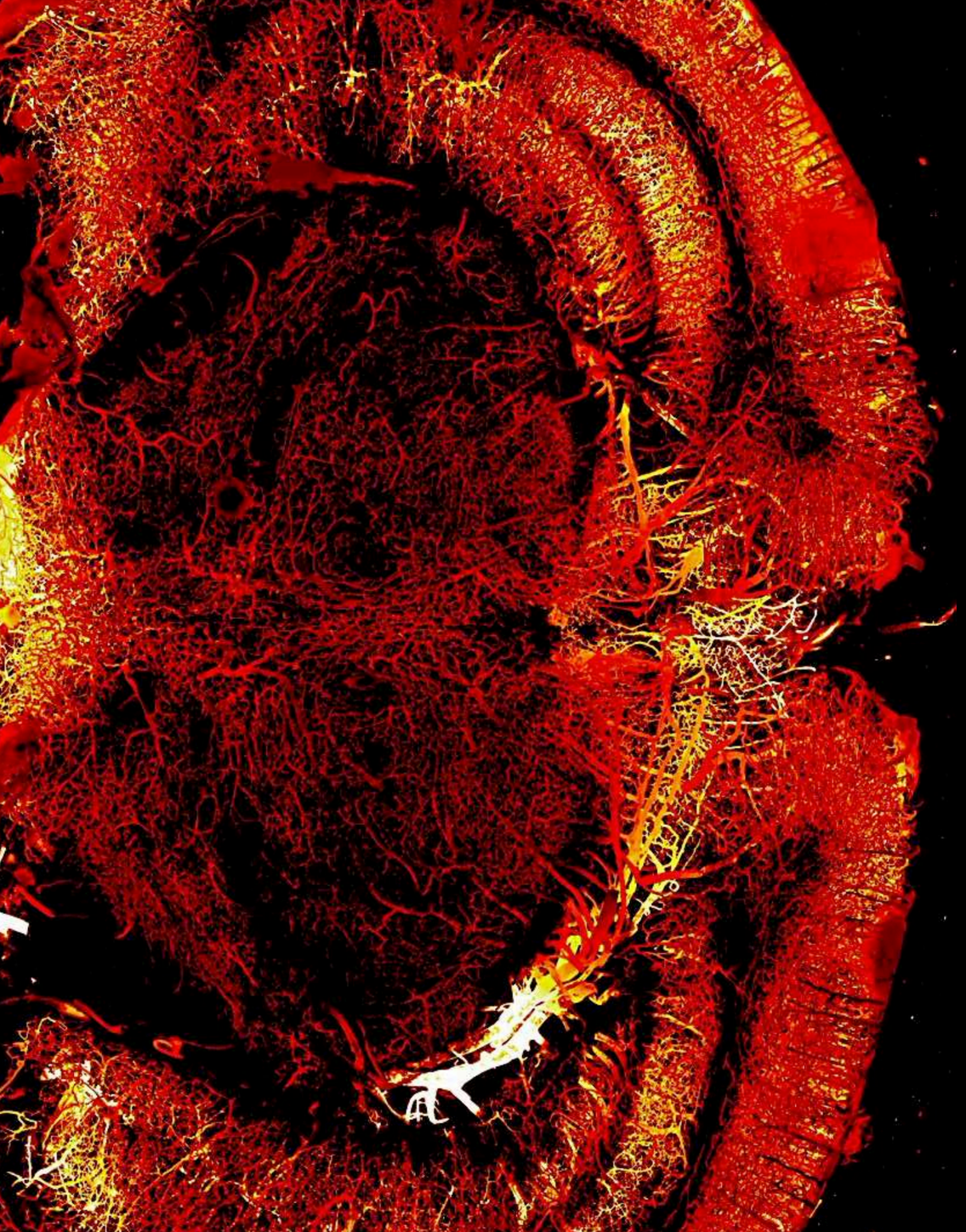
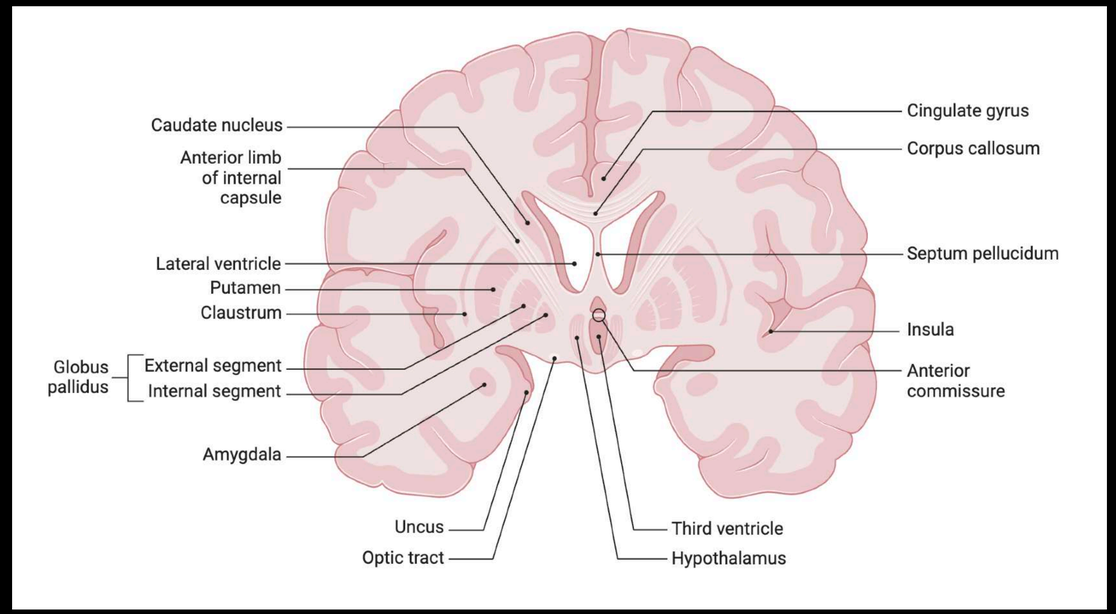
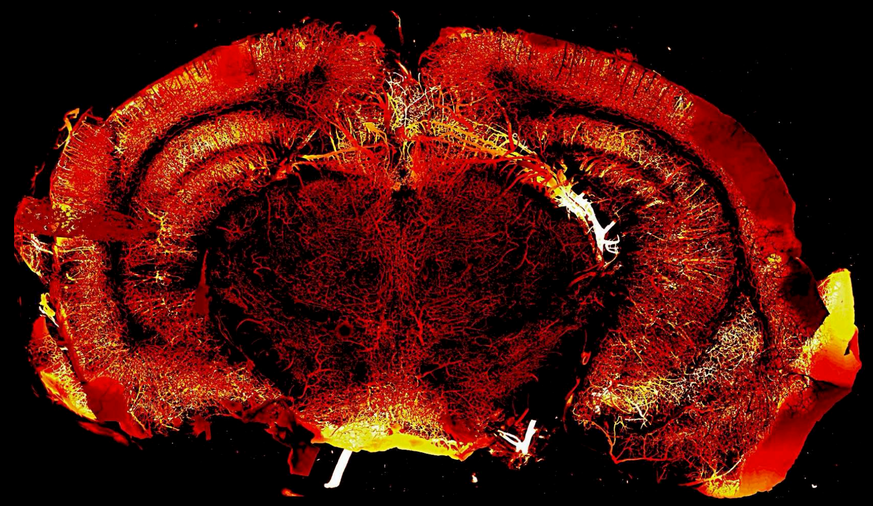


OPTOGENETICS

control Nature with light

Steve Knutson December 12, 2023



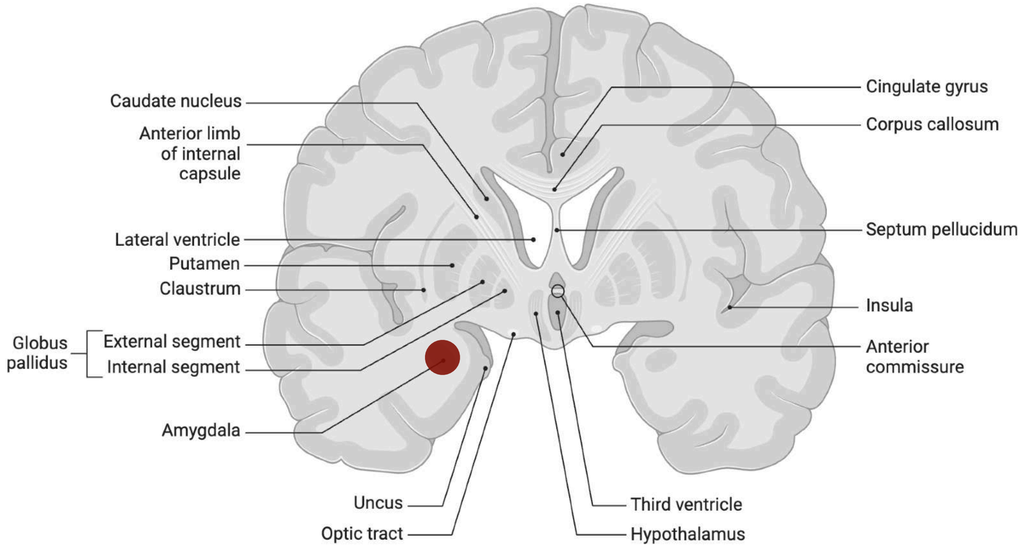


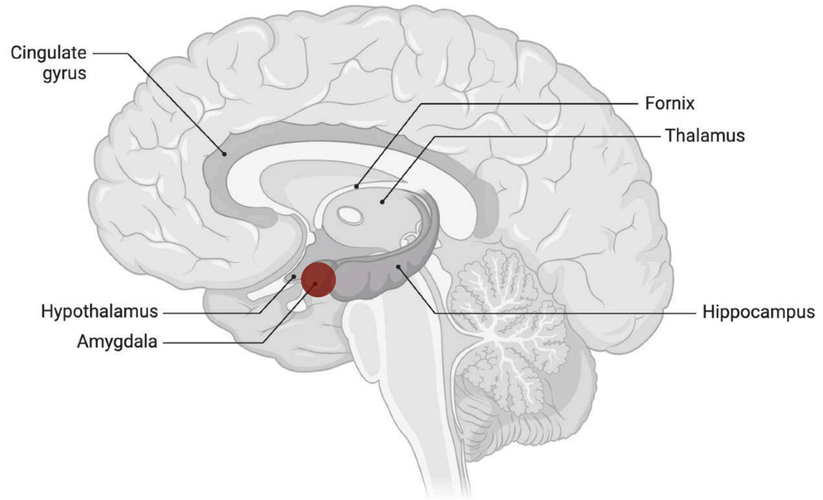
Francis Crick, 1977

"…the major challenge facing neuroscience is the need to **control one type of cell in the brain** while leaving others unaltered. Electrical stimuli cannot meet this challenge - they activate all circuitry without distinguishing between different cell types, and their signals cannot turn off neurons with precision. Drugs are not specific enough either, and they are much slower than the natural operating speed of the brain.…

Light activation may be the only answer."

2017



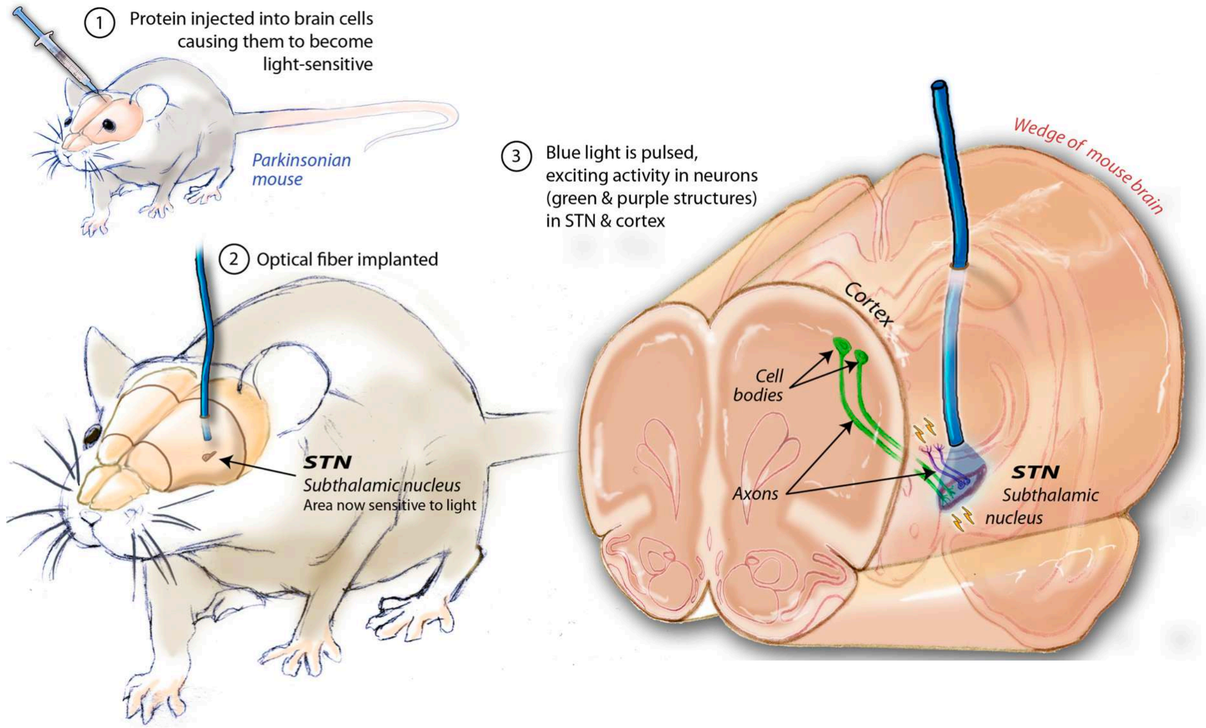


Yale School of Medicine

*where is **violence** located in the brain?*

U.S. Department *of* Defense

2017


1 Protein injected into brain cells causing them to become light-sensitive
Parkinsonian mouse
2 Optical fiber implanted
STN
Subthalamic nucleus
Area now sensitive to light
3 Blue light is pulsed, exciting activity in neurons (green & purple structures) in STN & cortex
Wedge of mouse brain
Cortex
Cell bodies
Axons
STN
Subthalamic nucleus

"Jonesy"

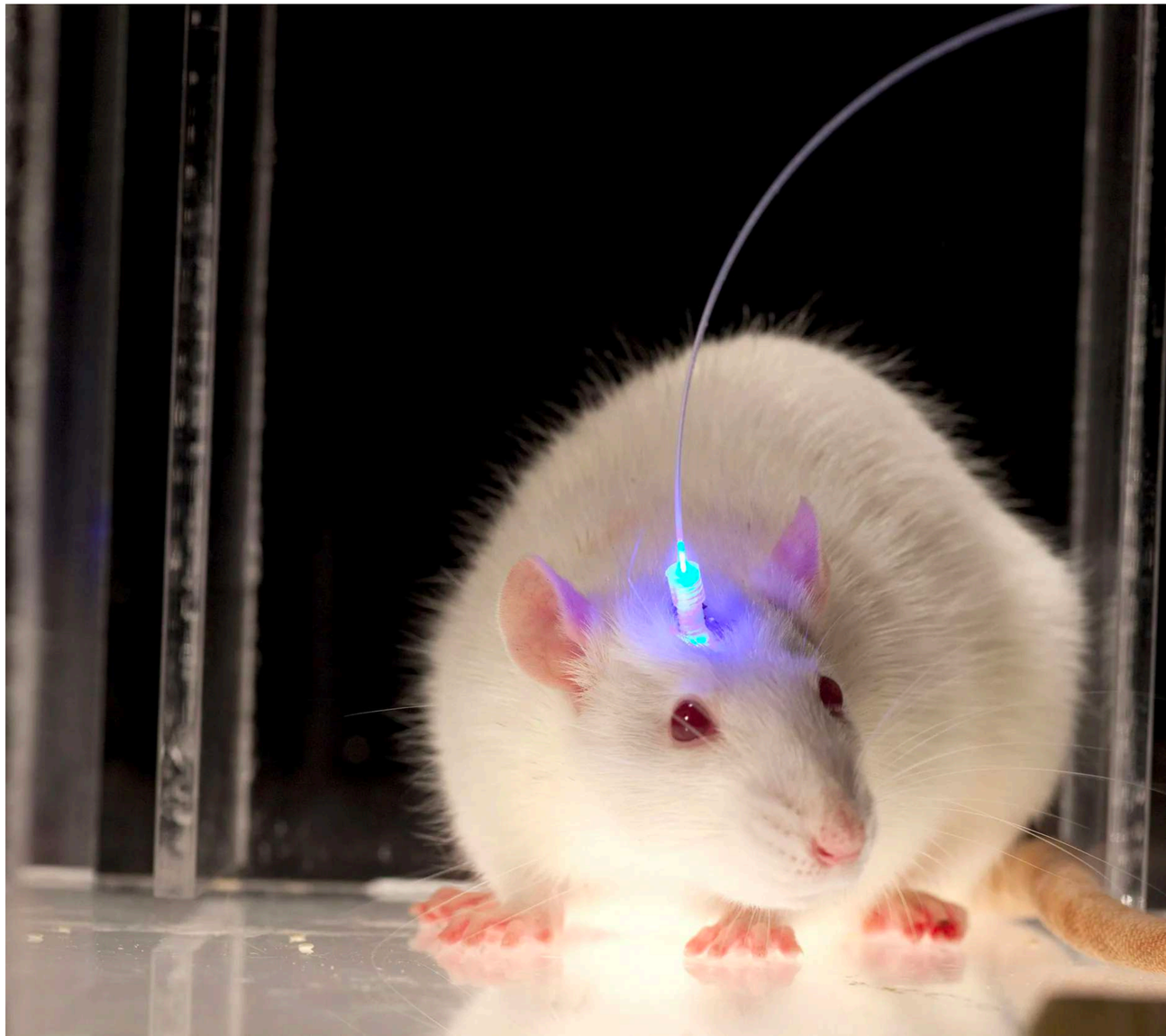
Optogenetics

nature

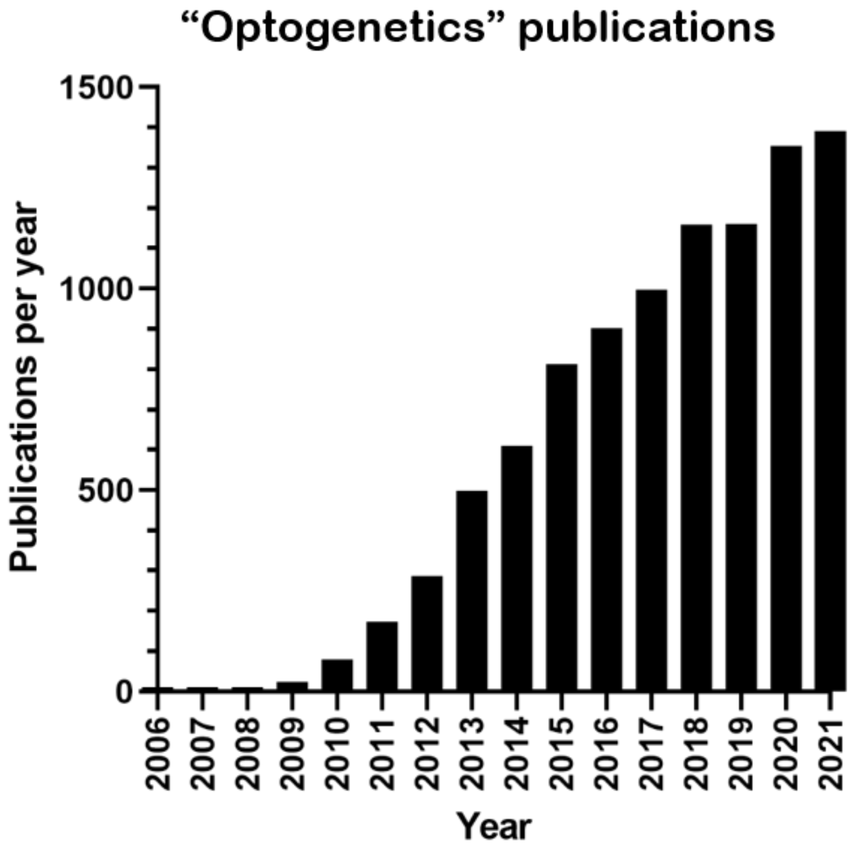
Method of the Year (2010)

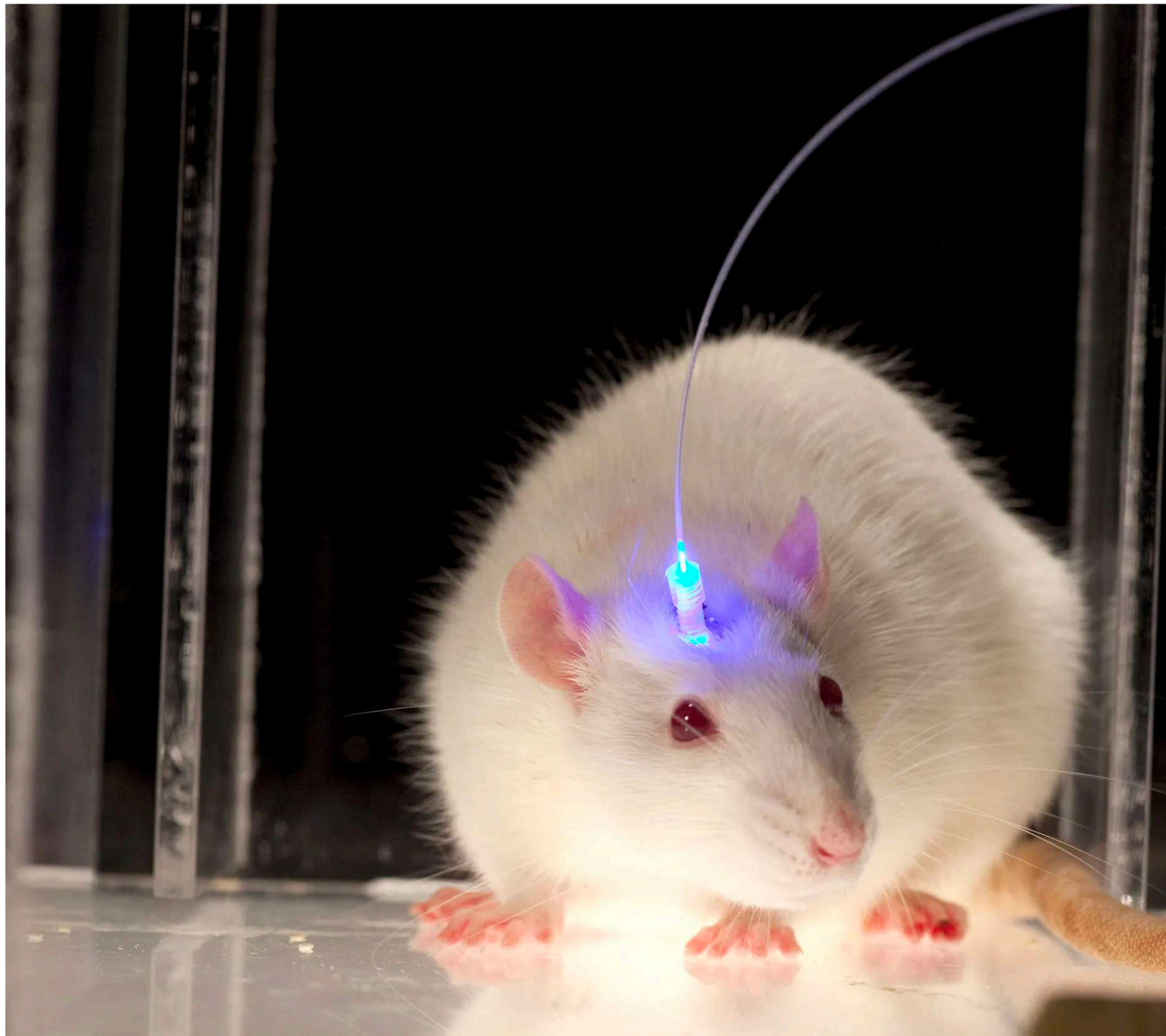
Science

Biggest Breakthrough (2013)



Optogenetics





Optogenetics

TIME
HEALTH • THE BRAIN

Noninvasive Brain Control Is Real — and That's Good

THE NEW YORKER

LIGHTING THE BRAIN

TIME
HEALTH • THE BRAIN

Erasing Bad Memories May Soon Be Possible

The NEW ENGLAND JOURNAL of MEDICINE

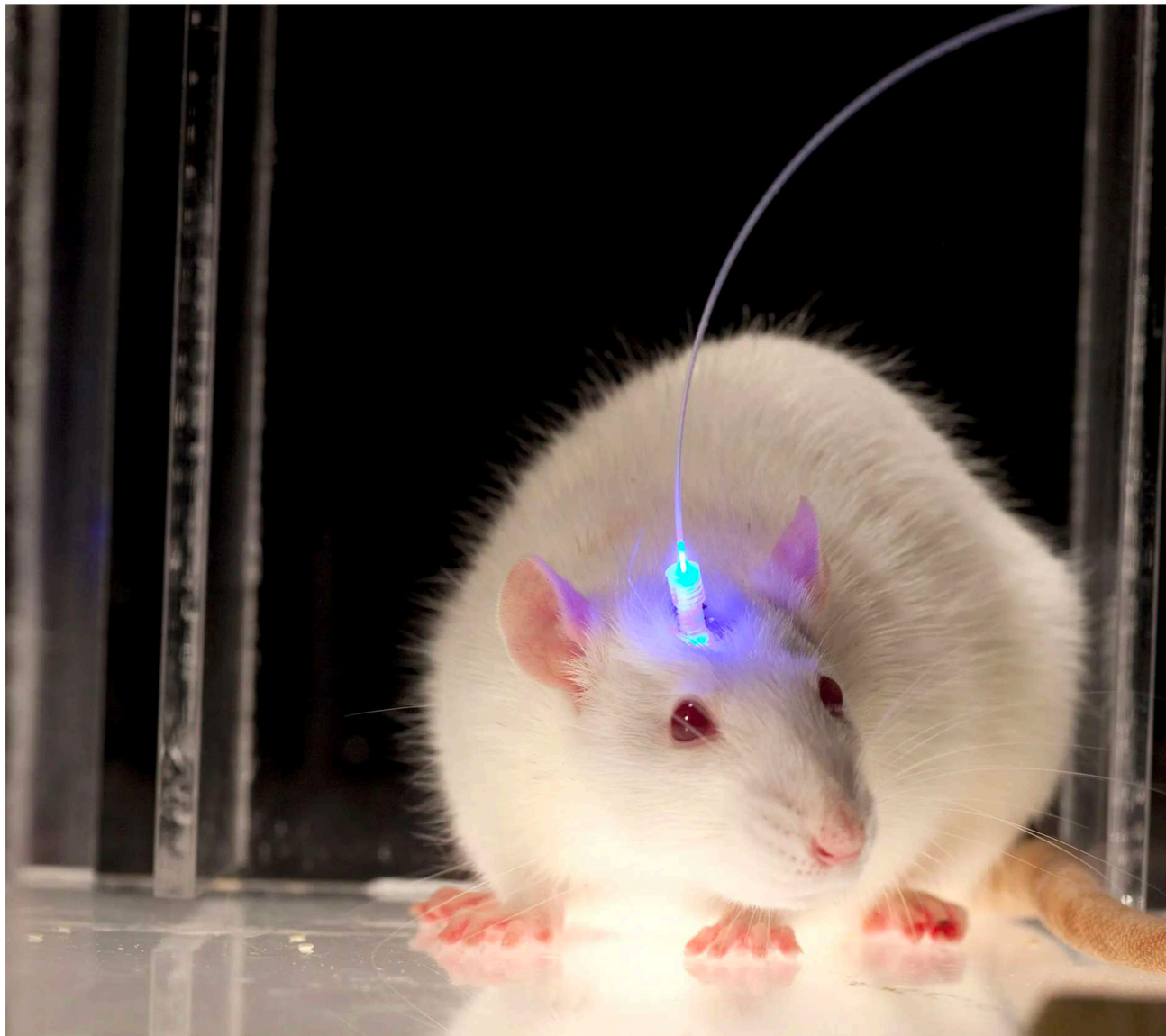
Optogenetics — The Might of Light

The New York Times

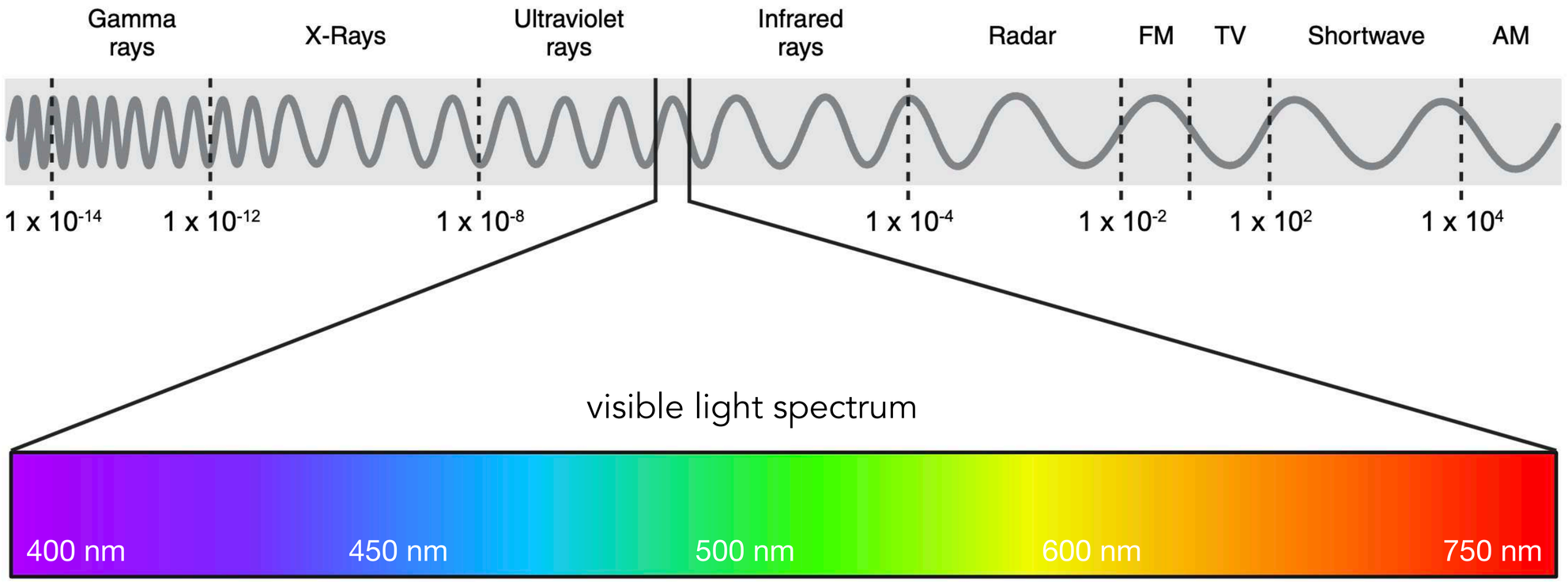
Brain Control in a Flash of Light

Science

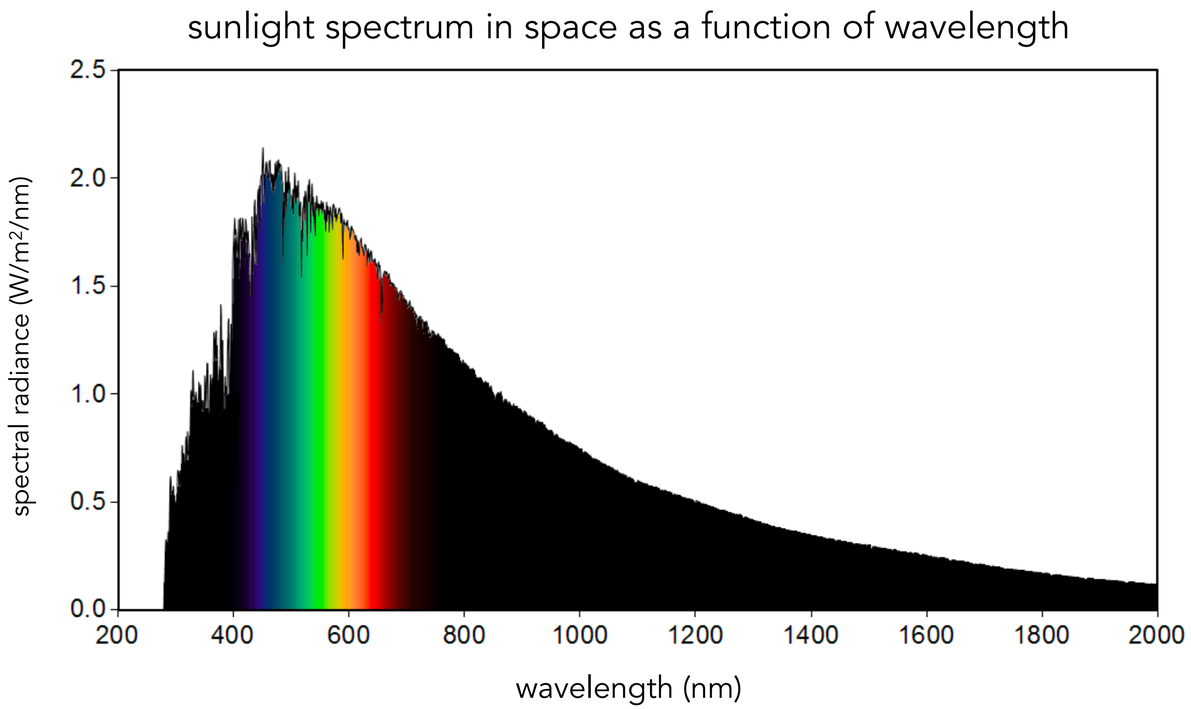
Efforts to control monkey brains get a boost

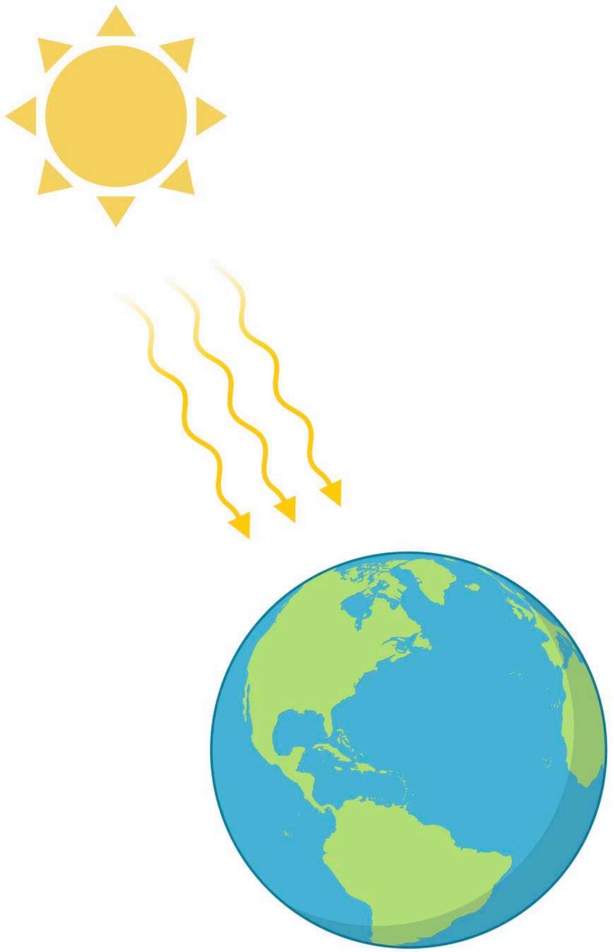


Sources of light/energy on Earth

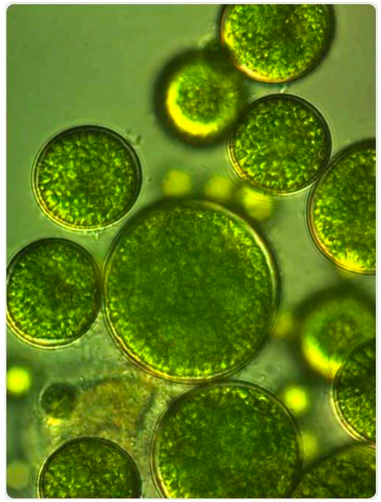
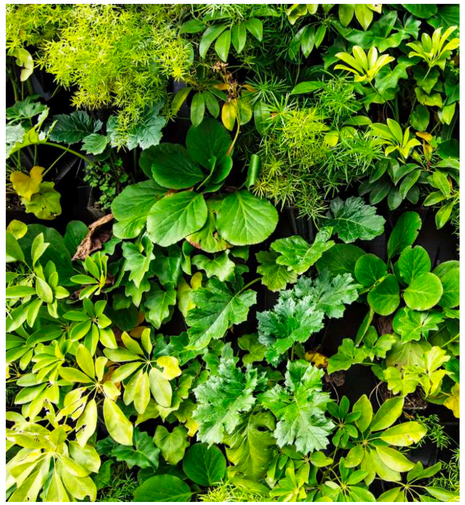


Sources of light/energy on Earth



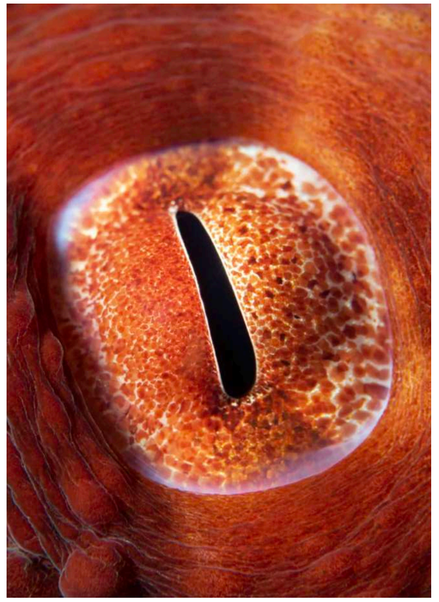


The ability to “sense” visible light evolved everywhere

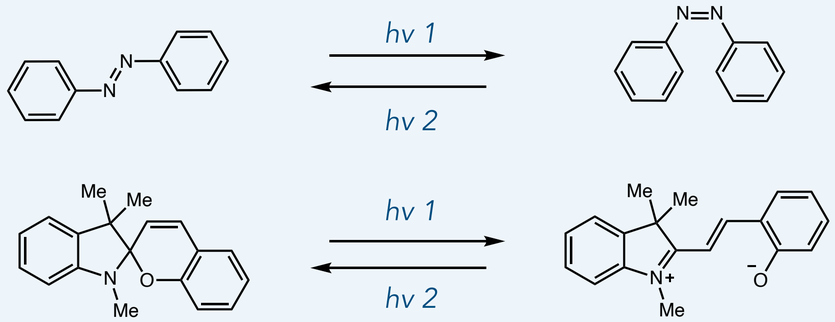




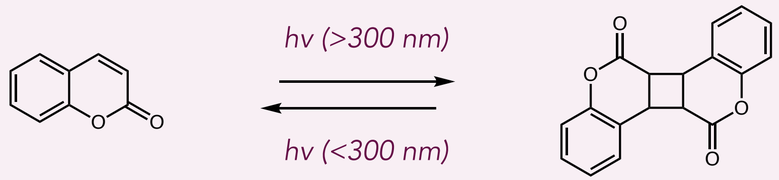


Organic photochemistry

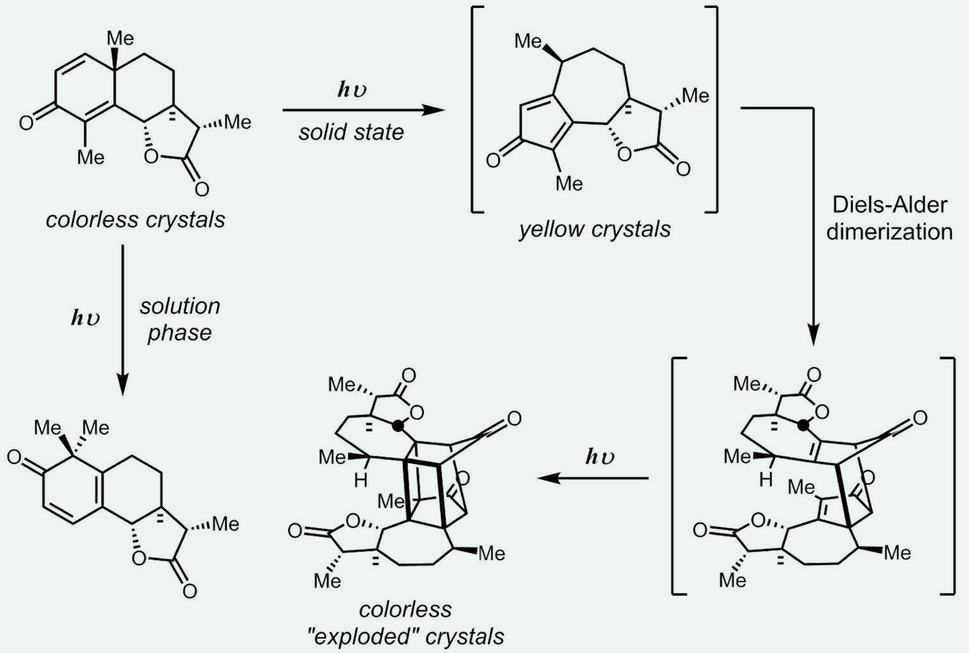
photoisomerization



photodimerization

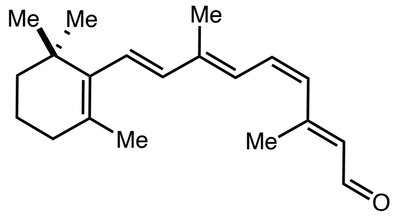


photoconversion of Santonin (1834)

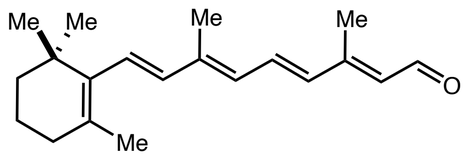


Organic photochemistry + protein = **light biosensor**

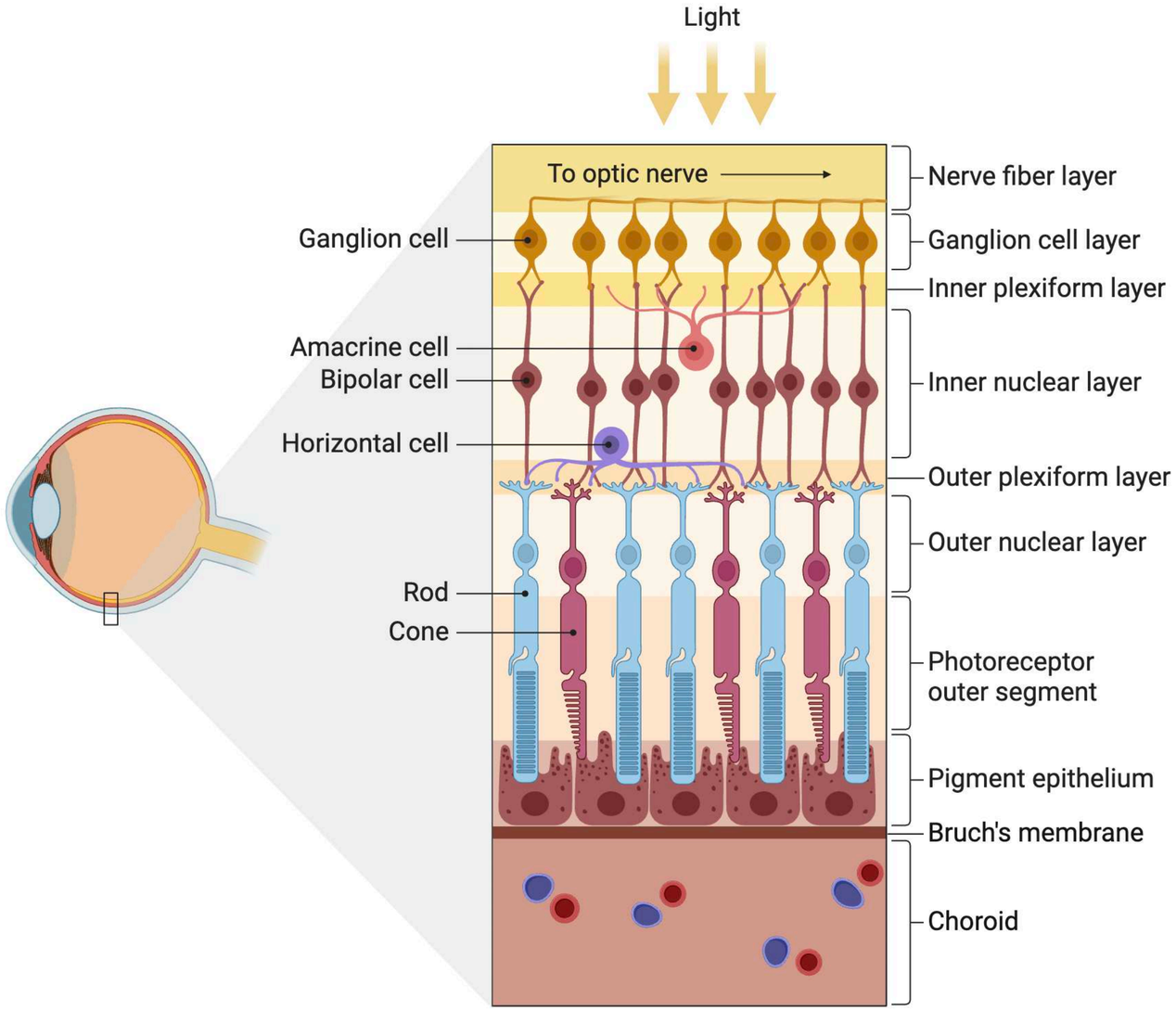
retinal (11-cis)



hv
~400-450nm

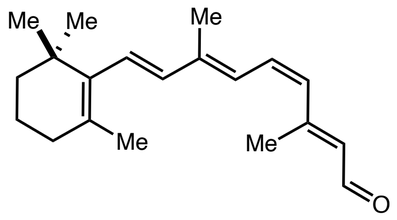


retinal (all trans)

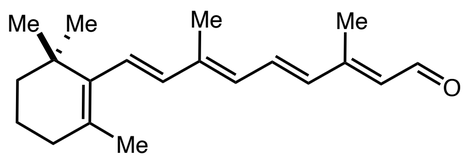


Organic photochemistry + protein = light biosensor

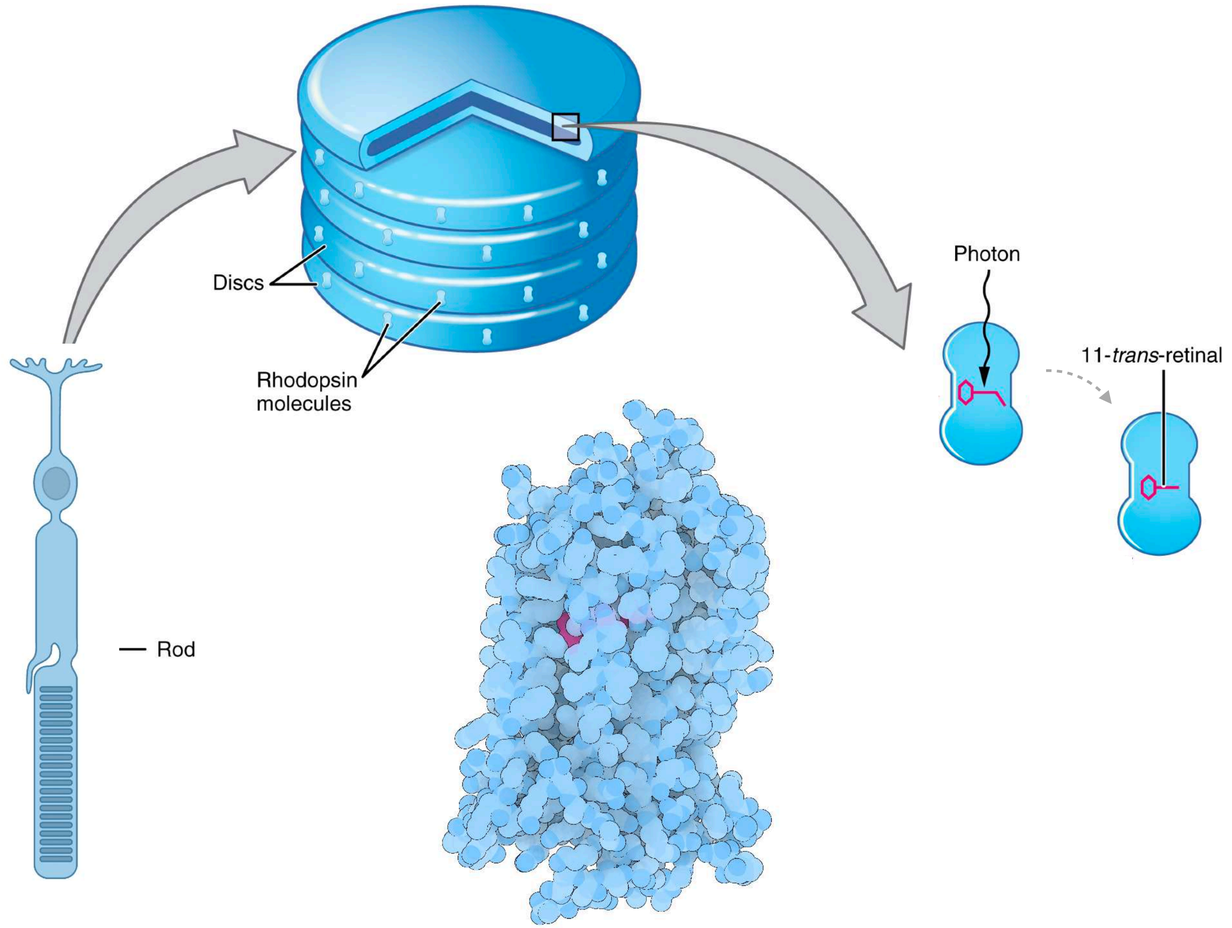
retinal (11-cis)



hv
~400-450nm

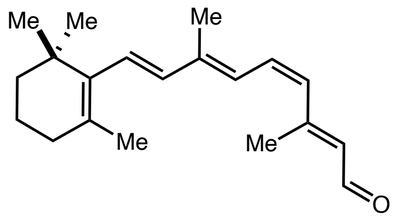


retinal (all trans)

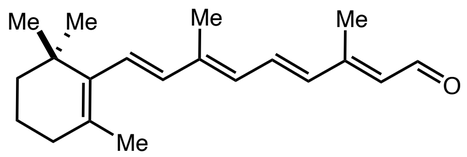


Organic photochemistry + protein = light **biosensor**

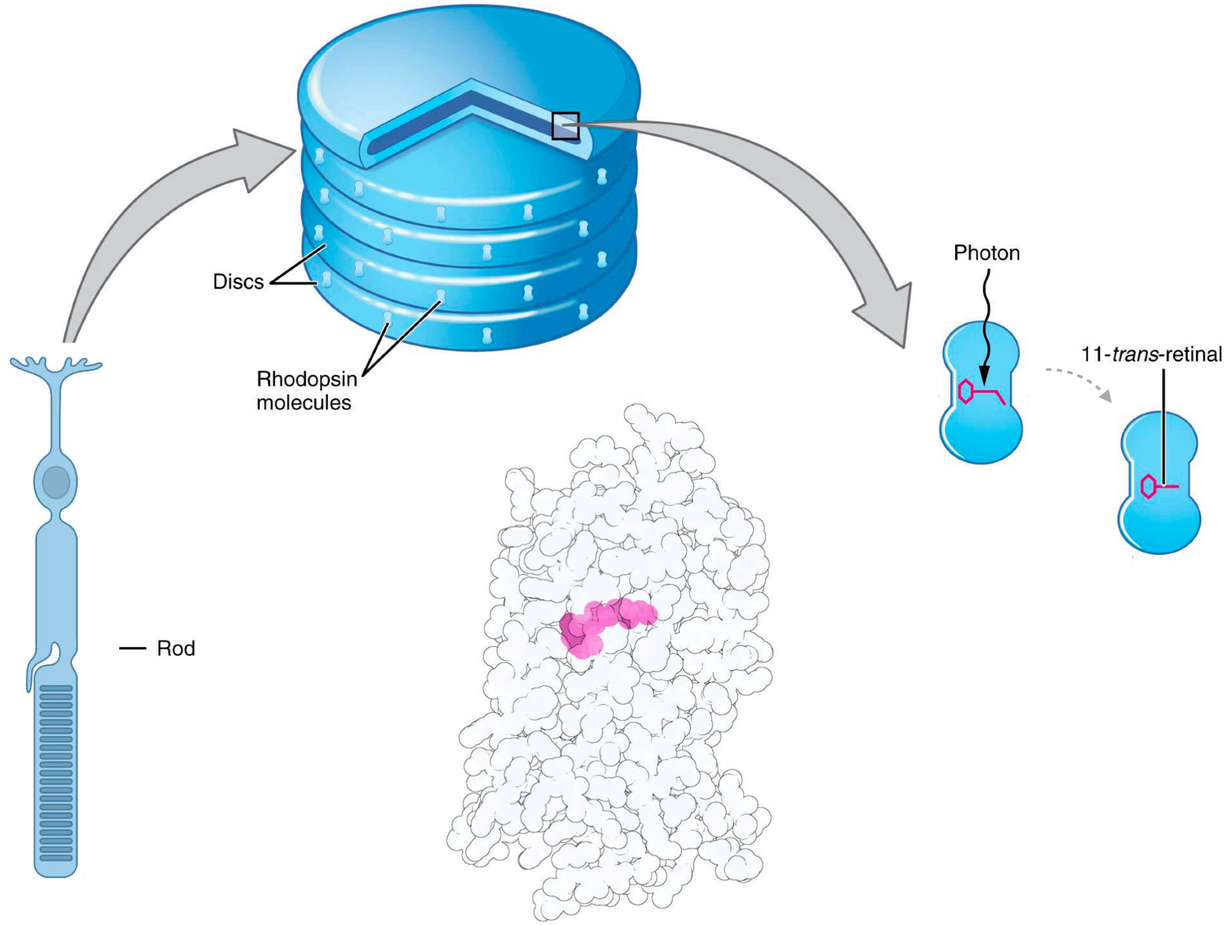
retinal (11-cis)



hv
~400-450nm



retinal (all trans)



Organic photochemistry + protein = light **biosensor**

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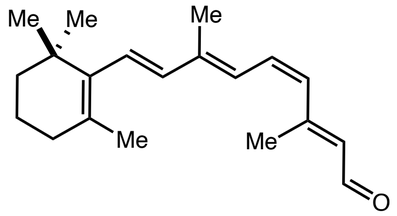
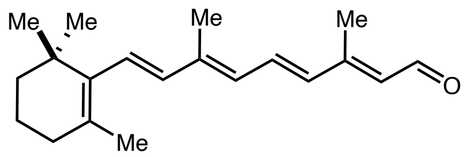
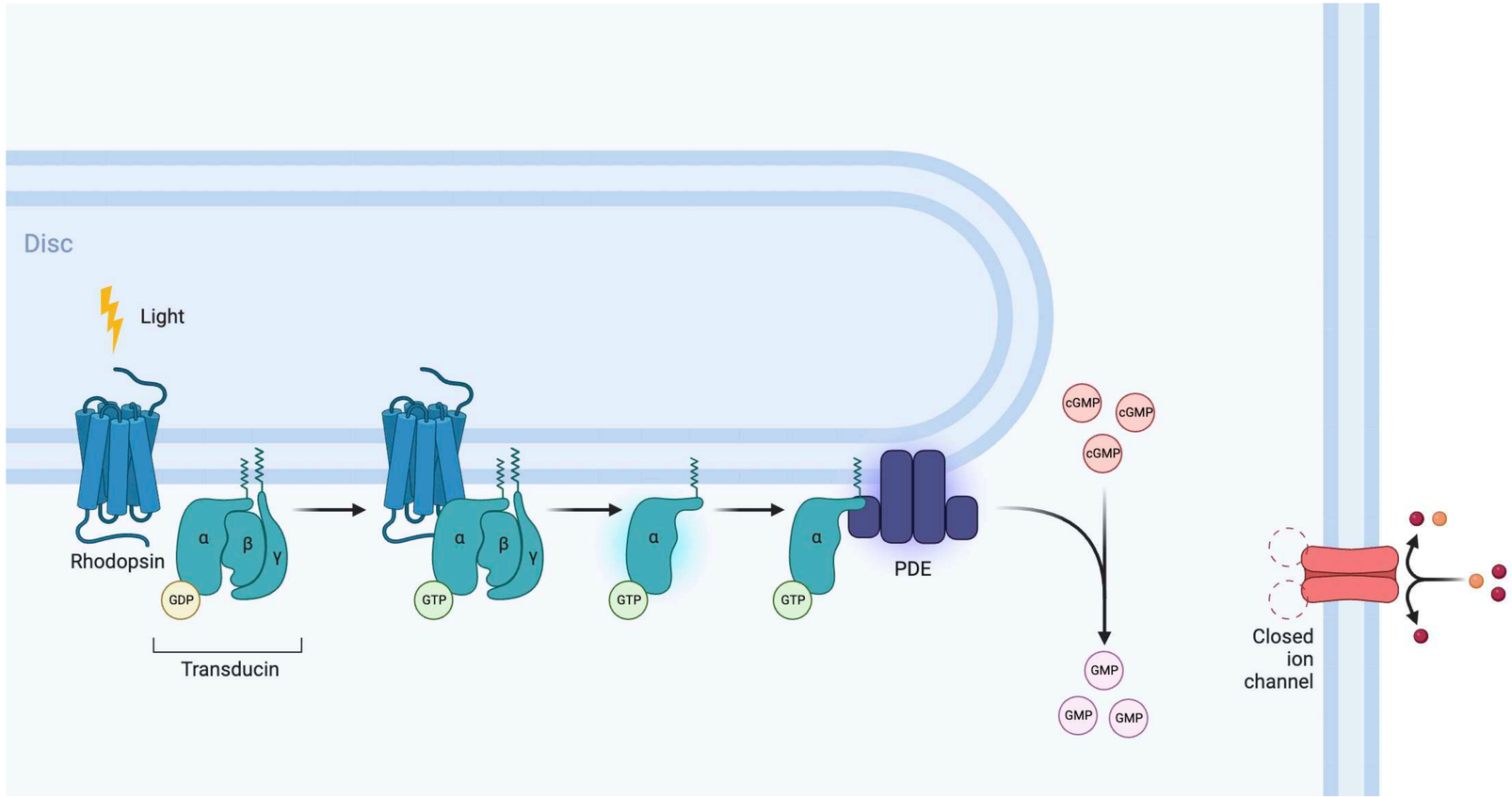


hv
~400-450nm

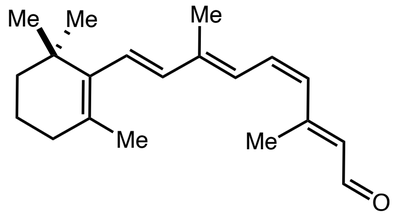


retinal (all trans)

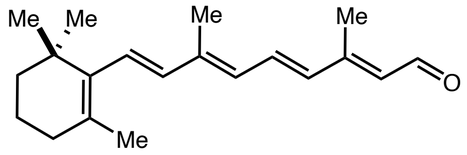


Organic photochemistry + protein = light biosensor

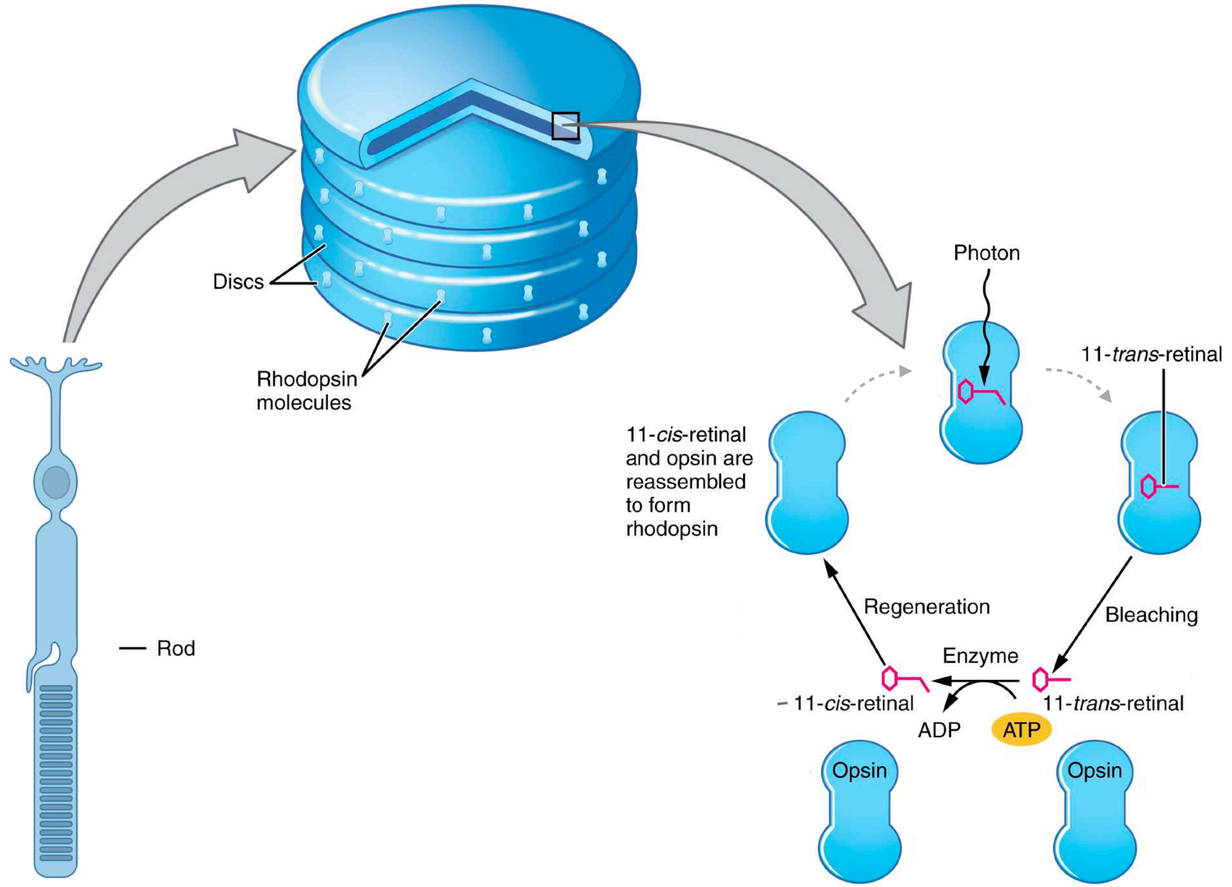
retinal (11-cis)



hv
~400-450nm

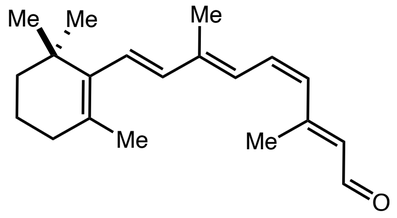


retinal (all trans)

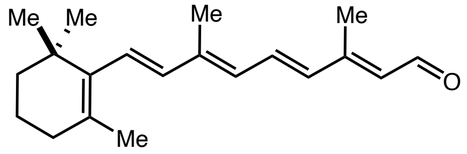


Organic photochemistry + protein = light **biosensor**

retinal (11-cis)



hv
~400-450nm

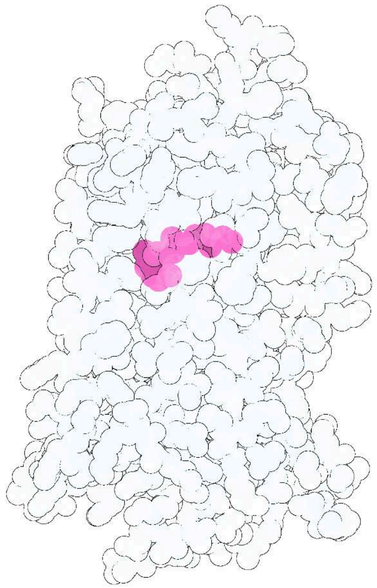


retinal (all trans)

rhodopsin

G-protein coupled receptor

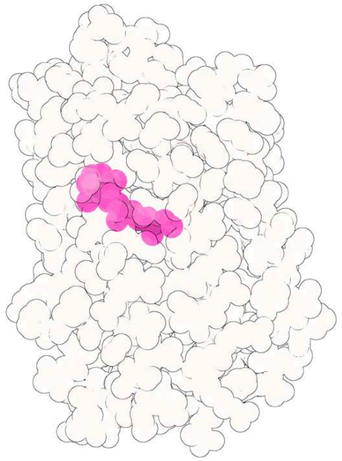
~500 MYA



bacteriorhodopsin

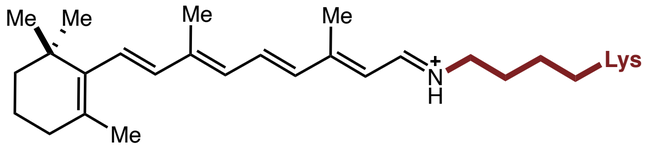
ion channel

~3 BYA

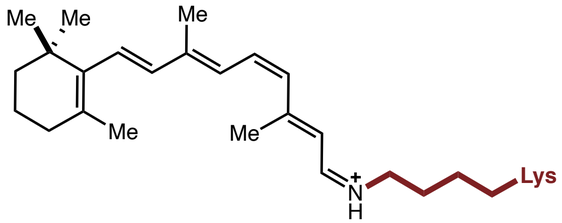


Organic photochemistry + protein = light **biosensor**

retinal (all trans)



hv
~400-450nm

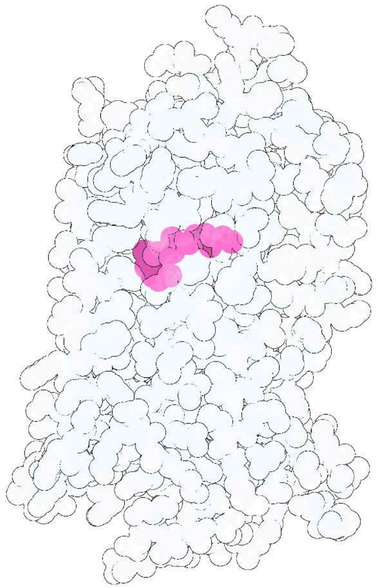


retinal (11-cis)

rhodopsin

G-protein coupled receptor

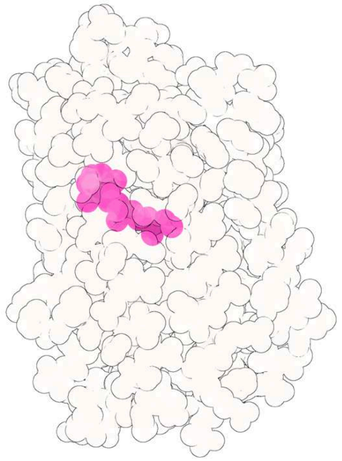
~500 MYA



bacteriorhodopsin

ion channel

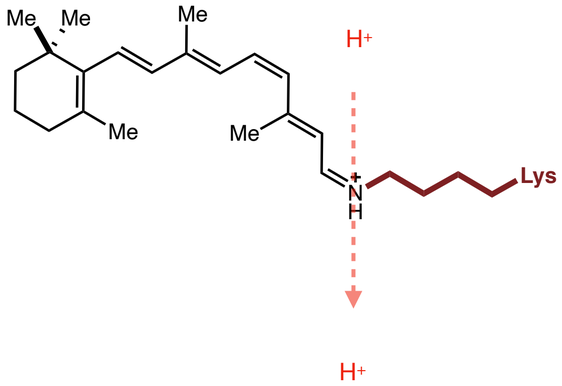
~3 BYA



Organic photochemistry + protein = light **biosensor**

retinal (all trans)

hv
~400-450nm



rhodopsin

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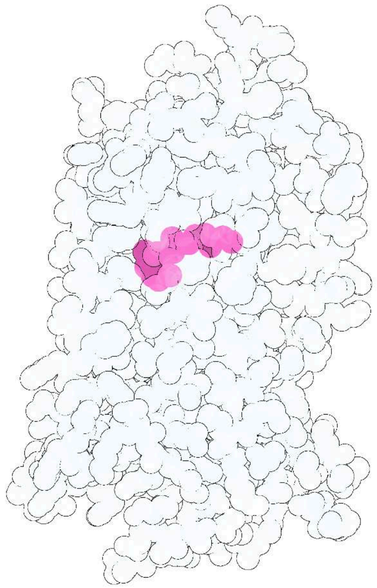
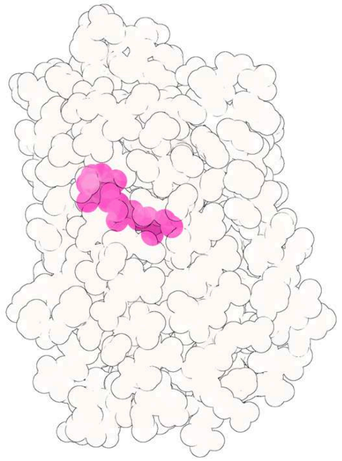


bacteriorhodopsin

ion channel

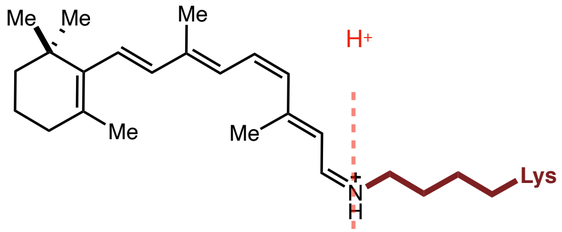
~3 BYA



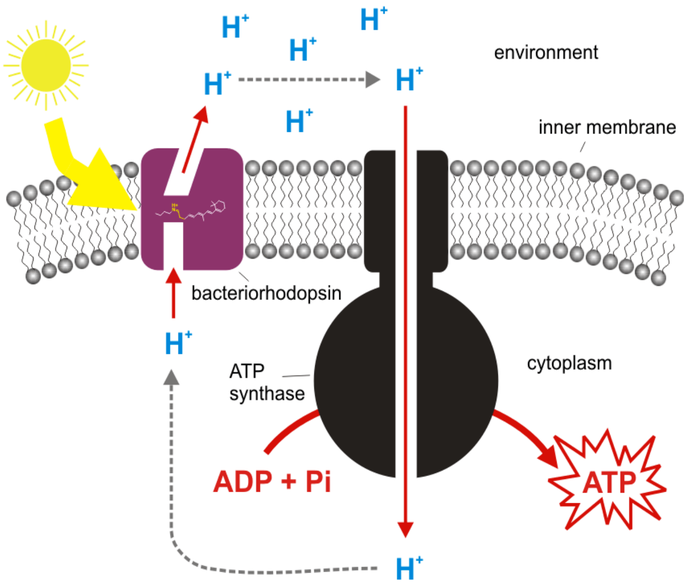
Organic photochemistry + protein = light biosensor

retinal (all trans)

hv
~400-450nm



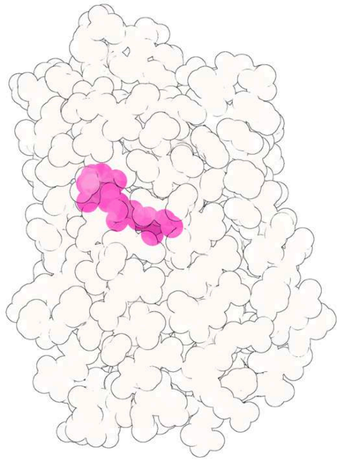
H+



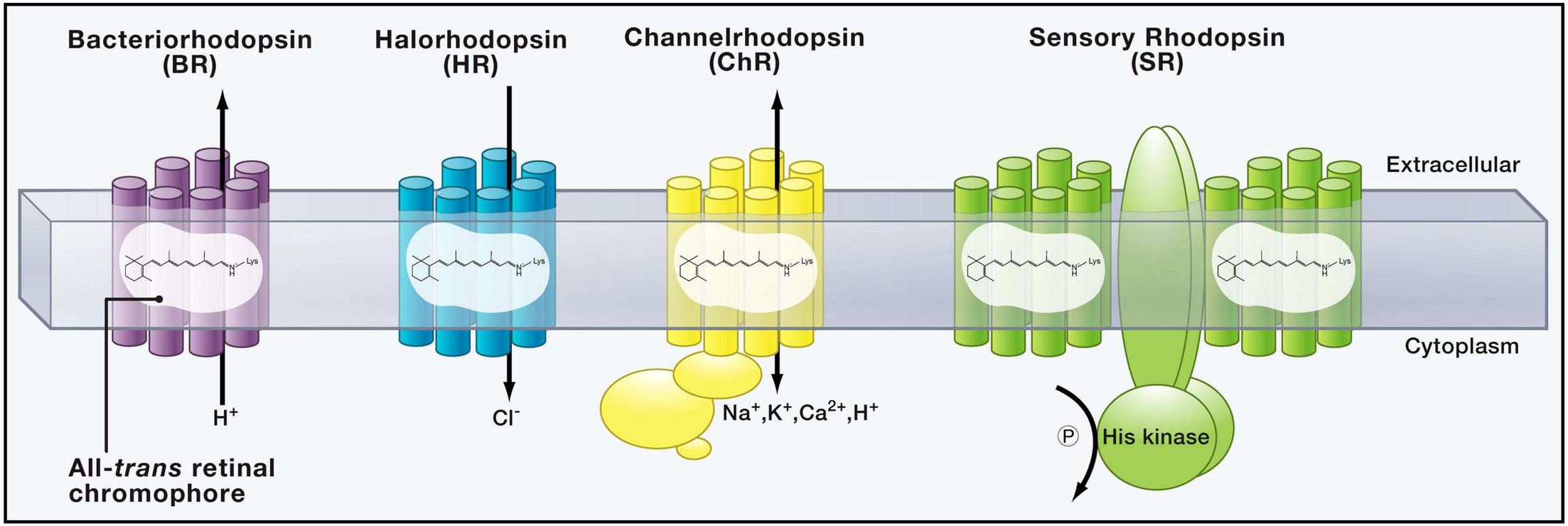
bacteriorhodopsin

ion channel

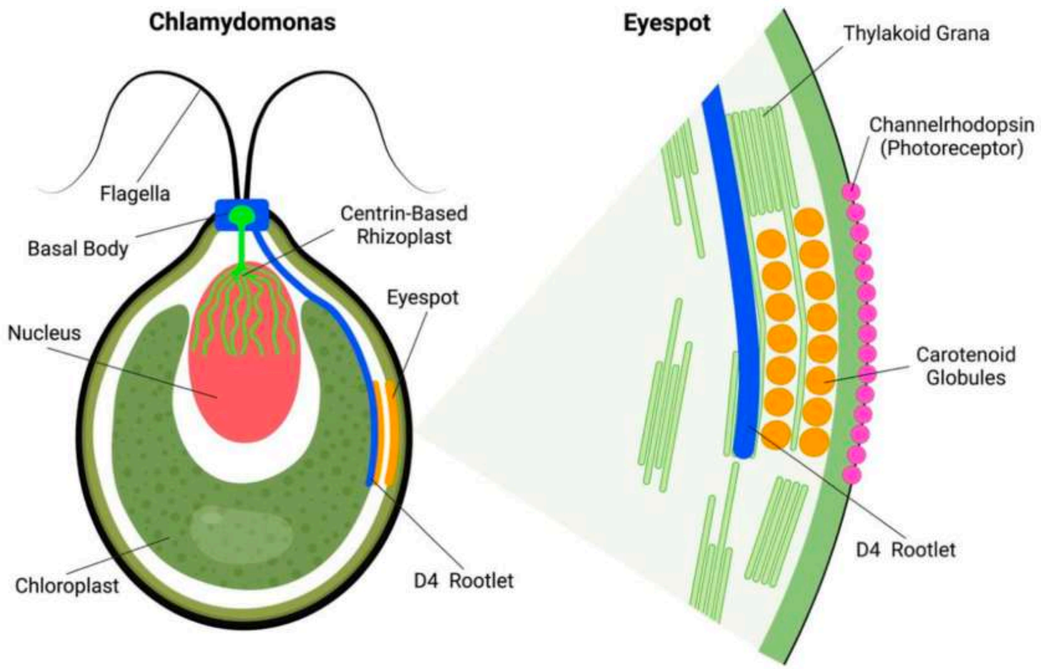
~3 BYA

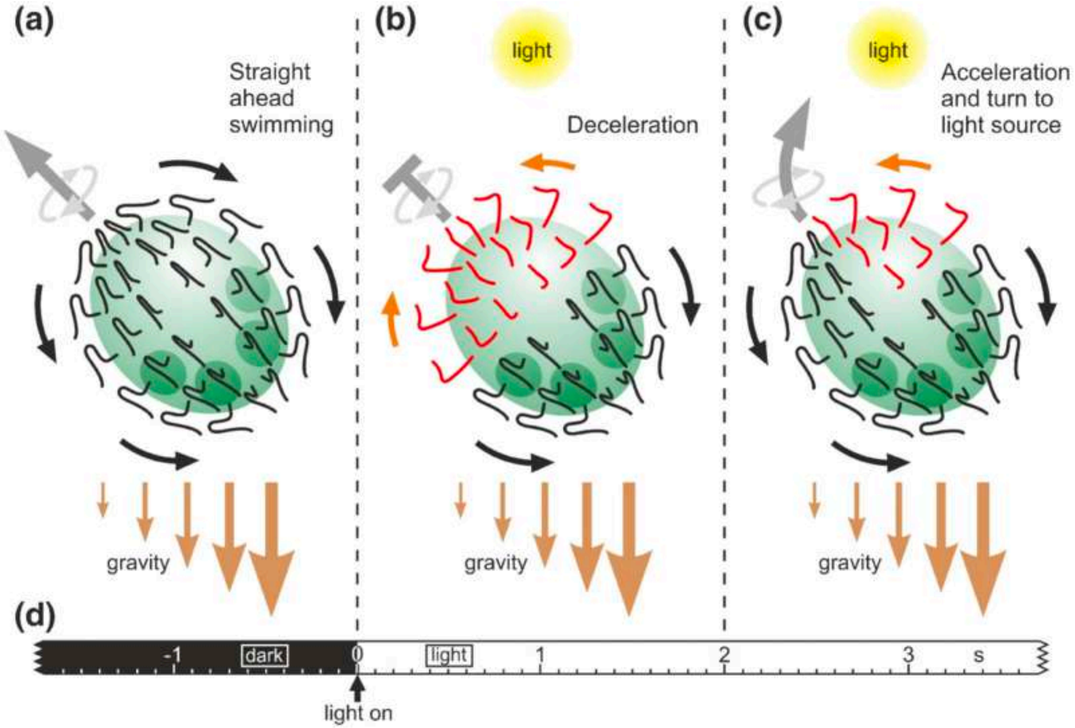


The channelrhodopsin family

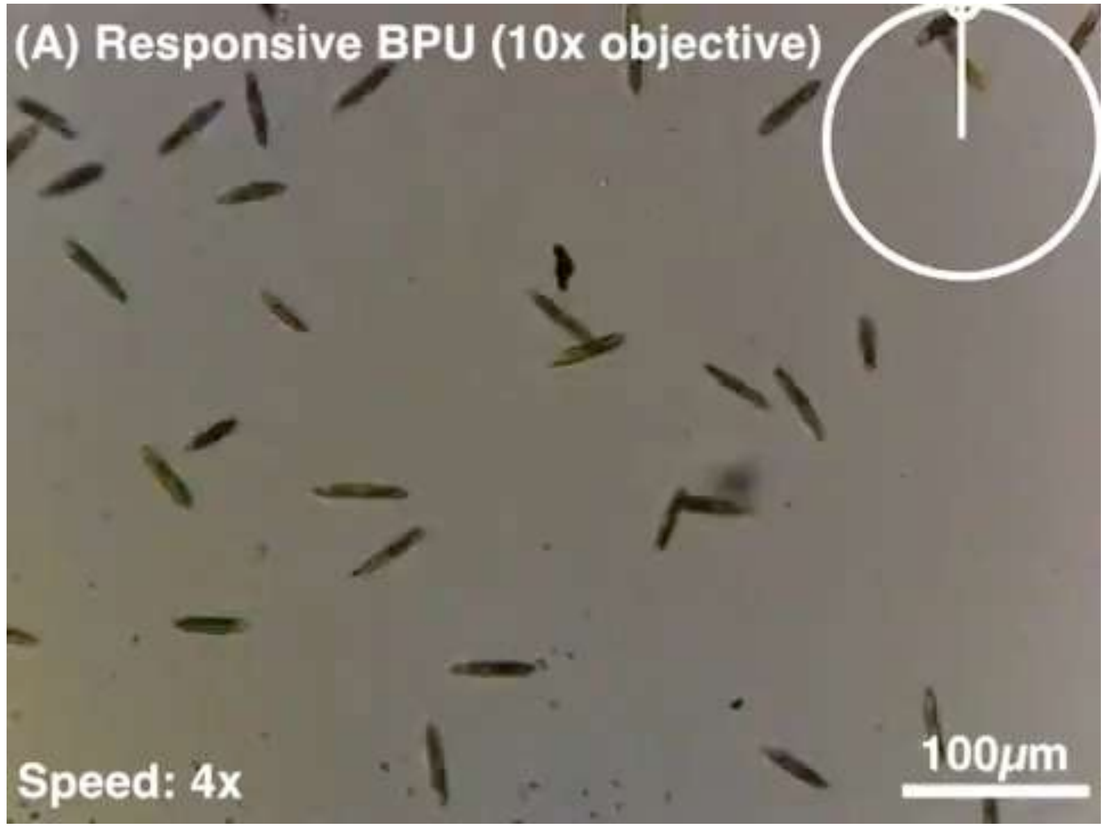


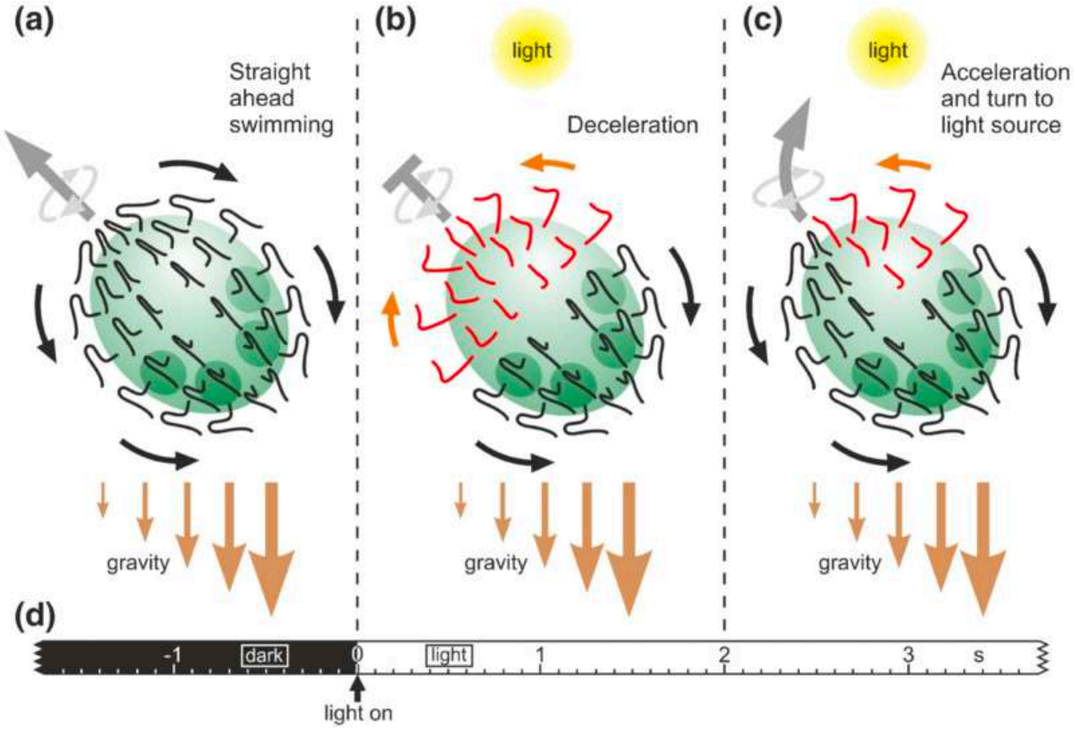
Phototaxis


Chlamydomonas
Flagella
Basal Body
Centrin-Based Rhizoplast
Eyespot
Nucleus
Chloroplast
D4 Rootlet
Eyespot
Thylakoid Grana
Channelrhodopsin (Photoreceptor)
Carotenoid Globules
D4 Rootlet


(a)
Straight ahead swimming
gravity
(b)
light
Deceleration
gravity
(c)
light
Acceleration and turn to light source
gravity
(d)
-1
dark
0
light
1
2
3
s
light on

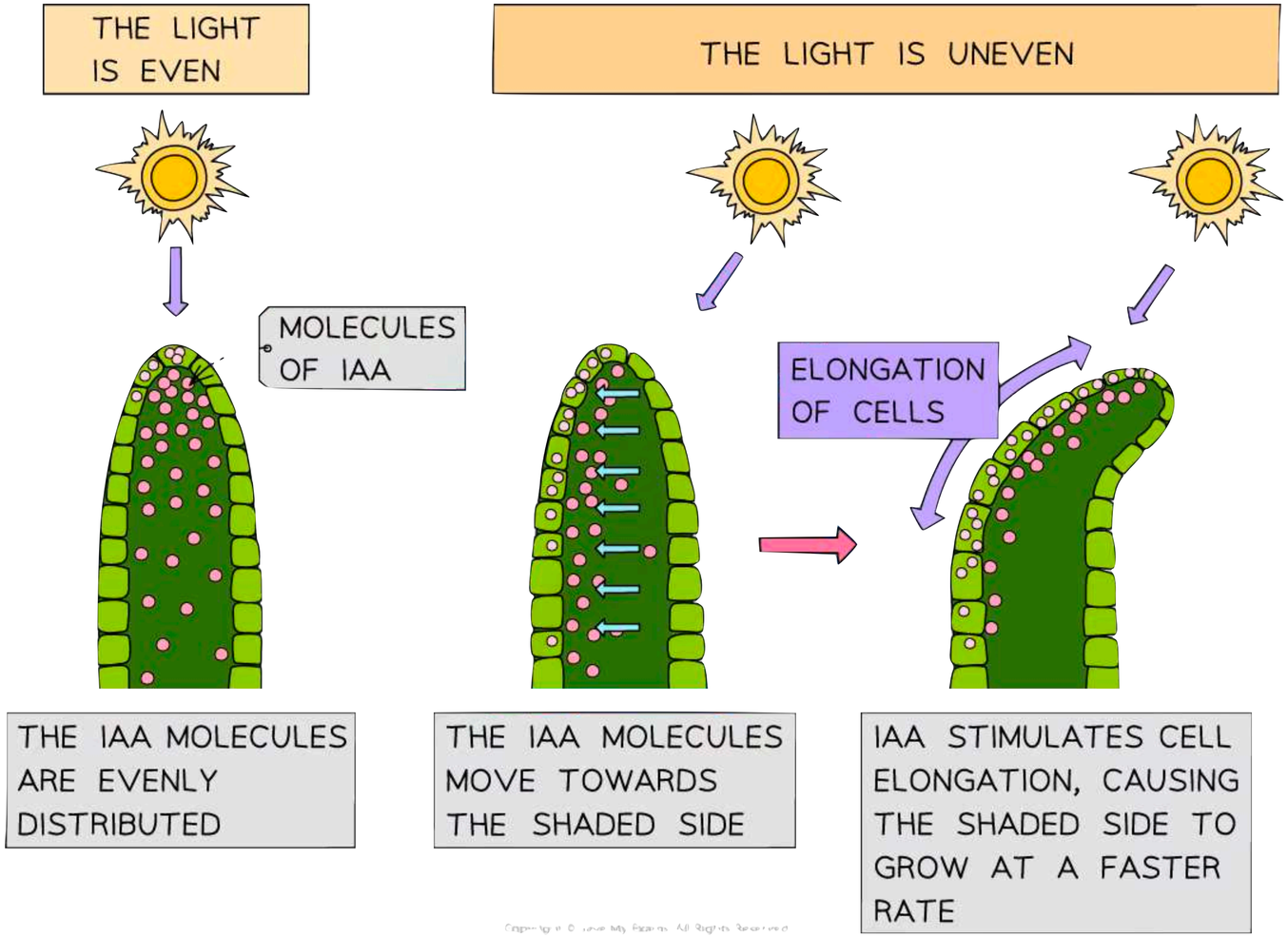
Phototaxis


(A) Responsive BPU (10x objective)
Speed: 4x
100μm

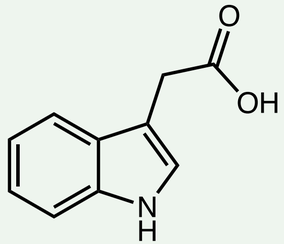

(a)
Straight ahead swimming
(b)
light
Deceleration
(c)
light
Acceleration and turn to light source
gravity
gravity
gravity
(d)
-1
dark
0
light
1
2
3
s
light on

Phototropism

Phototropism

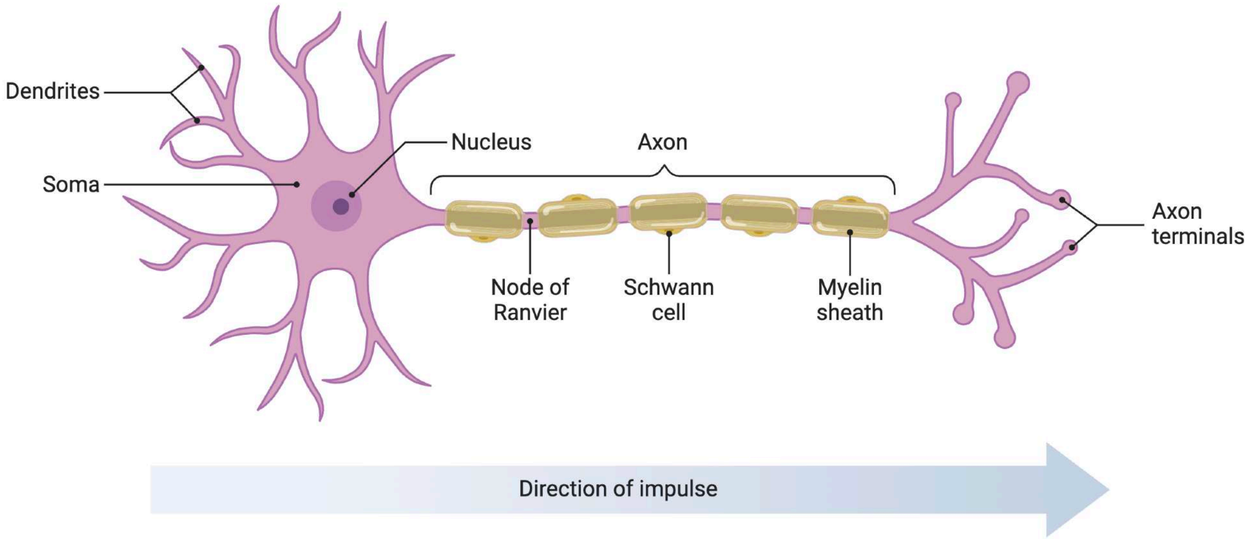


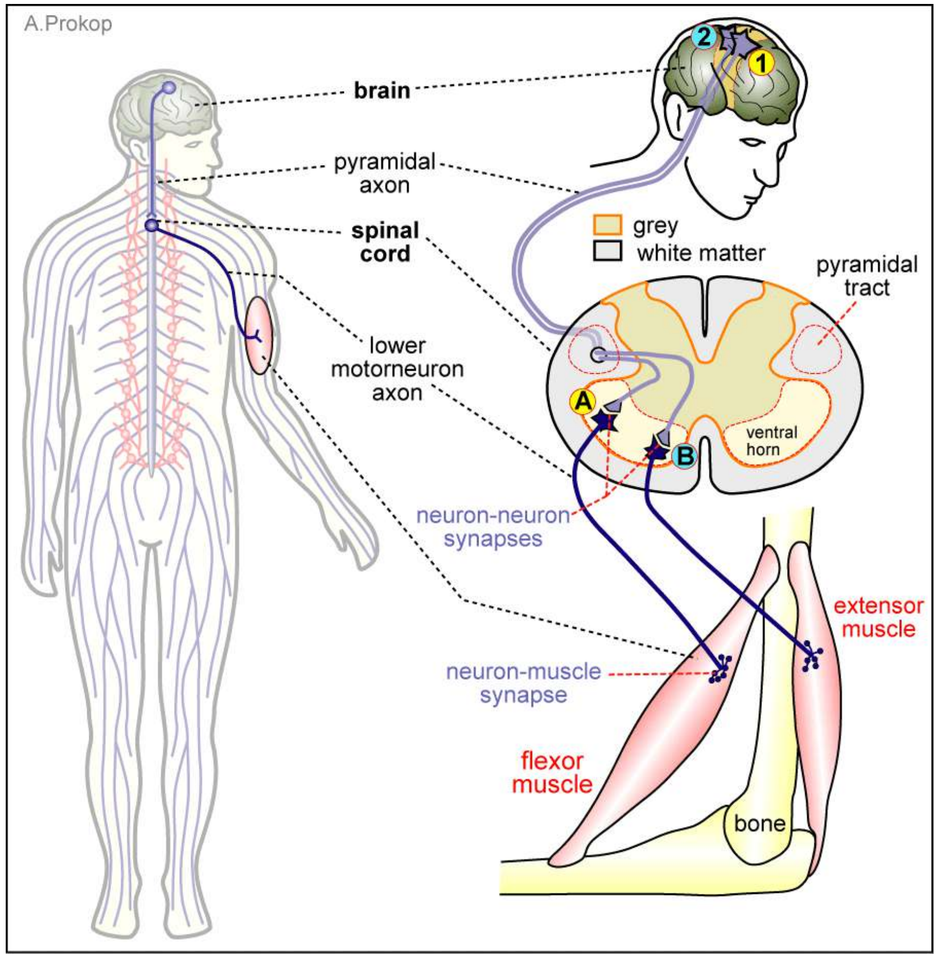
indole acetic acid (IAA)



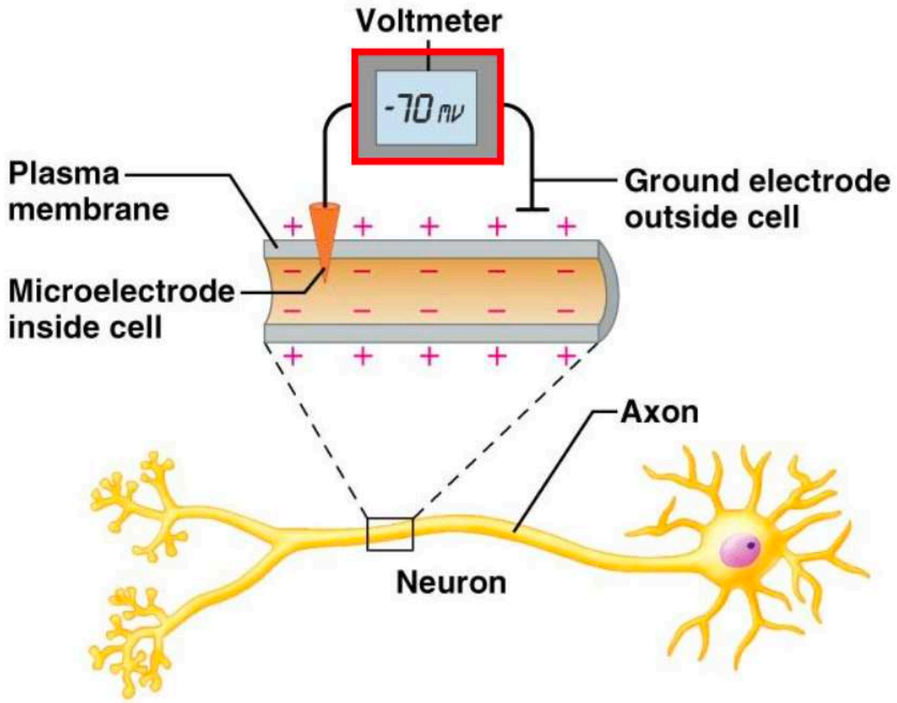
Fundamental principles in neurology

the neuron

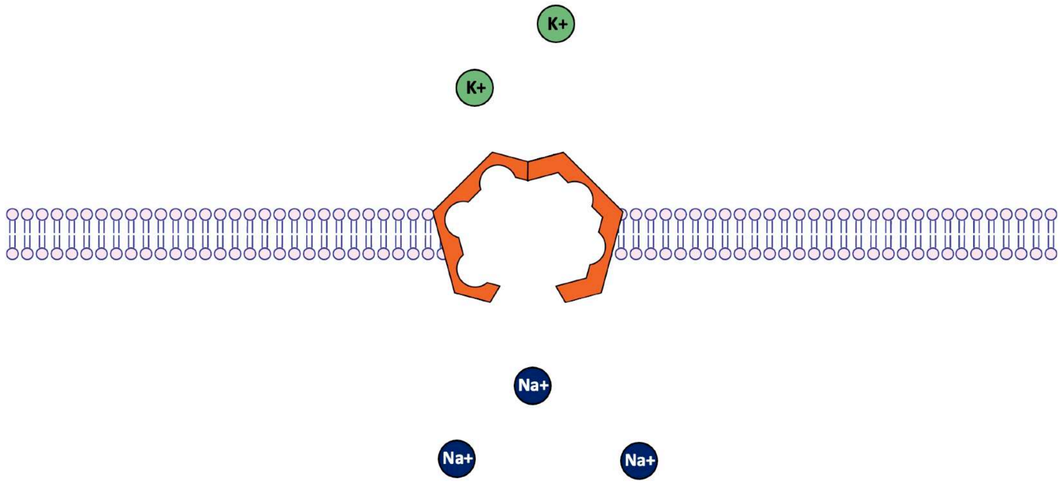




Electrochemical gradients in neuronal conduction



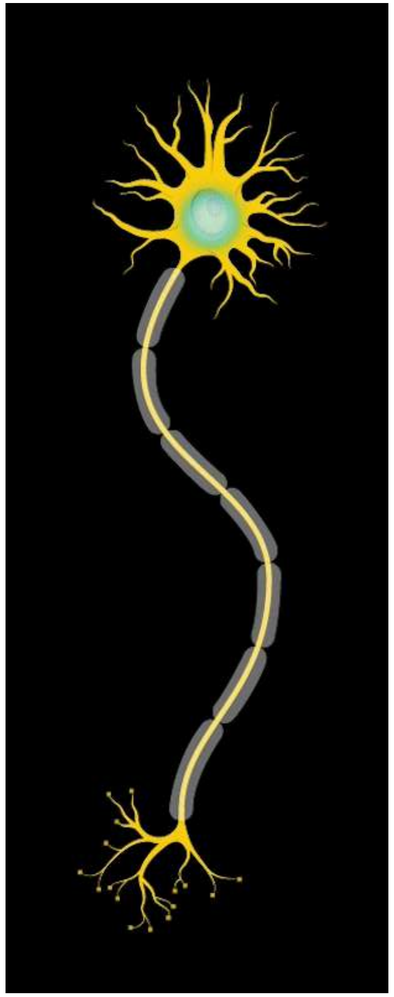
net output: 3 sodium ions

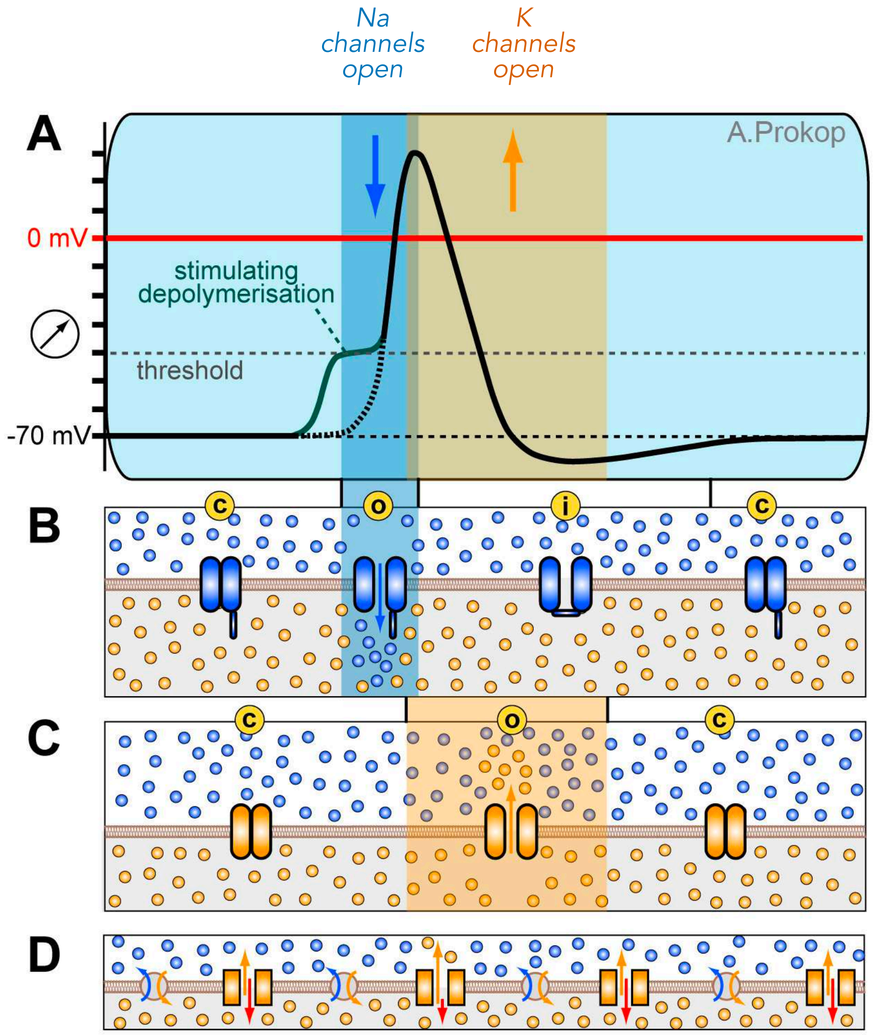


net input: 2 potassium ions

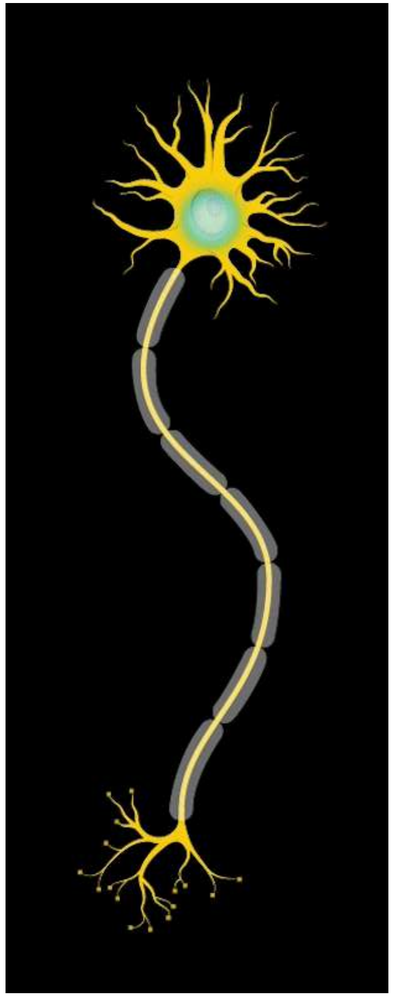
buildup of negative charge inside neuron

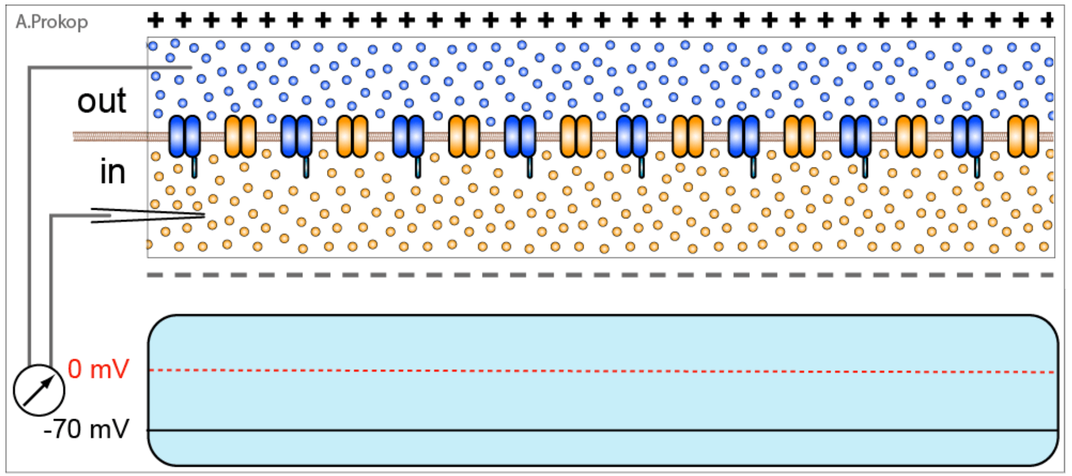
Electrochemical gradients in neuronal conduction

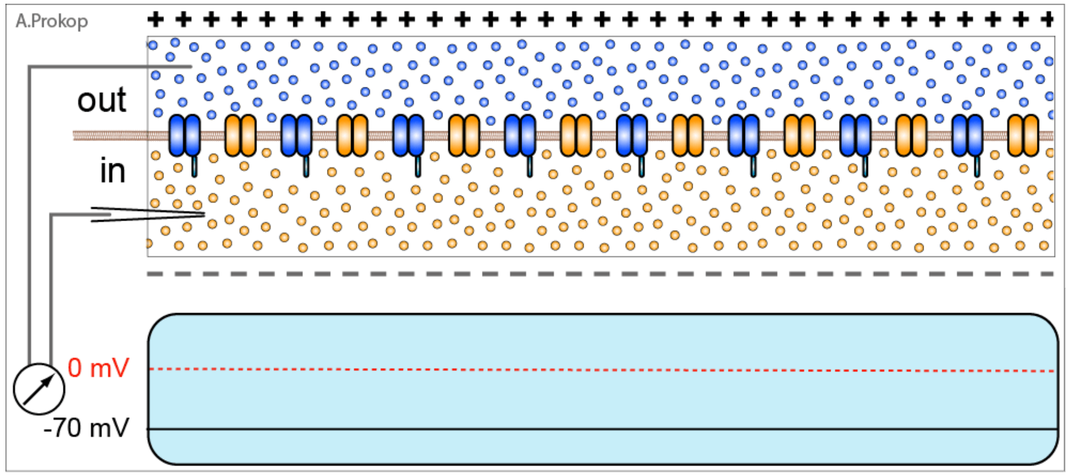




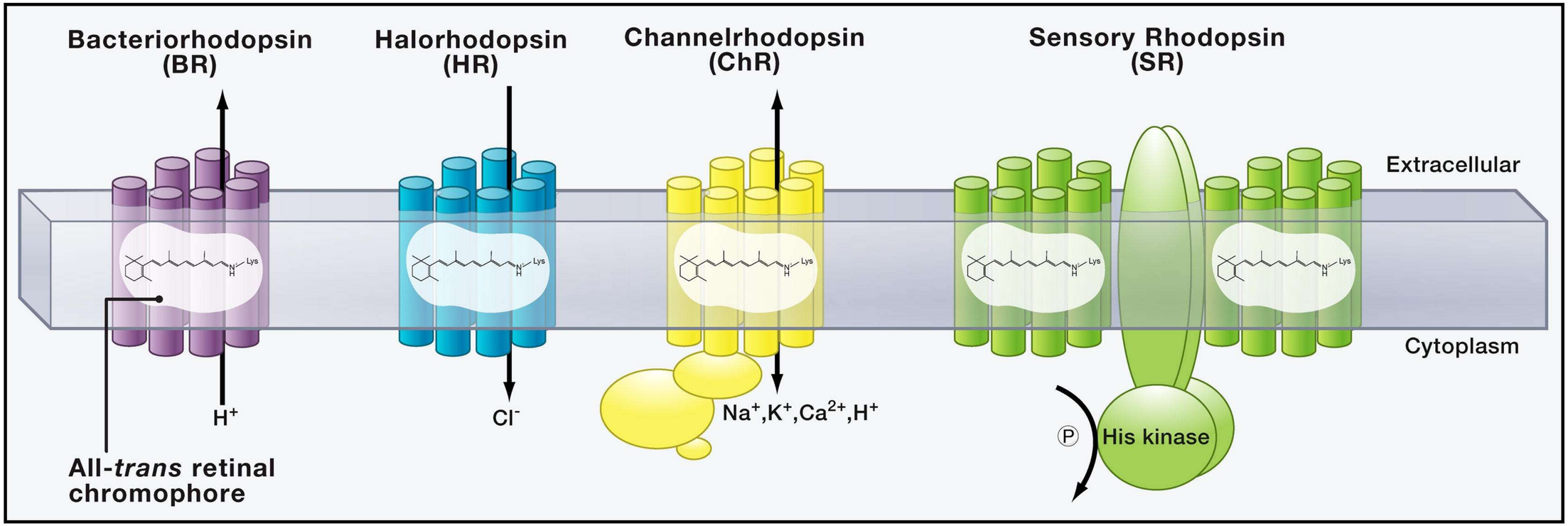
Electrochemical gradients in neuronal conduction



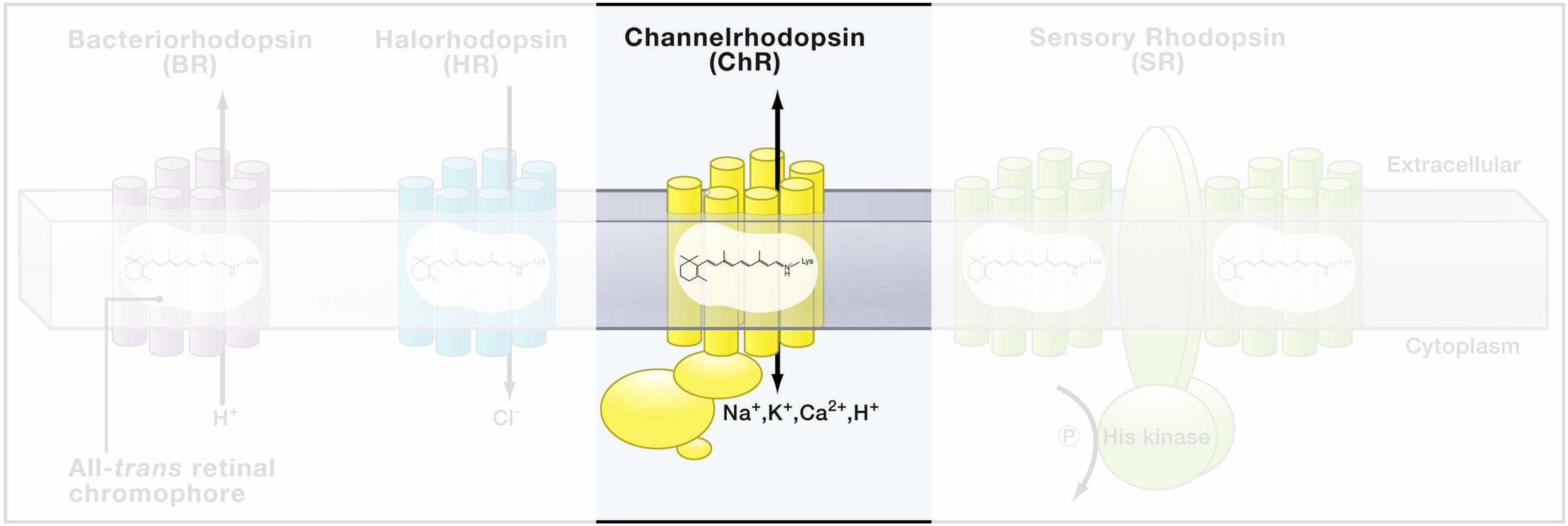




Goal: implement light-activated ion channels to activate neurons



Goal: implement light-activated ion channels to activate neurons

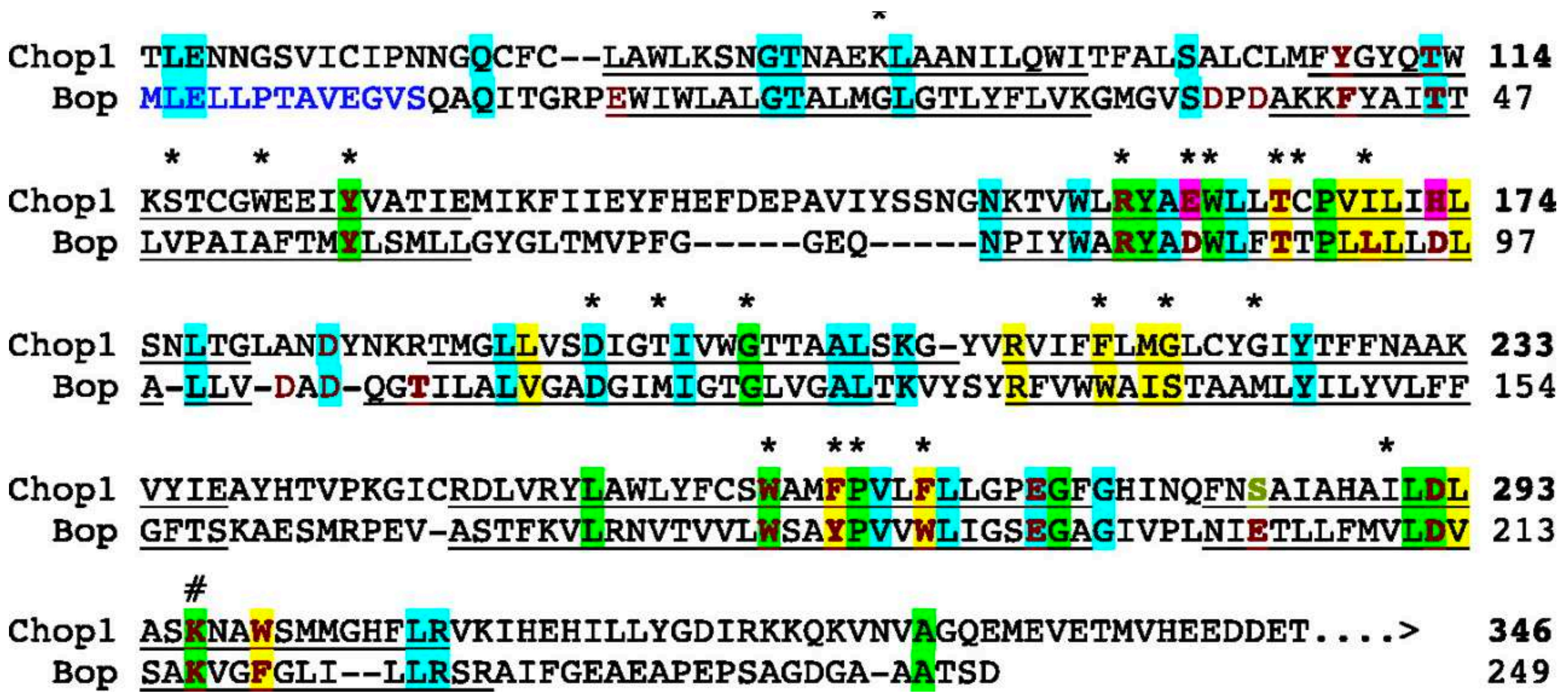


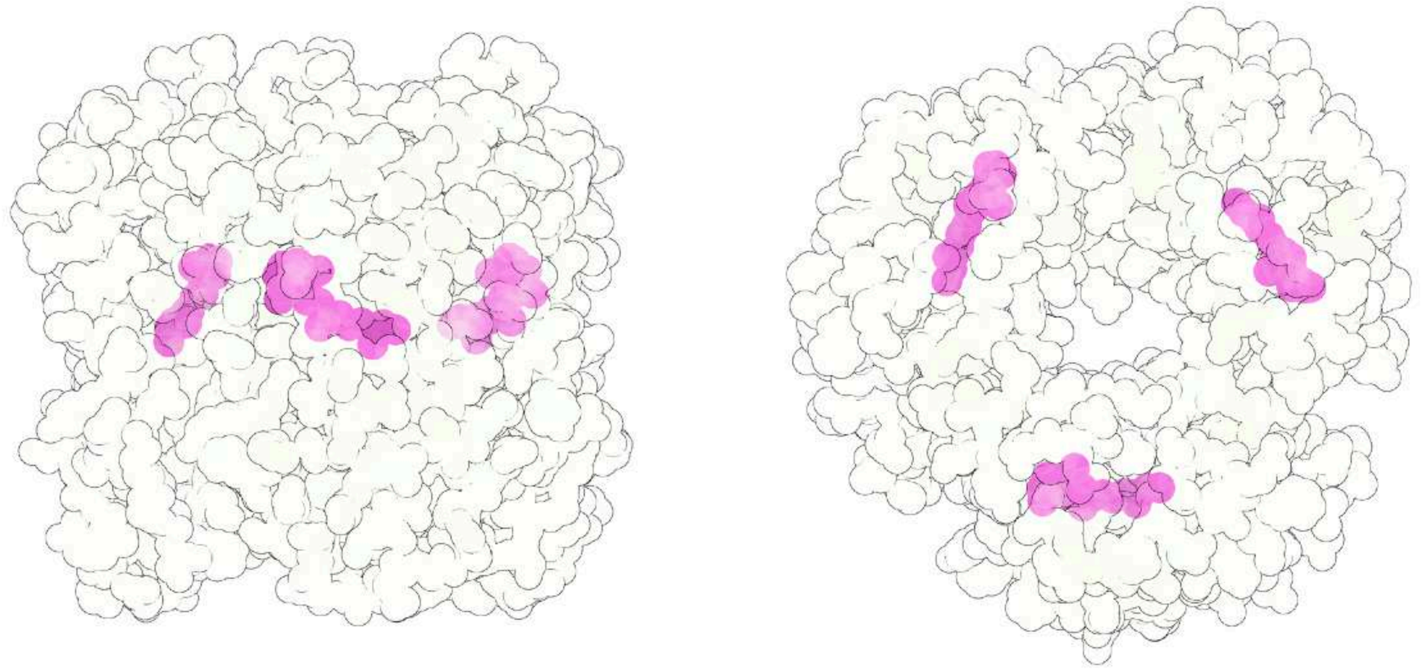
Channelrhodopsin cloning and *in vitro* characterization

Peter Hegemann

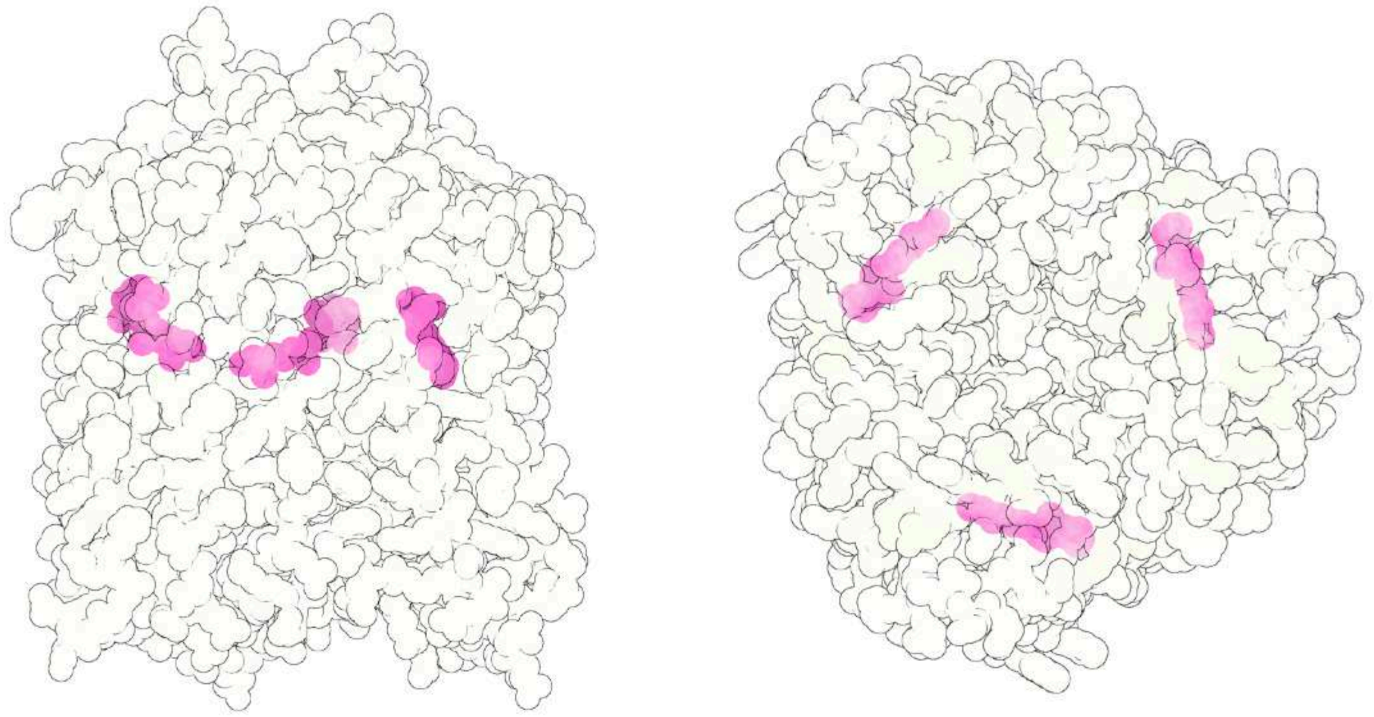
Georg Nagel

Chlamydomonas reinhardtii
(green algae)


Chop1 TLENNGSVICIPNNGQCFC--LAWLKSNGTNAEKLAANILQWITFALSALCLMFYGYQTW 114
Bop MLELLPTAVEGVSQAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT 47
Chop1 KSTCGWEEIYVATIEMIKFIIEYFHEFDEPAVIYSSNGNKTVWLRYAEWLLTCPVILIHL 174
Bop LVPAIAFTMYLSMLLGYGLTMVPFG-----GEQ-----NPIYWARYADWLFTTPLLLLDL 97
Chop1 SNLTGLANDYNKRTMGLLVSDIGTIVWGTTAALSKG-YVRVIFFLMGLCYGIYTFFNAAK 233
Bop A-LLV-DAD-QGTILALVGADGIMIGTGLVGALTKVYSYRFVWWAISTAAMLYILYVLFF 154
Chop1 VYIEAYHTVPKGICRDLVRYLAWLYFCSWAMFPVLFLLGPEGFGHINQFNSAIAHAILDL 293
Bop GFTSKAESMRPEV-ASTFKVLRNVTVVLWSAYPVVWLIGSEGAGIVPLNIETLLFMVLDV 213
Chop1 ASKNAWSMMGHFLRVKIHEHILLYGDIRKKQKVNVAGQEMEVETMVHEEDDET....> 346
Bop SAKVGFGLI--LLRSRAIFGEAEAPEPSAGDGA-AATSD 249



bacteriorhodopsin



channelrhodopsin (algae)

Science, **2002**, *296*(5577), 2395-2398.

Channelrhodopsin cloning and *in vitro* characterization

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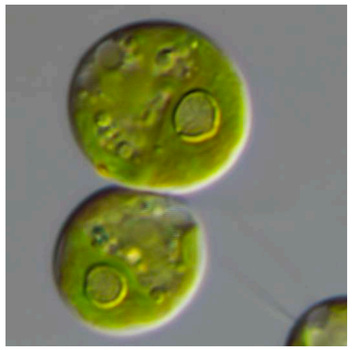
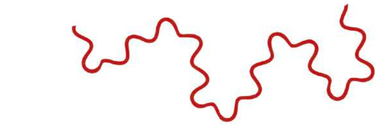
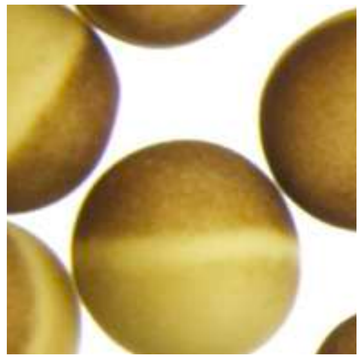
Georg Nagel



Chlamydomonas reinhardtii
(green algae)



channelrhodopsin RNA



Xenopus lavis oocyte
(frog egg)

Science, **2002**, *296*(5577), 2395-2398.

Channelrhodopsin cloning and *in vitro* characterization

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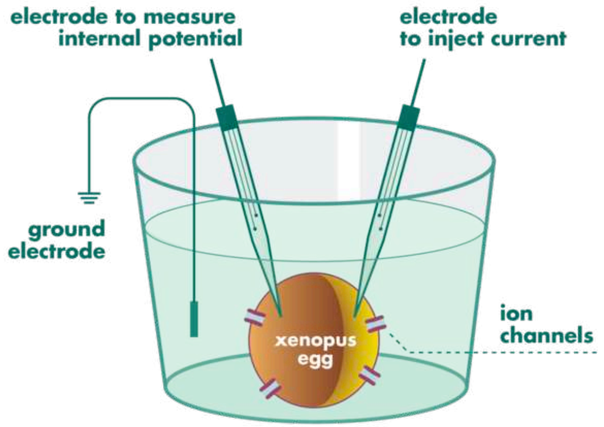

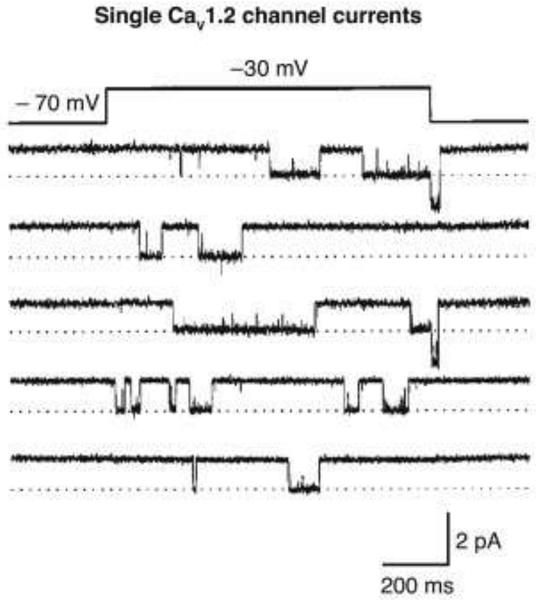

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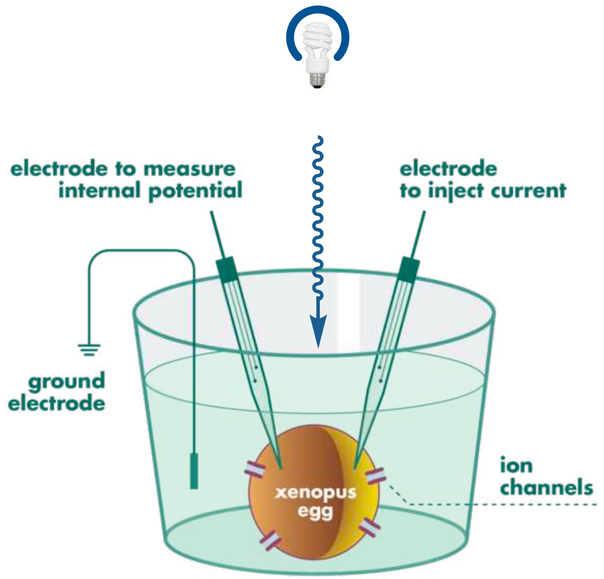
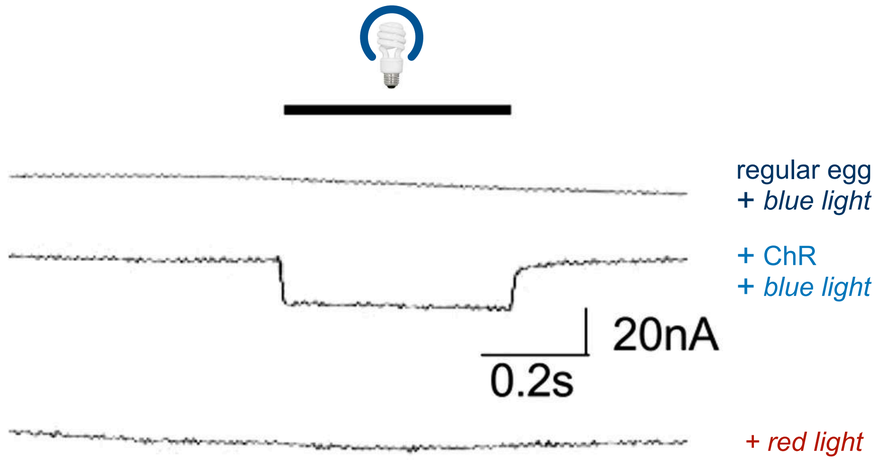




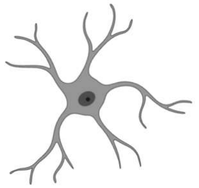
Science, **2002**, *296*(5577), 2395-2398.

Channelrhodopsin insertion into neurons

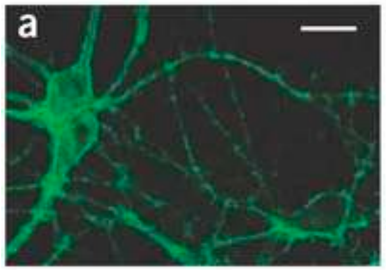
Karl Deisseroth

Georg Nagel

virus containing channelrhodopsin DNA

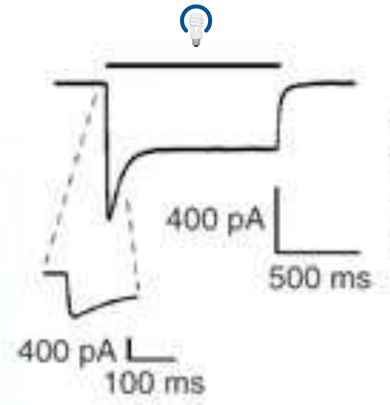


rat hippocampal neurons

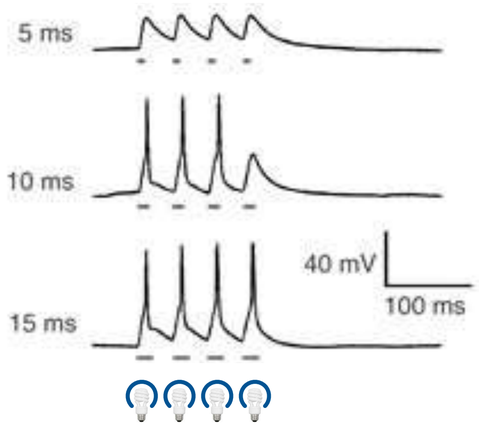


ChR expression

depolarization and repolarization



pulsed activation

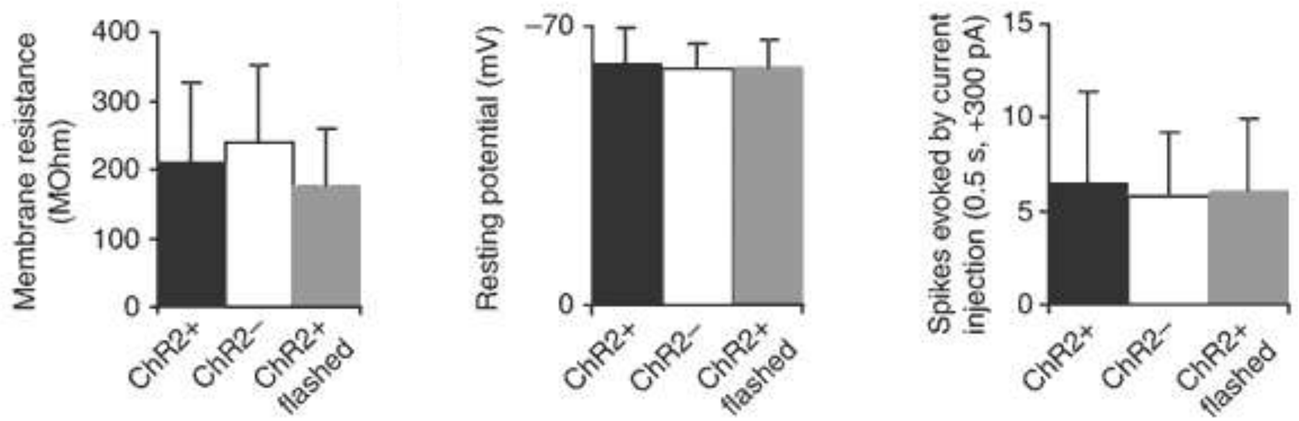


Nature Neuroscience, **2005**, *8*(9), 1263-1268.

ChR+ neurons behave just like regular neurons

Karl Deisseroth

Georg Nagel

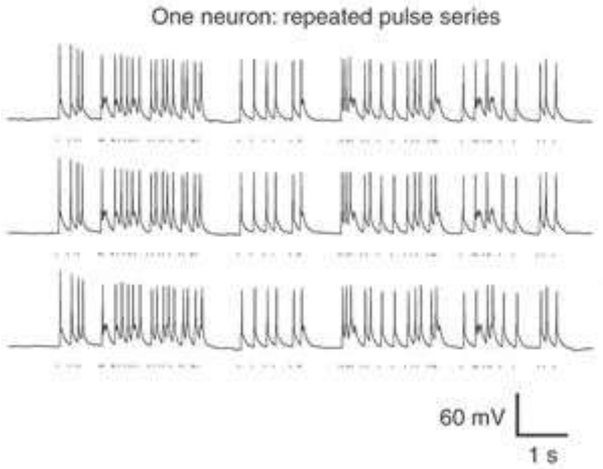


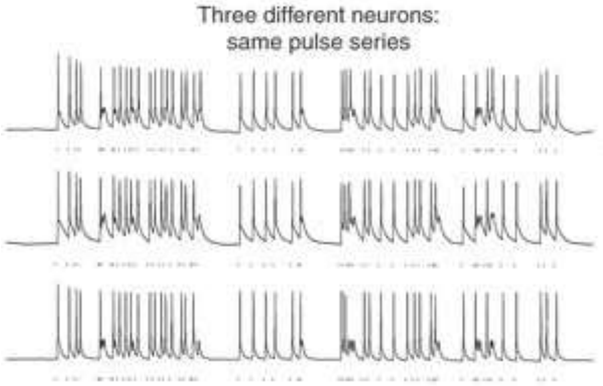
Nature Neuroscience, **2005**, *8*(9), 1263-1268.

Repeated optical control of neuroactivation

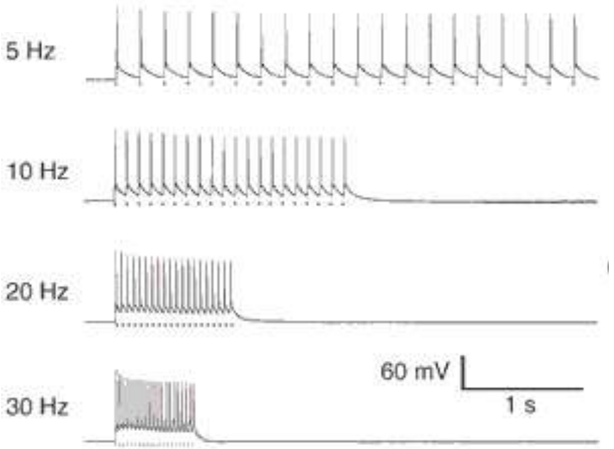
Karl Deisseroth

Georg Nagel





super fast pulsed activation and reactivation

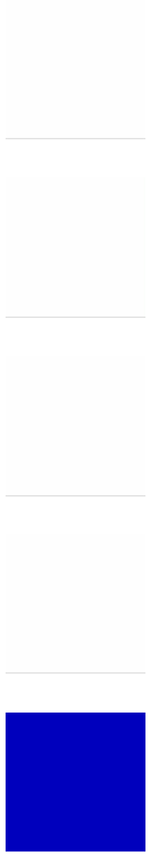


Nature Neuroscience, **2005**, *8*(9), 1263-1268.

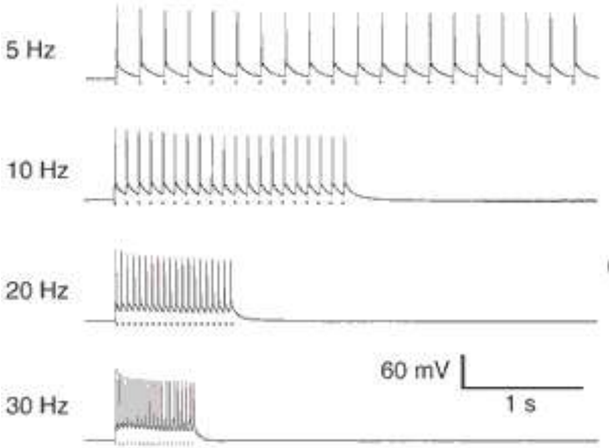
Repeated optical control of neuroactivation

Karl Deisseroth

Georg Nagel



super fast pulsed activation and reactivation

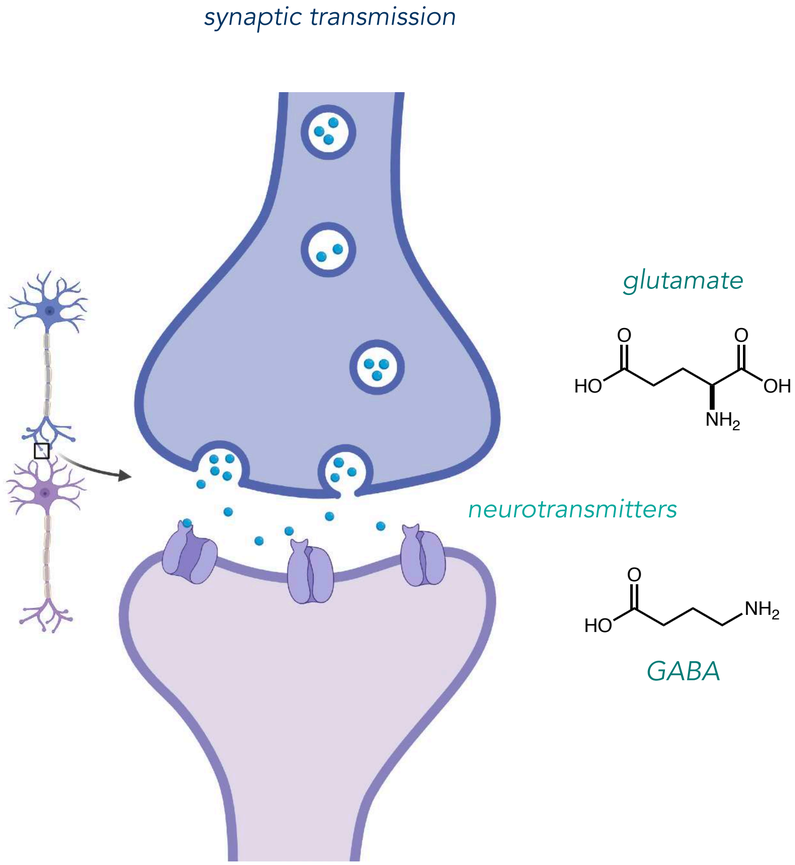


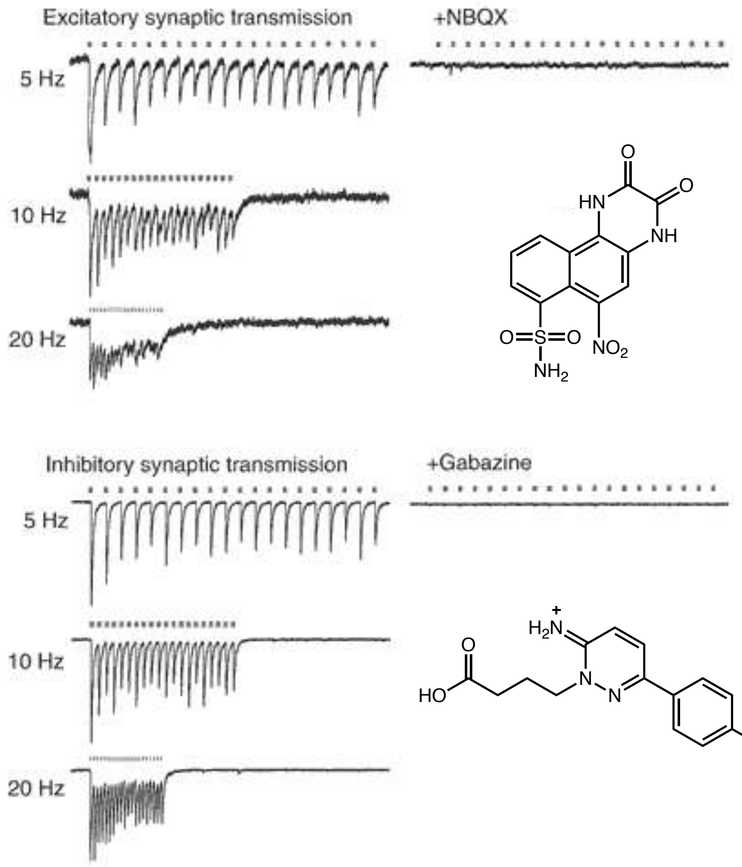
Nature Neuroscience, **2005**, *8*(9), 1263-1268.

Optical control over synaptic transmission

Karl Deisseroth

Georg Nagel





Nature Neuroscience, **2005**, *8*(9), 1263-1268.

In vivo optogenetics

Susanna Lima

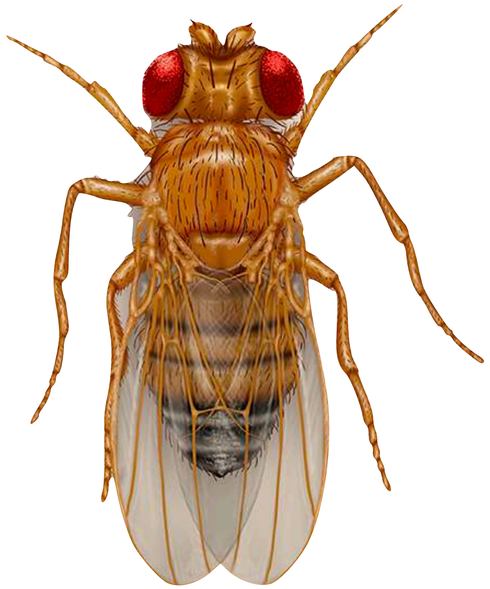
Gero Miessenböck

Cell

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

Drosophila melanogaster
(fruit fly)



Cell, **2005**, *121*(1), 141-152.

In vivo optogenetics

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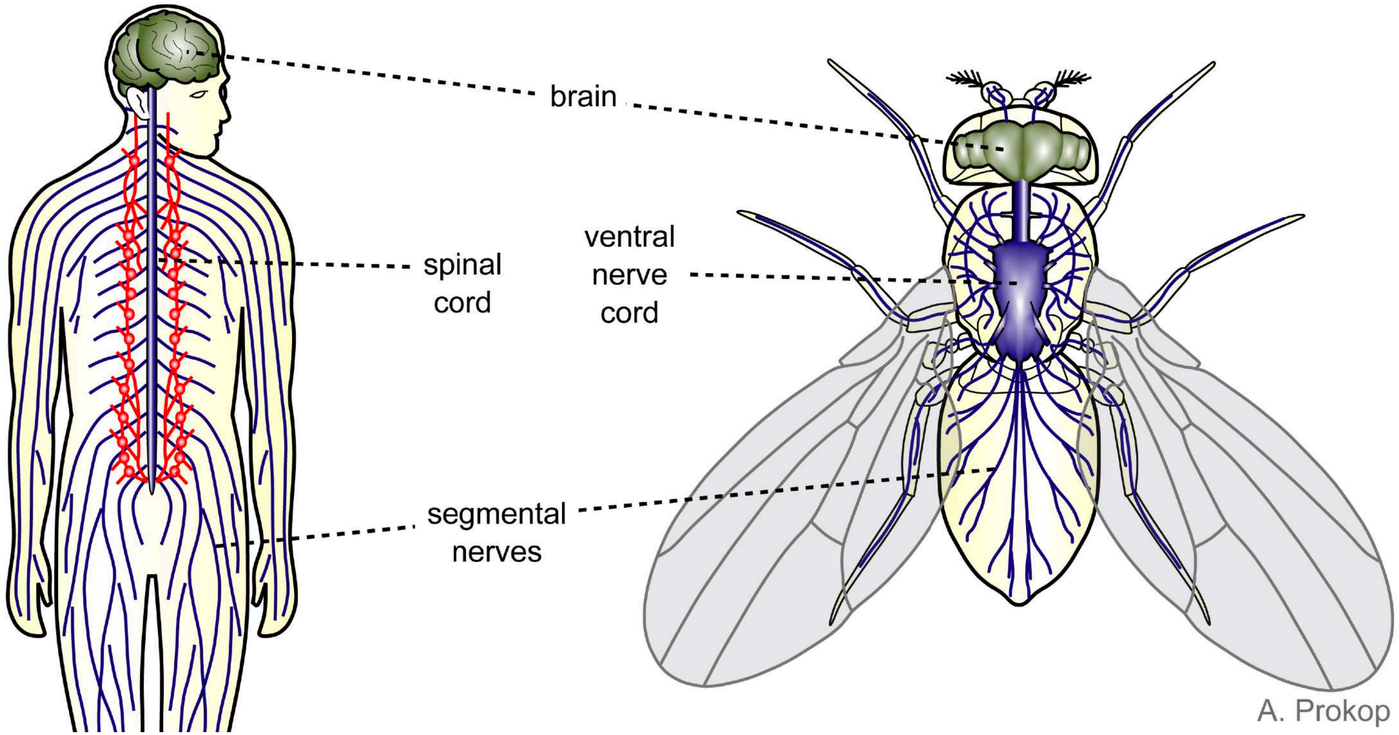
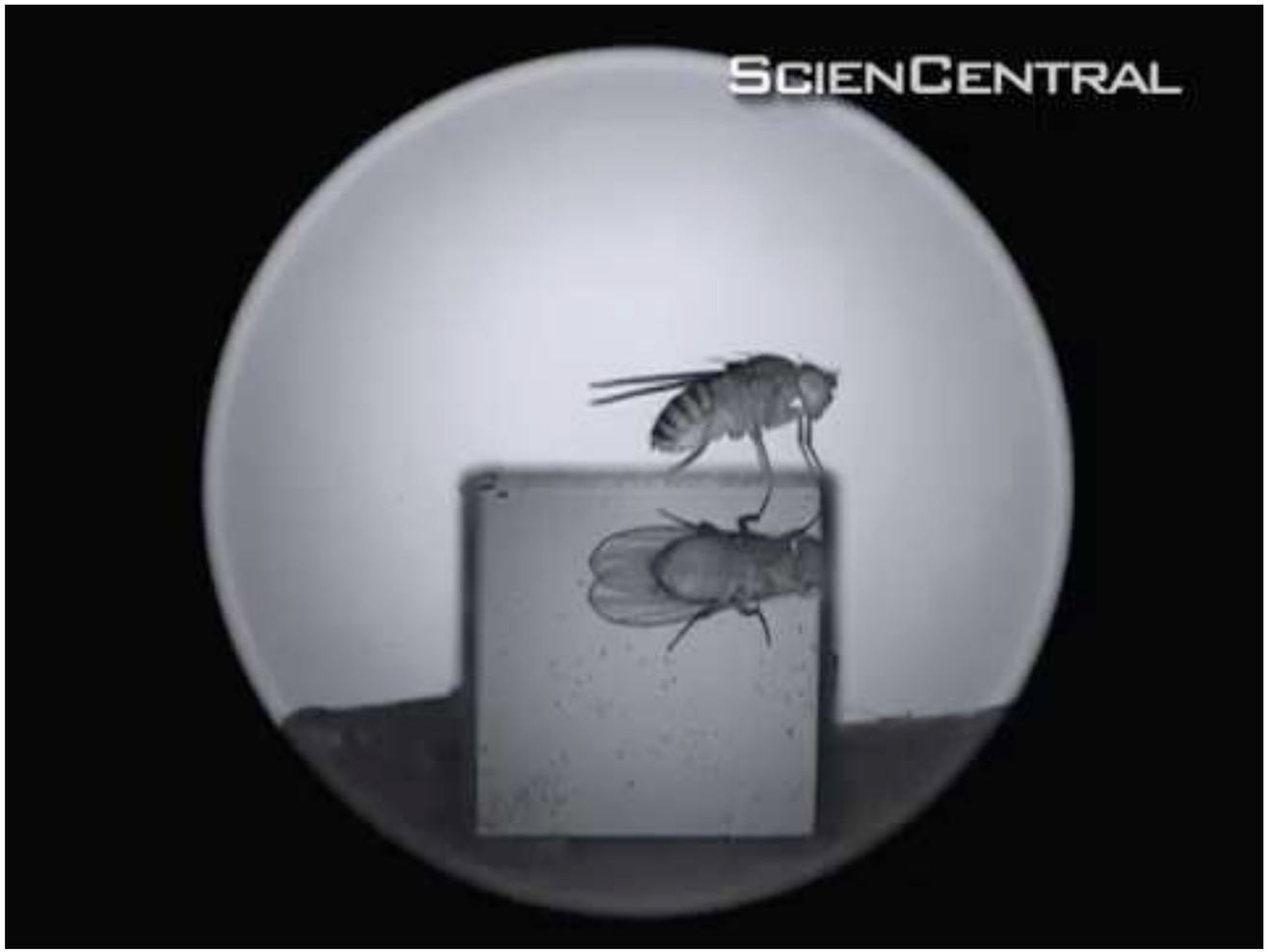


Cell, **2005**, *121*(1), 141-152.

Mapping and controlling the *flight* response

Susanna Lima

Gero Miessenböck



Cell, **2005**, *121*(1), 141-152.

Mapping and controlling the *flight* response

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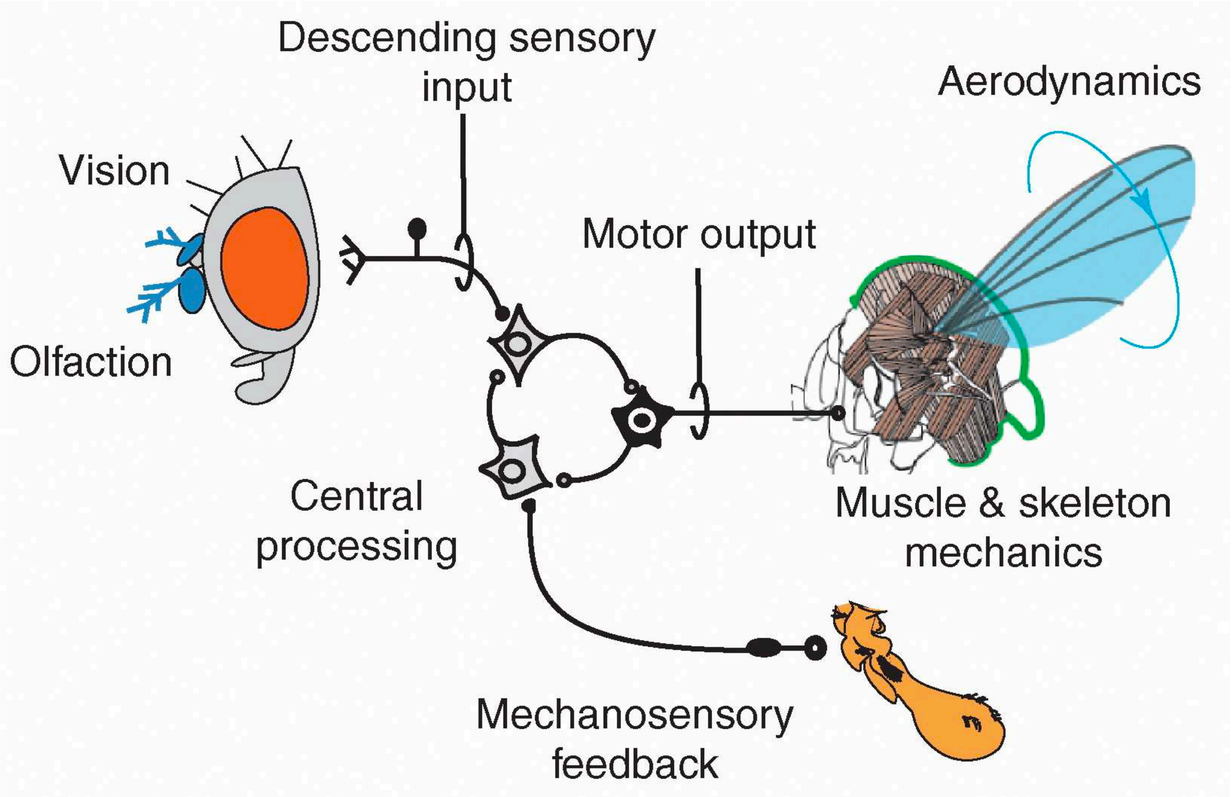
Gero Miessenböck



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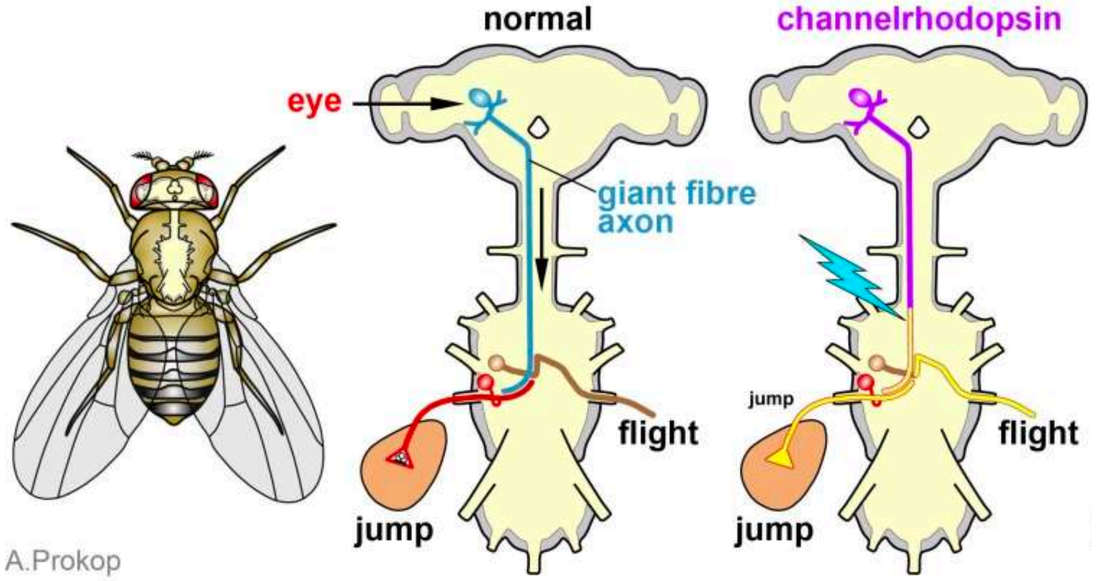

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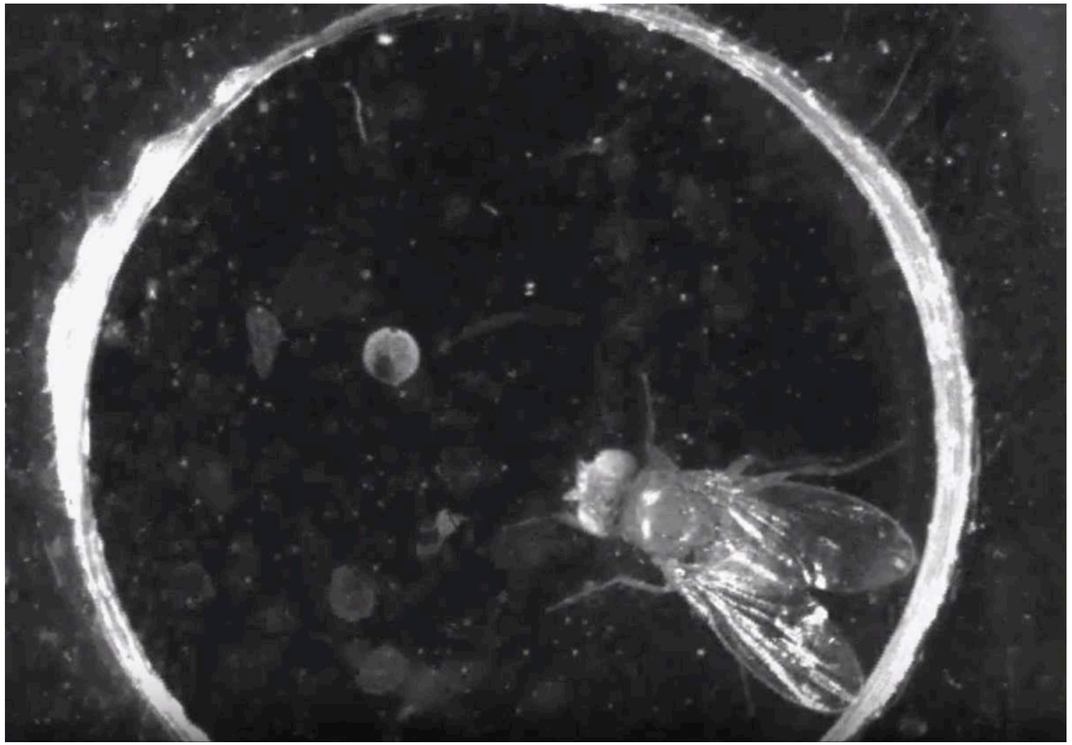
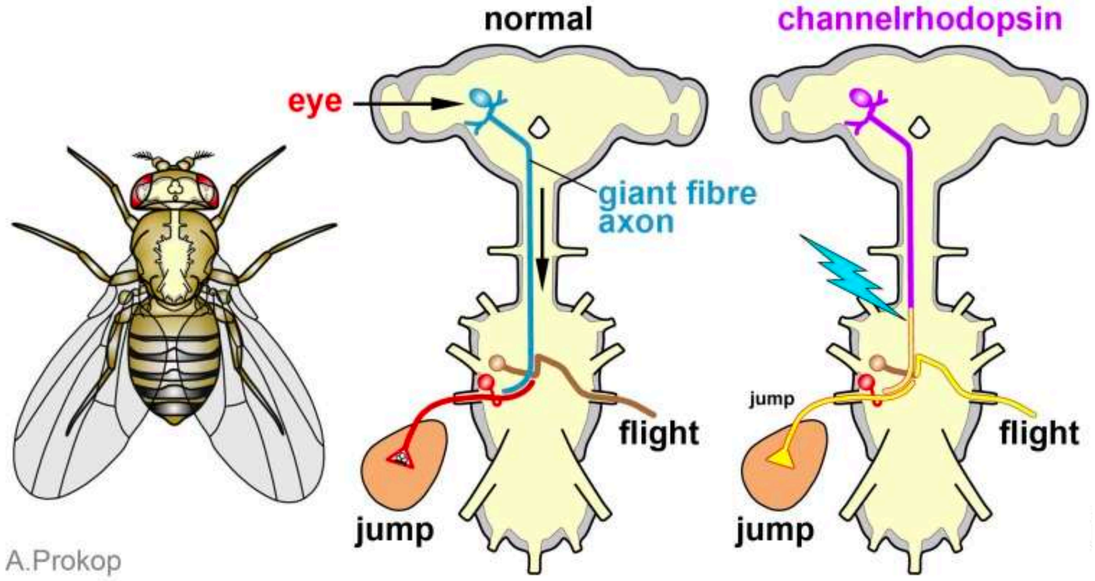
Mapping and controlling the *flight* response





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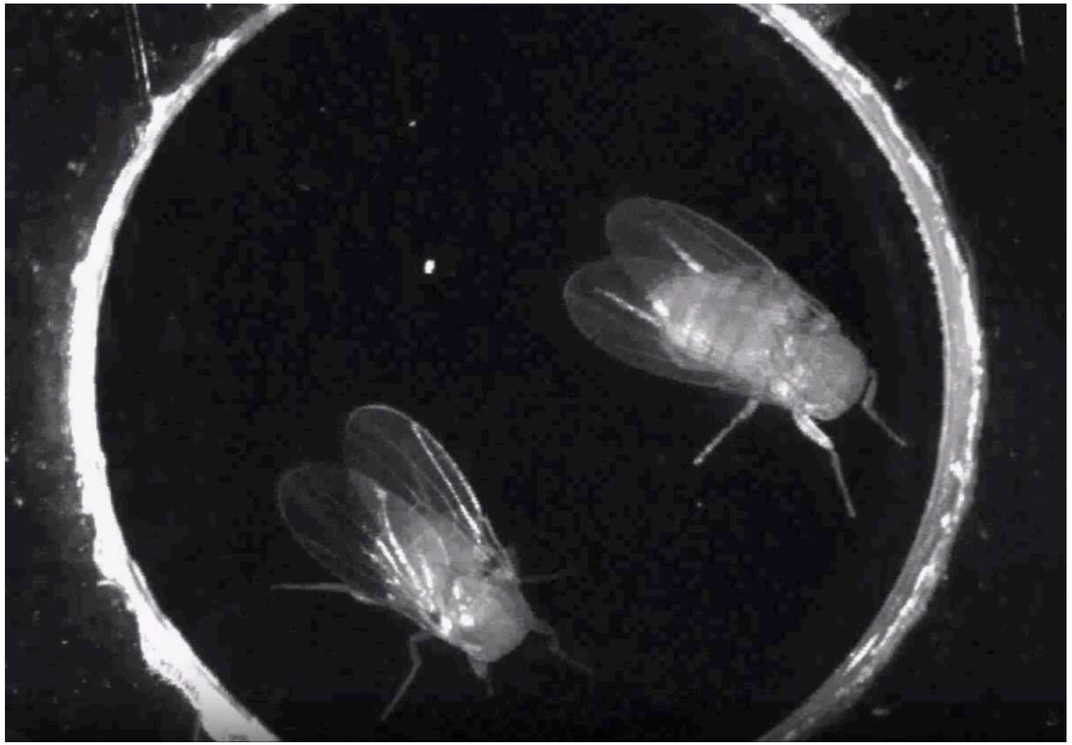
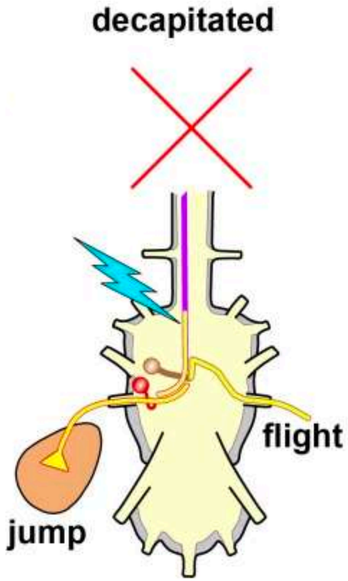
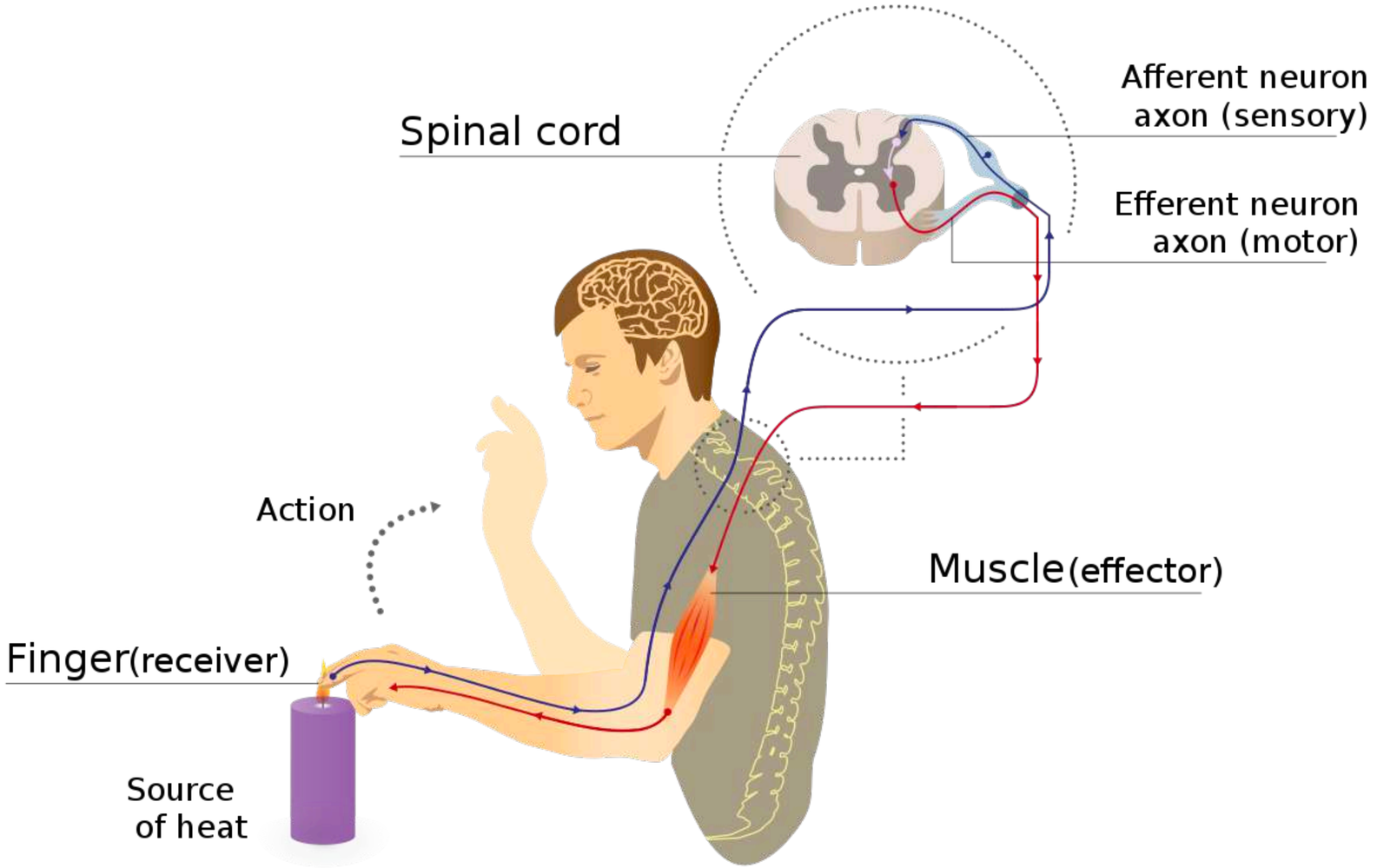
Mapping and controlling the *flight* response





Cell, **2005**, *121*(1), 141-152.

Mapping **reflex arcs** with optogenetics

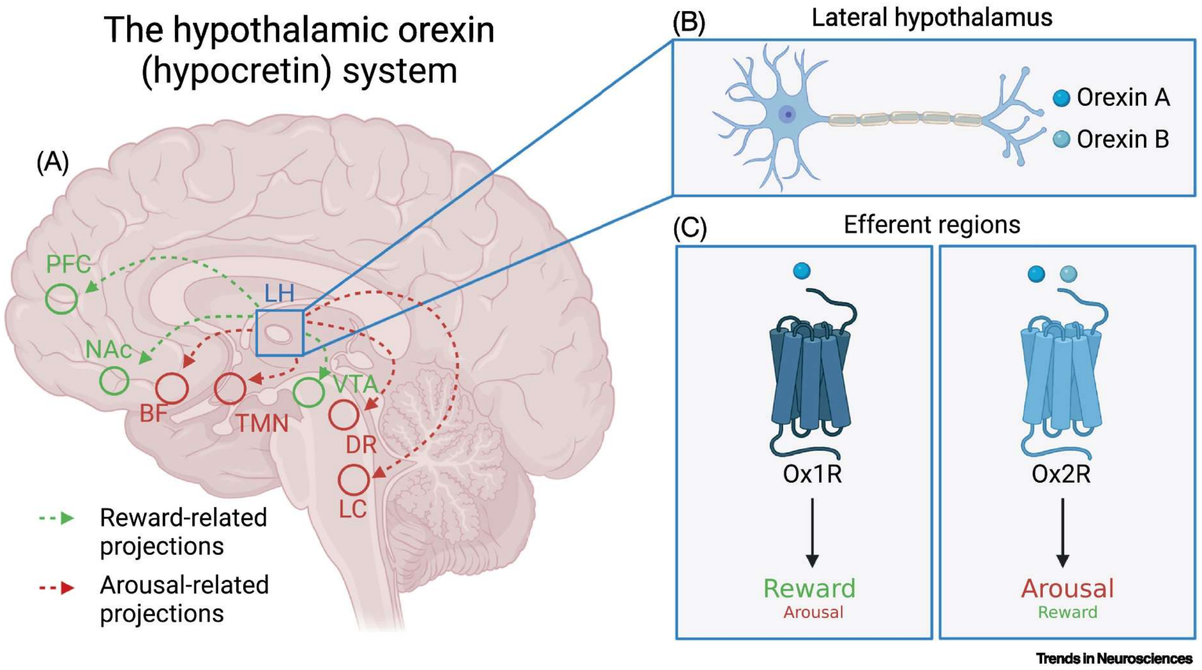


Cell, **2005**, *121*(1), 141-152.

Understanding **wake/sleep** cycles with optogenetics

Karl Deisseroth

Luis de Lecea



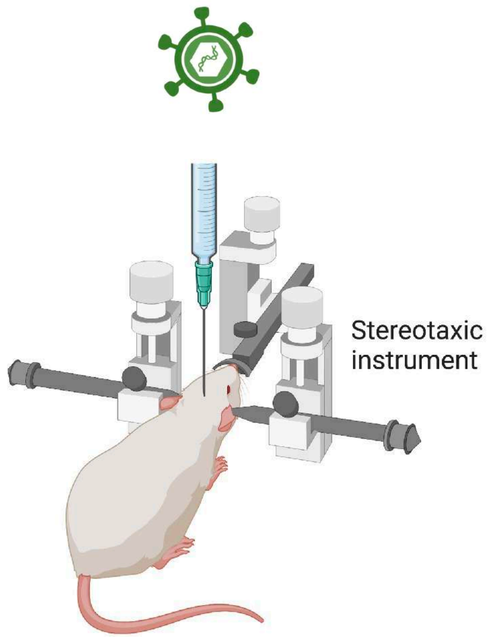
Nature, **2007**, *450*(7168), 420-424.

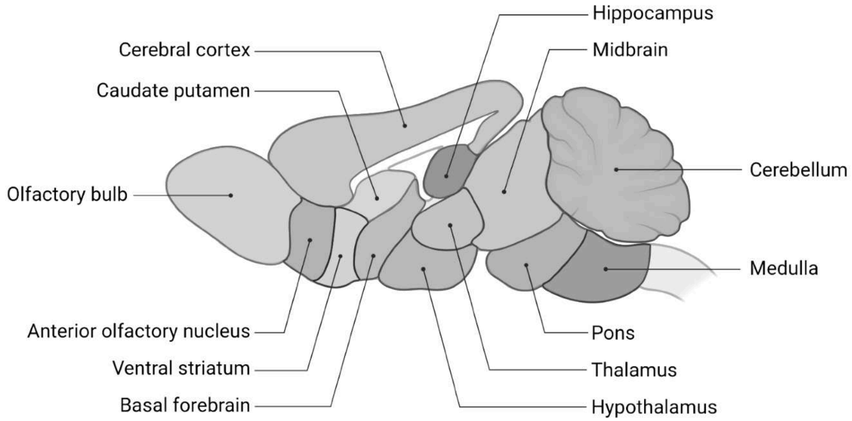
Understanding **wake/sleep** cycles with optogenetics

Karl Deisseroth

Luis de Lecea

virus containing channelrhodopsin DNA





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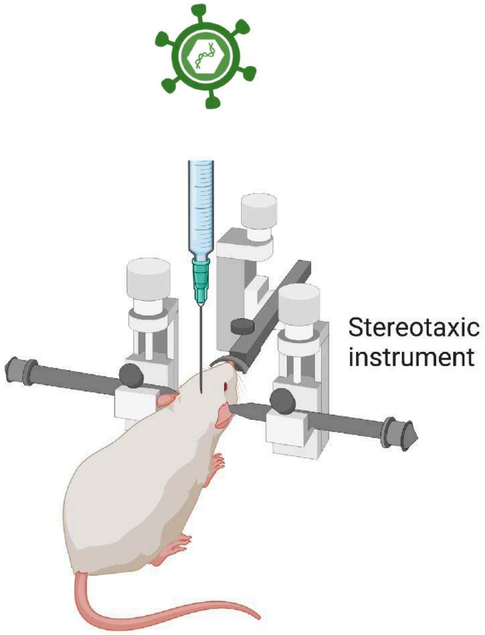

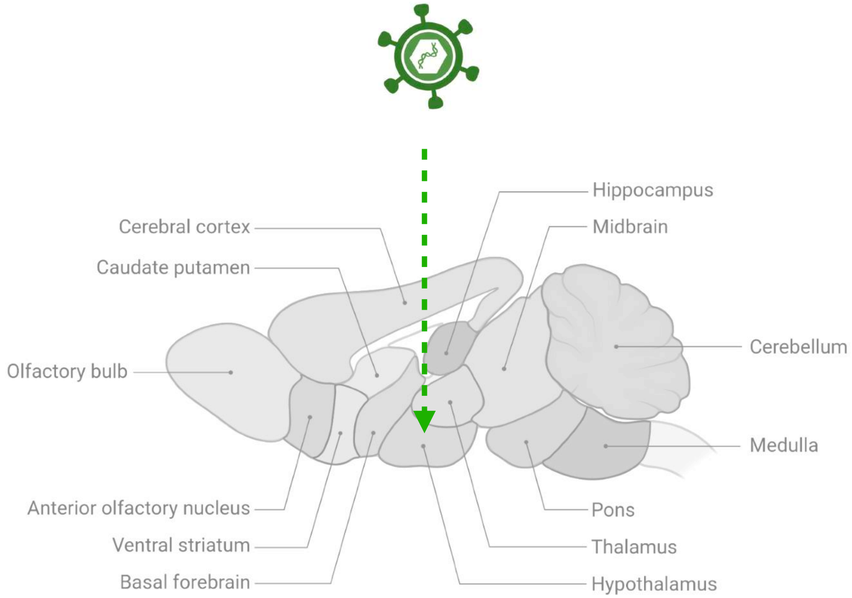

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Understanding **wake/sleep** cycles with optogenetics

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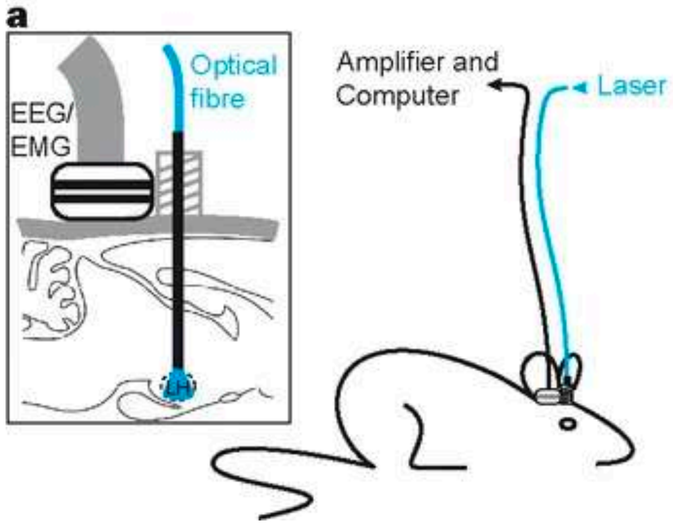
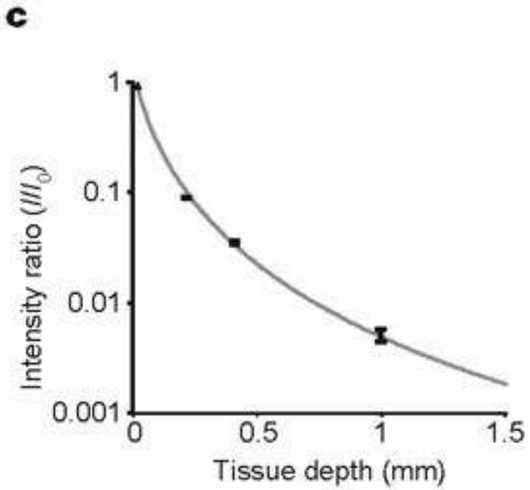
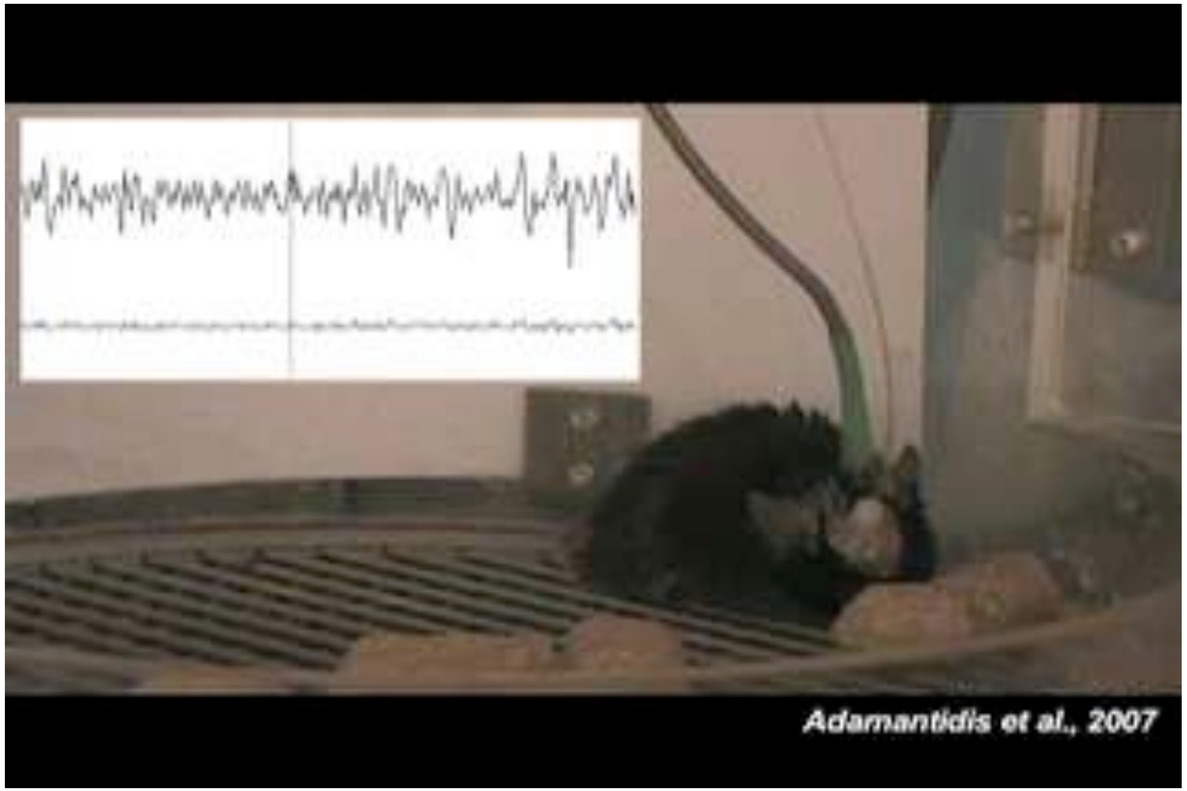
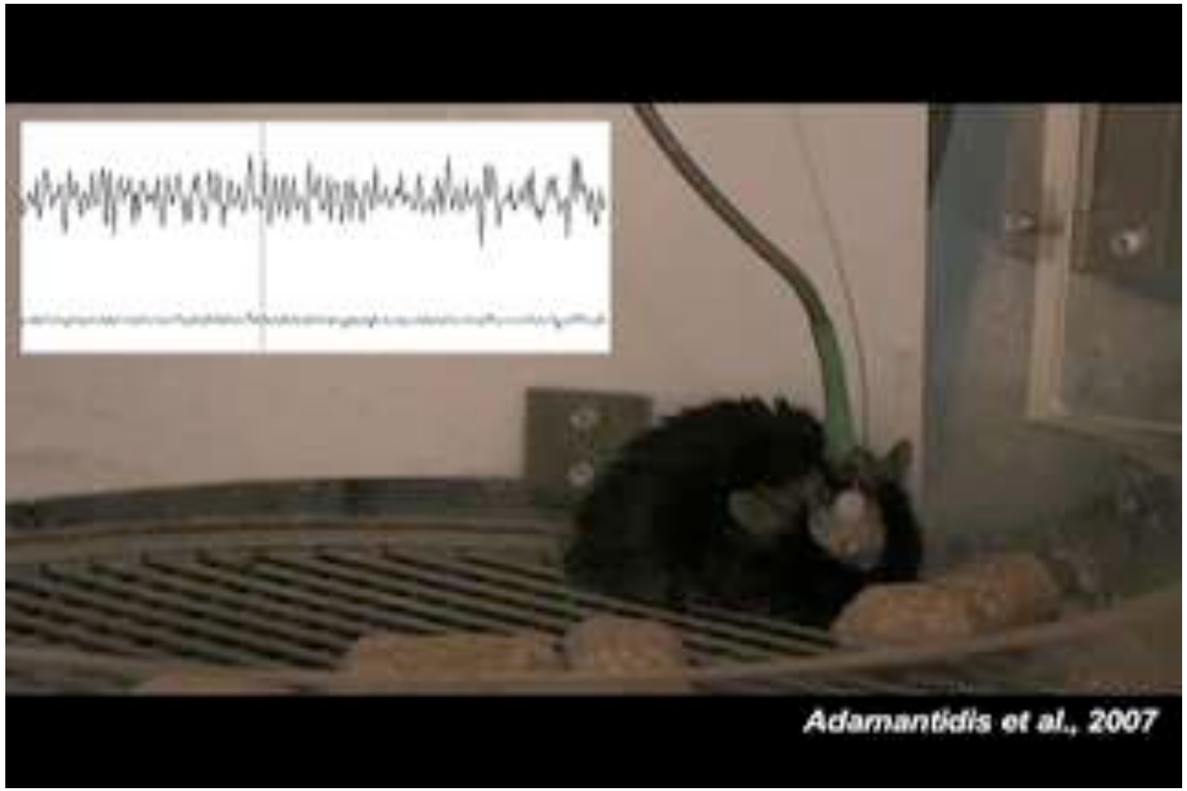




Nature, **2007**, *450*(7168), 420-424.

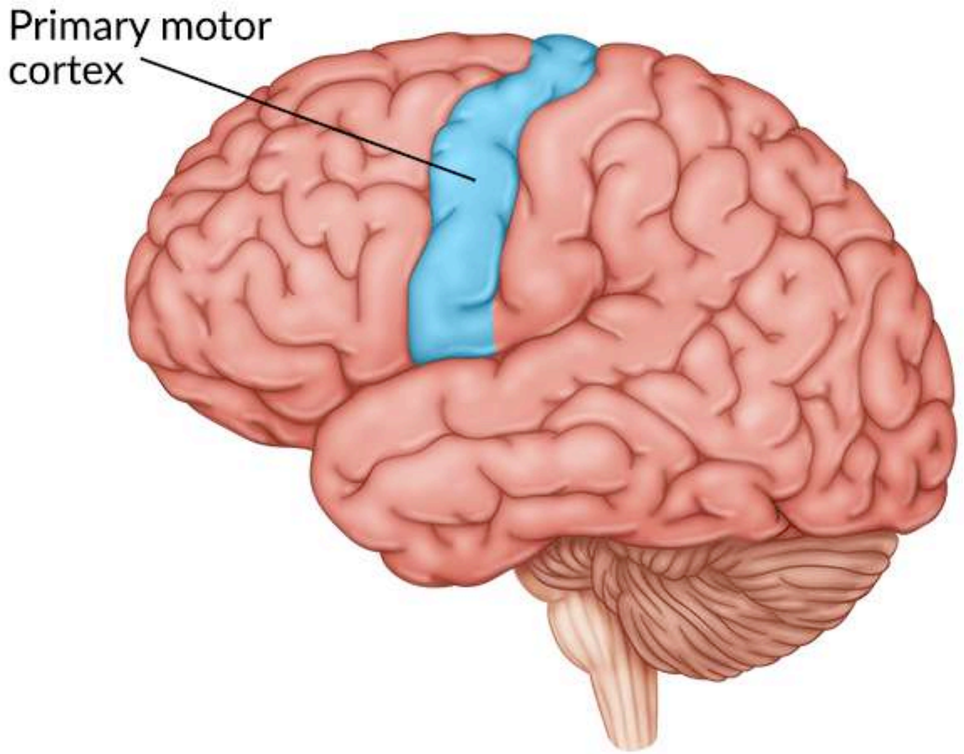
Understanding **wake/sleep** cycles with optogenetics

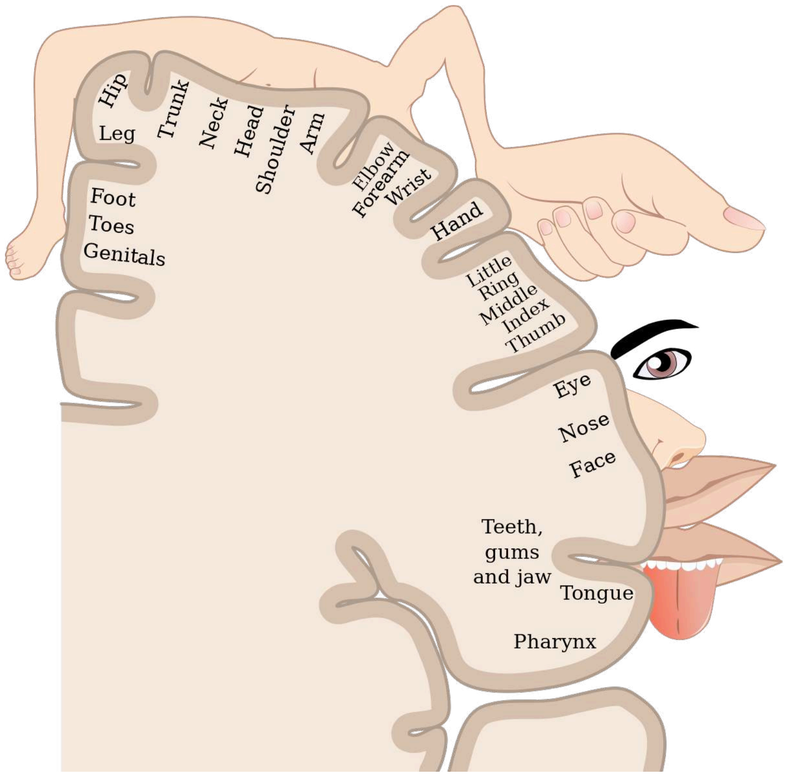




Nature, **2007**, *450*(7168), 420-424.

Mapping the **motor cortex** with optogenetics



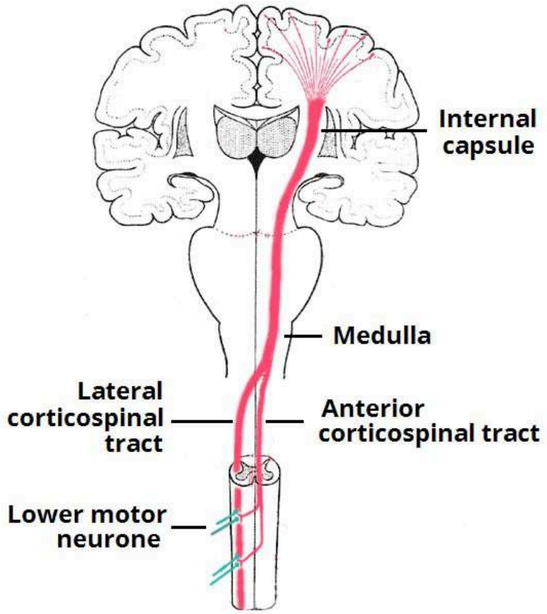


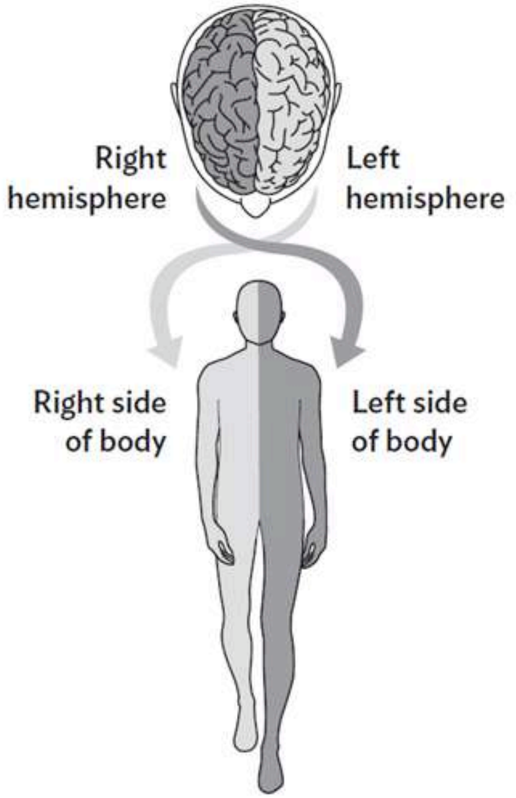
J Neuroscience, **2008** 27(52), 14231-14238.

Mapping the **motor cortex** with optogenetics

Karl Deisseroth

Viviana Gradinaru





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Mapping the **motor cortex** with optogenetics

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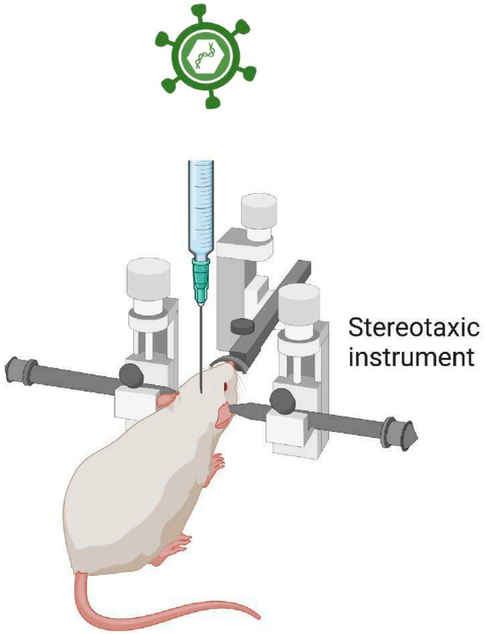
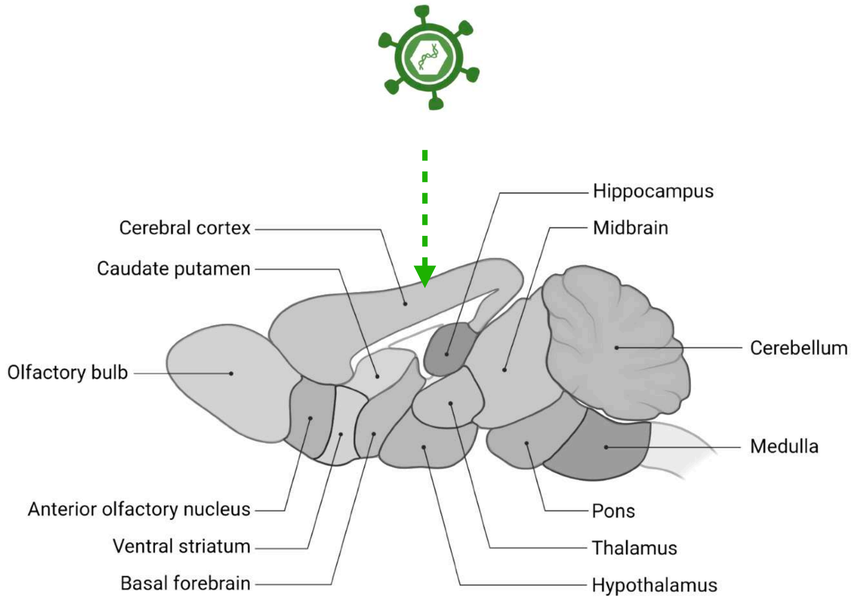
virus containing channelrhodopsin DNA





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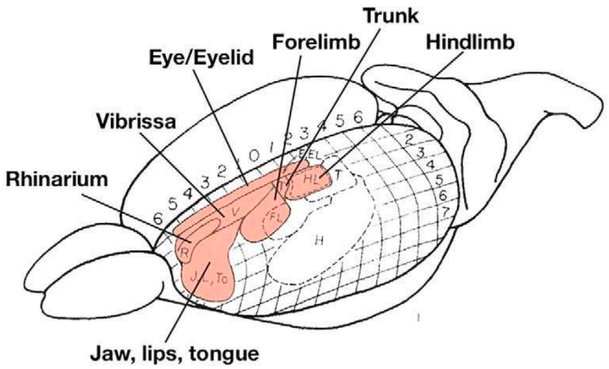

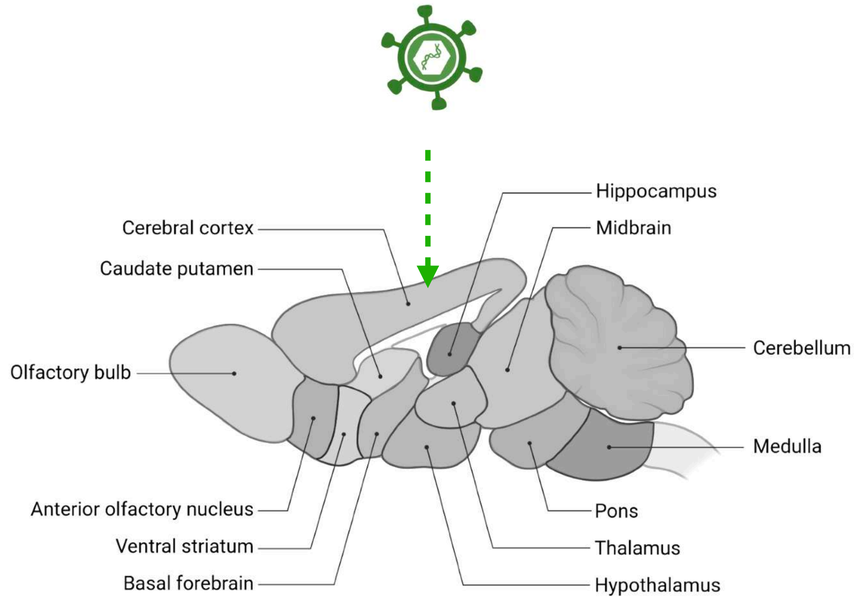

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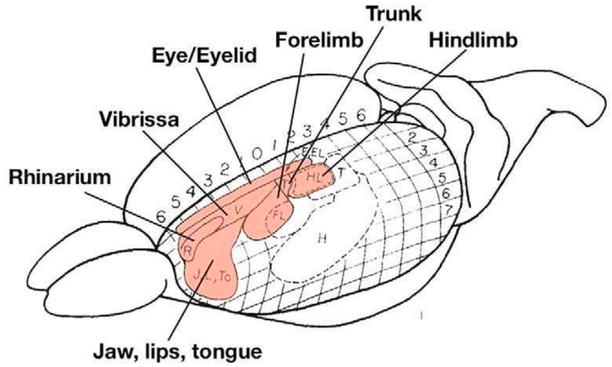
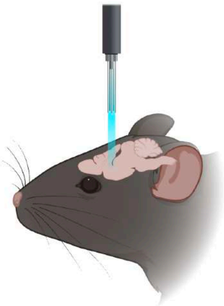


right motor cortex
= left body movement



J Neuroscience, **2008** 27(52), 14231-14238.

Object <u>fascination</u> in the hypothalamus

Daesoo Kim

Sae-Geun Park[1,3]

Yong-Cheol Jeong[1,3]

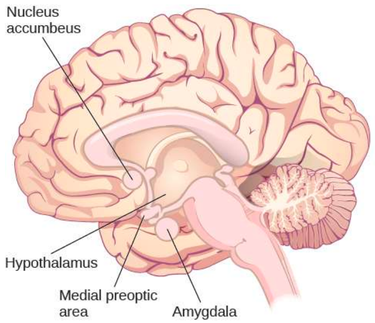
Dae-Gun Kim[1,2,3]

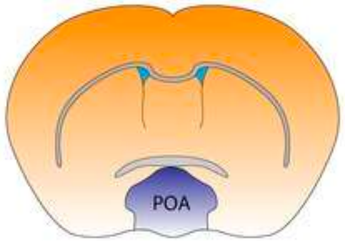
Phill-Seung Lee[2*]

Nature Neuroscience, **2018**, 21(3), 364-372.

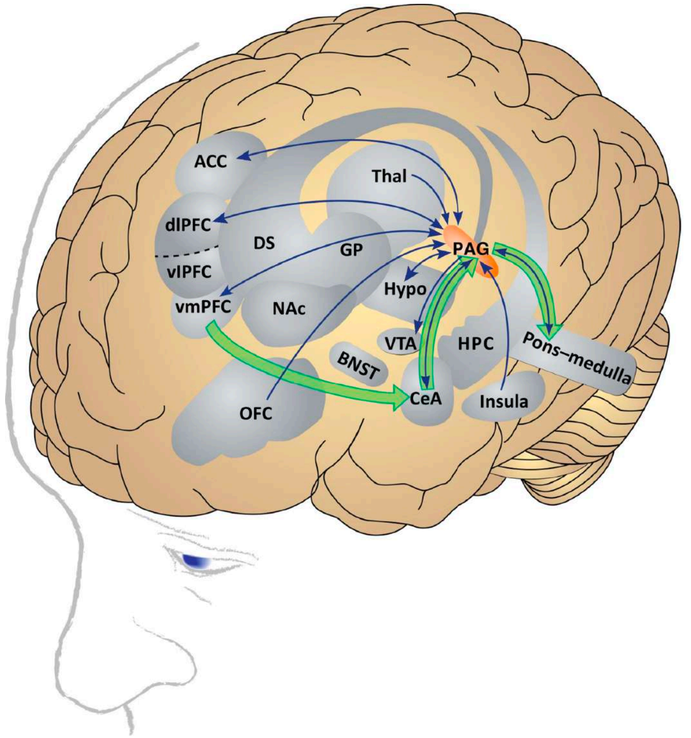
Object <u>fascination</u> in the hypothalamus

pre-optic area (POA)



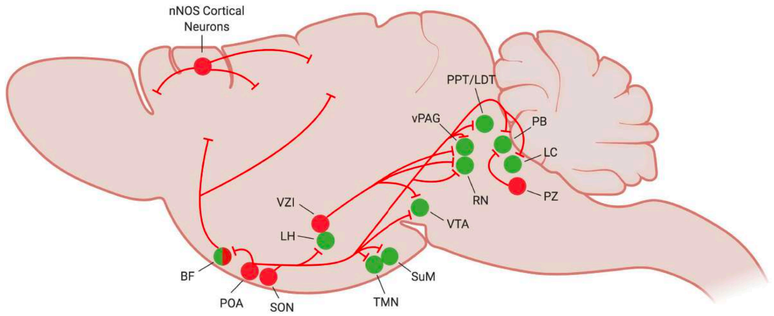


ventral periaqueductal gray region (vPAG)

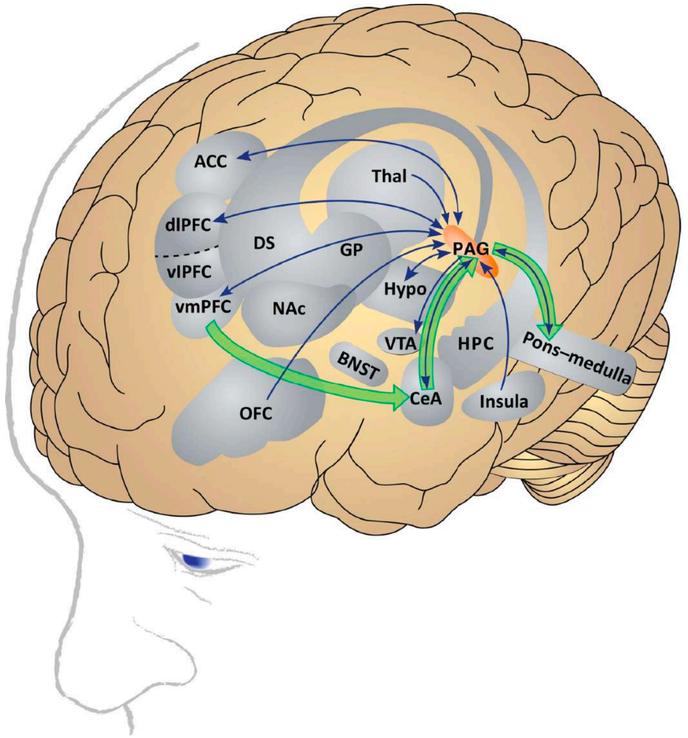


Nature Neuroscience, **2018**, 21(3), 364-372.

Object <u>fascination</u> in the hypothalamus

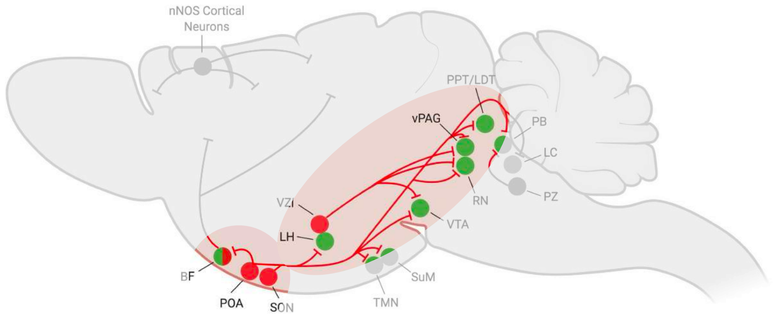


ventral periaqueductal gray region (vPAG)

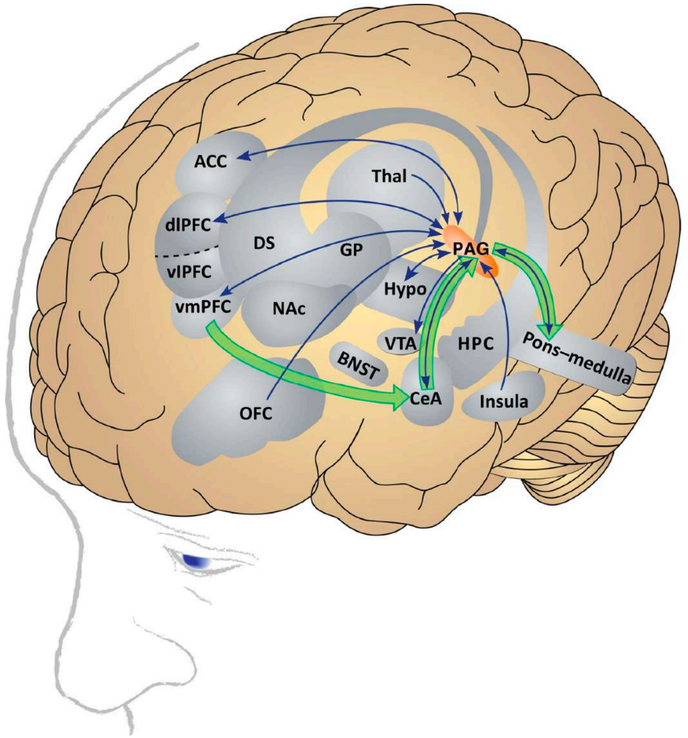


Nature Neuroscience, **2018**, 21(3), 364-372.

Object <u>fascination</u> in the hypothalamus



ventral periaqueductal gray region (vPAG)



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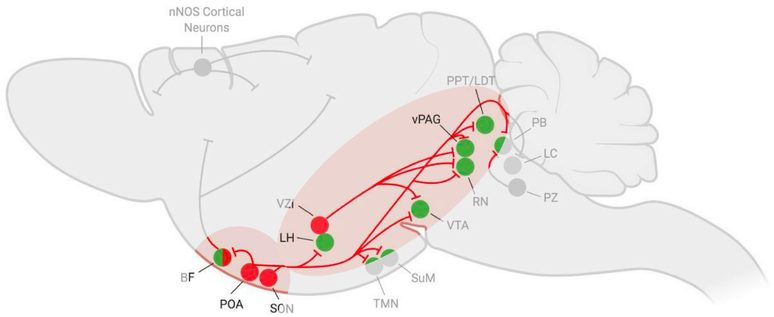
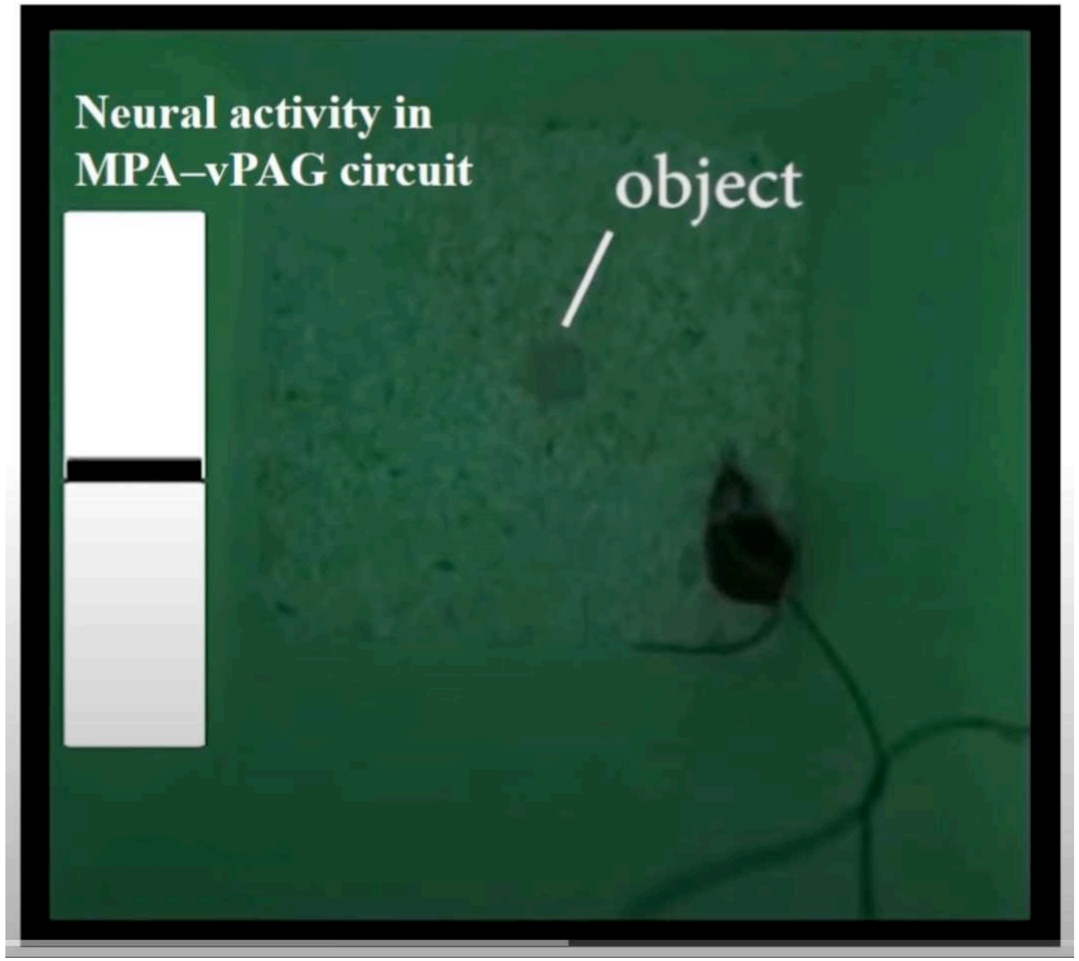
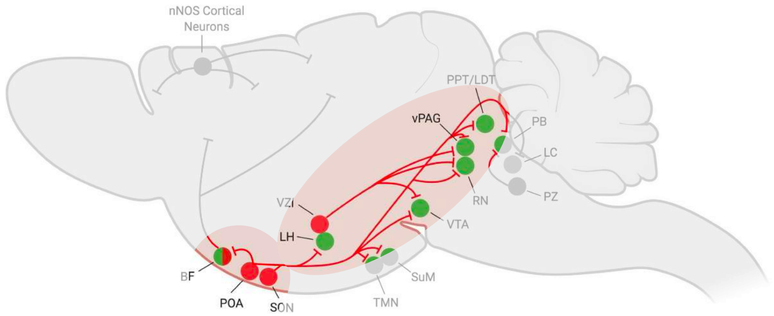
Object <u>fascination</u> in the hypothalamus

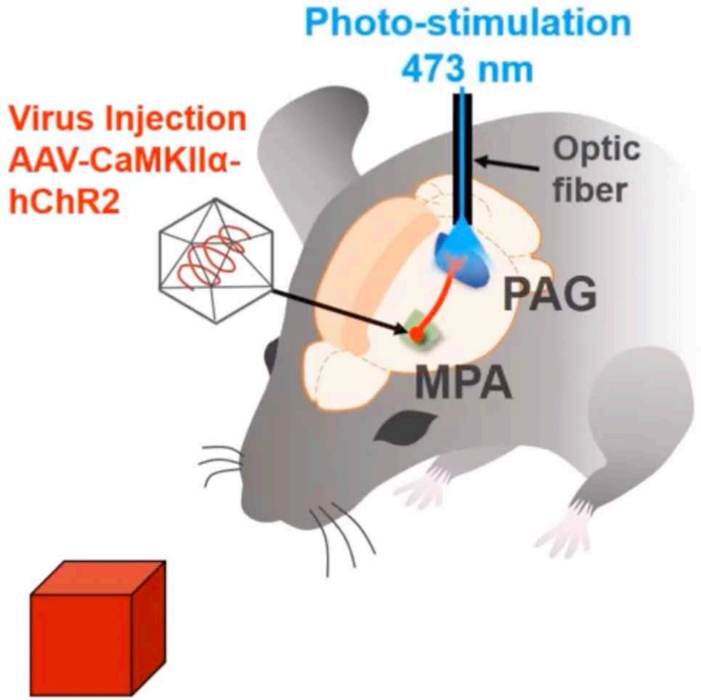




Nature Neuroscience, **2018**, 21(3), 364-372.

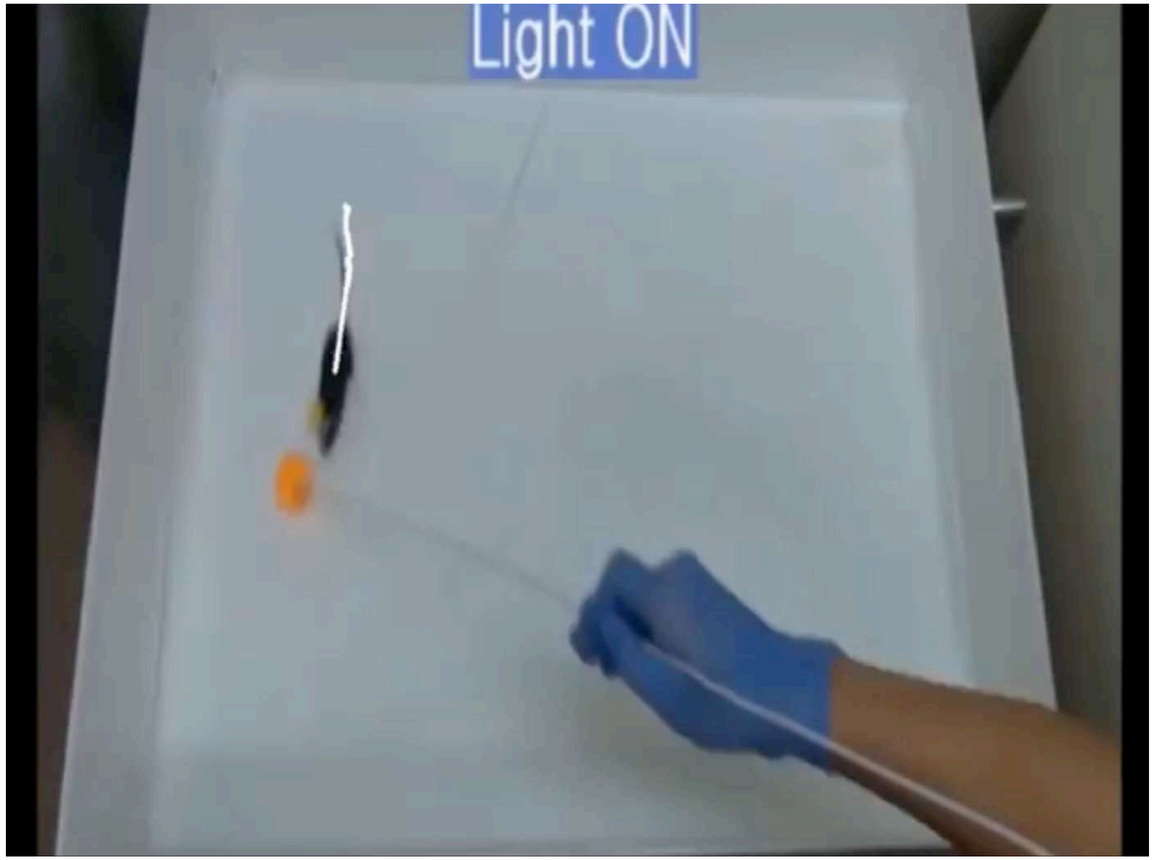
Object <u>fascination</u> *induced with light*

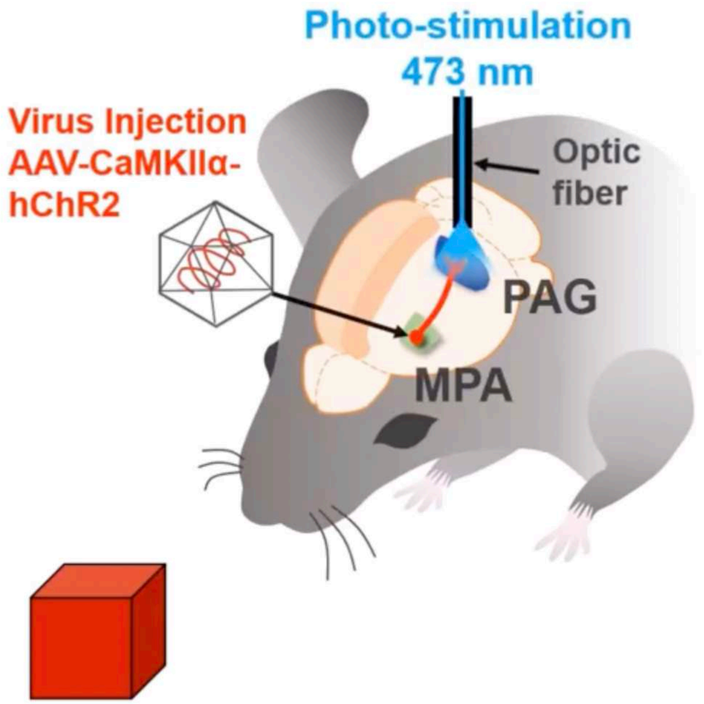




Nature Neuroscience, **2018**, 21(3), 364-372.

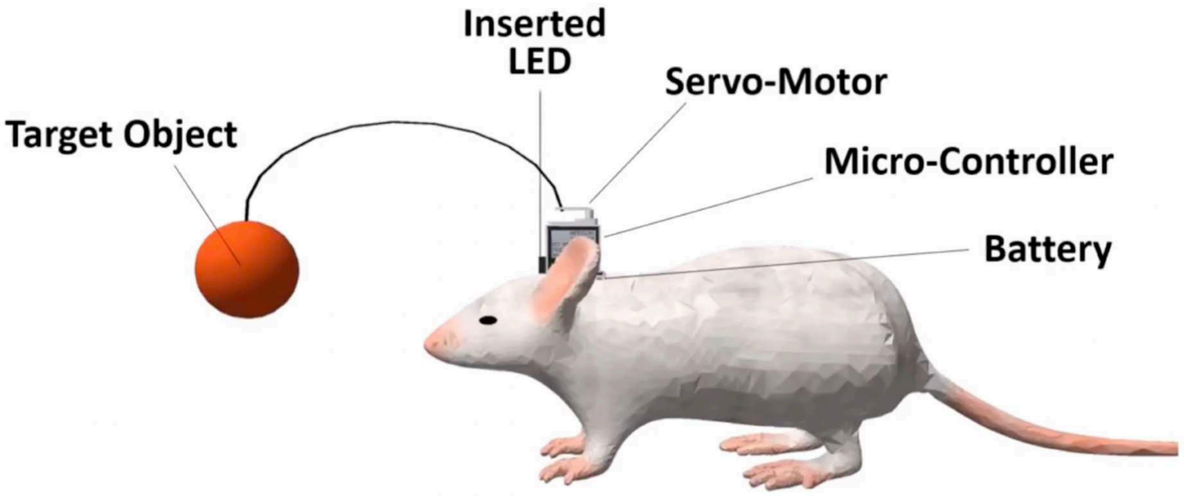
Object <u>fascination</u> *induced with light*

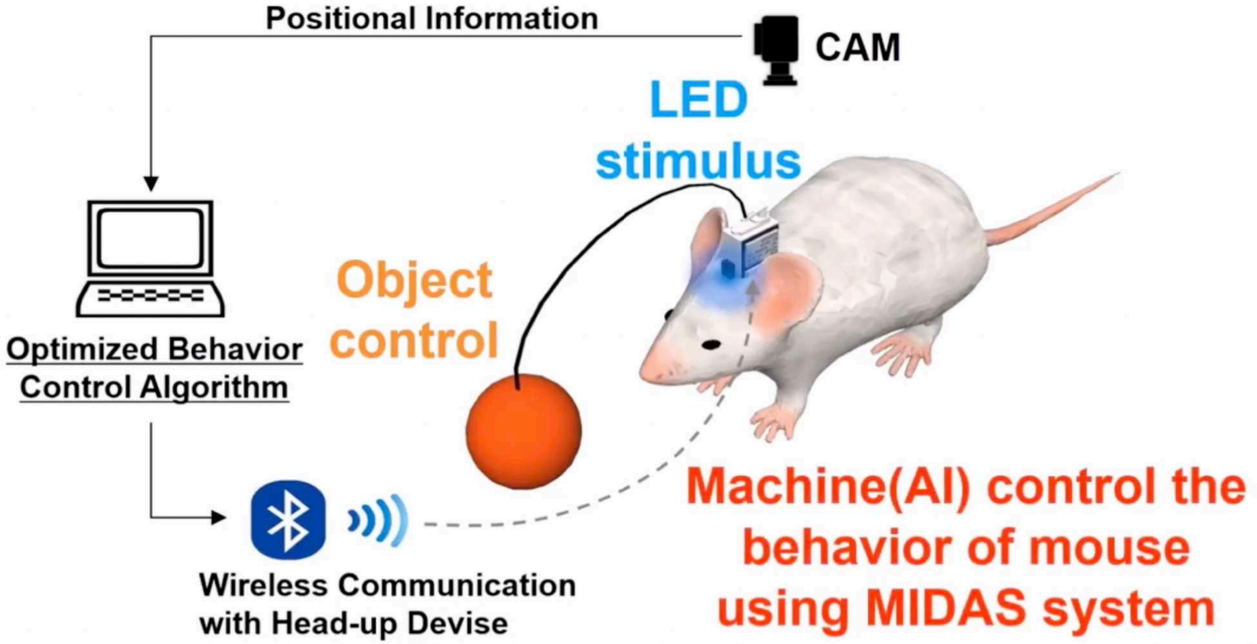




Nature Neuroscience, **2018**, 21(3), 364-372.

Controlling mouse movement with optogenetic object fascination





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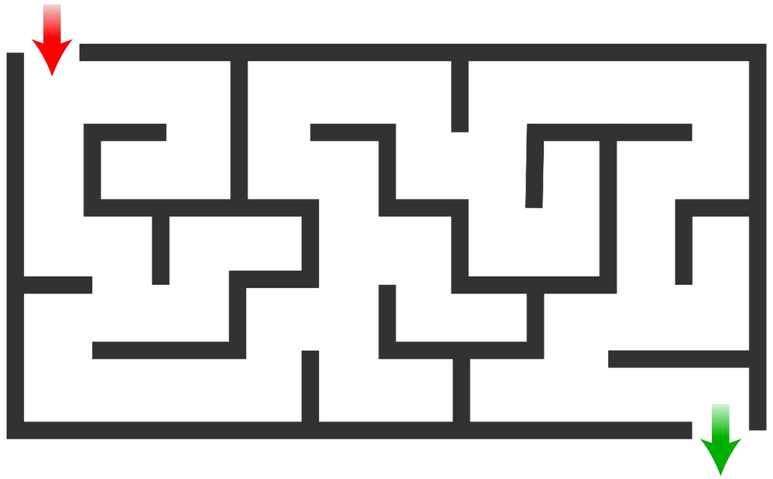
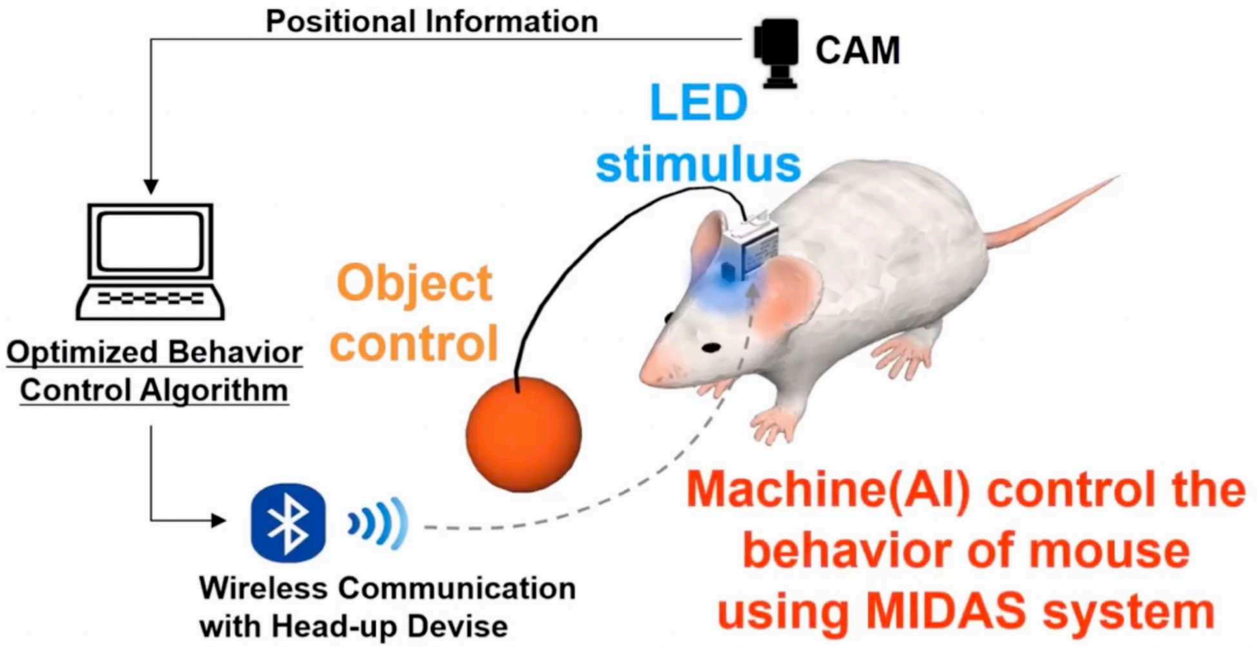
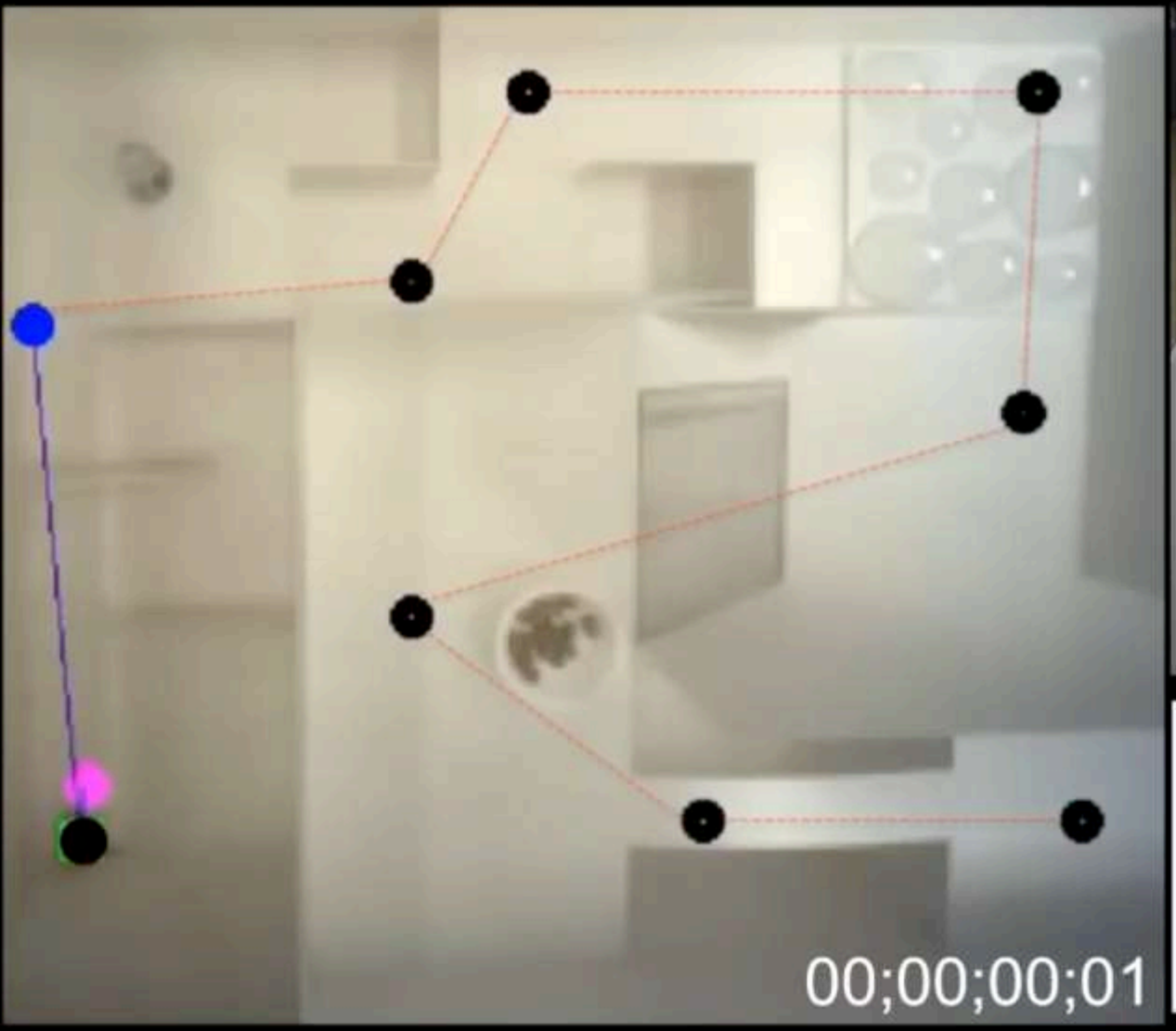
Controlling mouse movement with optogenetic object fascination

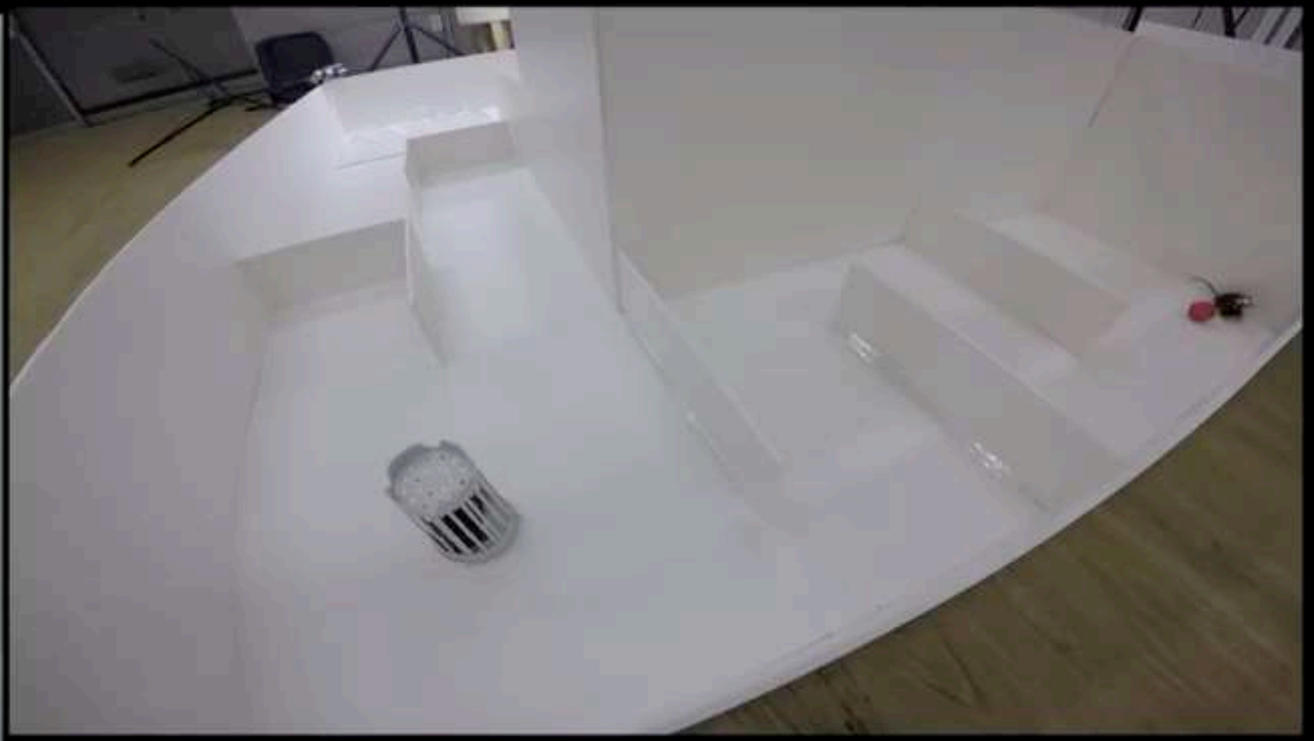


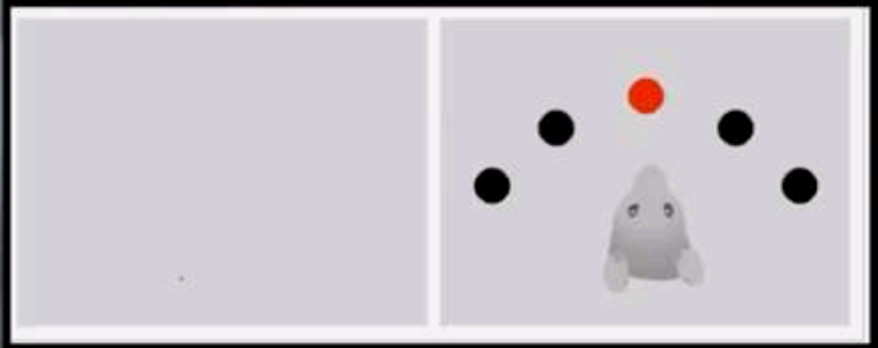


Nature Neuroscience, **2018**, 21(3), 364-372.

Automated 3D-maze navigation
by using object-chasing behavior in mice







Aniborg

KAIST

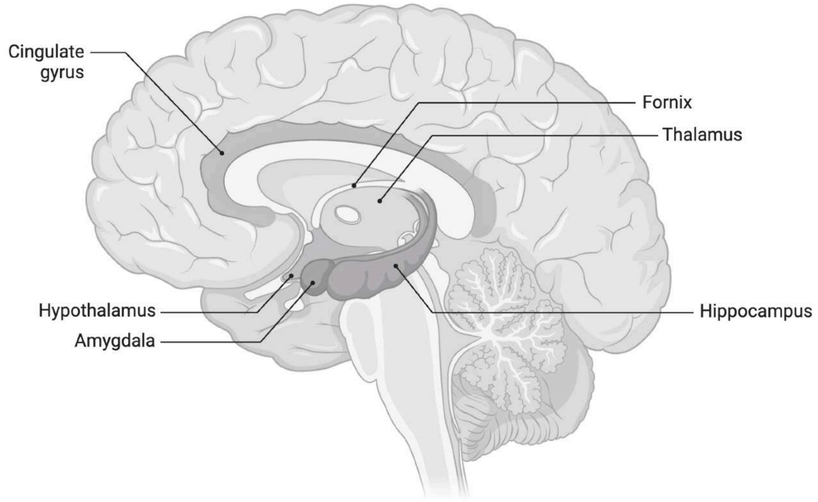
Guiding
without light stimulation

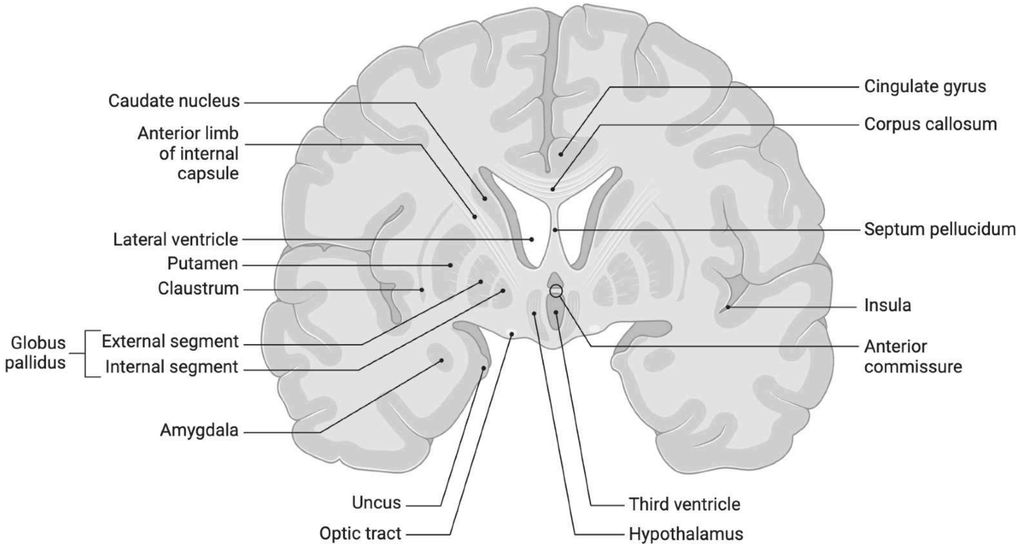
Guiding
with light stimulation

Mapping/tagging memories with optogenetics

what is memory?

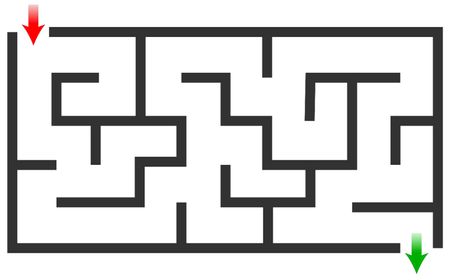
where and how is it stored?





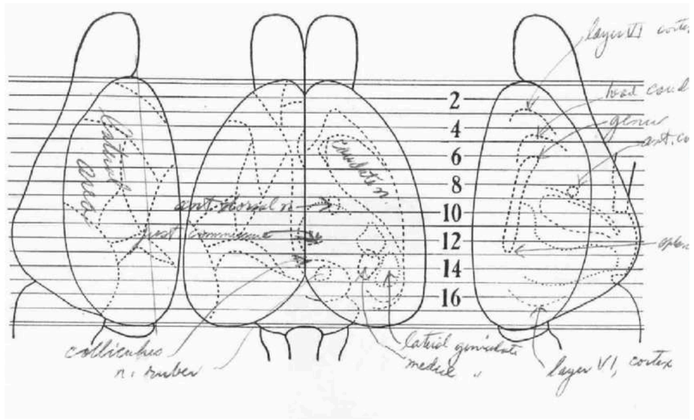
Searching for the **engram**

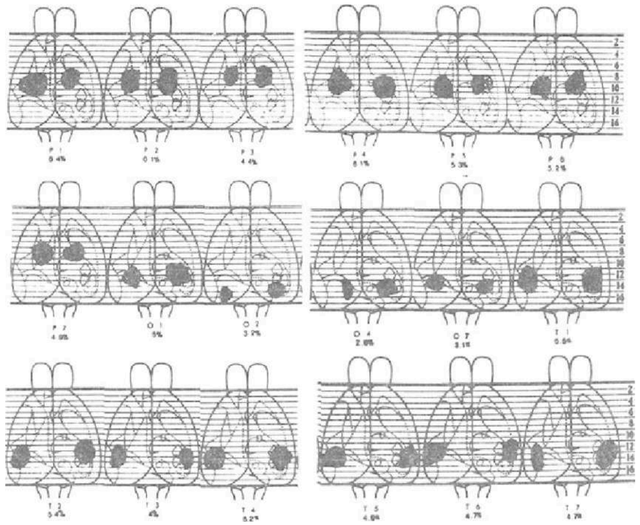
Karl Lashley



1. *tissue amount mattered (>10-15% mass)*

2. *location made no difference*





Searching for the **engram**

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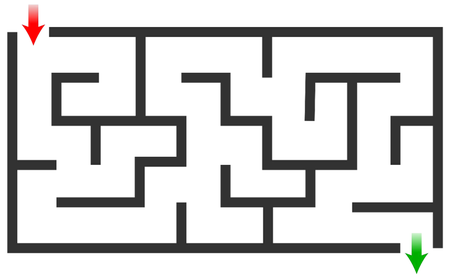
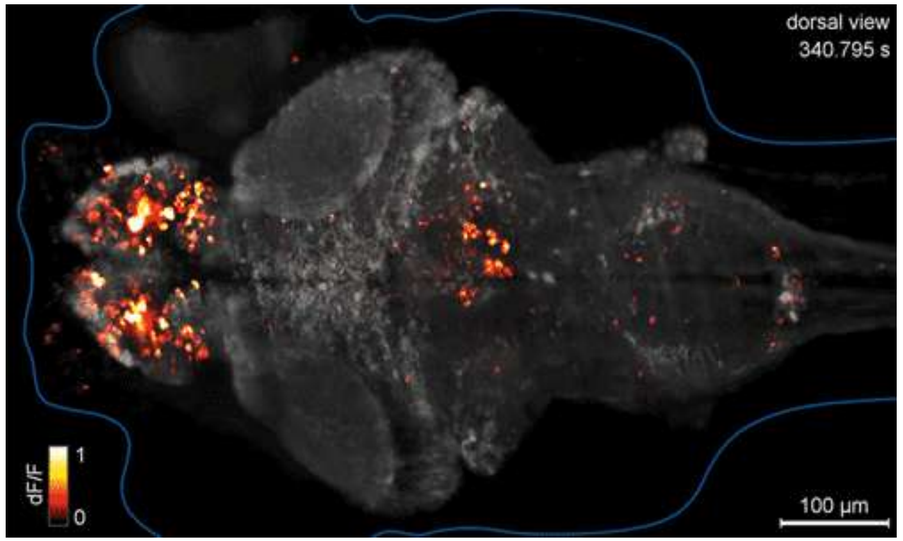
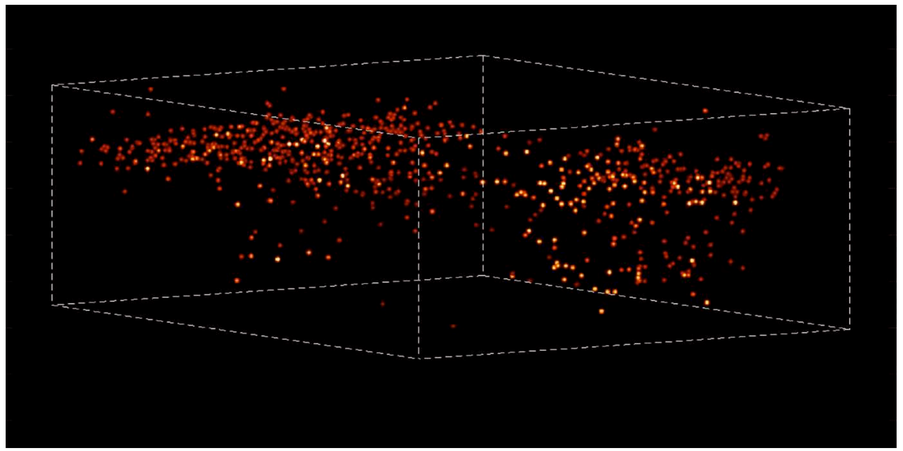


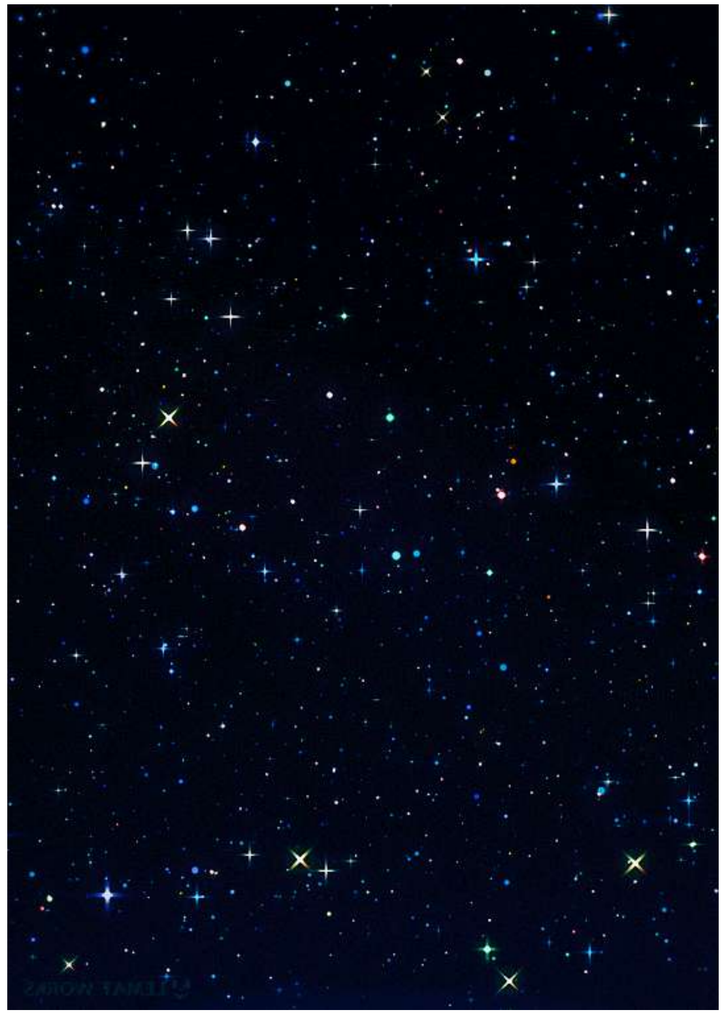
1. *tissue amount mattered (>10-15% mass)*
2. *location made no difference*

1. ***mass action*** *learning/memory is distributed across the brain*
2. ***equipotentiality*** *different regions can compensate for damage*

A memory is a collection of neurons







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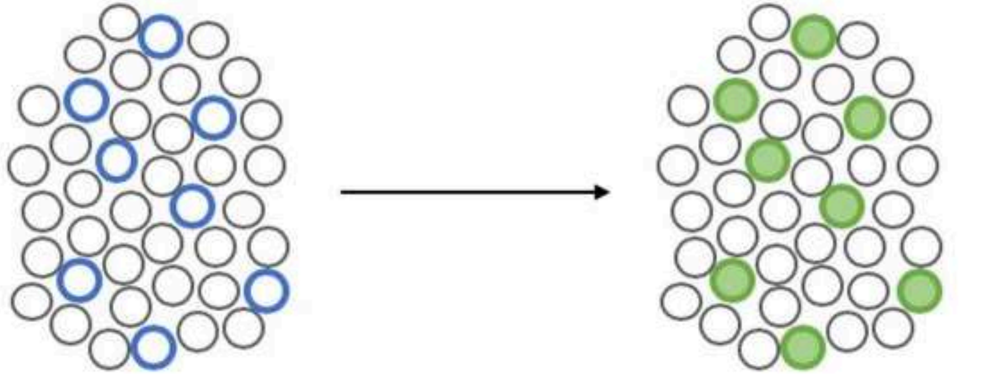
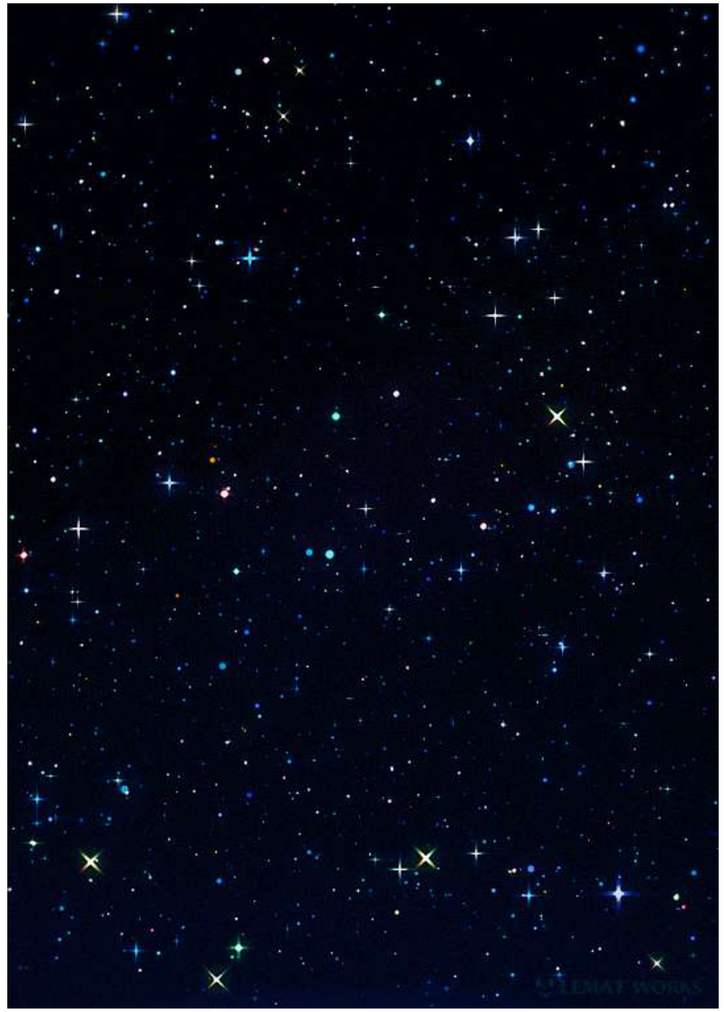


Neurons with relatively higher excitability at the time of training

Neurons with relatively higher excitability are preferentially allocated to an engram



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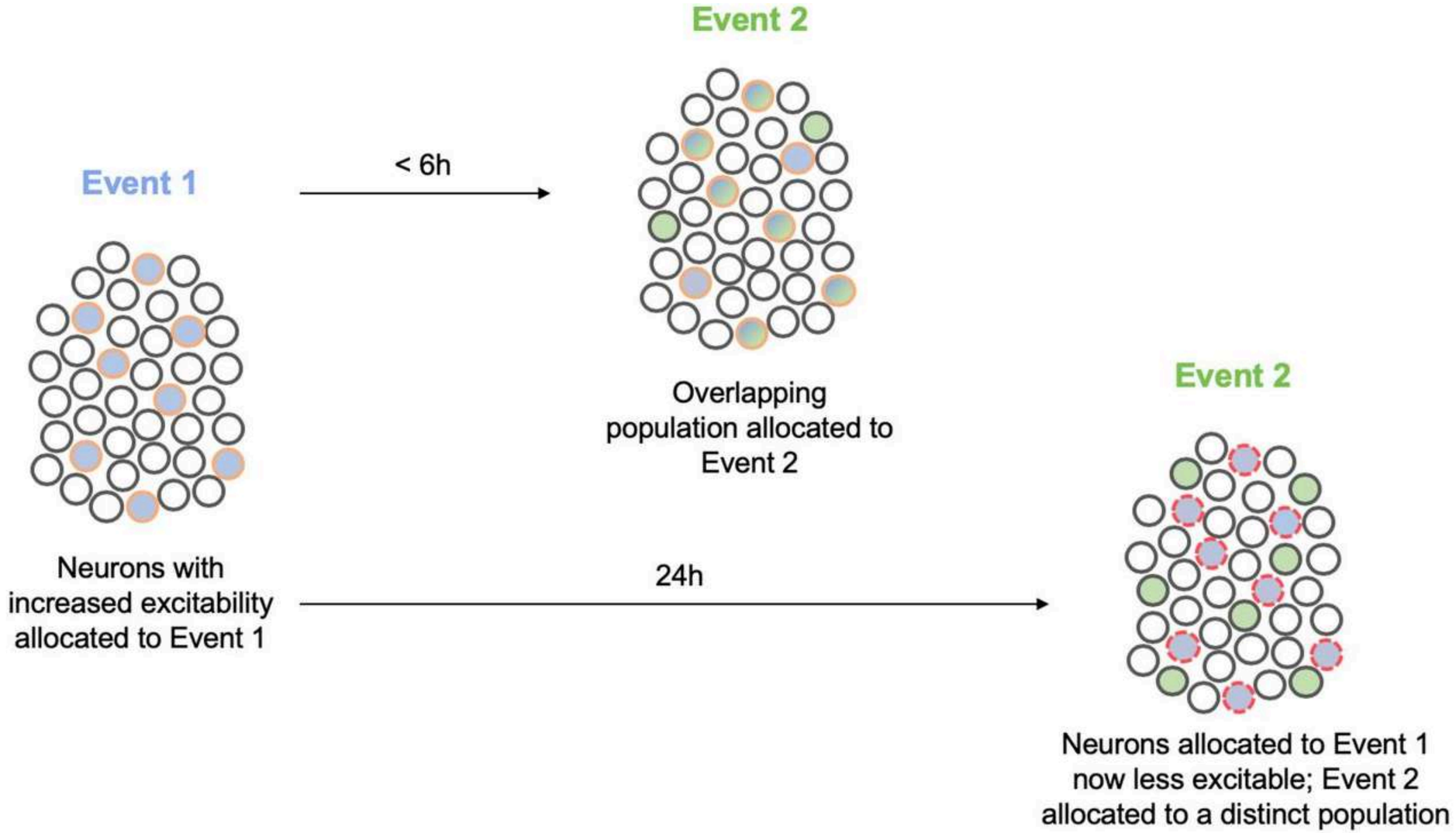
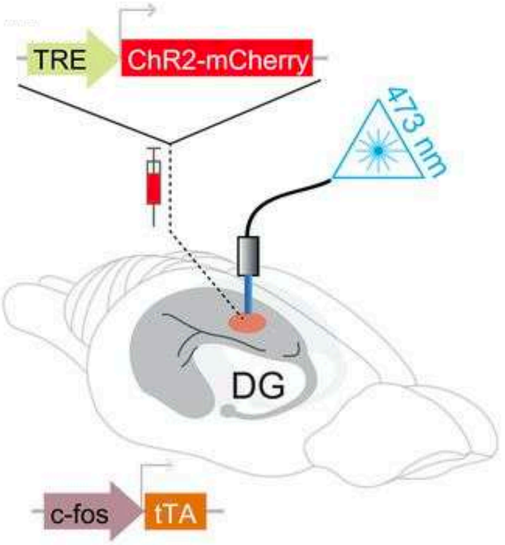
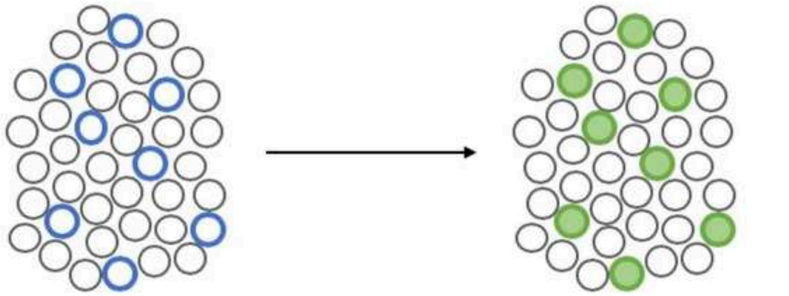


Mapping/tagging memories with optogenetics

inducible ChR expression





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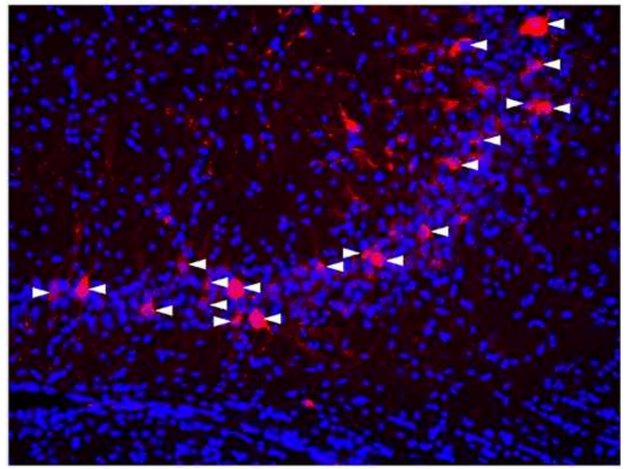
ChR expression

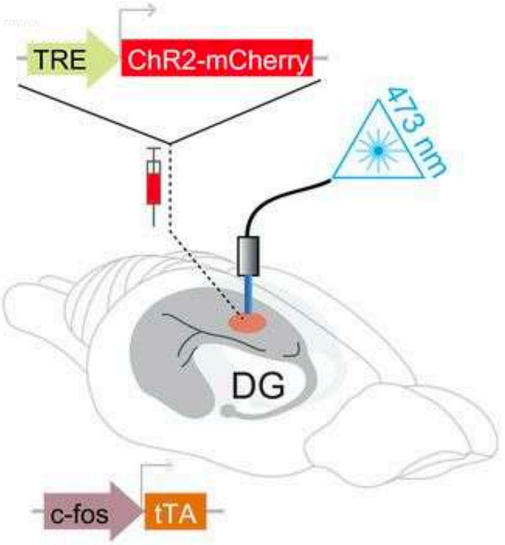


only neurons active during an event will express channelrhodopsin

Science, **2020,** *367*(6473), eaaw4325.

Mapping/tagging memories with optogenetics

inducible ChR expression



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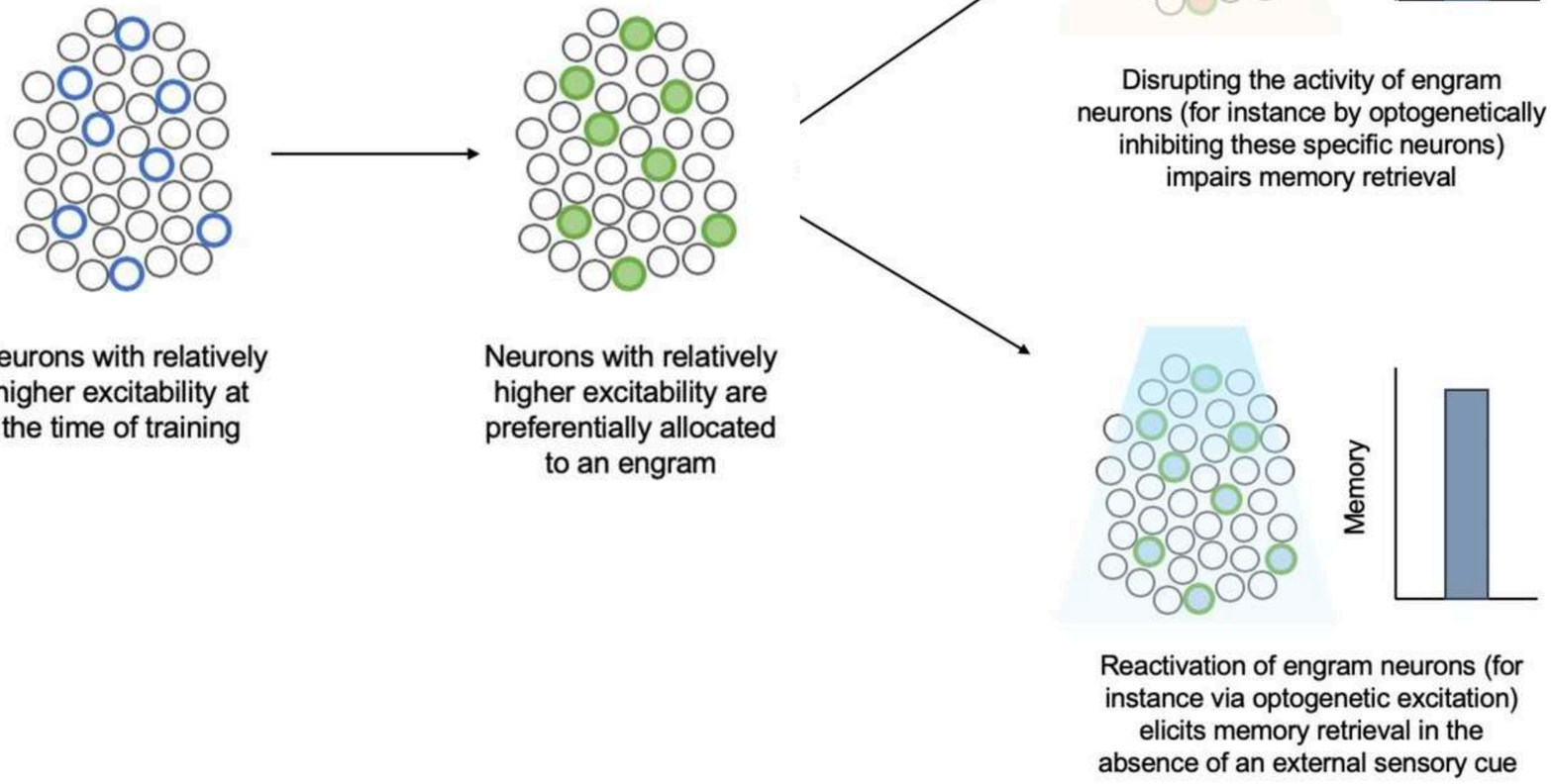
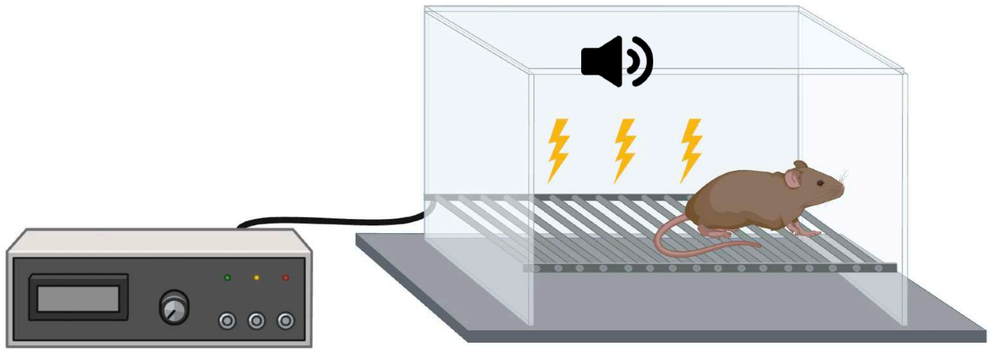


Science, **2020,** *367*(6473), eaaw4325.

Memory manipulation worklfow

fear conditioning

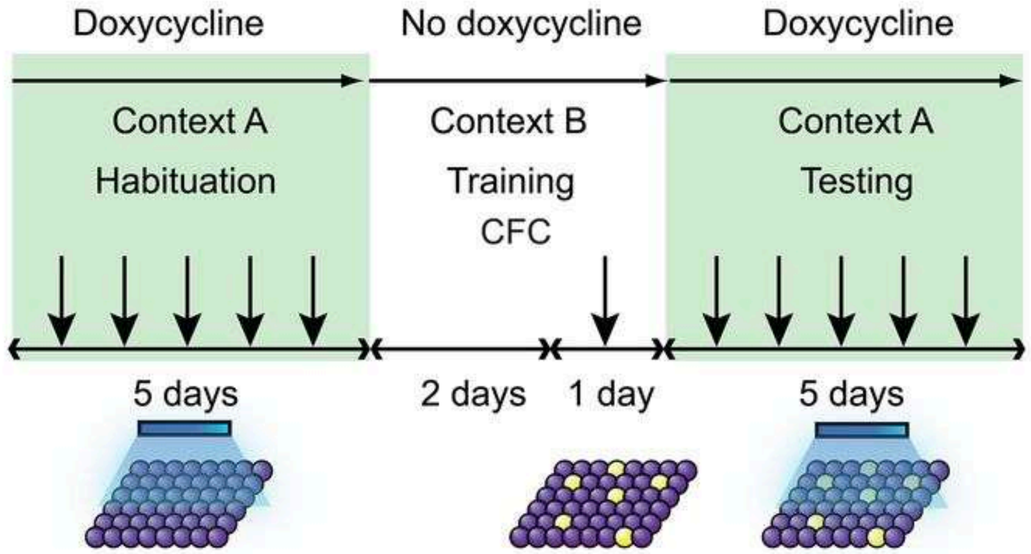


the bad memory:

audible cue followed by shock

now blue-light activatable

even in absence of cue



Science, **2020,** *367*(6473), eaaw4325.

Memory manipulation worklfow

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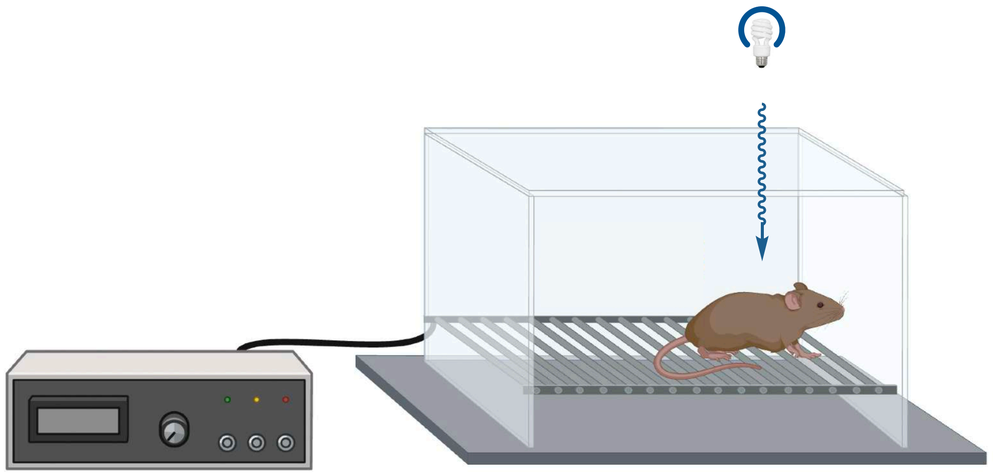
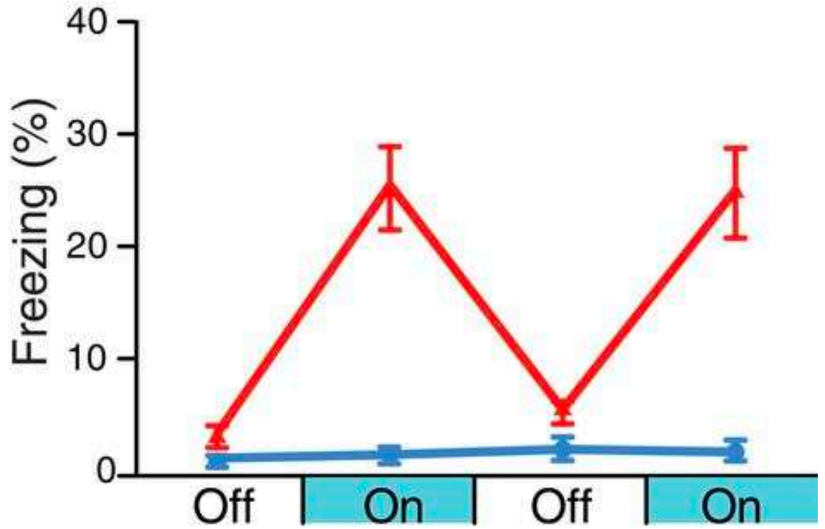


the bad memory:

now blue-light activatable

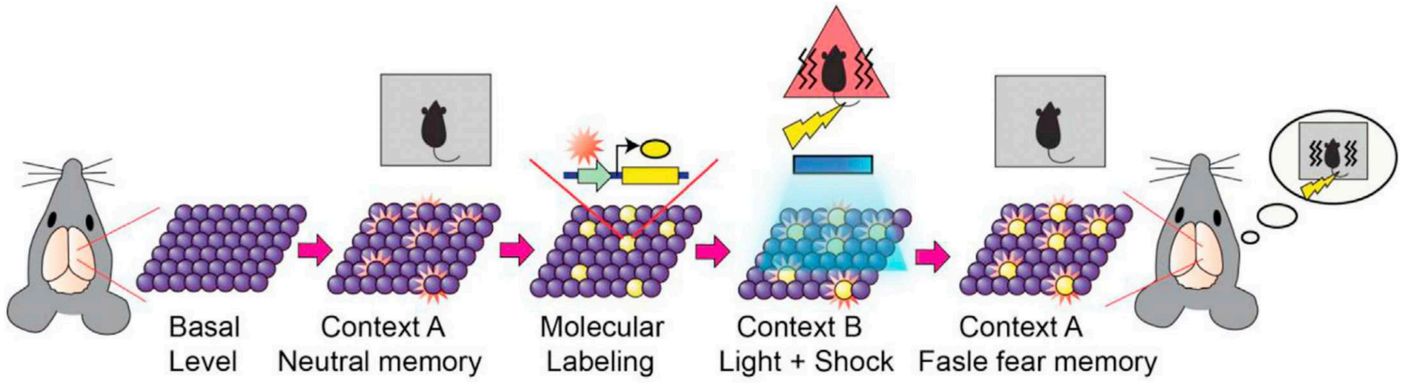
even in absence of cue

blue-light activated memory recall



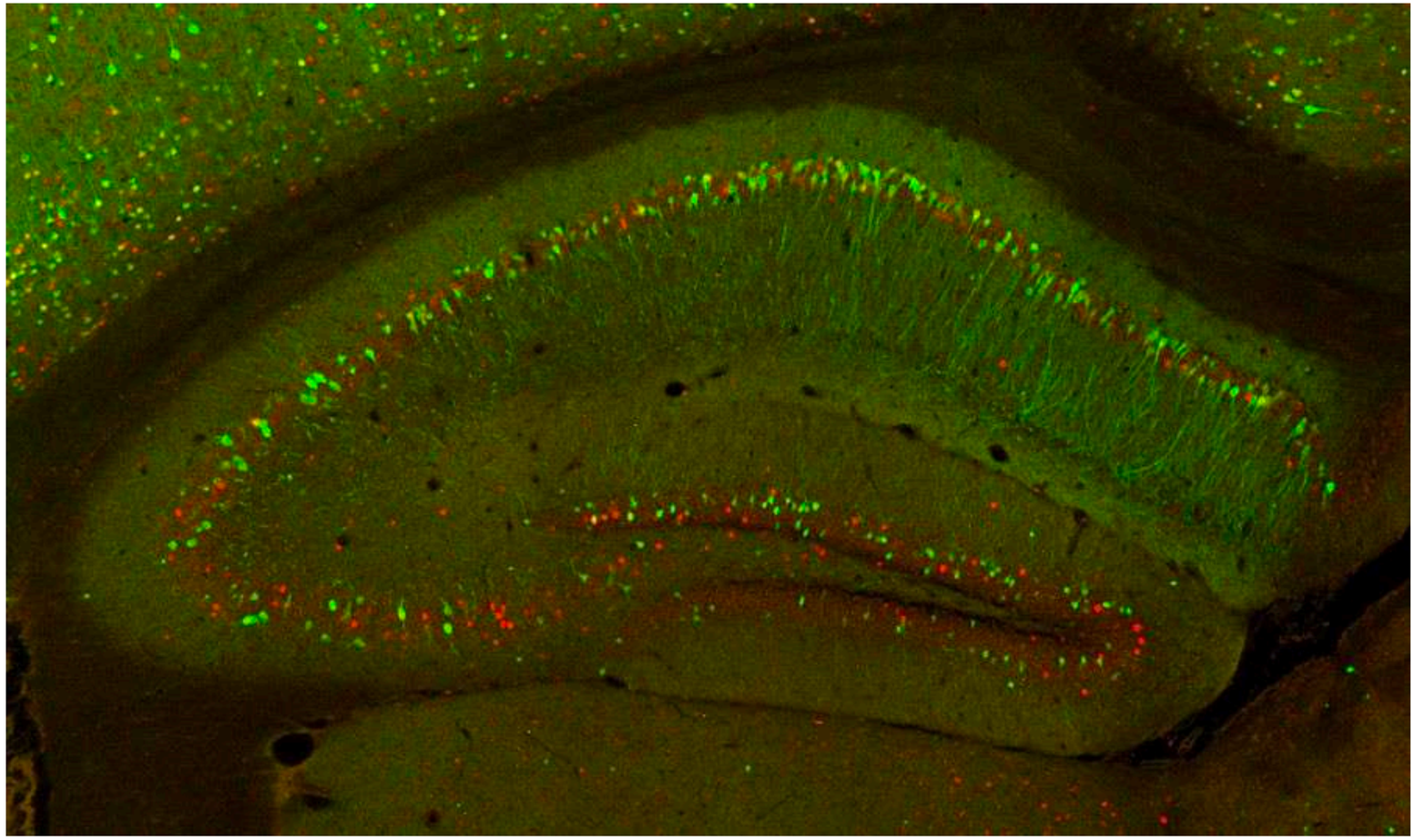
Science, **2020,** *367*(6473), eaaw4325.

Optogenetic inception of **false** memories

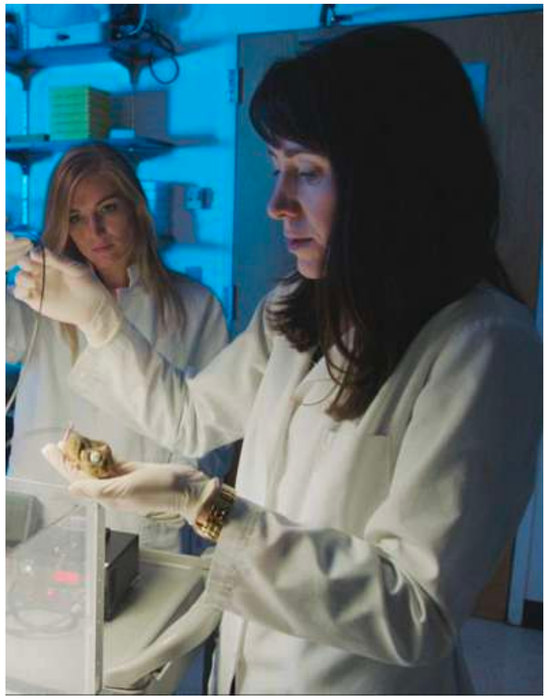


Christine Denny

Optogenetic inception of **false** memories



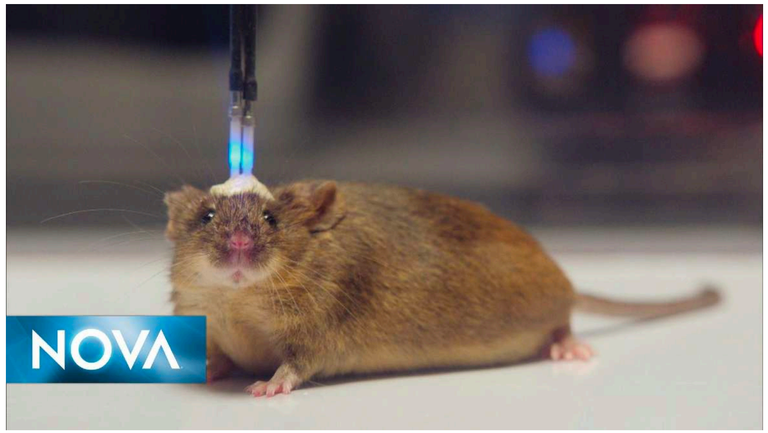
memory-encoding neurons *memory-retrieving neurons*



Christine Denny

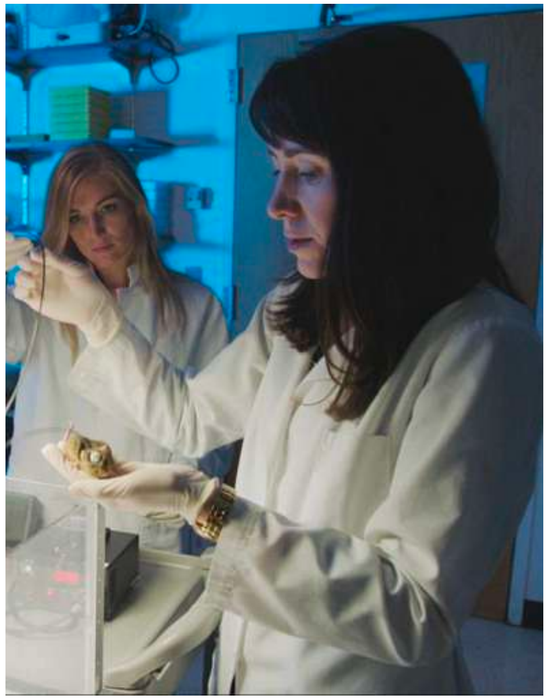
Optogenetic inception of **false** memories

Memory Hackers | Manipulating Memories with Optogenetics



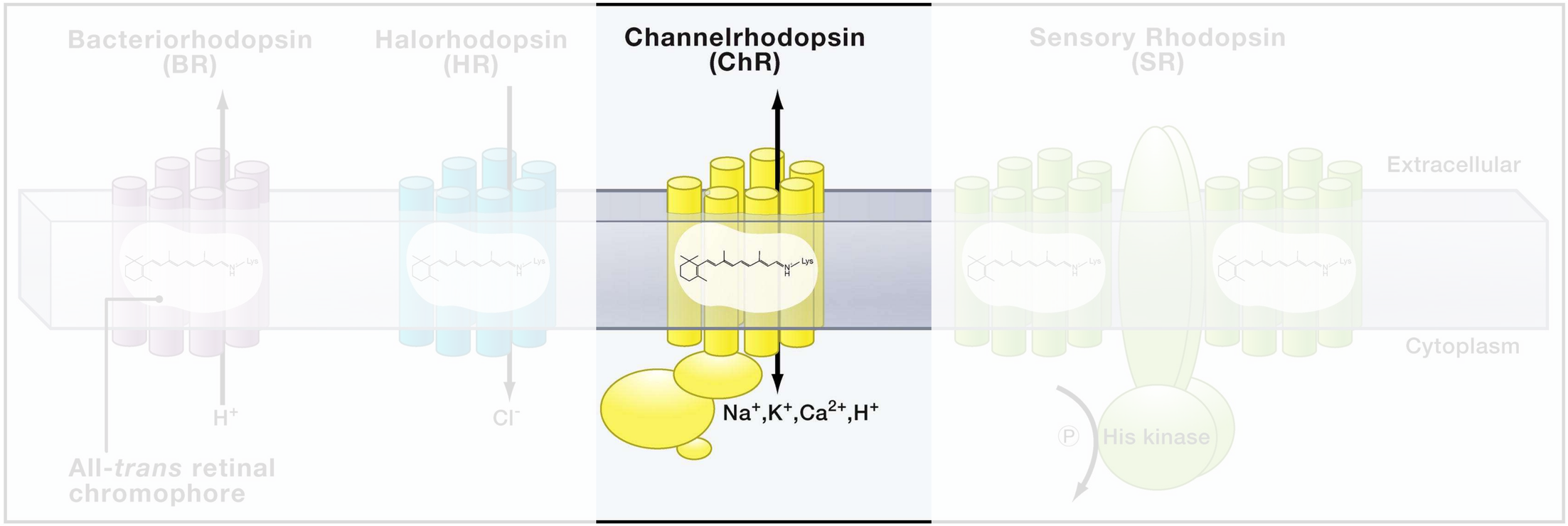
The Memory Hunter

The pioneering research of neuroscientist Christine Ann Denny ’05, MS'06, could have life-altering implications for people suffering with everything from Alzheimer’s disease to post-traumatic stress disorder.

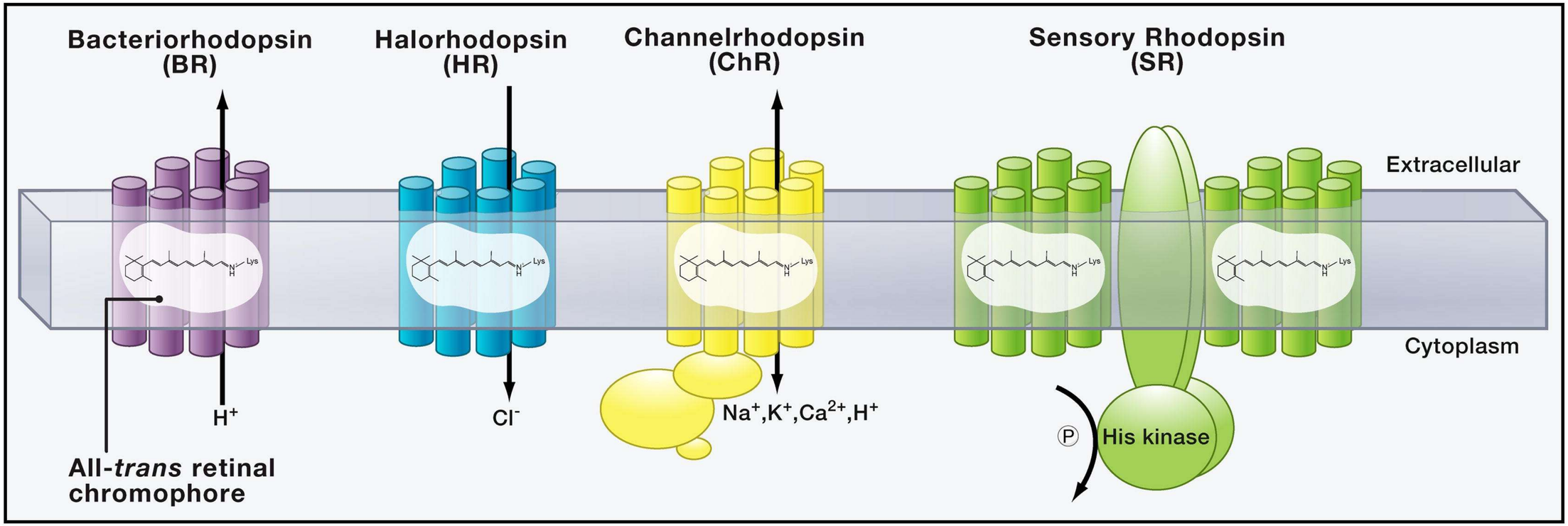


Christine Denny

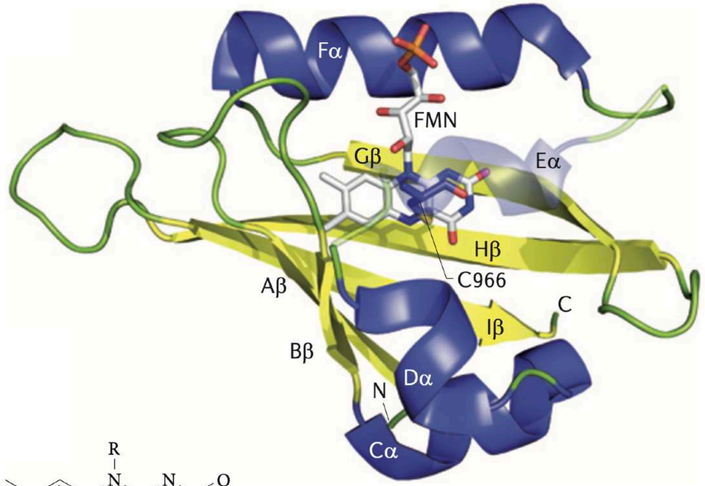
Next-generation optogenetic tools

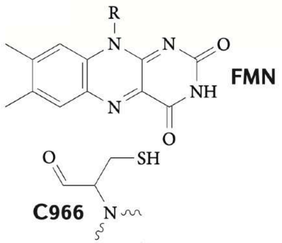


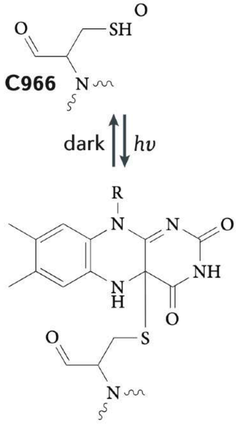
Next-generation optogenetic tools

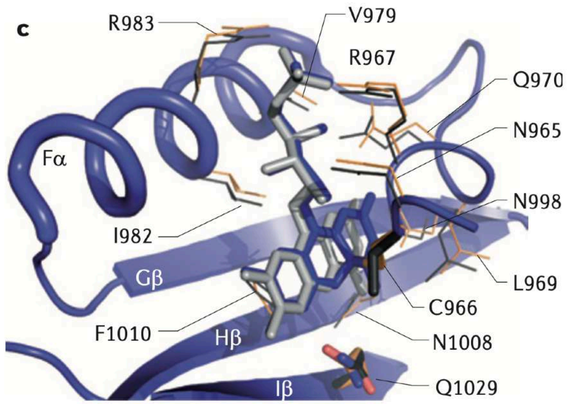


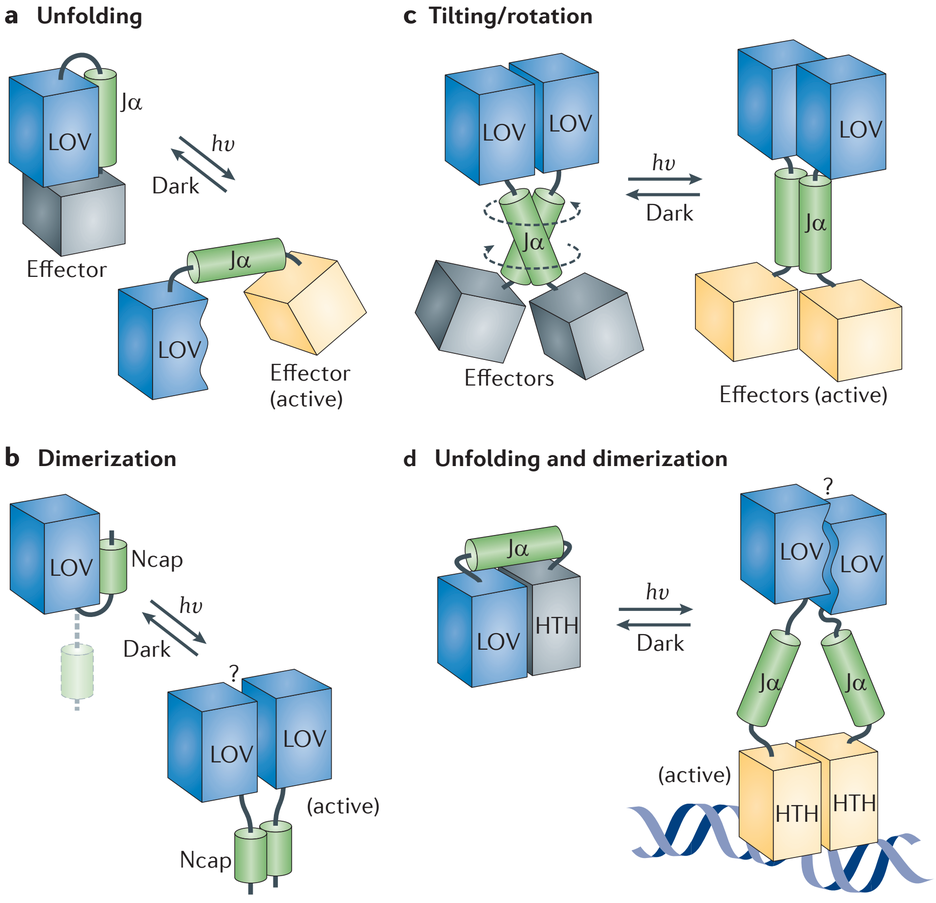
Light-oxygen-voltage (**LOV**) domains



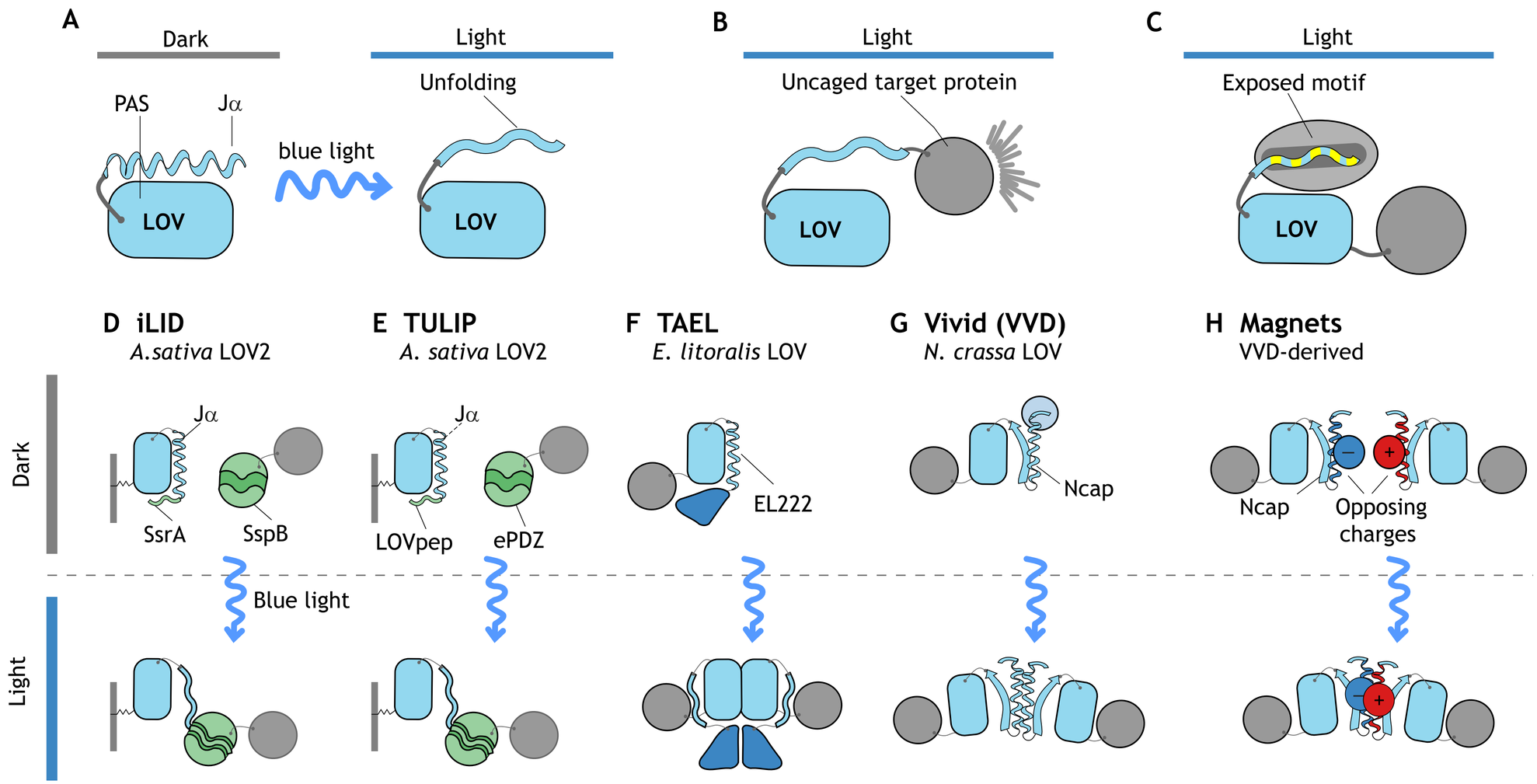






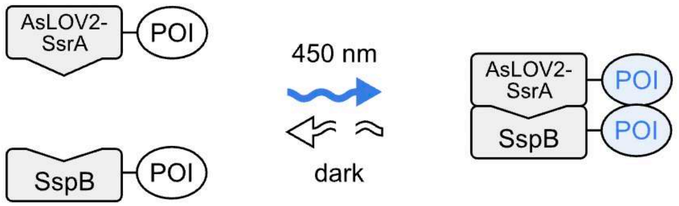


LOV-based optogenetic switches

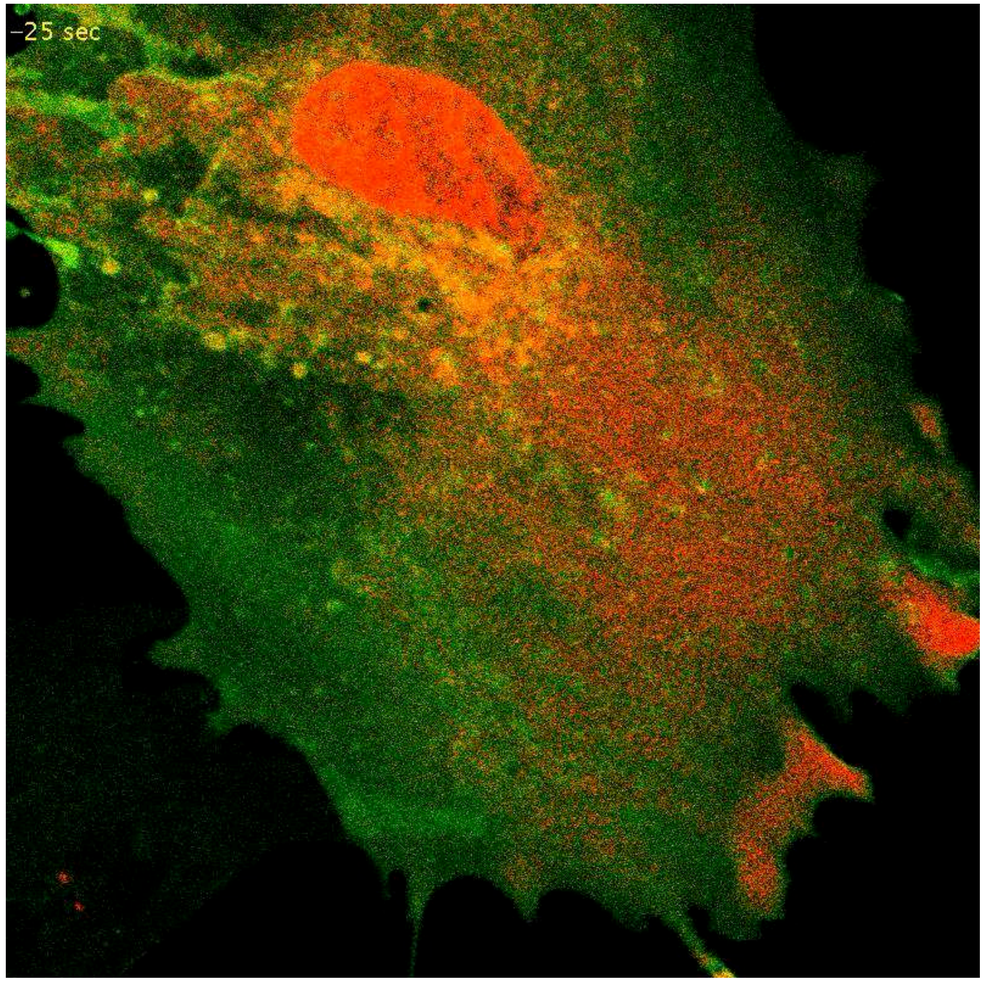


Light-activated **dimerization**

iLID



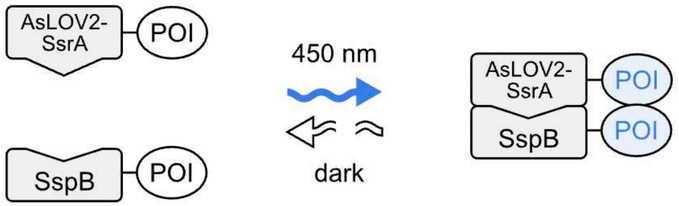
Photoreceptor	AsLOV2–SsrA
Binding partner	SspB
Cofactor	FMN
Source organism	*Avena sativa*
Mode of action	heterodimerization
Excitation wavelength	450 nm
Reversion wavelength	dark
Excitation time	seconds
Reversion time	seconds to minutes (variants available)



Proc. Natl. Acad. Sci., **2017**, *112*(1), 112-117.

Light-activated **dimerization**

iLID



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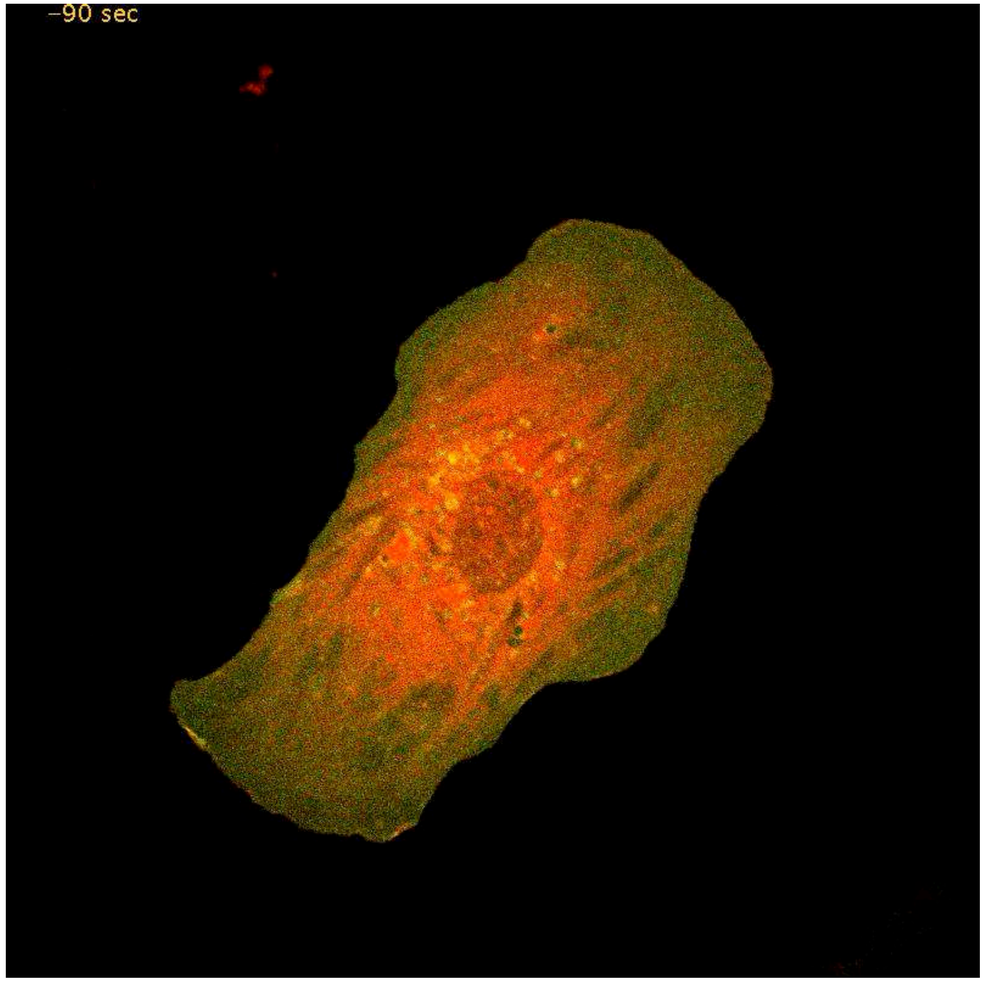

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Light-activated **dimerization**

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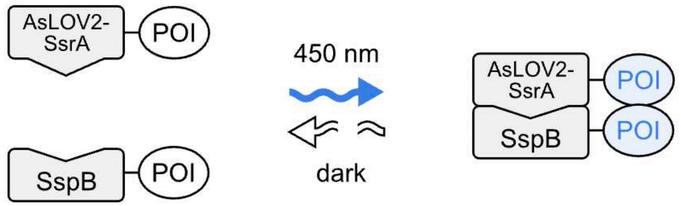

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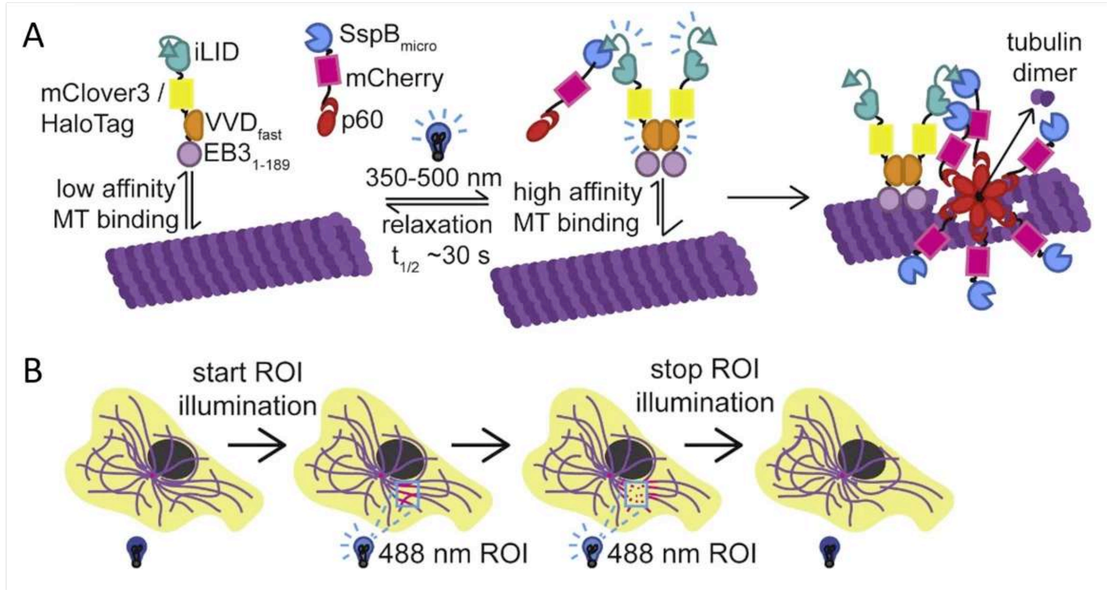
Binding partner	SspB
Cofactor	FMN
Source organism	*Avena sativa*
Mode of action	heterodimerization
Excitation wavelength	450 nm
Reversion wavelength	dark
Excitation time	seconds
Reversion time	seconds to minutes (variants available)

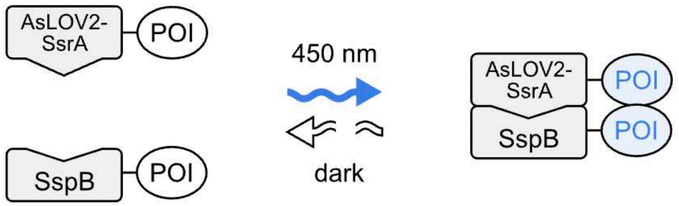
optical control over microtubule/cytoskeleton



Current Biology, **2022**, *32*(21), 4660-4674.

Light-activated **dimerization**

iLID



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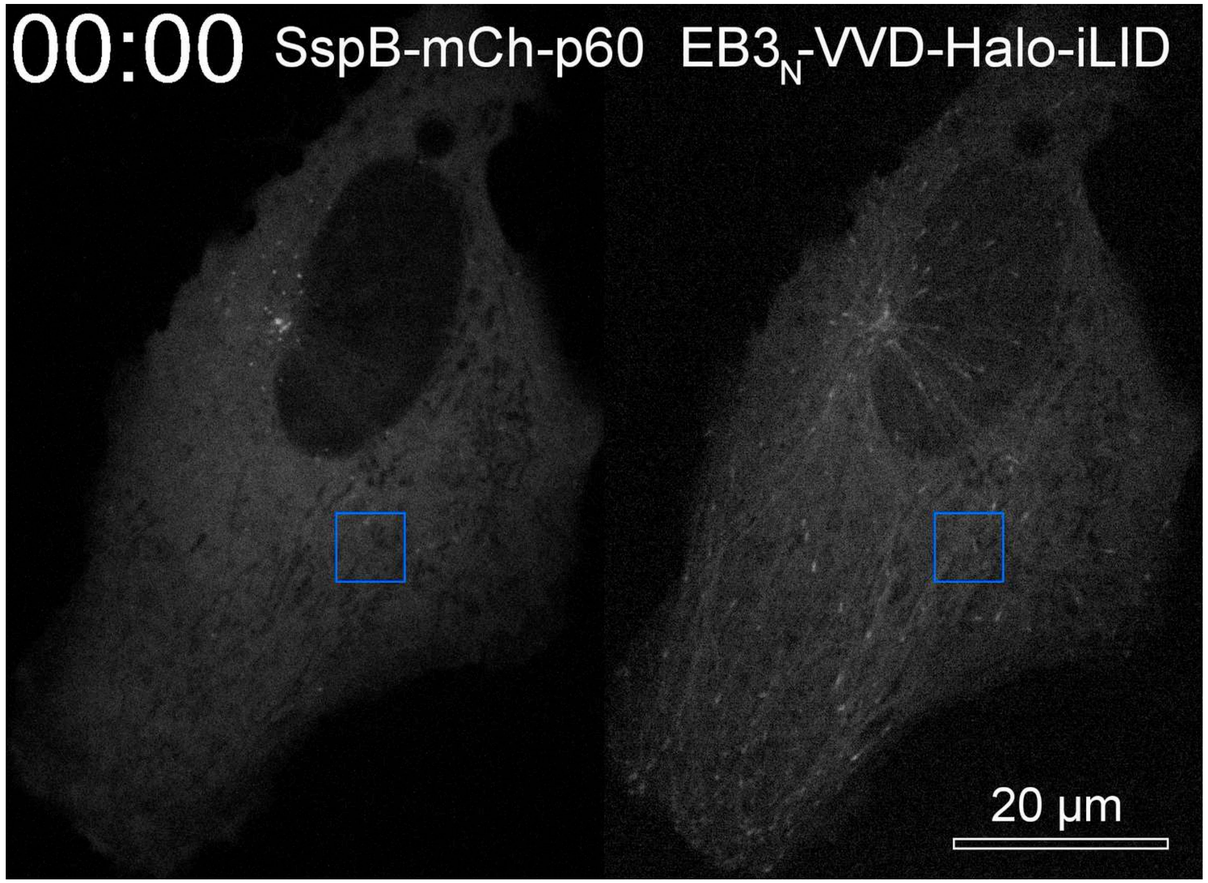
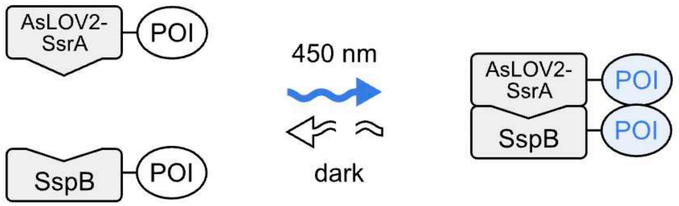
Binding partner	SspB
Cofactor	FMN
Source organism	*Avena sativa*
Mode of action	heterodimerization
Excitation wavelength	450 nm
Reversion wavelength	dark
Excitation time	seconds
Reversion time	seconds to minutes (variants available)



Current Biology, **2022**, *32*(21), 4660-4674.

Light-activated **dimerization**

iLID



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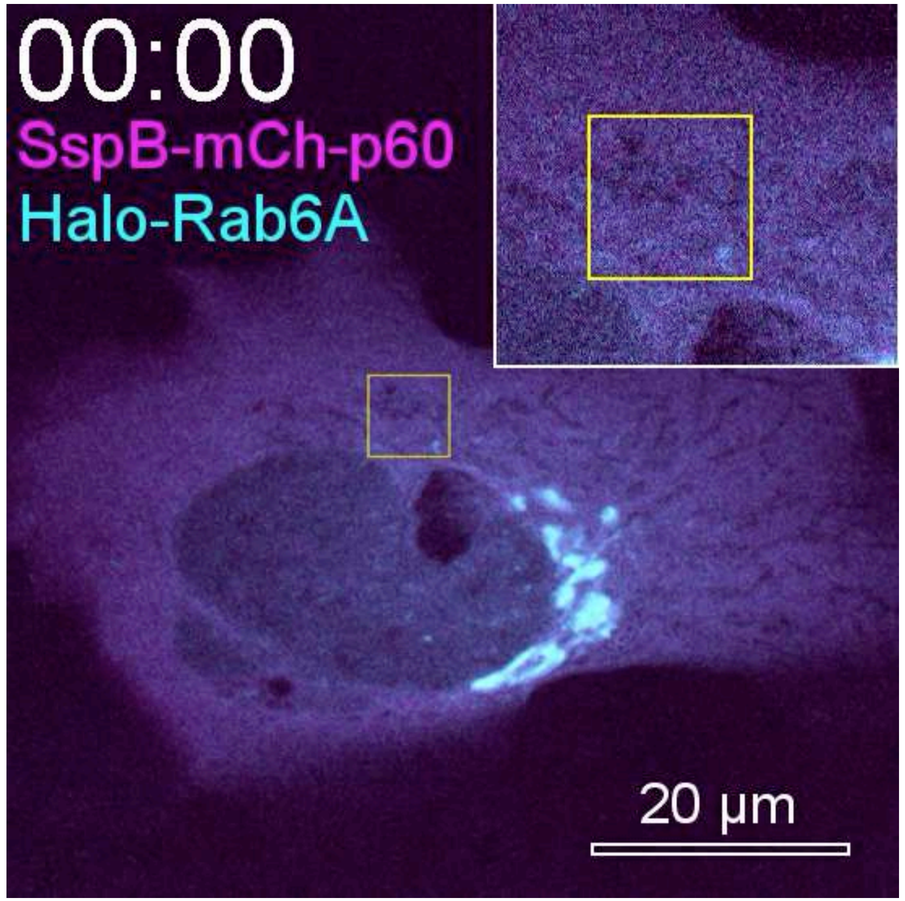
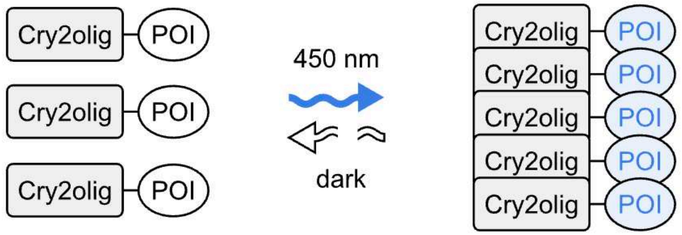


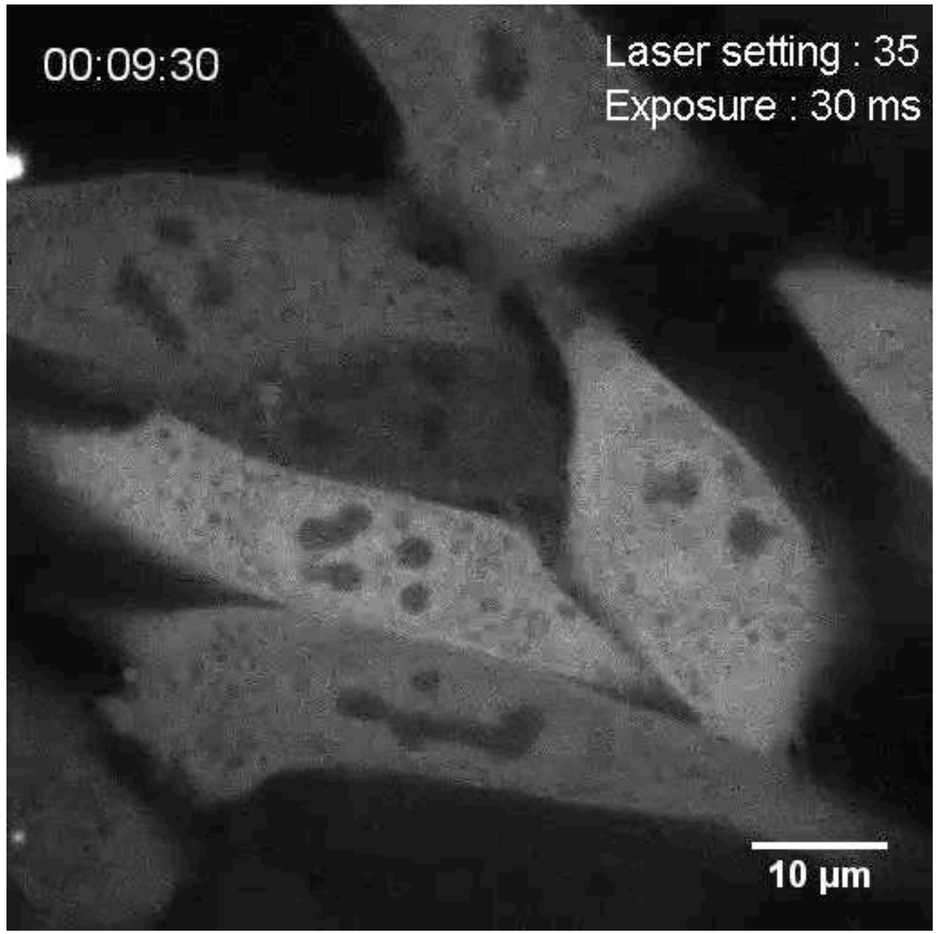
Current Biology, **2022**, *32*(21), 4660-4674.

Light-activated **phase separation**

CRY2olig

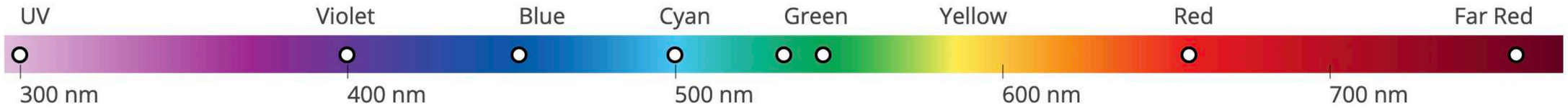


Photoreceptor	CRY2olig
Binding partner	/
Cofactor	FAD
Source organism	*Arabidopsis thaliana*
Mode of action	homooligomerization
Excitation wavelength	450 nm
Reversion wavelength	dark
Excitation time	seconds
Reversion time	minutes



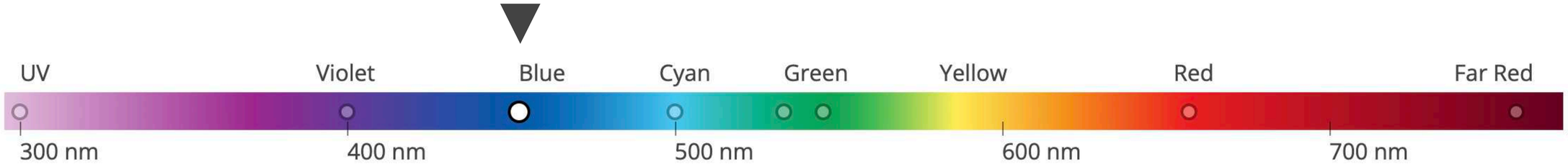
Cell, **2017**, *168(1)*, 159-171.

Taste the optogenetic rainbow



UV receptors
Cyanobacteriochromes
BLUF domains
LOV domains
Cryptochromes
Fluorescent proteins
Cobalamin-binding domains
Phytochromes

Taste the optogenetic rainbow



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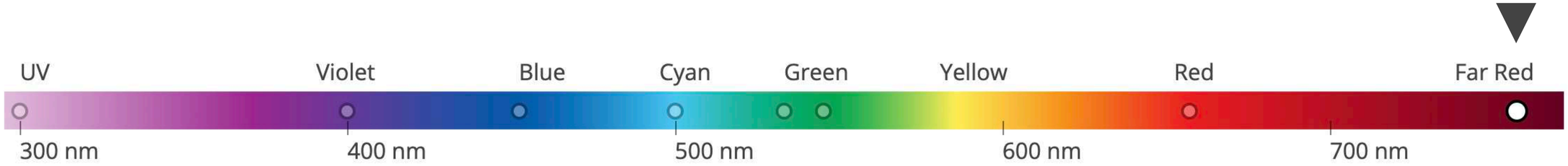
Taste the optogenetic rainbow



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BLUF domains
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Fluorescent proteins
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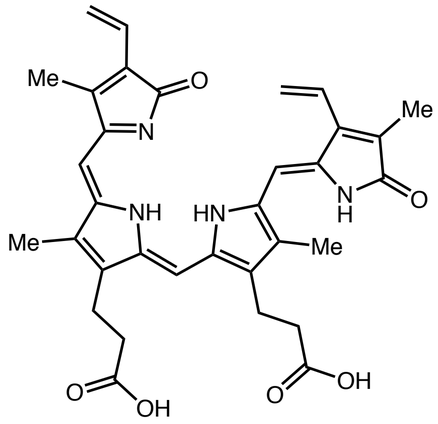
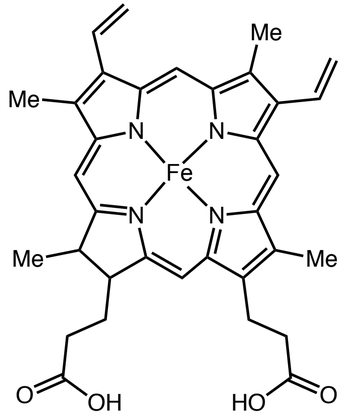


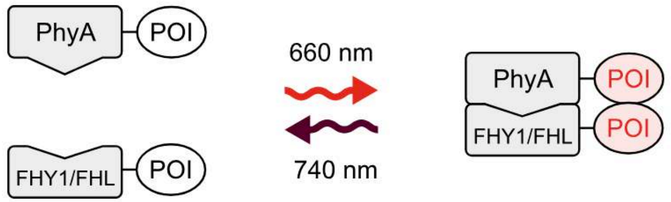
biliverdin



heme

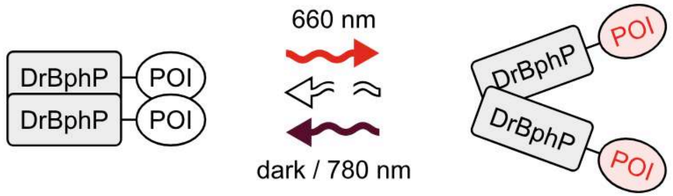
Taste the optogenetic rainbow

PhyA/FHY1 & PhyA/FHL



Photoreceptor	PhyA
Binding partner	FHY1 or FHL
Cofactor	PCB
Source organism	*Arabidopsis thaliana*
Mode of action	heterodimerization
Excitation wavelength	660 nm
Reversion wavelength	740 nm
Excitation time	? Add information
Reversion time	? Add information

DrBphP



Photoreceptor	DrBphP
Binding partner	/
Cofactor	Biliverdin
Source organism	*Deinococcus radiodurans*
Mode of action	homodimerization, dissociation
Excitation wavelength	660 nm
Reversion wavelength	780 nm, dark
Excitation time	? Add information
Reversion time	? Add information

Choose your **fighter**

I would like to use an optogenetic switch...

...that I can fuse to my proteins of interest. | ...with a predefined function.

Mode of switch action:

- [ ] Homodimerization
- [ ] Heterodimerization
- [ ] Oligomerization (clustering)
- [x] Dissociation
- [ ] Shielding/caging
- [ ] Photocleavage

of the proteins of interest.

Color of the switch-inducing light:

- [ ] UV
- [ ] Violet
- [ ] Blue
- [ ] Cyan
- [x] Green
- [ ] Red
- [ ] Near infrared

- [ ] Display only the switches that can be actively reversed from excited to ground state.

- [ ] My optogenetic application requires deep tissue penetration of light.

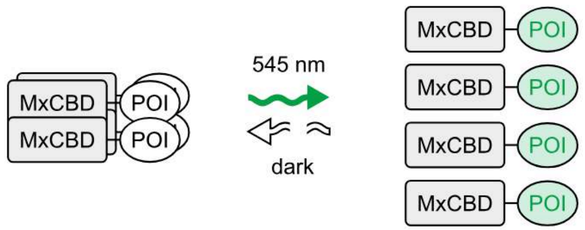
Display only the switches that have been previously tested in **any of** the following host cell lines / organisms:

Select the hosts

Choose your **fighter**

OptoBase

MxCBD

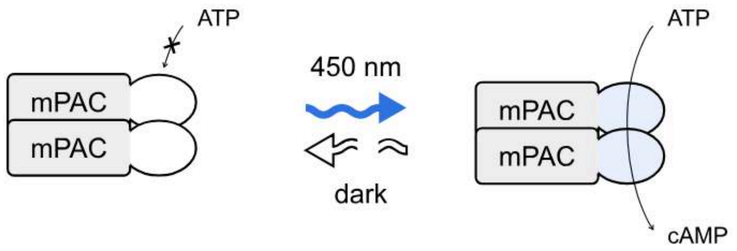


Photoreceptor	MxCBD
Binding partner	/
Cofactor	AdoCbl, MetCbl or CNCbl
Source organism	*Myxococcus xanthus*
Mode of action	homotetramerization, dissociation
Excitation wavelength	545 nm
Reversion wavelength	dark
Excitation time	? Add information
Reversion time	? Add information

Choose your **fighter**

OptoBase

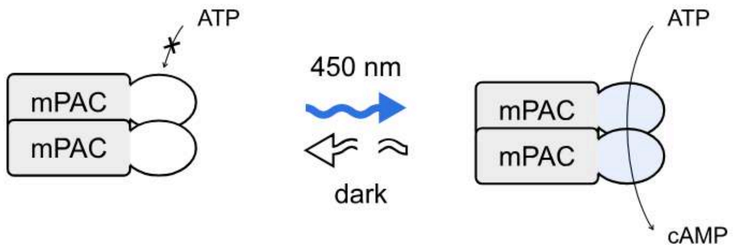
molecule synthesis



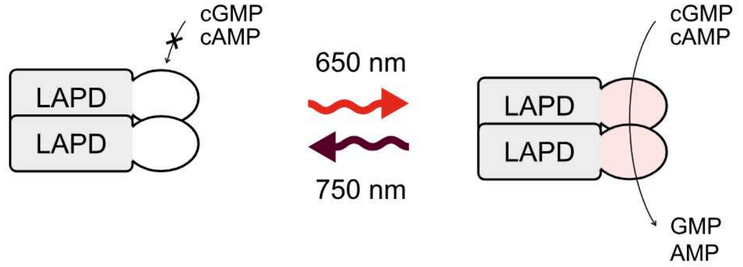
Choose your **fighter**

OptoBase

molecule synthesis



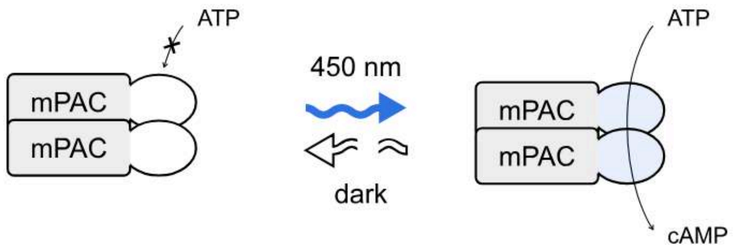
molecule degradation



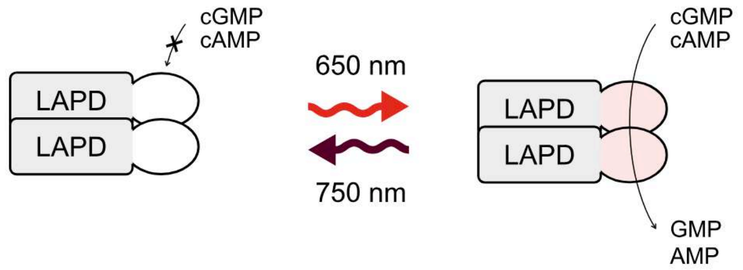
Choose your **fighter**

OptoBase

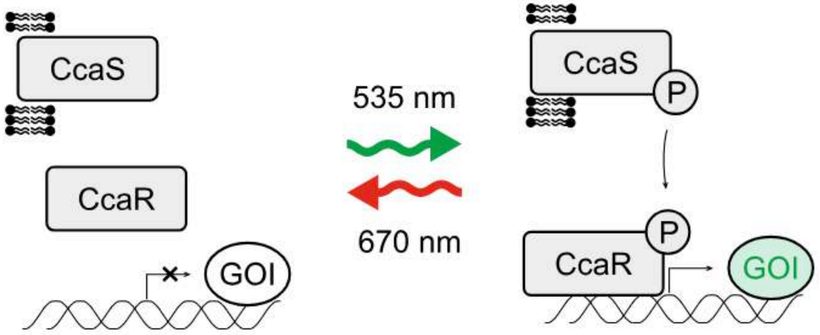
molecule synthesis



molecule degradation



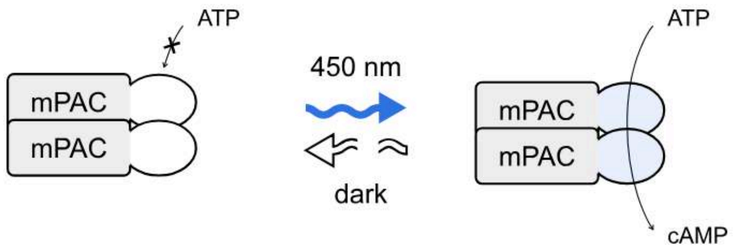
DNA binding/gene activation



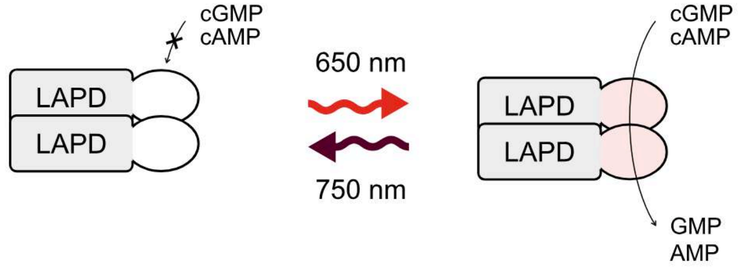
Choose your **fighter**

OptoBase

molecule synthesis



molecule degradation



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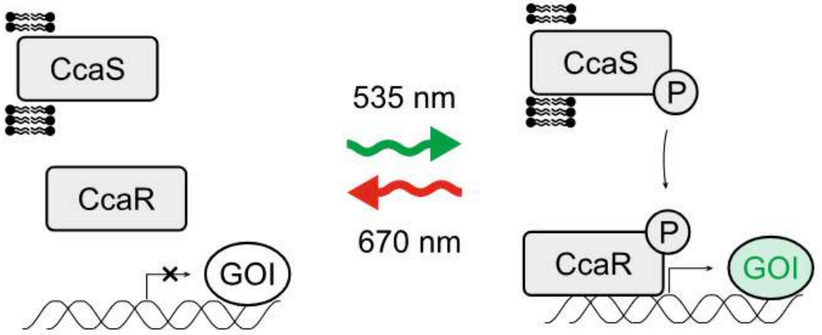
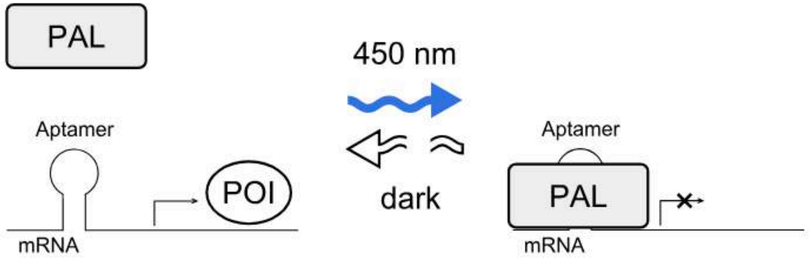
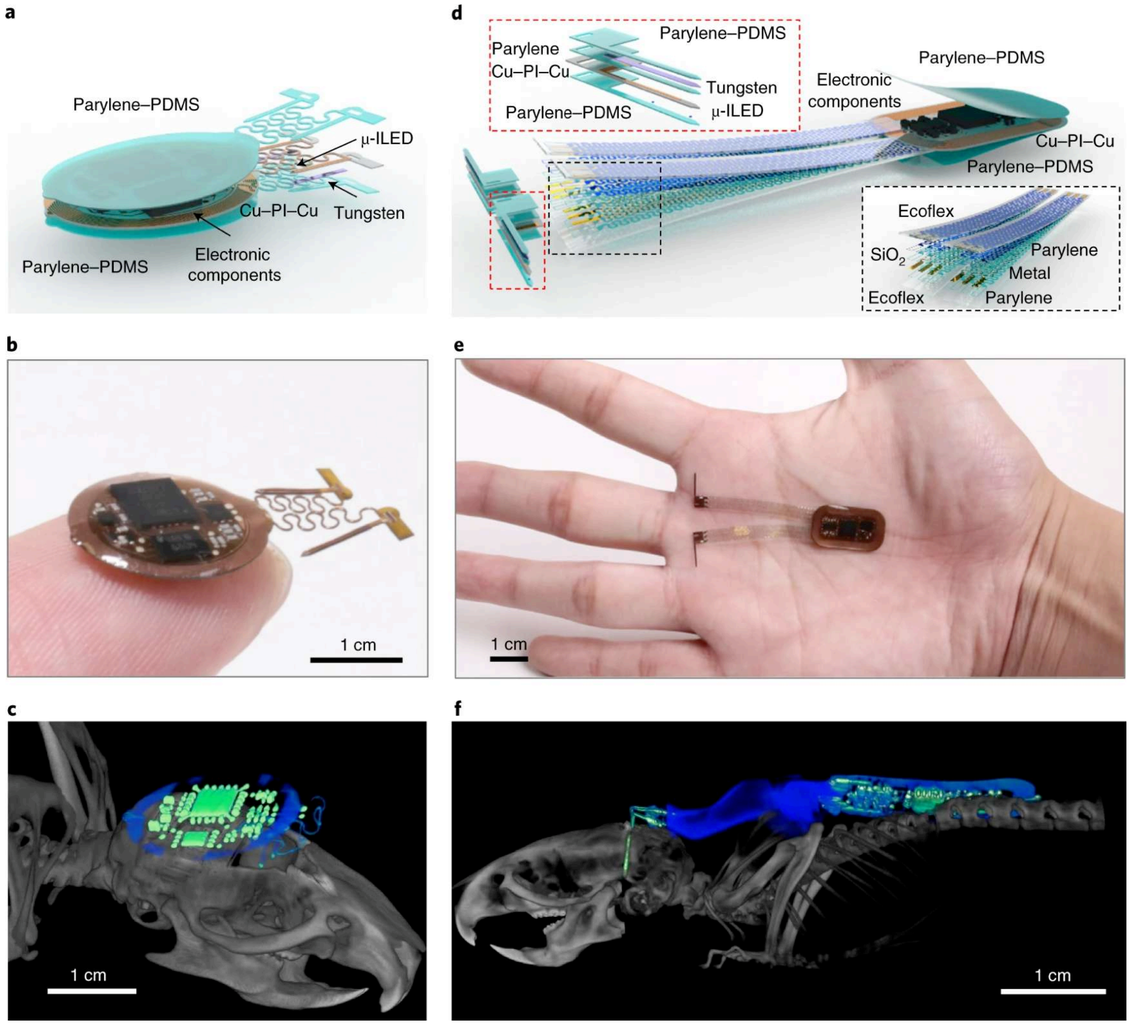
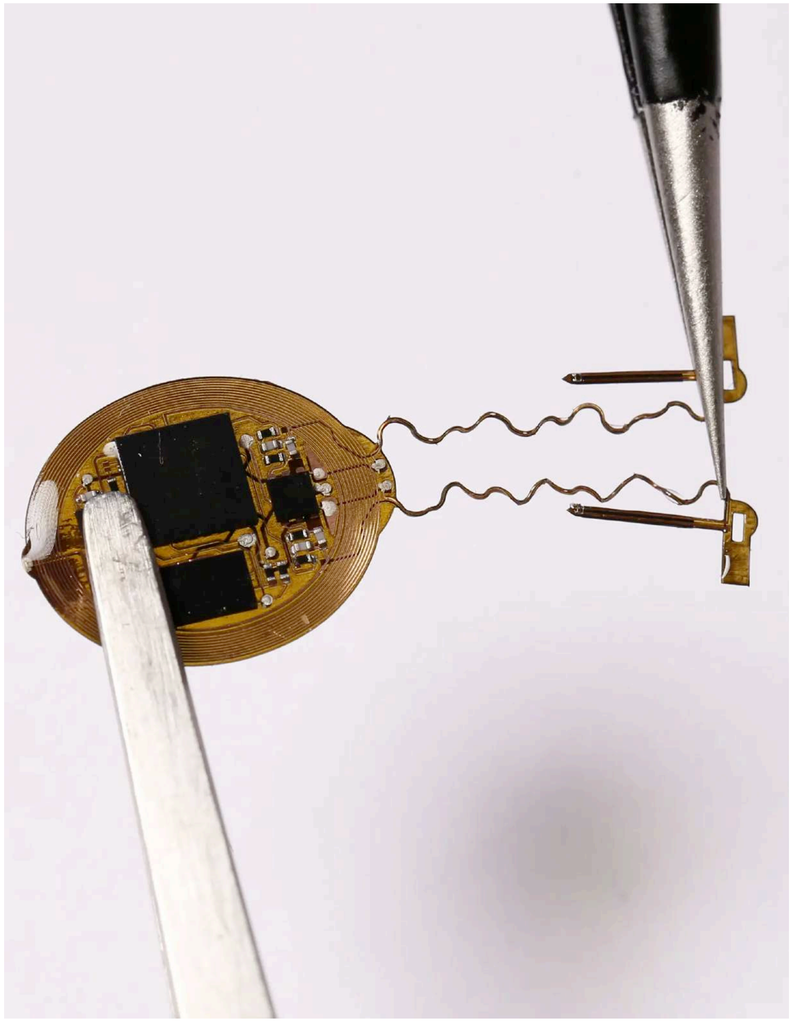


RNA binding/gene inhibition



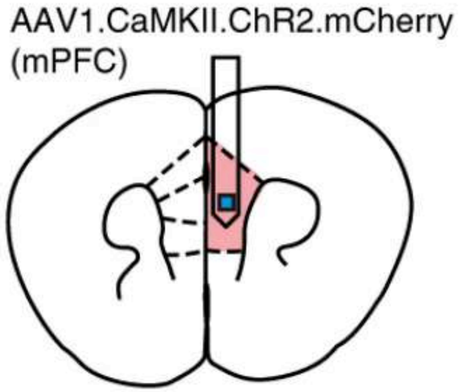
Optogenetic hardware upgrades

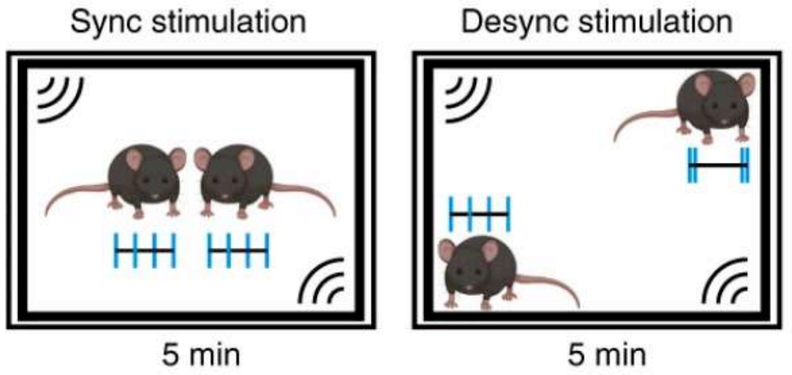




Nature neuroscience, **2021**, *24*(7), 1035-1045.

Optogenetic hardware upgrades





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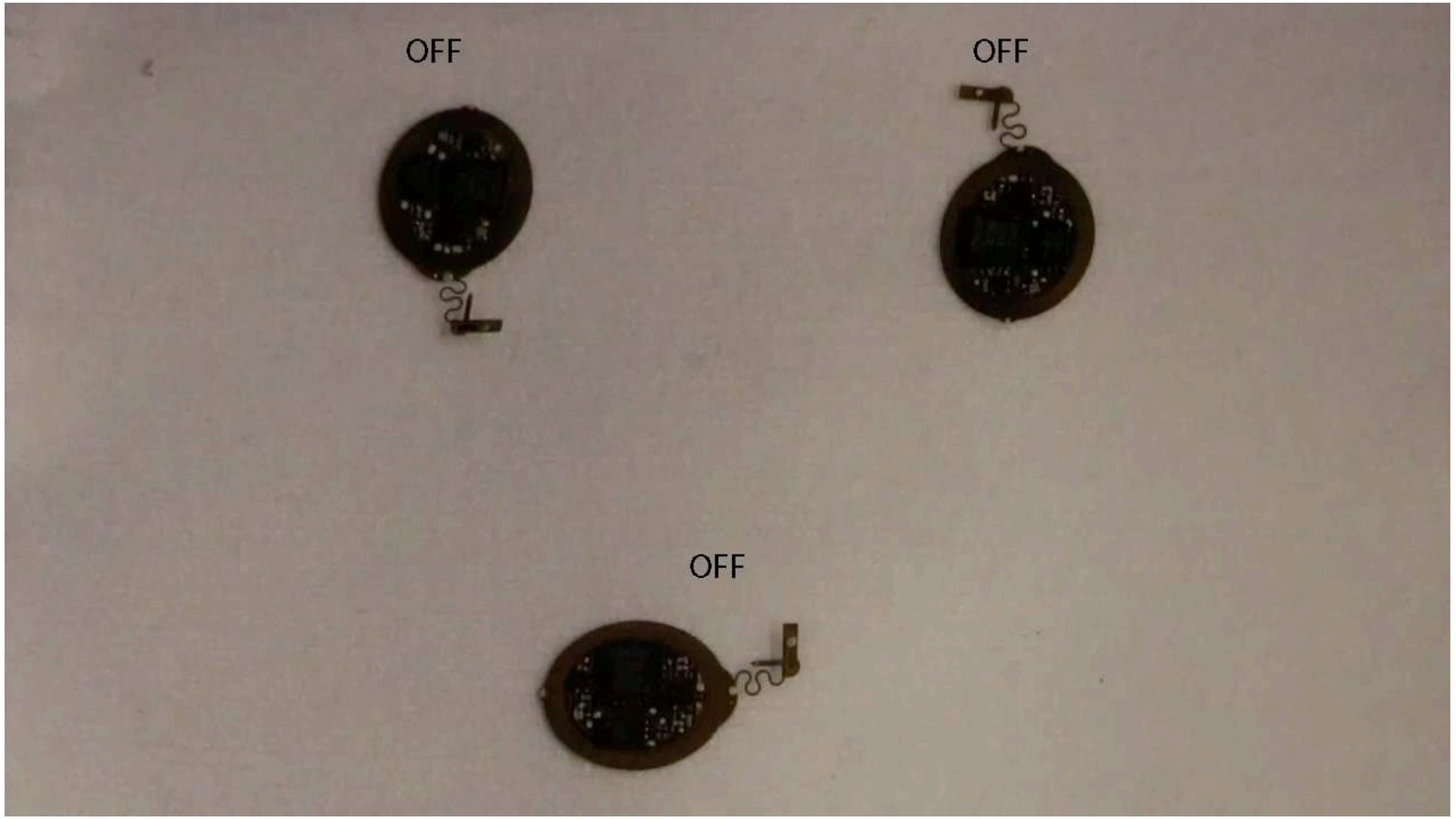
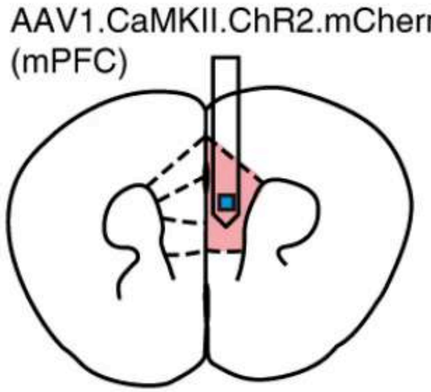
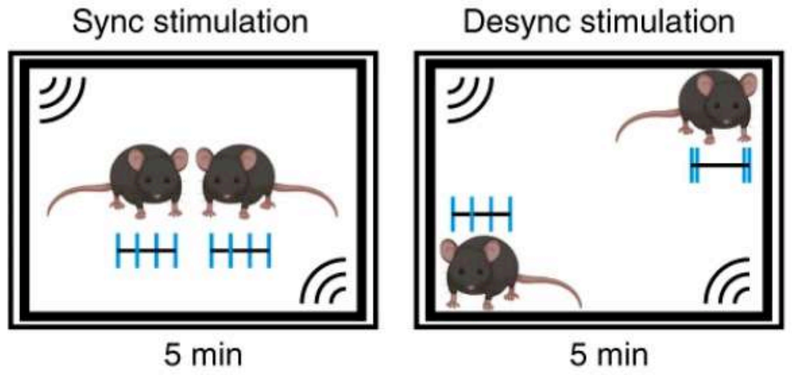
Optogenetic hardware upgrades







Nature neuroscience, **2021**, *24*(7), 1035-1045.

Optogenetic hardware upgrades

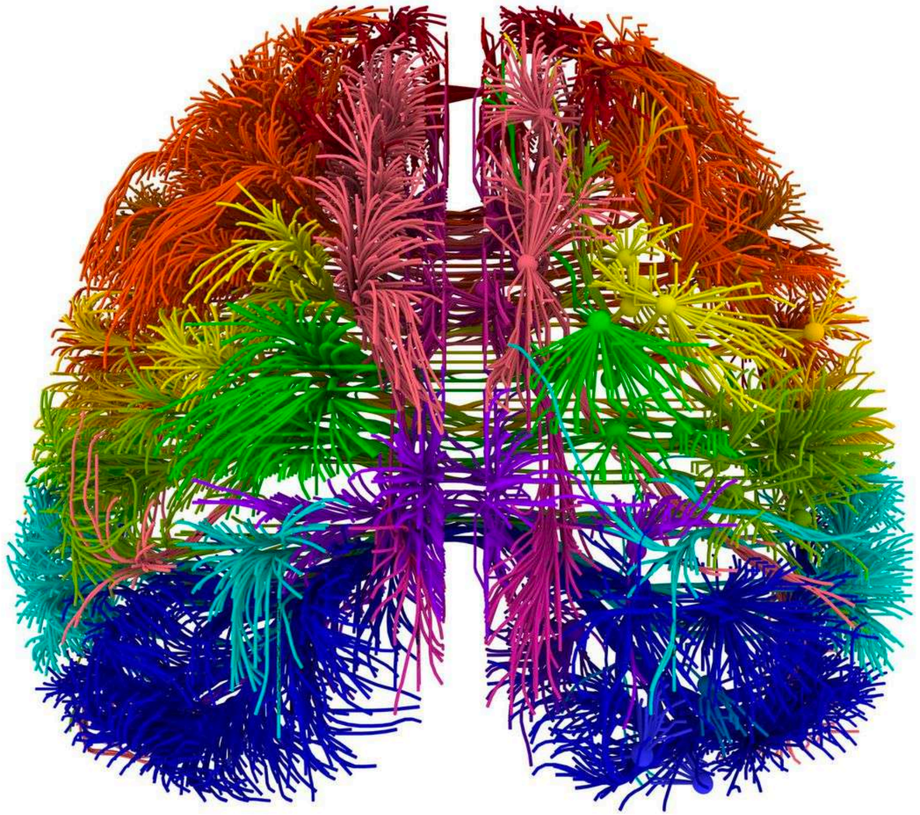
cortex activation synchronized

asynchronous

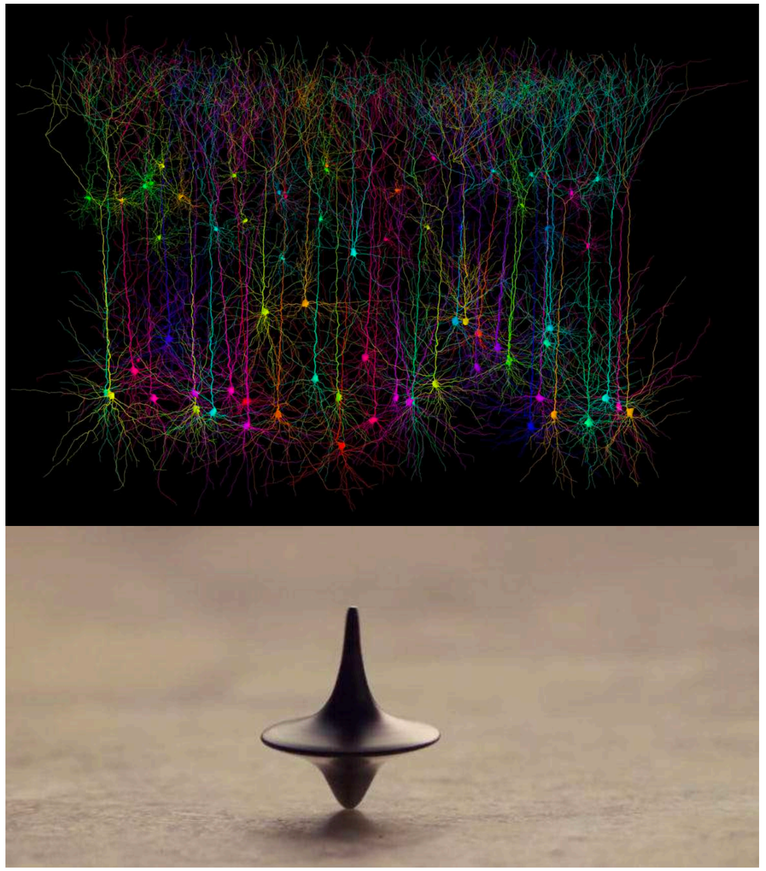
Nature neuroscience, **2021**, *24*(7), 1035-1045.

The optogenetic **outlook**

brain mapping/functional wiring

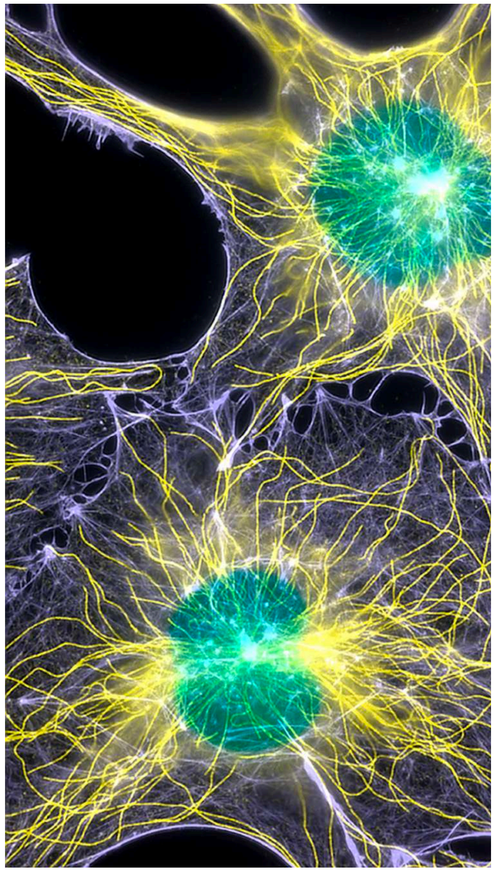


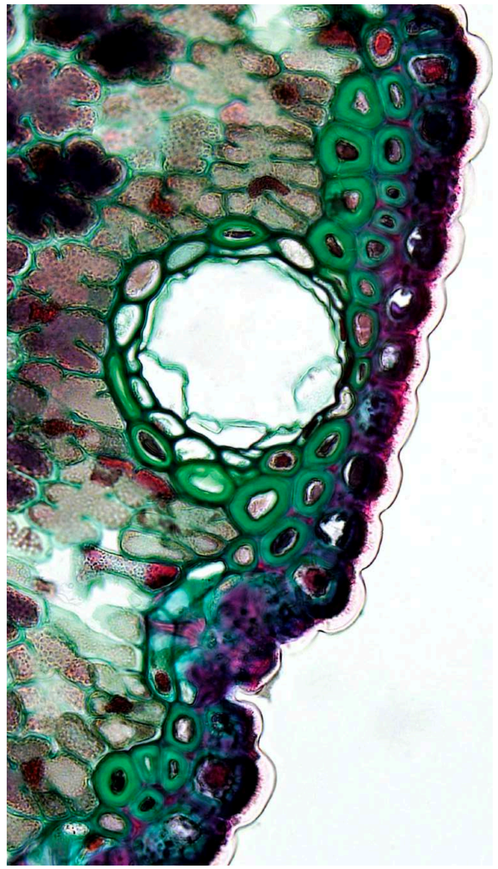
understanding/manipulating memory



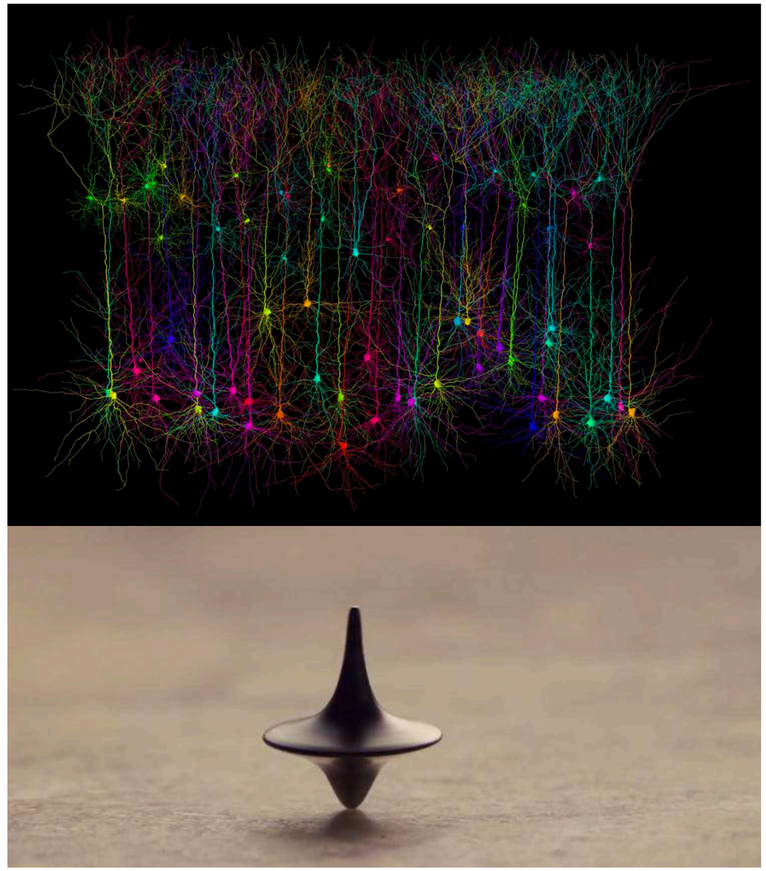
The optogenetic **outlook**

basic cell biology mechanisms



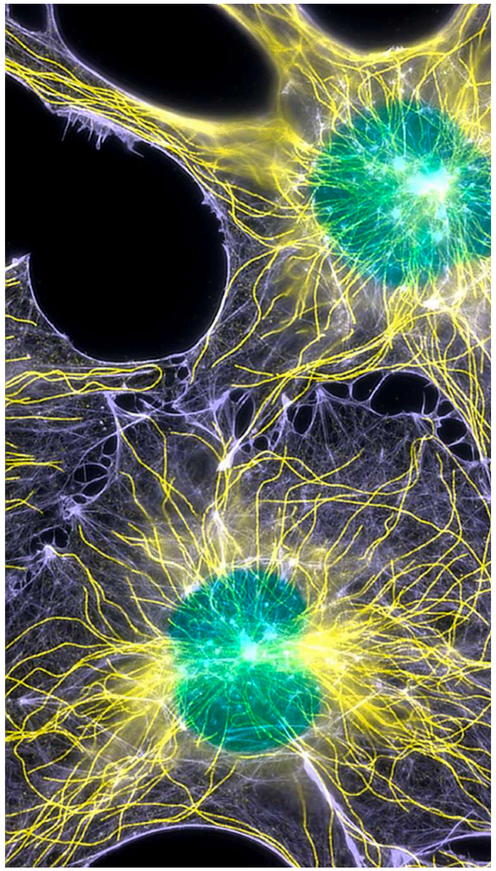


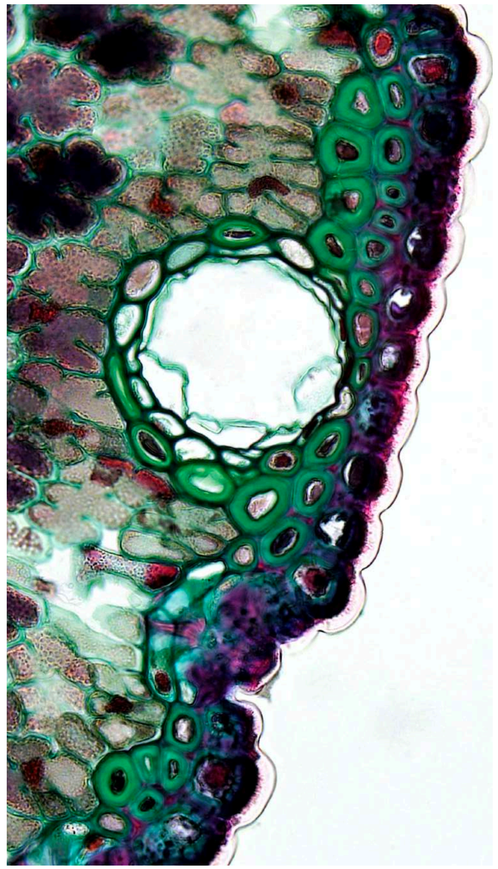
understanding/manipulating memory



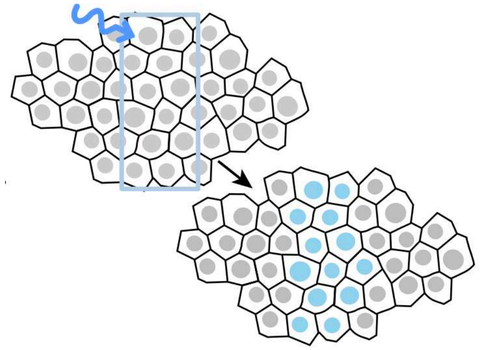
The optogenetic **outlook**

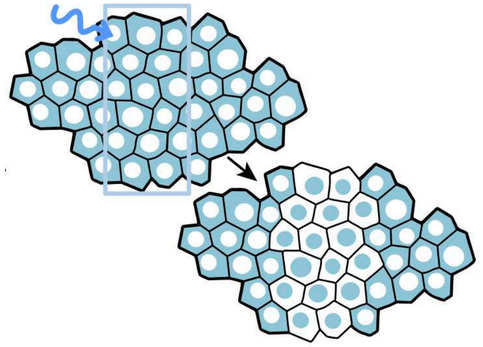
basic cell biology mechanisms





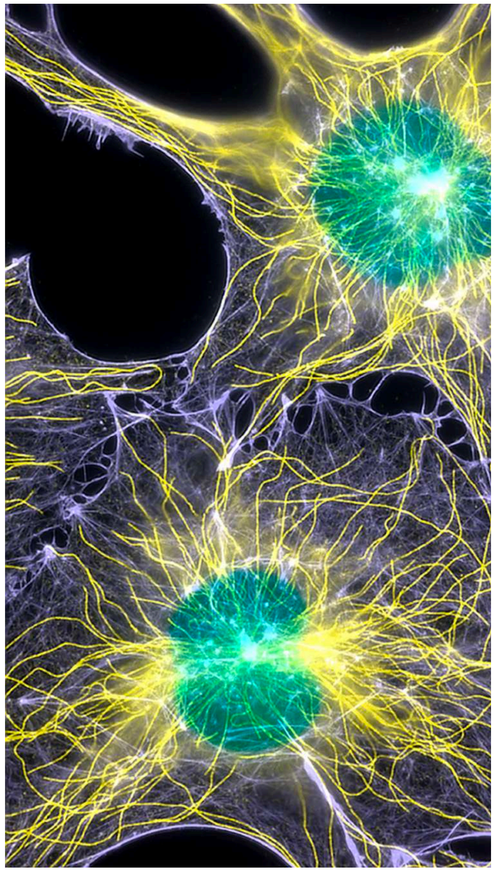
tissue and embryonic development

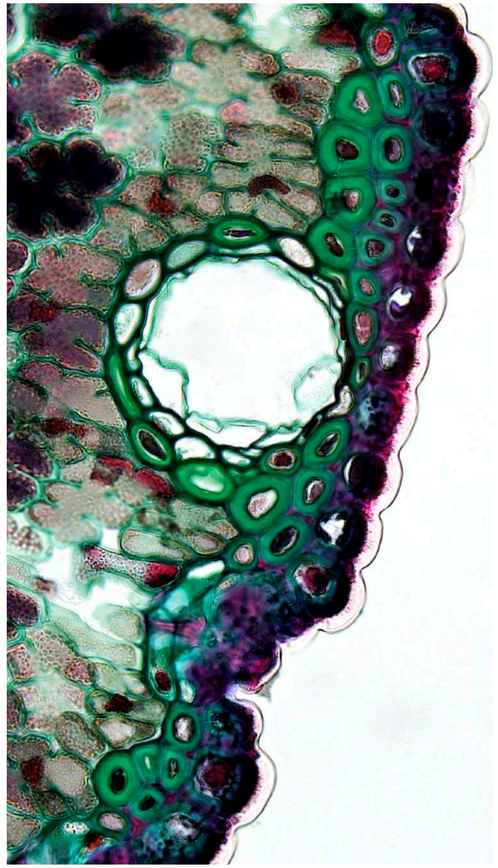




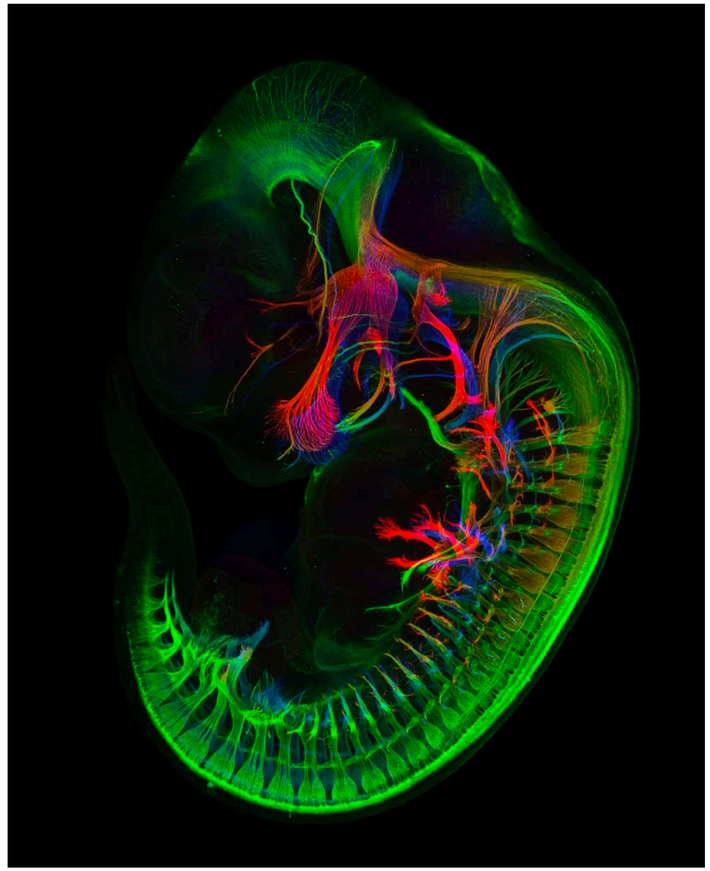
The optogenetic **outlook**

basic cell biology mechanisms

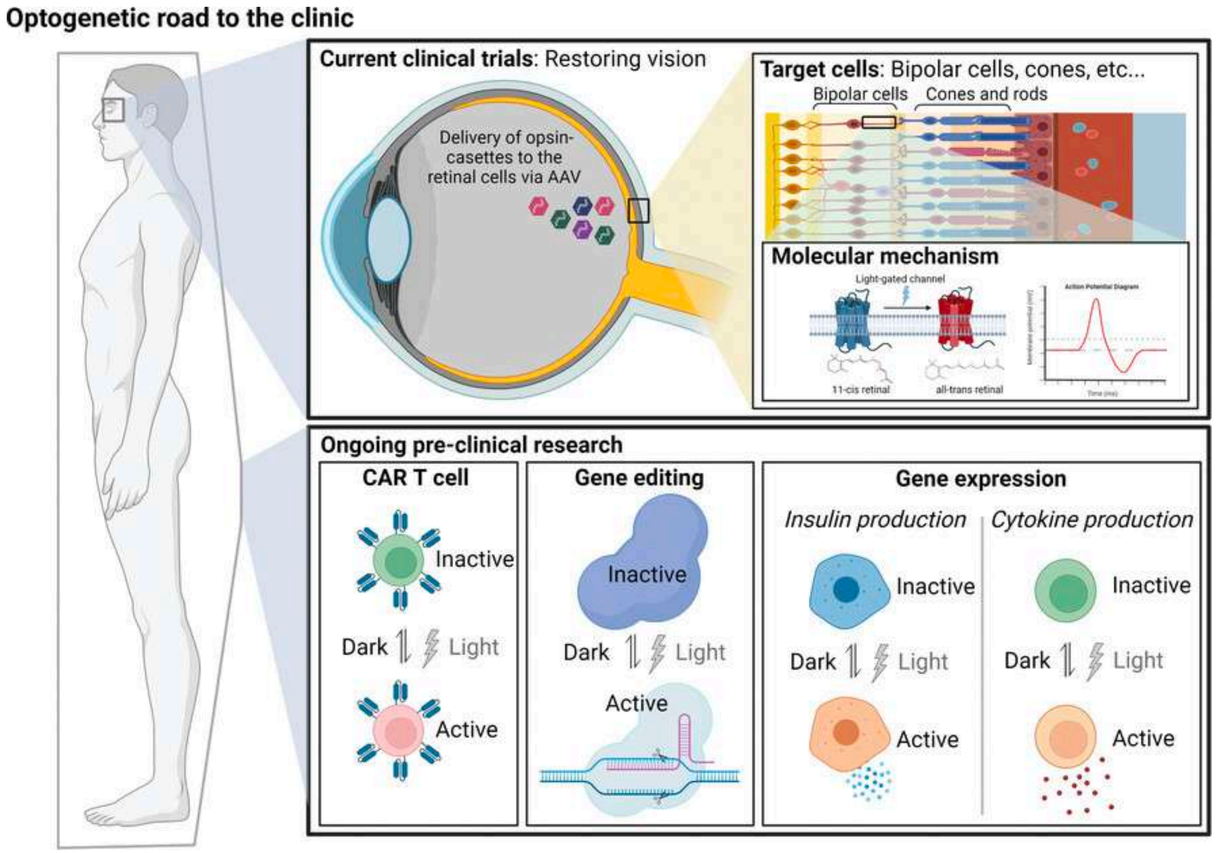




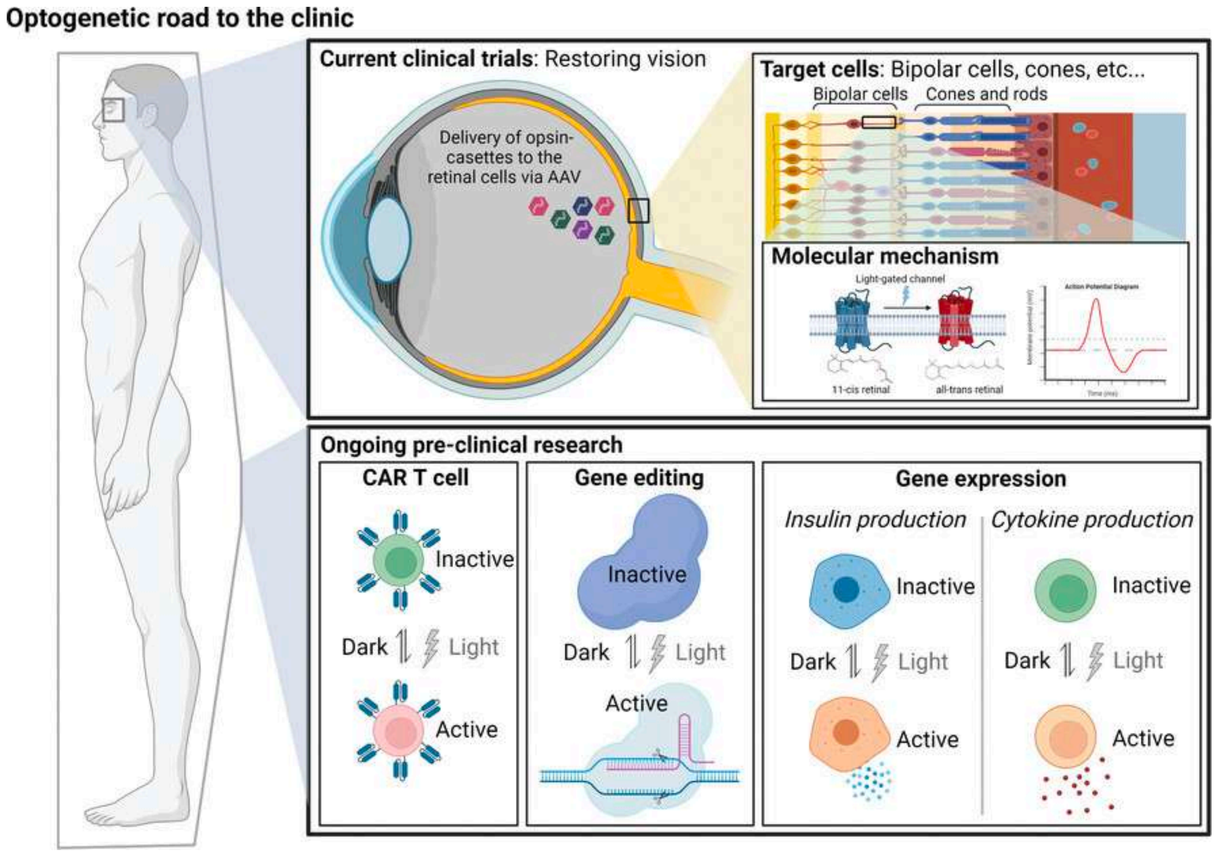
tissue and embryonic development

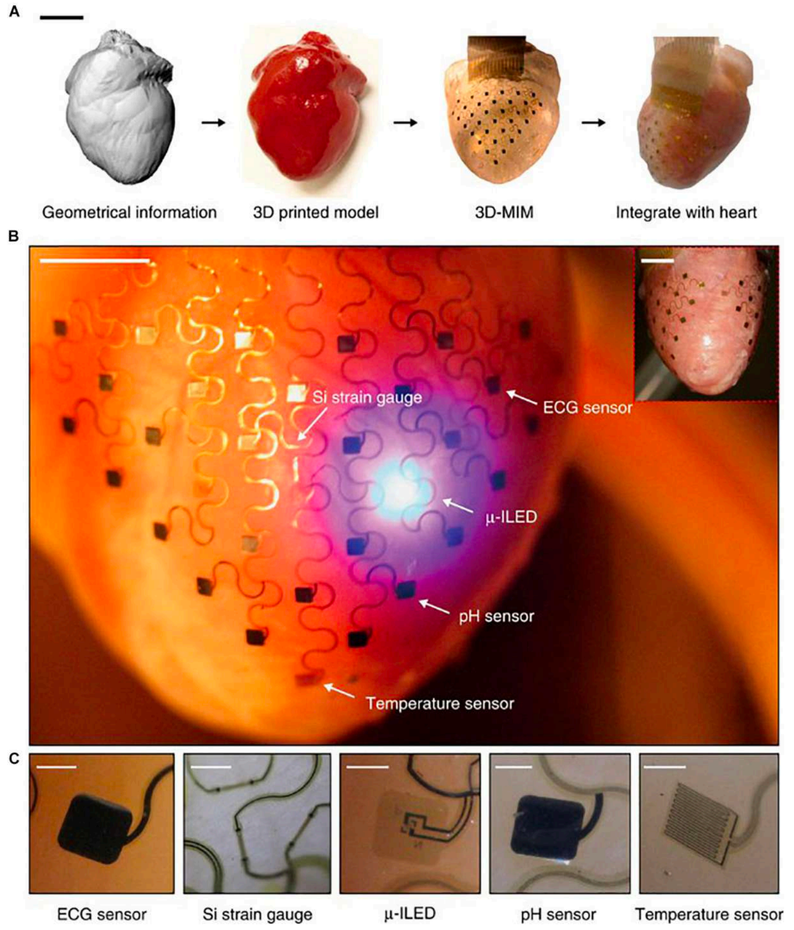


The optogenetic **outlook**



The optogenetic **outlook**





Thank you

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