Norepinephrine (NE), one of the major catecholamines in the brain, is involved in many physiological and behavioral processes such as stress and reward. Despite its important roles, NE remains largely unexplored compared to the other major central catecholamine, dopamine (DA). This is due in part to the diffuse distribution of NE projections throughout the brain and accessibility of NE neurons, complicating detection of the relatively low physiological NE concentrations. Recent studies have demonstrated that in vivo fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes can detect real time, subsecond changes of NE in the brains of both anesthetized and awake-behaving rats, offering a local view of NE regulation (release and clearance). Furthermore, these studies have revealed different regulatory mechanisms between NE and DA, and that these two catecholamines have functional differences as well. For the last decade, these fundamental studies provided new insights into the understudied roles of NE in reward/aversion processes, drug addiction, and related behavioral responses. However, there are still limitations in the electroanalytical determination of NE in vivo in terms of selectivity. Here, the current status of electrochemical measurements of NE and associated findings are highlighted, and remaining challenges are discussed. © The Author(s) 2018. Published by ECS. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/2.0091812jes]

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Noradrenergic Systems in the Brain

NE neurons in the locus coeruleus (LC) and nucleus of the solitary tract (NST), the primary sources of NE in the brain, project diffusely throughout the entire brain to areas such as the thalamus, hippocampus, hypothalamus, BNST, and cortex, however, dense terminal locations remain highly selective. Similar to other neurotransmitters, NE neurotransmission across synapses and in extracellular fluid is initiated by an electrical signal, the action potential, while presynaptic NE α-β-adrenergic autoreceptors and NE transporters (NET) regulate NE release and clearance, respectively (Fig. 1, right). The majority of released NE is rapidly taken back up into the axon terminal by NET, the most important clearance process, and is restored into storage vesicles or metabolized by catechol-O-methyltransferase (COMT). The released NE also reaches other cells by convective and/or diffusive mass transport, and modulates their activity through the activation of post-synaptic or functional NE receptors. Only a small portion of the NE is removed by diffusion away from the release sites, eventually affecting neighboring neurons. These are the primary processes of fast communication (ms range) between NE and other neurotransmitters through α- and β-adrenergic receptors (AR)(Fig. 1). It is noteworthy that current analytical methodologies including recently developed optical techniques (e.g. fluorescence false neurotransmitters) are still unable to quantify exocytotic release directly at a single synapse or junction in vivo, due to their prohibitively small size (~ hundreds nm) and difficulty of approaching them under in vivo conditions. Instead, in vivo electrochemical analysis enables us to determine the neurochemicals that diffuse away from their release sites at the microelectrode surface and make meaningful measurements of neurochemicals from an intact brain. Specif- ically, voltammetric techniques have been applied to the study of real-time dynamics of neurotransmitter release and its subsequent removal (clearance) from the extracellular space, providing a local view of neurotransmission and demonstrating site-specific variation.

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Currently, voltammetry coupled with microelectrodes has enabled spatially (μm) resolved recordings of rapid (ms) changes of various neurochemicals in a variety of biological preparations, spanning from exocytotic events at single cells to the human brain with nanomolar sensitivity.\textsuperscript{24,28,29}

**Electrochemical Methods Coupled with Micro-Sized Sensors for Characterizing in vivo NE**

**Electrochemical detection of neurochemicals with micro-sized electrodes.**—While the discovery of NE happened in the mid 1940s by Ulf von Euler, a breakthrough occurred in the 1970s when Ralph Adams and his colleagues pioneered the recording of electroactive biogenic amines and related substances in the extracellular fluid of an anesthetized rat brain using voltammetry (e.g. cyclic voltammetry and chronoamperometry) coupled with miniaturized carbon paste electrodes (50 to 200 μm in diameter).\textsuperscript{24,28,30,31} These in vivo electrochemical measurements overcame the drawbacks of in vitro assessments of tissue content for neurochemicals and their metabolites.\textsuperscript{27} Since that time, new and conventional electrochemical techniques (e.g. fast-scan cyclic voltammetry (FSCV), continuous potential amperometry, and various pulsing methods) coupled with bare and surface-modified microelectrodes and biosensors have been developed and/or modified to improve selectivity and sensitivity for the determination of neurochemicals in vivo and ex vivo.

In particular, these smaller electrodes allowed for faster electrochemical measurements to be taken and minimized problems caused by typical macro-sized electrodes at high scan rates. The microelectrode (≤ 10 μm radius) reduces the double layer capacity, which is proportional to the electrode area, and facilitates rapid changes in the electrode potential. In addition, its small size minimizes voltage distortion of voltammograms caused by \textit{iR} drop even in high resistance solutions.\textsuperscript{32,33} Such advantages of microelectrodes can reduce the time scale of voltammetric detection and provide faster kinetic measurements of subsecond changes of neurotransmission. As a result of these technological advances, in 1984 Wightman and Stamford successfully used in vivo FSCV for the study of DA regulation (release and clearance) through DA D2 autoreceptors and transporters (DAT) with pharmacological manipulations.\textsuperscript{37,34} Modern carbon-fiber microelectrodes for in vivo measurements are ∼ 5–10 μm in diameter and less than 150 μm in length, which cause minimal tissue damage and have the spatial resolution necessary to selectively determine extracellular neurotransmission in target brain areas without interference of neurotransmitters diffused from neighboring structures.\textsuperscript{16,24,28,35,36}

This high temporal (≤ 10 ms) and spatial (≤ 100 μm) resolution can reveal unique information about the rapid regulatory mechanisms of local neurotransmission and related substances in real time, as well as correlate these fluctuations with physiological and behavioral changes as well as disease pathology. Especially, FSCV in freely moving rat preparations has significantly contributed to our understanding of the roles of limbic DA in behaviors (e.g. goal directed behaviors), substance abuse (e.g. alcohol and drugs), and reward learning.\textsuperscript{7,42} For the last two decades, the application of ex vivo and in vivo FSCV coupled with carbon-fiber microelectrodes has been widely expanded to the fields of neuroscience and psychology for the monitoring of neurochemical regulation within discrete brain regions or single cells of a wide range of taxa ranging from insects to humans.\textsuperscript{24,28,29}

In **vivo electrochemical analysis of NE with carbon-fiber microelectrodes.**—In the 1980–1990s, François Gonon and his colleagues investigated NE in both the central and peripheral nervous systems using differential pulse voltammetry (DPV) coupled with carbon-fiber microelectrodes.\textsuperscript{34–45} At the same time, the Adams group demonstrated electrochemical measurements of NE in anesthetized rat brains using constant potential amperometry.\textsuperscript{46,47} They also attempted to understand the role of NE in stress by monitoring NE in small brain areas of awake rats in 1992.\textsuperscript{47} However, these voltammetric methods were unable to selectively determine NE in the presence of a variety of electroactive neurochemicals (e.g. catecholamine metabolites, pH changes and ascorbic acid). Despite the selectivity issues, FSCV can overcome many of the limitations from the early days of in vivo electrochemistry.\textsuperscript{12,24,28} The background-subtracted cyclic voltammograms from FSCV provide a “fingerprint” that identifies the recorded analyte(s), which enables NE and DA to be distinguished from their metabolites and other neurochemicals.\textsuperscript{28,48,49} Importantly, in the early 1990s, the Stamford group demonstrated that electrically evoked NE release in rat brain slices can be detected using FSCV.\textsuperscript{17,40} In 2000s, Mark Wightman and colleagues successfully measured sub-second changes of electrically evoked and naturally occurring NE release in small subregions (only a few hundred microns) of the thalamus and BNST of both anesthetized and awake behaving rats using FSCV coupled with carbon-fiber microelectrodes (Fig. 1).\textsuperscript{15,16,20,50} Tissue homogenate studies showed that over 91% of the catecholamine content in these brain areas is NE.\textsuperscript{16,18} In addition, anatomical (histological) and pharmacological evidence further confirmed that the predominant catecholamine was NE.\textsuperscript{16,51} They also investigated NE regulation in its terminals in the BNST using iontophoresis barrels (tip diameter, ∼ 1μm) attached to a carbon-fiber microelectrode. This allows for simultaneous detection of local NE transmission and quantification of iontophotically delivered NE α\textsubscript{2} autoreceptor and NET inhibitors.\textsuperscript{52} This method can accurately autocatalyze pharmacological agents to the NE terminals (> 100 μm away) without affecting neighboring structures, providing a new understanding of local NE regulatory mechanisms via NE terminal’s α\textsubscript{2} autoreceptors and NET.\textsuperscript{52}

Recently, the Wightman group provided new insights into the understudied roles of NE in learning, pain, drug withdrawal, and behavioral responses to environmental stimuli (e.g. food and light/sound).\textsuperscript{12,19,20,36,53} Importantly, these studies revealed that NE and DA in limbic structures are reciprocally modulated to process responses to both positive and negative stimuli. For example, NE increases in response to aversive stimuli such as tail pinch, noise, and a bitter taste, while DA decreases during exposure.\textsuperscript{19,20,54} These results suggest that NE and DA neurons crosstalk in order to maintain homeostasis. Their current findings of NE regulatory mechanisms are highlighted in the next section.

**Different Regulatory Mechanisms of NE and DA Transmission**

Despite the many anatomical and biochemical similarities between NE and DA in the brain, it is generally accepted that they have different biological functions. Wightman and his colleagues revealed striking differences in the dynamics of electrically evoked extracellular NE and DA release and clearance in different limbic structures, including the BNST and striatum.\textsuperscript{12,14,54,55} Figure 2 shows simultaneous concentration changes of NE in the BNST and DA in the striatum evoked
by electrical stimulation of the medial forebrain bundle (MFB) in the same anesthetized rat using multichannel FSCV.14 Because both NE and DA projections originating from their cell bodies travel via the MFB, non-selective electrical stimulation of the MFB activates these catecholamine neurons together.14,16,18 Such preparations take advantage of this for direct comparison of NE and DA regulatory mechanisms in the same animal under identical conditions (e.g. levels of anesthesia and drug doses).15,19 Both NE and DA concentrations measured at their oxidation potentials increase rapidly during electrical stimulation and afterwards decrease back to pre-stimulation basal levels. This time-dependent concentration increase during stimulation is due to the imbalance between release and uptake of catecholamine levels. This subsecond (phasic) changes of NE release (i.e. transients) in NE rich regions of the brain occur with a lower frequency (< 1 Hz).3,63 In contrast, naturally occurring NE concentration are hardly observed even after selective inhibition of NET and α2 autoreceptors.14,16,18 These distinct regulatory mechanisms of NE and DA may impact their different biological functions in the brain. Thus, future investigation into NE neurotransmission in different brain regions will facilitate our understanding of the distinct roles and functions of local NE circuits in animal behaviors and how dysregulation of these circuits contributes to many psychiatric disorders and substance abuse.

Limitations of Current Electroanalysis Techniques for the Study of Local NE and DA

Despite the advantages of in vivo voltammetric techniques, a major limitation in any electroanalysis method is the inability to distinguish NE selectively in the presence of DA due to their nearly identical redox potentials.16,66 NE differs from DA by having one more hydroxyl group in its structure and exhibits a slightly more positive oxidation potentials.15,18,65,67,69 Limitations of Current Electroanalysis Techniques for the Study of Local NE and DA

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Figure 2. Different regulation of electrically evoked NE and DA in the brain of the same anesthetized rat. The traces of BNST-NE and Striatum-DA concentration evoked by electrical stimulation measured at their oxidation potentials (0.7 V for NE and 0.65 V for DA). Electrical stimulation is indicated by the solid red bars under the traces.

Figure 3. Naturally occurring phasic DA release in the Striatum of an awake-behaving rat. Color plot (upper) for the voltammetric data, with current changes encoded in false color for DA in the striatum. The set composed of all background-subtracted cyclic voltammograms recorded for 15s. The white dashed line in the color plot indicates the potential of DA oxidation (~0.65 V). The trace (lower) shows Striatum-DA concentration changes. Insets: background-subtracted cyclic voltammogram is recorded at the time indicated by the asterisk (*). Arrows indicate phasic DA release.
However, the lower sensitivity may be enhanced by modification of traditional NE waveforms and scan rates coupled with analog background subtraction, as previous studies have demonstrated increased sensitivity of neurochemicals such as DA at carbon-fiber microelectrodes. For example, a “sawhorse” waveform with increasing scan rate decreased the limit of detection of electrically evoked DA with analog background subtraction to near 1.0 nM and improved the selectivity of in vivo adenosine detection in the presence of other neurochemicals (interferences) such as H$_2$O$_2$ and adenosine triphosphate (ATP).

While there have been numerous attempts to significantly separate DA and NE redox potentials using different parameters (e.g. waveforms and scan rates) through voltammetric techniques (including FSCV), a variety of surface-modified electrodes by electrochemical and chemical pretreatment, and statistics (e.g. chemometrics), they have been largely unsuccessful in vivo. To date, this limitation has restricted the use of electrochemical methods to brain areas that only contain significant concentrations of one catecholamine, NE or DA, such as the BNST and striatum, respectively. In addition, since voltammetric methods alone cannot distinguish between the two, pharmacological evidence by systemic administration of well-characterized pharmacological agents is limited to brain areas where only either NE or DA neurons are present. The verification of NE and/or DA by the use of pharmacological agents is limited to brain areas where only either NE or DA receptors/transporters are present. If targeted brain areas do not have dense catecholamine autoreceptors and transporters, or if both catecholamine transporters coexist (NET modulate DA clearance due to NET’s higher affinity for DA than for NE), it is almost impossible to determine whether the recorded signal is NE and/or DA. As such, little is currently known about (1) the regulatory mechanisms of local NE and DA in many brain regions where appreciable amounts of both catecholamines are present, including the medial prefrontal cortex and ventral pallidum, or (2) how individual catecholamine circuits impact cognition, memory and emotional processing.

Integration of FSCV with Optogenetic and Chemogenetic Techniques

In recent years, the selective targeting and controlling (excitation or inhibition) of specific neural circuits has been made possible with the development of new experimental genetic approaches, optogenetics and chemogenetics, without affecting other surrounding cells and fibers of passage. These techniques make use of combinatorial viral approaches and recombinase-driver lines. Briefly, optogenetics involves transduction of specific neuronal populations with genes encoding light-sensitive proteins called opsins (e.g. channelrhodopsin and halorhodopsin), which include membrane-associated ion channels and engineered G protein-coupled receptors (GPCRs) that can be modulated by light. Opsin-expressing neurons can be stimulated or inhibited by specific wavelengths of light from a laser or light-emitting diode (LED).

In contrast, chemogenetics allows for the excitation or inhibition of neuronal firing through activation of synthetic receptors such as GPCRs. These receptors are activated by synthetic chemical ligands (termed “designer drugs”) such as clozapine-N-oxide (CNO), which are generally biologically inert. These two genetic techniques offer their own distinct advantages. Optogenetics enables the manipulation of specific neural activity acutely and reversibly with millisecond temporal precision by delivering photostimulation directly to target cell bodies or terminals, allowing us to reveal causal relationships between manipulated neural activity, functional outcomes, and specific behaviors.

Compared to optogenetics, chemogenetics can control neural circuits in vivo with scalability and ease of application. This technique is ideal for longer periods of manipulation of neuronal activity ranging from minutes to days depending on the route of ligand delivery (via animal’s water or systemic injections), ligand concentration, and the pharmacokinetic properties of the synthetic ligand(s) used. Together, these genetic tools overcome many of the challenges of using non-selective conventional approaches (e.g. electrical stimulation, chemical lesion, and pharmacological agents) which often recruit neighboring neurons and unrelated terminal fields, to isolate and manipulate local NE and DA transmission. Pairing in vivo FSCV with targeted genetic tools will allow for the selective determination of local NE or DA transmission in brain areas receiving both NE and DA innervation by individually manipulating NE or DA neurons. Indeed, within the last decade, incorporating FSCV with optogenetics has been primarily adapted to facilitate the understanding of the functional roles of DA circuits in the striatum, as well as the behavioral outcomes associated with manipulations of these circuits.

Figure 5 shows that selective optical stimulation (40 Hz, 60 pulses) of channelrhodopsin-2 (ChR2) expressing midbrain DA neurons, evokes DA release in the striatum (Fig. 5). Blue light (473 nm) activates the ChR2, a blue light-sensitive ion channel, resulting in excitation of the DA neurons and subsequent DA release. Both the color plot and the individual background-subtracted voltammogram establish the signals are due to optically evoked DA.

Optically evoked [DA]$_{max}$ in the striatum measured at its oxidation potential (+0.68 V, dotted line) was much lower than non-selective electrically evoked [DA]$_{max}$ at optimal conditions (Fig. 2). This relatively lower [DA]$_{max}$ was observed because, unlike electrical stimulation, optically evoked DA release depends on other factors including the expression pattern of the opsin used to excite the neuron, and the efficacy of light stimulation. Non-selective electrical stimulation also activates other non-DA neurons including glutamatergic neurons, which may enhance terminal DA release. In addition, optogenetics affords better spatial resolution since the single light probe (optrode) is smaller and causes less tissue damage than bipolar electrical stimulating electrodes, which are widely used to non-selectively activate the catecholamine neurons. Such bipolar electrodes are composed of two stainless-steel electrodes, each of which have 0.2 mm outer diameter and are spaced ~1.0 mm apart.

In contrast to optogenetics, attempts to integrate FSCV with chemogenetics to study DA regulation have not been well documented. Our recent studies demonstrate how chemogenetic inhibition
Effects of chemogenetic inhibition and excitation of midbrain DA neurons on striatum-DA transmission. The electrically evoked DA concentration change is apparent in the color plot at the potential for its oxidation (+0.68 V, dotted line) and reduction (–0.21 V, solid line). The trace (dotted line) is shown at the potential at which DA is oxidized. Insets: background-subtracted cyclic voltammogram recorded at the evoked release. Light stimulation is indicated by the solid blue bar under the trace.

Figure 5. Optically evoked DA in the striatum of an anesthetized rat. The color plot composed of all background-subtracted cyclic voltammograms recorded for 15s before and after light stimulation of midbrain DA neurons (60 Hz, 40 pulses delivered at 0 s). DA concentration change is apparent in the color plot at the potential for its oxidation (+0.68 V, dotted line) and reduction (–0.21 V, solid line). The trace (dotted line) is shown at the potential at which DA is oxidized. Insets: background-subtracted cyclic voltammogram recorded at the evoked release. Light stimulation is indicated by the solid blue bar under the trace.

and activation of midbrain DA neurons modulates DA transmission in the striatum of anesthetized rats (Fig. 6). Chemogenetic excitation of midbrain DA neurons with ligand CNO (1.0 mg/kg, i.p.) enhanced DA release in the striatum evoked by electrical stimulation of the midbrain, while inhibition of the DA neurons decreased the electrically evoked DA release. To the best of our knowledge, integration of these combined FSCV and genetic approaches to NE neurons in vivo has not been reported yet. Importantly, the application to NE neurons is more challenging than with DA neurons, due to the difficulty in targeting the two major NE cell bodies in the LC and NST, which exhibit diffuse projections throughout the brain and are relatively smaller than the DA cell bodies. It is worth noting that regulation of optogenetically and chemogenetically evoked DA and NE depends on many factors, including light stimulation parameters (e.g. frequency and pulse duration) similar to non-selective electrical stimulation, and ligand (e.g. CNO) concentration, respectively. Further, transduction of the NE and DA cell body regions can vary greatly depending on the volume, titer, and serotype of the virus infused, all of which are not well established and vary from lab to lab. For example, we most often use the AAV2/10 serotype to deliver transgenes in the brain,10,90 because it produces a high transduction efficiency and is easily titrated compared to many other serotypes. Yet many laboratories use larger volumes of AAV2/1 or AAV2/5,91,92 which have also shown to be less efficient in transduction compared to AAV2/10.93,94

Accordingly, as a future direction, the guidelines and optimized conditions (e.g. stimulation conditions and viral transduction parameters) for these new genetic approaches in combination with electrochemical techniques need to be established in order to achieve reproducible, physiological NE transmission. In summary, determining NE regulation with electrochemical analysis or genetic techniques alone cannot elucidate the roles of the understudied local NE circuits in brain functions and behaviors. A novel combination of FSCV coupled with these genetic techniques will permit us to selectively determine (1) rapid local NE transmission and its distinct roles in behaviors even in the presence of both NE and DA inputs, (2) understudied local NE regulatory mechanisms, and (3) the distinct functional roles of these local NE circuits in complex physiological and behavioral phenomena.

Conclusions

Here, we have provided an overview of past and current electrochemical analysis of NE neurotransmission, NE’s distinct regulatory mechanisms and functional roles in the brain, and discussed the limitation of selectivity for the electrochemical determination of NE in the presence of DA. In the future, incorporating in vivo FSCV with targeted optogenetic and chemogenetic tools will enable us to monitor NE transmission selectively in brain areas of interest by individually manipulating NE neurons. This new approach will detail the distinct regulatory mechanisms of NE transmission in many discrete brain areas where NE and DA coexist, through both DA and NE receptors and transporters. These findings will provide the framework to (1) understand how individual local NE circuits are linked to various brain functions controlling physiological conditions as well as essential behavioral outputs, and (2) provide new insights into how the dysfunction of local NE systems contributes to the pathogenesis of common psychiatric and neurodegenerative diseases (e.g. depression, drug addiction, and Parkinson’s disease).

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References

1. G. Aston-Jones and J. D. Cohen, *Annu Rev Neurosci*, 28, 403 (2005).
2. S. J. Sara, *Nature Rev Neurosci*, 10, 211 (2009).
3. C. W. Berrie and B. D. Waterhouse, *Brain Res Rev*, 42, 33 (2003).
4. R. A. Wise, *Neuron*, 36, 220 (2002).
