Published in final edited form as:
Front Biosci. ; 13: 5479–5510.

Structural answers and persistent questions about how nicotinic receptors work

Gregg B. Wells

Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center, College Station, TX 77843-1114 USA

Abstract

The electron diffraction structure of nicotinic acetylcholine receptor (nAChR) from *Torpedo marmorata* and the X-ray crystallographic structure of acetylcholine binding protein (AChBP) are providing new answers to persistent questions about how nAChRs function as biophysical machines and as participants in cellular and systems physiology. New high-resolution information about nAChR structures might come from advances in crystallography and NMR, from extracellular domain nAChRs as high fidelity models, and from prokaryotic nicotinoid proteins. At the level of biophysics, structures of different nAChRs with different pharmacological profiles and kinetics will help describe how agonists and antagonists bind to orthosteric binding sites, how allosteric modulators affect function by binding outside these sites, how nAChRs control ion flow, and how large cytoplasmic domains affect function. At the level of cellular and systems physiology, structures of nAChRs will help characterize interactions with other cellular components, including lipids and trafficking and signaling proteins, and contribute to understanding the roles of nAChRs in addiction, neurodegeneration, and mental illness. Understanding nAChRs at an atomic level will be important for designing interventions for these pathologies.

Keywords

Acetylcholine; Addiction; Neurodegeneration; Nicotine; Protein Design; Protein Folding; Protein Structure; Cys-Loop Receptors; Review

2. INTRODUCTION

Nicotinic acetylcholine receptors (nAChRs) are integral-membrane, neurotransmitter-activated ion channels in the central and peripheral nervous systems that participate in signal transmission associated with the physiological release of acetylcholine (1). As cation-selective ion channels, they depolarize transmembrane voltage when they transition from the closed resting state to the open cation-conducting state by binding agonist. Continued exposure to agonist causes transition from the open state to the closed desensitized state. More than four decades of research have answered many structural questions about nAChRs. Reviews published across these decades reveal the progression of questions and answers (2–15). Current understanding of the structural basis of how nAChRs work is relatively advanced compared to other areas of ion channel biophysics (16–27). Despite this level of understanding, however, questions persist, not only about biophysical properties but also about the structural basis of how nAChRs affect cellular and systems physiology (28).

Send correspondence to: Gregg B. Wells, M.D., Ph.D., Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, 440 Reynolds Medical Building, 1114 TAMU, College Station, TX 77843-1114, USA. Tel: 979-458-8888, Fax: 979-847-9481, E-mail: gbwells@tamu.edu.

By reviewing research literature published primarily between 2003 and mid-2007, the goals of this review are to describe the current state of understanding of the structures of nAChRs and to describe questions for which atomic-level structural answers still await.

3. WHICH STRUCTURAL FEATURES DEFINE NACHRS AND OTHER CYS-LOOP RECEPTORS?

Mammalian genomes contain 16 nAChR subunits ($\alpha 1$ – $\alpha 7$, $\alpha 9$, $\alpha 10$, $\beta 1$ – $\beta 4$, δ , γ , ϵ ; $\alpha 8$ is found only in chicken). Primary sequence homology and the consensus sequence of the Cys loop (C-x-[LIVMFQ]-x-[LIVMF]-x(2)-[FY]-P-x-D-x(3)-C from reference (29)) are important unifying properties of the Cys-loop superfamily and its component families. In addition, an N-terminal extracellular domain containing the agonist binding site, four transmembrane domains (M1–M4), and an intracellular large cytoplasmic loop between M3 and M4 are unifying topological features. For nAChRs, α subunits contain extracellular tandem cysteine residues near the start of M1. Subunits assemble into pentamers to form homomeric or heteromeric ligand gated ion channels. The nAChRs are selective for cations, typically Na^+ and K^+ with varying levels of relative permeability for Ca^{2+} . For example, the Ca:Na permeability ratio is about 10 for rat $\alpha 7$ or $\alpha 7$ -containing nAChRs (30, 31) and about 0.2 for muscle-type nAChR from frog (*Rana pipiens*) (32).

The presence of two cysteines with a disulfide bond and separated by 13 amino acid residues with various degrees of conservation has been used as a defining structural characteristic for nAChR subunits from eukaryotes (33, 34) and, more generally, for Cys-loop receptor subunits (20, 22, 26). The Cys-loop receptor and subunit superfamily includes nAChRs and ionotropic 5-HT₃ receptors (5-HT₃R) (35, 36) (cation selective), ionotropic GABA receptors (37, 38) and glycine receptors (39–41) (anion selective), zinc-activated channels (42, 43), glutamate-gated GluCl chloride channels (44, 45), and serotonin-gated chloride channels (46, 47).

Using the Cys loop as a defining feature of Cys-loop proteins, however, might be overly restrictive for identifying proteins sharing overall structural homology. For example, requiring a Cys loop with 13 amino acid residues between cysteines as a defining feature excludes the acetylcholine binding protein (only 12 residues between cysteines in the Cys loop) (48, 49). Moreover, nicotinoid prokaryotic proteins contain no Cys loop (50, 51), although they are or are likely to be structurally similar to Cys-loop subunits.

The diversity of Cys-loop subunits and potential subunits across vertebrate, invertebrate (52–57), and prokaryotic genomes raises questions about which structural features are common to all Cys-loop subunits and which structural and functional features distinguish subgroups and individual receptors. Comparing structural (primary through quaternary structure) and functional properties can suggest which novel proteins belong to the Cys-loop superfamily regardless of the presence of a true Cys loop. Presently, comparing primary sequences probably is the best starting point for evaluating membership in the Cys-loop receptor family (Figure 1). Structural genomics of membrane and nonmembrane proteins, however, will provide means to identify candidates for membership in the Cys-loop superfamily based on higher order (secondary through quaternary) structural similarities even when primary sequence homology is insignificant (58–61).

4. WHAT IS KNOWN ABOUT THE STRUCTURE OF NACHRS?

The highest resolution structural information about nAChRs has come from electron diffraction of helical tubular crystals of nAChRs from *Torpedo marmorata* (4 Å resolution) and from X-ray crystallography of acetylcholine binding protein (AChBP) (about 2 Å

resolution). Extending the *Torpedo* nAChR structure to higher resolution with electron diffraction is constrained by disorder of the tubular crystals. Understanding nAChRs based on properties of AChBP is constrained by the absence in AChBP of a transmembrane domain and the absence of ion channel function. Determining atomic-resolution structures of nAChR subtypes beyond *Torpedo* is an important goal for the future. Structural comparisons among different subtypes will yield insights about distinctive patterns of ligand binding, ion channel behavior, and roles in physiology. This information also will guide the design of subtype specific drugs. X-ray crystallography is a promising approach but presently is hampered by the difficulty of obtaining large amounts of nAChRs for crystallization trials and the difficulty of crystallizing integral membrane proteins. NMR can produce information about both protein structure and dynamics but presently is limited by the maximum size of suitable proteins (about 100 kDa).

Model systems can continue to yield important information about structures of nAChRs. Extracellular domain nAChRs, which are high fidelity, pentameric models of full length nAChRs, might be more successful for crystallography if they can be crystallized without detergent and more successful for NMR because of their smaller size. Ion channel homologues from bacteria recently were recognized and might be easier to produce in large quantity for crystallography and NMR.

Static structures provide incomplete pictures of nAChRs. Intramolecular dynamics is essential for starting and stopping ion flow through nAChRs and might be important for intramembranous and cytoplasmic interactions between nAChRs and other proteins.

Understanding such dynamics will come not only from physical methods but also from computational methods like molecular dynamics simulations. The challenge for the future is to integrate structure and dynamics to explain how nAChRs work.

4.1. Electron microscopy and electron diffraction

Electron microscopy has contributed much information about the overall shape and internal structure of nAChRs (62–76). The most comprehensive and highest resolution structures have come from electron diffraction studies of muscle-type nAChRs in helical tubular crystals grown from membrane vesicles of *Torpedo marmorata*, an electric fish (77–88). The *Torpedo* nAChR structure at 4 Å resolution in the resting state is the best structure to date (Figure 2) and often is the foundation for homology modeling of other nAChRs (89, 90).

In this structure, the N-terminal extracellular domain of each subunit contains ten β -strands and one major α -helix, similar to the structure of the acetylcholine binding protein (AChBP) (Figure 3). Based on the AChBP structure, the subunit order in muscle-type receptors based on the locations of the principal and complementary faces of the binding sites is anticlockwise (α 1) γ (α 1) δ (β 1) when viewed from the extracellular compartment (Figure 2). Adjacent subunits interact through contacts in the N-terminal extracellular domains (mainly polar contacts), α -helical transmembrane domains (mainly hydrophobic contacts), and loops between transmembrane domains and the amphipathic MA helix (91) (containing both hydrophobic and hydrophilic properties) in the large cytoplasmic loop just before M4. The Cys loop and the β 1– β 2 loop of the N-terminal extracellular domain of a subunit interact with the short M2–M3 loop of the transmembrane domain. This interaction across domains might help open the channel in the agonist-bound conformation. In addition, the conformation of the two α 1 subunits is different from the conformation of the β 1, γ , and δ subunits. A change in the conformation of the α 1 subunits to match the conformation of β 1, γ , and δ subunits might be a feature of the gating mechanism that opens the pore. AChBP with a HEPES buffer molecule at the binding site (48) and the open channel of the *Torpedo* receptor after rapid spray-freezing with ACh (85) led to a model of structural transitions

from the closed to open state (90). A rearrangement within an $\alpha 1$ subunit potentially is associated with ligand binding and gating and closes its B and C loops onto the ligand binding site. Comparing the closed and open structures suggests that rotational movements within subunits contribute to channel opening. The extracellular and cytoplasmic vestibules (central spaces ringed by the subunits on the extracellular side and cytoplasmic side, respectively, of the transmembrane domain) of the *Torpedo* receptor are strongly electronegative, which likely contributes to cation selectivity of the open channel. Narrow windows in the walls of the cytoplasmic vestibule also appear to contribute to selectivity for ion charge and size and to regulation of the amount of current allowed through the pore. This structure combined with AChBP structures form the foundation for homology modeling of other nAChRs and modeling Cys-loop receptors, in general.

4.2. X-ray crystallography and X-ray diffraction

Although the supply of muscle-type nAChRs from natural sources is abundant, crystallizing nAChRs and most other integral membrane proteins is more difficult than crystallizing water-soluble proteins because of the presence of transmembrane domains (92, 93). X-ray diffraction of membranes from *Torpedo californica* electric organ provided estimates of the dimensions of the nAChRs (66). More recently, three-dimensional microcrystals of detergent-solubilized nAChRs from *Torpedo marmorata* and bound with α -bungarotoxin were grown in a lipidic matrix (94), a technique that has successfully crystallized other membrane proteins (95–97). These crystals can serve as seeds for growing larger crystals. In contrast to muscle-type nAChRs, obtaining sufficient quantities of native neuronal nAChRs for crystallization trials is considerably more difficult. Heterologous expression systems operated at laboratory scale produce only microgram quantities of nAChRs under favorable circumstances. Hundreds of milligrams of protein often are needed for successful crystallization trials, although nanotechnology techniques applied to protein crystallography might considerably reduce that amount of protein (98). Heterologous expression, however, probably is essential if site-specific mutations are to be studied in muscle-type or neuronal nAChRs. Such mutations, impossible to obtain from natural sources of nAChRs, likely will be useful to facilitate crystallization or test hypotheses about nAChR function.

The crystallographic structure at 1.94 Å of the extracellular domain of the mouse $\alpha 1$ subunit bound to α -bungarotoxin provided the first X-ray crystallography of a mammalian nAChR subunit and demonstrated advantages of recombinant production of nAChRs for crystallography (99). Important findings include structures of the main immunogenic region (MIR; an important target of autoantibodies in myasthenia gravis), the Cys loop, and the carbohydrate chain interacting with α -bungarotoxin. A hydrophilic domain with a water molecule forming a pocket within the subunit contrasted with a hydrophobic domain within the acetylcholine binding protein (see next section). This difference might be important for ion channel function of $\alpha 1$ in pentameric nAChRs.

4.3. AChBP structural studies

High resolution X-ray crystallographic information about the structure of nAChRs started to unfold in 2001 from an unexpected source: acetylcholine binding protein (AChBP) (100–104). Lacking transmembrane domains, this pentameric water-soluble protein was identified during a study of the effects of glia on synaptic transmission in the freshwater mollusk *Lymnaea stagnalis* (49). Its primary sequence homology with Cys-loop receptor subunits, formation of homomeric pentamers, and binding of nicotinic ligands in a pattern similar to $\alpha 7$ nAChRs supported its use as a water-soluble structural model for the N-terminal extracellular domain of nAChRs (48, 105). Each subunit contains an N-terminal α -helix, ten β -strands forming a β sandwich and an immunoglobulin fold (106), and two 3_{10} helices (107) (Figure 4). Ligand binding sites (occupied by HEPES in the crystal) are formed

between adjacent subunits by loops from one subunit (the principal face or plus side; Figure 4) and β -strands from the other subunit (complementary face or minus side; Figure 4). Loops from the plus side and β -strands and loop $\beta 8$ – $\beta 9$ from the minus side form interfaces between subunits. The Cys loop contains 14 residues instead of the usual 15 residues (48). The location of the Cys loop suggests a potential role for interaction between the Cys loop and transmembrane domains in nAChRs that might contribute to gating of the transmembrane domain. Residues of the subunit interfaces are not well conserved in the Cys-loop superfamily, a feature that might contribute to the selective association between subunits when forming pentamers.

Crystal structures of nicotinic ligands instead of HEPES bound to AChBP have provided more detail about the ligand binding site. The crystal structures of nicotine and carbamylcholine bound to AChBP suggest how nicotinic agonists bind to nAChRs. They also help explain the results of previously reported biochemical and electrophysiological experiments that probed the interactions of specific side chains with ligands (108, 109). These structures answered a long-standing question about the negatively charged site that interacted with the positive charge at the tertiary nitrogen of nicotine and quaternary nitrogen of carbamylcholine (110–112). The negatively charged site is formed by interaction with π electrons from the aromatic side chains of Trp143, Tyr192, Tyr185, and Trp53 and the backbone carbonyl of Trp143. Compared to the HEPES-bound structure, loop C of the principal binding site contracted around each ligand. Structures of AChBP with peptides that competitively inhibit nAChRs might resemble the resting state of nAChRs. AChBP with α -cobratoxin (113) showed movement of loop C away from the binding site compared to the AChBP structures with HEPES, nicotine, or carbamylcholine. Conformational changes of loop C might be important distinguishing features of resting, open, and desensitized states of nAChRs and might be an important target for designing drugs to affect different nAChR states.

The structures of AChBPs from different species and with different pharmacological properties have been the foundation for proposals about how nAChRs differ in pharmacology and how the channel gates. The second example of an AChBP was obtained from *Aplysia californica*, a saltwater mollusk (114). Comparison of the structures of the *Aplysia* AChBP without ligand, with nicotinic agonists (+)-epibatidine and lobeline, and with nicotinic antagonists α -conotoxin ImI and methyllycaconitine showed binding of the two agonists was associated with closing of loop C. In contrast, binding of the two antagonists was associated with an extended or open loop C (115). These results support the participation of loop C in the differing effects of agonists compared to competitive antagonists. The α -conotoxin PnIA (A10L D14K) (116) binds nonselectively to AChBP homologs from different mollusks. By comparison, α -conotoxin ImI (117) binds selectively to *Aplysia* AChBP and to $\alpha 7$ and $\alpha 3\beta 2$ nAChRs. The difference in structures and binding properties of these two conotoxins with AChBP homologs suggested protein contacts that might be important for ligands that are selective for specific nAChR subtypes. Galantamine and cocaine, positive allosteric modulators of nAChRs, bound deeply into subunit interfaces of *Aplysia* AChBP in the crystal structure without contacting the tip of loop C (118). These structures from homomeric *Aplysia* AChBP suggested these positive allosteric modulators bind at non- α subunit interfaces in heteromeric neuronal nAChRs. The third AChBP was obtained from *Bulinus truncatus*, a freshwater mollusk, and was crystallized with a CAPS buffer molecule in four of the five ligand binding sites (119). The backbone structures of AChBPs from *B. truncatus*, *L. stagnalis*, and *A. californica* were similar although pairwise comparisons show only 20% to 40% sequence identity. Differences in affinity for nicotinic ligands by the AChBP from *B. truncatus* compared to AChBP from *L. stagnalis* arose primarily from differences at three homologous positions in the primary sequences (119).

4.4. AChBP with methods other than X-ray crystallography

Biophysical methods other than X-ray crystallography have been applied to AChBP to understand energetics and dynamics of ligand binding and channel gating of nAChRs. The large amount of properly folded and water-soluble AChBP that can be heterologously expressed makes applying these methods to AChBP often easier than applying the methods to native or heterologously expressed neuronal nAChRs in the presence of membranes or detergents. Changes of intrinsic tryptophan fluorescence (five Trp residues on each subunit) were associated with ligand binding, making fluorescence spectroscopy a valuable tool for probing kinetics and structural changes of ligand binding (120). Fluorescent labels attached to AChBP through native and nonnative cysteine residues signaled the environments around the cysteines and the dynamics of distinctive changes in those environments caused by binding of different ligands (121, 122). Frictional coefficients and fluorescence anisotropy decay that measured hydrodynamic properties of the AChBP:α-bungarotoxin complex in the presence of bound and specifically labeled α-bungarotoxin suggested that this ligand was not rigidly bound but retained segmental flexibility (123). Absorption and fluorescence spectroscopy of benzylidene-ring substituted anabaseines bound to AChBPs from *L. stagnalis*, *A. californica*, and *B. truncatus* showed the extent of proton dissociation and the reduced flexibility of the bound ligand compared to free ligand (124). Molecular dynamics simulations and intrinsic tryptophan fluorescence in solution suggested changes in relative tryptophan conformations on loops C and D on adjacent subunits when ACh binds. These studies also identified closing of loop C over the opening to the binding site as another potentially important change with ligand binding. These effects of binding acetylcholine might be important for channel gating (125). Molecular dynamics and ligand docking simulations of AChBP and either *α*-tubocurarine or metocurine suggested two different orientations for these curare derivatives at the ligand binding site. This conclusion, supported by binding measurements with binding site mutations, indicated that apparently minor differences in ligand structure could significantly alter binding interactions between ligand and AChBP (126). Hydrogen-deuterium exchange mass spectrometry (127) and solution NMR (128) provided evidence that binding agonists induced conformation changes in loop C and loop F at the binding site that were different from the conformation changes induced by antagonists. Molecular dynamics modeling of AChBP with bound nicotine and carbamylcholine potentially augments conclusions from X-ray crystallographic structures and suggested that water molecules are present at discrete locations in the binding site and may participate in ligand binding (129).

4.5. Homology modeling of nAChRs based on AChBP and *Torpedo* nAChR

A frequently applied method to link findings about the known structure and dynamics of AChBP to undetermined structural and dynamic features of neuronal nAChRs is homology modeling (130). This method starts with alignment of primary sequences of AChBP and nAChR and then develops structural models of pentameric nAChR receptors based on structures of AChBP and nAChR from *Torpedo*. Models of nAChR subtypes α7 (131–135), α4β2 (131, 132), muscle-type (α1)₂(β1)γδ (131, 135), α4β4 (132), and α3β2 (132) have been reported. Homology models of nAChRs help generate hypotheses about ligand binding (131–133, 135), channel gating (134), and ion conduction (134) that can be tested experimentally and that guide interpretations of experiments. Homology models based on AChBP and *Torpedo* nAChR also have been developed for GABA_A (136, 137), GABA_C (138), 5-HT₃ (139, 140), and glycine (141–144) members of the Cys-loop receptor superfamily.

4.6. NMR studies

Solution NMR as a method of determining protein structure has the advantages that crystals are not needed and protein structure and dynamics can be determined under a range of

conditions, including physiological conditions. A major obstacle for applying solution NMR to neuronal nAChRs is the limitation of about 100 kDa in the molecular mass suitable for solution NMR (145). Other obstacles are the relatively large amount of protein needed (in the range of milligrams) and the need for ^{13}C and ^{15}N isotopic labeling of amino acids in a heterologous expression system. Peptide models of the primary face of ligand binding site of $\alpha 1$ subunits (146–149) and $\alpha 7$ subunits (150) have overcome these obstacles. Studying transmembrane domains in micelles instead of an entire nAChR also overcomes these obstacles while providing information relevant to the ion conduction path (151–153). The extracellular domain of $\alpha 1$ combines native-like structure and a size small enough (31 kDa) to be compatible with solution NMR (154).

Advances in protein NMR techniques in areas of isotopic labeling (155) and analysis of large (e.g., 900 kDa) proteins (156–159) promise to make solution NMR in the future more relevant to full length nAChRs. Solid-state NMR overcomes some of the size limitations of solution NMR and can be applied to nAChRs in their native membrane environment, which potentially maintains structural and dynamic features that depend on that environment (160). A solid-state NMR study of isotopically labeled antagonist neurotoxin II bound to *Torpedo* nAChR showed the toxin structure likely is similar in the free and bound state with the exception of an isoleucine (161). A study with solid-state NMR of isolated M1 peptides suggested strong protein-lipid interaction involving M1 from $\alpha 1$ in model membranes (162).

4.7. Extracellular domain nAChRs

The large N-terminal extracellular domain of nAChRs is an attractive candidate for structural studies. Conceptually similar to the AChBP, extracellular domains of nAChR subunits might form pentameric, water-soluble, high fidelity models for the extracellular domain of full length nAChRs. The size of extracellular domain nAChRs (120–150 kDa), although still beyond current NMR technology, is more compatible for NMR than is the size of full length nAChRs. Their potential for water solubility without detergents makes them better candidates than full length nAChRs for crystallization and biophysical methods intolerant of detergents or membranes.

The feasibility of extracellular domain nAChRs was supported by studies with muscle-type subunits (163–167), $\alpha 7$ subunits (168–171), and $\alpha 4$ and $\beta 2$ subunits (172). Extracellular domain muscle-type subunits without any transmembrane domains formed pentamers detected by electron microscopy (165). Extracellular domain $\alpha 7$ and $\alpha 4\beta 2$ nAChRs likely were pentamers and showed affinities for small and large ligands equal to the ligand affinities of the respective full length nAChRs (169, 172). Retaining M1 on the extracellular domain, however, was required for efficient expression of extracellular domain $\alpha 7$ and $\alpha 4\beta 2$ nAChRs. Based on this observation, preparing large amounts of water-soluble extracellular domain nAChRs might require a strategy that includes *in vitro* removal of M1 from extracellular domain nAChRs. The feasibility of such a strategy was supported by inserting a specific proteolysis site in extracellular domain $\alpha 7$ subunits between the extracellular domain and the N-terminus of M1 (169). This extracellular domain $\alpha 7$ subunit with M1 and a specific proteolysis site outside M1 formed pentamers and had ligand affinities equal to the ligand affinities of full length $\alpha 7$ nAChRs. Concatamers of extracellular domains with or without a C-terminal M1 domain offer another potential approach to water-soluble extracellular domain nAChRs that has been successful with full length $\alpha 4$ and $\beta 2$ subunits (173) and other Cys-loop receptors (174).

4.8. nAChRs and unnatural amino acids

Incorporating unnatural amino acids into ion channels as they are synthesized *in vivo* provides a powerful method beyond the spectrum of twenty natural amino acids to test

hypotheses about structure and function of nAChRs (175–178). Unnatural amino acids include side chains with chemical properties beyond properties of natural amino acids such as fluorescence and photochemical reactivity. Unnatural amino acids first were incorporated into muscle-type nAChRs expressed in *Xenopus* oocytes (179). Carboxyl, amino, and fluoro derivatives of Tyr190, Tyr198, and Phe198 at the agonist binding site changed the dose-response behavior for acetylcholine and the inhibition constant for *d*-tubocurarine. The advantages of fine-tuning chemical properties through unnatural amino acids was evident in a study of the role of hydrogen bonds involving Asp89 near the binding site in the mouse embryonic muscle-type nAChR (180). Substitution of nitro or keto groups in place of the carboxyl of aspartate suggested that Asp89 contributes not only hydrogen bonds and electrostatic charge (181) but also a favorable arrangement of dipoles. Fine-tuning of side chain properties at sites of tyrosine residues with tyrosine derivatives (e.g., fluoro, bromo, *p*-methyl, *p*-methoxy, and *m*-hydroxy derivatives) in 5-HT₃ receptors helped develop a model combining ligand binding and channel gating based on rearrangements of hydrogen bonds (182). Structure of the peptide backbone also can be altered by incorporating components beyond the natural amino acids. Replacing amino acids in M1 and M2 with α -hydroxy acids changed specific bonds in the backbone from peptide to ester bonds (183). The changes in electrophysiological behavior associated with these changes suggested that backbone hydrogen bonds in M1 and backbone conformation of M2 contribute to gating. Frameshift suppression (184) promises to open a route to incorporating *in vivo* multiple, different unnatural amino acids in a nAChR subunit (185). Reassigning sense codons to unnatural amino acids instead of depending on nonsense codons to introduce unnatural amino acids into proteins is an alternate approach that might lead to multiple different unnatural amino acids in proteins (186).

5. HOW DO LIGANDS BIND TO NACHRS?

The general structure of the agonist binding site, also called the orthosteric binding site (in contrast to an allosteric binding site), in nAChRs has been outlined by homology with AChBP (187–190). General principles, however, do not explain the diversity of ligand interactions and functional behaviors observed with the diversity of subtypes of nAChRs. Because the extent of structural homology between AChBP and specific types of nAChRs is uncertain at the atomic level, the structural information from AChBP is a starting point and not the final answer for questions about how nAChRs work. These questions will continue to be answered by combining biochemical, electrophysiological, pharmacological, and structural methods to nAChRs. Some of the questions are: Why does a given agonist show different affinities and different potencies for different nAChRs? Why do antagonists show different affinities for different nAChRs? Is a unique set of interactions between agonist and nAChR required for opening the channel? Which structural and chemical properties are required in a nicotinic agonist? How do competitive antagonists bind tightly to nAChRs and keep the channel from opening? How can a specific type of nAChR be maintained pharmacologically in its closed, opened, or desensitized states without affecting other types of nAChRs? How does the binding of small molecules at sites on nAChRs other than the agonist binding site affect function of nAChRs? Such sites are called allosteric binding sites.

This section concentrates on answers and persistent questions associated with ligand binding at the orthosteric binding site and at allosteric binding sites and with distant effects associated with ligand binding, namely, channel gating. The next section will consider in more detail how nAChRs gate.

5.1. Muscle nAChRs

Recent studies about how nicotinic ligands bind to muscle-type nAChRs have been based on curare derivatives, nicotine, epibatidine, choline derivatives, and trimethylammonium

derivatives. A ligand binding study of interactions between functional groups of *d*-tubocurarine and the γ subunit of fetal mouse muscle (($\alpha 1$) $_2\beta\gamma\delta$) nAChRs was interpreted with single model of *d*-tubocurarine in the ligand binding site (191). By comparison, modeling of ligand binding focused on the ϵ subunit of adult human muscle (($\alpha 1$) $_2\beta\epsilon\delta$) nAChRs produced a different orientation of *d*-tubocurarine in the ligand binding site and showed that methylation of *d*-tubocurarine could dramatically change predicted interactions between ligand and binding site (192, 189). Descriptions of ligand–nAChR interactions might need to consider differences arising from seemingly minor changes in ligand structure and in subtype and species of nAChRs.

The wide range of chemical modifications possible with unnatural amino acids and electrophysiological measurements indicated a potential range of interactions available to agonists at the ligand binding site of fetal muscle (($\alpha 1$) $_2\beta\gamma\delta$) nAChRs (193). Cation- π interaction with Trp $\alpha 149$ was important for ACh binding, hydrogen bonding to a backbone carbonyl was important for nicotine binding, and both cation- π interaction and hydrogen bonding to the backbone were important for epibatidine binding. Additional interactions between epibatidine and the desensitized ligand binding site were suggested from ligand binding to chimeras between γ and δ subunits (the binding site at the $\alpha\gamma$ interface binds epibatidine more tightly than does the site at the $\alpha\delta$ interface) (194). Photochemical labeling of *Torpedo* nAChRs (195, 196) with a benzoylcholine partial agonist might lead to understanding the basis for low efficacy of partial agonists and the development of photochemically-active full agonists with well-defined photoreactive intermediates (197).

Long, bisquaternary dicholine agonists like suberyldicholine (18.7 Å between quaternary nitrogens) appeared to bind to multiple sites on muscle-type nAChRs, suggesting that the extent of an agonist binding site depends on the agonist (198, 199). For the simpler alkyltrimethylammonium functional group of choline, length requirements of alkylthiol derivatives of the minimalist agonist tetramethylammonium tethered to cysteines supported the sufficiency of the single agonist binding site formed by aromatic side chains in the AChBP structure (200). This interpretation presumed that the AChBP structure described the open conformation of nAChRs. Identifying residues from muscle-type nAChRs potentially interacting with peptide inhibitors waglerin-1 (201), α -cobratoxin (202), a short-chain α -neurotoxin from *Naja oxiana* (203), and a short-chain α -neurotoxin from *Naja nigricollis* (204) was aided by comparisons with AChBP or *Torpedo* nAChR structures. Focusing on the dynamics of bound α -cobratoxin, the C-terminal domain of fluorescein derivatives of the antagonist appeared to be mobile and not bound to *Torpedo* nAChR by time-resolved fluorescence anisotropy. This result suggested that only the N-terminus interacts with the nAChR surface (205).

Besides structural descriptions of ligand binding, dissociation constants of low affinity agonists (206) and electrostatic effects important for ligand binding to *Torpedo* nAChRs (207) provided thermodynamic and energetic descriptions of ligand binding to muscle-type nAChRs. A model combining acetylcholine diffusion and binding, protein structure, reaction kinetics, and synaptic geometry at the neuromuscular junction incorporated the complex three-dimensional physiological environment for nAChRs (208). Continued development of such unifying models will help interpret and reveal physiological roles for atomic level details of nAChR structure and dynamics.

5.2. Neuronal nAChRs

Understanding ligand binding to $\alpha 4\beta 2$ and $\alpha 7$ -containing nAChRs is important because of the prominence of these subtypes in the human brain. Results from computer simulated docking and molecular modeling of nicotinic ligands suggested structure-activity relationships to explain how nicotine and deschloroepibatidine bind to $\alpha 4\beta 2$ nAChR (209).

Similar methods were applied to a variety of nicotinic ligands that distinguish between $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs (210, 211). Understanding binding to $\alpha 4\beta 2$ nAChRs is complicated by the existence of multiphasic binding or dose-response curves suggesting receptor populations with different relative $\alpha 4:\beta 2$ stoichiometries (212, 213, 173, 214, 215). In contrast to the multiphasic concentration-response curves of ACh, nicotine, and cytosine with $\alpha 4\beta 2$ nAChRs, binding of TC-02559 was monophasic (216). Similar to muscle-type nAChRs, comparing ligand binding to $\alpha 4\beta 2$ and $\alpha 7$ -containing nAChRs suggested the importance of subunit-specific local interactions at the binding site (217) as well as long-range electrostatic interactions (218). Residues of α -bungarotoxin, a long α -neurotoxin, that interact with $\alpha 7$ nAChRs are identical to or overlapping with the toxin residues that interact with muscle-type nAChRs (219). Chimeras of short α -neurotoxins, long α -neurotoxins, and κ -neurotoxins have helped identify regions of these toxins important for interaction with $\alpha 7$ nAChRs and for selectivity of κ -neurotoxins with $\alpha 3\beta 2$ nAChRs (220). The agonist properties of antihelminthic drug pyrantel with $\alpha 7$ nAChRs depend on interaction with Gln57 of the complementary face (221). Molecular dynamics simulations of the extracellular domain of $\alpha 7$ nAChRs have allowed more global assessment of predicted structure and dynamics induced by ligand binding (222, 223). High resolution X-ray crystal structures and physicochemical characterization of nicotinic ligands are important components of molecular dynamics and docking computations (224).

Understanding ligand binding to $\alpha 4\beta 2$ and $\alpha 7$ -containing nAChRs also helps develop subtype-specific drugs and, more narrowly, state-specific (e.g., the open state) drugs for a given subtype (225–233). Epibatidine has been the starting point for synthesizing derivatives with high affinity for $\alpha 4\beta 2$ nAChRs and low affinity for $\alpha 7$ -containing nAChRs (234–236) or low affinity for other $\beta 2$ -containing or $\beta 4$ -containing nAChRs (237–240). Epibatidine also has been the starting point for fluorescent derivatives that potentially will be useful for high-throughput screening and single-molecule analysis of binding (241). Derivatives of pyridine (242, 243); piperazines, diazepam, diazocanes, diazabicyclononanes, diazabicyclodecanes (244); and cytosine (245) also have shown promise for selectivity for $\alpha 4\beta 2$ nAChRs. For finer discrimination of subunit composition, a piperidine derivative selectively and noncompetitively blocked $\alpha 3$ - and $\alpha 4$ -containing nAChRs when only $\beta 2$ or $\beta 4$ subunits also were present. The presence of additional nonessential subunits $\alpha 5$, $\alpha 6$, or $\beta 3$, however, decreased inhibition by this blocker (246). Pyridine derivatives are the basis for $\alpha 4\beta 2$ -selective radioligands for detection by positron emission tomography (PET) (247). Drugs with high affinity for $\alpha 7$ -containing nAChRs (248, 249) and with minimal cross-reactivity with 5-HT₃ receptors (250) are targets for medicinal chemistry that might become therapies for Alzheimer disease and schizophrenia. In other contexts, insecticides for example, low affinity for human nAChRs is the goal. Understanding the structural basis of binding by the neonicotinoids class of compounds (e.g., imidacloprid and thiacloprid) specifically to insect nAChRs will lead to better insecticides with lower human toxicity (251, 252).

Conotoxins are an especially rich source of tools for achieving and analyzing subtype-specific interactions (253–259). Marine snails of the *Conus* genus produce these peptide toxins in their venom that target ligand-gated and voltage-gated ion channels. Members of the family of α - and αA -conotoxins contain multiple disulfide bonds and are competitive antagonists for nAChRs. Many α - and αA -conotoxins are selective for muscle-type and/or $\alpha 3$ - and $\alpha 7$ -containing nAChRs. With an estimated 50 to 200 peptide toxins produced by each *Conus* species (260), many useful toxins for nAChR research have yet to be characterized. Recently identified conotoxins are specific for different types of nAChRs: muscle-type nAChRs (261–263), $\alpha 3\beta 4$ nAChRs (264), $\alpha 3\beta 2$ and $\alpha 7$ nAChRs (265), $\alpha 7$ -containing nAChRs (266), and $\alpha 9\alpha 10$ nAChRs (267). Conotoxin antagonism against $\alpha 9\alpha 10$ nAChRs showed both a role of $\alpha 9\alpha 10$ nAChRs in neuropathic pain and the potential

therapeutic role of conotoxins (268). With $\alpha 3$ -containing nAChRs, α -conotoxin BuIA kinetically distinguished $\beta 2$ subunits from $\beta 4$ subunits because of much slower off-rates with $\beta 4$ subunits (269). Understanding structural features of conotoxins and of conotoxin interactions with muscle-type (270), $\alpha 7$ (271), and $\alpha 3\beta 2$ (271, 272) nAChRs might lead to targeted modifications of conotoxins for enhanced subunit selectivity or novel activities. For example, a benzoylphenylalanine derivative of a α -conotoxin GI was designed as a photoaffinity label for *Torpedo* nAChRs (273).

5.3. Allosteric binding

Beyond the orthosteric binding sites of nAChRs are allosteric binding sites (274–277). The concept of allosteric binding sites that modulate channel activity arises from observations that some compounds affect channel function stimulated by agonists. These compounds themselves do not have agonist activity nor are they competitive inhibitors of agonist binding at the orthosteric binding site. The interpretation of these observations is that binding of allosteric modulators to allosteric binding sites on nAChRs modifies the function of nAChRs. These binding sites, in principle, can be within the ion pore or can be within the extracellular, cytoplasmic, or transmembrane domains. Positive allosteric modulators increase agonist-induced activity; negative allosteric modulators decrease such activity. Because allosteric binding sites are outside the orthosteric binding site, allosteric modulators extend beyond the constraints of the orthosteric site the opportunities for developing subunit and subtype specific modulation.

Defining allosteric binding sites and the activity of allosteric modulators is important for understanding nAChR function and for drug development. Galantamine, a positive allosteric modulator of human $\alpha 7$ and $\alpha 4\beta 2$ nAChRs important for treatment of Alzheimer disease (278–280), binds at the subunit interface in AChBP without interacting with loop C. This result suggests this drug binds at non- α subunit interfaces in heteromeric neuronal nAChRs (118). In contrast, this drug was a partial agonist without allosteric activity on mouse muscle nAChRs (281). It was not a competitive inhibitor of nicotinic agonists, so the binding site of its agonist activity likely is distinct from the binding site of the typical nicotinic agonists ACh and carbachol. The negative allosteric effects of kynurenic acid might attenuate the positive allosteric effects of galantamine on $\alpha 7$ -containing nAChRs (282). A collection of derivatives of methyllycaconitine with negative allosteric modulator activity on $\alpha 3\beta 4$ nAChRs produced quantitative structure-activity relationship and pharmacophore models useful for detecting other negative allosteric modulators (283). Derivatives of (2-amino-5-keto)thiazole were positive allosteric modulators of $\alpha 2\beta 4$, $\alpha 4\beta 2$, $\alpha 4\beta 4$, and $\alpha 7$ nAChRs but did not potentiate $(\alpha 1)_2(\beta 1)\gamma\delta$, $\alpha 3\beta 2$, or $\alpha 3\beta 4$ nAChRs (284). Desformylflustrabromine showed promise as a positive allosteric modulator of $\alpha 4\beta 2$ nAChRs (285, 286). Four positive allosteric modulators of $\alpha 7$ nAChRs showed two classes of effects, raising the possibility that combinations of modulators could produce additional options for therapeutic effects on $\alpha 7$ -containing nAChRs *in vivo* (287). Crystal violet, a negative allosteric modulator, bound at a site overlapping the phencyclidine binding region in the resting and desensitized states of *Torpedo californica* nAChRs (288). Docking computations can suggest allosteric binding sites on nAChRs as was tested with positive allosteric modulators galanthamine, codeine, and serine and with $\alpha 7$, $\alpha 3\beta 4$, and $\alpha 4\beta 2$ nAChRs (289). Neurosteroids had allosteric effects on nAChRs (290) and GABA_A receptors (291) in the transmembrane domain. Mutations leading to autosomal dominant nocturnal frontal lobe epilepsy reduced the positive allosteric effect of Ca²⁺ on $\alpha 4\beta 2$ nAChRs (292, 293). Zn²⁺ also modulated the function of neuronal nAChRs (294, 295). Residues in $\alpha 4$ at the interface with $\beta 2$ or $\beta 4$ subunits might mediate this modulation by Zn²⁺ (296). This proposed location of the Zn²⁺ allosteric site might be structurally and functionally analogous to the site for benzodiazepine binding on GABA_A receptors.

5.4. Effects of post-translational modifications

N-linked glycosylation of the extracellular domain affects nAChRs during subunit assembly into mature nAChRs and during transport to the cell surface. The importance of glycosylation for these processes was demonstrated for *Torpedo* nAChRs (297–301) and $\alpha 7$ nAChRs (302). Glycosylation also affected desensitization and conductance of *Torpedo* nAChRs (303). Partial deglycosylation of purified *Torpedo californica* nAChRs did not appear to affect structure, internal dynamics as measured by $^1\text{H}/^2\text{H}$ exchange, or ability to desensitize with agonist (304). In contrast, deglycosylation adversely affected binding of α -bungarotoxin to the extracellular domain of human $\alpha 1$ (166). Establishing the cellular machinery for adding mammalian patterns of glycosylation to nAChRs produced in high-level expression systems like yeast (305–307) and baculovirus (308, 309) might be important for efficiently producing large amounts of human nAChRs for structural studies. Nitrosylation of tyrosines is another post-translational modification potentially affecting nAChRs (310).

6. WHAT ARE THE FUNCTIONALLY IMPORTANT DYNAMICS OF NACHRS?

6.1. How does the channel open and close?

One of the most important actions induced by agonist binding to nAChRs is opening the channel (i.e., the transmembrane domains) thus permitting the flow of cations. How does the channel open? A second question involving structure and dynamics of desensitization is, How does the channel close? The first question is being answered in terms of residues, secondary structures, and dynamics of nAChRs. Rotational movements within $\alpha 1$ subunits of muscle-type nAChRs that serve to open the channel were suggested by electron microscopy of resting and open *Torpedo* nAChRs (85, 90). As another possibility, tilting of the pore-lining M2 segments was suggested as a mechanism for opening the channel of a homomeric $\alpha 7$ -5-HT_{3A} chimeric receptor. This mechanism was based on Zn²⁺ binding to metal ion binding sites created by substituting histidine residues into M2 (311). Molecular dynamics simulations (312) suggested both types of motions might be contributing to channel opening in $\alpha 7$ nAChRs (313–318). Transient photoreactive accessibility of residues in M1, M2, and the M2–M3 loop of the δ subunit of *Torpedo* during gating was evidence of gating-specific and possibly subunit-specific transmembrane domain motions (319). A computational analysis of *Torpedo* nAChR using elastic networks theory suggested that symmetric quaternary twisting and asymmetric tilting of M2 combine to open the channel and that loops A and F and the large cytoplasmic loop also affected channel gating (320).

Different regions and residues of nAChR subunits have been implicated in channel gating. Loops $\beta 1$ – $\beta 2$, $\beta 6$ – $\beta 7$, and $\beta 8$ – $\beta 9$ at the interface between the extracellular domain and transmembrane domain have attracted much attention as potential transducers of the opening signal from the agonist binding site to the transmembrane domain. The successful construction of a chimeric ion channel gated by ACh and containing the AChBP extracellularly and the 5-HT_{3A} receptor sequence starting at M1 demonstrated the importance of the loops $\beta 1$ – $\beta 2$, $\beta 6$ – $\beta 7$, and $\beta 8$ – $\beta 9$ (321). Residues in loop $\beta 6$ – $\beta 7$ (Cys loop) and the M2–M3 loop influenced fast opening (322). Conserved proline and serine residues of the M2–M3 loop were coupled to the agonist binding site through a conserved glutamate on loop $\beta 1$ – $\beta 2$ and a conserved arginine on the C-terminal end of the $\beta 10$ strand of the $\alpha 1$ subunit (323, 324). Unnatural amino acid substitutions supported the importance of the conserved proline residue in the M2–M3 loop for channel gating in mouse 5-HT_{3A} receptors (325, 326). Adjacent residues within the $\beta 2$ strand of the $\beta 4$ subunit affected the coupling of binding and gating for the species selective agonist 5-(trifluoromethyl)-6-(1-methyl-azepan-4-yl)methyl-1*H*-quinolin-2-one (TMAQ) (327). Electrostatic interactions among three residues ($\alpha K145$, $\alpha D200$, and $\alpha Y190$) in the human $\alpha 1$ subunit at the

periphery of the agonist binding site (328) and interaction of two residue pairs at the α - δ (α Y127 and δ N41) and α - ϵ (α Y127 and ϵ N39) subunit interfaces (329) in muscle nAChRs affected gating.

The link between the agonist binding site and channel gating might not be restricted to a few residues. An investigation of charged residues within loops β 1- β 2, β 6- β 7, and β 8- β 9 of muscle nAChRs and GABA_A and glycine receptors concluded the overall distribution of electrostatic interactions rather than interactions between specific ion pairs was important at the gating interface between the extracellular domain and transmembrane domain (330). Analysis of covariance of amino acid residues in the multiple primary sequence alignment of Cys-loop receptors suggested highly coupled positions form a three-dimensional network connecting the agonist binding site to the transmembrane domain and to channel gating (331).

In α 7 nAChRs, a network of electrostatic interactions involving residues in loops β 1- β 2 and β 6- β 7 and the M2-M3 linker linked the agonist binding site to gating of the channel (332). The inner and outer layers of β sheets in the β -sandwich (Figure 3) of the extracellular domain participated in coupling agonist binding and channel gating in α 7 nAChRs (333, 334). Accessibility of inner layer residues to cysteine modification suggested that motions in addition to rotational motion within the extracellular domain might be important in the gating of α 7 nAChRs. The dynamics of the transition between closed and open states of muscle-type nAChRs has been characterized by rate equilibrium linear free energy relationship (REFER) analysis. This method compares changes in the opening rate to changes in the equilibrium between closed and open states when a specific position is mutated to several different amino acid types (335). The REFER analysis is interpreted to measure to what extent a given position in the primary sequence is in an open-like state when the nAChR reaches its transition state between closed and open. Positions near the agonist binding site were in an open-like state; positions in the transmembrane domain were in a closed-like state. Positions located between the agonist binding site and the transmembrane domain in the nAChR in three-dimensional space were in states with a gradient of contributions of open and closed states (336, 337). This spatial variation of open state character in the transition state suggested that, during the transition to an open channel, parts of the nAChR near the agonist binding site move before parts near the transmembrane domain. The apparently asynchronous movement of different parts of the nAChR during opening suggested a broad potential energy surface without sharp features (338, 339). Investigations involving REFER analysis have focused on the extracellular domain (340); on M2 (341, 342), M3 (343), and M4 (344) domains; and on the theoretical underpinnings of the interpretation (345-347).

Structural answers to the second question, about how the channel closes, have come from investigating where ion conduction is blocked along M2 in the resting and desensitized states. A site of such blocking of ion conduction, called a gate, was identified in the resting state in the middle of M2 in GABA_A (348) and 5HT₃ (349) receptors by scanning cysteine accessibility mutagenesis (SCAM) (350, 351). A gate located near the cytoplasmic end of M2 was suggested for nAChRs by SCAM (352, 353). Additional understanding of how to interpret SCAM experiments might reconcile these gate locations for different Cys-loop receptors (348). Different gates might act in different structural states of the channel. For example, ion channel block by choline affected a gate for the resting state in nAChRs but did not affect a gate for the desensitized state, implying the gate of the resting state is different from the gate of the desensitized state (354).

6.2. Pore structure and dynamics: How do ions traverse the membrane through nAChRs?

Experimental and computational studies focusing on the transmembrane domains, especially M2, are contributing information about the structure, dynamics, and ion permeation of the pore region (355). Molecular and Brownian dynamics computations of the transmembrane domain of *Torpedo* nAChR suggested that a hydrophobic region of the channel prevents cation flow in the closed state. For the open state, small changes in channel structure led to large changes in conductance (356–358). Molecular dynamics of M2 domains with spatially fixed M1, M3, and M4 domains showed asymmetrical dynamics in M2 and bending motions at this central hydrophobic region of M2 that could correspond to the hydrophobic gate (359). A hydrophobic region of M2 also might be the gate for ion flow for $\alpha 4\beta 2$ nAChRs (360) and $\alpha 7$ nAChRs (361), based on profiles of pore radius and water density from molecular dynamics calculations of the transmembrane domains. A model of a pore composed solely of M2 peptides from the δ subunit of *Torpedo marmorata* (362, 363) or rat (364) agreed with results from solid-state NMR and other experimental methods and might represent the closed form of the pore. Lysine substitutions at a single homologous position within the central hydrophobic region of M2 of the four subunits of human muscle-type nAChRs caused differing effects on open channel conductance. This finding suggested that the subunits at this position experience substantially different microenvironments and are unequally affected by transmembrane voltage (365). Substitutions of lysines at all positions and histidine and arginine at some positions of M2 in the δ subunit of mouse muscle-type nAChRs detected individual proton binding events of single open channels (366). These substitutions also identified the positions of δ M2 facing into the pore in closed and open states. These results suggested that rotation of M2 during opening is minimal. A point mutation at a conserved leucine near the middle of M2 of $\alpha 3$ affected gating in $\alpha 3\beta 4$ and $\alpha 3\beta 2$ nAChRs (367). Rhodamine labeling of a Cys residue substituted into the extracellular end of M2 of $\beta 1$ demonstrated the feasibility of time-resolved fluorescence for investigating the dynamics of channel opening (368).

In addition to understanding ion flow through the transmembrane domain, understanding how channel blockers interact with the transmembrane domain and with nAChR dynamics is important. For example, such understanding could guide the development of new and more specific channel blockers. The noncompetitive channel blocker chlorpromazine interacted with several M2 residues of muscle-type nAChR according to molecular dynamics simulations with the whole nAChR embedded in a lipid bilayer (369). Moreover, chlorpromazine also inhibited conformational changes in the nAChR that might be important for transitions between resting, open, and desensitized states.

Although M2 lines the ion conduction path, the other transmembrane domains indirectly might affect nAChR gating and conductance even though they do not directly interact with ion flow. For example, mutations at position 15' in the middle of M1 of different subunits in mouse muscle-type nAChRs affected kinetic parameters of gating in a subunit-specific manner. This finding suggested M1 contributes to gating (370). M1 peptide from *Torpedo californica* showed unexpected conformational flexibility in solid-state NMR experiments with phospholipids, possibly because of the effect of a conserved proline residue near the middle of M1 (371). Interaction between the transmembrane segments M1 and M2 in muscle nAChRs was found to affect gating (372). Molecular dynamics simulations of the transmembrane domain of muscle nAChR suggested that M4 plays a role in communicating effects from the membrane environment to M2 and then to channel gating (373). In this modeling, conformations of side chains of M2 contributed to channel gating. M4 appeared to link the lipid environment to nAChR gating (374). Electrostatic forces facilitating ion flow through the pore likely arise from the transmembrane domain and to a much lesser extent from longer range interactions from the extracellular domain or large cytoplasmic loop (375, 376).

Beyond gating and ion flow, the pore also participates in the structure of the nonconducting desensitized state. According to infrared spectroscopy, the α -helices of the transmembrane domain of *Torpedo californica* nAChRs were preferentially perpendicular to the membrane surface and showed no reorientation with desensitization (377). In general, however, the structure of the desensitized state is poorly understood.

7. WHAT IS THE STRUCTURAL BASIS FOR FUNCTIONS OF THE LARGE CYTOPLASMIC LOOP?

The amino acid sequence of the large cytoplasmic loop between M3 and M4 shows considerable diversity among different nAChR subunits. The functions of the large cytoplasmic loop, however, have not been studied as extensively as the functions of the extracellular domain or transmembrane domain. The structure of this loop was not seen in the 4 Å structure of *Torpedo* nAChR (90) and was presumed to be disordered.

Structural disorder is important to the functions of some proteins (378) and might contribute to various functions of large cytoplasmic loops in the resting, open, and desensitized states. Studies of the large cytoplasmic loop from the δ subunit from *Torpedo californica* and rat suggested the possibility of structural studies of this domain isolated from the remainder of the subunit or nAChR (379, 380). Residues in the large cytoplasmic loop near the intracellular end of M4 affected assembly of subunits and electrophysiological properties of nAChRs (381–384). Because of the sequence diversity of the large cytoplasmic loop, this region might be important for subunit-specific behavior and interactions with cellular components and physiology. The large cytoplasmic loop interacts with cytoplasmic transport machinery for trafficking nAChRs to synapses (385–389). Residues in M1 (390) and at the cytoplasmic and extracellular ends of M4 (391) of $\alpha 1$ affected assembly and targeting to the cell surface. At the cell membrane, the large cytoplasmic loop interacted with cytoskeleton (392–394). Phosphorylation of the large cytoplasmic loop (395, 396) affected desensitization (397, 398), expression (399, 400), and cytoskeletal interaction (401). Palmitoylation (402, 403) affected expression of $\alpha 7$ nAChRs (404). Future development of the structural description of how nAChRs work will need to include the dynamic structure of the large cytoplasmic loop, including posttranslational modifications and interactions with other proteins.

8. HOW DO CELLS REGULATE BIOSYNTHESIS AND FUNCTION OF NACHRS?

Considered broadly, elements of structural biology contribute to the function of nAChRs during their whole life cycle. Extending beyond nAChRs in isolation, these elements include the structural basis of genomic regulation and allelic variations; structures and dynamics important to subunit translation and assembly; and nAChR interactions with cellular elements, including other proteins and lipids.

8.1. Genomic regulation

Components of gene regulation for nAChRs (405, 406) include activation and repression elements (407), long range regulatory elements coordinating expression of nAChR gene clusters (408), cell-specific promoter activity (409), and transcription factors controlling nAChR expression (410). RNA splicing is known to affect transcripts for ϵ (411), $\alpha 7$ (412–415), insect $\alpha 6$ (416), and the glycine receptor β subunit (417). A functionally significant allelic variation of the *CHRNA7* gene for $\alpha 7$ was identified in a Japanese population (418).

8.2. Subunit assembly

Diverse combinations of neuronal subunits can assemble into diverse types of nAChRs with functional implications (419–423). Some details about the process of subunit assembly are known, but much remains to be discovered. Ultimately, a set of five stable subunit-subunit interfaces must form. What structural intermediates exist between primary sequences of amino acid residues and the final quaternary structures? What conformational changes convert one intermediate to the next? The process of subunit assembly is best understood for muscle-type nAChRs (424–426), where $(\alpha 1)_2(\beta 1)\delta\gamma$ and $(\alpha 1)_2(\beta 1)\delta\epsilon$ are endpoints. For neuronal nAChRs, the diversity of endpoints remains a subject of investigation (427, 428). Which subunits assemble together (429–431)? What is the stoichiometry of assembly for heteromeric nAChRs (432, 213, 433, 434)? Knowing structures of subunit-subunit interfaces might help decode rules that regulate which subunits can assemble to form nAChRs.

Subunit composition of nAChRs changes during development (435) and affects where the nAChRs are positioned within spatially complex neurons (436). Because neurons often produce several types of subunits that potentially can produce a diverse set of nAChRs, what factors influence the types of nAChRs that are produced from a diverse pool of subunits? Signals in the cellular environment can affect the outcome. For example, the inflammatory cytokines interleukin-1 β and tumor necrosis factor α affected the relative production of $\alpha 4\beta 2$ and $\alpha 4\beta 4$ nAChR from a pool of $\alpha 4$, $\beta 2$, and $\beta 4$ subunits (437).

8.3. Interactions with cellular components

In cells, nAChRs interact with other cellular components, notably proteins and lipids. These interactions affect expression, targeting, electrophysiological function, signaling, and survival (438). Rapsyn, originally identified as a 43 kDa protein associated with *Torpedo* nAChRs, mediated the effect of agrin and helps cluster muscle-type nAChRs (439–442). The tumor suppressor protein adenomatous polyposis coli (APC) participated in the signaling path downstream of agrin and the muscle-specific kinase MuSK for clustering nAChRs in muscle (443).

In neurons, proteins in the PSD-95/SAP90 family (444), splice variants of PSD93 (445), and APC (446) promoted the organization of nAChRs at synapses. The chaperone protein 14-3-3 η (447) and possibly other 14-3-3 isoforms (448) modified expression of $\alpha 4\beta 2$ nAChRs and changed the proportions of nAChRs with high and low agonist sensitivity (448). These effects possibly were mediated by interactions with a phosphorylated site in the large cytoplasmic loop of $\alpha 4$ subunits. Visinin-like protein-1 (VILIP-1), a protein sensor of intracellular calcium, also appeared to interact with the large cytoplasmic loop of $\alpha 4$ subunits and increased the surface expression of $\alpha 4\beta 2$ nAChRs (449). PICK1 likely bound to the large cytoplasmic loop of $\alpha 7$ and decreased clustering of $\alpha 7$ nAChRs (450).

RIC-3 is a transmembrane protein of the endoplasmic reticulum produced from the resistance to inhibitors of cholinesterase (*ric-3*) gene in *Caenorhabditis elegans* and found in humans, mice, and *Xenopus*. It has varying effects on expression of nAChRs. In *Xenopus* oocytes, RIC-3 increases the functional expression of $\alpha 7$ nAChRs but reduces the whole-cell currents from $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs and abolishes current from 5-HT $_3$ receptors (451). It had little effect on current from $\alpha 1$ glycine receptors (452). RIC-3 appears to interact with an isoleucine residue near the extracellular end of M1 to stop transport to the cell surface and with residues in an amphipathic helix (residues 410–427) in the large cytoplasmic loop of $\alpha 7$. The outcome is to increase the total number and the surface expression of $\alpha 7$ nAChRs (451, 453). In contrast to the mixed effects of RIC-3 on nAChRs expressed in *Xenopus* oocytes, co-expression of nAChR subunits and RIC-3 in human kidney tsA201 cells increased functional expression of $\alpha 7$, $\alpha 8$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 3\beta 2$, and

$\alpha 4\beta 4$ nAChRs. RIC-3, however, did not increase functional expression of $\alpha 9$ or $\alpha 9\alpha 10$ nAChRs (454). Association between RIC-3 and unassembled $\alpha 4$ subunits and $\beta 2$ subunits suggested RIC-3 interacts with nAChR subunits during assembly and maturation into nAChRs (454). Calnexin (455), a chaperone protein in the endoplasmic reticulum, also interacted with nAChR subunits during subunit folding and assembly (456–459). The ubiquitin-proteasome system helped regulate the number of active nAChRs by degrading subunits removed from the endoplasmic reticulum by the process of ER-associated degradation (ERAD) (460).

The gene *lynx1* originally was found in mice (461) and produces the protein lynx1 with sequence and structural similarity to snake neurotoxins like α -bungarotoxin. The protein lynx1 physically associated with $\alpha 7$ and $\alpha 4\beta 2$ nAChRs and modified the current through and enhanced desensitization of $\alpha 4\beta 2$ nAChRs (462). Deletion of *lynx1* in mice was expected to increase cholinergic function and did improve learning and memory (463). Deletion of *lynx1*, however, led to a vacuolar degeneration of the brain that was consistent with the presence of hypersensitive nAChRs. These results suggested that lynx1 helps modulate neuronal activity *in vivo*. Another member of the *ly-6/neurotoxin* gene superfamily, *lynx2*, was expressed at specific locations in the mouse nervous system during development (464). This gene superfamily has twenty-seven members in the human genome (465), some of which beyond *lynx1* might have roles in the nervous system.

Lipids directly interact with the transmembrane domain of nAChRs. These lipid-protein interactions not only affected ion conduction (466) but also might affect nAChR trafficking (467, 468).

9. WHY IS UNDERSTANDING NACHRS AS BIOPHYSICAL MACHINES RELEVANT TO QUESTIONS ABOUT NORMAL AND PATHOLOGICAL FUNCTION OF THE NERVOUS SYSTEM?

Understanding structures of nAChRs when interacting with other cellular components is important for understanding structural aspects of normal function and pathological function of nAChRs in addiction, neurodegeneration, and mental illness. For example, features of the structural mechanism of upregulating nAChRs by nicotine and other nicotinic ligands are beginning to emerge. Nicotine might interact as a protein folding chaperone with the extracellular domain leading to enhanced expression of $\alpha 4\beta 2$ nAChRs and other nAChRs (469–473, 421). Thermodynamics of ligand interaction at the binding site of $\alpha 4$ -containing nAChRs might explain increased assembly and upregulation of these nAChRs (474). Other proposed mechanisms of upregulation of $\alpha 4\beta 2$ nAChRs by nicotine include effects on nAChR trafficking (475) and stabilizing by nicotine of a form of the receptor that is more sensitive to activation (476). Understanding the mechanism of nAChR upregulation by nicotine will lead to methods for preventing and treating nicotine addiction. Interactions between nAChRs and 14-3-3 η might help control the activity of nAChRs, a potentially important feature in schizophrenia and neurodegeneration (447). Designing new drugs for diseases related to the function of nAChRs will benefit from a comprehensive and detailed understanding of the structural basis for nAChR function in cellular environments.

10. WHAT CAN WE LEARN FROM NACHRS ABOUT PROTEIN FOLDING AND DE NOVO DESIGN OF PROTEINS?

Lessons relating structure and function for nAChRs will contribute other pieces to the protein folding puzzle for integral membrane proteins (477, 478). The combined presence of aqueous phase, lipid phase, and surface charge on the lipid bilayer brings greater complexity

to protein folding for integral membrane proteins than is encountered with water-soluble proteins. Creating nAChRs containing unnatural amino acids (section 4.8) illustrates the potential of nAChRs with properties tailored by human imagination. Foreshadowed by nAChRs as biosensors (479–481), understanding how nAChRs are produced and work as biophysical machines under controlled conditions of single channel recording and in a complex cellular milieu will guide the development of proteins with novel functions.

Acknowledgments

Preparation of this review was supported by the National Institute on Drug Abuse, the National Institute of Neurological Disorders and Stroke, Philip Morris USA Inc. and Philip Morris International. René Anand graciously contributed comments on the manuscript.

Abbreviations

ACh	acetylcholine
AChBP	acetylcholine binding protein
APC	adenomatous polyposis coli
5-HT₃	5-hydroxytryptamine (serotonin)
GABA	γ -aminobutyric acid
GABA_A	γ -aminobutyric acid type A receptor
GABA_C	γ -aminobutyric acid type C receptor
M1–M4	transmembrane domains M1 through M4
MIR	main immunogenic region
nAChR	nicotinic acetylcholine receptor
NMR	nuclear magnetic resonance

References

1. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol.* 2007; 47:699–729. [PubMed: 17009926]
2. O'Brien RD, Eldefrawi ME, Eldefrawi AT. Isolation of acetylcholine receptors. *Annu Rev Pharmacol.* 1972; 12:19–34. [PubMed: 4339017]
3. Karlin A. The acetylcholine receptor: progress report. *Life Sci.* 1974; 14:1385–1415. [PubMed: 4597876]
4. Heidmann T, Changeux JP. Structural and functional properties of the acetylcholine receptor protein in its purified and membrane-bound states. *Annu Rev Biochem.* 1978; 47:317–357. [PubMed: 354498]
5. Popot JL, Changeux JP. Nicotinic receptor of acetylcholine: structure of an oligomeric integral membrane protein. *Physiol Rev.* 1984; 64:1162–1239. [PubMed: 6208568]
6. Galzi JL, Revah F, Bessis A, Changeux JP. Functional architecture of the nicotinic acetylcholine receptor: from electric organ to brain. *Annu Rev Pharmacol Toxicol.* 1991; 31:37–72. [PubMed: 2064379]
7. Changeux JP, Galzi JL, Devillers-Thiéry A, Bertrand D. The functional architecture of the acetylcholine nicotinic receptor explored by affinity labelling and site-directed mutagenesis. *Q Rev Biophys.* 1992; 25:395–432. [PubMed: 1293635]
8. Karlin A. Structure of nicotinic acetylcholine receptors. *Curr Opin Neurobiol.* 1993; 3:299–309. [PubMed: 8369624]

9. Karlin A, Akabas MH. Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. *Neuron*. 1995; 15:1231–1244. [PubMed: 8845149]
10. Le Novère N, Changeux JP. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. *J Mol Evol*. 1995; 40:155–172. [PubMed: 7699721]
11. Gotti C, Fornasari D, Clementi F. Human neuronal nicotinic receptors. *Prog Neurobiol*. 1997; 53:199–237. [PubMed: 9364611]
12. Lindstrom J. Nicotinic acetylcholine receptors in health and disease. *Mol Neurobiol*. 1997; 15:193–222. [PubMed: 9396010]
13. Lindstrom J, Peng X, Kuryatov A, Lee E, Anand R, Gerzanich V, Wang F, Wells G, Nelson M. Molecular and antigenic structure of nicotinic acetylcholine receptors. *Ann N Y Acad Sci*. 1998; 841:71–86. [PubMed: 9668222]
14. Corringer PJ, Le Novère N, Changeux JP. Nicotinic receptors at the amino acid level. *Annu Rev Pharmacol Toxicol*. 2000; 40:431–458. [PubMed: 10836143]
15. Lindstrom, J. The structures of neuronal nicotinic receptors. In: Clementi, F.; Fornasari, D.; Gotti, C., editors. *Neuronal Nicotinic Receptors*. Springer-Verlag; Berlin: 2000.
16. Karlin A. Emerging structure of the nicotinic acetylcholine receptors. *Nat Rev Neurosci*. 2002; 3:102–114. [PubMed: 11836518]
17. Colquhoun, D.; Shelley, C.; Hatton, C.; Unwin, N.; Sivilotti, L. *Nicotinic Acetylcholine Receptors*. In: Abraham, DJ., editor. *Burger's Medicinal Chemistry and Drug Discovery*. John Wiley; Hoboken, N.J: 2003.
18. Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. *Rev Physiol Biochem Pharmacol*. 2003; 147:1–46. [PubMed: 12783266]
19. Alkondon M, Albuquerque EX. The nicotinic acetylcholine receptor subtypes and their function in the hippocampus and cerebral cortex. *Prog Brain Res*. 2004; 145:109–120. [PubMed: 14650910]
20. Connolly CN, Wafford KA. The Cys-loop superfamily of ligand-gated ion channels: the impact of receptor structure on function. *Biochem Soc Trans*. 2004; 32:529–534. [PubMed: 15157178]
21. Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. *Prog Neurobiol*. 2004; 74:363–396. [PubMed: 15649582]
22. Lester HA, Dibas MI, Dahan DS, Leite JF, Dougherty DA. Cys-loop receptors: new twists and turns. *Trends Neurosci*. 2004; 27:329–336. [PubMed: 15165737]
23. Changeux JP. The visit of Professor Chen-Yuan Lee to the Pasteur Institute in 1970 and the use of a snake venom toxin to identify the acetylcholine receptor protein. *Toxin Reviews*. 2005; 24:15–41.
24. Changeux, J-P.; Edelman, SJ. *Nicotinic Acetylcholine Receptors: From Molecular Biology to Cognition*. Odile Jacob Publishing Corp.; New York, N.Y.: 2005.
25. Jensen AA, Frølund B, Liljefors T, Krogsgaard-Larsen P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J Med Chem*. 2005; 48:4705–4745. [PubMed: 16033252]
26. Sine SM, Engel AG. Recent advances in Cys-loop receptor structure and function. *Nature*. 2006; 440:448–455. [PubMed: 16554804]
27. Halliwell RF. A short history of the rise of the molecular pharmacology of ionotropic drug receptors. *Trends Pharmacol Sci*. 2007; 28:214–219. [PubMed: 17416425]
28. Sivilotti L, Colquhoun D. Acetylcholine receptors: too many channels, too few functions. *Science*. 1995; 269:1681–1682. [PubMed: 7569892]
29. Hulo N, Bairoch A, Bulliard V, Cerutti L, De Castro E, Langendijk-Genevaux PS, Pagni M, Sigrist CJ. The PROSITE database. *Nucleic Acids Res*. 2006; 34:D227–230. [PubMed: 16381852]
30. Séguéla P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain $\alpha 7$: a nicotinic cation channel highly permeable to calcium. *J Neurosci*. 1993; 13:596–604. [PubMed: 7678857]
31. Castro NG, Albuquerque EX. α -Bungarotoxin-sensitive hippocampal nicotinic receptor channel has a high calcium permeability. *Biophys J*. 1995; 68:516–524. [PubMed: 7696505]

32. Adams DJ, Dwyer TM, Hille B. The permeability of endplate channels to monovalent and divalent metal cations. *J Gen Physiol.* 1980; 75:493–510. [PubMed: 6247423]
33. Lukas RJ, Changeux JP, Le Novère N, Albuquerque EX, Balfour DJ, Berg DK, Bertrand D, Chiappinelli VA, Clarke PB, Collins AC, Dani JA, Grady SR, Kellar KJ, Lindstrom JM, Marks MJ, Quik M, Taylor PW, Wonnacott S. International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. *Pharmacol Rev.* 1999; 51:397–401. [PubMed: 10353988]
34. Alexander SP, Mathie A, Peters JA. Transmitter-gated channels. *Br J Pharmacol.* 2007; 150(Suppl 1):S82–S95. [PubMed: 17279072]
35. Reeves DC, Lummis SC. The molecular basis of the structure and function of the 5-HT₃ receptor: a model ligand-gated ion channel (review). *Mol Membr Biol.* 2002; 19:11–26. [PubMed: 11989819]
36. Thompson AJ, Lummis SC. 5-HT₃ receptors. *Curr Pharm Des.* 2006; 12:3615–3630. [PubMed: 17073663]
37. Akabas MH. GABA_A receptor structure-function studies: a reexamination in light of new acetylcholine receptor structures. *Int Rev Neurobiol.* 2004; 62:1–43. [PubMed: 15530567]
38. Sieghart W. Structure, pharmacology, and function of GABA_A receptor subtypes. *Adv Pharmacol.* 2006; 54:231–263. [PubMed: 17175817]
39. Cascio M. Structure and function of the glycine receptor and related nicotinic receptors. *J Biol Chem.* 2004; 279:19383–19386. [PubMed: 15023997]
40. Colquhoun D, Sivilotti LG. Function and structure in glycine receptors and some of their relatives. *Trends Neurosci.* 2004; 27:337–344. [PubMed: 15165738]
41. Betz H, Laube B. Glycine receptors: recent insights into their structural organization and functional diversity. *J Neurochem.* 2006; 97:1600–1610. [PubMed: 16805771]
42. Davies PA, Wang W, Hales TG, Kirkness EF. A novel class of ligand-gated ion channel is activated by Zn²⁺. *J Biol Chem.* 2003; 278:712–717. [PubMed: 12381728]
43. Houtani T, Munemoto Y, Kase M, Sakuma S, Tsutsumi T, Sugimoto T. Cloning and expression of ligand-gated ion-channel receptor L2 in central nervous system. *Biochem Biophys Res Commun.* 2005; 335:277–285. [PubMed: 16083862]
44. Wolstenholme AJ, Rogers AT. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology.* 2005; 131(Suppl):S85–S95. [PubMed: 16569295]
45. Sunesen M, de Carvalho LP, Dufresne V, Grailhe R, Savatier-Duclert N, Gibor G, Peretz A, Attali B, Changeux JP, Paas Y. Mechanism of Cl[−] selection by a glutamate-gated chloride (GluCl) receptor revealed through mutations in the selectivity filter. *J Biol Chem.* 2006; 281:14875–14881. [PubMed: 16527818]
46. Ranganathan R, Cannon SC, Horvitz HR. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in *C. elegans*. *Nature.* 2000; 408:470–475. [PubMed: 11100728]
47. Menard C, Horvitz HR, Cannon S. Chimeric mutations in the M2 segment of the 5-hydroxytryptamine-gated chloride channel MOD-1 define a minimal determinant of anion/cation permeability. *J Biol Chem.* 2005; 280:27502–27507. [PubMed: 15878844]
48. Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, Smit AB, Sixma TK. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature.* 2001; 411:269–276. [PubMed: 11357122]
49. Smit AB, Syed NI, Schaap D, van Minnen J, Klumperman J, Kits KS, Lodder H, van der Schors RC, van Elk R, Sorgedraeger B, Brejc K, Sixma TK, Geraerts WP. A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature.* 2001; 411:261–268. [PubMed: 11357121]
50. Tasneem A, Iyer LM, Jakobsson E, Aravind L. Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. *Genome Biol.* 2005; 6:R4. [PubMed: 15642096]
51. Bocquet N, Prado de Carvalho L, Cartaud J, Neyton J, Le Poupon C, Taly A, Grutter T, Changeux J-P, Corringer P-J. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. *Nature.* 2007; 445:116–119. [PubMed: 17167423]

52. Tomizawa M, Casida JE. Structure and diversity of insect nicotinic acetylcholine receptors. *Pest Manag Sci.* 2001; 57:914–922. [PubMed: 11695184]
53. Jones AK, Sattelle DB. Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, *Caenorhabditis elegans*. *Bioessays.* 2004; 26:39–49. [PubMed: 14696039]
54. van Nierop P, Keramidas A, Bertrand S, van Minnen J, Gouwenberg Y, Bertrand D, Smit AB. Identification of molluscan nicotinic acetylcholine receptor (nAChR) subunits involved in formation of cation- and anion-selective nAChRs. *J Neurosci.* 2005; 25:10617–10626. [PubMed: 16291934]
55. Dent JA. Evidence for a diverse Cys-loop ligand-gated ion channel superfamily in early bilateria. *J Mol Evol.* 2006; 62:523–535. [PubMed: 16586016]
56. Jones AK, Brown LA, Sattelle DB. Insect nicotinic acetylcholine receptor gene families: from genetic model organism to vector, pest and beneficial species. *Invert Neurosci.* 2007; 7:67–73. [PubMed: 17216517]
57. Jones AK, Davis P, Hodgkin J, Sattelle DB. The nicotinic acetylcholine receptor gene family of the nematode *Caenorhabditis elegans*: an update on nomenclature. *Invert Neurosci.* 2007; 7:129–131. [PubMed: 17503100]
58. Lindahl E, Elofsson A. Identification of related proteins on family, superfamily and fold level. *J Mol Biol.* 2000; 295:613–625. [PubMed: 10623551]
59. Chandonia JM, Brenner SE. The impact of structural genomics: expectations and outcomes. *Science.* 2006; 311:347–351. [PubMed: 16424331]
60. Lundstrom K. Structural genomics for membrane proteins. *Cell Mol Life Sci.* 2006; 63:2597–2607. [PubMed: 17013556]
61. Dobrovetsky E, Menendez J, Edwards AM, Koth CM. A robust purification strategy to accelerate membrane proteomics. *Methods.* 2007; 41:381–387. [PubMed: 17367710]
62. Cartaud J, Benedetti EL, Cohen JB, Meunier JC, Changeux JP. Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. *FEBS Lett.* 1973; 33:109–113. [PubMed: 4124671]
63. Nickel E, Potter LT. Ultrastructure of isolated membranes of *Torpedo* electric tissue. *Brain Res Bull.* 1973; 57:508–517.
64. Reed K, Vandlen R, Bode J, Duguid J, Raftery MA. Characterization of acetylcholine receptor-rich and acetylcholinesterase-rich membrane particles from *Torpedo californica* electroplax. *Arch Biochem Biophys.* 1975; 167:138–144. [PubMed: 1130789]
65. Chang RS, Potter LT, Smith DS. Postsynaptic membranes in the electric tissue of *Narcine*. IV. Isolation and characterization of the nicotinic receptor protein. *Tissue Cell.* 1977; 9:623–644. [PubMed: 416517]
66. Ross MJ, Klymkowsky MW, Agard DA, Stroud RM. Structural studies of a membrane-bound acetylcholine receptor from *Torpedo californica*. *J Mol Biol.* 1977; 116:635–659. [PubMed: 563472]
67. Cartaud J, Benedetti EL, Sobel A, Changeux JP. A morphological study of the cholinergic receptor protein from *Torpedo marmorata* in its membrane environment and in its detergent-extracted purified form. *J Cell Sci.* 1978; 29:313–337. [PubMed: 627610]
68. Schiebeler W, Hucho F. Membranes rich in acetylcholine receptor: characterization and reconstitution to excitable membranes from exogenous lipids. *Eur J Biochem.* 1978; 85:55–63. [PubMed: 639824]
69. Klymkowsky MW, Stroud RM. Immunospecific identification and three-dimensional structure of a membrane-bound acetylcholine receptor from *Torpedo californica*. *J Mol Biol.* 1979; 128:319–334. [PubMed: 439138]
70. Lindstrom J, Gullick W, Conti-Tronconi B, Ellisman M. Proteolytic nicking of the acetylcholine receptor. *Biochemistry.* 1980; 19:4791–4795. [PubMed: 7000183]
71. Zingsheim HP, Neugebauer DC, Barrantes FJ, Frank J. Structural details of membrane-bound acetylcholine receptor from *Torpedo marmorata*. *Proc Natl Acad Sci U S A.* 1980; 77:952–956. [PubMed: 6928692]
72. Kistler J, Stroud RM. Crystalline arrays of membrane-bound acetylcholine receptor. *Proc Natl Acad Sci U S A.* 1981; 78:3678–3682. [PubMed: 6943572]

73. Wise DS, Wall J, Karlin A. Relative locations of the β and δ chains of the acetylcholine receptor determined by electron microscopy of isolated receptor trimer. *J Biol Chem.* 1981; 256:12624–12627. [PubMed: 7309725]
74. Holtzman E, Wise D, Wall J, Karlin A. Electron microscopy of complexes of isolated acetylcholine receptor, biotinyl-toxin, and avidin. *Proc Natl Acad Sci U S A.* 1982; 79:310–314. [PubMed: 6952187]
75. Zingsheim HP, Neugebauer DC, Frank J, Hänicke W, Barrantes FJ. Dimeric arrangement and structure of the membrane-bound acetylcholine receptor studied by electron microscopy. *EMBO J.* 1982; 1:541–547. [PubMed: 7188351]
76. Karlin A, Holtzman E, Yodh N, Lobel P, Wall J, Hainfeld J. The arrangement of the subunits of the acetylcholine receptor of *Torpedo californica*. *J Biol Chem.* 1983; 258:6678–6681. [PubMed: 6853498]
77. Brisson A, Unwin PNT. Tubular crystals of acetylcholine receptor. *J Cell Biol.* 1984; 99:1202–1211. [PubMed: 6480689]
78. Brisson A, Unwin PNT. Quaternary structure of the acetylcholine receptor. *Nature.* 1985; 315:474–477. [PubMed: 4000275]
79. Kubalek E, Ralston S, Lindstrom J, Unwin N. Location of subunits within the acetylcholine receptor by electron image analysis of tubular crystals from *Torpedo marmorata*. *J Cell Biol.* 1987; 105:9–18. [PubMed: 3611197]
80. Toyoshima C, Unwin N. Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes. *Nature.* 1988; 336:247–250. [PubMed: 2461515]
81. Unwin N, Toyoshima C, Kubalek E. Arrangement of the acetylcholine receptor subunits in the resting and desensitized states, determined by cryoelectron microscopy of crystallized *Torpedo* postsynaptic membranes. *J Cell Biol.* 1988; 107:1123–1138. [PubMed: 3417777]
82. Toyoshima C, Unwin N. Three-dimensional structure of the acetylcholine receptor by cryoelectron microscopy and helical image reconstruction. *J Cell Biol.* 1990; 111:2623–2635. [PubMed: 2277076]
83. Unwin N. Nicotinic acetylcholine receptor at 9 Å resolution. *J Mol Biol.* 1993; 229:1101–1124. [PubMed: 8445638]
84. Beroukhi R, Unwin N. Three-dimensional location of the main immunogenic region of the acetylcholine receptor. *Neuron.* 1995; 15:323–331. [PubMed: 7544140]
85. Unwin N. Acetylcholine receptor channel imaged in the open state. *Nature.* 1995; 373:37–43. [PubMed: 7800037]
86. Unwin N. Projection structure of the nicotinic acetylcholine receptor: Distinct conformations of the α subunits. *J Mol Biol.* 1996; 257:586–596. [PubMed: 8648626]
87. Miyazawa A, Fujiyoshi Y, Stowell M, Unwin N. Nicotinic acetylcholine receptor at 4.6 Å resolution: transverse tunnels in the channel wall. *J Mol Biol.* 1999; 288:765–786. [PubMed: 10329178]
88. Unwin N, Miyazawa A, Li J, Fujiyoshi Y. Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the α subunits. *J Mol Biol.* 2002; 319:1165–1176. [PubMed: 12079355]
89. Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature.* 2003; 424:949–955. [PubMed: 12827192]
90. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J Mol Biol.* 2005; 346:967–989. [PubMed: 15701510]
91. Finer-Moore J, Stroud RM. Amphipathic analysis and possible formation of the ion channel in an acetylcholine receptor. *Proc Natl Acad Sci U S A.* 1984; 81:155–159. [PubMed: 6320162]
92. Werten PJ, Rémy HW, de Groot BL, Fotiadis D, Philippsen A, Stahlberg H, Grubmüller H, Engel A. Progress in the analysis of membrane protein structure and function. *FEBS Lett.* 2002; 529:65–72. [PubMed: 12354615]
93. Loll PJ. Membrane protein structural biology: the high throughput challenge. *J Struct Biol.* 2003; 142:144–153. [PubMed: 12718926]
94. Paas Y, Cartaud J, Recouvreur M, Grailhe R, Dufresne V, Pebay-Peyroula E, Landau EM, Changeux JP. Electron microscopic evidence for nucleation and growth of 3D acetylcholine

- receptor microcrystals in structured lipid-detergent matrices. *Proc Natl Acad Sci U S A*. 2003; 100:11309–11314. [PubMed: 13679581]
95. Landau EM, Rosenbusch JP. Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *Proc Natl Acad Sci U S A*. 1996; 93:14532–14535. [PubMed: 8962086]
 96. Nollert P. Lipidic cubic phases as matrices for membrane protein crystallization. *Methods*. 2004; 34:348–353. [PubMed: 15325652]
 97. Cherezov V, Clogston J, Papiz MZ, Caffrey M. Room to move: crystallizing membrane proteins in swollen lipidic mesophases. *J Mol Biol*. 2006; 357:1605–1618. [PubMed: 16490208]
 98. Pechkova E, Nicolini C. Protein nanocrystallography: a new approach to structural proteomics. *Trends Biotechnol*. 2004; 22:117–122. [PubMed: 15036861]
 99. Dellisanti CD, Yao Y, Stroud JC, Wang ZZ, Chen L. Crystal structure of the extracellular domain of nAChR $\alpha 1$ bound to α -bungarotoxin at 1.94 Å resolution. *Nat Neurosci*. 2007; 10:953–962. [PubMed: 17643119]
 100. Grutter T, Changeux JP. Nicotinic receptors in wonderland. *Trends Biochem Sci*. 2001; 26:459–463. [PubMed: 11504610]
 101. Brejc K, van Dijk WJ, Smit AB, Sixma TK. The 2.7 Å structure of AChBP, homologue of the ligand-binding domain of the nicotinic acetylcholine receptor. *Novartis Found Symp*. 2002; 245:22–32. [PubMed: 12027010]
 102. Sixma TK, Smit AB. Acetylcholine binding protein (AChBP): a secreted glial protein that provides a high-resolution model for the extracellular domain of pentameric ligand-gated ion channels. *Annu Rev Biophys Biomol Struct*. 2003; 32:311–334. [PubMed: 12695308]
 103. Smit AB, Brejc K, Syed N, Sixma TK. Structure and function of AChBP, homologue of the ligand-binding domain of the nicotinic acetylcholine receptor. *Ann N Y Acad Sci*. 2003; 998:81–92. [PubMed: 14592865]
 104. Smit AB, Celie PH, Kasheverov IE, Mordvintsev DY, van Nierop P, Bertrand D, Tsetlin V, Sixma TK. Acetylcholine-binding proteins: functional and structural homologs of nicotinic acetylcholine receptors. *J Mol Neurosci*. 2006; 30:9–10. [PubMed: 17192605]
 105. Dougherty DA, Lester HA. Neurobiology. Snails, synapses and smokers. *Nature*. 2001; 411:252–253. 255. [PubMed: 11357112]
 106. Bork P, Holm L, Sander C. The immunoglobulin fold. Structural classification, sequence patterns and common core. *J Mol Biol*. 1994; 242:309–320. [PubMed: 7932691]
 107. Toniolo C, Benedetti E. The polypeptide 3_{10} -helix. *Trends Biochem Sci*. 1991; 16:350–353. [PubMed: 1949158]
 108. Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK. Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. *Neuron*. 2004; 41:907–914. [PubMed: 15046723]
 109. Karlin A. A touching picture of nicotinic binding. *Neuron*. 2004; 41:841–842. [PubMed: 15046715]
 110. Dougherty DA, Stauffer DA. Acetylcholine binding by a synthetic receptor: implications for biological recognition. *Science*. 1990; 250:1558–1560. [PubMed: 2274786]
 111. Dougherty DA. Cation- π interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. *Science*. 1996; 271:163–168. [PubMed: 8539615]
 112. Zhong W, Gallivan JP, Zhang Y, Li L, Lester HA, Dougherty DA. From ab initio quantum mechanics to molecular neurobiology: a cation- π binding site in the nicotinic receptor. *Proc Natl Acad Sci U S A*. 1998; 95:12088–12093. [PubMed: 9770444]
 113. Bourne Y, Talley TT, Hansen SB, Taylor P, Marchot P. Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake α -neurotoxins and nicotinic receptors. *EMBO J*. 2005; 24:1512–1522. [PubMed: 15791209]
 114. Hansen SB, Talley TT, Radi Z, Taylor P. Structural and ligand recognition characteristics of an acetylcholine-binding protein from *Aplysia californica*. *J Biol Chem*. 2004; 279:24197–24202. [PubMed: 15069068]
 115. Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y. Structures of *Aplysia* AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. *EMBO J*. 2005; 24:3635–3646. [PubMed: 16193063]

116. Celie PH, Kasheverov IE, Mordvintsev DY, Hogg RC, van Nierop P, van Elk R, van Rossum-Fikkert SE, Zhmak MN, Bertrand D, Tsetlin V, Sixma TK, Smit AB. Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an α -conotoxin PnIA variant. *Nat Struct Mol Biol.* 2005; 12:582–588. [PubMed: 15951818]
117. Ulens C, Hogg RC, Celie PH, Bertrand D, Tsetlin V, Smit AB, Sixma TK. Structural determinants of selective α -conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. *Proc Natl Acad Sci U S A.* 2006; 103:3615–3620. [PubMed: 16505382]
118. Hansen SB, Taylor P. Galanthamine and non-competitive inhibitor binding to ACh-binding protein: evidence for a binding site on non- α -subunit interfaces of heteromeric neuronal nicotinic receptors. *J Mol Biol.* 2007; 369:895–901. [PubMed: 17481657]
119. Celie PH, Klaassen RV, van Rossum-Fikkert SE, van Elk R, van Nierop P, Smit AB, Sixma TK. Crystal structure of acetylcholine-binding protein from *Bulinus truncatus* reveals the conserved structural scaffold and sites of variation in nicotinic acetylcholine receptors. *J Biol Chem.* 2005; 280:26457–26466. [PubMed: 15899893]
120. Hansen SB, Radi Z, Talley TT, Molles BE, Deerinck T, Tsigelny I, Taylor P. Tryptophan fluorescence reveals conformational changes in the acetylcholine binding protein. *J Biol Chem.* 2002; 277:41299–41302. [PubMed: 12235129]
121. Hibbs RE, Talley TT, Taylor P. Acrylodan-conjugated cysteine side chains reveal conformational state and ligand site locations of the acetylcholine-binding protein. *J Biol Chem.* 2004; 279:28483–28491. [PubMed: 15117947]
122. Hibbs RE, Radi Z, Taylor P, Johnson DA. Influence of agonists and antagonists on the segmental motion of residues near the agonist binding pocket of the acetylcholine-binding protein. *J Biol Chem.* 2006; 281:39708–39718. [PubMed: 17068341]
123. Hibbs RE, Johnson DA, Shi J, Hansen SB, Taylor P. Structural dynamics of the α -neurotoxin-acetylcholine-binding protein complex: hydrodynamic and fluorescence anisotropy decay analyses. *Biochemistry.* 2005; 44:16602–16611. [PubMed: 16342951]
124. Talley TT, Yalda S, Ho KY, Tor Y, Soti FS, Kem WR, Taylor P. Spectroscopic analysis of benzylidene anabaseine complexes with acetylcholine binding proteins as models for ligand-nicotinic receptor interactions. *Biochemistry.* 2006; 45:8894–8902. [PubMed: 16846232]
125. Gao F, Bren N, Burghardt TP, Hansen S, Henchman RH, Taylor P, McCammon JA, Sine SM. Agonist-mediated conformational changes in acetylcholine-binding protein revealed by simulation and intrinsic tryptophan fluorescence. *J Biol Chem.* 2005; 280:8443–8451. [PubMed: 15591050]
126. Gao F, Bern N, Little A, Wang HL, Hansen SB, Talley TT, Taylor P, Sine SM. Curariform antagonists bind in different orientations to acetylcholine-binding protein. *J Biol Chem.* 2003; 278:23020–23026. [PubMed: 12682067]
127. Shi J, Koeppe JR, Komives EA, Taylor P. Ligand-induced conformational changes in the acetylcholine-binding protein analyzed by hydrogen-deuterium exchange mass spectrometry. *J Biol Chem.* 2006; 281:12170–12177. [PubMed: 16484218]
128. Gao F, Mer G, Tonelli M, Hansen SB, Burghardt TP, Taylor P, Sine SM. Solution NMR of acetylcholine binding protein reveals agonist-mediated conformational change of the C-loop. *Mol Pharmacol.* 2006; 70:1230–1235. [PubMed: 16847142]
129. Amiri S, Sansom MS, Biggin PC. Molecular dynamics studies of AChBP with nicotine and carbamylcholine: the role of water in the binding pocket. *Protein Eng Des Sel.* 2007; 20:353–359. [PubMed: 17595341]
130. Krieger, E.; Nabuurs, SB.; Vriend, G. Homology Modeling. In: Bourne, PE.; Weissig, H., editors. *Structural Bioinformatics.* Wiley-Liss; Hoboken, N.J.: 2003.
131. Le Novère N, Grutter T, Changeux JP. Models of the extracellular domain of the nicotinic receptors and of agonist- and Ca^{2+} binding sites. *Proc Natl Acad Sci U S A.* 2002; 99:3210–3215. [PubMed: 11867716]
132. Schapira M, Abagyan R, Totrov M. Structural model of nicotinic acetylcholine receptor isotypes bound to acetylcholine and nicotine. *BMC Struct Biol.* 2002; 2:1. [PubMed: 11860617]

133. Chou KC. Insights from modelling the 3D structure of the extracellular domain of $\alpha 7$ nicotinic acetylcholine receptor. *Biochem Biophys Res Commun.* 2004; 319:433–438. [PubMed: 15178425]
134. Amiri S, Tai K, Beckstein O, Biggin PC, Sansom MS. The $\alpha 7$ nicotinic acetylcholine receptor: molecular modelling, electrostatics, and energetics. *Mol Membr Biol.* 2005; 22:151–162. [PubMed: 16096259]
135. Mordvitsev DY, Polyak YL, Kuzmin DA, Levitsova OV, Tourleigh YV, Utkin YN, Shaitan KV, Tsetlin VI. Computer modeling of binding of diverse weak toxins to nicotinic acetylcholine receptors. *Comput Biol Chem.* 2007; 31:72–81. [PubMed: 17392029]
136. Ernst M, Brauchart D, Boresch S, Sieghart W. Comparative modeling of GABA_A receptors: limits, insights, future developments. *Neuroscience.* 2003; 119:933–943. [PubMed: 12831854]
137. Ernst M, Bruckner S, Boresch S, Sieghart W. Comparative models of GABA_A receptor extracellular and transmembrane domains: important insights in pharmacology and function. *Mol Pharmacol.* 2005; 68:1291–1300. [PubMed: 16103045]
138. Harrison NJ, Lummis SC. Molecular modeling of the GABA_C receptor ligand-binding domain. *J Mol Model.* 2006; 12:317–324. [PubMed: 16249935]
139. Reeves DC, Sayed MF, Chau PL, Price KL, Lummis SC. Prediction of 5-HT₃ receptor agonist-binding residues using homology modeling. *Biophys J.* 2003; 84:2338–2344. [PubMed: 12668442]
140. Thompson AJ, Price KL, Reeves DC, Chan SL, Chau PL, Lummis SC. Locating an antagonist in the 5-HT₃ receptor binding site using modeling and radioligand binding. *J Biol Chem.* 2005; 280:20476–20482. [PubMed: 15781467]
141. Bertaccini EJ, Shapiro J, Brutlag DL, Trudell JR. Homology modeling of a human glycine $\alpha 1$ receptor reveals a plausible anesthetic binding site. *J Chem Inf Model.* 2005; 45:128–135. [PubMed: 15667138]
142. Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H, Laube B. The β subunit determines the ligand binding properties of synaptic glycine receptors. *Neuron.* 2005; 45:727–739. [PubMed: 15748848]
143. Cheng MH, Cascio M, Coalson RD. Homology modeling and molecular dynamics simulations of the $\alpha 1$ glycine receptor reveals different states of the channel. *Proteins.* 2007; 68:581–593. [PubMed: 17469203]
144. Speranskiy K, Cascio M, Kurnikova M. Homology modeling and molecular dynamics simulations of the glycine receptor ligand binding domain. *Proteins.* 2007; 67:950–960. [PubMed: 17357155]
145. Tzakos AG, Grace CR, Lukavsky PJ, Riek R. NMR techniques for very large proteins and RNAs in solution. *Annu Rev Biophys Biomol Struct.* 2006; 35:319–342. [PubMed: 16689639]
146. Basus VJ, Song G, Hawrot E. NMR solution structure of an α -bungarotoxin/nicotinic receptor peptide complex. *Biochemistry.* 1993; 32:12290–12298. [PubMed: 8241115]
147. Gentile LN, Basus VJ, Shi QL, Hawrot E. Preliminary two-dimensional [¹H]NMR characterization of the complex formed between an 18-amino acid peptide fragment of the α -subunit of the nicotinic acetylcholine receptor and α -bungarotoxin. *Ann N Y Acad Sci.* 1995; 757:222–237. [PubMed: 7611677]
148. Zeng H, Moise L, Grant MA, Hawrot E. The solution structure of the complex formed between α -bungarotoxin and an 18-mer cognate peptide derived from the $\alpha 1$ subunit of the nicotinic acetylcholine receptor from *Torpedo californica*. *J Biol Chem.* 2001; 276:22930–22940. [PubMed: 11312275]
149. Zeng H, Hawrot E. NMR-based binding screen and structural analysis of the complex formed between α -cobratoxin and an 18-mer cognate peptide derived from the $\alpha 1$ subunit of the nicotinic acetylcholine receptor from *Torpedo californica*. *J Biol Chem.* 2002; 277:37439–37445. [PubMed: 12133834]
150. Moise L, Piserchio A, Basus VJ, Hawrot E. NMR structural analysis of α -bungarotoxin and its complex with the principal α -neurotoxin-binding sequence on the $\alpha 7$ subunit of a neuronal nicotinic acetylcholine receptor. *J Biol Chem.* 2002; 277:12406–12417. [PubMed: 11790782]

151. Montal M, Opella SJ. The structure of the M2 channel-lining segment from the nicotinic acetylcholine receptor. *Biochim Biophys Acta*. 2002; 1565:287–293. [PubMed: 12409201]
152. Yushmanov VE, Xu Y, Tang P. NMR structure and dynamics of the second transmembrane domain of the neuronal acetylcholine receptor $\beta 2$ subunit. *Biochemistry*. 2003; 42:13058–13065. [PubMed: 14596621]
153. Bondarenko V, Xu Y, Tang P. Structure of the first transmembrane domain of the neuronal acetylcholine receptor $\beta 2$ subunit. *Biophys J*. 2007; 92:1616–1622. [PubMed: 17142275]
154. Yao Y, Wang J, Viroonchatapan N, Samson A, Chill J, Rothe E, Anglister J, Wang ZZ. Yeast expression and NMR analysis of the extracellular domain of muscle nicotinic acetylcholine receptor α subunit. *J Biol Chem*. 2002; 277:12613–12621. [PubMed: 11812776]
155. Kainosho M, Torizawa T, Iwashita Y, Terauchi T, Mei A, Guntert P. Optimal isotope labelling for NMR protein structure determinations. *Nature*. 2006; 440:52–57. [PubMed: 16511487]
156. Fiaux J, Bertelsen EB, Horwich AL, Wüthrich K. NMR analysis of a 900K GroEL-GroES complex. *Nature*. 2002; 418:207–211. [PubMed: 12110894]
157. Foster MP, McElroy CA, Amero CD. Solution NMR of large molecules and assemblies. *Biochemistry*. 2007; 46:331–340. [PubMed: 17209543]
158. Sprangers R, Kay LE. Quantitative dynamics and binding studies of the 20S proteasome by NMR. *Nature*. 2007; 445:618–622. [PubMed: 17237764]
159. Sprangers R, Velyvis A, Kay LE. Solution NMR of supramolecular complexes: providing new insights into function. *Nat Methods*. 2007; 4:697–703. [PubMed: 17762877]
160. Williamson PT, Meier BH, Watts A. Structural and functional studies of the nicotinic acetylcholine receptor by solid-state NMR. *Eur Biophys J*. 2004; 33:247–254. [PubMed: 14740198]
161. Krabben L, van Rossum BJ, Castellani F, Bocharov E, Schulga AA, Arseniev AS, Weise C, Hucho F, Oschkinat H. Towards structure determination of neurotoxin II bound to nicotinic acetylcholine receptor: a solid-state NMR approach. *FEBS Lett*. 2004; 564:319–324. [PubMed: 15111116]
162. De Planque MR, Rijkers DT, Liskamp RM, Separovic F. The $\alpha M1$ transmembrane segment of the nicotinic acetylcholine receptor interacts strongly with model membranes. *Magn Reson Chem*. 2004; 42:148–154. [PubMed: 14745794]
163. Wang ZZ, Hardy SF, Hall ZW. Membrane tethering enables an extracellular domain of the acetylcholine receptor α subunit to form a heterodimeric ligand-binding site. *J Cell Biol*. 1996; 135:809–817. [PubMed: 8909552]
164. Wang ZZ, Hardy SF, Hall ZW. Assembly of the nicotinic acetylcholine receptor. The first transmembrane domains of truncated α and δ subunits are required for heterodimer formation *in vivo*. *J Biol Chem*. 1996; 271:27575–27584. [PubMed: 8910344]
165. Tierney ML, Unwin N. Electron microscopic evidence for the assembly of soluble pentameric extracellular domains of the nicotinic acetylcholine receptor. *J Mol Biol*. 2000; 303:185–196. [PubMed: 11023785]
166. Psaridi-Linardaki L, Mamalaki A, Remoundos M, Tzartos SJ. Expression of soluble ligand- and antibody-binding extracellular domain of human muscle acetylcholine receptor α subunit in yeast *Pichia pastoris*. Role of glycosylation in α -bungarotoxin binding. *J Biol Chem*. 2002; 277:26980–26986. [PubMed: 12015305]
167. Kostelidou K, Trakas N, Zouridakis M, Bitzopoulou K, Sotiriadis A, Gavra I, Tzartos SJ. Expression and characterization of soluble forms of the extracellular domains of the β , γ and ϵ subunits of the human muscle acetylcholine receptor. *FEBS J*. 2006; 273:3557–3568. [PubMed: 16884496]
168. West AP, Bjorkman PJ, Dougherty DA, Lester HA. Expression and circular dichroism studies of the extracellular domain of the α subunit of the nicotinic acetylcholine receptor. *J Biol Chem*. 1997; 272:25468–25473. [PubMed: 9325259]
169. Wells GB, Anand R, Wang F, Lindstrom J. Water-soluble nicotinic acetylcholine receptor formed by $\alpha 7$ subunit extracellular domains. *J Biol Chem*. 1998; 273:964–973. [PubMed: 9422757]

170. Tsetlin VI, Dergousova NI, Azeeva EA, Kryukova EV, Kudelina IA, Shibanova ED, Kasheverov IE, Methfessel C. Refolding of the *Escherichia coli* expressed extracellular domain of $\alpha 7$ nicotinic acetylcholine receptor. *Eur J Biochem.* 2002; 269:2801–2809. [PubMed: 12047391]
171. Avramopoulou V, Mamalaki A, Tzartos SJ. Soluble, oligomeric, and ligand-binding extracellular domain of the human $\alpha 7$ acetylcholine receptor expressed in yeast: replacement of the hydrophobic cysteine loop by the hydrophilic loop of the ACh-binding protein enhances protein solubility. *J Biol Chem.* 2004; 279:38287–38293. [PubMed: 15226316]
172. Person AM, Bills KL, Liu H, Botting SK, Lindstrom J, Wells GB. Extracellular domain nicotinic acetylcholine receptors formed by $\alpha 4$ and $\beta 2$ subunits. *J Biol Chem.* 2005; 280:39990–40002. [PubMed: 16174636]
173. Zhou Y, Nelson ME, Kuryatov A, Choi C, Cooper J, Lindstrom J. Human $\alpha 4\beta 2$ acetylcholine receptors formed from linked subunits. *J Neurosci.* 2003; 23:9004–9015. [PubMed: 14534234]
174. Ericksen SS, Boileau AJ. Tandem couture: cys-loop receptor concatamer insights and caveats. *Mol Neurobiol.* 2007; 35:113–128. [PubMed: 17519509]
175. Dougherty DA. Unnatural amino acids as probes of protein structure and function. *Curr Opin Chem Biol.* 2000; 4:645–652. [PubMed: 11102869]
176. Beene DL, Dougherty DA, Lester HA. Unnatural amino acid mutagenesis in mapping ion channel function. *Curr Opin Neurobiol.* 2003; 13:264–270. [PubMed: 12850209]
177. England PM. Unnatural amino acid mutagenesis: a precise tool for probing protein structure and function. *Biochemistry.* 2004; 43:11623–11629. [PubMed: 15362846]
178. Wang L, Xie J, Schultz PG. Expanding the genetic code. *Annu Rev Biophys Biomol Struct.* 2006; 35:225–249. [PubMed: 16689635]
179. Nowak MW, Kearney PC, Sampson JR, Saks ME, Labarca CG, Silverman SK, Zhong W, Thorson J, Abelson JN, Davidson N, Schultz PG, Dougherty DA, Lester HA. Nicotinic receptor binding site probed with unnatural amino acid incorporation in intact cells. *Science.* 1995; 268:439–442. [PubMed: 7716551]
180. Cashin AL, Torrice MM, McMenimen KA, Lester HA, Dougherty DA. Chemical-scale studies on the role of a conserved aspartate in preorganizing the agonist binding site of the nicotinic acetylcholine receptor. *Biochemistry.* 2007; 46:630–639. [PubMed: 17223685]
181. Lee WY, Sine SM. Invariant aspartic acid in muscle nicotinic receptor contributes selectively to the kinetics of agonist binding. *J Gen Physiol.* 2004; 124:555–567. [PubMed: 15504901]
182. Beene DL, Price KL, Lester HA, Dougherty DA, Lummis SC. Tyrosine residues that control binding and gating in the 5-hydroxytryptamine₃ receptor revealed by unnatural amino acid mutagenesis. *J Neurosci.* 2004; 24:9097–9104. [PubMed: 15483128]
183. England PM, Zhang Y, Dougherty DA, Lester HA. Backbone mutations in transmembrane domains of a ligand-gated ion channel: implications for the mechanism of gating. *Cell.* 1999; 96:89–98. [PubMed: 9989500]
184. Hohsaka T, Ashizuka Y, Taira H, Murakami H, Sisido M. Incorporation of nonnatural amino acids into proteins by using various four-base codons in an *Escherichia coli* *in vitro* translation system. *Biochemistry.* 2001; 40:11060–11064. [PubMed: 11551202]
185. Rodriguez EA, Lester HA, Dougherty DA. *In vivo* incorporation of multiple unnatural amino acids through nonsense and frameshift suppression. *Proc Natl Acad Sci U S A.* 2006; 103:8650–8655. [PubMed: 16728509]
186. Link AJ, Tirrell DA. Reassignment of sense codons *in vivo*. *Methods.* 2005; 36:291–298. [PubMed: 16076455]
187. Sine SM. The nicotinic receptor ligand binding domain. *J Neurobiol.* 2002; 53:431–446. [PubMed: 12436411]
188. Grutter T, Le Novère N, Changeux JP. Rational understanding of nicotinic receptors drug binding. *Curr Top Med Chem.* 2004; 4:645–650. [PubMed: 14965300]
189. Sine SM, Wang HL, Gao F. Toward atomic-scale understanding of ligand recognition in the muscle nicotinic receptor. *Curr Med Chem.* 2004; 11:559–567. [PubMed: 15032604]
190. Dutertre S, Lewis RJ. Toxin insights into nicotinic acetylcholine receptors. *Biochem Pharmacol.* 2006; 72:661–670. [PubMed: 16716265]

191. Willcockson IU, Hong A, Whisenant RP, Edwards JB, Wang H, Sarkar HK, Pedersen SE. Orientation of *d*-tubocurarine in the muscle nicotinic acetylcholine receptor-binding site. *J Biol Chem.* 2002; 277:42249–42258. [PubMed: 12196523]
192. Wang HL, Gao F, Bren N, Sine SM. Curariform antagonists bind in different orientations to the nicotinic receptor ligand binding domain. *J Biol Chem.* 2003; 278:32284–32291. [PubMed: 12799358]
193. Cashin AL, Petersson EJ, Lester HA, Dougherty DA. Using physical chemistry to differentiate nicotinic from cholinergic agonists at the nicotinic acetylcholine receptor. *J Am Chem Soc.* 2005; 127:350–356. [PubMed: 15631485]
194. Pennington RA, Gao F, Sine SM, Prince RJ. Structural basis for epibatidine selectivity at desensitized nicotinic receptors. *Mol Pharmacol.* 2005; 67:123–131. [PubMed: 15496507]
195. Mourot A, Grutter T, Goeldner M, Kotzyba-Hibert F. Dynamic structural investigations on the *Torpedo* nicotinic acetylcholine receptor by time-resolved photoaffinity labeling. *Chembiochem.* 2006; 7:570–583. [PubMed: 16538695]
196. Vodovozova EL. Photoaffinity labeling and its application in structural biology. *Biochemistry (Mosc).* 2007; 72:1–20. [PubMed: 17309432]
197. Nirthanan S, Ziebell MR, Chiara DC, Hong F, Cohen JB. Photolabeling the *Torpedo* nicotinic acetylcholine receptor with 4-azido-2,3,5,6-tetrafluorobenzoylcholine, a partial agonist. *Biochemistry.* 2005; 44:13447–13456. [PubMed: 16216068]
198. Kapur A, Davies M, Dryden WF, Dunn SM. Tryptophan 86 of the α subunit in the *Torpedo* nicotinic acetylcholine receptor is important for channel activation by the bisquaternary ligand suberyldicholine. *Biochemistry.* 2006; 45:10337–10343. [PubMed: 16922509]
199. Carter CR, Cao L, Kawai H, Smith PA, Dryden WF, Raftery MA, Dunn SM. Chain length dependence of the interactions of bisquaternary ligands with the *Torpedo* nicotinic acetylcholine receptor. *Biochem Pharmacol.* 2007; 73:417–426. [PubMed: 17118342]
200. Stewart DS, Chiara DC, Cohen JB. Mapping the structural requirements for nicotinic acetylcholine receptor activation by using tethered alkyltrimethylammonium agonists and antagonists. *Biochemistry.* 2006; 45:10641–10653. [PubMed: 16939216]
201. Molles BE, Rezai P, Kline EF, McArdle JJ, Sine SM, Taylor P. Identification of residues at the α and ϵ subunit interfaces mediating species selectivity of wagnerin-1 for nicotinic acetylcholine receptors. *J Biol Chem.* 2002; 277:5433–5440. [PubMed: 11724791]
202. Konstantakaki M, Changeux JP, Taly A. Docking of α -cobratoxin suggests a basal conformation of the nicotinic receptor. *Biochem Biophys Res Commun.* 2007; 359:413–418. [PubMed: 17555709]
203. Mordvintsev DY, Polyak YL, Levtsova OV, Tourleigh YV, Kasheverov IE, Shaitan KV, Utkin YN, Tsetlin VI. A model for short α -neurotoxin bound to nicotinic acetylcholine receptor from *Torpedo californica*: comparison with long-chain α -neurotoxins and α -conotoxins. *Comput Biol Chem.* 2005; 29:398–411. [PubMed: 16290328]
204. Kessler P, Thai R, Beau F, Tarride JL, Ménez A. Photocrosslinking/label transfer: A key step in mapping short α -neurotoxin binding site on nicotinic acetylcholine receptor. *Bioconjug Chem.* 2006; 17:1482–1491. [PubMed: 17105227]
205. Johnson DA. C-terminus of a long α -neurotoxin is highly mobile when bound to the nicotinic acetylcholine receptor: a time-resolved fluorescence anisotropy approach. *Biophys Chem.* 2005; 116:213–218. [PubMed: 15894420]
206. Purohit Y, Grosman C. Estimating binding affinities of the nicotinic receptor for low-efficacy ligands using mixtures of agonists and two-dimensional concentration-response relationships. *J Gen Physiol.* 2006; 127:719–735. [PubMed: 16735756]
207. Meltzer RH, Thompson E, Soman KV, Song XZ, Ebalunode JO, Wensel TG, Briggs JM, Pedersen SE. Electrostatic steering at acetylcholine binding sites. *Biophys J.* 2006; 91:1302–1314. [PubMed: 16751247]
208. Cheng Y, Suen JK, Radi Z, Bond SD, Holst MJ, McCammon JA. Continuum simulations of acetylcholine diffusion with reaction-determined boundaries in neuromuscular junction models. *Biophys Chem.* 2007; 127:129–139. [PubMed: 17307283]

209. Huang X, Zheng F, Crooks PA, Dwoskin LP, Zhan CG. Modeling multiple species of nicotine and deschloroepibatidine interacting with $\alpha 4\beta 2$ nicotinic acetylcholine receptor: from microscopic binding to phenomenological binding affinity. *J Am Chem Soc.* 2005; 127:14401–14414. [PubMed: 16218635]
210. Bisson WH, Scapozza L, Westera G, Mu L, Schubiger PA. Ligand selectivity for the acetylcholine binding site of the rat $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nicotinic subtypes investigated by molecular docking. *J Med Chem.* 2005; 48:5123–5130. [PubMed: 16078832]
211. Yuan H, Petukhov PA. Computational evidence for the ligand selectivity to the $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nicotinic acetylcholine receptors. *Bioorg Med Chem.* 2006; 14:7936–7942. [PubMed: 16919961]
212. Zwart R, Vijverberg HP. Four pharmacologically distinct subtypes of $\alpha 4\beta 2$ nicotinic acetylcholine receptor expressed in *Xenopus laevis* oocytes. *Mol Pharmacol.* 1998; 54:1124–1131. [PubMed: 9855643]
213. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *Mol Pharmacol.* 2003; 63:332–341. [PubMed: 12527804]
214. Khiroug SS, Khiroug L, Yakel JL. Rat nicotinic acetylcholine receptor $\alpha 2\beta 2$ channels: comparison of functional properties with $\alpha 4\beta 2$ channels in *Xenopus* oocytes. *Neuroscience.* 2004; 124:817–822. [PubMed: 15026122]
215. López-Hernández GY, Sánchez-Padilla J, Ortiz-Acevedo A, Lizardi-Ortiz J, Salas-Vincenty J, Rojas LV, Lasalde-Dominicci JA. Nicotine-induced up-regulation and desensitization of $\alpha 4\beta 2$ neuronal nicotinic receptors depend on subunit ratio. *J Biol Chem.* 2004; 279:38007–38015. [PubMed: 15247303]
216. Zwart R, Broad LM, Xi Q, Lee M, Moroni M, Bermudez I, Sher E. 5-I A-85380 and TC-2559 differentially activate heterologously expressed $\alpha 4\beta 2$ nicotinic receptors. *Eur J Pharmacol.* 2006; 539:10–17. [PubMed: 16674940]
217. Horenstein NA, McCormack TJ, Stokes C, Ren K, Papke RL. Reversal of agonist selectivity by mutations of conserved amino acids in the binding site of nicotinic acetylcholine receptors. *J Biol Chem.* 2007; 282:5899–5909. [PubMed: 17189260]
218. Huang X, Zheng F, Chen X, Crooks PA, Dwoskin LP, Zhan CG. Modeling subtype-selective agonists binding with $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptors: effects of local binding and long-range electrostatic interactions. *J Med Chem.* 2006; 49:7661–7674. [PubMed: 17181149]
219. Marinou M, Tzartos SJ. Identification of regions involved in the binding of α -bungarotoxin to the human $\alpha 7$ neuronal nicotinic acetylcholine receptor using synthetic peptides. *Biochem J.* 2003; 372:543–554. [PubMed: 12614199]
220. Lyukmanova EN, Shenkarev ZO, Schulga AA, Ermolyuk YS, Mordvintsev DY, Utkin YN, Shoulepkov MA, Hogg R, Bertrand D, Dolgikh DA, Tsetlin VI, Kirpichnikov MP. Bacterial expression, NMR and electrophysiology analysis of chimeric short/long-chain α -neurotoxins acting on neuronal nicotinic receptors. *J Biol Chem.* 2007; 282:24784–24791. [PubMed: 17576769]
221. Bartos M, Rayes D, Bouzat C. Molecular determinants of pyrantel selectivity in nicotinic receptors. *Mol Pharmacol.* 2006; 70:1307–1318. [PubMed: 16825485]
222. Henchman RH, Wang HL, Sine SM, Taylor P, McCammon JA. Asymmetric structural motions of the homomeric $\alpha 7$ nicotinic receptor ligand binding domain revealed by molecular dynamics simulation. *Biophys J.* 2003; 85:3007–3018. [PubMed: 14581202]
223. Henchman RH, Wang HL, Sine SM, Taylor P, McCammon JA. Ligand-induced conformational change in the $\alpha 7$ nicotinic receptor ligand binding domain. *Biophys J.* 2005; 88:2564–2576. [PubMed: 15665135]
224. Locati A, Berthelot M, Evain M, Lebreton J, Le Questel JY, Mathé-Allainmat M, Planchat A, Renault E, Graton J. The exceptional hydrogen-bond properties of neutral and protonated lobeline. *J Phys Chem A.* 2007; 111:6397–6405. [PubMed: 17585846]
225. Carroll FI. Epibatidine structure-activity relationships. *Bioorg Med Chem Lett.* 2004; 14:1889–1896. [PubMed: 15050621]
226. Glennon RA. Medicinal chemistry of $\alpha 4\beta 2$ nicotinic cholinergic receptor ligands. *Prog Med Chem.* 2004; 42:55–123. [PubMed: 15003719]

227. Taylor P, Hansen SB, Talley TT, Hibbs RE, Radic Z. Contemporary paradigms for cholinergic ligand design guided by biological structure. *Bioorg Med Chem Lett*. 2004; 14:1875–1877. [PubMed: 15050619]
228. Cassels BK, Bermúdez I, Dajas F, Abin-Carriquiry JA, Wonnacott S. From ligand design to therapeutic efficacy: the challenge for nicotinic receptor research. *Drug Discov Today*. 2005; 10:1657–1665. [PubMed: 16376826]
229. Gotti C, Riganti L, Vailati S, Clementi F. Brain neuronal nicotinic receptors as new targets for drug discovery. *Curr Pharm Des*. 2006; 12:407–428. [PubMed: 16472136]
230. Mazurov A, Hauser T, Miller CH. Selective $\alpha 7$ nicotinic acetylcholine receptor ligands. *Curr Med Chem*. 2006; 13:1567–1584. [PubMed: 16787204]
231. Yogeewari P, Sriram D, Ratan Bal T, Thirumurugan R. Epibatidine and its analogues as nicotinic acetylcholine receptor agonist: an update. *Nat Prod Res*. 2006; 20:497–505. [PubMed: 16644549]
232. Broad LM, Sher E, Astles PC, Zwart R, O'Neill MJ. Selective $\alpha 7$ nicotinic acetylcholine receptor ligands for the treatment of neuropsychiatric diseases. *Drugs of the Future*. 2007; 32:161–170.
233. Romanelli MN, Gratteri P, Guandalini L, Martini E, Bonaccini C, Gualtieri F. Central nicotinic receptors: Structure, function, ligands, and therapeutic potential. *ChemMedChem*. 2007; 2:746–767. [PubMed: 17295372]
234. Carroll FI, Ware R, Brieady LE, Navarro HA, Damaj MI, Martin BR. Synthesis, nicotinic acetylcholine receptor binding, and antinociceptive properties of 2'-fluoro-3'-(substituted phenyl)deschloroepibatidine analogues. Novel nicotinic antagonist. *J Med Chem*. 2004; 47:4588–4594. [PubMed: 15317468]
235. Carroll FI, Ma W, Yokota Y, Lee JR, Brieady LE, Navarro HA, Damaj MI, Martin BR. Synthesis, nicotinic acetylcholine receptor binding, and antinociceptive properties of 3'-substituted deschloroepibatidine analogues. Novel nicotinic antagonists. *J Med Chem*. 2005; 48:1221–1228. [PubMed: 15715488]
236. Bunnelle WH, Daanen JF, Ryther KB, Schrimpf MR, Dart MJ, Gelain A, Meyer MD, Frost JM, Anderson DJ, Buckley M, Curzon P, Cao YJ, Puttfarcken P, Searle X, Ji J, Putman CB, Surowy C, Toma L, Barlocco D. Structure-activity studies and analgesic efficacy of N-(3-pyridinyl)-bridged bicyclic diamines, exceptionally potent agonists at nicotinic acetylcholine receptors. *J Med Chem*. 2007; 50:3627–3644. [PubMed: 17585748]
237. Avalos M, Parker MJ, Maddox FN, Carroll FI, Luetje CW. Effects of pyridine ring substitutions on affinity, efficacy, and subtype selectivity of neuronal nicotinic receptor agonist epibatidine. *J Pharmacol Exp Ther*. 2002; 302:1246–1252. [PubMed: 12183686]
238. Huang Y, Zhu Z, Xiao Y, Laruelle M. Epibatidine analogues as selective ligands for the $\alpha_x\beta_2$ -containing subtypes of nicotinic acetylcholine receptors. *Bioorg Med Chem Lett*. 2005; 15:4385–4388. [PubMed: 16039849]
239. Frost JM, Bunnelle WH, Tietje KR, Anderson DJ, Rueter LE, Curzon P, Surowy CS, Ji J, Daanen JF, Kohlhaas KL, Buckley MJ, Henry RF, Dyhring T, Ahring PK, Meyer MD. Synthesis and structure-activity relationships of 3,8-diazabicyclo[4.2.0]octane ligands, potent nicotinic acetylcholine receptor agonists. *J Med Chem*. 2006; 49:7843–7853. [PubMed: 17181167]
240. White R, Malpass JR, Handa S, Richard Baker S, Broad LM, Folly L, Mogg A. Epibatidine isomers and analogues: structure-activity relationships. *Bioorg Med Chem Lett*. 2006; 16:5493–5497. [PubMed: 16934977]
241. Grandl J, Sakr E, Kotzyba-Hibert F, Krieger F, Bertrand S, Bertrand D, Vogel H, Goeldner M, Hovius R. Fluorescent epibatidine agonists for neuronal and muscle-type nicotinic acetylcholine receptors. *Angew Chem Int Ed Engl*. 2007; 46:3505–3508. [PubMed: 17385777]
242. Dukat M, Ramunno A, Banzi R, Damaj MI, Martin B, Glennon RA. 3-(2-Aminoethyl)pyridine analogs as $\alpha 4\beta 2$ nicotinic cholinergic receptor ligands. *Bioorg Med Chem Lett*. 2005; 15:4308–4312. [PubMed: 16039854]
243. Xiao Y, Fan H, Musachio JL, Wei ZL, Chellappan SK, Kozikowski AP, Kellar KJ. Sazetidine-A, a novel ligand that desensitizes $\alpha 4\beta 2$ nicotinic acetylcholine receptors without activating them. *Mol Pharmacol*. 2006; 70:1454–1460. [PubMed: 16857741]

244. Audouze K, Nielsen EO, Olsen GM, Ahring P, Jørgensen TD, Peters D, Liljefors T, Balle T. New ligands with affinity for the $\alpha 4\beta 2$ subtype of nicotinic acetylcholine receptors. Synthesis, receptor binding, and 3D-QSAR modeling. *J Med Chem.* 2006; 49:3159–3171. [PubMed: 16722635]
245. Kozikowski AP, Chellappan SK, Xiao Y, Bajjuri KM, Yuan H, Kellar KJ, Petukhov PA. Chemical medicine: Novel 10-substituted cytosine derivatives with increased selectivity for $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *ChemMedChem.* 2007; 2:1157–1161. [PubMed: 17530728]
246. Papke RL, Buhr JD, Francis MM, Choi KI, Thinschmidt JS, Horenstein NA. The effects of subunit composition on the inhibition of nicotinic receptors by the amphipathic blocker 2,2,6,6-tetramethylpiperidin-4-yl heptanoate. *Mol Pharmacol.* 2005; 67:1977–1990. [PubMed: 15761116]
247. Kozikowski AP, Chellappan SK, Henderson D, Fulton R, Giboureau N, Xiao Y, Wei ZL, Guilloteau D, Emond P, Dolle F, Kellar KJ, Kassiou M. Acetylenic pyridines for use in PET imaging of nicotinic receptors. *ChemMedChem.* 2007; 2:54–57. [PubMed: 17096450]
248. Biton B, Bergis OE, Galli F, Nedelec A, Lochead AW, Jegham S, Godet D, Lanneau C, Santamaria R, Chesney F, Léonardon J, Granger P, Debono MW, Bohme GA, Sgard F, Besnard F, Graham D, Coste A, Oblin A, Curet O, Vigé X, Voltz C, Rouquier L, Souilhac J, Santucci V, Gueudet C, Françon D, Steinberg R, Griebel G, Oury-Donat F, George P, Avenet P, Scatton B. SSR180711, a novel selective $\alpha 7$ nicotinic receptor partial agonist: (1) binding and functional profile. *Neuropsychopharmacology.* 2007; 32:1–16. [PubMed: 17019409]
249. Ivy Carroll F, Ma W, Navarro HA, Abraham P, Wolckenhauer SA, Damaj MI, Martin BR. Synthesis, nicotinic acetylcholine receptor binding, antinociceptive and seizure properties of methyllycaconitine analogs. *Bioorg Med Chem.* 2007; 15:678–685. [PubMed: 17098430]
250. Baker SR, Boot J, Brunavs M, Dobson D, Green R, Hayhurst L, Keenan M, Wallace L. High affinity ligands for the $\alpha 7$ nicotinic receptor that show no cross-reactivity with the 5-HT₃ receptor. *Bioorg Med Chem Lett.* 2005; 15:4727–4730. [PubMed: 16165358]
251. Tomizawa M, Talley TT, Maltby D, Durkin KA, Medzihradsky KF, Burlingame AL, Taylor P, Casida JE. Mapping the elusive neonicotinoid binding site. *Proc Natl Acad Sci U S A.* 2007; 104:9075–9080. [PubMed: 17485662]
252. Wang Y, Cheng J, Qian X, Li Z. Actions between neonicotinoids and key residues of insect nAChR based on an ab initio quantum chemistry study: hydrogen bonding and cooperative π - π interaction. *Bioorg Med Chem.* 2007; 15:2624–2630. [PubMed: 17300943]
253. McIntosh JM, Santos AD, Olivera BM. *Conus* peptides targeted to specific nicotinic acetylcholine receptor subtypes. *Annu Rev Biochem.* 1999; 68:59–88. [PubMed: 10872444]
254. Olivera BM. *Conus* venom peptides: Reflections from the biology of clades and species. *Annu Rev Ecol Syst.* 2002; 33:25–42.
255. Lewis RJ. Conotoxins as selective inhibitors of neuronal ion channels, receptors and transporters. *IUBMB Life.* 2004; 56:89–93. [PubMed: 15085932]
256. Nicke A, Wonnacott S, Lewis RJ. α -Conotoxins as tools for the elucidation of structure and function of neuronal nicotinic acetylcholine receptor subtypes. *Eur J Biochem.* 2004; 271:2305–2319. [PubMed: 15182346]
257. Terlau H, Olivera BM. *Conus* venoms: a rich source of novel ion channel-targeted peptides. *Physiol Rev.* 2004; 84:41–68. [PubMed: 14715910]
258. Janes RW. α -Conotoxins as selective probes for nicotinic acetylcholine receptor subclasses. *Curr Opin Pharmacol.* 2005; 5:280–292. [PubMed: 15907916]
259. Norton RS, Olivera BM. Conotoxins down under. *Toxicon.* 2006; 48:780–798. [PubMed: 16952384]
260. Olivera BM, Rivier J, Clark C, Ramilo CA, Corpuz GP, Abogadie FC, Mena EE, Woodward SR, Hillyard DR, Cruz LJ. Diversity of *Conus* neuropeptides. *Science.* 1990; 249:257–263. [PubMed: 2165278]
261. Teichert RW, Rivier J, Torres J, Dykert J, Miller C, Olivera BM. A uniquely selective inhibitor of the mammalian fetal neuromuscular nicotinic acetylcholine receptor. *J Neurosci.* 2005; 25:732–736. [PubMed: 15659611]

262. Liu L, Chew G, Hawrot E, Chi C, Wang C. Two potent $\alpha 3/5$ conotoxins from piscivorous *Conus achatinus*. *Acta Biochim Biophys Sin* (Shanghai). 2007; 39:438–444. [PubMed: 17558449]
263. López-Vera E, Jacobsen RB, Ellison M, Olivera BM, Teichert RW. A novel alpha conotoxin (α -PIB) isolated from *C. purpurascens* is selective for skeletal muscle nicotinic acetylcholine receptors. *Toxicon*. 2007; 49:1193–1199. [PubMed: 17382984]
264. Imperial JS, Bansal PS, Alewood PF, Daly NL, Craik DJ, Sporning A, Terlau H, López-Vera E, Bandyopadhyay PK, Olivera BM. A novel conotoxin inhibitor of Kv1.6 channel and nAChR subtypes defines a new superfamily of conotoxins. *Biochemistry*. 2006; 45:8331–8340. [PubMed: 16819832]
265. Loughnan M, Nicke A, Jones A, Schroeder CI, Nevin ST, Adams DJ, Alewood PF, Lewis RJ. Identification of a novel class of nicotinic receptor antagonists: dimeric conotoxins VxXIIA, VxXIIB, and VxXIIC from *Conus vexillum*. *J Biol Chem*. 2006; 281:24745–24755. [PubMed: 16790424]
266. Whiteaker P, Christensen S, Yoshikami D, Dowell C, Watkins M, Gulyas J, Rivier J, Olivera BM, McIntosh JM. Discovery, synthesis, and structure activity of a highly selective $\alpha 7$ nicotinic acetylcholine receptor antagonist. *Biochemistry*. 2007; 46:6628–6638. [PubMed: 17497892]
267. Ellison M, Haberlandt C, Gomez-Casati ME, Watkins M, Elgoyhen AB, McIntosh JM, Olivera BM. α -RgIA: a novel conotoxin that specifically and potently blocks the $\alpha 9\alpha 10$ nAChR. *Biochemistry*. 2006; 45:1511–1517. [PubMed: 16445293]
268. Vincler M, Wittenauer S, Parker R, Ellison M, Olivera BM, McIntosh JM. Molecular mechanism for analgesia involving specific antagonism of $\alpha 9\alpha 10$ nicotinic acetylcholine receptors. *Proc Natl Acad Sci U S A*. 2006; 103:17880–17884. [PubMed: 17101979]
269. Azam L, Dowell C, Watkins M, Stitzel JA, Olivera BM, McIntosh JM. α -Conotoxin BuIA, a novel peptide from *Conus bullatus*, distinguishes among neuronal nicotinic acetylcholine receptors. *J Biol Chem*. 2005; 280:80–87. [PubMed: 15520009]
270. Cortez L, Marino-Buslje C, de Jiménez Bonino MB, Hellman U. Interactions between α -conotoxin MI and the *Torpedo marmorata* receptor α - δ interface. *Biochem Biophys Res Commun*. 2007; 355:275–279. [PubMed: 17292857]
271. Dutertre S, Nicke A, Tyndall JD, Lewis RJ. Determination of α -conotoxin binding modes on neuronal nicotinic acetylcholine receptors. *J Mol Recognit*. 2004; 17:339–347. [PubMed: 15227641]
272. Dutertre S, Nicke A, Lewis RJ. $\beta 2$ subunit contribution to $4/7$ α -conotoxin binding to the nicotinic acetylcholine receptor. *J Biol Chem*. 2005; 280:30460–30468. [PubMed: 15929983]
273. Kasheverov IE, Chiara DC, Zhmak MN, Maslennikov IV, Pashkov VS, Arseniev AS, Utkin YN, Cohen JB, Tsetlin VI. α -Conotoxin GI benzoylphenylalanine derivatives. ^1H -NMR structures and photoaffinity labeling of the *Torpedo californica* nicotinic acetylcholine receptor. *FEBS J*. 2006; 273:1373–1388. [PubMed: 16689926]
274. Maelicke A, Schrattenholz A, Samochocki M, Radina M, Albuquerque EX. Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease. *Behav Brain Res*. 2000; 113:199–206. [PubMed: 10942046]
275. Pereira EF, Hilmas C, Santos MD, Alkondon M, Maelicke A, Albuquerque EX. Unconventional ligands and modulators of nicotinic receptors. *J Neurobiol*. 2002; 53:479–500. [PubMed: 12436414]
276. Hogg RC, Buisson B, Bertrand D. Allosteric modulation of ligand-gated ion channels. *Biochem Pharmacol*. 2005; 70:1267–1276. [PubMed: 16043127]
277. Arias HR, Bhumireddy P, Bouzat C. Molecular mechanisms and binding site locations for noncompetitive antagonists of nicotinic acetylcholine receptors. *Int J Biochem Cell Biol*. 2006; 38:1254–1276. [PubMed: 16520081]
278. Schrattenholz A, Pereira EF, Roth U, Weber KH, Albuquerque EX, Maelicke A. Agonist responses of neuronal nicotinic acetylcholine receptors are potentiated by a novel class of allosterically acting ligands. *Mol Pharmacol*. 1996; 49:1–6. [PubMed: 8569694]
279. Samochocki M, Zerlin M, Jostock R, Groot Kormelink PJ, Luyten WH, Albuquerque EX, Maelicke A. Galantamine is an allosterically potentiating ligand of the human $\alpha 4/\beta 2$ nAChR. *Acta Neurol Scand*. 2000; 102(s176):68–73.

280. Texidó L, Ros E, Martín-Satué M, López S, Aleu J, Marsal J, Solsona C. Effect of galantamine on the human $\alpha 7$ neuronal nicotinic acetylcholine receptor, the *Torpedo* nicotinic acetylcholine receptor and spontaneous cholinergic synaptic activity. *Br J Pharmacol*. 2005; 145:672–678. [PubMed: 15834443]
281. Akk G, Steinbach JH. Galantamine activates muscle-type nicotinic acetylcholine receptors without binding to the acetylcholine-binding site. *J Neurosci*. 2005; 25:1992–2001. [PubMed: 15728839]
282. Lopes C, Pereira EF, Wu HQ, Purushottamachar P, Njar V, Schwarcz R, Albuquerque EX. Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at $\alpha 7^*$ nicotinic receptors. *J Pharmacol Exp Ther*. 2007; 322:48–58. [PubMed: 17446300]
283. McKay DB, Chang C, Gonzalez-Cestari TF, McKay SB, El-Hajj RA, Bryant DL, Zhu MX, Swaan PW, Arason KM, Pulipaka AB, Orac CM, Bergmeier SC. Analogs of methyllycaconitine as novel noncompetitive inhibitors of nicotinic receptors: pharmacological characterization, computational modeling, and pharmacophore development. *Mol Pharmacol*. 2007; 71:1288–1297. [PubMed: 17308033]
284. Broad LM, Zwart R, Pearson KH, Lee M, Wallace L, McPhie GI, Emkey R, Hollinshead SP, Dell CP, Baker SR, Sher E. Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. *J Pharmacol Exp Ther*. 2006; 318:1108–1117. [PubMed: 16738207]
285. Sala F, Mulet J, Reddy KP, Bernal JA, Wikman P, Valor LM, Peters L, König GM, Criado M, Sala S. Potentiation of human $\alpha 4\beta 2$ neuronal nicotinic receptors by a *Flustra foliacea* metabolite. *Neurosci Lett*. 2005; 373:144–149. [PubMed: 15567570]
286. Kim JS, Padnya A, Weltzin M, Edmonds BW, Schulte MK, Glennon RA. Synthesis of desformylflustrabromine and its evaluation as an $\alpha 4\beta 2$ and $\alpha 7$ nACh receptor modulator. *Bioorg Med Chem Lett*. 2007; 17:4855–4860. [PubMed: 17604168]
287. Grønlien JH, Håkerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J. Distinct profiles of $\alpha 7$ nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol Pharmacol*. 2007; 72:715–724. [PubMed: 17565004]
288. Arias HR, Bhumireddy P, Spitzmaul G, Trudell JR, Bouzat C. Molecular mechanisms and binding site location for the noncompetitive antagonist crystal violet on nicotinic acetylcholine receptors. *Biochemistry*. 2006; 45:2014–2026. [PubMed: 16475790]
289. Iorga B, Herlem D, Barre E, Guillou C. Acetylcholine nicotinic receptors: finding the putative binding site of allosteric modulators using the “blind docking” approach. *J Mol Model*. 2006; 12:366–372. [PubMed: 16372175]
290. Nievas GA, Barrantes FJ, Antollini SS. Conformation-sensitive steroid and fatty acid sites in the transmembrane domain of the nicotinic acetylcholine receptor. *Biochemistry*. 2007; 46:3503–3512. [PubMed: 17319650]
291. Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate GABA_A receptors through two discrete transmembrane sites. *Nature*. 2006; 444:486–489. [PubMed: 17108970]
292. Rodrigues-Pinguet N, Jia L, Li M, Figl A, Klaassen A, Truong A, Lester HA, Cohen BN. Five ADNFLE mutations reduce the Ca^{2+} dependence of the mammalian $\alpha 4\beta 2$ acetylcholine response. *J Physiol*. 2003; 550:11–26. [PubMed: 12754307]
293. Rodrigues-Pinguet NO, Pinguet TJ, Figl A, Lester HA, Cohen BN. Mutations linked to autosomal dominant nocturnal frontal lobe epilepsy affect allosteric Ca^{2+} activation of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. *Mol Pharmacol*. 2005; 68:487–501. [PubMed: 15901849]
294. García-Colunga J, González-Herrera M, Miledi R. Modulation of $\alpha 2\beta 4$ neuronal nicotinic acetylcholine receptors by zinc. *Neuroreport*. 2001; 12:147–150. [PubMed: 11201076]
295. Hsiao B, Dweck D, Luetje CW. Subunit-dependent modulation of neuronal nicotinic receptors by zinc. *J Neurosci*. 2001; 21:1848–1856. [PubMed: 11245669]
296. Hsiao B, Mihalak KB, Repicky SE, Everhart D, Mederos AH, Malhotra A, Luetje CW. Determinants of zinc potentiation on the $\alpha 4$ subunit of neuronal nicotinic receptors. *Mol Pharmacol*. 2006; 69:27–36. [PubMed: 16189299]

297. Sumikawa K, Miledi R. Assembly and *N*-glycosylation of all ACh receptor subunits are required for their efficient insertion into plasma membranes. *Mol Brain Res.* 1989; 5:183–192. [PubMed: 2725195]
298. Gehle VM, Sumikawa K. Site-directed mutagenesis of the conserved *N*-glycosylation site on the nicotinic acetylcholine receptor subunits. *Brain Res Mol Brain Res.* 1991; 11:17–25. [PubMed: 1662742]
299. Gehle VM, Walcott EC, Nishizaki T, Sumikawa K. *N*-glycosylation at the conserved sites ensures the expression of properly folded functional ACh receptors. *Brain Res Mol Brain Res.* 1997; 45:219–229. [PubMed: 9149096]
300. Ramanathan VK, Hall ZW. Altered glycosylation sites of the δ subunit of the acetylcholine receptor (AChR) reduce $\alpha\delta$ association and receptor assembly. *J Biol Chem.* 1999; 274:20513–20520. [PubMed: 10400680]
301. Wanamaker CP, Green WN. *N*-linked glycosylation is required for nicotinic receptor assembly but not for subunit associations with calnexin. *J Biol Chem.* 2005; 280:33800–33810. [PubMed: 16091366]
302. Chen D, Dang H, Patrick JW. Contributions of *N*-linked glycosylation to the expression of a functional $\alpha 7$ -nicotinic receptor in *Xenopus* oocytes. *J Neurochem.* 1998; 70:349–357. [PubMed: 9422381]
303. Nishizaki T. *N*-glycosylation sites on the nicotinic ACh receptor subunits regulate receptor channel desensitization and conductance. *Brain Res Mol Brain Res.* 2003; 114:172–176. [PubMed: 12829329]
304. daCosta CJ, Kaiser DE, Baenziger JE. Role of glycosylation and membrane environment in nicotinic acetylcholine receptor stability. *Biophys J.* 2005; 88:1755–1764. [PubMed: 15626708]
305. Hamilton SR, Bobrowicz P, Bobrowicz B, Davidson RC, Li H, Mitchell T, Nett JH, Rausch S, Stadheim TA, Wischnewski H, Wildt S, Gerngross TU. Production of complex human glycoproteins in yeast. *Science.* 2003; 301:1244–1246. [PubMed: 12947202]
306. Wildt S, Gerngross TU. The humanization of *N*-glycosylation pathways in yeast. *Nat Rev Microbiol.* 2005; 3:119–128. [PubMed: 15685223]
307. Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang Y, Rios S, Bobrowicz P, Stadheim TA, Li H, Choi BK, Hopkins D, Wischnewski H, Roser J, Mitchell T, Strawbridge RR, Hoopes J, Wildt S, Gerngross TU. Humanization of yeast to produce complex terminally sialylated glycoproteins. *Science.* 2006; 313:1441–1443. [PubMed: 16960007]
308. Seo NS, Hollister JR, Jarvis DL. Mammalian glycosyltransferase expression allows sialoglycoprotein production by baculovirus-infected insect cells. *Protein Expr Purif.* 2001; 22:234–241. [PubMed: 11437599]
309. Harrison RL, Jarvis DL. Protein *N*-glycosylation in the baculovirus-insect cell expression system and engineering of insect cells to produce “mammalianized” recombinant glycoproteins. *Adv Virus Res.* 2006; 68:159–191. [PubMed: 16997012]
310. Négrerie M, Martin JL, Nghim HO. Functionality of nitrated acetylcholine receptor: the two-step formation of nitrotyrosines reveals their differential role in effectors binding. *FEBS Lett.* 2005; 579:2643–2647. [PubMed: 15862303]
311. Paas Y, Gibor G, Grailhe R, Savatier-Duclert N, Dufresne V, Sunesen M, de Carvalho LP, Changeux JP, Attali B. Pore conformations and gating mechanism of a Cys-loop receptor. *Proc Natl Acad Sci U S A.* 2005; 102:15877–15882. [PubMed: 16247006]
312. Kandt C, Ash WL, Tieleman DP. Setting up and running molecular dynamics simulations of membrane proteins. *Methods.* 2007; 41:475–488. [PubMed: 17367719]
313. Law RJ, Henschman RH, McCammon JA. A gating mechanism proposed from a simulation of a human $\alpha 7$ nicotinic acetylcholine receptor. *Proc Natl Acad Sci U S A.* 2005; 102:6813–6818. [PubMed: 15857954]
314. Taly A, Delarue M, Grutter T, Nilges M, Le Novre N, Corringer PJ, Changeux JP. Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys J.* 2005; 88:3954–3965. [PubMed: 15805177]

315. Cheng X, Lu B, Grant B, Law RJ, McCammon JA. Channel opening motion of $\alpha 7$ nicotinic acetylcholine receptor as suggested by normal mode analysis. *J Mol Biol.* 2006; 355:310–324. [PubMed: 16307758]
316. Cheng X, Wang H, Grant B, Sine SM, McCammon JA. Targeted molecular dynamics study of C-loop closure and channel gating in nicotinic receptors. *PLoS Comput Biol.* 2006; 2:e134. [PubMed: 17009865]
317. Taly A, Corringer P-J, Grutter T, Prado de Carvalho L, Karplus M, Changeux J-P. Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. *Proc Natl Acad Sci U S A.* 2006; 103:16965–16970. [PubMed: 17077146]
318. Cheng X, Ivanov I, Wang H, Sine SM, McCammon JA. Nanosecond time scale conformational dynamics of the human $\alpha 7$ nicotinic acetylcholine receptor. *Biophys J.* 2007; 93:2622–2634. [PubMed: 17573436]
319. Arevalo E, Chiara DC, Forman SA, Cohen JB, Miller KW. Gating-enhanced accessibility of hydrophobic sites within the transmembrane region of the nicotinic acetylcholine receptor's δ -subunit. A time-resolved photolabeling study. *J Biol Chem.* 2005; 280:13631–13640. [PubMed: 15664985]
320. Szarecka A, Xu Y, Tang P. Dynamics of heteropentameric nicotinic acetylcholine receptor: Implications of the gating mechanism. *Proteins.* 2007; 68:948–960. [PubMed: 17546671]
321. Bouzat C, Gumilar F, Spitzmaul G, Wang HL, Rayes D, Hansen SB, Taylor P, Sine SM. Coupling of agonist binding to channel gating in an ACh-binding protein linked to an ion channel. *Nature.* 2004; 430:896–900. [PubMed: 15318223]
322. Grutter T, de Carvalho LP, Dufresne V, Taly A, Edelstein SJ, Changeux JP. Molecular tuning of fast gating in pentameric ligand-gated ion channels. *Proc Natl Acad Sci U S A.* 2005; 102:18207–18212. [PubMed: 16319224]
323. Czajkowski C. Neurobiology: triggers for channel opening. *Nature.* 2005; 438:167–168. [PubMed: 16281019]
324. Lee WY, Sine SM. Principal pathway coupling agonist binding to channel gating in nicotinic receptors. *Nature.* 2005; 438:243–247. [PubMed: 16281039]
325. Lummis SC, Beene DL, Lee LW, Lester HA, Broadhurst RW, Dougherty DA. *Cis-trans* isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. *Nature.* 2005; 438:248–252. [PubMed: 16281040]
326. Andreotti AH. Opening the pore hinges on proline. *Nat Chem Biol.* 2006; 2:13–14. [PubMed: 16408084]
327. Young GT, Broad LM, Zwart R, Astles PC, Bodkin M, Sher E, Millar NS. Species selectivity of a nicotinic acetylcholine receptor agonist is conferred by two adjacent extracellular $\beta 4$ amino acids that are implicated in the coupling of binding to channel gating. *Mol Pharmacol.* 2007; 71:389–397. [PubMed: 17065235]
328. Mukhtasimova N, Free C, Sine SM. Initial coupling of binding to gating mediated by conserved residues in the muscle nicotinic receptor. *J Gen Physiol.* 2005; 126:23–39. [PubMed: 15955875]
329. Mukhtasimova N, Sine SM. An intersubunit trigger of channel gating in the muscle nicotinic receptor. *J Neurosci.* 2007; 27:4110–4119. [PubMed: 17428989]
330. Xiu X, Hanek AP, Wang J, Lester HA, Dougherty DA. A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. *J Biol Chem.* 2005; 280:41655–41666. [PubMed: 16216879]
331. Chen Y, Reilly K, Chang Y. Evolutionarily conserved allosteric network in the Cys loop family of ligand-gated ion channels revealed by statistical covariance analyses. *J Biol Chem.* 2006; 281:18184–18192. [PubMed: 16595655]
332. Sala F, Mulet J, Sala S, Gerber S, Criado M. Charged amino acids of the N-terminal domain are involved in coupling binding and gating in $\alpha 7$ nicotinic receptors. *J Biol Chem.* 2005; 280:6642–6647. [PubMed: 15611071]
333. McLaughlin JT, Fu J, Sproul AD, Rosenberg RL. Role of the outer β -sheet in divalent cation modulation of $\alpha 7$ nicotinic receptors. *Mol Pharmacol.* 2006; 70:16–22. [PubMed: 16533908]
334. McLaughlin JT, Fu J, Rosenberg RL. Agonist-driven conformational changes in the inner β -sheet of $\alpha 7$ nicotinic receptors. *Mol Pharmacol.* 2007; 71:1312–1318. [PubMed: 17325129]

335. Auerbach A. Life at the top: the transition state of AChR gating. *Sci STKE*. 2003; 2003:re11 . [PubMed: 12824477]
336. Grosman C, Zhou M, Auerbach A. Mapping the conformational wave of acetylcholine receptor channel gating. *Nature*. 2000; 403:773–776. [PubMed: 10693806]
337. Grosman C. Free-energy landscapes of ion-channel gating are malleable: changes in the number of bound ligands are accompanied by changes in the location of the transition state in acetylcholine-receptor channels. *Biochemistry*. 2003; 42:14977–14987. [PubMed: 14674774]
338. Auerbach A. Gating of acetylcholine receptor channels: brownian motion across a broad transition state. *Proc Natl Acad Sci U S A*. 2005; 102:1408–1412. [PubMed: 15665102]
339. Mitra A, Tascione R, Auerbach A, Licht S. Plasticity of acetylcholine receptor gating motions via rate-energy relationships. *Biophys J*. 2005; 89:3071–3078. [PubMed: 16113115]
340. Chakrapani S, Bailey TD, Auerbach A. Gating dynamics of the acetylcholine receptor extracellular domain. *J Gen Physiol*. 2004; 123:341–356. [PubMed: 15051806]
341. Mitra A, Cymes GD, Auerbach A. Dynamics of the acetylcholine receptor pore at the gating transition state. *Proc Natl Acad Sci U S A*. 2005; 102:15069–15074. [PubMed: 16217024]
342. Purohit P, Mitra A, Auerbach A. A stepwise mechanism for acetylcholine receptor channel gating. *Nature*. 2007; 446:930–933. [PubMed: 17443187]
343. Cadugan DJ, Auerbach A. Conformational dynamics of the α M3 transmembrane helix during acetylcholine receptor channel gating. *Biophys J*. 2007; 93:859–865. [PubMed: 17513382]
344. Mitra A, Bailey TD, Auerbach AL. Structural dynamics of the M4 transmembrane segment during acetylcholine receptor gating. *Structure*. 2004; 12:1909–1918. [PubMed: 15458639]
345. Chakrapani S, Auerbach A. A speed limit for conformational change of an allosteric membrane protein. *Proc Natl Acad Sci U S A*. 2005; 102:87–92. [PubMed: 15618401]
346. Colquhoun D. From shut to open: what can we learn from linear free energy relationships? *Biophys J*. 2005; 89:3673–3675. [PubMed: 16319077]
347. Zhou Y, Pearson JE, Auerbach A. Φ -value analysis of a linear, sequential reaction mechanism: theory and application to ion channel gating. *Biophys J*. 2005; 89:3680–3685. [PubMed: 16183877]
348. Bali M, Akabas MH. The location of a closed channel gate in the GABA_A receptor channel. *J Gen Physiol*. 2007; 129:145–159. [PubMed: 17227918]
349. Panicker S, Cruz H, Arrabit C, Slesinger PA. Evidence for a centrally located gate in the pore of a serotonin-gated ion channel. *J Neurosci*. 2002; 22:1629–1639. [PubMed: 11880493]
350. Akabas MH, Stauffer DA, Xu M, Karlin A. Acetylcholine receptor channel structure probed in cysteine-substitution mutants. *Science*. 1992; 258:307–310. [PubMed: 1384130]
351. Karlin A, Akabas MH. Substituted-cysteine accessibility method. *Methods Enzymol*. 1998; 293:123–145. [PubMed: 9711606]
352. Wilson GG, Karlin A. The location of the gate in the acetylcholine receptor channel. *Neuron*. 1998; 20:1269–1281. [PubMed: 9655513]
353. Zhang H, Karlin A. Contribution of the β subunit M2 segment to the ion-conducting pathway of the acetylcholine receptor. *Biochemistry*. 1998; 37:7952–7964. [PubMed: 9609688]
354. Purohit Y, Grosman C. Block of muscle nicotinic receptors by choline suggests that the activation and desensitization gates act as distinct molecular entities. *J Gen Physiol*. 2006; 127:703–717. [PubMed: 16735755]
355. Keramidas A, Moorhouse AJ, Schofield PR, Barry PH. Ligand-gated ion channels: mechanisms underlying ion selectivity. *Prog Biophys Mol Biol*. 2004; 86:161–204. [PubMed: 15288758]
356. Corry B. Theoretical conformation of the closed and open states of the acetylcholine receptor channel. *Biochim Biophys Acta*. 2004; 1663:2–5. [PubMed: 15157602]
357. Beckstein O, Sansom MS. A hydrophobic gate in an ion channel: the closed state of the nicotinic acetylcholine receptor. *Phys Biol*. 2006; 3:147–159. [PubMed: 16829701]
358. Corry B. An energy-efficient gating mechanism in the acetylcholine receptor channel suggested by molecular and Brownian dynamics. *Biophys J*. 2006; 90:799–810. [PubMed: 16284265]

359. Hung A, Tai K, Sansom MS. Molecular dynamics simulation of the M2 helices within the nicotinic acetylcholine receptor transmembrane domain: structure and collective motions. *Biophys J*. 2005; 88:3321–3333. [PubMed: 15722430]
360. Saladino AC, Xu Y, Tang P. Homology modeling and molecular dynamics simulations of transmembrane domain structure of human neuronal nicotinic acetylcholine receptor. *Biophys J*. 2005; 88:1009–1017. [PubMed: 15574706]
361. Ivanov I, Cheng X, Sine SM, McCammon JA. Barriers to ion translocation in cationic and anionic receptors from the Cys-loop family. *J Am Chem Soc*. 2007; 129:8217–8224. [PubMed: 17552523]
362. Kim S, Chamberlain AK, Bowie JU. A simple method for modeling transmembrane helix oligomers. *J Mol Biol*. 2003; 329:831–840. [PubMed: 12787681]
363. Kim S, Chamberlain AK, Bowie JU. A model of the closed form of the nicotinic acetylcholine receptor M2 channel pore. *Biophys J*. 2004; 87:792–799. [PubMed: 15298888]
364. Saiz L, Klein ML. The transmembrane domain of the acetylcholine receptor: insights from simulations on synthetic peptide models. *Biophys J*. 2005; 88:959–970. [PubMed: 15556982]
365. Grandl J, Danelon C, Hovius R, Vogel H. Functional asymmetry of transmembrane segments in nicotinic acetylcholine receptors. *Eur Biophys J*. 2006; 35:685–693. [PubMed: 16838147]
366. Cymes GD, Ni Y, Grosman C. Probing ion-channel pores one proton at a time. *Nature*. 2005; 438:975–980. [PubMed: 16355215]
367. Nieves-Cintrón M, Caballero-Rivera D, Navedo MF, Lasalde-Dominicci JA. Contribution of valine 7' of TMD2 to gating of neuronal $\alpha 3$ receptor subtypes. *J Neurosci Res*. 2006; 84:1778–1788. [PubMed: 17044037]
368. Dahan DS, Dibas MI, Petersson EJ, Auyeung VC, Chanda B, Bezanilla F, Dougherty DA, Lester HA. A fluorophore attached to nicotinic acetylcholine receptor $\beta M2$ detects productive binding of agonist to the $\alpha \delta$ site. *Proc Natl Acad Sci U S A*. 2004; 101:10195–10200. [PubMed: 15218096]
369. Xu Y, Barrantes FJ, Shen J, Luo X, Zhu W, Chen K, Jiang H. Blocking of the nicotinic acetylcholine receptor ion channel by chlorpromazine, a noncompetitive inhibitor: A molecular dynamics simulation study. *J Phys Chem B*. 2006; 110:20640–20648. [PubMed: 17034254]
370. Spitzmaul G, Corradi J, Bouzat C. Mechanistic contributions of residues in the M1 transmembrane domain of the nicotinic receptor to channel gating. *Mol Membr Biol*. 2004; 21:39–50. [PubMed: 14668137]
371. de Planque MR, Rijkers DT, Fletcher JI, Liskamp RM, Separovic F. The $\alpha M1$ segment of the nicotinic acetylcholine receptor exhibits conformational flexibility in a membrane environment. *Biochim Biophys Acta*. 2004; 1665:40–47. [PubMed: 15471569]
372. Corradi J, Spitzmaul G, De Rosa MJ, Costabel M, Bouzat C. Role of pairwise interactions between M1 and M2 domains of the nicotinic receptor in channel gating. *Biophys J*. 2007; 92:76–86. [PubMed: 17028140]
373. Xu Y, Barrantes FJ, Luo X, Chen K, Shen J, Jiang H. Conformational dynamics of the nicotinic acetylcholine receptor channel: a 35-ns molecular dynamics simulation study. *J Am Chem Soc*. 2005; 127:1291–1299. [PubMed: 15669869]
374. de Almeida RF, Loura LM, Prieto M, Watts A, Fedorov A, Barrantes FJ. Structure and dynamics of the $\gamma M4$ transmembrane domain of the acetylcholine receptor in lipid bilayers: insights into receptor assembly and function. *Mol Membr Biol*. 2006; 23:305–315. [PubMed: 16923724]
375. Meltzer RH, Lurtz MM, Wensel TG, Pedersen SE. Nicotinic acetylcholine receptor channel electrostatics determined by diffusion-enhanced luminescence energy transfer. *Biophys J*. 2006; 91:1315–1324. [PubMed: 16751249]
376. Meltzer RH, Vila-Carriales W, Ebalunode JO, Briggs JM, Pedersen SE. Computed pore potentials of the nicotinic acetylcholine receptor. *Biophys J*. 2006; 91:1325–1335. [PubMed: 16751248]
377. Hill DG, Baenziger JE. The net orientation of nicotinic receptor transmembrane α -helices in the resting and desensitized states. *Biophys J*. 2006; 91:705–714. [PubMed: 16648164]
378. Dyson HJ, Wright PE. Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol*. 2005; 6:197–208. [PubMed: 15738986]

379. Kottwitz D, Kukhtina V, Dergousova N, Alexeev T, Utkin Y, Tsetlin V, Hucho F. Intracellular domains of the δ -subunits of *Torpedo* and rat acetylcholine receptors--expression, purification, and characterization. *Protein Expr Purif*. 2004; 38:237–247. [PubMed: 15555939]
380. Kukhtina V, Kottwitz D, Strauss H, Heise B, Chebotareva N, Tsetlin V, Hucho F. Intracellular domain of nicotinic acetylcholine receptor: the importance of being unfolded. *J Neurochem*. 2006; 97(Suppl 1):63–67. [PubMed: 16635251]
381. Yu XM, Hall ZW. A sequence in the main cytoplasmic loop of the α subunit is required for assembly of mouse muscle nicotinic acetylcholine receptor. *Neuron*. 1994; 13:247–255. [PubMed: 8043279]
382. Valor LM, Mulet J, Sala F, Sala S, Ballesta JJ, Criado M. Role of the large cytoplasmic loop of the $\alpha 7$ neuronal nicotinic acetylcholine receptor subunit in receptor expression and function. *Biochemistry*. 2002; 41:7931–7938. [PubMed: 12069582]
383. Kuo YP, Xu L, Eaton JB, Zhao L, Wu J, Lukas RJ. Roles for nicotinic acetylcholine receptor subunit large cytoplasmic loop sequences in receptor expression and function. *J Pharmacol Exp Ther*. 2005; 314:455–466. [PubMed: 15833891]
384. Castelán F, Mulet J, Aldea M, Sala S, Sala F, Criado M. Cytoplasmic regions adjacent to the M3 and M4 transmembrane segments influence expression and function of $\alpha 7$ nicotinic acetylcholine receptors. A study with single amino acid mutants. *J Neurochem*. 2007; 100:406–415. [PubMed: 17076762]
385. Williams BM, Temburni MK, Levey MS, Bertrand S, Bertrand D, Jacob MH. The long internal loop of the $\alpha 3$ subunit targets nAChRs to subdomains within individual synapses on neurons *in vivo*. *Nat Neurosci*. 1998; 1:557–562. [PubMed: 10196562]
386. Temburni MK, Blitzblau RC, Jacob MH. Receptor targeting and heterogeneity at interneuronal nicotinic cholinergic synapses *in vivo*. *J Physiol*. 2000; 525(Pt 1):21–29. [PubMed: 10811721]
387. Keller SH, Lindstrom J, Ellisman M, Taylor P. Adjacent basic amino acid residues recognized by the COPI complex and ubiquitination govern endoplasmic reticulum to cell surface trafficking of the nicotinic acetylcholine receptor α -subunit. *J Biol Chem*. 2001; 276:18384–18391. [PubMed: 11279119]
388. Ren XQ, Cheng SB, Treuil MW, Mukherjee J, Rao J, Braunewell KH, Lindstrom JM, Anand R. Structural determinants of $\alpha 4\beta 2$ nicotinic acetylcholine receptor trafficking. *J Neurosci*. 2005; 25:6676–6686. [PubMed: 16014729]
389. Xu J, Zhu Y, Heinemann SF. Identification of sequence motifs that target neuronal nicotinic receptors to dendrites and axons. *J Neurosci*. 2006; 26:9780–9793. [PubMed: 16988049]
390. Wang JM, Zhang L, Yao Y, Viroonchatapan N, Rothe E, Wang ZZ. A transmembrane motif governs the surface trafficking of nicotinic acetylcholine receptors. *Nat Neurosci*. 2002; 5:963–970. [PubMed: 12219096]
391. Roccamo AM, Barrantes FJ. Charged amino acid motifs flanking each extreme of the $\alpha M4$ transmembrane domain are involved in assembly and cell-surface targeting of the muscle nicotinic acetylcholine receptor. *J Neurosci Res*. 2007; 85:285–293. [PubMed: 17131427]
392. Bencherif M, Lukas RJ. Cytochalasin modulation of nicotinic cholinergic receptor expression and muscarinic receptor function in human TE671/RD cells: a possible functional role of the cytoskeleton. *J Neurochem*. 1993; 61:852–864. [PubMed: 8360687]
393. Yu XM, Hall ZW. The role of the cytoplasmic domains of individual subunits of the acetylcholine receptor in 43 kDa protein-induced clustering in COS cells. *J Neurosci*. 1994; 14:785–795. [PubMed: 8301361]
394. Shoop RD, Yamada N, Berg DK. Cytoskeletal links of neuronal acetylcholine receptors containing $\alpha 7$ subunits. *J Neurosci*. 2000; 20:4021–4029. [PubMed: 10818137]
395. Pacheco MA, Pastoor TE, Wecker L. Phosphorylation of the $\alpha 4$ subunit of human $\alpha 4\beta 2$ nicotinic receptors: role of cAMP-dependent protein kinase (PKA) and protein kinase C (PKC). *Brain Res Mol Brain Res*. 2003; 114:65–72. [PubMed: 12782394]
396. Wiesner A, Fuhrer C. Regulation of nicotinic acetylcholine receptors by tyrosine kinases in the peripheral and central nervous system: same players, different roles. *Cell Mol Life Sci*. 2006; 63:2818–2828. [PubMed: 17086381]

397. Huganir RL, Delcour AH, Greengard P, Hess GP. Phosphorylation of the nicotinic acetylcholine receptor regulates its rate of desensitization. *Nature*. 1986; 321:774–776. [PubMed: 2423885]
398. Fenster CP, Beckman ML, Parker JC, Sheffield EB, Whitworth TL, Quick MW, Lester RA. Regulation of $\alpha 4\beta 2$ nicotinic receptor desensitization by calcium and protein kinase C. *Mol Pharmacol*. 1999; 55:432–443. [PubMed: 10051526]
399. Wang K, Hackett JT, Cox ME, Van Hoek M, Lindstrom JM, Parsons SJ. Regulation of the neuronal nicotinic acetylcholine receptor by SRC family tyrosine kinases. *J Biol Chem*. 2004; 279:8779–8786. [PubMed: 14679211]
400. Cho CH, Song W, Leitzell K, Teo E, Meleth AD, Quick MW, Lester RA. Rapid upregulation of $\alpha 7$ nicotinic acetylcholine receptors by tyrosine dephosphorylation. *J Neurosci*. 2005; 25:3712–3723. [PubMed: 15814802]
401. Colledge M, Froehner SC. Tyrosine phosphorylation of nicotinic acetylcholine receptor mediates Grb2 binding. *J Neurosci*. 1997; 17:5038–5045. [PubMed: 9185541]
402. Huang K, El-Husseini A. Modulation of neuronal protein trafficking and function by palmitoylation. *Curr Opin Neurobiol*. 2005; 15:527–535. [PubMed: 16125924]
403. Drisdell RC, Alexander JK, Sayeed A, Green WN. Assays of protein palmitoylation. *Methods*. 2006; 40:127–134. [PubMed: 17012024]
404. Drisdell RC, Manzana E, Green WN. The role of palmitoylation in functional expression of nicotinic $\alpha 7$ receptors. *J Neurosci*. 2004; 24:10502–10510. [PubMed: 15548665]
405. Deneris ES. Transcriptional regulation of neuronal nAChR subunit genes. In: Clementi, F.; Fornasari, D.; Gotti, C., editors. *Neuronal Nicotinic Receptors*. Springer-Verlag; Berlin: 2000.
406. Matter, J.-M.; Ballivet, M. Gene structure and transcriptional regulation of the neuronal nicotinic acetylcholine receptors. In: Clementi, F.; Fornasari, D.; Gotti, C., editors. *Neuronal Nicotinic Receptors*. Springer-Verlag; Berlin: 2000.
407. Valor LM, Castillo M, Ortiz JA, Criado M. Transcriptional regulation by activation and repression elements located at the 5′-noncoding region of the human $\alpha 9$ nicotinic receptor subunit gene. *J Biol Chem*. 2003; 278:37249–37255. [PubMed: 12860975]
408. Xu X, Scott MM, Deneris ES. Shared long-range regulatory elements coordinate expression of a gene cluster encoding nicotinic receptor heteromeric subtypes. *Mol Cell Biol*. 2006; 26:5636–5649. [PubMed: 16847319]
409. Danthi S, Boyd RT. Cell specificity of a rat neuronal nicotinic acetylcholine receptor $\alpha 7$ subunit gene promoter. *Neurosci Lett*. 2006; 400:63–68. [PubMed: 16546320]
410. Benfante R, Flora A, Di Lascio S, Cargnin F, Longhi R, Colombo S, Clementi F, Fornasari D. Transcription factor PHOX2A regulates the human $\alpha 3$ nicotinic receptor subunit gene promoter. *J Biol Chem*. 2007; 282:13290–13302. [PubMed: 17344216]
411. Koishi K, McLennan IS. A murine specific splicing variant of the acetylcholine receptor ϵ -subunit gene transcript. *Biochim Biophys Acta*. 1998; 1401:315–318. [PubMed: 9540821]
412. Garcia-Guzmán M, Sala F, Sala S, Campos-Caro A, Stühmer W, Gutiérrez LM, Criado M. α -Bungarotoxin-sensitive nicotinic receptors on bovine chromaffin cells: molecular cloning, functional expression and alternative splicing of the $\alpha 7$ subunit. *Eur J Neurosci*. 1995; 7:647–655. [PubMed: 7620615]
413. Saragoza PA, Modir JG, Goel N, French KL, Li L, Nowak MW, Stitzel JA. Identification of an alternatively processed nicotinic receptor $\alpha 7$ subunit RNA in mouse brain. *Brain Res Mol Brain Res*. 2003; 117:15–26. [PubMed: 14499477]
414. Severance EG, Cuevas J. Distribution and synaptic localization of nicotinic acetylcholine receptors containing a novel $\alpha 7$ subunit isoform in embryonic rat cortical neurons. *Neurosci Lett*. 2004; 372:104–109. [PubMed: 15531097]
415. Severance EG, Zhang H, Cruz Y, Pakhlevaniants S, Hadley SH, Amin J, Wecker L, Reed C, Cuevas J. The $\alpha 7$ nicotinic acetylcholine receptor subunit exists in two isoforms that contribute to functional ligand-gated ion channels. *Mol Pharmacol*. 2004; 66:420–429. [PubMed: 15322233]
416. Jin Y, Tian N, Cao J, Liang J, Yang Z, Lv J. RNA editing and alternative splicing of the insect nAChR subunit $\alpha 6$ transcript: evolutionary conservation, divergence and regulation. *BMC Evol Biol*. 2007; 7:98. [PubMed: 17597521]

417. Oertel J, Villmann C, Kettenmann H, Kirchhoff F, Becker CM. A novel glycine receptor β subunit splice variant predicts an unorthodox transmembrane topology. Assembly into heteromeric receptor complexes. *J Biol Chem.* 2007; 282:2798–2807. [PubMed: 17145751]
418. Tsuneki H, Kobayashi S, Takagi K, Kagawa S, Tsunoda M, Murata M, Matsuoka T, Wada T, Kurachi M, Kimura I, Sasaoka T. Novel G423S mutation of human $\alpha 7$ nicotinic receptor promotes agonist-induced desensitization by a protein kinase C-dependent mechanism. *Mol Pharmacol.* 2007; 71:777–786. [PubMed: 17132684]
419. Nelson ME, Lindstrom J. Single channel properties of human $\alpha 3$ AChRs: impact of $\beta 2$, $\beta 4$ and $\alpha 5$ subunits. *J Physiol.* 1999; 516(Pt 3):657–678. [PubMed: 10200416]
420. Broadbent S, Groot-Kormelink PJ, Krashia PA, Harkness PC, Millar NS, Beato M, Sivilotti LG. Incorporation of the $\beta 3$ subunit has a dominant-negative effect on the function of recombinant central-type neuronal nicotinic receptors. *Mol Pharmacol.* 2006; 70:1350–1357. [PubMed: 16822928]
421. Tumkosit P, Kuryatov A, Luo J, Lindstrom J. $\beta 3$ subunits promote expression and nicotine-induced up-regulation of human nicotinic $\alpha 6^*$ nicotinic acetylcholine receptors expressed in transfected cell lines. *Mol Pharmacol.* 2006; 70:1358–1368. [PubMed: 16835356]
422. Wu J, Liu Q, Yu K, Hu J, Kuo YP, Segerberg M, John PA, Lukas RJ. Roles of nicotinic acetylcholine receptor β subunits in function of human $\alpha 4$ -containing nicotinic receptors. *J Physiol.* 2006; 576:103–118. [PubMed: 16825297]
423. Tapia L, Kuryatov A, Lindstrom J. Ca^{2+} permeability of the $(\alpha 4)_3(\beta 2)_2$ stoichiometry greatly exceeds that of $(\alpha 4)_2(\beta 2)_3$ human acetylcholine receptors. *Mol Pharmacol.* 2007; 71:769–776. [PubMed: 17132685]
424. Keller SH, Taylor P. Determinants responsible for assembly of the nicotinic acetylcholine receptor. *J Gen Physiol.* 1999; 113:171–176. [PubMed: 9925816]
425. Millar NS. Assembly and subunit diversity of nicotinic acetylcholine receptors. *Biochem Soc Trans.* 2003; 31:869–874. [PubMed: 12887324]
426. Wanamaker CP, Christianson JC, Green WN. Regulation of nicotinic acetylcholine receptor assembly. *Ann N Y Acad Sci.* 2003; 998:66–80. [PubMed: 14592864]
427. Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci.* 2006; 27:482–491. [PubMed: 16876883]
428. Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. Heterogeneity and complexity of native brain nicotinic receptors. *Biochem Pharmacol.* 2007; 74:1102–1111. [PubMed: 17597586]
429. Nelson ME, Wang F, Kuryatov A, Choi CH, Gerzanich V, Lindstrom J. Functional properties of human nicotinic AChRs expressed by IMR-32 neuroblastoma cells resemble those of $\alpha 3\beta 4$ AChRs expressed in permanently transfected HEK cells. *J Gen Physiol.* 2001; 118:563–582. [PubMed: 11696612]
430. Khiroug SS, Harkness PC, Lamb PW, Sudweeks SN, Khiroug L, Millar NS, Yakel JL. Rat nicotinic ACh receptor $\alpha 7$ and $\beta 2$ subunits co-assemble to form functional heteromeric nicotinic receptor channels. *J Physiol.* 2002; 540:425–434. [PubMed: 11956333]
431. Marritt AM, Cox BC, Yasuda RP, McIntosh JM, Xiao Y, Wolfe BB, Kellar KJ. Nicotinic cholinergic receptors in the rat retina: simple and mixed heteromeric subtypes. *Mol Pharmacol.* 2005; 68:1656–1668. [PubMed: 16129735]
432. Anand R, Conroy WG, Schoepfer R, Whiting P, Lindstrom J. Neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes have a pentameric quaternary structure. *J Biol Chem.* 1991; 266:11192–11198. [PubMed: 2040627]
433. Plazas PV, Katz E, Gomez-Casati ME, Bouzat C, Elgoyhen AB. Stoichiometry of the $\alpha 9\alpha 10$ nicotinic cholinergic receptor. *J Neurosci.* 2005; 25:10905–10912. [PubMed: 16306403]
434. Drenan RM, Nashmi R, Imoukhuede PI, Just H, McKinney S, Lester HA. Subcellular trafficking, pentameric assembly and subunit stoichiometry of neuronal nicotinic ACh receptors containing fluorescently-labeled $\alpha 6$ and $\beta 3$ subunits. *Mol Pharmacol.* 2008; 73:27–41. [PubMed: 17932221]
435. Moretti M, Vailati S, Zoli M, Lippi G, Riganti L, Longhi R, Viegi A, Clementi F, Gotti C. Nicotinic acetylcholine receptor subtypes expression during rat retina development and their regulation by visual experience. *Mol Pharmacol.* 2004; 66:85–96. [PubMed: 15213299]

436. Gotti C, Moretti M, Zanardi A, Gaimarri A, Champtiaux N, Changeux JP, Whiteaker P, Marks MJ, Clementi F, Zoli M. Heterogeneity and selective targeting of neuronal nicotinic acetylcholine receptor (nAChR) subtypes expressed on retinal afferents of the superior colliculus and lateral geniculate nucleus: identification of a new native nAChR subtype $\alpha 3\beta 2$ ($\alpha 5$ or $\beta 3$) enriched in retinocollicular afferents. *Mol Pharmacol*. 2005; 68:1162–1171. [PubMed: 16049166]
437. Gahring LC, Days EL, Kaasch T, Gonzalez de Mendoza M, Owen L, Persiyanov K, Rogers SW. Pro-inflammatory cytokines modify neuronal nicotinic acetylcholine receptor assembly. *J Neuroimmunol*. 2005; 166:88–101. [PubMed: 16024094]
438. Gaimarri A, Moretti M, Riganti L, Zanardi A, Clementi F, Gotti C. Regulation of neuronal nicotinic receptor traffic and expression. *Brain Res Rev*. 2007; 55:134–143. [PubMed: 17383007]
439. Feng G, Steinbach JH, Sanes JR. Rapsyn clusters neuronal acetylcholine receptors but is inessential for formation of an interneuronal cholinergic synapse. *J Neurosci*. 1998; 18:4166–4176. [PubMed: 9592096]
440. Conroy WG, Berg DK. Rapsyn variants in ciliary ganglia and their possible effects on clustering of nicotinic receptors. *J Neurochem*. 1999; 73:1399–1408. [PubMed: 10501183]
441. Bruneau E, Akaaboune M. The dynamics of the rapsyn scaffolding protein at individual acetylcholine receptor clusters. *J Biol Chem*. 2007; 282:9932–9940. [PubMed: 17283077]
442. Zhang B, Luo S, Dong XP, Zhang X, Liu C, Luo Z, Xiong WC, Mei L. β -catenin regulates acetylcholine receptor clustering in muscle cells through interaction with rapsyn. *J Neurosci*. 2007; 27:3968–3973. [PubMed: 17428970]
443. Wang J, Jing Z, Zhang L, Zhou G, Braun J, Yao Y, Wang ZZ. Regulation of acetylcholine receptor clustering by the tumor suppressor APC. *Nat Neurosci*. 2003; 6:1017–1018. [PubMed: 14502292]
444. Conroy WG, Liu Z, Nai Q, Coggan JS, Berg DK. PDZ-containing proteins provide a functional postsynaptic scaffold for nicotinic receptors in neurons. *Neuron*. 2003; 38:759–771. [PubMed: 12797960]
445. Parker MJ, Zhao S, Bredt DS, Sanes JR, Feng G. PSD93 regulates synaptic stability at neuronal cholinergic synapses. *J Neurosci*. 2004; 24:378–388. [PubMed: 14724236]
446. Temburni MK, Rosenberg MM, Pathak N, McConnell R, Jacob MH. Neuronal nicotinic synapse assembly requires the adenomatous polyposis coli tumor suppressor protein. *J Neurosci*. 2004; 24:6776–6784. [PubMed: 15282282]
447. Jeanclos EM, Lin L, Treuil MW, Rao J, DeCoster MA, Anand R. The chaperone protein 14-3-3 η interacts with the nicotinic acetylcholine receptor $\alpha 4$ subunit. Evidence for a dynamic role in subunit stabilization. *J Biol Chem*. 2001; 276:28281–28290. [PubMed: 11352901]
448. Exley R, Moroni M, Sasdelli F, Houlihan LM, Lukas RJ, Sher E, Zwart R, Bermudez I. Chaperone protein 14-3-3 and protein kinase A increase the relative abundance of low agonist sensitivity human $\alpha 4\beta 2$ nicotinic acetylcholine receptors in *Xenopus* oocytes. *J Neurochem*. 2006; 98:876–885. [PubMed: 16787419]
449. Lin L, Jeanclos EM, Treuil M, Braunewell KH, Gundelfinger ED, Anand R. The calcium sensor protein visinin-like protein-1 modulates the surface expression and agonist sensitivity of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. *J Biol Chem*. 2002; 277:41872–41878. [PubMed: 12202488]
450. Baer K, Bürli T, Huh KH, Wiesner A, Erb-Vögtli S, Göckeritz-Dujmovic D, Moransard M, Nishimune A, Rees MI, Henley JM, Fritschy JM, Fuhrer C. PICK1 interacts with $\alpha 7$ neuronal nicotinic acetylcholine receptors and controls their clustering. *Mol Cell Neurosci*. 2007; 35:339–355. [PubMed: 17467288]
451. Castillo M, Mulet J, Gutiérrez LM, Ortiz JA, Castelán F, Gerber S, Sala S, Sala F, Criado M. Dual role of the RIC-3 protein in trafficking of serotonin and nicotinic acetylcholine receptors. *J Biol Chem*. 2005; 280:27062–27068. [PubMed: 15927954]
452. Halevi S, Yassin L, Eshel M, Sala F, Sala S, Criado M, Treinin M. Conservation within the RIC-3 gene family. Effectors of mammalian nicotinic acetylcholine receptor expression. *J Biol Chem*. 2003; 278:34411–34417. [PubMed: 12821669]

453. Castillo M, Mulet J, Gutierrez LM, Ortiz JA, Castelan F, Gerber S, Sala S, Sala F, Criado M. Role of the RIC-3 protein in trafficking of serotonin and nicotinic acetylcholine receptors. *J Mol Neurosci.* 2006; 30:153–156. [PubMed: 17192664]
454. Lansdell SJ, Gee VJ, Harkness PC, Doward AI, Baker ER, Gibb AJ, Millar NS. RIC-3 enhances functional expression of multiple nicotinic acetylcholine receptor subtypes in mammalian cells. *Mol Pharmacol.* 2005; 68:1431–1438. [PubMed: 16120769]
455. Williams DB. Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. *J Cell Sci.* 2006; 119:615–623. [PubMed: 16467570]
456. Gelman MS, Chang W, Thomas DY, Bergeron JJM, Prives JM. Role of the endoplasmic reticulum chaperone calnexin in subunit folding and assembly of nicotinic acetylcholine receptors. *J Biol Chem.* 1995; 270:15085–15092. [PubMed: 7797492]
457. Keller SH, Lindstrom J, Taylor P. Involvement of the chaperone protein calnexin and the acetylcholine receptor β -subunit in the assembly and cell surface expression of the receptor. *J Biol Chem.* 1996; 271:22871–22877. [PubMed: 8798466]
458. Chang W, Gelman MS, Prives JM. Calnexin-dependent enhancement of nicotinic acetylcholine receptor assembly and surface expression. *J Biol Chem.* 1997; 272:28925–28932. [PubMed: 9360963]
459. Wanamaker CP, Green WN. ER chaperones stabilize nicotinic receptor subunits and regulate receptor assembly. *J Biol Chem.* 2007; 282:31113–31123. [PubMed: 17728248]
460. Christianson JC, Green WN. Regulation of nicotinic receptor expression by the ubiquitin-proteasome system. *EMBO J.* 2004; 23:4156–4165. [PubMed: 15483627]
461. Miwa JM, Ibañez-Tallon I, Crabtree GW, Sánchez R, Šali A, Role LW, Heintz N. *lynx1*, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. *Neuron.* 1999; 23:105–114. [PubMed: 10402197]
462. Ibañez-Tallon I, Miwa JM, Wang HL, Adams NC, Crabtree GW, Sine SM, Heintz N. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin *lynx1*. *Neuron.* 2002; 33:893–903. [PubMed: 11906696]
463. Miwa JM, Stevens TR, King SL, Caldarone BJ, Ibanez-Tallon I, Xiao C, Fitzsimonds RM, Pavlides C, Lester HA, Picciotto MR, Heintz N. The prototoxin *lynx1* acts on nicotinic acetylcholine receptors to balance neuronal activity and survival *in vivo*. *Neuron.* 2006; 51:587–600. [PubMed: 16950157]
464. Dessaud E, Salaün D, Gayet O, Chabbert M, deLapeyrière O. Identification of *lynx2*, a novel member of the *ly-6/neurotoxin* superfamily, expressed in neuronal subpopulations during mouse development. *Mol Cell Neurosci.* 2006; 31:232–242. [PubMed: 16236524]
465. Mallya M, Campbell RD, Aguado B. Characterization of the five novel Ly-6 superfamily members encoded in the MHC, and detection of cells expressing their potential ligands. *Protein Sci.* 2006; 15:2244–2256. [PubMed: 17008713]
466. Guzmán GR, Ortiz-Acevedo A, Ricardo A, Rojas LV, Lasalde-Dominicci JA. The polarity of lipid-exposed residues contributes to the functional differences between *Torpedo* and muscle-type nicotinic receptors. *J Membr Biol.* 2006; 214:131–138. [PubMed: 17530159]
467. Pediconi MF, Gallegos CE, de Los Santos EB, Barrantes FJ. Metabolic cholesterol depletion hinders cell-surface trafficking of the nicotinic acetylcholine receptor. *Neuroscience.* 2004; 128:239–249. [PubMed: 15350637]
468. Baier CJ, Barrantes FJ. Sphingolipids are necessary for nicotinic acetylcholine receptor export in the early secretory pathway. *J Neurochem.* 2007; 101:1072–1084. [PubMed: 17437537]
469. Sallette J, Bohler S, Benoit P, Soudant M, Pons S, Le Novère N, Changeux JP, Corringer PJ. An extracellular protein microdomain controls up-regulation of neuronal nicotinic acetylcholine receptors by nicotine. *J Biol Chem.* 2004; 279:18767–18775. [PubMed: 14764595]
470. Kuryatov A, Luo J, Cooper J, Lindstrom J. Nicotine acts as a pharmacological chaperone to up-regulate human $\alpha 4\beta 2$ acetylcholine receptors. *Mol Pharmacol.* 2005; 68:1839–1851. [PubMed: 16183856]
471. Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, Changeux J-P, Corringer P-J. Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron.* 2005; 46:595–607. [PubMed: 15944128]

472. Corringer PJ, Sallette J, Changeux JP. Nicotine enhances intracellular nicotinic receptor maturation: a novel mechanism of neural plasticity? *J Physiol Paris*. 2006; 99:162–171. [PubMed: 16458492]
473. Kishi M, Steinbach JH. Role of the agonist binding site in up-regulation of neuronal nicotinic $\alpha_4\beta_2$ receptors. *Mol Pharmacol*. 2006; 70:2037–2044. [PubMed: 16966476]
474. Nashmi R, Lester H. Cell autonomy, receptor autonomy, and thermodynamics in nicotine receptor up-regulation. *Biochem Pharmacol*. 2007; 74:1145–1154. [PubMed: 17662697]
475. Darsow T, Booker TK, Piña-Crespo JC, Heinemann SF. Exocytic trafficking is required for nicotine-induced up-regulation of $\alpha_4\beta_2$ nicotinic acetylcholine receptors. *J Biol Chem*. 2005; 280:18311–18320. [PubMed: 15741168]
476. Vallejo YF, Buisson B, Bertrand D, Green WN. Chronic nicotine exposure upregulates nicotinic receptors by a novel mechanism. *J Neurosci*. 2005; 25:5563–5572. [PubMed: 15944384]
477. Bowie JU. Solving the membrane protein folding problem. *Nature*. 2005; 438:581–589. [PubMed: 16319877]
478. Barth P, Schonbrun J, Baker D. Toward high-resolution prediction and design of transmembrane helical protein structures. *Proc Natl Acad Sci U S A*. 2007; 104:15682–15687. [PubMed: 17905872]
479. Subrahmanyam S, Piletsky SA, Turner AP. Application of natural receptors in sensors and assays. *Anal Chem*. 2002; 74:3942–3951. [PubMed: 12199559]
480. Ide T, Ichikawa T. A novel method for artificial lipid-bilayer formation. *Biosens Bioelectron*. 2005; 21:672–677. [PubMed: 16202882]
481. Vockenroth IK, Atanasova PP, Long JR, Jenkins AT, Knoll W, Köper I. Functional incorporation of the pore forming segment of AChR M2 into tethered bilayer lipid membranes. *Biochim Biophys Acta*. 2007; 1768:1114–1120. [PubMed: 17368423]
482. Lindstrom JM. Nicotinic acetylcholine receptors of muscles and nerves: comparison of their structures, functional roles, and vulnerability to pathology. *Ann N Y Acad Sci*. 2003; 998:41–52. [PubMed: 14592862]

Figure 1.

Multiple sequence alignment by ClustalW of the N-terminal extracellular domains from the $\alpha 1$ subunit from *Torpedo marmorata*, the AChBP subunit from *Lymnaea stagnalis*, and all 16 human nAChR subunits. The residue numbers shown at the ends of the lines include the residues of the N-terminal signal sequences. The alignment includes a portion of the N-terminal end of transmembrane domain M1. Annotations above the blocks of sequences show structural features of the extracellular domain. The red labels $\alpha 1$ and $\alpha 2$ show α -helices; $\eta 1$ shows a 3_{10} -helix, which is slightly more tightly coiled than a α -helix (a 3.6_{13} -helix in this nomenclature) (107). The blue labels $\beta 1$ through $\beta 10$ show β -sheets. The green labels A through F show loops of the principal face (A, B, and C) and complementary face (D, E, and F) of the orthosteric binding site of *Torpedo marmorata* (14, 24). The Cys loop is labeled with an orange dotted line. Color code for amino acid residues: red for small and hydrophobic residues, excluding tyrosine (AVFPMLW); blue for acidic residues (DE); black for basic residues (RK); green for hydroxyl and amine residues and others (STYHCNGQ).

Figure 2.

Ribbon diagram of the pentameric structure of the nAChR from *Torpedo marmorata*. Color scheme of the five subunits from N terminus through C terminus of each subunit: $\alpha 1$ -blue to cyan; $\beta 1$ -cyan to green; δ -green to yellow; $\alpha 1$ -yellow to orange; γ -orange to red. A. View from the extracellular side of the nAChR and looking along the path of ion travel into a cell. The N terminal α -helix of the extracellular domain of each subunit is closest to the viewer. The five subunits are labeled. The subunit order is anticlockwise ($\alpha 1$) γ ($\alpha 1$) δ ($\beta 1$) when viewed from the extracellular compartment. B. View in the plane of the transmembrane domains. The adjacent $\alpha 1$ and δ subunits at the bottom of panel A are in front in panel B. The top third of panel B shows the five extracellular domains. The middle third of panel B is the plane of the membrane and shows the twenty α -helical transmembrane domains (four from each subunit). The bottom third of panel B shows the five amphipathic intracellular MA helices from the large cytoplasmic loops of the five subunits. Electron diffraction did not identify the structure of the portion of the large cytoplasmic loop connecting M3 to amphipathic intracellular helix MA for any of the five subunits. Except for the MA helices, therefore, the large cytoplasmic loops do not appear in this figure. The MA helices precede the M4 helices and are below the plane of the transmembrane domains. These images were created from Protein Data Bank accession 2bg9 (90).

Figure 3.

Ribbon diagram of a single $\alpha 1$ subunit of the nAChR from *Torpedo marmorata* viewed in the plane of the transmembrane domains. Starting with violet at the N-terminus of the ribbon, the color of the ribbon proceeds through the colors of the rainbow ending with red at the C-terminus. The extracellular α -helix is labeled $\alpha 1$. The β -strands 1, 2, 3, 5, 5', 6, 6', and 8 (labels in red) form the inner sheet of the β -sandwich core; β -strands 4, 7, 9, and 10 (labels in pink) form the outer sheet of the β -sandwich core. The transmembrane helices are labeled M1, M2, M3, and M4. MA is the amphipathic intracellular helix in the large cytoplasmic loop near M4. The N-terminus and C-terminus are labeled N and C, respectively. The loops labeled A loop, B loop, and C loop belong to the structure of the

principal face of the orthosteric binding site (14, 24). Strand 2, the loop between strands 5' and 6, and the loop between strands 8 and 9 form the complementary face components D, E, and F, respectively, of the orthosteric binding site (Figure 1). The Cys loop is the 15-residue sequence beginning and ending with disulfide-bonded cysteines that gives the name to the Cys-loop superfamily of receptors. The main immunogenic region (MIR) is a major focus of the autoimmune response leading to myasthenia gravis (482). The structure of the large cytoplasmic loop connecting M3 to MA, which precedes M4, was not identified by electron diffraction and does not appear in this figure. This image was created from Protein Data Bank accession 2bg9 (90).

Figure 4. Ribbon diagram of nicotine bound to two subunits of the pentameric AChBP from *Lymnaea stagnalis*. Nicotine is shown as an orange-colored, space-filling model. The loops labeled A, B, and C from the green subunit contribute to the principal face of the orthosteric binding site hosting nicotine. β -strands 2, 5, 5', 6, 6' and the β 8– β 9 loop from the blue subunit contribute to the complementary face of the orthosteric binding site hosting nicotine. This image was created from Protein Data Bank accession 1uw6 (108).