Bioluminescence-Optogenetics

17

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Abstract

In this chapter, we introduce a relatively new, emerging method for molecular neuromodulation—bioluminescence-optogenetics. Bioluminescence-optogenetics is mediated by luminopsin fusion proteins—light-sensing opsins fused to light-emitting luciferases. We describe their structures and working mechanisms and discuss their unique benefits over conventional optogenetics and chemogenetics. We also summarize applications of bioluminescence-optogenetics in various neurological disease models in rodents.

Keywords

Chemogenetics · Luciferase · Luciferin · Channelrhodopsin · Halorhodopsin

Abbreviations

AAV	Adeno-associated virus
ANT	Anterior nucleus of the thalamus
BRET	Bioluminescence resonance energy transfer
CNO	Clozapine-*N*-oxide
*Cr*ChR2	*Chlamydomonas* channelrhodopsin 2
CTZ	Coelenterazine
DG	Dentate gyrus
DREADD	Designer receptors exclusively activated by designer drugs
eLMO	Enhanced luminopsin
FLuc	Firefly luciferase
GLuc	*Gaussia* luciferase
iLMO	Inhibitory luminopsin
LED	Light-emitting diode
LMO	Luminopsin
LOV	Light-oxygen-voltage sensing protein
*Np*HR	*Natronomonas pharaonis* halorhodopsin
PD	Parkinson's disease
PPI	Protein–protein interaction
PSAM	Pharmacologically selective actuator molecule
PTZ	Pentylenetetrazol
RLuc	*Renilla* luciferase
SFLMO	Step-function luminopsin
SFO	Step-function opsin
*V*ChR1	*Volvox* channelrhodopsin 1

Genetic approaches to enable manipulation of neuronal activity constitute a fundamental class of methods in the basic neuroscientist's armamentarium. Furthermore, some of these methods may even hold potential for application in the clinical

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H. Yawo et al. (eds.), *Optogenetics*, Advances in Experimental Medicine and Biology 1293,
https://doi.org/10.1007/978-981-15-8763-4_17

setting. Specifically, in the past decade, optogenetics and chemogenetics have become workhorses in many laboratories to control activity in genetically predefined populations of neurons. To this end, optogenetics utilizes targeted expression of light-sensitive ion channels and pumps, known as opsins, that allow for modulation of neuronal activity with high temporal and spatial precision, facilitated by highly controlled delivery of photostimulation. However, the application of optogenetics is limited in two critical ways. First the light used to excite most opsins (visible wavelength range) is highly scattered and absorbed in brain tissue, significantly limiting its ability to penetrate and disperse. Second, the light source is external, necessitating delivery to the targeted tissue (often through a fiber optic). Consequently, light dispersion, and hence, neuronal control, is confined to a relatively small number of neurons located at the tip of an optical fiber. Thus, it is challenging to manipulate neurons in multiple locations and/or large brain areas as this would require a cumbersome number of fibers to achieve sufficient coverage. Chemogenetics overcomes this limitation through targeted expression of artificial metabotropic (e.g., DREADDs; designer receptors exclusively activated by designer drugs) and ionotropic (e.g., PSAMs; pharmacologically selective actuator modules) receptors that were engineered to be activated by foreign ligands, which can be introduced systemically for brain-wide spread (Sternson and Roth 2014). Without the need for implanted hardware, simultaneous modulation of multiple brain regions can be more readily achieved. However, chemogenetics does not provide precise temporal or spatial control of neuronal activity, as the activation of the chemogenetic actuators depends on diffusion and clearance of the activating molecule in the brain, a passive process difficult to control. The focus of this chapter will be on an emerging genetic neuromodulatory approach, bioluminescence-optogenetics, which combines the benefits and advantages of these two mainstream genetic techniques for controlling neural activity.

17.1 Luminopsin Fusion Proteins

Bioluminescence-optogenetics utilizes luminopsin fusion proteins (LMOs), which are combinations of light-sensing opsins and light-emitting luciferases (Fig. 17.1). LMOs are activated by cognate substrates of the luciferase enzyme, luciferin, which cross the blood–brain barrier. They generate biological light or bioluminescence through an enzymatic reaction, thus providing an internal light source for opsins (Fig. 17.2, left). This approach solves the limitation of light delivery into the brain for opsin activation, as light is generated in the vicinity of the opsin internally, obviating the need for implanted hardware. Of note, LMOs retain the capability to be activated by external light, similar to the conventional optogenetic approach, as the opsin moiety is exactly the same as commonly used in optogenetic probes (Fig. 17.2, right).

Bioluminescence is an inherently dim light source compared to external light sources used for conventional optogenetics (e.g., lasers and LEDs). Since the first publication in 2013 (Berglund et al. 2013), the efficacy of LMOs has been progressively improved by combining luciferases with higher bioluminescence emission and opsins with higher light sensitivity (Fig. 17.3). To date, about a dozen LMOs have been created by combining various opsins with luciferases (Table 17.1), and the arsenal of bioluminescence-optogenetics is readily expandable through engineering of new LMOs, particularly with the continued discovery and development of novel opsins and luciferases. In theory, any luciferase can be coupled to any kind of opsin as long as there is sufficient light emission and spectral overlap between the two for bioluminescent activation. In practice, the currently available LMOs all utilize marine luciferases, either *Gaussia* luciferase (GLuc; Fig. 17.1, left) or *Renilla* luciferase (RLuc; Fig. 17.1, right), both of which catalyze marine luciferin, coelenterazine (CTZ). The polarity of the LMO action is determined by the electrophysiological properties of the coupled opsin and can be either excitatory (Fig. 17.1, left) or inhibitory

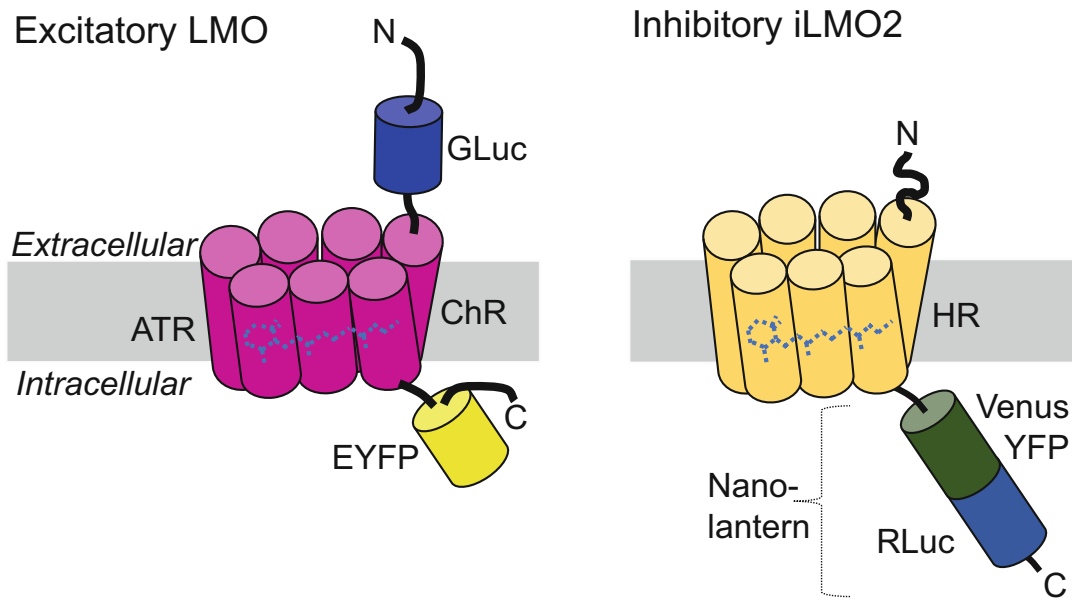

Fig. 17.1 The molecular structure of luminopsins (LMOs). (Left) In excitatory LMOs, *Gaussia* luciferase (GLuc) is attached to the N-terminus of a light-gated cation channel, channelrhodopsin (ChR), placing the luciferase outside the cell. The channel is tagged with enhanced yellow fluorescent protein (EYFP) at the C-terminus for convenient identification of LMO-expressing cells. All-*trans*-retinal (ATR) is the chromophore within ChR. (Right) In inhibitory iLMO2, *Renilla* luciferase (RLuc)-based Nano-lantern is attached to the C-terminus of a light-driven chloride pump, halorhodopsin (HR), placing the luciferase inside the cell

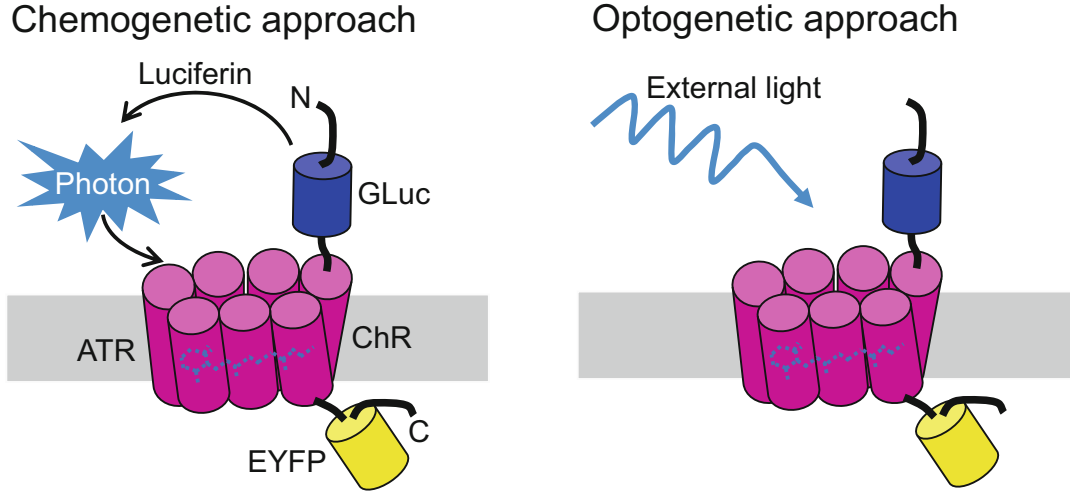

Fig. 17.2 Bimodal luminopsins. Light needed for activation of LMOs is internally produced when a luciferase catalyzes its substrate, analogous to chemogenetics (left). The same molecule can be activated by external light, similar to conventional optogenetics (right)

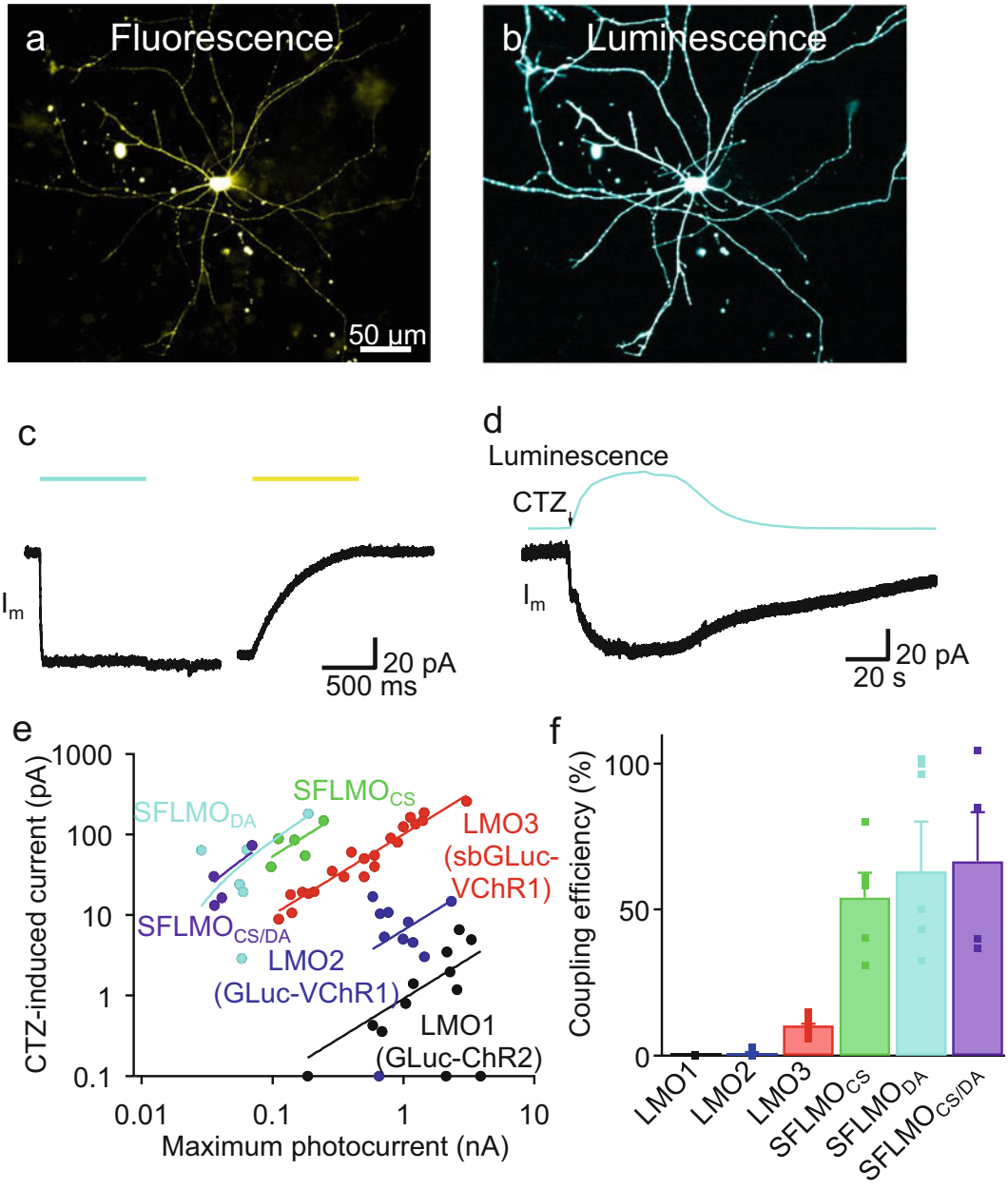

Fig. 17.3 Bioluminescence-induced photocurrent and its efficiency in neurons. a and b. $SFLMO_{DA}$-transfected neurons in fluorescence (**a**) and bioluminescence (**b**). The EYFP tag in the LMO was imaged through a green filter cube in the microscope. Bioluminescence was induced by CTZ (100μM) and imaged without a filter cube. Exposure time was 100 ms and 1 s, respectively. (**c**, **d**) Representative photocurrents (I_m) in response to physical (**c**) and biological light (**d**). The SFO_{DA} moiety in the LMO was activated by 480 nm light (422μW/mm^2; blue bar) and subsequently deactivated by 575 nm light (902μW/mm^2: yellow bar; **c**). CTZ (100μM; arrow) was bath-applied to the same cell, which induced bioluminescence and, in turn, bioluminescence-induced photocurrent (**d**). (**e**) Relationships between lamp-induced photocurrent and bioluminescence-induced photocurrent. Each cell was challenged by physical blue light from a lamp at a saturating intensity (> 2 mW/mm^2; 480 nm) and saturating concentration of CTZ (100μM) and the amplitudes of photocurrent induced in the two modes were plotted. $n = 12$ (LMO1), 10 (LMO2), 21 (LMO3), 5 ($SFLMO_{CS}$), 6 ($SFLMO_{DA}$), and 4 ($SFLMO_{CS/DA}$) cells. Data with SFLMOs were fitted with log-log linear regressions. (**f**) Coupling efficiency of SFLMOs. CTZ-induced photocurrent was divided by lamp-induced photocurrent in each cell and the means and SEMs were calculated for each variant of LMOs. $n = 12$ (LMO1), 10 (LMO2), 21 (LMO3), 5 ($SFLMO_{CS}$), 6 ($SFLMO_{DA}$), and 4 ($SFLMO_{CS/DA}$) cells. Data with LMO1, 2, and 3 were replotted from a previous publication (Berglund et al. 2016). The figure was reproduced from Berglund et al. (2020)

Table 17.1 Currently available luminopsin variants

Opsin \ Luciferase			GLuc	sbGLuc	GLuc/M23	Nano-lantern
Inhibitory	H^+ pump	Mac		iLMO[3]		
	Cl^- pump	NpHR				iLMO2[2]
	Cl^- channel	iChloC			iLMO4[4]	
Excitatory	Non-selective cation channel	ChR2	LMO1[1]			
		VChR1	LMO2[1]	LMO3[3]	LMO4[4]	
		SFO_{CS}		$SFLMO_{CS}$[5]		
		SFO_{DA}		$SFLMO_{DA}$[5]		
		$SFO_{CS/DA}$		$SFLMO_{CS/DA}$[5]		

The background color of luciferase and opsin moieties indicates emission and excitation spectra, respectively. Luminopsin molecules in black and red indicate inhibitory and excitatory ones, respectively. References: [1]Berglund et al. (2013); [2]Tung et al. (2015); [3]Berglund et al. (2016); [4]Park et al. (2020); [5]Berglund et al. (2020)

(Fig. 17.1, right). LMOs can be selectively expressed through targeted delivery of viruses (e.g., intracranial injection of adeno-associated virus; AAV) encoding the LMO construct under control of various promoters/enhancers, enabling neuromodulation of specific neuronal cell types.

17.2 Excitatory Luminopsins

In the first LMO (Berglund et al. 2013), wild-type GLuc was fused to *Chlamydomonas* channelrhodopsin 2 (*Cr*ChR2), based on the spectral overlap of blue emission in GLuc with blue excitation in *Cr*ChR2. The resulting molecule, LMO1, established the proof of concept for bioluminescence-optogenetics, namely activation of opsin by bioluminescence, albeit with dismissible efficacy: Only a fraction of the opsin moiety (~0.1%) was activatable by bioluminescence when CTZ was applied, compared to a saturating intensity of physical light from an arc lamp (defined as 100% efficacy). To improve efficacy, the authors employed a different opsin with superior light sensitivity, *Volvox* channelrhodopsin 1 (*V*ChR1), to create LMO2. Although *V*ChR1 is often times touted as redshifted *Cr*ChR2 due to its optimal excitation with green light, the authors capitalized on its superior sensitivity even in the blue spectrum and showed that *V*ChR1 could indeed improve efficacy up to 1%.

In a follow-up paper (Berglund et al. 2016), the same group generated the next LMO iteration, LMO3, by replacing the wild-type GLuc in LMO2 with a triple point mutated variant that is longer lasting and almost 10 times more bioluminescent (Welsh et al. 2009). The resulting LMO3 was 10 times more efficacious for bioluminescent activation than LMO2 and was able to elicit action potential firing in vitro and in vivo, when expressed in the rodent brains (Figs. 17.3b, c, d). Moreover, LMO3 activation was able to induce a specific behavioral change, namely rotational behavior, when expressed unilaterally in the substantia nigra.

Park et al. (2020) further bolstered this trend by coupling the brightest blue-emitting luciferase so far reported, the M23 variant of GLuc (Lindberg et al. 2013), with *V*ChR1, yielding LMO4. The authors showed that efficacy of activation by bioluminescence was improved in this new addition compared to previous iterations of the LMO family. Armed with this improved LMO, the authors showed that control of

neuronal activity was more efficient in vitro in cultured neurons (single unit activity) as well as in vivo in awake behaving rats (rotational behavior).

To improve efficacy and versatility of LMOs, Berglund et al. (2020) took a complementary approach as a workaround for inherently dim bioluminescence, instead replacing the opsin moiety with *Cr*ChR2 variants of enhanced light sensitivity and slower deactivation, step-function opsins (SFOs) and tested its efficacy in culture neurons in vitro (Figs. 17.3a, b). These longer lasting SFOs have the added ability of deactivation through application of longer wavelength light (Fig. 17.3c). The new luminopsins, termed step-function luminopsins (SFLMOs), were more efficiently activated by bioluminescence than previous LMO1–3 iterations (Figs. 17.3d–f). The authors further showed that SFLMOs could, as expected, control neuronal activity in vitro and rotational behavior in awake rats. In addition, SFLMOs offered an additional layer of controllability through deactivation by light of longer wavelength than generated by the luciferase, which can be applied using traditional optogenetic methods.

All aforementioned variants of LMOs are comprised of three transgenes, namely a marine luciferase for bioluminescence, a microbial opsin for photocurrent, and a hydrozoan fluorescent protein for fluorescent tagging of expressing cells. As in the case of opsins for conventional optogenetics, these foreign membrane proteins can be improperly processed in mammalian neurons and form cytosolic protein aggregates. To facilitate proper membrane targeting, Zhang et al. (2020) inserted the membrane trafficking signal found in a neuronal membrane protein, $K_{ir}2.1$, between the opsin and the fluorescent protein tag as previously used in enhancing expression of conventional optogenetic opsins (Gradinaru et al. 2008; Zhao et al. 2008). The resulting enhanced luminopsin 3 (eLMO3) demonstrated significantly reduced aggregation as well as significantly improved surface expression in vitro (primary mouse embryonic neuronal culture) and in vivo (AAV transduced mouse somatosensory cortex) as compared with its LMO3 predecessor. Additionally, in the same in vivo preparation, eLMO3 activation by CTZ more reliably elicited whisker-touching behavior.

17.3 Inhibitory Luminopsins

So far, four inhibitory luminopsins (iLMOs) based on hyperpolarizing opsins have been reported (Table 17.1, top). iLMO1 and iLMO2 (Fig. 17.1, right) (Tung et al. 2015) are fusion proteins of the light-driven chloride pump, *Natronomonas pharaonis* halorhodopsin (*Np*HR), with engineered RLucs, Tag red fluorescent protein (RFP)-RLuc and Nano-lantern (RLuc fused to Venus yellow fluorescent protein) (Saito et al. 2012), respectively. iLMO (without numbering) was a fusion protein of the slow-burn variant of GLuc with a light-driven proton pump from *Leptosphaeria maculans* (Berglund et al. 2016). iLMO4 (Park et al. 2020) contains the M23 variant of GLuc coupled with the improved chloride-conducting channelrhodopsin, iChloC (Wietek et al. 2015). These inhibitory LMOs have been demonstrated to induce neuronal inhibition in vitro and in vivo (both single unit activity and corresponding behavioral changes) in the presence of CTZ. A note on nomenclature: an iLMO3 variant was never published.

17.4 Fusion Vs. Co-Expression

The configuration of LMO as a single fusion protein provides convenience when expressing in cells (e.g., circumventing the need for coinfection with AAVs separately encoding the opsin and luciferase) as well as efficient activation of an opsin through bioluminescence, such as observed in SFLMOs. Multiple lines of evidence support that this highly efficient energy transfer from GLuc to SFO within the LMO molecule is mediated by radiationless bioluminescence resonance energy transfer (BRET), indicating that the fusion protein configuration is critical for bioluminescent activation of ChR-based LMOs (Berglund et al. 2020).

In contrast, *Np*HR has been demonstrated to be activated by a co-expressed luciferase without fusion, including firefly luciferase (FLuc) and RLuc (Land et al. 2014; Tung et al. 2015). While this energy transfer may not be as efficient, this functionality may offer the possibility for more flexible and creative usage of bioluminescence-optogenetics, such as transsynaptic activation by expressing a luciferase presynaptically and an opsin postsynaptically.

17.5 Luminopsin Substrate—Coelenterazine (CTZ)

Unlike DREADDs, which utilizes G-protein signaling, LMOs do not rely on any innate biochemical signaling cascades. Luciferins are completely foreign to mammals and other nonluminous species and do not have any distinct targets in the brain other than the exogenously expressed luciferase. However, luciferins, both CTZ for RLuc and GLuc and D-luciferin for FLuc, are known substrates for the ABC family transporters (Pichler et al. 2004; Zhang et al. 2007), whose activity may limit bioavailability of luciferins in the brain as they are expressed at the blood–brain barrier.

All the available toxicological data indicates that *CTZ poses no harm to mammals*; in contrast, clozapine-*N*-oxide (CNO)—the substrate for DREADDs—metabolizes to clozapine, an antipsychotic. No CTZ-induced cytotoxicity was observed in various mammalian cell lines (except for rat hepatocytes; Dubuisson et al. (2000)) at 100μM (Dubuisson et al. 2005), a concentration higher than estimated levels in the serum when systemically injected. Indeed, in addition to our publications, CTZ and luciferase have been widely used in oncological studies. Despite wide usage we are unaware of any documented adverse effects of CTZ administration. In fact, rodents treated with an excessively high dosage (>1 g/kg oral) of a CTZ derivative manifested no signs of acute or chronic toxicity (Dubuisson et al. 2005). Rather, therapeutic merits of CTZ, which is a natural antioxidant, have been suggested (Dubuisson et al. 2005), particularly as bioluminescence is an oxidation process.

CTZ is hydrophobic and therefore needs to be dissolved in a solvent first before diluting into aqueous solution for delivery. The resulting solution can be delivered to the brain systemically via various routes, including intraperitoneal, intravenous (jugular vein catheter or tail vein), intracortical, and intranasal injection. Among those, intranasal delivery is of special note. This rather inconspicuous route provides effectiveness and convenience for systemic administration of CTZ (Andreu et al. 2010). Yu et al. (2019) and Zhang et al. (2020) capitalized on the proximity of the nose and the brain for efficient delivery of CTZ to activate LMO3 in intracranial nervous tissue. While CTZ is expected to exert no significant off-target effects in the brain, certain solvents may affect the physiology of brain cells. Using neuronal firing recorded in a multi-electrode array in vitro as a readout, Prakash et al. (2020) systematically examined the effect of different preparations of CTZ in various solvents for a variety of LMOs. Their thorough study is a convenient guide for new luminopsin users to choose from different preparations of CTZ to avoid potential artifacts.

As there are many components to bioluminescence-optogenetics aside from the expression of the luminopsin—including the CTZ substrate and its metabolite (oxidized CTZ or coelenteramide), the solvents for CTZ, and resulting bioluminescence—it is possible that any component could impact neuronal activity indirectly through unexpected mechanisms, independent of the mechanism of bioluminescence-optogenetics. Therefore, to establish specificity of the approach, Gomez-Ramirez et al. (2020) tested each component systematically and demonstrated that neither CTZ, coelenteramide, nor solvent alone was sufficient to change neuronal activity. They further demonstrated that changes in neuronal activity were directly proportional to the amount of bioluminescence emitted. To rule out the effect of bioluminescence itself on neuronal activity, they employed a null mutated luminopsin with a point mutation that rendered the channel moiety nonfunctional. This elaborate negative control experiment utilizes opsins without phototransduction and perhaps should be more widely adopted in conventional

optogenetics. Thus, they provided strong evidence that the effect of LMOs was indeed caused by the changes in membrane potential induced by the opsin moiety gated by bioluminescence.

17.6 In Vivo Bioluminescence Imaging

One of the useful features of bioluminescence-optogenetics that is somewhat obvious, but nevertheless useful, is its byproduct bioluminescence. Bioluminescence emission can be used to noninvasively gauge expression of luminopsin molecules and/or pharmacodynamics of CTZ in vivo—a unique advantage of bioluminescence-optogenetics over conventional optogenetics and chemogenetics. Indeed bioluminescence emission from LMO molecules is strong enough to be observed through the intact skull and skin (Birkner et al. 2014; Tung et al. 2016). To illustrate this point, we expressed iLMO2 in the mouse primary visual cortex via AAV and observed transcranial/transcutaneous

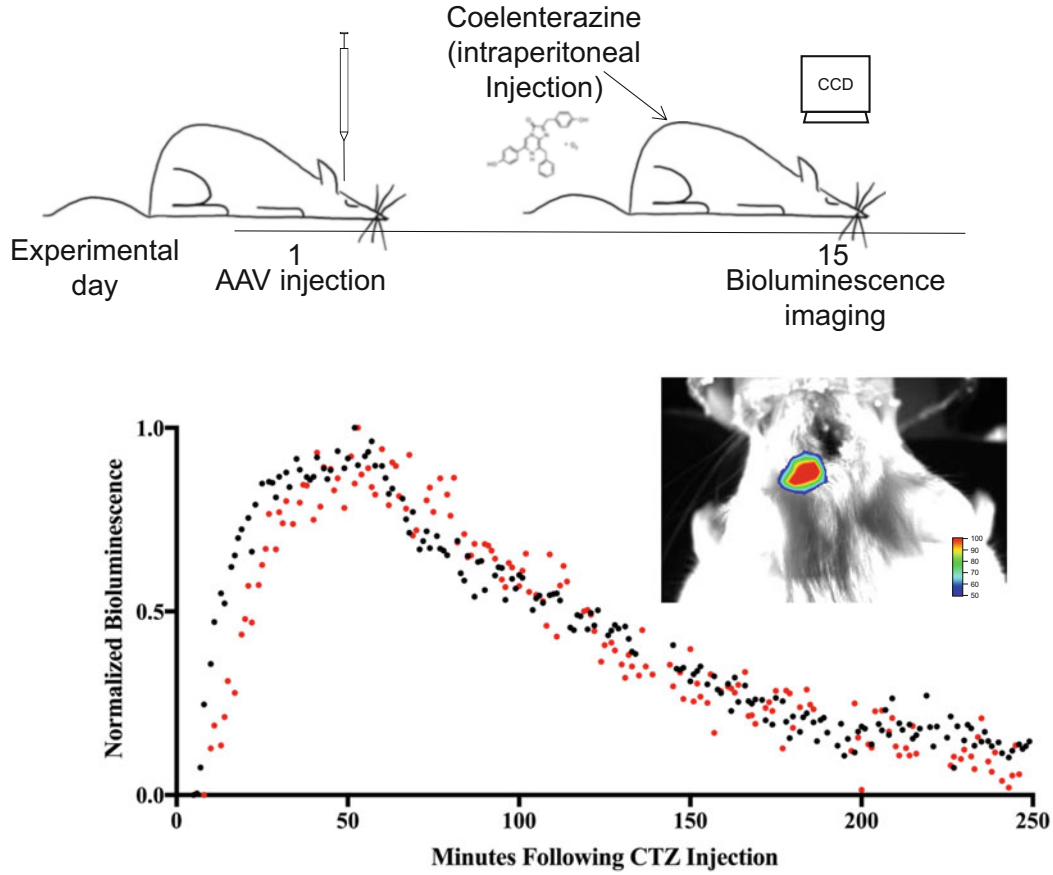

Fig. 17.4 In vivo bioluminescence imaging using LMO. An AAV vector carrying the iLMO2 gene was injected into the adult mouse primary visual cortex. After a wait period to allow for sufficient gene expression (15 days), we conducted transcranial/transcutaneous in vivo bioluminescence imaging (top). CTZ was injected intraperitoneally (10 mg/kg) and bioluminescence was observed over 4 h (bottom). Peak luminescence occurred at 52.5 ± 0.5 min with luminescence greater than half of the maximum occurring from 18.5 ± 5.5 to 111.5 ± 3.5 min (mean ± standard error). Imaging was performed in separate subjects (n = 2; subject indicated by color). Bioluminescence was observed in the posterior brain where the visual cortex resides (inset)

Table 17.2 Comparison of genetic neuromodulatory approaches

Feature \ Method	Optogenetics	Chemogenetics	Bioluminescence-optogenetics
Trigger	External light	Exogenous chemical	External light or BRET
Orthogonal multiplexing	Wavelength	Substrate	Substrate
Mechanism	Ionotropic	Ionotropic or metabotropic	Ionotropic
Hardware	Required	Independent	Optional
Region of Influence	Small	Variable	Variable
Kinetics	Fast	Slow	Fast or slow
Off-target effects	Low	Possible	Low
Intrinsic activation monitoring	Inferred from light intensity and opacity of the tissue	None	Bioluminescence

bioluminescence using a charge-coupled device camera after intraperitoneal injections of CTZ under anesthesia with isoflurane (Fig. 17.4). It took about 50 min for bioluminescence to reach its peak, with greater than half maximum luminescence occurring for 90 min (between 20 and 110 min postinjection) and with detectable luminescence observable for over 4 h. A repeat experiment in a separate animal resulted in almost identical bioluminescence, indicating reproducibility.

17.7 Comparison with Conventional Optogenetics and Chemogenetics

Bioluminescence-optogenetics was conceived to overcome the limitations of conventional optogenetics by leveraging a chemogenetic approach. Hence, a direct comparison of these three neuromodulatory modalities is warranted (Table 17.2). All three of these genetically encoded techniques enable highly specific neuromodulation when compared with conventional electrical stimulation, particularly with respect to their ability to target select cell types, facilitating circuit-informed modulation. Optogenetics has the advantage of temporal and spatial precision (i.e., control of the pattern and amount of light delivered and control of tissue modulated through placement of the fiber optics). However, optogenetics is limited for those same reasons, as external and implanted hardware are necessitated, which carries with it the risk for neural tissue injury, infection, hardware damage, and malfunction. Such limitations can constrain experimental design and limit its potential for translatability. Additionally, light dispersion is somewhat limited at sub-ablative power, making it difficult to conduct brain-wide and multiple-site neuromodulation as each brain region/nuclei would require a separate and/or multiple fiber placements to achieve adequate coverage.

Chemogenetics circumvents many of these limitations as it can be employed in a multinodal or even brain-wide fashion. Chemogenic substrates can be delivered systemically and will eventually disperse to all parts of the nervous

system; hence, wherever a chemogenetic receptor is present, the tissue can be modulated without the need for implanted hardware. However, herein lies a main limitation of chemogenetics: its temporal and spatial precision. The timing of neuromodulation is dependent on the dispersion and clearance of the substrate, a difficult process to control. Of course, one could consider more local delivery of substrate through cannulation and a pump, but that negates its advantage of hardware independence. Additionally, the most ubiquitously used chemogenic agents, DREADDs—which leverage endogenous metabotropic signaling cascades of which already exists in mammalian cells—can be confounded by endogenously occurring cellular processes and regulation. In a similar vein, these signaling cascades are also not exclusive to neuronal excitability and may have additional unintended downstream effects in some cell types. Furthermore, triggering activity through metabotropic receptors has slower response than ionotropic channels used in optogenetics. Moreover, the traditional substrate of DREADDs, CNO, metabolizes to the psychoactive molecule clozapine, which could lead to side effects.

Bioluminescence-optogenetics offers the advantages of both of these techniques, as LMOs can be activated through both approaches, activation of the opsin moiety directly through external light (in a traditional optogenetics sense) or activation of the opsin via BRET secondary to substrate binding (more akin to chemogenetics). Nevertheless, LMOs still have some of the same limitations as these other approaches, depending on the method chosen for activation. Importantly though, LMOs offer flexibility in experimental design to leverage the advantages of each system. In terms of key differences with DREADDs: LMOs are ionotropic which affords faster kinetics of activation than conventional chemogenetics; they do not rely on endogenous signaling cascades, so they are less likely to cause unintended effects; and the luminopsin substrate, CTZ, has not been demonstrated to have any toxicity or unwanted side effects. There are also several variants of effective luciferin, each with different kinetics, which offers more flexibility in experimental design. Moreover, LMOs enable users to spatiotemporally track their action through directly observing bioluminescence, the recording of which can be performed noninvasively.

17.8 Application in Neurological Disease Models in Rodents

Bioluminescence-optogenetics has been applied in various neurological disease models in rodents. To establish a mechanism of action for the therapeutic effects of physical exercise on peripheral nerve injury, Jaiswal et al. (2020) used iLMO2 (Fig. 17.1, right) to block the lower motor neuron activity thought to mediate the effect. Specifically, they injected an AAV carrying a Cre recombinase-dependent iLMO2 construct intramuscularly in the hindlimbs of transgenic mice expressing Cre under control of a motoneuron-specific promoter. The authors demonstrated that electrically evoked potentials in the muscles were reduced after application of CTZ in a time-dependent manner, confirming substrate-dependent inhibition of motoneurons by iLMO2. Using the same approach in a peripheral nerve injury model in mice, pretreatment with CTZ before each exercise session diminished functional recovery after the injury, suggesting a critical role of activity of motoneurons in this experimental paradigm. Their study exemplifies a simple but powerful approach using LMOs.

In an effort to lay the foundation for future treatments for epilepsy, Tung et al. (2018) utilized iLMO2 as a multi-nodal neuromodulatory therapeutic agent in a rat acute seizure model, induced by intraperitoneal injection of the chemoconvulsant pentylenetetrazol (PTZ). Targeting the circuit of Papez thought to mediate seizure generation in this model using AAV delivery, the authors expressed iLMO2 in multiple nuclei within this circuit, specifically granule cells in the dentate gyrus of the hippocampus (DG) and/or putative glutamatergic projection neurons in the anterior nucleus of the thalamus (ANT). iLMO2's efficacy in suppressing the induced seizures was assessed primarily through behavioral seizure outcomes. The authors observed a statistically significant decrease in

seizure duration compared with vehicle control when inhibiting a single nucleus bilaterally with iLMO2, either DG or ANT. Interestingly, when inhibiting both the DG and ANT with iLMO2 simultaneously, the authors observed a more pronounced seizure suppression than had been observed with inhibition of either nuclei alone. Seizure duration was decreased as well as Racine score, a measure of behavioral seizure severity. With iLMO2 in both DG and ANT, the majority of animals treated with CTZ showed the least severe form of seizure (Racine score 1), an effect not observed in control animals when vehicle was injected instead of CTZ. These results raise the prospect that better seizure suppression can be achieved using a circuit based multi-nodal neuromodulatory approach, exemplifying the advantage of multifocal targeting through the chemogenetic mode of bioluminescence-optogenetics.

As development of a potential therapeutic approach for Parkinson's disease (PD), Zenchak et al. (2020) used LMOs in conjunction with stem cell therapy in a genetic model of PD in mice. The authors first engineered mouse embryonic stem cells stably expressing LMO3. These cells were differentiated into neural precursors and then transplanted into the striatum of the mutant mice, which exhibit various neurological sequelae including motor deficits. When transplanted cells were chronically activated by daily injection of CTZ, the authors observed marked recovery of motor functions, indicating that increasing activity of neural precursors through LMO3 had therapeutic benefits in this rodent model of PD, while animals that received transplanted cells but did not receive CTZ failed to demonstrate a recovery of motor function.

Yu et al. (2019) took a similar combinatory stem cell/gene therapy for a mouse model of stroke. They first engineered induced pluripotent stem cells stably expressing LMO3, which they then differentiated into neuroprogenitor cells and transplanted into ischemic somatosensory cortex in mice. The transplanted cells were stimulated daily via intranasal delivery of CTZ. Using various physiological, biochemical, and histological assays, the authors demonstrated that neural circuits and connections were markedly repaired in the animals that received this combinatory treatment as compared with the control animals that received the cell transplantation but not CTZ. More importantly, those stroke model animals that received the transplanted cells and CTZ demonstrated significant improvements in behavioral tasks that require tactile sensation as compared with those that received neither or CTZ alone. The study suggests that chronic stimulation of neuroprogenitors through LMOs can provide pro-survival and pro-regenerative microenvironments that facilitate neural repair for the transplanted cells. Chemogenetic treatments of transplanted cells are an attractive alternative to optogenetic manipulations as transplanted cells may migrate and spread within the host brain, and thus may not be able to be targeted completely with conventional optogenetics, particularly given the limited spread of physical light within the tissue. Bioluminescence-optogenetics will ensure manipulation of transplanted cells even in such a scenario, as CTZ spreads brain-wide, far better than physical light delivered through fiber optics.

17.9 Potential beyond Rhodopsin-Based Optogenetics

It is important to note that, whereas we have focused our discussion specifically on bioluminescence-mediated activation of rhodopsins and their chromophore, retinal, there is great potential for bioluminescence to be employed in other optogenetic methods. We can imagine bioluminescent enzymes fused to photoactivated adenylyl cyclase to regulate intracellular cyclic adenosine monophosphate production (Schroder-Lang et al. 2007) as well as to various optogenetic systems that incorporate chromophores other than retinal, such as phycocyanobilin (Levskaya et al. 2009) and riboflavin-derived chromophores (Konermann et al. 2013). Phycocyanobilin-based systems are particularly interesting as they natively operate in the tissue-penetrating red/far-red spectrum (Tischer and Weiner 2014). Such systems may

be complemented by Akaluc (Iwano et al. 2018), a near-infrared emitting luciferase engineered from FLuc for bioluminescence-mediated activation.

Indeed, bioluminescence has already been deployed in one of these non-rhodopsin/non-retinal approaches. One such innovation is a variant on optogenetically gated transcriptional reporting of cellular protein–protein interaction (PPI) (Kim et al. 2019). The original use of this optogenetic method employed tagging a protein of interest with light-oxygen-voltage sensing protein (LOV) fused with a transcription factor for a reporter protein, separated by a proteolytic domain specific for a protease that would be tagged to another protein. This enables an AND logic for interaction of these two proteins of interest, for only in the presence of the correct wavelength of light to activate the LOV would the proteolytic domain be revealed such that it could be cleaved by the protease when the two proteins are close enough to interact. Thus, a researcher could regulate the precise timing of when this PPI would be reported. However, this method was prone to type 1 error: even without a PPI, if there was a high concentration of the protein tagged with the luciferase (during the light on phase), the proteolytic domain would be cleaved. Using a BRET gating mechanism researchers were able to overcome this limitation. Specifically, they added a luciferase tag to the protein they were already tagging with the protease, thus allowing the AND gate to be regulated by the presence of luciferin. The key advantage this afforded was that the proteins would need to be close enough not only for proteolytic cleavage to happen but also for BRET to occur. Thus, even if there were a high concentration of the protein tagged with the protease and luciferase (in the presence of luciferin), if a PPI were not occurring BRET could not occur, which greatly improved the reliability of this reporter system.

17.10 Concluding Remarks

This chapter provides an introduction to bioluminescence-optogenetics, specifically highlighting its versatility and advantages, from its potential for continued development to its growing application as a neuromodulatory approach to investigate disease and shed light on potential therapeutic avenues. As the field of bioluminescence-optogenetics continues to grow, we look forward to the innovative and creative uses the expanding user base will develop.

Acknowledgments This work was supported by NSF CBET-1512826 (KB/REG), NIH F31NS115479 (MAS), R21NS112948 (REG), DOD W81XWH1910776 (REG), and the Mirowski Family Foundation (REG).

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