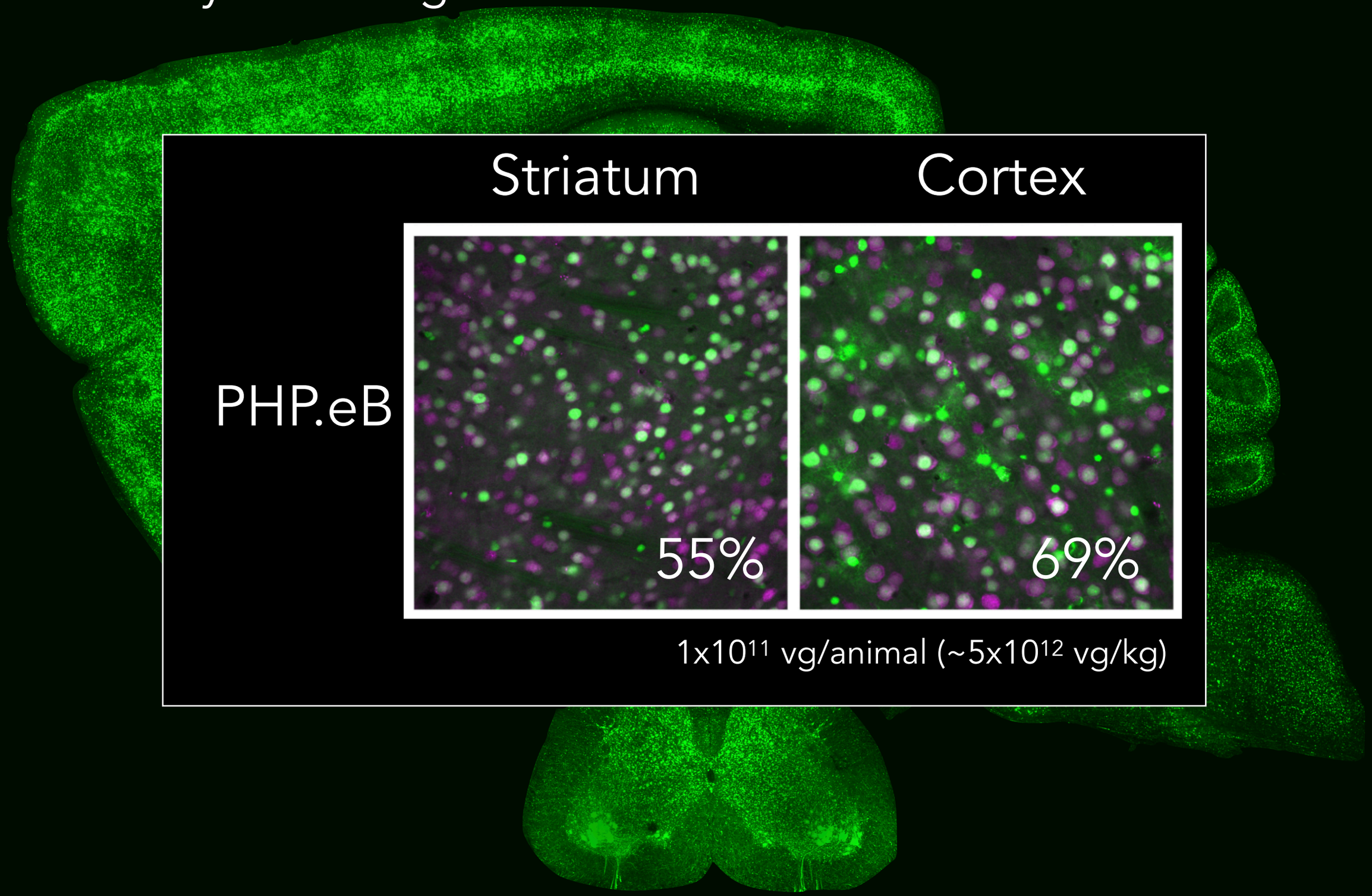
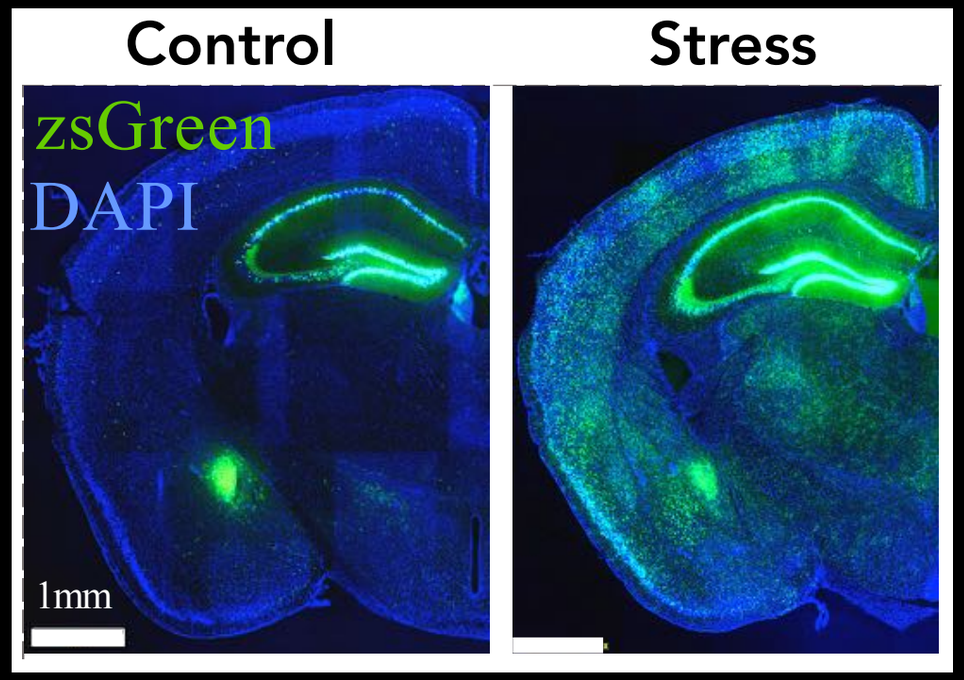
AAV-PHP.eB enables efficient transduction of neurons and astrocytes throughout the CNS at lower virus doses

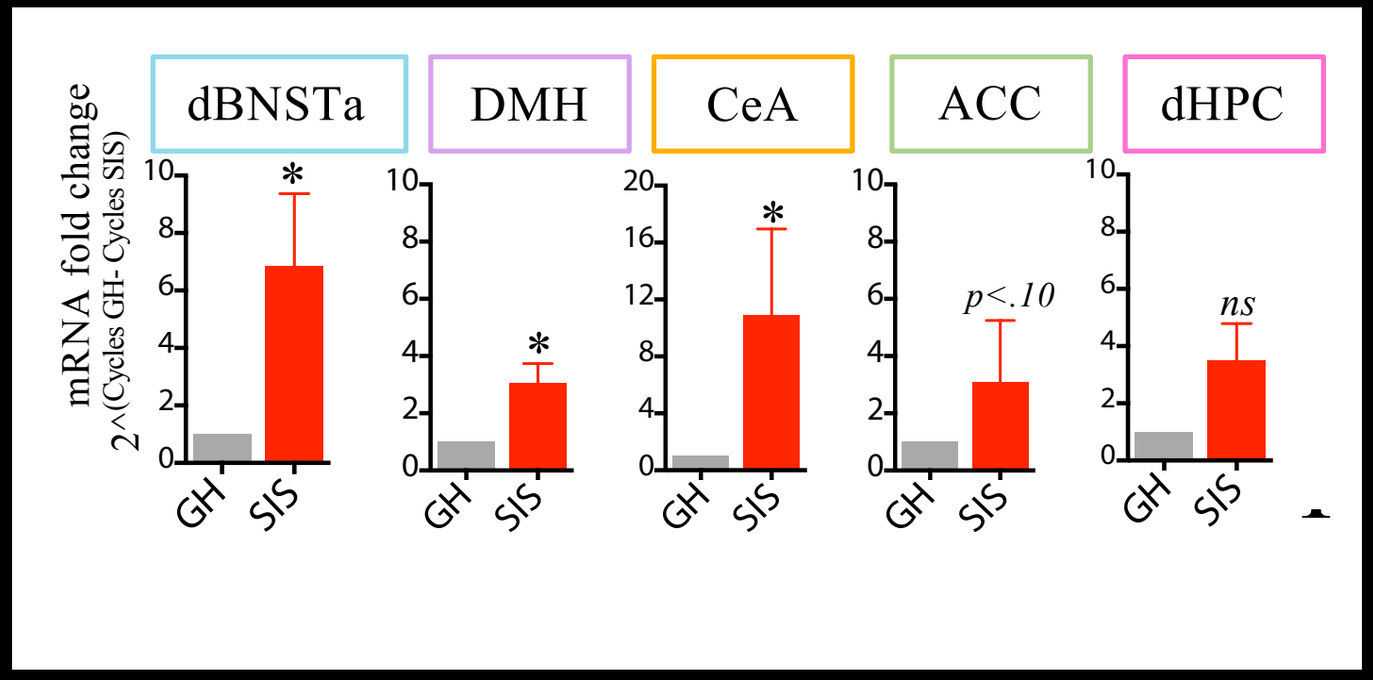


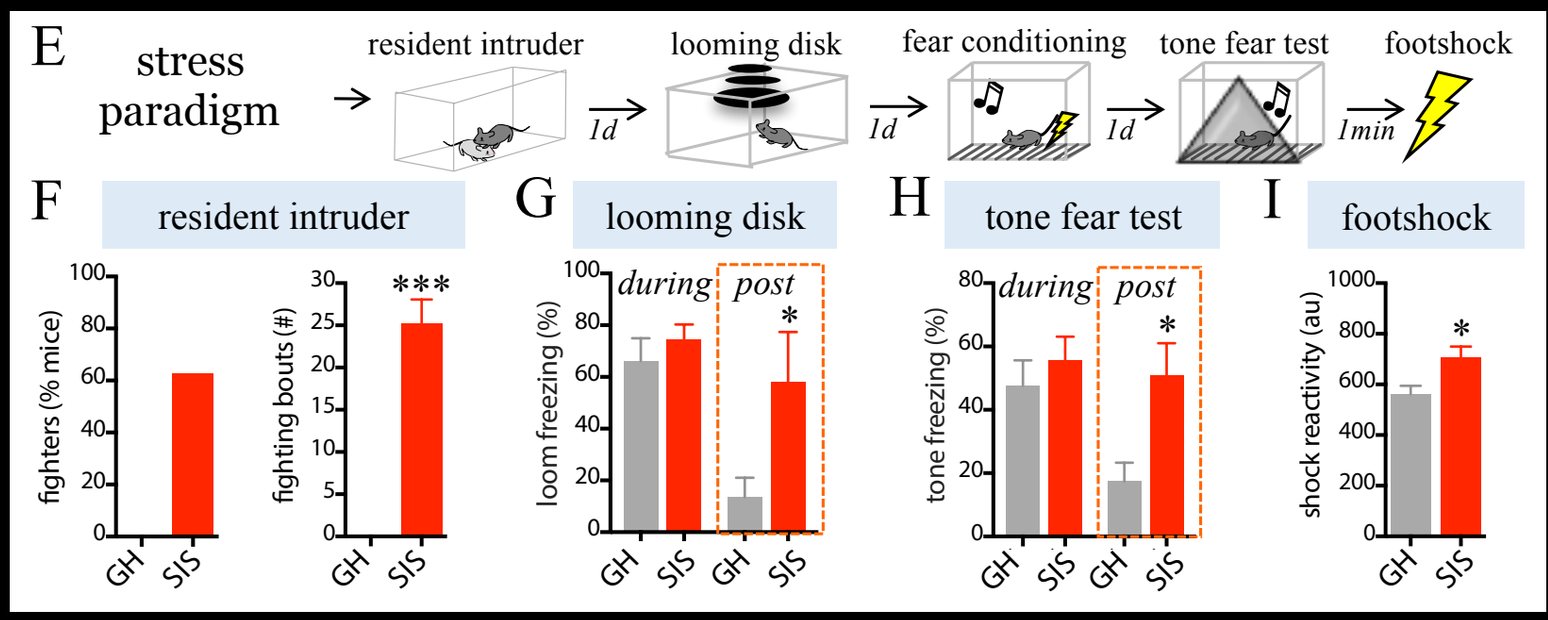
Chan *et al.* Nature Neurosci 2017

Chronic social isolation stress induces widespread increases in the Tac2 neuropeptide

Prolonged social isolation stress causes a dramatic upregulation of the Tac2 neuropeptide

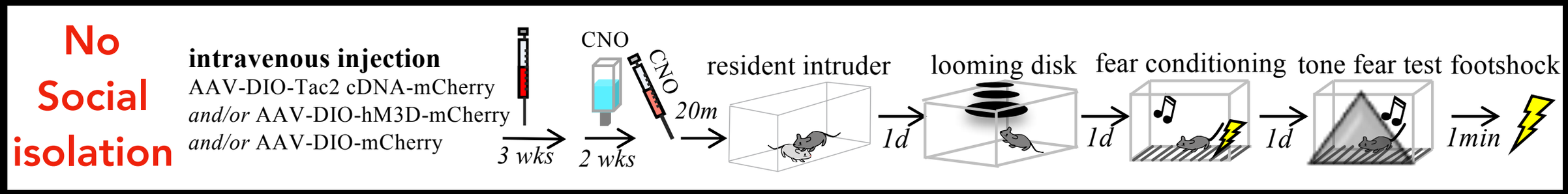


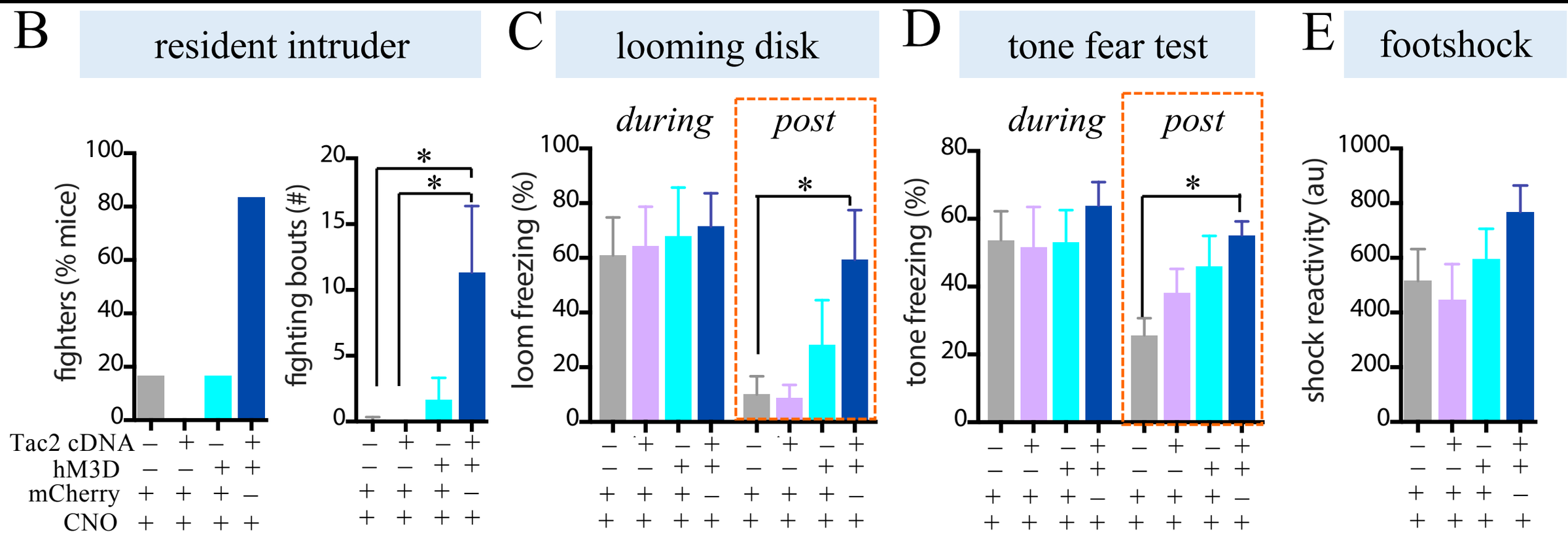




Moriel Zelikowsky et al. Cell 2018 (collaboration with D. Anderson lab - Caltech)

Can the effects of the stress be mimicked through circuit modulation using chemogenetics?

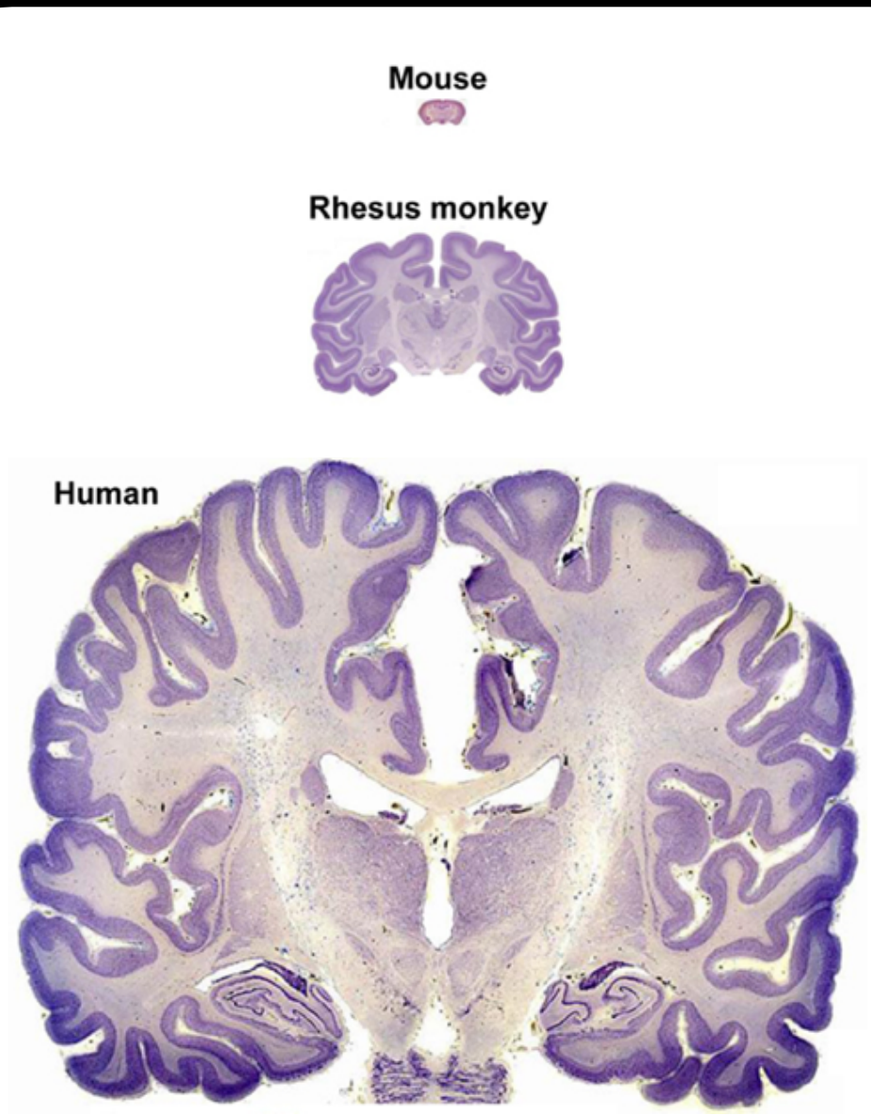




Moriel Zelikowsky et al. Cell 2018 (collaboration with D. Anderson lab - Caltech)

Will the PHP.B vectors work beyond the mouse?

Challenges for gene delivery to the CNS



adapted from DeFelipe – Front. Neuroanat., 15 May 2011

Objectives

- Develop vehicles for safe and efficient noninvasive CNS-wide gene transfer
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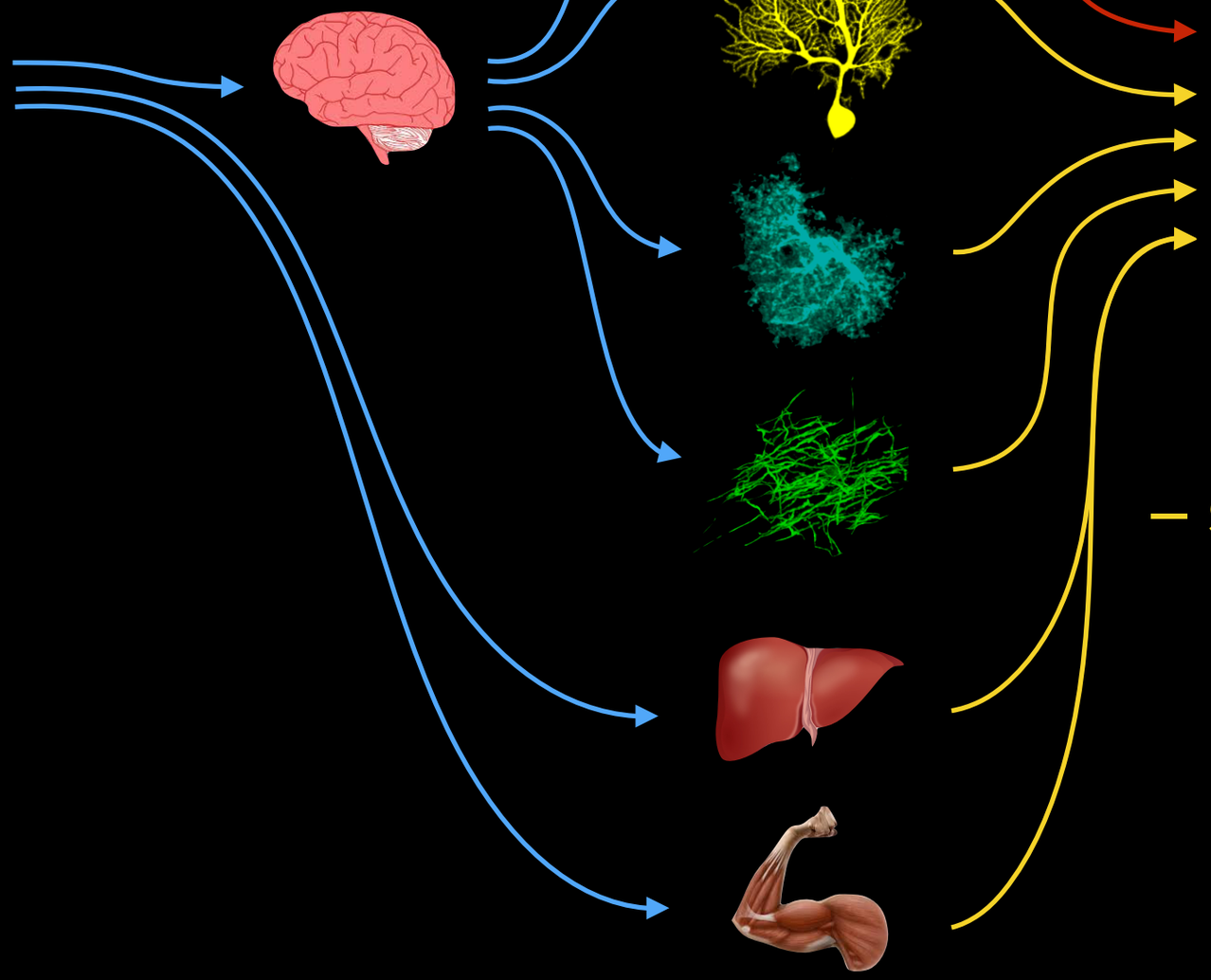
- Develop improved intersectional strategies for specific circuits
- Avoid immune responses to foreign transgenes and viral components

CREATE2.0: *in vivo-in silico* selection for improved AAV variants

parallel selections

AAV capsid libraries



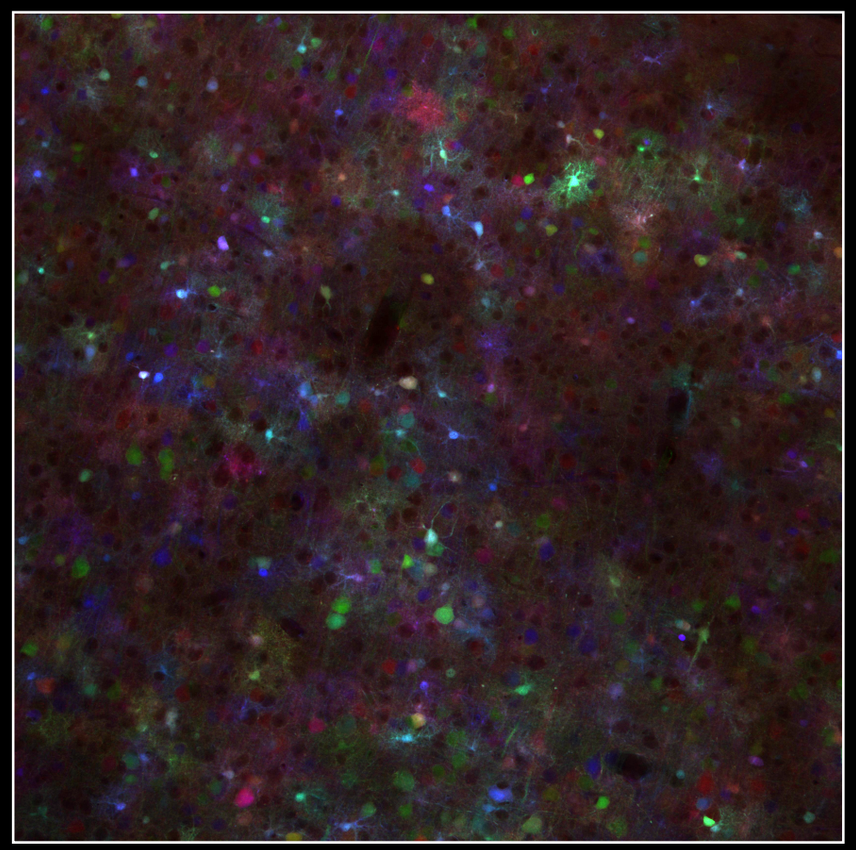
+ selective pressure

cell type selectivity

– selective pressure

Further evolution of AAV-PHP.B for cell type specificity

astrocytes

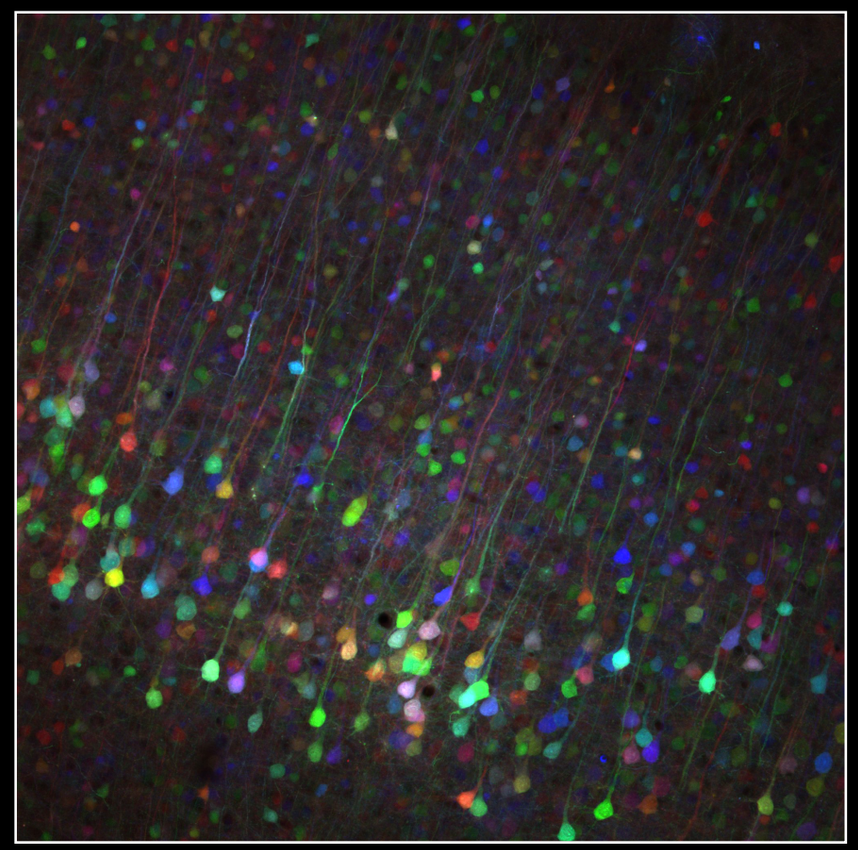


AAV-PHP.B2A

3AA ← PHP.B → 3AA

AAV-CAG-mTurquois2
AAV-CAG-mNeonGreen
AAV-CAG-mRuby2

neurons



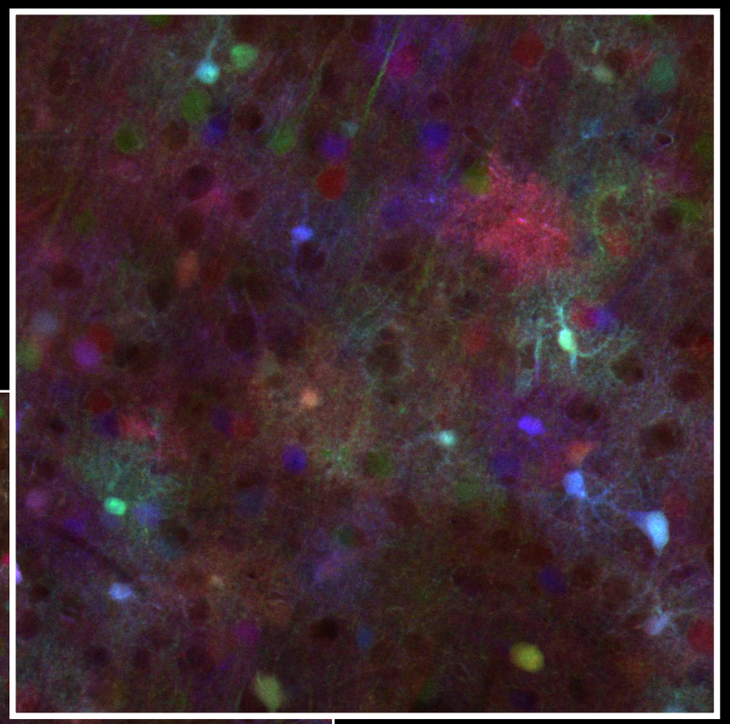
AAV-PHP.B2N

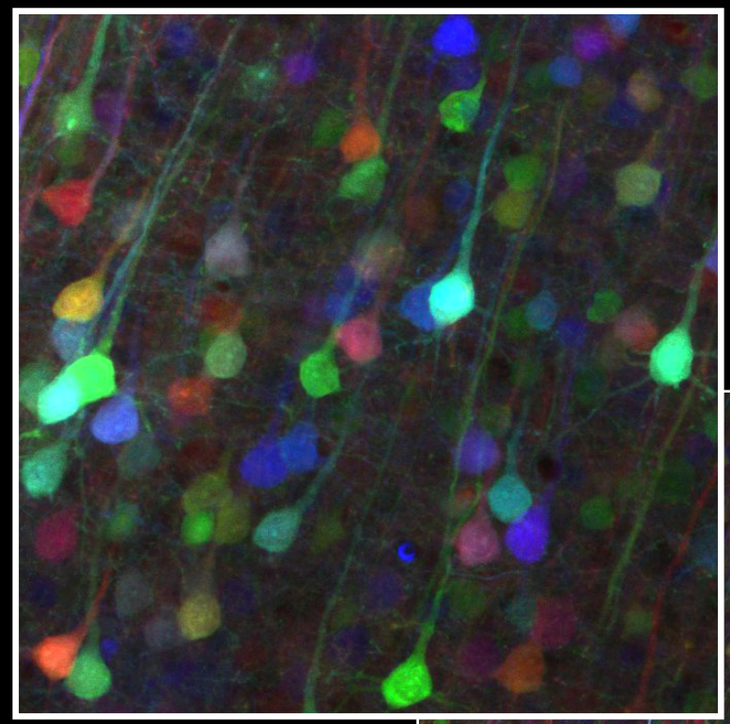
Deverman *et al.* unpublished

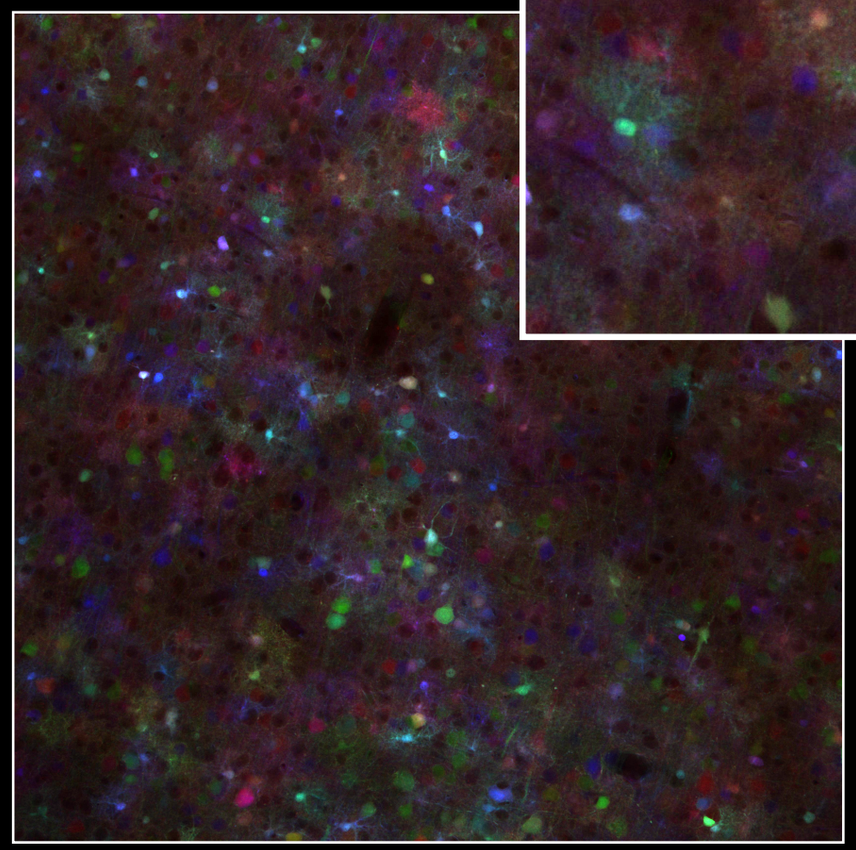
Further evolution of AAV-PHP.B for cell type specificity

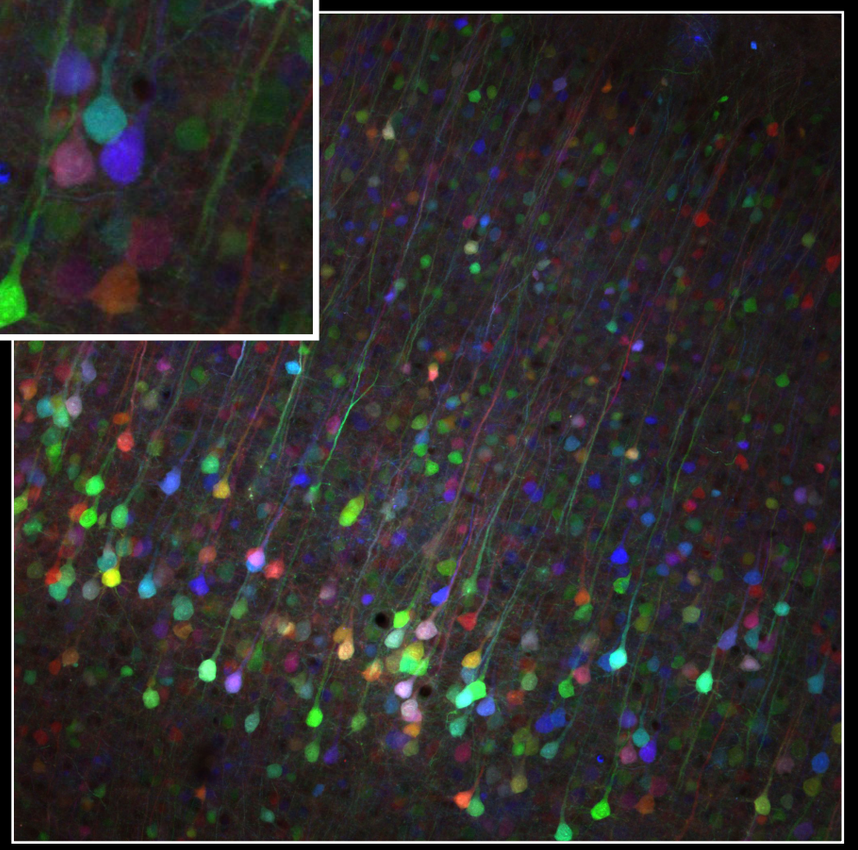
astrocytes

neurons









3AA ← PHP.B → 3AA

AAV-CAG-mTurquois2
AAV-CAG-mNeonGreen
AAV-CAG-mRuby2

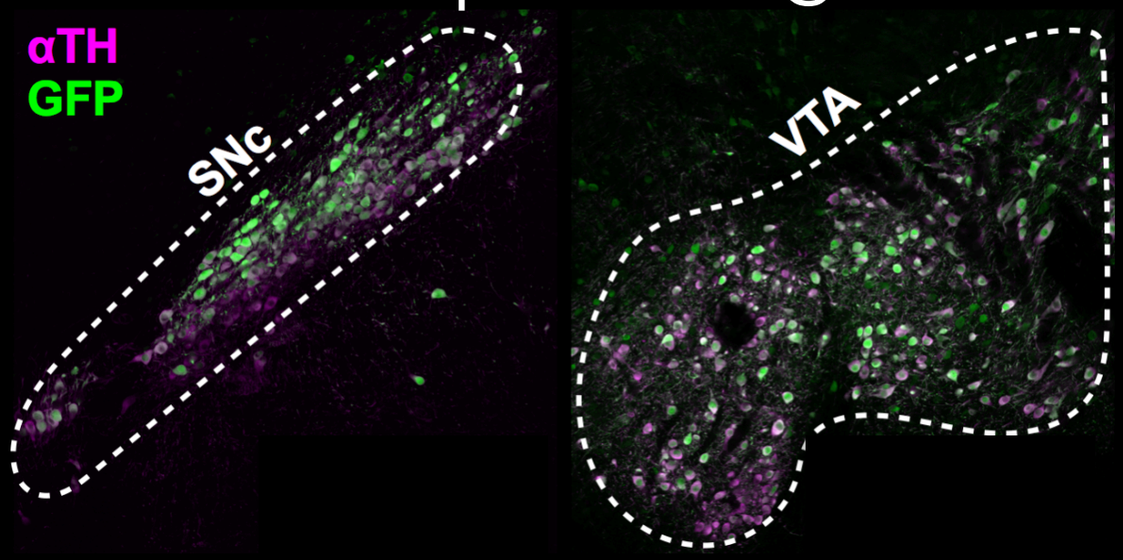
AAV-PHP.B2A

AAV-PHP.B2N

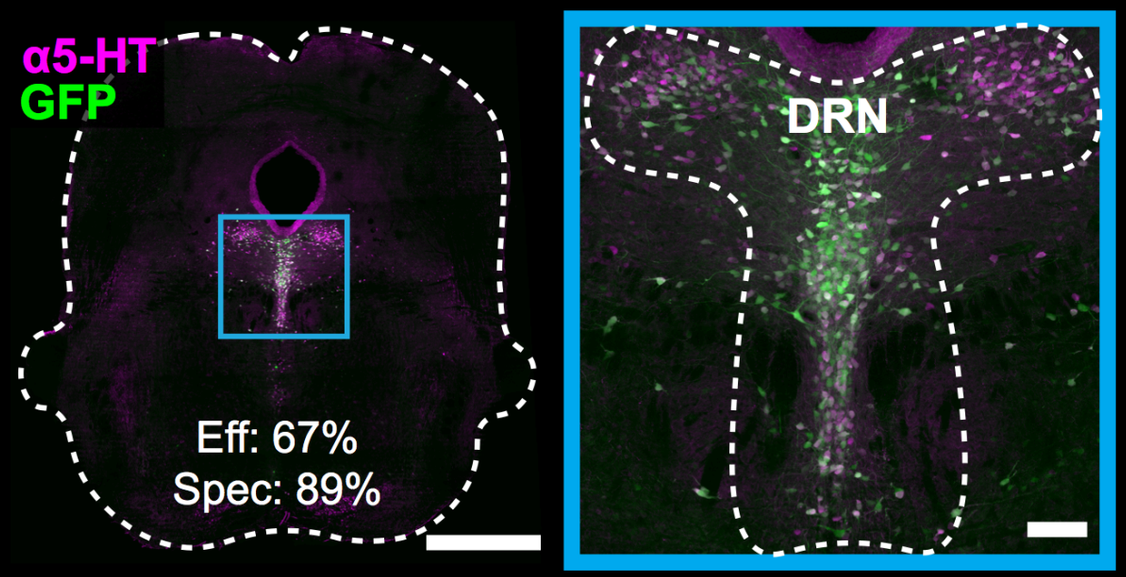
Deverman *et al.* unpublished

Achieving cell type-restricted expression with systemically delivered AAVs

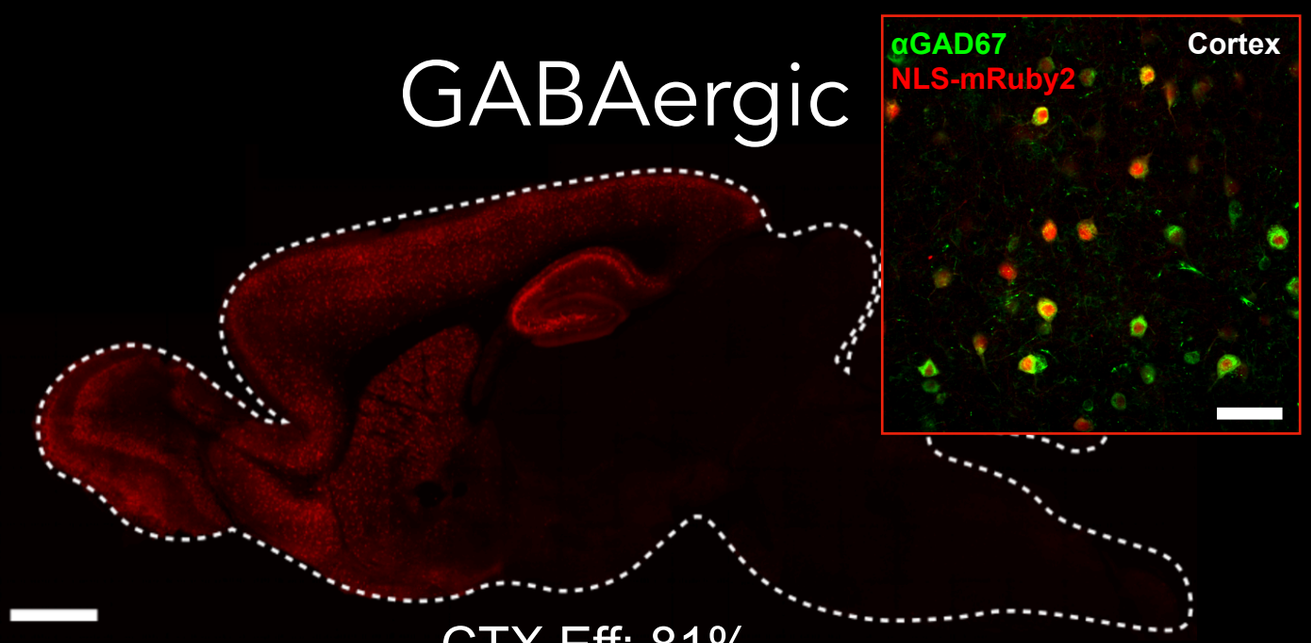
dopaminergic



serotonergic

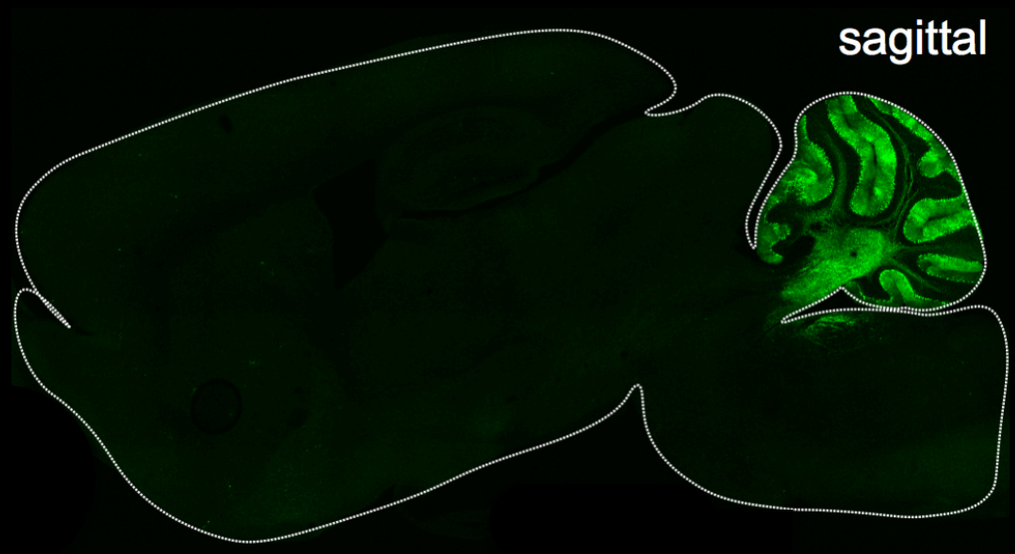


GABAergic



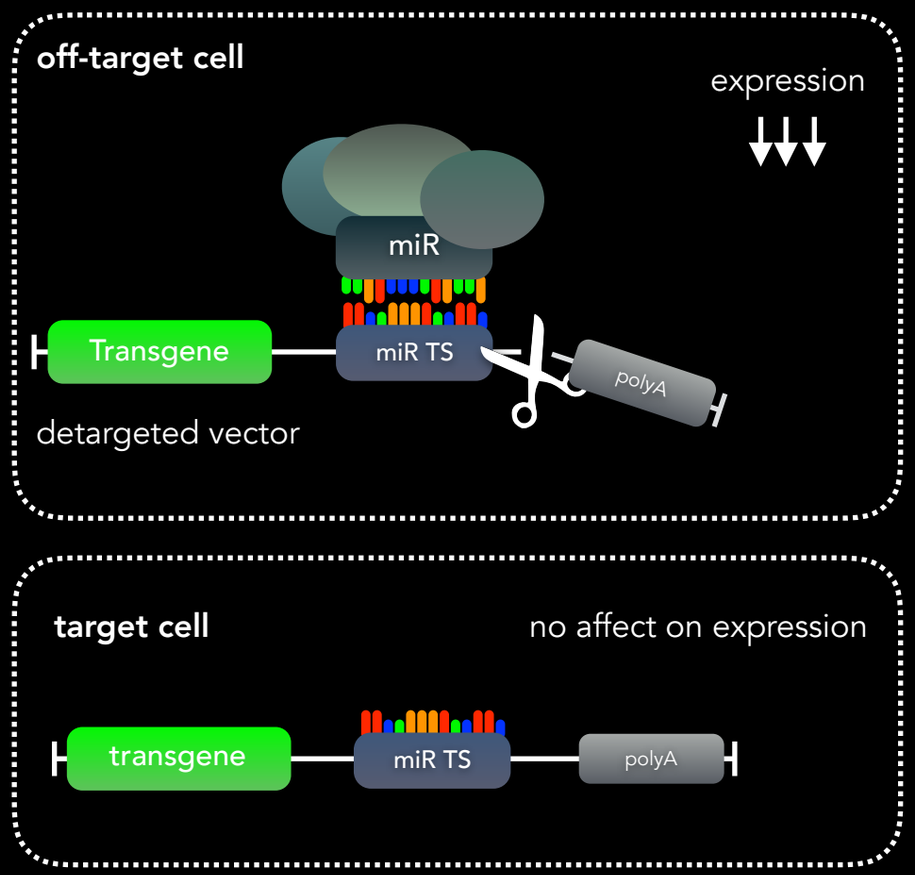
CTX Eff: 81%
CTX Spec: 87%

Purkinje neurons



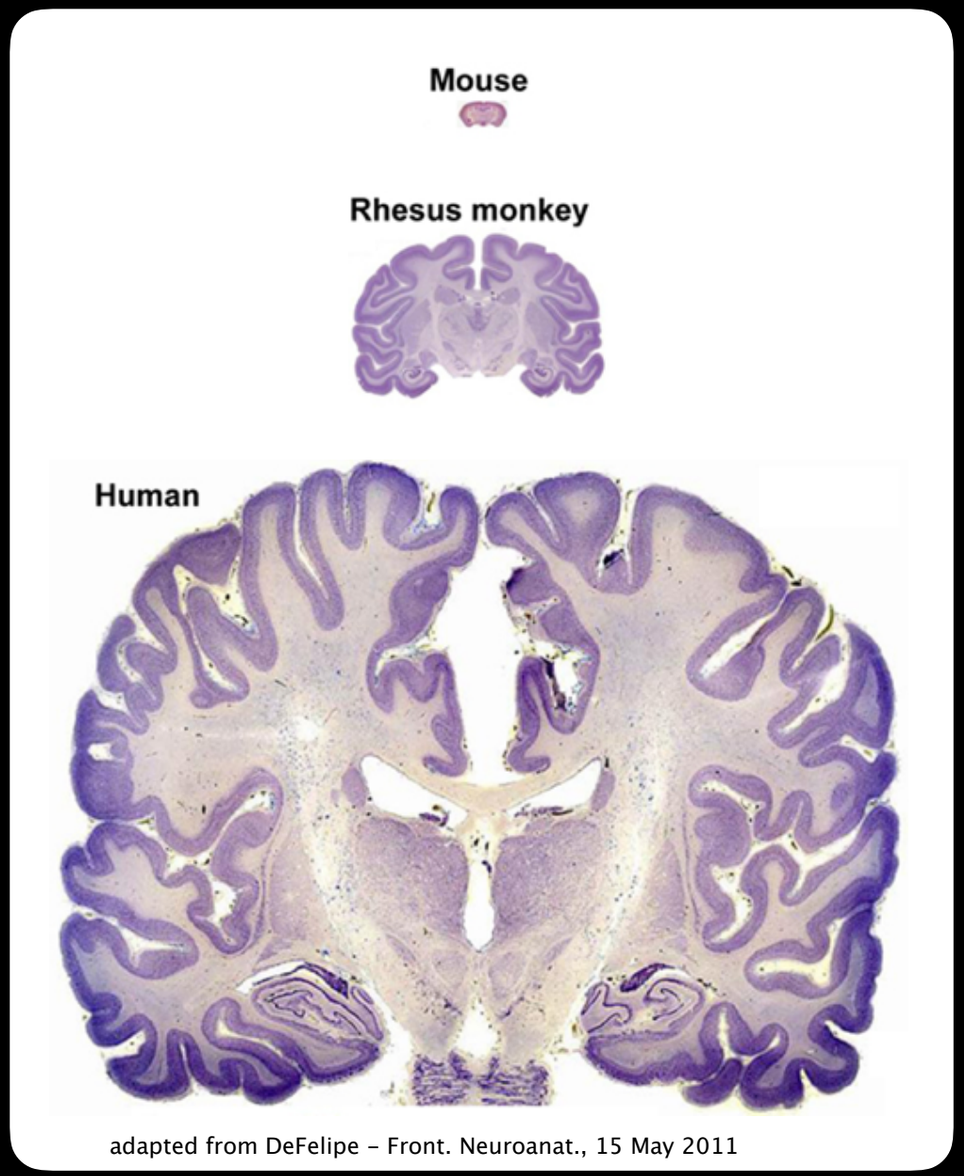
Chan *et al.* Nature Neurosci 2017

MiRNA binding sites added to the 3'UTR can be used to reduce expression in off-target cell types



MiRNA BS	Expression	BS Sequence	Reference
miR-122	Liver hepatocytes	ACAAACACCATTGTCACACTCCA	Xie et al 2011
miR-1	Skeletal muscle	ATACATACTTCTTTACATTCCA	Xie et al 2011
miR-124	Differentiated neurons	CCGTAAGTGGCGCACGGAAT	Karali et al 2011
miR-128-3p	Excitatory neurons	AAAGAGACCGGTTCACTGTGA	Keaveney et al 2018
miR-142-3p	Antigen presenting cells	TCCATAAAGTAGGAAACACTACA	Majowicz et al 2013
miR-181	Inner and middle retina	ACTCACCGACAGGTTGAA	Kay et al 2013
miR-221-3p	Excitatory neurons	GAAACCCAGCAGACAATGTAGCT	Keaveney et al 2018
miR-204	Retinal pigmented epithelium	TCCGTATCCTACTGTTTCCCTT	Karali et al 2011

Challenges for gene delivery to the CNS



adapted from DeFelipe – Front. Neuroanat., 15 May 2011

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- Avoid immune responses to foreign transgenes and viral components

Efficient and selective expression of genetically encoded modulators and sensors in defined cell types in genetically intractable animals

AAV capsid engineering

Tropism A

Tropism B

AAV genome

promoter — DREADDs — miR BSs

Sensors

gene regulatory elements

New safer Intersectional strategies

AAV2-retro

MNM 008

retrograde inducer

inducible Effector eg, DREADD

AAV

Acknowledgements

Stanley Center Vector Engineering

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NIH

Beckman Institute

CHDI

Hereditary Disease Foundation

Caltech-City of Hope Biomedical Initiative

Voyager Therapeutics