13. Photo-control of biological function

Optogenetics

Optogenetics

The genesis

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nature neuroscience — TECHNICAL REPORT

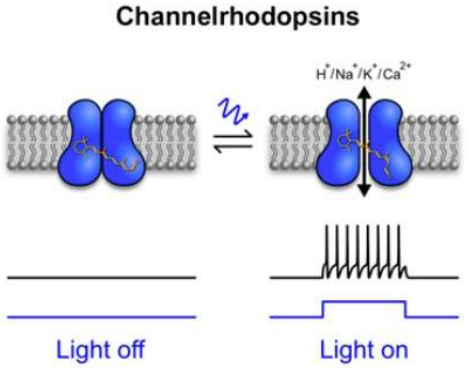
Millisecond-timescale, genetically targeted optical control of neural activity

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

Nature Neurosci. **2005**, 1263

Optogenetics

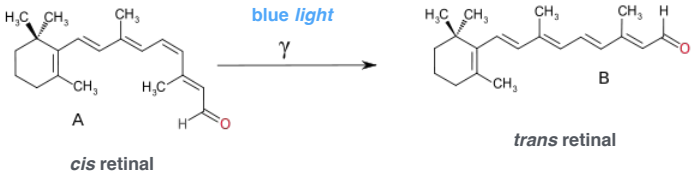
Microbial **opsins** are **Light-gated ion channels**



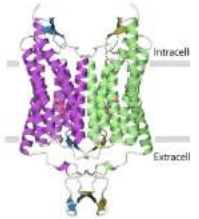
Knoepfel *Progr. Brain Res.* **2012**, *196*, 1-28

Optogenetics

Channelrhodopsins (ChR)

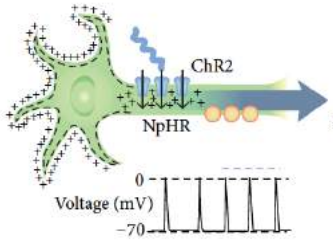


cis **retinal** ***trans*** **retinal**



Nureki, Deisseroth *Nature* **2012**, *482*, 7385

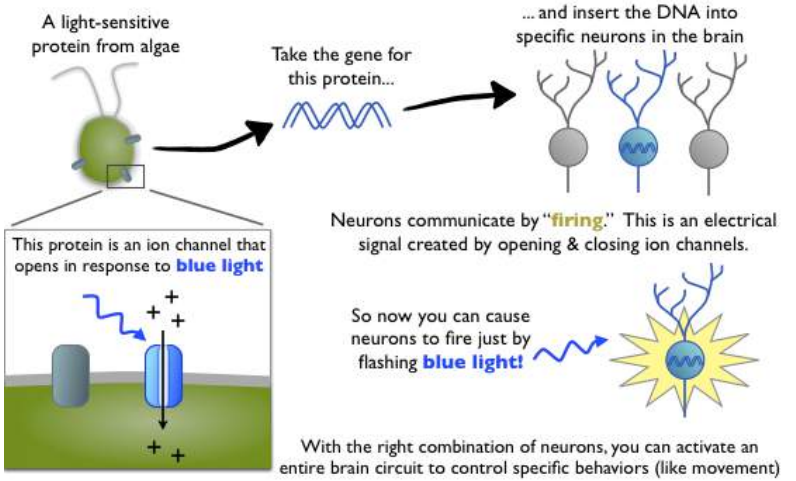
Optogenetics



ChR is a light-gated cation channel derived from algae that conducts cations, including sodium ions, in a light-dependent manner. Because the inward flow of sodium ions changes the electrochemical gradient and triggers neuron firing, neurons expressing ChRs can be optically controlled with high temporal precision within systems as complex as freely moving mammals.

Ishihara *Int. J. Photoenergy* **2014**, 1

Optogenetics



Neurobyn **2014**, 1

Optogenetics

Seminal paper

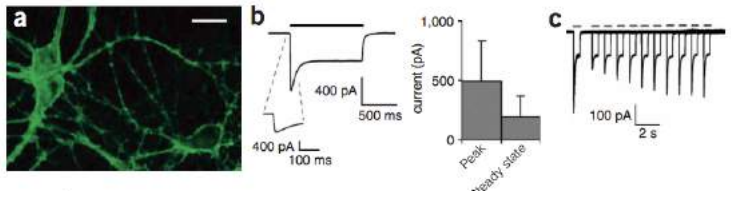
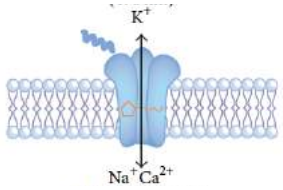


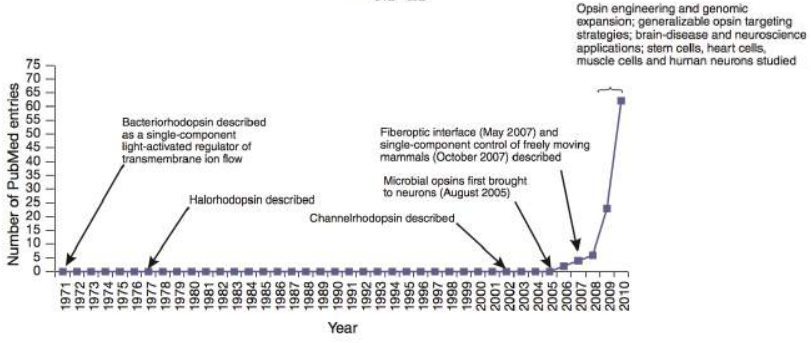
Figure 1 ChR2 enables light-driven neuron spiking. (a) Hippocampal neurons expressing ChR2-YFP (scale bar 30 μm). (b) Left, inward current in voltage-clamped neuron evoked by 1 s of GFP-wavelength light (indicated by black bar); right, population data (right; mean ± s.d. plotted throughout; $n = 18$). Inset, expanded initial phase of the current transient. (c) Ten overlaid

Nature Neurosci. **2005**, 1263

Optogenetics

The genesis





Deisseroth *Nature Meth.* **2011**, *8*, 27

Optogenetics

A comprehensive definition of optogenetics

The term "**optogenetics**" is a bit of a misnomer as it does not involve any interaction between light and the genome. But coming up with this label was definitely a smart move judging from how quickly it was adopted by the research community. The term is now firmly established both in the scientific literature and in the popular media, but its usage has not yet crystallized around a common acceptation.

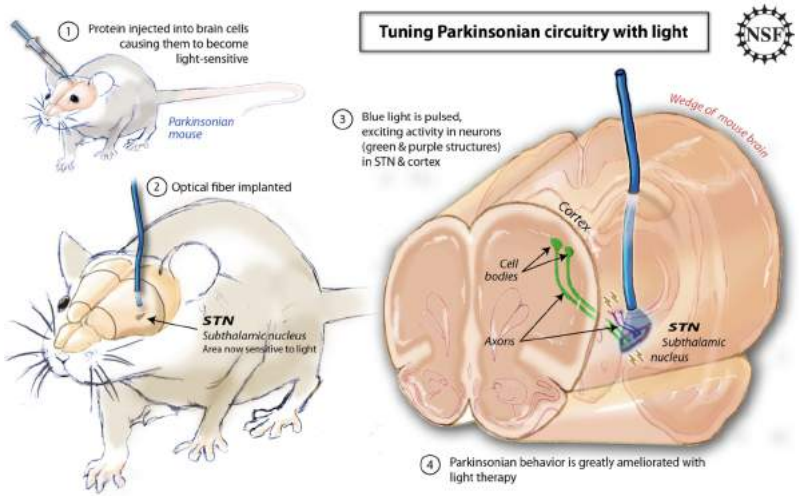
Etymologically, "**optogenetics**" simply refers to the **combination of optical and genetic approaches** and implicitly designates all strategies using genetically addressable light-sensitive tools to study biological systems. As a consequence, the term should seize on 20 years of utilization of FPs, including for simply labeling cells and proteins. More reasonably, optogenetics can designate the use of genetically addressable photosensitive elements not as inert dyes but as environmentally sensitive fluorophores (in which light emission is affected by identified factors) and/or as active agents (which can transduce optical energy into biophysical effects). This definition encompasses both monitoring and control strategies. We believe that a narrower acceptation of the word is unjustified."Optogenetics" should encompass the use of both control tools and reporters.

Which control tools and which reporters should be included in this definition? In the broadest sense, **optogenetic tools do not need to be fully genetically encoded but only genetically "addressable.**" This means that proteins requiring an exogenous cofactor to function can also be considered as "optogenetic" as long as their expression can be restricted to certain groups of cells. This definition includes a range of photochemical approaches where proteins are engineered to bind to a given photochromic ligand. It also encompasses the use of photoreceptor proteins in organisms lacking their specific chromophore. In such cases, the chromo- phore molecule has to be added exogenously (e.g., retinal in invertebrates for channelrhodopsin-based applications and bilin in nonplant organisms for phytochrome-based applications).

Overall, we wish to conclude that a comprehensive definition of optogenetics might be the following: optogenetics is the combination of optical and molecular strategies to monitor and control designated molecular and cellular activities in living tissues and cells using genetically addressable photosensitive tools.

Knoepfel *Progr. Brain Res.* **2012**, *196*, 1-28

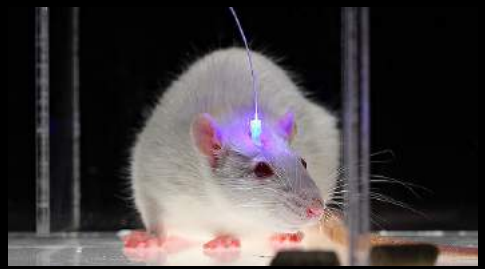
Optogenetics



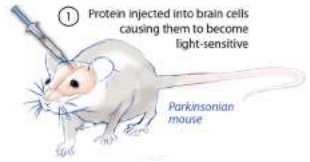
NSF *Press release, Apr. 16*, **2009**

13. Photo-control of biological function

Optogenetics



Optogenetics



How do you inject a gene in a living organism?

CRE/LOX system

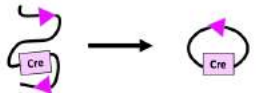
The Cre/lox system is one of the most powerful tools in the mouse geneticist's toolbox. It enables them to generate tissue-specific and inducible knockouts and thereby have exquisite control over the **location** and **timing** of gene expression – important stuff when deleting a certain gene everywhere or during development leads to an embryonic lethal phenotype. And, it can be used to turn transgene expression on or off, track individual cells or cell lineages, generate inversions or translocations, and report gene expression.

Cre/lox for dummies, Jackson Lab, **2011**

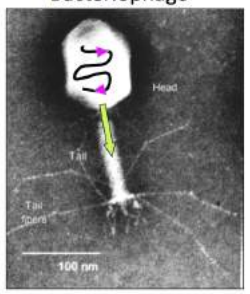
A Revolutionary Genetic Tool

Cre-lox system

- Natural part of P1 bacteriophage viral life cycle
- Viral DNA injected into bacteria, circularized using Cre-*lox*, and replicated for development of new viruses



Bacteriophage



Site-specific recombinase technology

A single enzyme, **Cre recombinase**, recombines a pair of short target sequences called the *Lox* sequences. This system can be implemented without inserting any extra supporting proteins or sequences. The Cre enzyme and the original *Lox* site called the ***LoxP* sequence** are derived from bacteriophage P1.

The Jackson Laboratory

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Other Channel Opsins

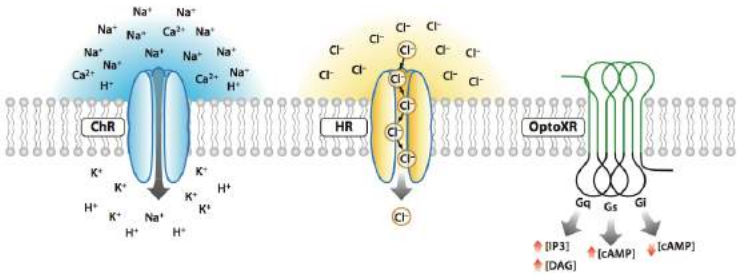
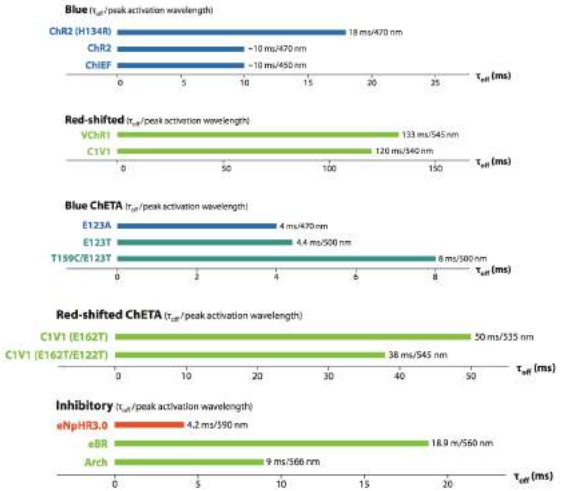


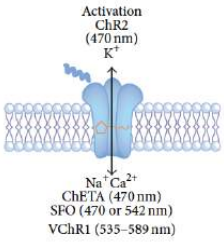
Figure 1

Optogenetic tool families. Channelrhodopsins conduct cations and depolarize neurons upon illumination (*left*). Halorhodopsins conduct chloride ions into the cytoplasm upon yellow light illumination (*center*). OptoXRs are rhodopsin-GPCR (G protein–coupled receptor) chimeras that respond to green (500 nm) light with activation of the biological functions dictated by the intracellular loops used in the hybrid (*right*).

Deisseroth *Annu. Rev. Neurosci.* **2011**, 389-412

Kinetic properties of optogenetic tools





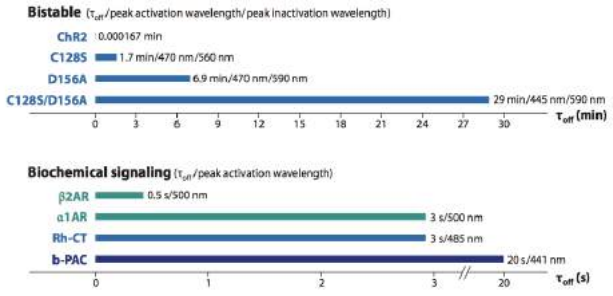
timescales of milliseconds

Deisseroth *Annu. Rev. Neurosci.* **2011**, 389-412

Optogenetics

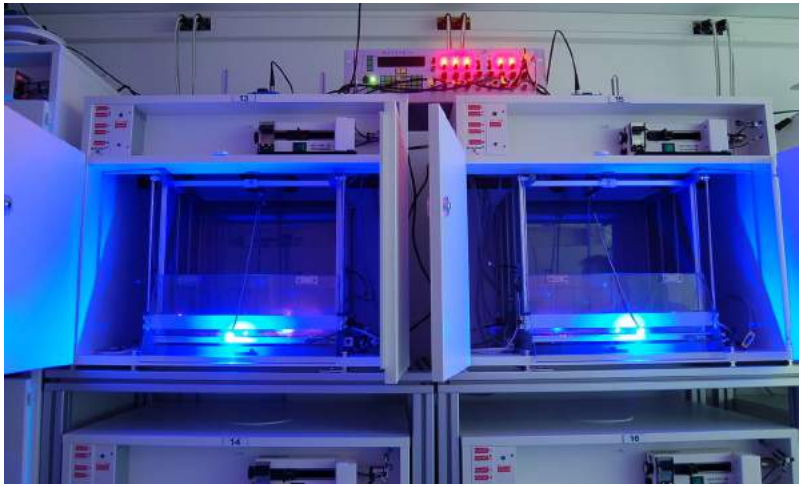
Kinetic properties of biochemical interactions

timescales of minutes



Deisseroth *Annu. Rev. Neurosci.* **2011**, 389-412

Optogenetics



Cages for rat equipped of optogenetics leds commutators which permit in vivo to study animal behavior during optogenetics' stimulations. (wikipedia, dec. 2015)