Targeting neurons and photons for optogenetics

Adam M Packer[1], Botond Roska[2] & Michael Häusser[1]

tea talk
july 11, 2013

srini turaga
sturaga@gatsby.ucl.ac.uk

opto•genetics

- targeting *which* neurons to activate and *when*

- light
- genetics (transgenics, viruses)

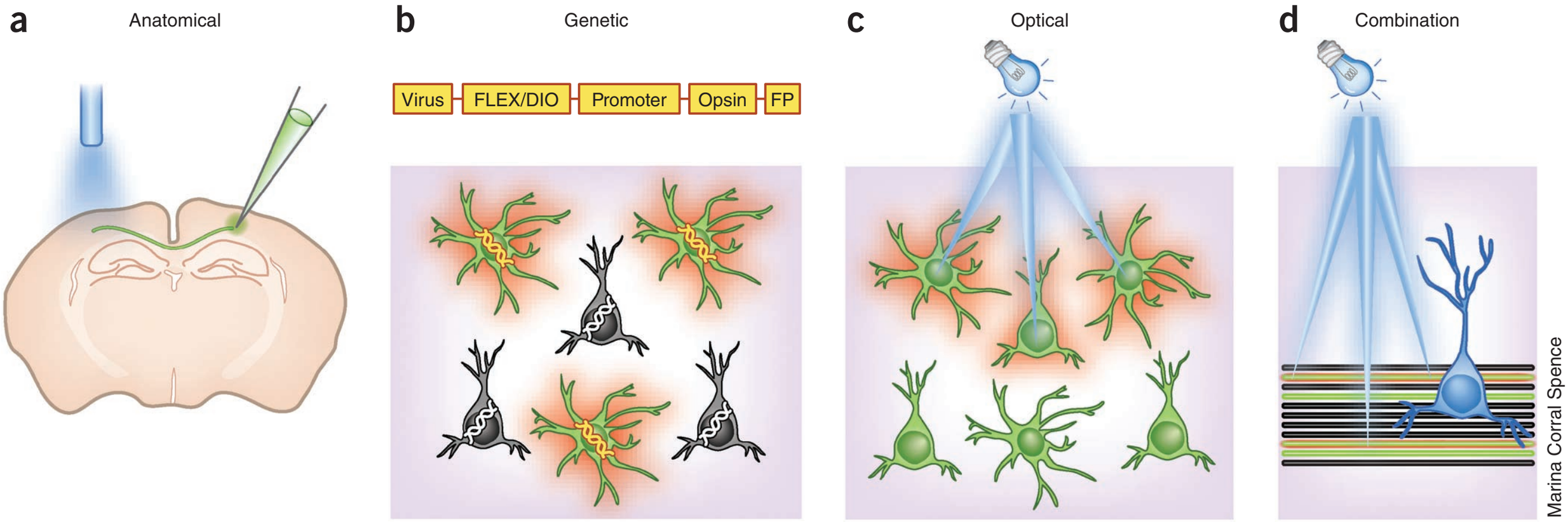

Figure 1 Intersectional strategies for targeting optogenetic manipulation. (**a**) Physical delivery of virus to a given anatomical location can exploit or uncover circuit connectivity patterns either by making use of axonal projections or by using viruses that are able to cross one or more synapses. (**b**) Cell types can be addressed if the cell type of interest has a known genetic identity. (**c**) Directing the illumination source to a given set of cells or even individual neurons and processes is useful when the targets of interest are separated in space relative to the spatial resolution of the technique used. (**d**) These three strategies can be combined, as shown in this example, in which axons of a particular cell class projecting to a subcellular domain of a neuron are photostimulated at different distances from the neuron.

- -genetics

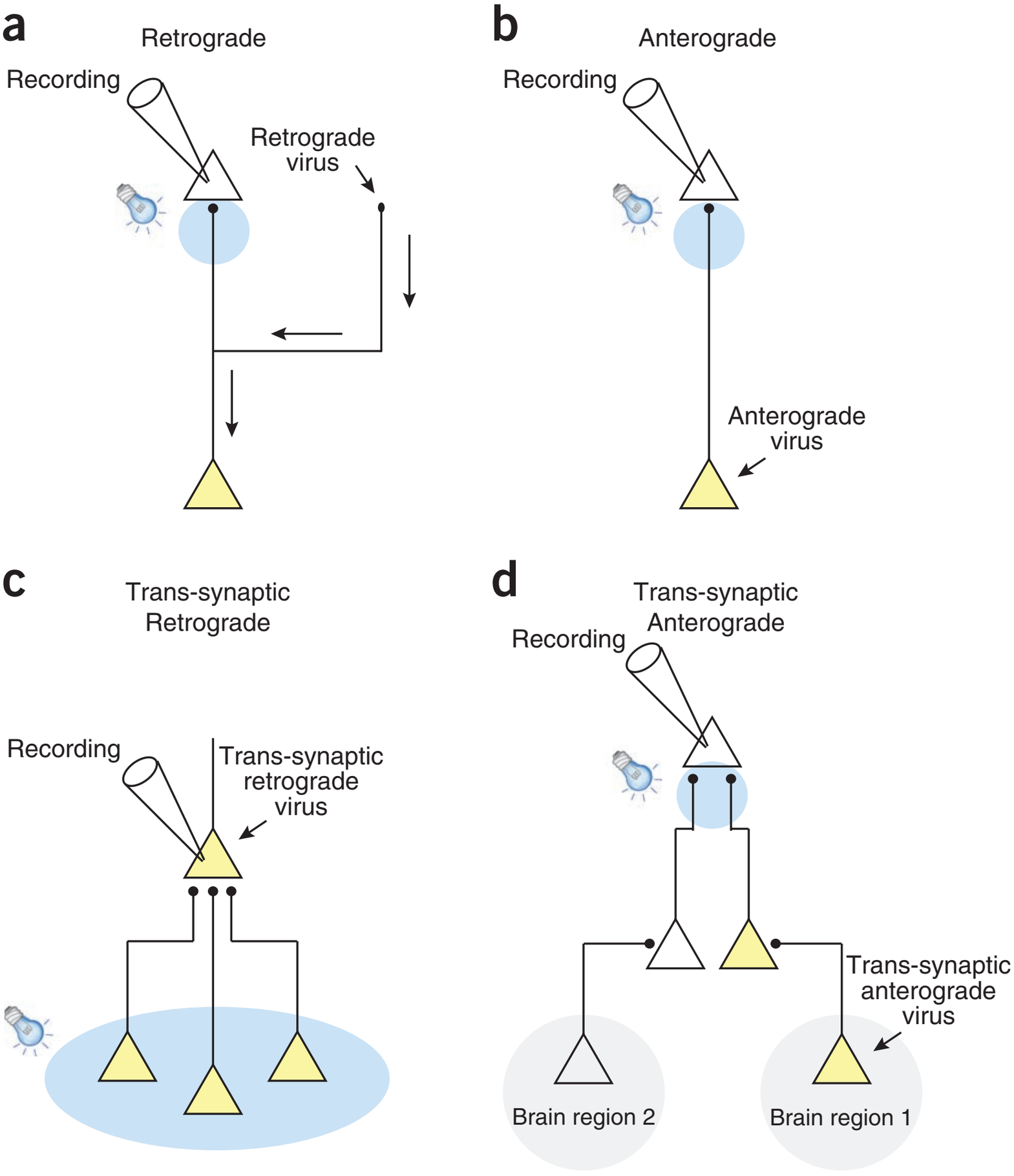

Figure 2 Viral targeting of optogenetic tools using knowledge of circuit connectivity. Schematic illustration of different strategies for targeting optogenetic tools to specific cell types based on their connectivity pattern. Neurons expressing an optogenetic tool are indicated in yellow, arrows next to cellular processes indicate the direction of viral spread, and the location of light stimulation is shown in blue. (**a**) Use of a retrograde virus with targeted virus injection to an axon projection region. (**b**) Use of an anterograde virus with targeted virus injection to the somatic region. (**c**) Use of a trans-synaptic retrograde virus starting from virus introduction (or infection) of a single postsynaptic cell, which leads to optogene expression in monosynaptically connected presynaptic partners. (**d**) Use of a trans-synaptic anterograde virus starting from virus injection in a given brain region to cause optogene expression in synaptically connected downstream neurons.

viral control of opto-gene expression

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics
- replication competent?
  - uncontrolled replication toxic for long-term studies

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics
- replication competent?
  - uncontrolled replication toxic for long-term studies
- lipid coated (rabies, lenti+vesicular stomatitis, herpes)?
  - lipid coating decreases virus diffusion

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics
- replication competent?
  - uncontrolled replication toxic for long-term studies
- lipid coated (rabies, lenti+vesicular stomatitis, herpes)?
  - lipid coating decreases virus diffusion
- envelope protein affinity?
  - cell type? axons? soma? dendrite?

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics
- replication competent?
  - uncontrolled replication toxic for long-term studies
- lipid coated (rabies, lenti+vesicular stomatitis, herpes)?
  - lipid coating decreases virus diffusion
- envelope protein affinity?
  - cell type? axons? soma? dendrite?
- promoter?
  - strength? cell type?

viral control of opto-gene expression

- DNA (AAV, HSV) or RNA (rabies)?
  - conditional expression with DNA viruses & Cre/Flp transgenics
- replication competent?
  - uncontrolled replication toxic for long-term studies
- lipid coated (rabies, lenti+vesicular stomatitis, herpes)?
  - lipid coating decreases virus diffusion
- envelope protein affinity?
  - cell type? axons? soma? dendrite?
- promoter?
  - strength? cell type?
- subcellular targeting?
  - soma? axons?

other genetic possibilities

- single-cell electroporation
- activity dependent expression
  - *Fos* promoter, conditioned on tetracycline
- light sensitive promoters (hsp, LightOn)

- opto-

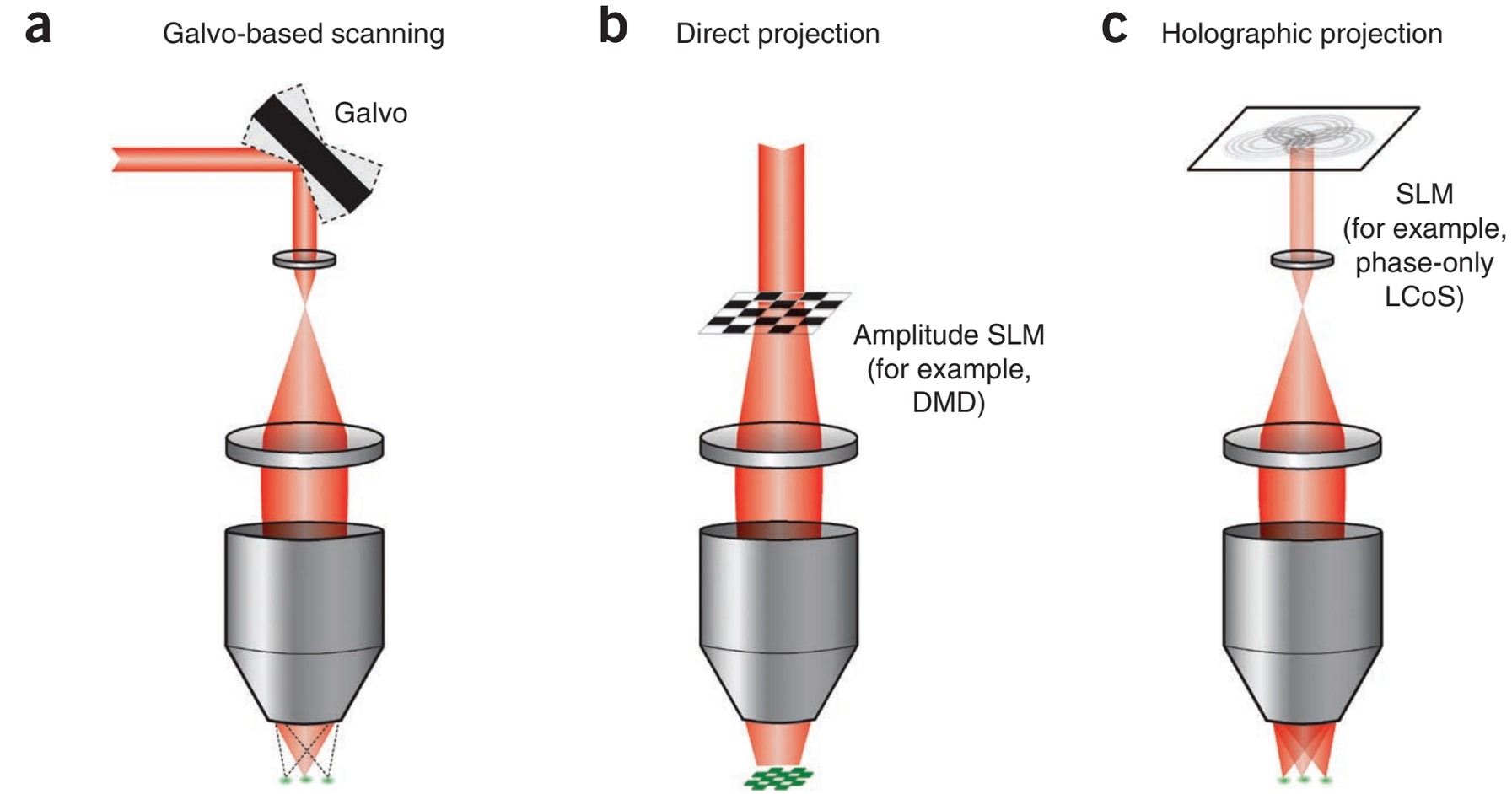

Figure 4 Patterned illumination strategies. (**a**) Top, pointing a single beam with galvanometer (galvo) mirrors is the most straightforward implementation of directing a focused beam of light onto different locations within a sample. Bottom, this approach is particularly useful for mapping studies[91] in which independent activation of small, localized subsets of labeled neurons or axons is desired for readout by downstream neurons. (**b**) Top, pointing multiple beams with a digital micromirror device[92]. Bottom, this enables more complex patterns of activation across large areas of tissue, which has proven useful in studies of retinal circuitry[63] and zebrafish behavior[93]. (**c**) Top, creating holographic patterns with a spatial light modulator combines the power of generating multiple individual beamlets with high efficiency in directing power into those beamlets. Bottom, this enables multi-site activation[70,76] when combined with two-photon excitation (see **Fig. 5**).

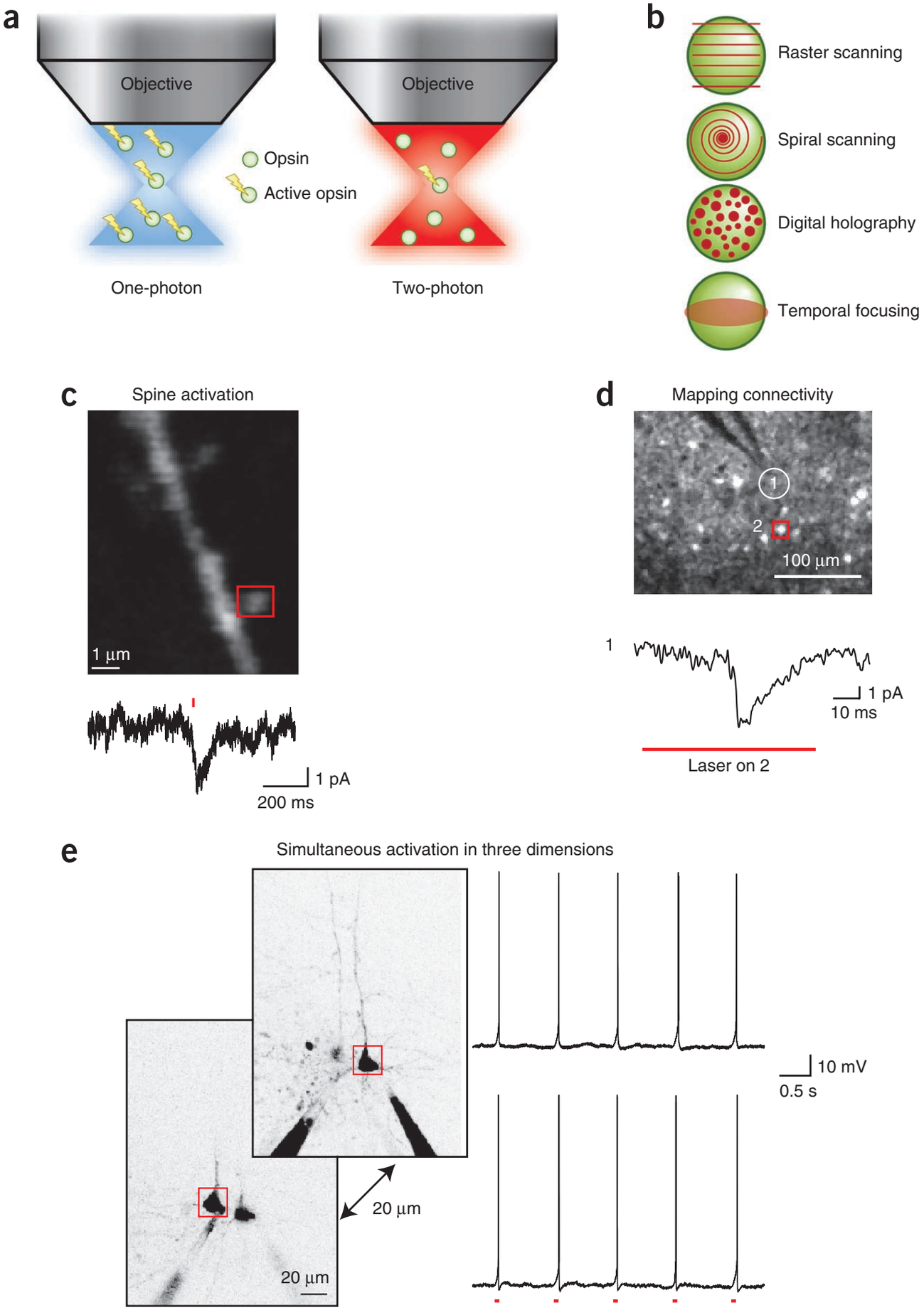

Figure 5 One-photon versus two-photon activation strategies: from spines to circuits. (**a**) In one-photon excitation (left), opsin molecules illuminated above and below the focal plane of interest are excited. In two-photon excitation (right), generally only opsin molecules in the focal plane are excited (but see ref. 68), leading to optical sectioning that allows activation to be restricted to the particular neurons of interest. (**b**) Spatiotemporal patterns for illuminating neurons with two-photon beams require different power budgets and yield different spatial and temporal resolutions (see **Table 1**). (**c**) Two-photon point stimulation of a dendritic spine on a neuron expressing C1V1 (top panel) generates current detectable at the soma (bottom trace). (**d**) Two-photon raster-scanning of neuron 2 (top panel, red box) during electrophysiological recording from neuron 1 (white circle, top panel and bottom trace) indicates that neuron 2 is monosynaptically connected to neuron 1. (**e**) Simultaneous action potential generation in two neurons in three dimensions using a spatial light modulator to generate separate laser beamlets over each neuron. Data in panels **c**–**e** adapted from ref. 76.

Table 1 Comparison of light targeting strategies

Targeted light strategy	Number of neurons addressed	Pros	Cons	Biological questions addressed	Representative references
1P full field	100–1,000	Many neurons activated simultaneously, high temporal resolution	Low spatial resolution using viral transfection	Circuit analysis of cell types	44,94
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	31
1P fiber-optic	100–1,000	Can be used in freely moving animals	Low spatial resolution	Effect of cell types on behavior	95
1P directed beam	10–100	Spatial resolution ~50 μm	Cannot activate individual neurons	Mapping anatomical features of cell types and projections	20,96
1P DMD	100–1,000	Commercially available	Low spatial resolution	Effect of activation of cell types in spatial patterns	63,64,93,97
1P SLM	100–1,000	Holographic patterns enable photostimulation in three dimensions	Low spatial resolution	Effect of activation of cell types in spatial patterns	98,99
2P directed beam	1	Single cell spatial resolution	Only one neuron at a time	Mapping inputs from individual neurons	68,75,76,100
2P SLM	~50	High-resolution holographic patterns can activate multiple individual neurons	Low temporal resolution	Manipulation of neural coding at the individual neuron level	70,76
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	69,70
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

1P, one-photon; 2P, two-photon.

the future...

- all optical measurement and control
- better control of more neurons
- better viral/genetic targeting