New approaches to simultaneously drive and measure neuronal activity

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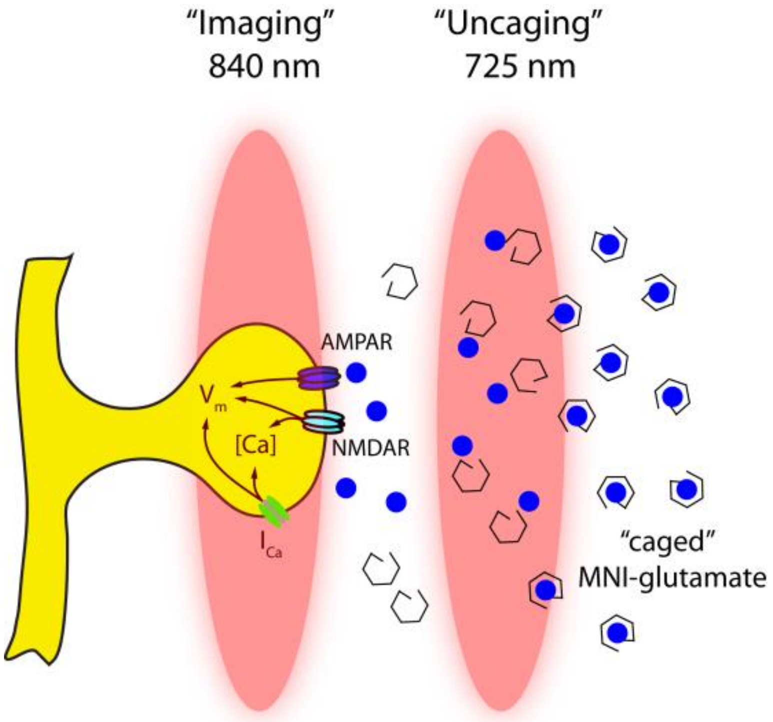
1) Simultaneous electrophysiology and Ca2+ imaging driven by neurotransmitter uncaging

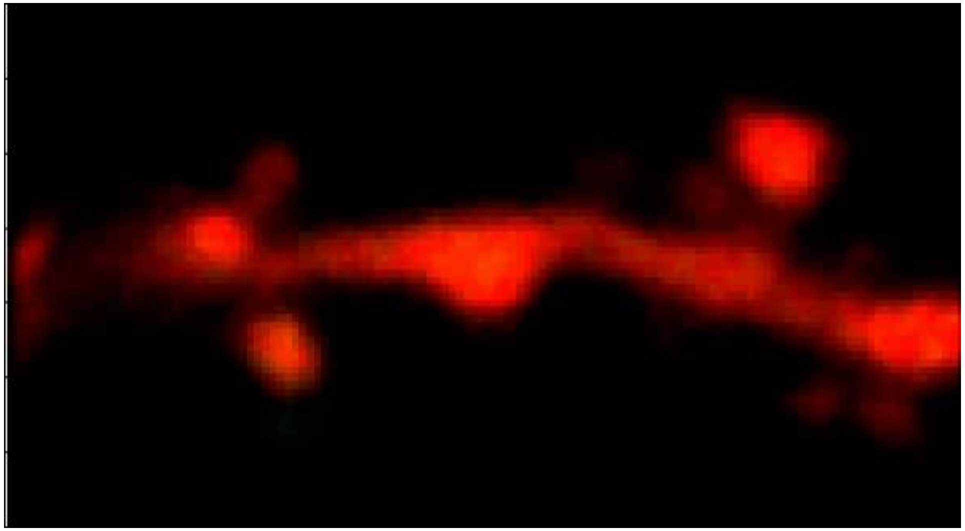
2) Optopatch: excitation/inhibition and readout of neuronal activity using light only

3) Holographic activation of neurons at cellular resolution combined with in vivo Ca2+ imaging

Simultaneous electrophysiology and Ca2+ imaging driven by neurotransmitter uncaging

Goal: maximal control: what neurotransmitter is released, where exactly it is released, read out both electrical and biochemical signal

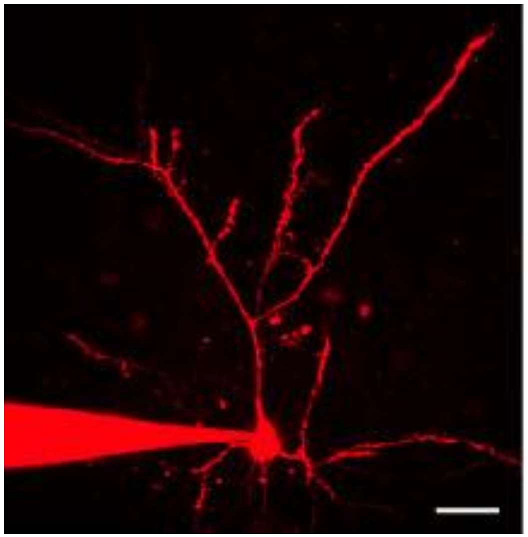




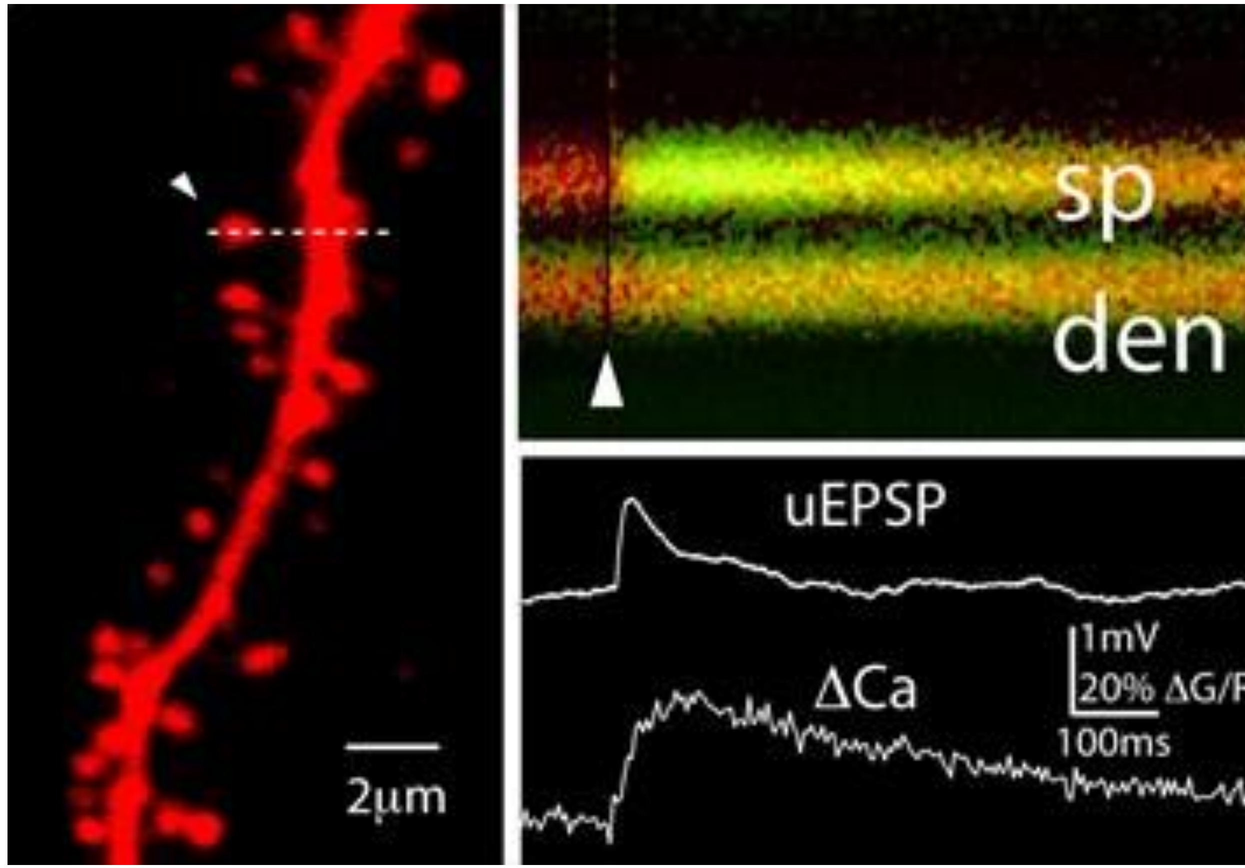
Microscope you need for this is a beast!

2 x Ti-Sapphire laser
2 x light path (including Pockels cell for beam control)
2 x GaAsP detectors (single synapse = very few photons)
Low noise patch clamp rig (2 x makes financial sense)
Needs complete darkness

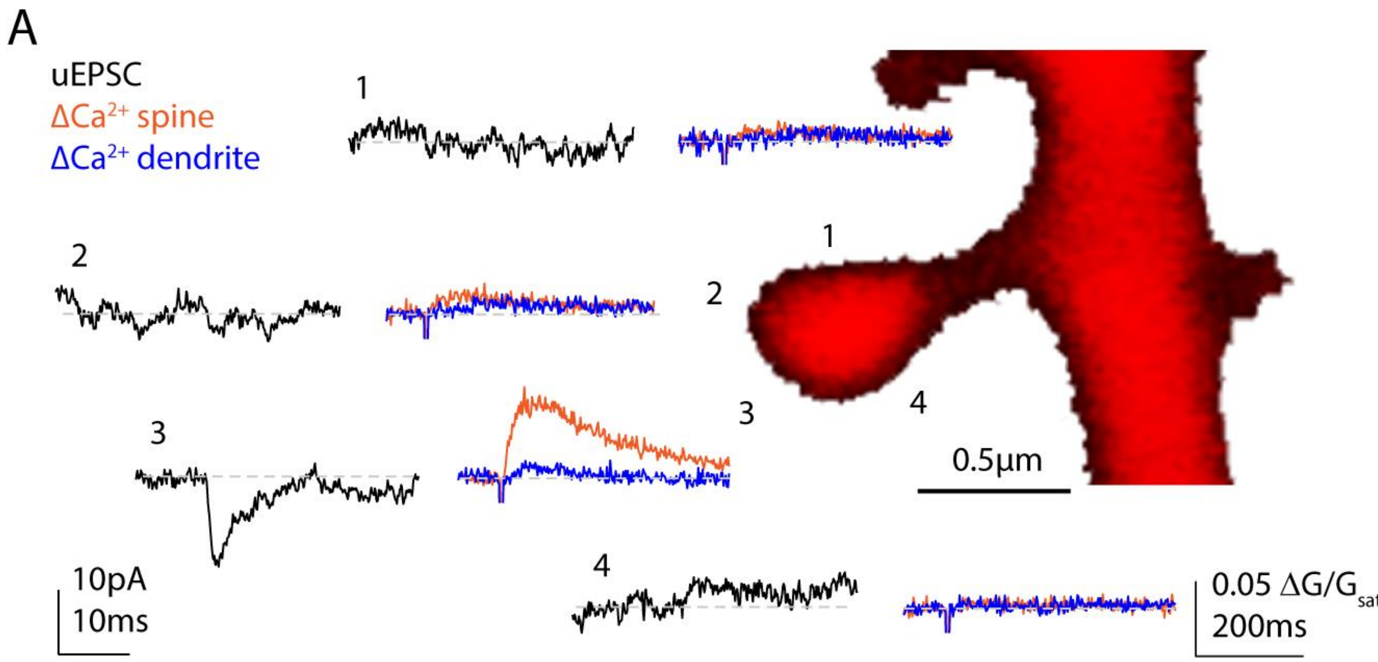
Combined whole-cell recording, Ca2+ imaging and uncaging



Cell filled with:
Alexa 594 (red)
Fluo 4 (green)

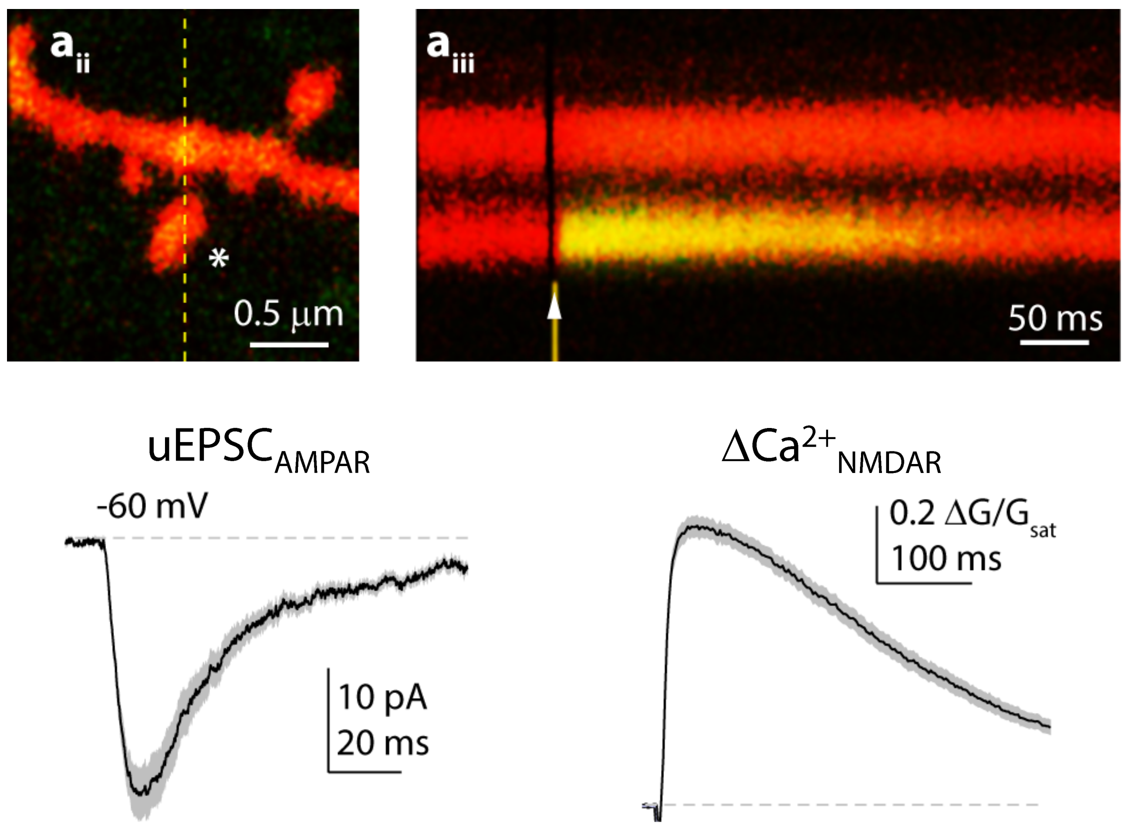


Spatial precision of glutamate uncaging



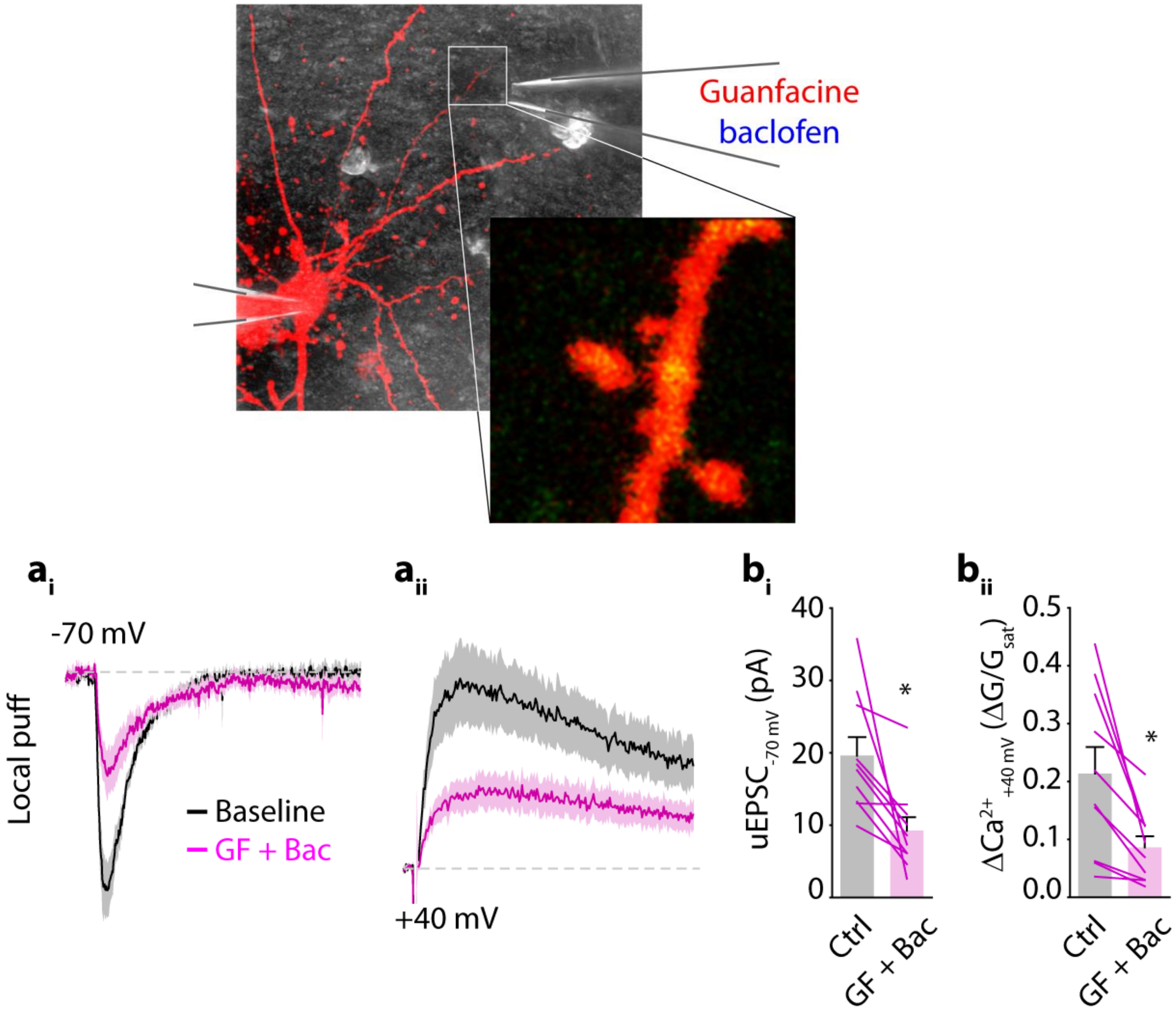
Glutamate Receptor Modulation Is Restricted to Synaptic Microdomains. Lur 2015.

Example: isolate postsynaptic responses to study glutamatergic transmission



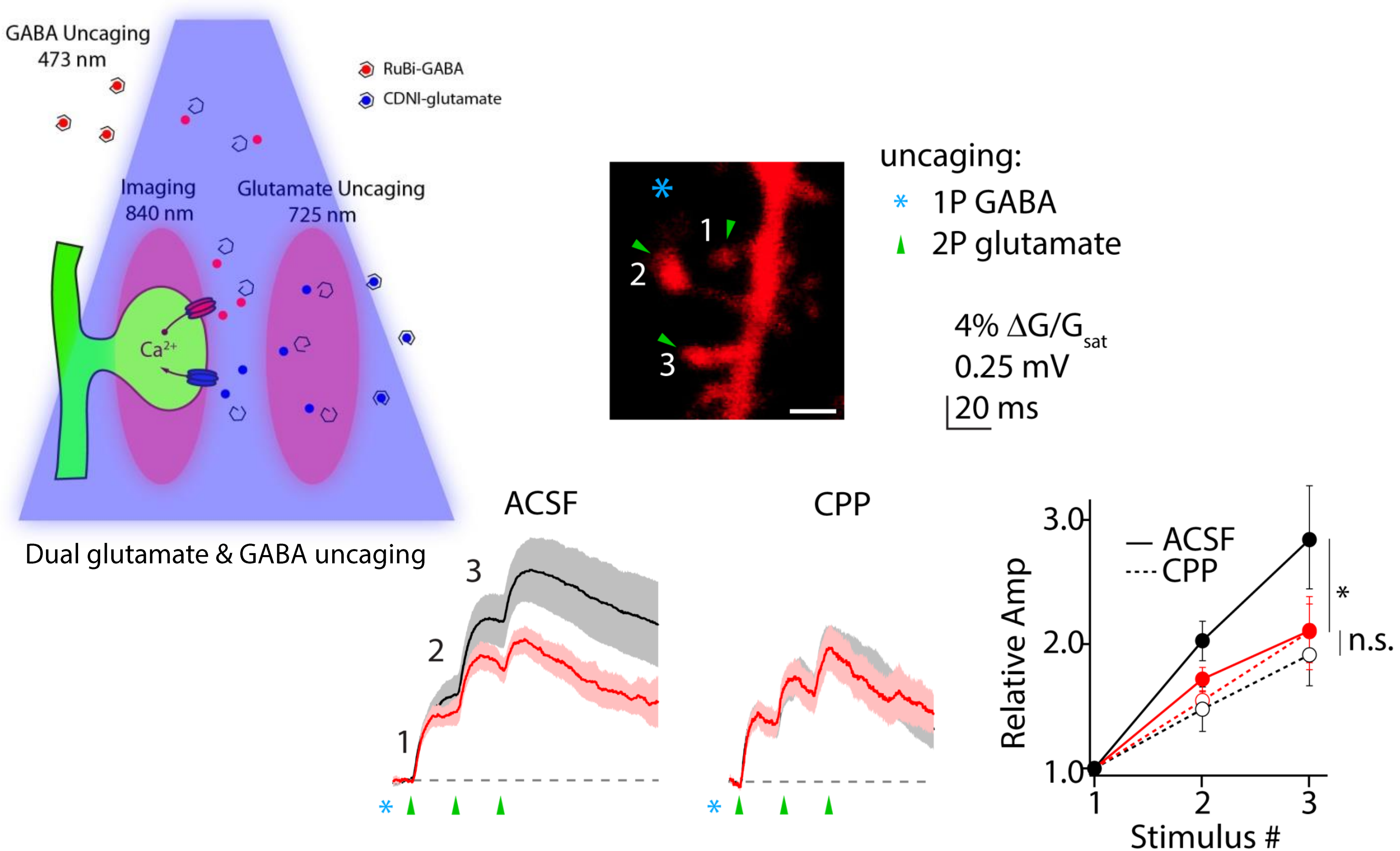
Glutamate Receptor Modulation Is Restricted to Synaptic Microdomains. Lur 2015.

Local neuromodulation of excitatory synapses



Glutamate Receptor Modulation Is Restricted to Synaptic Microdomains. Lur 2015.

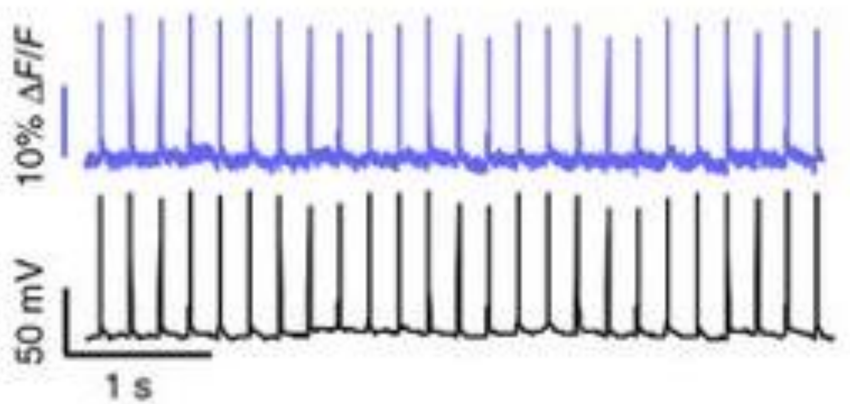
Dual-color uncaging: cooperation between neurotransmitters

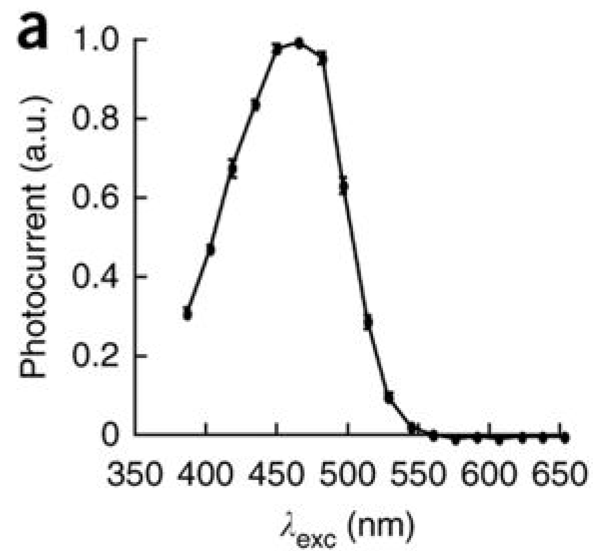


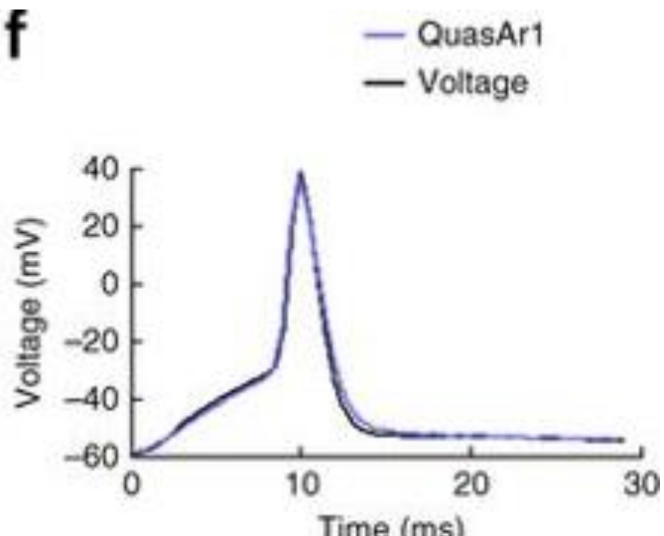
Compartmentalization of GABAergic inhibition by dendritic spines. Chiu 2013.

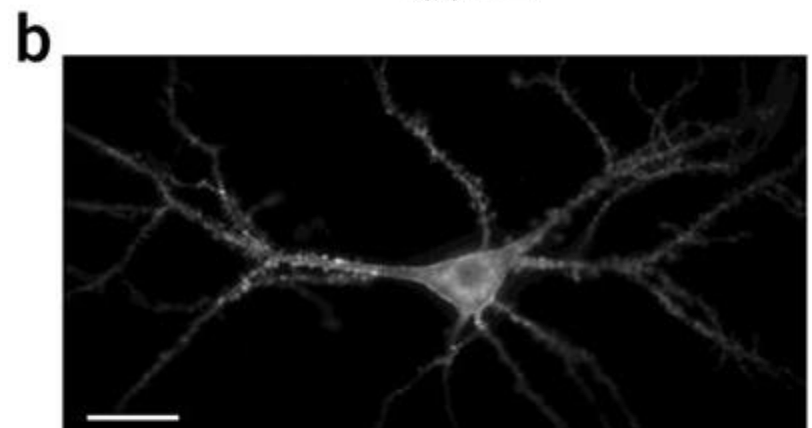
Optopatch: excitation/inhibition and readout of neuronal activity using light only

Co-express an optical indicator and actuator in the same cell
Voltage indicator: QuasArs (mutated from Arch voltage indicator)
Light driven actuator: CheRiff (a fresh-water green algae derived ChR2 variant)

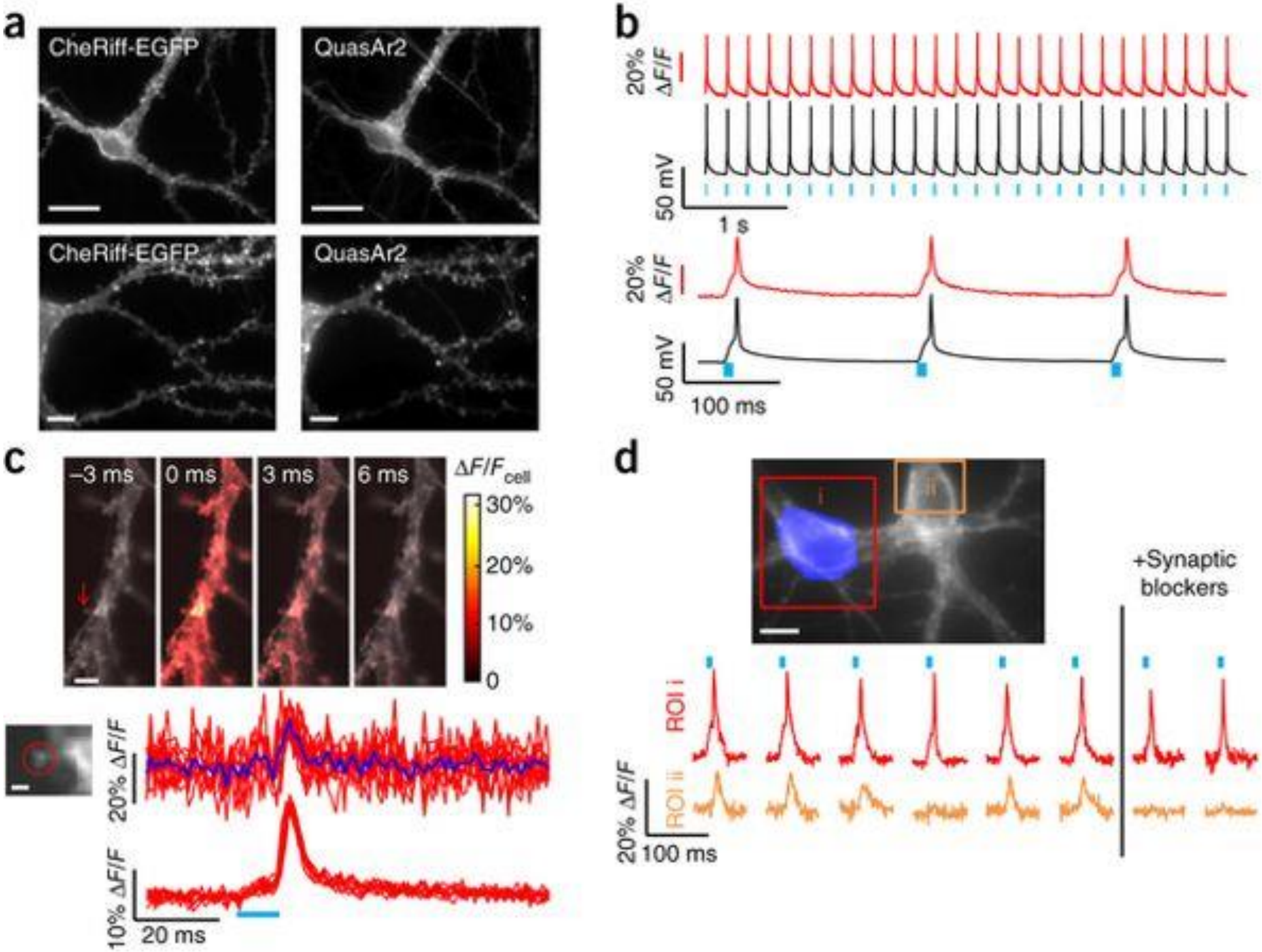






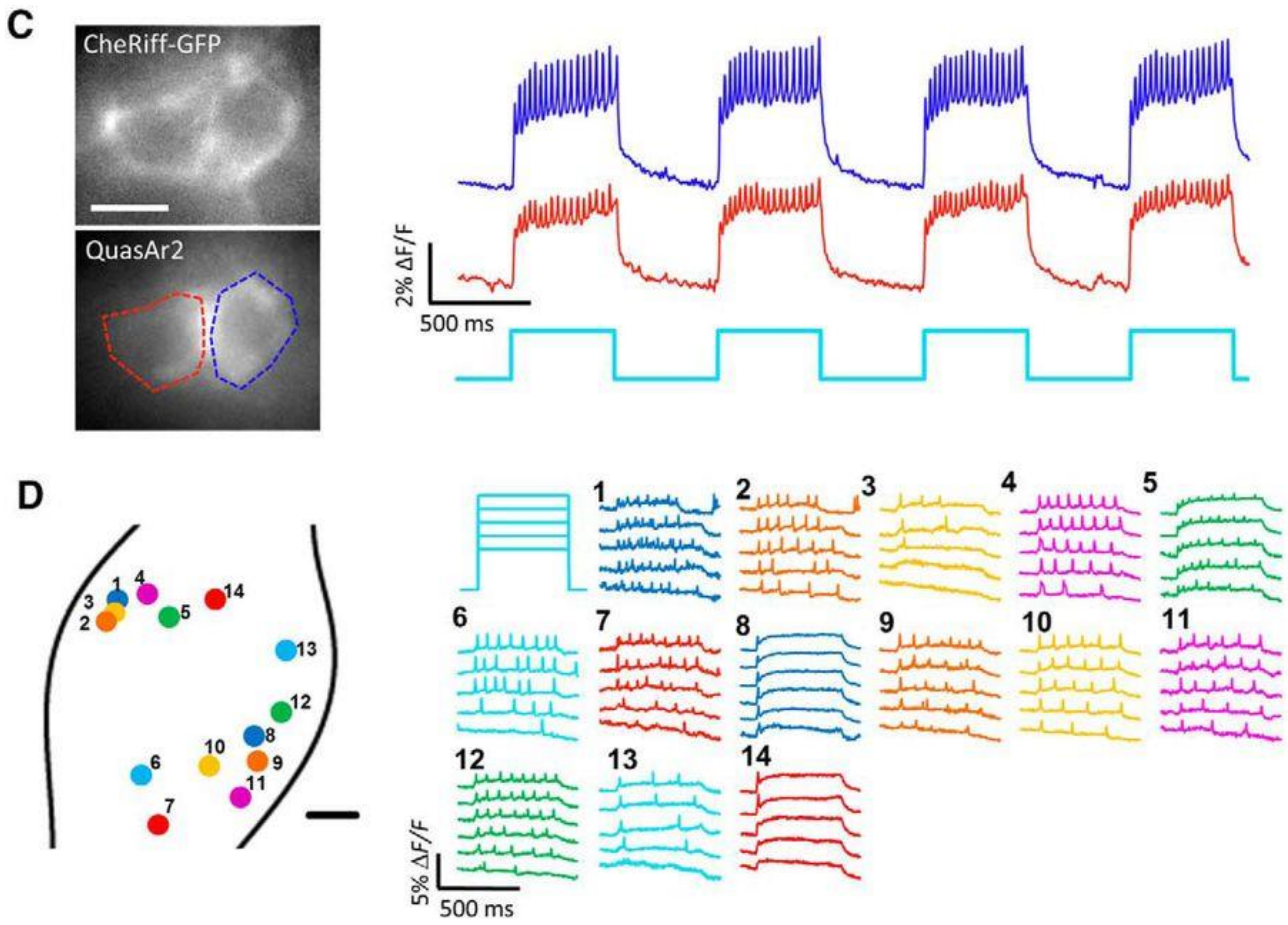


High-fidelity optical stimulation and recording



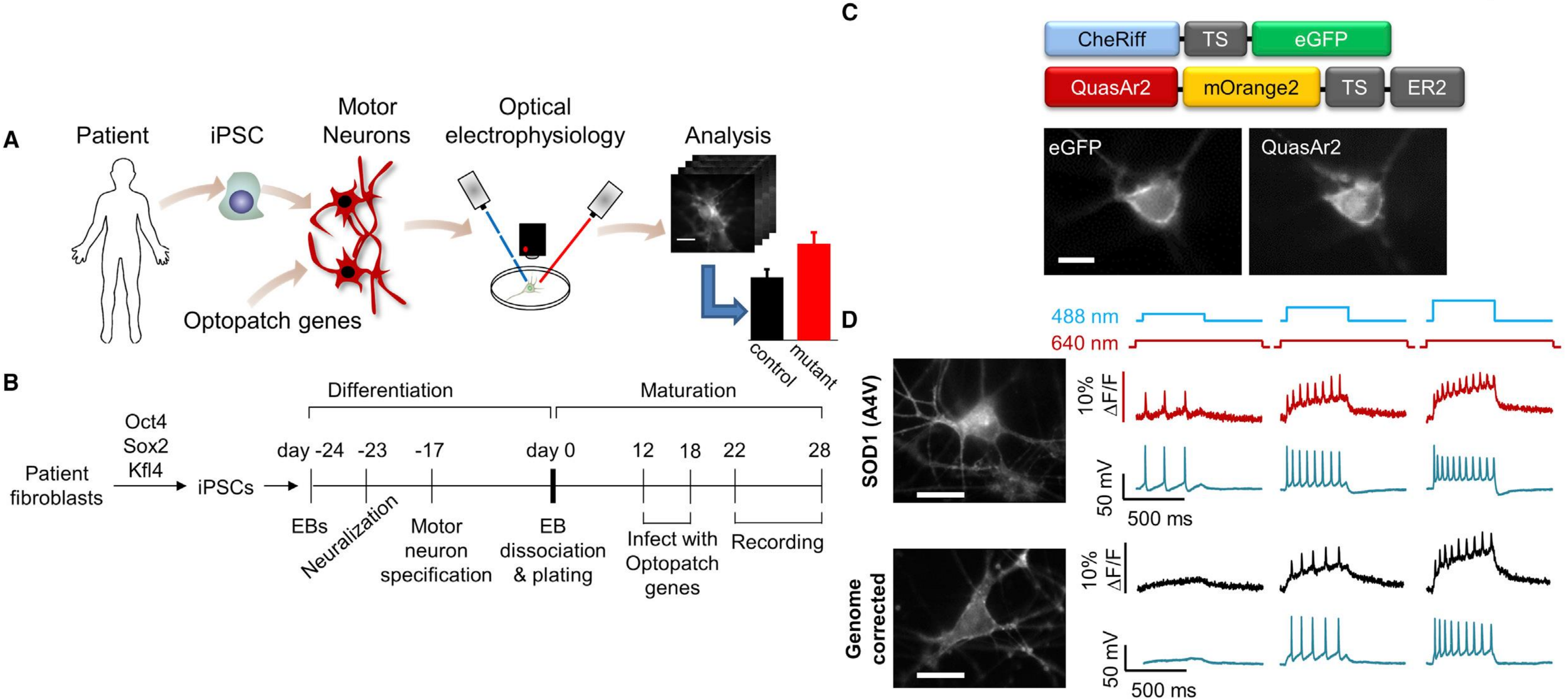
All-optical electrophysiology in mammalian neurons using engineered microbial rhodopsins. Hochbaum 2014.

Optopatch: in vivo – nodose ganglion imaging



Genetically Targeted All-Optical Electrophysiology with a Transgenic Cre-Dependent Optopatch Mouse. Luo 2016

Optopatch: high-throughput functional characterization of patient derived neurons

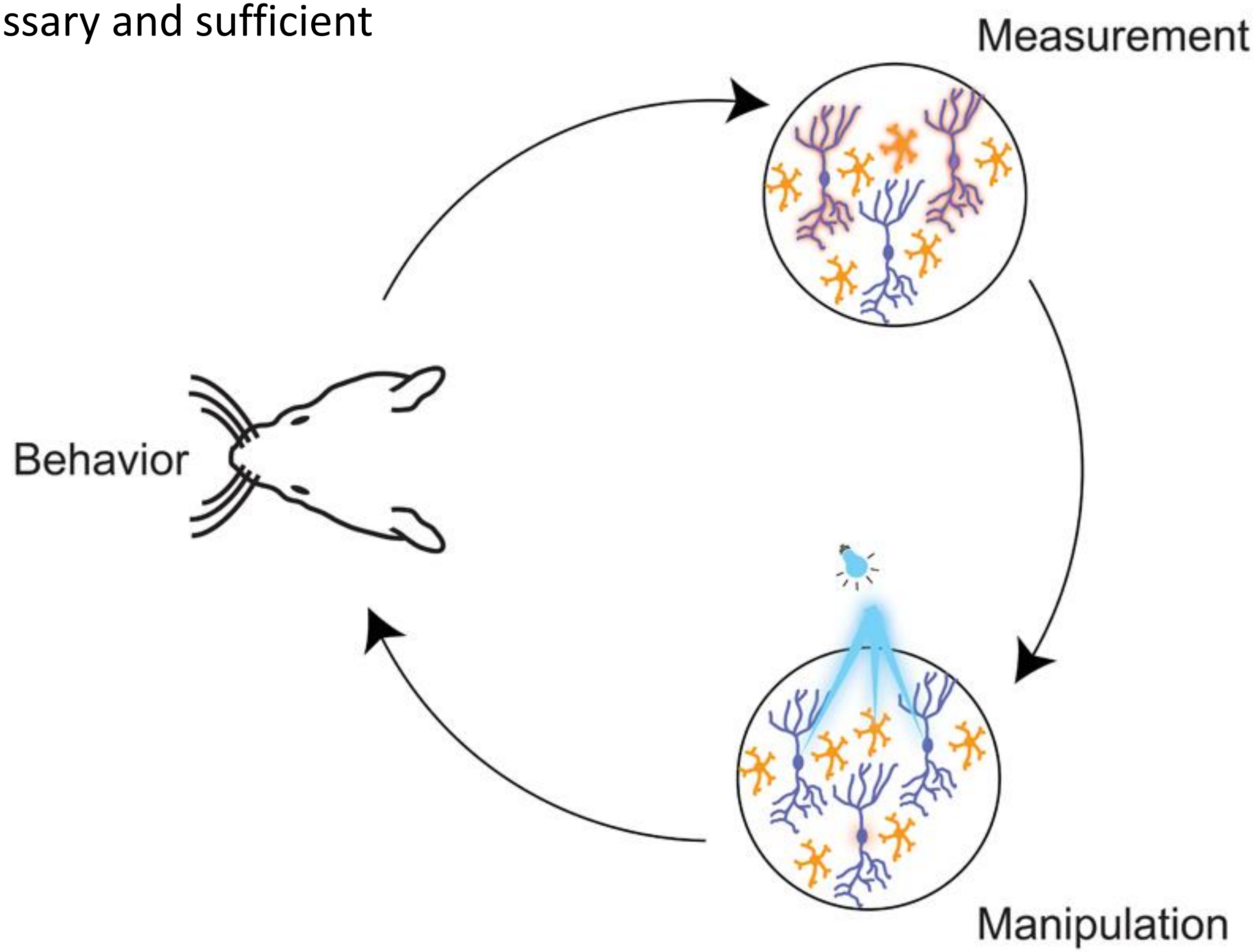


All-Optical Electrophysiology for High-Throughput Functional Characterization of a Human iPSC-Derived Motor Neuron Model of ALS

Kiskinis 2018

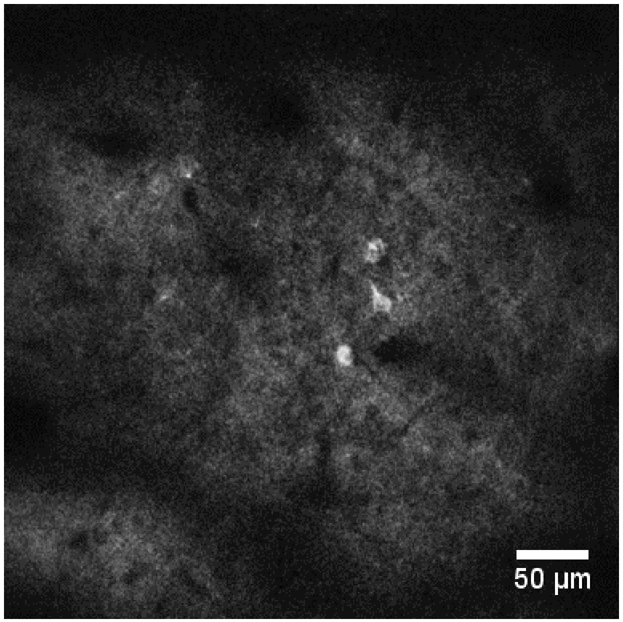
Holographic activation at cellular resolution combined with Ca2+ imaging in vivo

Goal: identify what neurons in what sequence generate behavior, then activate same cells in same sequence to reproduce it
-> necessary and sufficient

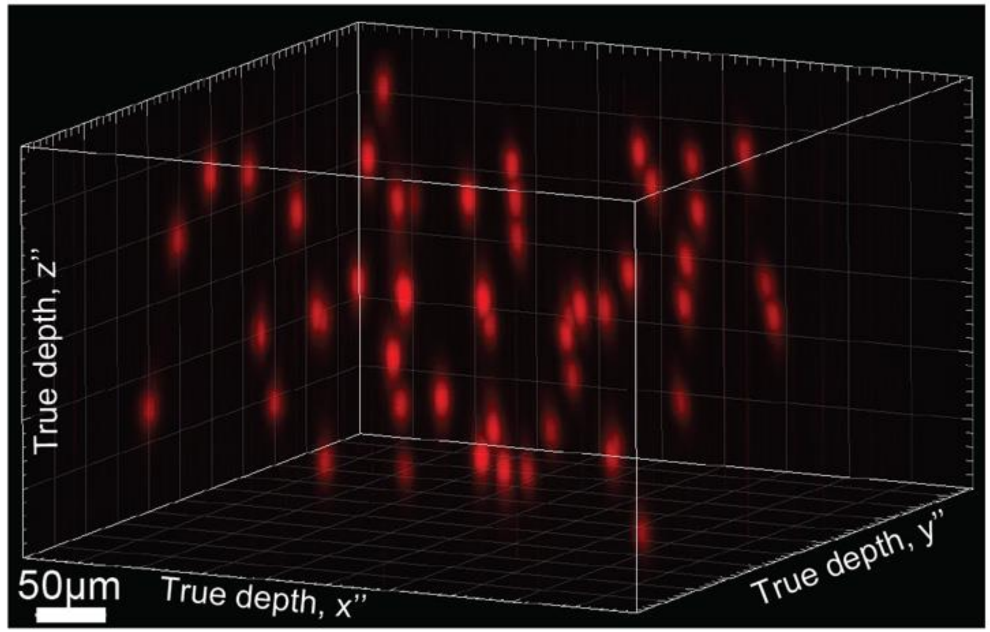


Targeting neurons and photons for optogenetics. Packer 2013

Behavior → Measure neurons → Activate neurons → Behavior

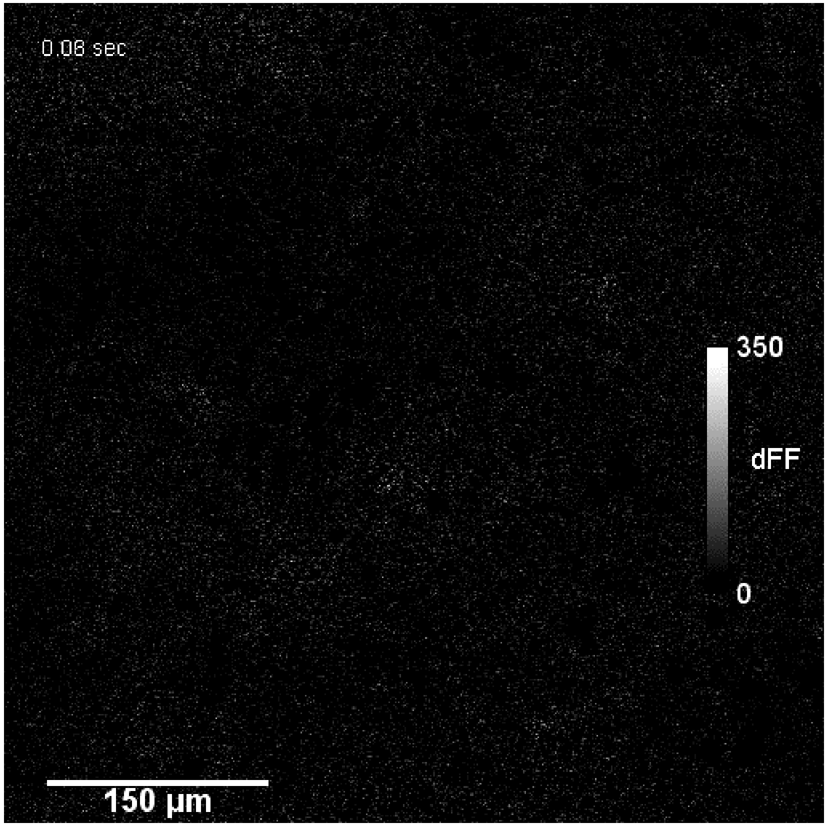


Striman 2016



Pegard 2017

Holographic activation at cellular resolution combined with Ca2+ imaging in vivo



Imaging: GCaMP6s at 30 Hz
Photostimulating: groups of 10 cells, 1 second between stimuli.
Movie: dFF, average of 10 trials, photostim artifact removed, and sped up 2x realtime.
Unpublished from Robert Lees, James Rowland, and Adam Harris (Packer Lab)

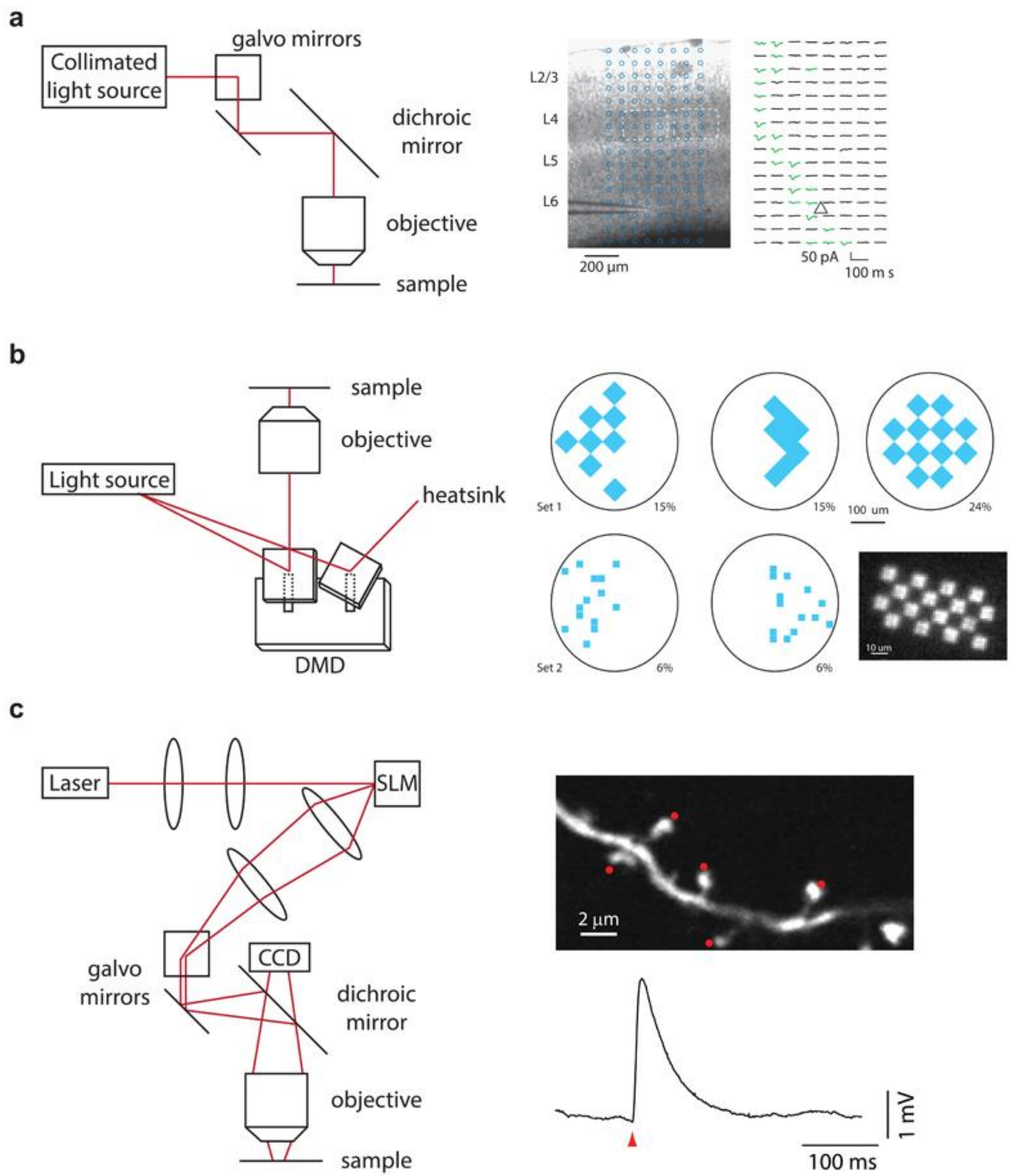
History

Targeted light strategy	Number of neurons addressed	Pros	Cons	Biological questions addressed	Representative references
1P full field	100 - 1000	Many neurons activated simultaneously, high temporal resolution	Low spatial resolution using viral transfection	Circuit analysis of cell types	47, 52
1P full field + sparse labeling	1 - 100	High spatial and temporal resolution; can identify cells individually	Only suitable for low numbers of neurons	Single to many-neuron computation	34
1P fiber	100 - 1000	Can be used in freely moving animals	Low spatial resolution	Effect of cell types on behavior	104
1P directed beam	10 - 100	Spatial resolution ~50 µm	Cannot activate individual neurons	Mapping anatomical features of cell types and projections	25, 105
1P DMD	100 - 1000	Commercially available	Low spatial resolution	Effect of activation of cell types in spatial patterns	71, 72, 103, 106
1P SLM	100 - 1000	Holographic patterns enable photostimulation in three dimensions	Low spatial resolution	Effect of activation of cell types in spatial patterns	107, 108
2P directed beam	1	Single cell spatial resolution	Only one neuron at a time	Mapping inputs from individual neurons	76, 83, 84, 109
2P SLM	~50	High-resolution holographic patterns can activate multiple individual neurons	Low temporal resolution	Manipulation of neural coding at the individual neuron level	78, 84
2P temporal focusing	1 - 10	High spatial and temporal resolution: can activate multiple individual neurons	Few neurons at a time given high laser power required for each neuron	Manipulation of neural coding at the individual neuron level	77, 78
2P AOD	1 - ?	High spatial and temporal resolution: can activate multiple neurons sequentially over very short intervals	Untested	Manipulation of neural coding at the individual neuron level	None

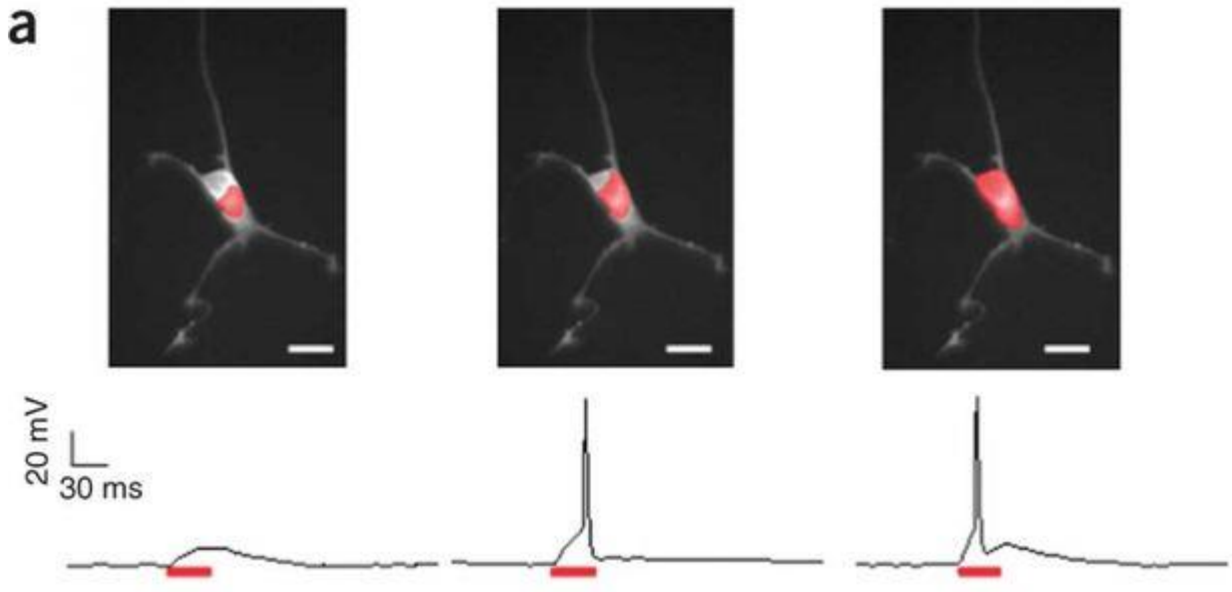
Targeting neurons and photons for optogenetics. Packer 2013

Evolution of optical approaches

<u>Pioneers</u>
Rafael Yuste
Valentina Emiliani
Michael Hausser
Hillel Adesnik



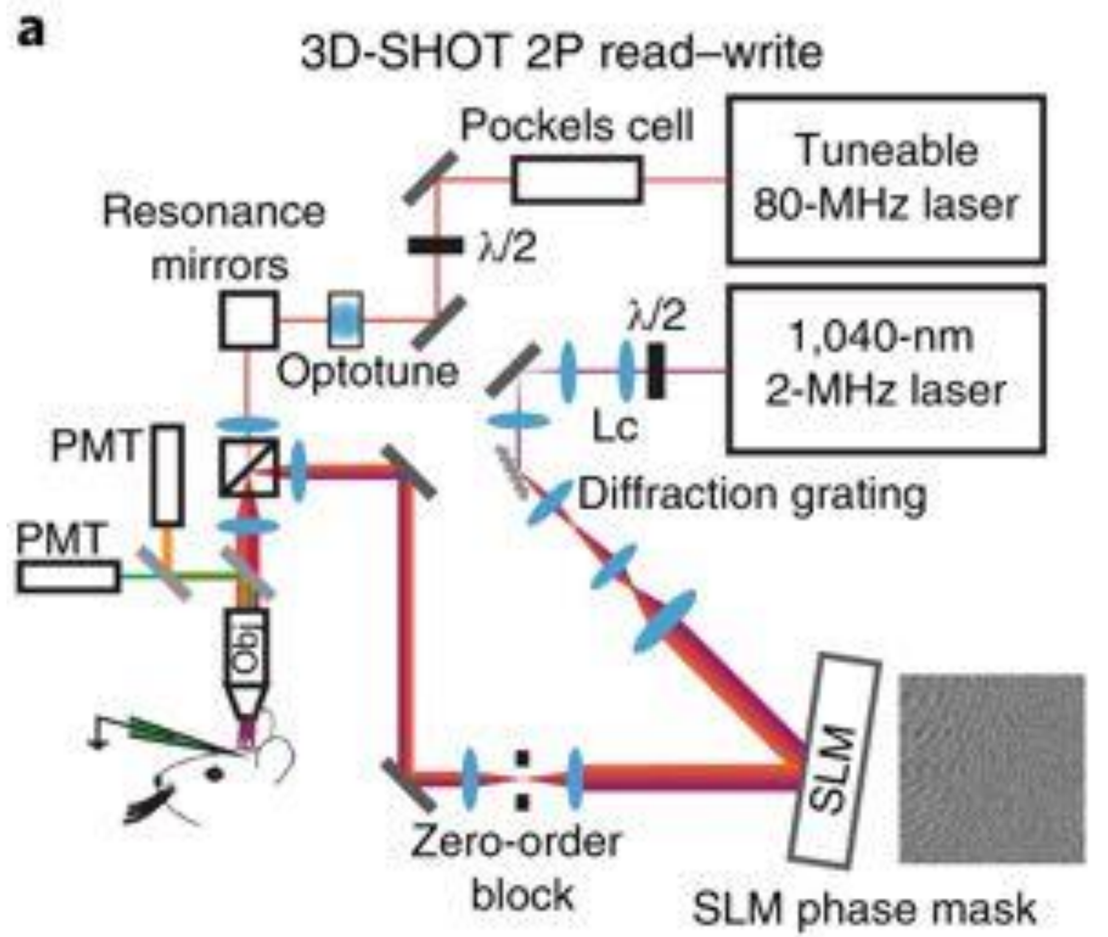
The importance of sculpting light



Scanless two-photon excitation of channelrhodopsin-2. Papagiakoumou 2010

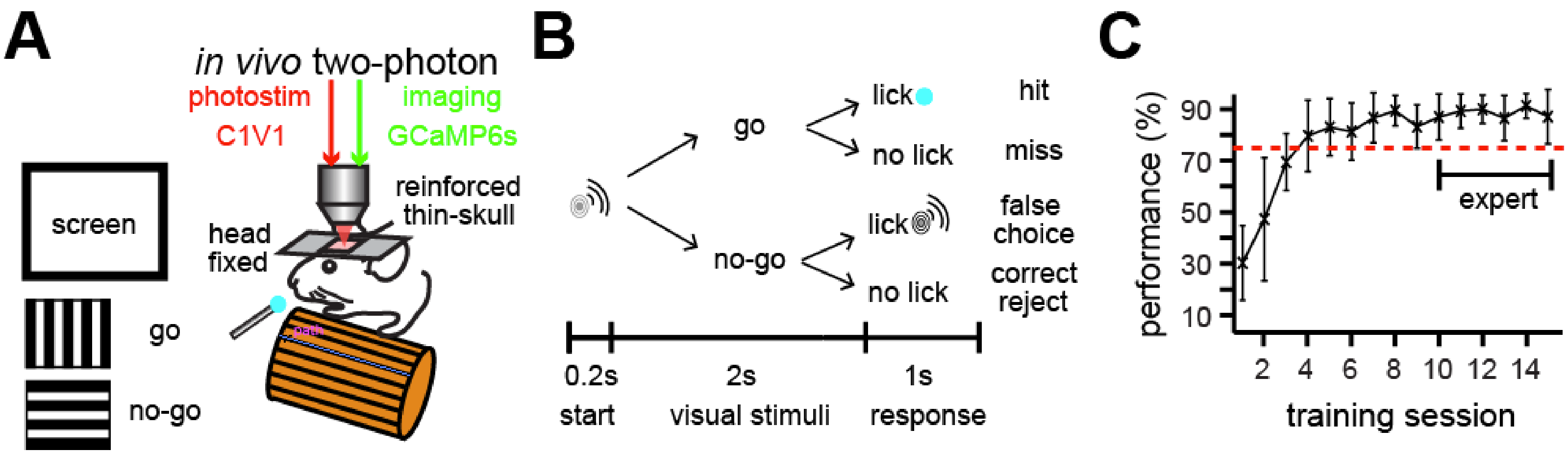
Complex optical setups for optical read-write

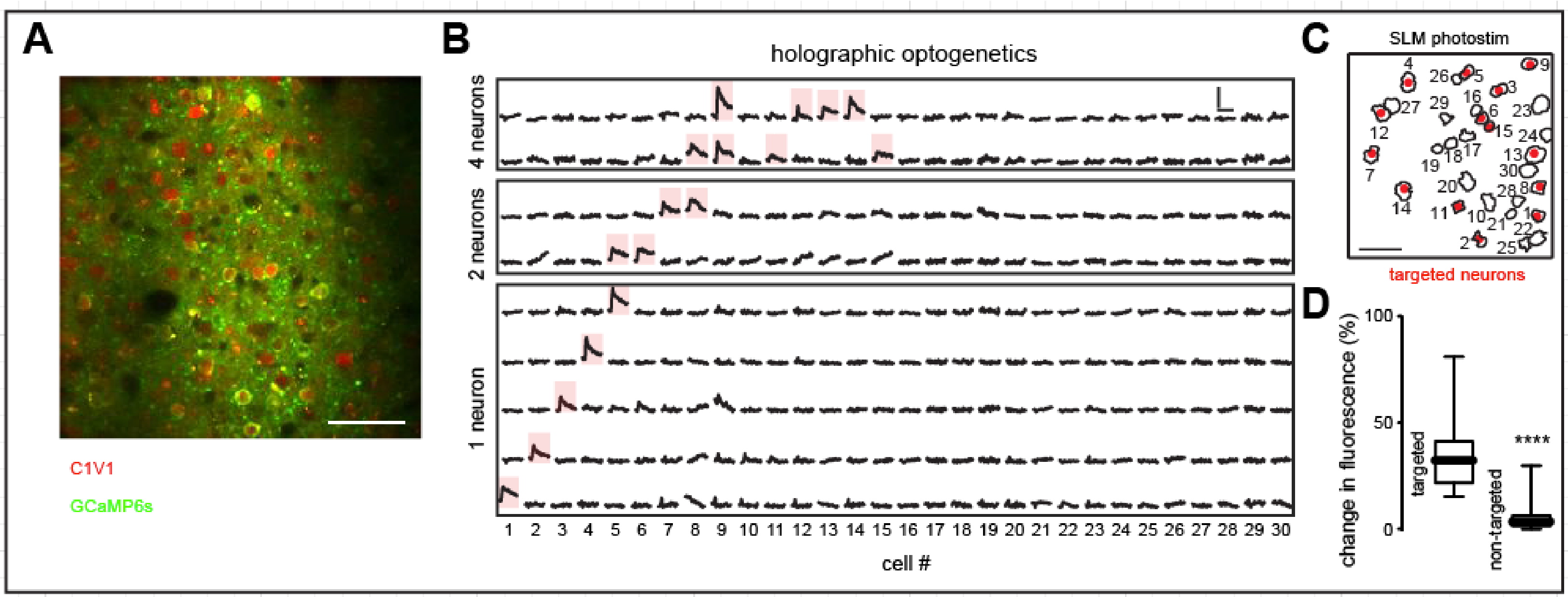
Read: GCaMPs
Write: ST-Chronos



Precise multimodal optical control of neural ensemble activity Mardinly 2018

Holographic stimulation in action





Controlling Visually Guided Behavior by Holographic Recalling of Cortical Ensembles. Carrillo-Reid 2019.

The dream experiment