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Course „Neural Circuits of motivated behavior“

Lecture: Techniques to study neural circuits (Week 2)

Olexiy Kochubey

Thursday 27.02.2025

Outlook

I. Animal models and genetic tools to study neural circuits

a) transgenic animal models and DNA recombinase systems

b) viral vectors in circuit neuroscience

II. Methods for manipulating activity of neural circuits

a) electric stimulation

b) light-controlled actuators: optogenetics

c) ligand-controlled actuators: chemogenetics

III. Electrophysiological methods of measuring neural circuit activity in-vivo

a) principles of extracellular spike recordings and analysis

b) modern recording techniques

IV. Optical methods of measuring neural circuit activity

a) basics of Ca^{2+} imaging

b) example configurations of optical recordings

I. Animal models and genetic tools to study neural circuits

Ia. Animal models in Neurobiology

Why do we need animal models and can not do everything on human?

- Human brain is very complex
- Mechanistic brain studies require invasive in-vivo or ex-vivo approaches
- Behavioral experiments on humans would often be problematic due to ethical reasons
- Some animals are unique in certain behaviors, or have unique sensory systems etc., thus can not be substituted (e.g. ants, bees, snakes, song birds, barn owls, elephants, dolphins…)

A generic "wish list" for the laboratory animal models

- Relatively small, easy-to-manipulate genome
- Short generation cycle to facilitate genetic manipulations
- Complex behavior paradigms
- Relatively large neurons and brain structures, possibility to record from during behavior

Ia. Widely used animal models in studies of neural circuits and behavior

	Advantages	Drawbacks
C. elegans (nematode) 	▪ Short life cycle ▪ Simple nervous system (302 neurons)	▪ Too simple nervous system and behavior
D. melanogaster (fruit fly) 	▪ Short life cycle, easy maintenance ▪ Relatively simple nervous system ▪ Complex behavior	▪ Too far from mammals (anatomy, physiology, behavior…)
Danio rerio (zebrafish) 	▪ Short life cycle ▪ Transparent body, suitable for imaging ▪ Relatively complex behavior	▪ Too far from mammals (anatomy, physiology, behavior…)
Laboratoty rodents 	▪ Mammals: brain anatomy, physiology, diseases – analogous to human ▪ Accessible genetics ▪ Brain studies technically accessible ▪ Complex behaviors ▪ Relatively short life cycle	▪ Life cycle still rather long ▪ Expensive maintenance ▪ Ethical issues
Non-human primates 	▪ Closest possible to human ▪ Very complex behaviors	▪ Long life cycle ▪ Difficult access to genetics ▪ Very expensive maintenance ▪ Long lasting experiments ▪ Ethical issues

Ia. Laboratory mice

Class: *Mammalia*

Order: *Rodentia*

Family: *Muridae*

Genus: *Mus*

Species: *Mus musculus (domestic mouse)*

Some facts

- Maximum life span: *3-4 years*
- Adult body weight: *25-30 g*
-
- Weaning age: *19-21 days*
- Sexual maturity: *6-8 weeks*

- Female estrous cycle: *4-5 days*
- Gestation period: *19-21 days*
- Litter size: *6-8 pups*
- Effective fertility: *up to ~ 1-1.5 years*

- Mouse brain volume: *~500 mm^3* (3x and 2800x smaller than rat's and human's)

Why mouse is a very important animal model in neurobiology?

- Similar structure and function of the nervous system to that in human
- Share many neurological diseases and their genetic mechanisms with humans
- Many standard behavioral paradigms work in mice
- Genomic manipulations (knock-ins, knock-outs...) are well established
- Numerous inbred strains and transgenic mouse lines were generated and are available worldwide

Ia. Site-specific gene editing using DNA recombinases

Why is this important?

- For studying specific brain circuits / neuron types, one needs cell-type specific control of gene expression

A DNA editing tool

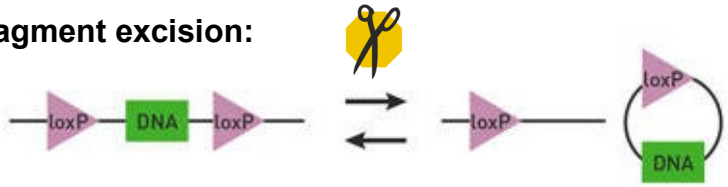
Cre recombinase (38 kDa)
(from bacteriophage P1)

loxP site sequence (34 bp)

ATAACTTCGTATA–NNNTANNN–TATACGAAGTTAT

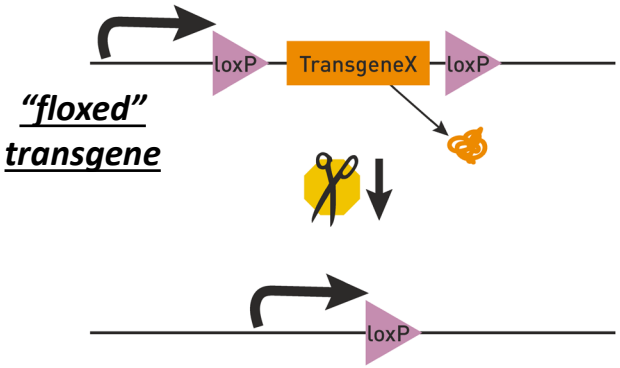
DNA editing modes by Cre recombinase

DNA fragment excision:

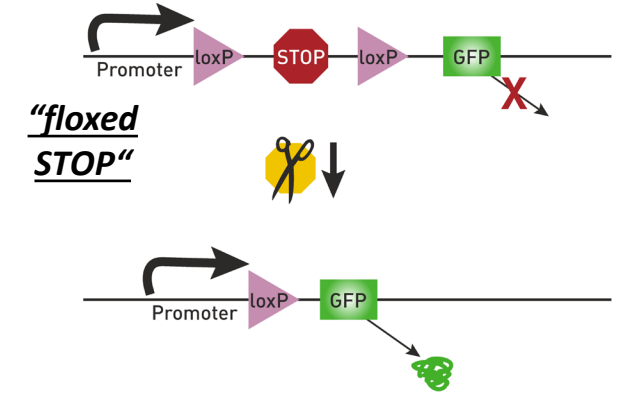


DNA fragment inversion:

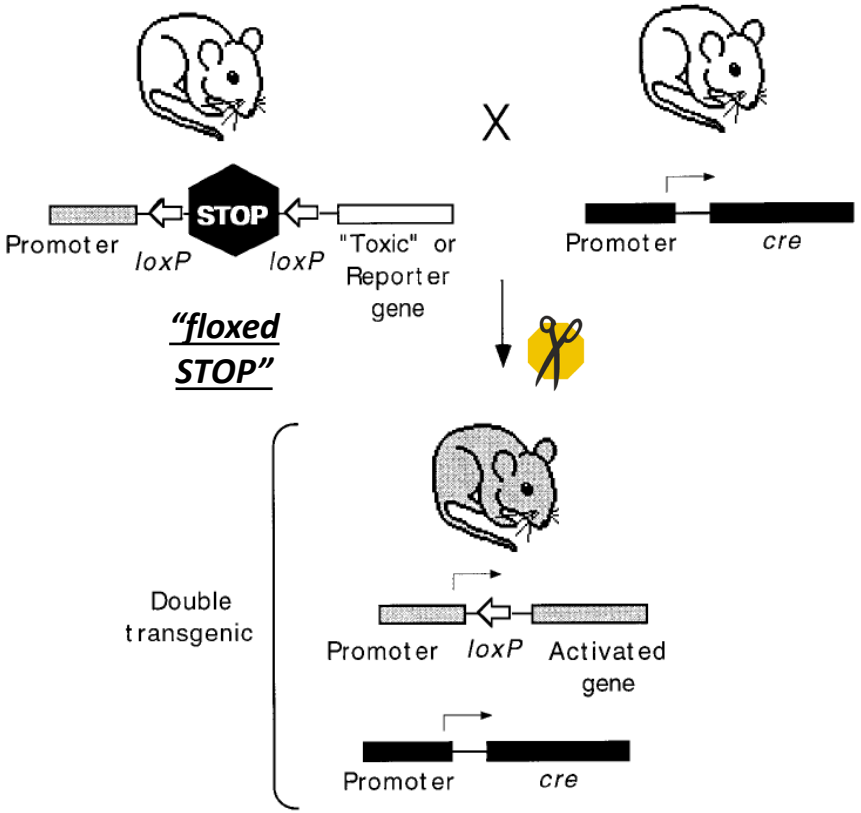
Turning OFF gene expression:



Turning ON gene expression:

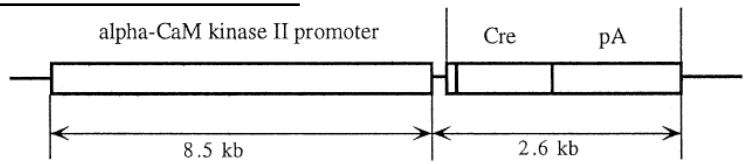


Ia. Conditional expression of a reporter gene using Cre-lox system

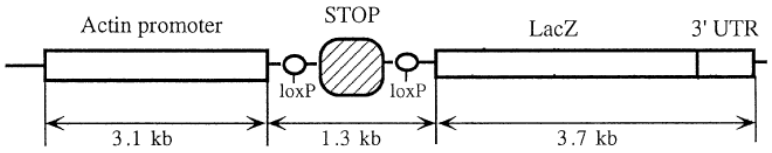


Sauer B., *Methods* 1998

CaMKII-Cre mouse line

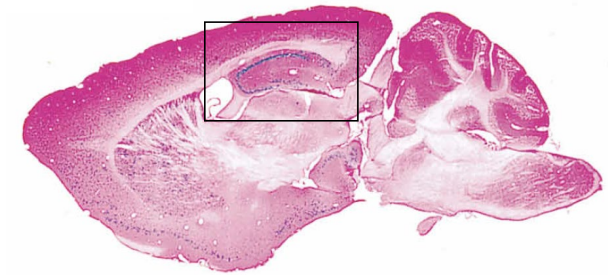


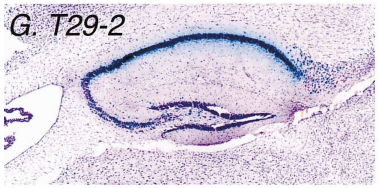
lacZ reporter "floxed STOP" mouse line

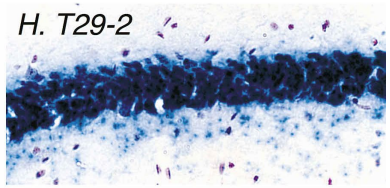


genetic X cross

Turning on lacZ expression in CaMKII positive neurons (e.g. in hippocampus)





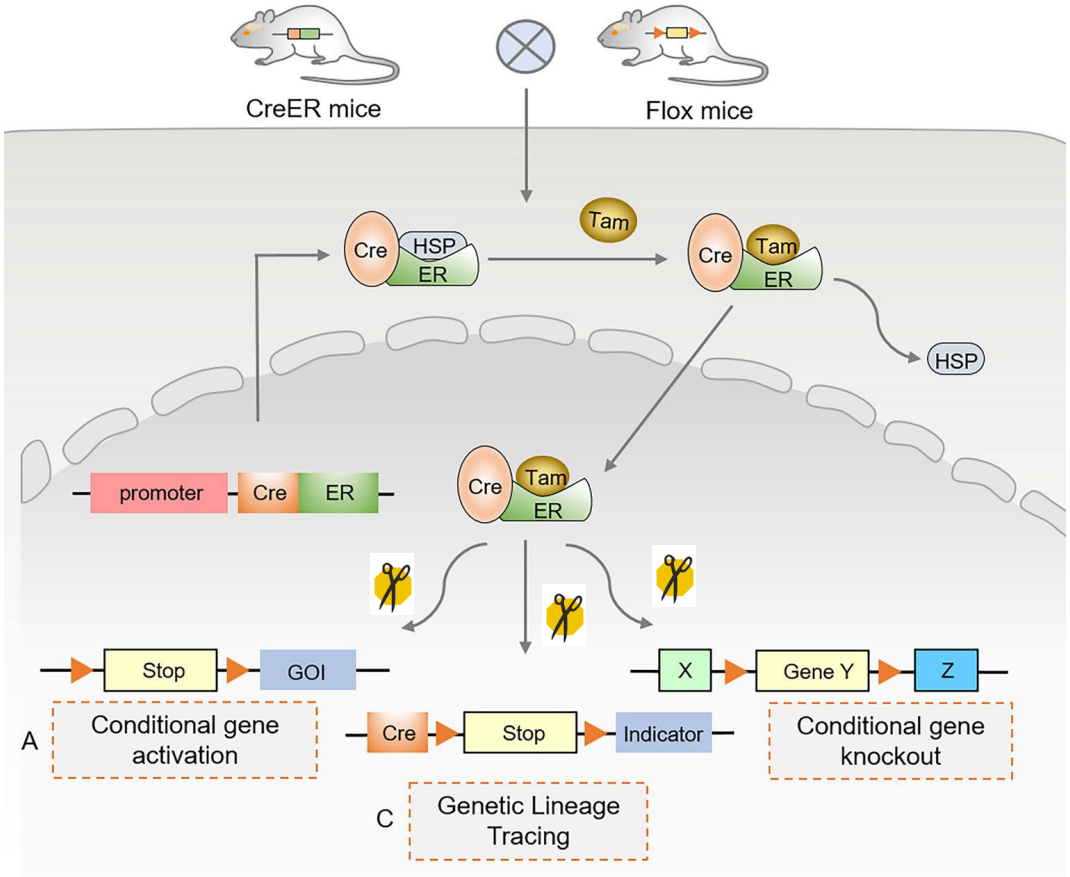


Tsien et al., *Cell* 1996

Ia. Inducible (= timed) Cre-lox recombination systems

Why important?

- Some genes express in development- or functional state-dependent manner, thus need a “catch”
- Inducible system (CreER^{T2}) allows defining a time window during which recombination occurs



ER = **mutated human estrogen receptor (G400V/M543A/L544A)**

Tam = **tamoxifen**

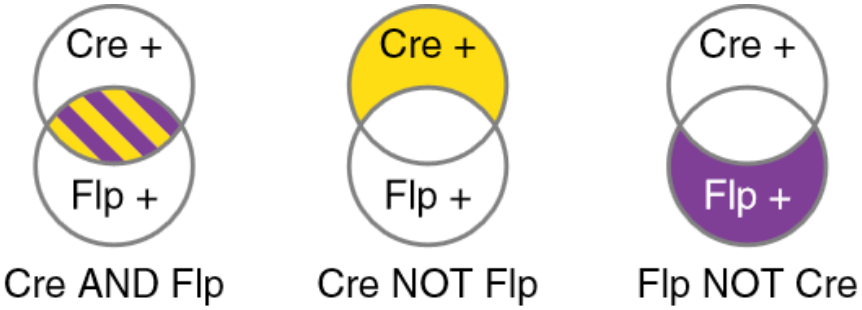
HSP = **heat shock protein 90**

Feil et al., *Methods Mol Biol*, 2009; Wang et al., *Front Cardiovasc Med* 2023

Ia. Flp-recombinase: like Cre- but different DNA recognition sites

Flp DNA-recombinase from *Saccharomyces cerevisiae:*

- "FRT" recognition site (34 bp): GAAGTTCCTATTC*tctagaaa*GAATAGGAACTTC
- FLPe mutant is thermo-resistant (used in mouse experiments due to higher stability)
- DNA editing principles similar to Cre-recombinase: excision, inversion, insertion, translocation
- ***Can be combined with Cre in a genetic cross to co-label two cell types (two different genes)***

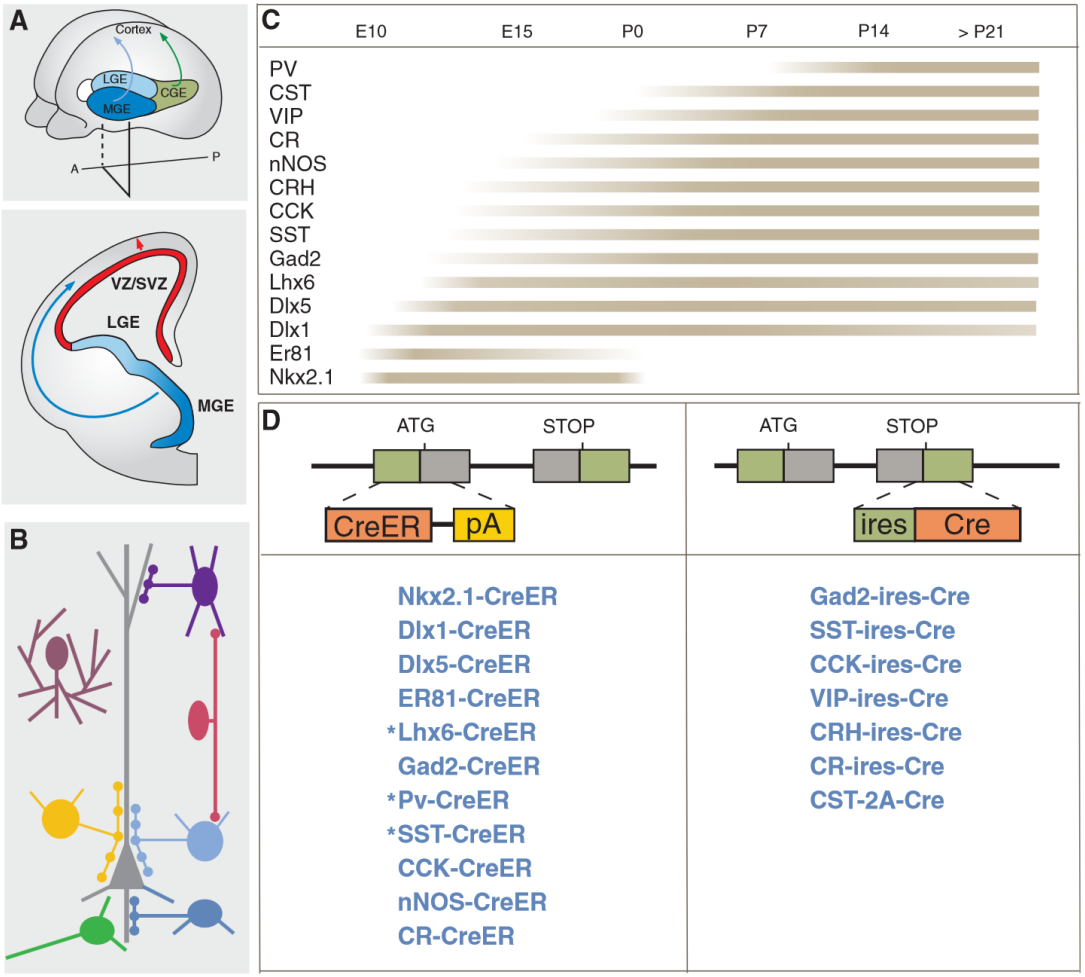


=> see also Fenno et al., Nature Methods 2014, for **"Intersect" technique** using Cre- and Flp- together

Ia. Numerous Cre- and Flp-recombinase knock-in mouse lines are now available

Example (see also Lecture of Week 1, development of inhibitory circuits):

resource library of **inhibitory neuronal subtypes** and transcription factors expressed in their progenitor cells



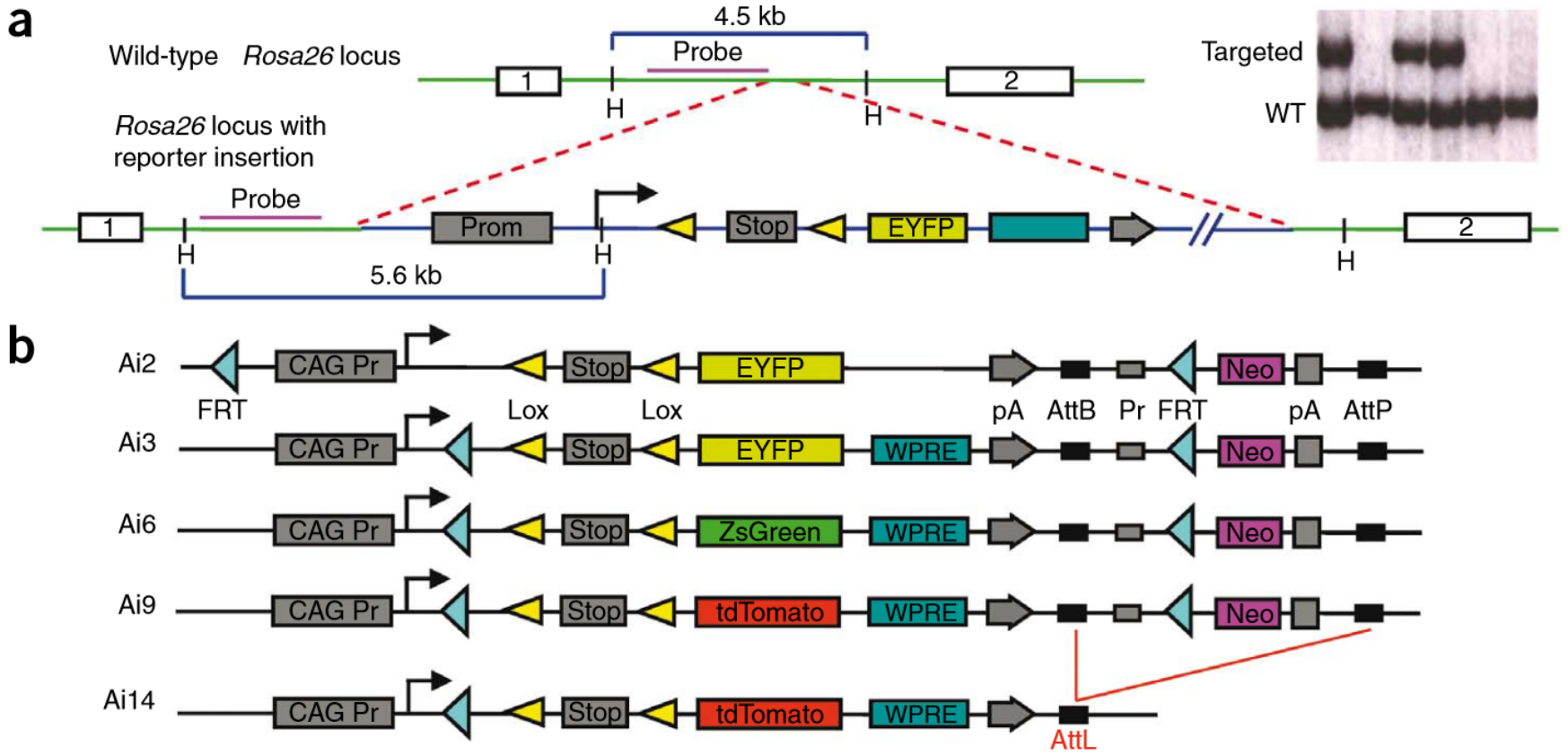
Databases:

https://www.creportal.org/
https://www.jax.org/
https://www.gensat.org/

…many lines were used in Allen Brain Connectivity Atlas

Taniguchi et al., *Neuron*, 2011

Ia. …as well as many reporter mouse lines were created



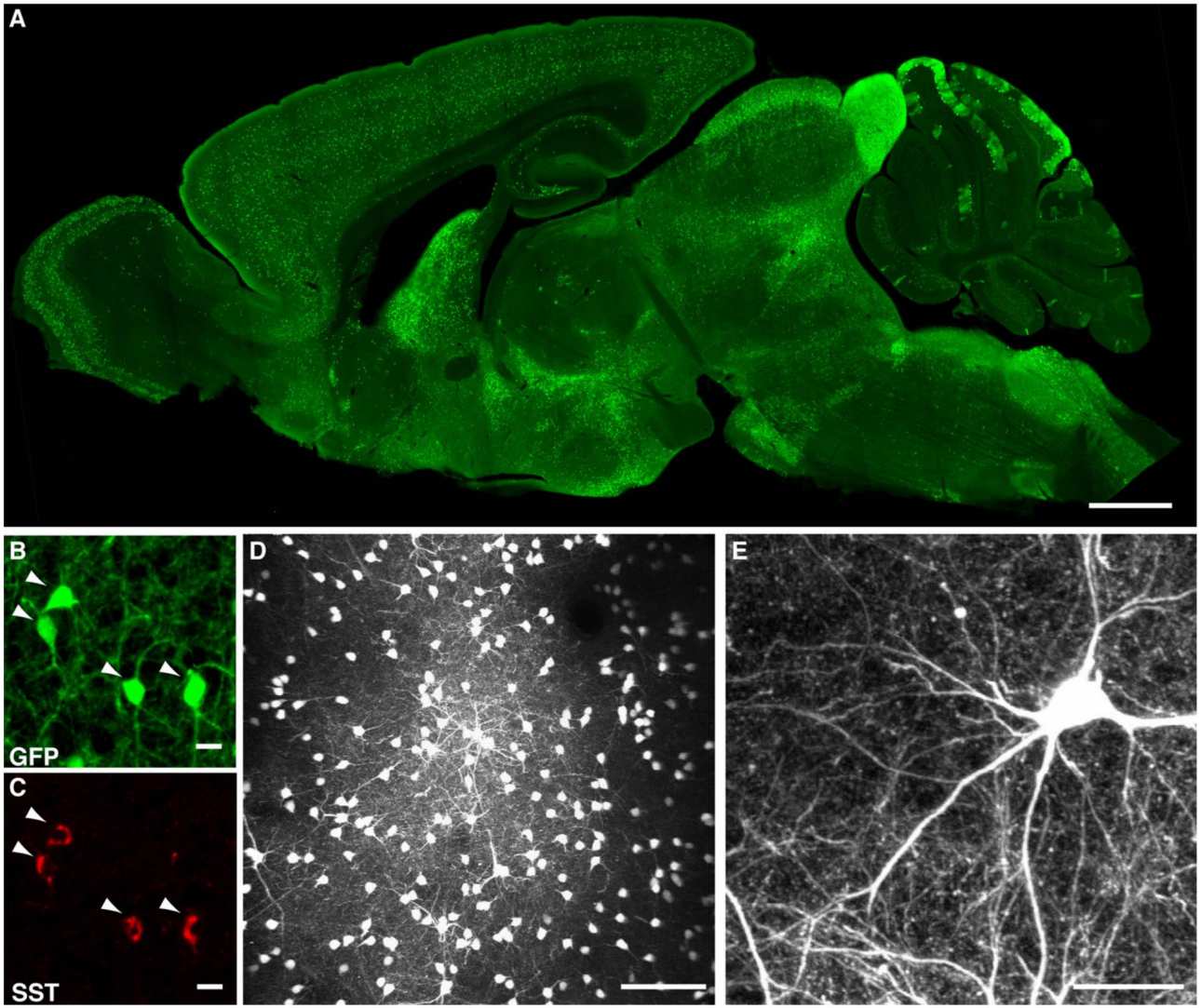
Madisen et al., *Nat Neurosci*, 2010, 2012

Which other reporters exist and can be useful?

- Optogenetic / chemogenetic activity actuators
- Fluorescent Ca^{2+}-indicators
- Immediate early gene expression tagging

Ia. Example of Cre-lox conditional expression of fluorescent reporter

SST-ires-Cre x RCE-loxP double transgenic (SST = somatostatin, marker of one of inhibitory neuron types)



Taniguchi et al., *Neuron*, 2011

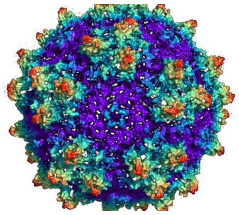
Ib. Viral vectors in neuroscience research

Ib. Viral vectors went „viral“ in circuit neuroscience

Why are these important?

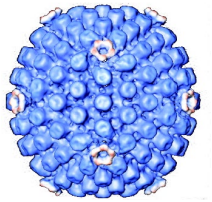
- Viral vectors provide better spatial & temporal specificity as compared to transgenic lines
- Have useful features like anterograde/retrograde uptake, trans-synaptic labelling, etc.
- Allow for a great flexibility of the experimental designs
- Fast and cheap to produce/handle/maintain as compared to transgenic mouse lines

Recombinant adeno-associated viruses (rAAV):



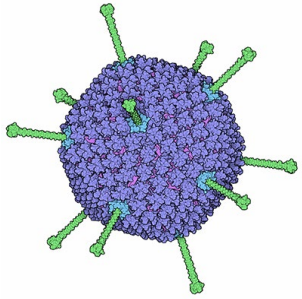
Herpes simplex viruses:

- fast expresssion onset
- retrograde or anterograde transsynaptic labeling
- **Biosafety level 2**



Adenoviruses:

- fast expresssion onset
- retrograde labeling
- large cloning capacity
- **Biosafety level 2**

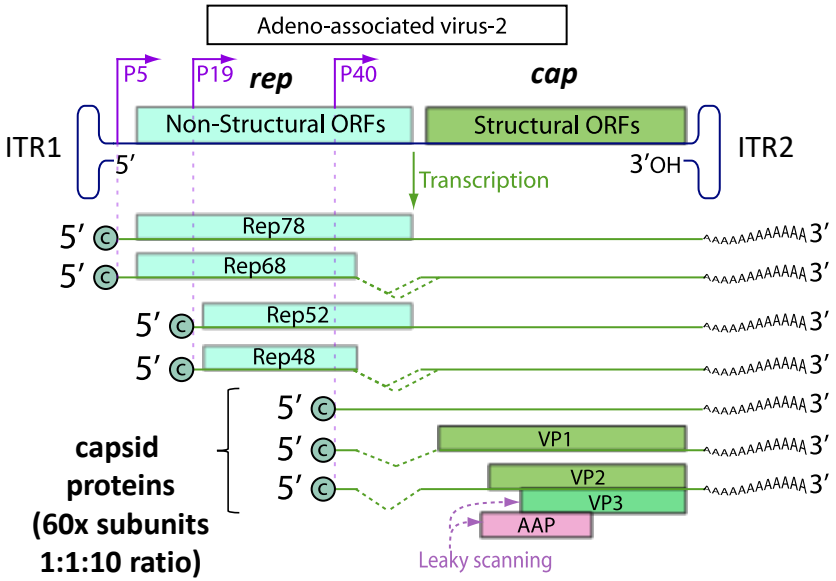


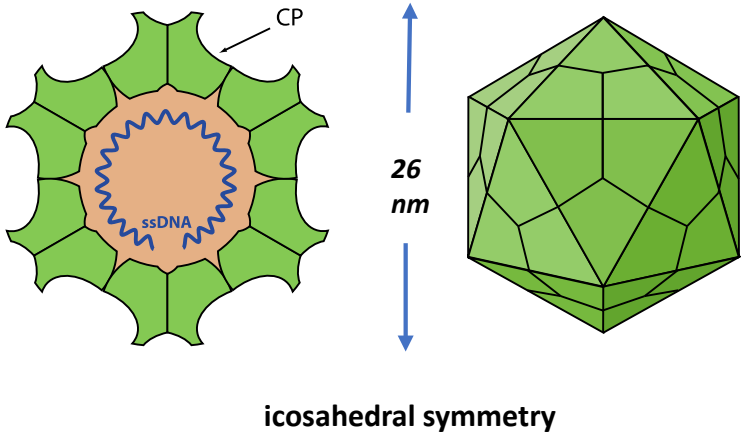
Pseudotyped rabies viruses:

Ib. rAAVs: accessible, versatile and the most popular tool in circuit neuroscience

Adeno-associated viruses:

- non-enveloped ssDNA virus (*Parvoviridae*) naturally asymptomatically occurring also in human (BSL1)
- wildtype viruses rely on other viruses (like adenoviruses) for replication





icosahedral symmetry

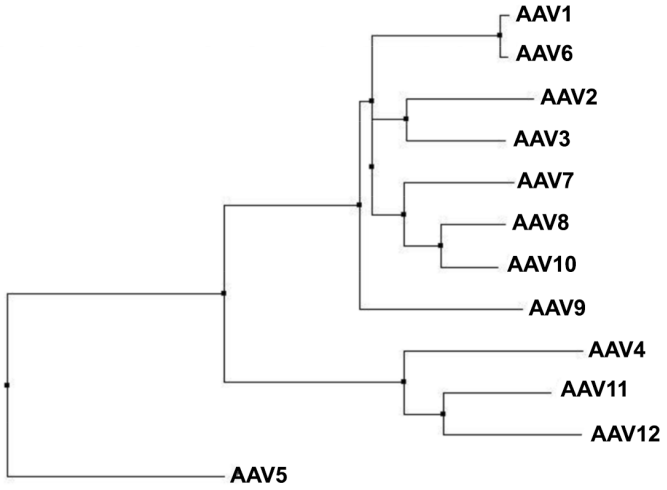
https://viralzone.expasy.org/226 Wörner et al., *Nat Commun*, 2021

Ib. AAV serotypes

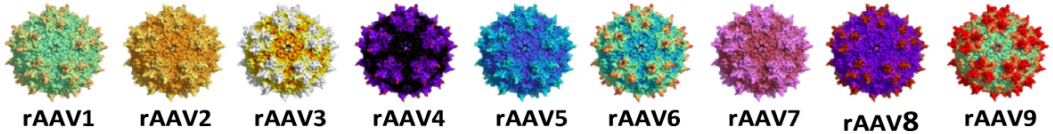
Sequences of VP1, VP2, VP3 capsid proteins define AAV serotype

Sequence homology (%) between serotypes 1-12

	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9	AAV10	AAV11	AAV12
AAV1	100%	83%	86%	64%	59%	**99%**	85%	84%	82%	85%	67%	61%
AAV2	83%	100%	87%	61%	58%	83%	82%	83%	82%	84%	63%	60%
AAV3	86%	87%	100%	63%	58%	87%	84%	85%	83%	85%	65%	61%
AAV4	64%	61%	63%	100%	53%	63%	64%	64%	63%	64%	82%	79%
AAV5	59%	58%	58%	53%	100%	59%	59%	58%	57%	57%	53%	53%
AAV6	**99%**	83%	87%	63%	59%	100%	85%	84%	82%	85%	66%	61%
AAV7	85%	82%	84%	64%	59%	85%	100%	88%	81%	88%	67%	62%
AAV8	84%	83%	85%	64%	58%	84%	88%	100%	85%	**93%**	66%	62%
AAV9	82%	82%	83%	63%	57%	82%	81%	85%	100%	86%	64%	60%
AAV10	85%	84%	85%	64%	57%	85%	88%	**93%**	86%	100%	67%	61%
AAV11	67%	63%	65%	82%	53%	66%	67%	66%	64%	67%	100%	84%
AAV12	61%	60%	61%	79%	53%	61%	62%	62%	60%	61%	84%	100%



Serotypes define interactions with the cell surface receptors, and thus – viral *tropism*



	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

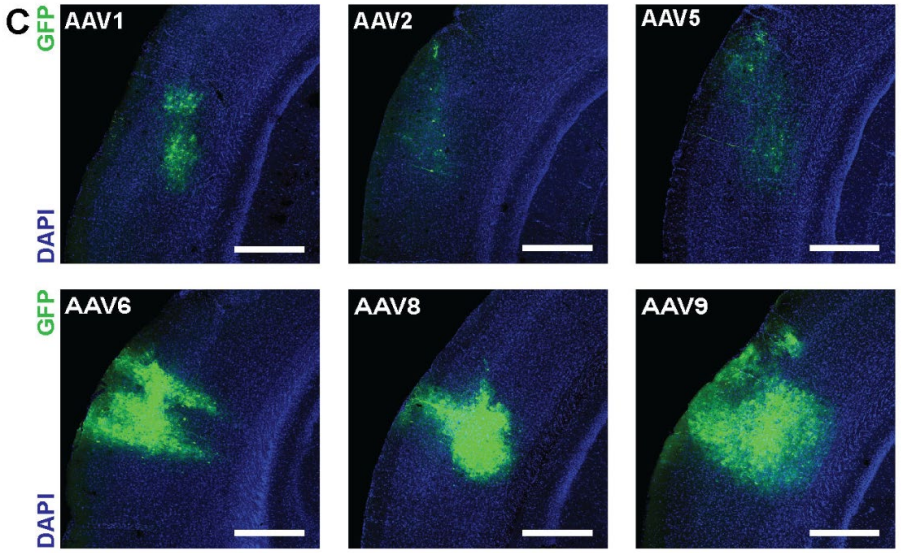
Vance et al., *in: Gene Therapy - Principles and Challenges,* 2015

Ib. AAV serotypes

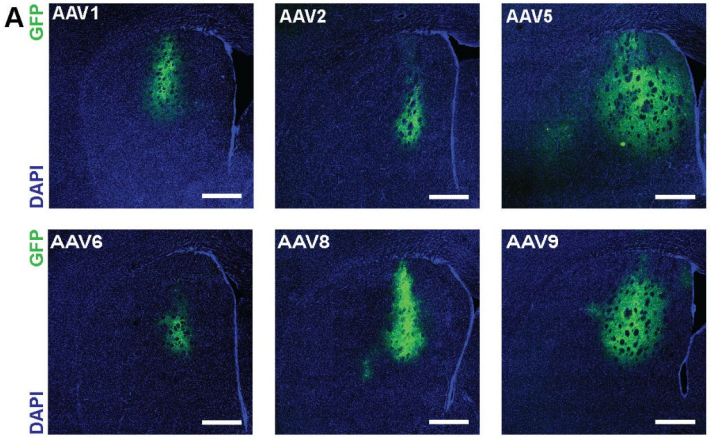
Why AAV serotypes are important?

- Serotype defines tropism to different cell types by selectivity of extracellular receptors
- Serotype affects intracellular fate of the virion (trafficking steps)
- New serotypes with advantageous properties can be engineered

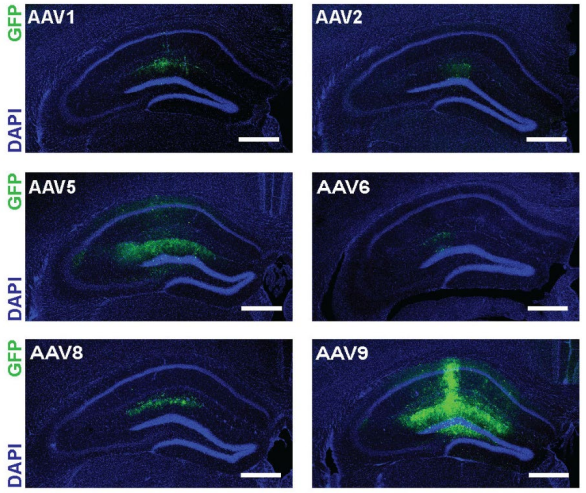
Cortex



Striatum



Hippocampus

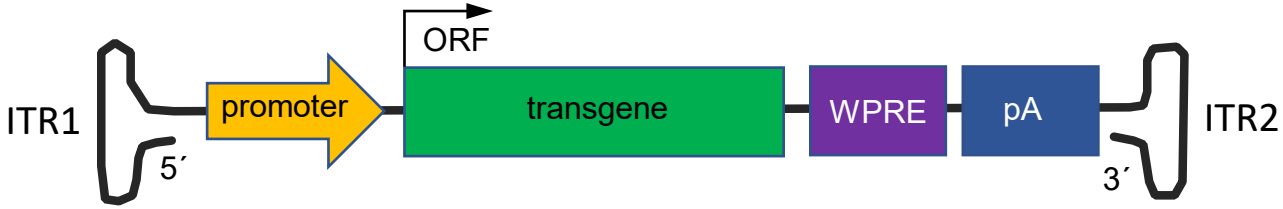


Injected: 80 nl AAV per site, titer: $9.6 \cdot 10^{11}$ virions/ml

Aschauer et al., *PLoS ONE,* 2013

Ib. rAAV engineering (simplified)

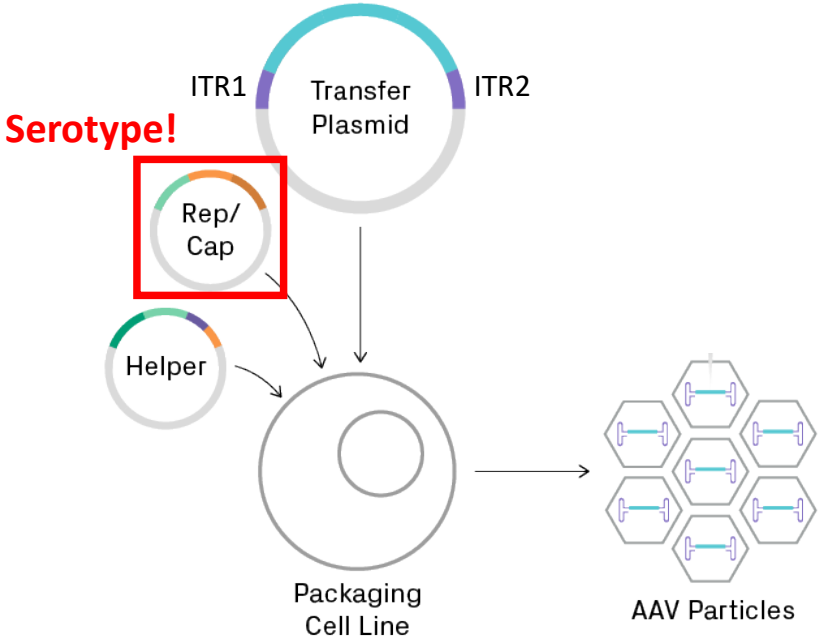
Step 1. Replace endogenous *rep* and *cap* genes with the expression cassette to obtain transfer plasmid



Promoters widely used in brain research

Name	Origin	Properties
hSyn1	Human synapsin1 gene	Neuron-specific
CamKIIα	Human/mouse CamKIIa	Pyramidal neurons (but not exclusively)
GFAP	Glial fibrillary acidic protein	Astrocyte-specific
CMV	Cytomegalovirus enhancer	Strong ubiquitous (neurons + glia)
CBA	Chicken beta-actin	Strong ubiquitous
CAG	CMV and CBA hybrid	Strong ubiquitous
EF1α	Translation elongation factor 1	Moderate ubiquitous

Step 2. Grow viral particles in cell lines trans-complementing viral genome

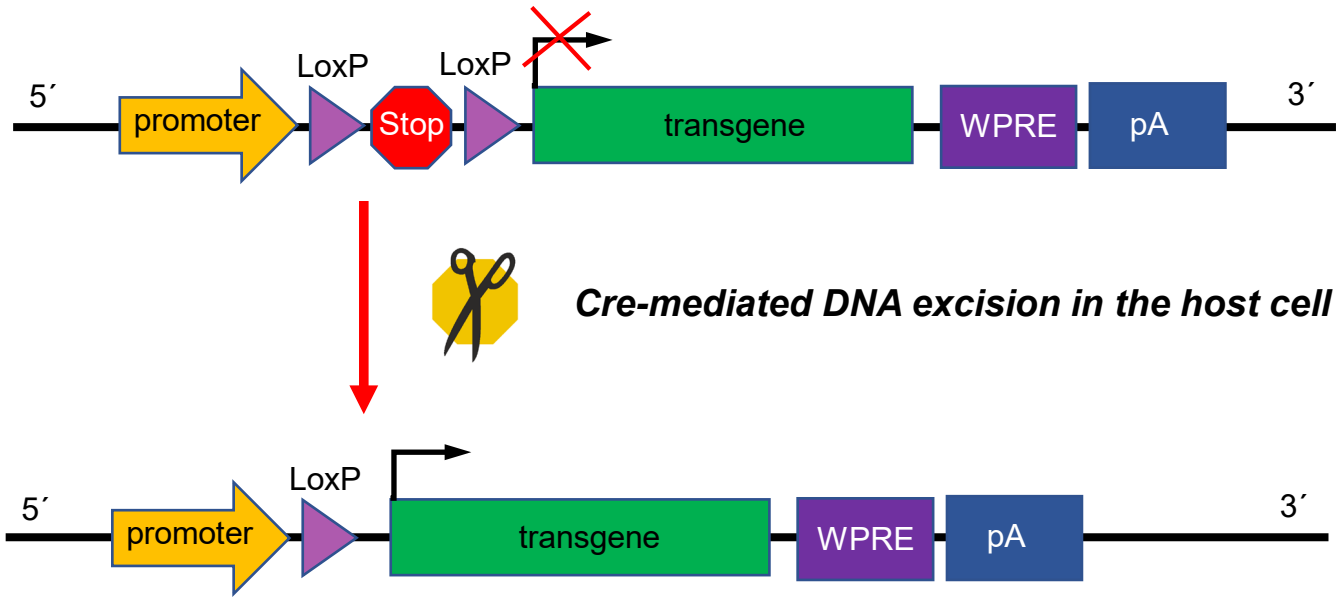


Ib. rAAV engineering: conditional transgene expression

What is it good for?

- Restrict expression of viral transgene to a genetically-defined (by Cre or Flp) neuronal population
- DNA-recombinases can themselves be delivered by another virus (very flexible designs possible)

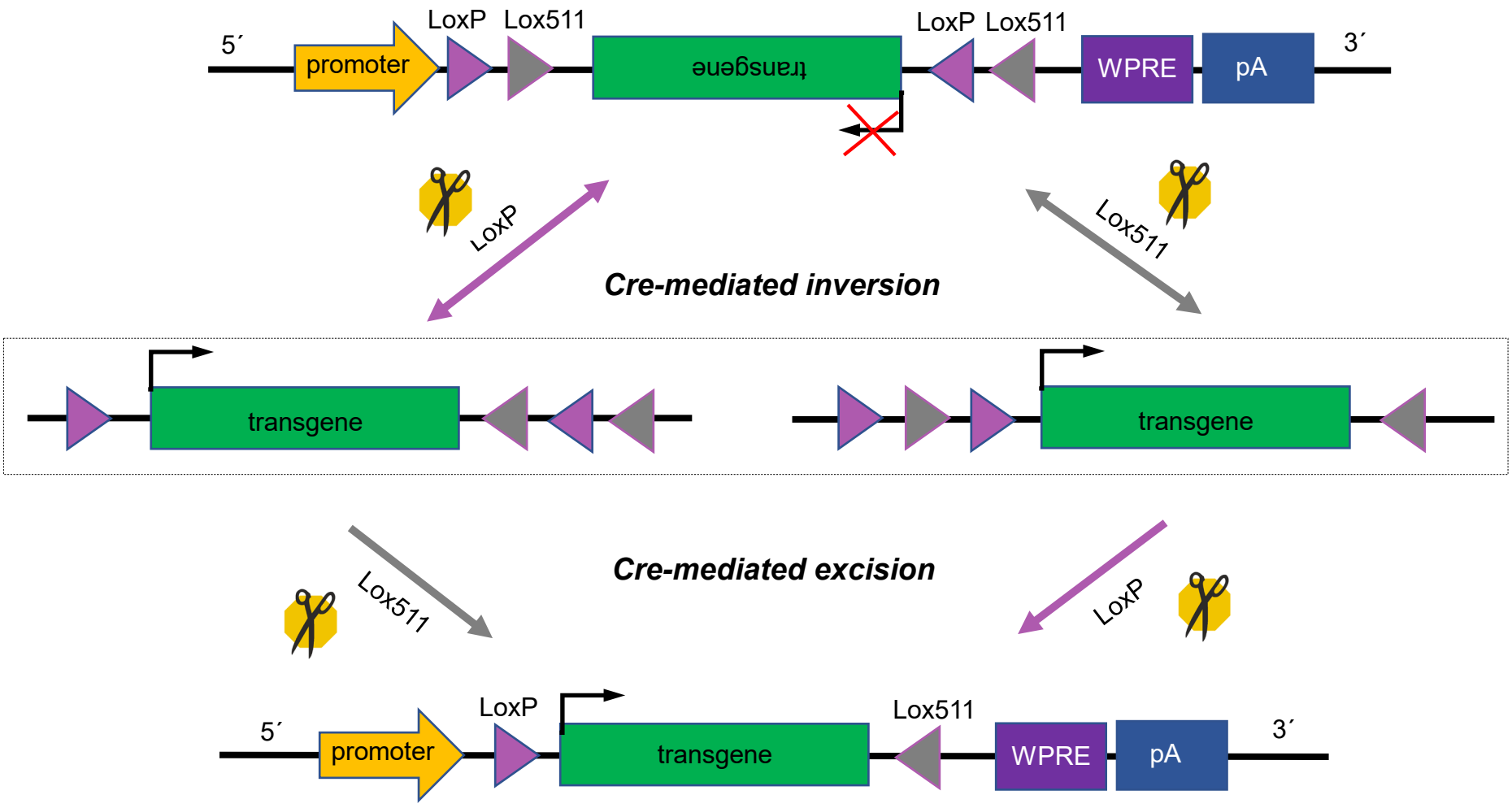
Approach 1. Use a "floxed STOP" a.k.a. "LSL":



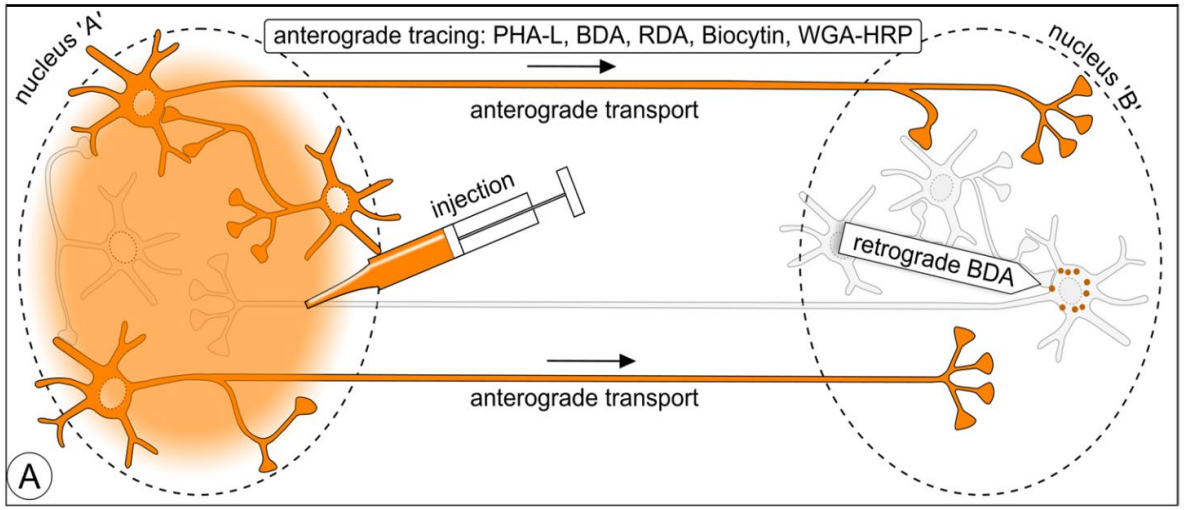
…but LSL-based AAVs sometimes have "leaky" transgene expression…

Ib. rAAV engineering: conditional transgene expression

Approach 2. Use a "FLEx" a.k.a. "DIO" switch (Schnütgen et al., *Nat Biotech* 2003); also possible with Flp !



Ib. Anterograde vs retrograde tracing of neuronal connectivity

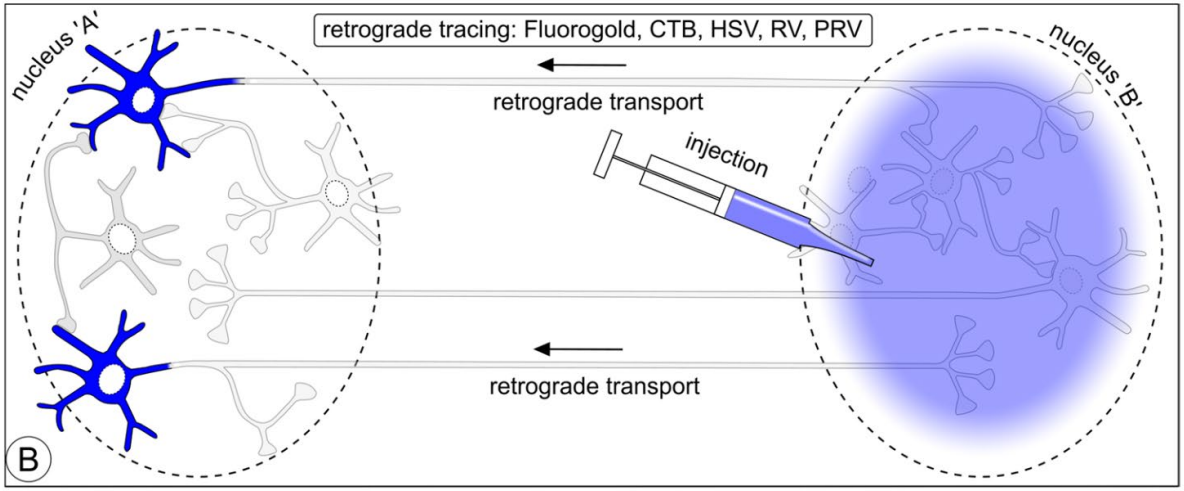


Anterograde labels

PHA-L = *Phaseolus vulgaris* Leucoagglutinin

BDA or RDA = Biotin or Rhodamine Dextran Amine

WGA-HRP = Wheat Germ Agglutinine – Horse Reddish Peroxidase



Retrograde labels

CTB = Cholera Toxin subunit B

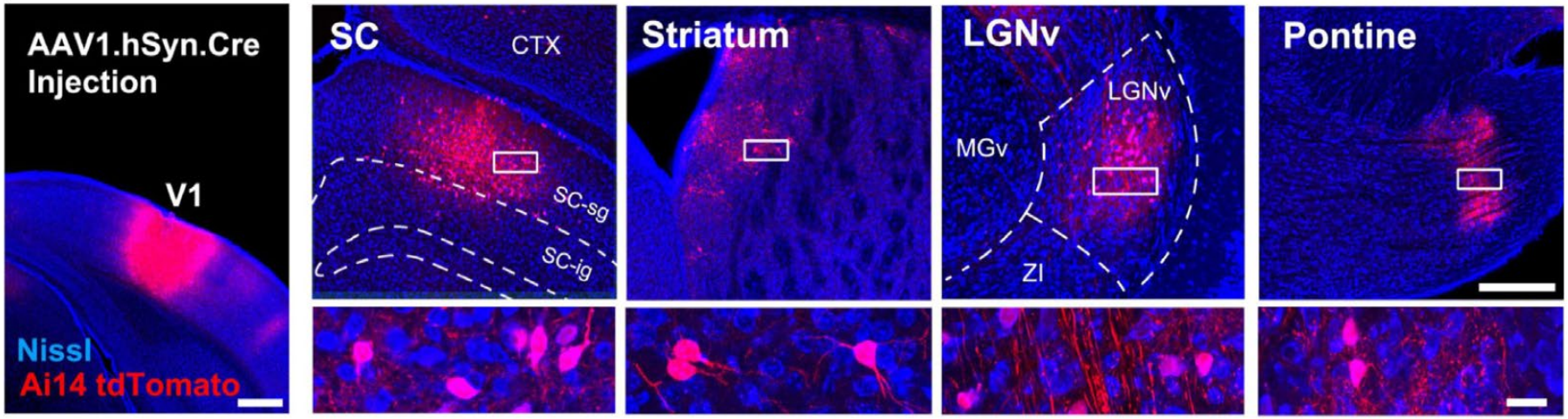
HSV = Herpes Simplex Virus

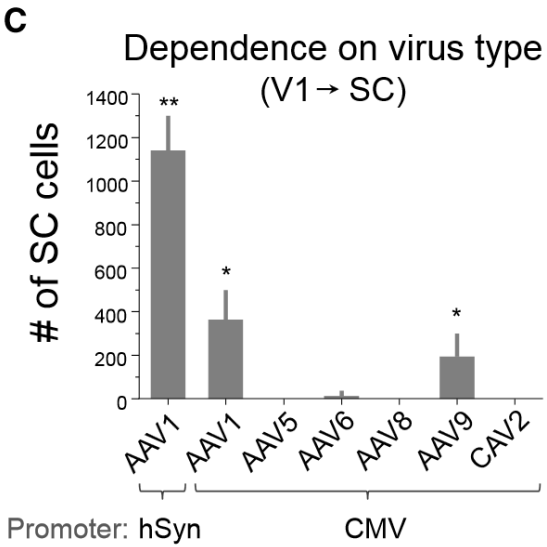
RV or PRV = Rabies or Pseudorabies Virus

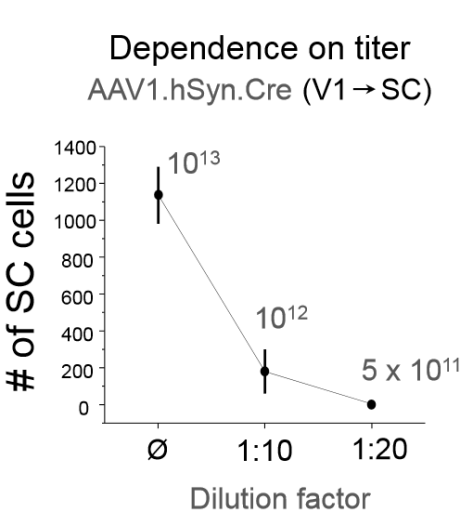
Lanciego and Wouterlood, *Brain Struct Funct,* 2020

Ib. Special features of AAV serotypes: AAV1 as anterograde TRANS-synaptic tracer

Mouse Ai14 (Rosa26-lsl-tdTomato reporter). AAV1-hSyn1-Cre jumps to distant projection areas



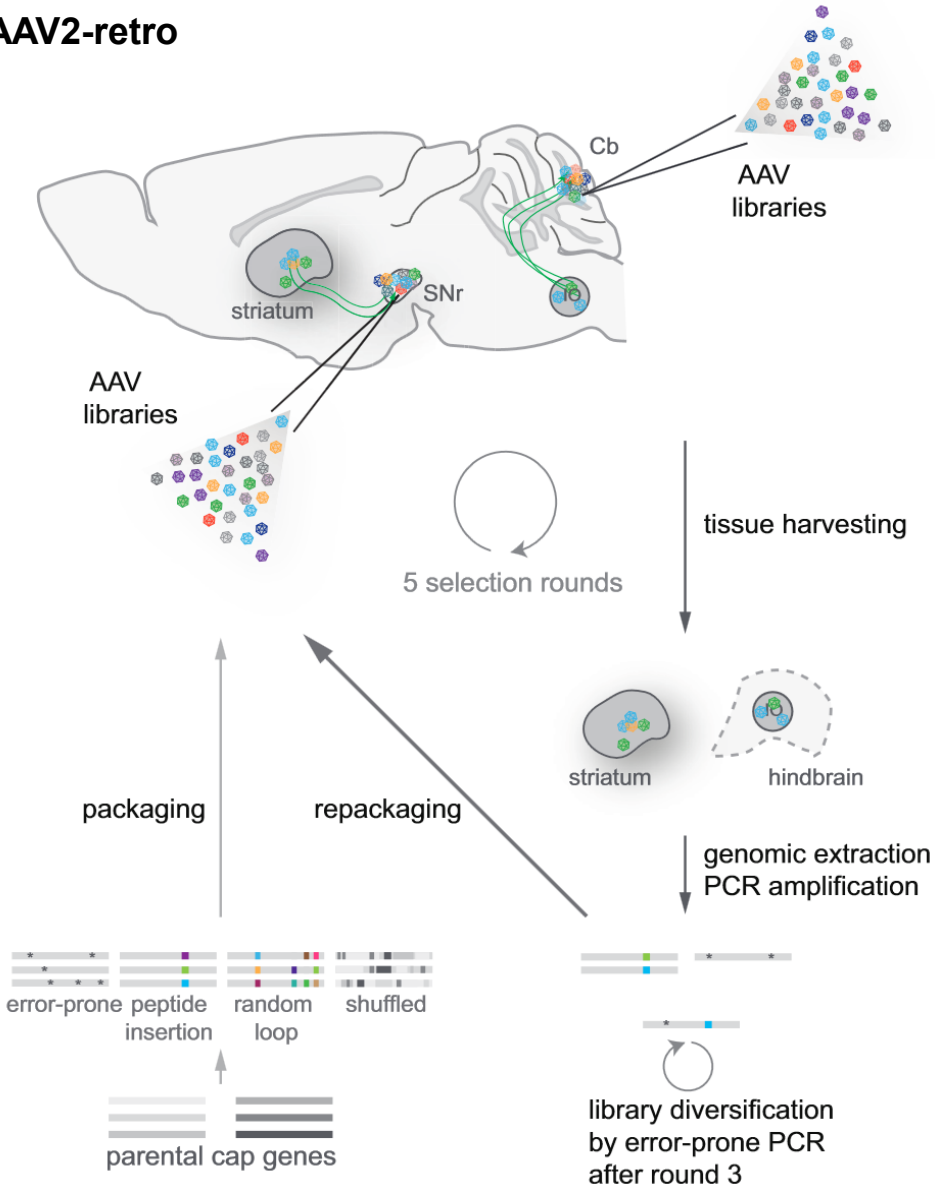




Zingg et al., *Neuron,* 2017

Ib. Special features of AAV serotypes: retroAAV, a designed retrograde tracer

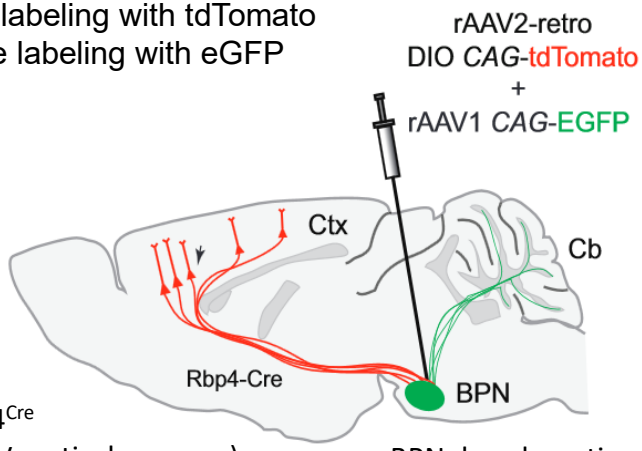
Directed evolution of AAV2-retro



Tervo et al., *Neuron,* 2016

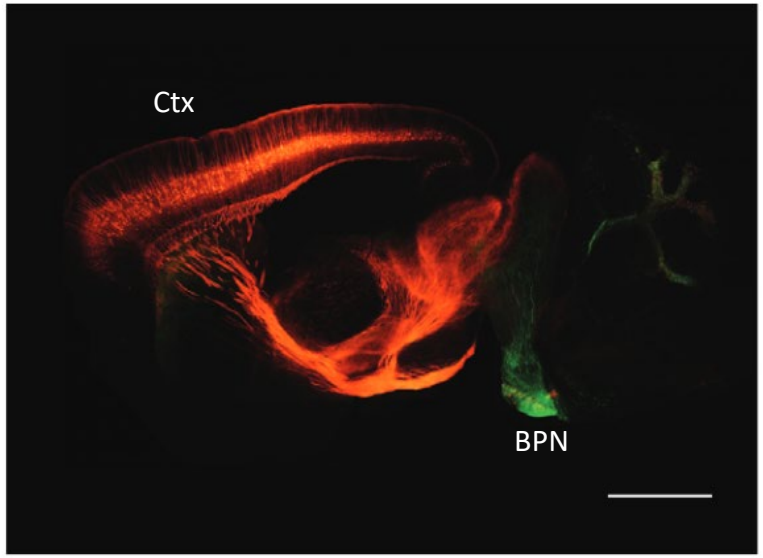
Ib. Special features of AAV serotypes: retroAAV, a designed retrograde tracer

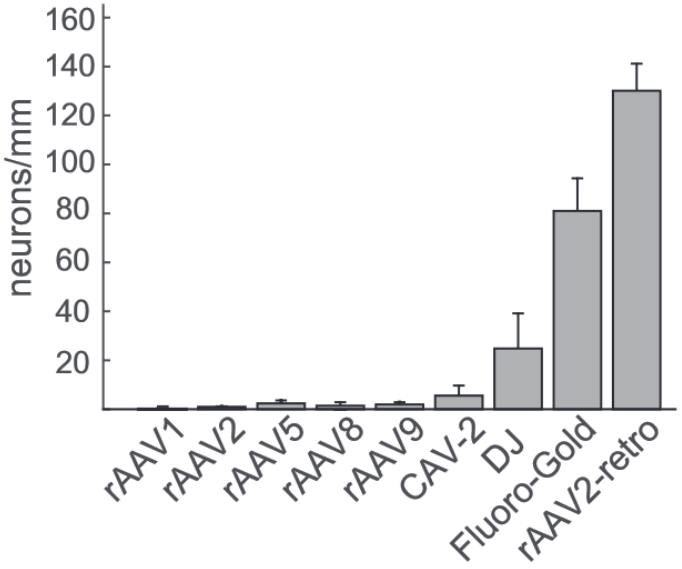
Retrograde labeling with tdTomato
Anterograde labeling with eGFP



Mouse: Rbp4^{Cre}
(Cre in layer V cortical neurons)

BPN=basal pontine nuclei





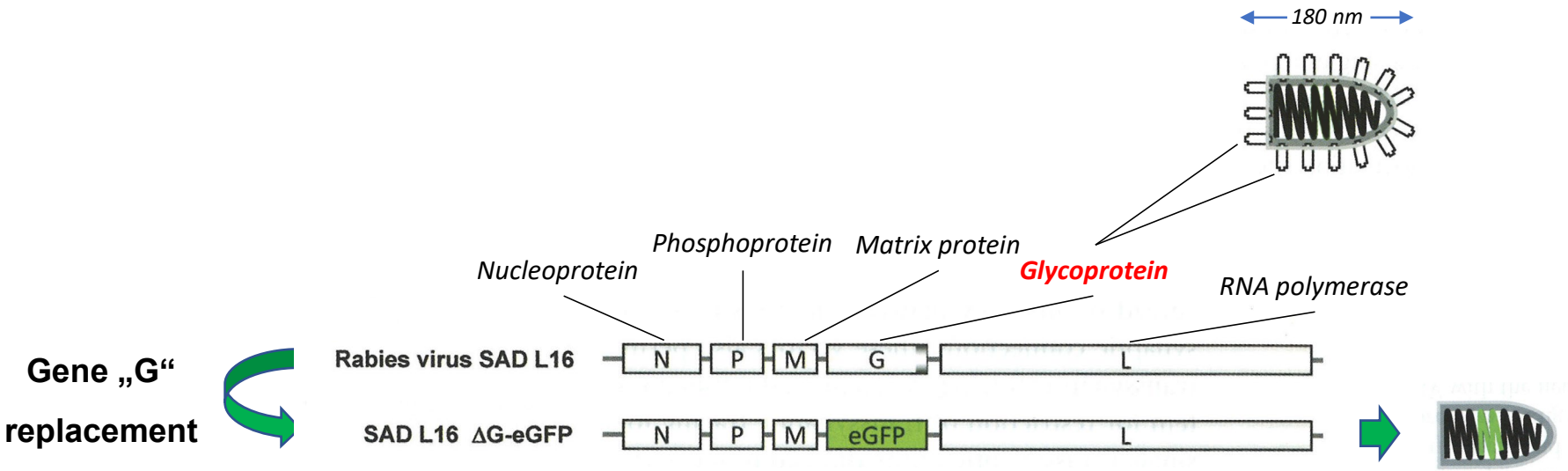
Tervo et al., *Neuron,* 2016

Ib. Pseudotyped rabies viral vectors for retrograde TRANS-synaptic labelling

Ib. Pseudotyped rabies virus: a tool for retrograde trans-synaptic labelling

Rabies virus:

- highly neurotropic, enveloped RNA virus of the *Rhabdoviridae* family
- Viral glycoprotein „G“ forms spikes and defines neurotropism

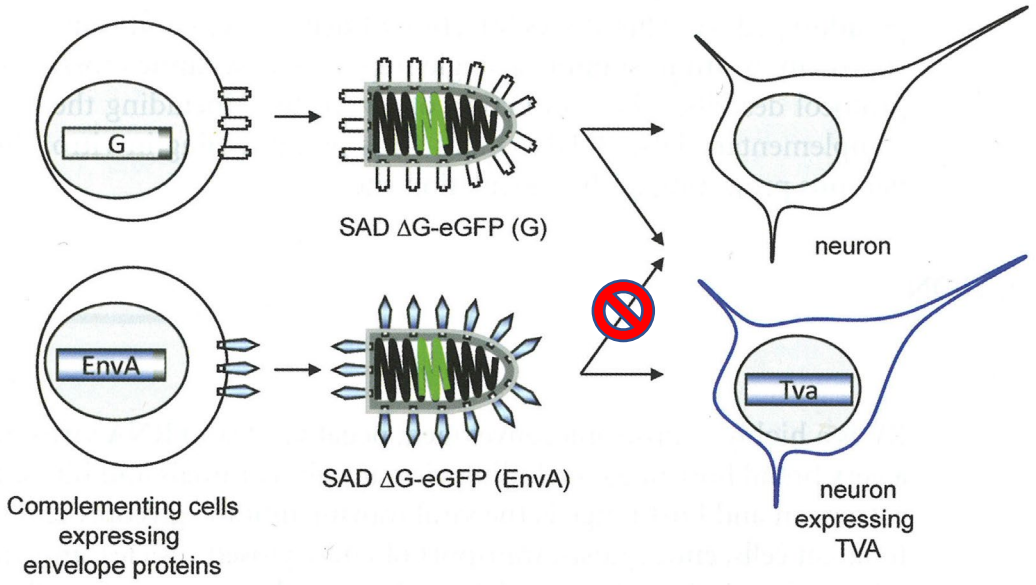


Hagendorf and Conzelmann, *in: Imaging in Neuroscience*, 2011

Ib. Pseudotyped rabies virus: a tool for retrograde trans-synaptic labelling

Pseudotyping = changing envelope protein (spikes) to achive required specificity of infection

EnvA =
Envelope protein of Avian sarcoma leucosis virus



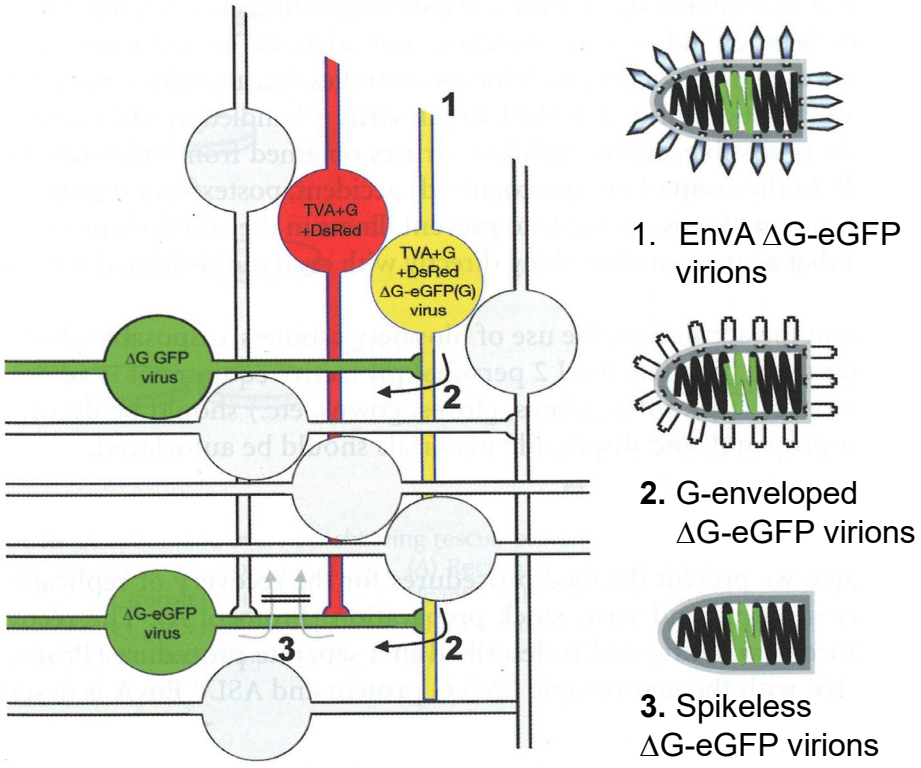
TVA =
avian receptor for EnvA

Hagendorf and Conzelmann, *in: Imaging in Neuroscience*, 2011

Ib. Pseudotyped rabies virus: a tool for retrograde trans-synaptic labelling

Experimental strategy:

- Transduce neurons of interest with AAV expressing TVA + G glycoprotein + **DsRed (marker)**
- Introduce EnvA-pseudotyped **ΔG-eGFP** virions
- In ~5-7 days „starter" neurons co-express eGFP+DsRed, and their presynaptic neurons express eGFP
- Retrograde spread of rabies virus is limited to only one trans-synaptic jump



Hagendorf and Conzelmann, *in: Imaging in Neuroscience*, 2011

II. Methods for manipulating activity of neural circuits

IIa. Manipulation of neuronal activity

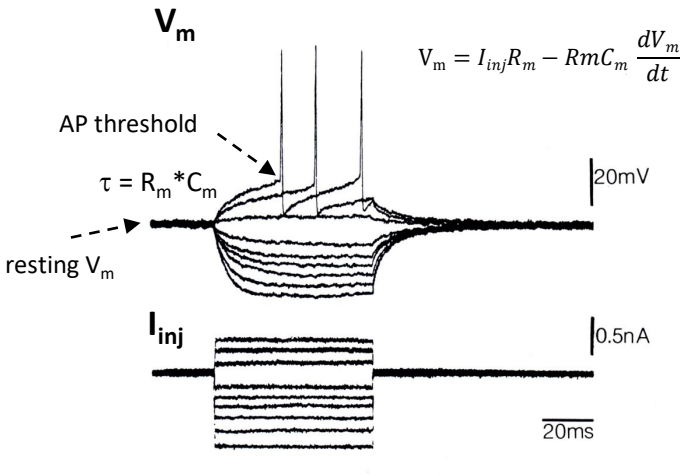
Why do we need to manipulate neuronal activity?

- To study the functional role of a certain neuronal population in sensory processing, memory, behavior…
- Sufficient role of a neuronal ensemble: if stimulated, generates expected response / behavior
- Necessary role of a neuronal ensemble: if inhibited, the expected response / behavior is suppressed

IIa. Manipulation of neuronal activity: electric stimulation

Current-clamp whole-cell recording in brain slice:

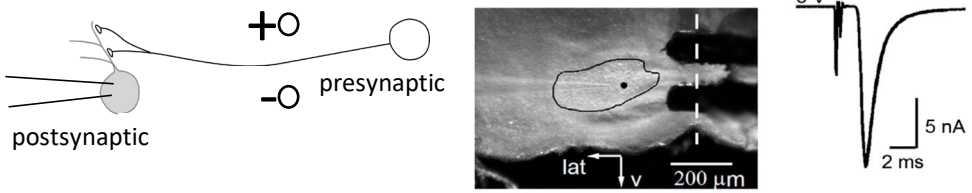
- direct depolarization of membrane by current injections
- one cell at a time
- hard to implement in-vivo



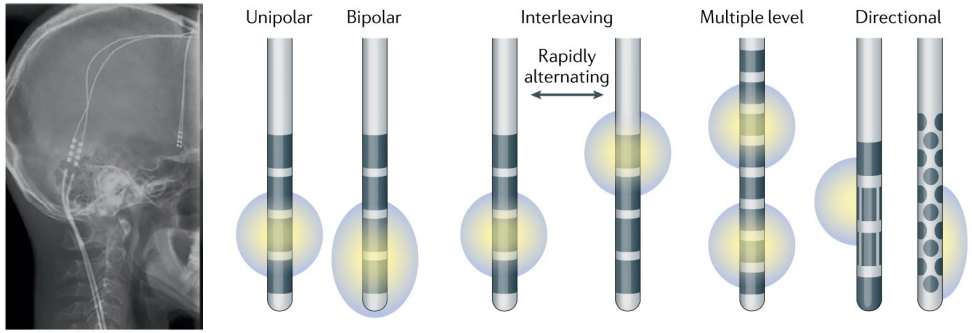
Extracellular stimulation electrodes:

- cell membrane depolarized by transient electric fields
- can be used in slices to stimulate axonal bundles
- can be used in-vivo (e.g. deep brain stimulation)

Bipolar electrode in brain slices



Deep Brain Stimulation (DBS) electrodes

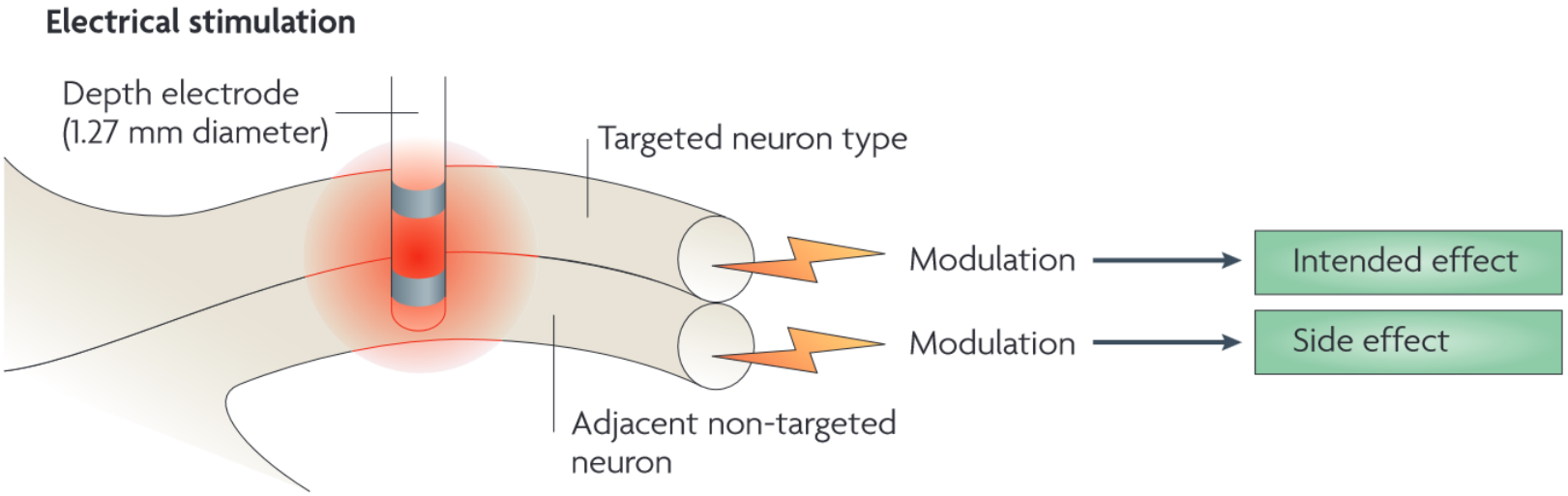


Krauss et al., *Nat Rev Neurol*, 2021

IIa. Manipulation of neuronal activity: electrical vs optical

Why electric manipulations are limited?

- Despite most physiological, they provide no selectivity for genetically defined cell populations
- Hard to *inhibit* a certain cell population with electric stimulation (use of refractory effects)



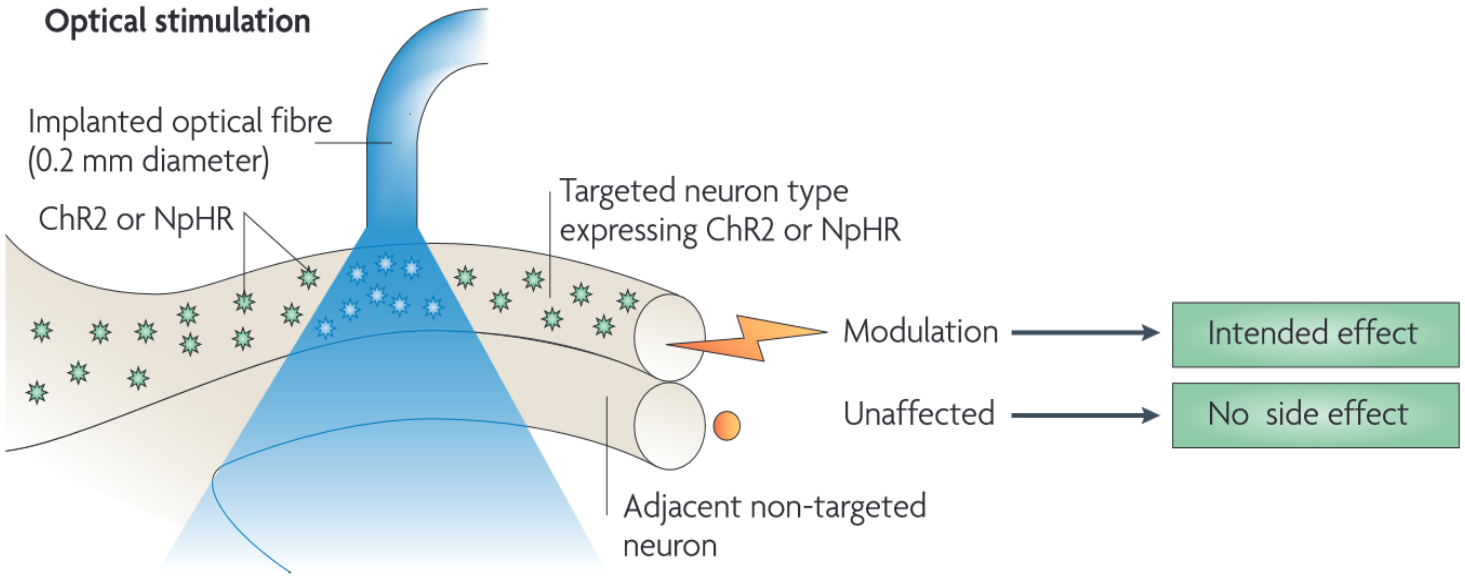
Zhang et al., *Nat Rev Neurosci*, 2007

IIa. Manipulation of neuronal activity: electrical vs optical

Optogenetics

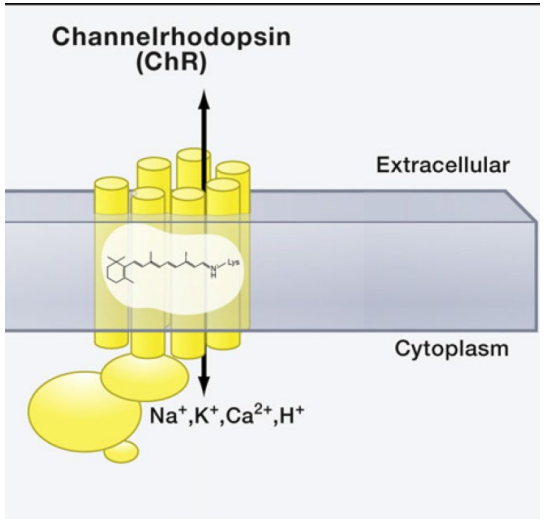
„Opto“:
- Use of electrogenic opsins (= light receptors) excited by incident photons

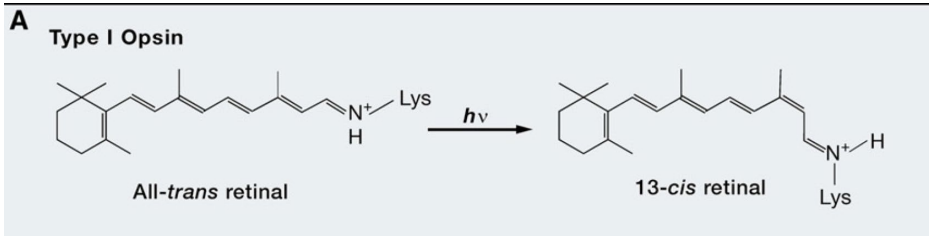
„Genetics“:
- Opsins are genetically targeted to defined cell population (e.g. conditional Cre-dependent expression)

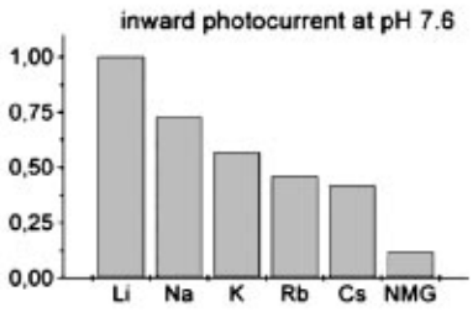


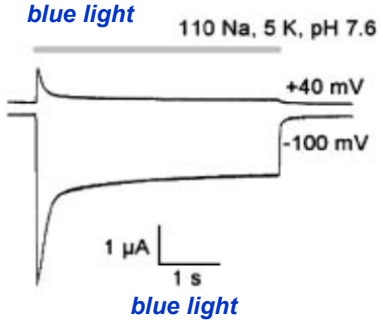
Zhang et al., *Nat Rev Neurosci*, 2007

IIb. Discovery of the Channelrhodopsin2 from green algae *Chlamydomonas reinhardtii*

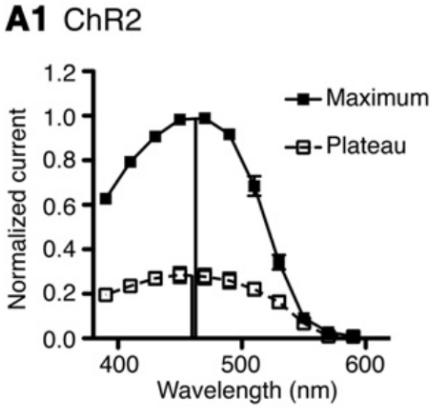


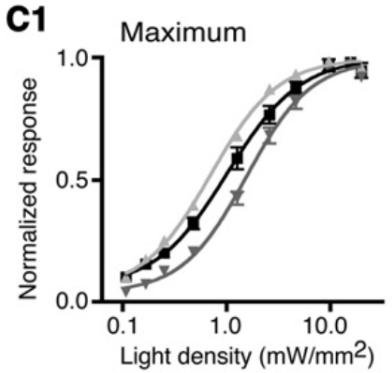






Nagel et al., *PNAS*, 2003 **(Ernst Bamberg lab)**



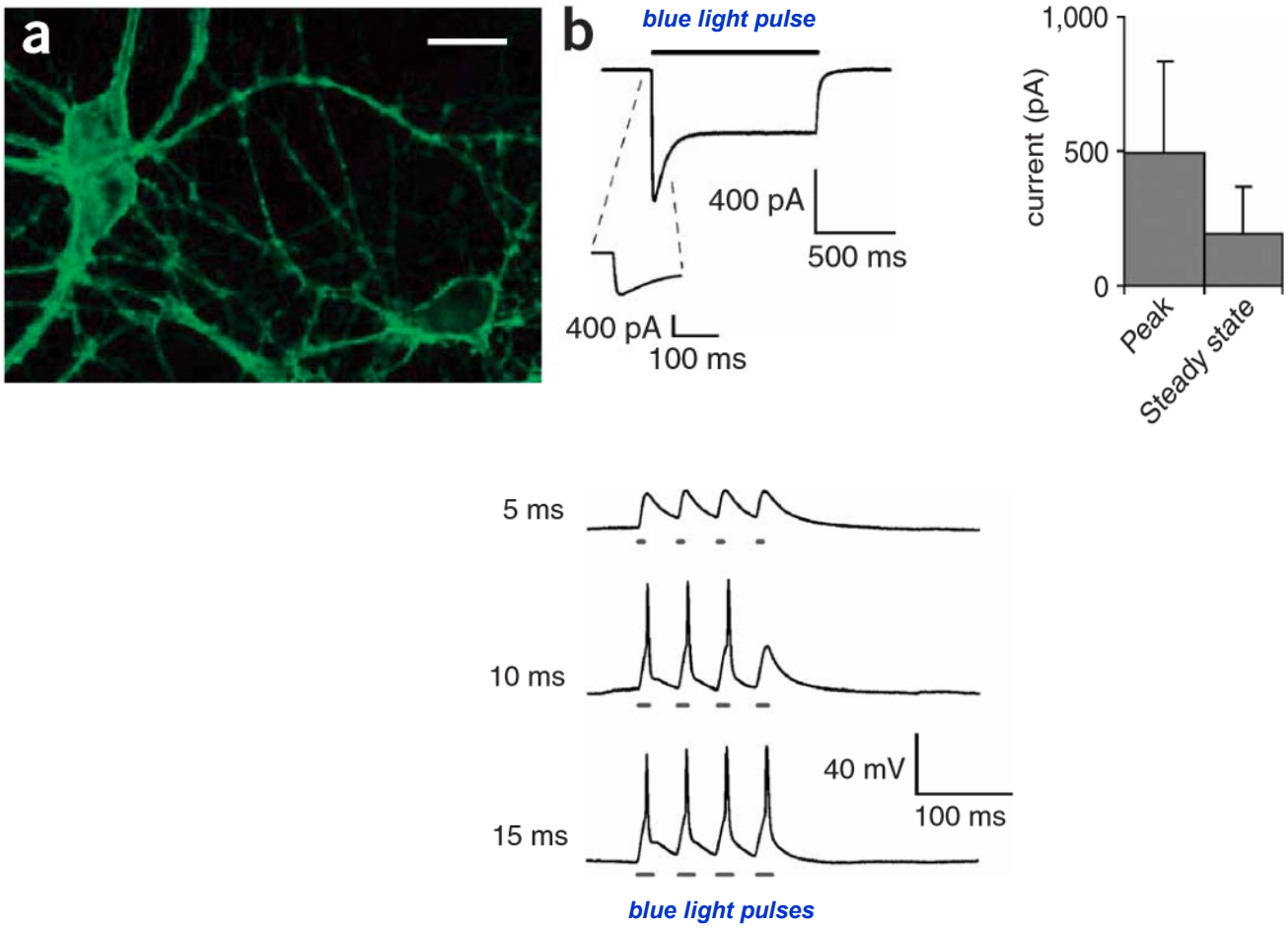


- ChR2 absorption spectrum ~ λ = 470 nm (blue light)
- A non-selective cation channel (V_{rev} ~0 mV)
- => generates inward current; depolarizing

Lin et al., *Biophys J*, 2009

IIb. Manipulation of neuronal activity: excitatory optogenetics

ChR2-eYFP expressed in hippocampal neurons

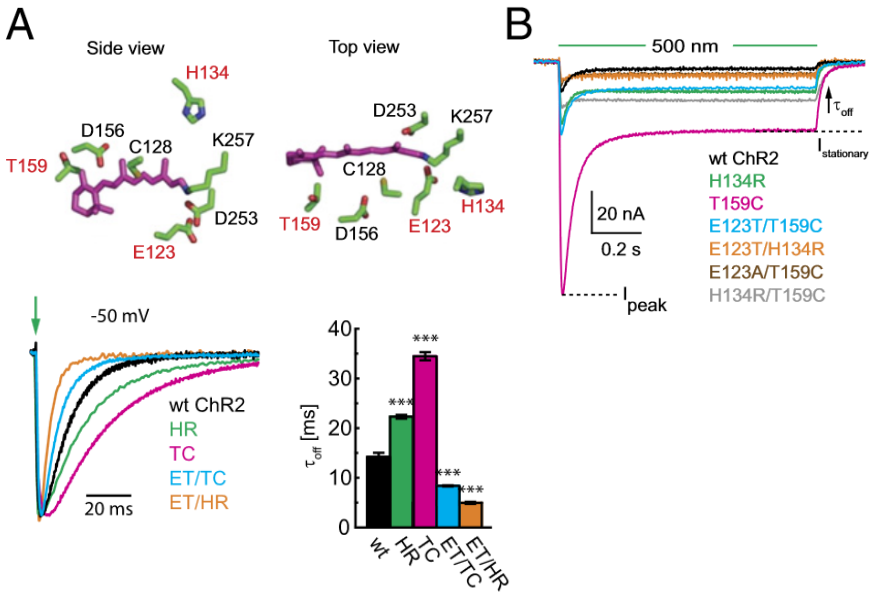


Boyden et al., *Nat Neurosci*, 2005 **(Karl Deisseroth lab)**

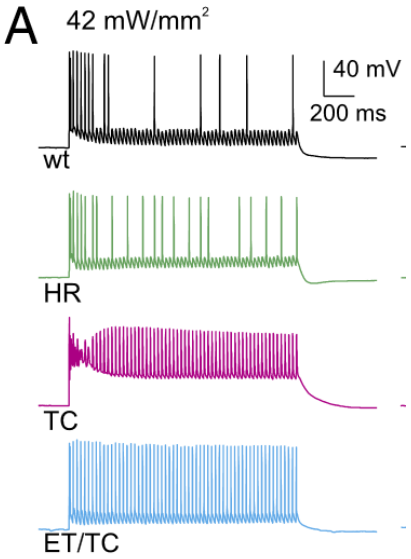
- Blue light pulses can trigger neuronal AP firing

IIb. Manipulation of neuronal activity: excitatory optogenetics

Engineering mutations to change ChR2 properties…

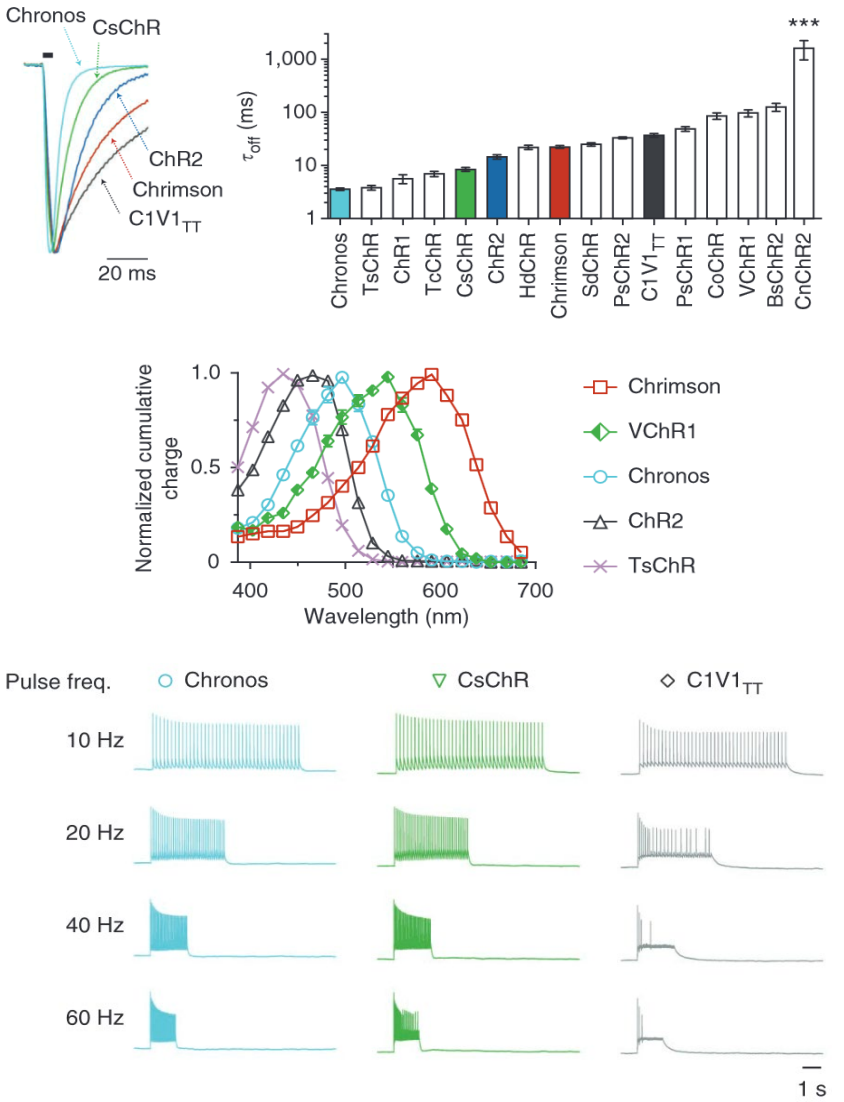


…affecting its performance to stimulate neurons



Berndt et al., *PNAS*, 2011

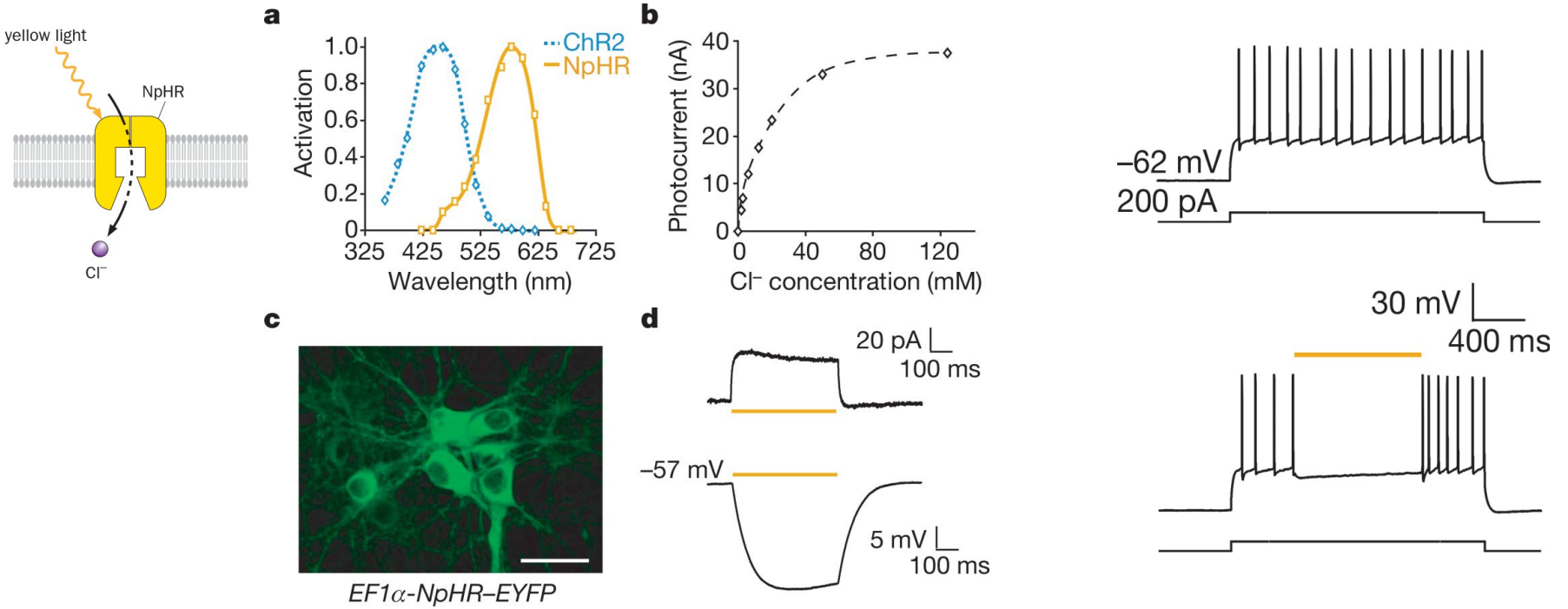
Opsins from different species, with different properties…



Klapoetke et al., *Nat Methods*, 2014

IIc. Manipulation of neuronal activity: inhibitory optogenetics

NpHR (Halorhodopsin) isolated from an archaea *Natronomonas pharaonis*



- NpHR absorption spectrum ~ λ = 590 nm (yellow light)
- Current depends on Cl^- (**a chloride pump, not a channel**!)
- => generates outward current; hyperpolarizing

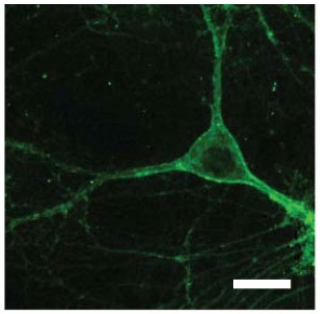
- yellow light can interrupt ongoing AP-firing
- => inhibitory opsin

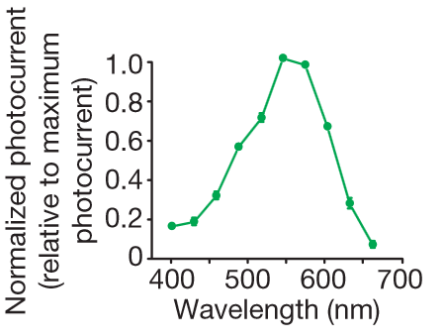
Zhang et al., *Nature* 2007 **(Karl Deisseroth lab)**

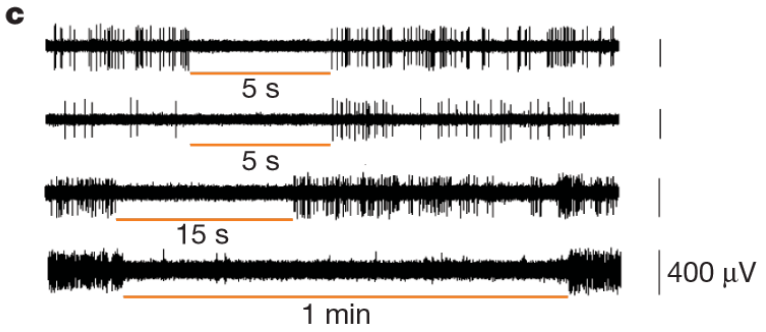
IIc. Manipulation of neuronal activity: inhibitory optogenetics

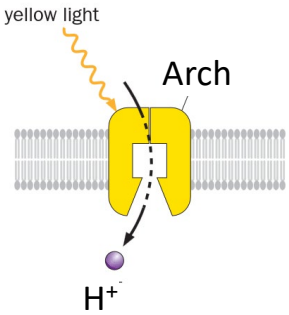
Arch (Archaeorhodopsin-3) isolated from an archaea *Halorubrum sodomense*

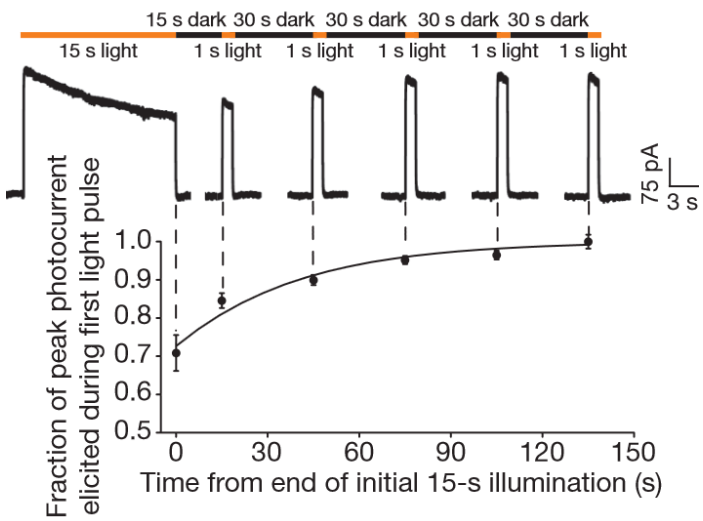
Arch-eGFP expressed in a hippocampal neuron









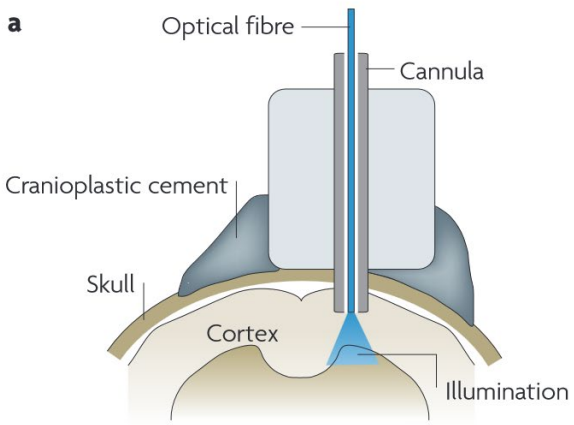


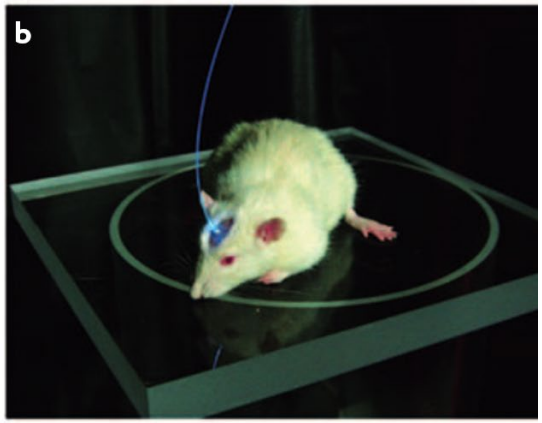
- yellow light can interrupt ongoing AP-firing
- => inhibitory opsin

- Arch absorption spectrum ~ λ = 580 nm (yellow light)
- Current depends on H^+ (**a proton pump, not a channel**!)
- => generates outward current; hyperpolarizing

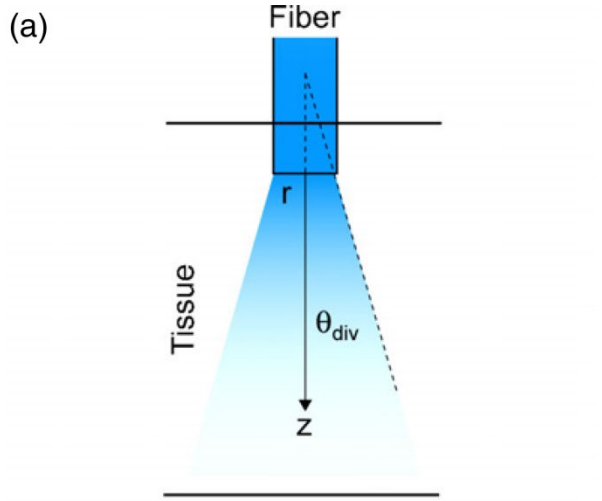
Chow et al., *Nature* 2010 **(Edward Boyden lab)**

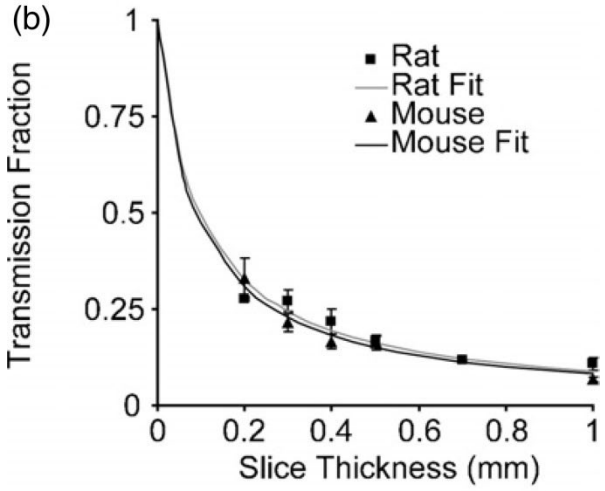
IIc. Light delivery for in-vivo optogenetic applications





Zhang et al., *Nat Rev Neurosci*, 2007





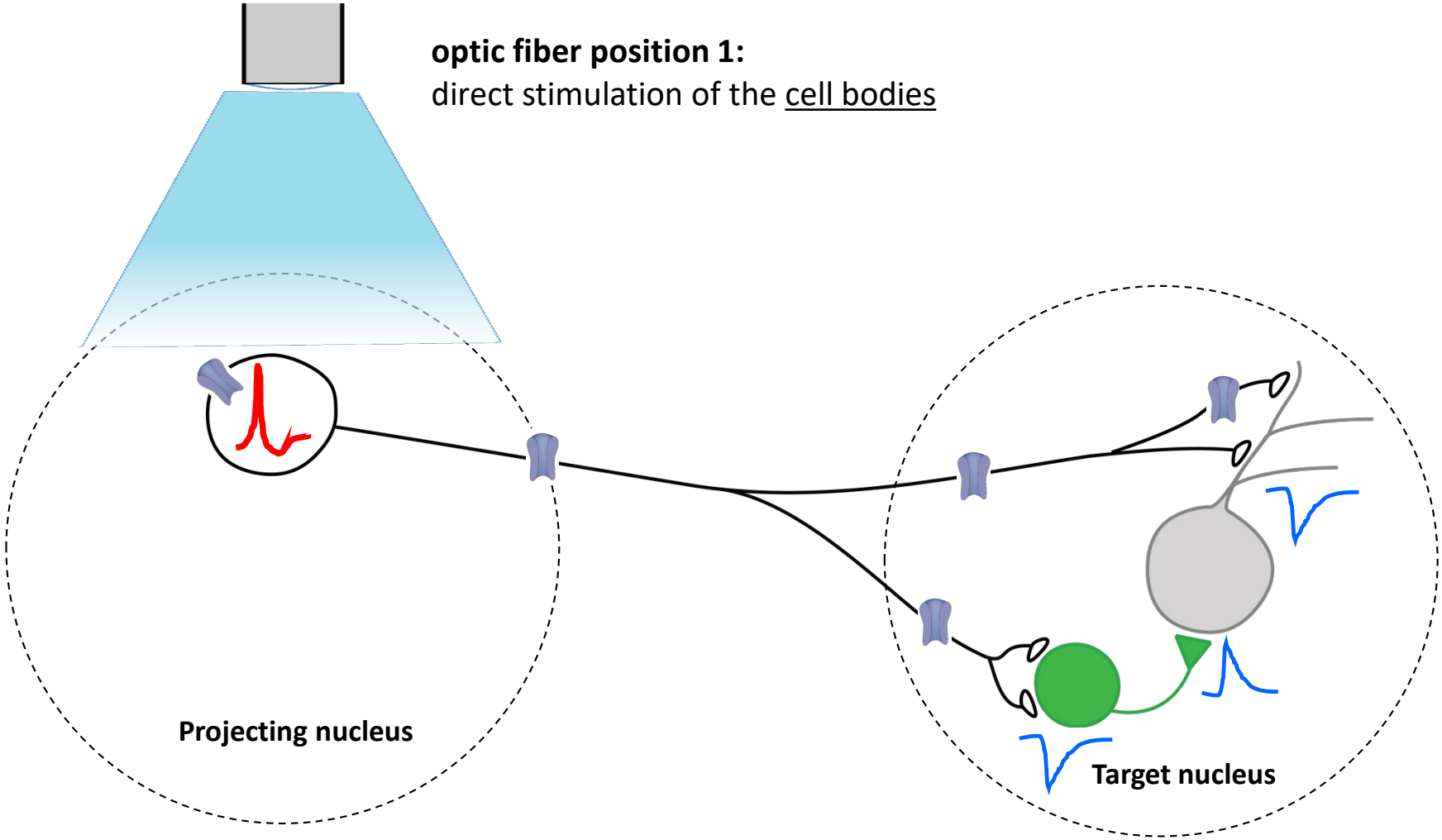
- Optical fiber core diameter ~200 µm
- Numerical aperture 0.39-0.5

=> Illuminates a conical volume ~ 500 µm diameter
… up to 500 µm from the fiber

Aravanis et al. *Neural Eng* 2007

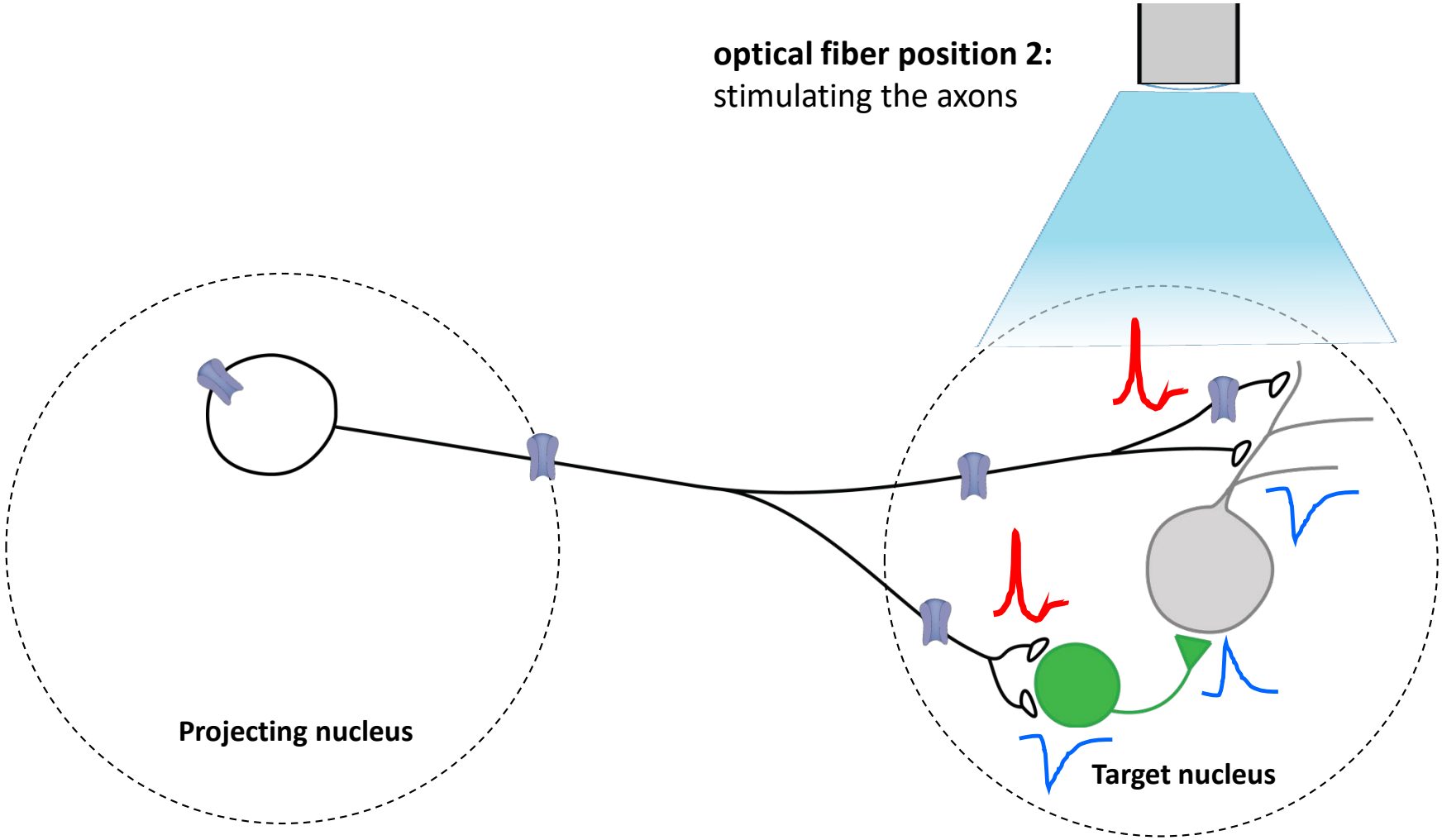
IIc. Optogenetics: flexibility of experimental designs

optic fiber position 1:
direct stimulation of the cell bodies



- Optic fiber can be placed above the projecting nucleus *in-vivo* to manipulate neurons at the soma level

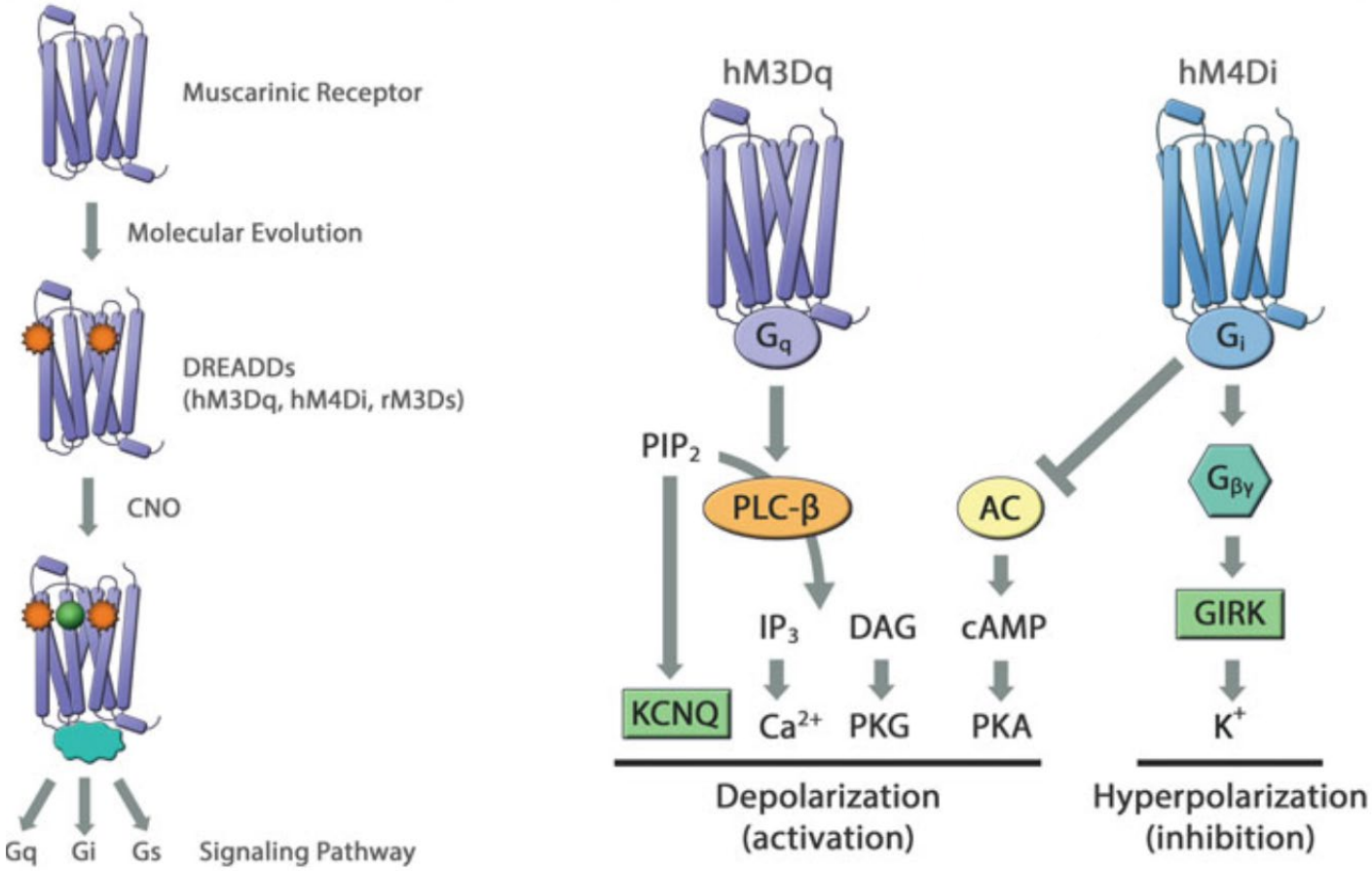
IIc. Optogenetics: flexibility of experimental designs

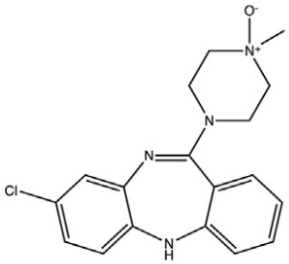


- Optic fiber can be placed in the target nucleus *in-vivo:* only transduced axons will be manipulated
- Slices of target nucleus can be made & surrounding (transduced) axons can be stimulated optically

IId. Ligand-controlled „chemogenetic“ actuators

DREADDs (**D**esigner **R**eceptors **E**xclusively **A**ctivated by **D**esigner **D**rugs)





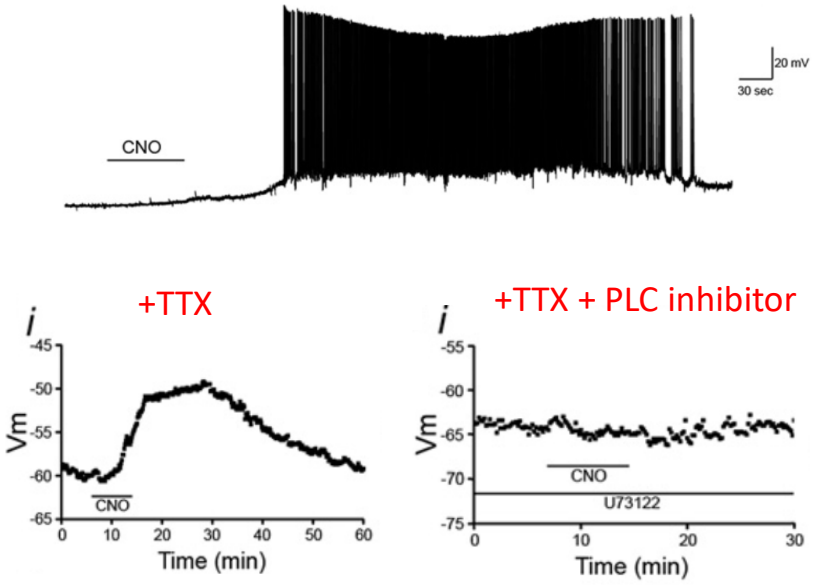
CNO = clozapine N-oxide

Attention: effect of DREADDs like hM3Dq on neuronal excitability is indirect thus not straightforward

Bryan Roth, *Neuron* 2016;
Michaelides and Hurd, *in: Neuroscience in the 21st century*, 2015

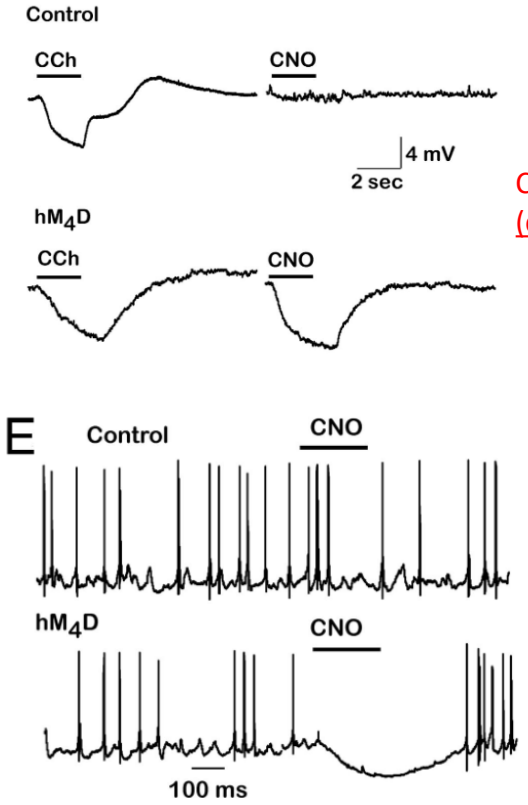
IId. Ligand-controlled „chemogenetic“ actuators

hM3D$_q$ in neurons from a knock-in mouse



Alexander et al., *Neuron* 2009

hM4D$_i$ in a transfected hippocampal neuron



CCh = carbachol (cholinergic agonist)

Armbruster et al., *PNAS* 2007

Pros and cons versus optogenetics

- Better suitable for longer-lasting manipulations
- Poor spatial resolution (*but this can be an advantage*); very poor temporal resolution (by CNO)
- Indirect action via second messengers is hard to control (e.g. hM3D$_q$ leads to PLC activation…)

=> Task for the Exercise session: read and discuss the paper on another type of optogenetic silencing tool, an inhibitory mosquito-derived rhodopsin eOPN3, better suitable for silencing activity in axonal terminals

NeuroResource

Neuron

Efficient optogenetic silencing of neurotransmitter release with a mosquito rhodopsin

Authors

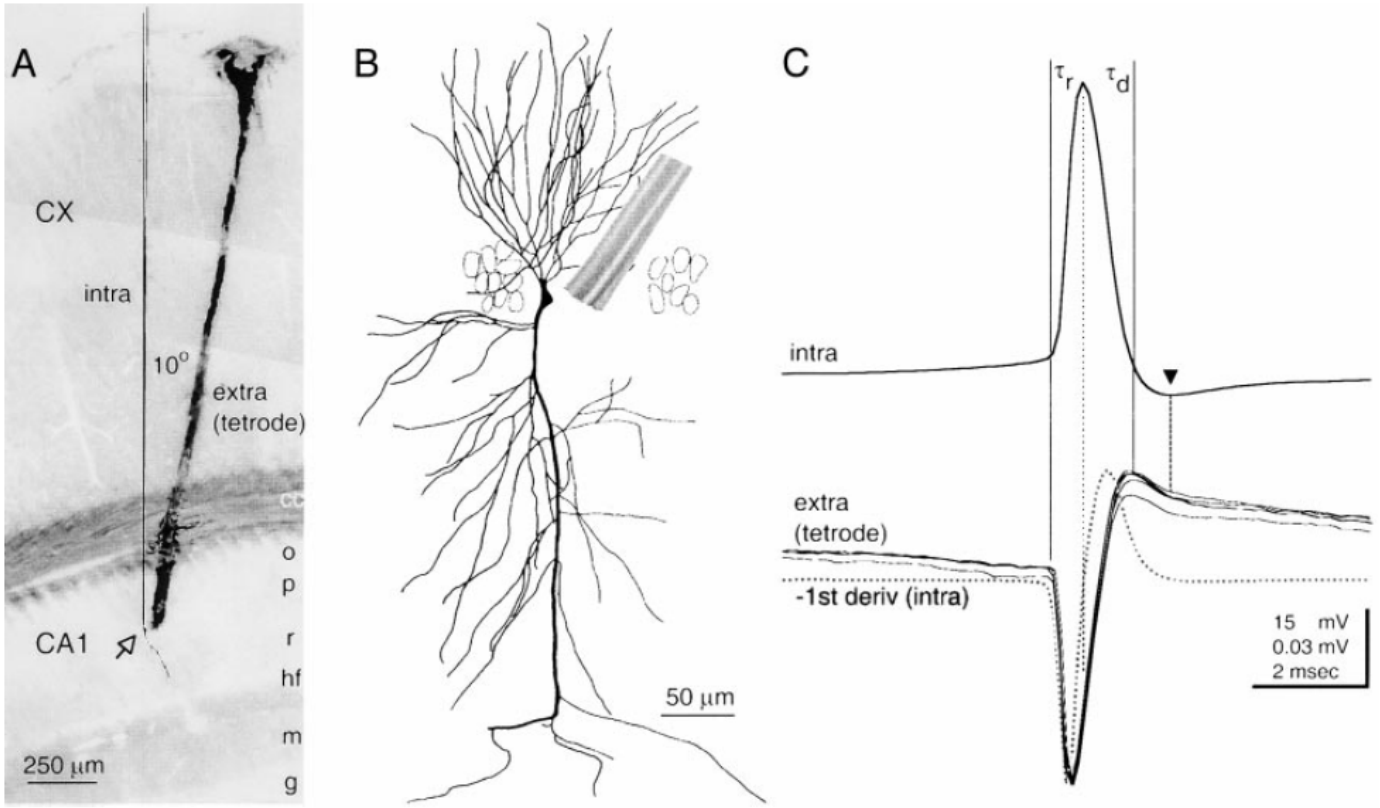
Mathias Mahn, Inbar Saraf-Sinik, Pritish Patil, ..., Benjamin R. Rost, J. Simon Wiegert, Ofer Yizhar

III. Electrophysiological methods for measuring neural circuit activity in-vivo

III. Neuronal spike recordings using extracellular electrodes

How does it work?

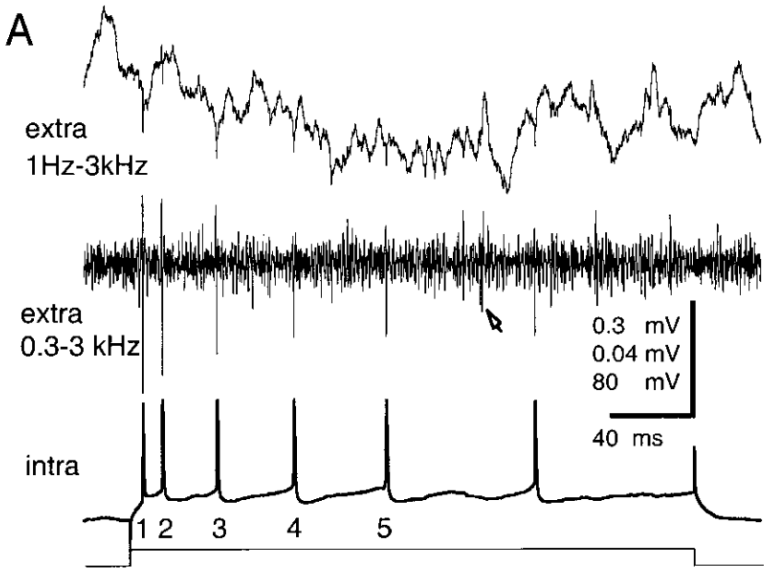
- Need a metal electrode (Ø 10-25 μm, 50-150 kΩ impedance @ 1 kHz), connected to a high R_{in} amplifier
- Close to the body of AP-generating neuron, extracellular currents are proportional to $[-dV_m(t)/dt]$
- Due to the low extracellular resistivity, the amplitude of the signal is ~1000 fold lower than intracellular AP



Henze et al., *J Neurophysiol* 2000

III. Neuronal spike recordings using extracellular electrodes

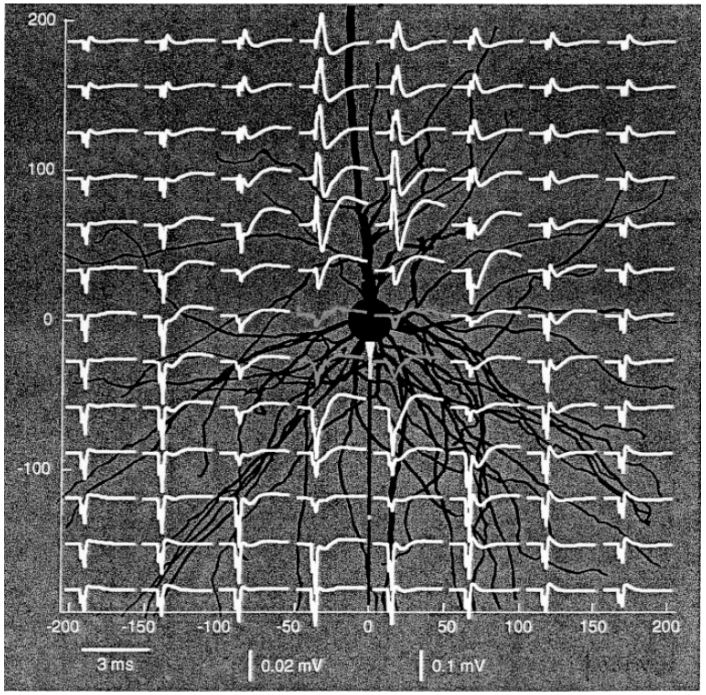
How the real traces look like



Henze et al., *J. Neurophysiol* 2000

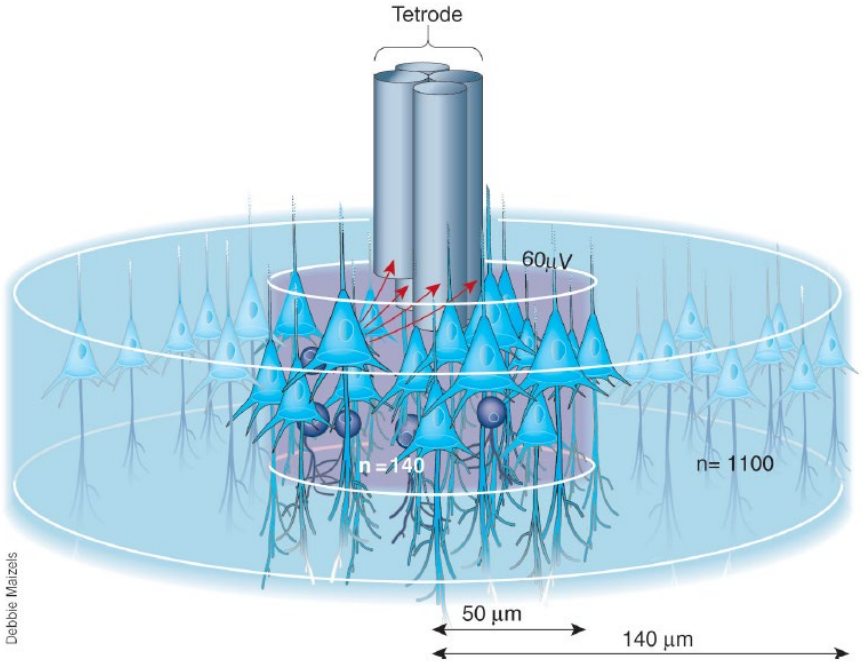
Modelled extracellular waveform

as a function of electrode position

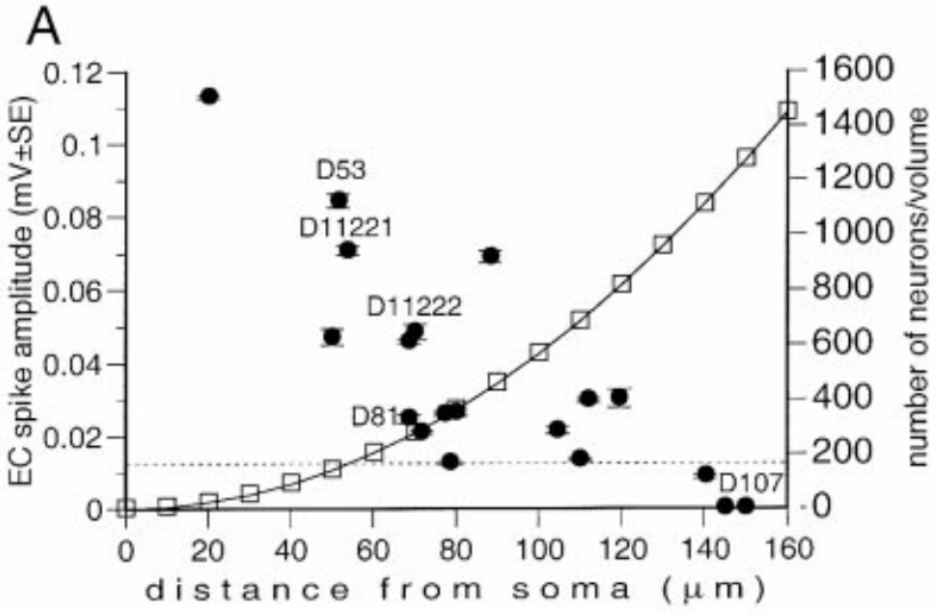


G. Holt & C. Koch, *J. Comp Neurosci* 1999

III. Neuronal spike recordings using extracellular electrodes



Buzsáki, *Nat. Neurosci* 2004



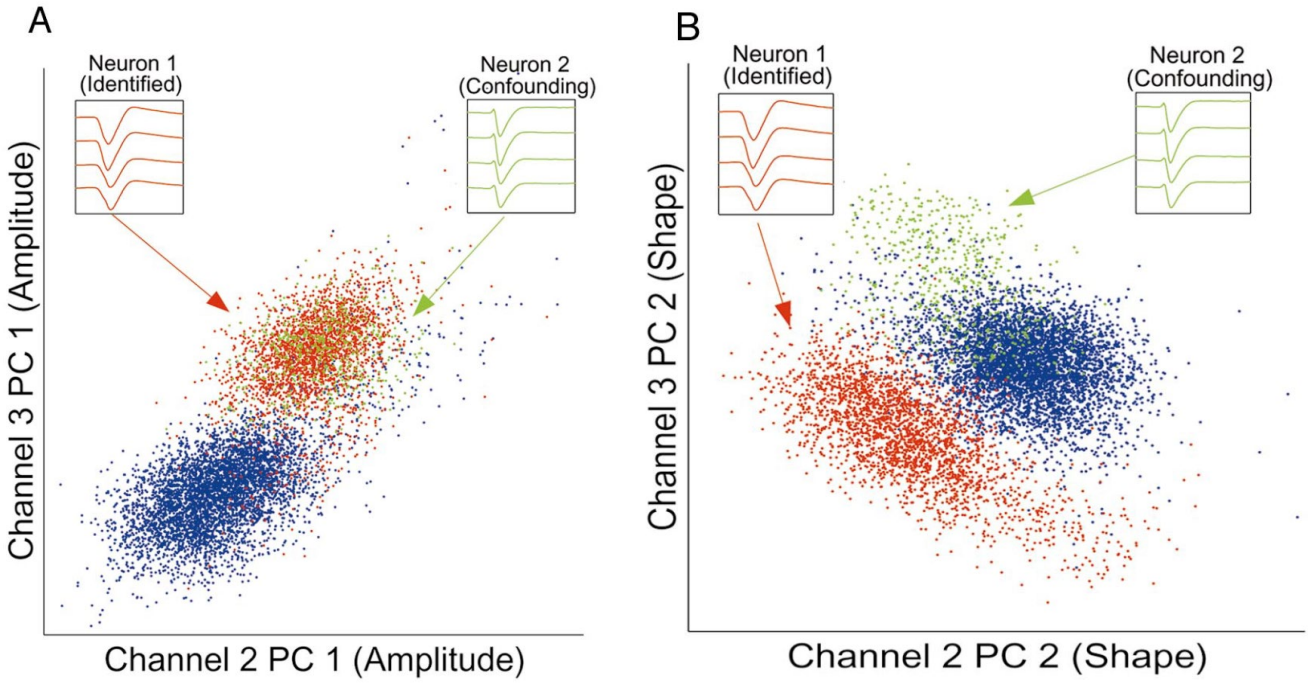
Henze et al., *J. Neurophysiol* 2000

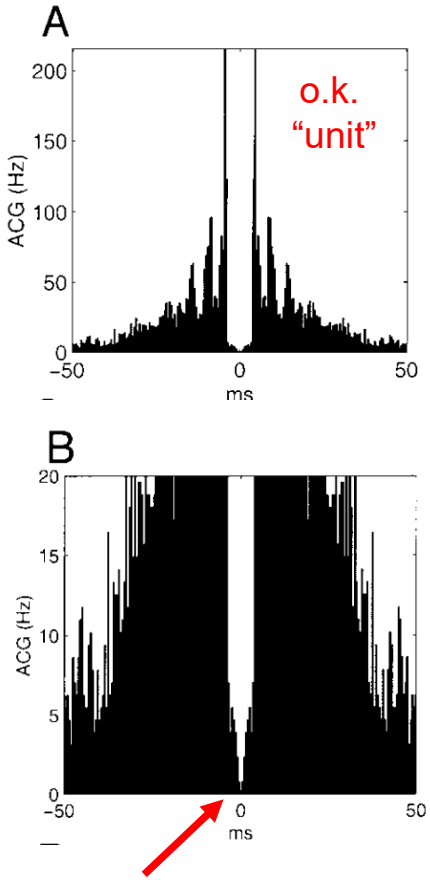
III. Analysis of extracellular spikes to separate recorded neurons

Analysis of tetrode recordings

- Filter the recorded traces (band-pass 0.3-3 kHz); detect spikes on all 4 channels to get the timestamps
- Use dimensionality reduction approaches to analyse and cluster spike waveform parameters (e.g. PCA)
- Do quality control to filter out „single units“ from „multi-units“ (e.g. autocorrelograms and other criteria)

Analysis of only spike amplitudes is not sufficient, need to account for shapes

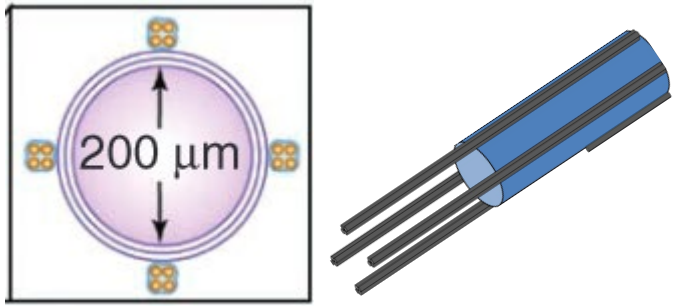




Refractory interval <2 ms is not empty
=> The “unit” is contaminated by spikes from other cells

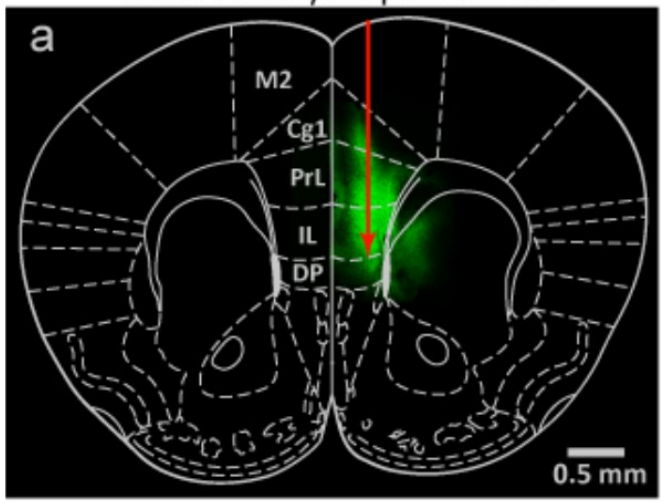
Harris et al., *J. Neurophysiol* 2000

III. Optrode recordings: identification of tagged neurons

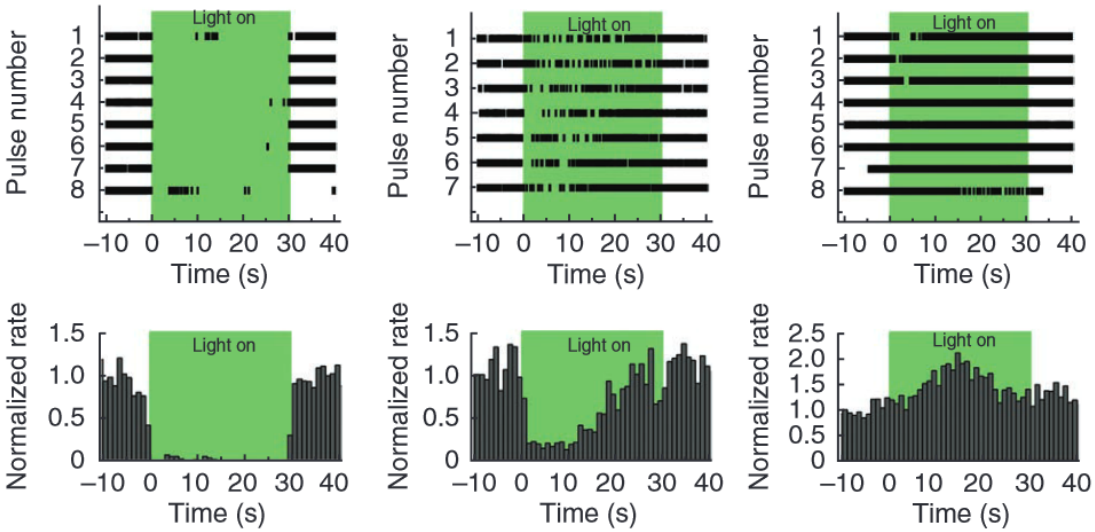


(inhibitory Halorhodopsin)

WT: AAV5::hSyn::NpHR3.0::EYFP



Identified types of "units":



Directly inhibited

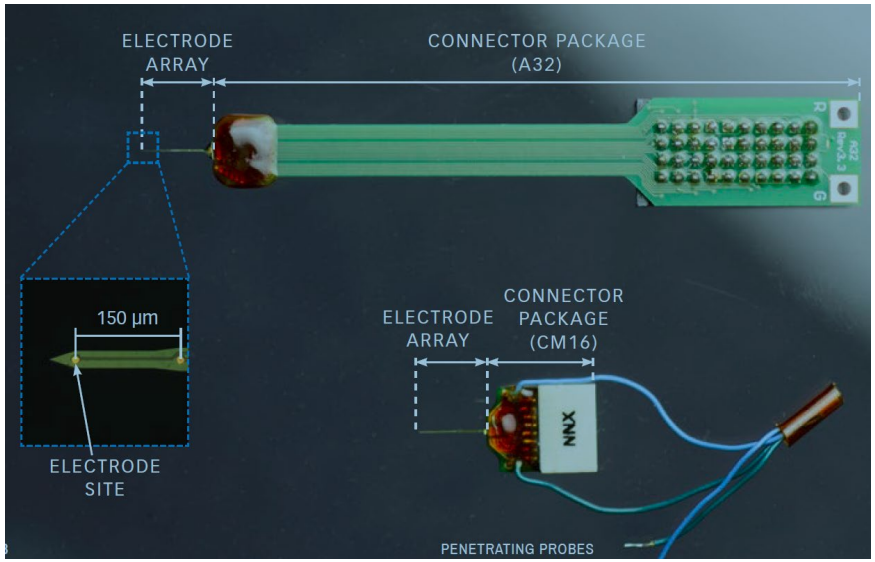
Transiently inhibited

Excited (disinhibited)

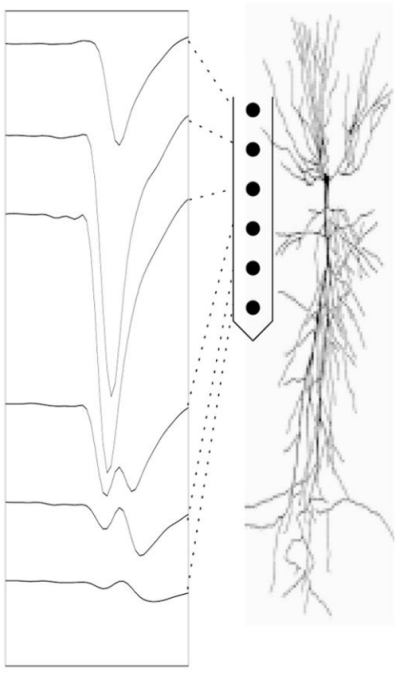
Anikeeva et al., *Nat Neurosci* 2011

- „Directly inhibited" units are most likely those expressing Halorhodopsin, i.e. „identified"

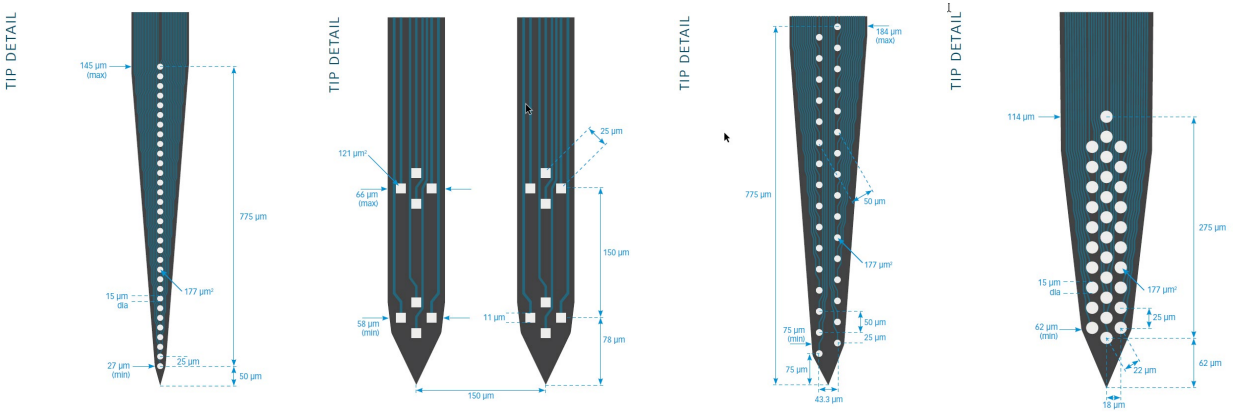
III. Silicone probes: extension of optrode recording technique



Data analysis must take into account the locations (neighborship) of recording sites



Harris et al., *J. Neurophysiol* 2000

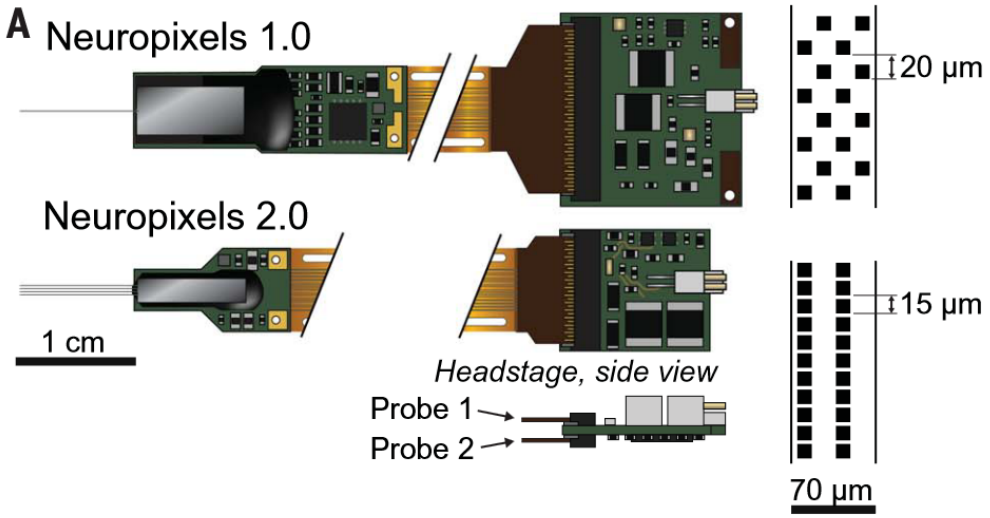


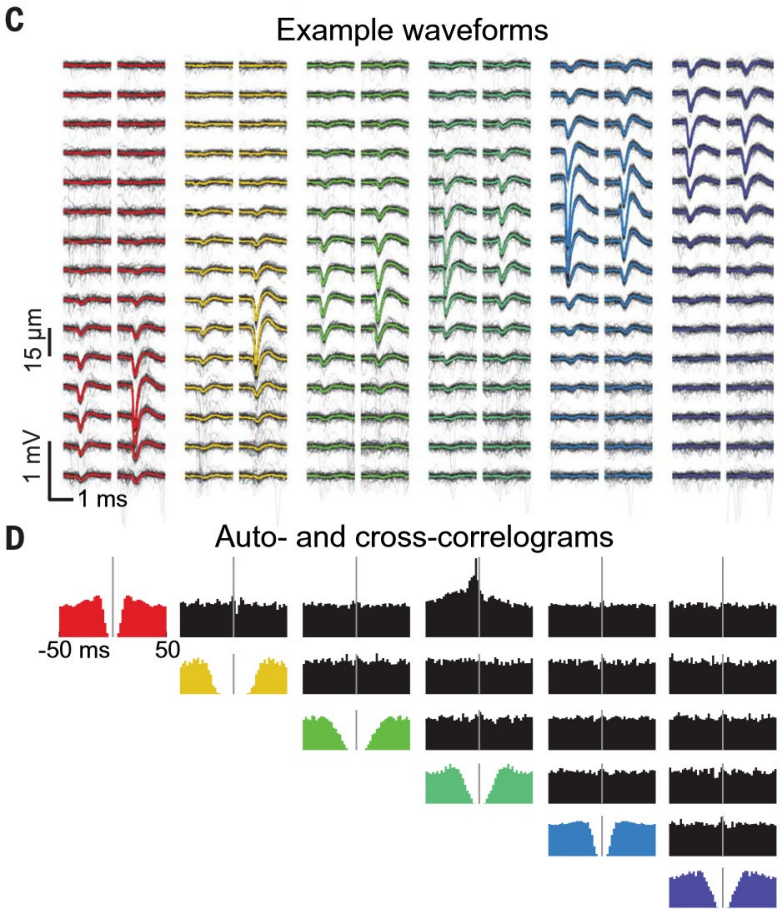
NeuroNexus probes catalog (neuronexus.com)

III. Neuropixels probe: the state-of-the-art high-throughput version of silicone probes

What are the advantage?

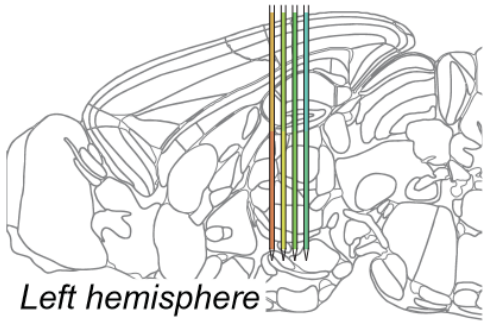
- Classical silicone probes require N parallel amplifier channels, specialized connectors and cables (n=4..256)
- Thus, increased thoughput of recordings led to increased bulkiness of the setup and the price (×N min)
- Neuropixels probes have 384-5120 (!) recording sites, a local pre-amplifier, a multiplexer, and USB interface

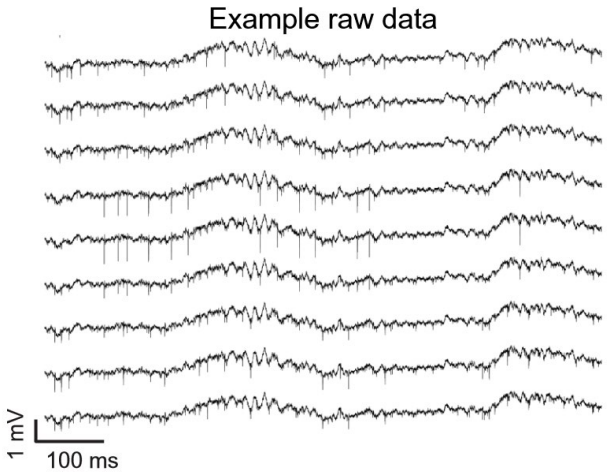




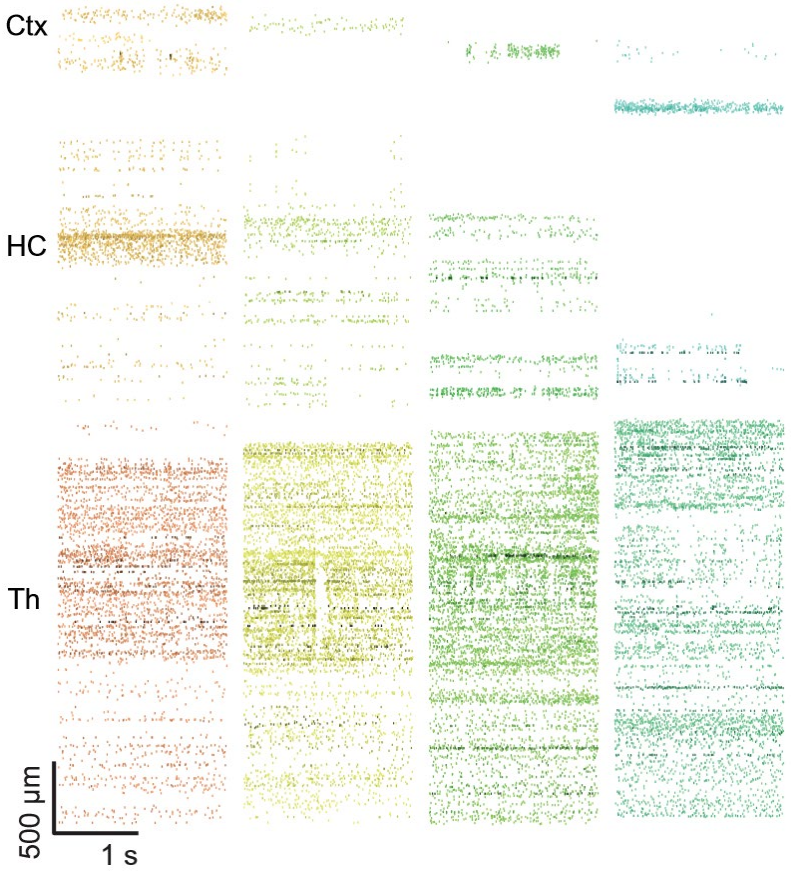
Steinmetz et al., *Science* 2021

III. Neuropixels probe: the state-of-the-art high-throughput version of silicone probes





Neuronal spiking from 6144 sites



Steinmetz et al., *Science* 2021

Some technical facts

- Shank lengths: 10 mm; cross-section 70x24 μm
- Recording sites 12x12 μm made of porous TiN, impedance ~150 kΩ
- Sampling frequency 30 kHz per site; data rate ~23 Mb/s

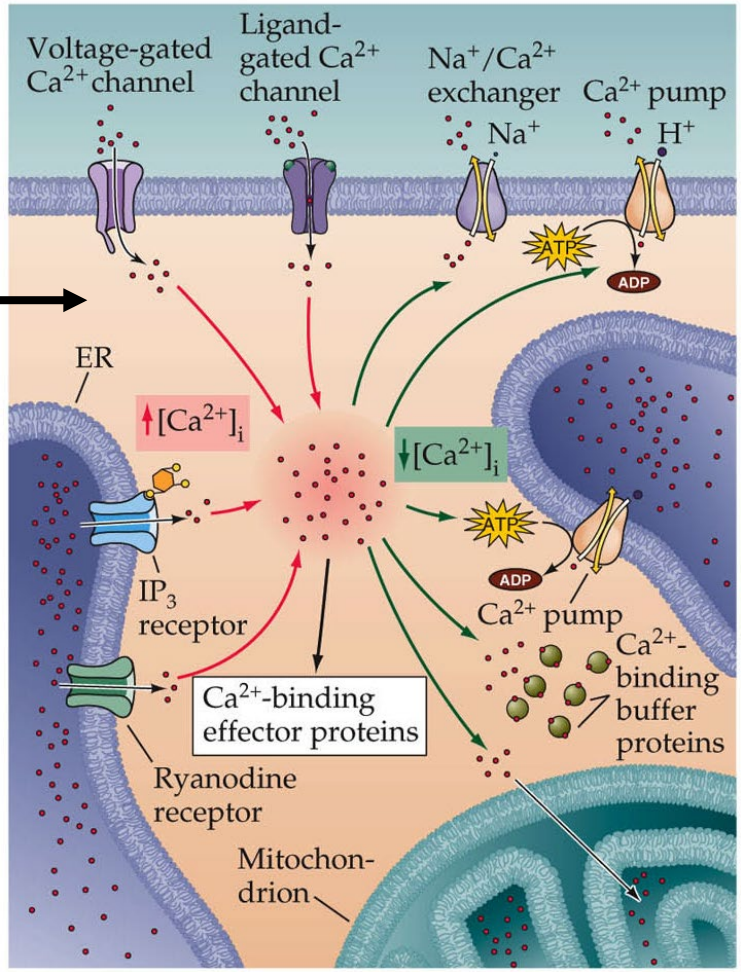
IV. Optical methods of measuring neural circuit activity: GECI

IV. Ca^{2+} dynamics in neuronal cells

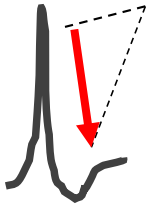
extracellular space

Cytosol
resting $[Ca^{2+}]_i$
~ 50 nM

Intracellular stores & membranes



AP



V_m repolarization
=> main Ca^{2+} influx

1) cell membrane depolarizes upon AP firing (Na^+ channels)

2) depolarization activates Ca-channels

3) Ca^{2+} influx occurs during AP repolarization (K^+ channels) when the driving force for Ca^{2+} influx increases, and while the Ca-channels are still open

4) free cytosolic $[Ca^{2+}]_i$ rises quickly (miliseconds) to ~ tens of μM range (at a peak); Ca^{2+} ions rapidly bind to intrinsic buffer molecules

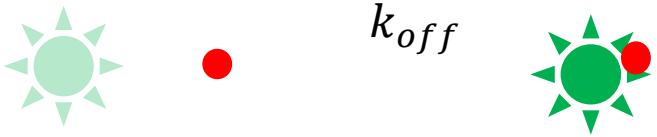
5) after the AP firing ceases, extrusion system slowly (seconds) removes excess Ca^{2+} to reach the resting $[Ca^{2+}]_i$ set point

IV. Ca^{2+} dynamics in neuronal cells

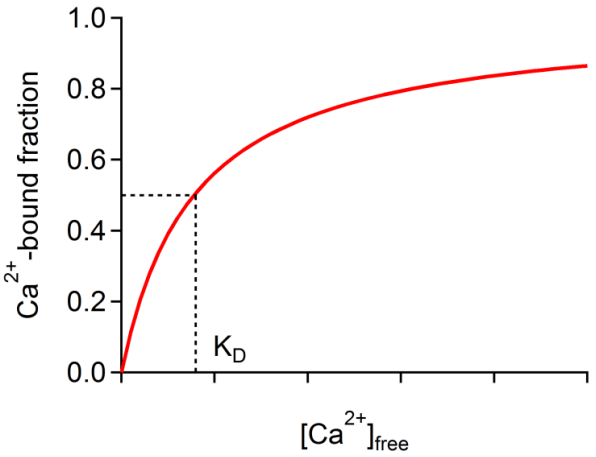
What is the practical significance?

- Increase in free cytosolic $[Ca^{2+}]_i$ is a good correlate of neuronal firing activity
- Fluorescent Ca^{2+} sensors (called [B] here) can be used to indirectly readout the rate of neuronal activity

$$B + Ca^{2+} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} BCa^{2+}$$



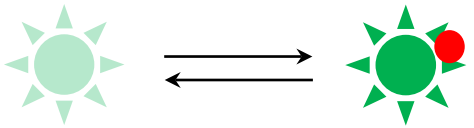
$$[B]_{free} + [BCa^{2+}] = [B]_{tot}$$



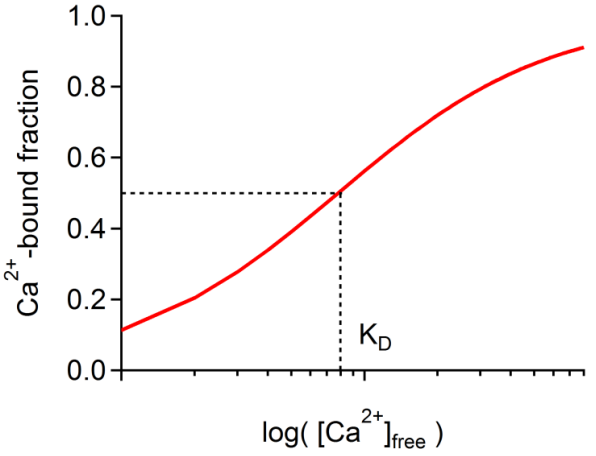
Equlilibrium Ca^{2+}-bound fraction (α) of the dye:

$$\alpha = \frac{[BCa^{2+}]}{[B]_{tot}} = \frac{[Ca^{2+}]_{free}}{[Ca^{2+}]_{free} + K_D}; \left(K_D = \frac{k_{off}}{k_{on}}\right)$$

Fluorescence readout:

f_0 (dark)  f_1 (bright)

$$F \cong ((1-\alpha)\cdot f_0 + \alpha \cdot f_1)\cdot [B]_{tot}$$

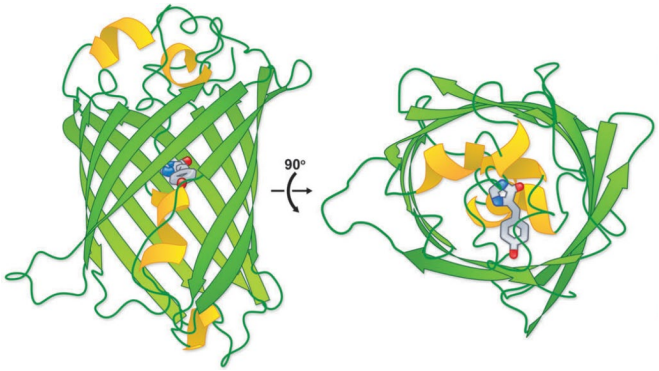


IV. Genetically Encoded Calcium Indicators (GECI)

Why GECI (and not small-molecule synthetic indicators) are particularly suitable for *in-vivo* studies?

- GECI can be expressed (e.g. by viruses) in genetically defined cell populations
- GECI are naturally biocompatible, show long-term stability, low toxicity, good fluorescent properties etc.

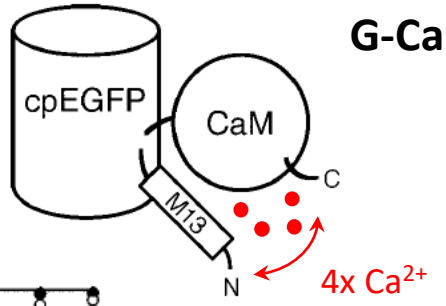
Aequoria victoria

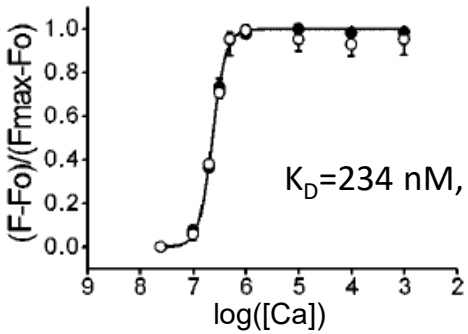


Örmö et al., *Science* 1996; Yang et al. *Nat Biotech* 1996

M13: a fragment of myosin light chain kinase
cpEGPF: circularly permuted EGFP moiety
CaM: calmodulin

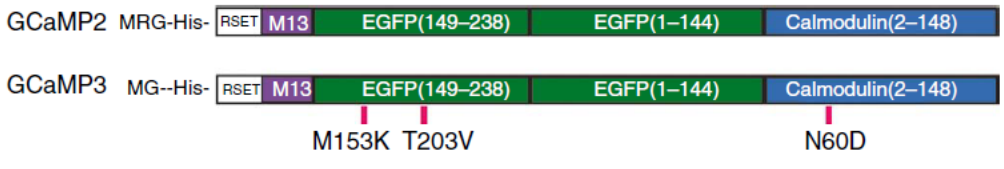
G-CaMP





Nakai et al., *Nat Biotechnol* 2001

IV. Targeted evolution of GCaMP GECI

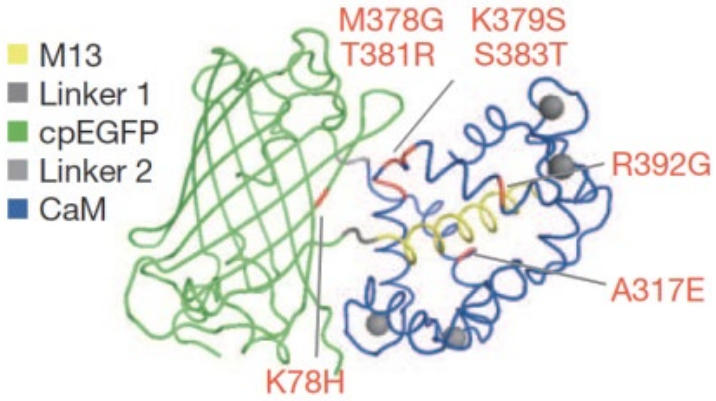


	cpEGFP	CaM			
GCaMP6s	K78H		T381R	S383T	R392G
GCaMP6m		M378G K379S	T381R	S383T	R392G
GCaMP6f		A317E	T381R	S383T	R392G

- **dynamic range**
- **brightness**
- **Ca^{2+} affinity (K_D) & kinetics**

Sensor	Dynamic range (F_{max}/F_{min})	K_d (nM)	Hill coefficient	k_{off} (s^{-1})
GCaMP3	13.5±0.7	345±17	2.54±0.04	2.57
GCaMP5G	45.4±0.9	447±10	2.46±0.04	2.52
GCaMP6s	63.2±3.1	144±4	2.90±0.17	1.12
GCaMP6m	38.1±1.8	167±3	2.96±0.04	2.06
GCaMP6f	51.8±2.8	375±14	2.27±0.10	3.93

GCaMP6 family *(nowadays, GCaMP7 and GCaMP8 are available)*



Tian et al., *Nature* 2009; Akerboom et al., *J Neurosci* 2012; Chen et al., *Nature* 2013

IV. GCaMP signals in AP firing neurons

Sensor	Dynamic range (F_{max}/ F_{min})	K_d (nM)	Hill coefficient	k_{off} (s^{-1})
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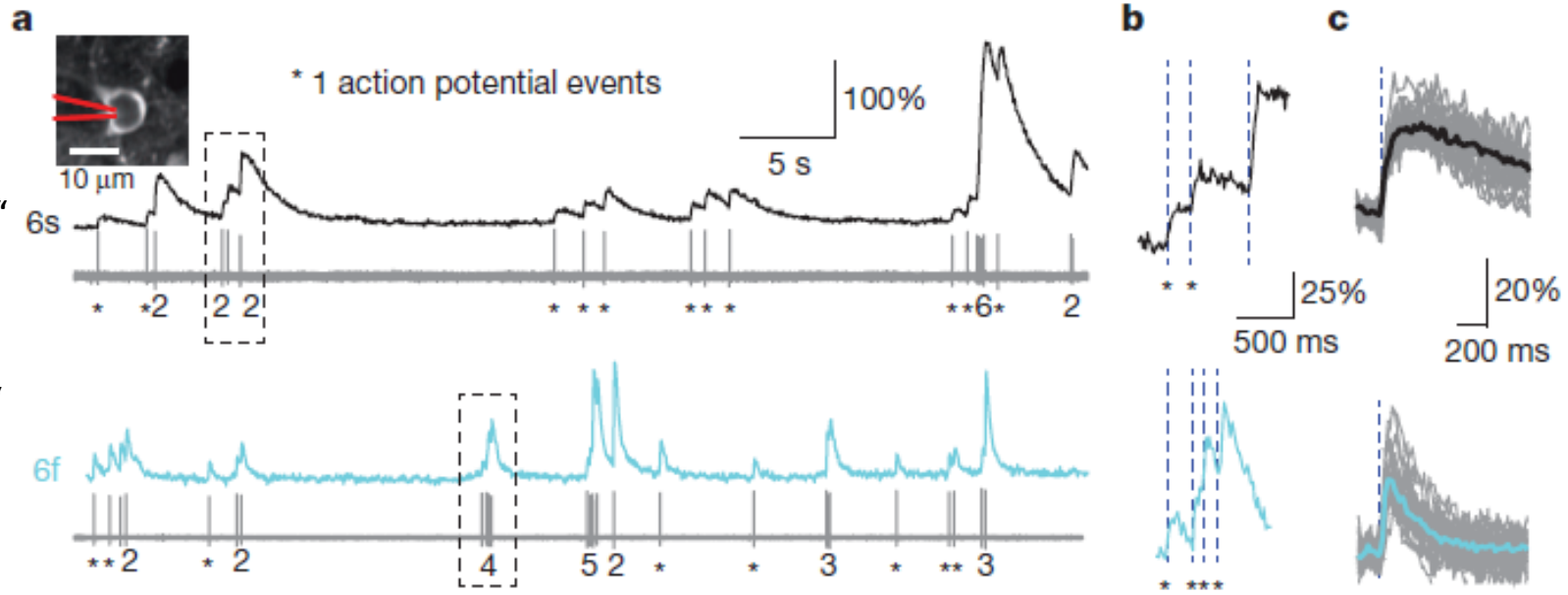
GCaMP6m	38.1±1.8	167±3	2.96±0.04	2.06
GCaMP6f	51.8±2.8	375±14	2.27±0.10	3.93

GCaMP6s „slow“ (K_D=144 nM)

GCaMP6f „fast“ (K_D=375 nM)



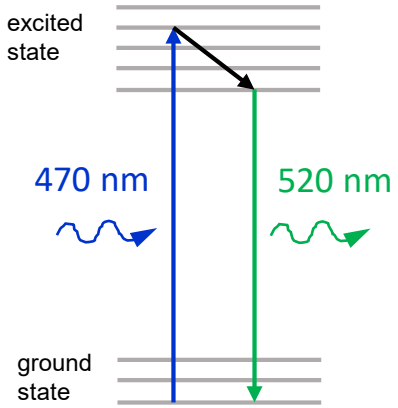
Tian et al., *Nature* 2009; Akerboom et al., *J Neurosci* 2012; Chen et al., *Nature* 2013

IV. Optical methods of measuring neural circuit activity: imaging techniques

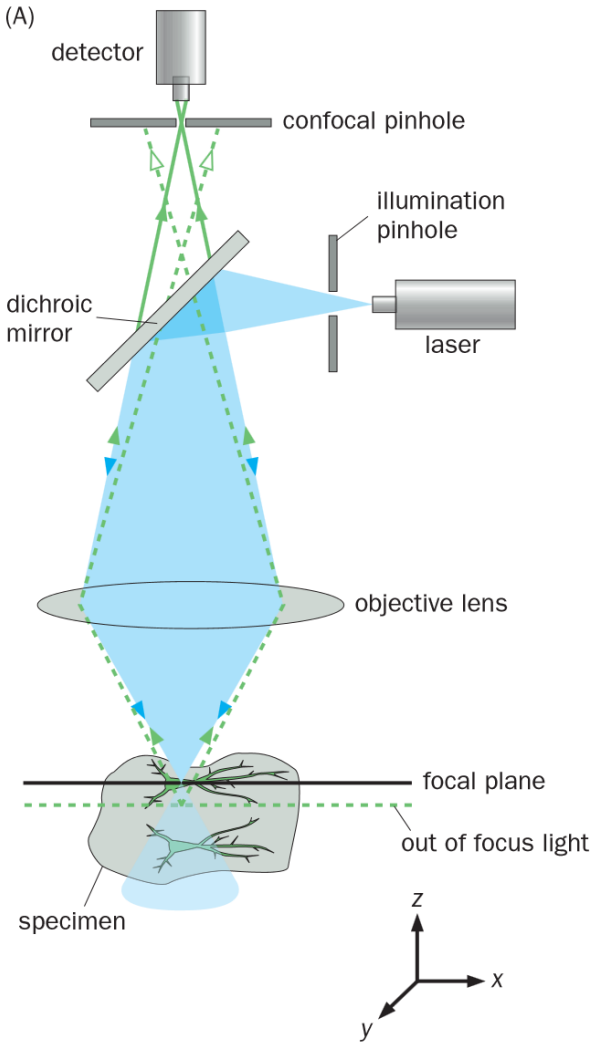
IV. Single-photon fluorescent microscopy

Single-photon excitation process

- Easy to implement and cheap
- Excitation light scatters a lot (problem for deep >200μm imaging)
- A lot of out-of-focus fluorescence out-of-focus bleaching

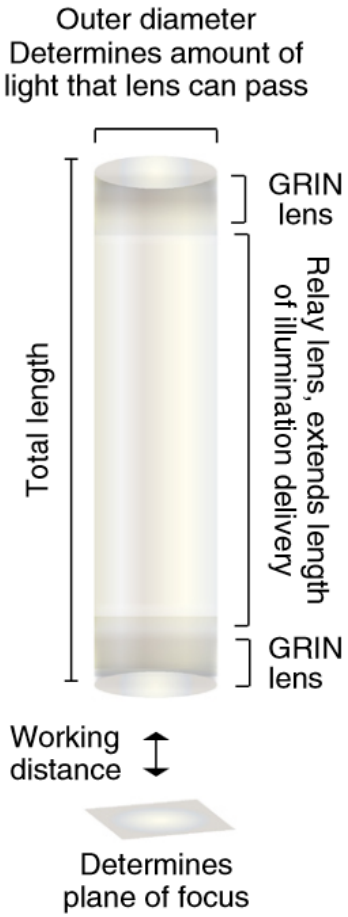


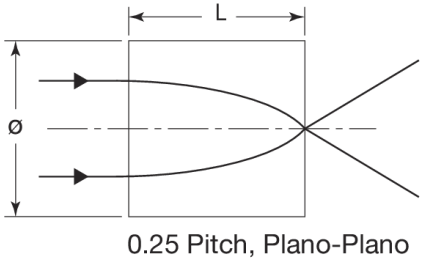
Jablonski A. *Nature* 1933

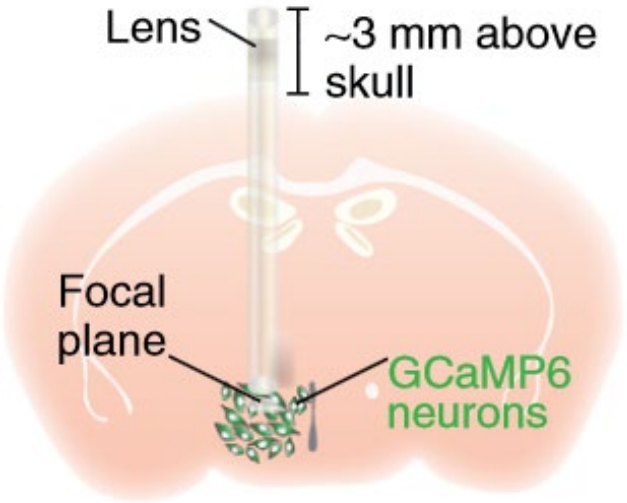


Liqun Luo, *Principles of Neurobiology*, 2015

IV. Deep brain Ca^{2+} imaging using microendoscopes





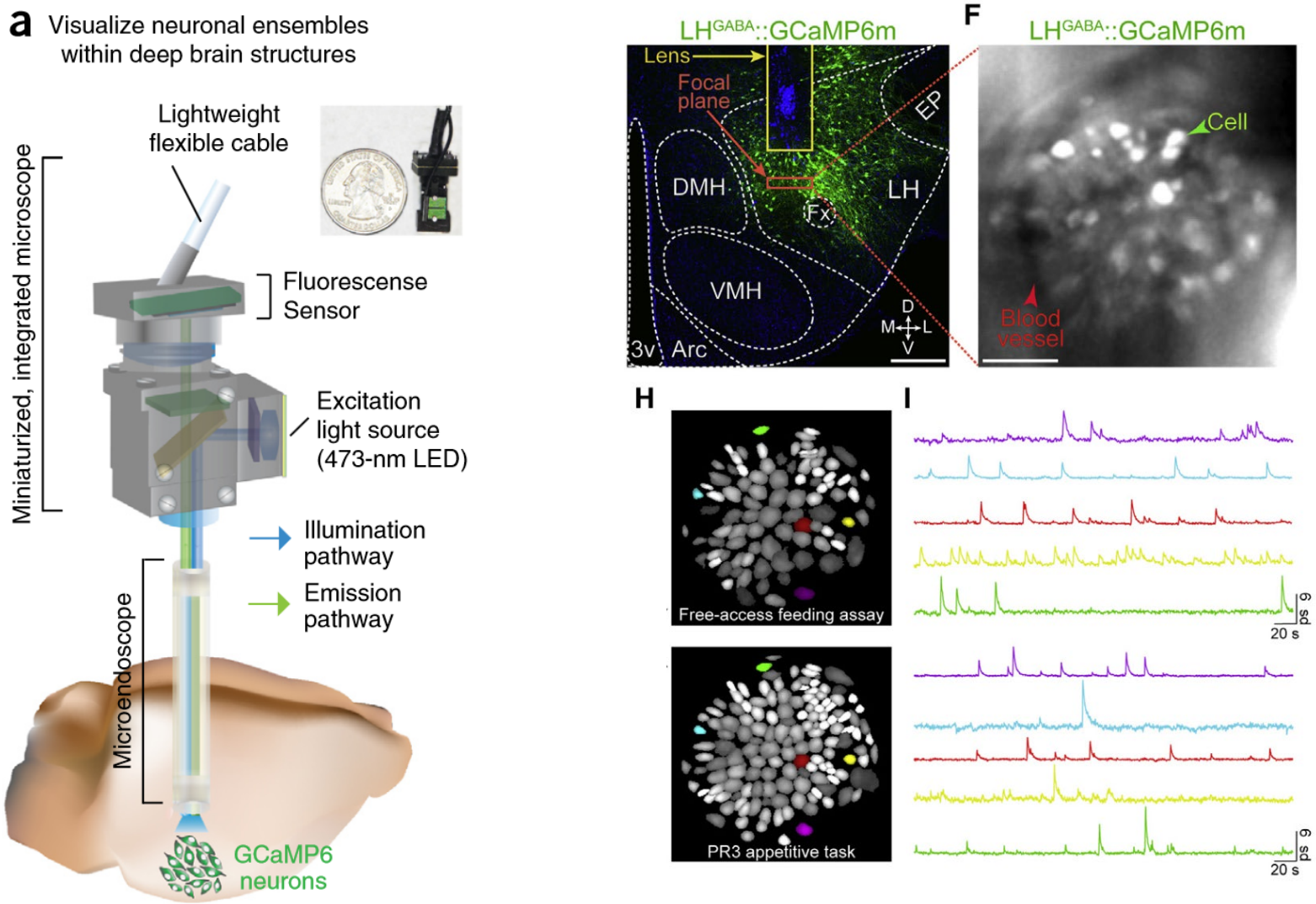


Gradient **R**efractive **IN**dex

GRIN lens

Resendez et al., *Nature Protocols*, 2016

IV. Deep brain Ca^{2+} imaging using microendoscopes

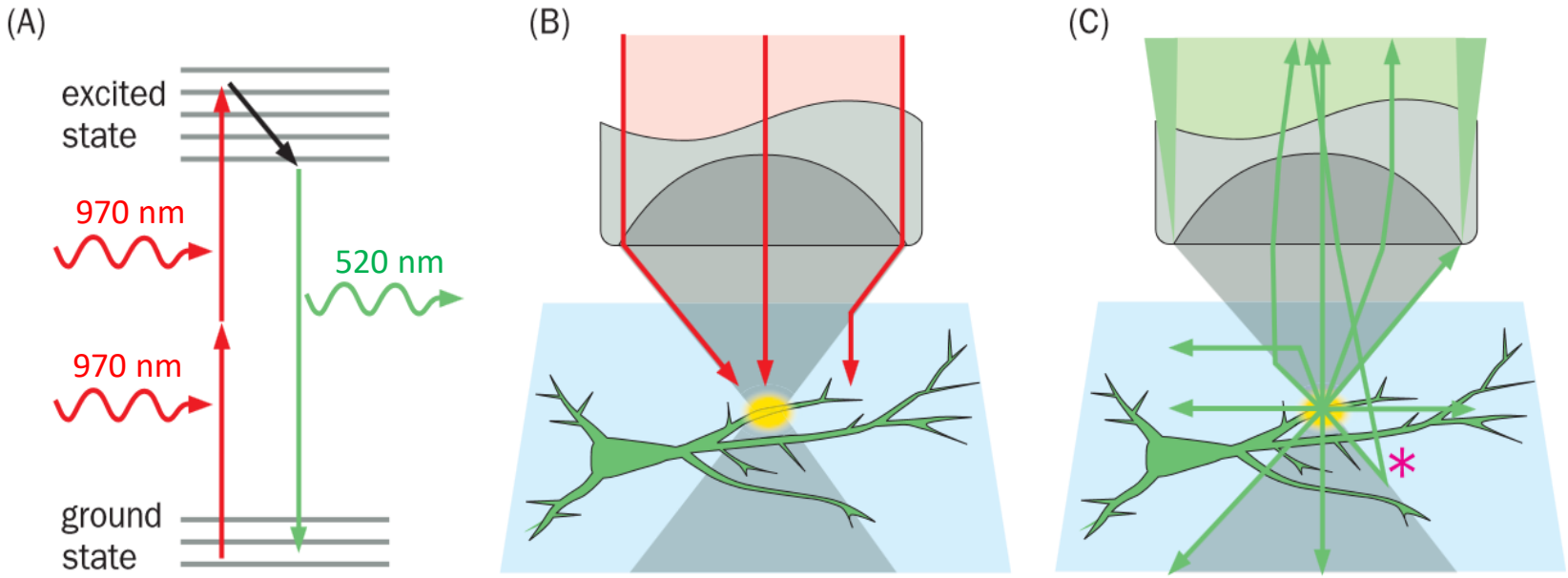


Ghosh et al., *Nat Methods*, 2011; Resendez et al., *Nat Protocols*, 2016; Jennings et al., *Cell*, 2015

IV. Two-photon fluorescent microscopy

Two-photon excitation process

- Excitation is restricted to a single spot (0.6 μm ⟷ , 4.2 μm ↕ FWHM)
- Near infra-red excitation light penetrates deep into tissue without much scattering
- Allows wide (mesoscale) imaging of hundreds of neurons located up to 1 mm deep into the brain
- (-) Relatively technically complex and expensive to implement, but
- (-) Most often limited to fixed-head applications



Liqun Luo, *Principles of Neurobiology*, 2015

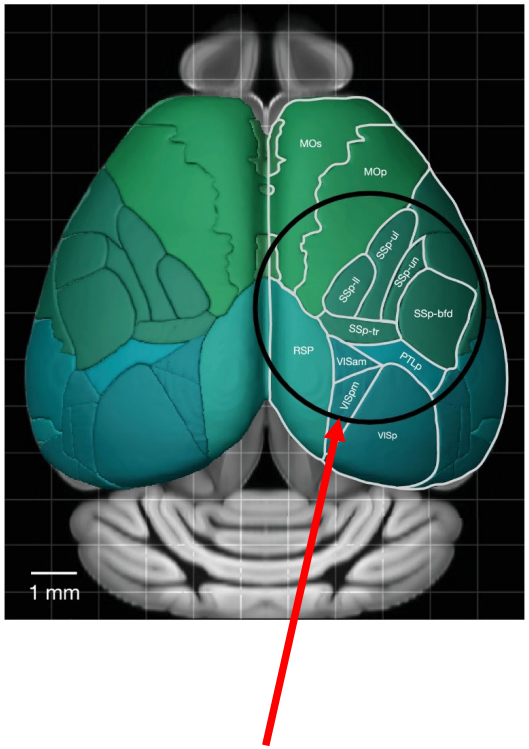
IV. Two-photon fluorescent microscopy for Ca^{2+} imaging

Mouse strain: GP5.17

(Thy1::GCaMP6f)

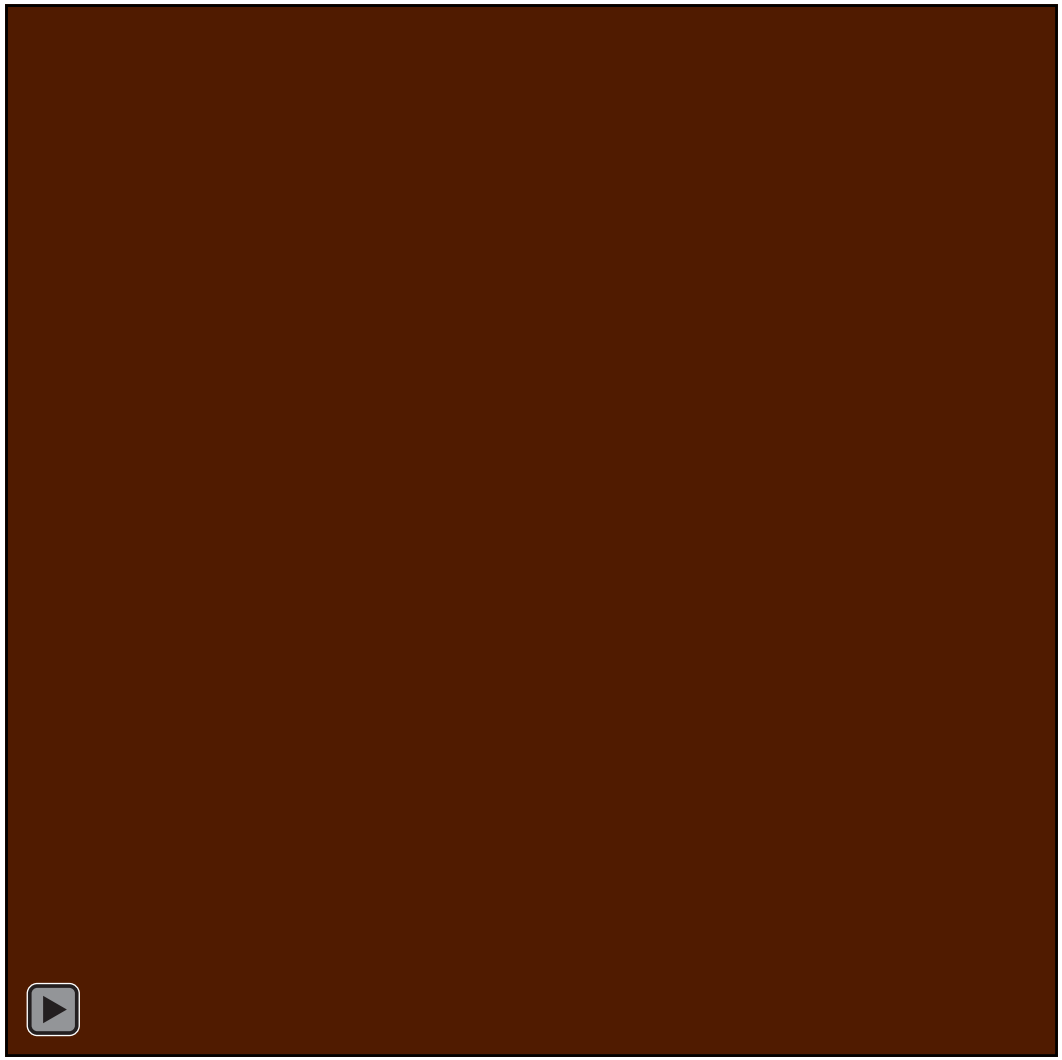
=> GCaMP6f expression in

excitatory cortical neurons



field of view in D-shaped cranial window

(= bone removed, replaced by glass)



Sofroniew et al., *eLife*, 2016 (Svoboda lab)

Background reading

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8. Resendez, S.L., Jennings, J.H., Ung, R.L., Namboodiri, V.M., Zhou, Z.C., Otis, J.M., Nomura, H., McHenry, J.A., Kosyk, O., and Stuber, G.D. (2016). **Visualization of cortical, subcortical and deep brain neural circuit dynamics during naturalistic mammalian behavior with head-mounted microscopes and chronically implanted lenses**. *Nat Protoc 11*, 566–597. 10.1038/nprot.2016.021.