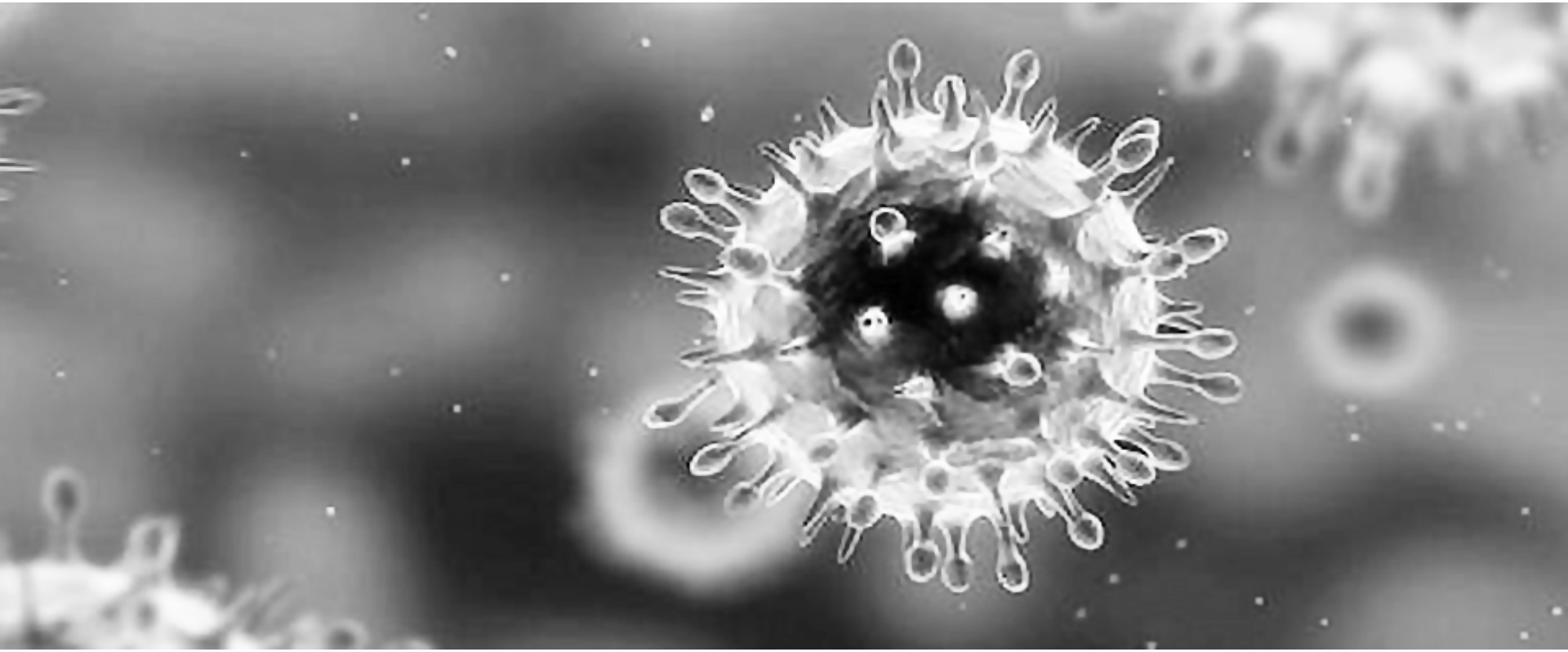
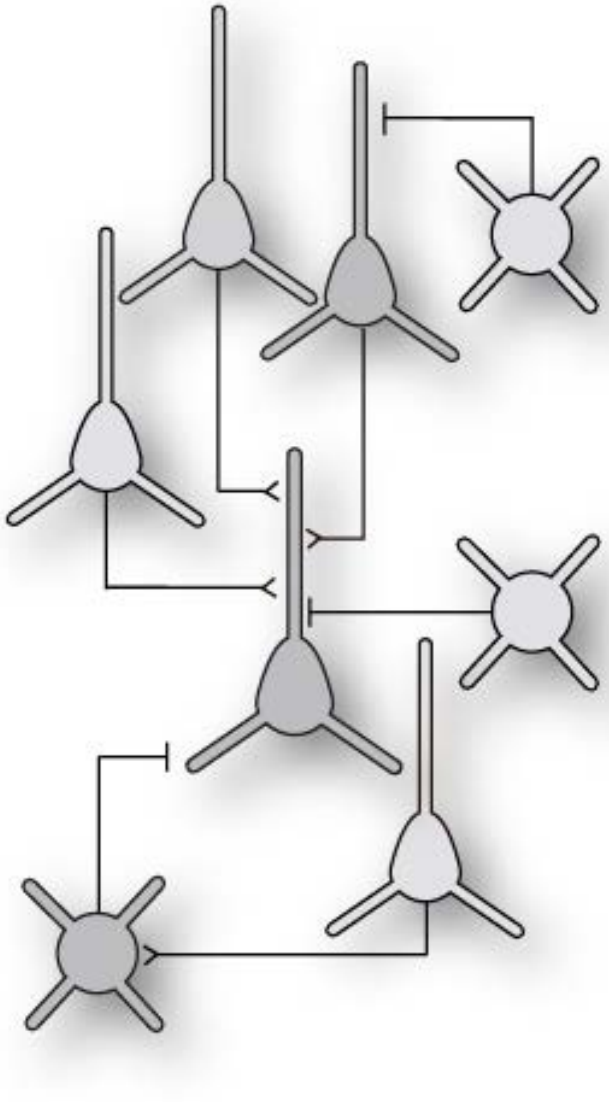
Viral vectors for neural circuit analysis

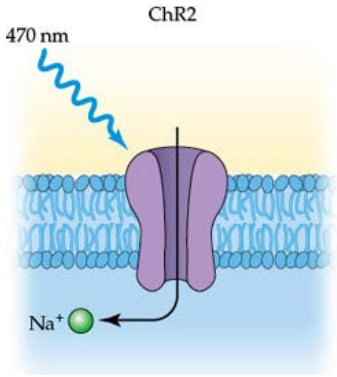


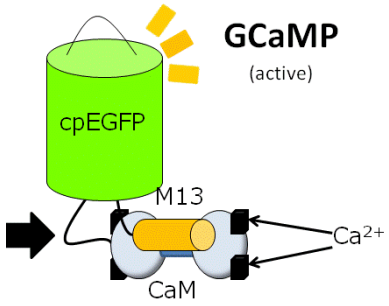
Sainsbury Wellcome Centre

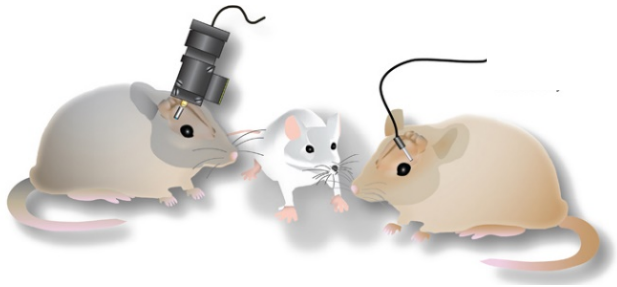
Andy Murray
a.murray@ucl.ac.uk

Gaining experimental access to neural circuits

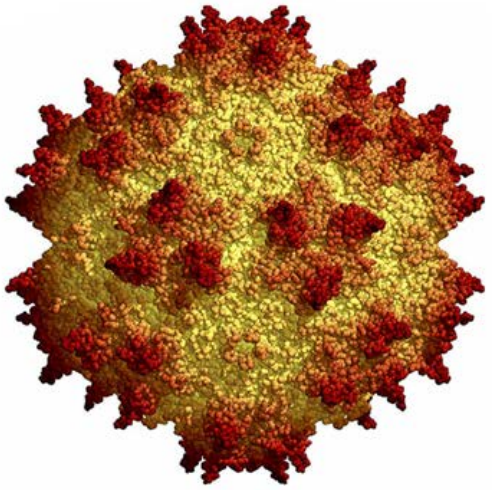


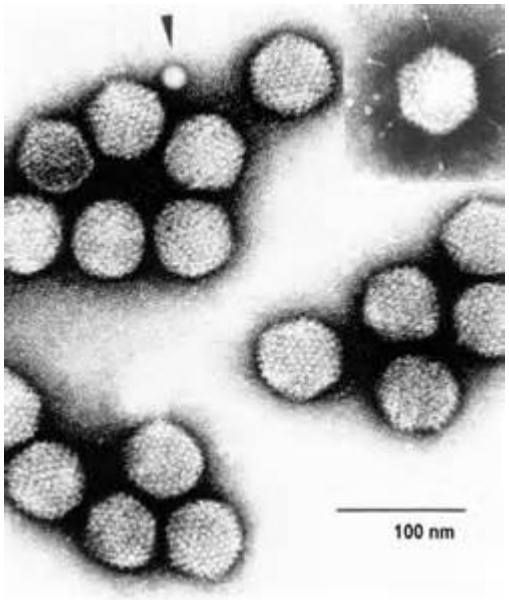






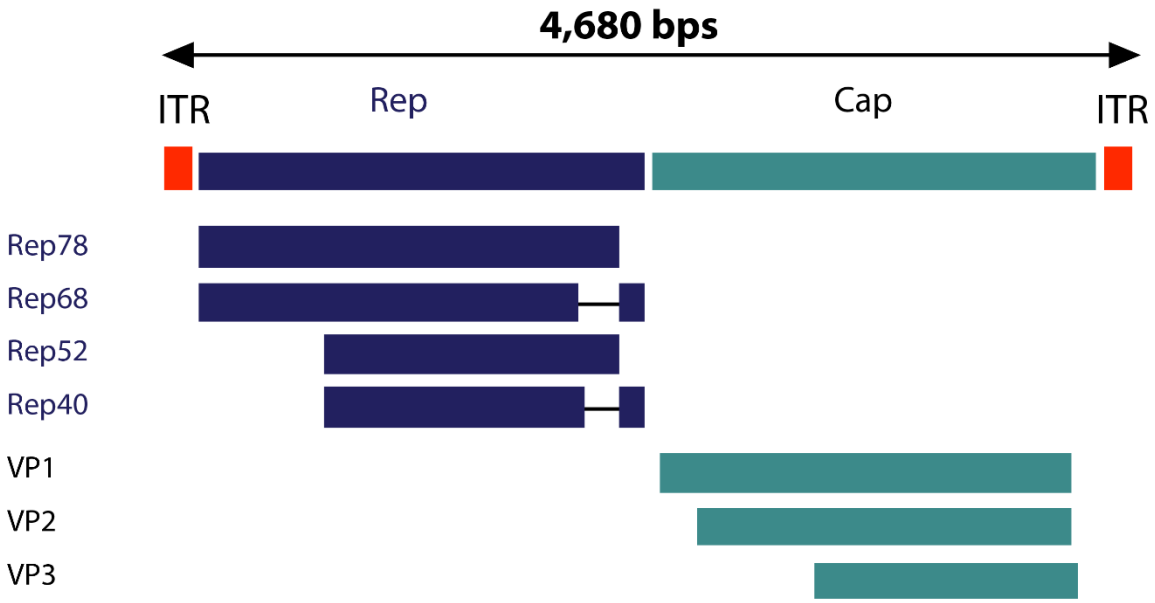
Adeno-associated virus (AAV)





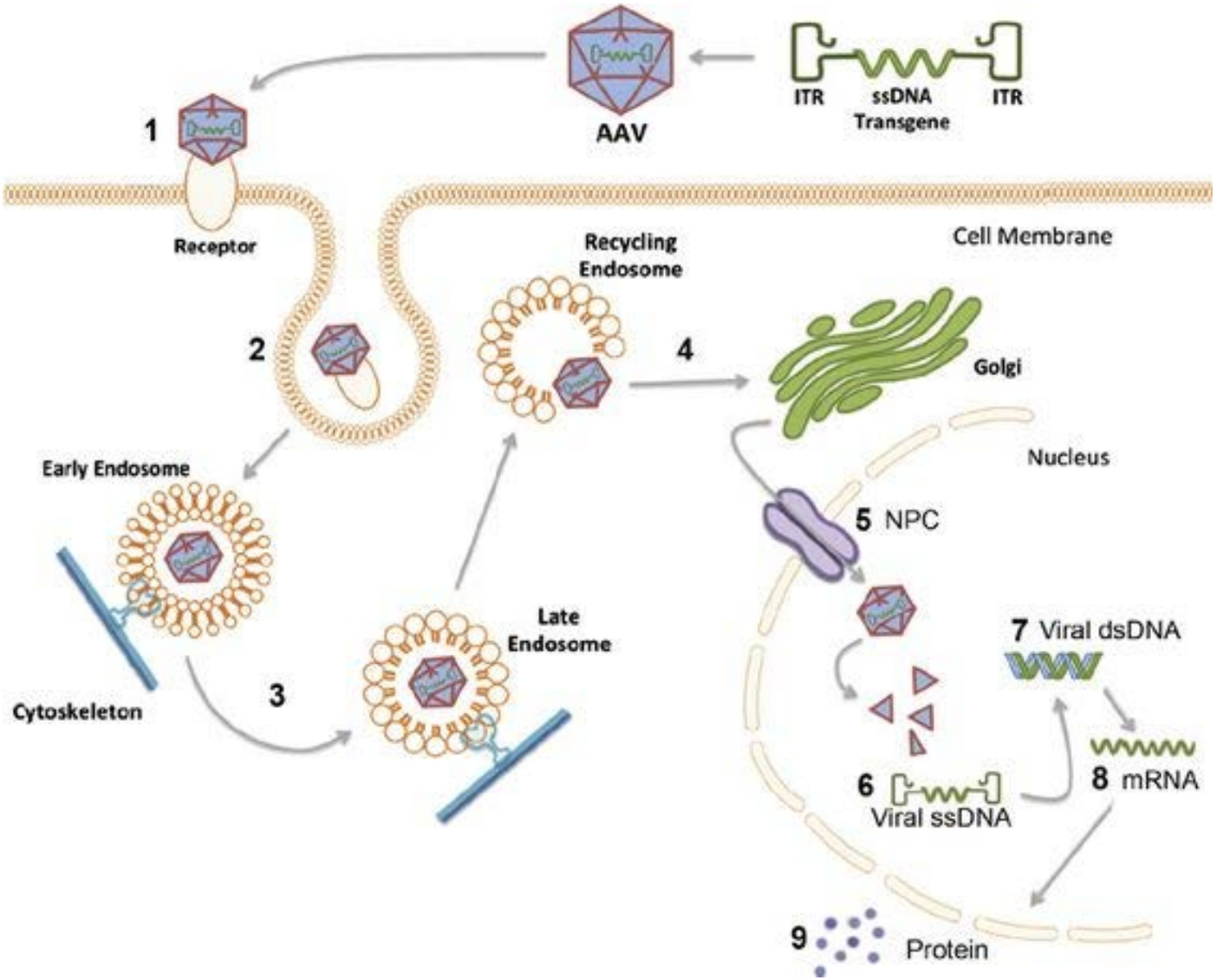
Adeno-associated virus (AAV)

AAV2 Genome Map



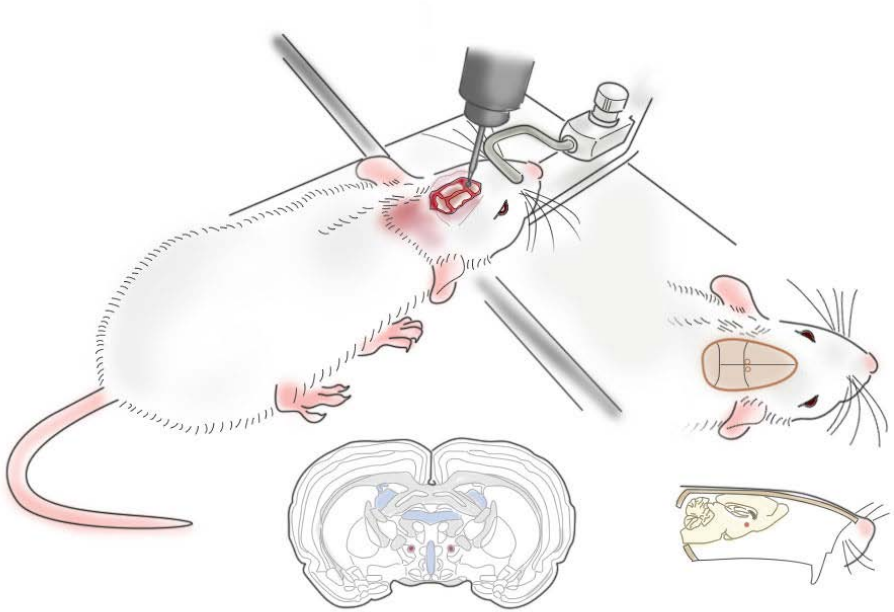
Rep78/68: involved in genome replication
Rep52/40: involved in genome packaging.
VP 1/2/3: capsid proteins.

Adeno-associated virus (AAV)

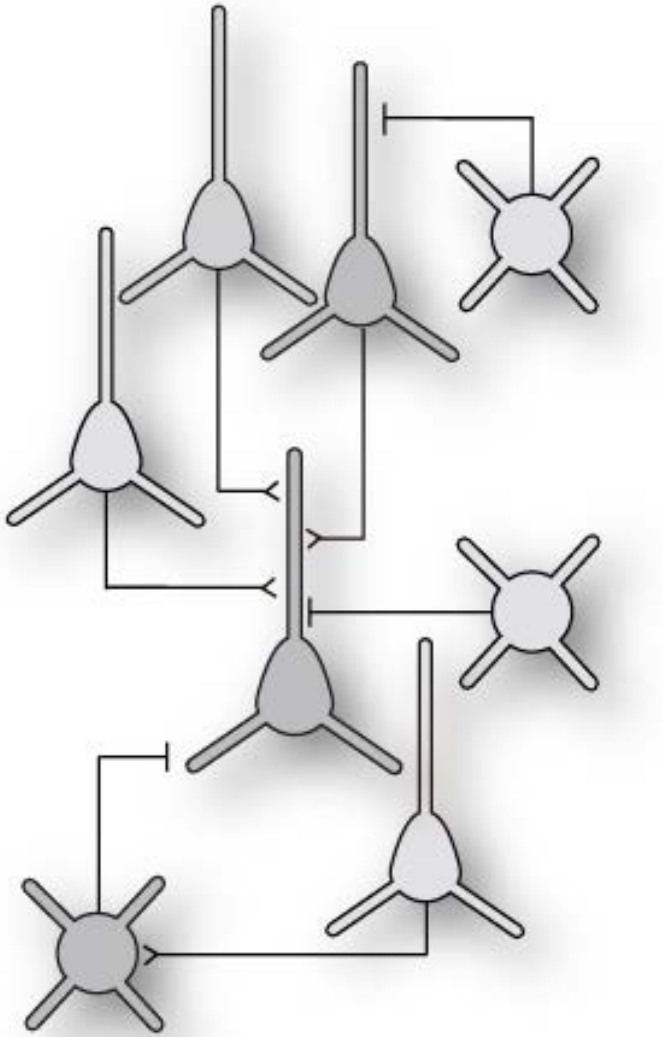


Recombinant AAV (rAAV)

4.9 Kb

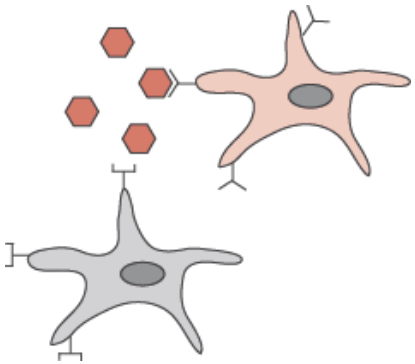


rAAV – Targeting the right cell type – how?



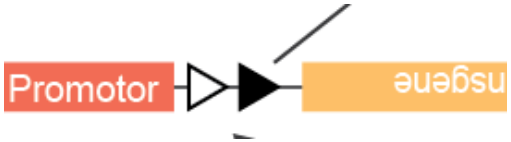
How do we target specific neuron types with AAV?

1. capsid tropism

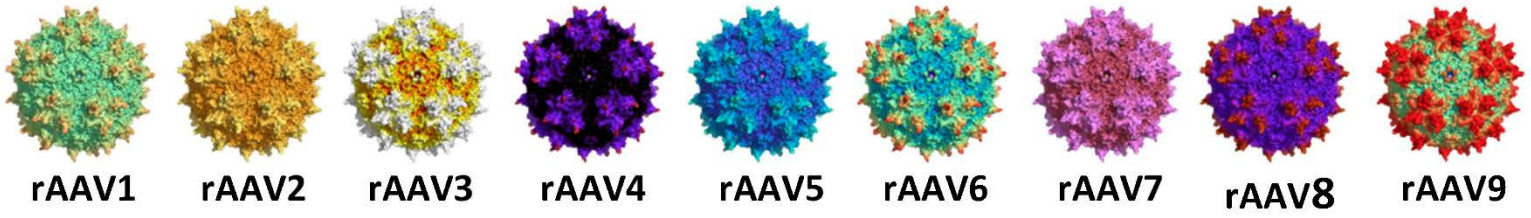


2. selective promotor

3. recombinase expression



rAAV capsid tropism – natural variants



	rAAV1	rAAV2	rAAV3	rAAV4	rAAV5	rAAV6	rAAV7	rAAV8	rAAV9
Primary receptor	N-linked sialic acid	HSPG	HSPG	O-linked sialic acid	N-linked sialic acid	N-linked sialic acid; HSPG	unknown	unknown	N-linked galactose
Secondary receptor	unknown	FGFR1, HGFR, integrins, CD9, LamR	FGFR1, HGFR, LamR	unknown	PDGFR	EGFR	unknown	LamR	LamR

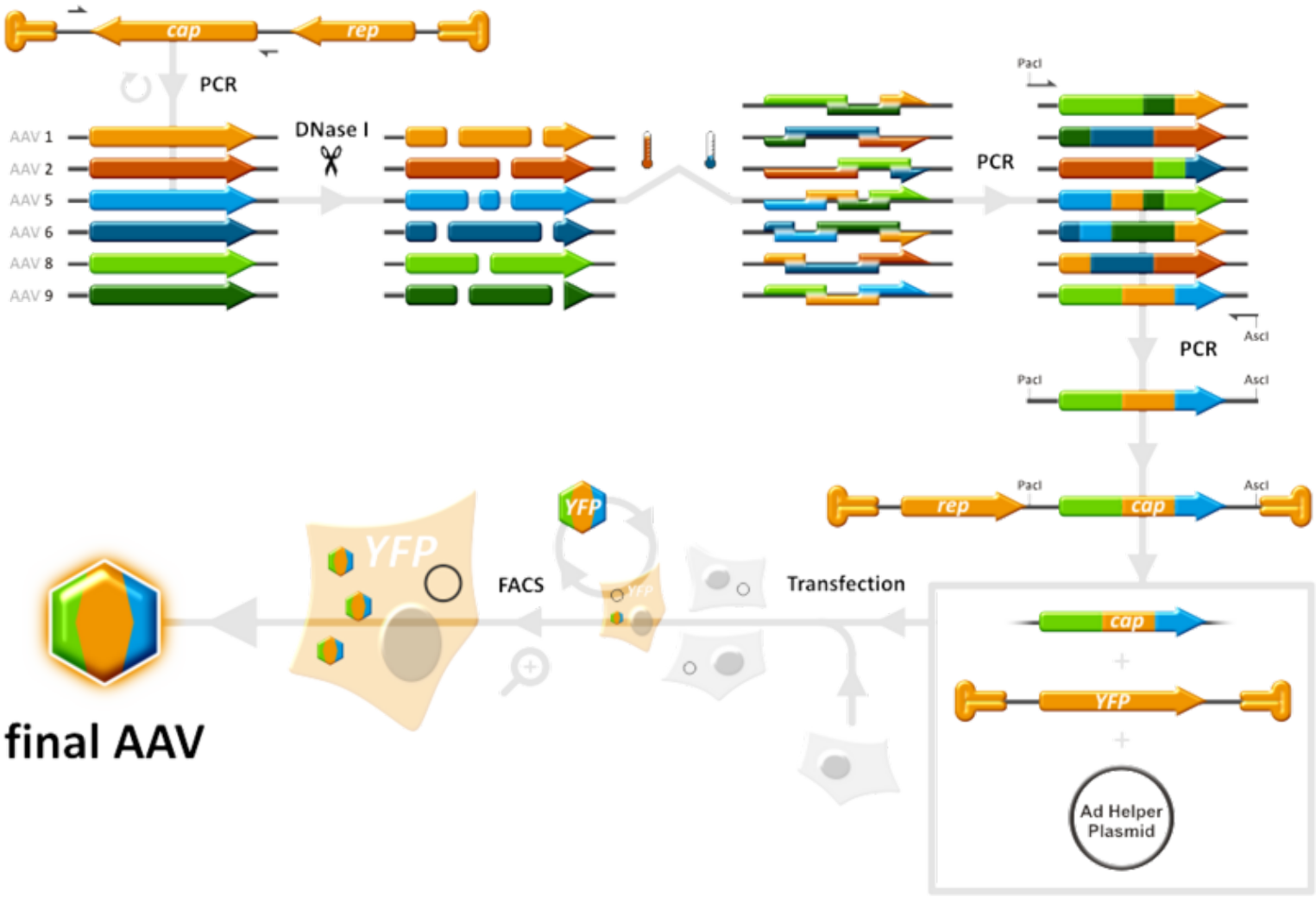
Vance et al., 2015

rAAV capsid tropism – natural variants

Serotype	AAV2/1	AAVDJ8	AAV9
cortex	+++	+++	+++
cerebellum	++	-/+	+
hippocampus	+++	+++	++
olfactory bulb	+++	++	+
striatum	+	+	+
substantia nigra	-/+	+	++

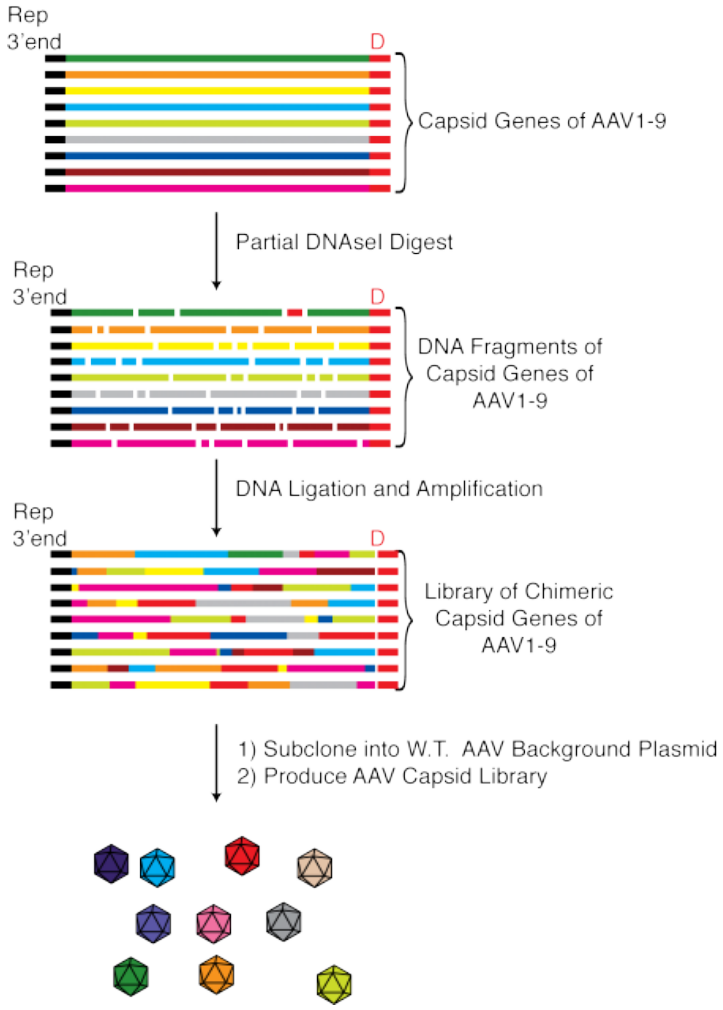
https://doi.org/10.1371/journal.pone.0188830.t001

rAAV capsid tropism – engineered variants



http://2010.igem.org/Team:Heidelberg/Notebook/Capsid_Shuffling/Homology_Based

rAAV capsid tropism – engineered variants



rAAV capsid tropism – engineered variants

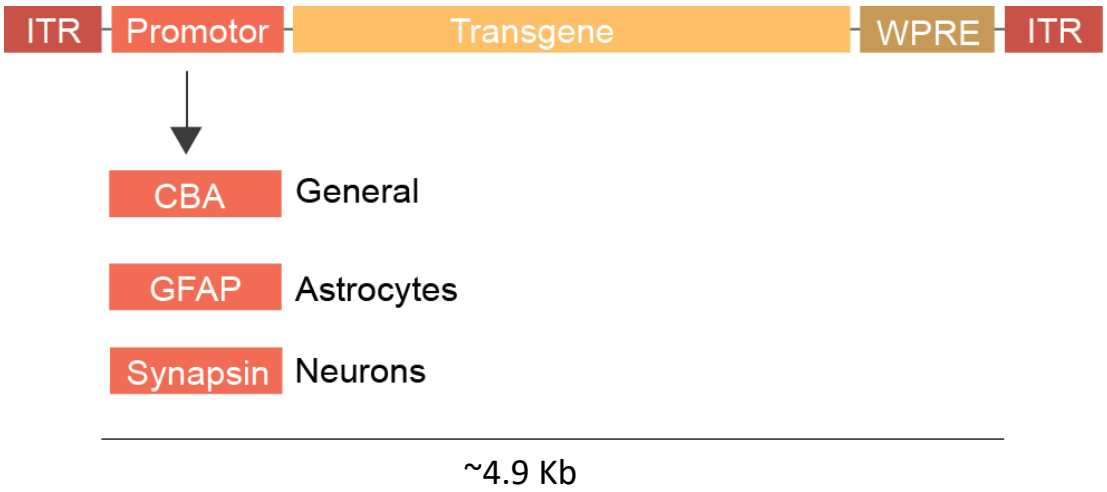
AAV-DJ (Grimm et al., J. Virol. 2008)
- Broad neuronal tropism

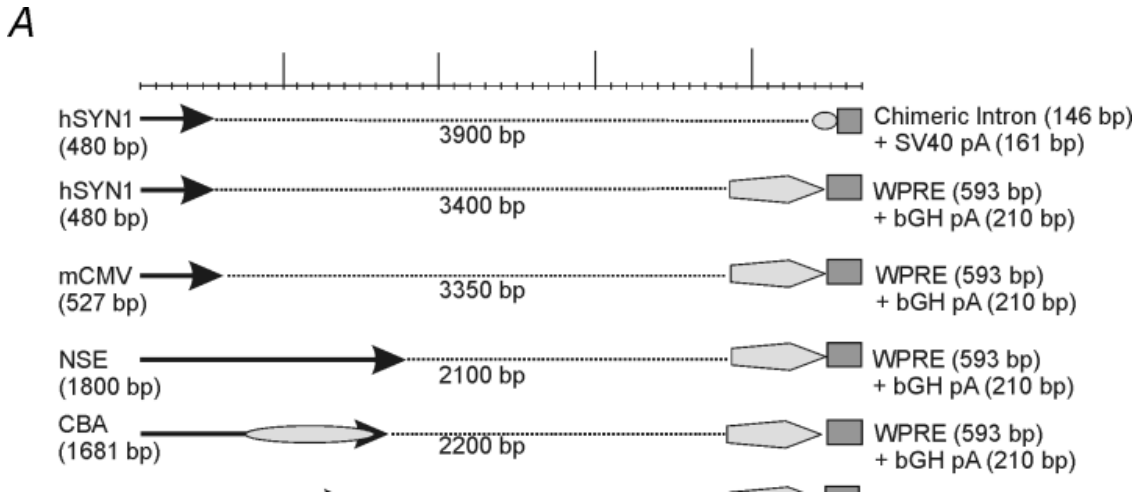
AAV-Retro (Tervo et al., Neuron. 2016)
-Infects neuronal terminals

AAV-Php.eB (Deverman et al., Nat. Biotech. 2016)
-Infects CNS after venous injection

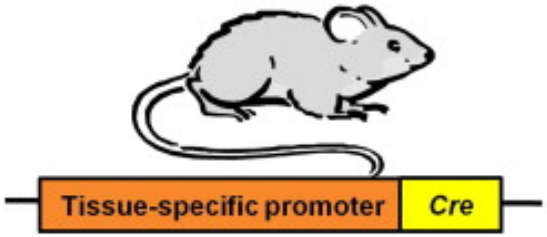
AAV-PhP.S (Deverman et al., Nat. Biotech. 2016)
-Infects PNS after venous injection

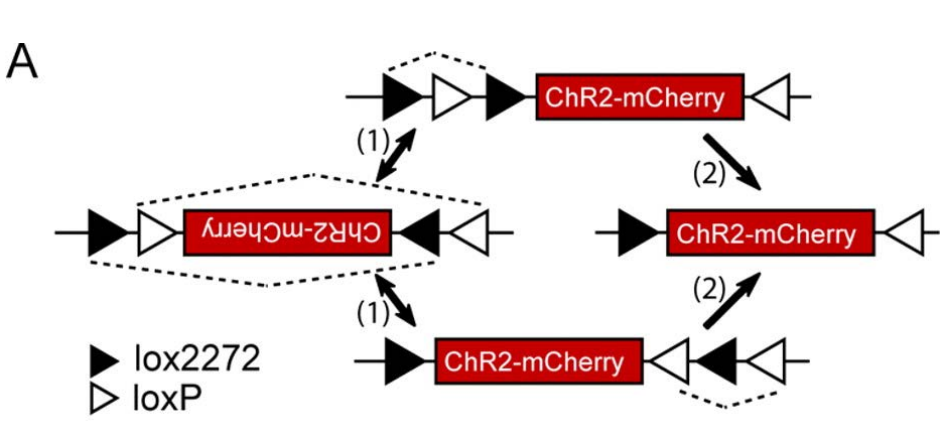
rAAV – selective promoters

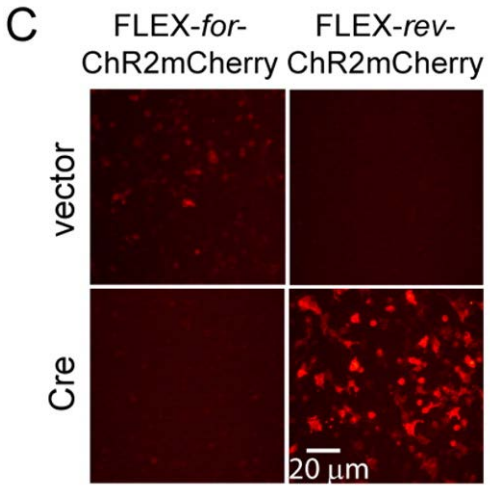




rAAV – recombinase expression



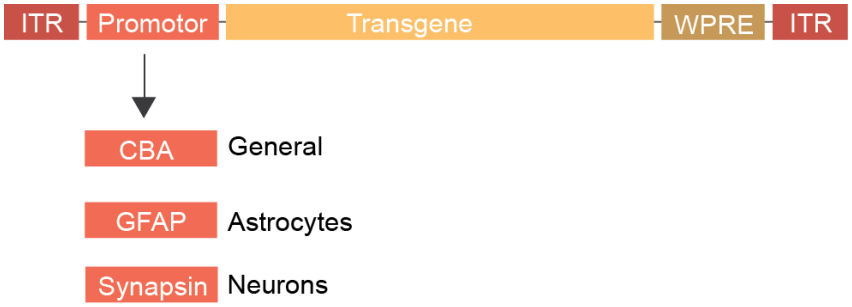




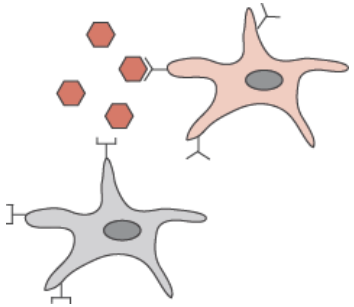
Atasoy et al., 2008

How do we target specific neuron types with AAV?

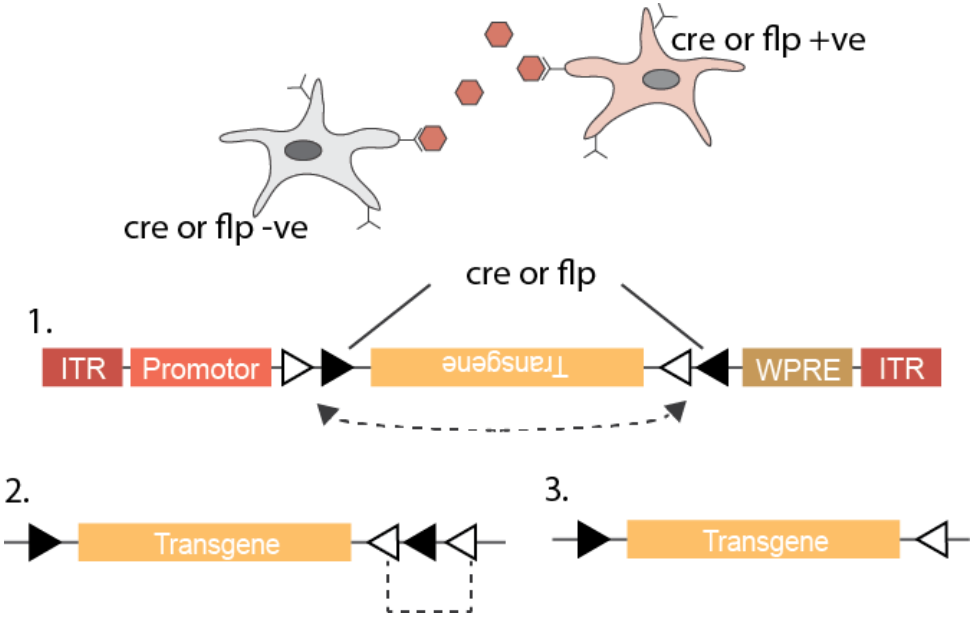
promoter



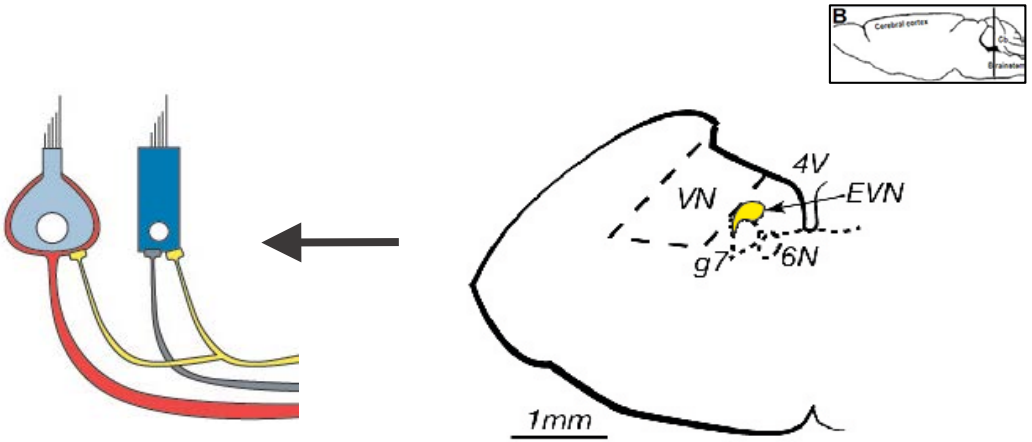
capsid tropism

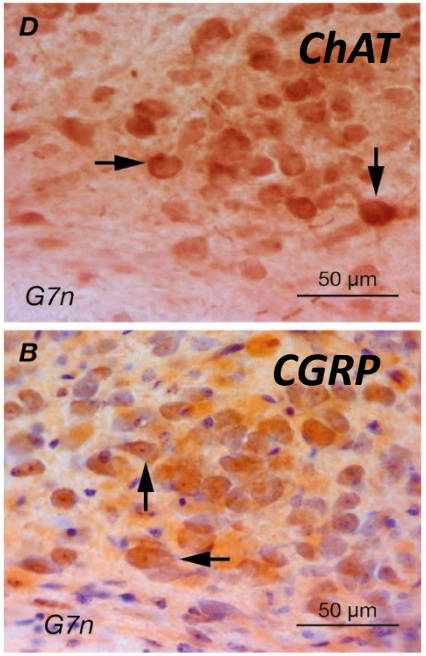


recombinase expression



Intersectional targeting with AAVs

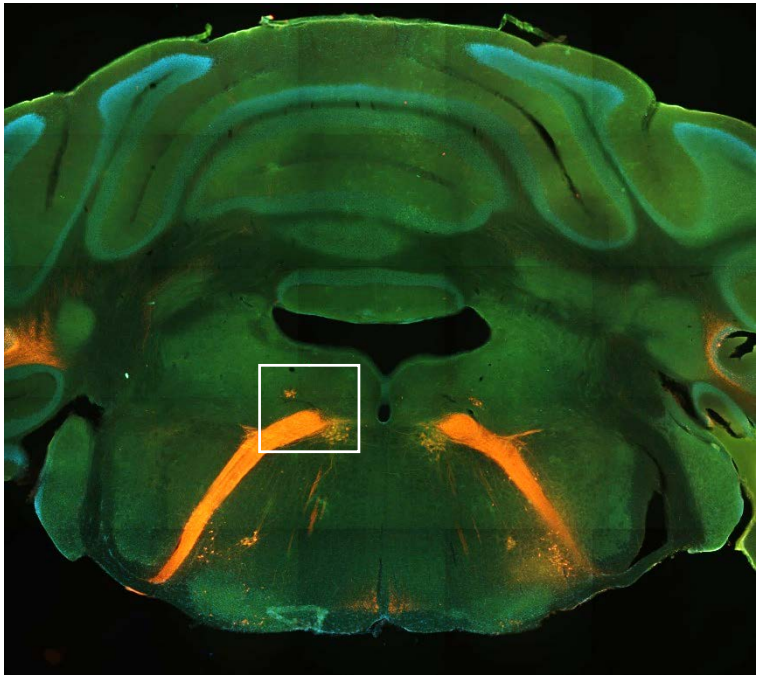


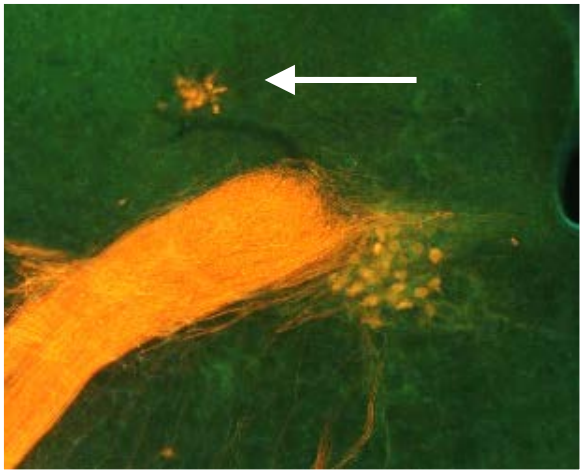


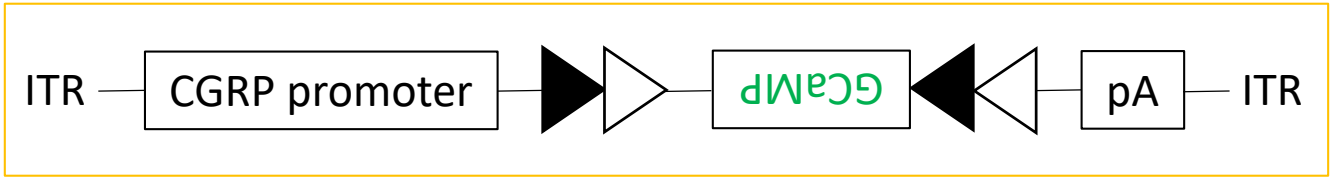
Victoria Tung

Intersectional targeting with AAVs

ChAT::Cre; tdTomato

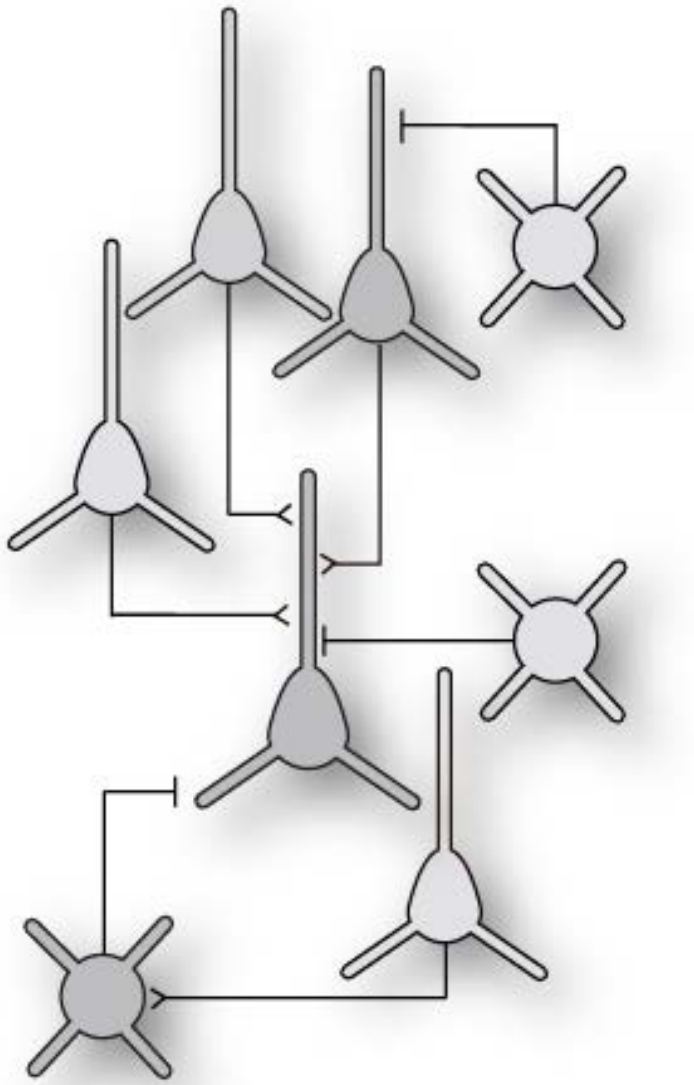




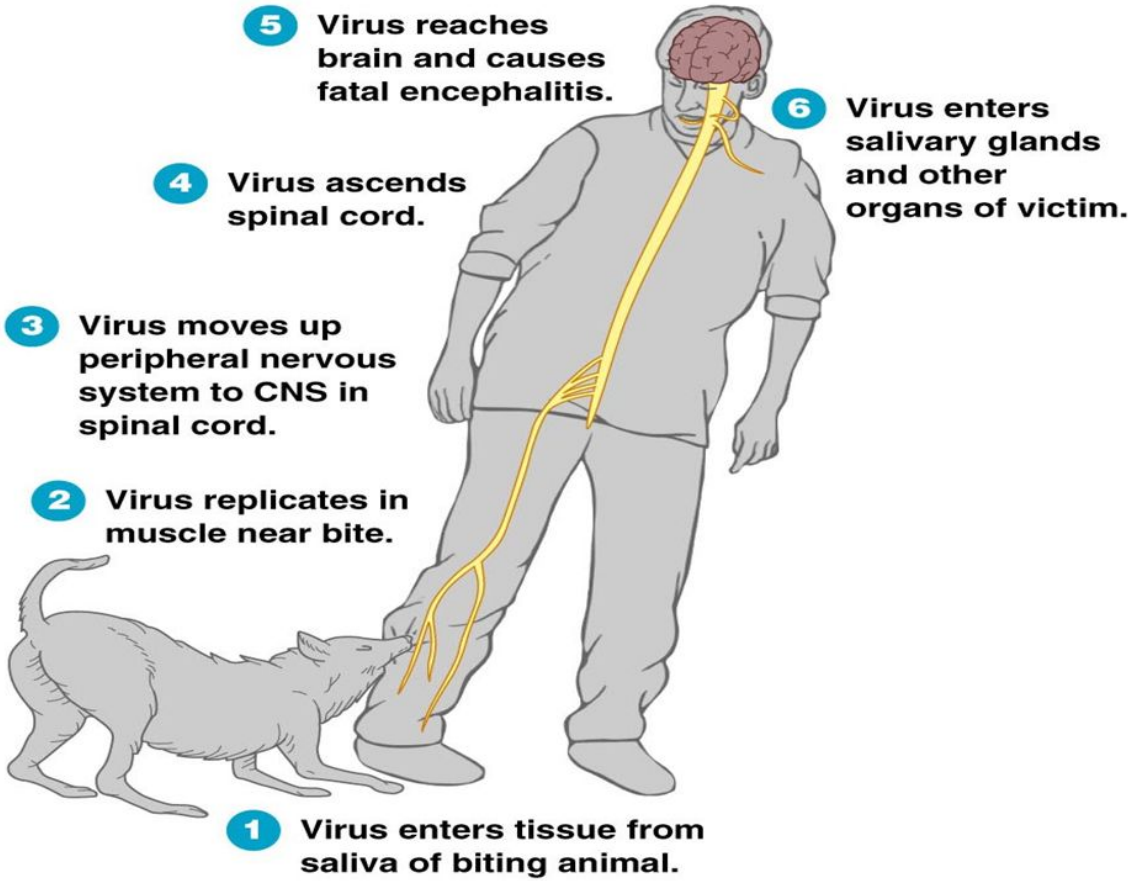


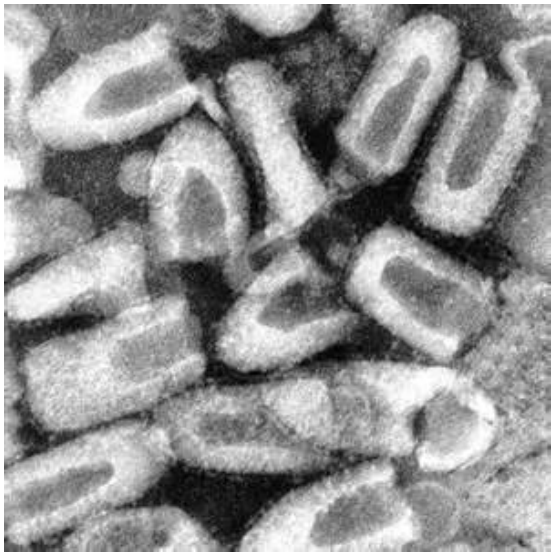
Victoria Tung

Defining inputs to genetically identified neurons

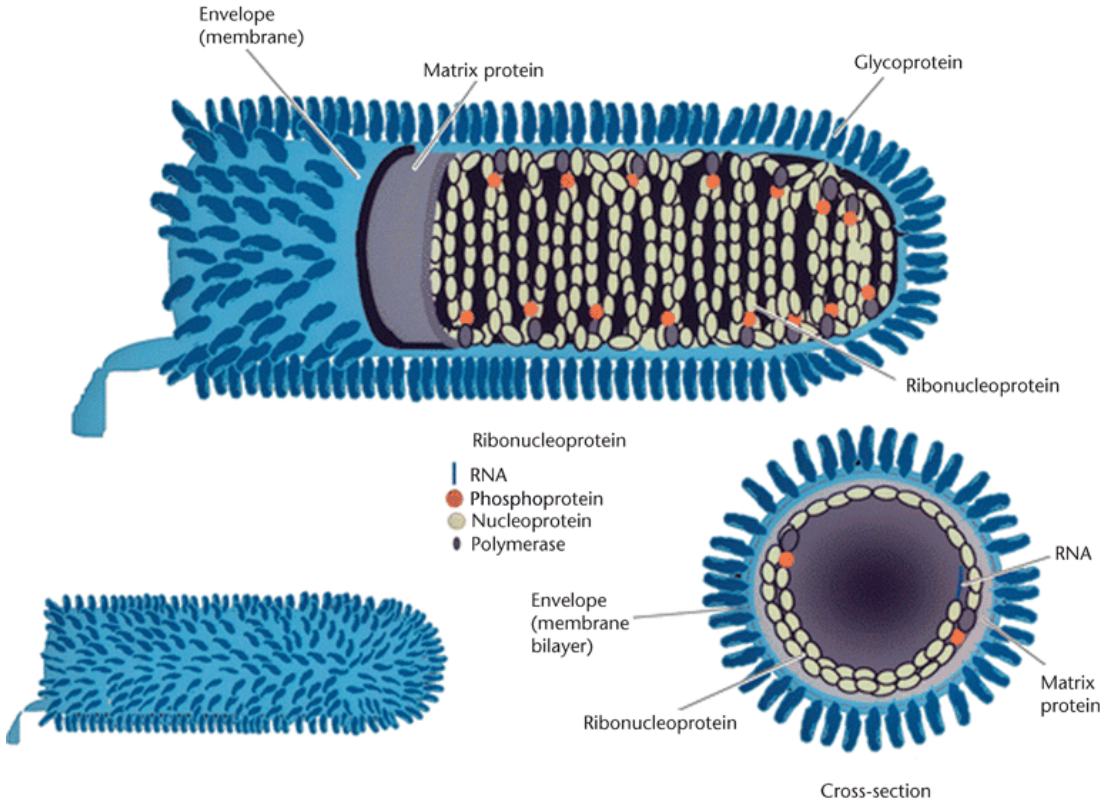


Rabies virus as a retrograde transsynaptic tracer

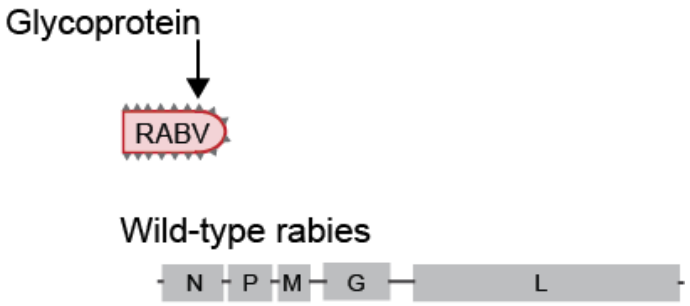


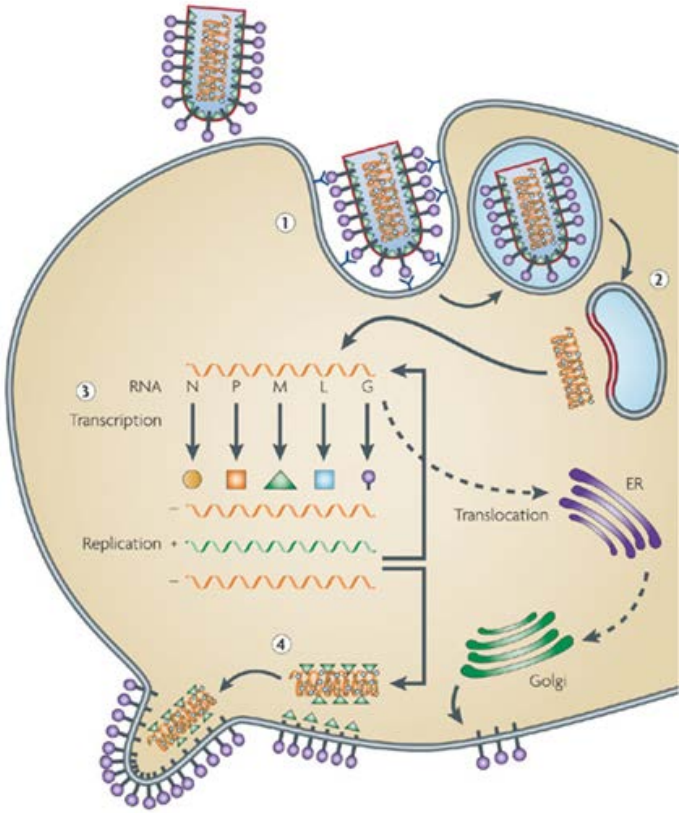


Rabies virus as a retrograde transsynaptic tracer



Monosynaptic tracing with rabies virus

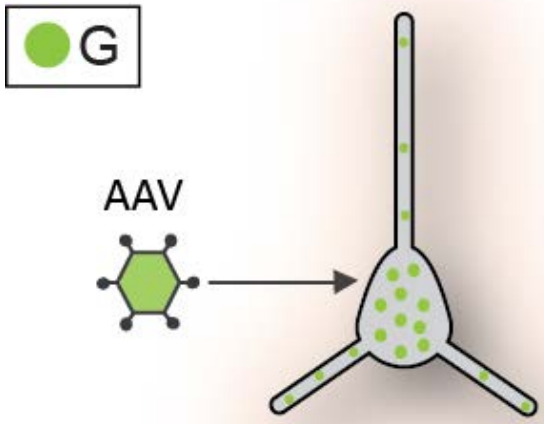




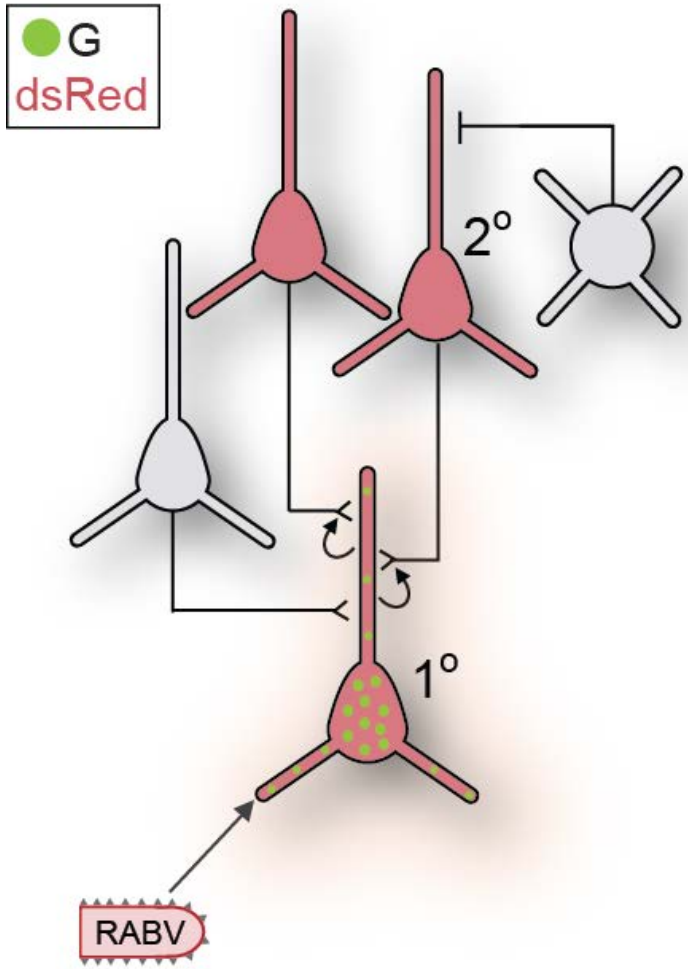
Schnell et al., 2010

Transsynaptic tracing with rabies virus

glycoprotein complementation

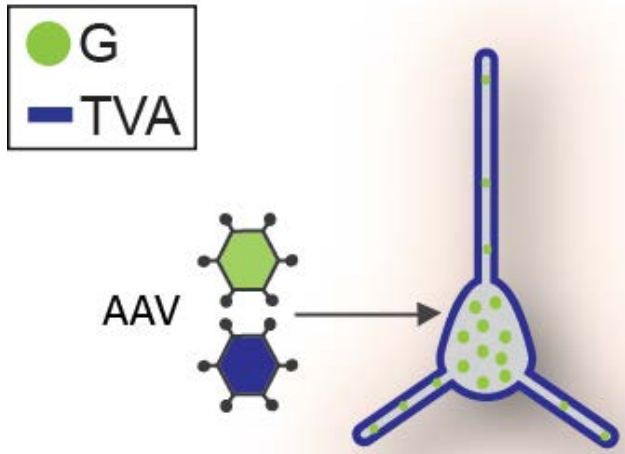


Transsynaptic tracing with rabies virus



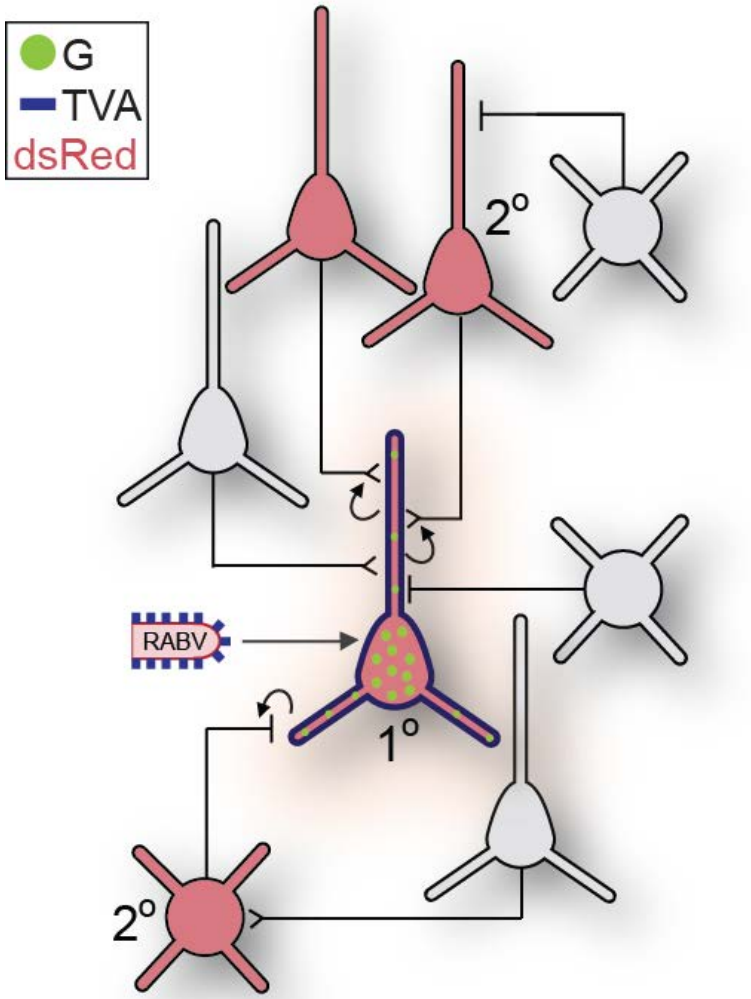
Transsynaptic tracing with rabies virus

targeting initial infection with EnvA/TVA

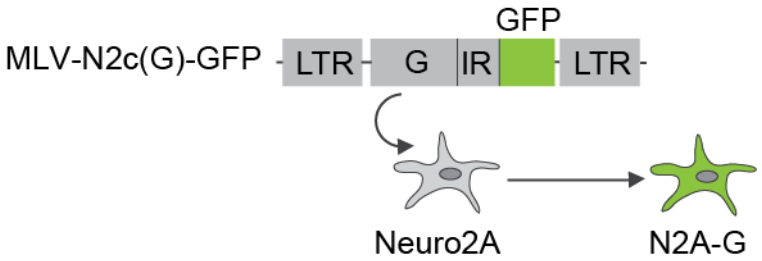


Transsynaptic tracing with rabies virus

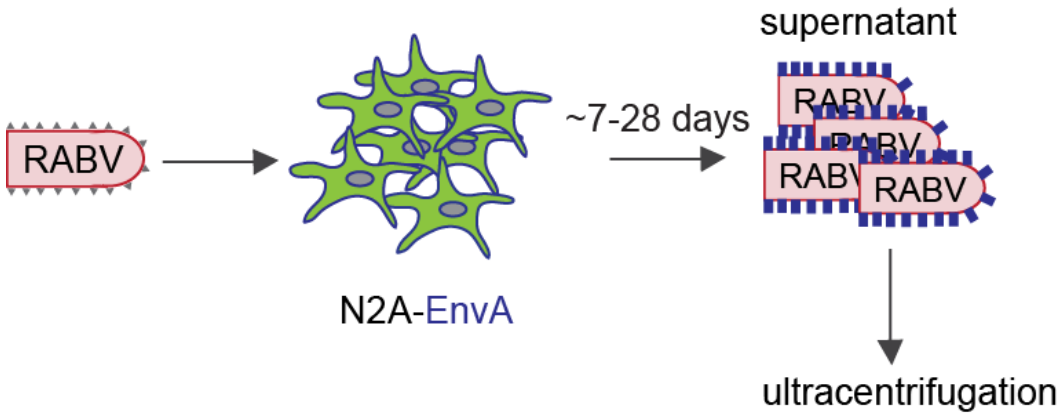
targeting initial infection with EnvA/TVA

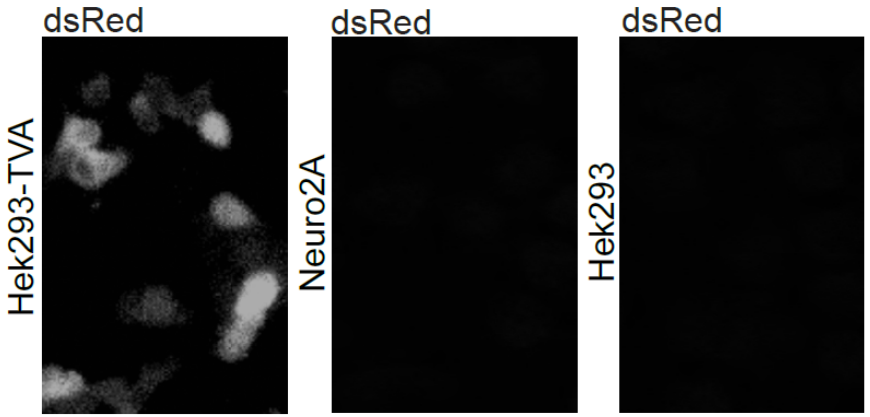


Producing rabies vectors in the lab

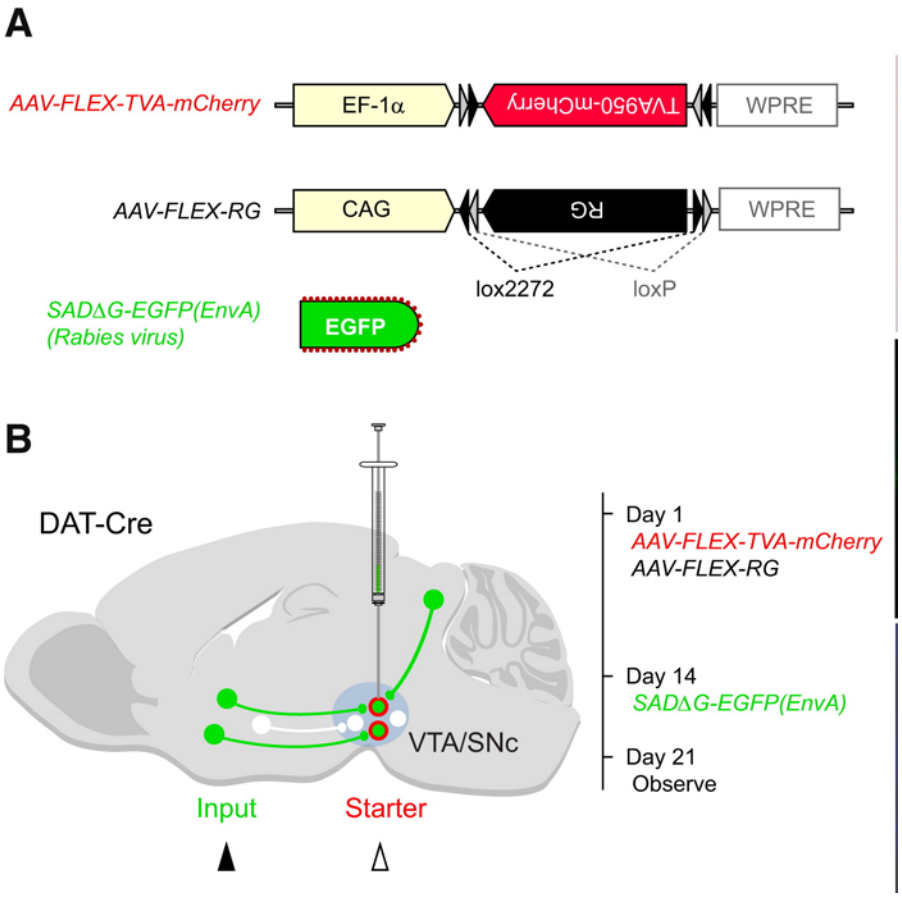


EnvA-pseudotyping of rabies virus



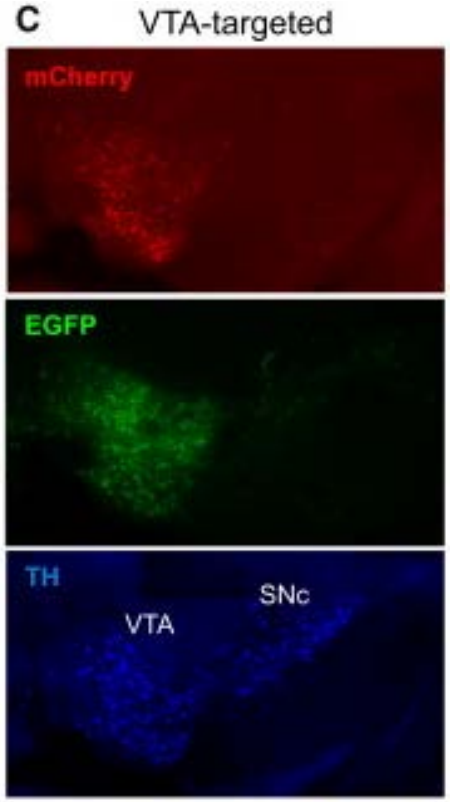


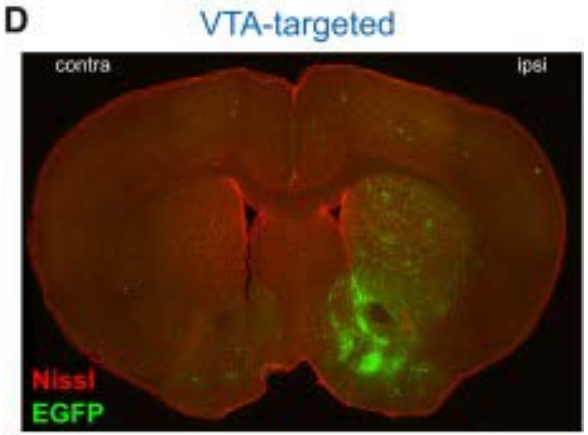
Circuit mapping with rabies virus

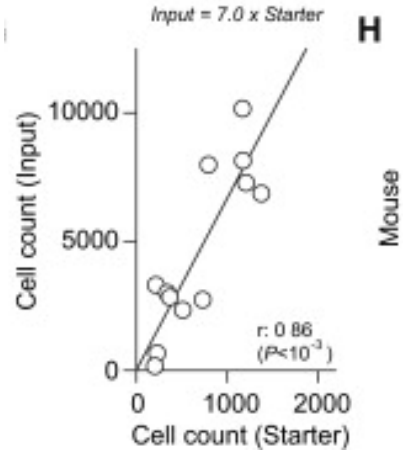


Watabe-Uchida et al., 2012

Circuit mapping with rabies virus



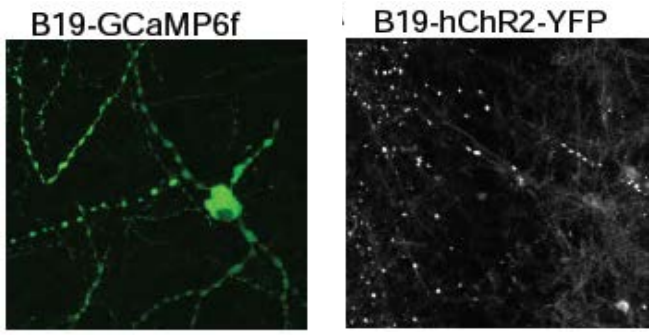




Watabe-Uchida et al., 2012

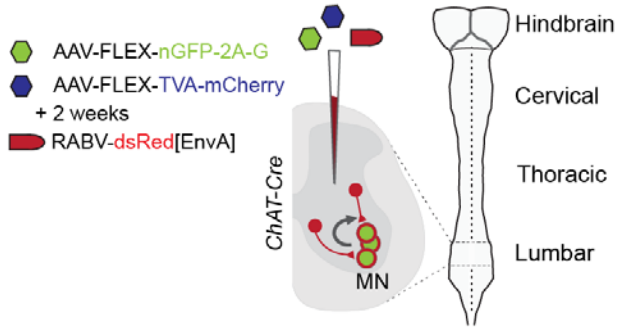
Disadvantages of rabies tracing

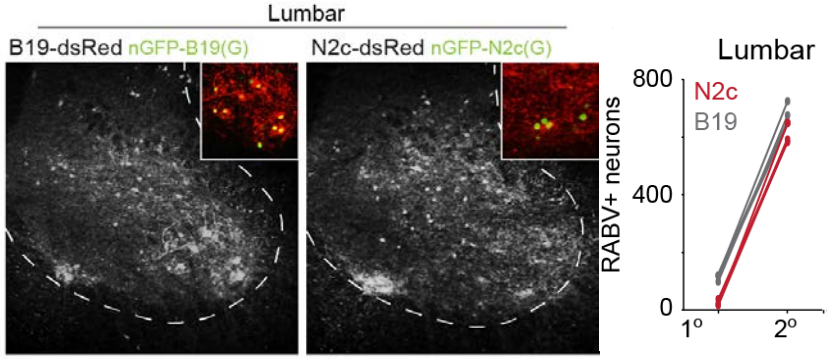
Toxicity

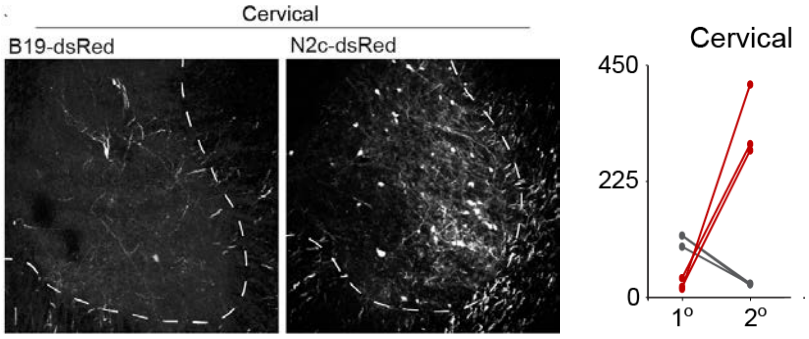


Low transsynaptic spread

Different strains of rabies virus have different properties

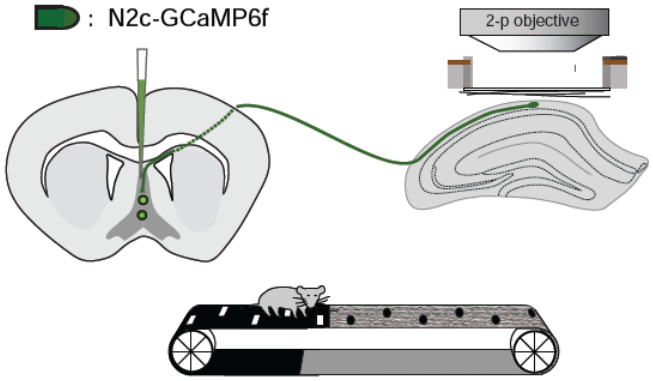




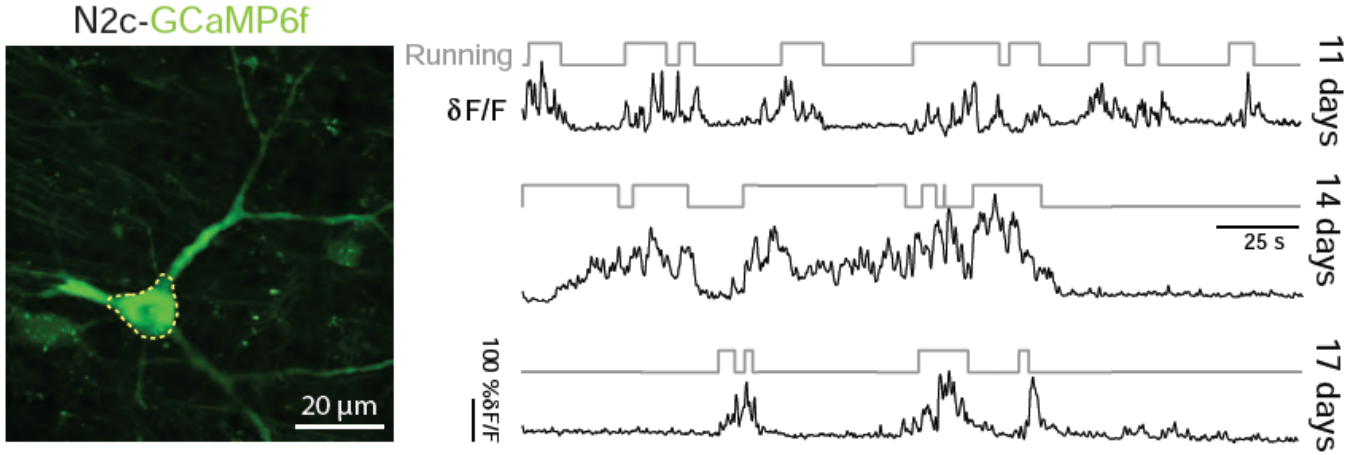


Monitoring neural activity with rabies

Experimental design

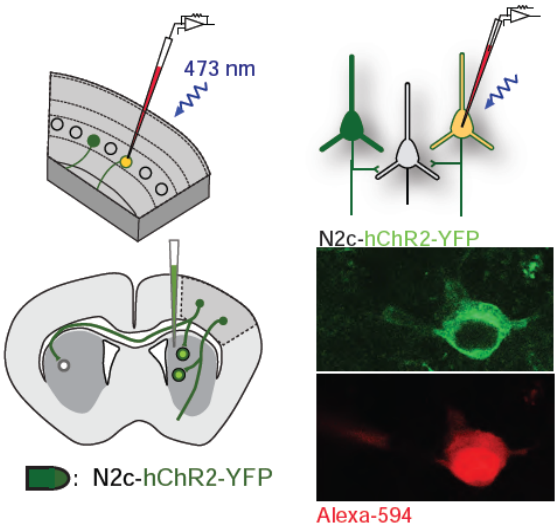


Measuring calcium transients in hippocampus

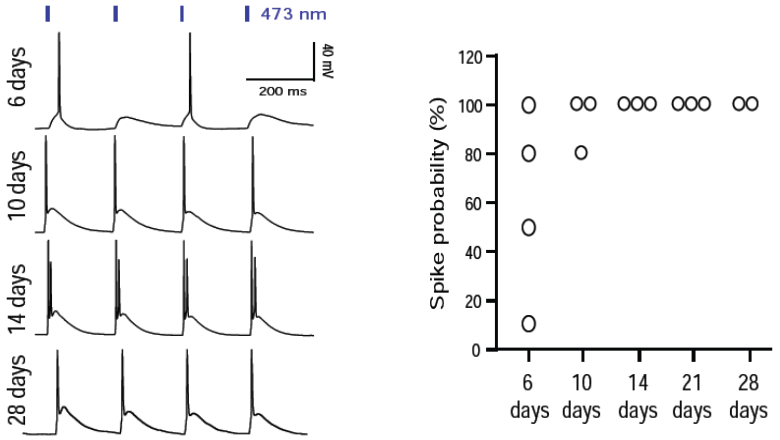


Channelrhodopsin stimulation with N2c rabies virus

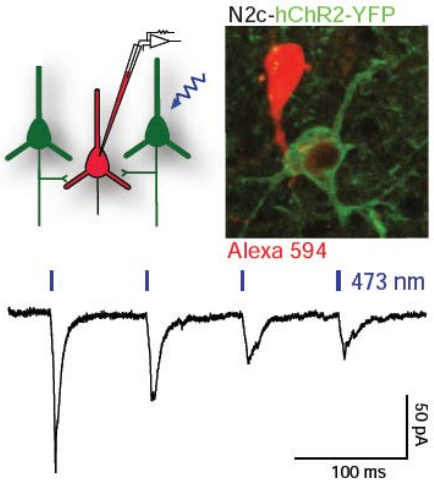
Experimental design



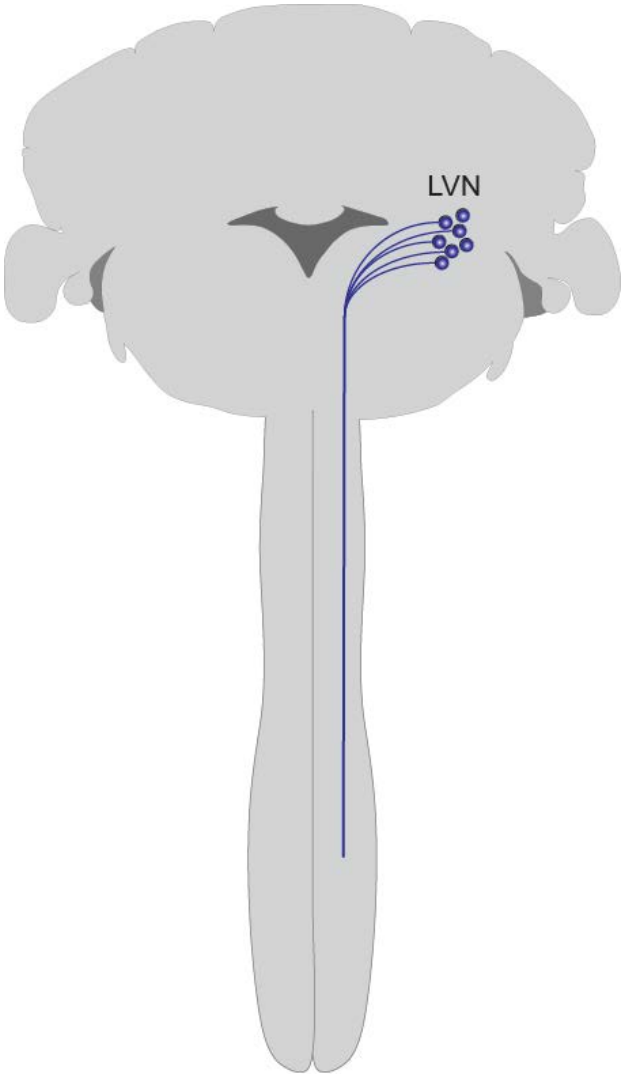
Photostimulation



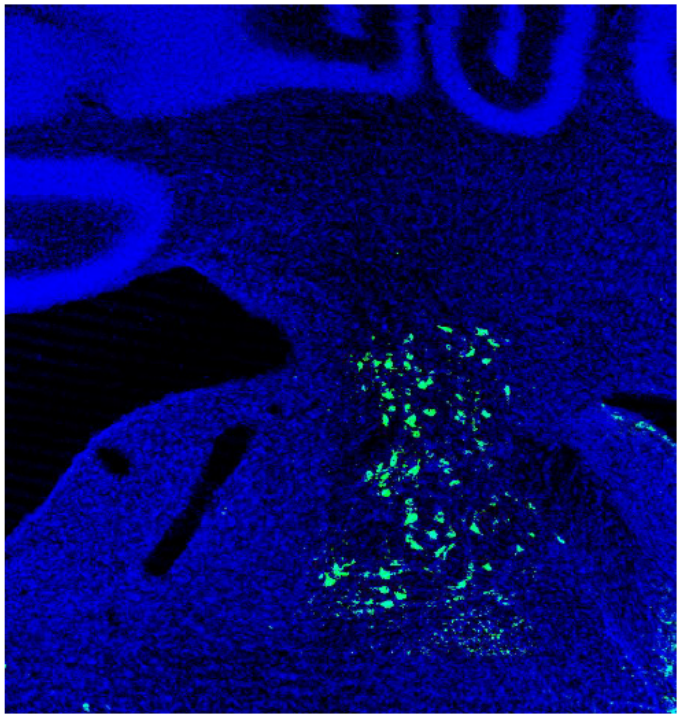
Generation of postsynaptic potentials



Identifying monosynaptic inputs to LVN-spinal neurons

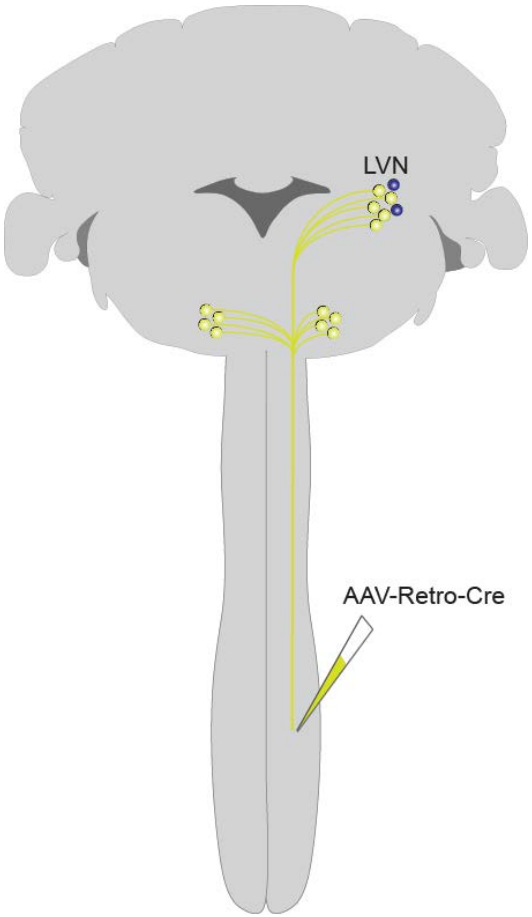


Nissl Fluorogold (lumbar cord)

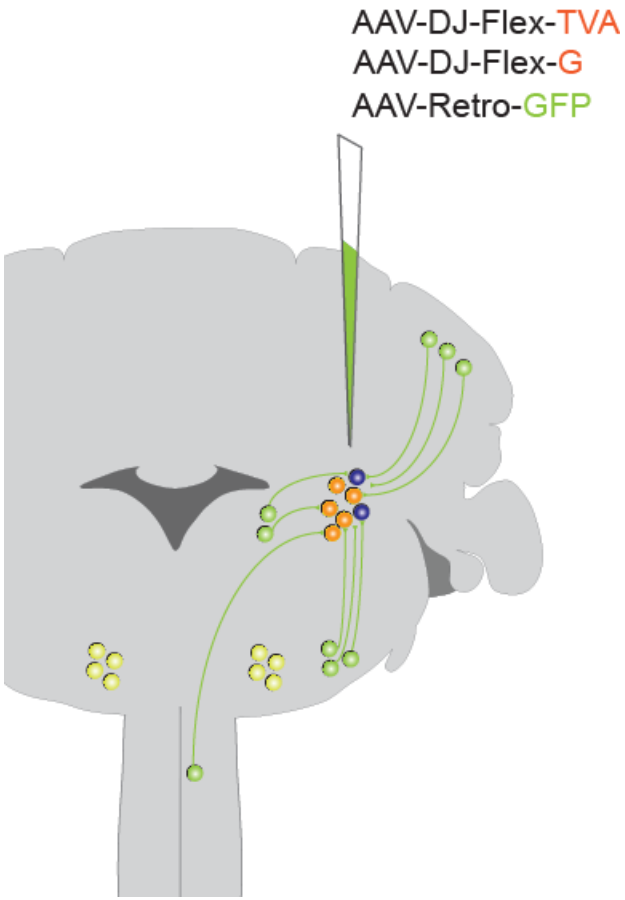
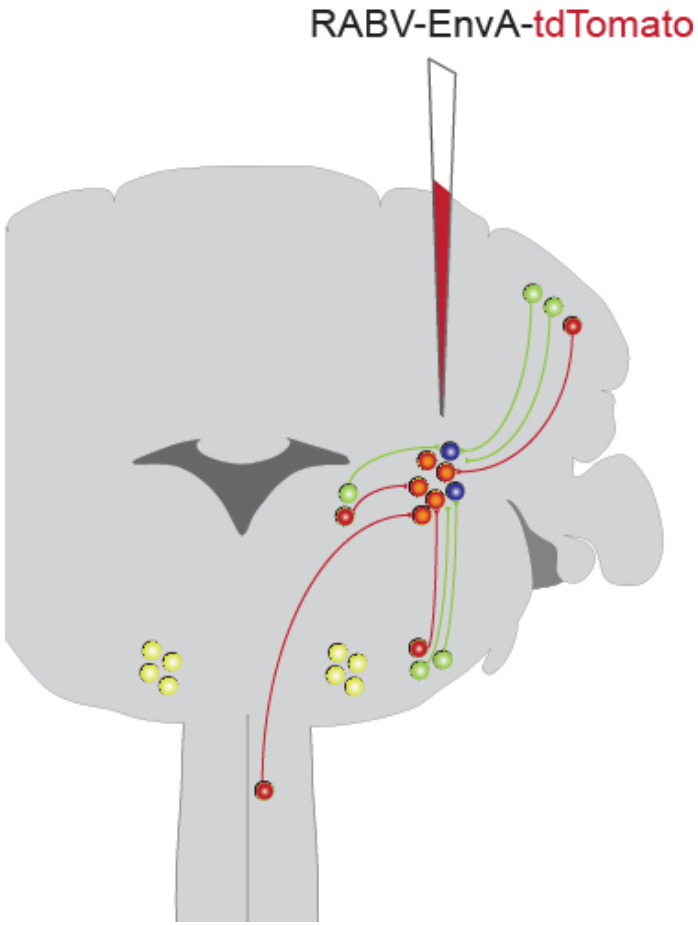
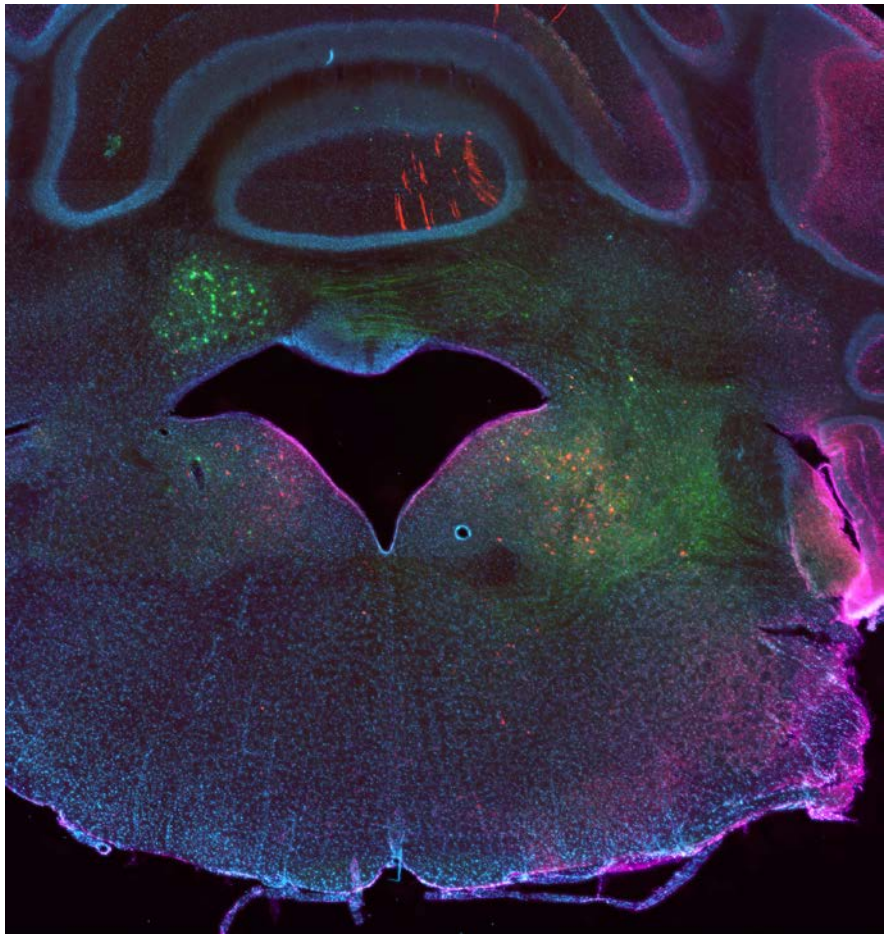


- ✗ Genetic marker
- ✓ Projection pattern
- ✓ Stereotaxic coordinates

Identifying direct and indirect inputs to LVN-spinal neurons



Identifying direct and indirect inputs to LVN-spinal neurons



Identifying direct and indirect inputs to LVN-spinal neurons