Modern Biotechnology

Module 4: Optogenetics technologies

2018.4.11

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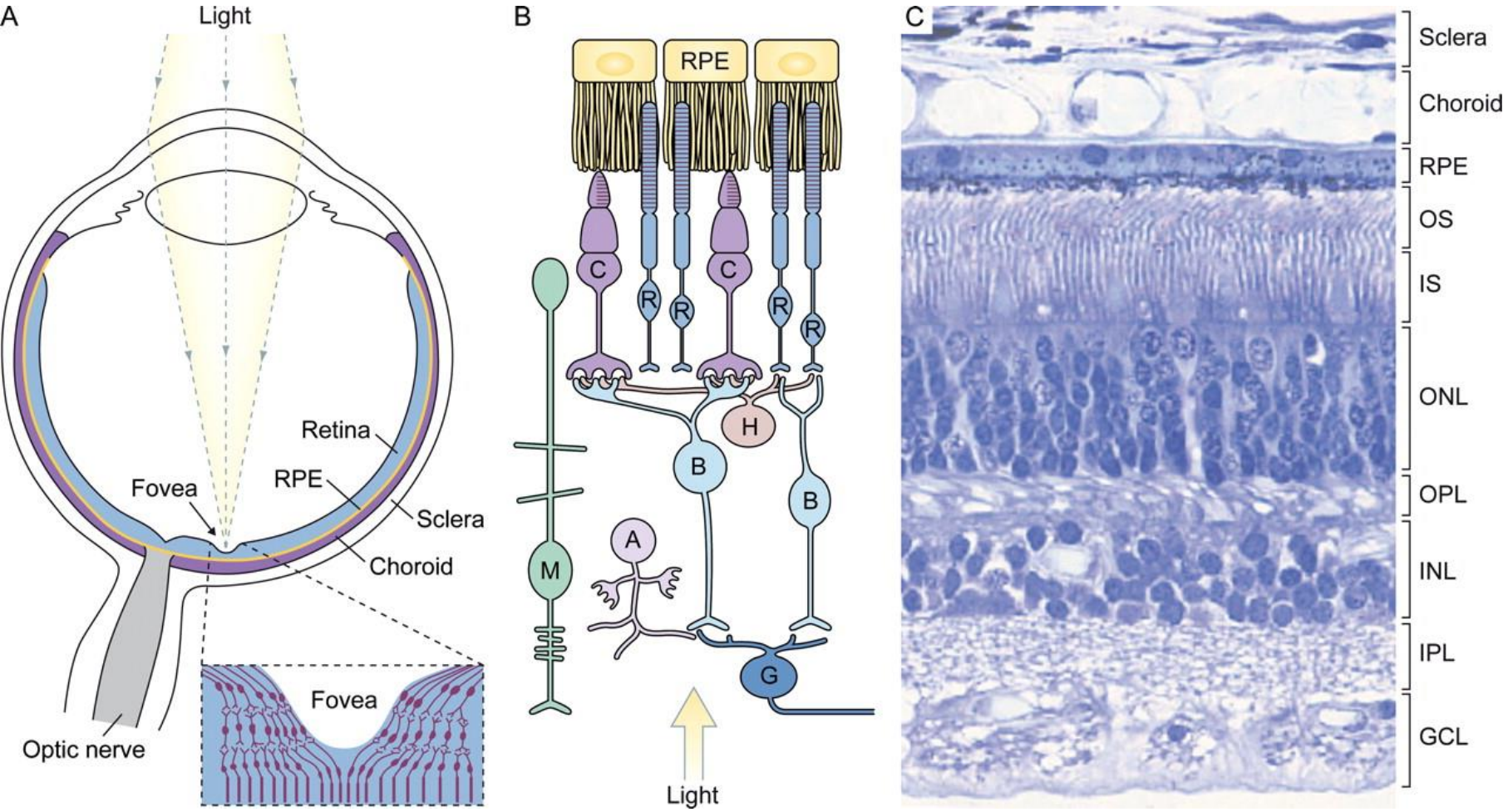
Outline

1. How do we sense light?
2. How do microbes sense light?
3. Definition and application of optogenetics
4. Elements of genetics systems
5. Elements of optical systems
6. Examples of optogenetics application

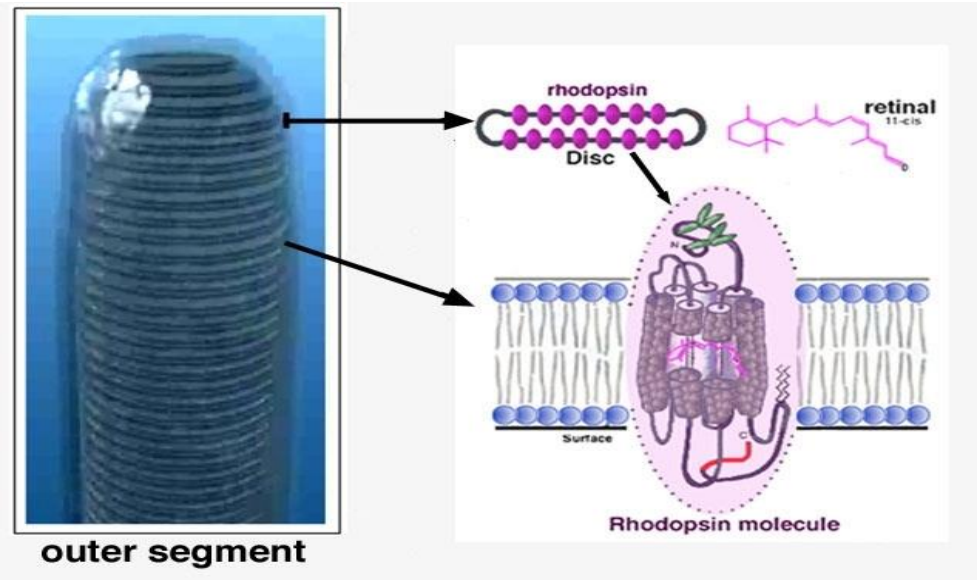
1. How do we sense light: the visual sense organ.

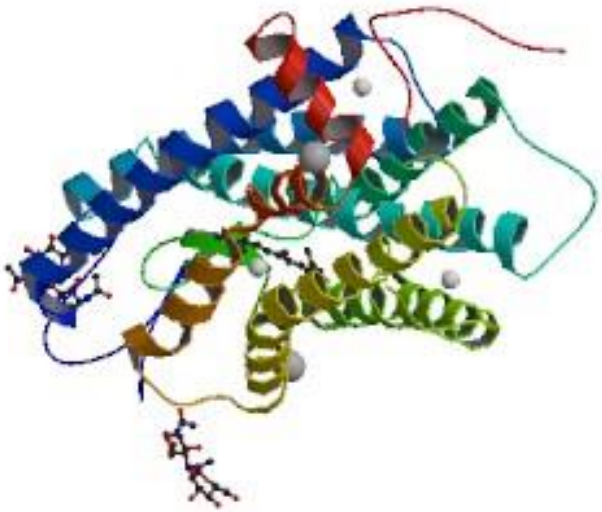


Sung C, Chuang J. JCB 2010;190:953-963

Rhodopsin: the photoreceptor

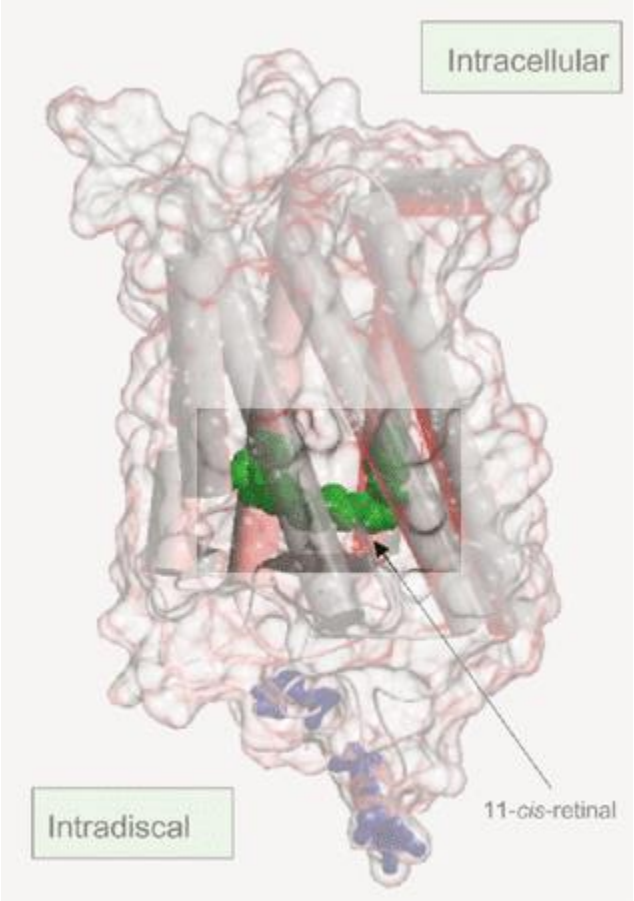
- Rhodopsin is found in the rods that are located in the eye
- Rods are composed of stacked disks
- Rhodopsin is densely packed into each disk
- Rods are responsible for black and white vision
- Rhodopsin works best at dim light, responsible for night vision (too much light will saturate the protein)



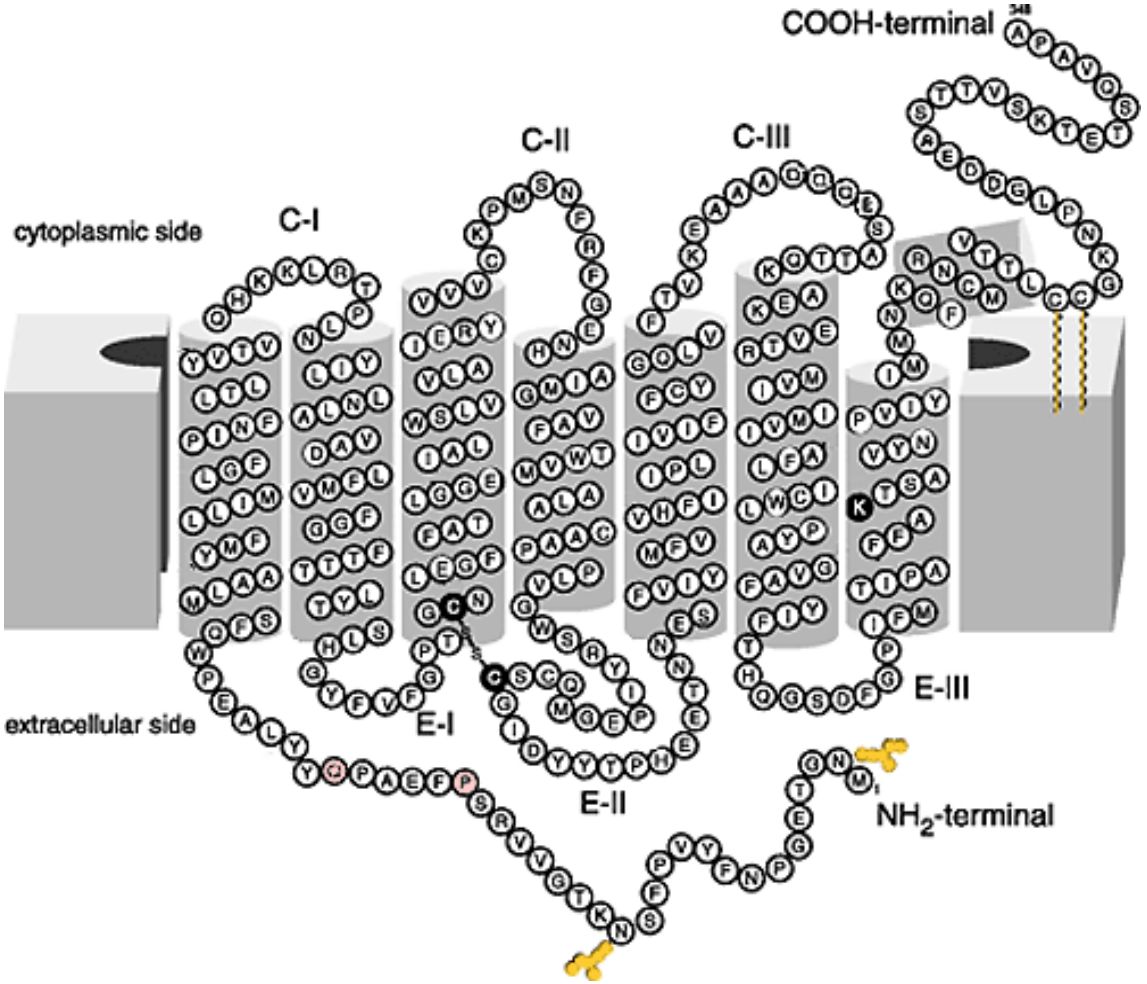


Opsin & Retinal

- Rhodopsin is made up of the protein opsin with the chromophore, retinal, covalently attached
- The linkage occurs at Lys-296



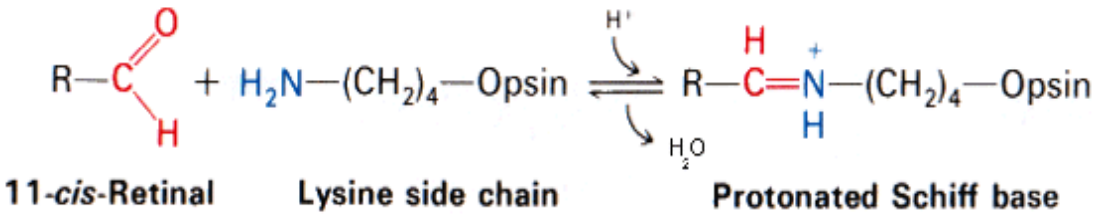
Rhodopsin Structure

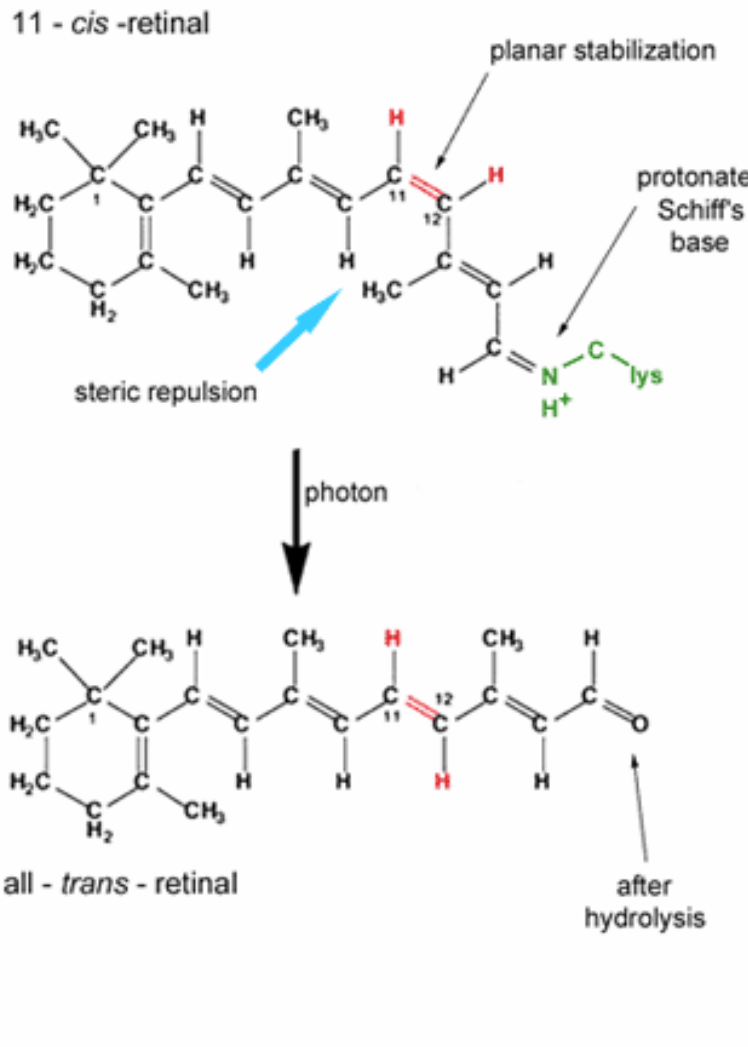


- 348 amino acids, 40 kD
- 7 transmembrane alpha helices connected by six loops of varying lengths
- N-terminal glycosylated
- Cys-322 and Cys-323 attach to membrane by palmitic acid, to form the 8th helix.
- Functional monomer, however, can form dimers

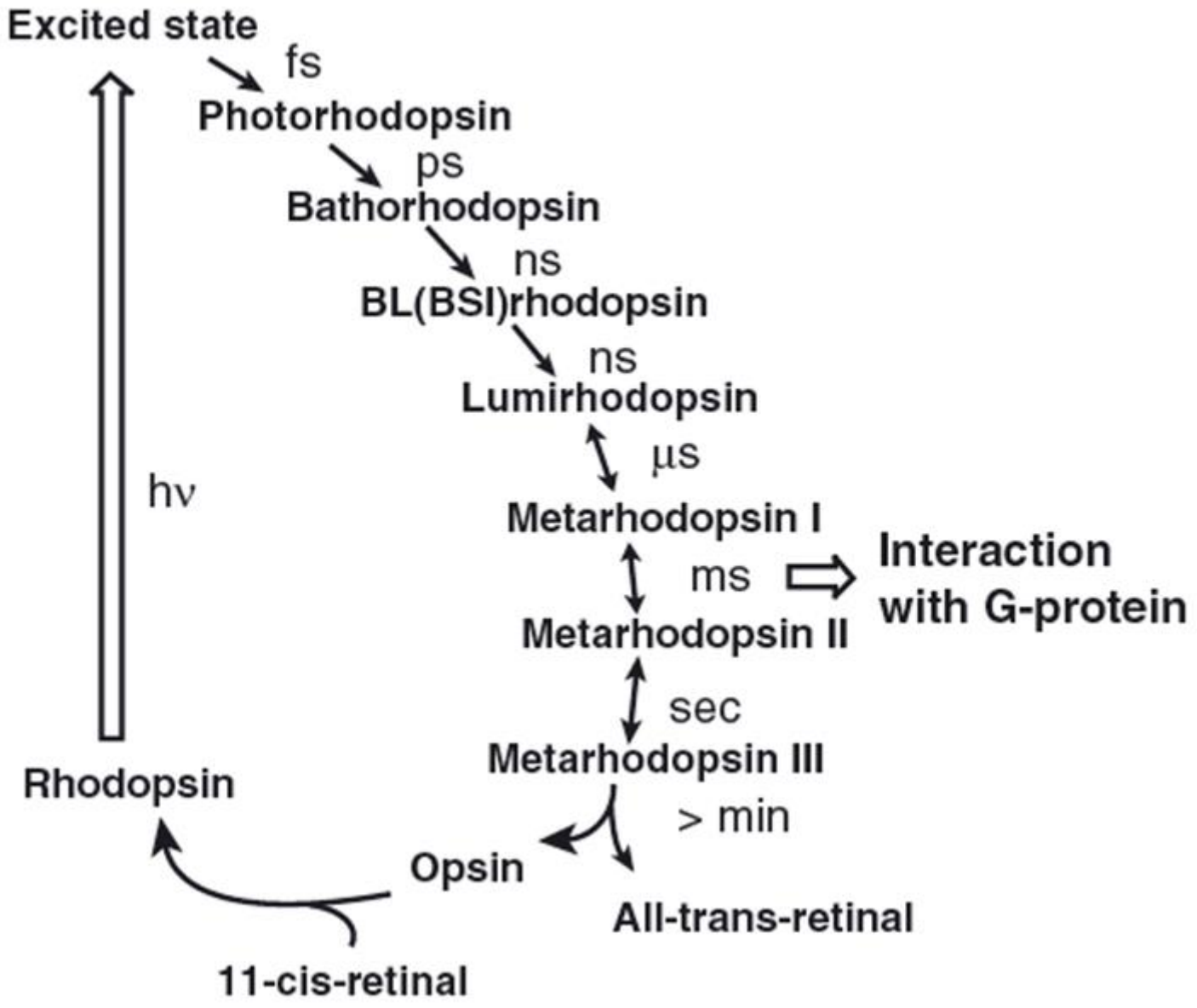
Retinal

- Retinal is a derivative of Vitamin A, which is a derivative of beta-carotene
- Isomerization of cis-retinal to trans-retinal by light causes a conformation change in rhodopsin which triggers a signal
- Rhodopsin absorbs at green-blue light (500 nm) which makes the protein appear reddish-purple



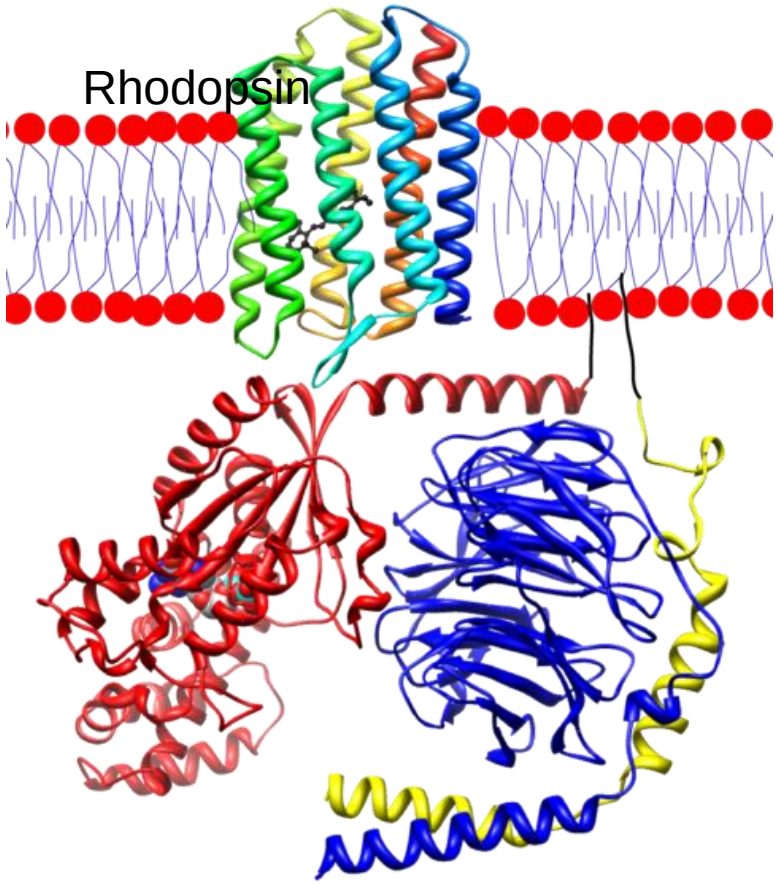


Isomerization of retinal by light induced orderly sequence conformational changes of rhodopsin



Rhodopsin: a G-protein coupled receptor (GPCR)

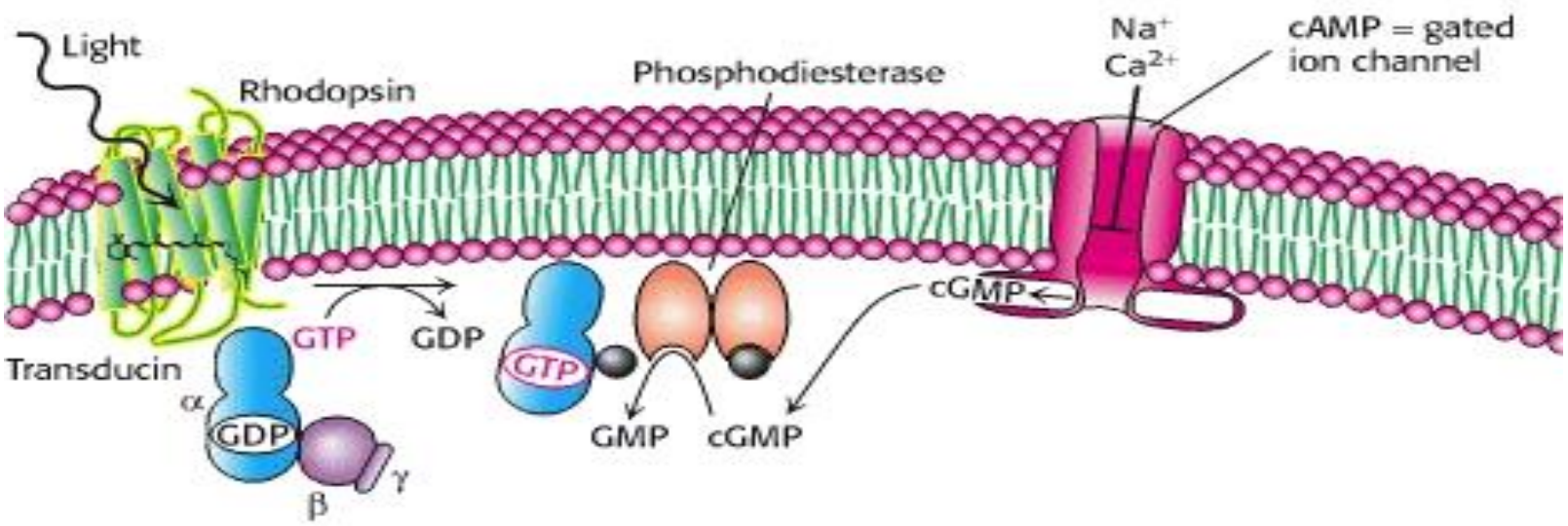
- 7 transmembrane receptor that sense molecules outside the cell and activate inside signal transduction pathways and cellular responses (Rhodopsin)
- Found only in eukaryotes
- GPCR activates a G-protein (Transducin) by exchanging its GDP for GTP
- GPCR is bound to a G-protein while in its inactive state
- Once a GPCR is active, the G-protein detaches
- G-protein is made up of 3 subunits (Gα, Gβ, and Gγ)
- Once G-protein is activated, the Gα subunit activates another protein (phosphodiesterase) and detaches from the other two subunits



Transducin: Gα is red, Gβ is blue, and Gγ is yellow

Phototransduction Pathway

- Light activates Rhodopsin which activates Transducin by exchanging its GDP for GTP
- When active, Transducin's alpha subunit dissociates from the other two subunits
- The active Transducin then activates a membrane bound protein called phosphodiesterase
- Phosphodiesterase hydrolyzes cGMP
- The hydrolyzes of cGMP, leads to the ion channel closing and the initiation of an action potential
- To return back to the inactive state Rhodopsin Kinase phosphorylates the cytosolic tail of rhodopsin which inhibits the activation of transducin
- Arrestin then binds to the phosphorylated rhodopsin further inhibiting activity



2. How do microbes sense light: light-activated channel/pump/enzyme

pump ion channel receptor kinase

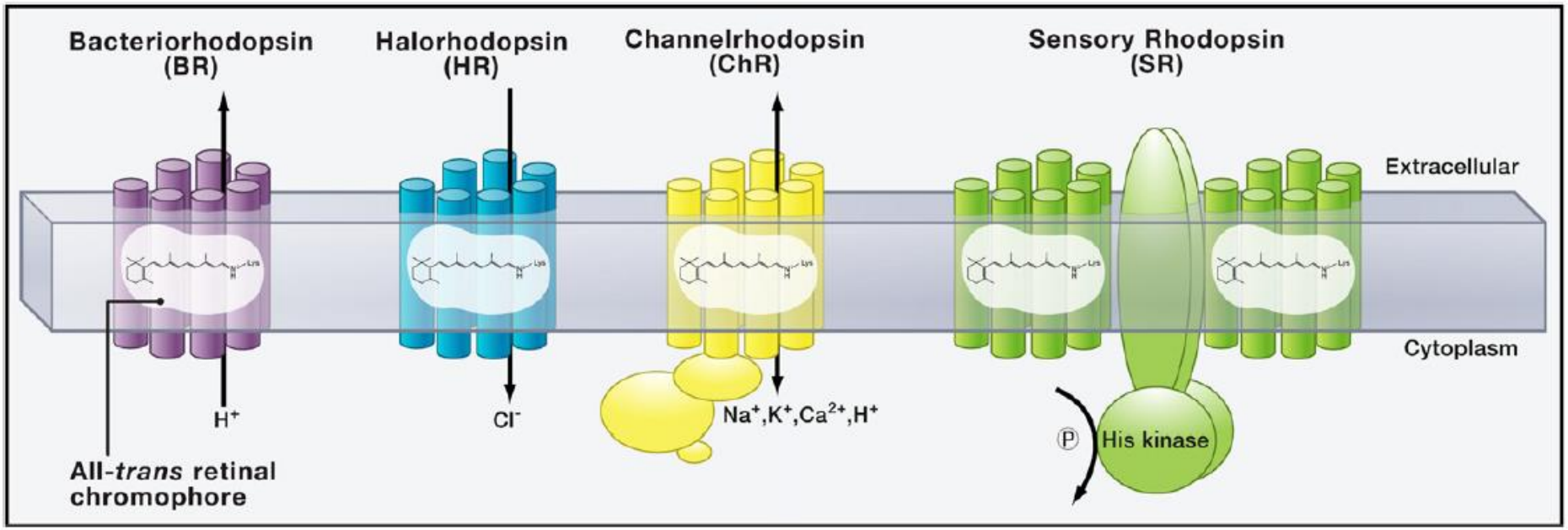
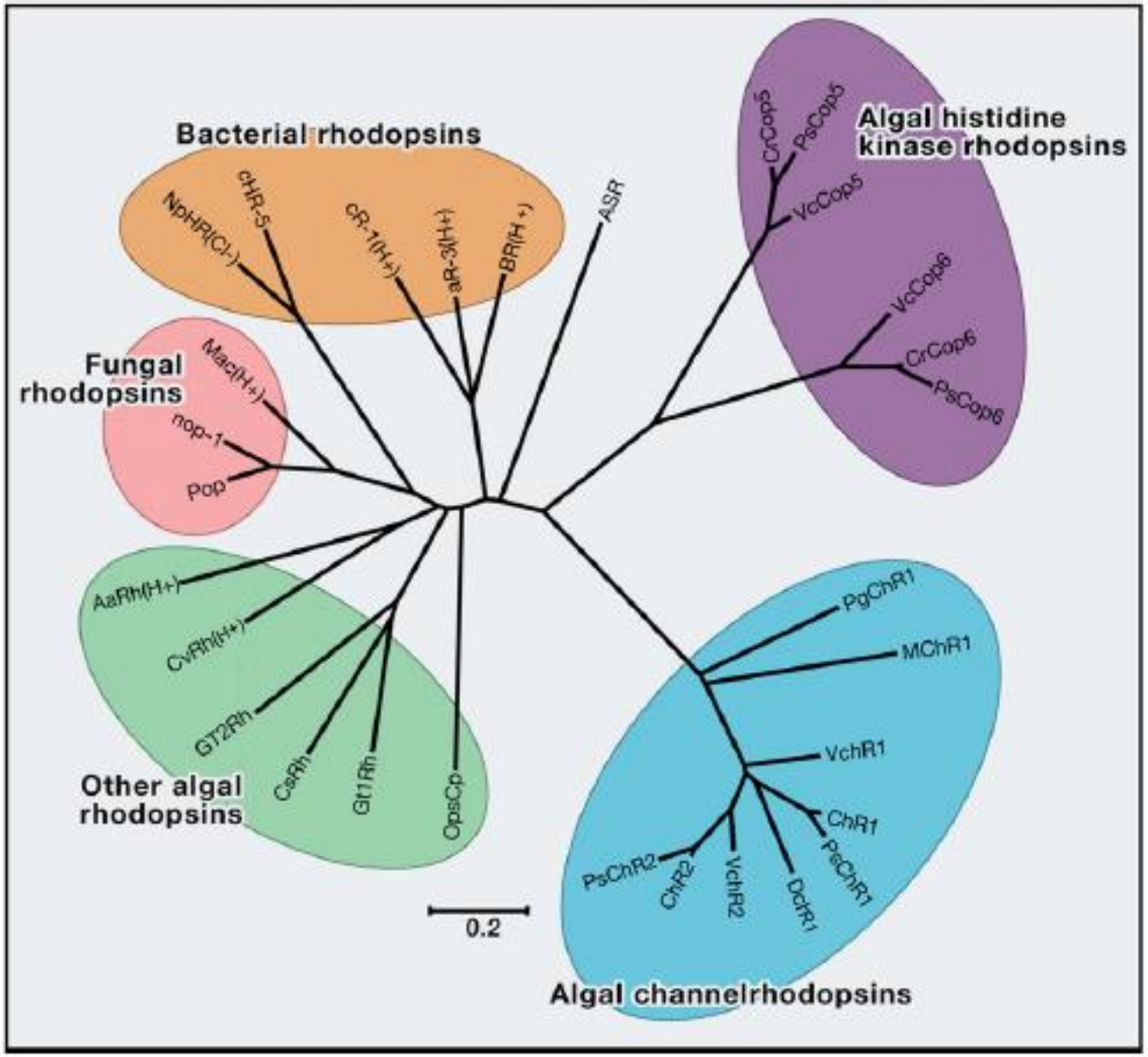


Figure 1. Type I Microbial Rhodopsins

BR (and PRs) pump protons from the cytoplasm to the extracellular medium, and HRs pump chloride into the cytoplasm; all three hyperpolarize the cell. SRs lack TM ion transport in the presence of the His kinase transducer protein Htr; and algal ChRs conduct cations across the membrane in both directions but always along the electrochemical gradient of the transported ions. In SRs and ChRs, proton translocation within the protein is linked to efficient photocycle progression, but these protons are not necessarily exchanged between the intra- and extracellular spaces.

Phylogenetic tree of microbial Opsins



3. Definition of optogenetics

Optogenetics is a technology that allows targeted, fast control of precisely defined events in biological systems.

By delivering optical control at the speed (millisecond-scale) and with the precision (cell type–specific) required for biological processing, optogenetic approaches have opened new landscapes for the study of biology, both in health and disease.

3. Definition of optogenetics

Optogenetics is the combination of genetic and optical methods to achieve gain or loss of function of well-defined events in specific cells of living tissue.

In the broadest sense, optogenetics encompasses a core technology—targetable control tools that respond to light and deliver effector function—and enabling technologies for:
(i) delivering light into tissues under investigation,
(ii) targeting the control tools to cells of interest, and
(iii) obtaining compatible readouts and performing analysis, such as targeted imaging or electrical recording of evoked activity.

Importance of optogenetics

Although it arose from neuroscience, optogenetics addresses a much broader need: the need to control defined events in defined cell types at defined times in intact systems.

Such analyses are important because cellular events are typically meaningful only in the context of other events occurring in the rest of the tissue, the organism and the environment as a whole.

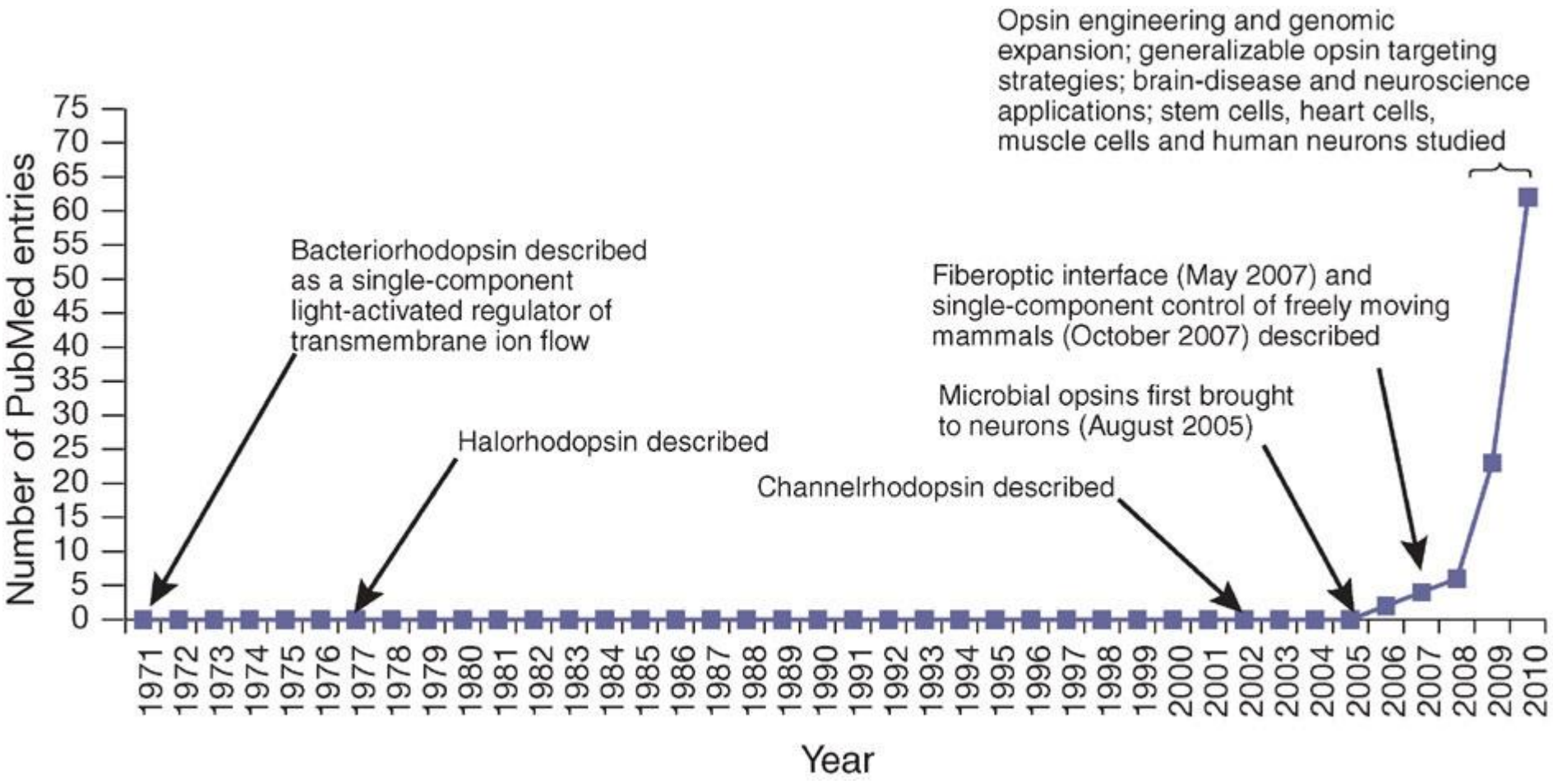
History of optogenetics

In 1979 Francis Crick: the major challenge facing neuroscience was to control one type of neuron in the brain while leaving others unaltered. He speculated light as the control tool.

In 1971, Stoeckenius and Oesterhelt discovered that bacteriorhodopsin, an light-activated proton pump. Later others were discovered: halorhodopsin in 1977; channelrhodopsin in 2002.

It took more than 30 years for neuroscientists to bring the two fields together. In 2005, Boyden and Dreisseroth introduced a microbial opsin gene to neuron so that it became precisely responsive to light.

Timeline of optogenetics development



Comparing classic electrode stimulation with light stimulation: high spatial resolution

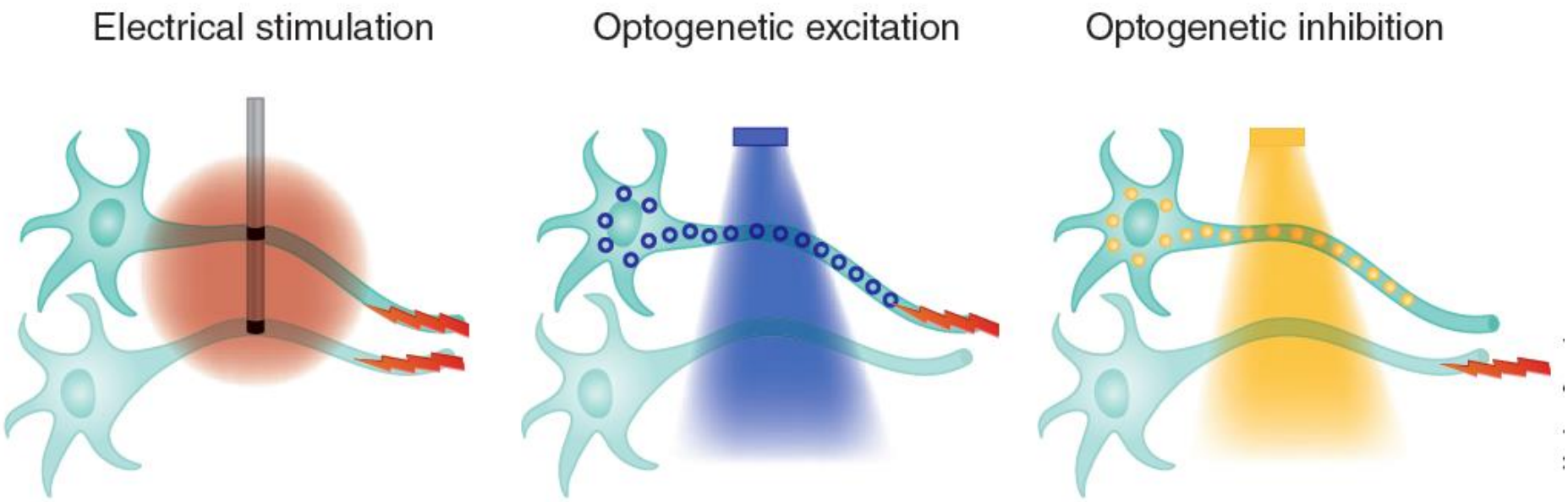


Figure 2 | Principle of optogenetics in neuroscience. Targeted excitation (as with a blue light–activated channelrhodopsin) or inhibition (as with a yellow light–activated halorhodopsin), conferring cellular specificity and even projection specificity not feasible with electrodes while maintaining high temporal (action-potential scale) precision.

Implications

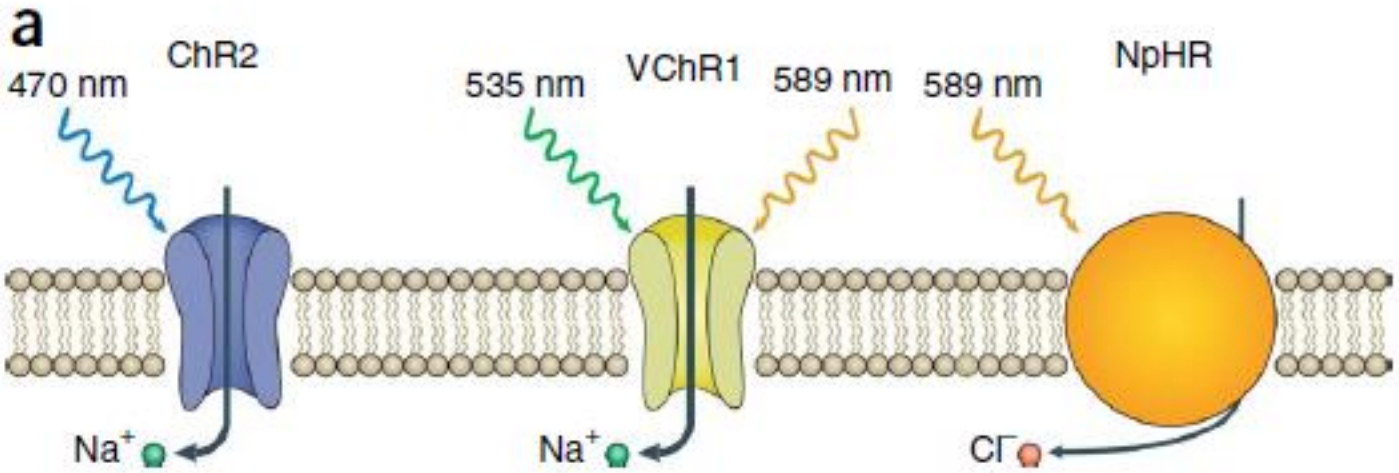
Optogenetic tools have now changed the way neuroscience is conducted.

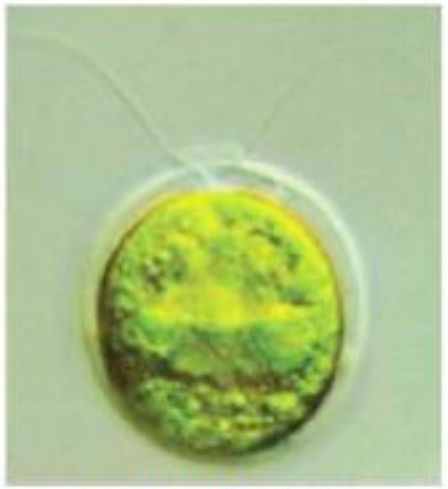
Obtaining precise causal control in intact systems as complex as behaving mammals is certainly important in neuroscience, and in other fields of biology.

Historically, it has not been possible to deliver causal, temporally precise gain or loss of function in one type of brain cell or in a defined projection from one brain region to another.

4. Elements of genetic tools:

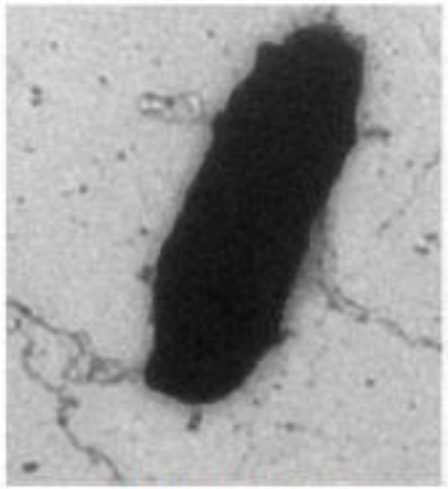
natural occurring effectors and their microbial sources





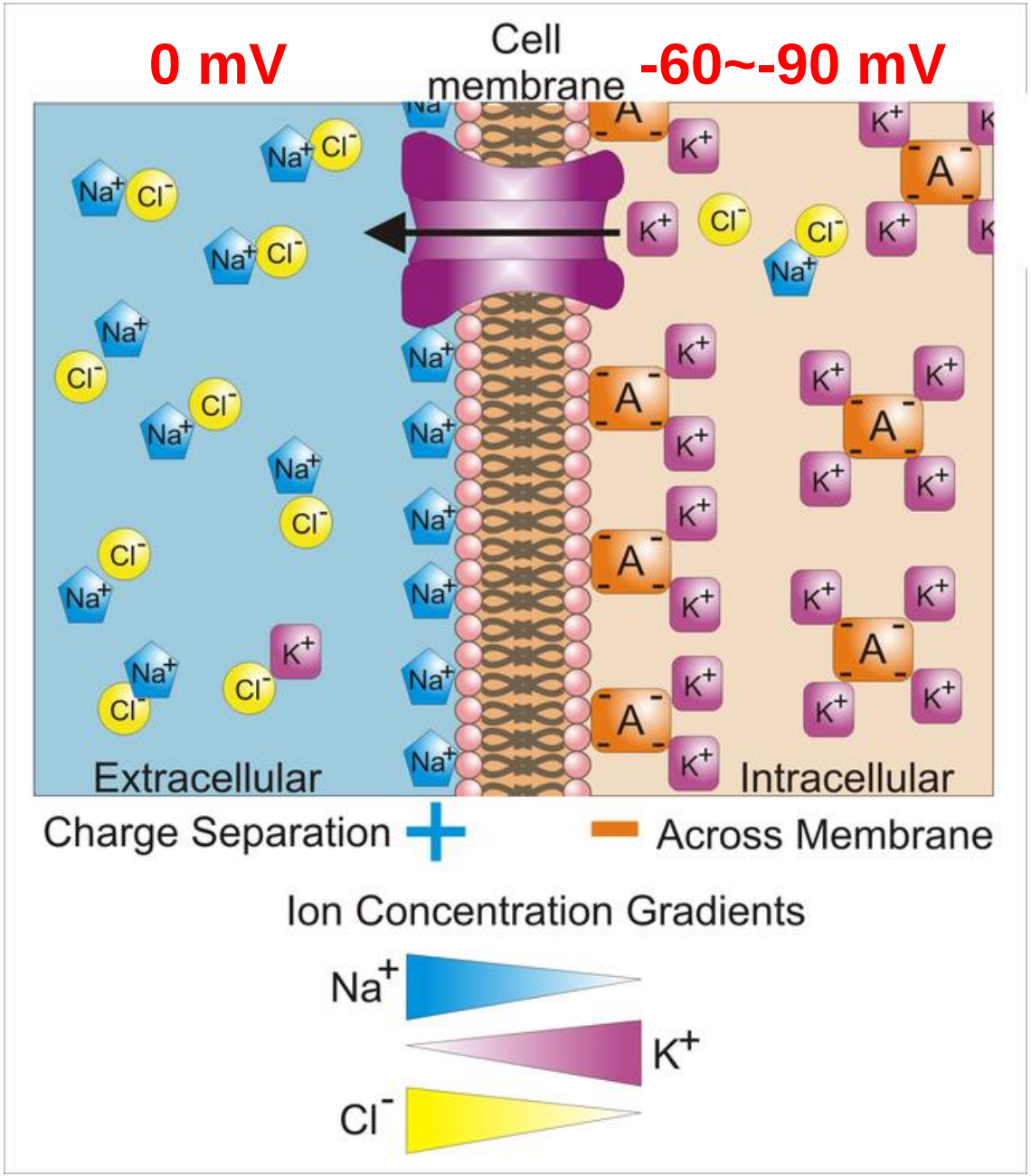
Chlamydomonas reinhardtii

Volvox carteri

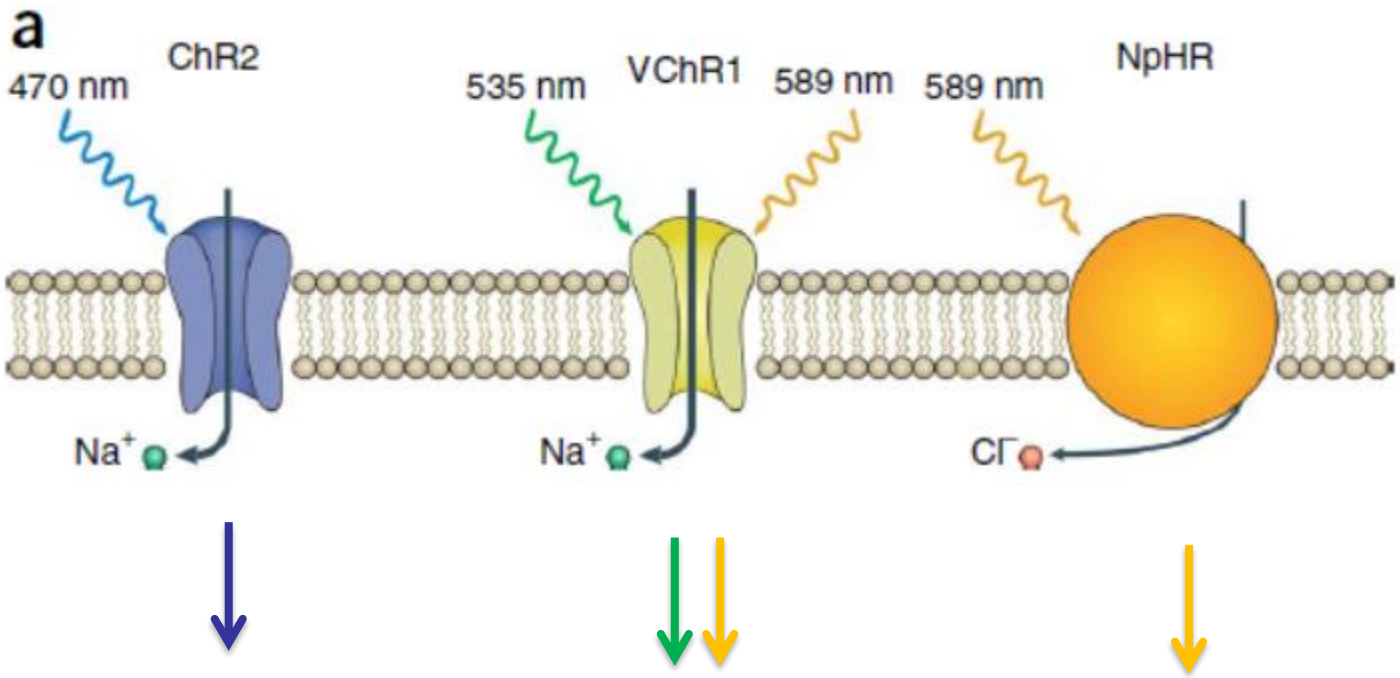


Natronomonas pharaonis

Ion concentration and resting membrane potential



The effect of light-activated ion channels and pumps on membrane potential



Depolarize

Depolarize

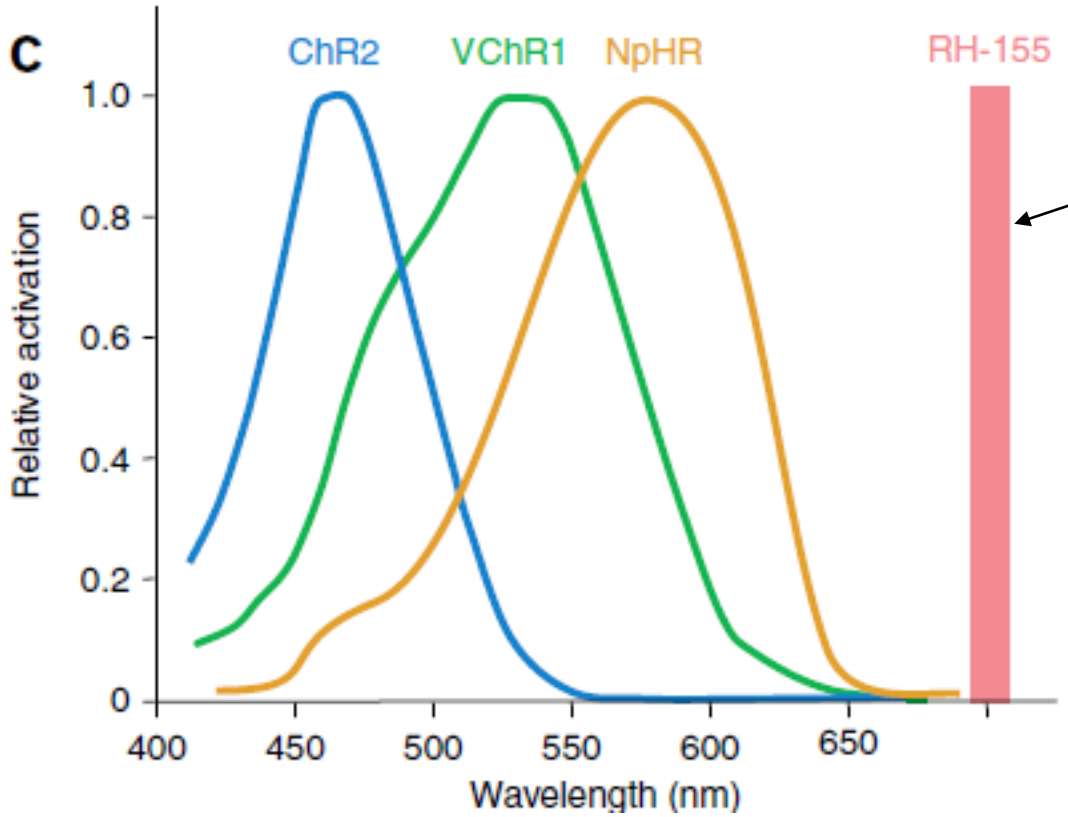
Hyperpolarize

Excitation

Excitation

Inhibition

Spectra separation: enabling combinatory control

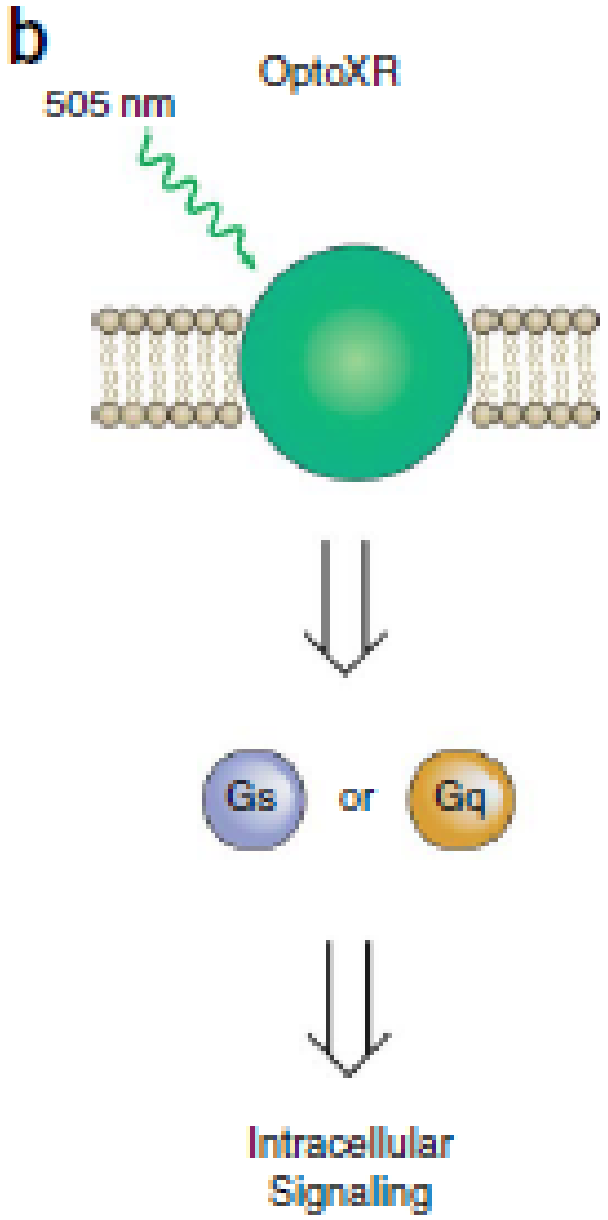


Membrane potential dye

Engineered synthetic rhodopsin

The chimera of bovine rhodopsin and intracellular loops of another GPCRs:

For optical control of well-defined intracellular biochemical signally



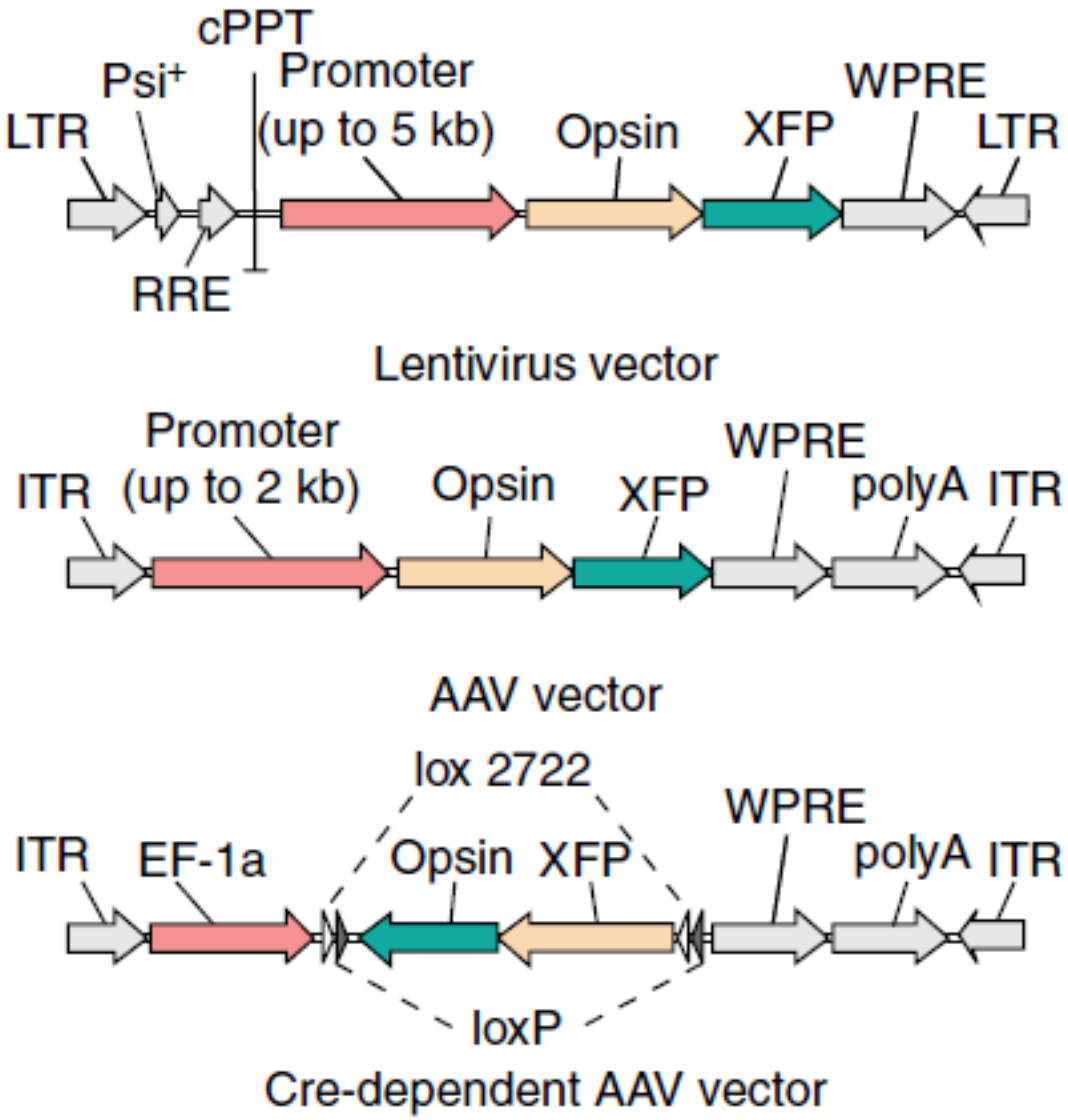
Desired functional properties

- Fast response time (ms):
  - precise temporal control via fast modulation of light source
- Combinatory control:
  - Parts with activation and inhibition actions exhibit different spectra properties

Genetic integration of parts

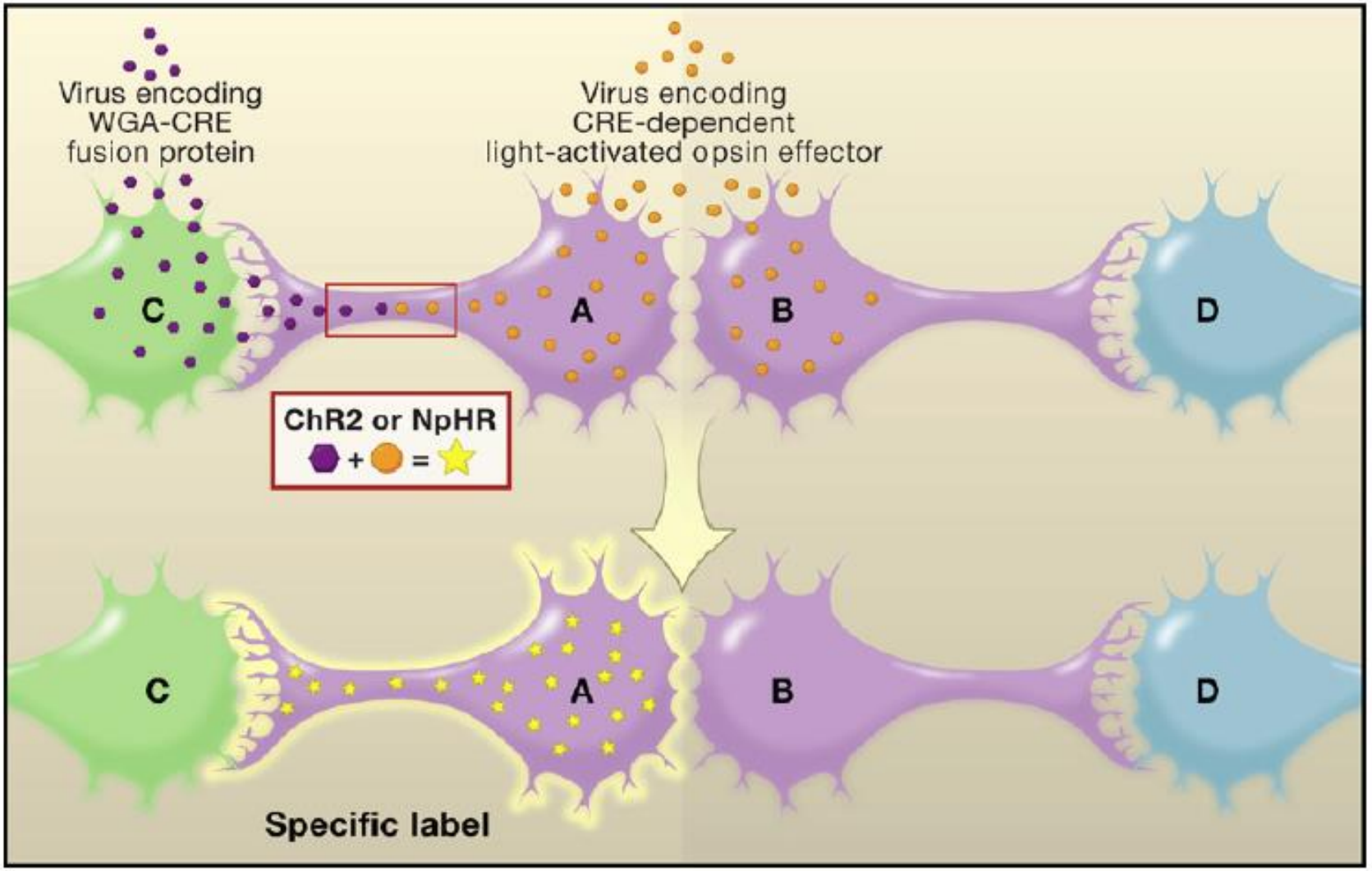
- Transgene: all cell types via cell type-specific promoter, higher specificity; time-consuming, low expression level
- Lentivirus: fast, high expression; compromising cell type specificity
- Adeno associated virus (AAV): fast, high expression; compromising cell type specificity

Viral vector designs



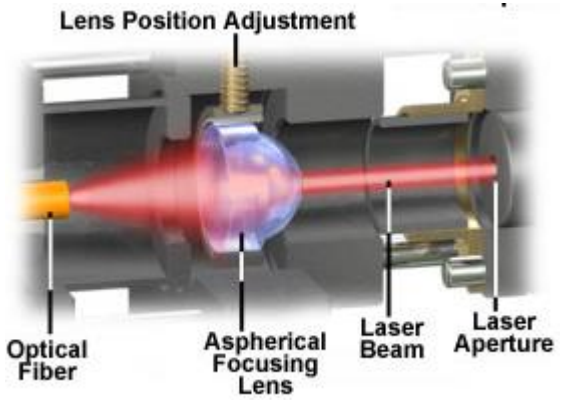
Cre enable additional temporal control

Trans synaptic trafficking of virus enabled the detection of neuron connectivity: spatial control



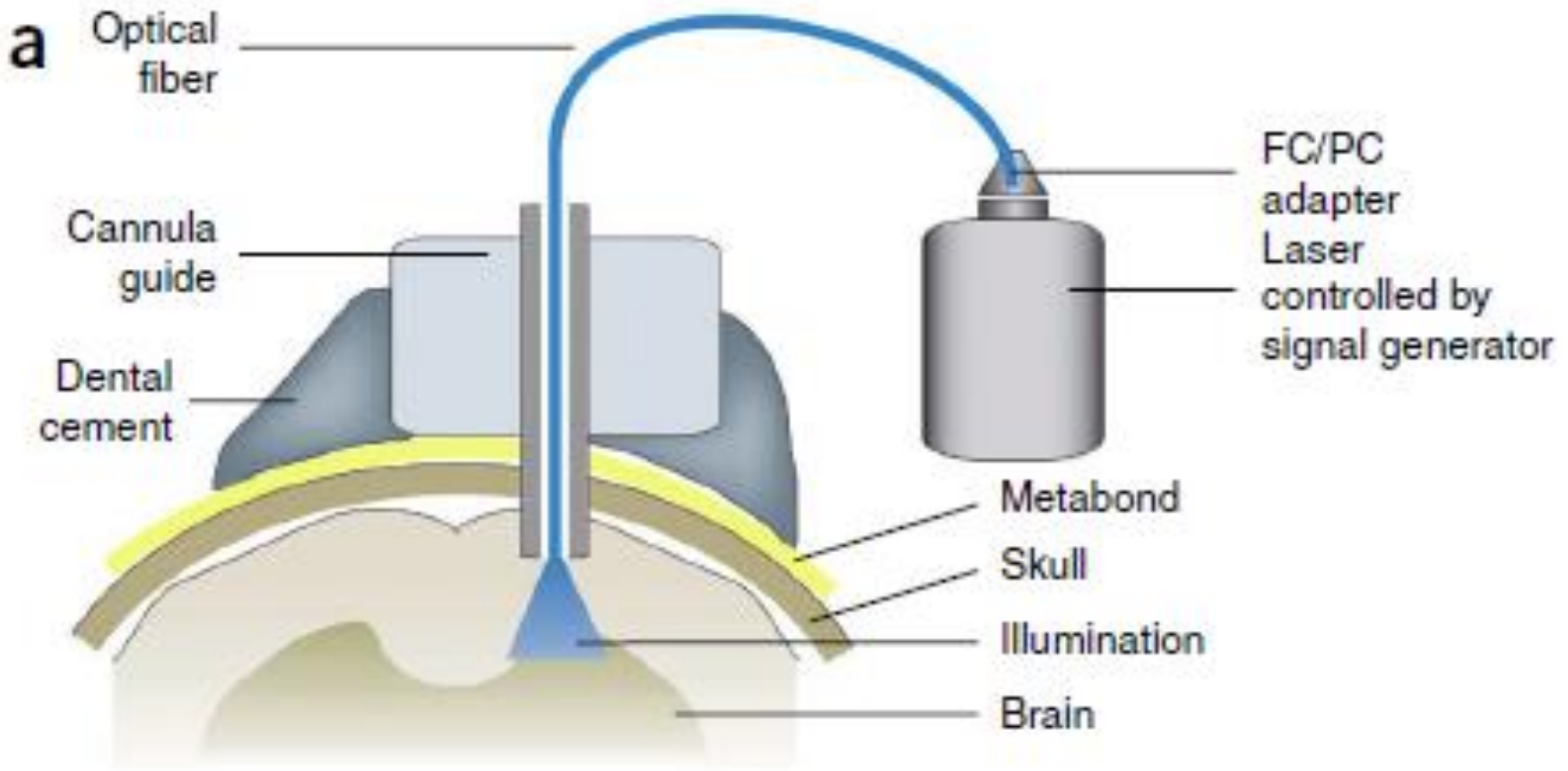
5. Elements of optical tools:

1. Optical fiber:
   low lateral spatial resolution
   Worst axial resolution

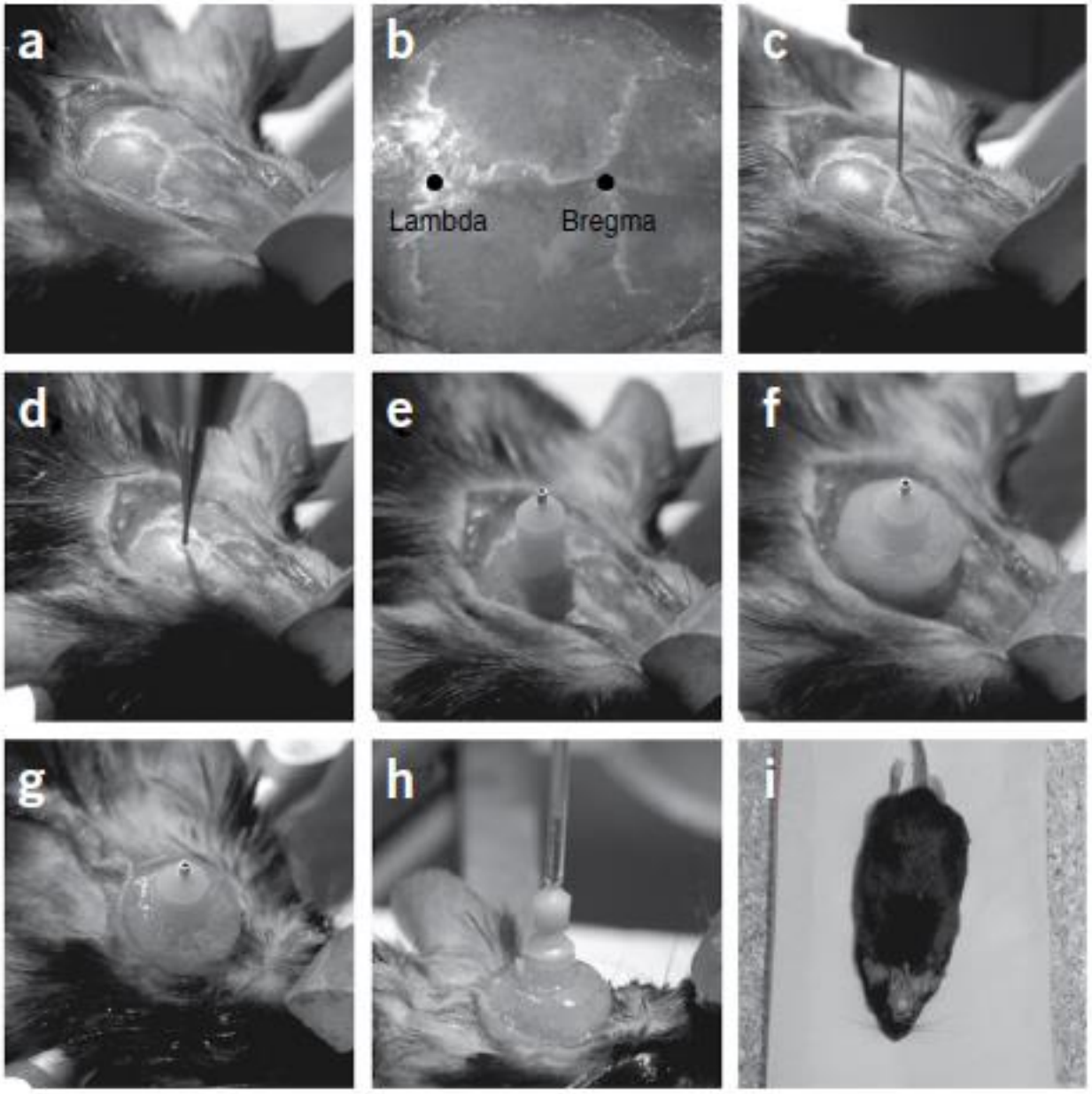


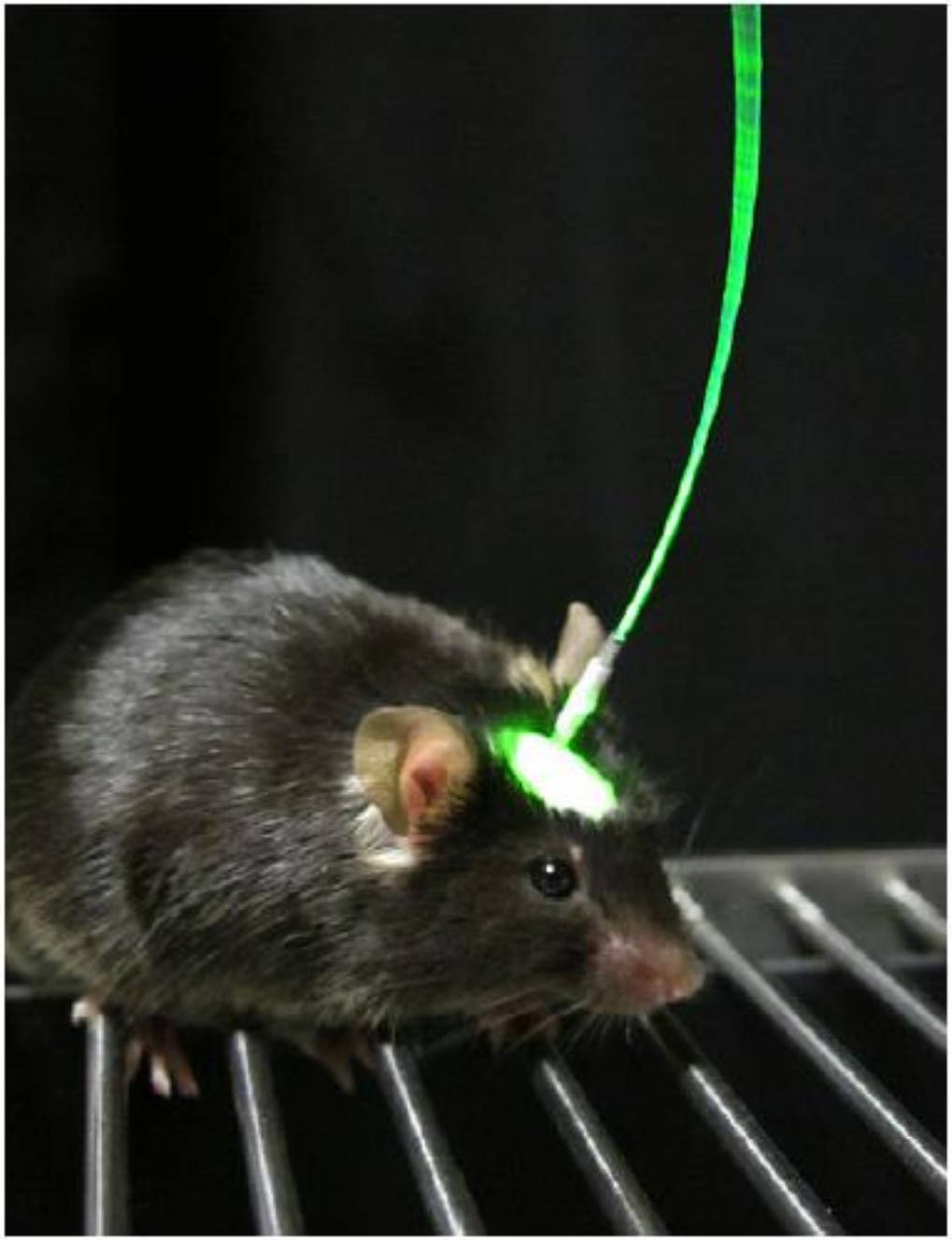
2. Combination of optical fiber and tetrode electrode:
   spatial resolution enhanced by electrode verification of neuron type
3. Structure illumination
   Simultaneously stimulate multiple locations, good lateral resolution, OK axial resolution
4. Two-photon confocal microscopy
   good lateral and axial resolution

Optical fiber: great temporal control



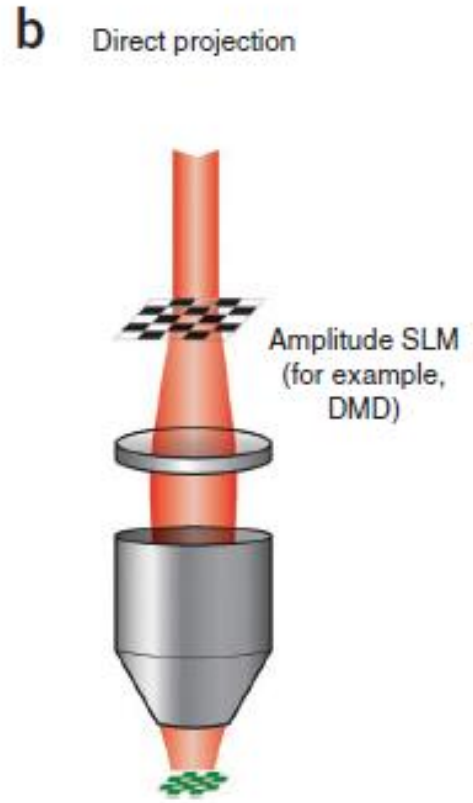
Stereotactic implantation of the cannula guide

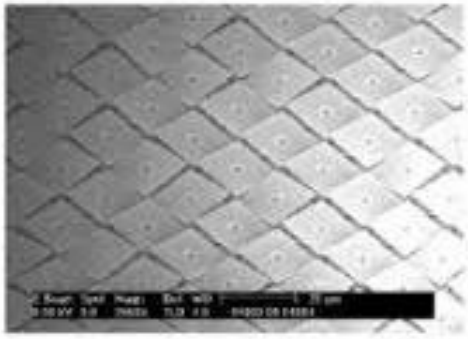


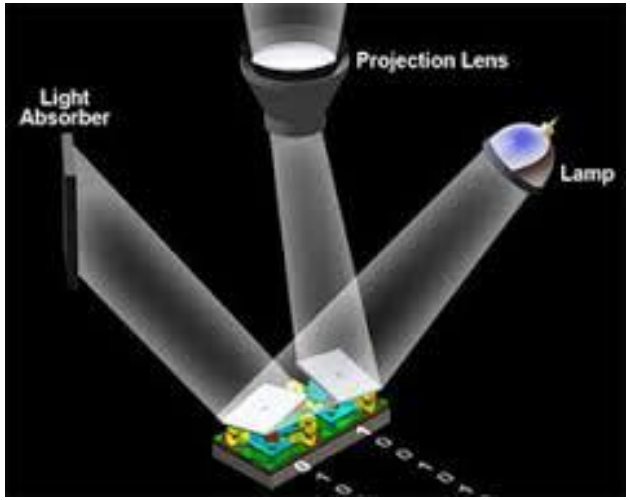


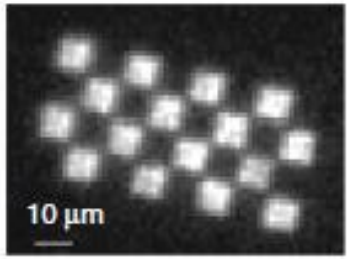
Structure illumination Mirror

- Digital mirror array (DMD)
- Simultaneously deliver light to independent area of arbitrary shape distributed over a large region
- Low light intensity (hinder two photon application)



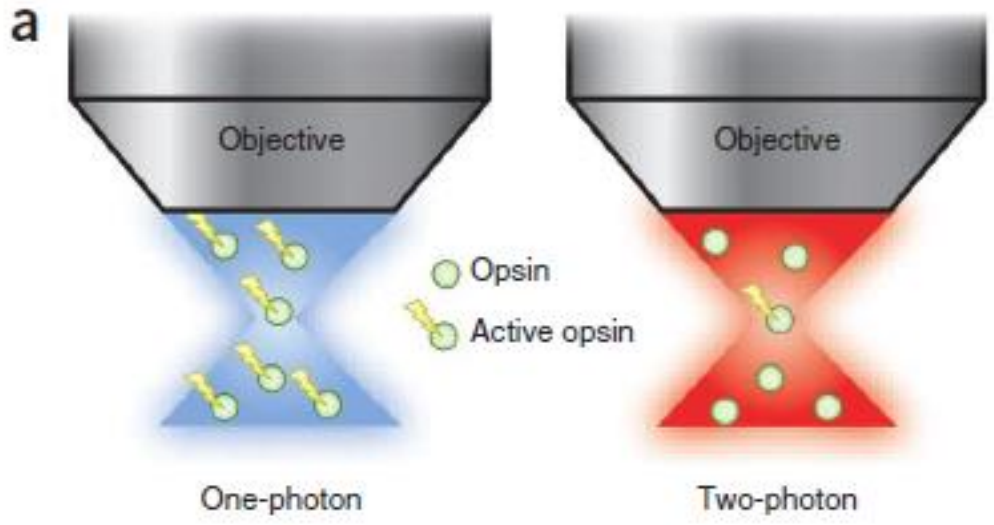


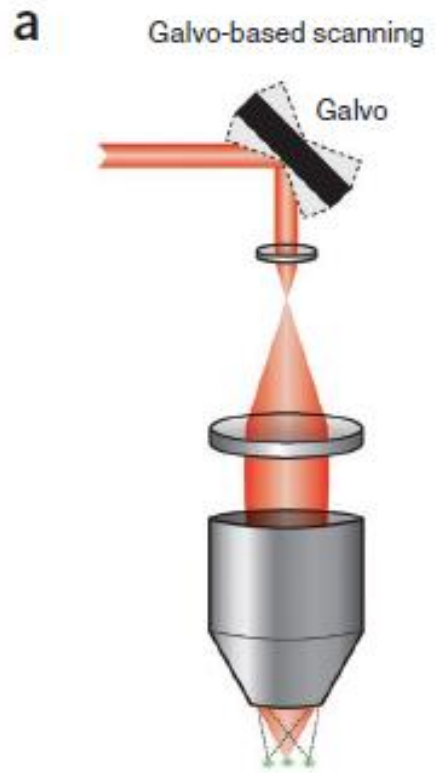


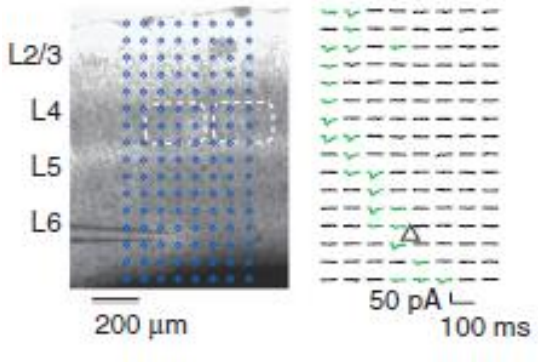


Two photon activation

- Galvo mirror control the location of excitation: arbitrary ROIs
- Two photon improves the z-axis precision

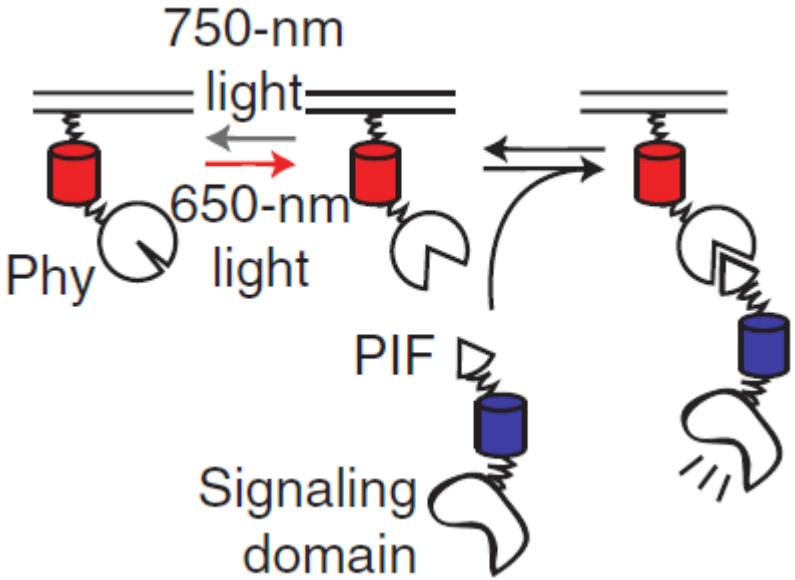




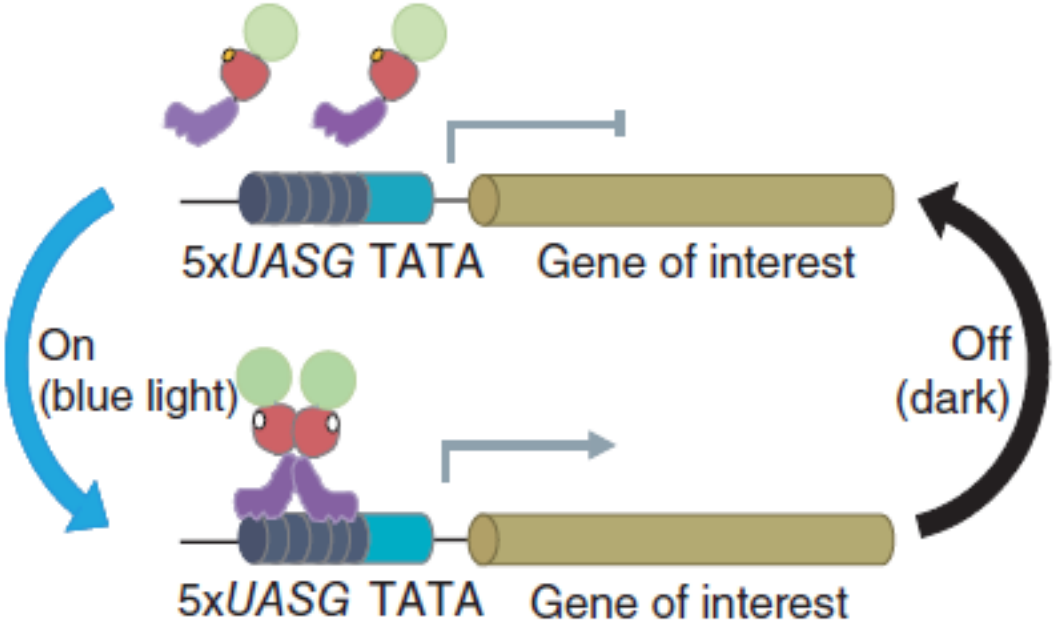


Other non ion channel optogenetic tools

The light-gated interaction between fragments of two plant proteins--Phy and PIF could be utilized to control the location of signal protein



Toettcher 2012 Nat Methods



Wang 2012 Nat Methods

The light-induced dimerization of a fungus protein VVD could be used to control the transcriptions

Combined strategy for neuron manipulation

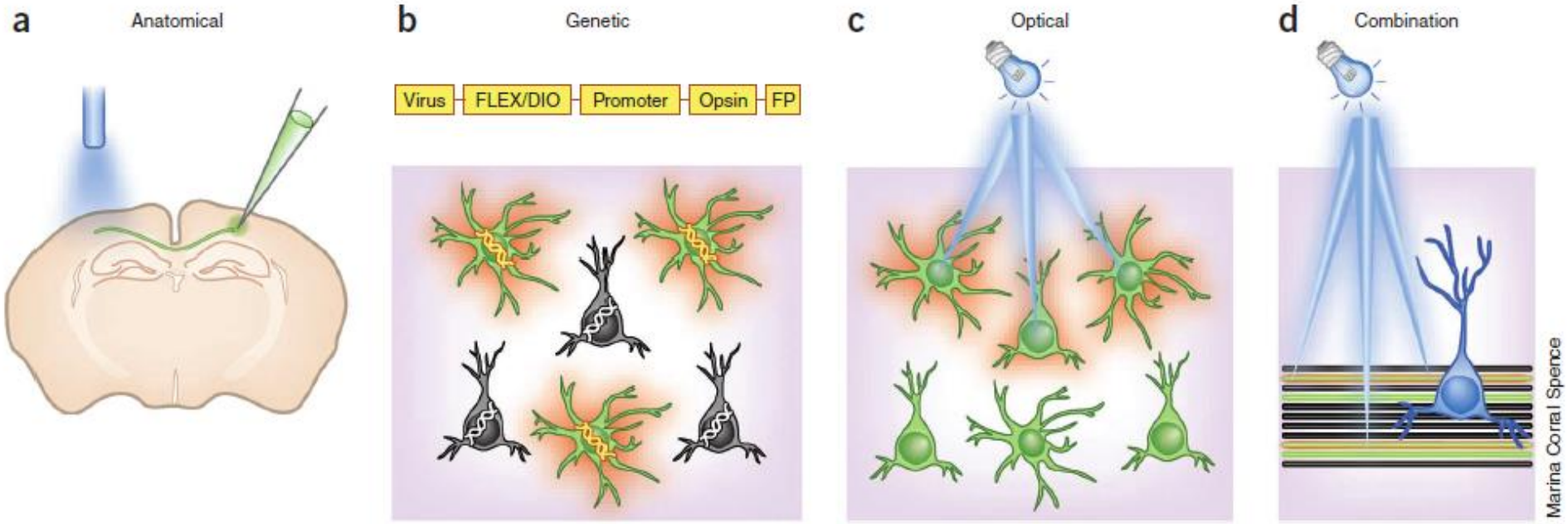


Figure 1 Intersectional strategies for targeting optogenetic manipulation. (**a**) Physical delivery of virus to a given anatomical location can exploit or uncover circuit connectivity patterns either by making use of axonal projections or by using viruses that are able to cross one or more synapses. (**b**) Cell types can be addressed if the cell type of interest has a known genetic identity. (**c**) Directing the illumination source to a given set of cells or even individual neurons and processes is useful when the targets of interest are separated in space relative to the spatial resolution of the technique used. (**d**) These three strategies can be combined, as shown in this example, in which axons of a particular cell class projecting to a subcellular domain of a neuron are photostimulated at different distances from the neuron.

Ultimate goal

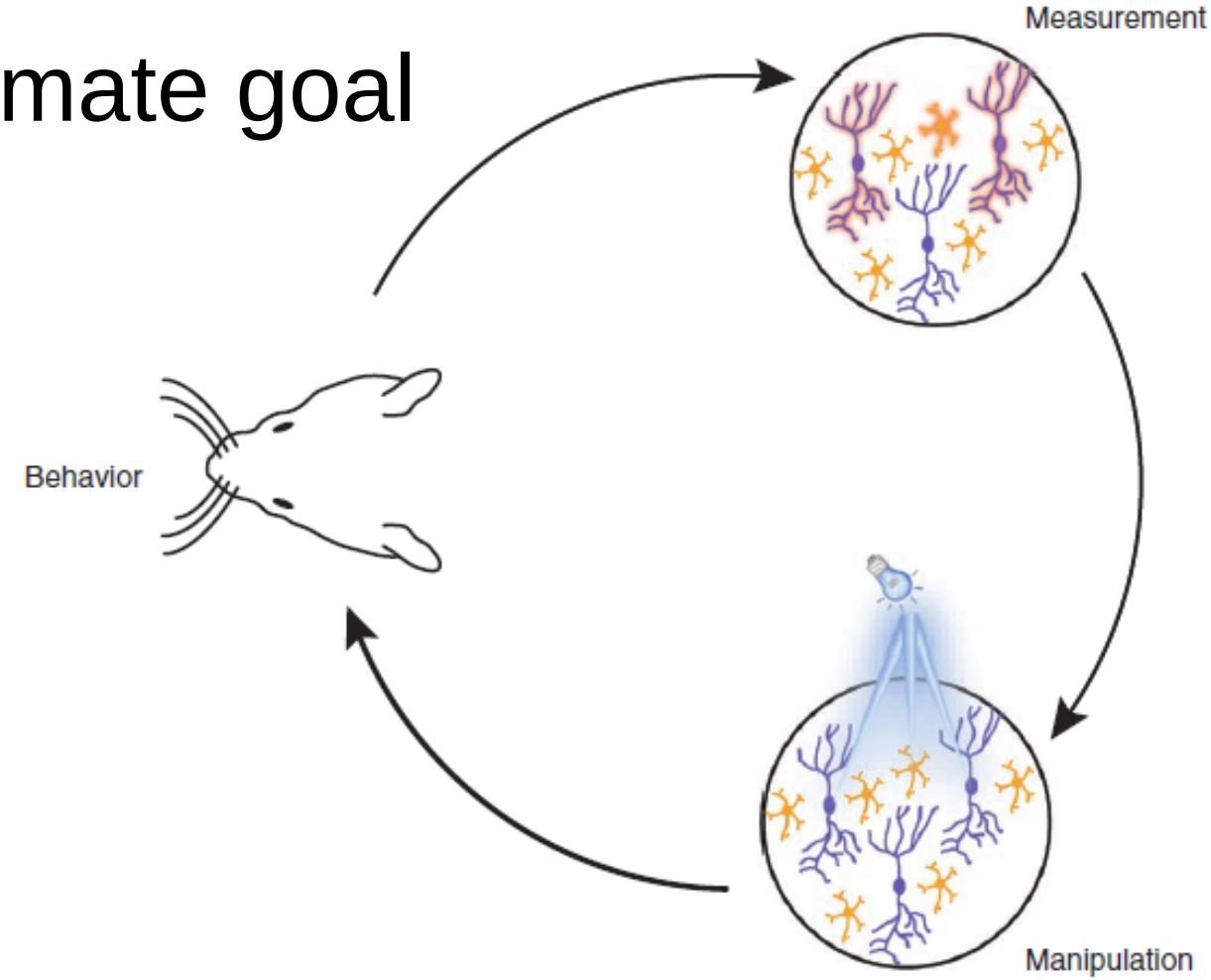


Figure 6 Using targeted optogenetics to enable 'dream experiments'. A schematic illustration of how 'targeted optogenetics' can be used to probe the neural code in a cortical circuit. The figure highlights the close interplay that is necessary between behavioral experiments, optical readout of patterns of activity and replay of the same patterns in the 'right' neurons using optogenetics. Targeted optogenetics allows the precision of temporal patterns and the precise membership of the neuronal ensemble to be tested directly to investigate their importance for the neural code driving the behavior.

6. Example 1: fast modulation of membrane potential

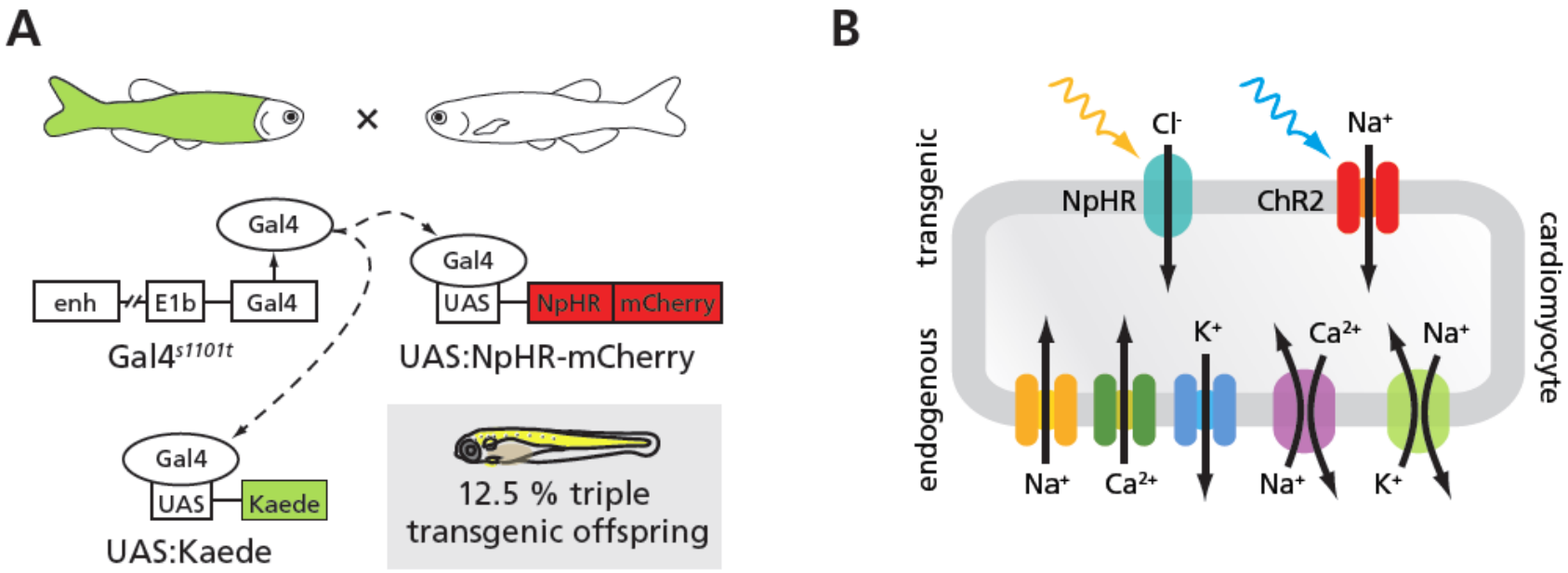
Optogenetic Control of Cardiac Function

Aristides B. Arrenberg,[1,3] Didier Y. R. Stainier,[2*] Herwig Baier,[1] Jan Huisken[2,4]

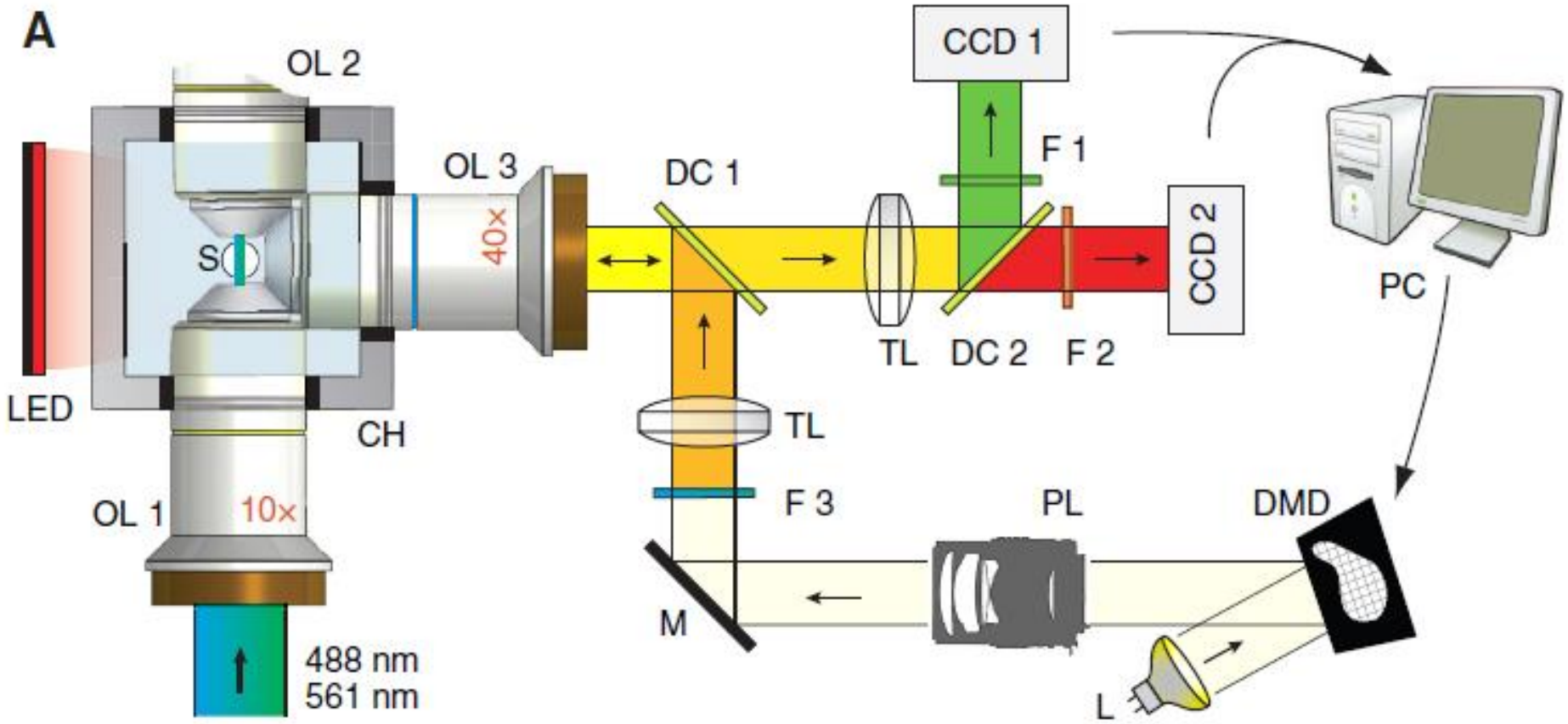
Science 2010, vol 330,971

Perturbation of a genetic encoded, optically controller pacemaker in zebrafish heart.

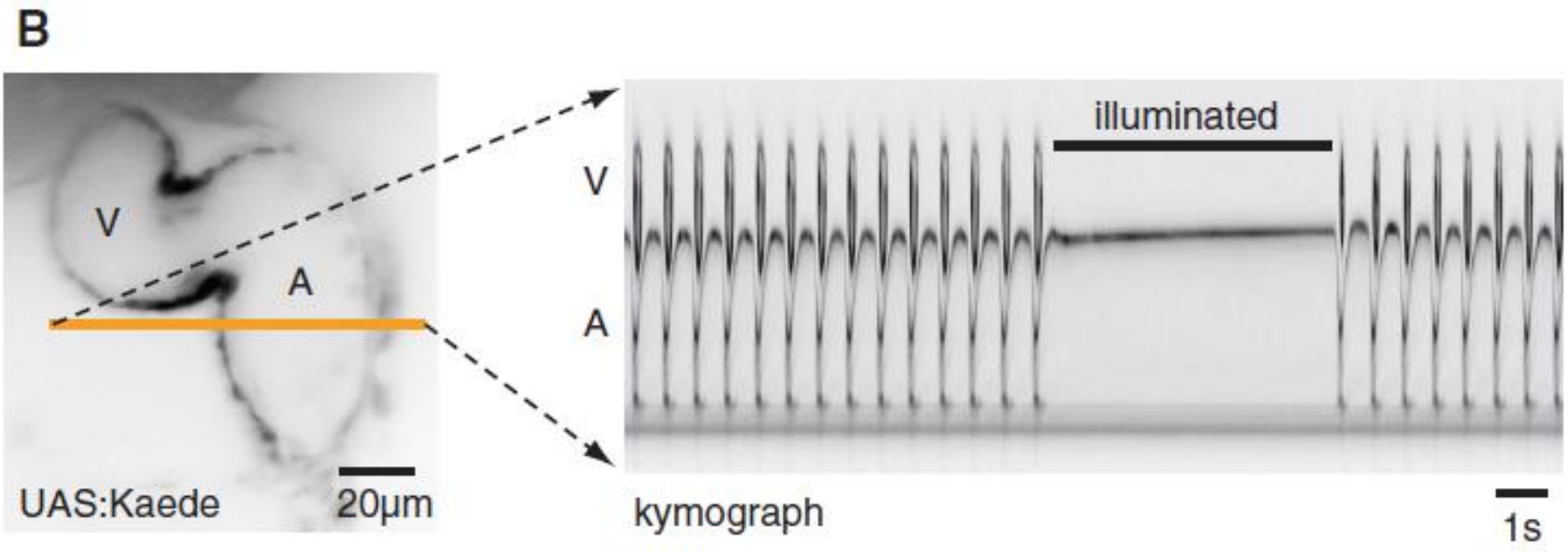
Zebrafish expressing NpHR and ChR2



Light sheet imaging and SLM activation

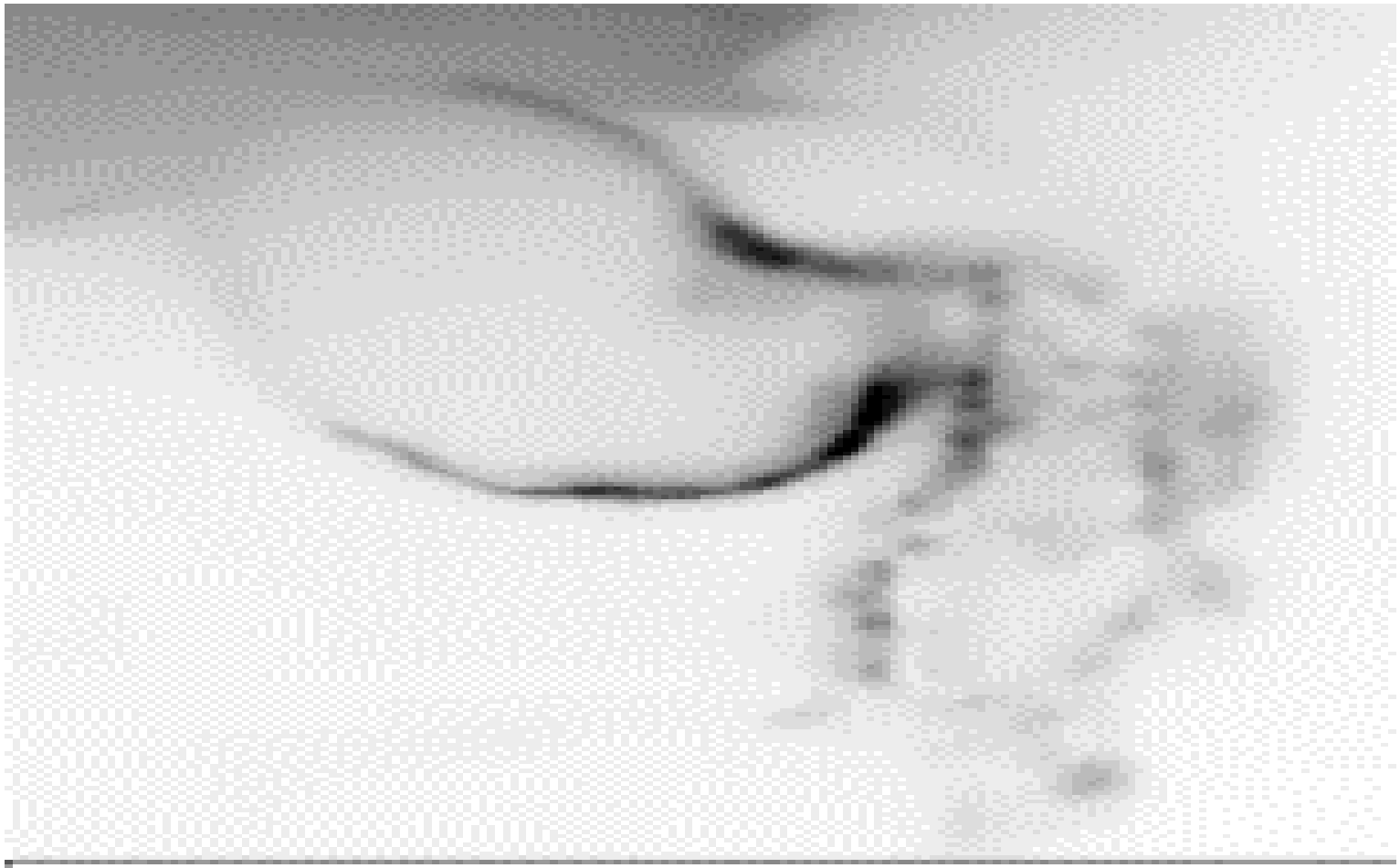


Automatic mapping of cardiac pacemaker using patterned illumination

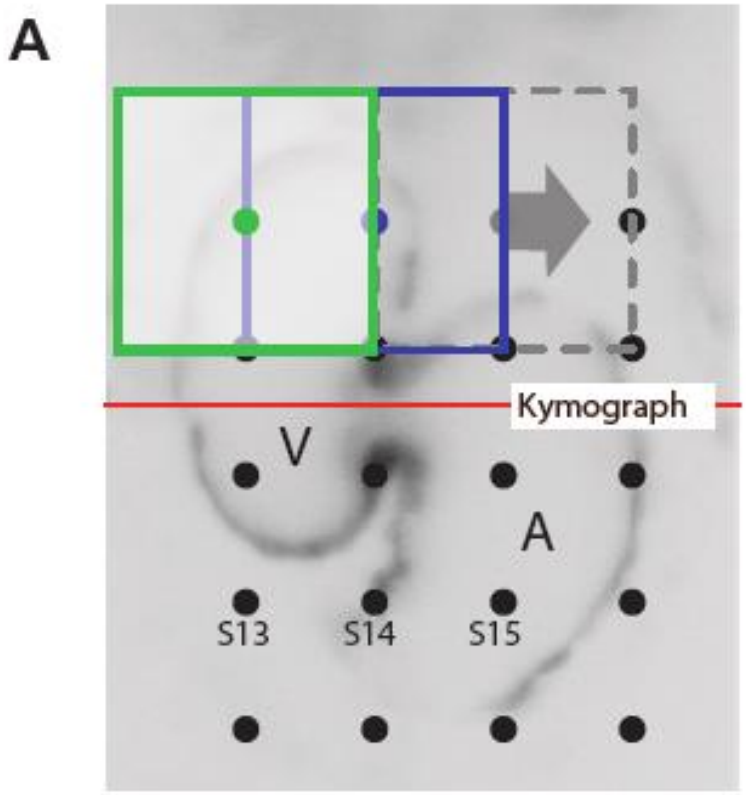


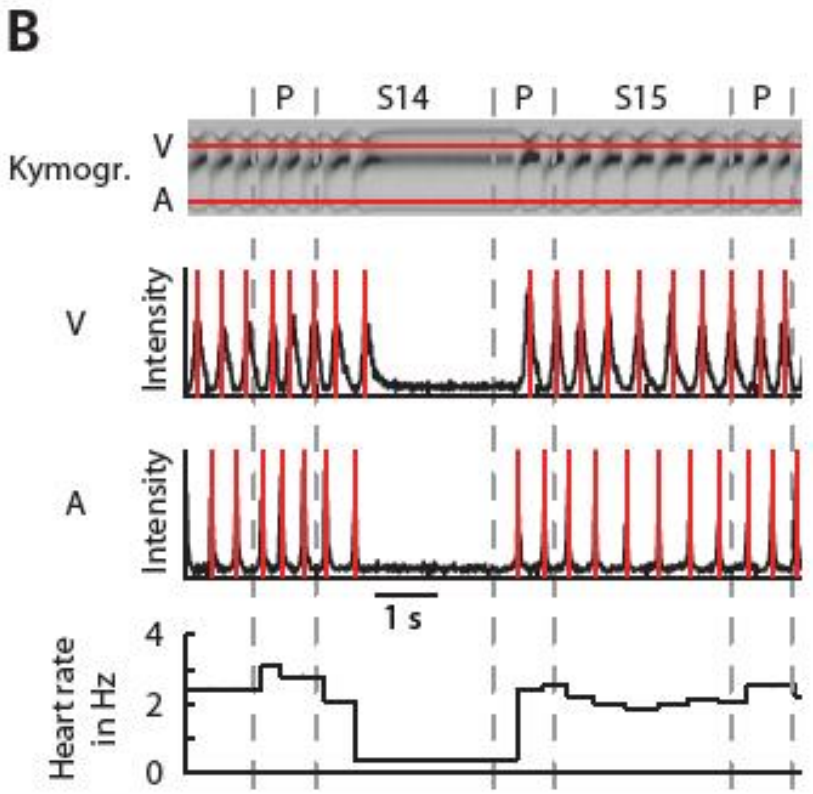
A 3-dpf zebrafish heart expressing NpHR-mCherry stopped beating when illuminated with orange light and recovered instantaneously afterward. The kymograph shows the motion of the heart wall along the highlighted line.

NpHR activation induces cardiac arrest

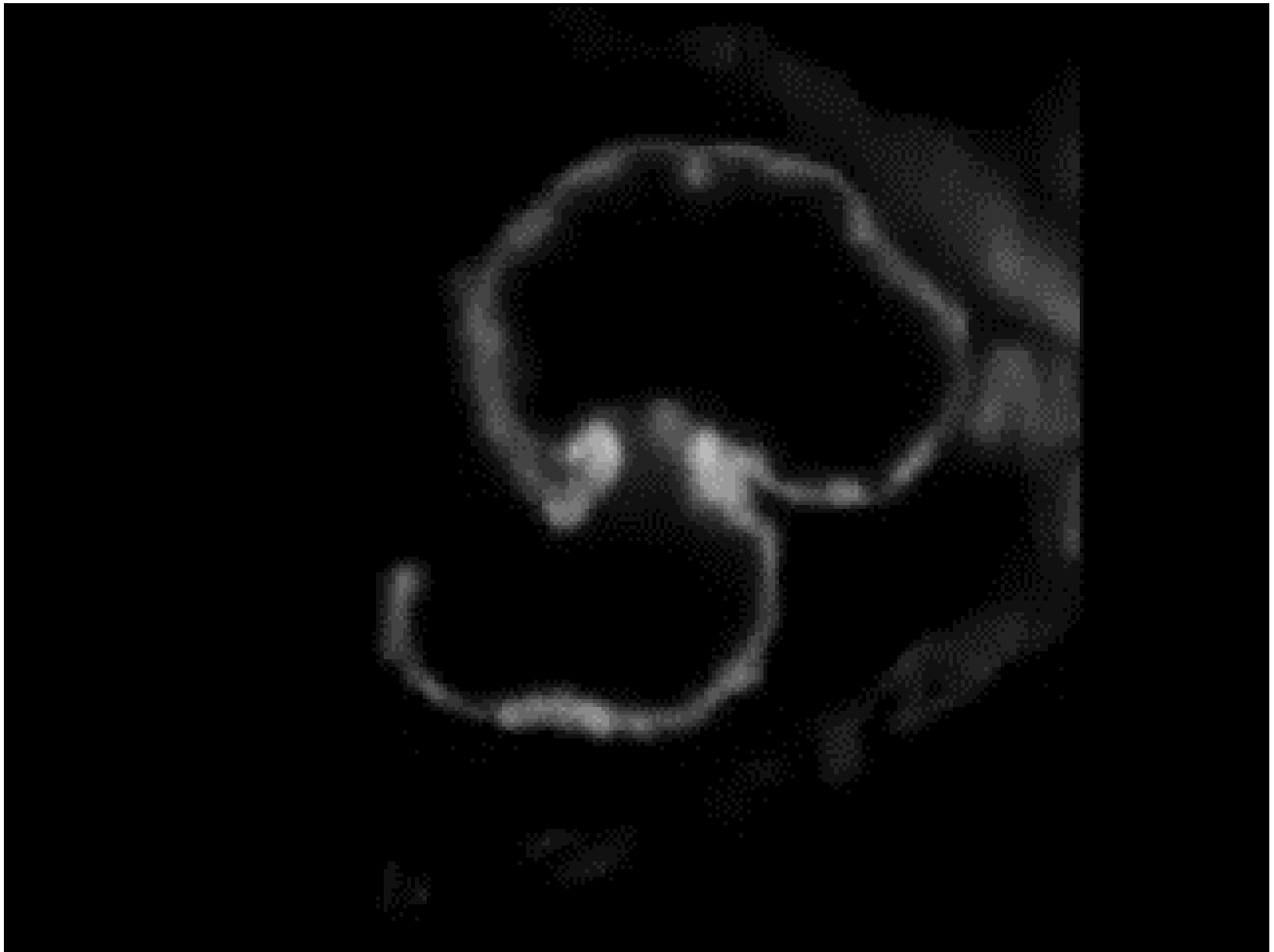


Automated mapping of the cardiac pacemaker using patterned illumination and image analysis.

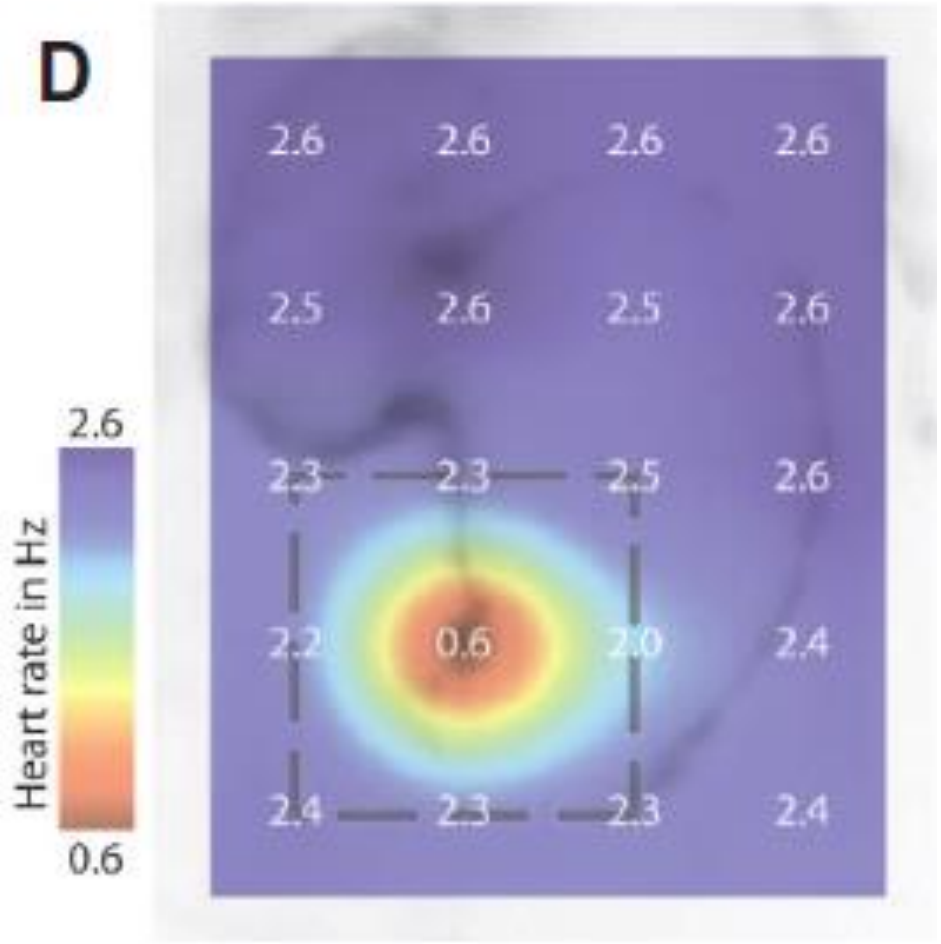




Automatic mapping of a 3 dpf heart

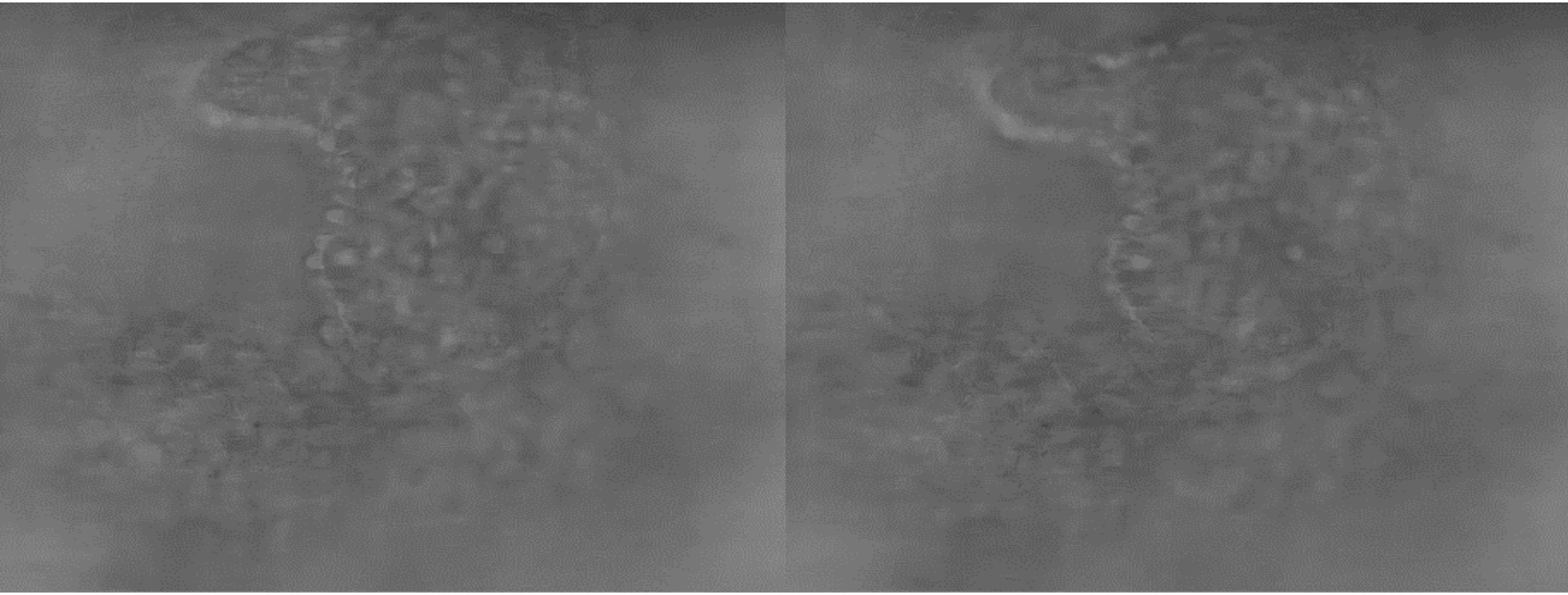


The observed heart rate after illumination

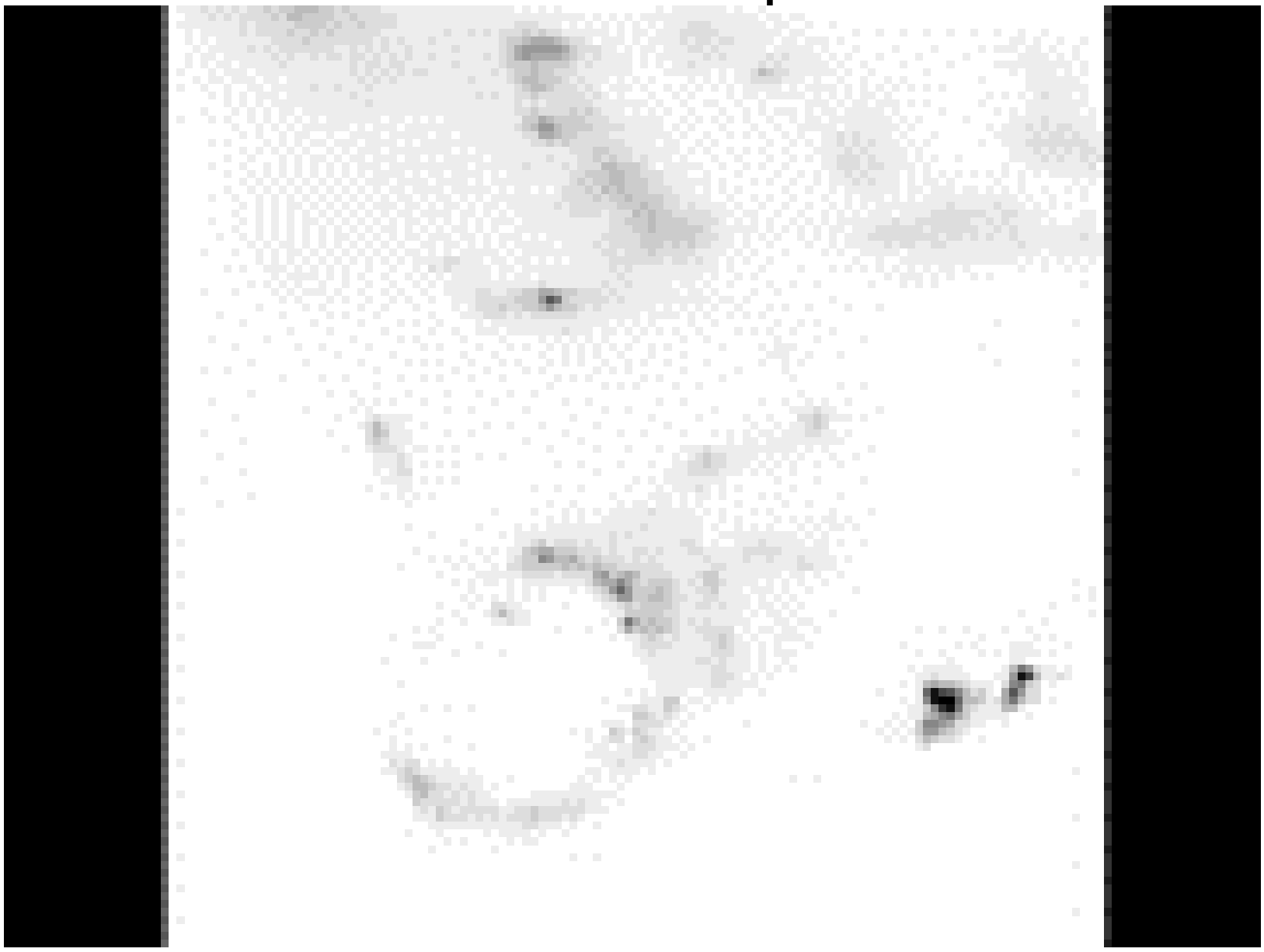


The pacemaker cells are located in the dorsal-right side of the inflow ring.

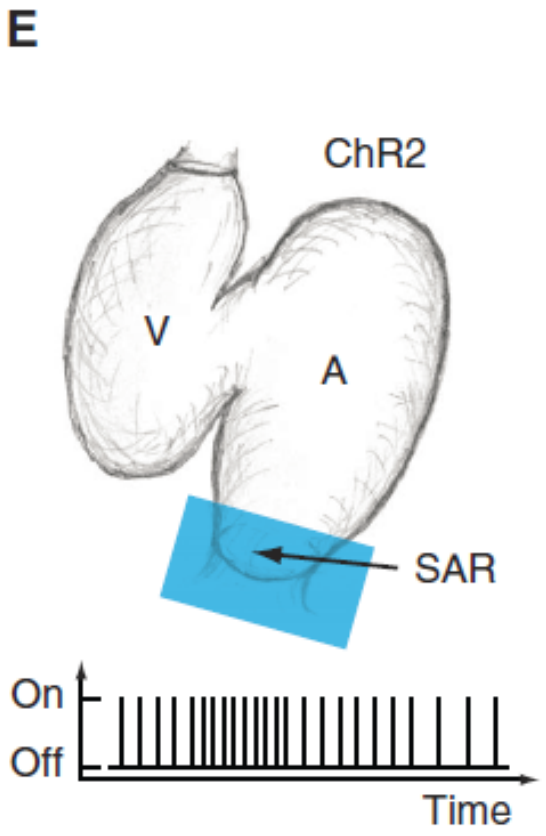
Fine tune the localization of pace maker cell

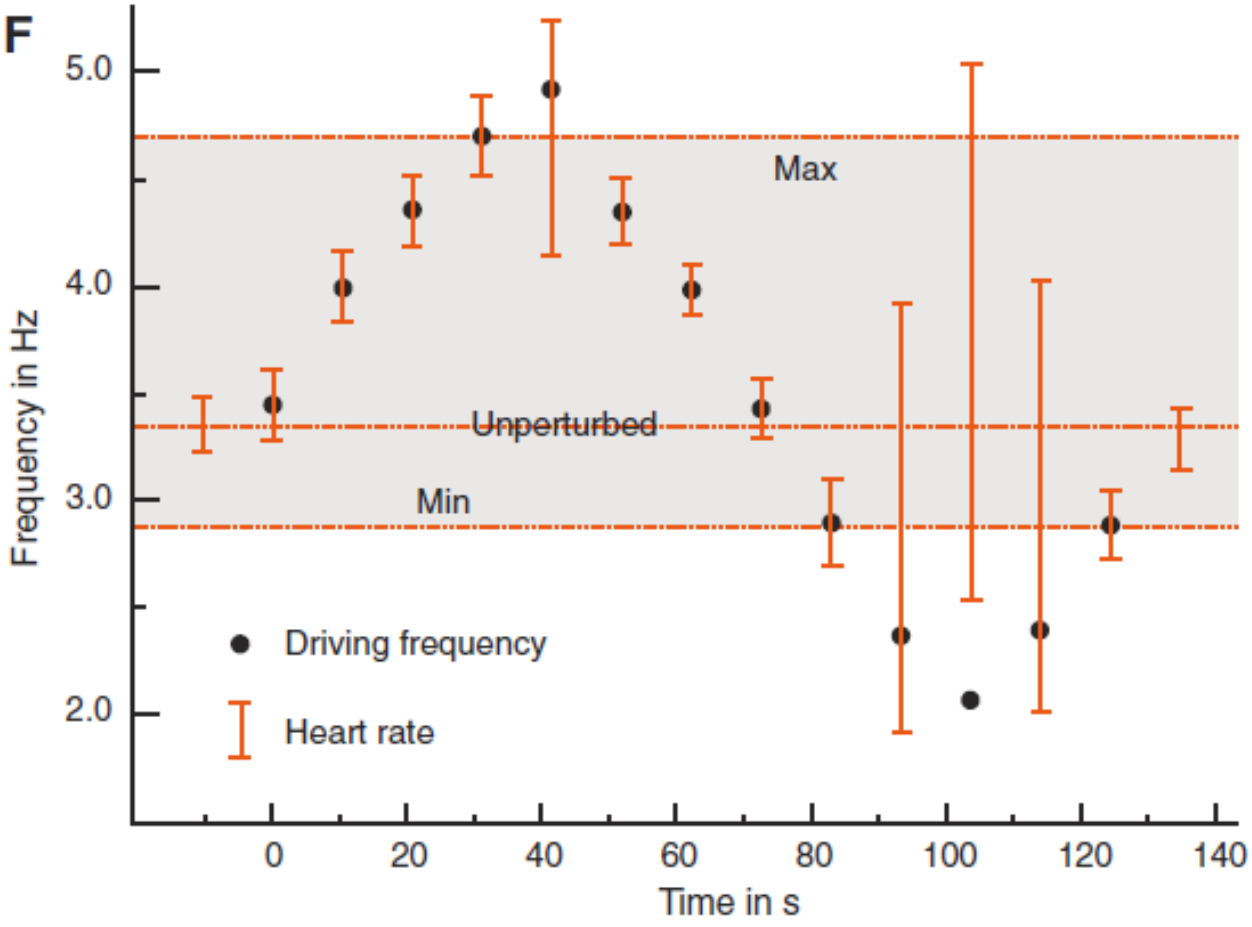


Using light pulse and ChR2 (depolarization) to control heart rate at pace marker



Using light pulse and ChR2 (depolarization) to control heart rate at pace marker





6. Example 2: persistent modulation of transcription

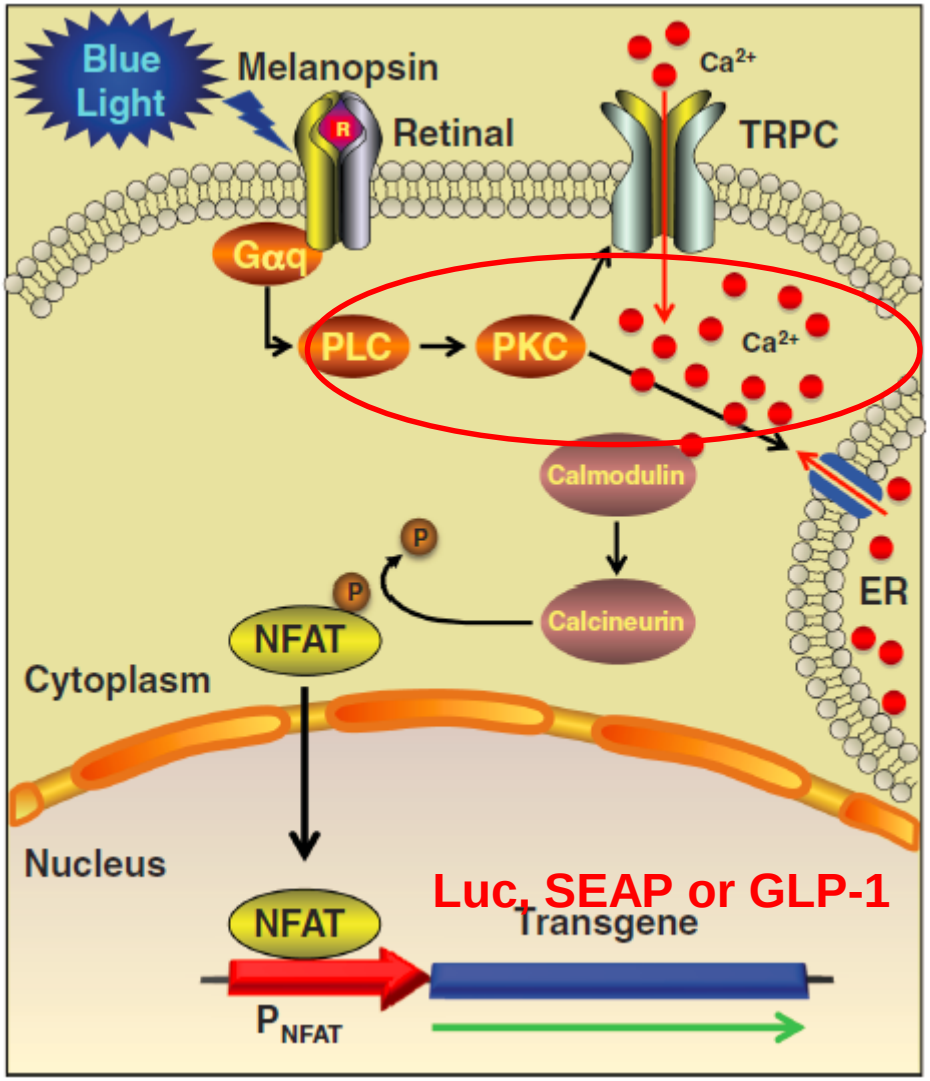
A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice

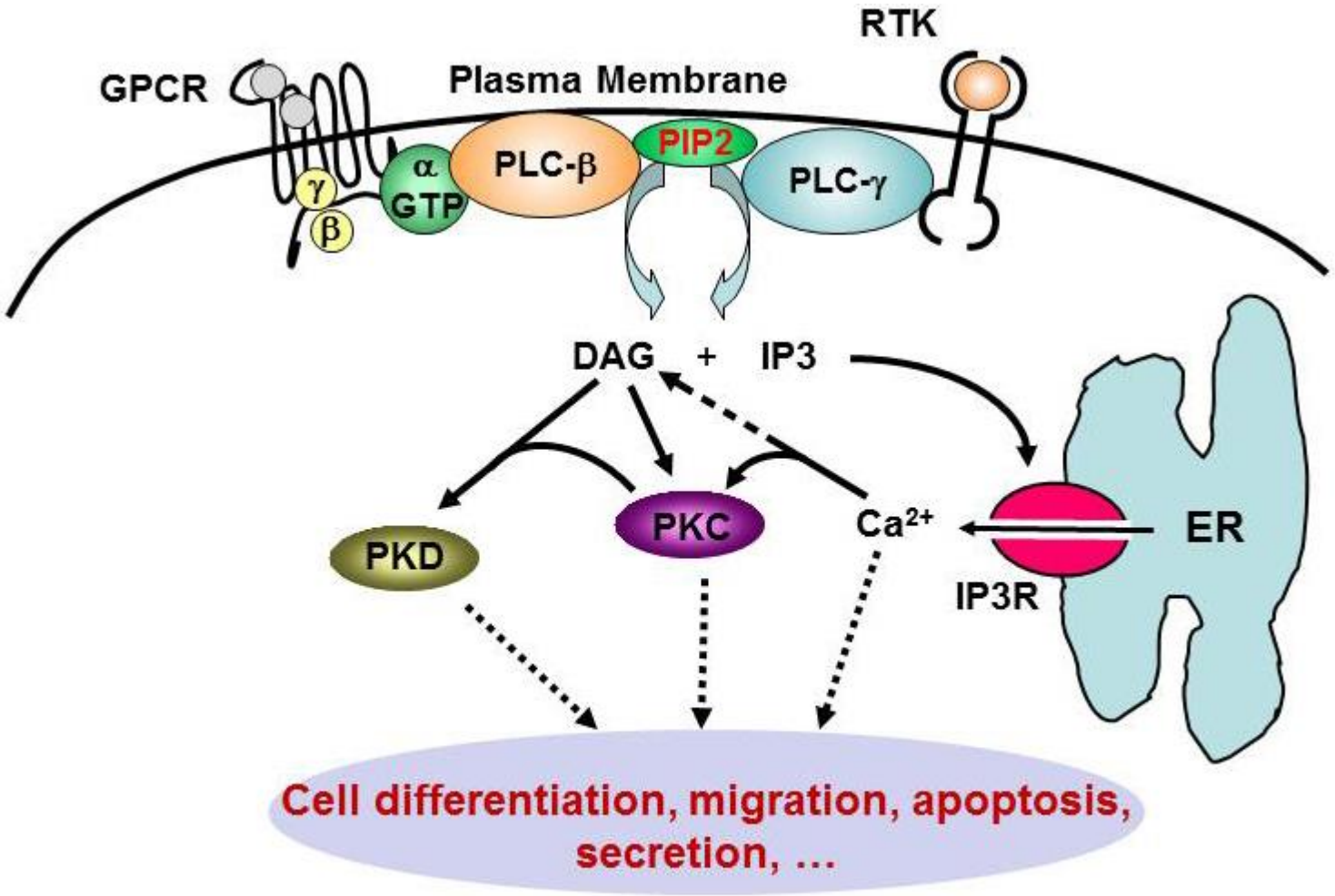
Haifeng Ye,[1] Marie Daoud-El Baba,[2] Ren-Wang Peng,[1] Martin Fussenegger[1,3]*

Science 2011, vol 332,1565

Activation of Melanopsin and transcriptional activity by NFAT could be used to modulate endocrine function in animal.

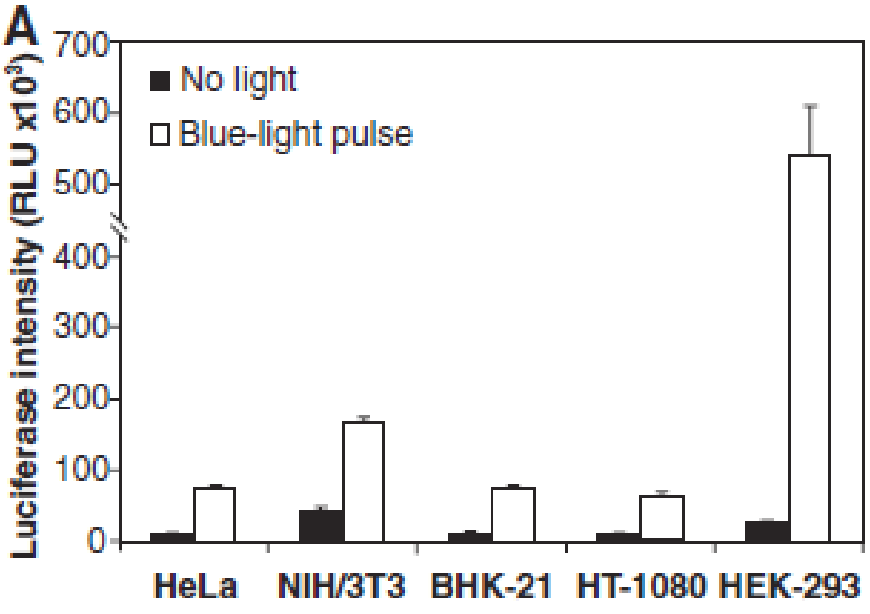
Molecular basis of synthetic biology approach to modulate gene expression with blue light

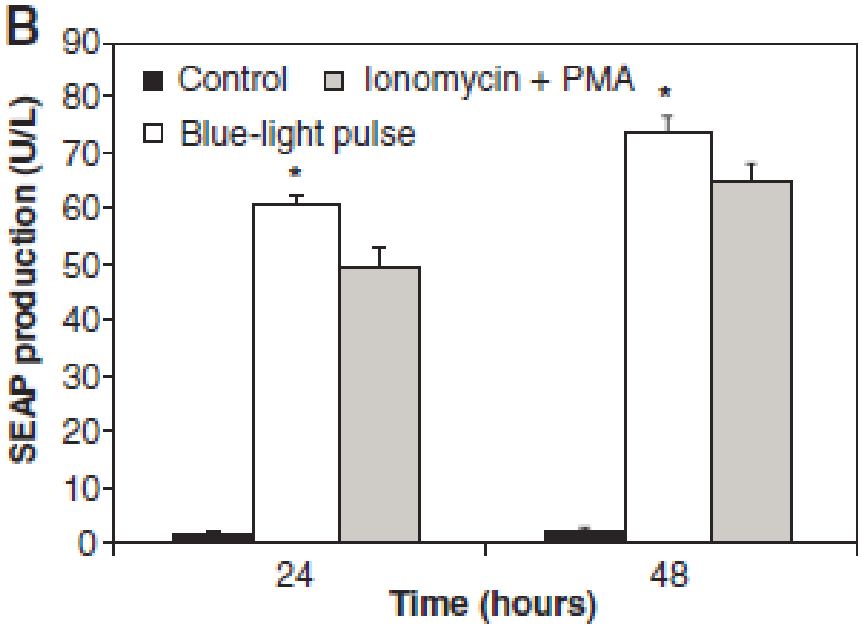




Signaling pathways of IP3 and DAG generation

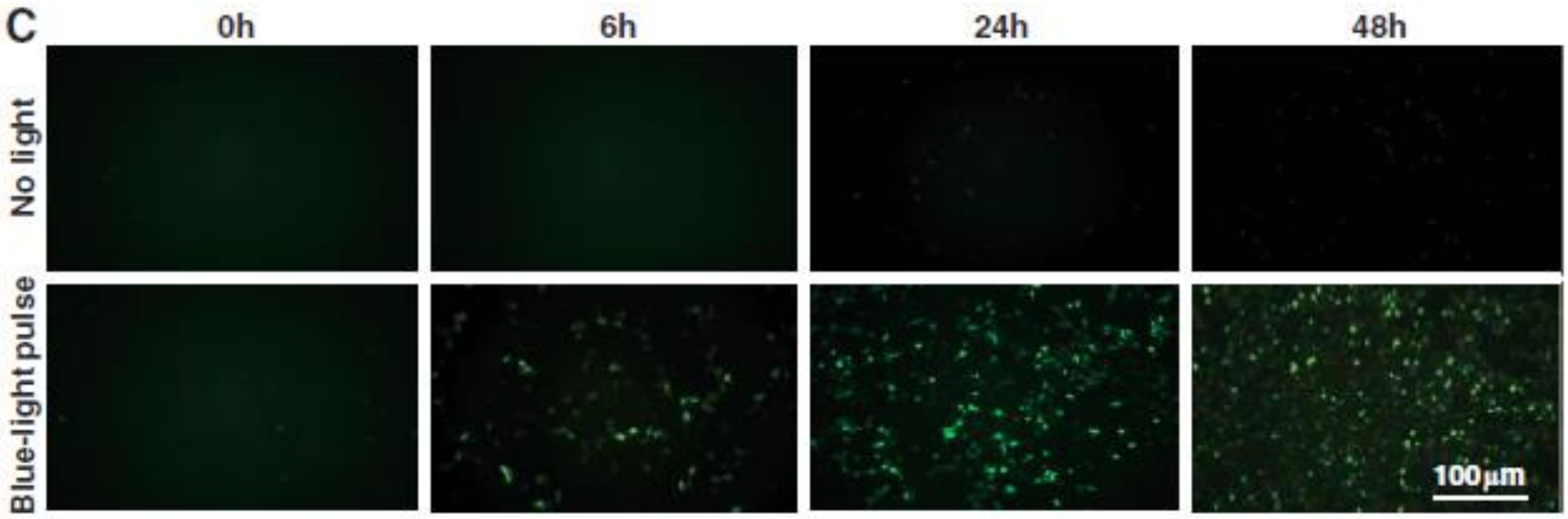
In vitro validation of the light induction system with luciferase activities



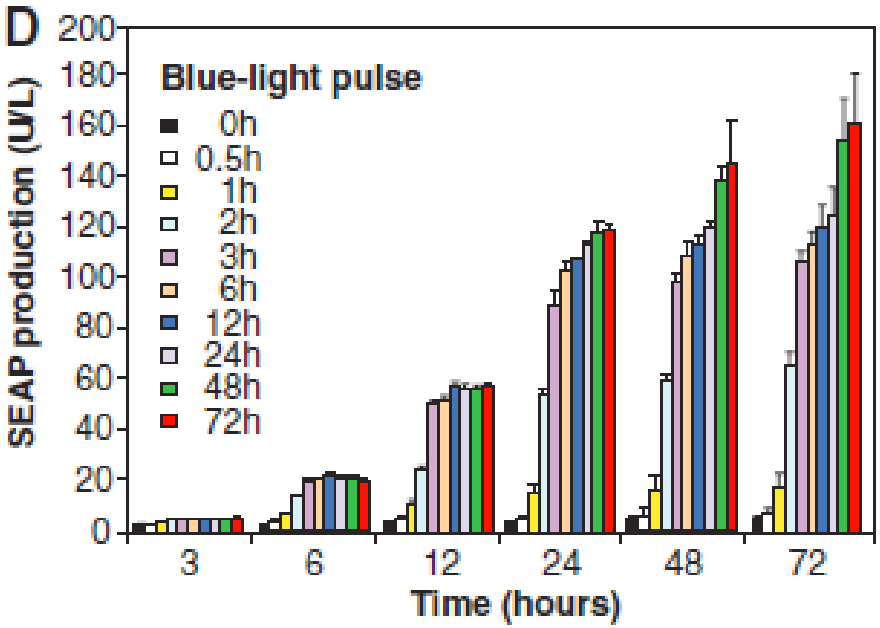


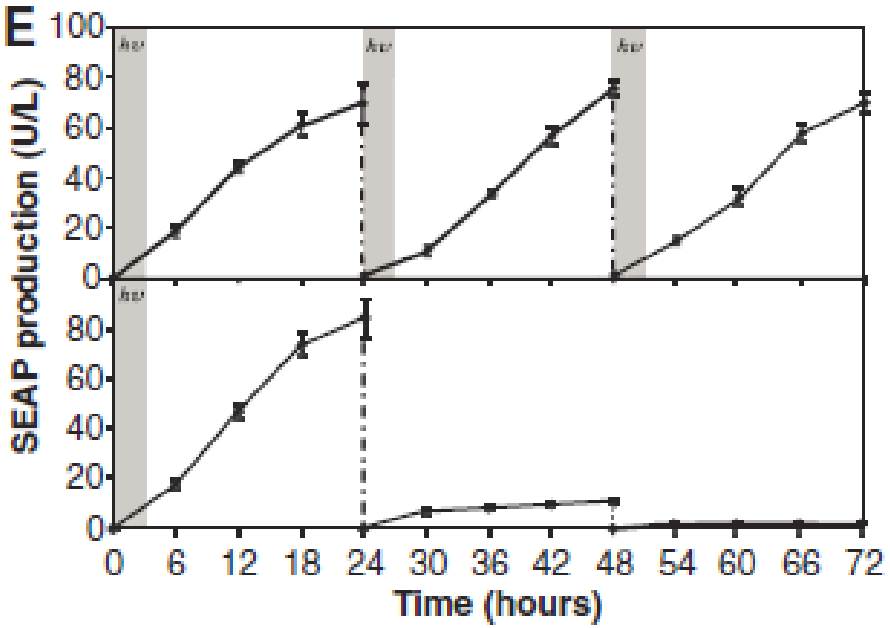
Ionomycin: raise $[Ca^{++}]_{cytosol}$
PMA: activate PKC

In vitro validation of the light induction system with YFP in HEK293 cell



Quantification of the dynamics of light induction system with SEAP in HEK293 cell





Subcutaneous implantation of engineered HEK expression GLP-1 could help alleviate blood glucose with light induction