TOPICAL REVIEW

Gating of nicotinic ACh receptors; new insights into structural transitions triggered by agonist binding that induce channel opening

Elaine A. Gay and Jerrel L. Yakel

Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, PO Box 12233, Research Triangle Park, NC 27709, USA

Nicotinic acetylcholine receptors (nAChRs) are in the superfamily of Cys-loop ligand-gated ion channels, and are pentameric assemblies of five subunits, with each subunit arranged around the central ion-conducting pore. The binding of ACh to the extracellular interface between two subunits induces channel opening. With the recent 4 Å resolution of the *Torpedo* nAChR, and the crystal structure of the related molluscan ACh binding protein, much has been learned about the structure of the ligand binding domain and the channel pore, as well as major structural rearrangements that may confer channel opening. For example, the putative pathway coupling agonist binding to channel gating may include a major rearrangement of the C-loop within the ligand binding pocket, and the disruption of a salt bridge between an arginine residue at the end of the β10 strand and a glutamate residue in the β1–β2 linker. Here we will review and discuss the latest structural findings aiming to further refine the transduction pathway linking binding to gating for the nAChR channels, and discuss similarities and differences among the different members of this Cys-loop superfamily of receptors.

(Resubmitted 6 August 2007; accepted after revision 3 September 2007; first published online 6 September 2007)
Corresponding author J. L. Yakel: NIEHS, F2-08, PO Box 12233, 111 T.W. Alexander Drive, Research Triangle Park, N.C. 27709, USA. Email: yakel@niehs.nih.gov

The structure of the nAChR ligand binding domain

Nicotinic acetylcholine receptors (nAChRs) are in the superfamily of Cys-loop ligand-gated ion channels that also include the serotonin 5-HT_3, $GABA_A$ and $GABA_C$, and glycine receptors. These channels are pentameric assemblies of five subunits that can be either homomeric (α subunits only) or heteromeric (both α and β for neuronal receptors and α, β, γ and δ for muscle or *Torpedo* receptors) (for review see Corringer *et al.* 2000; Giniatullin *et al.* 2005; Unwin, 2005; Sine & Engel, 2006). From decades of investigation, there is a good deal known about the structure and function of nAChRs using a variety of techniques including: electron microscopy (on 2D arrays of receptors from *Torpedo marmorata*), biochemistry, chemical labelling, site-directed mutagenesis and electrophysiology (for review see Lester *et al.* 2004; Unwin, 2005). However, two major advancements in the last few years have significantly increased our understanding of the structure/function characteristics of the nAChR ligand binding domain (LBD). First, the cloning and characterization of the molluscan ACh-binding protein (AChBP: Brejc *et al.* 2001; Smit *et al.* 2001) was a landmark event. The AChBP was found to be a pentamer analogous to the extracellular LBD of the Cys-loop family of receptors, and has the ability to bind nAChR ligands. The AChBP is a soluble protein and does not contain an ion channel pore or intracellular domains. However, when the AChBP is attached to the pore domain of the serotonin 5-HT_{3A} receptor, ACh can activate the opening of this hybrid channel (Bouzat *et al.* 2004). The second major advancement has been the refined 4 Å resolution electron microscopy structure of the *Torpedo* nAChR (Unwin, 2005). This structure provided a complete picture of the nAChR in near-physiological conditions.

The major structural feature of the LBD is two sets of β strands sandwiched together with discrete loop regions between strands (Fig. 1). Two distinct structural regions appear to be responsible for ligand binding and subsequent transduction of receptor activation to the channel pore. First, the transmitter binding site, at the interface of two subunits, is composed of a pocket of aromatic and hydrophobic resides from both the principal and complimentary subunits, and is capped by the C-loop. Second, the transition zone is made up of several loops that come into close contact with the transmembrane

 DOI: 10.1113/jphysiol.2007.142554

region (transmembrane domains M1 to M4) including: the Cys-loop, the $\beta1$–$\beta2$ linker, the $\beta8$–$\beta9$ linker, the $\beta10$–M1 linker and the M2–M3 linker (from the transmembrane domain). These structural elements link the LBD to the pore region (M2) where gating of the channel is thought to occur (Fig. 1).

Structures of various states of the nAChR: closed, open and desensitized

To understand how the nAChR is functioning, it is important to know the structure of the receptor in its various states: the closed state (in the absence of agonist), the open state and the desensitized state (high-affinity ligand-bound but non-conducting state of the channel). The superposition of all crystal structures of the different species of AChBP to date, with a variety of ligands (both agonists and antagonists) and buffer molecules, fall into two groups (Dutertre & Lewis, 2006; Ulens *et al.* 2006). The first configuration seen with multiple toxin antagonists and some buffer molecules has the C-loop in an 'open' configuration, corresponding to the closed or resting state of the channel. The second configuration, seen when agonists are bound, has the C-loop in a 'closed' configuration, presumably the open or desensitized state of the receptor. In comparisons of agonist *versus* antagonist-bound crystal structures, the $\beta1$–$\beta2$ linker, the Cys-loop, and the $\beta8$–$\beta9$ linker all move, suggesting changes may be due to receptor activation. However, some variations may also result from differences between the species of AChBP used for crystallization (Unwin, 2005; Ulens *et al.* 2006). In addition, the inactive

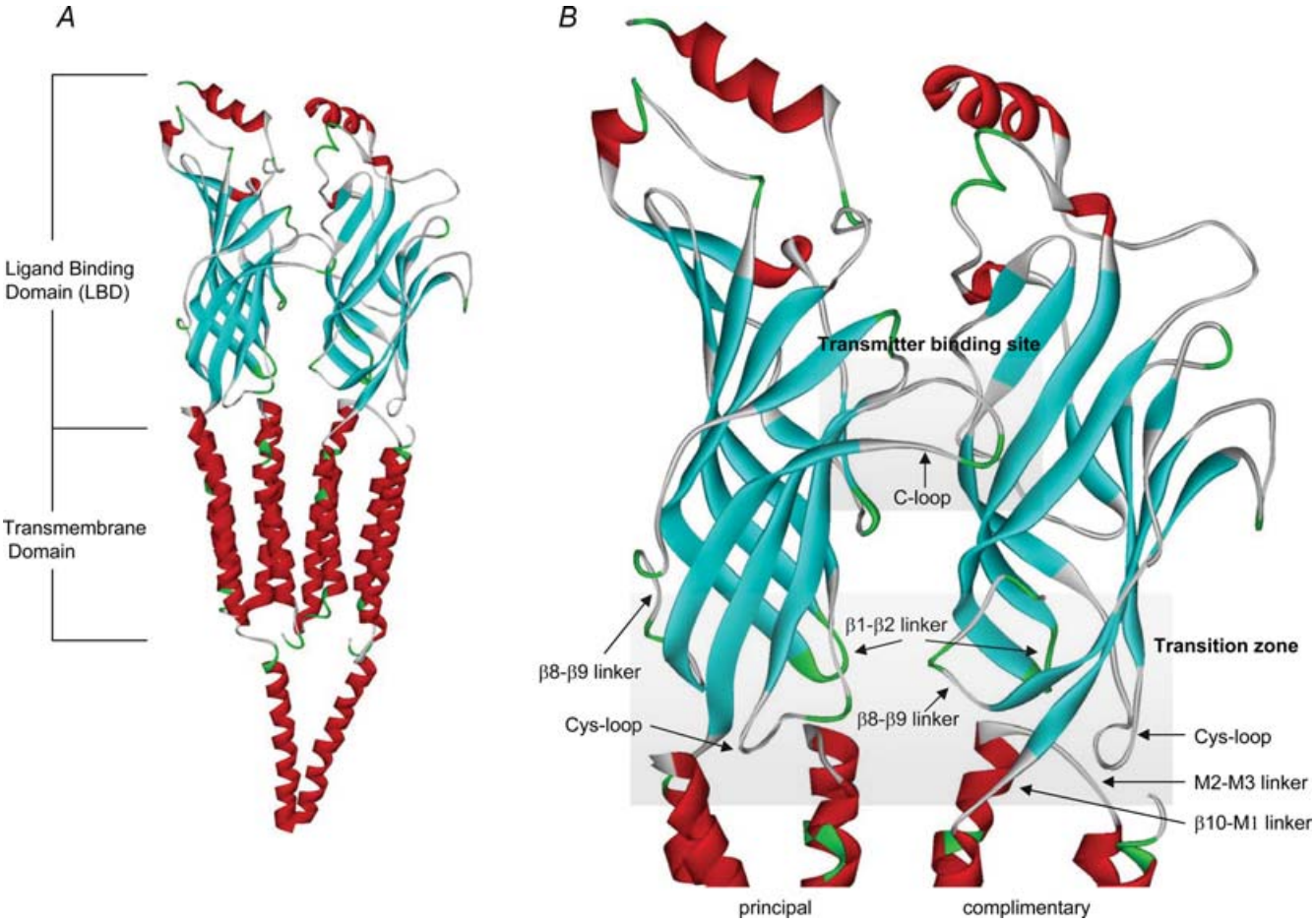

Figure 1. Ribbon diagrams of two subunits of the *Torpedo* nAChR (PDB:2BG9)
A, the α helicies are shown in red and the β strands in blue. The extracellular ligand binding domain (LBD) and transmembrane domains are highlighted. *B*, two major structural elements of the LBD are shown: the transmitter binding site and the transition zone. The transmitter binding site is composed of a cluster of aromatic residues from both the principal and complimentary subunits and is capped by the C-loop. The transition domain consists of several loops including: Cys-loop, $\beta1$–$\beta2$ linker, $\beta8$–$\beta9$ linker, $\beta10$–M1 linker and the M2–M3 linker. These loops are involved in converting structural changes at the transmitter binding site down to the pore domain and inducing channel gating.

state of the *Torpedo* nAChR resembles antagonist-bound AChBP structures, with the C-loop in an open position, and similar relative locations of the $\beta1$–$\beta2$ linker and Cys-loop (Unwin, 2005).

Additionally, the currently accepted structure of the nAChR implies more global movements of the extracellular subunits. For the *Torpedo* nAChR, comparison of α *versus* non-α subunits indicated a clockwise rotation of $\sim$10 deg of the inner β strands. The two α subunits appear to be in a 'distorted' conformation, while the non-α subunits are in a more 'relaxed' conformation. Also, the non-α subunits appear to resemble the homomeric AChBP structures. Unwin suggests that upon agonist binding, the α subunits rotate to the non-α subunit conformation (creating a more uniform structure), and that this movement could lead to gating of the channel by displacing the $\beta1$–$\beta2$ linker, thereby affecting M2 and the channel pore (Unwin, 2005).

It has not yet been determined whether the AChBP structures with agonist bound are in either the open or desensitized receptor state, or even the agonist-bound closed state. It has been difficult to gain structural information for the nAChRs in the open *versus* desensitized state because after the addition of agonist, receptors open and then desensitize with very different rates (from milliseconds, to seconds and minutes) depending on the subtype of nAChRs (Giniatullin *et al.* 2005). However, the original Unwin structure (9 Å) is presumed to be in the open configuration because it was determined after only a 5 ms exposure to ACh (Unwin, 1995). In their comparison of multiple AChBP crystal structures, Dutertre & Lewis (2006) suggest that there are no noticeable structural differences between open and desensitized states. Ulens *et al.* (2006) attempted to distinguish the desensitized state of the AChBP by evaluating crystal structures containing α conotoxins that, according to functional data, were thought to favour the desensitized state of nAChRs, i.e. ImI and a mutant form of PnIA. However, crystals of these toxins with AChBP did not indicate significant differences from other antagonist-bound structures.

While we are beginning to understand the structural variations between different states, we still lack definitive structures for the open and desensitized states of the nAChR (including the double ligand-bound closed and open states that most likely correspond to the receptor conformations assayed in functional studies) and other members of the Cys-loop ligand-gated ion channel family. Finally, based on a variety of functional data, multiple open and desensitized receptor states are expected to exist. Therefore, it is unlikely that crystal structures alone will be able to provide a complete understanding of nAChR motion upon ligand binding and channel gating, and thus caution must be taken when interpreting the data.

Recently, Dellisanti *et al.* (2007) reported a 1.94 Å resolution crystal structure of the mouse muscle $\alpha1$ nAChR subunit bound to α-bungarotoxin. This structure is of a mutated single monomer of the extracellular domain, and provides useful comparisons with previous nAChR and AChBP structures, as well as interesting new results suggesting additional important structural features. When comparing this structure to that of the structures of AChBP and the *Torpedo* nAChR, they superimpose very well. Consistent with functional studies, the structure of the $\alpha1$ extracellular domain indicates that K145 and D200 have a direct electrostatic interaction in this probably closed state of the receptor. Amino acid interactions in the transition zone are also similar, including a close proximity between R209 and E45. In addition, two new structural features are highlighted, a water molecule buried in the core of the subunit, and a well-ordered carbohydrate chain on the outside of the α subunit. Functional studies mutating the hydrophilic amino acids that interact with the water molecule suggest that this cavity may be important in channel gating. In addition, single channel experiments with deglycosylated receptors suggest that the carbohydrate chain may also regulate channel gating, as well as α-bungarotoxin binding. Both of these findings provide new avenues of investigation into the molecular mechanism controlling nAChR function.

Structural transitions during gating

Both the more recent structural data highlighted above, and a myriad of functional data over the last two decades, have provided significant insight into the transition state of nAChRs. As discussed above, in the AChBP, the binding of agonist to the ligand binding pocket results in a major rearrangement of the C-loop into the closed or capped position. Depending on whether an agonist or antagonist binds, the C-loop can swing by as much as 11 Å (Hansen *et al.* 2005). After ligand binding, a conserved tyrosine residue (Y185, for *Lymnaea* AChBP) in the C-loop is drawn closer to a conserved lysine residue (K139) in the $\beta7$ strand (Fig. 2*B*), breaking or weakening a previous interaction between this lysine and an aspartate residue (D194) in the $\beta10$ strand (K145 and D200 in the mouse muscle $\alpha1$ subunit) (Sine & Engel, 2006). A variety of functional data over the years has suggested that movements such as these around the transmitter binding domain might propagate through the rigid β strands to cause rearrangements within the transition zone. These in turn may interact with the M2–M3 linker to cause channel opening (Lester *et al.* 2004). Recently, Lee & Sine (2005) proposed that agonist binding to the muscle nAChR can lead to the disruption of a salt bridge between an arginine residue at the end of the $\beta10$ strand (R209) and a glutamate residue (E45) in the $\beta1$–$\beta2$ linker (Fig. 2*C*). These residues are conserved

among the various members of the Cys-loop ligand-gated ion channel family, and therefore suggest a common transduction mechanism between ligand binding and channel gating (Corringer *et al.* 2000; Absalom *et al.* 2003; Kash *et al.* 2003, 2004; Schofield *et al.* 2004; Xiu *et al.* 2005; Mercado & Czajkowski, 2006). In addition, for the 5-HT_3 receptor, Lummis *et al.* (2005) have proposed that structural changes induced by ligand binding lead to the *cis–trans* isomerization of a conserved proline residue (P*) on the M2–M3 linker and subsequent channel opening. However, although such a proline exists for nAChRs, no similar proline exists in either the GABA or glycine members of this superfamily (Kash *et al.* 2003, 2004). Instead, electrostatic and hydrophobic interactions might be responsible for gating of these receptors (Lee & Sine, 2005; Sine & Engel, 2006). Thus, it has been proposed that upon agonist binding, the C-loop is pulled into to a 'closed' position leading to an interaction between K139 and Y185, a disruption of a salt bridge between the β10 strand and the β1–β2 linker, followed by the isomerization of a proline residue on the M2–M3 linker, leading to channel opening (Sine & Engel, 2006).

Other regions of the receptor have also been proposed to be involved in the transition between ligand binding and channel gating (Lee & Sine, 2005; Mukhtasimova *et al.* 2005). For the chick α7 nAChR, various regions

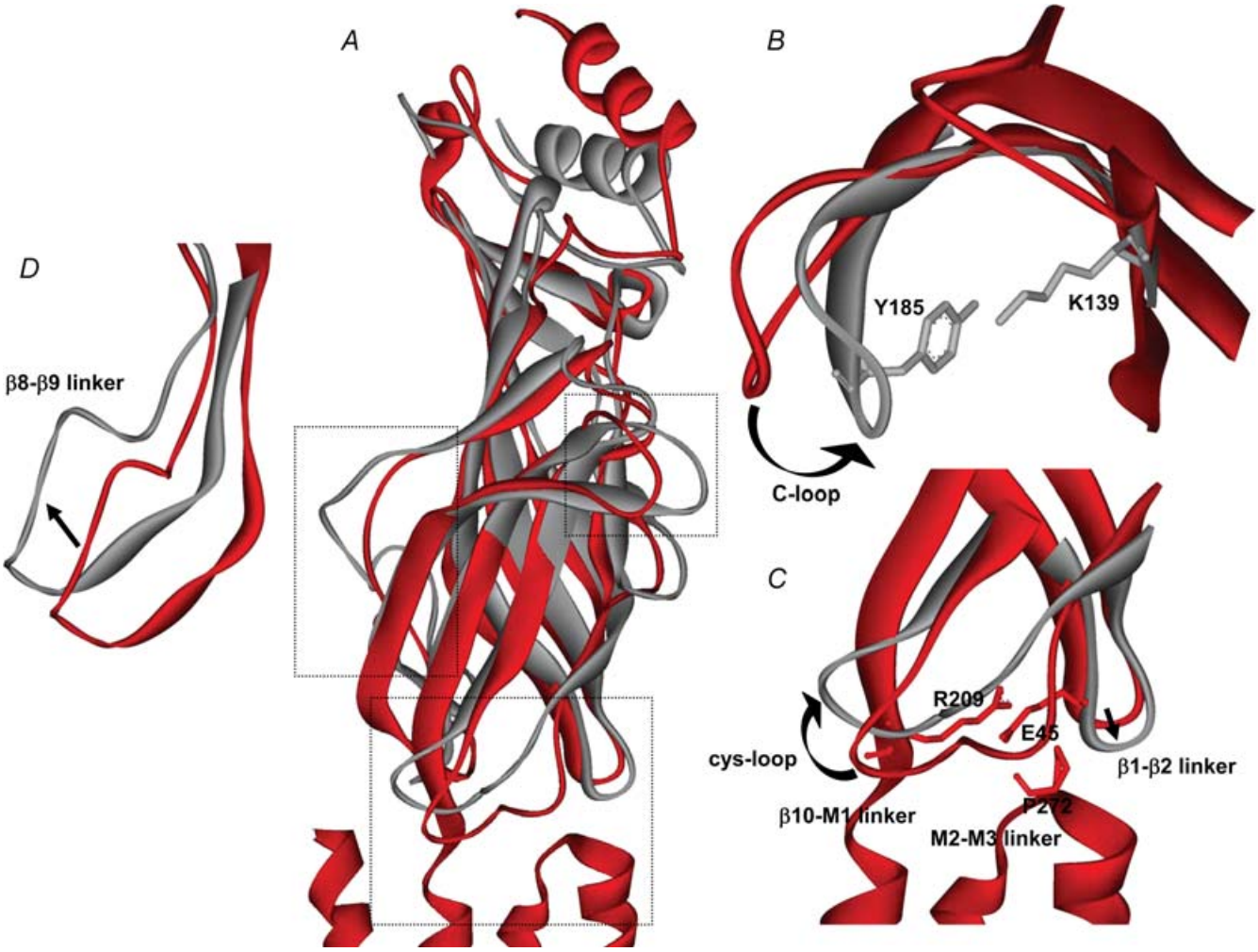

Figure 2. Possible movements within the ligand binding domain upon agonist binding
A, the closed receptor state (*Torpedo* nAChR α subunit; PDB:2BG9) is in red and a possible active or desensitized receptor state (*Lymnaea* AChBP bound to carbamylcholine; PDB:1UV6) for the LBD is in grey. Boxes indicate areas that are expanded in *B–D*. *B*, upon agonist binding, the C-loop moves in toward the channel, bringing Y185 and K139 (AChBP) into close contact, breaking a previous interaction between K139 and D194 (not shown). *C*, movement of the β1–β2 linker and the β10–M1 linker leads to the disruption of a salt-bridge between E45 and R209 (*Torpedo* nAChR), allowing for the possible isomerization of P272 (*Torpedo* nAChR) in some Cys-loop receptors. In addition, the Cys-loop may shift position in the presence of agonist. *D*, the β8–β9 linker also is thought to shift in a clockwise direction. These cartoons are derived from overlays of current crystal structures and while these are suggested movements based on agonist binding to the nAChR, some of these changes could be species related.

in the outer β strands were investigated (Lyford *et al.* 2003; McLaughlin *et al.* 2006). A glutamate residue in the β8–β9 linker (E172), a known site for modulation by divalent cations (Galzi *et al.* 1996), was found to undergo agonist-dependent movements during receptor activation (Lyford *et al.* 2003; Sine & Engel, 2006). Similarly, using fluorescence anisotropy decay to study the segmental motion of side chains in AChBP, Hibbs *et al.* (2006) demonstrated that agonists (but not antagonists) induced changes in conformational dynamics in the β8–β9 linker.

Above are highlights of recent insights into the structural mechanisms of receptor transitions during gating from an array of functional studies on nAChRs or other members in the Cys-loop ligand-gated ion channel family. While not covered in detail in this review, there have been a large number of studies combining a number of techniques (including receptor point mutations, ligand binding, pharmacological modifications and single channel analysis) that have provided information about possible transition states of nAChRs. It is important to understand that these functional studies give us a framework within which the structural data of various receptor states can be understood. For example, the extensive work of Auerbach and colleagues has provided an overall picture of the sequential nature of receptor gating for muscle nAChRs. By making point mutations in both the LBD and the pore domain, followed by measurements of rate-equilibrium free energy relationships (Zhou *et al.* 2005), they have been able to suggest blocks of coordinated motions starting with the β4–β5 linker, the β7–β8 linker, and the C-loop (which increases affinity for agonists), through the transition zone (the Cys-loop and the β1–β2 linker), to the pore region (M2) and gating of the channel (Grosman *et al.* 2000; Chakrapani *et al.* 2004; Purohit *et al.* 2007). This conformational wave propagates throughout the nAChR via Brownian motion in $\sim$1 μs (Grosman *et al.* 2000; Chakrapani & Auerbach, 2005), and provides a more complete view of nAChR gating than that which exists from crystal structures alone. In addition, functional studies have suggested movements that occur during the transition from the closed to open state of the nAChR that have yet to be identified through comparison of crystal structures (e.g. the β4–β5 linker, which contains the A-loop (Purohit *et al.* 2007)).

Structural transitions during desensitization

This review is focused primarily on movements within the extracellular domain of nAChRs during gating. However, there is a significant body of work that has focused on identifying key residues within the pore domain of nAChRs involved in desensitization, the high-affinity, ligand-bound but non-conducting state of the channel reached during sustained agonist application (Giniatullin *et al.* 2005). First of all, there are probably multiple functional desensitized states, the properties of which can depend on various factors including subunit make-up and modulation by local factors (including signal transduction cascades; Giniatullin *et al.* 2005). In addition, it is important to keep in mind that if receptor mutants display altered desensitization kinetics, this does not necessarily indicate that such sites are the structural motifs responsible for desensitization since macroscopic desensitization kinetics can be affected by not only the microscopic desensitization rate constants, but also by agonist binding and channel gating (Giniatullin *et al.* 2005).

Auerbach & Akk (1998) first suggested that there are two separate gates in the nAChR, a resting gate and a desensitization gate. Wilson & Karlin (2001), who were studying structural changes in mouse muscle nAChR in resting, open and desensitized states utilizing electrophysiology and site-directed mutagenesis, suggested a resting state gate near the mouth of the channel, and a desensitization gate further up into the M2 region near to the 9′ location. The 9′ location (residue L247 in the chick α7 nAChR), within the pore domain (M2), was previously shown to control fast desensitization (Revah *et al.* 1991; Giniatullin *et al.* 2005). The 9′ mutation dramatically decreased desensitization onset and increased agonist affinity, which was suggested to be due to the stabilization of a conducting, desensitized state of the receptor (Revah *et al.* 1991).

Besides the pore domain, regions in the extracellular domain of nAChRs might also be involved in receptor desensitization. Recently we found that mutating W55 of the rat α7 nAChR subunit to alanine dramatically slowed the rate of onset of desensitization; kinetic modelling indicated that the rate of transition of the receptor from the open to the desensitized state decreased by $>$ 30-fold, and the rate of recovery from desensitization increased by $\sim$2-fold (Gay EA, Giniatullin R, Skorinkin A & Yakel J, unpublished data). Interestingly, this W residue is within the β2 strand and is considered one of the possible aromatic residues that make up the ligand binding pocket. Up until now, identification of individual residues involved in desensitization of the α7 nAChR has been limited to the pore domain. However, the use of chimeric receptors has demonstrated that regions in the extracellular domain of non-α7 nAChRs are involved in defining receptor desensitization kinetics (Bohler *et al.* 2001; Giniatullin *et al.* 2005). For the 5-HT_3 receptor channel, Reeves *et al.* (2005) proposed that recovery from desensitization may require reformation of an interaction between the β1–β2 linker and the M2–M3 linker. Therefore, for the nAChRs, any structural change that interferes with the reformation of this β1–β2 linker/M2–M3 linker interaction might have an affect on the kinetics of desensitization.

Interestingly ImI and a mutant form of PnIA, which are antagonists at wildtype α7 receptors, can activate the

non-desensitizing α7-L247T nAChR mutant, signifying that they may stabilize a 'desensitized' state of this receptor (Ulens *et al.* 2006). In addition, when exposed to these peptides, this 9′ mutant activates and then desensitizes in the continued presence of ligand. These data suggest there are multiple desensitized states of the α7 nAChR, one of which involves the 9′ location in the M2 pore domain, while others may involve different amino acids in the LBD such as W55.

Molecular dynamic simulations

A series of molecular dynamic simulations of the α7 nAChR have provided corroborating insight into the structural rearrangements that may occur during agonist binding and subsequent channel gating (Henchman *et al.* 2005; Law *et al.* 2005; Taly *et al.* 2005; Cheng *et al.* 2006). For example, simulations of a model α7 nAChR predict a closer interaction between K145 and Y188 (equivalent to K139 and Y185 in the AChBP; Fig. 2*B*) after C-loop closure. In addition when the C-loop moves into the ligand-bound conformation, this induced an up- and outward movement of the lower part of the β10 strand, which initially broke the salt-bridge between R206 and E45, but which then reformed a more stable hydrogen bond between these amino acids (Cheng *et al.* 2006). In this simulation, after about 4 ns, there was a ∼10 deg rotation of M2–M3 linker, resulting in an increase in pore size from ∼1.9 to ∼3.0 Å. Additionally, Henchman *et al.* (2005) observe global outward movements of the bottom half of the LBD with agonists. Overall, these simulations suggest that only small movements are necessary to produce significant changes in channel gating as the process is energy -efficient and easily modulated by agonist binding and unbinding.

In conclusion, the exact nature of the structure of the Cys-loop ligand-gated ion channel subunits, and the movements/rearrangements observed during and after ligand binding, gating and desensitization are still unknown. Nevertheless, a general hypothesis has emerged that indicates agonist binding induces closure of the C-loop, which is conveyed to the M2 pore region, resulting in channel opening. Thus, the transduction pathway involves many regions of the channel. With the continued use of a variety of experimental, structural and modelling techniques, including the recent crystallization of the extracellular domain of the mouse muscle α1 nAChR subunit (Dellisanti *et al.* 2007), major advances are expected in the near future.

References

Absalom NL, Lewis TM, Kaplan W, Pierce KD & Schofield PR (2003). Role of charged residues in coupling ligand binding and channel activation in the extracellular domain of the glycine receptor. *J Biol Chem* **278**, 50151–50157.

Auerbach A & Akk G (1998). Desensitization of mouse nicotinic acetylcholine receptor channels. A two-gate mechanism. *J Gen Physiol* **112**, 181–197.

Bohler S, Gay S, Bertrand S, Corringer PJ, Edelstein SJ, Changeux JP & Bertrand D (2001). Desensitization of neuronal nicotinic acetylcholine receptors conferred by N-terminal segments of the β2 subunit. *Biochemistry* **40**, 2066–2074.

Bouzat C, Gumilar F, Spitzmaul G, Wang HL, Rayes D, Hansen SB, Taylor P & Sine SM (2004). Coupling of agonist binding to channel gating in an ACh-binding protein linked to an ion channel. *Nature* **430**, 896–900.

Brejc K, Van Dijk WJ, Klaassen RV, Schuurmans M, Van Der Oost J, Smit AB & Sixma TK (2001). Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **411**, 269–276.

Chakrapani S & Auerbach A (2005). A speed limit for conformational change of an allosteric membrane protein. *Proc Natl Acad Sci U S A* **102**, 87–92.

Chakrapani S, Bailey TD & Auerbach A (2004). Gating dynamics of the acetylcholine receptor extracellular domain. *J Gen Physiol* **123**, 341–356.

Cheng X, Lu B, Grant B, Law RJ & McCammon JA (2006). Channel opening motion of α7 nicotinic acetylcholine receptor as suggested by normal mode analysis. *J Mol Biol* **355**, 310–324.

Corringer PJ, Le Novere N & Changeux JP (2000). Nicotinic receptors at the amino acid level. *Annu Rev Pharmacol Toxicol* **40**, 431–458.

Dellisanti CD, Yao Y, Stroud JC, Wang ZZ & Chen L (2007). Crystal structure of the extracellular domain of nAChR α1 bound to α-bungarotoxin at 1.94 A resolution. *Nat Neurosci* **10**, 953–962.

Dutertre S & Lewis RJ (2006). Toxin insights into nicotinic acetylcholine receptors. *Biochem Pharmacol* **72**, 661–670.

Galzi JL, Bertrand S, Corringer PJ, Changeux JP & Bertrand D (1996). Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor. *EMBO J* **15**, 5824–5832.

Giniatullin R, Nistri A & Yakel JL (2005). Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. *Trends Neurosci* **28**, 371–378.

Grosman C, Zhou M & Auerbach A (2000). Mapping the conformational wave of acetylcholine receptor channel gating. *Nature* **403**, 773–776.

Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P & Bourne Y (2005). Structures of *Aplysia* AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. *EMBO J* **24**, 3635–3646.

Henchman RH, Wang HL, Sine SM, Taylor P & McCammon JA (2005). Ligand-induced conformational change in the α7 nicotinic receptor ligand binding domain. *Biophys J* **88**, 2564–2576.

Hibbs RE, Johnson DA, Shi J & Taylor P (2006). Structural dynamics of the acetylcholine binding protein: hydrodynamic and fluorescence anisotropy decay analyses. *J Mol Neurosci* **30**, 73–74.

Kash TL, Jenkins A, Kelley JC, Trudell JR & Harrison NL (2003). Coupling of agonist binding to channel gating in the $GABA_A$ receptor. *Nature* **421**, 272–275.

Kash TL, Kim T, Trudell JR & Harrison NL (2004). Evaluation of a proposed mechanism of ligand-gated ion channel activation in the GABAA and glycine receptors. *Neurosci Lett* **371**, 230–234.

Law RJ, Henchman RH & McCammon JA (2005). A gating mechanism proposed from a simulation of a human $\alpha 7$ nicotinic acetylcholine receptor. *Proc Natl Acad Sci U S A* **102**, 6813–6818.

Lee WY & Sine SM (2005). Principal pathway coupling agonist binding to channel gating in nicotinic receptors. *Nature* **438**, 243–247.

Lester HA, Dibas MI, Dahan DS, Leite JF & Dougherty DA (2004). Cys-loop receptors: new twists and turns. *Trends Neurosci* **27**, 329–336.

Lummis SC, Beene DL, Lee LW, Lester HA, Broadhurst RW & Dougherty DA (2005). Cis-trans isomerization at a proline opens the pore of a neurotransmitter-gated ion channel. *Nature* **438**, 248–252.

Lyford LK, Sproul AD, Eddins D, McLaughlin JT & Rosenberg RL (2003). Agonist-induced conformational changes in the extracellular domain of a $\alpha 7$ nicotinic acetylcholine receptors. *Mol Pharmacol* **64**, 650–658.

McLaughlin JT, Fu J, Sproul AD & Rosenberg RL (2006). Role of the outer β-sheet in divalent cation modulation of $\alpha 7$ nicotinic receptors. *Mol Pharmacol* **70**, 16–22.

Mercado J & Czajkowski C (2006). Charged residues in the $\alpha 1$ and $\beta 2$ pre-M1 regions involved in GABAA receptor activation. *J Neurosci* **26**, 2031–2040.

Mukhtasimova N, Free C & Sine SM (2005). Initial coupling of binding to gating mediated by conserved residues in the muscle nicotinic receptor. *J Gen Physiol* **126**, 23–39.

Purohit P, Mitra A & Auerbach A (2007). A stepwise mechanism for acetylcholine receptor channel gating. *Nature* **446**, 930–933.

Reeves DC, Jansen M, Bali M, Lemster T & Akabas MH (2005). A role for the $\beta 1$-$\beta 2$ loop in the gating of 5-HT3 receptors. *J Neurosci* **25**, 9358–9366.

Revah F, Bertrand D, Galzi JL, Devillers-Thiery A, Mulle C, Hussy N, Bertrand S, Ballivet M & Changeux JP (1991). Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* **353**, 846–849.

Schofield CM, Trudell JR & Harrison NL (2004). Alanine-scanning mutagenesis in the signature disulfide loop of the glycine receptor $\alpha 1$ subunit: critical residues for activation and modulation. *Biochemistry* **43**, 10058–10063.

Sine SM & Engel AG (2006). Recent advances in Cys-loop receptor structure and function. *Nature* **440**, 448–455.

Smit AB, Syed NI, Schaap D, Van Minnen J, Klumperman J, Kits KS, Lodder H, Van Der Schors RC, Van Elk R, Sorgedrager B, Brejc K, Sixma TK & Geraerts WP (2001). A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature* **411**, 261–268.

Taly A, Delarue M, Grutter T, Nilges M, Le Novere N, Corringer PJ & Changeux JP (2005). Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys J* **88**, 3954–3965.

Ulens C, Hogg RC, Celie PH, Bertrand D, Tsetlin V, Smit AB & Sixma TK (2006). Structural determinants of selective α-conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. *Proc Natl Acad Sci U S A* **103**, 3615–3620.

Unwin N (1995). Acetylcholine receptor channel imaged in the open state. *Nature* **373**, 37–43.

Unwin N (2005). Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol* **346**, 967–989.

Wilson G & Karlin A (2001). Acetylcholine receptor channel structure in the resting, open, and desensitized states probed with the substituted-cysteine-accessibility method. *Proc Natl Acad Sci U S A* **98**, 1241–1248.

Xiu X, Hanek AP, Wang J, Lester HA & Dougherty DA (2005). A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. *J Biol Chem* **280**, 41655–41666.

Zhou Y, Pearson JE & Auerbach A (2005). Φ-Value analysis of a linear, sequential reaction mechanism: theory and application to ion channel gating. *Biophys J* **89**, 3680–3685.

Acknowledgements

We would like to thank C. Erxleben and S. Dudek for advice in preparing the manuscript. Research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.