Forebrain cholinergic signaling: Wired and phasic, not tonic, and causing behavior.

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Abstract

Previous evidence in support of a slowly acting (scale of 100s of seconds) and volume-transmitted component of cholinergic signaling was based largely on studies using measures of brain acetylcholine (ACh) levels which required several minutes to generate a single data point and typically employed AChEsterase inhibitors (AChEIs) to foster ACh measurement. Moreover, collecting such data points in correlation with relatively stable behavioral states has further supported the view that extracellular ACh levels vary at a relatively slow rate. Here we argue that forebrain cholinergic signaling is exclusively phasic (milliseconds to perhaps seconds), unlikely to be volume-transmitted, and that previous neurochemical evidence and associated behavioral correlates may be re-interpreted in terms of integrated phasic cholinergic activity and specific behavioral and cognitive operations. The highly potent catalytic enzyme for ACh, AChE, prevents the presence of an “ambient” extracellular ACh level and thus renders it unlikely that ACh influences target regions via relatively slow changes in extracellular ACh concentrations. Real-time amperometric recordings of cholinergic signaling have suggested a specific function of rapid, phasic or transient cholinergic signaling in attentional contexts. Optogenetic studies support a causal relationship between these transients on behavior. Combined electrochemical and neurophysiological recordings revealed that the powerful behavioral control by cholinergic transients involves the generation of high-frequency oscillations. Such oscillations are thought to recruit efferent circuitry to (re)activate dormant task sets. Evidence showing the impact of genetic variations of the capacity of cholinergic synapses likewise can be interpreted in terms of their impact on the ability to sustain generation of repeated phasic cholinergic signals, as opposed to effects on ambient ACh levels. Further, while notions of slowly-changing, sleep stage-associated variations in extracellular ACh levels and their functions are widely accepted, the evidence is in fact fairly limited. An alternative hypothesis offers a role for high-frequency cholinergic transient signaling during REM sleep. By employing a theoretical framework that focuses on the phasic and causative characteristics and functions of cholinergic signaling, results from human cognitive neuroscience studies of cholinergic function may be substantially clarified and simplified. Compared to the current treatment of cholinergic deficits using AChEIs, the conceptualization of forebrain cholinergic signaling as wired, phasic, and causative predicts that drugs that either rescue transient presynaptic signaling, or amplify or rescue the postsynaptic impact of phasic signals, will be more efficacious in treating age- and dementia-related cognitive and cognitive-motor disorders.

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Introduction: ACh as a phasic modulator

Traditional descriptions of the anatomical organization of the basal forebrain cholinergic projections to telencephalic regions emphasize the hallmarks of a neuromodulatory system. These hallmarks include the presence of a relatively small number of soma in the basal forebrain giving rise to a relatively large innervation space, a limited topographical organization of cholinergic projections, a substantial degree of axonal collateralization, and the presence of extra-synaptic, or non-classical, receptors and, by implication, volume-transmission. Consequently, theories of cholinergic function have centered around the functions of slowly changing extracellular ACh levels (Yu and Dayan, 2002) and the role of volume transmission in, for example, primary visual cortex function (e.g., Lean et al., 2019). The main goal of this article is to critically probe these traditional descriptions, including our own prior interpretations (e.g., St Peters et al., 2011), that ACh acts by slowly (over 100 of seconds or even minutes) affecting the excitability of widespread target regions, and thereby primarily modulating relatively global functions such as “arousal”. As an alternative, we discuss the evidence in support of the view that ACh mediates neuromodulatory effects based on highly phasic and probably largely synaptic signaling. This re-conceptualization of ACh signaling as phasic, synaptic, and behaviorally causal fosters the integration of diverse levels of analysis of cholinergic functions in rodents, non-human primates and humans, the development of computational models, and more effective approaches to the psychopharmacological development of pro-cholinergic treatments.

Although the traditional anatomical and functional characteristics of forebrain cholinergic systems have been challenged in recent years (references below), the functional classification of ACh as a neuromodulator has persisted. Neuromodulation, however, has resisted a conclusive neuroscientific, categorical definition. Neuromodulators cannot be conclusively distinguished from "classical neurotransmitters" on the basis of the presence of volume transmission (e.g., Okubo et al., 2010) or of a particular class of post-synaptic receptors (ionotropic as well as metabotropic receptors for ACh). A focus on the description of effects on neuronal states (e.g., Picciotto et al., 2012; Avery and Krichmar, 2017) likewise does not conclusively identify neuromodulators and dissociates them from “classical neurotransmitters” (see also Table 1 in Dayan, 2012). Instead, as noted by Dayan (2012), neuromodulators can act via anatomically differentiated pathways and on fast timescales to support selective computations. Below we describe the evidence challenging the idea of ACh neuromodulation as nonspecific, slow, and spatially diffuse, and new evidence consistent with this more modern, computationally-based view of neuromodulator function.

The catalytic power of acetylcholinesterase (AChE) supports spatially and temporally constrained cholinergic signaling

The catalytic power of the AChE has been called “amazing” (Quinn, 1987). Indeed, the rate of ACh hydrolysis is limited by the rate of ACh diffusion to the active site, rather than by how quickly AChE can break it down. Thus, proposals suggesting extra-synaptic, or volume, transmission, or of nano- to micromolar concentrations of “ambient extracellular ACh levels”, capable of reaching targets across tens of micrometers of extracellular space (Descarries, 1998), require postulating mechanisms that limit synaptic hydrolysis of ACh. The AChE is abundantly present in the dendrites, perikarya, axons, and synaptic clefts (Blotnick-Rubin and Anglister, 2018), with a minor exception of the relatively small number of bipolar cholinergic interneurons in the cortex which exhibit low levels of AChE (Levey et al., 1984). Although translational and post-translational AChE modifications have been demonstrated and associated with neuro- and psychopathologies (Meshorer and Soreq, 2006), we are not aware of evidence that such modifications limit ACh hydrolysis to the degree that they could support the presence of ambient extracellular ACh levels.

We explored the possibility that a portion of newly released ACh in cortex escapes hydrolysis by endogenous AChE, by measuring new extracellular choline production (a main product of ACh hydrolysis) with choline-sensitive electrodes. In addition, we (co-)immobilized AChE on these electrodes to potentially hydrolize ACh not hydrolyzed by endogenous AChE (Giuliano et al., 2008). *In vitro*, these electrodes were potentially able to detect “spared” extra-synaptic ACh at low femtomolar concentrations. In the cortex *in vivo,* we produced relatively large, non-physiological, potassium-driven waves of ACh release in order to optimize the possibility that a portion of such ACh escapes the endogenous AChE. However, even in such conditions, choline currents did not indicate that a portion of ACh “escaped” the endogenous AChE.

Related to the presence or absence of ambient extracellular ACh levels, the presence or absence of classical cholinergic synapses (in cortex) has remained in dispute (Umbriaco et al., 1994; Smiley et al., 1997; Descarries and Mechawar, 2000; Turrini et al., 2001). However, if ACh indeed is nearly completely hydrolyzed by endogenous AChE, a significant degree of volume transmission would appear unlikely. Moreover, different firing patterns of basal forebrain cholinergic neurons may support different spatial ACh release dynamics (e.g., Manns et al., 2000; Lee et al., 2005; Unal et al., 2012); however, for ACh to exert relatively distant effects, akin to effects of monoamines over several millimeters (Schneider et al., 1994; Puopolo et al., 2005), it would seem necessary to postulate additional regulatory constraints of the efficacy of AChE.

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It may be of limited usefulness to raise such a binary question ("volume or not") (see also Sarter et al., 2009), and conclusive experiments that could reject the presence of volume transmission do not appear straight forward. However, the cholinergic synapse seems exquisitely equipped to limit the spatial range of cholinergic signaling (see also Dunant and Gisiger, 2017). The recent demonstration that electrical stimulation yielded a very limited spread of activated (fluorescent) G-protein-coupled ACh receptors (Jing et al., 2018) is consistent with this view.

Cholinergic neurotransmission is also often viewed as temporally diffuse, exhibiting variations in release levels across minutes. The attribution of relatively long-lasting arousal states to different extracellular ACh levels has supported this view (e.g., Kametani and Kawamura, 1990; Marrosu et al., 1995). However, to a substantial degree, this view may be confounded by the limited temporal resolution of previously predominant methods for monitoring changes in extracellular ACh levels. Using microdialysis to collect ACh from the extracellular space typically yields samples containing pM to low nM concentrations which are close to the detection limit of traditional analytical methods. Thus, it has been necessary to collect samples over several minutes. Moreover, such collections typically occurred while an AChEI was reverse-dialyzed to artificially increase levels of recoverable ACh[1]. As a result, ACh levels were long considered to vary at the scale of minutes by methodological default.

However, the catalytic power of the AChE limits, at a superb speed and efficacy, the duration of ACh action. The minutes-scale of view of cholinergic neurotransmission is also challenged by evidence from experiments using newer methods that allow real-time monitoring of ACh release. Using amperometric measures of evoked choline currents, demonstrated to reflect newly released and hydrolyzed ACh (Parikh and Sarter, 2006), we observed phasic, or "transient" cholinergic activity in the prefrontal cortex of rats performing a signal detection task. Such transients reliably predicted "switch hits" – correct signal detections following either a long temporal delay or a perceived nonsignal trial (i.e., after a correct rejection or miss (Parikh et al., 2007; Howe et al., 2013; Howe et al., 2017). These transients did not occur for other trial types, including correct rejections, misses, or hits following other hits (there were too few false alarms to analyze). We further demonstrated that the behavioral power of cholinergic transients is due to the generation

[1]If it is correct that the AChE effectively limits, or even prevents, the presence of extracellular ACh concentrations, it would need to be postulated that the successful recovery of ACh by microdialysis, in the absence of an AChE-inhibitor in the perfusion medium (Himmelheber et al., 1998; Herzog et al., 2003; Chang et al., 2006), results from the protection of ACh from the AChE by the glia barrier formed in response to the probe penetration injury (Jaquins-Gerstl and Michael, 2009; for more discussion of such technical issues see Sarter and Kim, 2015).

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of high frequency oscillations in cortex, requiring muscarinic M1 acetylcholine receptor (mAChR) stimulation (Howe et al., 2017).

Critically, optogenetic studies (Gritton et al., 2016) demonstrated that cholinergic transients cause behavior: Optogenetic inhibition of such transients during signal trials reduced hits, but did not affect correct rejections, similar to the effects of cholinergic lesions (McGaughy et al., 1996). Moreover, optogenetic generation of cholinergic transients during cued trials, which therefore coincided with, or substituted the occasional absence of, endogenously-generated transients, increased detection rates (or hits). However, the most conclusive evidence for the causal power of cholinergic transients comes from the effects of optogenetically-generated cholinergic transients during non-cue (or blank) trials - in which normally no such transients are observed. Evoked transients in such trials drastically increased the rate of false alarms (incorrect reports of a signal) from around 20% to nearly 50% (Gritton et al., 2016).

Further experimentation will be needed to disambiguate the precise computation driven by cholinergic transients. The task circumstances in which they have been demonstrated thus far suggest two possibilities: One is a cholinergic reduction of “expected uncertainty” (Yu and Dayan, 2005) about the presence of a signal, to the point that invalid cholinergic transients can instill relative certainty about the presence of a signal even in blank (nonsignal) trials. The other is the (re)activation of the dormant task set associated with the signal (due to the long monitoring periods and that the signal occurs on 50% of trials, the participant spends most of the time in the ‘nonsignal’ state). This latter possibility is supported both by the isolation of the transient to signals occurring either after long temporal delays or (perceived) nonsignal trials, as well as fMRI data from humans performing a parallel task (Howe et al., 2013): The ‘shift-hits’ associated with cholinergic transients primarily activated a prefrontal region associated with switching from external (monitoring) to internal (task-set retrieval) processing (Burgess et al., 2005; Chun and Johnson, 2011). Regardless, either interpretation replaces the traditional view describing ACh in terms of functionality of relatively undefined variations “states” related to presumed extracellular “levels” of ACh with more specific operations determined by the presence or absence of discrete cholinergic transients.

Anatomical foundations of locally-specific cholinergic signaling

Contemporary neuroanatomical research has revealed a heretofore unexpected degree of anatomical and functional parcellation of basal forebrain cholinergic neurons and a highly topographical organization of the basal forebrain cholinergic projection system, including complex relationships between basal forebrain afferent with efferent projection patterns (Zaborszky et al.,

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2008; Zaborszky et al., 2015b; Zaborszky et al., 2015a; Gielow and Zaborszky, 2017; Huppé-Gourgues et al., 2018; Lean et al., 2019). Combined with a limited degree of axonal collateralization (Price and Stern, 1983), this evidence suggests a neuronal projection system that can support regionally discrete cholinergic stimulation (see also Chavez and Zaborszky, 2017). The presence of neuronal subpopulations and topographic projections also supports proposals about cholinergic modules which can selectively modulate information processing in individual cortical areas and layers (see also Tingley et al., 2015).

The relatively high density of cholinergic contacts, relative to axonal lengths and neuron number (Mechawar et al., 2000), would seem to significantly limit the spatial selectivity of cholinergic function. However, further differentiation of cholinergic actions may be derived from the presence of target area-specific organization of microcircuits, involving diverse and regionally-specific populations of interneurons (e.g., Xiang et al., 1998; Eggermann and Feldmeyer, 2009; Chen et al., 2015). Moreover, evidence indicating neuronal activity-dependent cholinergic modulation of dendritic computation (e.g., Williams and Fletcher, 2018), and region-specific wiring of cholinergic terminals, in part via heteroreceptors expressed at cholinergic terminals (Lambe et al., 2003; Parikh et al., 2008; Parikh et al., 2010; Poorthuis et al., 2013), offer additional mechanisms for differentiated, locally-specific cholinergic signaling. Together, the previous view that ACh acts uniformly across large regions to, for example, “enhance cortical arousal”, seems increasingly obsolete.

Cholinergic “tone”: an intuitive, method-derived but unneeded concept?

As already mentioned, the traditionally dominating view that ACh acts relatively slowly to influence widespread target regions has been based in part on evidence obtained by using microdialysis to monitor extracellular ACh levels. Because of the limited sensitivity of conventional analytical methods, dialysates needed to be collected over several minutes in order to yield detectable concentrations of ACh in the sample (see also Footnote 1). Thus, by definition, data obtained from this method have suggested the functionality of slowly-changing levels of cholinergic tone (e.g., Savage, 2012; Coppola et al., 2016; Lecrux et al., 2017). Correlations between slowly-changing ACh levels with slowly-changing brain (arousal) states (e.g., Anaclet et al., 2015; Xu et al., 2015; Zant et al., 2016; Teles-Grilo Ruivo et al., 2017; Yang et al., 2017) have further supported the view that variations in “tonic” ACh levels are functional.

We repeatedly reported elevated ACh levels, based on the analysis of dialysate samples collected over 5-10 min and compared to pre-task baselines, in rats performing the very same sustained attention task which more recently yielded the demonstration of cue detection-associated

cholinergic transients (above). Moreover, such ACh levels were particularly high in the presence of a distractor that suppressed performance, and higher levels of ACh levels were correlated with better post-distractor performance recovery (St Peters et al., 2011). We interpreted these elevations as indicative of relatively better top-down control of attention, including task- and response rule-maintenance and performance compliance which support relatively better performance during and after the distractor period. In contrast, the unstable performance of rats with a neuronally-limited capacity for elevating cholinergic signaling was attributed to relatively weaker capacities for top-down control (Sarter and Paolone, 2011; Paolone et al., 2013; Sarter and Phillips, 2018). Other investigators reported similar findings and offered comparable conclusions from experiments in rodents performing tasks measuring similar or related cognitive-behavioral functions (Passetti et al., 2000; McGaughy et al., 2002).

Above we argued that the cholinergic synapse is designed and wired to support highly phasic cholinergic signaling. This view raises the question of whether dialysate-derived tonic ACh levels reflect the integration of transients. Because the dimensions of the neurochemical measures obtained from microdialysis versus enzyme-coated microelectrodes and amperometry cannot be readily unified, and because the measurement compartments and terminal fields monitored by these two methods differ rather profoundly (microdialysis probe insertion-induced millimeter-sized cavity versus reactions of enzyme immobilized on a micrometer-sized, relatively slim electrode; (e.g., Fig. 2 in Howe et al., 2017), a direct test of this possibility has remained elusive. To complicate the issue further, the amperometric method is optimized for the measurement of transients and probably not capable of tracking slow or tonic changes in ACh (should those exist), largely because hydrolyzed choline spikes are rapidly cleared by cholinergic synapses and also diffuse into the interstitial space.

For a test of the possibility that dialysis-derived tonic ACh levels represent integrated cholinergic transients, we measured choline currents using amperometry and ACh levels using microdialysis in (necessarily separate groups of) rats performing a cued appetitive response task. In this task, amperometrically measured choline spikes occur in trials in which rats indicated behaviorally that they detected a cue which predicted subsequent reward delivery. Measures were obtained from prefrontal cortex and from motor cortex. To compare amperometric data with ACh levels measured in 8-min dialysate collections, we expressed both types of data dimension-free and collapsed transient amplitudes over 8-min periods (methods and results are detailed in Supplemental Data in Parikh et al., 2007). Statistical comparisons between these two data sets

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indicate the absence of a significant difference, suggesting that microdialysis levels were e reproduced by folding transient data into time bins which matched the dialysis collection intervals.

Several caveats are important here. First, it should be acknowledged that we originally interpreted some aspects of this data, particularly the spatially-specific nature of cue-evoked transients (exclusively in mPFC and not motor cortex) versus the equivalent results for mPFC and motor cortex using either the microdialysis or re-analyzed amperometric data, as supporting different timescale mechanisms. However, although cue-evoked transients were confined to mPFC, amperometric activity did occur in motor cortex as well at various points in the trial – interestingly, the patterns suggested they may occur during shifts in motor behavior (e.g., from grooming to rearing). This leads to the second question of how transients could be integrated to lead to microdialysis results in light of the fast, highly-efficient action of AChE. As noted above, this may be related to the glial barrier created in response to the microdialysis probe penetration injury (Footnote 1).

In short, the evidence that transient signaling is sufficient to describe forebrain cholinergic signaling is currently tentative, but appears to be at least quantitatively possible. More critically, the evidence for longer-timescale action is methodologically problematic, and on first principles appears contradictory to the known efficiency of AChE. Definitive evidence on this point likely awaits further methodological development. However, to test the potential strength of a 'phasic only' conceptualization, below we assess the usefulness of this hypothesis in the context of evidence from research on arousal states and neurophysiological and cognitive neuroscience studies.

Arousal states: Cholinergic tone versus phasic ACh

It has been widely accepted that forebrain cholinergic tone is elevated during REM sleep, and that ACh levels in that stage are nearly comparable with levels seen in the awake state. Indeed, evidence indicating arousal-state associated ACh levels has remained a major source of support for the idea of a cholinergic tone. However, this may once again be at least partially a methodological artifact.

The primary evidence comes from classical studies which preceded even the availability of microdialysis. Sealed chambers were placed onto the pial surface of the cortex of anesthetized and immobilized animals and perfused with AChEIs to prevent ACh hydrolysis. Individual samples were collected over 10-15 min periods. ACh levels in these samples, in response to electrical stimulation of the reticular formation, formed the basis of the notion that arousing events increase

cortical ACh levels (Celesia and Jasper, 1966; Phillis, 1968; Szerb, 1971). Subsequent microdialysis studies measured extracellular ACh levels in 5-60-min dialysate samples. Results from these studies seemed to confirm that ACh levels were higher during wakefulness and paradoxical sleep when compared with slow-wave-sleep (Kametani and Kawamura, 1990, 1991; Marrosu et al., 1995; Jimenez-Capdeville and Dykes, 1996). However, as noted above, these measurements occurred in the presence of AChE inhibitors in the perfusion fluid to prevent ACh hydrolysis.

Overall, the available evidence showing elevated cholinergic tone during arousal states seems unexpectedly limited and is largely based on older methods which relied on inhibiting ACh hydrolysis and which, by default, generated measurement time points incapable of revealing underlying potential phasic release patterns – patterns that are predicted based on the neurophysiological activity of cholinergic soma during paradoxical sleep and wakefulness. In particular, recordings from cholinergic neurons in the basal forebrain indicate phasic, high frequency bursts during wake and REM sleep stages, that is, activity on a time scale that mirrors the time scale of transient ACh release events (Lee et al., 2005). It will be important to measure transient generation during sleep stages in future studies and to determine whether integrated transients reproduce the minute-based elevations seen in studies using microdialysis.

Genetic CHT variants, transients and attentional control

The capacity of the neuronal choline transporter (CHT) to import choline into cholinergic terminals is essential for, and the rate-limiting step of, ACh synthesis and release (for reviews see Okuda and Haga, 2003; Ferguson and Blakely, 2004; Sarter and Parikh, 2005). In both rodents and humans, sub-capacity variants of the CHT that limit elevations of cholinergic activity are associated with attentional vulnerabilities (English et al., 2009; Paolone et al., 2013; Parikh et al., 2013; Berry et al., 2014; Sarter et al., 2016). These vulnerabilities have been interpreted in terms of relatively weak attentional control mechanisms that are revealed, in part, by heightened distractibility (e.g., Kim et al., 2017b; Sarter and Lustig, 2019). As this interpretational framework considers relatively weak attentional control in these populations as a psychological trait, our focus on phasic or transient ACh release raises the question of how variations in such phasic signaling can support the expression of a relatively complex and stable cognitive style.

As noted above, there are (at least) two potential interpretations of the computation performed by cholinergic transients. Each suggests a slightly different solution to this question. The first, that cholinergic transients reduce “expected uncertainty” about the presence of the signal, suggests that the subcapacity CHT variants should result in either a failure to generate cholinergic

transients, or to generate transients with a reduced amplitude, and that this in turn would reduce decisional certainty about the presence of the signal. The resulting conservative bias ("saying no") would further impair detection performance and impede the capacity for shifting between internal and external attention (Chun et al., 2011)

The second possibility takes into account that (at least in humans) the transients associated with "shift-hits" are observed in anterior PFC, whereas activity associated with responding to distraction and other attentional challenges occurs in more dorsolateral/ventrolateral regions, especially along the border of right middle and inferior frontal gyri. A wide range of evidence from patient studies, task-based neuroimaging, and functional and anatomical connectivity studies point to this latter region as a major "hub" for interactions between attention networks, and its importance in motivated cognitive control (see review by Lustig and Sarter, 2016; see discussion in Berry et al., 2017, as well as many others). More specifically, right middle/inferior frontal gyrus has been discussed as critical for coordinating the network-level neural representation of (rMFG) relevant task sets, so that cognition and behavior are driven by these goal-relevant task sets, rather than being stimulus-driven (e.g., Braver et al., 2009).

Importantly, maintaining representations in working memory - including task-set representations - does not require tonic neuronal firing. Instead, they can be maintained by shifts in synaptic weights or coordinated variability and oscillatory behavior (e.g., Dehaene et al., 1998; Lustig et al., 2007; Sadaghiani et al., 2015; Schmitz and Duncan, 2018). Explicit activity may only be required only during the initial acquisition, to recover the task set after an error, or to 'protect' the representation in the face of competing inputs (see especially the discussion in Dehaene et al., 1998) or more occasionally to 'refresh' the representation as stochastic variability among components in the network gradually cause them to fall out of synch (Lustig et al., 2009).

These considerations concerning a second possible interpretational framework for cholinergic transients predict that reduced CHT function primarily results in behavioral deficits when repeated interference from irrelevant stimuli or task sets requires frequent, rapid refreshing of the relevant task set representation. Notably, this is the pattern shown by humans with a genetically reduced-capacity CHT: They fail to activate the right medial frontal gyrus in response to attentional challenge, but have preserved signal detection performance (Berry et al., 2015). However, they show a specific vulnerability to ongoing, salient external distractors both in the laboratory and everyday life (Berry et al., 2014).

In other words, as would be predicted by a CHT that limits the rate at which choline can be transported for the synthesis of ACh, humans expressing a reduced capacity variant of the CHT

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appear to have a specific deficit when repeated cholinergic transients are required with relatively short intervals between successive transients. A similar pattern was shown in Parkinson's patients with primarily cortical (rather than thalamic) cholinergic degeneration: They showed preserved signal detection (Kim et al., 2017a) but specific vulnerabilities in the face of external distractors (Kim et al., 2017b). We recognize that, at some point the distinction between "closely spaced cholinergic transients" and "tonic neuronal firing" may be difficult to empirically discern. However, there are critical conceptual distinctions: By this view the frequency of cholinergic activity is driven quantitatively by situational needs to refresh the task-set representation in the face of interference, rather than being a qualitatively different physiological "mode" (see also Fiebelkorn and Kastner, 2019).

This view appears to be consistent with a recent computational model suggesting that ACh acts to optimize a single computation that enhances multiple dimensions of the responses of cortical neurons (firing rate, rate variability, and correlated variability) to attention-demanding cues (termed "normalization"). This model predicts that loss of ACh-mediated normalization involves detection losses and enhanced distractibility (Schmitz and Duncan, 2018). As we argue herein, this model likewise does not require assuming that multiple time scales of cholinergic neurotransmission mediate dissociable cognitive functions of ACh.

AChEIs and cholinergic transients

AChEis not only have been used to elevate extracellular ACh levels in order to recover detectable levels of ACh in neurochemical studies, and therefore to support notions about the functionality of tonic levels of ACh (above), but also as a pharmacological tool in human studies to determine effects of elevated ACh levels on cognitive functions. The synaptic effects of AChEIs are insufficiently described merely in terms of elevated ACh levels. AChEIs elevate extracellular ACh levels by several 100 to several 1000% (e.g., Giacobini et al., 1996; Scali et al., 2002; Noori et al., 2012). As ACh stimulates presynaptic autoinhibitory muscarinic acetylcholine receptors (mAChRs), such strikingly elevated ACh levels are expected to silence presynaptic ACh release (e.g., Yan and Surmeier, 1996). At the same time, postsynaptic mAChRs and nAChRs, including receptors which may be located at distant extrasynaptic sites, experience presumably non-physiological levels of stimulation. It is extremely difficult to see how the resulting scenario - blockade of presynaptic signaling combined with excessive postsynaptic stimulation - can preserve and even enhance cholinergic information processing. These complexities may explain the limited therapeutic efficacy of AChEIs for treating age- and neurodegeneration-related cognitive impairments (e.g., Courtney et al., 2004; Maher-Edwards et al., 2011).

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The effects of AChEIs on pre- and postsynaptic signaling, particularly in interaction with endogenously generated transients, are not known (see also Basselin et al., 2009). Because the presence of ACEIs invalidates the amperometric measurement scheme used for recording cholinergic transients, this issue presently resists investigation. However, the finding that AChEIs sharpen the response of the visual cortex to visual stimulation, both in terms of fmri-based BOLD response (Silver et al., 2008) and the size of the receptive field of neurons in this region (Roberts et al., 2005), suggests that AChEIs, based on unknown mechanisms, can amplify spatially and temporally restricted cholinergic signaling (see also Runfeldt et al., 2014). Perhaps for this reason, AChEIs have been found occasionally to produce beneficial effects on attentional performance (e.g., Bentley et al., 2004; Rokem et al., 2010; Bauer et al., 2012; Gratton et al., 2017). New methods, such as G-protein-coupled ACh sensors (Jing et al., 2018) may be capable of shedding light on the effects of AChEI on phasic ACh release events.

Conclusions

Traditional assumptions about relatively lasting brain states controlled by the forebrain cholinergic system have coalesced with traditional neurochemical methods which generate minute-based measures of cholinergic activity and sample from relatively large neuronal spaces. The widespread uses of AChEIs to optimize ACh measures and as a pharmacological tool have further cemented the view that tonic (scale of 100s of seconds) changes in extracellular ACh levels mediate relatively large-scale cognitive functions (such as arousal or top-down attentional control). Based on the demonstration of the presence and functions of fast, phasic or “transient” cholinergic signaling, here we argue that cholinergic signaling and functions can be sufficiently described by the presence of cholinergic transients which mediate a single computation that, behaviorally, favors the detection of behaviorally significant cues in attentional settings, specifically when such detection involves shifts between modes of attention (e.g., intrinsic to extrinsic, or monitoring to cue-oriented responding). The interpretation of evidence from behavioral, neurophysiological as well as human imaging studies on the role of cholinergic signaling will be more constrained and eventually heuristically more powerful by abandoning intuitive notions about “tonic” cholinergic control of brain states and focusing instead on the role of fast cholinergic signaling for precise computational processes. Moreover, the search for effective pro-cholinergic, pro-cognitive treatments may benefit significantly from moving away from drugs the effects of which conform with views about tonic cholinergic activity and function, such as AChEIs, to drugs that enhance and rescue transient cholinergic signaling or their post-

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414 synaptic processing (e.g., Howe et al., 2010; Moran et al., 2018; Uslaner et al., 2018; Kucinski et
415 al., 2019).

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