RECEPTORES CYS-LOOP: MECANISMOS MOLECULARES DE ACTIVACIÓN Y MODULACIÓN POR FÁRMACOS NEUROACTIVOS

RESUMEN

Los receptores Cys-loop pertenecen a la familia de canales iónicos pentaméricos activados por ligandos (pLGICs). Se expresan ampliamente en el sistema nervioso, donde ejercen un rol vital en la comunicación neuronal. Están involucrados en los procesos de aprendizaje, memoria, movimiento, entre otros. Se han asociado alteraciones en la funcionalidad de estos receptores con una gran variedad de desórdenes neurológicos, tales como enfermedad de Alzheimer, enfermedad de Parkinson, epilepsia, síndromes miasténicos, esquizofrenia y depresión. Por ello, los receptores Cys-loop son importantes blancos farmacológicos. En consecuencia, consideramos que el conocimiento de los mecanismos moleculares que conducen a su activación y disfunción es de suma relevancia.

Los receptores Cys-loop están formados por un dominio extracelular, que contiene los sitios de unión de agonista, y un dominio transmembrana, que forma el poro iónico. La interfase entre ambos dominios, llamada región de acoplamiento, desempeña un rol clave en la propagación de los cambios conformacionales que se inician con la unión del agonista en la región extracelular y culminan con la apertura del poro iónico a nivel transmembranal.

En este trabajo de Tesis Doctoral estudiamos dos regiones claves en el proceso de activación de los receptores Cys-loop: el sitio de unión de agonista, donde comienza la respuesta, y la interfase entre los dominios extracelular y transmembrana o región de acoplamiento. Utilizamos receptores homopentaméricos que por estar compuestos por cinco subunidades iguales, poseen cinco sitios de unión de agonista y cinco regiones de acoplamiento idénticas. Los receptores homoméricos surgieron más tempranamente en la escala evolutiva por lo que presentan características estructurales y funcionales comunes a todos los miembros Cys-loop, y son, por lo tanto, modelos útiles para el estudio de los receptores de esta familia.

En el Capítulo I de esta Tesis determinamos el número de regiones de acoplamiento necesario para la activación de los receptores Cys-loop y su relación con los sitios de unión de agonista. Para ello, utilizamos como modelo de receptor homopentamérico al receptor quimérico α7-5HT$_3$A, compuesto por secuencias del receptor α7 en su dominio extracelular y secuencias del receptor 5-HT$_3$A en su dominio transmembrana, el que ha sido ampliamente utilizado como modelo de α7.

Para conocer la contribución de cada una de las cinco regiones de acoplamiento a la estabilidad de canal abierto del receptor α7-5HT$_3$A, empleamos nuestra estrategia experimental denominada *electrical fingerprinting*. Según esta estrategia, co-transfectamos células con una subunidad conteniendo la región de acoplamiento activa y otra subunidad conteniendo la región de acoplamiento inactiva, una de ellas conteniendo además mutaciones reporteras de conductancia. De esta forma, logramos expresar en membrana receptores con distinto número de regiones de acoplamiento funcionales que son identificados mediante registros de *patch-clamp* de canal único. Gracias a la presencia de las mutaciones reporteras de conductancia, la medición de la amplitud de cada apertura nos permitió conocer la estequiometria del receptor, es decir, el número de subunidades con región de acoplamiento funcional que tiene el receptor pentamérico que dio origen a esa apertura. Determinamos la duración de los eventos de apertura provenientes de receptores con distinto número de regiones de acoplamiento funcionales, que constituye una medida de la estabilidad de canal abierto. Encontramos que cada región de acoplamiento contribuye en forma independiente y simétrica a la estabilidad del canal abierto y que son necesarias las cinco regiones de acoplamiento funcionales para lograr la óptima activación del receptor. Demostramos además que la presencia de una sola región de acoplamiento funcional en el pentámero es suficiente para lograr la activación pero no permite mantener el canal abierto en su tiempo óptimo.

Además generamos receptores α7-5HT$_3$A mutantes, que contenían distinto número de sitios de unión de agonista y regiones de acoplamiento funcionales. Esta estrategia nos permitió establecer los requisitos estructurales mínimos que logran la activación del receptor, así como también los requerimientos estructurales que conducen a la máxima estabilidad del estado abierto. Encontramos que el receptor es capaz de responder al agonista mediante la ocupación de un único sitio si este se encuentra formado por dos subunidades con regiones de acoplamiento funcionales. Sin embargo, para lograr la óptima activación y duración máxima del canal abierto, el receptor modelo utilizado requiere de tres sitios de unión de agonista funcionales y sus cinco regiones de acoplamiento intactas.

En el Capítulo II, estudiamos la activación del receptor neuronal α7 en condiciones de sub-ocupación de sus cinco sitios de unión de agonista. Este receptor se localiza principalmente en sitios distantes a los sitios de síntesis y liberación de acetilcolina (ACh), por lo que la ACh, o su producto colina, deben difundir y unirse a receptores α7 distantes. Este mecanismo colinérgico no sináptico predice que el grado de ocupación de los receptores α7 sería bajo en condiciones fisiológicas.

Para estudiar la activación del receptor α7 en condiciones de sub-ocupación de sus sitios de agonista, realizamos ensayos electrofisiológicos y medimos la duración del canal abierto de receptores individuales que presentan un único sitio de unión de agonista

funcional, y la comparamos con la de receptores que tienen sus cinco sitios funcionales. Para conocer el número de sitios de unión de agonista funcionales empleamos nuevamente la estrategia *electrical fingerprinting*. Esta estrategia requiere la medición exacta de la amplitud. Teniendo en cuenta que los receptores α7 presentan aperturas de duración breve que no permiten la resolución de su máxima amplitud, los estudios electrofisiológicos se realizaron sobre receptores α7 mutados o en presencia de potenciadores que aumentan la duración del canal abierto. En este trabajo, demostramos que la estabilidad del canal abierto de receptores α7 que presentan un único sitio de unión de agonista funcional es la misma que la de los receptores que presentan sus cinco sitios disponibles.

Por otro lado, cuando reemplazamos el dominio transmembrana del receptor α7 por el del receptor 5-HT_3A, encontramos que la duración del canal abierto se incrementa al aumentar el número de sitios ocupados por agonista. Este resultado demuestra por primera vez que el dominio extracelular no es el único determinante de la relación entre ocupación y estabilidad del canal abierto.

Por lo tanto, en este trabajo demostramos la capacidad del receptor α7 de activarse y producir respuestas máximas con la ocupación de un solo sitio de unión de agonista, propiedad que es única y exclusiva de este receptor dentro de todos los miembros de la familia de receptores Cys-loop. Este resultado posee además relevancia fisiológica dado que esta propiedad le permitiría al receptor adaptarse al mecanismo de transmisión no sináptico.

En su conjunto, los resultados que surgen de esta Tesis revelan una novedosa relación funcional entre dos dominios estructurales de estos receptores, el sitio de unión de agonista y la región de acoplamiento, y, además, contribuyen al conocimiento general del mecanismo de activación de los receptores de la familia Cys-loop.

CYS-LOOP RECEPTORS: MOLECULAR MECHANISMS OF ACTIVATION AND MODULATION BY NEUROACTIVE DRUGS

SUMMARY

Cys-loop receptors belong to the family of pentameric ligand-gated ion channels (pLGICs). They are widely expressed in the nervous system, where they exert a vital role in neuronal communication. They are involved in learning, memory, movement processes, among others. Functional disorders of these receptors have been associated with several neurological disorders, such as Alzheimer's disease, Parkinson's disease, epilepsy, myasthenic syndromes, schizophrenia and depression. Because Cys-loop receptors are important pharmacological targets for the development of therapies, the knowledge of the molecular mechanisms leading to activation and dysfunction of these receptors is of great importance.

Cys-loop receptors contain an extracellular domain that carries the agonist binding sites and a transmembrane region that forms the ion pore. The interface between both domains, named as the coupling region, plays a key role in the propagation of the conformational changes from the binding site at the extracellular domain to the pore, located at the transmembrane region.

In this Thesis, we studied two key regions that are essential for the activation process of Cys-loop receptors: the agonist binding site, where the response begins, and the interface between the extracellular and transmembrane domains or coupling region. We used homopentameric receptors that contain five identical subunits, and therefore five identical agonist binding sites and coupling regions. Because homomeric receptors appeared earlier on the evolutionary scale, they present structural and functional features that are common to all Cys-loop members, and are therefore useful models for the study of this receptor family.

In Chapter I of this Thesis we studied the number of coupling regions necessary for Cys-loop receptor activation and evaluated the functional relationship of this domain with the agonist binding sites. To this end, we used a model of homopentameric receptor, the α7-5HT$_3$A chimeric receptor, which contains α7 sequences in the extracellular domain and 5-HT$_3$A sequences in the transmembrane domain.

To determine the contribution of each of the five coupling regions to the stability of the open channel, we used our experimental strategy which is called electrical fingerprinting. For this strategy, cells were co-transfected with a subunit with an active coupling region and another subunit with an inactive coupling region, one of which carrying reporter

conductance mutations, to generate receptors with different number of functional coupling regions. Next, we performed single-channel recordings to identify functional receptors using the patch-clamp technique. Due to the introduction of reporter conductance mutations, the measurement of the amplitude of each opening event allowed us to know receptor stoichiometry, i.e., the number of subunits with functional coupling region present in the pentameric receptor which originated the event. We measured open channel duration of receptors with different numbers of functional coupling regions, which indicates the open channel stability. We found that each coupling region contributes independently and symmetrically to open channel stability. We showed that five coupling regions are necessary to achieve optimal receptor activation and that the presence of only one functional coupling region is sufficient for receptor activation, but with reduced open channel duration.

Furthermore, we constructed α7-5HT$_3$A mutant receptors, containing different number of agonist binding sites and functional coupling regions. This strategy allowed us to establish the minimum structural requirements for receptor activation as well as the structural requirements for maximal open channel stability. We found that α7-5HT$_3$A receptors are capable of responding to agonist by occupying a single agonist binding site, only if this site is formed by two subunits carrying functional coupling regions. However, to achieve optimal activation and maximal open channel duration, the model receptor requires three functional agonist binding sites and five functional coupling regions.

In Chapter II, we studied α7 neuronal receptor activation under sub-occupancy conditions of its five agonist binding sites. In the brain, this receptor is mainly located at distant sites from the sites of synthesis and release of acetylcholine (ACh), so ACh, or its product choline, diffuse to bind distant α7 receptors. This non-synaptic cholinergic mechanism predicts that the degree of α7 receptor occupancy is low under physiological conditions.

To study α7 activation under sub-occupancy conditions we performed single-channel recordings and measured open channel duration of receptors with only one functional agonist binding site, and compared it with that of receptors containing their five intact agonist binding sites. To know the number of agonist binding sites, we employed again the electrical fingerprinting strategy. This strategy requires accurate measurement of open channel amplitude. Because the brief duration of α7 opening events do not allow full amplitude resolution, single-channel recordings were performed in either α7 mutant receptors or in the presence of potentiators that increase open channel duration. In this work, we demonstrated that open channel stability of receptors with a single agonist binding site is the same as that of receptors containing five functional sites.

Moreover, when we replaced the transmembrane domain of α7 receptors by that of 5-HT$_3$A receptor, we found that open channel lifetime increases as the number of sites

occupied by agonist increases. This result shows for the first time that the extracellular domain is not the only determinant of the relationship between occupancy and open channel stability.

Therefore, in this work we demonstrated the ability of α7 receptor for activation and eliciting maximal responses with occupancy of only one agonist binding site, a property that is unique for α7 among all members of the Cys-loop family. This result has a physiological relevance since this property would allow α7 receptors to adapt to their non-synaptic mechanism.

Taken together, the results that emerge from this Thesis reveal a novel functional relationship between two structural domains, the agonist binding site and the coupling region, and contribute to the general knowledge of the activation mechanism of Cys-loop receptors.

BIBLIOGRAFÍA

Absalom NL, Schofield PR, Lewis TM (2009) Pore structure of the Cys-loop ligand-gated ion channels. Neurochem Res 34:1805-1815.

Agnati LF, Leo G, Zanardi A, Genedani S, Rivera A, Fuxe K, Guidolin D (2006) Volume transmission and wiring transmission from cellular to molecular networks: history and perspectives. Acta Physiol (Oxf) 187:329-344.

Akk G, Steinbach JH (2000) Structural elements near the C-terminus are responsible for changes in nicotinic receptor gating kinetics following patch excision. J Physiol 527 Pt 3:405-417.

Almen MS, Nordstrom KJ, Fredriksson R, Schioth HB (2009) Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biol 7:50.

Andersen N, Corradi J, Bartos M, Sine SM, Bouzat C (2011) Functional relationships between agonist binding sites and coupling regions of homomeric Cys-loop receptors. J Neurosci 31:3662-3669.

Andersson U, Tracey KJ (2012) Reflex principles of immunological homeostasis. Annu Rev Immunol 30:313-335.

Arias HR (2000) Localization of agonist and competitive antagonist binding sites on nicotinic acetylcholine receptors. Neurochem Int 36:595-645.

Arias HR (2009) Is the inhibition of nicotinic acetylcholine receptors by bupropion involved in its clinical actions? Int J Biochem Cell Biol 41:2098-2108.

Arias HR, Bhumireddy P (2005) Anesthetics as chemical tools to study the structure and function of nicotinic acetylcholine receptors. Curr Protein Pept Sci 6:451-472.

Arias HR, Bhumireddy P, Bouzat C (2006) Molecular mechanisms and binding site locations for noncompetitive antagonists of nicotinic acetylcholine receptors. Int J Biochem Cell Biol 38:1254-1276.

Arias HR, Gumilar F, Rosenberg A, Targowska-Duda KM, Feuerbach D, Jozwiak K, Moaddel R, Wainer IW, Bouzat C (2009) Interaction of bupropion with muscle-type nicotinic acetylcholine receptors in different conformational states. Biochemistry 48:4506-4518.

Auerbach A (2013) The energy and work of a ligand-gated ion channel. J Mol Biol 425:1461-1475.

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

Barnes NM, Hales TG, Lummis SC, Peters JA (2009) The 5-HT3 receptor--the relationship between structure and function. Neuropharmacology 56:273-284.

Bartos M, Corradi J, Bouzat C (2009) Structural basis of activation of cys-loop receptors: the extracellular-transmembrane interface as a coupling region. Mol Neurobiol 40:236-252.

Baumann SW, Baur R, Sigel E (2003) Individual properties of the two functional agonist sites in GABA(A) receptors. J Neurosci 23:11158-11166.

Beato M, Groot-Kormelink PJ, Colquhoun D, Sivilotti LG (2004) The activation mechanism of alpha1 homomeric glycine receptors. J Neurosci 24:895-906.

Beckstein O, Sansom MS (2004) The influence of geometry, surface character, and flexibility on the permeation of ions and water through biological pores. Phys Biol 1:42-52.

Beckstein O, Sansom MS (2006) A hydrophobic gate in an ion channel: the closed state of the nicotinic acetylcholine receptor. Phys Biol 3:147-159.

Benallegue N, Mazzaferro S, Alcaino C, Bermudez I (2013) The additional ACh binding site at the alpha4(+)/alpha4(-) interface of the (alpha4beta2)2alpha4 nicotinic ACh receptor contributes to desensitization. Br J Pharmacol 170:304-316.

Bernal JA, Mulet J, Castillo M, Criado M, Sala F, Sala S (2009a) Single-channel study of the binding-gating coupling in the slowly desensitizing chimeric alpha7-5HT3A receptor. FEBS Lett 583:1045-1051.

Bernal JA, Mulet J, Castillo M, Criado M, Sala S, Sala F (2009b) Binding-gating coupling in a nondesensitizing alpha7 nicotinic receptor A single channel pharmacological study. Biochim Biophys Acta 1788:410-416.

Bertrand D, Gopalakrishnan M (2007) Allosteric modulation of nicotinic acetylcholine receptors. Biochem Pharmacol 74:1155-1163.

Blum AP, Van Arnam EB, German LA, Lester HA, Dougherty DA (2013) Binding interactions with the

complementary subunit of nicotinic receptors. J Biol Chem 288:6991-6997.

Bocquet N, Nury H, Baaden M, Le PC, Changeux JP, Delarue M, Corringer PJ (2009) X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457:111-114.

Bourne Y, Talley TT, Hansen SB, Taylor P, Marchot P (2005) Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake alpha-neurotoxins and nicotinic receptors. EMBO J 24:1512-1522.

Bouzat C. (1996) Ephedrine blocks wild-type and long-lived mutant acetylcholine receptor channels. Neuroreport 8:317-321.

Bouzat C (2012) New insights into the structural bases of activation of Cys-loop receptors. J Physiol Paris 106:23-33.

Bouzat C, Barrantes F, Sine S (2000) Nicotinic receptor fourth transmembrane domain: hydrogen bonding by conserved threonine contributes to channel gating kinetics. J Gen Physiol 115:663-672.

Bouzat C, Bartos M, Corradi J, Sine SM (2008) The interface between extracellular and transmembrane domains of homomeric Cys-loop receptors governs open-channel lifetime and rate of desensitization. J Neurosci 28:7808-7819.

Bouzat C, Bren N, Sine SM (1994) Structural basis of the different gating kinetics of fetal and adult acetylcholine receptors. Neuron 13:1395-1402.

Bouzat C, Gumilar F, del Carmen EM, Sine SM (2002) Subunit-selective contribution to channel gating of the M4 domain of the nicotinic receptor. Biophys J 82:1920-1929.

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.

Boyd GW, Low P, Dunlop JI, Robertson LA, Vardy A, Lambert JJ, Peters JA, Connolly CN (2002) Assembly and cell surface expression of homomeric and heteromeric 5-HT3 receptors: the role of oligomerization and chaperone proteins. Mol Cell Neurosci 21:38-50.

Brannigan G, LeBard DN, Henin J, Eckenhoff RG, Klein ML (2010) Multiple binding sites for the general anesthetic isoflurane identified in the nicotinic acetylcholine receptor transmembrane domain. Proc Natl Acad Sci U S A 107:14122-14127.

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

Broad LM, Zwart R, Pearson KH, Lee M, Wallace L, McPhie GI, Emkey R, Hollinshead SP, Dell CP, Baker SR, Sher E (2006) Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators. J Pharmacol Exp Ther 318:1108-1117.

Brown WEL, Hill AV (1923) The oxygen-dissociation curve of blood, and its thermodynamical basis. Proc R Soc Lond B Biol Sci 94:297-334.

Burli T, Baer K, Ewers H, Sidler C, Fuhrer C, Fritschy JM (2010) Single particle tracking of alpha7 nicotinic AChR in hippocampal neurons reveals regulated confinement at glutamatergic and GABAergic perisynaptic sites. PLoS ONE 5:e11507.

Carland JE, Cooper MA, Sugiharto S, Jeong HJ, Lewis TM, Barry PH, Peters JA, Lambert JJ, Moorhouse AJ (2009) Characterization of the effects of charged residues in the intracellular loop on ion permeation in alpha1 glycine receptor channels. J Biol Chem 284:2023-2030.

Castillo M, Mulet J, Bernal JA, Criado M, Sala F, Sala S (2006) Improved gating of a chimeric alpha7-5HT3A receptor upon mutations at the M2-M3 extracellular loop. FEBS Lett 580:256-260.

Celie PH, Kasheverov IE, Mordvintsev DY, Hogg RC, van NP, van ER, van Rossum-Fikkert SE, Zhmak MN, Bertrand D, Tsetlin V, Sixma TK, Smit AB (2005a) Crystal structure of nicotinic acetylcholine receptor homolog AChBP in complex with an alpha-conotoxin PnIA variant. Nat Struct Mol Biol 12:582-588.

Celie PH, Klaassen RV, van Rossum-Fikkert SE, van ER, van NP, Smit AB, Sixma TK (2005b) 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 280:26457-26466.

Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK (2004) Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. Neuron 41:907-914.

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

Changeux JP, Taly A (2008) Nicotinic receptors, allosteric proteins and medicine. Trends Mol Med 14:93-102.

Cheng X, Ivanov I, Wang H, Sine SM, McCammon JA (2007) Nanosecond-timescale conformational dynamics of the human alpha7 nicotinic acetylcholine receptor. Biophys J 93:2622-2634.

Cheng X, Wang H, Grant B, Sine SM, McCammon JA (2006) Targeted molecular dynamics study of C-loop closure and channel gating in nicotinic receptors. PLoS Comput Biol 2:e134.

Chiara DC, Middleton RE, Cohen JB (1998) Identification of tryptophan 55 as the primary site of [3H]nicotine photoincorporation in the gamma-subunit of the Torpedo nicotinic acetylcholine receptor. FEBS Lett 423:223-226.

Cho CH, Song W, Leitzell K, Teo E, Meleth AD, Quick MW, Lester RA (2005) Rapid upregulation of alpha7 nicotinic acetylcholine receptors by tyrosine dephosphorylation. J Neurosci 25:3712-3723.

Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56:2-5.

Colquhoun D. (2006) Agonist-activated ion channels. Br J Pharmacol 147 Suppl 1:S17-S26.

Colquhoun D, Sivilotti LG (2004) Function and structure in glycine receptors and some of their relatives. Trends Neurosci 27:337-344.

Contant C, Umbriaco D, Garcia S, Watkins KC, Descarries L (1996) Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. Neuroscience 71:937-947.

Corradi J, Andersen N, Bouzat C (2011) A novel mechanism of modulation of 5-HT(3)A receptors by hydrocortisone. Biophys J 100:42-51.

Corradi J, Gumilar F, Bouzat C (2009) Single-channel kinetic analysis for activation and desensitization of homomeric 5-HT(3)A receptors. Biophys J 97:1335-1345.

Corringer PJ, Poitevin F, Prevost MS, Sauguet L, Delarue M, Changeux JP (2012) Structure and pharmacology of pentameric receptor channels: from bacteria to brain. Structure 20:941-956.

Corry B (2006) An energy-efficient gating mechanism in the acetylcholine receptor channel suggested by molecular and Brownian dynamics. Biophys J 90:799-810.

Cymes GD, Grosman C (2008) Pore-opening mechanism of the nicotinic acetylcholine receptor evinced by proton transfer. Nat Struct Mol Biol 15:389-396.

daCosta CJ, Baenziger JE (2013) Gating of pentameric ligand-gated ion channels: structural insights and ambiguities. Structure 21:1271-1283.

daCosta CJ, Free CR, Corradi J, Bouzat C, Sine SM (2011) Single-channel and structural foundations of neuronal alpha7 acetylcholine receptor potentiation. J Neurosci 31:13870-13879.

daCosta CJ, Sine SM (2013) Stoichiometry for drug potentiation of a pentameric ion channel. Proc Natl Acad Sci U S A 110:6595-6600.

Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:699-729.

De Rosa MJ, Dionisio L, Agriello E, Bouzat C, Esandi MC (2009) Alpha 7 nicotinic acetylcholine receptor modulates lymphocyte activation. Life Sci 85:444-449.

Deeb TZ, Carland JE, Cooper MA, Livesey MR, Lambert JJ, Peters JA, Hales TG (2007) Dynamic modification of a mutant cytoplasmic cysteine residue modulates the conductance of the human 5-HT3A receptor. J Biol Chem 282:6172-6182.

Dellisanti CD, Yao Y, Stroud JC, Wang ZZ, Chen L (2007) Crystal structure of the extracellular domain of nAChR alpha1 bound to alpha-bungarotoxin at 1.94 A resolution. Nat Neurosci 10:953-962.

Descarries L, Gisiger V, Steriade M (1997) Diffuse transmission by acetylcholine in the CNS. Prog Neurobiol 53:603-625.

Dilger JP, Brett RS, Lesko LA (1992) Effects of isoflurane on acetylcholine receptor channels. 1. Single-channel currents. Mol Pharmacol 41:127-133.

Dilger JP, Liu Y (1992) Desensitization of acetylcholine receptors in BC3H-1 cells. Pflugers Arch 420:479-485.

Durisic N, Godin AG, Wever CM, Heyes CD, Lakadamyali M, Dent JA (2012) Stoichiometry of the human glycine receptor revealed by direct subunit counting. J Neurosci 32:12915-12920.

Dutertre S, Becker CM, Betz H (2012a) Inhibitory glycine receptors: an update. J Biol Chem 287:40216-40223.

Dutertre S, Drwal M, Laube B, Betz H (2012b) Probing the pharmacological properties of distinct subunit

interfaces within heteromeric glycine receptors reveals a functional betabeta agonist-binding site. J Neurochem 122:38-47.

Eisele JL, Bertrand S, Galzi JL, villers-Thiery A, Changeux JP, Bertrand D (1993) Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. Nature 366:479-483.

Everitt AB, Seymour VA, Curmi J, Laver DR, Gage PW, Tierney ML (2009) Protein interactions involving the gamma2 large cytoplasmic loop of GABA(A) receptors modulate conductance. FASEB J 23:4361-4369.

Fabian-Fine R, Skehel P, Errington ML, Davies HA, Sher E, Stewart MG, Fine A (2001) Ultrastructural distribution of the alpha7 nicotinic acetylcholine receptor subunit in rat hippocampus. J Neurosci 21:7993-8003.

Faghih R, Gopalakrishnan M, Briggs CA (2008) Allosteric modulators of the alpha7 nicotinic acetylcholine receptor. J Med Chem 51:701-712.

Faghih R, Gopalakrishnan SM, Gronlien JH, Malysz J, Briggs CA, Wetterstrand C, Ween H, Curtis MP, Sarris KA, Gfesser GA, El-Kouhen R, Robb HM, Radek RJ, Marsh KC, Bunnelle WH, Gopalakrishnan M (2009) Discovery of 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)benzenesulfonamide (A-867744) as a novel positive allosteric modulator of the alpha7 nicotinic acetylcholine receptor. J Med Chem 52:3377-3384.

Filatov GN, White MM (1995) The role of conserved leucines in the M2 domain of the acetylcholine receptor in channel gating. Mol Pharmacol 48:379-384.

Formenti A, De SA (2000) Effects of extracellular Ca2+ on membrane and seal resistance in patch-clamped rat thalamic and sensory ganglion neurons. Neurosci Lett 279:49-52.

Fritsch S, Ivanov I, Wang H, Cheng X (2011) Ion selectivity mechanism in a bacterial pentameric ligand-gated ion channel. Biophys J 100:390-398.

Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Diaz-Cabiale Z, Rivera A, Ferraro L, Tanganelli S, Tarakanov AO, Garriga P, Narvaez JA, Ciruela F, Guescini M, Agnati LF (2012) Extrasynaptic neurotransmission in the modulation of brain function. Focus on the striatal neuronal-glial networks. Front Physiol 3:136.

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.

Gotti C, Clementi F (2004) Neuronal nicotinic receptors: from structure to pathology. Prog Neurobiol 74:363-396.

Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I, Moretti M, Pedrazzi P, Pucci L, Zoli M (2009) Structural and functional diversity of native brain neuronal nicotinic receptors. Biochem Pharmacol 78:703-711.

Gotti C, Zoli M, Clementi F (2006) Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol Sci 27:482-491.

Gronlien JH, Hakerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J (2007) Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. Mol Pharmacol 72:715-724.

Grosman C, Salamone FN, Sine SM, Auerbach A (2000) The extracellular linker of muscle acetylcholine receptor channels is a gating control element. J Gen Physiol 116:327-340.

Grutter T, de Carvalho LP, Dufresne V, Taly A, Edelstein SJ, Changeux JP (2005) Molecular tuning of fast gating in pentameric ligand-gated ion channels. Proc Natl Acad Sci U S A 102:18207-18212.

Gumilar F, Arias HR, Spitzmaul G, Bouzat C (2003) Molecular mechanisms of inhibition of nicotinic acetylcholine receptors by tricyclic antidepressants. Neuropharmacology 45:964-976.

Gumilar F, Bouzat C (2008) Tricyclic antidepressants inhibit homomeric Cys-loop receptors by acting at different conformational states. Eur J Pharmacol 584:30-39.

Hales TG, Dunlop JI, Deeb TZ, Carland JE, Kelley SP, Lambert JJ, Peters JA (2006) Common determinants of single channel conductance within the large cytoplasmic loop of 5-hydroxytryptamine type 3 and alpha4beta2 nicotinic acetylcholine receptors. J Biol Chem 281:8062-8071.

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85-100.

Hamouda AK, Kimm T, Cohen JB (2013) Physostigmine and galanthamine bind in the presence of agonist at the canonical and noncanonical subunit interfaces of a nicotinic acetylcholine receptor. J Neurosci 33:485-494.

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.

Hansen SB, Taylor P (2007) Galanthamine and non-competitive inhibitor binding to ACh-binding protein: evidence for a binding site on non-alpha-subunit interfaces of heteromeric neuronal nicotinic receptors. J Mol Biol 369:895-901.

Hansen SB, Wang HL, Taylor P, Sine SM (2008) An ion selectivity filter in the extracellular domain of Cys-loop receptors reveals determinants for ion conductance. J Biol Chem 283:36066-36070.

Haydar SN, Dunlop J (2010) Neuronal nicotinic acetylcholine receptors - targets for the development of drugs to treat cognitive impairment associated with schizophrenia and Alzheimer's disease. Curr Top Med Chem 10:144-152.

Hibbs RE, Gouaux E (2011) Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature 474:54-60.

Hibbs RE, Sulzenbacher G, Shi J, Talley TT, Conrod S, Kem WR, Taylor P, Marchot P, Bourne Y (2009) Structural determinants for interaction of partial agonists with acetylcholine binding protein and neuronal alpha7 nicotinic acetylcholine receptor. EMBO J 28:3040-3051.

Hilf RJ, Dutzler R (2008) X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature 452:375-379.

Hilf RJ, Dutzler R (2009a) A prokaryotic perspective on pentameric ligand-gated ion channel structure. Curr Opin Struct Biol 19:418-424.

Hilf RJ, Dutzler R (2009b) Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. Nature 457:115-118.

Hill AV (1910) The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curve. J Physiol 40:IV-VII.

Hogg RC, Bertrand D (2007) Partial agonists as therapeutic agents at neuronal nicotinic acetylcholine receptors. Biochem Pharmacol 73:459-468.

Hogg RC, Buisson B, Bertrand D (2005) Allosteric modulation of ligand-gated ion channels. Biochem Pharmacol 70:1267-1276.

Hu XQ, Sun H, Peoples RW, Hong R, Zhang L (2006) An interaction involving an arginine residue in the cytoplasmic domain of the 5-HT3A receptor contributes to receptor desensitization mechanism. J Biol Chem 281:21781-21788.

Hu XQ, Zhang L, Stewart RR, Weight FF (2003) Arginine 222 in the pre-transmembrane domain 1 of 5-HT3A receptors links agonist binding to channel gating. J Biol Chem 278:46583-46589.

Hummer G, Rasaiah JC, Noworyta JP (2001) Water conduction through the hydrophobic channel of a carbon nanotube. Nature 414:188-190.

Hurst R, Rollema H, Bertrand D (2013) Nicotinic acetylcholine receptors: from basic science to therapeutics. Pharmacol Ther 137:22-54.

Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, Rutherford-Root KL, Berkenpas MB, Hoffmann WE, Piotrowski DW, Groppi VE, Allaman G, Ogier R, Bertrand S, Bertrand D, Arneric SP (2005) A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci 25:4396-4405.

Ivanov I, Cheng X, Sine SM, McCammon JA (2007) Barriers to ion translocation in cationic and anionic receptors from the Cys-loop family. J Am Chem Soc 129:8217-8224.

Jaldety Y, Glick Y, Orr-Urtreger A, Ickowicz D, Gerber D, Breitbart H (2012) Sperm epidermal growth factor receptor (EGFR) mediates alpha7 acetylcholine receptor (AChR) activation to promote fertilization. J Biol Chem 287:22328-22340.

Jha A, Auerbach A (2010) Acetylcholine receptor channels activated by a single agonist molecule. Biophys J 98:1840-1846.

Jha A, Cadugan DJ, Purohit P, Auerbach A (2007) Acetylcholine receptor gating at extracellular transmembrane domain interface: the cys-loop and M2-M3 linker. J Gen Physiol 130:547-558.

Karlin A. (1967) On the application of "a plausible model" of allosteric proteins to the receptor for acetylcholine. J Theor Biol 16:306-320.

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.

Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ, Peters JA (2003) A cytoplasmic region determines single-channel conductance in 5-HT3 receptors. Nature 424:321-324.

Keramidas A, Lynch JW (2013) An outline of desensitization in pentameric ligand-gated ion channel receptors. Cell Mol Life Sci 70:1241-1253.

Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP, Bertrand D (1998) Ivermectin: a positive allosteric effector of the alpha7 neuronal nicotinic acetylcholine receptor. Mol Pharmacol 53:283-294.

Labarca C, Nowak MW, Zhang H, Tang L, Deshpande P, Lester HA (1995) Channel gating governed symmetrically by conserved leucine residues in the M2 domain of nicotinic receptors. Nature 376:514-516.

Lape R, Colquhoun D, Sivilotti LG (2008) On the nature of partial agonism in the nicotinic receptor superfamily. Nature.

Law RJ, Henchman RH, McCammon JA (2005) A gating mechanism proposed from a simulation of a human alpha7 nicotinic acetylcholine receptor. Proc Natl Acad Sci U S A 102:6813-6818.

Le Novere N., Changeux JP (1995) Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. J Mol Evol 40:155-172.

Lee WY, Free CR, Sine SM (2008) Nicotinic receptor interloop proline anchors beta1-beta2 and Cys loops in coupling agonist binding to channel gating. J Gen Physiol 132:265-278.

Lee WY, Free CR, Sine SM (2009) Binding to gating transduction in nicotinic receptors: Cys-loop energetically couples to pre-M1 and M2-M3 regions. J Neurosci 29:3189-3199.

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

Lendvai B, Kassai F, Szajli A, Nemethy Z (2013) alpha7 nicotinic acetylcholine receptors and their role in cognition. Brain Res Bull 93:86-96.

Lendvai B, Vizi ES (2008) Nonsynaptic chemical transmission through nicotinic acetylcholine receptors. Physiol Rev 88:333-349.

Li SX, Huang S, Bren N, Noridomi K, Dellisanti CD, Sine SM, Chen L (2011) Ligand-binding domain of an alpha7-nicotinic receptor chimera and its complex with agonist. Nat Neurosci 14:1253-1259.

Liu Y, Dilger JP (1991) Opening rate of acetylcholine receptor channels. Biophys J 60:424-432.

Livesey MR, Cooper MA, Deeb TZ, Carland JE, Kozuska J, Hales TG, Lambert JJ, Peters JA (2008) Structural Determinants of Ca2+ Permeability and Conduction in the Human 5-Hydroxytryptamine Type 3A Receptor. J Biol Chem 283:19301-19313.

Livesey MR, Cooper MA, Lambert JJ, Peters JA (2011) Rings of charge within the extracellular vestibule influence ion permeation of the 5-HT3A receptor. J Biol Chem 286:16008-16017.

Lopes C, Pereira EF, Wu HQ, Purushottamachar P, Njar V, Schwarcz R, Albuquerque EX (2007) Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at alpha7* nicotinic receptors. J Pharmacol Exp Ther 322:48-58.

Lummis SC (2012) 5-HT(3) receptors. J Biol Chem 287:40239-40245.

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 alpha 7 nicotinic acetylcholine receptors. Mol Pharmacol 64:650-658.

Lynch JW (2009) Native glycine receptor subtypes and their physiological roles. Neuropharmacology 56:303-309.

Malysz J, Gronlien JH, Anderson DJ, Hakerud M, Thorin-Hagene K, Ween H, Wetterstrand C, Briggs CA, Faghih R, Bunnelle WH, Gopalakrishnan M (2009) In vitro pharmacological characterization of a novel allosteric modulator of alpha 7 neuronal acetylcholine receptor, 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)benzenesulfonamide (A-867744), exhibiting unique pharmacological profile. J Pharmacol Exp Ther 330:257-267.

Mazzaferro S, Benallegue N, Carbone A, Gasparri F, Vijayan R, Biggin PC, Moroni M, Bermudez I (2011) Additional acetylcholine (ACh) binding site at alpha4/alpha4 interface of (alpha4beta2)2alpha4 nicotinic receptor influences agonist sensitivity. J Biol Chem 286:31043-31054.

McLaughlin JT, Fu J, Rosenberg RL (2007) Agonist-driven conformational changes in the inner beta-sheet of alpha7 nicotinic receptors. Mol Pharmacol 71:1312-1318.

Melzer N, Villmann C, Becker K, Harvey K, Harvey RJ, Vogel N, Kluck CJ, Kneussel M, Becker CM (2010) Multifunctional basic motif in the glycine receptor

intracellular domain induces subunit-specific sorting. J Biol Chem 285:3730-3739.

Mike A, Castro NG, Albuquerque EX (2000) Choline and acetylcholine have similar kinetic properties of activation and desensitization on the alpha7 nicotinic receptors in rat hippocampal neurons. Brain Res 882:155-168.

Millar NS (2008) RIC-3: a nicotinic acetylcholine receptor chaperone. Br J Pharmacol 153 Suppl 1:S177-S183.

Millar NS, Gotti C (2009) Diversity of vertebrate nicotinic acetylcholine receptors. Neuropharmacology 56:237-246.

Miller C (1989) Genetic manipulation of ion channels: a new approach to structure and mechanism. Neuron 2:1195-1205.

Miller PS, Smart TG (2010) Binding, activation and modulation of Cys-loop receptors. Trends Pharmacol Sci 31:161-174.

Mitra A, Bailey TD, Auerbach AL (2004) Structural dynamics of the M4 transmembrane segment during acetylcholine receptor gating. Structure (Camb) 12:1909-1918.

Miwa JM, Freedman R, Lester HA (2011) Neural systems governed by nicotinic acetylcholine receptors: emerging hypotheses. Neuron 70:20-33.

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. Nature 423:949-955.

Monod J, WYMAN J, Changeux JP (1965) On the nature of allosteric transitions: a plausible model. J Mol Biol 12:88-118.

Moroni M, Meyer JO, Lahmann C, Sivilotti LG (2011) In glycine and GABA(A) channels, different subunits contribute asymmetrically to channel conductance via residues in the extracellular domain. J Biol Chem 286:13414-13422.

Mowrey D, Chen Q, Liang Y, Liang J, Xu Y, Tang P (2013) Signal transduction pathways in the pentameric ligand-gated ion channels. PLoS ONE 8:e64326.

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.

Mukhtasimova N, Lee WY, Wang HL, Sine SM (2009) Detection and trapping of intermediate states priming nicotinic receptor channel opening. Nature 459:451-454.

Nayak TK, Purohit PG, Auerbach A (2012) The intrinsic energy of the gating isomerization of a neuromuscular acetylcholine receptor channel. J Gen Physiol 139:349-358.

Nemecz A, Taylor P (2011) Creating an alpha7 nicotinic acetylcholine recognition domain from the acetylcholine-binding protein: crystallographic and ligand selectivity analyses. J Biol Chem 286:42555-42565.

Ng HJ, Whittemore ER, Tran MB, Hogenkamp DJ, Broide RS, Johnstone TB, Zheng L, Stevens KE, Gee KW (2007) Nootropic alpha7 nicotinic receptor allosteric modulator derived from GABAA receptor modulators. Proc Natl Acad Sci U S A 104:8059-8064.

Nury H, Bocquet N, Le PC, Raynal B, Haouz A, Corringer PJ, Delarue M (2010) Crystal structure of the extracellular domain of a bacterial ligand-gated ion channel. J Mol Biol 395:1114-1127.

Nys M, Kesters D, Ulens C (2013) Structural insights into Cys-loop receptor function and ligand recognition. Biochem Pharmacol 86:1042-1053.

O'Leary ME, White MM (1992) Mutational analysis of ligand-induced activation of the Torpedo acetylcholine receptor. J Biol Chem 267:8360-8365.

Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev 60:243-260.

Ortells MO, Lunt GG (1995) Evolutionary history of the ligand-gated ion-channel superfamily of receptors. Trends Neurosci 18:121-127.

Overington JP, Al-Lazikani B, Hopkins AL (2006) How many drug targets are there? Nat Rev Drug Discov 5:993-996.

Paas Y, Gibor G, Grailhe R, Savatier-Duclert N, Dufresne V, Sunesen M, de Carvalho LP, Changeux JP, Attali B (2005) Pore conformations and gating mechanism of a Cys-loop receptor. Proc Natl Acad Sci U S A 102:15877-15882.

Papke RL, Meyer E, Nutter T, Uteshev VV (2000) alpha7 receptor-selective agonists and modes of alpha7 receptor activation. Eur J Pharmacol 393:179-195.

Papke RL, Porter Papke JK (2002) Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. Br J Pharmacol 137:49-61.

Pear WS, Nolan GP, Scott ML, Baltimore D (1993) Production of high-titer helper-free retroviruses by transient transfection. Proc Natl Acad Sci U S A 90:8392-8396.

Peters JA, Cooper MA, Carland JE, Livesey MR, Hales TG, Lambert JJ (2010) Novel structural determinants of single channel conductance and ion selectivity in 5-hydroxytryptamine type 3 and nicotinic acetylcholine receptors. J Physiol 588:587-596.

Peters JA, Kelley SP, Dunlop JI, Kirkness EF, Hales TG, Lambert JJ (2004) The 5-hydroxytryptamine type 3 (5-HT3) receptor reveals a novel determinant of single-channel conductance. Biochem Soc Trans 32:547-552.

Priel A, Gil Z, Moy VT, Magleby KL, Silberberg SD (2007) Ionic requirements for membrane-glass adhesion and giga seal formation in patch-clamp recording. Biophys J 92:3893-3900.

Purohit P, Auerbach A (2007) Acetylcholine receptor gating at extracellular transmembrane domain interface: the "pre-M1" linker. J Gen Physiol 130:559-568.

Puskar NL, Xiu X, Lester HA, Dougherty DA (2011) Two neuronal nicotinic acetylcholine receptors, alpha4beta4 and alpha7, show differential agonist binding modes. J Biol Chem 286:14618-14627.

Rayes D, De Rosa MJ, Sine SM, Bouzat C (2009) Number and locations of agonist binding sites required to activate homomeric Cys-loop receptors. J Neurosci 29:6022-6032.

Rayes D, Spitzmaul G, Sine SM, Bouzat C (2005) Single-channel kinetic analysis of chimeric alpha7-5HT3A receptors. Mol Pharmacol 68:1475-1483.

Ren J, Qin C, Hu F, Tan J, Qiu L, Zhao S, Feng G, Luo M (2011) Habenula "cholinergic" neurons co-release glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. Neuron 69:445-452.

Rucktooa P, Smit AB, Sixma TK (2009) Insight in nAChR subtype selectivity from AChBP crystal structures. Biochem Pharmacol 78:777-787.

Sarter M, Parikh V, Howe WM (2009) Phasic acetylcholine release and the volume transmission hypothesis: time to move on. Nat Rev Neurosci 10:383-390.

Sauguet L, Poitevin F, Murail S, Van RC, Moraga-Cid G, Malherbe L, Thompson AW, Koehl P, Corringer PJ, Baaden M, Delarue M (2013) Structural basis for ion permeation mechanism in pentameric ligand-gated ion channels. EMBO J 32:728-741.

Shan Q, Han L, Lynch JW (2012) Distinct properties of glycine receptor beta+/alpha- interface: unambiguously characterizing heteromeric interface reconstituted in homomeric protein. J Biol Chem 287:21244-21252.

Sharma G, Vijayaraghavan S (2002) Nicotinic receptor signaling in nonexcitable cells. J Neurobiol 53:524-534.

Sharma G, Vijayaraghavan S (2008) Nicotinic Receptors: Role in Addiction and Other Disorders of the Brain. Subst Abuse 2008:81.

Shen XM, Ohno K, Tsujino A, Brengman JM, Gingold M, Sine SM, Engel AG (2003) Mutation causing severe myasthenia reveals functional asymmetry of AChR signature cystine loops in agonist binding and gating. J Clin Invest 111:497-505.

Sigworth FJ, Sine SM (1987) Data transformations for improved display and fitting of single-channel dwell time histograms. Biophys J 52:1047-1054.

Sine S.M. (2002) The nicotinic receptor ligand binding domain. J Neurobiol 53:431-446.

Sine SM (1993) Molecular dissection of subunit interfaces in the acetylcholine receptor: identification of residues that determine curare selectivity. Proc Natl Acad Sci U S A 90:9436-9440.

Sine SM (2012) End-plate acetylcholine receptor: structure, mechanism, pharmacology, and disease. Physiol Rev 92:1189-1234.

Sine SM, Claudio T, Sigworth FJ (1990) Activation of Torpedo acetylcholine receptors expressed in mouse fibroblasts. Single channel current kinetics reveal distinct agonist binding affinities. J Gen Physiol 96:395-437.

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

Sine SM, Quiram P, Papanikolaou F, Kreienkamp HJ, Taylor P (1994) Conserved tyrosines in the alpha subunit of the nicotinic acetylcholine receptor stabilize quaternary ammonium groups of agonists and curariform antagonists. J Biol Chem 269:8808-8816.

Sine SM, Wang HL, Hansen S, Taylor P (2010) On the origin of ion selectivity in the Cys-loop receptor family. J Mol Neurosci 40:70-76.

Solt K, Ruesch D, Forman SA, Davies PA, Raines DE (2007) Differential effects of serotonin and dopamine on human 5-HT3A receptor kinetics: interpretation within an allosteric kinetic model. J Neurosci 27:13151-13160.

Spitzmaul G, Dilger JP, Bouzat C (2001) The noncompetitive inhibitor quinacrine modifies the desensitization kinetics of muscle acetylcholine receptors. Mol Pharmacol 60:235-243.

Spitzmaul G, Gumilar F, Dilger JP, Bouzat C (2009) The local anaesthetics proadifen and adiphenine inhibit nicotinic receptors by different molecular mechanisms. Br J Pharmacol 157:804-817.

Spitzmaul GF, Esandi MC, Bouzat C (1999) Amphetamine acts as a channel blocker of the acetylcholine receptor. Neuroreport 10:2175-2181.

Sun YG, Pita-Almenar JD, Wu CS, Renger JJ, Uebele VN, Lu HC, Beierlein M (2013) Biphasic cholinergic synaptic transmission controls action potential activity in thalamic reticular nucleus neurons. J Neurosci 33:2048-2059.

Swope SL, Moss SJ, Raymond LA, Huganir RL (1999) Regulation of ligand-gated ion channels by protein phosphorylation. Adv Second Messenger Phosphoprotein Res 33:49-78.

Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP (2009) Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 8:733-750.

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

Tasneem A, Iyer LM, Jakobsson E, Aravind L (2005) Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. Genome Biol 6:R4.

Thompson AJ, Lester HA, Lummis SC (2010) The structural basis of function in Cys-loop receptors. Q Rev Biophys 43:449-499.

Thomsen MS, Hansen HH, Timmerman DB, Mikkelsen JD (2010) Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: from animal models to human pathophysiology. Curr Pharm Des 16:323-343.

Timmermann DB, Gronlien JH, Kohlhaas KL, Nielsen EO, Dam E, Jorgensen TD, Ahring PK, Peters D, Holst D, Christensen JK, Malysz J, Briggs CA, Gopalakrishnan M, Olsen GM (2007) An allosteric modulator of the alpha7 nicotinic acetylcholine receptor possessing cognition-enhancing properties in vivo. J Pharmacol Exp Ther 323:294-307.

Ulens C, Akdemir A, Jongejan A, van ER, Bertrand S, Perrakis A, Leurs R, Smit AB, Sixma TK, Bertrand D, de Esch IJ (2009) Use of acetylcholine binding protein in the search for novel alpha7 nicotinic receptor ligands. In silico docking, pharmacological screening, and X-ray analysis. J Med Chem 52:2372-2383.

Unwin N. (1993) Nicotinic acetylcholine receptor at 9 A resolution. J Mol Biol 229:1101-1124.

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

Unwin N, Fujiyoshi Y (2012) Gating movement of acetylcholine receptor caught by plunge-freezing. J Mol Biol 422:617-634.

Vizi ES, Fekete A, Karoly R, Mike A (2010) Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. Br J Pharmacol 160:785-809.

Wallace TL, Bertrand D (2013) Alpha7 neuronal nicotinic receptors as a drug target in schizophrenia. Expert Opin Ther Targets 17:139-155.

Wallace TL, Porter RH (2011) Targeting the nicotinic alpha7 acetylcholine receptor to enhance cognition in disease. Biochem Pharmacol 82:891-903.

Wang HL, Cheng X, Taylor P, McCammon JA, Sine SM (2008) Control of cation permeation through the nicotinic receptor channel. PLoS Comput Biol 4:e41.

Wang HL, Toghraee R, Papke D, Cheng XL, McCammon JA, Ravaioli U, Sine SM (2009) Single-channel current through nicotinic receptor produced by closure of binding site C-loop. Biophys J 96:3582-3590.

Wang J, Lester HA, Dougherty DA (2007) Establishing an ion pair interaction in the homomeric rho1 gamma-aminobutyric acid type A receptor that contributes to the gating pathway. J Biol Chem 282:26210-26216.

Wang K, Hackett JT, Cox ME, Van HM, Lindstrom JM, Parsons SJ (2004) Regulation of the neuronal nicotinic acetylcholine receptor by SRC family tyrosine kinases. J Biol Chem 279:8779-8786.

Wang Q, Lynch JW (2011) Activation and desensitization induce distinct conformational changes at the extracellular-transmembrane domain interface of the glycine receptor. J Biol Chem 286:38814-38824.

Wiesner A, Fuhrer C (2006) Regulation of nicotinic acetylcholine receptors by tyrosine kinases in the peripheral and central nervous system: same players, different roles. Cell Mol Life Sci 63:2818-2828.

Williams DK, Stokes C, Horenstein NA, Papke RL (2011a) The effective opening of nicotinic acetylcholine receptors with single agonist binding sites. J Gen Physiol 137:369-384.

Williams DK, Wang J, Papke RL (2011b) Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. Biochem Pharmacol 82:915-930.

Williams ME, Burton B, Urrutia A, Shcherbatko A, Chavez-Noriega LE, Cohen CJ, Aiyar J (2005) Ric-3 promotes functional expression of the nicotinic acetylcholine receptor alpha7 subunit in mammalian cells. J Biol Chem 280:1257-1263.

Xiao Y, Hammond PS, Mazurov AA, Yohannes D (2012) Multiple interaction regions in the orthosteric ligand binding domain of the alpha7 neuronal nicotinic acetylcholine receptor. J Chem Inf Model 52:3064-3073.

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.

Xiu X, Puskar NL, Shanata JA, Lester HA, Dougherty DA (2009) Nicotine binding to brain receptors requires a strong cation-pi interaction. Nature 458:534-537.

Yakel JL (2010) Gating of nicotinic ACh receptors: latest insights into ligand binding and function. J Physiol 588:597-602.

Yang Z, Taran E, Webb TI, Lynch JW (2012) Stoichiometry and subunit arrangement of alpha1beta glycine receptors as determined by atomic force microscopy. Biochemistry 51:5229-5231.

Yu XM, Hall ZW (1994) A sequence in the main cytoplasmic loop of the alpha subunit is required for assembly of mouse muscle nicotinic acetylcholine receptor. Neuron 13:247-255.

Zhang J, Xue F, Liu Y, Yang H, Wang X (2013) The structural mechanism of the cys-loop receptor desensitization. Mol Neurobiol 48:97-108.

Zhang J, Xue F, Whiteaker P, Li C, Wu W, Shen B, Huang Y, Lukas RJ, Chang Y (2011) Desensitization of alpha7 nicotinic receptor is governed by coupling strength relative to gate tightness. J Biol Chem 286:25331-25340.

Zhu B.T. (2005) Mechanistic explanation for the unique pharmacologic properties of receptor partial agonists. Biomed Pharmacother 59:76-89.

Zhu F, Hummer G (2010) Pore opening and closing of a pentameric ligand-gated ion channel. Proc Natl Acad Sci U S A 107:19814-19819.

Zoli M, Jansson A, Sykova E, Agnati LF, Fuxe K (1999) Volume transmission in the CNS and its relevance for neuropsychopharmacology. Trends Pharmacol Sci 20:142-150.

Zouridakis M, Zisimopoulou P, Poulas K, Tzartos SJ (2009) Recent advances in understanding the structure of nicotinic acetylcholine receptors. IUBMB Life 61:407-423.

Zwart R, De FG, Broad LM, McPhie GI, Pearson KH, Baldwinson T, Sher E (2002) 5-Hydroxyindole potentiates human alpha 7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices. Neuropharmacology 43:374-384.