Therapeutic Potential of α7 Nicotinic Acetylcholine Receptors

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Abstract—Progress in the fields of neuroscience and molecular biology has identified the forebrain cholinergic system as being important in many higher order brain functions. Further analysis of the genes encoding the nicotinic acetylcholine receptors (nAChRs) has highlighted, in particular, the role of α7 nAChRs in these higher order brain functions as evidenced by their peculiar physiologic and pharmacological properties. As
this receptor has gained the attention of scientists from academia and industry, our knowledge of its roles in various brain and bodily functions has increased immensely. We have also seen the development of small molecules that have further refined our understanding of the roles of a7 nAChRs, and these molecules have begun to be tested in clinical trials for several indications. Although a large body of data has confirmed a role of a7 nAChRs in cognition, the translation of small molecules affecting a7 nAChRs into therapeutics has to date only progressed to the stage of testing in clinical trials. Notably, however, most recent human genetic and biochemical studies are further underscoring the crucial role of a7 nAChRs and associated genes in multiple organ systems and disease states. The aim of this review is to discuss our current knowledge of a7 nAChRs and their relevance as a target in specific functional systems and disease states.

I. Introduction

Although nicotine is the most widely used “recreational drug” for more than 400 years, our understanding of its effects on the brain remains limited. With the introduction in 1929 of electroencephalography, nicotine exposure was found to cause modifications of brain electrical activity but provided no insights into the possible mechanisms underlying these modifications (reviewed in Yamamoto and Domino, 1965). In the first intracellular recordings conducted in brain slices, nicotine exposure failed to demonstrate significant activity; thus it was concluded that nicotine did not mimic the agonist acetylcholine (ACh). Histologic investigations carried out at approximately the same time revealed, however, surprising results illustrating that the toxin from the snake Bungarus multicintus (a-bungarotoxin, a-Btx), which displays a high affinity for muscle nAChRs, abundantly labeled specific regions in brain slices and that this labeling was displaced by incubation with nicotine (Clarke et al., 1985). Two possibilities could explain the discrepancy between electrophysiological and histologic studies, either a nonspecific binding of a-Btx with no functional significance occurred or the absence of a functional signal detected by intracellular recording resulted in a false negative finding.

Cloning of the neuromuscular junction nAChRs marked a turning point for the investigation of ligand-gated ion channels and offered the possibilities for investigation of related proteins at the proteomic and genomic levels. In addition, the availability of complementary DNA and mRNA allowed reconstitution of functional proteins in host systems such as Xenopus laevis oocytes or cell lines (Miledi et al., 1982; Barnard et al., 1982; Ballivet et al., 1988; Gopalakrishnan et al., 1995). Several genes encoding for nAChRs were identified from different invertebrate and vertebrate species. Genes encoding for the neuronal nAChRs were identified and subsequently classified as CHRNA and CHRM (CHRNA2–10 and CHRB2–4) and their chromosomal localizations were identified in the human genome, as illustrated by the human karyotype shown in Fig. 1A. CHRNA2–10, encoding the α2–10 subunits of neuronal nAChRs, were defined as sharing homology with the gene encoding for the α1 nAChR subunit of the neuromuscular junction and, more specifically, in the extracellular domain with the presence of two adjacent cysteines corresponding to amino acids 192–193 in the Torpedo marmorata sequence, from which the α1 nAChR subunit was cloned (Devillers-Thiery et al., 1979).

In the case of the adult form of the neuromuscular junction nAChR, ACh binds at the interface between α and δ or α and ε subunits (Pedersen and Cohen, 1990; Changeux and Edelstein, 1994; Karlin and Akabas, 1995). Subsequently, genes encoding for neuronal nAChRs were isolated simultaneously in avians (chick) and in the rat and were initially divided into α and β subunits according to the presence of two adjacent cysteines equivalent to those observed in the α1 subunit of the muscle receptors. Functional receptors were observed only with coexpression of α and β subunits, suggesting that the α subunit harbors the principal component for the ACh binding site, whereas the β subunit was considered as a constitutive subunit. However, since these initial studies, it has been shown that both the α and the β subunits contribute to the formation of the ligand-binding domain (LBD) in the neuronal nAChRs in which ACh binds at the interface between an α and an adjacent subunit.

ABBREVIATIONS: ACh, acetylcholine; AChBP, acetylcholine binding protein; AKI, acute kidney injury; AD, Alzheimer’s disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APP, amyloid precursor protein; ASD, autism spectrum disorder; αβ, amyloid-β; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; BD, bipolar disorder; bp, base pair; BP, break point; a-Btx, a-bungarotoxin; [11C]CHIBA-1001, 1,4-diazabicyclo[3.2.2]nonane analog 4-[11C]methylphenyl 2,5-diazabicyclo[3.2.2]noane-2-carbocylate; CD, Crohn’s disease; CKD, chronic kidney disease; CNS, central nervous system; CREEB, cAMP response element-binding protein; DBA, Dilute Brown Non-Agouti; BACE1, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, N-methyl-D-aspartate; NOR, novel object recognition; OA, osteoarthritis; PEM, positive allosteric modulator; PD, Parkinson’s disease; PET, positron emission tomography; FK, pharmacokinetic; S9-GSK, serine 9 on glycogen synthase kinase 3β; SANS, Scale for the Assessment of Negative Symptoms; SAR, structure-activity relationship; SNP, single nucleotide polymorphism; SPECT, single photon positron emission tomography; STATS, signal transducer and activator of transcription 3; TMD, transmembrane domain; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNFa, tumor necrosis factor alpha; UC, ulcerative colitis.
First identified in avian and, almost simultaneously, in rat, the \textit{CHRNA7} gene encoding for the $\alpha 7$ nAChR subunit, which exhibits unique structural features, initially proved difficult to express in a reconstituted system (Couturier et al., 1990; Séguéla et al., 1993). It was, however, immediately recognized that the $\alpha 7$ nAChR subunit shared a sufficient degree of structural similarity with the neuromuscular junction $\alpha 1$ nAChR subunit, to which $\alpha$-Btx also binds, and thus the $\alpha 7$ nAChRs could be the high affinity $\alpha$-Btx binding sites in the brain (Couturier et al., 1990). Moreover, the $\alpha 7$ nAChR subunit was the first to yield functional receptors when expressed in isolation, indicating that they can form functional homomers (Couturier et al., 1990; Séguéla et al., 1993). Confirmation that $\alpha$-Btx labeling corresponded to $\alpha 7$ nAChR expression was later obtained with $\alpha 7$ nAChR knockout mice that lacked $\alpha$-Btx labeling (Orr-Urtreger et al., 1997).

This initial and determinant step marked a turning point in our understanding of nAChRs and opened new opportunities and challenges to discover the contribution and relevance of the various nAChR subtypes in the

\textbf{Fig. 1.} The \textit{CHRNA7} gene, its duplication and microdeletion. (A) Human karyotype with the localization of \textit{CHRNA7} on chromosome 15. (B) Schematic representation of \textit{CHRNA7} and its duplication \textit{CHRFAM7A}. (C) Karyotype of a person showing a microdeletion on chromosome 15 labeled with fluorescence in situ hybridization (FISH) with a probe specific for \textit{CHRNA7} and a control probe reveals the presence of only one copy of the \textit{CHRNA7} gene. (D) FISH analysis of another patient reveals a higher signal (see inset) indicative of the triplication of \textit{CHRNA7} and illustrates the variability in the chromosomal region 15q13.3. FISH images were kindly provided by Dr. C. Schaaf (see also Schaaf, 2014; Soler-Alfonso et al., 2014).
brain and, more generally, in other organ systems. In this review, we shall discuss the current knowledge of the functions of \( \alpha 7 \) nAChRs and examine the development of new ligands designed to address therapeutic needs for Alzheimer’s disease (AD), cognitive impairment in schizophrenia, or other diseases.

II. From Gene to Function

A. The CHRNA7 Gene and Its Variants

As previously mentioned, cloning of the \( \alpha 7 \) nAChR subunit from chick (Couturier et al., 1990) and rat (Séguelé et al., 1993) was achieved in the early 1990s, followed shortly by the cloning of the human CHRNA7 gene (Doucette-Stamm et al., 1993) and determination of its chromosomal location (Fig. 1) (Chini et al., 1994). Sequence alignment of CHRNA7 with the genes for the other known nAChR subunits revealed certain similarities, but also differences. Comparisons across species revealed that CHRNA7 was conserved throughout evolution and that its ancestor gene could be traced to approximately one million years ago (Le Novère and Changeux, 1995). The consensus protein structure has the typical signature of nAChRs with four putative transmembrane domains (TMD I–IV) and a large cytoplasmic domain between TMD III and IV, resulting in a mature protein of \( \sim 55–60 \) kDa (Seto et al., 1981; Whiting et al., 1987; Gotti et al., 1991). The intracellular domain of the \( \alpha 7 \) nAChR was subsequently reported to play important physiologic roles, including receptor targeting or phosphorylation. Mapped in humans to chromosome 15, CHRNA7 encodes a mature protein of 479 amino acids and displays multiple unusual features, including putative regions that resemble known transcription factors.

CHRNA7 mRNA was shown to be widely expressed at low levels in post mortem human brain, but with highest expression in hippocampus and thalamus. Thalamic sensory nuclei to include the lateral and medial geniculate nuclei had high expression levels of CHRNA7 mRNA. The medial geniculate nucleus is the relay center between the inferior colliculus and the auditory cortex, and these regions have been implicated in processing of sensory information (Breese et al., 1997). As expression of CHRNA7 transcripts revealed regions matching the \( \alpha \)-Btx binding site comparable to the \( \alpha 1 \) nAChR subunit, this confirmed the initial observation that \( \alpha 7 \) nAChR subunits yield a receptor that binds with high affinity to this snake toxin. CHRNA7 transcripts were also highly expressed in the prefrontal cortex and other brain regions contributing to sensory and information processing (Musso et al., 2007; Parikh et al., 2010; Wallace and Bertrand, 2013b). The reduction of CHRNA7 transcripts observed in schizophrenia patients prompted the idea that this receptor might play a determinant role in this pathology (Freedman et al., 1995). From the clinical observation, it was noted that sensory gating is altered in schizophrenia, and given the high level of expression of \( \alpha 7 \) nAChRs in brain regions known to contribute to auditory processing, it was hypothesized that stimulation of these receptors might improve the patient’s condition. In healthy volunteers, sensory gating to auditory cues (two brief tones or clicks) is characterized by an attenuation of the evoked potential response to the second stimulus. In patients with schizophrenia, attenuation of the evoked potential response to the second stimulus is reduced (Freedman et al., 1996). As early as 1998, experiments conducted in animal models revealed that \( \alpha 7 \) nAChR agonists were able to normalize this sensory gating deficit by restoring attenuation to the second stimulus (Stevens et al., 1998). Reinforcing the hypothesis of a critical role of \( \alpha 7 \) nAChRs in ameliorating the sensory processing deficits in schizophrenia, these experiments stimulated further studies that have led to the discovery of multiple properties of \( \alpha 7 \) nAChRs and the related protein products.

At about the same time, genetic studies unveiled possible splice variants of CHRNA7 and a partially duplicated gene located upstream in close proximity of CHRNA7, coined dup \( \alpha 7 \) (Chini et al., 1994; Gault et al., 1998). Although the relevance of splice variants in \( \alpha 7 \) nAChR function still needs to be fully characterized, further efforts have been dedicated to more detailed analysis of the duplicate gene, which corresponds to exons 5-10 of CHRNA7 and is located 1.6 Mb centromeric to CHRNA7, which is now referred to as CHRFAM7A (for a recent detailed review, see Sinkus et al., 2015). The corresponding protein, dup \( \alpha 7 \), is widely expressed, but in lower levels than \( \alpha 7 \) nAChRs throughout the body but elevated in peripheral leukocytes (Villiger et al., 2002; Benfante et al., 2011, Costantini et al., 2015). Although so far no specific function has been directly associated with CHRFAM7A, it was proposed that it might regulate \( \alpha 7 \) nAChR activities. Coexpression experiments conducted in a heterologous expression system confirmed this hypothesis and revealed that dup \( \alpha 7 \) subunits when coexpressed can assemble with \( \alpha 7 \) nAChR subunits to alter the functionality of these receptors (Araud et al., 2011; de Lucas-Cerrillo et al., 2011). Despite the limitation inherent in heterologous expression systems, these results indicate that the dup \( \alpha 7 \) subunit, and its high level of variation in the human population, might play a role in modulating \( \alpha 7 \) nAChR expression and/or function. A further complexity observed in CHRFAM7A is the presence of a 2-base pair (bp) deletion that has been associated with the sensory gating deficit in a subpopulation of schizophrenia patients (Sinkus et al., 2009).

Further genetic analysis showed that the \( \alpha 7 \) nAChR protein contains sequences corresponding to Sp1, AP-2, Egr-1, and CREB transcription factors that might play a role in its regulation (Gault et al., 1998). Variants in the promoter region of CHRNA7 were also identified and associated with differential expression levels of \( \alpha 7 \) nAChR protein. Because these variants were associated
with symptoms of schizophrenia or the sensory gating deficit, it was proposed that dysregulation of α7 nAChR expression might be at the origin of the disease. Most recent genetic data have highlighted that the region of chromosome 15 that encompasses CHRNA7 is one of the most unstable within the human genome (Zafranski et al., 2010; Schaaf, 2014). As described by these authors, break points referred to as BP1–BP6 are observed in this region of the chromosome. Paracentric inversion between BP4 and BP5 is frequent in the population and was proposed to be associated with clinical features including mental retardation and seizures (Chini et al., 1994; Sharp et al., 2008). In turn, these break points can predispose to nonallelic homologous recombination, resulting in recurrent reciprocal BP4–BP5 microdeletions and microduplications. Copy number variants of CHRNA7 have been reported, leading to multiple classes of microduplications varying in size from 350 kb to 1.6 Mb (Schaaf, 2014). The microdeletions are now recognized as chromosome 15q13.3 deletion syndrome (OMIM #612001). Because they are rather prevalent and often accompanied by multiple neurodevelopmental and intellectual disabilities, it was recommended that testing for such microdeletions should be conducted in individuals showing unexplained intellectual disability, seizures, and/or mild dysmorphic features (Deutsch et al., 2011). Psychiatric disorders have been associated with this microdeletion syndrome, which is characterized by a 2-Mb deletion in 15q13.3 and shown to be inherited in 75% of patients with this syndrome (Masurel-Paulet et al., 2010). Although microdeletions in chromosome 15q13.3 generally encompass several genes and therefore render difficult the interpretation of the pathologic mechanisms, the identification in a smaller number of patients with homozygous microdeletion of CHRNA7 is starting to shed new light on the pathology associated with the absence of α7 nAChRs (Shinawi et al., 2009; Hoppman-Chaney et al., 2013; Le Pichon et al., 2013). Identification of the missing CHRNA7 can be performed using fluorescence in situ hybridization (FISH) analysis as illustrated in Fig. 1. Additional relevance for the role of α7 nAChRs was recently identified with the finding of triplicate expression of CHRNA7 that is associated with both impairment in cognition and neuropsychiatric phenotypes in a three-generation pedigree (Soler-Alfonso et al., 2014). These data illustrate that imbalance of the expression levels of α7 nAChRs with either a reduced expression or an excessive expression is associated with neurologic impairment and underscores the need for tight regulation of CHRNA7 expression (Liao et al., 2011; Cubells et al., 2011; Mikhail et al., 2011). Chromatin analysis of 15q11.2-13.3 has revealed the CHRNA7 transcript to be significantly diminished in the cerebral cortex of patients with autism and Rett syndrome (Yasui et al., 2011) as well as bipolar disorder, further supporting the hypothesis that reduced α7 nAChR expression may result in abnormal brain development and function. However, what is missing is the detection of the CHRFAM7A transcript, but as noted in a recent review the similarity of this gene with CHRNA7 renders difficult the query in genome-wide association studies. Nonetheless, CHRFAM7A was recently proposed to be associated with several cognitive deficits (Sinkus et al., 2015).

Data presented in Table 1 illustrate a series of CHRNA7 single nucleotide polymorphisms (SNPs) that have been shown to positively correlate with various disorders. A significant association of SNP rs8028396 with smoking, as well as with smoking in schizophrenia, in a non-Hispanic Caucasian population was observed (Stephens et al., 2012; Neri et al., 2012). SNP rs3087545 was first shown to be significantly associated to schizophrenia (Stephens et al., 2009) and also influenced differences in synaptic activity, as detected by functional magnetic resonance imaging, between patients with schizophrenia and control subjects performing an auditory oddball task (Tregellas et al., 2010). A Korean population-based study on the development of either schizophrenia or bipolar disorder (BD) demonstrated that SNPs rs2337506, rs6494223, and rs12916879 exhibited only a marginal association with BD (Joo et al., 2010) and that SNP rs6494223 was associated with a protective effect in BD (Ancin et al., 2010) and impaired attention in patients with euthymic bipolar disorder (Ancin et al., 2011). Additionally, in two separate studies in patients with AD in Northern Ireland, SNP rs6494233 was significantly associated with delusional symptoms (Carson et al., 2008a), whereas SNPs rs1514246, rs2337506, and rs8027814 were significantly associated with a reduced risk of AD (Carson et al., 2008b). There have been a number of other genetic association studies between CHRNA7 SNPs and various disease states, which all

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**TABLE 1**

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<th>SNP</th>
<th>Disease</th>
<th>Author</th>
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<tr>
<td>rs8028396</td>
<td>Smoking and smoking in schizophrenia</td>
<td>Neri et al., 2012; Stephens et al., 2012</td>
</tr>
<tr>
<td>rs3087545</td>
<td>Perinatal P50 inhibition; Schizophrenia and improvement in functional magnetic resonance imaging</td>
<td>Stephens et al., 2009; Tregellas et al., 2010; Ross et al., 2013</td>
</tr>
<tr>
<td>rs6494233</td>
<td>Delusional symptoms in AD</td>
<td>Carson et al., 2008a</td>
</tr>
<tr>
<td>rs2337506</td>
<td>Protective with respect to risk of AD</td>
<td>Carson et al., 2008b</td>
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<td>rs8027814</td>
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failed to show any significant association (Iwata et al., 2007; Joo et al., 2010; Neri et al., 2012; Cabranes et al., 2013).

Recently, patent WO 2013/057687 demonstrated the potential value that genetic analysis can afford by identifying responders of \( \alpha 7 \) nAChR agonist therapy (Arias et al., 2013). Genetic variations in 15q24, possessing certain “indicative SNPs,” have been unexpectedly accurate in predicting responsiveness of a patient with cognitive impairment to treatment with an \( \alpha 7 \) nAChR agonist. These “indicative SNPs” reside in genomic coordinates of either \( CHRNA5 \) or \( CHRNA3 \), encoding for the \( \alpha 5 \) or \( \alpha 3 \) nAChR subunit, respectively. In particular, patients could be more responsive to an \( \alpha 7 \) nAChR agonist if they carried \( CHRNA5 \) SNP rs55854698-T or SNP rs16969968 and/or a SNP forming a haplotype with them, or the \( CHRNA3 \) SNP rs6495308 or SNP rs1051730, or even a haplotype forming with either of these SNPs. Thus before treatment, a genotyping analysis of a patient afflicted with cognitive impairment that includes SNP rs55854698, for example, might predict whether treatment with an \( \alpha 7 \) nAChR ligand will be efficacious in that particular patient. To further expand, patients that were homozygous for \( CHRNA5 \) SNP rs55853698T showed a significant response to an administered \( \alpha 7 \) nAChR agonist versus placebo on visual learning and memory parameters \( (P < 0.015) \). These data further illustrate the complexity of the genetic relationship between \( \alpha 7 \) nAChR SNPs and SNPs for other nAChR subunits but also offer the promises for determining patients carrying influential SNPs to design the best treatment regimen for them.

Although these results highlight the complexity in genetic analysis of \( CHRNA7 \) and of the 15q13.3 chromosomal locus, the complexity is further increased with the presence of \( CHRFAM7A \). However, these complexities clearly point to a determinant role of \( \alpha 7 \) nAChRs in human brain function and that altered expression of \( \alpha 7 \) nAChRs is associated with cognitive impairment and neuropsychological deficits. SNP analyses are leading to the potential of personalized medicine for patients treated with \( \alpha 7 \) nAChR ligands, once these ligands become approved therapies.

Results from the genetic analysis conducted so far indicate that the chromosome region harboring the gene encoding for the \( \alpha 7 \) nAChR subunit presents an unusual complexity with 1) a duplication of exons 5–10 of \( CHRNA7 \) as the \( CHRFAM7A \) gene that encodes for the \( \alpha 7 \) dup protein; 2) important variability of the chromosome in this region due to homologous recombination resulting in inversions, microdeletions, and microduplications; and 3) variations in the promoter region of \( CHRNA7 \). All of these modifications appear to affect expression levels of \( \alpha 7 \) nAChR protein, suggesting the importance of tightly regulated expression for the normal function of \( \alpha 7 \) nAChRs. Furthermore, dysregulation of \( \alpha 7 \) nAChR expression has been linked to a number of clinical disorders, all with a component of cognitive dysfunction, and suggests the importance of \( \alpha 7 \) nAChRs in cognitive performance.

B. Functional Properties of \( \alpha 7 \) Nicotinic Acetylcholine Receivers

The most prominent functional features of \( \alpha 7 \) nAChRs expressed in heterologous systems are 1) their high sensitivity to \( \alpha \)-Btx and methyllycaconitine (MLA); 2) their ability to form functional channels in the absence of other nAChR subunits; 3) their fast desensitizing channels (millisecond time scale) upon prolonged application of agonist; 4) their enhanced permeability to calcium (\( \text{Ca}^{2+} \)) ions; and 5) their strong inward rectification (Couturier et al., 1990; Séguela et al., 1993; Bertrand et al., 1993; Peng et al., 1994; Puchacz et al., 1994). \( \text{Ca}^{2+} \) permeability of homomeric \( \alpha 7 \) nAChRs was measured to be at least fourfold higher than that reported for \( N \)-methyl-d-aspartate (NMDA) glutamate receptors (Mayer and Westbrook, 1987) in various species (avian, rat, or human) (Séguela et al., 1993; Bertrand et al., 1993; Fucile, 2004; Gilbert et al., 2009). The investigation of these unique functional features became more feasible upon the development of the stable expression of human \( \alpha 7 \) nAChR subunits in a human cell line, HEK293, which has been shown to be devoid of endogenous nAChR subtype expression (Gopalakrishnan et al., 1995). These studies revealed that stably expressed homomeric human \( \alpha 7 \) nAChR subunits had an affinity for \( \alpha \)-Btx binding with comparable currents and fast kinetics to those expressed in \( Xenopus \) oocytes (Peng et al., 1994). Further studies in this stable cell line, using voltage-clamp or fast ratiometric intracellular imaging (Delbono et al., 1997), were able to confirm that homomeric \( \alpha 7 \) nAChRs, unlike the heteromeric nAChR subtypes, exhibit a higher \( \text{Ca}^{2+} \) permeability relative to monovalent cations and to support previous work in \( Xenopus \) oocytes (Bertrand et al., 1993; Séguela et al., 1995; Sands et al., 1995) in which the intracellular \( \text{Ca}^{2+} \) rises within 10 ms with a mean peak concentration of 356 nM, the importance of which will be discussed later. Mutagenesis studies revealed that the \( \text{Ca}^{2+} \) permeability of \( \alpha 7 \) nAChRs is controlled by distinct amino acids strategically positioned at each end of the pore forming the channel, which are unique to this nAChR subtype (Galzi et al., 1992; Bertrand et al., 1993).

Determination of the concentration-response curves to ACh and nicotine revealed that \( \alpha 7 \) nAChRs displayed a higher sensitivity for nicotine than for ACh and that both agonists caused a fast and rapid desensitization of the receptors. The half activation concentration \( (\text{EC}_{50}) \) of \( \alpha 7 \) nAChRs by ACh is about 200 \( \mu \)M, and at this concentration, the response consists of a large inward current, with a maximum amplitude at about 10 ms (Puchacz et al., 1994; Gopalakrishnan et al., 1995). Intracellular recordings conducted in brain slices.
confirmed that native $\alpha 7$ nAChRs exhibited comparable physiologic and pharmacological properties as receptors expressed in heterologous systems (Alkondon et al., 1996). Moreover, these native receptors were selectively blocked by low concentrations of $\alpha$-Btx and MLA. The rapid desensitization has often been proposed to represent a protective mechanism, preventing excessive Ca$^{2+}$ influx into the cell that could prove cytotoxic. Evidence for such a hypothesis was provided by the observation that mutations in the second TMD, which reduce receptor desensitization, are associated with increased cell death and are lethal in an animal model (Orr-Urtreger et al., 2000; Lukas et al., 2001). An interesting parallel can be drawn with the $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which have fast activation and desensitization comparable with those of the $\alpha 7$ nAChRs (Traynelis et al., 2010). AMPA receptors are further subdivided into Ca$^{2+}$-permeable and Ca$^{2+}$-impermeable, and it was shown that Ca$^{2+}$-permeable receptors play a pivotal role in synaptic plasticity, triggered by the increase of the intracellular Ca$^{2+}$ (Liu and Cull-Candy, 2000; Malinow and Malenka, 2002). Similar to AMPA receptors, a growing body of evidence has revealed that $\alpha 7$ nAChRs also contribute to the modulation of synaptic and cellular functions and influence the release of other neurotransmitters (Dani and Bertrand, 2007; Albuquerque et al., 2009). Diverse studies have revealed the contribution of $\alpha 7$ nAChRs in fast neurotransmission, especially in the hippocampus where these receptors might participate in memory processes (Hefft et al., 1999; Grybko et al., 2010, 2011). Contributions of $\alpha 7$ nAChRs to neuronal network functions are not restricted to the hippocampus and cerebral cortex but are also observed in the cerebellum, where these receptors were shown, both in vitro and in vivo, to cause a switch from long-term depression to long-term potentiation (LTP) (Prestori et al., 2013). These studies indicate that ACh in the extracellular space reached sufficient concentrations to activate $\alpha 7$ nAChRs and that, despite its short response time, this receptor can contribute to brain signaling.

The strong inward rectification, which is a hallmark of all neuronal nAChR subtypes, implied that $\alpha 7$ nAChRs can contribute to signal processing only if the cell’s membrane potential is below $-40$ mV. Site-directed mutagenesis conducted in the second TMD that lines the channel pore has shown that rectification is associated with the glutamate residue at the opening of the channel and that mutation to an aspartic acid was sufficient to abolish rectification (Forster and Bertrand, 1995). The inward rectification was associated with the blockade caused by intracellular magnesium in a mechanism that strikingly resembled that observed for NMDA receptors (Nowak et al., 1984; Kupper et al., 1998). Finally, it is important to note the high degree of conservation of the glutamate residue across species, suggesting that inward rectification is a relevant property that has been maintained throughout evolution (Forster and Bertrand, 1995). Although the relevance of these properties of $\alpha 7$ nAChRs to drug discovery has not been thoroughly evaluated, it should be pointed out that they represent additional and yet unexplored possibilities for $\alpha 7$ nAChR modulation.

Given their capacity to reconstitute functional homomeric channels, the $\alpha 7$ nAChRs have been a workhorse for structure-function studies of four TMD proteins because it has been sufficient to mutate a single amino acid to influence the entire complex in the TMD or channel domain. These studies also pointed out numerous features of $\alpha 7$ nAChRs and confirmed that a small pocket localized at the border between two adjacent subunits constituted the orthosteric LBD (Galzi et al., 1991). Since then, many studies have attempted to resolve the three-dimensional structures of $\alpha 7$ nAChRs and how the LBD is formed (Andersen et al., 2013). A further confirmation of the clear structural determinants, with a distinct extracellular domain and TMD, was obtained using chimeras between receptors from different families. Namely, based on sequence alignments it was proposed that homologies between the serotoninergic 5-HT$_{3A}$ receptor and $\alpha 7$ nAChR were sufficient to allow the creation of a chimera containing the extracellular domain of the $\alpha 7$ nAChR and the channel domain of the 5-HT$_{3A}$ receptor that maintained the functionality of a ligand-gated ion channel (Eiselé et al., 1993). This chimeric construct yielded functional receptors that displayed the pharmacological profile of an $\alpha 7$ nAChR and the channel properties of a 5-HT$_{3A}$ receptor (Eiselé et al., 1993). Marking a turning point in our understanding of the functional domains governing the properties of four TMD receptors, this approach was successfully used in many studies to identify defined properties, such as the binding site for allosteric modulators of the $\alpha 7$ nAChR (Bertrand et al., 2008). These data also suggested that the N-terminal domain of the $\alpha 7$ nAChR, which is in the extracellular space, can be thought of as an independent structure that is functionally coupled to the channel domain. Structural analysis suggested that this domain, referred to as the LBD, resulted from the assembly of $\beta$-sheets in a barrel-like manner. Confirmation of this hypothesis was obtained with the discovery of the acetylcholine binding protein (AChBP) (Brejc et al., 2001). Initially identified in _Lymnea stagnalis_, this water-soluble protein forms a high-affinity complex that binds ACh, thereby contributing to the homeostasis of the ligand in the invertebrate nervous system (Smit et al., 2001). Sharing a high degree of sequence homology with the N-terminal domain of nAChRs, this protein was amenable to crystallization and provided the first atomic resolution image of the LBD (Brejc et al., 2001). Since this initial work, crystallization of AChBP from several invertebrates, including _Aplysia californica_ and _Capitella_
teleta, with different ligands, has been used as a tool to
direct more efficient drug development (Hansen et al.,
2005; Smit et al., 2006; Gao et al., 2006; Rucktooa et al.,
2009; Sander et al., 2010; Nemez and Taylor, 2011;
Kombo et al., 2012; Billen et al., 2012). Despite these
interesting results, important differences remained
between a ligand bound to AChBP versus to α7 nAChRs,
as shown in studies that attempted to link the develop-
ment of new α7 nAChR ligands based on predictions
from AChBP binding (Ulens et al., 2009; Akdemir et al.,
2011). Using site-directed mutagenesis, efforts were
dedicated to modifying the structure of the AChBP to
mimic as much as possible the human α7 nAChR
extracellular domain while maintaining protein solu-
bility (Nemez and Taylor, 2011). This work fully
confirmed that the multiple interactions were made
between a ligand and the interface of two adjacent α7
nAChR subunits. Crystal structures obtained for different
ligands bound to AChBP led to the hypothesis that
the N-terminal domain of the α7 nAChR subunit under-
goes conformational changes upon binding of a full
agonist to include the closure of the loop-C, which
contains the two adjacent cysteines in the α nAChR
subunits, whereas partial agonists and antagonists
maintained the loop-C in a more open conformation
(Hibbs et al., 2009; Brams et al., 2011a).

Providing a valuable insight into the N-terminal
domain of α7 nAChRs, crystal structure data were
heavily used to dissect the structure-function relationship
between ligand binding and channel activity. Complimented
by experiments using site-directed mutagenesis and/or
disulfide reagents to modify accessibility to the
cysteines in the LBD, data further highlighted the role of
key amino acid residues that are thought to contribute
to the pharmacophore (Papke et al., 2011a). Computer
modeling and docking of molecules based on known
structures rapidly expanded the number of molecules
that demonstrated selectivity for α7 nAChRs, as illustrat-
ed in the medicinal chemistry literature (Li et al.,
2010; Ghiron et al., 2010; Schrimpf et al., 2012; Kombo
et al., 2012; Zanaletti et al., 2012). Computer modeling
further pointed to the role of fractional hydrophobicity/
hydrophilicity of the LBD and the contribution of the
cation-π interaction of the ligand’s basic nitrogen atom
with tryptophan 149 of the LBD (Kombo and Bencherif,
2013). The LBD interaction with a ligand is, however,
probably more complex, as revealed by studies conducted
using nonnatural amino acids that point to an unusual
interaction of varenicline with α7 nAChRs and the
absence of a cation-π interaction with a tryptophan
residue (Van Arnam et al., 2013). This study further
revealed that the leucine at position 119 and tryptophan
at position 55 are important for the binding of large
molecules such as epibatidine or varenicline but are
dispensable for binding of smaller molecules such as
ACh. Consistent with the initial observations, all studies
confirmed that ligands are binding at the interface
between an α7 nAChR subunit and its adjacent subunit,
which is a point that must be considered when assessing
the properties of α7 subunit-containing heteromeric
nAChRs. It recently became evident that it is necessary
to evaluate ligand binding and the accompanied small
changes in the conformation of the LBD, as well as the
properties of the channel domain and its interaction with
the N-terminal domain, to understand the activation of
the receptor and to evaluate the properties of the channel
domain and its interaction with the N-terminal domain.

In early years of research in the field, a novel and
powerful approach to examine the domain properties of
the channel was the utilization of site-directed mutagen-
esis, which allowed study of the physiologic consequences
of the substitution of single or multiple amino acid
residues. Mutations conducted in the channel domains
confirmed the relevance of the amino acids residues that
are pointing outward toward the ionic pore as critical for
ionic selectivity. Although it would go beyond the scope
of this work to review in detail the multitude of mutations
that were designed and tested, it is of relevance to mention
the mutation, initially called L247T with reference to the
chick amino acid sequence, and subsequently called L9’T
with reference to its position in the channel domain
(Bertrand et al., 1992). The exchange of this leucine, which
is in the upper third of the channel domain, for a shorter
amino acid caused three major effects: 1) a profound
modification of receptor desensitization; 2) an increase of
nAChRs. It recently became evident that it is necessary
of 200-fold in sensitivity to ACh; and 3) a change in the
pharmaceutical profile of some compounds, with com-
petitive antagonists becoming agonists. Although at first
surprising, these three properties are closely related and
can be explained by considering that the mutation
changed the gating properties of the channel in the
desensitized state. Independent of the scientific interest
in understanding the different transition states occurring
upon activation and desensitization of α7 nAChRs, this
mutation offered a window into the binding properties
of α7 nAChRs in a desensitized state (Revah et al., 1991;
Bertrand et al., 1992). The observation that competitive
inhibitors of neuronal nAChRs, such as dihydro-beta-
erythroidine (DHβE) or d-tubocurarine become agonists
in L9’T mutant α7 nAChRs indicated that these molecules
can bind with high affinity and selectivity, but because
they stabilize a desensitized state, they act as competi-
tive inhibitors. By demonstrating without ambiguity
the existence of multiple states showing selective pharma-
coalogical profiles, this mutant offers an additional advan-
tage. Namely, the L9’T mutant α7 nAChR allowed the
determination of which state a given compound was
stabilizing and its affinity. Finally, by providing a func-
tional window on the desensitized states, the L9’T mutant
α7 nAChR provided insight into the discrepancy between
binding experiments yielding high affinities for a given
agonist and low levels of activity revealed by electrophys-
ology. To understand these differences and how the L9’T
mutant α7 nAChR could permit the resolution of this
conundrum, it should be recalled that binding experiments are generally conducted at equilibrium, that is, with sustained exposure to the ligand of interest, whereas functional assays are monitoring the receptor activation using brief agonist test pulses. Because \(\alpha_7\) nAChRs are desensitized by sustained exposure to agonists (see Fig. 2), it becomes evident that binding and functional experiments are challenging two different states of \(\alpha_7\) nAChRs, with the desensitized state showing a higher affinity for compounds than the active state. The availability of the L9'T mutant \(\alpha_7\) nAChR or equivalent mutants permitted investigations of how compounds are activating and desensitizing \(\alpha_7\) nAChRs in an unprecedented manner.

As indicated above, the \(\alpha_7\) nAChR contains a long amino acid sequence between TMD III and TMD IV that faces the intracellular cytoplasm and has been shown to contain consensus phosphorylation sites (Charpantier et al., 2005). Based on this observation it was proposed that compounds known to alter phosphorylation might modify the properties of \(\alpha_7\) nAChRs. Indeed, exposure to the general kinase inhibitor genistein caused a concentration-dependent, substantial increase in the current evoked by ACh test pulses. Effects of genistein or specific Src-family kinase inhibitors were observed both on reconstituted \(\alpha_7\) nAChRs expressed in Xenopus oocytes or cell lines as well as on native receptors and confirmed that activity of \(\alpha_7\) nAChRs can be modulated by phosphorylation (Charpantier et al., 2005). More recent experiments confirmed the relevance of T cell receptors in the phosphorylation and modulation of \(\alpha_7\) nAChRs in the brain (Komal et al., 2014). Activation of T cell receptors, expressed in neocortical neurons,

![Fig. 2](image_url). Activation and desensitization of the \(\alpha_7\) nAChRs. (A) Activation of \(\alpha_7\) nAChRs by brief pulses of nicotine at increasing concentrations compared with a saturating ACh test pulse. Plot of the peak inward current, as a function of the logarithm of the nicotine concentration, yields a typical concentration-response curve as shown by ○ (C). Data were normalized versus the current evoked by 1.28 mM ACh. (B) Sustained exposure to nicotine inhibits the current evoked by a brief ACh test pulse (100 \(\mu\)M). Plot of the peak ACh-evoked current as a function of the logarithm of nicotine concentration yields the inhibition curve shown by the ● (C). Inhibition was scaled to 50% of the maximal response corresponding to the fraction of current evoked by 100 \(\mu\)M ACh. Continuous curves through the data points are the best fits obtained with a Hill equation (D. Bertrand, personal communication).
caused a reduction in the firing rate of layer I interneurons of frontal cortex. The prominent role of α7 nAChRs in the frontal cortex and their modulation by phosphorylation opens new strategic avenues in drug discovery.

In the absence of a crystal structure of the entire α7 nAChR, comparison with other known and related structures and computer modeling represented the best alternative to gain further insight into α7 nAChR function. The discovery of four TMD-related channels in bacteria that offer the possibility of crystallization, marked an advance in our understanding (Bocquet et al., 2007; Hilf and Dutzler, 2009; Bocquet et al., 2009). Largely confirming previous hypotheses, these crystal structures pointed out the critical role of the interface between the LBD and the channel domain and, more specifically, of the short segment delimited by TMD II and TMD III (Lee et al., 2009; Bouzat, 2012; Calimet et al., 2013).

Expression studies of α7 nAChRs in recombinant systems revealed that expression of this nAChR subunit alone results in functional homomeric receptors, which display a fast activation, rapid desensitization, and are not functional in the absence of Ca2+. Structure-function analysis displayed a fast activation, rapid desensitization, and are not functional in the absence of Ca2+. Expression studies of α7 nAChRs in recombinant systems revealed that expression of this nAChR subunit alone results in functional homomeric receptors, which display a fast activation, rapid desensitization, and are not functional in the absence of Ca2+. Structure-function analysis confirmed that α7 nAChRs result from the assembly of five subunits with the orthosteric LBD formed at the interface between adjacent subunits. The pore domain lies in the center of the protein assembly and is lined by the second TMD. Site-directed mutagenesis and formation of chimeras with portions of the α7 nAChR protein have been used to demonstrate how α7 nAChR function can be modulated by compounds acting in the extracellular or transmembrane domain and by phosphorylation in the intracellular domain.

C. The α7 Nicotinic Acetylcholine Receptor as a Prototypical Allosteric Protein

Introduced in the 1960s, the term allosteric model (Monod et al., 1963) was proposed to describe the fact that proteins can have different conformations and that binding of a ligand can preferentially stabilize a given state. Initially developed to describe enzymatic reactions, this concept was soon extended to signal transduction proteins such as metabotropic receptors or ligand-gated ion channels (Changeux and Edelstein, 2005). Moreover, the model accounted for the fact that allosteric proteins comprise the main, or orthosteric, binding site and possibly other, or allosteric, sites where molecules interact. The term allosteric was coined from the Greek etymology, with “allos” meaning other and “stereos” indicating the object. In such a model, molecules that bind to allosteric sites can influence the protein transitions and thereby favor or inhibit the stabilization in the active state (Bertrand and Gopalakrishnan, 2007).

Molecules that favor stabilization in the active state increased the protein activity and are called positive allosteric modulators (PAMs), whereas molecules that favor the stabilization in a nonactive state are defined as negative allosteric modulators. One important consequence of allosteric modulation is the alteration on the receptor’s pharmacological profile. Namely, exposure to a PAM might change the profile of an agonist from partial to full agonist, as illustrated by the influence of ivermectin on the concentration-response relationship of α7 nAChRs to 1,1-dimethyl-4-phenylpiperazinium (Krause et al., 1998). Conversely, exposure to a negative allosteric modulator is expected to reduce activity and efficacy of compounds, as exemplified in engineered α7 nAChRs containing the point mutation M253L in which ivermectin becomes a negative allosteric modulator (Collins and Millar, 2010).

α7 nAChRs display typical characteristics of allosteric proteins; that is, they are activated or stabilized in the active state by orthosteric ligands such as ACh, and these orthosteric ligands can be allosterically modulated by other molecules. The first example of α7 nAChR allosteric modulation was provided by the finding that ivermectin increased the receptor’s apparent affinity and amplitude of the current evoked by ACh (Krause et al., 1998). Several molecules active at α7 nAChRs have since then been identified and divided in two groups. Type-I modulators are compounds such as 5-hydroxy indole (Zwart et al., 2002) or NS-1738 [1-(5-chloro-2-hydroxy-phenyl)-3-(2-chloro-5 trifluoromethylphenyl)-urea] (Timmermann et al., 2007), which increased the sensitivity of the receptor but do not modify its response time course and Type-II modulators such as PNU-120596 [1-(5-chloro-2,4-dimethoxy-phenyl)-3-(2-chloro-5 trifluoromethylphenyl)-urea] (Hurst et al., 2005), which modulated α7 nAChR activity and reduced or abolished desensitization. Use of chimeric receptors comprised of the extracellular domain of the α7 nAChR and TMD and C-terminal domain of the 5-HT3A receptor allowed examination of the critical protein determinants required for modulation by Type-I and Type-II PAMs (Bertrand et al., 2008). Conservation of the activity of the Type-I modulator NS-1738 at the α7-5-HT3 receptor chimera indicated that this compound must bind in the extracellular domain of the receptor, whereas the loss of activity of PNU-120596 suggested that this Type-II modulator probably interacts with the α7 nAChR TMD or intracellular domain (Bertrand et al., 2008). Since these initial studies, effects of Type-I and Type-II α7 nAChR PAMs have been widely studied in various models of cognitive impairment and neuroprotection (refer to Table 4).

Functional properties of the α7 nAChRs can be modulated by molecules that bind at a site different from the LBD. These molecules, also called allosteric modulators, can have positive or negative actions depending on the state that is preferentially stabilized. PAMs were further subdivided in Type-I and Type-II depending on their effects on receptor desensitization.
D. The α7 Nicotinic Acetylcholine Receptor Heteromers

Although most experiments, including those using immunoprecipitation, point to the existence of homomeric α7 nAChRs, the expression of multiple genes encoding for the neuronal nAChRs exist in the same cell. For example, it is known that SH-SY5Y cells express α3, α5, β2, β4, and at a low level α7 nAChR subunits but that overexpression of α7 nAChR subunits yielded robust currents attributable to α7 nAChRs alone that were quite distinct from those observed in naive SH-SY5Y cells (Lukas et al., 1993; Puchacz et al., 1994). Since then, several studies have attempted to determine if α7 nAChR subunits assemble purely as homomers or if, in some cases, α7 nAChR subunits could assemble with other nAChR subunits. Experiments were conducted to identify neurons that did not express other nAChR subunits, and recently it was proposed that the hypothalamic histaminergic tuberomammillary neurons expressed α7 nAChR subunits but no detectable levels of mRNA encoding for other nAChR subunits (Tischkau et al., 2014). These data suggested that bona fide α7 nAChR homomers expressed in neurons show similar properties to those observed in overexpressed cell systems.

Alternative strategies pursued by other laboratories attempted to resolve the question of possible heteromerization of α7 nAChR subunits by coexpression with other nAChR subunits types. As cells expressing α7 nAChR subunits often also expressed β4 subunits, experiments of coexpression of α7 nAChR subunits with other nAChR subunits (α2, α3, α4, and β4) were conducted by immunopurification and Western blot analysis (Criado et al., 2012). The results indicated that β4 nAChR subunits can coassemble with α7 nAChR subunits but that the pharmacological properties of the resulting receptors showed no significant differences in the response time course or pharmacological profile from homomeric α7 nAChRs, except for a minor difference in the sensitivity to cytisine (Criado et al., 2012). Additional studies examined the localization of α7 with β2 nAChR subunits in brain stem and hippocampus and suggested that coassembly of these subunits have a developmental role and influence the occurrence of sudden infant death syndrome (Machaalani et al., 2011; Machaalani et al., 2010). A word of caution should, however, be given, because these studies were conducted with antibodies and it is known that such labeling might be inaccurate (Moser et al., 2007; Jones and Wonnacott, 2005). However, additional studies have found that neurons in the basal forebrain and hippocampus expressed α7β2 nAChRs and that this expression resulted in a heteromeric nAChR that is functionally and pharmacologically distinct from homomeric α7 nAChRs, with slower desensitization rates and increased sensitivity to DHβE, a selective antagonist of β2-containing nAChRs (Liu et al., 2012; Liu et al., 2009). Furthermore, these publications discussed a greater sensitivity of α7β2 nAChRs than α7 nAChRs to the amyloid-β (Aβ) peptide.

The possible incorporation of other nAChR subunits types with α7 nAChR subunits into the pentameric complex raises questions about the formation of the LBD. Recalling that ACh binds at the interface between two adjacent α7 nAChR subunits, novel and distinct subunit sites might be formed at the interface of α7 and other nAChR subunits. To tackle this question, it was shown that α7 and β2 nAChR subunits can assemble into functional heteromers that have pharmacological properties resembling α7 nAChR homomers but with lower maximal amplitudes of agonist-evoked currents (Murray et al., 2012). Evidence for heteromeric assembly included colocalization of tagged subunits using laser scanning confocal microscopy, fluorescence Förster resonance energy transfer, and total internal reflection fluorescence microscopy. Electrophysiological evaluation demonstrated no significant difference between α7β2 nAChR heteromers and α7 nAChR homomers in the pharmacological sensitivity to agonists. A significant difference was, however, found with the competitive antagonist DHβE. In agreement with this observation, the use of cysteine mutants indicated that this ligand does not bind in a functionally productive manner at the interface between α7 and β2 nAChR subunits (Murray et al., 2012), suggesting that only the α7-α7 nAChR subunit interface is able to activate the α7β2 nAChR heteromers and produce the ACh-evoked response. This result supports the observation that α7β2 nAChR heteromers show reduced maximal amplitudes of inward currents. Recently, a more in-depth interrogation of the unique pharmacology of the rat α7β2 nAChR heteromers expressed in Xenopus oocytes showed that nonselective α7 nAChR agonists such as varenicline, nicotine, and cytisine became less efficacious in α7β2 nAChR heteromers and selective agonists of β2 subunit-containing nAChRs such as ABT-089 (1-{(2-methyl-3-(2-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide), SSR-108911 1,4-diazabicyclo[3.2.2]nonane-4-carboxylic acid, 4-bromophenyl ester, TC-5619 (N-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide), and encencline (EVP-6124; (R)-(7-chloro-N-quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide) exhibited comparable potencies with heteromeric α7β2 nAChRs as seen with homomeric α7 nAChRs but had a significantly reduced efficacy. Interestingly, there was no decrease in the efficacy of ACh with α7β2 nAChRs compared with α7 nAChRs; and the loss of efficacy for varenicline with α7β2 nAChRs was less than for cytisine and the selective α7 nAChR agonists. Although these results are not the final word on the pharmacology and function of heteromeric
α7 nAChRs, they clearly demonstrate that differential expression in the brain must be taken into account and that this diversity might offer additional strategies in the design of active compounds.

Although the α7 nAChRs were initially thought to be expressed only as homopentamers, more recent evidence points to the possibility of heteromeric assembly of α7 subunits with another nAChR subunit such as β2, β4, or dup α7 (discussed in section II.A). This further complexity must be taken into account when examining the role of α7-containing nAChRs in brain function.

E. Effects of Chronic Exposure of α7 Nicotinic Acetylcholine Receptors

One important property of the α7 nAChR to take into consideration in the concept of drug design is receptor desensitization. Namely, although brief exposures to agonists activate the receptors, sustained exposures to agonists are known to inhibit their subsequent responses by desensitization. Moreover, as illustrated in Fig. 2, concentrations causing inhibition are of orders of magnitude lower than those evoking a response, with limited or no overlap between the desensitization and activation curves. From this observation, it follows that sustained administration of an α7 nAChR agonist is unlikely to evoke a sustained response of the receptors. This raises the question whether ligands produce an efficacious effect by preferentially activating or inhibiting α7 nAChRs. Moreover, in clinical settings, longer and cumulative exposures need to be examined to assess if the therapeutic effects of a molecule do not cause tachyphylaxis, the loss of effect of a medication.

The observation of an increase in ligand binding in smokers’ brains revealed another puzzling property of nAChRs (Perry et al., 1999). Subsequently, the term receptor upregulation was coined to describe the apparent increase in receptor numbers, and further examination revealed that this process is not identical for all nAChR subtypes. Moreover, upregulation of heteromeric receptors such as the α4β2 nAChR depends upon multiple factors (Govind et al., 2012). A fast increase in α-Btx binding was observed upon nicotine exposure, suggesting the incorporation of additional α7 nAChRs into the membrane in chick ganglia (Berg et al., 2006). Similarly, it was concluded that potentiation of the α7 nAChR-mediated current observed in Xenopus oocytes and rat hippocampal interneurons after treatment with the tyrosine kinase inhibitor genistein was associated with an increase in receptor number (Cho et al., 2005). An alternative conclusion was, however, presented in other experiments, suggesting that genistein might act by an allosteric mechanism rather than due to an increase in receptor numbers (Charpantier et al., 2005). Nicotine exposure caused an increase in α-Btx binding in mouse prefrontal cortex that was attributed to reduced proteasome-dependent degradation due to the accumulation of ubiquitinated α7 nAChR subunits (Rezvani et al., 2007). In addition, these studies complement past efforts demonstrating that ultra-low exposure to α7 nAChR partial agonists result in sustained efficacy in models of working memory because of upregulation of cell surface α7 nAChRs (Werkheiser et al., 2011) or because of reduced turnover from inhibition of proteasomal activity (Rezvani et al., 2007).

Evidence obtained from animal models suggest that exposure to these α7 nAChR agonists, GTS-21 (3-(2,4-dimethoxybenzylidene)anabaseine) (Stevens et al., 2010), ABT-107 (5-(6-([3R]-1-azabicyclo[2.2.2]oct-3-yl)-oxy) pyridazin-3-yl)-1H-indole) (Bitner et al., 2010), RG3487 (N-([3S]-1-azabicyclo[2.2.2]oct-3-yl)-1H-indazole-3-carboxamide hydrochloride) (Wallace et al., 2011), and encencine (van Goethem et al., 2015), caused no tachyphylaxis or receptor upregulation. Maintained efficacy and absence of tachyphylaxis of α7 nAChR agonists has also been observed in Phase 1 and Phase 2 clinical trials, suggesting that α7 nAChR agonists may be good candidates for long-term treatment of cognitive disorders (Olincy et al., 2006; Freedman et al., 2008; Preskorn et al., 2014; Keefe et al., 2015). On closer examination of both the preclinical and clinical studies, it is apparent that efficacious concentrations of α7 nAChR agonists are extremely low, below those required to activate α7 nAChRs alone and below those that desensitize α7 nAChRs. The finding that at low concentrations, α7 nAChR agonists can increase the amplitude of ACh responses at α7 nAChRs expressed in cells has suggested that α7 nAChR agonists in vivo may similarly be acting in conjunction with the native ligand ACh. In vitro, the effect of α7 nAChR agonists on enhancing the activity of ACh has been termed “priming” (Quik et al., 1997; Papke et al., 2011b) and has also been observed with high-affinity α7 nAChR agonists (Wallace et al., 2011; Prckaerts et al., 2012). Although the underlying mechanisms of priming have not been fully revealed, a suggested mechanism has been described for the neuromuscular junction nAChR, in which priming may represent the stabilization of receptor conformations by ACh that are more easily activated by subsequent exposure to another molecule of ACh (Mukhtasimova et al., 2009). Alternatively, an α7 nAChR agonist may act to synchronize better receptor activation upon exposure to ACh. Additional work is required to describe better priming in vitro and to establish whether similar mechanisms are in effect in vivo. Collectively, these data point out the need for additional analyses before reaching a conclusion on the complexity of mechanisms underlying the maintenance of receptor density in the membrane.

Sustained exposure of α7 nAChRs to an agonist such as nicotine causes a profound desensitization that can block the response to ACh. Sustained exposures to an α7 nAChR agonist can, however, modify the fraction of receptor available. Although upregulation, that is an increase in the receptor number, was observed for some molecules and experimental conditions in vivo, another possible mechanism by which α7 nAChR agonists are
efficacious in vivo may be by priming of the receptor. This second mechanism may explain the absence of tachyphylaxis observed with different molecules rendering them attractive therapeutics.

**F. Modulation of α7 Nicotinic Acetylcholine Receptor Signaling**

The high Ca\(^{2+}\) permeability of the α7 nAChR is suggestive of a role downstream in events such as intracellular signaling, neurite outgrowth, synaptic transmission, modulation of neurotrophic factors, and excitotoxic processes (Role and Berg, 1996; Albuquerque et al., 1997). The neuroprotective effect of ligands acting at α7 nAChRs was supported by various studies (de Fiebre et al., 1995; Donnelly-Roberts et al., 1996; Meyer et al., 1998b). The mode of entry, but more importantly, the level of intracellular Ca\(^{2+}\) achieved upon activation of α7 nAChRs is pivotal to the outcome of downstream signaling events. Studies conducted in a stably transfected HEK293 cell line expressing α7 nAChRs revealed that stimulation elicited a significant increase in intracellular Ca\(^{2+}\) (~350 nM) (Delbono et al., 1997). Experiments conducted with the Type-II α7 nAChR PAM PNU-120596 caused an increase in intracellular Ca\(^{2+}\) concentration but was not cytotoxic in either PC12 cells or neurons from rat primary cultures (Hu et al., 2009). According to the Ca\(^{2+}\) set-point theory for cell survival (Franklin and Johnson, 1992), activation of α7 nAChRs yielded an intracellular Ca\(^{2+}\) concentration increase in the range of 200–400 nM, which permitted cell survival without the aid from trophic factors. Thus, in contrast to glutamate receptors, in which prolonged activation was shown to provoke cell death, activation of α7 nAChRs should be neuroprotective. This, as well as the importance of Ca\(^{2+}\) homeostasis to neuroprotection, was subsequently shown in diverse preparations using specific ligands of α7 nAChRs (Verkhratsky and Toescu, 1998; Donnelly-Roberts and Brioni, 1999) through the coupling of Ca\(^{2+}\) regulation to second messenger pathways (Berg and Conroy, 2002). Neuroprotective effects caused by exposure to SEN12333 (5-morpholin-4-yl-pentanoic acid (4-pyridin-3-yl-phenyl)-amide), a specific ligand of α7 nAChRs (Verkhratsky and Toescu, 1998; Verkhratsky et al., 1999), revealed that stimulation elicited a significant increase in intracellular Ca\(^{2+}\) concentration (~350 nM) (Verkhratsky et al., 1999; Verkhratsky and Toescu, 1998). Stimulation of α7 nAChRs 1037 induced an increase in phosphorylation of α7 nAChR agonist induced an increase in phosphorylation of serine 9 on glycogen synthase kinase 3β (S9-GSKβ), which was not observed in α7 nAChR knockout mice (Bitner et al., 2009). GSKβ has been shown to be a predominant kinase in AD, involved in tau hyperphosphorylation (Grimes and Jope, 2001). Additionally, A-582941 continuously infused subcutaneously to a steady-state exposure in Tg2576 mice, an AD model of amyloid precursor protein overexpression, significantly increased phosphorylation of S9-GSKβ in hippocampus and in a double transgenic AD mouse (TAPP, tau P301L X amyloid precursor protein) decreased phosphorylation of tau (Bitner et al., 2009). Increased phosphorylation of ERK1/2, CREB, and S9-GSKβ and decreased phosphorylation of tau were replicated with another α7 nAChR agonist of another chemotype (ABT-107) (Bitner et al., 2010). In addition, acute administration of A-582941 also dose dependently amplified both c-Fos and Arc, cytoskeletal proteins, in regions of brain involved in attention and working memory, although only in juvenile but not adult rats (Thomsen et al., 2008). The data collectively support the hypothesis that brain exposure to α7 nAChR agonists, shown to demonstrate cognitive enhancement, may be acting through these modulatory signaling pathways via an increase in protective Ca\(^{2+}\) concentrations and, furthermore, that through GSKβ inhibition, this neuroprotection may also have the potential to be disease modifying.

In vitro studies using PC12 cells have also shown that several α7 nAChR agonists, in the presence of the...
α7 nAChR, PNU-120596, all increased ERK1/2 phosphorylation, as well as phosphorylation of upstream kinases, p38 MAP kinase and mitogen-activated protein kinase kinase1/2, that have been linked to synaptic plasticity and cognition (Gubbins et al., 2010). Other studies using SH-SY5Y cells have also shown that PNU-282987 can rescue cells from apoptosis induced by rotenone and oligomycin A in a dose-dependent manner when applied after the insult (Parada et al., 2010). It was further demonstrated through use of specific kinase inhibitors that this rescue was activating HO-1 via the JAK1/Akt pathway to afford neuroprotection.

Neuroprotective effects caused by α7 nAChR agonists offer a promising possibility of disease modification, in addition to symptomatic treatment, for patients with cognitive deficits associated with neurodegeneration. Neuroprotection was shown to result from different mechanisms including the increase of intracellular Ca2+ and/or stimulation of second messenger cascades. Offering new therapeutic avenues, neuroprotection remains one of the important challenges in the field of α7 nAChRs.

G. Expression and Function of α7 Nicotinic Acetylcholine Receptors in Brain

To comprehend the role of α7 nAChRs in central nervous system (CNS) function, it is necessary to first examine which areas of the brain express them. As described above, the most reliable labeling strategies are the use of α7 nAChR-specific radiolabeled ligands such as α-Btx, MLA, A-585539, CHIBA-1001, AZ11637326, A-582941, A-844606, NS14492, or NS14490 (Han et al., 2003; Tribollet et al., 2004; Anderson et al., 2008; Hashimoto et al., 2008; Toyohara et al., 2010; Ettrup et al., 2011; Maier et al., 2011; Rötering et al., 2014). An alternative approach is to detect α7 nAChR expression levels by measuring the levels of CHRNA7 mRNA, as was performed in monkey brain (Han et al., 2000) and in post mortem human brain (Breese et al., 1997). Altogether, these data from mRNA expression and α-Btx binding indicate that α7 nAChRs are widely expressed in the brain and are at especially high densities in hippocampus, cerebral cortex, lateral and medial geniculate nuclei, reticular thalamic nucleus, basilar pontine nuclei, diagonal band of Broca, nucleus basalis of Meynert, inferior olive, posterior hypothalamus, mammillary bodies, dorsal nucleus of the vagus, and cerebellum (Breese et al., 1997). In vivo imaging using positron emission tomography (PET) tracers, such as CHIBA-1001, confirmed a similar distribution of α7 nAChRs (Tanibuchi et al., 2010). Although these studies provided a general distribution of α7 nAChRs, a fine cellular and subcellular localization of these receptors is required to examine their possible physiologic role. Successful subcellular localization of α7 nAChRs was obtained using fluorescent α-Btx or immunogold with an anti-α7 nAChR antibody and revealed that in hippocampus, α7 nAChRs are highly expressed presynaptically or perisynaptically, suggesting that these receptors might be involved in a critical role in modulation of the release of other neurotransmitters (Fabian-Fine et al., 2001; Jones and Wonnacott, 2004). The presynaptic role of α7 nAChRs was confirmed by the observation that their stimulation induced the release of other neurotransmitters (Girod et al., 2000; Rousseau et al., 2005; Zhu et al., 2005; Biton et al., 2007; Livingstone et al., 2009; Sydserff et al., 2009; Parikh et al., 2010; Huang et al., 2014a,b; Koranda et al., 2014).

A slightly different picture emerges when examining the electrophysiological properties of neurons using intracellular recordings and probing the response to specific cholinergic compounds. Recordings performed in hippocampal slices revealed the importance of α7 nAChRs on inhibitory interneurons, as illustrated by the sensitivity to different cholinergic agonists and allosteric modulators (Buhler and Dunwiddie, 2001; Christophe et al., 2002; Hurst et al., 2005; Charpantier et al., 2005). Projections from the basal forebrain innervate the cortex, and release of ACh was found to stimulate α7 and non-α7 nAChRs that are preferentially expressed on interneurons of the different cortical layers (Arroyo et al., 2012). MLA blocked fast neurotransmission indicative of the α7 nAChR contribution, whereas slow, non-α7 nAChR transmission was blocked by DHβE, with some cells showing a dual fast and slow component (Arroyo et al., 2012; Bennett et al., 2012). Cholinergic modulation of interneuronal activity was shown to participate in signal processing and the formation of LTP when the presynaptic cell was stimulated and postsynaptic cell was hyperpolarized (Grigoli et al., 2013). The initial evidence of a modulatory role for α7 nAChRs in synaptic activity was obtained in rat hippocampal CA1 pyramidal neurons by examining spontaneous activity and the effect of stimulation of the Schaffer collaterals (Alkondon et al., 1998, 2000; Frazier et al., 1998; Heft et al., 1999).

Figure 3 illustrates the localization and laminar localization of α7 nAChR expression in the cerebral cortex obtained by detailed electrophysiological recordings and pharmacological testing (Poorthuis et al., 2013; Arroyo et al., 2014). The fast and slow activity caused by ACh release observed in different neuronal types indicates that these cells must be expressing different nAChR subtypes that might be colocalized or expressed in distinct subcellular areas such as post- and/or perisynaptically. The imbalance of fast and slow cholinergic signals might result in different functional outcomes. For example, when considering the effects of an acetylcholinesterase inhibitor, increased half-life of ACh in the synaptic cleft and its vicinity is expected to result in a larger effect at the non-α7 nAChR component of the ACh-evoked response to cause a weighting toward tonic ACh activity. Exposure to an α7 nAChR antagonist such as MLA should result in diminished α7 nAChR activity.
and a similar imbalance between the fast and slow transients. In contrast, an α7 nAChR agonist could induce a weighting favoring fast versus slow transients as long as the agonist was not present at desensitizing concentrations. These data point to the necessity of establishing a better understanding of the respective contribution of different nAChRs subtypes at the cellular and circuit levels, especially when considering compounds like acetylcholinesterase inhibitors that have effects on multiple nAChR subtypes and nAChR subtype-selective compounds that can similarly alter the balance of nAChR subtype activity.

Expression of α7 nAChRs is not restricted to neurons but was also shown both histologically and functionally to be on glial cell types (Vélez-Fort et al., 2009), including astrocytes (Sharma and Vijayaraghavan, 2001), oligodendrocytes, and microglia (Shytle et al., 2004). Stimulation of the α7 nAChRs on glia caused many physiologically relevant activities that need to be taken into account when evaluating effects of molecules targeting α7 nAChRs.

Widely expressed in the brain, α7 nAChRs were shown to be localized pre- and postsynaptically. Presynaptic receptors were shown to modulate the release of neurotransmitter at both excitatory (glutamate) or inhibitory (GABA) neurons. Postsynaptic and extrasynaptic α7 nAChRs can also modulate neuronal activity and participate in neurotransmission. In addition, α7 nAChRs were also shown to be expressed by glial cells, but their role on these cell types remains to be clarified.

**III. Ligands Active at α7 Nicotinic Acetylcholine Receptors**

**A. Structure-Activity Relationship of α7 Nicotinic Acetylcholine Receptor Agonists**

Over the past two decades, medicinal chemistry has remarkably expanded the development of compounds acting via α7 nAChR agonism by agonists and PAMs. Extensive basic and clinical research studies were conducted and yielded numerous active and selective molecules that are reviewed in this section. The major attention will be kept on developing the various chemotypes that were brought up to clinical trials and are summarized in Table 2. The majority of α7 nAChR agonists described to date are comprised of the quinuclidine moiety, which encompasses structures such as the spirooxazolidinones, and quinuclidine carbamates, amides, and ethers. GTS-21 (DMXBA), one of the first reported ligands differing from nicotine to show binding specificity for the α7 nAChR, was described as a functionally selective partial agonist in comparison with ACh (Hunter et al., 1994; Briggs et al., 1995; Meyer et al., 1998a). This compound is not solely selective for α7 nAChRs and also binds to α4β2 nAChRs, but with a lower affinity. Over the years, GTS-21 has been characterized extensively both in vitro and in vivo and is one of the first molecules that was advanced into early phase clinical trials. However, newer ligands, stemming from scaffolds, such as the azaadamantane (ABT-126) and quinuclidine (encenicline, TC-5619, MEM3454/RG3487, and AQW051 [(R)-3-(6-p-tolyl-pyridin-3-ylxoyo)-1-aza-bicyclo(2.2.2)octane]), have expanded the chemical space also to include additional azabicyclic tertiary amine templates.

The most explored class of ligands for α7 nAChR is the quinuclidine amine-based moiety. AR-R17779 (–)-spiro[1-azabicyclo[2.2.2]octane-3,5’-oxazolidin-2’-one], a spirooxazolidinone, from the group at AstraZeneca (London, United Kingdom) was another early molecule in this chemical space (Mullen et al., 2000). Several research groups followed with expansion on this particular series to result in structurally diverse and selective compounds that were active preclinically. However, the challenges of these initial compounds in this series remained, which involved cross reactivity with the 5-HT3 receptors and limited penetration into the CNS. Astra-Zeneca further developed the structure-activity relationship (SAR) in the spirooxazolidinone series with identification of AZD0328 [(2’R)-spiro-[1-azabicyclo[2.2.2]octane-3,2’(3’H)-furo[2,3-b]pyridine] d-tartrate], a spirofuropyridine, that has been characterized in preclinical models (Syderff et al., 2009). AZD0328 was the first spirooxazolidine analog with good selectivity and potency for α7 nAChRs (Ki = 3 nM) and showed favorable pharmacokinetic (PK) properties sufficient for advancement into clinical studies. This compound acted as a partial agonist at rat and human
α7 nAChR agonists entered clinical trials and in discovery phase

<table>
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<tr>
<th>Compounds</th>
<th>Therapeutic Indications</th>
<th>Development Status</th>
<th>Company</th>
<th>Reference</th>
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<tr>
<td>GTS-21</td>
<td>Alzheimer's disease</td>
<td>Phase 2</td>
<td>Comentis</td>
<td>Briggs et al., 1995; Azuma et al., 1996, 1999; Mahnir et al., 1998; Li et al., 1999; Adams et al., 2000; Crutch, 2000; Bruchfeld et al., 2010; Loram et al., 2010; Vukelic et al., 2013</td>
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<td>Alzheimer's disease</td>
<td>Phase 1</td>
<td>Pfizer</td>
<td>Bodnar et al., 2005; Ishikawa and Hashimoto, 2011; Stegemann et al., 2013</td>
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<td>Discovery</td>
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<td>Memory/Roche</td>
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(continued)
α7 nAChRs and displayed an EC$_{50}$ of 338 nM and an efficacy of 64.7%; however, this compound also activated 5-HT$_3$ receptors with an EC$_{50}$ of 474 nM but an efficacy of only 12% (Sydserff et al., 2009). In vivo, this compound exhibited activity in a variety of preclinical models including novel object recognition (NOR) in mouse, reversal of short-term memory deficits in fimbria-fornix-lesioned rats, and improvement in working memory in the spatial delayed response task in rhesus monkey (Sydserff et al., 2009; Castner et al., 2011; Werkheiser et al., 2011).

Quinuclidine and ring expansion analogs have been extended by modification of functional linkers such as heteroaryls, amides, and carbamates. Pharmacia/Pfizer (Groton, CT) developed and reported on one of the earlier compounds in this series with PNU-282987, a quinuclidine benzamide (Bodnar et al., 2005). PNU-282987 showed both selectivity and potency as an α7 nAChR agonist (K$_i$ = 29 nM), retaining full agonist efficacy relative to nicotine in functional assays (Hajós et al., 2005). PNU-282987 has been thoroughly characterized both in vitro and in vivo, demonstrating the restoration of P50 gating deficits in rodents, and has served as a tool molecule to advance basic research efforts (Hajós et al., 2005; Vicens et al., 2010; McLean et al., 2011). However, a major drawback of this compound was the interaction with the human ether à go-go-related gene (hERG) channel, which could represent a major cardiovascular risk (Walker et al., 2006). Thus, to improve the selectivity and to diminish functional activity at the hERG channel, an analog of PNU-282987 was designed that demonstrated improved absorption, distribution, metabolism, and excretion properties, reduced hERG activity, as well as an adequate therapeutic index. This analog, PHA-543613 (N-([3R]-1-azabicyclo[2.2.2]oct-3-yl)furo[2,3-c]pyridine-5-carboxamide) (K$_i$ = 9 nM) (Acker et al., 2008), advanced into Phase 1 clinical studies. Recently, encenicline, a potent quinuclidine amide analog reported by FORUM (formerly EnVivo, Waltham, MA), demonstrated partial agonist activity at α7 nAChRs (K$_i$ = 10 nM) and antagonist activity at 5-HT$_3$ receptors (IC$_{50}$ < 10 nM) (Prickaerts et al., 2012). Encenicline demonstrated preclinical procognitive effects in rat NOR, as well as efficacy in Phase 1 and Phase 2 trials in normal volunteers and patients with schizophrenia (Barbier et al., 2015; Keefe et al., 2015), and is now in Phase 3 trials for treatment of cognitive impairment in schizophrenia and Alzheimer’s disease. Like the amide series from FORUM, MEM3454 (RG3487) also exhibited antagonism at the 5-HT$_3$ receptor and produced procognitive effects in rat NOR and in aged mouse and aged rat in a water maze (Wallace et al., 2011). As expected for an α7 nAChR agonist, RG3487 increased dopamine and ACh release in the rat hippocampus and prefrontal cortex (Huang et al., 2014b). Similarly, encenicline increased dopamine and ACh, as well as glutamate release in the prefrontal cortex (Huang et al., 2014a). Tested in clinical trials in schizophrenia patients, RG3487 showed no significant improvement of the cognitive deficit associated with schizophrenia, but patients with moderate negative symptoms exhibited a significant improvement in their symptoms (Umbricht et al., 2014). Novartis (Basel, Switzerland) also recently disclosed a quinuclidine ether α7 nAChR agonist, AQW051 (Di Paolo et al., 2014; Feuerbach et al., 2015). In vitro assessment of AQW051 at α7 nAChRs expressed in Xenopus oocytes revealed that this compound displayed an EC$_{50}$ of 7.5 μM but acted as a partial agonist, evoking only 75% of the ACh-evoked current. This compound inhibited the 5-HT$_3$ receptors with an IC$_{50}$ of 19 μM, demonstrating a better α7 nAChR/5-HT$_3$ selectivity profile than RG3487 and encenicline (Feuerbach et al., 2015). This compound also demonstrated a sufficient PK profile, with rapid CNS permeability, and activity in exploration in the rat social recognition model and an improved mouse sensory gating profile (DBA/2). Another amide quinuclidine compound (Targacept’s [Winston-Salem, NC] TC-5619) demonstrated potency and selectivity for α7 nAChRs (net
current EC$_{50}$ = 33 nM), with full efficacy relative to ACh in *Xenopus* oocytes (Hauser et al., 2009). TC-5619 (α7 nAChR $K_i = 1$ nM) exhibited superior in vitro selectivity against human 5-HT$_3$ receptors (IC$_{50} > 10$ μM). This compound also exhibited adequate in vivo properties, including PK profile and rapid CNS permeability as well being active in rat NOR and in mouse social exploration (Table 2).

Additionally, structural diversity began to emerge in the literature from this series within the amine portion of α7 nAChRs agonists. However, some of this diversity also led to azabicyclic amines that exhibited activity as ligands for other nAChR subtypes such as the 4β2 nAChR. Several alternate amines were recently published with α7 nAChR activity and include the diazabicyclooctanorbornanes such as SSR180711 (Biton et al., 2007; Pichat et al., 2007), the octahydropyrrolo[3,4-c]pyrrole A-582941 (Tietje et al., 2008), and the (3R,5R)-1-azabicyclo-[3.2.1]oct-3-yl-carboxamide PHA-709829 (Acker et al., 2008) and ABT-107 (Bitner et al., 2010; Malysz et al., 2010). The selective partial agonist SSR180711 is a 1,4-diazabicyclo[3.2.2]nonane carbamate derivative ($K_i = 50$ nM, EC$_{50} = 800$ nM) active in NOR, Morris water maze, and an MK-801-induced memory deficit model (Pichat et al., 2007). Researchers at AbbVie (formerly Abbott, North Chicago, IL) described A-582941, a novel biaryl diamine α7 nAChR agonist (Buccafusco et al., 2007; Tietje et al., 2008). A-582941 exhibited good affinity for the human α7 nAChRs ($K_i = 16.7$ nM), with partial agonist efficacy but with a favorable PK profile and CNS penetration. In addition, A-582941 improved cognitive performance in assays, such as the monkey delayed matching-to-sample task and rat social recognition (Buccafusco et al., 2007) and mouse inhibitory avoidance, and normalized sensory gating deficits induced by MLA in rats (Tietje et al., 2008). Overall, the large number of potent and selective α7 nAChR agonists that have been synthesized provide insight into the in vivo pharmacology and absorption, distribution, metabolism, and excretion properties, which has been instrumental in advancement of compounds into clinical trials over the years. However, biomarkers that can monitor target engagement have not been fully developed to accompany these clinical trials. Early work in this area has suggested that endophenotypes associated with schizophrenia, such as event-related electroencephalographic deficits in mismatch negativity, P300 (Preskorn et al., 2014) and P50 (Olincy et al., 2006), are improved by α7 nAChR agonists and that these endophenotypes might serve as biomarkers of target engagement in future clinical studies.

Functional expression of α7 nAChRs in recombinant systems has highlighted the similarities and differences between the α7 and other nAChR subtypes. These findings stimulated the search for α7 nAChR-specific ligands and led to the discovery of families of molecules showing selective α7 nAChR agonist activity in the micromolar range. Compounds with a quinuclidine moiety were extensively researched and yielded the discovery of several molecules that have progressed into clinical trials.

1. The Clinical Trials. To test the utility of α7 nAChR agonists for enhancing cognitive function and ultimately to assess the utility of α7 nAChR agonists in treating cognitive deficits, several compounds such as GTS-21, RG3487, encenicine, TC-5619, ABT-126, and AQQW051 have advanced into clinical trials (see Table 2). Despite strong preclinical evidence supporting profunctional effects, clinical results thus far have been limited. The paucity of results may be attributable to confounding issues that include 1) properties of compounds that may not exhibit adequate receptor selectivity and functional profile; 2) inadequate PK, particularly, CNS exposure to achieve necessary receptor occupancy and pharmacodynamic effects; and 3) clinical trial design issues. For example, the GTS-21 Phase 2 trial failed to show significant effects on cognition. Specifically, performance on the six domains of the MATRICS Consensus Cognitive Battery (MCCB) did not differ between GTS-21 and placebo, although a significant effect of GTS-21 treatment was observed on the Scale for the Assessment of Negative Symptoms (SANS) total score (Freedman et al., 2008). However, it should be noted that GTS-21 is not a prototypical α7 nAChR agonist. GTS-21 has higher affinity at 4β2 nAChRs, where it is a functional antagonist compared with its interaction with α7 nAChRs ($K_i = 650$ nM at rat and 2000 nM at human) with only weak agonist efficacy (6% efficacy relative to ACh) (Briggs et al., 1997). Thus, in the dose range used in the clinic, GTS-21 is more likely to interact with 4β2 than α7 nAChRs, and it is misleading to conclude that the lack of clinical benefit can be attributed to the α7 nAChR pharmacology. Subsequently, a number of α7 nAChR agonists/5-HT$_3$ receptor antagonists have been advanced to the clinic. For example, RG3487 has relatively equal affinities at α7 nAChRs ($K_i = 6$ nM) and 5-HT$_3$ receptors ($K_i = 2$ nM) and showed improvement in episodic secondary memory in a healthy volunteer study, although the cognitive enhancing effects were not confirmed using the MCCB composite score in a double-blind, placebo-controlled Phase 2 study in schizophrenia (Wallace and Porter, 2011; Umbricht et al., 2014). Although the lack of efficacy in this clinical trial might have different origins, one additional complexity relies on the methodology and assessment scale used. In this respect it should be recalled that few studies share the same measurement scales and that so far there is no agreement on the best method to be used for a given symptom domain in schizophrenia. Interestingly, a common trait emerges, with some of these studies demonstrating an improvement on the SANS (Umbricht et al., 2014; Keefe et al., 2015); although Walling et al. (2015) showed no benefit of TC-5619 on the SANS. Indicative of the fact that α7
nAChR agonists have not only reached the brain and have also modified the SANS scale, improvement in negative symptoms could be used as a kind of biomarker. Moreover, as negative symptoms are very important in the day-to-day functioning of schizophrenic patients, a drug that could reduce them would still be beneficial, even if there is no improvement in the cognitive symptoms.

More recently, positive signals of efficacy have been reported in schizophrenia with other α7 nAChR/5-HT3 receptor ligands. Tropisetron, an α7 nAChR agonist/5-HT3 receptor antagonist (Ki = 6.9 nM for α7 nAChRs and Ki = 5.3 nM for 5-HT3 receptors), which is primarily used as an antiemetic, was reported to improve sustained attention and to significantly improve sensory gating in a randomized, double-blind, placebo-controlled study in schizophrenia (Shiina et al., 2010). More recently, FORUM reported an improvement in cognitive deficits with encenicline, another α7 nAChR agonist/5-HT3 receptor antagonist using the CogState battery, with trends for improvement on the MCCB composite score, as well as improvement in the SANS in a Phase 2, double-blind, placebo-controlled clinical trial in schizophrenia that included smokers and nonsmokers (Keefe et al., 2015). The importance of trial design was underscored in this study by the secondary analysis in which the consistency in time of day of cognitive testing was critical to demonstrating efficacy with the MCCB (Hufford et al., 2014). TC-5619, an α7 nAChR agonist without 5-HT3 receptor antagonist activity, was found to improve cognitive dysfunction as assessed by the Groton Maze Learning Task of the CogState Schizophrenia Battery and to reduce negative symptoms in a 12-week study in schizophrenia (smokers and nonsmokers) (Lieberman et al., 2013). However, a second Phase 2 trial of negative symptoms and cognition failed to find any benefit of TC-5619 in schizophrenia (Walling et al., 2015). Collectively, these recent clinical results with compounds that exhibited improved receptor selectivity and enhanced PK profiles further validated the approach of activating α7 nAChRs as a therapeutic target.

Clinical trials conducted with α7 nAChR agonists showed promising results in most of the Phase 1 studies, with compounds demonstrating safety and efficacy in normal healthy volunteers. Cardiovascular events, such as reported for PNU-282987, marked, however, a halt of clinical studies for compounds from this class. Paucity of effects or lack of sufficient selectivity between α7 nAChRs and 5-HT3 receptors might be the cause of other discontinued compounds in clinical development. In the absence of clear reports, it is difficult to discuss the limited success of α7 nAChR agonists in clinical studies reported so far. However, it should be noted that Phase 3 clinical studies of encenicline, an α7 nAChR agonist/5-HT3 antagonist, are currently in progress, with two trials for Alzheimer’s disease as well as two trials for cognitive impairment in schizophrenia. The high degree of expression of α7 nAChRs in the brain and the relevance of cholinergic function to cognition suggest that α7 nAChR selective compounds may still become therapeutics for cognitive impairment. The success or failure of the encenicline Phase 3 studies will likely influence the likelihood that other α7 nAChR agonists are developed in the near future, regardless of how compelling the preclinical evidence is for the α7 nAChR as a target for treating cognitive impairment.

B. From the Crystal Structure to Developments in Structure-Activity Relationship

The discovery of the water-soluble AChBP, which allows the preparation of high resolution crystal structures, initially reported by Sixma and collaborators (Brejc et al., 2001; Sixma and Smit, 2003), marked a turning point in SAR research of compounds targeted at the α7 nAChR. Since then, other proteins sharing similar structure and functions have been identified in invertebrates and provide additional and valuable information about the ACh LBD (Hansen et al., 2005; Hibbs et al., 2009; Sander et al., 2010). The crystal structure of AChBP bound to different molecules brought new insights to the understanding of protein-ligand interactions and suggested that the LBD differentially changes conformation upon binding of an agonist or an antagonist (Hibbs et al., 2009; Brams et al., 2011a). Crystal structures have been obtained for epibatidine bound α7 nAChR/AChBP chimeras, with three additional Aplysia californica AChBP mutants showing further structural features with a variety of nicotinic ligands. These constructs may provide realistic templates for structure-aided drug design. Homology models of this type, coupled with docking studies and regional analysis, have become a tool for the rational design of new, selective nAChR ligands. Sequence analysis of AChBPs revealed that these proteins share about 20–26% overall sequence identity to the nAChR extracellular domains, but homologies within the orthosteric LBD are higher.

Studies comparing results obtained using the AChBP with functional assays for α7 and α4β2 nAChRs revealed some limitations, because some molecules known to bind with high affinity to AChBP had no activity on receptors expressed in heterologous systems (Ulens et al., 2009). Nonetheless, AChBP provides a useful template for the identification of novel molecules that were subsequently reported to have high affinity for the 5-HT3 receptor and α7 nAChR (Akkemir et al., 2011; Akdemir et al., 2012; Armishaw et al., 2009; Bourne et al., 2010; Brans et al., 2011b).

Recent publications of the GABA A and 5-HT3 receptor structures illustrate the feasibility of crystallization of members of the cys-loop family of ligand-gated ion channels and suggest that the three-dimensional structure of the α7 nAChR might be resolved in the near future (Hassaine et al., 2014; Miller and Aricescu,
Progressive refinement of the three-dimensional protein structure at the molecular level is expected to expand our understanding of the relationship between ligand binding and functional activity of α7 nAChRs.

Although crystal structure of a full α7 nAChR has not been achieved, progress made with related proteins such as the 5-HT3 receptor or AChBP has brought our understanding of the nAChRs one step further. Crystalization of the α7 nAChR is expected to shine a new light on ligand-protein interactions and is expected to open the discovery to new and more specific molecules.

C. Target Engagement and Overview of the Development of Biomarkers

Research in the nicotinic field provided, over the years, extensive data to support that α7 nAChRs play a pathophysiological role in several psychiatric and neurologic disorders, such as schizophrenia, AD, anxiety, depression, drug addiction, and autism. Altogether, this propelled α7 nAChRs as an attractive target for the design of selective molecules desired for a number of therapeutic indications. Moreover, it has been demonstrated in post mortem human brain samples that α7 nAChRs levels are altered in schizophrenia and AD patients. This finding generated interest in studies to image and assess alterations in α7 nAChRs levels in living brains of patients with such neuropsychiatric disorders. In this respect, it would also be relevant to measure in the intact brain and possibly in neuropsychiatric patients, the receptor occupancy of potential therapeutic α7 nAChR drugs that would provide an assessment of target engagement. A number of radioligands were synthesized by different laboratories to undertake such a measurement and to provide quantitatively the distribution of α7 nAChRs in the human brain through the utilization of PET tracer and single photon emission computed tomography (SPECT) (see Table 3). However, challenges ensued with such radioligand development due to lack of lead chemical structures that provided properties of high affinity and strategically attached functional groups to be labeled with PET and SPECT radioisotopes. It is important to stress that chemistry of ligands suitable for PET or SPECT imaging requires the possibility of rapidly inserting the desired radioactive group in the last step of synthesis, which seriously increases the challenge in the design of new molecules. In 2008, the 1,4-diazabicyclo[3.2.2]nonane analog 4-[11C]methylphenyl 2,5-diazabicyclo[3.2.2]nonane-2-carboxylate ([11C]CHIBA-1001) was developed by CHIBA University (Tokyo, Japan), and its selective uptake was confirmed in the conscious monkey brain by PET (Hashimoto et al., 2008).

To date, [11C]CHIBA-1001 has been the sole PET ligand accessible for clinical trials to monitor in the human brain α7 nAChR images. Although [11C]CHIBA-1001 exhibited adequate properties as an α7 nAChR imaging tool, its development for human studies remains unlikely. Recently, a novel series of octahydropyrrolo[3,4-c]pyrrole moieties were described by AbbVie (formerly Abbott) as ligands for nAChRs (Briggs et al., 2008; Tietje et al., 2008). Two octahydropyrrolo[3,4-c]pyrrole derivatives were characterized in the literature to be selective α7 nAChRs agonists adequate for labeling with $^{11}$C, 2-methyl-5-[6-phenylpyridazine-3-yl]octahydropyrrolo[3,4-c]pyrrole (A-582941) and 2-(5-methyl-hexahydropyrrolo[3,4-c]pyrrol-2-yl)-xanthene-9-one (A-844606) (Toyohara et al., 2010). These compounds possessed required properties of potency and selectivity indispensible for PET. A-582941 displaced specifically the radioligand $^{[3]H}$A-585539 binding to α7 nAChR membranes from both rat brain and human frontal cortex with $K_i$ values of 10.8 and 17 nM, respectively (Anderson et al., 2008). A-582941 competed with the specific binding of $^{[3]H}$MLA to rat brain membranes with a $K_i$ of 88 nM and exhibited much lower affinity for the α4β2 nAChR subtype, as measured using $^{[3]H}$cytisine binding to rat brain membranes ($K_i > 100,000$ nM). In addition, A-582941 (10 μM) did not exhibit any significant affinity for 78 other targets in a Cerep panel analysis, with the sole exception of 5-HT3 receptors, in which $^{[3]H}$BRL 43694 (granisetron) binding was displaced. The $K_i$ value of A-582941 for 5-HT3 receptors was 150 nM, which translates into a 15-fold higher $K_i$ than for α7 nAChRs (Tietje et al., 2008).

Over the past decade, there has been a considerable effort to expand the development of α7 nAChR ligands, with more than 20 compounds radiolabeled for PET and SPECT, but previous efforts by several research groups to develop a clinically viable α7 nAChR tracer for PET or SPECT have proven unsuccessful. None of these radioligands had sufficiently high specific binding at α7 nAChRs in vivo. Even [11C]CHIBA-1001, the recent PET radioligand for human subjects, exhibited a low α7 nAChR binding affinity and poor in vivo selectivity.

In vivo imaging of the α7 nAChR distribution, possibly up to the subcellular level, represents one of the indispensable steps toward a better understanding of these receptors in brain function. Development of radioligands, specific for α7 nAChRs and amenable to PET or SPECT studies is an enormous challenge that was brought one step further with molecules that were examined in primates. Indicative of positive outcomes, these pioneering studies are opening additional strategies to evaluate the contribution of the cholinergic system in brain function.

D. The Chemical Strategy for α7 Nicotinic Acetylcholine Receptor Positive Allosteric Modulators

In recent years, medicinal chemistry has evolved to include the synthesis of allosteric modulators active at α7 nAChRs with a focus on the identification of novel chemical entities. In this review, we will highlight such ligands as illustrated in Table 4, which are those most extensively characterized in the literature and have
served as tools to make a significant advancement in the 
\( \alpha^7 \) nAChR field. These compounds embody a new series 
of ligands that modulate the activity of \( \alpha^7 \) nAChRs and 
thus provide a potentially new therapeutic opportunity 
to treat \( \alpha^7 \) nAChR-associated cognitive deficits in 
schizophrenia. Initial work in the area of allosteric 
modulation of \( \alpha^7 \) nAChRs was accomplished using 
ivermectin or 5-hydroxyindole (Krause et al., 1998;
Zwart et al., 2002). The disclosure of the selective 
\( \alpha^7 \) nAChR Type-II PAM PNU-120596 provided the essen-
tial selective tool to exploit the mechanistic potential of 
such ligands (Hurst et al., 2005). Using an engineered 
variant of the human \( \alpha^7 \) nAChR and ACh-evoked 
inward currents in hippocampal interneurons, PNU-
120596 increased agonist-evoked Ca\(^{2+} \) flux (Hurst et al.,
2005). This compound also suppressed desensitization 
when tested in vitro and exhibited robust activity in the 
in vivo amphetamine-induced P50 gating deficit model. 
Experiments conducted in aged rodents and nonhuman 
primates showed that low doses of PNU-120596 augmented 
the effects of the acetylcholinesterase inhibitor 
donepezil on learning and memory (Callahan et al.,
2013). The positive effects were observed with low doses 
of PNU-120596 that were otherwise ineffective when 
applied alone and indicated that this drug combination 
was acting by potentiating \( \alpha^7 \) nAChRs, as confirmed by 
inhibition of the effects by MLA (Callahan et al., 2013).

More recently, the SAR within this chemical series 
was determined to be narrow, but the series contained 
compounds with improved potency, physicochemical 
properties, and PK when tested in vivo. A biaryl urea 
series was identified by the group at NeuroSearch 
(Copenhagen, Denmark), with NS1738 as an example 
of such a compound from the series (Timmermann et al.,
2007). NS1738 was also reported to enhance agonist 
potency, as well as the efficacy. Although this compound 
has limited CNS penetration, it exhibited the ability to 
rescue scopolamine-induced deficits in acquisition of 
a water maze learning task in rats and enhance 
performance in rat social recognition (Thomsen et al.,
2011). Interestingly, a team at the University of 
California-Irvine synthesized another molecule that is 
a kind of chimera between compounds active at the 
GABA\(_A\) receptor and at the \( \alpha^7 \) nAChR, by taking 
advantage of sequence homologies between these recep-
tors. From a library screen of modulators of the GABA\(_A\) 
receptor, the group identified a class of compounds 
highlighted by compound 6 (XY4083) (Ng et al., 2007). 
XY4083 exhibited properties of an \( \alpha^7 \) nAChR PAM but,
unlike PNU-120596, did not significantly prolong the

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<tr>
<th>Compounds</th>
<th>Species Tested</th>
<th>Company</th>
<th>Reference</th>
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<tbody>
<tr>
<td>[( ^{11} \text{C} )]-CHBA-1001</td>
<td>Rodent and Pig</td>
<td>Neurosearch</td>
<td>Ettrup et al., 2011</td>
</tr>
<tr>
<td>[( ^{11} \text{C} )]-NS-14492</td>
<td>Rodent</td>
<td>AbbVie</td>
<td>Horti et al., 2013</td>
</tr>
<tr>
<td>[( ^{11} \text{C} )]-A-833834</td>
<td>Rodent</td>
<td>Astra-Zeneca</td>
<td>Gordon et al., 2010; Maier et al., 2011</td>
</tr>
<tr>
<td>[( ^{18} \text{F} )]-AZ11637326</td>
<td>Rodent and Human</td>
<td>Johns Hopkins</td>
<td>Wong et al., 2014</td>
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<th>Compounds</th>
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<tr>
<td>[( ^{18} \text{F} )]-ASEM</td>
<td>Rodent and Human</td>
<td>Johns Hopkins</td>
<td>Wong et al., 2014</td>
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response time course. This compound reversed sensory gating deficits in rodents and improved working memory. Eli Lilly (Indianapolis, IN) and Johnson and Johnson (New Brunswick, NJ) identified from high throughput screenings a series of thiazole derivatives as $\alpha 7$ nAChR PAMs. LY-2087101, a (2-amino-5-keto) thiazole compound (Broad et al., 2006), was derived from that series and exhibited activity at the various brain subtypes of nAChRs and thus was not selective for $\alpha 7$ nAChRs (Young et al., 2008). Other compounds in the series related to LY-2087101 exhibited an enhancement in potency and maximal efficacy at both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs. JNJ1930942 (2-[[4-fluoro-3-(trifluoromethyl)phenyl]amino]-4-(4-pyridinyl)-5-thiazolemethanol) was reported to be selective for $\alpha 7$ nAChRs (Dinklo et al., 2011). This compound enhanced the peak agonist-evoked current amplitude, and similar to PNU-120596, was classified as a Type-II PAM with slowed desensitization kinetics. JNJ1930942 improved sensory gating of auditory evoked potentials in DBA/2 mice. More
recently, Roche (Basel, Switzerland) described another PAM selective for \( \alpha_7 \) nAChRs, RO5126946, or 5-chloro-N-[(1S,3R)-2,2-dimethyl-3-(4-sulfamoyl-phenyl)-cyclopropyl]-2-methoxy-benzamide), which is also classified as a Type-II PAM and displayed effects both in vitro and in vivo (Sahdeo et al., 2014). It was concluded and reported that subtle substitutions in this chemical series translate to profound effects on selectivity among the homomeric and heteromeric nAChR subtypes.

The finding that \( \alpha_7 \) nAChR activity can be modulated by different molecules suggested that PAMs represent an additional therapeutic possibility. The identification of Type-I and Type-II PAMs that were classified according to their effects on receptor desensitization unveiled the broad possibilities offered by allosteric modulation of \( \alpha_7 \) nAChRs. Although efforts in the search for additional PAMs are still in their infancy, it is clear that this strategy will be further exploited in the near future.

### IV. The Relevance of \( \alpha_7 \) Nicotinic Acetylcholine Receptors in Diseases

Normal brain function relies on precise connectivity and equilibrium between neuronal activities from multiple sources. Although there are numerous examples of single amino acid mutations causing altered brain function, the profound modifications caused by mutation in the AMPA receptor regulatory protein \( \gamma_2 \) (or stargazin) are particularly noteworthy (Osten and Stern-Bach, 2006). Mutant stargazin alters trafficking of AMPA receptors and leads to a complex phenotype, which includes changes in AMPA receptor function, aberrant pyramidal cell orientation, and epilepsy. During development, as cells are progressively migrating and differentiating, neurotransmitters are synthesized and released to aid in the establishment of neuronal connections. Expression of receptors is therefore one of the primary steps taking place early in development, and *CHRNA7* mRNA is found early in several nuclei that receive sensory information in the human fetal brain (Agulhon et al., 1999). Improper function of these receptors might therefore alter the organization of the brain. Although our knowledge of the relationship between \( \alpha_7 \) nAChRs and brain development is rather limited, developments in imaging technologies, combined with genetic associations and clinical phenotypes are paving the way to future research, as illustrated by the computer analysis of multiple genes contributing to schizophrenia (Gilman et al., 2012).

The aim of this section is to review the most prominent correlations found between *CHRNA7* and neurologic and psychiatric diseases. In the view of the determinant properties of \( \alpha_7 \) nAChRs and their implication in brain development, it is probable that additional diseases associated with this receptor will be discovered as progress is made in small patient cohorts or even in the case of pedigree studies (see Table 5).

#### A. Alzheimer’s Disease

In the United States alone, AD has become a devastating neurologic disorder affecting over 5 million patients and is the major form of dementia in the aging population. First identified in 1901 in a 51-year-old patient showing presenile dementia, this case allowed Dr. Alois Alzheimer to study the evolution of a disease that initiated with loss of short-term memory and was followed by progressive loss of cognitive function. Autopsy and histologic observations of this patient’s brain revealed a peculiar formation of amyloid plaques and neurofibrillary tangles that were subsequently used as the post mortem diagnostic criteria of the disease (Simchowicz, 1911).

Initiating with mild cognitive impairment (MCI) and progressing to loss of short-term memory, the symptoms of AD disable patients in their day-to-day functioning and pose an enormous burden on patients’ relatives and caregivers. It is therefore of no surprise that the finding of a possible relationship between histologic evidence and cognitive decline triggered immediate attention. Since these initial studies, replication of the clinical and histologic observations were made by other laboratories.

<table>
<thead>
<tr>
<th>Disease</th>
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<tr>
<td>Auditory dating deficits in families with schizophrenia and smokers with schizophrenia</td>
<td>Freedman et al., 1996; Leonard et al., 2002; Gault et al., 2003; Mexal et al., 2010; Flomen et al., 2013 Soler-Alfonso et al., 2014</td>
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<td>Tripllication in three generation pedigree of cognitive impairment and neuropsychiatric phenotype</td>
<td>Adler et al., 1998 Biley et al., 2002 Elmalie et al., 1997; Rozycka et al., 2013 Barabash et al., 2009; Heinzen et al., 2010 Swaminathan et al., 2011, 2012a,b Feher et al., 2009 Neubauer et al., 1998 Allen-Brady et al., 2010; Yasui et al., 2011 Mikhail et al., 2011 Masurel-Paulet et al., 2010; Szafranski et al., 2010</td>
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<tr>
<td>Inherited phenotypic trait of schizophrenia</td>
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<td>Neuropsychiatric disorders</td>
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<td>Epilepsy</td>
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<td>AD susceptibility; MCI risk and conversion to AD</td>
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<td>AD, Dementia with DLB or Pick’s</td>
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<td>Benign rolendic epilepsy</td>
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<tr>
<td>Autism and Rett Syndrome</td>
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<tr>
<td>Developmental Delay, Mental Retardation and/or ASD</td>
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<td>Microdeletion Syndrome</td>
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The decrease in [3H]acetylcholine and [3H]nicotine binding in AD was correlated with reduced cholinergic activity and a reduction in [3H]acetylcholine and [3H]nicotine binding in AD (Whitehouse and Au, 1986). Studies of the effects of blockade of the cholinergic system in young healthy subjects further supported the relationship between cognitive function and the cholinergic system (Bartus et al., 1982). In view of these observations, it was proposed that use of a compound that would block acetylcholinesterase activity might be beneficial in reducing the cognitive deficit in AD, which eventually led to the treatment of patients suffering from MCI and age-related decline with acetylcholinesterase inhibitors (see for review Tan et al., 2014). In view of the parallel reductions in ACh and nicotine binding sites in cerebral cortex, experiments were conducted to determine if nicotine could be a suitable treatment to restore cognitive function. Exposure to nicotine alleviated deficits in attention and information processing associated with AD (Wesnes and Warburton, 1984; Sahakian et al., 1989; Newhouse et al., 2012). The positive effects of nicotine treatment observed in these studies were initially attributed to nicotine interacting with the high-affinity α4β2 nAChRs. However, given the lack of robustness of α4β2 nAChR agonists in improving cognition, attention was refocused on other nAChR subtypes and more specifically on α7 nAChRs. Additional correlation between these receptors and AD was provided by the observation of a reduced expression of α7 nAChR labeling in post mortem AD cortex (Burghaus et al., 2000).

To further examine the putative relationship between α7 nAChRs and AD, it is important to recall key findings obtained in different models. Following the hypothesis that the increase of Aβ1-42 might be one of the determinant factors causing AD, experiments were conducted to determine whether this peptide interacted with α7 nAChRs and the consequences of this interaction. As a first step, studies examined if Aβ1-42 peptide could bind to nAChRs and more specifically to α7 nAChRs. Some studies report a direct interaction between Aβ1-42 and α7 nAChRs from mouse and human, suggesting a binding interaction (Wang et al., 2000, 2009). An absence of a correlation between α7 nAChR binding and the presence of Aβ plaques was also reported in other studies (Ikonomovic et al., 2009). Moreover, it was suggested that Aβ might disrupt the membrane structure and indirectly affect α7 nAChR expression and packing of lipids (Small et al., 2007). Appropriate caution must however be maintained, because false positives might be generated when evaluating protein expression using commercially available antibodies (Herber et al., 2004; Jones and Wonnacott, 2005). Experiments conducted with competitive antagonists such as α-Btx or MLA have found opposing results: an increase in expression of α7 nAChRs thought to compensate for the loss of ACh in AD (Counts et al., 2007; Liu et al., 2013) and reduced expression of α7 nAChRs in other studies (Burghaus et al., 2000). A lower level of α7 nAChR expression on neurons, at the same time as expression was higher on astrocytes, was also reported in sporadic and familial AD (Yu et al., 2005). Although determination of the level of expression and possible affinity between the Aβ1-42 and α7 nAChRs constitutes the first step toward the understanding of a possible correlation between Aβ1-42 and cholinergic functions, these studies rely on cross-sectional analyses and are therefore lacking the indispensable longitudinal
aspect that would be required to analyze the correlation between these parameters with the evolution of the disease in individual patients. Functional experiments aimed at examining the effects of Aβ$_{1-42}$ on the activities of the α7 nAChRs, together with pharmacological intervention with α7 nAChR agonists, provide an alternative to evaluate the role of the cholinergic system in AD. Initially, patch-clamp experiments conducted on rat hippocampal neurons in culture showed that exposure to rat Aβ$_{1-42}$ inhibited in a dose-dependent manner the current evoked by ACh and that this inhibition was noncompetitive (Liu et al., 2001). One difficulty in functional studies concerns the sequence and oligomerization of the Aβ used. Namely, species, sequence and peptide length (e.g., Aβ$_{1-40}$ versus Aβ$_{1-42}$, etc.) differences have been reported and, more importantly, Aβ oligomerization is difficult to control and probably represents a crucial variable that cannot be analyzed when comparing effects reported from different laboratories. This is illustrated by considering opposite results obtained in two laboratories reporting 1) activation of α7 nAChRs by exposure to 10 pM Aβ$_{1-42}$ (Dineley et al., 2002; Tong et al., 2011) and 2) absence of effect reported for apparently comparable experimental conditions (Small et al., 2007). Use of chimeric approaches and intracellular calcium measurements suggest that Aβ$_{1-42}$ interacts with the tyrosine residue Tyr-188 of α7 nAChRs (Tong et al., 2011). Evidence for the effects of Aβ on α7 nAChRs in animal models was obtained using different approaches. For example, it was shown that intraventricular injection of Aβ$_{1-42}$ inhibited the pressor response of the heart rate caused by choline infusion (Li and Buccafusco, 2004). Further agreement for an interaction between α7 nAChRs and Aβ was obtained in studies relying on selective α7 nAChR agonists (Wang et al., 2010b; Chen et al., 2010; Inestrosa et al., 2013). A further complexity might reside in the structural differences between homomeric α7 and heteromeric α7β2 nAChRs, as shown by intracellular recording in brain slices and from nAChRs expressed in Xenopus oocytes (Liu et al., 2009, 2012). The apparent contradictory observation of an exacerbation of AD effects in α7 nAChR knockout mice (Hernandez et al., 2010) versus improvement observed in another model (Dziewczapolski et al., 2009) can be reconciled when considering the stage and conditions. More specifically, if it is thought that stimulation of α7 nAChRs is neuroprotective, then it is understandable that inhibition of these receptors will exacerbate Aβ neurotoxicity, but inhibition of α7 nAChRs may also cause impairment of cognitive function. A balance between neuroprotection and reduction in cholinergic neurotransmission must therefore be taken into account when considering the effects and requires long-term studies.

Experiments conducted with Aβ$_{40}$ that contains the mutation E22G corresponding to the so-called “Artic Aβ” variant revealed that this polypeptide binds to α7 nAChRs and inhibits their function, as measured by Ca$^{2+}$ influx in CHO-K1 cells expressing α7 nAChRs (Ju et al., 2014). Unlike other Aβ mutations, such as the E22Q, which gives rise to a highly distinct phenotype with amyloid angiopathy leading to recurrent hemorrhage, the E22G mutation causes only cognitive deficits (Nilsberth et al., 2001). Although differences observed between distinct Aβ protein products highlight the complexity of interactions of these amyloid peptides, results obtained with α7 nAChRs suggest a plausible specific interaction that is therapeutically worthwhile pursuing.

Additionally, α7 nAChR stimulation could potentially play a role in rescuing presynaptic deficits in AD as a result of decreasing levels of β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) (Vassar et al., 2009; Kandalepas and Vassar, 2014; Yan and Vassar, 2014). In 2010, an intriguing study examined the connection between activation of α7 nAChRs in BACE1 knockout mice, showing restoration of paired-pulsed facilitation from mossy fiber-to-CA3 synapses, which is reflective of deficits in presynaptic release (Wang et al., 2010a). Second, activation of α7 nAChRs also restored LTP deficits at these terminals in BACE1 knockout mice. Both of these restorations by α7 nAChR agonists, in this case by nicotine and PNU-282987, were selective because pretreatment with 100 nM α-Btx blocked the agonist actions (Wang et al., 2010a).

One mechanistic insight afforded by this study was that this α7 nAChR-induced restoration in BACE1 knockout mice was due to one of the hallmark characteristics of α7 nAChR stimulation, which is elevation of protective levels of intracellular Ca$^{2+}$ and the concomitant increase in glutamate release from synaptic endings that affected downstream intracellular signaling cascades such as the ERK pathway (Dickinson et al., 2008). These studies clearly suggest that the combination of an α7 nAChR agonist with a BACE1 inhibitor may be an attractive approach to tackling the complexity in treating AD.

In view of the unique procognitive properties of α7 nAChR agonists and their neuroprotective activity in different models, it is reasonable to speculate that stimulation of α7 nAChRs could be disease modifying rather than solely compensate for cognitive decline. Thought of as the “missing link” between the histopathological hallmarks of amyloid or phosphorylated tau and the clinical manifestation of AD, α7 nAChRs constitute an important area of research (reviewed in Bencherif and Lippiello, 2010; Parri et al., 2011).

The safety and efficacy of the α7 selective agonist ABT-126 observed in a Phase 2 clinical trial conducted on subjects with mild-to-moderate AD dementia was recently published (Gault et al., 2015). Although this study failed to demonstrate statistically significant improvements with doses up to 25 mg; exposure-response analysis suggested that additional data with
higher concentrations should be performed either as mono or add-on therapy.

There are also other forms of dementia that may benefit therapeutically from an \( \alpha7 \) nAChR agonist. MCI is clinically defined as a patient having problems with memory with or without cognitive deficits that interfere with daily function (Petersen et al., 2001). Unfortunately, 10–15% of these patients annually will convert to dementia, particularly to AD (Petersen, 2009). A genetic study suggested that the CHRNA7 might act as a modifier gene in patients with MCI in protection from conversion to AD (Barabash et al., 2009). A recent study demonstrated that in a 6-month study, transdermal nicotine improved measures of attention, memory, and mental processing in nonsmoking MCI patients, although not in the global impression clinical rating scale (Newhouse et al., 2012).

Dementia with Lewy bodies (DLB) is the second most prevalent form of dementia, and in DLB the psychiatric symptoms, mainly visual hallucinations, emerge earlier than in AD. Thus these patients use more resources to cope with the disease and as a consequence early intervention may prove beneficial in quality of life for these patients and their caregivers (Mollenhauer et al., 2010). Interestingly, visual hallucinations and delusional misidentification associated with DLB were correlated with lower \( \alpha\)-Btx binding in the temporal cortex, indicative of \( \alpha7 \) nAChR localization, and not with \([^{3}H]\)-epibatidine binding, indicative of \( \alpha4\beta2 \) nAChR localization (Rei et al., 2000; Court et al., 2001). As nAChRs are also strongly expressed in the visual system, including the retina, the lateral geniculate nucleus and visual cortex, it would be of value to know if the density of receptors is also modified in other brain areas. As listed in Table 5, the CHRFAM7A gene without the 2-bp deletion has been shown to be significantly correlated with AD (\( P = 0.011 \)) and DLB (\( P = 0.001 \)), and Pick’s disease (\( P < 0.0001 \)) compared with healthy controls (Swaminathan et al., 2011, 2012a). Patients with DLB or PD with dementia share the same profile of dopaminergic and cholinergic deficits with widespread reductions in choline acetyltransferase-positive neurons in DLB and PD with dementia compared with PD alone (Rei et al., 2000; Fujishiro et al., 2006). If lower functional \( \alpha7 \) nAChRs are present in these other forms of dementia, then the therapeutic use of a potent \( \alpha7 \) nAChR agonist could be potentially beneficial as well.

Numerous observations point to a critical role of the cholinergic system in AD, as well as in other dementing illnesses. This is best illustrated by the fact that today acetylcholinesterase inhibitors represent one of the few tools available for the treatment of dementia. Development of \( \alpha7 \) nAChR agonists and the observation that these molecules not only can be precognitive but also neuroprotective has strengthened the need to pursue research into their utility in the treatment of AD. Because no disease-modifying treatment of AD has yet been developed, the hope is that \( \alpha7 \) nAChR agonists may fill this role, if only partially as a treatment to substantially slow disease progression, if not halt it entirely. Current Phase 3 clinical trials conducted with the \( \alpha7 \) nAChR agonist/5-HT\( _{3} \) antagonist enencine are hoped to mark a turning point in AD treatment. While time will tell if enencine can be successfully marketed, it is likely that \( \alpha7 \) nAChRs represent a promising target for the treatment of cognitive impairment in AD and may also alter the course of the disease.

### B. Schizophrenia

Affecting about 1% of the population or more than 50 million people worldwide, schizophrenia is a life-long severely disabling mental disorder characterized by deficits in thought processes, perception, and emotional responsiveness. Schizophrenia onset typically occurs in early adulthood and symptoms such as cognitive impairment and reduced attention might be indicative of altered brain function prior to the first psychotic episode and diagnosis. The role of \( \alpha7 \) nAChRs in this disease was recently thoroughly reviewed and supported by a biology that correlates with the pathophysiology of the disease and the involvement of cholinergic nuclei and, more specifically, \( \alpha7 \) nAChRs (Wallace and Bertrand, 2013a; Freedman, 2014). From its revised and expanded characterization by the Swiss psychiatrist Paul Eugen Bleuler (1857–1939), who coined the term schizophrenia, it was recognized that basic symptoms included “negative symptoms” with disorganized speech, thinking, affective incongruence, and withdrawal from reality and “positive symptoms” with tactile, auditory, visual, and gustatory hallucinations and delusions (Pearlson and Ford, 2014). Additionally, cognitive impairment in multiple domains is a core feature of schizophrenia and accounts for much of the continued disability and poor functional outcomes in patients after management of the positive symptoms (Green et al., 2004; Nuechterlein et al., 2011). Although impressive progress in treating positive symptoms has been made with the discovery of typical and atypical antipsychotics, it is now recognized that these molecules are ineffective at treating negative and cognitive symptoms (Kay and Singh, 1989; Chue and Lalonde, 2014; Keefe, 2014). Schizophrenia, as with many other brain disorders, is certainly multifactorial and includes environmental etiologies; however, as shown by recent twin studies, there are genetic factors predisposing for the development of schizophrenia (Cardno and Owen, 2014). The need for better molecules that would be efficacious for negative and cognitive symptoms is therefore tremendous and should be readily evaluated.

Without entering into a detailed review of all the elements linking \( \alpha7 \) nAChRs and schizophrenia, it is worthwhile recalling that a large percentage of patients are smokers and that their cigarette consumption is about twice that of smokers in the general population.
This prompted the idea that nicotine intake from smoking might constitute an attempt to self-medicate and that nicotine could reduce some of the symptoms. Post mortem examination of brain tissue revealed a reduction of α-Btx binding sites up to 50% in schizophrenic patients (Freedman et al., 1995; Marutle et al., 2001). Subsequent analysis of smoking versus non-smoking schizophrenics confirmed the reduced α7 nAChR expression at the cell surface but concluded that smoking might cause some compensation for this deficit (Leonard et al., 2000). Genetic studies conducted by different laboratories indicated that mutations in the CHRNA7 gene, its promoter region, or CHRFAM7A are associated with schizophrenia (Riley et al., 2000; Raux et al., 2002; Martin et al., 2007; Sinkus et al., 2009; Stephens et al., 2009; Bakanidze et al., 2013). A survey of the literature on schizophrenia and genetic association revealed that several studies failed to detect any association with CHRNA7 or CHRFAM7A, which is indicative of the rarity of these mutations in the schizophrenic population, the complexity of schizophrenia, and the need for a better classification of the disease (Petrovsky et al., 2009). Because schizophrenia is probably multifactorial, influenced by both environmental factors and genetics, with many other genes identified as linked to the disease, it would not be surprising that absence of an association would be observed in certain patient cohorts.

Because the first manifestations of the disease often occur in late adolescence, it would be of interest to identify biomarkers that could allow detection and possible treatment of the disease before the appearance of overt clinical symptoms. Interestingly, studies conducted in children have shown that delay in P50 inhibition is associated with attention problems during maturation (Ross et al., 2010). In view of the fact that amniotic choline activates fetal α7 nAChRs and facilitates the development of central inhibition, it was hypothesized that choline supplementation during pregnancy might improve child development (Ross et al., 2010). Randomized, placebo-controlled clinical trials conducted on more than 100 volunteers revealed that 76% of the children treated with phosphatidylcholine during development showed suppression of the P50 response at the 5th postnatal week, a marker of central inhibition, versus 43% in placebo-treated infants. Although the difference progressively diminished between treated and placebo groups by the 13th week, perinatal choline treatment might help in the development of central inhibition (Ross et al., 2013). In addition, studies examining children with autism spectrum disorder (ASD) have shown a reduction in the latency to evoke a P50 response, as well as a reduction in prepulse inhibition to a startle response, similar to that observed in schizophrenia, and suggests a role for α7 nAChR agonist therapy for this condition (Deutsch et al., 2010).

The importance of the cholinergic system was largely documented in animal models that supported the determinant role of α7 nAChRs by studying the effects of molecules specific for this receptor subtype. Exposure to α7 nAChR agonists or PAMs was found to restore cognitive impairments caused by pharmacological treatments. Consistent results obtained in different animal species ranging from rodent to nonhuman primates further strengthened the role of α7 nAChRs in cholinergic system functioning. Preclinical studies have demonstrated the efficacy of several α7 nAChR agonists in an N40 sensory gating model (Hashimoto et al., 2005; Simosky et al., 2008; Wildeboer-Andrud and Stevens, 2011), in particular ABT-107 (Radek et al., 2012). Other molecules such as TC-5619, SSR-180711, A-582941, or encenicline also demonstrated efficacy in cognitive models (Pichat et al., 2007; Tietje et al., 2008; Mazurov et al., 2012; Prickaerts et al., 2012). Although application of an α7 nAChR agonist alone was ineffective in a DBA/2 mouse model of sensorimotor gating (prepulse inhibition), it increased the effects of haloperidol or risperidone (Kohlhaas et al., 2012). TC-5619 showed a statistically significant effect on executive function based on measurements for the Groton Maze Learning Task but not on other cognitive domains (Lieberman et al., 2013). However, secondary measurements for negative symptoms demonstrated efficacy in TC-5619-treated patients with schizophrenia, and these effects were more pronounced in smokers than non-smokers (Lieberman et al., 2013). However, a second Phase 2 trial with TC-5619 failed to demonstrate any benefit in alleviating either negative or cognitive symptoms (Walling et al., 2015). The logical next steps for proof-of-concept were obtained in early clinical studies using α7 nAChR agonists (Olincy et al., 2006; Tregellas et al., 2011; Barbier et al., 2015; Preskorn et al., 2014). Although a more detailed discussion of these clinical trials is presented in section III, it is important to note that studies of the 5-HT_3 receptor antagonist tropisemtrion, which also acts as an agonist at α7 nAChRs, caused a normalization of the P50 response in schizophrenic patients (Shiina et al., 2010; Zhang et al., 2012).

The complex modifications observed in behavior and multiple higher cognitive functions are, however, unlikely to be caused by the modification of the α7 nAChR pathway alone but might implicate some interdependence with other neurotransmitter systems. Contribution of the glutamatergic system and more specifically the hypofunction of the NMDA receptor is one of the hypotheses that is preponderantly discussed in the field of schizophrenia (Veerman et al., 2014). This was supported by the observation that exposure of healthy subjects to NMDA antagonists such as ketamine causes schizophrenic-like symptoms (Adler et al., 1999). A possible cross-interaction between NMDA receptor and α7 nAChR neurotransmission would therefore represent a plausible mechanism that would explain results.
observed with these two classes of receptors. Speaking in favor of a cross-interaction, the following facts must be considered. First, important evidence comes from the pharmacological interactions observed in rodent and monkey in which impairment caused by ketamine or MK-801 exposure could be improved by the α7 nAChR agonist GTS-21 (Cannon et al., 2013; Callahan et al., 2014). Similarly, in a mouse model, tropisetron improved the cognitive deficits caused by phencyclidine (Hashimoto et al., 2006). The α7 nAChR agonists encenicline and TC-5619 have also been shown to reverse phencyclidine-induced deficits that mimic negative-like symptoms of schizophrenia in mice (Pedersen et al., 2014). Deficits induced by the open channel blocker MK-801 can be reversed by α7 nAChR agonists as well as by α7 nAChR PAMs (Jones et al., 2014). The use of a specific α7 nAChR agonist is probably necessary, as nicotine, a non-selective ligand, could not restore the ketamine-induced deficits in humans (D’Souza et al., 2012). Interestingly, however, nicotine was able to alleviate ketamine-induced sensory and memory impairment and improve attention in subjects displaying a predisposition for auditory hallucinations/delusions (Knott et al., 2012). The reports in subjects displaying a predisposition for auditory sensory and memory impairment and improve attention however, to be replicated and extended to understand the contribution of α7 nAChRs or α7 dup in cognitive impairments and the population of patients that might benefit from treatment with α7 nAChR agonists.

C. Autism

Autism spectrum disorders (ASDs), which include autistic disorder, Asperger’s disorder, and pervasive developmental disorder, are defined by socialization and communication deficits and a need for preservation of “sameness.” Initially considered in 1943 as the manifestation of a single affliction, autism and schizophrenia became classified as two separate diseases, although they shared several clinical features, such as social withdrawal, communication impairment, and poor eye contact (Barneveld et al., 2011). With a prevalence of up to 2/1000 individuals, autism is about four times more frequent in men than women and shows up to 90% genetic inheritability (Casey et al., 2012). Despite evidence for genetic transmission, genome-wide analysis performed on large cohorts has failed to identify associations with specific genes or mutations, suggesting that variants exert only a weak effect on ASD (Anney et al., 2012). An alternative genetic analysis conducted on more than a thousand patients was aimed at elucidating why a disease that is highly heritable cannot be associated with a given gene or set of genes (Casey et al., 2012). In rare cases, autism is associated with agents that cause birth defects, but the impressive increase in autism of about 30% between 2012 and 2014 suggests that environmental causes might be at the origin of ASD (Neggers, 2014). Similar observations were made in a meta-analysis of results from an Asian population (Feng et al., 2013). Questions about recent increases in ASD have been raised because this might reflect a difference
in diagnostic practice and government-subsidized financial incentives for named diagnoses more than a real increase in the frequency of ASD.

With regard to the cholinergic system, neuropathological abnormalities in the basal forebrain cholinergic nuclei originally indicated that dysfunction of cholinergic neurotransmission may be involved in the etiology of autism (Ray et al., 2005). Neurochemical studies have shown a loss of α7 nAChRs in thalamus (specifically the paraventricular nucleus and nucleus reuniens) in autistic versus control brains, suggesting a role in the relay of information from the periphery to cerebral cortex and in modulation of cortical outputs (Lee et al., 2002; Martin-Ruiz et al., 2004). Data from this small sample suggest that cholinergic dysfunction in the thalamus of persons with autism may contribute to disrupted regulation of sensory input to the cerebral cortex and disturbance of the processing of emotions and cognition. A 15q11.2-13.3 chromatin analysis revealed alterations in epigenetic regulation resulting in reduced transcription of CHRNA7 in human frontal cortex in ASD. CHRNA7 transcripts were high in normal subjects less than 1 year of age and declined until about 20 years of age. Interestingly, the postnatal decline in CHRNA7 levels around 1 to 2 years of age corresponds to the onset of autistic symptoms (Yasui et al., 2011). This study also revealed an association between ASD and genomic instability at chromosome 15q13.3 due to recurrent microdeletions, also resulting in a reduced level of CHRNA7 transcripts in human frontal cortex in ASD. CHRNA7 transcripts were high in normal subjects less than 1 year of age and declined until about 20 years of age. Interestingly, the postnatal decline in CHRNA7 levels around 1 to 2 years of age corresponds to the onset of autistic symptoms (Yasui et al., 2011). Modifications of the cholinergic system are expected to affect multiple neurotransmitter systems in brain regions affected in ASD, therapeutically targeting this receptor may be beneficial.

Another challenging hypothesis has been suggested that autism, schizophrenia, and attention deficit disorder are part of a single neurologic disorder corresponding to a spectrum of imbalances in neurotransmission (Lippiello, 2006). Because α7 nAChRs modulate a number of neurotransmitter systems in brain regions affected in ASD, therapeutically targeting this receptor may be beneficial.

Although the linkage between autism and a genetic cause remained elusive for a long time, the newest findings reported for the CHRNA7 locus on chromosome 15 provide a stimulating hypothesis. Complementing other observations of cholinergic and α7 nAChR deficits in autism, these genetic studies are pointing to a determinant role of α7 nAChRs in cognitive impairment associated with autism. Further confirmation for the correlation between α7 nAChRs and autism will be provided by progress in genome-wide association studies and will determine whether future development of new treatments for ASD will include α7 nAChR agonists.

### D. Microdeletion Syndrome

The orphan disease of 15q13.3 microdeletion syndrome (see Table 6) was first described in 2008 (Sharp et al., 2008), and since then, using whole genomic microarray analysis (International Schizophrenia Consortium, 2008; Stefansson et al., 2008; Masurel-Paulet et al., 2010), over 150 individuals worldwide with a frequency of 1/30,000–1/40,000 have been identified that carry these deletions. Several studies have been conducted that demonstrate an association between these microdeletions and neurodevelopmental phenotypes such as bipolar depression, epilepsy, schizophrenia (Stefansson et al., 2008), and autism, as well as developmental delays, mental retardation, and variable facial and digital dysmorphisms (Sharp et al., 2008; Lowther et al., 2015). As described in detail in **section II**, the area of this microdeletion in 15q13.3 contains the previously mentioned break points of BP4 and BP5 that flank the CHRNA7 gene (OMIM #118511) (Deutsch et al., 2011). It was also recently suggested that a haploinsufficiency of CHRNA7 is causative for the

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<th>Disease</th>
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<td>Epilepsy</td>
<td>Taske et al., 2002; Sharp et al., 2008; Stefansson et al., 2008; Helbig et al., 2009; Dibbens et al., 2009</td>
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<td>Schizophrenia</td>
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<td>Recurrent rage outbursts</td>
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<td>Microdeletion Syndrome</td>
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various phenotypes connected to the 15q13.3 microdeletion syndrome (Szafranski et al., 2010). In a review of the literature on genetic data derived from screening samples with genomic arrays, it was concluded that the clinical presentations of 15q13.3 microdeletion syndrome were due to haploinsufficiency of the CHRNA7 gene and that there is a logical rationale to test α7 nAChR agonists and PAMs for potential therapeutic efficacy (Deutsch et al., 2011; Schauf, 2014). Another review of patient samples and literature suggested that CHRNA7 could potentially be a susceptibility factor for both juvenile myoclonic epilepsy and childhood centro-temporal spikes and epilepsy (Masurel-Paulet et al., 2010). It was suggested that genetic screening and identification of the 15q13.3 microdeletions could result in early intervention with special education and speech therapy, instead of waiting for symptoms to fully manifest (Deutsch et al., 2011). For example, a patient suffering from 15q13.3 deletion syndrome and also presenting with recurrent episodes of aggressive rage outbursts showed a dramatic decline in such events upon treatment with galantamine, an α7 PAM and acetylcholinesterase inhibitor, combined with behavioral management (Cubells et al., 2011) to suggest the potential of this approach.

It was further suggested that clinical intervention could also theoretically be possible with first, the identification of patients expressing this microdeletion, followed by the administration of an α7 nAChR agonist alone or in combination therapy with an α7 nAChR PAM in cases such as these in which there are decreased functional α7 nAChRs (Deutsch et al., 2011; Schauf, 2014). A preclinical proof-of-concept study for testing the efficacy of α7 nAChR agonists and PAMs in 15q13.3 microdeletion syndrome became feasible with the recent development of a mouse model that captures the main characteristics of the human syndrome, such as schizophrenia-like auditory processing deficits, increased seizure susceptibility, and increased body weight (Feigin et al., 2014). This model may provide a way to test compounds preclinically for their ability to ameliorate symptoms in microdeletion syndrome and establish whether clinical trials with α7 nAChR agonists or PAMs are warranted for microdeletion syndrome.

The recently discovered 15q13.3 microdeletion syndrome is marking a new step in the understanding of the role of α7 nAChRs and cholinergic function in neurodevelopmental disorders. The combination of the development of better analysis of allelic expression with appropriate cognitive assessment will provide the indispensable tools to identify the syndrome early and in time for effective therapeutic intervention. The development of an animal model will aid in the determination of whether patients with 15q13.3 microdeletion syndrome may be candidates for α7 nAChR agonist or PAM treatment.

E. Parkinson’s Disease

Parkinson’s disease (PD) is a neurodegenerative disease that is characterized by hypokinetie rigid syndrome or paralysis agitans because of the tremor observed in the extremities (Lees, 2007). Historically recognized, this disease began to gain the attention of clinicians only at the turn of the 19th century with the description by James Parkinson in his essay on the Shaking Palsy published in 1817 to provide one of the earliest descriptions of the clinical symptoms of a disorder now known as Parkinson’s disease. Since then, it was shown that PD is the consequence of degeneration in the ventrolateral tier of the substantia nigra pars compacta (Brigo et al., 2014; Grosset et al., 2014; Niccolini et al., 2014; Stoeossi et al., 2014). The observation that exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes clinical symptoms that resemble PD suggested that lesions caused by this toxic agent could be used for animal models of PD (Javitch et al., 1984). However, this molecule does not show the same toxicity in human and rodents, and the model of choice retained for rats and mice is the local injection of 6-hydroxy-dopamine into the brain regions containing dopaminergic cell bodies, axons, or synaptic terminals (Perese et al., 1989).

Degeneration of dopaminergic neurons causes multiple symptoms including hyposomnia, visual hallucinations, olfactory dysfunction, anxiety, cognitive disorders, and motor control deficits (Lees, 2007). As for most neurodegenerative diseases, prevalence increases with age and most recent epidemiologic studies indicate a prevalence of PD of about 0.5% at the age of 65 years (Wright Willis et al., 2010; Schneider and Obeso, 2015). Genome-wide association studies of large PD cohorts revealed that several genes might contribute to PD, with an important role for α-synuclein, and that mutations in the Parkin gene are associated with an early age of onset of PD (Ramanan and Saykin, 2013).

In the extrapyramidal system there is a balance between the dopaminergic projection from the substantia nigra pars compacta onto the striatum and the corticostriatal inputs that impacts the striatum’s projection to the thalamus and the feedback loop from the thalamus back to the cortex. Dysfunction of the dopaminergic projection from the substantia nigra directly impacts upon the function of the striatum and therefore on the cortical interactions with different brain nuclei. The substantia nigra is an important player in particular for control of eye movement, motor planning, reward seeking, or learning. Moreover, as the thalamus can be considered as a relay between the sensory systems and the cortex, indirect impairment of the thalamus caused by the dysfunction of the substantia nigra alters sensory perceptions (Müller et al., 2013; Barter et al., 2014).
The observation that PD is associated with a specific degeneration of dopaminergic neurons suggested that adding exogenous dopamine might counterbalance the dysfunction of the substantia nigra. Successful introduction in the 1960s of levodopa, a brain permeable precursor of dopamine, marked a turning point in the treatment of PD (Katzenschlager and Lees, 2002). Long-term treatment of PD with levodopa, however, includes significant side effects with motor dyskinesias, visual hallucinations, etc. Statistical analysis of PD cohorts showed, however, no toxicity associated with levodopa treatments or the acceleration of symptoms (Simuni and Stern, 1999). Alternative treatments include deep brain electrical or magnetic stimulation, which are thought to stimulate the remaining neurons in the substantia nigra (Sanghera et al., 2004; Chang et al., 2011). Although pharmacological treatments and stimulation represent a significant advance for patients, these therapies fail to change the course or the progression of the disease.

Based on the observation of a lower prevalence of PD in smokers, it was proposed that nicotine may exert a neuroprotective and possibly beneficial effect (Neafzy et al., 1968; Gorell et al., 1999; Quik, 2004). Experimental evidence in animal models suggesting that nicotine increased production of neurotrophic factors further supported this hypothesis (Maggio et al., 1998). Albeit other factors, including caffeine consumption, were proposed to be associated with a reduction of PD prevalence, the protective effects of nicotine were replicated in many studies. Furthermore, a reduction of the olfactory impairment in PD was observed in PD-affected smokers (Lucassen et al., 2014).

Although the mechanisms by which nicotine could reduce the susceptibility to PD may involve different nAChRs subtypes, we shall focus the discussion on the possible role of $\alpha_7$ nAChRs. Examination of $\alpha$-Btx binding in the monkey substantia nigra revealed moderate labeling, indicative of $\alpha_7$ nAChR expression (Han et al., 2003; Kulak and Schneider, 2004). In monkeys, low doses of MPTP that did not cause motor symptoms resulted in an increase in $\alpha$-Btx binding, whereas a change in $\alpha$-Btx binding was not observed in animals treated with an acute high dose of MPTP or long-term escalating doses of MPTP, in which PD-like motor symptoms were present (Kulak and Schneider, 2004). This was interpreted as reflecting a compensatory elevation of $\alpha_7$ nAChR expression in response to the low doses of MPTP and could participate in minimizing the dysfunction caused by this molecule. Interestingly in the same study, the low doses of MPTP caused cognitive impairments, and brain regions involved in cognition (e.g., hippocampus, prefrontal cortex and cingulate gyrus) did not show increases in $\alpha$-Btx binding.

In contrast to the monkey, $\alpha$-Btx binding in human revealed a high level of labeling in the substantia nigra pars compacta, indicating that progressive atrophy of this brain area could affect $\alpha_7$ nAChR neurotransmission (Court et al., 2000). Analysis of the cortical distribution of nAChRs, conducted with Western blots, revealed a reduced expression of both the $\alpha_4$$\$&$\alpha_2$ and $\alpha_7$ nAChRs in PD patients (Burghaus et al., 2003), suggesting that a reduction of nAChR-mediated neurotransmission might aggravate the cognitive deficits associated with PD. In addition, impairment of cholinergic neurotransmission in the premotor and motor areas could contribute to the bradykinesia (slowness of movement) or other movement-associated functions. Simulation of $\alpha_7$ nAChRs might therefore produce neuroprotective effects and represent an important strategy to minimize the cognitive decline or impaired motor functions associated with PD.

Further analysis of the distribution of $\alpha_7$ nAChRs in human brain revealed an additional important expression of these receptors in cerebellum, both post mortem and in vivo (Court et al., 2000; Toyohara et al., 2009). In view of the determinant role of the cerebellum in motor coordination as well as in cognitive processes, it will be of value to review if, and how, $\alpha_7$ nAChRs are altered in the cerebellum of PD patients.

As indicated above, long-term treatment with levodopa causes multiple side effects including dyskinesia. Stimulation of nicotinic receptors reduced PD-associated dyskinesia by interaction with multiple receptor subtypes including $\alpha_7$ nAChRs (Quik et al., 2013; Zhang et al., 2013, 2014). In agreement with these observations, although nicotine reduced apomorphine-induced rotational behavior in hemiparkinsonian rats injected unilaterally with 6-hydroxydopamine, rotational behavior worsened during exposure to the nicotinic blocker mecamylamine (Han and Wang, 2007). Different doses of nicotine, capable of producing activation or desensitization, indicated that the rotational behavior was only affected by desensitizing doses of nicotine, not by nAChR activation. The specific $\alpha_7$ nAChR agonist ABT-107 at 0.03–1 mg/kg decreased levodopa-induced dyskinesias in parkinsonian monkeys (Zhang et al., 2014). Similar effects were reported for another selective $\alpha_7$ nAChR agonist, AQW051 (15 mg/kg) (Di Paolo et al., 2014), indicating that this therapeutic strategy can apply to higher mammals and may be beneficial in humans. It remains, however, to be determined whether activating or desensitizing concentrations of a selective $\alpha_7$ nAChR agonist will be required in humans, because ABT-107 was efficacious at activating and desensitizing doses and AQW051 at only desensitizing doses in the MPTP monkey model (Bitner et al., 2010; Feuerbach et al., 2015). If desensitizing concentrations are required, the effects on dyskinesia of $\alpha_7$ nAChR agonists may be achieved at the expense of improvements in cognition.

PD is characterized first by movement disorders that are thought to be caused initially by the neurodegeneration of the dopaminergic neurons in the substantia nigra. PD is also accompanied by cholinergic deficits and cognitive impairment in some patients.
The negative association between PD and smoking provided the first clues for the possible contribution of nAChRs in this disease. Most recent evidence is now pointing to the role of α7 nAChRs in treating levodopa-induced dyskinesias and cognitive impairment.

**F. Nonneuronal Related Diseases**

The discoveries that the neuromuscular junction is a chemical synapse and that these synaptic events are mediated by nAChRs suggested that such neurotransmitter receptors were restricted to the nervous system. However, expression of mRNAs in different tissues surprisingly revealed that genes encoding for ligand-gated ion channels are not exclusively expressed in neurons but can also be expressed in many other cell types. As an example, NMDA receptors are also expressed in red blood cells (Makhro et al., 2013). However, when considering biologic questions from a broader perspective, it became obvious that cells from different tissues might exploit the vast repertoire of membrane proteins encoded in the genome. In this respect, it is interesting to note that nAChRs are widely expressed throughout the body and that α7 nAChRs are thought to play a role in many physiologic functions outside the CNS. In this section we shall discuss evidence for CHRNA7 expression in different tissues, the possible role(s) of α7 nAChRs in nonneuronal cells, and their validity as a therapeutic target for nonneuronal related diseases.

1. **Cancer.** Cancer is characterized by abnormal cell division and proliferation and, sometimes, by migration of a small number of cells to form metastases (http://www.cancer.gov). The main categories of cancer include:

   **Carcinoma** - cancer that originates in the skin or in tissues that line or cover internal organs, (i.e., cells of epithelial origin). Numerous subtypes of carcinoma exist to include adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, and transitional cell carcinoma.

   **Sarcoma** - cancer that originates in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissues (i.e., cells of mesenchymal origin).

   **Leukemia** - cancer that originates in blood-forming tissues such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

   **Lymphoma and myeloma** - cancers that originate in the cells of the immune system.

   **Central nervous system cancers** – cancers that originate in the tissues of the brain and spinal cord and are neuronal or glial in origin.

   Whereas it would be beyond the scope of this work to review all forms of cancer, their origins, outcomes, and the possible role of nAChRs, it is important to delineate the main features that characterize cancer and the development of tumors. It is generally accepted that cancer begins by a modification of DNA that triggers abnormal cell division. Furthermore, when, in normal conditions, cells begin aberrant division they undergo apoptosis and cancer cells are not stopped and can initiate further divisions. Importantly, as the tumor grows, its general metabolism increases, requiring more oxygen and nutrients, and is accompanied by a neovascularization to support its metabolic requirements. Cells that initiate migration and will ultimately cause metastases are thought to be changing fate and, temporarily, returning to a more embryonic cell type with the capacity of migration through lymphatic and blood vessels to other organs.

   Smoking-associated lung cancers with more than about 200,000 new cases per year in the United States alone and more than 150,000 associated deaths per year, are the most obvious cancers linked to nicotine. Lung cancers are subdivided into non-small cell lung cancers and small cell lung cancers, according to histologic observations (DeSantis et al., 2014). The devastating link between tobacco products and cancer results from the association of carcinogenic compounds such as nitrosamines N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyrdyl)-1-butanoic acid together with nicotine (Hecht, 2003; West et al., 2003). Although there is still debate about the carcinogenicity of nicotine itself, recent evidence points to a possible effect of nicotine on long-term mini-organ cultures (Ginzkey et al., 2014). Additional evidence for nicotine-induced mutations was provided by the recent analysis of nicotine and oxidative stress in lung cancer (Bavarva et al., 2014). There is good agreement, however, on the fact that nicotine promotes cell survival and division, which are key factors for tumor growth and can precipitate development of lung cancer in susceptible individuals (Davis et al., 2009; Schaal and Chellappan, 2014). Furthermore, the antiapoptotic activity of nicotine, which is beneficial in other conditions like neurodegenerative diseases, is considered to be deleterious in lung cancer (Zeidler et al., 2007; Egleton et al., 2008; Schuller, 2012). An additional and determining factor in tumor formation associated with nicotine is its angiogenic activity of promoting neovascularization (Cooke and Ghebremariam, 2008;Dasgupta and Chellappan, 2006; Arias et al., 2009; Lee and Cooke, 2012). A susceptibility locus for lung cancer and nAChRs was found on chromosome 15 but mapped to 15q25, which corresponds to the position of the CHRNA5 gene encoding for the α5 nAChR subunit (Hung et al., 2008). Expression of CHRNA7 was, however, observed in human lung, indicating that α7 nAChRs might contribute to the regulation of lung epithelia (Plummer et al., 2005).

   Data pointing to the determinant role of nicotine in the development of lung cancer have prompted numerous studies using culture models and shown that proliferation could be reduced by use of nAChR antagonists...
Nicotine is associated with several forms of cancer in which the common mechanisms of cell migration, tumor growth, etc., observed in lung cancer are replicated. For example, nicotine stimulated cell proliferation and increased cell survival in colon cancers, and these effects were inhibited by α-Btx (Cucina et al., 2012; Dinicola et al., 2013). In pancreatic cancers, nicotine was also shown to stimulate the production of membrane-bound mucin, a protein known to induce tumor initiation and development by modulating cellular growth, differentiation, transformation, adhesion, invasion, and immune surveillance (Kunigal et al., 2012; Momi et al., 2013).

Additional insights in the biochemical pathways implicated in nicotine-associated cancer are discussed in a recent review (Grando, 2014).

Although smoking-associated cancer is well acknowledged, the direct contribution of nicotine as a carcinogenic compound remains rather tenuous. Nonetheless, differential expression of nAChRs was reported in several forms of cancer with notably overexpression of the α7 nAChRs. Because inhibition of these receptors was shown to reduce tumor growth, neovascularization, and metastasis, an α7 nAChR antagonist might broaden the therapeutic possibilities to fight some forms of cancer.

G. α7 Nicotinic Acetylcholine Receptors and the Immune Connection

Inflammation caused by excessive cytokine production is the culprit in many diseases, leading to both human morbidity and mortality; however, at the same time inflammation is an important part of the defense mechanism that is critical for survival. The innate immune system is activated during infection and injuries by initiating the release of proinflammatory cytokines such as interleukin-6 and tumor necrosis factor alpha (TNFα) to control infection and promote tissue repair (Medzhitov, 2008). However, a balance must be maintained, because an excessive release could lead to a more severe inflammatory state and spread of inflammation (Nathan, 2002). Neural reflex circuits that detect peripheral inflammation provide regulatory feedback via specific nerve signals to suppress the innate immune response (Olofsson et al., 2012). The autonomic nervous system has been shown to regulate this cytokine production via neural pathways involving the vagus nerve, which provides parasympathetic innervation to most organ systems, with ACh being the principle neurotransmitter connecting the nervous system to the immune system. Collectively, these connections form the “cholinergic anti-inflammatory pathway” (Wang et al., 2003). A detailed review of the neural circuits involved in the reflex control over the innate immune response has been published elsewhere (Tracey, 2009).

About 10 years ago, it was found, through use of antisense and pull-down experiments, that the cholinergic control of inflammation depended on the α7 nAChR subunit (Wang et al., 2003). Experiments conducted with α7 nAChR knockout mice confirmed the essential role of these receptors in the cholinergic anti-inflammatory pathway, with these mice exhibiting higher TNFα serum levels over wild-type mice 2 hours post lipopolysaccharide (LPS) injection (Wang et al., 2003). The vagus nerve has been shown to be a vital component in this pathway, involving transduction of vagal signals via the celiac and superior mesenteric ganglia to result in release of norepinephrine in the spleen (Vida et al., 2011) and in inhibition of proinflammatory cytokines through α7 nAChR signaling on macrophages (Wang et al., 2003; Sun et al., 2013). Additional studies involving experimental models of local and systemic inflammation used electrical stimulation of the vagus nerve or cholinergic drugs acting via α7 nAChRs to modulate inflammation and showed that stimulation of α7 nAChRs results in blockade of endothelial cell activation and the subsequent recruitment of leukocytes during inflammation (Saeed et al., 2005).

Stimulation of α7 nAChRs in a murine macrophage cell line by the agonists nicotine GTS-21 and choline have all been shown to inhibit LPS-induced TNFα and high-mobility group box 1 (HMGB1) protein release to improve survival in experimental sepsis (Wang et al., 2004), which supports the role of α7 nAChR agonists as anti-inflammatory agents. It was initially postulated and later confirmed that in macrophages and other inflammatory-related cells α7 nAChRs interact with janus kinase 2 (JAK2) to stimulate and phosphorylate the anti-inflammatory signal transducer and activator of transcription 3 (STAT3), a key component of the
STAT3-NF-κB cascade, which dimerizes to decrease proinflammatory cytokines, such as TNFα, HMGB1, and interleukin (de Jonge and Ulloa, 2007; Marrero and Bencherif, 2009). It was also demonstrated that stimulation of the vagus nerve attenuates inflammation in macrophages of STAT3 knockout mice (de Jonge and Ulloa, 2007). Recent data have further expanded this mechanism to demonstrate that α7 nAChR activation significantly induced microRNA, miR-124, a potential modulator in the anti-inflammatory response (Sun et al., 2013).

Outside the CNS, α7 nAChRs are thought to have a pivotal role in peripheral immune cells via the cholinergic anti-inflammatory pathway, with the potential for therapeutic intervention in several inflammatory diseases such as endotoxemia (Rosas-Ballina et al., 2008), rheumatoid arthritis (van Maanen et al., 2009, 2010), artherosclerosis (Bencherif et al., 2011), and inflammatory bowel disease (Sandborn, 1999). The α7 nAChR subunit is widely expressed in immune cells including monocytes (Matsunaga et al., 2001), macrophages (Rosas-Ballina et al., 2008), B and T cells (Fujii et al., 2008), and dendritic cells (Aicher et al., 2003). More recently, α7 nAChR subunit expression has been found within the celiac and superior mesenteric ganglia and splenic nerve fibers, key components of the vagus nerve signaling pathway to the spleen (Downs et al., 2014).

Stimulation of the vagus nerve was found to induce multiple effects, including a reduction of inflammation. Results accumulated over the past 10 years point to a role of α7 nAChRs in the regulation of inflammatory pathways, and it was proposed that ACh released during vagus nerve stimulation mediates its effects through stimulation of this nAChR subtype. As vagus nerve stimulation effects were mimicked by α7 nAChR agonists, this opens new strategies for the treatment of inflammation with α7 nAChR agonists.

1. Sepsis. Severe sepsis is the third leading cause of death in most developed countries and accounts for >9% of overall deaths in the United States alone (Ulloa, 2005). Sepsis has been defined as systemic inflammation in response to an infection, whereas "severe sepsis" refers to organ dysfunction observed during systemic inflammation with or without an associated infection (Ulloa and Tracey, 2005). To date there are no approved drugs specific for the treatment of sepsis, a costly illness that is poorly understood (Deutschman and Tracey, 2014). It is known that severe sepsis involves a flood of cytokine production, including TNFα, interleukin-1β, interleukin-10, interferon gamma, and HMGB1 (Bencherif, 2009; Bencherif et al., 2011), in which HMGB1 is a late lethal mediator of sepsis (Kim et al., 2014b). This flood of cytokines involves the participation of intracellular and extracellular "danger signals" that activate inflammasomes to mediate the release of these cytokines and involves both P2X7 receptors and α7 nAChRs (Deutschman and Tracey, 2014). This reaction in sepsis is extreme such that an anti-TNFα antibody approach has met with limited success due to the fact that this disease involves multiple cytokines and requires a treatment that would have a broad effect.

As mentioned in the introduction of this section, a selective α7 nAChR agonist, acting through the cholinergic anti-inflammatory pathway, could be more efficient at inhibiting production of proinflammatory cytokines by preventing the phosphorylation of STAT3 and in turn preventing activation of the NF-κB pathway (Peña et al., 2010). It has been shown that administration of nicotine to mice after cecal ligation and puncture or LPS treatment significantly reduced serum levels of HMGB1 (Huston and Tracey, 2011) by suppressing Toll-like receptor expression through activation of the PI3K/Akt pathway (Kim et al., 2014b). It has been reported that HMGB1 has a deleterious effect on epithelial and endothelial barriers, causing a failure at these sites of protection and making the events of sepsis result in organ as well as mitochondrial dysfunction by depleting cells of ATP needed to support survival (Deutschman and Tracey, 2014). A recent paper demonstrated how α7 nAChR signaling inhibits the events of inflammasome activation, as well as the release of mitochondrial DNA, through the use of a selective agonist or vagus nerve stimulation, all of which pointed to a direct role of α7 nAChRs expressed on mitochondria in this cascade of events (Lu et al., 2014). Further evidence was provided in α7 nAChR knockout mice where there was a failure of inhibition of inflammasome activation with vagal nerve stimulation (Lu et al., 2014). Collective understanding of these studies and events may eventually lead to a more efficacious method of treatment of the complications of sepsis.

Defined as a systemic response to inflammation, sepsis is expected to benefit from the findings that inflammation may be downregulated through the cholinergic anti-inflammatory pathway. This pathway can be activated by stimulation of the vagus nerve and the response is mediated through α7 nAChRs. Further study of the role of α7 nAChR activation in sepsis will yield a better understanding of mechanisms to curb inflammasome activation and perturbation of mitochondrial activity.

2. Inflammatory Bowel Disease, Focus on Ulcerative Colitis. Inflammatory bowel disease (IBD), which encompasses Crohn’s disease (CD) and ulcerative colitis (UC), affects approximately 3.6 million patients in the United States and Europe combined (Loftus, 2004). Unlike CD, UC is a disease of nonsmokers, exhibiting a fivefold increased risk, whereas smoking decreased the risk of disease and had a favorable effect on disease course and severity (Harries et al., 1982). Conventional treatments include corticosteroids and aminosalicylates but only 60–80% of IBD sufferers achieve remission with these drugs, however, not without
comparisons due to unwanted side effects. UC has been associated with altered STAT3 expression and phosphorylation, and it is possible that an $\alpha 7$ nAChR agonist can reverse that alteration in the STAT3 pathway (Wang et al., 2004). Thus, therapeutic intervention with new small molecule $\alpha 7$ nAChR agonists has potential that would be greatly needed.

It has been shown that nicotine, given by transdermal or enema delivery, attenuated disease in colitis models induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) or dextran sulfate sodium (DSS), as well as in clinical trials, with improvements in histologic and global clinical scores of colitis (Pullan et al., 1994). However, several clinical trials using nicotine have led to mixed results because of the limitations of associated side effects (McGilligan et al., 2007). In a TNBS-induced colitis model, anabaseine (GTS-21), an $\alpha 7$ nAChR agonist, showed less tissue damage, less myeloperoxidase activity, less TNF$\alpha$ production in the colon, and inhibited NF-κB activation (Bai et al., 2007). Other studies have shown that acetylcholinesterase inhibitors effectively modulate the acute colonic inflammation in colitis possibly by an increase in the cholinergic tone of the colon (Miceli and Jacobson, 2003). Vagal nerve stimulation via ACh also ameliorates disease activity (van der Zanden et al., 2009). In this same study, it has been shown that vagotomized $\alpha 7$ nAChR knockout mice display more severe colitis than wild-type mice. More recently, nicotine suppressed hyperexcitability of colonic dorsal root ganglion neurons in DSS-induced colitis, which was attenuated by the $\alpha 7$ nAChR antagonist MLA (Abdrakhmanova et al., 2010). In the same study, nicotine failed to suppress hyperexcitability of dorsal root ganglion neurons in $\alpha 7$ nAChR knockout mice. In a 2011 study, nicotine attenuated oxazolone-induced colitis (a model of UC) but worsened TNBS-induced colitis (a model of CD), and these opposing effects were dependent on the differential expression of $\alpha 7$ nAChRs on CD4$^+$ T cells in the colon, with more than two-thirds of CD4$^+$ T cells expressing $\alpha 7$ nAChRs in the UC model versus less than 3% of CD4$^+$ T cells in the CD model (Galitovskiy et al., 2011). This study also associated the improvement in the UC model with an immunosuppressive effect of increasing CD25$^+$Foxp3$^+$ regulatory T cells and reducing Th17 cells versus in the CD model in which the Th17 pro-inflammatory cells were increased. More recently, it was shown that the dose and route of administration of nicotine was critical to the protective outcome in the DSS-induced colitis model, with administration of low (12.5–50 μg/ml), but not high, doses of oral nicotine in DSS-treated mice having a significant effect on decreasing disease severity, histologic scores of damage and TNF$\alpha$ levels in colon tissue (AlSharari et al., 2013). These data are consistent with a role for activation rather than desensitization of $\alpha 7$ nAChRs in the DSS-induced colitis model.

It will be interesting if a selective $\alpha 7$ nAChR agonist can attenuate the severity of colitis and reduce the unwanted side effects of other therapies, as well as clarify the mixed results that have been associated with transdermal nicotine. It will also be of significance if the human-specific CHRFAM7A gene, shown to be expressed in human intestinal epithelial cells (Dang et al., 2015), plays a mechanistic role in regulating $\alpha 7$ nAChR activity in IBD.

Inflammatory bowel disease (IBD) affects a growing number of people throughout the world. Although the etiology of IBD in most cases remains elusive, the need for additional treatment strategies that could, at least, attenuate the effects is rapidly increasing, because current therapies are not effective in all patients and have unwanted side effects. The observation that stimulation of the vagus nerve can attenuate the symptoms, together with results from animal models, suggests that activation of $\alpha 7$ nAChRs might prove beneficial for the treatment of IBD.

3. $\alpha 7$ Nicotinic Acetylcholine Receptors and Osteoarthritis. Smoking-associated side effects reveal a plethora of activity of nicotine on different organ systems. For example, smoking is thought to affect bone healing (Truntzer et al., 2015; Miller, 2014) and represents a detrimental factor when considering dental implants (Baig and Rajan, 2007; Heitz-Mayfield and Huynh-Ba, 2009). In other studies it is suggested that nicotine might reduce or prevent osteoarthritis (OA) (Gullahorn et al., 2005), a finding that is supported by analysis of total knee replacement surgery in large Chinese populations where there was a 51% reduction in the number of smokers versus nonsmokers requiring surgery (Leung et al., 2014). However, a less clear image emerges from other studies, indicating no positive reduction in OA associated with smoking (Wilder et al., 2003). Because it was shown that nicotine exerts both positive and negative modulatory effects as a function of its concentration (Rothem et al., 2009), differences in conclusions from the various studies might depend upon the amount of circulating nicotine in the patient’s blood.

Inflammation is an important factor in OA, and the role of cholinergic modulation of the inflammatory pathway provides insight into the understanding of the complex mechanisms underlying OA. Examination of the inflamed synovium from OA patients revealed expression of $\alpha 7$ nAChRs predominantly in the intimal lining in vivo, as well as in fibroblast-like synoviocytes in vitro (van Maanen et al., 2009). Nicotine treatment decreased infiltration of inflammatory cells into the synovial tissue and bone erosion in a DBA/1 mouse model of collagen-induced arthritis (van Maanen et al., 2010). As a complement to these observations, $\alpha 7$ nAChR knockout mice present an exacerbated response in the collagen-induced arthritis model (van Maanen et al., 2010). The presence of choline acetyltransferase activities...
mRNA and marked expression of α7 nAChRs was reported in the pannus of the knee joint, supporting the hypothesis that this nAChR subtype is involved in OA (Forsgren, 2012).

Because nicotine interacts with many nAChRs subtypes, it is important to examine the effects of selective α7 nAChR compounds including agonists and PAMs. The attenuation of cytokine release in stimulated whole blood cells isolated from patients with OA by nicotine or GT-21 supports the hypothesis of a predominant role of α7 nAChRs in the inflammatory response (Bruchfeld et al., 2010). A similar conclusion was reached from experiments conducted with the α7 nAChR agonist AR-R17779, which attenuated the elevation in TNFα in the blood and synovial tissue, delayed the onset of disease, and was protective against joint destruction (van Maanen et al., 2009).

Joint afflictions are a serious cause of morbidity, with effects lasting for decades. Improving OA would have broad consequences for the general population and more specifically for the elderly. Given the strong link between inflammation and OA, it is readily understood that molecules acting on inflammatory pathways will be beneficial for this disease. Preliminary data obtained with α7 nAChR agonist tool compounds suggest that stimulation of these receptors represents a novel strategy to treat OA that needs to be followed up with more potent α7 nAChR agonists displaying better drug-like properties.

H. α7 Nicotinic Acetylcholine Receptors and Cardiac Function

Atherosclerosis is an additional inflammatory condition that is a major contributor to cardiovascular disease and often leads to sudden cardiac arrest or myocardial infarction. In the progression of atherosclerosis, inflammatory cells are engaged to release a host of proinflammatory cytokines, chemokines, adhesion molecules, and growth factors (Libby, 2002). α7 nAChRs are expressed on rat atrium and localized to the endothelial layer (Mazloom et al., 2013) and in the intracardiac ganglion (Cuevas and Adams, 1994; Cuevas and Adams, 1996). As previously mentioned, the α7 nAChRs are also expressed on immune cells and are key mediators in the cholinergic anti-inflammatory pathway in which α7 nAChRs become activated via release of ACh from the vagus nerve to attenuate the inflammatory response that occurs by activation of the JAK2-STAT3 and suppression of the NF-κB pathways (Wang et al., 2004). In a recent study using infusion of angiotensin II to induce atherosclerosis in apolipoprotein E knockout mice, it was demonstrated that AR-R17779, a selective α7 nAChR agonist, attenuated atherosclerosis as well as abdominal aortic aneurysms via decreased interleukin-6 and interleukin-1β gene expression in aortic tissue (Hashimoto et al., 2014). In addition, AR-R17779 also decreased atherosclerotic plaque build-up and total cholesterol and triglyceride levels as well as lowering blood pressure in these mice. This group plans to study this compound using α7 nAChR/apolipoprotein E double knockout mice to establish a more definitive connection between α7 nAChRs and atherosclerosis. Chronic hypertension has also been shown to contribute to end-organ damage and a study tested if α7 nAChR dysfunctional signaling is also involved. In this study, α7 nAChR knockout mice were used in a two-kidney one-clip hypertension model, which caused the release of proinflammatory cytokines and more severe organ damage than in wild-type mice (Li et al., 2011). In addition, the α7 nAChR agonist PNU-282987 was used to chronically treat spontaneously hypertensive rats and shown to attenuate the inflammatory effects induced in this hypertension model (Li et al., 2011).

In contrast, the role of α7 nAChRs in heart rate variability (HRV) was examined in endotoxemic rats because HRV, a reflection of the strength of vagus nerve signaling, has been used as a noninvasive measure of sepsis (Mazloom et al., 2013). In this study, the α7 nAChR agonist PHA-543613 was unable to prevent the reduction in HRV that occurs in endotoxemic rats but was able to modulate the effect of LPS on body temperature, supporting past research that demonstrates a tonic role for α7 nAChRs in systemic inflammation (Andersson and Tracey, 2012; Mazloom et al., 2013). However, pretreatment with the α7 nAChR antagonist, MLA, was able to further reduce HRV and induce a febrile response in endotoxemic rats but had no effect on either measure in naive rats. Future analysis with selective α7 nAChR agonists or α7 nAChR knock-out mice will continue to provide insight into the role of α7 nAChRs in heart rate dynamics during sepsis.

Cardiovascular diseases are recognized to be one of the major factors causing premature death, with atherosclerosis being one of multiple contributory factors. Atherosclerosis is often closely related to inflammation and would therefore represent another possible target for α7 nAChR specific compounds.

I. The Role of α7 Nicotinic Acetylcholine Receptors in Renal Function

Data from the Centers for Disease Control and Prevention reveal that more than 20 million Americans may have kidney disease, with the risk increasing each year. Kidney disease falls into several categories but the focus here will be on the two main forms. The sudden loss of kidney function is referred to as acute kidney injury (AKI), also known as acute renal failure, which can occur due to traumatic injury, damage from shock or sepsis, damage from drugs or toxins, obstruction, or sudden reduction in blood flow. When kidney damage and decreased function last longer than 3 months, it is referred to as chronic kidney disease (CKD) and is a devastating illness that has recently increased at an alarming rate and now affects over 13% of the
population (Couser et al., 2011), caused primarily by diabetes (type 1 and 2) and high blood pressure. The involvement of α7 nAChRs in renal disease was shown in a study that found nicotine pretreatment protected mice from renal dysfunction in a dose-dependent manner and that this protection was absent in α7 nAChR knockout mice (Sadis et al., 2007). Subsequent studies showed that α7 nAChRs are strongly expressed in kidney cortex with the highest protein levels in the proximal tubules compared with the distal tubules (Rezonzew et al., 2012). Flow cytometry on human kidney cells and cell lines demonstrated the expression of α7, α4, and β2 nAChR subunits, with α7 nAChR being higher than the other two subunits in the proximal tubule epithelial cell line (HK-2) and renal glomerular endothelial cells (Chatterjee et al., 2012). Collectively, these studies place α7 nAChRs in areas affected by renal disease.

Severe sepsis and septic shock are major contributing factors leading to AKI, because the kidney is the main target of the proinflammatory assault. AKI is also a major issue for many intensive care patients, accounting for more than 50% of patients affected with AKI (Zarjou and Agarwal, 2011), and to date there is no effective treatment. Sepsis-induced AKI has a pathogenesis that involves multiple pathways and requires a therapeutic approach that would attack this complicated cascade of events. An α7 nAChR agonist approach, involving the cholinergic anti-inflammatory pathway, may be beneficial. In an LPS-induced AKI sepsis model, nicotine and GTS-21 attenuated kidney injury with a reduction in serum TNFα levels as well as in kidney levels of TNFα, chemokine CCL2, and chemokine CXCL10. Both nicotine and GTS-21 also improved renal function as measured by blood-urea-nitrogen levels and leukocyte infiltration (Chatterjee et al., 2012). Mice with LPS-induced AKI exhibited significantly enhanced renal proteasome activity compared with saline control mice, but treatment with nicotine or GTS-21 attenuated both the ATP-dependent and independent renal proteasome activity induced by LPS (Chatterjee et al., 2012). It was concluded by these studies that proteasome inhibition preserves the inhibitor of kappa B alpha-NF-κB complex and that α7 nAChR agonists regulate proteasome activity, which supports previous studies demonstrating that α7 nAChR agonism regulates protein turnover by partial inhibition of proteasome activity (Rezvani et al., 2007). In addition, many kidney diseases have been associated with altered STAT3 expression and phosphorylation (Liu et al., 2014) and it is possible that α7 nAChR agonists may reverse that alteration, because they have been shown to be neuroprotective through the JAK2/STAT3 and NF-κB pathways (de Jonge and Ulloa, 2007; Marrero and Bencherif, 2009).

Ischemia-reperfusion (I/R) injury, another model of human AKI with the characteristic signs of kidney inflammation (i.e., infiltration of circulating immune cells and renal dysfunction), was also investigated with use of α7 nAChR agonists for efficacy in preclinical models. Pretreatment with nicotine or GTS-21 attenuated acute tubular injury and renal dysfunction in the I/R model in rats but was not significantly protective if the agonists were administered 2 hours after the onset of the injury (Yeboah et al., 2008). This study also demonstrated that functional α7 nAChRs were detected in rat tubular epithelial cells, which are also involved in the inflammatory response of this AKI model, and suggested a local cholinergic effect. More recently, an innovative approach demonstrated that an ultrasound treatment 24 hours before I/R also prevented renal injury in mice (Gigliotti et al., 2013). It was further shown that cytisine, an α7 nAChR agonist, also triggered this protective effect. The protective effect of ultrasound pretreatment was abrogated in α7 nAChR knockout mice or by α-Btx treatment in wild-type mice, suggesting involvement of the splenic “cholinergic anti-inflammatory pathway” and activation of α7 nAChRs in causing the reduction in the inflammation in this injury model. Splenectomy and adoptive transfer showed that the spleen and CD4+ T cells mediated the protective effects of ultrasound pretreatment. This study revealed the importance of an intact spleen for the efficacy of α7 nAChR agonists, because activation of adrenergic receptors on the spleen activated CD4+ T cells to stimulate the production of ACh, triggering the activation of α7 nAChRs and the accompanying anti-inflammatory response (Gigliotti et al., 2013). This study clearly demonstrated the role of α7 nAChRs in AKI, as well as the importance of the spleen versus a direct effect on the kidney.

Cigarette smoking has been shown to be a factor associated with high risk for several chronic diseases, with CKD being no exception, because cigarette smoking has contributed to the progression of this disease (Orth, 2000, 2002). In the well-validated 5/6 nephrectomized model of human CKD disease, rats exhibited increased proteinuria and increased oxidative stress, and chronic nicotine pretreatment of the rats further exacerbated the proteinuria and oxidative stress (Rezonzew et al., 2012). Pretreatment with the α7 nAChR antagonist MLA significantly improved the glomerular injury score as well as reduced oxidative stress. This result is in contrast to the beneficial effects of α7 nAChR agonists in AKI, and these opposing effects in AKI versus CKD models may parallel the contrasting effects of nicotinic agonists in UC and CD models of IBD. However, more research is needed in this area with the use of safer and more selective agonists than nicotine. In addition, studies need to be performed across species, age, and sex to ensure consistent results and to account for the sex differences noted in both preclinical (Kang et al., 2014) as well as clinical studies demonstrating that sex plays a role in the prediction of renal decline (Halbesma et al., 2008). From preclinical studies, the sex difference.
resulted in greater renovascular vasodilation in female rat kidney tissue with the suggestion that it could be facilitated by estrogen having a direct effect on \( \alpha 7 \) nAChR downstream signaling (El-Mas et al., 2011).

Overall, the studies mentioned in this section highlight the recent interest in \( \alpha 7 \) nAChRs and kidney-related diseases. The association between smoking and CKD is illustrative of the presence of \( \alpha 7 \) nAChRs in the kidney and their relevance in renal function. Preliminary data from animal models indicate, however, that by reducing inflammation, \( \alpha 7 \) nAChR agonists should be beneficial for certain kidney diseases, most notably AKI. On the contrary, it was found that treatment with \( \alpha 7 \) nAChR antagonists showed beneficial outcomes in other renal conditions such as CKD. Refined characterization and classification of the kidney disease and design of adequate treatment regimens with \( \alpha 7 \) nAChR agonists or antagonists may allow novel approaches for treating renal disorders.

V. Conclusions

Our understanding of the nervous system has been advanced by DNA cloning and sequencing that has allowed the discovery of entire families of genes encoding for neurotransmitter receptors. The nAChRs are a good example of the diversity existing within a single receptor family. Given its structure and particular physiologic properties, which include a high Ca\(^{2+} \) receptor family. Given its structure and particular binding for neurotransmitter receptors. The nAChRs are allowed the discovery of entire families of genes encoding for the nervous system. This has resulted in greater interest in \( \alpha 7 \) nAChRs and kidney-related diseases. The association between smoking and CKD is illustrative of the presence of \( \alpha 7 \) nAChRs in the kidney and their relevance in renal function. Preliminary data from animal models indicate, however, that by reducing inflammation, \( \alpha 7 \) nAChR agonists should be beneficial for certain kidney diseases, most notably AKI. On the contrary, it was found that treatment with \( \alpha 7 \) nAChR antagonists showed beneficial outcomes in other renal conditions such as CKD. Refined characterization and classification of the kidney disease and design of adequate treatment regimens with \( \alpha 7 \) nAChR agonists or antagonists may allow novel approaches for treating renal disorders.

Our understanding of the nervous system has been advanced by DNA cloning and sequencing that has allowed the discovery of entire families of genes encoding for neurotransmitter receptors. The nAChRs are a good example of the diversity existing within a single receptor family. Given its structure and particular physiologic properties, which include a high Ca\(^{2+} \) permeability, the \( \alpha 7 \) nAChR has been the center of attention of many laboratories, opening up speculation about its physiologic role in cognitive processes and its contribution in neurologic and psychiatric diseases.

Structure-function studies conducted at the homeric \( \alpha 7 \) nAChRs concluded that a functional complex is composed of five subunits arranged around an axis of pseudosymmetry with the ion channel in the center. Each subunit spans the membrane four times, with the N and C termini facing the extracellular domain. The channel pore is made by the assembly of the five identical \( \alpha 7 \) subunits in a barrel-like manner and lined by the second TMD. Specific cationic selectivity of the \( \alpha 7 \) nAChR is provided by the specificity of the amino acids of the second TMD that are pointing toward the ionic pore. The LBD, where ACh binds, lies at the interface between two adjacent \( \alpha 7 \) subunits, and, given its homomeric structure, the \( \alpha 7 \) nAChRs display five identical LBDs. Recent studies, however, indicate that in some conditions (in vivo and in vitro), \( \alpha 7 \) subunits can assemble with another subunit, with heteromeric \( \alpha 7 \) nAChRs containing \( \beta 2 \) or \( \alpha 7 \) dup subunits, which have been described. These heteromeric nAChRs may display different physiologic or pharmacological characteristics.

Recent detailed genetic studies have demonstrated the complex nature of the chromosome localization of \( CHRNA7 \), the gene encoding for the \( \alpha 7 \) nAChR, and the presence in human of a duplication of exons 5-10 in \( CHRFAM7 \) on the same chromosome arm that encodes the dup \( \alpha 7 \) protein. Most recent work using different techniques combined with FISH examination have surprisingly revealed an unforeseen degree of variation at this gene locus in the human population. Moreover a correlation emerges between these variants in chromosome 15 and cognitive performance in patients with a variety of neurologic and psychiatric disorders. Deletion of \( CHRNA7 \) observed in some patients correlates with severe cognitive impairment. These genetic findings further support the hypothesis of the involvement of \( \alpha 7 \) nAChRs in cognition that was initially suggested by studies with \( \alpha 7 \) nAChR agonists and PAMs.

To exploit the promise offered by selectively targeting \( \alpha 7 \) nAChRs, efforts were dedicated to the identification of selective \( \alpha 7 \) nAChR agonists to serve as new pharmacological tools and as therapeutic interventions. These studies conducted by several laboratories yielded a panoply of small molecules displaying exquisite binding selectivity for \( \alpha 7 \) nAChRs. After the identification of PNU-282987, chemical structures containing a quinuclidine moiety represent the majority of compounds, showing sufficient discrimination of \( \alpha 7 \) versus other nAChR subtypes. The close structural homology among the ligand-gated ion channels offered an additional challenge because many molecules displaying agonistic properties at the \( \alpha 7 \) nAChR acted as antagonists of the 5-HT\(_3\) receptor. Nonetheless, molecules such as RG3487 and encenicline (EVP-6124) displayed adequate profiles to be introduced into clinical trials. Some success was achieved in reducing activity at the 5-HT\(_3\) receptor with \( \alpha 7 \) nAChR agonists such as TC-5619, AQW051, and ABT-126, which were also studied in clinical trials through Phase 2.

Because \( \alpha 7 \) nAChR agonists were primarily targeted as cognitive enhancers, novel chemical compounds were mainly tested in AD or schizophrenic patients. One of the difficulties encountered in clinical studies relates to the cognitive tests employed to examine the efficacy of \( \alpha 7 \) nAChR agonists and the multiplicity of available tests (e.g., ADAS-Cog 13, Cog-State, RBANS, MCCB, etc.) and the lack of consensus to date on the most effective testing instruments. Nonetheless, progress made with the \( \alpha 7 \) nAChR agonist encenicline, which is now in Phase 3 clinical trials for AD and schizophrenia, illustrates the continued hope that use of an \( \alpha 7 \) nAChR agonist will be beneficial in the treatment of cognitive deficits in these disorders.

Development of new molecules acting at \( \alpha 7 \) nAChRs is not limited to direct acting agonists and antagonists, but data from the literature illustrate the feasibility of identifying allosteric modulators. Binding at a site distinct from the ACh orthosteric site, these compounds are able to modulate receptor function by potentiating or inhibiting the response to a ligand and, in some cases, to reduce or suppress agonist-induced desensitization. Preclinical studies have already shown the potential of...
these molecules as cognitive enhancers, and it is likely that new and even more active compounds will be identified in the near future.

Moreover, although initial studies were largely focused on the CNS, where dysfunction of α7 nAChRs has been linked to cognitive deficits in numerous neurologic and psychiatric diseases, numerous publications have collectively demonstrated the pivotal role of α7 nAChRs in other organ systems, particularly the immune system. Obtaining a thorough understanding of the α7 nAChR is indispensable for the development of better personalized therapy and, despite the difficulties, efforts in the discovery of new ligands active at α7 nAChRs are expected to provide new therapeutic avenues for the treatment of neurologic and immunologic diseases.

Based on current knowledge it can be foreseen that molecules targeting α7 nAChRs may have a broad range of therapeutic applications ranging from brain to different organs or to the immune system. Providing the first review of the pleiotropic role of α7 nAChRs throughout the body, we have attempted to underline the therapeutic potential of this cholinergic receptor and the benefits as a therapeutic target for a variety of disease indications that it has to offer.

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Wrote or contributed to the writing of the manuscript: Lee, Donnelly-Roberts, Flood, Magar, and Bertrand.

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Therapeutic Potential of α7 nAChRs


Therapeutic Potential of \(\alpha 7\) nAChRs


