Crossing the Blood-Brain-Barrier for Brain-Wide Anatomical and Functional Mapping with novel AAVs and CLARITY

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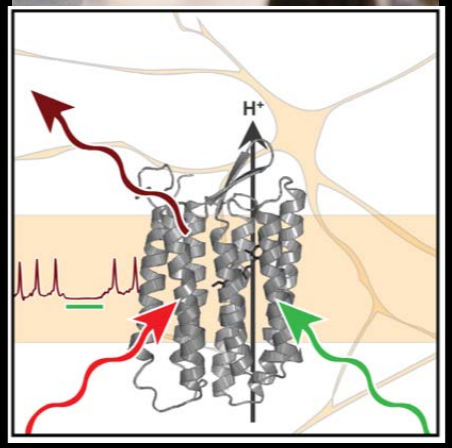
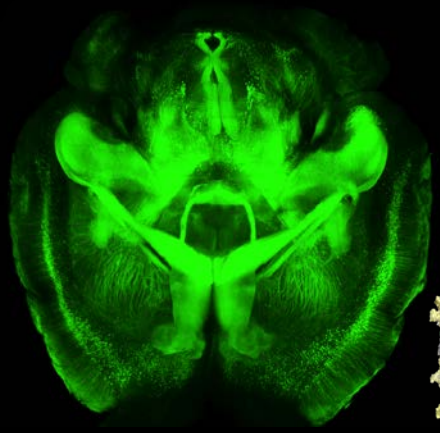
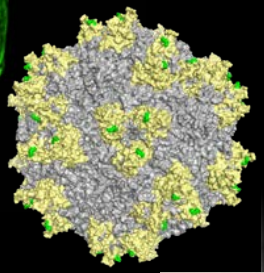
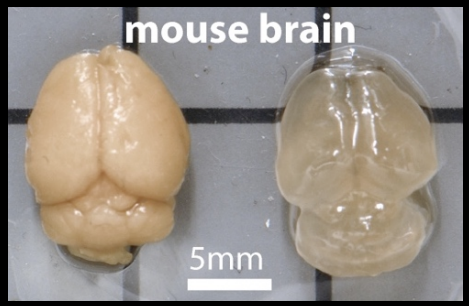
Assistant Professor of Biology and Biological Engineering
Heritage Principal Investigator



Optogenetics



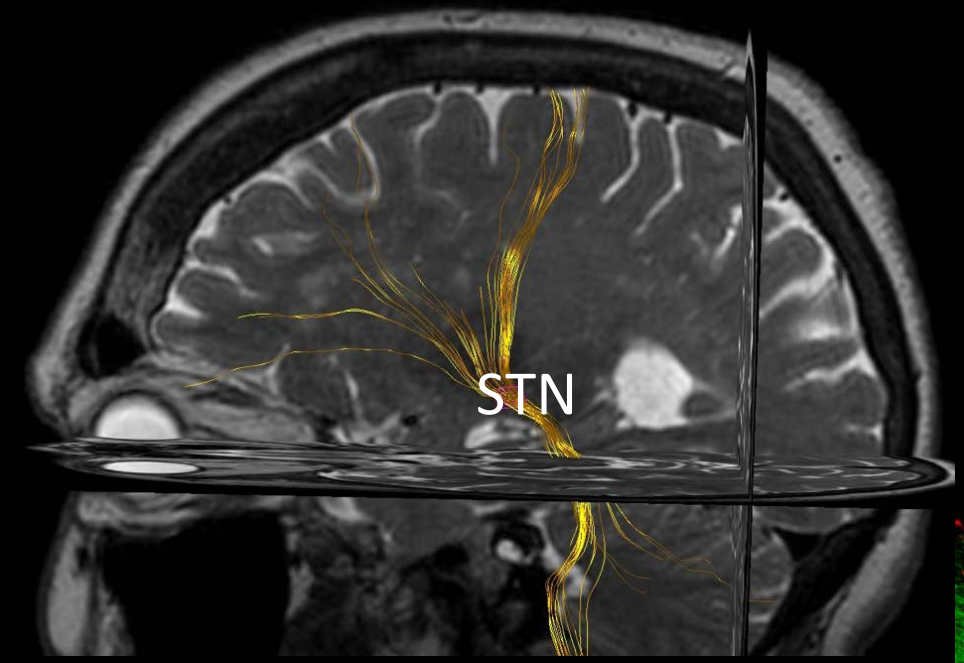


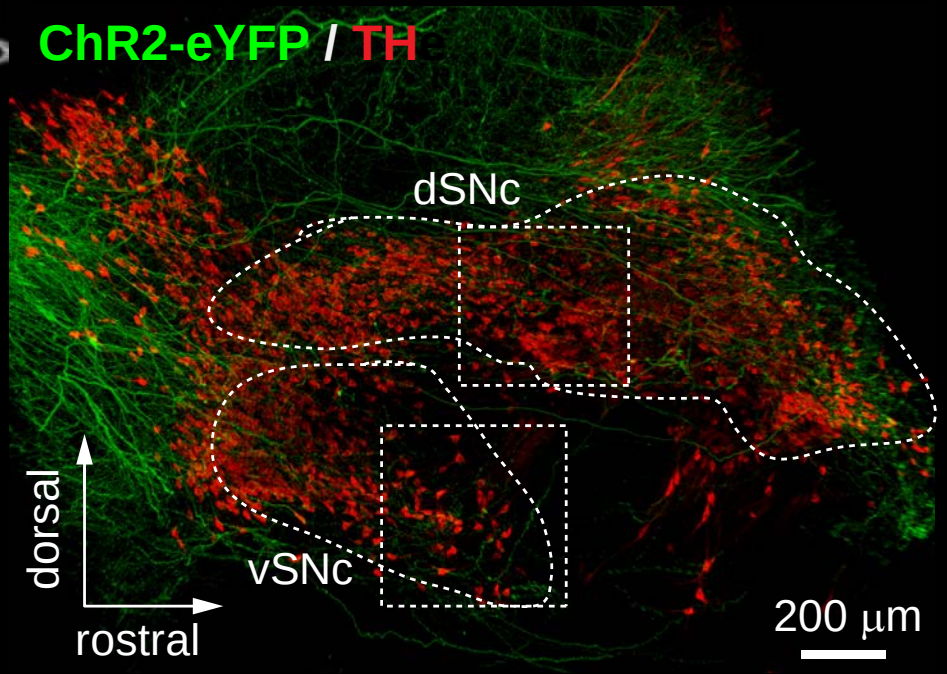


CLARITY &
delivery vectors

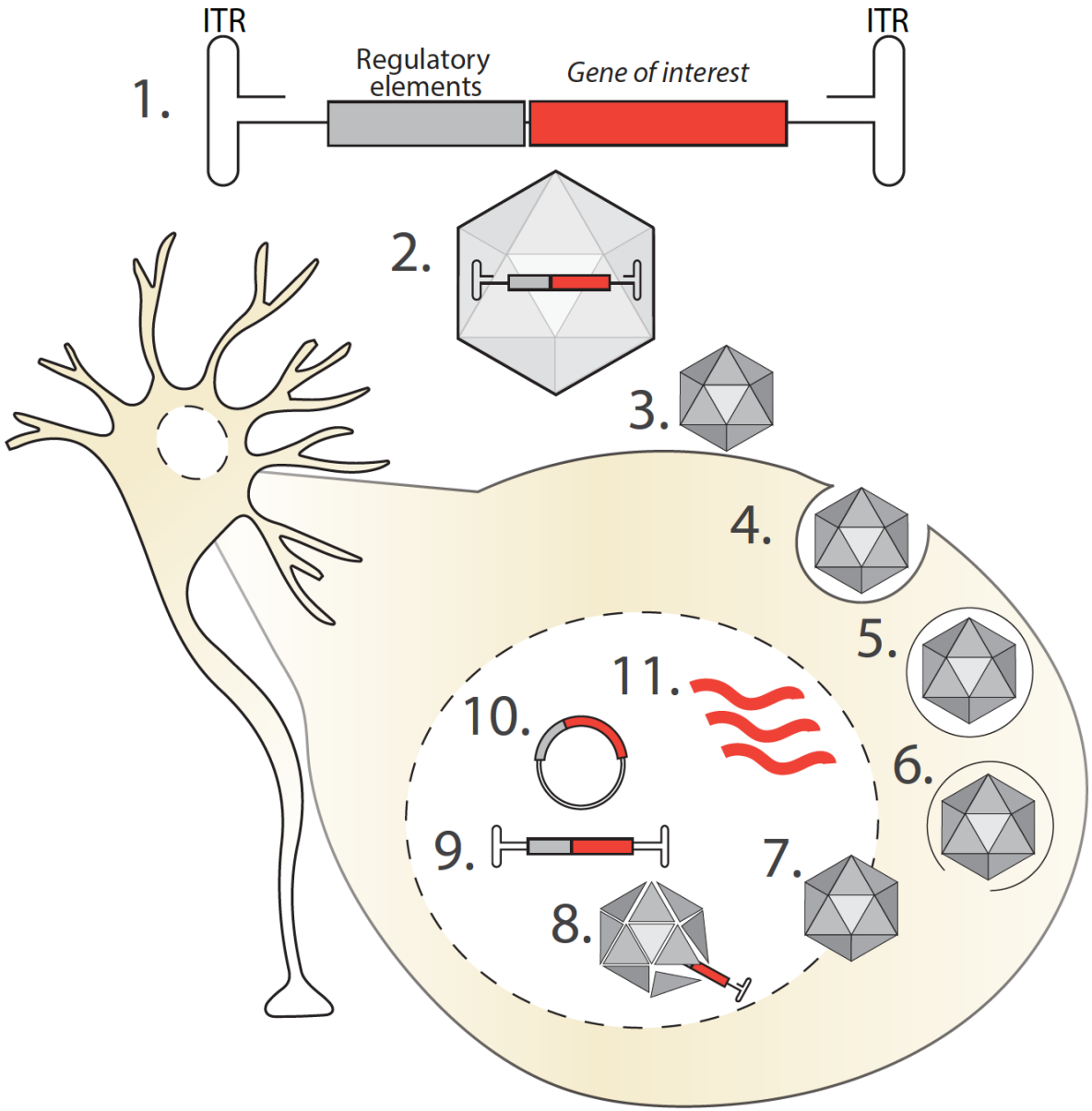
Deep Brain Stimulation

Delivering Genes across the Blood-Brain-Barrier and Mapping Projections Brain-Wide: AAV engineering and Tissue Clearing

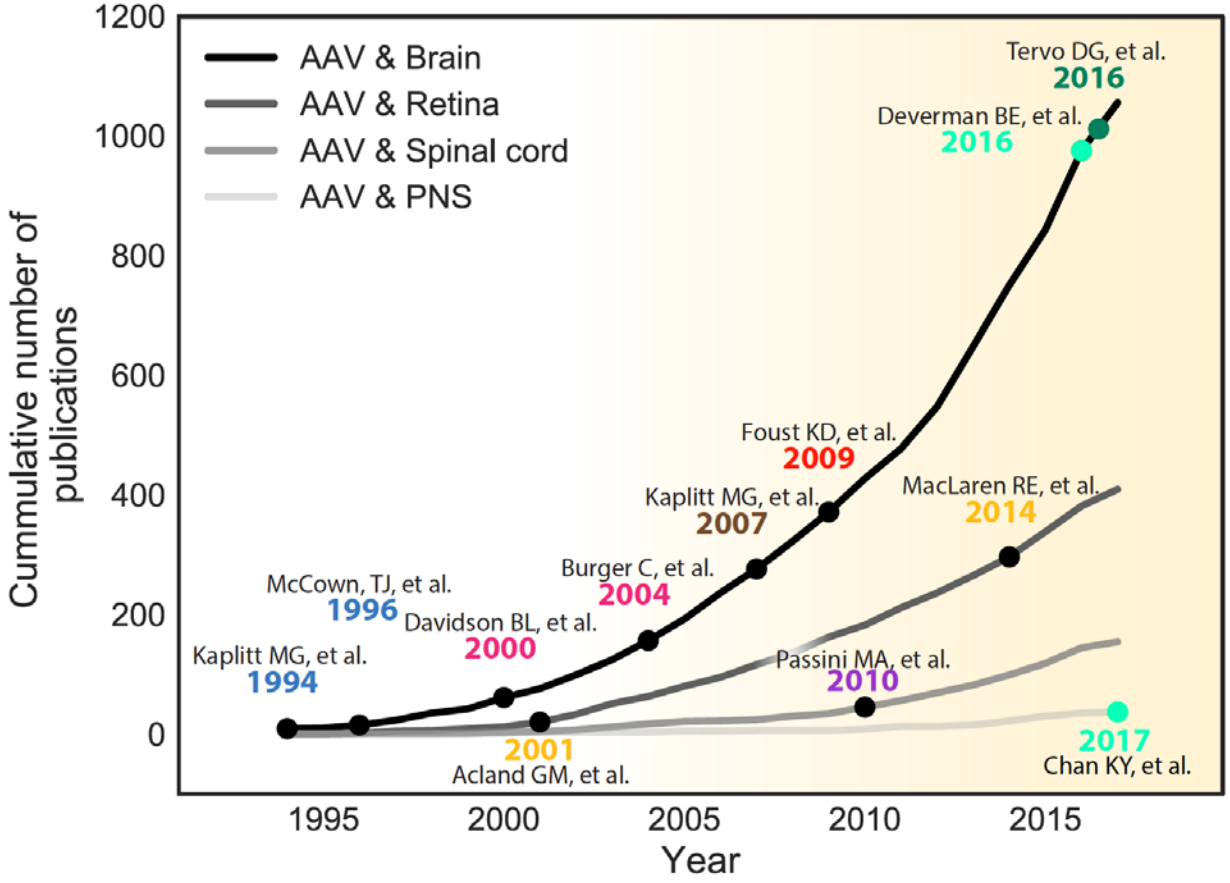




rAAV transduction of a neuron



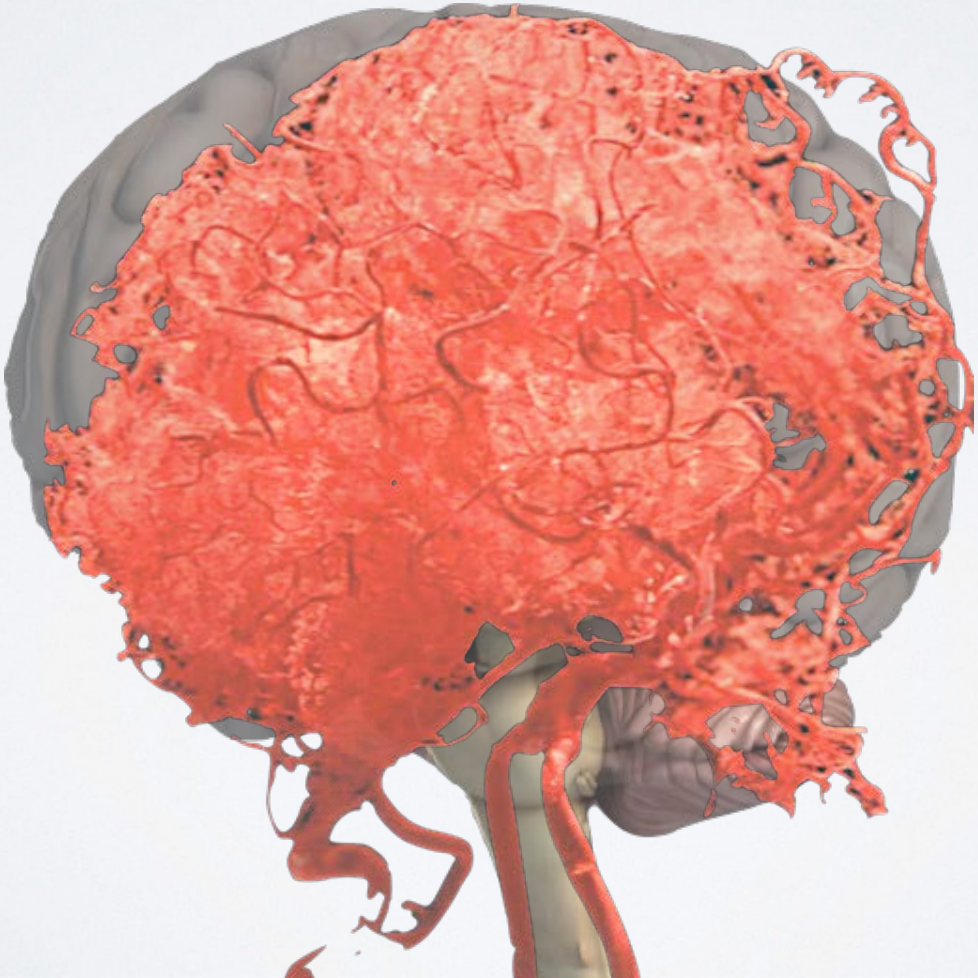
Overview of AAV use in the nervous system



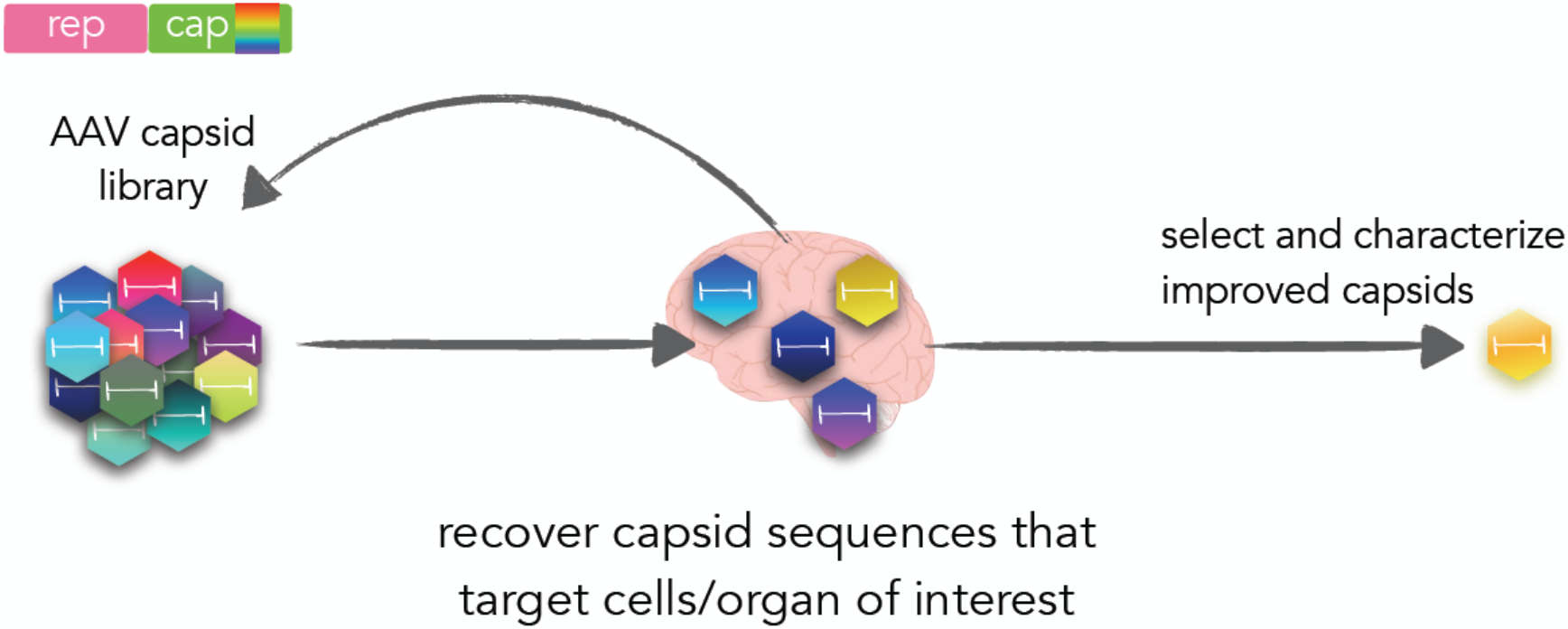
1. First demonstrations of rAAVs in the brain
2. Comparison of rAAV serotypes' gene transfer, tropsim, & distribution in the CNS
3. Phase I trial: AAVs for Parkinson's disease
4. CNS-wide transduciton via systemic injection of AAV in neonatal mice
5. Retinal gene therapy
6. Spinal cord gene therapy
7. Designer rAAVs for systemic delivery to the CNS and PNS: r*AAV-PHP.B, PHP.eB, & PHP.S*
8. Designer rAAV for efficient retrograde trafficking: r*AAV2-retro*

Engineering Vectors for Efficient Delivery of Therapeutics to the Whole Brain

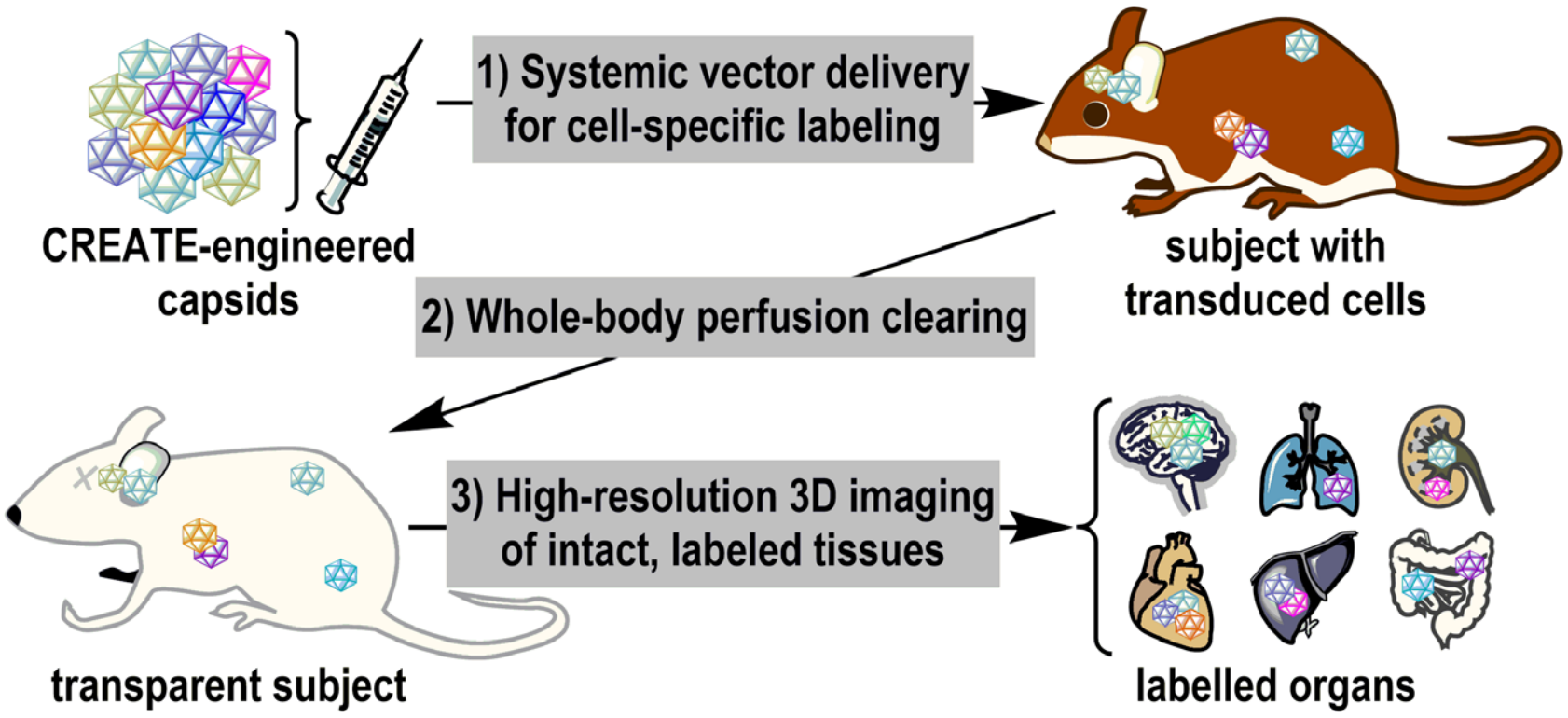
Agents of interest: neuroprotective growth factors, labels for circuit anatomy to inform DBS



In vivo selection for AAV capsids with more desirable properties

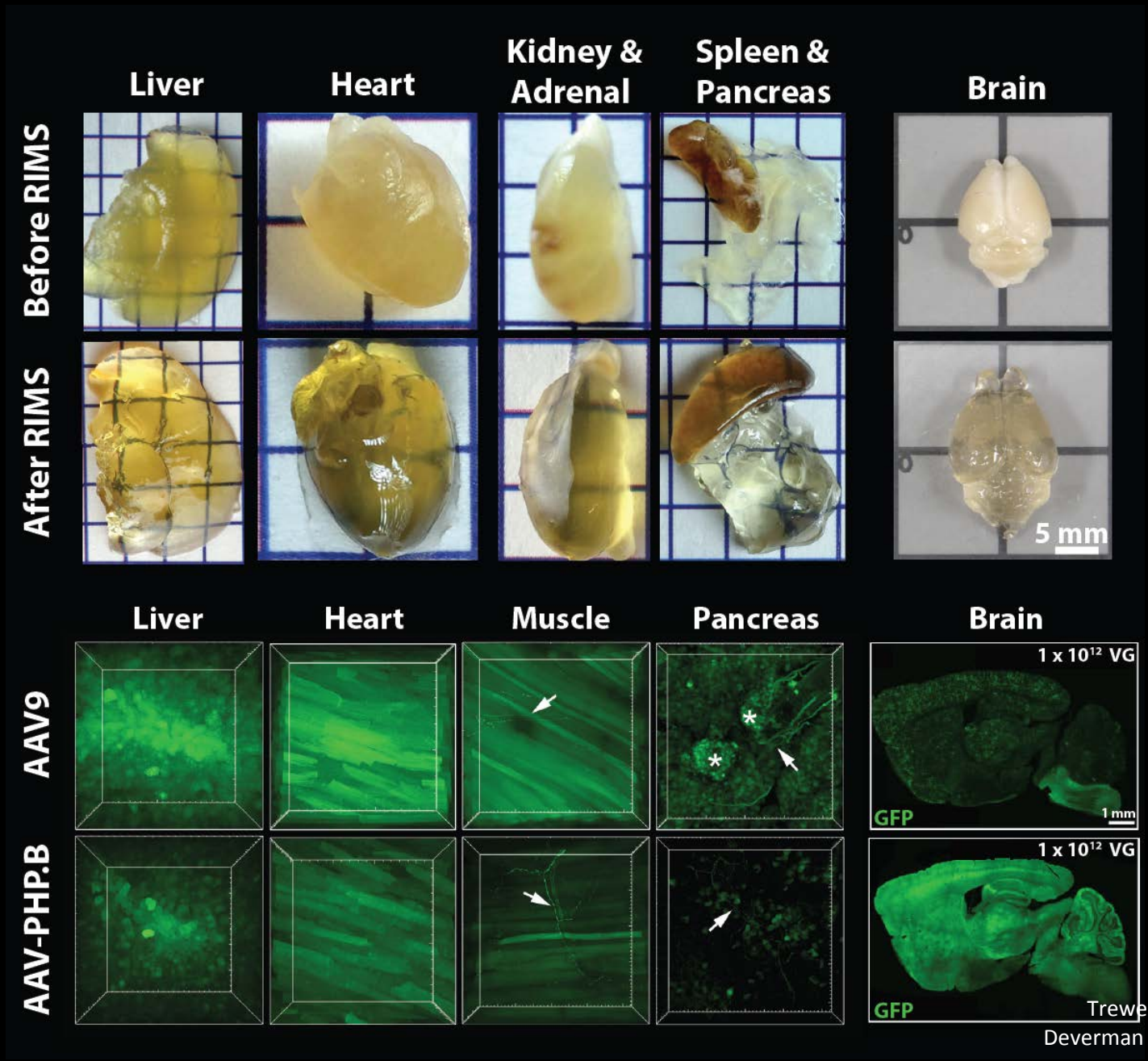


Whole-body PARS for viral biodistribution



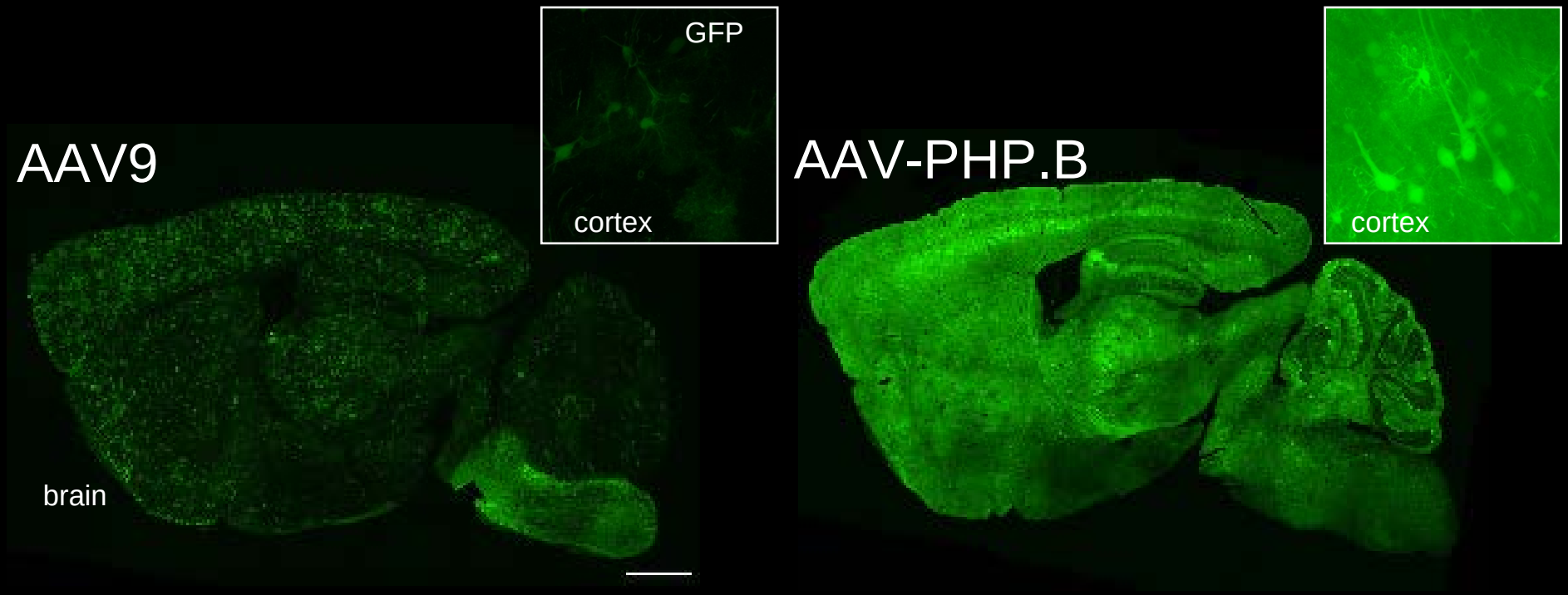
PARS = Perfusion-Assisted Agent Release in Situ

Whole-body tissue clearing by PARS for viral biodistribution



Yang *et al*. (Cell, 2014)
Treweek *et al*. (Nat.Prot, 2015)
Deverman *et al. (Nat.Biotech,*2016)

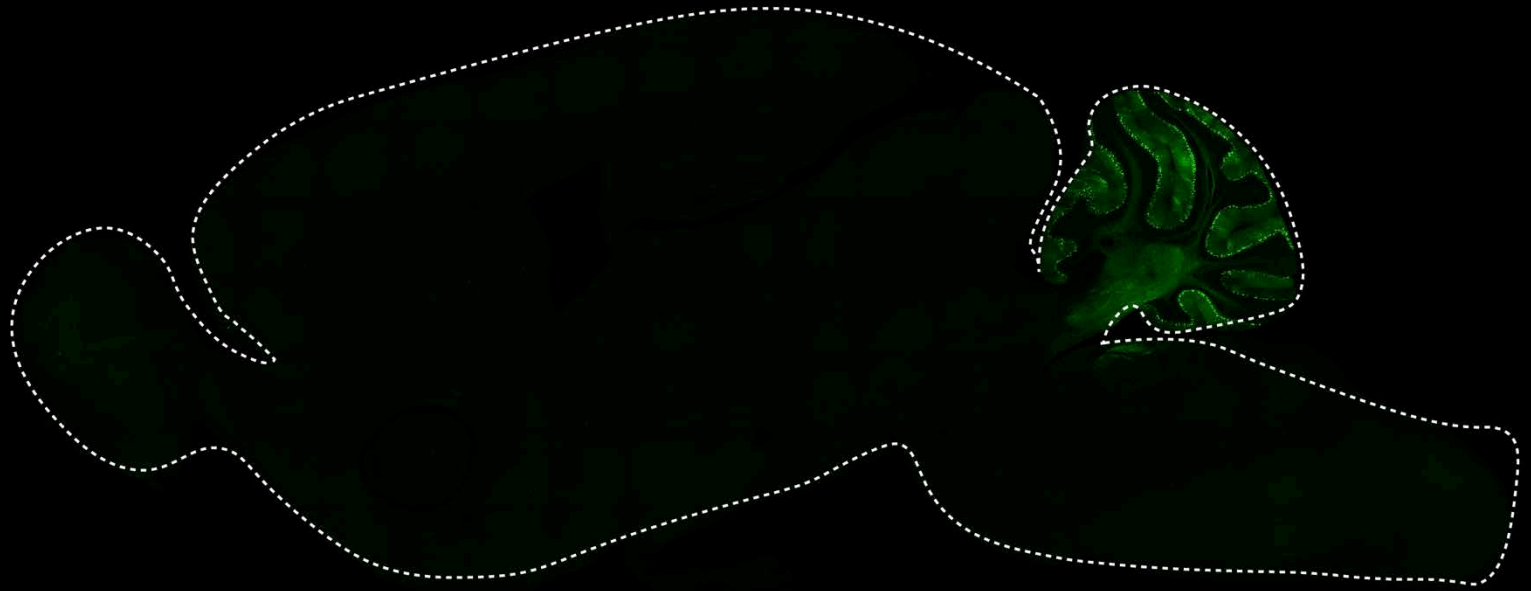
Brain-wide gene transfer via systemic delivery in adult rodent



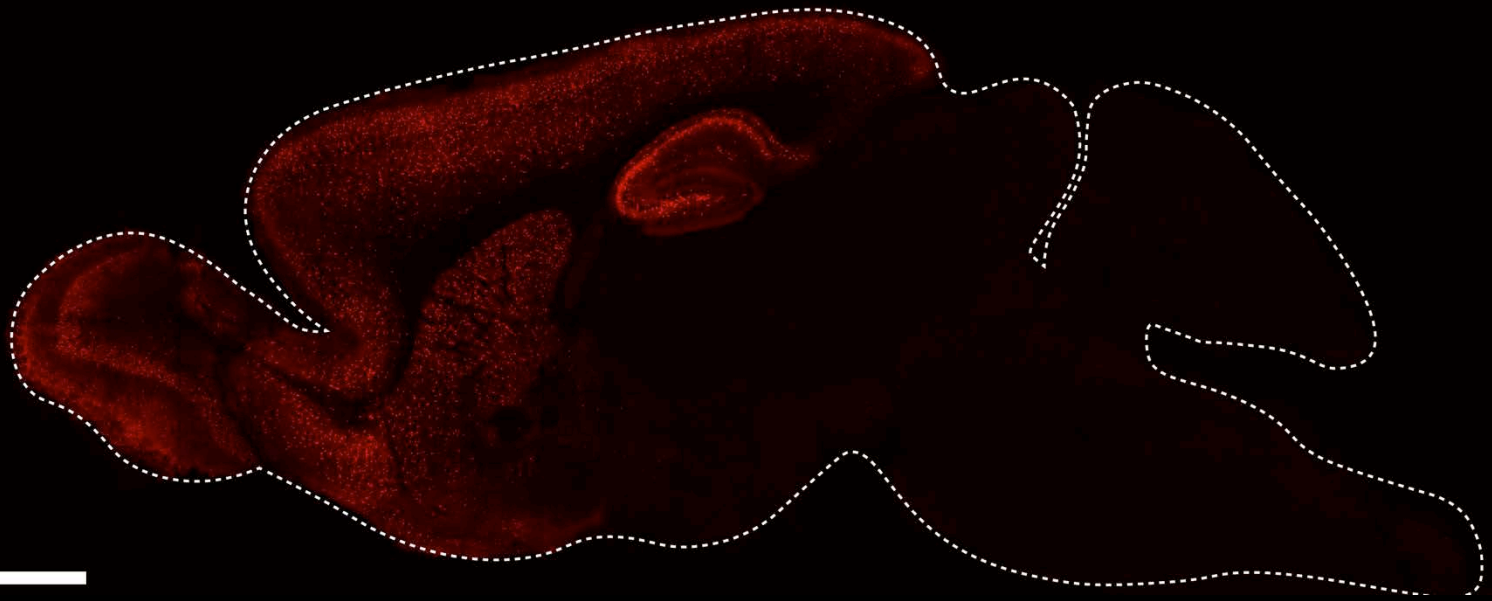
Deverman et al, Nature Biotechnology, 2016

Long Lasting Expression

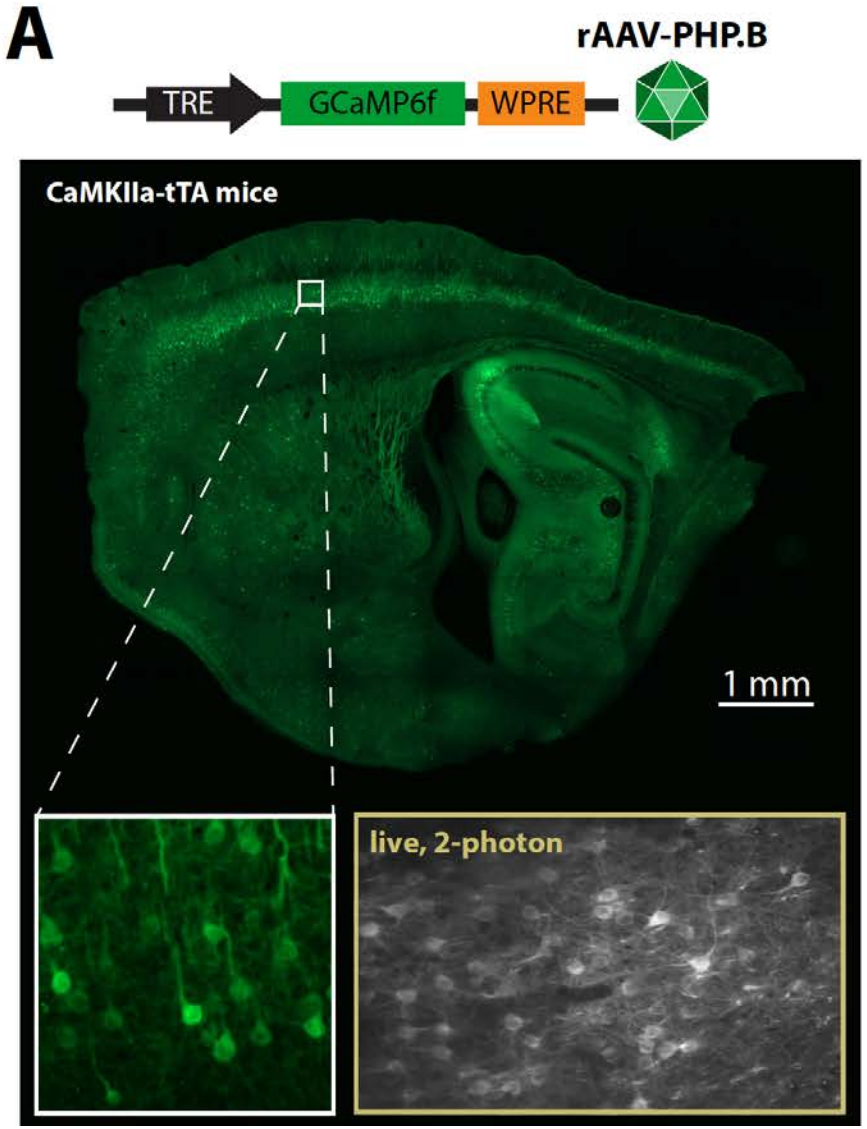
AAV-PHP.eB delivering a vector with a Purkinje cell type-specific promoter driving GFP

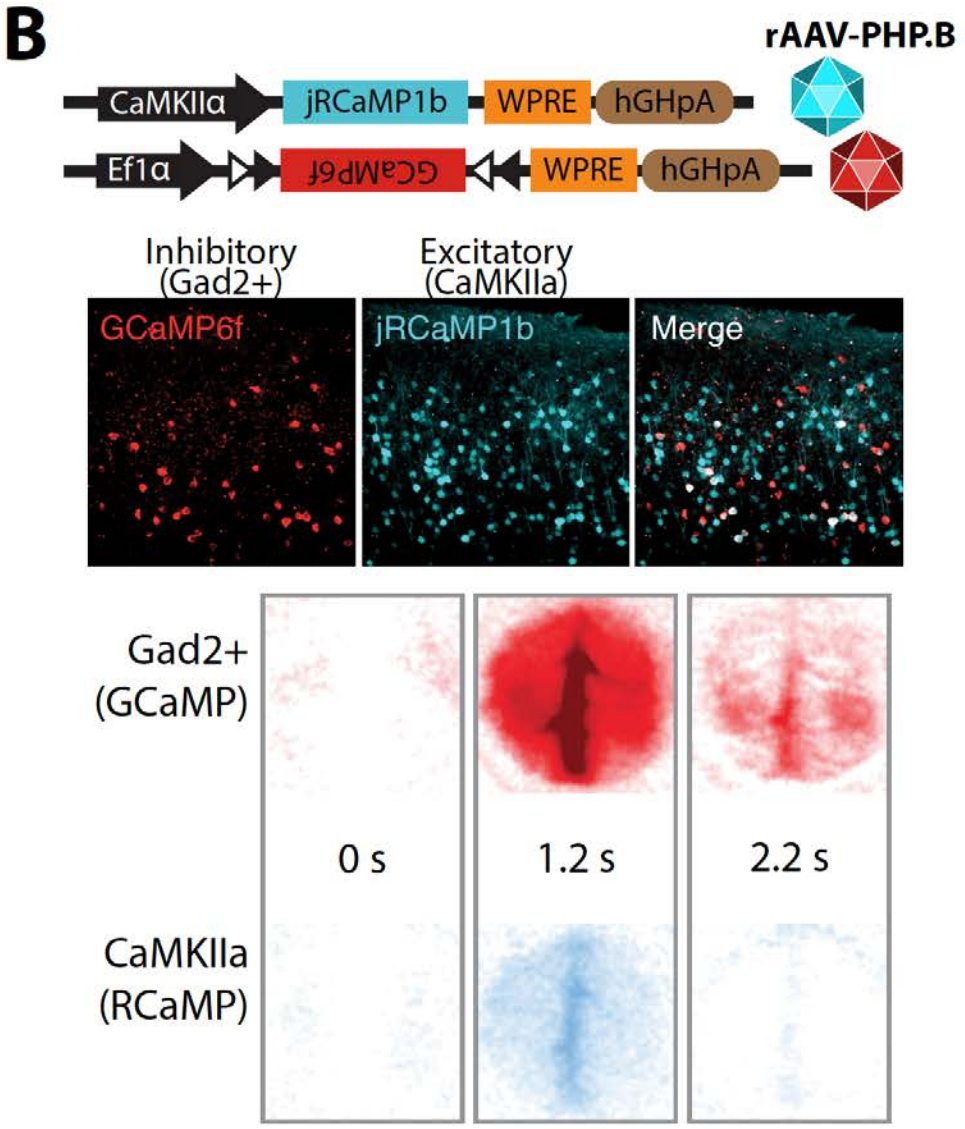


AAV-PHP.eB delivering a vector with an inhibitory enhancer specific to the forebrain driving mRuby2



Widespread AAV-mediated delivery for neuronal activity dynamics recording during behavior

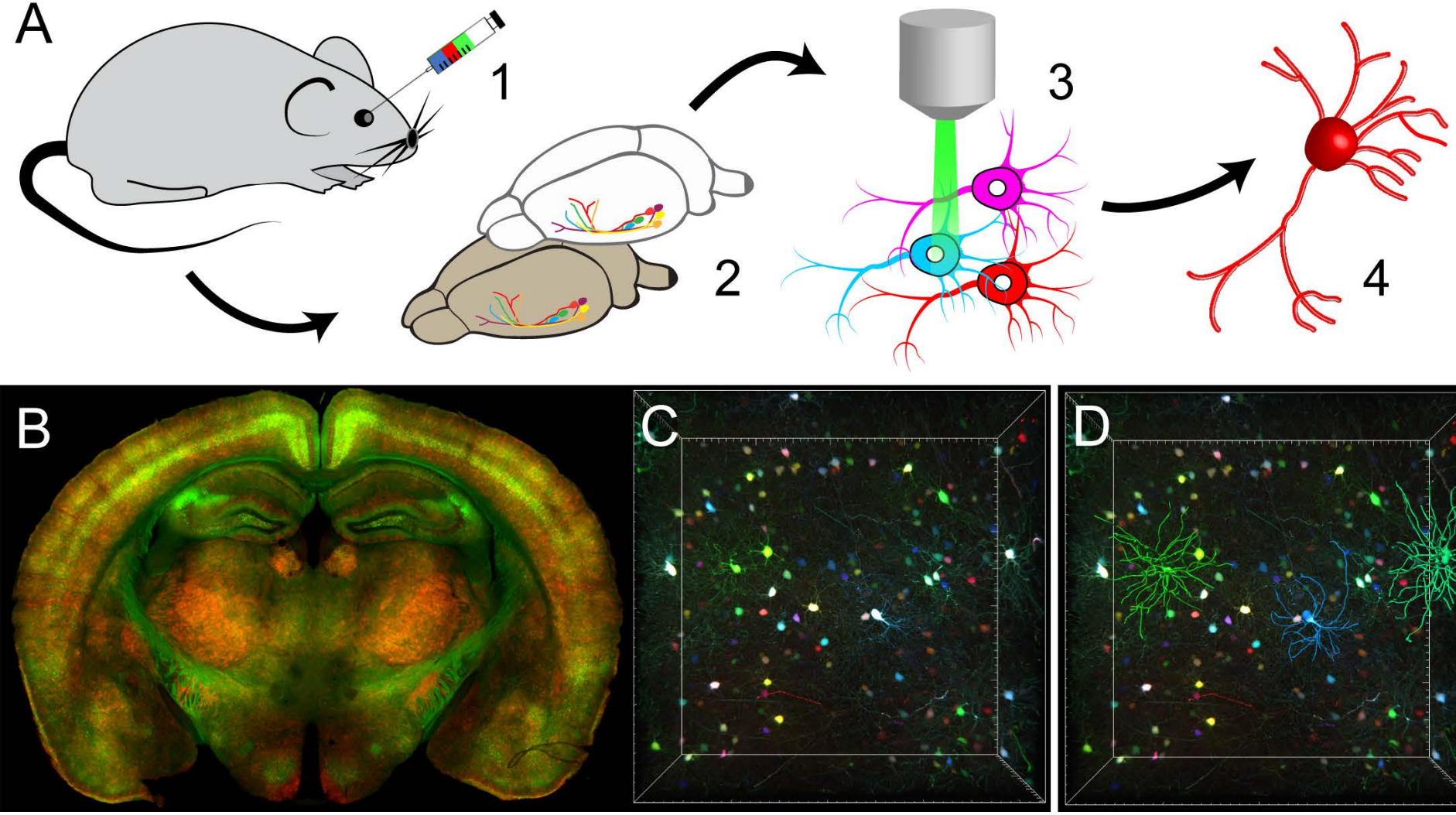




A: Gradinaru/Tsao Labs at Caltech

B: Allen et al, Neuron 2017

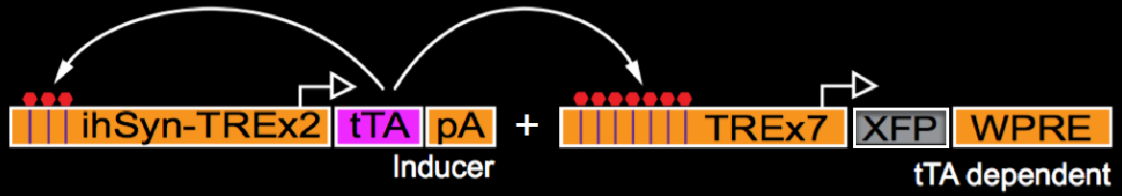
Broadly transducing AAVs permit brainwide transgene expression and facilitate neurite tracing.

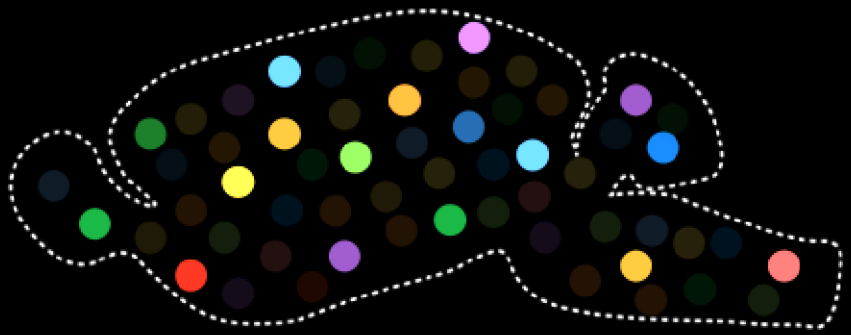


A two-component approach decouples color diversity from labeling density

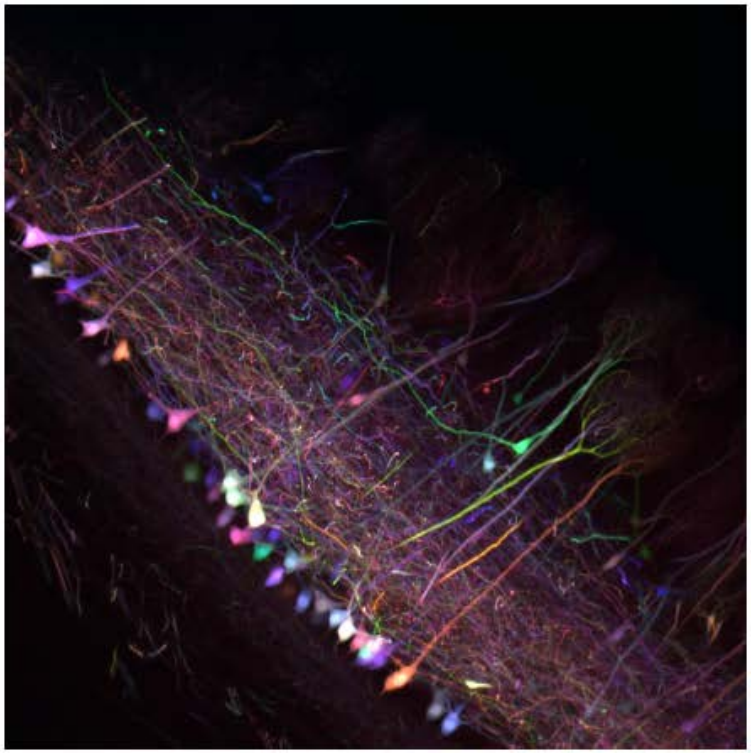
Deliver XFPs at a high dose to with none of them expressing to provide high color diversity

Delivered at a low dose to to sparsely turn on expression

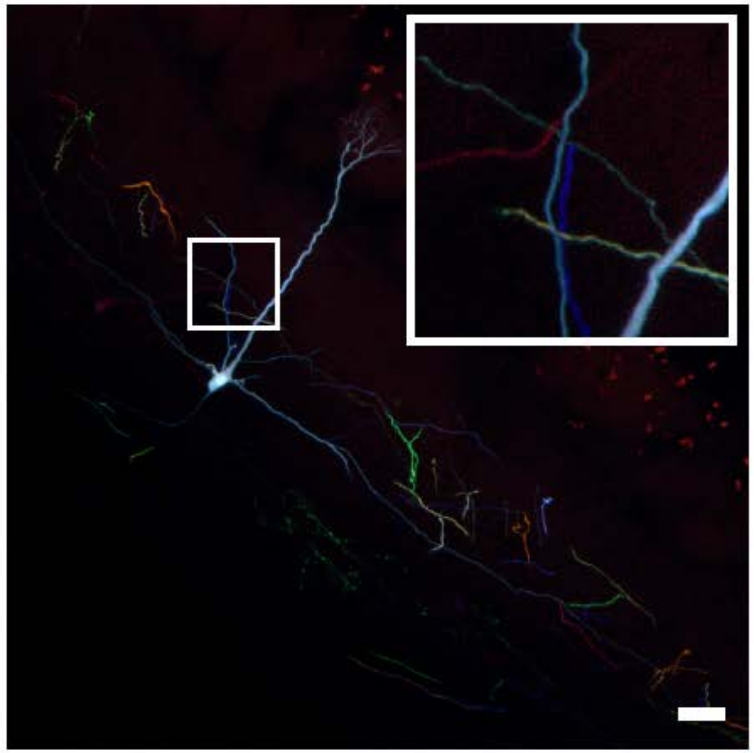




Sparse Stochastic Multicolor Labelling for ***Genetic*** Tracing

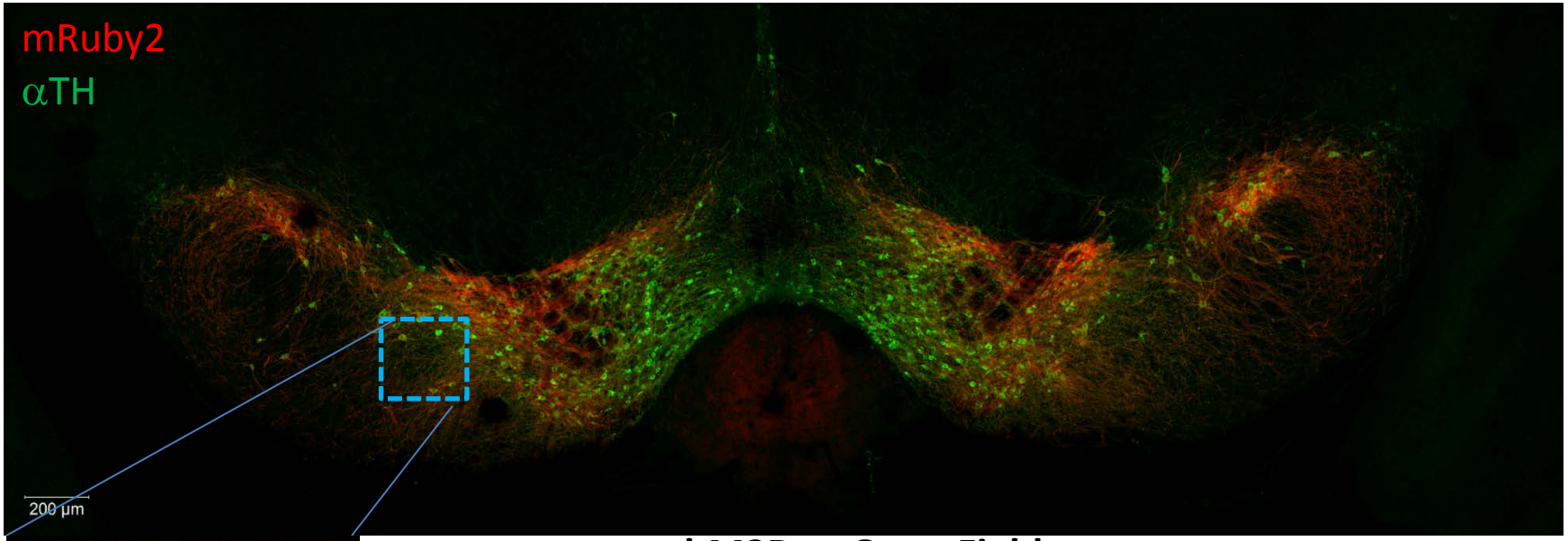


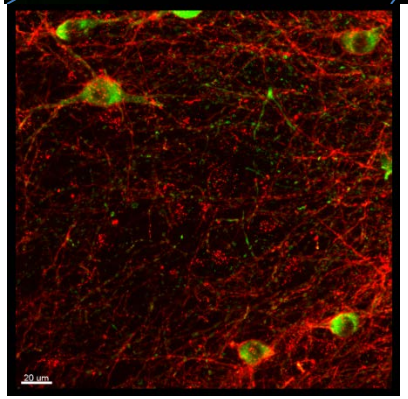
High dose inducer



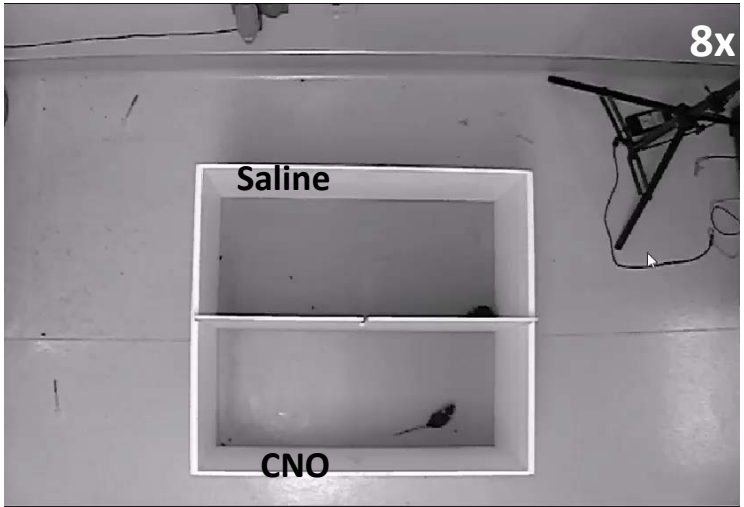
Low dose inducer

Noninvasive behavioral control:
modulation of dopamine neurons with systemic AAVS and DREADDS changes locomotion





hM3Dq - Open Field



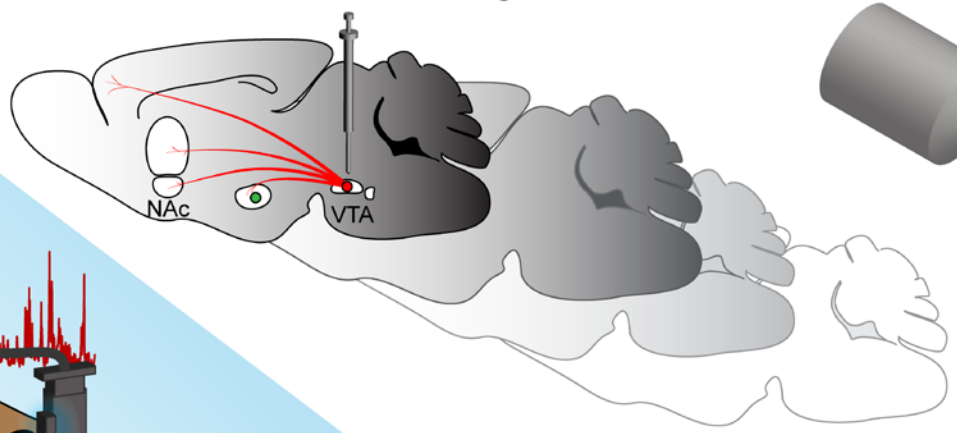
Systemic PHP.eB in
TH-Cre+ animals
delivers
hSyn-DIO-hM3Dq-mRuby2

($5x10^{11}$ vg, IHC at 3.5 weeks)

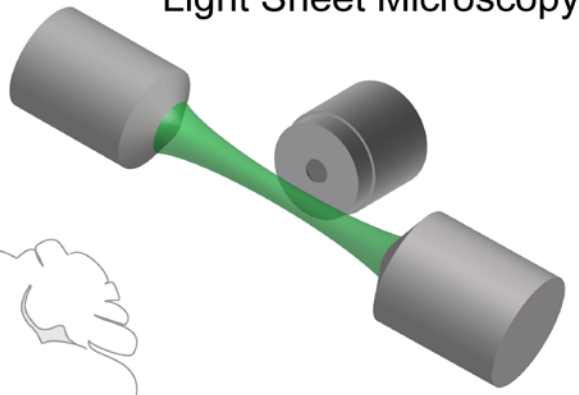
Anatomical Analysis and Tracing

Activity Monitoring and Control

Viral Vector-Based Labelling Methods

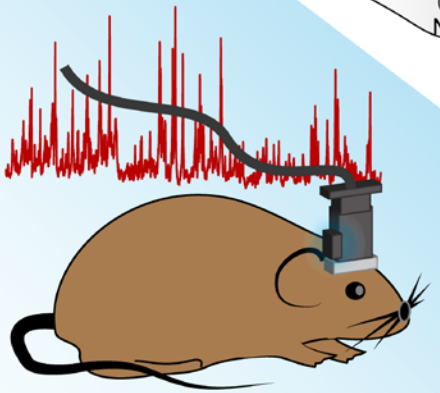


Light Sheet Microscopy



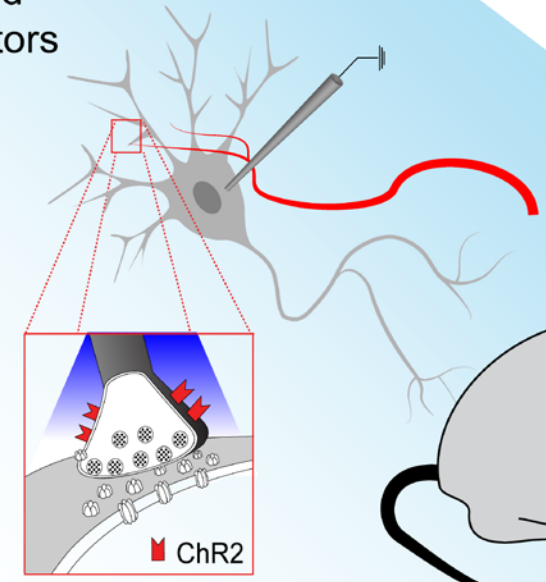
Tissue Clearing

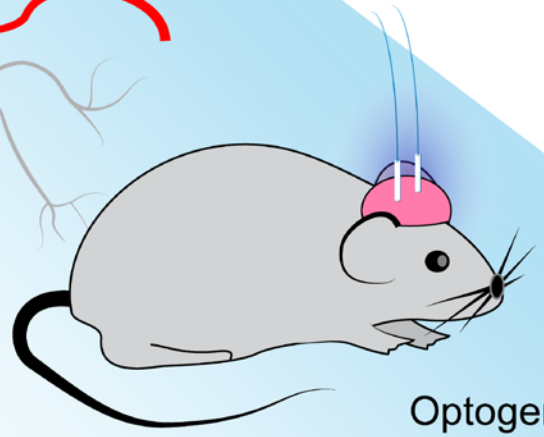
Multicolor Labelling



Genetically Encoded
Neural Activity Indicators

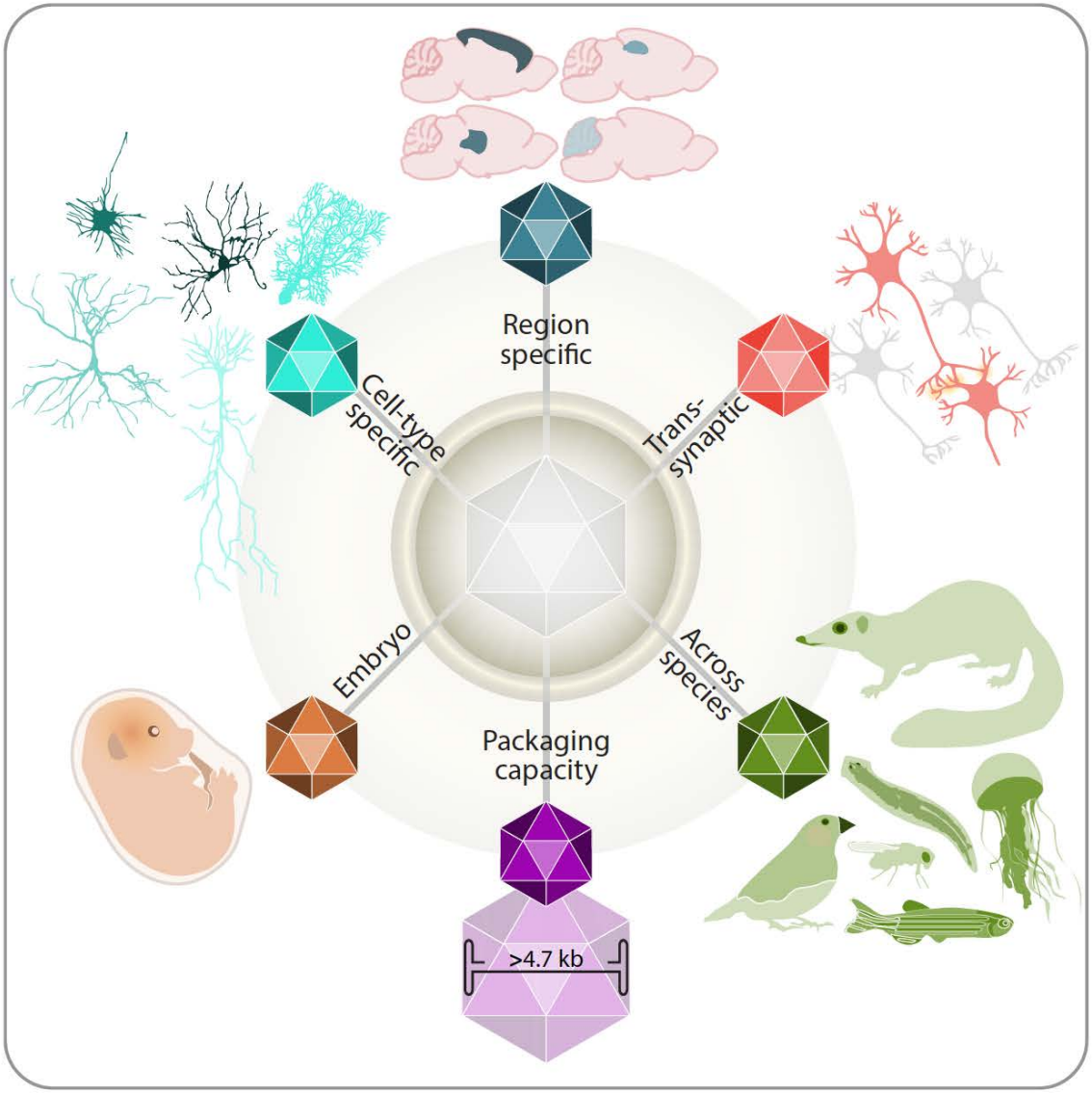
Chemogenetics



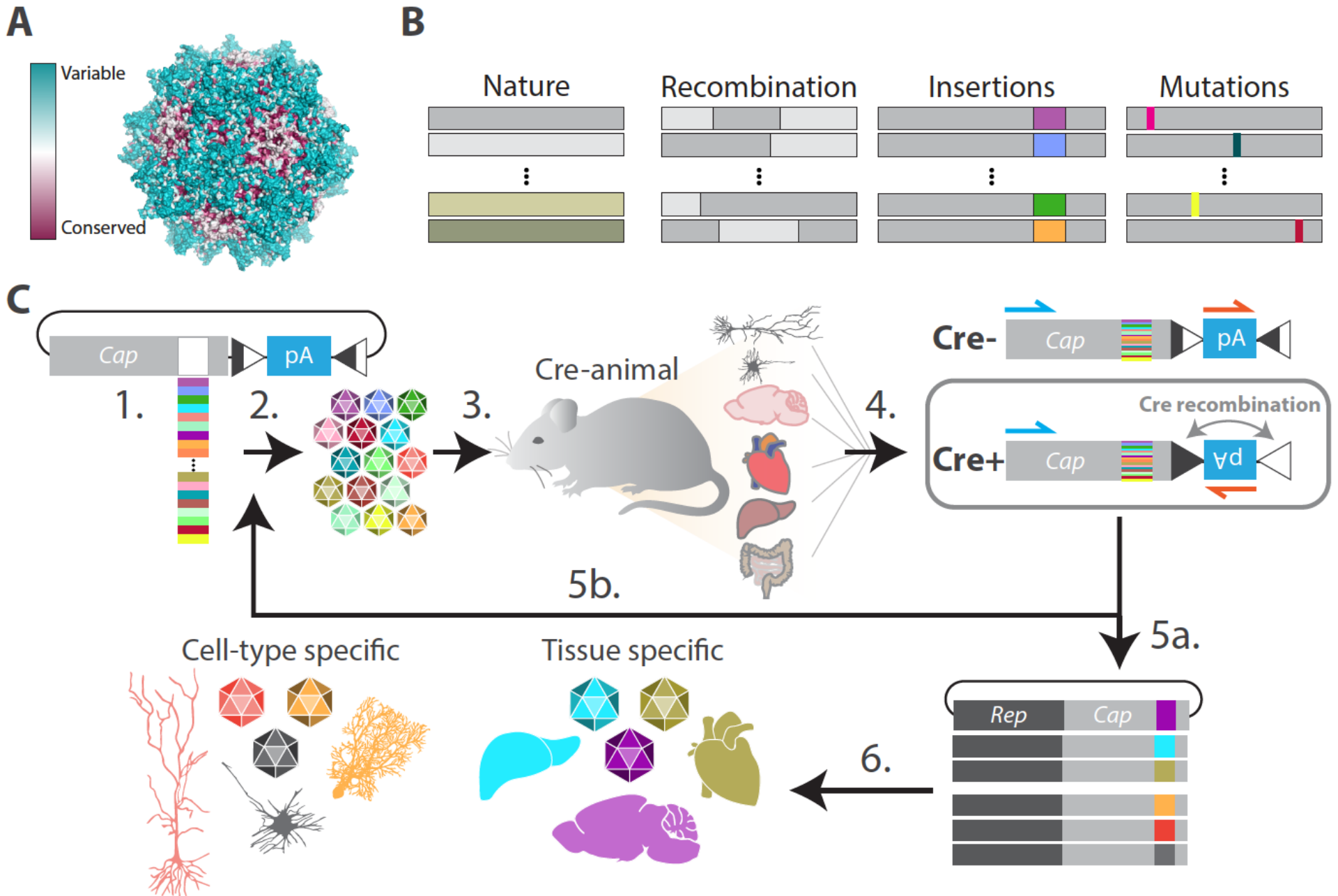


Optogenetics

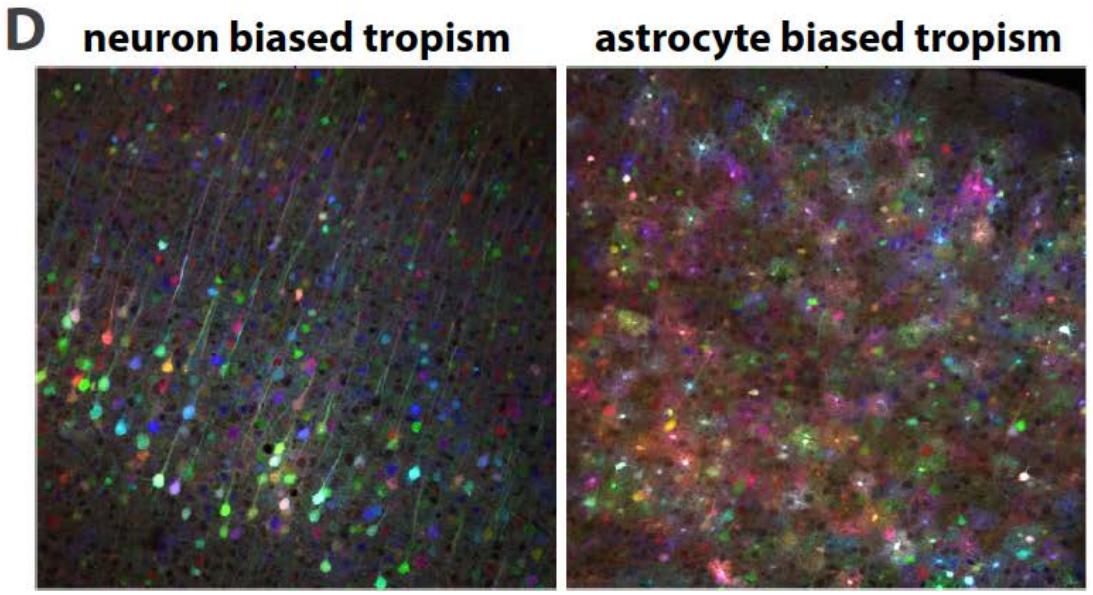
AAVs – potential developments

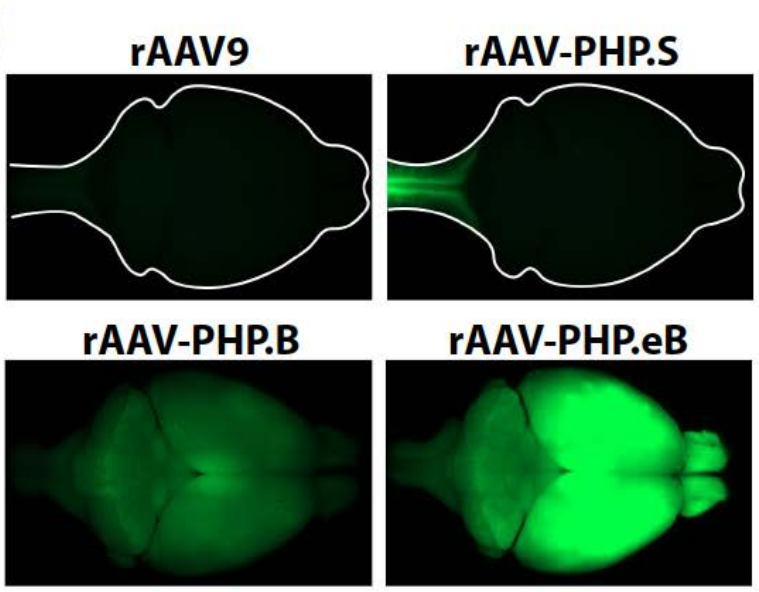


Engineering designer AAVs for neuroscience



Systemic AAVs





Visualizing the Activity and Anatomy of Brain Circuits: Optogenetics, Tissue Clearing, Viral Vectors

Gradinaru Lab, Caltech

Claire Bedbrook, Nick Flytzanis
Ken Chan, Priya Kumar, Ryan Cho
Michael Altermatt, Xiaozhe Ding
Jenny Treweek, Alon Greenbaum
Collin Challis, Min Jee Jang
Rosemary Challis, Ben Deverman
Elliott Robinson, Anat Kahan,
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- **Cheng Xiao, Chunyi Zhou**
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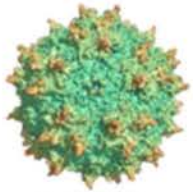
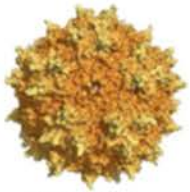
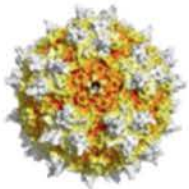
NIH Director's New Innovator, **PECASE,** SPARC
Foundations: Moore, Beckman, Curci, Sloan, Pew,
Heritage Medical Foundation

CLOVER: CLARITY, OPTOGENETICS AND VECTOR ENGINEERING RESEAR
CENTER @ THE BECKMAN INSTITUTE CALTECH
PI: Viviana Gradinaru
Director: Ben Deverman (bd@caltech.edu)
http://www.beckmaninstitute.caltech.edu/clover.shtml

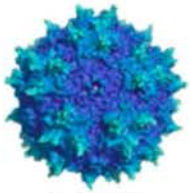
Current applications of AAVs: Pre-clinical (1st row) and Clinical (2nd row)

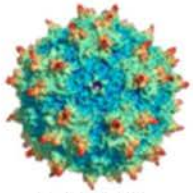

AAV1

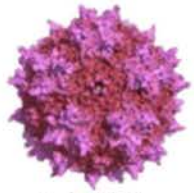

AAV2

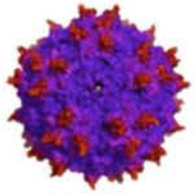

AAV3

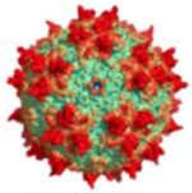

AAV4


AAV5


AAV6


AAV7

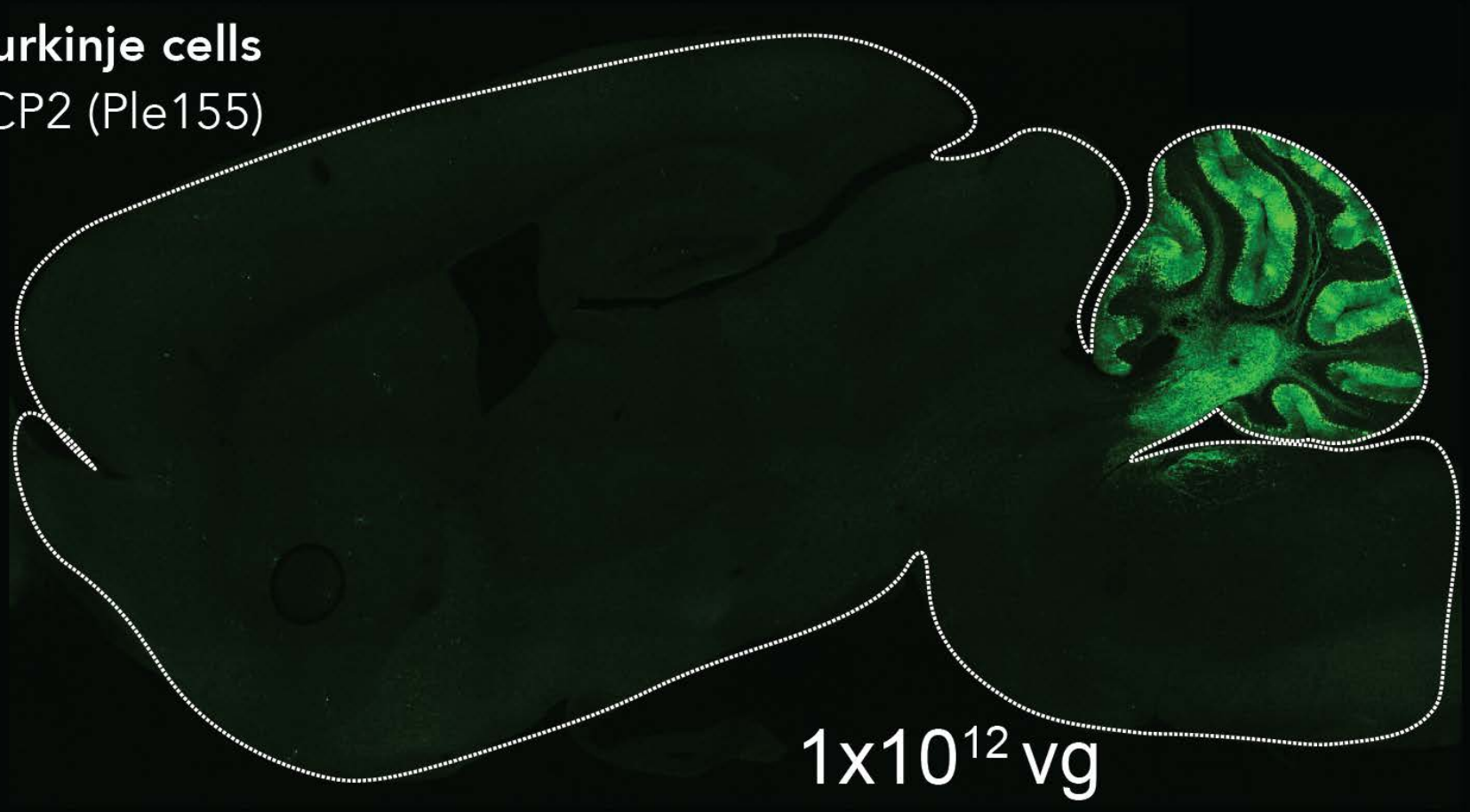

AAV8


AAV9

Heart disease Muscular dystrophy	Various animal model studies	Liver cancer	Mucopoly-saccharidosis type VII Familial ALS RPE65 deficient vision loss	Globoid cell leukodystrophy Human immunodeficiency	Duchenne muscular dystrophy		Hemophilia A Familial hyper-cholesterolemia Glycogen storage disease type II	Heart failure CNS disorders
Lipoprotein lipase deficiency Heart disease	Cystic fibrosis Hemophilia B Leber's congenital amourosis Parkinson's disease Canavan disease		RPE65 deficient disease	Hemophilia A			Hemophilia B	Spinal muscular atrophy

Chen H, WJMG, 2015

Purkinje cell restricted expression with IV AAV-PHP.eB and the PCP2 promoter

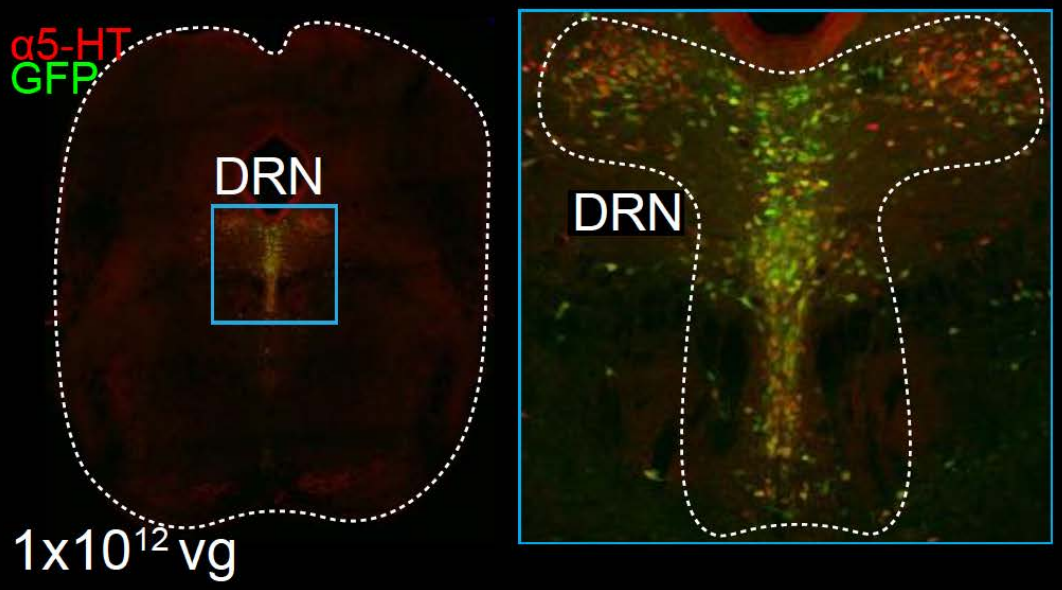


Chan et al, Nature Neuroscience , 2017

Achieving cell type-restricted expression with systemically delivered AAVs

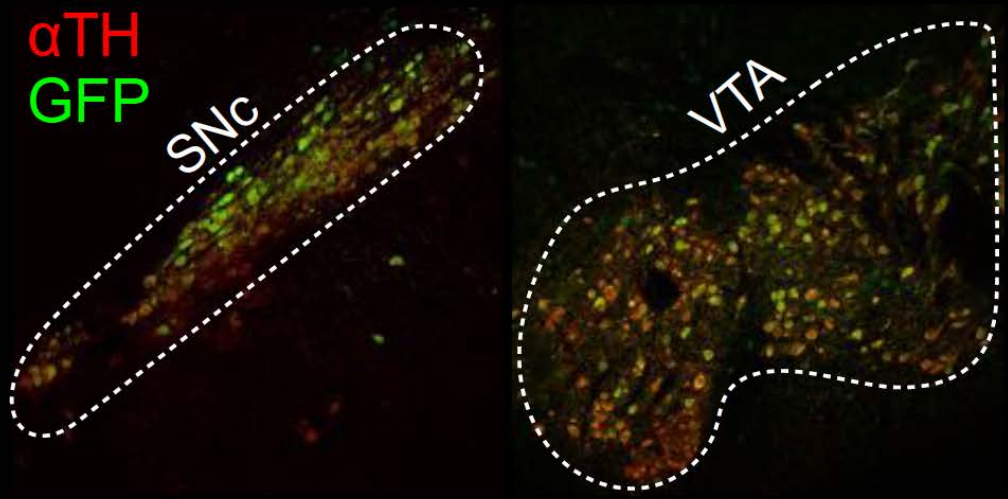
serotinergic neurons

FEV (Ple67)



dopaminergic neurons

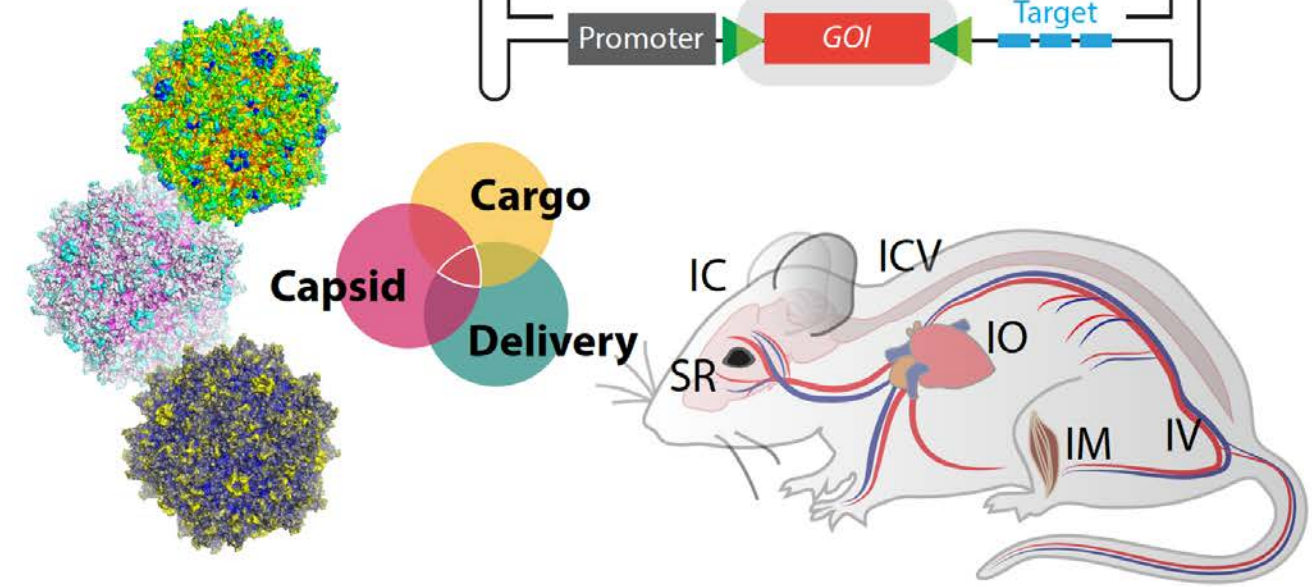
mTH



Chan et al, Nature Neuroscience , 2017

Methods for cell type-restricted expression in the CNS

A. Implementation



B. Delivery: Direct intracranial

Cargo: DIO-FP / Vip-Cre transgenic
Capsid: rAAV5