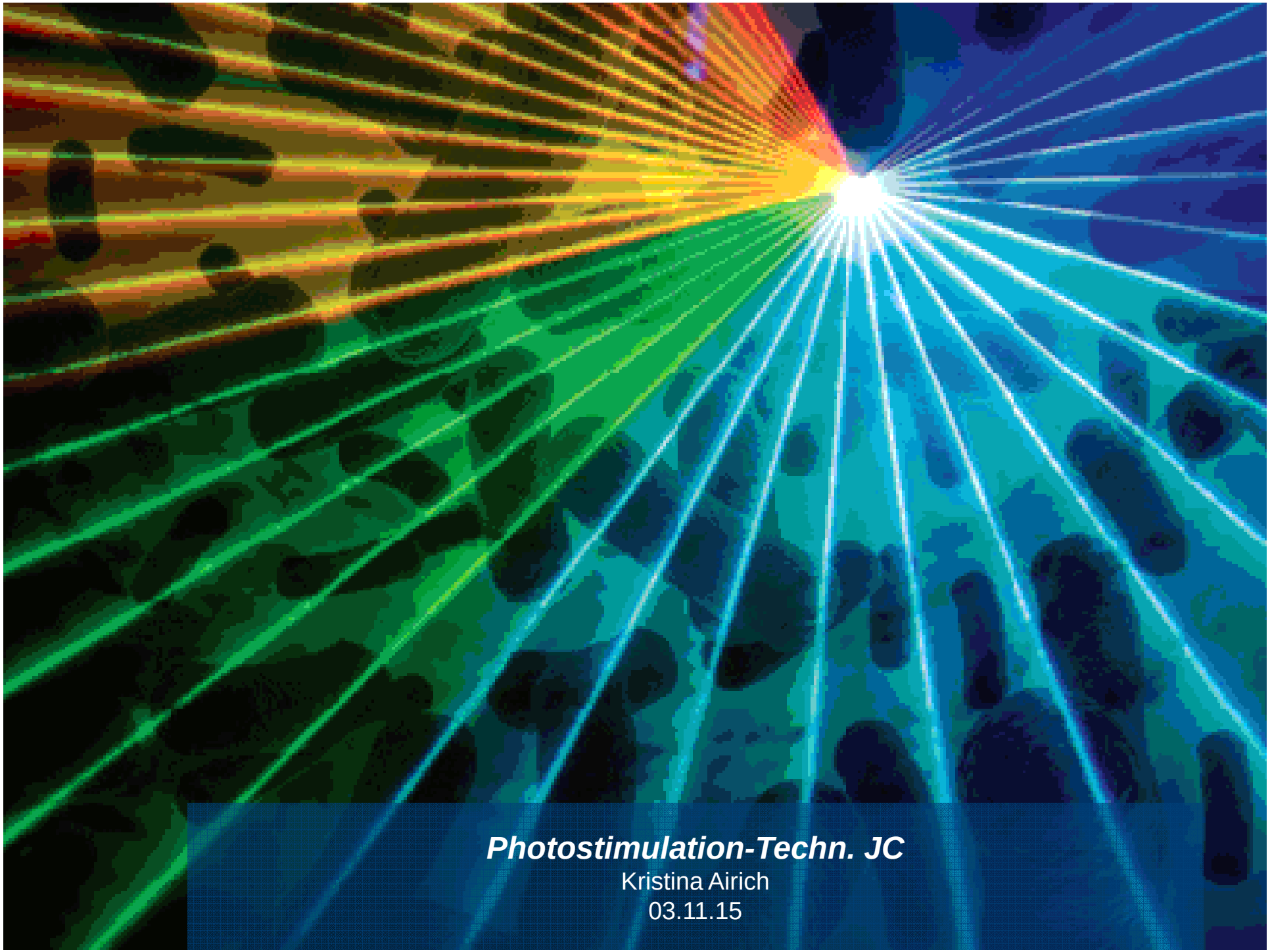
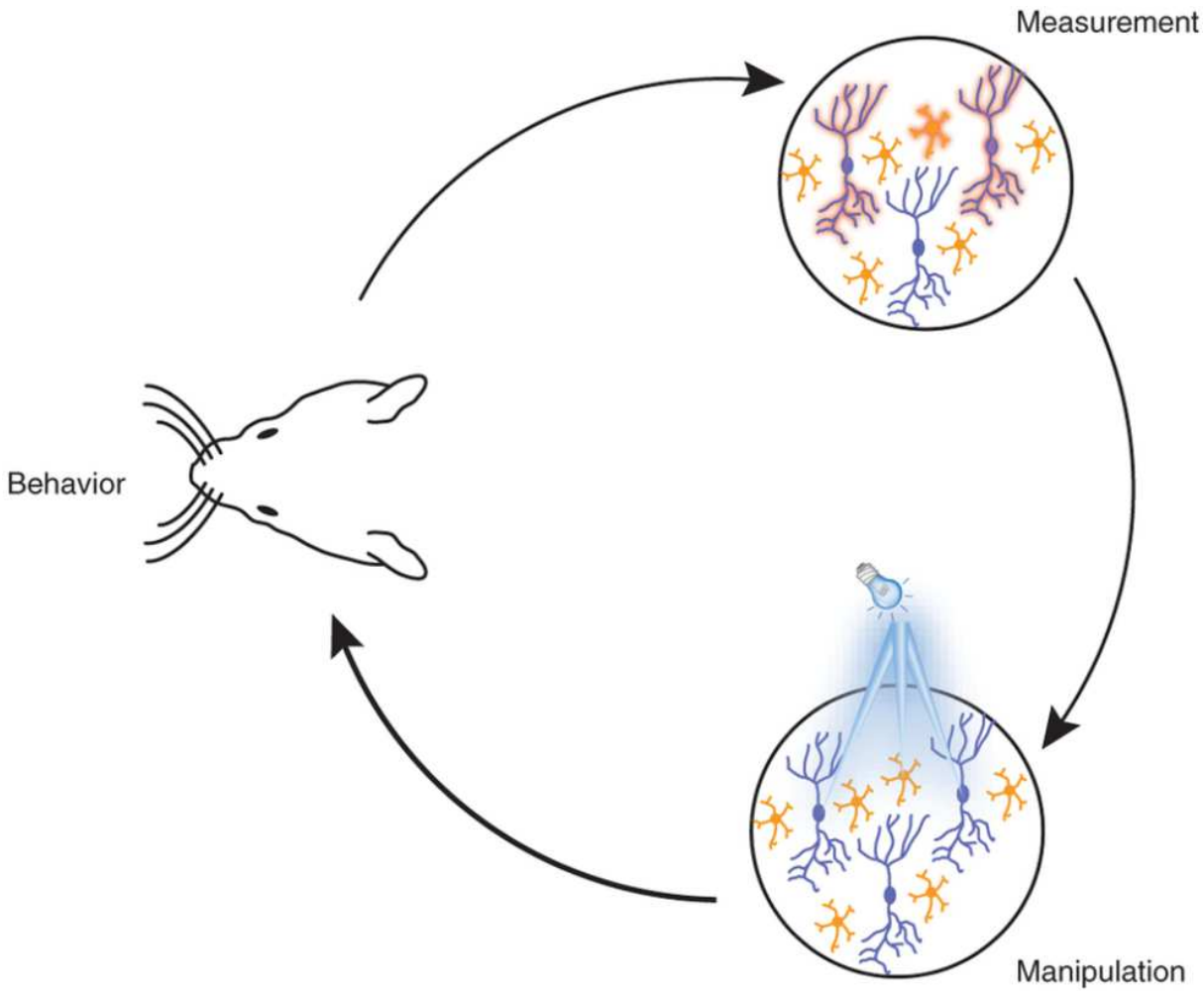


Photostimulation-Techn. JC

Kristina Airich

03.11.15

Goal

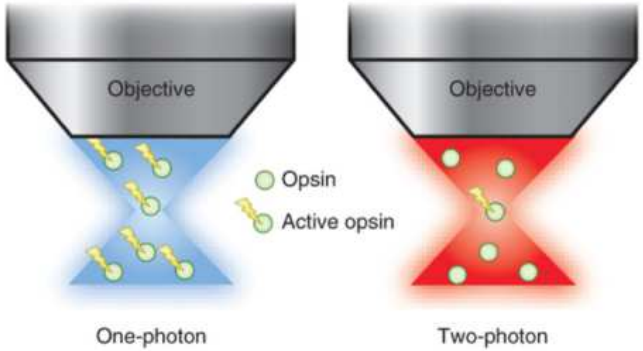


A. Packer,2013

Limitations

1P widefield excitation
➔ substan no cellular resolution

2P imaging
➔only *in vitro*



A. Packer,2013

Combi optical and electrophys.
➔ Artifacts during recording
➔ lack of spatial resolution(extracellular electrodes)
Brain tissue at depth
➔decofused light (scattering)

Probes & sensors sensitive to visible wavelength light
➔ overlap excitation and emission wavelengths
➔ data loss (interrupt fluorescence)

From the past

in the speed and precision of single-cell activation through refinement of the ability to visualize and selectively illuminate individual cells to allow the playback of specific neuronal population codes. The creation of a method to simultaneously perform optogenetic activation and achieve calcium imaging at a single-cell or even dendritic-spine resolution in a freely moving mammal would also be an enormous advance for the field.

It is intriguing to speculate that a very specific (and now testable) neural circuit dysfunction (such as an imbalance in excitation and inhibition) could be causally involved in multiple psychiatric diseases including anxiety, depression, addiction, schizophrenia and autism. Variation in precise symptomatology from disease to disease could be more closely linked to variation in the role of the affected circuit than to fundamentally distinct principles of the pathologically altered neural activity propagation. In particular, given the high rate of co-morbidity among the various mental illnesses and the shared symptomatology between individual diseases, identification of such simplifying themes and unifying theories by optogenetic or other means is one of the most pressing needs and exciting avenues of research into neurological and psychiatric disease.

A. Packer,2013

Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution *in vivo*

Adam M Packer, Lloyd E Russell, Henry W P Dalgleish & Michael Häusser

Affiliations | **Contributions** | **Corresponding author**

Nature Methods **12**, 140–146 (2015) | doi:10.1038/nmeth.3217
Received 03 August 2014 | Accepted 24 November 2014 | Published online 22 December 2014 | Corrected online **06 February 2015**
Corrigendum (July, 2015)

Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields

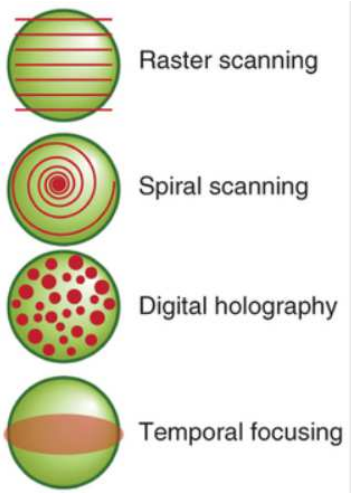
John Peter Rickgauer, Karl Deisseroth & David W Tank

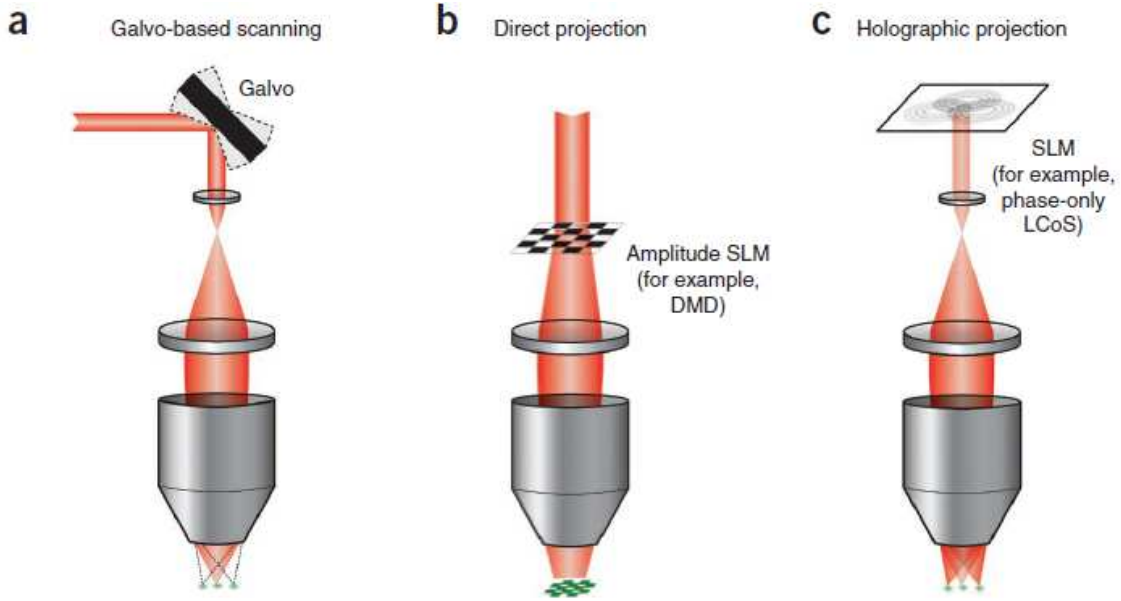
Affiliations | **Contributions** | **Corresponding author**

Nature Neuroscience **17**, 1816–1824 (2014) | doi:10.1038/nn.3866
Received 16 July 2014 | Accepted 15 October 2014 | Published online 17 November 2014

Simultaneous Imaging

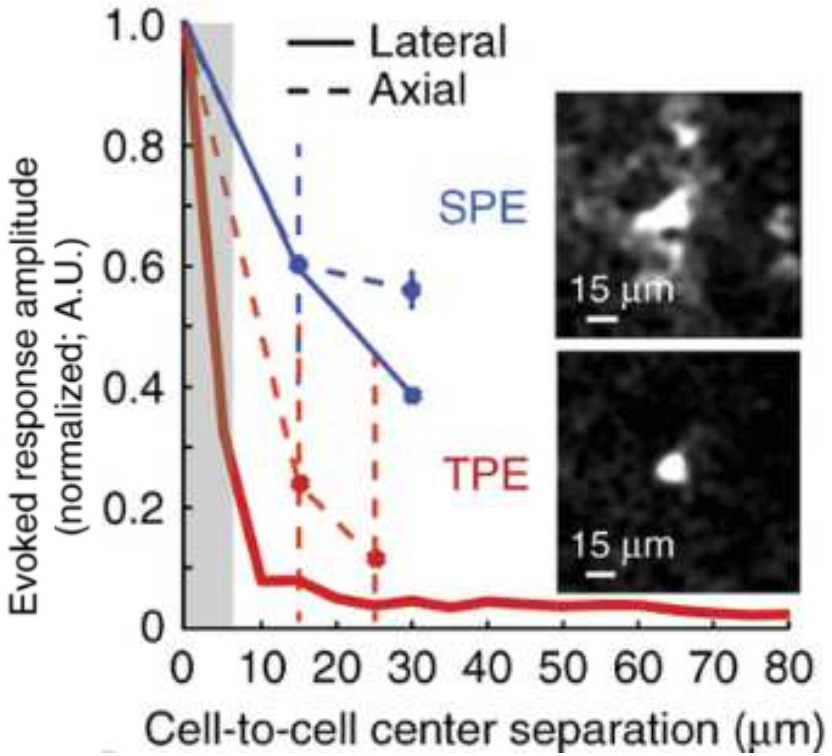
+SLM-based (spatial light modulator) beam reflecting/splitting & generating a single spot
+ Galvanometer mirror
+Temporal focusing
+Spiral scanning (programmed patterns of photostimulation)





A. Packer,2013

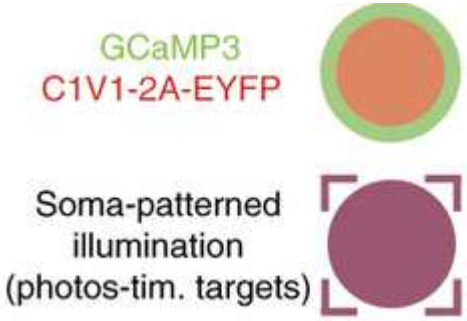
infrared TPE can separately stimulate cells in densely labeled tissue compared to visible-light SPE

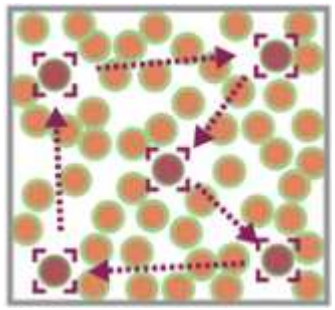


response profiles show the possibility to use TPE at low power

J.P. Rickgauer,2014

Temporal Focusing

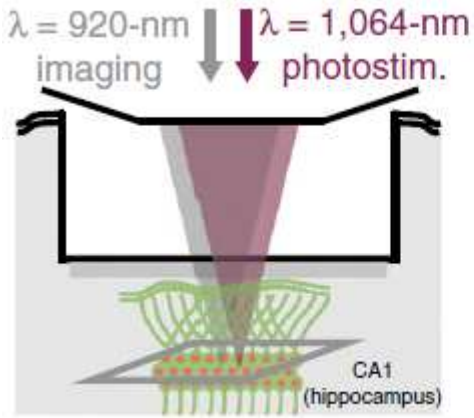


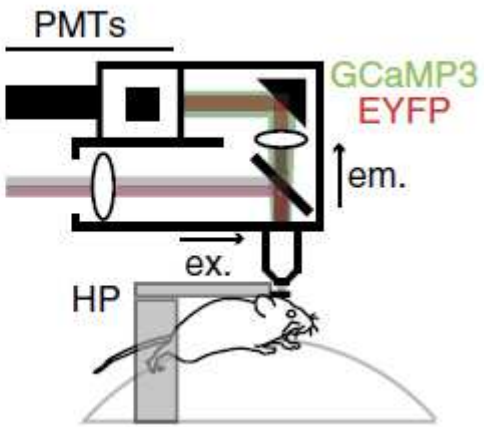


TPE imaging=>selected targets for TPE photostimulation
rapid repositioning of the stimulation spot stimulation at multiple locations

J.P. Rickgauer,2014

two spectrally separated infrared TPE laser scaning systems for both fluorescence imaging and photostimulation



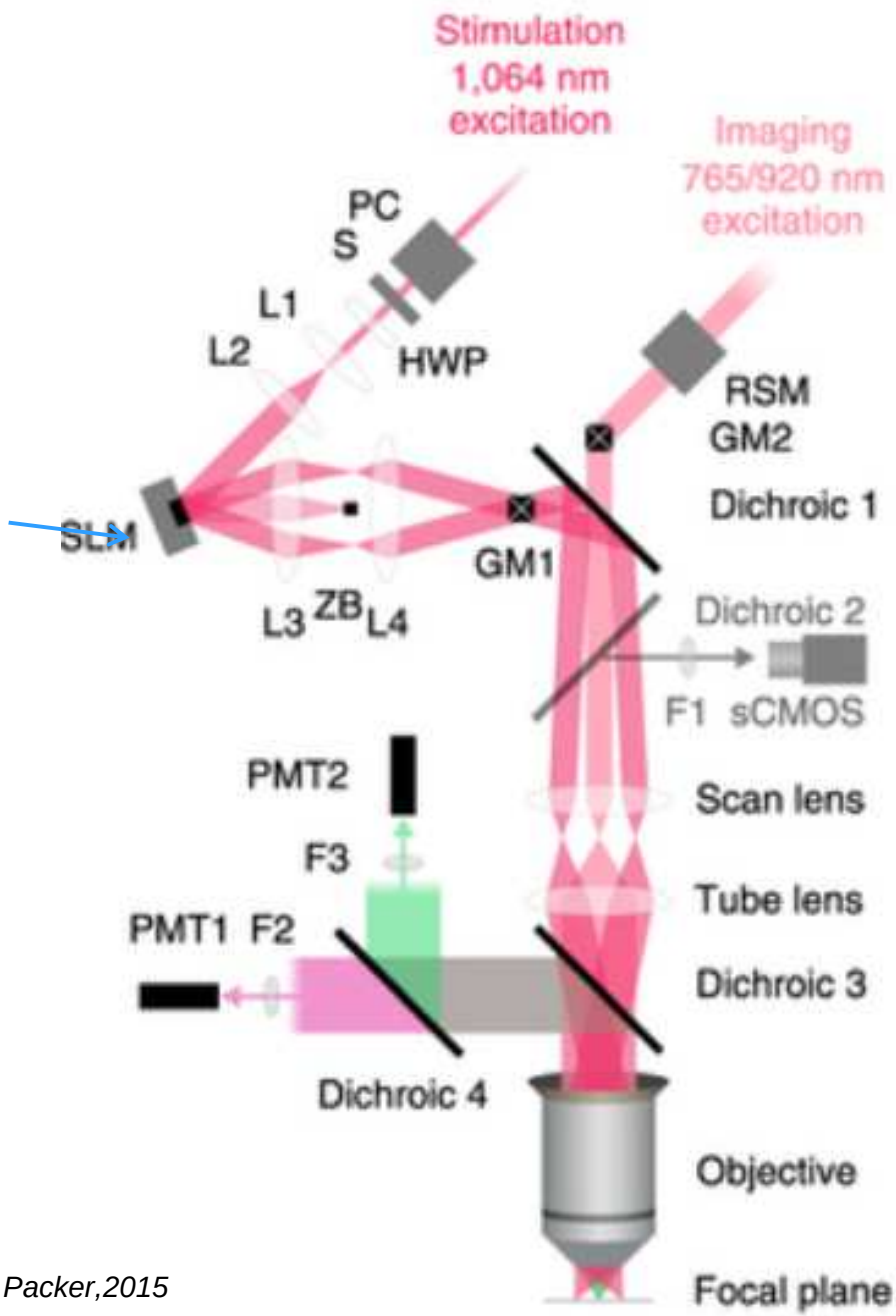


opsin photocycle time: long
excited-state lifetime (fluorophore): short
➔different molecular probes in one
➔ continuous optical recording

J.P. Rickgauer,2014

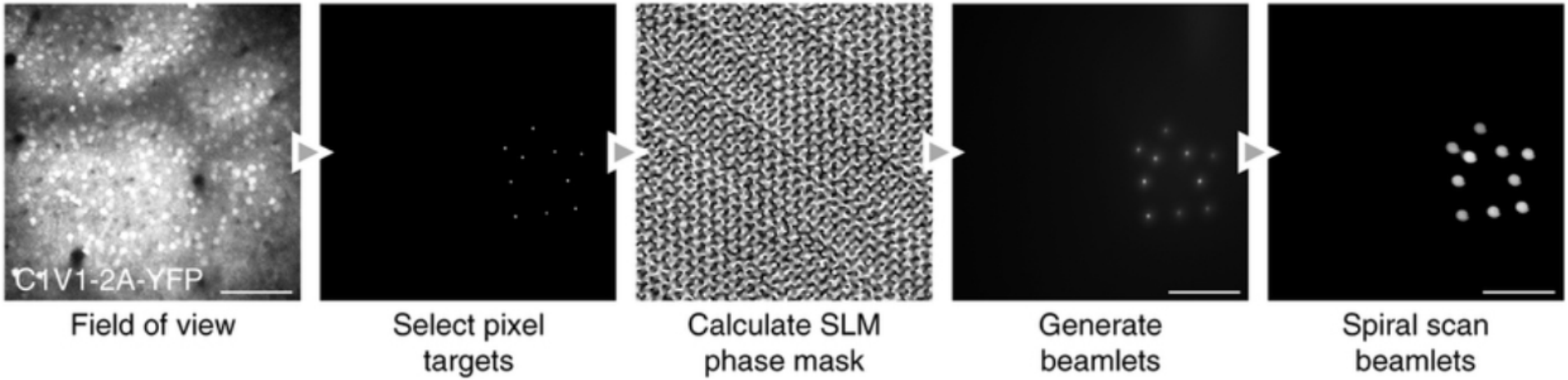
SLM-based 2P patterned stimulationt

- Two-photon, dual-beampath, resonant scanning microscope
- SLM and galvanometer mirrors➔tIndependent manipulation of laser➔rapid repositionings



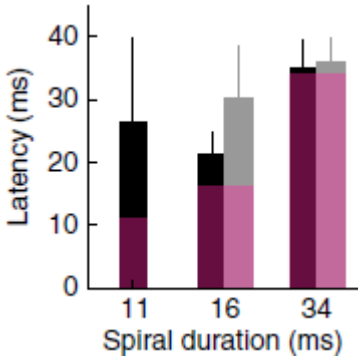
A. Packer,2015

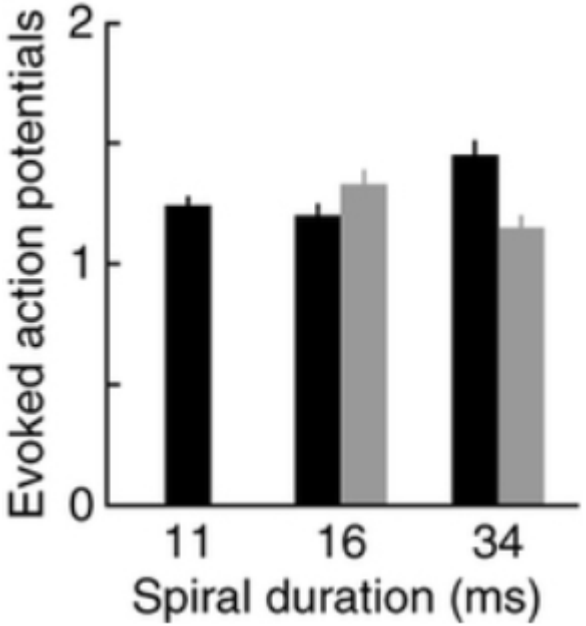
Multiple locations protocol



targeting spiral stimulation to multiple locations

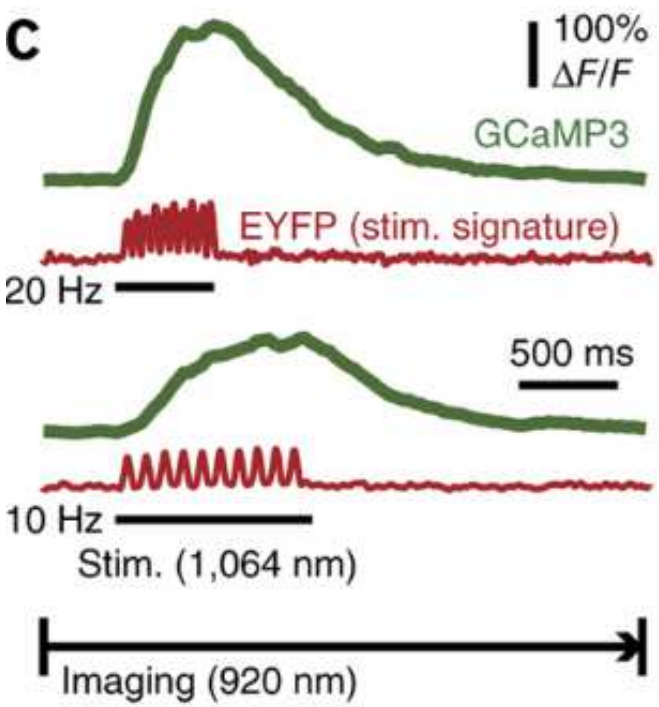
optically generated action potentials with low jitter
➔precisely timed action potentials in multiple neurons





A. Packer,2015

Artifact management

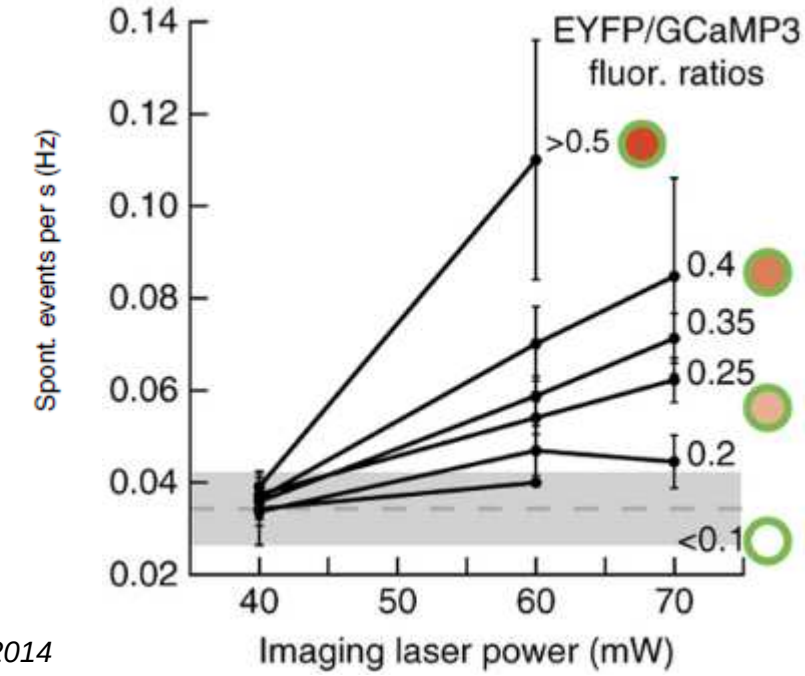


GCaMP3 transients separable from optical stimulation artifacts

infrared light-blocking filters
➔ light shielded from photodetectors & excited fluorescence
➔ No estimation and subtraction needed

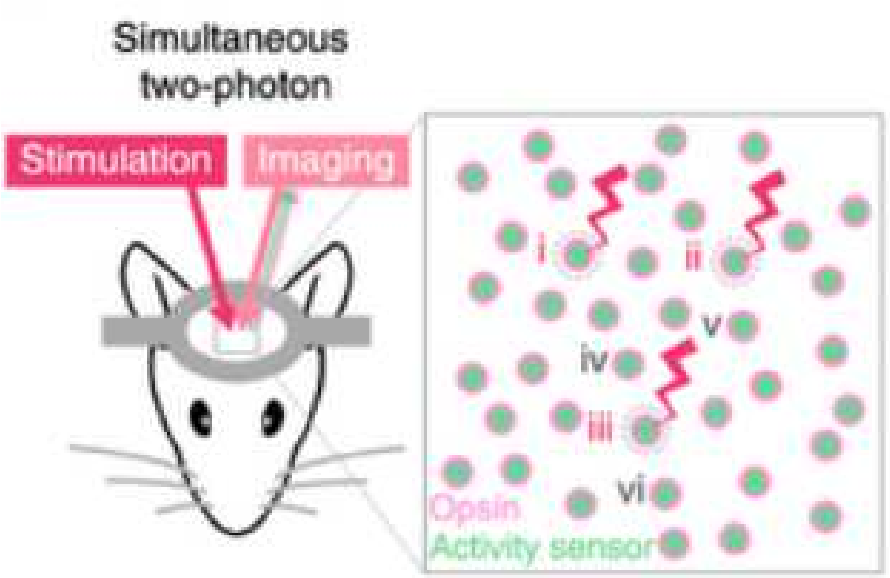
low-power stimulation pulses➔ detect subthreshold activity
visible as fluorescence transients

define a range for imaging

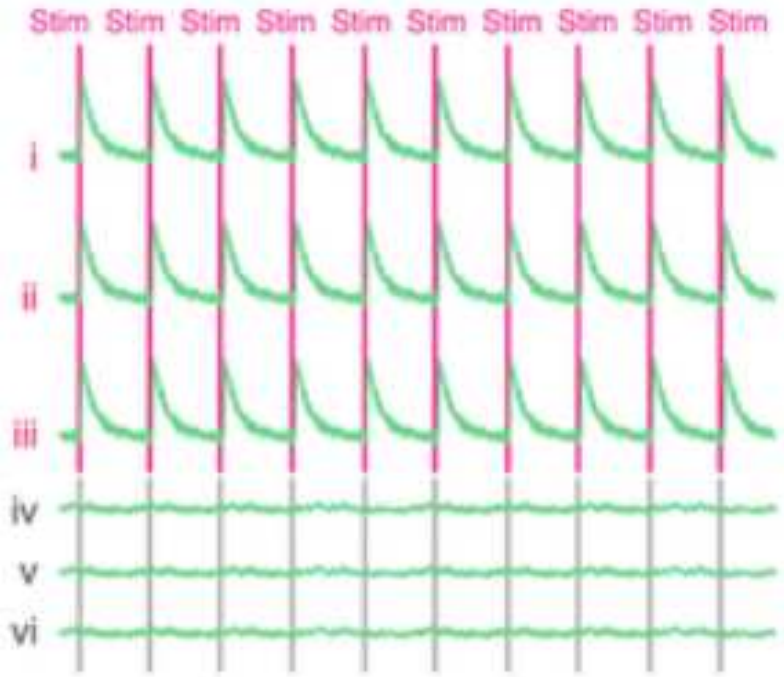


J.P. Rickgauer,2014

Spatial Resolution



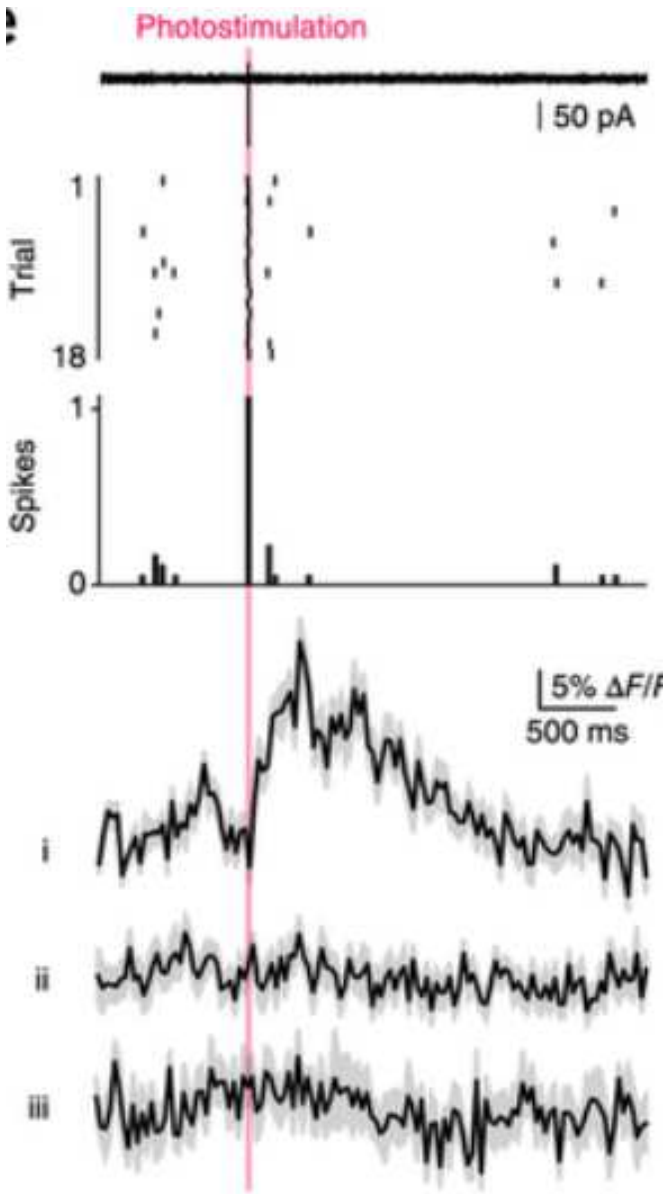
Opsin ➔ photostimulation
Calcium sensor ➔ optical readout



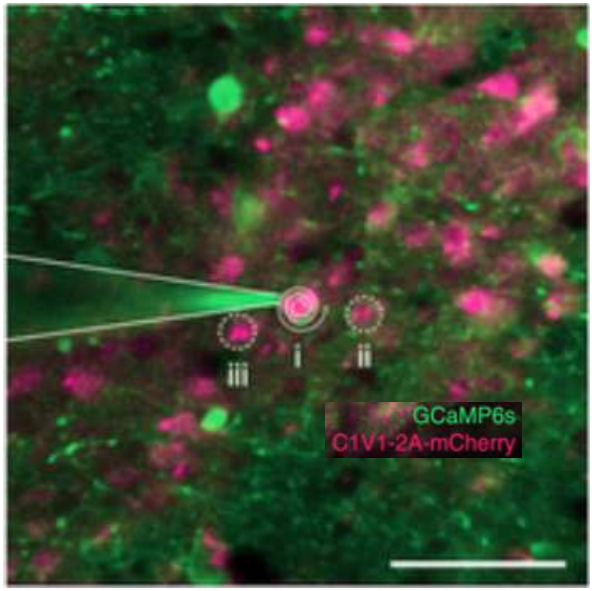
recording & photostimulation of user-selected neurons

A. Packer,2015

Simultaneous recording & imaging



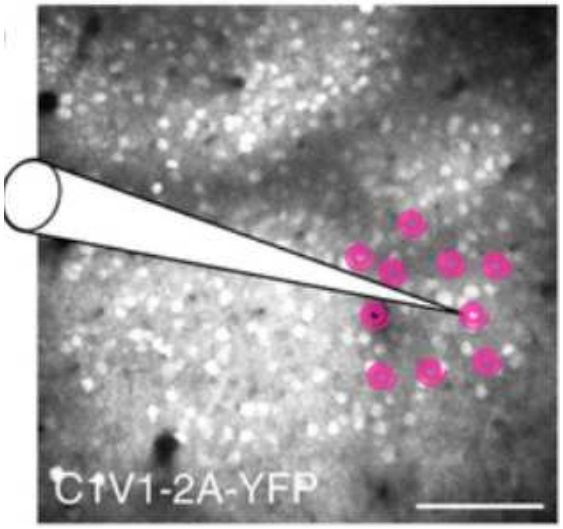
two-photon targeted cell-attached patch-clamp recording & optogenetic stimulation in a spiral pattern



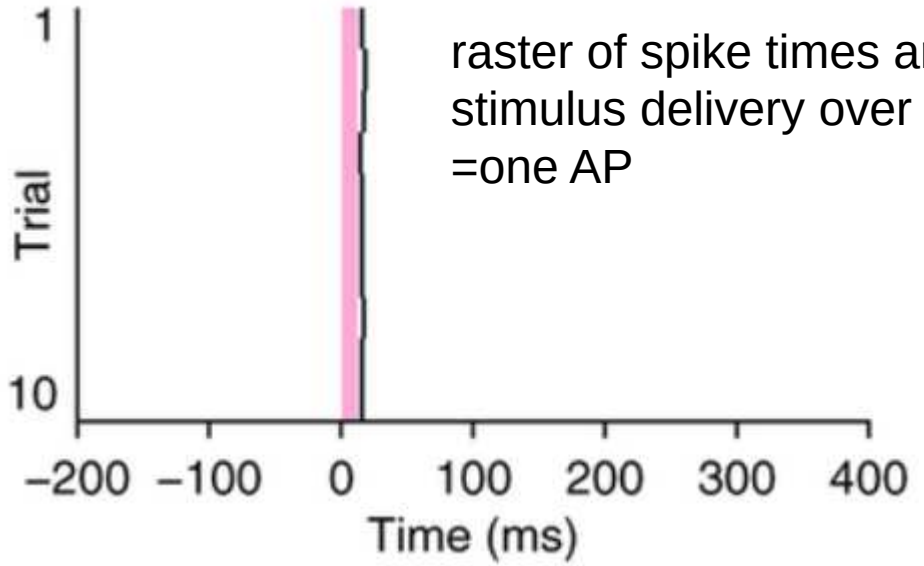
high-speed calcium imaging recordings

A. Packer,2015

AP recording and stimulating



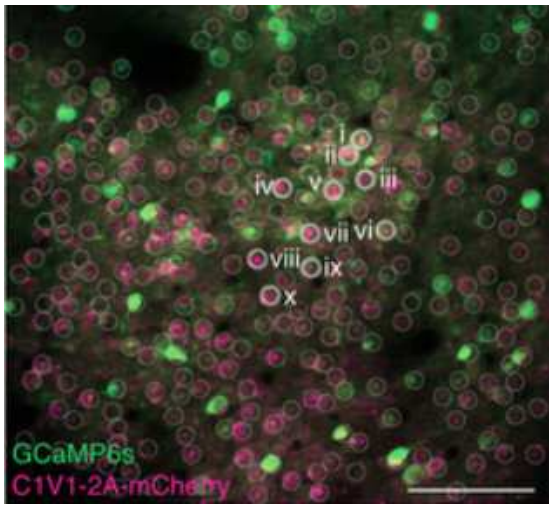
cell-attached patch-clamp recording configuration ➔multiple locations were photostimulated while AP generation recorded in one

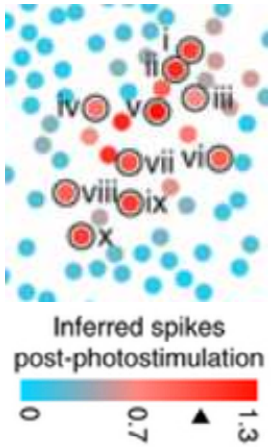


raster of spike times around stimulus delivery over ten trials =one AP

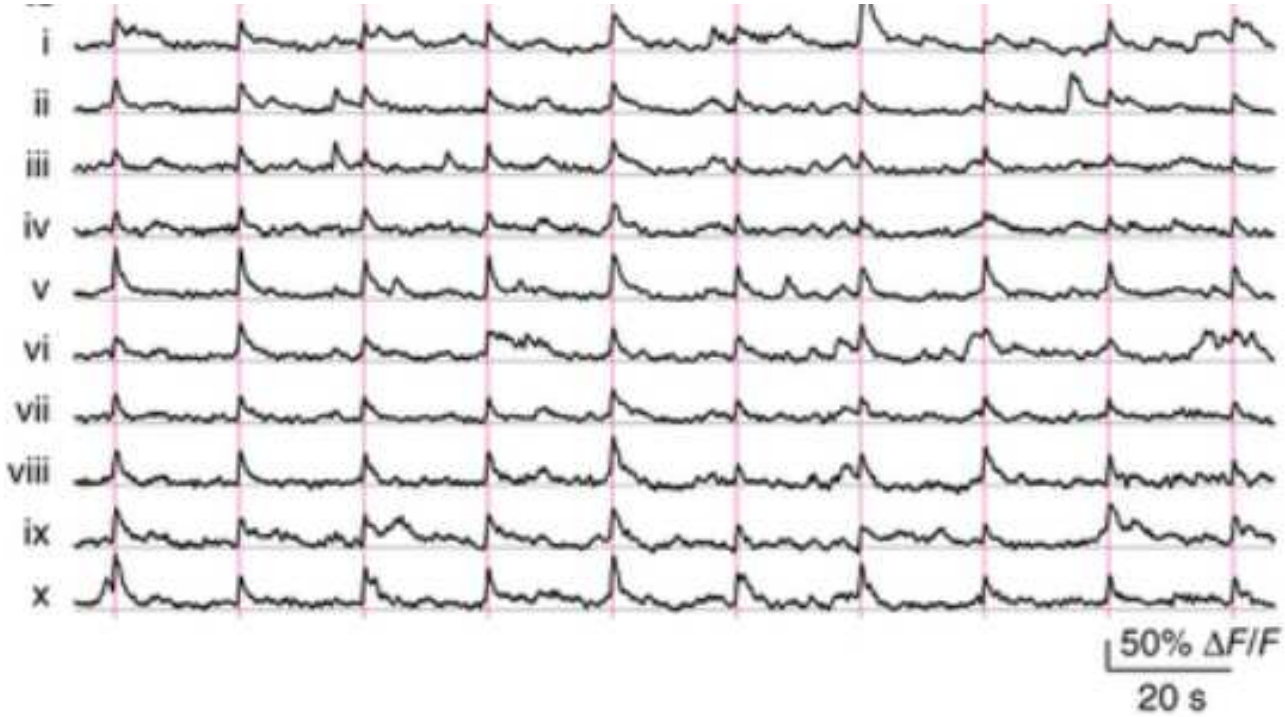
A. Packer,2015

Stimulation strength





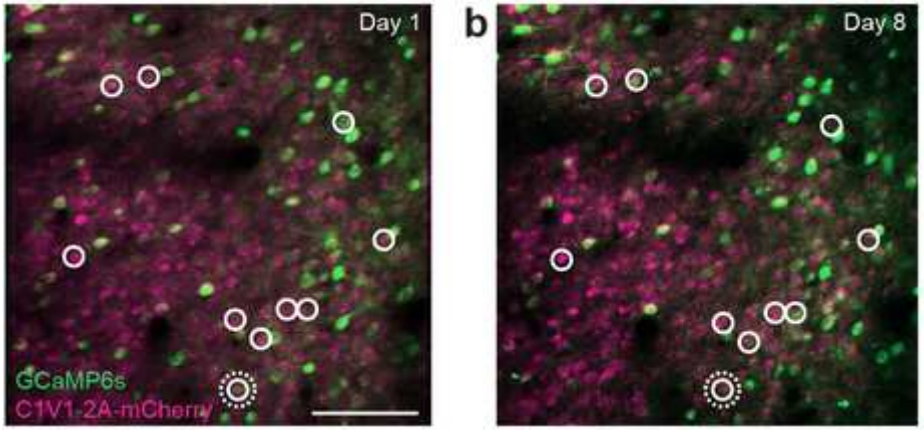
view of 10 individual target neurons & strength of photostimulation➔ Input integration in polulation
inferred spikes immediately post-stimulation
290 surrounded neurons were imaged.



Calcium transients =response

A. Packer,2015

Long term



A. Packer,2015

the same ten neurons 1 week later and photostimulated them again
➔ Longterm observations

Summary

- spatiotemporally precise action potentials in defined neurons
- simultaneously manipulating and recording their responses (activity) and many others in the same field of view.
- optically generating action potentials in individual neurons with low jitter
- high-throughput, flexible and long-term optical imaging

Exp. Procedure I

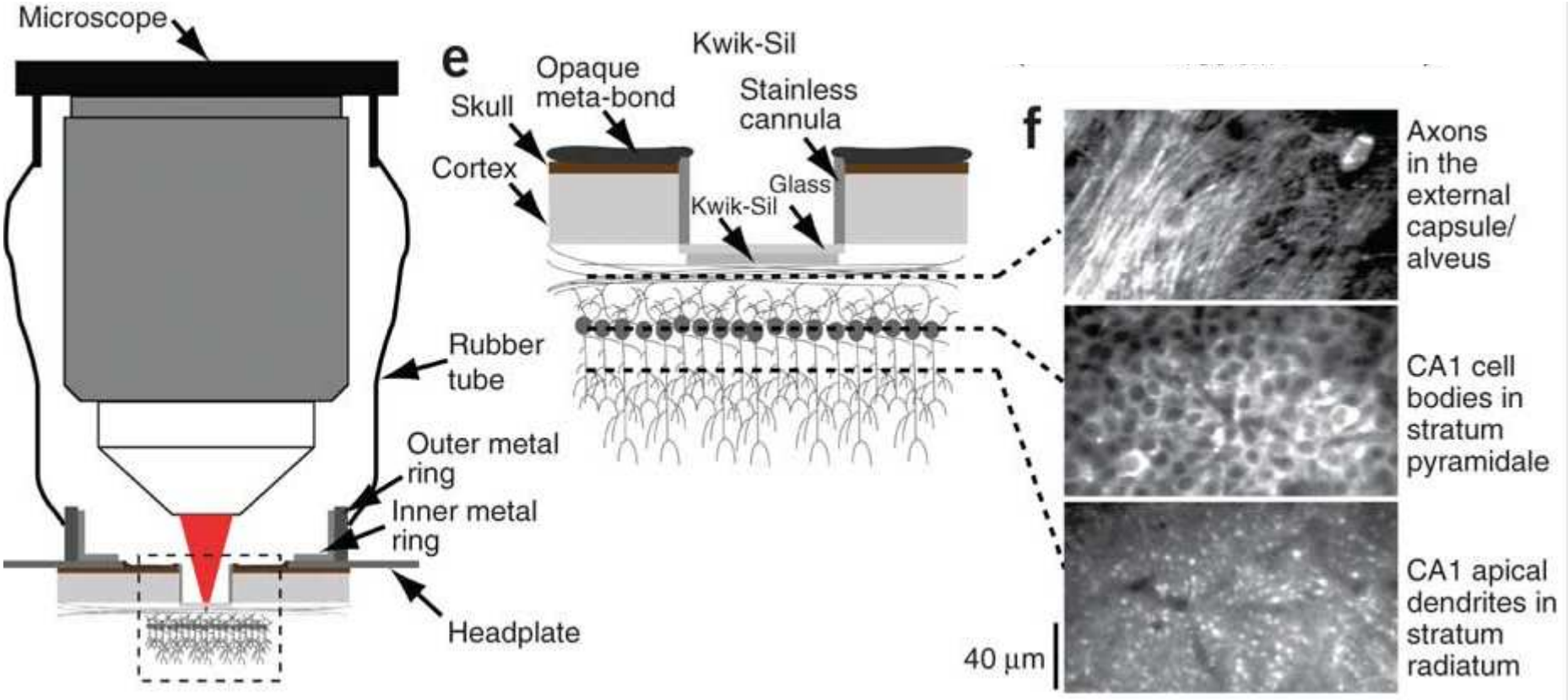
1. cellular resolution imaging

➔ Two-photon microscopy + calcium-sensitive dyes (TPE, SLM)

2. imaging more than a mm beneath the cortical surface

➔Chronic imaging window

2. Chronical window



D. Dombeck,2013

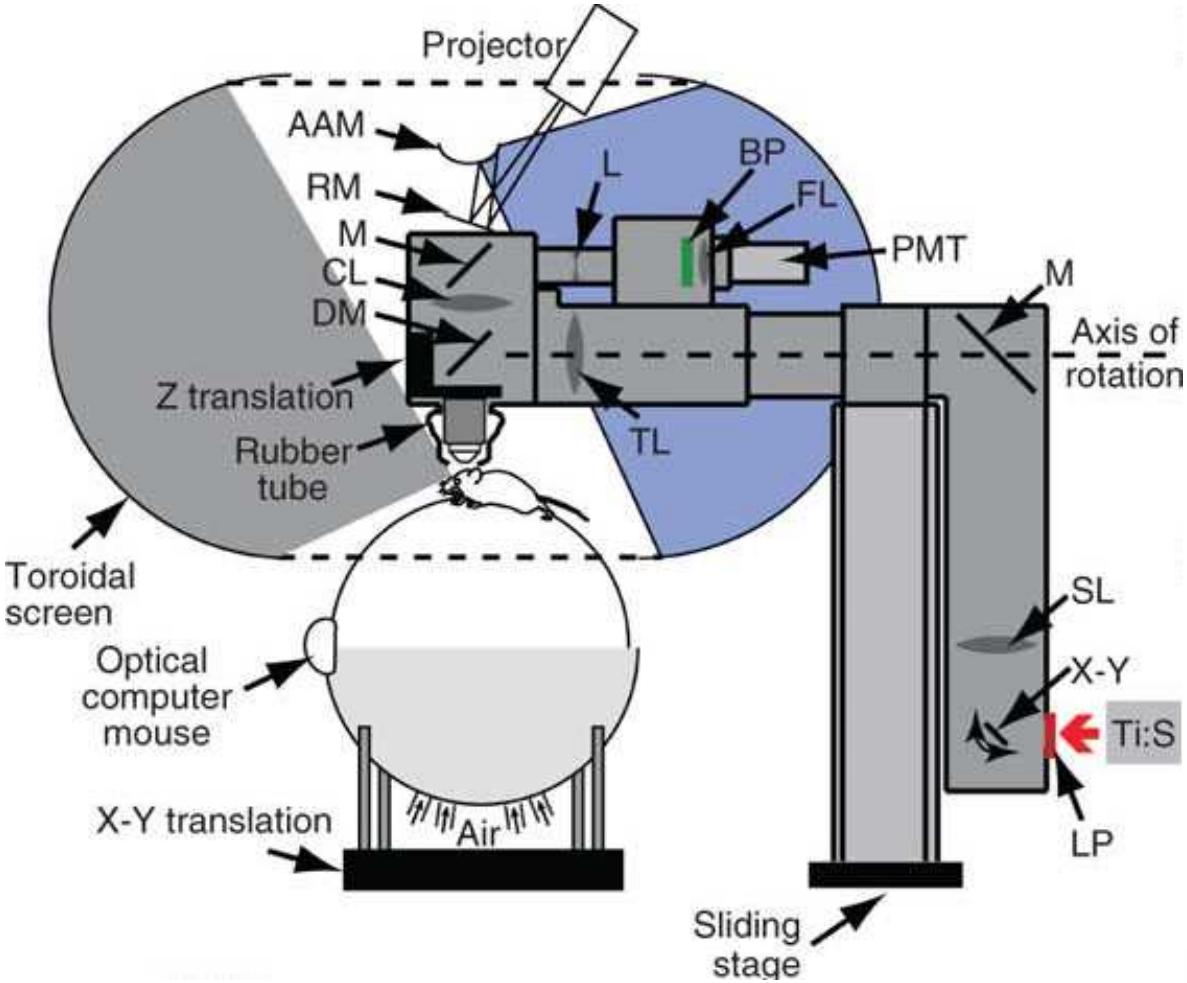
Exp. Procedure II

1. cellular resolution imaging (mobile mouse)
➔ Two-photon microscopy in combination with calcium-sensitive dyes

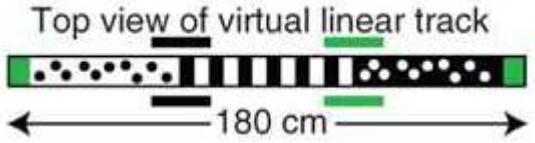
2. imaging more than a mm beneath the cortical surface
➔Chronic functional imaging window

3. imaging that is compatible with navigation behavior
➔virtual reality system

3. Virtual Reality System

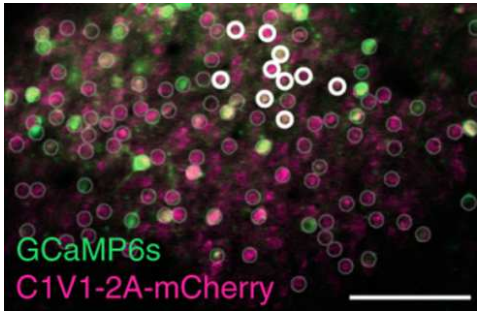


D. Dombeck,2013

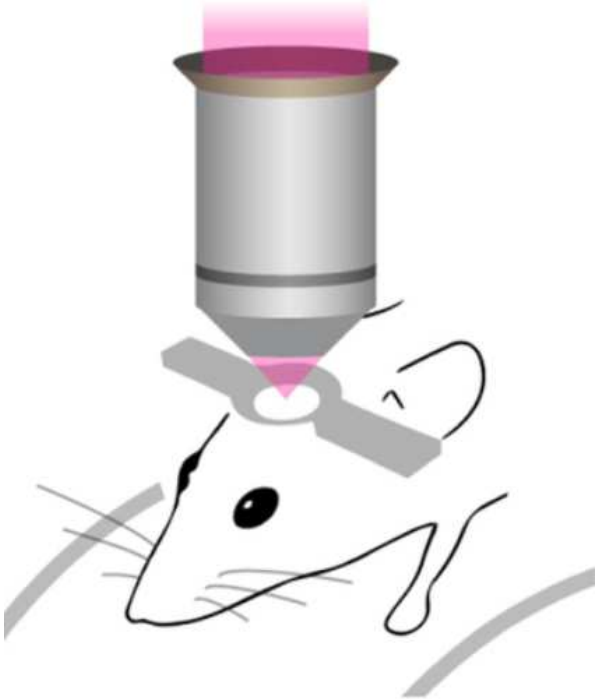


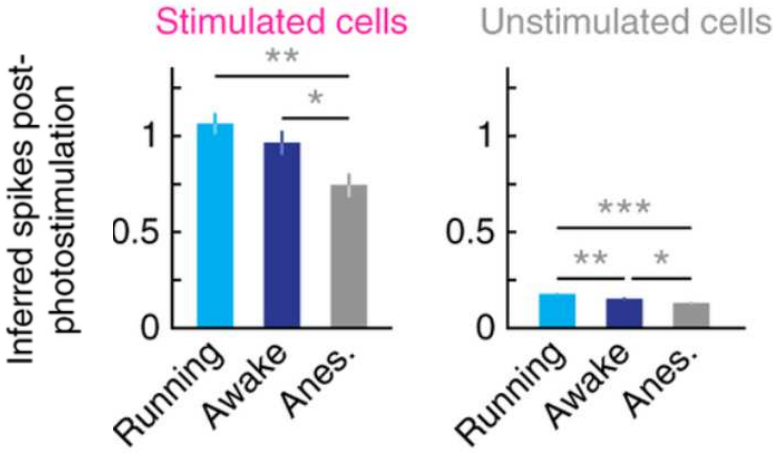
https://www.youtube.com/watch?v=1DJOTEDBA2c

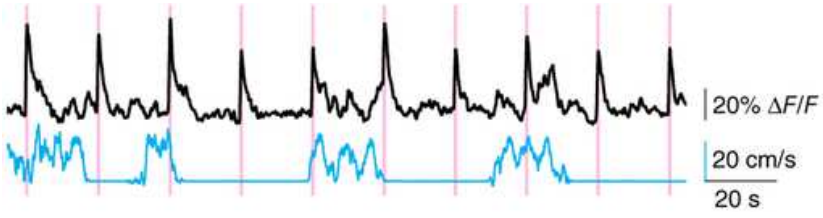
Dependence on behavioural state



behavioral states modulate the responsiveness of neural circuits compare the local network response to photostimulation







calcium transient(simultaneous stimulation: 10 neurons)

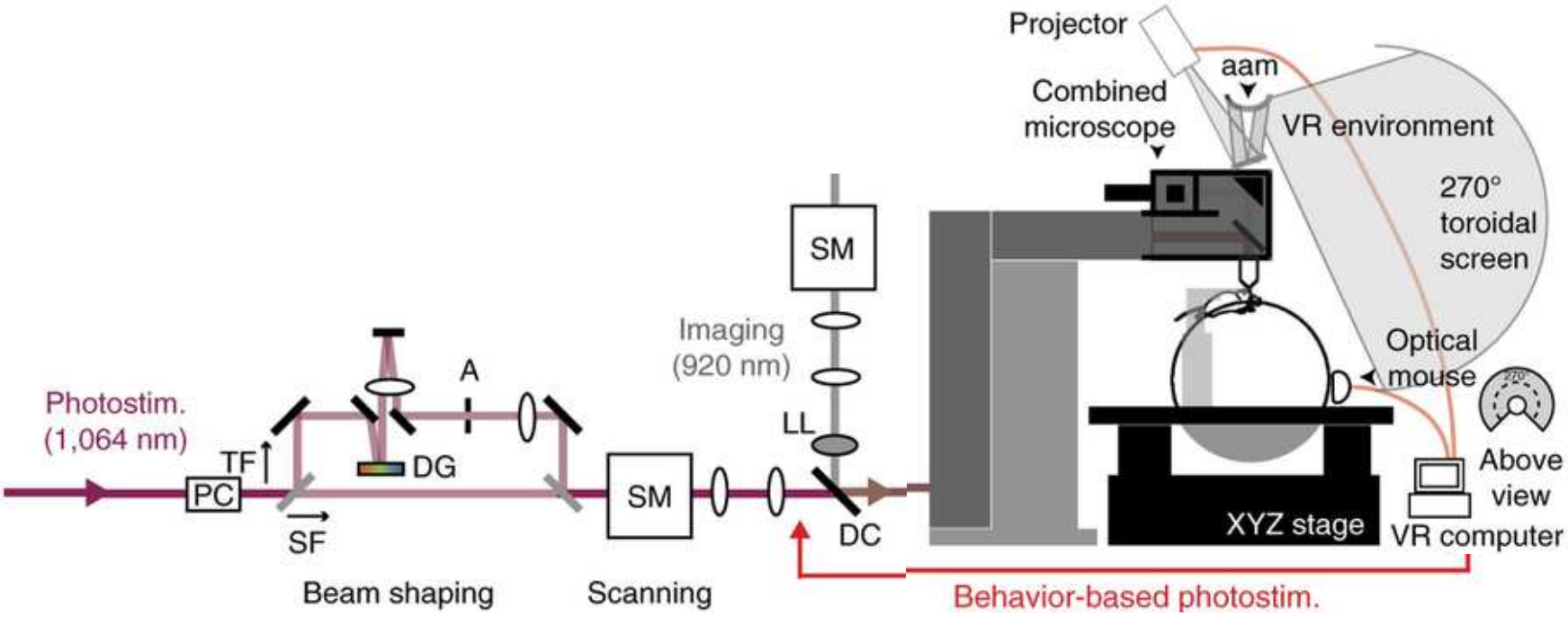
running speed

A. Packer,2015

VR system-combination

inject virus ➔ implant optical window➔ image

➔trained to perform visually guided behaviors
➔large-scale optical recordings
➔characterize



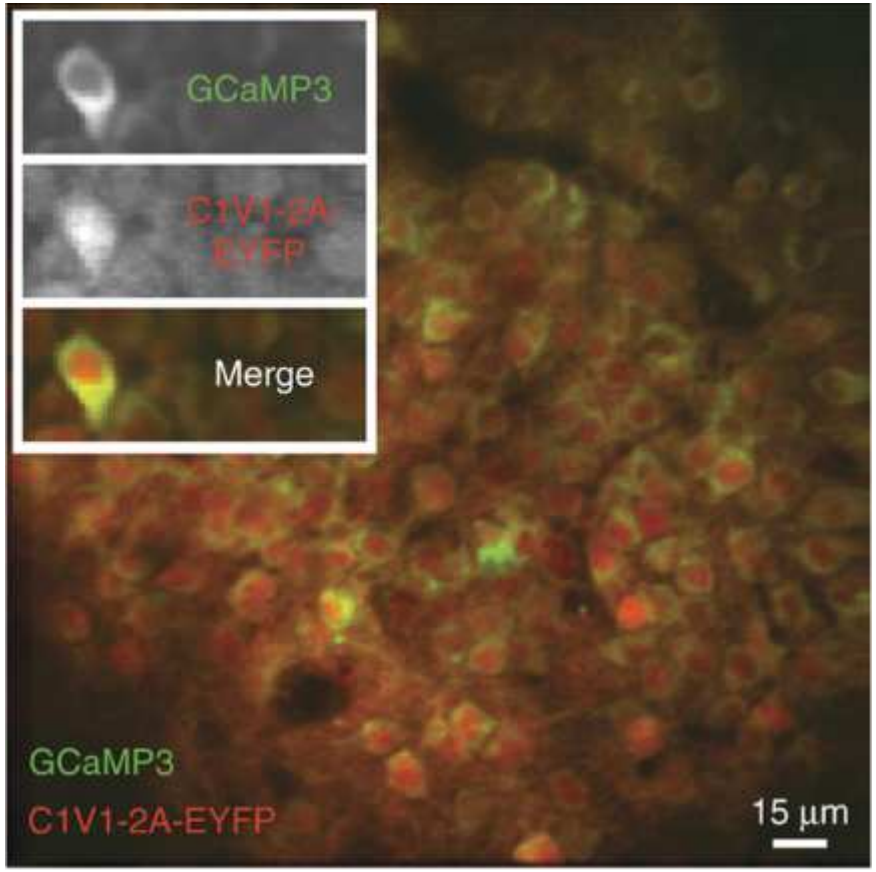
A. Packer,2013

Exp. Proof-of-Concept

Task: Measure and then manipulate patterns of task-modulated activity in single neurons

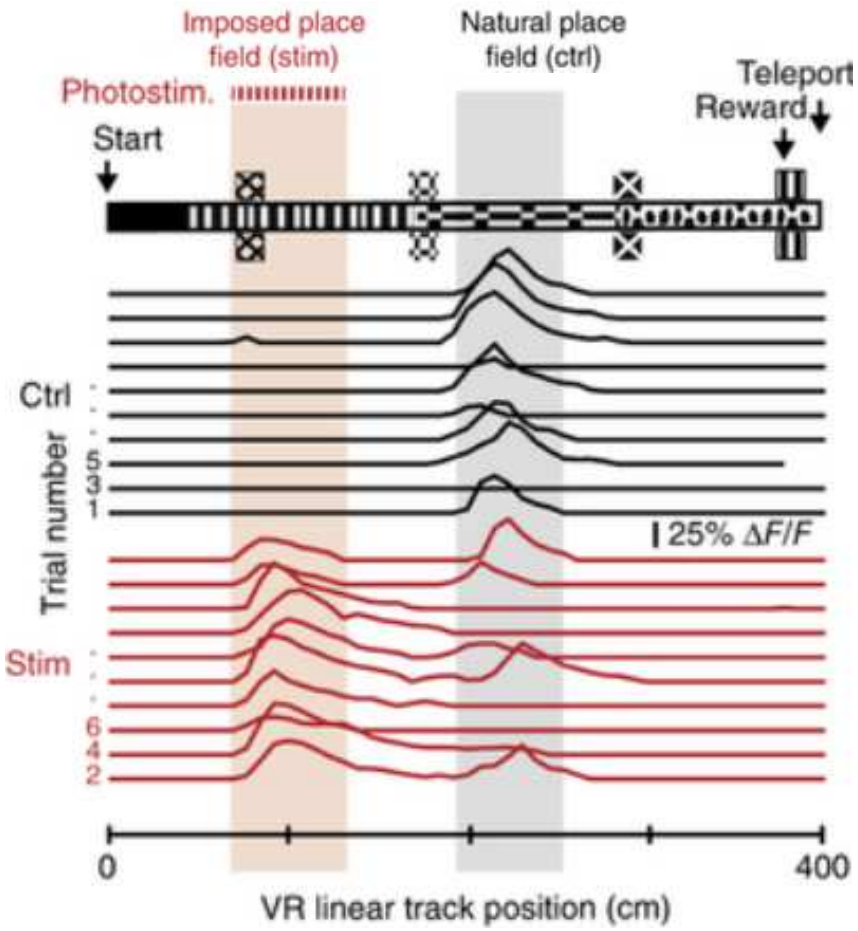
Approach: optically map neurons expressing the probe-sensor (inject virus) pair in CA1 pyramidal population
➔ spatial behavior in a VR environment
➔ detect place cells or silent cells
➔trained to perform visually guided behaviors
➔stimulated & recording task-driven dynamics
➔VR software to compute 'gate' signals
➔characterize neuron population activity.

virus-mediated expression of the probe in CA1 and transgenic expression of the sensor



J.P. Rickgauer,2014

Experiment



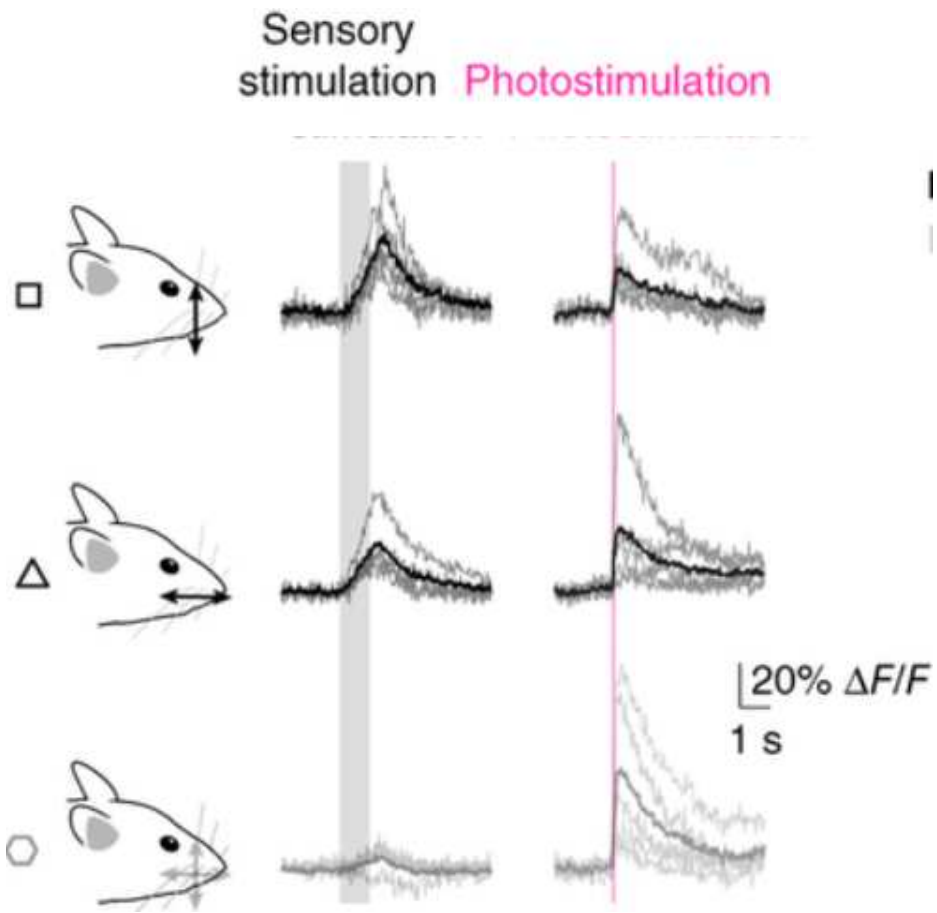
- natural firing field is later (225 cm)➔ produced place field-like activity

- 'imposed field' activity in this example also suppressed activity in the natural place field of that cell

J.P. Rickgauer,2014

➔impose patterns of activity in single cells that mimic natural patterns of activity during the same task.

Stimulation orientation

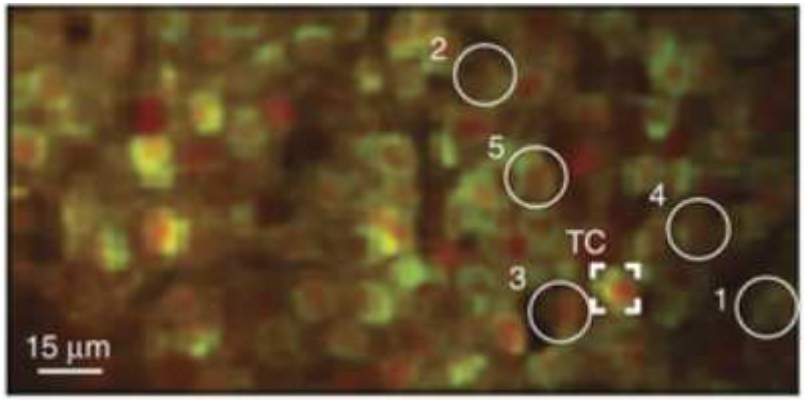


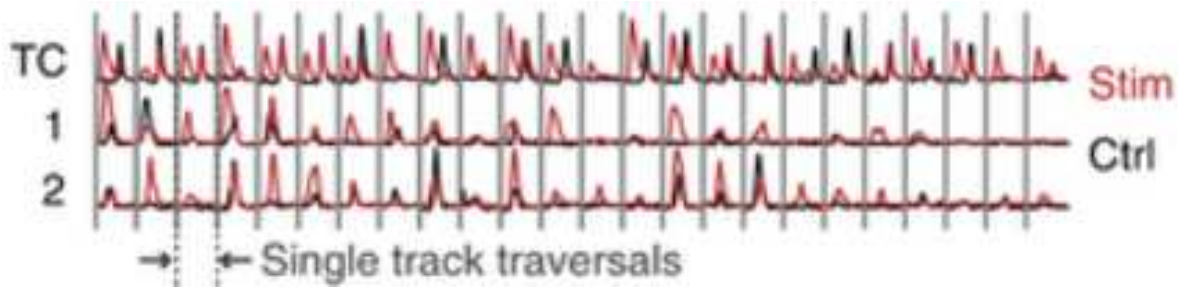
responded strongly or weakly to particular stimulation orientations

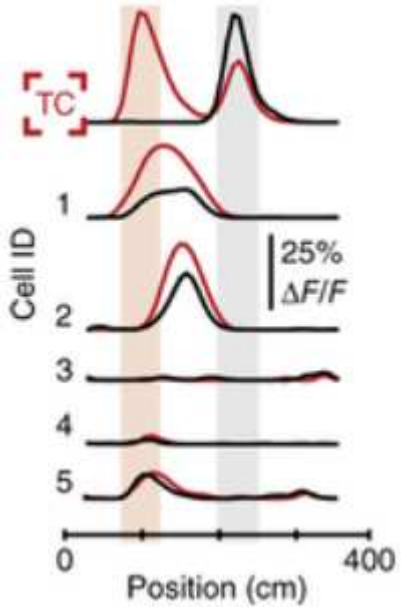
A. Packer,2014

Secondary Effect

Stimulation trials that evoked activity in this place cell produced a significant change in the task-modulated activity



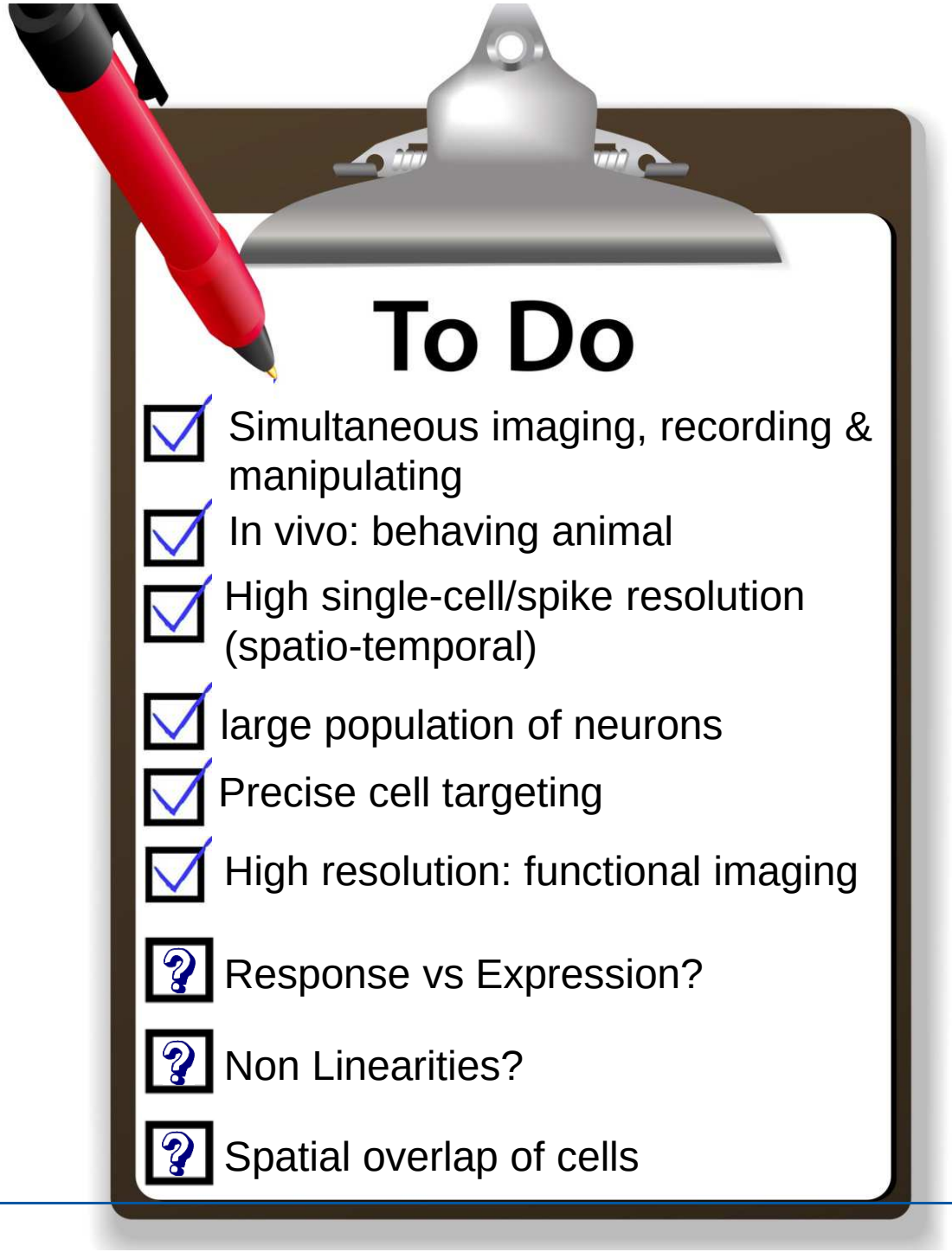




J.P. Rickgauer,2014

Discussion

- Some neurons expressed strong seonsor, but showed reduced responses to photostimulation
- Response depends too much on the expression of the sensor
- Linear non-negative deconvolution algorithm used
- Consider non-linearities: indicator saturation, Ca2¨+ binding, diffusional equilibrium, Ca2+]*i* and fluorescence readouts different from *F*/*F*
- define a specific range for every device feature and for every animal separately?
- Spatial overlap in intensity pattern


To Do
Simultaneous imaging, recording & manipulating
In vivo: behaving animal
High single-cell/spike resolution (spatio-temporal)
large population of neurons
Precise cell targeting
High resolution: functional imaging
Response vs Expression?
Non Linearities?
Spatial overlap of cells

Outlook

- create a new class of experiments to examine behavioral substrates
  - neurai microcircuits by allowing cell-specific perturbation of activity
  - pairing stimulation with periods in a behavior
  - estimate the range of AP firing changes by reference to the same population, during the same behavior
  - anatomical distribution of spatial information
  - map firing fields and subthreshold inputs
  - long-term changes in receptive fields.
  - mimic activity whole set of experiments
  - provide a better SNR, estimations


THANK
YOU

http://www.jove.com/video/50885/two-photon-calcium-imaging-mice-navigating-virtual-reality

https://www.youtube.com/watch?v=1DJOTEDBA2c