The use of *Drosophila* in neuroscience research

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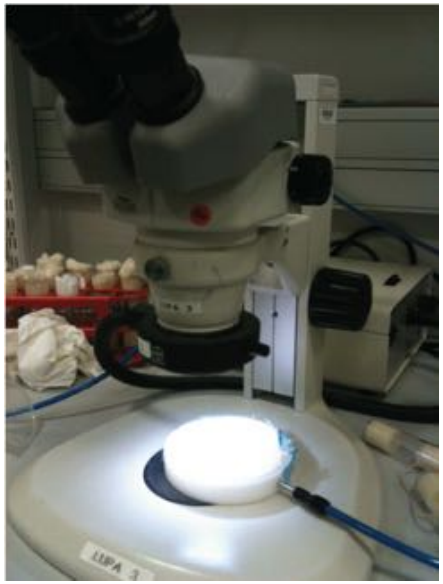
EXPERIMENTAL MODELS IN NEUROSCIENCE

Drosophila as a research model

- Studied for ~100 years
- Short life cycle
- Many available mutants and tools
- Easy to breed

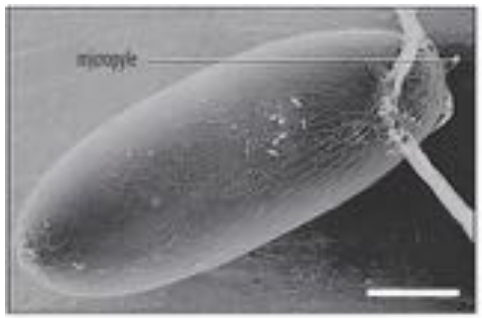


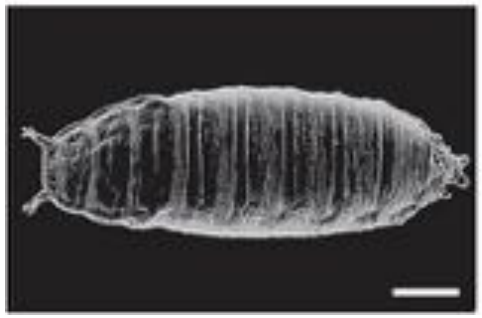
Advantages of using Drosophila in neurosciences:

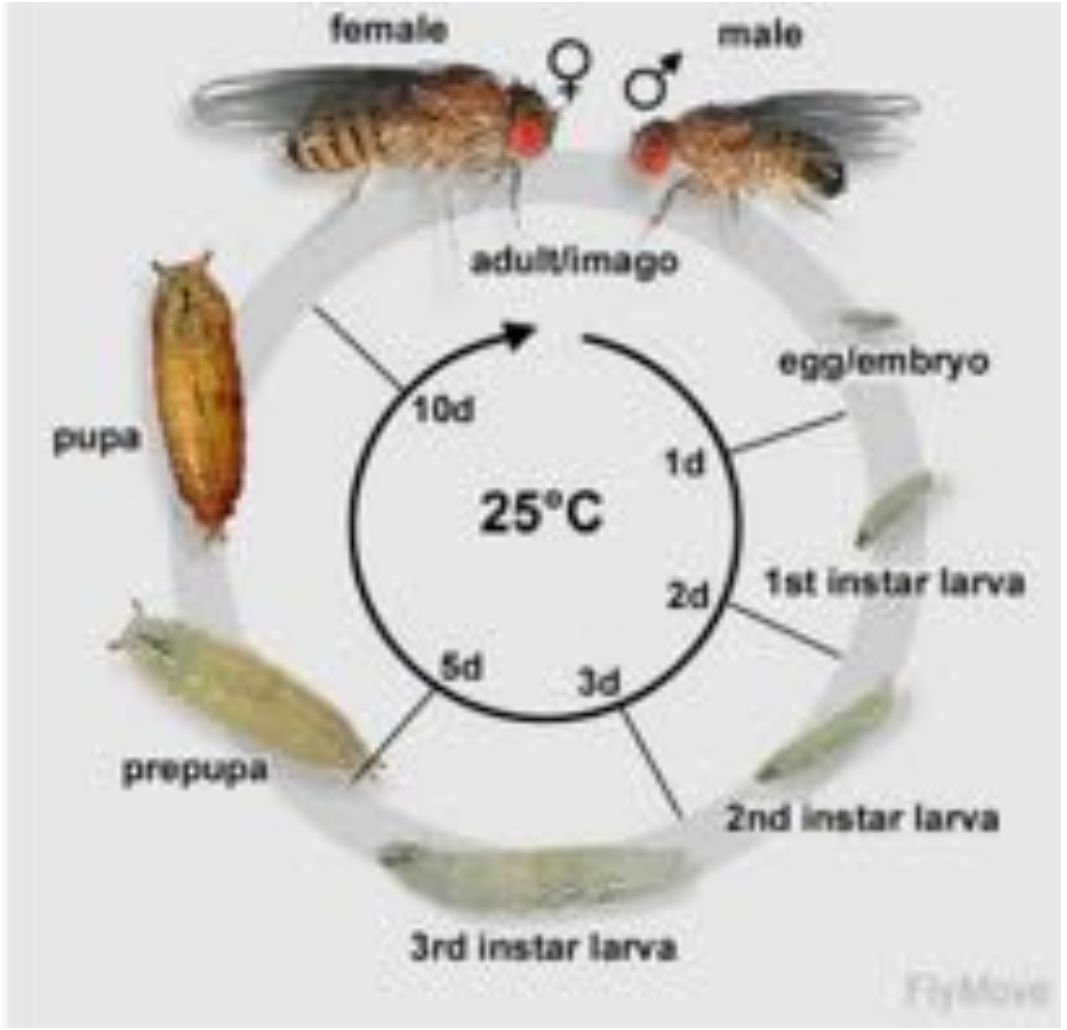
- High degree of conservation with mammals
- Known morphology (including cellular)
- Relatively accessible structures
- From cell biology to behavior
- Smaller genetic redundancy
- A large repertoire of genetic tools (for example null alleles)
- A large collection of manipulation tools (for example optogenetics)
- A set of stereotyped behaviors (locomotion; circadian rhythms, etc)
- Sophisticated quantification tools

If you need a metric for the success of a model organism…

micropyle






female
male
adult/imago
egg/embryo
1st instar larva
2nd instar larva
3rd instar larva
prepupa
pupa
25°C
1d
2d
3d
5d
10d

Thomas Hunt Morgan

A.H. Sturtevant: The first genetic map:

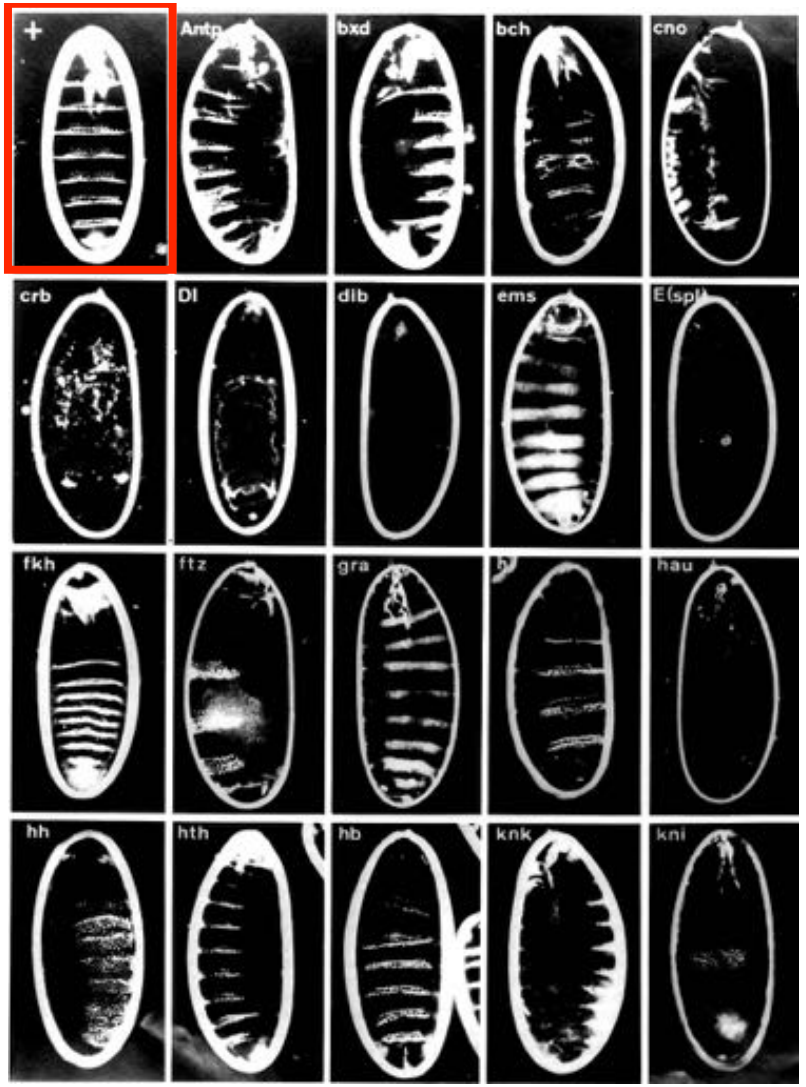
Sturtevant, A. H. 1913. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. *Journal of Experimental Zoology*. 14: 43-59.

THE LINEAR ARRANGEMENT OF SIX SEX-LINKED FACTORS IN DROSOPHILA, AS SHOWN BY THEIR MODE OF ASSOCIATION

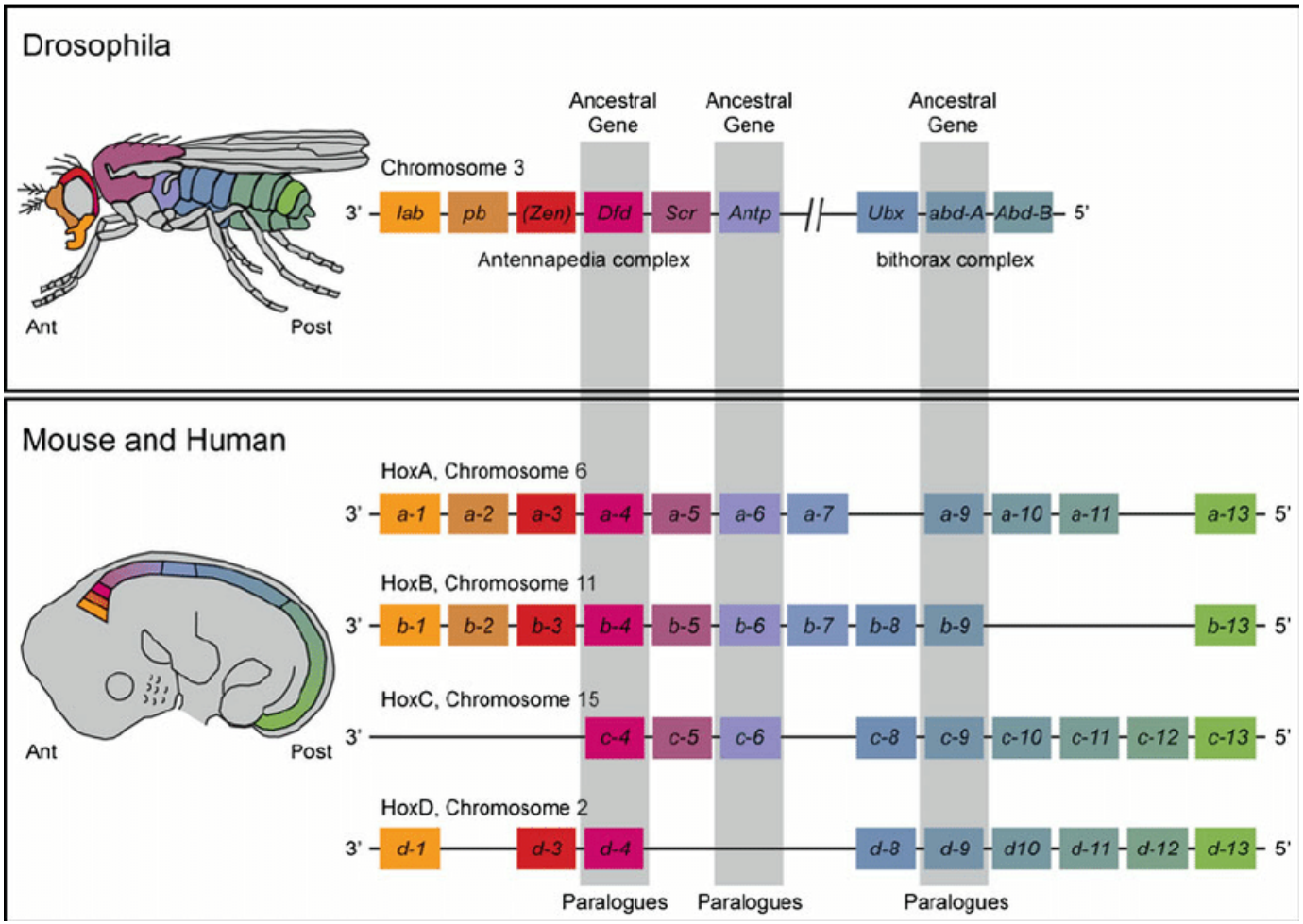
A. H. STURTEVANT

wildtype

Wieschaus and
Nüsslein-Volhard
looked for mutants
that affect the
fly body plan



Drosophila homeotic genes are conserved



Some *Drosophila* genetic trickery

Wild-type

Ultrabitorax

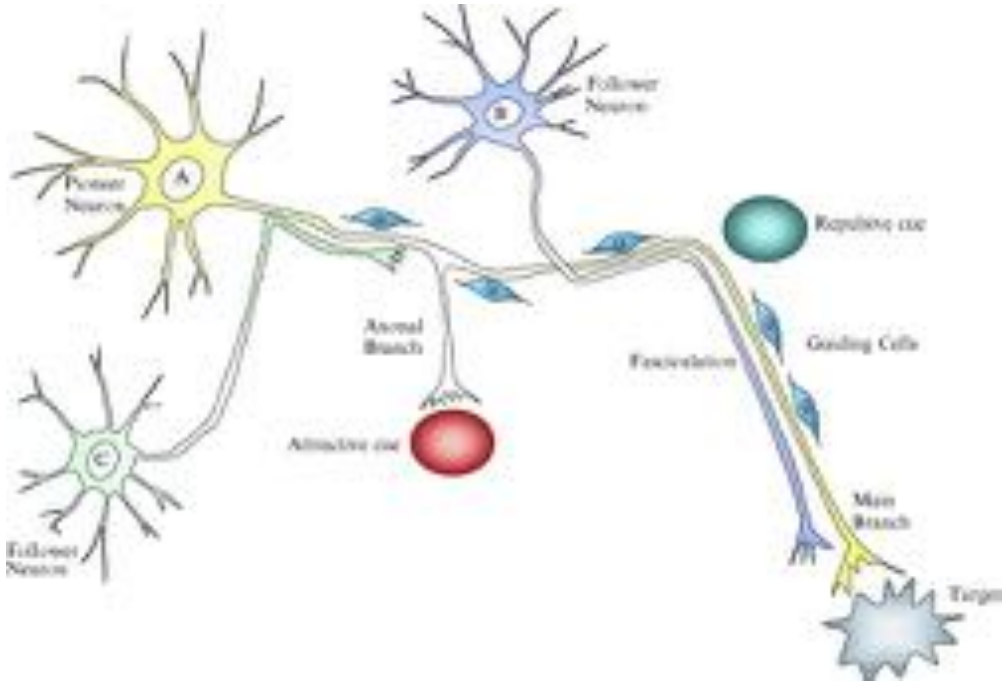
Antennopedia

Lewis E. A gene complex controlling segmentation in *Drosophila*. *Nature* **276,** 565–570 (1978)

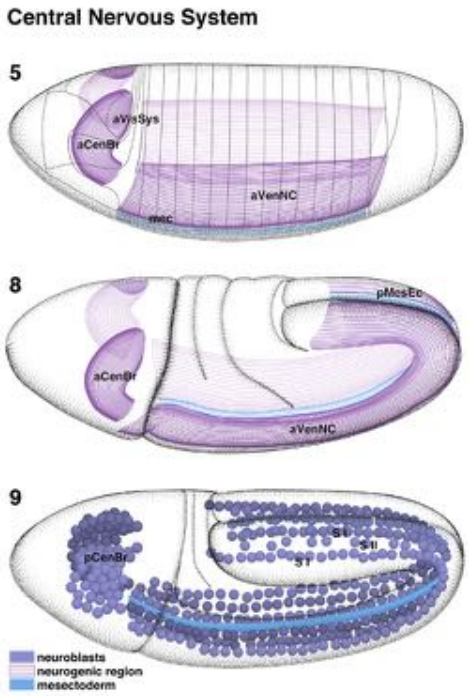
Lewis E. Report on the mutants Antennapedia-Bacon and Antennapedia-Yu. Drosoph Inf Serv 1956; 30:76.

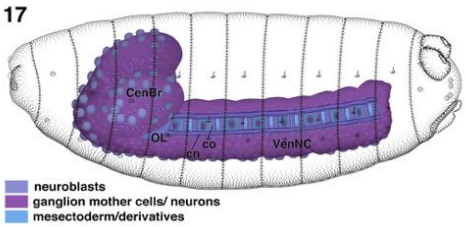
The use of *Drosophila* in biomedical research, some examples.

Axon guidance



Axon guidance





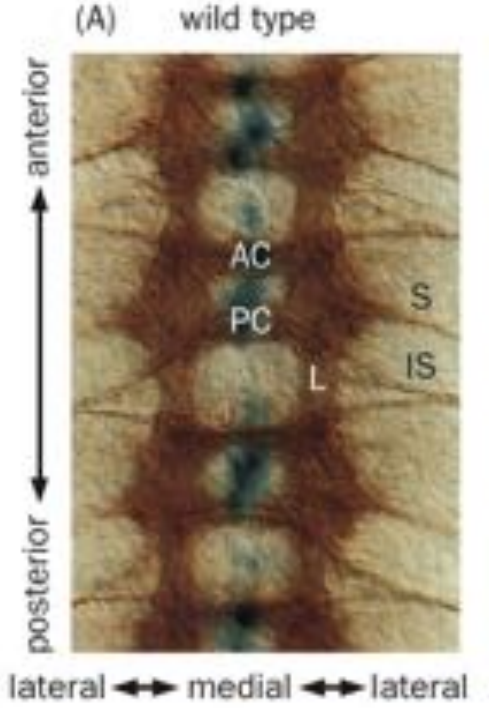


Figure 7-12 Principles of Neurobiology | © Garland Scien

Crossing the midline: Combinatorial actions of guidance receptors specify axon trajectory choice

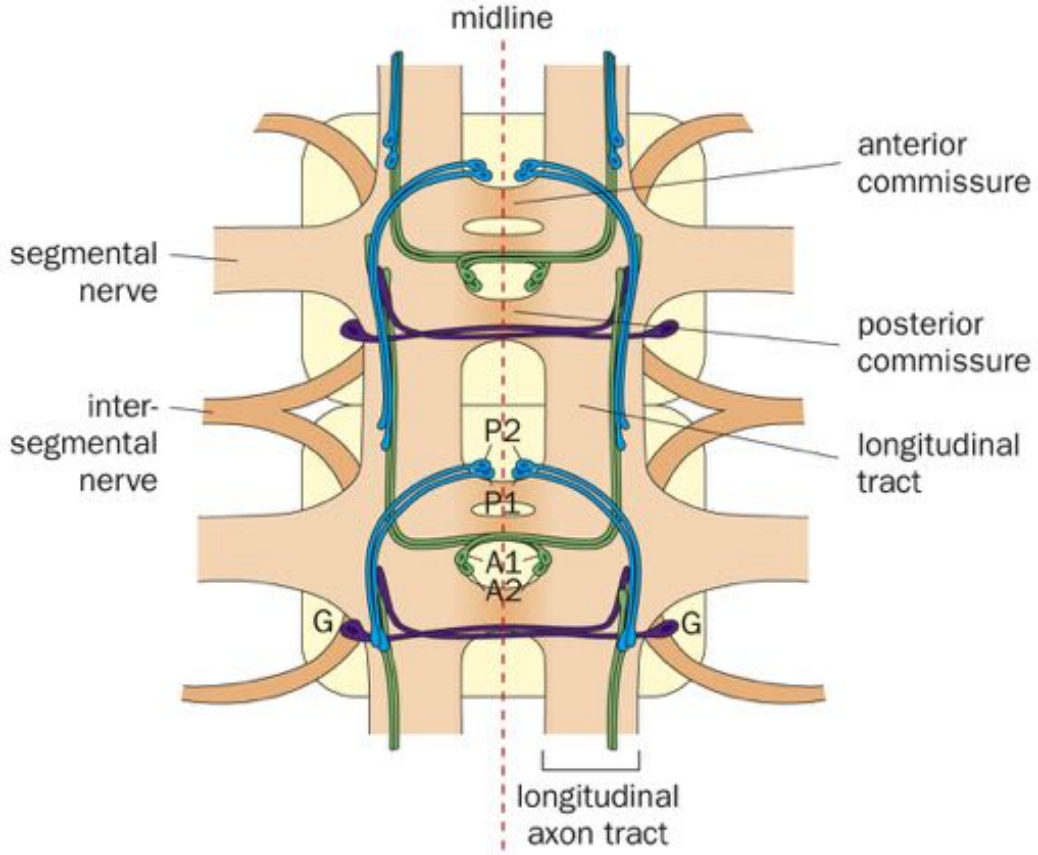
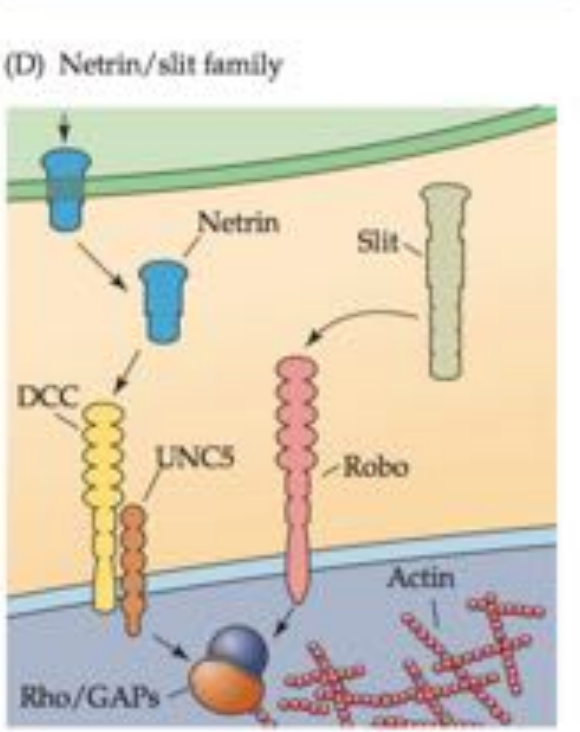


Figure 7-11 Principles of Neurobiology (© Garland Science 2016)



Crossing the midline: Combinatorial actions of guidance receptors specify axon trajectory choice

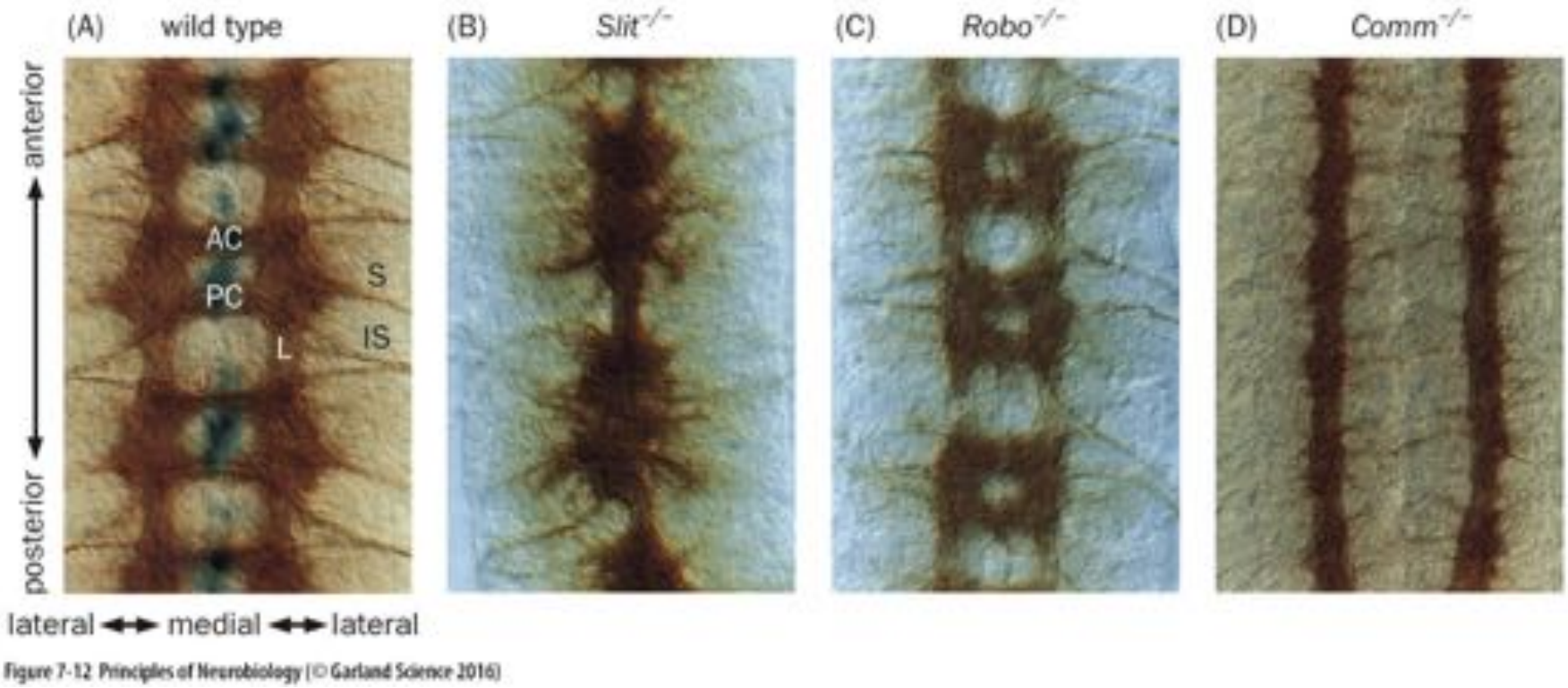
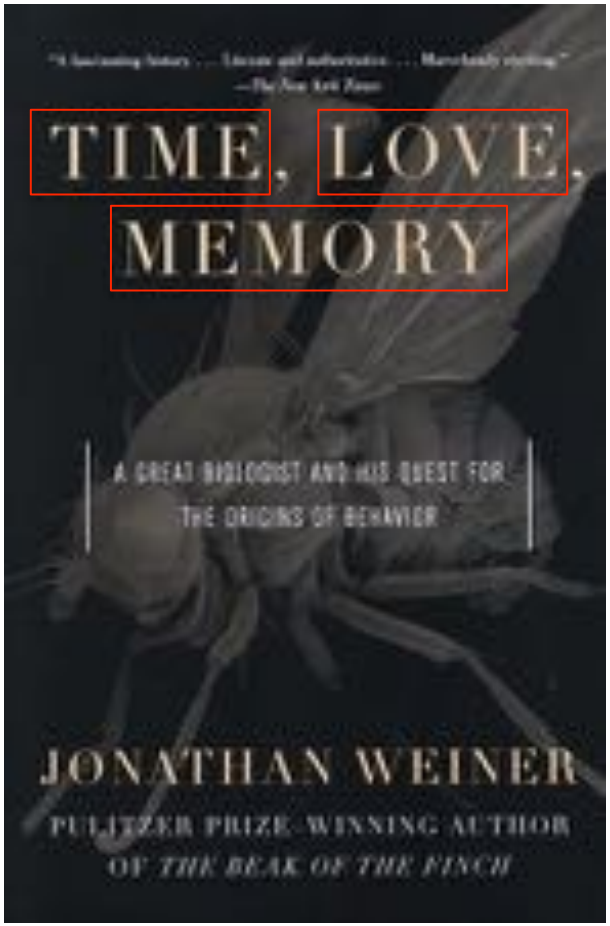


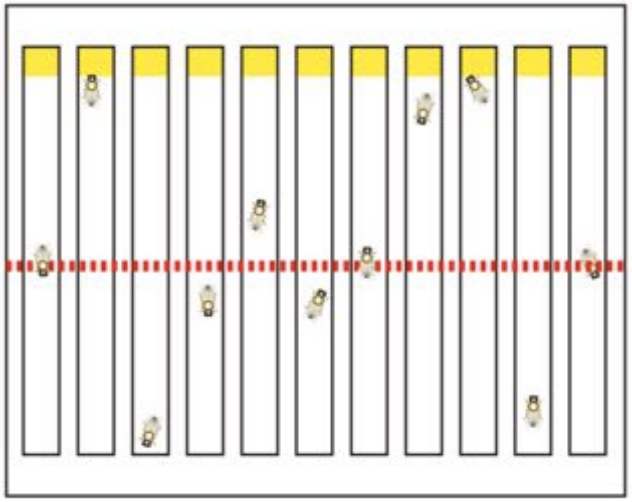
Figure 7-12 Principles of Neurobiology (© Garland Science 2016)

Seeger M, Tear G, Ferres-Marco D, Goodman CS. Mutations affecting growth cone guidance in Drosophila: genes necessary for guidance toward or away from the midline. Neuron. 1993 Mar;10(3): 409-26.

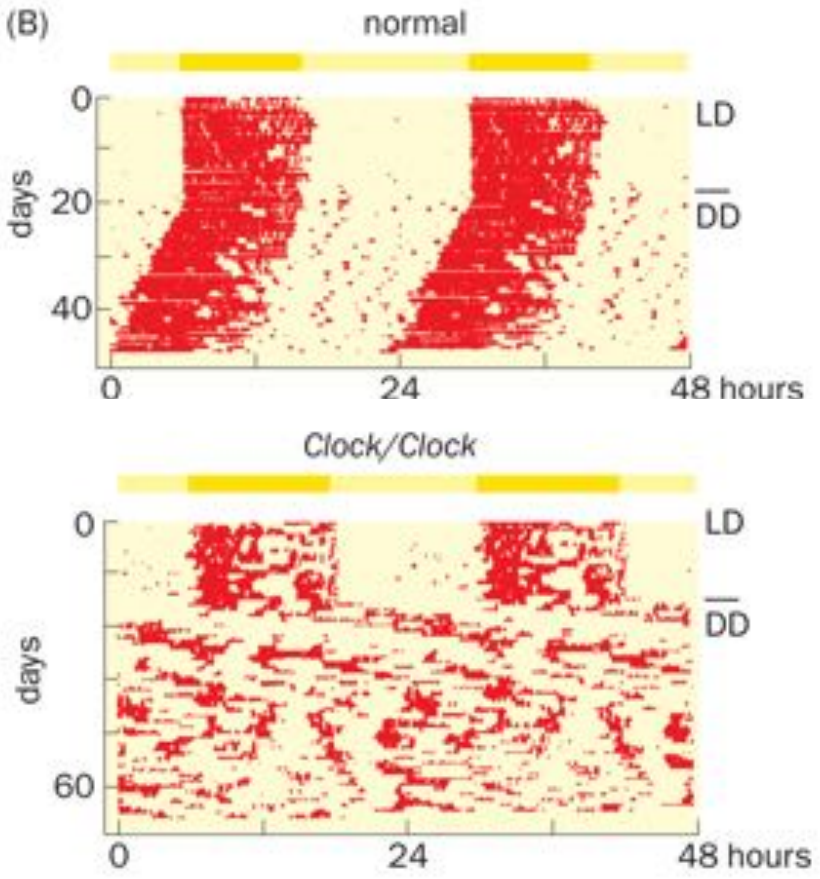
Seymour Benzer (October 15, 1921 – November 30, 2007)



Time…actually circadiam rithms

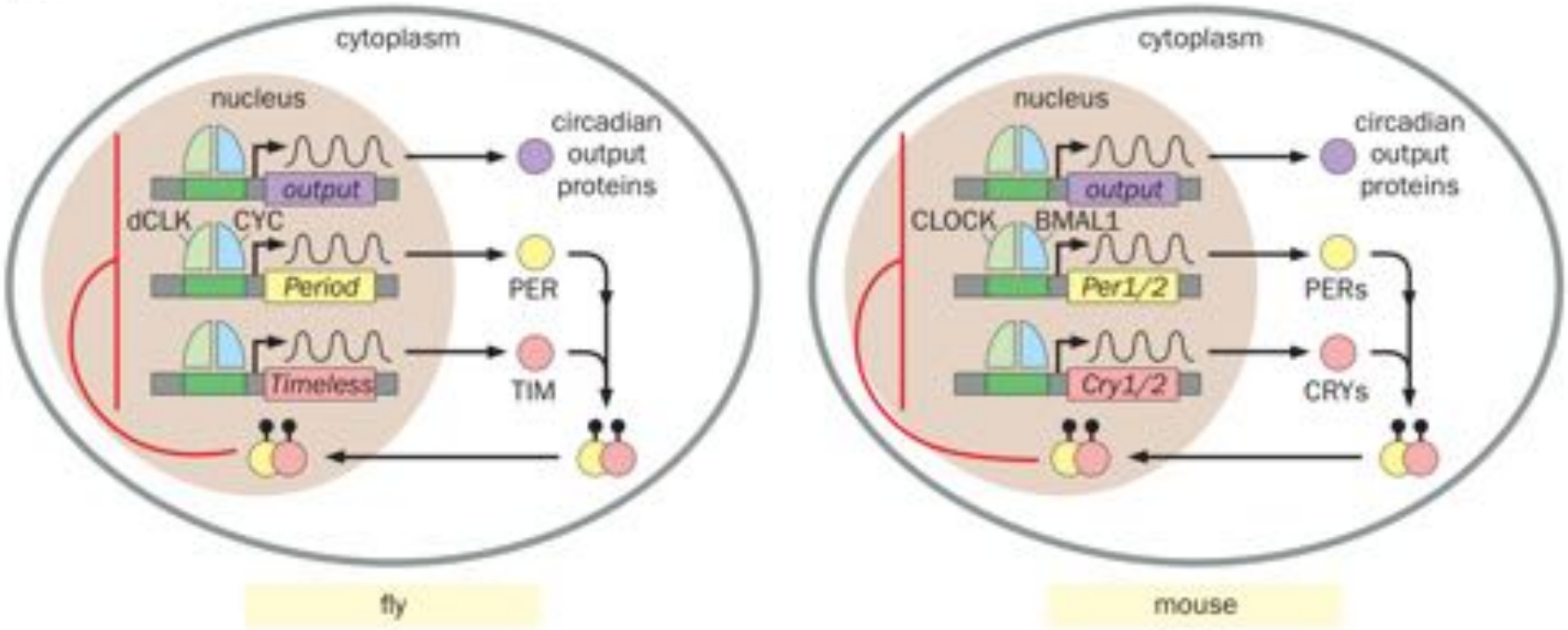


Adapted from Vosshall, 2007



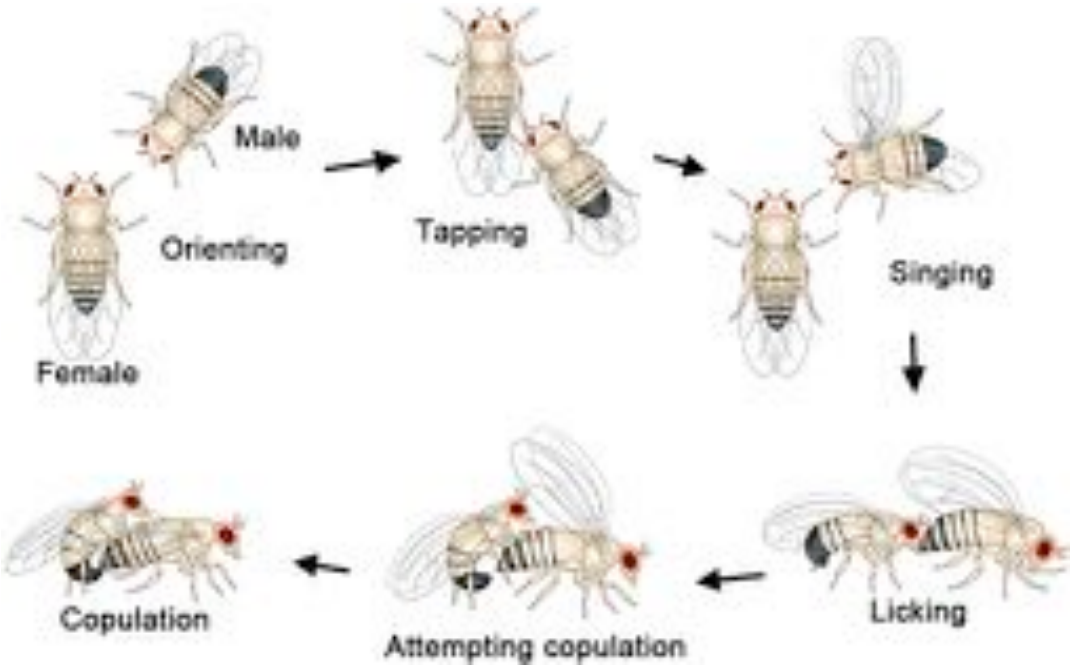
…and “period” and “timeless”

Ritmos Circadianos – feedback transcricional autoinibitório

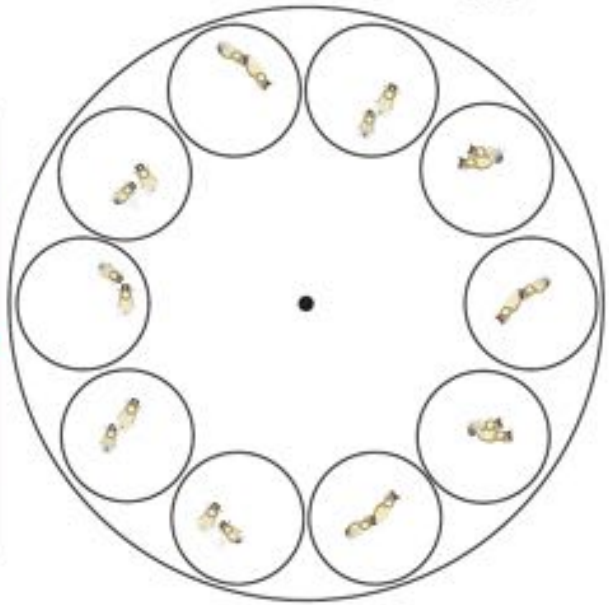


Love…actually sex

Courtship behaviour



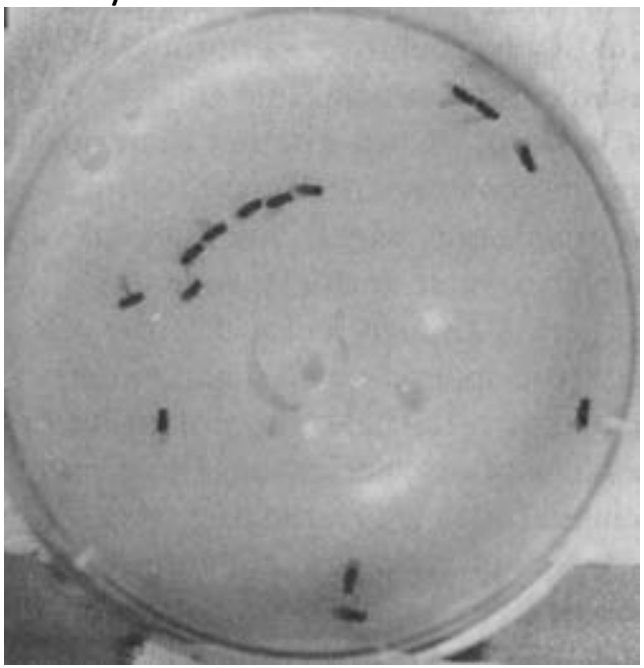
Courtship behaviour arenas



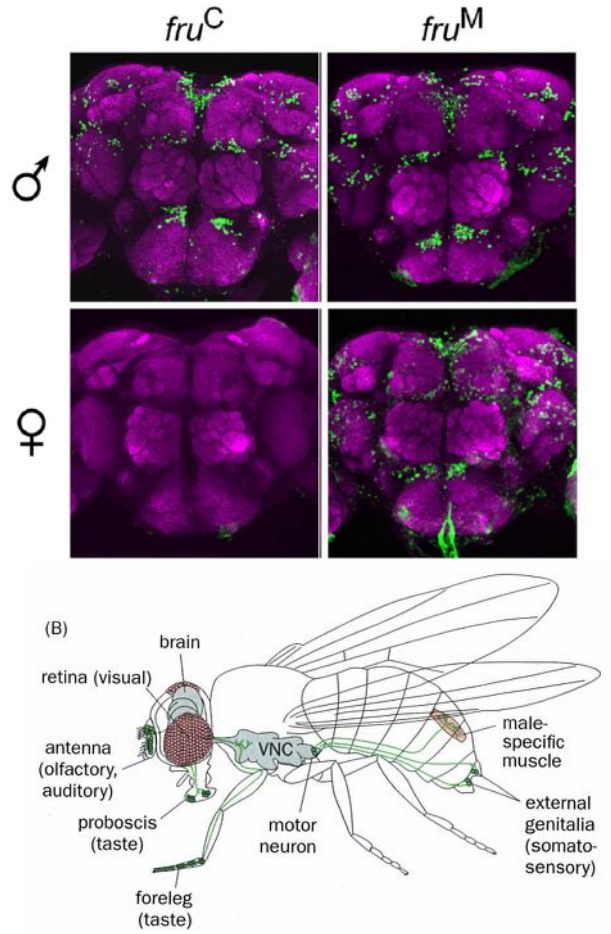
Adapted from Vosshall, 2007

Role of fruitless gene

Fru1/Df



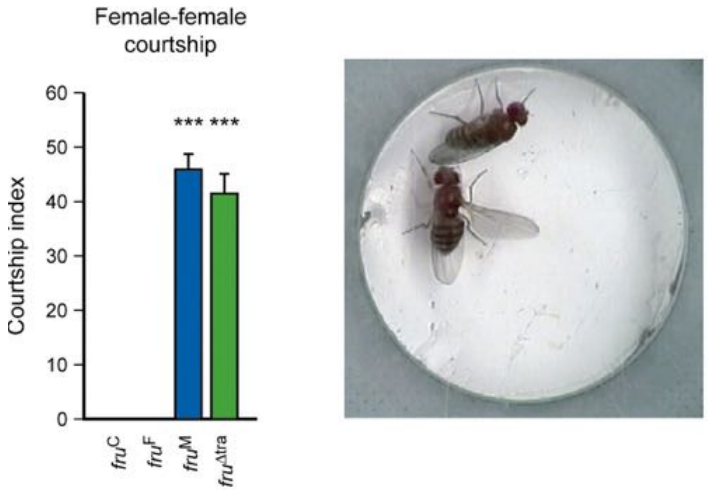
Hall 1994, Science



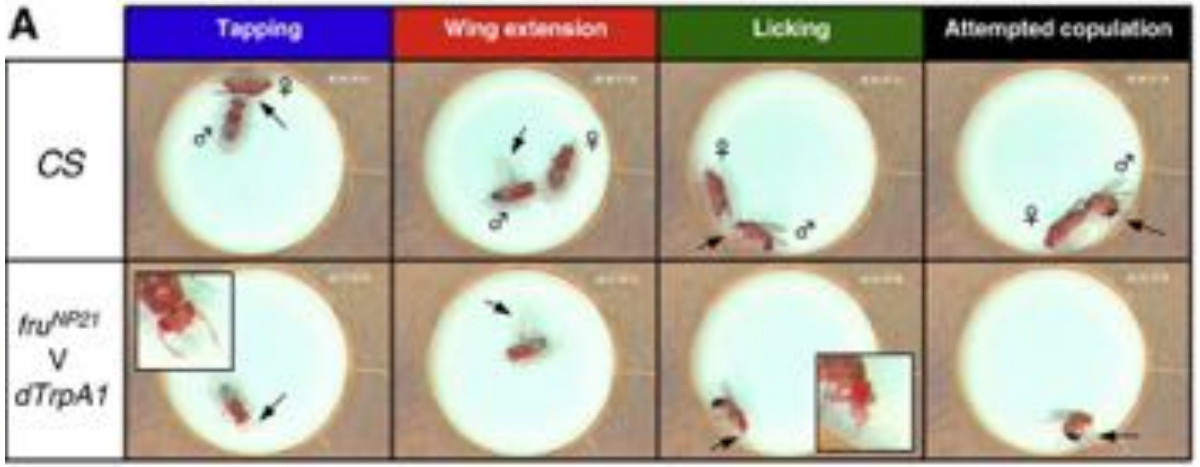
Demir and Dickson, *fruitless* Splicing Specifies Male Courtship Behavior in *Drosophila* Cell, Vol. 121, 785–794, June 3, 2005

Role of fruitless gene

fruitless loss-of-function



fruitless gain-of-function

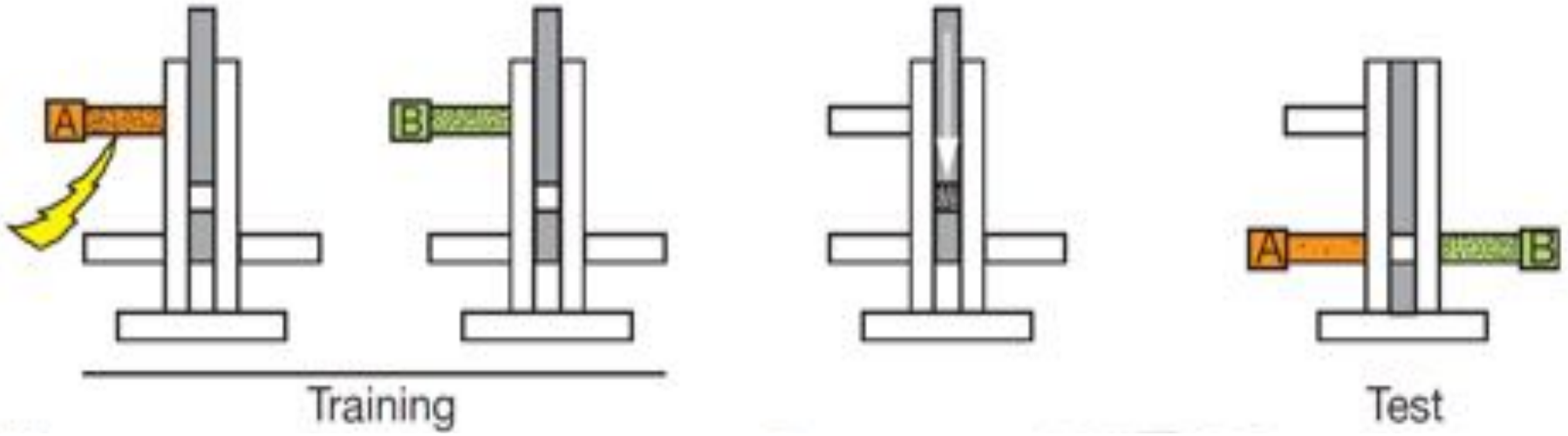


Kohatsu et al. Female contact activates male-specific interneurons that trigger stereotypic courtship behavior in Drosophila. Neuron. 2011

Demir and Dickson, *fruitless* Splicing Specifies Male Courtship Behavior in *Drosophila* Cell, Vol. 121, 785–794, June 3, 2005

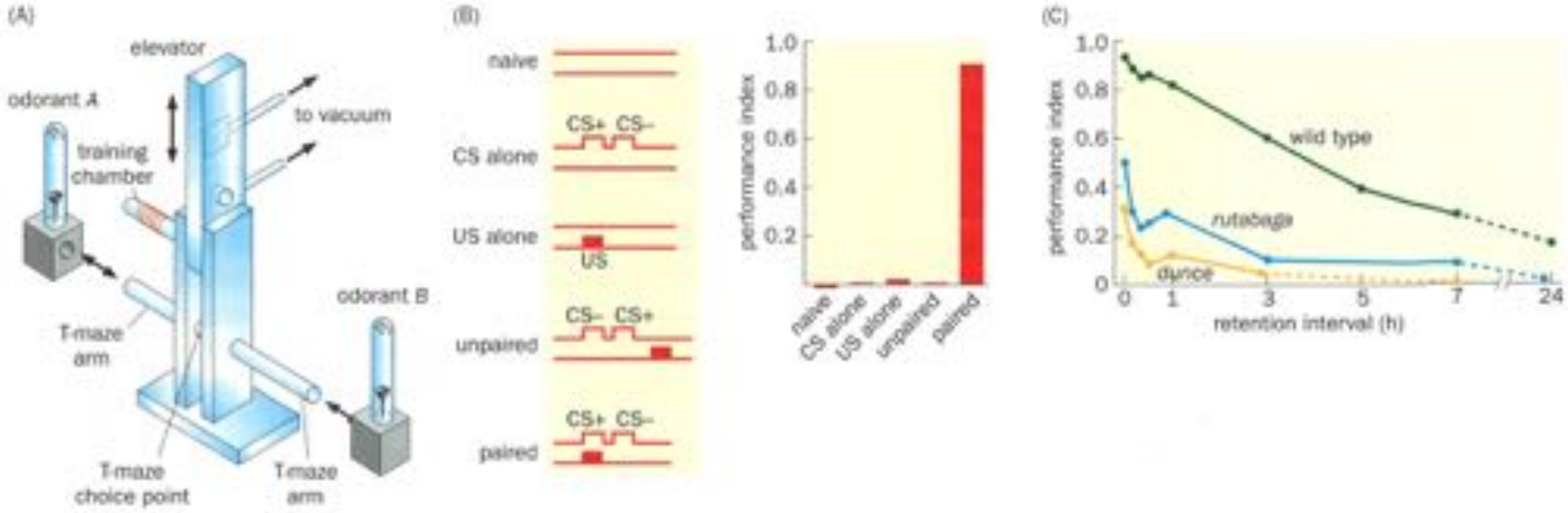
Memory…yes, actually memory

A T-maze assay for olfactory aversive conditioning



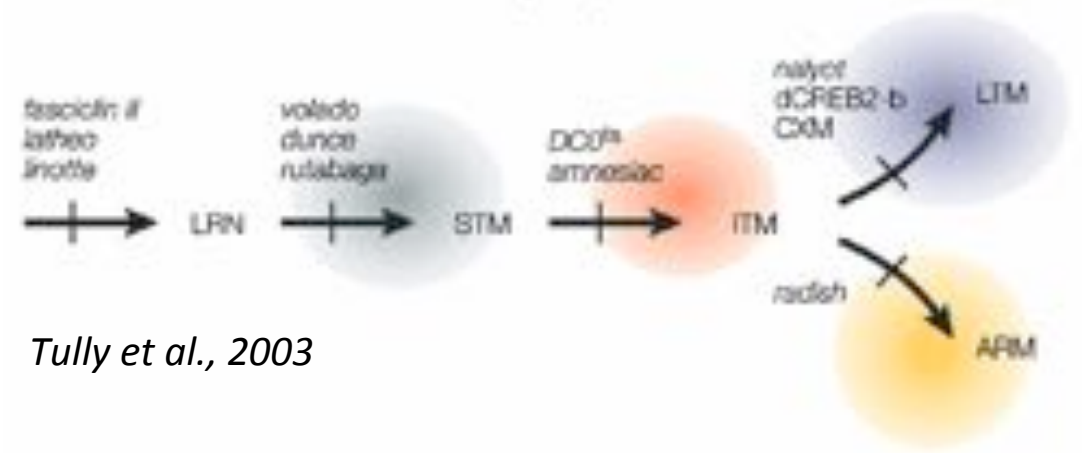
Adapted from Vosshall, 2007

Identification of learning and memory genes



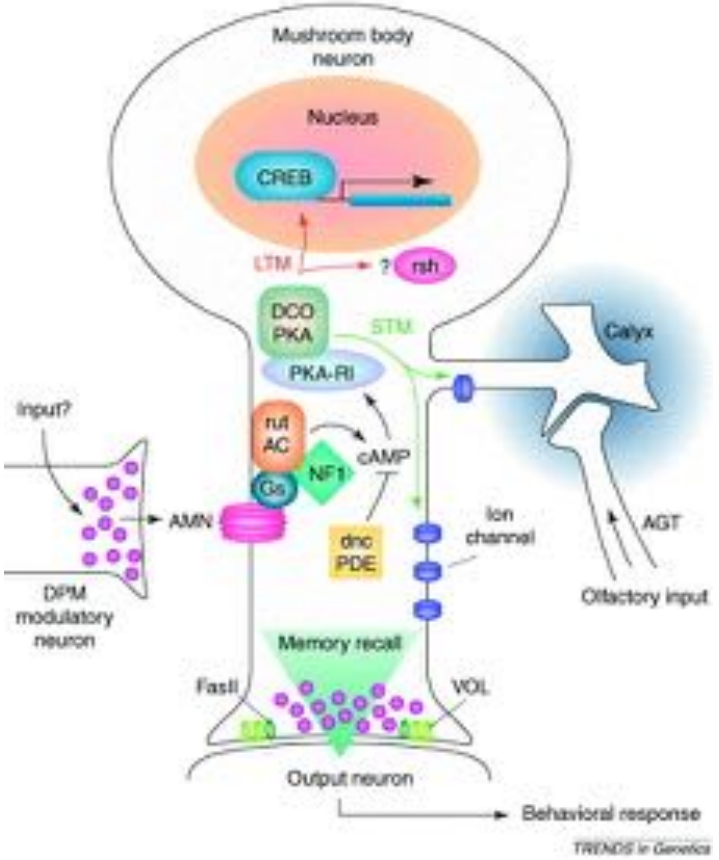
Adapter from Tully and Quinn 1985 and Yudai et al. 1976

Genetic control of learning and memory



Tully et al., 2003

The Drosophila mushroom body, shown in the MB010B-GAL4 line. Courtesy of Katrin Vogt.



Trends in Genetics 2001 17, 719-726DOI: (10.1016/S0168-9525(01)02526-4)

And many other behaviors…

EMOTIONS

ALCOHOL CONSUMPTION

MOTOR CONTROL

SLEEP

EJACULATION

REGURGITATION

PAIN

DEPRESSION

GROUP BEHAVIOUR

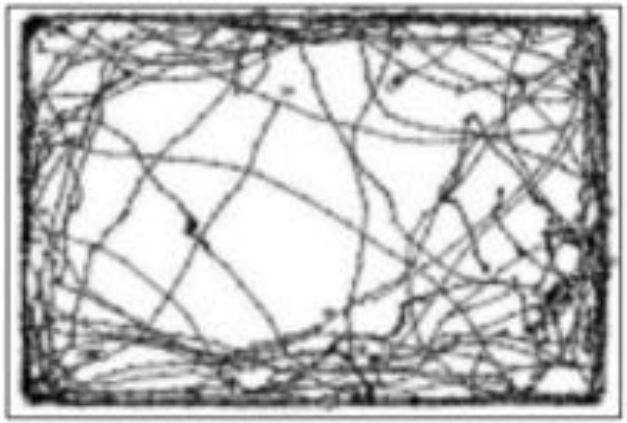
FREEZING BEHAVIOUR

HUNGER

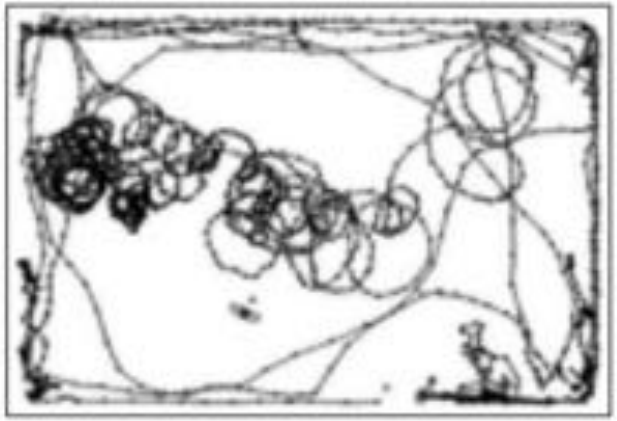
AGRESSION

You can even study cocaine addiction!

sober



On coke



Neuronal manipulation by Optogenetics

A prologue

The impact of molecular biology on neuroscience

Francis Crick, OM FRS

The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

How our brains work is one of the major u
techniques of molecular biology that are al
result of the human genome project many n
vely influence the progress of neuroscience.
what their difficulties are, in the hope tha
tools.

deserves immediate and serious attention.

A major first step, then, is to identify the many different types of neuron existing in the cerebral cortex and other parts of the brain. One of the next requirements (as discussed above) is to be able to turn the firing of one or more types of neuron on and off in the alert animal in a rapid manner. The ideal signal would be light, probably at an infrared wavelength to allow the light to penetrate far enough. This seems rather far-fetched but it is conceivable that molecular biologists

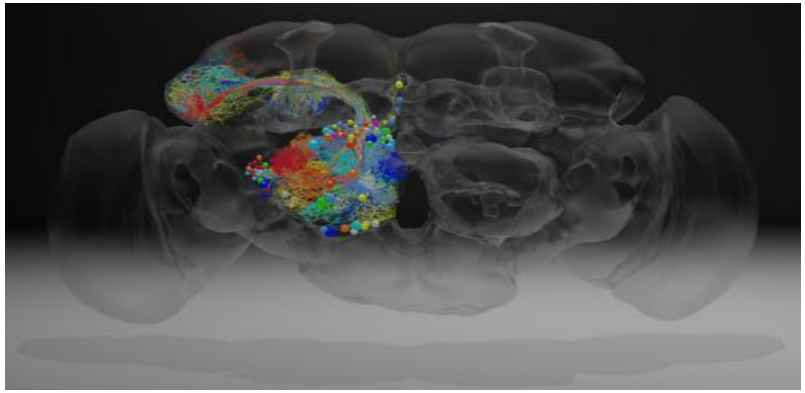
Francis Crick, The impact of molecular biology on neuroscience., Philos Trans R Soc Lond B Biol Sci. 1999

Some definitions:

Optogenetics: A set of techniques that allow the manipulation of neuronal populations using light.

- Uses heterologous channels genetically encoded in a set of neurons
- Uses light to trigger channel opening with high temporal resolution
- Effects include gain and loss of neuronal activity

Manipulation



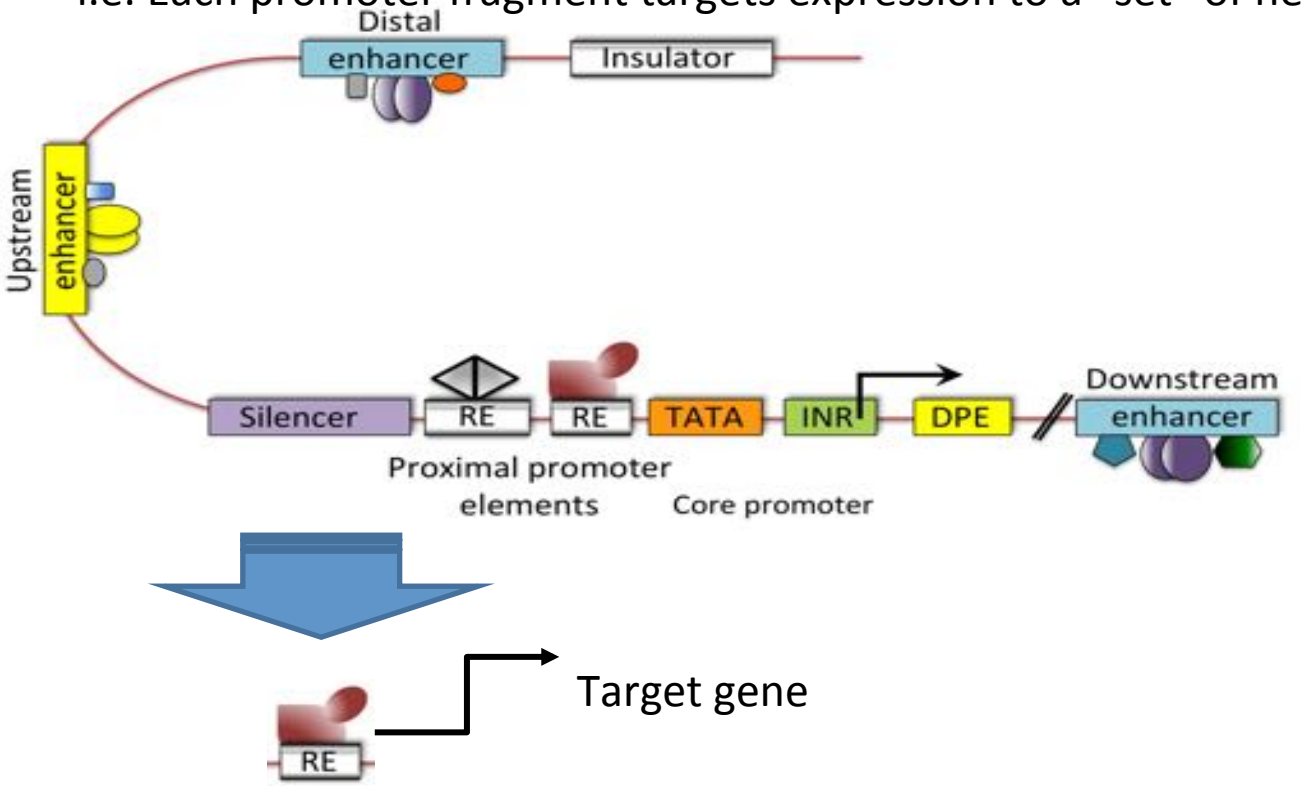
Behavior?

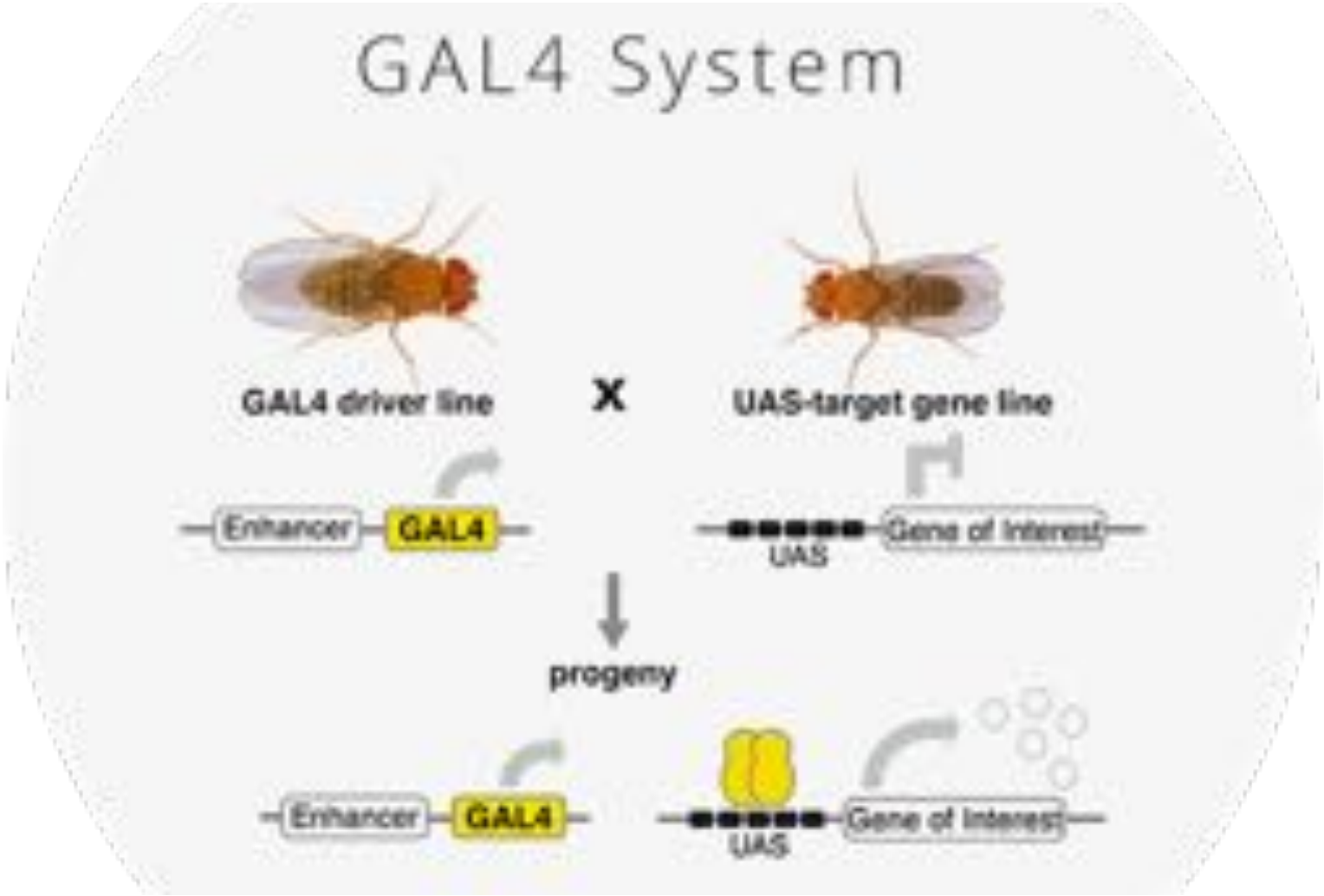
(aggression; courtship; feeding; movement; choice..)

Optogenetics: 10 years after ChR2 in neurons - Views from the community ; in Nature Neuroscience 18(9):1202-12 · August 2015

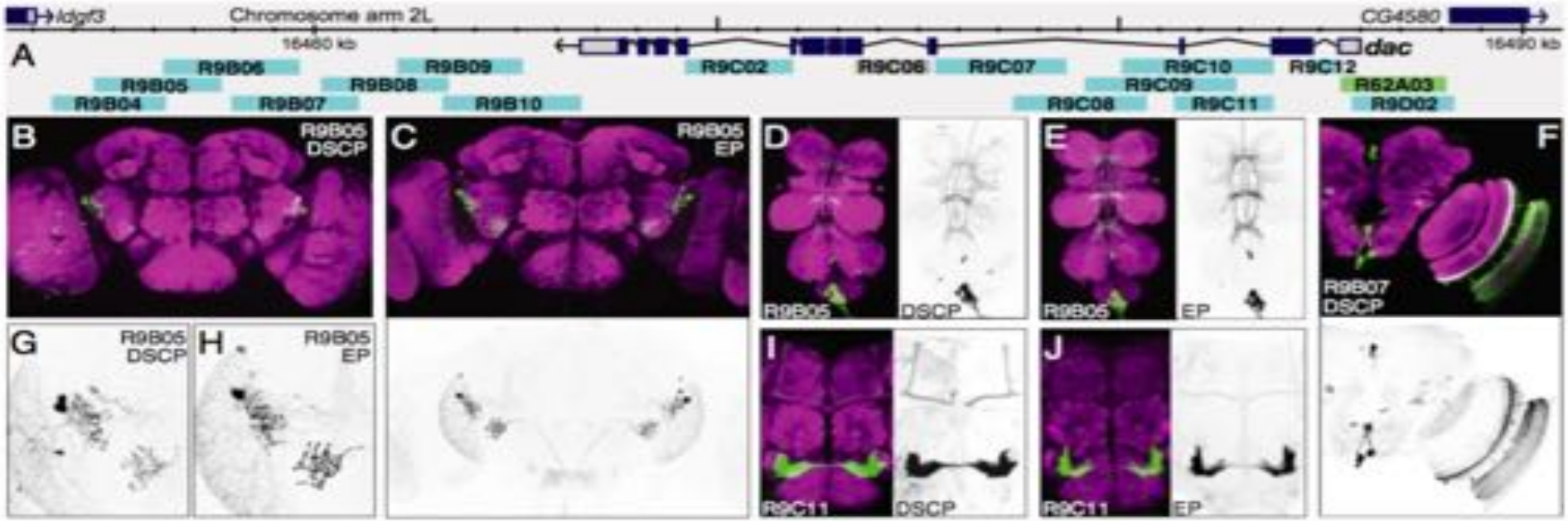
What's the "genetics" from?

Each cell has a transcriptional/genetic signature.
i.e. Each promoter fragment targets expression to a "set" of neurons.




GAL4 System
GAL4 driver line
X
UAS-target gene line
Enhancer
GAL4
UAS
Gene of Interest
progeny
Enhancer
GAL4
UAS
Gene of Interest

Driving expression with (almost) single cell resolution



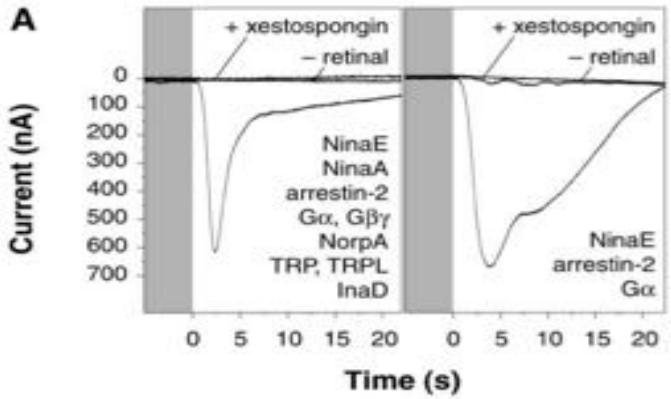
FlyLight Pfeiffer et al., Proc. Natl. Acad. Sci. USA 105, 9715-9720.

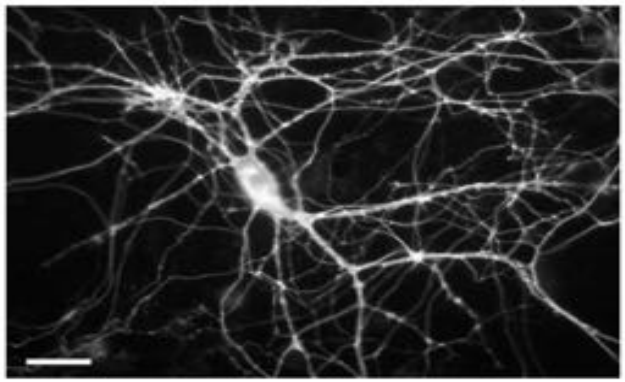
Back to the opto from optogenetics

Neuron. 2002 Jan 3;33(1):15-22.

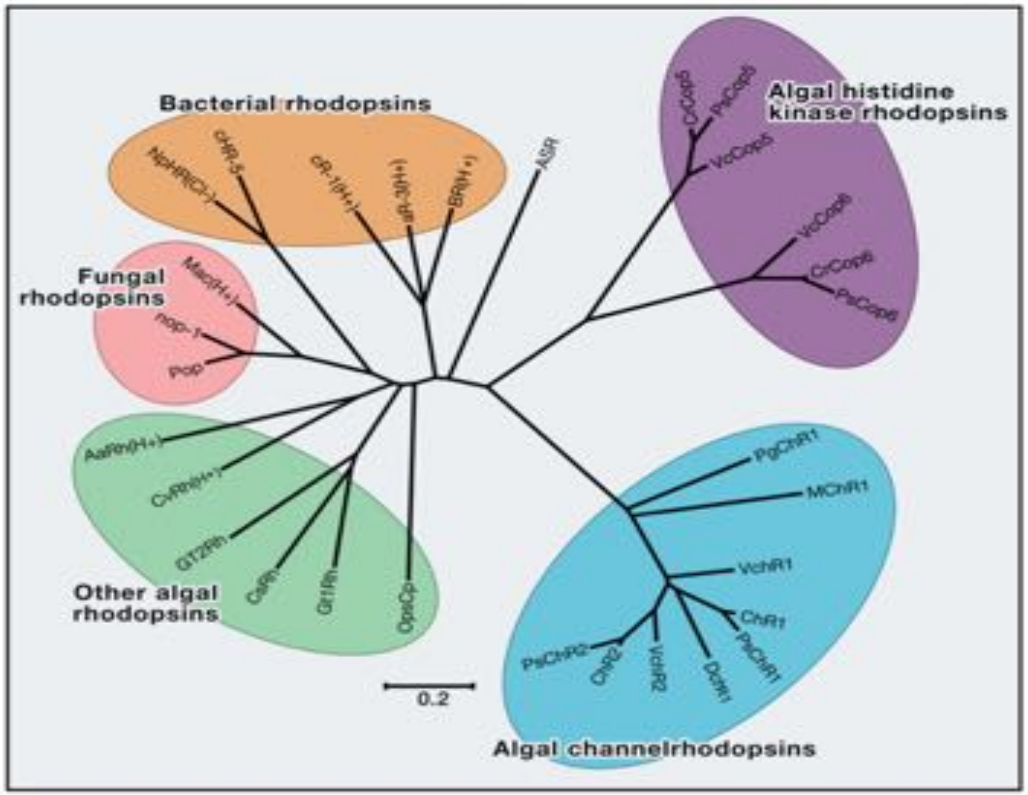
Selective photostimulation of genetically chARGed neurons.

Zemelman BV[1], Lee GA, Ng M, Miesenböck G.



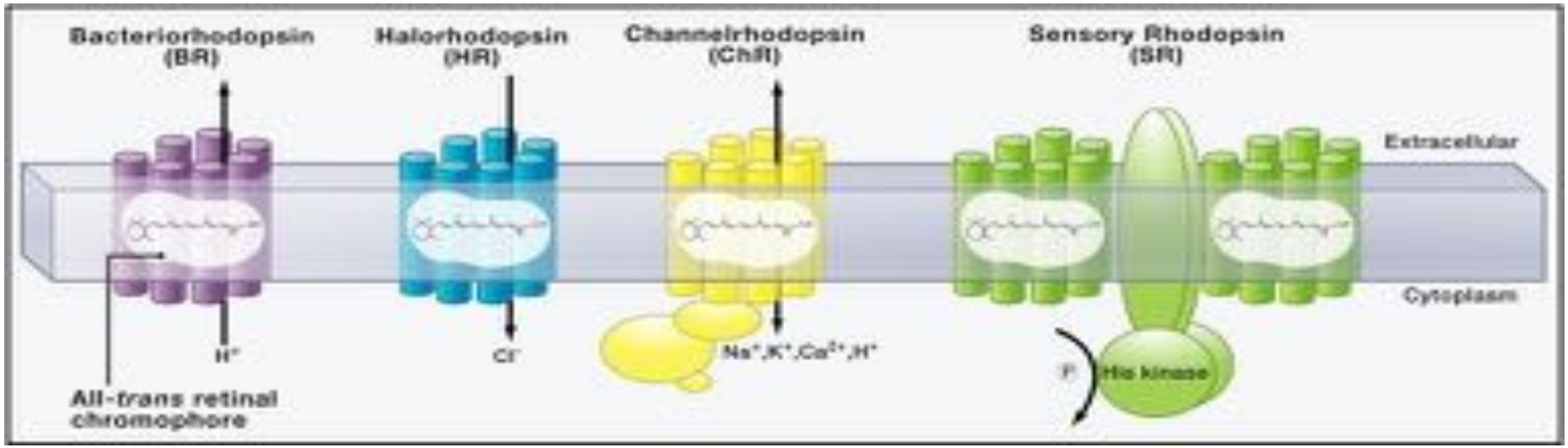


The magic ingredient: Opsins, Light-sensitive transmembrane proteins



Zhang et al, The microbial opsin family of optogenetic tools. Cell. 2011 Dec 23;147(7)

The magic ingredient: Opsins, Light-sensitive transmembrane proteins



Zhang et al, The microbial opsin family of optogenetic tools. Cell. 2011 Dec 23;147(7)

nature neuroscience

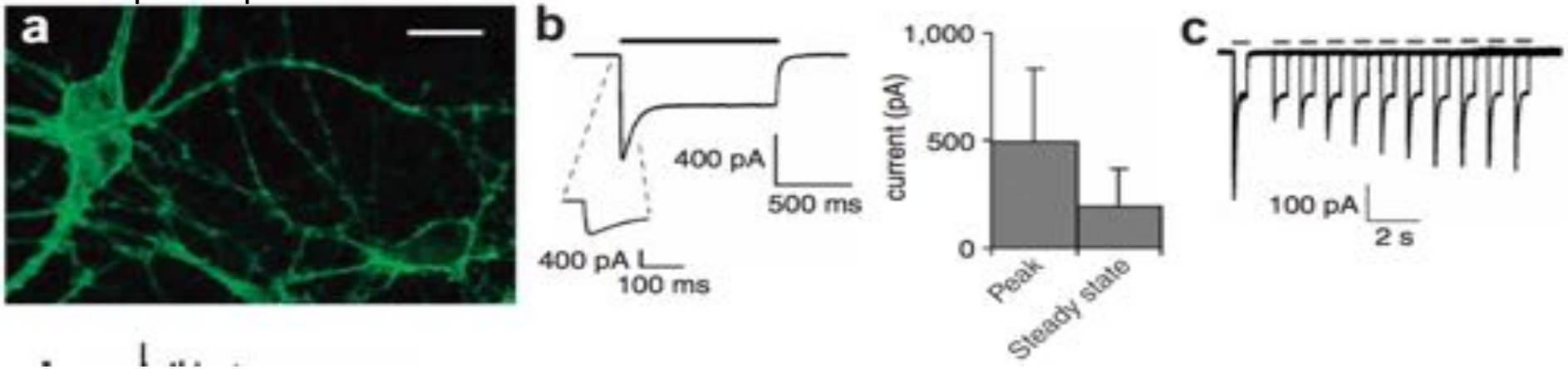
TECHNICAL REPORT

Millisecond-timescale, genetically targeted optical control of neural activity

Edward S Boyden[1], Feng Zhang[1], Ernst Bamberg[2,3], Georg Nagel[2,5] & Karl Deisseroth[1,4]

NATURE NEUROSCIENCE VOLUME 8 | NUMBER 9 | SEPTEMBER 2005

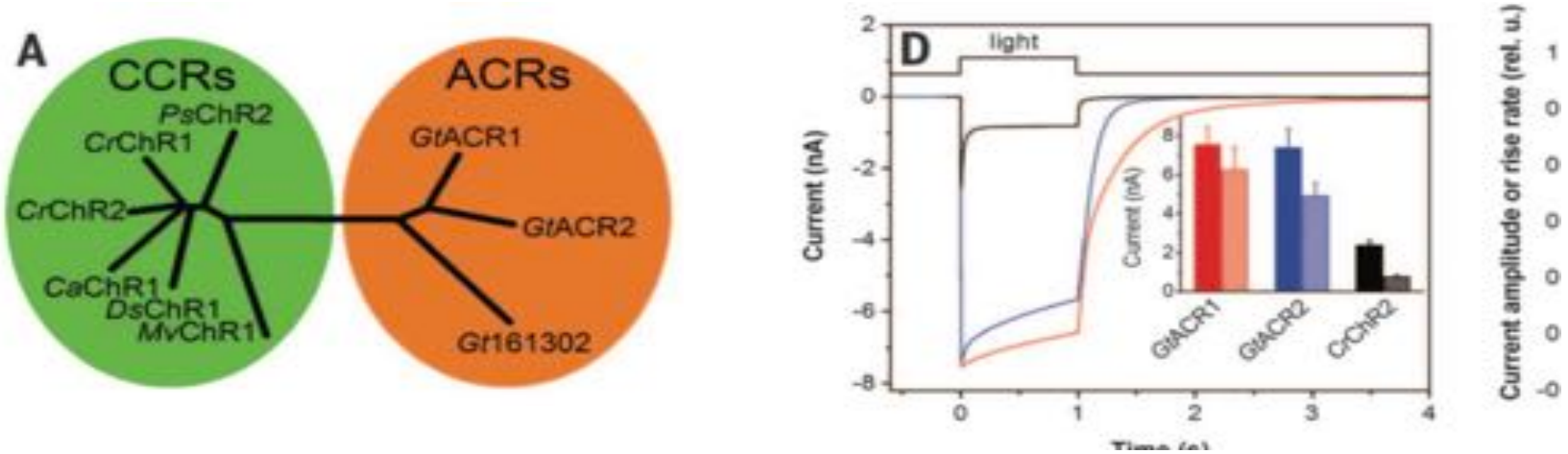
Hipocampal neurons > ChR2:YFP



NEUROSCIENCE

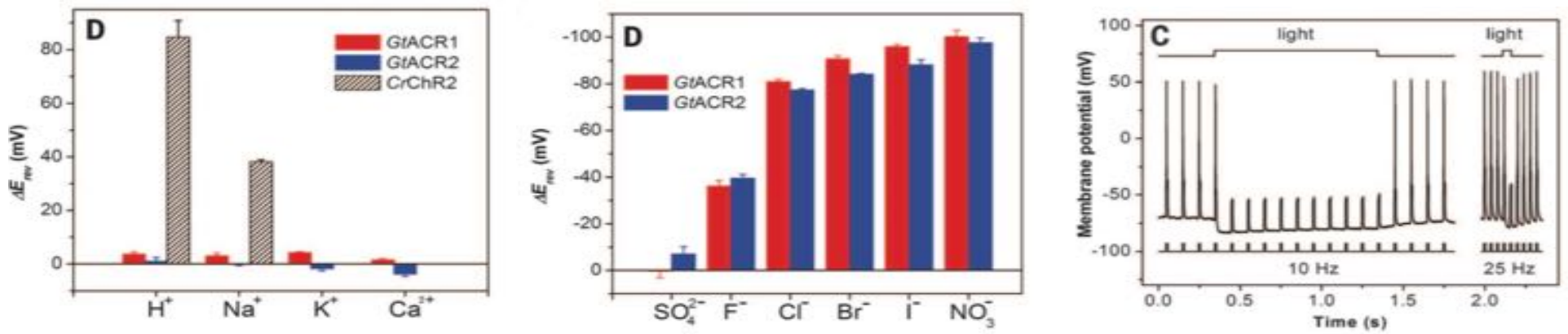
Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics

Elena G. Govorunova,[1] Oleg A. Sineshchekov,[1] Roger Janz,[2]
Xiaoqin Liu,[2] John L. Spudich[1]*



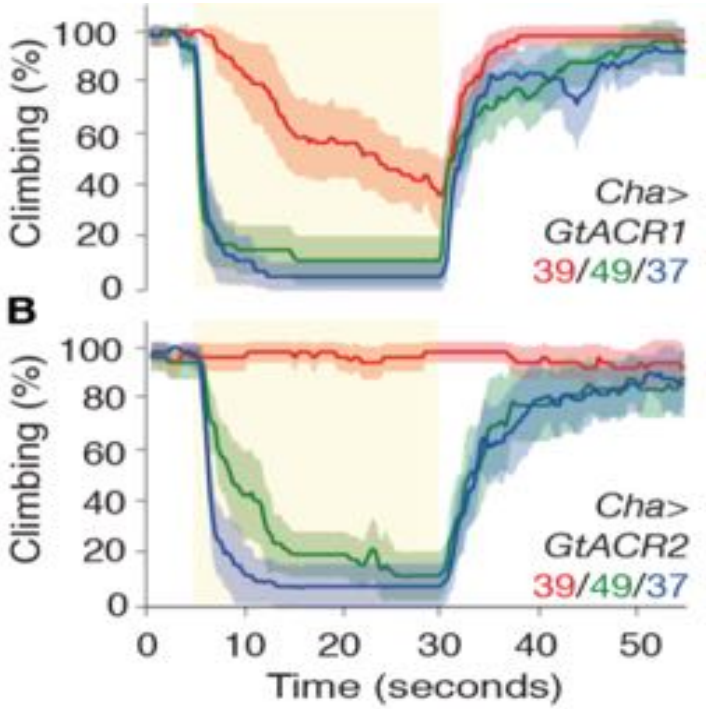
Govorunova et al, Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics, Science. Aug 7 (2015)

GtACR is a anion specific light-dependent channel



Govorunova et al, Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics, Science. Aug 7 (2015)

In vivo application of GtACR



Mohammad et al., Optogenetic inhibition of behavior with anion channelrhodopsins. Nat Method

Optogenetics:

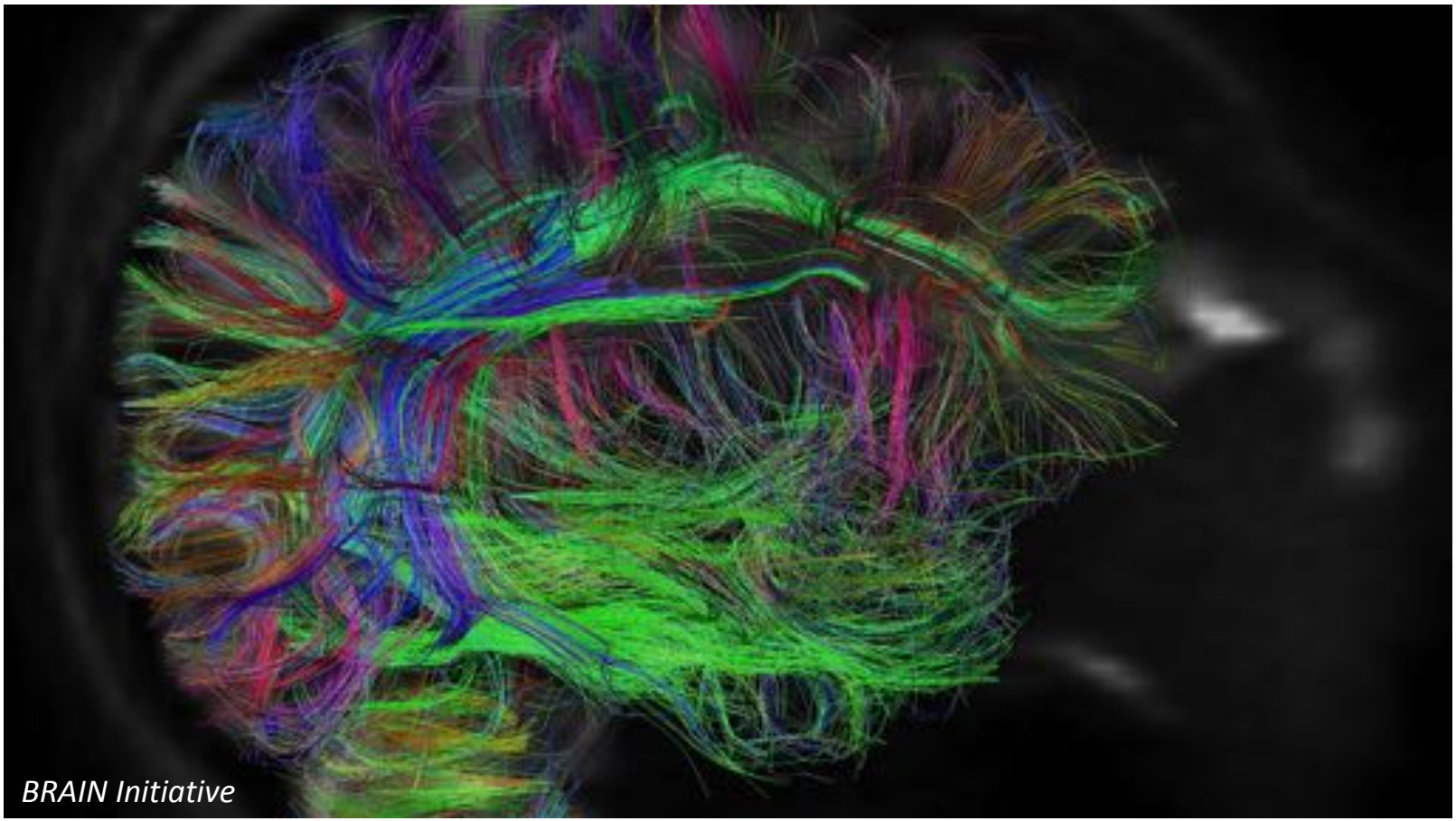
Advantages:

- Less invasive then traditional electrical stimulation
- Strong temporal control
- Targeted expression of channel rhodopsins, via specific promoters
- In vivo effects

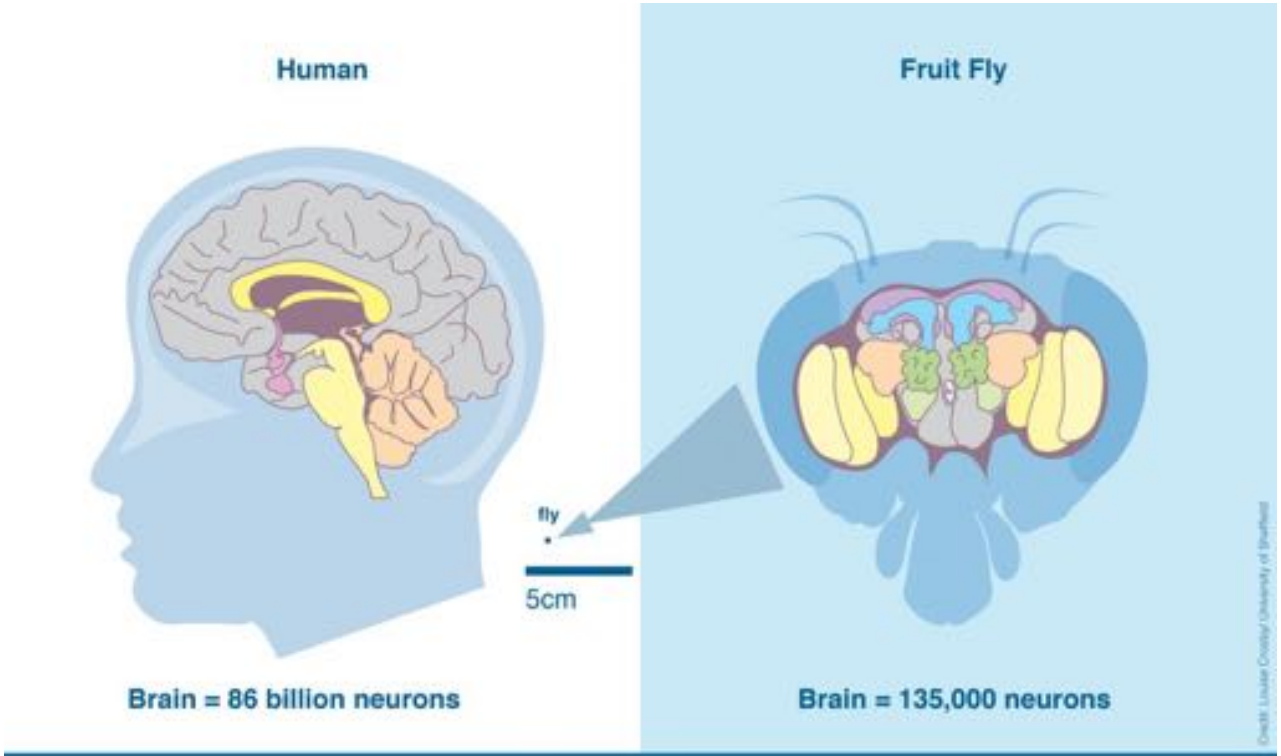
Disadvantages:

- Depends on transgenic lines/vector for rhodopsin expression
- Requires building a stimulation setup
- Depends on light penetration
- Not all cell types are responsive
- May depend on surgeries
- May display some toxicity

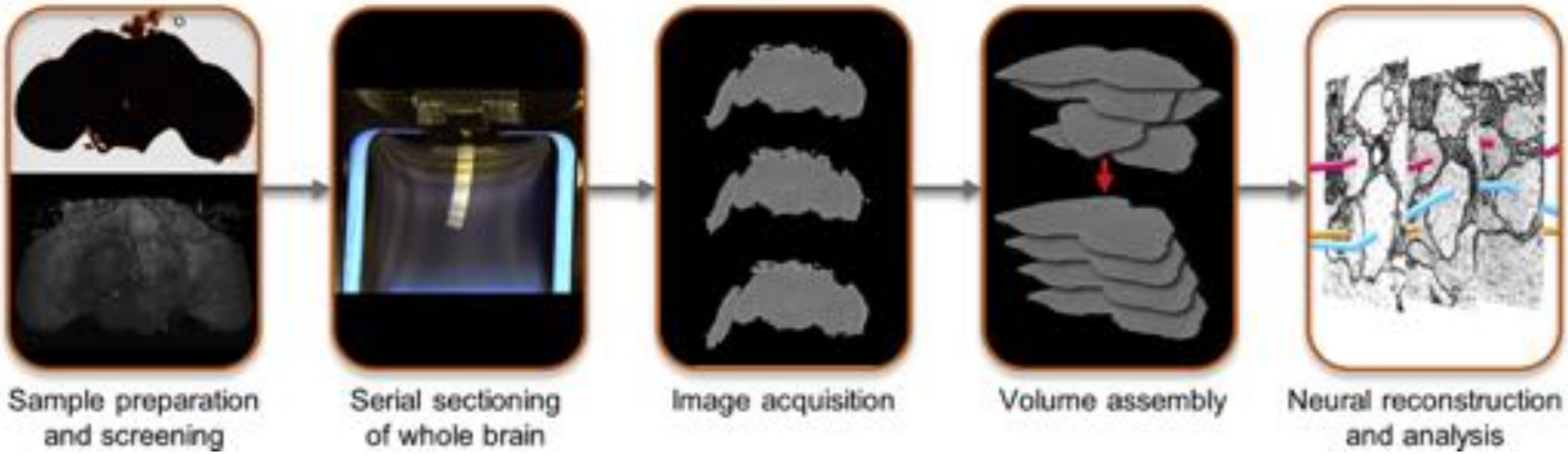
Establishing a connectome of the human brain



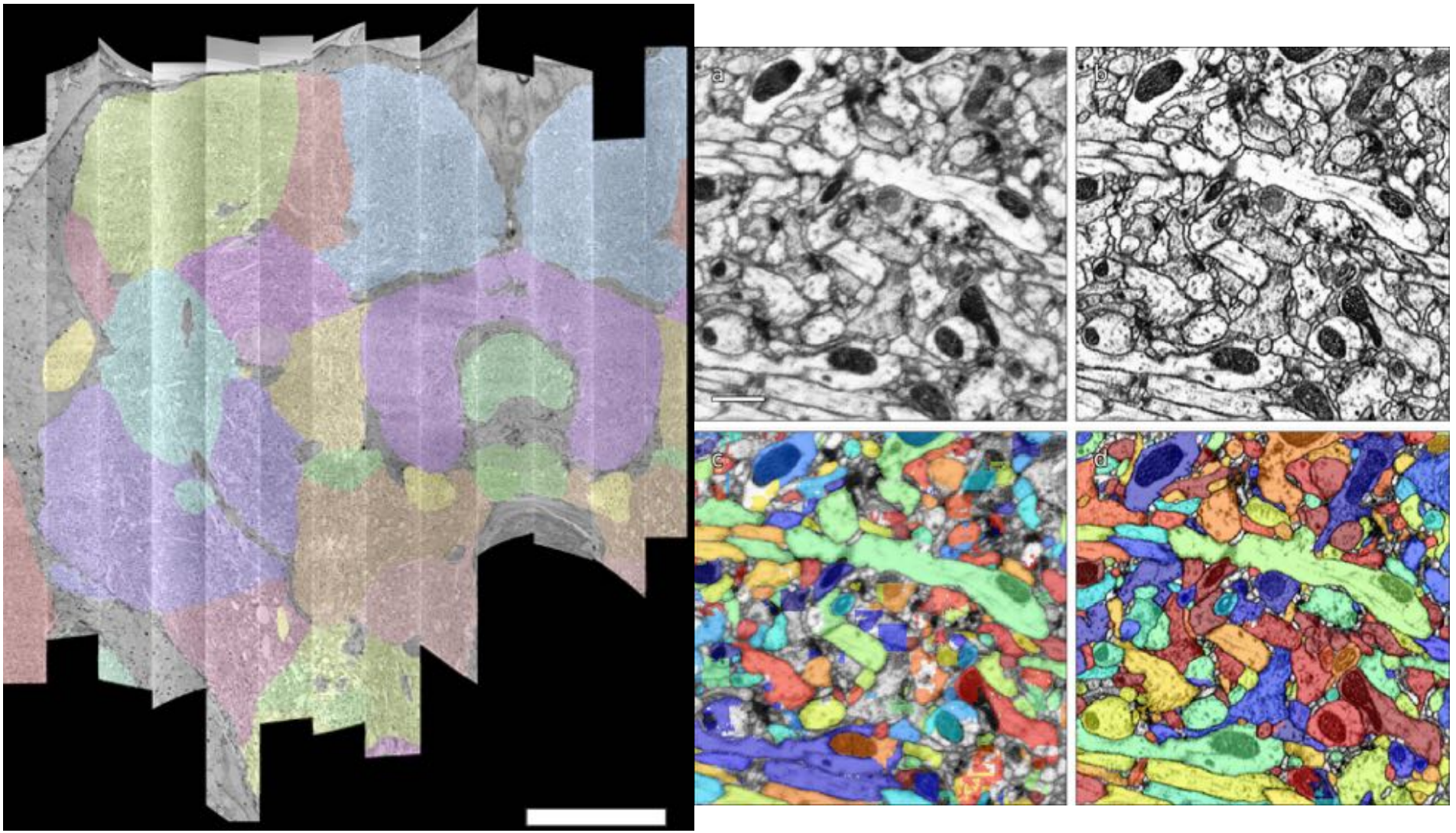
Starting point: the fly brain

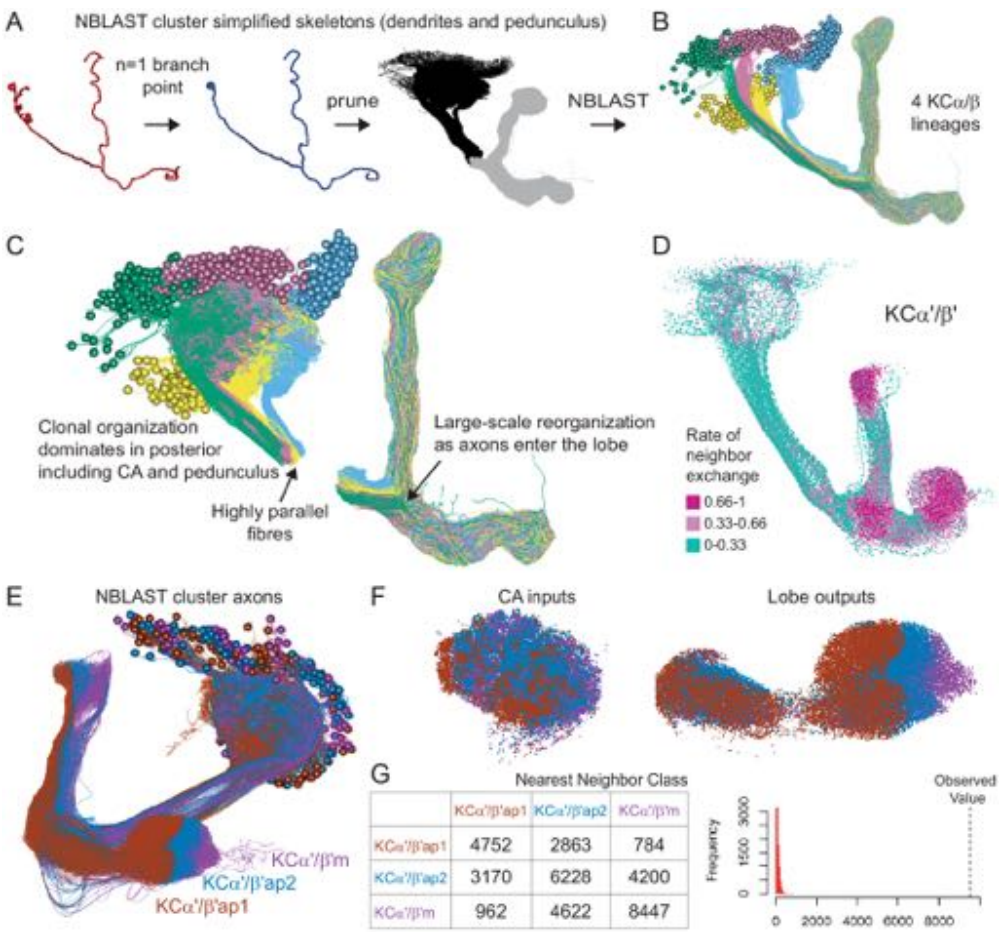


Starting point: the fly brain



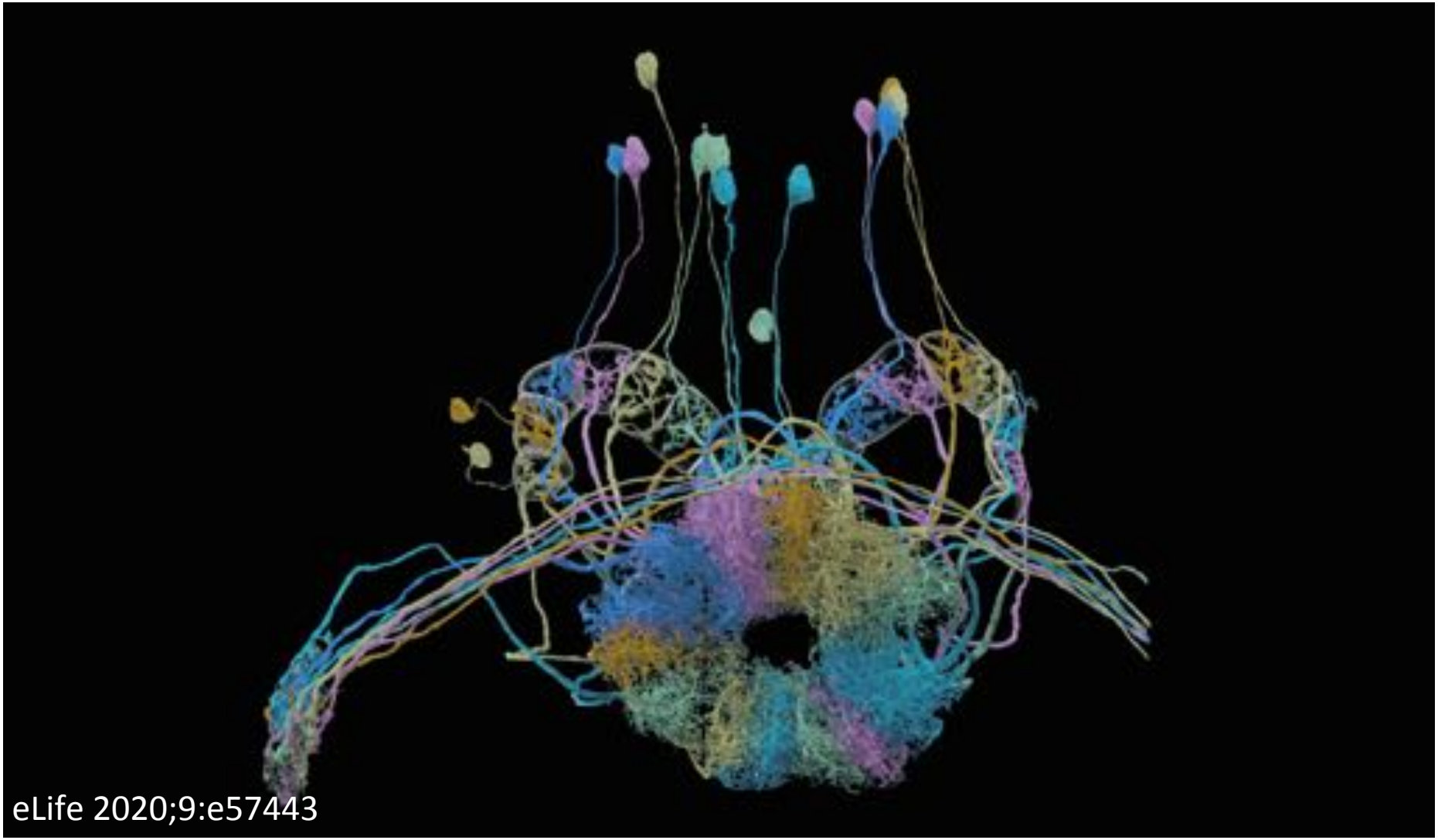
Cell 2018 174730-743.e22DOI: (10.1016/j.cell.2018.06.019)


a
b
c
d



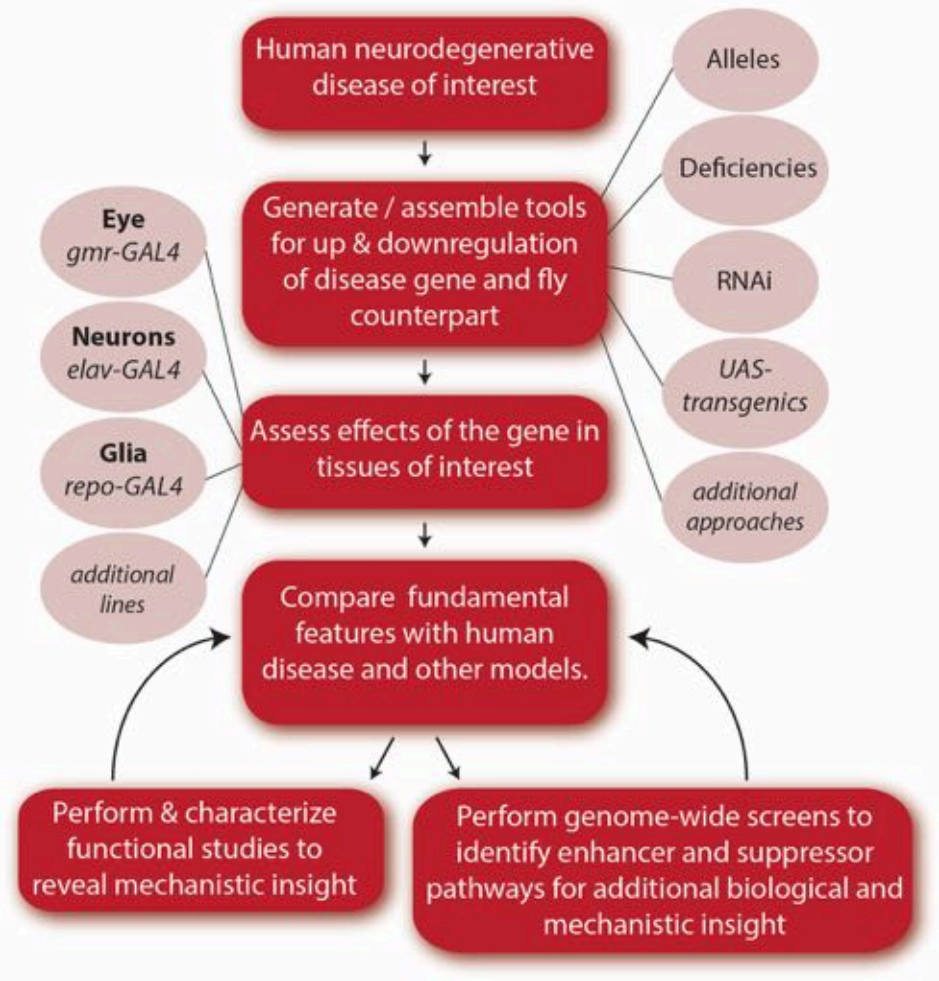
	KCα'/β'ap1	KCα'/β'ap2	KCα'/β'm
KCα'/β'ap1	4752	2863	784
KCα'/β'ap2	3170	6228	4200
KCα'/β'm	962	4622	8447

eLife 2020;9:e57443

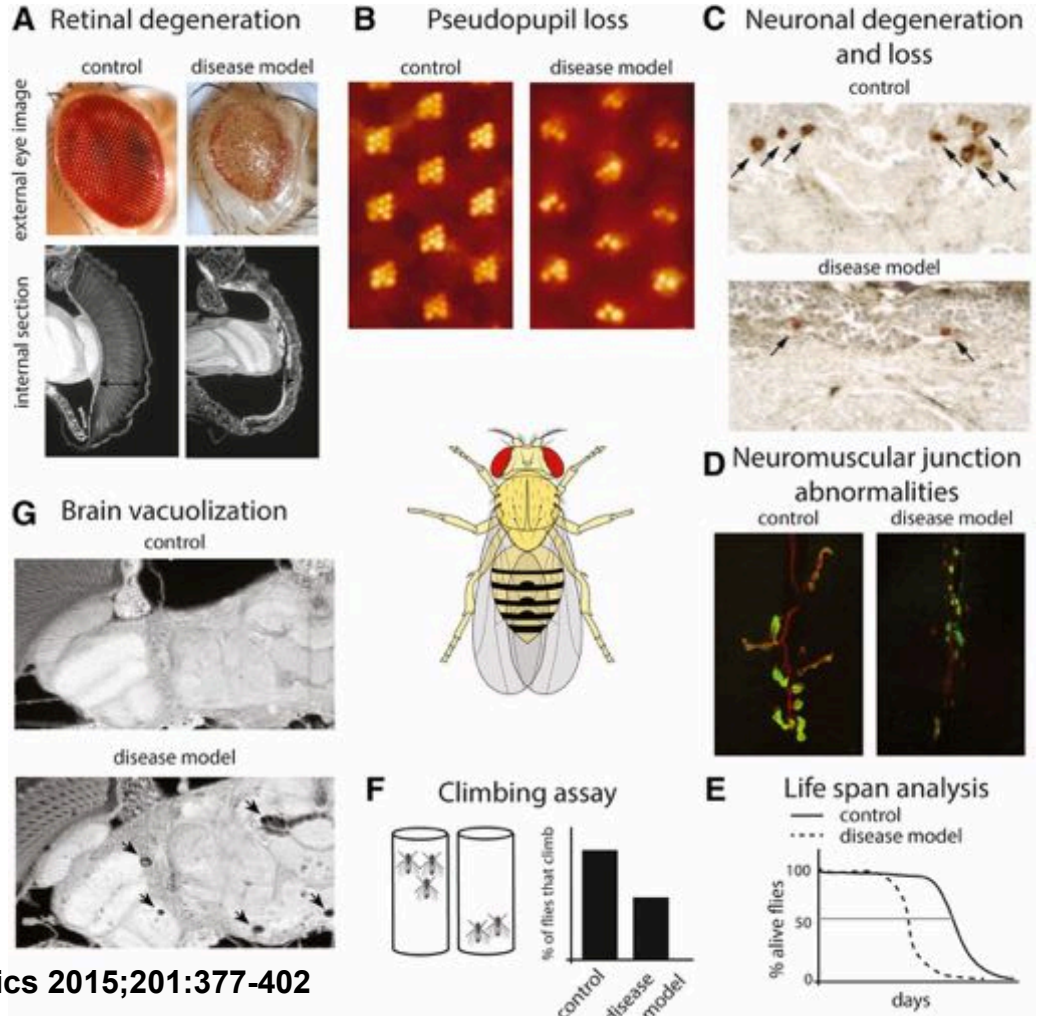

eLife 2020;9:e57443

Drosophila as a model to study neurodegeneration

Steps for investigating a *Drosophila* model for a human neurodegenerative disease.



Examples of robust assays to assess neural degeneration and dysfunction in Drosophila.



eeanne McGurk et al. Genetics 2015;201:377-402

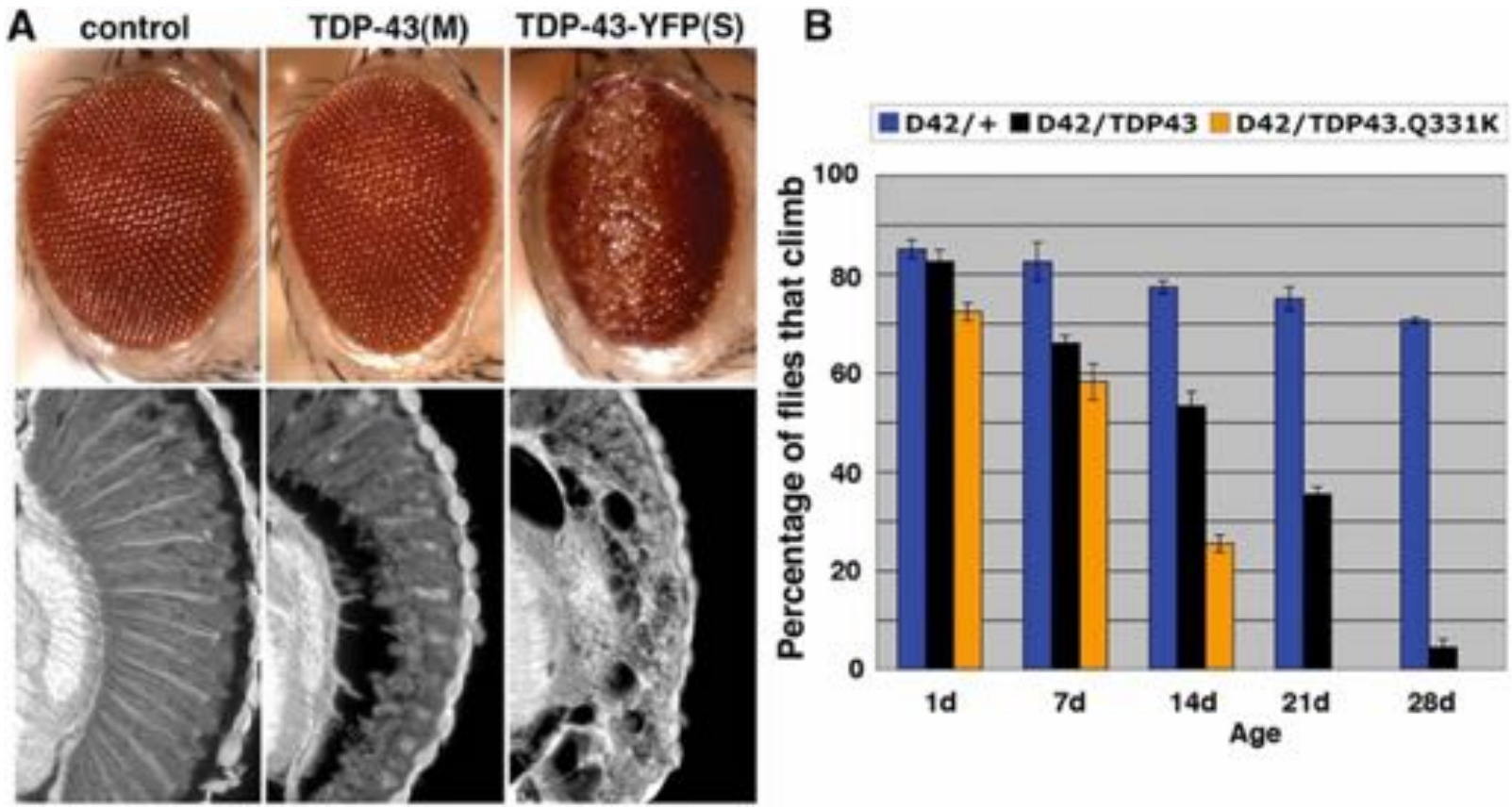
Vol 466|26 August 2010|doi:10.1038/nature09320 nature

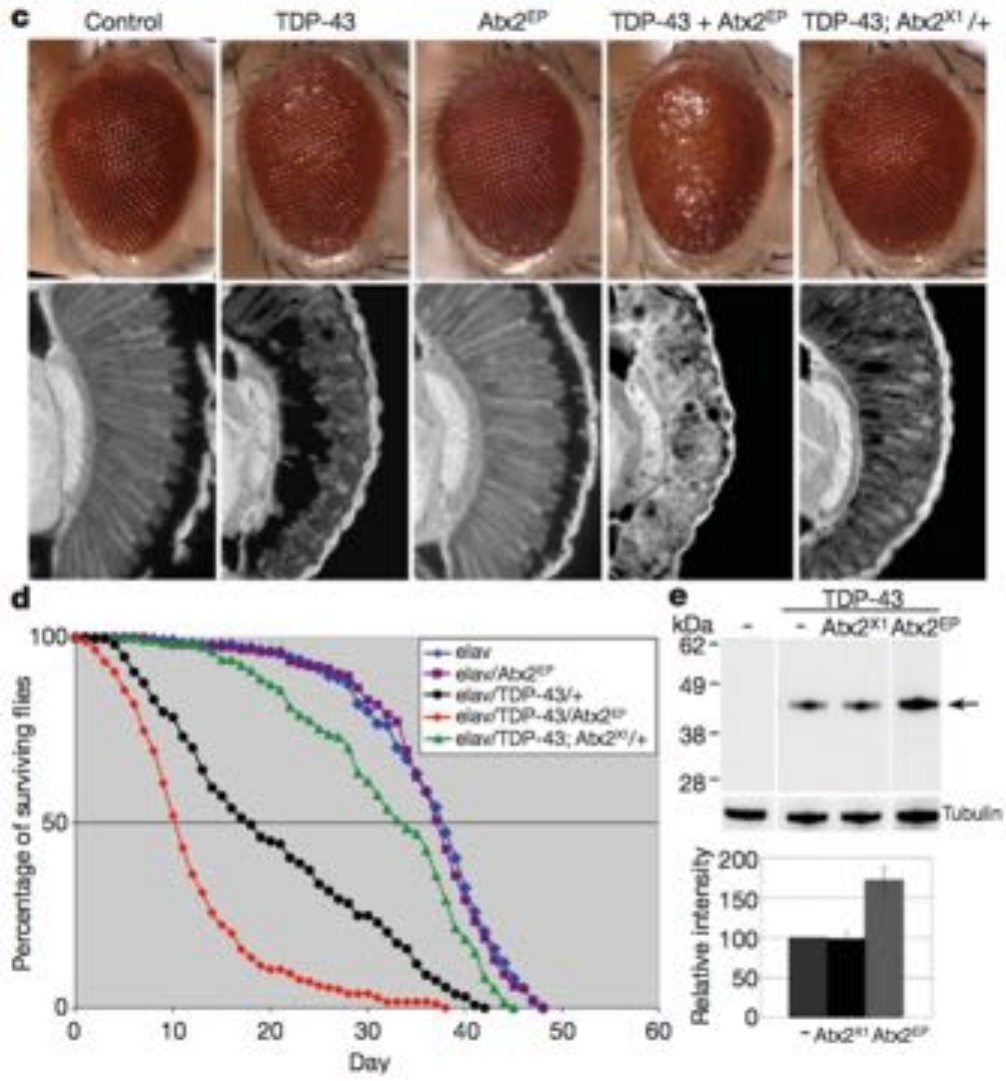
ARTICLES

Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS

Andrew C. Elden[1]*, Hyung-Jun Kim[2]*, Michael P. Hart[1]*, Alice S. Chen-Plotkin[3,4]*, Brian S. Johnson[1], Xiaodong Fang[1], Maria Armakola[1], Felix Geser[3], Robert Greene[3], Min Min Lu[1], Arun Padmanabhan[1], Dana Clay-Falcone[3], Leo McCluskey[4], Lauren Elman[4], Denise Juhr[5], Peter J. Gruber[5], Udo Rüb[6], Georg Auburger[7], John Q. Trojanowski[3], Virginia M.-Y. Lee[3], Vivianna M. Van Deerlin[3], Nancy M. Bonini[2] & Aaron D. Gitler[1]

TDP-43 toxicity in Drosophila leads to eye degeneration and loss of climbing ability.




c
Control
TDP-43
Atx2EP
TDP-43 + Atx2EP
TDP-43; Atx2X1 /+
d
Percentage of surviving flies
100
50
0
0
10
20
30
40
50
60
Day
elav
elav/Atx2EP
elav/TDP-43/+
elav/TDP-43/Atx2EP
elav/TDP-43; Atx2X1/+
e
kDa
-
TDP-43
-
Atx2X1
Atx2EP
62
49
38
28
Tubulin
Relative intensity
200
150
100
50
0
-
Atx2X1
Atx2EP

Advantages of using Drosophila in neurosciences:

- High degree of conservation with mammals
- Known morphology (including cellular)
- Relatively accessible structures
- From cell biology to behavior
- Smaller genetic redundancy
- A large repertoire of genetic tools (for example null alleles)
- A large collection of manipulation tools (for example optogenetics)
- A set of stereotyped behaviors (locomotion; circadian rhythms, etc)
- Sophisticated quantification tools

THANK YOU!

Further readings:
https://www.theguardian.com/science/2017/oct/07/fruit-fly-fascination-nobel-prizes-genetics
https://droso4schools.wordpress.com/why-fly/

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