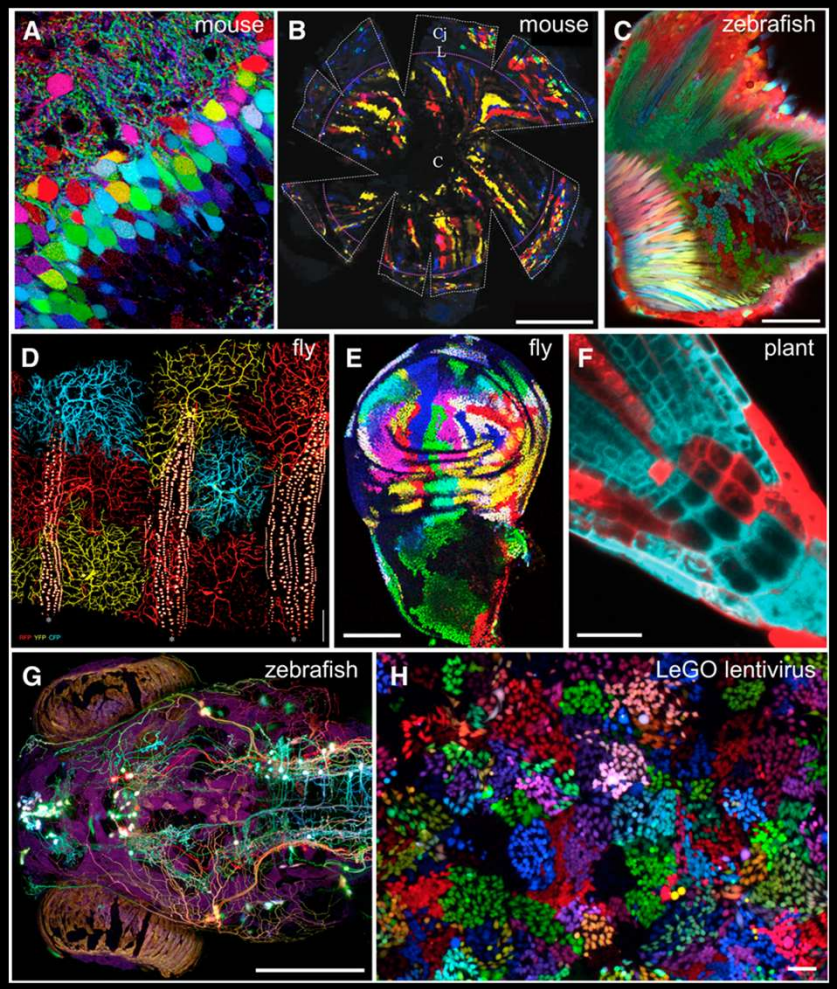
«Μοντέλα μυών και καινοτόμες τεχνικές για τη μελέτη του Νευρικού Συστήματος»



Myrto Denaxa
16/12/2025

Talk outline:

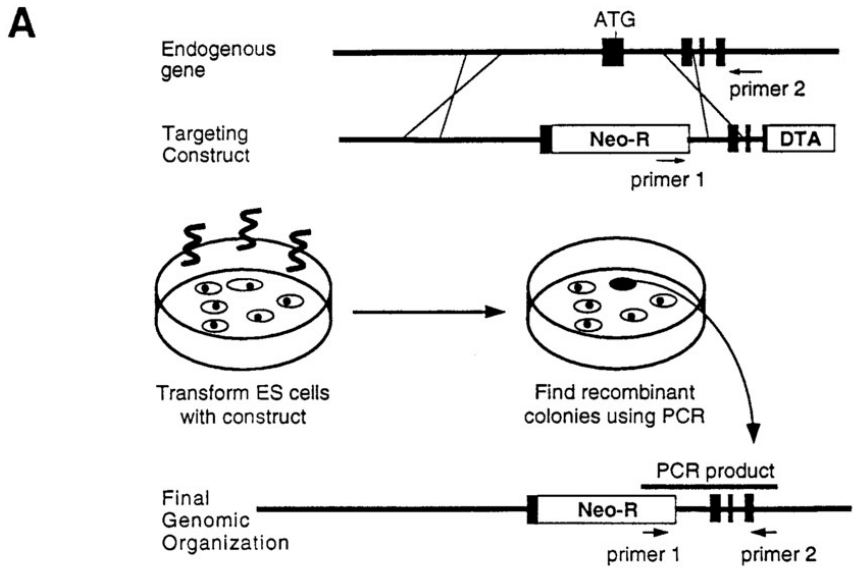
- ***Making and Using Genetically Modified Mice***
- ***Gene Delivery Strategies***
- ***Visualizing Neural Structures***
- ***Manipulating Neural Activity***

Making and Using Transgenic Organisms:

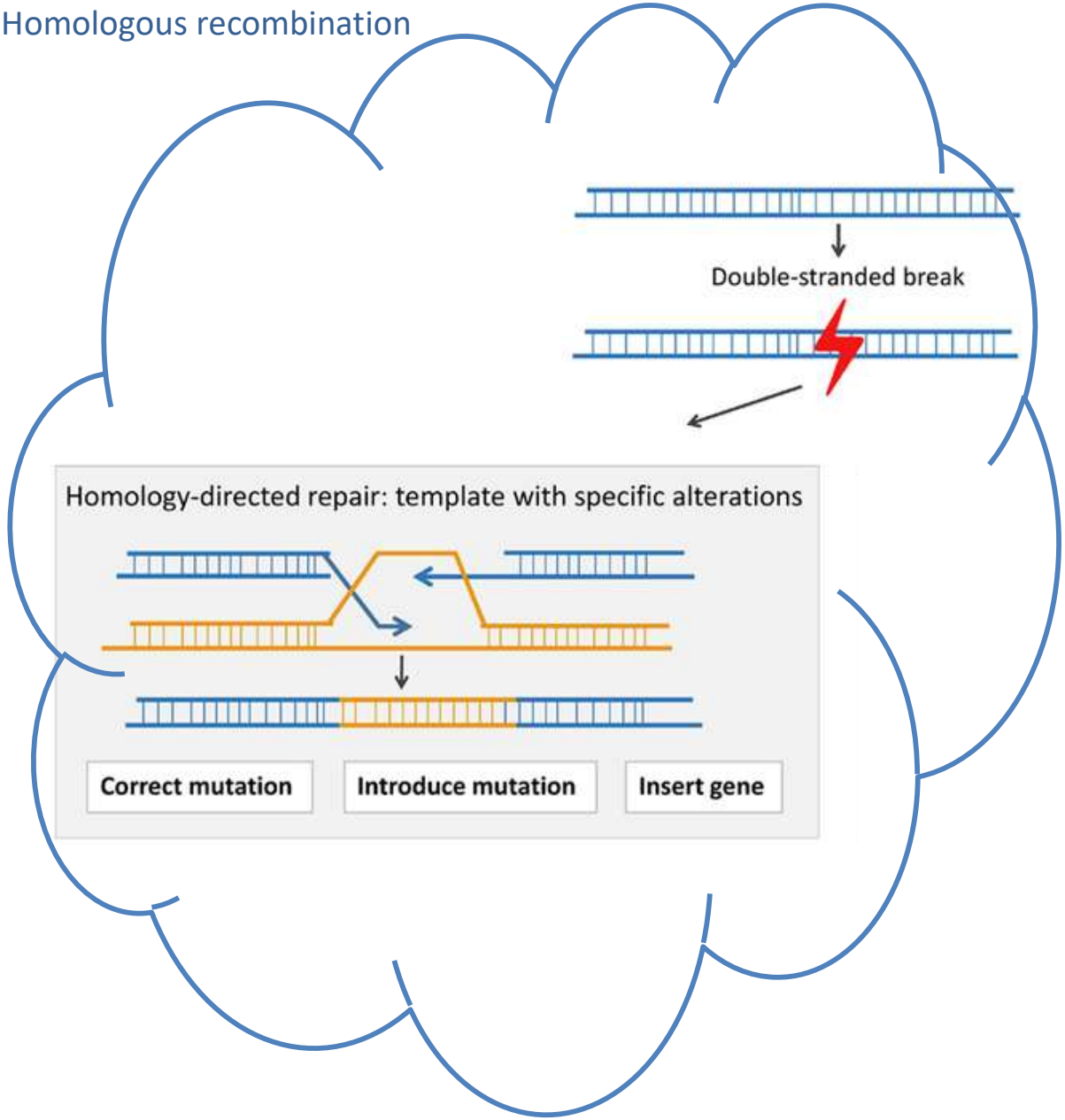
Describe common transgenes used in modern neuroscience research and explain how these genes can be restricted to specific cell types at specific times.

- **Common transgenes in neuroscience:** KO mice, KI mice: Fluorescent Reporter or any other protein of interest
- **Binary transgenic systems:** conditional KO mice (Cre/lox, Flp/Frt)

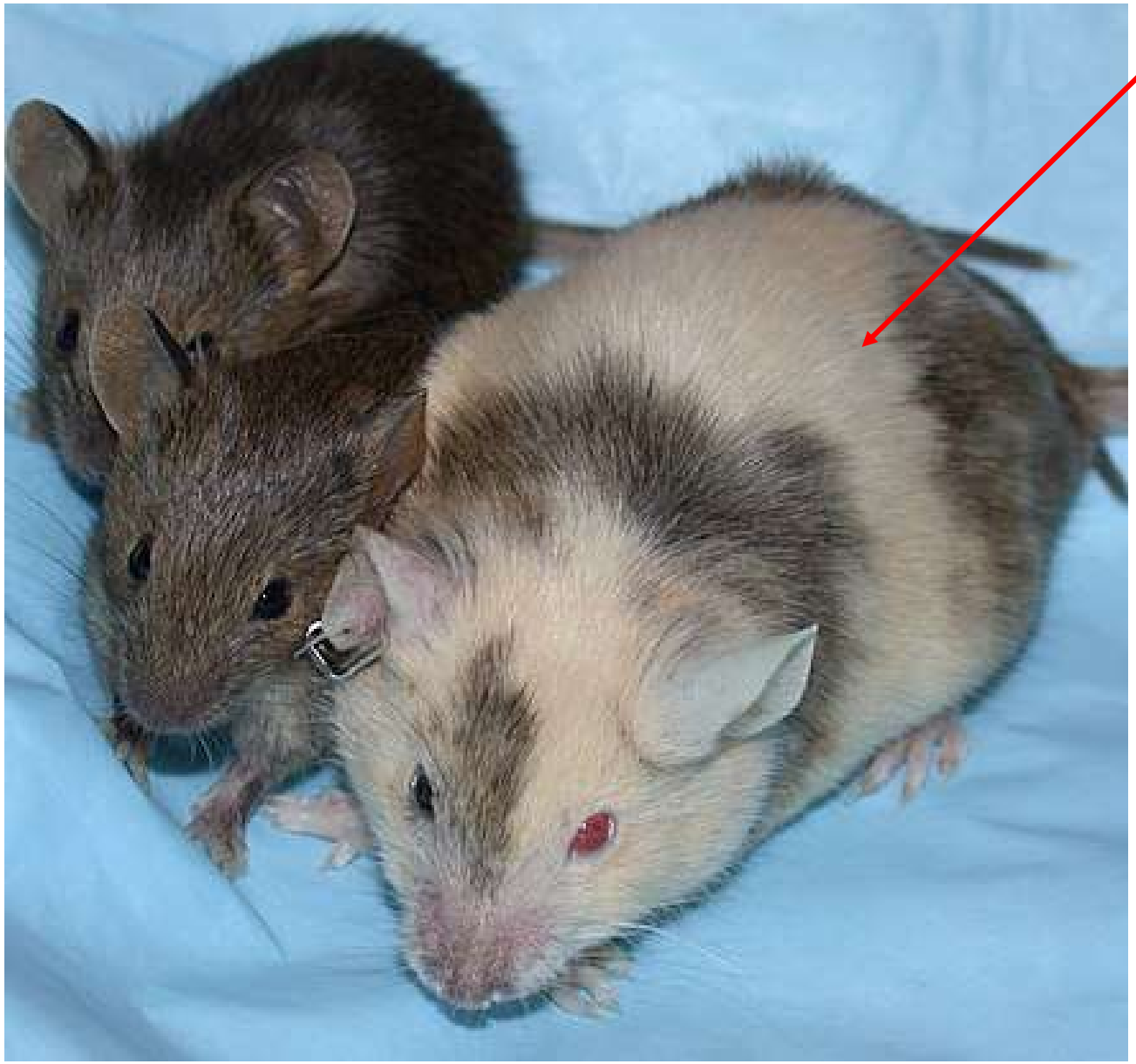
The procedure for making transgenic mice

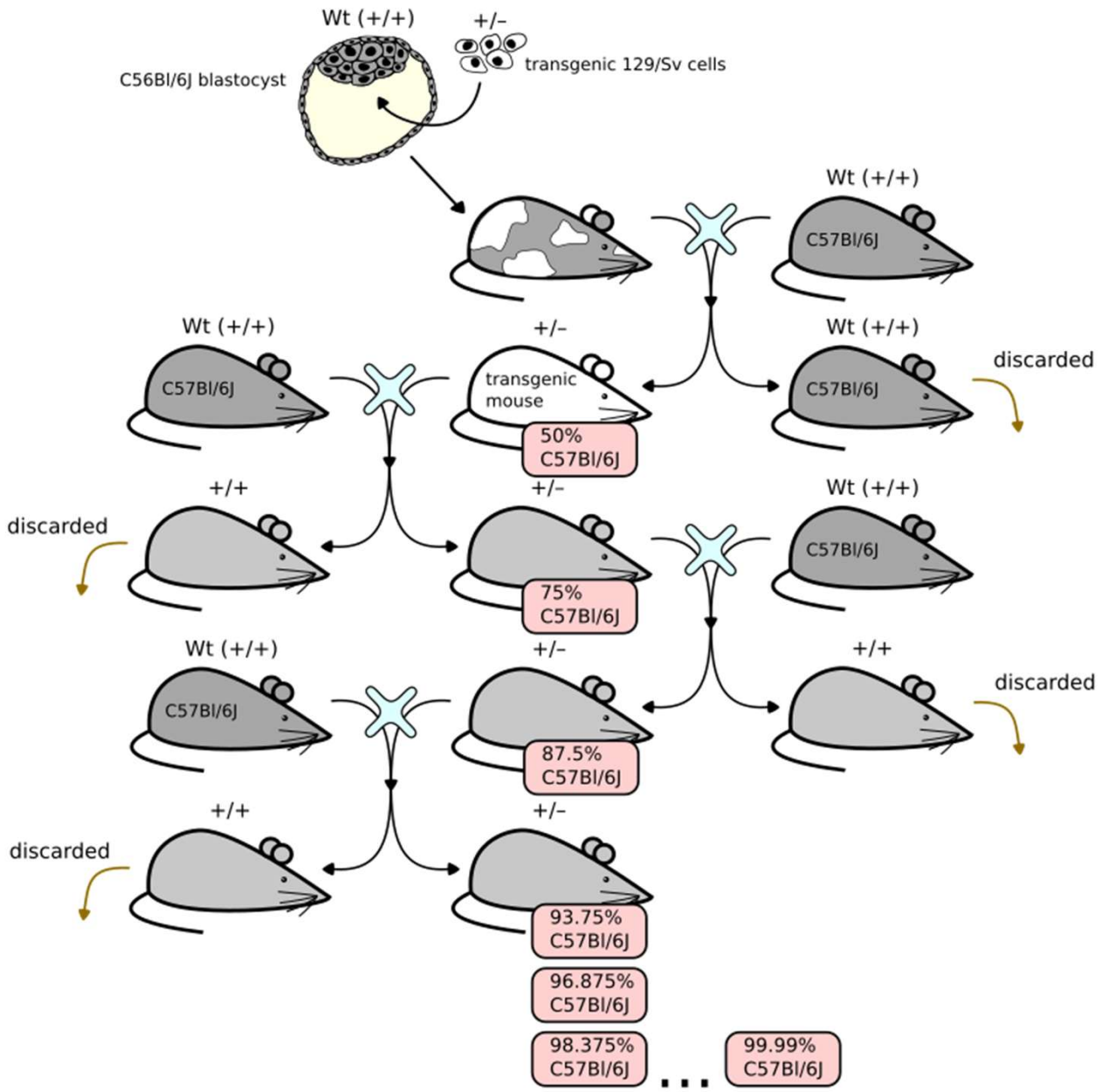


Homologous recombination



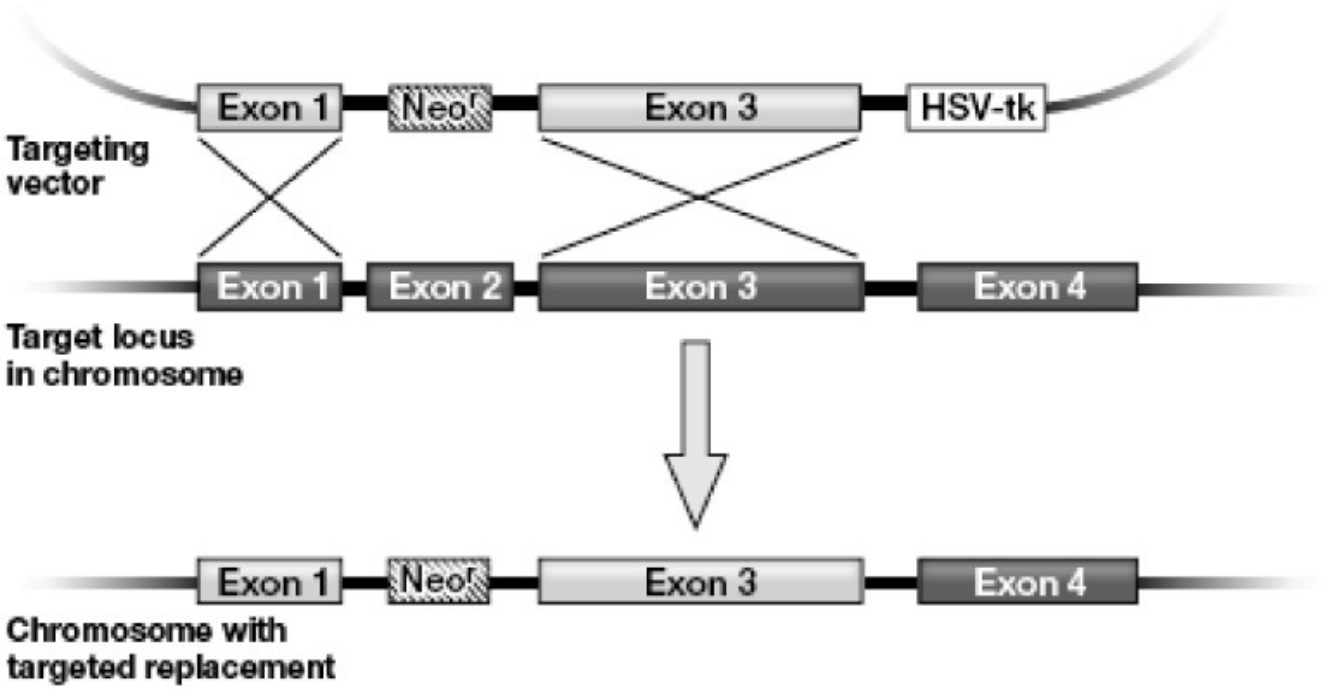
Chimeric mouse

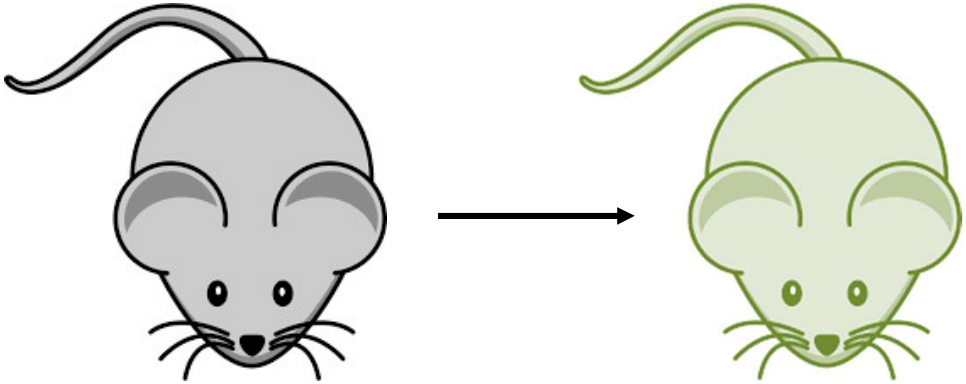



Wt (+/+)
+/–
C56Bl/6J blastocyst
transgenic 129/Sv cells
Wt (+/+)
C57Bl/6J
Wt (+/+)
C57Bl/6J
+/–
transgenic mouse
50% C57Bl/6J
Wt (+/+)
C57Bl/6J
discarded
+/+
discarded
+/–
75% C57Bl/6J
Wt (+/+)
C57Bl/6J
Wt (+/+)
C57Bl/6J
+/–
87.5% C57Bl/6J
+/+
discarded
+/+
discarded
+/–
93.75% C57Bl/6J
96.875% C57Bl/6J
98.375% C57Bl/6J
...
99.99% C57Bl/6J

Making and Using Transgenic Organisms:

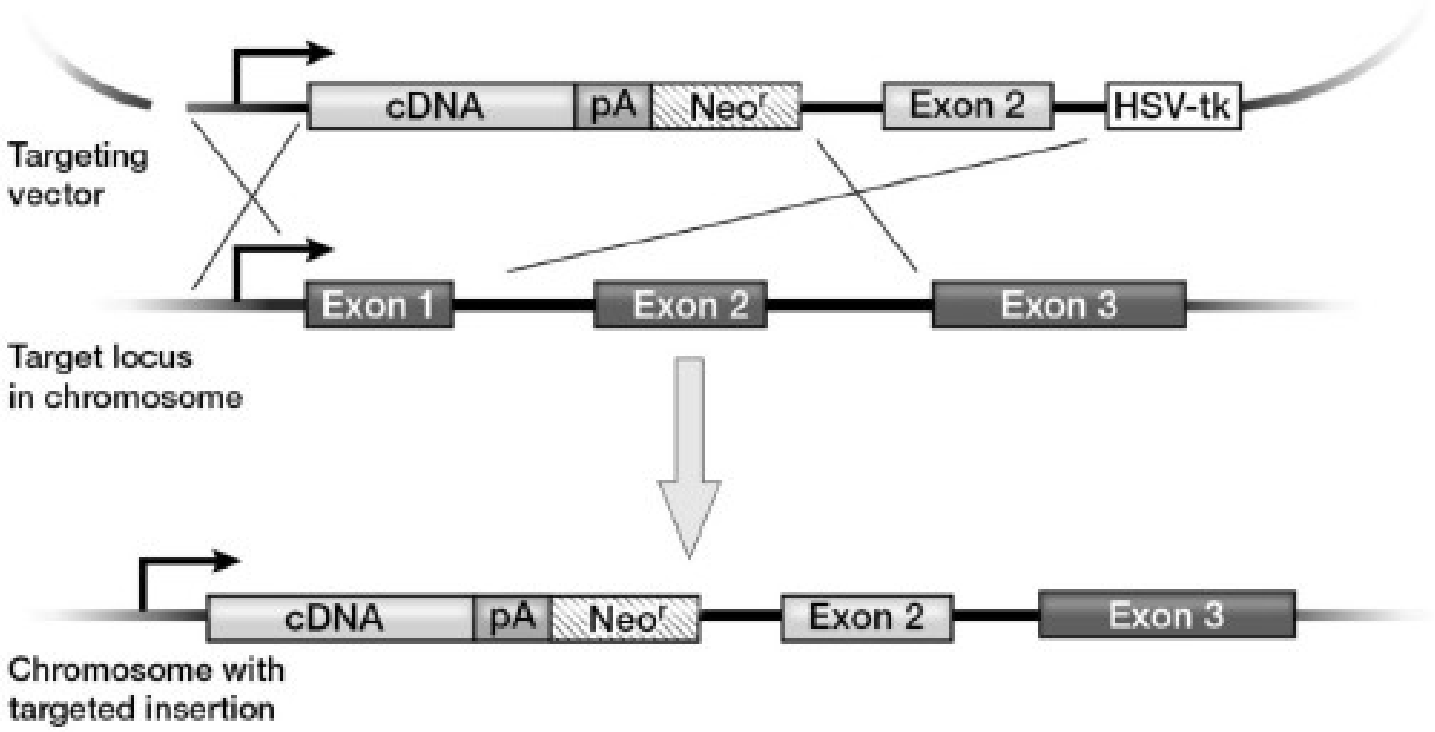
KO mice

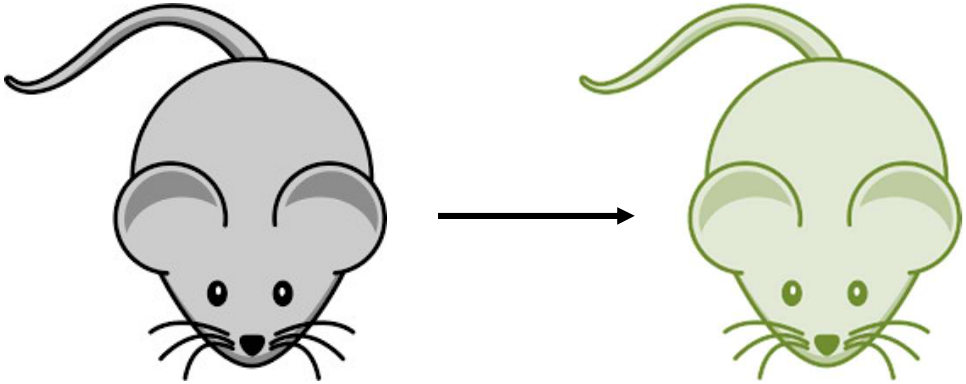




Making and Using Transgenic Organisms:

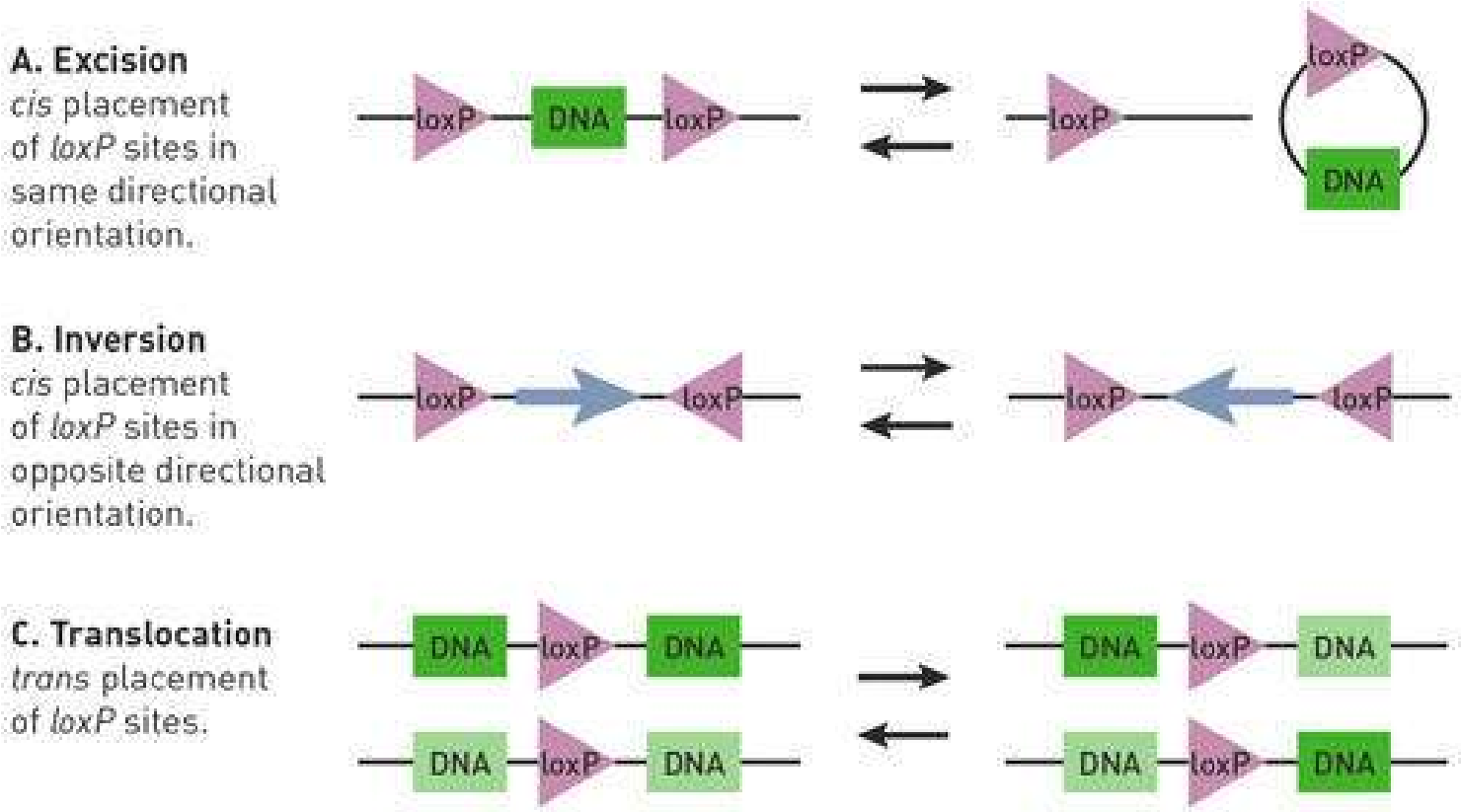
KI mice



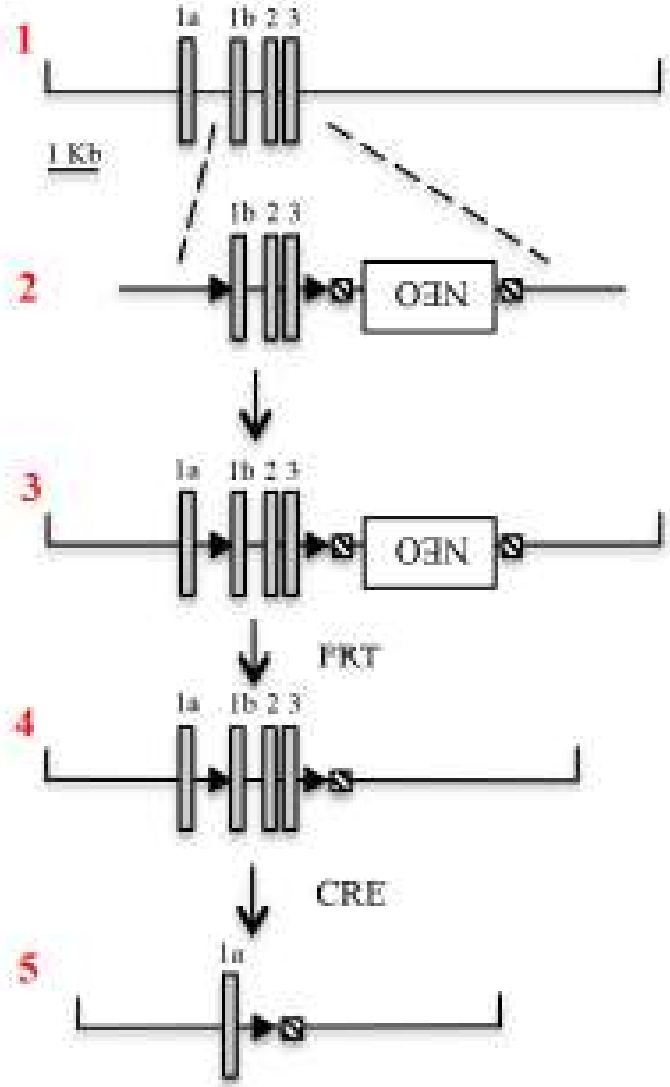


Making and Using Transgenic Organisms:

CRE-loxP recombination system



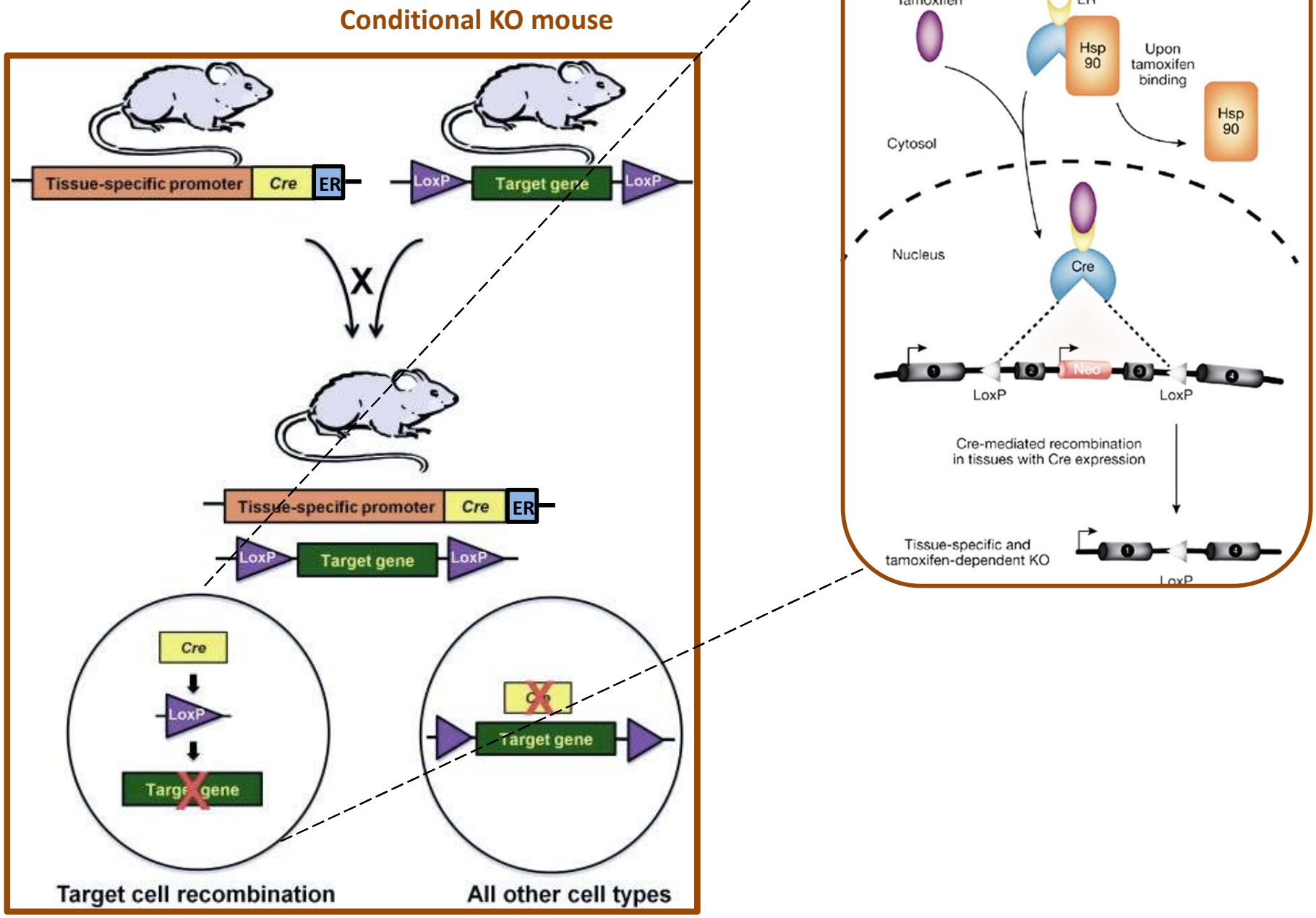
Making and Using Transgenic Organisms:
Conditional KO mice (1)



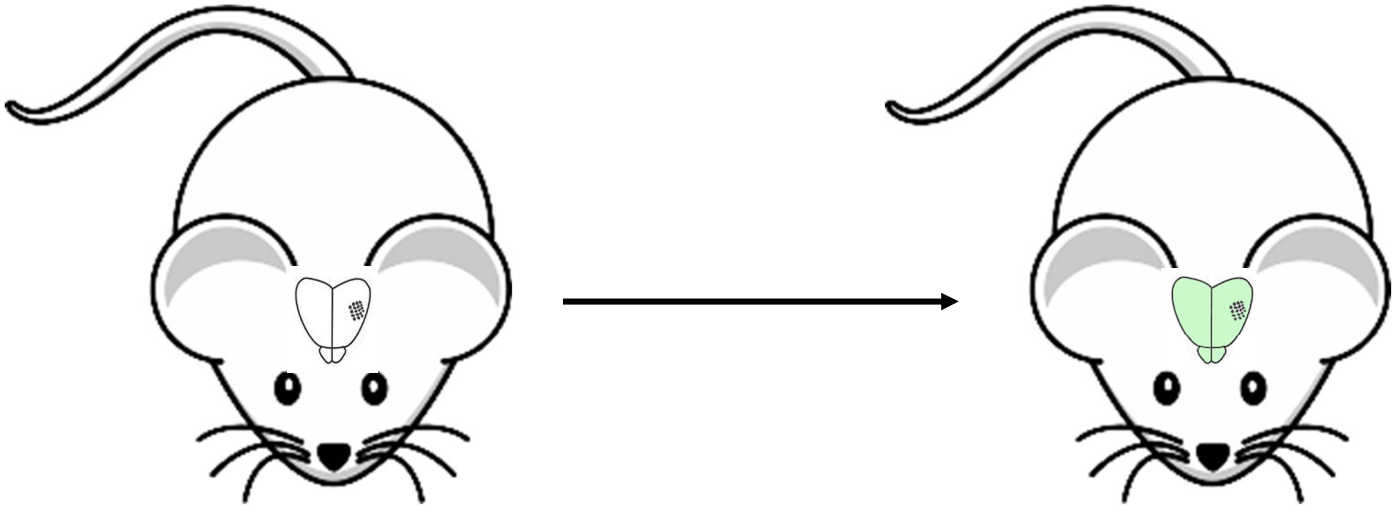
LoxP sites

Flox sites

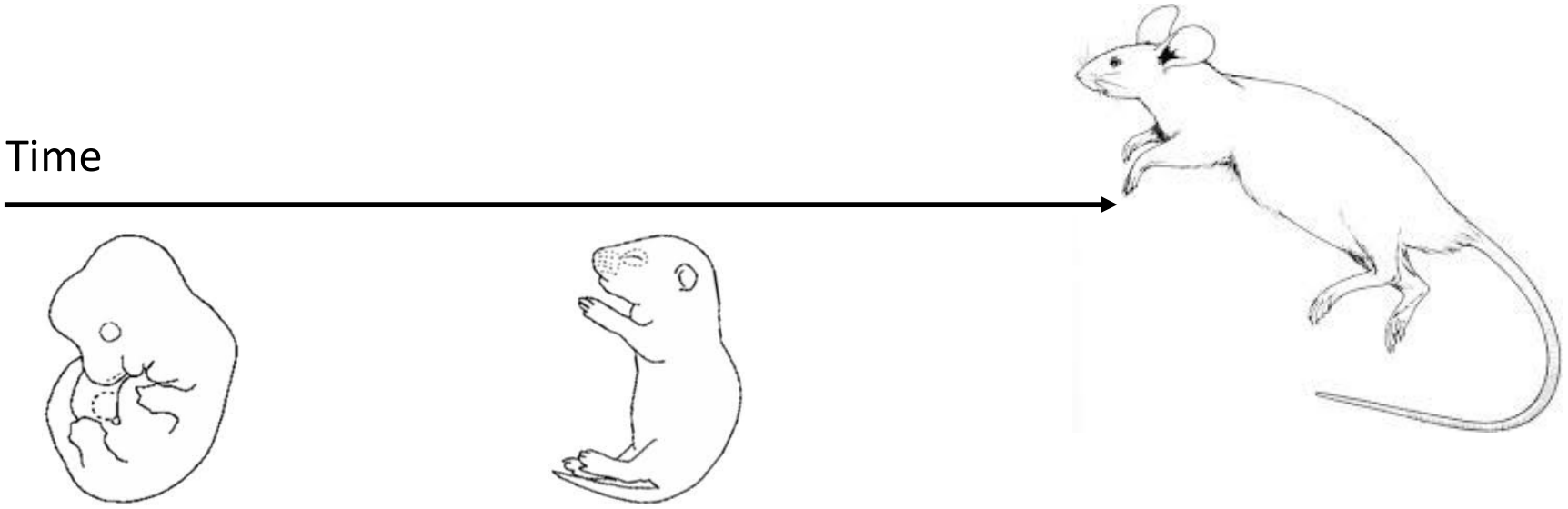
Making and Using Transgenic Organisms:
Conditional KO mice (2)

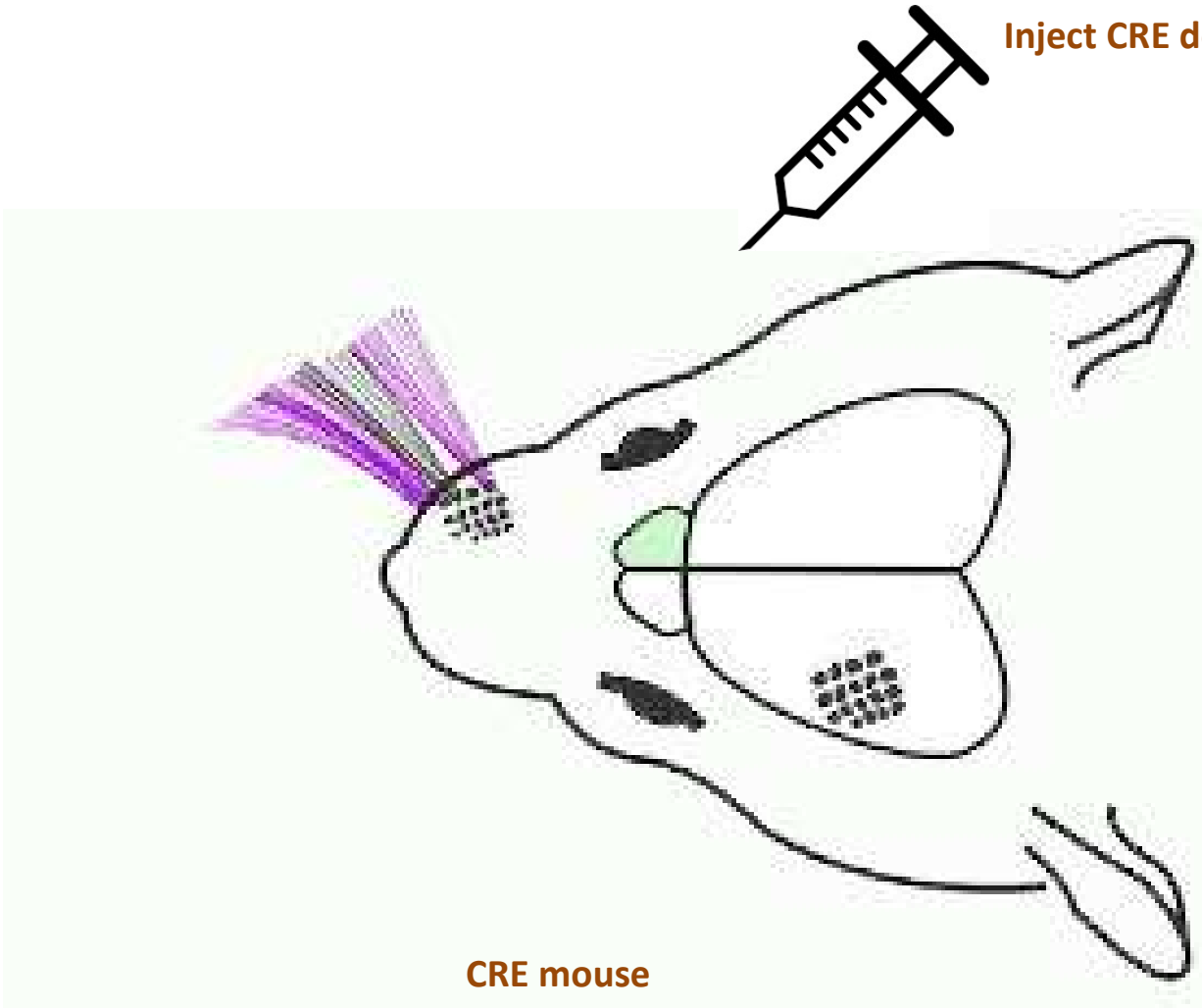


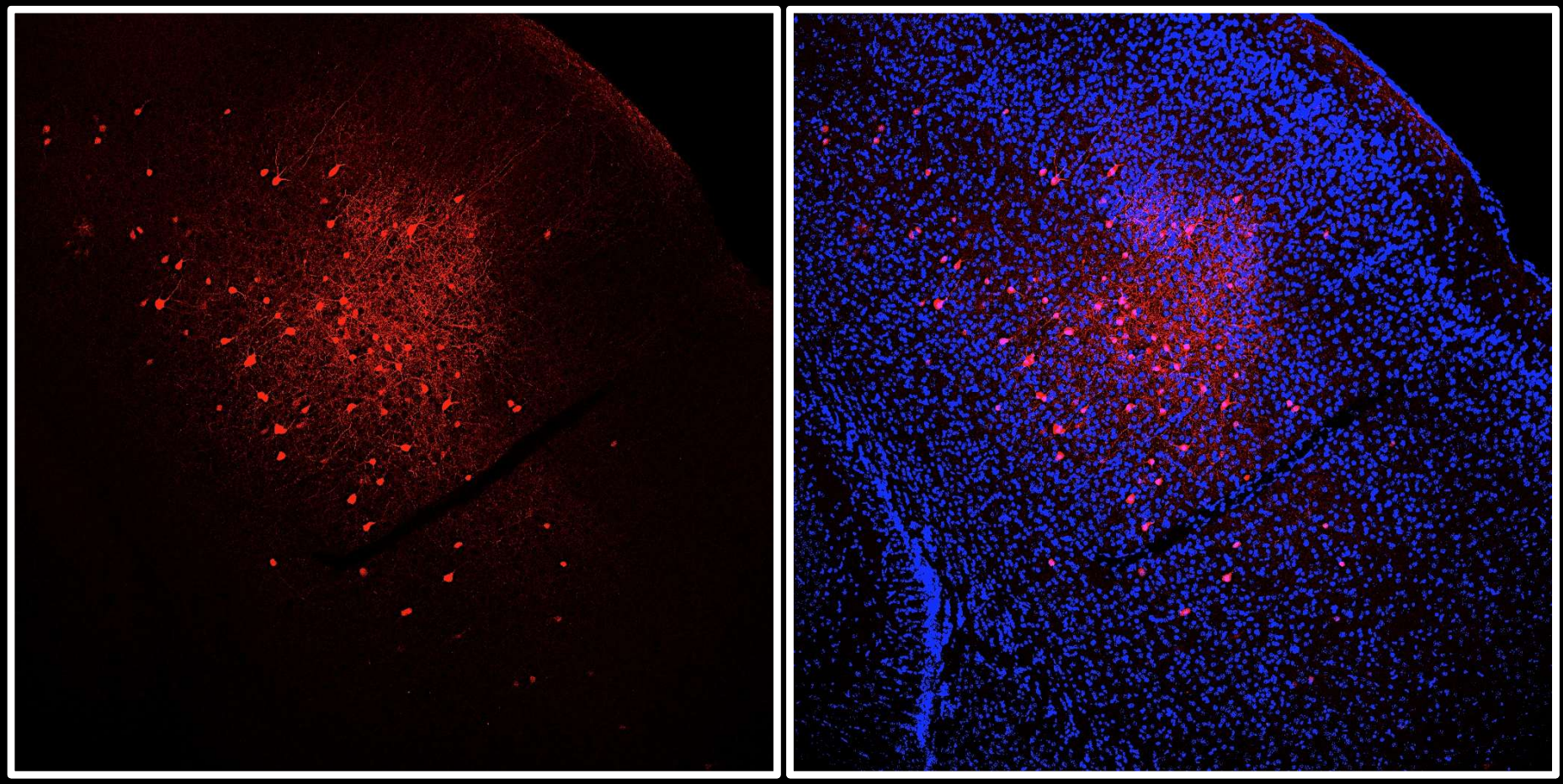
Conditional KO mouse



Tamoxifen Inducible Conditional KO mouse



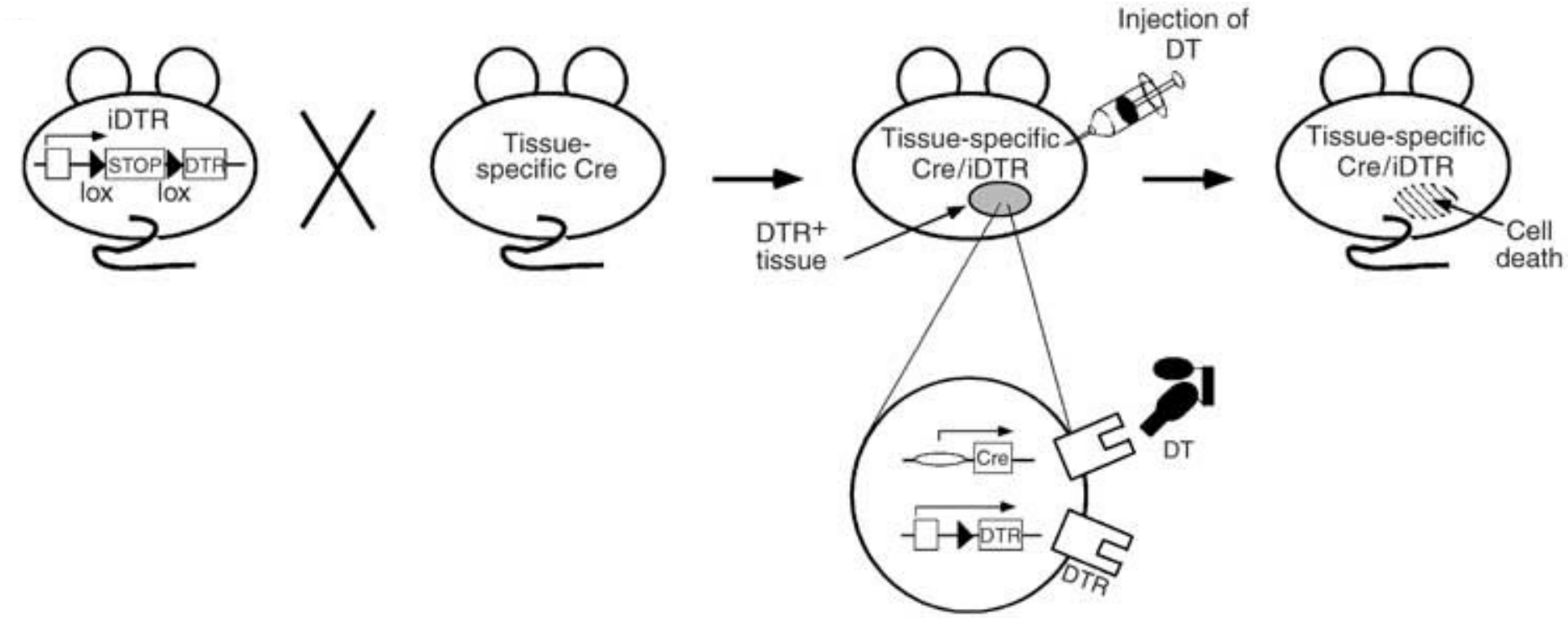

Inject CRE dependent reporter
CRE mouse



Nkx2.1CRE;AAV8FlexRFP DAPI

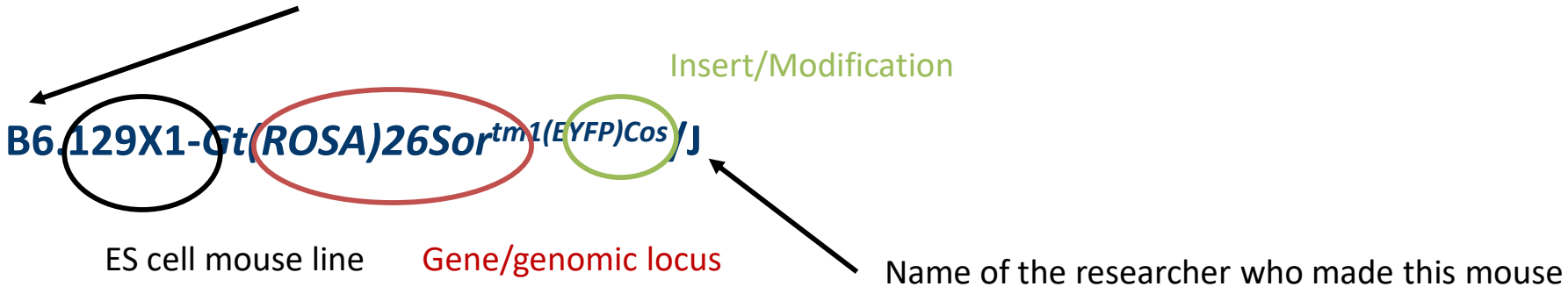
Making and Using Transgenic Organisms:

Conditional KI mouse used to ablate neurons: Diphtheria toxin receptor mouse



Transgenic mouse nomenclature:

Mouse Line background (blastocyst-backcrosses)



KO mouse: X

X^- mutant allele

X^+ wt allele

KI mouse: X ^{YFP} protein you have inserted in the X gene locus

Cre mouse lines: X^{CRE}

Cre inducible mouse lines: X ^{CREERT2}

Conditional KO mouse: X^f or X^{fl}

Gene Delivery Strategies :

The ultimate goal of creating a DNA construct is to deliver the manipulated sequence into living cells so that the endogenous cellular machinery can transcribe and translate the sequence into functional proteins. A neuroscientist might want to deliver a DNA sequence into a population of cells in culture, in a brain slice, or in the brain of a living animal. We will describe common methods of delivering recombinant DNA into cells in each of these environments.

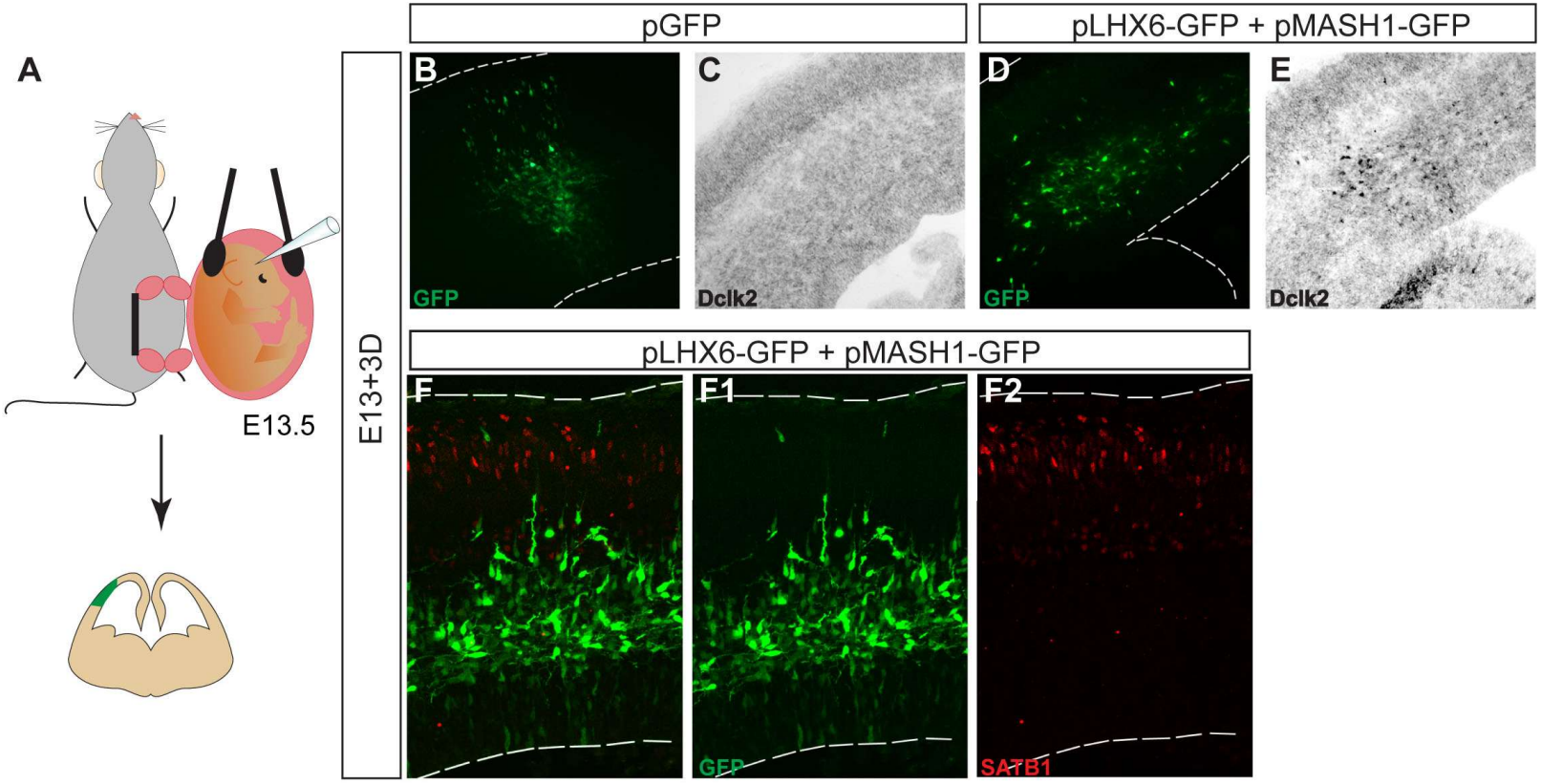
• **Physical gene delivery:** microinjection, electroporation

~~• **Chemical gene delivery:** calcium phosphate transfection, lipid-based transfection~~

• **Viral gene delivery:** adenovirus, adeno-associated virus (AAV), lentivirus, herpes simplex virus (HSV), canine adenovirus (CAV)

Gene Delivery Strategies:

Physical gene delivery: electroporation/in utero electroporation



Embryos: Ultrasound guided electroporation

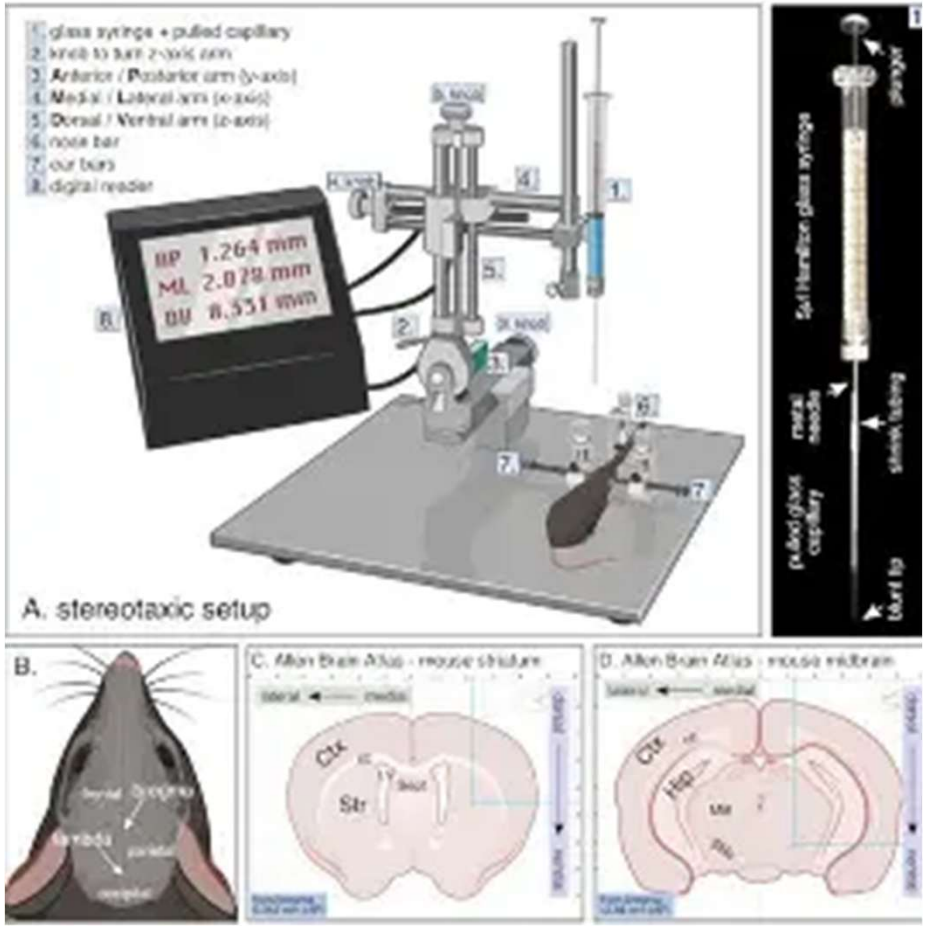
https://www.jove.com/video/2047/ultrasound-guided-microinjection-into-mouse-forebrain-utero-at

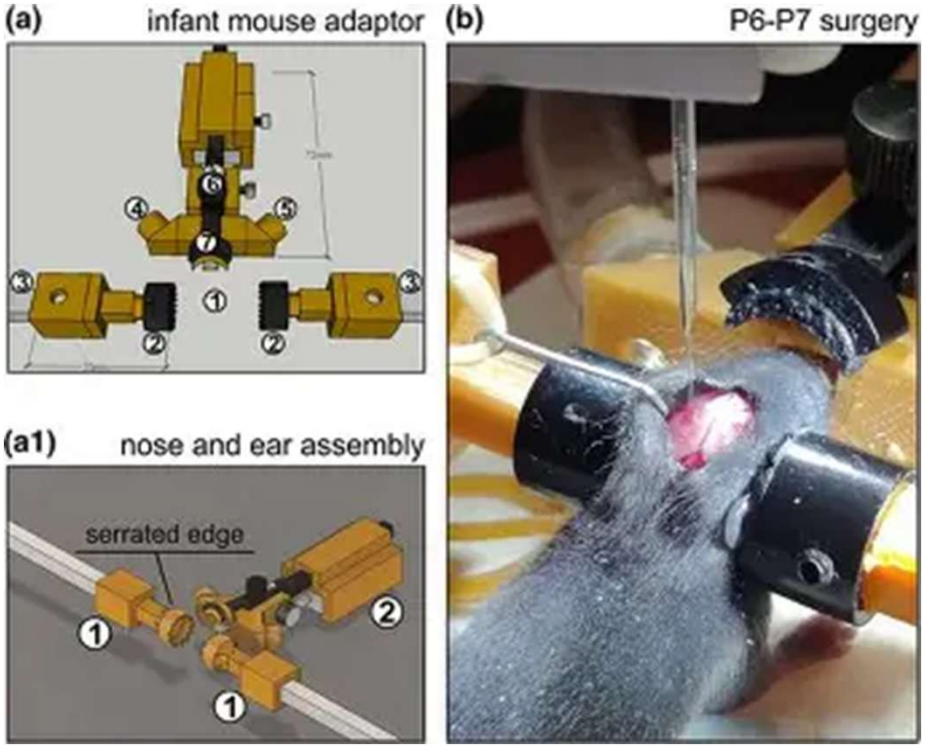
Embryos: Ultrasound guided

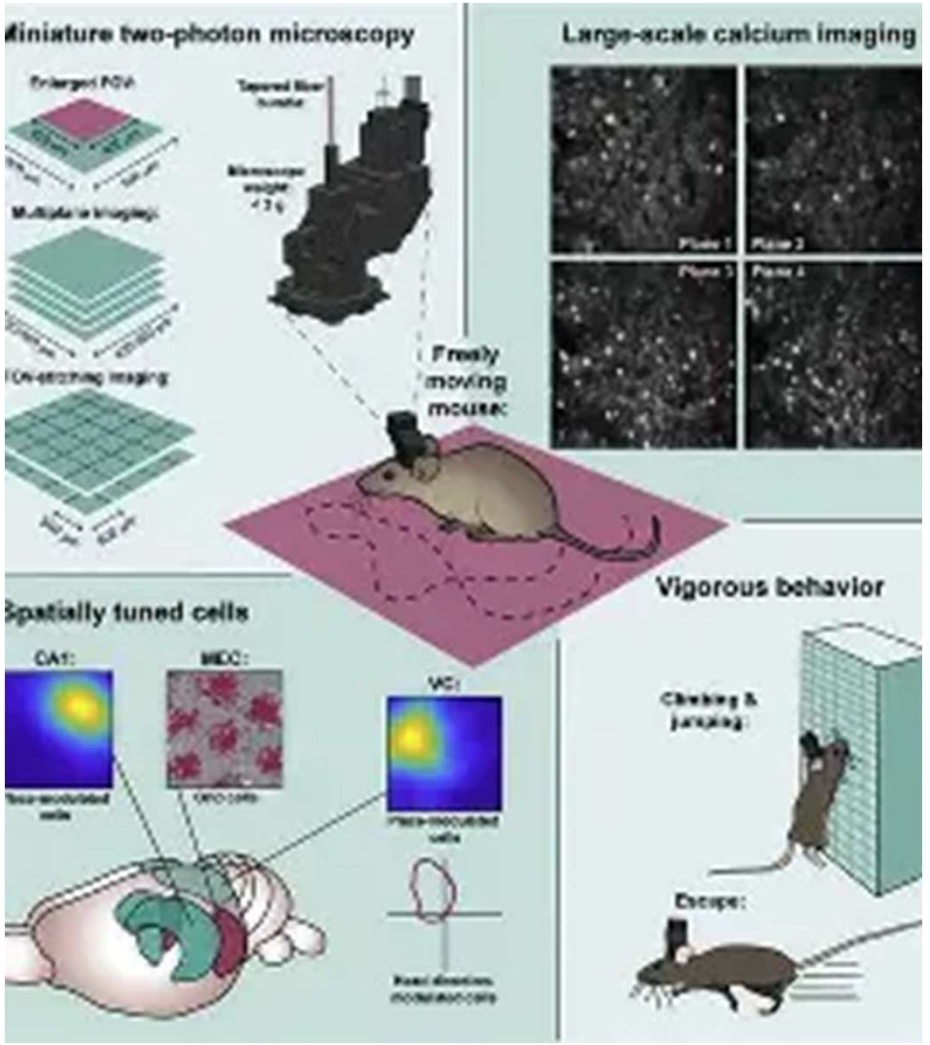
Early postnatal pups: Intracranial Injections (no surgery)

Late postnatal-Adult mice: Stereotactic Injections (surgery)

Late postnatal-Adult mice: Stereotactic Injections (surgery)






Miniature two-photon microscopy
Large-scale calcium imaging
Freely moving mouse
Spatially tuned cells
CA1:
MEC:
VC:
Vigorous behavior
Climbing & jumping:
Escape:

Questions

Visualizing Neural Structures:

Techniques used to investigate the structure and connectivity of the nervous system. Using these techniques, it is possible to classify cells based on location, morphology, gene/protein expression profiles, and connections with other cells.

- Visualizing gross cellular morphology/gene;protein expression:

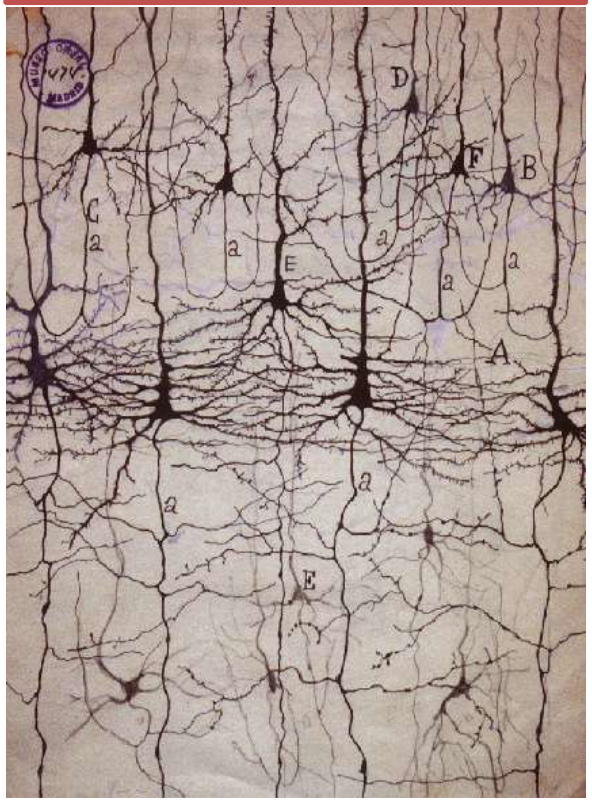
- Golgi staining (histochemistry)
- Immunohistochemistry (proteins and reporter genes)
- mRNA (mRNA in situ hybridization, RNAscope ISH)

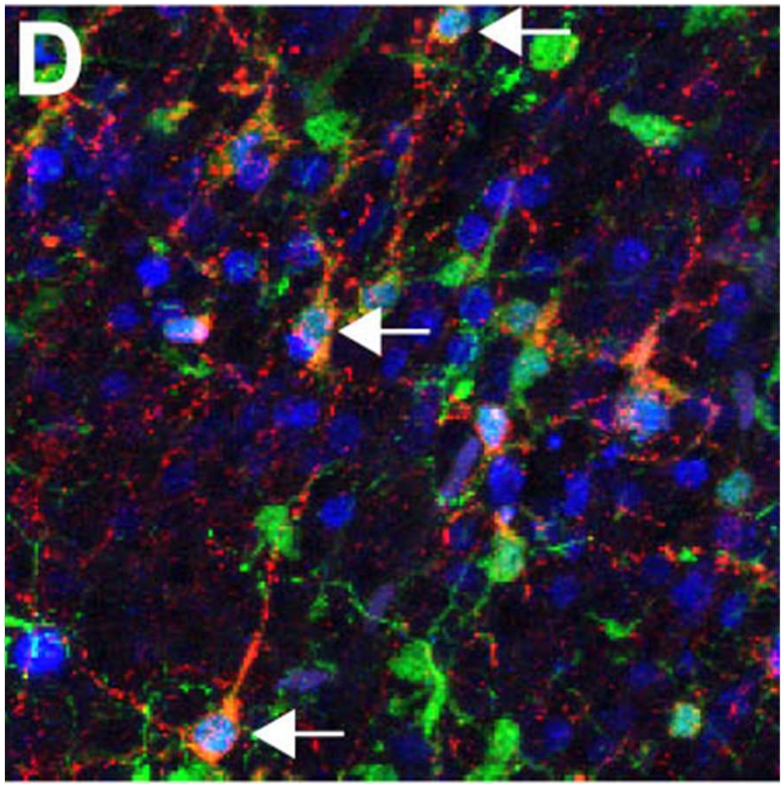
- Visualizing neural circuitry and connection between different brain regions, whole brain imaging:

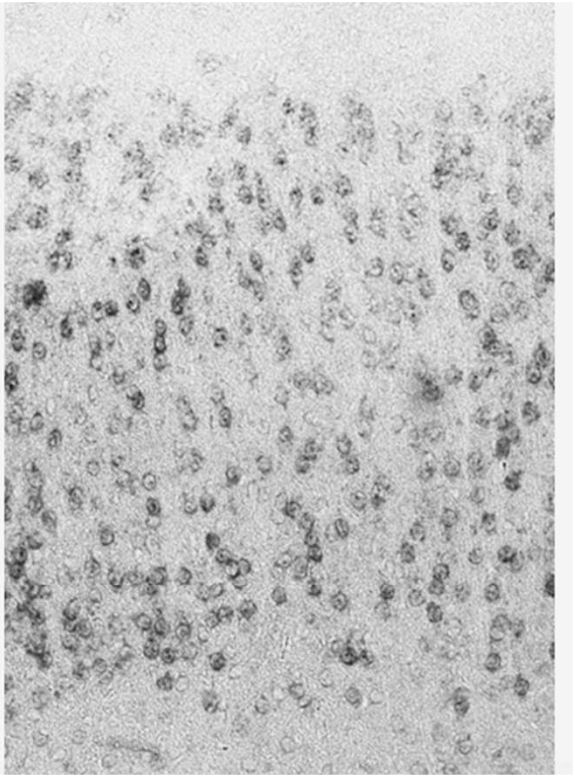
- Lipophilic tracers (DiI, DiO)
- Transgenic Mice/Reporter genes
- Brainbow technology
- Viruses (Rabies)
- Clarity, iDisco technology

Visualizing gross cellular morphology/ gene;protein expression (1)

Golgi staining	Immunohistochemistry (3)	mRNA *in situ*







Santiago Ramon y Cajal, 1899

Visualizing neural circuitry and connection between different brain regions, whole brain imaging

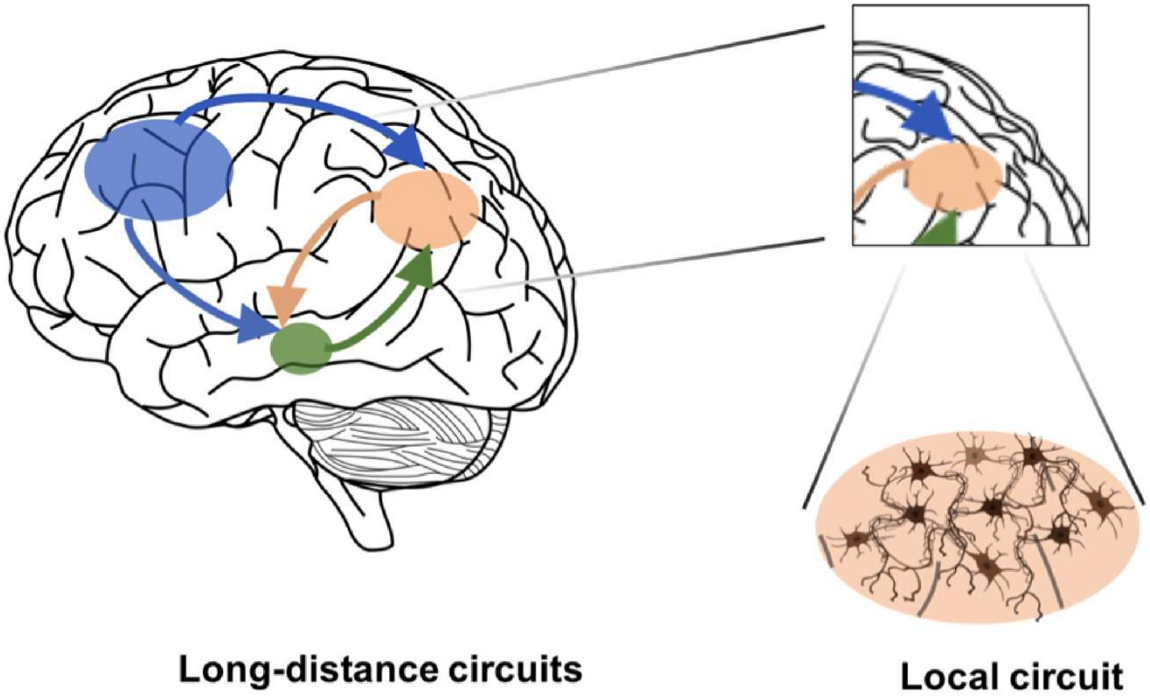


Figure 1: Examples of neuronal circuits.

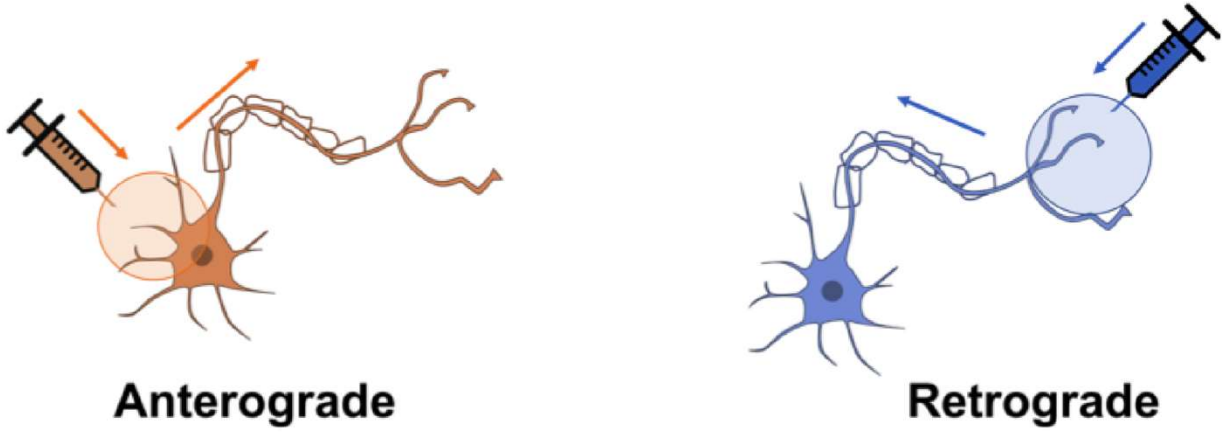
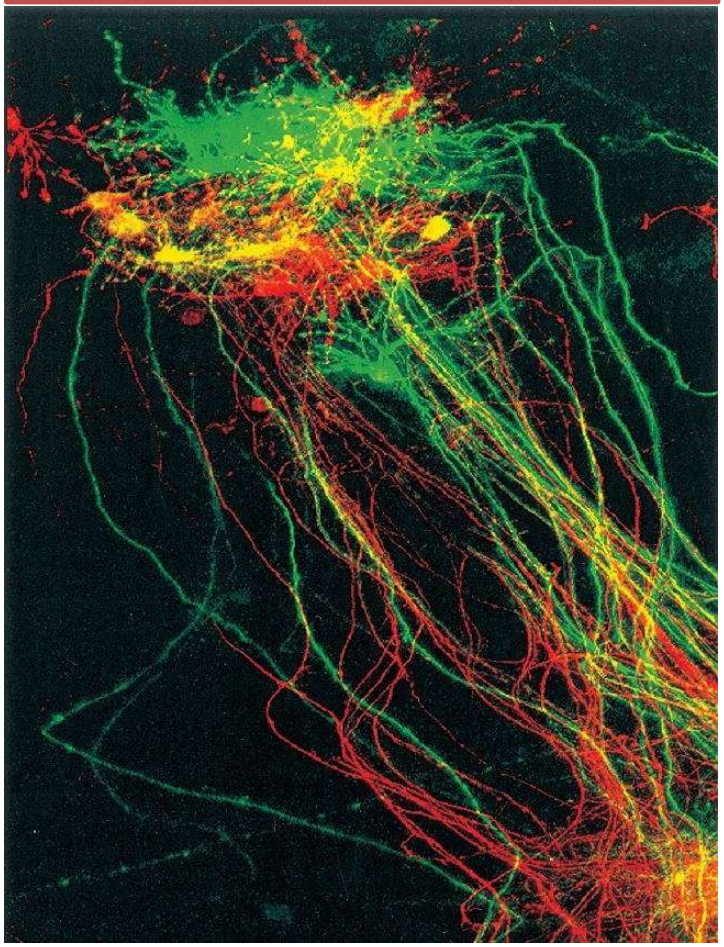
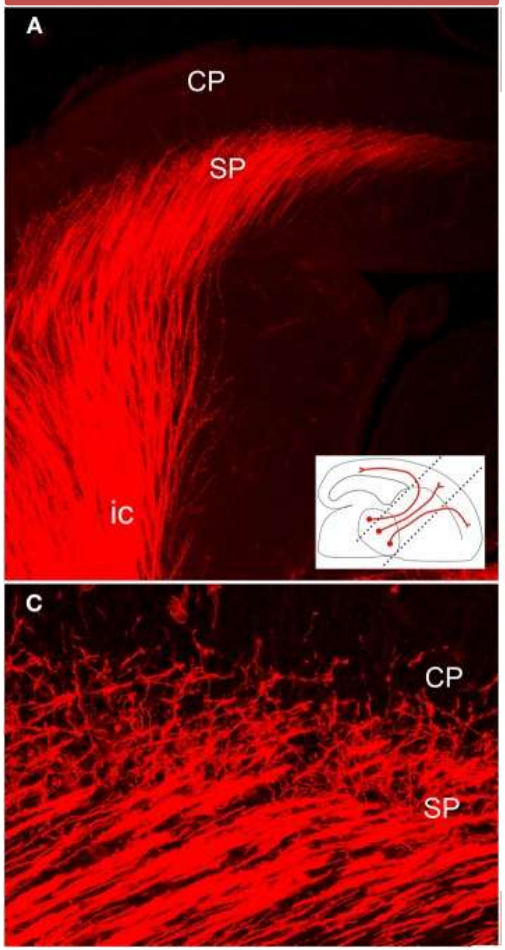


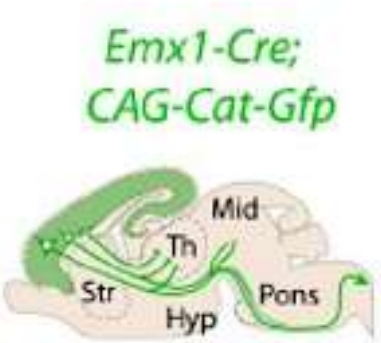
Figure 2: Directions of neuronal tracing: The tracer spreads as shown by the arrows, either away from the cell body (anterograde) or towards it (retrograde).

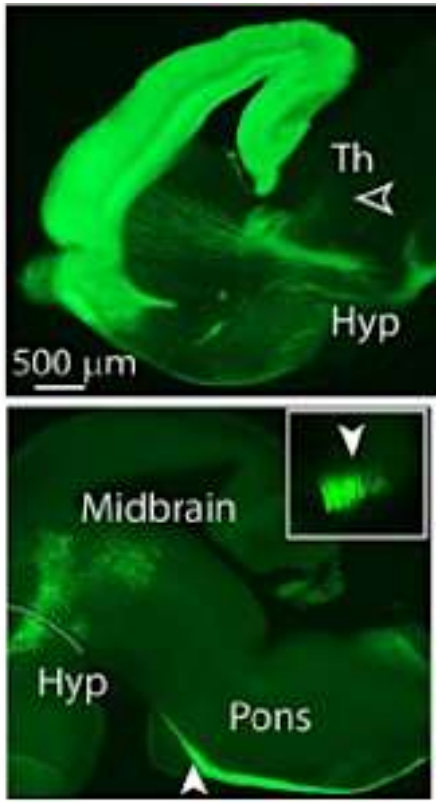
Visualizing neural circuitry and connection between different brain regions, whole brain imaging

DiI, DiD labeling	DiI labeling	Trangenic mice/GFP reporter









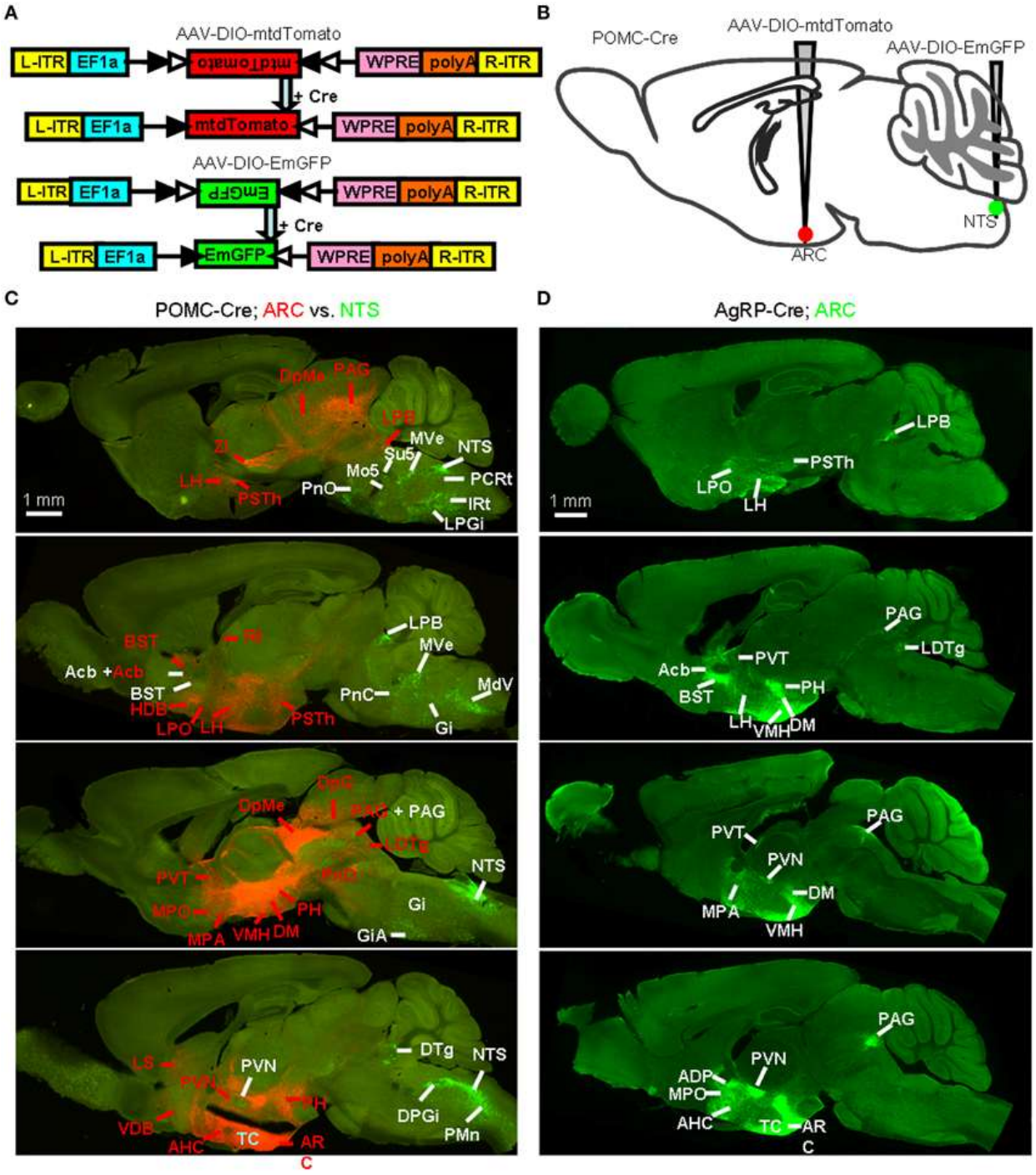
Kwan et al., 2008

Viral gene delivery: adenovirus, adeno-associated virus (AAV), lentivirus, herpes simplex virus (HSV), canine adenovirus (CAV)

Summary of viruses of interest to neuroscientists:

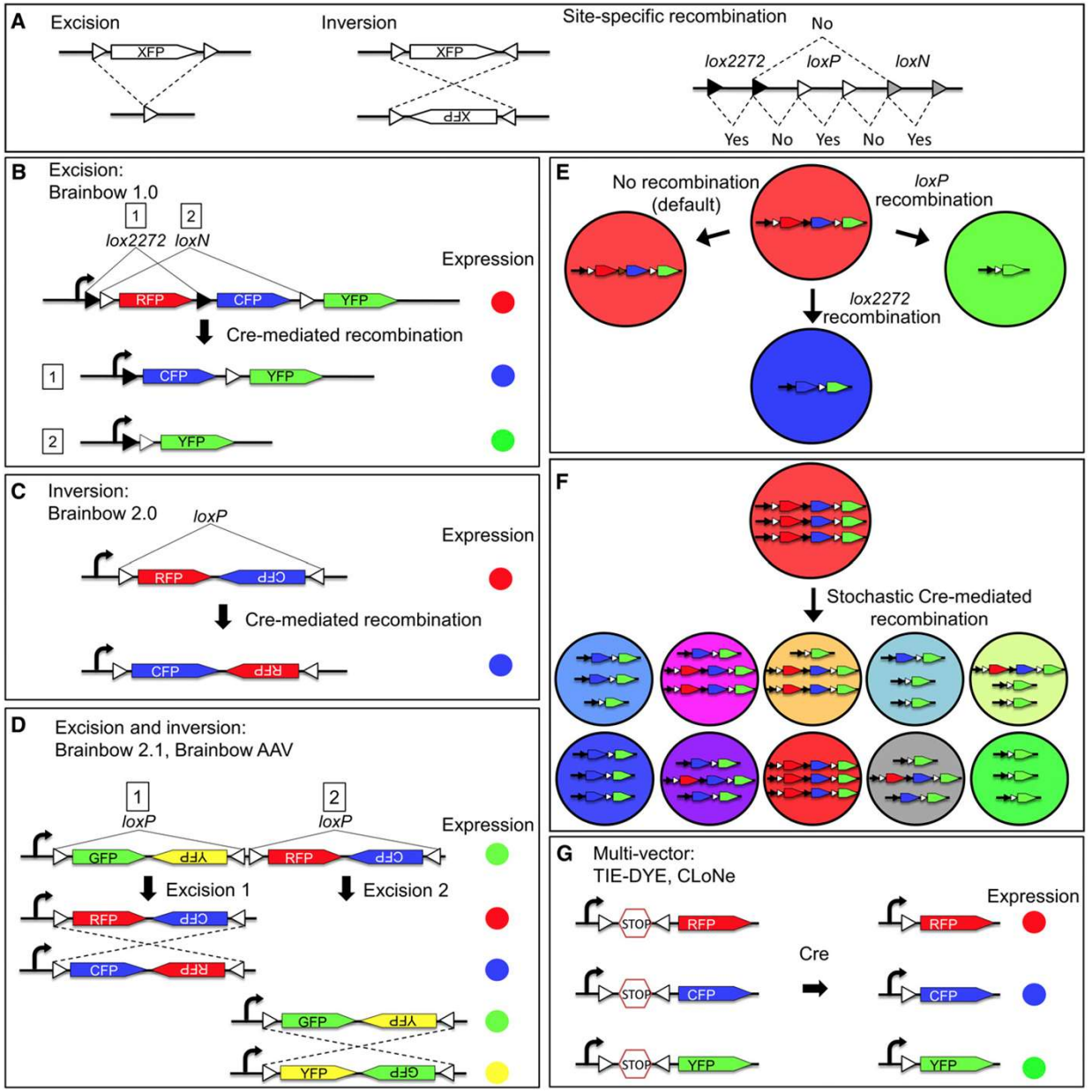
Family	Genome	Size	Titer	Characteristics	Bio-safety level
Retroviridae gamma-retrovirus lentivirus	ssRNA	9-12Kb	10^7-10^9 cfu/ml	Viral genome integrates into host genome Dividing cells, stable expression Non dividing cells, low expression	BSL1-2
Parvoviridae adeno-associated virus (AAV)	ssDNA	4.8Kb	10^{12}-10^{13} gc/ml	Do not integrate Stable expression, small genome size	BSL1
Adenoviridae Ad5 CAV	dsDNA	36Kb	10^{10}-10^{12} vg/ml	Do not integrate For rapid and transient expression, inflammation	BSL2
Herpesviridae Pseudorabies virus (PRV)	dsDNA	144 Kb	10^7-10^9 cfu/ml	Large and complex genomes Transynaptic tracing, gene transfer, rapid expression	BSL2
Rhabdoviridae Rabies virus (RABV)	ssRNA	12Kb	10^8-10^9 cfu/ml	Simple genome Genome can not recombined with CRE or FLP recombinases	BSL1-2

Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Neurotropic Viruses/AAV Viruses



Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Brainbow technology

Principles of brainbow labeling

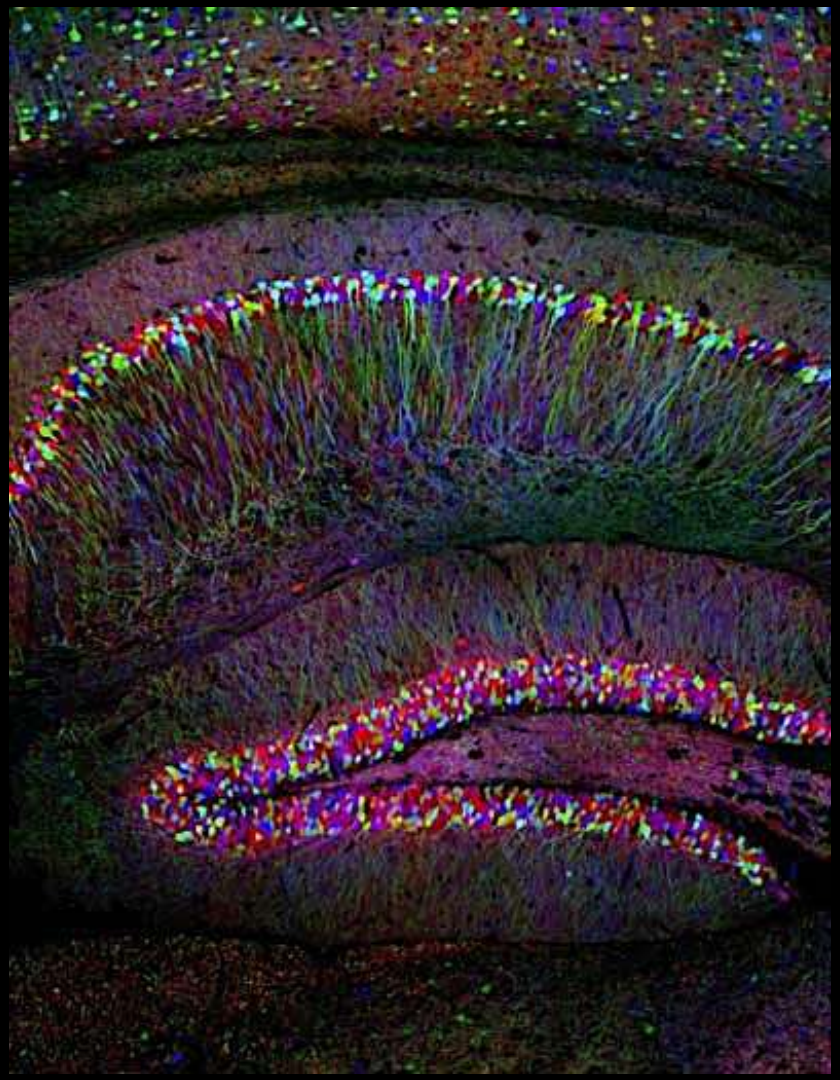


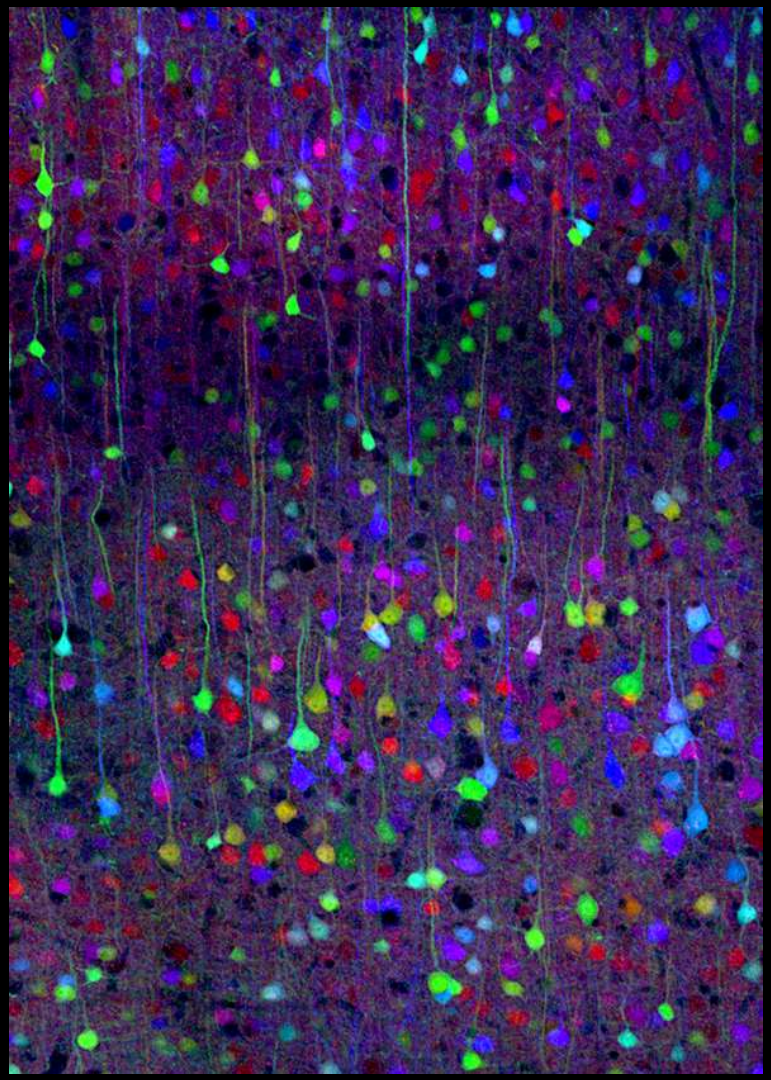
Weissman and Pan, 2015

Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Brainbow technology

Organism	Latin name	Promoter	Transgenic lines
Mouse	Mus musculus	Neuronal	Brainbow 1.0/1.1/2.0/2.1 (Livet et al. 2007),
			Brainbow 3.0/3.1a/3.2a, Flpbow 1/3a,
			Autobowb (Cai et al. 2013)
		Ubiquitous	R26-Confettib (Snippert et al. 2010)
			R26-Rainbow (Rinkevich et al. 2011)
			Rainbow (Tabansky et al. 2013)
			MAGICc (Loulier et al. 2014)
Zebrafish	Danio rerio	Gal4 inducible	Ubow (Ghigo et al. 2013) Brainbow (Robles et al. 2013)
			Zebrabow (Pan et al. 2013)
		Ubiquitous	PriZm (Gupta and Poss 2012)
Fruit fly	Drosophila melanogaster	Gal4 inducible	Zebrabow (Pan et al. 2013) dBrainbowb (Hampel et al. 2011)
			Flybow1.0/1.1/2.0a (Hadjiecono mou et al. 2011)

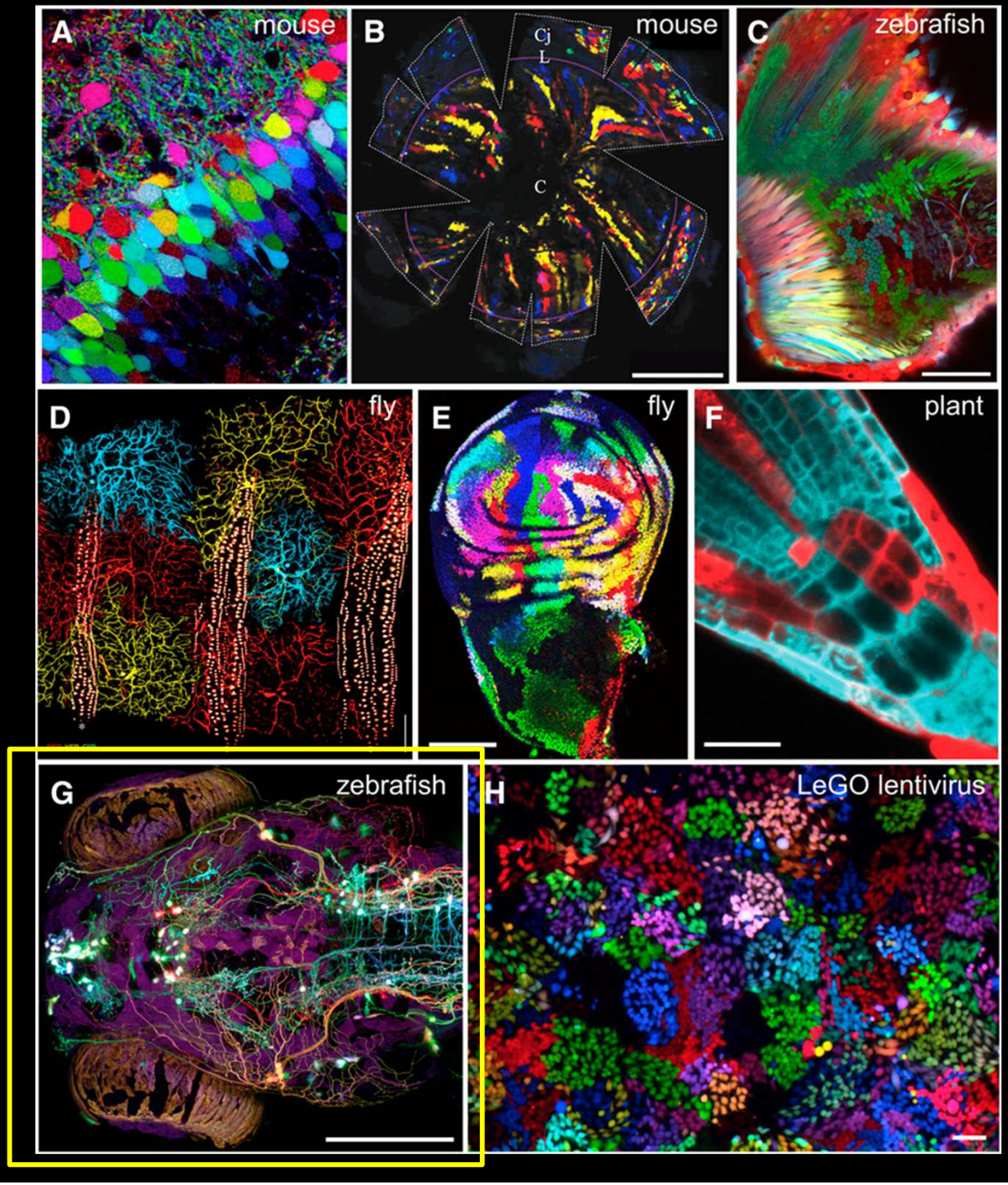
Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Brainbow technology





Livet et al., 2007

Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Brainbow technology



Weissman and Pan, 2015

Visualizing neural circuitry and connection between different brain regions, whole brain imaging

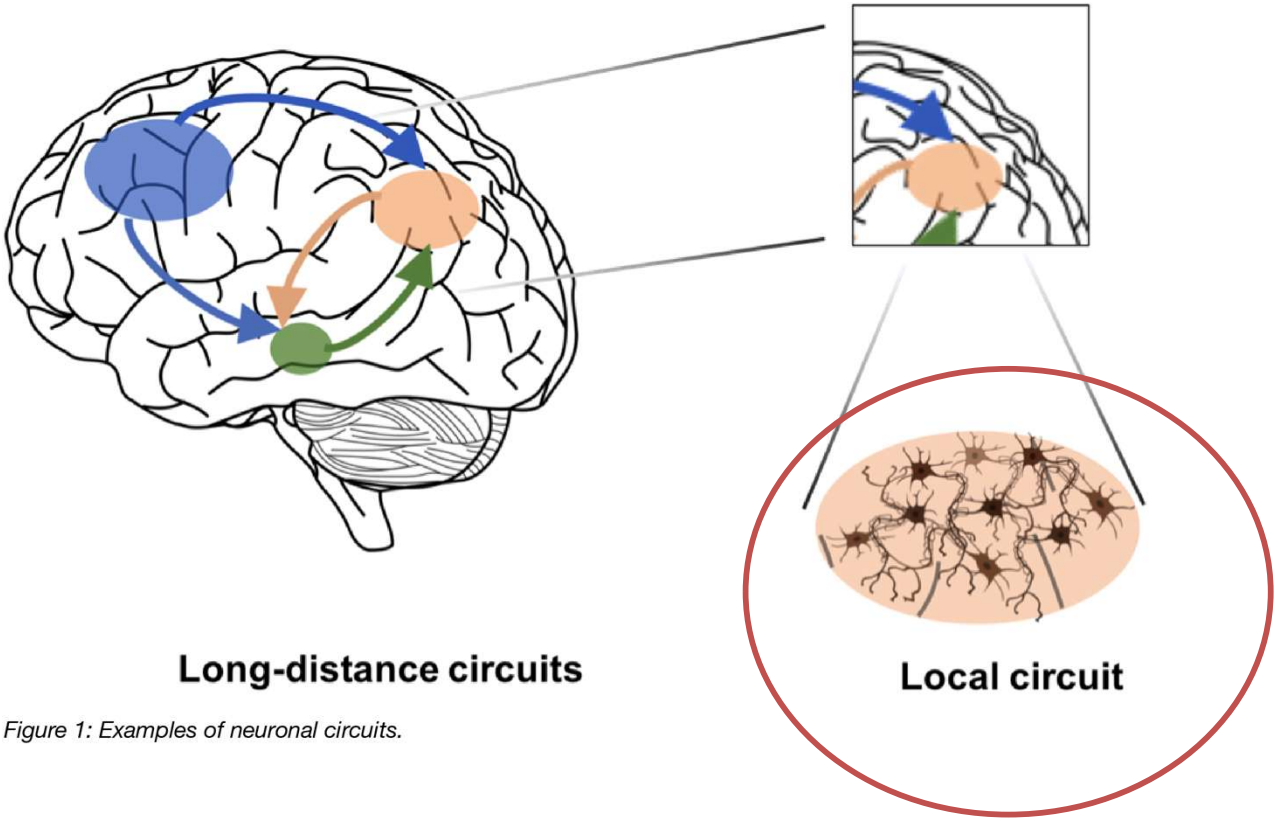


Figure 1: Examples of neuronal circuits.

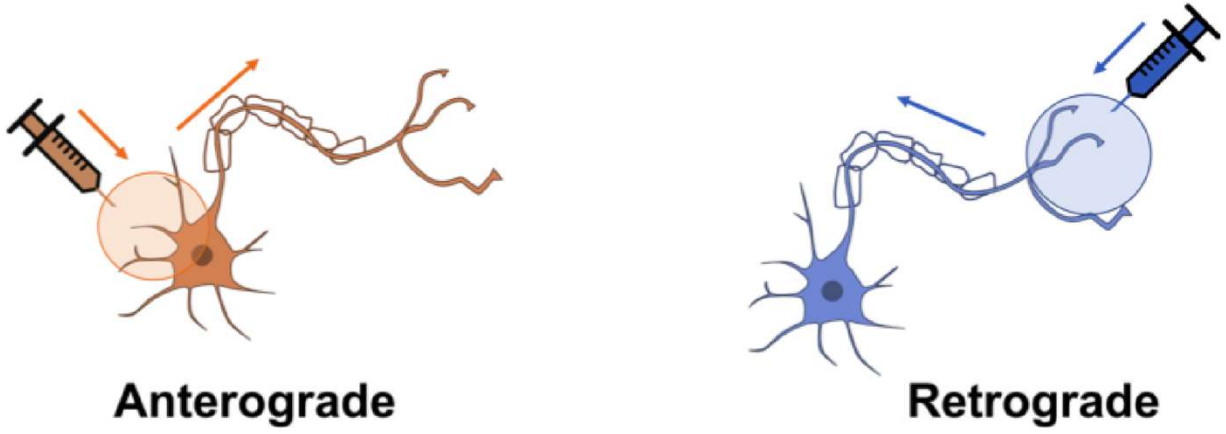


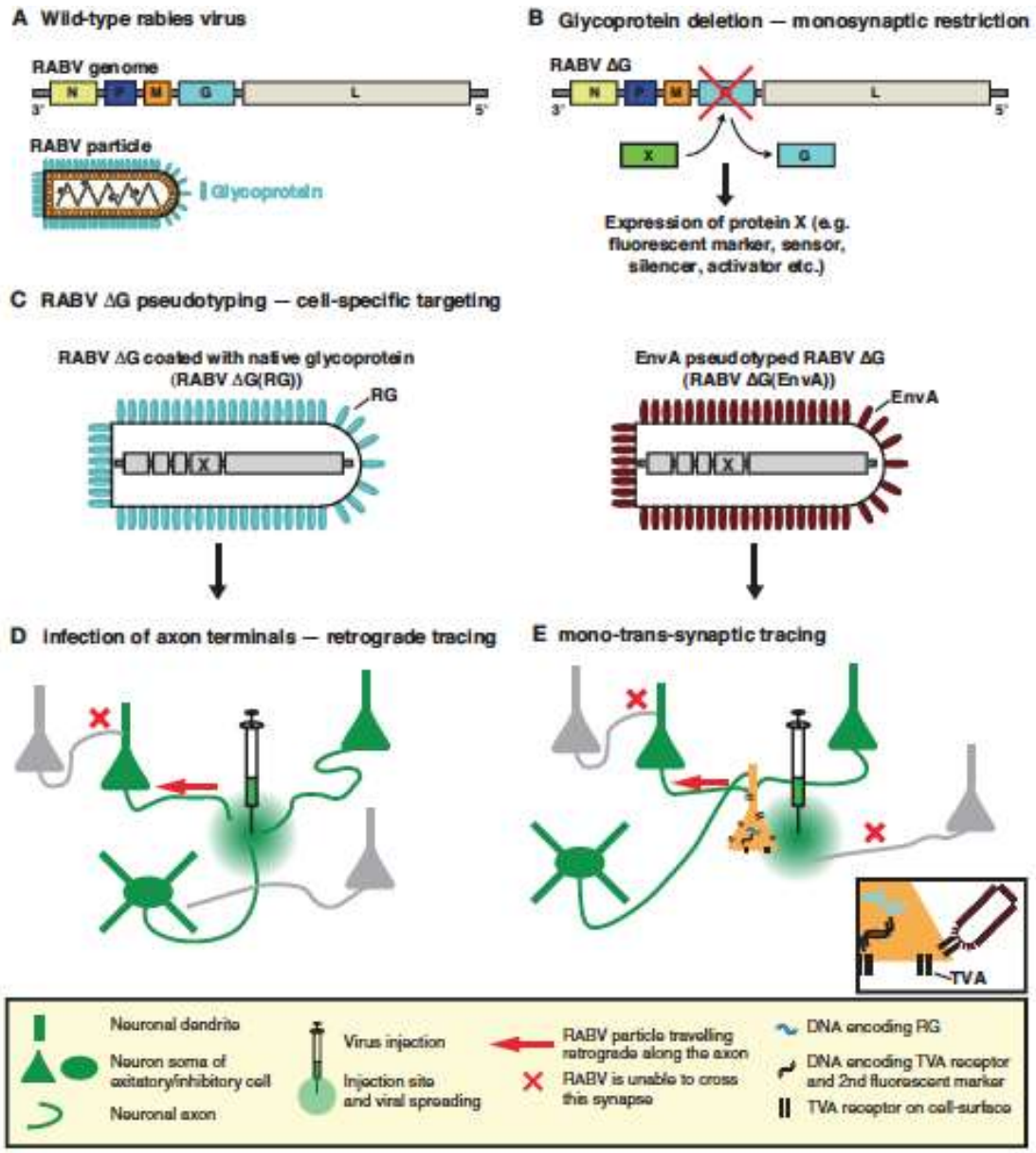
Figure 2: Directions of neuronal tracing: The tracer spreads as shown by the arrows, either away from the cell body (anterograde) or towards it (retrograde).

Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Neurotropic Viruses

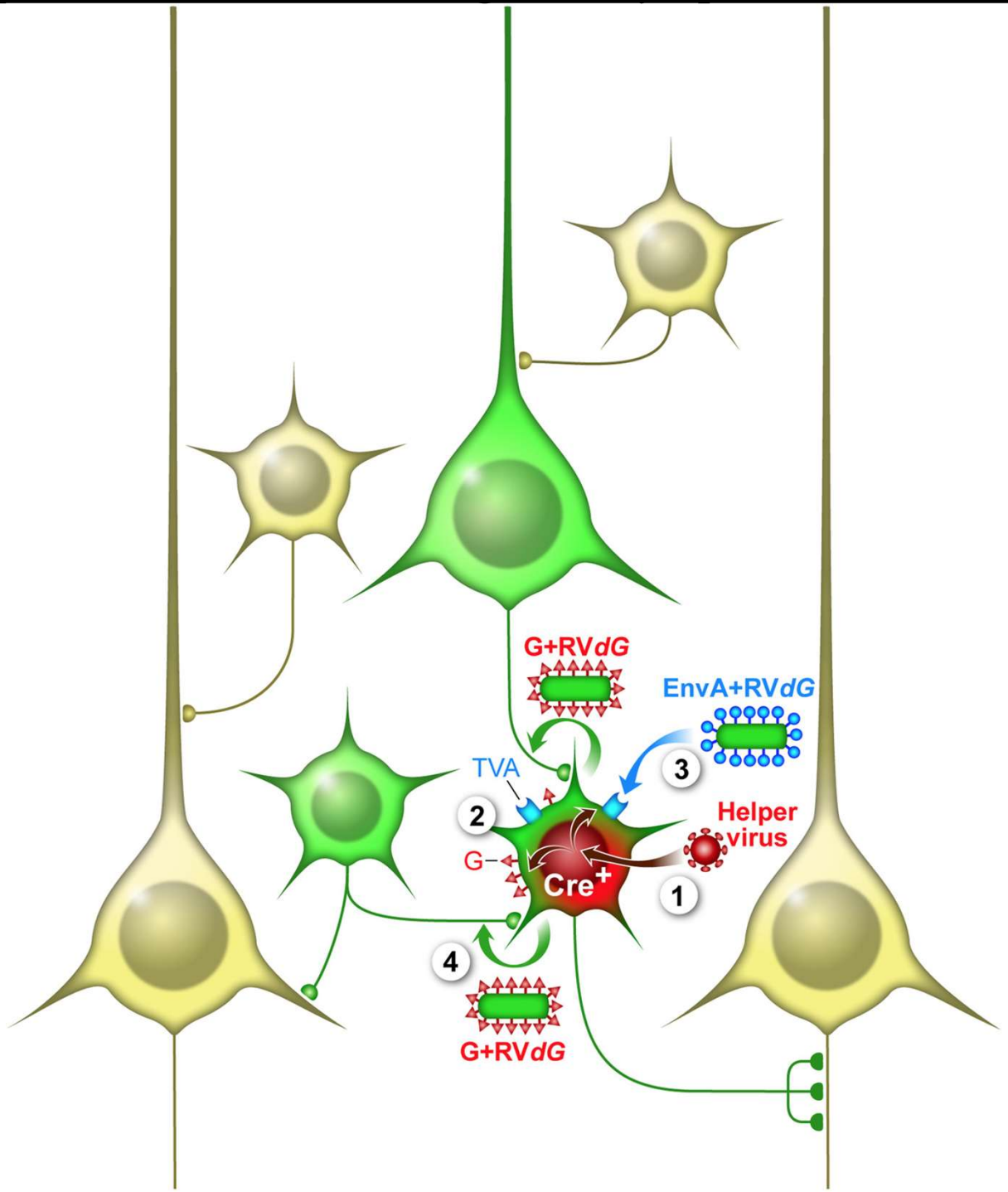
Summary of viruses of interest to neuroscientists:

Family	Genome	Size	Titer	Characteristics	Bio-safety level
Retroviridae gamma-retrovirus lentivirus	ssRNA	9-12Kb	10^7-10^9 cfu/ml	Viral genome integrates into host genome Dividing cells, stable expression Non dividing cells, low expression	BSL1-2
Parvoviridae adeno-associated virus (AAV)	ssDNA	4.8Kb	10^{12}-10^{13} gc/ml	Do not integrate Stable expression, small genome size	BSL1
Adenoviridae Ad5 CAV	dsDNA	36Kb	10^{10}-10^{12} vg/ml	Do not integrate For rapid and transient expression, inflammation	BSL2
Herpesviridae Pseudorabies virus (PRV)	dsDNA	144 Kb	10^7-10^9 cfu/ml	Large and complex genomes Transynaptic tracing, gene transfer, rapid expression	BSL2
Rhabdoviridae Rabies virus (RABV)	ssRNA	12Kb	10^8-10^9 cfu/ml	Simple genome Genome can not recombined with CRE or FLP recombinases	BSL1-2

Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Neurotropic Viruses/ Circuit Tracing with Glycoprotein-Deleted Rabies Viruses

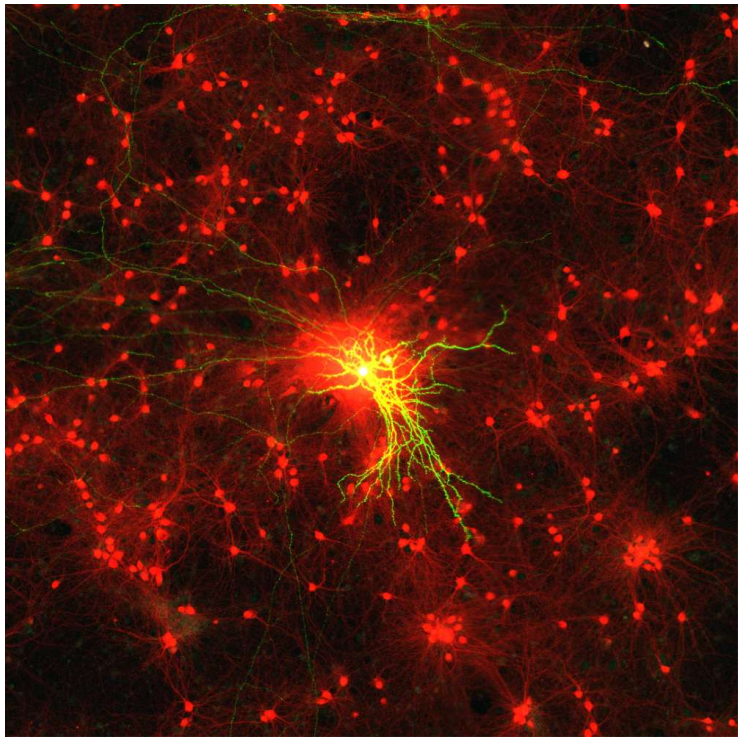


Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Neurotropic Viruses/ Circuit Tracing with Glycoprotein-Deleted Rabies Viruses

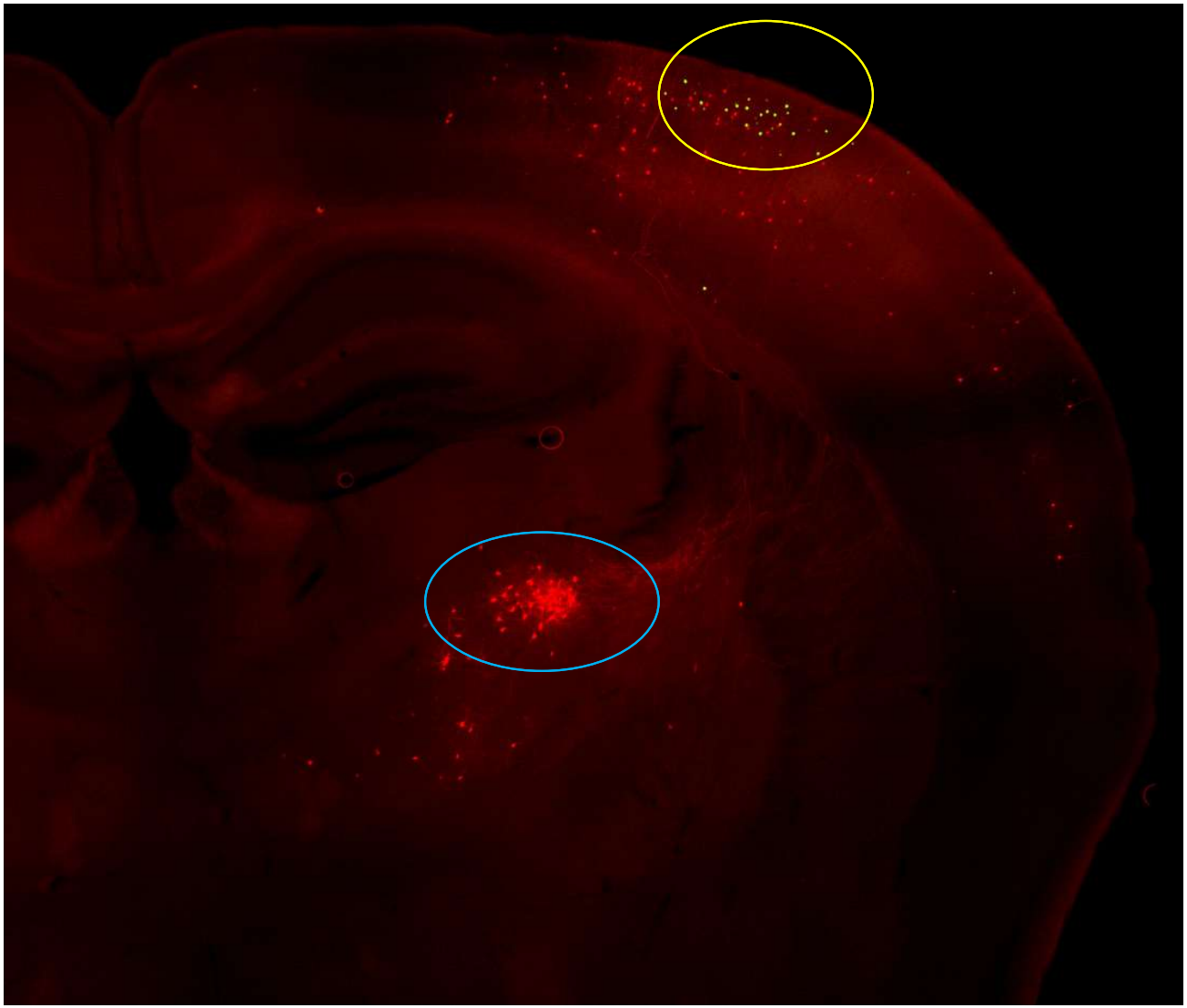


Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Neurotropic Viruses/Circuit Tracing with Glycoprotein-Deleted Rabies Viruses

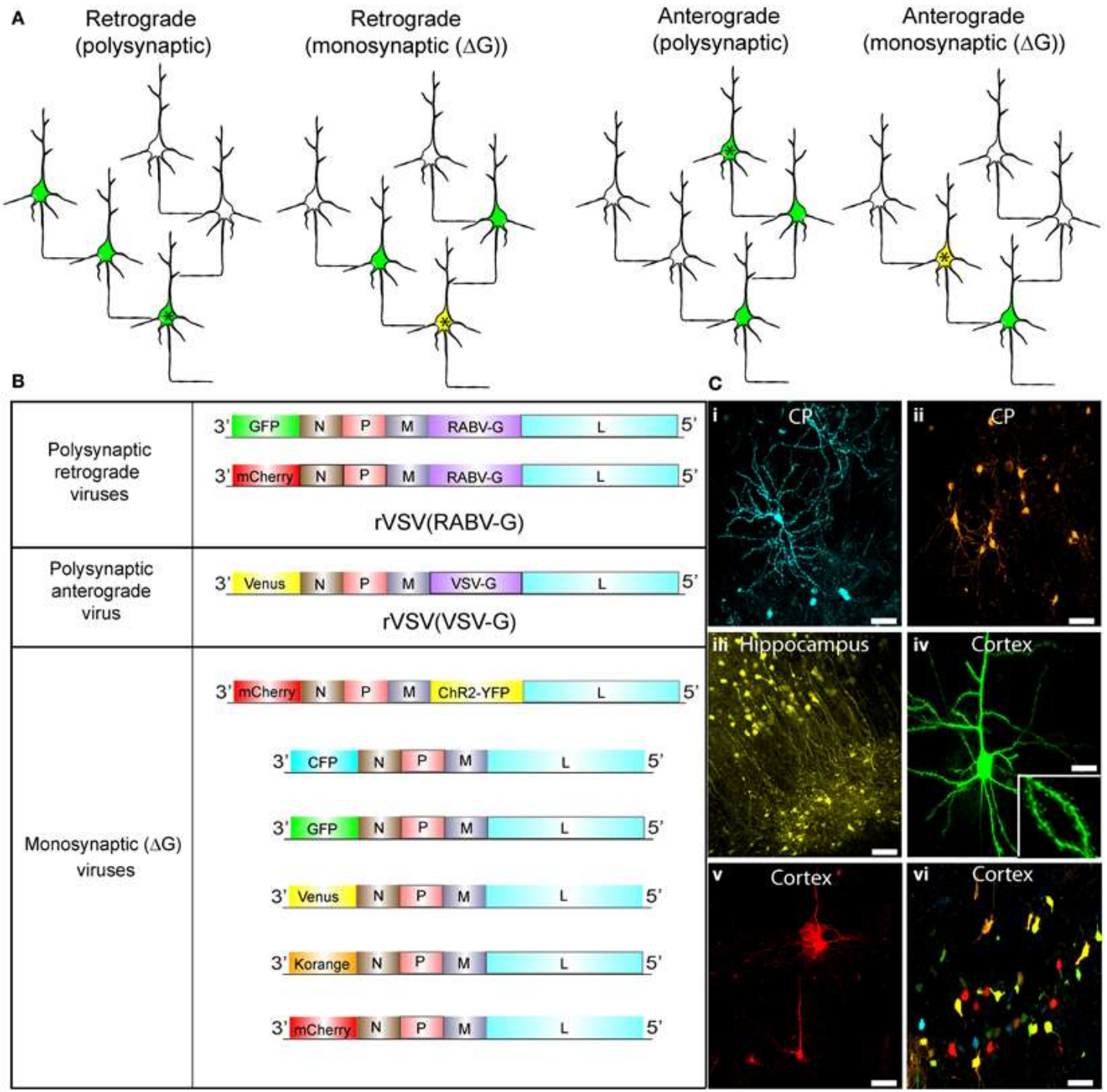
Infected neuronal culture



In vivo tracing/ In utero cortical electroporation



Unpublished data from Polleux lab

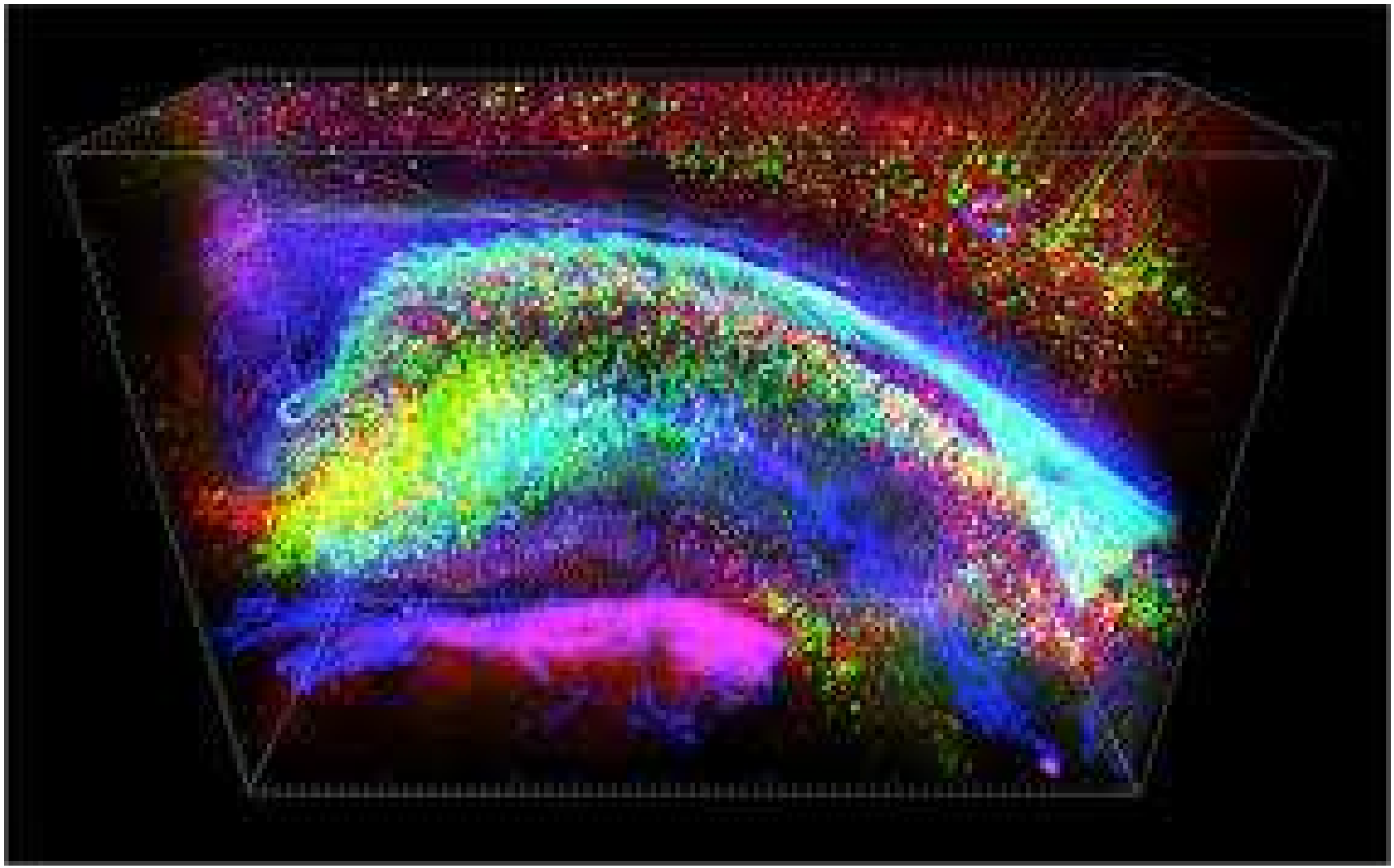


Beier et al., 2013

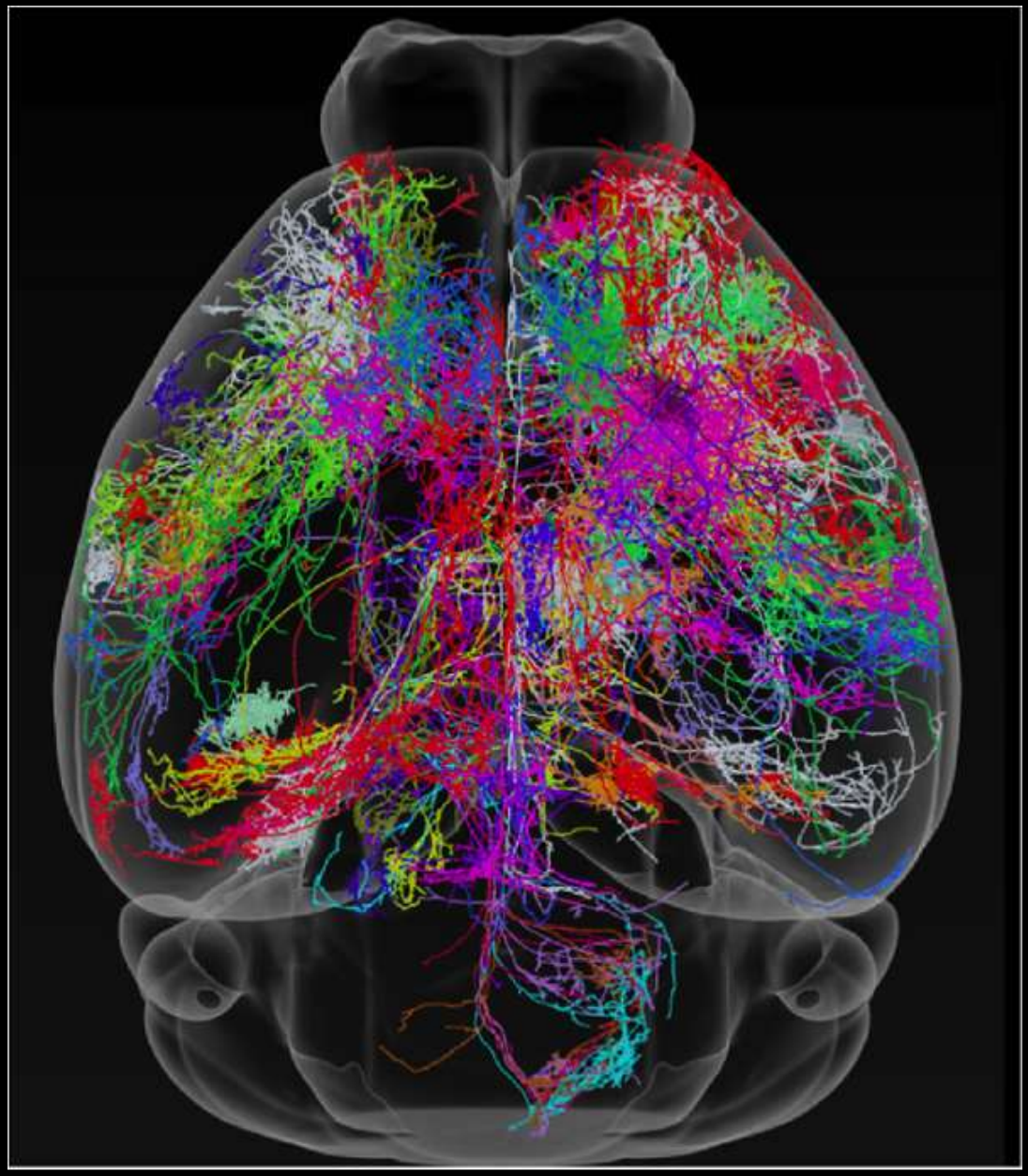
Visualizing neural circuitry and connection between different brain regions, whole brain imaging: Tissue Clearing/3D Imaging

Tissue Clearing Methods:

- ***ScaleA2*** **(Hama et al., 2011)**
- ***3DISCO*** **(Erturk et al., 2012a, 2012b, 2014)**
- ***ClearT2*** **(Kuwajima et al., 2013)**
- ***SeeDB*** **(Ke et al., 2013)**
- ***CLARITY*** **(Chung et al., 2013, Yang et al., 2014)**
- ***CUBIC*** **(Susaki et al., 2014)**
- ***iDISCO*** **(Renier et al., 2014)**
- ***ACT-PRESTO*** **(Lee et al. 2016)**



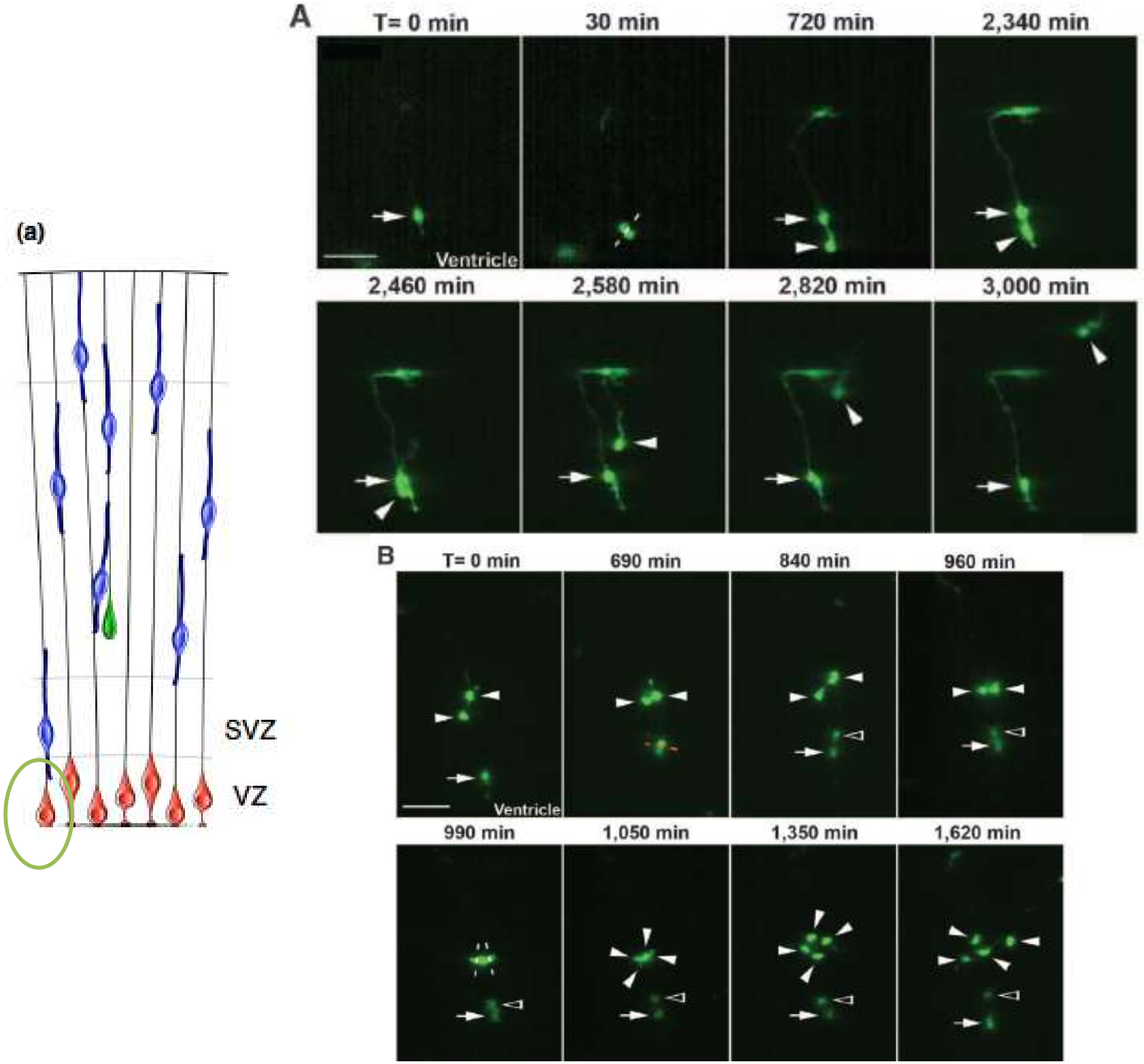
- http://www.chunglabresources.com/cl1/



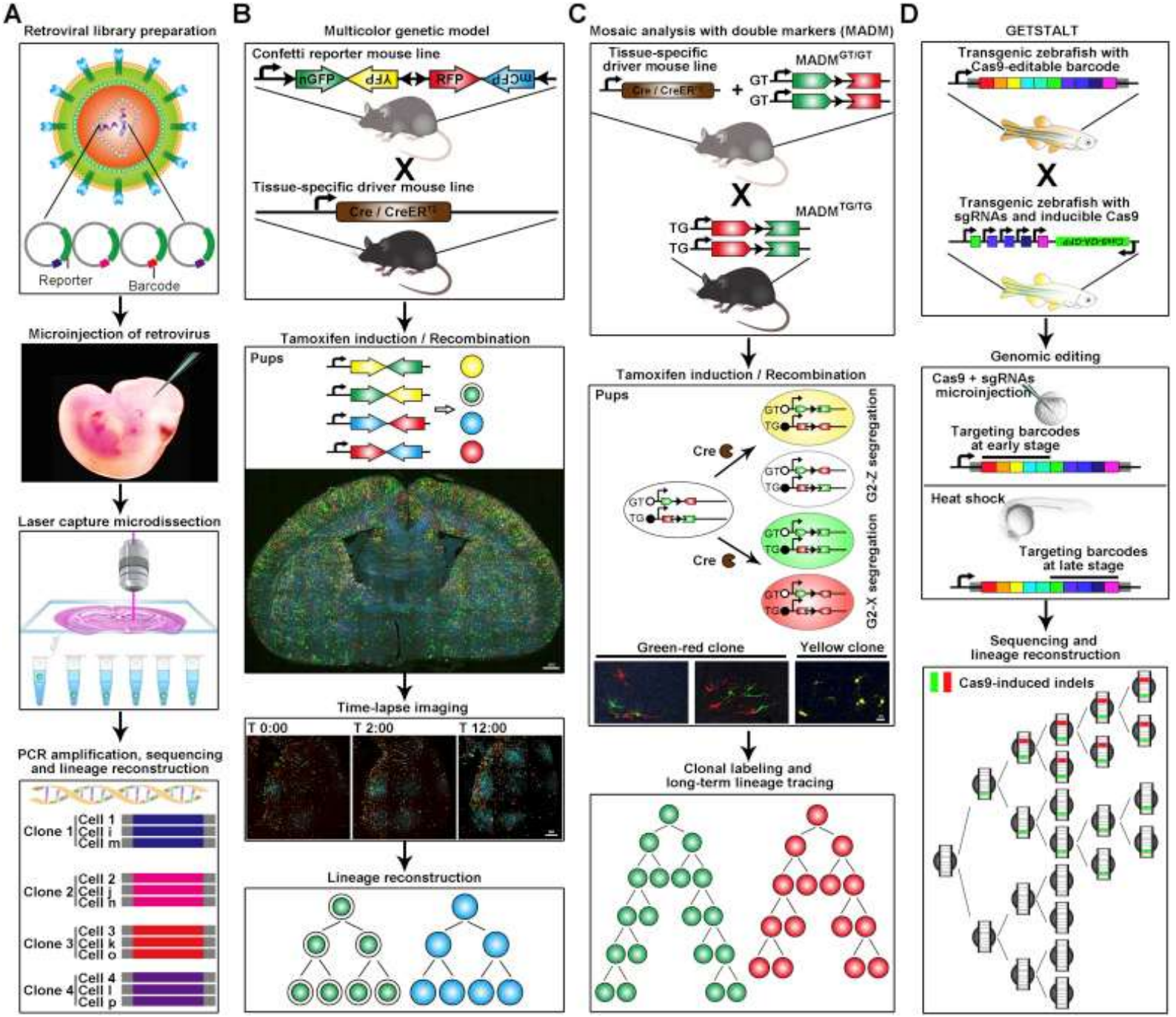
Viral gene delivery: adenovirus, adeno-associated virus (AAV), lentivirus, herpes simplex virus (HSV), canine adenovirus (CAV)

Summary of viruses of interest to neuroscientists:

Family	Genome	Size	Titer	Characteristics	Bio-safety level
Retroviridae gamma-retrovirus lentivirus	ssRNA	9-12Kb	10^7-10^9 cfu/ml	Viral genome integrates into host genome Dividing cells, stable expression Non dividing cells, low expression	BSL1-2
Parvoviridae adeno-associated virus (AAV)	ssDNA	4.8Kb	10^{12}-10^{13} gc/ml	Do not integrate Stable expression, small genome size	BSL1
Adenoviridae Ad5 CAV	dsDNA	36Kb	10^{10}-10^{12} vg/ml	Do not integrate For rapid and transient expression, inflammation	BSL2
Herpesviridae Pseudorabies virus (PRV)	dsDNA	144 Kb	10^7-10^9 cfu/ml	Large and complex genomes Transynaptic tracing, gene transfer, rapid expression	BSL2
Rhabdoviridae Rabies virus (RABV)	ssRNA	12Kb	10^8-10^9 cfu/ml	Simple genome Genome can not recombined with CRE or FLP recombinases	BSL1-2


A
T= 0 min
30 min
720 min
2,340 min
Ventricle
2,460 min
2,580 min
2,820 min
3,000 min
(a)
SVZ
VZ
B
T= 0 min
690 min
840 min
960 min
Ventricle
990 min
1,050 min
1,350 min
1,620 min

Multiplexed strategies for lineage tracing and clonal analysis.



doi.org/10.1016/j.cr.2018.09.001

Questions

Manipulating Neural Activity:

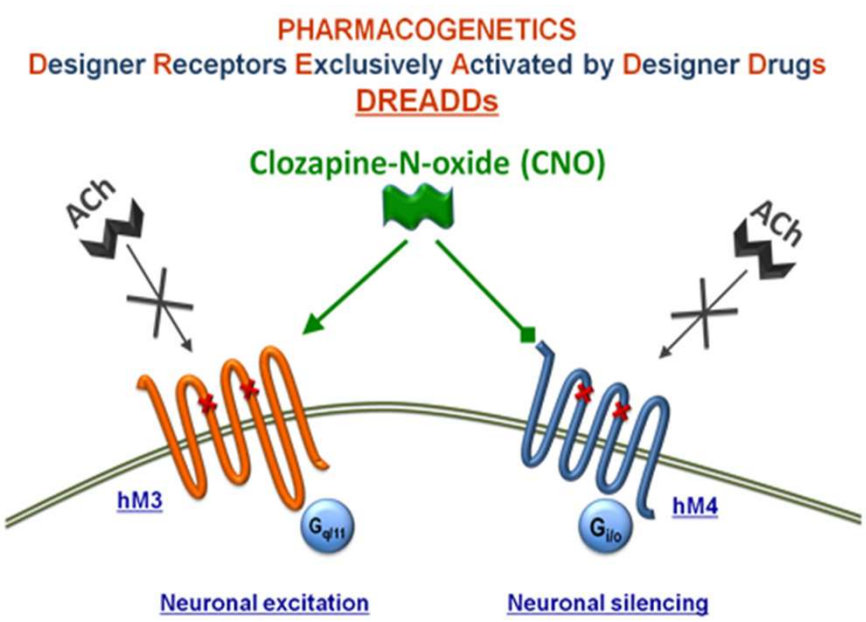
Approaches to artificially inducing or suppressing electrical activity in neurons in order to study neuron function at the cellular or the network level.

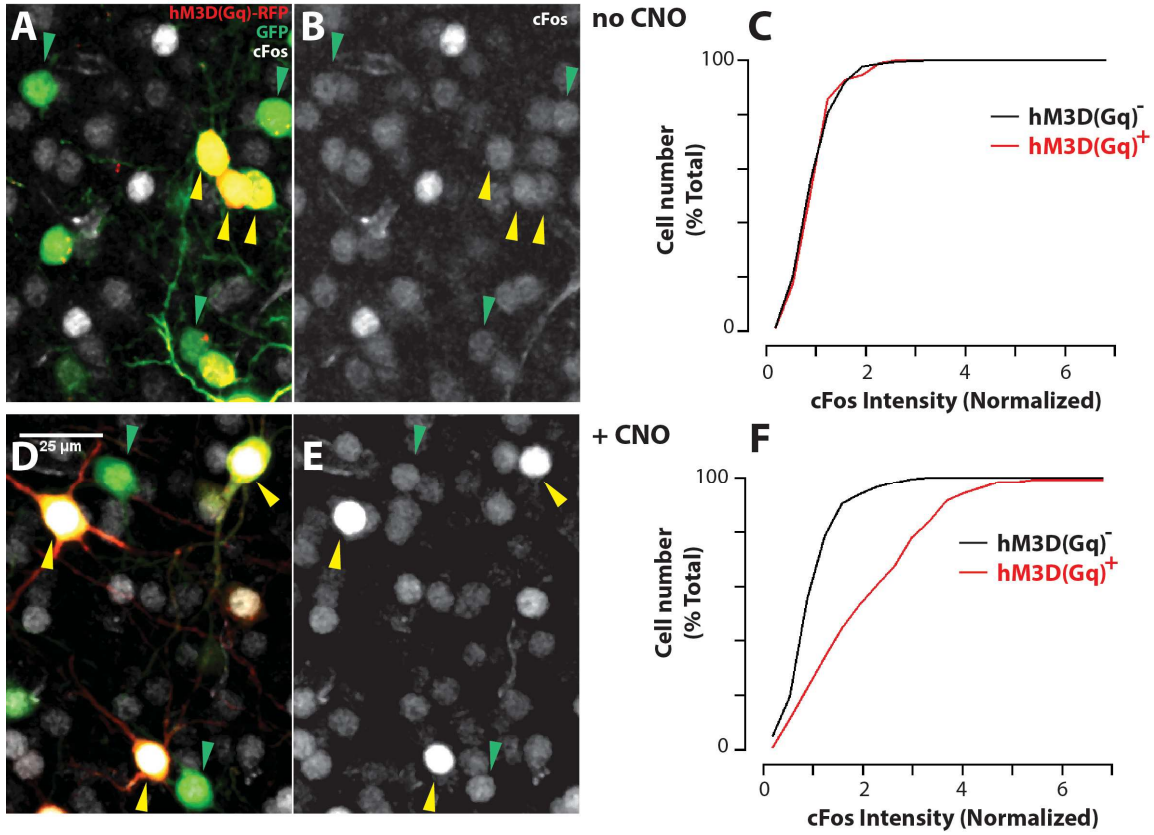
- **Pharmacological maniPhysical manipulation:** ablation, thermal cooling
- **Electrical manipulation:** microstimulation, electrolytic lesions
- **pulation:** agonists, antagonists, drug delivery methods
- **Genetic manipulation:** perturbation of endogenous genes, expression of genes to ablate neurons

- **Pharmacogenetics:** designer receptors exclusively activated by designer drugs (DREADDs: hM3Dq, hM4Di)
- **Optogenetics:** channelrhodopsin-2 (ChR2), halorhodopsin (NpHR), archaerhodopsin (Arch), light delivery methods

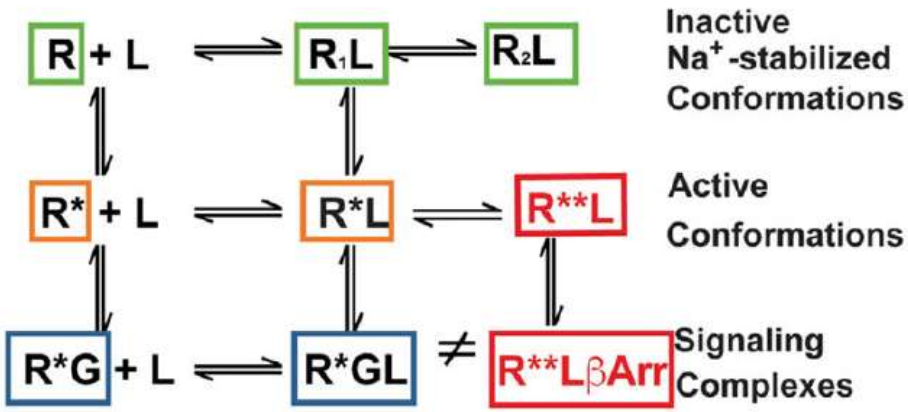
Manipulating Neural Activity:

Pharmacogenetics: designer receptors exclusively activated by designer drugs (DREADDs: hM3Dq, hM4Di)





R=Receptor; L=Ligand; G=G protein; βArr=βArrestin



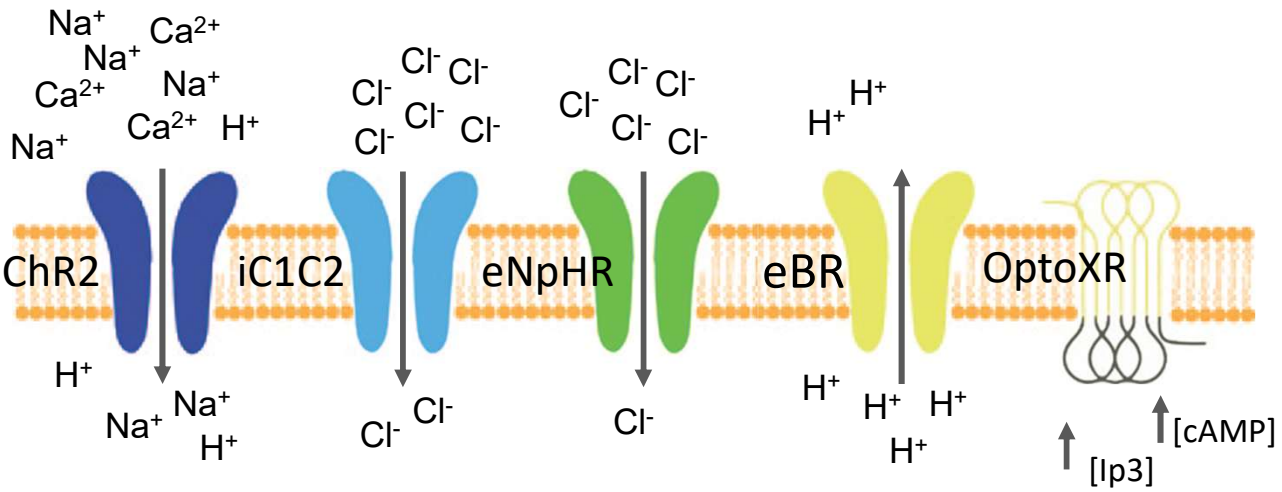
G protein signaling

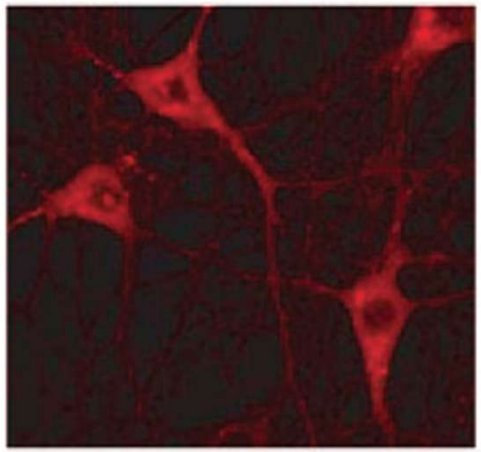
↑ Ca++, ↑cAMP, ↑GIRK
Gq Gs Gi

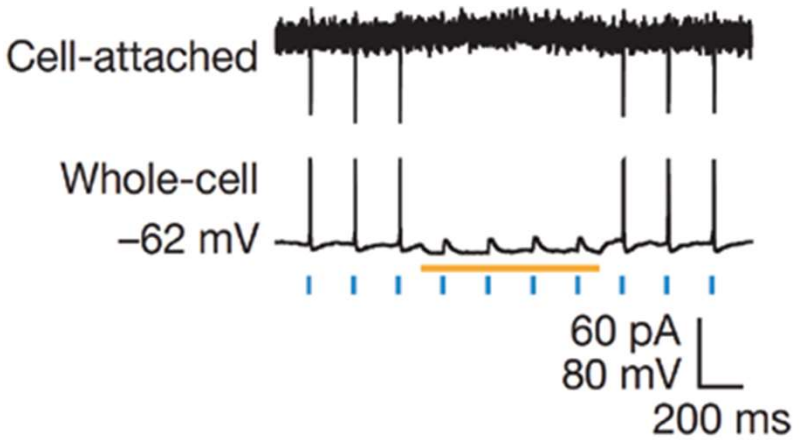
β-Arrestin signaling
↑pERK, transcription

Manipulating Neural Activity:

Optogenetics: channelrhodopsin-2 (ChR2), halorhodopsin (NpHR)







Guru et al., 2015

http://www.nature.com/collections/tqxhytcpwh/video

Questions