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Review

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.

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Contents

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 (α1–α10, β1–β4, 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-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.

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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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 α2, α3, α4, or α6 subunits can combine in binary fashion with β2 or β4 subunits to form ligand-binding and/or functional nAChRs (e.g., α4β2-nAChRs). β3 and α5 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 α4β2α5- or α3β4α5-nAChRs (found naturally expressed). They also can contribute to formation of quaternary complexes that contain more than one of the α2–4 or α6 subunits or that contain both β2 or β4 subunits (for example, α4α6β2β3- or α3β2β4α5-nAChRs). In addition, mammalian muscle-type nAChRs are quaternary complexes composed of α1, β1, δ and either γ (fetal) or ε (adult) subunits. By contrast, phylogentically ancient nAChR α7 subunits are able to form functional homopentamers, the simplest possible prototype for a ligand-gated ion channel. Although nAChR α9 subunits also are able to form functional homomers with modest channel activity, function is markedly enhanced when they and α10 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 α7 subunits (α7-nAChRs) are the most abundant curaremimetic neurotoxin-binding nAChRs in the brain. nAChRs containing α4 and β2 subunits (α4β2*-nAChRs) are the most abundant high affinity nicotine-binding nAChRs in the brain. However, other, less abundant nAChRs (e.g., α3*-nAChRs, α6*-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 α4β2*-nAChR, having two α4β2 subunit cassettes thought to provide an α4:β2 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, α4β2*-nAChR having 2 α4 subunits and 3 β2 subunits [$(\alpha4)_2(\beta2)_3$-nAChR; i.e., having a β2 subunit in the "fifth" or "accessory" position] have higher sensitivity for many nicotinic agonists than "low sensitivity" $(\alpha4)_3(\beta2)_2$-nAChR having an α4 subunit in the accessory position. Moreover, wild-card subunits α5 or β3 can occupy the fifth position, creating $(\alpha4)_2(\beta2)_2\alpha5$- or $(\alpha4)_2(\beta2)_2\beta3$-nAChR having yet again distinctive pharmacological character. It is likely that further diversity exists in other complexes that contain, for example, α4 and α6 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. α4*-nAChRs in the brain

nAChRs that bind radiolabeled nicotine with the highest affinity contain α4 subunits (α4*-nAChR; see reviews and/or tables by [1–7]. Immunoassays have shown that the predominant, naturally expressed form of α4*-nAChRs in the vertebrate brain contains α4 and β2 subunits (α4β2-nAChRs) [25,26]. α4β2-nAChRs have been implicated in nicotine self-administration, reward, and dependence, and in diseases such as Alzheimer's and epilepsy [1–5,27–33]. α4 subunits can also assemble with β4 subunits to form α4β4-nAChRs that have comparably high nicotine affinity [34–36]. β4 subunit mRNA colocalizes with α4 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, α4 subunits can have more than one type of assembly partner [39–56]. For example, nAChRs containing α4, β2, and α5 subunits are expressed naturally, and heterologously-expressed α4β2α5-nAChRs have interesting properties distinct from those of simpler α4β2-nAChR. To the first approximation, properties of heterologously expressed α4*-nAChRs and naturally-expressed α4*-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 α4β2α5- or α4β2β3-nAChR isoforms containing the indicated subunits in the accessory position, more work on heterologously expressed α4*-nAChRs is warranted, as are careful comparisons of properties of these α4*-nAChRs of defined subunit composition(s) with properties of naturally expressed α4*-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 α4 subunits [26,61,63,64]. Upregulation seems to reflect nicotine-mediated, "chaperone-like" facilitation of α4 and β2 subunit assembly that is manifest as

stabilization of α4β2-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 α4β2-nAChR function also occurs on chronic nicotine exposure not only in the brain but also in a variety of heterologous expression systems and for α4β2-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. α7*-nAChRs in the brain

Another prominent nAChR subtype found in vertebrate central and autonomic nervous systems contains α7 subunits (α7-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 α7-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 α7 genes as well as some native form of Bgt-binding nAChRs [68,92,93]. Knock out of the α7 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 α7 gene products.

By virtue of their unique subcellular localizations, channel kinetics and Ca^{2+} permeability, α7-nAChRs appear to have novel functional roles in addition to (i.e., distinct from) the mediation of classical excitatory neurotransmission. For example, Bgt-sensitive α7-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 α7-nAChRs.

Table 1
Different properties and locations of homomeric and heteromeric α7*-nAChR. Studies of acutely dissociated, dopaminergic neurons from the ventral tegmental area (VTA) indicate their expression of homomeric α7-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 α7β2-nAChR that are absent in nAChR β2 subunit knock-out mice [90,91]. Some of the properties distinguishing these α7- and α7β2-nAChR are summarized here.

	α7β2 nAChRs in VDB	α7 nAChRs in VTA
Choline response		
Rising time (ms)	72.1 ± 9.1	29.1 ± 2.9 (n = 12)***
Decay time (ms)	28.6 ± 2.8	10.2 ± 1.5 (n = 12)***
IC50 (nM)		
Methyllycaconitine	0.7	0.4
Dihydro-β-erythroidine	200	>100,000
$A\beta_{1-42}$ inhibition (%)		
1 nM	35 ± 8	7 ± 3**
10 nM	70 ± 9	10 ± 10**

** $p < 0.01$ for comparisons between α7β2- and α7-nAChRs.
*** $p < 0.001$ for comparisons between α7β2- and α7-nAChRs.

Because of their homomeric nature in heterologous expression systems, α7-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 α7-nAChR homomers, perhaps reflecting the phylogenitically ancient status of α7 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 α7-nAChRs are similar to those of native Bgt-binding nAChRs in cells or tissues that express α7 subunits [85,105–107], suggesting the possibility that many if not all nAChRs containing α7 subunits exist as homomers. Some studies of native α7-nAChRs also suggest that they exist as homomers [2–5,108,109].

However, some nAChRs in neurons have been postulated to contain α7 plus other kinds of subunit [5,13,110–112]. For instance, heterologous expression work has indicated that nAChR α7 and β2 subunits can assemble together to form heteromeric, functional channels [113], and histological studies have shown that there is co-expression of nAChR α7 and β2 subunits in most forebrain cholinergic neurons [110]. We discovered a pharmacologically-unusual nAChR subtype that is naturally expressed in the rat basal forebrain (Table 1), and we obtained evidence that this subtype is a heteropentameric α7β2-nAChR that is absent in nAChR β2 subunit knock-out mice [91]. Interestingly, these α7β2-nAChRs exhibit high sensitivity to pathological levels of β-amyloid [91]. These data suggest that whereas most α7-nAChRs are formed as homomeric pentamers, others may exist as heteromers, including possible α7β2-nAChR pentamers (Fig. 1). The fact that oligomeric amyloid-β seems to have high affinity for α7β2-nAChRs expressed in the brain region that is damaged early in Alzheimer's disease may have implications for disease etiopathogenesis.

2.3. α6*-nAChRs in the brain

α6 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 α6*-nAChRs play critical roles in nicotine

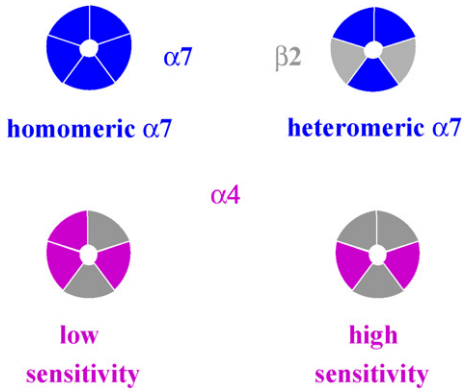

Fig. 1. Schematic illustrations are shown as viewed from synaptic space of selected nicotinic acetylcholine receptor (nAChR) subtypes, all of which are assembled as pentamers of subunits forming a rosette with a central pore, which is the ion channel. In the upper part of the figure are shown the presumed structures of: left – a homomeric α7-nAChR composed only of α7 subunits (blue) as found, for example, in the ventral tegmental area; right – a heteromeric α7β2-nAChR composed of α7 subunits (blue) and β2 subunits (gray) as found, for example, on basal forebrain cholinergic neurons except in mice lacking β2 subunits [91]. The heteromeric, presumably α7β2-nAChR subtype displays elevated sensitivity to functional blockade by β-amyloid or dihydro-β-erythrodine than seen for homomeric α7-nAChRs [91]. In the lower part of the figure are shown isoforms of α4β2-nAChRs having a 3:2 (left) or a 2:3 (right) ratio of α4:β2 subunits (α4 subunits, lavender). $(\alpha4)_3(\beta2)_2$-nAChRs have comparatively low sensitivity to nicotinic agonists when compared to "high sensitivity," $(\alpha4)_2(\beta2)_3$-nAChRs [9–12]. Studies based on heterozygotic mice with lower gene doses for nAChR α4 subunits indicate that proportions of functional expression of high sensitivity $(\alpha4)_2(\beta2)_3$-nAChRs are decreased and that the opposite occurs in nAChR subunit β2+/− heterozygotic mice, suggesting that natural expression of these two isoforms occurs, could be regulated, and has physiological relevance [143].

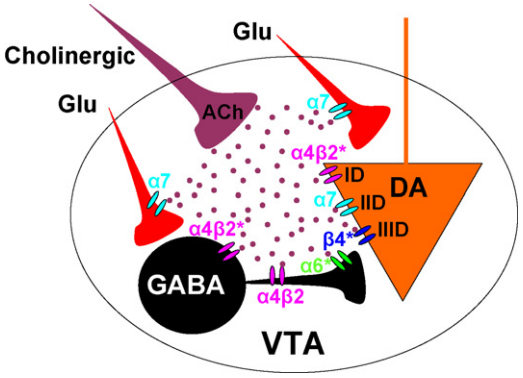

Fig. 2. Diagram indicating some of the complexity in nicotinic acetylcholine receptor (nAChR) expression across cell types and at different locations relative to the synapse, using the ventral tegmental area (VTA) as an example. It has been known for some time that α7-nAChRs (turquoise) are present on 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, α4β2-nAChRs (lavender), α7-nAChRs (turquoise), or β4*-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 α6*-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 α4β2-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.

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 α6 subunits, these subunits were of "orphan" status. Kuryatov et al. [118] found functional expression of human α6 plus β4 plus β3 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 α6*-like nAChRs [119,120]. Using transgenic mice expressing gain-of-function nAChR α6 subunits, the presence of functional α6*-nAChRs on ventral tegmental area (VTA) DAergic neurons has been reported [121]. Recently, we reported a novel discovery that functional α6*-nAChRs are located on GABAergic presynaptic boutons associated with VTA DAergic neurons, where these α6*-nAChRs mediate nicotinic modulation of GABA release onto those DAergic neurons [122]. This adds to literature indicative of many roles for nAChRs 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 α6*-nAChR investigations. Initially, α-CtxMII was thought to be highly selective for α3β2*-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 α3β2 subunit interface, the α-CtxMII-sensitive receptors were identified as α3β2*-nAChRs, but in α3 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-α3*-nAChRs account for most α-CtxMII-sensitive nAChRs. The α6 subunit represented a likely alternate component of ^{125}I-α-CtxMII binding sites since it is closely related to the α3 subunit, and residues in α3 subunits found to be critical for α-CtxMII sensitivity [125] are conserved in α6 subunits.

Other "α3β2-specific" antagonists, such as neuronal- or kappa-bungarotoxin and α-CtxPnIA, have also turned out to block α6*-nAChRs [129]. The sensitivity of α6*-nAChRs to α-CtxMII and the colocalization of α6 and β3 with β2 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 α6 subunit gene deletion [130], and a β3 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 α6*-nAChRs, but one limitation is that this compound poorly distinguishes between α6β2*- and α3β2*-nAChR subtypes. The more selective antagonist of α6*-nAChR, α-CtxPIA [132–134] Exhibits 75-f old higher affinity for rat α6β2*-nAChRs than for rat α3β2*-nAChRs [134].

There is an emerging consensus that nAChR α6 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 α6*-nAChRs play roles in nicotine dependence. These same brain regions in which α6 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 α6*-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 α6*-nAChRs are candidates for mediation of these effects [116].

There is good evidence that α6*-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 (α4α6β2β3 subtype) or two (α6β2β3 subtype) α-CtxMII-sensitive ACh binding sites indicating that pre-synaptic α6*-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 α3β2- and α6β2-nAChRs on superior colliculus neurons, and these receptors are likely located on pre-synaptic terminals of GABAergic neurons where they modulate GABA release. Interestingly, Champtiaux et al. [130] demonstrated that the majority of DAergic cell-body nAChR function in the VTA is mediated by non-α6*-nAChRs. Most of the α6*-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 α6β2* subtype, because treatment with α6β2*-selective nAChR antagonists (either α-CtxMII or α-CtxPIA; 1 nM) or deletion of the nAChR β2 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 further review of that work please see Letchworth and Whiteaker in this volume for a current perspective 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 α4 and β2 are expressed could be due to expression as α4 or β2 or other isoforms of α4β2-nAChRs (Fig. 1) [143] could explain puzzling, earlier findings. Perhaps more importantly, consideration needs to be given to how high sensitivity and low sensitivity α4β2-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 α7β2-nAChRs highly sensitive to β-amyloid [91] might alter thinking about roles for nAChRs in that disease. Discovery of α6*-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.

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