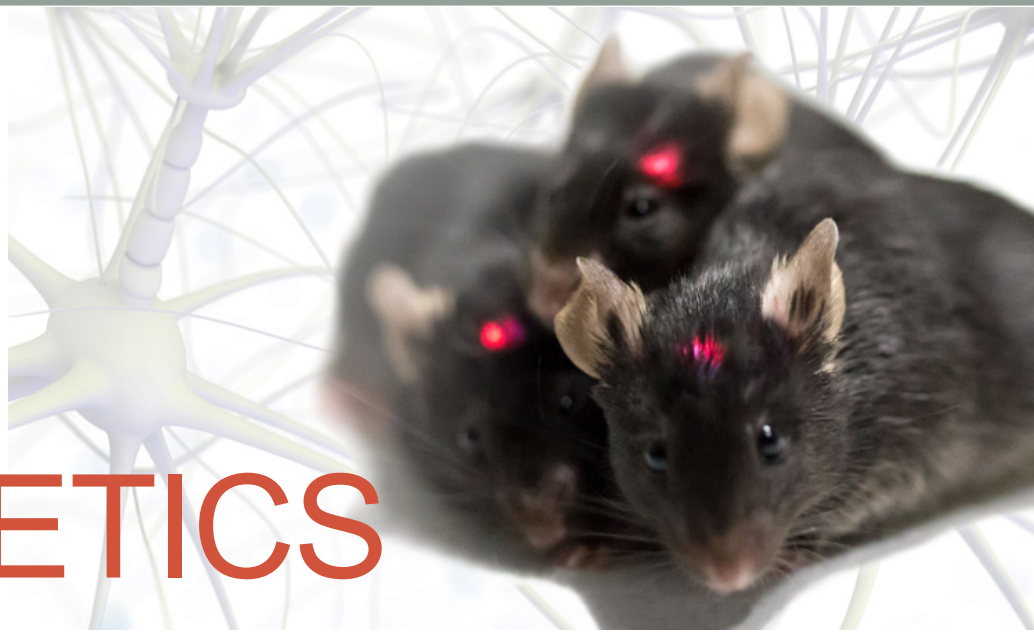


OPTOGENETICS

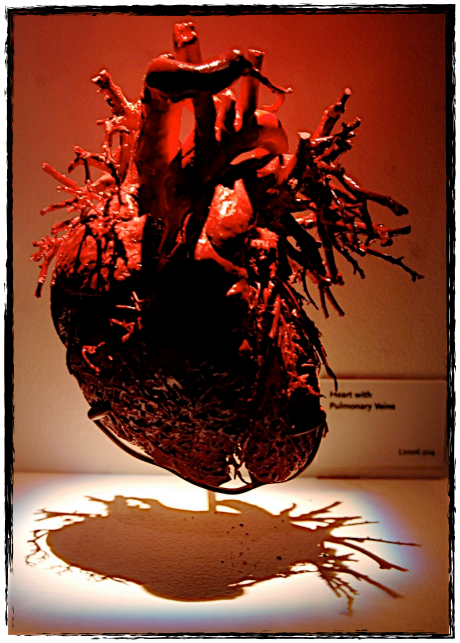
Shinning light on neuronal circuits

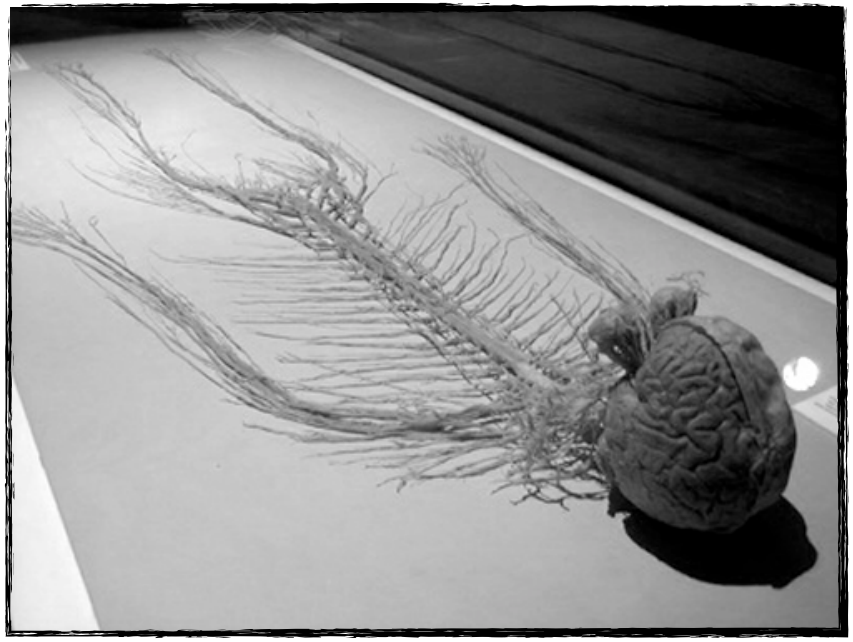
João Peça,
jpeca@cnc.uc.pt

Café com Física
UC | Fev 2019

CENTER FOR NEUROSCIENCE
AND CELL BIOLOGY
UNIVERSITY OF COIMBRA
PORTUGAL

What controls behavior?

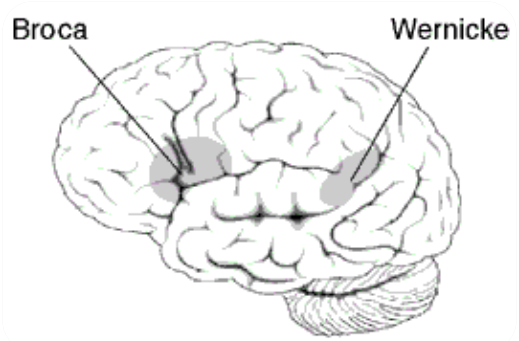




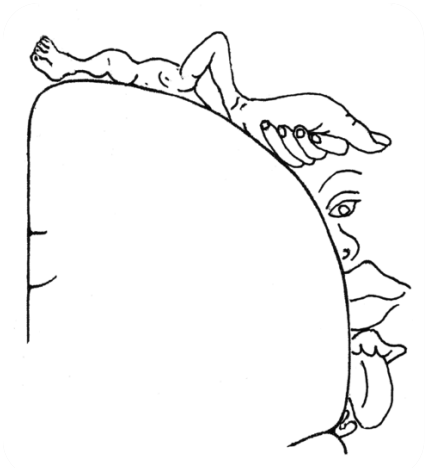
What is the Hegemonikon

Neuronal circuits

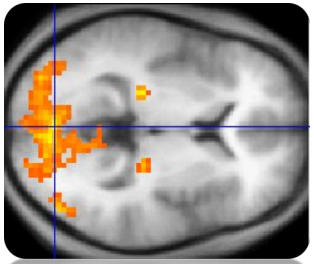
Joseph Gall
1810-

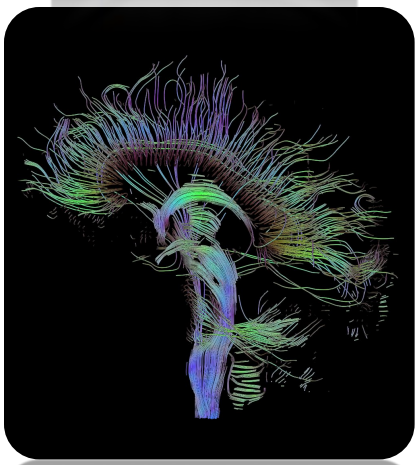


Paul Broca, 1861



Wilder Penfield, 1950





Ramachandran
Damásio
Logothetis
…

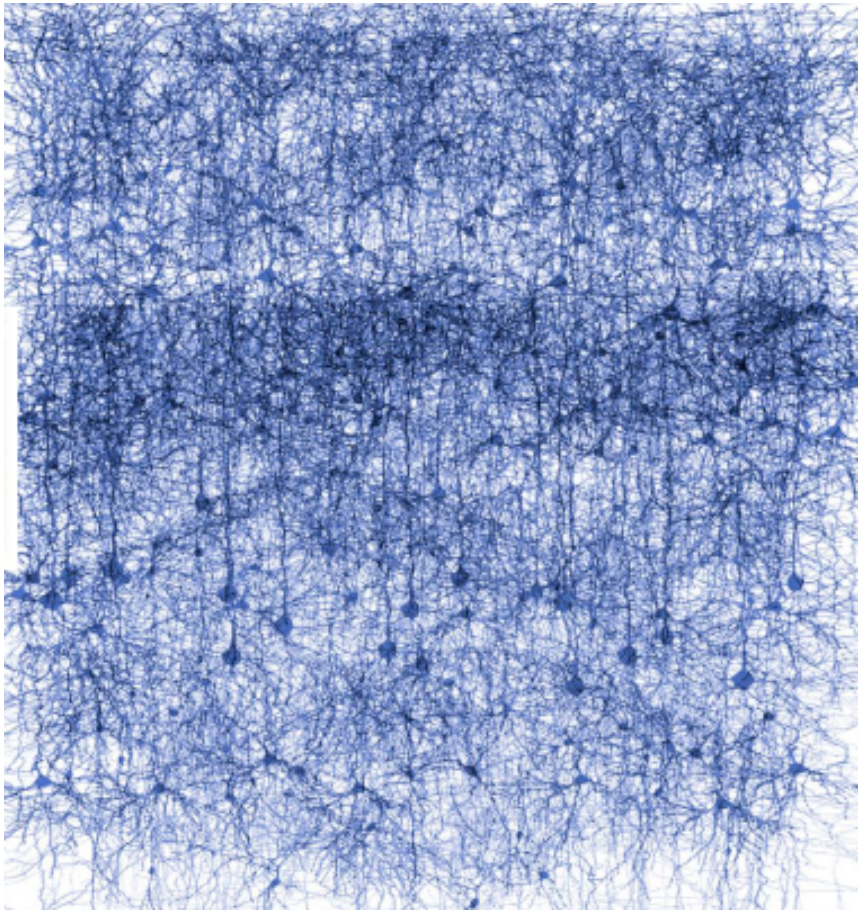
Crick 1979

it. For example, a method that would make it possible to inject one neuron with a substance that would then clearly stain all the neurons connected to it, and no others, would be invaluable. So would a method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered.

Visualization → Control

The Brain, a Scientific American Book (1979)

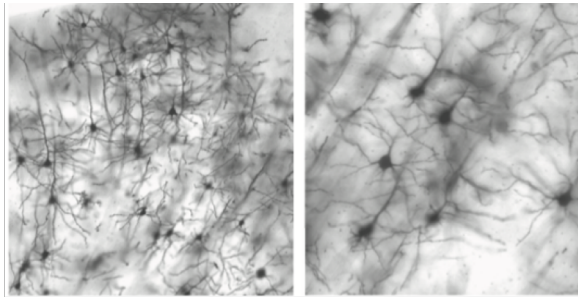
Complexity of the brain

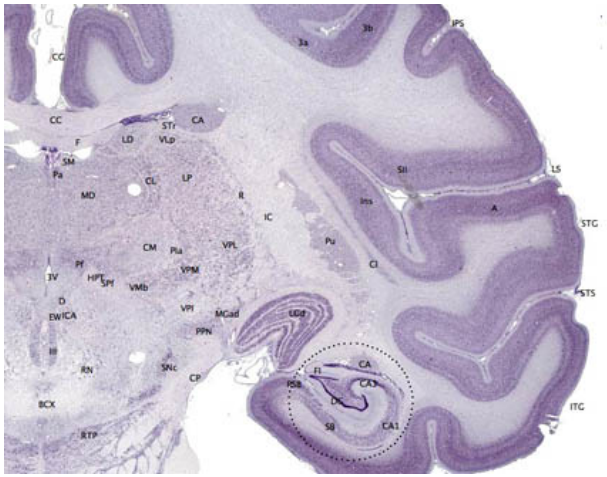


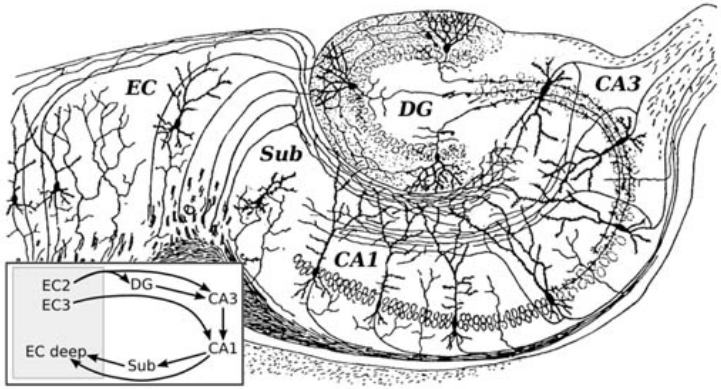
Only 1 in 100 neurons is shown here

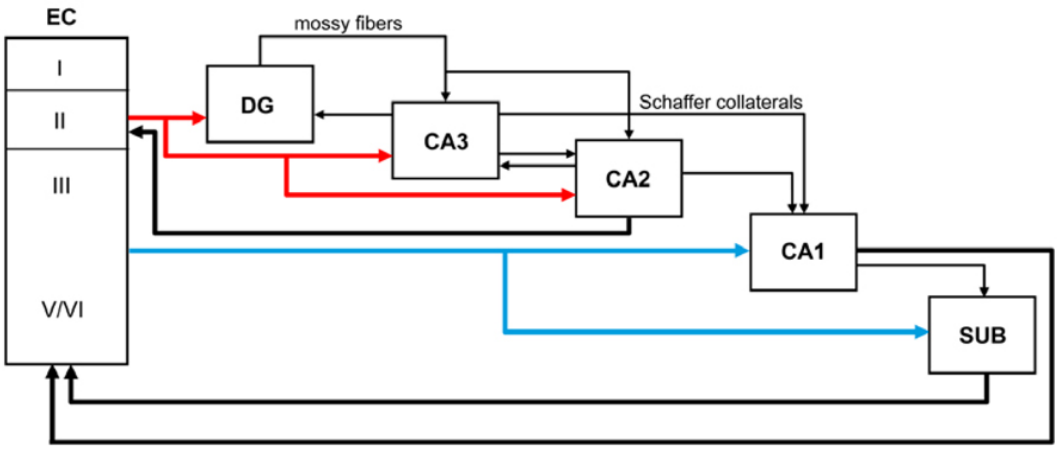
H. Markram, Blue Brain project

From Golgi to Diagrams









Circuit reconstruction allows for detailed on information flow and architecture

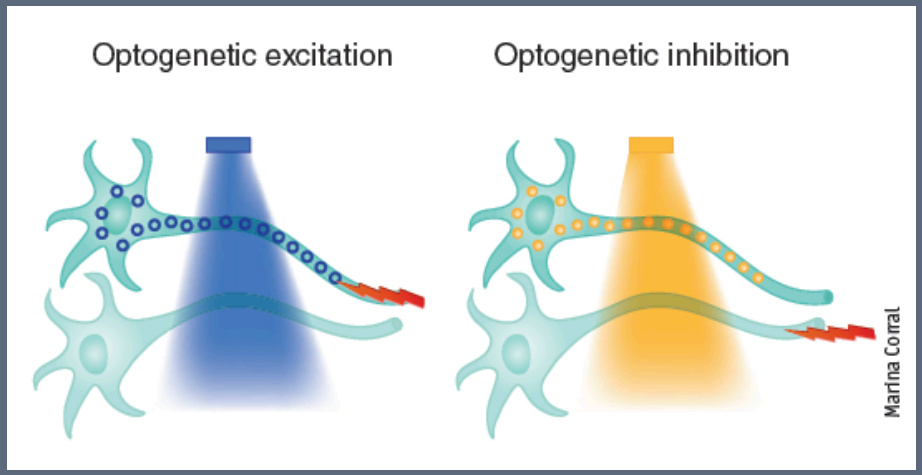
Llorens-Martin, M., et al. (2014). Front Neuroanat **8**: 38.

Cartography of the brain

Mapping functional circuitry in the nervous system is a major goal for both cellular and system neuroscience

- *Many methods have been developed to map functional connectivity of neural circuitry:*
  - *Electrophysiological stimulation*
  - *Glutamate uncaging*
  - *Ligand-gated ion channel coupled with photo-uncaging (P2X receptor)*

- *An ideal technique would require*
  - *Rapid photoactivation*
  - *Precise temporal control*
  - *High spatial resolution*
  - *Genetic targetability*



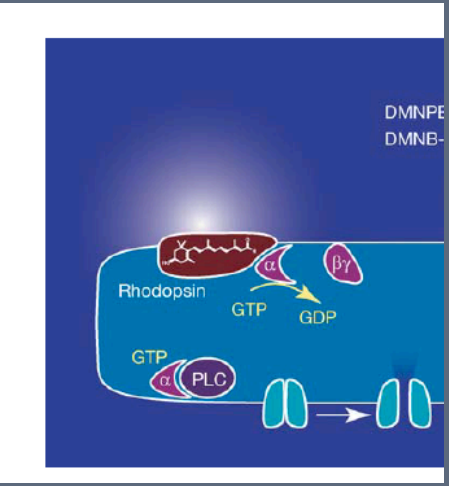
“3-step” optogenetics

Coexpression of the Drosophila photoreceptor genes encoding arrestin-2, rhodopsin, and the α subunit of the cognate heterotrimeric G protein—an explosive combination we term “chARGe”—sensitizes generalist vertebrate neurons

Neuron, Vol. 33, 15–22, January 3, 2002, Copyright ©2002 by Cell Press

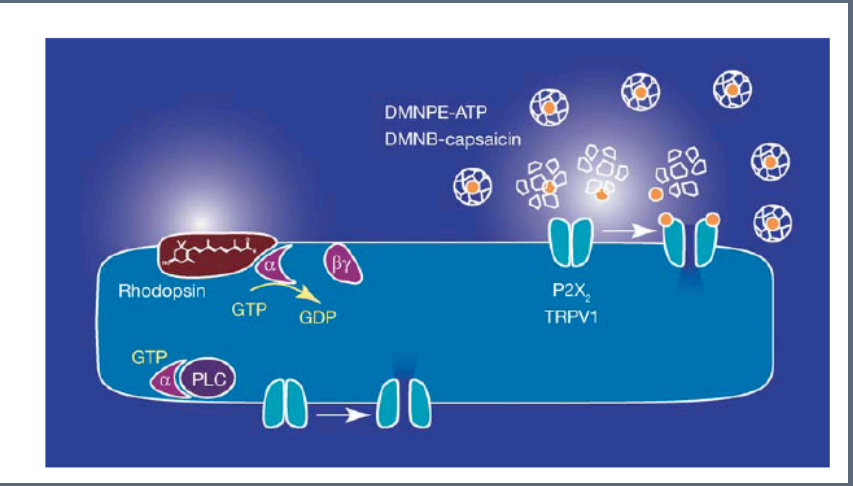
Selective Photostimulation of Genetically ChARGed Neurons

Neurotechnique



“2-step” optogenetics

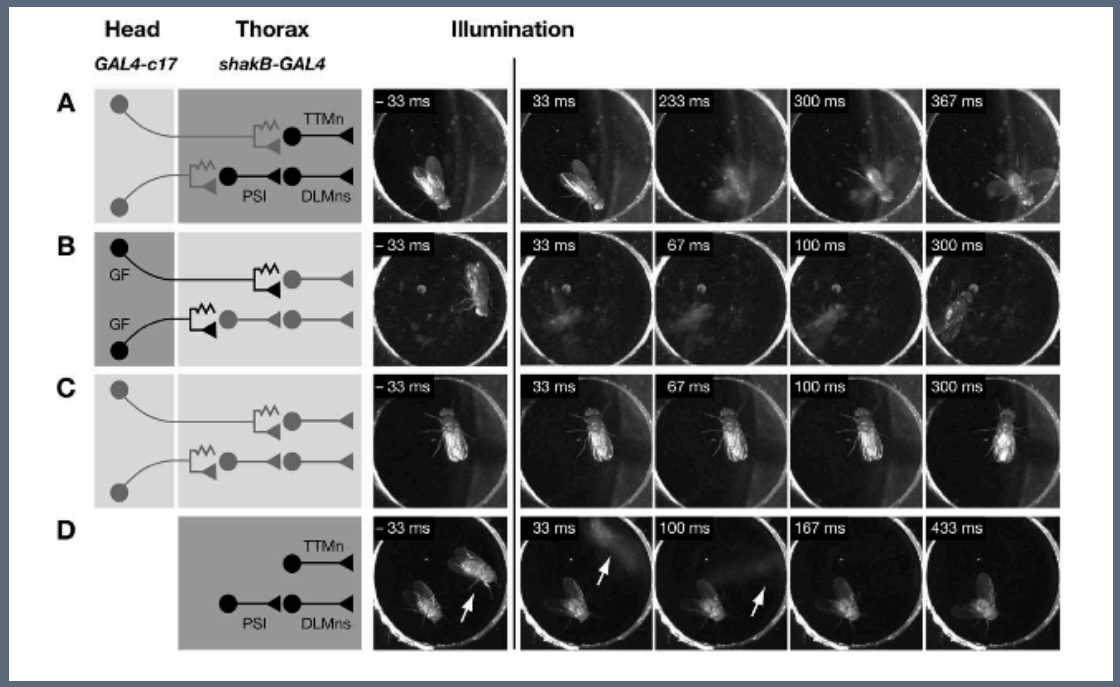
Because the fly genome lacks **purinoceptor** sequences (Littleton and Ganetzky, 2000), photoreleased ATP is expected to act selectively on the genetically designated targets.

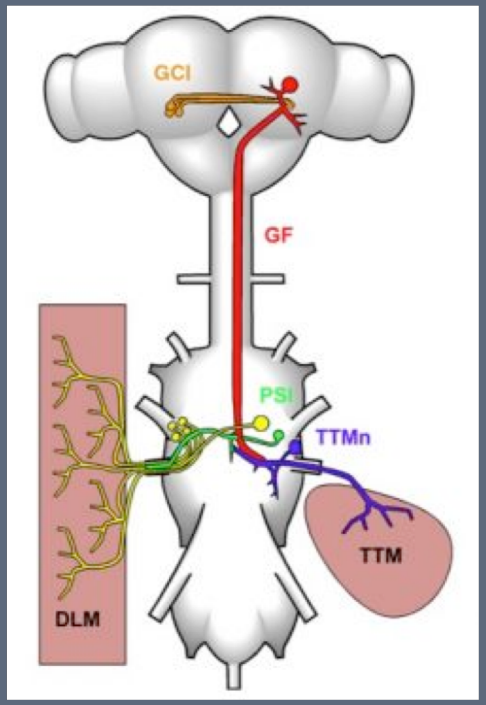


“2-step” optogenetics in action

Remote Control of Behavior through Genetically Targeted Photostimulation of Neurons

Susana Q. Lima and Gero Miesenböck*
Department of Cell Biology
Yale University School of Medicine
333 Cedar Street
New Haven, Connecticut 06520

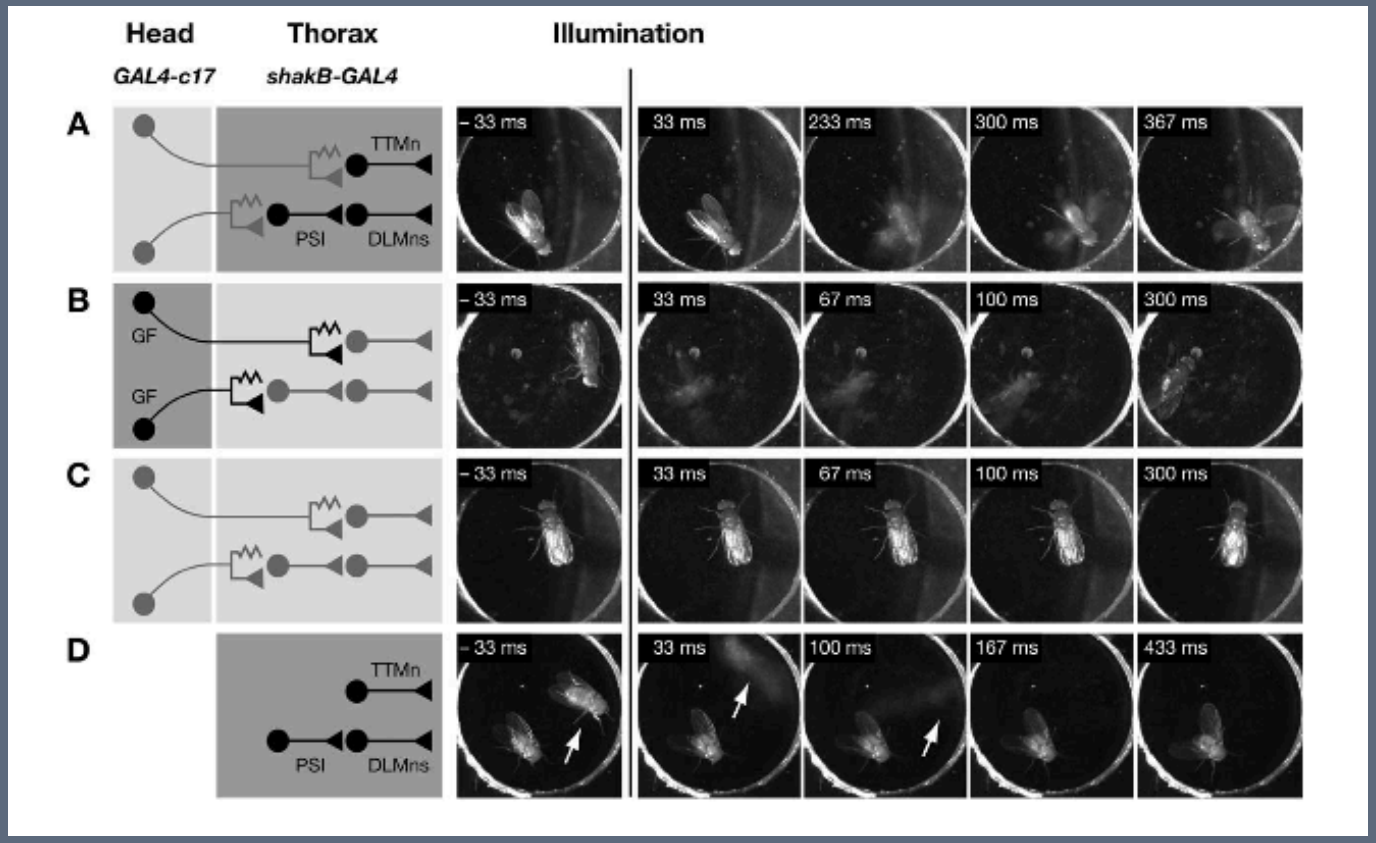


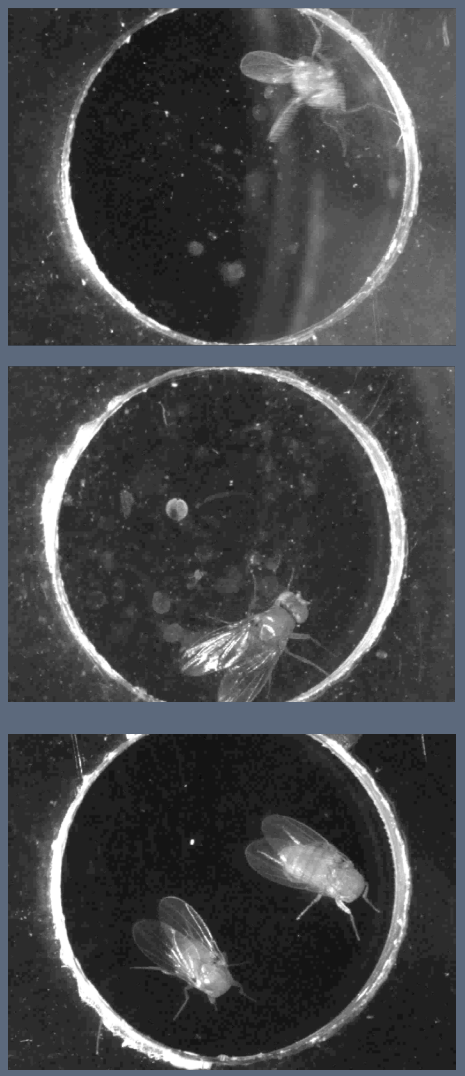


http://www.sussex.ac.uk/lifesci/baconlab/research/giantfibre

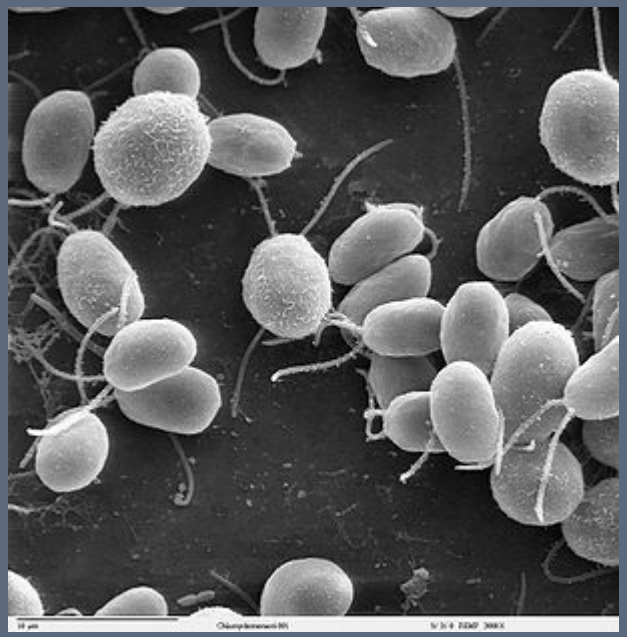
Giant fiber (GF) neurons in the brain
Thoracic ganglion contains TTMn, PSI, and DLMns

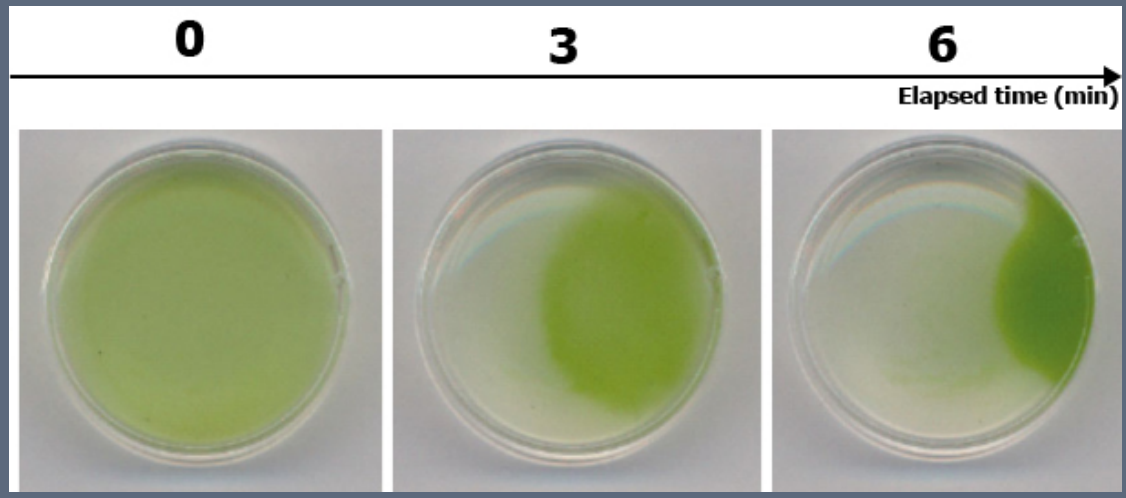
“2-step” optogenetics



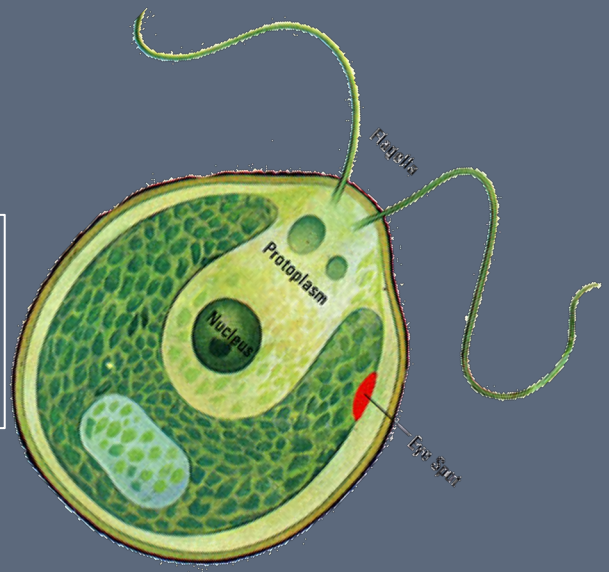


“Algae Vision”





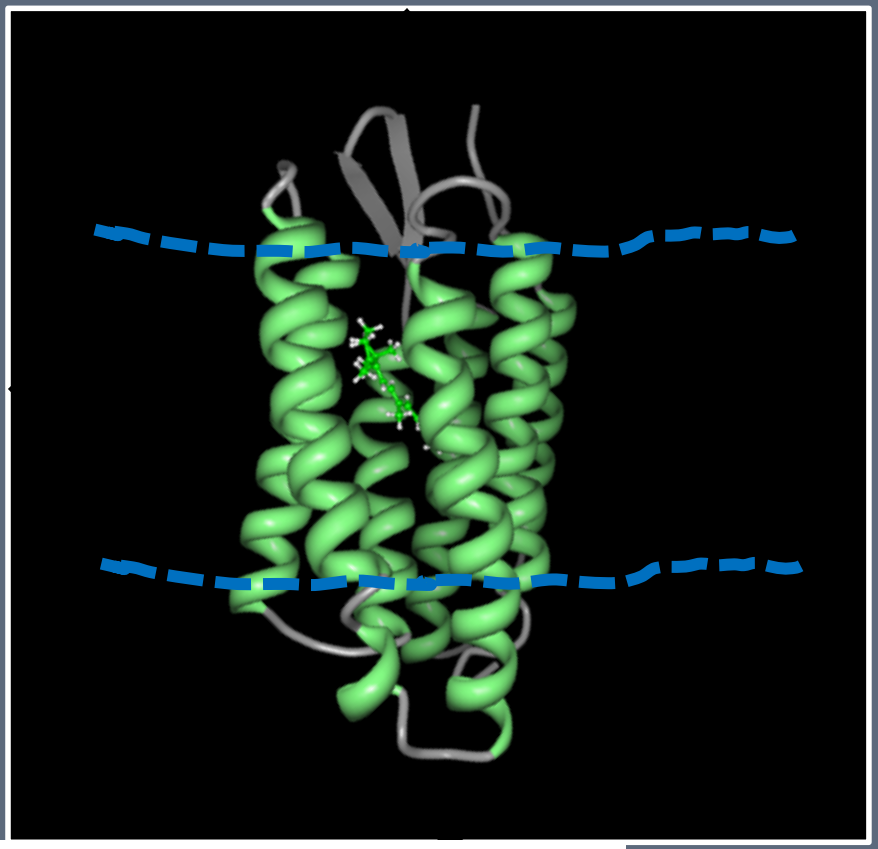
Chlamydomonas reinhardtii **microalgae shows phototaxic behavior**

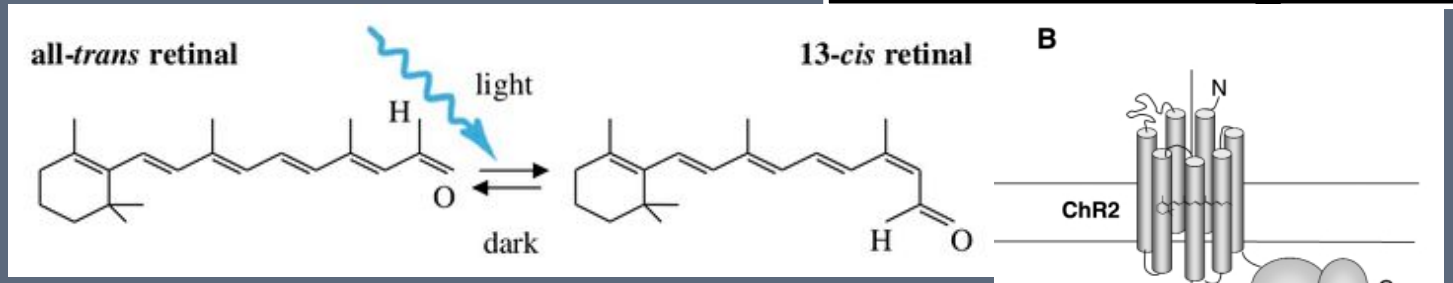


Channelrhodopsin-1: A Light-Gated Proton Channel in Green Algae

Georg Nagel,[1]* Doris Ollig,[1] Markus Fuhrmann,[2]
Suneel Kateriya,[2] Anna Maria Musti,[3] Ernst Bamberg,[1]
Peter Hegemann[2]

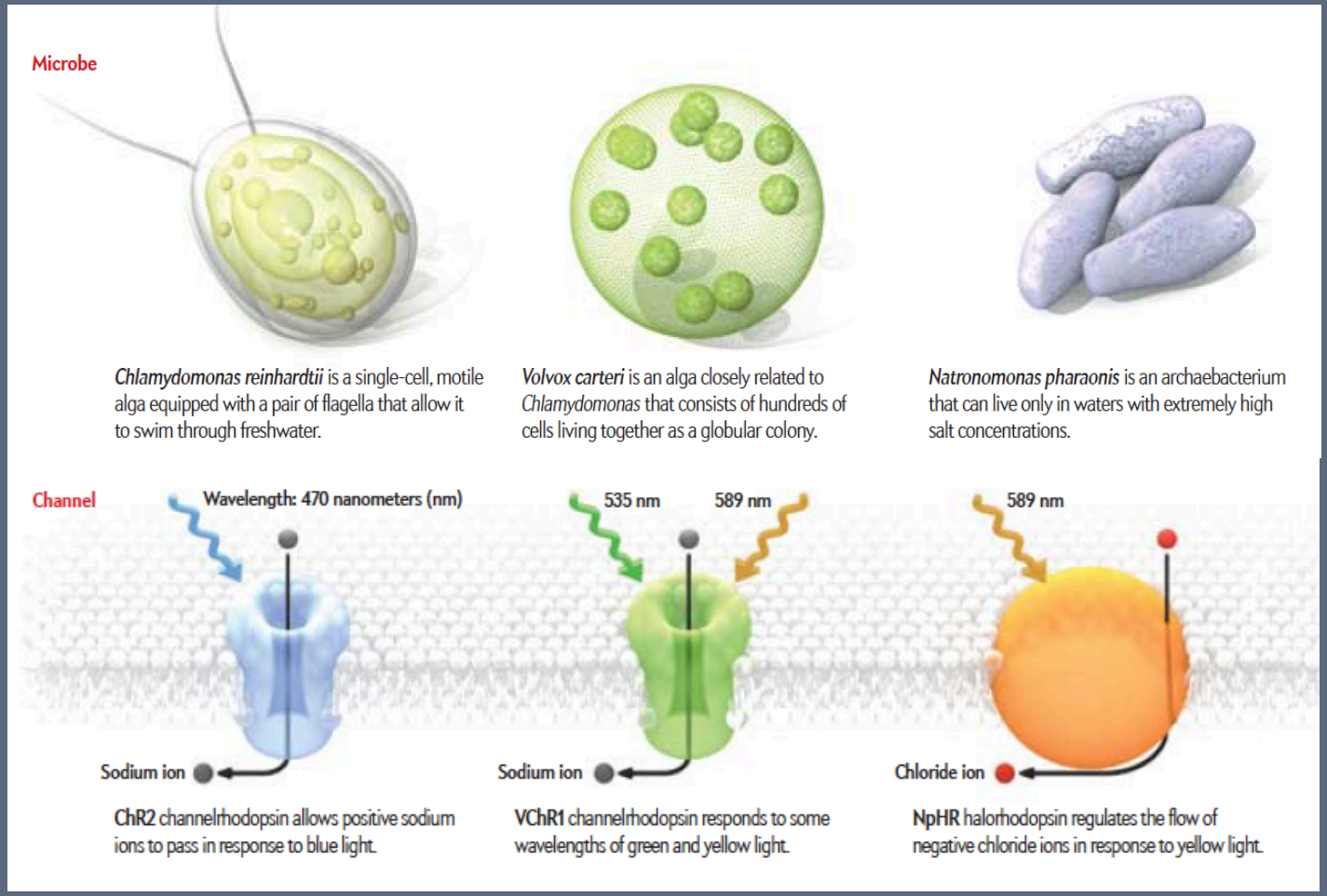
Phototaxis and photophobic responses of green algae are mediated by rhodopsins with microbial-type chromophores. We report a complementary DNA sequence in the green alga *Chlamydomonas reinhardtii* that encodes a microbial opsin-related protein, which we term Channelopsin-1. The hydrophobic core region of the protein shows homology to the light-activated proton pump bacteriorhodopsin. Expression of Channelopsin-1, or only the hydrophobic core, in *Xenopus laevis* oocytes in the presence of all-*trans* retinal produces a light-gated conductance that shows characteristics of a channel selectively permeable for protons. We suggest that Channelrhodopsins are involved in phototaxis of green algae.





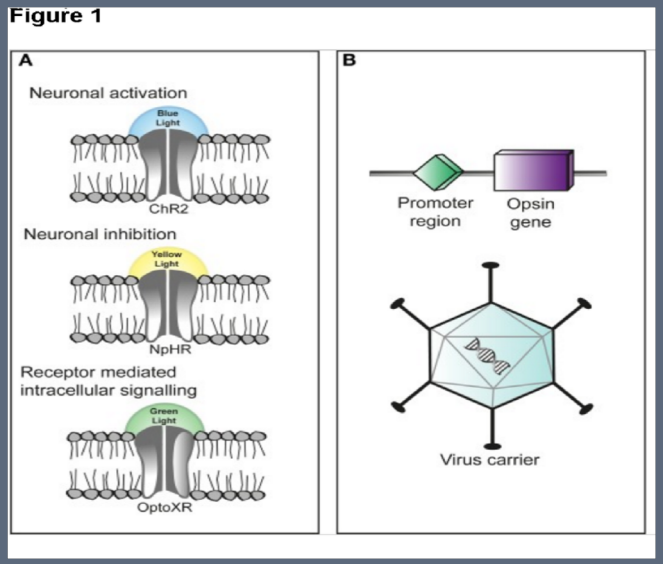
Nagel et al., 2002 and 2003 (Science,PNAS)

Other channels and pumps

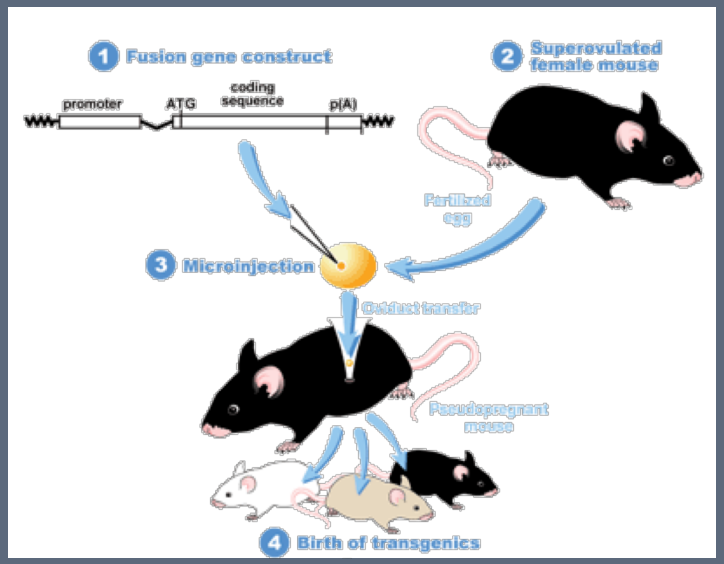


How to start optogenetics experiments?

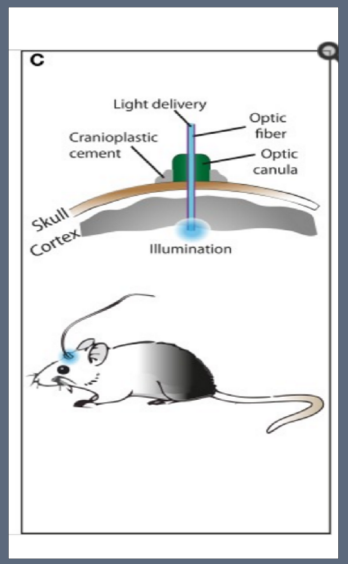
Gene therapy based



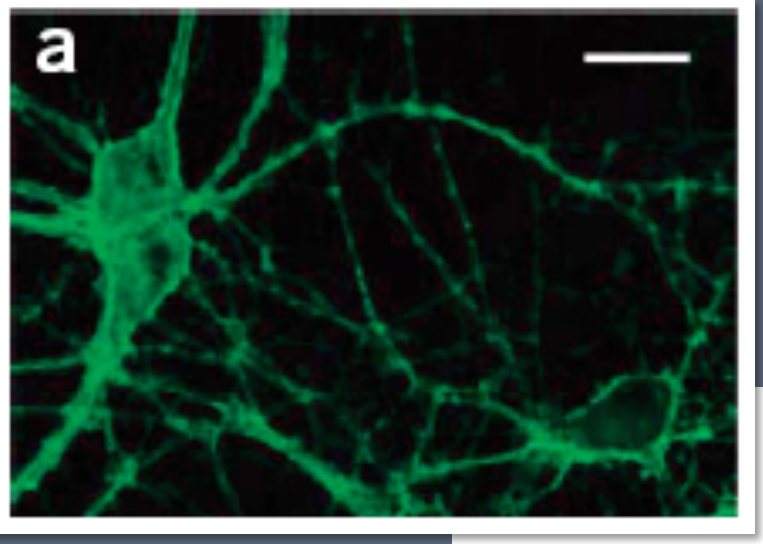
Transgene based

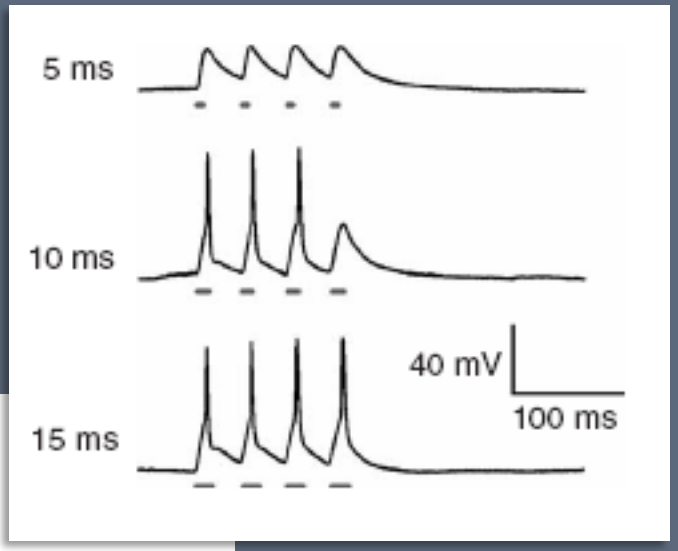


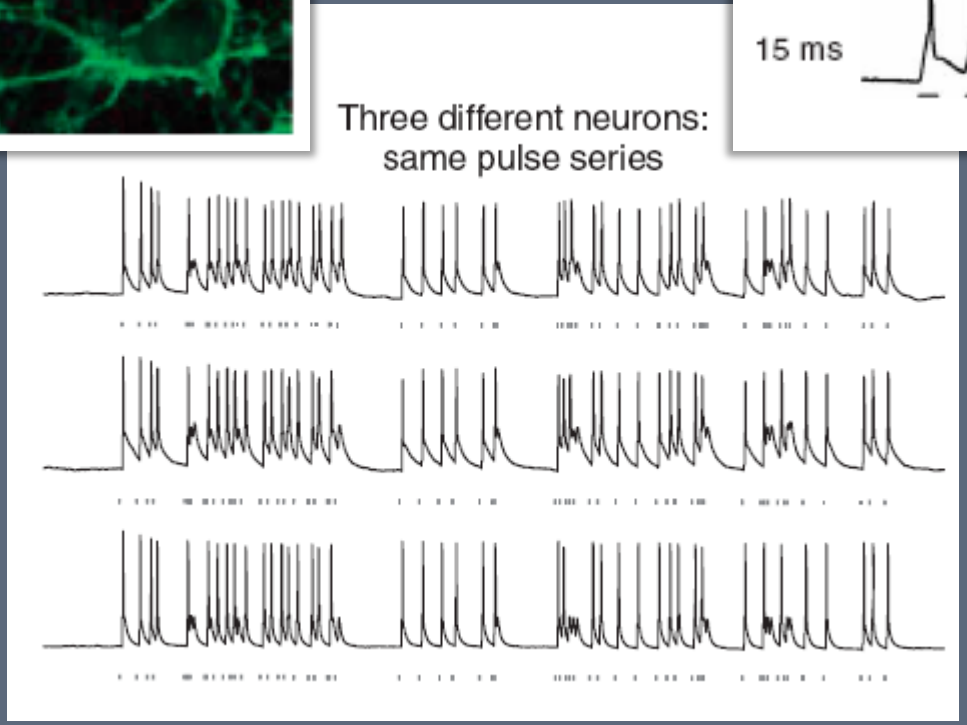
Activation



Typical in vitro result

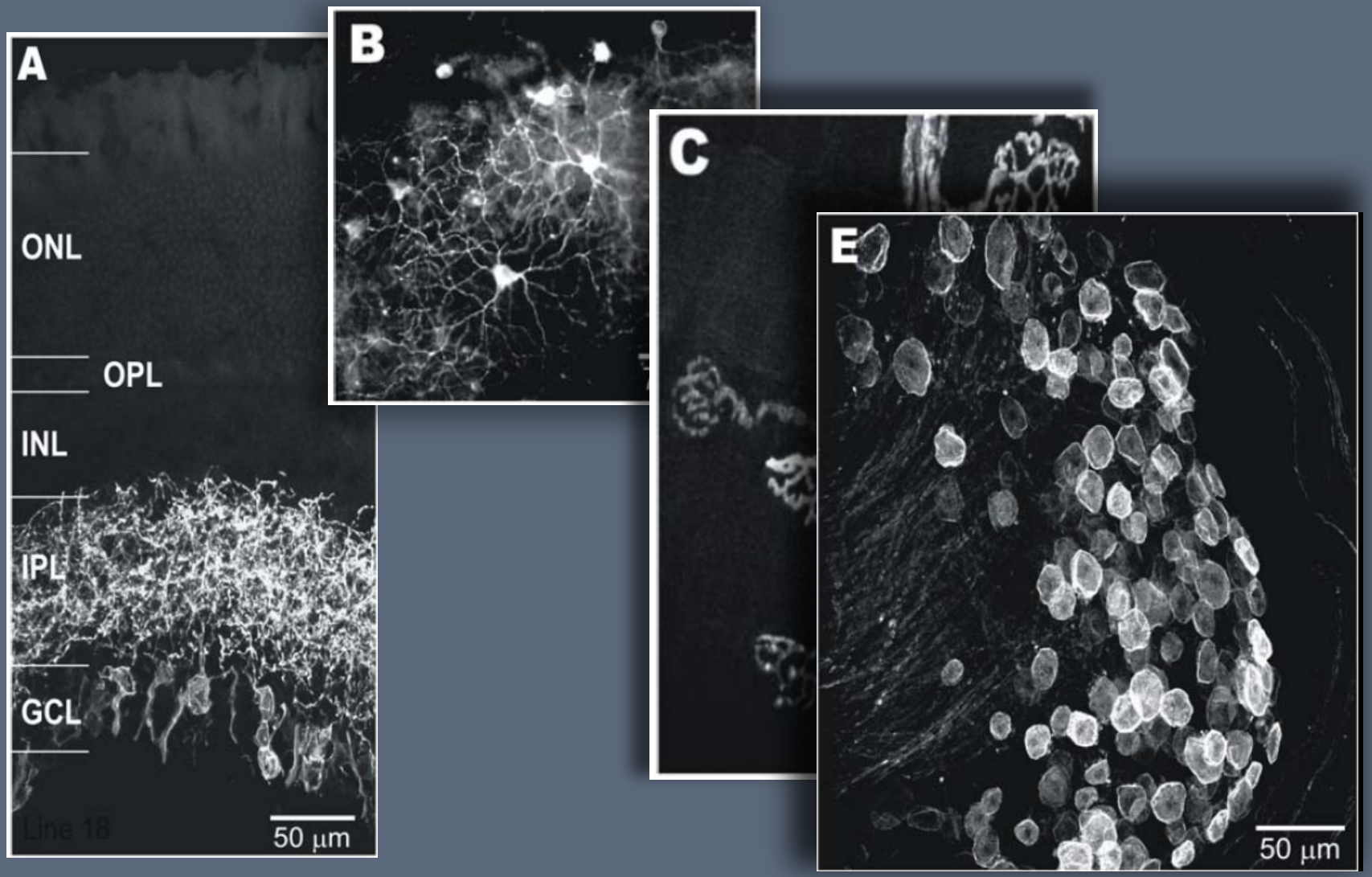




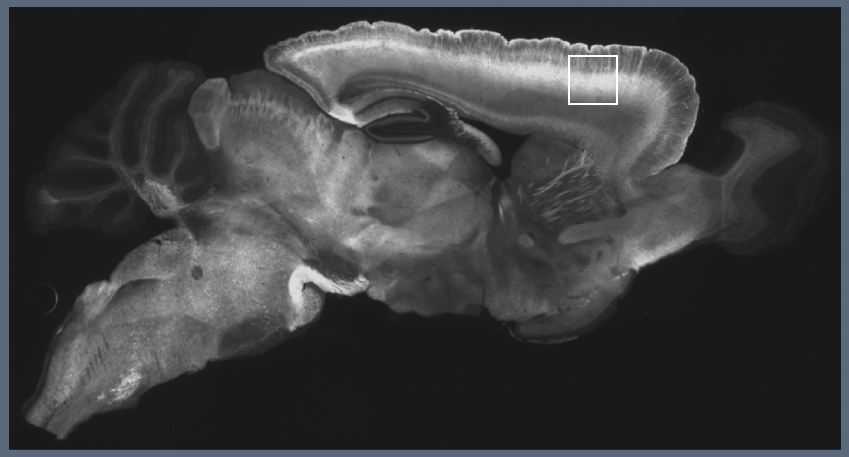


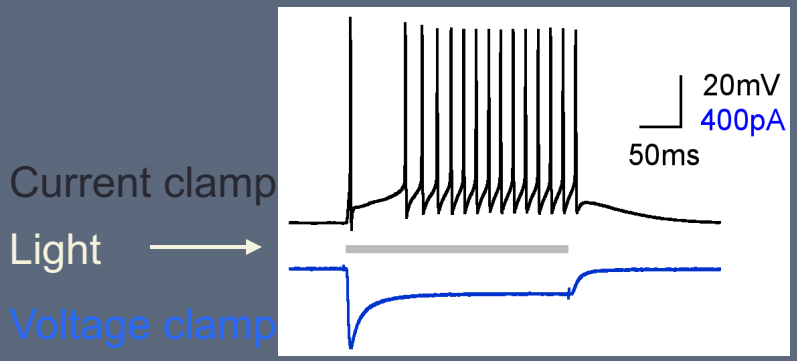
Boyden et al., 2005

Characterization of ChR2 transgenic lines

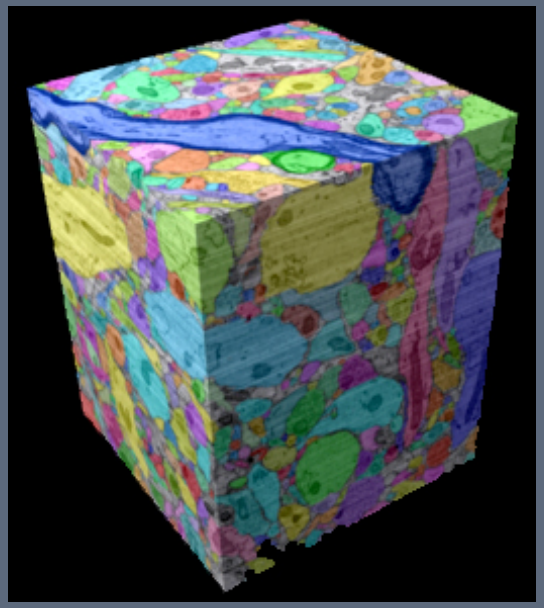


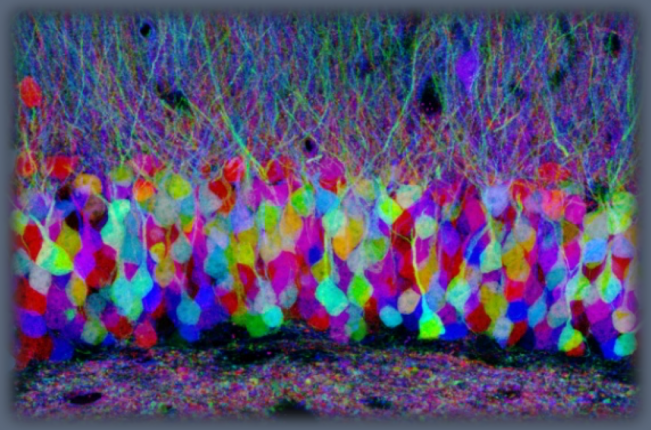
ChR2-mice positive neurons can be activated by light without exogenous retinal

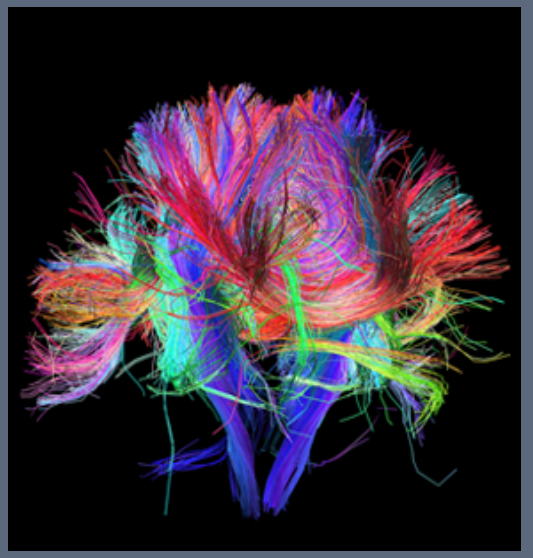




ChR2-assisted dissection of Microcircuits

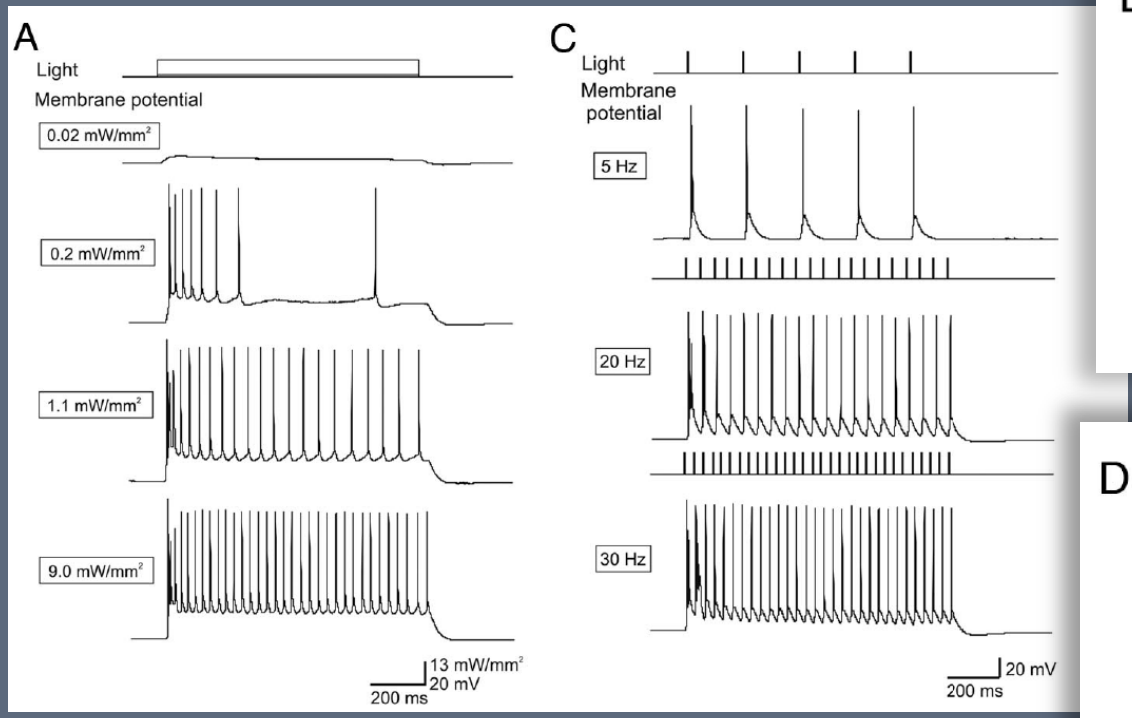


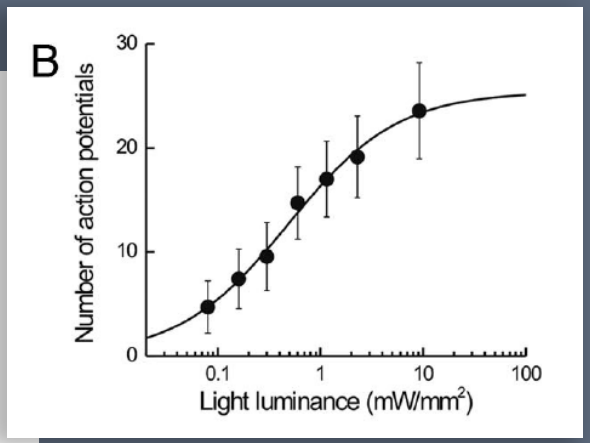


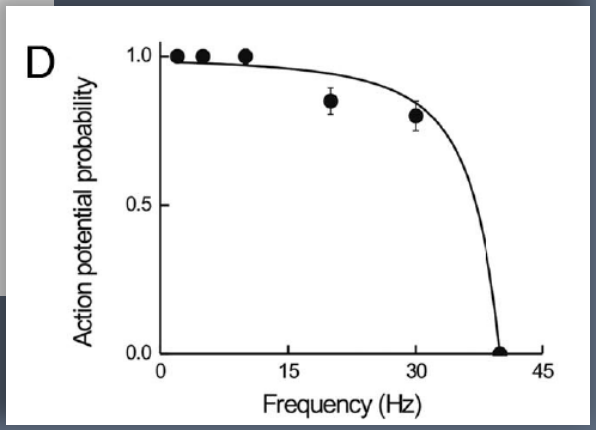


High-speed mapping of synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice

Illumination controls number and frequency of action potentials

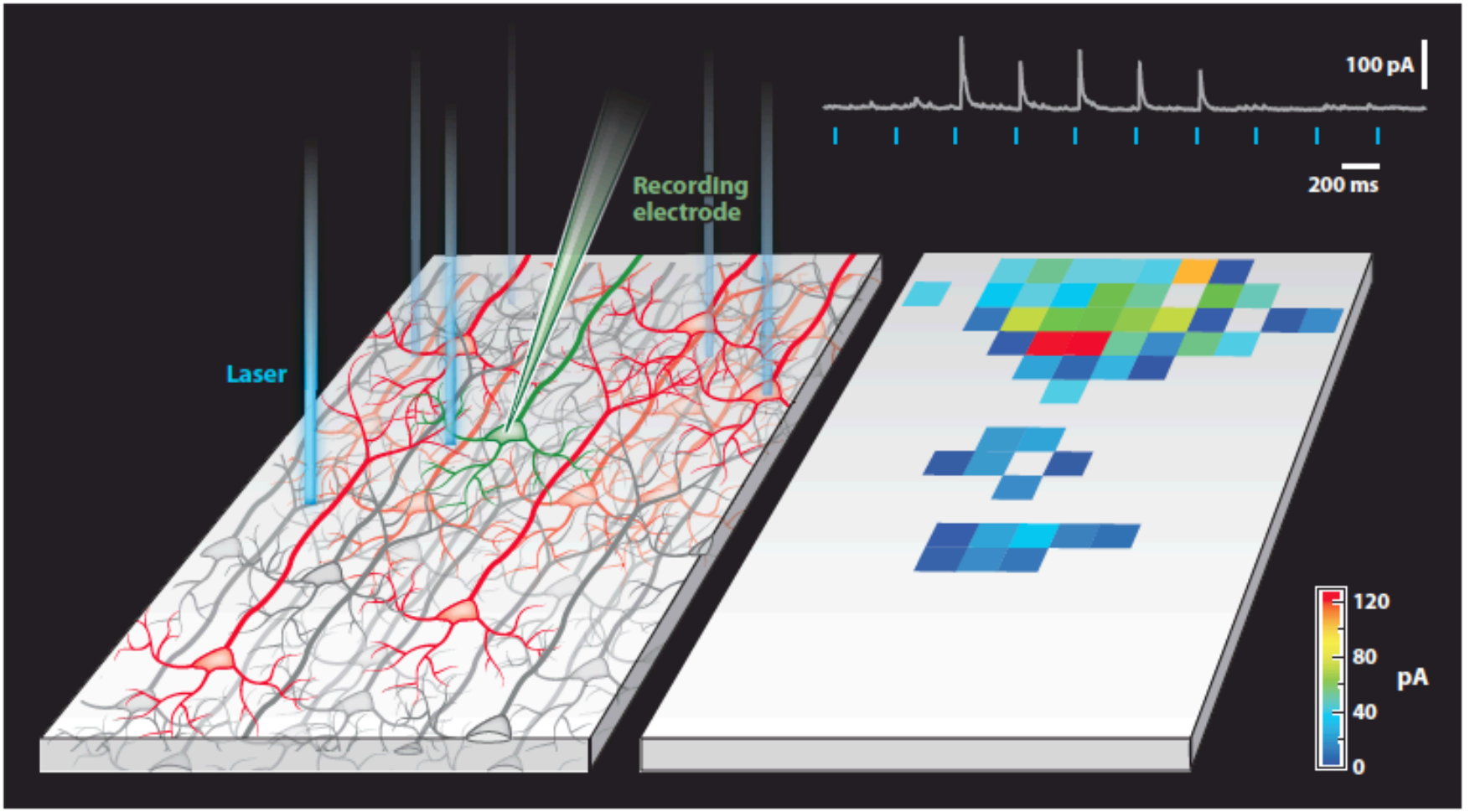






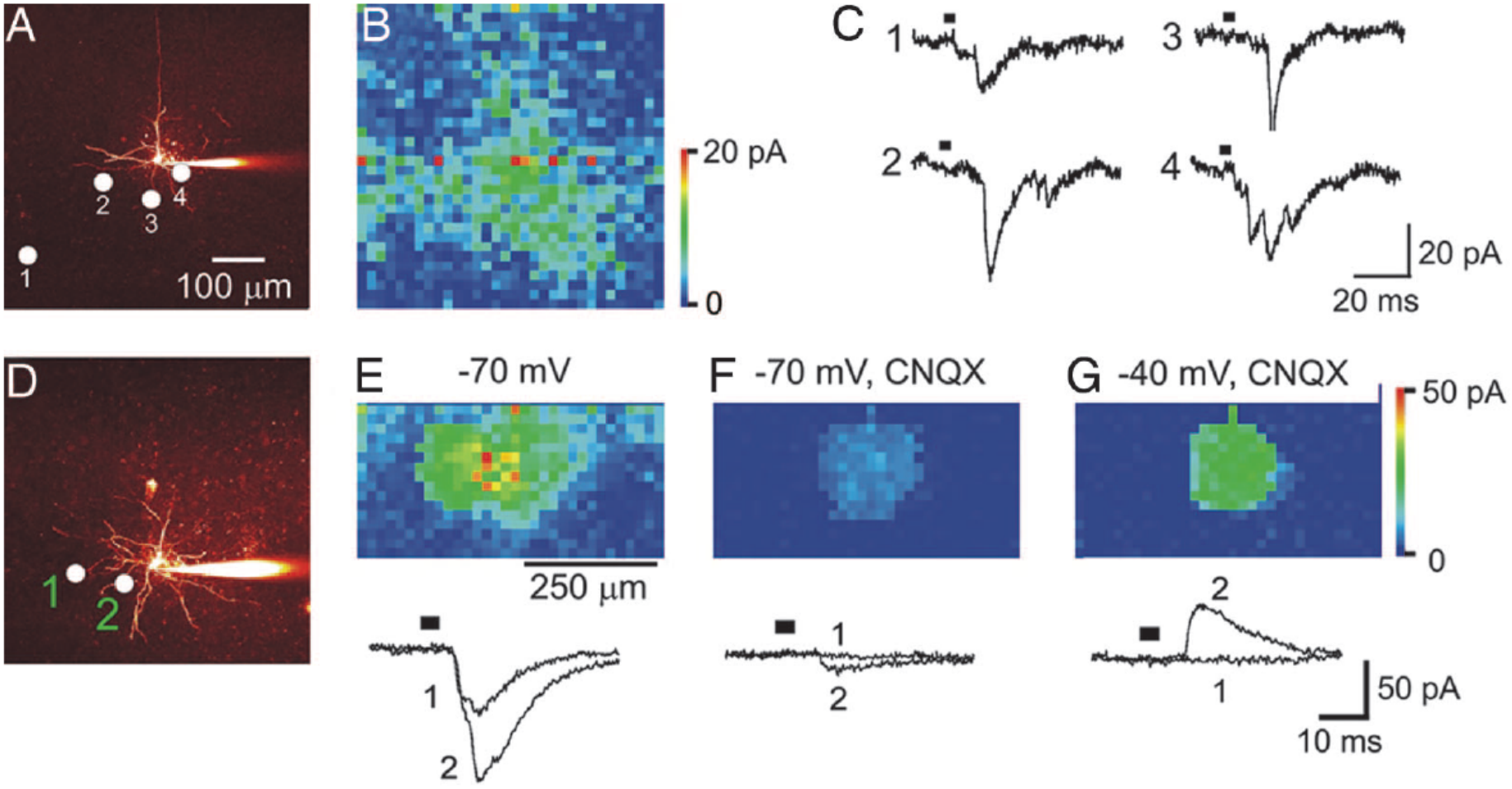
Wang, H*.,Peça, J.*, Matsuzaki, M., et al. (2007). Proc Natl Acad Sci U S A **104**(19): 8143-8148.

Mapping microcircuits



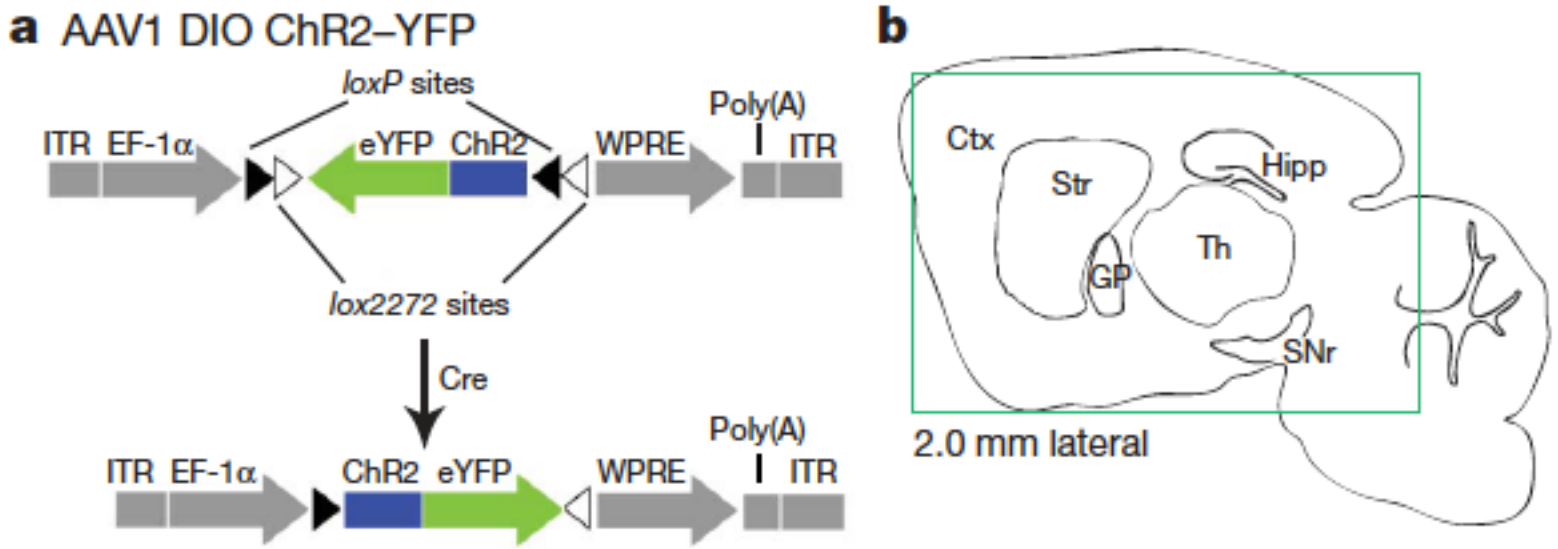
Miesenbock, G. Annual Review of Cell and Developmental Biology (2011)

Properties of cortical microcircuits

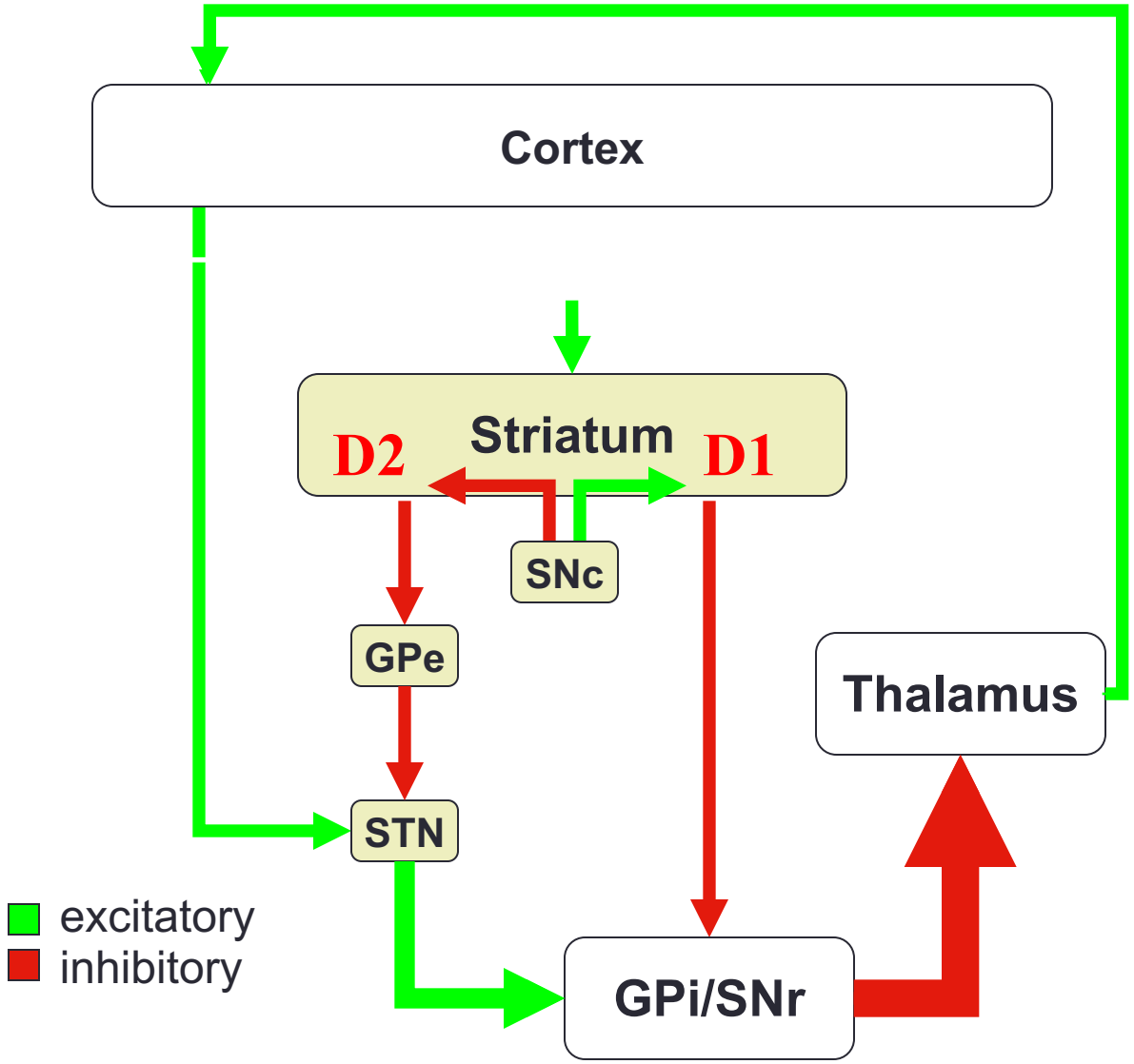


Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry

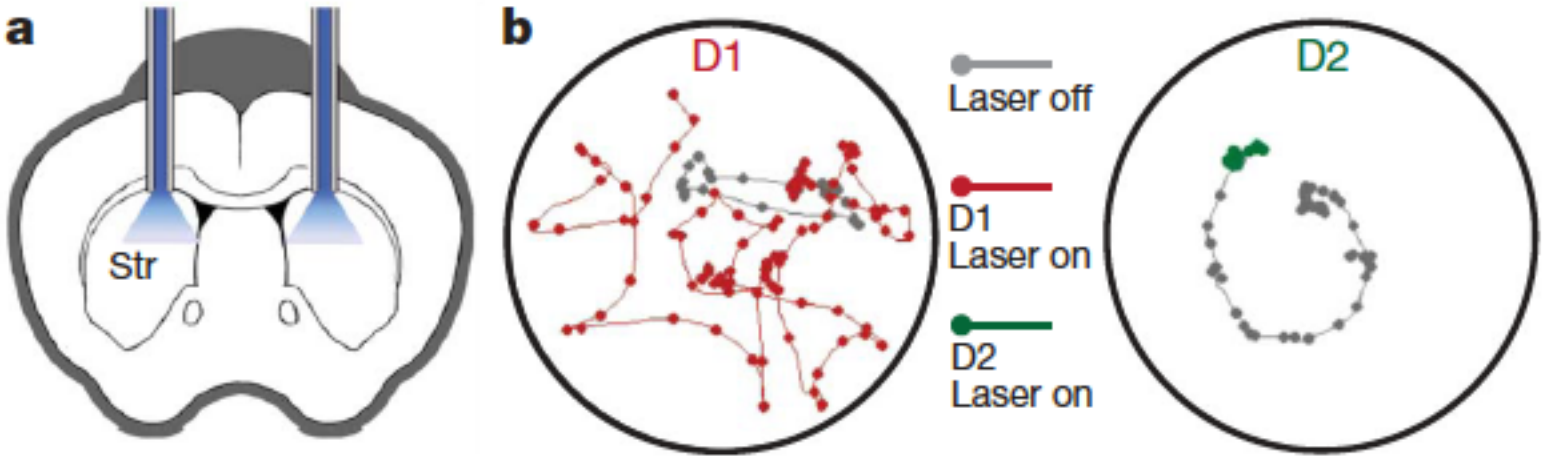
Alexxai V. Kravitz[1], Benjamin S. Freeze[1,4,5], Philip R. L. Parker[1,3], Kenneth Kay[1,5], Myo T. Thwin[1], Karl Deisseroth[6] & Anatol C. Kreitzer[1,2,3,4,5]



Cortico-striatal-thalamic-cortical loop



In vivo regulation of motor behavior



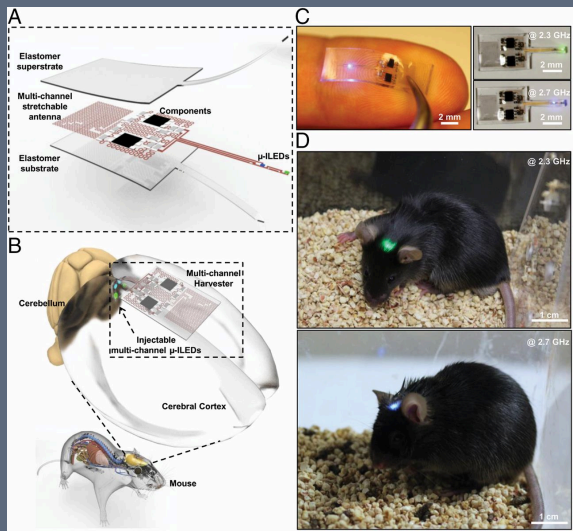
Kravitz et al, Nature 2010

Movie S2:
Bilateral illumination of indirect pathway

Kreitzer lab, 2010

Summary

- Optogenetics allows precise control of neuronal circuits
- Several studies have looked at the role of specific brain regions in neuropsychiatric disorders
- It is possible to test predictions on neuronal function
- Optogenetics allows an understanding of the role of specific neuron types
- Limitations:
  - Spatial limitations
  - Invasive
  - Requires gene therapy



OPTOGENETICS