ELSEVIER

Brain Research Reviews 26 (1998) 198–216

BRAIN RESEARCH REVIEWS

Brain nicotinic receptors: structure and regulation, role in learning and reinforcement [1]

Jean-Pierre Changeux *, Daniel Bertrand [2], Pierre-Jean Corringer, Stanislas Dehaene [3], Stuart Edelstein [4], Clément Léna, Nicolas Le Novère, Lisa Marubio, Marina Picciotto [5], Michele Zoli [6]

Neurobiologie Moléculaire, CNRS URA 1284, Institut Pasteur, 25–28 rue du Dr. Roux, 75724, Paris Cedex 15, France

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 ionic channels, intracellular second messengers and the relevant enzymes, cell surface adhesion molecules, or growth and trophic factors [21,78,81,52,79]. 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 organisation, 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 with 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]).

Keywords: Nicotinic receptor; Structure; Learning; Reinforcement

Contents

* Corresponding author.

[1] Published on the World Wide Web on 24 October 1997.

[2] Present address: CHU, Dept. of Physiology, 1, rue Michael Servet, CH 1211 Geneve 4, Switzerland

[3] Present address: INSERM U 334, SHFJ CEA, 4 Place du Gl. Leclerc, 91401 Orsay Cedex, France

[4] Present address: Dept. de Biochimie, Université de Genève, Quai Ernest-Ansermet 30, CH-1211 Geneve 4, Switzerland.

[5] Present address: Dept. of Psychiatry, Yale University Medical School, Connecticut Mental Health Center, 34 Park Street, New Haven, Connecticut 06508, USA.

[6] Present address: Dipartimiento di Scienze Biomediche, Sezione di Fisiologia, Universita di Modena, Via Campi 287, I-41100 Modena, Italy.

0165-0173/98/$19.00
PII S0165-0173(97)00040-4

1. The acetylcholine nicotinic receptor: an allosteric membrane protein mediating signal transduction and its regulation.

1.1. Identification of the nAChR

The nAChR was initially identified as a *protein* from fish electric organ [27,23,91,141] as a heteropentamer of about 300 000 MW with an $[\alpha 1_2.\beta 1.\gamma/\epsilon.\delta]$ 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 ($\alpha 2$–$\alpha 9$) and share with electric organ $\alpha 1$ 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 ($\beta 2$–$\beta 4$). All nAChR subunits share a similar hydropathy profile, with two hydrophilic domains and four hydrophobic domains (M1–M4) about 20 amino acids long. Parallel *affinity labeling* (with *Torpedo* nAChR) and *site-directed mutagenesis* experiments (primarily with the $\alpha 7$ 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 [68,80,100,83,7,92].

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 $\alpha 9$ subunit which, in *Xenopus* oocyte reconstitution experiments, forms homooligomeric nAChR able to respond to ACh. Yet, the $\alpha 9$-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 $\alpha 7$ and $\alpha 8$ 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 diverged from their invertebrate counterparts. Structural gene duplications and tissue-specific promoter switches then led to muscle $\alpha 1$, $\beta 1$, and $\delta/\gamma/\epsilon$-subunits clades which form $[\alpha 1_2.\beta 1.\gamma/\epsilon.\delta]$ 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 $\beta 2/\beta 4$ subunits probably occurred more than 800 MYA, that for $\alpha 5/\beta 3$ and the rest of the subunit genes slightly later. Finally, the duplications yielding $\alpha 2$ and $\alpha 4$, in one branch, and $\alpha 3$ and $\alpha 6$, in another branch, may have taken place, respectively, more than 620 MYA and 500 MYA, followed by the most recent $\beta 2/\beta 4$ 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-

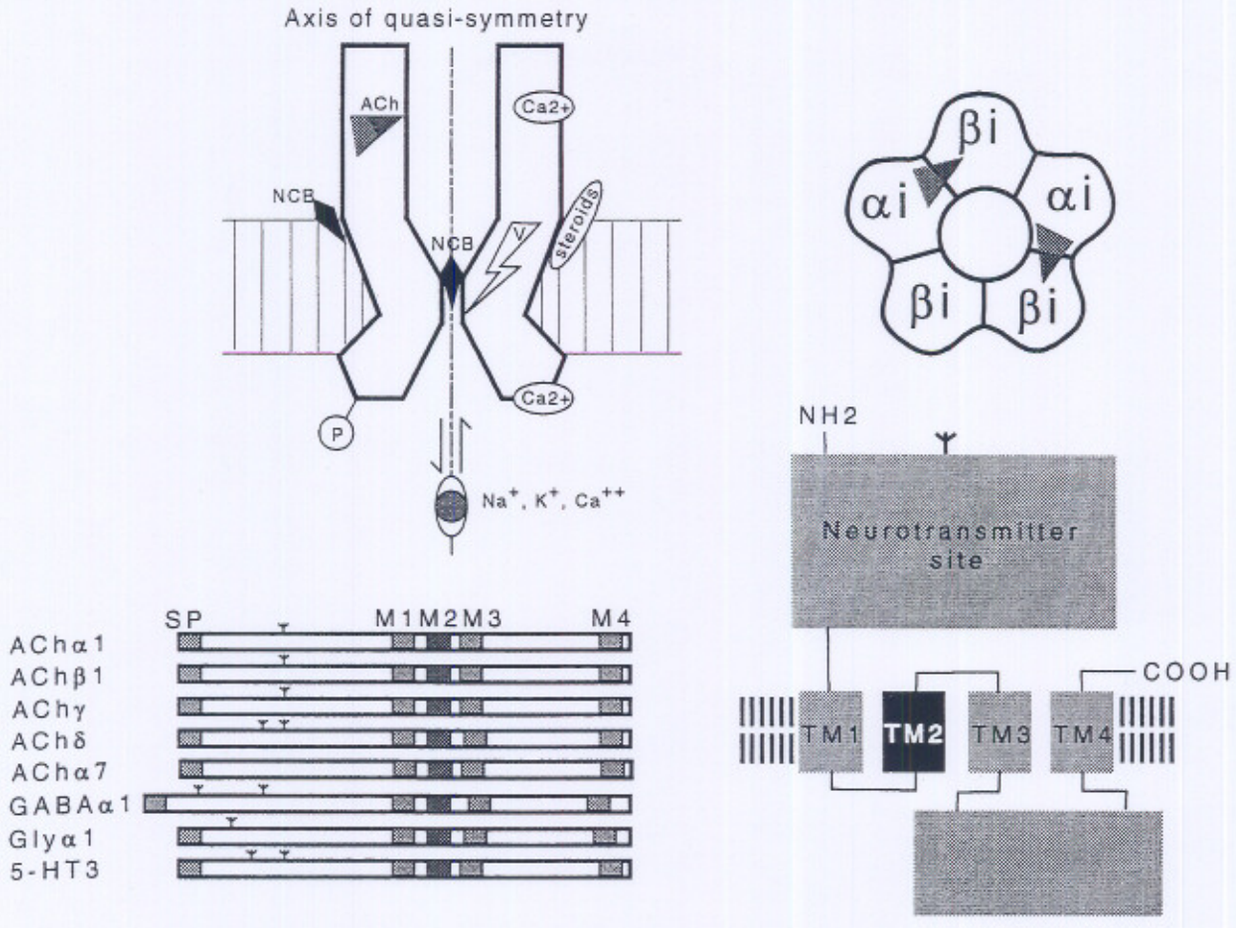

Fig. 1. Models of the quaternary structure of muscle and neuronal acetylcholine receptors (modified from [7]). Top left: schematic cross section showing the active site (ACh) and the ion channel together with various allosteric sites for noncompetitive blockers (NCB), Ca^{2+}, steroids, phosphorylation and voltage-sensing (V). Top right: front view of a model of neuronal nicotinic receptors with 2 α and 3 β subunits. Bottom left: sequence homologies and sequence domains within the ligand-gated ion channels of the nicotinic receptor family. Bottom right: putative transmembrane organizations of each subunit (M1, M2, M3, M4 are transmembrane segments).

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 ($\alpha2\beta2$, $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, etc.) or more ($\alpha3\beta4\alpha5$) ($\alpha1\beta2\alpha5$), ($\alpha3\beta2\beta4\alpha5$) 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 $\alpha4\beta2$ subunits exhibits a preferential order of affinity for ACh or nicotine > DMPP > cytisine, in contrast to cytisine > nicotine > ACh > DMPP for rat $\alpha4\beta4$ or nicotine > cytisine > DMPP > ACh for rat $\alpha7$ (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/δ 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 ($\alpha7$ to $\alpha9$), 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 $\alpha4\beta2$ or $\alpha3\beta4$ possess functionally equivalent ligand binding sites at $\alpha4/\beta2$ or $\alpha3/\beta4$ 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 $\alpha3\alpha5\beta4$ [155,161,37,138] or even more such as $\alpha3\beta2\beta4\alpha5$ [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 $\alpha7/\alpha8$

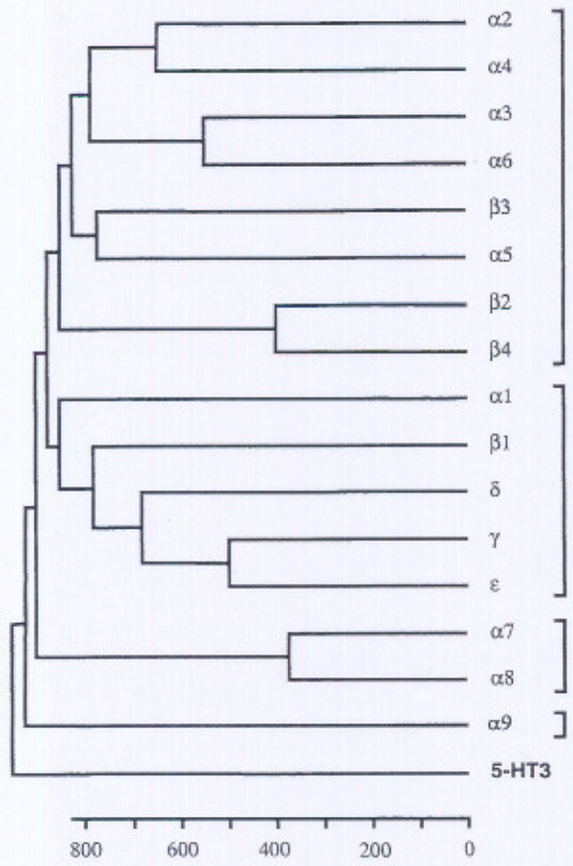

Fig. 2. Simplified version of the evolutionary tree of acetylcholine nicotinic receptors subunits (in million years) (modified from [98]).

subunits form homooligomers which exhibit a pCa^{2+}/pNa^{+} higher than 15. Subunits $\alpha 2$ to $\alpha 6$ and $\beta 2$ to $\beta 4$ form heterooligomeric channels with a pCa^{2+}/pNa^{+} ranging between 0.5 and 2.5. In contrast, the muscle receptors $\alpha 1_2.\beta_1.\gamma/\epsilon.\delta$ 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, $\alpha 3\beta 2$ containing oligomers desensitize 10 times faster than $\alpha 3\beta 4$ or $\alpha 4\beta 4$ oligomers, but $\alpha 7$ 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 $\alpha 2$ mRNA distribution is restricted to the *spiriformis lateralis* nucleus in the chick [41,116] and to the interpeduncular nucleus in the rat [160]. Besides, $\alpha 3$ and $\beta 4$ mRNA are detected only in autonomic ganglia and in a few central nuclei (such as retina, habenular nuclei, pineal gland, nucleus of the *tractus solitarius*, etc.) [160,50]; the distribution of $\alpha 6$ and $\beta 3$ mRNA is limited to somatosensory ganglia and to several central locations, such as the central catecholaminergic nuclei [47,99]; $\alpha 5$ mRNA, though more largely distributed, remains expressed at medium levels in a limited number of areas [159]. In contrast, $\alpha 4$ 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 $\beta 2$ 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 $\alpha 4\beta 2$-subunits [136], whereas ^{125}I α-bungarotoxin binding [32] parallels the distribution of $\alpha 7$ mRNA that is restricted to a few layers of cerebral cortex, to the hypothalamus, hippocampus, inferior colluliculus, and to a few brain stem nuclei in the rat [147].

In the course of embryonic and postnatal development, the $\alpha 3$, $\alpha 4$, $\beta 2$ and $\beta 4$ 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. $\alpha 3$ vs. $\alpha 4$ 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 ($R \rightleftharpoons T$) 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 $L = T/R$). 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 two allosteric 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 ($\alpha 7$–$\alpha 9$ homooligomers, 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).

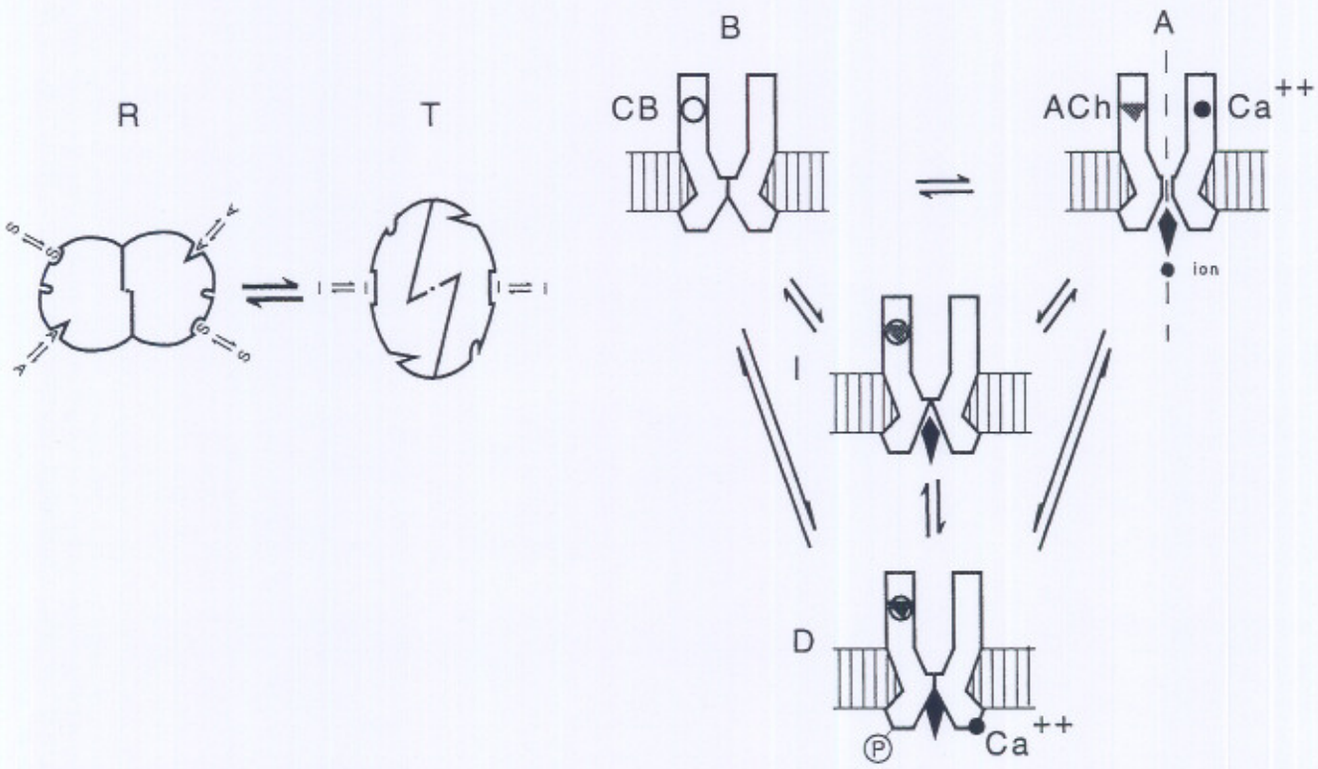

Fig. 3. Diagrammatic representation of the two-state allosteric model for regulatory enzymes (Monod–Wyman–Changeux) (left) and of its extension to ligand-gated ion channels as a multistate allosteric network (right) (modified from [18,26,63]); R and T are respectively the relaxed — high affinity for substrates (S) and activators (A) — and constrained — high affinity for inhibitors (I) — states of the two-sate model. B, A, I 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.

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 microsacs [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 δ subunit to the covalent labeling by DDF increases upon stabilization of the D state by the non-competitive blocker meproadifen, while that of the γ-subunit decreases. Moreover, under the same conditions, the contribution of loops A and B from the 'principal component' of the α-subunits increases up to six-fold relative to loop C, a finding consistent with the higher affinity of the D state for nicotinic ligands compared to resting and active states [63,67]. As found with classical allosteric proteins (see [133,85]), the binding domains for nicotinic 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 $R \rightleftharpoons T$ transition. Consistent with this view, perturbation of the subunit interactions, for instance by omitting the γ or the ϵ-subunit in muscle nAChR [$\alpha\beta\delta$-AChR], causes a striking increase of the frequency of spontaneous channel openings [88]. The straightforward interpretation of this observation (which is, 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 ($L > 20\,000$) 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 [139] (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 nicotinic *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 of 80 pS distinct from the 40 pS of the wild type (WT); moreover, dihydro-βerythroidine, 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 in 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 (γ-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 8×10^5 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 ([38], 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 chimera 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 $\alpha 7$ G152 in the observed change of the isomerization constant [38]. Interestingly, mutation of its homolog in human muscle $\alpha 1$ 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 $5\text{-}HT_3$ receptors (see [63,144]) and possibly to glutamate receptors [130].

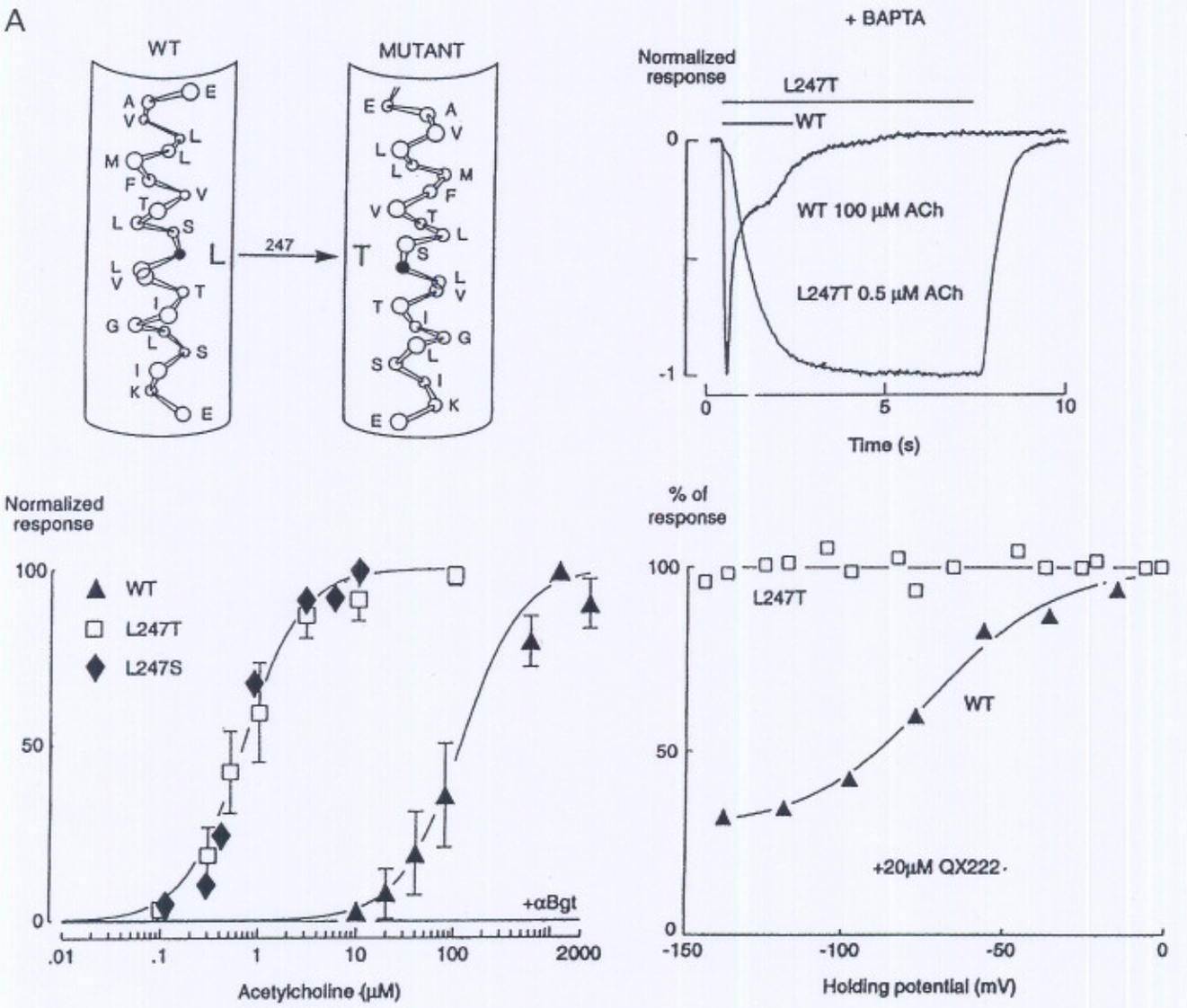

Fig. 4. Pleiotropic phenotype of the point mutant $\alpha 7$ L247T homooligomer and interpretation of its properties in terms of the allosteric model (from [139,11]). (A) Top left: diagrammatic representation of the M2 channel domain pointing to the L247T mutation in the equatorial part. Top right: compared 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 (DHβE). Right column: interpretation of the L247T data on the basis of a selective opening of the channel in a desensitized state. CB. competitive blocker.

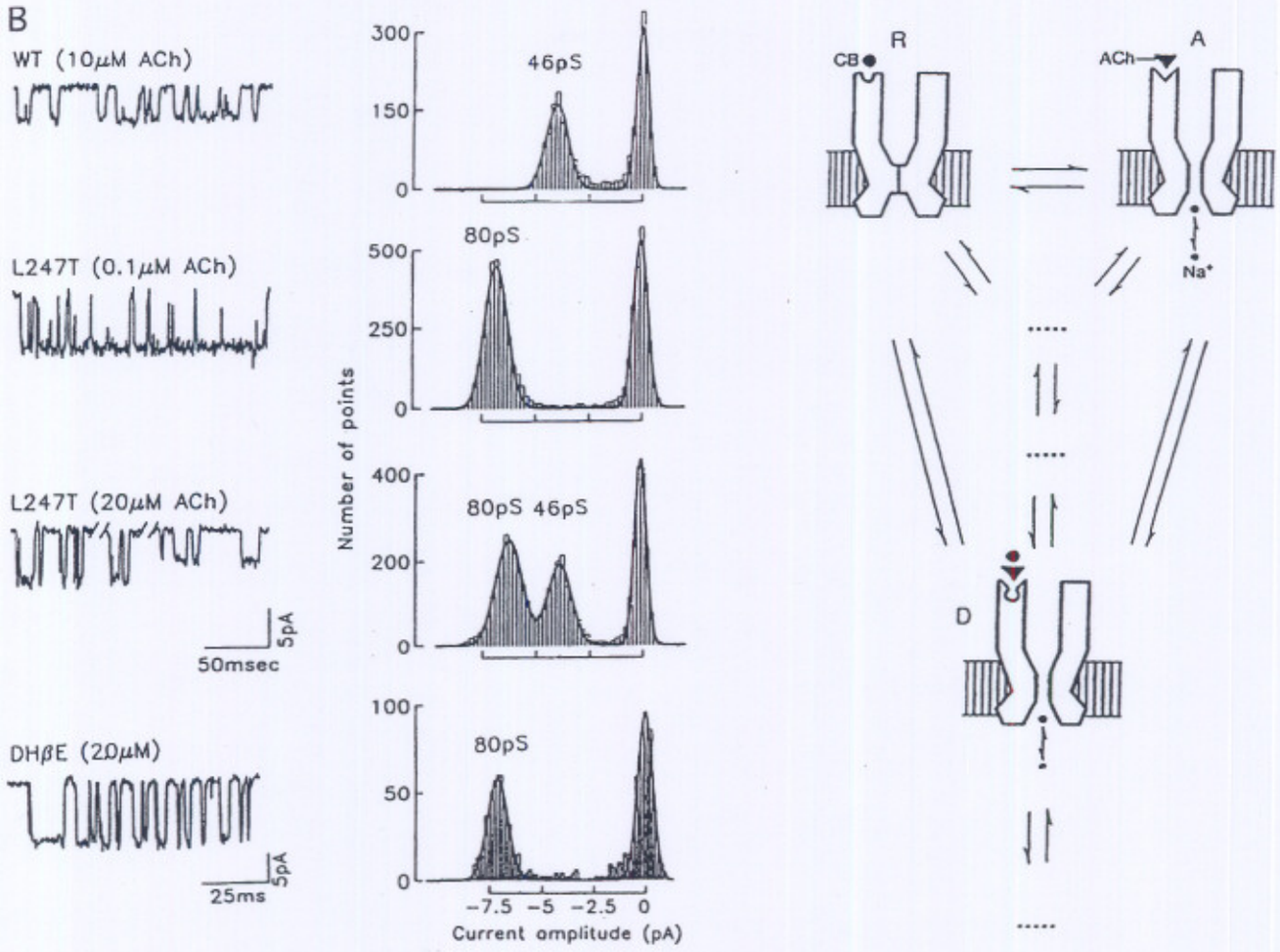

Fig. 4 (continued).

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]). Ca^{2+} ions which exhibit a high permeation through the neuronal nAChR channel [120,9] also act as positive effectors of neuronal nAChR [119,156]. Single channel recordings show that potentiation by Ca^{2+} ions results from an increased opening frequency of ACh-activated channels. Moreover, among divalent cations, Ba^{2+} and Sr^{2+} exert a positive effect, but not Mg^{2+}. In other words, Ca^{2+} 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 alter all features of potentiation. Moreover, insertion of the AA161–172 sequence into the Ca^{2+} insensitive 5-HT_3 receptor results in a receptor activated by 5HT and potentiated by Ca^{2+} ions [64]. The Ca^{2+} 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 Ca^{2+} ions primarily affect the isomerization constant (L) between the resting and open channel conformations of the nAChR [63,64].

Variations in the external Ca^{2+} 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 Ca^{2+} ions on the nAChR, involving both external Ca^{2+} binding sites and Ca^{2+} influx through its ionic channel.

Moreover, nAChRs carry binding sites on their external surface for noncompetitive blockers [23], steroids [13,154] physostigmine [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 $\alpha 7$ nAChR at least, relies upon the presence of a charged residue within the ionic pore where intracellular Mg^{2+} might bind and block the channel [60,1,110,82,142].

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 [5,53]) and, thus, in a metaphoric 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 Mg^{2+} [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 Mg^{2+} block of the channel. This model is indeed parsimonious: only two states (B and A) are required. However, the voltage-sensitive Mg^{2+} block (or homolog) is rarely encountered in other species of ligand-gated ion channels (cationic or anionic). 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 [76,25]. Analysis of the molecular mechanisms involved in LTP or LTD 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 10^{15} 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 $\beta 2$-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, preterminal 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 $\leqslant$ cytisine $>$ ACh $>$ DMPP;

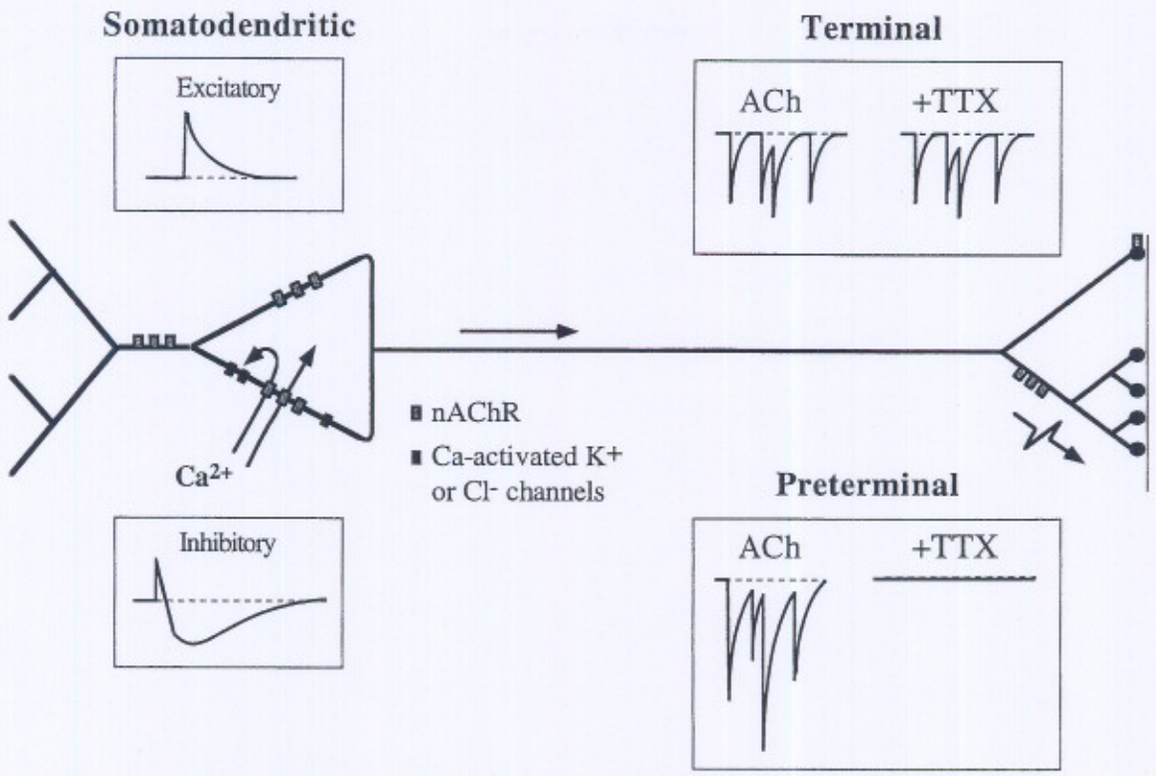

Fig. 5. Schematic representation of the compartmentalized surface distribution of the nAChR in a neuron evaluated by electrophysiological techniques (modified from [29]).

inhibition by hexamethonium and dihydro β-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 $\beta 2$ subunit, while, in habenular neurons, the cytisine to nicotine ratio did not significantly change, thus demonstrating the dominant contribution of the $\beta 2$-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 synaptosomal 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 dorsolateral 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 $\beta 2$-subunit knock-out mice, indicating that the $\beta 2$-subunit is a necessary component of the nAChR implicated. The nicotine enhancement requires high external Ca^{2+} concentrations. In the ventro basal nucleus, Cd^{2+} ions, which efficiently block high threshold voltage-sensitive Ca^{2+} channels, inhibit nAChR-mediated presynaptic facilitation. In contrast, in the dorso-lateral geniculate nucleus, Cd^{2+} does not show this effect and nicotine enhances Ca^{2+}-dependent GABA release apparently without depolarizing the nerve terminal. This observation supports the view that Ca^{2+} 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, muscarinic 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 afferent axons due 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.

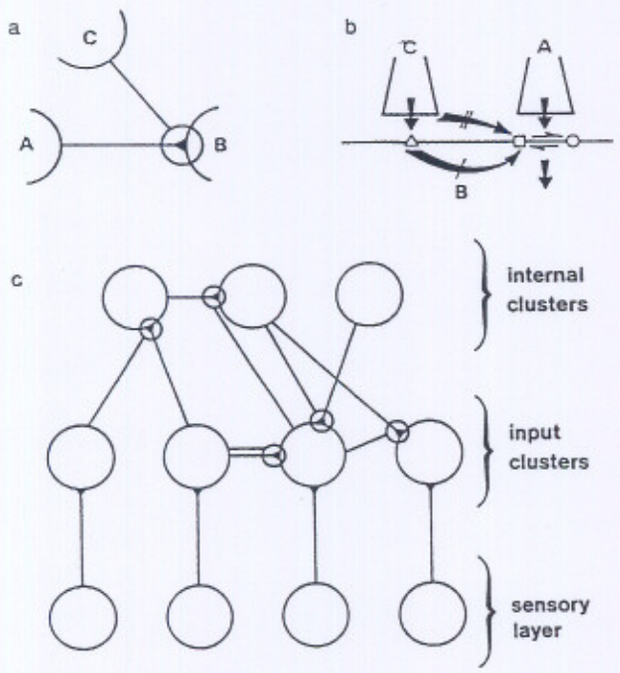

Fig. 6. The concept of synaptic triad (from [44]).

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 nAChR 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

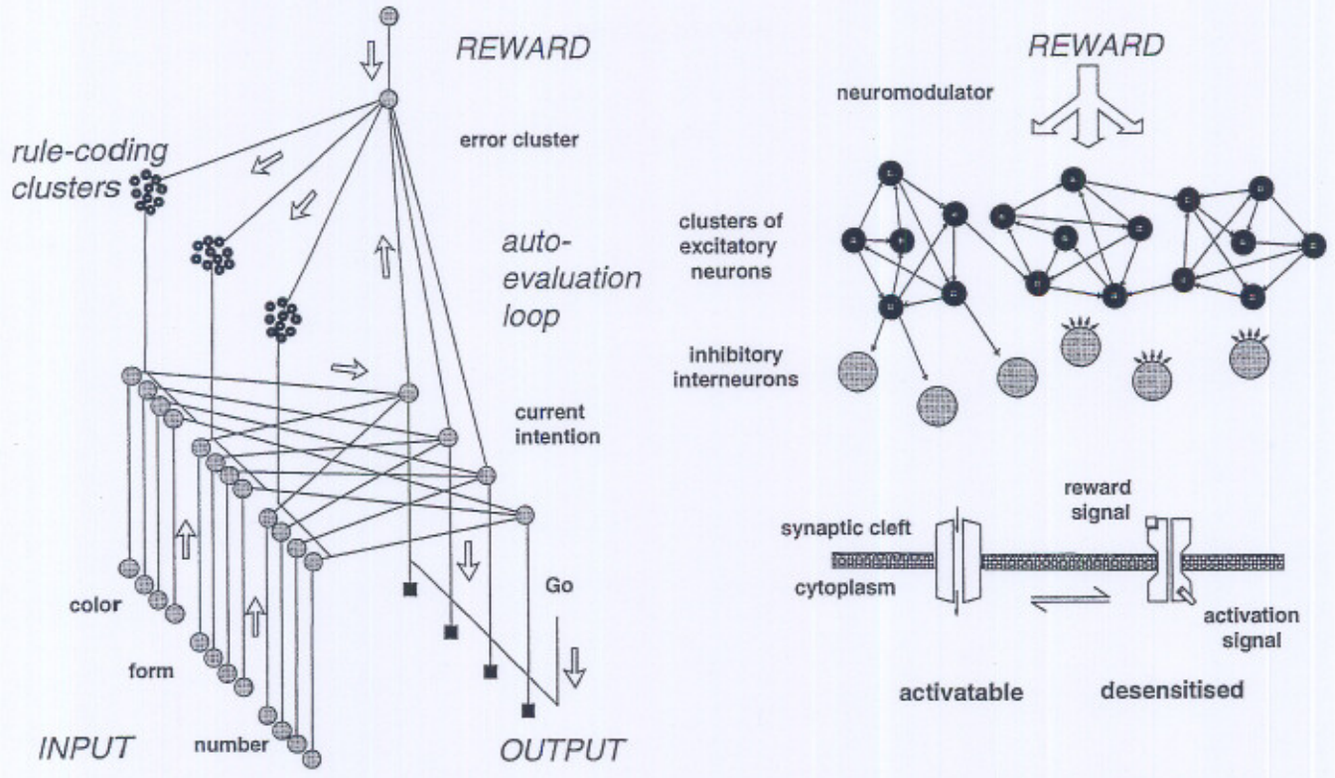

Fig. 7. Potential role of allosteric receptors as time-coincidence detectors and their integration within a formal neuronal organism able to perform delayed-response tasks [42,43]. Left: formal organism able to successfully pass the Wisconsin card sorting test. Right: diagrammatic representation of the set of rule coding clusters operating as a 'generator of diversity': the cluster of neurons active at the moment the reward is received (top) becomes stabilized by selective stabilization of an allosteric receptor conformation (bottom) binding jointly the reward signal and the activation signal.

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* defined as neuronal pathways either *externally* driven or *internally* elicited via auto-evaluation 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 in the model 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 [165]. 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-β-erythroidine, were assayed in a delayed 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 significant 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 addition, 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 ($\beta2^{-}/^{-}$) 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 ^{3}H-nicotine binding measured in both extracts and in situ by receptor autoradiography. The β2-subunit containing nAChR, thus contributes to the high-affinity binding sites for ^{3}H nicotine (most probably together with the α4-subunit whose distribution closely parallels that of the β2-subunit) [168]. As mentioned above, the electrophysiological response to nicotine of the neurons from the anterior thalamus (which express very high levels of β2 and α4-subunit mRNA) are absent in the mutant.

The $\beta2^{-}/^{-}$ mice tested for the retention of an avoidance 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 $\beta2^{-}/^{-}$ mice. The effect of low nicotine levels on passive avoidance learning is thus mediated by a β2-subunit containing nAChR [136]. Moreover, preliminary observations support the view that slight modifications in the learning behavior of $\beta2^{-}/^{-}$ 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 $\beta2^{-}/^{-}$ mice for their performance 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

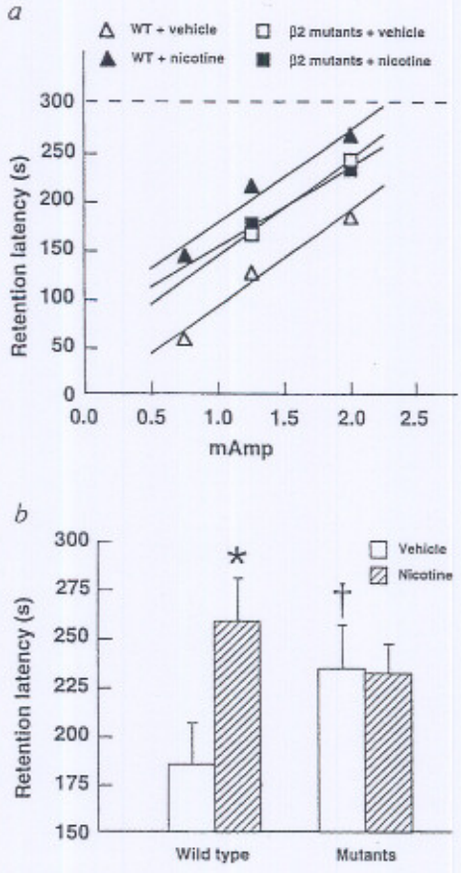

Fig. 8. Compared performances of $\beta2^{-}/^{-}$ mice and their wild type siblings on the passive avoidance task (from [136]).

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 transmission may only become apparent during aging when, for instance, compensation by other neural 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 proposed 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 memory, 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 [40]. 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 *fos*-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 $\beta2^{-/-}$ mice, dopamine release in the striatum is no longer stimulated by nicotine and nicotine self-administrative is dramatically impaired. These results support the notion that $\beta2$-subunit-containing high affinity nAChRs contribute to the reinforcing effects of nicotine.

In situ binding and hybridization studies reveal labeling in catecholaminergic nuclei for $\beta2$-subunit mRNA and high-affinity nicotine binding, but also for $\alpha6$ and $\beta3$ mRNAs which are very abundant in aminergic neurons [99]. It has thus been proposed, as a working hypothesis, that $\alpha6\beta2\beta3$ 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

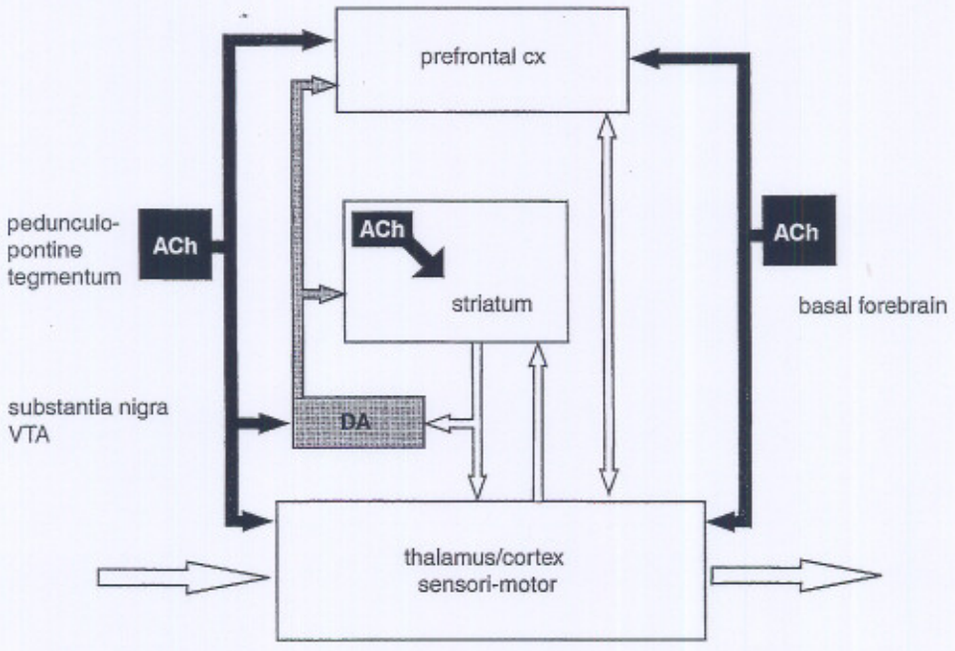

Fig. 9. Plausible contribution of cholinergic nicotinic pathways to reinforcement mechanism and to their high order regulation.

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 [108]. 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 $\alpha 4$ 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.

Acknowledgements

This work was supported by grants from the Collège de France, the Centre National de la Recherche Scientifique, the Association Française contre les Myopathies, Direction des Recherches et Etudes Techniques, Tobacco Research and the EU Biotech and Biomed Programs.

References

[1] E.X. Albuquerque, E. Pereira, M. Alkondon, A. Schrattenholz, A. Maelicke, Minireview: Nicotinic acetylcholine receptors on hippocampal neurons: Distribution on the neuronal surface and modulation of receptor activity, J. Recept. Signal Transduct Res. 17 (1997) 243–266.

[2] L.F. Agnati, M. Zoli, I. Strömberg, K. Fuxe, Intercellular communication in the brain: wiring versus volume transmission, Neuroscience 69 (1995) 711–726.

[3] J. Altman, B.J. Everitt, S. Glautier, A. Markun, D. Nutt, R. Oretti, G.D. Philipps, T.W. Robbins, The biological, social and clinical bases of drug addiction: commentary and debate, Psychopharmacology 125 (1996) 285–345.

[4] M. Amador, J. Dani, Mechanisms for modulation of nicotinic acetylcholine receptors that can influence synaptic transmission, J. Neurosci. 15 (1995) 4525–4532.

[5] P. Andersen, O. Hvalby, B. Paulsen, T. Hökfelt (Eds.), Memory concepts Basic and Clinical aspects, Excerpta Medica, Amsterdam, 1993.

[6] D. Barford, L.N. Johnson, The allosteric transition of glycogen phosphorylase, Nature 340 (1989) 609–616.

[7] D. Bertrand, J.P. Changeux, Nicotinic receptor: an allosteric protein specialized for intercellular communication, Seminars Neurosci. 7 (1995) 75–90.

[8] D. Bertrand, M. Ballivet, D. Rungger, Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in Xenopus oocytes, Proc. Natl. Acad. Sci. USA 87 (1990) 1993–1997.
[9] D. Bertrand, J.L. Galzi, A. Devillers-Thiéry, S. Bertrand, J.P. Changeux, Stratification of the channel domain in neurotransmitter receptors, Current Opinion in Cell Biol. 5 (1993) 688–693.
[10] D. Bertrand, J.L. Galzi, A. Devillers-Thiéry, S. Bertrand, J.P. Changeux, Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal alpha7 nicotinic receptor, Proc. Nat. Acad. Sci. USA 90 (1993) 6971–6975.
[11] D. Bertrand, A. Devillers-Thiéry, F. Revah, J.L. Galzi, N. Hussy, C. Mulle, S. Bertrand, M. Ballivet, J.P. Changeux, Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain, Proc. Natl. Acad. Sci. USA 89 (1992) 1261–1265.
[12] S. Bertrand, A. Devillers-Thiéry, E. Palma, B. Buisson, S. Edelstein, P.J. Corringer, J.P. Changeux, D. Bertrand, Paradoxical allosteric effects of competitive inhibitors on neuronal alpha 7 nicotinic receptor mutants, NeuroReport (1997) in press.
[13] D. Bertrand, S. Valera, S. Bertrand, M. Ballivet, D. Rungger, Steroids inhibit nicotinic acetylcholine receptors, NeuroReport 2 (1991) 277–280.
[14] A. Bessis, N. Champtiaux, L. Chatelin, J.P. Changeux, The neuron-restrictive silencer element (NRSE): a dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the brain, Proc. Natl. Acad. Sci. USA 94 (1997) 5906–5911.
[15] J. Boulter, K. Evans, D. Goldman, G. Martin, D. Treco, S. Heinemann, J. Patrick, Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor alpha-subunit, Nature 319 (1986) 368–374.
[16] N.D. Boyd, J.B. Cohen, Kinetics of binding of ^{3}H acetylcholine and ^{3}H carbamylcholine to Torpedo postsynaptic membranes: slow conformational transitions of the cholinergic receptor, Biochemistry 19 (1980) 5344–5358.
[17] J.P. Changeux, The feedback control mechanism of biosynthetic L-threonine deaminase by L-isoleucine, Cold Spring Harbor. Symp., Quant. Biol 26 (1961) 313–318.
[18] J.P. Changeux, Sur les propriétés allostériques de la L-thréonine deaminase de biosynthèse VI. Discussion générale, Bull Soc. Chim. Biol. 47 (1965) 281–300.
[19] J.P. Changeux, Responses of acetylcholinesterase from Torpedo marmorata to salts and curarizing drugs, Mol. Pharmacol. 2 (1966) 369–392.
[20] J.P. Changeux, Remarks on the symmetry and cooperative properties of biological membranes, Nobel Symposium: Symmetry and functions in Biological Systems at the Macromolecular level, Wiley, New York, 1969, pp. 235–256.
[21] J.P. Changeux, L'Homme Neuronal, Fayard, Paris (1983); English translation: Neuronal man, Princeton University Press (1985 and 1997).
[22] J.P. Changeux, Functional architecture and dynamics of the nicotinic acetylcholine receptor: an allosteric ligand-gated ion channel, Fidia Research Foundation Neuroscience Award Lectures, Vol. 4, 1990, pp. 21–168.
[23] J.P. Changeux, A critical view of neuronal models of learning and memory. Memory concepts. Basic and clinical aspects. Novo Nordisk Foundation, Copenhagen, Danemark, Elsevier, Amsterdam, 1993, pp. 413–433.
[24] J.P. Changeux, Neurotransmitter receptors in the changing brain: allosteric transitions, gene expression and pathology at the molecular level, Nobel Symposium: Individual Development over the Lifespan: Biological and Psychosocial Perspectives, Cambridge University Press, 1996, pp. 107–138.
[25] J.P. Changeux, T. Heidmann, Allosteric receptors and molecular models of learning. In: Synaptic Function. Wiley, New York, 1987, pp. 549–604.
[26] J.P. Changeux, A. Devillers-Thiéry, P. Chemouilli, Acetylcholine receptor: an allosteric protein, Science 225 (1984) 1335–1345.
[27] J.P. Changeux, M. Kasai, C.Y. Lee, The use of a snake venom toxin to characterize the cholinergic receptor protein, Proc. Natl. Acad. Sci. USA. 67 (1970) 1241–1247.
[28] J.P. Changeux, J.P. Thiéry, Y. Tung, C. Kittel, On the cooperativity of biological membranes, Proc. Nat. Acad. Sci. USA 57 (1967) 335–341.
[29] J.P. Changeux, A. Bessis, J.P. Bourgeois, P.J. Corringer, A. Devillers-Thiéry, J.L. Eiselé, M. Kerszberg, C. Léna, N. Le Novère, M. Picciotto, M. Zoli, Nicotinic Receptors and Brain Plasticity. Function and Dysfunction in the nervous system. Cold Spring Harb. Symp. Quant. Biol. Volume 61, Cold Spring Harb. Laboratory Press, 1996, pp. 343–362
[30] P.S. Churchland, T. Sejnowski, The computational brain. MIT Press, Cambridge Mass., 1992.
[31] P.B.S. Clarke, Nicotinic receptors in mammalian brain: localization and relation to cholinergic function, Prog. Brain Res. 98 (1993) 77–83.
[32] P.B.S. Clarke, R.D. Schwartz, S.M. Paul, C.B. Pert, A. Pert, Nicotinic binding in rat brain: autoradiographic comparison of [^{3}H]acetylcholine, [^{3}H]nicotine, and [^{125}I]-alpha-bungarotoxin, J. Neurosci. 5 (1985) 1307–1315.
[33] D. Colquhoun, H.P. Rang, The relation between clasical and cooperative models for drug action in drug receptors, Macmillan, London, 1973.
[34] D. Colquhoun, B. Sakmann, Fast-events in single-channel currents activated by acetylcholine and its analogues at the frog muscle endplate, J. Physiol. 369 (1985) 501–557.
[35] J.G. Connolly, A.J. Gibb, D. Colquhoun, Heterogeneity of neuronal nicotinic acetylcholine receptors in thin slices of rat medial habenula, J. Physiol. 484 (1995) 87–105.
[36] W.G. Conroy, D.K. Berg, Neurons can maintain multiple classes of nicotinic acetylcholine receptors distinguished by different subunit compositions, J. Biol. Chem. (1995) 4424–4431.
[37] W.G. Conroy, A.B. Vernallis, D.K. Berg, The alpha 5 product assembles with multiple acetylcholine receptor subunits to form distinctive receptor subtypes in brain, Neuron 9 (1992) 679–691.
[38] P.J. Corringer, S. Bertrand, S. Bohler, S. Edelstein, J.P. Changeux, D. Bertrand, Identification of critical elements modulating desensitization of neuronal nicotinic receptors (1997) (submitted).
[39] P.J. Corringer, J. Galzi L, J.L. Eiselé, S. Bertrand, J.P. Changeux, D. Bertrand, Identification of a new component of the agonist binding site of the nicotinic alpha 7 homooligomeric receptor, J. Biol. Chem. 270 (1995) 11749–11752.
[40] J. Dani, S. Heinemann, Molecular and cellular aspects of nicotine abuse, Neuron 16 (1996) 905–908.
[41] P. Daubas, A. Devillers-Thiéry, B. Geoffroy, S. Martinez, A. Bessis, J.P. Changeux, Differential expression of the neuronal acetylcholine receptor 2 subunit gene during chick brain development as detected by in situ hybridization and cDNA amplification, Neuron 5 (1990) 49–60.
[42] S. Dehaene, J.P. Changeux, A simple model of prefrontal cortex function in delayed-response tasks, J. Cogn. Neurosci. 1 (1989) 244–261.
[43] S. Dehaene, J.P. Changeux, The Wisconsin card sorting test: theoretical analysis and simulation of a reasoning task in a model neuronal network, Cereb. Cortex 1 (1991) 62–79.
[44] S. Dehaene, J.P. Changeux, J.P. Nadal, Neural networks that learn temporal sequences by selection, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 2727–2731.
[45] S. Dehaene, J.P. Changeux, A hierarchical neuronal network for planning behavior, Proc. Natl. Acad. Sci. U.S.A. (1997) in press.
[46] J. Del Castillo, B. Katz, A study of curare action with an electrical micro-method, Proc. R. Soc. Lond. (Biol.) 146 (1957) 339–356.
[47] E.S. Deneris, J. Boulter, L.W. Swanson, J. Patrick, S. Heinemann, Beta 3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain, J. Biol. Chem. 264 (1989) 6268–6272.
[48] M. Dennis, J. Giraudat, F. Kotzyba-Hibert, M. Goeldner, C. Hirth,

J.Y. Chang, C. Lazure, M. Chrétien, J.P. Changeux, Amino acids of theTorpedo marmorata acetylcholine receptor subunit labeled by a photoaffinity ligand for the acetylcholine binding site, Biochemistry 27 (1988) 2346–2357.

[49] A. Devillers-Thiéry, J.L. Galzi, S. Bertrand, J.P. Changeux, D. Bertrand, Stratified organization of the nicotinic acetylcholine receptor channel, NeuroReport 3 (1992) 1001–1004.

[50] K. Dineley-Miller, J. Patrick, Gene transcripts for the nicotinic acetylcholine receptor subunit beta4 are distributed in multiple areas of the rat central nervous system, Mol. Brain Res. 16 (1992) 339–344.

[51] J.E. Dowling, Information processing by local circuits: The vertebrate retina as a model system. In: F.O. Schmitt, F.G. Worden (Eds.), The Neurosciences: Fourth Study Program, MIT Press, Cambridge, Mass, 1979, pp. 163–181.

[52] G. Edelman, Neural Darwinism, Basic Books, New York, 1987.

[53] G. Edelman, G. Tononi, Selection and development: the brain as a complex system, Behavioral, Neurobiological and Psychosocial perspectives, Cambridge University Press, 1996, pp. 107–138.

[54] S.J. Edelstein, An allosteric mechanism for the acetylcholine receptor, Biochem. Biophys. Res. Commun. 48 (1972) 1160–1165.

[55] S. Edelstein, J.P. Changeux, Allosteric proteins after 30 years: the binding and state functions of the neuronal alpha 7 nicotinic acetylcholine receptor, Experientia 52 (1996) 1083–1090.

[56] S. Edelstein, J.P. Changeux, Allosteric transitions of the acetylcholine receptor. Linkage Thermodynamics of Macromolecular Interactions, Advances in Protein Chemistry (1997) (in press).

[57] S. Edelstein, O. Schaad, J.P. Changeux, Myasthenic nicotinic receptor mutant interpreted in terms of the allosteric model, C.R. Acad. Sci. Paris (1997) in press.

[58] S. Edelstein, O. Schaad, E. Henry, D. Bertrand, J.P. Changeux, A kinetic mechanism for nicotinic acetylcholine receptors based on multiple allosteric transitions, Biol. Cybern. 75 (1996) 361–379.

[59] J.L. Eiselé, S. Bertrand, J.L. Galzi, A. Devillers-Thiéry, J.P. Changeux, D. Bertrand, Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities, Nature 366 (1993) 479–483.

[60] I. Forster, D. Bertrand, Inward rectification of neuronal nicotinic acetylcholine receptors investigated by using the homomeric $\alpha 7$ receptor, Proc. R. Soc. Lond. 260 (1995) 139–148.

[61] J.M. Fuster, The prefrontal cortex: Anatomy, physiology, and neuropsychology of the frontal lobe, Raven Press, New York, 1989.

[62] J.L. Galzi, J.P. Changeux, Neuronal nicotinic receptors: molecular organization and regulations, Neuropharmacology 34 (1995) 563–582.

[63] J.L. Galzi, S.J. Edelstein, J.P. Changeux, The multiple phenotypes of allosteric receptor mutants, Proc. Natl. Acad. Sci. USA 93 (1996) 1853–1858.

[64] J.L. Galzi, S. Bertrand, P.J. Corringer, J.P. Changeux, D. Bertrand, Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor, EMBO J. 15 (1996) 5824–5832.

[65] J.L. Galzi, D. Bertrand, A. Devillers-Thiéry, F. Revah, S. Bertrand, J.P. Changeux, Functional significance of aromatic amino acids from three peptide loops of the alpha 7 neuronal nicotinic receptor site investigated by site-directed mutagenesis, FEBS Lett. 294 (1991) 198–202.

[66] J.L. Galzi, A. Devillers-Thiery, N. Hussy, S. Bertrand, J.P. Changeux, D. Bertrand, Mutations in the ion channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic, Nature 359 (1992) 500–505.

[67] J.L. Galzi, F. Revah, F. Bouet, A. Ménez, M. Goeldner, C. Hirth, J.P. Changeux, Allosteric transitions of the acetylcholine receptor probed at the amino acid level with a photolabile cholinergic ligand, Proc. Natl. Acad. Sci. USA 88 (1991) 5051–5055.

[68] J. Giraudat, M. Dennis, T. Heidmann, J.Y. Chang, J.P. Changeux, Structure of the high affinity site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the delta subunit is labeled by [^{3}H]-chlorpromazine, Proc. Natl. Acad. Sci. USA 83 (1986) 2719–2723.

[69] J. Giraudat, M. Dennis, T. Heidmann, P.Y. Haumont, F. Lederer, J.P. Changeux, Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: [^{3}H] chlorpromazine labels homologous residues in the beta and delta chains, Biochemistry 26 (1987) 2410–2418.

[70] P.S. Goldman-Rakic, Cellular basis of working memory, Neuron 14 (1995) 477–485.

[71] S. Granon, B. Poucet, C. Thinus-Blanc, J.P. Changeux, C. Vidal, Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection, and effortful processing, Psychopharmacology 119 (1995) 139–144.

[72] S. Grillner, P. Wallen, L. Brodin, Neuronal network generating locomotor behavior in lamprey: circuitry, transmitters, membrane properties and simulation, Ann. Rev. Neurosc. 14 (1991) 169–199.

[73] T. Heidmann, J.P. Changeux, Fast kinetic studies on the interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from *T. marmorata*, Eur. J. Biochem. 94 (1979) 255–279.

[74] T. Heidmann, J.P. Changeux, Fast kinetic studies on the allosteric interactions between acetylcholine receptor and local anesthetic binding sites, Eur. J. Biochem. 94 (1979) 281–296.

[75] T. Heidmann, J.P Changeux, Interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from Torpedo marmorata in the millisecond time range: resolution of an 'intermediate' conformational transition and evidence for positive cooperative effects, Biochem. Biophys. Res. Commun. 97 (1980) 889–896.

[76] T. Heidmann, J.P. Changeux, Un modèle moléculaire de régulation d'efficacité d'un synapse chimique au niveau postsynaptique, C. R. Acad. Sci. Paris 3. 295 (1982) 665–670.

[77] J.A. Hill, M. Zoli, J.P. Bourgeois, J.P. Changeux, Immunocytochemical localization of a neuronal nicotinic receptor: the beta2 subunit, J. Neurosci. 13 (1993) 1551–1568.

[78] B. Hille, Ionic channel of excitable membranes. M.A. Sunderland (Ed.), Sinauer, 1984.

[79] T. Hökfelt, Neuropeptides in perspective: the last ten years, Neuron 7 (1991) 867–879.

[80] F. Hucho, W. Oberthür, F. Lottspeich, 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.

[81] R.L. Huganir, P. Greengard, Regulation of neurotransmitter receptor desensibilization by protein phosphorylation, Neuron 5 (1990) 555–567.

[82] C.K. Ifune, J.H. Steinbach, Inward rectification of acetylcholine-elicited currents in rat phacochromocytoma cells, J. Physiol. Lond. 457 (1992) 143–165.

[83] K. Imoto, C. Busch, B. Sackmann, M. Mishina, T. Konno, J. Nakai, H. Bujo, Y. Mori, K. Fukuda, S. Numa, Rings of negatively charged amino acids determine the acetylchoine receptor channel conductance, Nature 335 (1988) 645–648.

[84] A. Imperato, A. Mulas, G. Di Chiara, Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats, Eur. J. Pharmacol. 132 (1986) 337–338.

[85] S. Iwata, K. Kamata, S. Yoshida, T. Minowa, T. Ohta, T and R states in the crystals of bacterial L-lactate dehydrogenase reveal the mechanism of an allosteric control, Nature Structural Biology 1 (1994) 176–184.

[86] M.B. Jackson, Spontaneous openings of the acetylcholine receptor channel, Proc. Natl. Acad. Sci. USA 81 (1984) 3901–3904.

[87] M.B. Jackson, Kinetics of unliganded acetylcholine receptor channel, Proc. Natl. Acad. Sci. USA 81 (1986) 3901–3904.

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

and delta-subunits, Pflügers Arch. Eur. J. Physiol. 417 (1990) 129–135.
[89] P.N. Kao, A.J. Dwork, R.R.J. Kaldany, M. Silver L, J. Widemann, J. Stein, A. Karlin, Identification of the alpha-subunit half-cystine specifically labeled by an affinity reagent for acetylcholine receptor binding site, J. Biol. Chem. 259 (1984) 11662–11665.
[90] A. Karlin, On the application of 'a plausible model' of allosteric proteins to the receptor for acetylcholine, J. Theoret. Biol. 16 (1967) 306–320.
[91] A. Karlin, Structure of nicotinic acetylcholine receptors, Curr. Op. Neurobiol. 3 (1993) 299–309.
[92] A. Karlin, M. Akabas, Toward a structural basis for the function of the nicotinic acetylcholine receptors and their cousins, Neuron 15 (1996) 1231–1244.
[93] M. Kerszberg, J.P. Changeux, A model for reading morphogenetic gradients: autocatalysis and competition at the gene level, Proc. Natl. Acad. Sci. USA 91 (1994) 5823–5827.
[94] D. Koshland, G. Nemethy, D. Filmer, Comparison of experimental binding data and theoretical models in proteins containing subunits, Biochemistry 5 (1966) 365–385.
[95] C. Léna, J.P. Changeux, Allosteric modulations of the nicotinic acetylcholine receptor, Trends Neurosci. 16 (1993) 181–186.
[96] C. Léna, J.P. Changeux, Role of calcium ions in nicotinic facilitation of GABA release in mouse thalamus, J. Neurosci. 17 (1997) 576–585.
[97] C. Léna, J.P. Changeux, C. Mulle, Evidence for 'Preterminal' nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus, J. Neurosci. 13 (1993) 2680–2688.
[98] N. Le Novère, J.P. Changeux, Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells, J. Mol. Evol. 40 (1995) 155–172.
[99] N. Le Novère, M. Zoli, J.P. Changeux, Neuronal nicotinic receptor alpha-6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain, J. Neurosci. 8 (1996) 2428–2439.
[100] R.J. Leonard, C.G. Labarca, P. Charnet, N. Davidson, H.A. Lester, Evidence that the M2 membrane-spanning region lines the ion channel pore of the nicotinic receptor, Science 242 (1988) 1578–1581.
[101] E.D. Levin, Nicotinic systems and cognitive function, Psychopharmacology 108 (1992) 417–431.
[102] X.M. Li, M. Zoli, U.B. Finnman, N. Le Novère, J.P. Changeux, K. Fuxe, A single (−) nicotine injection causes change with a time delay in the affinity of striatal D2 receptors for antagonist, but not for agonist, nor in the D2 receptor mRNA levels in the rat substantia nigra, Brain Res. 679 (1995) 157–167.
[103] A.R. Luria, Higher cortical functions in man, Basic Books, New York, 1966.
[104] D.S. MacGehee, L. Role, Presynaptic ionotropic receptors, Cur. Op. Neurobiol. 6 (1996) 342–349.
[105] D.S. McGehee, M. Heath, S. Gelber, P. Devay, L.W. Role, Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors, Science 269 (1995) 1692–1697.
[106] L.L. McMahon, K.W. Yoon, V.A. Chiappinelli, Nicotinic receptor activation facilitates GABAergic neurotransmission in the avian lateral spiriform nucleus, Neuroscience 59 (1994) 689–698.
[107] L.L. McMahon, K.W. Yoon, V.A. Chiappinelli, Electrophysiological evidence for presynaptic nicotinic receptors in the avian ventral lateral geniculate nucleus, J. Neurophysiol. 71 (1994) 826–829.
[108] R. Maldonado, A. Salardi, O. Valverde, T.A. Samad, B. Roques, E. Borrelli, Absence of opiate rewarding effects in mice lacking dopamine D2 receptors, Nature (in press).
[109] M.J. Marks, J.R. Pauly, S.D. Gross, E.S. Deneris, I. Hermans-Borgmeyer, S.F. Heinemann, A.C. Collins, Nicotine binding and nicotinic receptor subunit RNA after chronic treatment, J. Neurosci. 12 (1992) 2765–2784.
[110] A. Mathie, D. Colquhoun, S.G. Cull-Candy, Rectification of currents activated by nicotinic acetylcholine receptors in rat sympathetic ganglion cells, J. Physiol. Lond. 427 (1990) 625–655.
[111] E. Merlo Pich, S.R. Pagliusi, M. Tessari, D. Talabot-Ayer, R. Hooft van Huijsduijnen, C. Chiamulera, Common neural substrates for the addictive properties of nicotine and cocaine, Science 275 (1997) 83–86.
[112] M.J. Marks, J.R. Pauly, S.D. Gross, E.S. Deneris, I. Hermans-Borgmeyer, S.F. Heinemann, A.C. Collins, Nicotine binding and nicotinic receptor subunit RNA after chronic treatment, J. Neurosci. 12 (1992) 2765–2784.
[113] J. Monod, J.P. Changeux, F. Jacob, Allosteric proteins and cellular control systems, J. Mol. Biol. 6 (1963) 306.
[114] J. Monod, J. Wyman, J.P. Changeux, On the nature of allosteric transitions: a plausible model, J. Mol. Biol. 12 (1965) 88–118.
[115] P.R. Montague, P. Dayan, T.J. Sejnowski, A framework for mesencephalic dopamine systems based on predictive Hebbian learning, J. Neurosci. 16 (1996) 1936–1947.
[116] R.G.M. Morris, F. Schenk, F. Tweedie, L.E. Jarrard, Ibotenate lesions of hippocampus and/or subiculum: dissociating components of allocentric spatial learning, Eur. J. Neurosci. 2 (1990) 1016–1028.
[117] H. Muirhead, M.F. Perutz, Structure of hemoglobin. A three dimensional Fourier synthesis of reduced human hemoglobin at 5.5 Å resolution, Nature (1963) 633–638.
[118] C. Mulle, J.P. Changeux, A novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques, J. Neurosci. 10 (1990) 169–175.
[119] C. Mulle, C. Léna, J.P. Changeux, Potentiation of nicotinic receptor response by external calcium in rat central neurons, Neuron 8 (1992) 937–945.
[120] C. Mulle, D. Choquet, H. Korn, J.P. Changeux, Calcium influx through nicotinic receptor in rat central neurons: Its relevance to cellular regulation, Neuron 8 (1992) 135–143.
[121] C. Mulle, C. Vidal, P. Benoit, J.P. Changeux, Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo interpeduncular system, J. Neurosci. 11 (1991) 2588–2597.
[122] D. Nachmansohn, Chemical and molecular basis of nerve activity, Academic Press, New York, 1959, p. 235.
[123] K. Nakazawa, ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons, J. Neurosci. 14 (1994) 740–750.
[124] R.R. Neubig, J.B. Cohen, Conformations of *Torpedo* acetylcholine receptor associated with ion transport and desensitization, Biochemistry 21 (1982) 3460–3467.
[125] A. Nordberg, B. Winblad, Reduced number of ^{3}H-nicotine and ^{3}H-acetylcholine binding sites in the frontal cortex of Alzheimer brains, Neurosci. Lett. 72 (1986) 307–318.
[126] S. Numa, A molecular view of neurotransmitter receptors and ionic channels, Harvey Lecture Ser. 83 (1989) 121–165.
[127] S. Numa, M. Noda, H. Takahashi, T. Tanabe, M. Toyosato, Y. Furutani, S. Kikyotani, Molecular structure of the nicotinic acetylcholine receptor, Cold Spring Harb. Symp. Quant. Biol. 48 (1983) 57–69.
[128] M. Ortells, G. Lunt, Evolutionary history of the ligand-gated ion channel superfamily of receptors, Trends Neurosci. 18 (1995) 121–127.
[129] R.E. Oswald, J.P. Changeux, Crosslinking of alpha-bungarotoxin to the acetylcholine receptor fromTorpedo marmorata by ultraviolet light irradiation, FEBS Lett. 139 (1982) 225–229.
[130] Y. Paas, The macro- and microarchitectures of the ligand-binding domain of glutamate receptors, Trends Neurosci. (1997) in press.
[131] S.E. Pedersen, J.B. Cohen, D-tubocurarine binding sites are located at the alpha–gamma and alpha–delta subunit interfaces of the nicotinic acetylcholine receptor, Proc. Natl. Acad. Sci. USA 87 (1990) 2785–2789.
[132] E.F. Pereira, M. Alkondon, T. Tano, N.G. Castro, F.M. Froes, R.

Rozental, R.S. Aronstam, A. Schrattenholz, A. Maelicke, E.X. Albuquerque, A novel agonist binding site on nicotinic acetylcholine receptors, J. Recept. Res. 13 (1993) 413–436.
[133] M.F. Perutz, Mechanisms of cooperativity and allosteric regulation in proteins, Quart. Rev. Biophys. 22 (1989) 139–236.
[134] M. Picciotto, M. Zoli, V. Zachariou, J.P. Changeux, Contribution of nicotinic acetylcholine receptors containing the β2-subunit to the behavioural effects of nicotine, Biochem. Soc. Trans. 25 (1997) in press.
[135] M. Picciotto, M. Zoli, R. Rimondini, C. Léna, L. Marubio, E. Merlo Pich, K. Fuxe, J.P. Changeux, β-2 subunit containing acetylcholine receptors are involved in the reinforcing properties of nicotine, Nature (1997) in press.
[136] M. Picciotto, M. Zoli, C. Léna, A. Bessis, Y. Lallemand, N. Le Novère, P. Vincent, E. Merlo Pich, P. Brûlet, J.P. Changeux, Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain, Nature 374 (1995) 65–67.
[137] F.E. Pontieri, G. Tanda, F. Orzi, G. Di Chiara, Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs, Nature 382 (1996) 255–257.
[138] J. Ramirez-Latorre, C.R. Yu, X. Qu, F. Perin, A. Karlin, L. Role, Functional contributions of alpha 5 subunit to neuronal acetylcholine receptor channels, Nature 380 (1996) 347–351.
[139] F. Révah, D. Bertrand, J.L. Galzi, A. Devillers-Thiéry, C. Mulle, N. Hussy, S. Bertrand, M. Ballivet, J.P. Changeux, Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor, Nature 353 (1991) 846–849.
[140] L.W. Role, Diversity in primary structure and function of neuronal nicotinic acetylcholine receptor channels, Current Opinion in Neurobiol. 2 (1992) 254–262.
[141] L.W. Role, D.K. Berg, Nicotinic receptors in the development and modulation of CNS synapses, Neuron 16 (1996) 1077–1085.
[142] S.B. Sands, A.C.S. Costa, J.W. Patrick, Barium permeability of neuronal nicotinic receptor alpha 7 expressed in Xenopus oocytes, Biophys. J. 65 (1993) 2614–2621.
[143] T. Schirmer, P.R. Evans, Structural basis of the allosteric behavior of phosphofructokinase, Nature 343 (1990) 140–145.
[144] P.R. Schoffield, J.W. Lynch, S. Rajendra, K.D. Pierce, C.A. Handford, P.H. Barry, Molecular and genetic insights into ligand binding and signal transduction at the inhibitory glycine receptor, Cold Spring Harbor Symp. Quant. Biol. 61 (1997) 333–342.
[145] W. Schulz, P. Dayan, R. Montague, A neural substrate of prediction and reward, Science 275 (1997) 1593–1599.
[146] T. Shallice, From neuropsychology to mental structure, Cambridge University Press, 1988.
[147] P. Séguéla, J. Wadiche, K. Dineley-Miller, J.A. Dani, J.W. Patrick, Molecular cloning, functional properties and distribution of rat brain alpha7: A nicotinic cation channel highly permeable to calcium, J. Neurosci. 13 (1993) 596–604.
[148] S.M. Sine, H.J. Kreienkamp, N. Bren, R. Maeda, P. Taylor, Molecular dissection of subunit interfaces in the acetylcholine receptor: identification of determinants of alpha-conotoxin M1 selectivity, Neuron 15 (1995) 205–211.
[149] G. Stafford, R.E. Oswald, G. Weiland, The beta subunit of neuronal nicotinic acetylcholine receptors is a determinant of the affinity for substance P inhibition, Mol. Pharmacol. 45 (1994) 758–762.
[150] O.K. Steinlein, A. Magnusson, J. Stoodt, S. Bertrand, S. Weiland, S.F. Berkovic, K.O. Nakken, P. Propping, D. Bertrand, An insertion mutation of the CHRNA4 gene in a familial autosomal dominant nocturnal frontal lobe epilepsy, Human Mol. Gen. 6 (1997) 943–947.
[151] O. Steinlein, J. Mulley, P. Propping, R. Wallace, H. Philipps, G. Sutherland, I. Scheffer, S. Berkovic, A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy, Nature Genet. 11 (1995) 201–203.
[152] D. Umbriaco, K.C. Watkins, L. Descarries, C. Cozzari, B.K. Hartman, Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections, J. Comp. Neurol. 348 (1994) 351–373.
[153] N. Unwin, Acetylcholine receptor channel imaged in the open state, Nature 373 (1995) 37–43.
[154] S. Valera, M. Ballivet, D. Bertrand, Progesterone modulates a neuronal nicotinic acetylcholine receptor, Proc. Nat. Acad. Sci. USA 89 (1992) 9949–9953.
[155] A.B. Vernallis, W.G. Conroy, D.K. Berg, Neurons assemble acetylcholine receptors with as many as three kinds of subunits and can segregate subunits among receptor subtypes, Neuron 10 (1993) 451–464.
[156] S. Vernino, M. Amador, C.W. Luetje, J. Patrick, J.A. Dani, Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors, Neuron 8 (1992) 127–134.
[157] C. Vidal, J.P. Changeux, Pharmacological profile of nicotinic acetylcholine receptors in the rat prefrontal cortex: an electrophysiological study in slice preparation, Neuroscience 29 (1989) 261–270.
[158] C. Vidal, J.P. Changeux, Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro, Neuroscience 56 (1993) 23–32.
[159] E. Wada, D. McKinnon, S. Heinemann, J. Patrick, L.W. Swanson, The distribution of mRNA encoded by a new member of the neuronal nicotinic acetylcholine receptor gene family (α_5) in the rat central nervous system, Brain Res. 526 (1990) 45–53.
[160] E. Wada, K. Wada, J. Boulter, E. Deneris, S. Heinemann, J. Patrick, L.W. Swanson, Distribution of alpha2, alpha3, alpha4, and beta2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat, J. Comp. Neurol. 284 (1989) 314–335.
[161] F. Wang, V. Gerzanich, G.B. Wells, R. Anand, X. Peng, K. Keyser, J. Lindstrom, Assembly of human neuronal nicotinic receptor alpha 5 subunits with alpha 3, beta 2 and beta 4 subunits, J. Biol. Chem. 271 (1996) 17656–17665.
[162] U. Warpman, A. Nordberg, Epibatidine and ABT 418 reveal selective losses of alpha4 beta 2 nicotinic receptors in Alzheimer brains, NeuroReport 6 (1995) 2419–2423.
[163] T.C. Westfall, Effect of nicotine and other drugs on the release of [^{3}H]norepinephrine and [^{14}C]dopamine in rat brain striatum and hypothalamus slices, Neuropharmacology 13 (1974) 1025–1032.
[164] H. Wigstrom, B. Gustafsson, On long-lasting potentiation in the hippocampus: a proposed mechanism for its dependence on coincident pre- and postsynaptic activity, Acta Physiol. Scan. 123 (1985) 519–522.
[165] J. Winkler, S.T. Suhr, F.H. Gage, L.J. Thai, L.J. Fisher, Essential role of neocortical acetylcholine in spatial memory, Nature 375 (1995) 484–487.
[166] S. Wonnacott, Presynaptic nicotinic ACh receptors, Trends Neurosci. 20 (1997) 92–98.
[167] M. Zoli, L.F. Agnati, P. Hedlund, X.M. Li, S. Ferré, K. Fuxe, Receptor-receptor interactions as an integrative mechanism in nerve cells, Mol. Neurob. 7 (1993) 293–334.
[168] M. Zoli, N. Le Novère, J. Hill, J.P. Changeux, Developmental regulation of nicotinic receptor subunit mRNAs in the rat central and peripheral nervous system, J. Neurosci. 15 (1995) 1912–1939.
[169] M. Zoli, M. Picciotto, R. Ferrari, J.P. Changeux, A spatial learning deficit in aged mice lacking high-affinity nicotinic receptors, Soc. Neurosci. Abstr. vol. 23 (1997).