

optogenetics

gatsby tea talk
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optogenetics

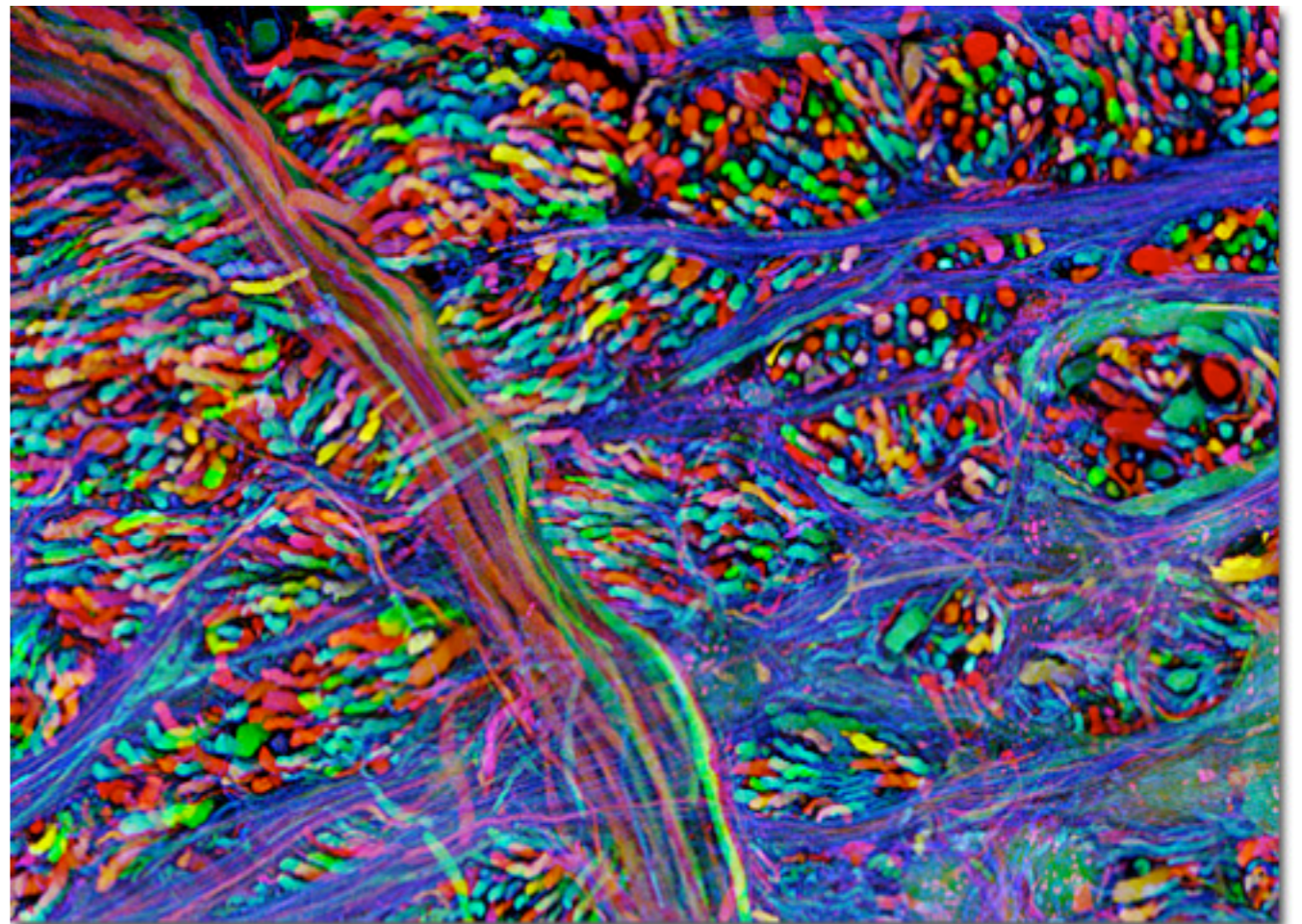
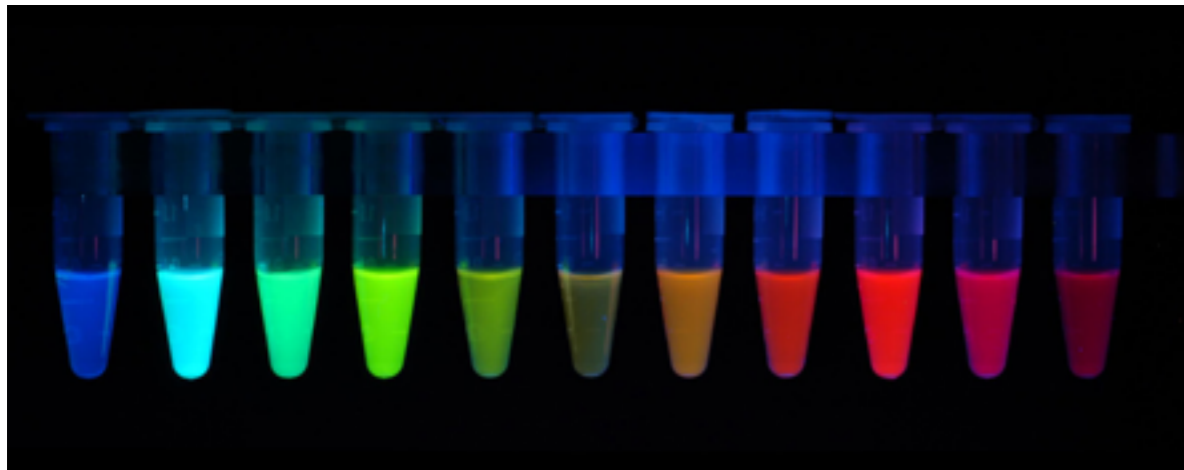
- optical imaging
- optical control
- genetic targeting

optogenetics

- **what?**
imaging structure activity
control membrane potential, etc
- **where & when?**
single cells
genetically identified populations
spatially localized populations
recently active populations
recently plastic populations
- **how?**
proteins, ion channels, pumps, rhodopsins, fluorescence
viruses
promoters
transgenic lines

optical imaging

- what can we image?
- structure
 - inorganic dyes
 - GFP, YFP, mCherry
 - brainbow

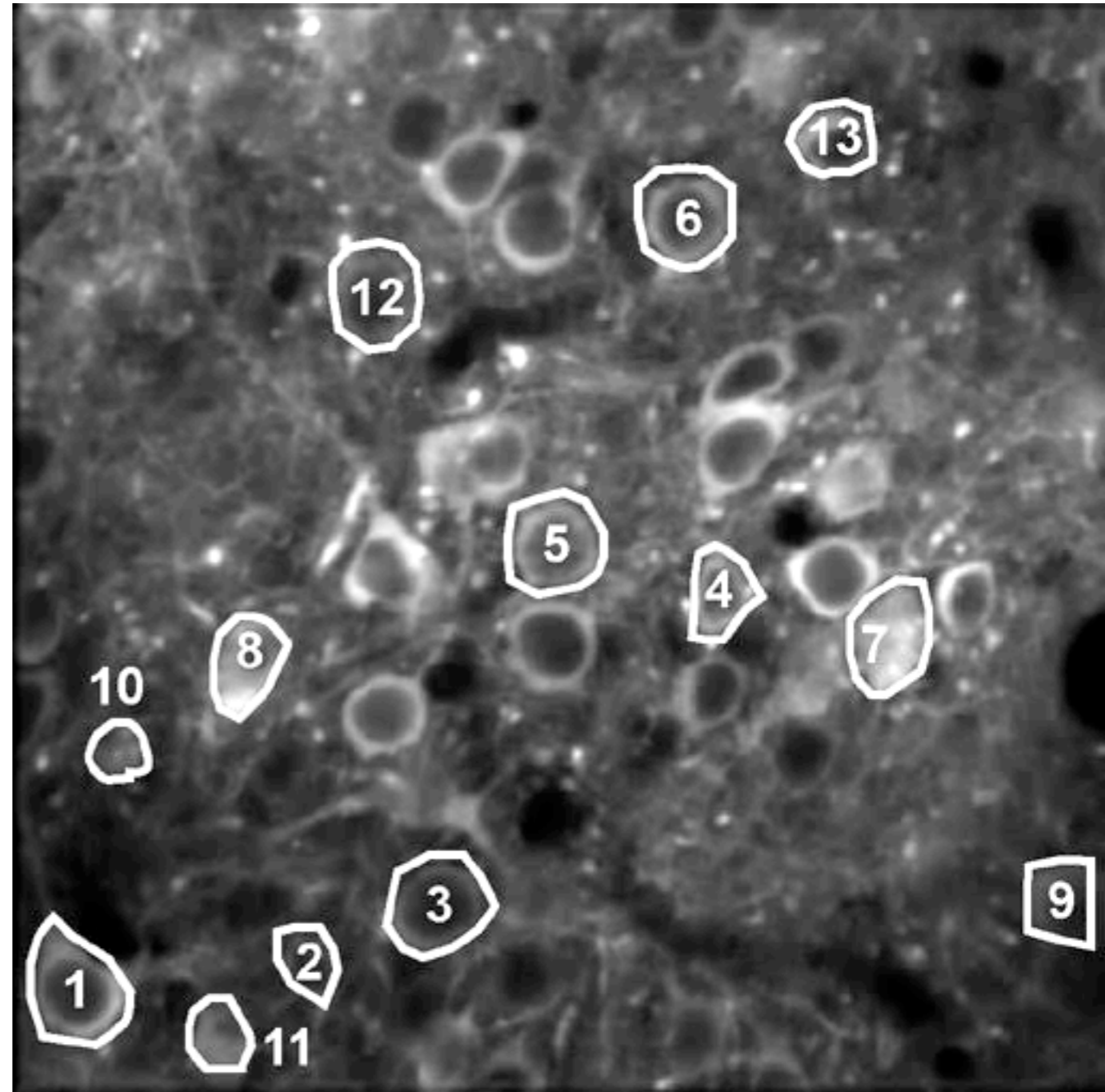


optical imaging

- what can we image?
- neural activity
 - membrane voltage
voltage sensitive dyes; *VSD*
 - intracellular calcium
concentration
synthetic dyes (Oregon
Green Bapta-1; *OGB1*)
genetically encoded calcium
indicators (*GEI*)
Cameleon, *GCaMP1-GCaMP6*
 - pH, Cl⁻, cell cycle, etc

optical imaging

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optical imaging

- calcium imaging considerations
 - speed? kinetics Ca^{2+} and kinetics of Ca^{2+} sensor
 - dynamic range? when does the sensor saturate?
 - SNR? how bright is the fluorescence?

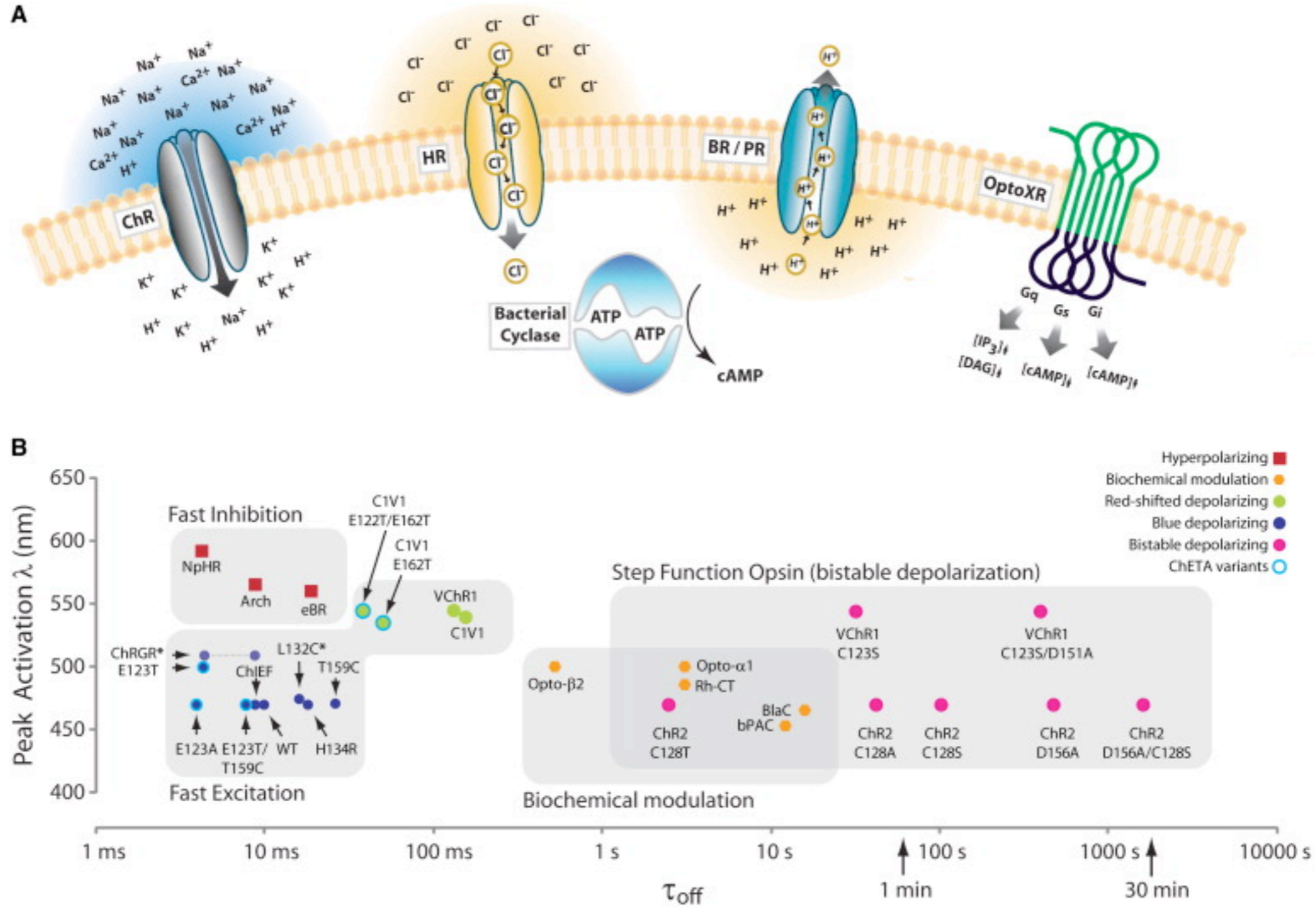
optical control

- what does it do?
 - excitation (depolarize neurons)
 - inhibition (hyperpolarize neurons)
 - other molecular pathways

optical control

- what timescale?
 - fast (millisecond, single AP)
ChR2, NpHR, Arch, Mac, CIVI
 - slow (step function opsins; SFO)
ChR2-SFO, VChR1-SFO
- what wavelength?

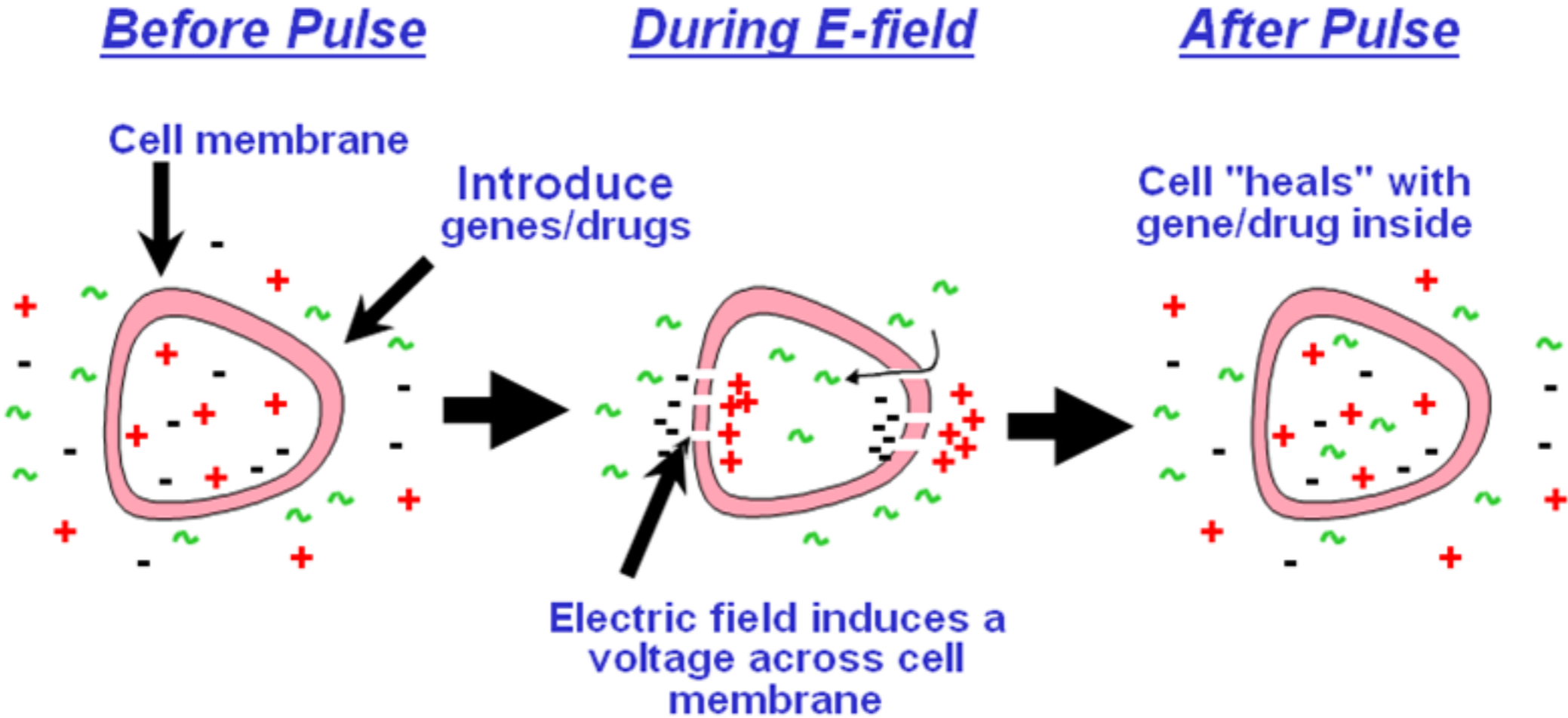
optical control



genetic delivery

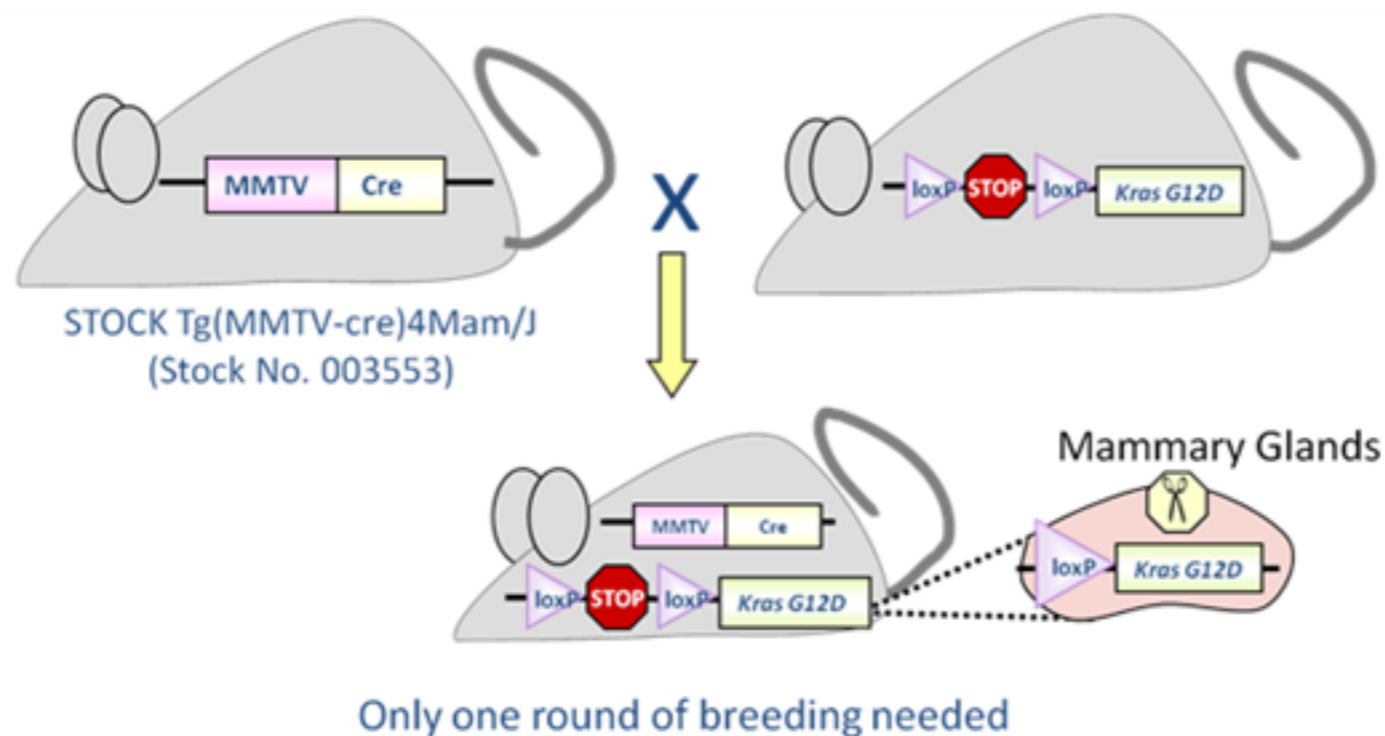
- how?
 - electroporation
 - transgenic cre-dependent expression
 - viral vector
 - virus + cre

electroporation



transgenic cre-lox system

- “Cre” recombinase: an enzyme that cuts DNA at “lox” sites
- many transgenic Cre mouse lines available
- Cre-ER is “inducible” by feeding the mice tamoxifen (an antibiotic)



viruses

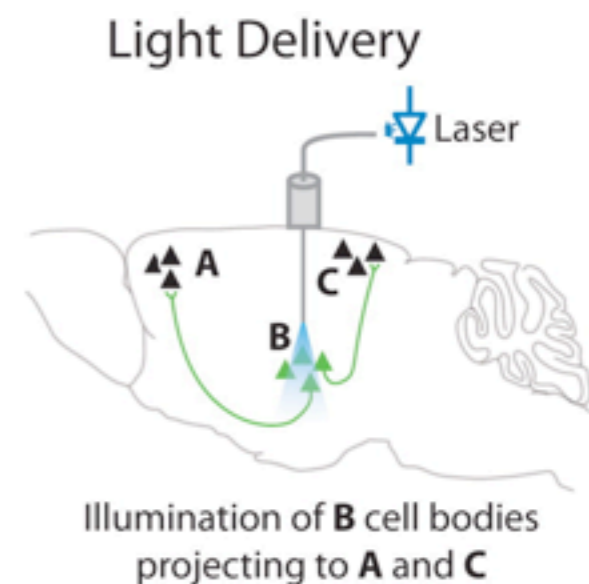
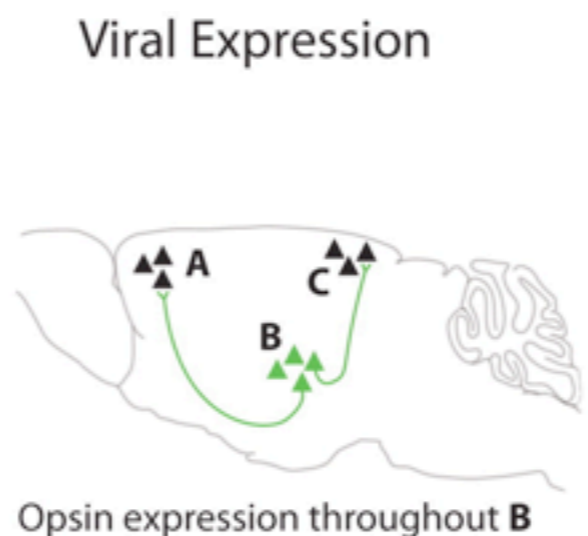
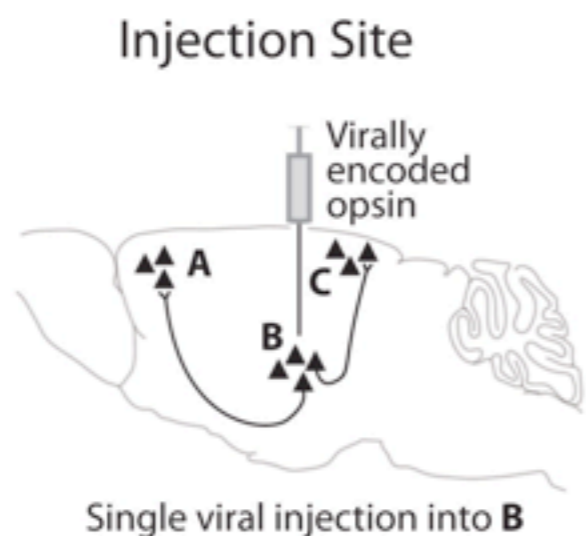
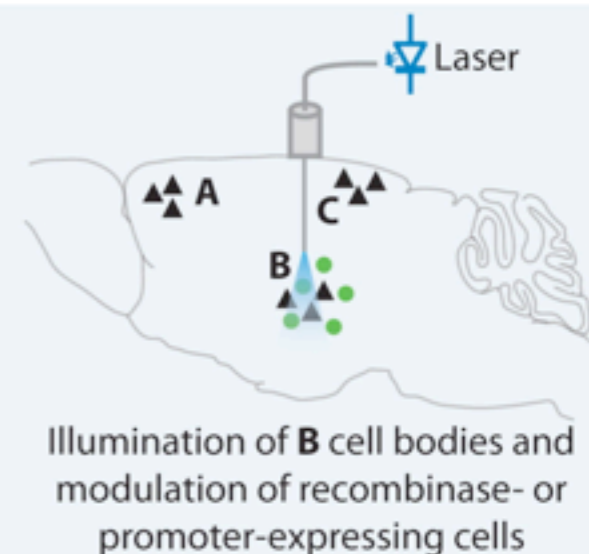
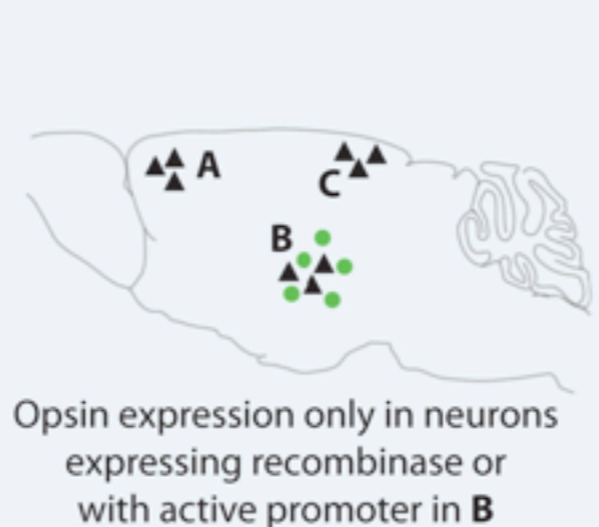
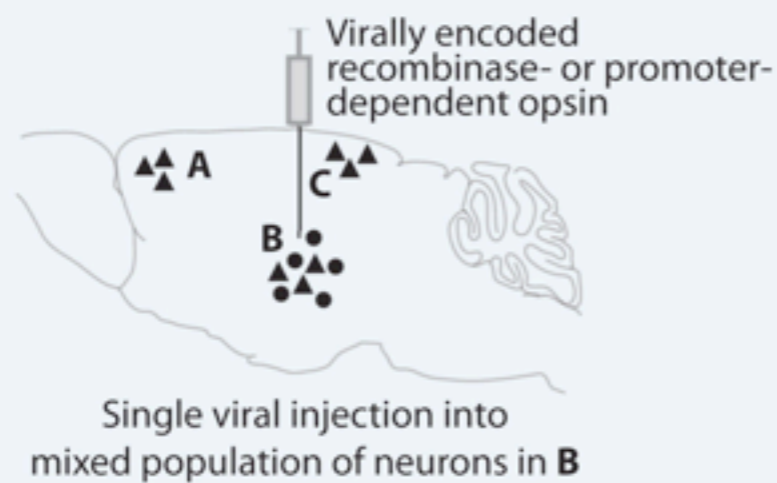
- lentivirus (LV)
- herpes simplex virus (HSV)
- adeno-associated virus (AAV)
- rabies/pseudorabies

viruses

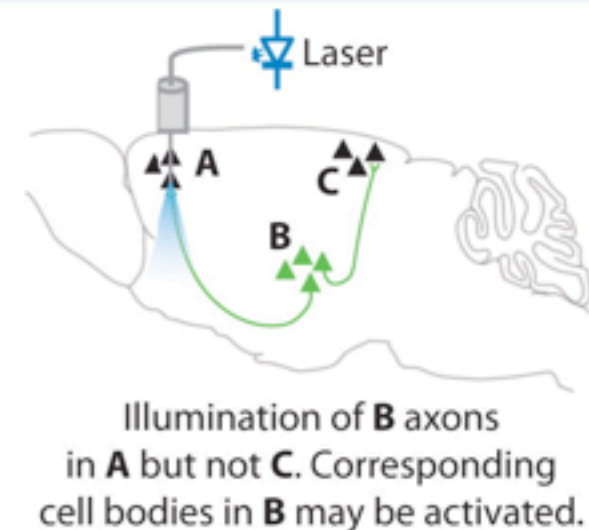
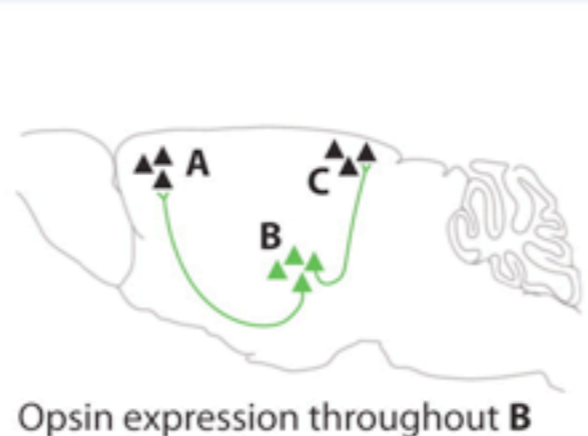
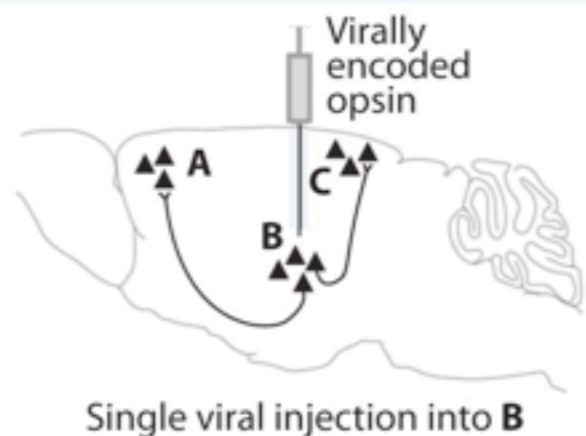
- considerations
 - payload size?
 - expression level
 - infection efficiency/bias?
 - pathogenicity?
 - retrovirus?

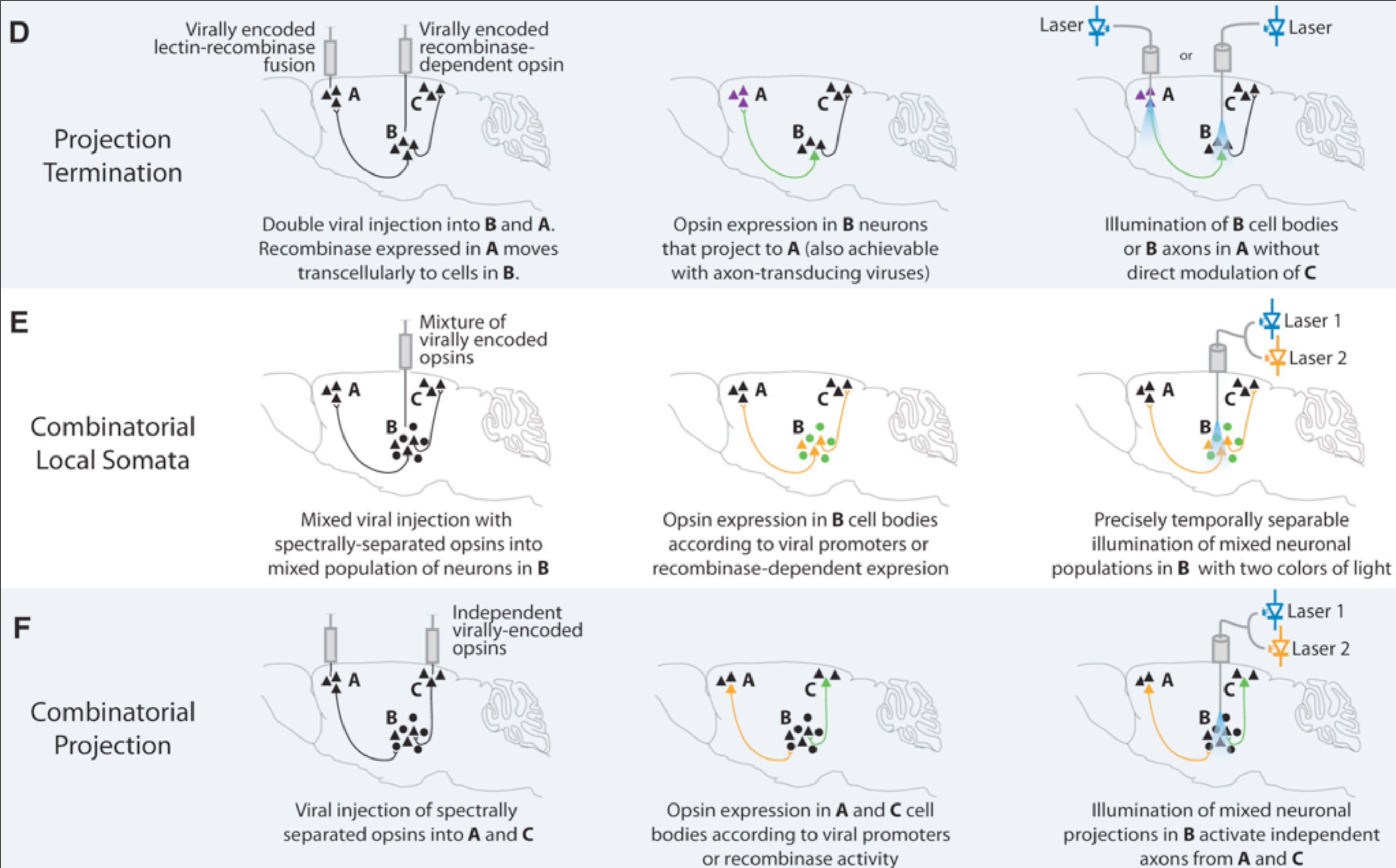
targeted genetic delivery

- where?
 - cell-type specific promoter
 - area specific promoter
 - local virus injection/electroporation

ALocal
Somata**B**Recombinase-
or promoter-
dependent**C**

Projection





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