

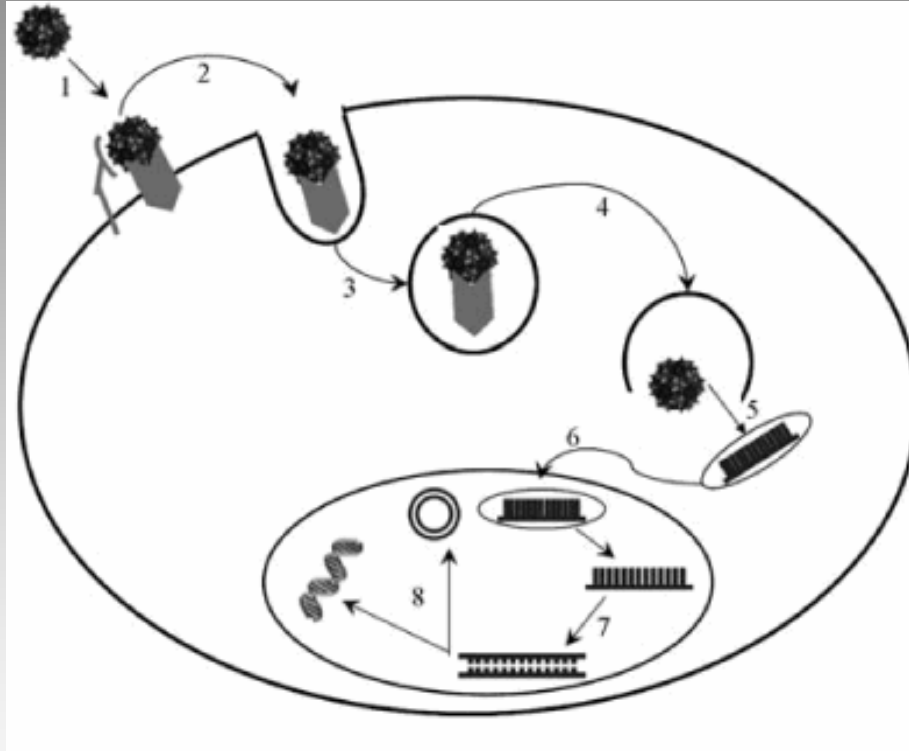
# Adeno-associated viral vectors and stereotaxic delivery

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# Adeno-Associated Virus

- Parvovirus family
- Linear ssDNA of either polarity
- Infects dividing and non-dividing cells
- Persists in episomal state in host nucleus
- Can infect large number of cell types
- ~4.7 kb wild type genome

# AAV Life Cycle



# Packaging Components of rAAV

1. Transfer vector: Inverted Terminal Repeats (ITR) flanking gene of interest
2. AAV helper plasmid expressing rep and cap proteins
3. Adenovirus helper plasmid expresses adenoviral proteins necessary for packaging



# AAV as a Viral Vector

## Strengths

- Low immune response
- Infects dividing and non-dividing cells
- Non-integrating
- Persistent expression
- Serotypes differentially infect cell types
- Various routes of delivery
- High titers

## Weaknesses

- Not ideal for dividing cells, DNA is lost through cell division
- Small genome allowing gene cassette of ~4.4kb
- Serotypes differentially infect cell types



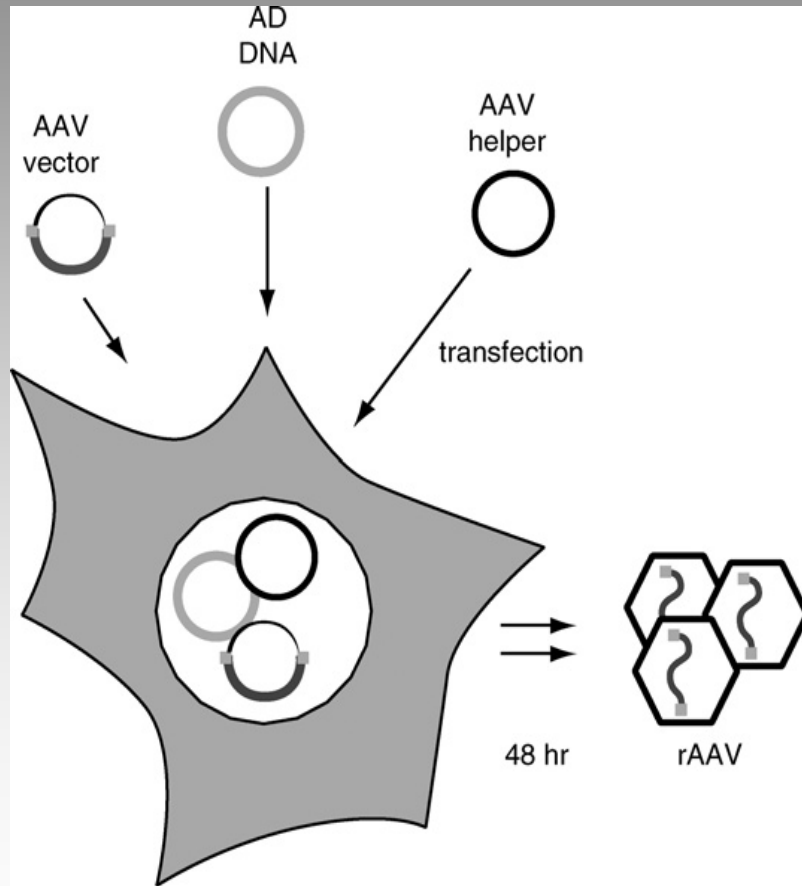
# AAV Serotypes

- AAV is subdivided into serotypes which exhibit different tissue tropism
- Serotypes are not tissue specific because they can transduce many different cell types
- Serotypes have higher affinities for certain cell types due to differences in capsid proteins

# AAV Serotypes Target Different Tissues

Tissue	Optimal Serotypes
Liver	AAV8, AAV9, AAVDJ
Skeletal Muscle	AAV1, AAV6-9
CNS	AAV1, AAV2, AAV5, AAV8, AAV9, AAVDJ
Photoreceptor Cells	AAV5
Lung	AAV9
Heart	AAV8
Pancreas	AAV8
Kidneys	AAV2

# rAAV Production



- Triple transfection
- Wait 48-72 hours
- Lyse cells by freeze/thaw or sonication
- CsCl gradient, iodixanol gradient or heparin column
- Dialyze fractions
- Titer by Taqman qPCR
- Titers  $>10^{12}$  vg/ml



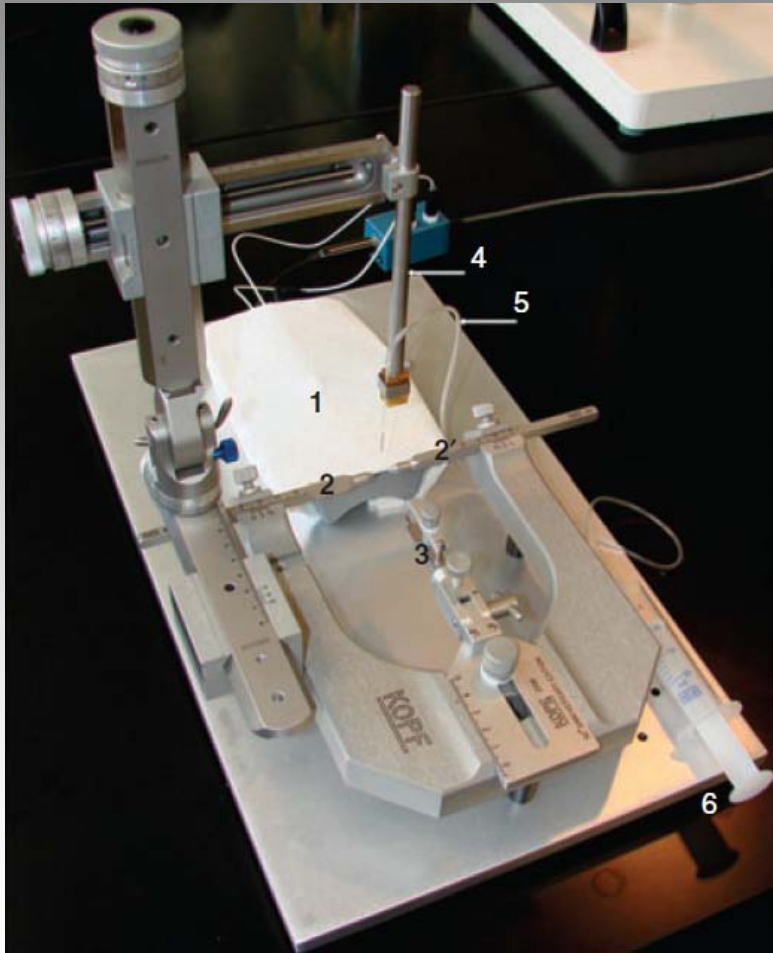
# Stereotaxic Delivery

- Good spatiotemporal control
- Any brain region or subpopulation of cells
- Inject at any postnatal date

## Common Uses

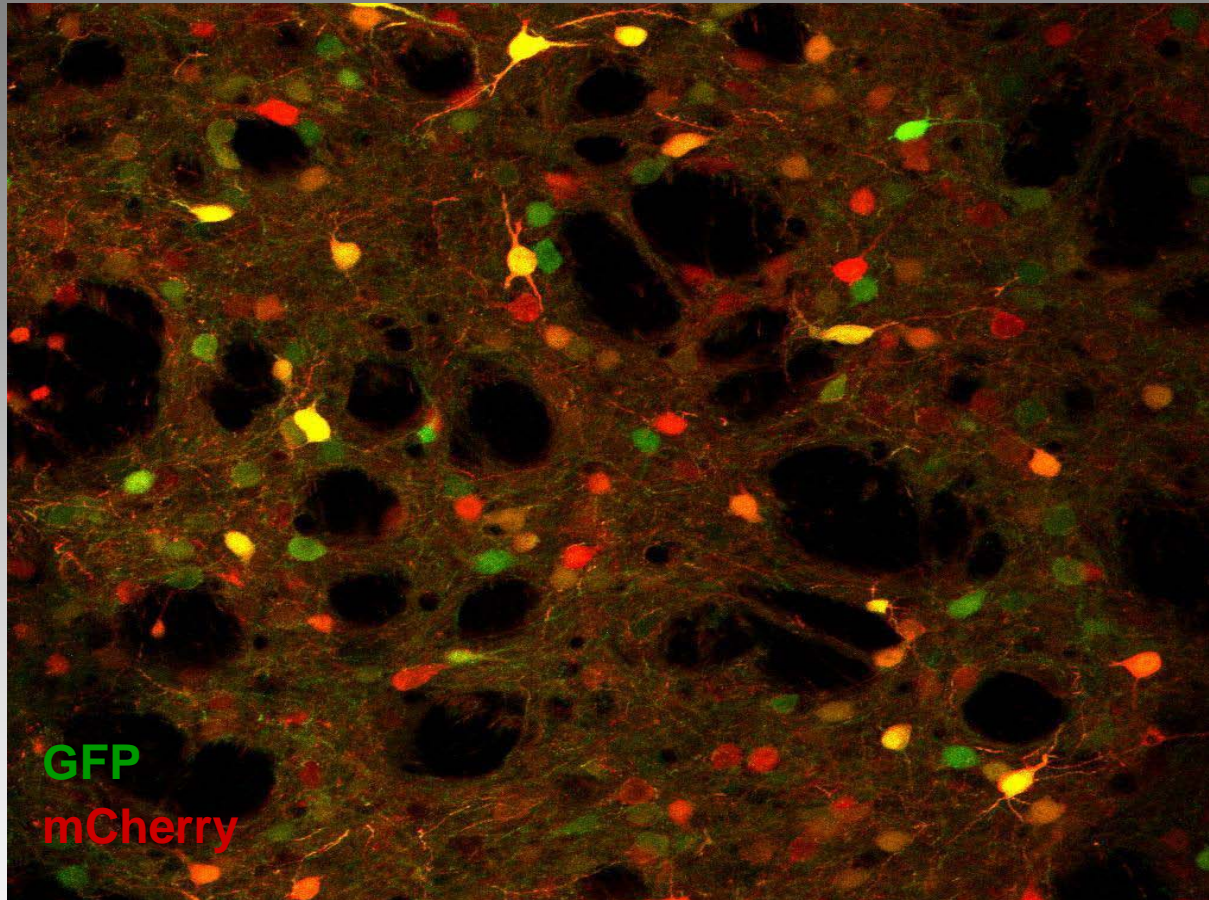
- Fluorescent labeling of cell populations
- Neuronal track tracing
- Viral mediated gene knockdown or over-expression
- Cell specific targeting using cre transgenic mice

# Stereotaxic injection protocol



- Anesthetize animal
- Secure rodent to stereotax, continue anesthesia
- Make incision and locate bregma
- Drill hole in skull at desired coordinate
- Load syringe with viral suspension
- Lower needle to proper coordinate
- Infuse viral particles at .1-.5 ul/min

# Intracerebral injection for protein over-expression



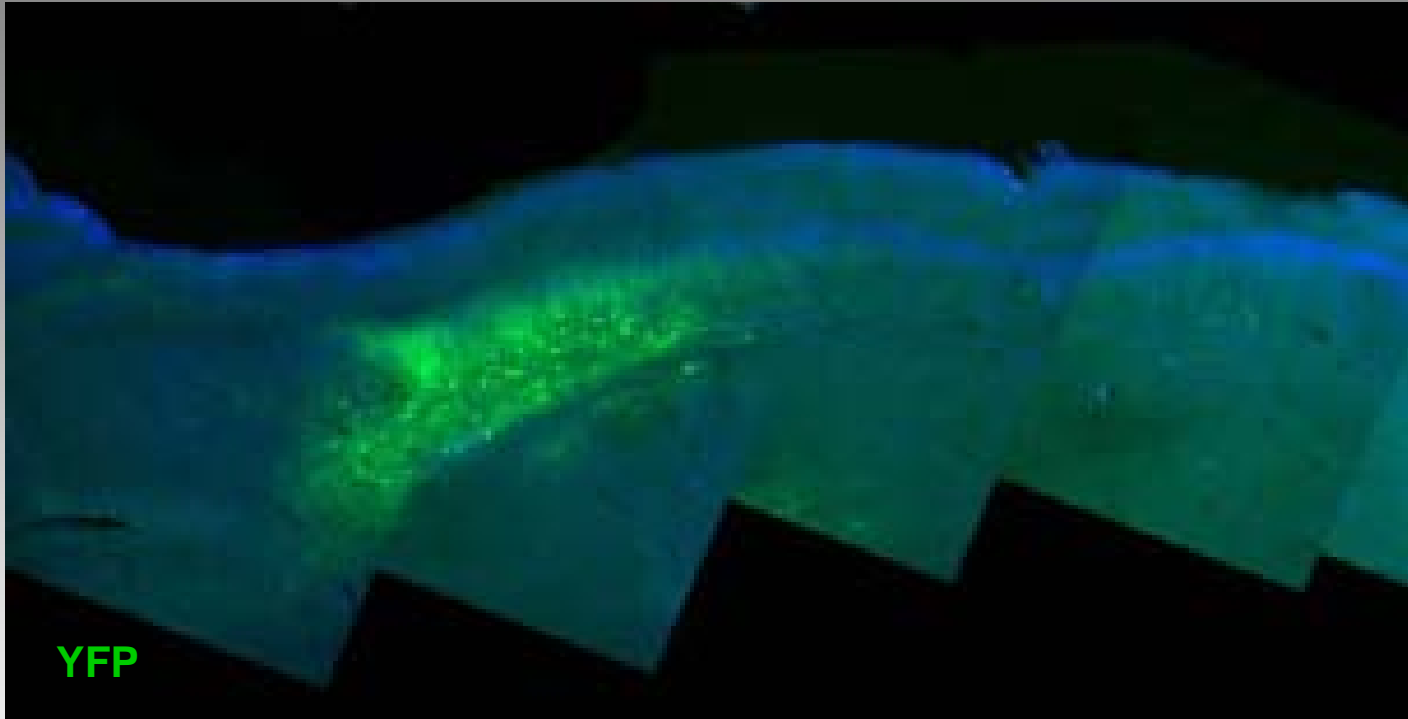
Serotype: AAV9

Protein Expressed: Girik1-2a-mCherry and Girik2-2a-GFP

Amount injected:  $10^9$  vg

Manufacturer: VVC

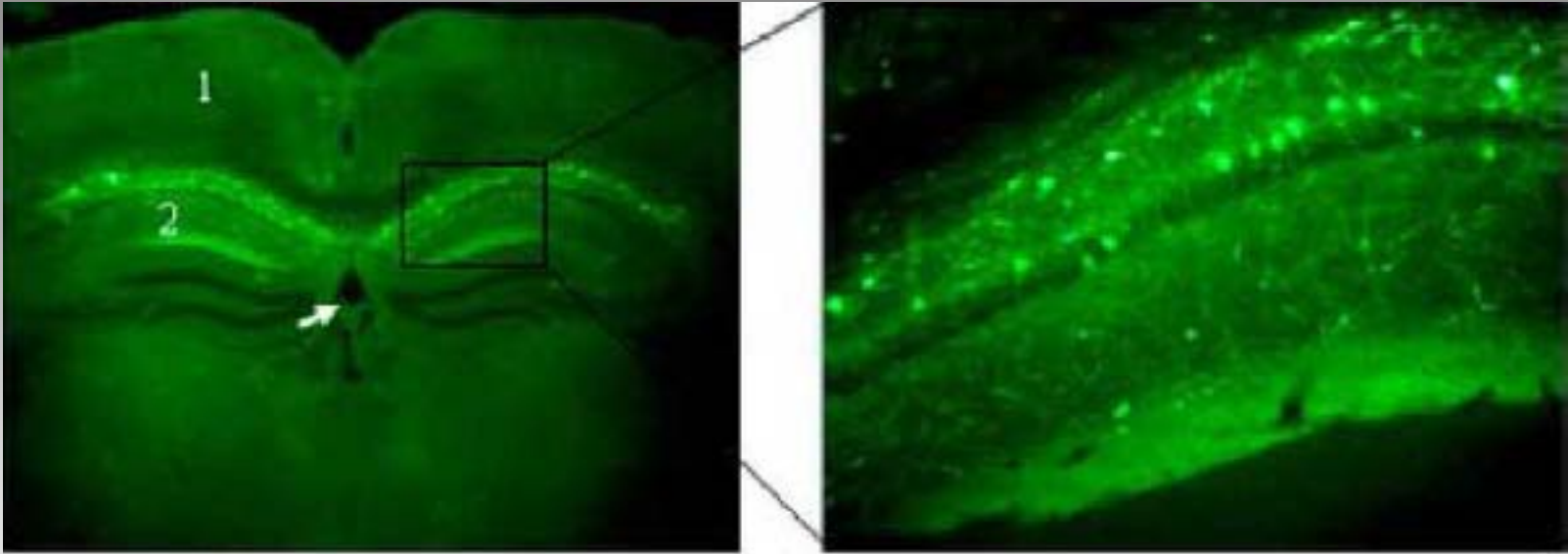
# Cell specific targeting using cre transgenic



Serotype: AAVDJ  
Protein expressed: ArchT-YFP  
Amount injected:  $10^9$  vg  
Manufacturer: VVC

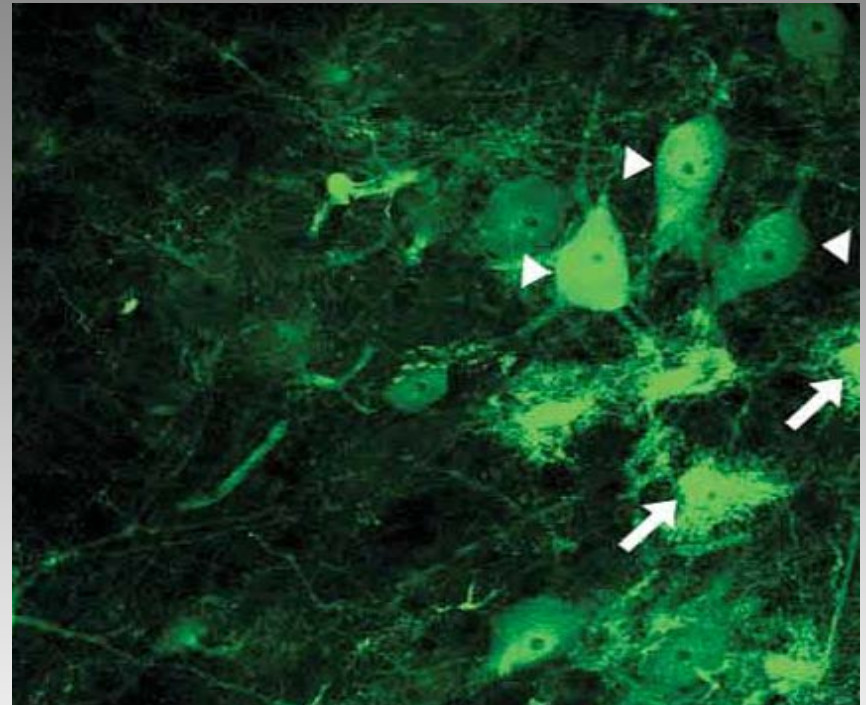
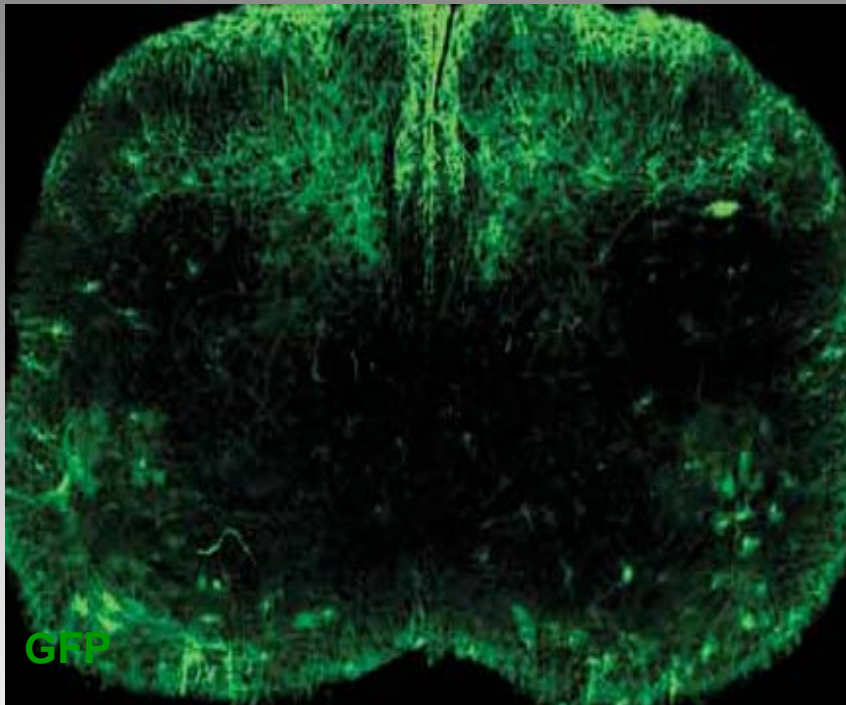
McGinley et al, unpublished

# Intraventricular Injection



Serotype: AAV2  
Protein Expressed: GFP  
Amount injected:  $10^8$  vg  
Manufacturer: UNC

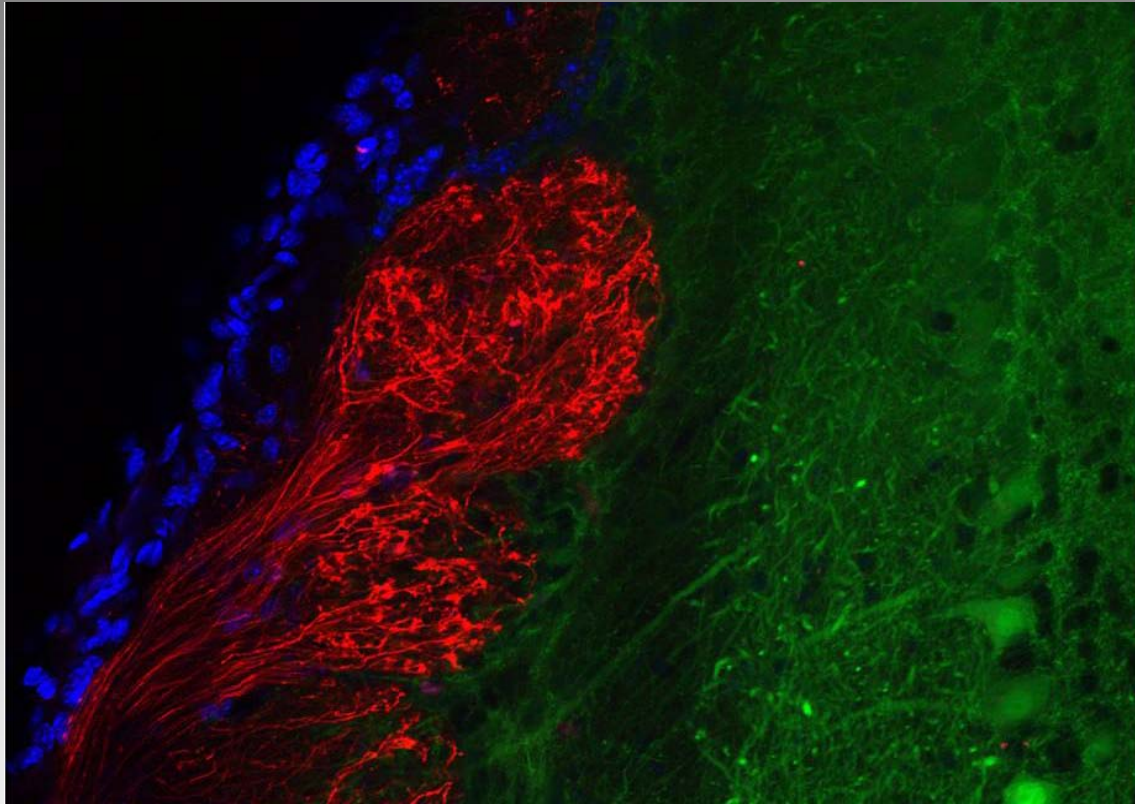
# Intravenous Injection



Serotype: AAV9  
Protein expressed: GFP  
Amount injected:  $4 \times 10^{11}$   
Manufacturer: UPenn

Foust et al., Nature Biotechnology, 2009

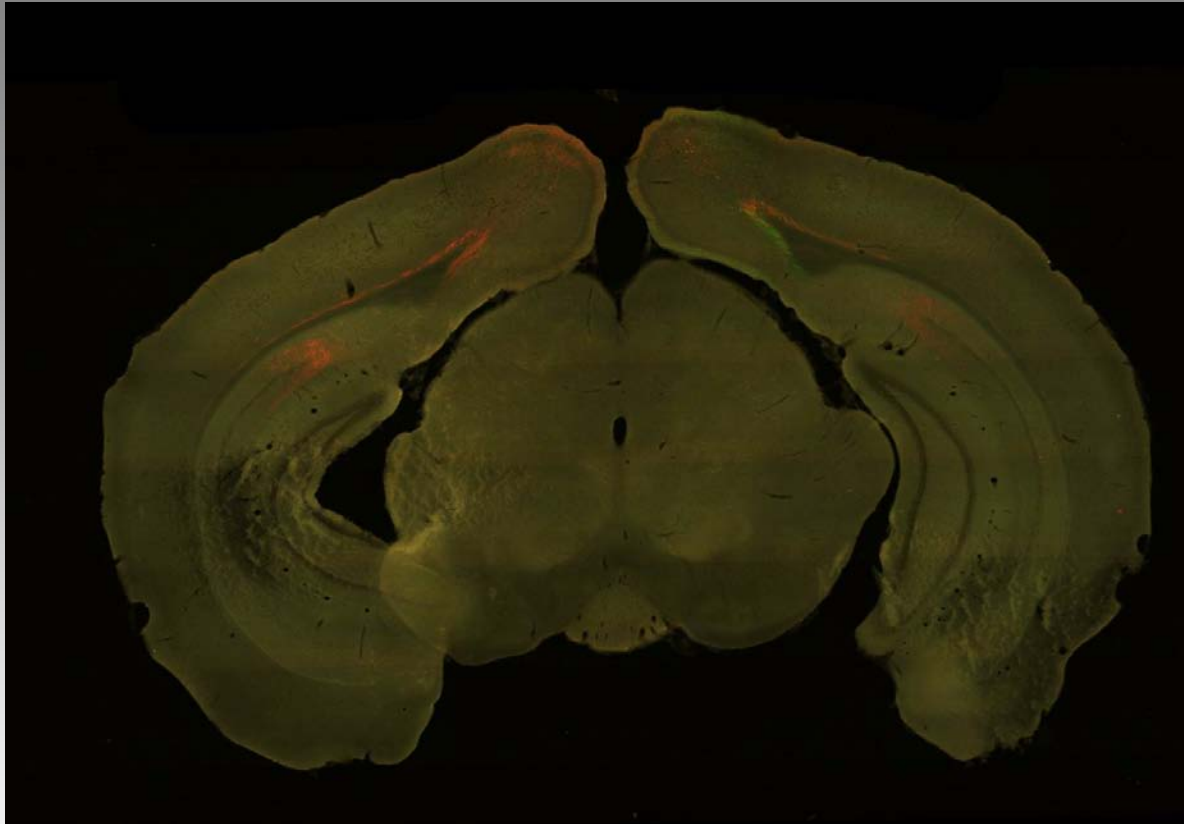
# Intranasal Injection



Serotype: AAV2  
Protein Expressed: dsRed  
Amount injected:  $10^{10}$  vg  
Manufacturer: VVC

Borisovska et al., unpublished

# Neuronal Track Tracing



Serotype: AAV2

Proteins expressed: GFP and mCherry

Amount injected:  $\sim 10^8$  vg

Manufacturer: UPenn



# Vollum Viral Core Services

- Free consultation
- Cloning, production and titering of AAV, lenti- and retro- viral particles
- Stereotaxic delivery of viral particles
- Contact: [washbure@ohsu.edu](mailto:washbure@ohsu.edu)