Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Drosophila toolbox II

Advanced genetic techniques to manipulate neuronal activity and to crack synaptic & modulatory neuronal circuits

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Am Hubland

In the lecture Drosophila toolbox II today, I'd like to......

- inform you about cutting edge techniques in genetic manipulation of the nervous system and neuronal tracing

- explain the mode of action of genetic tools for neuronal manipulation and tracing

- show the power of combining genetic with optical techniques (microscopy) to understand the brain

- demonstrate the usefulness, power and beauty of the *Drosophila* brain as a genetically tractable model in neuroscience

Learning outcomes:

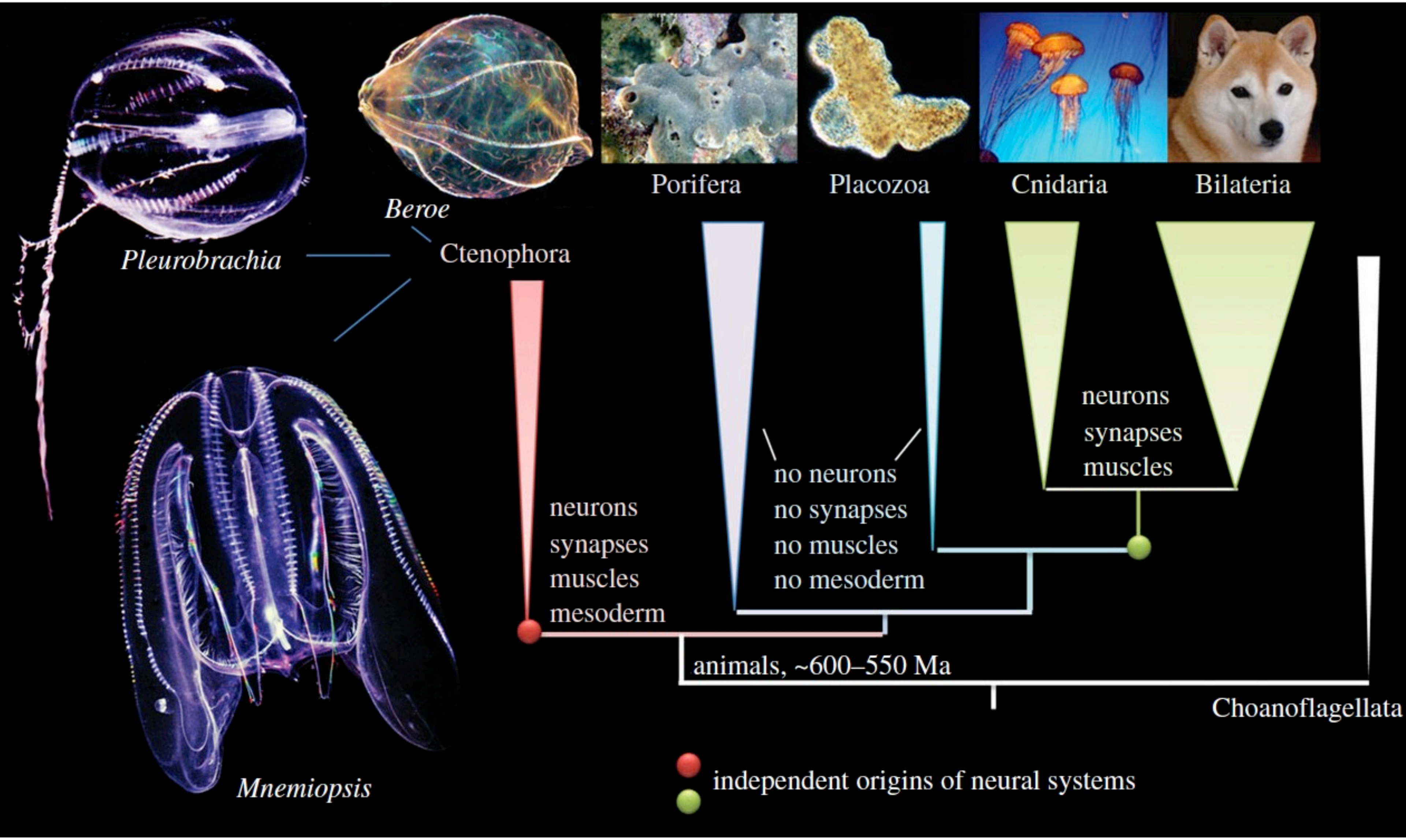
- you should understand the working principles of binary expression systems
- you should understand the principles of genetic manipulation of neuronal activity
- you should understand the principles of genetic tracing of neuronal circuits
- you should understand the principles of genetically encoded activity sensors

- don't bother too much about the applied examples, they just serve to give biological context

Why have brains evolved?
What is the basic function of a brain?

The main function of brains is to coordinate behaviour!

The nervous system may have been invented twice during evolution



Moroz & Kohn 2016 Phil T Roy Soc

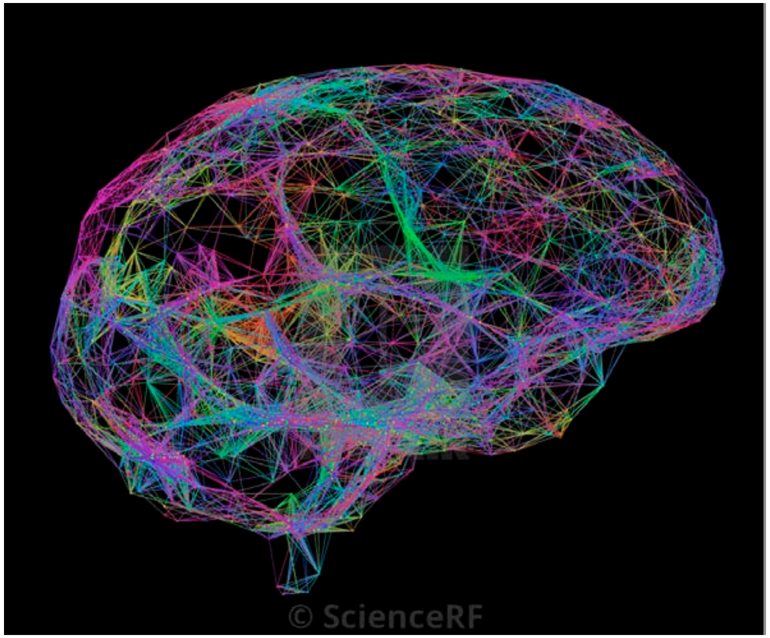
How does the brain control behaviour?

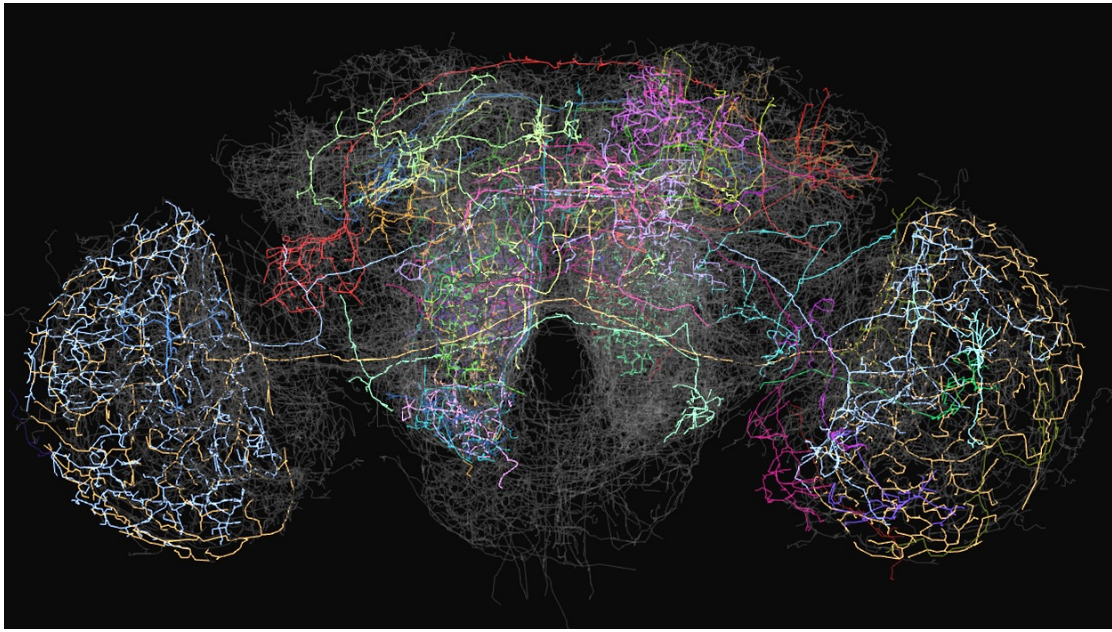
genes
↓
neurones
↓
neuronal networks
↓
brain
↓
effectors
↓
behaviour

How does the brain control behaviour?

genes	*molecular level*
↓ neurones ↓ neuronal networks ↓ brain	*cellular level*
↓ effectors	*organismic level*
↓ behaviour	*evolutionary-ecological level*

Understanding the brain requires understanding of neuronal circuits





PhD project offer in sociology: Describe the complete personal networks within a defined society – 2 options, 4 years time

Würzburg

120.000 inhabitants

- one mayor
- few clubs
- one Indian restaurant
- one University
- one major basketball team

Earth

8.000.000.000 inhabitants

- 1.000.000+ mayors
- 1.000.000+ clubs
- 1.000.000+ Indian restaurant
- 10.000+ Universities
- 100.000+ major basketball teams

Scientific project offer in neurobiology: Describe the complete neuronal network within a brain– 2 options, 10+ years time

brain of a fruit fly
120.000 neurons

brain of a human
20.000.000.000 neurons

- keeping fruit flies is very cheap and easy
- flies offer an outstanding genetic toolbox
- flies typically have „one neuron for each job“
- for most genes, flies have only one copy
- flies have to perform complex behaviours and can fulfill amazing tasks

The small brain of a fly controls sophisticated behaviours

sleep, feeding
social interaction

courtship, mating and aggression

defensive and escape behaviour

flight control and navigation

Content of this lecture:

- connectomics
- modern genetic techniques to dissect neuronal connectivity
- modern genetic techniques to image neuronal activity
- modern genetic techniques to probe the functionality of neuronal connectivity
    - constitutive silencing and activation
    - conditional silencing and activation
- methods to narrow down genetic manipulation to “single“ neurons

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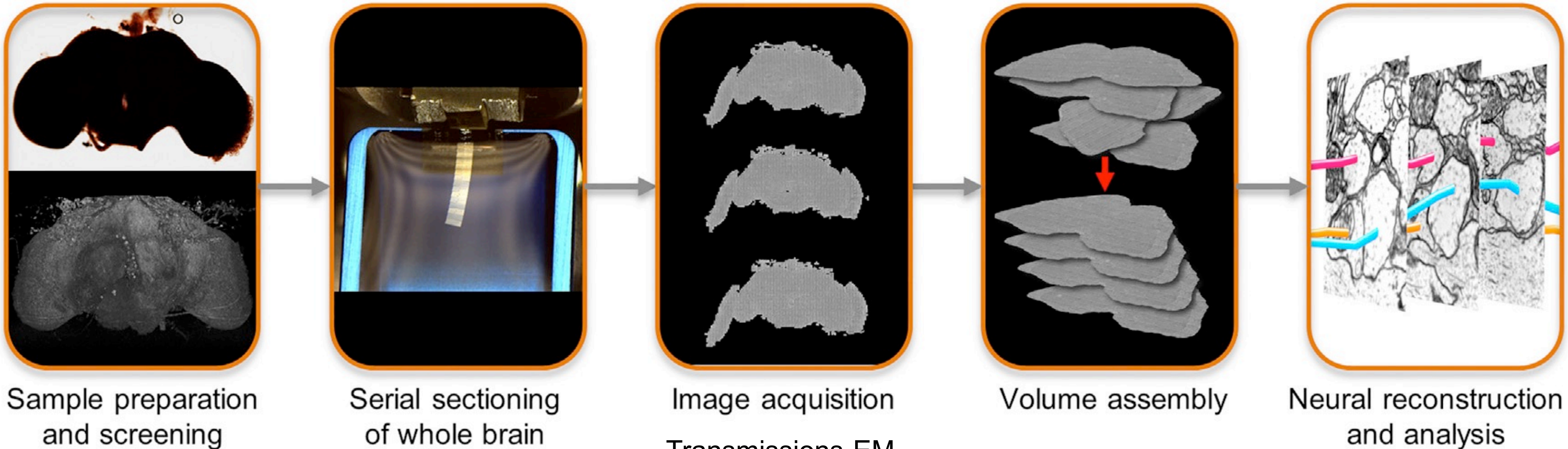
Connectomics

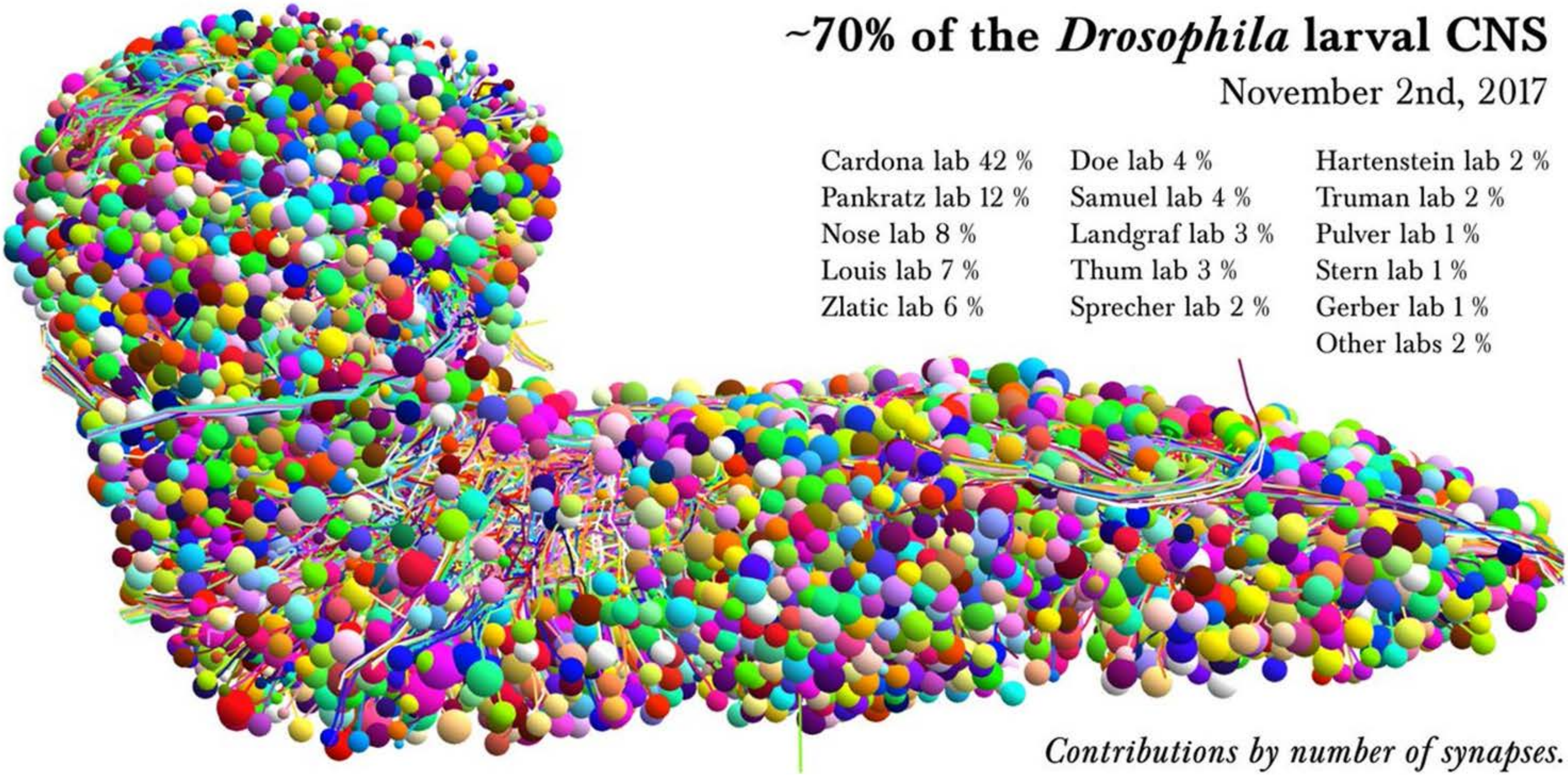


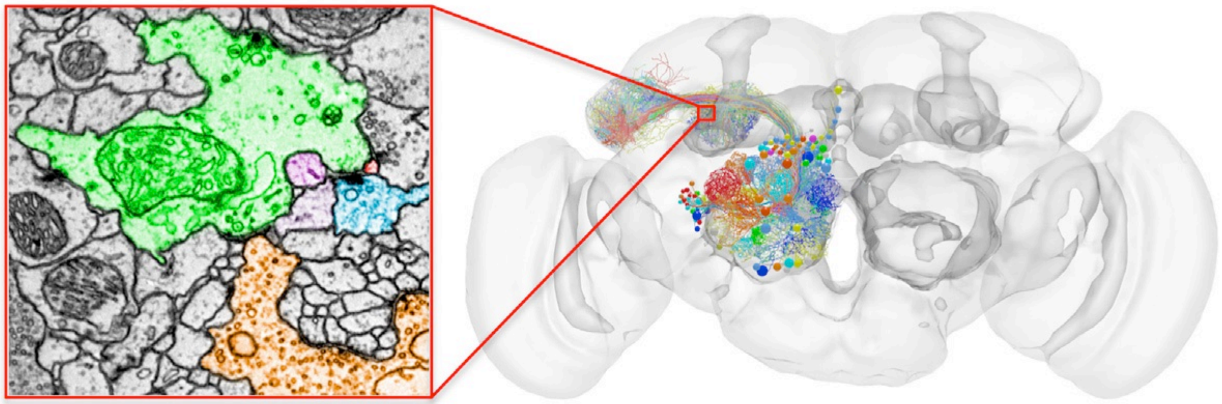
Transmissions-EM
(serial blockface-EM)

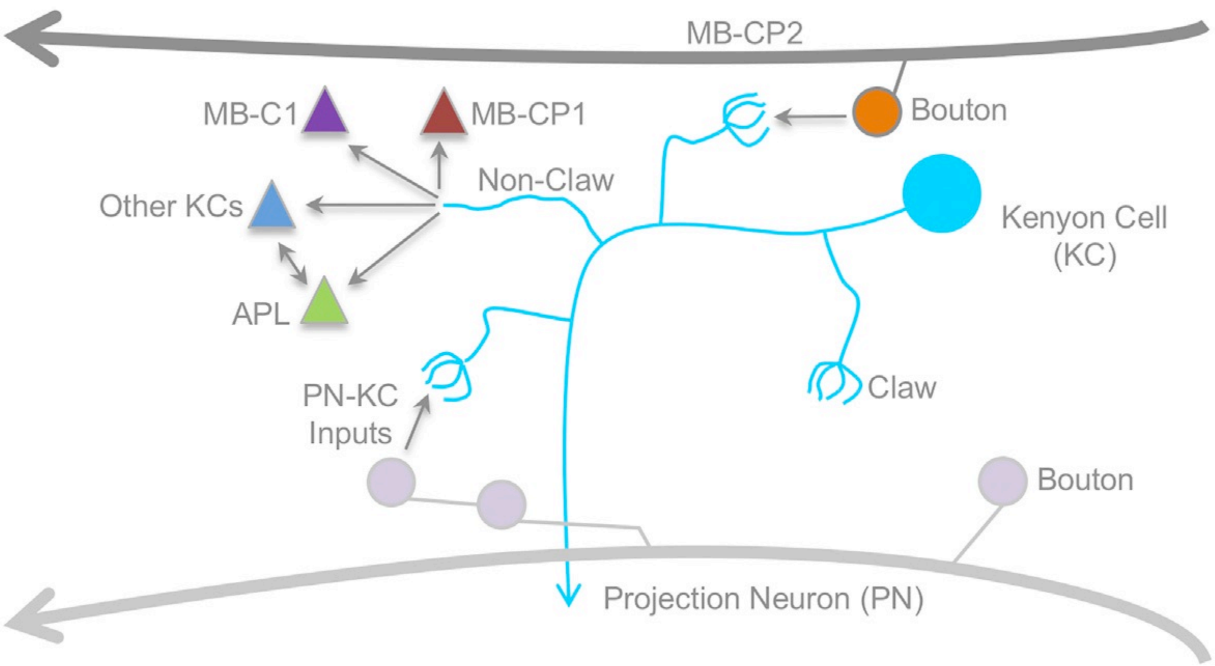
Zheng et al. 2018 Cell

- 1986 connectome for *C. elegans* (302 neurons)
- 2018 connectome for the larval CNS of *Drosophila* (ca 20.000 Neurone)
- 2020 connectome for the brain/hemibrain of *Drosophila* (ca. 135.000 Neurone)
- connectome for parts of the zebrafish/mouse brain are in progress

Connectomics of the larval fruit fly CNS

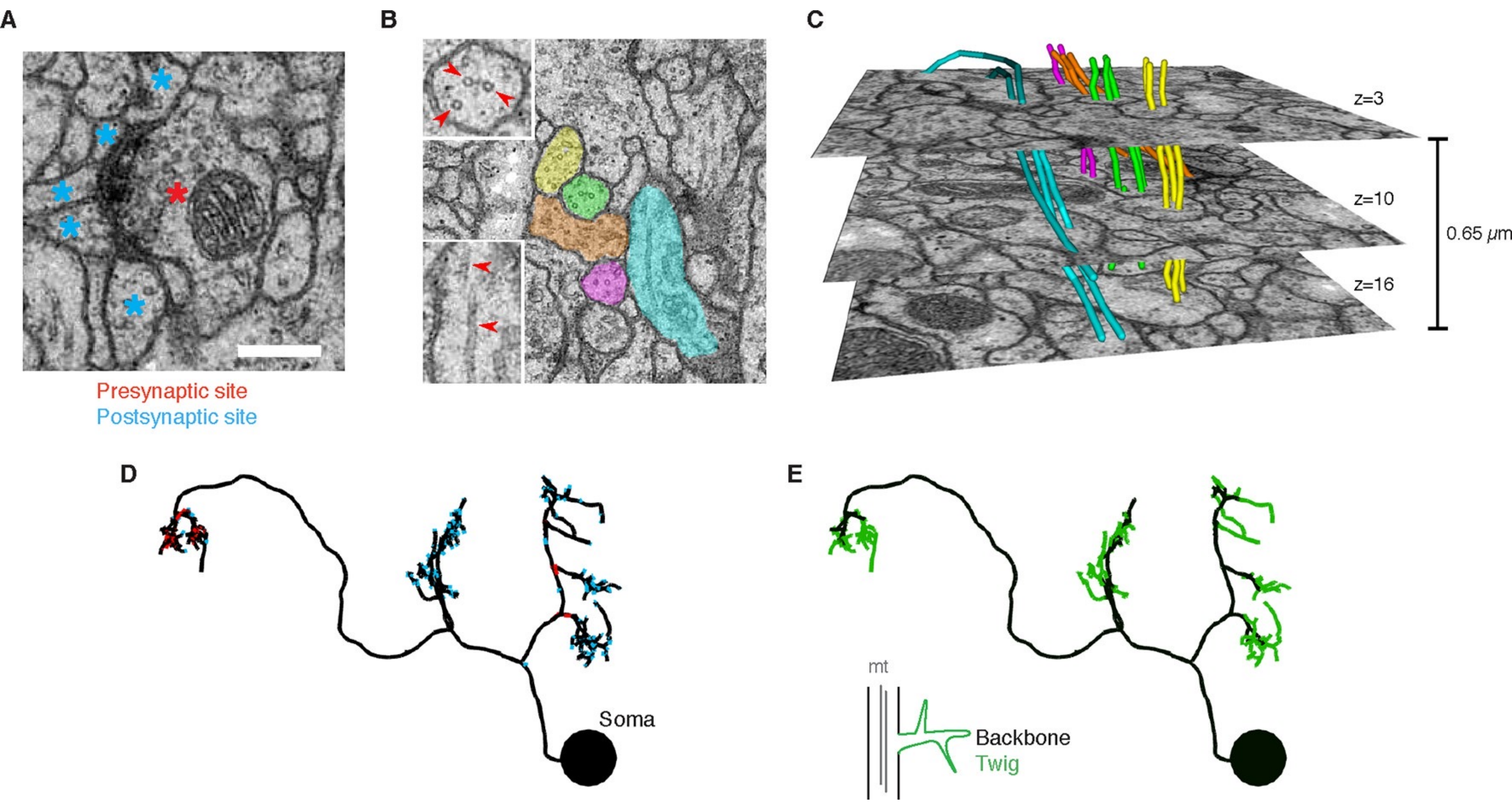






Zheng et al. 2018 Cell

serial reconstructions lead to skeleton models



Schneider-Mizell et al. 2016 eLife

serial reconstructions lead to skeleton models

If you want to check it out:

https://eyewire.org/explore

https://neuprint.janelia.org

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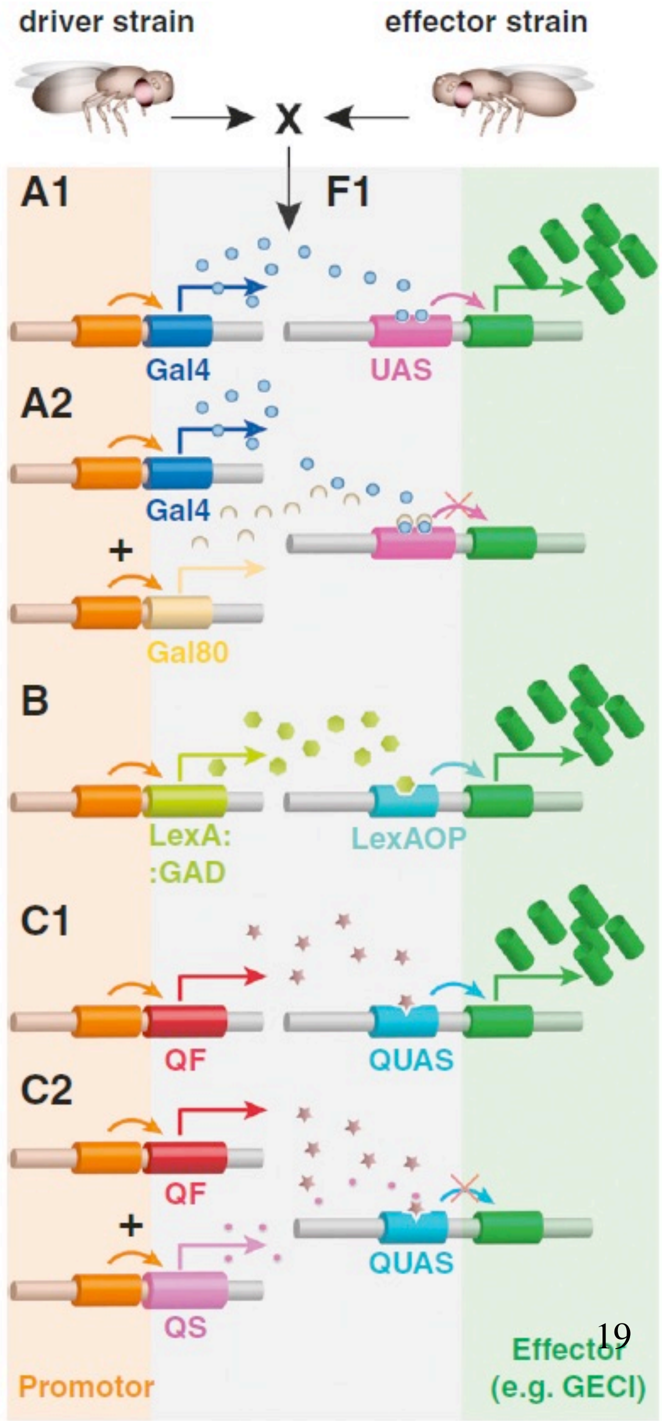
Orthogonal expression systems – expanding the GAL4-UAS-system



Riemensperger et al. (2012) Biochim Biophys Acta

Genetic techniques to dissect neuronal circuits

Q:
What are in- and outputs of neuron X/circuit Y?
Which neurons are pre- and postsynaptic of neuron X/circuit Y?

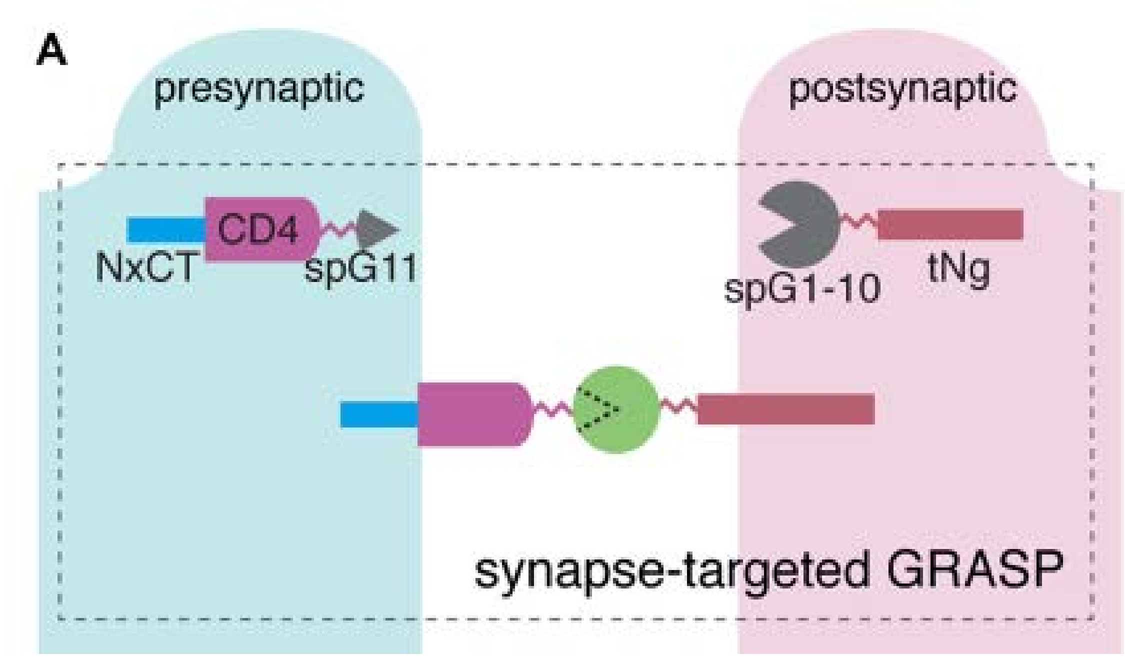
genetically trace the anatomy, connectivity and activity of gene X/ circuit Y

measure using appropriate confocal microscopy methods

Genetic analysis of synaptic connectivity: GRASP

GRASP = GFP Reconstitution Across Synaptic Partners

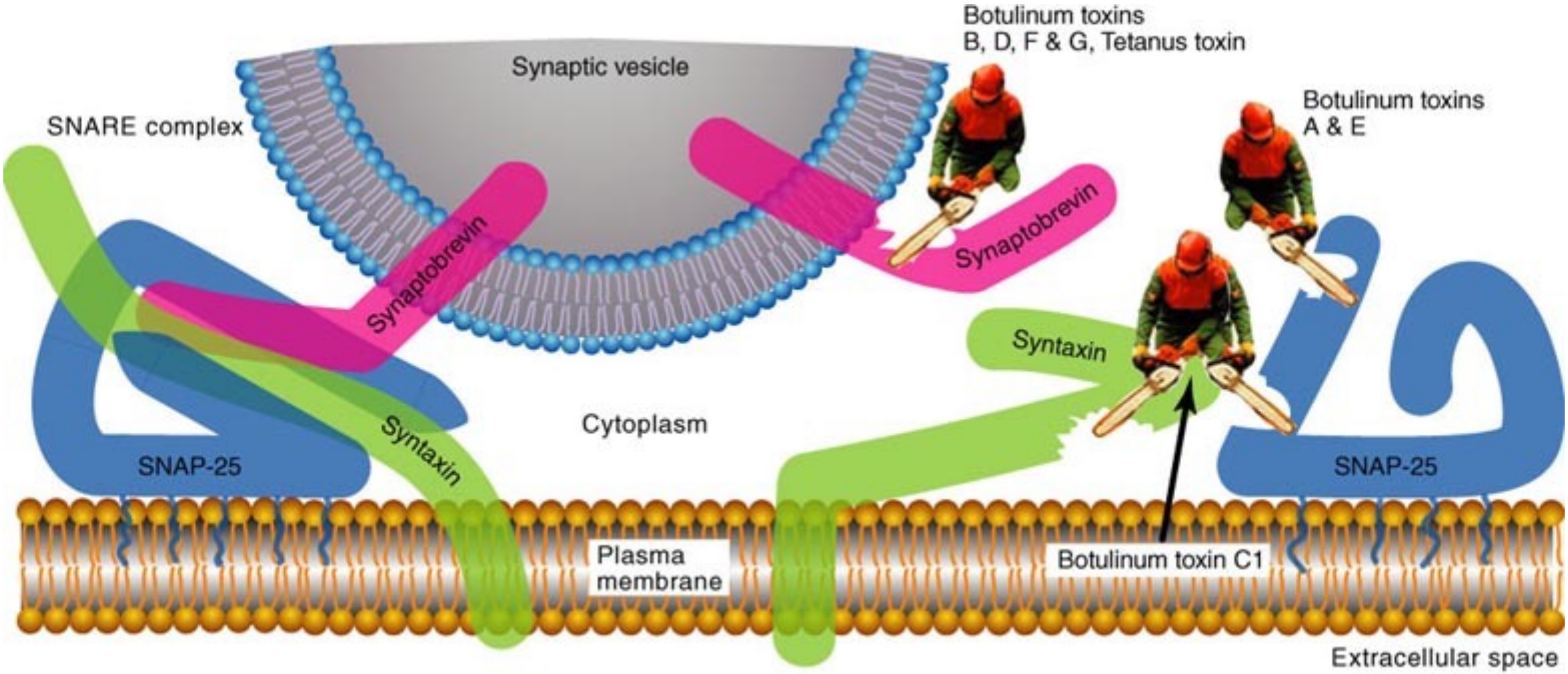
- based on two parts of the GFP molecule: splitGFP1-10 und splitGFP11
- first developed for *C. elegans*, also used in *Drosophila*, zebrafish and mouse



activity-independent
GRASP

Excursion: SNARE proteins and synaptic vesicle fusion

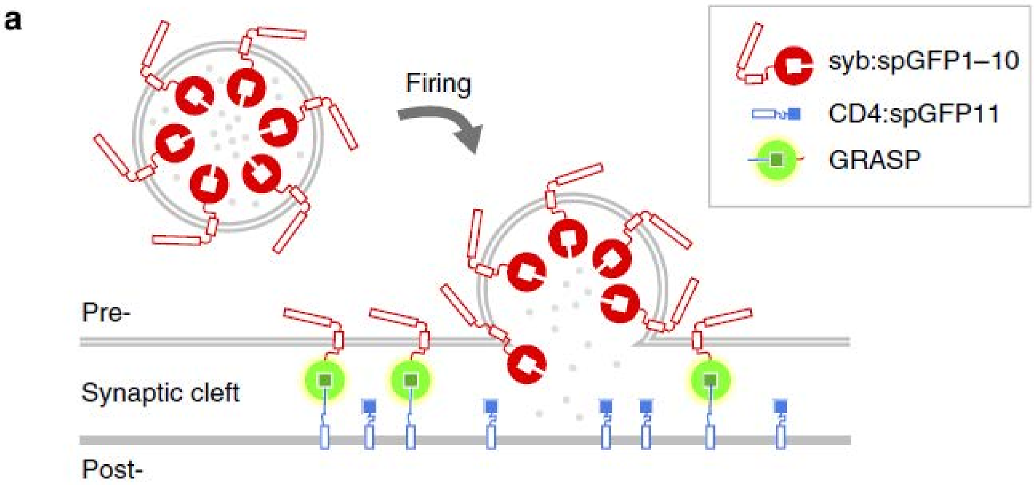
- vesicular (v) & “target“-membrane bound (t) SNARE proteines are essential for membrane fusion during synaptic vesicle release



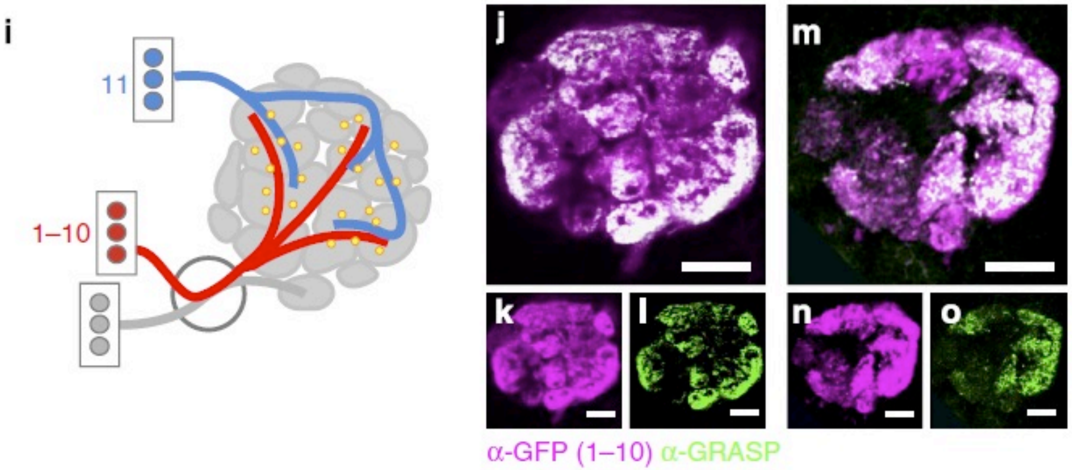
Genetic analysis of synaptic connectivity: syb-GRASP

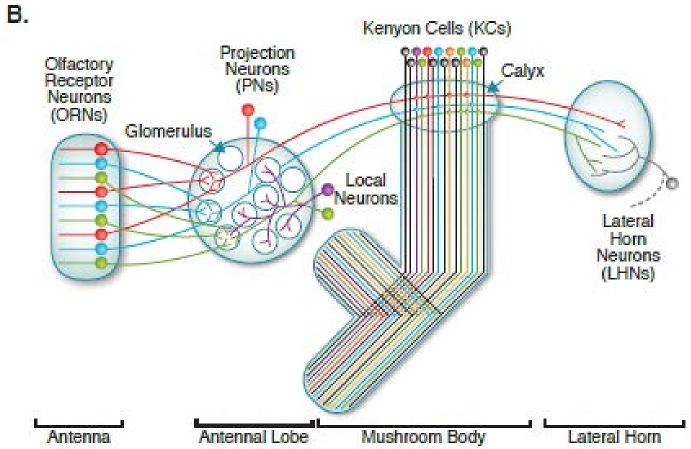
<u>two components:</u>

- Synaptobrevin-splitGFP1-10 (syb:spGFP1-10)
- CD4-splitGFP11 (CD4:spGFP11)



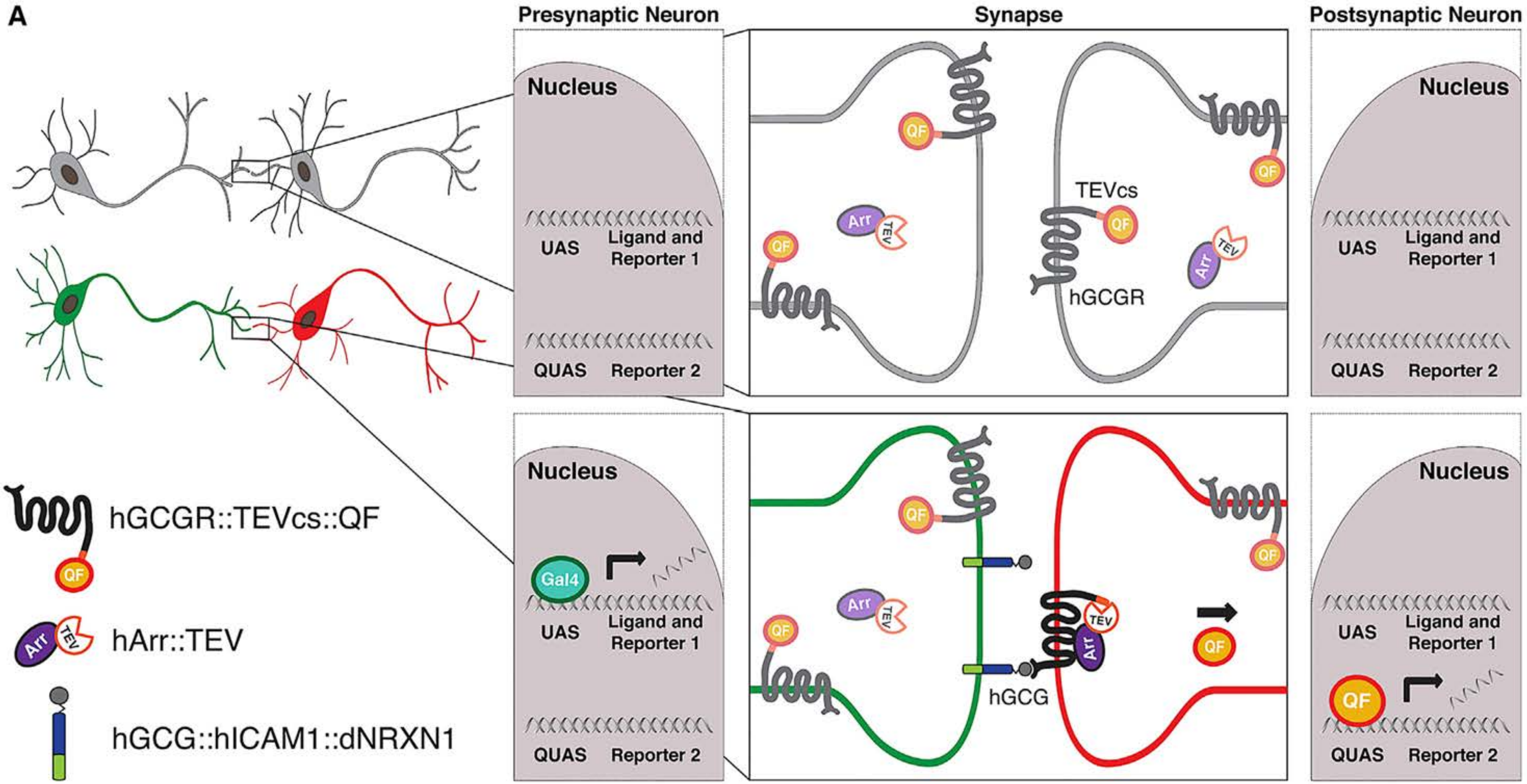
activity-dependent
GRASP





Macpherson et al. 2015 Nature Commun

Trans-tango – genetic tracing of pre- to post-synaptic connections

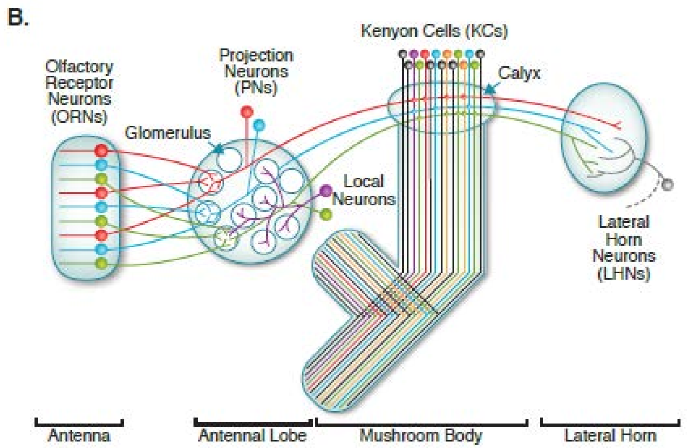


TEV= N1a protease of the tobacco etch virus
hArr = human β-arrestin2
hGCG = human glucagon
reporter 1 = GFP

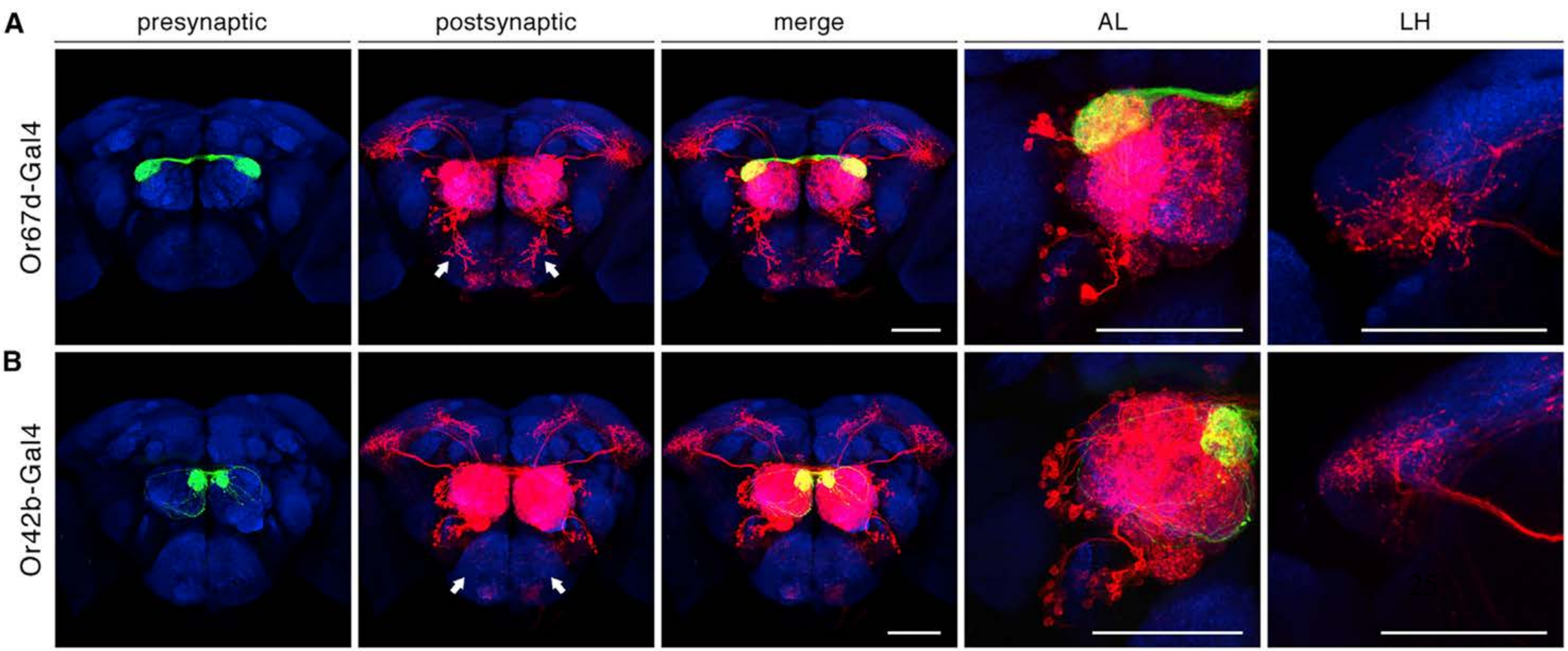
hICAM1 = human cell-adhesion meolcuel
dNRXN1 = *Drosophila* synaptic protein (neurexin)
hGCGR = human glucagon receptor
reporter 2 = mCherry (an RFP variant)

Talay et al. 2017 Neuron

Trans-tango – genetic tracing of pre- to post-synaptic connections

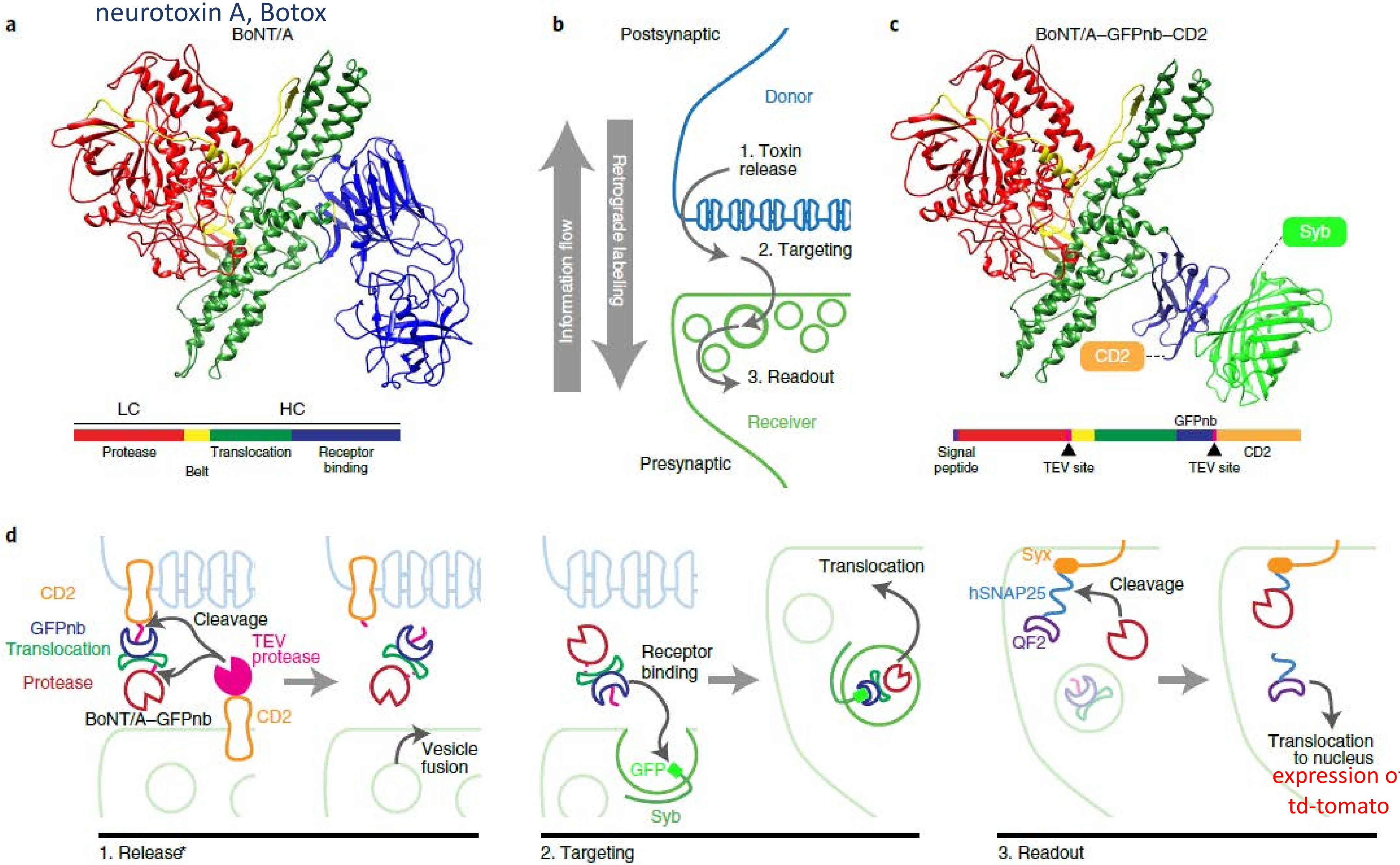


Talay et al. 2017 Neuron



BAcTrace – genetic tracing of post- to pre-synaptic connections

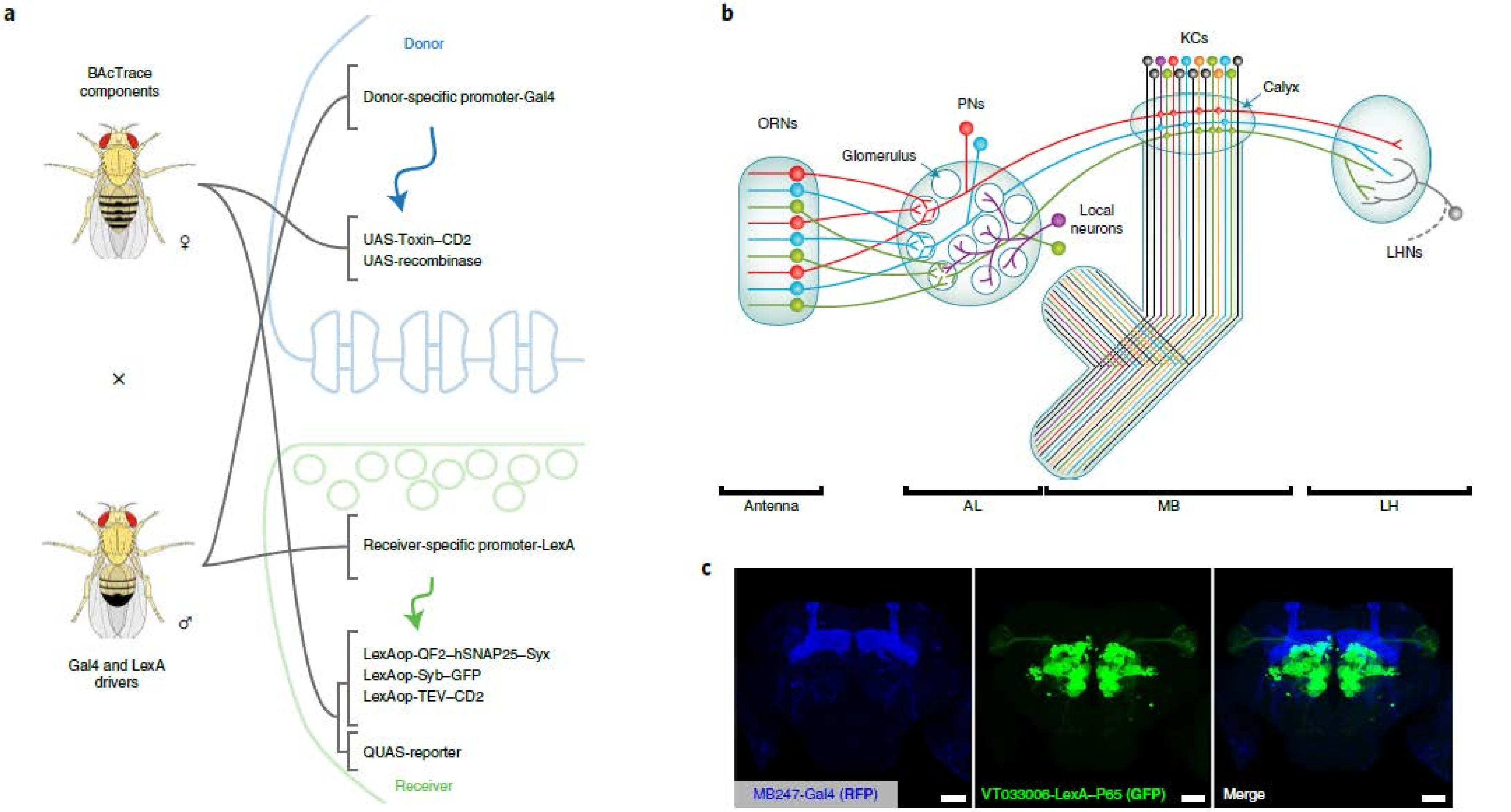
Clostridium botulinum
neurotoxin A, Botox



BAcTrace (Botulinum-Activated Tracer)

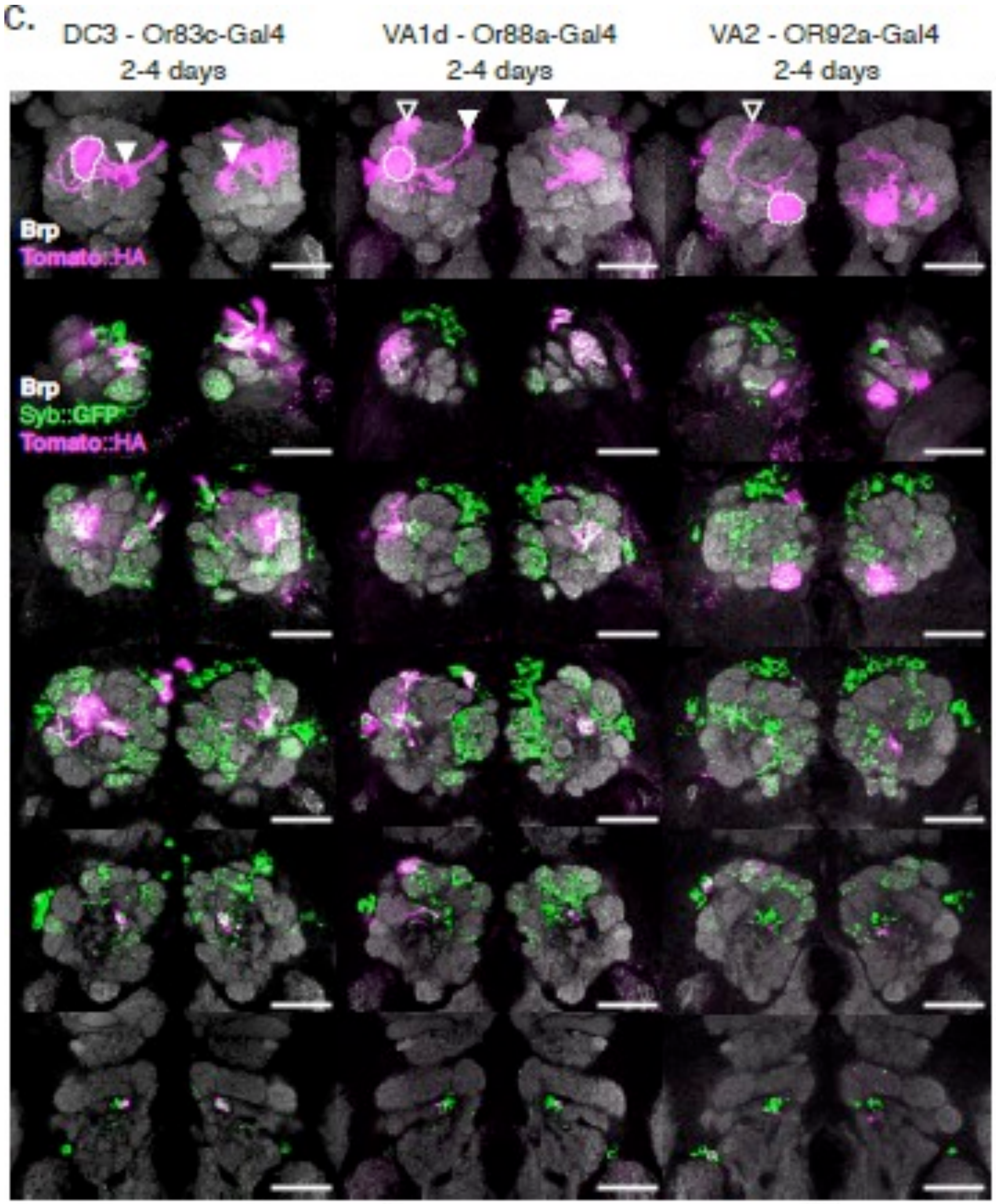
Cachero et al. 2020 Nature Methods

BAcTrace – genetic tracing of post- to pre-synaptic connections



Cachero et al. 2020 Nature Methods

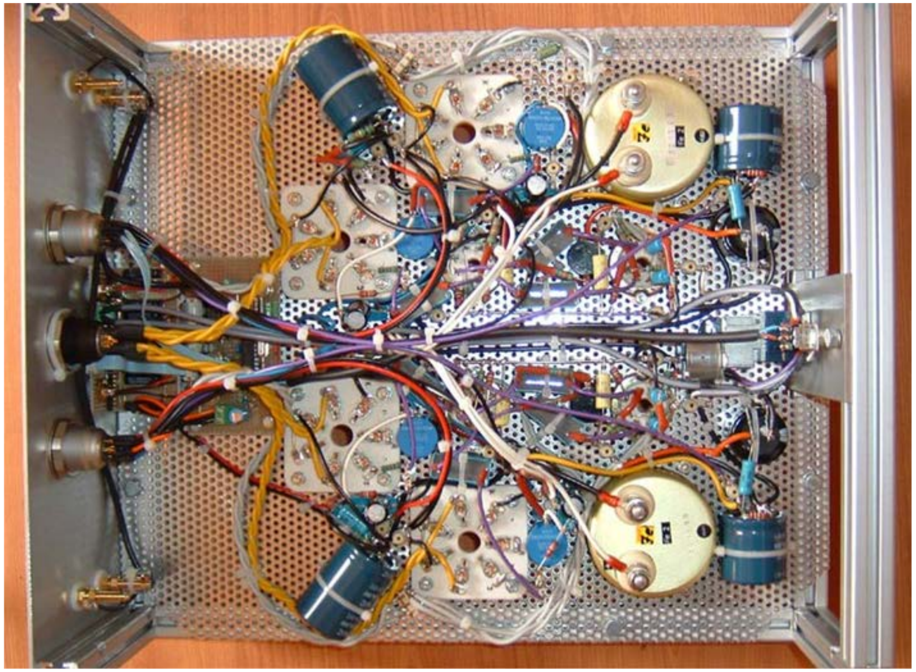
BAcTrace – genetic tracing of post- to pre-synaptic connections



Cachero et al. 2020 Nature Methods

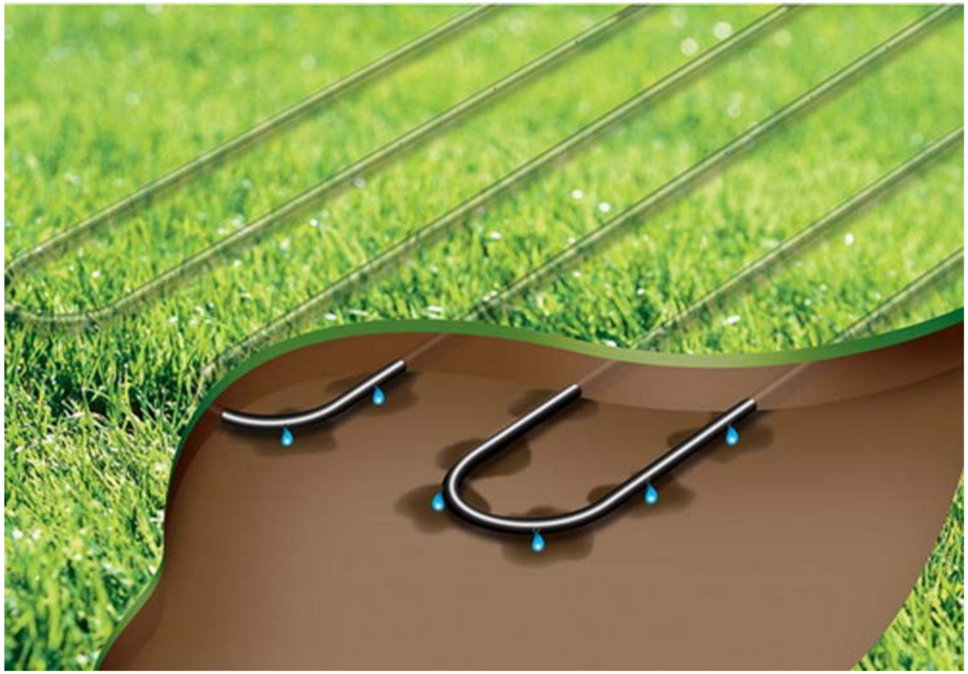
Complication: neuromodulators (=biogenic amines & neuropeptides don't act via synapses

wiring transmission



Specificity by wiring

volume transmission

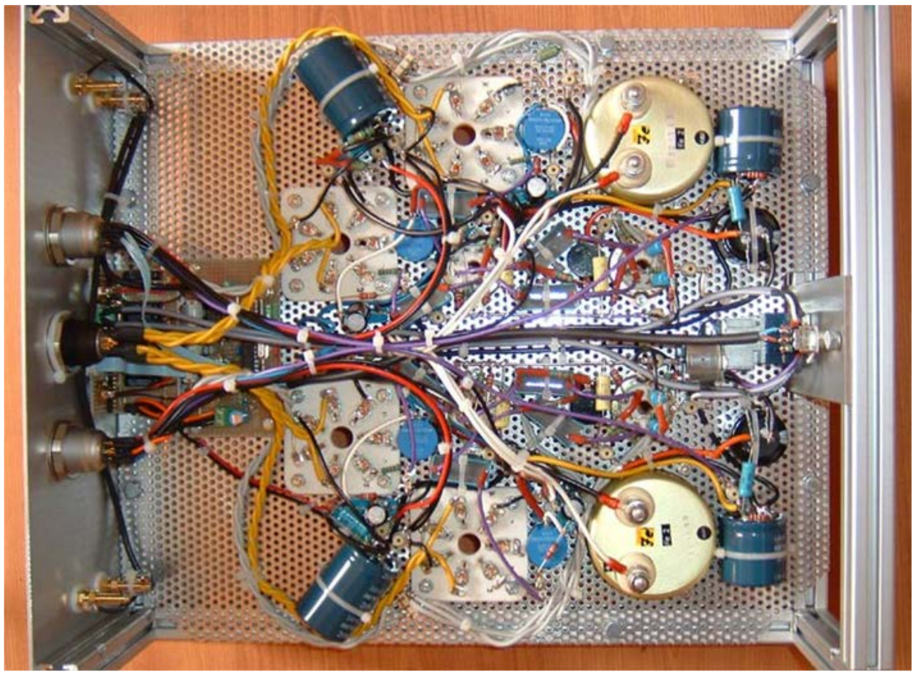


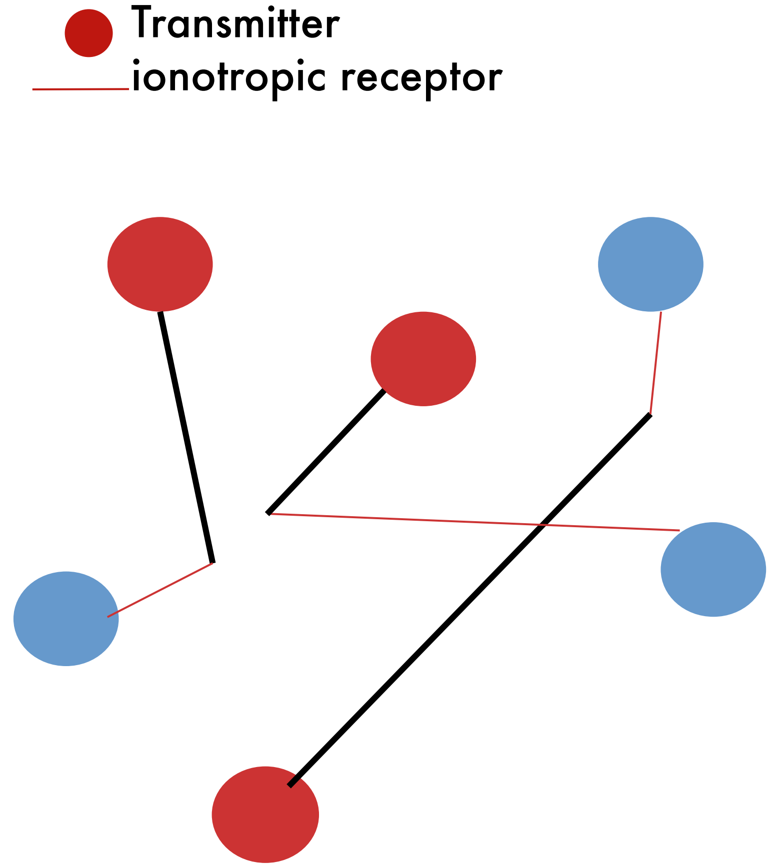
Specificity by receptors

Synaptic signalling vs. volume transmission are the extreme ends of neuronal signalling

Fast synaptic transmission and electrical synapses

Specificity by wiring

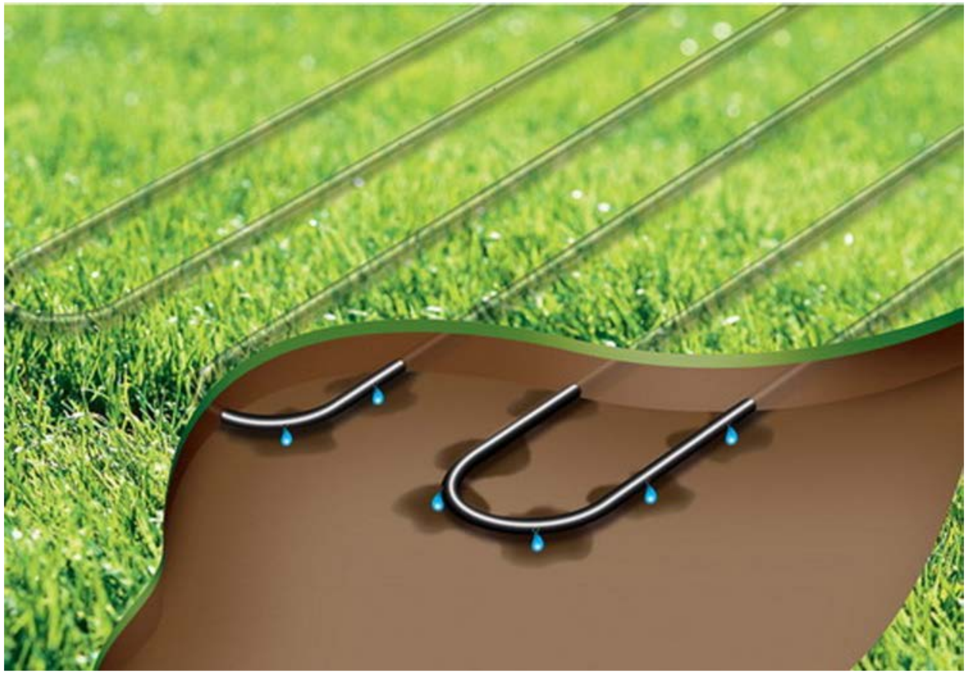


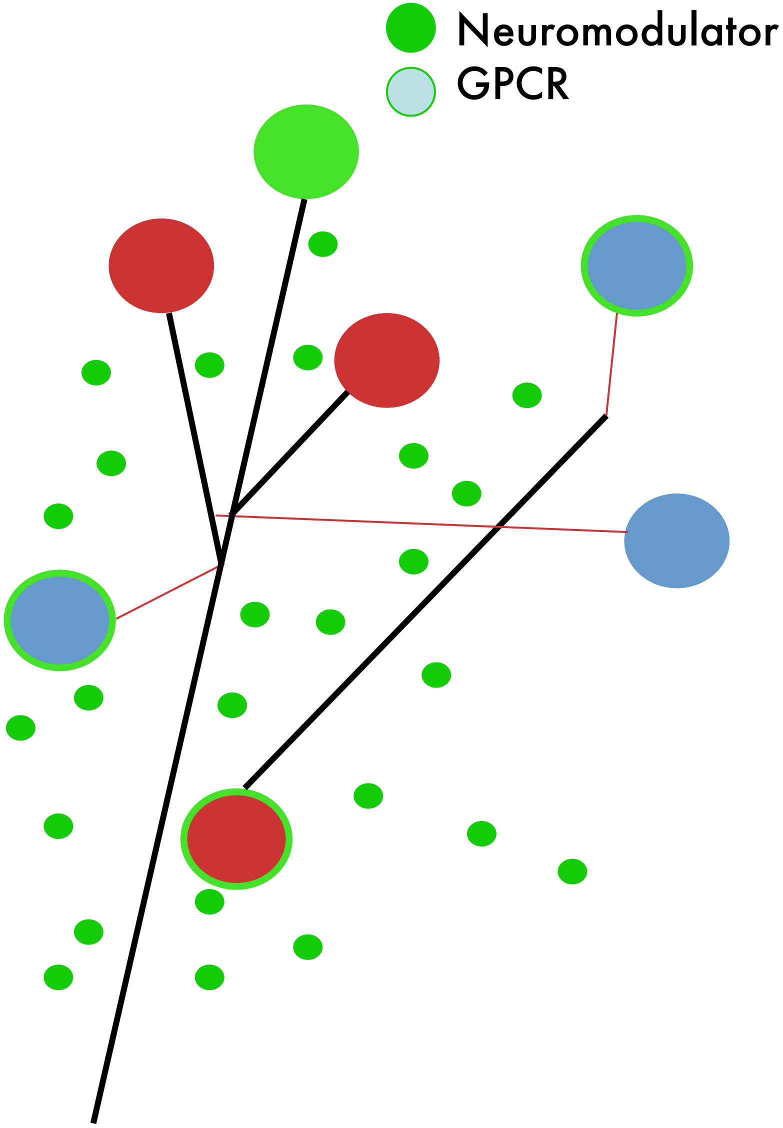


•"private communication" restricted to the synapse

•ionotropic receptors are limited to increasing the permeability of the cell
•membrane to certain ions

Neuromodulators act parasynaptically or via volume transmission

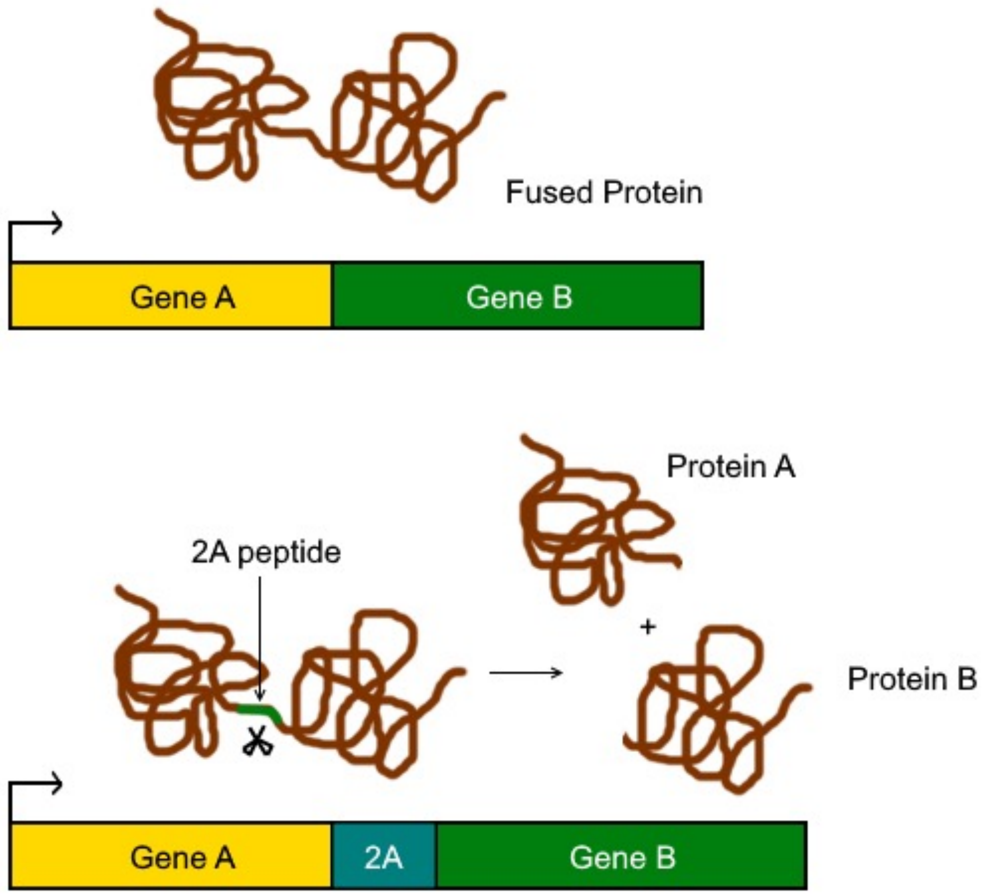




- Specificity via receptors
- information sent to whole neuron populations
- paracrine "tissue hormones"

Chemoconnectomics relies on T2A ribosomal skipping - Trojan triplet T2A-GAL4s

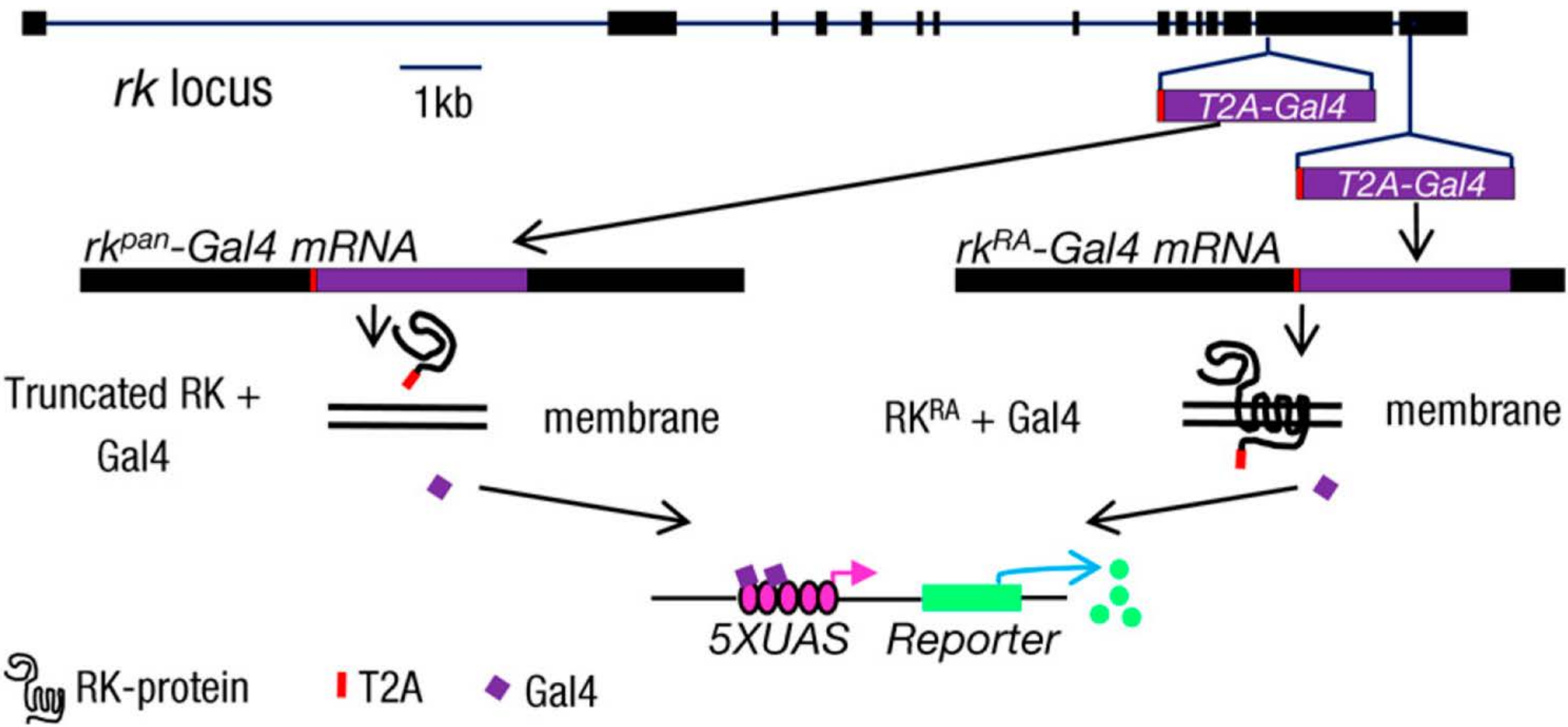
- viral T2A sequence induces a „jump“ of the ribosome during translation



Wikipedia

T2A ribosomal skipping - Trojan triplet T2A-GAL4s

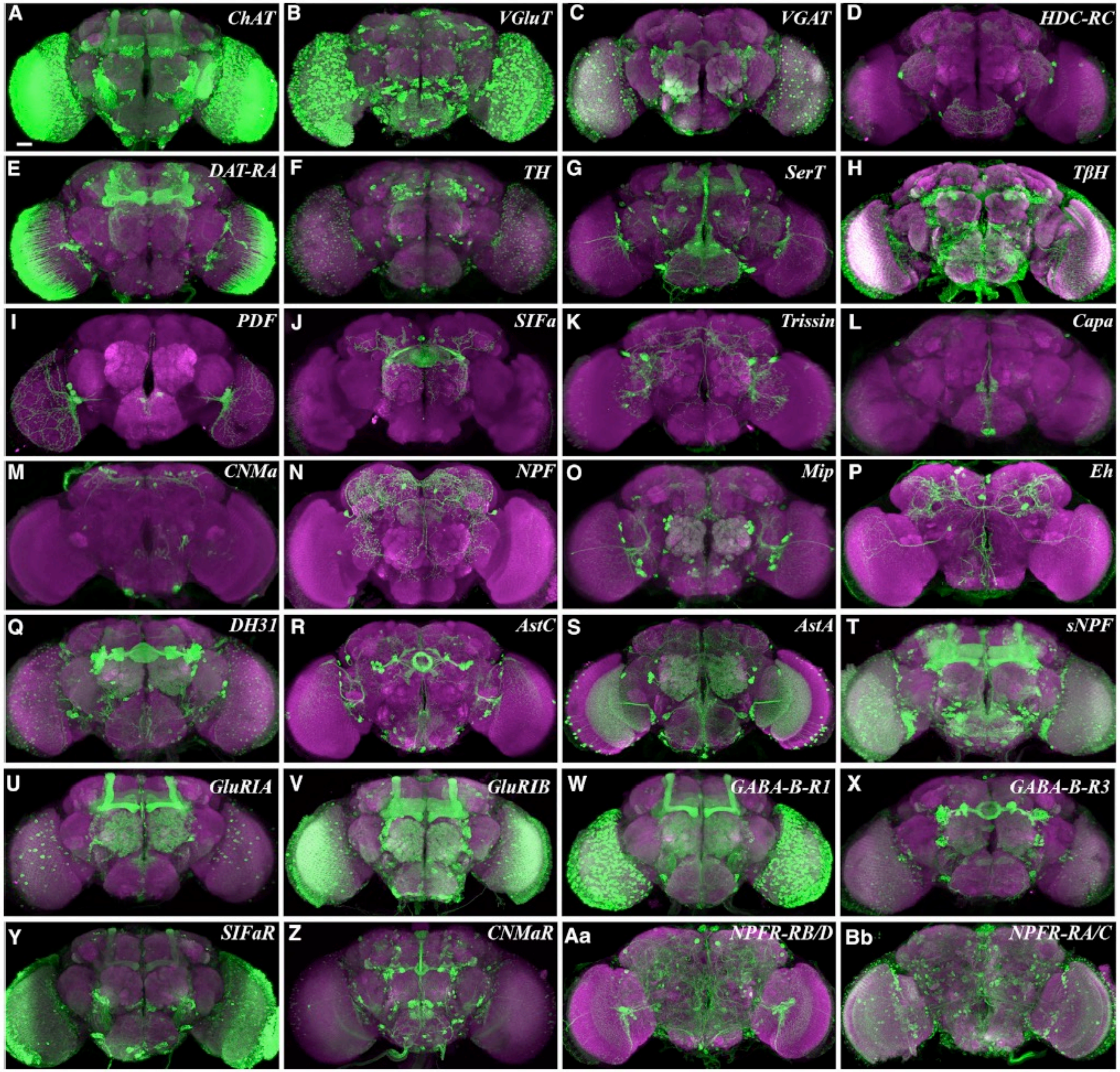
- genomic in-gene insertion of T2A-Gal4 via CRISPR-Cas9



Vorteil: Das GAL4-Protein wird orts- und mengenspezifisch wie das Zielgen exprimiert, da es im gleichen genomischen Locus sitzt.

Diao & White (2012) Genetics

Chemoconnectomics



Bowen et al. (2019) Neuron

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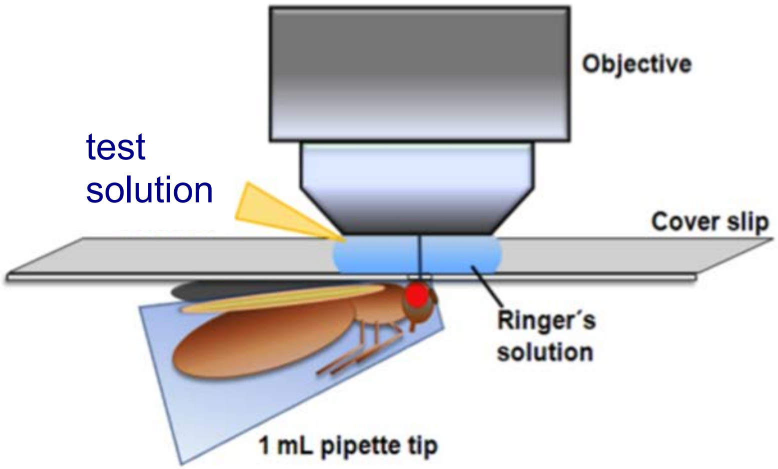
Live cell imaging in the brain of intact *Drosophila*

<u>That is needed:</u>

•a fluorescent microscope

•a sensitive camera

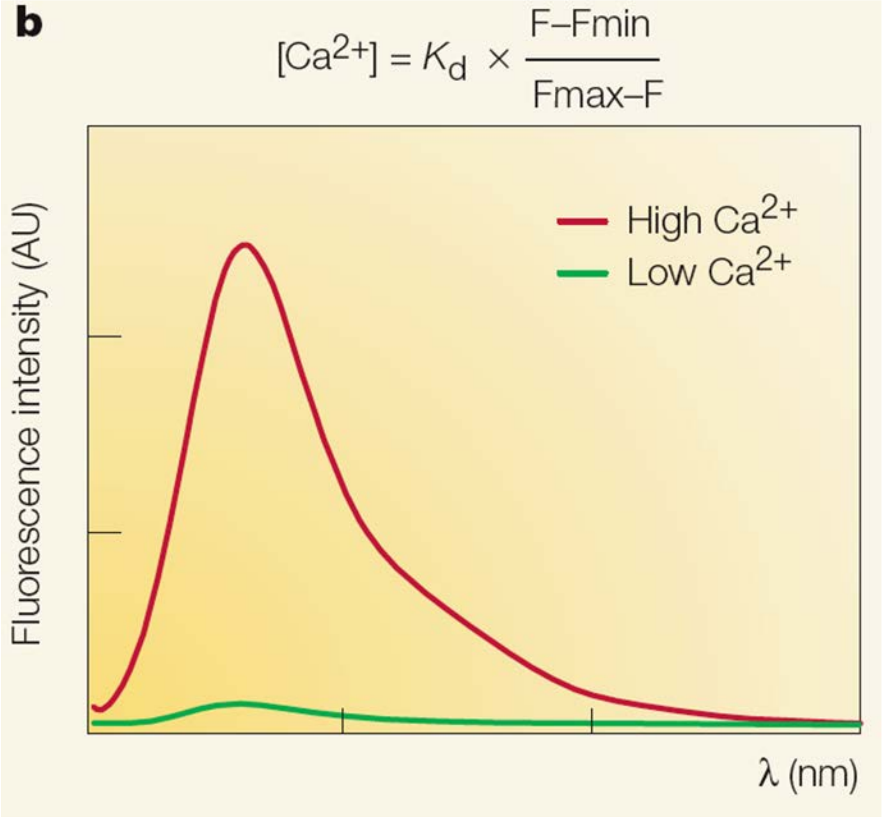
•a fly expressing a genetically encoded activity sensor



Christiansen et al. 2011 J Neurosci

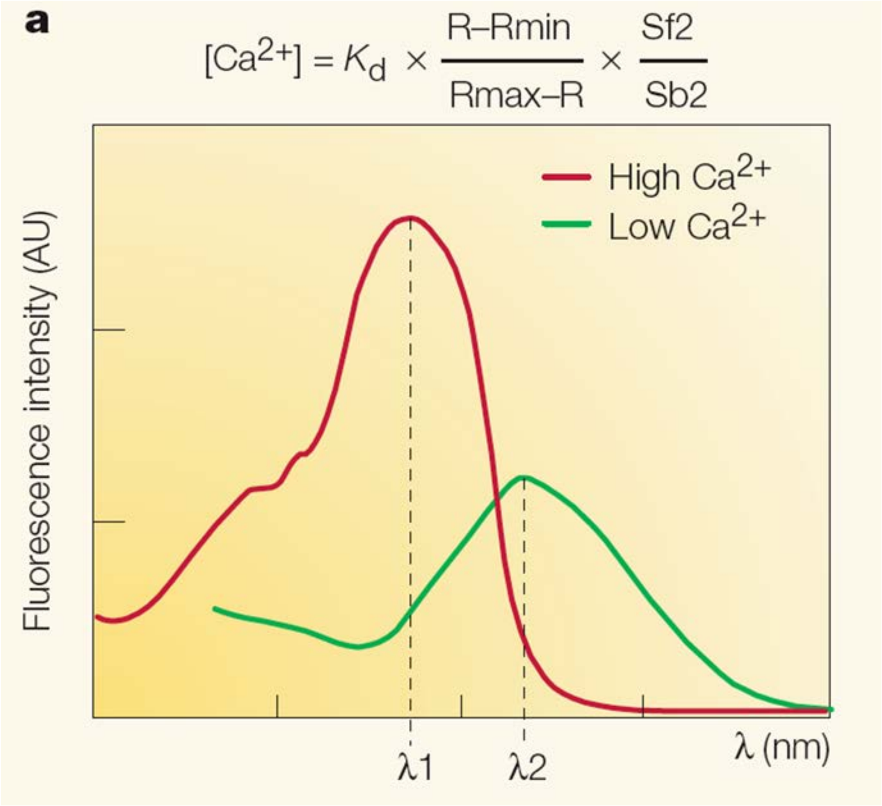
In general, two types of indicators can be distinguished:

non-ratiometric indicators



- good change in signal intensity
- prone to movement artefacts

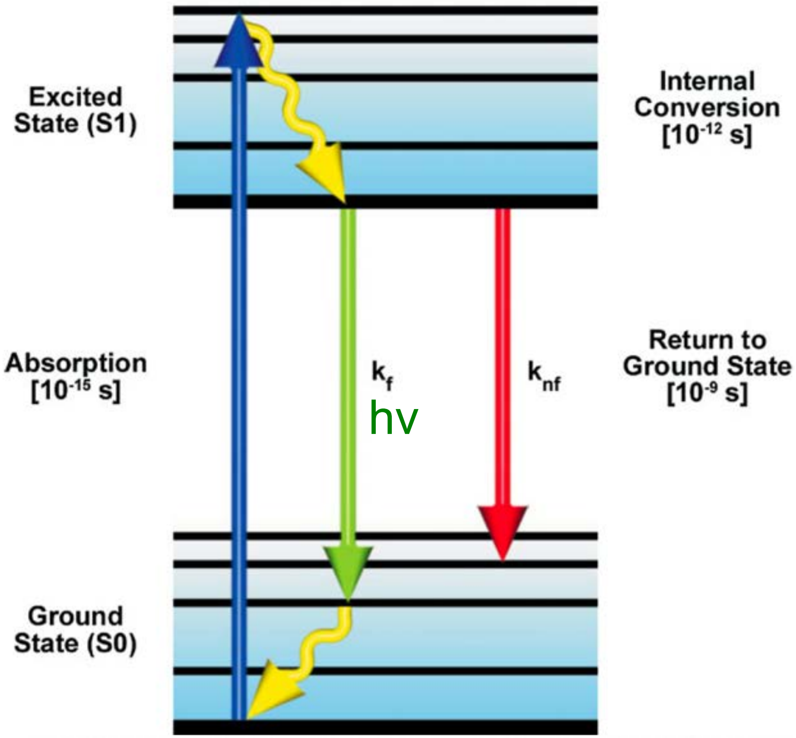
ratiometric indicators

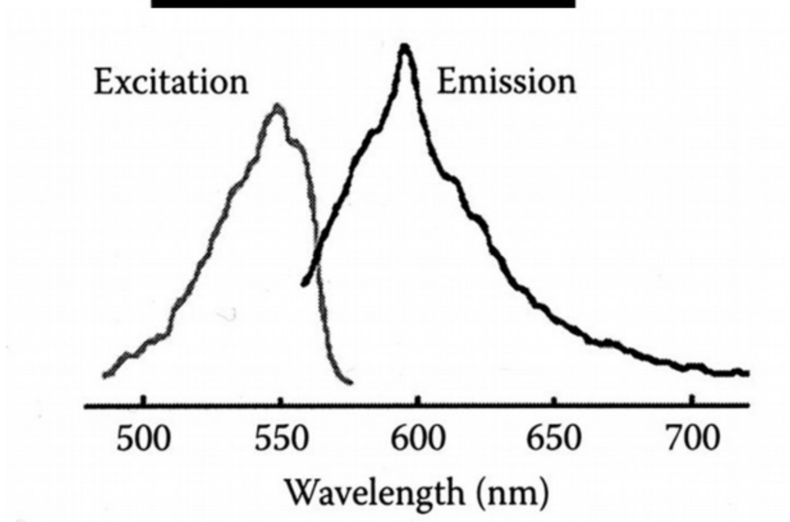


- good signal-to-noise ratio
- no movement artefacts

Recap: Fluorescence

Simplified Perrin-Jablonski-Diagram for a fluorescent molecule



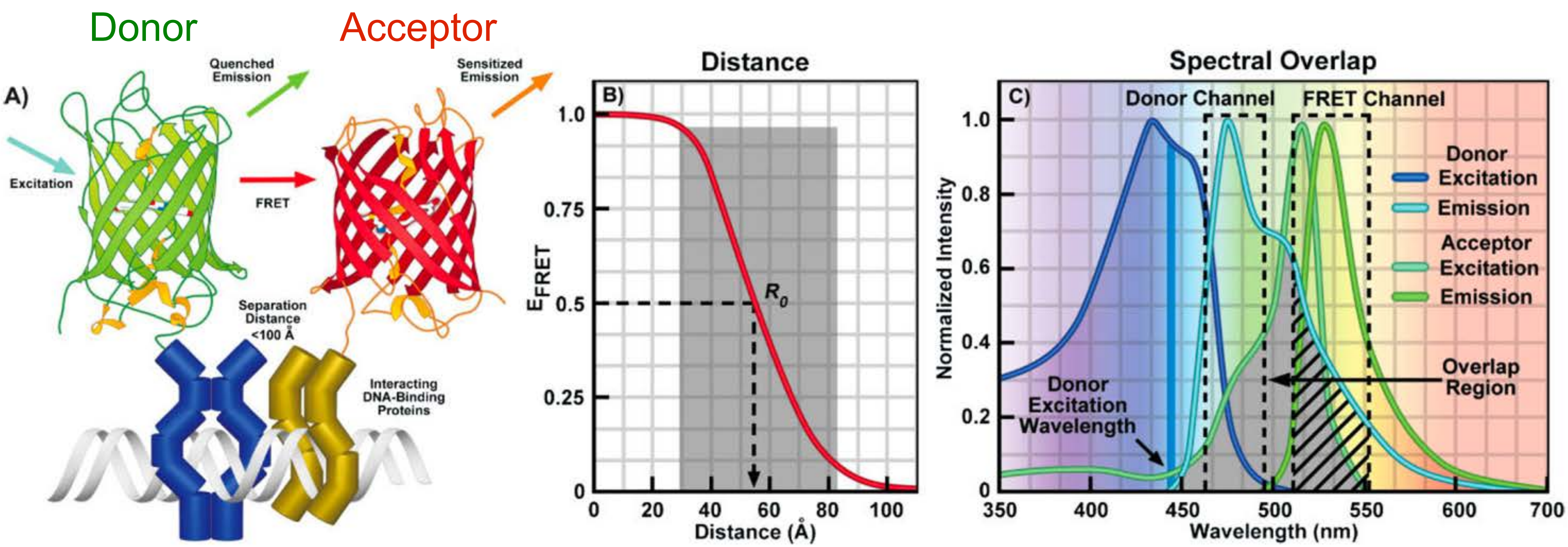


due to thermal energy loss ( ):

$\lambda_{emission} > \lambda_{excitation}$

Day & Davidson (2012) Bioessays

XFP-based Fluorescence Resonance Energy Transfer (FRET)



$$E_{\mathrm{FRET}} = \frac{R_0^6}{(R_0^6 + r^6)}$$

R_0 = Förster distance

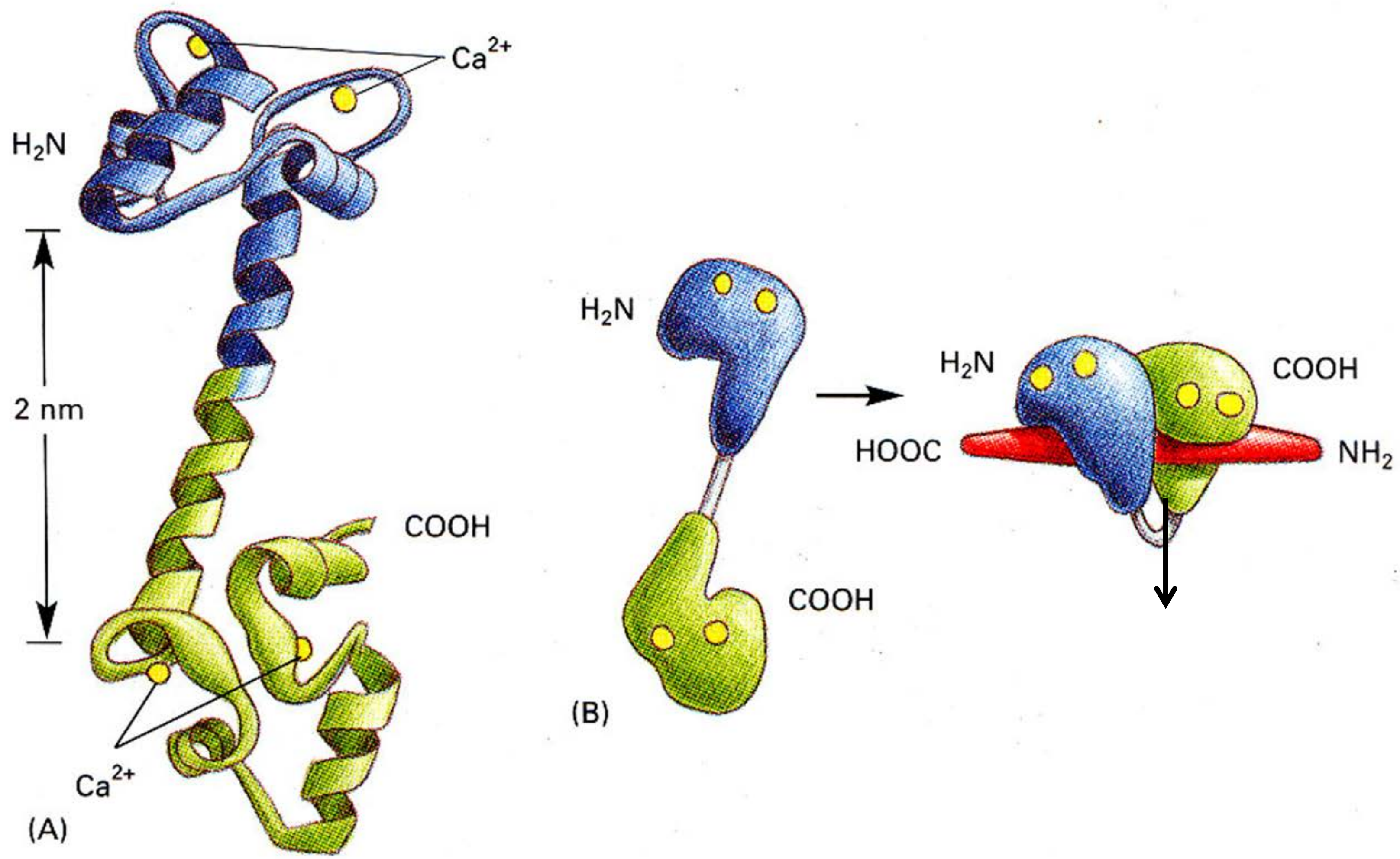
The energy of the excited donor is not emitted as light (photons), but transferred by electrical dipol interactions (resonance) that only take place in very close apposition of donor and acceptor

Day & Davidson (2012) Bioessays

Several GECIs are based on Calmodulin

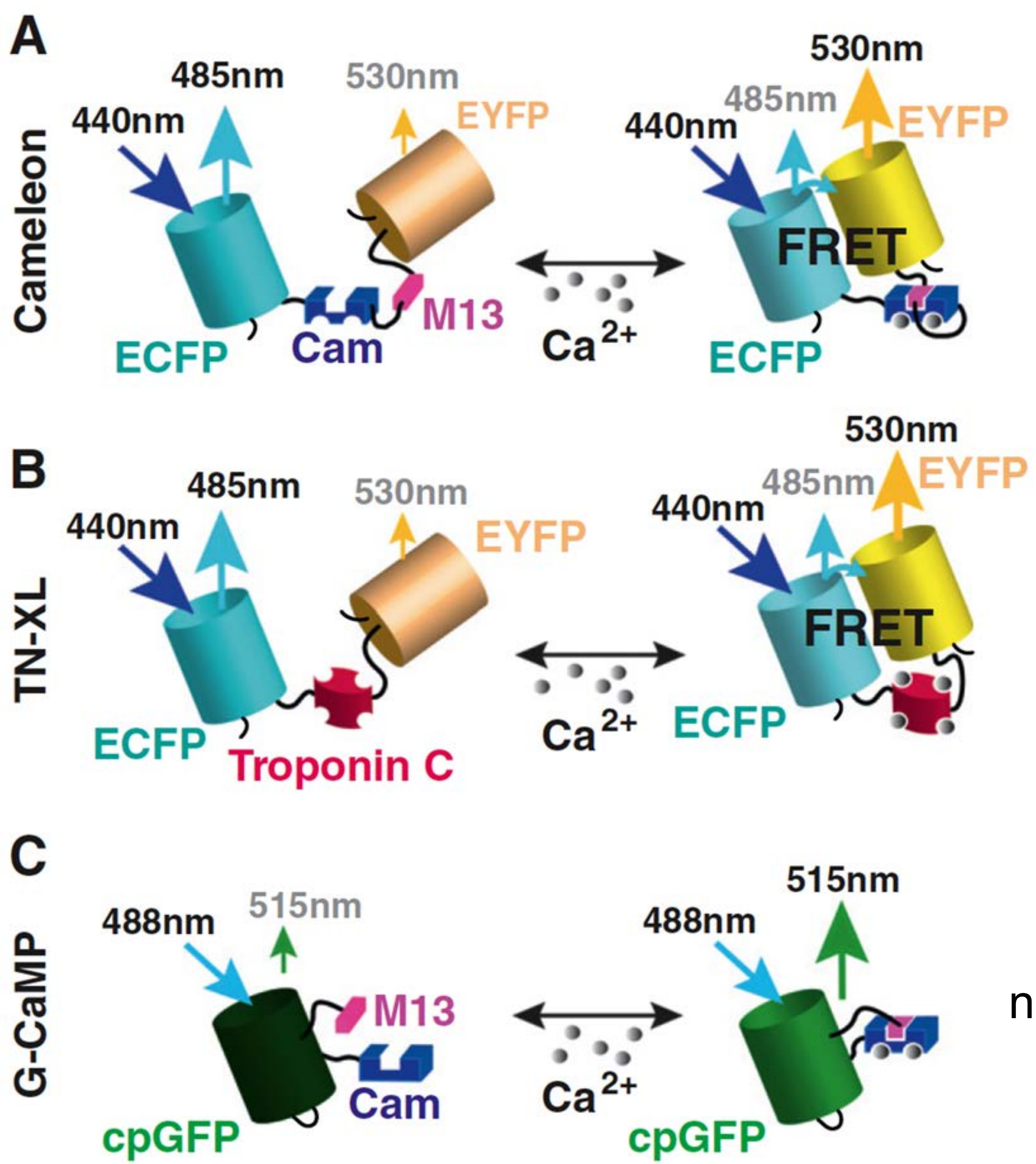
CaM
without bound Ca^{2+}

CaM
with bound Ca^{2+}



Activation of protein kinases etc.

Several GECIs are based on Calmodulin



ratiometric

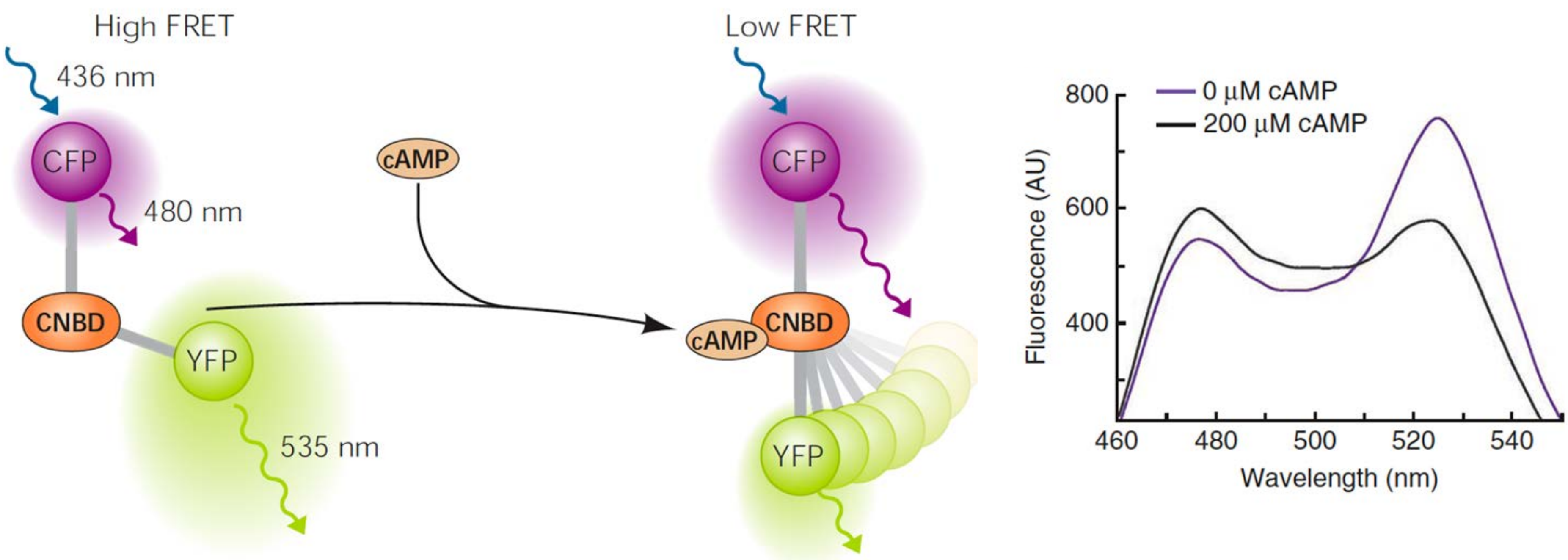
ratiometric

non-ratiometric

Riemensperger et al. (2012) Biochim Biophys Acta

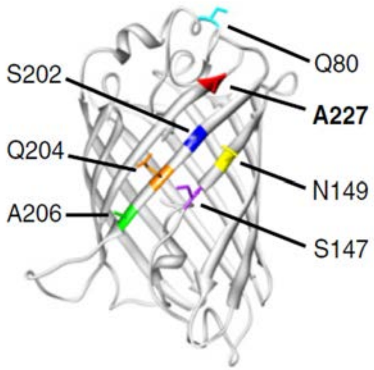
Epac-camps: a FRET-based genetically encoded cAMP indicator

Epac-camps: based on the "Exchange protein directly activated by adenylate cyclase

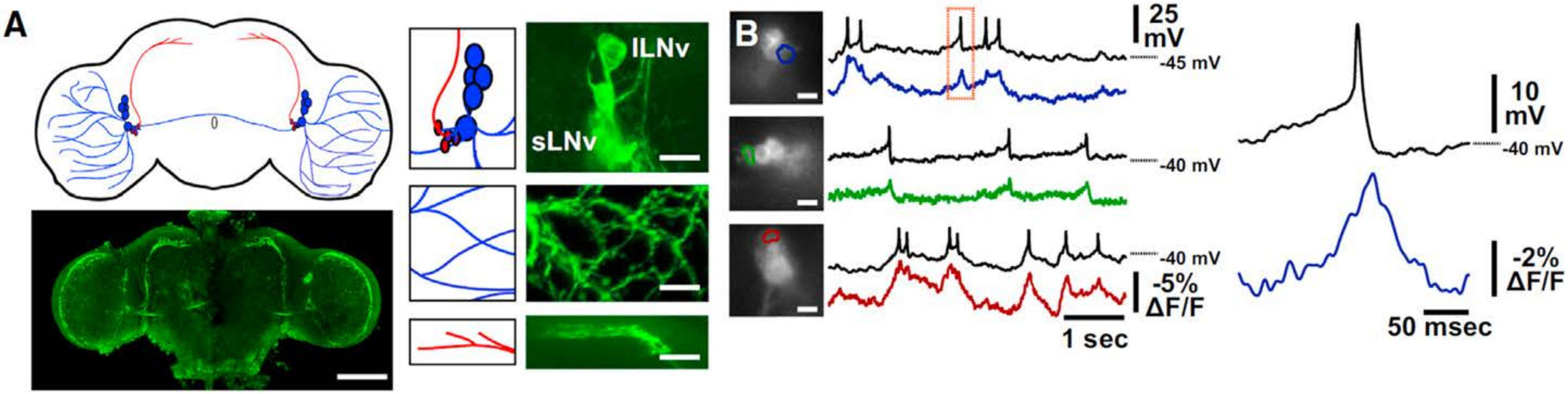


Nikolaev & Lohse (2006) Physiology
Börner et al (2011) Nature Protocols

Optical electrophysiology in *Drosophila*: genetically encoded voltage indicators (GEVIs)



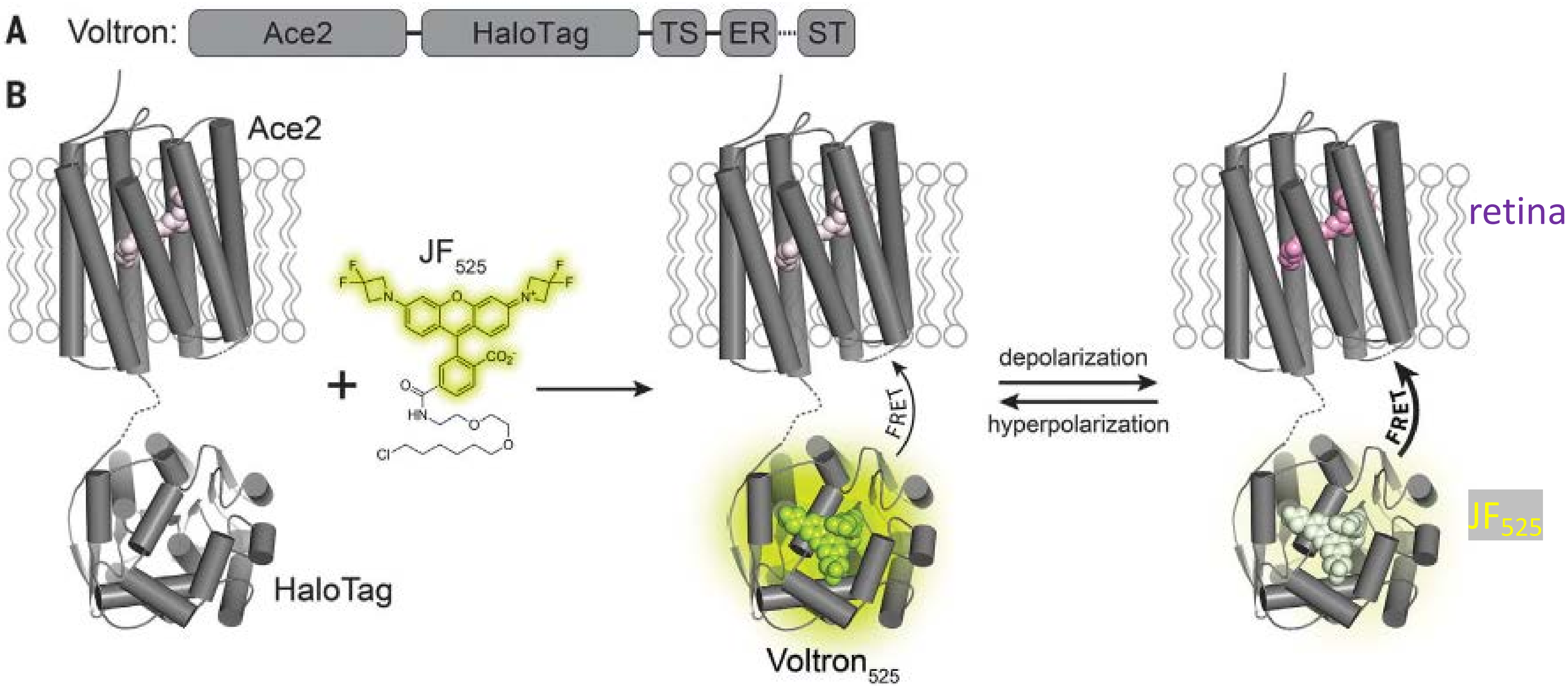
ArcLight
a voltage-sensitive GFP



pdf-GAL4<UAS-ArcLight

Jin et al. (2012) Neuron
Cao et al (2013) Cell

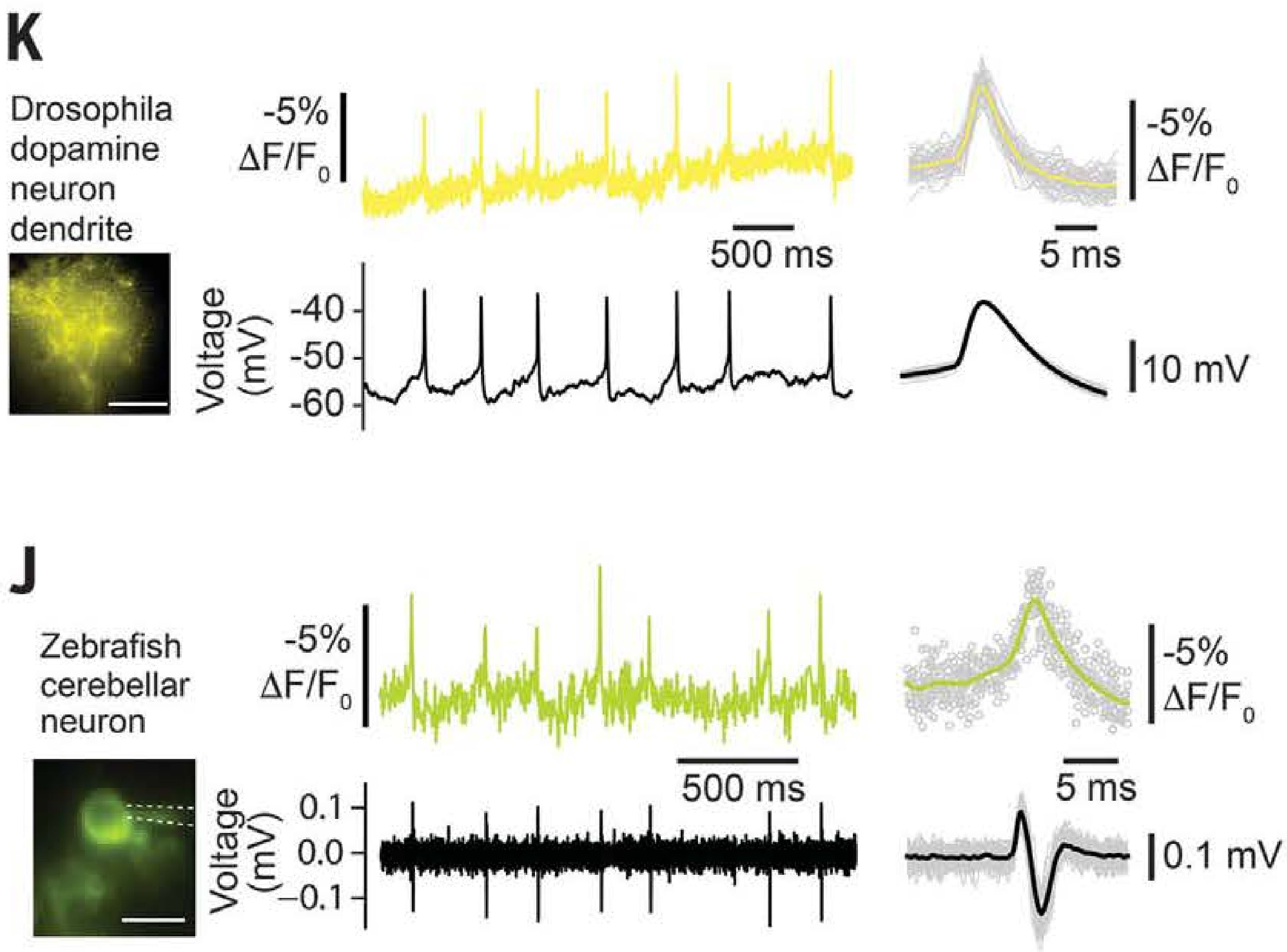
A new generation of indicators: chemigenetic indicators



Ace2 = voltage-sensitive microbial rhodopsin domain
HaloTag = dye-capture protein, binds the chemical fluorophore JF_{525}

Abd el Fattah et al. 2019 Science

A new generation of indicators: chemigenetic indicators



- increased brightness & photostability
- allows imaging of many neurons in large brain areas

Abd el Fattah et al. 2019 Science

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Neurogenetics can be used to dissect neuronal circuits

Q: What is the function of neuron X/circuit Y, say, in behaviour A?

genetically manipulate gene X/ circuit Y

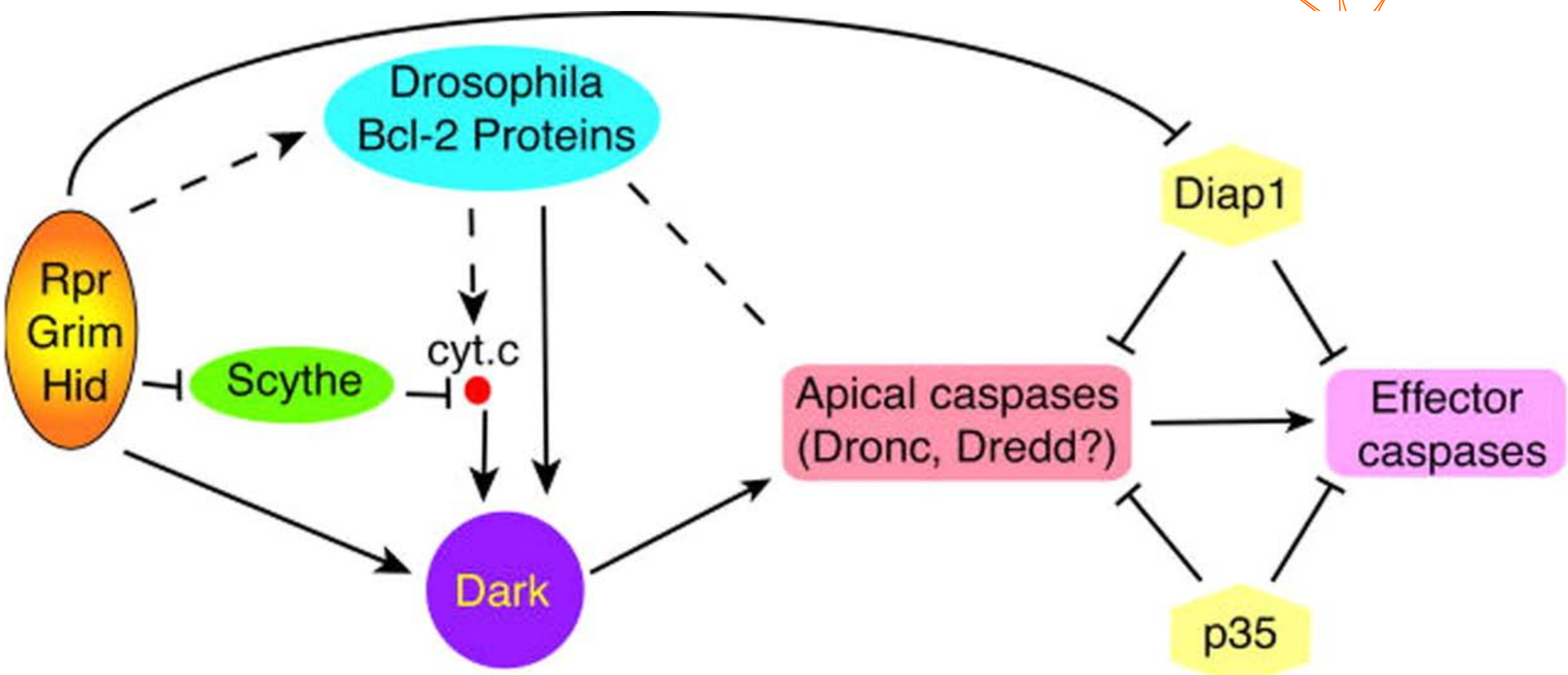
↓

Test effect in behavioural assay for A

How to manipulate neuronal activity: genetic ablation

cell ablation by cell-specific ectopic expression of the preapoptotic genes

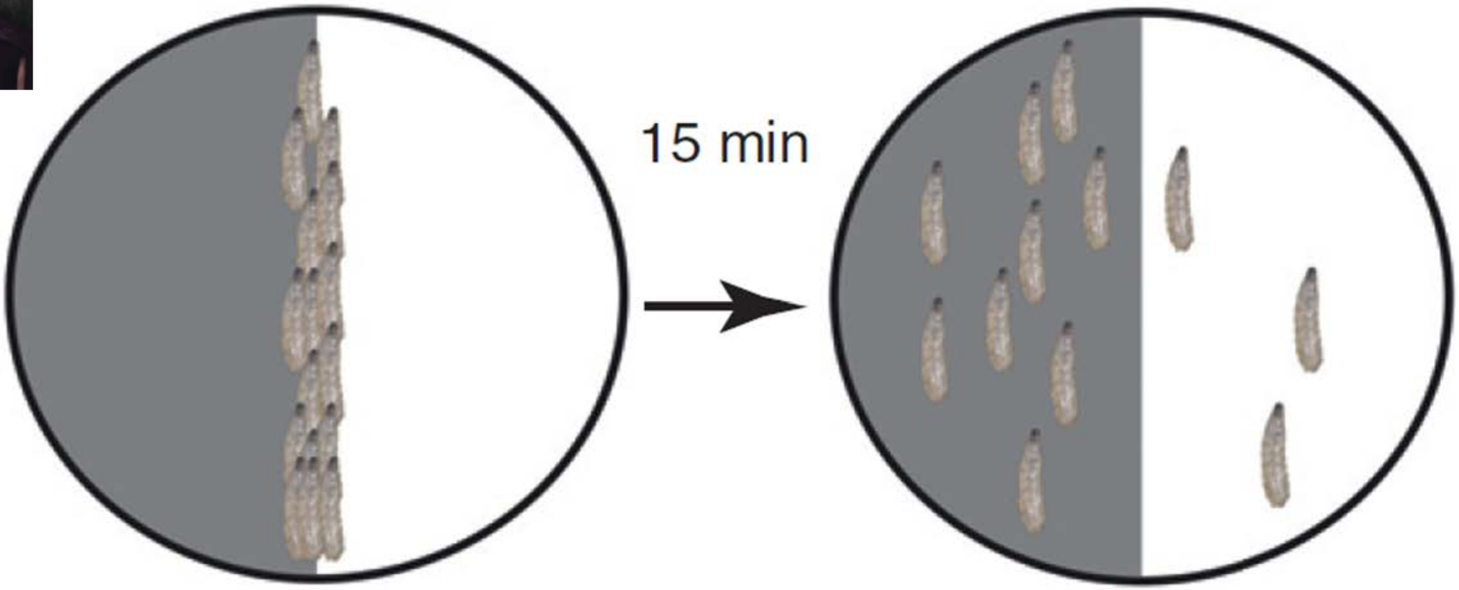
- •UAS-hid
- •UAS-rpr
- •UAS-grim



Chen & Abrams 2000 J Cell Biol

Larval light avoidance as a simple example for a behavioural read-out

- light avoidance (negative phototaxis)
- manipulation of cholinergic photoceptor cells via *IGMR-Gal4*

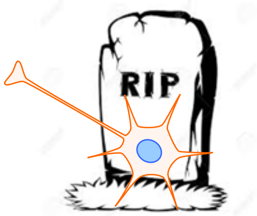


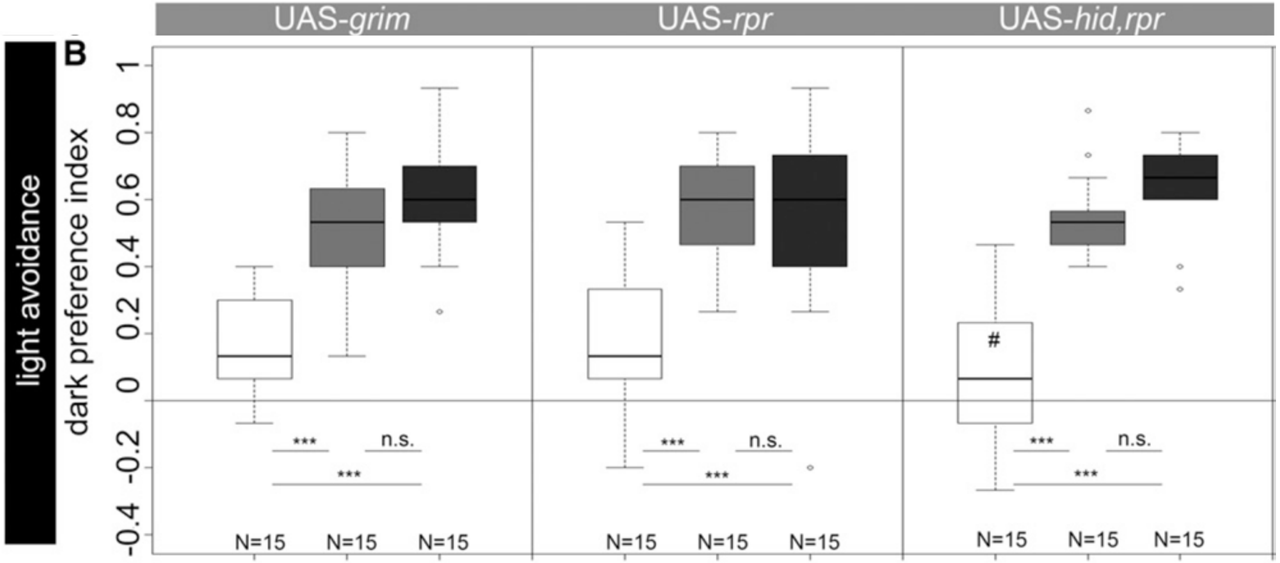
calculate “dark preference index”
1 = all larvae are in the dark
0 = 50% of larvae in the dar
-1= all larvae are on the bright side (as you hopefully are..)

Keene & Sprecher 2012 Trends Neurosci

How to manipulate neuronal activity: genetic ablation

cell ablation by cell-specific ectopic expression of the preapoptotic genes
•UAS-hid
•UAS-rpr
•UAS-grim

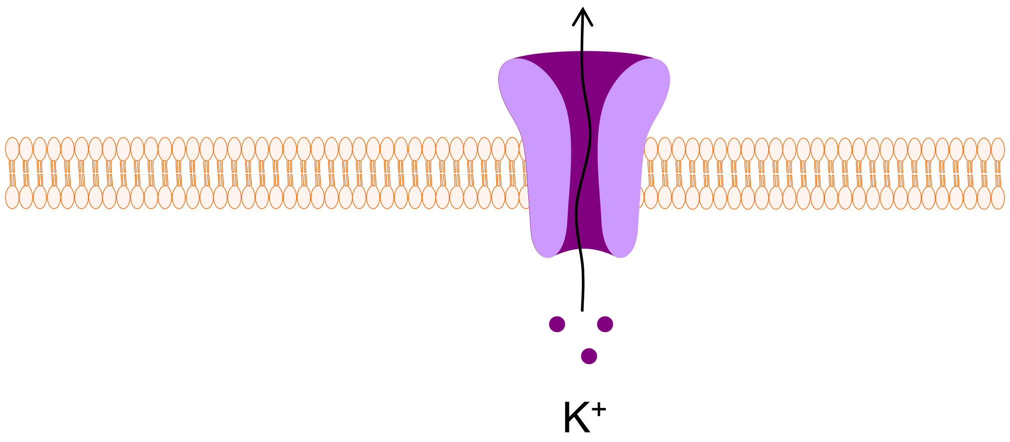


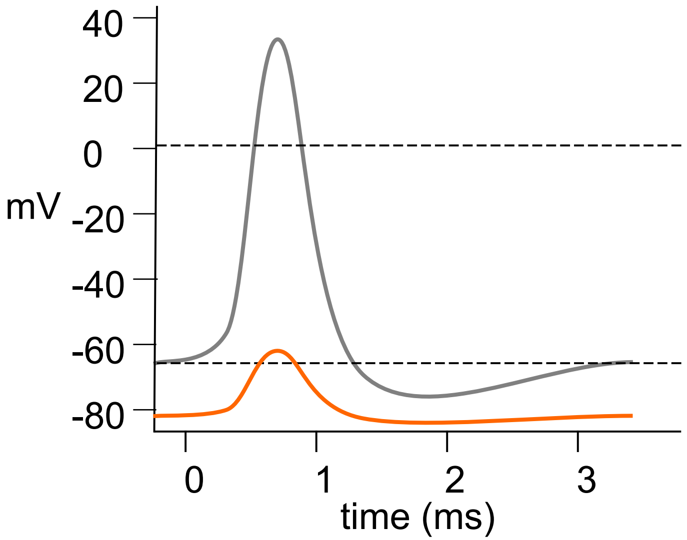


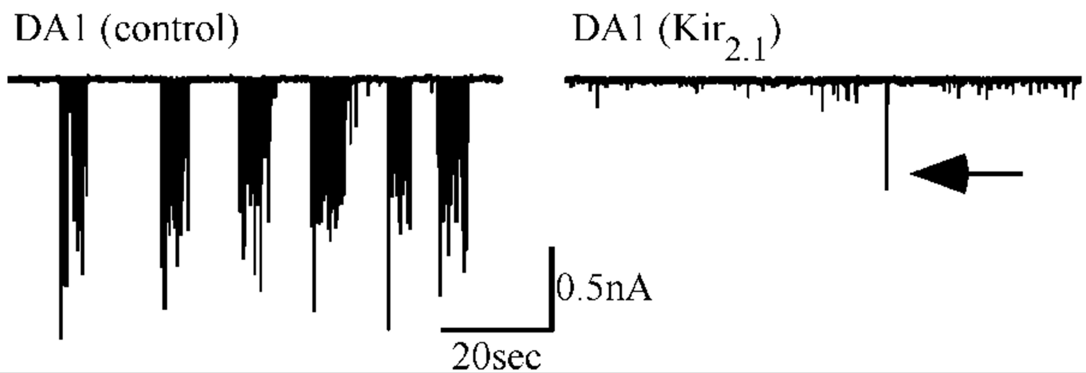
Pauls et al. 2015 Genetics

How to manipulate neuronal activity: electrical silencing

- ectopic expression of rectifying K^+ channels (K_{ir}) leads to hyperpolarisation

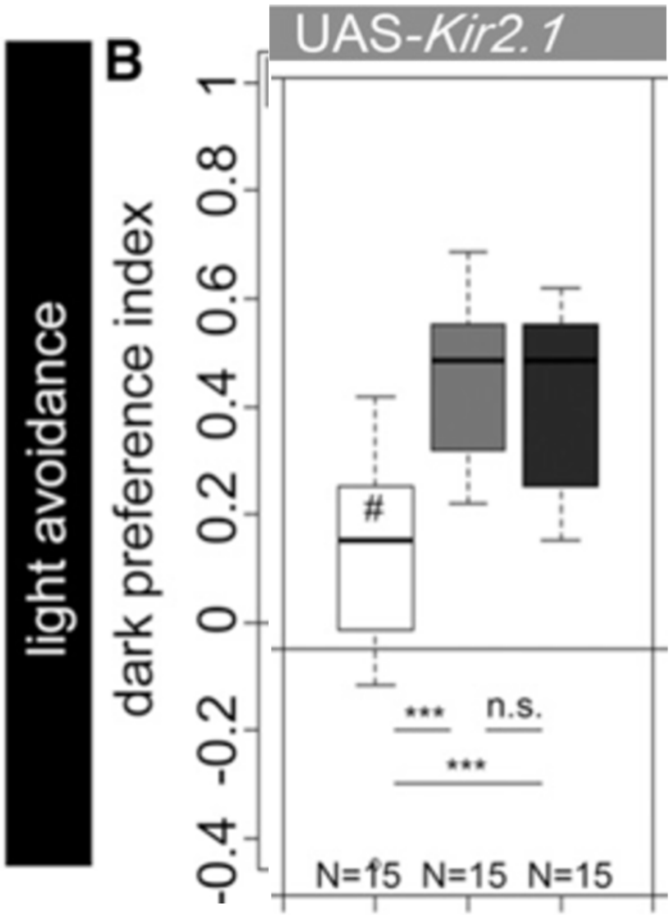






Baines et al. 2001 J Neurosci

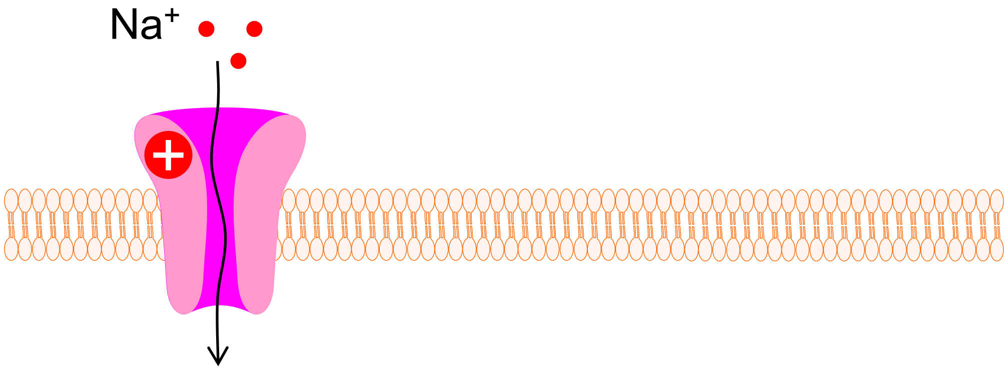
How to manipulate neuronal activity: genetic silencing

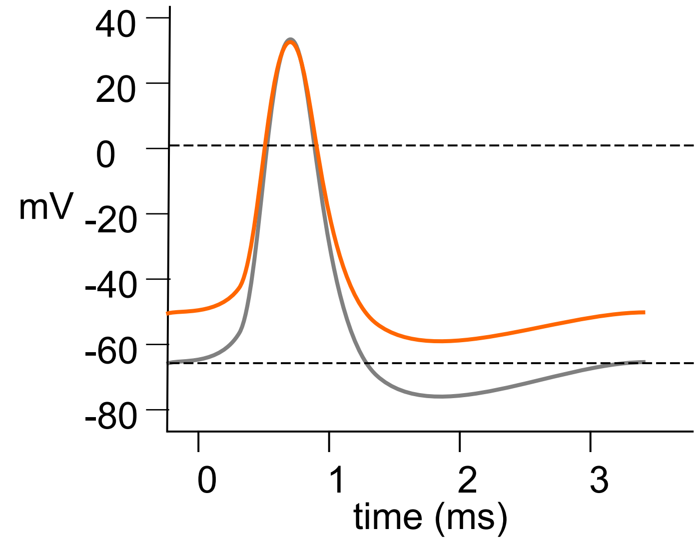


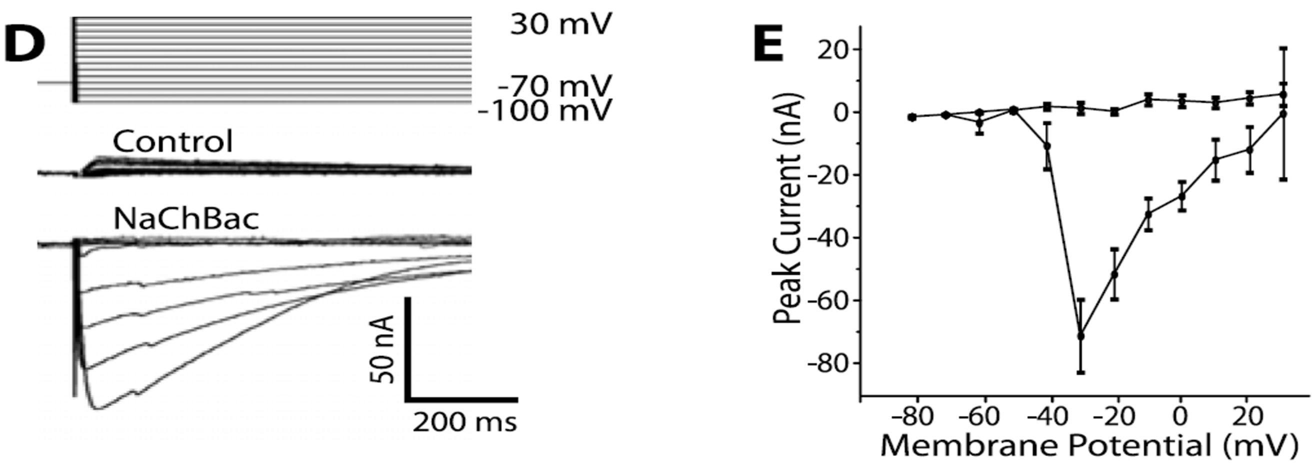
Pauls et al. 2015 Genetics

How to manipulate neuronal activity: genetic activation

- ectopic expression of Na^+ channels lead to increased excitability







Nitabach et al. 2006 J Neurosci

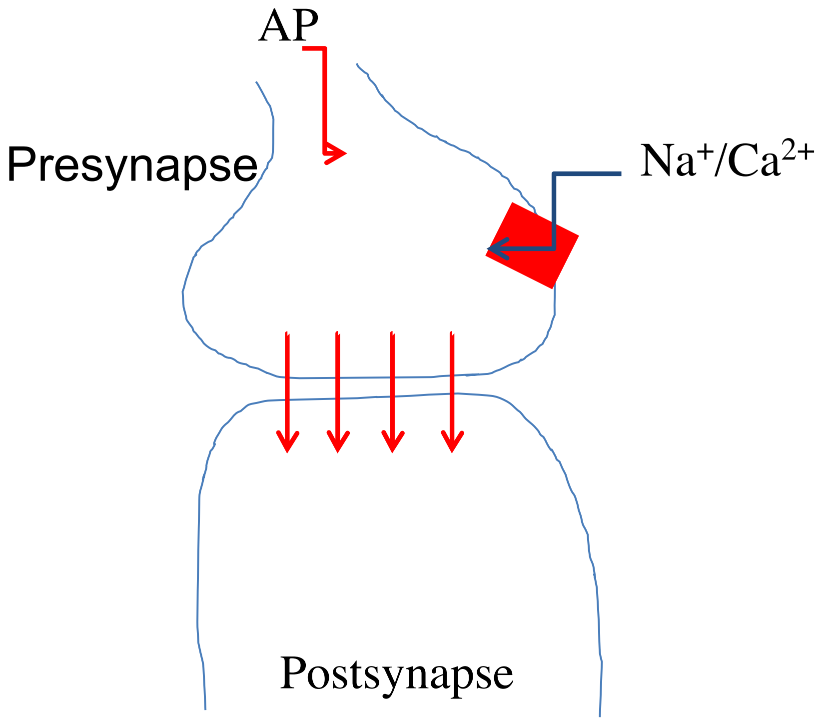
Content of this lecture:

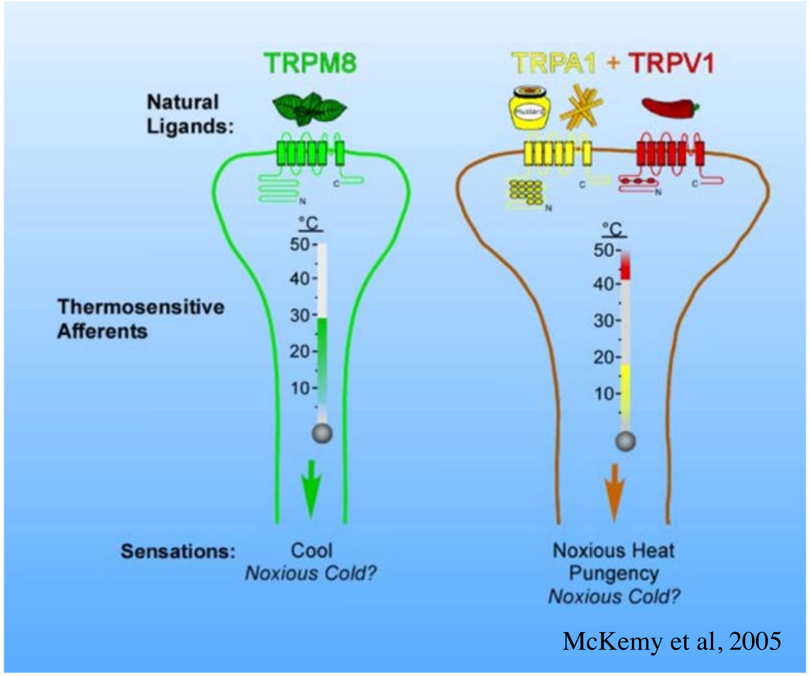
- connectomics
- modern genetic techniques to dissect neuronal connectivity
- modern genetic techniques to image neuronal activity
- modern genetic techniques to probe the functionality of neuronal connectivity
    - constitutive silencing and activation
    - conditional silencing and activation
- methods to narrow down genetic manipulation to “single“ neurons

Genetic tools to conditionally activate neurons in *Drosophila*

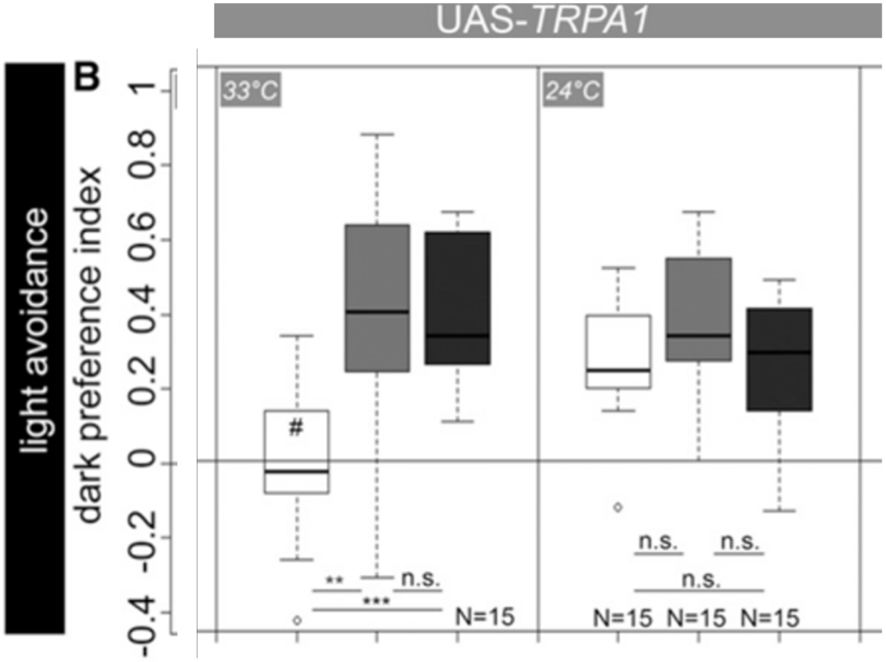
Thermogenetic tools:

- •UAS-TRPA1 - activation via a heat-sensitive cation channel
- •UAS-TRPM8 - activation via a cold-sensitive cation channel





How to manipulate neuronal activity: genetic activation



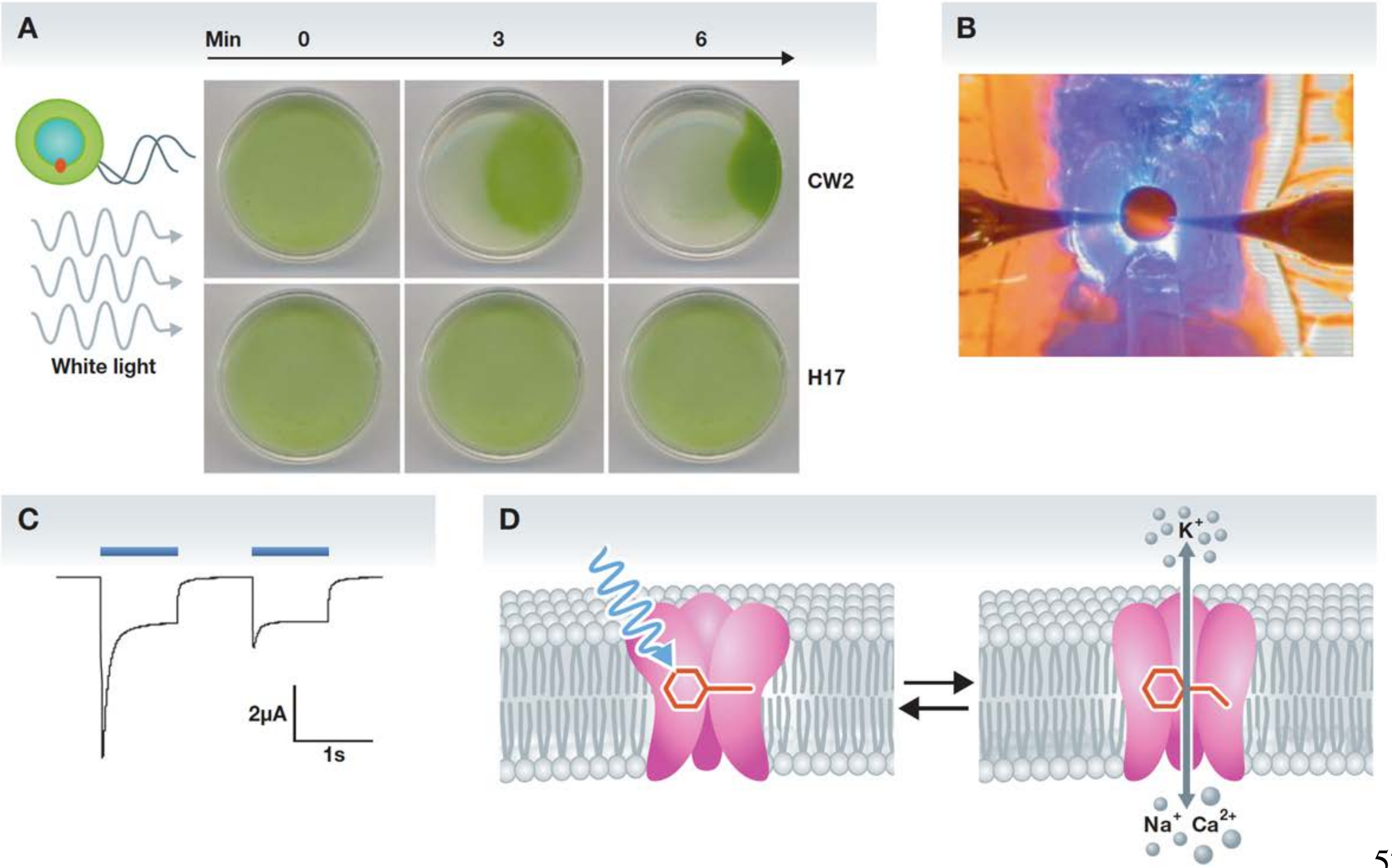
Pauls et al. 2015 Genetics

Optogenetics to conditionally activate neurons in *Drosophila*

• Optogenetic tools:

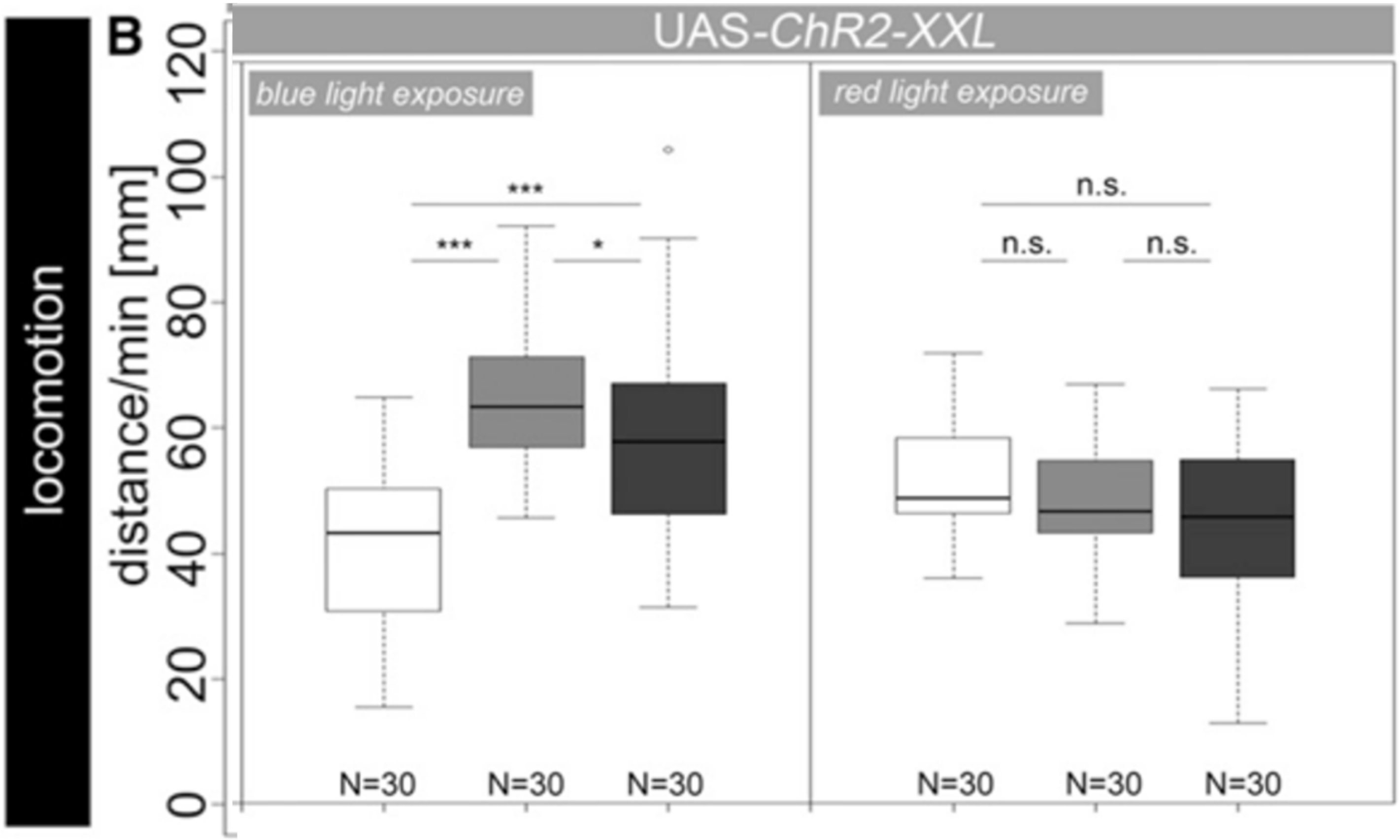
• neuronal activation via a light-sensitive algal rhodopsin-based cation channel

• example UAS-channelrhodopsin 2-XXL



Nagel & Hegemann 2013 EMBO Mol Med
Dawydow et al. 2014 PNAS

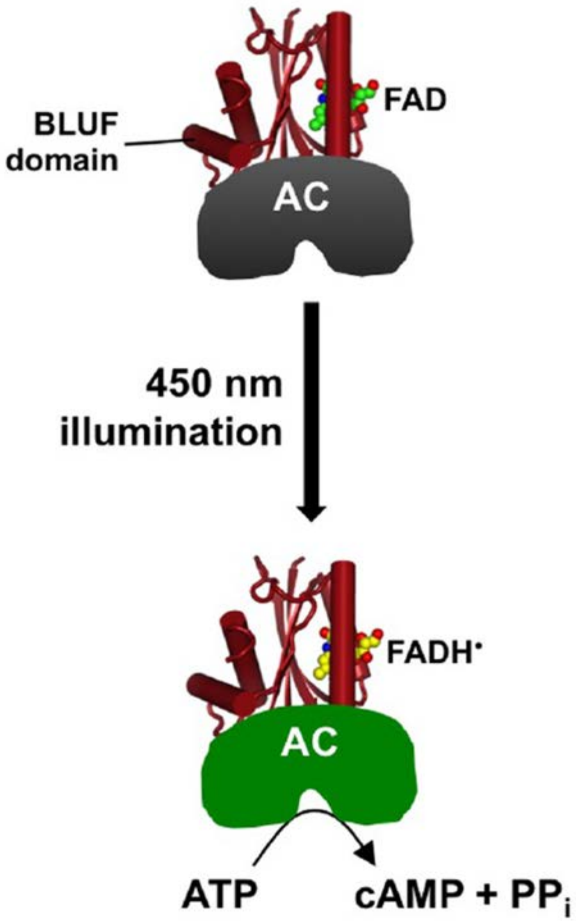
How to manipulate neuronal activity: genetic conditional activation



Pauls et al. 2015 Genetics

How to manipulate neuronal activity: optogenetic activation of cAMP signalling

Beggiatoa photo-active
adenylyl cyclase



Patel & Gold 2015 Front Pharmacol

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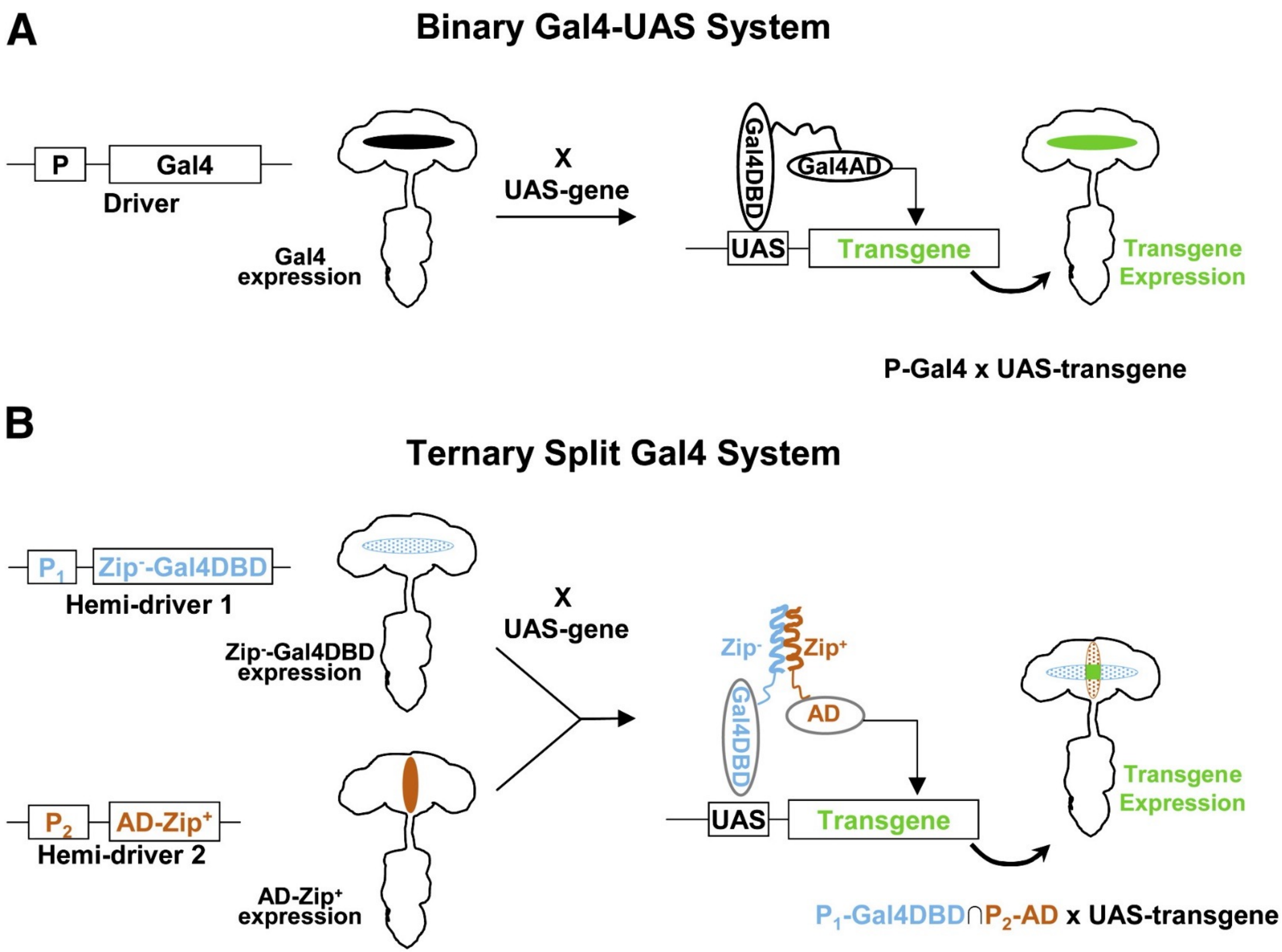
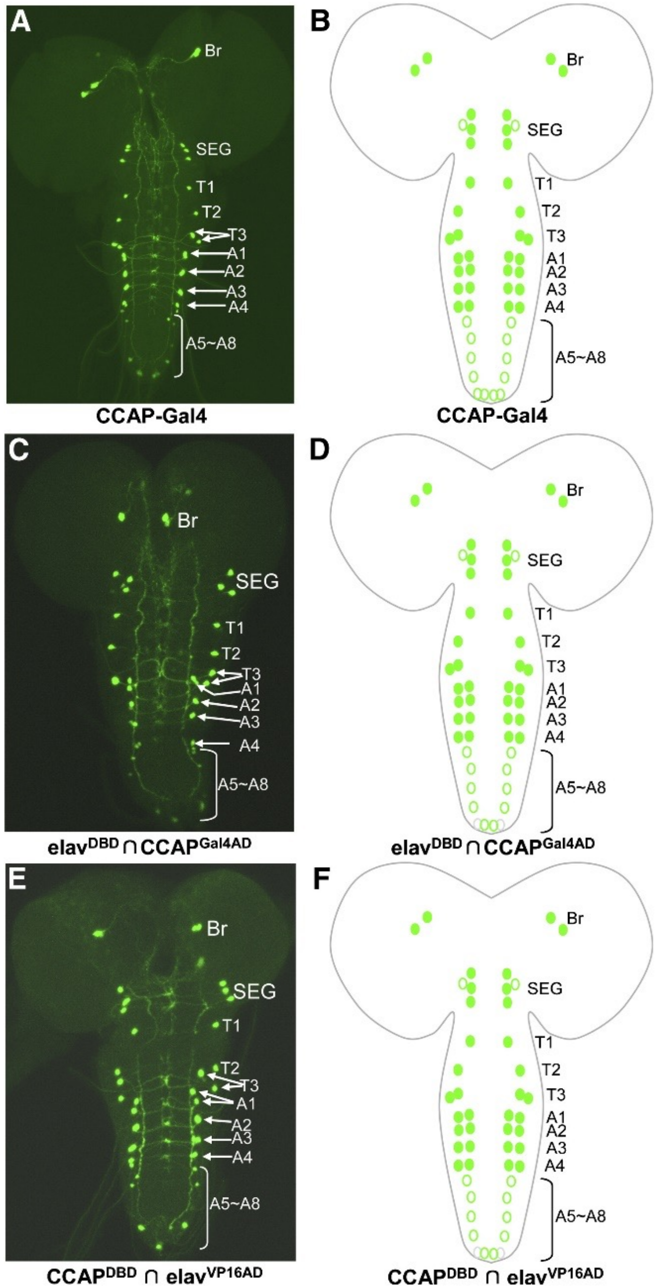
Split-Gal4 system allows to manipulate very small sets of neurons



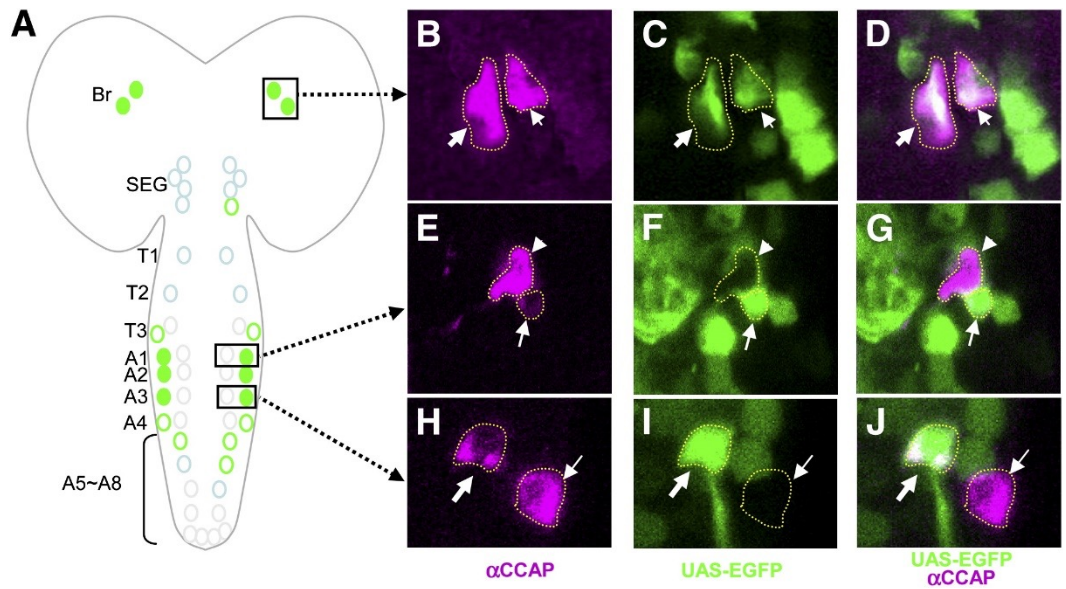
Luan et al. 2006 Neuron

Split-Gal4 system allows to manipulate very small sets of neurons



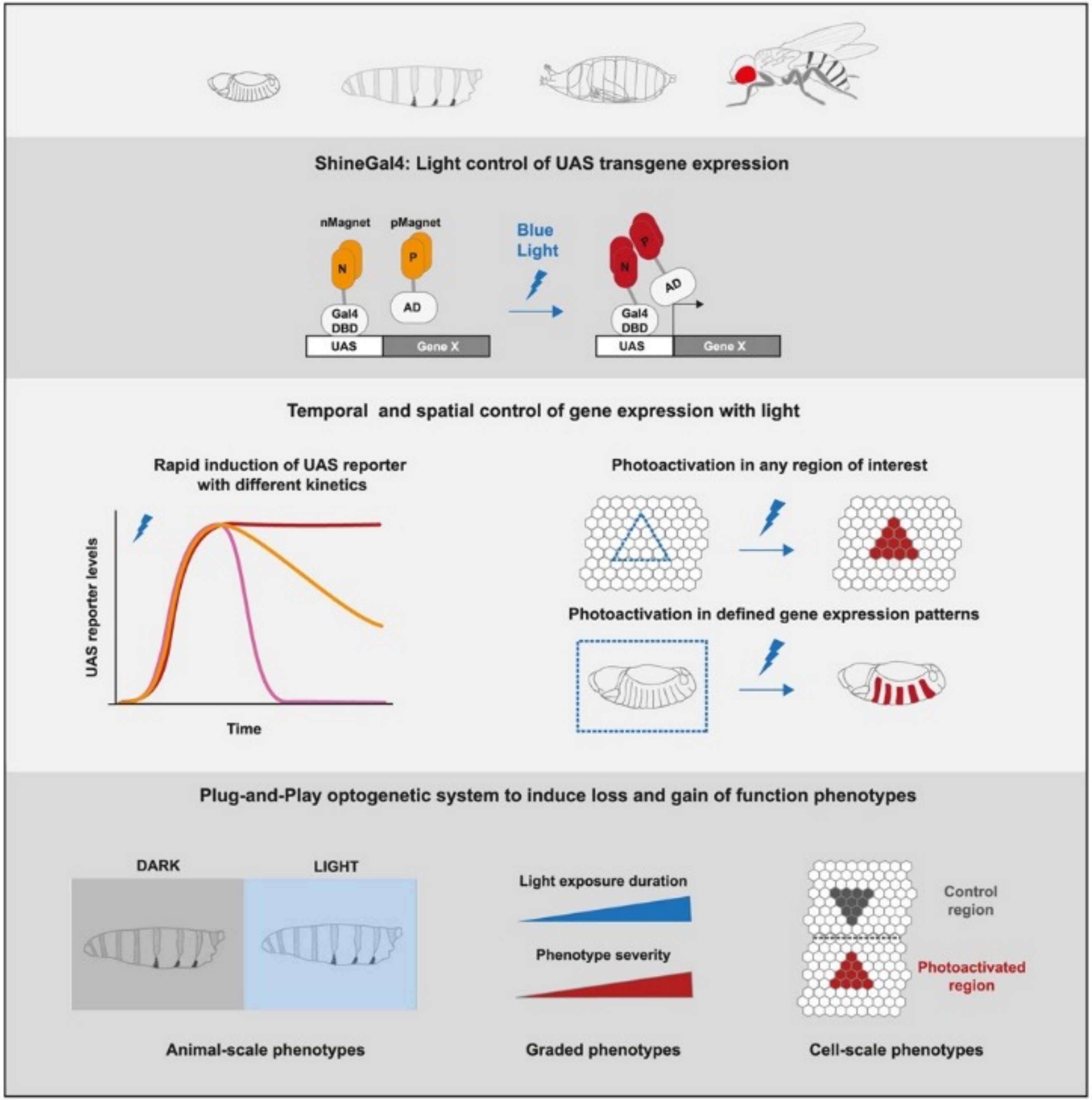
$ChAT^{DBD} \cap CCAP^{GAL4AD}$

- labels those CCAP neurons that use acetylcholine as transmitter (made by choline acetyl transferase (ChAT))

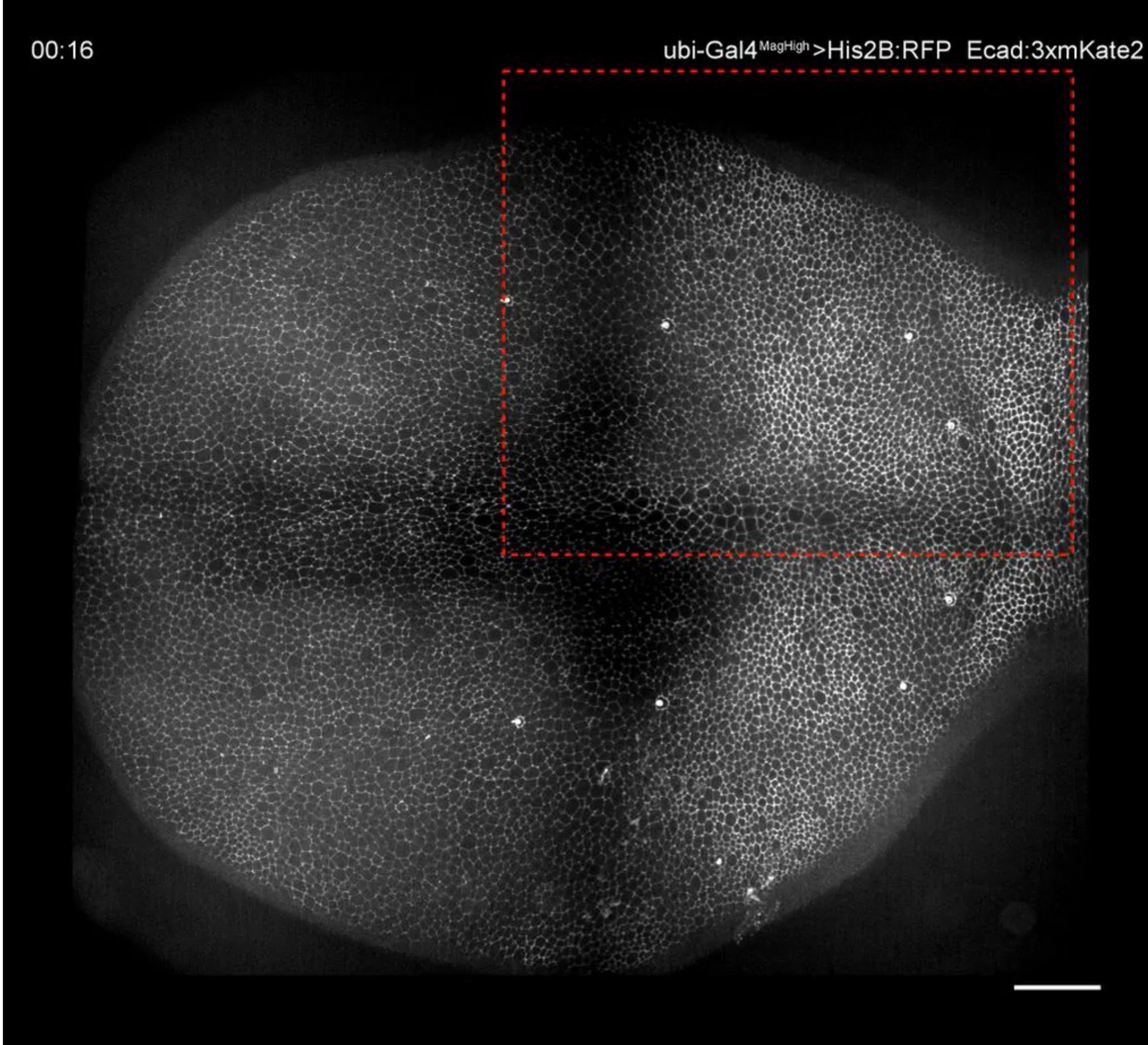


Luan et al. 2006 Neuron

Shine-Gal4: optogenetic manipulation of small sets of neurons

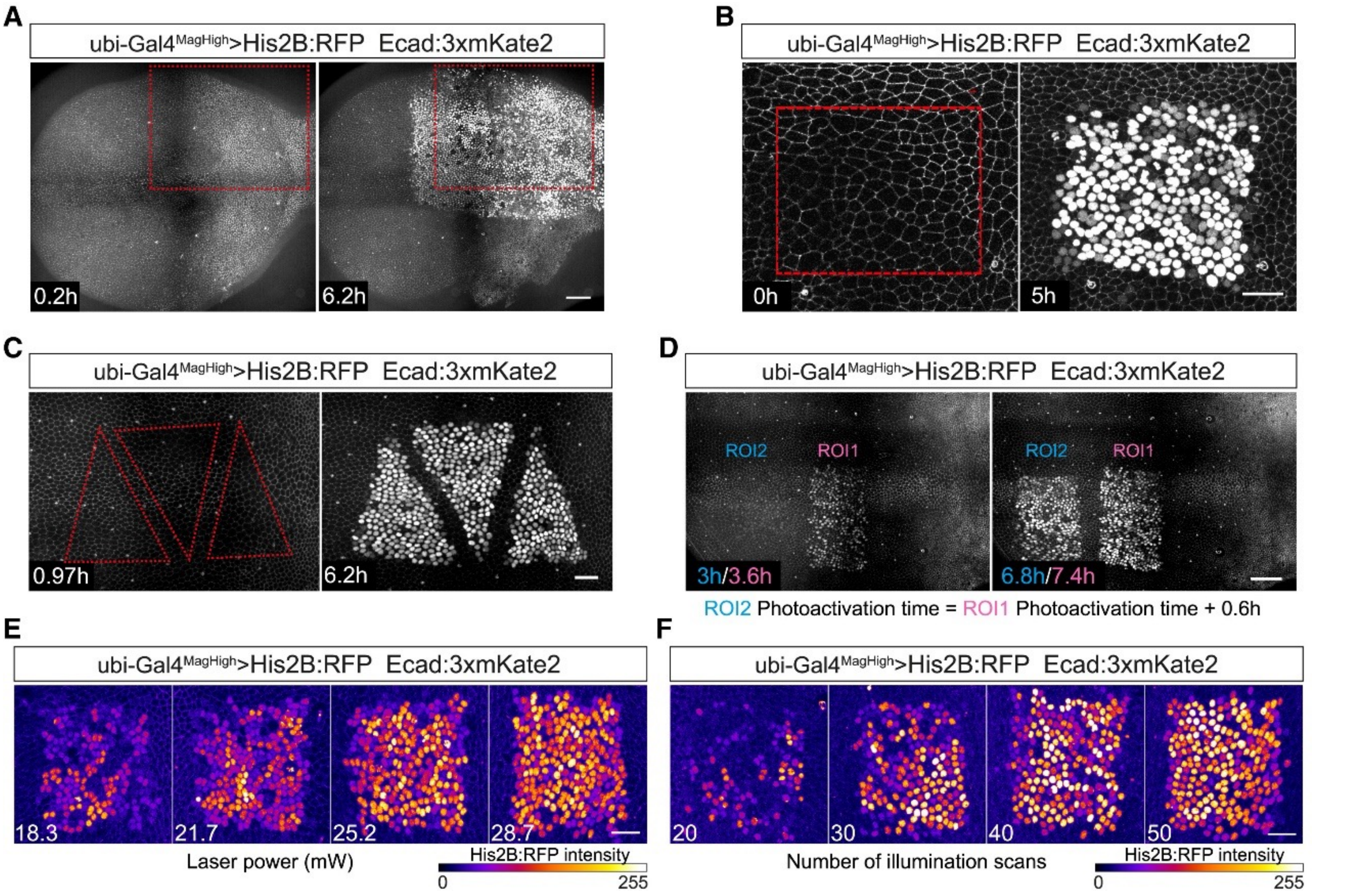


Pietro et al. 2021 Devel Cell



l Cell

Shine-Gal4: optogenetic manipulation of small sets of neurons



Pietro et al. 2021 Devel Cell

Summary

If you want to which neurons control specific behaviour A during your PhD:

- activate/inhibit neuronal activity in candidate neurons
- do behavioural screen

↓

- identify the neurons in the hemibrain/ connectome data bank
- trace pre- and postsynaptic partners

↓

- image neuronal activity in the circuit in behaving intact flies

↓

- be happy 😀 and publish