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INVITED REVIEW

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Cholinergic modulation of the cortical neuronal network

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Abstract Acetylcholine (ACh) is an important neurotransmitter of the CNS that binds both nicotinic and muscarinic receptors to exert its action. However, the mechanisms underlying the effects of cholinergic receptors have still not been completely elucidated. Central cholinergic neurons, mainly located in basal forebrain, send their projections to different structures including the cortex. The cortical innervation is diffuse and roughly topographic, which has prompted some authors to suspect a modulating role of ACh on the activity of the cortical network rather than a direct synaptic role. The cholinergic system is implicated in functional, behavioural and pathological states including cognitive function, nicotine addiction, Alzheimer's disease, Tourette's syndrome, epilepsies and schizophrenia. As these processes depend on the activation of glutamatergic and GABAergic systems, the cholinergic terminals must exert their effects via the modulation of excitatory and/or inhibitory neurotransmission. However, the understanding of cholinergic modulation is complex because it is the result of a mixture of positive and negative modulation, implying that there are various types, or even subtypes, of cholinergic receptors. In this review, we summarize the current knowledge on central cholinergic systems (projections and receptors) and then aim to focus on the implications for ACh in the modulation of cortical neuronal activity.

Keywords Acetylcholine · Cortical network · Excitation · Inhibition · Nicotinic and muscarinic receptors

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Introduction

Acetylcholine (ACh) acts in many cognitive functions, such as excitability [198], attention [24, 188], learning [62, 131], memory [74, 167], the stress response [145], wakefulness and sleep [81, 82], and cortical modulation of sensory information [48, 130, 150, 157]. There is evidence that these actions are exerted by controlling the signal/noise ratio in sensory processing [173]. The study of ACh in the mnemonic process shows that ACh plays a role in the first stages of learning (in acquisition) and not during the recall process [131]. Elsewhere, ACh is implied in the spatial working memory [150]. The stress response induces ACh release in the forebrain. This ACh release is responsible for physiological and emotional responses, in particular through its action on the hypothalamic-pituitary system [145]. At the sensory level, in the rat auditory cortex, cortical ACh can modify cortical responses facilitating thalamo-cortical auditory synaptic transmission [128]. Indeed, in this way, ACh facilitates the detection and discrimination of tones [167]. In the rat somatosensory cortex, ACh plays a part in the organization and modification of receptive fields [85], in particular by enlarging them [42]. In the visual cortex, ACh facilitates the neuronal response to visual stimulation, and seems to increase orientation selectivity in simple cells, as well as orientation selectivity and direction selectivity in complex cells (see for review [174]). In associational areas, ACh mediates the conditioned response of the cortical cells and thus contributes to the enhanced processing of behaviourally significant stimuli [152].

Degeneration of the cholinergic system of the basal forebrain occurs in many diseases in addition to Alzheimer's disease, including Parkinson's disease [9, 50], Creutzfeldt-Jakob disease [8, 29], Down syndrome [201], Korsakoff's syndrome [9], amyotrophic lateral sclerosis-parkinsonism-dementia complex [140], progressive supranuclear palsy [165, 177], olivopontocerebellar atrophy [92] and dementia pugilistica [180]. All these disorders are characterized by various degrees of cognitive impairment.

These observations suggest a selective effect of ACh on the processing of sensory stimuli and therefore a phasic effect of ACh restricted to a defined set of cortical neurons and/or local circuits.

The cholinergic system in the brain

Cholinergic forebrain projections are classified into six main central pathways (Ch1–Ch6) linked to the origin of the nuclei where the cholinergic fibres arise [126]. Cholinergic nuclei from the septum (Ch1) and the vertical limb of the diagonal band (Ch2) project only on the hippocampus whereas pedunculopontinus nucleus (part of Ch5) and laterodorsal tegmental nucleus (Ch6) from the brainstem project on the thalamus. Cholinergic nuclei from the lateral part of the horizontal limb of the diagonal band (Ch3) project to the olfactory bulb. The pathway innervating the cortex (Ch4) mainly originates from the nucleus basalis magnocellular (NBM, named the nucleus basalis of Meynert in the human species) [84, 126] (Fig. 1). The cholinergic fibres of the NBM project to the cortex with a topographic organization according to a rostrocaudal, ventrodorsal and mediolateral gradient [15, 61, 110, 111, 124, 199]. In contrast to primates, the rat nuclei included in these cholinergic pathways are not well delimitated. In the rat (Fig. 1), Ch4 pathway groups originate from several other nuclei, in addition to the NBM, such as the substantia innominata [15, 98, 194], diagonal band nucleus [76], nucleus ansa lenticularis [15] and a part of the magnocellular preoptic nucleus [15]. In 1989, Butcher and Semba [27] accounted for confusions in the nomenclature of nuclei of the basal forebrain. Various authors used different terms for the same structure as well as the same term to describe different structures. Moreover, it seems very difficult to generalize the traditional nomenclature from one species to another. However, the NBM is generally associated with the Ch4 pathway. In addition, the labelling of choline acetyltransferase in the cortex suggested the presence of few cholinergic bipolar intracortical interneurons [53, 103]. The majority of these interneurons (88%) are co-labelled for GABA [13] and vasoactive intestinal polypeptide (VIP) [34] and are mainly concentrated in layer II/III of the cortex [147]. However, their existence in, their implications for and their contribution to the cortical neuronal network are open to discussion [26, 187].

Among the population of neurons of the NBM projecting to the cortex, 30% to 35% are GABAergic neurons [63], whose axons preferentially connect cortical GABAergic interneurons [66] to disinhibit them [83]. The remaining neurons release ACh in the cortex and this release can be regulated at two levels: either by modulating the activity of cholinergic neurons in the NBM or by modulating cholinergic terminals at the cortical level. In NBM, cholinergic neurons are under the control of GABAergic interneurons [134] and glutamatergic neurons [58]. At the cortical level, released GABA can diffuse extrasynaptically in substantial amounts and activate $GABA_A$ receptors on cholinergic terminals and then inhibit ACh release [118]. Moreover, GABAergic innervation can also activate the release of newly synthesized ACh [16] or can suppress the inhibition of ACh release through two successive GABAergic synapses (one GABAergic neuron originating from the NBM and one cortical GABAergic interneuron) [150].

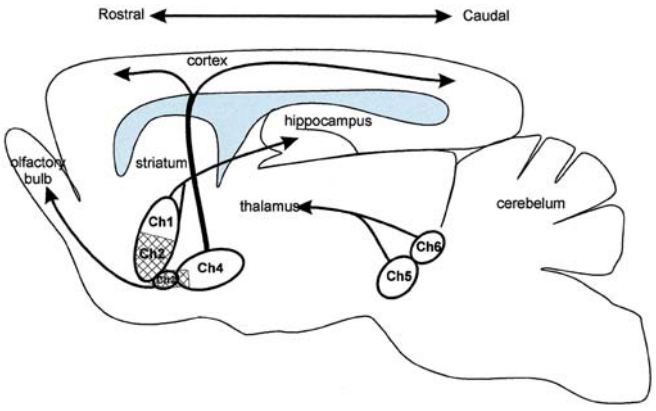

Fig. 1 Rat cholinergic central pathways. *Hatched area*: diagonal band (modified from ref [126])

Other neurotransmitters may interfere with the regulation of cortical ACh release; such as, dopamine acting through activation of cholinergic neurons in the NBM [41]; serotonin, according to the subtype of activated serotonin receptor modulating the activity of cholinergic neurons in the NBM and the release of ACh by cholinergic terminals in the cortex [78]; norepinephrine, acting as a tonic inhibitor of ACh release in the cortex [178]; and cholecystokinin stimulating ACh cortical release [116]. Finally, the presence of autoreceptors on cortical cholinergic fibres would also permit the possible autoregulation of the cholinergic system [102].

In young rats, cholinergic denervation of the neocortex by specific immunolesion reduced the size of the cortex [160] and delayed the differentiation of pyramidal neurons in the whole cortex [79]. These observations suggest that cholinergic innervation of the cortex does not have specific cortical target area. In fact, cholinergic fibres can be found in all cortical areas and layers [125, 127], with the density of cholinergic fibres differing from one area to another, and from one layer to another [113], although the accurate topographic organization of cholinergic terminals is not clear. For instance, electrophysiological studies show than 92% of cells in the visual cortex respond to application of exogenous ACh [173].

To summarize, these data do not support the selective ACh activation of the cortical network by cholinergic fibres but are in favour of a global modulating action of ACh on cortical functions via effects independent of cortical areas and layers [61, 123]. So, the specific effects of ACh observed on cognitive functions do not seem to be due to precise innervation and might be attributed to at least the specific distribution of both nicotinic and muscarinic cholinergic receptors.

Central nicotinic receptors

Nicotinic receptors are part of the ionotropic receptor family and they are found in both peripheral and central nervous system (PNS and CNS respectively). In the CNS, the various types of nicotinic receptors display a particular anatomic distribution, and have specific pharmacological and physiological profiles. While their structures and functional properties have been extensively studied, their subunit structures and their physiological roles are not totally understood.

Structure

Nicotinic receptors were first isolated from fish electric organs (torpedo, eel) and skeletal muscles of mammals. Muscular nicotinic receptors (250 kDa) are made up of five individual protein subunits, (2α, 1β, 1δ, 1ε or 1γ embryonic subunit). Each subunit contains four transmembrane domains (20 amino acids, M1–M4) and two extracellular hydrophilic segments (N- and C-terminals). The M2 domain of each subunit contributes to the pore responsible for cation (Ca^{2+}, Na^{+}, K^{+}) permeability [68]. The two subunits α, implied in the muscle nicotinic receptor (named $\alpha 1$) can be identified by a pair of cysteine residues in the N-terminus and play a role in the agonist site [86], so two agonist molecules must bind in order to activate the ligand-gated ion channel. The subunits of neuronal nicotinic receptors, like their muscular counterparts, have extracellular C- and N-terminal domains and four hydrophobic transmembrane segments (M1–M4). The amino acid sequences of the neuronal nicotinic receptor subunits show, in the same species, only 40% to 50% homology with muscular nicotinic receptor sequences [166]. This difference between the subunits is mainly due to the variability of the size and sequence of the intracellular loop between M3 and M4 segments, which contains putative sites of phosphorylation [166]. Neuronal nicotinic receptors are assumed to have a pentameric structure composed of two subunit types. Nine α subunits (α_2 to α_{10}) and three β subunits (β_2 to β_4) have been identified [19, 36, 37, 43, 44, 51, 56, 57, 144, 168, 169, 172, 189]. Classic stoichiometry of the neuronal receptor is most often 2α subunits for 3β subunits, but functional homomers α_7 [36], α_8 [69] and α_9 [56] have been observed. α_{10} is always associated with α_9 mainly in cochlear cells [57]. β subunits will not, alone, form functional receptors [20]. Receptors formed by three different types of subunits, including most often α_5 subunits, have been also described [93]. Finally, nicotinic neuronal receptors present a variety of subtypes (see [112] for nomenclature) due to several possible combinations between subunits (up to 1728 possible receptor subtypes, according to [175]). This diversity implies different kinetics of desensitization, conductance states (5–45 pS) and open times (0.1–8 ms) [166].

Localization

The expression of the subunits of nicotinic receptors has been studied using mainly in situ hybridization approaches. These studies have shown that during the development of the CNS, some subunits of nicotinic receptors (α_3, β_2, α_4, β_4, α_7) can be expressed at very early stages of embryonic development (E11 in rats) before the formation of morphologically differentiated synapses [205]. In the sensory primary cortex, α_7 is present during development but disappears at adult age [23]. In the adult rat brain, the distribution of mRNA subunits is differential: α_2 mRNA is mainly detected in the interpeduncular nucleus and in the deep layer of the cortex [191], and α_9 mRNA is mainly express in the outer cochlear cells [57]. α_8 mRNA (discovered in chick brain) is not present in mammals. α_5, α_6 and β_3 subunits have a restricted distribution: α_5 mRNA has been found in the hippocampus, the substantia nigra pars compacta, the ventral tegmental area, the interpeduncular nucleus and in layer VIb of the cortex [190]; α_6 and β_3 mRNA are particularly abundant in the somatosensory ganglia and seem associated with catecholaminergic neurons [101]. α_3 mRNA is mainly expressed in the thalamus, in the interpeduncular nucleus and in layers IV and V of the cortex [191]. β_4 mRNA is detected in the hippocampus, in the medial habenula, in the interpeduncular nucleus, in the olfactory area, in the cerebellum and in the locus coerulus [45]. In the cortex, β_4 mRNA is abundant in layer IV and can also be detected in layers I–III [45]. α_4 and β_2 mRNA subunits are expressed in the majority of cerebral structures and in all layers of the cortex [191]. Elsewhere, the use of α-bungarotoxin, a specific marker, shows that α_7 subtype is mainly present in the hippocampus and in the hypothalamus [166]. The results of immunohistochemistry and radioligand binding experiments correlate well with the localization of mRNA transcripts for α_4, β_2 and α_7 subunits by in situ hybridization but show variations in the expression of each subunit [47, 77, 105, 141].

So it seems that most nicotinic receptors contain either α_4 and β_2 subunits or the α_7 subunit (see for reviews [39, 166]).

Pharmacology

The diversity of nicotinic receptors implies a difference in their selectivity for and sensitivity to nicotinic agonists and antagonists [52] and a difference in the permeability of their cationic channel [32].

Agonists

The relative agonist potencies obtained for nicotinic receptors expressed in *Xenopus* oocytes are defined elsewhere [108, 172] and are shown in Table 1:

Table 1 The relative agonist potencies obtained for nicotinic receptors expressed in *Xenopus* oocytes. (*DMPP* 1,1-Dimethyl-4-phenylpiperazinium)

Subunits	Affinity order
$\alpha_2\beta_2$	Nicotine>ACh/DMPP>cytisine
$\alpha_2\beta_4$	Cytisine>nicotine>ACh>DMPP
$\alpha_3\beta_2$	ACh/DMPP>nicotine>cytisine
$\alpha_3\beta_4$	Cytisine>nicotine/ACh/DMPP
$\alpha_4\beta_2$	ACh/nicotine>DMPP>cytosine
$\alpha_4\beta_4$	Cytisine>nicotine>ACh>DMPP
α_7	Nicotine>cytisine>DMPP>ACh

Antagonists

Curare, the alkaloid extracted from the plant *Chondodendron tuberculosum*, is the traditional antagonist of nicotinic receptors of the PNS. It is not efficient against central nicotinic receptors, contrary to its derivate, d-tubocurarine. However, this latter antagonist is non-selective, affecting both 5-HT_3, glycine and $GABA_A$ ionotropic receptors in addition to the nicotinic receptor [161].

Dihydro-β-erythroidine (DHBE), an alkaloid isolated from the seeds of *Erythrina*, binds in a competitive manner the receptor with strong affinity for α_3-, α_4- and β_2-containing receptor [52]. $\alpha_3\beta_2$ and $\alpha_4\beta_2$ receptors can be blocked at submicromolar concentrations (100 nM for hippocampus neurons, [3]). However, DHβE is 10 to 50 times less active on the α_7 and $\alpha_3\beta_4$ receptors [33].

N-methyllycaconitine (MLA), a diterpene alkaloid produced by seeds of *Delphinium brownii*, is a selectively competitive antagonist of neuronal α_7 receptors and is one of the most powerful non-peptidic antagonists known [52]. It can block this receptor at concentrations lower than 1 nM [5], but blocks $\alpha_6\beta_2$* receptors with a K_i of 30 nM and other nicotinic receptors at higher concentrations [133].

α-Bungarotoxin and n-bungarotoxin are toxins extracted from the venom of the snake *Bungarus multicinctus*. α-Bungarotoxin blocks the agonist-binding site of the α_7 (and α_8 and α_9) homopentameric receptor with an affinity (K_i) of 1 nM [40, 109]. n-Bungarotoxin is selective for $\alpha_3\beta_2$ and $\alpha_4\beta_2$ receptors at concentrations 10 nM and 1 μM, respectively [109].

Mecamylamine is a non-selective, non-competitive channel blocker of nicotinic receptors [11]. At high concentrations (>100 μM), it can act on *N*-methyl-D-aspartate (NMDA) receptors [146].

However, the lack of very selective antagonists prevents a precise characterization of the different subtypes of nicotinic receptors.

Allosteric ligands

Non-competitive allosteric ligands can be activators or inhibitors of nicotinic receptors (Fig. 2). When they are activators such as serotonin [170], they increase the opening time of the channel and the ionic conductance [151]. Members of the organophosphorus class of anticholinesterases are nicotinic receptor activators [106] whereas other anticholinesterase compounds (galantamine, physotigmine and tacrine) have been observed to be inhibitors of nicotinic receptors of the rat striatum [35], or as activators of nicotinic receptors in natural murine and human neurons [115], nicotinic receptors expressed in transfected fibroblasts cell line M10 [176], or murine and human cell lines [115]. Inhibitory allosteric ligands may act on two sites, one with high affinity and the other with low affinity. These ligands block the nicotinic receptors without affecting the binding of ACh to its site [99]. Anaesthetics, ethanol and barbiturates are allosteric ligand inhibitors [148]. Other binding sites on the nicotinic receptor have been described: (1) the steroid site causes desensitization of the receptor [14] when activated by progesterone, corticosterone or dexamethasone; (2) the dihydropyridine site, activated by L-type Ca^{2+} channel antagonists, blocks activation of the nicotinic receptor [107].

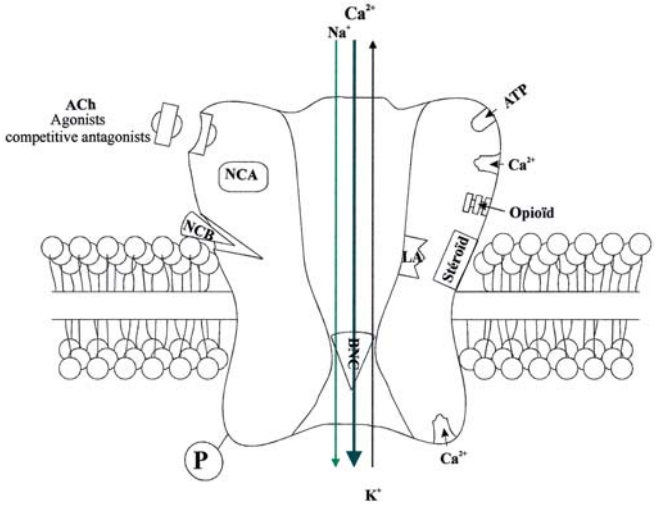

Fig. 2 Scheme of the central nicotinic receptor. (modified from [32, 148]). (*LA* Local anesthetic, *NCA* non-competitive activator, *NCB* non-competitive blocker, *P* phosphorylation site)

The nicotinic receptor, mainly permeable to Na^+ and Ca^{2+} ions, is allosterically modulated by Ca^{2+} ions. Ca^{2+} binding sites are located in the extracellular N-terminal and when the site is occupied by Ca^{2+} ions, ACh-mediated currents are potentiated in a voltage-insensitive manner [138] and there is an increase in channel opening probability [1, 6, 183]. In addition, there are two intracellular Ca^{2+} binding sites [39, 59], which mediate a voltage-dependent reduction in conductance [148].

Function

Nicotinic receptors are permeable to Na^+, K^+ and Ca^{2+} ions. However, the neuronal nicotinic receptor subtypes are highly permeable to Ca^{2+} with a Ca^{2+}/Na^+ permeabil-

ity ratio higher than 1 (10 for α7 nicotinic receptors [137, 172]) whereas the ratio is only 0.1 to 0.3 for the muscular nicotinic receptor [137, 184]. Thus, the neuronal nicotinic receptor can cause a marked increase in the intracellular Ca^{2+} concentration, enough to influence cellular Ca^{2+}-sensitive processes [137], for example the activation of K^+-dependent Ca^{2+} currents or the transcription of early genes [166]. This important increase in the intracellular Ca^{2+} concentration at hyperpolarized potentials is of interest when voltage-dependent Ca^{2+} channels are not activated. So neuronal nicotinic receptors may act in synaptic plasticity, in addition to the activation of voltage-dependent Ca^{2+} channels and NMDA receptors [121, 137, 185]. Finally, a neurotrophic role during early development in the formation of synapses [162] and neurite retraction [155] has also been attributed to α7 receptors (see for reviews [22, 179]).

The neuronal nicotinic receptors are located in a presynaptic position where they can modulate neurotransmitter release (see for reviews [39, 122, 197]). Their activation will cause the intracellular Ca^{2+} concentration to vary, and it is known that the chemical release of neurotransmitter depends mainly on these local Ca^{2+} variations at the site of release. Nicotinic receptors also occur at the postsynaptic level in different structures [64, 65] and were recently found in the cortex of ferret [161], rat [154, 200] and humans [4] where their activation induces a fast cationic inward current. Extrasynaptic nicotinic receptors, generally assigned as preterminal receptors, may modulate neuronal functions [104] such as the release of neurotransmitter or local excitability [39]. In fact, in interpeduncular nucleus GABAergic interneurons, the activation of these receptors induces a spike discharge leading to the enhancement of GABA release [100].

Central muscarinic receptors

The clinical use of gallamine as an adjunct to anaesthetics has revealed the presence of muscarinic receptors [159]. Then, using the affinity of a muscarinic antagonist, pirenzepine, Hammer et al. [73] showed the existence of various subtypes of muscarinic receptor and distinguished two classes: "M1" receptors with a strong affinity for pirenzepine and "M2" receptors with an intermediate and a low affinity for pirenzepine.

Structure

The muscarinic receptors are metabotropic. Five cloned genes, called m1 to m5, have been characterized [17, 95, 96] and generate five types of muscarinic receptor proteins named M1 to M5. The receptor is a single glycoprotein with seven transmembrane helices, an extracellular N-terminus containing glycosylation sites, an intracellular C-terminus and a large cytoplasmic domain between transmembrane segments 5 and 6 (see for review [80]). The site of ACh binding has not been clearly identified [38, 195]. The intracellular G protein binding site implies the second and the third cytoplasmic loops [72, 195]. Each subtype of receptor is related to different G proteins, which can modulate, either directly or by a second messenger, the activation of ionic channels (Fig. 3).

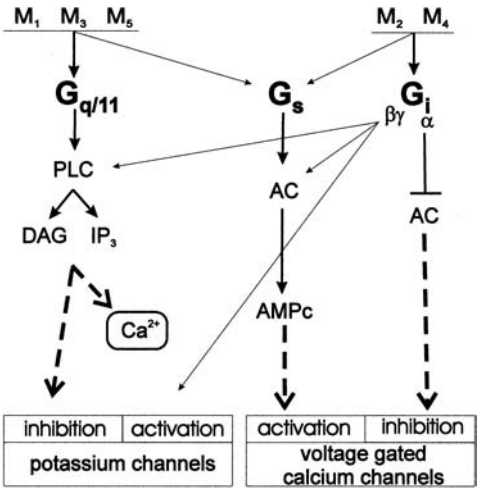

Fig. 3 Metabolic pathways associated with the activation of muscarinic receptors (modified from [143])

The family of the M1-like receptors defined by Hammer et al. [73] comprises the M1, M3 and M5 subtypes, and the family of the M2-like receptors comprises the M2 and M4 subtypes. Generally, the M1-like receptors stimulate, via a pertussis-toxin-insensitive G-protein ($G_{q/11}$), the phosphoinositol pathway, which can close K^+ channels thus leading to cell depolarization. The M2-like receptors inhibit adenylate cyclase via a pertussis-toxin-sensitive G-protein (G_i) leading to the inhibition of voltage-gated Ca^{2+} channels [31, 54]. However, these specificities are not absolute, and the mechanisms of muscarinic receptor-mediated signal transduction are more complex because of cross-over between signalling pathways [31, 60, 143].

Localization

Localization of muscarinic receptors was studied by autoradiography of the CNS with [^{3}H]propylbenzilylcholine. This technique revealed their presence in various structures, such as olfactory anterior nucleus, olfactory tubercle, hippocampus, hypothalamic supraoptic nucleus, nucleus accumbens and cortex [163]. The dendritic area of the hippocampus, of the striatum, of the nucleus accumbens and of the cortex [88, 97, 117] and many cranial nerves, sensory and motor nerves [192], were labelled significantly.

In situ hybridization applied to the rat brain revealed that m1 mRNA is mainly localized in the telencephalon and particularly in the cortex, the striatum and the hippocampus [21]. m2 mRNA, which is less abundant, is

especially found in subcortical nuclei [25] whereas m3 mRNA is also localized in the telencephalon and in some thalamic nuclei. m4 mRNA is prevalent in the striatum, in the cortex and in the hippocampus [21]. m5 mRNA is present in the hippocampus, in the striatum, in the cortex, in the ventral tegmental area [186] and in the substantia nigra pars compacta where it co-locates with the dopamine D2 receptor [193].

The use of monoclonal M35 antibodies allows the localization of muscarinic receptors without differentiating between subtypes [181]. The brain areas most strongly labelled are the olfactory tubercle, the striatum and the interpeduncular nucleus. The cerebellum is also slightly labelled. Muscarinic receptors were also found on glial cells and blood vessels of the brain [181]. In the neocortex, a clear laminar distribution was observed with a strong labelling of layer V and sometimes of layers II/III. However, each area could have a particular distribution, as in the parietal cortex where layers III and VIb are strongly labelled [163, 181]. Muscarinic receptors have also been found to co-localize with nicotinic receptors on pyramidal neurons of the rat neocortex [182] but not often on cortical interneurons [204].

The M35 antibody has the same affinity for all subtypes of muscarinic receptor [28] and for a long time specific antibodies against the various subtypes were usable only for immunoprecipitation [196]. Consequently, the precise localization of the various subtypes remains poorly known. A preliminary study [102] shows that the M1 subtype is present in all cortical layers whereas the subtypes M2 and M4 are less abundant. Moreover, the labelling of the M2 subtype is highly correlated with the labelling of cholinergic neurons. Finally, the M5 subtype seems to be preferentially localized in the superficial layers of the cortex [158].

Pharmacology

Agonists

To date, muscarine (an alkaloid extracted from poisonous mushroom *Amanita muscaria)* is the main pharmacological tool used to activate specifically muscarinic receptors and there is no agonist with a specific selectivity for one particular subtype [31].

Antagonists

Atropine, a well known alkaloid extracted from the plant *Atropa belladonna*, is a non-selective muscarinic antagonist. Moreover, this antagonist has, at high concentrations, non-specific effects on other receptors. To differentiate muscarinic receptor subtypes, only a few antagonists have been introduced [55]. Pirenzepine is used at low concentrations as a specific antagonist of the M1 family [73]. Methoctramine and AFDX-116 [11–2({-[(diethylamino)methyl]-1-piperidinyl}-acetyl)-5,11-dihydro-6H-pyrido(2,3-b) (1,4)-benzodiazepine-one] [132], are more selective for the M2 family [55] whereas 4-DAMP [4-diphenylacetoxy-*N*-(2-chloroethyl)-piperidine hydrochloride] seems to be more specific for the M3 subtype [12]. MT-7, a toxin purified from the venom of *Dendroaspis angusticeps*, inhibits, with high selectivity, the M1 subtype [2, 142] and it is the most specific antagonist for any subtype of muscarinic receptor [31]. The lack of selective agonists and the paucity of highly selective antagonists are major problems impeding the characterization of the different subtypes of muscarinic receptors.

Functions

Several studies have localized muscarinic receptors at peri-, extra-, pre- and postsynaptic levels on pyramidal neurons and non-pyramidal neurons [31, 135, 136, 164]. Postsynaptic muscarinic receptors induce a depolarization of neurons by inhibiting different K^+ currents: (1) the rectifying outward current I_m (m for muscarinic), which is voltage dependent and insensitive to Ca^{2+}, and activated at the resting potential [46, 94, 120], (2) Ca^{2+}- and voltage-dependent I_{AHP} current (AHP for afterhyperpolarization) which is responsible for the slow post-hyperpolarization [120], (3) the leak current (I_{leak}), independent of the potential and Ca^{2+} [114], (4) the voltage-dependent potassium current (I_k) [202]. However, postsynaptic receptors can also act by inhibiting Ca^{2+} channels in the hippocampus [67]. It is generally accepted that the muscarinic receptors of M1/M3 subtype are located at the postsynaptic level [102, 136, 153]. The localization of the M2/M4 receptors is less well defined but was traditionally recognized at the presynaptic level as an autoreceptor implied in negative feedback [49, 102, 203] or as a heteroreceptor regulating synaptic transmission by acting on Ca^{2+} channels [171, 181].

For a long time, muscarinic M5 subtype receptors were considered non-functional because little was known about their localization, their binding properties and their physiological functions. Reever et al. [158], using an exclusion labelling technique, showed that the M5 subtype was distinct from the others, with a preferential localization in the superficial layers of the cortex. These preliminary results suggested that the M5 subtype has a significant and independent role in modulating the cortical network. However, specific ligands need to be developed in order to explore the physiological function of M5 receptors.

Cholinergic modulation of the synaptic response

To study the intimate mechanisms underlying the cognitive functions implicating ACh, many authors have worked on the modulatory role of ACh on the cortical [129, 149] networks of mammals. The cortical network is mainly composed of extracortical fibres and of pyramidal and non-pyramidal interconnected neurons. In a given

cortical neuron, the synaptic response is complex, resulting from an interaction between both excitatory inputs, which are mainly glutamatergic, and inhibitory inputs, mainly GABAergic. To understand the modulatory role of ACh in the synaptic response, a pharmacological dissection has been done in order to study the excitatory and inhibitory components of the response independently. The release of glutamate seems to be essentially increased by the activation of presynaptic nicotinic receptors in rat prefrontal cortex [71, 185]. Muscarinic agonist application also increases glutamate release. Agonists activate postsynaptic muscarinic receptors, which induces a short hyperpolarization followed by a slow depolarization of cortical pyramidal neurons [30, 87, 119, 120]. The fast hyperpolarization is due to the depolarization of GABA interneurons making synapses with pyramidal neurons, whereas slow depolarization is obtained mainly by depression of the M current, which can be activated only at the depolarized potentials and by the removal of I_{AHP} current responsible for the AHP [120].

The release of GABA has been also reported to be increased by the activation of presynaptic nicotinic receptors in hippocampus [156]. Recent studies have demonstrated that nicotine could act on postsynaptic nicotinic receptors to induce an excitatory current on interneurons [4, 154, 161, 200]. Moreover, activation of postsynaptic muscarinic receptors can induce a fast excitation of GABA interneurons by changing their membrane potential [120], leading to an increase of GABA release [87].

However, application of muscarinic agonists can also remove both excitatory and inhibitory transmission [7, 90, 139] by a presynaptic [90] or a postsynaptic mechanism. In this latter case, the activation of these receptors may induce a hyperpolarization of neurons [89] as first reported to occur in the rat parabrachial nucleus [54].

All these results were obtained by application of exogenous ACh or other agonists and do not allow conclusions to be drawn about the effects of endogenous ACh. Moreover, pharmacological dissection of the synaptic response in these experiments did not reveal any information about ACh's modulatory effect on the functional integrative signal (i.e. the interaction between excitation and inhibition).

In the auditory cortex, Metherate and Ashe [129] attempt to examine how spontaneously released ACh acts on synaptic potentials, using an anticholinesterase compound. They concluded that ACh depresses synaptic potentials mediated by both glutamate and GABA. In another study using the muscarinic antagonist atropine, an endogenous muscarinic component was identified in the evoked synaptic response [10]. However, numerous questions still remain with respect to the endogenous cholinergic modulation of the interaction between excitation and inhibition.

In an attempt to contribute to a new approach to answer these questions, we proposed an analysis based on the continuous measurement of conductance variation in response to synaptic activations [18]. Taking into account that the reversal potential of excitatory signals is 0 mV and of inhibitory signals is −80 mV, this variation of conductance is linearly decomposed into its excitatory (glutamatergic) and inhibitory (GABAergic) components assuming that no voltage-dependent current was activated in the recorded neuron. The decomposition is made with an algorithm [two equations with two unknown values, the excitatory (G_{exc}) and the inhibitory (G_{inh}) conductances] based on the values of the conductance $\Delta G(t)$ and of the apparent reversal potential recorded in the soma at any time of the response. This method allows us to explain how synaptic inhibition interacts with synaptic excitation during a synaptic response, independently of the blockade of one of the components (excitation or inhibition). Then, the modulatory effects of endogenous cortical ACh, released by electrical stimulation of cholinergic afferents, on this interaction can be studied. Figure 4 illustrates the potential of this method. In order to study the muscarinic modulation of the synaptic integration by endogenous ACh, we perfused atropine (10 μM) following stimulation of synaptic afferents, including cholinergic ones. The synaptic response induced by the electrical stimulation of layer I was recorded in a pyramidal neuron of layer V by whole-cell patch-clamp recording from cortical slices in control conditions and after atropine application. Analysis of the recording current shows that the total conductance of the response remains unchanged. However, the decomposition method revealed that the amplitude of inhibitory conductance decreased whereas the amplitude of excitatory conductance increased. One consequence of these changes was a large increase in the amplitude of the depolarization on the voltage trace. So, we can hypothesize that endogenous ACh has opposite effects on GABAergic interneurons versus glutamatergic neurons, to decrease the excitability of the recorded neuron, but this analysis does not permit us to identify the precise level of the modulation of the excitation and/or the inhibition in a polysynaptic network. However, the opposite effects of ACh could be explained by the activation of different subtypes of muscarinic receptors on GABAergic and glutamatergic neurons. A specific investigation of the involvement of nicotinic and muscarinic receptors in the modulation of the cortical network is underway, using selective pharmacological tools (unpublished results).

To date, all data concerning cholinergic modulation in the brain have revealed that it is the result of a mixture of positive and negative modulations, implying that each type of cholinergic receptor has a different location. What is the physiological significance of these modulations?

Functional explanations of these cholinergic modulations have been proposed by Kimura [89], who considers the synaptic inputs activated and implied in the synaptic response. Electrophysiological studies performed on the pyriform cortex [149] and on slices of somatosensory cortex [70], or studies carried out by optical measurements in the visual cortex [91] show that ACh removes the excitation and the inhibition induced by intracortical innervation. In contrast, excitation due to the activation of

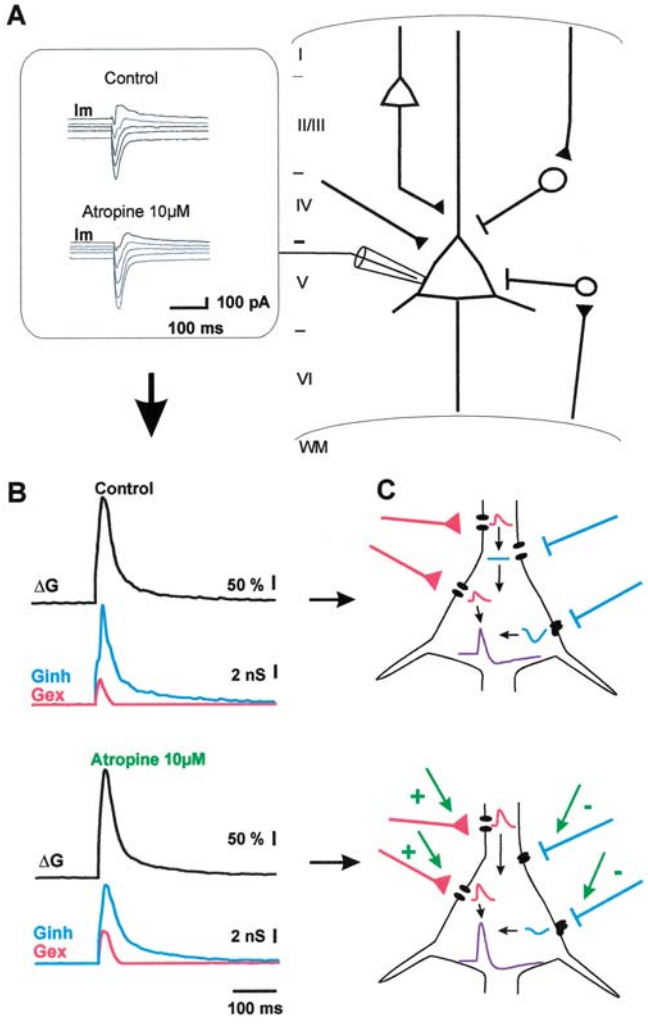

Fig. 4A–C New approach for the analysis of a synaptic response. **A** (*right*) Schematic of the rat cortical network including GABAergic and glutamatergic neurons. Recordings are made from the pyramidal neuron of layer V following electrical stimulation of afferents. **A** (*left*) Current synaptic responses at five holding potentials in control conditions and after the application of 10 µM atropine. **B** Corresponding total conductance changes (ΔG, *black trace*) under control conditions and after atropine application. Inhibitory (G_{inh}, *blue trace*) and excitatory (G_{exc}, *red trace*) conductances responsible for total conductance change. Atropine induced an increase in G_{exc} and a decrease in G_{inh}. **C** Hypothetical representation of synaptic integration under control conditions (*top*) and in the presence of atropine (*bottom*). Glutamatergic excitatory postsynaptic potentials are represented in *red*. Excitation of GABAergic afferents induces either a shunting inhibition mediated by activation of $GABA_A$ receptors (*blue line*), or an inhibitory postsynaptic potential mediated by activation of $GABA_B$ receptors (*blue curve*). *Purple trace* is the voltage response resulting from the synaptic integration. Atropine acting by inhibiting GABAergic neurons and activating glutamatergic neurons enhances the depolarizing phase of the synaptic response, leading to an increase in the excitability of the neuron

extracortical afferents is either insensitive to ACh [75], or even facilitated by ACh, in the case of the thalamic afferents [70]. Inhibition of the intracortical innervation seems to be specifically due to the activation of presynaptic muscarinic receptors [70] whereas nicotinic receptors activate the synaptic response in a different way, according to the location of the synaptic entries. The optical recordings show that the suppression of the excitation induced by white matter stimulation is variably significant, according to the target layer. Excitation recorded in the superficial layers (II/III) and deep layers (V, VI) is decreased by 40% to 50%, whereas it is decreased by 20% to 30% in the intermediate layers [91]. So it could be supposed that ACh shifts the network from a prevalently intracortical influence to a prevalently extracortical influence.

Hypotheses about the global action of ACh on the network have been made and numerous specific actions of ACh on its receptors have been found. However, the link between specific actions on receptors and the global action of ACh is still lacking. The diversity of both muscarinic and nicotinic receptors would certainly permit a fine modulation of the synaptic response. Few studies have been made to understand the role of the different subtypes of receptors and many questions are unresolved. If these various receptors subtypes have a differential localization on specific neurons, their activation might permit a precise modulation of neurons and a facilitation of certain pathways. Using the above-described analysis, we are attempting to identify the particular role and neuronal localization of different subtypes of both cholinergic receptors.

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References

1. Adams DJ, Nutter TJ (1992) Calcium permeability and modulation of nicotinic acetylcholine receptor-channels in rat parasympathetic neurons. J Physiol (Paris) 86:67–76
2. Adem A, Karlsson E (1997) Muscarinic receptor subtype selective toxins. Life Sci 60:1069–1076
3. Alkondon M, Albuquerque EX (1993) Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. Part I: Pharmacological and functional evidence for distinct structural subtypes. J Pharmacol Exp Ther 265:1455–1473
4. Alkondon M, Pereira EFR, Eisenberg HM, Albuquerque EX (2000) Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neural networks. J Neurosci 20:66–75
5. Alkondon M, Pereira EFR, Wannacott S, Albuquerque EX (1992) Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist. Mol Pharmacol 41:802–808
6. Amador M, Dani JA (1995) Mechanism for modulation of nicotinic acetylcholine receptors that can influence synaptic transmission. J Neurosci 15:4525–4532
7. Aramakis VB, Bandrowski AE, Ashe JH (1997) Muscarinic reduction of GABAergic synaptic potentials results in disinhibition of the AMPA/kainate-mediated EPSP in auditory cortex. Brain Res 758:107–117

8. Arendt T, Bigl V, Arendt A (1984) Neurone loss in the nucleus basalis of Meynert in Creutzfeldt-Jakob disease. Acta Neuropathol (Berl) 65:85–88
9. Arendt T, Bigl V, Arendt A, Tennstedt A (1983) Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. Acta Neuropathol (Berl) 61:101–108
10. Bandrowski AE, Moore SL, Ashe JH (2001) Cholinergic synaptic potentials in the supragranular layers of auditory cortex. Synapse 41:118–130
11. Banerjee S, Punzi JS, Kreilick K, Abood LG (1990) [^{3}H]Mecamylamine binding to rat brain membranes. Studies with mecamylamine and nicotine analogues. Biochem Pharmacol 40:2105–2110
12. Barlow RB, McMillen LS, Veale MA (1991) The use of 4-diphenylacetoxy-N-(2-chloroethyl)-piperidine (4-DAMP mustard) for estimating the apparent affinities of some agonists acting at muscarinic receptors in guinea-pig ileum. Br J Pharmacol 102:657–662
13. Bayraktar T, Staiger JF, Acsady L, Cozzari C, Freund TZ, Zilles K (1997) Co-localization of vasopressive intestinal polypeptide, γ-aminobutyric acid and choline acetyltransferase in neocortical interneurons of the adult rat. Brain Res 757:209–217
14. Bertrand D, Valera S, Bed S, Ballivet M, Rungger D (1991) Steroids inhibit nicotinic acetylcholine receptors. Neuroreport 2:277–280
15. Bigl V, Woolf NJ, Butcher LL (1982) Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital, and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis. Brain Res Bull 8:727–749
16. Bonanno G, Ruelle A, Andrioli GC, Raiteri M (1991) Cholinergic nerve terminals of human cerebral cortex possess a GABA transporter whose activation induces release of acetylcholine. Brain Res 539:191–195
17. Bonner TI, Buckley NJ, Young AC, Brann MR (1987) Identification of a family of muscarinic acetylcholine receptor genes. Science 237:527–532
18. Borg-Graham LJ, Monier C, Fregnac Y (1998) Visual input evokes transient and strong shunting inhibition in visual cortical neurons. Nature 393:369–373
19. Boulter J, O'Shea-Greenfield A, Duvoisin RM, Connolly JG, Wada E, Jensen A, Gardner PD, Ballivet M, Deneris ES, McKinnon D (1990) Alpha 3, alpha 5, and beta 4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. J Biol Chem 265:4472–4482
20. Boyd RT (1997) The molecular biology of neuronal nicotinic acetylcholine receptors. Crit Rev Toxicol 27:299–318
21. Brann MR, Buckley NJ, Bonner TI (1988) The striatum and cerebral cortex express different muscarinic receptor mRNAs. FEBS Lett 230:90–94
22. Broide RS, Leslie FM (1999) The alpha7 nicotinic acetylcholine receptor in neuronal plasticity. Mol Neurobiol 20:1–16
23. Broide RS, O'Connor LT, Smith MA, Smith JA, Leslie FM (1995) Developmental expression of alpha 7 neuronal nicotinic receptor messenger RNA in rat sensory cortex and thalamus. Neuroscience 67:83–89
24. Bucci DJ, Holland PC, Gallagher M (1998) Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. J Neurosci 18:8038–8046
25. Buckley NJ, Bonner TI, Brann MR (1988) Localization of a family of muscarinic receptor mRNAs in rat brain. J Neurosci 8:4646–4652
26. Butcher LL, Oh JD, Woolf NH (1993) Cholinergic neurons identified by *in situ* hybridization histochemistry. Prog Brain Res 98:1–8
27. Butcher LL, Semba K (1989) Reassessing the cholinergic basal forebrain: nomenclature schemata and concepts. Trends Neurosci 12:483–485
28. Carsi-Gabrenas JM, Van der Zee EA, Luiten PG, Potter LT (1997) Non-selectivity of the monoclonal antibody M35 for subtypes of muscarinic acetylcholine receptors. Brain Res Bull 44:25–31
29. Cartier L, Verdugo R, Vergara C, Galvez S (1989) The nucleus basalis of Meynert in 20 definite cases of Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatr 52:304–309
30. Caulfield MR (1993) Muscarinic receptors – characterization, coupling and function. Pharmacol Ther 58:319–379
31. Caulfield MP, Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmac Rev 50:279–290
32. Changeux JP, Bertrand D, Corringer P-J, Dehaene S, Edelstein S, Lena C, Le Novere N, Marubio N, Picciotto M, Zoli M (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. Brain Res Rev 26:198–216
33. Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliot KJ, Elliot EC (1997) Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha 2 beta 2, h alpha 2 beta 4, h alpha 3 beta 2, h alpha 3 beta 4, h alpha 4 beta 2, h alpha 4 beta 4 and h alpha 7 expressed in *Xenopus* oocytes. J Pharmacol Exp Ther 280:346–356
34. Chedotal A, Cozzari C, Faure MP, Hartman BK, Hamel E (1994) Distinct choline acetyltransferase (ChAT) and vasopressive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. Brain Res 646:181–193
35. Clarke PB, Reuben M, el-Bizri H (1994) Blockade of nicotinic responses by physostigmine, tacrine and other cholinesterase inhibitors in rat striatum. Br J Pharmacol 111:695–702
36. Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T, Ballivet M (1990) A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. Neuron 5:847–856
37. Couturier S, Erkman L, Valera S, Rungger D, Bertrand S, Boulter J, Ballivet M, Bertrand D (1990) Alpha 5, alpha 3, and non-alpha 3. Three clustered avian genes encoding neuronal nicotinic acetylcholine receptor-related subunits. J Biol Chem 265:17560–17567
38. Curtis CA, Wheatley M, Bansal S, Birdsall NJ, Eveleigh P, Pedder EK, Poyner D, Hulme EC (1989) Propylbenzilylcholine mustard labels an acidic residue in transmembrane helix 3 of the muscarinic receptor. J Biol Chem 264:489–495
39. Dani JA (2001) Overview of nicotinic receptors and their roles in the central nervous system. Biol Psychiatr 49:166–174
40. Davies AR, Hardick DJ, Blagbrough IS, Potter BV, Wolstenholme AJ, Wonnacott S (1999) Characterisation of the binding of [^{3}H]methyllycaconitine: a new radioligand for labelling alpha 7-type neuronal nicotinic acetylcholine receptors. Neuropharmacology 38:679–690
41. Day J, Fibiger HC (1992) Dopaminergic regulation of cortical cholinergic release. Synapse 12:281–286
42. Delacour J, Houcine O, Costa JC (1990) Evidence for a cholinergic mechanism of "learned" changes in the responses of barrel field neurons of the awake and undrugged rat. Neuroscience 34:1–8
43. Deneris ES, Boulter J, Swanson LW, Patrick J, Heinemann S (1989) Beta 3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. J Biol Chem 264:6268–6272
44. Deneris ES, Connolly J, Boulter J, Wada E, Wada K, Swanson LW, Patrick J, Heinemann S (1988) Primary structure and expression of beta 2: a novel subunit of neuronal nicotinic acetylcholine receptors. Neuron 1:45–54
45. Dineley-Miller K, Patrick J (1992) Gene transcripts for the nicotinic acetylcholine receptor subunit, beta4, are distributed in multiple areas of the rat central nervous system. Brain Res Mol Brain Res 16:339–344

46. Dodd J, Dingledine R, Kelly JS (1981) The excitatory action of acetylcholine on hippocampal neurones of the guinea pig and rat maintained in vitro. Brain Res 207:109–127
47. Dominguez del Toro E, Juiz JM, Peng X, Lindstrom J, Criado M (1994) Immunocytochemical localization of the alpha 7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. J Comp Neurol 349:325–342
48. Donoghue JP, Carroll KL (1987) Cholinergic modulation of sensory responses in rat primary somatic sensory cortex. Brain Res 408:367–371
49. Douglas CL, Baghdoyan HA, Lydic R (2001) M2 muscarinic autoreceptors modulate acetylcholine release in prefrontal cortex of C57BL/6 J mouse. J Pharmacol Exp Ther 299:960–966
50. Dubois B, Ruberg M, Javoy-Agid F, Ploska A, Agid Y (1983) A subcortico-cortical cholinergic system is affected in Parkinson's disease. Brain Res 288:213–218
51. Duvoisin RM, Deneris ES, Patrick J, Heinemann S (1989) The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: beta 4. Neuron 3:487–496
52. Dwoskin LP, Xu R, Ayers JT, Crooks PA (2000) Recent developments in neuronal nicotinic acetylcholine receptor antagonists. Exp Opin Ther Patents 10(10):1561–1581
53. Eckenstein F, Thoenen (1983) Cholinergic neurons in the rat cerebral cortex demonstrated by immunohistochemical localization of choline acetyltransferase. Neurosci Lett 36:211–215
54. Egan TM, North RA (1986) Acetylcholine hyperpolarizes central neurones by acting on an M2 muscarinic receptor. Nature 319:405–407
55. Ehlert FJ, Delen FM, Yun SH, Tran P (1991) Differential coupling of subtypes of the muscarinic receptor to signaling mechanisms in brain and peripheral tissues. Adv Exp Med Biol 287:301–312
56. Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S (1994) α_9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell 79:705–715
57. Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J (2001) Alpha 10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. Proc Natl Acad Sci USA 98:3501–3506
58. Fadel J, Sarter M, Bruno JP (2001) Basal forebrain glutamatergic modulation of cortical acetylcholine release. Synapse 39:201–212
59. Fairclough RH, Joseph R, Richman DP (1993) Imaging ligand binding sites on the *Torpedo* acetylcholine receptor. Ann N Y Acad Sci 681:113–125
60. Felder CC (1995) Muscarinic acetylcholine receptors: signal transduction through multiple effectors. FASEB J 9:619–625
61. Fibiger HC (1982) The organization and some projections of cholinergic neurons of the mammalian forebrain. Brain Res 257:327–388
62. Fine A, Hoyle C, Maclean CJ, Levatte TL, Baker HF, Ridley RM (1997) Learning impairments following injection of a selective cholinergic immunotoxin, ME20.4 IgG-saporin, into the basal nucleus of Meynert in monkeys. Neuroscience 81:331–343
63. Fisher RS, Buchwald NA, Hull CD, Levine MS (1988) GABAergic basal forebrain neurons project to the neocortex: the localization of glutamic acid decarboxylase and choline-acetyltransferase in feline corticopetal neurons. J Comp Neurol 272:489–502
64. Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV (1998) Synaptic potential mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. J Neurosci 18:8228–8235
65. Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV (1998) Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. J Neurosci 18(4):1187–1195
66. Freund TF, Meskenaite V (1992) Gamma-aminobutyric acid-containing basal forebrain neurons innervate inhibitory interneurons in the neocortex. Proc Natl Acad Sci USA 89:738–742
67. Gähwiler BH, Brown DA (1987) Muscarine affects calcium-currents in rat hippocampal pyramidal cells in vitro. Neurosci Lett 76:301–306
68. Galzi JL, Changeux JP (1995) Neuronal nicotinic receptors: molecular organization and regulations. Neuropharmacology 34:563–582
69. Gerzanich V, Anand R, Lindstrom J (1994) Homomers of alpha 8 and alpha 7 subunits of nicotinic receptors exhibit similar channel but contrasting binding site properties. Mol Pharmacol 45:212–220
70. Gil Z, Connors BW, Amitai V (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19:679–686
71. Gioanni Y, Rougeot C, Clarke PB, Lepouse C, Thierry AM, Vidal C (1999) Nicotinic receptors in the rat prefrontal cortex: increase in glutamate release and facilitation of mediodorsal thalamo-cortical transmission. Eur J Neurosci 11(1):18–30
72. Haga T, Haga K, Kameyama K, Nakata H (1993) Phosphorylation of muscarinic receptors: regulation by G proteins. Life Sci 52:421–428
73. Hammer R, Berrie CP, Birdsall NJ, Burgen AS, Hulme EC (1980) Pirenzepine distinguishes between different subclasses of muscarinic receptors. Nature 283:90–92
74. Hasselmo ME, Anderson BP, Bower JM (1992) Cholinergic modulation of cortical associative memory function. J Neurophysiol 67:1230–1246
75. Hasselmo ME, Bower JM (1992) Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex. J Neurophysiol 67:1222–1229
76. Henderson Z (1981) A projection from acetylcholinesterase-containing neurones in the diagonal band to the occipital cortex of the rat. Neuroscience 6:1081–1088
77. Hill JA Jr, Zoli M, Bourgeois JP, Changeux JP (1993) Immunocytochemical localization of a neuronal nicotinic receptor: the beta 2-subunit. J Neurosci 13:1551–1568
78. Hirano H, Day J, Fibiger HC (1995) Serotoninergic regulation of acetylcholine release in the rat frontal cortex. J Neurochem 65:1139–1145
79. Hohmann CF, Kwiterovich KK, Oster-Granite ML, Coyle JT (1991) Newborn basal forebrain lesions disrupt cortical cytodifferentiation as visualized by rapid Golgi staining. Cereb Cortex 1(2):143–157
80. Hulme EC, Birdsall NJ, Buckley NJ (1990) Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol 30:633–673
81. Jasper HH, Tessier J (1971) Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. Science 172:601–602
82. Jimenez-Capdeville ME, Dykes RW (1996) Changes in cortical acetylcholine release in the rat during day and night: differences between motor and sensory areas. Neuroscience 71:567 –579
83. Jimenez-Capdeville ME, Dykes RW, Myasnikov AA (1997) Differential control of cortical activity by the basal forebrain in rats: a role for both cholinergic and inhibitory influences. J Comp Neurol 381:53–67
84. Johnston MV, McKinney M, Coyle JT (1981) Neocortical cholinergic innervation: a description of extrinsic and intrinsic components in the rat. Exp Brain Res 43:159–172
85. Juliano SL, Ma W, Eslin D (1991) Cholinergic depletion prevents expansion of topographic maps in somatosensory cortex. Proc Natl Acad Sci USA 88:780–784
86. Kao PN, Karlin A (1986) Acetylcholine receptor binding site contains a disulfide cross-link between adjacent half-cystinyl residues. J Biol Chem 261:8085–8088

87. Kawagushi Y (1997) Selective cholinergic modulation of cortical GABAergic cell subtypes. J Neurophysiol 78:1746–1747
88. Kellar KJ, Martino AM, Hall DP Jr, Schwartz RD, Taylor RL (1985) High-affinity binding of [^{3}H]acetylcholine to muscarinic cholinergic receptors. J Neurosci 5:1577–1582
89. Kimura F (2000) Cholinergic modulation of cortical function: a hypothetical role in shifting the dynamics in cortical network. Neurosci Res 38:19–26
90. Kimura F, Baughman RW (1997) Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. J Neurophysiol 77:709–716
91. Kimura F, Fukuda M, Tsumoto T (1999) Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. Eur J Neurosci 11:3597–3609
92. Kish SJ, El-Awar M, Schut L, Leach L, Oscar-Berman M, Freedman M (1988) Cognitive deficits in olivopontocerebellar atrophy: implications for the cholinergic hypothesis of Alzheimer's dementia. Ann Neurol 24:200–206
93. Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP (2001) Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci 21:1452–1463
94. Krnjevic K, Pumain R, Renaud L (1971) The mechanism of excitation by acetylcholine in the cerebral cortex. J Physiol (Lond) 215:247–268
95. Kubo T, Fukuda K, Mikami A, Maeda A, Takahashi H, Mishina M, Haga T, Haga K, Ichiyama A, Kangawa K (1986) Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323:411–416
96. Kubo T, Maeda A, Sugimoto K, Akiba I, Mikami A, Takahashi H, Haga T, Haga K, Ichiyama A, Kangawa K (1986) Primary structure of porcine cardiac muscarinic acetylcholine receptor deduced from the cDNA sequence. FEBS Lett 209:367–372
97. Kuhar M, Yamamura HI (1976) Localization of cholinergic muscarinic receptors in rat brain by light microscopic radioautography. Brain Res 110:229–243
98. Lehmann J, Nagy JI, Atmadia S, Fibiger HC (1980) The nucleus basalis magnocellularis: the origin of a cholinergic projection to the neocortex of the rat. Neuroscience 5:1161–1174
99. Lena C, Changeux JP (1993) Allosteric modulations of the nicotinic acetylcholine receptor. Trends Neurosci 16:181–186
100. Lena C, Changeux JP, Mulle C (1993) Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. J Neurosci 13:2680–2688
101. Le Novere N, Zoli M, Changeux JP (1996) Neuronal nicotinic receptor alpha 6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. Eur J Neurosci 8:2428–2439
102. Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218–3226
103. Levey AI, Wainer BH, Rye DB, Mufson EJ, Mesulam MM (1984) Choline acetyltransferase-immunoreactive neurons intrinsic to rodent cortex and distinction from acetylcholinesterase-positive neurons. Neuroscience 13:341–353
104. Lindström J (1997) Nicotinic acetylcholine receptors in health and desease. Mol Neurobiol 15:193–222
105. Liu C, Nordberg A, Zhang X (1996) Differential co-expression of nicotinic acetylcholine receptor alpha 4 and beta 2 subunit genes in various regions of rat brain. Neuroreport 7:1645–1649
106. Liu JK, Kato T (1994) Effects of physostigmine on relative acetylcholine output induced by systemic treatment with scopolamine in an in vivo microdialyse of rat frontal cortex. Neurochem Int 24:589–596
107. Lopez MG, Fonteriz RI, Gandia L, de la Fuente M, Villarroya M, Garcia-Sancho J, Garcia AG (1993) The nicotinic acetylcholine receptor of the bovine chromaffin cell, a new target for dihydropyridines. Eur J Pharmacol 247:199–207
108. Luetje CW, Patrick J (1991) Both alpha- and beta-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. J Neurosci 11:837–845
109. Luetje CW, Wada K, Rogers S, Abramson SN, Tsuji K, Heinemann S, Patrick J (1990) Neurotoxins distinguish between different neuronal nicotinic acetylcholine receptor subunit combinations. J Neurochem 55:632–640
110. Luiten PG, Gaykema RP, Traber J, Spencer DG Jr (1987) Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported *Phaseolus vulgaris* leucoagglutinin. Brain Res 413:229–250
111. Luiten PG, Spencer DG Jr, Traber J, Gaykema RP (1985) The pattern of cortical projections from the intermediate parts of the magnocellular nucleus basalis in the rat demonstrated by tracing with *Phaseolus vulgaris*-leucoagglutinin. Neurosci Lett 57:137–142
112. Lukas RJ, Changeux JP, Le Novere N, Albuquerque EX, Balfour DJ, Berg DK, Bertrand D, Chiappinelli VA, Clarke PB, Collins AC, Dani JA, Grady SR, Kellar KJ, Lindstrom JM, Marks MJ, Quik M, Taylor PW, Wonnacott S (1999) International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. Pharmacol Rev 51:397–401
113. Lysakowski A, Wainer BH, Bruce G, Hersh LB (1989) An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. Neuroscience 28:291–336
114. Madison DV, Lancaster B, Nicoll RA (1987) Voltage clamp analysis of cholinergic action in the hippocampus. J Neurosci 7(3):733–741
115. Maelicke A, Schrattenholz A, Samochocki M, Radina M, Albuquerque EX (2000) Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease. Behav Brain Res 113:199–206
116. Magnani M, Mantovani P, Pepeu G (1984) Effect of cholecystokinin octapeptide and ceruletide on release of acetylcholine from cerebral cortex of the rat in vivo. Neuropharmacology 11:1305–1309
117. Mash DC, Potter LT (1986) Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. Neuroscience 19:551–564
118. Materi LM, Semba K (2001) Inhibition of synaptically evoked cortical acetylcholine release by intracortical glutamate: involvement of GABAergic neurons. Eur J Neurosci 14:38–46
119. McCormick DA (1992) Cellular mechanisms underlying cholinergic and noradrenergic modulation of neuronal firing mode in the cat and guinea pig dorsal lateral geniculate nucleus. J Neurosci 12:278–289
120. McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J Physiol (Lond) 375:169–194
121. McGehee DS (2002) Nicotinic receptors and hippocampal synaptic plasticity …it's all in the timing. Trends Neurosci 25:171–172
122. McGehee DS, Role LW (1996) Presynaptic ionotropic receptors. Curr Opin Neurobiol 6:342–349
123. Mechawar N, Cozzari C, Descarries L (2000) Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. J Comp Neurol 428:305–318
124. Mesulam MM, Geula C (1988) Nucleus basalis (Ch4) and cortical cholinergic innervation in the human brain: observations based on the distribution of acetylcholinesterase and choline acetyltransferase. J Comp Neurol 275:216–240
125. Mesulam MM, Hersh LB, Mash DC, Geula C (1992) Differential cholinergic innervation within functional subdivisions of the human cerebral cortex: a choline acetyltransferase study. J Comp Neurol 318:316–328

126. Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10:1185–1201
127. Mesulam MM, Volicer L, Marquis JK, Mufson EJ, Green RC (1986) Systematic regional differences in the cholinergic innervation of the primate cerebral cortex: distribution of enzyme activities and some behavioral implications. Ann Neurol 19:144–151
128. Metherate R, Ashe JH (1993) Nucleus basalis stimulation facilitates thalamocortical synaptic transmission in the rat auditory cortex. Synapse 14:132–143
129. Metherate R, Ashe JH (1995) Synaptic interactions involving acetylcholine, glutamate, and GABA in rat auditory cortex. Exp Brain Res 107:59–72
130. Metherate R, Cox CL, Ashe JH (1992) Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. J Neurosci 12:4701–4711
131. Miranda MI, Bermudez-Rattoni F (1999) Reversible inactivation of the nucleus basalis magnocellularis induces disruption of cortical acetylcholine release and acquisition, but not retrieval, of aversive memories. Proc Natl Acad Sci USA 96:6478–6482
132. Mochida S, Kobayashi H (1988) A novel muscarinic receptor antagonist AF-DX 116 differentially blocks slow inhibitory and slow excitatory postsynaptic potentials in the rabbit sympathetic ganglia. Life Sci 22(42):2195–2201
133. Mogg AJ, Whiteaker P, McIntosh JM, Marks M, Collins AC, Wonnacott S (2002) Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII- sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. J Pharmacol Exp Ther 302:197–204
134. Moore H, Sarter M, Bruno JP (1995) Bidirectional modulation of cortical acetylcholine efflux by infusion of benzodiazepine receptor ligands into the basal forebrain. Neurosci Lett 189:31–34
135. Mrzljak L, Levey AI, Belcher S, Goldman-Rakic PS (1998) Localization of the m2 muscarinic acetylcholine receptor protein and mRNA in cortical neurons of the normal and cholinergically deafferented rhesus monkey. J Comp Neurol 390(1):112–132
136. Mrzljak L, Levey AI, Goldman-Rakic PS (1993) Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission. Proc Natl Acad Sci USA 90(11):5194–5198
137. Mulle C, Choquet D, Korn H, Changeux JP (1992) Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. Neuron 8(1):135–143
138. Mulle C, Lena C, Changeux JP (1992) Potentiation of nicotinic receptor response by external calcium in rat central neurons. Neuron 8:937–945
139. Murakoshi T (1995) Cholinergic modulation of synaptic transmission in the rat visual cortex in vitro. Vision Res 35:25–35
140. Nakano I, Hirano A (1983) Neuron loss in the nucleus basalis of Meynert in parkinsonism-dementia complex of Guam. Ann Neurol 13:87–91
141. Nakayama H, Shioda S, Okuda H, Nakashima T, Nakai Y (1995) Immunocytochemical localization of nicotinic acetylcholine receptor in rat cerebral cortex. Brain Res Mol Brain Res 32:321–328
142. Nasman J, Jolkkonen M, Ammoun S, Karlsson E, Akerman KE (2000) Recombinant expression of a selective blocker of M(1) muscarinic receptors. Biochem Biophys Res Commun 271:435–439
143. Nathanson NM (2000) A multiplicity of muscarinic mechanisms: enough signaling pathways to take your breath away. Proc Natl Acad Sci USA 97:6245–6247
144. Nef P, Oneyser C, Alliod C, Couturier S, Ballivet M (1988) Genes expressed in the brain define three distinct neuronal nicotinic acetylcholine receptors. EMBO J 7:595–601
145. Newman MB, Nazian SJ, Sanberg PR, Diamond DM, Shytle RD (2001) Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat. Prog Neuropsychopharmacol Biol Psychiatr 25(3):609–620
146. O'Dell TJ, Christensen BN (1988) Mecamylamine is a selective non-competitive antagonist of N-methyl-D-aspartate and aspartate-induced currents in horizontal cells dissociated from the catfish retina. Neurosci Lett 94:93–98
147. Parnavelas JG, Kelly W, Franke E, Eckenstein F (1986) Cholinergic neurons and fibres in the rat visual cortex. J Neurocytol 15:329–336
148. Paterson D, Nordberg A (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 61:75–111
149. Patil MM, Hasselmo ME (1999) Modulation of inhibitory synaptic potentials in the pyriform cortex. J Neurophysiol 81:2103–2118
150. Pepeu G, Blandina P (1998) The acetylcholine, GABA, glutamate triangle in the rat forebrain. J Physiol (Paris) 92:351–355
151. Pereira EF, Reinhardt-Maelicke S, Schrattenholz A, Maelicke A, Albuquerque EX (1993) Identification and functional characterization of a new agonist site on nicotinic acetylcholine receptors of cultured hippocampal neurons. J Pharmacol Exp Ther 265:1474–1491
152. Pirch JH, Turco K, Rucker HK (1992) A role for acetylcholine in conditioning-related responses of rat frontal cortex neurons: microiontophoretic evidence. Brain Res 586:19–26
153. Porter AC, Bymaster FP, DeLapp NW, Yamada M, Wess J, Hamilton SE, Nathanson NM, Felder CC (2002) M1 muscarinic receptor signaling in mouse hippocampus and cortex. Brain Res 944:82–89
154. Porter JT, Cauli B, Tsuzuki K, Lambolez B, Rossier J, Audinat E (1999) Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. J Neurosci 19:5228–5235
155. Pugh PC, Berg DK (1994) Neuronal acetylcholine receptors that bind alpha-bungarotoxin mediate neurite retraction in a calcium-dependent manner. J Neurosci 14:889–896
156. Radcliffe KA, Fisher JL, Gray R, Dani JA (1999) Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. Ann N Y Acad Sci 868:591–610
157. Rasmusson DD, Dykes RW (1988) Long-term enhancement of evoked potentials in cat somatosensoriel cortex by co-activation of the basal forebrain and cutaneous receptors. Exp Brain Res 70:276–286
158. Reever CM, Ferrari-DiLeo G, Flynn DD (1997) The M5 (m5) receptor subtype: fact or fiction? Life Sci 60:1105–1112
159. Riker WF, Wescoe W (1951) The pharmacology of flaxedil with observations on certain analogues. Ann NY Acad Sci 54:373–392
160. Robertson RT, Gallardo KA, Claytor KJ, Ha DH, Ku KH, Yu BP, Lauterborn JC, Wiley RG, Yu J, Gall CM, Leslie FM (1998) Neonatal treatment with 192 IgG-saporin produces long-term forebrain cholinergic deficits and reduces dendritic branching and spine density of neocortical pyramidal neurons. Cereb Cortex 8:142–155
161. Roerig B, Nelson DA, Katz L (1997) Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT_3 receptors in developing visual cortex. J Neurosci 17:8353–8362
162. Role LW, Berg DK (1996) Nicotinic receptors in the development and modulation of CNS synapses. Neuron 16:1077–1085
163. Rotter A, Birdsall NJ, Burgen AS, Field PM, Hulme EC, Raisman G (1979) Muscarinic receptors in the central nervous system of the rat. I. Technique for autoradiographic localization of the binding of [^{3}H]propylbenzilylcholine mustard and its distribution in the forebrain. Brain Res 180(2):141–165
164. Rouse ST, Thomas TM, Levey AI (1997) Muscarinic acetylcholine receptor subtype, m2: diverse functional implications of differential synaptic localization. Life Sci 60:1031–1038

165. Ruberg M, Javoy-Agid F, Hirsch E, Scatton B, Lheureux R, Hauw J-J, Duyckaerts C, Gray F, Morel-Maroger A, Rascol A, Serdaru M, Agid Y (1985) Dopaminergic and cholinergic lesions in progressive supranuclear palsy. Ann Neurol 18:523–529
166. Sargent PB (1993) The diversity of neuronal nicotinic acetylcholine receptors. Annu Rev Neurosci 16:403–443
167. Sarter M, Bruno JP (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Rev 23:28–46
168. Schoepfer R, Conroy WG, Whiting P, Gore M, Lindstrom J (1990) Brain alpha-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. Neuron 5:35–48
169. Schoepfer R, Whiting P, Esch F, Blacher R, Shimasaki S, Lindstrom J (1988) cDNA clones coding for the structural subunit of a chicken brain nicotinic acetylcholine receptor. Neuron 1:241–248
170. Schrattenholz A, Pereira EF, Roth U, Weber KH, Albuquerque EX, Maelicke A (1996) Agonist responses of neuronal nicotinic acetylcholine receptors are potentiated by a novel class of allosterically acting ligands. Mol Pharmacol 49:1–6
171. Segal M (1989) Presynaptic cholinergic inhibition in hippocampal cultures. Synapse 4:305–312
172. Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW (1993) Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J Neurosci 13:596–604
173. Sillito AM, Kemp JA (1983) Cholinergic modulation of the functional organization of the cat visual cortex. Brain Res 289:143–155
174. Sillito AM, Murphy PC (1987) The cholinergic modulation of cortical function. In: Jones EG, Peters A (eds) Cerebral cortex, volume 6. Plenum, New York, pp 161–185
175. Steinlein OK (1998) New functions for nicotinic acetylcholine receptors ? Behav Brain Res 95:31–35
176. Svensson AL, Nordberg A (1996) Tacrine interacts with an allosteric activator site on alpha4beta2 nAChRs in M10 cells. Neuroreport 7:2201–2205
177. Tagliavini F, Pilleri G, Bouras C, Constantinidis J (1984) The basal nucleus of Meynert in patients with progressive supranuclear palsy. Neurosci Lett 44:37–42
178. Tellez S, Colpaert F, Marien M (1997) Acetylcholine release in the rat prefrontal cortex in vivo, modulation by α_2-adrenoceptor agonists and antagonists. J Neurochem 68:778–785
179. Torrao AS, Britto LR (2002) Neurotransmitter regulation of neural development: acetylcholine and nicotinic receptors. An Acad Bras Cienc 74:453–461
180. Uhl GR, McKinney M, Hedreen JC, White CL III, Coyle JT, Whitehouse PJ, Price DL (1982) Dementia pugilistica: loss of basal forebrain cholinergic neurons and cortical cholinergic markers (Abstract). Ann Neurol 12:99
181. Van der Zee EA, Luiten PG (1999) Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. Prog Neurobiol 58:409–471
182. Van der Zee EA, Streefland C, Strosberg AD, Schröder H, Luiten PGM (1992) Visualization of cholinoceptive neurons in the rat neocortex: colocalization of muscarinic and nicotinic acetylcholine receptors. Mol Brain Res 14:326–336
183. Vernino S, Amador M, Luetje CW, Patrick J, Dani JA (1992) Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron 8:127–134
184. Vernino S, Rogers M, Radcliffe KA, Dani JA (1994) Quantitative measurement of calcium flux through muscle and neuronal nicotinic acetylcholine receptors. J Neurosci 14(9):5514–5524
185. Vidal C, Changeux JP (1993) Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. Neuroscience 56:23–32
186. Vilaro MT, Palacios JM, Mengod G (1990) Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci Lett 114:154–159
187. Vogt BA (1991) The role of layer I in cortical function. In : Peters A (ed) Cerebral cortex, volume 9.Plenum, New York, pp 49–80.
188. Voytko ML, Olton DS, Richardson RT, Gorman LK, Tobin JR, Price DL (1994) Basal forebrain lesions in monkeys disrupt attention but not learning and memory. J Neurosci 14:167–186
189. Wada K, Ballivet M, Boulter J, Connolly J, Wada E, Deneris ES, Swanson LW, Heinemann S, Patrick J (1988) Functional expression of a new pharmacological subtype of brain nicotinic acetylcholine receptor. Science 240:330–334
190. Wada E, McKinnon D, Heinemann S, Patrick J, Swanson LW (1990) The distribution of mRNA encoded by a new member of the neuronal nicotinic acetylcholine receptor gene family (alpha 5) in the rat central nervous system. Brain Res 526:45–53
191. Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW (1989) Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. J Comp Neurol 284:314–335
192. Wamsley JK, Lewis MS, Young WS 3rd, Kuhar MJ (1981) Autoradiographic localization of muscarinic cholinergic receptors in rat brainstem. J Neurosci 1:176–191
193. Weiner DM, Levey AI, Brann MR (1990) Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc Natl Acad Sci USA 87:7050–7054
194. Wenk H, Bigl V, Meyer U (1980) Cholinergic projections from magnocellular nuclei of the basal forebrain to cortical areas in rats. Brain Res 2:295–316
195. Wess J, Blin N, Mutschler E, Bluml K (1995) Muscarinic acetylcholine receptors: structural basis of ligand binding and G protein coupling. Life Sci 56:915–922
196. Wolfe BB, Yasuda RP (1995) Development of selective antisera for muscarinic cholinergic receptor subtypes. Ann N Y Acad Sci 757:186–193
197. Wonnacott S (1997) Presynaptic nicotinic ACh receptor. Trends Neurosci 20:92–98
198. Woody CD, Gruen E (1987) Acetylcholine reduces net outward currents measured *in vivo* with single electrode voltage clamp techniques in neurons of the motor cortex of cats. Brain Res 424:193–198
199. Woolf NJ (1991) Cholinergic systems in mammalian brain and spinal cord. Prog Neurobiol 37:475–524
200. Xiang Z, Huguenard JR, Prince DA (1998) Cholinergic switching within neocortical inhibitory networks. Science 281:985–988
201. Yates CM, Simpson J, Maloney AFJ, Gordon A, Reid AH (1980) Alzheimer-like cholinergic deficiency in Down syndrome. Lancet 2:979
202. Zhang L, Weiner JL, Carlen PL (1992) Muscarinic potentiation of IK in hippocampal neurons: electrophysiological characterization of the signal transduction pathway. J Neurosci 12(11):4510–4520
203. Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J (2002) Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. J Neurosci 22:1709–1717
204. Zilles K, Schroder H, Schroder U, Horvath E, Werner L, Luiten PG, Maelicke A, Strosberg AD (1989) Distribution of cholinergic receptors in the rat and human neocortex. EXS 57:212–228
205. Zoli M, Le Novere N, Hill JA Jr, Changeux JP (1995) Developmental regulation of nicotinic ACh receptor subunit mRNAs in the rat central and peripheral nervous systems. J Neurosci 15:1912–1939