
Brain nicotinic receptors: structure and regulation, role in learning and reinforcement

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Abstract

The introduction, in the late sixties, of the concepts and methods of molecular biology to the study of the nervous system had a profound impact on the field, primarily through the identification of its basic molecular components. These structures include, for example, the elementary units of the synapse: neurotransmitters, neuropeptides and their receptors, but also ion channels, intracellular second messengers and the relevant enzymes, cell surface adhesion molecules, or growth and trophic factors [21,74,81,52,79]. Attempts to establish appropriate causal relationships between these molecular components, the actual organisation of neural networks, and a defined behavior, nevertheless, still must overcome many difficulties. A first problem is the recognition of the minimum levels of organization, from the molecular, cellular, or multicellular (circuit) to the higher cognitive levels, that determine the given physiological and/or behavioral performance under investigation. A common difficulty (and potential source of errors of interpretation) is to relate a cognitive function to a network organization which does not possess the required structural complexity and vice-versa. Another problem is to distinguish, among the components of the system, those which are actually necessary and those which, taken together, suffice for a given behavior to take place. Identification of such a minimal set of building blocks may receive decisive insights from the elaboration of neurally plausible formal models that bring together, within a single and coherent ‘artificial organism’, the neuronal network, the circulating activity, and the behavior they determine (see [42,43,45,72,30]). In this communication, we shall attempt, still in a preliminary fashion, to bring together: (1) our recent knowledge on the molecular biology of brain nicotinic receptors (nAChRs) and their allosteric properties and (2) integrated behaviors, such as cognitive learning, investigated for instance by delayed-response or passive avoidance tasks that are likely to involve nAChRs in particular at the level of reinforcement (or reward) mechanisms (see [18,29,135]). © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Nicotinic receptor; Structure; Learning; Reinforcement

Contents

1. The acetylcholine nicotinic receptor: an allosteric membrane protein mediating signal transduction and its regulation .................................................. 199
   1.1. Identification of the nAChR .................................................................................. 199
   1.2. Structural and functional diversity of nAChR ......................................................... 199
   1.3. Allosteric transitions of the nAChR: models and experimental evidence .............. 201

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1.1. Identification of the nAChR

The nAChR was initially identified as a protein from electric fish electric organ [27,23,91,141] as a heteropentamer of about 300,000 MW with an [α1, β1, γ, δ] stoichiometry. Extensive biochemical studies, including reconstitution experiments, have shown that this single molecular species carries two ACh binding sites located at interfaces between subunits, a unique ion channel along its transmembrane axis of pseudo-symmetry, and all the structural elements that mediate their coupling in the course of the activation and desensitization processes [24,92,153] (Fig. 1).

Application of recombinant DNA techniques to the nAChR in the early 80’s revealed close homologies between nAChR subunit sequences from electric organ and skeletal muscle from higher vertebrates including humans [126,127] and subsequently between muscle and neuronal nAChRs [15,141]. To date, the sequences of 10 neuronal nAChR subunits have been established [141]. Eight are designated as α-subunits (α2–α9) and share with electric organ α1 subunit a pair of adjacent cysteines at positions 192 and 193 in Torpedo [89,91], while the others are referred to as non-α or β-subunits (β2–β4). All nAChR subunits share a similar hydropathy profile, with two hydrophobic domains and four hydrophilic domains (M1–M4) about 20 amino acids long. Parallel affinity labeling (with Torpedo nAChR) and site-directed mutagenesis experiments (primarily with the α7 neuronal subunit expressed in Xenopus oocytes, but also with Torpedo and muscle nAChRs) have demonstrated that for neuronal (as well as for muscle) nAChR: (1) the large N-terminal hydrophilic domain carries the multiple loops [48,62] of the neurotransmitter binding site; (2) the highly variable C-terminal hydrophilic domain faces the cytoplasm, where it can be phosphorylated [81]; and (3) the transmembrane segment M2 forms the wall of the ion channel [86,88,100,83,7,92].

As observed for electric organ and muscle nAChR, neuronal nAChRs are pentameric oligomers that undergo transitions for activation and desensitization. Yet, striking differences exist in the structure and mode of assembly of their subunits, their physiological and pharmacological properties, and their distribution in the brain.

1.2. Structural and functional diversity of nAChR

Analysis of the sequence of the known nAChR subunits indicates that these subunit genes share a common origin and have a long phylogenetic history. The reconstituted phylogenetic tree [98,128,29] places an early divergence from the α9 subunit which, in Xenopus oocyte reconstitution experiments, forms homooligomeric nAChR able to respond to ACh. Yet, the α9-subunit nAChR is inhibited by α-bungarotoxin, as well as by both nicotine and muscarine, and thus exhibits an atypical nicotinic/muscarinic pharmacology. Subsequent divergences have taken place with the vertebrate neuronal α7 and α8 subunits which also form 'low affinity' homopentamers inhibited by α-bungarotoxin. The vertebrate muscle and 'high affinity' neuronal nAChR subunits comprise a large group that, around 1000 million years ago (MYA), may have diversified from their invertebrate counterparts. Structural gene duplications and tissue-specific promoter switches then led to muscle α1, β1, and δ/γ/ε subunits clades which form [α1, β1, γ, δ] complexes and to the 'high affinity' neuronal subunit subfamily, which emerged at the beginning of the chordate phylum. Segregation of the ancestral gene for the β2/β4 subunits probably occurred more than 800 MYA, that for α5/β3 and the rest of the subunit genes slightly later. Finally, the duplications yielding α2 and α4, in one branch, and α3 and α6, in another branch, may have taken place, respectively, more than 620 MYA and 500 MYA, followed by the most recent β2/β4 bifurcation, a little less than 420 MYA. This last duplication coincides with the split between the teleost and the tetrapod lineages and thus accompanies an increased complexity of the brain associated with terrestrial life, together with a correlative diversification of the cholinergic path-
ways. It is quite remarkable that no known duplication of nAChR genes took place over the course of the dramatic increase of brain complexity that characterizes the recent evolution of mammals (Fig. 2).

Multiple combinations of both α-type and β-type subunits form a wide variety of functional heterooligomers, with subunits of two (α2β2, α3β2, α4β2, α3β4, etc.) or more (α3β4α5, α1β2α5, α3β2β4α5) different types [141,161]. These multiple combinations of nAChR subunits possess distinct pharmacological and physiological properties. For instance, nAChR reconstituted in Xenopus oocytes from rat α4β2 subunits exhibits a preferential order of affinity for ACh or nicotine > DMPP > cysrine, in contrast to cysrine > nicotine > ACh > DMPP for rat α4β4 or nicotine > cysrine > DMPP > ACh for rat α7 (ref. in [140]). Photo-affinity labeling and site directed mutagenesis studies have demonstrated that in electric organ and muscle nAChR, the two binding sites are located at the α/γ and α/β interfaces and that the non-α 'complementary' component accounts for their difference in binding properties [129,131,39]. In the case of homopentameric neuronal receptors (α7 to α9), each subunit contributes both a 'principal component' (made up of at least three loops, A, B, and C) at one interface and a 'complementary component' (made up of at least two loops, D and E) at the other interface [39]. It is anticipated that heteropentameric neuronal nAChRs such as α4β2 or α3β4 possess functionally equivalent ligand binding sites at α4/β2 or α3/β4 interfaces with the complementary component provided by the β-type subunit. More complex situations are expected for oligomers comprising three types of subunits such as α3α5β4 [155,161,37,138] or even more such as α3β2β4α5 [36]. The resulting diversity of subunit interfaces most likely accounts for the observed pharmacological diversity of the multiple heterooligomeric receptor species identified (see [99,29] for discussion).

A similar diversity takes place for the ion channel. The relative permeabilities for cations vary with the nature and combination of subunits forming the receptor. The α7/α8
subunits form homo-oligomers which exhibit a pCa\(^{2+}/pNa^+\) higher than 15. Subunits α2 to α6 and β2 to β4 form heterooligomeric channels with a pCa\(^{2+}/pNa^+\) ranging between 0.5 and 2.5. In contrast, the muscle receptors α1, β1, γ, ε, and δ are the least permeable to Ca\(^{2+}\), exhibiting a pCa\(^{2+}/pNa^+\) of about 0.2 [9,7].

Also, the rate of desensitization varies with subunit composition. For instance, α3β2 containing oligomers desensitize 10 times faster than α3β4 or α4β4 oligomers, but α7 homooligomers desensitize even faster [140].

The distribution of neuronal nAChR subunits also strikingly differs from one subunit to the other. In situ hybridization reveals that α2 mRNA distribution is restricted to the spiriformis lateralis nucleus in the chick [41,116] and to the interpeduncular nucleus in the rat [160]. Besides, α3 and β4 mRNA are detected only in autonomic ganglia and in a few central nuclei (such as retina, habenular nuclei, pincel gland, nucleus of the tractus solitarius, etc.) [160,50]; the distribution of α6 and β3 mRNA is limited to somatosensory ganglia and to several central locations, such as the central catecholaminergic nuclei [47,59]; α5 mRNA, though more largely distributed, remains expressed at medium levels in a limited number of areas [159]. In contrast, α4 is widely distributed, in the inner ear cochlear and vestibular ganglia and in the whole brain, except striatum, hippocampus and cerebellum, being particularly concentrated in the thalamus [160]. Finally β2 mRNA and protein are present in the entire nervous system [160,77].

High-affinity \(^3\)H nicotine binding [32] coincides largely with the distribution of the α4β2-subunits [136], whereas \(^125\)I α-bungarotoxin binding [32] parallels the distribution of α7 mRNA that is restricted to a few layers of cerebral cortex, to the hypothalamus, hippocampus, inferior colliculus, and to a few brain stem nuclei in the rat [147].

In the course of embryonic and postnatal development, the α3, α4, β2 and β4 mRNAs appear very early, mostly at rat E11, a stage at which the first neurons differentiate [168]. Later in development some subunit mRNA levels decline, while others increase (e.g., α3 vs. α4 in cerebral cortex) [168]. A challenge for the years to come is to relate the properties of the various nAChR physiological responses recorded in vivo, at the level of a particular neural pathway, to a defined combination of subunits, thus paving the way to a ‘circuit-targeted’ molecular pharmacology.

1.3. Allosteric transitions of the nAChr: models and experimental evidence

The mechanisms by which ACh causes the fast opening of the ion channel and its slow desensitization was, in the past, modeled in terms analogous to those used for enzyme reactions [46,122]. Meanwhile, experiments on bacterial and eukaryotic regulatory enzymes [113] and on hemoglobin [117,133] led to the proposal that the concept of ‘allosteric’ site [17,113] and transition [114] may apply to the processes of signal transduction and of its regulation mediated by neurotransmitter receptors [19,20,28,90,54,55,124].

The initial concept of the allosteric site referred to regulatory sites topologically distinct from the site(s) of biological activity (e.g. catalysis) [17,113]; their proposed function was to selectively bind the regulatory ligands and to cause a modification of properties of the biologically active site(s), in an indirect manner i.e. via a conformational change or allosteric transition of the protein. In a subsequent step and to account, in addition, for cooperative effects between identical ligand binding sites, allosteric proteins were viewed as ‘closed microcrystals’, or oligomers, composed of a finite number of identical subunits and as a consequence possessing at least one axis of symmetry [114]. Allosteric interactions between identical and different binding sites were then postulated to be mediated by a concerted all-or-none (R ^= T) transition between discrete conformational states with different ligand binding properties. The transition was further postulated to preserve the symmetry of the oligomer and the two-states were assumed to exist, in reversible equilibrium, prior to ligand binding (with an isomerization constant L ^= R). The diverse ligands active on the system would then differentially and selectively stabilize the state for
which they display a preferential affinity: the active state for activators (or agonists), the silent inactive, resting state, for inhibitors (or antagonists) [114] (Fig. 3). Many of the straightforward predictions of this simple minimal scheme have been validated by structural studies on regulatory enzymes and hemoglobin [133,6,143]. Particularly striking in this respect is the three-dimensional structural resolution at 2.5 Å of the twoallosteric states (R and T) of a bacterial L-lactate dehydrogenase within the same crystal lattice that clearly demonstrates a conservation of symmetry in the course of the allosteric transition [85].

Yet, the possible occurrence of locally ‘induced’ reorganizations of the ligand binding site complementary to the actual structure of the ligand molecule have been reported in a few instances (for discussion, see [56,133]). They were taken as evidence in favor of an alternative sequential mechanism according to which the conformational transition which mediates signal transduction requires the prior binding of, and is thus ‘induced’ by, the regulatory ligand [94].

The extension of the two-state allosteric model to membrane-bound pharmacological receptors and in particular to nAChRs [20,28,90,33,73–75,16,67,55,124] relies upon structural and functional analogies with classical globular allosteric proteins, yet with several characteristic features.

(1) The nAChRs are transmembrane oligomers carrying several categories of topographically distinct sites but composed of equivalent, though most often non-identical, subunits with an axis of five-fold pseudo-symmetry perpendicular to the plane of the membrane (α7-α9 homomultimers, however, form symmetrical oligomers). (2) Consistent with the two-state model, ACh as well as other nicotinic agonists cause all-or-none openings and closings of the ion channel in the μs to ms time range with intrinsic conductance and ionic selectivities independent of the structure of the agonist. Furthermore, these openings may occur, spontaneously, in the absence of ACh [86–88]. (The alternative sequential-type model [46,34] on the other hand, assumes that the ion channel opens exclusively when two ligands simultaneously occupy the two sites, and thus does not account for the occurrence of these spontaneous openings).

Moreover, at variance with classical allosteric proteins, nAChR, and other ligand-gated ion channels, may undergo multiple conformational transitions ‘en cascade’, in slower time ranges (0.1 s to min) from resting (B) and active (A) states toward distinct conformations, which include closed desensitized (D) states with high affinity for agonists and antagonists, thereby forming what one may call an allosteric network of conformational states [63] (Fig. 3).

![Diagrammatic representation of the two-state allosteric model for regulatory enzymes (Monod–Wyman–Changeux) left and of its extension to ligand-gated ion channels as a multisite allosteric network (right) (modified from [18,26,63]). R and T are respectively the relaxed — high affinity for substances (S) and activators (A) — and constrained — high affinity for inhibitors (I) — states of the two-state model. B, A, and D are respectively B, the basal, resting, closed state; A, the active, open channel; I and D, the fast and slowly desensitized refractory and high affinity states of nicotinic receptor.](image-url)
A considerable body of in vitro fast measurements, in particular by rapid fluorescent or radioactive ligand binding and by ion flux recordings with the same population of membrane microsomes [22], together with in vivo electrophysiological recordings [55,56] is indeed consistent with the extended version of the allosteric scheme and moreover can be fitted quantitatively by the basic equations of the model [58,57].

In agreement with a modulation of the quaternary (and tertiary) structure of the nAChR oligomer, affinity labeling experiments (for example) with the competitive antagonist dimethylaminobenzene diazonium fluoroborate (DDF) under rapid mixing conditions with Torpedo receptor-rich membranes [67] reveal striking reorganizations of the ACh binding sites in the course of the transition from the resting to the desensitized states. Consistent with a location of the binding site at the interface between subunits, the contribution of the 5 subunit to the covalent labeling by DDF increases upon stabilization of the D state by the non-competitive blocker meprap啡素, while the 6 subunit decreases. Moreover, under the same conditions, the contribution of loops A and B to the principal component of the subunits increases up to six-fold relative to loop C, a finding consistent with the higher affinity of the D state for nicotine ligands compared to resting and active states [63,67]. As found with classical allosteric proteins (see [133,85]), the binding domains for nicotine ligands occupy a strategic position at the interfaces between subunits where they probe, but also control, conformational transitions that affect the quaternary structure of the receptor oligomers.

Also, the original allosteric model [114] underlines the critical role of the constraints imposed by the quaternary structure upon the $R \leftrightarrow T$ transition. Consistent with this view, perturbation of the subunit interactions, for instance by omitting the $\gamma$ or the $\epsilon$-subunit in muscle nAChR [168, AChR1], causes a striking increase of the frequency of spontaneous channel openings [88]. The straightforward interpretation of this observation (which, of course, also valid for point mutated receptors) is that the isomerization constant ($L$) between active and resting states which, in the wild type, strongly favors the resting state ($L > 20000$) decreases by several orders of magnitudes in the altered receptor [57,12,88].

1.4. Pleiotropic mutations in the channel and active site domains interpreted in terms of allosteric transitions

The structure–function relationships of ion channels and receptors have been explored by site-directed mutagenesis in a rather rigid framework. The implicit assumption has systematically been that the mutated amino acid causes a phenotype that is directly determined by the properties of the site to which it contributes being carried by some kind of “frozen” protein structure (see [78,83]). Indeed, for instance, mutations within the transmembrane segment M2 alter the ionic specificity of the channel, cationic vs. anionic [66] or Ca$^{2+}$ vs. Na$^+$ [10], together with its intrinsic conductance [83,100]. It was thus rather unexpected to discover [129] (Fig. 4) that in $\alpha 7$ homooligomers mutation of leucine 247, a hydrophobic amino acid from the channel domain M2 — initially identified by labeling with chlorpromazine in Torpedo [69] and conserved in all members of the nAChR family — dramatically increases the apparent affinity for nicotine agonists up to 200-fold. The Leu 247 Thr mutation causes, in addition, a loss of desensitization, a resistance to the channel blocker QX 222, and the appearance of a novel conductance state at 80 pS distinct from the 40 pS of the wild type (WT); moreover, dihydro-β-erythroidine, a competitive antagonist of the WT becomes a full agonist that exclusively activates the high conductance state [139,11,49]. A simple interpretation of this paradoxical phenotype is again based upon the four-state allosteric scheme. It assumes that mutations of the leucine ring at position 247 render the channel permeable to cations at least one of the desensitized, high affinity states. All features of the pleiotropic phenotype of L247 T mutant would simply result from the activation of an open desensitized state ($\gamma$-phenotype).

Mutation at another hydrophobic ring within M2, Val 251, causes a similar phenotype (loss of desensitization, enhanced affinity for agonist), but with a weaker agonistic response to dihydro-β-erythroidine [49]. In this case, an alteration of the isomerization constant, $L$, from the resting to the active state (a decrease from about $8 \times 10^5$ in the WT to about 20 in the mutant) gives a better fit of the data [63] (L phenotype). In other words, the pleiotropic character of channel mutants can be accounted for by alterations of, either the intrinsic properties of one or more of the receptor conformations, or the isomerization constant of one or more of the conformational equilibrium, within the framework of the allosteric scheme (see [63,57,56]) (Fig. 4).

At the level of the nicotinic binding site domain, mutations of affinity labeled residues (or their homologs) cause, in general, reduced apparent affinity for agonists, but with unchanged cooperativity and maximal current amplitude [65]. Attempts to identify the structural elements that account for the differences in binding properties between the high and low affinity neuronal nAChR modified this view and led to the extension of the concept of allosteric transitions to the interpretation of active site properties (138), see also [148]. Microchimeras were constructed in particular with fragments of loop B (AA 151–155) from the high-affinity $\alpha 4$ subunit (which associates with the $\beta 2$ subunit) and introduced into the low affinity $\alpha 7$ subunit. On the other hand, the chimeric $\alpha 7$ receptor homooligomers exhibited an approximately 100-fold increase in affinity for both nicotine and acetylcholine in equilibrium binding measurements: on the other hand, the electro-
physiological recordings revealed that the apparent affinity for agonists only increased 3–4 fold, while the concentration required to desensitize the mutant chimeras dramatically decreased (20- to 50-fold). Quantitative analysis of the data revealed that the most plausible interpretation of this apparent "gain of function" is not a "local" change of binding properties of the active site but an alteration of the isomerization constant leading to the desensitized D state (L phenotype). Point mutation analysis further showed a critical contribution of the residue α7 G152 in the observed change of the isomerization constant [38]. Interestingly, mutation of its homolog in human muscle α1 subunit (G153S) causes a genetically transmissible 'slow channel' myasthenic syndrome [148].

Thus, point mutations within both the channel [139] and the active site domain [148,38] as well as alteration of the subunit composition [88] cause highly pleiotropic phenotypes for which analysis leads to interpretations consistent with the view that the many functions subsumed by nAChR molecule are 'linked' by major allosteric transitions which globally affect the 3D organisation of the receptor molecule. The same conclusion applies to glycine, GABA_A and 5-HT_3 receptors (see [63,144]) and possibly to glutamate receptors [130].

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**Fig. 4.** Pleiotropic phenotype of the point mutant α7 L247T homomultimer and interpretation of its properties in terms of the allosteric model (from [139,110]). (A) Top left: diagrammatic representation of the α2β1 channel domain pointing to the L247T mutation in the equatorial part. Top right: compared responses of WT and L247T illustrating the loss of desensitization in the L247T mutant. Bottom left: compared dose–response curves for acetylcholine of WT and L247T showing an increase of apparent affinity by about two orders of magnitude for L247T. Bottom right: loss of sensitivity of L247T to inhibition by the channel blocker QX 222. (B) Left column: single channel recordings illustrating the presence of a novel conductance state of 80 pS in L247T and its selective activation by low ACh concentration (0.1 μM) and by dihydro-β-erythroidine (DHE). Right column: interpretation of the L247T data on the basis of a selective opening of the channel in a desensitized state. CB: competitive blocker.
1.5. Multiple binding sites for allosteric effectors on nAChR and a potential role of nAChR as a 'coincidence detector'

The nAChRs carry, in addition to the nicotinic ligand binding sites, a variety of topographically distinct allosteric sites (see [95,7]). Ca²⁺ ions which exhibit a high permeation through the neuronal nAChR channel [120,9] also act as positive effectors of neuronal nAChR [119,136]. Single channel recordings show that potentiation by Ca²⁺ ions results from an increased opening frequency of ACh-activated channels. Moreover, among divalent cations, Ba²⁺ and Sr²⁺ exert a positive effect, but not Mg²⁺. In other words, Ca²⁺ potentiates nAChR response upon binding to specific external regulatory sites [119]. The regulatory sites involved are located in the large synaptic amino terminal hydrophilic domain [59] within the AA166–172 stretch [64]. Mutation of amino acids in this canonical domain alters all features of potentiation. Moreover, insertion of the AA161–172 sequence into the Ca²⁺ insensitive 5-HT₃ receptor results in a receptor activated by SHT and potentiated by Ca²⁺ ions [64]. The Ca²⁺ regulatory site is located in close proximity to loop E of the acetylcholine binding site [39]. The data are consistent with a mechanism according to which Ca²⁺ ions primarily affect the isomerization constant (L) between the resting and open channel conformations of the nAChR [63,64].

Variations in the external Ca²⁺ concentration (in the millimolar range) have been noted in several brain regions and in ganglionic sympathetic neurons, during periods of high synaptic activity [4]. These observations render plausible a mechanism for efficient short-term regulation of nicotinic transmission by the action of Ca²⁺ ions on the nAChR, involving both external Ca²⁺ binding sites and Ca²⁺ influx through its ionic channel.

Moreover, nAChRs carry binding sites on their external surface for noncompetitive blockers [23], steroids [13,154], phystostigmine [132], substance P [149], ATP [123] and, on their internal surface, possess phosphorylation sites [79,81].

Finally, current–voltage relationships established for native as well as reconstituted nAChR reveal that, at variance with muscle nAChR, all known combinations of neuronal nAChR display strong outward rectification [8].
Under these conditions, synaptic currents will not be detected when the cell is depolarized. In other words, the neuronal nAChR behaves as a voltage-sensing device which, in the case of the a7 nAChR at least, relies upon the presence of a charged residue within the ion pore where intracellular Mg²⁺ might bind and block the channel [60,110,82,142].

Thus, on the basis of the occurrence of multiple regulatory sites and of voltage-sensing properties which are linked by a dominant allosteric transition between activatable versus refractory conformations of the receptor, it was postulated that the differential stabilization of one of these conformations of the receptor would determine the efficacy of the synapse at the postsynaptic level [76]. Depending on the initial balance between conformations, the regulation might either be a potentiation or a depression. Moreover, continuing this speculative point of view, it was suggested that nAChRs and allosteric receptors in general, may serve as detectors of time-coincidence [76,23,56] (ref. in [5,52]) and, thus, in a metaphorical sense, may serve as building blocks of a 'chemical Hebb synapse'.

A popular model for near-coincidence detection between pre- and postsynaptic excitation has been suggested in recent years for the glutamate-NMDA receptor on the basis of the voltage-dependent block of the ion channel by Mg²⁺ [164,5]. The presynaptically released glutamate activates the NMDA receptor channel only if, at the same moment, the postsynaptic membrane is sufficiently depolarized to release the Mg²⁺-block of the channel. This model is indeed parsimonious: only two states (B and A) are required. However, the voltage-sensitive Mg²⁺ block (or homolog) is rarely encountered in other species of ligand-gated ion channels (caution!). Moreover, a large majority of ligand-gated ion channels display desensitization and/or potentiation with a variety of kinetic features. In addition, because of their transmembrane disposition, these receptors carry sites on both their synaptic and cytoplasmic sides, offering the opportunity to 'link together', through the membrane, multiple convergent signals and, in particular, pre- and postsynaptic signals.

A more general model [76,56] would simply result from the ability of these allosteric receptors: (1) to recognize several convergent signals at the level of their multiple active and allosteric binding sites and (2) to integrate their effects, within a given time window, as a result of a major, highly cooperative, allosteric transition. Simulation experiments indeed illustrate that, using the values of the parameters determined with *Torpedo* nAChR, changes of efficiency following a Hebbian rule could theoretically be obtained which may last seconds or even minutes [76,35]. Analysis of the molecular mechanisms involved in LTD or LTP in *Aplysia and Drosophila* learning might lead to a test of these models despite the fact that many of these processes may involve several different (ionotropic or metabotropic) receptors [see Kandel, Andersen (this symposium) and [55], for discussion].

2. Nicotinic receptors integrated within neuronal networks: cognitive learning and reinforcement

2.1. nAChRs within neuronal networks

The relationship between the uni-dimensional relative simplicity of the genome (about 200,000 genes) and the three-dimensional extreme complexity of the brain (about 10¹⁵ synapses) is not straightforward. The aphorism one gene—one enzyme can in no way become ‘one gene— one synapse’ or ‘one gene—one behavior’. ‘We know that it is not possible to assign an integrated cerebral function to a single center, a single neurotransmitter, or a single receptor... The action of many genes ‘converge’ on a given brain structure, and a single gene may have ‘divergent’ effects on several different structures’ [21]. The spatial and temporal unfolding of gene transcription through networks of intercellular and intracellular signalling pathways, transcription factors, and promoter elements during development [93] results in the formation of defined patterns of genes expressed in the adult brain.

Moreover, in the particular case of nAChR subunit genes (for review, see [29,14]) a given nerve cell may express different combinations of such genes. More complex and still largely unknown, post-transcriptional processes are thus necessary to target the ‘right combination’ of nAChR subunits to the ‘right place’ in the cell and thus within the particular network to which the neuron belongs. Therefore, several levels of organisation have to be considered: (1) the subcellular compartmentalization of diverse functional species of nAChR using in this case electrophysiological techniques; (2) the still rather speculative concept of ‘synaptic triad’ which relies upon the occurrence of postsynaptic allosteric receptors; (3) multiple-level networks which may plausibly account for cognitive learning tasks involving reinforcement (or reward processes) and are under scrutiny by (4) following the learning behavior of nAChR β2-subunit knock-out mice.

2.2. The subcellular compartmentalization of nAChR oligomers

Nicotinic agonists elicit electrophysiological responses from brain neurons at the level of somatodendritic, perisynaptic, and terminal compartments (Fig. 5).

The somatodendritic membrane of many neurons in the brain generates fast inward currents [31,141]. Single channel events may even be recorded using patch-clamp techniques upon application of acetylcholine (or nicotine) either from freshly dissociated neurons of juvenile rats [118,121,119,120] or from thin slices from the adult rat or mouse brain [35,136]. As anticipated from in situ hybridization experiments, the pharmacological profile of the dominant single channels [118,121] and the whole cell response [35,136] recorded from the soma of medial habenula neurons (nicotine < cytisine > ACh > DMPP-
inhibition by hexamethonium and dihydroβ-erythroidine but insensitivity to α- and neuronal bungarotoxin differ from that of neurons from various thalamic regions (nicotine > DMPP > cytisine). Moreover, no response to nicotine could be found in thalamic neurons in mutant mice lacking the β2 subunit, while, in habenular neurons, the cytisine to nicotine ratio did not significantly change, thus demonstrating the dominant contribution of the β2 subunit to the nicotinic response in the somas of thalamic neurons [135,136].

In another approach, whole cell recordings [97] in thin slices and on acutely isolated interpeduncular neurons that have retained synaptic contacts attached to their cell body show that nicotine dramatically increases the frequency of large GABAergic currents in these cells. Yet, rather unexpectedly, the Na⁺ spike blocker tetrodotoxin (TTX) was found to inhibit this effect. As the specificity of TTX is strictly limited to Na⁺ channels, these channels must intervene between the nAChR and the GABA release site. The location of these nAChRs thus differs from both the ‘somatic’ and the ‘terminal’ receptors described in synaptic preparations that are insensitive to TTX [166]. The term preterminal [97] was used to specify the distribution of these nAChRs on the axon (see also [122,106,107,104]).

It was also observed that TTX insensitive functional nAChR are present on axon terminals in the brain [166,106,105,96]. The direct action of nAChRs located on these terminals was recorded using the patch clamp technique on thin slices of the ventrobasal complex and dorso-lateral geniculate nucleus from mouse sensory thalamus [96]. In these preparations, nicotinic agonists enhanced the frequency of miniature GABA currents and decreased the failure rate of evoked inhibitory synaptic currents. Both effects disappeared in β2-subunit knock-out mice, indicating that the β2-subunit is a necessary component of the nAChR implicated. The nicotine enhancement requires high external Ca²⁺ concentrations. In the ventrobasal nucleus, Ca²⁺ ions, which efficiently block high threshold voltage-sensitive Ca²⁺ channels, inhibit nAChR-mediated presynaptic facilitation. In contrast, in the dorso-lateral geniculate nucleus, Ca²⁺ does not show this effect and nicotine enhances Ca²⁺-dependent GABA release apparently without depolarizing the nerve terminal. This observation supports the view that Ca²⁺ influx through the nAChR channel may directly contribute to nicotine facilitation of synaptic (here inhibitory) transmission [96].

Finally, it was noted that in the neocortex ionophoretic application of nicotine increases the amplitude of the postsynaptic potentials evoked by stimulation of the superficial cortical layers (14% of the cells). In contrast, muscarnic agonists decrease the amplitude of the postsynaptic response. In all instances, the early postsynaptic potentials were glutamatergic [157]. Thus, presynaptic nAChRs may potentiate both inhibitory GABAergic and excitatory glutamatergic synapses, a finding recently confirmed and extended in different systems [105,104].
The exact intervention of these preterminal and terminal nAChRs in identified physiological processes remains elusive. One possibility (among others) is that they contribute to a global (volume) control of neurotransmitter release by a general depolarisation of axon terminals to ACh released in a paracrine manner [2].

2.3. The concept of synaptic triads and multi-level model networks for cognitive learning tasks

The common view of neurotransmitter receptors is that they mediate signal transduction in the postsynaptic membrane of a chemical synapse; yet, allosteric receptors introduced into networks of formal neurons may further be exploited to model interactions between synapses. The simplest theoretical example is the synaptic triad that was initially introduced in a formal network developed to account for song acquisition by birds [44] (Fig. 6). The device is made up of three neurons, A and C converging on a common neuron B, and allosteric receptors are introduced in the postsynaptic membrane of the A-B synapse. In such a triad the efficacy of a synapse of neuron A on neuron B is postulated to be influenced by a third neuron, C, called a modulator. If the synapse A-B is excitatory and its postsynaptic allosteric receptor exists spontaneously in a 'refractory' conformation, then synapse C-B will be able to switch the postsynaptic receptor of synapse A-B to an 'activatable' state by releasing a diffusible chemical messenger (see Fig. 6). As a consequence, synapse C-B must be active before synapse A-B, and with a determined time delay, for signals to be transmitted by synapse A-B, thereby creating a mechanism for time-sequence detection and production [44]. In addition, since the postsynaptic receptor of synapse A-B may follow an 'allosteric Hebb rule' [76,26] then a short-term modification of synaptic efficacy can, in principle, take place at this level if time-coincidence is achieved. Introduction of this rule leads to the differentiation of sequence detecting neurons and to the stabilisation of ongoing temporal sequences.

It is of interest that triads, composed of a dopamine terminal and of a presumed excitatory input together with a spine of a pyramidal cell have been identified in prefrontal cortex [70]. Similar devices have also been recognized in retina [51] and assumed for elementary learning in simple systems such as Aplysia ganglia (Kandel) or vertebrate cerebellum (Ito), although in a different conceptual framework [23]. At this time, synaptic triads with cholinergic terminals and postsynaptic nAChRs have not been identified, but their occurrence appears plausible [152].

Synaptic triads may serve as building blocks to elaborate more complex but at this stage still formal models of 'artificial organisms' which accomplish cognitive learning tasks (Fig. 7). As expected, their architecture requires to be operative network organizations on a larger scale.

(1) Delayed-response tasks were initially designed with higher vertebrates and humans to test for the acquisition of cognitive patterns (or rules of behavior) that selectively engage the prefrontal cortex (review [103,146,61,70]). In these tasks, the subject is asked to select between two rules of behavior, for instance, one according to the position of a cue presented before a delay period, the other according to its identity. The reinforcement or reward given during the learning period specifies and stores in memory the rule that the organism subsequently follows during the test.

The formal neural architectures that successfully perform the task [42,43] comprise two hierarchically nested levels of organisation: a low, sensori-motor, routine level consisting of neurons with modifiable synaptic weights, and a higher, cognitive level containing neural units coding for the rules or programs of behavior. Each cluster is formalized as a set of hundreds of neurons densely interconnected by excitatory synapses that may exist in at least two self-sustained states of activity. Clusters are linked together by 'axon bundles' that are modulated locally by synaptic triads [44].

The clusters are linked by lateral inhibitory connections in such a way that when one rule-coding unit is spontaneously active, the others are in the low activity or silent state. In the course of learning, the layer of rule-coding units plays the role of a generator of diversity: the activity of each particular cluster spontaneously changes and alternates at random with time, in such a way that the organism is able to test successively one (or the other) of the dimension rules against its environment; a search by trial-and-error takes place. Then, a positive reward is delivered from the external environment when the formal organism

![Fig. 6. The concept of synaptic triad (from [44]).](image)
produces a motor output congruent with the rule to be learned: the particular cluster active at this precise moment is selected (Fig. 7).

(2) A particularly original aspect of these models is the implementation of positive and/or negative reinforcement (or reward) mechanisms as neuronal pathways either externally driven or internally elicited via autoevaluation loops with reference to stored memories [43]. Mobilisation of these reinforcement pathways is viewed as causing the volume release of reinforcement signals such as dopamine, norepinephrine, acetylcholine, or coexisting messenger peptides that directly and/or indirectly behave as effectors of allosteric receptors. In the situation where the positively reinforcing signal is released within the time window during which a defined cluster is currently active, it would stabilize the ongoing activity by changing the synaptic efficacies of the active cluster by a Hebbian modulation of allosteric receptors, thereby increasing the probability of selecting the same output again in subsequent trials. Negative reinforcement, in contrast, would destabilize current activity at the synaptic level, thereby resetting the system to a random trial-and-error mode [42,43,53,115,145].

These theoretical and still highly speculative attempts to model architectures capable of carrying out cognitive learning tasks may appear far fetched in a basically experimental approach to neurotransmitter receptor function. Yet, they illustrate the minimal complexity of network architectures required for an organism to accomplish such tasks. Further they show that if allosteric receptors are assumed to play a crucial role as coincidence detectors, they must be distributed at strategic positions within the network architecture at which their dynamic properties may control, in a bottom-up manner, the dynamics of the behavioral response. Within this conceptual framework a possible contribution of brain nAChRs in established learning tasks was therefore experimentally examined.

2.4. Contribution of brain nAChRs to cognitive tasks

Acetylcholine is an important participant in the maintenance of cognitive functions; lesions of forebrain cholinergic nuclei and pathways alter memory, and, more specifically, working memory and attentional processes that rely on the integrity of the prefrontal cortex and are deeply affected in patients with Alzheimer’s disease [145]. Some contributions of ACh to memory are mediated by muscarinic receptors, but nicotine selectively improves memory and attention via nAChRs [101] and nicotine binding sites significantly decrease (compared to muscarinic receptor sites) in the neocortex of Alzheimer patients [125]. To evaluate the contribution of nAChRs to these functions
(but also to test the theoretical models presented above),
the effects of two nicotinic antagonists, neuronal bungarotoxin
and dihydro-B-erythroidine, were assessed in a de-
layed matching-to-sample (MTS) task in the rat [71]. A
less difficult task, the non-matching to sample task
(NMTS), was used as a control, since it depends on
the natural tendency of the rat to spontaneously alternate, on
a second run, to the branch it did not visit the first time.
Interestingly, injections of neuronal bungarotoxin into the
prelimbic area of the prefrontal cortex produced a signif-
ificant decrease in working memory performance in the MTS
but not in the NMTS task. These findings are consistent
with the observation that nACHR mediates enhancement of
glutamate release in the prefrontal cortex [158]. In ad-
dition, they bring direct support to the notion that nACHR
selectively contributes to high level cognitive tasks.

Another series of experiments were carried out with
mice deficient in the neuronal β2-subunit [134,136]. The
homozygous mutant mice (β2−/−) do not display any
obvious physical or behavioral abnormalities. The loss of
β2-subunit immunoreactivity and mRNA is accompanied
by a complete loss of high affinity β-nicotinic binding
measured in both extracts and in situ by receptor auto-
radiography. The β2-subunit containing nACHR, thus
contributes to the high-affinity binding sites for β-nicotine
(most probably together with the α4-subunit whose distri-
bution closely parallels that of the β2-subunit) [168]. As
mentioned above, the electrophysiological response to
nicotinic of the neurons from the anterior thalamus (which
express very high levels of β2 and α4-subunit mRNA)
are absent in the mutant.

The β2−/− mice tested for the retention of an avoid-
ance task showed marked differences compared to their
non-mutant siblings. Nicotine (0.01 mg/kg) consistently
enhanced retention in WT animals but was completely
ineffective in β2−/− mice. The effect of low nicotine
levels on passive avoidance learning is thus mediated by a
β2-subunit containing nACHR [136]. Moreover, prelimi-
inary observations support the view that slight modific-
ations in the learning behavior of β2−/− mice take place
in the absence of nicotine: the mice spontaneously display
longer retention latencies, as if they were able to perform
better than their non mutant litter-mates [136] (Fig. 8).
This observation would mean that β2-subunit containing
nACHR mediates endogenous effects of ACh in the brain
under defined behavioral conditions.

We have also investigated β2−/− mice for their per-
formance on a spatial learning task (the Morris water
maze) [169]. This task has been shown to be highly
sensitive to cholinergic deficits and to be impaired during
normal aging in rodents. Both adult (6–8 month old) and
aged (24 month old) animals were therefore tested. The
animals were trained for 10 days to find a hidden platform
in the Morris water maze. Whereas adult mutant animals
and their wild type siblings performed identically on this
task, aged mutant mice showed a significant impairment in
both place learning curve and place recall with respect to
their wild type siblings. On the other hand, no significant
difference in performance was observed when the platform
was visible, demonstrating that the mutant and wild type
animals had similar sensory-motor ability and motivation.
These data demonstrate that lack of high affinity nACHRs
leads to an impairment in spatial learning and memory.
In addition, they indicate that deficits in nicotinic transmis-
sion may only become apparent during aging when, for
instance, compensation by other neuronal systems becomes
less effective. At variance with the delayed response tasks,
neither the passive avoidance task nor the Morris water
maze test belong to the category of ‘high level’ cognitive
behaviours and thus may be used to challenge the pro-
posed model [42,43]. Additional studies are needed which
‘link together’ the molecular level to the higher cognitive
network level.

Several major neural systems have been, in the past,
reported to contribute jointly to drug dependence and
addiction, as well as to cognitive processes such as mem-
ory, subjective attribution, and craving [3]. The theoretical
approach mentioned above for cognitive learning, which
places are important functional role upon the ascending
reward systems, interestingly, converges with an issue of
important social dimensions, the neural basis of drug addiction. Indeed, the dopamine systems from the ventral segmental area (VTA) to the ventral striatum (nucleus accumbens) and prefrontal cortex and from substantia nigra to the dorsal striatum (caudate, putamen) are considered as the main anatomical substrates for drug-seeking behavior and for the reinforcing effects of cocaine, amphetamine, morphine, and nicotine [111,3]. Other pathways involved include the descending connections via the central gray which may mediate aversive aspects of drug dependence and the cholinergic neurons from the basal forebrain (such as the nucleus basalis of Meynert in humans) which may modulate cortical arousal and contribute to mnemonic and subjective consequences of drug reinforcement [3]. Nicotine, at concentrations which maintain self-administration, enhances extracellular dopamine release (measured by microdialysis) in the nucleus accumbens [163,84], a finding consistent with the notion that modulation of dopamine release is a major neurochemical effect of nicotine administration [49]. Nicotine also stimulates local glucose utilisation [137] in a manner qualitatively similar to that of strongly addictive drugs. Moreover, under conditions of self-administration of intravenous cocaine, the pattern of activation of brain regions followed by fox-related antigen mapping (nucleus accumbens, medial prefrontal cortex, medial caudate areas) parallels that found with cocaine [111]. Interestingly, recent results [134,135] indicate that in anesthetized β2−/− mice, dopamine release in the striatum is no longer stimulated by nicotine and nicotine self-administration is dramatically impaired. These results support the notion that β2-subunit-containing high affinity nACHRs contribute to the reinforcing effects of nicotine.

In situ binding and hybridization studies reveal labeling in catecholaminergic nuclei for β2-subunit mRNA and high-affinity nicotine binding, but also for α6 and β3 mRNAs which are very abundant in amine neurons [99]. It has thus been proposed, as a working hypothesis, that α6β2β3 nACHRs are candidates for the mediation of nicotine-enhanced stimulation of catecholamine release and nicotine reinforcement [99].

The molecular and cellular loci of the plastic changes that account for tolerance and addiction remain to be identified [40]. In the case of nicotine addiction, possible targets include, as mentioned earlier, the high affinity nACHR molecule itself. Its short and long-term desensitization properties may contribute to some transient aspects of tolerance, while posttranscriptional increases of nACHR protein without changes in the levels of nACHR mRNA may contribute to their long-term features [109,112]. Other possibilities, alternatively and/or in addition, are interactions between nACHR and dopamine receptors [167,102]. For instance, a single subcutaneous administration of nicotine causes a significant decrease in the affinity of a selective dopamine D2 receptor antagonist in striatal membranes [102]. In addition to molecular mechanisms involving the nACHR protein itself, interactions between nACHRs and dopaminergic receptors may thus play a crucial role. This possibility, of course, warrants additional investigation. In any case, dissection of the mechanisms underlying nicotine reinforcement, using the methods offered by recombinant DNA technologies, has begun. An important

![Fig. 9. Plausible contribution of cholinergic nicotinic pathways to reinforcement mechanism and to their high order regulation.](image-url)
issue, among others, is to what extent such mechanisms share components with the rewarding effects of other drugs like cocaine or morphine, but differ when food is used as reward [106]. As mentioned, they might equally be helpful in testing the model proposed for cognitive learning tasks and in particular in further specifying an eventual "global" role of acetylcholine nicotinic pathways as high order regulation of dopaminergic reinforcement pathways (Fig. 9). Possible contribution of the allosteric properties of the nAChR (or other neurotransmitter receptors and second messenger systems) in such global regulations could be tested with knock-in mice carrying identified mutations in nAChR subunit genes altered in their specific binding sites and/or allosteric properties.

3. Conclusions

From electric organ and muscle to brain, the diverse nAChR species identified follow common structural rules that, in most cases, are also shared with other receptor channels (e.g., for GABA, glycine, serotonin and possibly glutamate, although the biochemistry of glutamate receptors is still fragmentary). They are allosteric membrane proteins, yet with distinctive properties. These transmembrane heteropentamers carry topographically distinct sites for a variety of categories of ligands and undergo multiple conformational transitions, which account for fast signal transduction and its ‘higher order’ slow regulation through desensitization and/or potentiation.

The multiple combinations of nAChR subunits form a wide diversity of nAChR oligomers which differ in their pharmacological specificity, their activation and desensitization properties, and their distribution in the brain at the regional, cellular and subcellular levels. Their precise location at strategic positions in neuronal networks, in particular at the level of axon terminals (inhibitory and excitatory), may be expected to play a decisive role in the mode of transmission contributed by acetylcholine in the brain. The possibility exists that nAChRs take part in both classical wiring transmission at authentic anatomical synapses [152] and paracrine ‘volume’ transmission for more global neuromodulatory actions [2]. The restricted distribution of nAChR oligomers may also have a profound impact on the plasticity of neuronal networks, in particular via both Ca\(^{2+}\) potentiation and Ca\(^{2+}\) influx through activated nAChR channels. The nAChR-mediated Ca\(^{2+}\) entry may for instance regulate the efficiency of other ligand-gated channels and ionic channels, enhance intracellular phosphorylation–dephosphorylation reactions, and, as a consequence, modify both the excitability of nerve cells and their ability to ‘integrate’ synaptic and/or paracrine signals.

Biochemical and molecular studies on brain nAChRs have opened many new lines of research concerning cognitive processes. In this respect, the attempts to model formal, though neurally plausible, networks that accomplish cognitive tasks (such as the delayed-response tasks) have at least the virtue of demonstrating that understanding the role of neurotransmitter receptors in learning requires, first, knowledge of their intrinsic functional properties, but also as emphasized above, the identification of their precise localization within a rather complex, though defined, architecture that includes, in particular, several levels of organisation, multiple sets of interconnected ‘groups’ of neurons, and a critical accessibility and modulation by reinforcement mechanisms (Fig. 9).

Finally, nAChR structural properties and distribution have been directly (or indirectly) implicated in major brain dysfunctions. For instance, in Alzheimer’s disease patients, degeneration of cholinergic neurons and a differential loss of high affinity nicotine binding takes place while nicotine improves their performances in memory tasks [101,162]. Genetic studies with families suffering from autosomal dominant nocturnal frontal lobe epilepsy reveal that missense mutations in the α4 subunit gene are associated with the disease [151,150]. Also, as discussed here, high affinity nAChRs have been implicated in nicotine addiction [40]. The contributions of nAChRs to brain functions and dysfunctions are already more numerous than expected and many more may be anticipated.

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References


[48] M. Dennis, J. Ginmattar, P. Kortvysolak, M. Goldfarb, C. Hirn,


[80] F. Hueso, W. Obertühr, F. Leutespeich, The ion channel of the nicotinic acetylcholine receptor is formed by the homologous helices M2 of the receptor subunits, FEBS Lett. 205 (1986) 137–142.


[88] M.B. Jackson, K. Inoto, M. Mishina, T. Konno, S. Numa, B. Sackmann, Spontaneous and agonist-induced openings of an acetylcholine receptor channel composed of bovine muscle alpha-, beta-


