Virus engineering for neuroscience

Ian Wickersham
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10/16/2014

Why viruses?

- “Cell-type-specific” expression -> targeting based on gene expression
- “Circuit-specific” expression -> targeting based on synaptic connections

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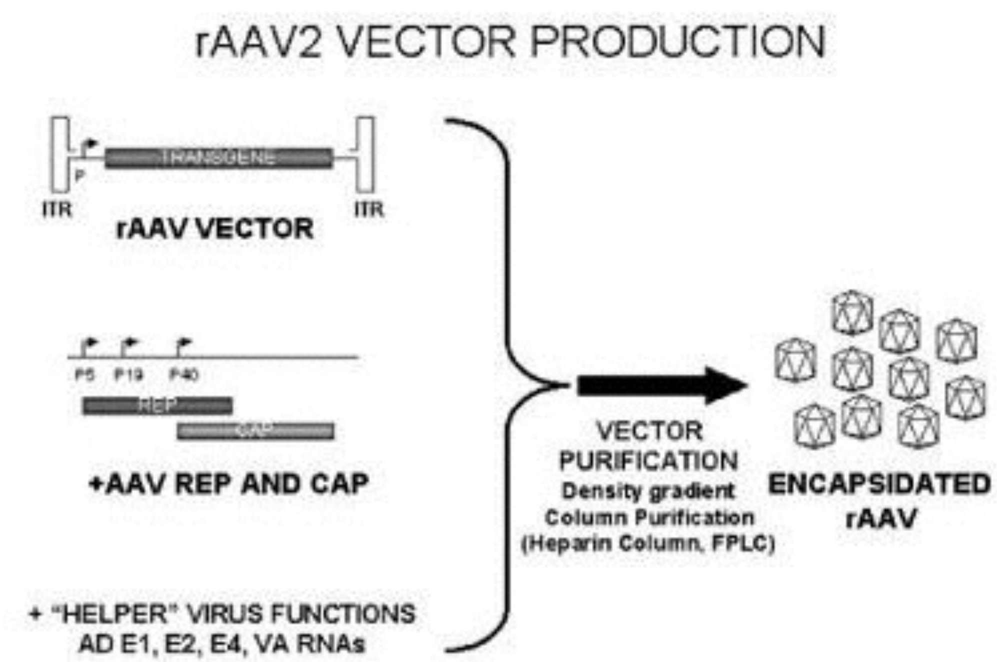
AAV: workhorse for transgene delivery

- high (but slow) expression levels, nontoxic
- specialist cores make high quality preps
- packaged as different "serotypes" (strains); s "tropism" (which cells it infects)
- small packaging capacity.



Reprinted by permission from Macmillan Publishers Ltd: *Gene Therapy* © 2003. Source: Sun, J. Y., V. Anand-Jawa, et al. "Immune Responses to Adeno-associated Virus and its Recombinant Vectors." *Gene Therapy* 10, no. 11 (2003): 964–76.

- expression of two genes from same virus is not high
- nonenveloped virus so can't be easily recoated with other viruses' envelope proteins
- DNA genome -> can be made Cre (or Flp, etc.) dependent

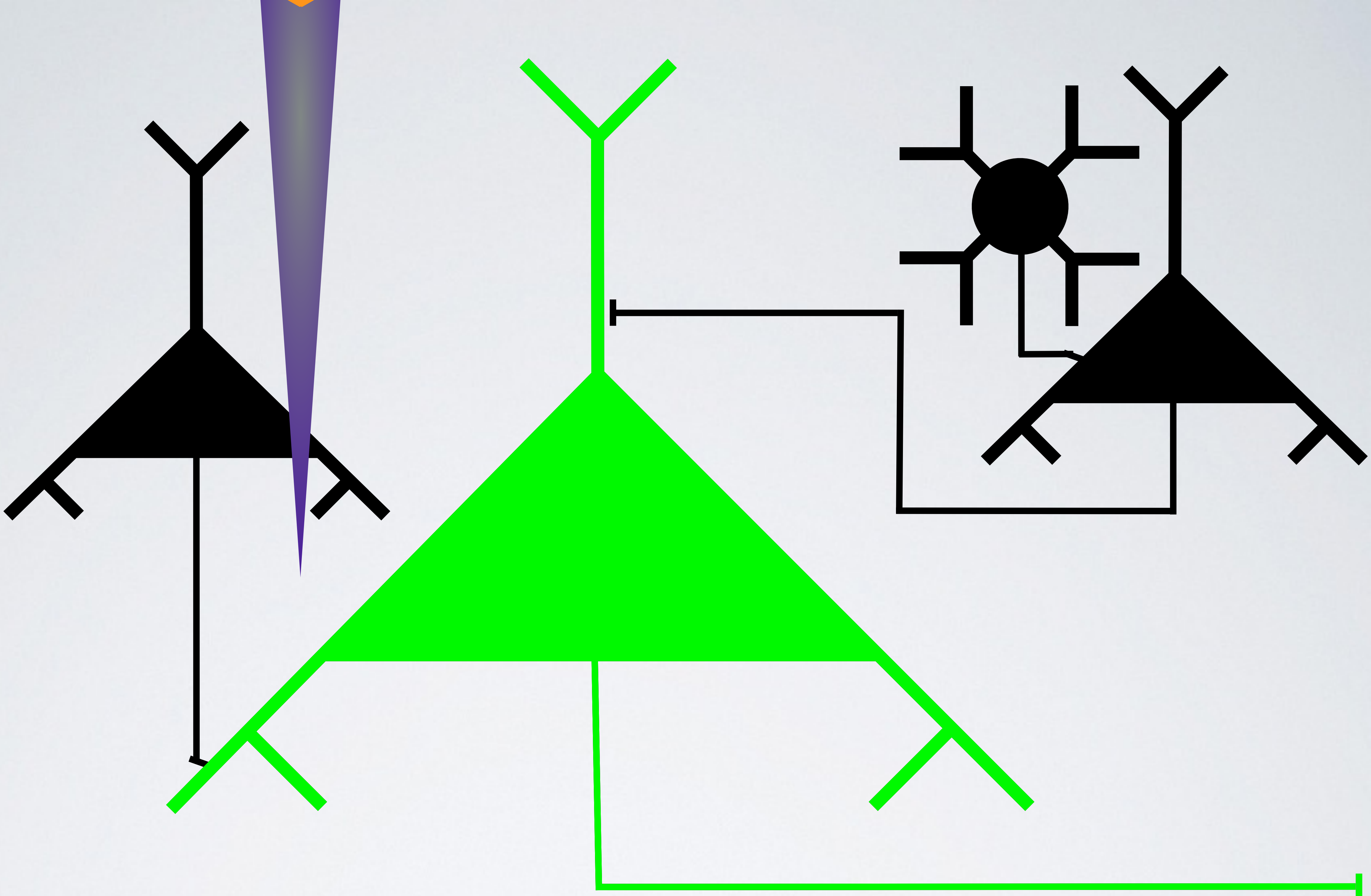
AAV: workhorse for transgene delivery

- almost no viral sequences left in vector genome
- components of vector genome: ITRs, promoter, (kozak sequence), transgene, woodchuck posttranscriptional regulatory element, polyadenylation signal

- promoters: CAG, synapsin-1, EF1a, CamKII…
- genome must be <4.7 kB including ITRs

Targeting using promoters unsuccessful for many neuron types

- Interneuron subtypes in particular
- Cre lines method of choice



CURRENT STANDARD:
CRE MICE + AAV-FLEX

ITR	Prom	lox1	lox2	GFP	lox1	lox2	WPRE	pA	ITR

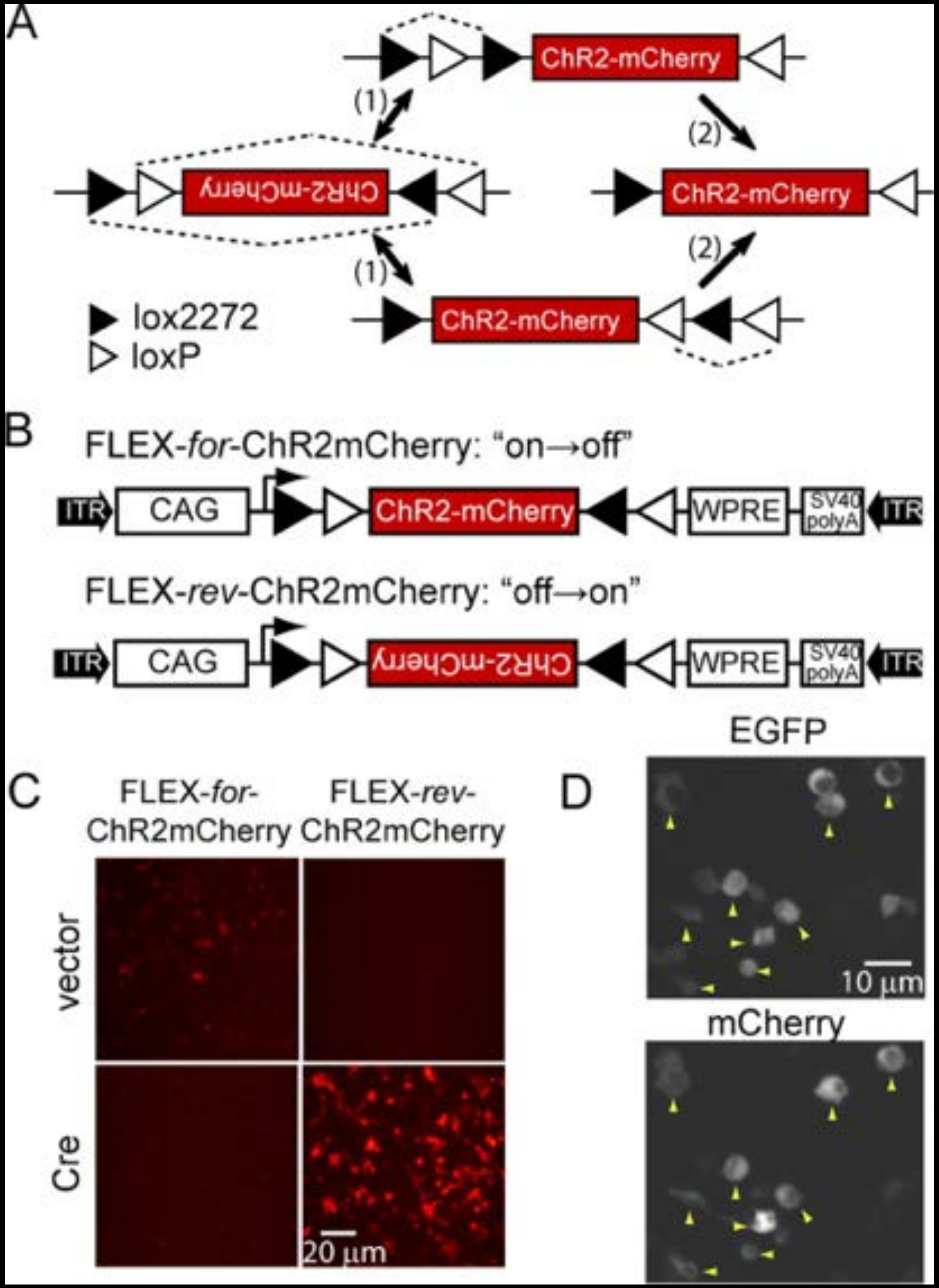
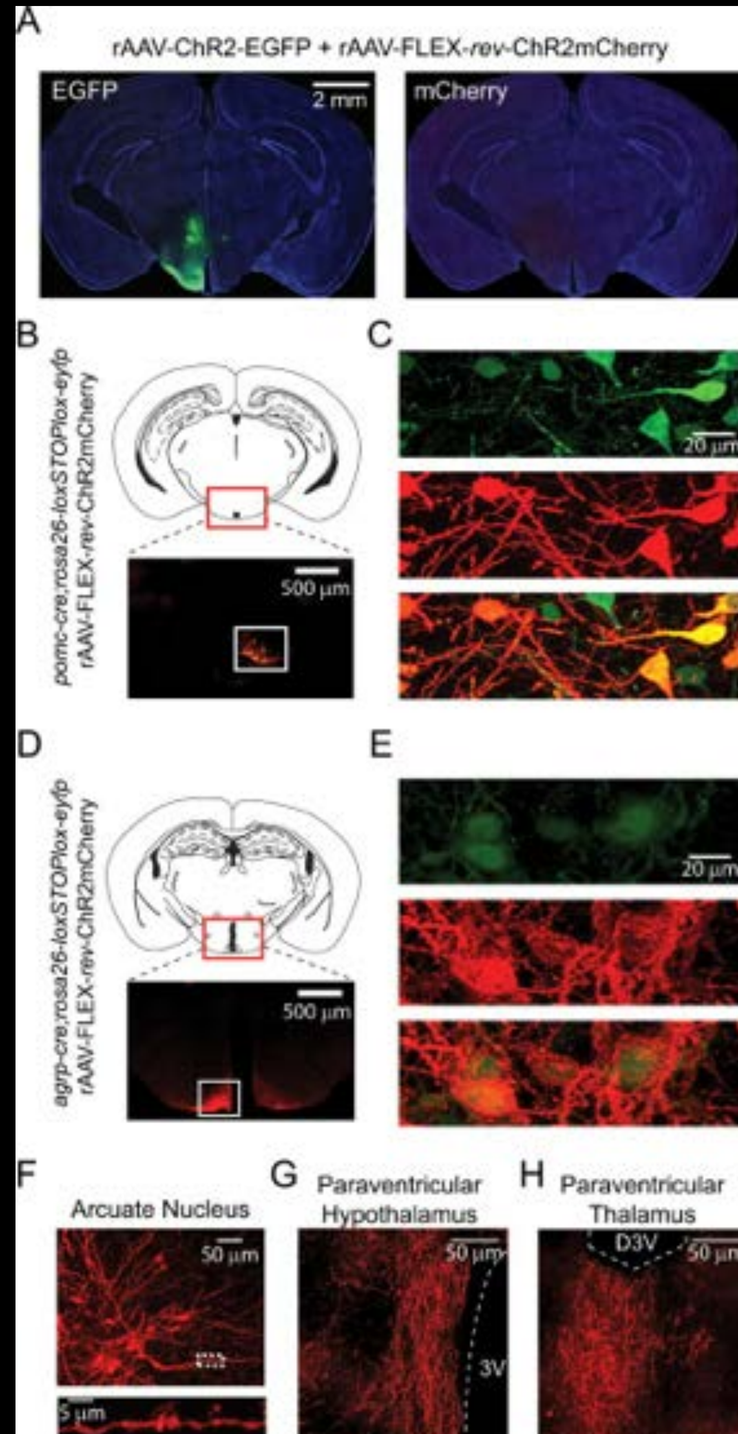


Figure 1.

Design and characterization of a FLEX switch for ChR2mCherry. A, FLEX switch recombination sequence for stable inversion proceeds in two steps: (1) inversion followed by (2) excision. loxP and lox2272 are orthogonal recombination sites. B, Construct design for FLEX-for-ChR2mCherry and FLEX-rev-ChR2mCherry. CAG, CMV enhancer/β-globin chimeric promoter; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element; ITR, inverted terminal repeat. C, Images showing mCherry fluorescence in HEK 293 cells for FLEX-for-ChR2mCherry and FLEX-rev-ChR2mCherry in the presence and absence of Cre. D, Colocalization of EGFP and mCherry fluorescence (yellow arrowheads) in HEK 293 cells cotransfected with FLEX-rev-ChR2mCherry and Cre-IRES-EGFP.

Atasoy, Aponte et al. '08



Atasoy, Deniz, Yexica Aponte, et al. "A FLEX Switch Targets Channelrhodopsin-2 to Multiple Cell Types for Imaging and Long-range Circuit Mapping." *The Journal of Neuroscience* 28, no. 28 (2008): 7025–30. CC license BY-NC-SA.

Figure 2.

Cre-dependent ChR2mCherry expression in transgenic mice using rAAV-FLEX-rev-ChR2mCherry. A, Distribution of fluorescent neurons resulting from a large coinjection (150 nl) of rAAV-ChR2-EGFP and rAAV-FLEX-rev-ChR2mCherry into the hypothalamus of wild-type mice. Extensive fluorescence from EGFP (left) but no fluorescence from mCherry in brain slices (right) shows the absence of background expression with rAAV-FLEX-rev-ChR2mCherry. The background image of the slice was obtained from 4′,6′-diamidino-2-phenylindole fluorescence. B, Top, Schematic showing location of the imaged area in caudal arcuate nucleus. Bottom, mCherry fluorescence only in the arcuate nucleus after a large injection of rAAV-FLEX-rev-ChR2mCherry into the hypothalamus of pomc-cre;rosa26-loxSTOPlox-eyfp mice. Compare distribution of fluorescence with A. C, Colocalization of mCherry and EYFP fluorescence in arcuate nucleus. D, E, Similar to B and C; in this case, agrp-cre;rosa26-loxSTOPlox-eyfp mice were used with rAAV-FLEX-rev-ChR2mCherry virus injections. F, Top, Image showing neuron morphology from the arcuate nucleus of labeled POMC neurons. Bottom, Higher-magnification image of boxed area. G, H, Axonal projections of AGRP neurons infected with rAAV-FLEX-rev-ChR2mCherry. Strong axonal labeling was observed in the paraventricular nucleus of the hypothalamus (G) and paraventricular thalamus (H). 3V, Third ventricle; D3V, dorsal third ventricle.

Atasoy, Aponte et al. '08

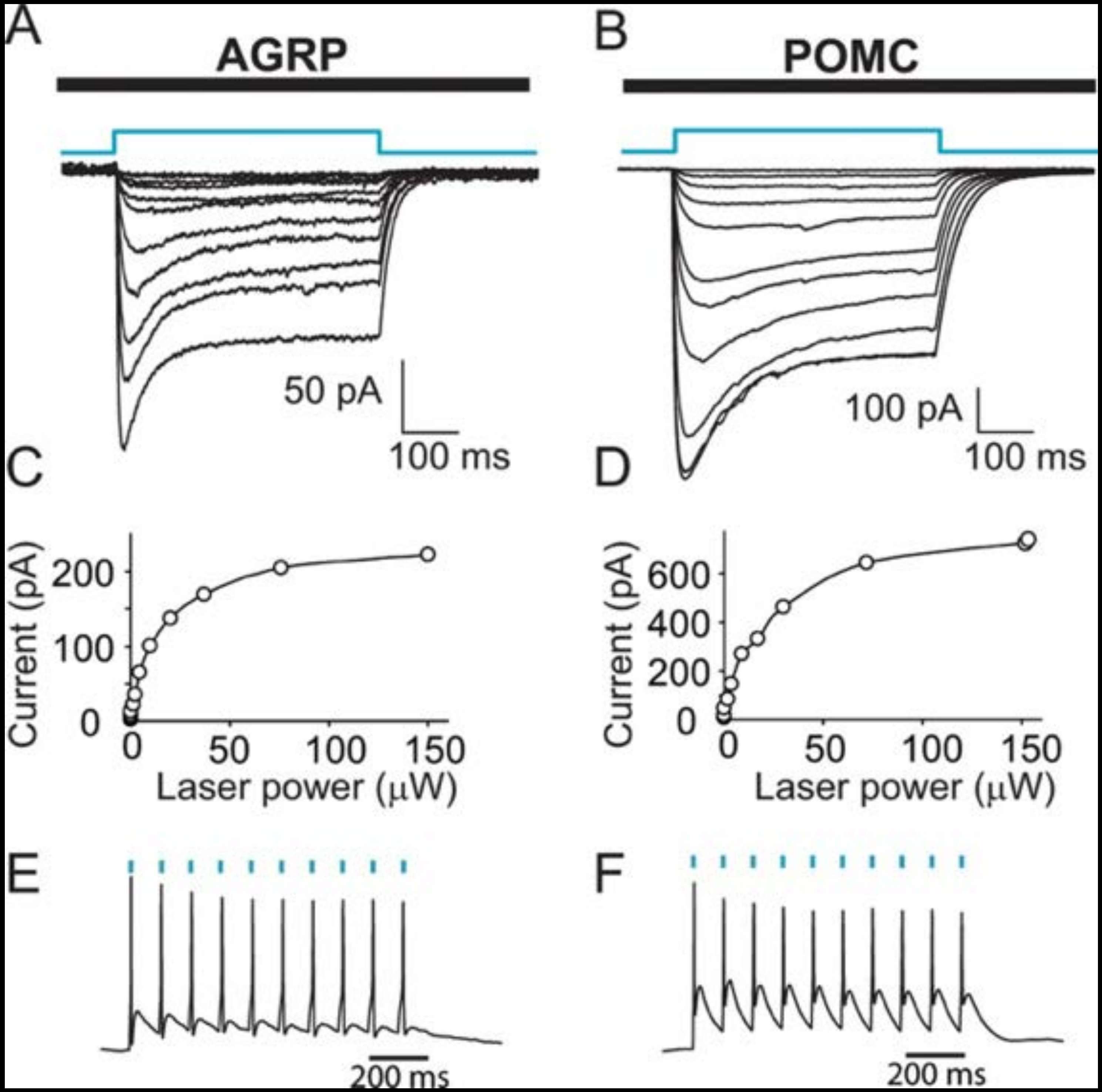


Figure 3.

Photostimulation of AGRP and POMC neurons in the hypothalamus. A, B, Whole-cell voltage-clamp recordings from mCherry positive neurons in hypothalamic slices from agrp-cre or pomc-cre mice infected with rAAV-FLEX-rev-ChR2mCherry. Light pulses (500 ms) of varying power elicited ChR2mCherry-mediated inward currents. C, D, The peak current is plotted as a function of laser power for AGRP (C) and POMC (D) neurons. E, F, Perisomatic repetitive stimulation with 1 ms light pulses at 10 Hz in AGRP (E) and POMC (F) neurons. Blue dashes mark timing of light flashes.

Atasoy, Aponte et al. '08

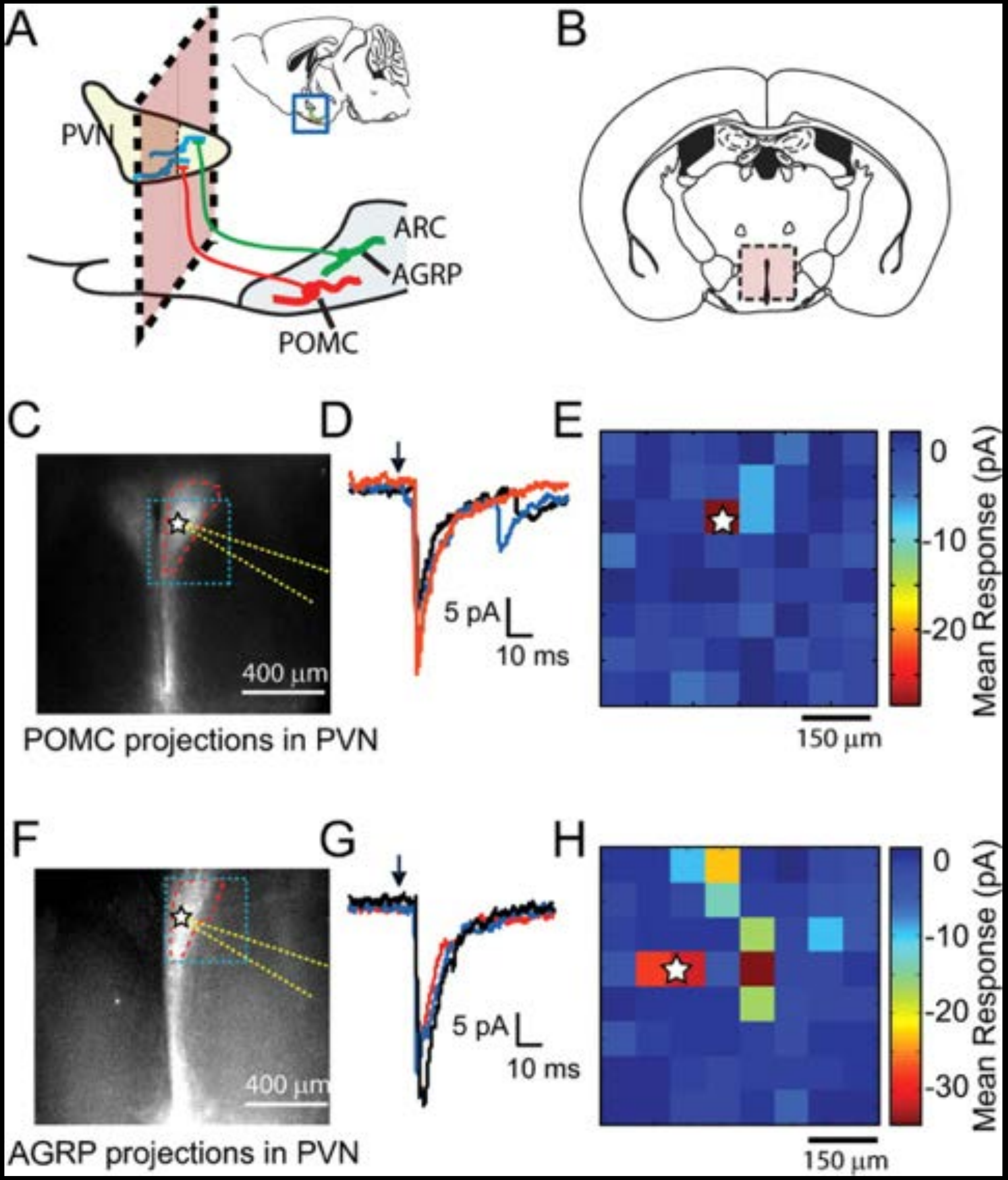


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Figure 4.

Channelrhodopsin-assisted circuit mapping for hypothalamic neuronal circuits: AGRP→PVN and POMC→PVN. A, Diagram of a sagittal hypothalamic section depicting anatomy of connections between ARC and PVN. The pink box denotes the plane of the coronal slice. B, Coronal slices containing PVN, but not arcuate nucleus, were used for whole-cell voltage-clamp recordings from PVN neurons. C, Fluorescence image showing POMC axonal projections to PVN. Blue box outlines region of laser stimulation in E, PVN boundary is outlined in red, location of recorded cell body is marked by a star, and recording pipette is outlined in yellow. D, Overlay of POMC→PVN IPSCs resulting from three photostimulation trials at a site perisomatic to a voltage-clamped PVN neuron. E, Synaptic input map shows mean current responses over 100 ms time window as a color map in voltage-clamped PVN neuron resulting from LSPS of axons originating from POMC neurons. The position of the soma is marked with a star. F–H, Similar to C–E, but in this case, projections arise from AGRP neurons.

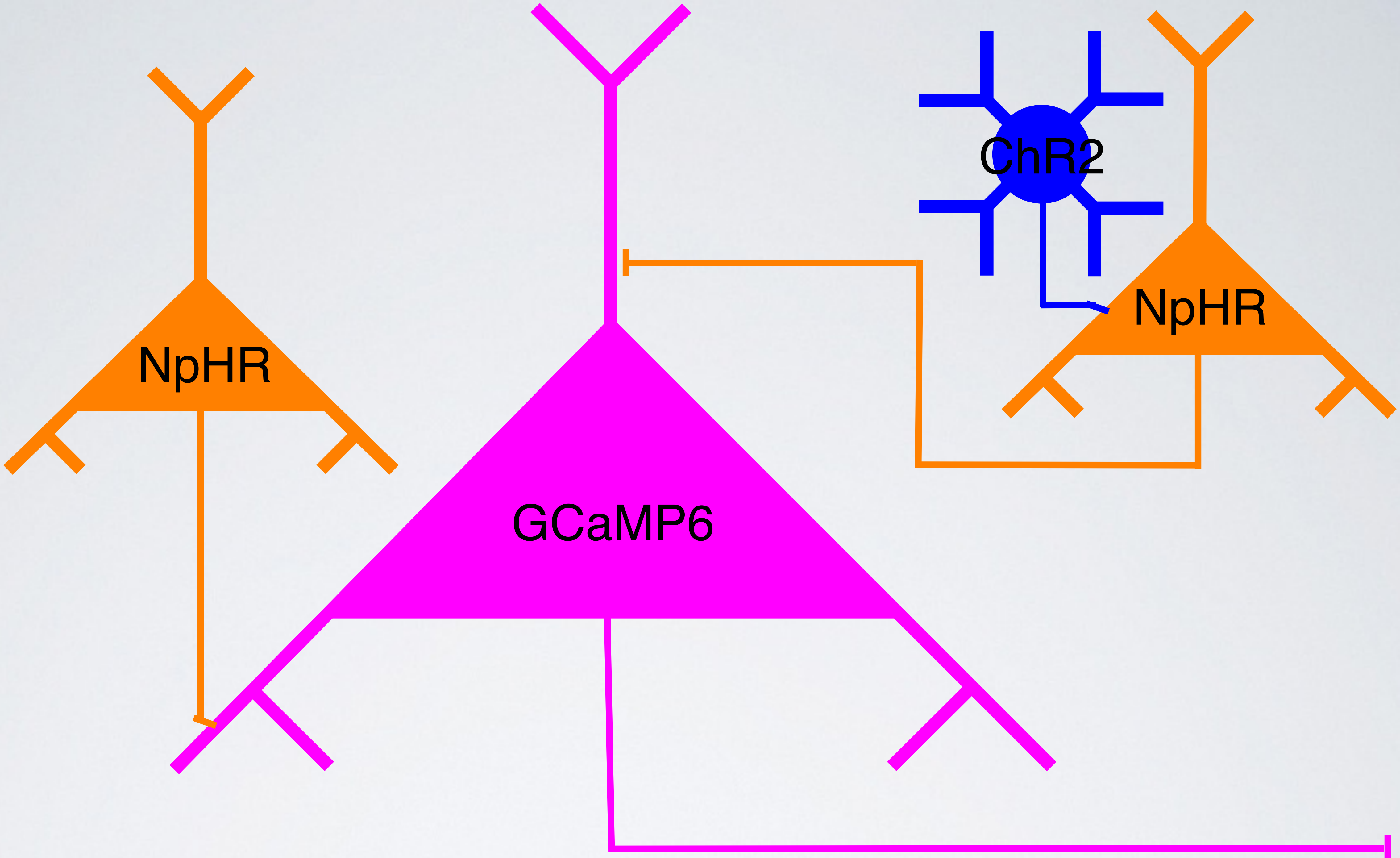
Atasoy, Aponte et al. ‘08

Cre mice/rats: effective but limiting

- Only practical for targeting one or two cell types at a time
- Precludes use in most other species
- Mouse lines expensive to create and maintain, crossing takes time

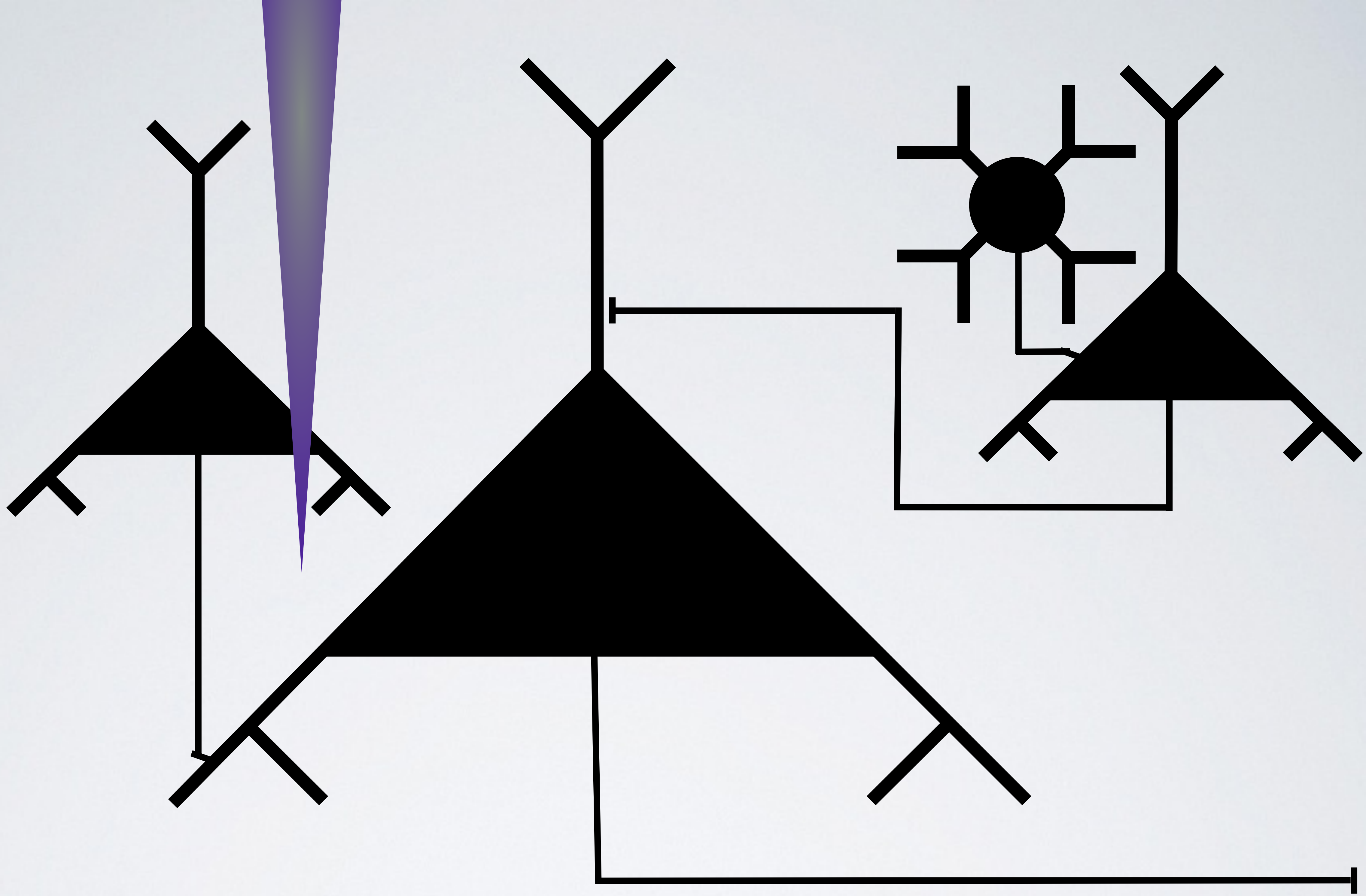
How to achieve highly multiplexed investigation?

- Opsins: ChR2, NpHR, Arch, ArchT, Chrimson, Chronos, iC1C2, JAWS…
- Indicators: GCaMP6, ArcLight, ASAP1, B-GECO1, R-GECO1, R-CaMP1.07…
- Not to mention Cas9, dominant negative mutants, GRASP…
- But crossing mouse lines to achieve progeny expressing n recombinases (Cre, FlpO, KD, B2, B3…) does not scale well.



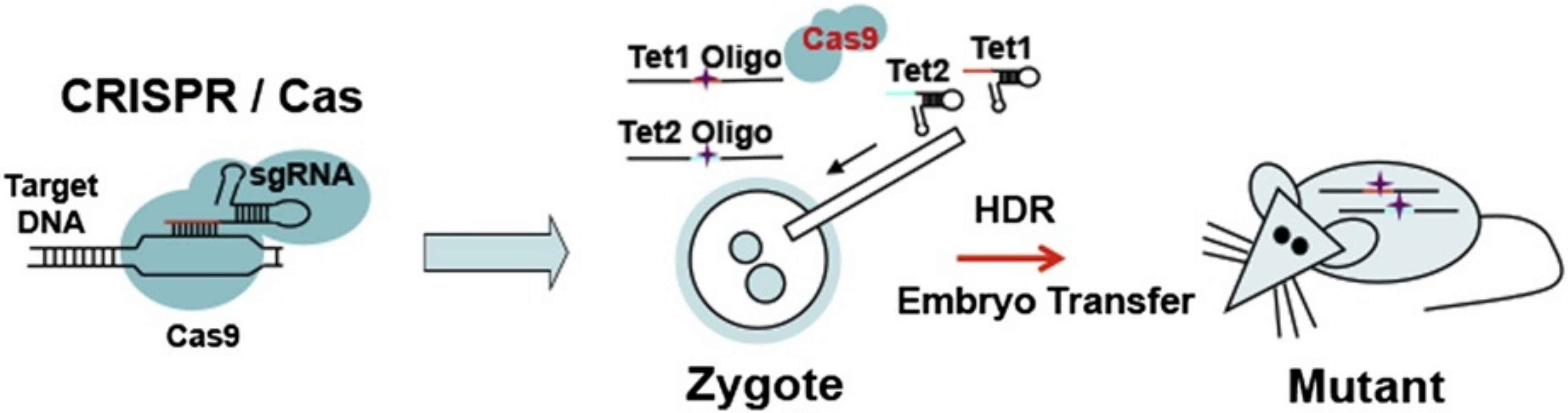
MULTIPLEXED OPTOGENETICS

Goal: cell-type-specific transgene expression in wild-type animals of any species



MULTIPLEXED OPTOGENETICS

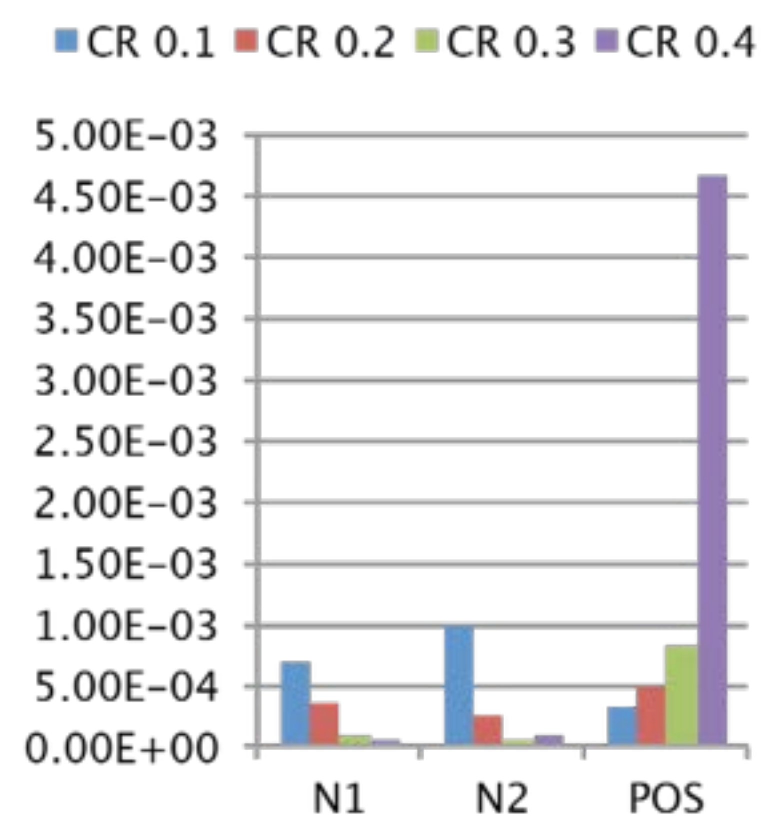
CRISPR/Cas9: potential for “somatic knock-ins”?



Source: Wang, Haoyi, Hui Yang, et al. "One-step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-mediated Genome Engineering." *Cell* 153, no. 4 (2013): 910–18.

In progress: system for selective expression in **cortical interneuronal subtypes**

- targeting major categories of cortical & hippocampal interneurons
- NSF grant



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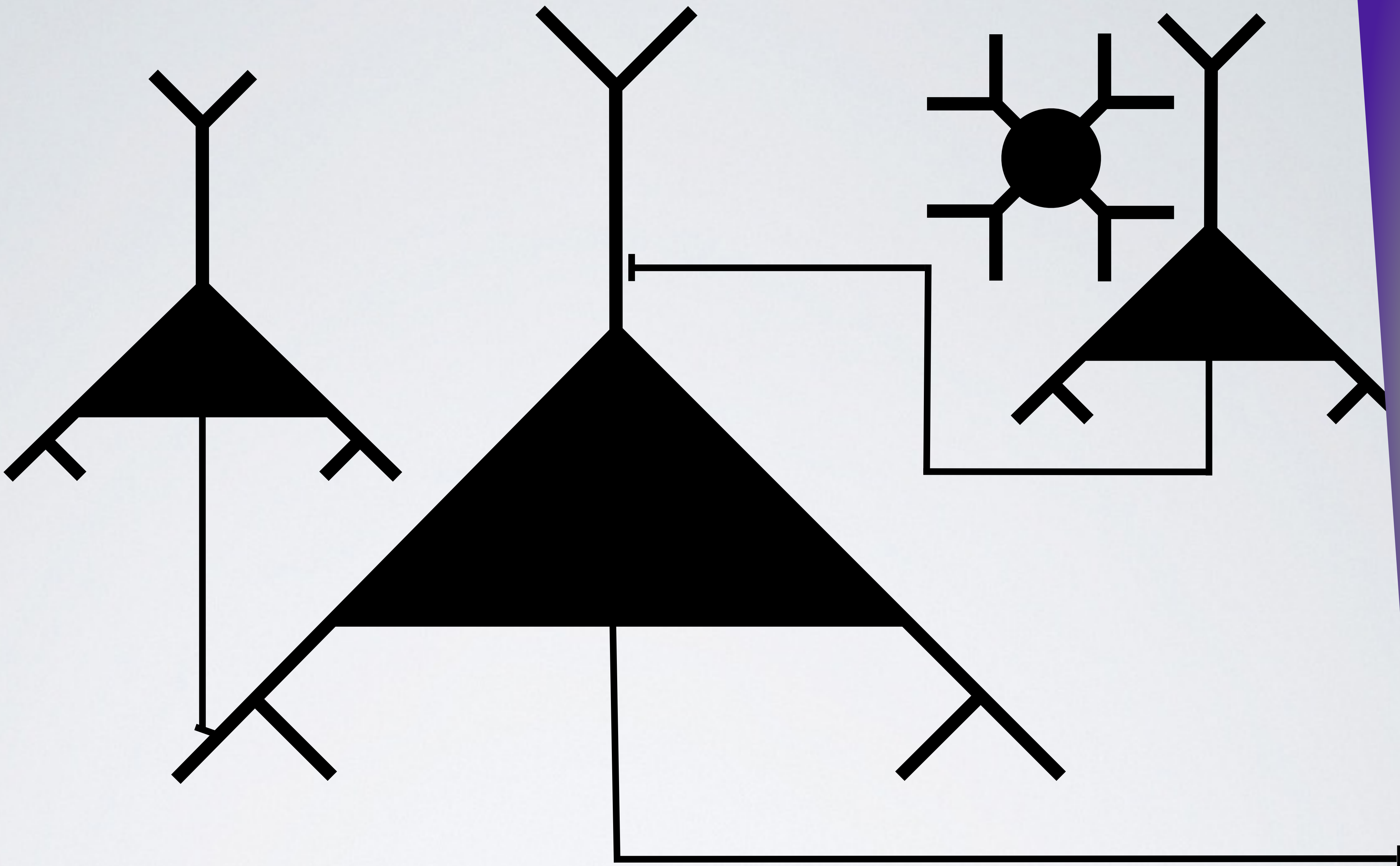
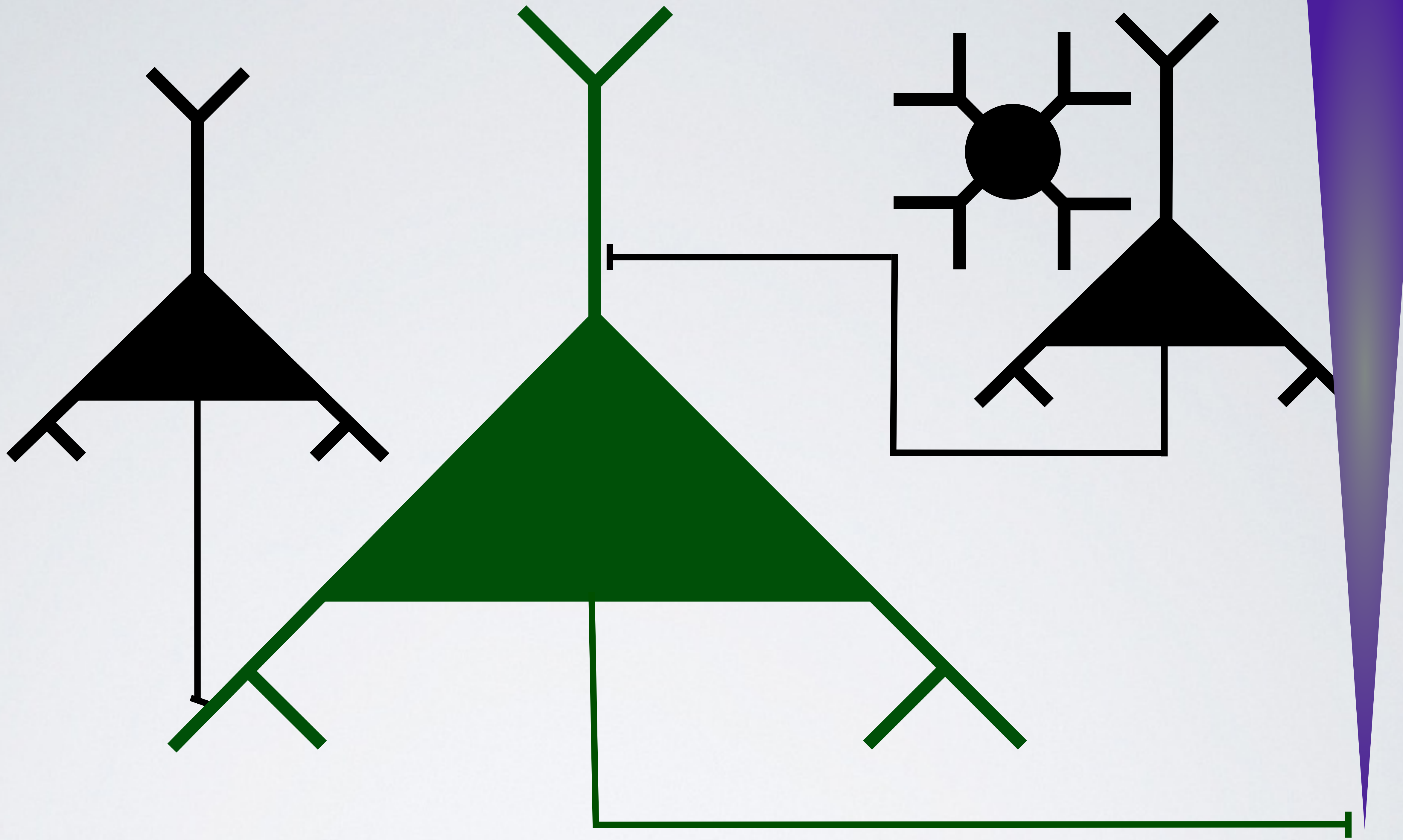


RETROGRADE TARGETING: DELIVERY VIA AXONS



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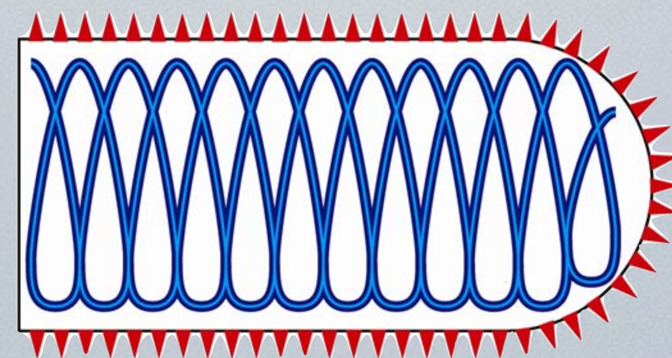
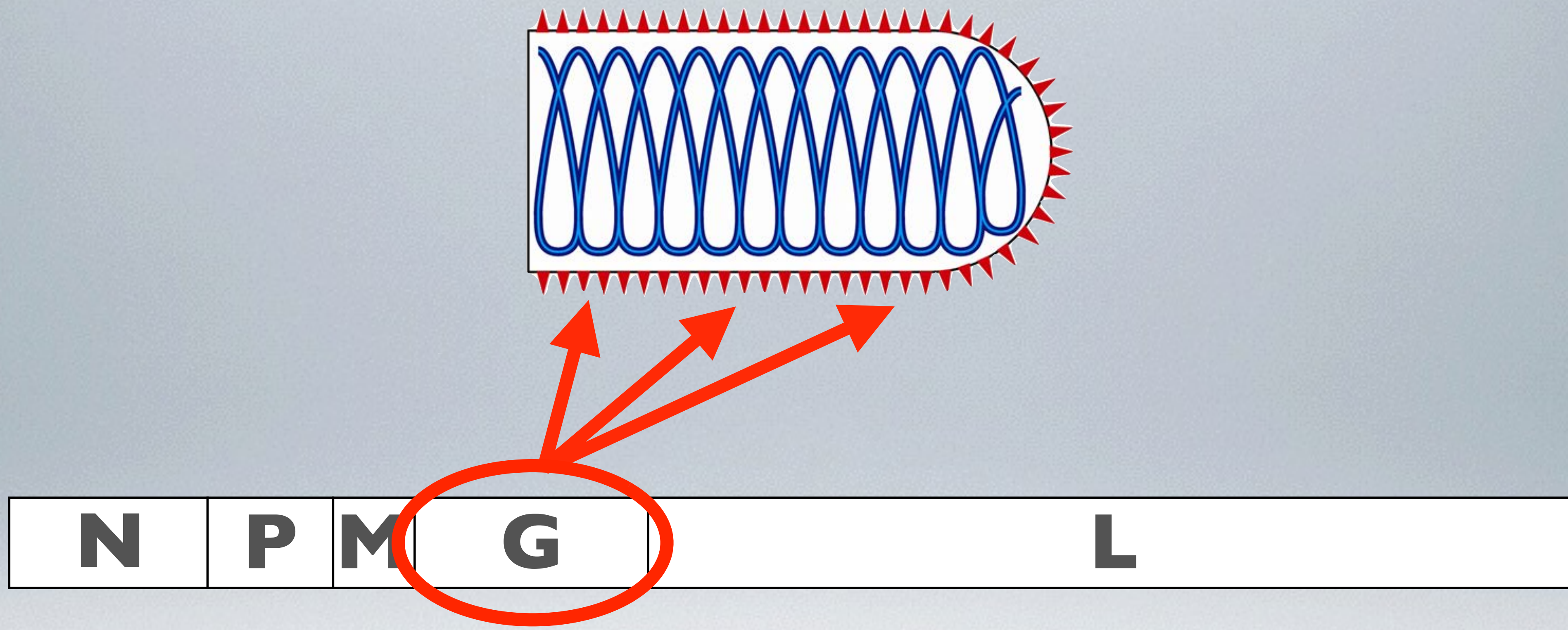


RABIES VIRUS

Wickersham et al. 2007a



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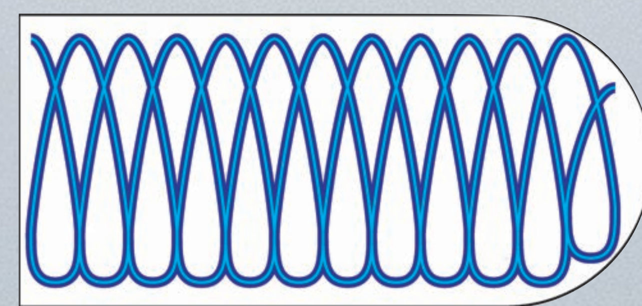

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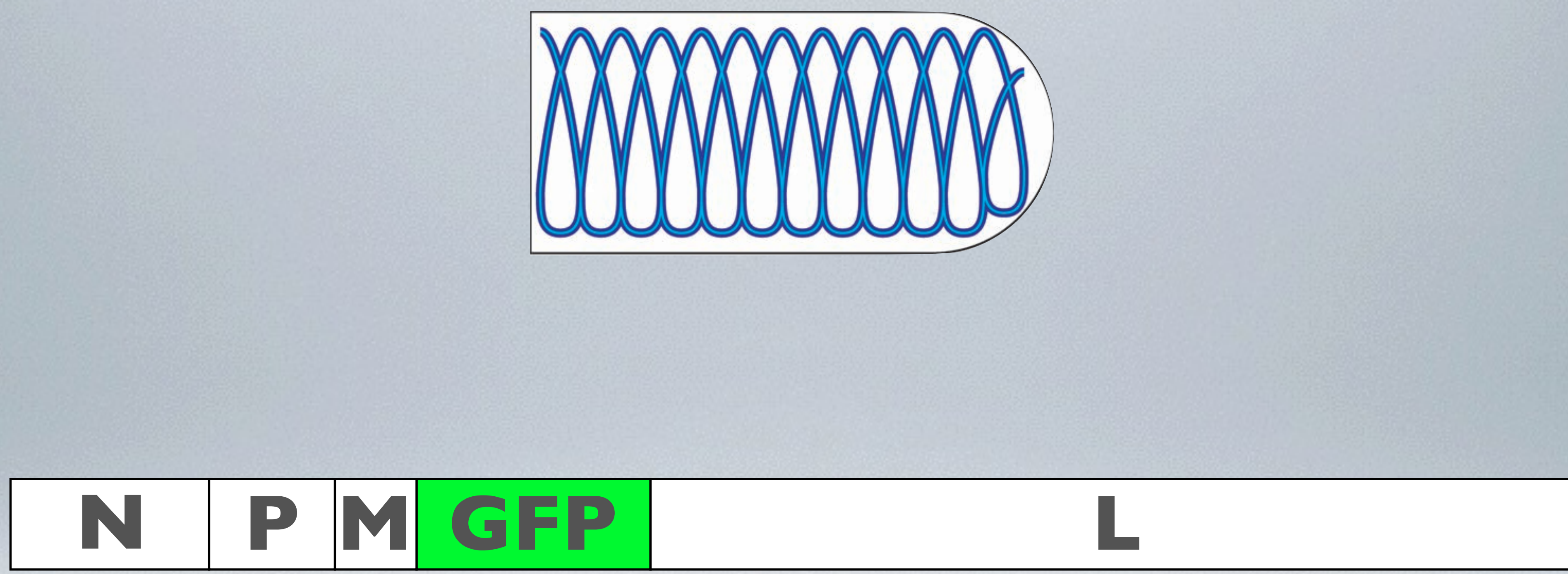

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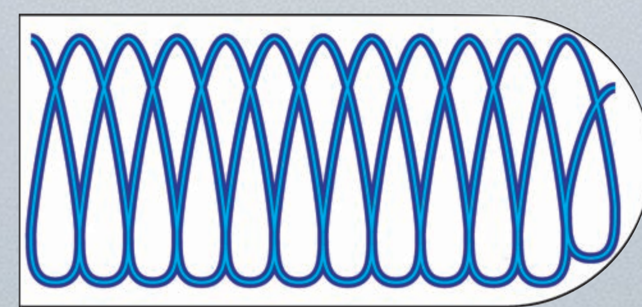
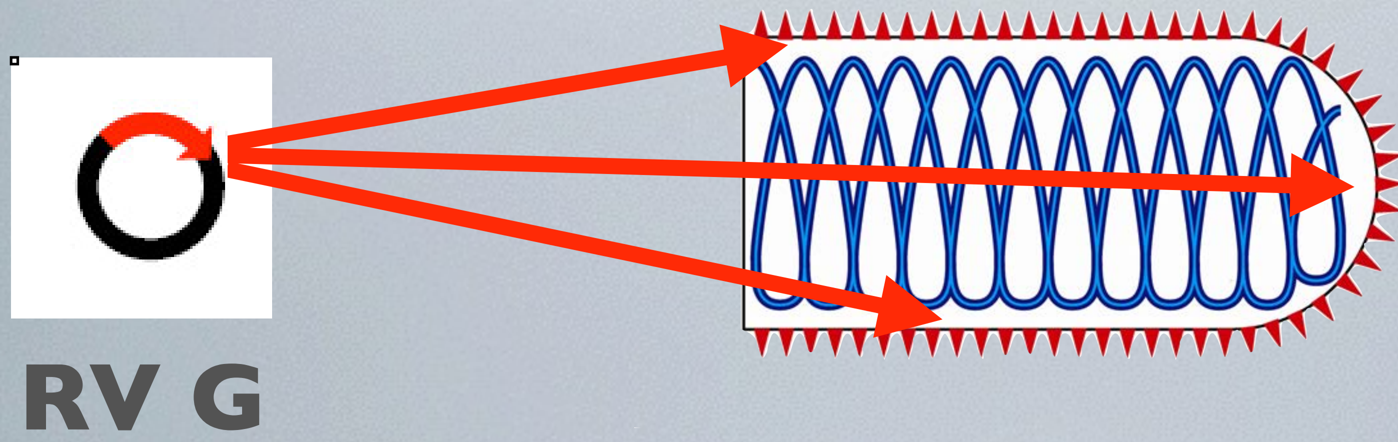


RABIES VIRUS

- carries own polymerase; can NOT use exogenous promoters, but strong expression in all cell types

Wickersham et al. 2007a



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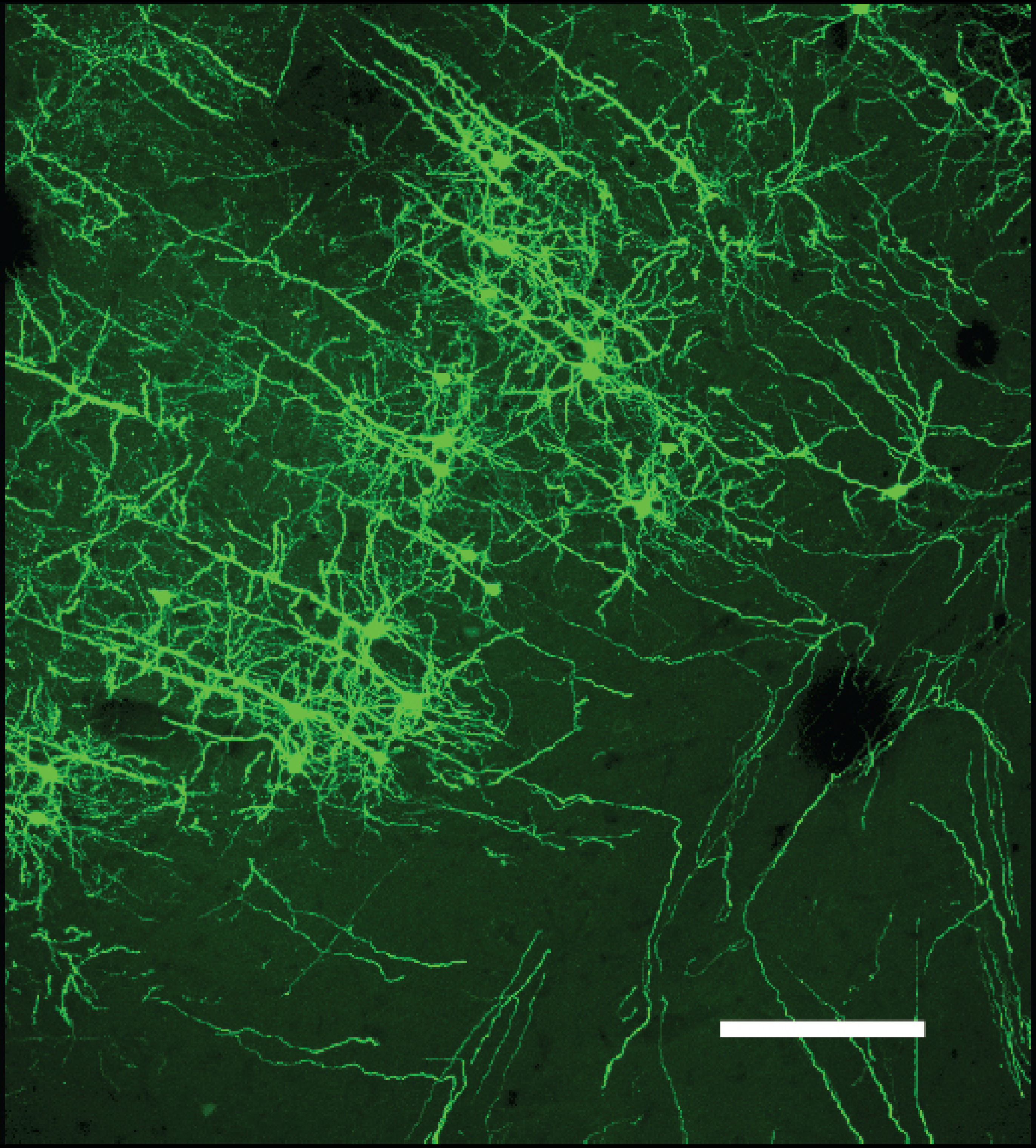
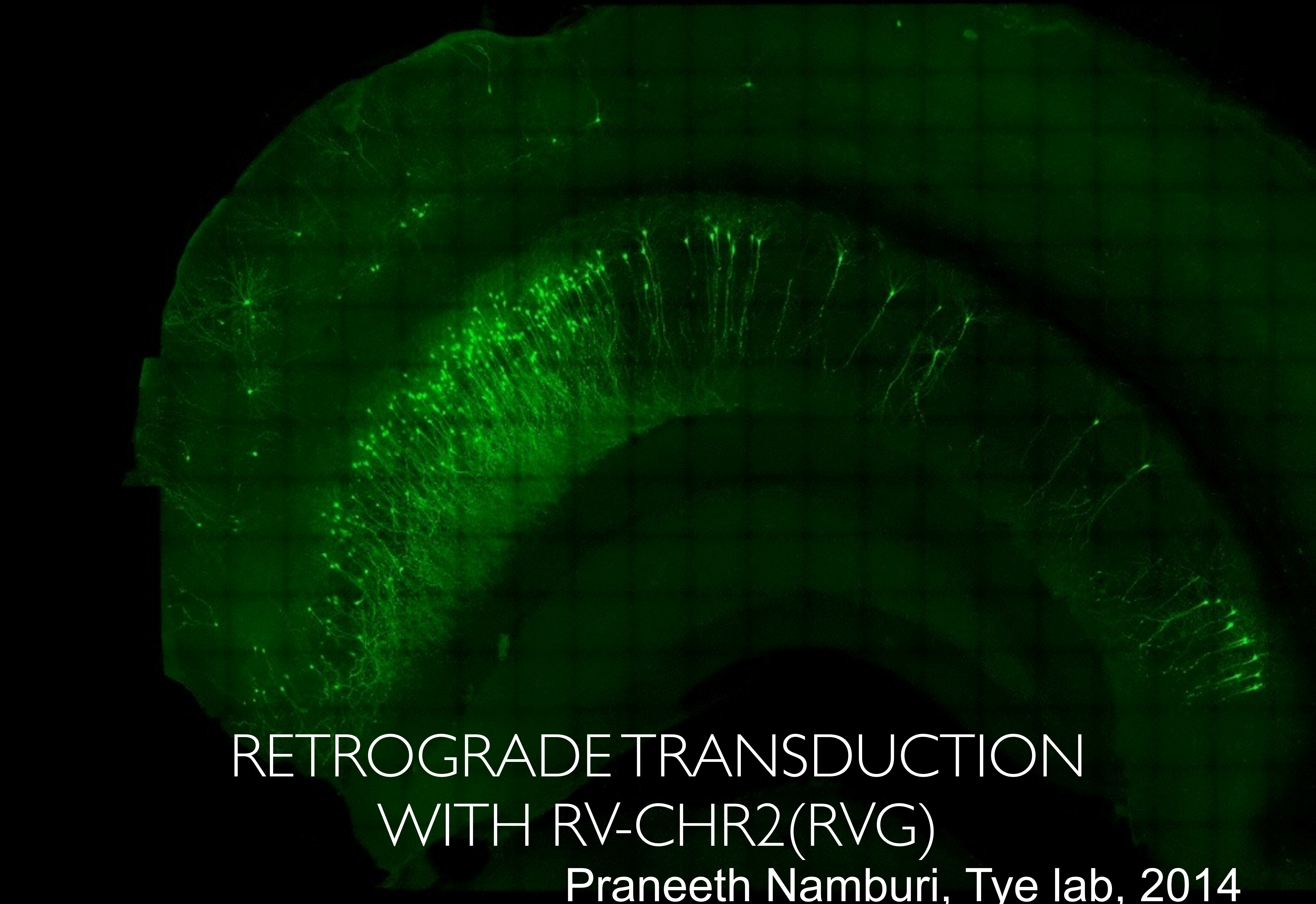
Wickersham et al. 2007a



RETROGRADE TARGETING WITH A RABIES VIRAL VECTOR

Wickersham et al. 2007a

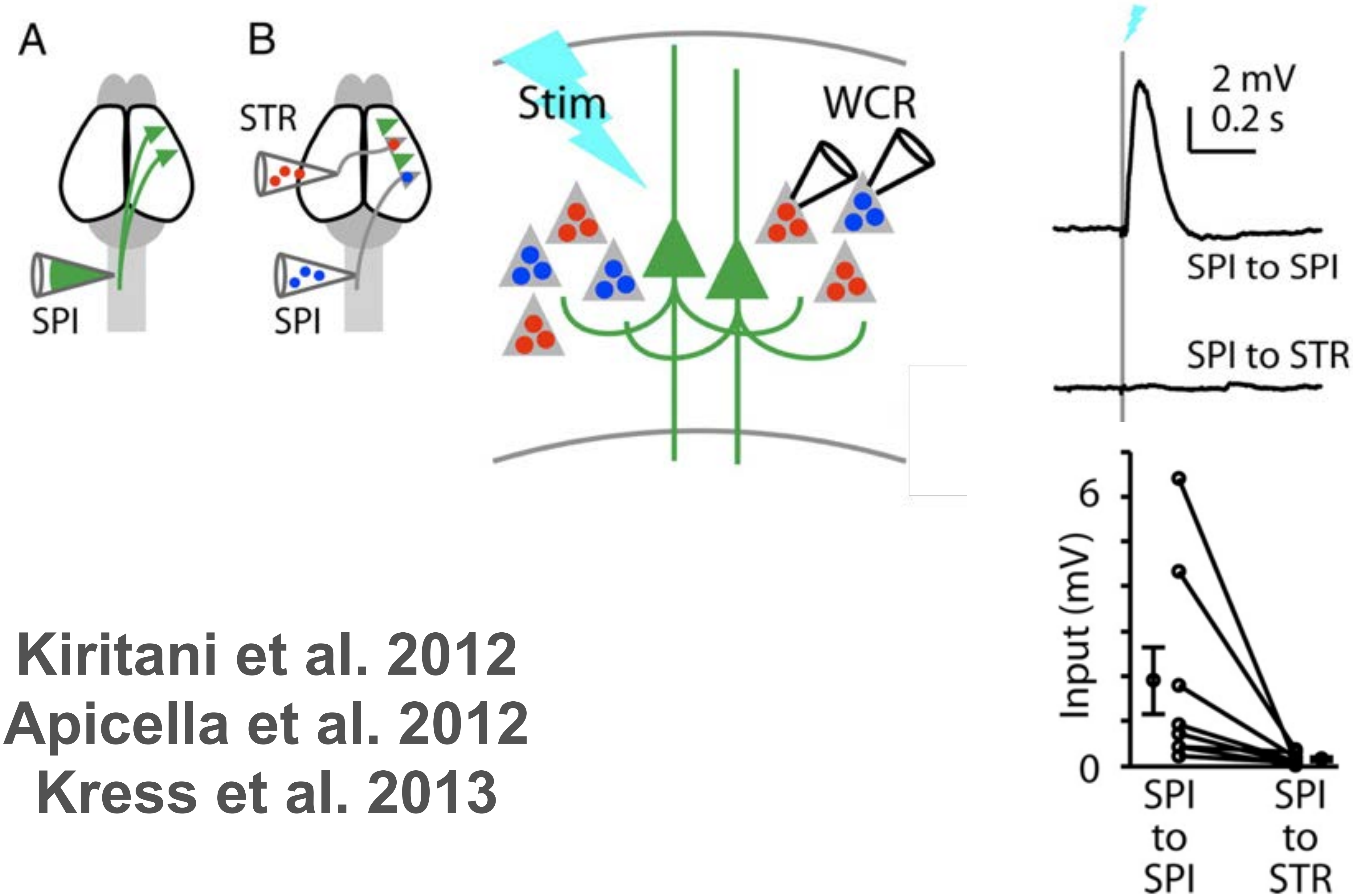
Source: Wickersham, Ian R., Stefan Finke, et al. "Retrograde Neuronal Tracing with a Deletion-mutant Rabies Virus." *Nature Methods* 4, no. 1 (2007): 47–49.



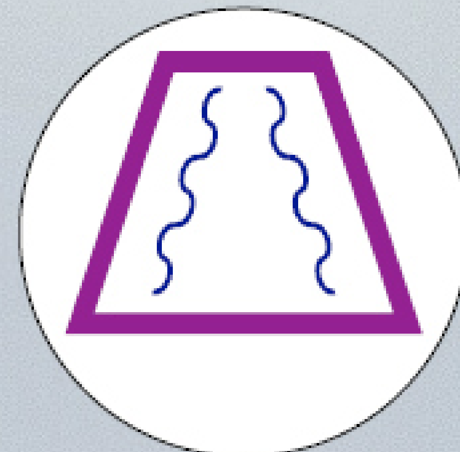
RETROGRADE TRANSDUCTION WITH RV-CHR2(RVG)

Praneeth Namburi, Tye lab, 2014

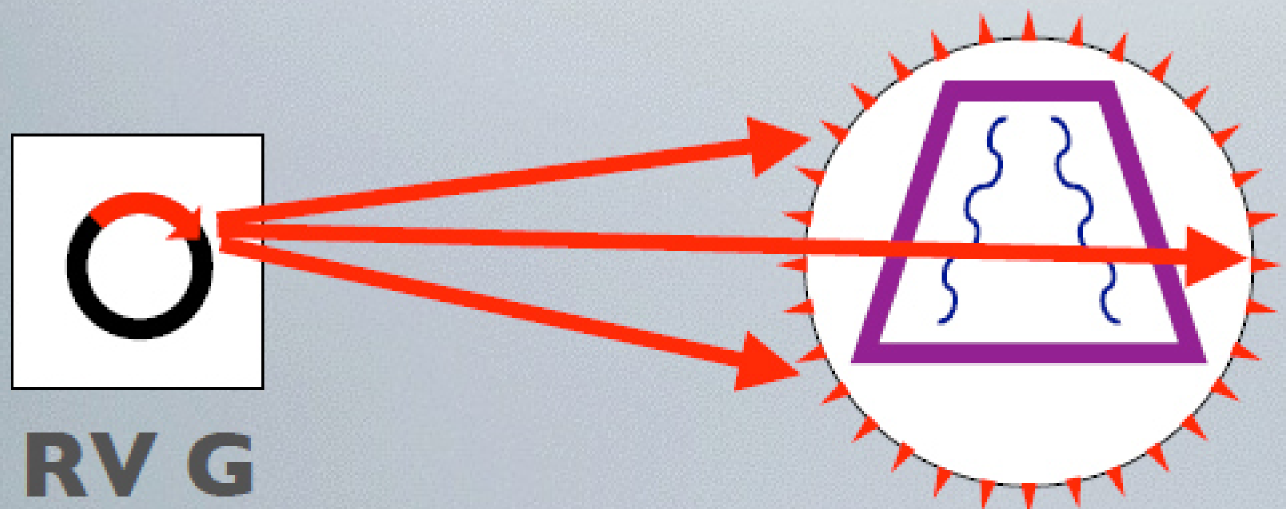
Retrograde delivery of ChR2 using RV for patch confirmation of connectivity



Kiritani et al. 2012
Apicella et al. 2012
Kress et al. 2013



LENTIVIRUS



LENTIVIRUS

WITH RV ENVELOPE, RETROGRADELY INFECTIOUS

Mazarakis et al. 2001
Wickersham et al. 2007a
Kato et al. 2011

Figure removed due to copyright restrictions.
Please see Figure 1A from Wickersham, Ian R., Heather A. Sullivan, et al. "Lentiviral Vectors for Retrograde Delivery of Recombinases and Transactivators." *Cold Spring Harbor Protocols* 2015, no. 4 (2015): pdb–prot075879.

RETROGRADE INFECTION WITH LV-CRE(RVG)

Wickersham et al. in press

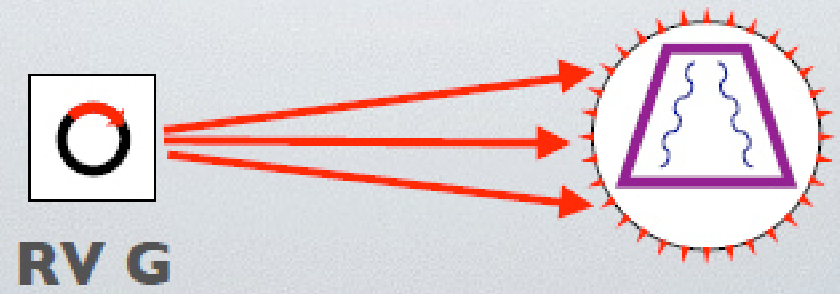
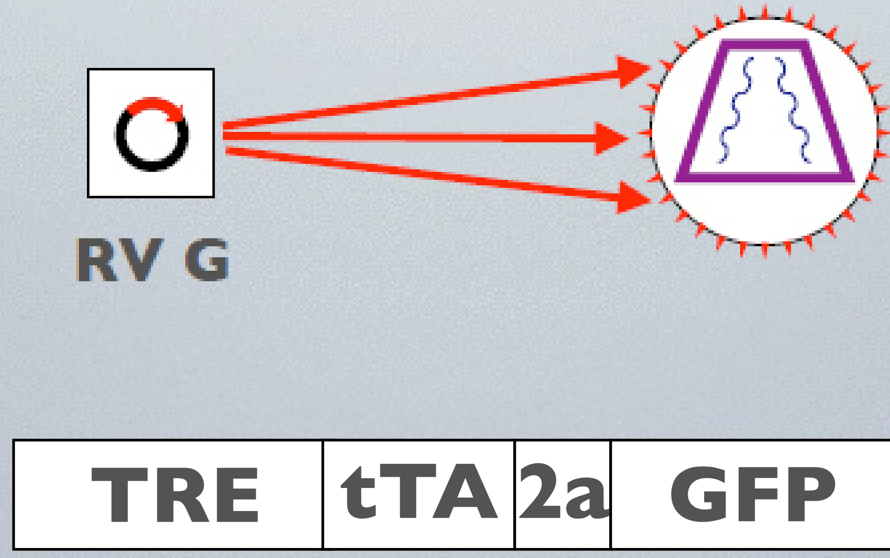


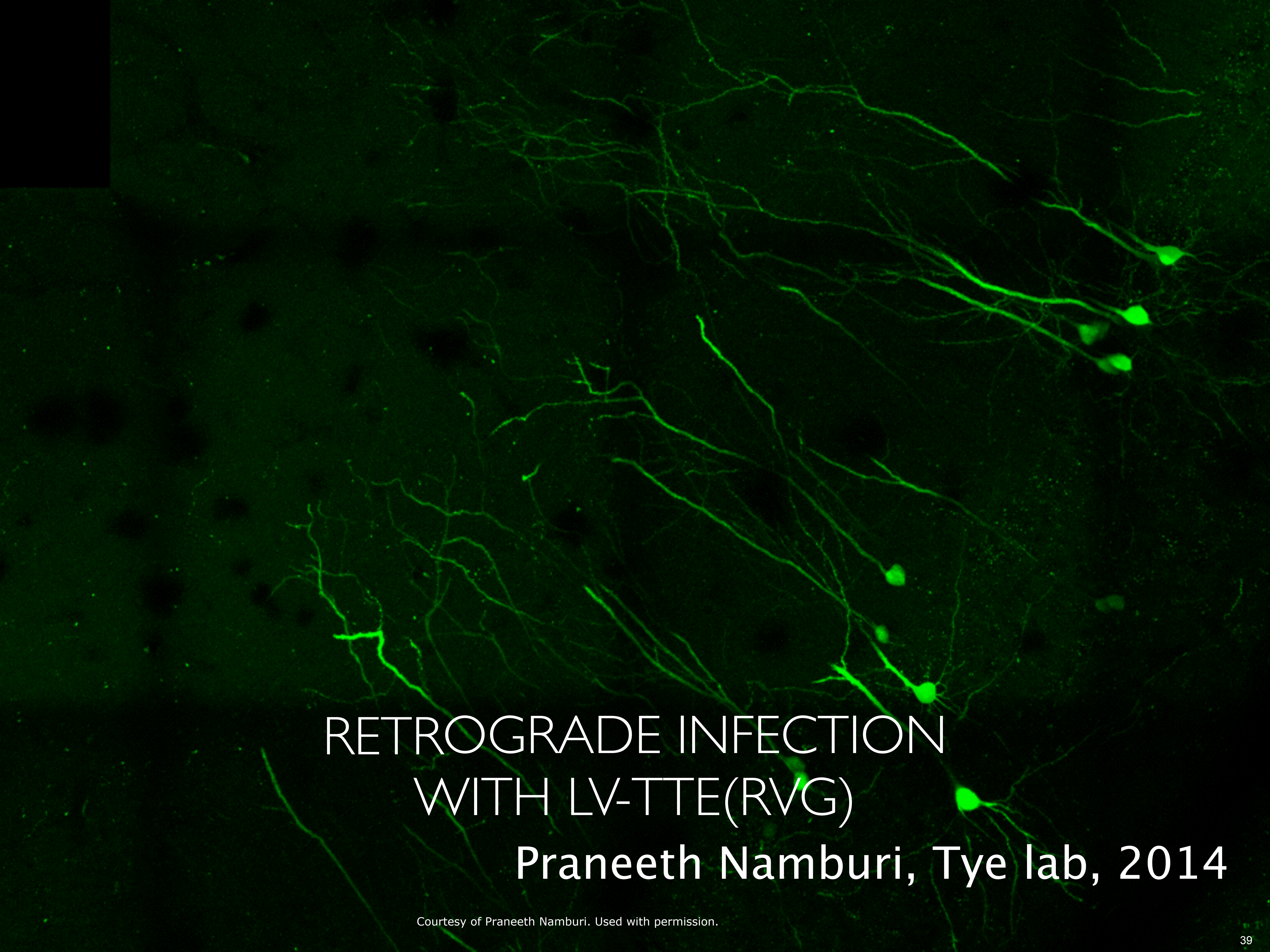
Figure removed due to copyright restrictions.
Please see figure 2A from Cetin, Ali, and Edward M. Callaway. "Optical Control of Retrogradely Infected Neurons using Drug-regulated "TLoop" Lentiviral Vectors." *Journal of Neurophysiology* 111, no. 10 (2014): 2150–59.

"T-LOOP" LENTIS: HIGH, FAST, TET-REPRESSIBLE EXPRESSION FROM SINGLE COMPACT CASSETTE

Cetin & Callaway '14



LV-TTE(RVG)



RETROGRADE INFECTION WITH LV-TTE(RVG)

Praneeth Namburi, Tye lab, 2014

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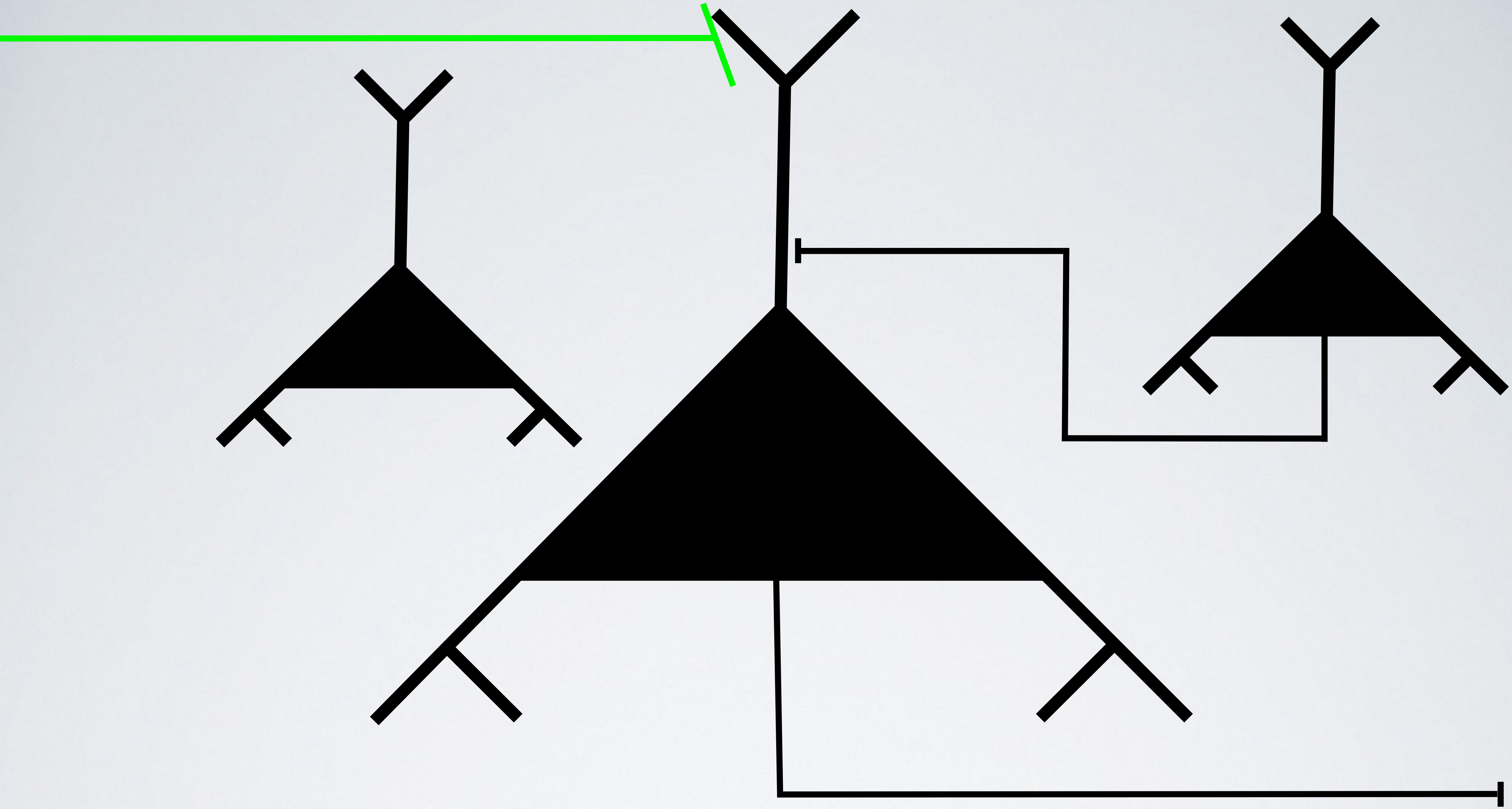
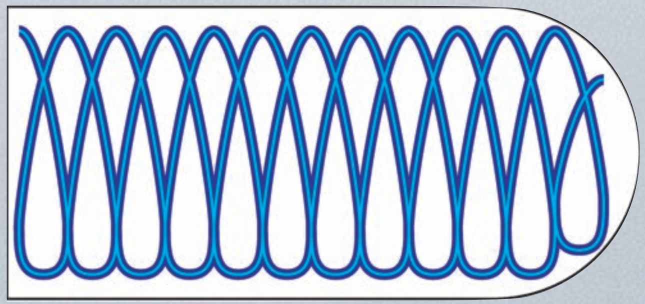


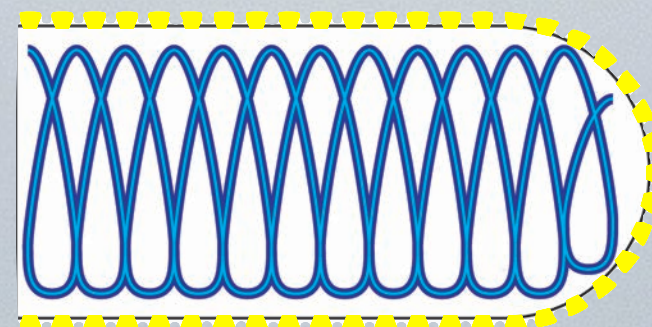
ANTEROGRADE TARGETING: DELIVERY TO AXONS VIA SOMATA



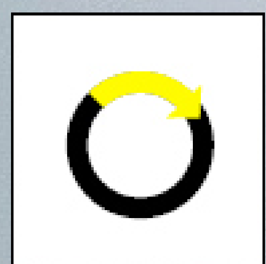
- RNA genome -> can NOT be made Cre (or Flp, etc.) dependent
- enveloped virus -> can be easily recoated with other viruses' envelope proteins

Wickersham et al. 2013

VSV G



Wickersham et al. 2013



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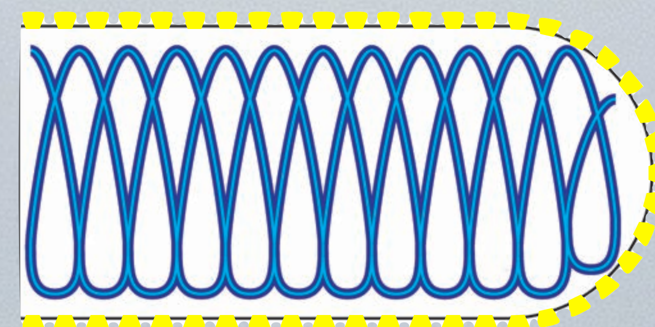
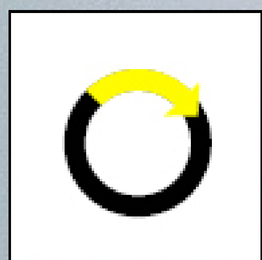


Wickersham et al. 2013



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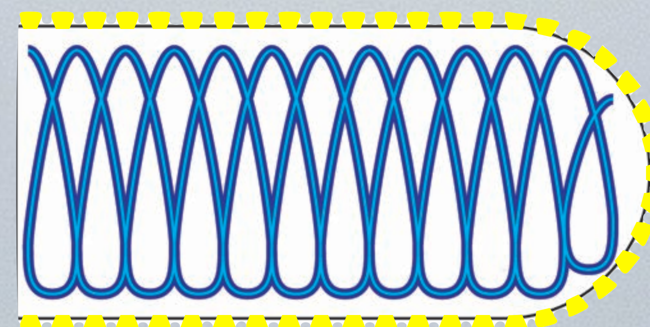
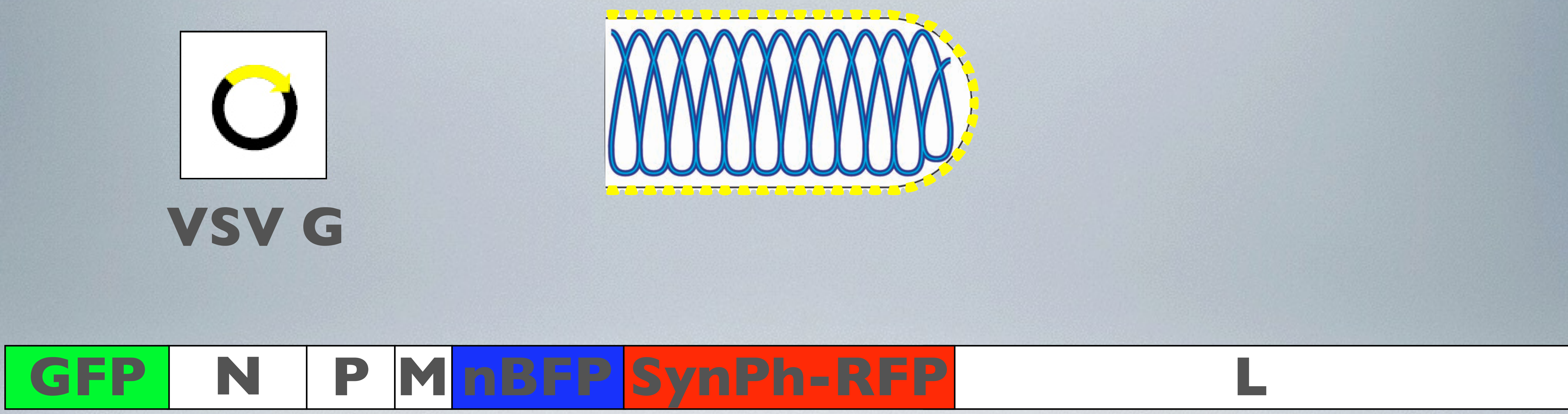


Wickersham et al. 2013



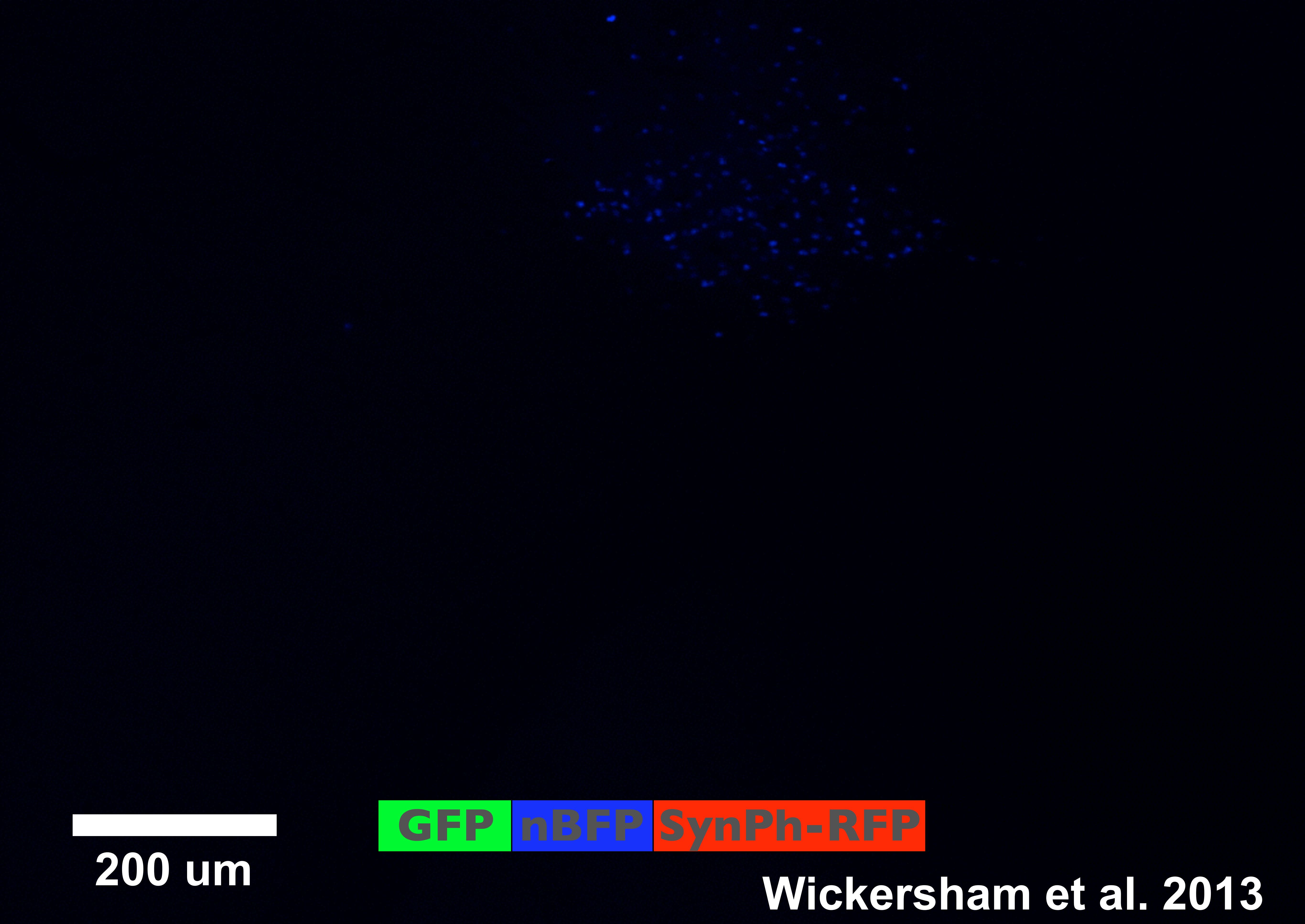
Wickersham et al. 2013

200 um

GFP nBFP SynPh-RFP

Wickersham et al. 2013

Source: Wickersham, Ian R., Heather A. Sullivan, et al. "Axonal and Subcellular Labelling using Modified Rabies Viral Vectors." *Nature Communications* 4 (2013).



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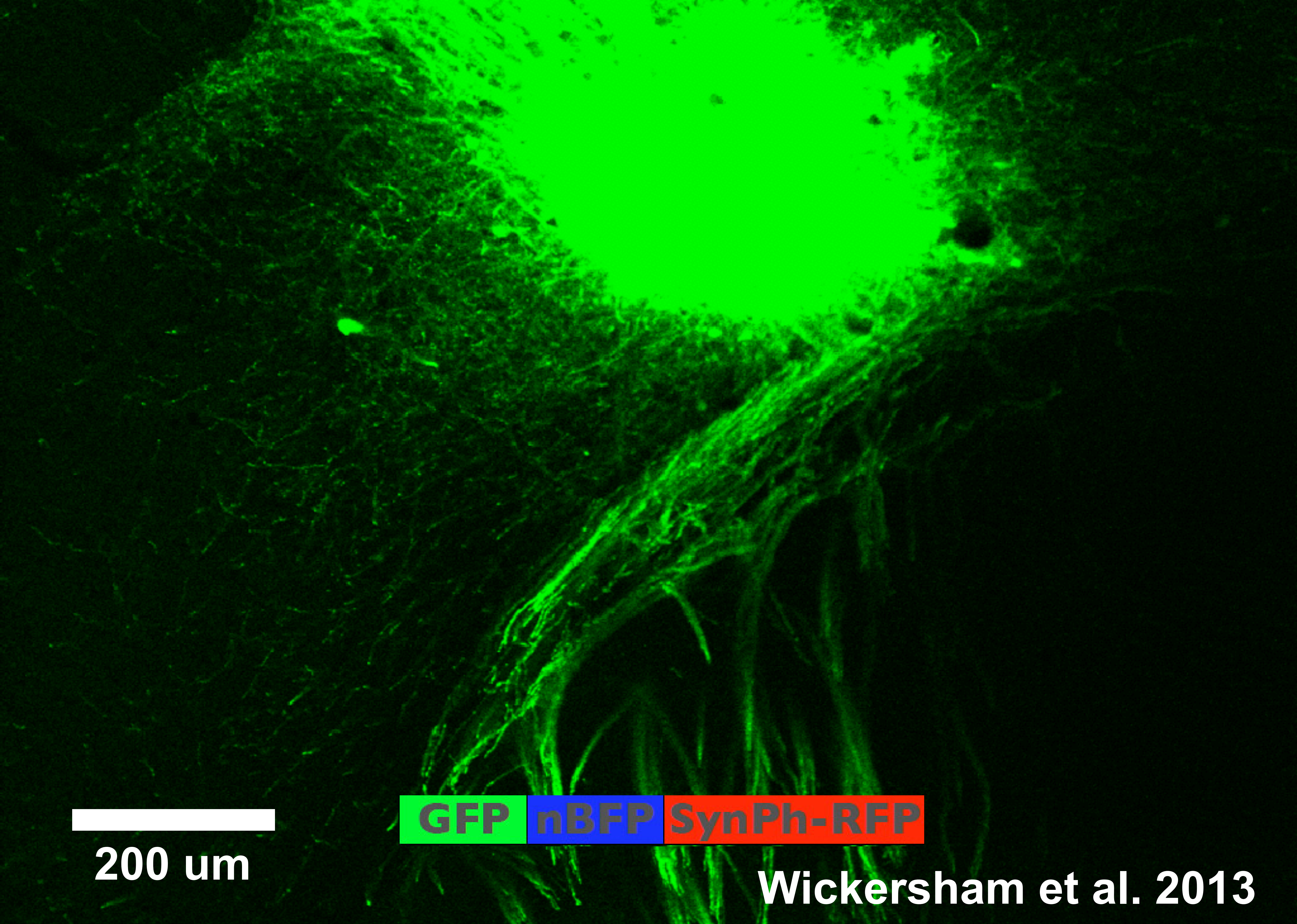

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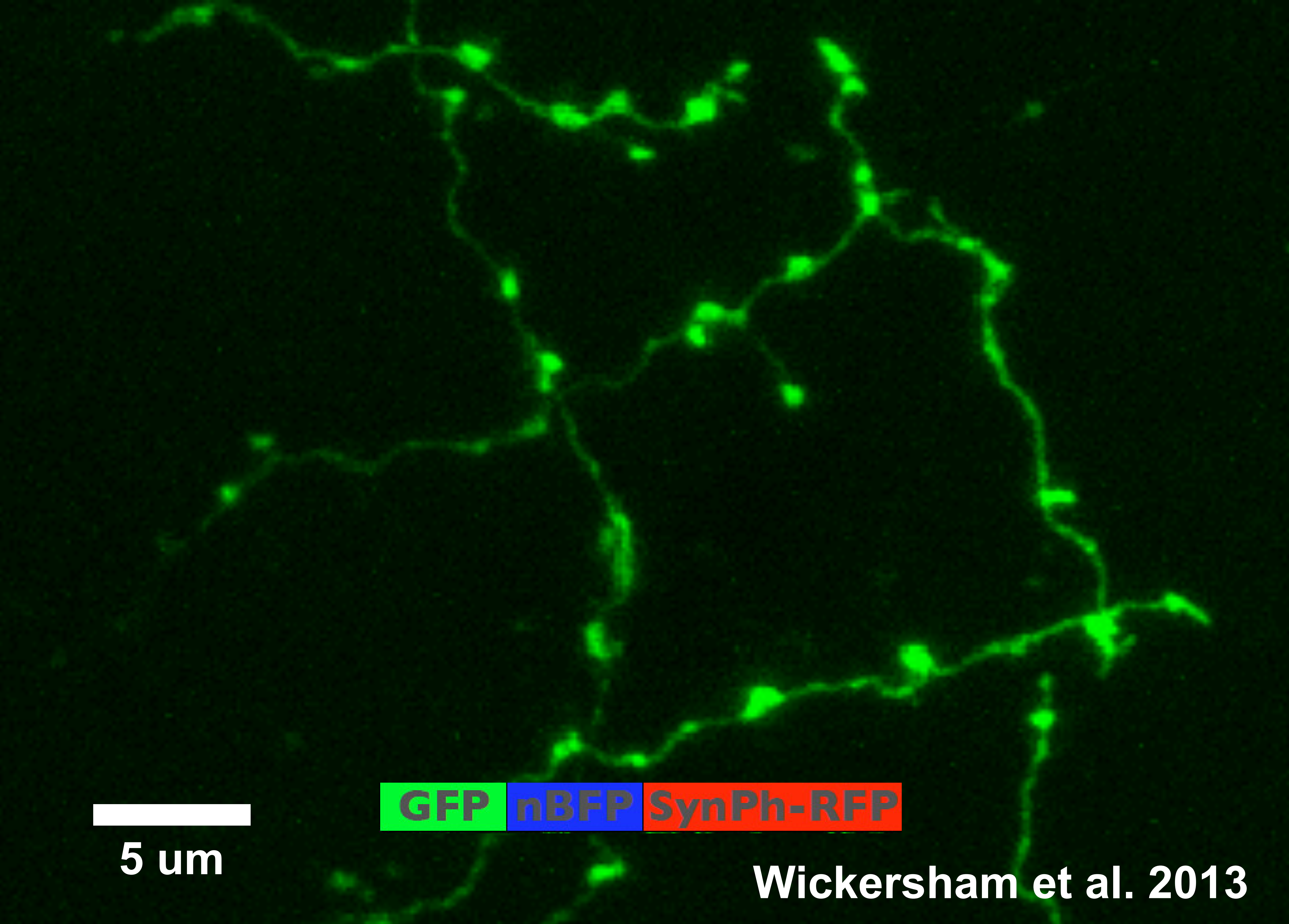

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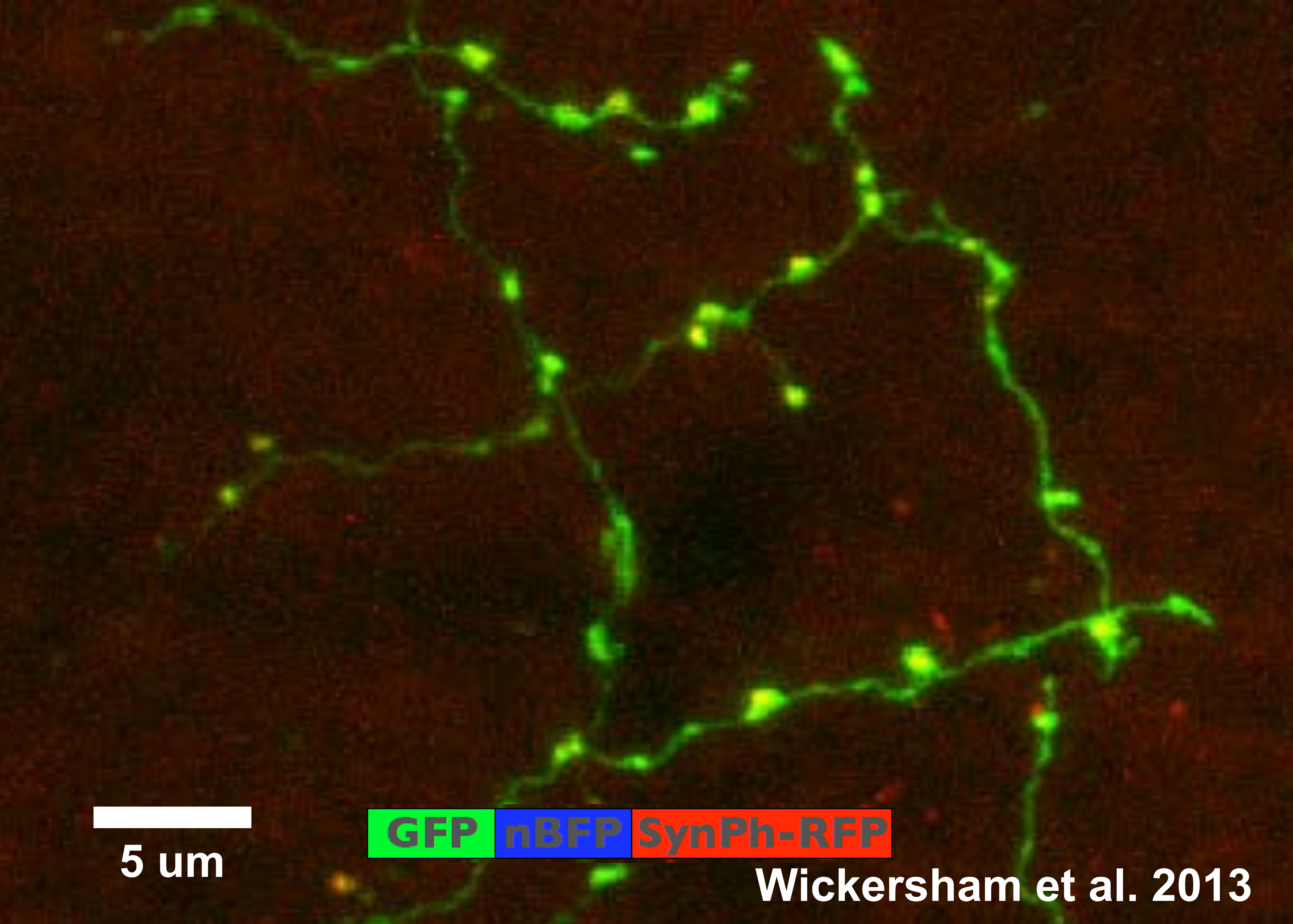

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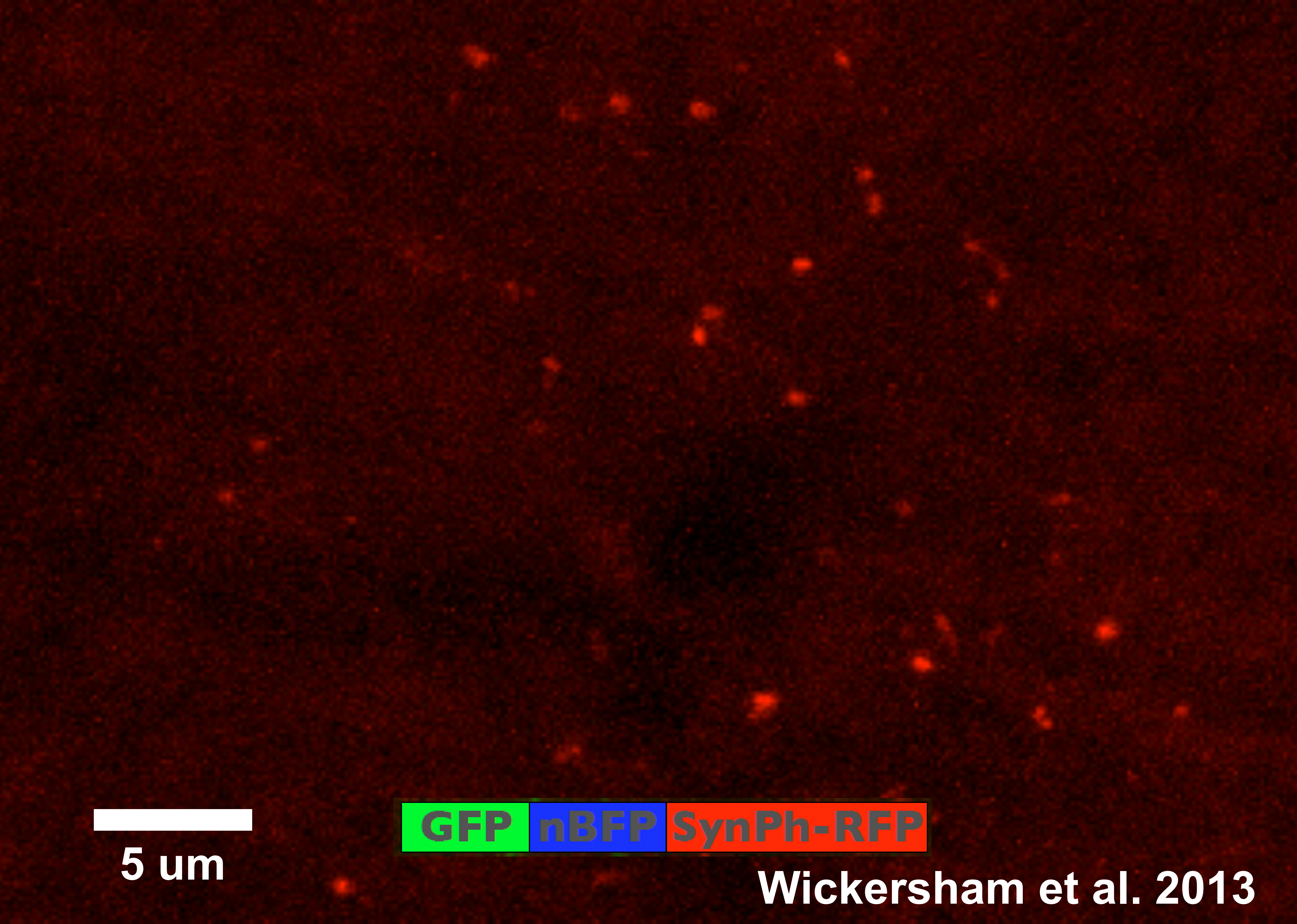
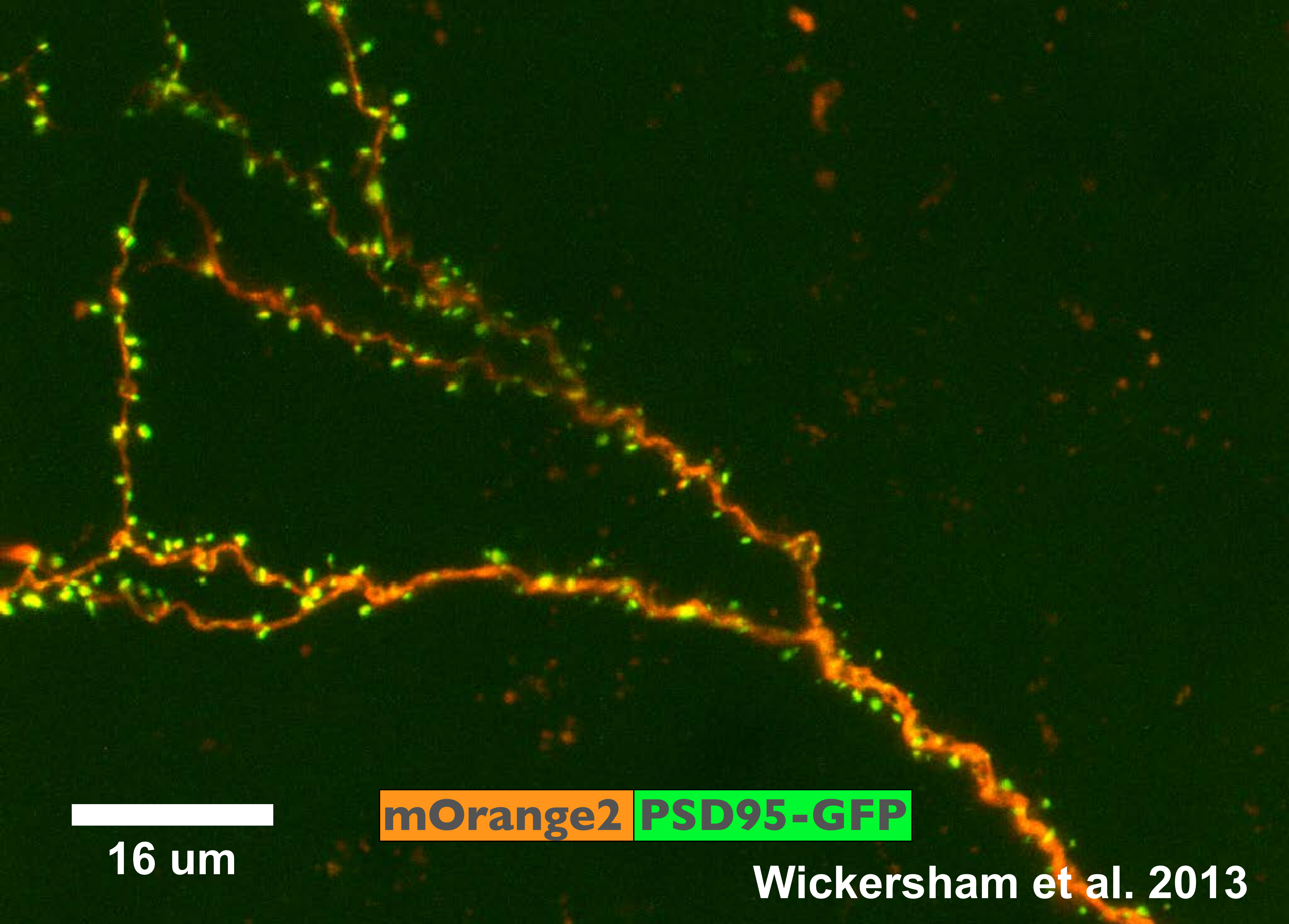

GFP
nBFP
SynPh-RFP
5 um
Wickersham et al. 2013



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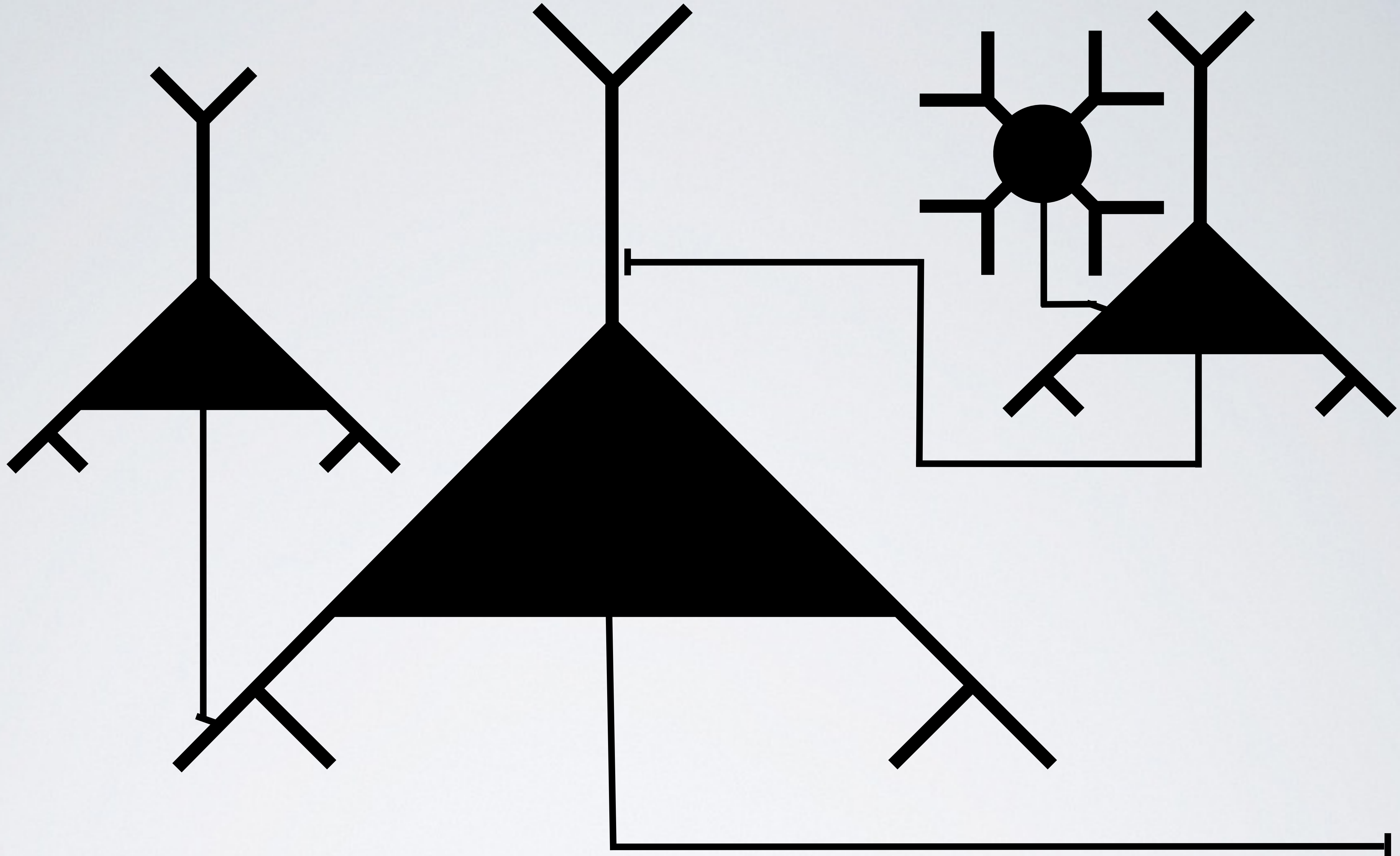
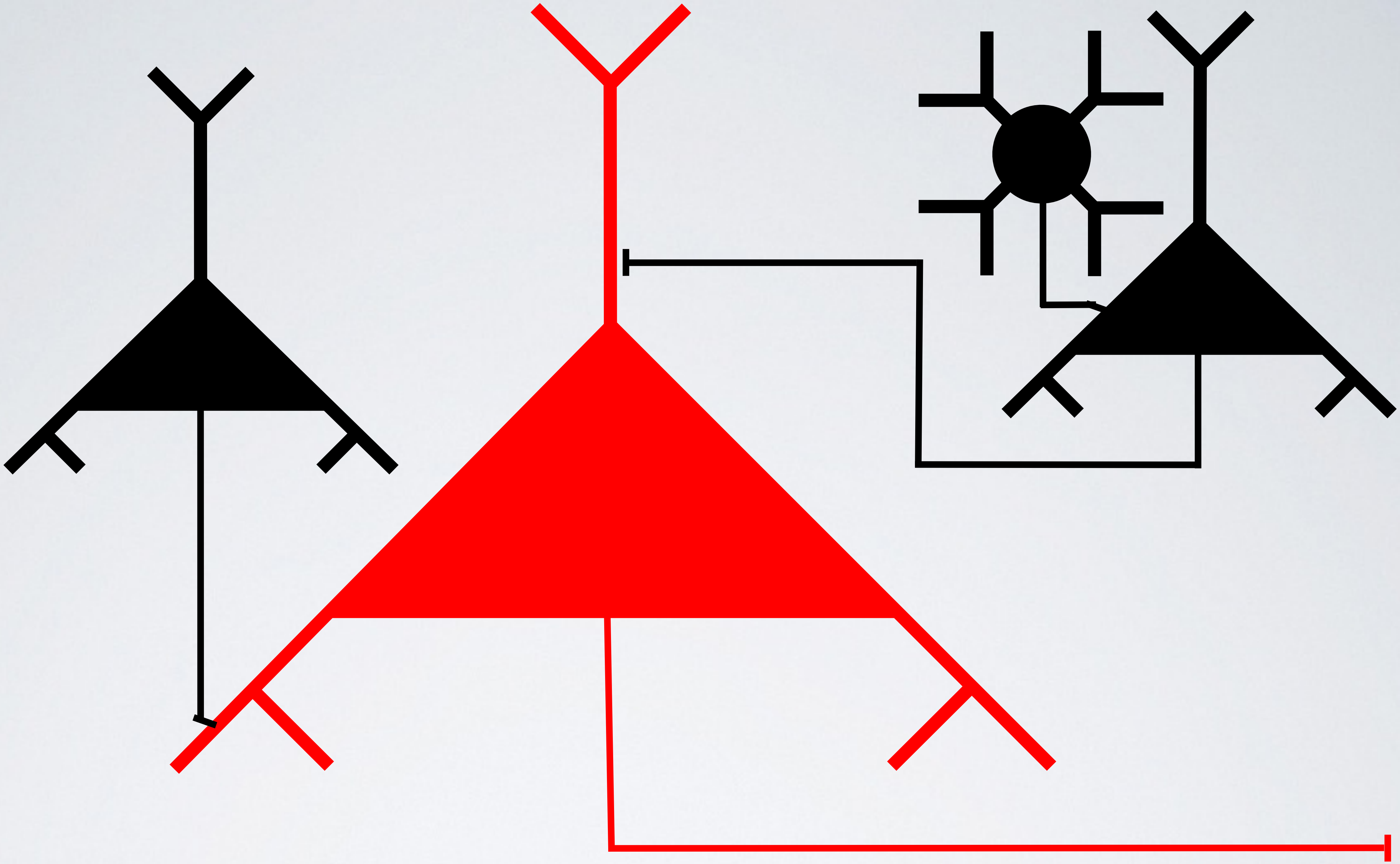
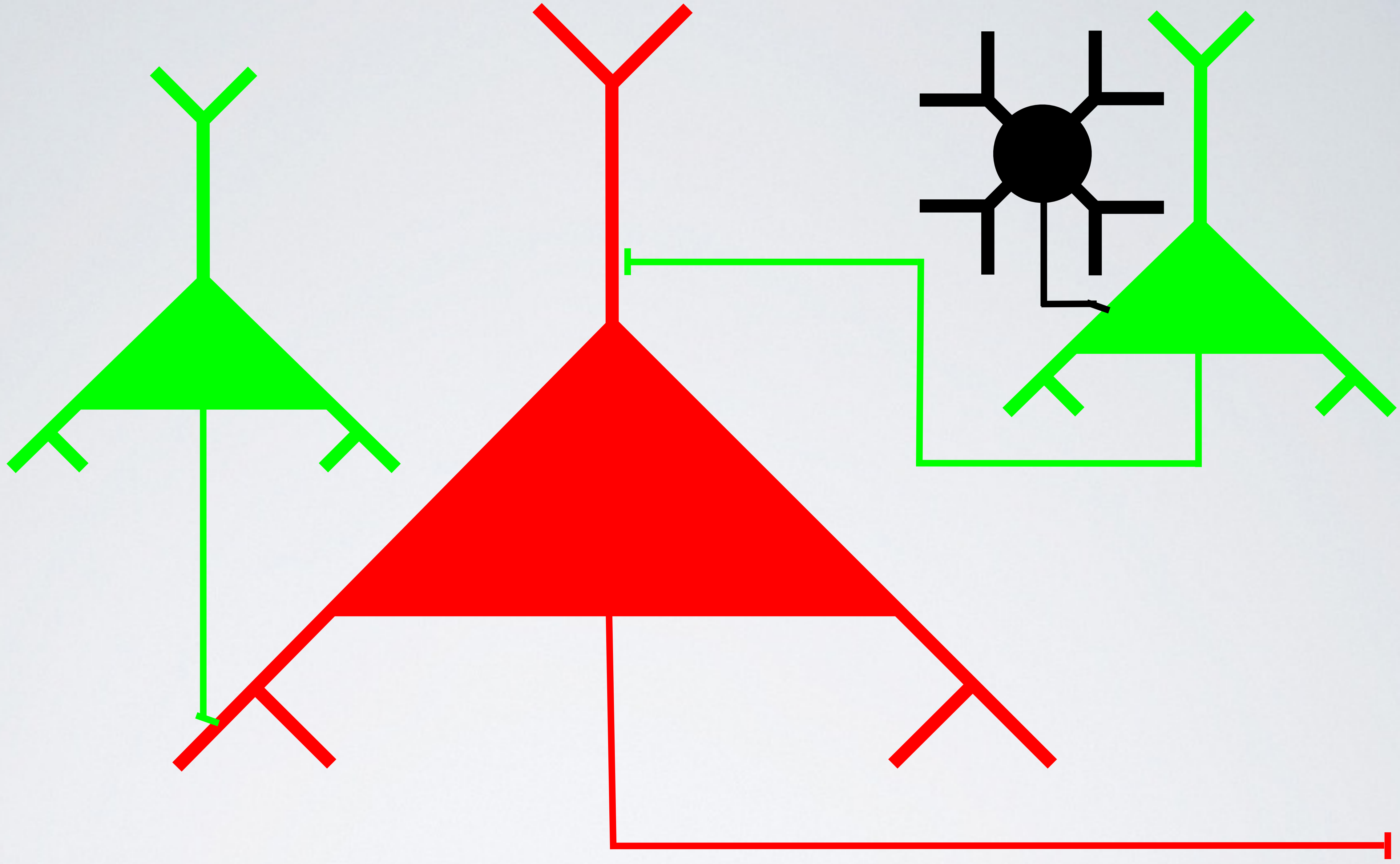
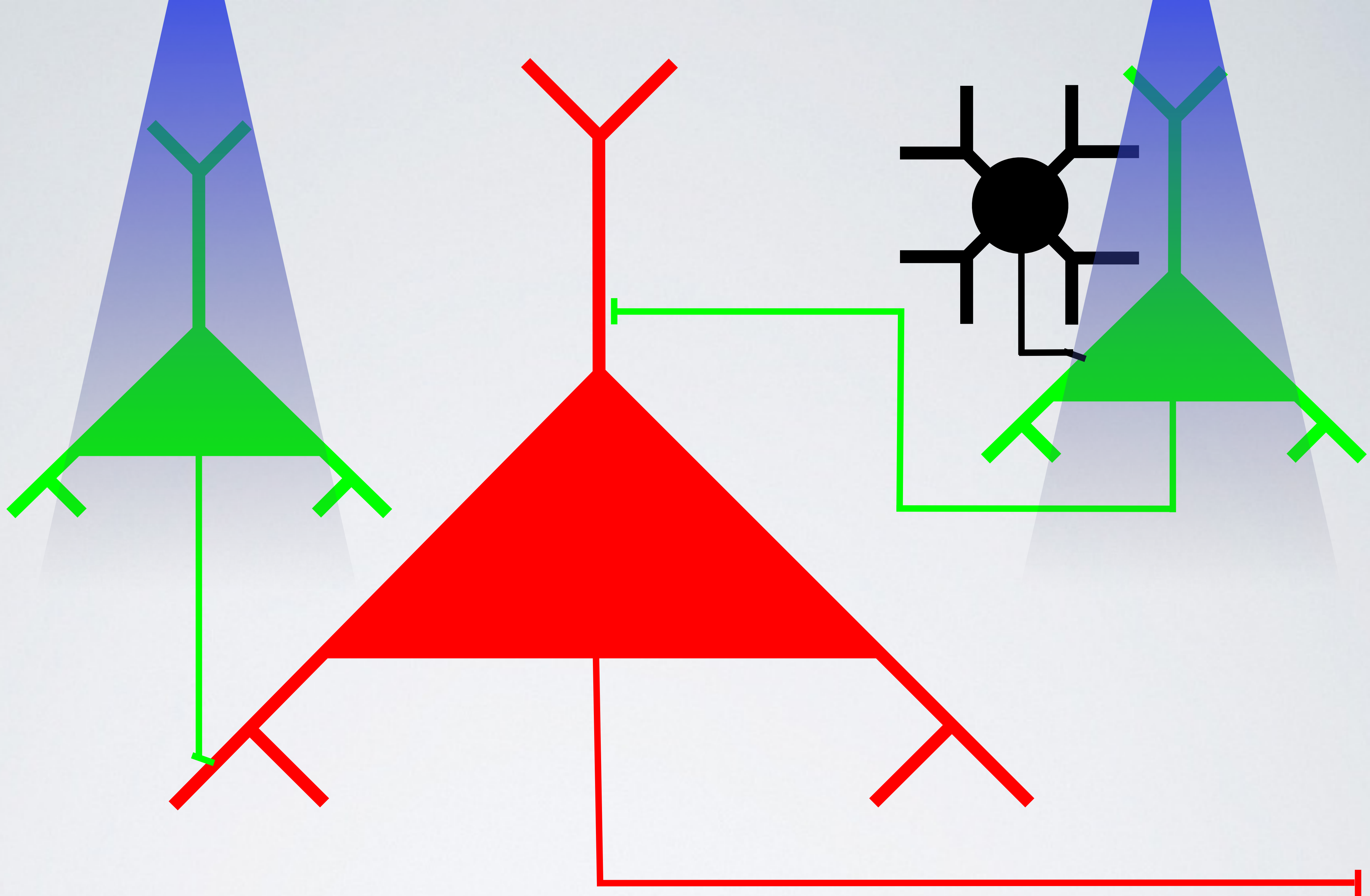
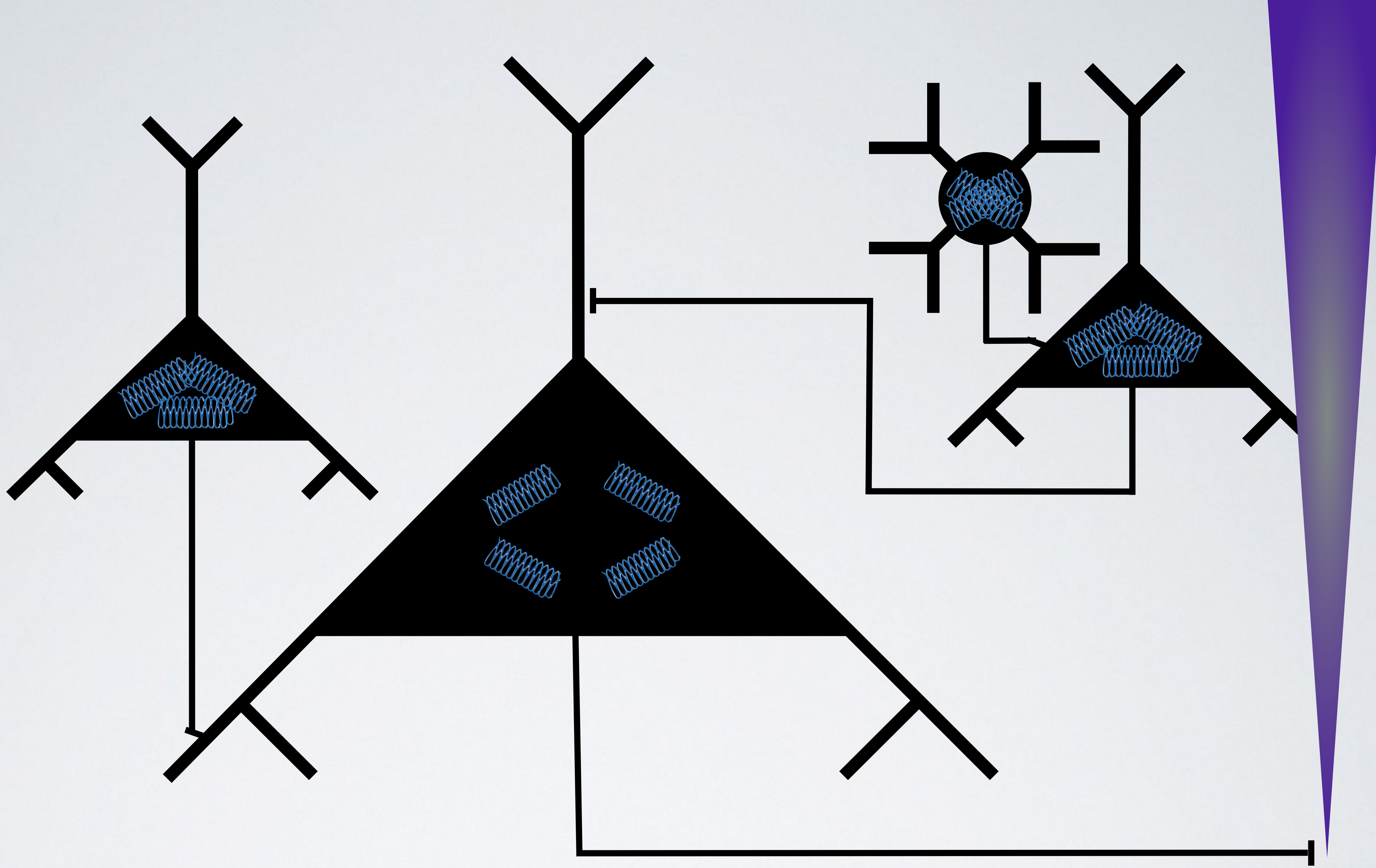


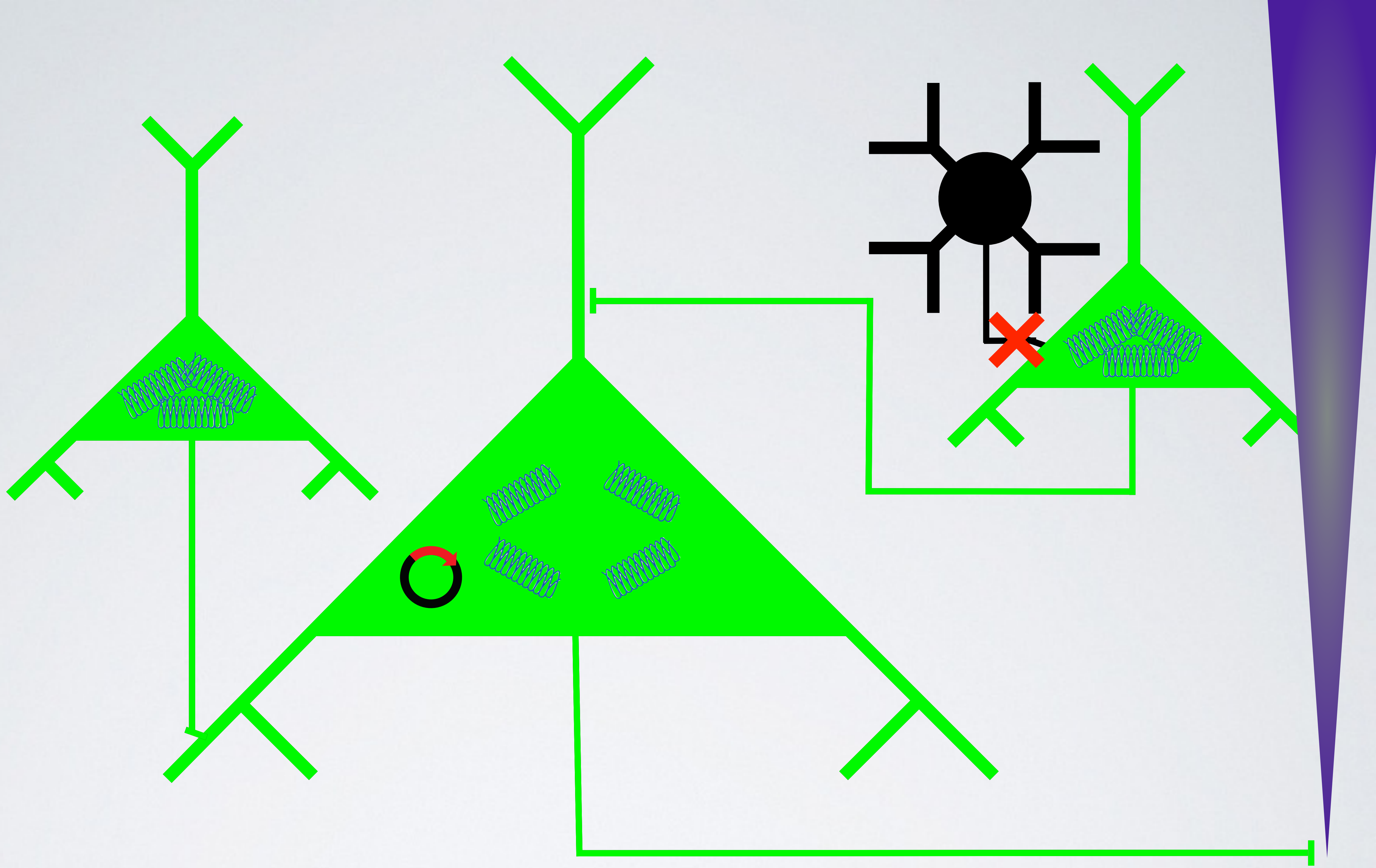






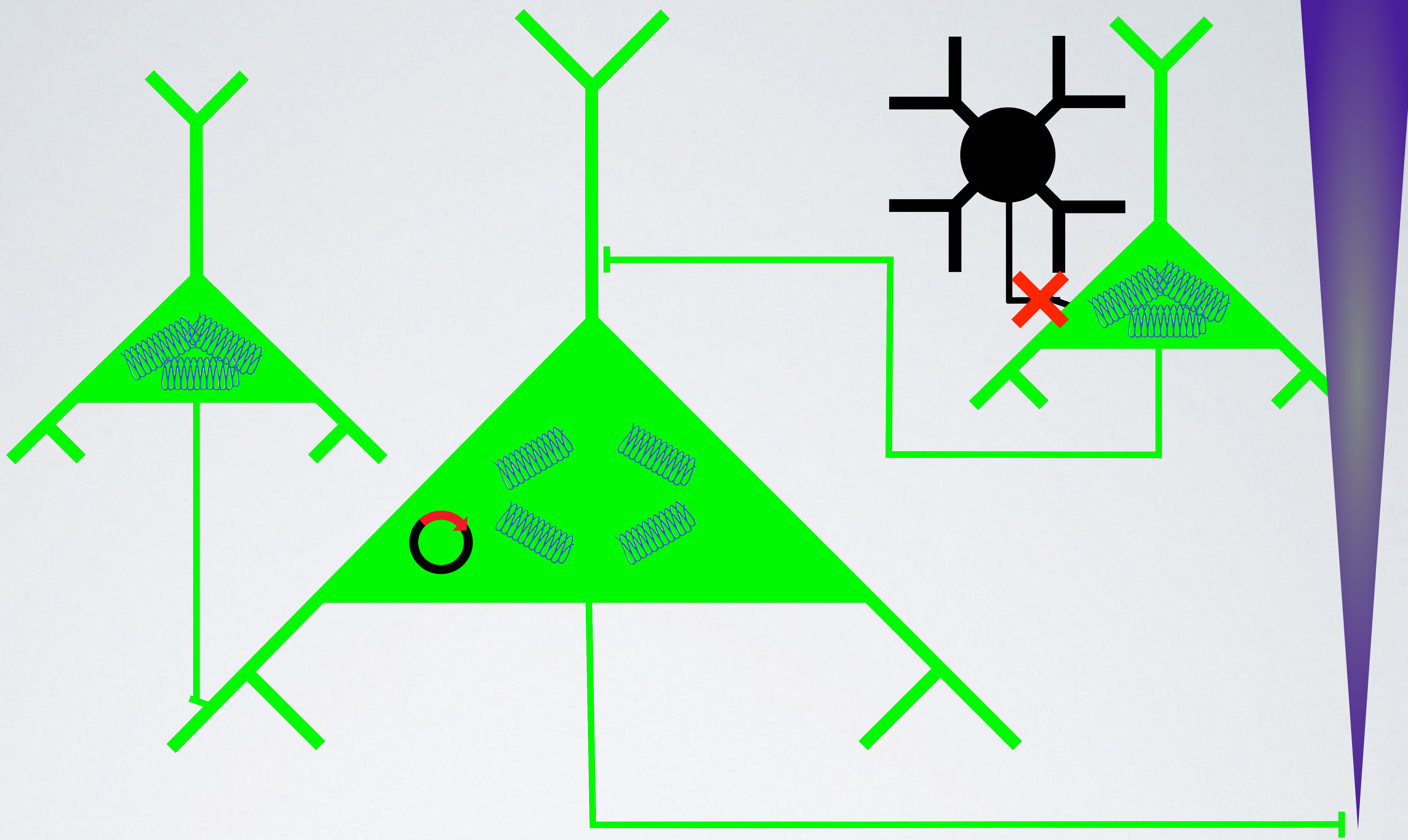


RABIES VIRUS

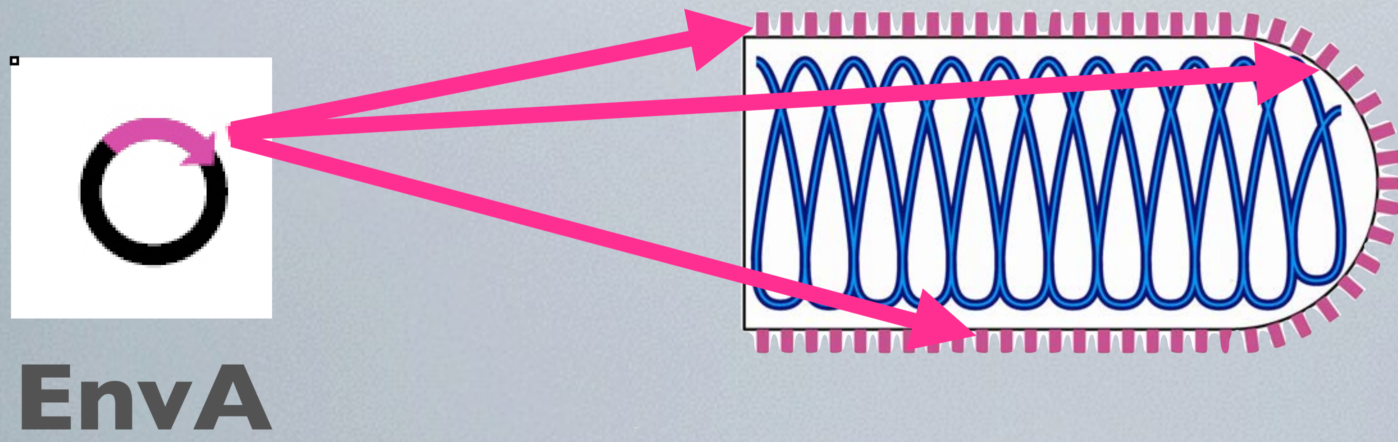


MONOSYNAPTIC TRACING

Wickersham et al. 2007b

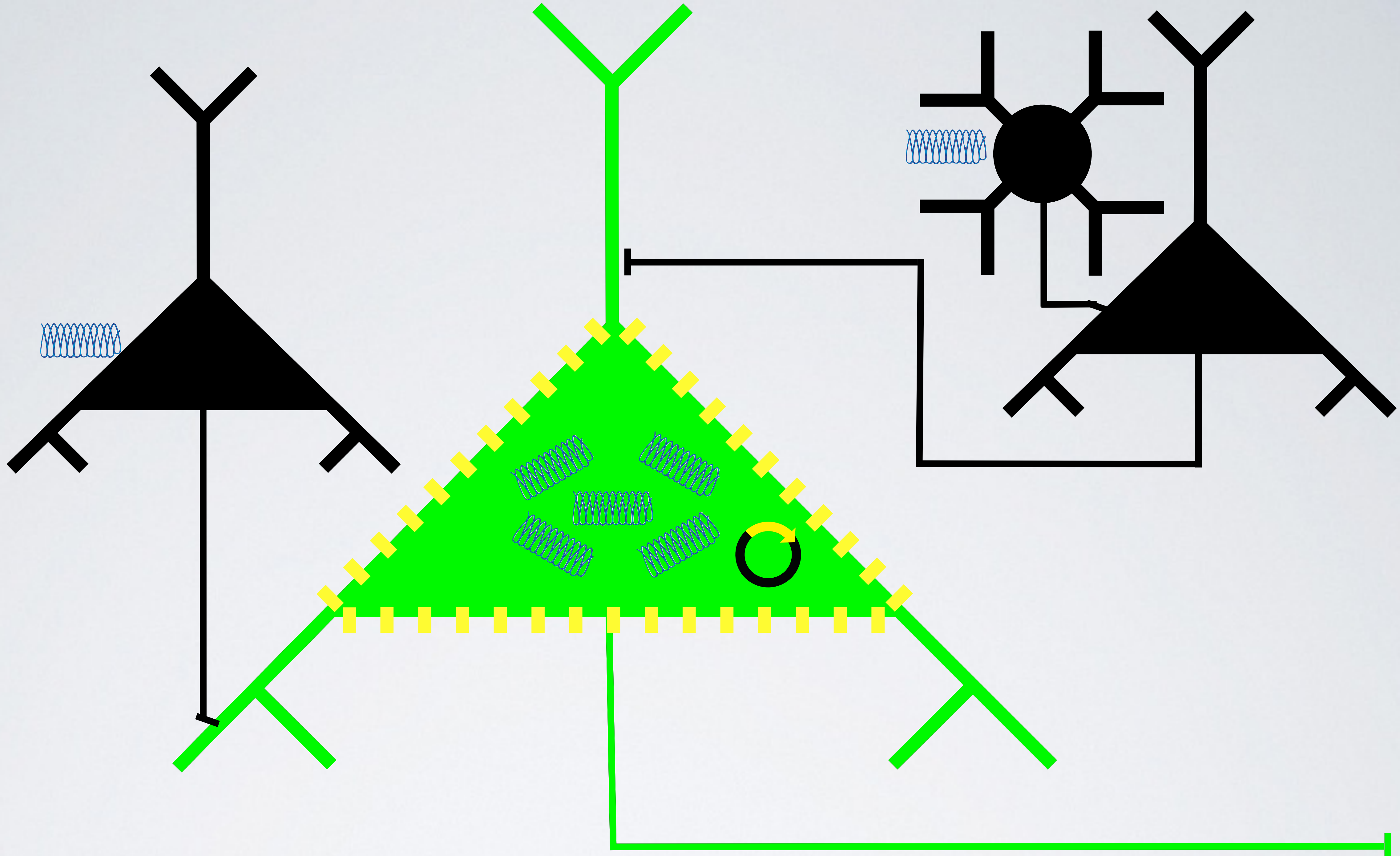


MONOSYNAPTIC TRACING USING RETROGRADE COINFECTION



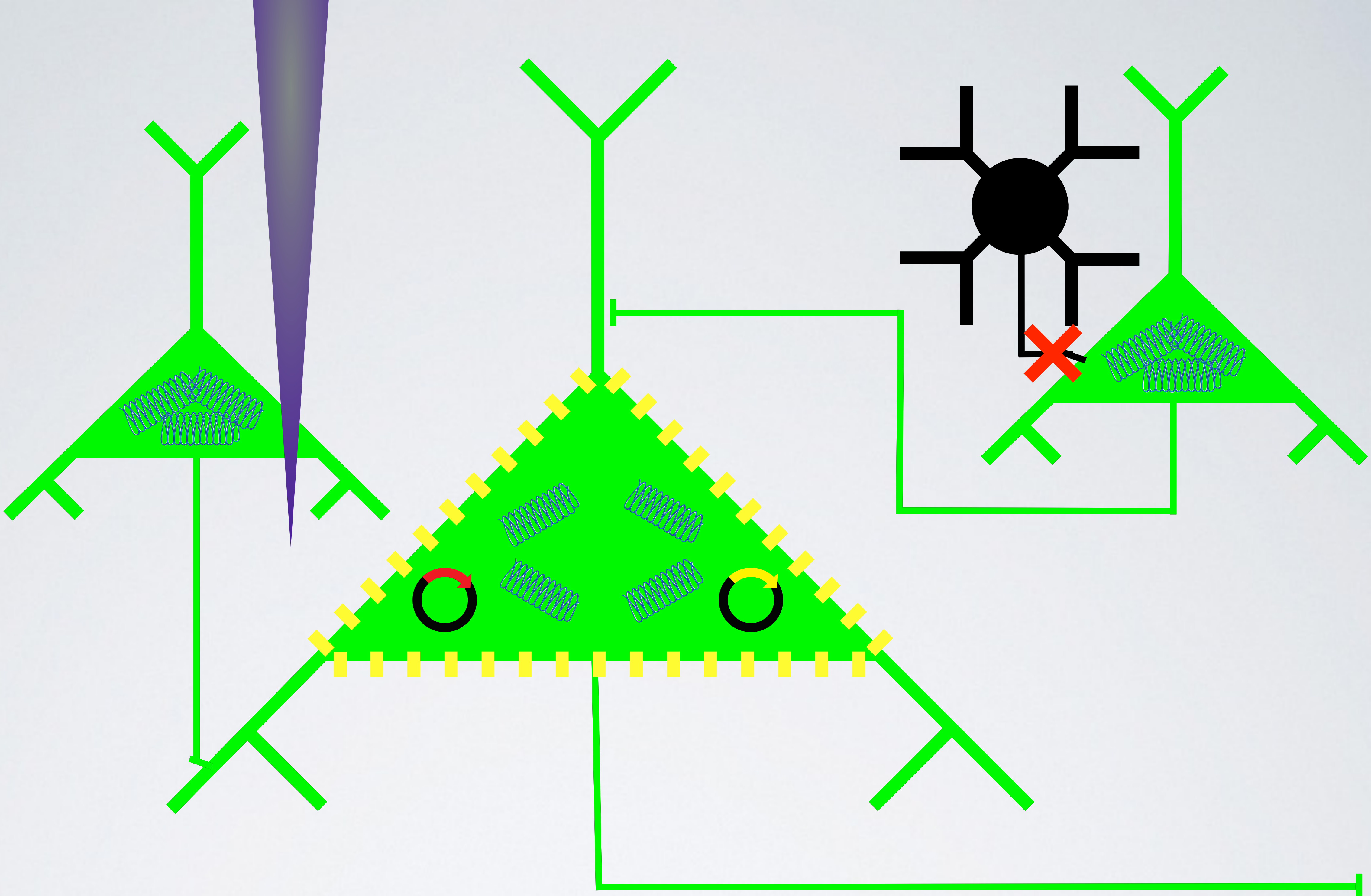
TARGETING INFECTION WITH ENVA/TVA

Wickersham et al. 2007b

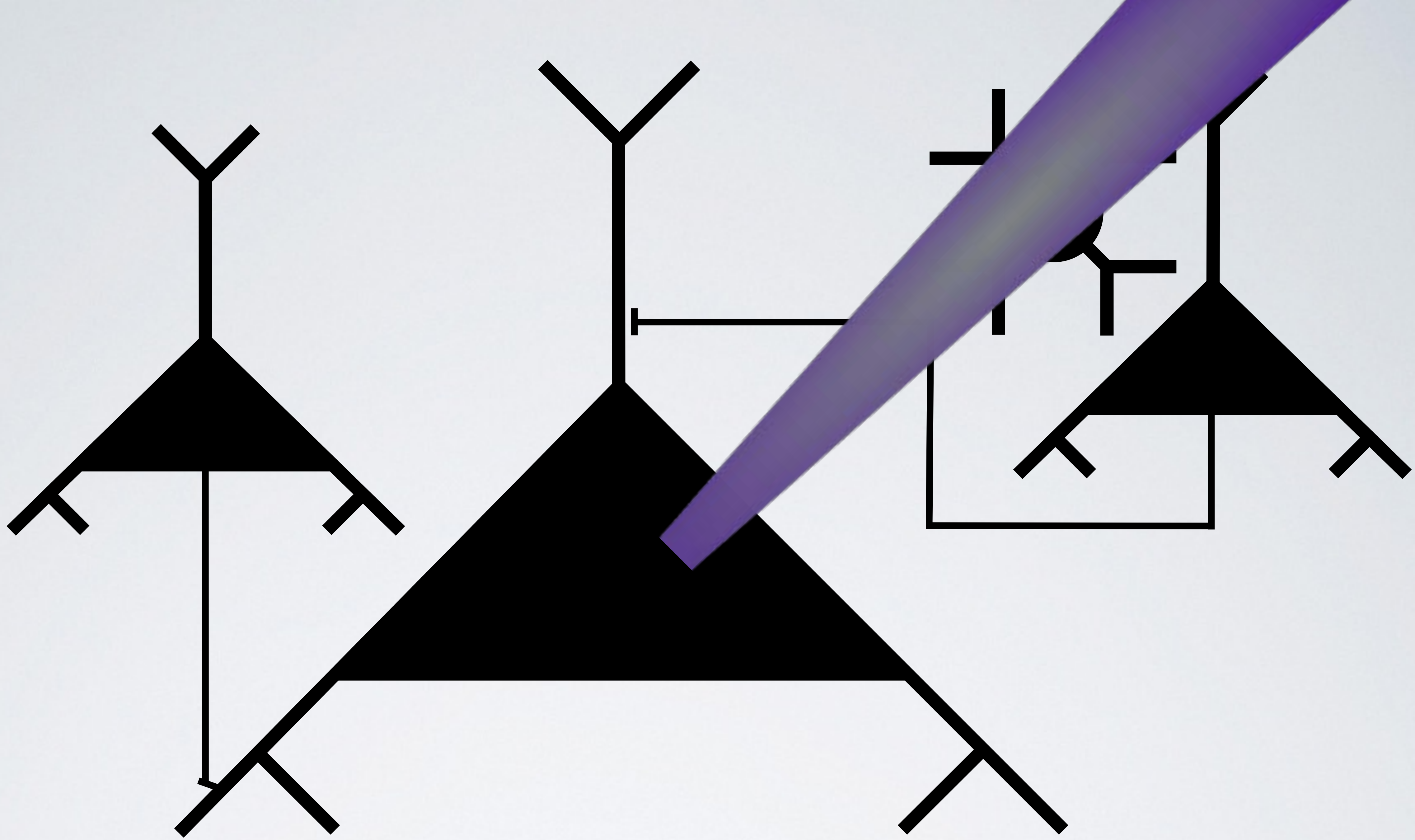


TARGETING INFECTION WITH ENVA/TVA

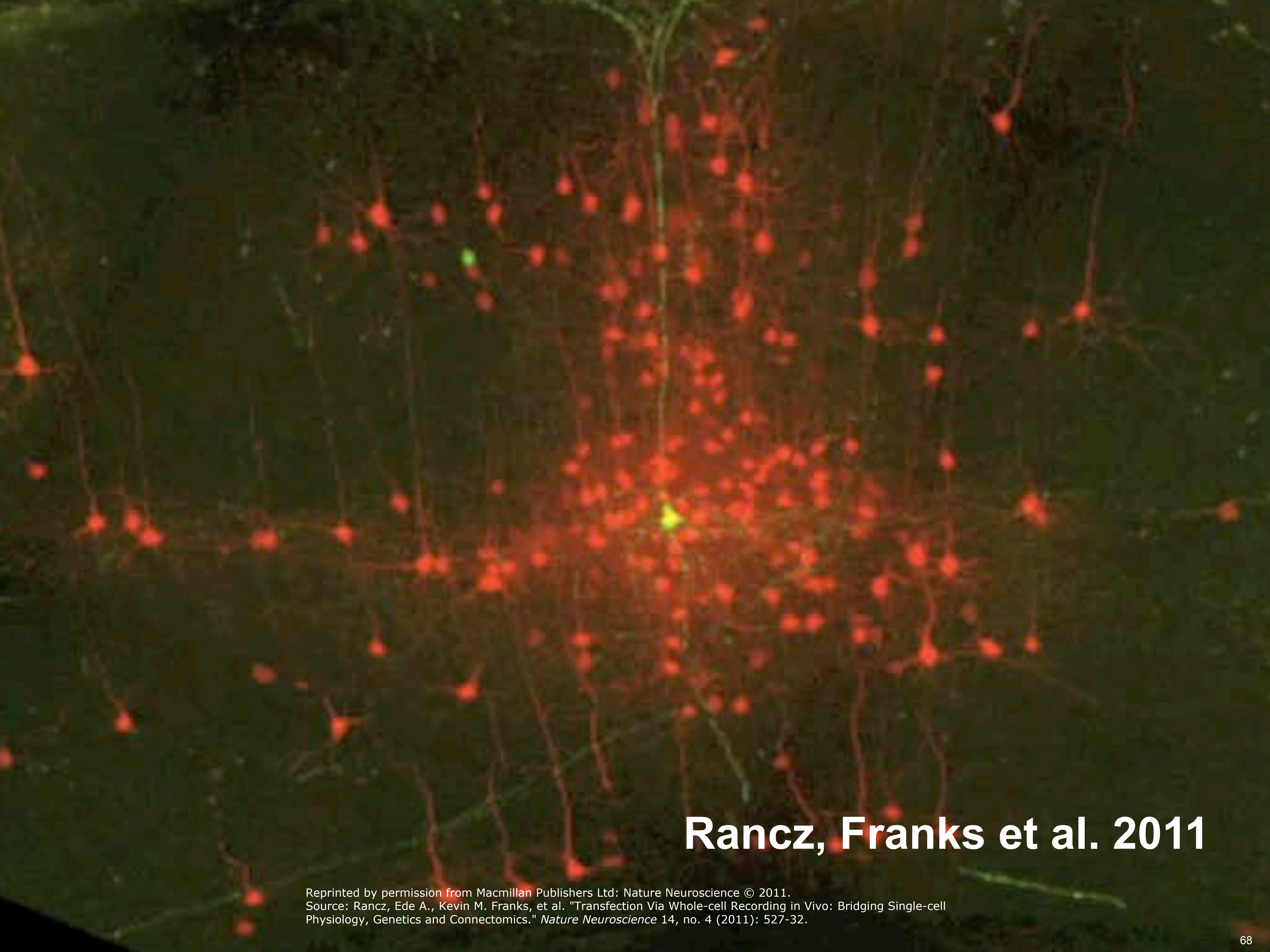
Wickersham et al. 2007b



Wickersham et al. 2007b

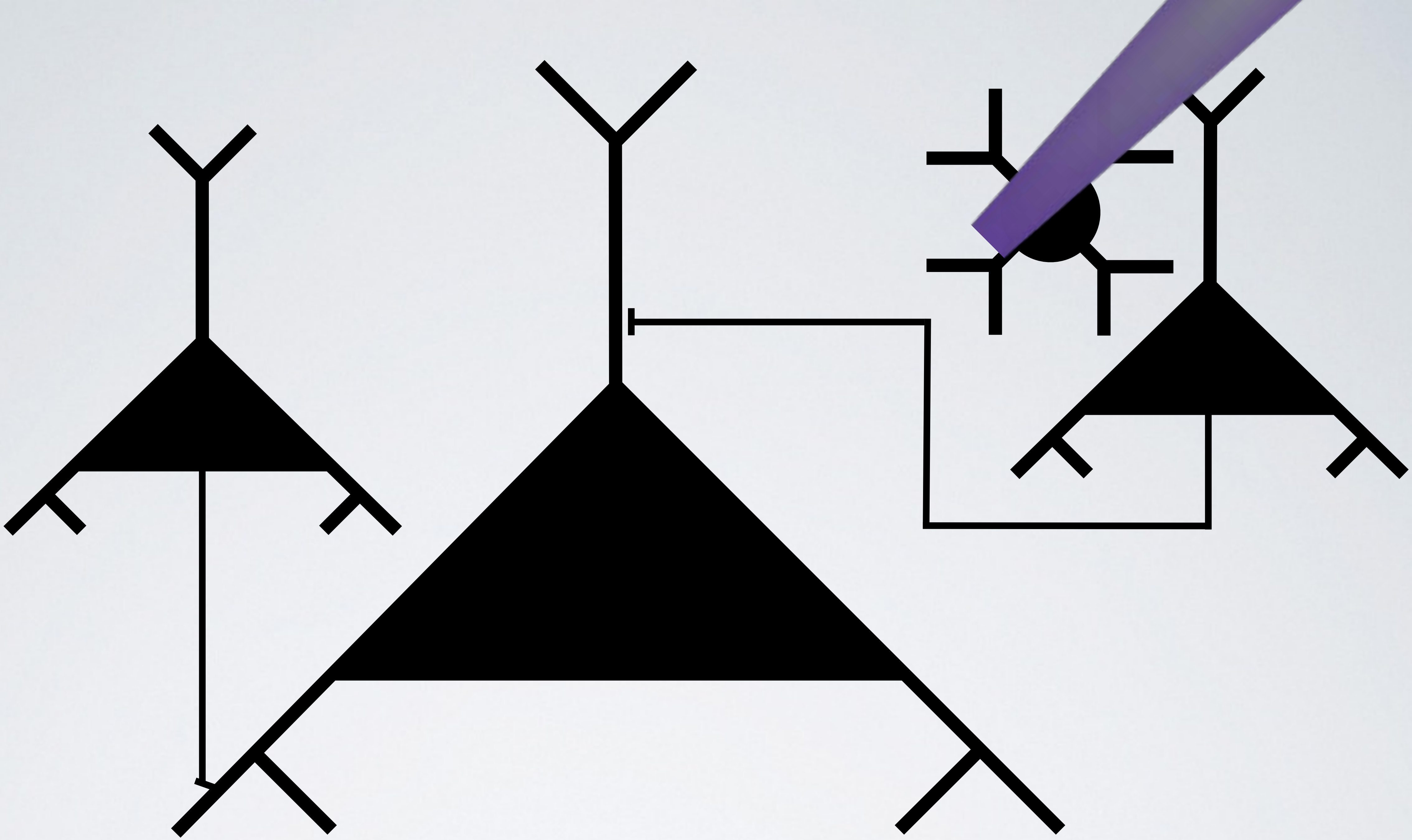


Marshel et al. 2010
Rancz, Franks et al. 2011
Velez-Fort et al. 2014



Rancz, Franks et al. 2011

Source: Rancz, Ede A., Kevin M. Franks, et al. "Transfection Via Whole-cell Recording in Vivo: Bridging Single-cell Physiology, Genetics and Connectomics." *Nature Neuroscience* 14, no. 4 (2011): 527-32.



Kodandaramaiah, Suhasa B., Giovanni Talei Franzesi, et al. "Automated Whole-cell Patch-clamp Electrophysiology of Neurons in Vivo." *Nature Methods* 9, no. 6 (2012): 585–87.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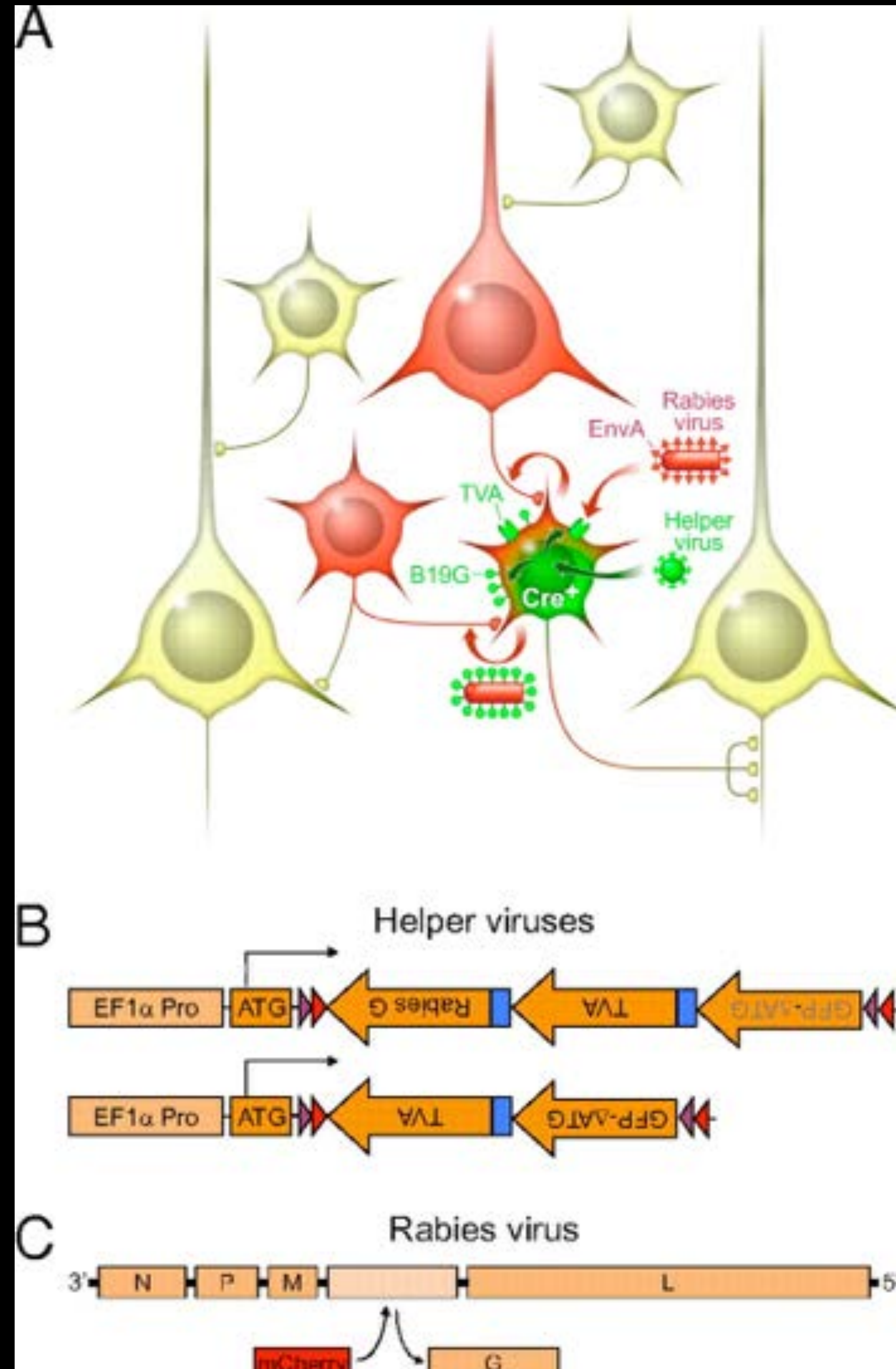
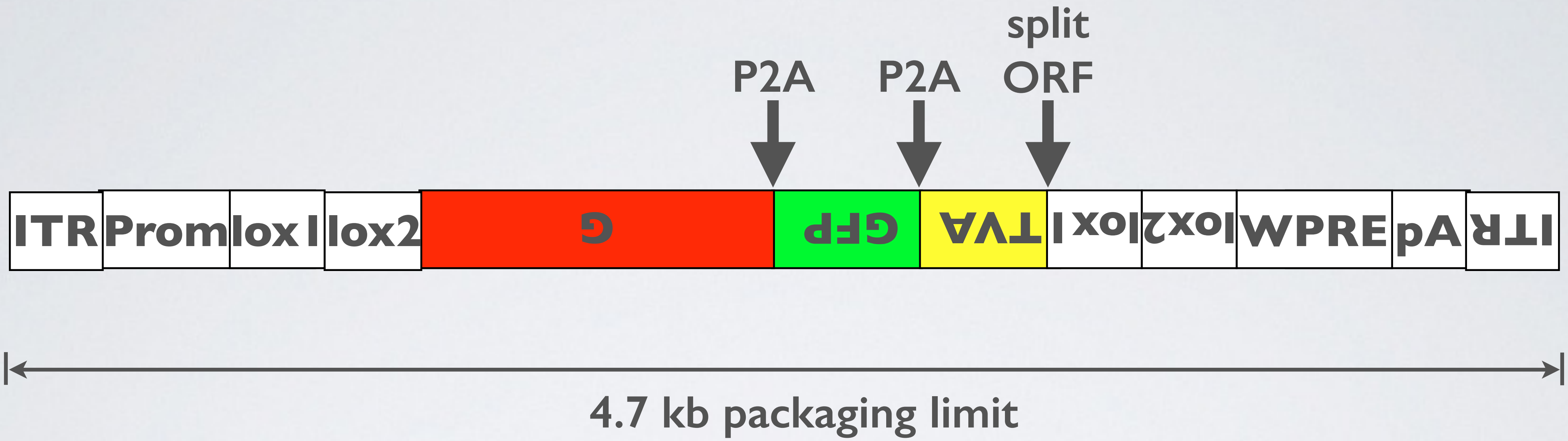
PIPETTE
HIGH PRESSURE/SUCTION



TARGETING CELL TYPES WITH AAV-FLEX-TVA-G + RV(ENVA)

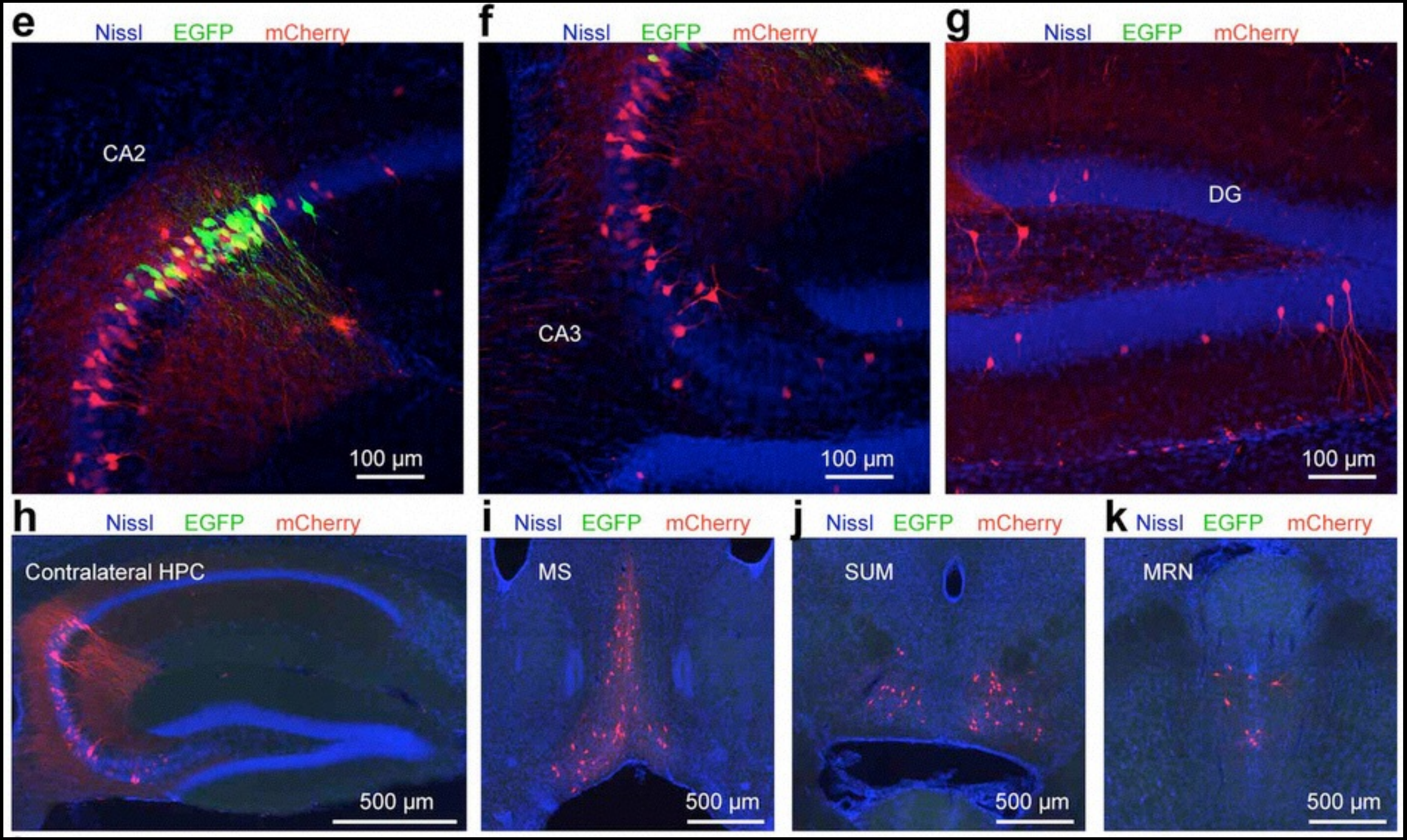
Wall et al. '10



deposited with
Addgene
UNC vector core
UPenn vector core

Source: Kohara, Keigo, Michele Pignatelli, et al. "Cell Type-specific Genetic and Optogenetic Tools Reveal Hippocampal CA2 Circuits." *Nature Neuroscience* 17, no. 2 (2014): 269–79.

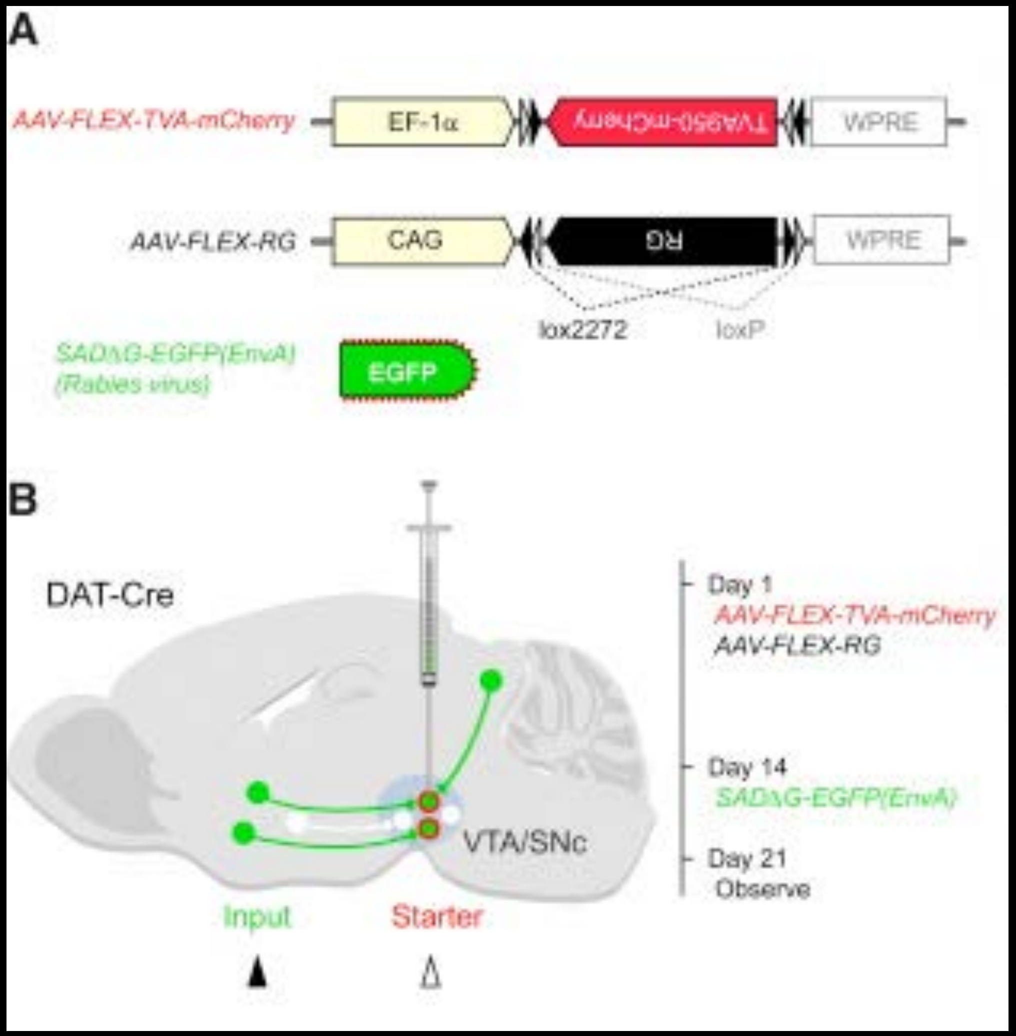
Kohara et al. 2014



Source: Kohara, Keigo, Michele Pignatelli, et al. "Cell Type-specific Genetic and Optogenetic Tools Reveal Hippocampal CA2 Circuits." *Nature Neuroscience* 17, no. 2 (2014): 269–79.

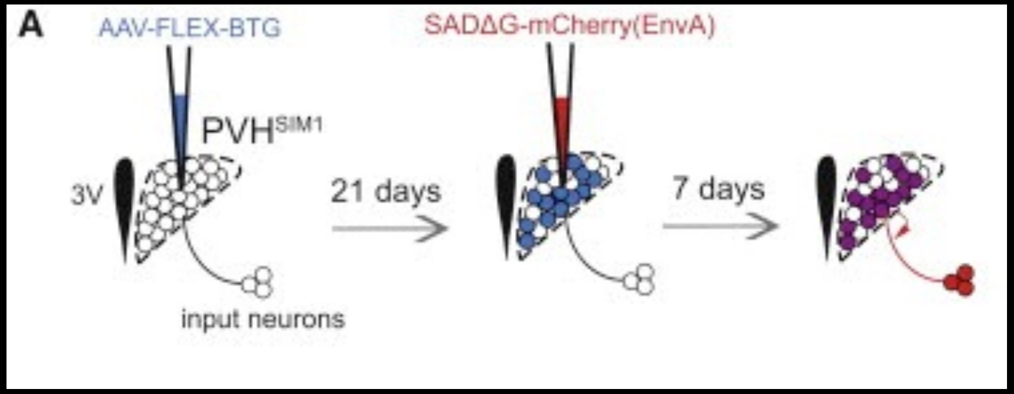
Kohara et al. 2014

Watabe-Uchida... & Uchida '12
Hitti & Siegelbaum '14
Miyamichi...& Mizrahi '14
Krashes...& Lowell '14



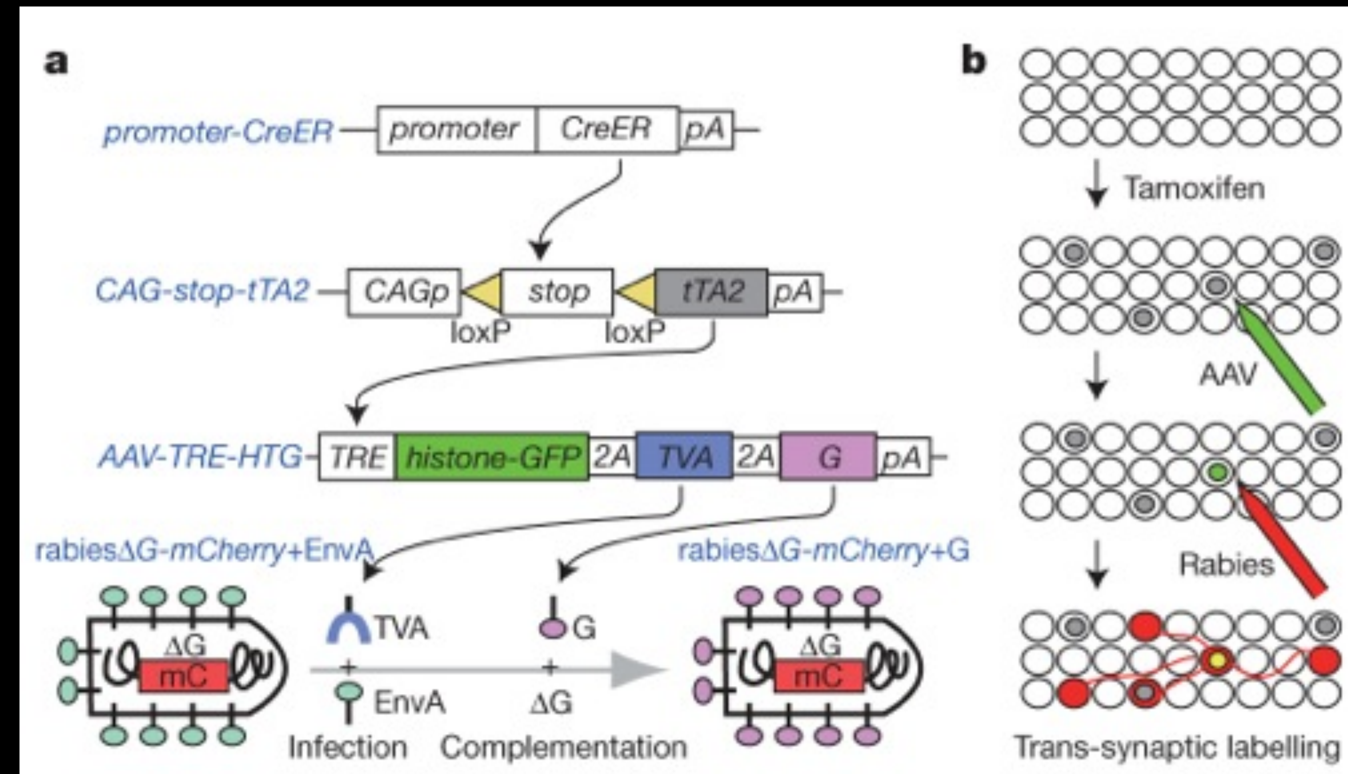
Source: Watabe-Uchida, Mitsuko, Lisa Zhu, et al. "Whole-brain Mapping of Direct Inputs to Midbrain Dopamine Neurons." *Neuron* 74, no. 5 (2012): 858–73.

Betley...& Sternson '13



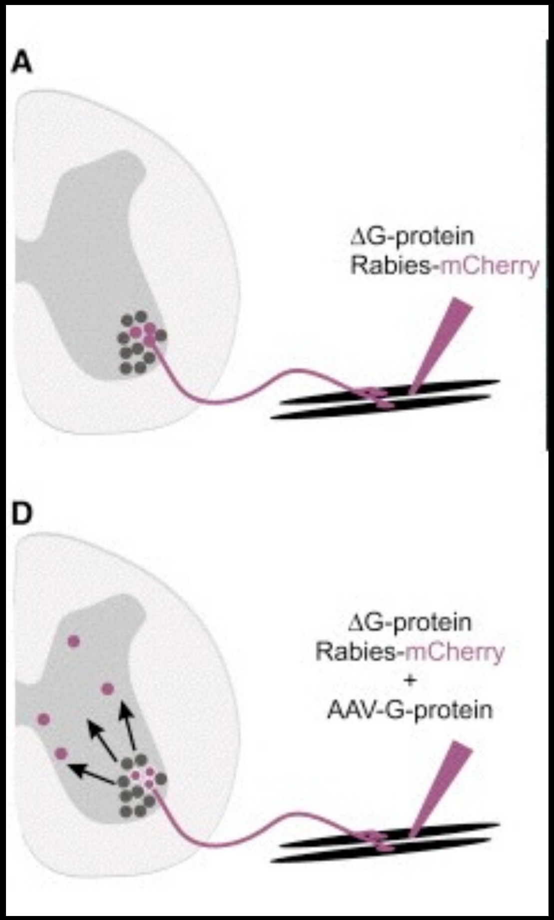
Source: Betley, J. Nicholas, Zhen Fang Huang Cao, et al. "Parallel, Redundant Circuit Organization for Homeostatic Control of Feeding Behavior." *Cell* 155, no. 6 (2013): 1337–50.

Miyamichi...& Luo '11
Fu...& Stryker '14



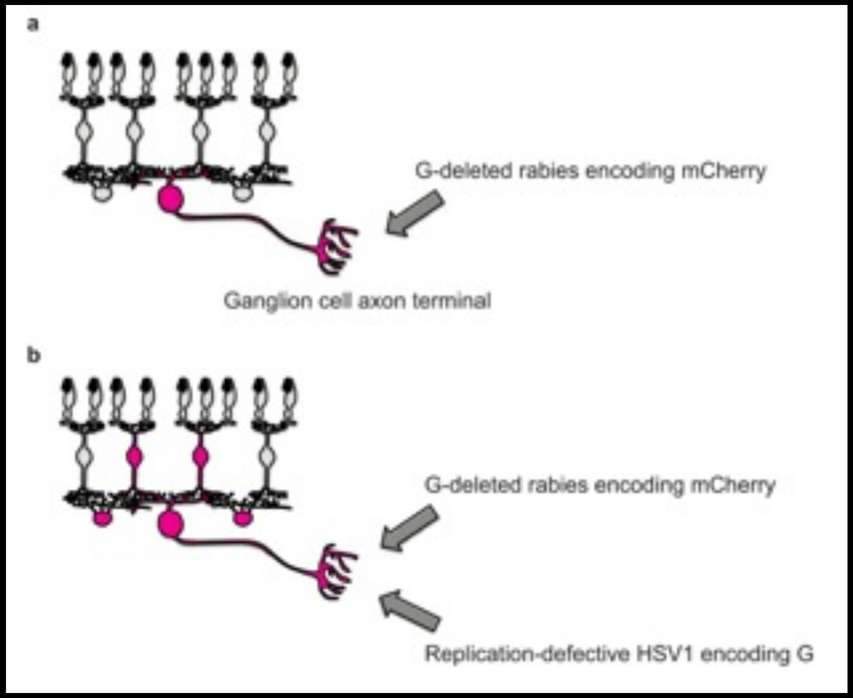
Source: Miyamichi, Kazunari, Fernando Amat, et al. "Cortical Representations of Olfactory Input by Trans-synaptic Tracing." *Nature* 472, no. 7342 (2011): 191–96.

Stepien...& Arber '10
Tripodi...& Arber '11
Pivetta...& Arber '14
Levine...& Pfaff '14



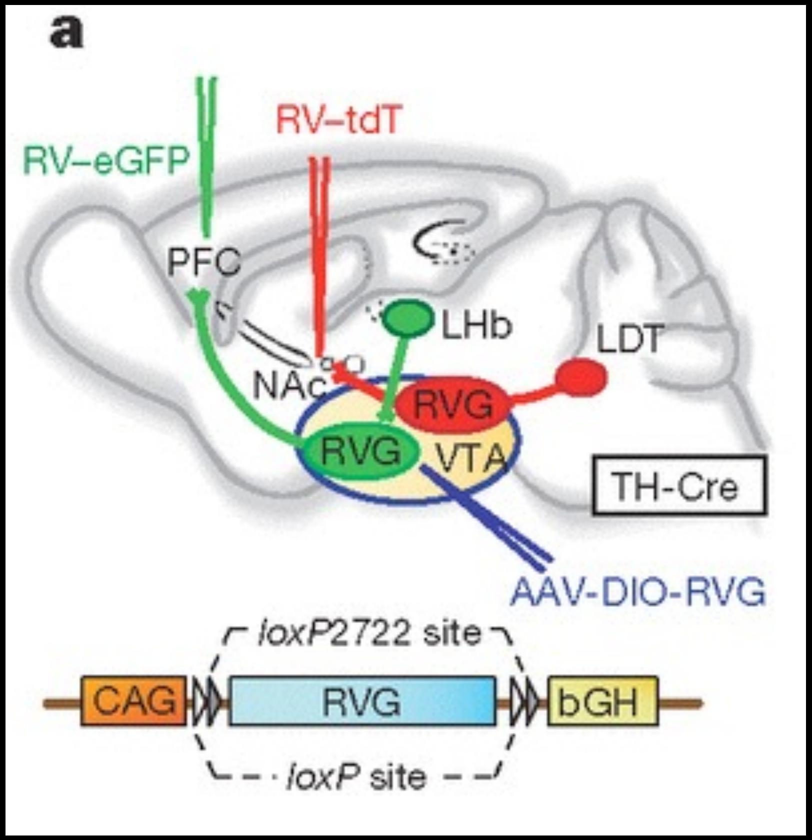
Source: Stepien, Anna E., Marco Tripodi, et al. "Monosynaptic Rabies Virus Reveals Premotor Network Organization and Synaptic Specificity of Cholinergic Partition Cells." *Neuron* 68, no. 3 (2010): 456–72.

Yonehara...& Roska '11



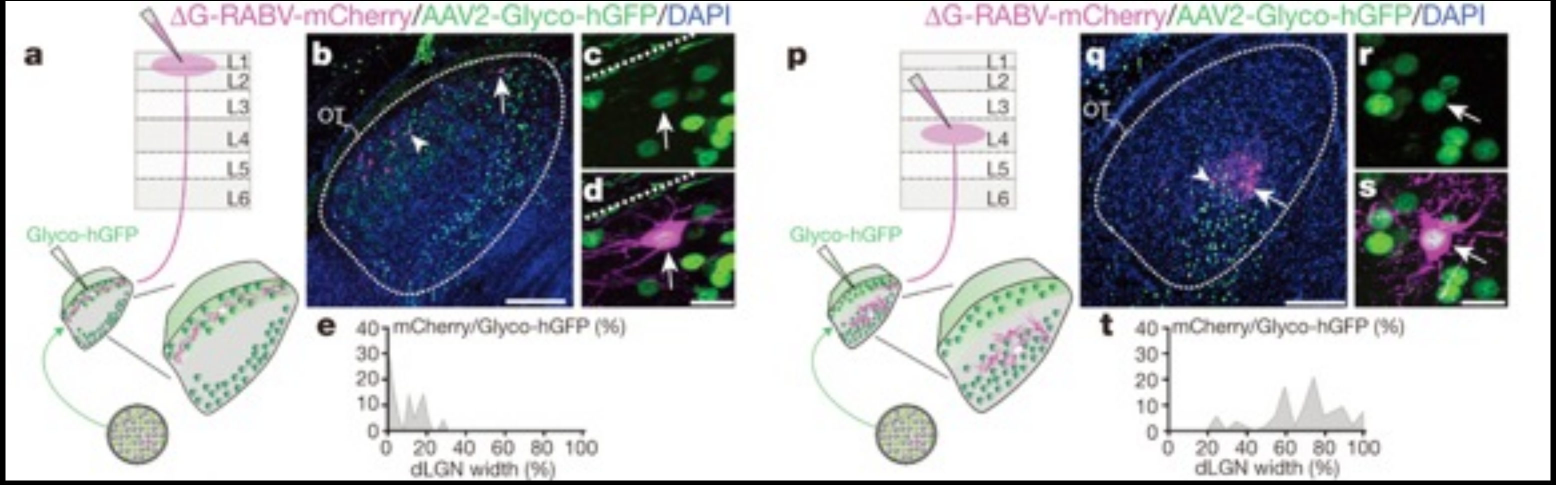
Source: Yonehara, Keisuke, Kamill Balint, et al. "Spatially Asymmetric Reorganization of Inhibition Establishes a Motion-sensitive Circuit." *Nature* 469, no. 7330 (2011): 407–10.

Lammel, Lim…
& Malenka '13



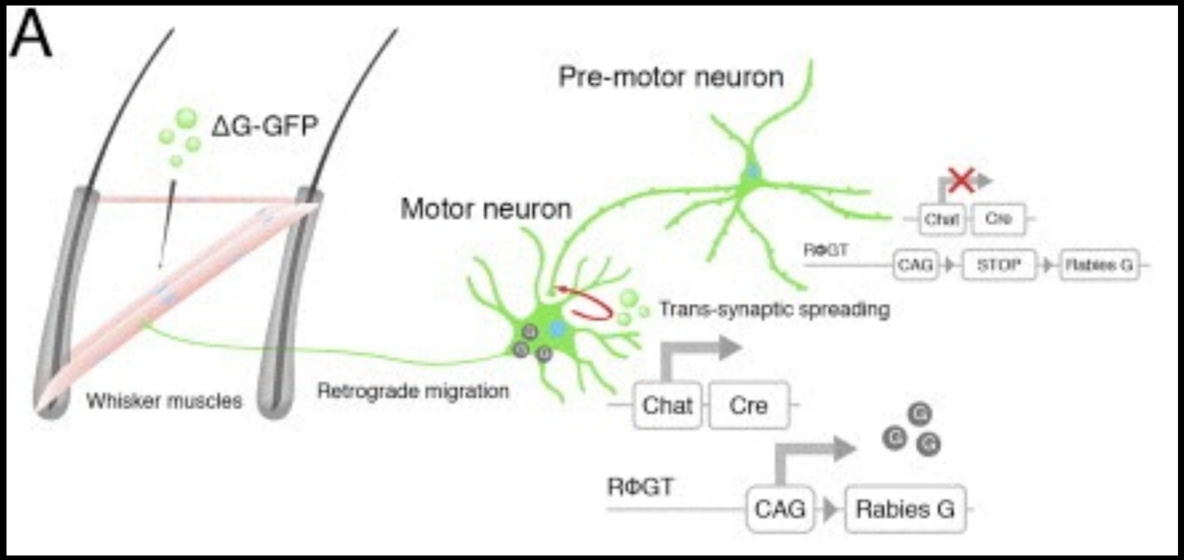
Source: Lammel, Stephan, Byung Kook Lim, et al. "Input-specific Control of Reward and Aversion in the Ventral Tegmental Area." *Nature* 491, no. 7423 (2012): 212–17.

Cruz-Martin...
& Huberman '14



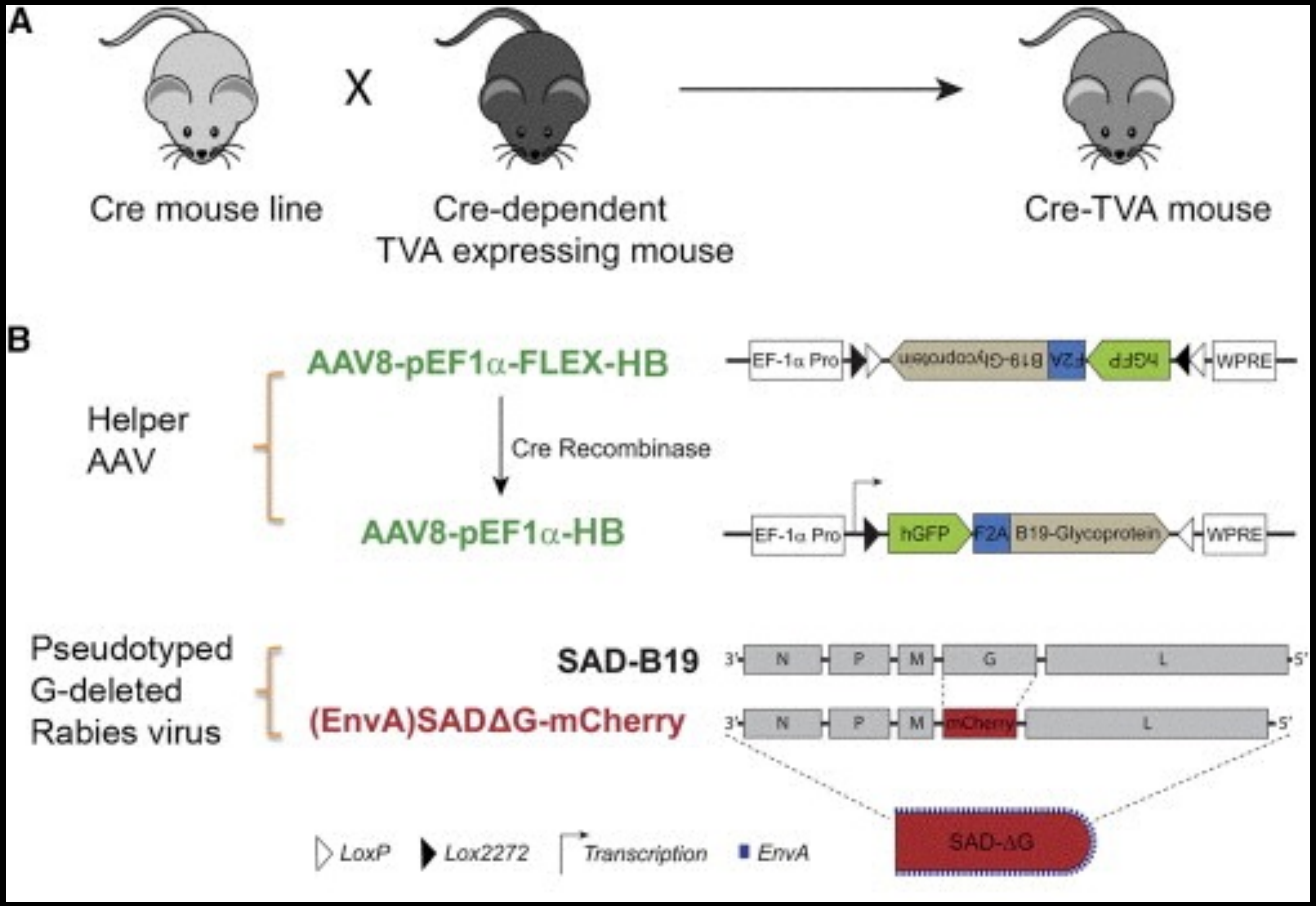
Source:Cruz-Martín, Alberto, Rana N. El-Danaf, et al. "A Dedicated Circuit Links Direction-selective Retinal Ganglion Cells to the Primary Visual Cortex." *Nature* 507, no. 7492 (2014): 358–61.

Takatoh...& Wang '13



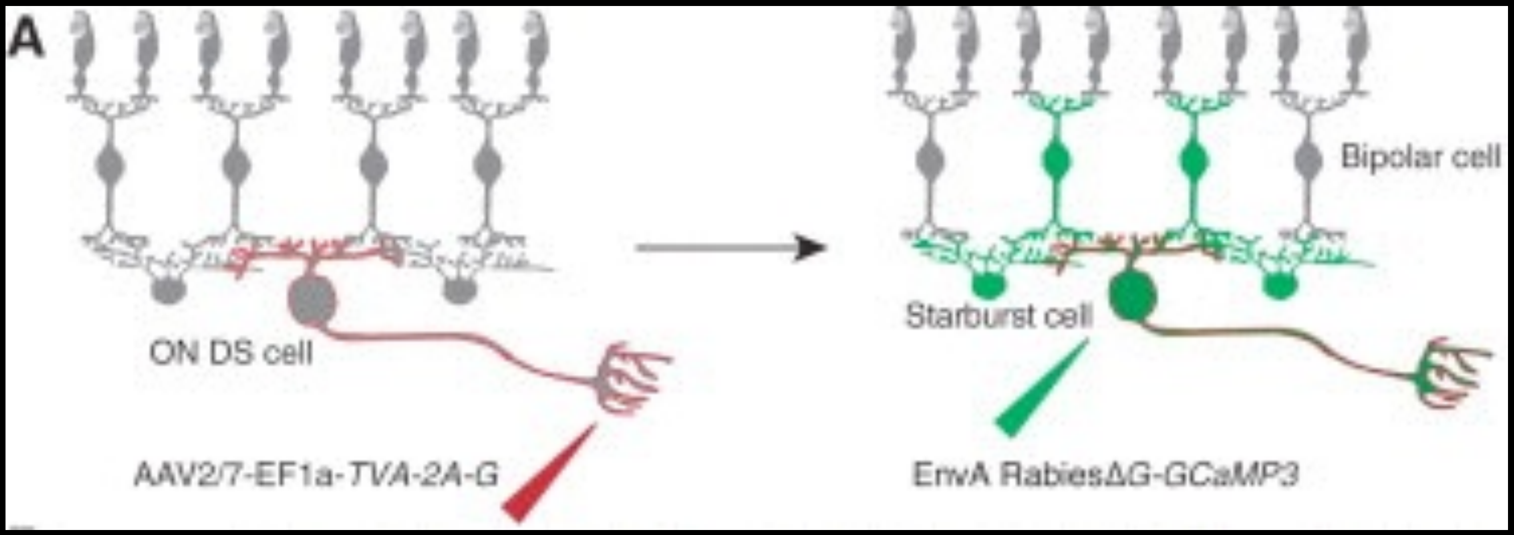
Source: Takatoh, Jun, Anders Nelson, et al. "New Modules are Added to Vibrissal Premotor Circuitry with the Emergence of Exploratory Whisking." *Neuron* 77, no. 2 (2013): 346–60.

Sun...& Xu '14



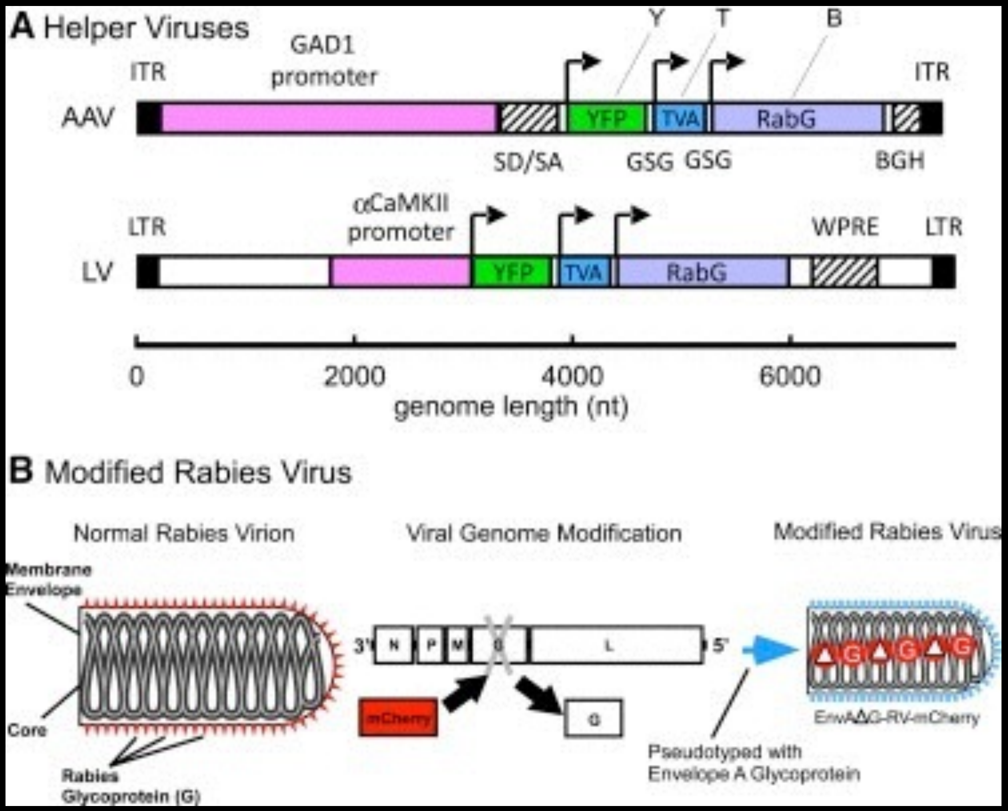
Source: Sun, Yanjun, Amanda Q. Nguyen, et al. "Cell-type-specific Circuit Connectivity of Hippocampal CA1 Revealed Through Cre-dependent Rabies Tracing." *Cell Reports* 7, no. 1 (2014): 269–80.

Yonehara, Farrow... & Roska '13



Source: Yonehara, Keisuke, Karl Farrow, et al. "The First Stage of Cardinal Direction Selectivity is Localized to the Dendrites of Retinal Ganglion Cells." *Neuron* 79, no. 6 (2013): 1078–85.

Liu...& Lyon '13

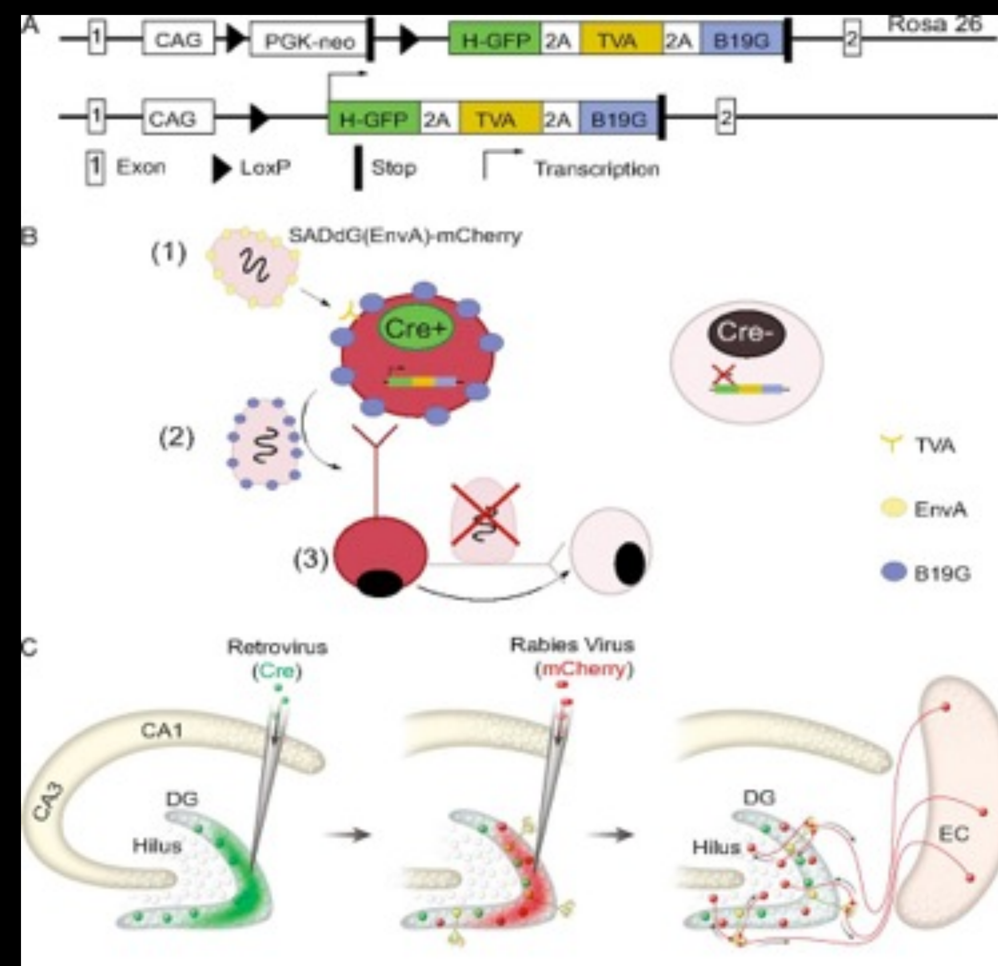


Source: Liu, Yong-Jun, Markus U. Ehrengruber, et al. "Tracing Inputs to Inhibitory or Excitatory Neurons of Mouse and Cat visual Cortex with a Targeted Rabies Virus." *Current Biology* 23, no. 18 (2013): 1746–55.

Garcia...& Arenkiel ’12

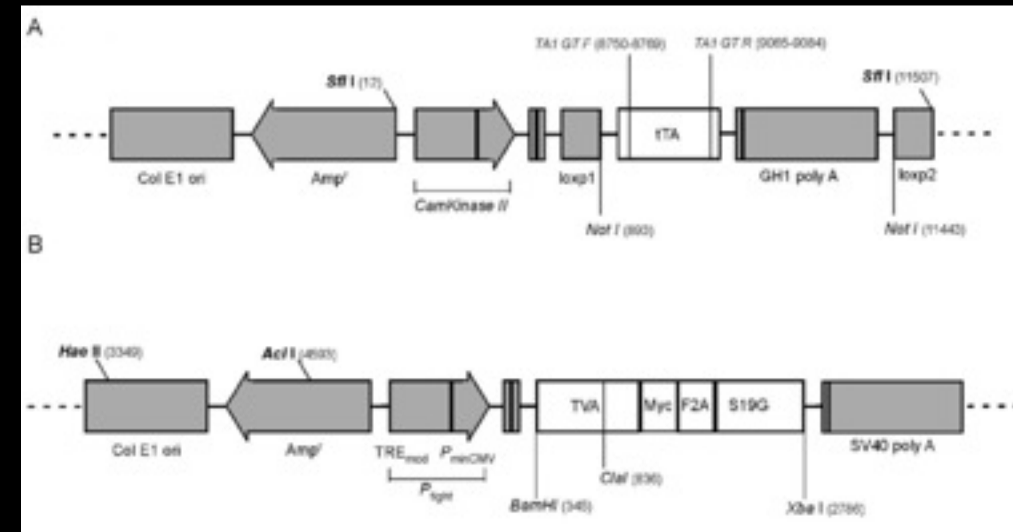
Figure removed due to copyright restrictions.
Please see Figure 1 from Garcia, Isabella, Cynthia Kim, et al. "Genetic Strategies to Investigate Neuronal Circuit Properties using Stem Cell-derived Neurons." *Frontiers in Cellular Neuroscience* 6 (2012).

Li...& Gage ‘13

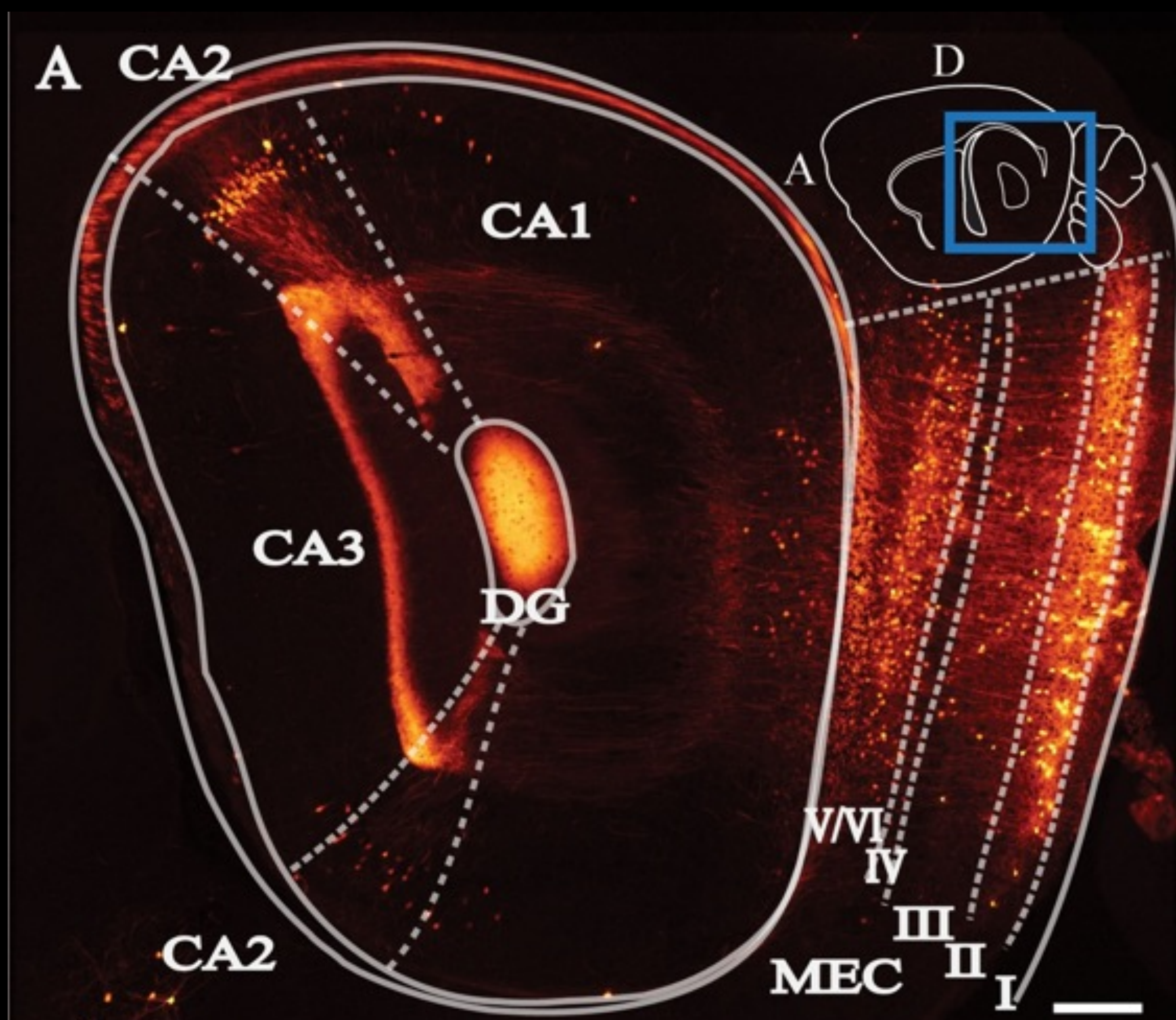


Source: Li, Yan, Floor J. Stam, et al. "Molecular Layer Perforant Path-associated Cells Contribute to Feed-forward Inhibition in the Adult Dentate Gyrus." *Proceedings of the National Academy of Sciences* 110, no. 22 (2013): 9106–11.

Weible...& Kentros ’10
Rowland...& Kentros ’13

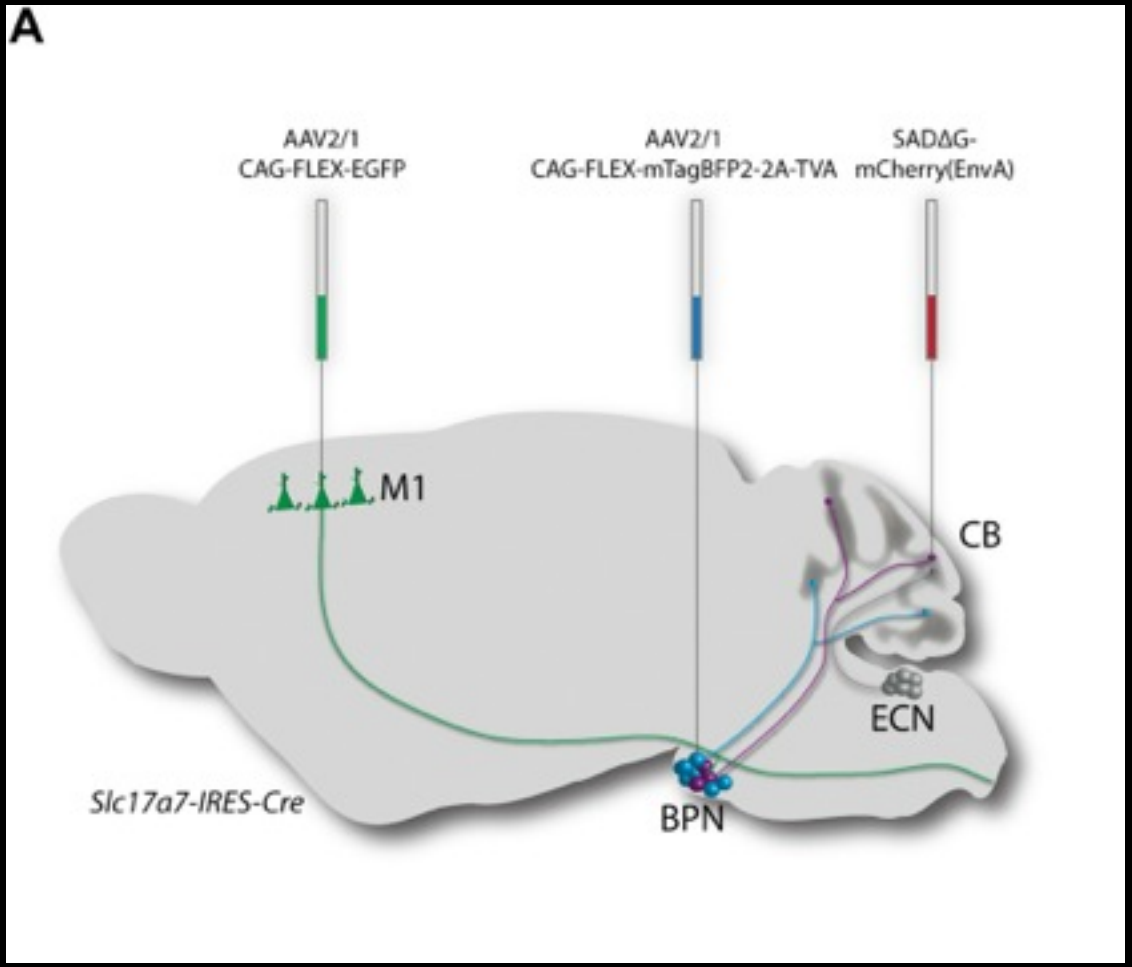


Weible, Aldis P., Leslie Schwarcz, et al. "Transgenic Targeting of Recombinant Rabies Virus Reveals Monosynaptic Connectivity of Specific Neurons." *The Journal of Neuroscience* 30, no. 49 (2010): 16509–13. CC license BY-NC-SA.

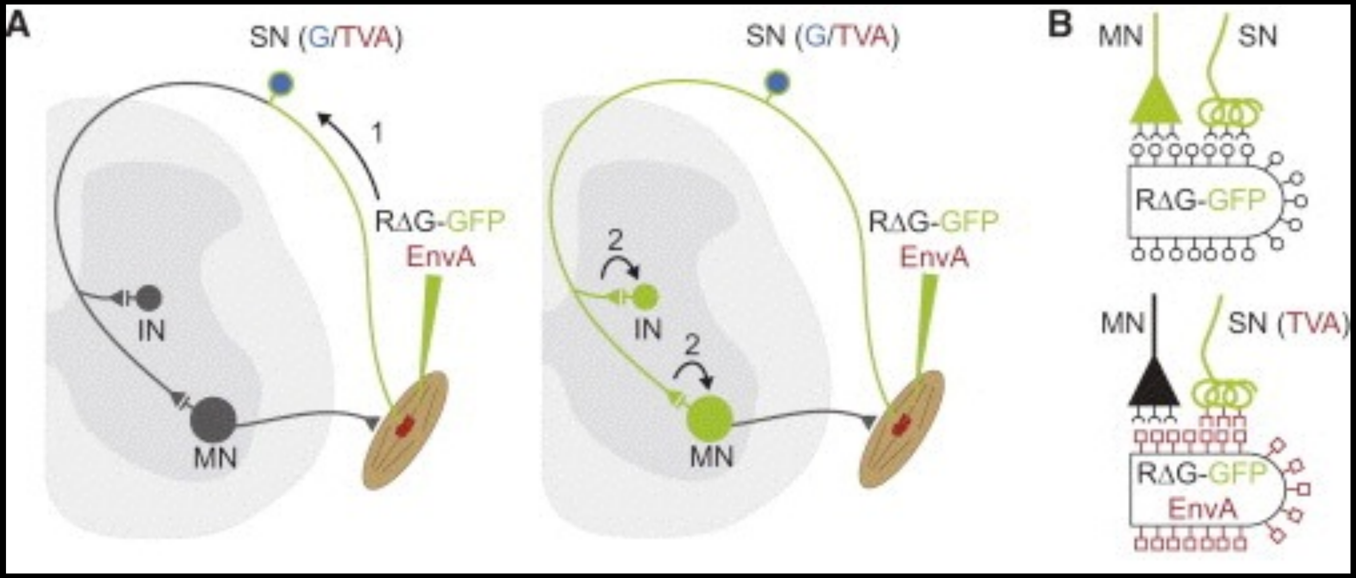


Rowland, David C., Aldis P. Weible, et al. "Transgenically Targeted Rabies Virus Demonstrates a Major Monosynaptic Projection from Hippocampal Area CA2 to Medial Entorhinal Layer II Neurons." *The Journal of Neuroscience* 33, no. 37 (2013): 14889–98. CC license BY-NC-SA.

Huang...& Hantman ’13



Zampieri...& Murray '14



Source: Zampieri, Niccolò, Thomas M. Jessell, et al. "Mapping Sensory Circuits by Anterograde Transsynaptic Transfer of Recombinant Rabies Virus." *Neuron* 81, no. 4 (2014): 766–78.

All are using first-generation system

Vector evolution: LV vs RV

RV:

RV G

LV:

Figure removed due to copyright restrictions.
Please see Figure 1 from Cockrell, Adam S., and Tal Kafri. "Gene Delivery by Lentivirus Vectors." *Molecular Biotechnology* 36, no. 3 (2007): 184–204.

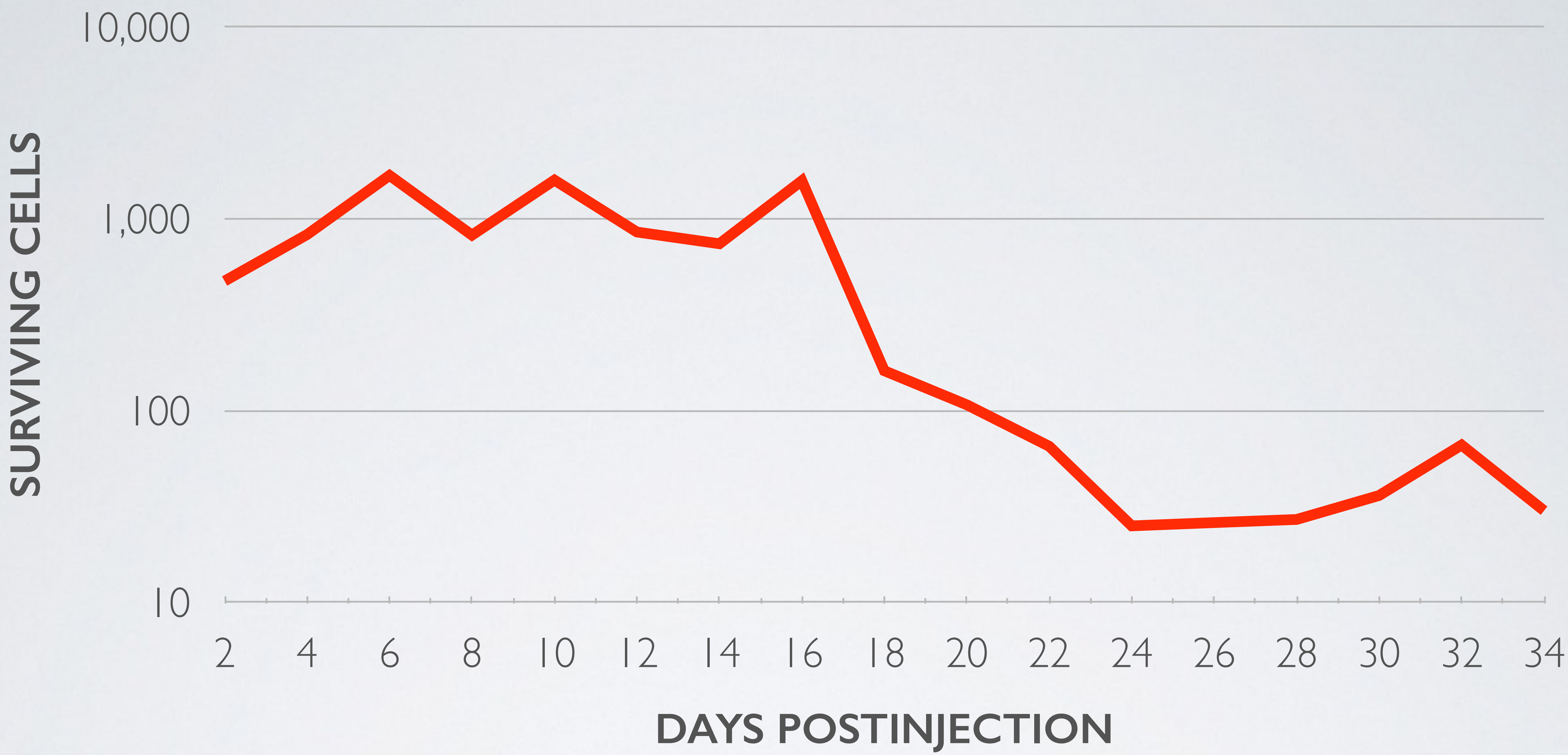
Major limitations of first-generation monosynaptic tracing

1) Only retrograde

2) Typically labels only a fraction of presumed inputs

3) Double labeling of inputs to two populations not effective

4) **Cytotoxic** - doesn’t allow long-term studies (imaging, gene knockout, cognitive and behavioral paradigms…)



RV TOXICITY

Source: Wickersham, Ian R., Stefan Finke, et al. "Retrograde Neuronal Tracing with a Deletion-mutant Rabies Virus." *Nature Methods* 4, no. 1 (2007): 47–49.

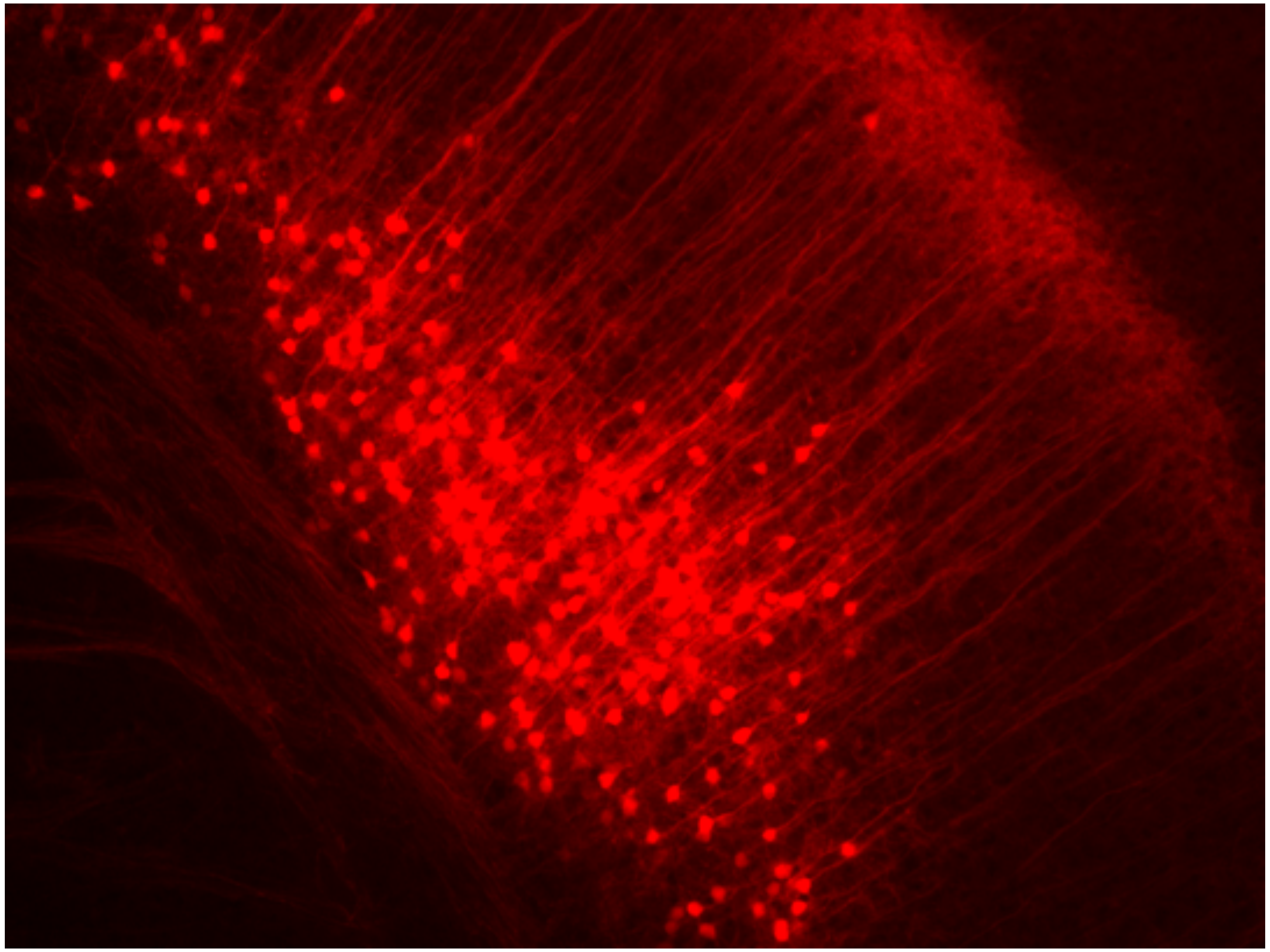
In progress: system for **nontoxic** monosynaptic tracing

- for long-term monitoring & manipulation of identified synaptically connected neurons

- RV based

- progressing well:

- NIMH grant



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ID	Plasmid	Experimental Purpose	Status
52490	pRVdG-4BFP2	Expresses mTagBFP2 Edit	Submitted
52496	pRVdG-4Halo3Y	Expresses eNpHR 3.0-EYFP Edit	Submitted
52497	pRVdG-4GCaMP6s	Expresses GCaMP6s Edit	Submitted
52498	pRVdG-4BFP2-5postmGRASP	Expresses mTagBFP2 and postsynaptic mGRASP component Edit	Submitted
52499	pRVdG-4RFP-5premGRASP	Expresses TagRFP-T and presynaptic mGRASP component Edit	Submitted
59325	pRVdG-4ArchT-EGFP	Expresses ArchT-EGFP Edit	Submitted
59326	pRVdG-4ArchT-mCherry	Expresses ArchT-mCherry Edit	Submitted
59327	pRVdG-4Halo3-mCherry	Expresses eNpHR 3.0-mCherry Edit	Submitted
59328	pRVdG-4ChR2-mCherry	Expresses ChR2-mCherry Edit	Submitted
59329	pAAV-CAG-FLEX-splitTVA950	Expresses splitTVA950 Edit	Submitted
59330	pAAV-CAG-FLEX-splitTVA800	Expresses splitTVA800 Edit	Submitted
59331	pAAV-CAG-FLEX-EGFP	Expresses EGFP Edit	Submitted
59332	pAAV-CAG-FLEX-splitTVA-EGFP	Expresses splitTVA-P2A-EGFP Edit	Submitted
59333	pAAV-synP-FLEX-EGFP-B19G	Expresses EGFP-P2A-B19G Edit	Submitted

Resources

- “A Plasmid Editor”
- addgene.org
- NCBI BLAST
- neb.com
- epochlifescience.com
- UPenn vector core (AAV)
- UNC vector core (AAV)
- MIT vector core (HSV)
- Salk vector core (RV)
- Duke vector core (RV)
- jaxmice.jax.org

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Praneeth Namburi
Kay Tye
Klaus Conzelmann
Matthias Schnell
Carlos Lois
Ed Boyden
Sebastian Seung
Ed Callaway
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Inder Verma

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Alfonso Apicella
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MIT Department of Brain & Cognitive Sciences
Simons Center for the Social Brain
NSF BRAIN Initiative
NIMH BRAIN Initiative
NINDS BRAIN Initiative

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