Life Sciences 167 (2016) 1–5

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

# Proteins brighten the brain

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ARTICLE INFO

*Article history:*
Received 14 March 2016
Received in revised form 9 October 2016
Accepted 21 October 2016
Available online 22 October 2016

*Keywords:*
Optogenetic
Bioluminescence
Neurological disorders

ABSTRACT

Through selective activation/inhibition or dissection of neuronal circuits, optogenetic tools have raised hopes for a better understanding of neuropsychiatric mechanisms and therapeutic targets for various disorders. Although, overcoming serious limitations result in from conventional neuronal circuit study, this method has its own imperfections, such as optogenetic modulation of neural activity, using an internal, animal-generated, light source. In this review, limitations of external light delivery systems and possible approaches for using internal light sources in laboratory animals and perhaps, human being, are being addressed.



**Contents**



## 1. Introduction

Alternations in the structure and function of the nervous system are related to many neurological and psychiatric diseases. To achieve a circuit-level insight of these illnesses, with an acceptable temporal and spatial specificity, optogenetic method is a reasonable approach [1,2]. Using opsins and different modalities of light delivery devices in rodent models, researchers are now probing associational as well as projectional pathways and identifying regions, associated with Parkinson's disease, epilepsy, etc. [3,4].

Despite the eye-catching growth in knowledge and methods of this technology in recent years, implementing this modality as a therapeutic measure has it long way to go. A rather easy cell targeting with mice Cre line - and probably harder application in humans-, possible immunological responses to the transgene and the viral vector, ethical concerns in applying microbial transgene into a human, and finally, pervasive limitations of light delivery systems in traditional optogenetics.

In this paper, limitations of external light delivery systems and possible approaches for using internal light sources in laboratory animals and humans, are reviewed.

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http://dx.doi.org/10.1016/j.lfs.2016.10.024
0024-3205/

## 2. Challenges toward using external light sources and wireless optogenetics approaches

External light sources (laser or LED) i.e. the regular optogenetics approaches, are invasive and performed in rather unnatural circumstances, as they must be connected to the animal *via* fixed cannula and constraining it's movements. While optical rotatory joints provide a rather free movement, they are not convenient within an open-topped behavioral apparatus, home-cage illumination, or any structure with an enclosed top. Physical bonding of fiber optic cables to a static skeletal structure, e.g. skull and its external fixtures, could be destroyed by the animal, a cage mate, or an unintentional damage from housing. Besides, nearby neural tissue injury can occur, during fiber insertion or coupling [1,2]. Moreover, in behavioral studies, external light source could induce light-evoked behaviors such as fear, distract the animal, and probably induce increased locomotion [5]. Studying subjects with a social component, including depression or anxiety or experiments that involve mazes or other types of complex movement, further identifies the importance to control for the aberrant consequences of external light stimulation. Moreover, in experiments with two or more animals, fiber optic cables restrict their interaction [5].

Currently, methods to perform experiments on freely behaving animals are scarce [6–9]. These systems often have poor spatial resolution, which limits their functionality, besides restricting the natural behavior of the subject by the tethered optical fiber. In addition, the mechanical reliability of most optogenetic neural probes suffers from optoelectronic integration, as most of them integrate rigid silica fibers [5].

The first fully internal method of light delivery was described by Montgomery et al. [10]. This easy-to-construct device is two orders of magnitude, smaller and lighter than previous wireless systems. The internal device has a radio frequency (RF)-powered LED, applied for wireless optogenetic stimulation of spatially challenging and highly mobile areas essentially the brain, spinal cord, or peripheral nerve endings. Though less efficient, LEDs or driving the blue LED with higher powers, increase both general heating of the animal by the radiofrequency field, and local tissue heating at the site of LED. In addition, using hard materials and geometrically thick designs of this device, restrict its potential for chronic biocompatibility and integration with soft tissues of the nervous system [11]. In an attempt, Park et al. designed a miniaturized, fully implantable, thinner (by a factor of 5), softer (by a factor of 10,000) and more stretchable (by a factor of 10) optoelectronic system. Without optimization around specific cages or animal body types, this device enables strong operation and large transmission range, therefore, facilitating experiments in wider areas of the body in long run [11].

There is an increased demand for optical devices to deliver light not only on to the surface, but also into deeper sub-regions of the brain. Biomedical optical techniques have extremely limited penetration depth, as live tissues are optically turbid with strong light absorption and scattering [12]. Although the type of tissue and the light wavelength effect depth limit, accessible depth is typically around one millimeter or less [13]. Additionally, high-intensity light sources for delivering light deep into the brain could cause accidental damage near the surface, where light energy is high [4]. In recent years, time reversal of ultrasonically encoded light (TRUE) - which combines the ultrasonic modulation of diffused coherent light with optical phase conjugation, attaining dynamic focusing of light into a scattering medium [14] - techniques have been employed to break this limit [14–16]. Even though, there were speed limitations that prevents them from *in vivo* applications. On the other hand, insertion of light guides or optic fibers to access deep brain structures is technically demanding and causes damage to brain tissue. The application of miniature LEDs can minimize damage, while it also makes the implants difficult to fabricate [5,11].

Further complex light delivery systems are required to control the distribution of light in the three dimensional structure of the brain. One possible approach is using a matrix of waveguide arrays that are integrated on a single substrate to build a prosthetic device control the light distribution at a different depth [17]. Although the relatively large size of the probe and the complex optics are needed to couple light into the channel waveguide in large arrays, which are the major obstacles of this approach [18].

Another challenge toward external light devices is the limited number and locations of photo-stimulated neurons, a result of light attenuation in brain tissue due to light scattering and limited volume of neurons [19]. To correct this limitation, multiple LEDs could be precisely placed on the implant, to target different brain regions simultaneously, or different layers of layered structures such as the cerebral cortex [5]. Implantation of multiple light sources and stimulation of multiple locations can increase the number of stimulated neurons but is often impractical.

## 3. Internal light and optogenetics

There are numerous different bioluminescence systems, production of light through biological processes, in nature [20]. Core molecules in bioluminescent reactions are an enzyme (luciferase) and its substrate (luciferin), which can be oxidized by the enzyme and generate an excited molecule, emitting visible light rays [21].

With the current limitations of external light sources, bioluminescence systems to produce light, can be considered a promising light source system, to generate internal light stimulation.

Variants of luciferases have served in the context of optogenetics [3,4,19,22]. Berglund et al. [19] developed a chimeric light-generating activated ion channel, a fusion protein of luciferase and light-activated ion channel (ChR). In order to produce larger photocurrents, *Gaussia* luciferase (GLuc) was fused with Volvox Channelrhodopsin-1 (VChR), which was able to activate ChR, allowing modulation of neuronal activity. The same group assessed applicability of luminopsins *in vivo* in mice by applying substrate intravenously. The fusion protein successfully reached into neuronal membranes, generating *in vivo* bioluminescence. Thus, they demonstrated that activation of ChR by bioluminescence is sufficient to affect behavior [23]. Next, they addressed whether integrating brighter versions of GLuc with luminopsins, would yield to either supra-threshold depolarization or effective silencing of action potentials, when complied with the appropriate optogenetic elements [4]. They employed slow-burn GLuc (sbGLuc) in excitatory luminopsin, which increased the half-life of light emission 10 times over that of wild-type GLuc, preserving the intensity of the luminescence signal at each time. Applying superluminescent GLuc (slGLuc) to inhibitory luminopsin, improved quantum yield, and resulted in faster turnover rates, culminating in 10 times improved bio-luminescence intensities [4]. It should be noted that luminopsin would face the depolarization block, when neurons are over depolarized.

In another experiment, Land et al. [22] designed an adenoviral construct, containing firefly luciferase gene, with an EF1 promoter to activate halorhodopsin channels and suppress neural activity in the striatum of mice without an external light source. The FOS activity was reduced significantly in mice co-infected with halorhodopsin and luciferase, compared to the control group infected with halorhodopsin only. Interestingly, neuronal activity was greatly reduced in behaving animals after luciferin administration and amphetamine-induced locomotor activity declined in halorhodopsin/luciferase mice pre-injected with luciferin compared to controls. These results implied that, luciferase has the ability to generate enough light *in vivo* to activate halorhodopsin, inhibit neural activity and adjust behavior. It should be noted that luciferase operate a very high energy consuming process to create light, by hydrolyzing eight ATP molecules for releasing a single photon of green light [24]. Dual operation of opsins and luciferin may place additional energy demand in neurons. However, this is avoided by using marine luciferases [3].

Moreover Tung et al. [3] designed inhibitory luminopsins by characterizing the bioluminescence emission properties of the various luciferase proteins, and selecting the most suitable ones to couple with an