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Naturally-expressed nicotinic acetylcholine receptor subtypes

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Abstract

Nicotinic acetylcholine receptors (nAChRs) warrant attention, as they play many critical roles in brain and body function and have been implicated in a number of neurological and psychiatric disorders, including nicotine dependence. nAChRs are composed as diverse subtypes containing specific combinations of genetically-distinct subunits and that have different functional properties, distributions, and pharmacological profiles. There had been confidence that the rules that define ranges of assembly partners for specific subunits were well-established, especially for the more prominent nAChR subtypes. However, we review here some newer findings indicating that nAChRs having largely the same, major subunits exist as isoforms with unexpectedly different properties. Moreover, we also summarize our own studies indicating that novel nAChR subtypes exist and/or have distributions not heretofore described. Importantly, the nAChRs that exist as new isoforms or subtypes or have interesting distributions require alteration in thinking about their roles in health and disease.

Keywords

nicotine; nicotinic receptor; acetylcholine; Alzheimer's disease; drug dependence

1. INTRODUCTION

nAChRs are prototypical members of the ligand-gated ion channel superfamily of neurotransmitter receptors. nAChRs represent both classic and contemporary models for the establishment of concepts pertaining to mechanisms of drug action, synaptic transmission, and structural/functional diversity of transmembrane signaling molecules (see reviews [1–7]). nAChRs are found throughout the nervous system (e.g., in muscle, autonomic and sensory ganglia, and the CNS). They are very important, because they play many critical roles in brain and body function, making it logical that nAChRs also are implicated in a number of neurological and psychiatric disorders, as they are in nicotine dependence. nAChRs exist as multiple, diverse subtypes composed as pentamers of unique combinations from a family of at least seventeen ($\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, γ , δ , ϵ) similar, but genetically-distinct, subunits. nAChR subtypes are named according to their known subunit composition (using an “*” to indicate possible additional assembly partners; [6]). Each subunit gene has a unique promoter, even though some are collected in a cluster, suggesting a means for cell-

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specific expression. There also are unique protein sequence elements for each, especially in the large, cytoplasmic loop, suggesting means for differential post-translational control of subunit trafficking. There is evidence for specificity of targeting of nAChR subunit proteins and the relevant nAChR assemblies to sub- or peri-synaptic destinations in somatodendritic domains, but also down axons to pre-terminal or synaptic terminal locations. Although many nAChR subtypes are possible in theory, there seem to be some rules that define and limit the number of viable subunit combinations. Most of these nAChR subtypes appear to exist as heteropentamers containing two or more different kinds of subunit. 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., $\alpha 4\beta 2$ -nAChRs). $\beta 3$ and $\alpha 5$ subunits are “wild-cards” not able to form nAChRs alone or with any other single type of subunit. However, they are capable of integrating into complexes with two other subunit types found in binary complexes to form distinctive, trinary complexes (such as $\alpha 4\beta 2\alpha 5$ - or $\alpha 3\beta 4\alpha 5$ -nAChRs (found naturally expressed). They also can contribute to formation of quaternary complexes that contain more than one of the $\alpha 2$ -4 or $\alpha 6$ subunits or that contain both $\beta 2$ or $\beta 4$ subunits (for example, $\alpha 4\alpha 6\beta 2\beta 3$ - or $\alpha 3\beta 2\beta 4\alpha 5$ -nAChRs). In addition, mammalian muscle-type nAChRs are quaternary complexes composed of $\alpha 1$, $\beta 1$, δ and either γ (fetal) or ϵ (adult) subunits. By contrast, phylogenetically ancient nAChR $\alpha 7$ subunits are able to form functional homopentamers, the simplest possible prototype for a ligand-gated ion channel. Although nAChR $\alpha 9$ subunits also are able to form functional homomers with modest channel activity, function is markedly enhanced when they and $\alpha 10$ subunits co-assemble to form a novel binary complex [8] (note that these subunits and the unusual nAChRs they constitute are not substantially expressed in the brain). nAChRs containing $\alpha 7$ subunits ($\alpha 7$ -nAChRs) are the most abundant curaremimetic neurotoxin-binding nAChRs in the brain. nAChRs containing $\alpha 4$ and $\beta 2$ subunits ($\alpha 4\beta 2^*$ -nAChRs) are the most abundant high affinity nicotine-binding nAChRs in the brain. However, other, less abundant nAChRs (e.g., $\alpha 3^*$ -nAChRs, $\alpha 6^*$ -nAChRs) must exist and may play important physiological roles.

Nevertheless, the field has been somewhat altered by realization that the lack of two-fold symmetry in pentameric assemblies allows for more diversity across nAChR subtypes than heretofore realized. More recent work has indicated that even for $\alpha 4\beta 2^*$ -nAChR, having two $\alpha 4\beta 2$ subunit cassettes thought to provide an $\alpha 4:\beta 2$ subunit interface where nicotinic agonists bind to gate channel opening, there exist unique isoforms that have different subunits occupying the “fifth” or “accessory” position in the pentamer [9–12]. Remarkably, the pharmacological properties of these isoforms can be quite different. For example, $\alpha 4\beta 2^*$ -nAChR having 2 $\alpha 4$ subunits and 3 $\beta 2$ subunits [$(\alpha 4)_2(\beta 2)_3$ -nAChR; i.e., having a $\beta 2$ subunit in the “fifth” or “accessory” position] have higher sensitivity for many nicotinic agonists than “low sensitivity” $(\alpha 4)_3(\beta 2)_2$ -nAChR having an $\alpha 4$ subunit in the accessory position. Moreover, wild-card subunits $\alpha 5$ or $\beta 3$ can occupy the fifth position, creating $(\alpha 4)_2(\beta 2)_2\alpha 5$ - or $(\alpha 4)_2(\beta 2)_2\beta 3$ -nAChR having yet again distinctive pharmacological character. It is likely that further diversity exists in other complexes that contain, for example, $\alpha 4$ and $\alpha 6$ subunits. The characterization of these isoforms presents new and larger challenges than before. Although the physiological implications of this unexpectedly broader diversity are currently poorly understood, they are bound to influence our understanding of phenomena such as nicotine dependence as well as strategies for translation of nAChR drug discovery to treatment of neuropsychiatric disorders.

Functionally, nAChRs in the brain play roles not only in the mediation of classical, excitatory, cholinergic neurotransmission at selected loci, but also and perhaps more globally in the modulation of neurotransmission by other chemical messengers, including glutamate, GABA, the monoamines dopamine, norepinephrine and serotonin, and acetylcholine (ACh) itself [2–5, 13–17]. This means that some nAChR subtypes have

postsynaptic (or peri-synaptic), somatodendritic localizations, whereas others have pre-synaptic dispositions (i.e., on neuronal terminals). However, care should be exercised in calling some nAChRs according to their disposition in synaptic space. Indeed, so called “pre-synaptic” nAChRs that reside on nerve terminals and that perhaps locally modulate neurotransmitter release might actually be called “post-synaptic” if they lie under cholinergic nerve endings. It probably is wise to speak of nAChRs with respect to their location on soma, dendrites, nerve terminals, or even processes slightly distal to nerve terminals. Moreover, some nAChRs have been implicated in processes such as the structuring and maintenance of neurites and synapses [18–20] and even in modulation of neuronal viability/death [21–24]. Thus, nAChR subtypes in the brain play complex and interesting roles in modulation of the chemical milieu of the brain, in completion of neuronal circuits, and perhaps in development and architecture of synapses. In this review, we summarize some of the recent progress in studies of naturally-expressed nAChR subtypes in the brain and their function, and we highlight just some of the possible roles for nAChRs in diseases.

2. DISCUSSION

2.1 $\alpha 4^*$ -nAChRs in the brain

nAChRs that bind radiolabeled nicotine with the highest affinity contain $\alpha 4$ subunits ($\alpha 4^*$ -nAChR; see reviews and/or tables by [1–7]. Immunoassays have shown that the predominant, naturally expressed form of $\alpha 4^*$ -nAChRs in the vertebrate brain contains $\alpha 4$ and $\beta 2$ subunits ($\alpha 4\beta 2$ -nAChRs) [25, 26]. $\alpha 4\beta 2$ -nAChRs have been implicated in nicotine self-administration, reward, and dependence, and in diseases such as Alzheimer's and epilepsy [1–5, 27–33]. $\alpha 4$ subunits can also assemble with $\beta 4$ subunits to form $\alpha 4\beta 4$ -nAChRs that have comparably high nicotine affinity [34–36]. $\beta 4$ subunit mRNA colocalizes with $\alpha 4$ subunit mRNA in many brain regions [37–38] that could be involved in complex behaviors including nicotine dependence. Moreover, in addition to existence in “binary” nAChR complexes containing two types of subunits, $\alpha 4$ subunits can have more than one type of assembly partner [39–56]. For example, nAChRs containing $\alpha 4$, $\beta 2$, and $\alpha 5$ subunits are expressed naturally, and heterologously-expressed $\alpha 4\beta 2\alpha 5$ -nAChRs have interesting properties distinct from those of simpler $\alpha 4\beta 2$ -nAChR. To the first approximation, properties of heterologously expressed $\alpha 4^*$ -nAChRs and naturally-expressed $\alpha 4^*$ -nAChRs of the same composition (as best can be ascertained) are very similar (op. cit.). However, given their evident physiological importance and their potential to form as diverse combinations of subunits, including $\alpha 4\beta 2\alpha 5$ - or $\alpha 4\beta 2\beta 3$ -nAChR isoforms containing the indicated subunits in the accessory position, more work on heterologously expressed $\alpha 4^*$ -nAChRs is warranted, as are careful comparisons of properties of these $\alpha 4^*$ -nAChRs of defined subunit composition(s) with properties of naturally expressed $\alpha 4^*$ -nAChRs in vertebrate brain neurons.

Considerable attention has been given to effects of chronic nicotine exposure on nAChRs because of relevance to habitual use of tobacco products [57] but information about these effects remains incomplete. Chronic nicotine exposure for periods of days induces increases in numbers of nAChR-like radioligand binding sites (or, in some studies, subunit polypeptides) in human or non-human animal brain or in cells from a variety of primary or continuous culture systems [2–5, 26, 58–62]. Although these binding sites can be in intracellular pools of possible assembly intermediates and not always on functional, cell surface nAChRs, the magnitude of their upregulation varies considerably across experiments and experimental systems, and concentration-response studies show that nAChR subtypes most sensitive to nicotine-induced upregulation include $\alpha 4$ subunits [26, 61, 63–64]. Upregulation seems to reflect nicotine-mediated, “chaperone-like” facilitation of $\alpha 4$ and $\beta 2$ subunit assembly that is manifest as stabilization of $\alpha 4\beta 2$ -nAChR assembly intermediates in

a state or states detectable by radioagonist binding assays [64]. In terms of functional effects, nicotine acts acutely much in the way that ACh does, causing opening of nAChR channels. However, many studies indicate that nicotine exposure for seconds-minutes-hours leads to losses in nAChR function. Again, there is great diversity in experimental systems used and in magnitudes, kinetics, and dose-dependence of effects, partly reflecting variations in techniques and diversity in experimental systems used [2–6, 61, 65–68]. There seems to be a consensus that there is at least one stage of reversible functional loss on shorter-term (seconds-minutes) nicotine exposure called "desensitization," and that a more slowly reversible (or irreversible?) loss of nAChR function occurs on longer-term (tens of minutes-hours) nicotine exposure via a process called "persistent inactivation" by some [61, 68–69]. Desensitization or persistent inactivation of a particular nAChR subtype occur at much lower concentrations of nicotine and for shorter exposure times than necessary to induce upregulation of binding sites, and acute function or persistent inactivation can be blocked under conditions that allow upregulation to occur, suggesting distinction between these processes [61]. There seemed to be a consensus that persistent inactivation of $\alpha 4\beta 2$ -nAChR function also occurs on chronic nicotine exposure not only in the brain but also in a variety of heterologous expression systems and for $\alpha 4\beta 2$ -nAChRs from various species including humans [70–73]. Perhaps importantly, most studies examining effects of chronic nicotine exposure in vivo or in vitro typically use 10 days or shorter exposure. Because human smokers are exposed to nicotine for months-years, laboratory work to date might be providing a misleading view of effects on nAChR expression related to smoking behavior.

2.2 $\alpha 7$ -nAChRs in the brain

Another prominent nAChR subtype found in vertebrate central and autonomic nervous systems contains $\alpha 7$ subunits ($\alpha 7$ -nAChRs). These sites have been known to exist for many years based on their ability to bind the curaremimetic neurotoxin, α -bungarotoxin (Bgt) [74–78]. They long have been known to exhibit many of the biochemical and pharmacological features of true nAChRs, to have brain distributions sub- or peri-synaptic to cholinergic terminals, to have levels of expression sensitive to chronic nicotine exposure and/or modification of cholinergic inputs, and to reveal hints of functional significance in electrophysiological studies. However, their physiological relevance was elusive, and their functional study was confounded until heterologous expression studies of $\alpha 7$ -nAChR composed as homomers revealed unusually rapid, agonist-induced, calcium ion-permeable channel opening and inactivation [79–83]. Subsequently, renewed searches for functions of natural Bgt-binding nAChRs uncovered short-lived, nicotine-gated, toxin-sensitive, inward currents and/or elevations of intracellular Ca^{2+} in chick autonomic neurons [84], in human ganglionic neuron-like clonal cells [85], or in rat CNS neurons [16, 40–44, 86–91]. Many of the cell types naturally expressing Bgt-sensitive, functional nAChRs have been shown to express $\alpha 7$ genes as well as some native form of Bgt-binding nAChRs [68, 92–93]. Knock out of the $\alpha 7$ gene leads to absence of Bgt-binding nAChRs in cell lines or in mice [85, 94]. Thus, correlations have been drawn between the expression of a major form of Bgt-binding nAChRs and $\alpha 7$ gene products.

By virtue of their unique subcellular localizations, channel kinetics and Ca^{2+} permeability, $\alpha 7$ -nAChRs appear to have novel functional roles in addition to (i.e., distinct from) the mediation of classical excitatory neurotransmission. For example, Bgt-sensitive $\alpha 7$ -nAChRs have been implicated in processes such as vicinal control of neurotransmitter release [7, 14], development and maintenance of neurites and synapses [18–20], long-term potentiation [95–96], seizures [97], and neuronal viability/death [21–24]. These intriguing findings underscore the need for further characterization of functional $\alpha 7$ -nAChRs.

Because of their homomeric nature in heterologous expression systems, $\alpha 7$ -nAChRs and related chimeric receptors have proven to be very useful in mutagenesis-based studies that

have provisionally identified structures and residues contributing to ligand binding, subunit interactions, and/or lining the ligand-gated ion channel [98–104]. These $\alpha 7$ -nAChR homomers, perhaps reflecting the phylogenetically ancient status of $\alpha 7$ subunits, represent one of the simplest possible prototypes for elucidation of nAChR structure and function, and they are ideally suited for studies of nAChR structure-function relationships. Bgt-sensitive, functional or ligand binding activities of heterologously-expressed, homomeric $\alpha 7$ -nAChRs are similar to those of native Bgt-binding nAChRs in cells or tissues that express $\alpha 7$ subunits [85, 105–107], suggesting the possibility that many if not all nAChRs containing $\alpha 7$ subunits exist as homomers. Some studies of native $\alpha 7$ -nAChRs also suggest that they exist as homomers [2–5, 108–109].

However, some nAChRs in neurons have been postulated to contain $\alpha 7$ plus other kinds of subunit [5, 13, 110–112]. For instance, heterologous expression work has indicated that nAChR $\alpha 7$ and $\beta 2$ subunits can assemble together to form heteromeric, functional channels [113], and histological studies have shown that there is co-expression of nAChR $\alpha 7$ and $\beta 2$ subunits in most forebrain cholinergic neurons [110]. We discovered a pharmacologically-unusual nAChR subtype that is naturally expressed in the rat basal forebrain, and we obtained evidence that this subtype is a heteropentameric $\alpha 7\beta 2$ -nAChR that is absent in nAChR $\beta 2$ subunit knock-out mice [91]. Interestingly, these $\alpha 7\beta 2$ -nAChRs exhibit high sensitivity to pathological levels of β -amyloid [91]. These data suggest that whereas most $\alpha 7$ -nAChRs are formed as homomeric pentamers, others may exist as heteromers, including possible $\alpha 7\beta 2$ -nAChR pentamers (Fig. 1). The fact that oligomeric amyloid- β seems to have high affinity for $\alpha 7\beta 2$ -nAChRs expressed in the brain region that is damaged early in Alzheimer's disease may have implications for disease etiopathogenesis.

2.3 $\alpha 6^*$ -nAChRs in the brain

$\alpha 6$ subunits are not widely expressed in the brain, but they are prevalent in midbrain dopaminergic (DAergic) regions associated with pleasure, reward, and mood control (Fig. 2) [114–115], suggesting that $\alpha 6^*$ -nAChRs play critical roles in nicotine dependence and in the ability to modulate mood and emotion attributed to nicotine [116]. Until a report from Lindstrom's laboratory [117] of heterologous expression in oocytes of nAChRs containing $\alpha 6$ subunits, these subunits were of "orphan" status. Kuryatov et al. [118] found functional expression of human $\alpha 6$ plus $\beta 4$ plus $\beta 3$ subunits in an oocyte system, but functional expression in that system was poor for other combinations and was poor for responses to nicotine relative to those for ACh. More recent results suggest success in using alternative strategies to express $\alpha 6^*$ -like nAChRs [119–120]. Using transgenic mice expressing gain-of-function nAChR $\alpha 6$ subunits, the presence of functional $\alpha 6^*$ -nAChRs on ventral tegmental area (VTA) DAergic neurons has been reported [121]. Recently, we reported a novel discovery that functional $\alpha 6^*$ -nAChRs are located on GABAergic presynaptic boutons associated with VTA DAergic neurons, where these $\alpha 6^*$ -nAChRs mediate nicotinic modulation of GABA release onto those DAergic neurons [122]. This adds to literature indicative of many roles for nAChR in direct and indirect modulation of DAergic neuronal function.

The development of α -conotoxins (α -Ctx) as tools for investigating neuronal nAChRs [123] has provided much-needed assistance to $\alpha 6^*$ -nAChR investigations. Initially, α -CtxMII was thought to be highly selective for $\alpha 3\beta 2^*$ -nAChRs [124] and became a useful tool for investigating receptor structure [125]. Based on sensitivity to α -CtxMII, pre-synaptic nAChRs on striatal DAergic terminals were divided into two classes: " α -CtxMII-sensitive" and " α -CtxMII-resistant" [126–127]. Because α -CtxMII was expected to block any nAChRs containing the $\alpha 3\beta 2$ subunit interface, the α -CtxMII-sensitive receptors were identified as $\alpha 3\beta 2^*$ -nAChRs, but in $\alpha 3$ knockout mice, the majority of ^{125}I - α -CtxMII binding (including in terminal regions of VTA/substantia nigra (SN) DAergic projections) is not eliminated

[128], suggesting that non- $\alpha 3^*$ -nAChRs account for most α -CtxMII-sensitive nAChRs. The $\alpha 6$ subunit represented a likely alternate component of ^{125}I - α -CtxMII binding sites since it is closely related to the $\alpha 3$ subunit, and residues in $\alpha 3$ subunits found to be critical for α -CtxMII sensitivity [125] are conserved in $\alpha 6$ subunits.

Other " $\alpha 3\beta 2$ -specific" antagonists, such as neuronal- or kappa-bungarotoxin and α -CtxPnIA, have also turned out to block $\alpha 6^*$ -nAChRs [129]. The sensitivity of $\alpha 6^*$ -nAChRs to α -CtxMII and the colocalization of $\alpha 6$ and $\beta 3$ with $\beta 2$ subunits in VTA DAergic neurons further suggested that the α -CtxMII-sensitive component of nicotine-mediated DA release might contain these subunits. Indeed, striatal ^{125}I - α -CtxMII binding was eliminated upon $\alpha 6$ subunit gene deletion [130], and a $\beta 3$ subunit-null mutation drastically reduced (although it did not abolish) expression of these receptors [131]. Thus, α -CtxMII appears to be a useful tool for recognizing naturally-expressed $\alpha 6^*$ -nAChRs, but one limitation is that this compound poorly distinguishes between $\alpha 6\beta 2^*$ - and $\alpha 3\beta 2^*$ -nAChR subtypes. The more selective antagonist of $\alpha 6^*$ -nAChR, α -CtxPIA [132–134] exhibits 75-fold higher affinity for rat $\alpha 6\beta 2^*$ -nAChRs than for rat $\alpha 3\beta 2^*$ -nAChRs [134].

There is an emerging consensus that nAChR $\alpha 6$ subunit mRNA and proteins are distributed in brain regions thought to be involved in reward and drug reinforcement, in theory being involved in DA release [135]. This makes the hypothesis particularly intriguing that $\alpha 6^*$ -nAChRs play roles in nicotine dependence. These same brain regions in which $\alpha 6$ messages and proteins have been found also are implicated in attention, mood control, and neurological or psychiatric disorders, including anxiety, depression, and Parkinson's disease [116]. Other studies from our laboratory [136–137] and from others [138–139] suggest interactions between antidepressants and some nAChR subtypes, but specific studies examining $\alpha 6^*$ -nAChRs have not yet been performed. The high incidence of tobacco use by mentally ill individuals, coupled with reports of the mood-stabilizing effects of nicotine in tobacco users, implicate nAChRs in the control of mood and emotion, and $\alpha 6^*$ -nAChRs are candidates for mediation of these effects [116].

There is good evidence that $\alpha 6^*$ -nAChRs, in particular, modulate neurotransmitter release in multiple brain regions. Based on studies using α -CtxMII in combination with nAChR subunit-null mutant mice, Salminen et al. [140] determined that striatal pre-synaptic nAChRs mediating DA release contain one ($\alpha 4\alpha 6\beta 2\beta 3$ subtype) or two ($\alpha 6\beta 2\beta 3$ subtype) α -CtxMII-sensitive ACh binding sites indicating that pre-synaptic $\alpha 6^*$ -nAChRs contribute to nicotinic modulation of DA release in the striatum. These subunit assignments were confirmed using immunochemical approaches by Gotti et al. [131]. Also, Endo et al. [141] found naturally-expressed $\alpha 3\beta 2$ - and $\alpha 6\beta 2$ -nAChRs on superior colliculus neurons, and these receptors are likely located on pre-synaptic terminals of GABAergic neurons where they modulate GABA release. Interestingly, Champiaux et al. [130] demonstrated that the majority of DAergic cell-body nAChR function in the VTA is mediated by non- $\alpha 6^*$ -nAChRs. Most of the $\alpha 6^*$ -nAChRs synthesized in VTA DAergic cell bodies appear to be transported to cell terminal regions (such as striatum and nucleus accumbens) [131]. However, our recent findings indicate that there also are functional nAChRs located on GABAergic, pre-synaptic boutons associated with these VTA DAergic cell bodies (Fig. 2) [122]. Agonist activation of these nAChRs results in increased inhibitory post synaptic currents (IPSCs) measured at DAergic cell bodies. Our data indicate that these receptors are predominantly of the $\alpha 6\beta 2^*$ subtype, because treatment with $\alpha 6\beta 2^*$ -selective nAChR antagonists (either α -CtxMII or α -CtxPIA; 1 nM) or deletion of the nAChR $\beta 2$ subunit abolishes nicotine-induced increases in inhibitory post-synaptic currents [122].

2.4 Studies of heterologously expressed nAChRs

Given the complexity of subunit composition (types and stoichiometries) in nAChR subtypes, it might be expected that heterologous expression in oocytes or cell lines might be a boon. However, although there have been some successes (see above and [2, 5, 142], it has not been easy to develop cell lines that stably express specific nAChR subtypes. Moreover, when expression is driven by introduction of loose subunits, each encoded by its own vector, investigators are at the mercy of cell with regard to the mixture of closed assemblies. There is some success and significant promise in work using concatenated subunits to allow investigators to control and specify subunit stoichiometries and arrangements, but we will likely benefit from review of that work in the near future rather than here, allowing the field to further develop.

2.5 Conclusions

It is clear that naturally-expressed nAChR diversity is broader than heretofore suspected, bringing new challenges to receptor characterization. However, great opportunities and new ideas with profound translational implications come from this. For example, the realization that distinctive pharmacological properties in native nAChRs in regions where $\alpha 4$ and $\beta 2$ are expressed could be due to expression as $\alpha 4$ or $\beta 2$ or other isoforms of $\alpha 4\beta 2$ -nAChRs (Fig. 1) [143] could explain puzzling, earlier findings. Perhaps more importantly, consideration needs to be given to how high sensitivity and low sensitivity $\alpha 4\beta 2$ -nAChRs in humans would respond to nicotine at pharmacological concentrations attained in smokers, interstitial fluid acetylcholine that is present at concentrations that rival those of nicotine in smokers, or synaptic acetylcholine that is one hundred times more concentrated or greater. The finding that the brain region affected early in Alzheimer's disease contains a unique, heteropentameric $\alpha 7\beta 2$ -nAChRs highly sensitive to β -amyloid [91] might alter thinking about roles for nAChRs in that disease. Discovery of $\alpha 6^*$ -nAChRs on GABA terminals associated with VTA DAergic neuronal soma [122] brings a new dimension to our understanding of nicotine's effects and nAChR's roles in drug dependence. It is likely that additional findings will also emerge, contributing to further evolution in our understanding about nAChRs and their physiological, pathological and pharmacological roles.

Non-standard abbreviations

nAChR(s)	nicotinic acetylcholine receptor(s)
VTA	ventral tegmental area
DAergic	dopaminergic
DA	dopamine
SN	substantia nigra
ACh	acetylcholine
IPSC(s)	inhibitory post synaptic current(s)
Bgt	α -bungarotoxin
αCtx	α -conotoxin

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Fig. 1.

Schematic illustrations are shown as viewed from synaptic space of selected nicotinic acetylcholine receptor (nAChR) subtypes, all of which are assembled as pentamers of subunits forming a rosette with a central pore, which is the ion channel. In the upper part of the figure are shown the presumed structures of: left - a homomeric $\alpha 7$ -nAChR composed only of $\alpha 7$ subunits (blue) as found, for example, in the ventral tegmental area; right - a heteromeric $\alpha 7\beta 2$ -nAChR composed of $\alpha 7$ subunits (blue) and $\beta 2$ subunits (gray) as found, for example, on basal forebrain cholinergic neurons except in mice lacking $\beta 2$ subunits [91]. The heteromeric, presumably $\alpha 7\beta 2$ -nAChR subtype displays elevated sensitivity to functional blockade by β -amyloid or dihydro- β -erythrodine than seen for homomeric $\alpha 7$ -nAChRs [91]. In the lower part of the figure are shown isoforms of $\alpha 4\beta 2$ -nAChRs having a 3:2 (left) or a 2:3 (right) ratio of $\alpha 4$: $\beta 2$ subunits ($\alpha 4$ subunits, lavender). ($\alpha 4$)₃($\beta 2$)₂-nAChRs have comparatively low sensitivity to nicotinic agonists when compared to “high sensitivity,” ($\alpha 4$)₂($\beta 2$)₃-nAChRs [9–12]. Studies based on heterozygotic mice with lower gene doses for nAChR $\alpha 4$ subunits indicate that proportions of functional expression of high sensitivity ($\alpha 4$)₂($\beta 2$)₃-nAChRs are decreased and that the opposite occurs in nAChR subunit $\beta 2$ +/- heterozygotic mice, suggesting that natural expression of these two isoforms occurs, could be regulated, and has physiological relevance [143].

Fig. 2.

Diagram indicating some of the complexity in nicotinic acetylcholine receptor (nAChR) expression across cell types and at different locations relative to the synapse, using the ventral tegmental area (VTA) as an example. It has been known for some time that $\alpha 7$ -nAChRs (turquoise) are present on glutamatergic nerve terminals (Glu, red) synapsing on dopaminergic (DA) cell bodies (and perhaps dendrites; DA, orange triangle) in the VTA [2–5]. Moreover, different nAChRs, presumably responding to acetylcholine released from cholinergic nerve terminals (purple) have a variety of distributions on DA neurons in the VTA. In fact, it recently became clear that functional nAChR phenotypes allow “binning” of VTA DA type ID, type IID and type IIID neuronal subsets predominantly expressing, respectively, $\alpha 4\beta 2$ -nAChRs (lavender), $\alpha 7$ -nAChRs (turquoise), or $\beta 4^*$ -nAChRs (blue) on soma and/or dendrites [90]. This means that there isn’t just one kind of VTA DA neuron, at least with respect to nAChR expression profiles. Whether these VTA DA functional nAChR phenotypes change with nicotine exposure and/or are on VTA VDA neurons that also receive distinctive inputs or have different targets remains to be determined. Moreover, newly appreciated is the presence of $\alpha 6^*$ -nAChRs (green) not only on VTA DA nerve terminals ending in the NAc and perhaps on VTA soma (not shown in this illustration) [121, 130–131], but also on GABAergic boutons (black) associated with VTA DA neuronal soma and/or proximal dendrites [122]. In addition, on GABAergic terminals, pre-terminals and/or soma, there are $\alpha 4\beta 2$ -nAChRs [144]. The balance between activation and desensitization or persistent inactivation of different nAChR subtypes on VTA nerve terminals ending on DA

neurons and on those neurons and their own terminals likely dictates VTA DA neuronal activity relevant to nicotine dependence and perhaps to cholinergic control of dependence on other agents as well as more generally in mood and reward.

TABLE 1

Different properties and locations of homomeric and heteromeric $\alpha 7^*$ -nAChR. Studies of acutely dissociated, dopaminergic neurons from the ventral tegmental area (VTA) indicate their expression of homomeric $\alpha 7$ -nAChR, whereas studies using similar techniques and cholinergic neurons from the ventral aspect of the nucleus of the diagonal band (VDB) are consistent with expression there of heteromeric $\alpha 7\beta 2$ -nAChR that are absent in nAChR $\beta 2$ subunit knock-out mice [90–91]. Some of the properties distinguishing these $\alpha 7$ - and $\alpha 7\beta 2$ -nAChR are summarized here.

	$\alpha 7\beta 2$ nAChRs in VDB	$\alpha 7$ nAChRs in VTA
Choline response		
Rising time (ms)	72.1 $\pm$ 9.1	29.1 $\pm$ 2.9 (n=12) ***
Decay time (ms)	28.6 $\pm$ 2.8	10.2 $\pm$ 1.5 (n=12) ***
IC50 (nM)		
Methyllycaconitine	0.7	0.4
Dihydro- β -erythroidine	200	>100,000
A β_{1-42} inhibition (%)		
1 nM	35 $\pm$ 8	7 $\pm$ 3 **
10 nM	70 $\pm$ 9	10 $\pm$ 10 **

Note:

**
p<0.01,

p<0.001 for comparisons between $\alpha 7\beta 2$ - and $\alpha 7$ -nAChRs.