Comparison Between Opsins Based on Wavelength of Laser

مقارنة بين Opsins على أساس الطول الموجي لليزر

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Abstract
This work is an optogenetics investigation in order to compare opsins that associated with neural tasks inside organisms. The elementary goal of the proposed work is comparing opsins depending on utilized laser parameters (wavelength). Laser wavelength is an elementary base to distinguish these opsins, the used model was Adult white Swiss Bulb mouse handled with diverse laser wavelengths via optical fiber. The proposed study confirmed that opsins influence on mouse`s brain can lead to determine particular opsins depending on mouse`s behaviors. Also there is an influence on neural tasks due to variation of power consumption in other words performance of opsins was affected by this variation.
Keywords*:* Stimulation, Inhibition, Sensors, light sensitive protein.

الخلاصة

هذا العمل هو تحقيق علم البصريات الوراثي من أجل مقارنة opsins المرتبطة بالمهام العصبية داخل الكائنات الحية. الهدف الأساسي للعمل المقترح هو مقارنة opsins اعتمادًا على الليزر المستخدمة (الطول الموجي). الطول الموجي لليزر هو قاعدة أولية للتمييز بين هذه opsins ، والنموذج المستخدم كان فأرًا أبيض سويسريًا للبالغين تم التعامل معه بأطوال موجات ليزر متنوعة عبر الألياف الضوئية. أكدت الدراسة المقترحة أن تأثير opsins على دماغ الفأر يمكن أن يؤدي إلى تحديد opsins معينة اعتمادًا على سلوكيات الفأر. هناك أيضًا تأثير على المهام العصبية بسبب اختلاف استهلاك الطاقة بمعنى آخر تأثر أداء opsins بهذا الاختلاف.
الكلمات المفتاحية : تحفيز, تثبيط, حساسات, بروتين حساس للضوء.

1. Introduction

One of cutting edge technologies which has been occupied large amount of attention is optogenetics, it is considered an incredibly advantageous biological procedure engages light employment for managing cells in organisms, normally nerve cells which subjected to genetic modification in order to show sensitivity of light via tiny outlets which form the external cover (membrane) of proteins in living cells. The main goal of this integration between light and genetics is monitoring and ruling behaviors of nerve cells inside organism tissues and investigating the influence extent of light as shown in the figure 1[1,2].

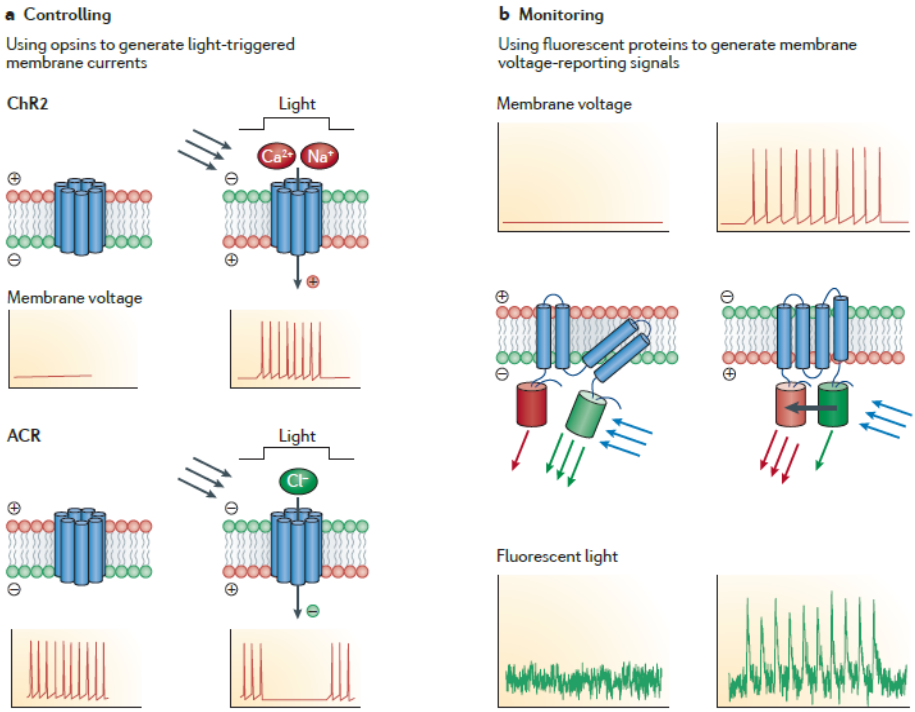

Figure 1. Monitoring and ruling neural tasks using light

Handling procedure of Optogenetics offers high accuracy throughout very short time that enables researchers to maintain rate with rapid biologically processing. In order to investigate neural code, because of optogenetics has dealt with nerve cells that requires high speed performance to permit adding or removal of accurate action patterns inside brain cells of mammals. Wide range of investigations have been performed involving optogentics utilization in diverse fields such activation nerve cells using laser, returning fully defected vision to sightless individuals using a type of optogentics actuators as well as light behaviors of green algae [3,4].

Applications of Optogentics have been conducted powerfully and precisely for controlling numerous features of neural task. Genetic and optical methods applied combined in order to perform firm spatial and physical managing kinds of nerve cells in the brain of organisms, an innovative progress that will permit specialists to attain standard understanding of neural circuit task associated with manners of animals. As nerve cells are then elucidated with light of an appropriate frequency they will be temporarily triggered or inhibited, perhaps signaling trail will be adjusted, relying on type of opsin which is selected for expression [5, 6].

Transgenic animals have been utilized to accomplish the above mentioned process, spatially limited light application permits for additional modification so as to target precise area of brain. Light might be applied in a wide range of physical models for optimally effect neuronal task also may be limited to precise short behavioral periods. Optogenetics instruments primary employed for controlling neuronal tasks many years ago [7], they have been widespread expanded and currently include an enormous collection of proteins that permit controlling neural activity regardless timescales, controlling of biochemical activity inside cell, as well as others such as control of neural task synchronizing with optical monitoring [7].

Optogentics sensors are responsible for optical tracing of neuronal tasks, they include calcium indicator (GCaMP **)** , vesicular release (synapto-pHluorin) and others .On the other hand , Optogentics actuators including numerous types of light-sensitive proteins such as channel rhodopsin , halo rhodopsin, archaerhodopsin as well as others , all of them have performed neuronal control. Actuators have been utilized to stimulate single or numerous actions which can be arranged into ordinary prickle series or formed pseudo-random. Both of sensors and actuators are classified

as light -sensitive proteins (opsins), these proteins represent essential part of living cells in all organisms [8,9].

The main objective of the proposed work is to distinguish various types of opsins using laser parameters (wavelength). The environment of research is neurons of mouse`s brain which is considered so appropriate model to investigate reactions (behavior s) of mouse when it is subjected to laser beams in order to perform (control) neural tasks such as stimulation or inhibition. The comprehensive organization of this article as follows: section2 explains groups of opsins, section 3 describes research methodology, results & discussions exhibited in section 4 and section 5 reviews the conclusion of this article.

2. Groups of Opsins

Wholly, opsins have been classified into two main groups: microbial opsins (Group I) and vertebrate opsins (Group II). Opsin diversity has been further subdivided to form up to 22 sub-groups located in the vertebrates alone [10, 11, 12]. Group I opsins are existing in prokaryotic microbial organisms such as bacteria and algae, and are formed of a single cover surrounds protein constituent and it works as a gate way. Such opsins are employed by microorganisms in order to accomplish range of tasks for instance direction-finding resources of energy and avoiding risky atmospheres, in addition to managing concentrations of a range of ions. Whereas Group II opsins are existing in mammal cells also are principally exploited for vision and adjusting circadian pulses. These opsins are G protein-attached receptors and commence a signaling throughout activation, and as a result they generate alterations slowly in neural actions as a compared with Group I. Group I opsins have two distinct features that make them more compatible in many applications of genetic engineering, first one is simplicity of composition (one constituent protein) and secondly their rapider kinetics [13, 14].

Two Groups of opsins necessitate a variety of vitamin called retinal; it is responsible for isomerization process leading to absorption of a photon. At what time retinal connects to the opsin, the resultant composition be converted into light sensitive, when a photon hits retinal opsin integration that will cause isomerization, thus this process will stimulate a significant alteration in the opsin. An alteration in external cover of protein (membrane), as well as activation or inhibition of neuronal action will be occurred. Consequently, retinal is a vital factor for using optogenetic actuators efficiently. Opportunely, for the premature evidence via experimental practices, retinal is found adequately in neural tissues of mammals to allow utilizing of optogenetic tools exclusive of supplement process except some cases such as Drosophila it requires to addition of retinal [15, 16]. Optogentics actuators can be employed into operation modes; stimulation mode where nerve cells have been triggered to take action due to light effect and inhibition mode which is a reserve process of stimulation as shown in the figure 2.

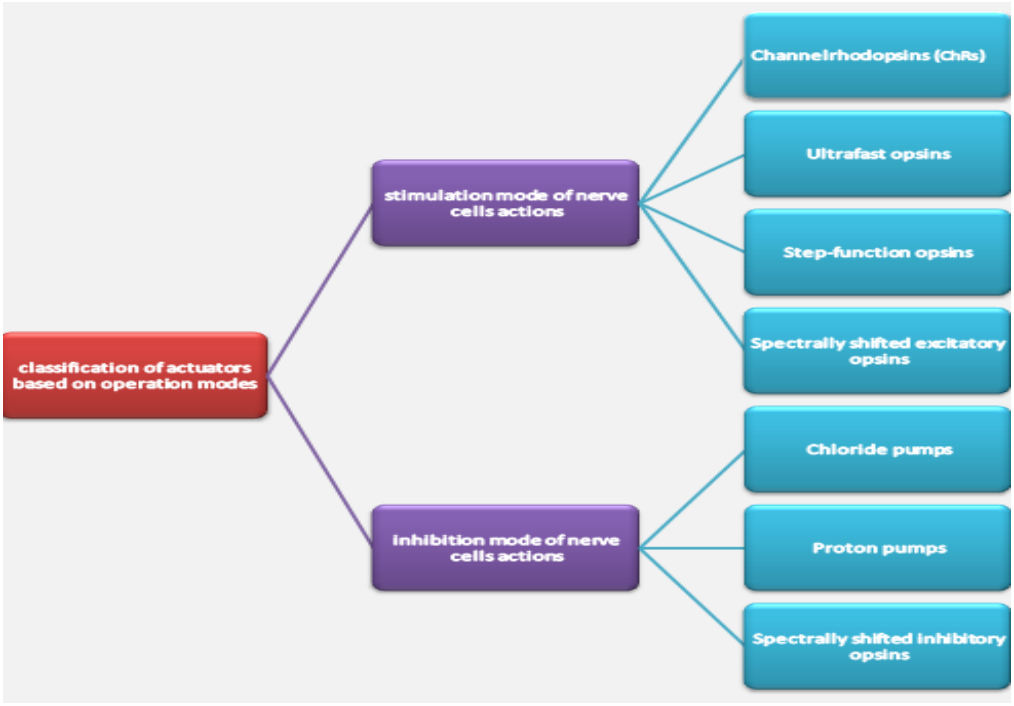

Figure 2. Operation modes of opsins including stimulation and inhibition

Channel rhodopsin's (ChRs) are considered one of most common actuators has been used in neural stimulation, when ChRs are elucidated with light it works as a gate way for passing ions and thus cell will be depolarized. On the other hand, Proton pump is an efficient inhibitor nerve cell via pushing protons away from cell, and it has a number of traits which qualify it to perform rapid inhibition [17, 18].

3. Research Methodology

3.1 Methodology criteria

The process of dealing brain with light efficiently stills a complex research, because it requires so carful implementation in order to avoid harming living cells due to large amount of used light. An appropriate power density should be less than 10 mW/mm^2 to achieve neural task properly, however opsins are able to diminish intensity of used light due to high sensitivity [19, 20]. A choice a laser for optogenetics application is rather insubstantial, also controlled by a number of factors shown below:

a) Wavelength of laser

A harmonization between laser wavelength and opsin sensitivity is an essential requirement to employ laser appropriately in optogentics application, laser has diverse wavelengths such as 561, 594 and 473 nm.

b) Required power for proper stimulation

The source of laser should supply adequate output power in order to stimulate a particular opsin properly. Consequently, laser ought to compensate power losses throughout fiber transmission system. Additionally, power requires being modifiable, because light necessities for producing photocurrents might diverge significantly according to examined organisms, such as animals due to diversity of expression intensity which involves opsin.

c) Reliable Power Steadiness

Continues high performance of laser requires power steadiness throughout duration of handling

which might takes number of hours . Hence, the upper limit of allowable power deviation is not more 2%. Additionally, it is so essential that laser`s waveforms are identical during handling with high frequencies in order to accomplish reliable treatment [21, 22].
Comprehensively, lasers utilized in optogenetics have been categorized generally into Diode Pumped Solid State lasers and laser diodes. The two categories are reliable and high performance in applications of optogentics, they have been operated properly even with high temperature of atmosphere Diode Pumped Solid State lasers (DPSSs) are influential and applied in wide range of fields involve optogentics. DPSSs are accessible in numerous wavelengths within visible range, wavelengths such as 532 and 561 nm classically employed. DPSSs are fundamentally restricted by their structure which influences pumping process to release a laser in a laser opening [23, 24]. DPSSs have operated steadily with highest power. Designs of DPSS has handled diverse energetic powers, they are influenced by modulation ability. On the other hand, DPSSs which have been subjected to direct modulation usually they lack adequate steadiness of waveforms cycles. Consequently, DPSSs employed for optogenetics require a compact modulation device that permits devices to run constantly with high reliability. DPSSs are considered so sufficient optically, due to direct activation of the pump grin into functional absorption band of the laser. This process reduces surplus losses in crystalline pattern of laser; optical efficiency reaches to 70%. Choosing combination of the handled objects facilitates construction of laser diodes to wide range of wavelengths (600 nm – 30 μm) as shown in the figure 3.

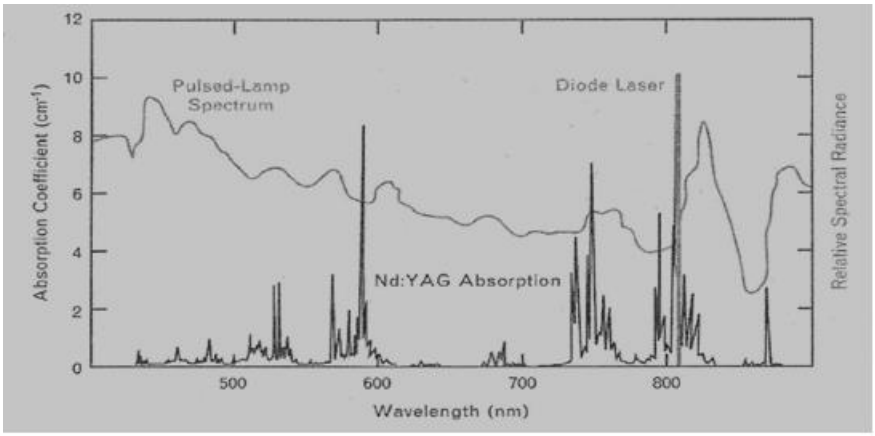

Figure 3. Range of DPSS laser wavelengths

On the other hand, laser diodes are most affordable and accurate laser sources. An elementary feature of laser diodes is an attribute temperature of these devices. Each apparatus of laser has a specific temperature, which corresponds to thermal steadiness of diode laser. Laser diode with higher characteristic temperatures is not affected extremely by thermal alterations of the neighboring atmosphere [25, 26].

3.2 Method of investigation

Normally handling neurons by laser will run particular opsins of both hemispheres of mouse`s brain to interact with applied laser. Adult white Swiss Balb mouse 4 months old, 430g approximately shown in figure 4 is utilized in this study. It is worthy to mentioning that animal belongs to Iraqi Research Center of Cancer and Medical genetics Baghdad- Iraq, it is preserved under appropriate circumstances including room temperature, food as well as continuous monitoring. A manipulation medium used in this study is a single optical fiber is coupled laser and implanted at neighboring neurons in brain of mouse. Mouse`s behaviors are stated before (normal condition) and throughout laser handling. Three different images are used to investigate reaction (behavior) of mouse in terms of laser wavelengths. In each round of experiment image is mounted at three places next to external wall of test transparent cylinder with 75 cm diameter.

4. Results and Discussions

The images are black cat, black rat and bread piece, physical reactions of mouse in each case are running, stopping and catching cylinder wall. For each laser wavelength , the mouse`s behavior is assumed corresponding to specific group of opsins in neurons. Thus, these opsins are classified based on behaviors and distances (cycles) which are done by mouse inside cylinder. A digital camera coupled with computer is used to monitor mouse`s reaction and calculate number of cycles (distances). Table 1 illustrates full obtained information involve the proposed work. The duration stated behaviors for each range of laser wavelength is three minutes and in order to avoid possible defection of brain tissues or any harmful effect experiment is conducted throughout month approximately to present adequate recovery duration to mouse after each round. A number of cycles are considered to calculate distances even with straight paths.

Figure. 4Adult white Swiss Bulb mouse

Table 1 Mouse`s behaviors according to laser wavelengths and normal conditions

Image	Mouse`s Behaviors					
	Normal Condition	Ranges of Laser Wavelength (nm)				
	No Laser	430 - 470	470 - 490	490 - 510	510 - 530	530 - 580
Black Cat	Fast run + stop 23.5 cycle	Walk + stop + run 10.3 cycle	run + stop 11.1 cycle	Fast run + stop 14.2 cycle	Fast run + stop 17.2 cycle	Fast run + stop 24.32 cycle
Black Rat	Run+ stop 14.6 cycle	Walk + stop 7.2 cycle	run + stop 8..25 cycle	Fast run + stop 10.13 cycle	Fast run + stop 12.17 cycle	Fast run + stop 15.1 cycle
Bread Piece	Run + walk + stop 8.7 cycle	Fast run + stop 10. 15 cycle	Run + stop 11.19 cycle	Run + stop 9.84 cycle	Run + stop 9.62 cycle	Run + stop 9.33 cycle
		A		B	C	D

Based on above obtained results, four groups of diverse behaviors can be determined in terms of used wavelengths. Physical outputs of Group A expressed different behaviors from them in normal conditions, that means ordinary behaviors have been changed extremely due to influence of both ranges of laser wavelengths. For Group B, there is a difference in outputs from Group A, in other words behaviors started to vary slightly. Whereas Group C indicates noticeable differences in results thus mouse returned to normal reactions towards three images. Finally, with Group D behaviors are matched extremely with normal conditions. Consequently, the proposed study assumes there are four groups of opsins responsible for these behaviors. Hence, Group A corresponds to opposite normal behaviors or inhibition opsins, Group B represents other opsins which named in this work pre stimulation opsins. In addition to opsins of Group C which are closer exceedingly and they are named advanced pre stimulation opsins; Group D opsins are associated with stimulation mode of neurons as shown in table 1. Unlike a lot of researches which are

depending on previous information involve neural tasks which correlated with opsins, in this study opsins engaged neurons of particular neural task (behavior) are determined by physical conduct which involved laser wavelength. Such categories in this study are compared with mentioned opsins in other literatures as shown in table 2.

Table 2 Comparison proposed work with other literatures

proposed opsins & wavelengths (nm)		opsins in other literatures & wavelengths (nm)	reference number	
Group A - inhibition	(430 – 470), (470 – 490)	ChR2 , ChR2(H134R) - inhibition	470	26
Group B -pre stimulation	(490 – 510)	ChRGR - Fast Excitatory	505	27
Group C- advanced pre stimulation	(510 – 530)	C1V1 ChETA (E162T) - Fast Excitatory	530	28
Group D - normal behavior (stimulation)	(530 600)	VChR1-SFOs - Bistable Modulation	560	28
		Arch/ArchT - stimulation	566	29
		eNpHR3.0 - Inhibitory	590	30

From above table the proposed four divisions of opsins correspond to other four in diverse literature, power consumption is within (50 – 70 mW), then with same ranges of laser wavelengths average of power dissipation adjusted to 90 mW. Under this condition, both of Groups B and C interfered extremely thus power consumption influenced on performances of opsins in addition to wavelengths. Finally, duration of handling with laser adjusted from 3to 15 minutes however there is no noticeable difference in mouse`s behaviors.

5. Conclusion

According to the proposed work physical feedbacks of freely moving animal illustrate features of opsins involve neurons of brain; variation of laser wavelength can lead to stimulation or inhibition neural tasks as shown with adult white Swiss Bulb mouse in addition to power consumption of used laser which can perform interference between behaviors (opsins). Whereas the duration change of treatment with laser has no effect in the proposed work.

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