



Interpreting Chemical Neurotransmission in Vivo: Techniques, Time Scales, and Theories

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ABSTRACT: Monitoring neurotransmitter levels is a major research strategy for determining the functions of neuronal systems, specifically the ascending neuromodulator systems. In this Viewpoint, we consider the impact of different methods for recording extracellular neurotransmitter levels in vivo on theories concerning the signaling mode(s) and functions of these neuronal systems. As exemplified by evidence from experiments using different methods to measure acetylcholine (ACh) signaling, both neuromodulatory and deterministic functions have been attributed to cholinergic activity. Technical and experimental advances now allow determination of the validity of such dual-signaling theories.

KEYWORDS: *Acetylcholine, dopamine, serotonin, noradrenaline, microdialysis, electrochemistry, neuromodulation*

Decoding neuronal information processing involves establishing relationships between behavioral-cognitive processes and presynaptic neurotransmitter release as well as postsynaptic activity patterns. Moreover, coding at presynaptic neuronal soma may reveal dissociations with synaptic neurotransmitter release patterns and thereby begin to reveal the impact of the integration of terminals into target circuitry. However, establishing such a triad of relationships has remained an elusive goal, in part because the brain's code for complex behavioral and cognitive operations has remained largely unknown, perhaps with the exception of primary perceptual mechanisms, and in part because monitoring synaptic neurotransmitter release in behaving animals continues to pose formidable challenges.

During the last quarter century, methods to study the functions of presynaptic neurotransmitter release have evolved from ex vivo measures of neurotransmitter content in tissue to in vivo neurochemical monitoring techniques. Increasingly smaller recording devices and the development of more sensitive analytical methods have continued to increase the spatial and temporal resolution of in vivo recordings of extracellular neurotransmitter levels.¹ Importantly, the differing temporal and spatial resolutions of individual methods are not merely technical constraints that invariably compel cautionary interpretation of changes in the extracellular concentrations of neurotransmitters, but these resolutions impose on the very conceptualization of the functions of neurochemically defined groups of neurons. Below, we will briefly illustrate this quandary with respect to research on forebrain cholinergic systems and then discuss how our preferred methods inevitably confirm or reject theories describing neuromodulatory (modulating the likelihood or strength of a behavioral or cognitive process and varying on the scale of tens of seconds to minutes) versus deterministic functions (evoking a behavioral or cognitive process and varying on the scale of hundreds of milliseconds) of neurotransmitter systems.

METHODS DETERMINE FUNCTIONAL CONCEPTS: CHOLINERGIC TRANSMISSION AS AN EXAMPLE

The cortical cholinergic input system is the densest among the neuromodulator systems projecting to cortex. Although there

are some regionally and layer specific characteristics, conventional anatomical descriptions uniformly have supported the notion of a diffusely organized neuromodulatory forebrain cholinergic system. Such a notion implies similarly unrefined functions modulated by such a neurotransmitter system, such as “readiness for cognition”, “gating of cortical information”, or even less defined functions such as “arousal”. Furthermore, ultrastructural evidence indicates a paucity of classical synapses, thereby confirming the view that volume neurotransmission is a corollary of such a neuromodulatory system. It needs to be noted, however, that evidence to the contrary has also been described and that the presence of fast ionotropic nicotinic ACh receptors suggests that the discussion about the presence or absence of volume transmission may be more productively reframed in terms of multiple and diverse modes of neurotransmission.²

Early studies using cortical cups and later microdialysis generated evidence in support of a cholinergic mediation of wakefulness, REM sleep, and “arousal”. In the 1990s, experiments using specific cholinotoxins and attention-taxing tasks began to demonstrate that specific attentional processes depend on cortical cholinergic activity. Results from microdialysis studies measuring extracellular ACh levels in the cortex of rats performing attention tasks indicated elevated extracellular ACh levels when compared to rats performing various behavioral control conditions. Owing to the need for collecting dialysate samples over minutes to obtain measurable levels of ACh using traditional HPLC-electrochemical detection methods, the interpretation of such elevated ACh levels, in terms of indexing high demands on attention, were supportive of the concept of ACh as neuromodulator.

Real-time voltammetric or amperometric measures of synaptic cholinergic release events generated evidence for the first time indicating non-neuromodulatory actions of ACh in the cortex. Cue-evoked cholinergic release events, termed

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Table 1. Components of Cholinergic Neurotransmission and Recording Methods

	cholinergic transients	cholinergic neuromodulation
time scale	0.2–0.5 s (rise time) to 4–6 s (peak levels)	tens of seconds to minutes, not a necessary confound of analytical limits ⁵
detected by choline-sensitive microelectrodes?	YES, because method is optimized for detecting choline spikes	probably NO, because slowly changing choline concentrations may be relatively small compared with stable extracellular choline concentrations (3–5 μ M) and/or the amperometric technique is not optimal for reliably detecting slowly changing currents
detected by microdialysis?	NO, because either below limit of detection and/or fully hydrolyzed	YES, because nonhydrolyzed “ambient” level and/or confounded by probe-evoked glia-derived diffusion barrier ⁶
functions	reducing cue detection uncertainty	higher levels improve attentional task compliance in situations taxing attentional control (reducing opportunity costs) ³

“transients”, were found to be associated with the detection of cues in certain trials. Furthermore, optogenetic generation of such transients “forced” the detection of cues, including false alarms in noncued trials. The collective evidence from these experiments suggested that, in the absence of cholinergic transients, subjects remain “stuck” in reporting the absence of a cue even though a cue had been presented. Thus, cholinergic transients exhibit deterministic functions.³

Additional evidence showed that cholinergic transients are unlikely to escape hydrolysis by acetylcholinesterase and thus it is unlikely that this type of cholinergic signal reaches extra-synaptic receptors. In other words, cholinergic transients do not appear to be volume-transmitted. Furthermore, these transients appear to be generated based on the integration of cholinergic terminals into cortical circuitry. In contrast, the generation of cholinergic transients may not depend on burst firing of cholinergic neurons situated in the basal forebrain.

■ NEUROMODULATION AND VOLUME TRANSMISSION, OR DETERMINISTIC SYNAPTIC SIGNALING, OR BOTH?

Similar to cholinergic neurotransmission, evidence from microdialysis and voltammetric recordings support both neuromodulatory and deterministic functions of dopamine, respectively. Because of the absence of feasible or practical electrochemical measurement schemes, similar conceptualizations about tonic versus phasic, or neuromodulatory versus deterministic functions have not yet clearly evolved for other neuromodulator systems. However, it seems fair to speculate that noradrenergic or serotonergic transients likewise exist, and that integrating the terminals from these seemingly diffusely organized neuronal systems into forebrain target circuitry underlies the generation of such transients. Expression of heteroreceptors by noradrenergic and serotonergic terminals is a key element of such integration.

Thus, the evolving forebrain may have “usurped” diffuse afferent projection systems to involve their inputs in the mediation of specific behavioral-cognitive processes. As a result, ACh, dopamine, noradrenaline, and serotonin signaling can be assumed to be highly localized, with transients associated with a specific behavioral or cognitive operations occurring specifically in certain regions, layers, and circuits. Furthermore, the essential component of transients, that is, the initial rise of the signal, can be predicted to occur on the scale of hundreds of milliseconds. Such transients likely are not volume-transmitted.

By contrast, measures of neurotransmitter release that have supported neuromodulatory functions raise important questions. As illustrated in Table 1 for ACh, the use of different techniques supporting different modes of transmission, and the confounding impact of the lower temporal resolution of microdialysis for conclusions in terms of neuromodulation, have

rendered a confusing and even conflicting literature. Views on the nature of synaptic signaling of a particular neurotransmitter appear to depend primarily on the nature of the technique used to measure extracellular concentrations.

The concern that minute-by-minute-based microdialysis data per se dictates an interpretation in terms of neuromodulation has often led to the reflexive dismissal of this technique. However, the time scale at which neuromodulation occurs is not clear. For some neurotransmitters, such as glutamate, amperometric recordings likewise suggested background variations on the scale of minutes (such minute resolution appears not to be feasible using amperometric measures of ACh; see Table 1). Extracellular levels of neuromodulators, measured by microdialysis, vary systematically with behavior, and thus, they index a neurobiologically significant phenomenon. When compared with peak amplitudes of transients measured by electrochemical methods, the typically much higher concentrations measured using microdialysis may serve to stimulate low-affinity and extra-synaptic receptors not reached by transient signals. Thus, microdialysis data support descriptions of entirely different models of neuronal communication than evidence obtained from voltammetric or amperometric recordings.

As analytical techniques continue to evolve and enable the analysis of microdialysis samples collected over just 1 min,⁴ experiments can be designed to test hypotheses concerning neuromodulatory functions and their temporal resolution. For example, hypothesizing that higher levels of cholinergic neuromodulation influence the propensity for staying-on-task despite accumulating opportunity costs,³ experiments can be designed to vary such costs over defined periods of time and to show whether neuromodulatory levels vary over such periods. Our finding that rats that exhibit low attentional control and low cholinergic neuromodulation spontaneously shift between good and poor levels of attentional performance on the scale of about 3 min⁵ supports the possibility that cholinergic neuromodulation takes place on the scale of tens of seconds to several minutes. In principle, the time scales of variations in neuromodulatory action are expected to vary as a function of task and context, rather than represent an invariant characteristic of a neurotransmitter.

While microdialysis remains an essential method for measuring at least one mode of neurotransmission of numerous neurotransmitters, we cannot ignore the possibility that the neuropathological and immunological effects of insertion of a microdialysis probe impact the nature of this measure.⁶ Clearly, fundamental questions persist about the space that is monitored by this method and how penetration injury alters the extracellular compartmentalization of analytes. We also cannot fully exclude the possibility that integrated phasic or transient release events eventually explain data obtained from microdialysis, at least for some neurotransmitters, thereby potentially

rejecting the presence of a neuromodulatory signaling mode. However, with respect to ACh, it appears unlikely that transients can be integrated to explain levels determined in microdialysis experiments, in part because we observe higher neuromodulatory levels in situations where there are fewer transients. Similarly, the discussion about the presence and interactions between multiple modes of dopaminergic transmission appears to continue.

■ WHERE TO GO FROM HERE?

As in any line of scientific inquiry, methods determine theories, and vice versa. For many neurotransmitters, we now have multiple methods for in vivo monitoring, and long-standing temporal resolution limits are being broken. Moreover, techniques to manipulate behavioral, cognitive, and neuronal processes have become vastly more sophisticated in recent years, allowing more conclusive tests of the presence and functions of diverse modes of neurotransmission. Using different recording methods with increasingly converging temporal resolution, and recording neurotransmitter levels in animals engaged in defined and hypothesis-driven behavioral and cognitive operations, evidence will be generated that arguably will get us closer to the true time scale(s) of presynaptic coding and thus to the true workings of the brain.

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