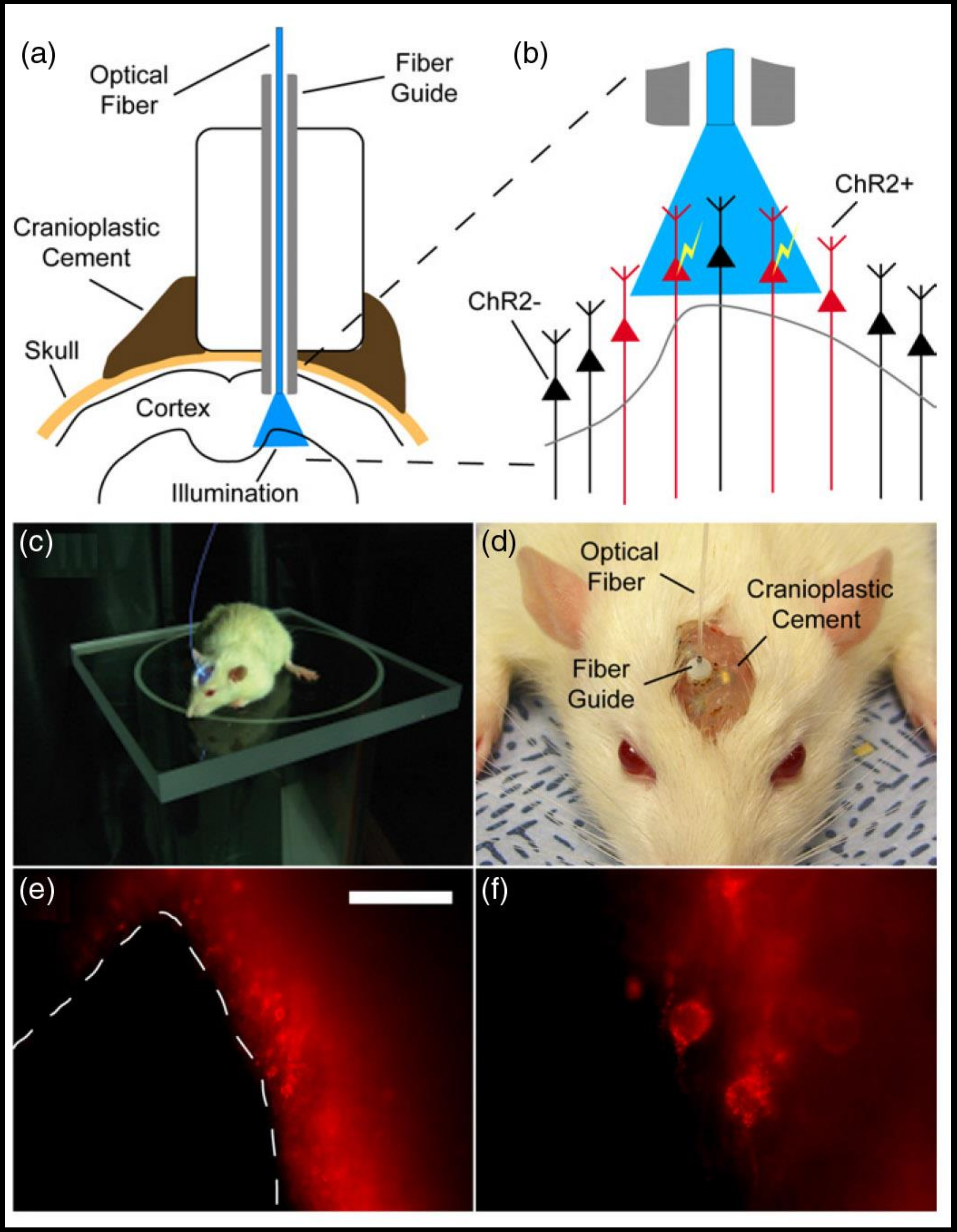
Inhibitory channel
(iC1C2)



Inhibitory SFO
(SwiChR)

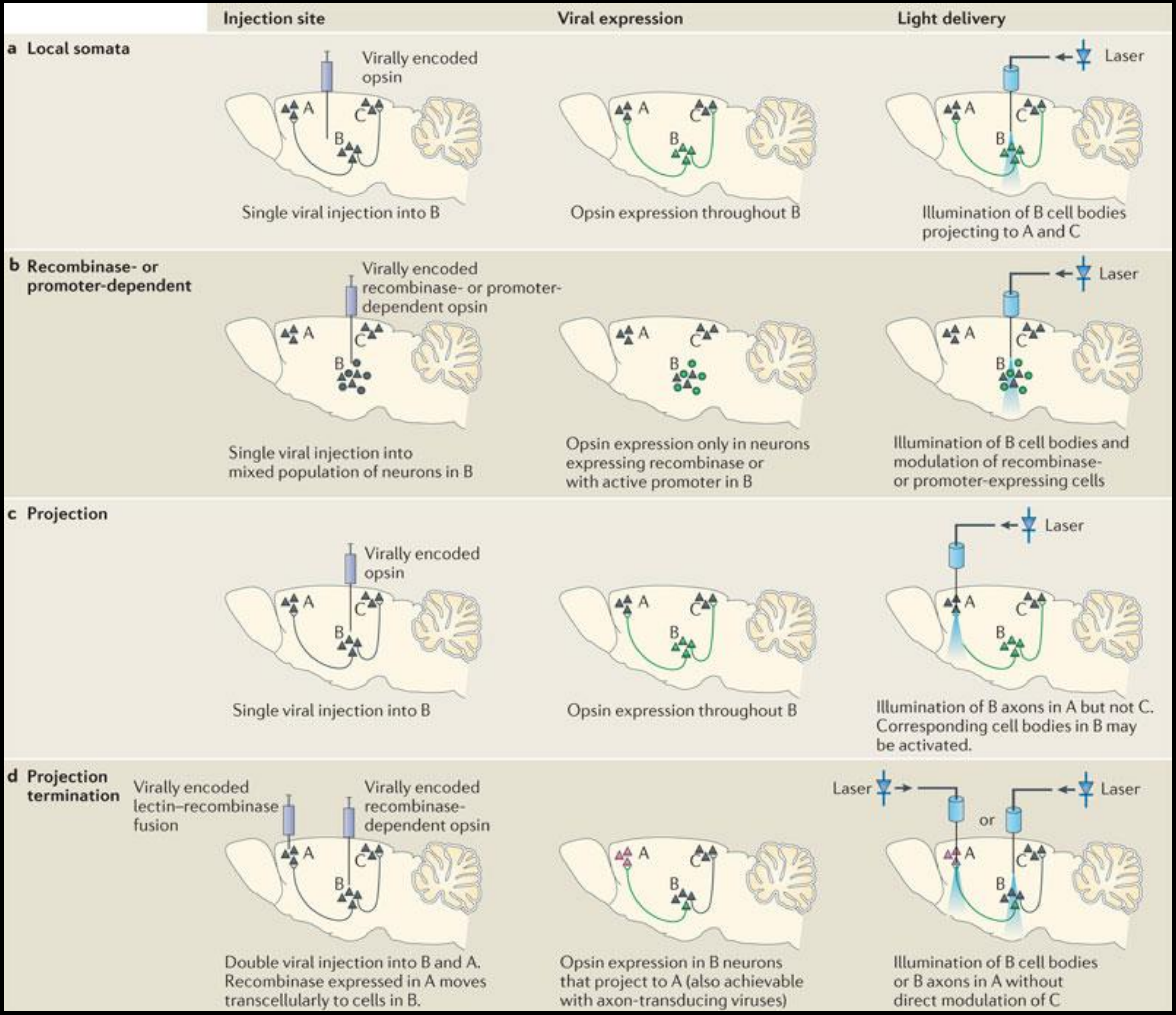
Tye and Deisseroth, Nat Rev Neurosci 2012

In Vivo Applications



Aravanis et al, J Neural Eng 2007

Optogenetic targeting strategies



Key Considerations in Designing Perturbation Experiments

- What effect does the manipulation have on the cell?
- What effect does the manipulation have on the circuit?

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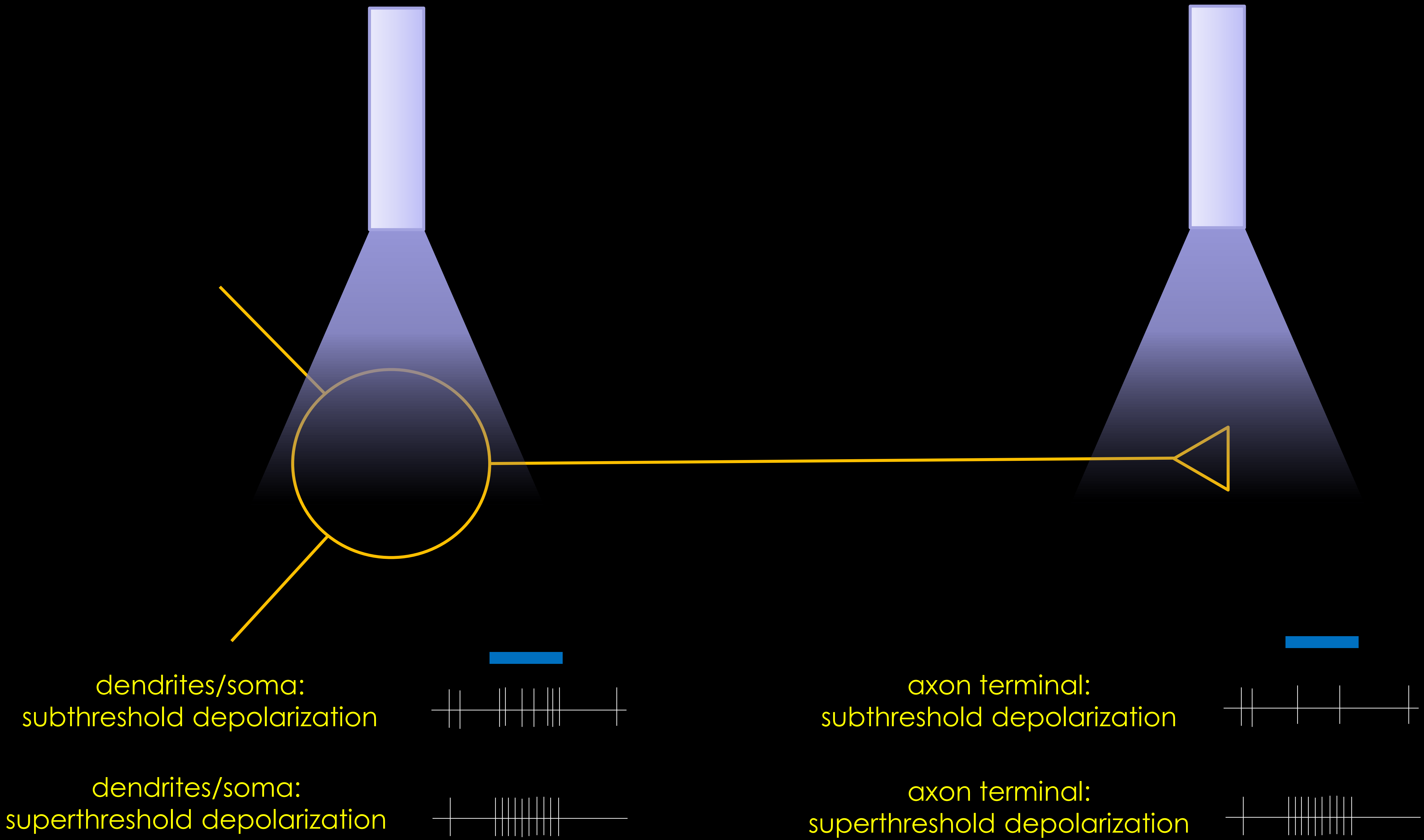
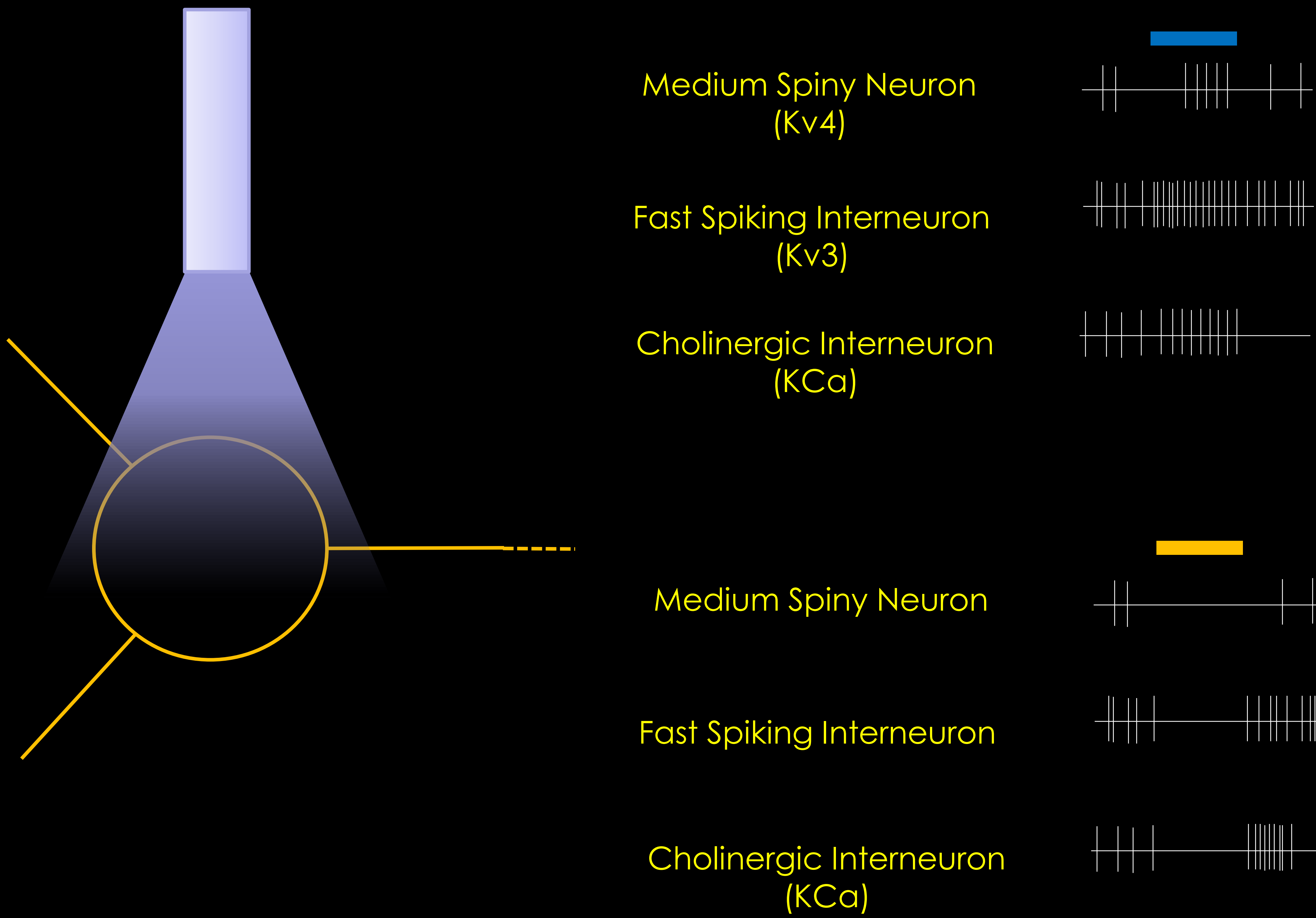
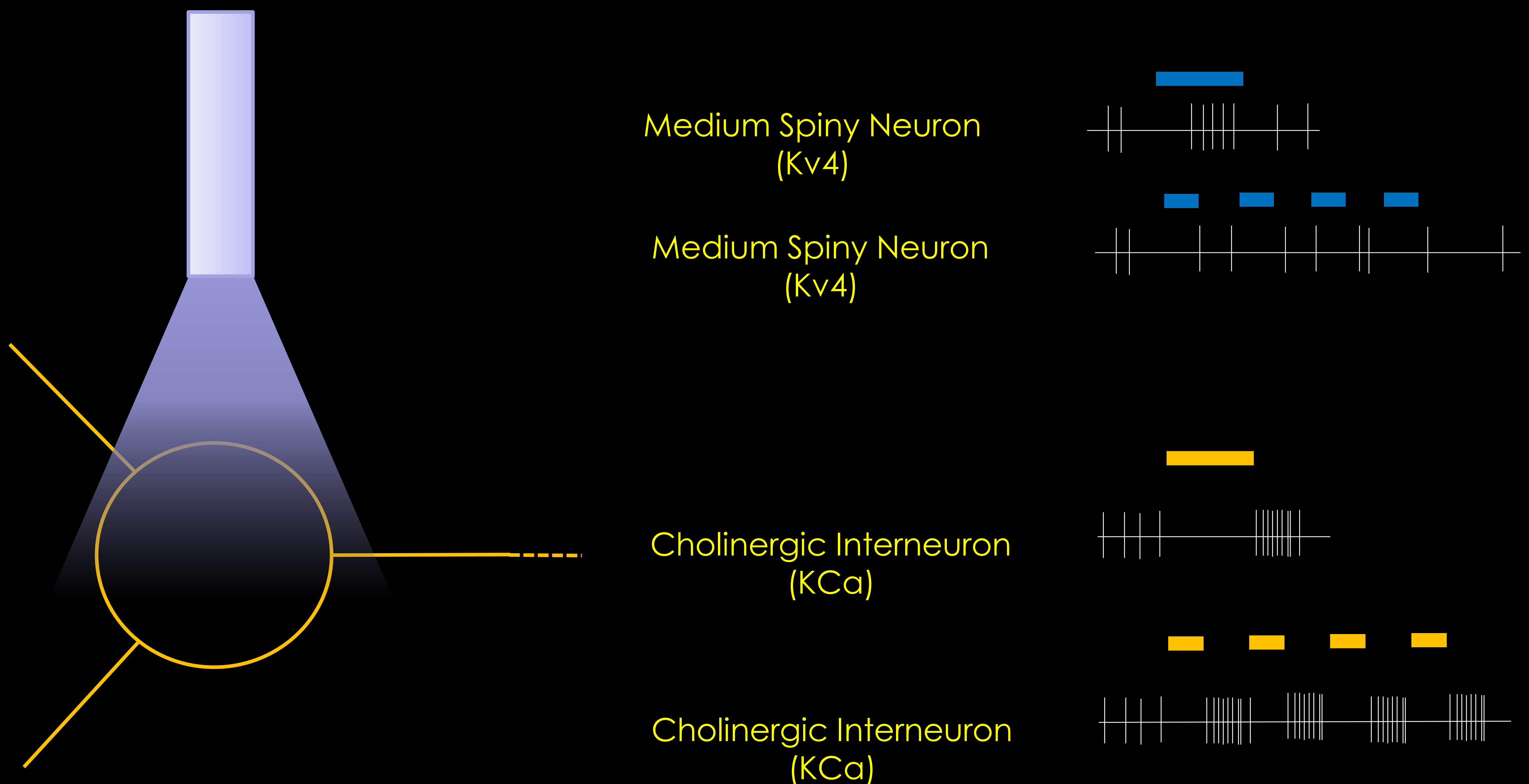
Cellular Effects of Optogenetic Manipulations Depend on Intensity and Locus



Cellular Effects of Optogenetic Manipulations Depend on Intrinsic Conductances and Firing



Cellular Effects of Optogenetic Manipulations Depend on Pattern of Illumination



Paradoxical Modulation by Optogenetic Effectors

- Suppression of spiking by activators: depolarization block
- Activation of spiking by eNpHR: dendritic loading of Cl^- yields depolarizing shift in E_{Cl}, GABA becomes depolarizing
- Activation of spiking by Arch: change in extracellular pH activates ASIC channels
- Increased spontaneous neurotransmitter release by Arch

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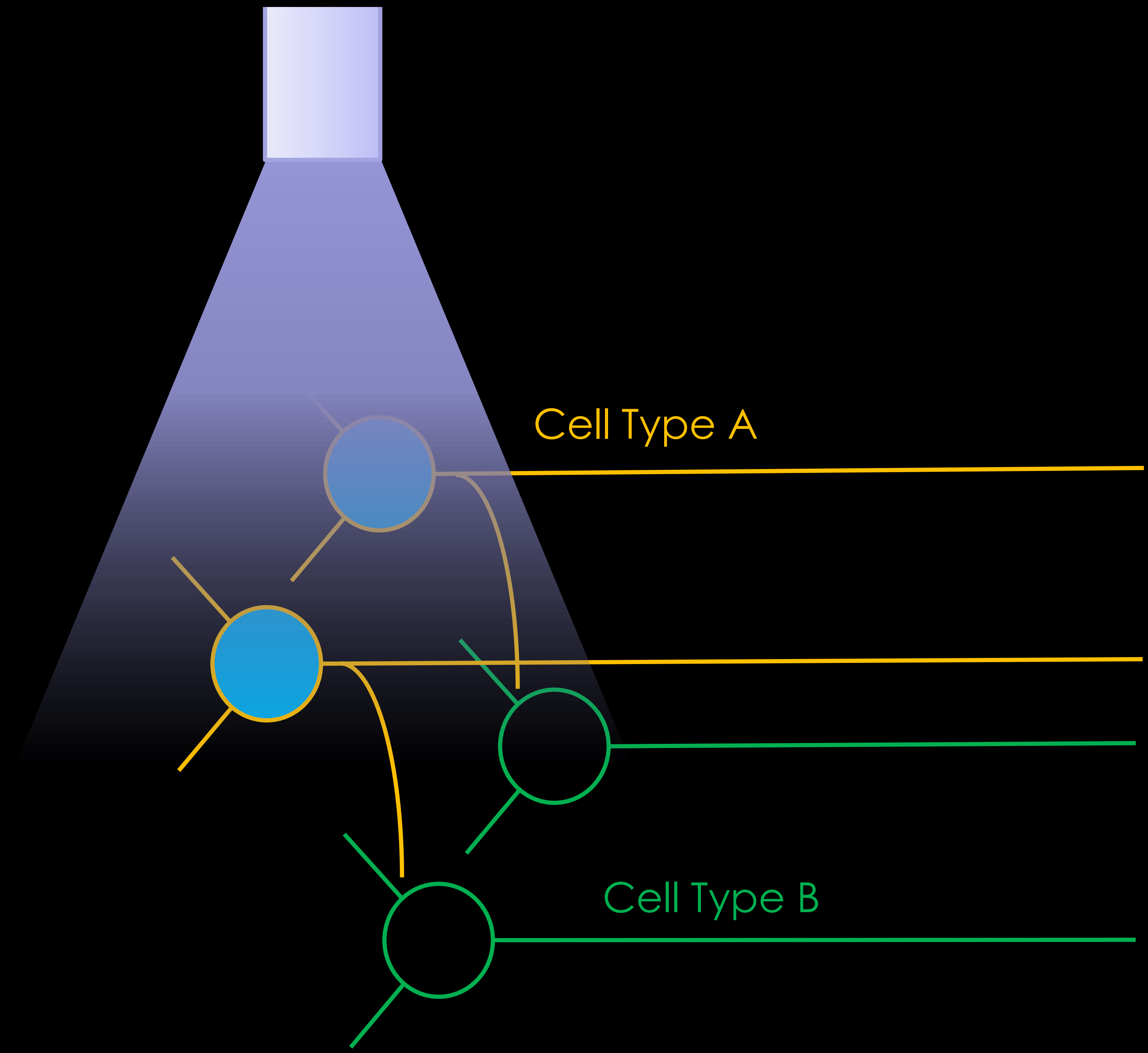
Activity Manipulation: Effects of Local Microcircuitry



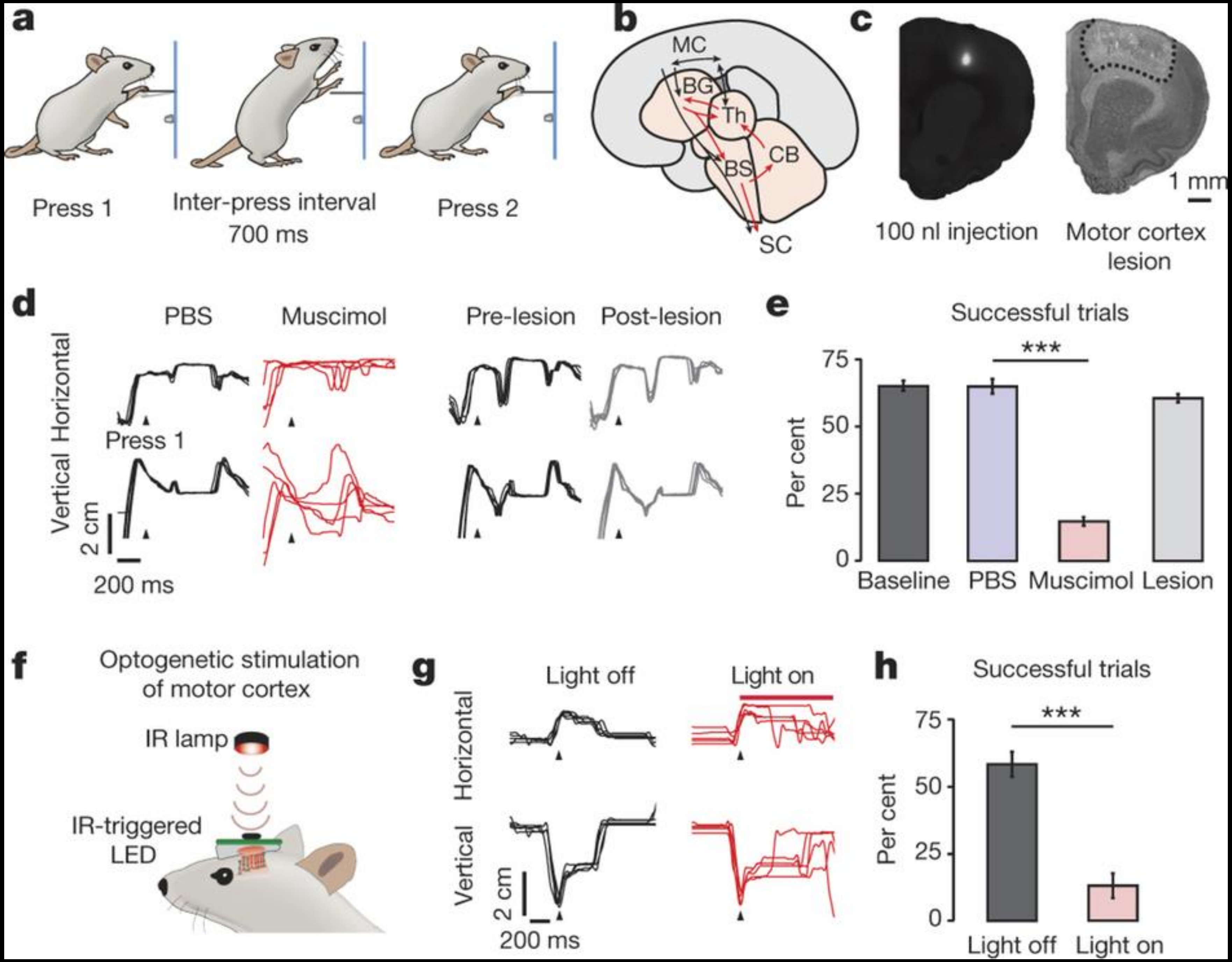
Scenario 1: Cell type A and B are glutamatergic.

ChR2 in cell type A

Behavior could be driven by either cell type A or B

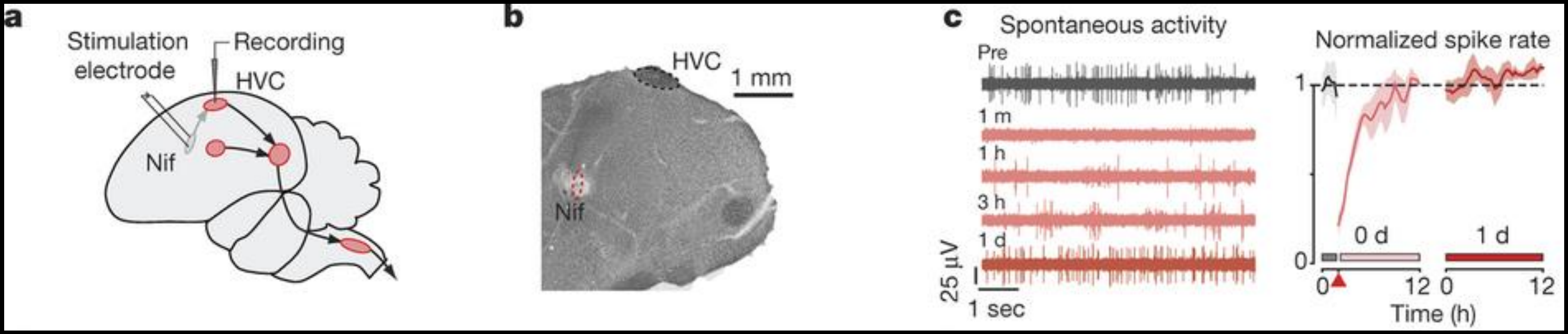
For a more complex analysis of optogenetic effects on circuit computation, see Phillips and Hasenstaub 2016

Activity Manipulation: Network Effects

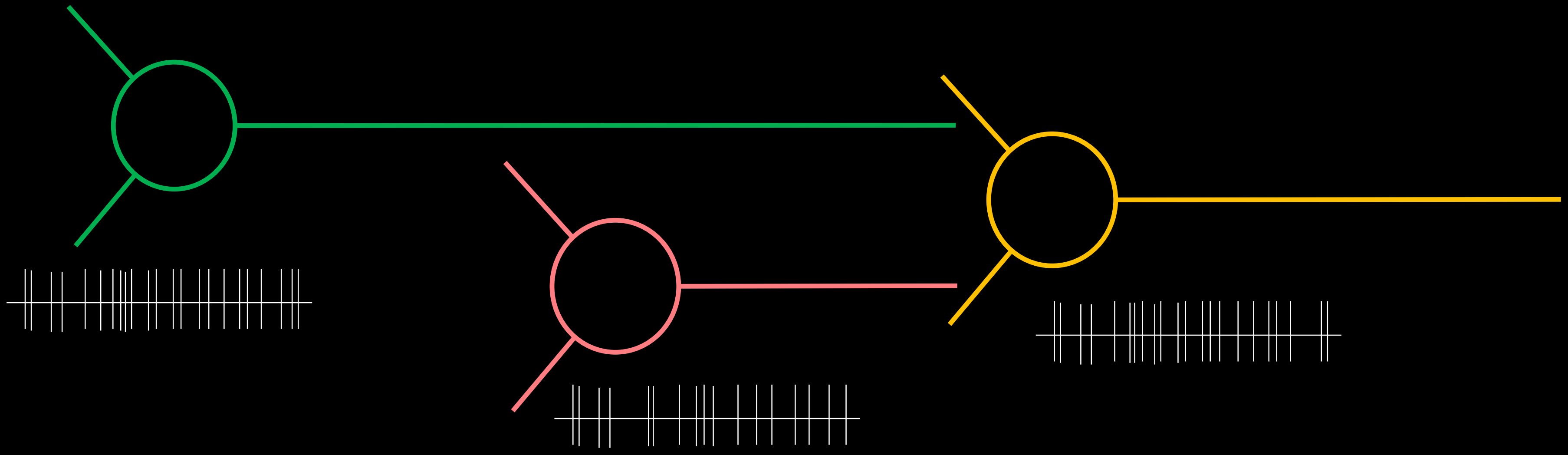


Otchy et al, Nature 2015

Activity Manipulation: Network Effects



Otchy et al, Nature 2015



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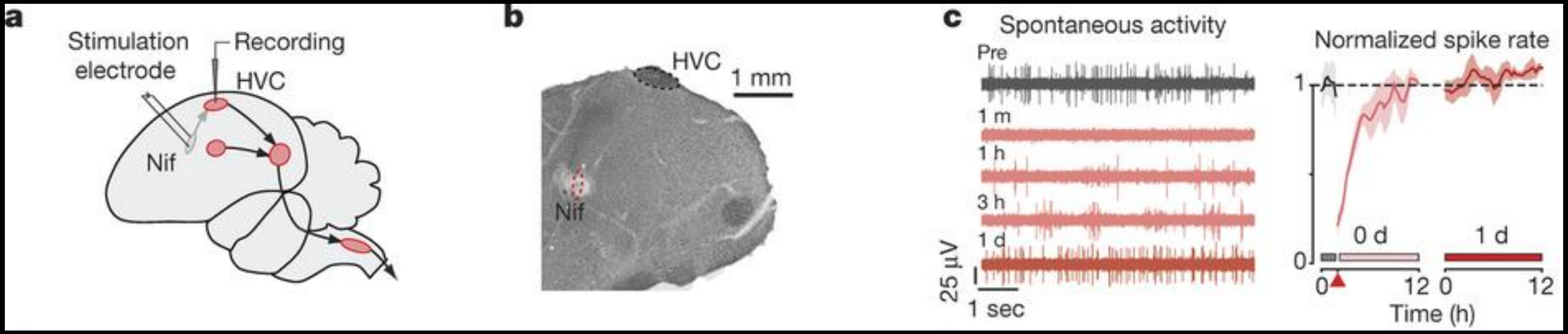
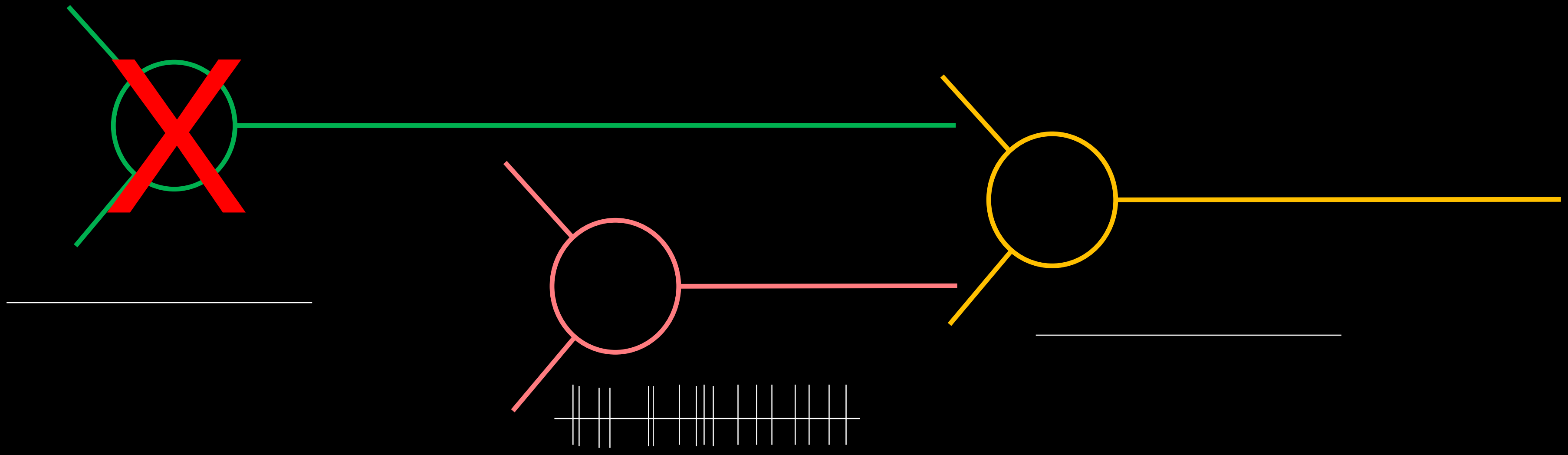


Otchy et al, Nature 2015



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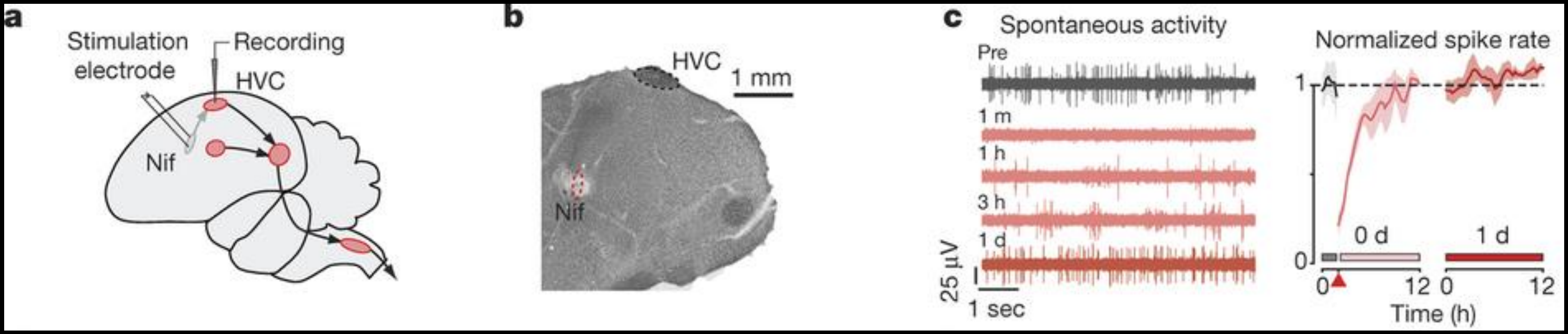

Otchy et al, Nature 2015