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Optogenetic and Chemogenetic Approaches To Advance Monitoring Molecules

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ABSTRACT: Fast-scan cyclic voltammetry (FSCV) is a high-resolution technique used to investigate neurotransmission *in vitro*, *ex vivo*, and *in vivo*. In this Viewpoint, I discuss how optogenetic and chemogenetic methods, when combined with FSCV, can impact and advance our understanding of neurotransmission and enable more detailed investigation of the roles of neurotransmitter systems in normal and disease states.

KEYWORDS: *Fast-scan cyclic voltammetry, dopamine, norepinephrine, serotonin, optogenetics, chemogenetics*

Direct measurement of neurotransmitter release and reuptake *in situ* is a powerful means to investigate chemical neurotransmission and to understand how information is processed under physiological and pathological conditions. Fast-scan cyclic voltammetry is an analytical method widely employed to study second-by-second changes in extracellular dopamine (DA) concentrations with high spatial resolution following electrical stimulation of neurons or behaviorally induced neurotransmitter release. Dopamine dynamics in subregions of the striatum have been effectively investigated using FSCV because of the high density of striatal DA terminals and the high DA release probability in these brain regions.

Despite the enormous utility of FSCV to investigate release and uptake of striatal DA, there are challenges to studying these phenomena within the striatum and in other brain regions. Pharmacological evidence from *ex vivo* (brain tissue slice) preparations and *in vivo* behaving animals indicates that single-pulse electrical stimulation releases neurotransmitters other than DA, including non-electroactive acetylcholine (Ach) and glutamate, which directly modulate subsequent DA release,[1−3] thus complicating data interpretation. Furthermore, the use of FSCV to detect electroactive neurotransmitters other than DA (e.g., serotonin, norepinephrine (NE)) presents difficulties largely associated with low levels of innervation and release occurring concomitantly with equal or greater amounts of DA. While strategies such as principal components analysis have been proposed to deconvolute voltammograms arising from complex mixtures of analytes, none of these have found widespread practical use. Indeed, principle components analysis of cyclic voltammograms arising from mixtures of DA and NE cannot resolve specific contributions from each of these neurotransmitters.

Recent advances in controlling the stimulation or silencing of neurons with genetic, spatial, and temporal precision are providing the means to address some of these challenges. The advent of optogenetic and chemogenetic tools for investigating circuit dynamics enables selective control over specific circuitry. Optogenetic methods involve transient or stable transduction of specific neuronal populations with genes encoding bacterial opsins, which are membrane-associated ion channels or pumps that can be stimulated by light and tagged with fluorescent proteins for visualization. The most commonly employed ospin for activating neurons is channelrhodopsin2 (ChR2), which causes an influx of Na^+ resulting in membrane depolarization and the firing of action potentials when stimulated by blue light (473 nm). By contrast, expressing archaerhodopsin enables yellow light (655 nm) triggering of H^+ efflux, membrane hyperpolarization, and neuronal silencing.

An orthogonal, yet related, strategy for selective neuronal excitation or silencing is carried out via selective expression of G-protein-coupled receptors known as designer receptors exclusively activated by designer drugs (DREADDs). These receptors have been "re-engineered" to recognize the synthetic small-molecule ligand clozapine-*N*-oxide (CNO). In experiments using DREADDs, specific populations of neurons are stimulated or silenced over longer time frames compared with light-triggered opsin activation. In both optogenetic and chemogenetic experiments, genetic selectivity is achieved using recombinase-dependent opsin/DREADD expression, in combination with neuron-specific recombinase expression, or the viral encoding of promoters recognized by specific cell types. Recently, we have begun to witness the implementation of optogenetic and chemogenetic strategies in conjunction with FSCV. Continued and widespread adoption of these combined tools is anticipated to facilitate future advances associated with decoding information processing associated with neurotransmitter signaling.

CONFOUNDING FACTORS OF ELECTRICAL STIMULATION

Even though the subsecond resolution associated with FSCV facilitates the observation of rapid neurotransmitter release events, this technique requires analytes to adsorb to carbon-fiber microelectrodes (CFMs), limiting voltammetric sampling rates to 10 Hz (but sometimes as high as 60 Hz). In contrast,

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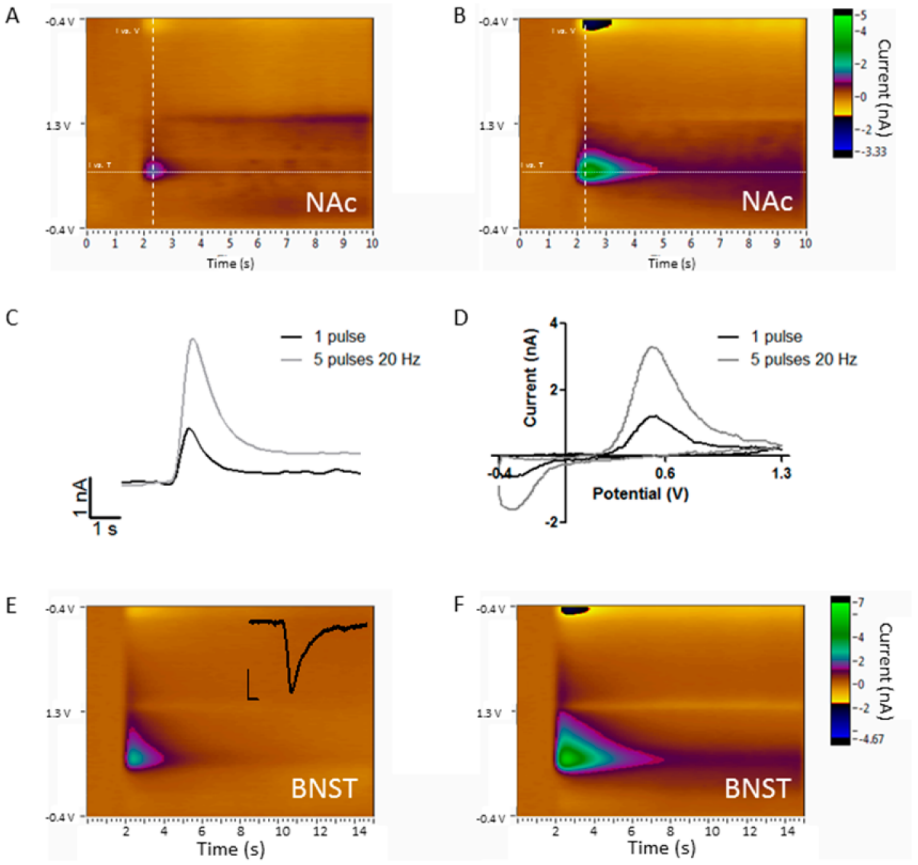

Figure 1. Optical stimulation of ChR2 virally expressed in VTA dopamine terminals demonstrates frequency dependent DA release in *ex vivo* slice preparations. Color plots of DA release in the nucleus accumbens (NAc) core in response to (A) a single 5 ms blue-light (473 nm) pulse vs (B) five 5 ms pulses at 20 Hz. White dashed lines demark the positions at which current vs time plots and cyclic voltammograms were obtained. (C) Current vs time plots and (D) cyclic voltammograms from the color plots in A and B. (E) Single pulse optical stimulation in the bed nucleus of the stria terminalis (BNST) of DA terminals expressing ChR2 releases both DA and glutamate (inset as measured by whole-cell voltage clamp, scale bar 20 pA by 5 ms.) (F) A five-pulse, 20-Hz stimulation in the same recording location as in panel E shows enhanced DA release.

uptake of biogenic amines occurs on the order of seconds. Due to the latter time scale, electrical stimulation, which can result in the release of multiple neurotransmitters, has ample time to modulate release probability (and potentially uptake) at biogenic amine synapses. In contrast to electrical stimulation, light-mediated stimulation of ChR2 enables selective stimulation of neurons. While using ChR2 to stimulate biogenic amine terminals eliminates direct activation of other neurons within slices or *in vivo*, it does not circumvent the activation of downstream biogenic amine heteroreceptors and/or autoreceptors. These receptors in turn may influence the release of other neurotransmitters within the associated microcircuitry.

As mentioned above, in brain tissue slices, recent evidence indicates that single-pulse electrical stimulation releases neurotransmitters that can impact DA release. Previously, experiments using nicotinic antagonists revealed that ACh released from cholinergic interneurons upon electrical stimulation potentiates DA release. Recent findings, furthermore, demonstrated that optical stimulation of cholinergic interneurons expressing ChR2 is sufficient to release DA via the activation of nicotinic receptors and independent of DA neurons firing action potentials.[2,3] Future use of optogenetic techniques in *ex vivo* preparations will reduce the contribution of within slice circuit events, affording a more precise examination of release mechanisms and machinery. Additionally, we now know that DA neurons can release GABA or glutamate and perhaps additional signaling molecules (e.g., ATP, H^+), in addition to dopamine (Figure 1E). Combining FSCV and whole-cell electrophysiology to measure neurotransmitter release with optogenetics for cell type selectivity allows us to dissect the contributions from individual neuronal populations. As such, it provides a means to test the role of multitransmitter release in both physiological and pathological conditions.

INVESTIGATING NEUROTRANSMITTER RELEASE MECHANISMS

In *ex vivo* electrophysiology, small stimulus trains are used to draw conclusions about synaptic mechanisms and to investigate whether pharmacologic treatment and/or plasticity alter presynaptic release. Electrical stimulation of DA terminals in striatal slices depletes most of the DA in the terminals, preventing the examination of multipulse stimulation in slices (see above). Optogenetically stimulating DA terminals in brain tissue slices, however, more closely resembles *in vivo* conditions where stimulated DA release scales with pulse number and stimulation frequency. By reconciling *in vivo* and *ex vivo* observations, optogenetic stimulation of terminals will permit high throughput analysis afforded by *ex vivo* recordings. As such, facile manipulation of presynaptic effectors genetically or pharmacologically (e.g., ion channels, G-protein coupled receptors, synaptic machinery) can be used to identify functional roles under physiological or pathological conditions

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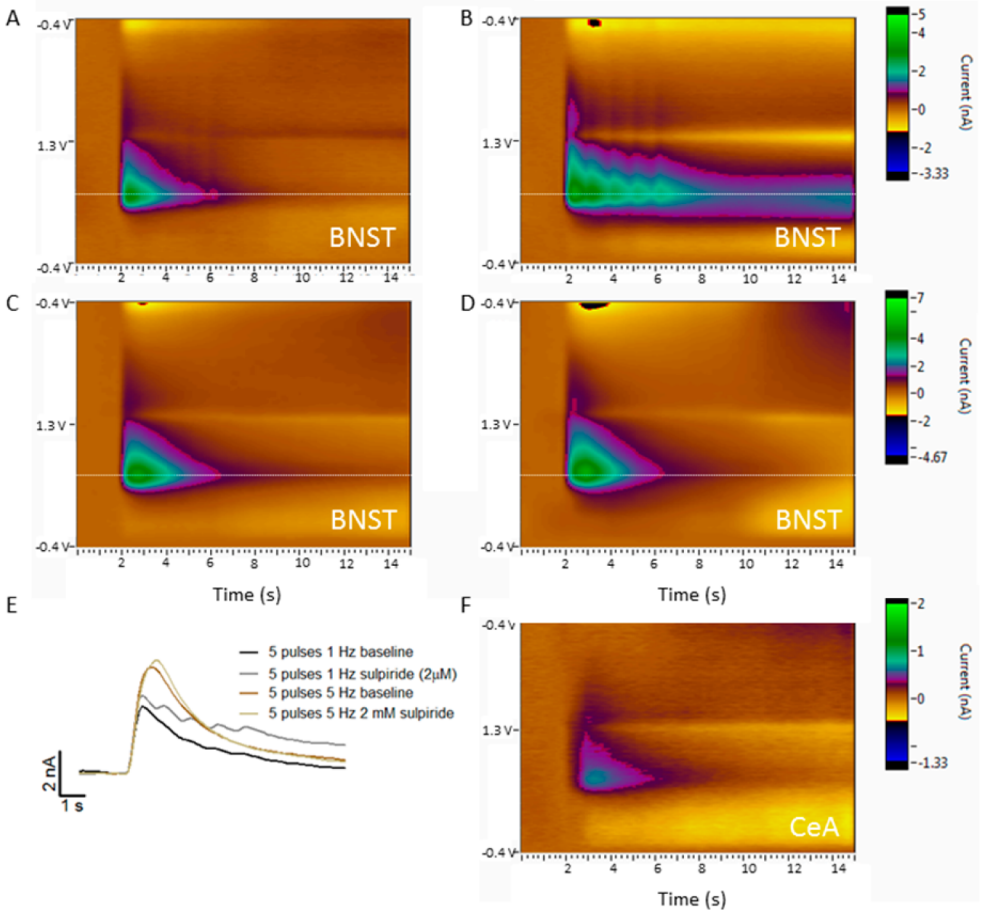

Figure 2. Representative experiments showing the effects of D2 antagonists to alter DA release in the BNST under low firing conditions. (A) A five-pulse, 1-Hz optical stimulation of VTA terminal DA release in the BNST under baseline conditions and (B) following application of 2 μM sulpiride to block D2 receptors. (C) A five-pulse, 5-Hz optical stimulation of VTA DA release in the BNST under baseline conditions (C) and following application of 2 μM sulpiride (D). (E) Current vs time traces from color plots in panels A–D. White dashed lines in panels A–D signify where voltammograms were obtained. (F) A 20-pulse, 20-Hz optical stimulation of VTA DA terminals in the central nucleus of the amygdala (CeA) releases detectable DA.

(Figures 1 and 2). Furthermore, combining FSCV with whole-cell electrophysiology will greatly enhance our ability to understand presynaptic functions (Figure 1E).

MIXED REGIONS

One issue with utilizing FSCV to study biogenic amines and other molecules is that this technique has its greatest utility in brain regions where the neurotransmitter being examined has dense innervation (e.g., the striatum for DA, the ventral bed nucleus of the stria terminalis for NE,[4] and the substantia nigra pars reticulate for serotonin) and little innervation from interfering species. Analyte identification is governed by the voltammetric signature of the analyte, which is related to the properties of the waveform being used. In many brain regions with co-innervation of multiple biogenic amine systems, however, it remains difficult to distinguish one analyte from another after electrical stimulation. This is particularly challenging for DA and NE in areas with moderate to high DA content (e.g., various subregions of the amygdala; Figure 2F) because DA and NE are detected using the same waveform. Furthermore, the hydroxyl group on the β-carbon of NE reduces adsorption at carbon-fiber microelectrodes between scans, thereby decreasing detection efficiency. Additionally, some regions have mixed DA/NE/serotonin innervation from multiple neuronal pathways that may be regulated in distinct fashions. By injecting recombinase-dependent virus to express ChR2 in specific nuclei (e.g., ventral tegmental area, locus coeruleus, dorsal raphe) under the control of biogenic amine driver lines (e.g., TH-Cre, DAT-Cre, SERT-Cre), researchers can selectively activate specific biogenic amine neurons. Thus, these tools may enable the dissection of contributions from individual pathways (e.g., periaquaductal gray DA vs ventral tegmental area DA, both of which innervate the dorsal bed nucleus of the stria terminalis), since it is likely there are pathway specific adaptations and modulation in various maladaptive states.

REGIONS WITH MODERATE TO LOW FIBER DENSITY

FSCV requires close electrode proximity (10 μm) to presynaptic release sites, making systematic recordings in areas with moderate/low fiber density challenging. ChR2 and other optogenetic/chemogenetic molecules are often coupled to a fluorophore that labels presynaptic terminals. Using a fluorescent microscope, investigators can target subregions of highest density in slices permitting more reliable recording strategies in areas with low innervation and increasing the probability of successful recordings. Additionally, in striatal and

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other tissues, fluorophore densities could aid in investigating disparities between areas of high and low biogenic amine innervation. These tools will further enable our understanding of how diffusion and other transport mechanisms, like organic cation transporters, which may be expressed by nonbiogenic amine cells, influence uptake mechanisms (Figure 2F).

EXAMINING RELEASE MECHANISMS

Chemogenetic strategies (DREADD G-protein-coupled receptors) differ from optogenetic technologies (cation/anion channels and proton pumps) by coupling to endogenous intracellular signaling pathways. Specific DREADDs couple to G_s, G_q, and G_i to manipulate cellular function. These tools not only activate or inhibit cells (akin to optogenetic strategies) but also have the ability to influence more discrete signaling events within cells. In addition, newer viral DREADD constructs target DREADD proteins to terminal regions[5] enabling chemogenetic control over cells with subcellular selectivity. This level of control will be particularly useful in conjunction with *ex vivo* slice FSCV preparations where neurotransmitter tone is limited. Additionally, the use of DREADDs will be an excellent way to control and to manipulate synapses during *in vivo* FSCV recordings in behaving animals.

CONCLUSIONS

Application of optogenetic and chemogenetic tools in conjunction with FSCV is anticipated to enhance our understanding of the neurobiology of biogenic amine signaling greatly and to provide insight into how biogenic amine (and other) synapses are altered under homeostatic and pathological conditions. Optogenetic and chemogenetic techniques do have drawbacks, as all technology has limitations. Recent studies have emphasized the need for multiple lines of converging evidence prior to data interpretation.[6] Additionally, optically activated channels are dependent on inherent kinetics (though most act within the range of phasic and tonic firing rates for biogenic amine neurons). Moreover, DREADDs have the potential to engage signaling mechanisms that are not observed under endogenous conditions. Nonetheless, we have significant gains to be made regarding circuit precision and mechanism from applying these strategies that will provide the basis for a new generation of research aimed at monitoring molecules in neural systems.

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