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Viewpoint

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Why Optogenetics Needs in Vivo Neurochemistry

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ABSTRACT: In neuroscience, the consequences of optogenetic manipulation are often studied using in vivo electrophysiology and by observing behavioral changes induced by light stimulation in genetically targeted rodents. In contrast, reports on the in vivo neurochemical effects of optogenetic stimulation are scarce despite the improving quality of analytical techniques available to monitor biochemical compounds involved in neurotransmission. This intriguing lack of neurochemical information suggests the existence of unknown or misunderstood factors hampering the expected rise of a novel specialty putatively be termed "neurochemical optogenetics".

KEYWORDS: *Optogenetics, in vivo neurochemistry, microdialysis, voltammetry, neurotransmitters*

The activation of neurons by an external stimulus, such as light, temperature, electrical pulse, or chemical signal, depends on their proteome and, in particular, the distribution of specific cell-surface receptors.[1] In both prokaryotes and eukaryotes, some proteins can be activated by light. Members of the opsin family, including retinal pigments in visual systems, are stimulated by visible light. Channel rhodopsins, discovered in the green algae *Chlamydomonas reinhardtii*, are examples of opsins coupled to sodium channels that increase cell excitability when exposed to blue light. Today, cells can be genetically engineered to express channel rhodopsins or other types of opsins such that they can be activated or silenced after exposure to specific wavelengths of light.[1] This approach, known as optogenetics, first reported by Boyden in 2005,[2] has surged in the field of neuroscience as represented by >1000 references in PubMed ("optogenetics" as the key word from 2005 to April 2015). The findings reported in these papers were mostly derived from electrophysiological recordings sometimes associated with behavioral observations during in vivo studies.

Brain function arises not only from the propagation of electrical waves—action potentials or field potentials—but also via intercellular signaling associated with the release of neurotransmitters targeting pre- and postsynaptic receptors present at synaptic clefts or the perisynaptic space. As such, one would have expected a rise in the number of optogenetics studies that included neurochemical monitoring similar to papers reporting electrophysiological findings in combination with optogenetic control of cell activity. Yet, this has not occurred. When again considering the period from 2005 to April 2015, of all articles published on "brain and electrophysiology" (6958) and articles published on "brain and microdialysis or electrochemistry/voltammetry" for neurochemistry approaches (4812), the electrophysiology/neurochemistry ratio was 60:40. If this ratio is extended to optogenetics, the number of papers combining optogenetics and neurochemistry would be expected to be roughly 400 instead of the 12 found when the keywords "brain", "optogenetic", and "microdialysis" or "electrochemistry" or "voltammetry" are entered into the PubMed database. Although approximate, this method of estimation yields a result very close to the actual number of published studies reporting the effects of optogenetic manipulations on neurotransmitter monitoring in living animals. At the time this Viewpoint was written, only six optogenetic studies were identified that included in vivo neurotransmitter monitoring. Moreover, only dopamine has been investigated using fast-scan cyclic voltammetry in combination with optogenetic targeting. This dearth is expected to lead to a limited knowledge of the neurobiological consequences of optogenetic manipulations.

The first paper reporting in vivo neurotransmitter release in the context of optogenetic stimulation was published by Budygin and co-workers in 2010.[3] In this study, the authors delivered adeno-associated virus into the rat substantia nigra (a brain region rich in dopaminergic cell bodies) to express channel rhodopsin-2, an excitatory channel permeable to sodium. The effects of blue-light stimulation on the nigrostriatal pathway were evaluated in the dorsal striatum of anesthetized rats by measuring changes in extracellular dopamine concentrations using carbon-fiber microelectrodes and fast-scan cyclic voltammetry. The authors determined the light-pulse parameters needed to produce dopamine release similar to that obtained with electrically evoked stimulation. In a subsequent paper, these authors performed optogenetic experiments in freely moving rats in combination with a behavioral task

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involving ethanol self-administration after viral manipulation to express channel rhodopsin in dopaminergic neurons in the ventral tegmental area.[4] Here, dopamine release triggered by optogenetic stimulation altered ethanol drinking behavior (Figure 1).

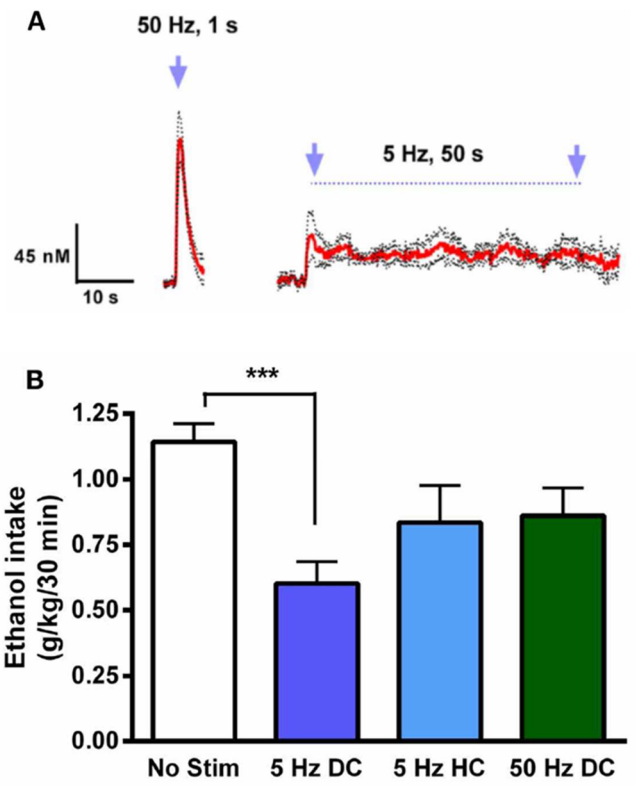

Figure 1. (A) Blue light activation of rat ventral tegmental (VTA) dopamine neurons mimics phasic and tonic dopamine release in nucleus accumbens. Dopamine concentration changes expressed as means ± SEMs (N = 5) were evoked by 50 light pulses at 50 Hz or 250 light pulses at 5 Hz via optical stimulation in the VTA. (B) Tonic dopamine release alters ethanol drinking only when optogenetic stimulation is applied in the drinking cage and not in the home cage. Bar graphs illustrate average values of total ethanol consumed (g/kg) across multiple optogenetic sessions performed in the drinking cage (DC) or home cage (HC). ***P < 0.001 compared with no stimulation. From the open access article by Bass et al., 2013, *Frontiers in Behavioral Neuroscience*.[4] With permission from the authors.

These examples illustrate that dopamine can be monitored during optogenetic stimulation using in vivo preparations, which are mandatory for behavioral studies. In addition to branching out to other neurotransmitter systems, next steps will also involve simultaneous monitoring of several neurotransmitters. Success in optogenetic neurochemical monitoring will require: (i) expertise in in vivo neurochemical techniques; (ii) the availability of methods enabling high sampling rates for behaviorally relevant monitoring of one or more neurotransmitters; and (iii) the implementation of controls to investigate the direct effects of laser light or heat on nontargeted endogenous ion-channels or biochemicals.

Improvements in the temporal, spatial, and chemical resolution of available neurochemical techniques continue to be made through the use of direct and indirect electrochemical sensors and microdialysis sampling coupled to ultrahigh performance liquid chromatography[5] or capillary electrophoresis. The available optogenetic/neurochemical publications indicate that high sampling rates can be used to detect variations in extracellular dopamine induced by light stimulation having short duration to mimic trains of electrical or behaviorally evoked stimulation.[3,4] Optogenetic modulation of chemical neurotransmission is theoretically possible for many different neurotransmitters including biogenic amines, amino acids, and neuropeptides, and for energy metabolites. Simultaneously monitoring several neurochemicals at high sampling rates will provide information on neurotransmitter interactions, co-release of neurotransmitters, or heteroregulation across neurotransmitter systems during optogenetic challenge. By altering neuronal activity, optogenetics may also affect energy demand. Thus, the effects of optogenetic stimulation on extracellular energy biomarkers will be important targets for future investigation.

The possibility for light-induced artifacts in neurochemical studies cannot be excluded as laser light can induce heat or other alterations in brain tissue, which is unwanted when studying neurotransmission during behavioral tasks. Even if artifacts due to light stimulation of nontargeted ion channels or dopamine transporters are reported to be negligible and not affecting in vivo extracellular dopamine levels,[3] perturbations of neurotransmitter systems via heat-sensitive TRPV channels will need to be verified.[6] In addition, as electrophysiologists (and also dopamine electrochemists) have carefully reduced the side effects of optogenetic stimulation on electrical signals (and dopamine release),[1] neurochemists using optogenetics to study other neurotransmitters will need to adopt similar biological criteria to validate neurochemical data when using microdialysis or biosensors. For instance, variations in extracellular neurotransmitters induced by an optogenetic challenge should be physiologically relevant and neurochemical variations after light stimulation should be consistent with opsin densities at cell surfaces (i.e., with light-induced opsin currents).[1]

Optogenetics will be useful in combination with in vivo neurochemical monitoring because the power of the former relies on the specificity of activation and/or inhibition of defined subpopulations of nerve cells as compared to the currently used means of neuronal stimulation (i.e., pharmacological, chemical, or electrical). Reciprocally, optogenetics needs neurochemistry. Electrophysiology approaches cannot provide direct, quantitative information on neurotransmitter signaling or metabolic alterations associated with optogenetic challenge. Exploring chemical neurotransmission in combination with optogenetic approaches will complement electrophysiology studies, enabling a clearer understanding of neural circuits and their control of behavior. An additional message to our colleagues beginning neurochemical experiments coupled with optogenetics is that now is the time to share good (and bad) news in this exciting and promising field.

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Notes

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