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Spotlight

A dLight-ful New View of Neuromodulation

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Neuromodulators such as dopamine can transform neural circuit function, but the mechanisms underlying such transformations are incompletely understood. A recent study introduced dLight1, a genetically encoded fluorescent dopamine indicator. dLight1 allows the optical measurement of dopamine sensed by isolated target circuits with high spatiotemporal resolution and has unique advantages for the study of neuromodulatory mechanisms.

One of the most remarkable aspects of the nervous system is its flexibility: neural circuits can adapt an organism's behavior to an astonishing array of conditions, including adaptations on short timescales that would be impossible to implement via circuit rewiring. A major mechanism for this fast adaptation is through neuromodulation: neuromodulators such as dopamine, serotonin, and norepinephrine can quickly but reversibly reconfigure neural circuits by altering the intrinsic and synaptic properties of their target neurons.

A major challenge in the study of neuromodulation has been the ability to detect neuromodulatory chemicals *in vivo* at high spatiotemporal resolution. As our ability to precisely monitor and manipulate neuronal activity *in vivo* – for example, using genetically encoded calcium indicators and optogenetics – has advanced in recent years, this need for neuromodulatory sensors has become even more apparent. The ability to precisely measure the release of neuromodulators such as dopamine would offer great insight into mechanisms of neuromodulatory action, especially when multiplexed with other emerging tools in neuroscience. To this end, Patriarchi *et al*. [1] engineered dLight1, a genetically encoded fluorescent dopamine indicator capable of sensing dopamine transients in awake, behaving animals.

Dopamine receptors are members of the G protein-coupled receptor (GPCR) superfamily, which have seven transmembrane domains. dLight1 was created by replacing the third intracellular loop on a human dopamine receptor (D1, D2, or D4) with a circularly permuted GFP (cpGFP) module from GCaMP6 (Figure 1A) [2]. The resulting GPCR binds dopamine, causing an increase in cpGFP fluorescence without activating downstream signaling cascades (cAMP). Thus, the expression of dLight1 does not appear to interfere with native GPCR signaling. Patriarchi *et al*. generated several versions of dLight1 (1–5) with varying dynamic ranges and affinities for dopamine, which could be useful for different types of studies. In general, they observed a tradeoff between dynamic range ($\Delta F/F_{max}$) and apparent affinity, although continued engineering of dLight1 may eventually overcome this limitation. Patriarchi *et al*. also generated a mutated version of dLight1 that does not bind dopamine, providing a reliable control for future experiments.

Because dLight1 is a fast, genetically encoded optical sensor, it offers several advantages over other approaches. Most notably, unlike microdialysis or fast-scan cyclic voltammetry (FSCV), dLight1 offers high temporal and spatial resolution as well as the potential to genetically isolate cells of interest for study. Additionally, while both microdialysis and FSCV largely reveal extrasynaptic neuromodulator dynamics, the membrane-bound nature of dLight1 means that it senses dopamine only in close proximity to the postsynaptic cell of interest. Although dLight1 is not confined to the synapse, high-resolution imaging of this sensor in combination with synaptic markers could differentiate between synaptic and extrasynaptic signals. Finally, because dLight1 is an optical sensor compatible with one- and two-photon imaging (Figure 1B), images acquired across days could be registered using structural markers, allowing longitudinal analyses of dopamine sensing in defined areas over multiday behavioral experiments.

Calcium imaging in dopamine neurons, using for instance genetically encoded calcium indicators (GECIs), has also emerged as a potential tool to monitor dopamine dynamics in a defined area. For example, Howe and Dombeck [3] used GECIs in combination with both fiber photometry and two-photon imaging to observe calcium signaling changes in striatum-projecting dopaminergic axons originating from the midbrain, and discovered phasic dopamine transients associated with locomotion. However, the measurement of calcium dynamics in dopaminergic axons does not map straightforwardly onto dopamine release dynamics. Several well-known mechanisms regulate dopamine release independently of action potential firing, including inhibition by presynaptic nicotinic acetylcholine receptors and dopamine D2 autoreceptors [4]. Additional changes in extracellular dopamine concentration resulting from action potential-independent mechanisms, such as changes in reuptake, would also not be

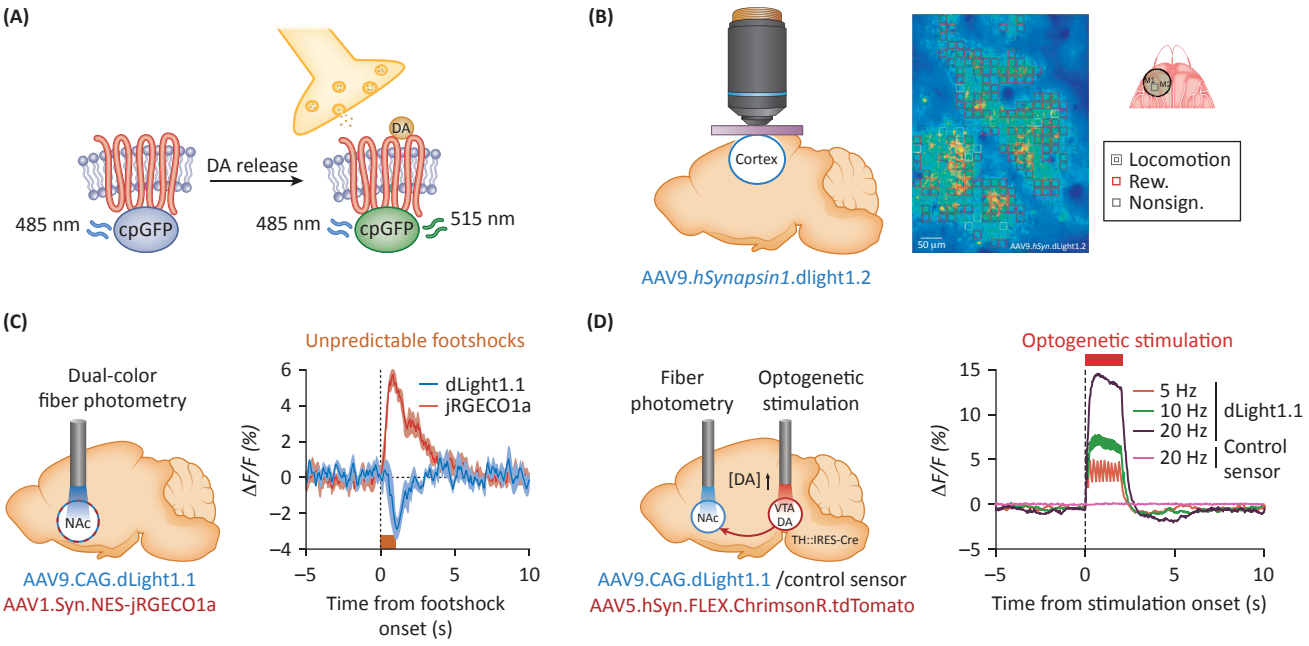

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Figure 1. Compatibility of dLight1 with Existing Optical Technologies. (A) dLight1 was created by replacing the third intracellular loop on a human dopamine receptor with a circularly permuted GFP (cpGFP) module. When dopamine (DA) is released by a nearby presynaptic terminal, it binds to dLight1, causing an increase in fluorescence of cpGFP. (B) An example of *in vivo* two-photon dLight1 imaging in cortex through a cranial window. The heatmap shows the pattern of dLight1.2 expression. Boxes show computationally defined regions of interest (ROIs) for analysis. Coloring of the ROIs indicates the associated activity observed during behavior (reward signaling, locomotion, or nonsignificant). (C) An example of dual-color fiber photometry in nucleus accumbens using dLight1.1 paired with the red calcium indicator jRGECO1a. When the mice are given unpredictable footshocks, dLight1 fluorescence decreases, whereas jRGECO1a fluorescence increases. (D) An example of fiber photometry measurement of dLight1.1 signals in nucleus accumbens paired with optogenetic stimulation of VTA dopamine neurons (which project to the nucleus accumbens) using the red-shifted excitatory opsin ChrimsonR. Stimulation of dopaminergic inputs to the nucleus accumbens leads to a frequency-dependent increase in dLight1 fluorescence. No increase in fluorescence is seen using the dLight1 control sensor. (B–D) adapted from Patriarchi *et al*. [1]. Reprinted with permission from AAAS.

detected by calcium imaging. Recent work from Josh Berke's laboratory emphasizes discrepancies between dopamine cell firing and measurements of extracellular dopamine using FSCV [5]. The new ability to image dopamine with dLight1 will allow neuroscientists to address these issues.

Perhaps one of the most versatile features of dLight1 lies in its built-in compatibility with existing technologies, including GECI imaging and optogenetics. Measuring dLight1 activity via detection of green fluorescence is compatible with simultaneous measuring of red GECIs such as jRGECO1a [6]. As a proof of concept, Patriarchi *et al*. show that by sensing both dopamine and calcium signals simultaneously in the same cells, one can differentiate between the presence of the neurotransmitter and its postsynaptic effects on cell firing. As an example, they found that subjecting mice to a footshock decreases dopamine signaling in the nucleus accumbens (detected using dLight1.1) but increases overall neuronal activity (detected using jRGECO1a; Figure 1C). These results are presumably due to decreased activation of G_i-coupled dopamine D2 receptors and demonstrate how the ability to specifically monitor neuromodulator levels will enrich our understanding of the downstream circuit effects of neuromodulation.

Patriarchi *et al*. further demonstrate the compatibility of dLight with optogenetics. They show that dLight1 can successfully measure dopamine released via optogenetic circuit control in awake, behaving mice using a red-shifted excitatory opsin, ChrimsonR (Figure 1D) [7]. This marriage of techniques promises significant insight into the relationship between circuit activity and neuromodulator effects in future experiments.

Notably, dLight1 is not the only fluorescent dopamine sensor under development. While this Spotlight was in proof, a similar GPCR-based sensor called $GRAB_{DA}$ was published, and its use additionally demonstrated in other model systems including fish and flies [8]. Other groups are developing single-wall carbon nanotube (SWNT)-based

probes, which offer excellent $\Delta F/F_{max}$ compared with current versions of dLight1 or $GRAB_{DA}$ and do not photobleach [9]. These SWNT-based probes may also offer advantages due to their near-IR emission wavelengths (in a window of low tissue absorption). However, SWNT-based probes are not genetically encoded and are largely excluded from the synaptic cleft due to their size. Selection of the best fluorescent dopamine probe will thus depend on the requirements of the particular experiment.

In conclusion, the dLight1 sensor developed by Patriarchi *et al*. is a major advance in the measurement of *in vivo* neuromodulator release given its optical readout, fast dynamics, and high dopamine affinity. Especially when used in combination with other methods, it will provide a powerful new view of the dopaminergic system *in vivo*. Further, the methods used by Patriarchi *et al*. to engineer dLight1 are generally suitable for the development of optical sensors based on other GPCRs. Thus, the development of dLight1 is a boon not only for dopamine research, but for the study of neuromodulation more broadly.

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Spotlight

Organic Electronics for Artificial Touch

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Artificial restoration of touch is an active area of research in neuroprosthetics. However, most approaches do not consider emulating the biological machinery they intend to replace. Recently, Kim *et al*. proposed a bioinspired artificial touch transducer that closely mimics the behavior of natural sensory afferents.

Somatosensation allows us to experience and interact with the world around us. It underlies a range of behaviors from relatively simple object interactions, such as picking up a glass of water, to the complex sensorimotor coordination of gracefully playing a nocturne by Chopin on the piano. However, for people with lost or degraded somatosensation, as is the case for an amputee or stroke survivor, even the simplest daily activities can be challenging and sometimes impossible [1,2]. Although sensory substitution (e.g., vibrotactile input elsewhere in the body) and visual feedback can provide gross information on the state of a prosthetic limb, they deliver an incomplete experience to the user. Recent efforts to enrich prosthetic sensory feedback aim to engage the remaining intact nervous system by directly stimulating peripheral nerves [1] or the somatosensory cortex in an attempt to evoke naturalistic sensations [3]. To restore naturalistic sensation, technologies must leverage the endogenous coding properties and scalability of biological sensory organs such that the brain and spinal cord can construct a rich representation of the environment (Figure 1).

In a recent issue of *Science*, Kim *et al*. report on the fabrication of an artificial sensory afferent (ASA) that has the potential to replicate biological signal transduction [4]. The device transduces pressure signals in a way that resembles a slowly adapting type 1 sensory afferent (SA1). When pressure is applied to the ASA, a series of elastomeric, carbon nanotube (CNT) microstructures compress, reducing the contact resistance. This CNT-based pressure sensor provides mechanical flexibility while also operating in a pressure range similar to that of receptors in the skin (1–80 kPa). This change in resistance then regulates the voltage on a ring oscillator, producing action potential-like signals at frequencies comparable to biological sensory afferent (BSA) firing rates (1–100 Hz). To mimic the electrochemical signaling inherent to neurons, voltage pulses from the ring oscillator then drive current flow in a synaptic transistor that is crucial for creating neuron-like signal transduction. Unlike standard complementary metal oxide semiconductor (CMOS) transistors, each input to a synaptic transistor imparts a chemical memory that can produce effects similar to paired pulse facilitation and synaptic depression [5].