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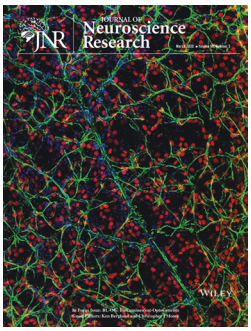
COMMENTARY



BL-OG: BioLuminescent-OptoGenetics

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In this issue of *In Focus* of the *Journal of Neuroscience Research* are two additional articles employing a relatively new methodology for manipulating neural activity, *BioLuminescent-OptoGenetics* or "BL-OG." This title captures the elemental features of a novel strategy increasingly being employed that has several unique benefits. First, BL-OG provides a high degree of flexibility, allowing drug-like chemogenetic control (created by injecting a luciferin whose photon output activates a neighboring optogenetic element) and direct optogenetic control in the same preparation, allowing sustained or immediate activation or suppression. In all instantiations published to date, this diverse combination of options is present within a single luminopsin (LMO) molecule. Second, BL-OG provides a temporally aligned report of activation, generating externally detectable photons as a by-product of its driving mechanism whenever its chemogenetic modulation is in effect. The BL-OG approach resolves the ambiguity inherent in all other drug-like approaches as to the timing of the arrival of any small molecule to its target, allowing the researcher direct confirmation of delivery.

The title "BL-OG" has been increasingly applied for the combined BioLuminescent-OptoGenetic approach. This term has been used and "published" in grant announcements and conference abstracts for several years, and is now appearing in published papers in this issue (e.g., Gomez-Ramirez, More, Friedman, Hochgeschwender, & Moore, 2019). This acronym not only captures the constituent elements of the strategy, it does so with an isomorphic dash, representing the amino-acid linker that connects a luciferase and an optogenetic element in an LMO. This naming convention also has the upside of being relatively memorable, a useful advantage for a novel approach. One notable drawback is the similarity between BL-OG and the commonly used term *blog*, a near parallel that yields a colorfully diverse set of returns when it is used in Internet searches on the topic.

The LMOs provide a single molecule encompassing all elements needed for the BL-OG strategy. These novel molecules comprise three transgenes, namely a luciferase to produce 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, the foreign membrane protein may not be processed properly, and can form protein aggregates inside mammalian neurons. To facilitate proper membrane targeting, Zhang et al. (2019) inserted the membrane trafficking signal found in a neural membrane protein, $K_{ir}2.1$, between the opsin and the fluorescent protein tag. The resulting enhanced Luminopsin 3 (eLMO3) showed significantly reduced protein aggregation and significantly improved surface expression, when quantified as bioluminescence from the luciferase moiety. Furthermore, it demonstrated enhanced photocurrent passage through the opsin moiety recorded in primary culture of mouse embryonic neurons. The utility of the improved LMO was further confirmed *in vivo* in the mouse brain, where the somatosensory cortex was transduced by an adeno-associated viral vector carrying the eLMO3 gene. Again, eLMO3 showed improved membrane targeting compared to its predecessor, and was able to elicit specific behavior, namely vibrissal touching behavior, more reliably when its substrate, coelenterazine (CTZ), was administered through intranasal delivery. Capitalizing on the proximity of the nose and the brain, the authors reaffirmed the effectiveness and convenience of this rather inconspicuous route for systemic administration of CTZ (Andreu et al., 2010; Yu et al., 2019).

The BL-OG reaction requires the substrate CTZ, delivered in a solvent that promotes its solubility, and creates "by-products" including the CTZ metabolite, coelenteramide, and resulting bioluminescence. Any of these necessary elements could in concept cause changes in neural activity, clouding the interpretation of the driver of the BL-OG effect. To test the specificity of the BL-OG approach, Gomez-Ramirez et al. (2019) tested each component systematically and showed that in reasonable concentrations neither CTZ, coelenteramide, solvent, nor bioluminescence alone changed neural activity in isolation. They further showed that changes in neural activity were directly proportional to the amount of bioluminescence emitted. Thus, they effectively proved that BL-OG effects are caused by membrane potential change through gating of the opsin moiety by bioluminescence. To rule out the effect of bioluminescence itself on neural activity, they employed an LMO with a point mutation that renders the channel moiety nonfunctional. This well-balanced negative control experiment provided a nearly complete emulation of the natural conditions without

TABLE 1 Nomenclatures of genetic methods for control of neural activity

Technique	Optogenetics	Chemogenetics	BL-OG
Representative proteins	Channelrhodopsins	DREADDs	Luminopsins
Activating modality	Light	Chemical	Light and chemical

phototransduction, and perhaps should be more widely adopted in conventional optogenetics.

Taken with the roster of papers in the prior commentary (Berglund & Gross, 2019), these new BL-OG papers further advance this technology, and underscore the utility and specificity of this approach (Table 1).

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