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Nicotinic Receptor Contributions to Smoking: Insights from Human Studies and Animal Models

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Abstract It is becoming increasingly evident that a variety of factors contribute to smoking behavior. Nicotine is a constituent of tobacco smoke that exerts its psychoactive effects via binding to nicotinic acetylcholine receptors (nAChRs) in brain. Human genetic studies have identified polymorphisms in nAChR genes, which predict vulnerability to risk for tobacco dependence. In vitro studies and animal models have identified the functional relevance of specific polymorphisms. Together with animal behavioral models, which parse behaviors believed to contribute to tobacco use in humans, these studies demonstrate that nicotine action at a diversity of nAChRs is important for expression of independent behavioral phenotypes, which support smoking behavior.

Keywords Tobacco · Nicotine · Addiction · Acetylcholine · Cholinergic · e-cigarettes

Nicotinic Acetylcholine Receptors

The primary addictive component identified in tobacco smoke is nicotine, which exerts its behavioral effects via interaction with nicotinic acetylcholine receptors (nAChRs). Broadly, nAChRs can be separated into two main categories: neuronal and muscle receptors. Muscle and neuronal nAChRs are pentameric transmembrane cation channels belonging to the superfamily of ligand-gated ion channels that include the GABA, 5-HT, and glycine receptors, but a different complement of subunits makes muscle and neuronal nAChRs responsive to different compounds. Muscle nAChRs consist of $\alpha1$, $\beta1$, γ, δ, and ε subunits, whereas neuronal nAChRs consist of $\alpha2$-10 and $\beta2$-4 (for a more detailed review of nAChR composition and function, see [1]). As most nicotine-associated behaviors are thought to be regulated in the CNS, neuronal nAChRs in the periphery would not make ideal drug targets.

The composition of the receptor and neuroanatomical localization adds to the specificity and complexity of cholinergic signaling by conveying differing pharmacologic characteristics. Heteromeric nAChRs ($\beta2$* and $\beta4$*; *denotes assembly with other subunits) are generally more sensitive to agonists, with some subtypes of $\beta2$*nAChRs demonstrating functional activity at nanomolar concentrations, whereas homomeric nAChRs ($\alpha7$, $\alpha9$, and $\alpha10$) generally require micromolar concentrations of agonist for their activation [1]. Following activation, nAChRs enter a desensitized (inactive) state and some heteromeric receptors show preferential desensitization at low concentrations of nicotine. As described below, diverse behavioral outcomes appear to be achieved by activation versus inhibition of nAChRs.

nAChRs are expressed in brain areas that regulate a variety of behaviors. $\beta2$*nAChRs (including two major subclasses $\alpha4\beta2$*- and $\alpha6\beta2$*nAChRs) and $\alpha7$ nAChRs are the most common nAChR subtypes in the CNS with complementary expression in the dorsal striatum, thalamus, and amygdala but with neuroanatomical overlap in the ventral tegmental area (VTA), cortex, hippocampus, and basal ganglia [2–4]. These brain areas regulate sensory transmission, learning and memory, emotion, and reward. The $\alpha6\beta2$*nAChRs are selectively expressed in catecholaminergic nuclei and enriched in the mesolimbic DA system, which is believed to support

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addictive drugs. α3β4*nAChRs have modest CNS expression but are enriched in the medial habenula (mHb) to interpeduncular nucleus (IPN) pathway with a small subset of these receptors containing the α5, i.e., α3α5β4 [5–7]. The mHb-IPN pathway regulates the mesolimbic system and is highly implicated in smoking phenotype. α3 and β4 nAChR subunits also form nAChRs in the ganglion, however, raising considerations about possible peripheral autonomic side effects that could result from drug targeting of α3β4*nAChRs. A small population of α3β2*nAChRs in the habenula and IPN may prove important for smoking phenotype, but there are currently limited tools to assess this.

nAChR Contributions to Smoking

β2*nAChRs

Although genome-wide association studies (GWAS) have failed to yield convincing evidence for β2 subunit polymorphisms that predict risk for tobacco dependence, candidate gene studies further show that polymorphisms in CHRNB2 are associated with the subjective effects of nicotine, Fagerstrōm Test for Nicotine Dependence (FTND) scores [8]; and varenicline, bupropion, and nicotine replacement therapy outcomes [9–11, 12, 13, 14]. Furthermore, GWAS, linkage analysis and candidate gene studies have greatly implicated CHRNA3, CHRNA4, CHRNA5, CHRNA6, and CHRNB3 [15, 16•, 17•, 18•, 19–22] nAChR subunit genes that assemble with β2 to make functional receptors (see Table 1). Of these, α4 (CHRNA4) and α6 (CHRNA6) primarily assemble with β2 in brain areas thought to regulate nicotine/tobacco reinforcement.

CHRNA4 and CHRNA6 variations are linked to tobacco dependence. Numerous studies assessing nicotine dependence demonstrate that multiple CHRNA4 polymorphisms, especially rs2236196, rs1044394, and rs1044396, are associated with increased FTND score, DSM-IV nicotine dependence symptoms, and cigarettes per day (CPD) [15, 20, 29–33]. Increased sensitivity to the subjective effects of nicotine and better cessation outcomes have also been associated with these CHRNA4 variants [13, 34]. Linkage analysis among a population of nicotine-dependent or non-dependent individuals reveals that rare CHRNA4 variants are protective against nicotine dependence. In addition, this study revealed that these variants are associated with altered β2*nAChR binding in the brain, as measured by SPECT imaging [35]. In vitro data indicate that these rare variants result in both increased expression and function of α4β2*nAChRs [36]. Although less studied than CHRNA4, recent evidence also implicates CHRNA6 polymorphisms in smoking behaviors and dependence. Risk for nicotine dependence has been associated with polymorphisms in CHRNA6, especially rs13277254, located upstream of the CHRNA6-CHRNB3 gene cluster [15, 28, 29, 31, 37–40]. A few studies have shown that variation in CHRN A6 is positively associated with smoking initiation, initial sensitivity, and positive subjective effects of nicotine that predict susceptibility to smoking [38, 41]. Furthermore, varenicline, a partial agonist of α4β2*nAChRs (including α4α6β2*nAChRs) is highly effective for promoting smoking cessation [42, 43] and reducing craving, withdrawal and pleasurable experiences associated with smoking [44–46] (but see discussion of varenicline agonist properties at α7 nAChRs below).

Imaging studies using a highly selective β2*nAChR competitive agonist, 5-iodo-A85830, demonstrate that the smoke from a single cigarette results in nicotine binding of more than 88 % of the β2*nAChRs in brains of smokers [47•]. Not only do β2*nAChRs appear to be highly relevant for smoking, nicotine/tobacco exposure also increases expression or function of these nAChRs [48]. Post mortem and imaging studies demonstrate that β2*nAChR binding is increased in human smokers, suggesting nicotine-induced upregulation of these receptors with receptor levels requiring weeks to return to levels observed in non-smokers [49–53]. Decreased α4β2*nAChR density in brains of smokers has also been associated with better cessation outcomes [54], further suggesting that β2*nAChRs support tobacco dependence.

α3*, α5*, β4*nAChRs

CHRNA3-CHRNA5-CHRNB4 genes, closely clustered on chromosome 15, encode the α3, α5, and β4 subunits of the nAChR and are often co-expressed and co-regulated. Initial GWAS have identified SNPs within this region as being associated with nicotine dependence [15, 16•, 17•, 18•]. Further candidate gene studies and meta-analyses have identified CHRNA3-CHRNA5-CHRNB4 SNPs associated with dependence [25–27], smoking initiation [23, 55, 56], and heavy smoking behavior [28, 57]. The most common SNPs identified are rs16969968 of CHRNA5 and rs578776 in CHRNA3 [15, 18•, 26–29, 55]. These particular SNPs are not in linkage disequilibrium and so appear to represent two independent gene clusters, producing haplotypes with distinct associations to nicotine dependence. The minor A allele of rs16969968 is considered a "risk" allele due to high frequency in the smoking population, whereas the minor G allele of rs578776 is expressed less frequently and thus considered to be protective [15]. Therefore, a combination of the rs16969968 A allele and rs578776 C allele is considered the haplotype with the most risk for nicotine dependence, with the opposite alleles conveying a protective effect.

Polymorphisms of the CHRNA3-CHRNA5-CHRNB4 cluster are known to have functional effects. The most commonly associated SNP of the CHRNA5 gene, rs16969968, results in a non-synonymous substitution of aspartic acid to

Table 1 Human genetics data linking nicotinic receptor genes to smoking

Gene	SNP	Phenotype	References
CHRNB2	rs2072658	Increased early subjective response to tobacco (negative physical; positive)	Ehringer et al. [9]; Hoft et al. [13]
	rs2072660	Increased FTND score (minor allele)	Wessel et al. 12]
	rs2072661 rs3811450	Decreased abstinence rates (minor allele); increased withdrawal symptoms (minor allele)	Conti et al. [10]; Perkins et al. [11]
	rs4262952	Increased odds of continuous abstinence with varenicline	King et al. [14]
CHRNB4	rs1948	Earlier age of smoking initiation (risk allele: CC)	Schlaepfer et al. [23]
CHRNA3	rs578776	Increased FTND score (risk allele: G); positive smoking status	Saccone et al. [15]; Hong et al. [24]
	rs6495308	Increased CPD (risk allele: T)	Berrettini et al. [16•];
	rs1051730	Increased FTND score (minor allele); increased CPD; elevated cotinine levels; positive smoking status	Thorgeisson et al. [17•]; Keskitalo et al. [25]; Chen et al. [26]; Hong et al. [24]; Munafo et al. [27]
	rs3743078	Increased CPD (risk allele: CC)	Stevens et al. [28]
CHRNA4	rs2229959	Increased early subjective response to tobacco (negative physical)	Hoft et al. [13]
	rs2236196	Increased FTND score; increased CPD; increased heaviness of smoking; rush/high; cognitive effects; abstinence rates	Saccone et al. [29, 15]; Li et al. [20]; Hutchison et al. [34]
	rs2273504	Increased FTND score; increased CPD; increased heaviness of smoking	Li et al. [20]; Saccone et al. [15]
	rs1044394	Increased FTND score; increased DSM-IV dependence symptoms	Han et al. [32]; Kamens et al. [33]
	rs1044396	Increased/decreased FTND score; smoking quantity; heaviness of smoking; DSM-IV dependence symptoms; cigarettes per day	Feng et al. [19]; Li et al. [20]; Han et al. [32]; Kamens et al. [33]
	rs1044397	Decreased FTND score	Feng et al. [19]
	rs3787137	Increased FTND score; increased CPD; increased heaviness of smoking	Li et al. [20]
	rs3746372	Increased CPD	Voineskos et al. [30]
	rs6122429	Increased self reports of nicotine reward	Hutchison et al. [34]
CHRNA5	rs16969968	Increased FTND score (risk allele: A); increased CPD; increased heaviness of smoking (risk allele A); increased risk of habitual smoking; elevated cotinine levels; increased subjective pleasure in early smoking; positive smoking status	Saccone et al. [29]; Bierut et al. [18]; Sherva et al. [55]; Stevens et al. [28]; Chen et al. [26]; Saccone et al. [15]; Grucza et al. [56]; Hong et al. [24]; Munafo et al. [27]
	rs514743	Earlier age of smoking initiation (risk allele: TT)	Schlaepfer at al. [23]
	rs55853698	Significant association with CPD	Liu et al. [57]
CHRNA6	rs13277254	Increased FTND score; increased DSM-IV dependence symptoms; increased CPD; earlier age of smoking initiation	Saccone et al. [15, 31]; Hoft et al. [37]; Thorgeirsson et al. [38]
	rs2304297	Increased FTND score; significant association with DSM-IV dependence symptoms; positive subjective response to nicotine	Saccone et al. [29]; Hoft et al. [37]; Zeiger et al. [41]
	rs7828365	Increased heaviness of smoking	Stevens et al. [28]
	rs9298628 rs2217732 rs13273442	Increased FTND score	Wang et al. [39]; Culverhouse et al. [40]
	rs892413	Increased FTND score; increased CPD	Wang et al. [39]
CHRNA7	rs1909884 rs904952 rs10438287	Significant association with FTND score; increased FTND score	Greenbaum et al. [62]; Philibert et al. [63]; Saccone et al. [31]

Table 1 (continued)

Gene	SNP	Phenotype	References
	rs12915265 rs6494212 rs904951 rs1913456 rs7178176	Increased dizziness at first inhalation	Pedneault et al. [65]

Abbreviations: Fagerström Test of Nicotine Dependence (FTND); cigarettes per day (CPD)

asparagine at position 398 (D398N) [18•, 29]. This substitution causes decreased ACh-evoked function at α5*nAChRs without altering expression in cultured cells [18•, 58]. fMRI studies have shown a reduced anterior cingulate cortex (ACC) to NAc connectivity in human subjects expressing the D398N substitution [24], which is associated with addiction severity. In mice, this substitution results in a partial loss of receptor function, with increased nicotine intake and decreased sensitivity to the rewarding properties of nicotine [59, 60]. These data suggest that the risk allele of the rs16969968 in the CHRNA5 gene decreases sensitivity to nicotine and increases the propensity for addiction. The risk allele of rs578776 within CHRNA3, however, lowers activation of the ACC [61] and decreases function of the ACC to thalamus pathway [24]. This reduced function is thought to be associated with feedback information about reward rather than anticipation and is more strongly associated with recent nicotine exposure than addiction severity. These studies implicate a role for α5*nAChRs in mediating the rewarding effects of nicotine, whereas α3*nAChRs appear to mediate feedback information about nicotine exposure, suggesting that the α3 nAChR subunit may be more involved in craving or withdrawal processes.

α7* nAChRs

Polymorphisms within the CHRNA7 gene encoding the α7 nAChR have been linked to smoking behavior in different populations but with varying results. SNPs of the CHRNA7 gene have been associated with nicotine dependence in women [62], whereas adoption studies found that a link was evident in male subjects but not females [63]. Likewise, a CHRNA7 and nicotine dependence relationship has been noted in African American individuals but not European Americans [31], with one study of a UK-based population finding no association [64]. Recent data has associated the CHRNA7 gene with an increased probability of dizziness at first inhalation [65]. Since increased sensitivity at initiation of smoking is positively linked to nicotine dependence [66], this provides some evidence that α7 nAChRs may be involved in initiation of smoking in healthy individuals. As mentioned above, varenicline may also promote smoking cessation, in part, via stimulation of α7 nAChRs [1, 67•]. However, the specific contribution of α7 nAChRs to varenicline smoking cessation effects in humans has not currently been elucidated.

The most notable association between the α7 nAChRs and smoking occurs in individuals suffering from schizophrenia. It is well established that tobacco use is more prevalent in individuals with schizophrenia diagnosis than in the general population [68, 69]. Smokers with schizophrenia not only smoke more cigarettes but also tend to extract more nicotine from a cigarette than healthy counterparts [70]. Variations of the CHRNA7 gene have been associated with smoking in this population [71–73]. There is approximately a 50 % reduction in expression of α7*nAChRs found in the brains of subjects with schizophrenia compared to healthy controls [74, 75]; as detailed in the animal model section below, reductions in α7 nAChR function may increase nicotine use and reward. One theory for reduced α7 nAChR expression is that a truncated duplicate α7 gene acts as a dominant negative to prevent expression of α7 nAChRs at the cellular membrane [76]. A self-medication hypothesis suggests that some individuals with schizophrenia smoke to relieve deficits in appropriate filtering of sensory stimuli [77, 78]. Polymorphisms at the gene locus for the α7 nAChR on chromosome 15 regulate these "P50" sensory deficits [79] and tobacco use counteracts this phenotype [77, 78].

nAChR Contributions to Addiction Phenotype: Animal Models

Reward and Reinforcement

Rodent studies have highly implicated β2*nAChRs in nicotine reward and reinforcement. Knockout mice with a null mutation of the β2 subunit (β2KO) fail to self-administer nicotine [80•, 81–83], do not show nicotine-conditioned place preference (CPP) [84] and do not show nicotine enhancement of conditioned reinforcement [85]. Similarly, local infusion of the β2*nAChR-selective antagonist, dihydro-beta-erythroidine (DHβE) into the VTA greatly attenuates nicotine self-administration in rats [86•]. β2KO mice also fail to show nicotine-stimulated locomotor activation, a behavior, which like nicotine reward and reinforcement

requires dopamine (DA) release [87]. Not surprisingly, in vitro studies combining genetic and pharmacological tools reveal that activation of β2*nAChRs is required for nicotine-induced DAergic neuron firing and NAc DA release [80•, 88]. Behaviorally, re-expression of β2 subunit in the mesolimbic DA pathway rescues nicotine-associated locomotor activity and acquisition of nicotine self-administration in β2KO mice [83, 89], suggesting that β2*nAChRs in this pathway are critical and sufficient for nicotine addiction-like phenotype (see Table 2).

α4 and α6 subunits, which require β2 for their assembly, are also critical for nicotine reward, reinforcement and nicotine-associated locomotor activation. α4KO mice do not exhibit nicotine CPP, do not self-administer nicotine [83, 91, 92], and exhibit blunted nicotine-stimulated DA release at baseline [83, 91, 92, 93]. In addition, α4β2*nAChR gain-of-function mice with a single-point mutation in the α4 subunit (L9A) show leftward shifts in nicotine CPP and associated DAergic neuron firing [94], suggesting that activation of α4*nAChRs is sufficient for nicotine reinforcement and reward. Similarly, α6KO mice fail to develop nicotine self-administration or nicotine CPP and delivery of selective α6β2*nAChR α-conotoxin MII antagonists (CTX) into the VTA or NAc blocks nicotine self-administration and CPP, suggesting that activation of mesolimbic α6β2*nAChRs is critical for nicotine reinforcement and reward [83, 91, 95–98]. Recent ex vivo studies suggest that α4α6β2*nAChRs make up a subclass of nAChRs in the VTA which are highly sensitive to physiologically relevant doses of nicotine [99], presumably due to binding at the α4–α6 interface. α6β2*nAChRs are thought to contribute to as much as 80 % of nicotine-stimulated DA release on NAc terminals [100]. Electrophysiological studies reveal that mice with a gain-of-function single-point mutation of the α6 subunit (L9S) are hypersensitive to endogenous ACh and nicotine, resulting in enhanced VTA DAergic neuron activity and DA release at terminals in the NAc compared to wild type mice, an effect blocked by CTX [101]. L9S mice show a parallel hyperlocomotor response to nicotine that appears to require the α4 subunit since L9S mice bred to have an α4 null mutation fail to show this phenotype [102].

Other nAChR subunits have also been implicated in nicotine reward and reinforcement. For example, α2KO and α5KO mice display increased nicotine self-administration compared to WT [103, 104]. When α5 is re-expressed in the mHb, nicotine self-administration returns to WT levels [103]. Mice overexpressing β4 show decreases in freely available nicotine intake, an effect that is rescued by mHb expression of the α5 variant, D398N [59, 60, 105]. These studies suggest that independent β4*- and α5*nAChRs work in opposition to regulate nicotine intake or that introduction of the α5 subunit into the α3β4*nAChR not only changes the properties of the receptor, as was discussed above [18•, 58–60], but also has a significant effect on nicotine-dependent behavioral outcomes.

Although early studies suggested that α7 nAChRs did not play a critical role in nicotine reinforcement or reward [83, 84], an accumulation of recent data suggest that low affinity α7 nAChRs work in opposition to β2*nAChRs, enhancing nicotine reinforcement and reward when α7 nAChRs are genetically or pharmacologically inhibited and reducing nicotine self-administration and nicotine CPP when α7 nAChRs are stimulated [106, 107]. Studies assessing methyllycaconitine (MLA), an α7 nAChR antagonist, effects on nicotine self-administration have returned mixed results [108, 109], perhaps because MLA has potency as an α6β2*nAChR antagonist [110]. Local infusion of a highly selective α7 antagonist peptide, α-conotoxin ArIB [V11L, V16D], into the NAc or ACC resulted in a nearly threefold increase in active lever pressing and breakpoints during a progressive ratio schedule of reinforcement suggesting that a loss of α7 nAChR function in these brain areas, such as that seen with schizophrenia, increases nicotine self-administration [106]. Nicotine-associated DA release is elevated in α7KO mice [111], which show leftward shifts in nicotine CPP [107] following systemic nicotine injection. By contrast, α7KO mice showed impaired oral nicotine self-administration during a two-bottle choice but only after 40 days of exposure suggesting that α7 nAChRs may differentially regulate initiation and maintenance of nicotine self-administration in α7KO mice [83, 112]. Rodent studies using α7-selective agonist compounds, however, show that both nicotine CPP, a subchronic paradigm [107], and nicotine self-administration following more chronic dosing [106] are inhibited when α7 nAChRs are stimulated.

Dependence

Nicotine dependence in rodent studies is characterized by physical and affective signs of withdrawal. This is generally achieved by providing continuous chronic or semi-chronic exposure to nicotine followed by removal of nicotine (spontaneous withdrawal) or by injection of a nAChR antagonist such as mecamylamine (MEC) (precipitated withdrawal). Physical nicotine withdrawal results in an increase of somatic signs [113–116] (e.g., paw tremor, body shakes, stretching, scratching, piloerection) as well as hyperalgesia [116, 117]. Affective signs of withdrawal include increases in anxiety behavior measured on the elevated plus maze (EPM) and light dark box [116, 118, 119] and a reduction in reward processing as indicated by increased reward thresholds in the intracranial self stimulation procedure (ICSS) [109, 115, 120–122].

Pharmacological and genetic studies have implicated β2*nAChRs in withdrawal behavior. DHβE-precipitated withdrawal results in somatic signs [114, 116, 120] and increased anxiety in the EPM [116] following chronic nicotine exposure. It is interesting that administration of the partial

Table 2 Pharmacological and genetic findings linking nAChR subunits to nicotine addiction phenotype

Subunit	Manipulation	Behavioral Outcome	Reference
β2	KO	Nicotine self-administration blocked (rescued by re-expression in VTA)	Picciotto et al. [80•]; Maskos et al. [81]; Besson et al. [82]; Pons et al. [83]
		Nicotine CPP blocked (not rescued by low-level re-expression in VTA)	Walters et al. [84]; Mineur et al. [89]
		Conditioned reinforcement blocked	Brunzell et al. [85]
		Nicotine locomotor activation blocked (rescued by low-level re-expression in VTA)	King et al. [87], Mineur et al. [89]
		Nicotine evoked DA release blocked	Zhou et al. [88]
		Loss of nicotine-stimulated DAergic neuron firing	Picciotto et al. [80•]
		Loss of anxiety-related behavior (EPM)	Jackson et al. [119]
		Loss of withdrawal-induced increases in anxiety (EPM)	Jackson et al. [119]
		Withdrawal-induced increases in somatic signs intact	Salas et al. [117]; Jackson et al. [119]
	DHβE	Nicotine self-administration blocked (infusion in VTA)	Corrigall et al. [86•]
		Nicotine CPP blocked	Walters et al. [84]
		Evoked DA release blocked	Zhou et al. [88]
		Anxiolytic (EPM; marble burying)	Anderson and Brunzell [131]
		Antidepressant-like (tail suspension; forced swim)	Andreasen et al. [139]
		Precipitates somatic signs of withdrawal	Epping-Jordan et al. [120]; Damaj et al. [116], Malin et al. [114]
		Precipitates withdrawal-induced increases in anxiety (EPM)	Jackson et al. [149]
		Precipitates withdrawal-induced increases in ICSS	Epping-Jordan et al. [120]
	Varenicline	Anxiolytic (marble burying, NIH)	Turner et al. [130]; Hussman et al. [132]
		Antidepressant-like (forced swim)	Rollema et al. [137]; Caldarone et al. [140]
		Reduces withdrawal-induced increases in ICSS thresholds	Igari et al. [123]
	ABT-089	Anxiolytic during nicotine withdrawal and anxiogenic in naïve mice (NIH)	Yohn et al. [133]
	Cytisine	Antidepressant-like (tail suspension; forced swim)	Mineur et al. [138]
	A-85380	Trained rats self-administer this selective agonist	Liu et al. [90]
		Antidepressant-like (forced swim)	Buckley et al. [136]; Caldarone et al. [140]
β3	KO	Decreased anxiety levels (EPM)	Booker et al. [144]
β4	KO	Decreased anxiety levels (EPM; light dark)	Salas et al. [143]; Semenova et al. [145]
		Reduced withdrawal-induced somatic signs and hyperalgesia	Salas et al. [117]; Stoker et al. [125]; Jackson et al. [127]
	α-CTX AuIB	Precipitates nicotine withdrawal-induced somatic signs	Jackson et al. [127]
α2	KO	Increased self-administration	Lotfipour et al. [104]
α4	KO	Nicotine self-administration blocked (rescued by re-expression in VTA) and blunted nicotine-stimulated DA release	Pons et al. [83]; Exley et al. [91]
		CPP blocked and blunted nicotine-stimulated DA release	McGranahan et al. [92]
		Blunted basal and nicotine-stimulated DA release	Marubio et al. [93]
		Nicotine-stimulated anxiolysis blocked	McGranahan et al. [92]
		Increased anxiety levels (EPM)	Ross et al. [134]
	L9S	Anxiogenic (EPM; mirrored chamber)	Labarca et al. [135]
	L9A	Hypersensitive to nicotine-stimulated DAergic neuron firing and nicotine CPP	Tapper et al. [94]
	Sazetidine	Anxiolytic (NIH)	Hussman et al. [132]
		Antidepressant (tail suspension; forced swim)	Turner et al. [130]; Caldarone et al. [140]
α5	KO	Increased nicotine self-administration	Fowler et al. [103]
		Reduced nicotine withdrawal-induced somatic signs	Jackson et al. [119]; Salas et al. [128]; Jackson et al. [127]
		Nicotine withdrawal-induced increases in anxiety intact (EPM)	Jackson et al. [119]

Table 2 (continued)

Subunit	Manipulation	Behavioral Outcome	Reference
α6	KO	Nicotine CPP blocked	Sanjakdar et al. [98]
		Nicotine self-administration blocked (rescued by re-expression in VTA) and blunted nicotine-stimulated DA release	Pons et al. [83]; Gotti et al. [97]; Exley et al. [91]
	L9S	Hypersensitive DAergic neuron firing and DA release	Drenan et al. [101]
		α4 co-expression required for hyperlocomotion	Drenan et al. [102]
	α-CTX MII α-CTX PIA	Nicotine CPP blocked	Jackson et al. [95]; Sanjakdar et al. [98]
		Nicotine self-administration blocked (infusion NAc and VTA)	Brunzell et al. [96]; Gotti et al. [97]
		Blocks nicotine-stimulated DAergic neuron firing	Liu et al. [99]
α7	KO	Leftward shift in nicotine CPP (enhanced at low doses)	Harenza et al. [107]
		Nicotine self-administration unaffected	Pons et al. [83]
		Nicotine-stimulated DA release increased, nicotine self-administration blunted	Besson et al. [111]
		Chronic oral nicotine intake decreased	Levin et al. [112]
		Anxiety-like behavior unaffected (EPM; light dark; open field)	Salas et al. [124]; Jackson et al. [119]
		Loss of nicotine withdrawal-induced increases in somatic signs	Jackson et al. [119]; Stoker et al. [125]
		Spontaneous nicotine withdrawal-induced increases in anxiety intact	Jackson et al. [119]
		MEC precipitated nicotine withdrawal-induced anxiety reduced	Jackson et al. [119]
		Leftward shift in MEC dose response curve, as measured by withdrawal-induced increases in ICSS thresholds	Stoker et al. [125]
	MLA	Nicotine self-administration unaffected	Grottick et al. [108]
		Nicotine self-administration blocked	Markou and Paterson [109]
		Reversed nicotine-induced anxiogenesis	Tucci et al. [146]
		Antidepressant (tail suspension; forced swim)	Andreasen et al. [139]
		Precipitates nicotine withdrawal-induced increases in somatic signs	Markou and Paterson [109]; Damaj et al. [116]; Salas et al. [124]
		No effect on nicotine withdrawal-induced increases in anxiety (EPM)	Damaj et al. [116]
		No effect on nicotine withdrawal-induced increases in ICSS thresholds	Markou and Paterson [109]
	α-CTX ArIB	Nicotine self-administration increased (NAc and ACC infusion)	Brunzell et al. [106]
	PHA-543613	Nicotine CPP blocked	Harenza et al. [107]
	PNU-282987	Nicotine self-administration blocked (NAc infusion)	Brunzell et al. [106]
		Increased anxiety levels	Pandya et al. [147]

Abbreviations: nicotinic acetylcholine receptor non-selective antagonist mecamylamine (MEC), semi-selective antagonist methyllycaconitine (MLA), selective antagonists dihydro-beta-erythroidine (DHβE), α conotoxin MII (α-CTX MII), PIA (α-CTX PIA), ArIB (α-CTX ArIB) and AuIB (α-CTX AuIB), selective partial agonists (cytisine, varenicline, sazetidine, ABT-089), selective agonists (A-85830; PHA-54613; PNU282987); leucine to serine (L9S) or leucine to alanine (L9A) single point mutation in pore forming domain resulting in gain-of-function phenotype; null mutation of subunit resulting in total "knock out" of the receptor (KO); brain areas tested include ventral tegmental area (VTA), nucleus accumbens (NAc) and anterior cingulate cortex (ACC); and behavioral assays tested include conditioned place preference (CPP), elevated plus maze (EPM), novelty induced hypophagia (NIH) and intracranial self stimulation (ICSS)

β2*nAChR agonist varenicline relieved increases in ICSS thresholds instigated by spontaneous nicotine withdrawal [123], presumably due to stimulation of β2*nAChRs since DHβE administration promotes withdrawal-induced increases in ICSS thresholds [120]. Contrary to pharmacological data, however, studies utilizing β2KO mice show that withdrawal-associated anxiety is absent in the β2KO mice but that somatic signs remain intact [117, 119], suggesting a strong role for β2*nAChRs in mediating the affective signs of nicotine withdrawal but indicating that β2*nAChR mediation of physical withdrawal symptoms requires further validation.

Studies assessing α7 nAChR contributions to withdrawal have utilized MLA and α7KO mice. MLA-precipitated nicotine withdrawal induces somatic withdrawal signs [109, 116, 124]. This is presumably due to MLA properties at α7 nAChRs since CTX antagonism of α6*nAChRs blocked

withdrawal-induced conditioned place aversion (CPA) and had no effect on somatic withdrawal measures [95]. In contrast, deletion of the α7 subunit blocked observation of somatic withdrawal [119, 125]. Together these data indicate a decisive role for α7 nAChRs in the expression of physical withdrawal. α7 nAChR-mediated affective signs, however, are somewhat inconclusive. Whereas MLA-precipitated withdrawal does not elevate anxiety in the EPM [116] or elevate ICSS thresholds following chronic nicotine exposure [109], studies using α7KO mice indicate a potential role of α7 nAChRs in affective withdrawal. Spontaneous withdrawal does not change anxiety in the α7KO compared to wild-type mice [119], however precipitated withdrawal with 2 mg/kg MEC results in reduced anxiety-like behavior in the EPM task [119]. Indeed, α7KO mice show elevated ICSS thresholds in response to precipitation of nicotine withdrawal at lower doses of MEC (1.5 mg/kg) than WT mice (3 and 6 mg/kg) [125], suggesting a leftward shift in the dose response curve for MEC effects rather than a withdrawal deficit in these mice. Since mRNA levels of other nAChR subunits are unchanged in the α7KO mouse [126], differences in responses to MEC are unlikely due to compensatory changes in other nAChRs but this does not preclude alterations in other neurotransmitter systems.

The habenula, a brain area enriched with α3β4*nAChRs and α5*nAChRs, is receiving increasing attention for its contributions to nicotine dependence. Genetic deletion of the β4 nAChR subunit is associated with reduced somatic withdrawal signs [117, 125, 127] and hyperalgesia [117]. Somatic signs of nicotine withdrawal can also be precipitated by intracerebroventricular (i.c.v) administration of AuIB, a selective α3β4 antagonist [127]. This effect is not altered by deletion of the α5 subunit, suggesting that α3α5β4*nAChRs are not critical for expression of physical withdrawal. Other (non-α3β4)α5*nAChRs may contribute to withdrawal, as deletion of the α5 subunit results in decreased somatic signs when withdrawal is precipitated with the non-specific nAChR antagonist, MEC [119, 127, 128]. α5KO studies suggest that α5*nAChRs do not contribute to withdrawal-associated increases in anxiety behavior [119]. These data suggest a role for independent α5* and β4*nAChRs in mediating physical signs of withdrawal, but further validation is required to confirm a role for these subunits in affective behavioral withdrawal signs.

Anxiety- and Depression-Like Behavior

Many smokers report that they smoke to relieve anxiety and there is a high concordance of anxiety and major depression diagnoses with smoking [129]. Although these are complex emotions that cannot be entirely assessed in animals, rodent models of anxiety and antidepressant efficacy suggest that nAChRs contribute to the biology of affective behaviors associated with nicotine use.

Unlike reward and reinforcement, where a preponderance of the evidence suggests that activation of β2*nAChRs is essential for these behaviors, an accumulation of rodent data indicate that inhibition of β2*nAChRs promotes anxiolysis-like behavior. The β2*nAChR antagonist, DHβE, and partial agonists varenicline, ABT-089, and sazetidine promote anxiolysis-like behavior in the EPM, marble burying, and conditioned inhibition tasks [130–133]. Low-dose nicotine mimics anxiolysis-like effects of DHβE, suggesting that desensitization of nAChRs by low doses of nicotine may decrease anxiety [131]. A study using mice lacking nAChR α4 subunits in the VTA showed that these mice failed to benefit from the anxiolytic-like effects of low-dose nicotine, suggesting that α4β2*nAChRs in the VTA are required for nicotine-induced anxiolysis in the EPM [92] (but see [134]). In contrast, L9A mice with gain-of-function α4β2*nAChRs show increased basal anxiety in the EPM [135] to suggest that stimulation of the α4*nAChRs is sufficient to promote anxiety, presumably in brain areas other than those that support nicotine reward and reinforcement.

Similarly, DHβE and the α4β2*nAChRs partial agonists varenicline, sazetidine, and cytisine have been shown to produce antidepressant-like effects in the forced swim and tail suspension tests in mice [130, 136–140]. Studies in knockout mice reveal that β2*nAChRs regulate the antidepressant-like efficacy of MEC and its potentiation of the classic antidepressant, amitriptyline [141, 142]. Curiously, recent data suggest that stimulation of α4β2*nAChRs promotes antidepressant effects of sazetidine [140]. Further data are necessary to determine whether stimulation or inhibition of α4β2*nAChRs may benefit smokers with depression.

Studies implicate other nAChR subunits in affective behavior. Mice with a null mutation of the β4 or β3 subunit show less basal anxiety-like behavior than wild-type mice in the EPM, light dark, and prepulse inhibition tasks [143–145], suggesting that cholinergic tone at these receptors may support anxiety phenotype. α7KO mice show similar basal anxiety levels as WT mice in open field, EPM, and light dark tests [119, 124]. Other studies show that intrahippocampal MLA reverses nicotine-induced anxiogenesis in the social interaction test [146] and that systemic administration of the selective α7 nAChR agonist, PNU-282987, increases anxiety-like behavior [147], suggesting that inhibition of α7 nAChRs may decrease anxiety behavior. Together, these studies suggest that the endogenous cholinergic system regulates emotive behaviors that could be targeted by nicotine in individuals who use tobacco products.

Summary and Therapeutic Implications

Although FDA-approved first-line smoking cessation drugs greatly improve quit outcomes, a limited number of smokers

are successful at quitting with currently available therapeutics [148]. A diversity of neuronal nAChRs may provide novel targets for assisting unique populations of smokers to quit. Human genetics studies have implicated a variety of nAChR subunits as contributing to risk for tobacco dependence phenotype. The strongest GWAS candidate thus far is CHRNA5. The α5 nAChR subunit affects agonist and antagonist binding affinity and potency, but as an accessory subunit does not contribute to agonist binding and therefore is not an ideal drug target. Large GWAS studies have relied primarily upon the FTND scores. Smaller gene-targeted studies have begun to assess alternate nAChR subunit contributions to a variety of behavioral phenotypes that support tobacco use. Where GWAS failed to identify strong associations of α4, α6, β2, or α7 with tobacco dependence, targeted gene studies have implicated variations in these subunits as contributing to smoking phenotype. This is relevant as these nAChR subunits assemble to make nAChRs that are targeted by the smoking cessation therapeutic, varenicline. Although genetic studies identify risk variants for tobacco dependence, they do not rule out the relevance of targets that do not show significant genetic variability across the populace. Human and animal preclinical laboratory studies are necessary to identify these alternative viable nAChR targets for smoking cessation and to establish a functional strategy for inhibition or stimulation of specific nAChR subtypes to promote a desired phenotypic effect. As with animal models, controlled human laboratory studies should strive to parse behaviors that are relevant to tobacco addiction in order to develop tailored treatments for individuals according to their motives for smoking. With clinical assessment tools to reliably identify motives for smoking, we can perhaps expect the best outcomes for identifying strategies for quitting.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Darlene Brunzell is the principal investigator of NIH grants R01 DA031289 "Nicotinic receptor contributions to affective behavior," UH2/UH3 TR000958 "Medication development of a novel therapeutic for smoking cessation," and Virginia Foundation for Healthy Youth Grant 8520893 "Exercise and environmental enrichment as a prevention strategy for nicotine use in adolescent males and females". Dr. Brunzell contributes as a co-investigator to NIH P30 DA033934 "Central Virginia Drug Abuse Core" to Dr. William L Dewey, R01 AG041161 "Novel bivalent multifunctional ligands towards Alzheimer's disease" to Dr. Shijun Zhang and NIAAA P50 AA022537 "Cross-species investigation of gene networks for ethanol-related behaviors" to Dr. Kenneth S. Kendler. Alexandra M. Stafford contributes to studies assessing nAChR contributions to behaviors relevant to smoking phenotype. She is supported by an NIDA training grant T32 DA 007027 to Dr. William L Dewey. Dr. Claire Dixon contributes to studies assessing nAChR contributions to behaviors relevant to smoking phenotype.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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