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Previews

It's lights out for presynaptic terminals

Xinyi Jenny He[1] and Matthew R. Banghart[1,*]
[1]Division of Biological Sciences, Neurobiology Section, University of California San Diego, La Jolla, CA 92093, USA
*Correspondence: mbanghart@ucsd.edu
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Reliable optogenetic tools for sustained, projection-specific presynaptic silencing have been elusive. Recently in *Neuron*, Mahn et al. (2021) and Copits et al. (2021) describe how the light-activated inhibitory GPCRs eOPN3 and PPO can be used to reversibly suppress synaptic transmission in mice.

Efforts to relate neural circuit connectivity to behavior can be driven by experimental approaches for perturbing synaptic communication between neurons. Optimal tools would provide rapid, reversible, and cell-type-specific inhibition of synaptic transmission in a manner that is reliable, minimally invasive, and compatible with *in vivo* optical and electrophysiological recording methods. Critically, as many neurons send axons to multiple sites in the brain, in order to discriminate between outputs to different target structures, this experimenter-controlled synaptic suppression would be projection-specific as well. While several good options for silencing neural activity and synaptic transmission are available, none of them meet all of the aforementioned criteria on their own. Optogenetic tools based on light-activated chloride channels, chloride pumps, and proton pumps have proven valuable for silencing action potentials using somato-dendritic illumination, especially for short periods of time (milliseconds to seconds). However, their application to axonal and presynaptic compartments has been riddled with issues related to poor efficacy, high light intensity requirements, and paradoxical excitatory effects related to the ensuing changes in intracellular ion concentrations (Wiegert et al., 2017).

In contrast, the chemogenetic tool hM4D strongly attenuates synaptic transmission by engaging endogenous G_i signaling pathways that inhibit synaptic vesicle release in response to the selective exogenous agonist clozapine-*N*-oxide (CNO) (Roth, 2016). Although hM4D avoids many of the problems that plague optogenetic silencers, the requirement to administer CNO through an implanted cannula to achieve projection specificity is cumbersome, disruptive to behavioral experiments, and slow to reverse. A more optimal approach might result from optogenetic control over metabotropic signaling. Indeed, chimeric G-protein-coupled receptors (GPCRs), constructed by merging mammalian GPCRs with rhodopsin, activate G-protein signaling in response to light, including signaling through the $G_{i/o}$ pathway (Spangler and Bruchas, 2017). Unfortunately, in contrast to light-gated pumps and channels, their exquisite photosensitivity renders them sensitive to ambient light, and their phototransduction cycles involve photobleaching (retinal dissociation and recycling), the latter of which greatly impedes repetitive photoactivation. Both features have rendered them rather impractical to implement in the laboratory.

In this and the previous issue of *Neuron*, two studies address this gap by exploring the ability of $G_{i/o}$-coupled non-visual rhodopsins to suppress synaptic output from mammalian neurons (Copits et al., 2021; Mahn et al., 2021). Mahn et al. (2021) describe eOPN3, a targeting-enhanced, mosquito-derived rhodopsin with mammalian homologs that are found in many different tissues. In parallel, Copits et al. (2021) report studies into PPO, a parapinopsin found in photoreceptor cells of the lamprey pineal organ. Like other optogenetic actuators, both eOPN3 and PPO are bistable opsins that retain the retinal chromophore, and both form functional photopigments without requiring the addition of exogenous retinal when expressed in mammalian cells. Using a combination of *in vitro* and *ex vivo* experiments, both groups establish that photoactivation of these inhibitory opsins can effectively suppress neuronal firing and synaptic transmission in mammalian neurons. Their mechanistic studies indicate that both opsins engage canonical components of the $G_{i/o}$ signaling pathway, including G-protein-coupled inward rectifier K^+ (GIRK) channels and voltage-gated Ca^{2+} channels (Figure 1A). Using *in vivo* electrophysiology and behavioral measurements, they go on to establish that local illumination of opsin-expressing synaptic terminals produces temporally precise, reversible inhibition of synaptic transmission.

Both studies commence with rigorous *in vitro* evaluation to establish that the opsins functionally engage inhibitory G proteins and GIRK channels and to determine their wavelength sensitivity. Whereas eOPN3 exhibits a broad action spectrum that peaks between 500 and 550 nm, PPO can be activated with either UV (365 nm) or blue (470 nm) light and then switched off with amber (590 nm) light (Figure 1B). From there, each group took different paths to probing the effects of opsin activation on synaptic transmission. Mahn et al. (2021) conducted an extensive series of experiments in autaptic hippocampal cultures to establish that eOPN3 can suppress neurotransmitter release through direct actions in presynaptic terminals. As a proxy for presynaptic inhibition, Copits et al. (2021) first determined that PPO can inhibit voltage-gated Ca^{2+} channel currents in cultured dorsal root ganglion neurons. Both groups showed that opsin activation produces effects that are comparable to activation of endogenous $GABA_B$ receptors with baclofen. Next, in hippocampal brain slices, Mahn et al. (2021) confirmed that eOPN3 inhibits synaptic transmission in response to green light (530 nm) and found that

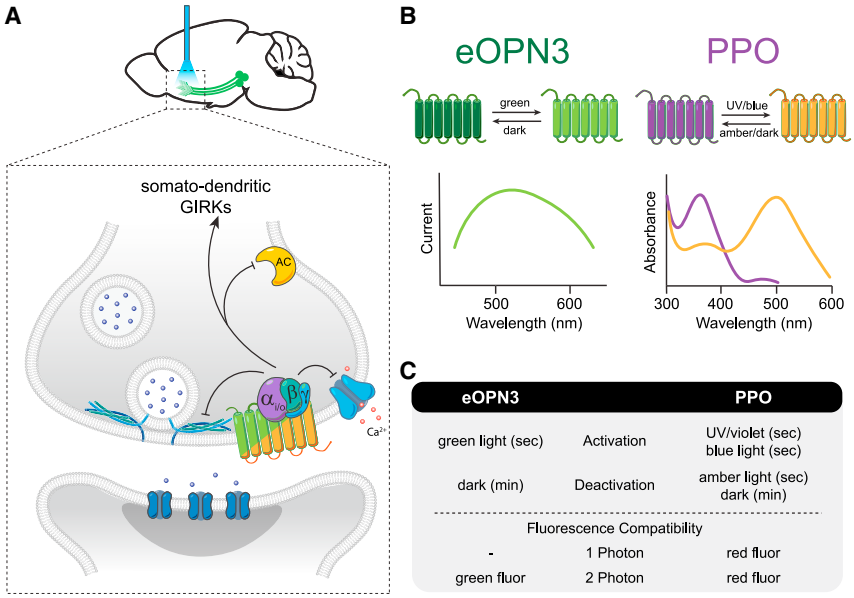

Figure 1. Key features of eOPN3 and PPO-mediated synaptic silencing
(A) Schematic illustration of presynaptic signaling mechanisms through which eOPN3 and PPO suppress neurotransmitter release.
(B) Spectral properties of eOPN3 and PPO.
(C) Summary of key properties that may guide experimental implementation.

local activation at single axonal boutons inhibits action-potential-driven presynaptic Ca^{2+} influx. Similarly, Copits et al. (2021) showed in brain slices that activation of PPO with blue light suppresses electrically evoked thalamocortical transmission as well as spontaneous GABA release in the nucleus accumbens (NAc).

Moving into *in vivo* electrophysiology but studying different neural projections, both groups found that local illumination of synaptic terminals from opsin-expressing neurons was able to rapidly and reversibly influence action potential firing in postsynaptic neurons. Using the red fluorescent dopamine sensor RdLight1, Copits et al. (2021) also showed that PPO activation can reversibly suppress dopamine release from ventral tegmental area (VTA) dopamine terminals in the NAc. To demonstrate that opsin-mediated photoinhibition of synaptic terminals can be used to study behavior, Mahn et al. (2021) quantified rotational movements in the open field in response to optical suppression of dopaminergic projections from the substantial nigra to the dorsomedial striatum. Consistent with dopamine's role in balancing striatal output, inhibiting nigrostriatal terminals with eOPN3 increased the ratio of ipsilateral to contralateral rotations. Copits et al. (2021) chose to study the effects of inhibiting VTA-to-NAc projections on motivated behaviors. PPO-mediated silencing of dopaminergic terminals in the NAc reduced the number of rewards obtained in fixed ratio and progressive ratio nose poke tasks, and pairing illumination with cocaine administration completely blocked the formation of a conditioned place preference. They further found that inhibition of glutamatergic projections from the basolateral amygdala to the NAc reversibly increased consumption of rewarding sucrose solution.

While both advances offer researchers powerful new tools for probing neural circuits, each opsin has unique features that affect its suitability for different experimental contexts (Figure 1C). For example, while the broad action spectrum of eOPN3 prohibits its use in most one-photon (1P) imaging experiments, Mahn et al., (2021) report minimal activation by two-photon (2P) excitation at wavelengths <980 nm, indicating that it is compatible with 2P imaging of green fluorophores. In contrast, PPO was shown to be compatible with 1P imaging of red fluorophores. Despite some photodeactivation in response to red fluorophore excitation, PPO can still be activated by blue (or UV) light, presumably due to the low-intensity illumination required for fluorescence imaging. Although PPO is not activated by 2P excitation beyond 1,000 nm, these wavelengths may similarly favor photodeactivation. Because both tools exhibit active states that last for minutes, imaging methods that rely on raster scanning could lead to the slow accumulation of active opsin. Further exploration of these tools in the context of common *in vivo* imaging modalities is necessary.

Another key feature that requires some consideration is reversal, as synapses can undergo long-term depression in response to activation of presynaptic $G_{i/o}$-coupled GPCRs (Atwood et al., 2014). Optical suppression of synaptic transmission by eOPN3 reversed in the dark with a time constant of ~5 min in multiple preparations. Although PPO's effects on synaptic output appear to largely reverse in the dark after excitation with pulsed blue light, the precise kinetics and extent of reversal in different contexts are less clear. Other work with PPO has employed ultraviolet-violet (360–405 nm) light for photoactivation, which produces a relatively stable state that can be rapidly switched off with longer wavelength light (Rodgers et al., 2021). Because blue light is absorbed by both forms of PPO, it may produce a mixture of photostates that favors activation yet remains prone to spontaneous relaxation in the dark. As low-cost violet and amber light sources are readily available, PPO users may wish to consider multiple optical configurations based on their unique experimental needs and constraints.

Although both eOPN3 and PPO are clearly powerful and advantageous tools, presynaptic $G_{i/o}$-coupled GPCRs may not be optimal for suppressing output from all synapses, as some terminals may lack appropriate signaling components or exhibit low sensitivity to $G_{i/o}$ signaling during high-frequency activity. Alternative approaches might involve targeting components of the presynaptic release machinery directly or engineering photoswitchable peptide toxins that block endogenous presynaptic Ca^{2+} channels (Schmidt et al., 2014; Liu et al., 2019). Another solution may result from

engineering a tightly regulated, high-conductance light-gated K^+ channel (Cosentino et al., 2015). Regardless of approach, the ability to precisely restrict the action of opto- and chemogenetic tools to synaptic connections between defined pre- and postsynaptic partners, perhaps by utilizing *trans*-synaptic interactions, remains an important future goal.

In summary, the validation of eOPN3 and PPO as tools for optogenetic silencing of synaptic transmission constitutes a major breakthrough that enables projection-specific inhibition with good temporal control and high reliability. Though neither tool is compatible with all imaging modalities, together they provide options for presynaptic silencing with 1P imaging of red fluorophores and 2P imaging of both green and red probes. The choice of opsin may ultimately come down to wavelength preference and empirical measures of efficacy in the experimental system under study.

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Neuronal intranuclear inclusion disease: Polyglycine protein is the culprit

Michelle E. Ehrlich[1] **and Lisa M. Ellerby**[2,*]
[1]Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[2]The Buck Institute for Research on Aging 8001 Redwood Blvd, Novato, CA 94945, USA
*Correspondence: lellerby@buckinstitute.org
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In this issue of *Neuron*, Boivin et al. (2021) show that a polyglycine-expanded protein, uN2CpolyG, is translated from an expansion of GGC repeats in the 5′ UTR of the *NOTCH2NLC* (Notch homolog 2 N-terminal-like C) gene, defining a new pathological mechanism for neuronal intranuclear inclusion diseases (NIID).

Neuronal intranuclear inclusion disease (NIID) is a rare sporadic or autosomal dominant multisystem neurodegenerative disease characterized by ubiquitin-immunopositive nuclear inclusions. Muscle and skin biopsies revealed eosinophilic intranuclear inclusions from NIID patients, and these were initially used to diagnose the disease. NIID presentation is highly variable but may include progressive cognitive impairments, tremor, reversible encephalitic episodes, alterations in the pyramidal tracts that innervate motor neurons of the spinal cord and brain stem, cerebellar ataxia, peripheral neuropathy, autonomic dysfunction, seizures, and muscle weakness, including the ocular muscles (Figure 1). There are also ocular changes: the outer retinal layer atrophies, but the inner retinal layer is preserved (Nakamura et al., 2020). The muscles of the eye are atrophied with bilateral miosis. NIID can affect the entire body, resulting in other symptoms, and intranuclear