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Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system

Antoine Taly, Pierre-Jean Corringer‡, Denis Guedin§, Pierre Lestage§ and Jean-Pierre Changeux||*

Abstract | Nicotinic receptors — a family of ligand-gated ion channels that mediate the effects of the neurotransmitter acetylcholine — are among the most well understood allosteric membrane proteins from a structural and functional perspective. There is also considerable interest in modulating nicotinic receptors to treat nervous-system disorders such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder and tobacco addiction. This article describes both recent advances in our understanding of the assembly, activity and conformational transitions of nicotinic receptors, as well as developments in the therapeutic application of nicotinic receptor ligands, with the aim of aiding novel drug discovery by bridging the gap between these two rapidly developing fields.

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Acetylcholine (ACh) was one of the first neurotransmitters to be discovered and is one of the most important in the central nervous system (CNS) and peripheral nervous system (PNS). ACh is produced by the enzyme choline acetyltransferase and its actions are mediated through two types of acetylcholine receptors (AChRs) — the G protein-coupled muscarinic AChRs and the nicotinic AChRs (nAChRs).

The nAChRs are ligand-gated ion channels that are present in both the PNS (at the skeletal neuromuscular junction and in the autonomic nervous system) and the CNS. They are integral membrane proteins involved in the rapid 'phasic' effects of ACh under conditions of brief release of this neurotransmitter that produce high local concentrations. nAChRs can also respond to low ACh concentrations, and are the target of tonically released ACh and of systemically applied pharmacological agents, including nicotine. Typically, activation of brain nAChRs results in enhanced release of various key neurotransmitters, including dopamine, serotonin, glutamate and GABA (γ-aminobutyric acid).

Knowledge of the atomic structure, functional organization and conformational transitions of the nAChRs has recently progressed to the extent that these receptors are now among the most well understood allosteric membrane proteins (that is, proteins that mediate signal transduction between topographically distinct sites, through a conformational change)[1]. This makes them an exceptional model to investigate the molecular mechanisms of drug–receptor interactions[2–7]. The acute effect of ACh consists of the fast opening (microsecond to millisecond range) of a cationic channel that is permeable to Na^+, K^+ and sometimes Ca^{2+} ions. Of key importance for drug design, chronic exposure to ACh or nicotinic drugs causes a gradual decrease in the rate of this ionic response (100 ms to minutes), leading to a high-affinity, desensitized, closed state of the receptor[5]. Many of the clinically used drugs that target nAChRs are administered for months, resulting in long-term changes in receptor properties and/or number. Chronic exposure to nicotine causes a striking increase, typically by twofold, in the total number of high-affinity receptors — a process termed upregulation[8].

There is considerable interest in modulating nAChRs to treat various nervous-system disorders, such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and tobacco addiction[9–11]. This article first examines recent advances in our understanding of the assembly, activity and conformational transitions of the nAChR[5,12], highlighting the relevance of these discoveries to the design of novel, improved drugs that target nAChRs. Recent developments in the therapeutic application of nicotinic receptor ligands in the treatment of various CNS disorders are

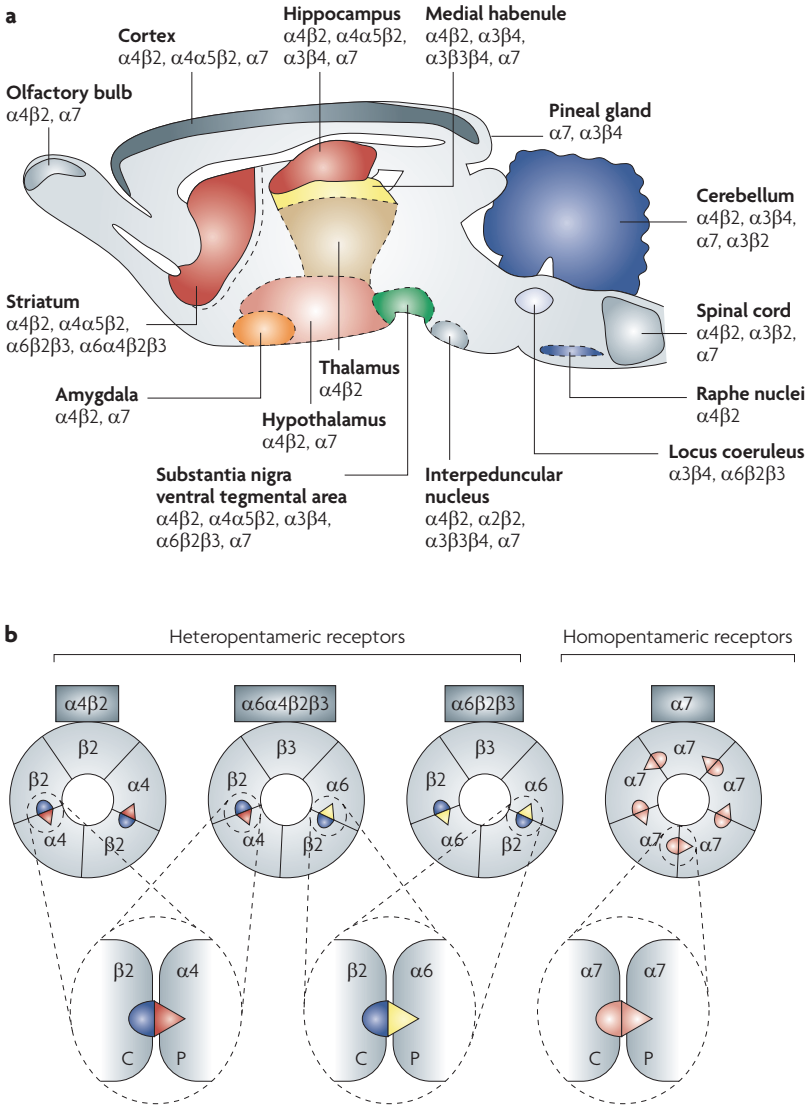


Figure 1 | **Variability of nicotinic binding sites and receptor subunits. a** | The various assemblies of nicotinic acetylcholine receptor subtypes are broadly distributed in the brain**. b |** Variability in pharmacological specificity of the nicotinic binding site at subunit interfaces results from the association of nicotinic receptor subunits into multiple oligomers**.** Triangles represent the 'principal' (P) side of the binding site, and semi-circles represent the 'complementary' (C) side. The receptors are named according to the subunits that form the binding site. Figure is reproduced, with permission, from REF. 280 © (2006) Elsevier.

then discussed. The article also aims to help bridge the gap between these two fields of research to inspire novel drug discovery.

nAChR structure at the atomic level

nAChRs are integral allosteric membrane proteins with a molecular mass of ~290 kDa, comprising five identical or homologous subunits symmetrically arranged around a central ionic channel. In mammals, there are several types of nAChRs, which differ in their subunit compositions according to their location in the body. The nAChR subunits are encoded by 17 genes. Of these, nine α-subunits and three β-subunits are expressed in the brain. The various pentameric nAChR subunit combinations differ in their pharmacological and kinetic properties, and in their localization[13,14] (FIG. 1).

The atomic structure of the nAChR is well characterized (BOX 1; FIGS 2,3). Each subunit consists of a large amino-terminal extracellular domain (ECD), a transmembrane domain (TMD) comprising four segments (TM1–TM4), and a variable cytoplasmic domain (FIG. 2A). There are 2–5 ACh-binding sites within the ECD, which are topographically distinct from the functionally linked cationic ion channel, located on the axis of symmetry of the TMD. The atomic structure of the ECD was first solved for the ACh-binding proteins (AChBPs) — soluble pentameric homologues of the nAChR ECD that were initially cloned from invertebrate snails[15–17]. Each AChBP molecule has five identical binding sites for ACh and nicotine, located at the boundary between subunits[15–17].

nAChR ACh-binding sites

The principal difficulty in targeting drugs to nAChRs in the brain arises from the broad diversity of nAChR homo-pentamers and hetero-pentamers (FIG. 1). Understanding the atomic structure of the binding site and of its complexes with various nicotinic ligands is of crucial importance in the design of novel agents that target defined nervous-system pathologies and receptor subtypes.

ACh-binding sites at subunit interfaces. The nicotinic binding site lies at the interface between an α-type subunit (the 'principal' component) and a non-α-subunit (the 'complementary' component)[5], except in homomeric nAChRs, such as α7 nAChRs (REF. 18) (FIG. 1b). The number of binding sites per pentamer therefore differs depending on its composition, from two (as in the muscle 2α1.1β.1γ.1δ nAChR) up to five (as in the neuronal α7 homopentamer)[13] (FIG. 1). Non-equivalent binding sites formed by different subunits that have different affinities for agonists and antagonists may coexist within a given oligomer. A diverse range of neuronal nAChR hetero-oligomers exist in the brain, which creates substantial challenges for targeted drug design[6].

Amino acids of the ACh-binding site. Several amino-acid residues contribute to the ACh-binding site[2,5,19–23] (FIG. 3a). They are grouped into sequence stretches referred to as loops A, B and C (the principal component) and D, E and F (the complementary component). These loops form a compact pocket within the AChBP structure, which is located at the centre of the interface of the ECD and capped by loop C[15–17,24–30]. The conserved residues of the binding site are the aromatic amino acids[5]: Y93 (loop A), W149 and Y151 (loop B), Y190 and Y198 (loop C), W55 and W57 (loop D) (numbering refers to loci in *Torpedo marmorata*). The bridged cysteine residues in loop C (C192 and C193) are present only in the α-subunits. These residues establish contacts with nicotinic ligands in the co-crystal of AChBP (FIG. 3b).

Oligomer
A particular category of protein that results from the assembly of identical or homologous subunits and possesses axes of symmetry.

Box 1 | **Atomic structure of nicotinic acetylcholine receptors (nAChRs)**

Early electron microscopy observation of the fish electric organ nAChR[253,254] revealed a cylinder of ~8 nm in diameter and ~16 nm in length which, when viewed from above, presents as a rosette of five subunits organized around a symmetrical five-fold axis that is perpendicular to the membrane plane. The images distinguish the extracellular domain (ECD), the transmembrane domain (TMD) and a cytoplasmic, intracellular domain. In the TMD[254,255], the four transmembrane segments fold in α-helices (TM1–TM4). The TM2 helix of each subunit borders the ion channel along the axis of symmetry and is surrounded by the TM1 and TM3 segments, which make a second ring of helices shielding the TM2 segment from the lipid bilayer (TM4 being located at the periphery of the bundle). Of importance for the pharmacologist is the prediction arising from these studies that TM1, TM3 and especially TM4 contact lipid molecules in the plasma membrane, which is in agreement with the labelling of these segments with hydrophobic probes[256] and the X-ray structure of the TMD[53,81] (see the section 'Allosteric modulatory sites' in the main text).

The X-ray structure of the ECD was solved using the acetylcholine (ACh)-binding proteins (AChBPs), which are soluble pentameric nAChR ECD homologues that were initially cloned from snails[15–17]. Each individual AChBP subunit folds as an immunoglobulin-like β-sandwich, composed of a six-strand inner sheet and a four-strand outer sheet. The sheets tightly interact with one another and are covalently connected by a cys-loop that includes two bridged cysteines and is present in all animal nAChRs[257]. Each AChBP molecule has five identical binding sites for ACh and nicotine, located at the boundary between subunits[15–17]. The crystal structure of a triple mutant of the ECD of the mouse nAChR α1 subunit, solved as a monomeric complex with α-bungarotoxin[258], can be successfully superimposed on the crystal structure of AChBP monomers, establishing AChBP as a reliable template of the ECD of the mammalian nAChR for drug design.

Bacterial homologues of nAChRs[257] are encoded by a heterogeneous family of genes. Those that have greatest sequence homology to nAChRs — the *Gloeobacter violaceus* pentameric ligand-gated ion channel (GLIC)[82] and the *Erwinia chrysanthemi* pentameric ligand-gated ion channel (ELIC)[81] — possess a compact structure and lack the first 20 residues that contain the amino-terminal α-helix in AChBP and nAChRs, the canonical disulphide bridge and the large cytoplasmic loop between TM3 and TM4 that is present in all nAChRs. GLIC, expressed as a homo-pentamer in human embryonic kidney (HEK) 293 cells and *Xenopus* oocytes, is a transmembrane cationic channel that is opened by extracellular protons and has slow kinetics of activation, does not show desensitization and has a single channel conductance of 8 pS[82]. This indicates that the cytoplasmic domain is not necessary for channel gating, which is also the case for the 5-hydroxytryptamine type 3 receptor and the GABA (γ-aminobutyric acid) type A receptor[259].

The crystal structure of ELIC at 3.3 Å resolution[81] and of GLIC at 2.9–3.1 Å[53,81] reveals a striking conservation of secondary and tertiary motifs that include the ECD β-sandwich and the four transmembrane α-helices, which together delineate a core structure that is conserved in the superfamily.

Interaction with ligands. The aromatic residues and disulphide bridge of the binding site have an electronegative character, and it was proposed that the positive charge carried by most nicotinic ligands would be neutralized by these residues[31,32]. The binding of ACh involves a cation–π interaction (a non-covalent interaction between a cation and an electron-rich π system) with the conserved tryptophan residue of loop B[33], making the ammonium centre of the agonist a key pharmacophore. In the case of nicotine, efficient cation–π interaction with this residue is correlated with high binding affinity, explaining the high affinity of nicotine for the α4β2 nAChR, in contrast to the low binding affinity of nicotine for the muscle-type nAChR[34]. The structure of co-crystals of AChBP with all agonists studied[16,17,24–29] shows that the tertiary or quaternary ammonium group binds in the centre of an 'aromatic box' consisting of the tyrosine and tryptophan residues from loops A, B, C and D. The distance of this ammonium group from the tryptophan residue in loop B is compatible with a cation–π interaction, a feature confirmed by a recent nuclear magnetic resonance (NMR) imaging study with ACh[35]. Antagonist binding to the nAChR is generally less well characterized.

Selectivity for nicotinic ligands. In general, the variable residues that contribute to the binding site are not included in the aromatic box and are found on both the principal and the complementary component[37,38]. This is the case for the amino acids that distinguish the α7 from the α4β2 receptors, the ligands for which are under intensive investigation by the pharmaceutical industry (discussed below). However, exceptions might exist and mutations of conserved residues in loop C may provide reversal of agonist selectivity[39].

Conformational changes of the binding site. Extensive X-ray studies on AChBP have shown that agonist binding is associated with a closed conformation of the capping loop C, whereas the ligand-free resting and antagonist-bound forms show a more open loop C conformation[40] (FIG. 3). However, when extrapolating from the results obtained with AChBP to the allosteric transitions of nAChRs — assuming agonist-bound versus antagonist-bound states represent resting, and active and desensitized states, respectively (discussed below) — caution should be taken. Indeed, in contrast to the conformational transition of the bacterial receptors (discussed below), there is no difference between the conformation of the β-sandwich (an immunoglobulin-like fold comprising two β-sheets) in the 'open' and 'closed' AChBP structures, suggesting that AChBP is in a structurally 'frozen' conformation[1,41].

The nAChR ion channel

Channel blockers are an important category of nicotinic drugs that exert valuable therapeutic actions in humans, from local anaesthesia to aiding smoking cessation (discussed below). They exert their effects in a manner distinct from that of competitive antagonists, which modulate the orthosteric binding site of the nAChR. Typically, channel blockers sterically occlude the channel pore (BOX 2; FIG. 2), preventing ion flux, but they have also been found to substantially alter the allosteric transitions of the nAChR[5].

The channel pore is located within the 20 α-helices bundle of the TMD, at the level of the α-helically folded TM2, in the axis of symmetry of the nAChR pentamers[5] (BOX 2; FIG. 2). Early affinity labelling experiments with channel blockers identified key residues located at positions 2, 6, 9, 13 and 20 within TM2 (REFS 2,42,43), which is consistent with the notion that the same side of each TM2 α-helix 'faces' the pore and that the walls of the channel consist of superimposed pentameric rings of homologous amino acids. The binding sites for the various channel blockers are distributed throughout the transmembrane channel, in particular within its gate (the mid-section stretch of hydrophobic residues) in the closed conformation, and at the entrance of the ion selectivity filter, which consists of rings of hydrophilic residues

Allosteric transition
The global conformational change that brings together the multiple topographically distinct sites carried by a regulatory protein.

Orthosteric binding site
The main biologically active site of a receptor, to which the cognate ligand binds.

at the cytoplasmic border. This region constitutes the constriction of the channel in the open conformation and is involved in monovalent-ion selection, possibly through a specific dehydration mechanism (BOX 2). It is still a largely unexplored target for drug design.

nAChR allosteric modulatory sites

Modulation of nAChR function has been achieved by a range of pharmacological agents that are termed allosteric modulators. These bind to protein regions other than the active site or the channel lumen. Positive and negative allosteric modulators exert activatory and inhibitory effects, respectively[44–46]. Notably, allosteric modulators typically have low intrinsic activity but provide selective potentiation or inhibition of physiological activity, without directly affecting the ongoing signalling processes. Moreover, their recently identified binding sites have revealed unexpected protein regions that regulate the allosteric transitions of the nAChR.

Allosteric modulation through 'non-agonist' binding interfaces. In heteropentameric neuronal nAChRs such as α4β2, ACh binding occurs at the orthosteric site at the α–β interface, but not at the homologous sites of other interfaces. The allosteric modulator, Zn^{2+}, can either potentiate (at the α–α interface) or inhibit (at the β–α interface) the α4β2 nAChR, depending on the Zn^{2+} concentration used[47,48]. These sites have also been proposed as the target of synthetic allosteric modulators. Using AChBP as a model, disruption of the cysteine bridge of loop C, performed to mimic a non-agonist binding interface, yields micromolar affinities for galantamine, strychnine, cocaine and morphine, and their interaction has been shown by X-ray crystallography[29]. The possible binding of allosteric modulators at interfaces that do not normally bind nicotinic ligands is reminiscent of the binding site through which benzodiazepines allosterically potentiate GABA type A ($GABA_A$) receptors[49].

Allosteric sites near the extracellular transmembrane interface. Most neuronal nAChRs, including α4β2 and α7, are potentiated by Ca^{2+} at millimolar concentrations[50]. Residues E18, E44 and especially E172 have been shown to be crucial for Ca^{2+}-elicited potentiation[50]. E44 and E172 are in close proximity to one another, at the opposite sides of the subunit interface of the ECD, near the TMD, whereas E18 is located in the upper part of the subunit[51]. This site has a key role in the structural reorganization of the outer β-sheet that occurs during receptor activation[52]. In parallel, a voltage-dependent inhibitory Zn^{2+}-binding site has been identified within the β2 subunit of the α4β2 nAChR[48]. These sites are plausible targets for nicotinic-drug design that have yet to be fully explored.

Allosteric sites in the transmembrane domain. The transmembrane domain of the nAChR is embedded with lipids and known to be the site of modulation by various hydrophobic molecules and lipids. The X-ray structure of the bacterial *Gloeobacter violaceus* pentameric ligand-gated ion channel (GLIC), a nAChR homologue, reveals lipid molecules intermingled with TM3–TM1 and TM4 (REF. 53). The nAChR interacts selectively with defined lipids, including cholesterol, and its function is dependent on the lipid composition of the membrane (reviewed in REFS 54–56). Cholesterol is known to be crucial to nAChR function, and it interacts within the transmembrane domain between TM1, TM3 and TM4 (REF. 57). Steroids that are structurally related to cholesterol inhibit nAChRs by interacting at the lipid–protein interface[58,59]. These steroid-binding sites might be homologous to those identified on $GABA_A$ receptors[60].

General anaesthetics are small hydrophobic compounds that typically allosterically inhibit nAChRs by binding to specific residues within small cavities of the TMD[61,62]. The pattern of binding to these residues differs in basal and desensitized conformations, further supporting the notion that these sites modulate the allosteric transitions of the nAChR.

The binding site on α7 nAChRs[63,64] for the positive allosteric modulators PNU-120596 and LY-2087101, has been tentatively identified within the transmembrane domain. It comprises five amino acids within TM1, TM2 and TM4 which, when mutated, substantially reduce the potentiation of α7 nAChRs by allosteric modulators (FIG. 4). These amino acids form an intrasubunit cavity that is located between the four TMDs and might be similar to the binding site for neurosteroids and volatile anaesthetics on members of the ligand-gated ion channel family, including $GABA_A$ and glycine receptors[65].

Allosteric sites in the cytoplasmic domain. The intracellular loop that links the TM3 and TM4 transmembrane segment is unique to eukaryotic receptors but remains a largely unexplored territory for drug design. With the exception of an identified α-helical segment known as the membrane-associated stretch[66], the cytoplasmic domain seems to be highly variable and without a defined structure.

The cytoplasmic domain has several phosphorylation sites that in muscle control desensitization[67] or end-plate localization mediated by agrin-induced tyrosine phosphorylation[68]. The cytoplasmic domain of the α4 nAChR subunit binds the scaffold protein 14-3-3η and the Ca^{2+} sensor visinin-like protein 1 (REF. 69), and the β2 subunit interacts with several cytoskeletal proteins, such as tubulin, dynamin and clathrin, and with G protein systems that are involved in intracellular signalling pathways[70]. Therefore, the cytoplasmic domain could have key functions in the regulation of neuronal nAChR localization, trafficking and upregulation, and so is a potential, though largely unexplored, target for drug design.

Allosteric transitions of nAChRs

The signal transduction mechanism that links the many topographically distinct sites of the nAChR has been proposed to be a global conformational change of the receptor protein, referred to as allosteric transition[1,12] (BOXES 3,4; FIG. 5). Allosteric transition needs to be considered in the design of nicotinic drugs, both orthosteric and allosteric, that have defined agonist, antagonist or partial agonist character. Over the past decade, molecular

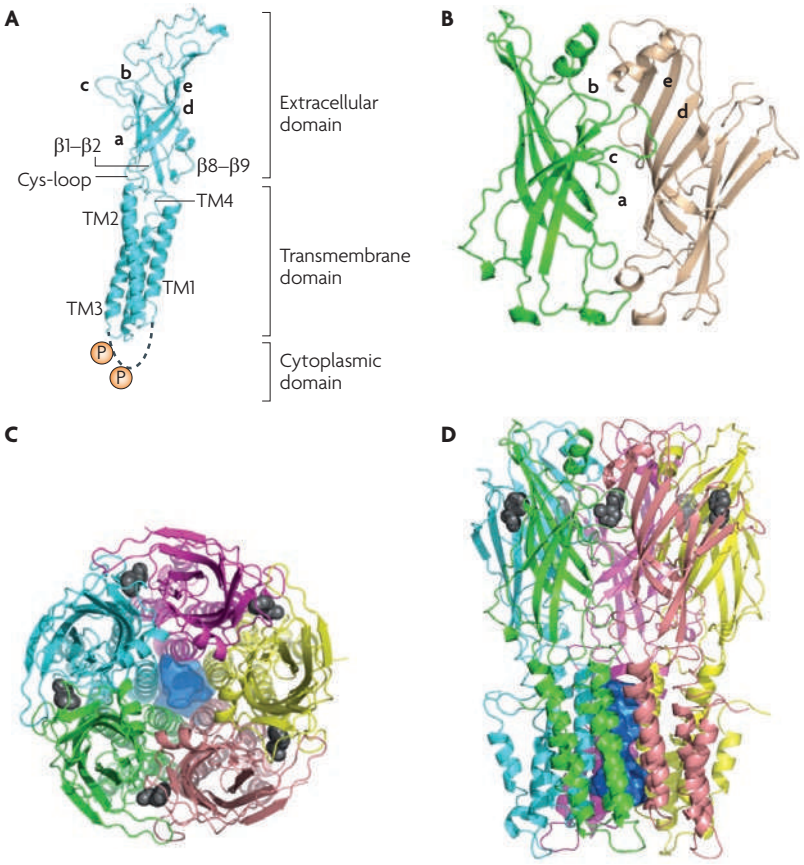


Figure 2 | **Model of the α7 nicotinic acetylcholine receptor (nAChR).** This was obtained by comparative modelling based on the homologue protein from *Erwinia chrysanthemi* (Protein Data Bank code 2VL0). **A** | Structure of one subunit of the α7 nAChR model. The cytoplasmic domain and its phosphorylation sites are schematically represented, as they are not present in the model. The 'loops' of the binding site (a–e) are labelled as well as the loops of the interface between the extracellular and transmembrane (TM) domains (cys-loop, β1–β2 and β8–β9). **B** | Close view of the acetylcholine-binding site. For clarity, only two monomers are represented. The loops of the binding site (a–e) are labelled. **C**,**D** | Top view (**C**) and side view (**D**) of the α7 nAChR pentamer, showing five nicotine molecules (dark grey) in the binding sites and the volume of the ion channel (dark blue).

mechanics studies and X-ray structural data have offered molecular mechanisms for the allosteric transition that mediates channel opening.

Asymmetrically 'induced' protein motion. Low-resolution electron microscope images (4–9 Å) of the *T. marmorata* muscle-type nAChR, suggested that ACh induced an asymmetrical distortion of the α-subunits to open the pore[71], in a process known as induced fit. These estimated motions are consistent with physiological data on muscle-type nAChRs[4,23].

Global allosteric mechanisms. Normal-mode analysis (NMA) gives an *in silico* decomposition of protein movements into discrete modes (the concerted motion of a set of atoms), which may be used to describe the slow (biologically relevant) transitions of proteins[72,73]. The application of this method to a model of α7 nAChR, based on the then available AChBP and electron microscope *T. marmorata* structures[74–76], shows that the lower-frequency mode corresponds to a global quaternary twist motion of the protein, resulting from a tilt of each subunit that causes anticlockwise motion in the upper part of the nAChR pentamer (FIG. 5a). This motion occurs concomitantly with a bending of the subunits at their ECD–TMD interface, a pore dilatation over its entire length and a structural reorganization of the ACh-binding site[74,75].

The twist mode accounts for key features of receptor channel opening and closing by agonists and antagonists, and for key features of naturally occurring mutations in the nAChRs, which cause autosomal dominant nocturnal frontal lobe epilepsy and congenital myasthenic syndromes, and alter gating properties of the channel[4,75]. The twist mode is stabilized in the resting conformation following binding of the antagonist cobratoxin[77–79].

The atomic mechanism of channel opening can be seen using the X-ray structures of the bacterial nAChR homologues *Erwinia chrysanthemi* pentameric ligand-gated ion channel (ELIC) and GLIC, stabilized in closed or open conformation[53,80–82] (FIG. 5b). Despite low ELIC–GLIC sequence identity (18%), their common core structure undergoes a quaternary twist, similar to that described above, that contributes to at least 29% of the closed to open transition, and each subunit undergoes tertiary deformations[53,80]. These deformations involve a substantial rearrangement of the subunit interfaces and a downward motion of the β1–β2 loop. This is apparently coupled to a tilt of the TM2 and TM3 segments and generates a wide opening in the upper part of the pore (from 2 to 12 Å diameter) (BOX 4).

Challenges for drug discovery

Docking studies. Docking studies were first based upon the high-resolution structures of AChBP with snake α-toxins and α7 nAChR[51,83], various antagonists[40,77,84–94], apolipoprotein E[85], conotoxin[89] and long-chain snake toxins[40]. Several studies of ligand docking carried out with nAChR ACh-binding site models[87,95–100] account for experimental data on the binding site residues and in particular the cation–π interaction[97], providing further docking sites for the design of nicotinic ligands.

Subunit diversity. The subunit diversity of brain nAChRs (nine α-subunits and three β-subunits) poses a substantial challenge to drug discovery. Indeed, the large number of nAChR homopentamers and heteropentamers exhibit distinct pharmacological, electrophysiological and kinetic properties and are differentially distributed throughout the nervous system.

Pharmacologists have grouped nicotinic drugs as ligands of α7 or α4β2 receptor subtypes on the basis of standard *in vitro* pharmacological assays (discussed below). However, difficulties with this simplified paradigm arise in the design of drugs that are targeted to nAChRs of the human brain. First, in primates, including humans, the α4 subunit in α4β2 may be substituted by α2 (REFS 101,102). Moreover, in the case of α4β2 receptors, and possibly also α2β2 receptors, different stoichiometries of the subunits may occur in the heteropentamer,

Induced fit
A conformational change in a receptor, proposed by Koshland, that is induced after a ligand is bound, with the consequence that the receptor conformation locally adapts to, and indefinitely varies with, the structure of the ligand.

Quaternary twist
A global rotational motion of the receptor protein, resulting from a tilt of each subunit, that leads to the opening of the ion channel and a structural reorganization of the acetylcholine-binding site.

resulting in different functional oligomers. For example, the (α4β2)2α4 receptor exhibits lower sensitivity to ACh and faster desensitization than (α4β2)2β2 (REFS 103,104). Furthermore, in addition to the principal (α2, α3, α4 and α6) and complementary (β2 and β4) subunits, 'accessory' subunits exist (α5, β3 and β4). Although these do not contribute to the ACh-binding sites, they may contribute to allosteric modulatory sites. In the mouse, *in vivo* deletion of various subunit genes[105,106] identified five functional nAChR subtypes in dopaminergic terminals in the striatum: α4α6β2β3, α6β2β3 and α6β2, which bind α-conotoxin MII with high affinity and have the highest sensitivity to nicotine, and α4β2 and α4α5β2, which do not bind conotoxin, are more numerous than the α6-containing subtypes and have lower affinity for nicotine[106]. Furthermore, various uncommon combinations of subunit subtypes, including α2, α3, α5 and β4, are expressed in the habenulo-interpeduncular system[14,107].

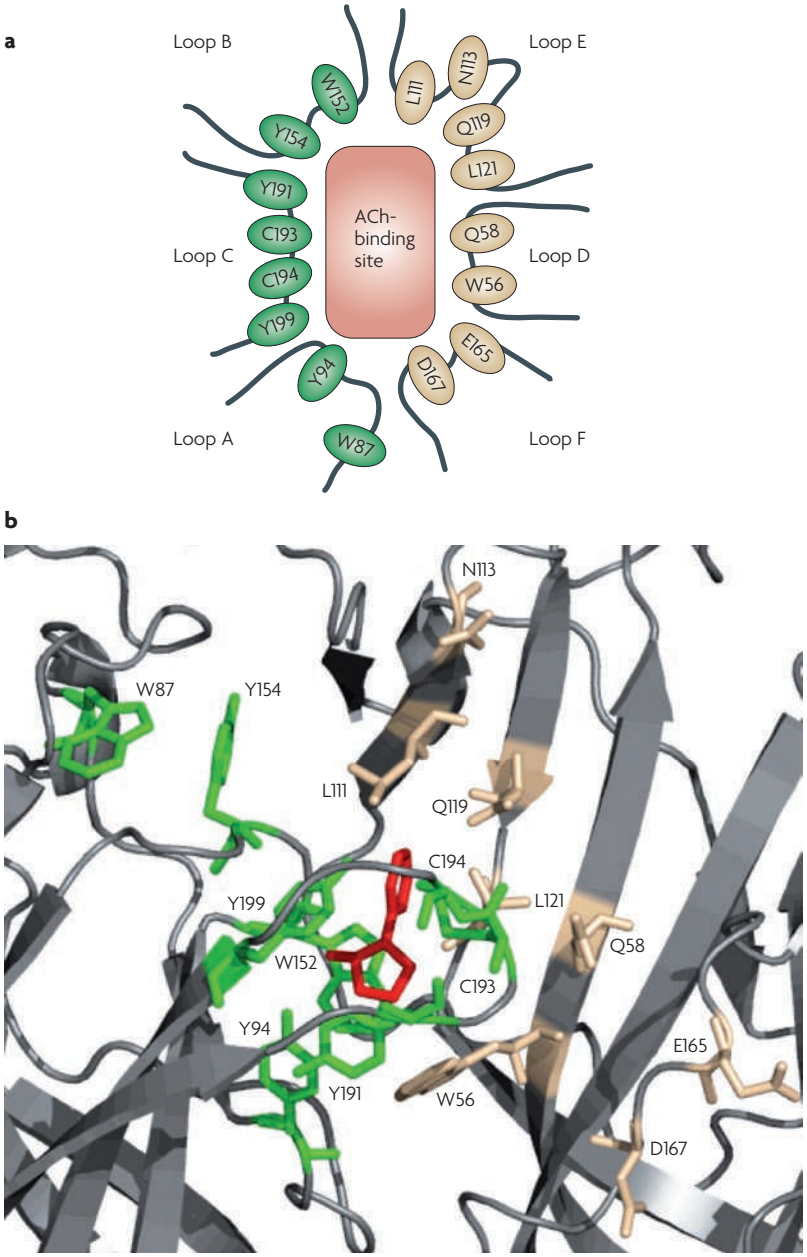


Figure 3 | **Structure of the acetylcholine (ACh)-binding site on the α7 nicotinic acetylcholine receptor (nAChR). a** | Schematic representation of the ACh-binding site, illustrating amino-acid residues that participate in ligand binding. The grouping of these amino-acid residues into loops A, B and C (the principal component) and loops D, E and F (the complementary component) are shown. **b** | Model of the α7 nAChR obtained by comparative modelling, using the X-ray crystallography structure of ACh-binding protein in the presence of nicotine as a template[26]. Residues identified by biochemical labelling are shown. For clarity, only two monomers are represented. Amino-acid residues of the principal side of the binding site are shown in green, and those of the complementary side are shown in beige. Nicotine is depicted in red.

Data from the X-ray structure analysis of these possible oligomers are insufficient to fully support drug design and, in this respect, comparative molecular modelling *in silico* may be of considerable use. Indeed, as discussed above, AChBP constitutes a suitable template to simulate the binding of ligands to nAChR and to tackle the problem of subunit diversity[108]. Three-dimensional models of binding sites with different subunit combinations have already been generated. Docking studies have successfully discriminated binding to α4β2 versus α7 nAChRs[87] and have led to the proposition that loop E may be responsible for the specific binding of AR-R17779 and GTS-21 to α7 nAChRs[109]. However, these methods are costly, and additional developments will probably be required to allow *in silico* screening of large libraries.

Implications of the allosteric transitions for drug design. The allosteric transition paradigm adds a new dimension to drug conception. In the past, both agonist and antagonist drugs were designed to fit a single conformation of the nAChR site. By contrast, the allosteric scheme implies that agonists and antagonists, as well as positive and negative modulators, select and stabilize structurally different conformations that may be modelled for the active and resting states — using, for example, the quaternary twist model[75,78,79]. Yet, this modelling is still not feasible for the desensitized state, which dominates drug-binding assays.

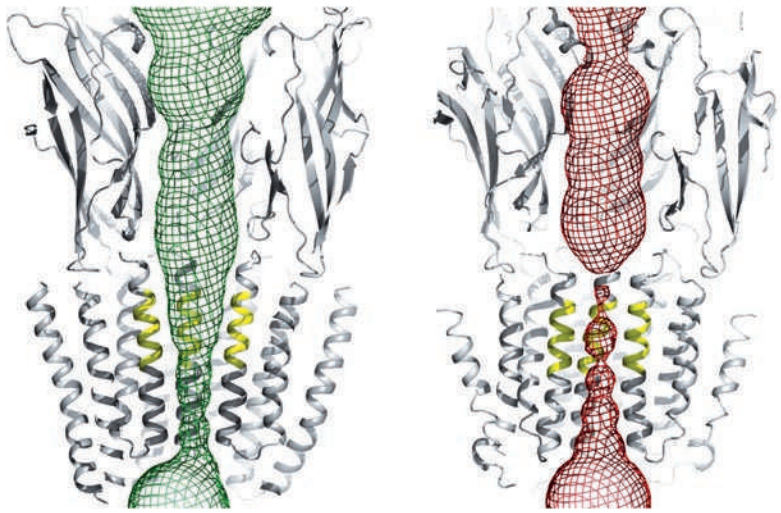
This paradigm also states that the diverse conformations of the nAChR may spontaneously exist in the absence of a ligand[12,110–112]. From a drug design perspective, this is of importance because it suggests the possibility that antagonists (both competitive and non-competitive) may not only inhibit the effect of agonists, but may also exhibit an intrinsic activity in blocking spontaneously open channels. The channel blockers quinidine and fluoxetine have proved beneficial in cases of congenital myasthenic syndromes in which nAChR channels are constitutively open[113]. Early docking studies with the antagonist α-bungarotoxin and small agonist molecules in the α7–AChBP model have revealed that both categories of compounds cannot be docked to a common conformation of the ACh-binding site[51,83], but that a substantial reorganization of the structure is necessary, such that the α-toxin antagonists dock exclusively when loop C is open[77]. The quaternary twist was found to be associated with binding site closure[75,114] and to be blocked by α-toxins[78,79], but conversely to be associated

Box 2 | **Channel selectivity and potential binding sites for blockers**

The nicotinic acetylcholine receptor (nAChR) channel is bordered by the transmembrane segment 2 (TM2) α-helix of each subunit, which together contribute rings of homologous residues to the ion permeation pathway[42,260] (reviewed in REFS 2,5) and are the binding site for channel blockers. Rings of negatively charged residues are found at both extremities of the channel, which contribute to cation translocation through electrostatic attraction[261]. The cytoplasmic border (consisting of residues at positions –2 to 2) of TM2 is the key region that selects monovalent cations over anions, as shown by numerous studies on chimeras of cationic and anionic channels[262–267]. Because this region is also the narrowest portion of the open channel, it was proposed to constitute the selectivity filter, which screens hydrated ions through a partial-dehydration mechanism[268].

The X-ray structure of the open *Gloeobacter violaceus* pentameric ligand-gated ion channel (GLIC)[53,80] clearly shows a homologous organization to that of nAChRs, and discloses a funnel shape that is widely opened at the outer end (6 Å radius), which progressively narrows to a constriction consisting of the 2′ ring, as expected. The 3 Å radius here is too narrow to allow permeation of a fully hydrated cation (reviewed in REF. 265). The hydrophobic T2′ of TM2 is flanked by the polar S6′ and E2′ rings. This cluster of the polar or charged side chains seems to be well located to transiently complex with cations, and may therefore partially substitute for water molecules, decreasing the energetic barrier that must be overcome for ion translocation. The case of divalent Ca^{2+} permeation is not fully understood. Mutation of several rings along TM2, including the intermediate E1′, have been shown to selectively interfere with Ca^{2+} transport[269].

The figure illustrates the ion permeation pathway of GLIC (left) and *Erwinia chrysanthemi* pentameric ligand-gated ion channel (ELIC) (right) bacterial receptors, shown in mesh representation over the entire length of the protein. Only three subunits of the extracellular domain and transmembrane domain are shown (in grey) for clarity. The ring of hydrophobic residues from TM2 are shown in yellow, at the level of which the pore is closed in ELIC.



with an increase in receptor affinity for nicotine[115]. The efficiency of allosteric models should be improved by the incorporation of the ELIC–GLIC X-ray data on the core ECD rotation, which is expected to yield a reorganization of the ligand-binding site in addition to and/or distinct from motion of loop C. This should enable the assignment of compounds from a chemical series to defined subunit interfaces, the assessment of their agonist or antagonist character, and may also be useful for the design of new agonist and antagonist drugs that are targeted to particular interfaces.

These strategies have proved to be successful for allosteric modulators[63], leading to the development of entirely new categories of pharmacological agents.

This allosteric mechanism offers an explanation for partial agonism — a phenomenon that is a major concern for drug design (discussed below). Early studies[116] raised the possibility that a given ligand might bind non-exclusively to both conformational states, yet with a preferential affinity for the active state[5]. An alternative possibility is that a given compound binds simultaneously (though with different affinities) to different sites on the nAChR, with opposite effects on the physiological response — as seen with suxamethonium, which behaves as an agonist at the ACh-binding site and a blocker of the open ion channel, on muscle nAChR[117]. Also, the kinetics of activation by a given agonist might be counteracted by a fast ongoing desensitization process, so that the amplitude of the response does not reach the maximum even if the compound is a full agonist[5]. Furthermore, the response to partial agonists might be limited by an early intermediate and silent conformational change, referred to as 'flipping', which is thought to occur while the channel is still closed[118,119]. Each of these possibilities should therefore be considered in drug design.

The recent progress in understanding nAChR structure and functional properties presents a considerable number of new opportunities for the development of novel CNS therapies.

Implications of the desensitized state for drug design. A consequence of the high-affinity desensitized state of the nAChR under prolonged exposure to nicotinic ligands is that equilibrium binding assays of nicotinic agonists usually select the desensitized rather than the active state. However, in some cases, desensitization may not only mean the prevention of an excess of ionotropic activity[120]; nicotinic receptors have been detected in molecular complexes with G proteins[68], arrestins[121,122] and protein kinases[123], and it might be that a second wave of signalling, of a metabotropic nature, follows the initial ionotropic signalling phase[124]. Evidence showing a pro-survival role of desensitization (for example, see REF. 125) might be interpreted according to this hypothesis, rather than on the basis of the desensitization preventing excess Ca^{2+} from entering the cell. However, considerable work remains to be done to explore these possibilities.

Nicotinic ligands and Alzheimer's disease

Changes in nAChR activity are emerging in a diverse range of CNS diseases, and there is considerable pharmaceutical interest in therapeutically targeting these receptors. Alzheimer's disease is characterized by progressive cognitive decline, accompanied by a loss of neurons and synapses — especially cholinergic synapses — in the basal forebrain, cerebral cortex and hippocampus[126] and by a substantial reduction in both muscarinic and nicotinic AChR expression[127]. In the cerebral cortex, the massive reduction in nAChRs in Alzheimer's disease[128–130] involves predominantly the α4β2 subtype, sparing the α7

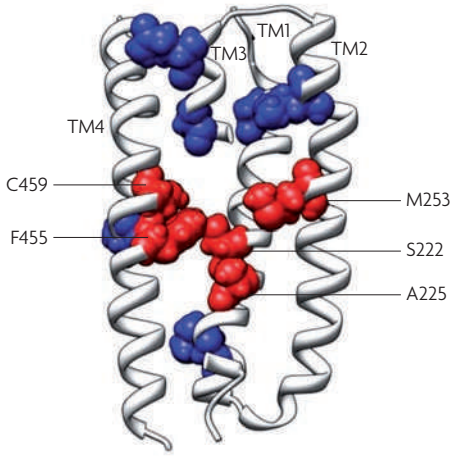


Figure 4 | **Potential binding sites for allosteric modulators.** The binding site for positive allosteric modulators of the α7 nicotinic acetylcholine receptor (namely, PNU-120596 and LY-2087101). The site is located in the transmembrane domain, between the four transmembrane segments (TM1–TM4). Residues in which mutation strongly affects the receptor potentiation are represented in red, and those in which mutation weakly affects receptor potentiation are represented in blue.

subtype[131]. By contrast, in the hippocampus, a loss of α7 nAChRs seems to predominate and to correlate with the progressive loss of cognitive function[132–136].

Early studies indicated that acute nicotine administration improved performance of patients with Alzheimer's disease in cognitive tasks, whereas acute administration of the non-competitive (channel blocker) antagonist mecamylamine resulted in dose-dependent impairment of performance in a battery of cognitive tasks[137–141] (summarized in REFS 142,143). Interestingly, the effect of mecamylamine was most pronounced in patients with Alzheimer's disease, intermediate in healthy elderly subjects, and least pronounced in healthy young subjects, suggesting that cholinergic transmission becomes increasingly limited with age and disease.

α7 nAChR ligands. Several lines of evidence indicate that many features of the procognitive activity of nicotine and its memory-enhancing potential are mediated by α7 nAChRs. First, there is a high level of expression of α7 nAChRs in the hippocampus, a region that is known to be involved in memory formation. Second, gene knockout and antisense studies have shown a role for α7 nAChRs in learning and memory[144–146], and specifically in attention and working–episodic memory[147,148]. Third, pharmacological studies have shown that a range of structurally diverse α7 nAChR-selective agonists or positive allosteric modulators improve the cognitive deficits that are associated with Alzheimer's disease.

As expected, AR-R17779 — a selective partial α7 nAChR agonist — improved scopolamine-elicited deficits in social recognition, and the 24-hour memory retention interval in unimpaired animals. Repeated doses of AR-R17779 enhanced long-term learning and attenuated working-memory deficits in rats. Two partial agonists of α7 nAChRs, GTS-21 (also a strong α4β2 antagonist) and MEM-3454 (also a strong 5-hydroxytryptamine type 3 receptor (5HT3) antagonist)[149] (TABLE 1), further showed a procognitive action and, in preclinical studies, MEM-3454 enhanced episodic, spatial and working memory. The procognitive effect of MEM-3454 on episodic memory was completely blocked by the α7-specific antagonist methyllycaconitine, establishing that the efficacy of MEM-3454 can be attributed to α7 nAChR binding. In a small Phase I clinical trial, GTS-21 improved episodic secondary memory tasks, including word recall, and picture and word recognition[150]. Similarly, MEM-3454 improved the 'quality of episodic secondary memory' score, which is a measure of episodic memory. As for episodic memory, working memory is impaired in Alzheimer's disease. MEM-3454 significantly improved the quality of working memory score in patients with Alzheimer's disease in Phase I trials. In a Phase II trial, the quality of working memory scores were also improved by MEM-3454, as was the ADAS–cog (Alzheimer's disease assessment scale–cognitive subscale) score. The ability of α7 nAChR activation to improve cognitive deficits has recently been reviewed in depth[149].

The therapeutic potential of α7 nAChR agonists or positive allosteric modulators (TABLE 1) is further increased by the fact that they may have neuroprotective properties, in particular towards amyloid-β (Aβ) toxicity (caused by the Aβ1–42 peptide). The α7 nAChR has previously been implicated in the *in vitro* neuroprotective effects of nicotine, using PC12 cells[151]. Choline, like nicotine, can protect neural cells from cytotoxicity that is induced by growth factor deprivation[152] or exposure to the glutamate analogue AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)[153]. As with nicotine, the weak α7 nAChR agonist GTS-21 is neuroprotective, specifically protecting against Aβ1–42-elicited neurotoxicity[154]. This effect is probably due to small, protracted increases in receptor-mediated Ca^{2+} influx. Importantly, high concentrations of GTS-21 reduced cell survival, underlining the possible risk of over-stimulation[152]. Studies have indicated that the neuroprotective action of α7 nAChR-specific agents arises from receptor inhibition or desensitization, rather than stimulation *per se,* leading some to suggest that α7 nAChR antagonists could be useful neuroprotective agents[155]. GTS-21 is a partial agonist with considerable residual inhibitory activity — a property that might account for its neuroprotective effects.

More recently, it has been reported that Aβ1–42 binds to rat and guinea pig α7 nAChRs with picomolar affinity[156]. Whether Aβ1–42 acts as an agonist or an antagonist at α7 nAChRs remains controversial[157–159]. Indeed, two recent papers contest the notion that Aβ1–42 binds to α7 nAChRs at all[160,161]. By contrast, deleting the α7 nAChR subunit in a mouse model of Alzheimer's disease that overexpresses a mutated form of the human amyloid precursor protein confers protection against memory loss and synaptic dysfunction, supporting a crucial role for α7 nAChR as a target[288]. Agonist activity could compromise cell viability through a prolonged stimulation of Ca^{2+} entry, whereas antagonism could prevent intrinsic

Box 3 | **Allosteric transitions of nAChRs**

Since the 1960s, the signal transduction mechanism that links the diverse, topographically distinct sites of the nicotinic acetylcholine receptor (nAChR) has been proposed to be a global conformational change of the molecule, referred to as allosteric transition[5,270]. Two principal types of models have been debated in this context[12,271]. A simple, stepwise 'instructive' scheme[272,273] postulates that the conformational change that results in an open ion channel occurs after the ligand is bound to the receptor site. The transition would therefore be induced by the neurotransmitter molecule and so the receptor would locally adapt to, and indefinitely vary with, the structure of the ligand. This model quantitatively fits most electrophysiological recordings, including patch clamp data[274], but does not provide a simple account for other receptor properties, such as spontaneous channel openings[12].

An alternative model (the Monod–Wyman–Changeux model[275]) posits that the receptor protein spontaneously undergoes reversible transitions between at least two discrete and global conformational states, even in the absence of agonist[1] and that a conformational selection, or shift in the conformers population, takes place in the presence of agonist[271]. The model accounts for signal transduction mediated by the nAChR between an active, open channel conformation that selectively binds agonists, and a resting, closed conformation that selectively binds the competitive antagonists. The model also accounts for the cooperative ligand binding that is attributed to the cooperative assembly of the protein from repeated subunits into a symmetrical oligomer. It therefore predicts that agonists (and positive allosteric modulators) and antagonists (and negative allosteric modulators) select and stabilize structurally different conformations. It also accounts for the spontaneous opening of the channel in the absence of acetylcholine and the unexpected gain-of-function effects of some of the pathological mutations of the receptor[12].

However, to account for desensitization, additional slowly accessible, high-affinity closed states have to be added[276]. The desensitized states are dominant in the standard conditions of the equilibrium binding assays that are currently used in drug testing, in parallel with electrophysiological recordings that characterize the active state of the receptor. Striking differences in specificities are observed between desensitized and active conformations. Compared with the active state, which binds agonists, the desensitized state generally binds both agonists and antagonists, but with a higher affinity principally for agonists[5]. Little is known about the structure of the desensitized state except that it differs from the active state primarily at the interface between extracellular and transmembrane domains[277] and that, at its cytoplasmic end, the closed channel is paradoxically more accessible to some non-competitive blockers[43,278].

cytoprotective mechanisms. Regardless of the exact effect of Aβ1–42 on receptor activity, it does seem to block the activation by nicotine and, consistent with the cytoprotective nature of this interaction, amyloid deposition limits neuroprotection[151]. This phenomenon may explain at least part of the neurotoxicity that is associated with Aβ1–42 (REF. 156).

An alternative mechanism has been proposed to explain how α7 nAChRs contribute to Aβ1–42 neural toxicity. Amyloid plaques form in the entorhinal cortex of patients with Alzheimer's disease and this region, which connects the neocortex and the hippocampus, plays a crucial part in memory. It has been suggested that plaques in this region represent the lytic remnants of degenerated, Aβ1–42-burdened pyramidal neurons, and that amyloid internalization depends on α7 nAChR-mediated Ca^{2+} entry[162]. Of interest, chronic nicotine treatment has been shown to reduce the plaque burden in animal models of Alzheimer's disease[123].

Several pharmacological options have therefore emerged for the development of novel α7 nAChR ligands as candidate drugs to treat Alzheimer's disease. Full agonists could provide neuroprotection and memory enhancement, resulting from desensitization of nAChRs. Desensitization should also be maintained to reduce the risk of toxicity. If cellular accumulation of Aβ1–42 contributes to its neurotoxicity, α7 nAChR agonists would be expected to have limited efficacy unless their affinity matches that of Aβ1–42, allowing the agonists to compete with Aβ1–42. Few full α7 nAChR agonists are in development for the treatment of Alzheimer's disease, in contrast to those that are targeted towards schizophrenia.

Partial agonists have received more drug development attention than full agonists. Partial agonists have many of the properties of agonists but, because of residual inhibition, the problems of toxicity that result from excessive stimulation and Ca^{2+} entry would not be anticipated to arise. Moreover, *in vitro* pharmacological properties of partial agonists indicated that, depending on the concentration of endogenous ACh, they can have either a facilitating or an inhibitory effect on the biological response[163].

The development of α7 nAChR antagonists has received only minor attention from the drug development sector. It is currently unclear whether reduced receptor activity through desensitization and antagonism produces the same functional responses. Antagonists might reduce the neuronal accumulation of Aβ1–42, but this may depend on the relative number of binding sites for the antagonist and Aβ1–42. Also, selective blockers of Ca^{2+} entry through the α7 channel may be considered in drug development efforts to prevent amyloid internalization.

Allosteric modulators currently occupy a central position in α7-targeted drug development because these ligands alter the amplitude, but not the pattern, of the physiological response. One potential disadvantage is that the modulation efficacy would decrease upon the progressive loss of endogenous ACh that occurs in Alzheimer's disease, as the destruction of cholinergic neurons proceeds. Allosteric modulators might be more useful for the treatment of schizophrenia, in which the endogenous agonist is present, and treatment with agonists would be advantageous in neurodegenerative disorders to counteract the decline in levels of the endogenous agonist[164].

α4β2 nAChR ligands. Based on evidence that α4β2 nAChRs have a role in cognitive function[144,165–169], compounds have been developed that target this receptor (TABLE 2). The aim is to identify agonists with improved safety and therapeutic profiles compared with nicotine, for the treatment of Alzheimer's disease. In particular, the positive cognitive effects of nicotine following acute exposure were not found in later trials with chronic exposure. This chronic–acute discrepancy in the effects of nicotine in Alzheimer's disease has also been seen in early clinical studies of the Abbott compound ABT-418 — a selective α4β2 full agonist and the first new chemical entity developed as a patch formulation. Having shown cognition-enhancing activity in a number of preclinical models, as well as in a small Phase II study for smoking cessation using well selected, pharmacodynamically active doses, the compound was advanced in 1993 to a larger Phase II Alzheimer's disease trial (in a chronic setting); the compound failed, but was successful when re-tried in an acute setting[9,170]. Nicotine performed similarly in the two trials.

Monod–Wyman–Changeux model
A molecular mechanism that was initially proposed in 1965 to account for the allosteric interactions mediated by a large body of regulatory enzymes. It posits that the protein oligomer spontaneously undergoes reversible transitions between at least two discrete and symmetrical conformational states, even in the absence of agonist, and that ligands selectively stabilize any one of these states through a process of conformational selection.

Box 4 | **Structural mechanism of ion channel opening**

The activation of nicotinic acetylcholine receptors (nAChRs) involves structural reorganizations that alter, in a concerted manner, the gate of the ion channel and the shape of the various binding pockets for allosteric effectors, notably the ACh-binding site. At present, no unifying model of the transition exists, but combined mutagenesis (reviewed in REF. 4), *in silico*[76], electron microscopy[71] and X-ray experiments[53,80,81] revealed key protein motions that underlie this process. X-ray and *in silico* data support the view that activation is concomitant with a global twist motion[53,76]. The reorganization of the ACh-binding site potentially involves a closing of the capping loop C[17], quaternary motions caused by both a rotation of the β-sandwich of each subunit[53] and, in the case of the heteromeric muscle-type nAChR, an asymmetric tertiary distortion of the two α-subunits relative to the non-α subunit, with the inner β-sheet rotating by 10° (REF. 71).

The transduction of these motions to the ion channel gate has been explored by mutagenesis studies at the level of a 'principal' pathway, which consists of a cluster of interacting residues bridging both the ACh-binding and ion channel sites in the three-dimensional structure. This pathway notably involves the outer sheet, which holds the allosteric site for Ca^{2+}-stimulated potentiation[279], as well as key loops located at the interface between the extracellular and transmembrane domains: the β1–β2 loop, the cys-loop and the TM2–TM3 loop. Electron microscopy data have suggested a 'pin into pocket' interaction at this level[254], but higher-resolution X-ray analysis shows that, in the course of activation, a downward motion of the β1–β2 loop is concomitant with a lateral motion of the TM2–TM3 loop away from the channel axis[53].

A large body of evidence indicates that the gate is located in the middle–upper part of the channel, at the level of three rings of hydrophobic residues that create a narrow barrier to permeant ions in the closed states of the channel[81,254]. X-ray structure analysis of bacterial nAChR homologues suggests that channel opening is caused by a global tilt of the TM2 helices, which widely opens the gate, in concert with the TM3 segment and TM2–TM3 loop — the TM1 and TM4 segments being comparatively fixed. The different motions of the various transmembrane segments produce considerable reorganization, notably at the level of allosteric-modulator-binding pockets that are thought to contribute to the binding of cholesterol, steroids, anaesthetics and synthetic compounds.

Phase II Alzheimer's disease clinical trials of ispronicline (also known as TC-1734/AZD-3480), an α4β2 full agonist (developed by AstraZeneca under licence from Targacept), have recently been completed. However, this compound failed to show improvement over placebo in a Phase IIB study. Consequently, the future of its clinical development is uncertain, and this is one of the reasons that led the pharmaceutical industry to shift towards nicotinic ligands exhibiting selectivity for α7 rather than α4β2 nAChRs.

ABT-418, discussed above, has also been found to be active in a limited human trial in attention deficit hyperactivity disorder (ADHD)[171]. A second compound, ABT-089, which is a partial agonist at α4β2 nAChRs, was also efficacious in ADHD[172].

Nicotinic ligands and schizophrenia

In addition to the obvious symptoms of hallucinations and delusions, patients with schizophrenia frequently suffer from cognitive symptoms, such as the inability to focus attention[173]. This deficit is increasingly perceived as central to the disease, as it precedes the manifestation of psychiatric symptoms. Impaired attention is caused by defective sensory gating, the process by which the brain adjusts its response to sensory stimuli. nAChRs are known to control sensory gating, and studies investigating the role of nAChRs in schizophrenia have focused primarily on α7 nAChRs. Sensory-gating deficits in patients with schizophrenia[174] have been linked to chromosome 15q14, proximal to the α7 locus[175,176]. In addition, a decrease in α7 nAChR density in the hippocampus of patients with schizophrenia has been reported[177]. Similarly, a low density of α7 nAChRs in inbred strains of mice is associated with poor gating[178]. Recently, the expression of a novel variant of α7 nAChR, CHRNA7-2 (cholinergic receptor, nicotinic, α7, variant 2), was found to be reduced below control levels in the prefrontal cortex of patients with schizophrenia[179].

Patients with schizophrenia[174] and DBA/2 mouse models[180,181] respond to nicotine administration with improved sensory gating, presumably through α7 nAChR activation[182,183]. The frequency of tobacco smoking is greater in patients with schizophrenia than in healthy subjects; furthermore, smoking abstinence impairs working memory, whereas the reinstatement of smoking improves performance. Typical antipsychotic drugs and the majority of atypical antipsychotic drugs have no effect on P50 auditory gating. However, the atypical antipsychotic drug clozapine normalizes auditory gating in DBA/2 mice — an effect which involves an α7 nAChR mechanism[181].

Two compounds that are currently in clinical use might have direct effects on the α7 nAChR. The anticholinesterase inhibitor galantamine has modulatory effects on α7 nAChR and was reportedly beneficial for patients with schizophrenia in a case study[184]. Similarly, topisetron, a 5HT3 antagonist marketed outside the United States as an anti-nausea drug, also has efficacy as an α7 nAChR agonist and increases the inhibition of P50 auditory gating in schizophrenia[185]. GTS-21, one of a series of compounds derived from anabaseine, an alkaloid found in marine worms, is a partial agonist of α7 nAChRs that improves memory-related behaviours in various paradigms and normalizes auditory gating[186]. It is the leading clinical candidate in the field of α7 nAChRs. Initially evaluated in normal subjects, GTS-21 was found to significantly improve attention and memory. In a second Phase I trial[187], GTS-21 normalized P50 auditory gating in patients with schizophrenia. Although the subsequent initial Phase II trial did show an improvement in the negative symptoms of schizophrenia, there was no cognitive effect[188], which may be due to the fact that GTS-21 is also a strong α4β2 antagonist, together with the occurrence of a significant learning bias during the trial[189,190]. Further proof of concept to support the development of α7 nAChR ligands might come from another group of candidates: Targacept's TC-5619, EnVivo's EVP-6124 and Sanofi–Aventis' SS-R180711.

Nicotinic ligands and smoking cessation

Current medications for smoking cessation, such as nicotine and bupropion (which has been classified as a non-competitive antagonist at rat α4β2 nAChRs), have limited efficacy[191], and the discovery and development of more effective drugs is needed. The molecular mechanisms of nicotine addiction are under investigation,

P50 auditory gating
The ability of a healthy organism to suppress the evoked response to an auditory stimulus that occurs 50 ms after a first stimulation.

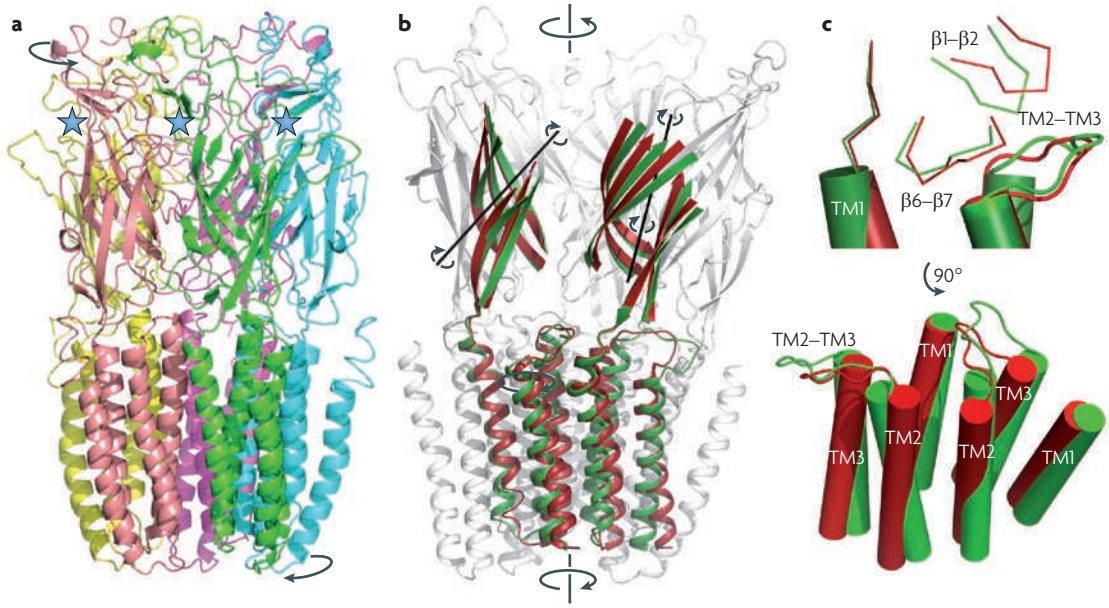


Figure 5 | **The nAChR gating mechanism. a** | The quaternary twist model for nicotinic acetylcholine receptor (nAChR) activation, inferred from *in silico* normal mode analysis[74–76] of a nAChR homology model (REF. 75). Stars indicate the location of the acetylcholine-binding site. **b**,**c** | Concerted transition of bacterial nAChR homologues, based on the comparison of *Erwinia chrysanthemi* pentameric ligand-gated ion channel (ELIC) and *Gloeobacter violaceus* pentameric ligand-gated ion channel (GLIC). Superimposition of ELIC (red, closed channel) and GLIC (green, open channel) illustrates a quaternary twist of the core structure and a rotation of the extracellular domain (**b**). The transmembrane helices of two subunits adjacent to the pore are represented as cylinders, illustrating a concerted motion of the TM2, TM3 and TM2–TM3 segments outside the pore (**c**, top). Concerted downward motion of the β1–β2 segment and outward motion of the TM2–TM3 segment causes pore opening (**c**, bottom).

and CNS nAChRs are known to be involved, together with several neurochemical systems[192], including the mesolimbic dopaminergic system[192–197]. In β2-knockout mice, nicotine self-administration and nicotine-elicited dopamine striatal release is abolished[198]. Nicotine self-administration is also reduced in rats by dihydro-β erythroidine (DHβE), a selective α4β2 antagonist[199]. In this context, partial agonists may substitute for the desired effects of nicotine and antagonize its reinforcing properties[163,200]. Varenicline (Chantix/Champix; Pfizer), the most recently approved drug for smoking cessation which is now on the market, is a partial agonist at α4β2 nAChRs, and a full agonist at α7 nAChRs (REF. 200). The introduction of Varenicline to the market consolidates the proof of concept for a safe nicotine-like compound and lends support to the use of synthetic nAChR agonists for other indications. However, the US Food and Drug Administration and the European Medicines Agency had noticed that serious neuropsychiatric symptoms (changes in mood and suicidal ideation) that are linked to varenicline might occur in some patients. In this respect, it should be noted that nicotine and smoking-cessation drugs are usually tested under short-term conditions, while it is becoming clear that long-term chronic exposure to nicotine (and possibly to nicotinic drugs in general) could modify the levels of α4β2 versus α7 nAChRs[201,202]. It is relevant that varenicline is a full α7 agonist.

New, more subtype-selective compounds may arise from the recent advances in the identification of nAChR subtypes that are involved in nicotine addiction[107,203]. For example, as discussed above, at least five functional nAChR subtypes have been identified in dopaminergic terminals in the striatum: α4α6β2β3, α6β2β3 and α6β2, which have the highest sensitivity to nicotine, and α4β2 and α4α5β2, which are more numerous than the α6-containing subtypes, yet with lower affinity for nicotine[105,106,204]. Deletion of α5 and α7 in mice decreases the physical signs of nicotine withdrawal[289]. Moreover, nAChRs containing β4, α2 and α5 in the habenulo-interpeduncular systems are necessary for nicotine withdrawal in mice[107]. Drugs might therefore be designed to target receptors containing the still unexplored α6 subunit, and also those with the so-called accessory subunits α2, α5 and β3. Interestingly, three independent studies have mapped susceptibility loci for lung cancer at nAChR genes: *CHRNA5*, *CHRNA3* and *CHRNB4* (REFS 205–207). It is not known whether these genes are related directly to intrinsic risks for lung cancer or indirectly to smoking, the known evitable cause of lung cancer. However, they include the gene encoding the α3 accessory subunit, which is expressed in dopaminergic neurons and might therefore be involved in nicotine sensitization and tolerance. β4 deletion abolishes withdrawal signs in the mouse[208], and α3 and β4 are also present in the

Table 1 | **α7 nAChR compounds in clinical development for CNS disorders**

Compound (company)	Primary indication	Type of agonist	Development stage
GTS-21 (REF. 150) (CoMentis)	Schizophrenia	Partial agonist	Phase II
SSR-180711 (REF. 281) (Sanofi–Aventis)	Alzheimer's disease	Partial agonist	Phase II*
MEM-3454‡/R-3487 (REF. 149) (Memory/Roche)	Alzheimer's disease	Partial agonist	Phase II
MEM-63908‡/R-4996 (Memory/Roche)	Alzheimer's disease	Partial agonist	Phase I
AZD-0328 (REF. 282) (AstraZeneca)	Alzheimer's disease	Partial agonist	Phase II
S-24795‡ (REF. 283) (Servier)	Alzheimer's disease	Partial agonist	Phase I*
TC-5619 (REF. 284) (Targacept)	Schizophrenia	Full agonist	Phase I
ABT-107‡ (Abbott)	Cognitive disorders	Not known	Phase I
EVP-6124‡ (EnVivo)	Schizophrenia	Not known	Phase II

*Trial discontinued. ‡Structure not disclosed. CNS, central nervous system; nAChR, nicotinic acetylcholine receptor.

PNS, supporting the view that they might be directly involved in craving and relapse — an area that is largely unexplored in the development of medications to assist with smoking cessation.

Nicotinic ligands and depression

The hypothesis that there is a hypercholinergic tone in major depressive disorder dates back to the early 1970s[209] and has been revived at the turn of this century[210], with a shift in focus towards nicotinic rather than muscarinic transmission. Meanwhile, a number of key antidepressants, such as fluoxetine (Prozac; Lilly), sertraline (Zoloft; Pfizer), paroxetine (Paxil/Seroxat; Novo Nordisk/GlaxoSmithKline), nefazodone, nisoxetine, citalopram (Celexa/Cipramil/Cipram; H. Lundbeck), nomifensine and vanoxerine[211–216] were shown to inhibit neuronal nAChRs, in addition to inhibiting selective monoamine reuptake. The agents have this effect in the low micromolar range, which is a considerably higher concentration than that needed to inhibit monoamine reuptake, but does correspond to the drug concentration in the brain at the time when these antidepressants begin to be therapeutically active. Using *in vivo* proton NMR imaging, levels of choline (the rate-limiting precursor to endogeneous ACh) were shown to be increased in the brains of patients with depression[217] and in the prefrontal cortex of adolescents with depression[218] compared with the control group.

Next, compounds that do not inhibit monoamine reuptake but affect various nAChRs subtypes through a general non-competitive blockade (for example, by mecamylamine), desensitization (for example, by nicotine) or antagonism, through either neutral antagonists (for example, DHβE (at α4β2) or methyllycaconitine (at α7)) or partial agonists (for example, cytisine), were studied either in mice[219–222] or in humans[223–225]. In all cases, inhibition of nAChR activity was beneficial. Moreover, in mice, the effect was synergistic with known antidepressants such as imipramine, citalopram, or reboxetine[219].

Targacept's Phase II clinical trial of mecamylamine as an add-on therapy to citalopram (a combination known as TRIDMAC) in partial or weak responders to citalopram, obtained positive results in 2006; similar results were observed in a smaller trial conducted by Yale University[226]. Meanwhile, Targacept has considered the fact that mecamylamine is a racemic mixture of two enantiomers (namely, the *S*-enantiomer, TC-5213, and the *R*-enantiomer, TC-5214) and that the α4β2 nAChR, as discussed above, is a mixture of oligomers that have different stoichiometries and pharmacological properties: (α4β2)2β2 (which has high sensitivity for both ACh and nicotine, and low Ca^{2+} permeability) and (α4β2)2α4 (which has low sensitivity for ACh and nicotine, and high Ca^{2+} permeability)[227]. Targacept showed that, although the *R*-enantiomer blocks both channel stoichiometries, the *S*-enantiomer blocks the low-sensitivity channels with greater efficacy than the *R*-enantiomer and enhances agonist-elicited activation of the high-sensitivity channels. They went on to show that, *in vivo*, selective activation of the high-sensitivity channels and inhibition of the low-sensitivity channels by the *S*-enantiomer displays greater efficacy in various antidepressant models than global inhibition by the *S*-enantiomer. As a consequence, the *R*-enantiomer TC-5214 is now in Phase II clinical trials for the treatment of depression. Targacept also has another selective α4β2 modulator, TC-2216, in Phase I clinical trials for both depression and anxiety disorders.

Nicotinic ligands and neuropathic pain

The analgesic properties of tobacco were recognized by Jean Nicot in the sixteenth century and, since then, the antinociceptive effect of nicotine and epibatidine acting on the inhibitory descending pain pathway have been extensively investigated. In α4- and β2-knockout mice, the responses of raphe neurons to nicotine is abolished, together with nicotine-elicited antinociception[228], and α4-hypersensitive knock-in mice show nicotine hypersensitivity in the supraspinal control (hot-plate assay), but not in the spinal control (tail flick assay)[229]. Also, α7- and non-α7-containing nicotinic receptors directly or indirectly (through GABAergic interneurons) modulate serotonin release in spinal cord slices[230]. However, the identity of the receptors that are responsible for the spinal control of nociception is currently unknown. In this process, the nicotine-induced antinociception seems to be mediated primarily by activation of calcium–calmodulin-dependent protein kinase 2, but this is not the case for supraspinal nociception control[229].

The first nicotinic receptor ligand to undergo Phase II clinical trials for analgesic activity was the potent Abbott compound ABT-594, a nAChR agonist that preferentially targets α4β2 (REFS 231–235). The compound allowed for the clinical proof of concept, but could not be developed further because of adverse effects such as emesis and nausea[236]. As these adverse effects seemed to be attributable to the activation of the ganglionic α3β4 nAChR receptors, Abbott undertook a search for more selective

Table 2 | **Non-α7 nAChR compounds in development for CNS disorders**

Compound (company)	Primary indication	Receptor subtype selectivity	Development stage
Varenicline[200] (Pfizer)	Smoking cessation	α7 (full agonist) α4β2 (partial agonist)	Launched
Dianicline[285] (Sanofi–Aventis)	Smoking cessation	α4β2 (partial agonist)	Phase II*
Lobeline[251]	Smoking cessation	α4β2 (partial agonist)	Phase III
ABT-089 (REF. 172) (Abbott)	Cognitive dysfunction	α4β2 (partial agonist)	Phase II
TC-1734 (REF. 286) (Targacept)	Alzheimer's disease	(α4β2)2β2 (full agonist) (α4β2)2α4 (partial agonist)	Phase II*
TC-5214 (REF. 287) (Targacept)	Depression	α4β2 (antagonist)	Phase II
S-38232‡ (Servier)	Alzheimer's disease	α4β2 (full agonist)	Phase I*
ABT-594‡ (REF. 231) (Abbott)	Pain	α4β2 (full agonist)	Phase I

*Trial discontinued. ‡Structure not disclosed. CNS, central nervous system; nAChR, nicotinic acetylcholine receptor.

α4β2 agonists, independently and in cooperation with Neurosearch. Both initiatives were successful: new compounds — A-366833 and ABT-894 — with improved α4β2 selectivity and a broad spectrum of analgesic efficacy without adverse effects were identified[236]. ABT-894 was chosen to go into Phase II clinical trials but the results were disappointing, and the development of this compound for neuropathic pain has been discontinued. The compound remains in clinical trials for ADHD.

Similarly, Targacept announced in a press release (Targacept provides update on TC-6499 and pain program in GlaxoSmithKline Alliance; see Further information) that development of its α4β2-specific compound TC-6499, in an alliance with GlaxoSmithKline, has been discontinued as the results of a Phase I multiple, rising-dose trial indicated that the compound is probably too subtype-specific to provide adequate efficacy. The precise selectivity profile of the compound remains to be determined.

Regarding the ascending afferent excitatory pain pathway, in which nicotinic antagonists are anticipated to have analgesic effects, a newly described subtype of nAChR, α9α10, seems to be expressed on dorsal root ganglia (in addition to the inner ear) and constitutes an *in vitro* target for new α-conotoxins, such as Vc1.1, RgIA, or It14a[237–241]. *In vivo*, these α-conotoxins display potent alleviation of allopathic pain[239,242,243] (and additionally reveal an endogeneous ACh activation of lymphocytes through α9α10 nAChRs, which are inhibited by these α-conotoxins). Yet their analgesic target might be a $GABA_B$ receptor located on the terminals of the ascending afferent nerves. Here, the α-conotoxins would act as agonists and suppress neurotransmission through a Src-mediated inhibition (probably by internalization) of the N-type $Ca_v2.2$ Ca^{2+} channels; the $GABA_B$ receptor has been shown to discriminate between the analgesic α-conotoxins and close analogues that are inactive on pain, whereas the α9α10 nAChR does not discriminate between them[244,245].

Nicotinic ligands, Parkinson's disease and ADHD

Despite great potential, no nicotinic receptor ligand has yet undergone Phase II clinical trials for Parkinson's disease. Nicotine facilitates dopamine release by acting at both somatodendritic and presynaptic nAChRs on mesolimbic[246,247] and nigrostriatal[247] neurons. For example, on nigrostriatal neurons, nicotine facilitates dopamine release at the somatodendritic level in the substantia nigra by driving the switch from a tonic firing of isolated 'spikes' to a phasic burst firing, and at the terminal level in the dorsal striatum through a process of 'contrast' enhancement and 'noise' elimination. Specifically, endogeneous ACh, tonically released by local, large cholinergic interneurons, maximizes the release probability of isolated dopaminergic spikes and consequently maximizes the subsequent use-dependent, short-term depression of release probability at rapidly successive pulses. Nicotinic receptor suppression — either through antagonists or desensitizing agonists, or physiologically through the action of dopamine itself — can therefore suppress release triggered by single stimuli but correspondingly facilitate release triggered by burst stimuli, thereby achieving a high-pass filtering of dopamine secretion, which suppresses the noise and enhances the contrast. This opens new perspectives for pharmacological nicotinic manipulation of dopamine release, but raises two important selectivity issues. First, within the cortico-striato-thalamic loop (which controls the motor cortex), the nicotinic manipulation must exert the desired contrast-enhancing action on the dorsal striatum (which inhibits the pallidum, itself inhibitory to the thalamus), but must not exert the same enhancing effect on the downstream pallidum itself (nicotine itself actually exerts strong pallidal activation and indeed does not relieve the symptoms of Parkinson's disease in humans[248]). Second, the nicotinic manipulation of dopamine release should be confined to the dorsal striatum and not extend to the ventral striatum, which is not easy to achieve. Targeting the α6 nAChR subunit, which is restricted to the striatum, could address the issue of avoiding the pallidum, but would not solve the problem of avoiding the ventral striatum while targeting the dorsal striatum. Another avenue that warrants further investigation has been provided by a careful comparison of nicotine and epibatidine, which shows that these compounds have opposite discriminations between the nigrostriatal and mesolimbic pathways[249].

ADHD, in contrast to Parkinson's disease, is an indication for which numerous compounds are undergoing Phase II clinical trials. Typically, these are the same compounds that are in clinical trials for Alzheimer's disease and/or schizophrenia. On the basis of preclinical data, α4β2 ligands in particular may be expected to be efficacious[250]. Three compounds are undergoing Phase II clinical trials for ADHD — namely, ABT-089, an α4β2 partial agonist from Abbott[251]; ABT-894, a subtype-selective agonist of undisclosed specificity, also from Abbott; and lobeline, an α4β2 and α7 partial agonist and α4β4 full agonist (which also acts also on vesicular monoamine transporter 2 (REF. 252)) that is under investigation by Yaupon Therapeutics.

Conclusions and future directions

This Review has attempted to begin to bridge the gap between the two rapidly progressing fields in nicotinic receptor research: the knowledge on the atomic structure, functional organization and conformational transitions of the nAChRs, and the development of nicotinic agents as novel therapeutics for nervous-system disorders by the pharmaceutical industry. A clear conclusion that may be drawn from this Review is that stronger interactions between the two fields should be established for the benefit of drug design. Receptor subunit diversity beyond the standard α4β2–α7 dichotomy should be further considered and additional subunit interfaces should be explored, particularly *in silico* following the emerging knowledge of the atomic three-dimensional structure of the ACh-binding site, the ion channel and the multiple allosteric sites, which vary with subunit diversity. Another important conclusion is that drug design should take into account the fact that targets are not fixed structural entities but are able to undergo discrete allosteric transitions. Such transitions are beginning to be understood at the atomic level, for interconvertible states of a receptor that have different conformations and even strikingly different specificities with respect to agonists and antagonists, channel blockers and allosteric modulators. These states are posited to correspond to the known open and closed receptor states, but also the desensitized and upregulated states, which were beyond the scope of this Review but for which pharmacological specificity is expected to differ from the active and resting states[8].

It is anticipated that these ongoing developments in chemical pharmacology will be of considerable help in the exploration of new sites as well as novel conformational transitions and dynamics of various classes of nAChRs. Such studies hold promise for the rational design of drugs based on the atomic structure of their receptors.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CHRNA3 | *CHRNA5* | *CHRNB4*
OMIM:
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Alzheimer's disease | attention deficit hyperactivity disorder | depression | schizophrenia

FURTHER INFORMATION

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