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Chapter

Modes of Acetylcholine Signaling in the Prefrontal Cortex: Implications for Cholinergic Dysfunction and Disorders

Matthew Fecik and Lisa M. Savage

Abstract

The forebrain cholinergic system is an important mediator of arousal, attention, memory, and other cognitive processes. Cholinergic signaling is typically divided into two patterns, tonic signaling, which involves sustained changes in ambient acetylcholine (ACh) tone over seconds to minutes, and phasic signaling, which involves fast changing, spatially specific release of ACh on a millisecond timescale. There is evidence to suggest unique functional roles for both types of signaling in the prefrontal cortex: phasic release of ACh is thought to be necessary for attentional processes, as well as cue detection, while tonic signaling is thought to be involved in regulating global arousal states and has been shown to increase with general cognitive demand. The differences between these two types of signaling may originate from electrophysiological properties of cholinergic cell types, distinct muscarinic and nicotinic receptor utilization and/or expression, and/or differential hydrolysis of ACh by acetylcholinesterase. This review will summarize the current views on the functional role of each type of signaling, while the contributions of ACh receptors, hydrolysis, and basal forebrain anatomy are examined. Additionally, the implications of these factors in ACh signaling will be examined in terms of cholinergic circuit dysfunction that occurs in neurodegenerative diseases.

Keywords: acetylcholine, prefrontal cortex, attention, arousal, Alzheimer's disease, alcohol use disorder

1. Introduction

While the role of acetylcholine (ACh) as a neuromodulatory regulator of REM sleep, global arousal states, and consciousness in the prefrontal cortex has been known for quite some time [1–3], the evidence that ACh also functions as a fast-acting neurotransmitter on a precise spatial and temporal scale to carry out specific cognitive operations has only begun to be explored within the past two decades, with progress accelerating significantly in recent years [4–6]. Studies elucidating short-duration phasic cholinergic signaling in the cortex have been made possible by the use of techniques
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for collecting temporally precise data on ACh in vivo, beginning with electrochemical detection [7], and more recently including optogenetics and fiber photometry [8–11]. Such techniques open exciting new possibilities to advance our understanding of cholinergic neurotransmission in the brain, and importantly, our understanding and treatment of brain disorders such as Alzheimer’s disease (AD) and alcohol use disorder (AUD).

ACh functions in the prefrontal cortex are based on a variety of factors such as cortical cytoarchitecture, receptor utilization, cholinesterase activity, and basal forebrain anatomy. It is therefore necessary to reevaluate our understanding of the literature on disorders of the basal forebrain through the lens of the tonic/phasic ACh signaling distinction. The goal of this review is to define what is currently known about tonic and phasic ACh signaling in the prefrontal cortex, how the heterogeneity between these two types of signaling may arise, review the differences in receptor utilization these two types of transmission require, evaluate the role of acetylcholinesterase activity in this distinction, and review some of the deficits to each type of transmission that occur in both AD and AUD.

2. Modes of ACh signaling and their roles in behavior

2.1 Tonic ACh signaling

Tonic signaling (sometimes referred to as volume transmission) represents the traditional conceptualization of cholinergic transmission in the central nervous system. In this form of signaling, ACh functions as a slow-acting neuromodulator that exerts its effects on timescales of several seconds to minutes or even hours. Neuromodulatory ACh originating from the basal forebrain has been shown to be involved in functions such as the transition between arousal states, REM sleep, and exploratory behavior [2, 12–15]. Tonic signaling tends to lack both spatial and temporal precision.

Early evidence suggested that “ACh efflux” was not only involved in global arousal states but also directly involved in attentional processes and reward-based learning [16–18]. An early study using in vivo microdialysis measured ACh efflux in the medial prefrontal cortex (mPFC) during a 5-choice serial reaction time task (5-CSRTT) and found that in male rats that were well trained on this task, there was increased ACh efflux, an effect that was attenuated in rats not previously trained [19]. Interestingly, they found that ACh efflux was positively correlated with the number of trials completed during the session in which the cue light was illuminated for 5 seconds every trial, compared to 0.5 or 0.25 seconds. The authors contribute that this rise is dependent on the fact that the longer stimulus may elongate the saliency of the food reward and novelty of the task, which has been shown to be dependent on ACh [18, 20]. However, a follow-up study demonstrated that the increase in ACh efflux was due to the performance of the task alone, as rats whose reinforcement was yoked to another subject’s performance rather than their own did not show the same increase in ACh efflux, despite also receiving the same pattern of rewards and being exposed to the cue stimuli [21]. Later literature would go on to implicate attentional control of behavior, particularly cue detection, as a cognitive function dependent on phasic ACh signaling, not general ACh tone [9, 22, 23].

Understanding of tonic cholinergic signaling came mostly from studies using in vivo microdialysis, in which cholinesterase activity is typically inhibited to ensure a
sufficient concentration of ACh for electrochemical detection. This leads to a potential misunderstanding of cholinergic activity in the frontal cortex, with some suggesting that ACh “tone” may even be a methodological artifact [4]. One of the reasons for such a conclusion stems from the seemingly hyperefficient action of AChE, which is the main enzyme involved in the hydrolysis of ACh in the synaptic cleft and therefore the termination of its action. However, it has been shown that a sufficient volume of ACh is able to transiently inhibit AChE [24, 25], leading to further increases in ACh that can potentially spill over into the extracellular fluid and increase cholinergic tone. Such a mechanism would involve a positive feedback loop in which significant quantities of ACh release can inhibit AChE, further increasing the concentration of extracellular ACh. However, this inhibition may be short-lived [24, 25], perhaps on the timescale of milliseconds, and therefore may cause small “pulses” of spillover without hindering fast synaptic phasic signaling at the point of inhibition.

However, the possibility that visual attention tasks require both intact tonic and phasic ACh signaling in the mPFC cannot be ruled out, and it is possible that performance on these tasks requires an alert attentional state governed by tonic signaling, which has been shown to mediate shifts in attention, as well as phasic signaling to encode for specific behavioral epochs and serve as an indicator of cue presentation. Such a mechanism would have been missed by the microdialysis approaches. Thus, the possibility that task performance is reliant on phasic signaling, while behavioral engagement is dependent on the ambient tonic signaling that is perpetually present in the cortex cannot be ruled out and the delineation of the two is a potential area of future research.

2.2 Phasic ACh signaling

Contrasted with tonic ACh signaling, phasic signaling involves much faster time scale circuit-specific release. Phasic signaling is thought to be wired, meaning that it involves the release of ACh from a single cell that innervates a single cortical cell in a highly specific manner [4]. Unlike tonic signaling, it does not involve spillover from the synapse into the extracellular fluid, rather it is constrained to the synapse. This type of signaling occurs on a much faster timescale—likely that of only hundreds of milliseconds. This type of signaling has been implicated in cortical control of attention, specifically cue detection—the cognitive process needed to determine whether a cue that signals a reward is present or not [6].

Based on work using mice, it has been demonstrated that projections originating in the nucleus basalis of Meynert (NbM) and substantia innominata (SI), which form the NbM complex, send their axons to the mPFC and are necessary for cue detection [6]. Particularly, it is thought that these projections are involved in the shift between vigilance and cue detection [6]. This is further demonstrated by the fact that disruption of the mPFC’s cholinergic innervation impairs cue detection, while disrupting projections to the other targets of the NbM, such as the motor cortex, yields no effect on this task [23]. Phasic ACh signaling in this circuit is likely the causal mediator of cue detection, as it has been shown that optogenetic stimulation of the NbM during a cue detection task improved performance during cued trials and increased the false alarm rate during non-cued trials, suggesting that millisecond timescale cholinergic signaling originating in the NbM is involved directly in the encoding of the representation of the cue in the prefrontal cortex in mice [9].

In addition, there is evidence to suggest that the basal forebrain is involved in the formation of stimulus associations in mice. Tu et al. [11] found that cholinergic...
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signaling during associative conditioning can affect the strength of the association formed in a time-dependent manner. Although the NbM is commonly cited as the primary source of cholinergic projections to the prelimbic cortex (PrL), and injection of retrograde tracer into the PrL revealed that the greatest source of cholinergic innervation originated in the horizontal diagonal band (HDB). Therefore, Tu et al. [11] targeted the PrL projections originating in the HDB for optogenetic manipulation and found that stimulation during the unconditioned stimulus impaired associative learning while inhibition facilitated it. Furthermore, they found that optogenetic stimulation during the conditioned stimulus did not affect the strength of associative learning, but inhibition lead to impaired learning. This was further accompanied by fiber photometry data that show that the level of excitation of the PrL correlates with the strength of the memory, such that ACh signaling during the unconditioned stimulus strengthened across sessions. These data suggest that phasic signaling is uniquely sensitive to timing, such that the functional role of the cholinergic projections from the HDB to the PrL region involves specifically timed excitation, further lending evidence for the role of ACh in encoding specific stimulus representations [11].

One of the ways that cholinergic signaling can be achieved on this timescale is through the actions of acetylcholinesterase (AChE), which is an incredibly effective hydrolytic enzyme, making the local regulation of AChE as one of the ways in which a degree of heterogeneity is introduced between tonic and phasic ACh signaling. Local expression of AChE may contribute to some of the anatomical heterogeneity between tonic and phasic signaling and makes it likely that phasic signaling would occur, due to its potent catalytic action. However, this has not been investigated thoroughly and represents a future area of inquiry. As cholinesterase activity is likely one of the most important regulators of spatially and temporally restricted ACh signaling in the prefrontal cortex, our understanding of its spatial distribution in the cortex is of paramount importance.

2.3 Tonic vs. phasic signaling: differing viewpoints on the distinction

The presence of such a highly potent catalytic mechanism such as the hydrolysis of ACh by AChE has led some to suggest that tonic ACh signaling in the forebrain is unlikely to affect behavior at all. In this view, the fact that the rate-limiting step of ACh hydrolysis is the diffusion of ACh into the synapse, and not the hydrolytic action of AChE itself, is evidence to suggest that ACh is unlikely to travel distances beyond the synapse and therefore changes in extracellular ACh concentration is unlikely to be a contributor to behavioral events [4]. However, others believe that the tonic/phasic distinction is an oversimplification and that ACh signaling most likely has both fast and slow components that both contribute to behavior. In this view, cholinergic signaling varies as a function of anatomy, receptor subtypes, and ACh hydrolysis—and therefore the concept of ACh tone may still have some functional role in behavior [26].

While the exact middle ground between these two viewpoints is yet to be determined, one study demonstrated both relevant tonic and phasic ACh signaling simultaneously in the PFC and dorsal hippocampus of mice in an attempt to differentiate between their functions. Using electrochemical choline biosensors, Teles-Grilo Ruivo et al. [15] demonstrated that tonic ACh signaling during sleep was highest exclusively during REM sleep that preceded wakefulness. They also demonstrated that tonic signaling was highest as the animal approached a reward in a randomized forced alternation T-maze. Additionally, they found that phasic ACh was associated with the presentation of the reward, with phasic signaling showing response halfwidths.
significantly shorter than during tonic signaling. Importantly, they demonstrated that both tonic and phasic signaling were highly coordinated between the PFC and the dorsal hippocampus, suggesting that not only does such a distinction in modes of ACh transmission exist in the cortex, but likely can be extended to the hippocampus as well. Should this be the case, the drivers of the distinction are likely to be driven by ubiquitous mechanisms such as cholinesterase activity, or some intrinsic property of basal forebrain anatomy. Ruivo et al. [15] suggest that their results demonstrate that tonic ACh signaling, especially during REM sleep, maybe prepare the mPFC and hippocampus for subsequent alertness and later attentional demands.

Additionally, there is electrophysiological evidence to suggest a dichotomy in cholinergic signaling in the basal forebrain. Unal et al. [27] demonstrated that there are two distinct populations of basal forebrain cholinergic neurons that are distinguished by their electrophysiological properties. Early firing neurons were more excitable, quicker firing, and had more pronounced refractory periods following firing, while later firing neurons were less excitable but more sustained in their ACh release. The authors suggested early firing cells may be involved in phasic signaling and therefore be important for attention, while the late firing cells may be involved in tonic signaling and therefore more important for global arousal states [27]. This suggests that the dichotomy has its roots in electrophysiological correlates.

The above work was later expanded on by Laszlovzsky and colleagues [28]. They recorded from cells in mice both in vivo and in vitro and determined that basal forebrain cholinergic neurons took one of two forms, excitable, burst-firing cells (Burst-BFCN) and less excitable, rhythmic cells (Reg-BFCN). They found Burst-BFCN cells to be more numerous than Reg-BFCN in both the NbM and the horizontal limb of the HDB and consisted of two subtypes, ones with regular inter-spike intervals which they refer to as Burst-BFCN-SB and ones with Poisson-like inter-spike intervals which they refer to as Burst-BFCN-PL. They found that Burst-BFCN cells showed cortical synchronicity and fired bursts of action potentials in response to both reward and punishment during an auditory cue detection task. Reg-BFCN cells, on the other hand, were found to have precise spikes after behavioral outcomes, mainly hits, but not false alarms, correct rejections, or misses. Furthermore, there was distinct anatomical heterogeneity amongst these cell types, with Burst-BFCN found in the anterior basal forebrain and Reg-BFCN cells found in the posterior division [28]. These findings offer a unique viewpoint on the current tonic/phasic debate. They contend that such a divide between tonic and phasic signaling does exist and has anatomical and electrophysiological origins. Such an explanation seems to suggest that tonic ACh signaling has a much greater role in cue detection and cognitive operations than would be suggested by Sarter and Lustig [4].

Recently, basal forebrain cholinergic neurons have also been categorized into neurons that express calbindin-D28K (D28K) protein (ChAT D28K+) and those that do not (ChAT D28K-). The expression of D28K across the basal forebrain nuclei ranges significantly. About 40% of ChAT neurons in the VDB co-stain D28K, relative to 30% in the MS, 16% in the HDB, and less than 2% in the NB [29]. ChAT+ neurons that also stain for D28K have fewer processes and a lower firing frequency. Interestingly, D28K is a Ca\(^{2+}\) binding protein that may function to protect cells from Ca\(^{2+}\)-dependent neurodegeneration [29]. This is supported by data that the D28K protein is decreased in cholinergic neurons as a function of aging and in AD [30, 31]. Data support that cholinergic neurons are a heterogeneous population of cells, and understanding the unique profiles of the subpopulations may lead to a better understanding of critical behavioral processes they are involved in.
The location of cholinergic neurons is also a predictor of differential function. Amongst the cholinergic nuclei that project to the cortex, the HDB (rostral BF), and NBM/SI (caudal BF), there is data to support differential function across the anatomical location. Early literature on the NbM to prefrontal cortex circuit suggests a limited degree of axon collateralization between individual projection neurons, which limits the crosstalk between cortical cells and layers [32]. Thus, these circuits seem to be suited for phasic signaling, such that limited collateralization allows for a degree of spatial specificity that is necessary for wired cholinergic transmission in the prefrontal cortex. However, there exists some heterogeneity across species in terms of the projection targets of the NbM, such that its innervation of the prelimbic and infralimbic cortex has been well characterized in mice, but the data seem a bit more nuanced in the case of rats, such that retrograde viral tracing does not show NbM innervation of the prelimbic or infralimbic cortices in this species, rather the HDB is the key region [33]. Likewise, there is data to suggest that the mouse prelimbic cortex receives its primary cholinergic innervation from the HDB as well [11].

Assessing cholinergic activity across the BF anteroposterior axis via calcium imaging and ACh-specific fiber photometry, it has been revealed that the more rostral cholinergic neurons (HDB) are responsive to pupil change, which marks arousal state, reward delivery, and reward omission. In contrast, cholinergic activity in the caudal region (NbM/SI) was more responsive to unconditioned cues, delivery of shock, and cues that predicted shock [34]. It is therefore possible that the projections of the NbM/ SI to the cortex may be important for salient cue detection and that the projections from the HDB to the prefrontal cortex are more critical for appetitive arousal states.

Cortical receptor location within the architectural layers of the cortex may also play a key role in the outcome of ACh signaling. In addition to the aforementioned factors, evidence for tonic and phasic ACh as distinct behaviorally relevant modes of neurotransmission come from the differential roles and actions of muscarinic and nicotinic ACh receptors within the cortex [31]. There is some evidence to suggest that fast nicotinic ACh receptor (nAChR) mediated signaling is essential for phasic ACh signaling in the cortex while muscarinic ACh receptors (mAChR) are involved in slow changes in ACh concentration such as during tonic signaling. However, such a distinction may not be so cut and dry, as there seems to be a degree of muscarinic activity needed to perform a cue detection task [35], which suggests that muscarinic receptor activity may be needed to regulate global arousal states and “prime” the prefrontal cortex for phasic cholinergic signaling.

3. The contribution of acetylcholine receptors in modulating tonic and phasic activity

3.1 Nicotinic receptors

Nicotinic ACh receptors (nAChR) in the cortex have been shown to be essential for a number of cognitive functions, such a top-down attentional control of behavior and general working memory [36, 37]. Given their fast-acting, ionotropic responses, nAChRs are thought to be involved in phasic ACh signaling. Furthermore, given their selective laminar expression throughout different regions of the cortex, it stands to reason that nAChRs would be selectively expressed in circuits where fast, wired ACh transmission is the norm, and in such a distribution that promotes spatially restrained and functionally heterogeneous responses to ACh signaling from the basal forebrain.
While there is evidence to suggest a role for the utilization of these receptors in phasic signaling, the distinction between the role of nAChR in the two modes of cholinergic neurotransmission seems to be much more nuanced.

Much of the evidence surrounding the role of nAChRs in fast synaptic ACh signaling is electrophysiological. It has been shown that the activation of nAChRs evokes short-latency depolarizing postsynaptic potentials in mouse neocortical pyramidal neurons [38], which has been suggested to function as laminar selectivity relative to muscarinic function. Furthermore, nAChR activation throughout the cortex seems to be layer specific, as Poorthuis et al. [39] showed that nAChR activation leads to inhibition of pyramidal cells in the mPFC in layers II and III, but enhanced excitability of layers V and VI. Interestingly, their data suggest that the response to ACh in this region is dependent on different subunits depending on the layer of the cortex. They demonstrated that pyramidal neurons in layer VI exhibit slow inward currents in the presence of ACh that are absent in β2 subunit deficient mice and were blocked by an antagonist for β2 containing nAChRs and were only occasionally accompanied by α7 mediated currents. Meanwhile, layer V mPFC pyramidal cells showed an attenuated response to ACh in α7 deficient mice as well as following the application of an α7 antagonist, but not in β2 deficient mice, suggesting differential expression of these subunits by layer. They also performed two-photon imaging in cortical pyramidal cells of each layer in β2 and α7 deficient mice and found that nAChR-induced neuronal activation is dependent on the β2 subunit when ACh changes in concentration slowly, suggesting that not only is the β2 subunit utilized during tonic signaling, but also potentially reveals an interesting anatomical phenomena in which layer VI, in which β2 containing nAChRs are most involved in, is involved in tonic signaling, while layer V may not be [39].

The idea that nicotinic receptors are involved in tonic ACh signaling is an interesting one as well, in that it would stand as evidence against the assumption nAChRs mediate phasic signaling exclusively, and that tonic signaling is mostly reliant on muscarinic receptors. In addition to its presumed role in the detection of ambient ACh, β2 subunit deletion has been shown to impair attentional performance in mice [40], suggesting a role of such receptor subtypes in phasic ACh signaling, or at least furthering the idea that attentional performance requires some changes in ambient ACh levels. Interestingly enough, the deletion of this subunit can also upregulate muscarinic receptor excitability to compensate in layer VI of the mPFC, which was also seen following the deletion of the o5 subunit as well [41]. The previously mentioned study by Poorthuis and colleagues [39] notably blocked muscarinic receptors to isolate neuronal responses due to nicotinic activation, meaning that such an increase in excitation was masked, and cholinergic tone that would have been detected primarily by muscarinic receptors may have instead bound to nicotinic receptors in layer VI. An alternative explanation is that such a mechanism may allow for further priming of the cortex to ACh signaling to make up for the reduction caused by subunit deletion, allowing for phasic-dependent cognitive processes to attempt to bounce back even in the presence of less cholinergic signaling.

In addition to the β2 subunit, it seems the o5 nAChR subunit is necessary for attention as well, as deletion of the Chrna5 gene, which encodes for the o5 subunit, impairs attention [42]. It was also demonstrated that the loss of Chrna5 delays cholinergic excitation, such that mPFC layer VI pyramidal cells from Chrna5 deficient mice show attenuated onset kinetics but unaffected response magnitude during optogenetic stimulation of cholinergic afferents in vitro and that enhancing nicotinic receptor affinity pharmacologically can actually restore the typical response to optogenetic
stimulation [8]. The authors suggest that α5 subunit containing nAChRs in the PFC may be necessary to define a critical window for cue detection, such that a delay to their onset results in the animal being unable to properly integrate detected stimuli via corticothalamic connections and ends up missing the cue altogether. Another interesting factor that may play into the α5 subunit’s role in phasic ACh signaling in cue detection is its relative rarity, in that its expression is much less prevalent than subunits such as β2 [43], and therefore may be more likely to facilitate spatially constrained wired transmission. However, this is yet to be investigated and represents a considerable gap in our understanding of the role of the α5 subunit in attention.

One further subtype that has been implicated in attention control, and by extension, the role of its dysfunction in cognitive decline, is the α7 nAChR which is composed entirely of α7 subunits. This particular subunit has been highly implicated in the cognitive decline associated with AD [44–46]. It has been shown that an α7 nAChR agonist was sufficient to enhance learning speed but not filter distracting information, which was instead enhanced by an α4/β2 agonist, with no effect on learning speed [47]. Additionally, it has been shown that performance on the 5-choice continuous performance task, a measure of attention in rodents, was improved following the administration of encenicline, a partial agonist to the α7 nAChR, in poor-performing rats [48]. Due to the fact the dysfunction of α7 has been demonstrated in Alzheimer’s disease, it is likely that this particular subtype of receptor is necessary for attentional processes in the cortex, in addition to other processes.

3.2 Muscarinic receptors

Muscarinic ACh receptors (mAChR) are thought to be mediators of tonic acetylcholine signaling, due to their longer duration of action via G-protein coupled mechanisms that require second messenger signaling. mAChRs are present in the prefrontal cortex both presynaptically and postsynaptically [49]. Thus, they would seem to be much more suited as receptors of tonic signaling, and likely their longer-lasting duration of action and the fact that a single muscarinic receptor can have amplified second messenger signaling lends itself to the idea that muscarinic receptors are involved in relatively slow timescale changes in ACh concentration in the prefrontal cortex. Similarly, such a mechanism would be suitable for the detection of relatively small concentrations of ACh present in the extracellular fluid during tonic transmission, as opposed to the much larger concentration present at the cholinergic synapse during phasic signaling [26].

However, there may be a degree of specificity as to which muscarinic receptor subtypes are involved in tonic signaling. It has been shown that the Gq-coupled M1 mAChR, but not the M3 or M5, is essential to the response of pyramidal neurons to tonic ACh [35], suggesting a role for only specific Gq-coupled receptors, not all of them. This is interesting, as the location of M1 receptors on the dendritic shaft and spines of cortical pyramidal cells provides a degree of anatomical evidence for volume transmission in the cortex, likely meaning that the M1 receptor’s anatomical distribution is what favors it toward the detection of ambient ACh over long distances due to the fact that cholinergic neurons are not known to make axo-axonic synapses [50]. These findings point to a specialized role of muscarinic receptors in detecting global, seconds-scale changes in ACh concentration indicative of perisynaptic signaling and tonic transmission.

As mentioned previously, muscarinic receptor activation may serve to “prime” the cortex for subsequent phasic signaling in such a way that both forms of signaling
are necessary for attentional processes to occur. A likely mechanism is via increases in cortical pyramidal cell excitability, in which muscarinic receptor activation has been shown to alter via the induction of LTD in these cells in the medial prefrontal cortex [51]. This is further supported by findings that mice that are deficient in the inhibitory M2 muscarinic receptor show enhanced attentional performance, despite having impaired object-location learning and spontaneous recognition memory [52], suggesting that while the Gq-coupled muscarinic receptors, such as M1, play an important role in cortical excitability, the activation of Gi-coupled receptors such as M2 may be detrimental to attentional performance. It seems likely that muscarinic receptor activation is needed for cue detection and sustained attentional processes, as a non-specific muscarinic receptor antagonist is sufficient to impair attentional performance on a two-choice visual discrimination task in mice [53]. Likewise, M1 receptor antagonism impairs performance on a divided attention task in rats, unlike antagonism of the M4 receptor which did not [54]. Additionally, the M1-positive allosteric modulator TAK-071 has been shown to rescue attentional performance in rats with basal forebrain cholinergic cell loss [55]. Such studies suggest that while muscarinic receptors may play a role in attentional processes, their role in phasic ACh signaling remains unclear.

While it seems that muscarinic receptors are needed for attentional processes due to their effects on global arousal states, the dichotomy of tonic signaling being dependent solely on muscarinic receptors while phasic signaling is dependent solely on nicotinic receptors is evidently an oversimplification. Likely, the mechanism by which attentional control occurs is reliant on both nAChRs and mAChRs, such that mAChRs are needed to orient the organism toward the object of their attention, while nAChRs, at least the α7 and α5 subtypes, are involved in the response of cortical neurons to short timescale phasic ACh release. Thus, both nAChRs and mAChRs seem necessary for phasic ACh signaling and its reliant cognitive operations such as cue detection. However, mAChRs on their own may be mediators of tonic ACh signaling, and thus a degree of tonic ACh efflux is needed to ready the prefrontal cortex for an orientation toward a specific stimulus. However, more research is needed to delineate the contributions of each receptor system to tonic and phasic signaling in the prefrontal cortex, though the way to do so does not seem immediately straightforward.

4. The role of acetylcholinesterase in tonic and phasic ACh signaling

One of the ways in which tonic and phasic ACh signaling are regulated is through the actions of AChE, the catalytic enzyme which hydrolyzes ACh and is thought to be the main determinant of the duration of cholinergic signaling. As stated before, AChE can hydrolyze ACh nearly as fast as the cell can release it, and thus the high catalytic activity of this enzyme is evidence itself of the presence of phasic ACh signaling in the prefrontal cortex. However, it has also been hypothesized that this is one of the reasons as to why tonic ACh signaling is unlikely to directly contribute to behavior at all. Sarter and Lustig [4] state that the fact that AChE is such an efficient hydrolyzer of ACh makes it unlikely for synaptic spillover to occur, and therefore tonic ACh as measured by in vivo microdialysis may be a methodological artifact due to the cholinesterase inhibitors perfused into the brain during the process. However, it has been shown that inhibition of AChE by an excess of ACh may occur via the formation of a ternary complex [25], which represents one mechanism by which high-ACh concentration can overwhelm AChE and spill over into the extracellular fluid. Therefore, a
central role of potentially heterogeneous utilization of AChE during tonic and phasic ACh signaling distinction must be considered.

In the brain, AChE is exclusively expressed as the tailed AChE\textsubscript{T} variant. This subunit is able to form an amphiphilic tetramer known as G\textsubscript{4} AChE, which is facilitated by and tethered to proline-rich membrane anchor (PRiMA), anchoring AChE to the presynaptic membrane [56, 57]. Acetylcholinesterase is co-expressed with markers of cholinergic neurons such as choline acetyltransferase (ChAT) \textit{in vivo} [57], suggesting that AChE is likely expressed in cholinergic cells before undergoing tetramerization \textit{via} PRiMA in the endoplasmic reticulum and being transported down the axon [58]. PRiMA likely links tailed G\textsubscript{4} AChE to the presynaptic membrane \textit{via} membrane rafts [59]. Interestingly, PRiMA shows robust co-expression with M1 muscarinic receptors, which are located postsynaptically, suggesting that AChE may originate primarily from cholinergic axons but also intrinsically from neurons in the cortex [60]. Additionally, this co-expression may have interesting functional implications, however, it may simply be due to the ubiquity of expression of the M1 receptor in basal forebrain target regions. To date, no studies have looked to colocalize PRiMA with nicotinic receptors, making any potential differences in PRiMA receptors between neurons expressing the two receptor types is unknown but represents a future area of inquiry.

Experimental manipulations to inhibit the endogenous action of AChE have been shown to cause attentional impairments, such as during a five-choice serial reaction time task in healthy rats [61], suggesting that inhibition of AChE in healthy subjects impairs behaviors likely dependent on phasic signaling. AChE knockout mice have been shown to exhibit a variety of motor deficits due to the role of peripheral AChE in muscle contraction and thermoregulation [62]. Farar et al. [63] characterized PRiMA KO mice on a number of motor and behavioral measures. They found that despite only very subtle motor impairments on the rotarod test and the wire task, these mice had a nearly 200–300-fold increase in extracellular ACh concentration in the striatum as measured \textit{via} in vivo microdialysis during anesthesia, with none of the thermoregulatory impairments seen in mice with a traditional AChE knockout. Additionally, these mice were not impaired on the Morris water maze. Interestingly, they found that the M1 muscarinic receptor was heavily downregulated across all areas of the brain measured, including by approximately 40% in the cortex, with no such decrease in the α\textsubscript{7} nAChR or β\textsubscript{2}-containing nAChRs. One interpretation of these results laid out by the authors is that these data serve as evidence for the hypothesis that AChE is primarily involved in regulating the extracellular ACh concentration, not terminating synaptic transmission [63].

Should that be the case, then it is possible that PRiMA knockout mice may have deficits in phasic ACh signaling, but not tonic signaling. Thus, the hypothesis may be the inverse of what was proposed by these authors, that AChE is primarily utilized at the cholinergic synapses of the prefrontal cortex to rapidly terminate ACh signaling, while ACh “tone” represents ACh that escapes this mechanism and is, therefore, less active during this form of signaling. This may represent a potential mechanism by which attentional and other cognitive impairments occur in disease states that alter AChE and disrupt the balance between ACh release and hydrolysis, such as Alzheimer’s disease, while leaving other functions that are dependent on tonic signaling unimpaired until later in the disease.

A likely mechanism for which AChE is inhibited at the synapse, as represented in Figure 1 is as such: an overabundance of ACh in the synaptic cleft during phasic signaling may lead to inhibition of AChE which allows acetylcholine to spill out of
the synapse and into the extracellular fluid. This ambient ACh would still be under the regulation of AChE, but the enzyme's location on the synaptic membrane would make it more difficult for extracellular acetylcholine to be hydrolyzed, allowing it to accumulate in the extracellular space. Therefore, AChE still regulates ACh tone, albeit mostly indirectly, through its regulation of phasic ACh signaling. Tonic ACh may also be released by non-synaptic terminals, perhaps by specialized basal forebrain cholinergic cells [27, 28], and it is the combination of these two mechanisms that are responsible for ambient ACh fluctuations.

5. ACh in brain disorders: circuit dysfunction

Reductions in markers for the cholinergic phenotype, such as the expression of ChAT, the enzyme necessary for ACh synthesis, are present throughout the basal forebrain following both AD and AUD, a consequence that is considered one of the hallmarks of both of these disorders in particular. These cholinergic deficits are present in two of the main basal forebrain circuits, the projections from the NbM complex (NbM, HDB, SI) to the cortical mantle, and the projections from the MS/DB to the hippocampus. In both disorders, there is a suppression in the basal forebrain cholinergic phenotype. In the case of AUD, there is evidence from animal models to suggest that these deficits may not be permanent and can be rescued via the use of voluntary wheel running exercise [64], the actions of neurotrophins [65], and the
AChE inhibitor galantamine [66]. In contrast, chronic treadmill exercise or voluntary wheel running has been shown to attenuate age-related reduction of cholinergic fibers in the cortex and hippocampus and improve some learning and memory outcomes but has minor effects on the number of ChAT [47] positive neurons [67–69]. However, exercise has been shown to improve ACh levels in the hippocampus in an Aβ1–42 peptide rat model [70]. This does suggest that deficits caused by both alcohol-related brain damage and AD may involve a reduction in functional cholinergic neurons, leading to reductions in overall ACh signaling, which can be rescued. It, therefore, seems likely that in both of these disorders there would be a disruption in either, or both, tonic and phasic ACh signaling in the brain.

The drivers of the selective neuropathological vulnerability of cholinergic neurons, across brain disorders, are their large size and extensive projections, which require high metabolic expenditures and trophic factors to maintain the considerable cytoskeletal surface, as well as the machinery for axonal transport over long distances. These morphological properties of cholinergic neurons increase their vulnerability to oxidative stress, neuroinflammation, and altered energy homeostasis that occurs during aging and disease states [71, 72].

Human clinical data have long supported the role of chronic heavy alcohol use leading to premature brain aging, as well as a risk for the development of dementia, including AD [73–77]. Furthermore, alcohol consumption has been linked to an increased risk of dementia in individuals with a genetic predisposition to AD [78, 79]. Recent data from preclinical studies demonstrate that the consequences of adult or developmental EtOH exposure resemble advanced brain aging or produces accelerated AD-related pathology in transgenic models with AD-related transgenes [80–83]. Advanced aging, AD, and AUD have some overlapping neuropathological sequelae: upregulation of proinflammatory markers, suppressed hippocampal neurogenesis, suppression of basal forebrain cholinergic phenotype, and altered pro- and mature NT levels—as well as a change in the ratio of Trk to p75NTRs [84–88]. A common pathway for cholinergic dysfunction in AD and AUD is the disruption of neurotrophins and their receptors (see Figure 2), which may drive additive effects of AD and AUD pathology.

5.1 Alzheimer’s disease and cholinergic dysfunction

The increase in AChE activity in AD has been known for some time, as many of the drugs currently available for the treatment of this disease target this enzyme and inhibit its activity [89–91]. Cholinesterase inhibition has been shown to increase cognitive performance on the Stroop task in human patients with AD, with the degree of inhibition directly correlating with performance [92]. In AD, AChE inhibitors prolong ACh action, as well as increase the uptake of NGF to improve cholinergic neuronal survival [93]. Furthermore, cholinesterase inhibitor therapy in AD improves cognitive performance by increasing the activation of frontal cortical circuits as determined by fMRI studies [94, 95]. However, only a subset of patients with AD is effectively responsive to AChE inhibitors, and cholinergic basal forebrain integrity is a key predictor of treatment success [96].

As mentioned previously, it is likely that AChE is needed for both tonic and phasic ACh signaling, but is a more immediate causal effector of phasic signaling. It is likely that tonic signaling is required to ready the cortex for phasic signaling, and AChE inhibition may also work to increase ACh tone and facilitate cognitive performance. However, there may be a degree of specificity for phasic signaling when it comes to this modulation. Should the hypothesis that phasic signaling is more immediately
dependent on AChE than tonic signaling be supported, then modulation of AChE should have more of an effect on phasic-dependent processes. However, since tonic signaling is likely needed to modulate phasic signaling and vice versa, disentangling these two may be difficult experimentally, since the effects that AChE inhibition may have on tonic signaling may occur after that of phasic signaling, but still on a relatively short pharmacological timescale.

Likely, a balance between ACh and AChE may be critical to the phasic ACh signaling needed for sustained attention. Thus, disorders such as AD disrupt attentional processes by dysregulation of AChE, leading to dysfunction of phasic ACh signaling in the cortex. It has been shown that presenilin-1 (PS1), the catalytic component of the γ-secretase complex and therefore the formation of amyloid β (Aβ) [97, 98] affects the processing of PRiMA, as was shown by the use of using hamster ovarian cells transfected with AChE_T and a PS1 conditional knockout mouse. They showed that γ-secretase inhibition led to an increase in both PRiMA and AChE_T and that G4 AChE was increased in the membrane rafts in the PFC of PS1 KO mice, representing a potential mechanism by which AD pathology imparts alterations to AChE function [99]. Furthermore, PS1 transgenic mice show increased AChE activity throughout the entirety of the basal forebrain, prior to any behavioral deficits, suggesting that AChE dysfunction may be one of the first steps in the cascade of pathology associated with AD [100]. This increase in AChE activity is typically the therapeutic target of cholinesterase-inhibiting drugs such as donepezil, one of the most widely used drugs for the treatment of AD.

Romberg et al. [101] tested 3xTgAD mice, a strain with APPsw, PS1M146V, and tauP301L mutations to recapitulate some of the major features of AD in humans, on...
a 5-choice serial reaction time task (5-CSRTT) and found that while the transgenic mice were able to match wild-type controls in performance early on in the task, they would later begin to show impairments across the duration of the task, specifically as stimulus duration was reduced, signifying deficits in sustaining attention. These deficits were rescued via the administration of donepezil, demonstrating that inhibiting the alterations to typical AChE activity was sufficient to restore similar functioning to wild-type mice, suggesting that this particular task is reliant on AChE to modulate cortical cholinergic activity [101]. It is likely that AD, through its upregulation of AChE activity, perturbs the frontocortical circuitry necessary for attention by disrupting phasic ACh signaling. Similarly, donepezil was able to reduce scopolamine-induced omissions during the 5-CSRTT, demonstrating that inhibiting AChE can compensate for muscarinic receptor antagonism during this task [102].

Nicotinic receptors have been heavily implicated in the pathogenesis of AD, which likely disrupts nicotinic-dependent signaling in the cortex, and as discussed above has been linked to phasic signaling. This dysfunction likely originates with the SK family of Ca\(^{2+}\) sensitive K\(^+\) channels, as treatment with a selective agonist of these channels improves the nAChR function [103]. Dysfunction with such circuitry is evident by the fact that impairments in sustained attention have been shown using a mouse model of AD [104], demonstrating likely deficits with phasic cholinergic deficits, which, as mentioned before, is at least partially dependent on the utilization of nAChRs in the prefrontal cortex [42, 48]. Additionally, there is some evidence to suggest a link between Aβ pathology and nicotinic dysfunction, as it has been shown that Aβ prevents nicotine-induced inhibitory signaling, but not excitatory signaling, in PFC pyramidal neurons in vitro [105], suggesting that Alzheimer’s disease pathology may be disrupting the balance of excitation and inhibition necessary for phasic ACh signaling in the PFC. The link between Aβ and nAChR function is further supported by data showing that infusion of Aβ increased α-bungarotoxin autoradiography binding to the α7 nAChR in the frontal cortex exclusively in animals that received weekly attentional stimulation, suggesting that α7 nAChR functionality may be impaired by Aβ, but regular activation of attentional circuitry can activate compensational mechanisms in both the cortex to attempt to restore regular functioning [106].

Similarly, within the cortex itself, there are deficits to muscarinic receptors as well, as M1 mAChRs are being considered as a potential target for treatment [107]. However, work using radioligand labeling of human participants with AD has demonstrated no changes in M1 labeling in the cortex in AD, only in the dentate gyrus [108]. It is possible that despite no changes in M1 expression in the cortex, there are still deficits to its typical function, as postmortem analysis of the brains of individuals with AD showed reduced G-protein coupling of the M1 receptor in the cortex, despite no change in its density [109], mirroring the radioligand results from Scarr et al. [108]. It is possible that impaired function of the M1 receptor in AD serves as an indicator of dysfunctional tonic ACh signaling in the cortex, but as mentioned previously, the relationship between muscarinic receptors and tonic signaling is not one-to-one, and there is likely some contribution of nicotinic receptors to tonic signaling dependent behaviors as well.

5.2 Alcohol use disorder and cholinergic dysfunction

The cholinergic pathology in AUD is similar to that of AD, with some overlap in the effects of binge ethanol exposure during adolescence and age-related cognitive decline [110]. It is therefore likely that alcohol-related damage to the basal forebrain
leads to dysfunction of tonic and phasic ACh signaling as well. Acute ethanol exposure in rats has been shown to lead to deficits in sustained attention, demonstrating that phasic ACh signaling in the cortex is likely dysregulated during intoxication [111], though the impairments due to acute ethanol likely have to do with the depressive effect of ethanol throughout the entirety of the brain. However, exposure to binge levels of ethanol in adolescence has been shown to lead to deficits that persist across the lifespan [112–114]. Adolescent intermittent ethanol (AIE) exposure has been shown to lead to reductions in ChAT immunostaining in the NbM in adulthood, an effect exclusive to rats that are exposed to ethanol in adolescence, but not adulthood [115]. This loss of ChAT has been shown to be rescued by galantamine, an AChE inhibitor [66]. Likely, this type of alcohol exposure leads to an upregulation of cholinesterase activity, leading to overactive hydrolysis of ACh that is making it difficult for precisely time-locked phasic ACh signaling to occur, similar to AD.

However, there is some evidence to suggest that overactive AChE is leading to some of these deficits in ways beyond its disruption of phasic ACh signaling. It has been shown that overactive AChE induces apoptosis in both living mice and cell cultures exposed to ethanol [116], suggesting that it is possible that ethanol may overstimulate AChE activity and lead to an apoptotic cascade. However, it is unclear how this relates to the rescue of alcohol-related deficits to cholinergic phenotype, as it has been shown that ChAT cells in the basal forebrain are not dead following exposure to binge levels of ethanol in adolescence, but rather are entering a quiescent state that can be rescued either via neurotrophins [65], cholinesterase inhibiting drugs [66], or voluntary wheel running exercise [64]. The relation between the quiescent state these cells take, and the apoptotic mechanism described is yet to be ascertained.

Similarly, AIE has been shown to have effects on tonic ACh signaling in the prefrontal cortex. Adolescent alcohol exposure has been shown to attenuate behaviorally relevant acetylcholine efflux in the PFC during a spontaneous alternation task, which was accompanied by reductions in ChAT in the NbM and the medial septum/diagonal band (MS/DB), suggesting that AIE disrupts innervation of the PFC by the basal forebrain, leading to a reduction in cholinergic tone [117]. Likely, ACh tone is needed during this task to induce a state of general arousal in which the animal is actively attenuating to extra-maze cues to determine the arms of the maze it has visited already and to avoid visiting the same arms consecutively [13]. Fernandez and Savage [112] also demonstrated parallel behavioral impairments, as rats exposed to AIE showed deficits on operant attention set-shifting task, a task that has been shown to be dependent on the mPFC [118]. The exact role of phasic ACh signaling during this task is yet to be investigated, but it is possible that the detection of a visual cue to indicate which of the two levers indicates reward is dependent on a similar mechanism to cue detection in the sustained attention task, and the fact that AIE rats are impaired on the shift from spatial side reinforcement to a visual cue determining reinforcement suggests that these two tasks may be dependent on similar mechanisms. However, more work is needed to determine whether phasic acetylcholine signaling is required for attention.

Other models of alcohol-related brain damage have shown similar effects on the cholinergic system and the prefrontal cortex. For example, adult rats either fed a pyrithiamine deficient diet (PTD), given access to ethanol in their drinking bottles (CET), or a combination of both (PTD-CET) were shown to have impaired spontaneous alternation behavior and reduced ACh efflux in the mPFC during this task, which was accompanied by a reduced latency to lever press during a set shift during operant attention set shifting despite no impairment in performance [119]. This suggests that
there are tonic ACh signaling deficits in this model, as demonstrated by the decreased ACh efflux, as well as possible phasic signaling deficits, as the increased amount of time needed to make a lever press in PTD, CET, and PTD-CET animals suggests that the time course of ACh signaling in PFC is being disrupted. The deficits seen during spontaneous alternation during PTD have been shown to be rescued by the AChE inhibitor tacrine [120], suggesting a role of AChE overexpression in the pathology seen in this disorder, suggesting that overactive AChE, mirroring what is seen in AD, is responsible for the tonic ACh deficits. Presumably, these deficits would extend to phasic ACh-dependent processes, but they were not tested here. The fact that cholinesterase inhibition has such an effect on tonic signaling is interesting, but not surprising, as while the mechanism proposed within this review suggests that AChE is more important for phasic signaling, it is nevertheless required to regulate ACh tone as well, exerting its actions both directly by hydrolyzing extracellular ACh and indirectly by regulating phasic signaling and synaptic spillover.

Alcohol-related brain damage and AD seem to converge on the cholinergic system, and the projections from the basal forebrain to the prefrontal cortex seem to be a set of circuits that show particular vulnerability to perturbations. Dysfunction of these circuits likely has effects on both tonic and phasic ACh signaling simultaneously, and it seems that, at the moment, it would be conceptually difficult to investigate an experimental manipulation that would affect one type of signaling but not the other. However, it may be possible to determine whether the time course of the impairments seen in these two types of signaling differ, such that perhaps phasic signaling is first affected by the early cholinergic deficits seen in AD, which later expands to tonic signaling deficits later on. This remains to be determined and represents a future direction for research into modes of ACh signaling.

6. Conclusions and future directions

As the distinction between the roles of tonic and phasic ACh signaling becomes clearer, it is important to understand the specific dysfunction that is occurring in both of these types of signaling in the cortex in disorders such as AD and AUD. It is likely that these two disorders have effects on both muscarinic and nicotinic ACh receptors and that these deficits are part of what is driving the dysregulation of the two types of ACh signaling in the cortex. Additionally, the role of AChE in this distinction cannot be overstated, as its role in hydrolyzing synaptic ACh may be one of the most important regulators of phasic ACh, with its inhibition by high ACh concentrations possibly leading to spillover into the extracellular space and therefore augmenting ACh tone that is usually otherwise due to a different set of basal forebrain cholinergic cells.

The availability of fluorescent ACh sensors for fiber photometry allows for the measurement of ACh activity in vivo on a second timescale. Studies in which changes to ACh signaling following alterations to normal AChE activity can be assessed and are needed to determine the exact role of this enzyme in cortical cholinergic synapses, and how its dysfunction relates to some of the deficits seen in common cholinergic disorders. Likewise, the contribution of alterations to typical nAChR and mAChR function in the PFC to phasic ACh signaling could be investigated using photometric methods. Studies looking directly into how tonic and phasic ACh utilize different cellular machinery seem to be elusive for now, but further breakthroughs in the field could lead to new avenues to address this gap in the literature.
Furthermore, reexamining AD and alcohol-related brain damage through the lens of the tonic phasic distinction may allow researchers to hone in on the exact mechanisms by which cholinergic dysfunction occurs in these diseases and therefore develop new treatments. A reconceptualization of these disorders in terms of the mounting evidence that ACh in the prefrontal cortex is phasic in regard to many of the cognitive symptoms seen in AD may lead us to a better understanding of how the current pharmacological treatments for these diseases work and how to improve them. Similarly, updating our understanding of alcohol-related brain damage using this new conceptualization of ACh signaling in the prefrontal cortex may lead the field into novel treatments and ways to prevent or even reverse cholinergic deficits that arise via exposure to heavy amounts of alcohol. Such advancements may be possible with a reevaluation of the way that ACh signaling in the cortex contributes to behavior.
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