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Heteromeric $\alpha 7\beta 2$ Nicotinic Acetylcholine Receptors in the Brain

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Abstract

The $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) is highly expressed in the brain, where it maintains various neuronal functions including (but not limited to) learning and memory. In addition, the protein expression levels of $\alpha 7$ nAChRs are altered in various brain disorders. The classic rule governing $\alpha 7$ nAChR assembly in the mammalian brain was that it was assembled from five $\alpha 7$ subunits to form a homomeric receptor pentamer. However, emerging evidence demonstrates the presence of heteromeric $\alpha 7$ nAChRs in heterologously expressed systems and naturally in brain neurons, where $\alpha 7$ subunits are co-assembled with $\beta 2$ subunits to form a novel type of $\alpha 7\beta 2$ nAChR. Interestingly, the $\alpha 7\beta 2$ nAChR exhibits distinctive function and pharmacology from traditional homomeric $\alpha 7$ nAChRs. We review recent advances in probing the distribution, function, pharmacology, pathophysiology, and stoichiometry of the heteromeric $\alpha 7\beta 2$ nAChR, which have provided new insights into the understanding of a novel target of cholinergic signaling.

Traditional Rule of Assembly of nAChRs

Nicotinic acetylcholine receptors (nAChRs) in mammals exist as a diverse family of channels composed of different combinations of subunits derived from at least 17 genes [1,2]. Functional nAChRs can be assembled as both heteromers containing α and β subunits, or as homomers containing only α subunits [1,2]. In the mammalian brain, the most abundant forms of nAChRs are the heteromeric $\alpha 4\beta 2$ nAChR and the homomeric $\alpha 7$ nAChR [3–7]. Homomeric $\alpha 7$ nAChRs are assembled with 5 $\alpha 7$ subunits, which are distinguished from the majority of other nAChR subtypes that require different subunit

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assembly partners (e.g., α plus β subunits). Naturally expressed $\alpha 7$ nAChRs might mediate classic excitatory neurotransmission at some loci, but they may also modulate the release of other neurotransmitters, neurite outgrowth, and even neuronal survival/death [8,9]. In addition, $\alpha 7$ nAChRs express not only on neurons but also on various types of non-neuronal cells including astrocytes and microglia [10,11] (Box 1). The $\alpha 7$ nAChR, as a target of nicotine action, also has been implicated in the development, differentiation, and pathophysiology of the nervous system [12,13]. In rat forebrain cholinergic neurons, $\alpha 7$ and $\beta 2$ are the predominant nAChR subunits, and they were found to colocalize [14]. Accumulating evidence demonstrates that these $\alpha 7$ and $\beta 2$ nAChR subunits can co-assemble to form functional $\alpha 7\beta 2$ nAChRs in heterologous expression systems, and also under natural conditions in the brain (Figure 1) [15,16]. Importantly, this novel type of heteromeric $\alpha 7\beta 2$ nAChR exhibits unique function, pharmacology, and pathophysiology, and likely is an important target for cholinergic modulation in neuronal function and disease.

Box 1

$\alpha 7$ nAChRs Express in Non-Neuronal Cells

The $\alpha 7$ nAChRs are widely expressed in the central and peripheral nervous systems, in neurons as well as in several nonneuronal cells. For example, emerging evidence demonstrates that $\alpha 7$ nAChRs are expressed in immune cells including macrophages/monocytes [56], dendritic cells [57], microglia [58] and astrocytes [10,11]. When the $\alpha 7$ nAChRs are activated, nicotine exhibits significant modulations in the production of proinflammatory cytokines, such as interleukin (IL)-1, TNF- α , and IL-1 β [59]. Therefore, the $\alpha 7$ nAChRs in the immune system play an important role in the regulation of multiple immune pathways and inflammatory responses [60,61]. The $\alpha 7$ nAChRs are also expressed in stem cells, where they likely mediate cholinergic signaling on these cells and regulate survival/apoptosis, proliferation, differentiation, and maturation [62,63]. Lastly, $\alpha 7$ nAChRs are also expressed in endothelial cells, bronchial epithelial cells, skin keratinocytes, and vascular smooth muscle cells, where these nAChRs are likely involved in the regulation of the function and pathology of these cells [64–66].

What is a heteromeric $\alpha 7$ nAChR?

Traditionally, neuronal nAChRs are classified into two subgroups based on their subunit combination: the heteromeric α and β pentamers (such as the $\alpha 4\beta 2$ nAChR), and the homomeric α pentamers (such as the $\alpha 7$ nAChR) (Figure 1). Here, the heteromeric $\alpha 7$ nAChR is defined as $\alpha 7$ nAChR subunits that are co-assembled with the $\beta 2$ nAChR subunit(s) to form a novel type of $\alpha 7\beta 2$ nAChR (Box 2).

Box 2

Heteromeric and Homomeric nAChRs

nAChRs are prototypical members of the cys-loop ligand-gated ion channel superfamily of neurotransmitter receptors. Neuronal nAChRs are found throughout the nervous system (e.g., in muscle, autonomic and sensory ganglia, and the CNS). nAChRs exist as

multiple, diverse subtypes composed as pentamers of unique combinations from a family of at least seventeen ($\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, δ , ϵ) similar, but genetically distinct, subunits [1]. There are two classic groups of nAChRs based on the pattern of their subunit combinations. Most of these nAChR subtypes appear to exist as heteropentamers containing two or more different types of subunits, called heteromeric nAChRs. For example, heterologous expression studies suggest that $\alpha 2$, $\alpha 3$, $\alpha 4$, or $\alpha 6$ subunits can combine in binary fashion with $\beta 2$ or $\beta 4$ subunits to form ligand-binding and/or functional nAChRs (e.g., the $\alpha 4\beta 2$ nAChR is abundantly expressed naturally in the brain) or in tertiary complexes containing more than one of these α or β subunit types. Another prominent nAChR subtype found in vertebrate central and autonomic nervous systems contains unique $\alpha 7$ subunits ($\alpha 7$ nAChR), called homomeric nAChRs. These two groups of nAChRs exhibit distinct function, pharmacology, and pathophysiology.

Evidence of Heteromeric $\alpha 7\beta 2$ nAChRs

The existence of heteromeric $\alpha 7\beta 2$ nAChRs has been shown in both heterologously expressed systems and native brain neurons by multiple experimental approaches. Early studies from Lindstrom and colleagues have demonstrated that chick retinal ganglion neurons express heteromeric $\alpha 7$ and $\alpha 8$ nAChRs that may include other α and/or β nAChR subunits [17–19]. Biochemical studies also support this possibility because the $\alpha 7$ protein can be immunoprecipitated with other subunits, although the identity of these subunits was not determined at that time [20]. Using a presynaptic preparation, McGehee *et al.* reported a high-affinity, α -bungarotoxin (α -Bgtx)-sensitive receptor which was one of the first hints of a possible heteromeric $\alpha 7^*$ nAChR based on differential α -Bgtx sensitivity [21]. Soon, the functional evidence for heteromeric $\alpha 7$ receptors was reported in embryonic chick sympathetic neurons [22] and in intracardiac ganglion neurons [23]. Collectively, these pioneering works suggest that chick peripheral neurons likely contain heteromeric $\alpha 7^*$ nAChRs. Initial gene expression studies demonstrated that nAChR $\alpha 7$ and $\beta 2$ subunits partially overlap on the cell somata of cultured hippocampal neurons [24]. Interestingly, Azam *et al.* reported that several cholinergic circuit brain regions predominantly expressed $\alpha 7$ and $\beta 2$ subunit mRNAs, rather than $\alpha 4$ mRNA, and that these $\alpha 7$ and $\beta 2$ subunit mRNAs are usually co-expressed [14], suggesting the possibility of $\alpha 7\beta 2$ nAChRs in cholinergically-relevant neurons of the mammalian brain. We transfected $\alpha 7$ and $\beta 2$ subunit mRNA into *Xenopus* oocytes and formed functional $\alpha 7$ -containing nAChRs ($\alpha 7^*$ nAChR) with different current kinetics and pharmacology than that of oocytes transfected with only $\alpha 7$ subunits [25]. In addition, we showed co-immunoprecipitation of $\alpha 7$ and $\beta 2$ subunits from cotransfected TSA201 cells, further suggesting the co-assembly of $\alpha 7\beta 2$ nAChRs. Subsequently, we reported findings consistent with a novel, naturally-occurring $\alpha 7^*$ nAChR subtype in rodent basal forebrain cholinergic neurons [26]. In these cells, $\alpha 7$ subunits were found to be co-expressed, colocalized and co-assembled with $\beta 2$ subunit(s), as demonstrated by RT-PCR, immunohistochemistry, $\alpha 7$ and $\beta 2$ subunit co-immunoprecipitation, and patch-clamp recordings (combined with pharmacological tools) using wild-type (WT) and nAChR $\beta 2$ knockout (KO) mice [26]. We and coworkers further confirmed this heteromeric $\alpha 7\beta 2$ pentamer using Förster resonance energy transfer (FRET) and total internal reflection fluorescence (TIRF) microscopy in cells of the human epithelial

cell line, SH-EP1, transfected with human $\alpha 7$ and $\beta 2$ subunits [27]. Recently, pharmacological characteristics of expressed $\alpha 7\beta 2$ nAChRs have been further profiled in *Xenopus* oocytes, demonstrating possibly different pharmacological properties compared to homomeric $\alpha 7$ nAChRs [15], although differences are frequently subtle for some compounds tested to date. In addition to rodents, $\alpha 7\beta 2$ nAChRs have also been found in human basal forebrain neurons [15] and cerebral cortical neurons [16]. Importantly, by using concatemeric technology, the $\alpha 7\beta 2$ nAChRs with different, completely defined stoichiometric combinations and subunit associations have been recently characterized [15]. Collectively, by using these experimental techniques (Box 3), accumulated evidence demonstrates that $\alpha 7$ and $\beta 2$ subunits can co-assemble to form a functional heteromeric $\alpha 7\beta 2$ nAChR in the CNS.

Box 3

Techniques Used To Study nAChR Assembly, Expression, and Function

Patch-Clamp Recording Technique

The patch-clamp technique is a laboratory technique in electrophysiology that allows the study of single or multiple ion channel activity and whole-cell currents in neurons. Owing to high sensitivity and the ability to perform rapid solution exchange, this technique is highly suitable for studying ligand-gated ion channel function, especially for $\alpha 7$ nAChR-mediated currents because of the rapid activation and desensitization of $\alpha 7$ nAChRs during ligand exposure.

Reverse Transcription PCR (RT-PCR)

RT-PCR is one variant of PCR which is commonly used in molecular biology to detect the level of RNA expression. RT-PCR can be used to qualitatively detect gene expression through the creation of cDNA from RNA transcripts. Although traditional PCR and the RT-PCR both produce multiple copies of specific amplified DNA sequences, the applications of the two techniques are fundamentally different. Traditional PCR is used to exponentially amplify target DNA sequences while RT-PCR is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement through the use of reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR. In addition to the qualitative study of gene expression, RT-PCR can be utilized for quantification of RNA expression, in both relative and absolute terms, by incorporating quantitative (q)PCR into the technique. The combined technique, described as quantitative RT-PCR or real-time RT-PCR, is considered to be the most powerful, sensitive, and quantitative assay for the detection of RNA levels.

Immunoprecipitation (IP)

IP is the technique of precipitating a protein antigen out of solution using an antibody that specifically recognizes and binds to that particular protein. This process can be used to isolate and concentrate a specific protein from a sample containing a mixture of thousands of different proteins. IP uses a secondary antibody (often immobilized to a solid phase) to crosslink the target and primary antibody complexes, forming a large multimolecular complex that can be easily separated from the liquid phase.

Immunoprecipitation of intact protein complexes (i.e., antigen together with any proteins or ligands that are bound to it) is termed co-immunoprecipitation (Co-IP). Co-IP works by selecting an antibody that specifically targets a known protein that is believed to be a member of a larger complex of proteins. By targeting this known member with an antibody it may become possible to pull the entire protein complex out of solution and thereby identify unknown members of the complex that interact with the target protein. Co-IP is a powerful technique frequently used by molecular biologists to study protein–protein interactions.

Fluorescence Resonance Energy Transfer (FRET)

The mechanism of FRET involves a donor chromophore, initially in its electronic excited state, which may transfer energy to an acceptor chromophore in nonradiative fashion through long-range dipole–dipole coupling. The efficiency of this energy transfer depends on the inverse 6th power of the distance between donor and acceptor, making FRET able to measure nanometer scale distance and the changes in distance. Measurements of FRET efficiency can be used to determine if two fluorophores are within a specific distance of each other. Such measurements are used as a research tool in fields including biology and chemistry.

Total Internal Reflection Fluorescence (TIRF) Microscopy

TIRF microscopy (TIRFM) employs a type of microscope with which a thin region of a specimen, usually less than 200 nm, can be observed. Prism-, objective-, and lightguide-based TIRFM produce the evanescent wave to selectively illuminate and excite fluorophores in a restricted region of the specimen immediately adjacent to the surface of a glass, silica, or plastic slide. The evanescent wave is generated only when the incident light is totally internally reflected at the surface/water or surface/specimen interface. The evanescent electromagnetic field decays exponentially away from the interface, and thus penetrates to a depth of only approximately 200 nm into the sample medium. Thus TIRFM enables selective visualization of surface regions such as the basal plasma membrane (which are about 7.5 nm in thickness) of cells. TIRF can also be used to observe the fluorescence of a single molecule, making it an important tool of biophysics and quantitative biology.

Receptor Concatemer Technology

nAChRs are natively assembled from individual subunits to produce functional pentameric assemblies. In native expression systems, a combination of intrinsic subunit properties and cellular chaperones are used to control subunit assembly. However, even under these circumstances, multiple receptor subtypes and/or stoichiometries can result. In heterologous expression systems the expression levels can be very different from those in native systems, and elements of the chaperoning system can be different or absent. Concatenated protein constructs encode multiple subunits (in this case of nAChRs) joined with short peptide linkers. In the case of fully concatenated pentameric nAChR constructs, this approach has been used to completely define subunit stoichiometry and subunit–subunit associations of nAChRs. This provides significant advantages in studying pure populations of desired nAChR subtypes, in probing the effects of particular

subunit compositions/assembly orders, and in performing detailed structure–function studies.

Functional Properties of Heteromeric $\alpha 7\beta 2$ nAChRs

The major functional feature of $\alpha 7\beta 2$ nAChRs is their slower (relative to homomeric $\alpha 7$ receptors) whole-cell current decay kinetics (Figure 2), which has been confirmed in heterologously transfected *Xenopus* oocytes [16,25,26,28], natively expressed mouse basal forebrain cholinergic neurons [26] and hippocampal interneurons [29], and functional $\alpha 7\beta 2$ nAChR concatemers [15]. Another functional property of $\alpha 7\beta 2$ nAChRs is their lower whole-cell current amplitude (relative to homomeric $\alpha 7$ receptors) to nAChR agonists, which has been observed both in heterologously expressed systems and natively expressed neurons. Consistent with these properties, the single-channel conductance of $\alpha 7$ -containing nAChRs is lower in hippocampal interneurons than in expressed homomeric $\alpha 7$ nAChRs [30]. However, cell-surface expression levels may also be an important contributor to the magnitude of the macroscopic currents observed. For example, concatemeric $\alpha 7\beta 2$ nAChRs expressed in *Xenopus* oocytes have larger agonist-induced current amplitudes compared to those measured from homomeric $\alpha 7$ nAChR concatemers [15]. The insertion of one $\beta 2$ subunit into the $\alpha 7$ concatemeric pentamer (at position 3) results in a threefold increase in current amplitude (compared to the homomeric $\alpha 7$ nAChR concatemer), and insertion of two $\beta 2$ subunits into the $\alpha 7$ pentamer (at positions 2 and 4) results in a twofold increase in current amplitude [15]. These increases in macroscopic current seem likely to be due to increased surface expression of concatemeric constructs containing the $\beta 2$ subunit. Co-expression of the $\alpha 7$ and $\beta 2$ subunits does not result in any significant change in the current–voltage curve [25]. In addition to the altered channel decay kinetics and amplitude between heteromeric $\alpha 7\beta 2$ nAChRs and homomeric $\alpha 7$ nAChRs in the CNS, it will be interesting to test other receptor/channel functions in the $\alpha 7\beta 2$ nAChRs such as single-channel properties and Ca^{2+} permeability in the future.

Pharmacological Properties of Heteromeric $\alpha 7\beta 2$ nAChRs

One of the more intriguing questions is whether the heteromeric $\alpha 7\beta 2$ nAChR exhibits an altered pharmacological profile compared to the homomeric $\alpha 7$ nAChR, including affinity and potency of nAChR agonists, antagonists, or allosteric modulators. Currently the accumulated data are still incomplete. Overall, often small changes in EC_{50} and IC_{50} values have been reported for multiple agonists and antagonists. However, these differences have often been inconsistent between reports examining the same compounds. This may reflect the difficulty of measuring subtle changes in pharmacological parameters, inconsistent subunit assembly order and/or stoichiometries between the various systems used, or a combination of both.

Agonists—Initial work demonstrates that the co-expression of the $\alpha 7$ and $\beta 2$ subunits in *Xenopus* oocytes results in significant changes in pharmacology compared to expressed homomeric $\alpha 7$ nAChRs. For example, whereas carbachol and choline are full or near-full agonists for the homomeric $\alpha 7$ nAChR, they are only partial agonists for heteromeric $\alpha 7\beta 2$ nAChRs [25]. In addition, the EC_{50} values for acetylcholine (ACh), carbachol, and choline

significantly increased when the $\beta 2$ subunit was co-expressed with the $\alpha 7$ subunit, suggesting lower receptor potency to nAChR agonists of the $\alpha 7\beta 2$ nAChR compared to the homomeric $\alpha 7$ nAChR. This feature of heteromeric $\alpha 7\beta 2$ nAChRs has been confirmed by several groups [15,27,28]. Impressively, Zwart *et al.* find that, except for ACh and epibatidine, which do not distinguish between the $\alpha 7\beta 2$ and the $\alpha 7$ nAChR, $\alpha 7\beta 2$ nAChRs display lower efficacy for 13 nAChR agonists tested, including three non-selective agonists (nicotine, cytosine, varenicline) and 10 selective $\alpha 7$ agonists (choline, carbamylcholine, DMAD, compound A, PNU-282978, SSR-108911, TC-1698, TC-7020, TC-5619, and EVP-6124) [28] (Table 1). In addition, they found that three selective $\alpha 4\beta 2$ nAChR agonists (ABT-089, sazetidine A, ispronnicline) fail to activate either the $\alpha 7$ or $\alpha 7\beta 2$ nAChRs [28]. These pharmacological features of $\alpha 7\beta 2$ nAChRs suggest that, while incorporation of $\beta 2$ subunits into the $\alpha 7$ subunit pentamer formed functional heteromeric $\alpha 7\beta 2$ nAChRs with altered function (compared to the homomeric $\alpha 7$ receptor), this does not shift the $\alpha 7$ nAChR to a 'β2-like' pharmacological profile. However, one of the most interesting findings is that some $\alpha 7$ partial agonists (e.g., compound A and DMBA) demonstrate significantly lower potency to activate $\alpha 7\beta 2$ nAChRs compared to $\alpha 7$ nAChRs, and were therefore functional antagonists of $\alpha 7\beta 2$ nAChRs. Therefore, compound A and DMBA may provide useful pharmacological tools to distinguish functional $\alpha 7\beta 2$ nAChRs from $\alpha 7$ nAChRs [28].

Antagonists—Most published works have found that $\alpha 7\beta 2$ and $\alpha 7$ nAChRs display similar antagonism by $\alpha 7$ -selective nAChR antagonists (e.g., MLA and α -Bgtx), which suggests that the predominant binding site for these antagonists for $\alpha 7\beta 2$ nAChRs are likely located at the $\alpha 7$ – $\alpha 7$ interface. By contrast, Murray *et al.* found a reduction in the MLA IC_{50} value (from 0.27 for $\alpha 7$ nAChR to 0.13 μM for $\alpha 7\beta 2$ nAChR) in oocytes expressing $\alpha 7\beta 2$ (1:10) nAChRs [27]. For the non- $\alpha 7$ nAChR antagonist, DH β E, which does not block homomeric $\alpha 7$ nAChRs, the $\alpha 7\beta 2$ receptors in rodent basal forebrain cholinergic neurons and hippocampal interneurons are sensitive to DH β E (while the homomeric $\alpha 7$ nAChRs expressed in midbrain neurons are not) [26,29]. For heterologously expressed $\alpha 7\beta 2$ nAChRs in oocytes, DH β E inhibition occurred only at the low concentration range, with an IC_{50} value of $4.58 \pm 0.4 \mu M$ and was well fitted by a single Hill equation with an nH value of 1.2 ± 0.1 [27]. However, recently two groups, using either individual or concatemerically expressed $\alpha 7\beta 2$ nAChRs in oocytes, have shown no difference in DH β E inhibition between $\alpha 7\beta 2$ and $\alpha 7$ nAChRs [15,28]. Furthermore, there was no difference in the inhibition to two other nAChR antagonists (mecamylamine and the snake venom α -toxin) among individual $\alpha 7$, concatemeric $\alpha 7$, and concatemeric $\alpha 7\beta 2$ nAChRs as well [15,28]. Table 2 summarizes the effects of nAChR antagonists on homomeric $\alpha 7$ and heteromeric $\alpha 7\beta 2$ nAChRs.

Positive Allosteric Modulators—Because as mentioned above it is likely that the activation of $\alpha 7\beta 2$ nAChRs by agonists is predominantly by action at the $\alpha 7$ – $\alpha 7$ interface, and that the $\alpha 7$ – $\beta 2$ interface seems to be different from the $\alpha 4$ – $\beta 2$ interface, the functional $\alpha 7$ – $\beta 2$ interface may be a target for $\alpha 7$ -selective positive allosteric modulator binding sites. While Murray *et al.* (2012) initially reported that $\alpha 7\beta 2$ and $\alpha 7$ nAChR-mediated currents were not differentially potentiated by the $\alpha 7$ nAChR positive allosteric modulator PNU-120596 [27], Thomsen *et al.* recently compared the effects of PNU-120596 on human subunit $\alpha 7$ and $\alpha 7\beta 2$ nAChRs heterologously expressed in *Xenopus* oocytes; they also

found similar potentiation of $\alpha 7$ and $\alpha 7\beta 2$ nAChR-mediated currents by this allosteric potentiator [16]. Therefore these results suggest that the site of action for the positive allosteric modulator PNU-120596 is likely at the $\alpha 7$ – $\alpha 7$ interface.

Distribution of Heteromeric $\alpha 7\beta 2$ nAChR in the CNS

Initial gene expression observations laid the groundwork for our following studies that provoked the hypothesis of the heteromeric $\alpha 7\beta 2$ nAChRs [14]. Combining patch-clamp electrophysiological and single-cell RT-PCR analysis, Yakel and colleagues reported a strong correlation between expression of the $\alpha 7$ and $\beta 2$ subunits in individual rat hippocampal interneurons [31], and Azam *et al.* found co-expression of nAChR $\alpha 7$ and $\beta 2$ subunit mRNA in most cholinergic-associated brain areas including forebrain cholinergic neurons [14]. Following these reports, we recognized that rodent basal forebrain cholinergic neurons express heteromeric $\alpha 7\beta 2$ nAChRs [26]. Later, we further confirmed that young mouse hippocampal interneurons also express functional $\alpha 7\beta 2$ nAChRs [29], which is consistent with previous reports that the $\alpha 7$ -containing nAChRs in rat hippocampal interneurons desensitized more slowly, and had a lower single-channel conductance, than homomeric $\alpha 7$ nAChRs [30,31]. However, Moretti *et al.* used α -Bgtx affinity purification and immunoprecipitation with anti- $\alpha 7$ subunit antibodies to isolate nAChRs containing $\alpha 7$ subunits from WT, $\alpha 7$ KO, or $\beta 2$ KO mouse brain samples; they demonstrated that the $\alpha 7\beta 2$ nAChRs were not detected in hippocampal tissues, but were detected in the forebrain, based on Western blot analysis of isolates using $\beta 2$ subunit-specific antibodies [15]. Recently, Thomsen *et al.* reported the presence of heteromeric $\alpha 7\beta 2$ nAChRs in human cerebral cortex [16]. These differences between studies may be due to genuine species differences or be a reflection of assay sensitivity because $\alpha 7\beta 2$ nAChRs constitute a small portion of the overall $\alpha 7$ subunit-containing nAChR population in all regions examined so far. However, because cholinergic forebrain neurons are few in number, it is likely that even the smaller population of $\alpha 7\beta 2$ nAChRs could be functionally relevant if they are located predominantly on only a small subset of cholinergic neurons (which project extensively to the rest of the brain). Using mouse cortical synaptosomes, Mehta *et al.* found that A β - and nicotine-induced Ca²⁺ responses appeared to largely involve $\alpha 7^*$ nAChRs, but the results using A β also indicated the possible presence of $\beta 2$ -containing receptors. In particular, by combining the use of the selective $\alpha 7$ nAChR antagonist, α -BgTx, with synaptosomes prepared from cortices of $\beta 2$ subunit KO mutants, the $\alpha 7^*$ nAChR-coupled responses from these mice were smaller in amplitude when exposed to nM doses of A β , similar to what was observed for pM doses of A β applied to control cortical preparations. This result suggests the possibility of $\alpha 7\beta 2^*$ nAChRs located in presynaptic terminals of cortical neurons [32]. In addition, Lykhmus *et al.* used a wide spectrum of nAChR subunit-specific antibodies, combined with nAChR $\alpha 7$ or $\beta 2$ KO mice, to demonstrate the presence of $\alpha 7\beta 2$ nAChRs in intracellular organelles (e.g., brain mitochondria) [33]. Although these $\alpha 7\beta 2$ nAChRs may not function as classical ion channels, at least for regulating mitochondrial pore formation, activating these $\alpha 7$ nAChRs should affect intra-mitochondrial kinases involved in MPTP formation [34], suggesting that these mitochondrial $\alpha 7\beta 2$ nAChRs play an important role in maintaining mitochondria resistance to apoptogenic factors. Taken together, the heteromeric $\alpha 7\beta 2$ nAChRs have been identified in several brain regions, in pre- and postsynaptic

compartments, as well as intracellular organelles such as mitochondria, where they may play an important role in mediating cholinergic modulations in brain function and diseases.

Assembly of Heteromeric $\alpha 7\beta 2$ nAChRs

After the identification of functional native $\alpha 7\beta 2$ nAChRs, the stoichiometry of this heteromeric nAChR was investigated in heterologous expression systems. One way to reveal the relationship of receptor stoichiometry and function is to express $\alpha 7$ and $\beta 2$ nAChR subunit mRNAs at different ratios. In early work, Khiroug *et al.* compared agonist affinity (i.e., EC_{50} values) of $\alpha 7\beta 2$ nAChRs with $\alpha 7$ and $\beta 2$ subunit injection ratios of 1:1 and 1:3, and found a doubled EC_{50} value when the $\beta 2$ subunit cDNA was increased; this suggested that the increased presence of the $\beta 2$ subunit ratio results in a decrease in affinity of the $\alpha 7\beta 2$ nAChR [25]. Furthermore, Zwart *et al.* compared receptor function between homomeric $\alpha 7$ and heteromeric $\alpha 7\beta 2$ nAChRs by nuclear injections of cDNAs in 1:3 and 1:10 ratios, and found a significant reduction of current amplitude and slowed current desensitization kinetics for the $\alpha 7\beta 2$ receptors, but with no difference in agonist affinity [28]. Both of these observations indicate that expression of functional $\alpha 7\beta 2$ nAChRs is possible with multiple subunit stoichiometries. Recently, the use of concatemeric technology to study the stoichiometry of $\alpha 7\beta 2$ nAChRs heterologously expressed in *Xenopus* oocytes has provided new insights, including direct confirmation of multiple functional $\alpha 7\beta 2$ nAChR stoichiometries. For example, Moretti *et al.* constructed the fully pentameric $\alpha 7^*$ nAChR concatemers from human nAChR subunit sequences, and compared the function and pharmacological profiles of these $\alpha 7^*$ nAChR concatemers (both homomeric $\alpha 7$ and heteromeric $\alpha 7\beta 2$) with the subunits arranged in the order $\alpha 7-\alpha 7-\alpha 7-\alpha 7-\alpha 7$ [$(\alpha 7)_5$ -nAChR homopentamer], $\alpha 7-\alpha 7-\beta 2-\alpha 7-\alpha 7$ [$(\alpha 7)_4(\beta 2)_1$ -nAChR], or $\alpha 7-\beta 2-\alpha 7-\beta 2-\alpha 7$ [$(\alpha 7)_3(\beta 2)_2$ -nAChR]. Each of the three concatemeric structures functionally expressed and exhibited ACh-induced currents. Compared to the homomeric $\alpha 7$ nAChRs from individual (i.e., unlinked) pentameric human $\alpha 7$ subunits, the concatemeric constructs had smaller current amplitudes, but both concatemeric heteromeric receptors [$(\alpha 7)_4(\beta 2)_1$ and $(\alpha 7)_3(\beta 2)_2$ nAChRs] had larger current amplitudes and slower decay kinetics (τ_{slow}) [15]. Pharmacologically, all three concatemeric constructs exhibited similar responses to different nAChR agonists; however, nicotine had significantly lower efficacy (normalized to that of ACh) at $(\alpha 7)_4(\beta 2)_1$ and $(\alpha 7)_3(\beta 2)_2$ $\alpha 7\beta 2$ nAChR concatemeric structures compared to $(\alpha 7)_5$ -nAChR concatemer or unlinked homomeric $\alpha 7$ nAChRs [15]. In addition, the antagonist pharmacology of $\alpha 7$ (MLA) or $\beta 2$ (DH β E) nAChRs were compared and found to be indistinguishable between the different $\alpha 7$ nAChRs [15]. Collectively, these concatemeric constructs provided new insights and improved understanding of the possible diverse stoichiometry of $\alpha 7\beta 2$ nAChRs, and these data confirm some functional and pharmacological characteristics of $\alpha 7\beta 2$ nAChRs previously reported in heterologous expression systems and native receptors in neurons. However, it should be noted that, while the subunit ratios and associations within concatemeric $\alpha 7\beta 2$ nAChRs are completely known, the same is not true of either natively expressed $\alpha 7\beta 2$ nAChRs or those assembled in artificial expression systems from unlinked subunits. It will be interesting and informative to further compare the function and pharmacology across receptors assembled in different ways.

Significance of the Heteromeric $\alpha 7\beta 2$ nAChR

Because homomeric $\alpha 7$ nAChRs are predominantly expressed throughout the CNS and nonneuronal system, we considered the possible significance of heteromeric $\alpha 7\beta 2$ nAChRs, and how might they function *in vivo* differently from homomeric $\alpha 7$ nAChRs.

Heteromeric $\alpha 7\beta 2$ nAChR Is a Sensitive Target for Amyloid β Peptides

Because native $\alpha 7\beta 2$ nAChRs were initially found in basal forebrain cholinergic neurons [26], this receptor may play an important role in the modulation of cholinergic signaling, and consequently brain cognitive function and disease pathology, such as in Alzheimer's disease (AD). Consistent with this, we initially found that the $\alpha 7\beta 2$ nAChRs in rodent basal forebrain cholinergic neurons exhibited high sensitivity to pathologically-relevant concentrations (e.g., 1 nM) of amyloid β peptide (e.g., oligomeric $A\beta_{1-42}$) [26], as compared to homomeric $\alpha 7$ nAChRs where much higher concentrations of $A\beta_{1-42}$ (e.g., 100 nM oligomeric) were needed for inhibition [35]. This finding was further confirmed by the sensitivity of heterologously expressed $\alpha 7\beta 2$ and $\alpha 7$ nAChRs in *Xenopus* oocytes; heteromeric $\alpha 7\beta 2$ nAChRs were much more sensitive to $A\beta_{1-42}$ inhibition than homomeric $\alpha 7$ -nAChRs [29]. Because the heteromeric $\alpha 7\beta 2$ nAChR in basal forebrain cholinergic neurons is a sensitive target for pathologically-relevant levels of $A\beta_{1-42}$ [36], this receptor may participate in the pathogenic process of AD. The mechanism that pathologically-relevant levels of $A\beta_{1-42}$ use to inhibit $\alpha 7\beta 2$ nAChRs in AD pathogenesis is still unclear, but there are at least two possibilities. First, the acute inhibition of $\alpha 7\beta 2$ nAChRs by $A\beta_{1-42}$ may directly lead to basal forebrain cholinergic signaling impairments in the hippocampus and subsequent learning and memory deficits [29]. Second, the $A\beta_{1-42}$ -sensitive $\alpha 7\beta 2$ nAChRs may mediate chronic upregulation of $\alpha 7$ -containing nAChRs because acute inhibition of $\alpha 7$ nAChRs triggers receptor upregulation [37–39]. In primary hippocampal neuronal cultures, we found that chronic treatment of neurons with $A\beta_{1-42}$ (100 nM, fibrils or oligomers) upregulated $\alpha 7$ nAChR surface expression and function, which can be abolished by $\alpha 7$ nAChR antagonists and was absent in cultures prepared from $\alpha 7$ nAChR KO [38]. This upregulation by $A\beta_{1-42}$ required a higher concentration (i.e., 100 nM) than we found was necessary to block the heteromeric $\alpha 7\beta 2$ receptors, suggesting that the receptors responsible for this upregulation were homomeric $\alpha 7$ nAChRs [38]. Moreover, using human neuroblastoma (SH-SY5Y) cells differentiated by the addition of 10 μ M retinoic acid to each dish, and incubating at 37 °C for 4 days to have cholinergic characteristics, we tested the effects of large $A\beta_{1-42}$ aggregates (100 nM) on $\alpha 7$ nAChR function and cell toxicity, and found that chronic exposure of $A\beta_{1-42}$ aggregates upregulated $\alpha 7$ nAChR function, and consequently resulted in cellular toxicity, which was prevented by pharmacological block of $\alpha 7$ (but not $\alpha 4\beta 2$) nAChRs [39]. These results suggest that $\alpha 7$ nAChRs (likely homomeric) play an important role in $A\beta_{1-42}$ -induced mediation of neuronal excitability as well as cellular toxicity. It will be interesting to examine the effects of lower doses of $A\beta$ exposure (e.g., 10 nM) on $\alpha 7^*$ nAChR expression and function in basal forebrain cholinergic neurons, where the heteromeric $\alpha 7\beta 2$ nAChRs are expressed.

Heteromeric $\alpha 7\beta 2$ nAChR Is a Sensitive Target for Volatile Anesthetic Agents

Neuronal nAChRs are important targets for anesthetic agents, in particular the heteromeric $\alpha 4\beta 2$ nAChR subtype; the homomeric $\alpha 7$ nAChRs are less sensitive to general anesthetic agents [40,41]. The mechanism of this differential sensitivity to anesthetic agents between $\alpha 4\beta 2$ and $\alpha 7$ nAChR is unclear. In previous studies using molecular dynamics simulations, Tang's group found that, although multiple anesthetic binding sites were observed in $\alpha 7$, $\alpha 4$ and $\beta 2$ subunits, anesthetic binding to a site at the interface between extracellular and transmembrane domains of the $\beta 2$ subunit produced a profound change in protein dynamics that was likely to affect channel function [42–45]. Based on these results, they proposed a hypothesis to explain the lower sensitivity of $\alpha 7$ nAChRs to general anesthetics, in which the susceptibility to anesthetic perturbation in the $\beta 2$ (but not $\alpha 7$) subunit underlies the functional sensitivity of $\alpha 4\beta 2$ nAChRs to volatile anesthetics [42]. To test this hypothesis, we examined the effects of isoflurane on native $\alpha 7\beta 2$ nAChRs expressed in rat basal forebrain cholinergic neurons and heterologously expressed $\alpha 7\beta 2$ nAChRs in *Xenopus* oocytes, and found that isoflurane significantly inhibited native $\alpha 7\beta 2$ nAChRs on basal forebrain cholinergic neurons but did not inhibit native $\alpha 7$ nAChRs on ventral tegmental area (VTA) dopamine neurons [46]. Similar results were also observed in heterologously transfected $\alpha 7\beta 2$ and $\alpha 7$ nAChRs in *Xenopus* oocytes [46]. These results clearly suggest that, compared to homomeric $\alpha 7$ nAChRs, the heteromeric $\alpha 7\beta 2$ nAChR is sensitive to anesthetic agents and therefore may play a role in the mediation of anesthetic effects in the CNS.

Inhibition of Heteromeric $\alpha 7\beta 2$ nAChRs by $\alpha 7$ nAChR Partial Agonists

As mentioned above, some $\alpha 7$ nAChR partial agonists (e.g., compound A and DMBA) may demonstrate significantly lower efficacy for $\alpha 7\beta 2$ nAChRs as compared to $\alpha 7$ nAChRs, and were therefore essentially functional antagonists for $\alpha 7\beta 2$ nAChRs (rather than partial agonists for $\alpha 7$ nAChRs), providing useful pharmacological tools to clearly distinguish functional $\alpha 7\beta 2$ from $\alpha 7$ nAChRs [28]. These findings are potentially highly significant because with such compounds one can selectively inhibit $\alpha 7\beta 2$ nAChRs, and this will provide a novel experimental approach to evaluate the impact of $\alpha 7\beta 2$ nAChRs in cholinergic signaling-modulated neural functions and disorders. However, given earlier-stated caveats concerning discrepancies in pharmacological parameters measured across multiple studies, perhaps reflecting differences in $\alpha 7\beta 2$ nAChR assembly across the experimental models used, it will be important to confirm these exciting findings.

Concluding Remarks

The $\alpha 7$ nAChR is one of most highly expressed nAChRs in the CNS and mediates crucial cholinergic signaling in a variety of brain functions and disorders. The $\alpha 7$ nAChR was classically considered to be a homomeric nAChR. However, this idea has been recently challenged by accumulating evidence that demonstrates that a heteromeric $\alpha 7$ nAChR can be co-assembled and functional when formed with the $\beta 2$ subunit in both heterologous expression systems and naturally in brain neurons. This novel type of $\alpha 7\beta 2$ nAChR exhibits distinctive pharmacology and function from traditional homomeric $\alpha 7$ nAChRs, suggesting that it may play an important and unique role in the physiology, pharmacology, and

pathophysiology in the CNS. However, there are several important questions about this novel type of $\alpha 7\beta 2$ nAChR that need to be addressed in future studies (see Outstanding Questions). For example, while some small (and sometimes inconsistent) pharmacological differences have been seen, agonists that are highly selective for $\alpha 7\beta 2$ nAChRs have yet to be discovered. Thus far, all $\alpha 7$ and $\alpha 4\beta 2$ nAChR-selective agonists have not shown significant selectivity between heteromeric $\alpha 7\beta 2$ and homomeric $\alpha 7$ nAChRs. This has been used to hypothesize that these agonists predominantly activate $\alpha 7^*$ nAChRs at the $\alpha 7$ – $\alpha 7$ subunit interface [15,27]. If this is the case, what role in ligand recognition (if there is any) may be played by the $\alpha 7$ – $\beta 2$ interface? Is it possible that some ligands yet to be discovered do in fact act at the $\alpha 7$ – $\beta 2$ interface to alter $\alpha 7\beta 2$ nAChR function and pharmacology? Second, what is the stoichiometry of native $\alpha 7\beta 2$ -nAChRs? Although the use of concatemers expressed in *Xenopus* oocytes provided insights into the understanding of $\alpha 7\beta 2$ nAChR stoichiometry, these completely defined assemblies are subtly different functionally and pharmacologically from native $\alpha 7\beta 2$ nAChRs in basal forebrain cholinergic neurons and hippocampal interneurons. Because fully concatemeric pentameric constructs faithfully replicate the properties of other nAChR subtypes (see for example [47–51]), this strongly suggests that concatemers with different ratios and assembly orders of $\alpha 7$ and $\beta 2$ subunits need to be investigated, and that rigorous control of subunit ratios in unlinked heterologous expression systems is needed. Third, are functional $\alpha 7\beta 2$ nAChRs expressed in other brain regions? Presently it has been shown that native $\alpha 7\beta 2$ nAChRs are expressed in rodent basal forebrain cholinergic neurons and hippocampal interneurons, and in human cerebral cortical neurons, but not in human cerebellar and hippocampal neurons (at least at levels detectable with the techniques used to date). Fourth, what if any role do these heteromeric $\alpha 7^*$ nAChRs play during development and in the pathogenesis of disease? For example, the fact that $\alpha 7\beta 2$ nAChRs are sensitive to pathologically-relevant levels of $A\beta_{1-42}$ suggests that these receptors may have a role in the pathogenesis of AD. Furthermore the development of selective $\alpha 7\beta 2$ nAChR ligands may help to address the role of these receptors in AD and other disorders and diseases linked to the $\alpha 7$ receptor (e.g., Parkinson's disease and schizophrenia) [52–55]. Fifth, the differences in Ca^{2+} permeability between $\alpha 7$ homomeric and $\alpha 7\beta 2$ heteromeric receptors have not been investigated. Even in the absence of a change in relative Ca^{2+} permeability, the significantly slower current kinetics might mean that significantly more overall Ca^{2+} influx will occur per stimulation. This could be an important difference with regard to the functional impact of the $\alpha 7\beta 2$ nAChRs. Sixth, are there other differences in functional properties (e.g., single-channel properties, conductance, and open probability) between $\alpha 7\beta 2$ and $\alpha 7$ nAChRs? This information will tell us more about the potential role of the $\alpha 7$ – $\beta 2$ interface and the influence of $\beta 2$ subunits on the properties of the $\alpha 7\beta 2$ nAChRs. Clearly, we are at an early stage in our attempts to parse the contribution of $\alpha 7\beta 2$ nAChRs in cholinergic modulation in brain function and disorders. Fortunately, the recent application of experimental tools (e.g., concatemeric technology, FRET) and early indications of (albeit somewhat incompletely/inconsistently characterized) novel $\alpha 7\beta 2$ nAChR selective compounds (e.g., amyloid- β , compound A, DH β E, and DMBA) are hopeful signs. It seems likely that further developments in these regards will drive new investigations designed to address these important questions in ever more rigorous and resolving ways. Finally, in addition to heteromeric $\alpha 7\beta 2$ nAChRs, other possible combinations of α or β subunits with the $\alpha 7$ subunit may form other types of heteromeric

receptors, such as $\alpha 7\beta 4$ or $\alpha 7\beta 3$ nAChRs. It may even be possible that $\alpha 7$ subunit can assemble with $\alpha 4\beta 2$ nAChRs to form a more complex $\alpha 7\alpha 4\beta 2$ nAChR with altered $\alpha 4\beta 2$ receptor function and pharmacology. All these possibilities should be tested in future studies.

Outstanding Questions

What role in ligand recognition (if there is any) may then be played by the $\alpha 7$ – $\beta 2$ interface? Is it possible that some ligands yet to be discovered do in fact act at the $\alpha 7$ – $\beta 2$ interface to alter $\alpha 7\beta 2$ nAChR function and pharmacology?

What is the stoichiometry of native $\alpha 7\beta 2$ nAChRs? Although the use of concatemers expressed in *Xenopus* oocytes provided insights into the understanding of $\alpha 7\beta 2$ nAChR stoichiometry, these completely defined assemblies are subtly different functionally and pharmacologically from native $\alpha 7\beta 2$ nAChRs in basal forebrain cholinergic neurons and hippocampal interneurons.

Are functional $\alpha 7\beta 2$ nAChRs expressed in other brain regions? Presently it has been shown that native $\alpha 7\beta 2$ nAChRs are expressed in rodent basal forebrain cholinergic neurons and hippocampal interneurons, as well as in human basal forebrain and cerebral cortical neurons.

What if any role do these heteromeric $\alpha 7^*$ nAChRs play during development and in the pathogenesis of disease? For example, the fact that $\alpha 7\beta 2$ nAChRs are sensitive to pathologically- relevant levels of $A\beta^{1-42}$ suggests that these receptors may have a role in the pathogenesis of AD. Furthermore, the development of selective $\alpha 7\beta 2$ nAChR ligands may help to address the role of these receptors in AD and other disorders and diseases linked to the $\alpha 7$ receptor.

Are there other differences in functional properties (e.g., single-channel properties, conductance, and open probability) between $\alpha 7\beta 2$ and $\alpha 7$ nAChRs? This information will tell us more about the potential role of the $\alpha 7$ – $\beta 2$ interface and the influence of $\beta 2$ subunits on the properties of the $\alpha 7\beta 2$ nAChRs.

Are there differences in Ca^{2+} permeability between $\alpha 7$ homomeric and $\alpha 7\beta 2$ heteromeric receptors? Even in the case of absence of a change in relative Ca^{2+} permeability, the significantly slower current kinetics might mean that significantly more overall Ca^{2+} influx will occur per stimulation. This could be an important difference with regard to the functional impact of the $\alpha 7\beta 2$ nAChRs.

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Trends

There is a novel type of heteromeric $\alpha 7\beta 2$ nAChR that is expressed in heterologous expression systems and naturally in brain neurons.

Compared to classic homomeric $\alpha 7$ nAChRs, the $\alpha 7\beta 2$ nAChR exhibits distinctive function and pharmacology.

Native $\alpha 7\beta 2$ nAChRs are specifically expressed in brain regions associated with learning and memory, such as basal forebrain and hippocampus, suggesting an important target for cholinergic modulation of brain cognitive function.

The $\alpha 7\beta 2$ nAChRs are highly sensitive to pathologically-relevant levels of amyloid β peptides, suggesting a new target for Alzheimer's disease pathogenesis and therapeutics.

Figure 1.

Cartoon Figure Showing Homomeric and Heteromeric $\alpha 7$ Nicotinic Acetylcholine Receptors (nAChRs). Yellow dots indicate the $\alpha 7$ - $\alpha 7$ interface for ligand binding sites, blue dots the $\alpha 7$ - $\beta 2$ interface, the orange dot indicates the $\beta 2$ - $\beta 2$ interface, and black dots the $\beta 2$ - $\alpha 7$ interface. It is important to note that the subunit ratio (s) and subunit-subunit associations within native $\alpha 7\beta 2$ nAChR subtypes are not yet known, although studies using heterologous expression systems indicate that multiple arrangements are compatible with $\alpha 7\beta 2$ nAChR function.

Figure 2.

Comparison of Current Kinetics Between $\alpha 7$ and $\alpha 7\beta 2$ nAChR-Mediated Currents. (A) Choline-induced inward currents in dissociated rat ventral tegmental areas (VTA) dopamine (DA) neuron (left) and vertical diagonal band of Broca (VDB) cholinergic (Cho) neuron (middle) demonstrate a slow current kinetic and wide current duration in VDB cholinergic neurons. (B) In transfected *Xenopus* oocytes, compared to homomeric $\alpha 7$ nAChRs, the $\alpha 7\beta 2(1:1)$ nAChR-mediated current shows small amplitude, a slow decay constant, and longer current duration.

Table 1
Pharmacological comparison of nAChR agonists between $\alpha 7$ and $\alpha 7\beta 2$ (1:10) expressed in *Xenopus* oocytes

Agonists	$\alpha 7$		$\alpha 7\beta 2$ (1:10)			Reference
	EC ₅₀ (μM)	E _{max} (%)	Hill	EC ₅₀ (μM)	E _{max} (%)	Hill
ACh	180.0	113	1.4	300.0 (1.7)	115 (1.0)	1.6
	113.0	106	1.2	170.0 (1.5)	107 (1.0)	1.7
Nicotine	36.0	87	2.2	123.0 (3.4)	45 (0.5)	1.5
Choline	3100.0	116	1.7	5200.0 (1.7)	37 (0.3)	1.1
	972.0	108	2.1	1355.0 (1.4)	75 (0.7)	1.7
CCh	800.0	90	1.4	1800.0 (2.3)	42 (0.5)	1.3
	779.0	118	1.6	923.0 (1.2)	76 (0.6)	1.4
Epibatidine	1.3	101	1.3	1.4 (1.0)	91 (0.9)	1.4
	0.5	98	2.7	2.6 (5.2)	99 (1.0)	1.0
Cytisine	28.0	71	2.3	113.0 (4.0)	16 (0.2)	1.5
	5.0	91	0.8	2.7 (0.5)	19 (0.2)	1.3
PNU-282987	1.4	100	0.6	11.0 (7.9)	32 (0.3)	0.7
TC-1698	0.4	106	1.1	32.0 (80.0)	14 (0.1)	0.8
TC-5619	0.9	111	0.8	50.0 (55.6)	34 (0.3)	1.2
TC-7020	1.2	80	0.9	32.0 (26.7)	14 (0.2)	0.8
EVP-6124	1.1	75	0.3	40.0 (36.4)	12 (0.2)	1.2
Compound-A	0.3	88	1.1	9.8 (32.7)	65 (0.7)	0.5
Compound-B						

Note: The numbers after EC₅₀ and E_{max} in $\alpha 7\beta 2$ (1:10) panel indicate the ratio of $\alpha 7\beta 2/\alpha 7\beta 2$.

Table 2
Pharmacological comparison of nAChR antagonists between $\alpha 7$ and $\alpha 7\beta 2$ oocytes

Agonists	$\alpha 7$						$\alpha 7\beta 2$			Reference
	IC ₅₀ (μM)	Bottom (%)	Hill	IC ₅₀ (μM)	Bottom (%)	Hill				
MLA (oocytes)	0.00038	0	2.2	0.00030 (0.8)	0 (0)	1.7 (1:10)	#21			
(oocytes)	0.00089		1.7	0.0013 (1.5)		1.6 (1:10)	#16			
(Native)	0.00019	0	1.4 (VTA)	0.0012 (0.6)	0 (0)	1.3 (VDB)	#19			
DHβE (oocytes)	9.7	0	1.9	5.5 (1.1)	0 (0)	1.2 (1:10)	#21			
(oocytes)	47		2.7	18 (0.4)		1.1 (1:10)	#16			
(Native)	>10	80	1.0 (VTA)	0.18	0 (0)	0.8 (VDB)	#19			
DMAB (oocytes)	24	49	2.0	9 (0.4)	15 (0.3)	1.9 (1:10)	#20			
Compound-A (oocytes)	n.d.	n.d.	n.d.	26	6	0.6 (1:10)	#20			

The n.d. means no detectable inhibition was found with Compound-A on $\alpha 7$ receptors.