


# Manipulating and probing nerve cells by light

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Depart. of psychology



# Presentation

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## PART 1 (theory)

- Manipulating and probing techniques
- Optogenetics - manipulating and probing nerve cells by light
- Optogenetics - methodology
- Optogenetics summary

## PART 2 (examples)

- Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Adamantidis et al. Nature 2007.
- Functional imaging of hippocampal place cells at cellular resolution during virtual navigation. Dombeck et al. Nature Neuroscience 2010

# Manipulating and probing techniques

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	<i>Methodology</i>	<i>Temporal scale (how fast?)</i>	<i>Spatial scale (how accurate?)</i>
<i>Manipulation</i>	Pharmacology	🕒	🕒
	Lesioning	🕒	🕒
	Electrical stimulation	🕒	🕒
<i>Imaging</i>	EEG	🕒	🕒
	MEG	🕒	🕒
	MRI	🕒	🕒

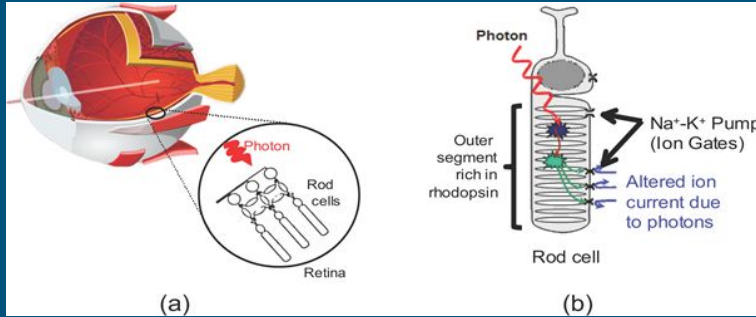
# Optogenetics - manipulating and probing nerve cells by light

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- Light could provide a rapid way to control and readout the activity of nerve cells (or any other cell), if
  - A) Cells would be able to respond to light
  - B) Cells would be able to send light if activated

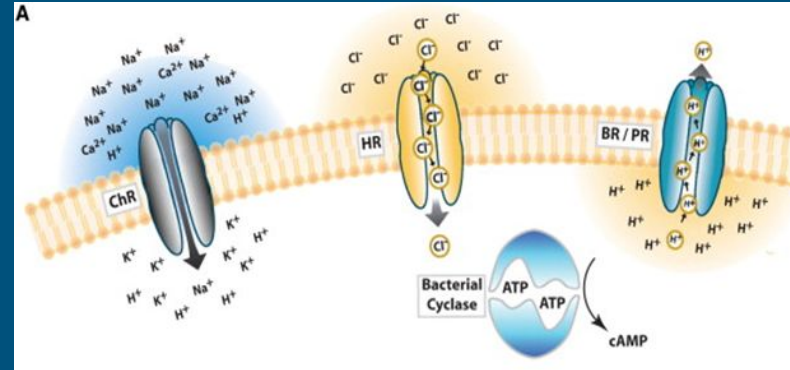
# Optogenetics - *manipulating* and probing nerve cells by light

- Our vision is based on rod and cone cells capability to react to light/photons (opsins)



<http://spie.org/x19173.xml> 2014

- Algae express specific ion pumps able to respond to light/photons (channel-, halo- and bacteriorhodopsin)



Yizhar et al., Neuron, 2011

# Optogenetics - *manipulating* and probing nerve cells by light

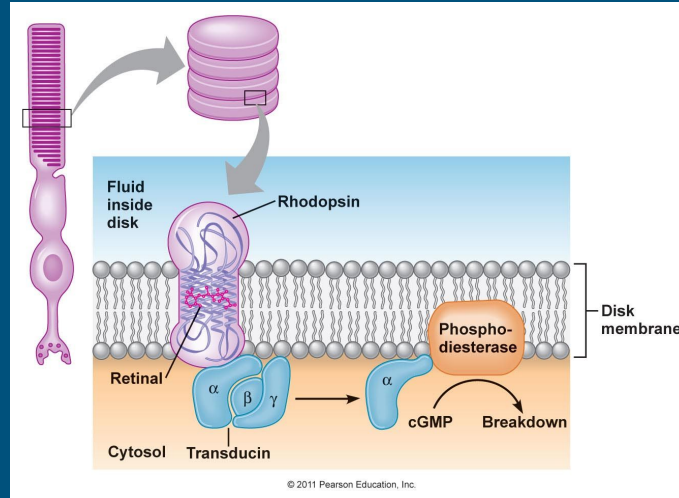
Photon causes modifications in retinal molecule

-> change in the opsin protein conformation

-> activation of the channel

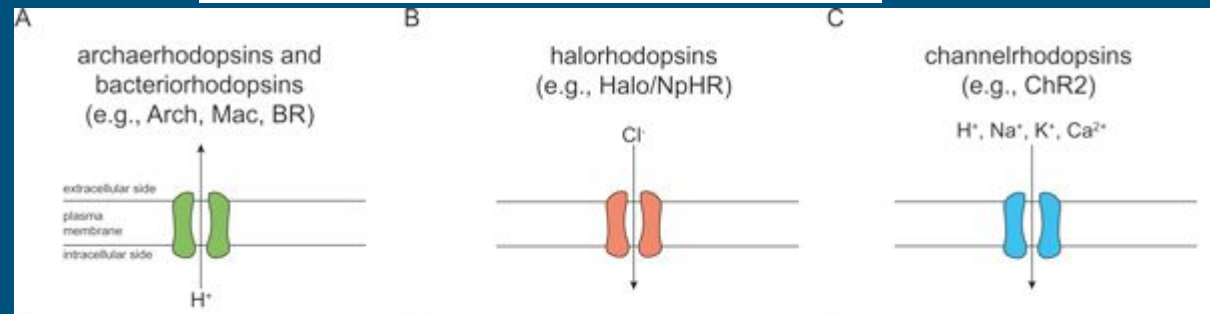
-> activation of the second messenger system

-> increase the probability of the cell be activated or inhibited



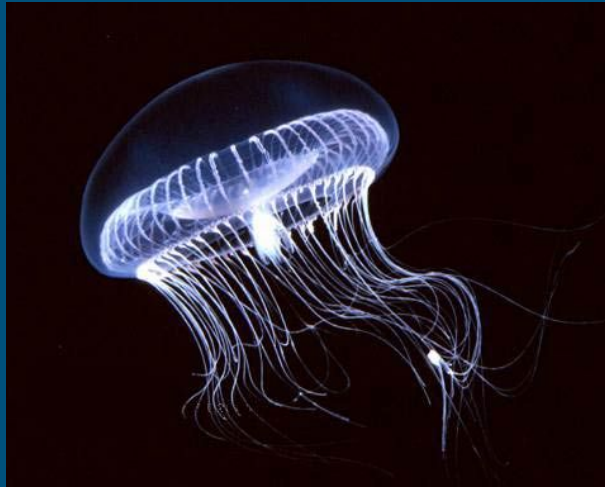
[https://droualb.faculty.mjc.edu/Course%20Materials/Physiology%20101/Chapter%20Notes/Fall%202007/chapter\\_10%20Fall%202007.htm](https://droualb.faculty.mjc.edu/Course%20Materials/Physiology%20101/Chapter%20Notes/Fall%202007/chapter_10%20Fall%202007.htm)

Boyden 2011



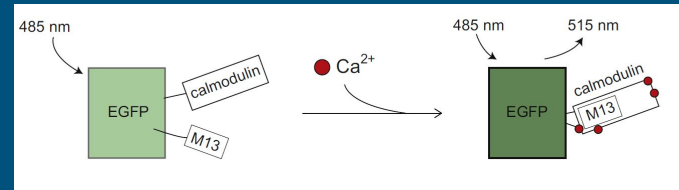
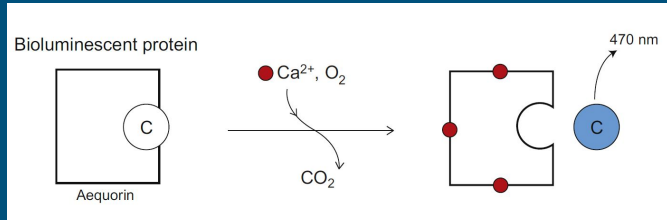
# Optogenetics - manipulating and *probing* nerve cells by light

- The Aequorea Victoria can produce flash of lights by releasing calcium to distract predators (bioluminescence)



# Optogenetics - manipulating and *probing* nerve cells by light

- Binding of calcium ions to aequorin leads to the oxidation of coelenterazine to coelenteramide. Coelenteramide relaxes to the ground state while emitting a photon of 470 nm
- After binding of calcium to GCaMP conformational intramolecular changes lead to an increase in the emitted fluorescence of 515 nm





# Optogenetics -definition

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“genetic targeting of specific neurons or proteins with optical technology for imaging or control of the targets within intact, living neural circuits” (Deisseroth et al., 2006).

# Optogenetics - methodology

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Isolate corresponding gene for  
the protein

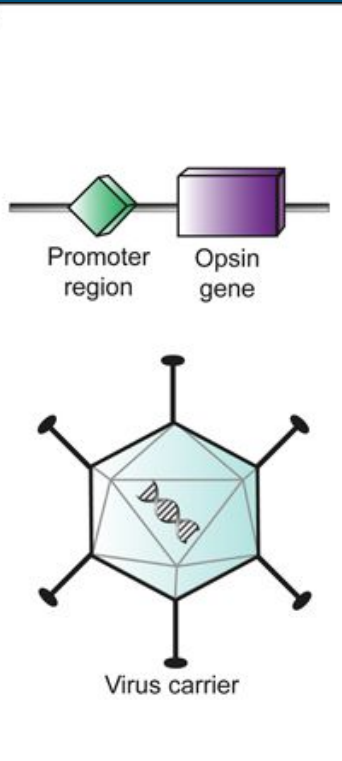


Transfer and merge the gene  
in the DNA of nerve cells

Stimulate the tissue with light

Read out the light signal

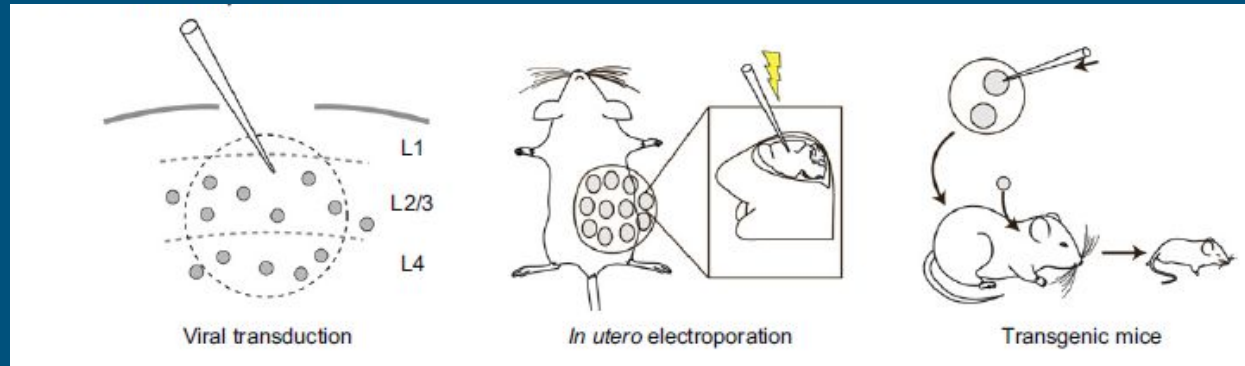
# Optogenetics - methodology transfer



Transfer by

1. viruses
2. Electroporation
3. Transgenic lines

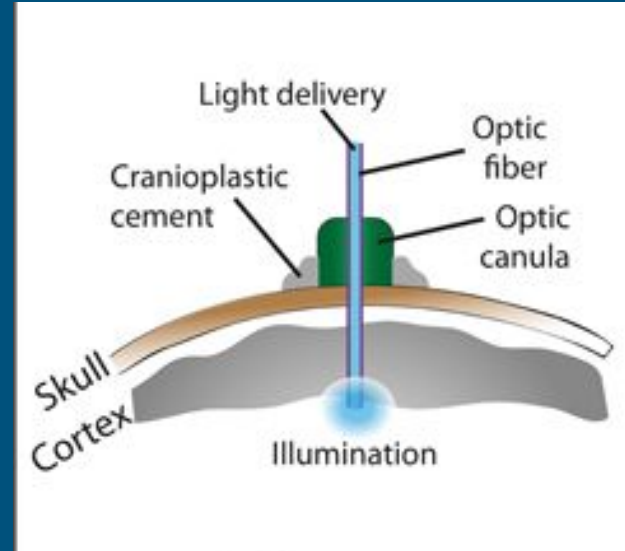
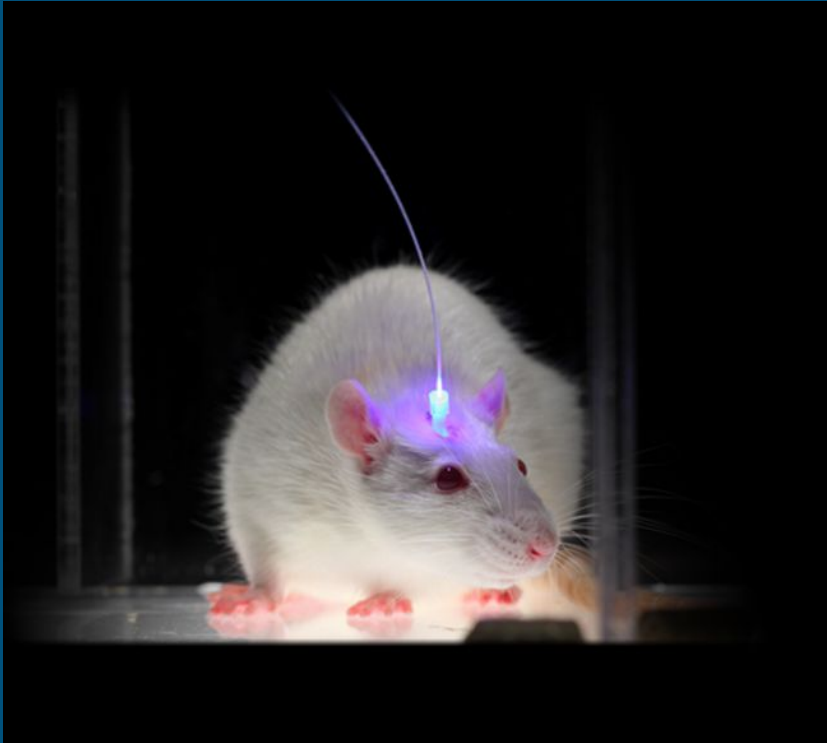
Allows transfer only to certain cells



Grienberger 2012  
Pama 2012

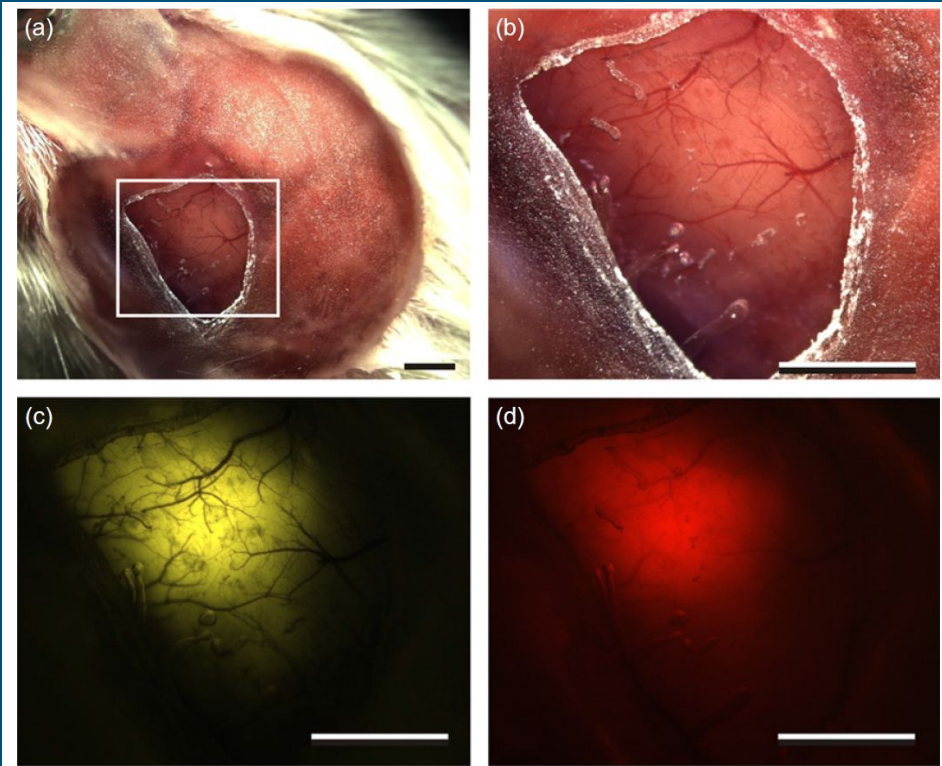
# Optogenetics - methodology illumination of the brain tissue

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<http://web.stanford.edu/group/dlab/optogenetics/>  
Pama 2012

# Optogenetics - methodology probing the brain

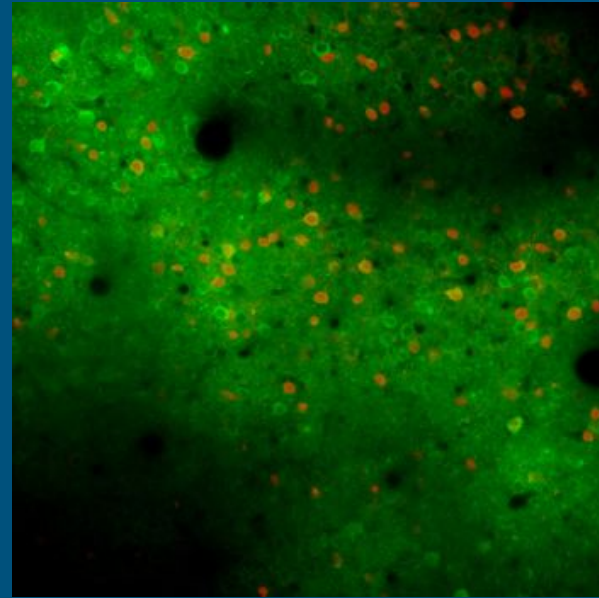
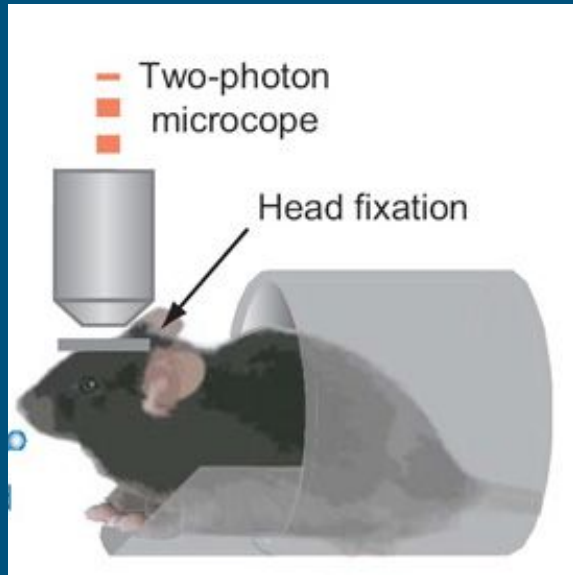


Wide field view of a mouse head after the embedment of a cover glass to the thinned bone.

Fluorescence images of a yellow-red VSFP variant captured through the glass window. Scale bars: 2mm.

# Optogenetics - methodology probing the brain

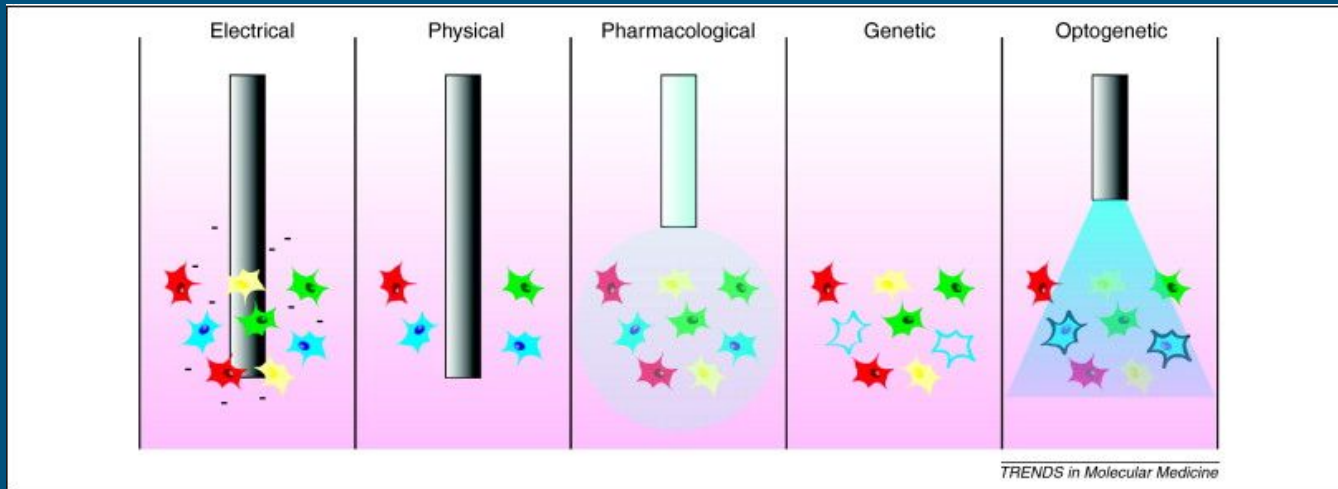
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# Optogenetics - summary

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Optogenetical methods allow temporally and spatially specific method to manipulate and probe neurons



# LETTERS

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## **Neural substrates of awakening probed with optogenetic control of hypocretin neurons**

Antoine R. Adamantidis<sup>1\*</sup>, Feng Zhang<sup>2\*</sup>, Alexander M. Aravanis<sup>2</sup>, Karl Deisseroth<sup>1,2</sup> & Luis de Lecea<sup>1</sup>

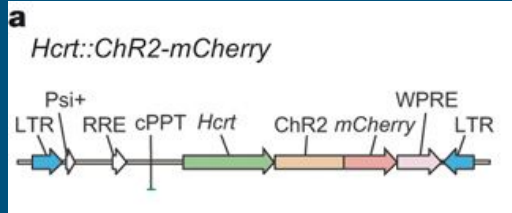


## Neural substrates of awakening probed with optogenetic control of hypocretin neurons

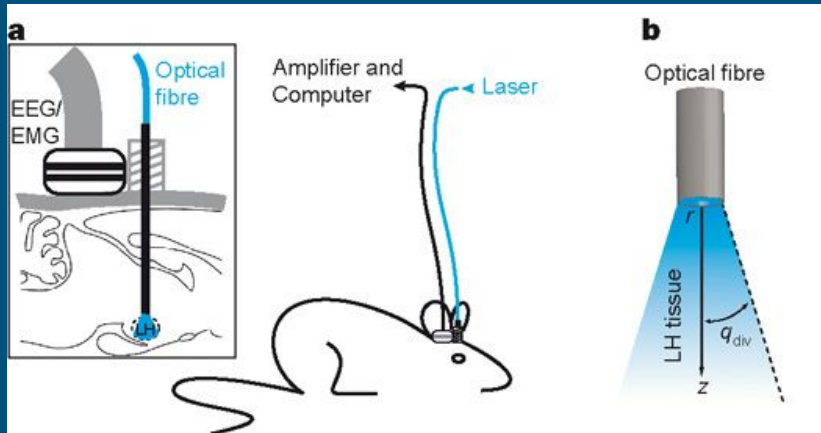
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- The neural underpinnings of sleep involve interactions between sleep-promoting areas and arousal systems
- Hypocretin-producing neurons are important for arousal stability and loss of Hcrt function has been linked to narcolepsy
  
- However, it is unknown whether electrical activity arising from Hcrt neurons is sufficient to drive awakening from sleep states or is simply correlated with it

# Neural substrates of awakening probed with optogenetic control of hypocretin neurons



Channelrhodopsin was isolated and transferred by viral vectors to hypocretin cells

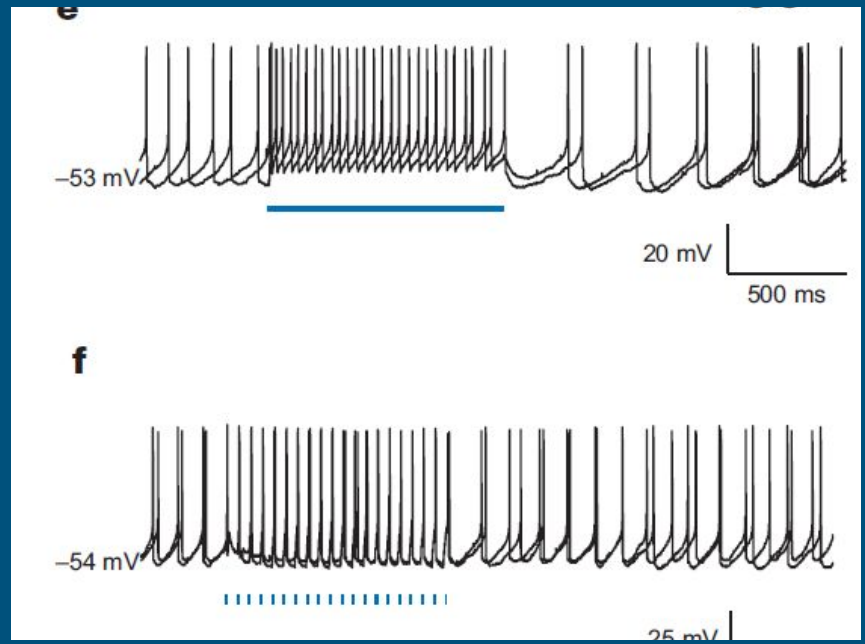


The neurons in the hypothalamus with channelrhodopsin were illuminated by laser

# Neural substrates of awakening probed with optogenetic control of hypocretin neurons



Light stimulation activated labeled hypocretin neurons



# Neural substrates of awakening probed with optogenetic control of hypocretin neurons

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Light stimulation of labeled hypocretin neurons reduced latency to wakefulness.

<http://www.nature.com/nature/journal/v450/n7168/extref/nature06310-s1.pdf>  
<http://www.nature.com/nature/journal/v450/n7168/suppinfo/nature06310.html>

# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

nature  
neuroscience

TECHNICAL REPORTS

Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

Daniel A Dombeck<sup>1</sup>, Christopher D Harvey<sup>1</sup>, Lin Tian<sup>2</sup>, Loren L Looger<sup>2</sup> & David W Tank<sup>1</sup>

# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

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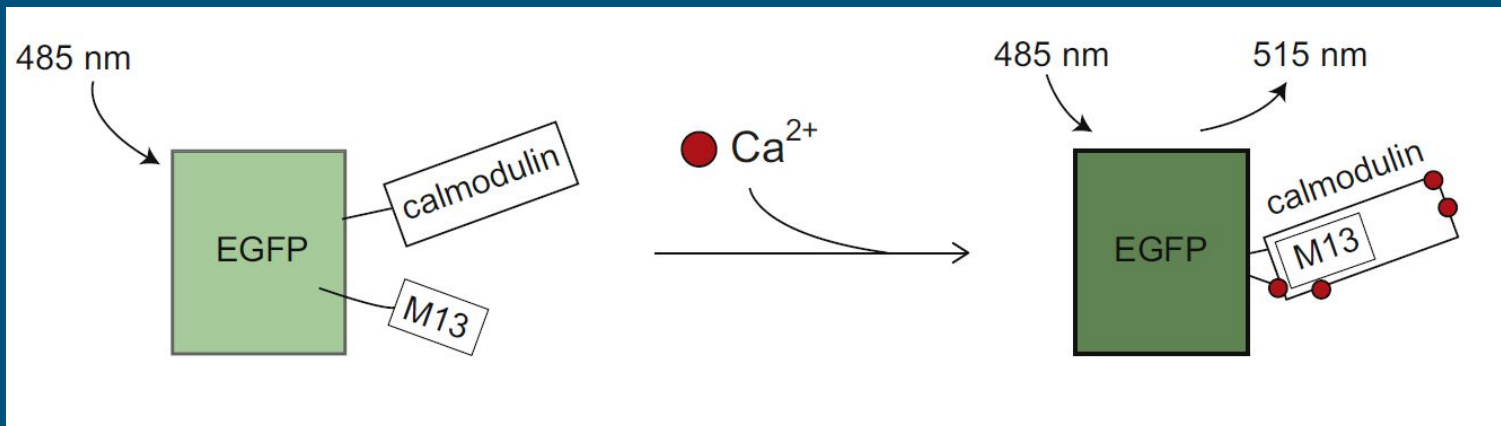
A **place cell** is a type of **pyramidal neuron** within the **hippocampus** that becomes active when an animal enters a particular place in its environment; this place is known as the place field.

- Before tetrode EEG recording of individual spiking activity
- Attempt to screen multiple neurons simultaneously

# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

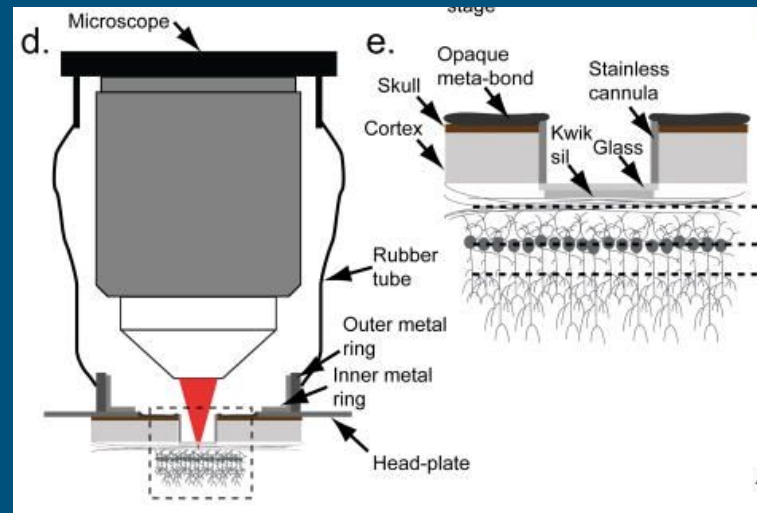
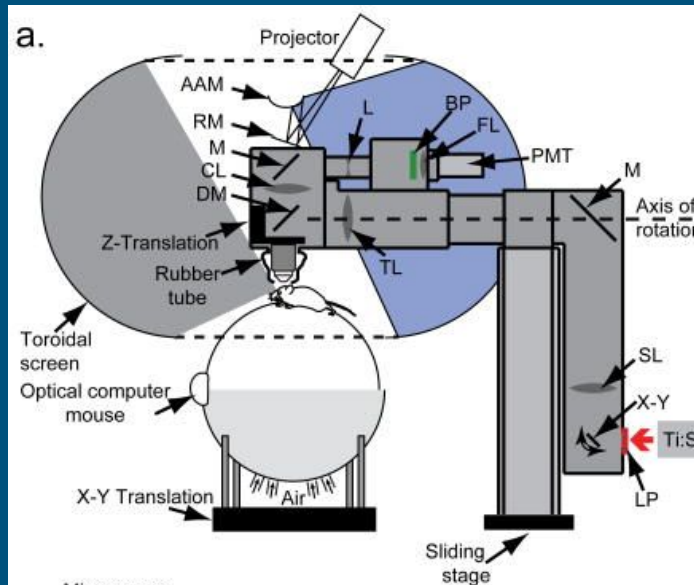
Calcium indicator was transferred by viral vector

AAV2/1-synapsin-1-GCaMP3



# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

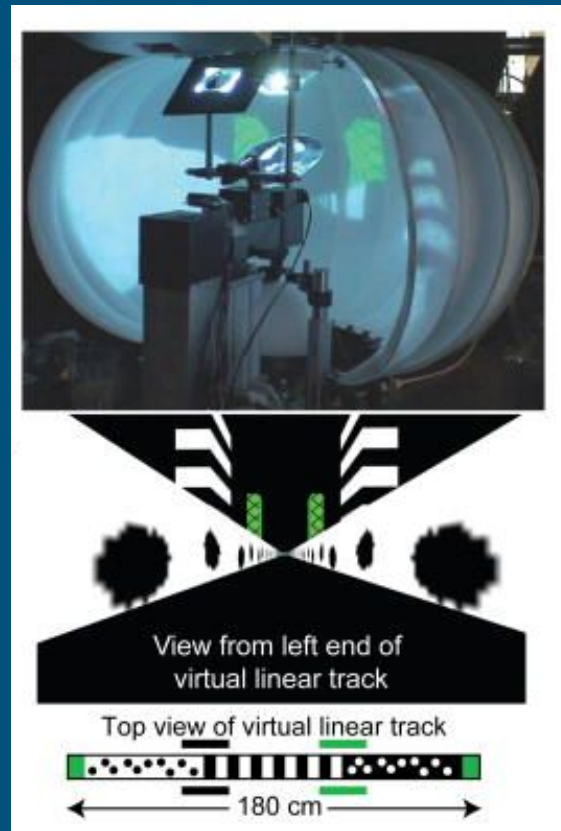
A glass window was implanted onto the skull and animal was attached to a two photon microscope



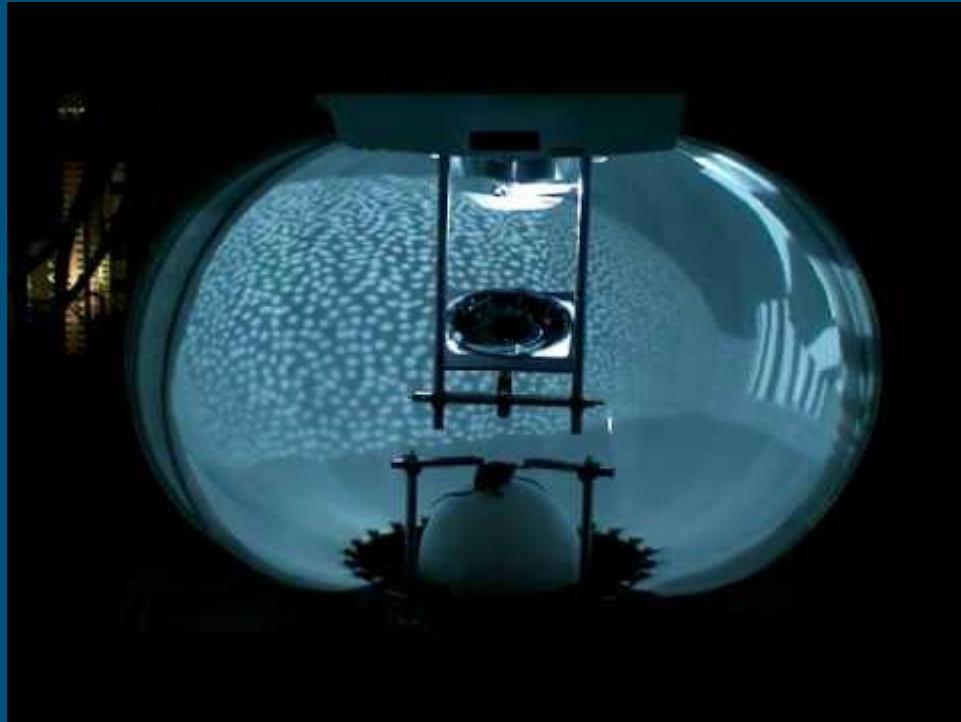


# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation

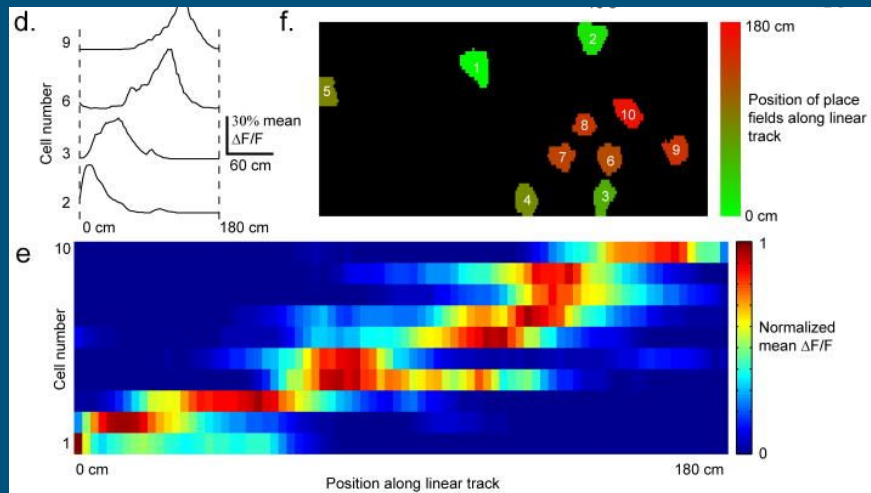
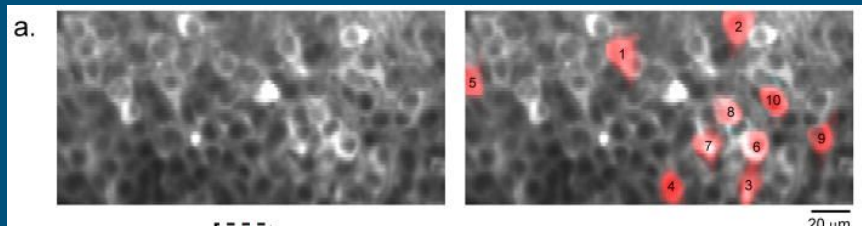
Animal was allowed to study virtual environment



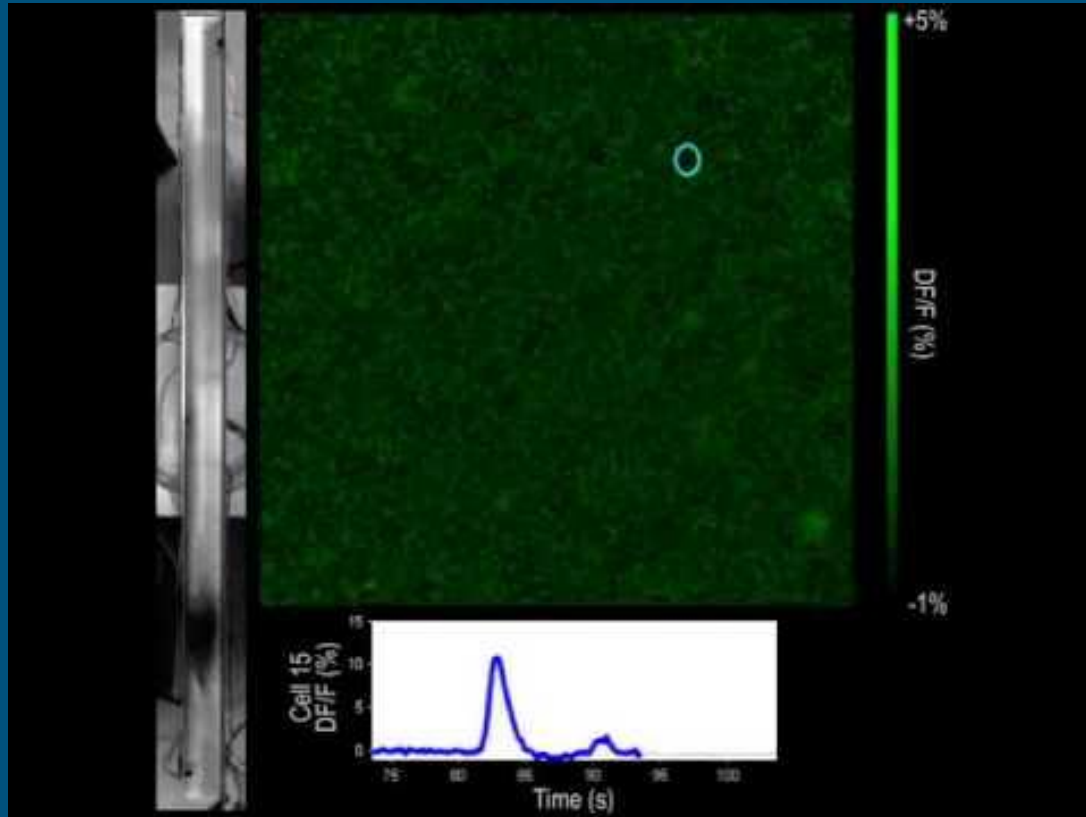
# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation



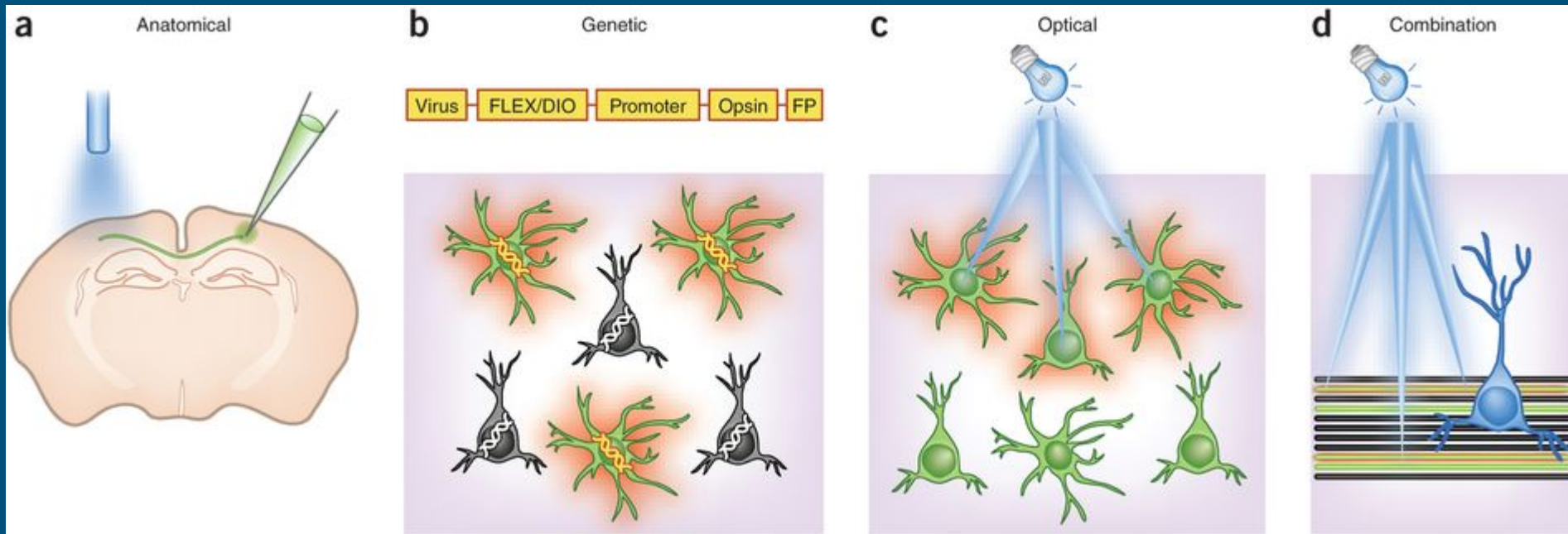
# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation



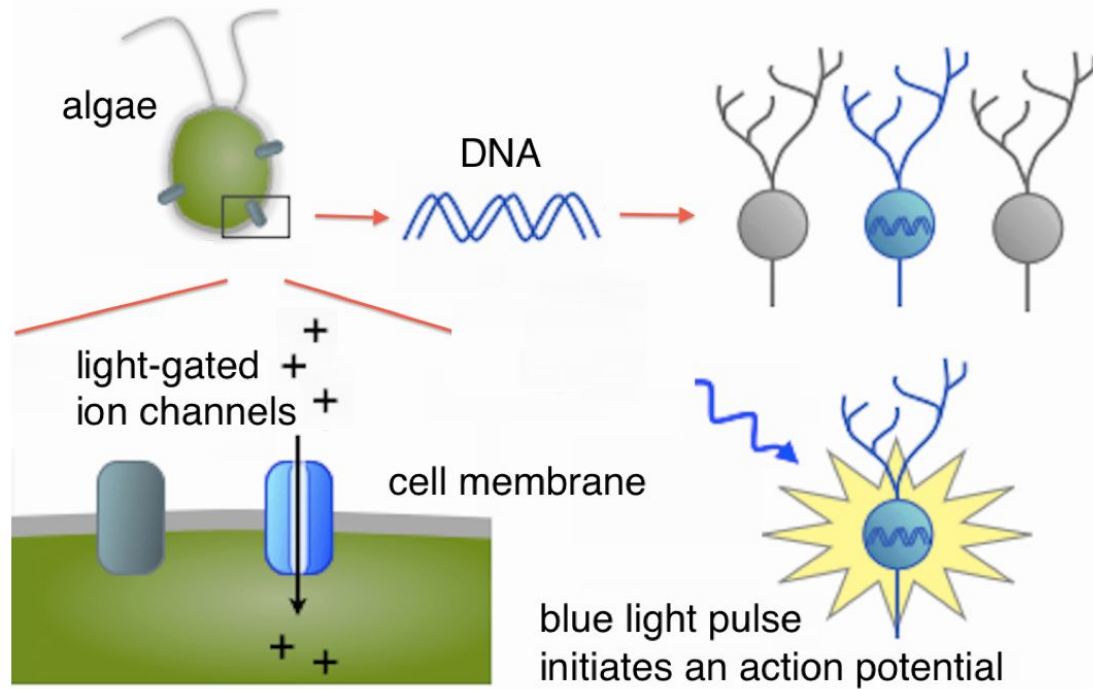
# Functional imaging of hippocampal place cells at cellular resolution during virtual navigation





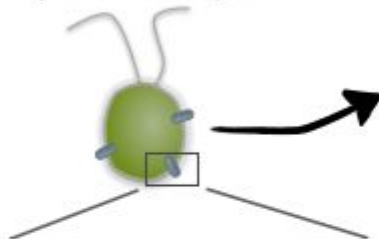


## Molecular Machines: Optogenetics & Gated Ion Channels



# How optogenetics works

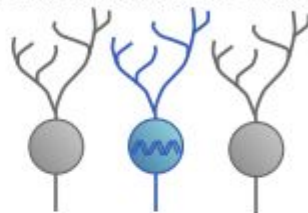
A light-sensitive protein from algae



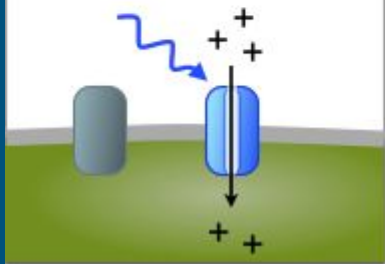
Take the gene for this protein...



... and insert the DNA into specific neurons in the brain



This protein is an ion channel that opens in response to **blue light**



Neurons communicate by "**firing**." This is an electrical signal created by opening & closing ion channels.

So now you can cause neurons to fire just by flashing **blue light!**



With the right combination of neurons, you can activate an entire brain circuit to control specific behaviors (like movement)