Optogenetics: the use of light-sensitive ion channels in neuroscience

Reporter: Weiliang Xing

Student ID: 108211104

Course: BME-503

Advisor: Emilia Entcheva

Contents

Abstract: In neuroscience field, both observation and perturbational methods are important for understanding the function of nervous system. During the last century, the observational methods have made great advances which leave the perturbation-al methods behind. The development and application of Optogenetics, however, are making up the gaps. By its reliable Optogenetical tools, or inserting light-sensitive channel proteins into the nervous cells, researchers can now trigger firing specific neurons under the stimulation of light. In this article, we will discuss the function and application of light-sensitive ion channels in neuroscience in details, which may provide useful insights into neuroscience, although Optogenetics is getting off to a flying start.

Key words: Optogenetics, light-sensitive ion channel, neuroscience.

1 Introduction

1.1 The definition of Optogenetics

From its birth in 2006[1] in the literature to the term commonly used in neuroscience as one perturbational method, Optogenetics refers to the combination of optical and genetical methods to achieve gain-of-function or loss-of-function of certain cells or tissues[2]. Karl Deisseroth in his review said that "Optogenetics is the combination of genetics and optics to control well-defined events within any specific cells of living tissue (not just those of the nervous system)."[3] However, the term "Optogenetics" is not as similar as other fields like optoelectronics, etc., which refers to interactions of light with electrons. Optogenetics, in another hand, is not defined by the interactions between light and genes; what is important is its effects of light on the protein products of genes[4], especially the light-sensitive channels. According to the 2019 revision of the Oxford English dictionary, Optogenetics is defined as "the branch of biotechnology which combines genetic engineering with optics to observe and control the function of genetically targeted groups of cells with light, often in the intact animal." In other words, Optogenetics is one kind of biochemical and optical control

techniques based on the input of certain or combined wavelength of light coupled to the output of cell's response for the functional discovery and/or realization.

1.2 The development of Optogenetics

The early development before this term birth in the literature may be back to an article published in 1979 Scientific American article written by Nobel winner Francis Crick. In this article, he suggested ''a method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered"[5].Till now, extracellular electrical manipulation cannot readily achieve true electrical excitation with less temporal precision, which results in the low specificity in certain cell type. The reason is obvious: they stimulate all the cells near the electrical field without distinguishing different cell types[6]. Crick then supposed that light might have such properties to realize such purpose. However, there was no clear method to make light as a tool in cell control, or make cells sensitive to light.

It always seems that scientific inspiration comes from other fields out of certain research. Scientists from another branch of biology-microbiology already knew that some microorganisms produce proteins positioned in the membrane which is sensitive to light to adjust environment. These proteins expressed by ''opsin'' genes seems to open the door of Optogenetics to some degree. In 1971 Walther Stoeckenius and Dieter Oesterhelt discovered one of these proteins, bacteriorhodopsin can be activated by green light, which is called afterward one kind of single-component ion pump Later the halorhodopsins and channelhodopsins were found in 1977 [7]and 2002[8].(Figure 1)
However, it still took almost 30 years to combine the two fields together into a new strategy for neuroscience research. During that time, different approaches without opsin genes were considered, like multicomponent strategies[9] involving different genes or combinations function. But the photocurrents were too slow and weak due to the toxicity of those foreign proteins.

A report in August 2005 made the single-component strategy possible into neuroscience. Feng Zhang, at el. first reported a microbial opsin gene without any other parts and neurons became precisely responsive to light [10].Soon after that, several additional reports followed and many new light-sensitive ion channel were found and used in

nervous study(Figure 2).Now, the single-component property of the microbial opsin system could provide temporal precision, specified targeting and applicability into neuroscience studies. Thousands of scientists are now learning how specific activity

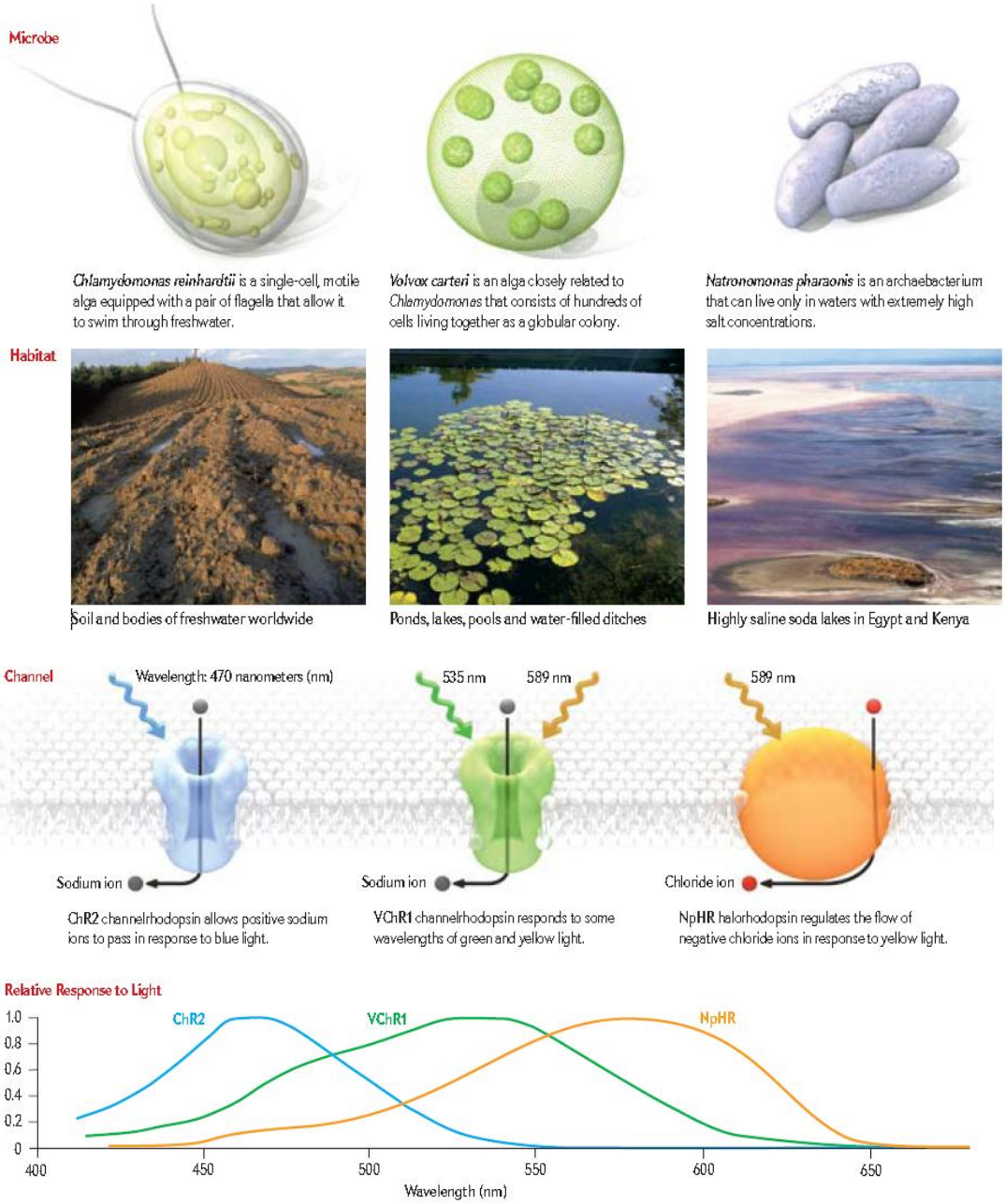

Figure 1. Certain algae or bacteria is sensitive to the light due to opsin proteins. The flow of electrically charged ions changes their response and metabolism when

illuminated. The opsin genes are the foundation of Optogenetics to control the activity patterns in targeted neurons. These proteins, which belong to ion channels, are ChR2, VChR1 and NpHR showed in the figure. They are stimulated by different wavelength by different ion flow pattern. (The figure is from[3])

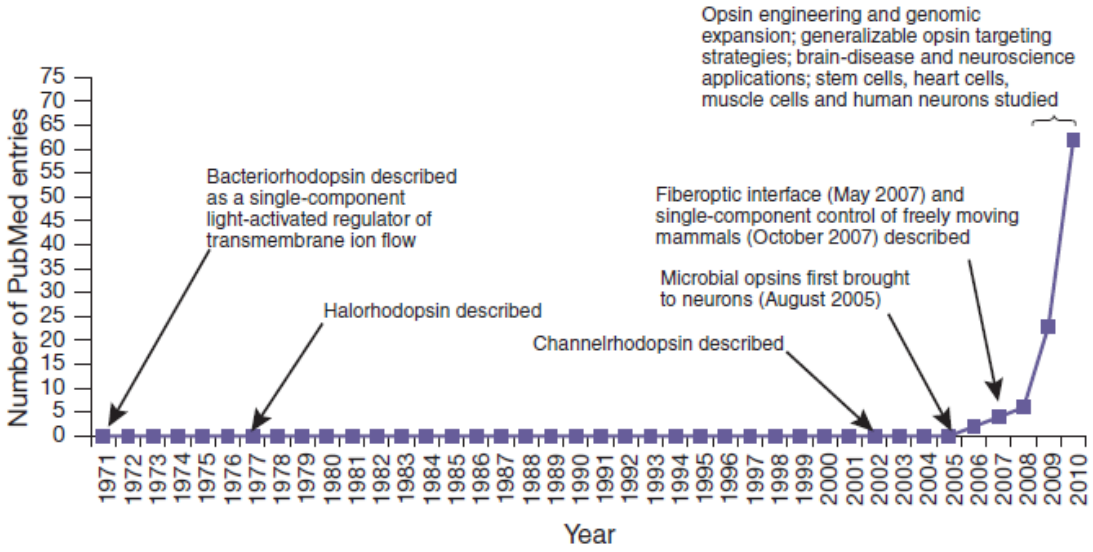

Figure 2. The Optogenetics' development in nearly 30 years. In the figure many milestones in this field are marked. The number indicates the publications referring to the word 'optogenetics' till 2010. (The figure is from [6])

patterns by selecting sets of neurons using optogenetic method lead to complex physiology and behavior in worms, flies, fish, birds, mice, rats and monkeys[3]. The work will bring new sights into human problems, including Parkinson's disease, depression, etc.

1.3 The principle of Optogenetics in neuroscience

Just as showed before, Optogenetics method controls well-defined events by optical ways. Its contents include the discovery and insertion of genes that express light-sensitive channel protein and the delivery of light into nervous system to make effects of certain cells or the whole tissue. The central work which Optogenetics depends on is the light-sensitive ion channels. Meanwhile, there are two classes of optogenetic devices: sensors and actuators, whose interaction with each other set the foundation of Optogenetics.

1.3.1 The general principle of light-sensitive ion channels

The light-sensitive ion channels belong to the microbial opsins (type I). Opsin proteins require retinal, a vitamin A-related cofactor that serves as the antenna for photons. When retinal is bound to it, the opsin protein are termed rhodopsins [11]. Retinal attaches to a conserved lysine residue of helix 7 to form a protonated retinal Schiff base (RSBH+). When absorbing a photon, the retinal isomerizes and triggers a sequence of conformational changes within the opsin partner. Then the opsin protein is 'opened' by illumination. Actually, opsin family encode seven-transmembrane structures. After photoisomerization, rhodopsins revert to the all-trans state[12]. Then the channels shuttle from the dark-adapted state through a stereotyped progression of functional and conformational states and then go back to dark-adapted state again.[13]The open of channels change the membrane potential of the cell and activate the cascade reaction which will lead to the signal process. The main process of specific ion channels are showed in Figure 3.

1.3.1.1 Bacteriorhodopsin (BR)

The first identified and best understood so far is the haloarchaeal proton pump bacteriohodopsin(BR)[14](Figure 3). Under low-oxygen conditions, BR is expressed in haloarchaeal membranes and serves as part of an alternative energy-production system, pumping protons from the cytoplasm to the extracellular medium for ATP synthesis [15].It may not belong to light-sensitive ion channels, but later on many light-gated pumps were found based on it due to their similar photcycles[16].

1.3.1.2 Halorhodopsin (HR)

The second class of opsin genes encode halorhodopsins (HR)(Figure 3). HR is a light-activated chloride pump first found in archaebacteria[17].The principle of HR is similar to that of BR. What is different is that it pumps chloride ions and the direction of transport is opposite to that of BR, from extracellular to the intracellular space[18].

1.3.1.3 Chanelrhodopsin (ChR)

The third class of opsin gene encodes channelrhodopsin (ChR)(Figure 3). It is found and demonstrated its light-activated ion-flux in the green algae Chlamydomonas reinhardtii [19]. ChR is highly homologous to BR and its effective cation channel pore is opened for

ion flux which depends on kinetics of channel closure rather than retinal isomerization [20].

.

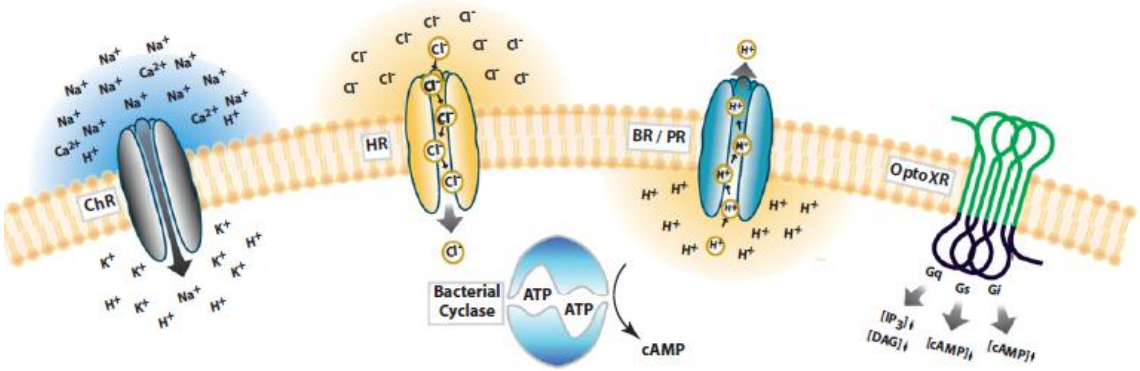

Figure 3 . The process of different ion channels . Channelrhodopsins conduct cations and depolarize neurons upon illumination (left). Halorhodopsins conduct chloride ions into the cytoplasm upon yellow light illumination (center). Bacteriorhodopsin conduct protons into the cytoplasm under low- oxygen(center).OptoXRs 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).(The figure is from [21])

1.3.2 The interaction of sensors and actuators in Optogenetics

In neuroscience field, optogenetic methods could work under the interaction of sensors and actuators. Sensors translate cell physiological signals into optical signals by certain protein expression like fluorescins: they make the cellular function visible; actuators transduce optical signals into physiological signals: they make cellular function control-lable [4]. The light-sensitive ion proteins are located on the actuators which accepted the optical stimulation. They build a complete response unit which shows researchers what are the nervous cells' function and how do they function under control (Figure 4).

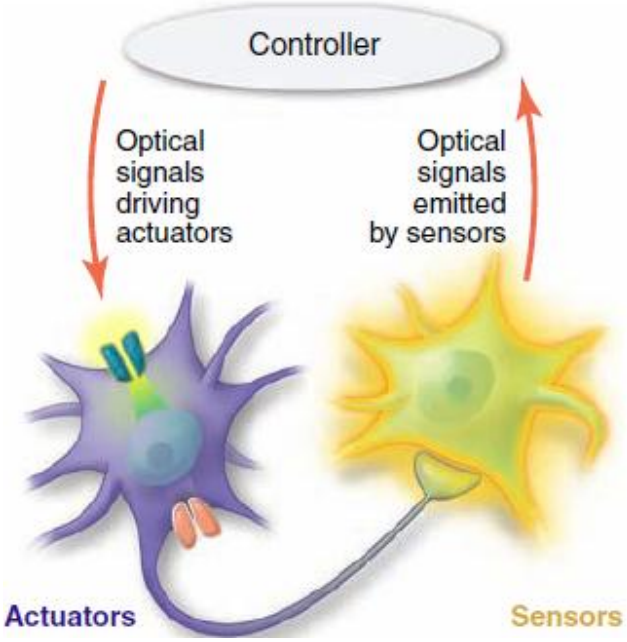

Figure 4. Sensors and actuators. Controller send optical stimulation to actuators, and then the ion channels of actuators are accepted and create de- or hyperpolarizing currents. At last the sensors report changes in membrane potential, intracellular calcium concentration or synaptic transmission by fluorescins' fluorescence response(the figure is from [4]).

2 The light-sensitive ion channels in neuroscience of Optogenetics

With the recent development of optogenetic strategies used into neuroscience, researchers could control distinct cell types by light, including enabling the non-invasive activation, inhibition, and modulation of specific neuronal populations in living animals with millisecond precision[22]. The light-sensitive ion channels take part in mainly the first two parts, which will be discussed in details.

2.1 Optogenetic excitation for neuroscience

2.1.1 ChR2 and its mutations

As noted above, Channelrhodopsin was introduced into hippocampal neurons in 2005 [10] and proved to confer millisecond precision control of neuronal spiking. After that a series of improvement of ChR2 are reported.ChR2 was initially improved by human codon optimization to replace histidine by arginine at position 134 to increase steady-state current size (ChR2-H134R)[23]. To overcome the barrier of Membrane-trafficking,

endoplasmic reticulum (ER)-export motifs were introduced to achieve high expression of halorhodopsins [24]. Then, the hybrid of ChR1 and ChR2 was found to improve expression for channelrhodopsins[25]. Then to solve the problems about the desensitization of ChR even in the presence of light, the chimeric opsins were found(e.g., CHIEF)[26].Another approach is to modify the residue E123 of ChR2 (ChR2-E123)to threonine or alanine; the result is ChETA[27],which reduces desensitization and closes the channel more quickly[11]. (Table 1)

When several new designs of ion channels are introduced to initiate precise spiking, anther requirement to simply alter the excitability of a target neuronal population appears. The latter one, however, pays more attention on the activity of neuronal population without specifically[11]. Then Berndt et al. developed the step-function opsins(SFOs)[28] to prolong the active state of the channel even after light-off compared to ChETA. Thus SFOs have inactivation time constants lasting for seconds and be activated by 10-ms pulse of blue light [28].Its improvement includes ChR2(D156 A) [29]. (Table 1)

2.1.2 VChR and its mutations

Another branch of optogenetical activation ion channel proteins would be VChR1, which is found from *Volvox carteri* [30]. VChR1 is activated by red light compared to ChR1. But it could create relatively small currents due to its low expression[11]. The improvement of VChR1 would be a chimeric opsin, C1V1, which combines parts of ChR1 [31], ChR2 and VChR1.The result is that C1V1 have both properties of VChR1 and ChR2: red-shifted activation and nanoampere-scale currents. (Table 1)

2.2 Optogenetic inhibition for neuroscience

2.2.1 NpHR and its mutations

Neuroscience study requires both activation and inhibition control of nervous cells. For this purpose, certain light-activated ion pumps could be used for inhibition. However, so far only one ion pump is efficient[32] : the ER trafficking-enhanced version of a halorhodopsin (NpHR) from *Natronomonas pbaraonis* [33][24, 34].NpHR acts to

hyperpolarize the targeted neurons by low activating chloride pump. From certain sense, NpHR is a true pump: it needs constant light stimulation[11]. To solve membrane trafficking problems, eNpHR2.0 and 3.0 appear to improve membrane targeting and higher currents[35]. (Table 1)

Table 1 light-sensitive ion channels in Optogenetics for neuroscience. The table shows the main characters of opsins which act as light-sensitive ion channels in neuroscience(the table is from [21]).

Opsin	Mechanism	Peak Activation λ	Off Kinetics (τ, ms)*
Blue/Green Fast Excitatory			
ChR2	Cation channel	470 nm	~10 ms
ChR2(H134R)	Cation channel	470 nm	18 ms
ChR2 (T159C)	Cation channel	470 nm	26 ms
ChR2 (L132C)	Cation channel	474 nm	16 ms*
ChETAs: ChR2(E123A) ChR2(E123T) ChR2(E123T/T159C)	Cation channel	470 nm (E123A) 490 nm (E123T)	4 ms (E123A) 4.4 ms (E123T) 8 ms (E123T/T159C)
ChIEF	Cation channel	450 nm	~10 ms
ChRGR	Cation channel	505 nm	4-5 ms* (8-10ms)
Yellow/Red Fast Excitatory			
VChR1	Cation channel	545 nm	133 ms
C1V1	Cation channel	540 nm	156 ms
C1V1 ChETA (E162T)	Cation channel	530 nm	58 ms
C1V1 ChETA (E122T/E162T)	Cation channel	535 nm	34 ms
Bistable Modulation			
ChR2-step function opsins (SFOs)	Cation channel	470 nm activation / 590 nm deactivation	2 s (C128T); 42 s (C128A) 1.7 min (C128S) 6.9 min (D156A) 29 min (128S/156A)
VChR1-SFOs	Cation channel	560 nm activation / 390 nm deactivation	32 s (C123S) 5 min (123S/151A)
Yellow/Red Inhibitory			
eNpHR3.0	Chloride pump	590 nm	4.2 ms
Green/Yellow Inhibitory*			
Arch/ArchT	Proton pump	566 nm	9 ms
eBR	Proton pump	540 nm	19 ms

3 Applications in neuroscience in diverse animal models

By far the application of optogenetics is still in its infancy mainly focusing on proof-of-principle studies in diverse animal models including mouse, rat, primate, *Caenorbabditis* elegans, fly and Zebrafish[11] depends on different study purpose. The application will be discussed below classified by main animal models.

3.1 Mouse

As Optogenetic methods become common in neuroscience recently, the mouse has been regarded as the most widely published Optogenetic model organism [11]. Mice owns a vast selection of transgenic lines expression Cre recombinase in specific neurons which could be used in Optogenetics[36]. Another advantage of mouse used in optogenetic field is the ability of the embryonic stem cells to express proteins engineered by optogenetic methods[37].

The first report to use channelrhodopsin in mouse was to exam the contribution of hypothalamic hypocretin neurons to sleep and wakefulness [38]. It was known during that time that the neural underpinning of sleep links with the interactions between sleep-promoting areas and arousal system in which Hypocretinn (Hcrt) plays an important part. While it was known that loss of Hcrt function has linked to narcolegpsy [39], whether the electrical activity from Hcrt neuron is sufficient to drive awakening is unknown. The reporters then targeted ChR2 into Hcrt cells in vivo of mouse to probe the impact of Hcrt neuron activity on sleep state transitions under the light stimulate. The result is obvious: under optogenetic photostimulation Hcrt neurons increases the probability of transition to wakefulness from either slow wave sleep or rapid eye movement sleep [38].

There are many researches in mouse model afterwards. Carter et al. modulate the locus coeruleus neurons bidirectionally by inserting multi-channels in mouse cell membrane[40]; Tsai et al. identified the causal relationships between activity patterns in VTA dopamine neurons and reward behavior in mice[41]; by stimulation of axonal terminals in the nucleus accumbens, investigators found that dopamine neurons corelease glutamate[42]. The

mechanisms about dopamine-modulated addiction was studied by inserting Cre-dependent ChR2 into mice genes[43]; by optogenetic methods, parvalbumin-expression fast-spiking interneurons are proved to involve in gamma oscillations and information processing in mouse prefrontal [44]. All in all, here are so many other researches focusing on mouse nervous system that have been boost Optogenetics to become a powerful tool in such area.

3.2 Rat

Due to their ability to complete complex behavioral missions and relative simplicity of their brains, rats become important for neuroscience by Optogenetics. Recently, blood oxygen level-dependent (BOLD) responses in fMRI was examined by optogenetic methods in rats [45]. The research focuses on identifying the BLOD signals by optogenetic tools on which functional magnetic resonance imaging (fMRI) depends to solve the controversy whether BOLD signals in a particular region can be caused by activation of local excitatory neurons [46].Stimulating ChR2 expression by light in excitatory neuronal cells creates enough BOLD response in local cortical targets as well as downstream thalamic regions. Then they developed a new technology called ofMRI approach which is suitable for functional circuit as well as global phenotyping of dysfunctional circuity [45].

Other researches focus on modulating rhythmic beating activity in rodent cardiomyocytes by ChR2 channels [47], modulating cardiovascular function[48] and blood pressure [49] in rats by ion channel optogenetic methods .

3.3 Primate

ChR2 delivery to cortical neurons of macaques in primate nervous system has been tried by Han et al. 2009[50]. In this research, investigators target the ChR2 specifically to excitatory neurons by lentivirus in primate. ChR2 was safely expressed and activation of excitatory neurons was observed. However, this report did not further discuss the behavioral responses in the primate by Optogenetics. Then inhibition ion channel eNpHR2.0 was introduced in to the retinas in 2010[51]. The results showed optogenetic efficacy in mearsurement linked to retinitis pigmentosa (RP), one kind of disease in which light-sensing cells degenerate in the retina. The efficiency of ChR2 in human embryonic stem cell-derived neurons is reported during that time [52].

3.4 Other models: *Caenorbabditis elegans*, fly and Zebrafish.

Lower animal models including invertebrate models are also investigated in optogenetic ways to apply light-sensitive ion channels into functional research in neuroscience. *Caenorbabditis elegans* enable large-scale investigation of various mutant strains for synaptic proteins [53]; it could be used for readout of neural activity combined with light stimulation [54] or control body wall muscle contraction bidirectionally with ChR2 and NpHR [55]. There are also many applications of Optogenetics in fly. ChR2 has been used to find the principle of the nociceptive response[56] and to investigate the innate escape response [57]. The advantage of fly model is that for them food supplement can provide enough retinal to drive ChR2 function. Through the fly experiment, the green peak activation wavelength of eNpHR 3.0 is detected [58]. The advantages of Zebrafish for optogenetic research in neuroscience are its short reproduction time and easy transduction into Zebrafish. Douglass et al. first use it to exam the role of somatosensory control of escape behavior by inserting ChR2 into single spikes[59]. Recent study shows that Zebrafish with eNpHR allow the tools to be easily targeted to specific neuronal subtypes [60].Another important application of ion channels in Zebrafish is to stimulate neurons that express endogenous light-activated proteins[60].

4 The future of optogenetic light-sensitive ion channels in neuroscience

Since the found of the first light-sensitive ion channel, the modification of the proteins has never stopped. Actually, the fidelity of optogenetic control via opsin gene expression is not optimal, with noise leading to missing action potentials or extra action potentials [10]. The modified opsins in the future enable high-fidelity control over high-frequency action potential in the neural system or bistable change in excitability and magnitude increased light sensitivity[6]. The better ion channels well prove importance of optogeneitcs in the application of neuroscience in the future in different ways. New channels will be activated by light ranging across, even beyond the visible spectrum such as UV light, which make non-invasive control possible. The main problems like membrane trafficking would be overcame better. In a word, more precise, diversity of light sensitivity and activation wavelength, better stability and efficient expressed light-sensitive ion channels will make Optogenetics one of the main technologies in neuroscience study in the future.

Reference

1. Greg Miller. Optogenetics:Shining New Light on Neural Circuits.Science,2006. 314 (5806):p.1674-1676.
2. Deisseroth, K., et al., *Next-generation optical technologies for illuminating genetically targeted brain circuits.* J Neurosci, 2006. **26**(41): p. 10380-6.
3. Deisseroth, K., *Controlling the Brain with Light.* Neuroscience, 2010: p. 6.
4. Miesenbock, G., *The optogenetic catechism.* Science, 2009. **326**(5951): p. 395-9.
5. Crick FH. 1979. Thinking about the brain. Sci. Am. 241:219–32
6. Deisseroth, K., *Optogenetics.* Nat Methods, 2011. **8**(1): p. 26-9.
7. Matsuno-Yagi, A. & Mukohata, Y. Biochem. Biophys. Res. Commun. 78, 237–243 (1977).
8. Nagel, G. et al. Science 296, 2395–2398 (2002).
9. Banghart, M., et al., *Light-activated ion channels for remote control of neuronal firing* Nat Neurosci, 2004. **7**(12): p. 1381-1386.
10. Boyden, E.S., et al., *Millisecond-timescale, genetically targeted optical control of neural activity.* Nat Neurosci, 2005. **8**(9): p. 1263-8.
11. Fenno, L., O. Yizhar, and K. Deisseroth, *The development and application of optogenetics.* Annu Rev Neurosci, 2011. **34**: p. 389-412.
12. Hofmann KP, Scheerer P, Hildebrand PW, Choe HW, Park JH,et al. 2009. A G protein-coupled receptor at work: the rhodopsin model. Trends Biochem. Sci. 34:540–52.
13. Bamann C, Gueta R, Kleinlogel S, Nagel G, Bamberg E. 2010.Structural guidance of the photocycle of channelrhodopsin-2 by an interhelical hydrogen bond. Biochemistry 49:267–78
14. Oesterhelt, D., and Stoeckenius, W. (1971). Rhodopsin-like protein from the purple membrane of Halobacterium halobium. Nat. New Biol. 233, 149–152.
15. Racker, E., and Stoeckenius, W. (1974). Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. J. Biol. Chem. 249, 662–663.
16. Be´ ja` , O., Spudich, E.N., Spudich, J.L., Leclerc, M., and DeLong, E.F. (2001). Proteorhodopsin phototrophy in the ocean. Nature 411, 786–789.
17. Matsuno-Yagi, A., and Mukohata, Y. (1977). Two possible roles of bacteriorhodopsin;a comparative study of strains of Halobacterium halobium differing in pigmentation. Biochem. Biophys. Res. Commun. 78, 237–243.
18. Essen, L.O. (2002). Halorhodopsin: light-driven ion pumping made simple? Curr. Opin. Struct. Biol. 12, 516–522.
19. Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E., and Hegemann, P. (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. Science 296, 2395–2398.
20. Feldbauer, K., Zimmermann, D., Pintschovius, V., Spitz, J., Bamann, C., and Bamberg, E. (2009). Channelrhodopsin-2 is a leaky proton pump. Proc. Natl. Acad. Sci. USA 106, 12317–12322.
21. Yizhar, O., et al., *Optogenetics in neural systems.* Neuron, 2011. **71**(1): p. 9-34.
22. Kravitz, A.V. and A.C. Kreitzer, *Optogenetic manipulation of neural circuitry in vivo.* Current Opinion in Neurobiology 2011(21): p. 433-439.

23. Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E, Gottschalk A. 2005. Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Curr.Biol. 15:2279–84
24. Gradinaru, V., K.R. Thompson, and K. Deisseroth, *eNpHR: a Natronomonas halorhodopsin enhanced for optogenetic applications.* Brain Cell Biol, 2008. **36**(1-4): p. 129-39.
25. Lin JY, Lin MZ, Steinbach P, Tsien RY. 2009. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys. J. 96:1803–14
26. Lin JY, Lin MZ, Steinbach P, Tsien RY. 2009. Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys. J. 96:1803–14
27. Gunaydin LA, Yizhar O, Berndt A, SohalVS, Deisseroth K, Hegemann P. 2010. Ultrafast optogenetic control. Nat. Neurosci. 13:387–92
28. Berndt A, Yizhar O, Gunaydin LA, Hegemann P, Deisseroth K. 2009. Bi-stable neural state switches. Nat. Neurosci. 12:229–34
29. BellmannD, Richardt A, Freyberger R, NuwalN, Schwarzel M, et al. 2010. Optogenetically induced olfactory stimulation in Drosophila larvae reveals the neuronal basis of odor-aversion behavior. Front. Behav. Neurosci. 4:27
30. Zhang F, Prigge M, Beyriere F, Tsunoda SP, Mattis J, et al. 2008. Red-shifted optogenetic excitation: a tool for fast neural control derived from Volvox carteri. Nat. Neurosci. 11:631–33
31. NagelG,Ollig D, Fuhrmann M, Kateriya S, Musti AM, et al. 2002. Channelrhodopsin-1: a light-gated proton channel in green algae. Science 296:2395–98
32. Tye K, Prakash R, Kim S-Y, Fenno LE, Grosenick L, et al. 2011. Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature. In press
33. Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, et al. 2010. Molecular and cellular approaches for diversifying and extending optogenetics. Cell 141:154–65
34. Kaneda, K., et al., *Selective optical control of synaptic transmission in the subcortical visual pathway by activation of viral vector-expressed halorhodopsin.* PLoS One, 2011. **6**(4): p. e18452.
35. Busskamp V, Duebel J, Balya D, Fradot M, Viney TJ, et al. 2010. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. Science 329:413–17
36. Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, et al. 2007. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J. Neurosci. 27:9817–23
37. Stroh A, Tsai HC, Ping Wang L, Zhang F, Kressel J, et al. 2010. Tracking stem cell differentiation in the setting of automated optogenetic stimulation. Stem Cells 29(1):78–88
38. Adamantidis, A.R., et al., *Neural substrates of awakening probed with optogenetic control of hypocretin neurons.* Nature, 2007. **450**(7168): p. 420-4.
39. Chemelli, R. M. et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98, 437–451 (1999).
40. Carter ME, Yizhar O, Chikahisa S, Nguyen H, Adamantidis A, et al. 2010. Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat. Neurosci. 13:1526–33
41. Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, et al. 2009. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324:1080–84

42. Tecuapetla F, Patel JC, Xenias H, English D, Tadros I, et al. 2010. Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. J. Neurosci. 30:7105–10
43. Brown MT, Bellone C, Mameli M, Labouebe G, Bocklisch C, et al. 2010. Drug-driven AMPA receptor redistribution mimicked by selective dopamine neuron stimulation. PLoS One 5:e15870
44. Sohal VS, Zhang F, Yizhar O, Deisseroth K. 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459:698–702
45. Lee, J.H., et al., *Global and local fMRI signals driven by neurons defined optogenetically by type and wiring.* Nature, 2010. **465**(7299): p. 788-92.
46. Logothetis, N. K., Pauls, J., Augath, M., Trinath, T. & Oeltermann, A.Neurophysiological investigation of the basis of the fMRI signal. Nature 412, 150–157 (2001).
47. Bruegmann T,Malan D, Hesse M, Beiert T, Fuegemann CJ, et al. 2010. Optogenetic control of heart muscle in vitro and in vivo. Nat. Methods 7:897–900
48. Abbott SB, Stornetta RL, Fortuna MG, Depuy SD, West GH, et al. 2009a. Photostimulation of retrotrapezoid nucleus phox2b-expressing neurons in vivo produces long-lasting activation of breathing in rats. J. Neurosci. 29:5806–19
49. Kanbar R, Stornetta RL, Cash DR, Lewis SJ, Guyenet PG. 2010. Photostimulation of Phox2b medullary neurons activates cardiorespiratory function in conscious rats.Am. J. Respir. Crit.CareMed. 182:1184–94
50. Han, X., et al., *Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain.* Neuron, 2009. **62**(2): p. 191-8.
51. Busskamp V, Duebel J, Balya D, Fradot M, Viney TJ, et al. 2010. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. Science 329:413–17
52. Weick JP, Johnson MA, Skroch SP, Williams JC, Deisseroth K, Zhang SC. 2010. Functional control of transplantable human ESC-derived neurons via optogenetic targeting. Stem Cells 28:2008–16
53. Liewald JF, Brauner M, Stephens GJ, BouhoursM, Schultheis C, et al. 2008. Optogenetic analysis of synaptic function. Nat. Methods 5:895–902
54. Guo ZV, Hart AC, Ramanathan S. 2009. Optical interrogation of neural circuits in Caenorhabditis elegans. Nat. Methods 6:891–96
55. Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, et al.2007a. Multimodal fast optical interrogation of neural circuitry. Nature 446:633–39
56. Hwang RY, Zhong L, Xu Y, Johnson T, Zhang F, et al. 2007. Nociceptive neurons protect Drosophila larvae from parasitoid wasps. Curr. Biol. 17:2105–16
57. Zimmermann G,Wang LP, Vaughan AG, Manoli DS, Zhang F, et al. 2009. Manipulation of an innate escape response in Drosophila: photoexcitation of acj6 neurons induces the escape response. PloS One 4:e5100
58. Xiang Y, Yuan Q, Vogt N, Looger LL, Jan LY, Jan YN. 2010. Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. Nature 468:921–26
59. Douglass AD, Kraves S, Deisseroth K, Schier AF, Engert F. 2008. Escape behavior elicited by single, channelrhodopsin-2-evoked spikes in zebrafish somatosensory neurons. Curr. Biol. 18:1133–37
60. Arrenberg AB, Del Bene F, Baier H. 2009. Optical control of zebrafish behavior with halorhodopsin. Proc. Natl. Acad. Sci. USA 106:17968–73