Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Spatiotemporal specificity in cholinergic control of neocortical function

William Muñoz and Bernardo Rudy

Cholinergic actions are critical for normal cortical cognitive functions. The release of acetylcholine (ACh) in neocortex and the impact of this neuromodulator on cortical computations exhibit remarkable spatiotemporal precision, as required for the regulation of behavioral processes underlying attention and learning. We discuss how the organization of the cholinergic projections to the cortex and their release properties might contribute to this specificity. We also review recent studies suggesting that the modulatory influences of ACh on the properties of cortical neurons can have the necessary temporal dynamic range, emphasizing evidence of powerful interneuron subtype-specific effects. We discuss areas that require further investigation and point to technical advances in molecular and genetic manipulations that promise to make headway in understanding the neural bases of cholinergic modulation of cortical cognitive operations.

Addresses
NYU Neuroscience Institute, NYU School of Medicine, Smilow Research Building Sixth Floor, 522 First Ave, NY, NY, 10016, United States

Corresponding author: Rudy, Bernardo (Bernardo.Rudy@nyumc.org)

Current Opinion in Neurobiology 2014, **26**:149–160

This review comes from a themed issue on **Inhibition: synapses, neurons and circuits**

Edited by **Gordon Fishell** and **Gábor Tamás**

For a complete overview see the Issue and the Editorial

Available online 15th March 2014

0959-4388/$ – see front matter,

http://dx.doi.org/10.1016/j.conb.2014.02.015

Introduction

Acetylcholine (ACh) release and the associated transformation of cortical networks as a result of its specific cellular actions on nicotinic and muscarinic receptors, play crucial roles in normal cognitive function. Interest on the cortical actions of ACh was first provoked by the effects of cholinergic drugs in humans: pharmacological activation of muscarinic cholinergic receptors produces delirium, while receptor blockade generates severe anterograde amnesia. Moreover, the dementia of Alzheimer's and Parkinson's diseases has been associated with the loss of cortical cholinergic innervation. While findings in humans, and a large body of experimental work in animal models, strongly implicate ACh in arousal, attention, sensory gating and memory processes, the precise systems and cellular level physiological bases of these modulations of cortical operations remain unknown.

Classical notions hold that the cholinergic system achieves this by releasing ACh diffusely across the cortical mantle, activating its receptors globally and producing slow responses. While this scheme might be applicable to behavioral fluctuations that are experienced over several minutes or longer such as arousal, it is hardly compatible with the experimentally observed properties of cholinergic influences on attention, sensory and motor responses or plasticity and learning. For instance:

(a) *Local* application of cholinergic antagonists and cholinergic denervation of *specific* cortical regions generates impairments in attention and learning, suggesting that cholinergic influence *within functionally distinct cortical areas* is crucial for specific behaviors [1••,2,3]. Conversely, nicotinic receptor knockout animals with cognitive deficits can be rescued when nicotinic receptors are re-expressed in *specific* cortical regions [4••] or in *specific* neuronal populations [5••].

(b) Studies of receptive field plasticity and memory emphasize the importance of the temporal interaction between cholinergic and sensory signals [6•]. Pairing cholinergic activation with a sensory stimulus triggers long-lasting enhancement of sensory-evoked responses if the two events coincide. Increasing time lags between the sensory and cholinergic signals abolishes the enhancement or even produces a depression of the conditioned responses [7,8].

(c) Optogenetic stimulation of cholinergic cells in the basal forebrain rapidly activates cortical networks (~126 ms latency, [9••]); much faster than presumed. The cholinergic actions on cortical neurons underlying this apparent reorganization of cortical dynamics must be even faster.

(d) Choline-sensitive electrochemistry has demonstrated phasic changes of ACh concentration in rats performing an attention task. These changes had a restricted cortical spatial distribution and precise temporal association with cue detection [10••].

These and other examples demonstrate that, contrary to the concept of global, slow broadcast, cholinergic signals and their resulting modulatory impact can regulate cortical dynamics and processing with remarkable spatiotemporal

precision. These properties allow the cholinergic system to participate in cortical processes that require that contextual cholinergic signals act in concert with local computations, such as the processing of relevant sensory inputs, in order to guide behavior. Here, we will review emerging data on the central question of how this *specificity* and *spatiotemporal range of function* might be supported by: (A) the functional organization of the cholinergic projections to the cortex, (B) the mode of transmission of ACh at cholinergic terminals, and (C) the cell-type specificity and dynamics of its actions on excitatory and inhibitory cortical neurons; factors outlined in Figure 1. We emphasize the actions of ACh on inhibitory neurons, as neuromodulation of inhibitory tone is posed to exert widespread network effects by virtue of the dense axonal arborization of GABAergic interneurons, the importance of these cells in shaping principal cell activity, and the observation that inhibitory tone can affect the induction of synaptic plasticity. Moreover, the existence of many distinct types of cortical interneurons, each with specific connectivity and impact on principal cell physiology, and their interneuron-specific cholinergic modulation, contributes to the diversity and spatiotemporal specificity of cholinergic modulation of cortical activity and functions.

Is localized ACh release supported by the functional organization of cholinergic projections?

Cholinergic projections to the neocortex arise from neurons in the nucleus basalis and associated magnocellular nuclei in the basal forebrain [11]. These projections have been classically thought to innervate the cortex diffusely, exerting global cholinergic control [12–15]. Nevertheless, hints of an organization scheme were found in the rough topographic layout of the rostrocaudal sequence of neurons in the basal forebrain, projecting to progressively medial-to-lateral cortical spaces [16–18]. Dual retrograde tracing suggested that while, as a whole, the projection system follows this trend, labeled regions of cholinergic cells in the basal forebrain still largely overlap when the dyes are injected in distant cortical areas [16,19]. Yet, the same experimental approach suggested that individual cholinergic cells innervate a restricted cortical space [16,18–21,22•]. Together, these findings led to a *mosaic* model in which cholinergic cells with localized terminal domains, project to different cortical regions than neighboring cells (Figure 1A). Even if focally activated, a system with this organization would be posed to have widespread effects across the cortex [19].

Recent experimental evidence indicates that this scheme is simplistic and incomplete. Importantly, it cannot account for the localized 'phasic' component of ACh release that has been measured in attending animals [10••]. An exciting proposal comes from re-examining with retrograde tracing the anatomical overlap of cholinergic neurons in relationship to the cortico-cortical interactions between their projection domains. Zaborszky and colleagues found that the degree of overlap of labeled neuron location within the basal forebrain is positively correlated to the connection strength between the different injected cortical regions [22•]. These finding suggest a shift in understanding from the *mosaic* to a *modular* organization of the cholinergic system (Figure 1A). In this new model, segregated bands of neighboring cholinergic cells do not project disparately across the cortical mantle, but instead possess defined cortical targets that are, in turn, functionally associated.

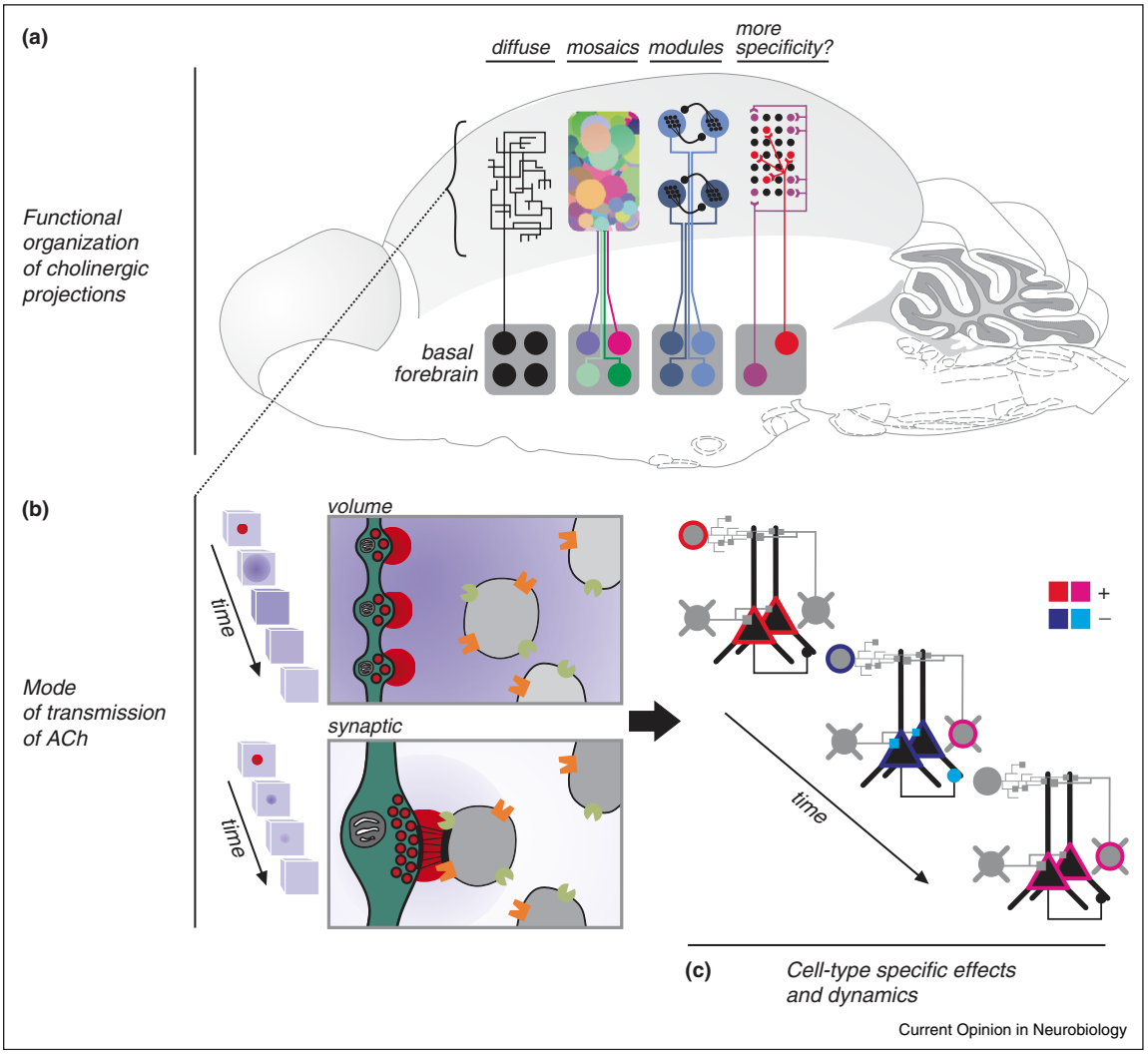
However, it is important to consider that these morphological inferences about basal forebrain cholinergic neurons come from double retrograde tracer experiments, which are associated with a number of caveats. *To date, completely reconstructed cholinergic neurons with the complete extent of their cortical terminal arborizations have not been reported.* The issues discussed here beg for a reappraisal of the anatomy and the specific connectivity of cells of the cholinergic projection system in the context of current genetic and molecular tools (Figure 1A).

Aside from anatomical considerations, ACh release might also be finely regulated, locally within terminal cortical areas, by a presynaptic mechanism. Based on electrophysiological and pharmacological evidence, it has been suggested that behavior-dependent glutamate release can lead to the activation of glutamate heteroreceptors on cholinergic axons, triggering ACh release [23,24]. In the model resulting from these observations, even a diffusely organized projection system might attain anatomical precision by virtue of the specificity of glutamatergic signaling. A similar glutamatergic presynaptic regulation has been suggested for mesolimbic dopamine release, wherein glutamate released from limbic and cortical axons has a *facilitating influence* on 'tonic' dopamine efflux [25,26].

Spatiotemporal specificity from the transmission mode of cholinergic terminals?

Current choline-sensitive electrochemistry can detect 'phasic' and 'tonic' changes in extracellular acetylcholine concentration in the scales of seconds and minutes, respectively [10••]. While these measurements are more precise than data obtained through traditional microdialysis, synaptic neurotransmission does not lie within the sensitivity range of this methodology. Indeed, whether ACh is released at classic synaptic junctions or from non-junctional sites has been a matter of much debate, and is an issue with profound implications for the spatiotemporal scale of cholinergic influences on cortical neurons. Ultrastructural inspection of presumed cholinergic release sites, or varicosities, have revealed conflicting pictures regarding their junctional specializations, from the nearly complete absence of synapses, to the moderate

Figure 1



Factors that control the spatiotemporal specificity of cholinergic actions in the cortex. The spatiotemporal precision of the cholinergic system is a function of a number of factors, three of which are discussed throughout this review. **(A)** First, the *functional organization of cholinergic projections to the cortex.* The spatial extent and specificity of individual and collective cholinergic cell innervation of the cortex is a major determinant of the spatial range of ACh release and impact. Anatomical investigation of these issues led to two organizational models (*diffuse and mosaic*) of the cortical cholinergic projections from the basal forebrain. These models fit with classical notions of diffuse and global cholinergic broadcast, as activation of either model system would lead to widespread cortical ACh modulation. However, recent anatomical reexamination suggests that the functional organization of these projections might follow a different principle. In the proposed *modular* model, neighboring cholinergic cells project to distinct cortical areas that are, in turn, functionally interconnected. Such an organization could mediate localized ACh release in specific cortical regions, as well as coordinate modulatory influences across computational pathways in the cortex. Modern anatomical and genetic approaches offer opportunities to continue delving into the organization and specificity of the cholinergic projections. For instance, whether there is specificity in the cortical postsynaptic targets of individual cholinergic cells remains elusive, and could be investigated with new tools and approaches that take advantage of advances in molecular genetics. Moreover, although the anatomical terminal innervation of the cholinergic system sets upper and lower spatial boundaries for cholinergic actions, understanding the functional scale of ACh impact requires that we consider the functional inputs to the cholinergic projection system, an issue that is not discussed here. Whether functional inputs activate this structure diffusely, or whether they focally drive circumscribed cholinergic projection cell groups remains to be investigated. **(B)** A second factor determining the spatiotemporal precision of cholinergic action is the *mode of transmission of ACh at cholinergic terminals* in the cortex. It has been argued that ACh is released from non-junctional sites, mediating slow and diffuse activation of cholinergic receptors over large cortical spaces (*volume transmission*). However, recent anatomical and electrophysiological data suggest that the point-to-point precision of classical synaptic transmission can be observed in the cortical cholinergic system, circumscribing fast cholinergic signaling to contacted cortical elements. **(C)** The third factor determining spatiotemporal precision is the *diversity and dynamics of the cell-type specific effects* of ACh. The spatiotemporal profile of ACh concentration interacts with the particular sensitivity, kinetics, and localization of diverse cholinergic receptors in postsynaptic targets. Together with cell-type specific expression of these receptors and downstream signaling cascades, these interactions determine the spatiotemporal coordination, interplay and predominance of a diversity of ACh effects in specific cortical cells and networks.

presence of subtle, synapse-like contacts, and to the existence of *bona fide* symmetric and asymmetric synapses [27–32]. Given that cholinergic signaling has been classically thought to act slowly and diffusely, reports indicating scarcity of synapses have been considered principal evidence supporting the concept of volume transmission: once ACh is released, it slowly diffuses until it reaches and influences a large extrasynaptic pool of receptors in wide cortical fields (Figure 1B; [33]).

However, reports that most cholinergic varicosities don't form synapses must be considered in light of a number of caveats. For instance, cholinergic synapses have been described as 'remarkably subtle and small, usually only identifiable in one or two sections,' and not showing prominent thickening of membrane appositions, suggesting that they may be rejected by some investigators because they fail to meet standard criteria for synapse classification [27]. Hence, determining whether a cholinergic varicosity forms a synapse or not may require complete serial analysis to verify that small junctions are not missed. Also, evidence that cholinergic varicosities are the preferential sites of synapse formation is lacking. In the case of innervation in the neostriatum by dopaminergic axons, it has shown that synapses are not preferentially formed at varicosities [34].

Are these small, synapse-like contacts functionally relevant? Given their meager appearance, one might argue that these contacts are not true synapses. However, the fact that these contact sites are often associated with accumulated vesicles argues against that [27,28,35•]. Furthermore, a recent study has demonstrated that these profiles are associated with postsynaptic specializations containing neuroligin 2 [35•]. Neuroligins are known to participate in trans-synaptic signaling complexes with presynaptic neurexins that result in the recruitment of synaptic proteins, and maturation of both pre- and postsynaptic specializations. On this basis, the authors argue that these contact sites should be considered synapses, as well as point out that such densely labeled neuroligin 2 sites in cholinergic terminals are encountered far more frequently than presumed.

It is evident that the predominance and physiological relevance of synaptic ACh release in the cortex needs to be reassessed.

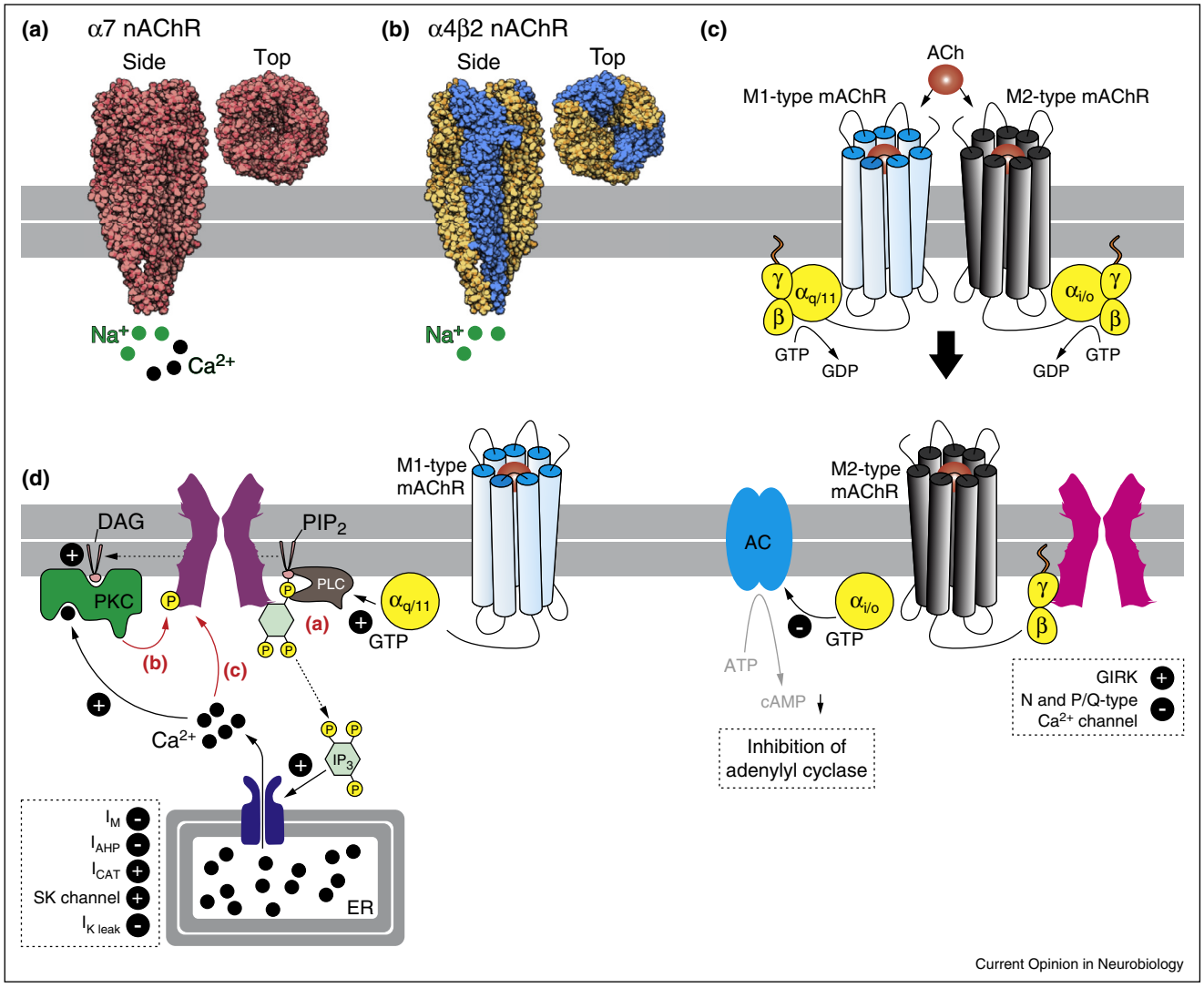
Diverse and specific cellular mechanisms of cholinergic control in neocortex.

Ultimately, the spatiotemporal range of cholinergic signals influences the cortex by activating nicotinic and muscarinic receptors that are expressed ubiquitously by different cortical neurons (Figures 2 and 3). Starting with the groundbreaking experiments of Krešimir Krnjevic, intense effort has gone into dissecting these cellular actions of ACh, with the goal of understanding the mechanisms of cholinergic modulation of network processing [36]. For the most part investigators have characterized cholinergic responses of cortical neurons in acute slices, resulting in the discovery of a large number of cellular effects of cholinergic action. These are summarized in Figure 3 and Table 1 (see also reviews [37,38]), and show that cholinergic signaling can increase or decrease neuronal excitability, as well as increase or decrease synaptic efficacy in a cell type-specific fashion.

Cholinergic effects on pyramidal cells (PCs) have been extensively studied, in different species and cortical areas, and there is general agreement that ACh enhances their excitability. Dampening effects have also been reported for pyramidal and spiny stellate cells, the glutamatergic cells that are the main recipients of sensory signals in layer 4 (Table 1). Much less understood are the effects of cholinergic modulation on GABAergic interneurons (INs), neurons that have critical roles in shaping network dynamics and functions [39–43]. This diverse population of local-circuit inhibitory cells selectively innervate specific postsynaptic domains of principal cells, with each cell type implicated in a particular operational role and hence cholinergic effects on these neurons are likely to be of major significance and represent a potential source of specificity. However, not only is our knowledge of cholinergic modulation of these neurons incomplete, but the literature is full of contradictory observations, stemming in part from difficulties in identifying interneuron subtypes (Table 1). In fact, an influential paper [44] found no correlation between subpopulations of INs defined on the basis of morphology and physiology and their responsiveness to neuromodulators. This lead to the view that either INs cannot be classified in a few discrete groups or specific subtypes do not have specific neuromodulatory responses.

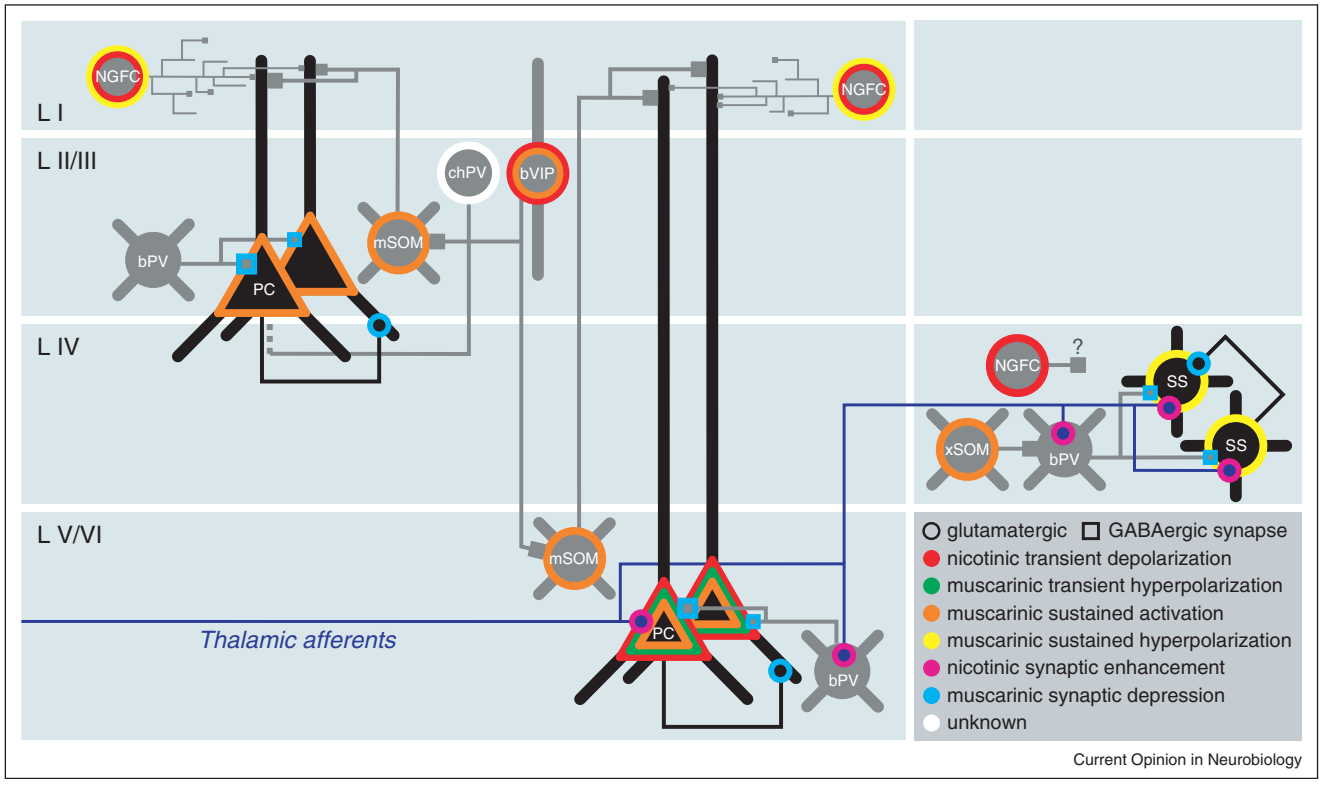
A different picture is now emerging (see also [45]). Work primarily in rodents has shown that the expression of certain molecular markers can serve as an initial platform to classify interneuron subgroups with defined developmental origins, anatomy, intrinsic electrophysiological properties, and connectivity. These markers include the calcium-binding protein parvalbumin (PV) and the neuropeptide somatostatin (SOM), which label non-overlapping interneuron subpopulations. PV cells comprise both fast spiking (FS) basket and chandelier cells, while the SOM subgroup includes at least two subtypes, (1) the Martinotti cells in layer 5 and in layers 2/3, that target the distal dendrites of PCs, and (2) layer 4 SOM interneurons that target mainly FS cells in the same layer [46]. Additionally, we recently showed that the expression of the ionotropic serotonin receptor (5-HT3aR) defines a third non-overlapping population of INs, and that PV, SST and 5-HT3aR-expressing INs together account for nearly 100% of all GABAergic cells in somatosensory cortex [47••,48,49]. The 5-HT3aR subgroup can be further divided in two subpopulations based on the

Figure 2



Ionotropic (nicotinic) and metabotropic (muscarinic) receptors mediate the effects of ACh. (a and b) Nicotinic AChRs are pentameric proteins consisting of a large variety of subunits. The subunit composition dictates channel function. Those expressed in the brain primarily exist as α7 homopentamers (a) or α4β2 heteropentamers, usually with a 2α, 3β stoichiometry (b) [38]. Shown are renderings of the side and top view of the receptors based on the closed structure of the Torpedo AChR (PDB code: 2bg9). The α4 subunit is colored blue, the β2 subunit yellow, and the α7 subunit red. These two nAChR subtypes display dramatically distinct kinetics and pharmacological properties [38]. Nicotinic responses are generally excitatory. Receptor activation produces transient depolarization due to the permeability of the ligand-gated channel with an equilibrium potential close to 0 mV. However, in addition to providing depolarization, α7 nAChRs can mediate slower cellular responses by virtue of the especially large Ca^{2+} permeability of these receptors. **(c)** Muscarinic receptors are G-protein coupled receptors (GPCRs), with the typical seven transmembrane domain structure of these proteins. Five different subtypes are known (M1-M5), of which four subtypes M1-M4 are the predominant ones in neocortex. Their functions depend on the signaling cascades that are initiated by the binding of ACh, which in turn largely depend on the subtype of heterotrimeric G protein associated with the receptor. M1, M3 and M5 (often referred to as M1-type) couple to Gq/11 G proteins, while M2 and M4 (often referred to as M2-type) are Gi/o-coupled receptors [57]. Upon binding of ACh to the receptor, the GDP associated with the G protein is exchanged for GTP. **(d)** Association with GTP produces the dissociation of the G protein. In the case of M1-type receptors the αq/11 subunit activates the enzyme phospholipase C β (PLC), which hydrolyses the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) resulting in the loss of PIP2 from the membrane and the production of diacyl glycerol (DAG) and inositol triphosphate (IP3). IP3 produces Ca^{2+} release from intracellular stores. DAG and Ca^{2+} activate protein kinase C (PKC). The loss of PIP2 (a), PKC-mediated phosphorylation of channels and other downstream targets (b) and Ca^{2+}-mediated signaling (c) produce downstream effects. In the case of M2-type receptors the αi/o subunit produces inhibition of adenyl cyclase resulting in a decrease of cAMP levels. The βγ subunit complex diffuses through the membrane and binds to G-protein activated inward rectifier K^+ (GIRK) channels activating them, or to N or P/Q type Ca^{2+} channels inhibiting them, or to other targets. Muscarinic effects can be excitatory or inhibitory depending on the targets of the signaling pathways activated by the receptor and can vary in different cells. They can also vary in a given cell at different times depending on the state of the cell. For instance to obtain IP3-dependent Ca^{2+} release, the Ca^{2+} stores must be full.

Figure 3



Nicotinic and muscarinic responses in neocortical neurons and their synapses. Cholinergic agonists regulate the function of neocortical neurons and their synapses in a cell-specific fashion. M1-type muscarinic modulation produces a sustained increase in the excitability of pyramidal neurons (PC) in supragranular and infragranular layers by inhibiting several types of K^+ channels (see Figure 2, Table 1). In addition, perisomatic ACh produces a transient hyperpolarization as a result of the activation of SK Ca^{2+}-activated K^+ channels that precedes the sustained depolarization and is seen predominantly in layer V PCs. A nicotinic mediated depolarization, capable of eliciting spiking, has also been reported in L5 PCs in some cortical areas (see Table 1). In contrast to the sustained activation of PCs, ACh produces a sustained hyperpolarization of spiny stellate cells (SS) in layer 4 of somatosensory cortex. It has been suggested that this hyperpolarization may serve to filter weak thalamocortical inputs and favor the activation of spiny stellate cells by stronger, more synchronous inputs. Muscarinic and nicotinic responses have also been observed on GABAergic neurons. Muscarinic agonists powerfully depolarize and increase the activity of SOM-expressing Martinotti (mSOM) cells in layers II/III and V/VI. These neurons have an ascending axon that targets and inhibits the distal dendrites of PCs. SOM-cells in L4 of somatosensory cortex (xSOM) differ in morphology and intrinsic firing properties from Martinotti cells. Their axons don't innervate L1 but profusely branch in L4, where they target local FS basket cells and are thus capable of disinhibiting this layer. These cells are also powerfully excited by muscarinic action [46]. Although there are contradictory observations regarding the effects of cholinergic modulation of the excitability of parvalbumin (PV)-expressing FS basket cells (bPV), most investigators agree there is no effect. The effect of ACh on chandelier cells (cPV), the second subtype of PV-interneuron has not been studied. In spite of their heterogeneity, all 5HT3aR IN subtypes are depolarized by ACh via nicotinic receptors. These interneuron group includes several subtypes, of which the two most prominent are illustrated in the figure: the neurogliaform cells (NGFC) which inhibit PCs and the bipolar/bitufted VIP INs (bVIP), which inhibit SOM INs and thus mediate disinhibition of excitatory neurons. There is evidence of muscarinic responses in some subpopulations within this family (Table 1), but they have not been studied in detail. In addition to these effects of cholinergic agonists on the excitability of cortical neurons, they have also been shown to modulate neurotransmitter release of several types of cortical synapses. Cholinergic agonists inhibit glutamate release from intracortical recurrent excitatory axons and GABA release from the terminals of FS basket cells (bPV) on excitatory neurons via activation of M2-type receptors; presumably by inhibiting N and P/Q type Ca^{2+} channels at the terminal. In contrast, cholinergic modulation increases thalamocortical inputs onto principal cells via nicotinic receptor modulation.

expression of the neuropeptide VIP: (1) VIP-expressing INs that include the bipolar, bitufted, CHAT-expressing cells, and (2) the non-VIP-expressing group that includes the neurogliaform cells. Based on their preferential localization in upper layers, it was suggested that 5HT3aR INs might be important mediators of top-down interareal processing, a hypothesis supported by recent observations [50•,51•,52–54].

Although much remains to be done, advances in our understanding of interneuron diversity and the use of these molecular markers as genetic entry points, allowing us to record and manipulate specific INs [55], is starting to reveal unique and specific ways in which ACh modulates distinct subtypes of INs in a given cortical area. Cholinergic modulation of these IN subtypes can trigger both inhibitory and disinhibitory effects with powerful impact

Table 1

Cholinergic actions on neocortical neurons and synapses

Cell type	Effect	ACh receptor	Cellular target of modulation
Pyramidal neurons[a]	Long lasting enhancement in excitability and responsiveness to depolarizing inputs (increased input resistance; slow, sustained voltage-dependent depolarization, and a reduction in spike frequency adaptation)	M1-type mAChR	Inhibition of the channels mediating I_M; I_{AHP}, the Ca^{2+}-activated K^+ current that mediates the slow AHP; and I_{Kleak}
Pyramidal neurons[b]	Increased slow afterdepolarization (ADP). Persistent activity.	M1-type mAChR	Activation of yet to be identified non-specific cationic channels (I_{CAT}).
LV pyramidal neurons[c]	Transient depolarization	nAChR	
Pyramidal neurons[d]	Transient hyperpolarization preceding the sustained depolarization	M1-type mAChR	Elevation of intracellular Ca^{2+} activates SK channels.
LIV spiny stellate cells in rat barrel cortex[e]	Persistent hyperpolarization	M2-type mAChR	Activation of GIRK K^+ channels
Intracortical excitatory synapses[f]	Inhibition of glutamate release	M2-type mAChR	Presumably by the membrane-delimited inhibition of N- and P/Q-type Ca^{2+} channels at the terminal
FS PV basket cells[g]	No effect on excitability	ACh, muscarine	
FS PV IN basket cell synapses on PCs[h]	Inhibition of GABA release	M2-type mAChR	Presumably by the membrane-delimited inhibition of N- and P/Q-type Ca^{2+} channels at the terminal
LII/III and V SOM INs[i]	Potent depolarization and long lasting increase in spiking	M1-type mAChR?	Unknown signaling pathway
LIV SOM INs[j]	Potent depolarization and long lasting increase in spiking	mAChRs	Unknown signaling pathway
SOM INs (LTS cells)[k]	Transient depolarization	nAChR	
VIP 5HT3aR INs[l]	Transient depolarization	Non α7 nAChR	
nonVIP 5HT3aR NGFCs[l]	Transient depolarization	α7 and non α7 nAChR	
nonVIP 5HT3aR nonNGFC[l]	Transient depolarization	nAChR	
nonVIP 5HT3aR NGFCs[m]	Hyperpolarization of NGFCs and suppression of GABAB-mediated inhibition of the apical dendrites of layer II/III pyramidal cells	M1-type mAChR	Elevation of intracellular Ca^{2+} activates SK channels.

[a] A muscarinic increase in the excitability of pyramidal neurons has been reported in many species, including humans [67,68], and in several cortical areas. The review by McCormick [69] covers this effect extensively (see also [70,71]). The muscarinic suppression of the M-type K^+ channels contributes voltage-dependence to the depolarization and enhances the responsiveness to synaptic inputs that reach the range of activation of these channels. There are indications that these effects have a slow onset and are sustained for long time periods (many minutes), but detailed quantitative data is lacking. Carr and Surmeier [72] suggested that inhibition of Kir2 inward rectifier K^+ channels contributes to the M1 receptor depolarization and enhanced summation of excitatory inputs in LV PCs in prefrontal cortex. [73] reported that carbachol reduced the persistent Na^+ current of LV PCs. McCormick and colleagues [69], as well as others also reported a transient inhibition of PCs, particularly in supragranular layers, that precedes the excitation and was suggested to be the result of a muscarinic activation of yet to be identified GABAergic neurons.

[b] Activation of nonspecific cationic channels inducing a slow afterdepolarization and long lasting persistent activity (up to 30 min) was reported in various cortical structures, including entorhinal and cingulate cortices. The underlying channels, perhaps members of the TRP family, have not been identified. According to some reports it is Ca^{2+}-dependent but other investigators have suggested it is Ca^{2+}-independent [74–79].

[c] [71,80–82].

[d] Gulledge and Stuart [62••] described a transient hyperpolarization of layer V PCs produced by the activation of perisomatic SK Ca^{2+}-activated K^+ channels that preceded the sustained depolarization of the neurons. The SK channels are activated by the Ca^{2+} released from intracellular stores as a result of the M1-type receptor-mediated production of IP3. In contrast to the activation of the PCs, which is observed in PCs in all layers and in all the cortical areas that have been investigated the hyperpolarization is more selective [83]. It is observed in rat L5 PCs in prefrontal, somatosensory and visual cortex, but is most robust in prefrontal cortex. On the other hand, PCs in layers II/III are much less responsive, except for deep layer III neurons of visual cortex. The same receptors, via a different signaling pathway, have been shown to *inhibit* SK channels in dendritic spines of hippocampal CA1 PCs by a casein kinase 2-dependent reduction of their Ca^{2+} sensitivity [84,85]. This inhibition enhances synaptic potentials and NMDA receptor mediated Ca^{2+} transients and facilitates the generation of LTP. It remains to be investigated whether this action also occurs in neocortical PCs.

[e] [70].

[f] [72,86–88].

[g] It has been reported in several cortical areas that cholinergic agonists have no effect on the somatodendritic excitability of PV FS cells identified based on physiological properties and post hoc IHC for PV [83,89] or single-cell RT-PCR [90], or using a mouse expressing GFP in PV neurons [83,89,90,91•]. However, Xiang *et al.* [92] reported that ACh hyperpolarizes electrophysiologically identified FS cells in layer V of visual cortex via muscarinic action, and Poorthuis et al., reported nicotinic responses in LII/III and LV FS INs in prefrontal cortex [80].

[h] Pronounced inhibition of GABA release at the presynaptic basket terminals of PV-expressing FS INs on PCs has been observed in somatosensory and insular cortices [91•,93], and Nunez *et al.* suggested that this effect, together with the muscarinic and nicotinic increase in excitability of the layer V PCs resulted in the generation of Ca^{2+} spikes and bursts of action potentials (APs) of the PCs when inputs in basal dendrites were stimulated [71]. Heterogeneous effects at FS cell terminals on INs, as well as on the terminals of non-identified 'non FS cells' on PCs were reported [91•,93].

[i] [46,94,95]. Mice expressing GFP in SOM neurons were used to identify IN subtype. The mAChR involved was not identified, however, a similar modulation was observed on hippocampal OLM cells, the equivalent of neocortical Martinotti cells, where it was shown to be mediated by an M1-type mAChR [96].

[j] Mice expressing GFP in SOM neurons [46], or the LTS firing pattern [46,94,95] were used to identify IN subtype.
[k] Contradictory results have been reported regarding nicotinic responses on SOM INs. [80,97] reported nicotinic effects on functionally identified SOM INs in mouse mPFC in layers II/III and V; but other studies of SOM INs have only seen muscarinic effects. The source of this discrepancy is not clear and may include age or cortical area differences.
[l] All 5HT3aR-expressing INs identified using a mouse expressing GFP in these neurons are depolarized by locally applied nicotine [47••] or the nicotinic agonist DMPP and using single cell RT-PCR to confirm the expression of 5HT3aR mRNA [98]. This group of GABAergic cells includes all layer I INs [47••], all of which have been independently shown to be depolarized via nicotinic receptors in response to locally applied ACh [83,99] or ACh optogenetically released from cholinergic afferents [59••]; and CCK-expressing INs with or without VIP [47••,90]. Layer I INs include the neurogliaform cells (NGFCs), which are also present in all layers, and all have nicotinic responses [47••]. The 5HT3aR group also includes the bipolar/bitufted VIP-expressing INs that are seen mainly in layers II/III. These neurons also show nicotinic depolarization [47••,59••,100]. Interestingly, these VIP INs have been shown to target mainly SOM INs, and hence their nicotinic activation is predicted to produce disinhibition of PCs (Figure 3; [50•,51•]). [83] reported nicotinic effects in nonidentified 'non fast spiking' (NFS) INs in layers II/III and V; this NFS INs are likely to be 5HT3aR INs. They also reported a muscarinic hyperpolarization of neurons expressing CCK. [89] reported muscarinic responses on INs identified by post hoc IHC as VIP+ or small CCK-expressing INs (depolarization) or large CCK-expressing (hyperpolarization followed by depolarization). Muscarinic modulation of different types of 5HT3aR neurons needs to be better characterized.
[m] Brombas *et al.* [101] found an M1-mediated hyperpolarization of NGFCs produced by the activation of SK Ca^{2+}-activated K^+ channels by the same signaling cascade as that producing hyperpolarization of pyramidal cells (see note d). The muscarinic inhibition of the NGFCs disinhibited the apical dendrites of layer II/III pyramidal neurons by removing the GABA B receptor-mediated inhibition of the apical dendrites produced by the GABA released by NGFCs.

on cortical dynamics and processing (Table 1, Figure 3; see also [56]).

Specificity resulting from the dynamics of cholinergic responses

The diversity of ACh effects on neocortical cells evokes a picture of staggering complexity, wherein multiple opposing and/or synergistic effects converge on the same neuron or on neurons that interact with each other, affecting network processing in ways that are difficult to predict (Figure 3, Table 1). In order to advance our understanding of the neural basis of cholinergic modulation of the cortex, it is critical to investigate *when the different cellular actions of ACh are engaged under physiological conditions* and what are their *predominance, interplay and dynamics* (Figure 1C). The understanding of these factors requires that we take into account the sensitivity of different cholinergic receptors to ACh, their kinetics and interactions with ACh release timing and duration, as well as their location with respect to the sources of ACh.

Receptor sensitivity to ACh. The ACh concentration to which receptors are exposed under physiological conditions is an issue of considerable importance, and may contribute to coordinating different cholinergic effects in time and space. This is because different receptors have different affinities for ACh. For instance, among the two most predominant types of nicotinic receptors, α7 homomeric receptors have a much lower affinity for ACh and faster desensitization kinetics than α4β2 nAChRs. The affinity for ACh also differs among different muscarinic receptors, and for a given receptor it depends on multiple factors [38,57]. Even for the same receptor and in the same cell, the modulation of different molecular targets can have different agonist sensitivities [58]. Therefore, under physiological conditions, different patterns of activity of cholinergic afferents may produce different responses; an issue that needs to be explored more thoroughly.

A related issue that may significantly affect the interpretation of experimental results is receptor desensitization as a result of too high or too prolonged exposure to the agonist, as occurs with uncontrolled puffing or bath application. In fact, most of the data in Figure 3 and Table 1 have been collected in this way, utilizing long exposures to ACh, or even non-physiological agonists, and at arbitrary concentrations, ranging from low μM to high mM. The high and broad expression of acetylcholinesterase, one of the most powerful enzymes, suggests that the system is built to allow only limited exposure to ACh.

Response dynamics. It is usually assumed that nicotinic actions are always fast and precise while muscarinic actions always slow, imprecise and sustained (Table 1). However, as we discuss below, both nicotinic and muscarinic responses can be fast and precise, as well as slow and sustained. The depolarization produced by the opening of the nicotinic channel produces the fastest responses, but the rates can vary depending on subunit composition. Homomeric receptors containing α7 subunits produce significantly faster responses than those mediated by non-α7 receptors, usually presumed to be α4B2, (e.g. rise time: 2.6 ms versus 14–35 ms; decay τ: 4.9 ms versus 190–218 ms, in [59••]; see review by [38]). However, it is important to be aware that α7 nicotinic receptors can also mediate slow cellular responses by virtue of their large calcium permeability, which can result in calcium-mediated signaling as in the case of some muscarinic responses (reviewed by [60,61]). The time course of muscarinic actions can also vary widely, depending on the signaling pathways involved. Those mediated by a membrane-delimited pathway (Gi/o; i.e. M2-type) are faster than those mediated by signaling cascades, which may involve the sequential activation of several enzymatic pathways (Figure 2). However, quantitative information on the kinetics of muscarinic responses on cortical neurons is sorely lacking.

In this context, the study by Gulledge and Stuart [62••] using fast pressure injection of ACh on PCs in cortical

slices, with a 12-ms solution exchange, is noteworthy in that it shows that there can be muscarinic responses that are much faster than usually presumed. The authors observed a transient (phasic) hyperpolarization in response to ACh, with a latency of ~344 ms, a rise time of 208 ms, and half-widths of 568 ms. The hyperpolarization was followed by a more sustained (several seconds long) depolarization, resembling the classical depolarizing response to tonic mAChR activation by prolonged agonist exposure (Table 1). Importantly, the slower depolarizing response also had a rise time of < 1 sec, and although the duration of ACh exposure required to fully produce this response was larger than that required to produce the hyperpolarization, it was still in the subsecond range (τ=139 ms versus 19 ms). Since both effects were apparently mediated by the same M1-type receptor, the results of this study also show that under physiological conditions different durations of ACh release might engage a different set of effects, even if mediated by the same receptor type within the same cell.

Furthermore, the study of Gu and Yakel [63••,64] suggests that not only is the absolute duration of ACh release important for its actions, but also the relative timing with respect to glutamatergic inputs. In hippocampal CA1 region, when cholinergic inputs were activated 100 or 10 ms *prior* to Schaffer collateral stimulation, this resulted in α7 receptor-dependent long-term potentiation (LTP) or short-term depression, respectively. However, when the cholinergic stimulation was delayed until 10 ms *after* the Schaffer collateral stimulation, a muscarinic receptor-dependent LTP was induced. These results demonstrate remarkable temporal precision of cholinergic actions and suggest complex interactions between the kinetics of receptor activation and the ongoing activity of the modulated cell.

A recent study in the reticular thalamic nucleus (TRN) provides another example of fast and precise nicotinic and muscarinic signaling. Sun *et al.* [65••] used electrical stimulation to evoke ACh release from cholinergic afferents in the TRN. Even a *single* 200 μs extracellular stimulus evoked a biphasic response consisting of an early nicotinic-mediated EPSC (nEPSC; probably α4β2 mediated) followed by a muscarinic-mediated IPSC (mIPSC). The nEPSC had a latency of ~3.5 ms; a 20–80% rise time of 10.8 ms and a decay time constant of 123.6 ms. The mIPSC that followed the nEPSC was a muscarinic response produced by the activation of G-protein-coupled inwardly rectifying potassium channels (GIRK), likely via a membrane-delimited pathway. It had a slower time course (latency, 31.7 ms; 20–80% rise time, 107.6 ms; decay time constant, 639 ms), but still had subsecond kinetics.

While there are indications that there are muscarinic responses which are much slower than those discussed here (Table 1) we lack quantitative data on their actual kinetics.

Cholinergic receptor localization with respect to ACh sources. Typical methods for agonist application hardly recapitulate the spatiotemporal patterns of ACh release, or the concentrations reached near the receptors under physiological conditions. Thus, the study of the neural mechanisms of cholinergic modulation will benefit from studies investigating the cellular responses to ACh released from cholinergic afferents, an issue that is increasingly being appreciated. The advent of optogenetics allows the use of light to specifically stimulate cholinergic afferents, an approach that seems particularly well suited for the neocortex and allows the study of cholinergic responses to a more physiological exposure of ACh. The pioneering studies by Gu and Yakel [63••] in the hippocampus, discussed earlier, and by Arroyo *et al.* [59••], using optogenetics to study nicotinic responses of interneurons in supragranular layers of visual cortex (Table 1) illustrate the merits of this approach.

Conclusions and perspectives

We summarized in this review emerging evidence on the sources of specificity and spatiotemporal diversity of the cortical cholinergic system that may explain the range of functions in which this versatile neurotransmitter-neuromodulator has been implicated. Throughout the review we pointed out some of the areas that need to be investigated. Recent advances bode well for significant progress in our understanding of the neural basis of cholinergic modulation of cortical function in the coming years. Optical activation of afferent cholinergic axons in acute slices, in order to expose neurons to physiologically relevant ACh concentrations, in the appropriate subcellular compartments, is particularly promising and can help dissect the dynamics of different cellular cholinergic effects.

Advances in *in vivo* recording in behaving animals will contribute to understanding when the different cellular actions of ACh are engaged under physiological conditions. This, together with the increase ability to manipulate specific molecular pathways in specific neuronal populations (in particular, genetically distinct groups of INs) afforded by modern molecular genetics, will help investigate the contribution of the various cellular effects of ACh to its network and behavioral actions. The use of channelrhodopsin to identify specific neuronal types during blind in vivo recording on behaving animals [66] will allow the characterization of the activity of specific neurons in the cortex and in the nucleus basalis. Combined with the ability to label and reconstruct recorded neurons (Munoz, W. et al., abstract in Soc Neurosci Abstr 2013, 71.17/OO9), it will be possible for the first time to unravel the relationship between the in

vivo firing properties of cholinergic neurons and their patterns of innervation of the cortex.

Acknowledgments

We wish to thank John Dani, Lorna Role, Misha Beierlein, and Mark Shapiro for information that was most helpful for the preparation of this review. We also wish to thank Gord Fishell, Robin Tremblay, Illya Kruglikov, Han Xu, Soohyun Lee, Lauren Bayer and other members of the Rudy and Fishell labs for many helpful discussions; and Abba Leffler for help with the rendering of the structure of the nicotinic receptors. Supported by NIH grants: R01NS30989 and P01NS074972 to BR and NRSA fellowship F31NS087919 to WM.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Herrero JL *et al.*: **Acetylcholine contributes through muscarinic receptors to attentional modulation in V1**. *Nature* 2008, **454**:1110-1114.
This elegant study in rhesus macaque monkeys performing an attention task demonstrates that local iontophoresis of muscarinic antagonists abolishes attentional modulation of the activity of V1 neurons and impairs performance.

2. Conner JM *et al.*: **Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning**. *Neuron* 2003, **38**:819-829.

3. Conner JM, Kulczycki M, Tuszynski MH: **Unique contributions of distinct cholinergic projections to motor cortical plasticity and learning**. *Cereb Cortex* 2010, **20**:2739-2748.

4. •• Guillem K *et al.*: **Nicotinic acetylcholine receptor beta2 subunits in the medial prefrontal cortex control attention**. *Science* 2011, **333**:888-891.
This study shows the remarkable result that re-expression of functional β2-subunit-containing nAChRs using a lentiviral vector in the prelimbic area of the PFC com-pletely restores the attentional deficit observed in mice with nAChR β2-subunit deletions.

5. •• King SL *et al.*: **Conditional expression in corticothalamic efferents reveals a developmental role for nicotinic acetylcholine receptors in modulation of passive avoidance behavior**. *J Neurosci* 2003, **23**:3837-3843.
This study explored the behavioral rescue of mice constitutively lacking the beta 2 nAChR subunit, which exhibit learning deficits, with an inducible, tetracycline-regulated transgenic system that allows restored beta2 expression specifically in corticothalamic cells. The study suggests an important, developmental role for beta2-containing nAChRs in corticothalamic axons and provides a potential neuroanatomical substrate for changes induced by prenatal nicotine exposure leading to attention deficit hyperactivity disorder and cognitive impairment.

6. • Froemke RC *et al.*: **Long-term modification of cortical synapses improves sensory perception**. *Nat Neurosci* 2013, **16**:79-88.
This study builds on the classical observations [7,8] that, in anesthetized rats, pairing a tone with stimulation of the nucleus basalis, but not unpaired stimulation, produces auditory cortical receptive field plasticity similar to that produced by behavioral learning. It shows that the long-lasting enhancement of paired tone-evoked activity influences the behavioral report of the paired tone in an auditory detection task.

7. Bakin JS, Weinberger NM: **Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis**. *Proc Natl Acad Sci U S A* 1996, **93**:11219-11224.

8. Rasmusson DD, Dykes RW: **Long-term enhancement of evoked potentials in cat somatosensory cortex produced by co-activation of the basal forebrain and cutaneous receptors**. *Exp Brain Res* 1988, **70**:276-286.

9. •• Pinto L *et al.*: **Fast modulation of visual perception by basal forebrain cholinergic neurons**. *Nat Neurosci* 2013, **16**:1857-1863.
This study employs optogenetic bimodal control of cholinergic neurons and their axons in cortex to demonstrate their influences in awake cortical dynamics, coding properties of V1 neurons, and the importance of cholinergic neuromodulation for the performance of a visual discrimination task. It demonstrates that cortical state transitions occur within ~100 ms of the onset of the stimulation.

10. •• Parikh V *et al.*: **Prefrontal acetylcholine release controls cue detection on multiple timescales**. *Neuron* 2007, **56**:141-154.
This study employs choline-sensitive microelectrodes to measure ACh release with subsecond resolution in rats doing an attention task. The paper demonstrates there is a phasic release of ACh associated with cue detection specifically in the medial prefrontal cortex, in addition to a diffuse and slower release phase.

11. Mesulam MM *et al.*: **Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6)**. *Neuroscience* 1983, **10**:1185-1201.

12. Adams CE *et al.*: **Basal forebrain neurons have axon collaterals that project to widely divergent cortical areas in the cat**. *Brain Res* 1986, **397**:365-371.

13. Boylan MK *et al.*: **Axonal branching of basal forebrain projections to the neocortex: a double-labeling study in the cat**. *Brain Res* 1986, **375**:176-181.

14. Fisher RS *et al.*: **Branched projections of pallidal and peripallidal neurons to neocortex and neostriatum: a double-labeling study in the cat**. *Brain Res* 1985, **326**:156-159.

15. McKinney M, Coyle JT, Hedreen JC: **Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system**. *J Comp Neurol* 1983, **217**:103-121.

16. Baskerville KA, Chang HT, Herron P: **Topography of cholinergic afferents from the nucleus basalis of Meynert to representational areas of sensorimotor cortices in the rat**. *J Comp Neurol* 1993, **335**:552-562.

17. Mesulam MM, Mufson EJ, Wainer BH: **Three-dimensional representation and cortical projection topography of the nucleus basalis (Ch4) in the macaque: concurrent demonstration of choline acetyltransferase and retrograde transport with a stabilized tetramethylbenzidine method for horseradish peroxidase**. *Brain Res* 1986, **367**:301-308.

18. Saper CB: **Organization of cerebral cortical afferent systems in the rat. II. Magnocellular basal nucleus**. *J Comp Neurol* 1984, **222**:313-342.

19. Bigl V, Woolf NJ, Butcher LL: **Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital, and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis**. *Brain Res Bull* 1982, **8**:727-749.

20. Price JL, Stern R: **Individual cells in the nucleus basalis – diagonal band complex have restricted axonal projections to the cerebral cortex in the rat**. *Brain Res* 1983, **269**:352-356.

21. Walker LC *et al.*: **Noncollateral projections of basal forebrain neurons to frontal and parietal neocortex in primates**. *Brain Res Bull* 1985, **15**:307-314.

22. • Zaborszky L *et al.*: **Neurons in the basal forebrain project to the cortex in a complex topographic organization that reflects corticocortical connectivity patterns: an experimental study based on retrograde tracing and 3D reconstruction**. *Cereb Cortex* 2013.
This study utilized retrograde tracing and 3D reconstruction to suggest that the neocortical projections from neurons in the nucleus basalis are not diffuse, but organized into either segregated or overlapping pools of projection neurons, and that the extent of overlap depends on the degree of connectivity between the cortical targets of these projecting neurons.

23. Parikh V *et al.*: **Glutamatergic contributions to nicotinic acetylcholine receptor agonist-evoked cholinergic transients in the prefrontal cortex**. *J Neurosci* 2008, **28**:3769-3780.

24. Hasselmo ME, Sarter M: **Modes and models of forebrain cholinergic neuromodulation of cognition**. *Neuropsychopharmacology* 2011, **36**:52-73.

25. Floresco SB *et al.*: **Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission**. *Nat Neurosci* 2003, **6**:968-973.

26. Grace AA: **Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia**. *Neuroscience* 1991, **41**:1-24.

27. Smiley JF, Morrell F, Mesulam MM: **Cholinergic synapses in human cerebral cortex: an ultrastructural study in serial sections**. *Exp Neurol* 1997, **144**:361-368.

28. Turrini P *et al.*: **Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy**. *Neuroscience* 2001, **105**:277-285.

29. De Lima AD, Singer W: **Cholinergic innervation of the cat striate cortex: a choline acetyltransferase immunocytochemical analysis**. *J Comp Neurol* 1986, **250**:324-338.

30. Umbriaco D *et al.*: **Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections**. *J Comp Neurol* 1994, **348**:351-373.

31. Mrzljak L *et al.*: **Cholinergic synaptic circuitry in the macaque prefrontal cortex**. *J Comp Neurol* 1995, **357**:603-617.

32. Aoki C, Kabak S: **Cholinergic terminals in the cat visual cortex: ultrastructural basis for interaction with glutamate-immunoreactive neurons and other cells**. *Vis Neurosci* 1992, **8**:177-191.

33. Descarries L, Gisiger V, Steriade M: **Diffuse transmission by acetylcholine in the CNS**. *Prog Neurobiol* 1997, **53**:603-625.

34. Groves PM, Linder JC, Young SJ: **5-Hydroxydopamine-labeled dopaminergic axons: three-dimensional reconstructions of axons, synapses and postsynaptic targets in rat neostriatum**. *Neuroscience* 1994, **58**:593-604.

35. • Takacs VT, Freund TF, Nyiri G: **Neuroligin 2 is expressed in synapses established by cholinergic cells in the mouse brain**. *PLoS ONE* 2013, **8**:e72450.
Using serial electron microscopic of sections double labeled for neuroligin 2 and choline acetyltransferase this study found neuroligin 2 in the postsynaptic membrane of cholinergic synapses in neocortex and other brain areas. Contact sites that were strongly labeled for neuroligin 2 did not resemble typical synapses. On the basis of these data, the paper suggests that cholinergic axons form synaptic connections much more frequently than what was previously recognized.

36. Krnjevic K, Pumain R, Renaud L: **The mechanism of excitation by acetylcholine in the cerebral cortex**. *J Physiol* 1971, **215**:247-268.

37. Thiele A: **Muscarinic signaling in the brain**. *Annu Rev Neurosci* 2013, **36**:271-294.

38. Dani JA, Bertrand D: **Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system**. *Annu Rev Pharmacol Toxicol* 2007, **47**:699-729.

39. Buzsaki G *et al.*: **Interneuron Diversity series: circuit complexity and axon wiring economy of cortical interneurons**. *Trends Neurosci* 2004, **27**:186-193.

40. Klausberger T, Somogyi P: **Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations**. *Science* 2008, **321**:53-57.

41. Isaacson JS, Scanziani M: **How inhibition shapes cortical activity**. *Neuron* 2011, **72**:231-243.

42. Petilla Interneuron Nomenclature G *et al.*: **Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex**. *Nat Rev Neurosci* 2008, **9**:557-568.

43. Fishell G, Rudy B: **Mechanisms of inhibition within the telencephalon: ''where the wild things are''**. *Annu Rev Neurosci* 2011, **34**:535-567.

44. Parra P, Gulyas AI, Miles R: **How many subtypes of inhibitory cells in the hippocampus?** *Neuron* 1998, **20**:983-993.

45. Lawrence JJ: **Cholinergic control of GABA release: emerging parallels between neocortex and hippocampus**. *Trends Neurosci* 2008, **31**:317-327.

46. Xu H *et al.*: **Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4**. *Neuron* 2013, **77**:155-167.

47. •• Lee S *et al.*: **The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors**. *J Neurosci* 2010, **30**:16796-16808.
This paper demonstrated that 5HT3a receptor-expressing neocortical interneurons do not overlap with PV and SOM-expressing populations and that together these three groups account for nearly 100% of all GABAergic INs. It also demonstrated that 5HT3 INs are enriched in superficial layers, where they represent the major IN population. All 5HT3 INs, independently of morphology or electrophysiological properties are depolarized by nicotine and the 5HT3 agonist mCPBG.

48. Batista-Brito R, Fishell G: **The developmental integration of cortical interneurons into a functional network**. *Curr Top Dev Biol* 2009, **87**:81-118.

49. Rudy B *et al.*: **Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons**. *Dev Neurobiol* 2011, **71**:45-61.

50. • Lee S *et al.*: **A disinhibitory circuit mediates motor integration in the somatosensory cortex**. *Nat Neurosci* 2013, **16**:1662-1670.
This study discovered a role for bipolar/bitufted 5HT3aR-VIP INs in mediating sensorimotor integration in barrel cortex and that these cells mediate disinhibition of PCs by inhibiting SOM interneurons.

51. • Pi HJ *et al.*: **Cortical interneurons that specialize in disinhibitory control**. *Nature* 2013, **503**:521-4.
These authors demonstrate that stimulation of VIP INs decreased the firing of SOM INs and some PV INs but increased the firing in pyramidal cells in both the auditory and the medial prefrontal cortices. The study showed that the disinhibition mediated by VIP neurons enhances the subpopulation of PCs that are responsive to tones in auditory cortex.

52. Palmer LM *et al.*: **The cellular basis of GABA(B)-mediated interhemispheric inhibition**. *Science* 2012, **335**:989-993.

53. Cruikshank SJ *et al.*: **Thalamic control of layer 1 circuits in prefrontal cortex**. *J Neurosci* 2012, **32**:17813-17823.

54. Gentet LJ *et al.*: **Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex**. *Nat Neurosci* 2012, **15**:607-612.

55. Taniguchi H *et al.*: **A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex**. *Neuron* 2011, **71**:995-1013.

56. Letzkus JJ *et al.*: **A disinhibitory microcircuit for associative fear learning in the auditory cortex**. *Nature* 2011, **480**:331-335.

57. Hulme EC, Birdsall NJ, Buckley NJ: **Muscarinic receptor subtypes**. *Annu Rev Pharmacol Toxicol* 1990, **30**:633-673.

58. Madison DV, Lancaster B, Nicoll RA: **Voltage clamp analysis of cholinergic action in the hippocampus**. *J Neurosci* 1987, **7**:733-741.

59. •• Arroyo S *et al.*: **Prolonged disynaptic inhibition in the cortex mediated by slow, non-alpha7 nicotinic excitation of a specific subset of cortical interneurons**. *J Neurosci* 2012, **32**:3859-3864.
This is the first paper to report the use of optogenetics to study cholinergic influences on neocortical neurons in acute slices. Nicotinic responses were observed on L1 INs and on layer II/III bipolar/bitufted INs, but not on FS cells. The responses mediated feed forward inhibition onto layer II/III PCs and FS neurons.

60. Shen JX, Yakel JL: **Nicotinic acetylcholine receptor-mediated calcium signaling in the nervous system**. *Acta Pharmacol Sin* 2009, **30**:673-680.

61. Berg DK, Conroy WG: **Nicotinic alpha 7 receptors: synaptic options and downstream signaling in neurons**. *J Neurobiol* 2002, **53**:512-523.

62. •• Gulledge AT, Stuart GJ: **Cholinergic inhibition of neocortical pyramidal neurons**. *J Neurosci* 2005, **25**:10308-10320.
This elegant study examined the effects of ACh application to layer 5 pyramidal cells and found that besides the classical sustained activation, there is a phasic hyperpolarization mediated by the same muscarinic receptor. Quantitative examination showed that the two muscarinic responses required different lengths of ACh exposure, but both were much less than 1 s.

63. •• Gu Z, Yakel JL: **Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity**. *Neuron* 2011, **71**:155-165.

In one of the first studies using optical release of ACh from cholinergic afferents expressing channelrhodopsin, this paper shows that septal cholinergic input, activated either by electrical stimulation or via the optogenetic approach, produces changes in hippocampal plasticity that depend on the timing of ACh release relative to the timing of glutamatergic inputs.

64. Berg DK: **Timing is everything, even for cholinergic control**. *Neuron* 2011, **71**:6-8.

65. •• Sun YG *et al.*: **Biphasic cholinergic synaptic transmission controls action potential activity in thalamic reticular nucleus neurons**. *J Neurosci* 2013, **33**:2048-2059.
This study utilized electrical stimulation to evoke ACh release from cholinergic afferents in the reticular thalamic nucleus (TRN). It demonstrates a remarkable precision of both nicotinic and muscarinic responses. Studies of unitary responses strongly suggest that the ACh mediating these responses is synaptic in origin and not the result of volume transmission.

66. Roux L *et al.*: **In vivo optogenetic identification and manipulation of GABAergic interneuron subtypes**. *Curr Opin Neurobiol* 2014, **26**:88-95.

67. Halliwell JV: **Cholinergic responses in human neocortical neurones**. *EXS* 1989, **57**:138-149.

68. McCormick DA, Williamson A: **Convergence and divergence of neurotransmitter action in human cerebral cortex**. *Proc Natl Acad Sci U S A* 1989, **86**:8098-8102.

69. McCormick DA: **Cellular mechanisms of cholinergic control of neocortical and thalamic neuronal excitability**. In *Brain Cholinergic Systems.* Edited by Steriade M, Biesold D. New York: Oxford University Press; 1990:236-264.

70. Eggermann E, Feldmeyer D: **Cholinergic filtering in the recurrent excitatory microcircuit of cortical layer 4**. *Proc Natl Acad Sci U S A* 2009, **106**:11753-11758.

71. Nunez A *et al.*: **Cholinergic-mediated response enhancement in barrel cortex layer V pyramidal neurons**. *J Neurophysiol* 2012, **108**:1656-1668.

72. Carr DB, Surmeier DJ: **M1 muscarinic receptor modulation of Kir2 channels enhances temporal summation of excitatory synaptic potentials in prefrontal cortex pyramidal neurons**. *J Neurophysiol* 2007, **97**:3432-3438.

73. Mittmann T, Alzheimer C: **Muscarinic inhibition of persistent Na^+ current in rat neocortical pyramidal neurons**. *J Neurophysiol* 1998, **79**:1579-1582.

74. Haj-Dahmane S, Andrade R: **Ionic mechanism of the slow afterdepolarization induced by muscarinic receptor activation in rat prefrontal cortex**. *J Neurophysiol* 1998, **80**:1197-1210.

75. Yan HD, Villalobos C, Andrade R: **TRPC channels mediate a muscarinic receptor-induced afterdepolarization in cerebral cortex**. *J Neurosci* 2009, **29**:10038-10046.

76. Egorov AV *et al.*: **Graded persistent activity in entorhinal cortex neurons**. *Nature* 2002, **420**:173-178.

77. Egorov AV *et al.*: **Ca^{2+}-independent muscarinic excitation of rat medial entorhinal cortex layer V neurons**. *Eur J Neurosci* 2003, **18**:3343-3351.

78. Rahman J, Berger T: **Persistent activity in layer 5 pyramidal neurons following cholinergic activation of mouse primary cortices**. *Eur J Neurosci* 2011, **34**:22-30.

79. Zhang Z, Seguela P: **Metabotropic induction of persistent activity in layers II/III of anterior cingulate cortex**. *Cereb Cortex* 2010, **20**:2948-2957.

80. Poorthuis RB *et al.*: **Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors**. *Cereb Cortex* 2013, **23**:148-161.

81. Poorthuis RB *et al.*: **Layer-specific interference with cholinergic signaling in the prefrontal cortex by smoking concentrations of nicotine**. *J Neurosci* 2013, **33**:4843-4853.

82. Zolles G *et al.*: **Functional expression of nicotinic acetylcholine receptors in rat neocortical layer 5 pyramidal cells**. *Cereb Cortex* 2009, **19**:1079-1091.

83. Gulledge AT *et al.*: **Heterogeneity of phasic cholinergic signaling in neocortical neurons**. *J Neurophysiol* 2007, **97**:2215-2229.

84. Giessel AJ, Sabatini BL: **M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels**. *Neuron* 2010, **68**:936-947.

85. Buchanan KA *et al.*: **Facilitation of long-term potentiation by muscarinic M(1) receptors is mediated by inhibition of SK channels**. *Neuron* 2010, **68**:948-963.

86. Gil Z, Connors BW, Amitai Y: **Differential regulation of neocortical synapses by neuromodulators and activity**. *Neuron* 1997, **19**:679-686.

87. Hasselmo ME, Bower JM: **Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex**. *J Neurophysiol* 1992, **67**:1222-1229.

88. Hasselmo ME, McGaughy J: **High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation**. *Prog Brain Res* 2004, **145**:207-231.

89. Kawaguchi Y: **Selective cholinergic modulation of cortical GABAergic cell subtypes**. *J Neurophysiol* 1997, **78**:1743-1747.

90. Porter JT *et al.*: **Selective excitation of subtypes of neocortical interneurons by nicotinic receptors**. *J Neurosci* 1999, **19**:5228-5235.

91. • Kruglikov I, Rudy B: **Perisomatic GABA release and thalamocortical integration onto neocortical excitatory cells are regulated by neuromodulators**. *Neuron* 2008, **58**:911-924.
This study found that cholinergic modulation inhibits GABA release from the terminals of FS INs on excitatory neurons via a membrane delimited muscarinic mechanism, but had no effect on the excitability of the neurons. The paper shows that the synaptic effect suppresses feed forward inhibition of thalamocortical inputs and acts together with the nicotinic enhancement of the thalamic input to promote thalamocortical transfer of sensory inputs.

92. Xiang Z, Huguenard JR, Prince DA: **Cholinergic switching within neocortical inhibitory networks**. *Science* 1998, **281**:985-988.

93. Yamamoto K *et al.*: **Postsynaptic cell type-dependent cholinergic regulation of GABAergic synaptic transmission in rat insular cortex**. *J Neurophysiol* 2010, **104**:1933-1945.

94. Fanselow EE, Richardson KA, Connors BW: **Selective, state-dependent activation of somatostatin-expressing inhibitory interneurons in mouse neocortex**. *J Neurophysiol* 2008, **100**:2640-2652.

95. Beierlein M, Gibson JR, Connors BW: **A network of electrically coupled interneurons drives synchronized inhibition in neocortex**. *Nat Neurosci* 2000, **3**:904-910.

96. Lawrence JJ *et al.*: **Cell type-specific dependence of muscarinic signalling in mouse hippocampal stratum oriens interneurones**. *J Physiol* 2006, **570(Pt 3)**:595-610.

97. Couey JJ *et al.*: **Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex**. *Neuron* 2007, **54**:73-87.

98. Ferezou I *et al.*: **5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons**. *J Neurosci* 2002, **22**:7389-7397.

99. Christophe E *et al.*: **Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons**. *J Neurophysiol* 2002, **88**:1318-1327.

100. Bennett C *et al.*: **Mechanisms generating dual-component nicotinic EPSCs in cortical interneurons**. *J Neurosci* 2012, **32**:17287-17296.

101. Brombas A, Fletcher LN, Williams SR: **Activity-dependent modulation of layer 1 inhibitory neocortical circuits by acetylcholine**. *J Neurosci* 2014, **34**:1932-1941.