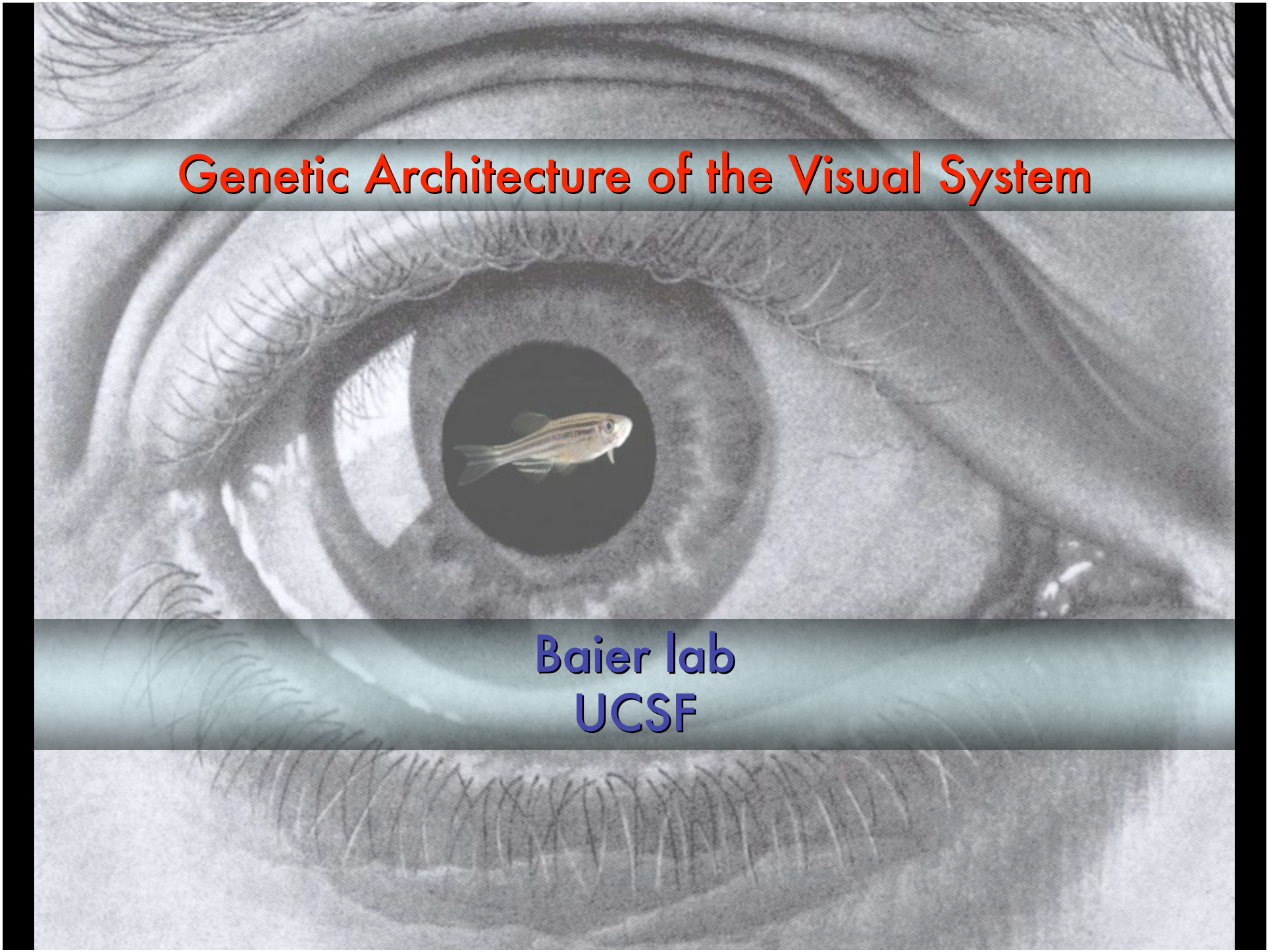
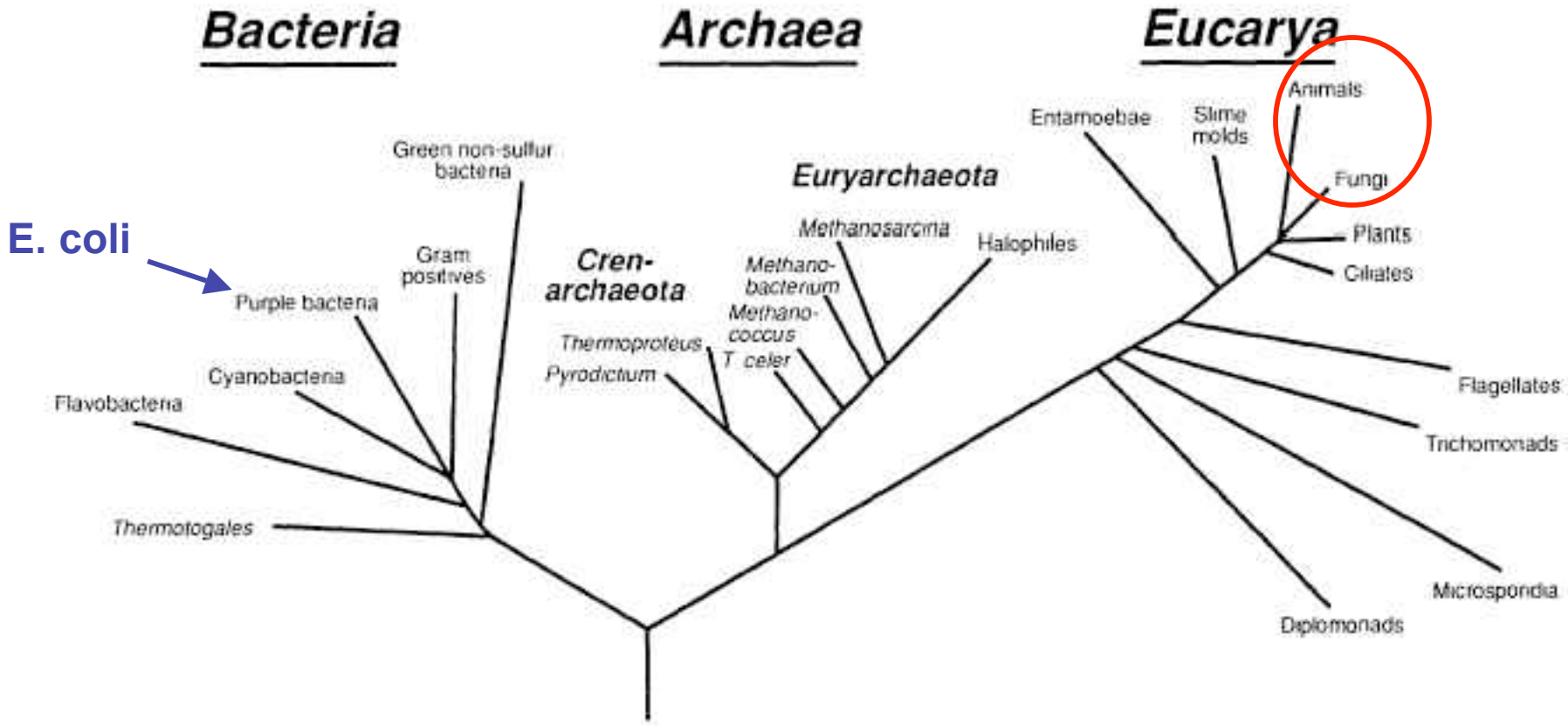
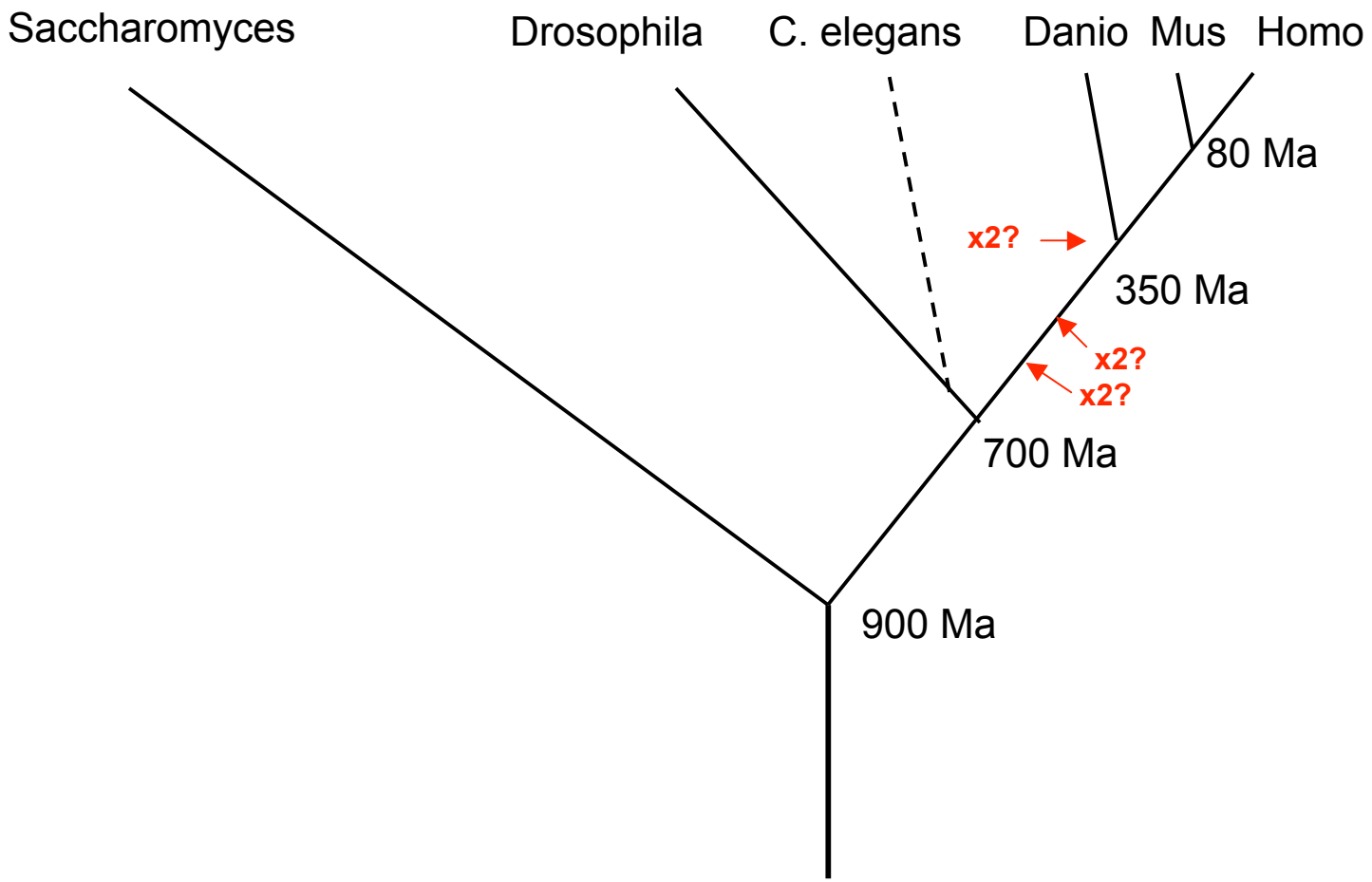

Genetic Architecture of the Visual System
Baier lab
UCSF

The Universal rRNA Tree



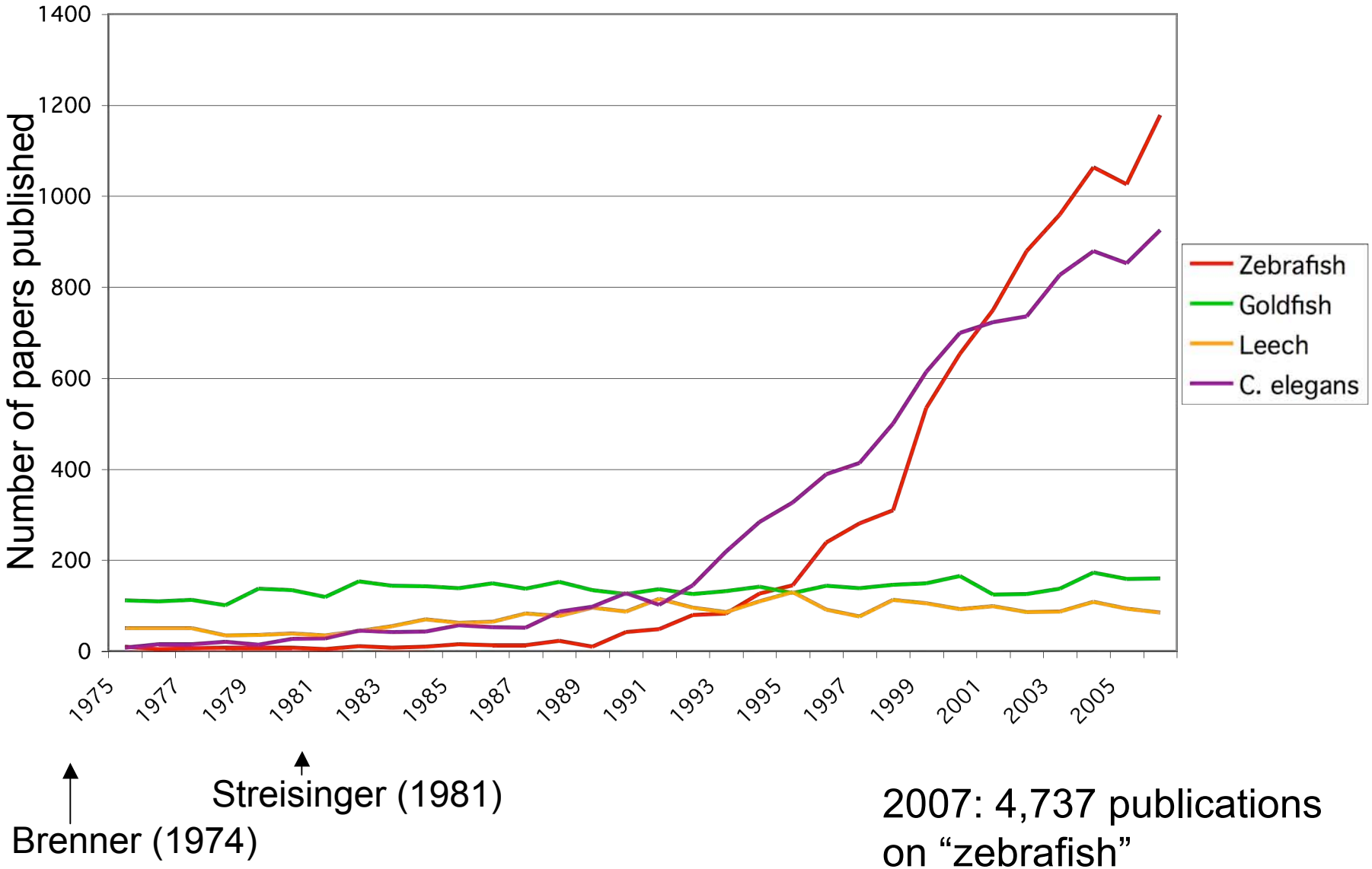
Wheelis et al. 1992 PNAS 89: 2930

Phylogeny of genetic model organisms



(Numbers are based on fossil evidence, molecular clocks, or best guesses.)

Explosion of research literature on genetic models



2007: 4,737 publications on “zebrafish”

Experimental advantages of zebrafish for neuroscience research

1) Genetics

2) Optics

3) Behavior

4) Chemistry

Outline of the talk

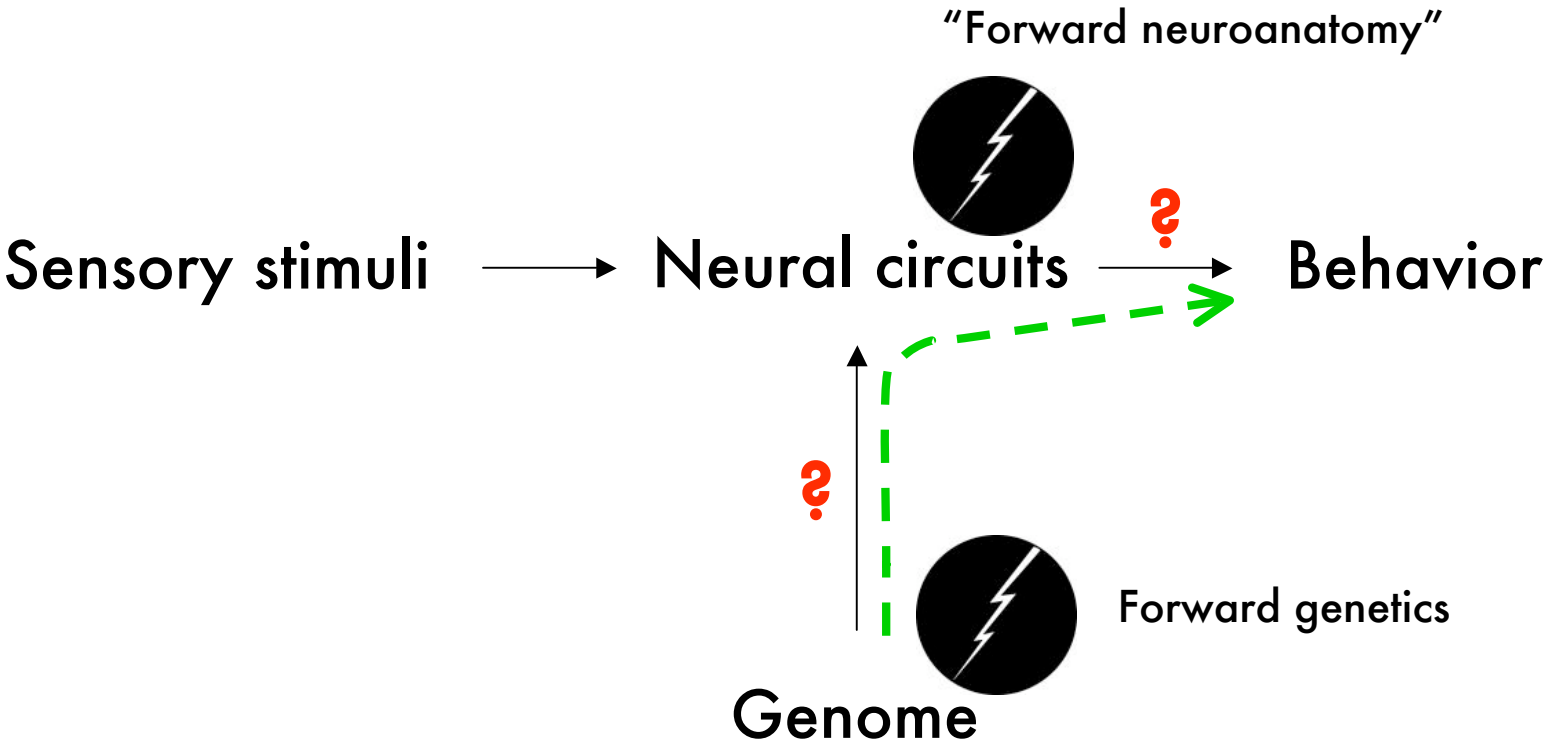
1) Genetic approaches to neural circuits & behavior

2) Assembly and function of retinotectal connections
(*two little stories*)

3) New tools for systematic functional dissection of circuits

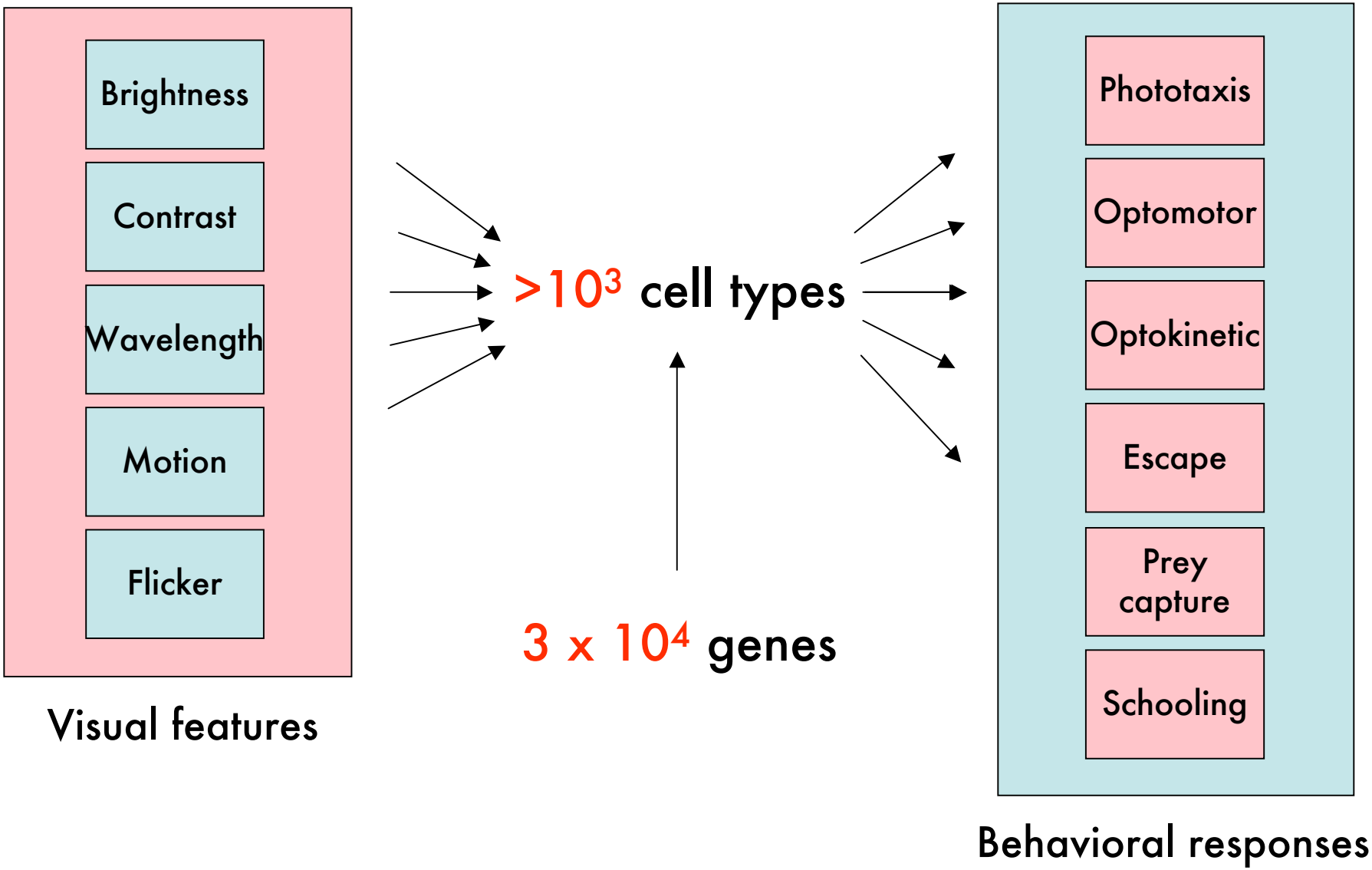
How can we untangle the brain?

Unbiased genetic approaches to circuit assembly & function

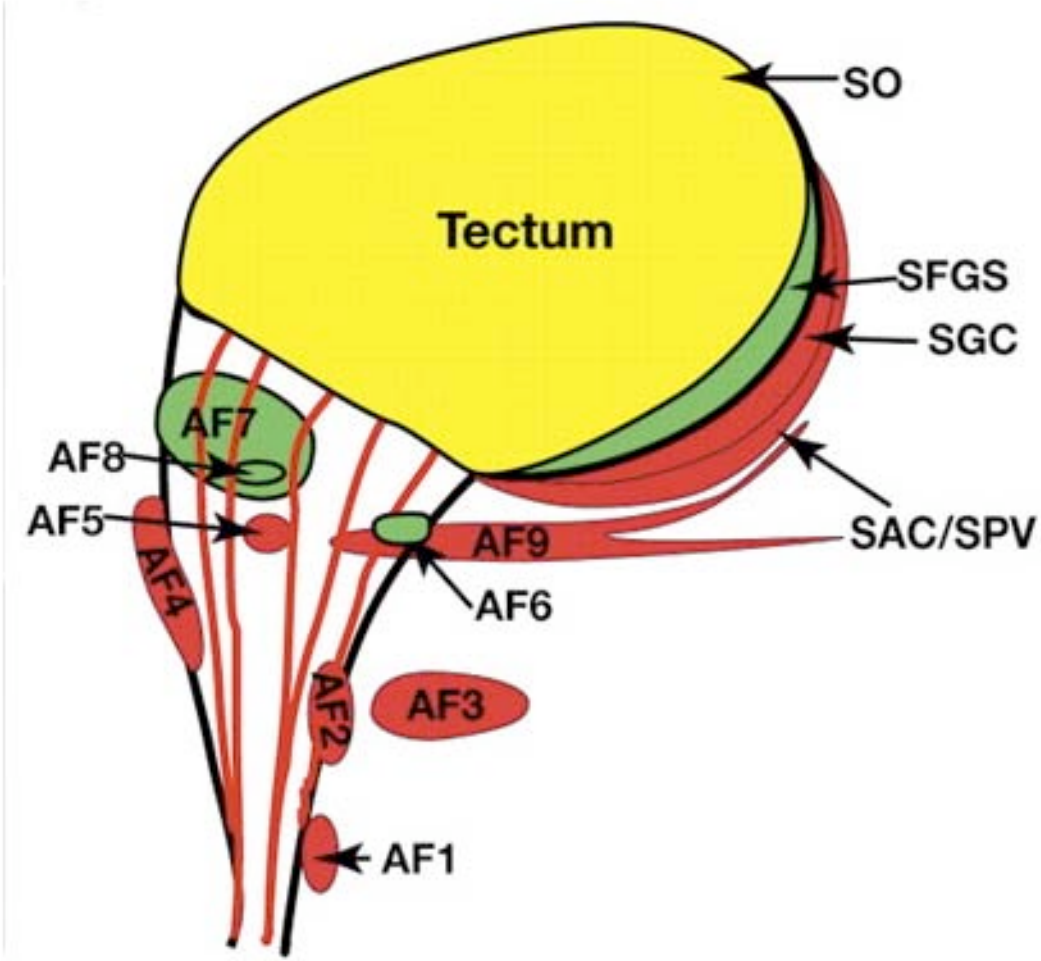


Phenotype-based systems neuroscience

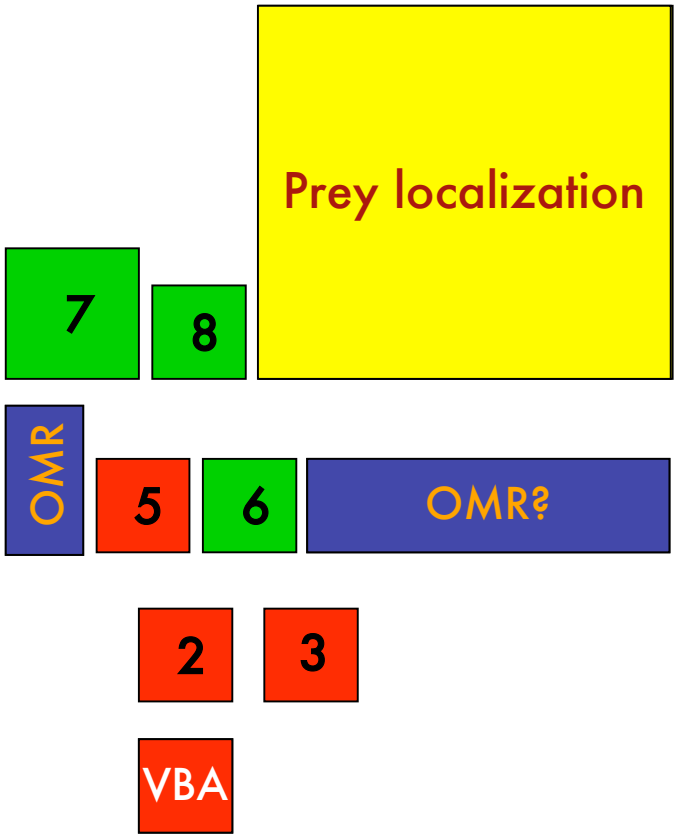
Zebrafish visual system



Ten visual pathways in zebrafish



From retina

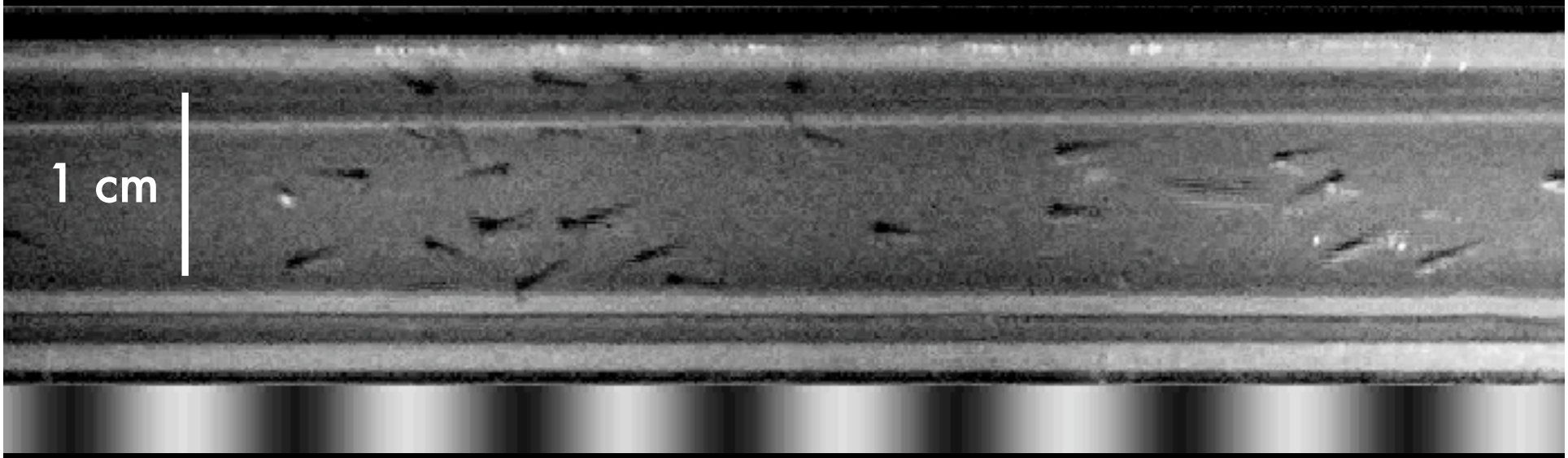


The zebrafish visual system develops on a tight schedule

Day 0: Fertilization
Day 1: Retinal ganglion cells born
Day 2: Retinal axons leave eye
Day 3: First visual responses
Day 4: Retinotectal map complete
Day 5: Optomotor behavior
Day 6: Visually guided prey capture

How are specific synaptic connections formed in such a short time?

Optomotor response (OMR)



2x speed

Mike Orger

High-throughput optomotor assay

Time

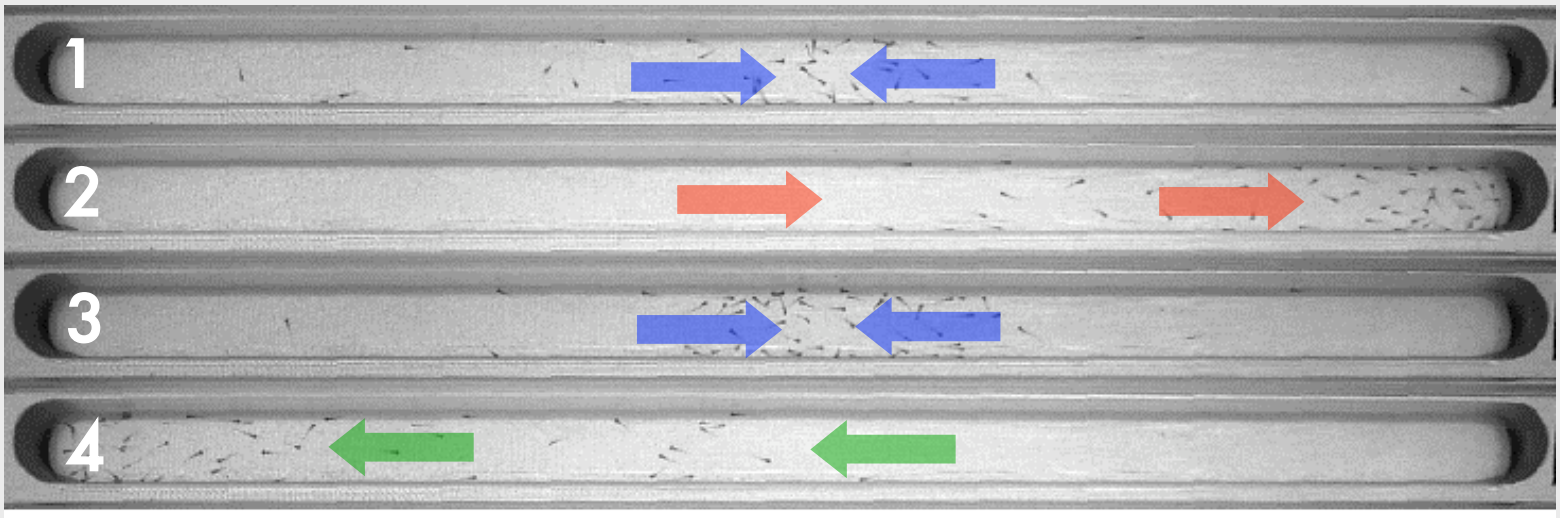
Strong motion stimulus

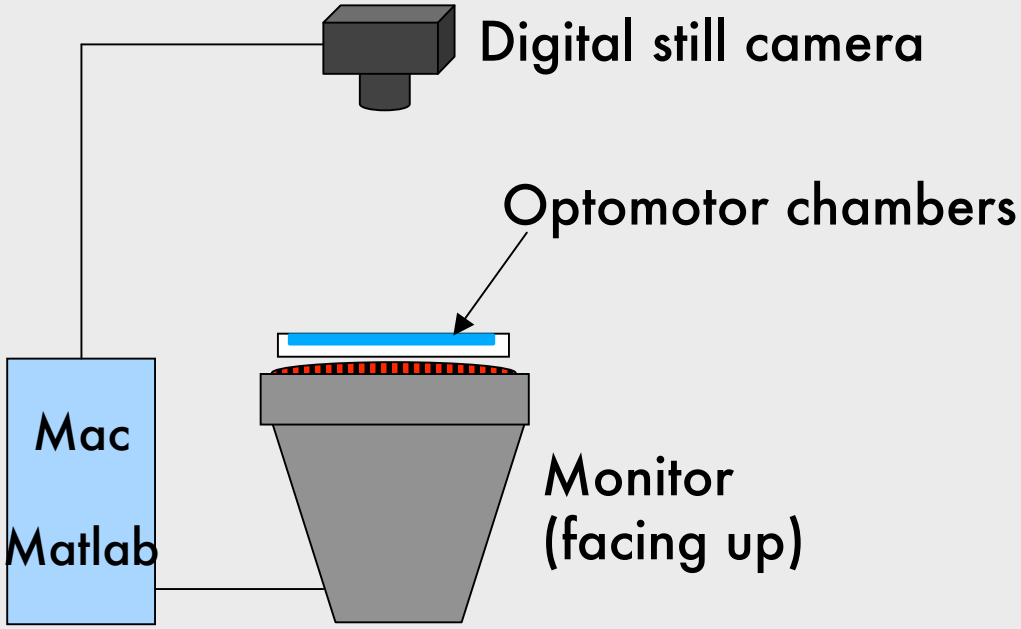
Test grating, e. g. high spatial frequency

Strong motion stimulus

Test grating, e. g. low contrast

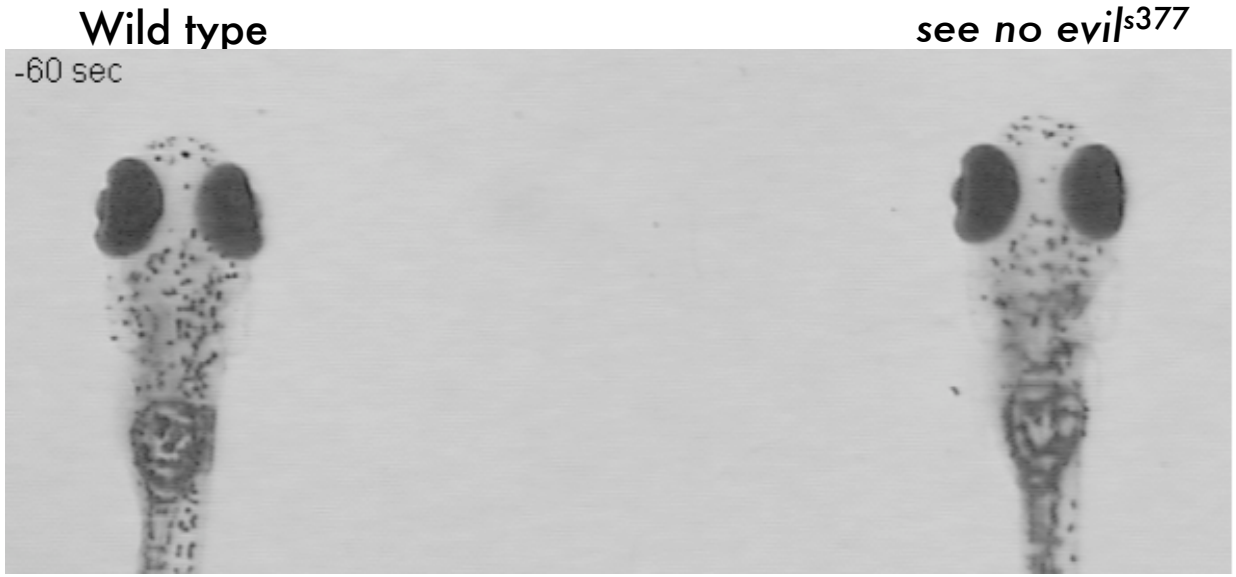
....

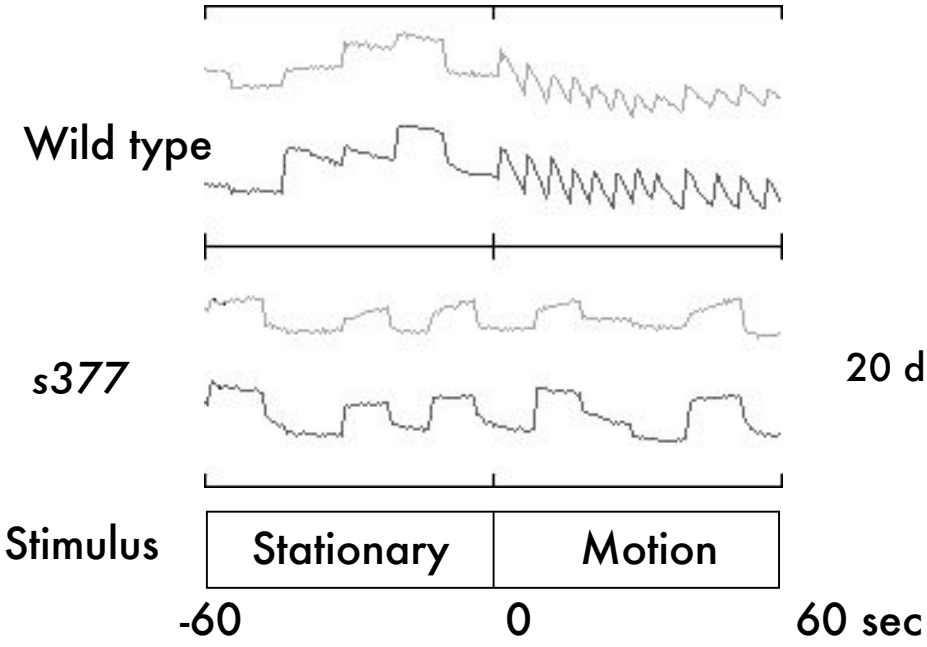


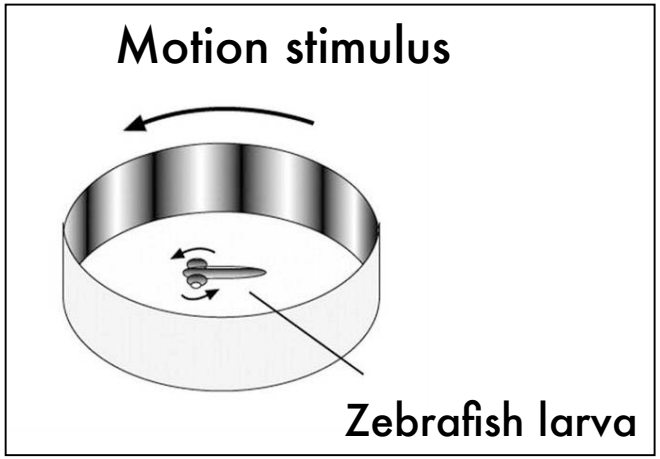


Mike Orger, Matt Smear

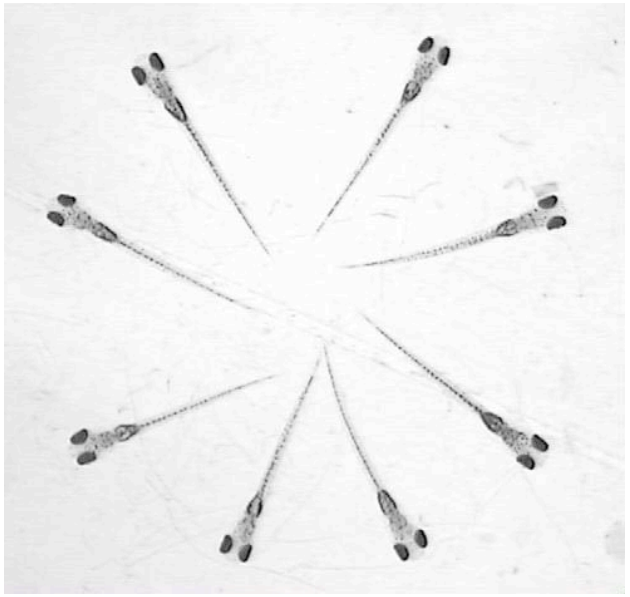
Optokinetic response (OKR)

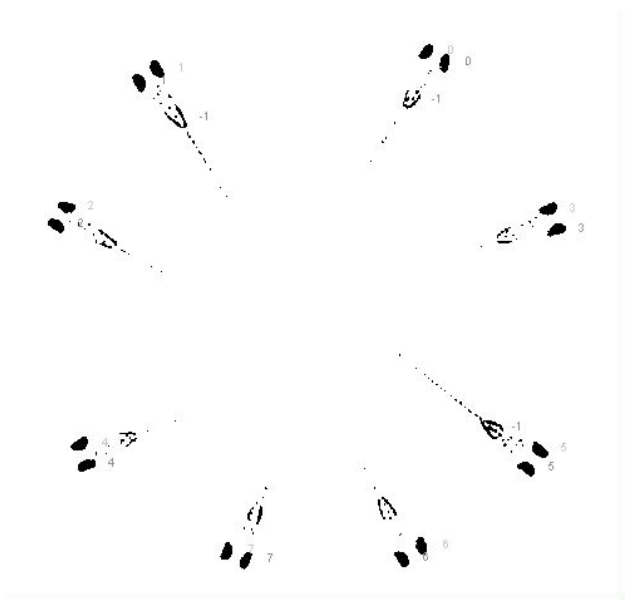


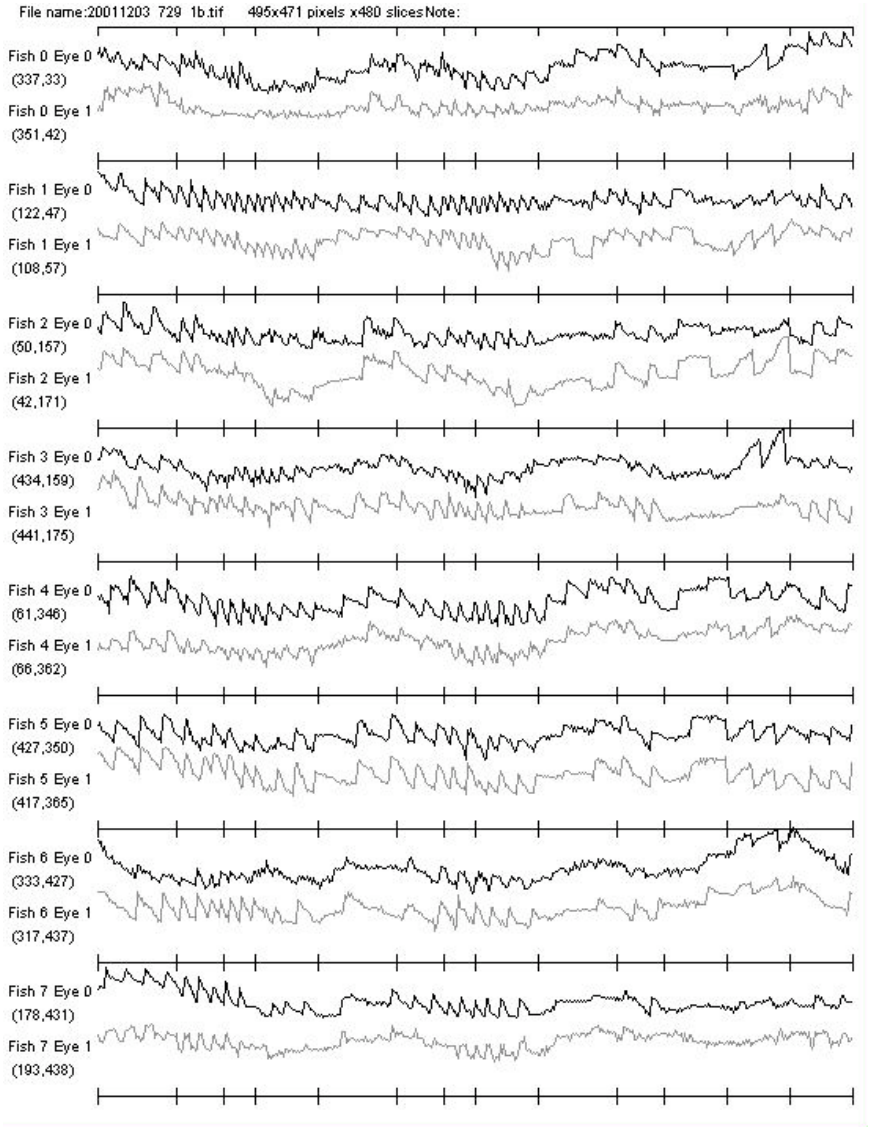




High-throughput OKR assay

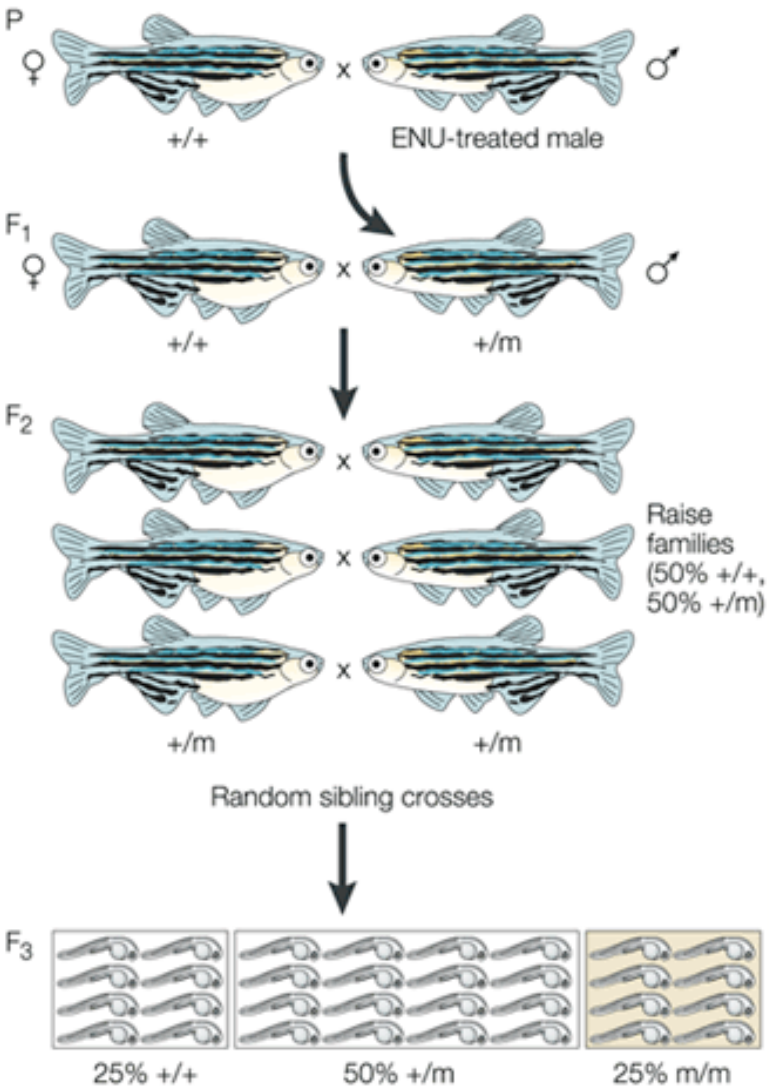




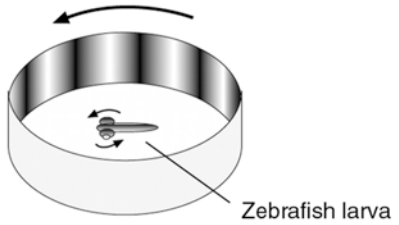


Akira Muto

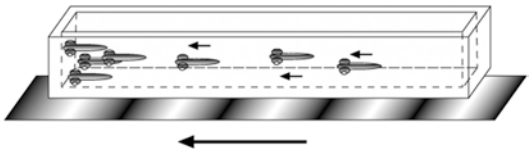
Large-scale ENU-mutagenesis screen for mutations disrupting visual responses



The optokinetic response (OKR)



The optomotor response (OMR)



1,988 F2 families (8,000 crosses; >1,000,000 fish larvae)

82 mutations in 55 genes

Degree of saturation: 25-40%

The zebrafish retinotectal map

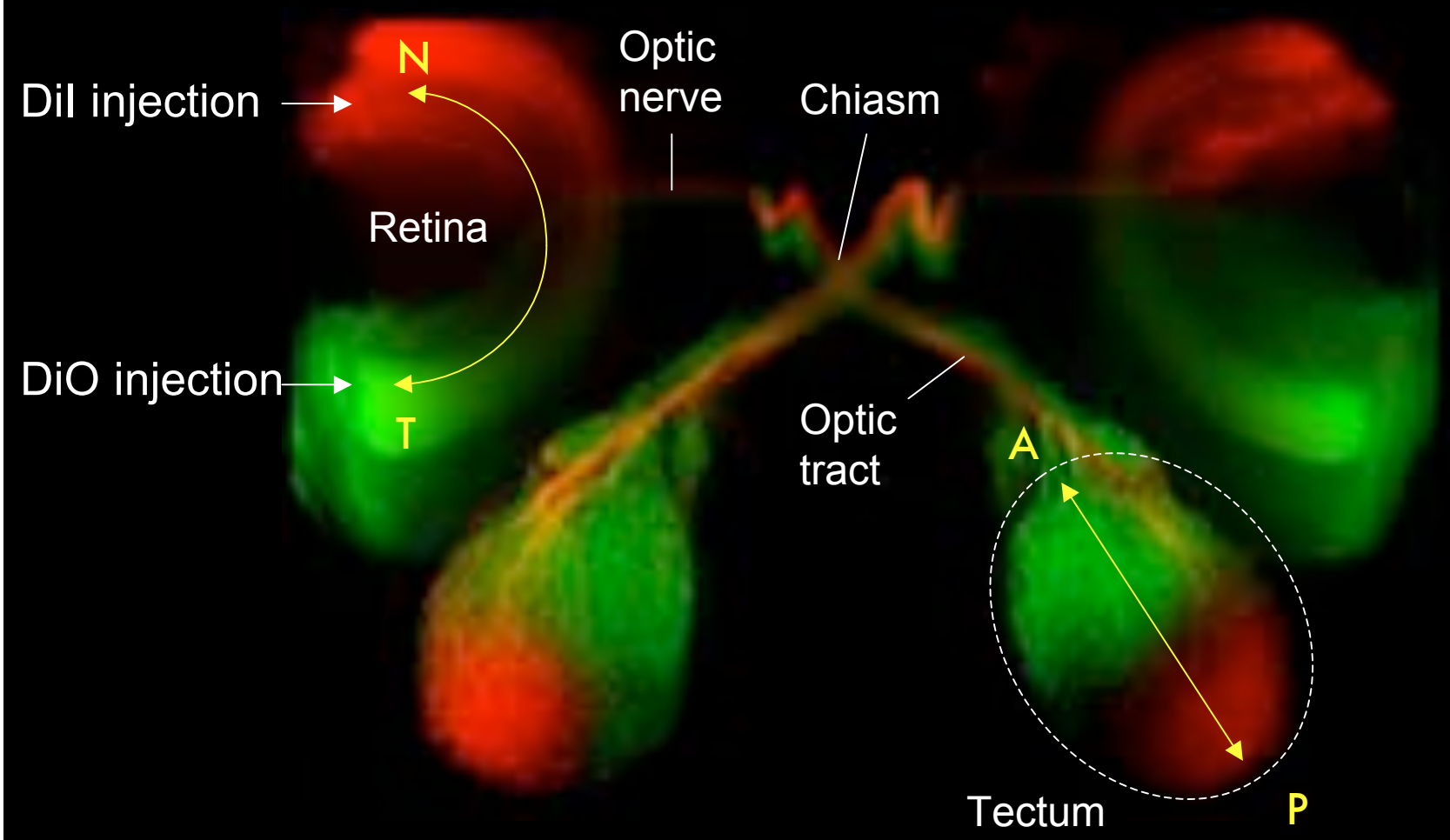
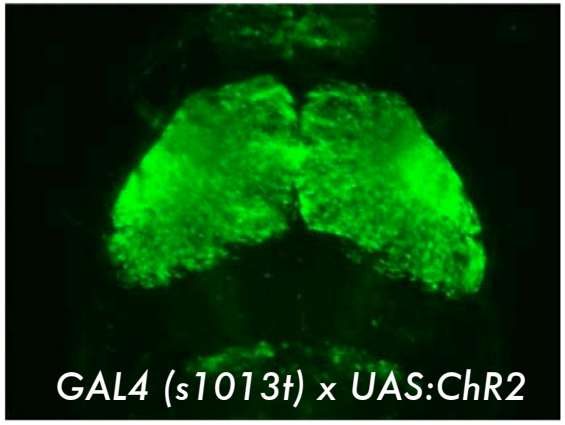
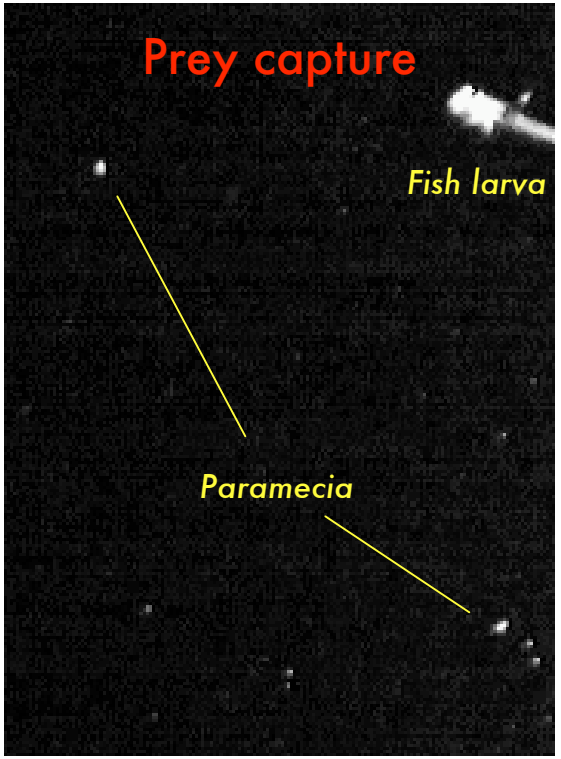


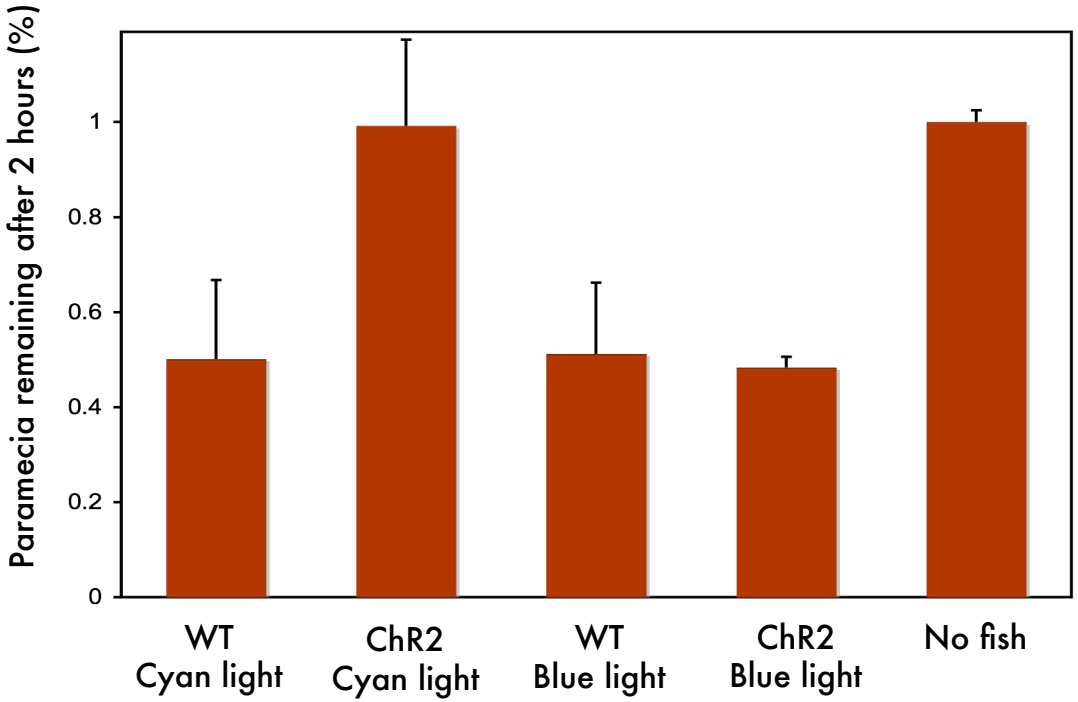
Image by Chi-Bin Chien

The retinotectal map is important for localizing small objects

- Tectum is required for prey capture (*not* for optomotor response) [Roeser et al., 2003; Gahtan et al., 2005]
- Precision of retinal axon targeting limits visual acuity [Smear et al., 2007]
- Perturbation of neuronal activity in the tectum with channelrhodopsin disrupts prey capture (*not* the optomotor response)

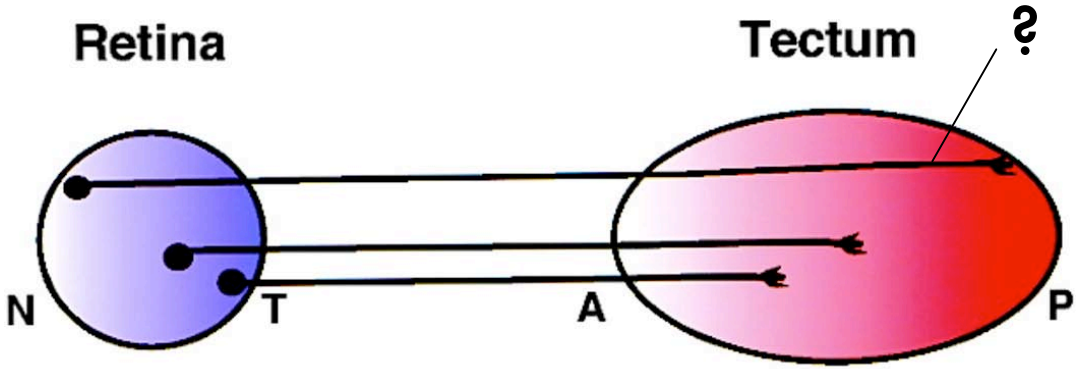




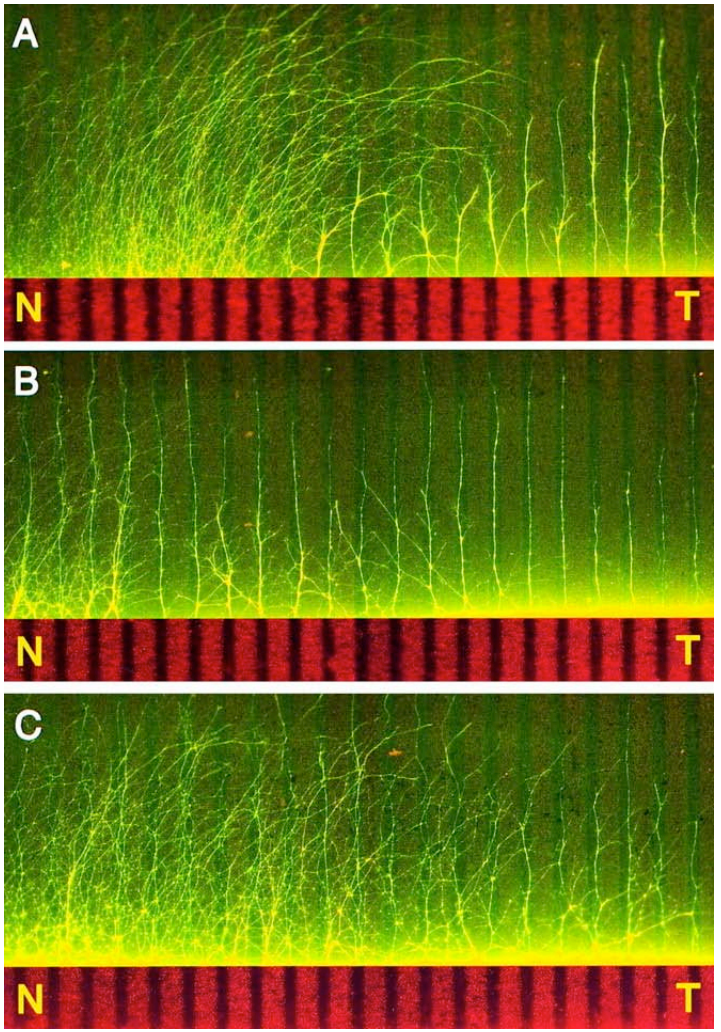


Filo Del Bene, Claire Wyart

What drives nasal axons into the posterior tectum?



Friedrich Bonhoeffer's stripe assay



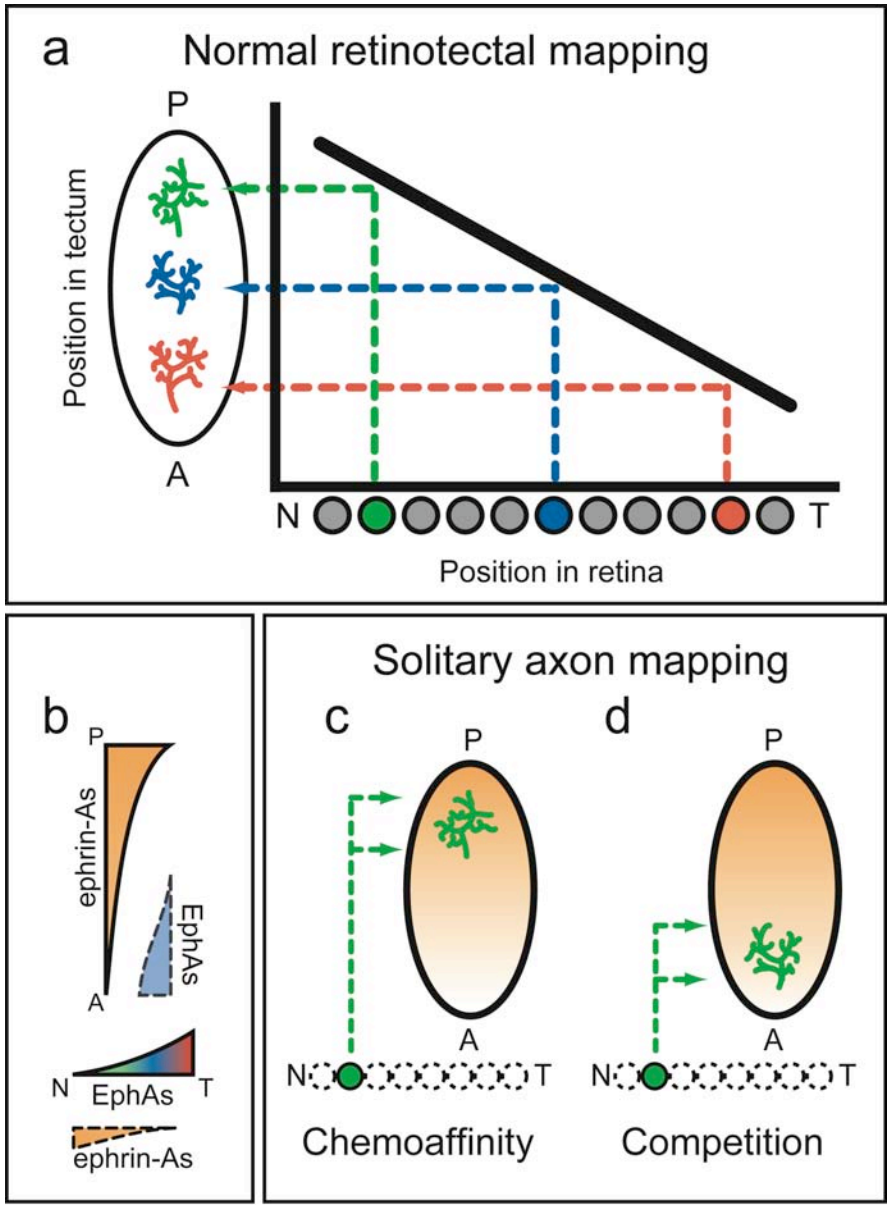
Monschau et al. (1997)

Two possibilities:

1) Axon-axon interactions (e.g., competition)

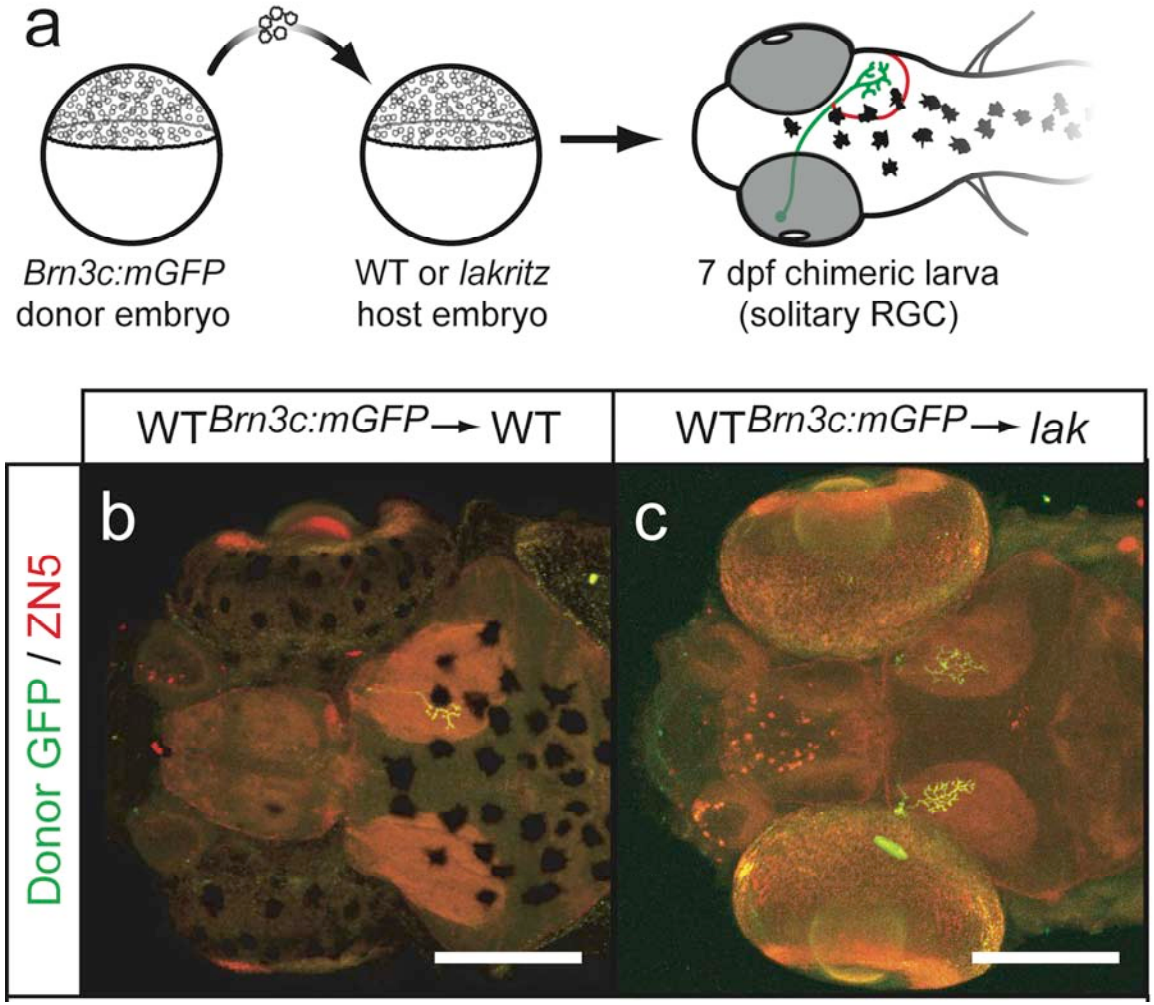
2) Pure chemoaffinity (e.g., a second gradient)

Where will a solitary axon go in the tectum?



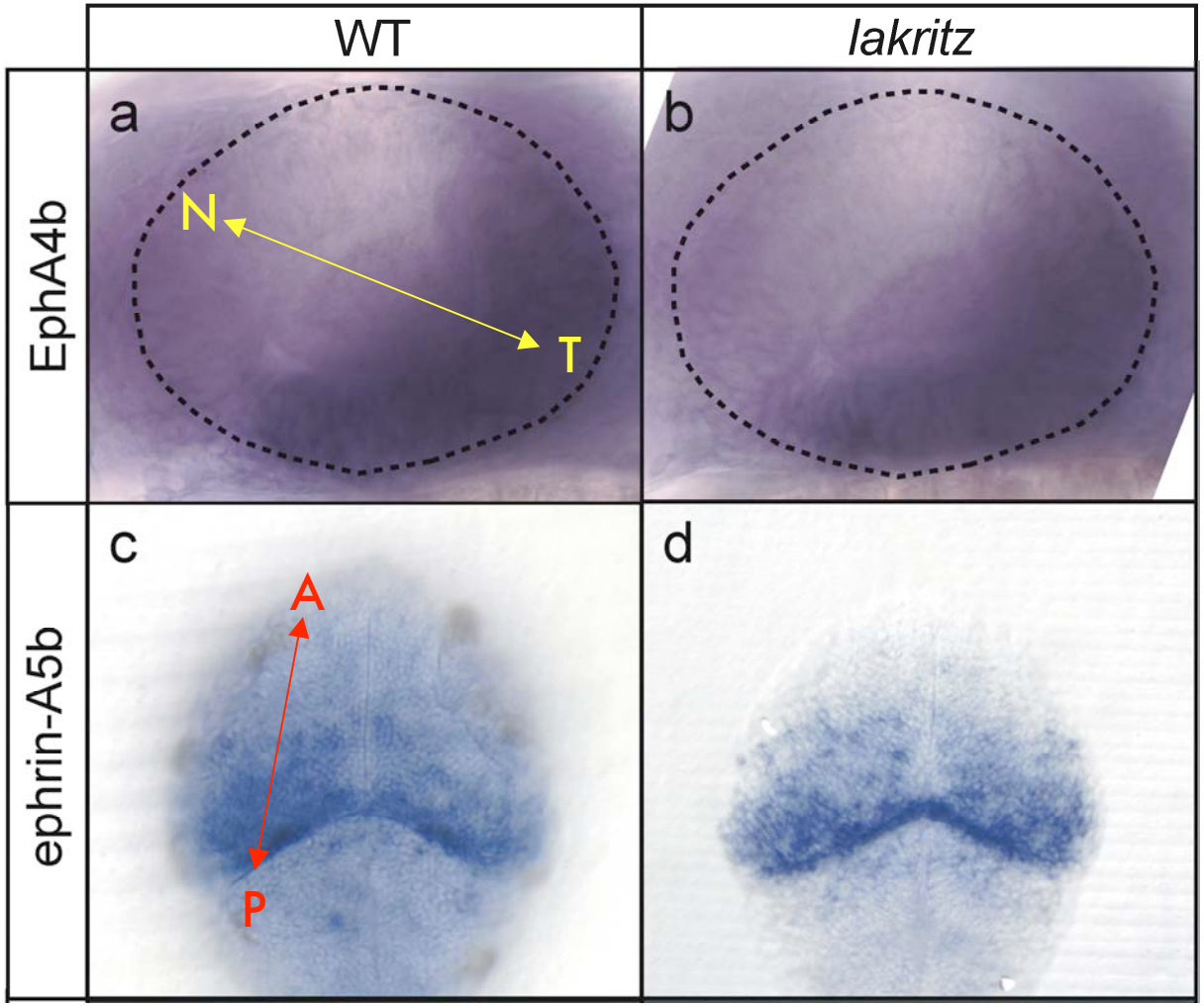
Nathan Gosse

Experiment: Create retina with just one ganglion cell

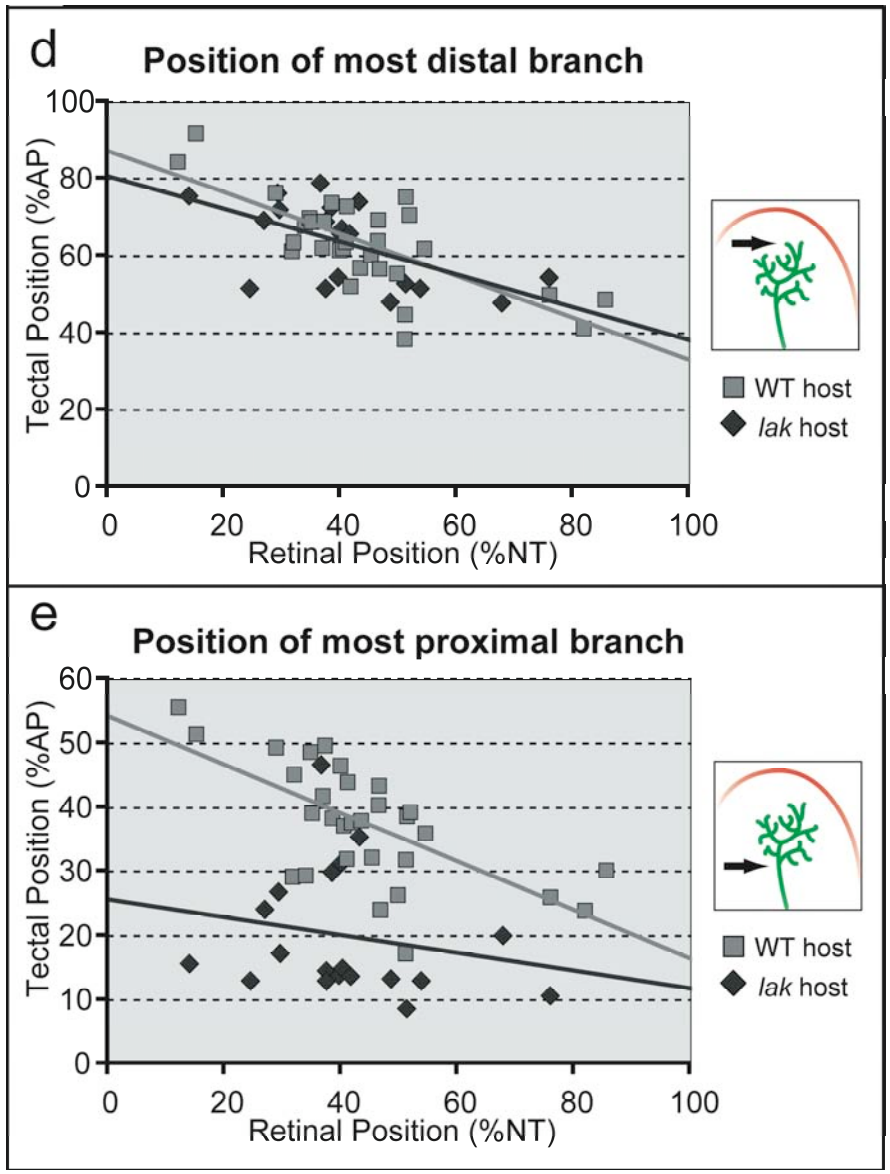


Zebrafish carrying the *lak* (*ath5*) mutation are unable to generate ganglion cells.

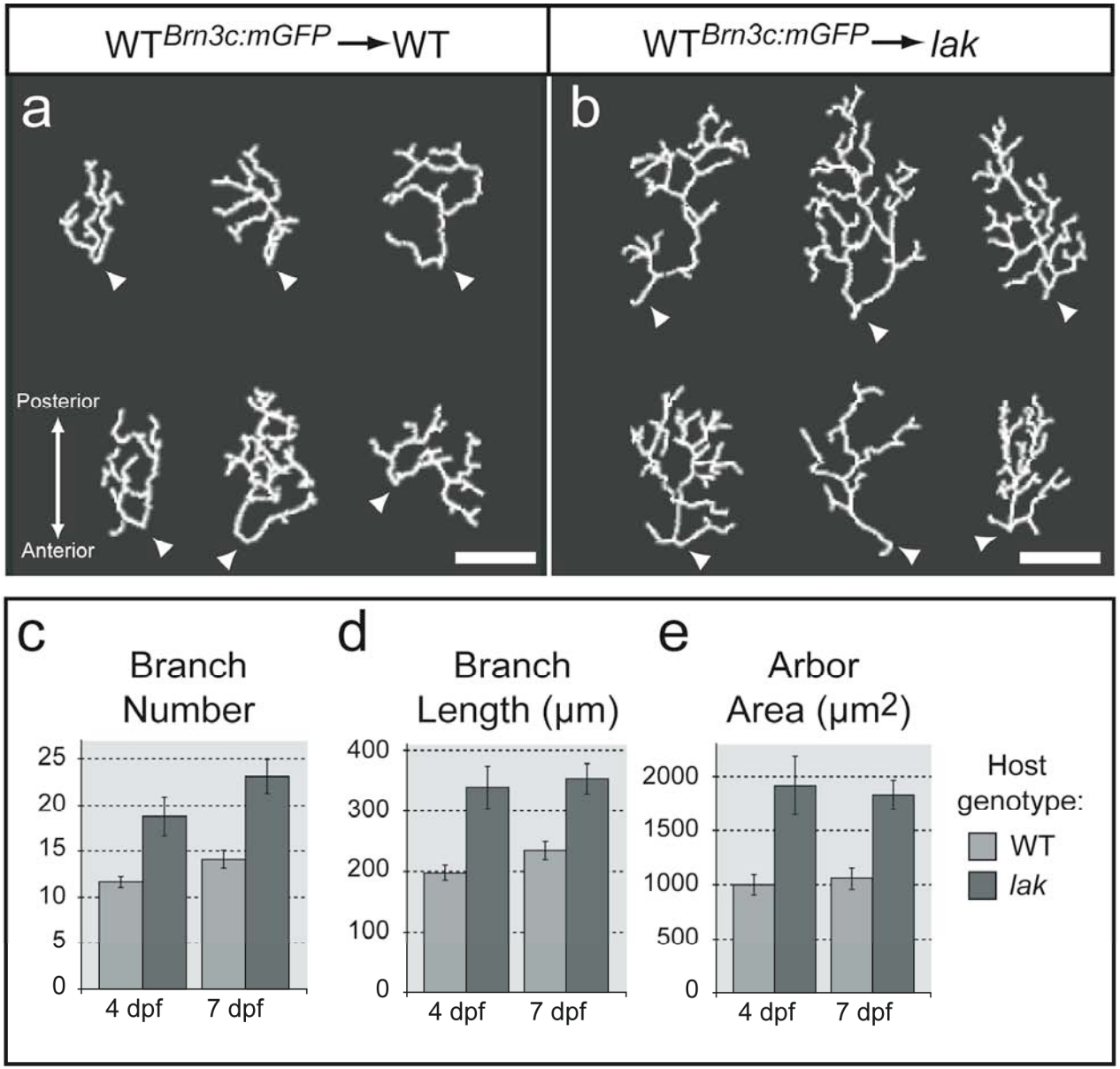
The *lak/ath5* retinal and tectal axes are properly patterned



Retinotopic order of solitary axons is maintained



Axon arbors grow larger and more complex in the absence of other axons



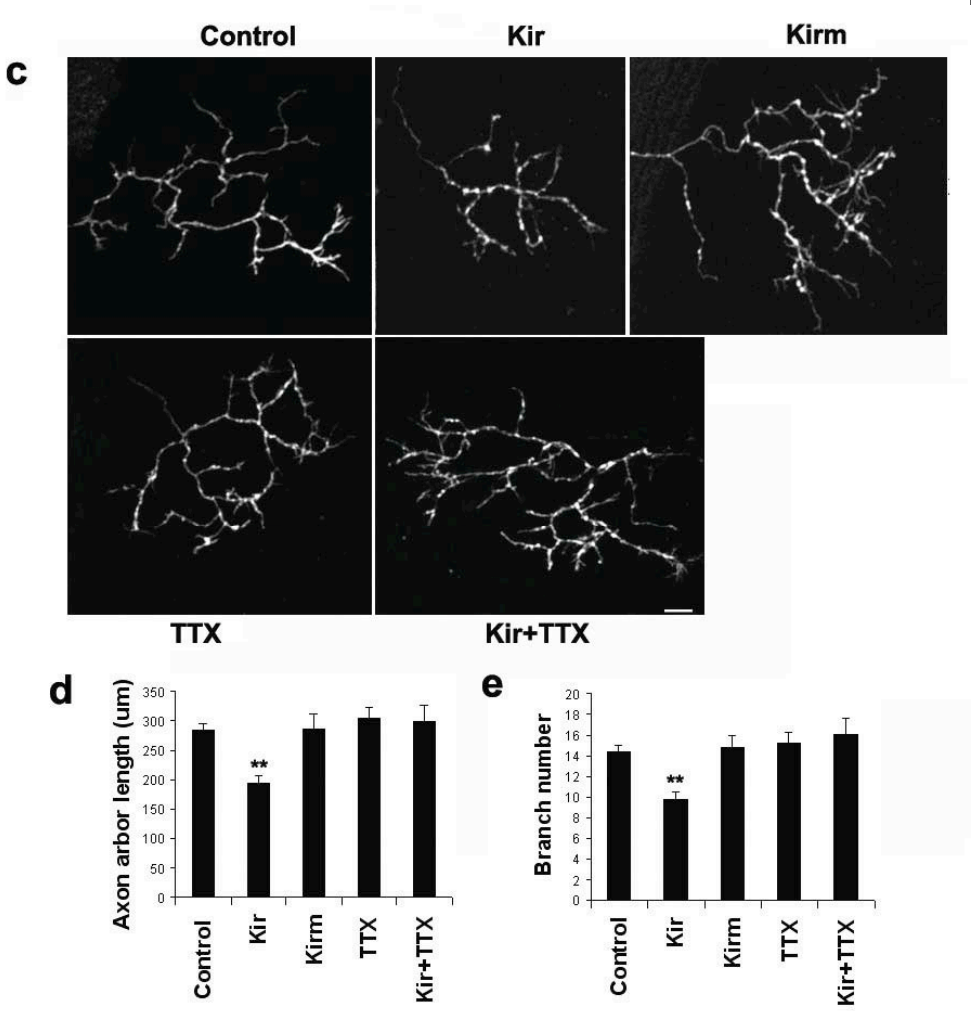
Triple role for activity in shaping axonal arbors

- Activity-based competition reduces arbor size of inactive axons among normally active axons (Hua et al., 2005).

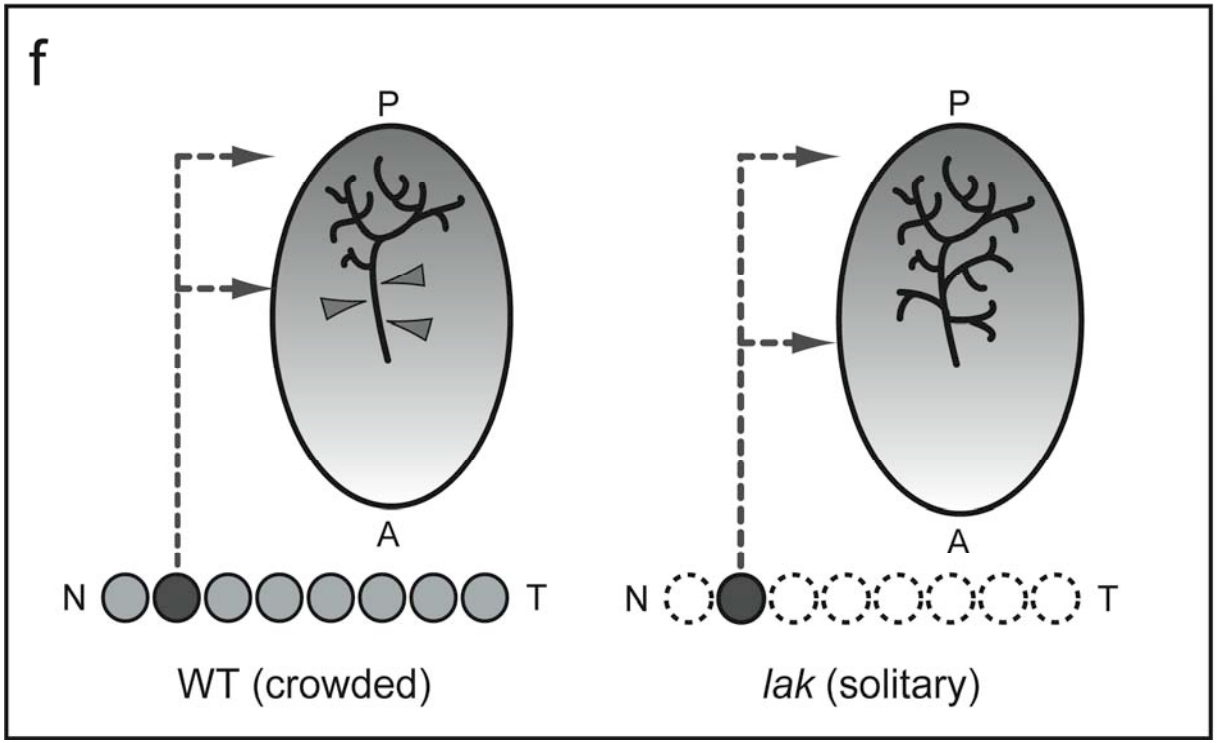
- Homeostasis increases arbor size of less active axons in *blu/vglut2a* mutants (Smear et al., 2007).

- Correlated activity stabilizes overlapping arbors by Hebbian mechanism (Poo and Cline labs).

Either lack of competition and/or homeostasis could produce the solitary-axon phenotype.



Summary of this part



- Solitary axons project to their retinotopically appropriate targets.
- Competition or other axon-axon interactions are *not required* for map formation.
- Axon-axon interactions have strong effects on axon arbor size.

Discussion points/Caveats

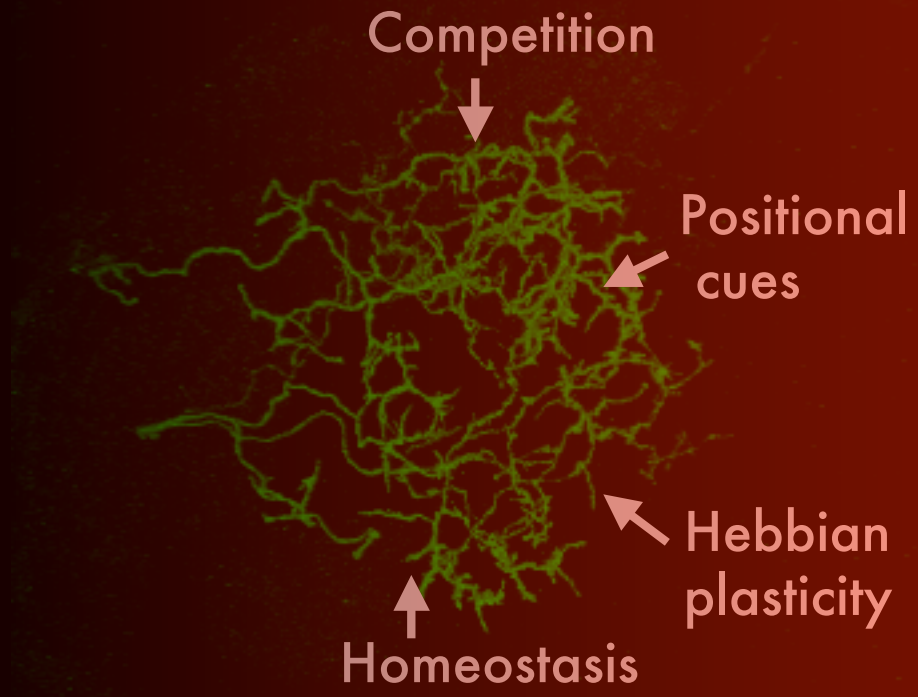
- Second gradient could be provided by EphA/ephrin-A signaling or other factors.

- Pathfinding to the tectum is not completely axon-autonomous (e. g., errors in the retina).

- Competition may still be important for mapping in crowded condition.

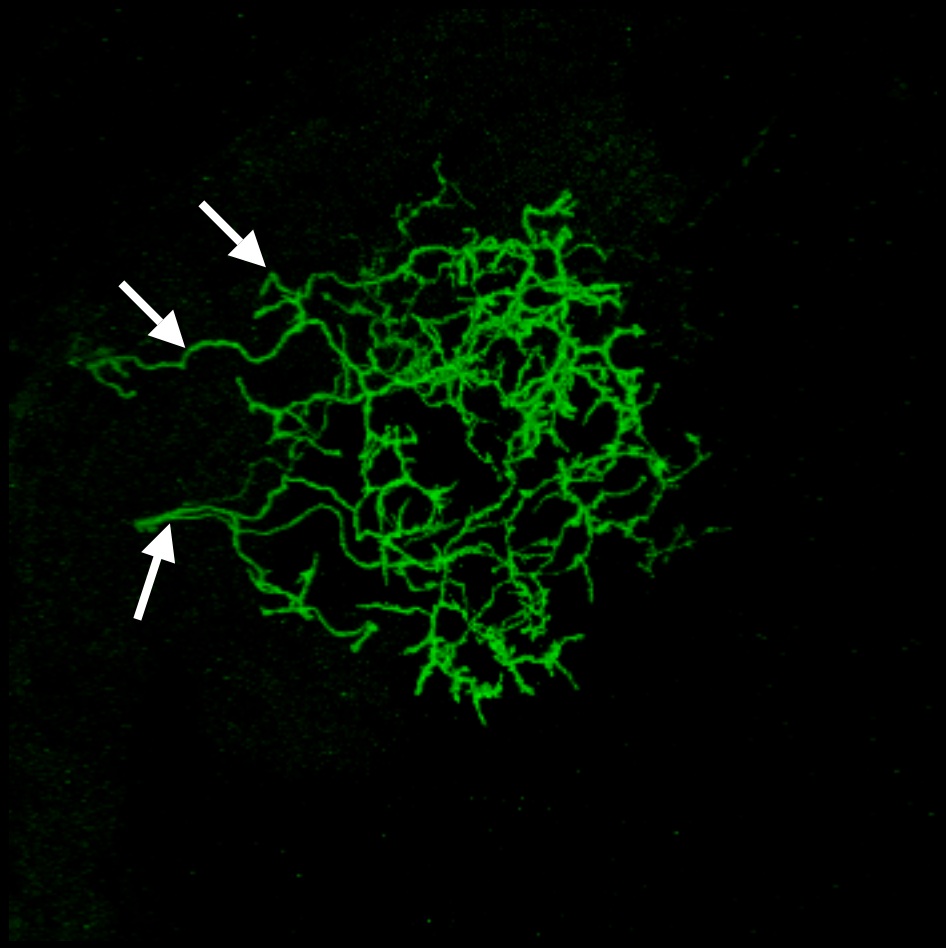
- Evolutionary view of species differences: Slow intrauterine development, delayed reliance on vision, and nocturnal lifestyle may have relaxed selection pressure on chemoaffinity mechanisms in mammals.

Retinotopic Mechanisms

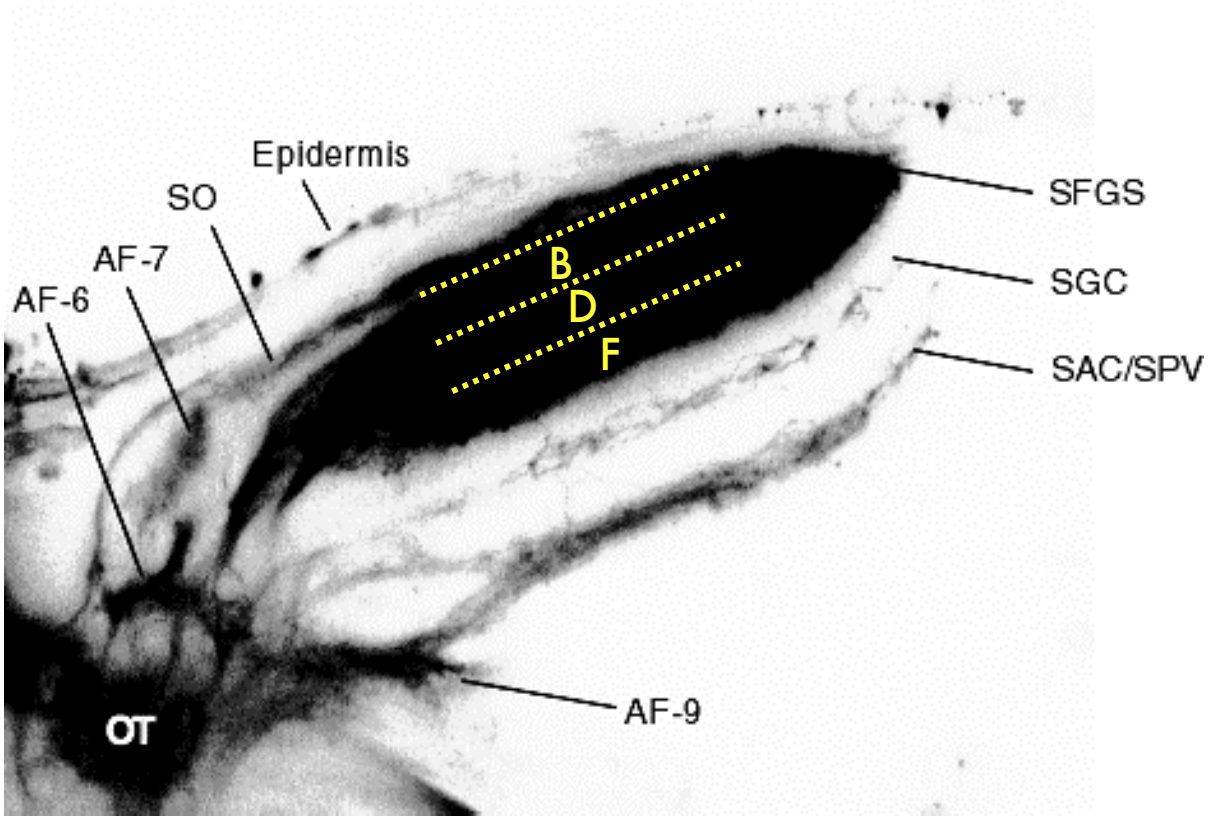


Ephrin-A

Planar organization of axonal arbors: Mechanisms?



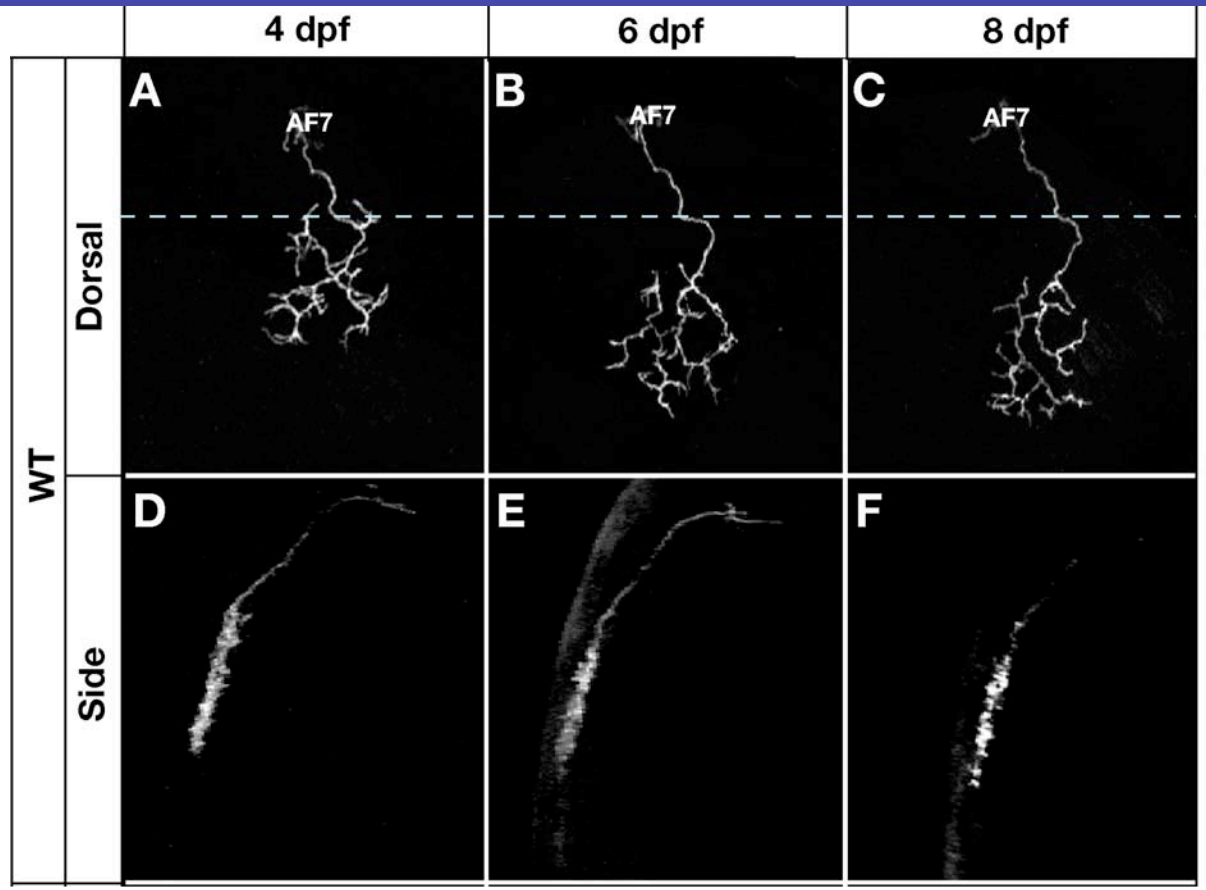
Retinal input to the tectum is organized in four layers



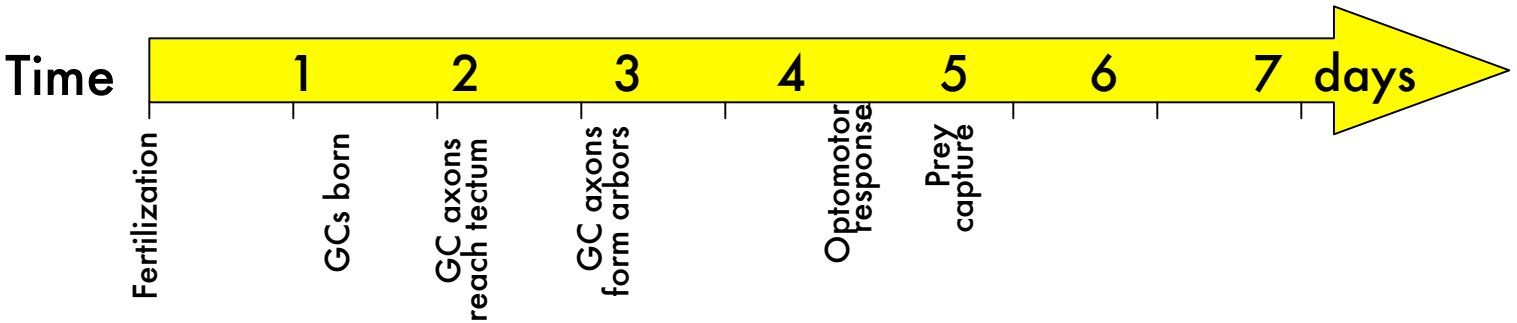
Whole-eye DiO fill of all ganglion cells

Tobias Roeser

Retinal axon arbors are targeted precisely and remain confined to one lamina during remodeling and shifting

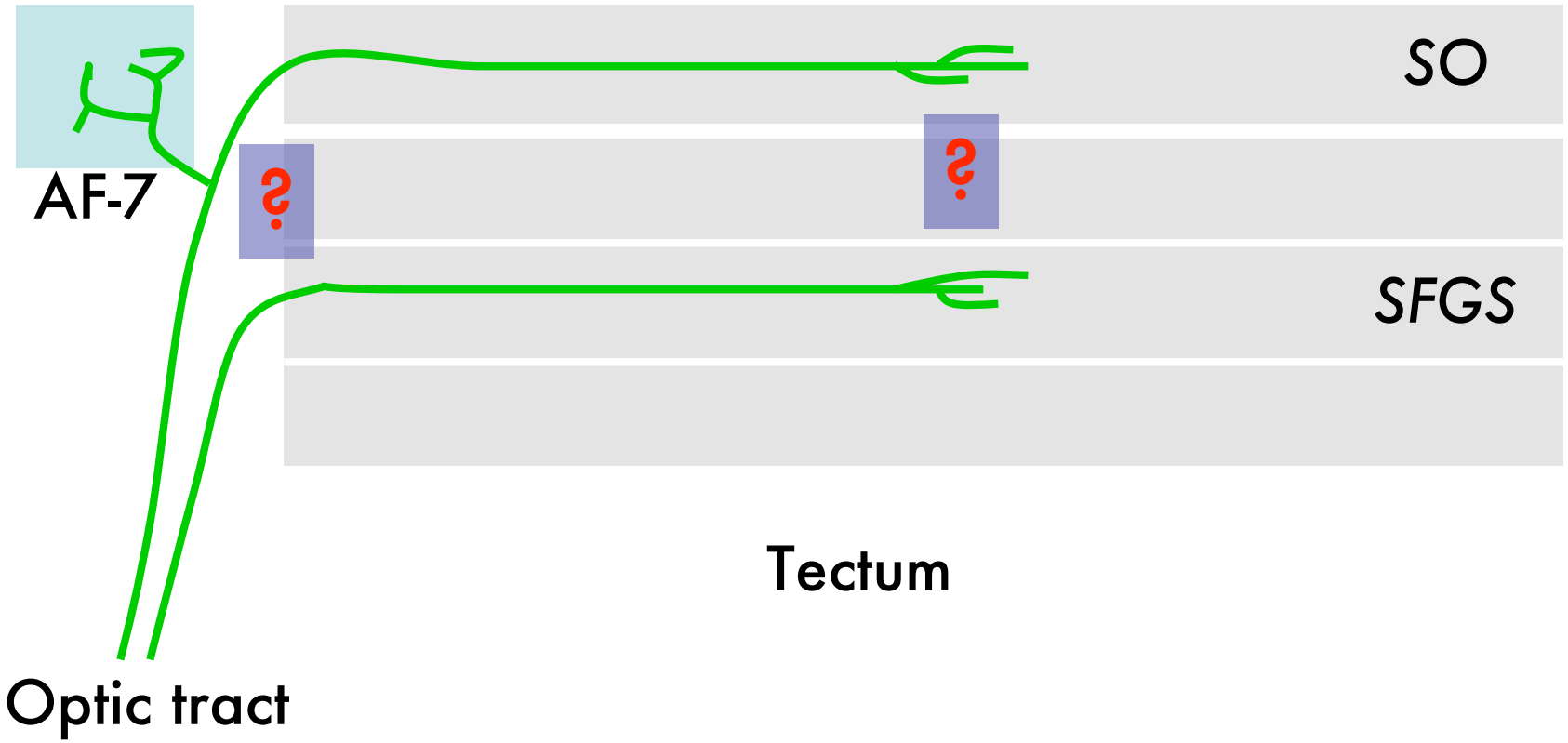


Variegated *Brn3c:GAL4; UAS:mGFP*

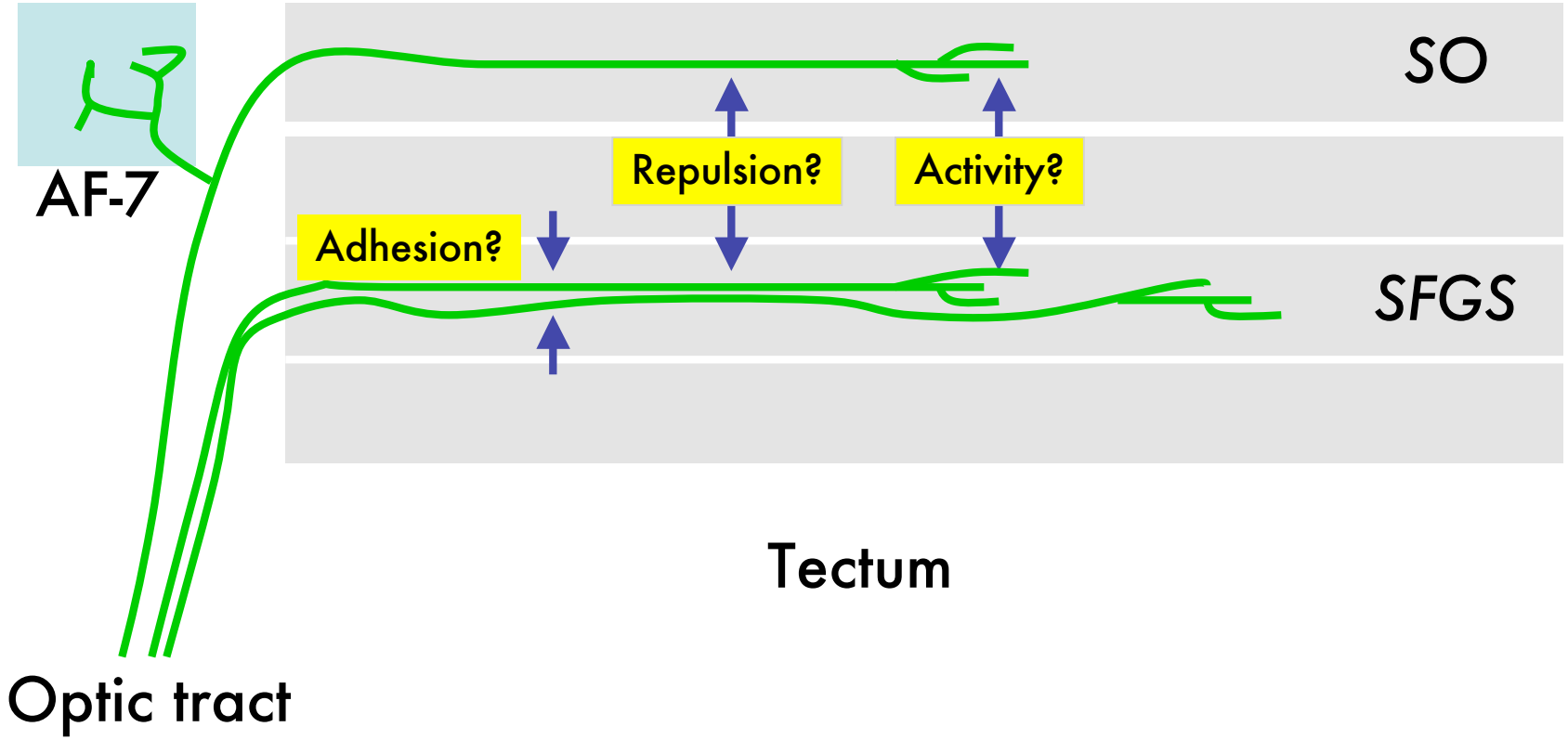


Tong Xiao

How is laminar specificity initiated and maintained?



Model 1: Lamina segregation by axon-axon interactions

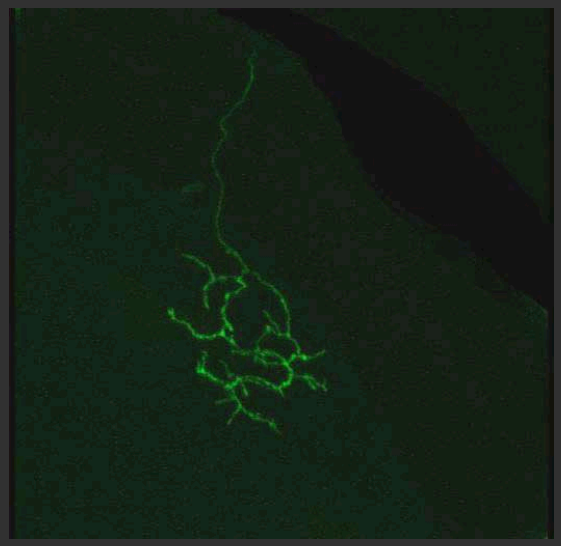


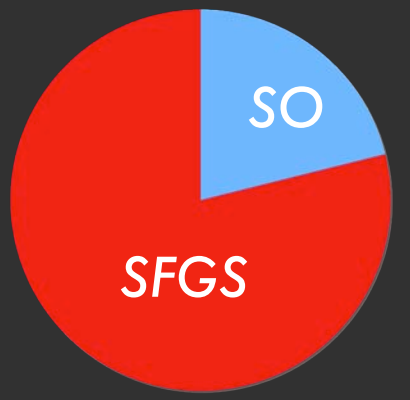
Precise lamina-specific targeting of solitary axons

Dorsal view

Side view

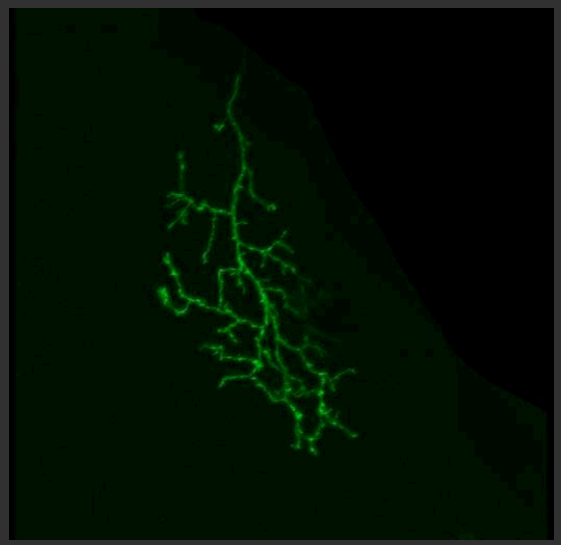
WT RGC in WT

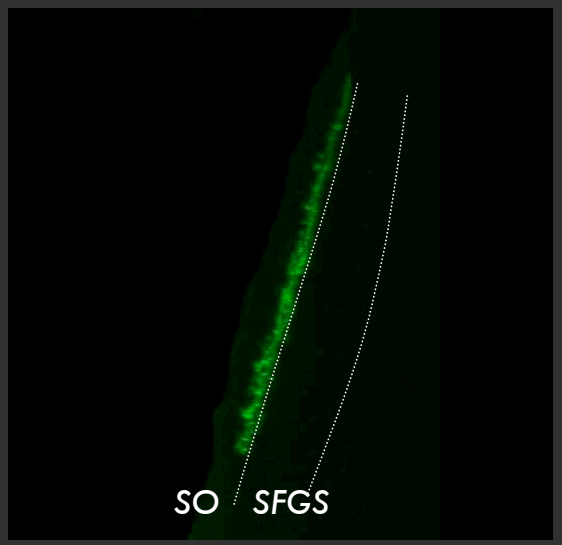


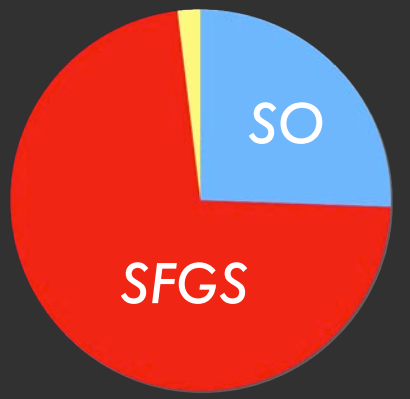


n = 104

WT RGC in *lakritz*



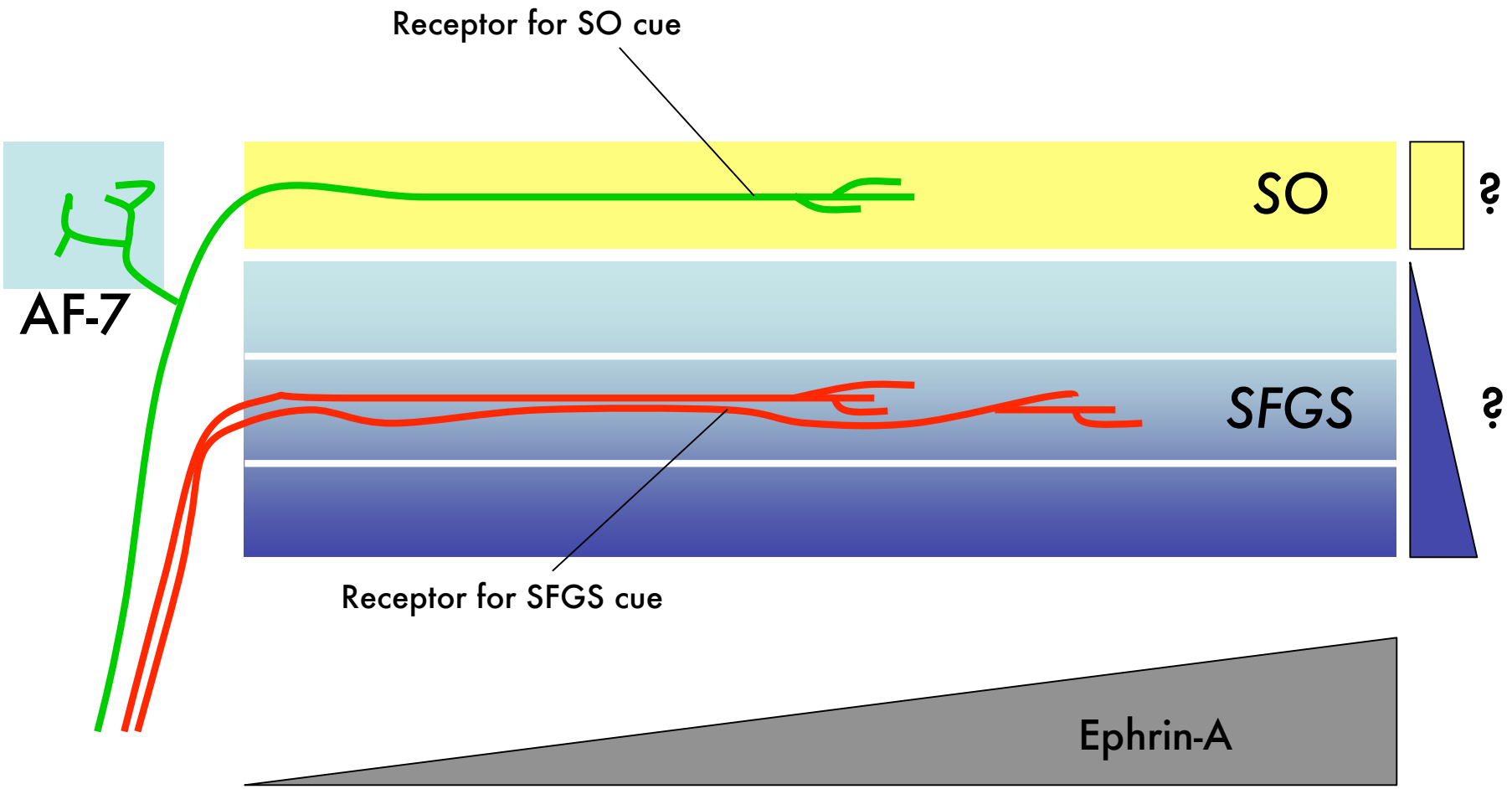




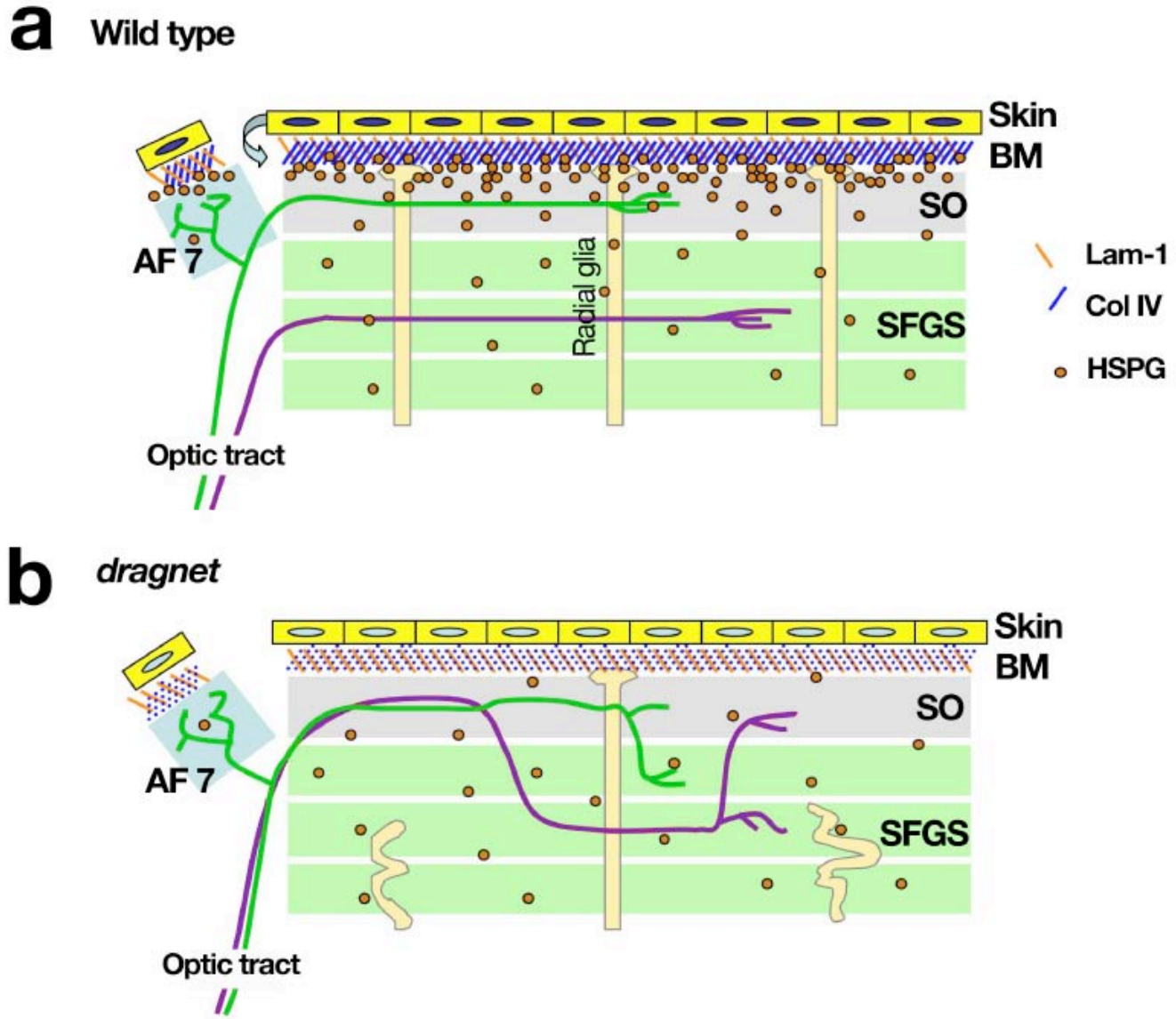
n = 51

Nathan Gosse

Model 2: Segregation by laminar positional cues in the tectum



Laminar guidance by secreted guidance cues (HSPG, Slit) anchored to the surface of the tectum



Conclusions

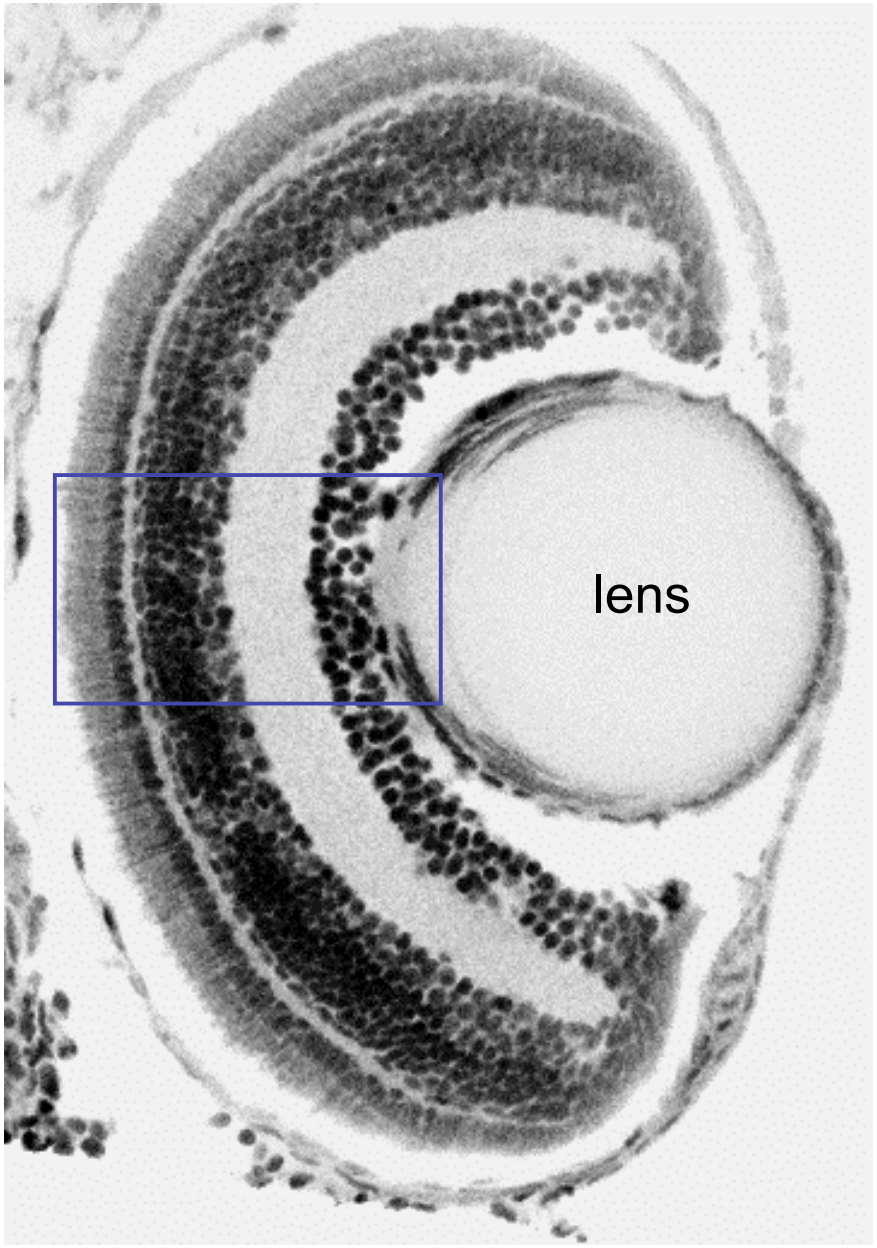
- Lamina-specific targeting is precise from the start; no evidence for exuberant branching and subsequent pruning.

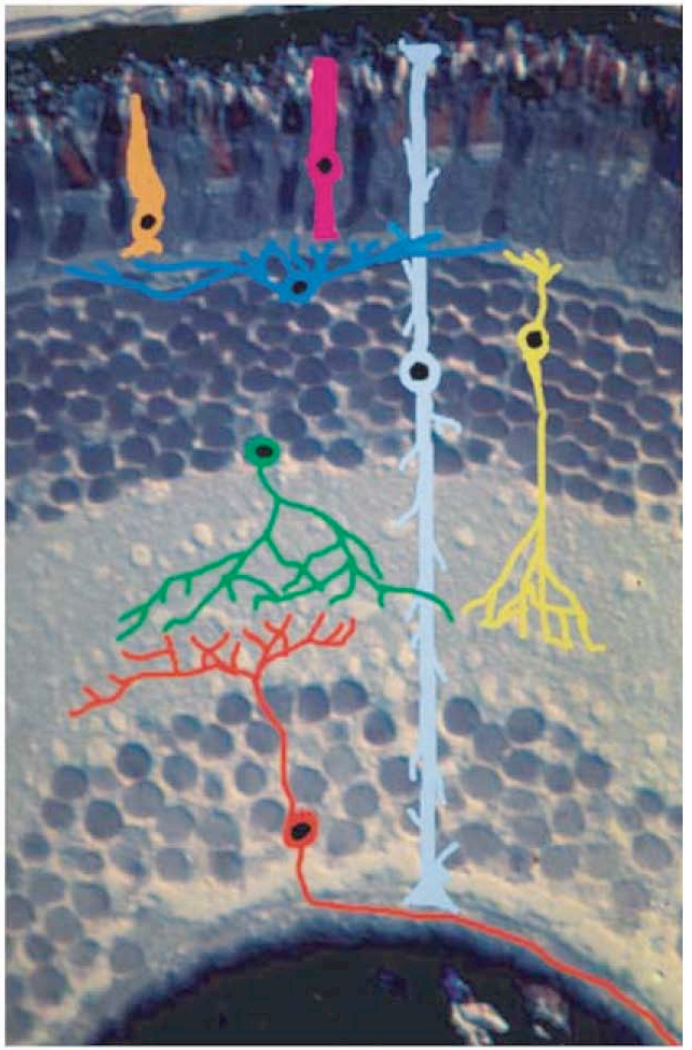
- Retinal ganglion-cell axons show laminar specificity in the tectum even in the absence of other axons.

- Mutations reveal the function of secreted molecules and axonal receptors in lamina-specific targeting.

- Axonal laminar specificity is essential for visual processing.

Assembly of synaptic layers in the retina

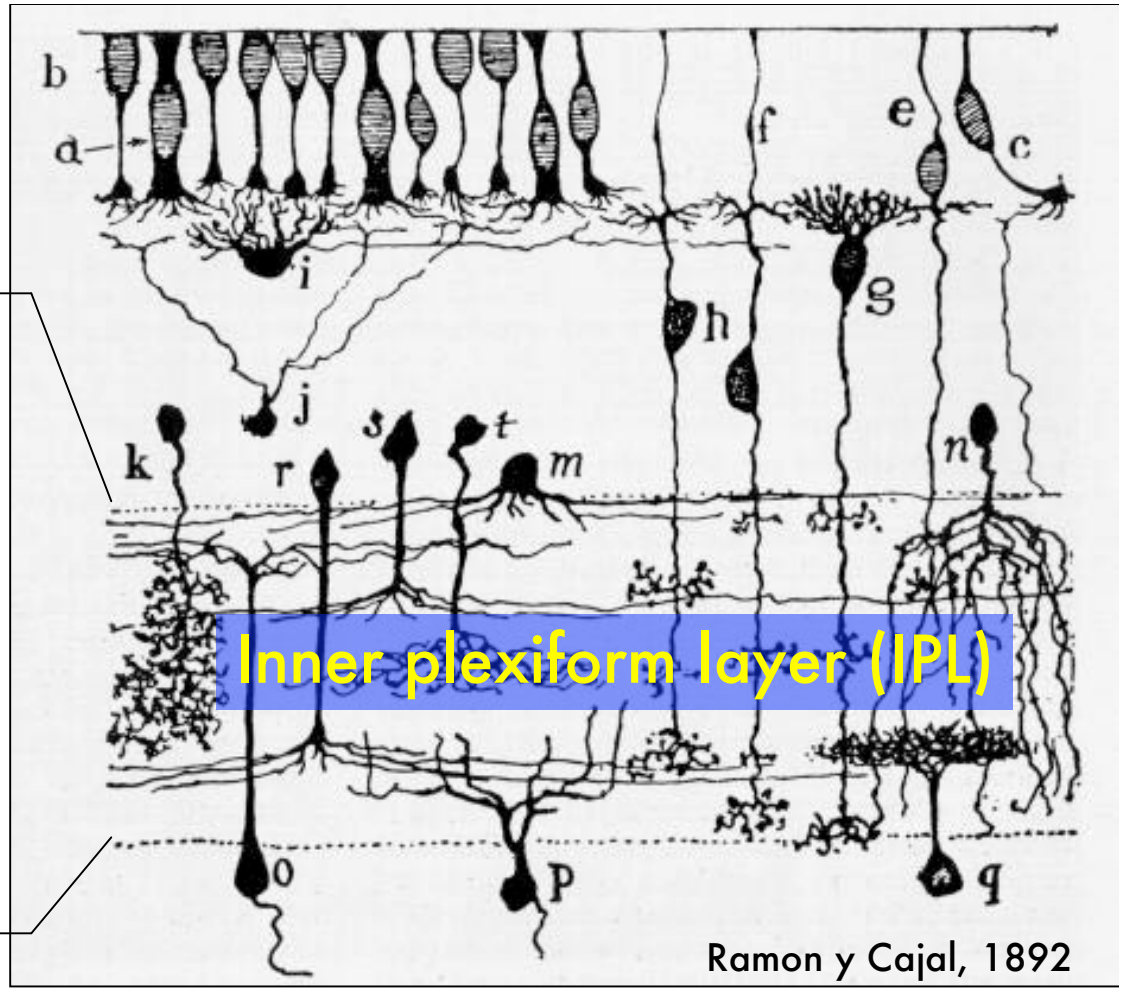




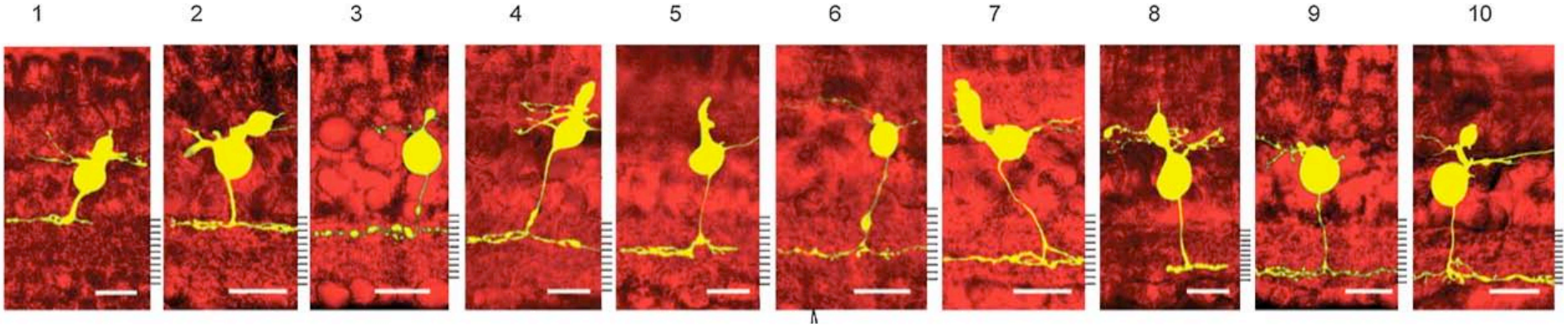
Bill Harris

The IPL is a stack of ca. 10 parallel circuits

1
2
3
4
5
6
7
8
9
10



Ramon y Cajal, 1892



Pang, Gao & Wu (J. Physiol., 2004)

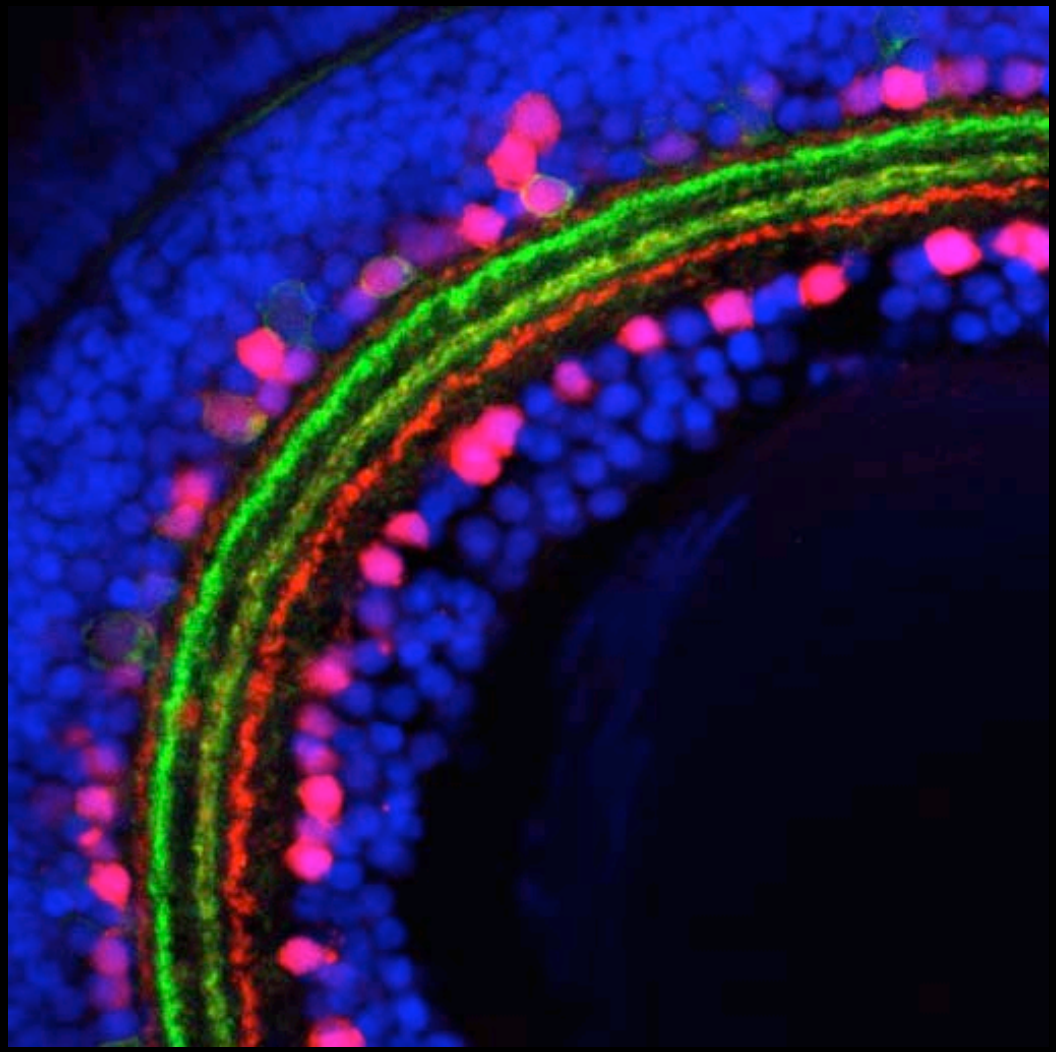
In the retina, the visual image is parsed into features by cells with distinct response properties

[The face of Frank Werblin; animated in original]

Channels of visual processing:

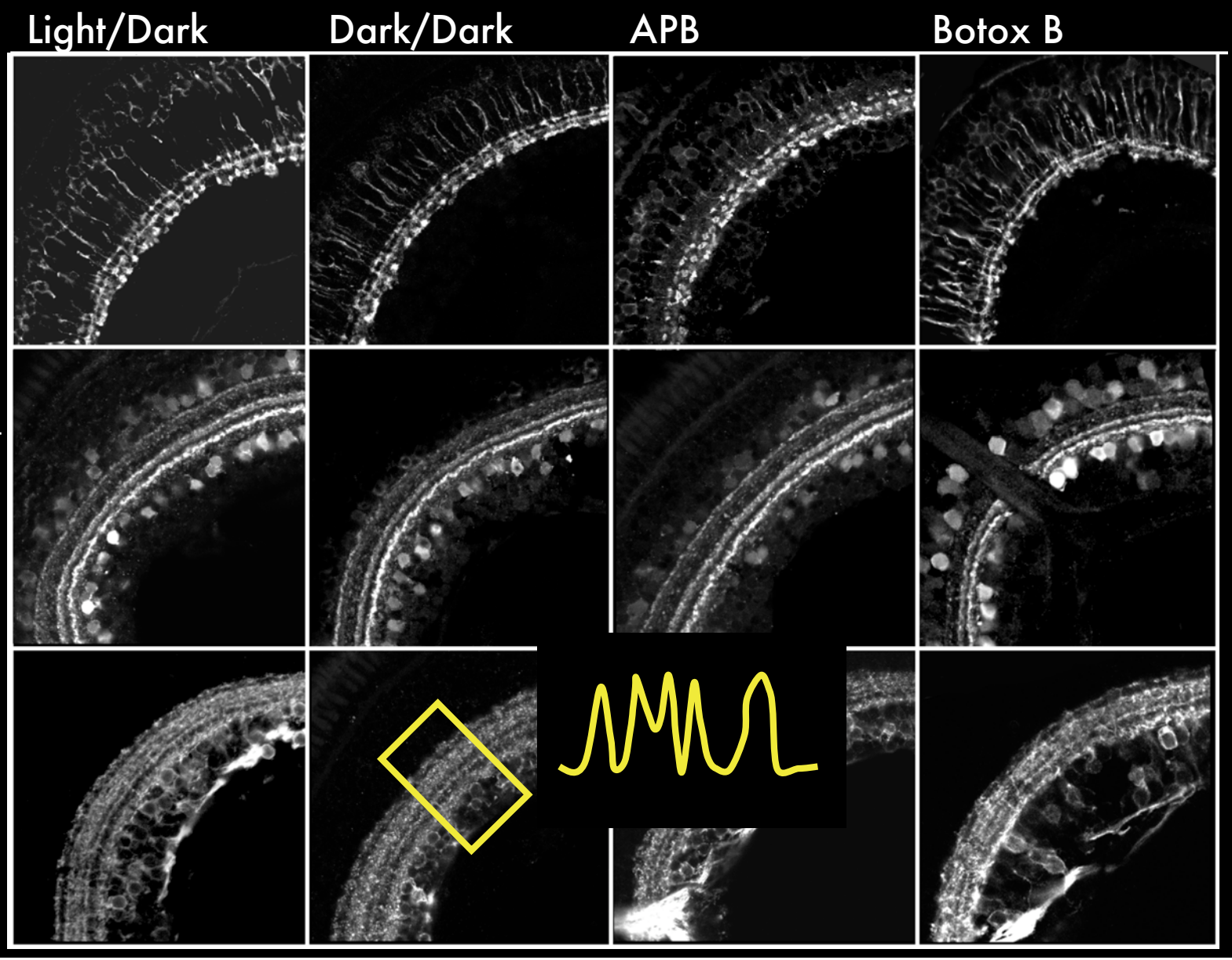
- brightness
- spatial contrast
- temporal contrast
- motion
- direction
- color

IPL sublaminae in zebrafish

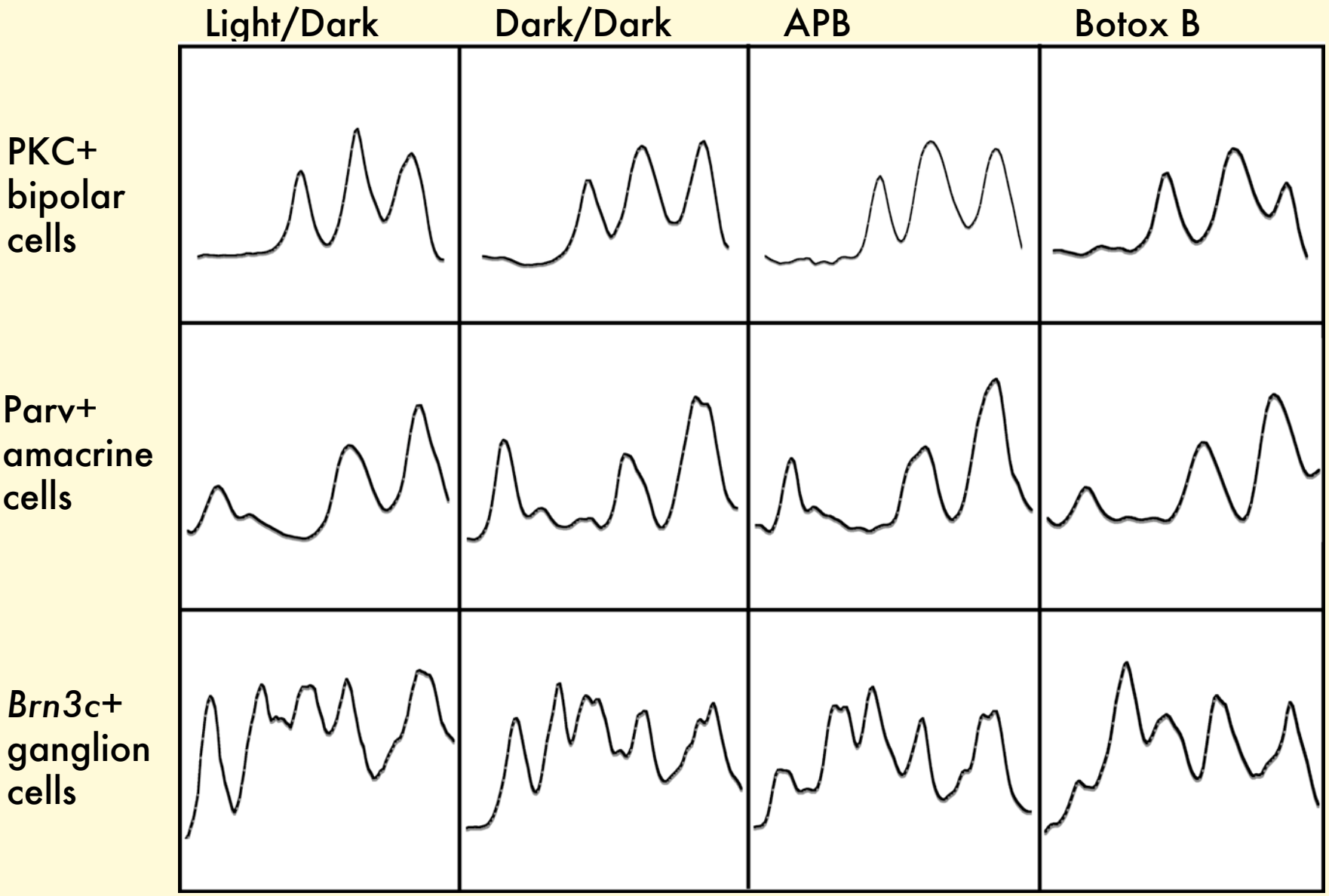


DAPI/*Pax6:mGFP*/Parvalbumin

Resilience of sublaminar circuitry to perturbations of activity



Resilience of sublaminar circuitry to perturbations of activity

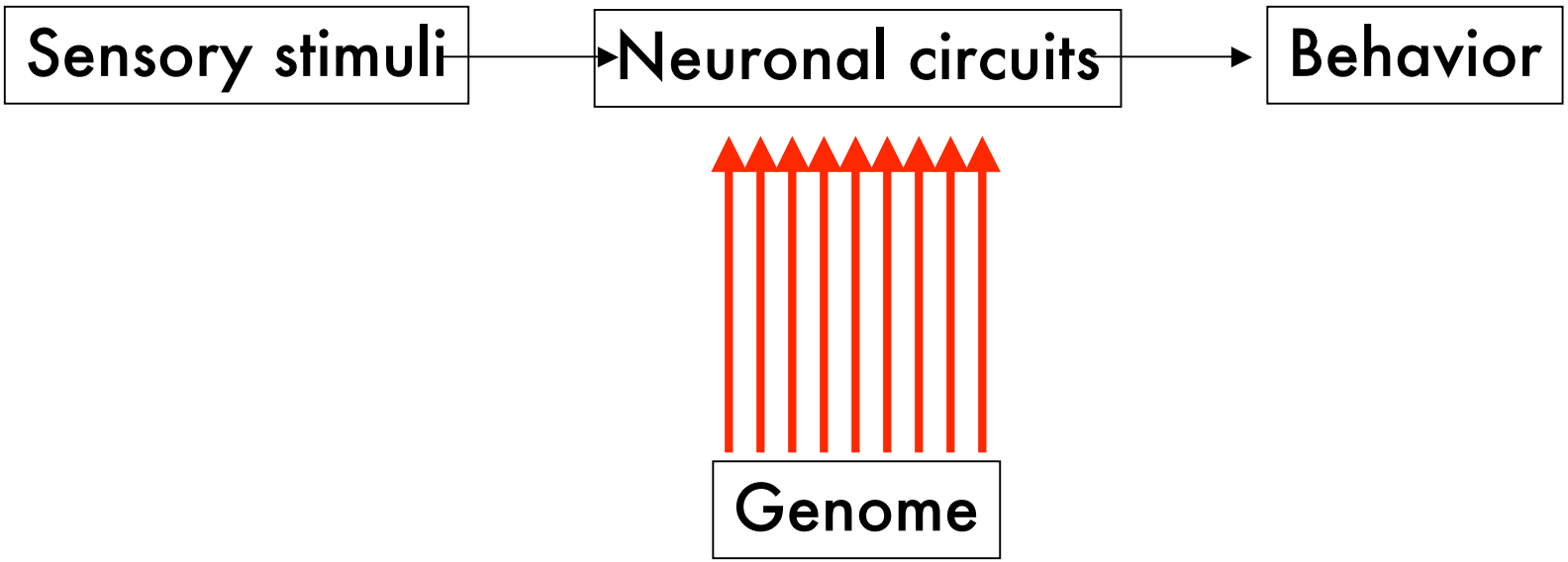


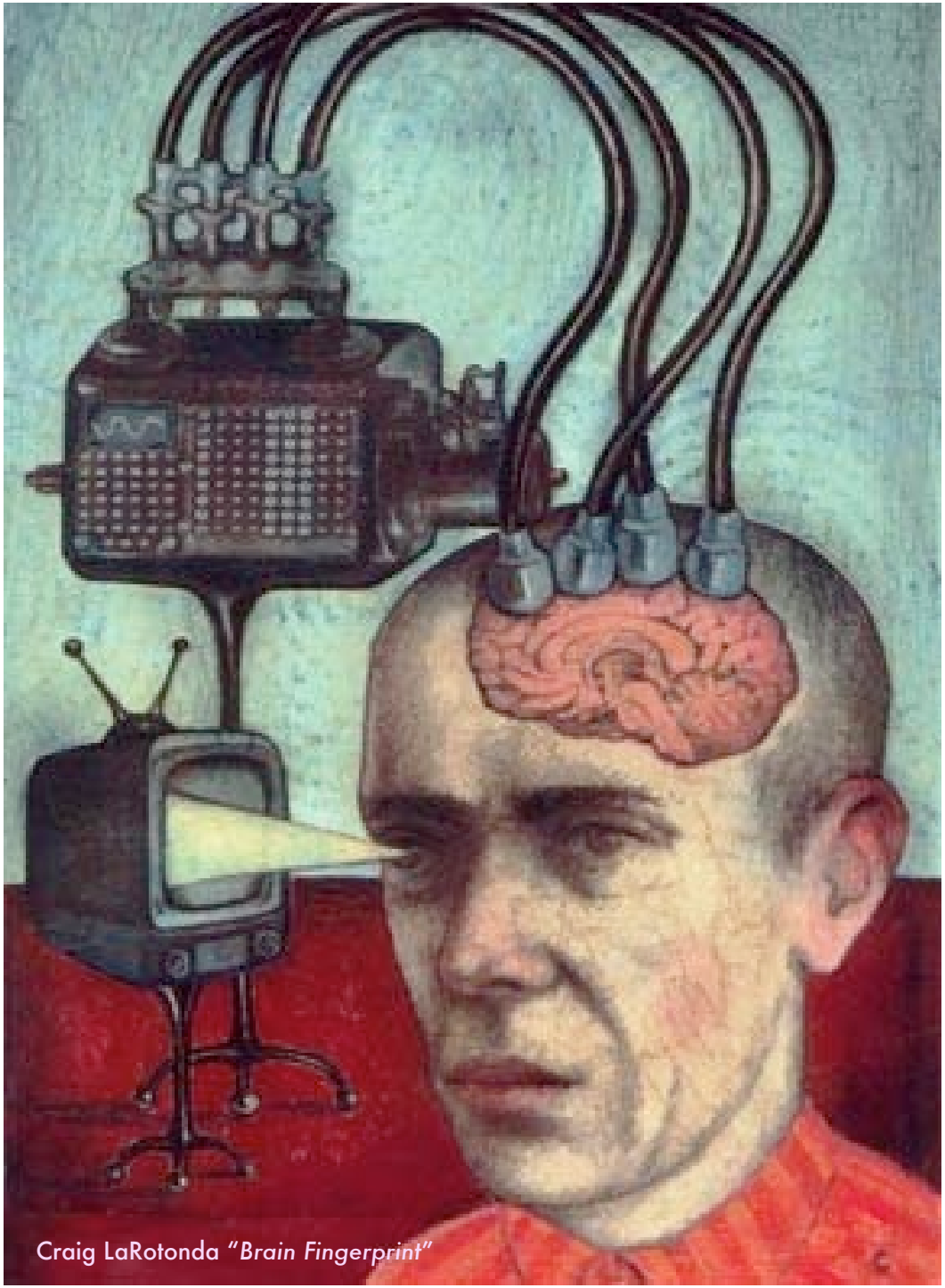
Conclusions

- Sublaminar targeting in the IPL is precise from the start; no evidence for exuberant branching and subsequent pruning.

- Lack of visual experience, lack of synaptic transmission, or imbalanced synaptic input do not prevent, delay, or otherwise alter synaptic sublamination.

Massive transfer of information from genes to synaptic circuitry





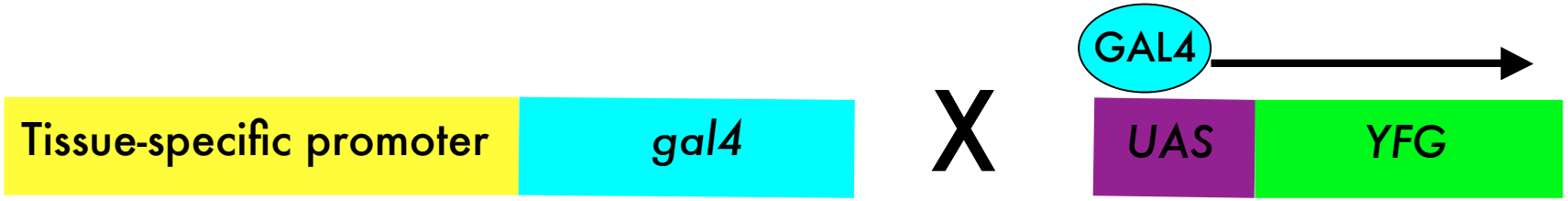
Remote optical control of neuronal activity

- Installation of a genetic switch in subsets of neurons by GAL4/UAS

- Optical manipulation of neural activity: channelrhodopsin, halorhodopsin, LiGluR, et al.

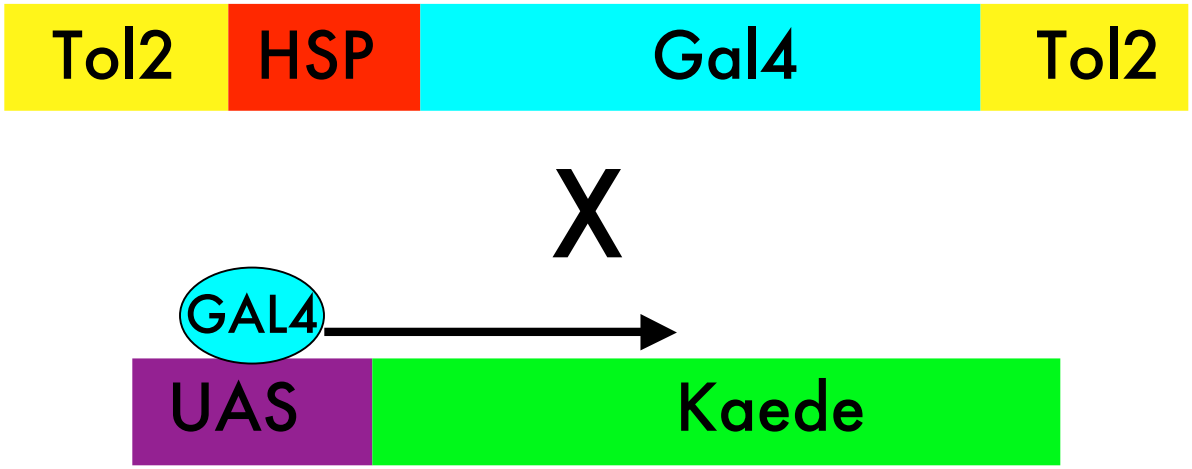
- Large-scale production of GAL4 enhancer-trap lines by Tol2 transposition.

GAL4/UAS

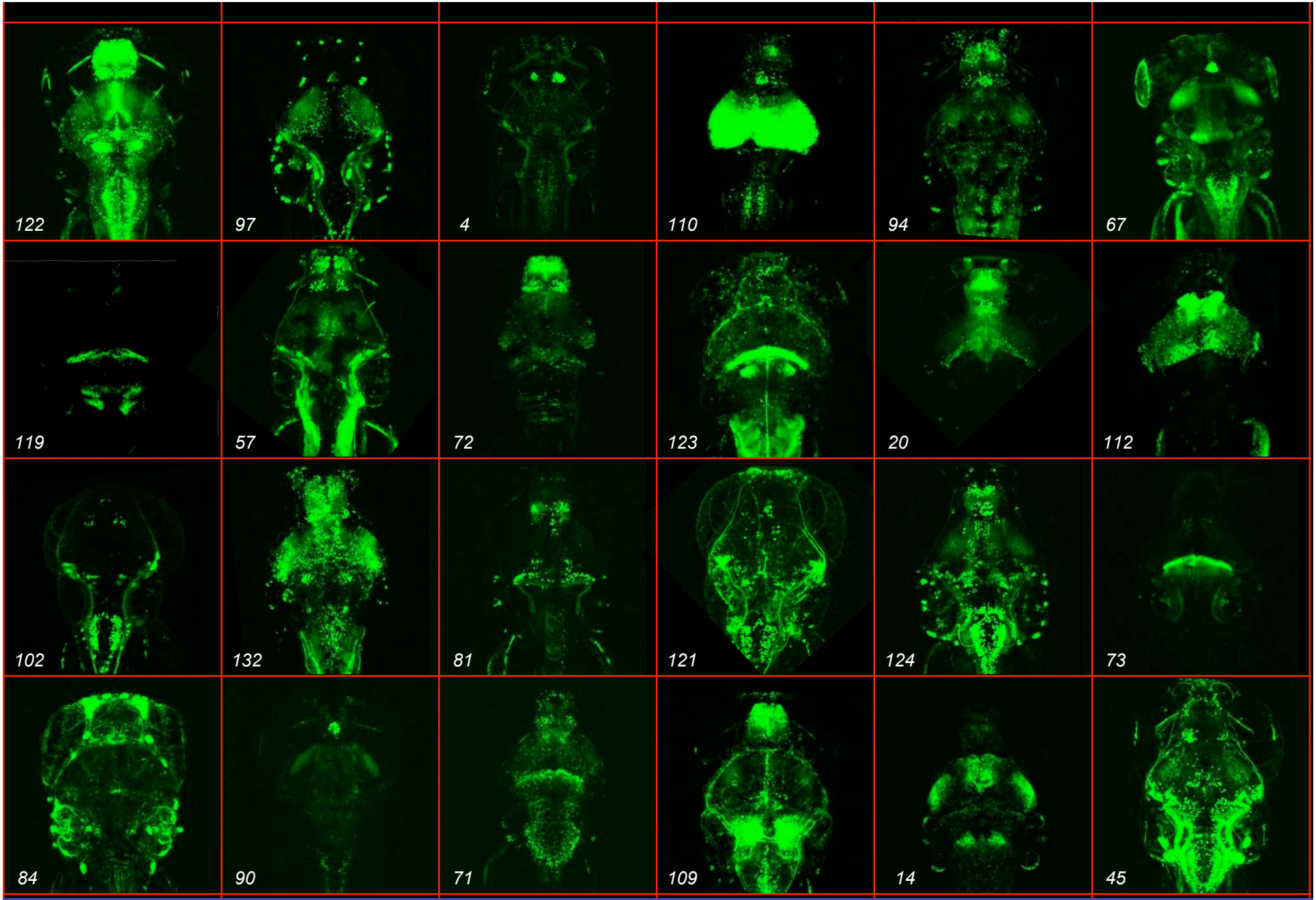


GFP, ChR2...

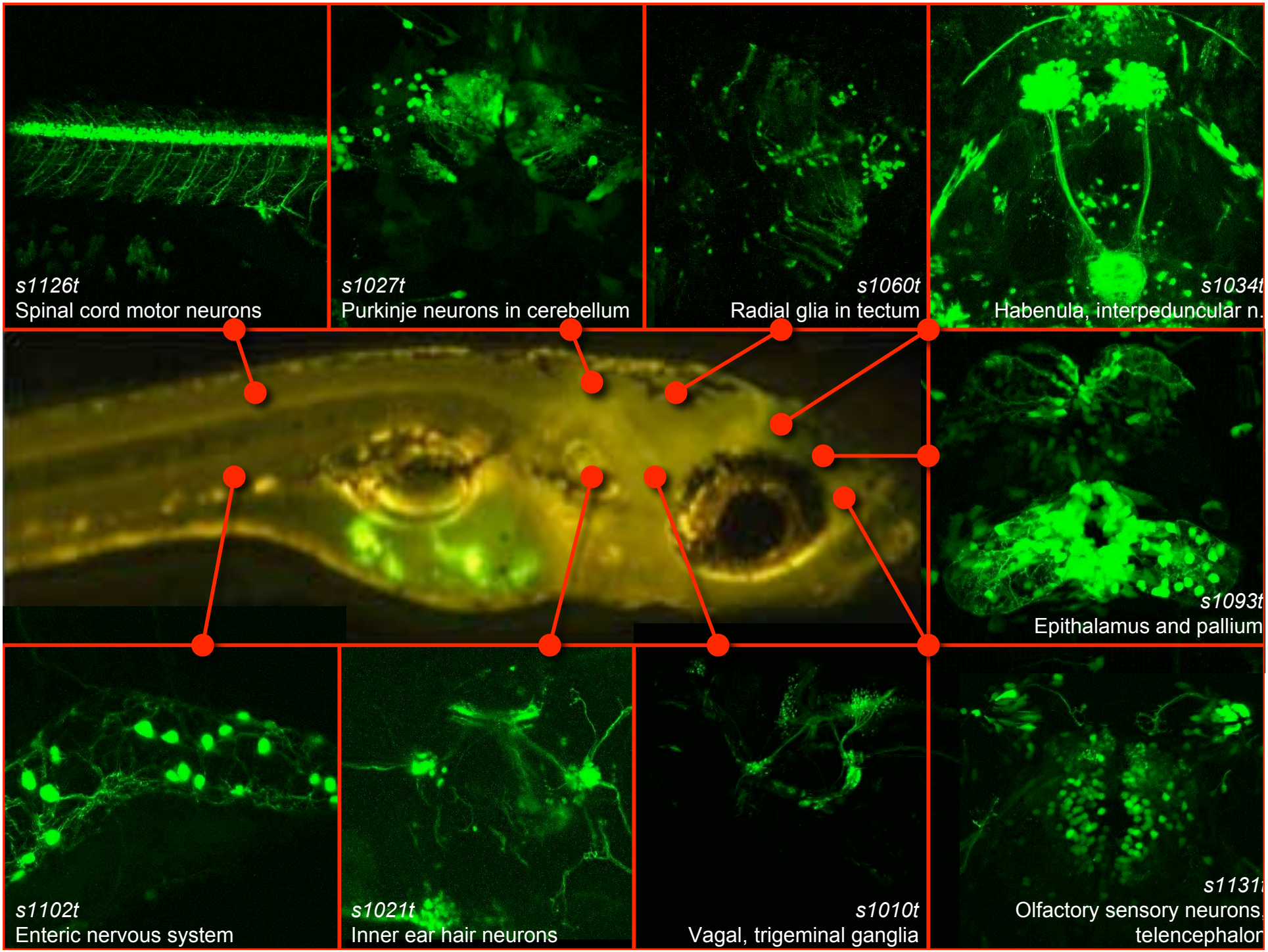
GAL4 Enhancer Trapping



- Inject GAL4 construct with transposase mRNA into 1-cell stage embryos
- Raise potential founders (F_0)
- Cross to UAS:Kaede to identify successful traps
- Raise offspring (F_1) to establish stable lines



A collection of 200 GAL4 enhancer trap patterns in the CNS


s1126t
Spinal cord motor neurons
s1027t
Purkinje neurons in cerebellum
s1060t
Radial glia in tectum
s1034t
Habenula, interpeduncular n.
s1093t
Epithalamus and pallium
s1102t
Enteric nervous system
s1021t
Inner ear hair neurons
s1010t
Vagal, trigeminal ganglia
s1131t
Olfactory sensory neurons,
telencephalon

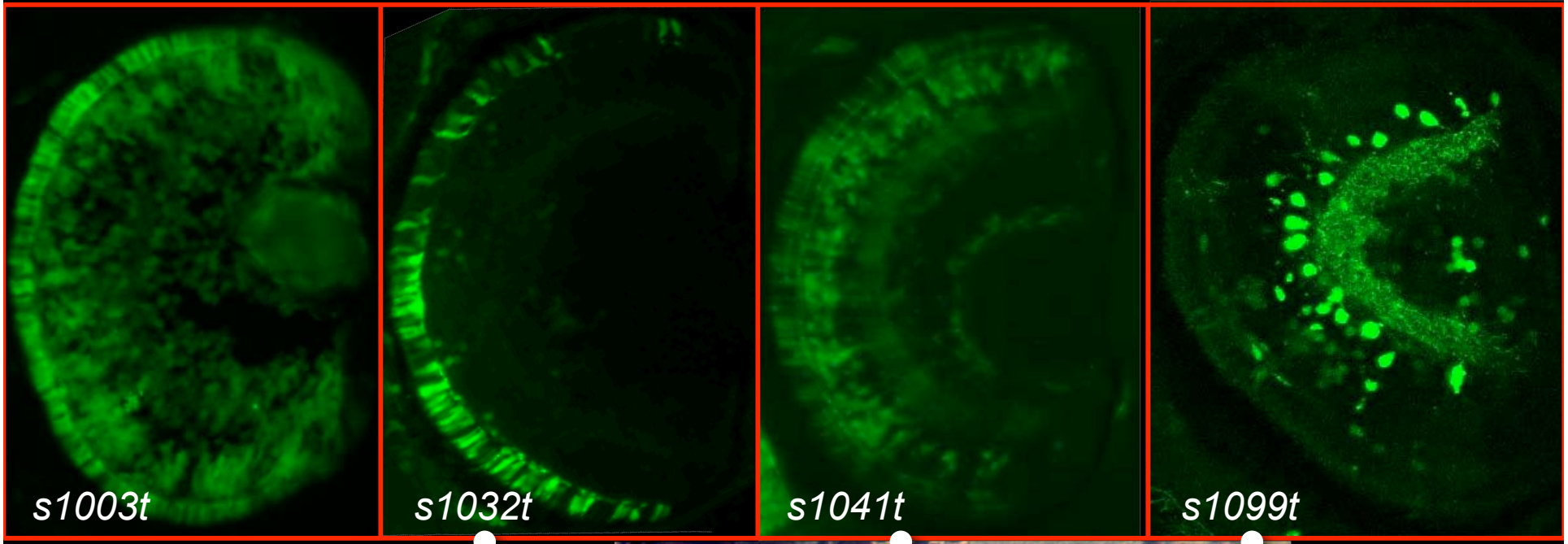
Retina-specific enhancer traps

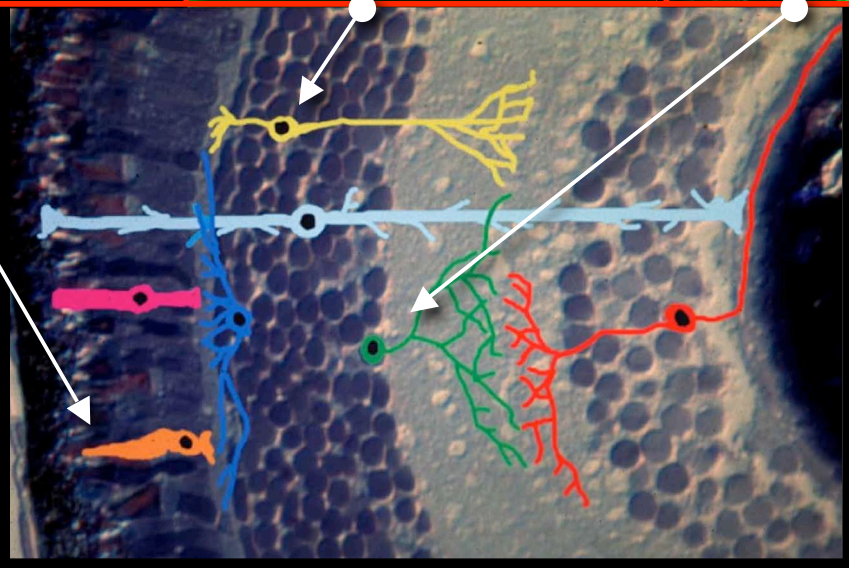
All retinal cells

Cone photoreceptors

Bipolar cells

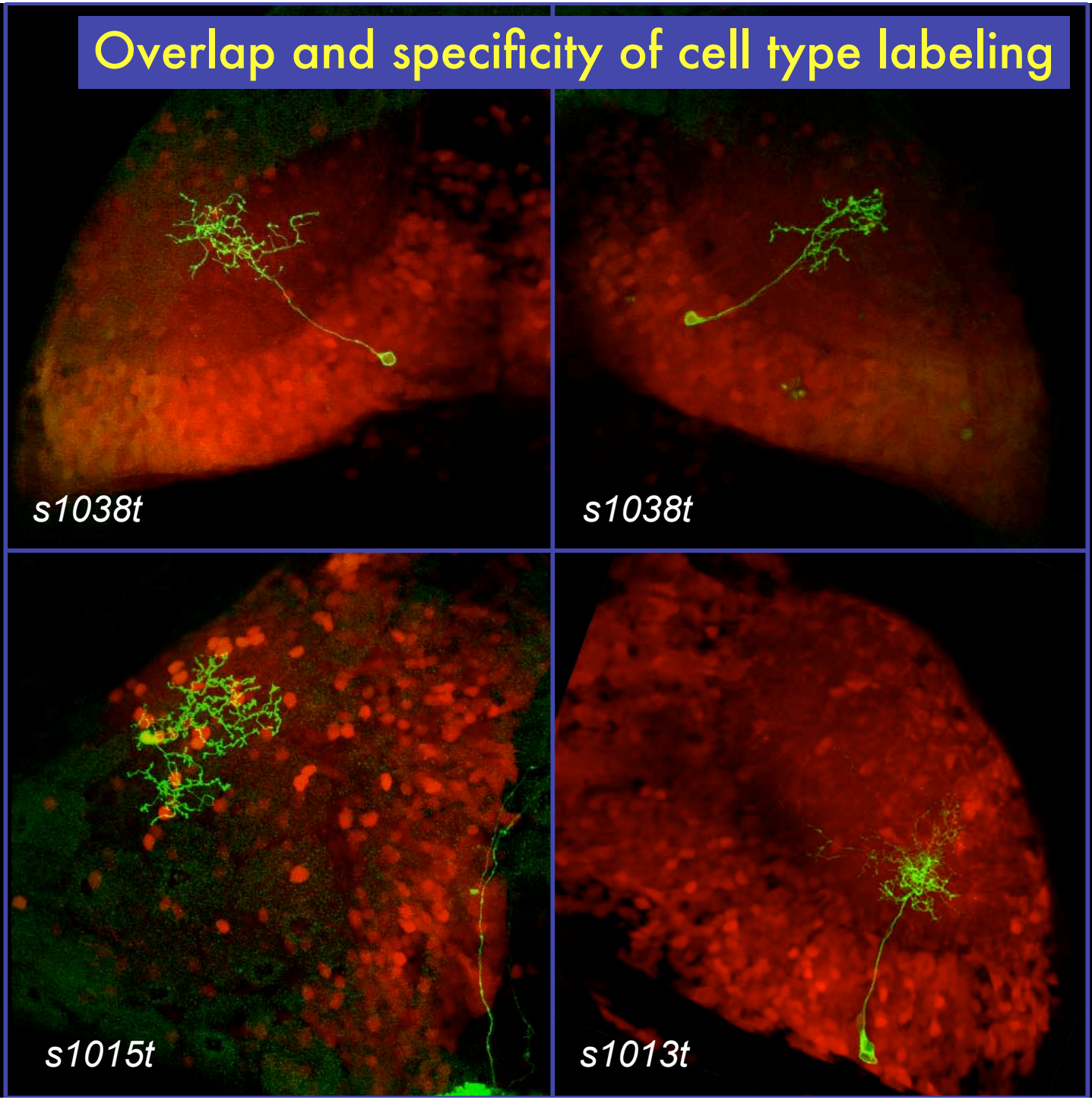
Subset of amacrines





Overlap and specificity of cell type labeling

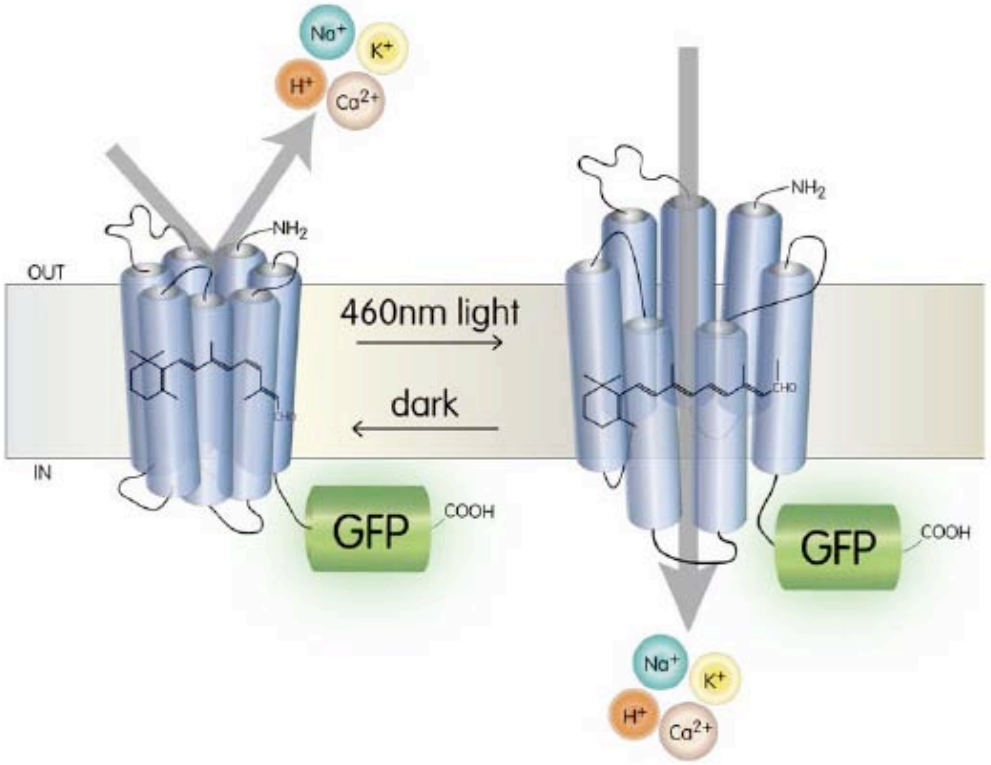
Et:GAL4; UAS:Kaede; UAS:mGFPvar
Kaede photoconverted / GFP



Channelrhodopsin-2

- ChR2 is a light-gated cation-selective membrane channel from *Chlamydomonas* (here tagged with mCherry).

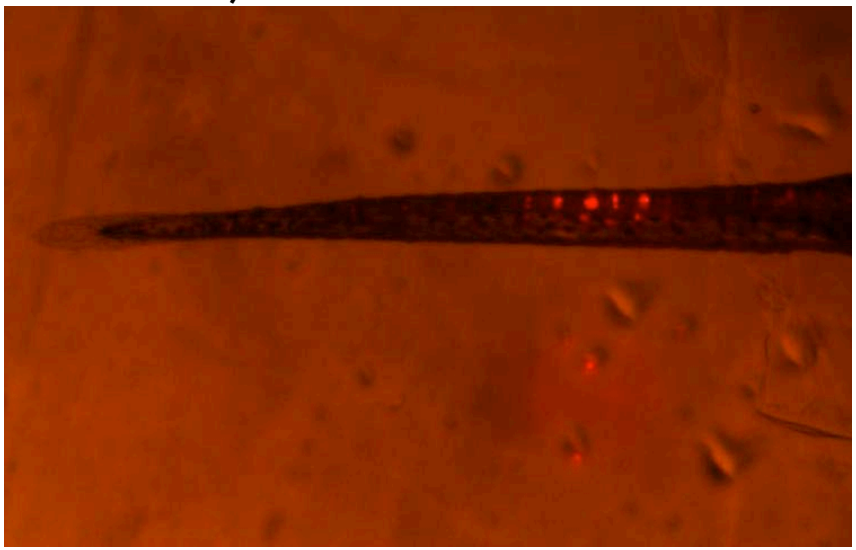
- Misexpression in *C. elegans* and *Drosophila* neurons has allowed optical control of simple behavior.



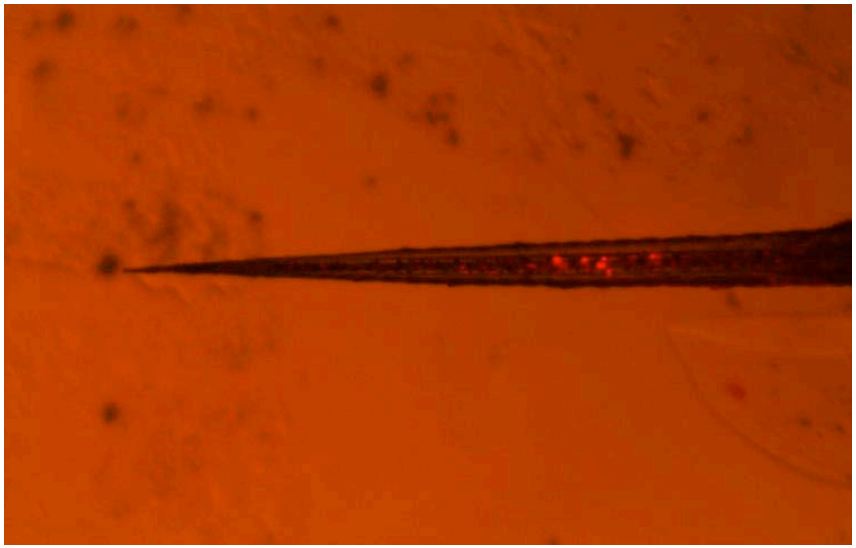
Flannery et al. 2006

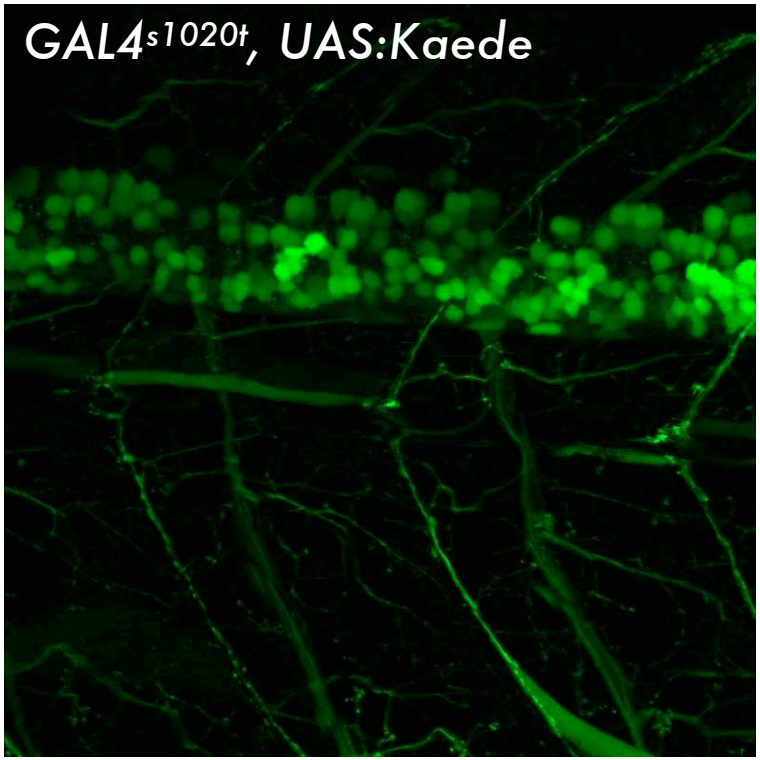
Channelrhodopsin in motoneurons: Tail movement elicited by local (200 µm), 1 sec, 460nm pulses

GAL4^{s1020t}, UAS:ChR2



Control: *GAL4^{s1020t}*





- Expression in primary and secondary motoneurons.
- 20 ms pulses sufficient
- Response in <100ms
- Accompanied by robust calcium responses

Filo Del Bene

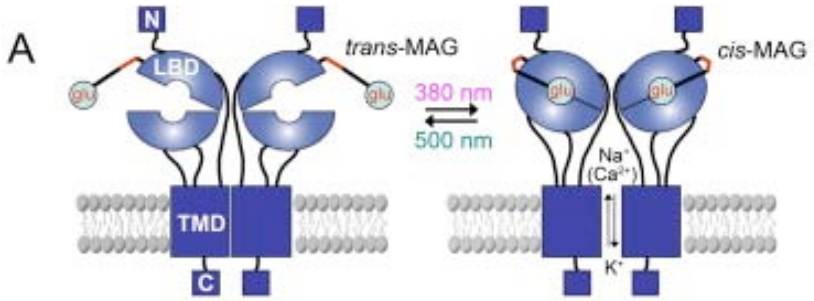
Light-gated glutamate receptor (LiGluR)

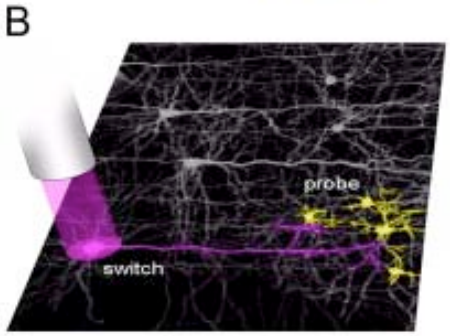
ON switch: 365 nm
OFF switch: 488 nm

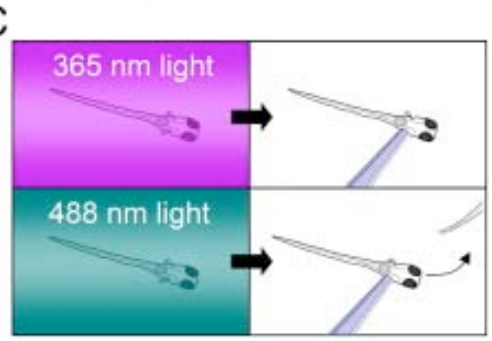
<u>Advantages for behavioral experiments:</u>

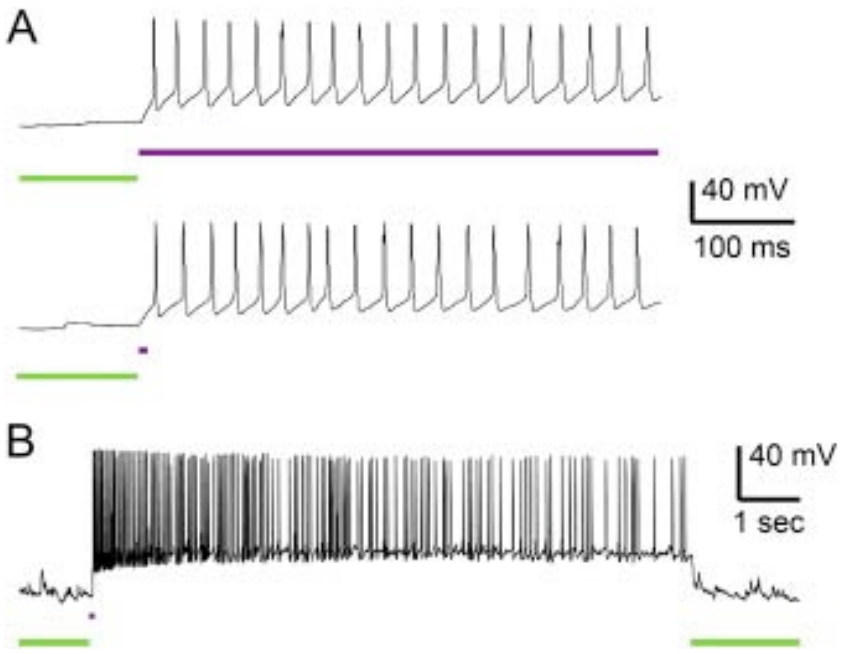
- MAG provides additional conditionality
- Precise timing
- Persistent activity in the dark

(low rate of spontaneous isomerizations)



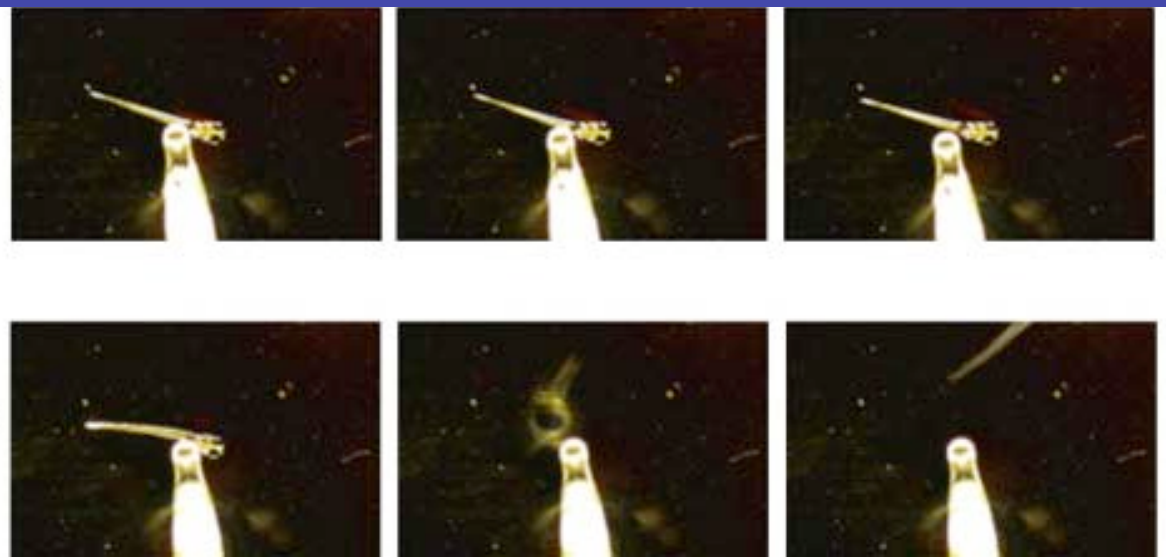






Szobota et al., *Neuron* (2007)

LiGluR in "sensory-specific" GAL4 line abolishes touch responses

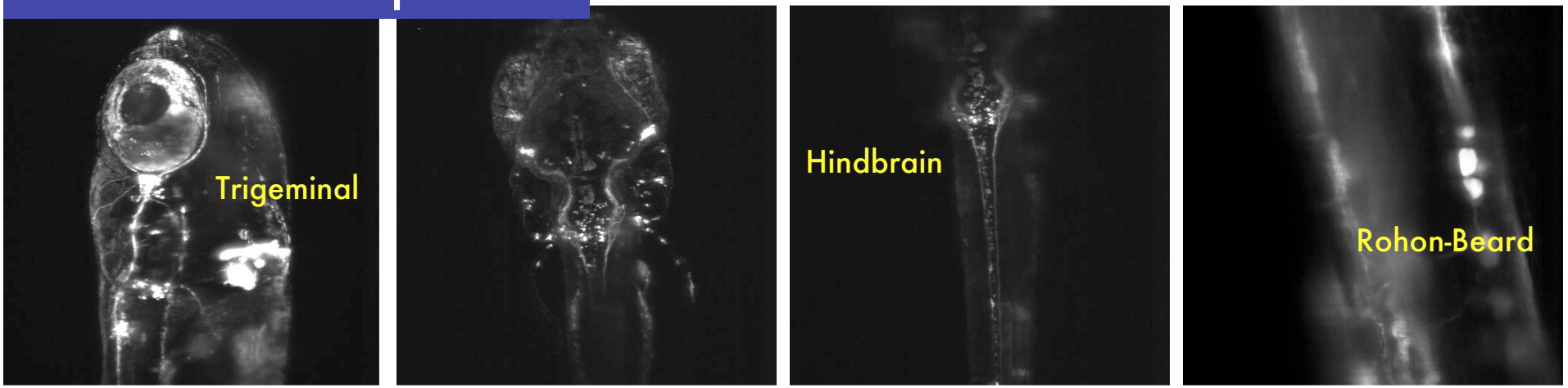


UAS:iGluR(L439C); MAG (1 mM) bath applied.

"If I were a fish, and somebody poked me in the side," (in this case, with a fine glass tip), Dr. Isacoff said, "I would escape." But when the translucent fish were strobed with violet light, the overstimulated creatures no longer detected being prodded. Blue-green light reversed the effect.

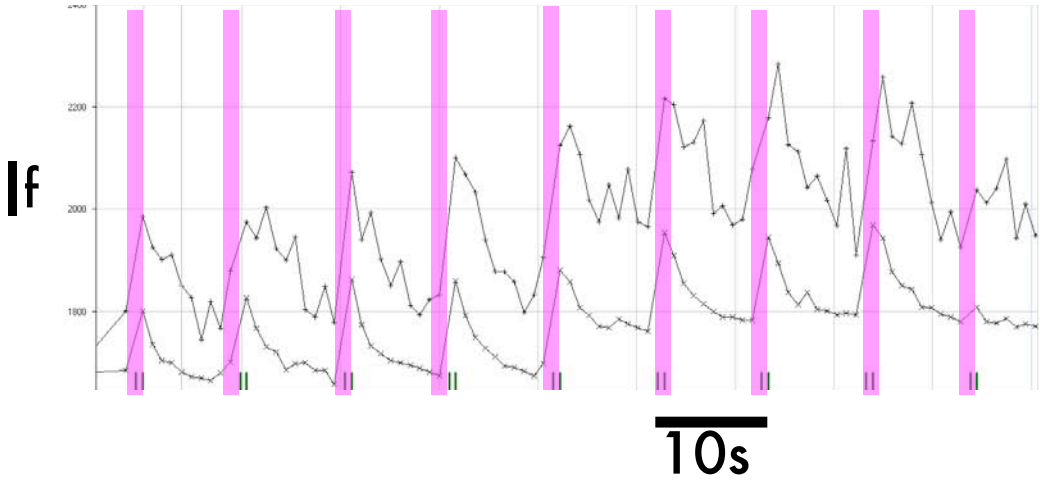
The New York Times
August 14, 2007

The *GAL4*$^{s102.1t}$ pattern:

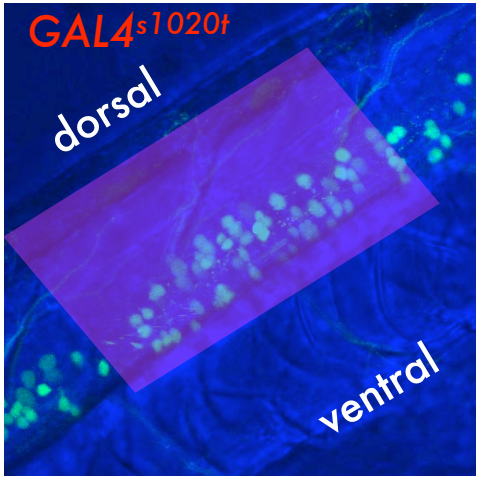


Szobota et al., *Neuron* (2007)

Calcium imaging confirms effects of LiGluR activation

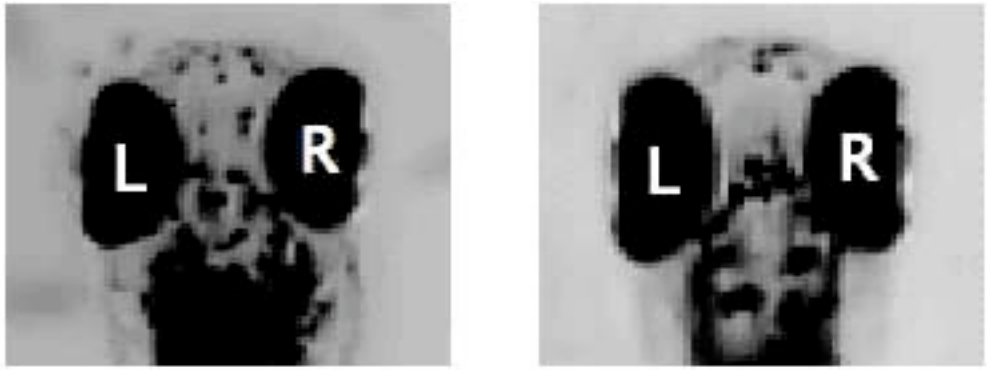


Spinal motoneurons



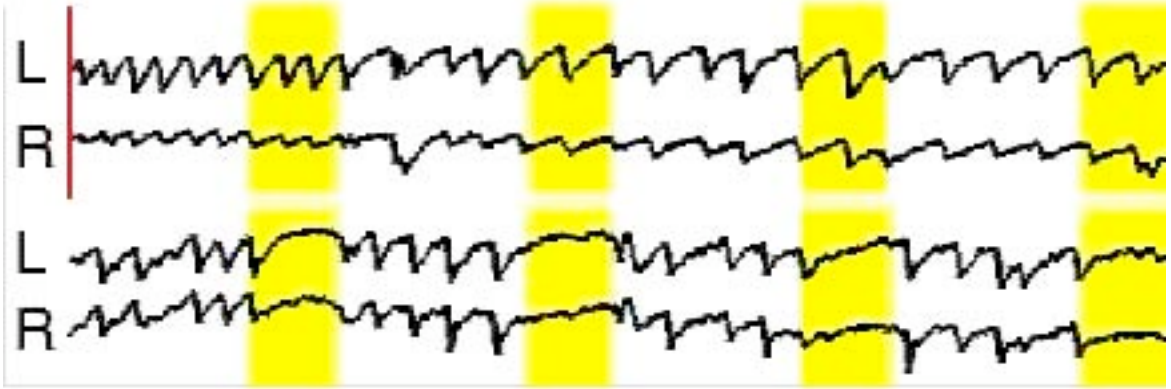
Erica Warp

Halorhodopsin activation with optical fiber blocks behavioral responses



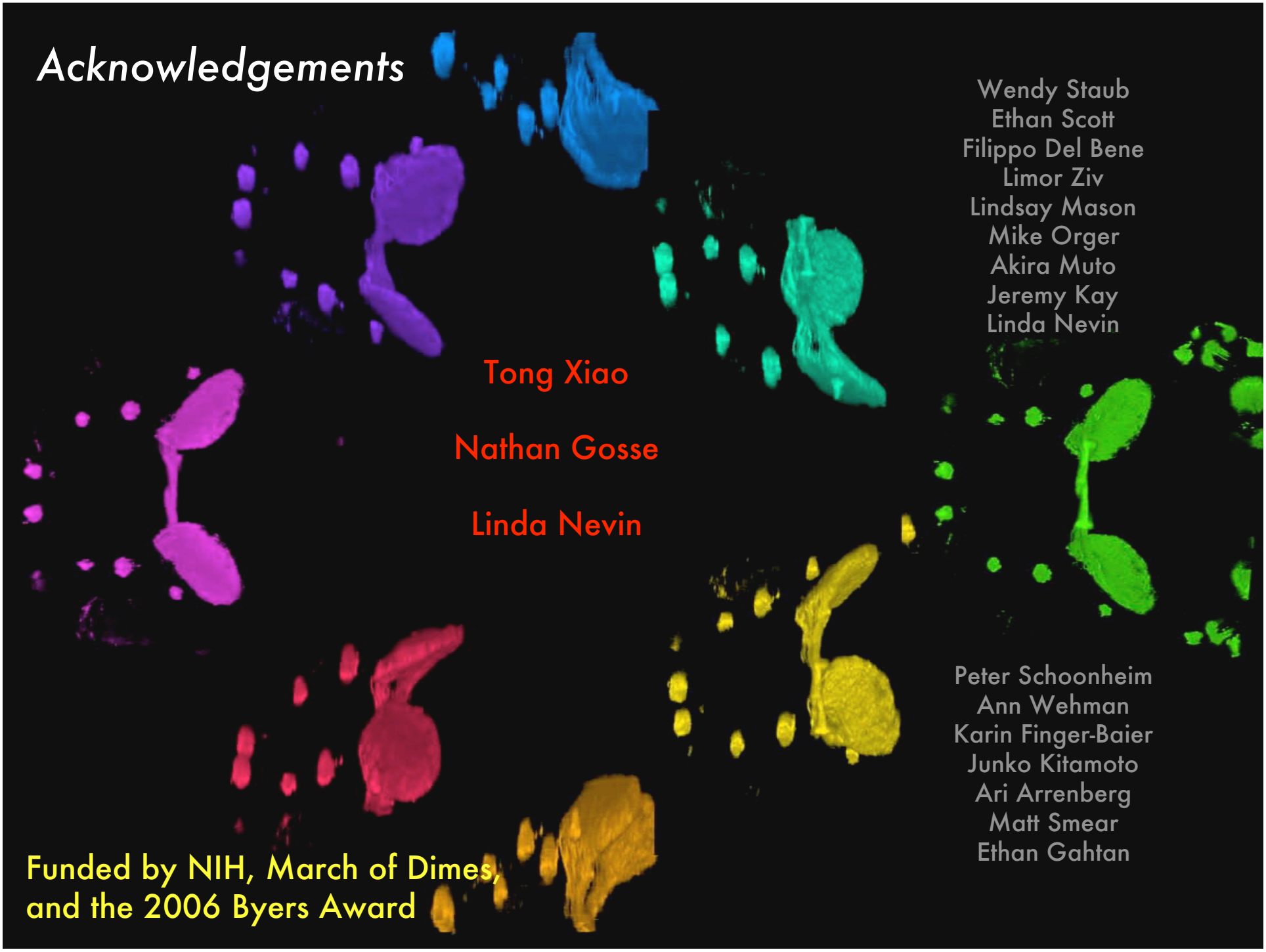
Non-expressor (control)

Pan-neuronal NpHR expressor



Ari Arrenberg

Acknowledgements



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