

The Genetic Components of Alcohol and Nicotine Co-Addiction: From Genes to Behavior

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Abstract: Co-occurrence of alcohol and nicotine addiction in humans is well documented and there is good evidence that common genes may contribute to both disorders. Although genetic factors contributing to tobacco and alcohol problem use have been well established through adoption, twin and family studies, specific genes remain to be identified and their mode of action elucidated. Recent work from human genetics studies has provided evidence that neuronal nicotinic acetylcholine receptors (nAChR) genes may have a role in mediating early behaviors that are risk factors for alcohol and nicotine dependence, such as age of initiation and early subjective responses to the drugs. Converging evidence suggests that the dopaminergic system is likely to be important in mediating the pleasurable feelings of reward when activated by nicotine and/or alcohol consumption. The nAChRs are important components of the dopaminergic reward system because some of the receptors have been shown to activate the release of dopamine, and mice lacking genes for specific nAChR gene subunits show altered behavioral responses to nicotine and alcohol. Furthermore, complex interactions between other neurotransmitter circuits including GABA, glutamate and serotonin may be modulated by nAChRs, leading researchers to study genes involved in neurobiology shared by different drugs. Future studies aimed at understanding the variation among these genes, and their corresponding functional implications, will help elucidate how natural variants in nicotinic receptor genes contribute to these common co-morbid disorders.

Keywords: Nicotine, alcohol, co-morbidity, genetics, nicotinic receptors, linkage, association, behavior, neurotransmitters.

INTRODUCTION

Nicotine and alcohol are two of the most widely used addictive drugs and both have hazardous health consequences resulting from their chronic use. Numerous studies have shown that alcohol use and smoking frequently co-occur and that both environmental and genetic factors contribute to the overlap between these two behaviors. A variety of studies using model systems have demonstrated that the neuronal nicotinic receptors (nAChRs) are a common site of action of nicotine and alcohol, in the sense that alcohol may modulate the pharmacological binding properties of nicotine at nAChRs [1-11]. Furthermore, the presence of several nAChR subtypes on dopaminergic nerve terminals implicates their potential involvement in the mesolimbic reward pathway, which may be a common component of many different types of addiction. Here we present a brief overview of the genetic underpinnings of alcohol and tobacco disorders, focusing on several human genetic associations between specific nAChR subunit genes and various alcohol and nicotine-related behaviors. Next, we present a comprehensive discussion of the biological role of nAChRs in mediating alcohol and nicotine responses. We conclude with our thoughts about the challenges still facing genetic studies of substance abuse research, and how those challenges may be overcome in the future.

COMMON GENES CONTRIBUTE TO ALCOHOL AND NICOTINE CO-ADDICTION

Numerous reports have provided evidence for high co-morbidity between smoking tobacco and alcohol use, whereby alcohol dependent individuals are more likely to be dependent on nicotine and vice versa [12-16]. There are several lines of evidence demonstrating that genetic factors are important for predicting long-term tobacco use and that significant genetic effects contribute to smoking, typically accounting for approximately 50% (28-84%) of the total variance [17-19]. Similarly, a variety of twin, adoption, and family studies have shown that genetic components play a role in the development of alcohol dependence [20-27]. More recent studies have provided data to support the theory that *common* genetic factors may contribute to the concurrent use of these two substances [19, 28-33]. Thus, there is a wealth of information suggesting that as much as half of the risk for nicotine and alcohol disorders may be mediated by genetic factors. Furthermore, the development of new analytical modeling techniques is beginning to help elucidate more detail about the environmental and etiological contributions to these two specific disorders and their co-occurrence.

HUMAN GENETIC STUDIES OF nAChR GENES

Recently, human genetic studies have provided evidence for a role of the nAChRs in the etiology of alcohol and tobacco co-addiction. Among the twelve subunit genes for neuronal nicotinic receptor subunits, those encoding the $\alpha 4$ and $\beta 2$ nAChR subunits (CHRNA4 and CHRNA2), have been the most widely studied in human populations, since the $\alpha 4\beta 2$ receptors are those most prevalent in the brain. The first line of evidence that natural variations in nAChR genes

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contributed to phenotypic variation in human disorders, came from a missense mutation in the CHRNA4 gene, that was associated with autosomal dominant nocturnal frontal lobe epilepsy in Australian families [34-38]. Similarly, a missense mutation (Val287Met) in the CHRNB2 gene was found to be associated with autosomal dominant nocturnal frontal lobe epilepsy in a Scottish family [39]. Because of the association between tobacco use and attention problems, the CHRNA4 gene has also been examined for possible association with attention deficit hyperactivity disorder, where one study did not find evidence for association [40], while a later study did find suggestive evidence [41].

Until recently, variations in genes encoding the neuronal nicotinic receptors have not been well studied for potential effects related to nicotine and alcohol abuse and dependence in humans. A partial summary of recent studies examining nicotine and alcohol behavioral association with nAChR human genes is presented in Table 1. Feng *et al.* [42] used the Fagerstrom Test for Nicotine Dependence (FTND) [43] and the Revised Tolerance Questionnaire (RTQ) [44] and examined six single nucleotide polymorphisms (SNPs) in CHRNA4 and four SNPs in CHRNB2. They reported an association between a haplotype in the CHRNA4 gene and nicotine dependence in Chinese men but no association between these phenotypes and CHRNB2. Similarly, Li *et al.* [45] detected an association between a different SNP in CHRNA4 and the heaviness of smoking index (HSI) from the FTND, but no evidence for association with CHRNB2. In fact, most studies of CHRNB2 variations have not found evidence for association with nicotine or alcohol addictions [42, 45-47]. Silverman *et al.* [47] screened for polymorphisms by sequencing pooled DNA samples, and identified five novel SNPs. Four of these, and their estimated haplotypes, were tested for association with smoking initiation and progression to nicotine dependence but no association was found [47]. Six other SNPs in the CHRNB2 gene showed no evidence for association in a separate study of individuals evaluated for smoking history and lifelong nicotine dependence [46]. However, two more recent reports have shown a possible role for this gene in initiation of smoking in women [48], and in early subjective response to smoking and alcohol [49].

The CHRNA6 and CHRNB3 genes are located next to each other on human chromosome 8 and code for the $\alpha 6$ and $\beta 3$ nAChR subunits, respectively, which form part of nAChRs abundant in the body of dopamine producing cells. The CHRNB3 gene was found recently to be a top candidate for nicotine dependence (as measured by FTND) in two studies from the Collaborative Genetic Study of Nicotine Dependence (COGEND). Using a whole genome association approach which examined over 2.4 millions SNPs, the SNP rs13277254 emerged as one of the most significant findings [50]. Using a candidate gene approach, whereby 3713 SNPs were genotyped in over 300 candidate genes, a strong association with nicotine dependence was seen with a different SNP in CHRNB3 (rs6474414) [51]. Additional evidence for an important role of variations in CHRNB3 and addiction phenotypes continues to grow. In a recent Colorado-based study using a clinical-based sample from the Center for Antisocial Drug Dependence (CADD), preliminary evidence for association between two SNPs (rs4950 and rs13280604) and early subjective response to nicotine was detected. Moreo-

ver, this finding was replicated with these specific SNPs in the separately ascertained National Longitudinal Study of Adolescent Health (Add Health), a community-based sample [52]. In this study, there was also evidence for an association between a SNP in CHRNA6 (rs2304297) and early subjective response to nicotine in both the selected CADD and community-based Add Health samples, suggesting that this genomic region containing CHRNA6 and CHRNB3 may be important in mediating early subjective response to nicotine.

On chromosome 15, a cluster of three phylogenetically conserved nAChR subunit genes includes CHRNA5, CHRNA3, and CHRNB4, which code for the $\alpha 5$, $\alpha 3$ and $\beta 4$ nAChR subunits respectively. In the candidate gene study of Saccone *et al.* [51], mentioned above, a non-synonymous SNP in CHRNA5 (rs16969968) was highly significant for association with nicotine dependence. Additionally, nominal significance between a SNP in CHRNA5 and nicotine dependence has also been reported in young Israeli women [48]. More recently, two linked SNPs (rs8023462 and rs1948) of the CHRNA5/A3/B4 gene cluster significantly predicted early age of initiation for tobacco in a clinical-based sample. These findings were then replicated in a separate population-representative sample, showing the same two SNPs to be associated with age of initiation for both tobacco and alcohol use [53]. Furthermore, Berrettini *et al.* [54] recently reported a significant association between natural variations in the CHRNA5/CHRNA3 genes and number of cigarettes per day in three independent samples of European origin. Thus, variations in CHRNA5/A3/B4 genes may influence behaviors that promote early age of experimentation with drugs and/or nicotine dependence. Clearly, evidence is accumulating for an important role of nAChR genes in mediating behaviors involved in tobacco and/or alcohol abuse and dependence. Although a variety of pharmacological and animal studies have provided support for involvement of these receptors in nicotine/alcohol action (next section below), few human studies have explored nicotine and/or alcohol phenotypes with the nAChR subunit genes. With greater appreciation of nAChRs diversity on one side and the sophistication of new genetic statistical analysis tools at the other, the elucidation of the molecular genetics of drug addictions is gaining momentum.

Despite these advances, however, there remain inconsistent findings in human genetic reports, likely due to differences in study samples, phenotypic measures, and genetic heterogeneity, whereby different genes may be important in different populations.

ROLE OF nAChRs IN ALCOHOL AND TOBACCO EFFECTS

Multiple neurotransmitters appear to orchestrate the reward profile of nicotine and alcohol addiction and co-addiction. Several lines of evidence from model systems using *Xenopus* oocytes [1, 2], rat electrophysiology [3-7], and mouse behavioral models [8-11] suggest that alcohol may modulate the pharmacological properties of nicotine binding at nAChRs, usually by enhancing receptor function. Since these receptors have been shown to activate release of dopamine, they are likely to be important for mediating the rewarding properties of the mesolimbic dopamine system [55, 56].

Table 1. Human Studies Implicating nAChR Genes in Alcohol and Tobacco Dependence

Phenotype	Gene(s)	Sample	Ref.
Smoking and nicotine dependence	CHRNA2 no association	Non-smokers, smokers, high ND smokers	Silverman, Neale <i>et al.</i> 2000
Smoking behavior and nicotine dependence	CHRNA2 no association	Individuals with smoking history available	Lueders, Hu <i>et al.</i> 2002
FTND, RTQ	CHRNA4	Males in families w/multiple nicotine addicted siblings	Feng, Nui <i>et al.</i> 2004
ND, SQ, HIS, FTND	CHRNA4, (no association for CHRNA2)	EA and AA families	Li, Beuten <i>et al.</i> 2005
SQ, HIS, FTND	CHRNA1, CHRM1 (AA only)	Mid-South Tobacco Family sample: EA and AA families	Lou, Ma <i>et al.</i> 2006
Nicotine dependence, smoking initiation	CHRNA2, CHRNA7, CHRNA9, CHRNA3	Israeli female students	Greenbaum, Kanyas <i>et al.</i> 2006
Subjective Effects, past month use	CHRNA4, CHRNA2	CADD	Ehringer, Clegg <i>et al.</i> 2007
FTND	CHRNA3, CHRNA5	Collaborative study on the Genetics of Nicotine Dependence (COGEND)	Bierut, Madden <i>et al.</i> 2007 Saccone, Hinrichs <i>et al.</i> 2007
Age of onset	CHRNA3, CHRNA4	CADD, National Youth Survey	Schlaepfer, Hoft <i>et al.</i> 2007
Early subjective response to tobacco	CHRNA6, CHRNA3	Center for Antisocial Drug Dependence (CADD), National Longitudinal Study of Adolescent Health (Add Health)	Zeiger, Haberstick <i>et al.</i> 2008.
Cigarettes per day	CHRNA5, CHRNA3	Three large European samples	Berrettini, Yuan <i>et al.</i> 2008

*FTND: Fagerstrom Test for Nicotine Dependence, RTQ: Revised Tolerance Questionnaire, SQ: Smoking Quantity, ND: Nicotine dependence, HIS: Heavyness of Smoking Index, EA: European American, AA: African American.

Here we first review the role of neuronal nicotinic acetylcholine receptors (nAChRs) and the mesolimbic dopamine system in the mediation of the behavioral and neurochemical effects of ethanol and/or nicotine in *ex vivo* and *in vivo* model systems. Subsequently, we highlight some of the main neurotransmitter systems that are modulated by nAChRs in the brain.

Neuronal nicotinic acetylcholine receptors belong to the large superfamily of ligand-gated ion channels that bind the endogenous neurotransmitter acetylcholine and the alkaloid nicotine, found in tobacco. A broad description of the nAChRs has been published in recent reviews [55, 57-59]. Briefly, nAChRs are formed by the pentameric association of α and β subunits, of which 12 nAChR subunits (α 2-10, β 2-4) have been identified in the brain. Different combinations of subunits generate subtypes of nAChRs with diverse functional and pharmacological properties, which *in vivo* may have selective roles in specific brain pathways. The main nAChR subtypes found in mammalian brain have been shown to be those containing the α 4 and β 2 subunits, which bind nicotine with high affinity, and the α -bungarotoxin-sensitive α 7 subunits, which form homomeric receptors [55]. The nAChRs found in the autonomic nervous system segregate and assemble into two distinct classes: α 7-containing receptors and heteromeric α 3 subunit-containing receptors, co-assembling with β 2 and β 4 with or without α 5 subunits [60]. The structure and localization of the nAChR subtypes have been elucidated using molecular techniques such as *in situ* hybridization, immunohistochemistry and imaging techniques including positron emission tomography (PET) and

single photon emission computed tomography (SPECT) [61]. Despite these complementary techniques, the lack of antibodies specific to the different subtypes of nAChRs and the possibility of false positives due to the use of PCR approaches, limit the verification and/or classification of the nAChR subtypes. In the future, the development of new technology and methodological approaches may facilitate better characterization and localization of specific subtypes.

The α 4 β 2 receptor subunits are encoded by the CHRNA4 and CHRNA2 genes (respectively) and expressed throughout the brain and spinal cord. They are principally located on presynaptic nerve terminals where they modulate the release of neurotransmitters including dopamine [56] and γ -aminobutyric acid (GABA) [62] (Fig. 1). These α 4 β 2 receptor subtypes, together with the α 7 homomeric receptor, are the most prevalent in the brain and the majority of work in the alcohol-nicotine field has focused on them. In fact, results from studies with *Xenopus* oocytes, mice and cerebral cortex cells grown in culture have confirmed that alcohol is able to stimulate the function of the naturally expressed α 4 β 2* receptors (where * indicates another subunit, often α 6 and/or β 3) and inhibit the action of the α 7 nAChRs [63].

Although not as well-studied, less abundant nAChR subtypes [64, 65] also appear to modulate some of the rewarding properties of alcohol and nicotine [66]. The main characteristic of these receptors is that they are blocked by a small peptide cone-snail toxin named α -Conotoxin MII. β 3 subunits are essential for correct assembly and stability of α 6-containing nAChRs in dopaminergic neurons as shown in

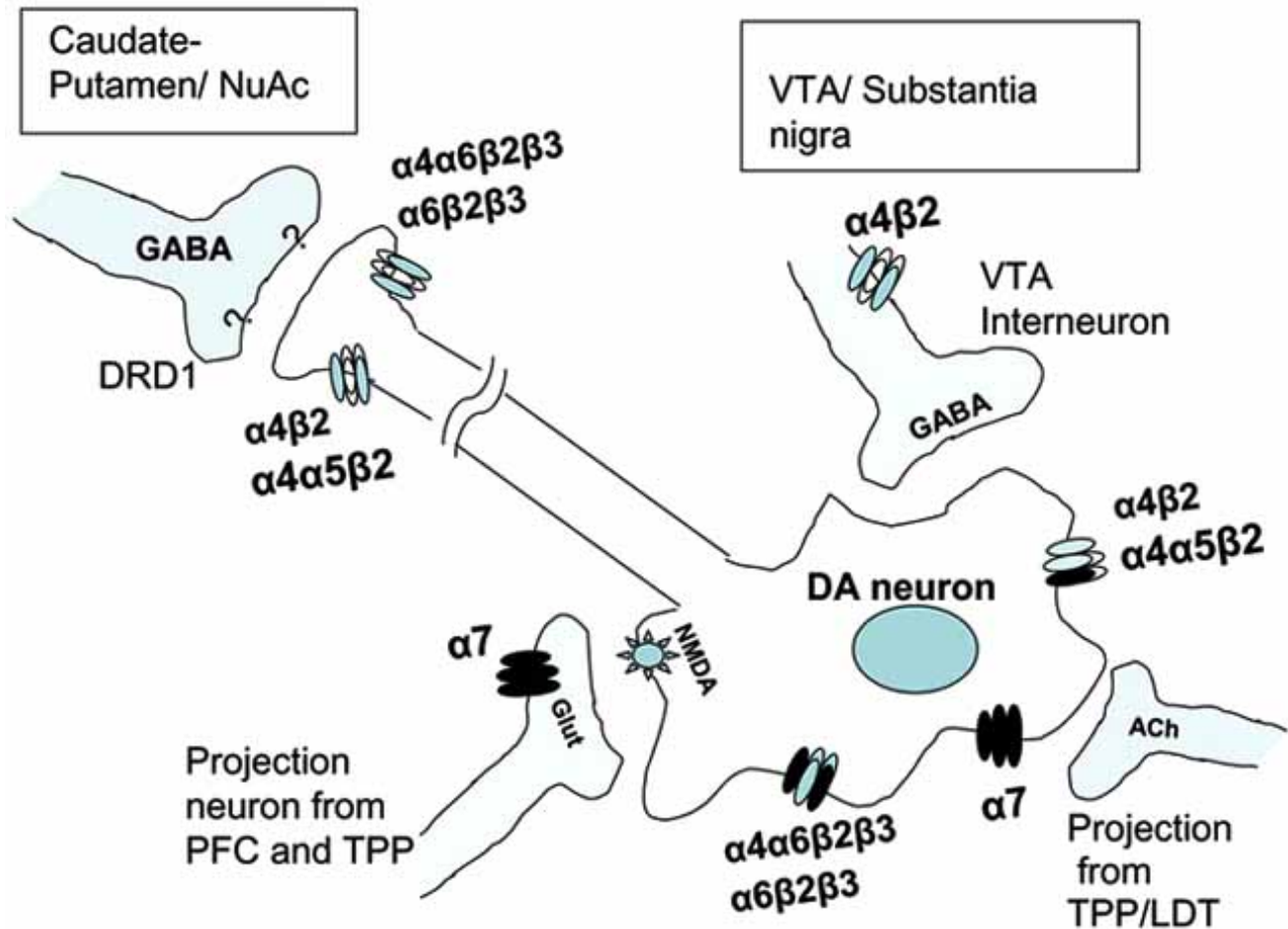


Fig. (1). Different combinations of nAChRs subunits modulate dopamine release in the mesolimbic reward pathway. The modulation of dopamine release in the VTA is mediated through presynaptic and preterminal nAChRs. Binding of nicotine or acetylcholine to the $\alpha 7$ receptors in glutamatergic terminals induce release of glutamate (Glut), which activates NMDA receptors in dopaminergic neurons resulting in activation of the release of dopamine. Cholinergic (ACh) input from mesopontine nuclei (TPP/LDT) also activate the release of dopamine in the VTA. Furthermore, desensitization of nAChRs of GABA releasing interneurons, also reduces the inhibition of dopamine release due to GABA, resulting in further induction of dopamine release in the striatum and nucleus accumbens. VTA; ventral tegmental area. PFC; Pre-frontal cortex. TPP; tegmental pedunculopontine nucleus. LDP; laterodorsal tegmental nucleus, NuAc; Nucleus accumbens. DRD1; Dopamine receptor 1. NMDA; *N*-methyl-D-aspartate.

recent studies using ligand-binding and immuno-purification techniques [67]. The nAChRs containing $\alpha 6$ and $\beta 3$ subunits are mainly expressed in midbrain dopamine neurons and in the locus coeruleus, which is a nucleus in the brain stem responsible for physiological responses to stress and panic.

Additional nAChR subunits including $\alpha 5$, $\alpha 3$ and $\beta 4$ have limited distribution in the brain (see below), but can represent a significant population of nAChRs with a critical function. These three subunits ($\alpha 5$, $\alpha 3$ and $\beta 4$) are encoded by a phylogenetically conserved cluster of nAChR subunit genes

(CHRNA5/A3/B4), important in fast cholinergic synaptic transmission. The three subunits are co-expressed in the adrenal medulla, autonomic ganglia and several structures of the brain including, the medial habenula, the interpeduncular nucleus and the inferior colliculus [68]. The significance of the subunit coexpression in these brain regions is not known but previous studies have shown that the habenulo-interpeduncular pathway exerts a tonic inhibitory influence on mesocortical, mesolimbic and mesostriatal dopaminergic neurons [69, 70]. Additionally, the $\alpha 5$ subunit is found in heteromeric receptors of the substantia nigra, ventral tegmental area (VTA), striatum, cortex and hippocampus, and the $\alpha 3\beta 4$ subunits are found together in hippocampus, medial habenula, pineal gland, cerebellum, locus coeruleus, substantia nigra and VTA [55, 71-73]. Therefore, when multiple α and β subunits are co-expressed, only some of the many possible combinations of nAChRs have been observed, for example the $\alpha 4\beta 2$, $\alpha 6\beta 3$ or $\alpha 3\beta 4$ -containing receptors are very common subunit assemblies. The precise determinants of these restricted assemblies of nAChRs and their preferential locations in the CNS are still not well understood.

NICOTINIC RECEPTORS AND THE DOPAMINERGIC SYSTEM

Neuronal nicotinic acetylcholine receptors (nAChRs) have been shown to activate the release of dopamine, making them important components of the dopaminergic reward system [55, 56]. Furthermore, several nAChR subtypes ($\alpha 4\alpha 6\beta 2\beta 3$, $\alpha 6\beta 2\beta 3$, $\alpha 6\beta 2$, $\alpha 4\beta 2$, and $\alpha 4\alpha 5\beta 2$) have been shown to be expressed on dopamine nerve terminals [74]. Converging evidence suggests that the dopaminergic system is likely to be important in mediating the pleasurable feelings of reward when activated by nicotine and /or alcohol consumption [71, 75-77]. Drug addiction is believed to involve plastic changes in neuronal systems associated with rewarding behaviors. The mesolimbic dopamine system includes three brain regions: the VTA, the nucleus accumbens (NAcc) and the prefrontal cortex (PFC). The VTA contains dopaminergic neurons and extensions from these cells lead into the nucleus accumbens and the prefrontal cortex, where the released dopamine activates other neurons (see Fig. 1 for a simplified diagram). The nAChRs located in the soma (body) of dopaminergic neurons of the VTA are able to excite these neurons directly leading to transient responses that are terminated by desensitization of nAChRs. Additionally, the stimulation and consequent desensitization of the gamma-aminobutyric acid (GABA)-containing neurons of the VTA, further contributes to the excitatory effect by eliminating the inhibitory effect of GABA. Another type of indirect modulation of dopamine release by nAChRs, is carried out by activation of $\alpha 7$ homomeric receptors on glutamatergic (Glut) terminals. This $\alpha 7$ activation triggers release of glutamine, which in turn stimulates ionotropic glutamate receptors (NMDA or *N*-methyl-D-aspartate) on dopaminergic neurons, leading to the induction of dopamine release [78]. Interestingly, elevated levels of dopamine have also been shown to modulate nAChRs in an area and subtype-dependent manner: Mice deficient in the dopamine transporter gene exhibit constitutively increased dopamine levels that result in significant modifications in nAChRs density and function [79]. This interaction highlights the inter-related modulatory effects that nAChRs have on dopaminergic

systems and vice versa. In other words, a localized increase in dopamine levels could account for significant adaptations in the cholinergic/nicotinic neurotransmission pathways.

Recent molecular biology results further underscore the importance of the interaction between nAChRs and dopaminergic systems in the etiology of nicotine and alcohol addiction. Inoue *et al.* [80] have reported synergistic enhancement of gene expression in co-cultures of VTA and NAcc neurons by nicotine and alcohol. Their results suggested that a novel cellular mechanism involving nAChRs, dopamine receptors, adenosine receptors and protein kinase A signaling, results in increased gene activation in cultured neurons exposed to alcohol and nicotine simultaneously. Furthermore, a recent report has demonstrated that simultaneous administration of alcohol and nicotine results in an additive dopamine release in the nucleus accumbens of rats, providing additional support for the importance of the mesolimbic "reward pathway" as a convergent response site for these two drugs [81, 82]. Thus, nicotine/ethanol interaction through co-activation of dopamine and adenosine receptors could contribute to the long term neuro-adaptations (*via* gene activation) that lead to the development of addiction.

ANIMAL STUDIES OF nAChRs

Animal studies with genetically modified rodents have focused on mimicking specific aspects of human addictions (self-administration, reward, withdrawal, locomotion activation) that can be easily measured in animals. In particular, genetically modified mouse models have been very important for the understanding of complex behaviors, related to addiction, relapse and reinstatement of drug dependence [83-85]. Here we present an overview of some of the results from animal models developed for studying co-addiction of alcohol and tobacco. Highlighting the importance of the dopaminergic reward pathway in mediating these behaviors, we focus on research using mice lacking individual nAChR genes to illustrate how these genes have become a key focus of nicotine and alcohol addiction research.

Drug self-administration or reinforcement is an important component of drug addiction that has been studied in animals. In fact, a recent publication [85] has shown strong experimental evidence for the hypothesis that a shared genetic determinant accounts for the nicotine and alcohol comorbidity. Le *et al.* [85] found that naive offspring of alcohol preferring rats self-administered larger amounts of nicotine intravenously and demonstrated more robust nicotine seeking behavior and relapse, compared to the offspring of alcohol non-preferring rats. These results point to nAChRs and the dopamine reward pathways as potential candidates for the interaction of alcohol and nicotine in the brain. However, alternative pathways or mechanisms that could explain how alcohol increases the reinforcing effects of nicotine and vice-versa need to be proposed and investigated. For example, the vast majority of schizophrenic individuals tend to be avid smokers, even when their treatment involves high doses of dopamine receptor blockers [86-89].

Additional research efforts have also focused on the issue of cross-tolerance between drugs. That is, chronic treatment with one drug results in reduced sensitivity for another. It is known that smokers appear to be less sensitive to the effects

of alcohol than non-smokers [29] but this cross-tolerance is difficult to mimic in animal models, mainly due to the differences in pharmacokinetics of both drugs (absorption, distribution, metabolism, etc). Some evidence of changes in $\alpha 4\beta 2$ receptor binding has been observed after chronic ethanol treatment in rats [90] and mice [91], suggesting that nicotinic receptors are involved in mediating cross-tolerance of alcohol and nicotine.

Quantitative trait locus (QTL) mapping analyses using recombinant inbred strains of mice with high (Inbred Long Sleep; ILS) and low (Inbred Short Sleep; ISS) sensitivity to ethanol have been used to identify common regions of the genome contributing to alcohol and nicotine sensitivity [92]. In order to search for candidate genes common to nicotine and alcohol sensitivity, researchers conducted a QTL analysis using the acoustic startle phenotype in these strains. The results led the researchers to a genomic region containing the $\alpha 4$ subunit gene (*Chrna4*) where a polymorphism within the coding region of the $\alpha 4$ nicotinic receptor subunit gene was identified. This polymorphism encodes for an amino acid substitution at position 529 (threonine in LS mice and alanine in SS mice) [93]. Further testing of a panel of recombinant inbred strains derived from the long sleep and short sleep strains of mice showed an association between this amino acid substitution and the effects of both alcohol and nicotine on the acoustic startle response [94]. Therefore, there is strong evidence from this animal model that the $\alpha 4$ nAChR subunit is likely to be important in modulating response to both substances, and a good candidate for pleiotropic effects contributing to the co-morbidity of alcohol and nicotine behaviors.

Further understanding of the role of the $\alpha 4\beta 2$ nAChRs was obtained using transgenic mice where either the gene coding for the $\alpha 4$ or the $\beta 2$ subunit of the receptor has been deleted ("knocked-out") from the genome and is no longer able to produce a protein. Mice with a $\beta 2$ deletion do not produce any nAChRs containing the $\beta 2$ subunit. That is, $\beta 2$ deficient mice do not produce $\alpha 4\beta 2$ receptors. Owens and colleagues [94] found that the mice deficient for the $\beta 2$ subunit exhibited less sensitivity to the alcohol-induced reduction of the acoustic startle response. Additional studies also indicated that the $\alpha 4\beta 2$ nAChRs are indeed involved in modulation of the effects of alcohol [8, 9].

Studies with $\alpha 7$ knock-out mice (the other nAChR most abundant in brain) have shown that deficiency of $\alpha 7$ homomeric nAChRs results in mice that, when exposed to alcohol, have less anxiety, increased alcohol-induced hypothermic response and longer duration of the alcohol-induced unconsciousness [95]. Interestingly, other measures of the mouse response to alcohol, such as acoustic startle, were not different between the mice lacking the $\alpha 7$ receptors and the control littermates, indicating that different nAChRs may be responsible for different behavioral responses to alcohol effects.

Regarding the $\alpha 6$ and $\beta 3$ receptor subunits, the α -Conotoxin MII snail toxin can be used to block $\alpha 6\beta 3$ -containing receptors and observe behavioral effects. For example, administration of α -Conotoxin MII to the rodent VTA brain region produced a diminished alcohol-induced release of dopamine and reduced stimulation of locomotor activity was

observed [66]. More recently, $\beta 3$ knock-out mice have been developed and studies have shown that this subunit is critical for the correct assembly, trafficking and stability of $\alpha 6$ -containing nAChRs in dopaminergic neurons. Further studies by Booker *et al.* [73] using $\beta 3$ -deficient mice, strongly suggested that the $\beta 3$ -containing nAChRs influence levels of anxiety and may be critical players in the continuation of smoking behaviors. Thus, $\alpha 6\beta 3$ -containing receptors emerge as a plausible common site for the interaction between nicotine and alcohol and could represent neurochemical targets for the development of drugs to treat alcoholism.

Animal models indicate that the $\beta 4$ subunit is important in the behavioral and physiological expression of anxiety in mice [96] and in the resistance to nicotine-induced seizures [97]. In particular, the role of the $\beta 4$ -containing nAChRs in mediating the signs of nicotine withdrawal has been addressed with the use of $\beta 4$ knock-out mice. Results from these studies have shown that the $\beta 4$ -deficient mice show decreased signs of nicotine withdrawal symptoms [97]. These data are of significant importance since $\beta 4$ -containing receptors appear to play a dominant role in the mediation of the negative reinforcement properties of nicotine.

In addition, the $\beta 4$ subunit is almost always co-expressed with the $\alpha 3$ subunit and both are necessary for normal autonomic function [98] but behavioral and physiological studies related to the $\alpha 3$ subunit have been difficult because mice deficient for this subunit rarely survive the weaning stages of life. However, mice that are heterozygous for the $\alpha 3$ deletion (+/- mice) are partially resistant to nicotine-induced seizures, similar to the $\beta 4$ deficient mice [97]. The behavioral effects of ethanol on these subunits have not been addressed, however, some evidence for the modulation of $\alpha 3\beta 4$ -containing nAChRs exists. Studies with rat nAChRs expressed in *Xenopus* oocytes performed a decade ago [99] concluded that the $\alpha 3\beta 4$ subunit combination may be especially sensitive to modulation by low ethanol concentrations. Furthermore, studies with adrenal PC12 cell lines also have shown that agonist responses in $\alpha 3\beta 4$ -containing nAChRs are sensitive to the effects of low concentrations of ethanol [5]. Thus, the $\alpha 3\beta 4$ -containing receptors could be contributors to the process of mutual reinforcement of alcohol and nicotine co-addiction.

Regarding the $\alpha 5$ subunit, experiments with $\alpha 5$ -deficient mice have shown that the presence of the $\alpha 5$ subunit is essential for the development of nicotine-induced seizures and hypolocomotion [96]. In fact, $\alpha 5$ -deficient mice show phenotypes very similar to the ones observed in $\beta 4$ -deficient mice. However, the rate of response to high doses of nicotine appears to be mediated by the $\alpha 5$ subunit, not the $\beta 4$ subunit [100]. The similar behavioral effects observed for these three subunits perhaps are not surprising since the genes for $\alpha 5$, $\alpha 3$ and $\beta 4$ subunits are clustered together in the genome and are likely to share regulatory elements [68].

INTERACTIONS OF NICOTINIC RECEPTORS WITH OTHER NEURONAL SYSTEMS

Alcohol and nicotine, at the pharmacological level, do not appear to share many characteristics: Nicotine is pro-convulsant, induces arousal and binds to specific cellular receptors. On the other hand, alcohol is anti-convulsant,

induces relaxation and appears to affect multiple receptors [101]. However, as mentioned earlier, the amount of tobacco smoked is positively correlated with the amount of alcohol consumed and the severity of alcohol dependence [30]. In animal models, chronic treatment with either ethanol or nicotine produces cross-tolerance to the other drug [102, 103]. Furthermore, a recent study examining the effects of nicotine and alcohol on fear conditioned learning has shown that these two drugs can each serve to ameliorate the negative effects of the other, which may be a mechanism contributing to their co-addiction [104].

The majority of the nAChRs are expressed on nerve terminals, where they function as modulators of the release of dopamine, GABA, norepinephrine, acetylcholine and glutamate [92]. Nicotine effects at the nAChRs are complex, because following activation, the nAChRs are quickly inactivated through a mechanism called desensitization. This inactivating mechanism could be the reason why chronic smokers no longer experience some of the effects of nicotine or, alternatively, it may generate a sensation that smokers crave. Since alcohol has been shown to interfere with this inactivation (desensitization) of nAChRs by nicotine [4, 5], this mechanism could contribute to the high prevalence of alcohol and nicotine co-morbidity.

Another mechanism that could explain the high alcohol consumption observed in smokers is based on the nicotinic modulation of GABA_A receptors after an ethanol challenge in rodents [105]. In these experiments, sub-chronic modulation of nicotinic receptors (pre-treatment with nicotine) attenuated the decline of dopamine in the nucleus accumbens of rats exposed to ethanol an hour earlier, thereby attenuating the sedative effects of alcohol and prolonging its stimulatory effects. Additional evidence of the ethanol potentiation of nAChR function comes from recent studies examining the release of GABA and glutamate neurotransmitters from rodent cortical bipolar neurons exposed to acetylcholine and ethanol [106].

The possible involvement of the glutamate (NMDA or *N*-methyl-D-aspartate) system in the behavioral interaction between nicotine and alcohol has also been recently explored in the rodent cerebellum [107]. In this study, the authors investigated the functional role of cerebellar NMDA in the ethanol and nicotine interaction using ethanol ataxia as the test response. An important observation in this study was the ability of intra-cerebellar nicotine administration to significantly attenuate ethanol ataxia, especially when intracerebellar NMDA was microinfused simultaneously. These results demonstrate the important function of nAChRs and NMDA receptors in the mediation of nicotine-ethanol motor behavioral interaction.

The relationship between the cholinergic and monoaminergic systems has also been documented [108]. It is of great interest to elucidate the subtypes of nAChRs responsible for the anxiety and depression behaviors related to smoking behaviors in humans. Studies with mutant mice support the role of nAChR subunits in behaviors related to anxiety and depression. For example, mice deficient in either the $\alpha 4$, $\beta 3$ or $\alpha 7$ subunits show less anxiety-like behaviors [57, 108]. In fact, tricyclic antidepressants, serotonin-selective

re-uptake inhibitors and the drug bupropion (atypical antidepressant) are all non-competitive antagonists of nAChRs. Moreover, the expression of nicotine-induced behavioral disinhibition observed in rodents can be counteracted by selective serotonin re-uptake inhibitors (citalopram) as well as by serotonin receptor (5-HT-1A) agonists that are also known to decrease ethanol consumption in rats and humans [109]. However, despite the well characterized connections between alcohol, nicotine depression and antidepressants, the role of nAChR as targets for depression-related behaviors is far from being understood.

In summary, a considerable amount of knowledge about the biology and function of nAChRs exists. The understanding of structural and functional subtypes of nAChRs and their specific distribution and pharmacology is rapidly improving, highlighting their role as mediators of addictive behaviors. Despite this expansion on studies of nAChRs, very little is known about human nAChRs at the molecular and pharmacological level.

DISCUSSION AND FUTURE DIRECTIONS

A growing body of research has provided evidence for the important role of genetic components in mediating the co-morbidity of alcohol and nicotine behaviors. Ultimately, the way humans cope with addictive drugs is really a combination of many environmental effects, multiple genes, and a large interaction between these factors. Certain genes may contribute to a greater likelihood of experimenting with and continuing to use drugs in high risk environments. Similarly, genetic constitution is likely to influence differential responses to drugs and whether or not certain individuals go on to continue to use, develop tolerance, and become dependent. Therefore, future behavioral genetic studies need to be aimed at developing approaches which can effectively model and understand potential gene-environment interactions contributing to the developmental stages in the etiology of addiction.

The mesolimbic dopamine system has been studied for many years as a key player in mediating pleasurable responses to drugs. Ongoing work provides evidence that neuronal nicotinic receptors are likely to be important components of this system, and may be a common site of action for nicotine and alcohol. Recent human association studies have provided strong support for an important role of these genes in nicotine behaviors, but few studies have yet examined alcohol phenotypes. Furthermore, the potential molecular functional consequences of specific human variations have not yet been studied, and this will be an important area of research for understanding potential differences in genetic regulatory mechanisms among individuals. One of the most significant advances enabling research of nicotinic receptors has been the development of a collection of mice which are deficient in specific nicotinic subunits and the ability to test these animals for behavioral responses to nicotine and alcohol. Future studies should include the development of double knock-out mice, which lack two or more specific subunits, and the development of mice with specific mutations in certain subunit genes informed by emerging findings from human genetic association studies. Additional results will also be expected from the use of conditional knock-out mice,

where the deletion of a specific subunit is controlled in a spatiotemporal manner, and the use of antisense oligonucleotide technologies to target specific neurons or areas of the brain. In addition, high resolution imaging techniques are emerging as state-of-the-art methods for studying addiction, and can include integration of genetic data to determine whether certain gene variants are associated with specific patterns of response in the brain [110]. Such translational research approaches are promising in their potential to yield significant insight into the molecular mechanisms of nAChRs and their role in drug-related behaviors.

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Key Learning Objectives:

- Family, twin, and adoption studies provide substantial evidence of a common genetic vulnerability to alcohol and nicotine addictions.
- Candidate gene and whole genome association studies are beginning to identify and replicate findings for nAChR genes that may underlie co-morbidity of these two drugs.
- Many pharmacological and animal studies have provided evidence for interactions of nicotine and alcohol within the central nervous system.
- Neuronal nicotinic acetylcholine receptors (nAChRs) represent a potential common site of action for nicotine and alcohol.
- The variety of subtypes, distribution, and properties of nAChRs are likely to be involved in the complex behavioral effects of alcohol and nicotine.
- Nicotine and alcohol also induce changes in many different neuronal pathways with connections to nAChRs, including the dopaminergic system, serotonergic pathways, opioid receptors, γ -aminobutyric acid receptors, and others.

Future Research Questions:

- Future work needs to focus on the developmental nature of these disorders by recruiting subjects prior to initiation of nicotine and alcohol use and continuing to follow individuals over their lifetime.
- New statistical approaches for analyzing possible gene-gene and gene-environment interactions will be essential for interpreting the information.
- Continued development of mouse behavioral models of nicotine and alcohol phenotypes, including translational approaches that target specific human DNA variations in transgenic mice, will be important in interpreting whether human gene associations lead to changes in behavior.
- Biological functional studies will be necessary in order to characterize the underlying molecular mechanisms of specific variations associated with these behaviors, potentially leading to improved prevention approaches and pharmacogenomic-based treatment approaches.
- Further development of non-invasive brain imaging techniques will aid in the elucidation of specific ligands and/or receptors in brain regions known to be associated with addiction phenotypes.

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