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Phasic acetylcholine release and the volume transmission hypothesis: time to move on

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Abstract

Traditional descriptions of the cortical cholinergic input system focused on the diffuse organization of cholinergic projections and the hypothesis that slowly changing levels of extracellular acetylcholine (ACh) mediate different arousal states. The ability of ACh to reach the extrasynaptic space (volume neurotransmission), as opposed to remaining confined to the synaptic cleft (wired neurotransmission), has been considered an integral component of this conceptualization. Recent studies demonstrated that phasic release of ACh, at the scale of seconds, mediates precisely defined cognitive operations. This characteristic of cholinergic neurotransmission is proposed to be of primary importance for understanding cholinergic function and developing treatments for cognitive disorders that result from abnormal cholinergic neurotransmission.

The entire cortical mantle is innervated by cholinergic neurons that originate in the nucleus basalis of Meynert, the substantia innominata and the horizontal limb of the diagonal band — all structures of the basal forebrain (BF) (FIG. 1). Traditionally, the cortical cholinergic input system has been categorized as the rostral component of the brain's ascending arousal systems, complementing the modulatory roles of, and interacting with, noradrenergic, serotonergic and other projection systems that broadly influence the readiness of the forebrain for input processing, wakefulness and somnolence[1]. However, more recent evidence has supported the more specific hypothesis that cortical cholinergic inputs mediate essential aspects of attentional information processing[2–9]. As a result, efforts to develop treatments for a wide range of cognitive disorders have focused on cholinomimetic approaches, particularly acetylcholinesterase (ACHE) inhibitors and agonists at muscarinic (m) and nicotinic (n) acetylcholine (ACh) receptors (AChRs)[10–12].

The anatomical organization of the cortical cholinergic input system seems to be largely consistent with the notion of a diffuse pathway (this article does not address the hippocampal cholinergic projection system or cholinergic projections to the amygdala). Tracing studies revealed a roughly ventrolateral, dorsomedial and rostrocaudal topographical organization of cholinergic BF projections but did not suggest a more precise topography that would indicate, for example, that adjacent neurons in the BF innervate adjacent regions in the cortex[13–16] (FIG. 1 b,c). Nearly all cortical layers and regions are innervated by BF cholinergic neurons[17], although the distribution of choline acetyltransferase (CHAT)- or ACHE-positive fibres in the

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cortex indicates differences in the density of the cholinergic innervation of specific layers[18–21] (FIG. 2). This seemingly diffuse organization of the cortical cholinergic input system has supported descriptions that it exerts general, uniform effects across the cortical hemispheres[20].

In contrast to other diffusely organized ascending systems, such as the ascending reticular systems of the brainstem, the axons of corticopetal cholinergic neurons (subcortical afferents that project to both cerebral hemispheres) do not seem to be extensively collateralized: individual neurons innervate a relatively small cortical field[22–24]. Thus, separate cortical regions, such as frontal and parietal regions, are not innervated by the same cholinergic neurons, suggesting that these regions may be differentially modulated by the cholinergic input system.

It has recently been proposed[14,15,25] that the corticopetal cholinergic system is less diffusely organized than was traditionally assumed (FIG. 1 b,c). In support of this hypothesis, it has been demonstrated that there are clusters of cholinergic cells in the BF[15,25,26] and that the BF receives modality-specific projections[27]. The morphological heterogeneity of BF cholinergic neurons (see REFS 28,29) and of their efferent and afferent projection systems, including the degree to which they exhibit a topographical organization, remains insufficiently understood[13]. For example, the finding that manipulations of the excitability of the nucleus accumbens affect prefrontal ACh release but not the release of ACh in parietal regions[30,31] does not correspond with traditional descriptions of the organization of this system: it is more consistent with views suggesting a refined anatomical or functional topographical organization of the BF corticopetal projection system.

Cholinergic transmission modes

Central to the debate about the organization and function of the cortical cholinergic input system is the question of whether cholinergic neurotransmission is restricted to classical synapses (wired transmission) or is capable of escaping the synapse to stimulate distant, extrasynaptic mAChRs and nAChRs (diffuse, paracrine, non-junctional or volume transmission)[32,33] (FIG. 3; TABLE 1). Despite the inconclusive evidence concerning the transmission mode that characterizes cortical ACh release, briefly reviewed below, contemporary models formalizing the functions of the BF cholinergic system and efforts to develop pro-cholinergic treatments have been based largely on the assumption that ACh is volume transmitted[7,10]. However, new evidence suggests that phasic transmission might have a central role in the cholinergic system.

Wired versus volume transmission

Some studies concluded that the great majority of cholinergic terminals in the cortex of rats and humans form synaptic contacts[34,35], supporting the notion that cholinergic transmission is mainly wired. By contrast, other studies that quantitatively analysed the ratio of cholinergic pre- to postsynaptic structures[36–40] demonstrated that mAChRs are present at non-cholinergic synapses[41], providing strong support for volume transmission of ACh. Furthermore, measures of basal ACh release obtained using microdialysis have been interpreted to indicate an extracellular ambient level of ACh[37], estimated to be in the high nanomolar to low micromolar range[42].

The reasons for the discrepancy between these studies remain unclear, and other studies have also produced apparently contradictory results. For example, the presence of extrasynaptic Ml and M2 mAChRs in the cortex with high affinity for ACh suggests volume transmission[41]. However, other studies that analysed the relationships between cholinergic innervation and the distribution of mAChRs suggested a close correspondence, indicative of wired transmission[18,43]. With respect to nAChRs in the cortex, the predominant presence of nAChRs

at presynaptic terminals of glutamatergic and other cortical afferents[44] has been suggested to be indicative of an extrasynaptic role and therefore volume transmission[45–47]. Furthermore, extrasynaptic α7 nAChRs have been demonstrated to be present in subcortical regions[46]. However, it is not clear whether the presence of heteroreceptors by default indicates the presence of non-junctional complexes, and whether the extrasynaptic location of these receptors, specifically α7 nAChRs, indicates volume transmission or unrelated signalling events[48–50].

As microdialysis probes are too large to enter the synaptic cleft, the recovery of ACh using microdialysis has been attributed to the presence of ACh in the extracellular space. However, the exquisite sensitivity of ACh levels collected by microdialysis to depolarization blockade by tetrodotoxin (for example, see [REF. 51]) suggests that ACh collected by this method both is tightly controlled by presynaptic activity and originates from synapses located extremely close to the microdialysis membrane. Furthermore, tetrodotoxin administration to ACHE-deficient mutant mice, with 100 times the normal level of basal ACh (4.6 nM instead of 556 nM), decreased ACh levels by 98%[52]. This finding illustrates that basal ACh levels measured by microdialysis closely reflect synaptic activity.

The interpretation that ACh recovered by microdialysis is indicative of volume transmission assumes that many of these presynaptic terminals form non-junctional release sites and/or are associated with concentrations of ACHE that are insufficient to completely hydrolyse newly released ACh. However, insertion of microdialysis probes results in oedema, haemorrhage, blood–brain barrier disruption, decreases in various enzymes associated with neurotransmitter synthesis and metabolism, intracellular changes, gliosis, neurodegeneration and lasting suppression of glucose metabolism[53–58]. Therefore, the ACh is in essence recovered from scarred tissue[57], and so the conclusion that microdialysis results demonstrate volume transmission must be treated with caution (see also [REFS 59,60]).

Acetylcholinesterase

The exceptional catalytic power of ACHE (one molecule of ACHE can hydrolyse 5,000 molecules of ACh per second[61–63]) and the presence of ACHE clusters at the synapse[64] have traditionally been cited in support of wired transmission of ACh. It has been suggested that as ACh dissociates from receptors it is so effectively hydrolysed by free ACHE units that it is virtually impossible that a single molecule of ACh may escape and activate another receptor[65]. The view that an enzyme that is characterized by such high catalytic power functions primarily to maintain a relatively stable extracellular ACh concentration contrasts boldly with the more traditional view that ACHE serves to rapidly and completely hydrolyse newly released ACh, thereby preventing spillover into the extrasynaptic space.

For volume transmission to occur, or for ACHE to have only a minor role in the immediate elimination of newly released ACh[37], the ACHE must be assumed to be limited in concentration, compartmentalized and/or regulated, and to thereby limit ACh metabolism. Much of our understanding of ACHE localization and enzymatic activity has been derived from work at the muscular endplate, where clusters of ACHE can be found. It has been suggested that the characteristics of ACHE localization at synapses in the brain might be sufficiently different to allow volume transmission[37]. It is not yet known whether this is the case, as evidence concerning the exact localization of synaptic, membrane-bound ACHE in the forebrain remains scarce. Likewise, the regulation and function of the soluble forms of synaptic ACHE are also poorly understood. Secreted, freely diffusing ACHE, although it constitutes a minor proportion of the overall ACHE in the brain, could hydrolyse ACh even if membrane-bound ACHE became saturated following a massive release event[66].

Using enzyme-selective microelectrodes and fixed-potential amperometry, it is possible to measure choline spikes that result from the hydrolysis of newly released ACh[67-71]. Likewise, by immobilizing ACHE on the surface of the recording electrode, in addition to choline oxidase (CHOX), ACh can be directly detected because it is hydrolysed on the recording surface. This adds to the choline signal detected by CHOX and yields larger currents than those obtained with recordings that detect choline only. As indicated by the results of calibration studies, electrodes coated with both ACHE and CHOX were able to detect low nanomolar concentrations of ACh, and co-immobilization of ACHE and CHOX did not affect the sensitivity of such recording sites to choline alone (for details see [REF. 72]). Massive depolarization events, triggered *in vivo* by application of KCl[72], did not result in the detection of larger current amplitudes by these doubly coated recording sites. ACh amounts at the recording sites were estimated at > 150 fmol. Although it cannot be excluded that lower levels of extracellular ACh exist and that electrodes equipped with more potent enzymes[73] would reveal this, the above studies should have detected a KCl-evoked extra-synaptic concentric wave of a millimolar concentration of ACh[37,40] in support of volume transmission if this were the case.

In summary, the evidence concerning the presence and the degree of volume transmission of ACh remains inconclusive. The ongoing debate is further complicated by the insufficiently and variably defined criteria and characteristics for both modes — wired and volume — of transmission (TABLE 1). It is possible that extracellular ACh diffuses only over short distances, thereby maintaining or establishing a fast form of non-synaptic transmission[45]. As we explain next, the exact mode of cholinergic neurotransmission may be of minor importance compared with the implications of new evidence that illustrates the phasic characteristics of functional ACh release.

Phasic cholinergic signalling

Here we discuss the potential functional implications of evidence which indicates that phasic cholinergic signals lasting seconds underlie the functions of the cortical cholinergic input system. This evidence *per se* does not reject the possibility that extrasynaptic cholinergic transmission takes place, particularly over short distances as described above[45]. However, it does question the functional significance of persistent ambient levels of extracellular ACh[37, 74].

The temporal resolution of studies that use microdialysis to measure ACh release is on the scale of minutes (BOX 1). Consequently, conclusions based on microdialysis data were consistent with the conventional characteristics of ACh acting as a neuromodulator, including slowly changing release levels and volume transmission. With the advent of enzyme-selective microelectrodes, the measurement of ACh concentrations with a sub-second resolution has become possible[68,69], and real-time measurements of ACh release *in vivo* have necessitated revisions of hypotheses concerning the regulation and function of the cortical cholinergic input system.

Recording prefrontal ACh release in animals performing a cued appetitive response task, we found that transient increases in ACh at the scale of seconds mediate cue-evoked attention to the reward ports (termed cue detection[9]; see BOX 1 for a paradigmatic illustration of such cholinergic transients). Furthermore, we measured ACh release using microdialysis in task-performing animals and demonstrated that the results, collected over an 8 min period, could be reproduced using the second-scale cholinergic transients by summing up and averaging these transients over 8 min periods (see supplemental data in [REF. 9]). Thus, the differential functions of the cortical cholinergic input system can be described by using different temporal units of behavioural and cognitive processes[8]. This evidence did not substantiate the presence

of a separate, independent mode of cholinergic neurotransmission characterized by slower, minute-scale changes in cholinergic activity.

Recent research has begun defining the local prefrontal circuitry that is responsible for generating phasic cholinergic signalling[44]. The evidence suggests that such cholinergic signals result from local intracortical glutamate–choline interactions, with glutamate signals that originate from thalamic afferents and stimulation of ionotropic glutamate receptors, presumably situated on cholinergic terminals, representing key steps in the generation of cholinergic transients.

Implications for neuropsychopharmacology

If fluctuations in basal extracellular ACh levels were functionally significant, ACHE inhibitors, by blocking the hydrolysis of ACh and thereby robustly elevating basal extracellular ACh levels, would be expected to markedly enhance cue detection processes and associated attentional performance. Likewise, drugs that act as mAChR agonists and mimick the tonic stimulation of these receptors that results from increased extracellular ACh levels should enhance the cognitive functions of healthy subjects and patients suffering from cognitive impairments. However, the efficacy of such cholinomimetic treatments has remained strikingly below expectation[75-77]. Although numerous mechanisms might contribute to the limited pro-cognitive effects of these compounds, such treatments are unlikely to augment, amplify or mimic the phasic characteristics of cholinergic activity that mediate cognitive operations, and thus they would not be expected to facilitate the cognitive functions that depend on cholinergic activity.

In contrast to pharmacological approaches that focus on elevating extracellular levels of ACh and direct stimulation of mAChRs, nAChR agonists, specifically α4β2*-selective nAChR agonists, generate phasic cholinergic signals and are proposed to robustly enhance cognitive functions[44]. Even the non-selective nAChR agonist nicotine, when given systemically to animals performing a cued appetitive response task, enhances the proportion of trials that involve cue detection and, mechanistically, augments the amplitude of the cholinergic transient that mediates cue detection[78]. Accumulating clinical evidence indicates that nAChR agonists enhance the cognitive abilities of a wide range of patients[79-81]. Although the overall evidence presently remains limited, nAChR agonists seem to act primarily by enhancing the temporally precisely orchestrated phasic cholinergic activity that mediates fundamental cognitive mechanisms.

Thus, the available neuropsychopharmacological evidence points to phasic ACh release being of crucial significance. The focus on developing cognition enhancers that amplify cholinergic transients represents a clear departure from the more traditional view that modifying persistent extracellular levels of ACh is a useful neuropsychopharmacological target.

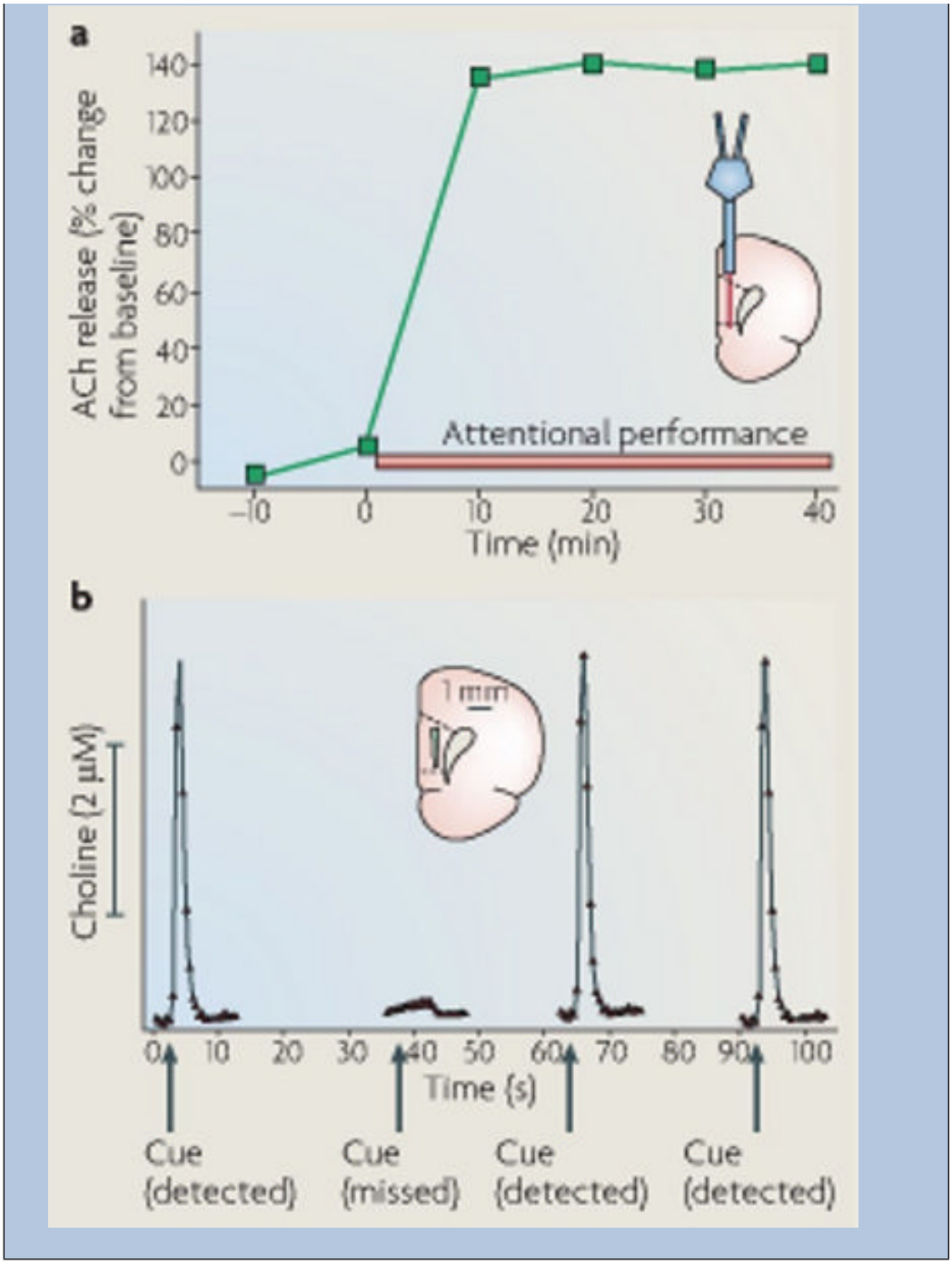
Different modes — time to move on?

The presence or absence of volume transmission and the degree, in terms of distance and time, of extrasynaptic effects of ACh remain unresolved issues. Indeed, it is difficult to conceive of an experiment that would conclusively reject the possibility that a proportion of ACh spills into the extracellular space. A more constrained version of volume transmission, involving fast extrasynaptic actions of ACh (as proposed in REF. 50), might be more plausible than the extreme hypothesis that fluctuations in extrasynaptic ACh levels are key to understanding cholinergic function. However, this debate seems to be less crucial for future research on the functions of cholinergic systems and for the development of pro-cognitive therapies than the implications of the phasic signalling characteristics of ACh that were found in recent experiments[8,9,44].

Box 1

ACh in attentional performance

During the performance of a sustained attention task, acetylcholine (ACh) release typically increases by 120–140% over the pre-task baseline. Part **a** of the figure shows a paradigmatic illustration of prefrontal ACh release, measured using *in vivo* microdialysis of animals performing an attentional task (the inset illustrates the placement of a microdialysis probe on a coronal section of the rat medial prefrontal cortex). Such increases are not observed in animals performing various control tasks. Because of the low temporal resolution of this technique (10 min collections were required to produce a single detectable data point), data from such studies are consistent with the conventional description of slowly changing levels of ACh, mediating 'arousal' states. If ACh release indeed had these characteristics, the question of whether, and to what degree, ACh is volume transmitted would be crucially important to hypotheses concerning the functions of this neuronal system. However, as illustrated in part **b** of the figure, our recent experiments using choline-sensitive microelectrodes (the placement of an electrode with four platinum- and enzyme-coated recording sites fabricated into its tip is illustrated in the inset) indicated that cholinergic activity occurs at the scale of seconds, and that transient increases in ACh release mediate the detection component of attention tasks. (Detection is defined as a cognitive process that involves the incorporation of a cue into the ongoing cognitive and behavioural process and therefore allows the cue to control behaviour; for details see [REF. 9].) The graph provides a paradigmatic illustration of the cholinergic transients that are evoked by a cue in trials that result in the detection of such a cue. Cues that fail to evoke such transients are missed (for actual data see [REF. 9]). We previously demonstrated that summing and averaging second-scale increases in cholinergic activity in task-performing animals statistically reproduced minute-scale ACh release data obtained using microdialysis (see the evidence described in the appendix of [REF. 9]). These highly orchestrated cholinergic transients are arguably a more important characteristic of cholinergic neurotransmission than the potential existence of volume transmission, rendering the question about the presence and degree of volume neurotransmission of secondary importance. Data in part **a** are modelled from data in [REF. 93].



As the functional significance of neurotransmission mode has also been debated with respect to several other neuromodulator systems[82-84], such as serotonergic and noradrenergic systems, our conclusions might generalize to these systems. For example, ultrastructural evidence indicated a high proportion of non-junctional neurotransmitter receptor complexes at serotonergic and noradrenergic terminals. Furthermore, the ability to measure release of these modulators using microdialysis has been interpreted as evidence for the existence of volume transmission[85,86]. However, it is intriguing to speculate that, similar to ACh, these neuromodulators code discrete information based on phasic release patterns. It may be further speculated that it is a common feature of all ascending neuromodulator systems that although

their anatomical organization reflects a diffuse and not obviously structured projection system, the regulation of their terminal activity by local (cortical) circuitry, involving heteroreceptors situated on their terminals, allowed the evolving forebrain to use the neuromodulator input to generate precisely orchestrated signals and mediate defined cognitive operations.

Concerning the mesolimbic dopamine system, there is strong evidence for the presence of both phasic and tonic neuronal activity, interactions between the two, and associated patterns of dopamine release (for example, see REFS 87,88). Moreover, differential functions of dopamine have been attributed to it acting at different timescales, ranging from quasi hormonal functions of tonic levels of dopamine release to reward and outcome processing by phasic release[89]. However, tonic release can also result from asynchronous firing of groups of dopaminergic neurons[90,91]. Furthermore, as the timescales that are applied to describe the release of a neurotransmitter are necessarily confounded by the temporal and spatial resolution as well as the sensitivity of detection techniques, the increasing use of electrochemical techniques to measure dopamine release will provide interesting new insights into the nature of functional dopamine release at the scale of seconds (for example, see REF. 92).

If transient release patterns are sufficient to explain the target area-specific cognitive operations that are mediated by ascending ‘neuromodulator’ systems, we could finally unchain ourselves from the misleading constraints of outdated descriptions of ‘arousal’ systems and their associated dichotomies, including phasic versus tonic and volume versus wired neurotransmission, and the classification of signalling molecules as neurotransmitters versus neuromodulators. Instead we could focus on determining how evolving forebrain circuits usurped these massive input systems. By modulating neurotransmission of these inputs based on heteroreceptors situated on their terminals and local microcircuitry contacting these terminals, forebrain target areas can use seemingly diffusely organized input systems to generate function-specific, transient changes in neurotransmitter release that initiate or foster the mediation of specific cognitive operations.

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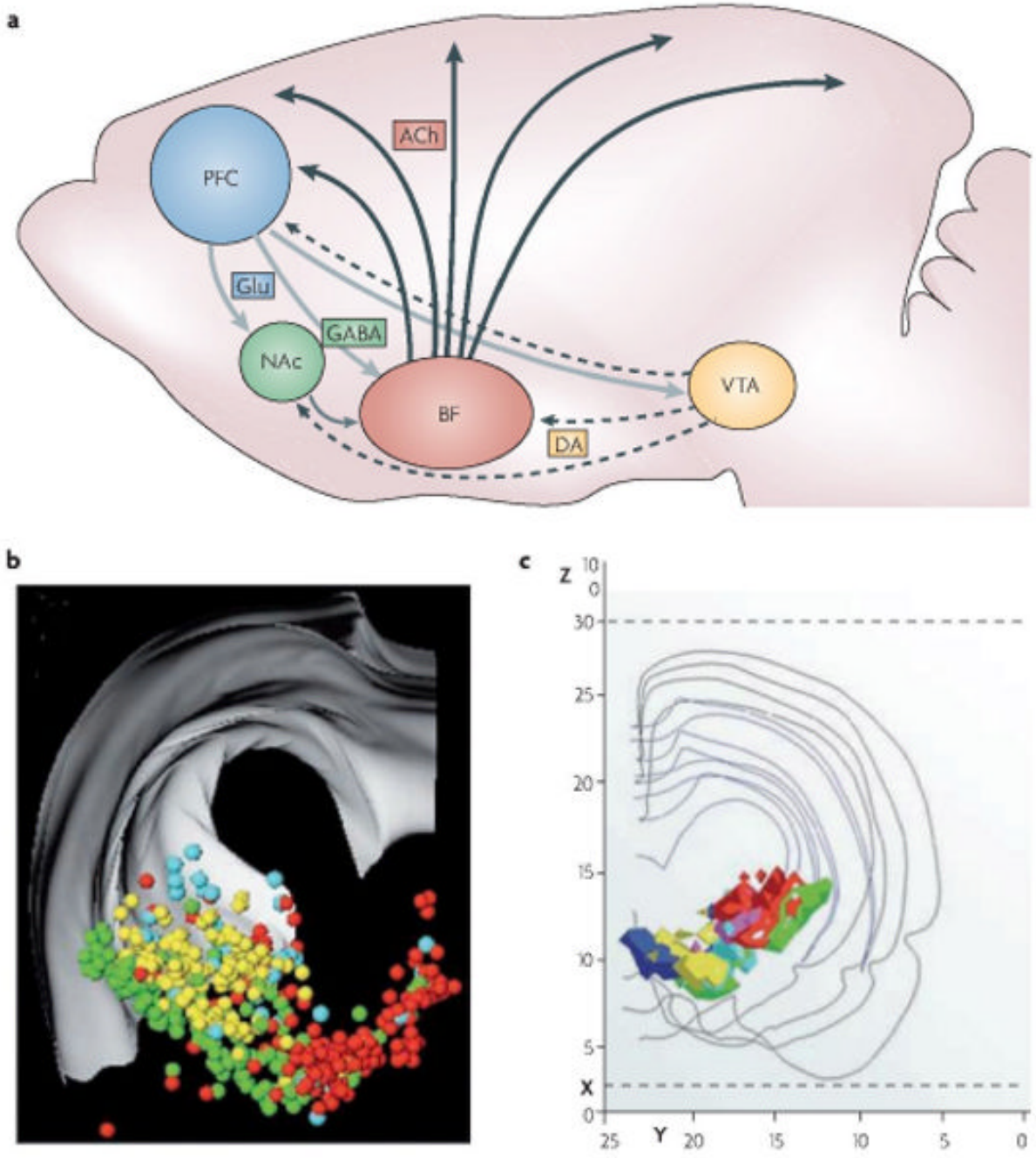

Figure 1. The cortical cholinergic input system

a | Basal forebrain (BF) efferent cholinergic projections to the entire cortical mantle, and the main telencephalic afferent projection systems of the BF (view at a sagittal section). Cholinergic neurons originate from the nucleus basalis of Meynert, the substantia innominata and the vertical and horizontal nuclei of the diagonal band of Broca (collectively termed the BF) and innervate all cortical areas and layers. The prefrontal cortex (PFC) is the only cortical region, in rodents and primates, that is known to project back to the BF both directly and indirectly (through the nucleus accumbens (NAc)). The BF, PFC and NAc are also all innervated by dopaminergic neurons from the ventral tegmental area (VTA), and these dopaminergic neurons in turn are contacted by PFC projections. This organization suggests a

profound control of the BF by the PFC. Not shown are brainstem projections to the BF. **b** | A composite map showing the three-dimensional distribution of cholinergic cells projecting to four arbitrarily defined mediolateral sectors of the neocortex. Cells projecting to different regions are colour-coded (medial: red; intermediary sector: blue and yellow; lateral parts of the neocortex: green). Note the relatively ordered rostromedial to caudolateral distribution of cells projecting to mediolaterally located cortical areas.**c** | A surface density-based render of the major organizational features in the BF (unit space: 400 × 400 × 50 μm; density threshold > 2 cells per voxel; the numbers along the z axis are the layers (sections) and the x and y values correspond to the voxel indices; for details see [REF. 14]). The colours of the units represent the brain regions that the cholinergic cells in those areas project to (blue: posteromedial cortex; yellow: medial prefrontal cortex; red: barrel cortex; green: posterior insular-perirhinal cortex; light blue: agranular insular-lateral orbital cortex; magenta: lateral frontal (motor) cortex). ACh, acetylcholine; GABA, γ-aminobutyric acid. Parts **b** and **c** are reproduced, with permission, from [REF. 14] © (2002) Springer.

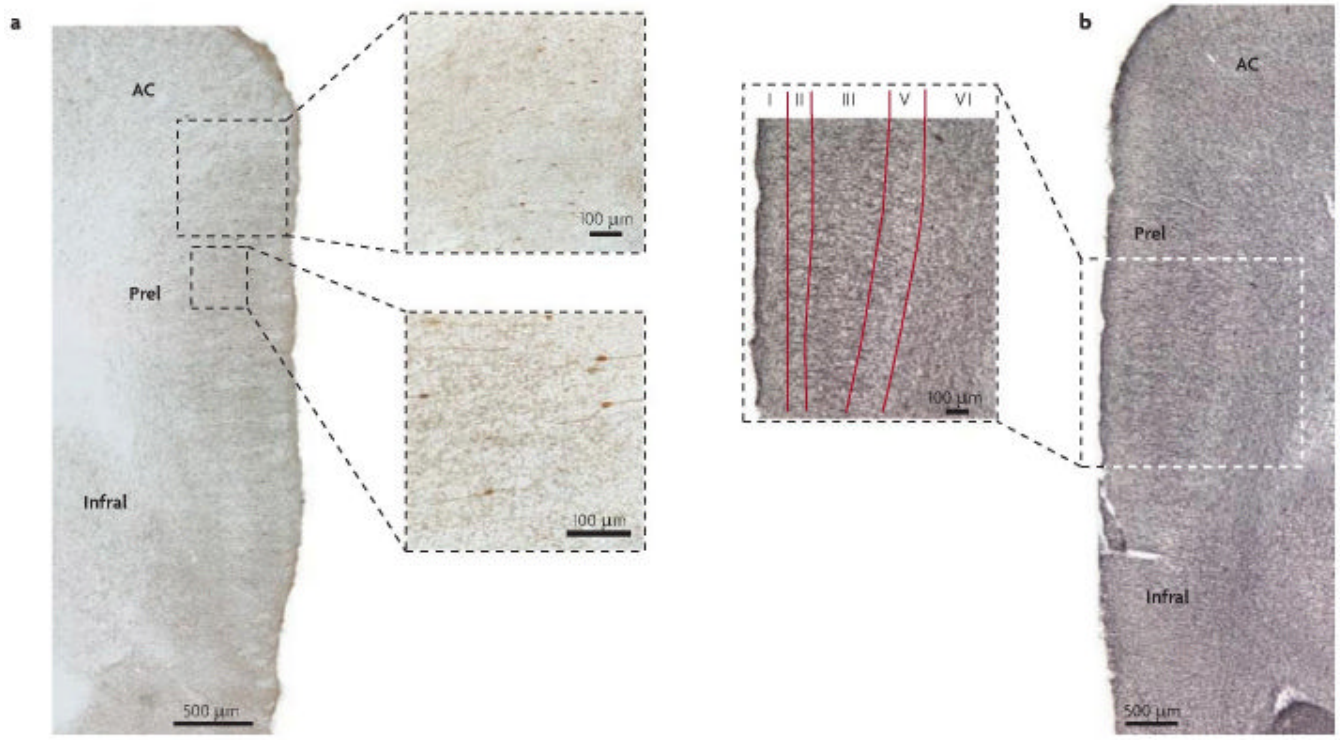

Figure 2. Cholinergic fibre distribution in the cortex

Coronal sections of the medial prefrontal cortex of the rat, visualized using choline acetyl-transferase (CHAT) immunohistochemistry (**a**) or a histochemical method for revealing acetylcholinesterase (ACHE)-positive fibres (**b**), are shown to illustrate the distribution of cholinergic fibres in the cortex. The low resolution sections in parts **a** and **b** show the anterior cingulate cortex (AC), the prelimbic cortex (Prel) and the infralimbic cortex (Infral); the expansions show photomicrographs of the stippled areas, with the cortical layers indicated for part **b** (note that in the rat the Prel is agranular (there is no layer IV)). CHAT immunoreactivity reveals fine varicose fibres and darkly stained bipolar interneurons with axons and dendrites that are organized perpendicularly to the pial surface. The phenotype of these neurons remains elusive[94]: they do not express p75 receptors and thus are unaffected by local infusions of the cholinotoxin 192 immunoglobulin G–saporin. Similarly, visualization of ACHE-positive fibres reveals dense cholinergic input in all layers. Except for some minor layer-specific organizational differences, the two methods reveal essentially similar patterns of cholinergic input (see also [REF. 18]). The density of cholinergic inputs is similar throughout the cortex, except that there are higher densities of cholinergic input to entorhinal and olfactory regions[17]. Part **b** is modified, with permission, from [REF. 72] © (2008) Elsevier.

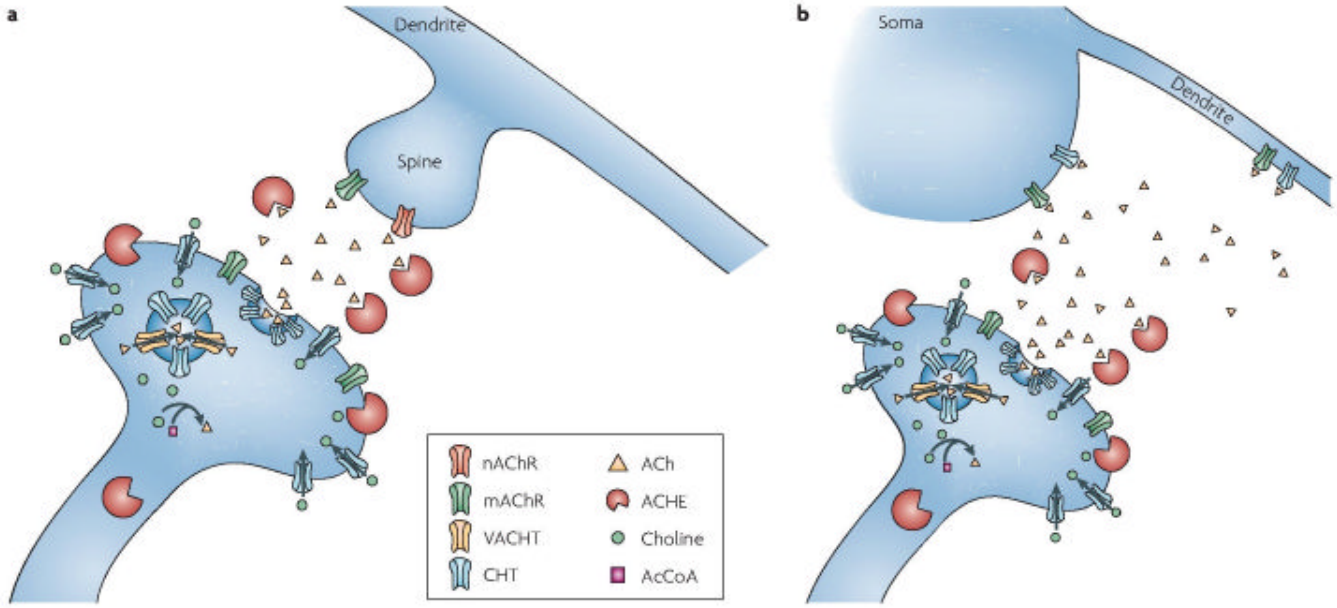

Figure 3. Major steps in the synthesis, release and metabolism of ACh, and the main characteristics of wired and volume transmission

Except for localized increases in choline resulting from acetylcholine (ACh) hydrolysis by acetylcholinesterase (ACHE), extracellular concentrations of choline are stable at ~4.85 μM[95]. To synthesize ACh, choline is transported into the terminal through choline transporter (CHT)[95]. In the terminal, choline acetyltransferase (CHAT) catalyses the synthesis of ACh from choline and acetyl CoA (AcCoA). The capacity of CHT is the most significant determinant of the rate of ACh synthesis. ACh is packed into vesicles by vesicular acetylcholine transporter (VACHT) and released on depolarization of the terminal. Following release, ACh can bind to nicotinic (n) and muscarinic (m) ACh receptors (AChRs) and is rapidly hydrolysed by ACHE to yield choline and acetate. In the wired model of cholinergic neurotransmission (**a**), the presence and high catalytic activity of ACHE restricts the neurotransmission to classic synapses or junctional complexes. By contrast, in the volume model of cholinergic neurotransmission (**b**), most presynaptic cholinergic terminals in the cortex do not form junctional complexes and so neurotransmission is mediated by ACh that escapes hydrolysis because of insufficient or regulated availability and/or activity of ACHE. This ACh reaches the extracellular space and can stimulate non-junctional nAChRs and mAChRs. As discussed in the main text, the generation of second-scale cholinergic transients seems to represent a more important characteristic of cholinergic neurotransmission than either mode of neurotransmission.

Table 1

Main characteristics of volume and wired neurotransmission

	Volume	Wired
Distribution of AChR and release sites	Non-junctional complex	Junctional complex
Transmission specificity	Transmission 'privacy' is limited to the specificity of the neurotransmitter and the selectivity of receptors	Transmission 'privacy' is based on the presence of a transmission channel dedicated to this neurotransmitter at this synapse
Ratio of pre- to postsynaptic sites	One source of neurotransmitter release affects many targets	One-to-one neurotransmission
Transmission timeline	Long transmission delay	Minimal transmission delay
Type of coded information	Produces widespread, general effects on 'arousal', 'readiness for processing', vigilance or somnolence	Inserts discrete and essential information into target circuits
Mimicking transmission mode	Effects of released neurotransmitter can typically be reproduced by administering direct agonists at target receptors or drugs that elevate extracellular levels of the neurotransmitter (for example, uptake inhibitors)	As direct receptor agonists or drugs that elevate extrasynaptic neurotransmitter levels do not reproduce the phasic pattern of neurotransmission that typically codes the information transferred to the target neuron, such drugs typically fail to reproduce the effects of endogenously released neurotransmitter
Energy costs	Low energy demands; opportunistic transmission along energy gradients is used for other purposes	Consumes more space and involves higher energy costs

AChR, acetylcholine receptor. Table is adapted from Agnati *et al.*[33].