BIOCHEMICAL PHARMACOLOGY 75 (2008) 323–333

available at www.sciencedirect.com

journal homepage: www.elsevier.com/locate/biochempharm

Genetics of nicotinic acetylcholine receptors: Relevance to nicotine addiction

Yann S. Mineur, Marina R. Picciotto *

Department of Psychiatry, Yale University School of Medicine, 34 Park Street, 3rd Floor Research, New Haven, CT 06508, USA

ARTICLE INFO

Article history:
Received 24 April 2007
Accepted 7 June 2007

Keywords:
Nicotine
Nicotinic
Addiction
Genetics
Mouse
Human

ABSTRACT

Human twin studies have suggested that there is a substantial genetic component underlying nicotine dependence, ongoing smoking and ability to quit. Similarly, animal studies have identified a number of genes and gene products that are critical for behaviors related to nicotine addiction. Classical genetic approaches, gene association studies and genetic engineering techniques have been used to identify the gene products involved in nicotine dependence. One class of genes involved in nicotine-related behavior is the family of nicotinic acetylcholine receptors (nAChRs). These receptors are the primary targets for nicotine in the brain. Genetic engineering studies in mice have identified a number of subunits that are critical for the ability of nicotine to activate the reward system in the brain, consisting of the dopaminergic cell bodies in the ventral tegmental area and their terminals in the nucleus accumbens and other portions of the mesolimbic system. In this review we will discuss the various lines of evidence suggesting that nAChRs may be involved in smoking behavior, and will review the human and animal studies that have been performed to date examining the genetic basis for nicotine dependence and smoking.

1. Introduction

Tobacco smoking is expected to be the single biggest cause of mortality worldwide by 2030, killing about 10 million people per year [1]. This projection may seem paradoxical given the increased public awareness about the danger of smoking; however, dependence on nicotine, the primary psychoactive component of tobacco, leads many people to continue to smoke despite their desire to quit. It is therefore critical to understand the genetic basis underlying the mechanisms involved in nicotine reinforcement and dependence, so that novel treatments can be developed to fight this epidemic.

The primary targets for nicotine are the nicotinic acetylcholine receptors (nAChRs), ligand-gated ion channels with a central cation pore. nAChRs are highly conserved across species [2], and are expressed in most tissues and organs, including the brain. nAChRs can be detected on presynaptic terminals, cell bodies and dendrites of many neuronal subtypes (see [3] for a review). The endogenous ligand for nAChRs is acetylcholine (ACh) [4], and activation of nAChRs can potentiate neurotransmitter release and neuronal excitability throughout the brain. As a result, nAChRs can modulate a large number of behaviors, ranging from basic physiological functions such as pain sensation, sleep pattern and feeding, to more complex processes involved in learning, affect and reward [4–10]. Moreover, nAChRs affect brain development, through their effects on synaptic transmission and plasticity [11], as well as aging, through their neuroprotective effects (for review, see [10]).

* *Corresponding author*. Tel.: +1 203 737 2041; fax: +1 203 737 2043.
E-mail address: marina.picciotto@yale.edu (M.R. Picciotto).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.06.010

2. Nicotinic receptor subtypes: localization, composition and pharmacology

Several homologous nAChR subtypes are expressed in distinct but overlapping patterns in the brain (reviewed in [12]). Each nAChR is composed of five subunits and can either be homomeric or heteromeric. While very close in structure, each receptor subtype is also unique based on its ligand selectivity, affinity, and pharmacokinetics. In mammals, contrary to what has been recently reported in insects whereby alternative splicing could potentially provide different subunits [13], each subunit is encoded by a single gene (α2–10 and β2–4 subunits encoded by CHRNA2–10 and CHRNB2–4) [12]. One major nAChR subclass is sensitive to α-bungarotoxin (α-BTX; homomeric and heteromeric receptors containing α7–α10 subunits), whereas αBTX-insensitive receptors are heteromeric and contain α2–α6 and β2–β4 subunits [7]. In the brain the most abundant and widespread subtypes are α7 and α4/β2-containing (α4/β2*, where '*' indicates other, as yet unidentified subunits) nAChRs [14,15]. Homomeric nAChRS are composed of α subunits with five ligand-binding sites (one at each subunit interface) while α/β-containing nAChRs have only two binding sites (at the α–β interface) [16]. Further, heteromeric nAChR composition obeys the ''rule of three'' defined in [12]: two α subunits defining the main binding sites; II: two complementary non-α subunits; III: a structural subunit not involved in acetylcholine binding.

The structure and localization of the different nAChR subtypes has been established using a number of complementary techniques, including *in situ* hybridization and PCR for specific subunit RNAs, immunoprecipitation for protein subunits, imaging by autoradiography, PET and SPECT, and functional electrophysiological or neurotransmitter release assays. A number of limitations are inherent to these different techniques (false positives, lack of quantification, limited specificity of antibodies and ligands, etc.) [17,18] and therefore studies using genetically modified animals have been very important in characterizing and/or confirming the localization, composition, pharmacology and functionality of the various nAChR subtypes [19,20]. Further, while a number of studies historically focused on the biochemical properties and molecular composition of nAChRs, the development of genetic animal models with knockout, knockin or selective expression of nAChR subunits has allowed for the investigation of the role of specific nAChRs in complex behaviors, including those related to addiction.

3. Genetic exploration of nicotinic receptor polymorphisms

3.1. *Family and twin studies*

Several twin studies as early as the 1950s concluded that smoking behavior is determined by underlying genetic factors [21–23]. It seems clear, however, that environmental actors are more critical for smoking initiation whereas genetic factors contribute strongly smoking persistence and difficulty in quitting [24,25].

The identification of the potential gene(s) and allelic variant(s) responsible for smoking has been very challenging: first, smoking behavior is not a single trait and involves exposure to the drug, habit formation, cued reinforcement, nicotine metabolism and withdrawal. Second, nicotine addiction is a complex trait, meaning that many genes with relatively small effects are likely to modify smoking behavior. Third, unpredictable interactions between genetic and environmental factors add another level of complexity. However, nicotine dependence is a very reliable and robust disorder in humans, and is easy to quantify compared to other complex brain disorders, thus leading to precise phenotypic profile that cannot be obtained in other models. Furthermore, the development of powerful large-scale genetic techniques and the cross-communication between several fields including animal studies, biochemical investigations and molecular biology, have provided several candidate genes for smoking. Nevertheless, the majority of the genes likely to be involved in smoking behaviors are still to be identified.

3.2. *Candidate gene approaches and single nucleotide polymorphism (SNP) analyses*

A promising hypothesis-driven approach to identifying genes involved in nicotine addiction involves identifying polymorphisms in genes known to interact directly with nicotine. The enzymes that metabolize nicotine and clear it from the blood stream have been particularly interesting targets. For instance, two studies have shown that polymorphisms in the cytochrome CYP2A6 is associated with smoking [26,27], although another study did not confirm this finding [28]. Polymorphisms in other cytochromes such as CYP2D6 did not reveal any association in several independent cohorts [29,30].

Since nAChRs are the primary targets for nicotine in the brain, several studies have looked for associations between SNPs in genes encoding nAChR subunits and smoking behavior. Analyses of SNPs in CHRNA4 and CHRNB2 subunits detected a haplotypic association with an α4 polymorphism but not a β2 polymorphism, and this association was only found in women [31]. Another study showed no correlation between β2 subunit polymorphisms and smoking [32]. This does not necessarily suggest that the α4/β2 nAChRs are not important for smoking behavior (see also ''animal models'' section), since the currently identified SNPs do not appear to result in functional changes in these nAChR subunits.

Recent studies have suggested that the less abundant β4* nAChRs could play a significant role in cigarette smoking [33]. The analysis of several variants of human CHRNB4 revealed that specific point mutations were responsible for differential sensitization of β4* nAChRs, possibly resulting in differential sensitivity to nicotine.

3.3. *Linkage analyses*

A number of whole-genome linkage analyses have been performed to identify novel genes associated with smoking and nicotine dependence. Numerous genomic loci have shown linkage with smoking-related behaviors. For instance, associations with smoking have been found for loci located on chromosomes 1, 2, 6, 9, 11, 14, 17, 18, 19, 21 [34–38]. In the case of the locus identified on chromosome 17, fine mapping coupled with targeted SNP analyses revealed that CHRNB1, the

gene encoding the muscle nAChR β1 subunit, was associated with smoking [39]. This outcome appears paradoxical as CHRNB1 is not thought to be expressed in the brain; however there may be a contribution of peripheral nAChRs to smoking behavior that is not yet understood. It is also possible that the same chromosomal region contains other genes that may also account for smoking-related behaviors [40]. Despite these advances, many of these loci do not show consistent linkage across different independent studies and cohorts, cannot be replicated, and/or lack power to undertake satisfactory meta-analyses [41]. There is therefore still a lot to be done to understand the genetic susceptibility to smoking. In addition, ethical concerns have been raised regarding the implications of identifying genetic susceptibility to smoking, and this has become the object of debate [42,43].

4. Modeling and testing nicotine addiction in animals

The American Psychiatric Association defines dependence (''addiction'') as persistent drug use despite the motivation to quit and withdrawal symptoms when the drug is not readily available [44]. In addition, relapse and reinstatement of drug dependence even after a long period of abstinence generally accompany the aforementioned symptoms While several of these symptoms can be modeled in animals, this clinical profile cannot be completely recapitulated (for instance, it is difficult to model the motivation to quit). In addition, dose–response relationships can very greatly across species, and this is particularly true for nicotine [45]. Instead, animal studies have focused on reproducing specific endophenotypes, which can be reliably measured in animals.

Like many other drugs of abuse, nicotine is believed to act in part through activation of the mesocorticolimbic system. Activation of nAChRs on dopaminergic neurons of the ventral tegmental area (VTA) increases their firing rate and stimulates dopamine (DA) release from their terminals in the nucleus accumbens [46–48]. Lesions of, or infusion of nicotinic antagonists into, the VTA can prevent the development of behaviors related to nicotine addiction [46,49].

Nicotine self-administration, a paradigm in which an animal can work to receive infusions of nicotine, is one test with relatively high face-validity for human nicotine intake (see [50] for a detailed review); however, concerns about the reproducibility of nicotine self-administration and its ability to predict the efficacy of therapeutics for smoking cessation have been raised [51–54].

Another paradigm, conditioned place preference (CPP), assesses the ability of a drug to induce an association between its rewarding effects and environmental cues. Response to smoking-related cues is a critical component of nicotine addiction in humans, and similar effects have been observed in animals [55]. There is a very narrow dose range for nicotine CPP in rodents [56], perhaps because rodents are highly susceptible to stress–nicotine interactions (for example, mice must be pre-handled to show nicotine-induced CPP [57]). It is believed that this procedure would decrease stress levels in the experimental animals which might diminish stress-induced behavioral confounds.

Locomotor activation is frequently used as a measure of psychostimulant effects related to stimulation of the dopaminergic system. Similarly, locomotor sensitization is thought to depend on neuroadaptations that occur in the mesolimbic DA system following repeated drug exposure [58]. Many studies have demonstrated that nicotine increases extracellular DA levels in the nucleus accumbens leading, in turn, to increased locomotor activity [58–61].

Over time, smokers become less sensitive to the effects of nicotine, a phenomenon known as tolerance. The mechanisms underlying nicotine tolerance are not completely clear because chronic nicotine can induce desensitization of nAChRs, but also results in nAChR upregulation [62]. Upregulation is still observed in abstinent smokers at least 14 days after smoking cessation [63], and tolerance can also be observed without changes in nicotine binding [64]. In animals, this pharmacological response can be assessed using nicotine hypothermia and hyperalgesia. When injected with nicotine, body temperature decreases, due in part to vasoconstriction. Repeated nicotine injection leads to tolerance to the hypothermic effects of nicotine but sensitization to pain induced by footshock or tail pinch [65]. While there are likely to be differences between human responses and animal models, partly due to difficulties in assessing this complex phenotype, upregulation of high-affinity nicotinic receptors is observed following chronic nicotine administration or tobacco use across species examined to date (see for instance, [66–69]).

Combining behavioral assays related to addiction with pharmacological and/or genetic manipulations of nAChRs can provide insight into the neurobiology of nicotine addiction and potential pharmacotherapies for smoking cessation.

5. Genetic animal models used to study the effects of nicotine

Genetically modified animals have been very valuable for identifying the physiological and behavioral roles of individual nAChR subtypes [5,9]. This has been particularly important because most pharmacological agents that modulate nAChR function are not specific for individual subtypes. Invertebrates such as *Drosophila* and *C. elegans* can be useful in studying the functional properties of nAChRs [13,70,71] because these organisms are easily genetically manipulated; however these invertebrate models do have limitations, such as less complex brain structures and different nAChR subtypes and expression patterns, as compared to mammalian species. Also, fundamental differences in nicotinic receptors (expression, pharmacology) have been observed across the animal kingdom, and this is particularly true between mammals and invertebrates [72]).

Genetically manipulated mice are more difficult to generate and maintain than flies or worms, but the mouse is a mammalian organism with a fully sequenced genome, and exhibits behaviors that can be related to human addiction. A genetically manipulated mouse line should show reproducibility of phenotypes across time and hopefully across laboratories [73]. Further, a genetic animal model should rely on the same basic underlying mechanisms involved in addiction-related phenotypes. It is worth noting, however, that animal

phenotypes that resemble human traits (face-validity) may not always reflect similar underlying mechanisms.

5.1. *Inbred/selected strains*

Inbred mouse strains, lines of mice that are genetically identical as a result of inbreeding, have been very useful in demonstrating the genetic basis for nicotine sensitivity, physiological response and behavior [74]. Comprehensive batteries of behavioral tests have been used to define the effects of nicotine across a large number of inbred strains [75], and have shown that response to nicotine cannot simply be explained by nAChR levels in the brain [76]. Further investigations have shown that mice selected for ethanol sensitivity also show differential susceptibility to nicotine-induced locomotor depression [77]. In addition, susceptibility to nicotine-induced seizures was inversely correlated with oral nicotine intake across several mouse strains, suggesting a possible protective mechanism against nicotine-induced toxicity [78].

Similar studies have been performed in inbred rat strains. The Roman High and Low Avoidance strains of rats [79] display differential responses to nicotine in various assays [80] including locomotor activation [81], although other responses to nicotine, such as avoidance behavior, were not different between the two lines [82]. Variability in function of the adrenergic system seems to underlie many of the differences between these two lines [83]. Similarly, the Flinders sensitive rat line [84] is hypersensitive to anticholinesterase blockers and shows alterations in central nAChR number and function [85,86].

As in human linkage studies, it has been difficult to identify specific genes responsible for differential responses to nicotine in these inbred lines. A point mutation in the α4 gene, found in DBA mice and not in C57BL/6 mice, has been linked to response to nicotine. This confirms that differences in $\alpha4^*$ nAChR function across mouse strains may not be attributed to variation in nAChR expression levels but rather, to the biophysical properties of polymorphic $\alpha4^*$ nAChRs [87].

The improvement in molecular techniques has permitted extensive molecular dissection of nAChR functions (see Fig. 1), including their roles in behaviors related to addiction. The first genetic modifications of nAChRs in mice were developed by deleting (“knocking out”, abbreviated KO) DNA sequences

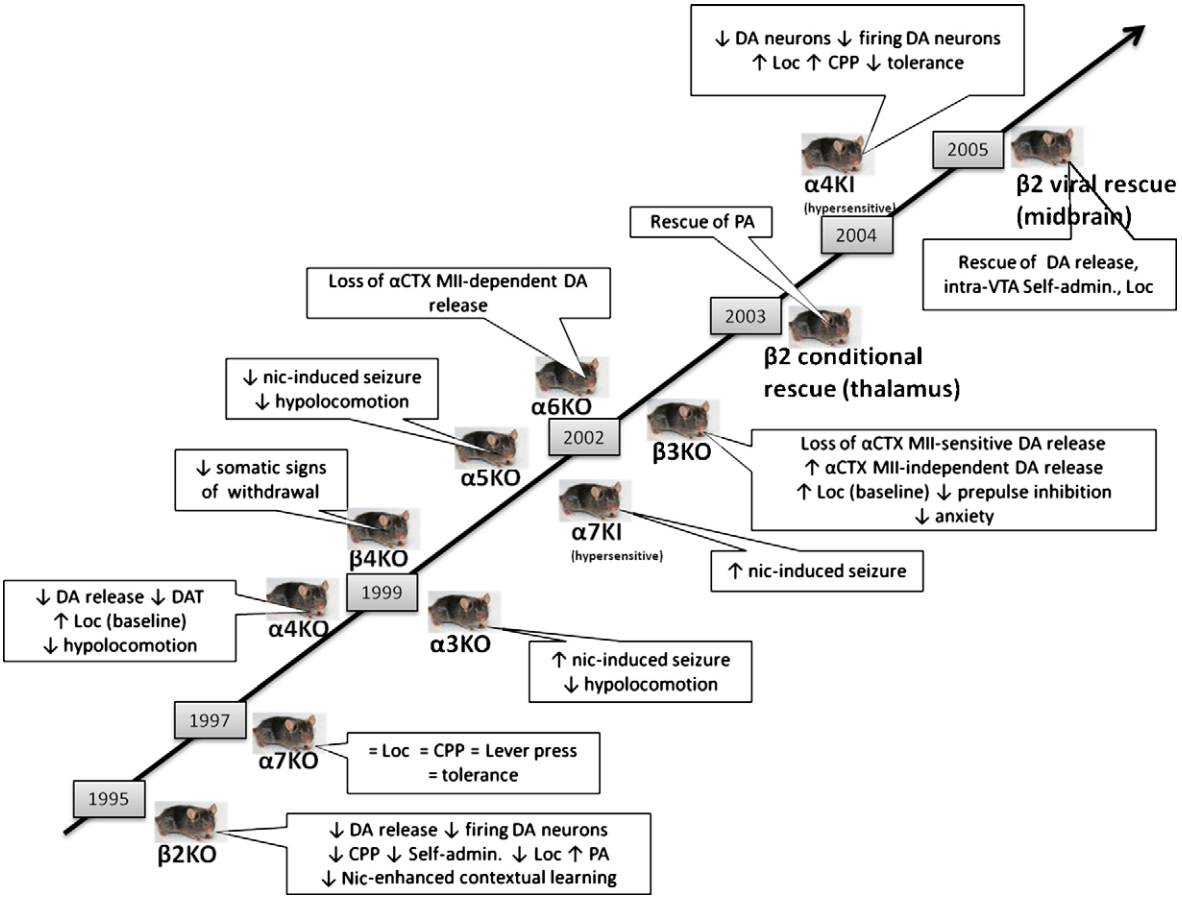

Fig. 1 – Genetically manipulated mouse models and their relevance for nicotine addiction. Timeline of the reported knockout or knockin of mutations for various nAChR subunits. Major phenotypes related to nicotine addiction are shown. Please note that the date for generation of the knockout lines may not match the date that the individual phenotypes were reported (see text for detailed paradigms and references). Abbreviations: ↑, increased compared to wild-type controls; ↓, decreased compared to wild-type controls; =, similar to wild-type controls; αCTX MII, α-conotoxin MII; CPP, nicotine-conditioned place preference; DA, dopamine; DAT, dopamine transporter; KO, knockout mouse line; KI, knockin mouse line; Loc, chronic nicotine-induced locomotor activation; Nic, nicotine; PA, passive avoidance; Self-admin., nicotine self-administration.

encoding individual nAChR subunits (see for instance [88,89]. While this approach defined links between specific nicotinic receptor subunits and behaviors related to nicotine addiction, constitutive knockout of a gene can result in molecular and physiological alterations, particularly during development [90]. In addition, nAChRs are expressed in many neuronal subtypes throughout the brain. Thus, KO mice lacking nAChR subunits in all cell types cannot be used to identify the anatomical locus of nAChR function. Thus, experiments using transgenic animals should always be interpreted with caution.

Targeted point mutations (''knockin'', abbreviated KI) in nAChR subunits have been used to alter the sensitivity of both of α4 [91,92] and α7 [93] subunits *in vivo*. Cell-specific and inducible transgenic expression systems can also provide information about the time in development or the neuronal subpopulations that are essential for particular behavioral effects of nicotine [94,95]. The use of viral vectors to express nAChR subunits in specific cells/brain loci on the background of a constitutive knockout has also been extremely powerful in identifying the anatomical basis for behavioral and neurochemical effects of nicotine [54,96]. Similarly, antisense oligonucleotides have been used to decrease the expression of specific nAChRs subunits in adulthood [97], although this approach has only been used for the α6 subunit.

5.2. *The β2 subunit [88]*

The β2-subunit, along with its partner α4, is the most widely expressed nAChR subunit in the brain [98]. β2* nAChRs have a high affinity for nicotine [88] and are essential for the control of dopaminergic neuron firing as they allow these neurons to switch from a resting to an active state [99]. In knockout mice lacking the β2 subunit, binding of α4/β2 agonists and antagonists is abolished [88,98], but the animals survive, are able to breed and appear grossly normal in most behavioral tasks [88]; however, most responses to nicotine appear to be absent in β2 subunit KO mice, including nicotine-induced DA release, nicotine-elicited increases in the firing rate of DA neurons and nicotine-elicited DA release in the striatum [100,101]. The inability of nicotine to stimulate the DA system in β2 KO mice is consistent with the absence of nicotine self-administration in these animals [100]. Several aspects of nicotine response associated with DA system activation are also abolished in β2 KO mice, including locomotor activation [95,102], nicotine discrimination [103], conditioned place preference [104] and increase in conditioned reinforcement following chronic nicotine exposure [105]. In addition, β2 KO animals have increased incentive for food reinforcement [105], and are not sensitive to the enhancing effects of nicotine or ethanol in contextual learning [106,107] and startle reflex [108].

Interestingly, the threshold for cocaine conditioned place preference is increased in β2 KO mice [109] and the ability of repeated cocaine exposure to result in sensitization of DA release is decreased in β2 KO mice treated with an α7 antagonist [110]. While there was a blunting of cocaine-induced CPP, β2 KO mice readily self-administer morphine, but not nicotine, into the VTA [54]. In contrast to acute measurements of nicotine reward and reinforcement, somatic signs of nicotine withdrawal are still observed in β2 KO mice [111,112].

Conditional rescue approaches have been important in defining the brain areas and times in development that β2* nAChRs are necessary for particular behaviors. For example, β2 KO mice show enhanced passive avoidance to a mild footshock [88] and tetracycline-inducible, localized transgenic rescue in corticothalamic glutamatergic neurons showed that this was due to a developmental role for β2* nAChRs [95]. In addition, viral vector-mediated re-expression of the β2 subunit in the VTA rescued DA release in the accumbens, neuronal firing in the VTA and intra-VTA nicotine self-administration [54], indicating that nAChRs in the VTA are critical for nicotine reinforcement during adulthood.

5.3. *The α4 subunit [113,114]*

The α4 subunit is the partner of the β2 subunit in the majority of heteromeric nAChRs in the brain. Animal studies have also shown that a functional polymorphism in the α4 subunit modulates nicotine intake in different mouse strains [115], possibly due to an alteration of the ratio between high and low affinity α4/β2* nAChRs [116,117].

Like β2 KO mice, α4 null-mutant mice do not show nicotine-elicited increase in striatal extracellular DA levels [118] and further abnormalities include failure to properly modulate dopaminergic transmission and impaired dopamine transporter levels [119]. These mice are also less sensitive to the locomotor depressant effects of nicotine while paradoxically, α4 KO mice had higher baseline locomotor activity compared to control animals. α6/β2* and α4β2* nAChRs appear to be the predominant nAChR subtypes that mediate endogenous cholinergic modulation of DA release [19]. Thus, increased function of α6/β2* nAChRs may be responsible for the baseline increase in locomotion in α4 KO mice.

Introduction of a point mutation resulting in a hypersensitive α4* nAChR receptor [91,120] resulted in nicotine-dependent locomotor sensitization and conditioned place preference at much lower doses of nicotine than were effective in wild-type mice [92]. This increase in sensitivity results in potential activation of these nAChRs by endogenous choline, and this may explain the loss of midbrain dopaminergic neurons in α4 knockin mouse lines [91,121]. α4 knockin mice also showed tolerance to a low dose of nicotine as measured by nicotine-induced hypothermia. These data strongly suggest that α4* nAChRs are both necessary and sufficient for behaviors related to nicotine addiction. Since the α4 subunit requires the β2 subunit for assembly, these studies using genetically modified mice suggest that α4/β2* nAChRs are critical for nicotine-related reward, motivation and tolerance.

5.4. *The α7 subunit [89]*

Like most of the other nAChR subunit KO mice, mice lacking the α7 subunit do not show gross anatomical or behavioral abnormalities [122]. The pharmacology and cellular responses of heteromeric nAChR subtypes are not altered in α7 KO mice [123]. There are, however, estrous cycle disturbances and lower rate of survival of pups born to α7 KO mothers [124]. Despite the broad expression of the α7 subunit in the brain, studies in α7 KO mice have not yet identified a role for the α7

subunit in nicotine reward and conditioning. Indeed, α7 KO animals show normal nicotine-conditioned place preference [104], lever-pressing behavior, nicotine-induced locomotor depression, and nicotine tolerance [125]. However, α7 KO animals do not show hyperalgesia following nicotine withdrawal [65]. It is clear that α7* nAChRs are important for long-term potentiation in the VTA [126], thus the absence of a phenotype related to nicotine reward in α7 KO mice may be due to compensation by other nAChR subunits. Consistent with this possibility, mice lacking both the α7 and the β2 subunits show greatly impaired passive avoidance learning [127].

Mice with a knockin of a point mutation resulting in hypersensitive α7 nAChRs are much more impaired than any of the knockout lines. Hypersensitive α7 knockin mice are barely viable since homozygotes die almost immediately after birth. Breeding of heterozygous animals showed that mice with hypersensitive α7 nAChR function showed nicotine-induced seizures at low doses (inversely correlated with oral nicotine intake in inbred strains of mice), whereas α7 null-mutants showed normal nicotine-induced seizure thresholds [128,129].

5.5. *The α3 subunit [130]*

The α3 subunit is expressed at high levels throughout the autonomic ganglia [131], in the medial habenula and superior colliculus [98] but low levels of CHRNA3 mRNA is also found in DA neurons [132–134]. α3 KO mice die very early, possibly due to autonomic dysfunction and impaired growth [130]. In addition, α3 KO mice show megacystis in the colon, which is analogous to the hypoperistalsis that has been observed in a human subject with an α3 deletion mutation [135]. α3 KO mice are resistant to nicotine-induced seizure and hypolocomotion [136]. Taken together, these data suggest that the α3 subunit is critical for ganglionic function but may also contribute to central responses to nicotine, such as nicotine-induced locomotor depression.

5.6. *The β4 subunit [137]*

The β4 subunit co-localizes with the α3 subunit in both the brain and the autonomic ganglia, but the β2 subunit may partially substitute for the loss of β4 in the autonomic ganglia since β4 KO mice were relatively normal, whereas β4/β2 double KO mice had a roughly similar phenotype to α3 KO animals [137]. These results confirm that α3/β4* nAChRs are the major nAChR subtype in the autonomic nervous system. β4* nAChRs appear to be responsible for the development of both somatic signs of nicotine withdrawal (such as increased grooming, chewing, scratching, and tremors) [111] and hyperalgesia [112], although the withdrawal syndrome in human smokers is normally characterized by more emotional processes, such as anhedonia and affective dysfunction, rather than somatic responses [138,139].

5.7. *The α5 subunit [140,141]*

α5 null-mutant mice do not show brain or anatomical abnormalities; moreover, these KO mice express normal levels of mRNAs encoding other nAChR subunits and have unchanged [^{125}I]-epibatidine and [^{125}I]α-bungarotoxin binding in the brain compared to wild-type animals. Despite having relatively unchanged nicotinic binding, α5 KO mice are less sensitive to nicotine-induced seizures and are also more resistant to the hypolocomotion induced by acute nicotine in specific mouse strains [140,141].

5.8. *The α6 [133] and β3 [142] subunits*

α6 and β3 knockout mice, like many other nAChR null-mutant lines, did not exhibit any obvious neurological or behavioral deficits, even in areas with high levels of expression, such as the visual and dopaminergic pathways [133]. One possibility is that expression of α4 subunits at least partially compensates for the loss of the α6 subunit in the dopamine system [133,142]. Knockout mouse studies were critical in demonstrating the that the α6, rather than the α3, subunit partners with β2 in mediating α-conotoxin MII-sensitive, nicotine-induced DA release [133], suggesting that α6* nAChRs play a potential role in addiction-related behaviors. In addition, the β3 subunit is likely to be important for assembly of α6* nAChRs, and regulates a-conotoxin MII binding sites and nicotine-mediated DA release [133,142].

6. Genetically manipulated mouse models: beyond addiction

The development of genetically modified animals with altered expression of nAChR subunit has identified many functions relevant to nicotine addiction; however, nicotinic receptors are involved in many functions in the nervous system, and also are important for basic physiological mechanisms.

It is clear that both anxiety and depression are related to smoking behavior in human subjects [9]. Consistent with a role for nAChRs in regulation of anxiety-like behavior, mice lacking either the α4, β3 or α7 subunits show less anxiety-like behaviors [122,143,144]. With respect to depression-like behavior, β2 KO mice do not respond to classical and atypical antidepressants, emphasizing the relationship between the cholinergic and the monoaminergic systems in mood disorders [145–147]. Interestingly, these mice are also less immobile in the forced swim and tail suspension tests at baseline, suggesting that β2* nAChRs may normally have a role in regulating mood.

7. Conclusions

Nicotine addiction is a continuing public health problem, and the need for therapeutics continues to drive research on nAChRs and their functions. To date, research into the role of human nAChR polymorphisms in smoking behavior has not resulted in significant associations, but this may be because functional alterations in nAChRs results in more profound dysfunction, such as nocturnal frontal lobe epilepsies [148,149] for a review see [150]. Limited power analyses, population variations and variable phenotypic definitions of nicotine dependence may also contribute to difficulty in identifying the

human genetics of vulnerability to smoking. Knockin mice bearing human ADNFLE mutations will be extremely useful in determining the functional consequences of these alterations in nAChR subunits, and may reveal neurochemical and behavioral consequences of these mutations that have not yet been examined in human subjects.

Genetically manipulated mice have been useful for identifying nAChR subtypes that are important for behaviors related to nicotine addiction. The combination of genetic and pharmacological techniques has highlighted specific targets for therapeutics to help people to quit smoking. In addition, the genetically manipulated mouse models have contributed to a greater understanding of the role of nAChRs in behaviors such as cognition and affect that may contribute to ongoing smoking, as well as to fundamental contributions of nAChRs to neuronal excitability. These studies have provided new targets for the investigation of nicotine-related mechanisms linked to addiction.

Acknowledgements

This work was supported by NIH grants DA13334/AA15632, DA14241 and DA00436.

REFERENCES

[1] World Health Organization. Updated projections of global mortality and burden of disease, 2002–2030: data sources methods and results; 2006.
[2] Le Novere N, Changeux JP. The ligand gated ion channel database. Nucleic Acids Res 1999;27:340–2.
[3] Dajas-Bailador F, Wonnacott S. Nicotinic acetylcholine receptors and the regulation of neuronal signalling. Trends Pharmacol Sci 2004;25:317–24.
[4] Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. Rev Physiol Biochem Pharmacol 2003;147:1–46.
[5] Picciotto MR, Caldarone BJ, King SL, Zachariou V. Nicotinic receptors in the brain. Links between molecular biology and behavior. Neuropsychopharmacology 2000;22:451–65.
[6] Wonnacott S, Kaiser S, Mogg A, Soliakov L, Jones IW. Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur J Pharmacol 2000;393:51–8.
[7] Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. Prog Neurobiol 2004;74:363–96.
[8] Hogg RC, Bertrand D. Nicotinic acetylcholine receptors as drug targets. Curr Drug Targets CNS Neurol Disord 2004;3:123–30.
[9] Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport 2002;13:1097–106.
[10] Picciotto MR, Zoli M. Nicotinic receptors in aging and dementia. J Neurobiol 2002;53:641–55.
[11] Berg DK, Conroy WG, Liu Z, Zago WM. Nicotinic signal transduction machinery. J Mol Neurosci 2006;30:149–52.
[12] Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. Trends Pharmacol Sci 2006;27:482–91.
[13] Sattelle DB, Jones AK, Sattelle BM, Matsuda K, Reenan R, Biggin PC. Edit, cut and paste in the nicotinic acetylcholine receptor gene family of *Drosophila* melanogaster. Bioessays 2005;27:366–76.
[14] Zoli M, Lena C, Picciotto MR, Changeux JP. Identification of four classes of brain nicotinic receptors using beta2 mutant mice. J Neurosci 1998;18:4461–72.
[15] Sargent PB. The diversity of neuronal nicotinic acetylcholine receptors. Annu Rev Neurosci 1993;16:403–43.
[16] Le Novere N, Corringer PJ, Changeux JP. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. J Neurobiol 2002;53:447–56.
[17] Moretti M, Vailati S, Zoli M, Lippi G, Riganti L, Longhi R, et al. Nicotinic acetylcholine receptor subtypes expression during rat retina development and their regulation by visual experience. Mol Pharmacol 2004;66:85–96.
[18] Le Novere N, Zoli M, Changeux JP. Neuronal nicotinic receptor alpha 6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. Eur J Neurosci 1996;8:2428–39.
[19] Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C, et al. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. J Neurosci 2003;23:7820–9.
[20] Jones IW, Wonnacott S. Why doesn't nicotinic ACh receptor immunoreactivity knock out? Trends Neurosci 2005;28:343–5.
[21] Fischer R. Lung cancer and cigarettes. Nature 1958;182:108.
[22] Carmelli D, Swan GE, Robinette D, Fabsitz R. Genetic influence on smoking—a study of male twins. N Engl J Med 1992;327:829–33.
[23] Edwards KL, Austin MA, Jarvik GP. Evidence for genetic influences on smoking in adult women twins. Clin Genet 1995;47:236–44.
[24] Heath AC, Cates R, Martin NG, Meyer J, Hewitt JK, Neale MC, et al. Genetic contribution to risk of smoking initiation: comparisons across birth cohorts and across cultures. J Subst Abuse 1993;5:221–46.
[25] Heath AC, Martin NG. Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. Addict Behav 1993;18:19–34.
[26] Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. Nature 1998;393:750.
[27] Tyndale RF, Sellers EM. Variable CYP2A6-mediated nicotine metabolism alters smoking behavior and risk. Drug Metab Dispos 2001;29:548–52.
[28] London SJ, Idle JR, Daly AK, Coetzee GA. Genetic variation of CYP2A6, smoking, and risk of cancer. Lancet 1999;353:898–9.
[29] Cholerton S, Boustead C, Taber H, Arpanahi A, Idle JR. CYP2D6 genotypes in cigarette smokers and non-tobacco users. Pharmacogenetics 1996;6:261–3.
[30] Saarikoski ST, Sata F, Husgafvel-Pursiainen K, Rautalahti M, Haukka J, Impivaara O, et al. CYP2D6 ultrarapid metabolizer genotype as a potential modifier of smoking behaviour. Pharmacogenetics 2000;10:5–10.
[31] Silverman MA, Neale MC, Sullivan PF, Harris-Kerr C, Wormley B, Sadek H, et al. Haplotypes of four novel single nucleotide polymorphisms in the nicotinic acetylcholine receptor beta2-subunit (CHRNB2) gene show no association with smoking initiation or nicotine dependence. Am J Med Genet 2000;96:646–53.
[32] Lueders KK, Hu S, McHugh L, Myakishev MV, Sirota LA, Hamer DH. Genetic and functional analysis of single nucleotide polymorphisms in the beta2-neuronal nicotinic acetylcholine receptor gene (CHRNB2). Nicotine Tob Res 2002;4:115–25.
[33] Liang Y, Salas R, Marubio L, Bercovich D, De Biasi M, Beaudet AL, et al. Functional polymorphisms in the

human beta4 subunit of nicotinic acetylcholine receptors. Neurogenetics 2005;6:37–44.
[34] Bergen AW, Korczak JF, Weissbecker KA, Goldstein AM. A genome-wide search for loci contributing to smoking and alcoholism. Genet Epidemiol 1999;17(Suppl. 1):S55–60.
[35] Duggirala R, Almasy L, Blangero J. Smoking behavior is under the influence of a major quantitative trait locus on human chromosome 5q. Genet Epidemiol 1999;17(Suppl. 1):S139–44.
[36] Gelernter J, Panhuysen C, Weiss R, Brady K, Poling J, Krauthammer M, et al. Genomewide linkage scan for nicotine dependence: identification of a chromosome 5 risk locus. Biol Psychiatry 2007;61:119–26.
[37] Gelernter J, Liu X, Hesselbrock V, Page GP, Goddard A, Zhang H. Results of a genomewide linkage scan: support for chromosomes 9 and 11 loci increasing risk for cigarette smoking. Am J Med Genet B Neuropsychiatr Genet 2004;128:94–101.
[38] Wang D, Ma JZ, Li MD. Mapping and verification of susceptibility loci for smoking quantity using permutation linkage analysis. Pharmacogenomics J 2005;5:166–72.
[39] Lou XY, Ma JZ, Payne TJ, Beuten J, Crew KM, Li MD. Gene-based analysis suggests association of the nicotinic acetylcholine receptor beta1 subunit (CHRNB1) and M1 muscarinic acetylcholine receptor (CHRM1) with vulnerability for nicotine dependence. Hum Genet 2006;120:381–9.
[40] Lou XY, Ma JZ, Sun D, Payne TJ, Li MD. Fine mapping of a linkage region on chromosome 17p13 reveals that GABARAP and DLG4 are associated with vulnerability to nicotine dependence in European-Americans. Hum Mol Genet 2007;16:142–53.
[41] Li MD, Ma JZ, Beuten J. Progress in searching for susceptibility loci and genes for smoking-related behaviour. Clin Genet 2004;66:382–92.
[42] Carlsten C, Burke W. Potential for genetics to promote public health: genetics research on smoking suggests caution about expectations. J Am Med Assoc 2006;296:2480–2.
[43] Bierut LJ, Cubells JF, Iacono WG, Li MD, Madden PA, Nelson EC, et al. Genetic research and smoking behavior. J Am Med Assoc 2007;297:809 (author reply 10).
[44] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. Washington, DC: American Psychiatric Association; 1994.
[45] Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, et al. Guidelines on nicotine dose selection for in vivo research. Psychopharmacology (Berl) 2007;190:269–319.
[46] Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res 1994;653:278–84.
[47] Picciotto MR, Corrigall WA. Neuronal systems underlying behaviors related to nicotine addiction: neural circuits and molecular genetics. J Neurosci 2002;22:3338–41.
[48] Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol 1989;40:191–225.
[49] Corrigall WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology (Berl) 1992;107:285–9.
[50] Corrigall WA. Nicotine self-administration in animals as a dependence model. Nicotine Tob Res 1999;1:11–20.
[51] Dar R, Frenk H. Do smokers self-administer pure nicotine? A review of the evidence. Psychopharmacology (Berl) 2004;173:18–26.
[52] Dar R, Frenk H. Nicotine may reinforce intravenous drug-taking behavior in drug users: a comment on Harvey et al. (2004). Psychopharmacology (Berl) 2005;179:516–7 (author reply 8–9).
[53] David V, Besson M, Changeux JP, Granon S, Cazala P. Reinforcing effects of nicotine microinjections into the ventral tegmental area of mice: dependence on cholinergic nicotinic and dopaminergic D1 receptors. Neuropharmacology 2006;50:1030–40.
[54] Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. Nature 2005;436:103–7.
[55] Le Foll B, Goldberg SR. Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. Trends Pharmacol Sci 2005;26:287–93.
[56] Picciotto MR. Nicotine as a modulator of behavior: beyond the inverted U. Trends Pharmacol Sci 2003;24:493–9.
[57] Grabus SD, Martin BR, Brown SE, Damaj MI. Nicotine place preference in the mouse: influences of prior handling, dose and strain and attenuation by nicotinic receptor antagonists. Psychopharmacology (Berl) 2006;184:456–63.
[58] Robinson TE, Becker JB. Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur J Pharmacol 1982;85:253–4.
[59] Balfour DJ, Benwell ME, Birrell CE, Kelly RJ, Al-Aloul M. Sensitization of the mesoaccumbens dopamine response to nicotine. Pharmacol Biochem Behav 1998;59:1021–30.
[60] Janhunen S, Tuominen RK, Piepponen TP, Ahtee L. Nicotine and epibatidine alter differently nomifensine-elevated dopamine output in the rat dorsal and ventral striatum. Eur J Pharmacol 2005;511:143–50.
[61] Hatchell PC, Collins AC. The influence of genotype and sex on behavioral sensitivity to nicotine in mice. Psychopharmacology (Berl) 1980;71:45–9.
[62] Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends Pharmacol Sci 1990;11:216–9.
[63] Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzler E, Frohlich E, Perry E, et al. Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. J Neurosci 2006;26:8707–14.
[64] McCallum SE, Caggiula AR, Booth S, Breese CR, Lee MJ, Donny EC, et al. Mecamylamine prevents tolerance but enhances whole brain [3H]epibatidine binding in response to repeated nicotine administration in rats. Psychopharmacology (Berl) 2000;150:1–8.
[65] Grabus SD, Martin BR, Batman AM, Tyndale RF, Sellers E, Damaj MI. Nicotine physical dependence and tolerance in the mouse following chronic oral administration. Psychopharmacology (Berl) 2005;178:183–92.
[66] Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J Pharmacol Exp Ther 1983;226:817–25.
[67] McCallum SE, Collins AC, Paylor R, Marks MJ. Deletion of the beta 2 nicotinic acetylcholine receptor subunit alters development of tolerance to nicotine and eliminates receptor upregulation. Psychopharmacology (Berl) 2006;184:314–27.
[68] Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ. Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. J Pharmacol Exp Ther 1999;289:1545–52.
[69] Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, et al. Effect of smoking history on [3H]nicotine binding in human postmortem brain. J Pharmacol Exp Ther 1997;282:7–13.
[70] Thany SH, Lenaers G, Raymond-Delpech V, Sattelle DB, Lapied B. Exploring the pharmacological properties of

insect nicotinic acetylcholine receptors. Trends Pharmacol Sci 2007;28:14–22.
[71] Jones AK, Sattelle DB. Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, *Caenorhabditis elegans*. Bioessays 2004;26:39–49.
[72] van Nierop P, Bertrand S, Munno DW, Gouwenberg Y, van Minnen J, Spafford JD, et al. Identification and functional expression of a family of nicotinic acetylcholine receptor subunits in the central nervous system of the mollusc Lymnaea stagnalis. J Biol Chem 2006;281:1680–91.
[73] Crabbe JC. Animal Model in Neurobehavioral Genetics: Methods for extimating Genetic Correlation. In: Jones C, Mormede P, editors. Neurobehavioral genetics: methods and applications. 1999.
[74] Hatchell PC, Collins AC. Influences of genotype and sex on behavioral tolerance to nicotine in mice. Pharmacol Biochem Behav 1977;6:25–30.
[75] Marks MJ, Romm E, Bealer SM, Collins AC. A test battery for measuring nicotine effects in mice. Pharmacol Biochem Behav 1985;23:325–30.
[76] Marks MJ, Burch JB, Collins AC. Genetics of nicotine response in four inbred strains of mice. J Pharmacol Exp Ther 1983;226:291–302.
[77] Phillips TJ, Burkhart-Kasch S, Gwiazdon CC, Crabbe JC. Acute sensitivity of FAST and SLOW mice to the effects of abused drugs on locomotor activity. J Pharmacol Exp Ther 1992;261:525–33.
[78] Robinson SF, Marks MJ, Collins AC. Inbred mouse strains vary in oral self-selection of nicotine. Psychopharmacology (Berl) 1996;124:332–9.
[79] Broadhurst PL, Bignami G. Correlative Effects of Psychogenetic Selection: a Study of the Roman High and Low Avoidance Strains of Rats. Behav Res Ther 1965;3: 273–80.
[80] Battig K, Driscoll P, Schlatter J, Uster HJ. Effects of nicotine on the exploratory locomotion patterns of female Roman high- and low-avoidance rats. Pharmacol Biochem Behav 1976;4:435–9.
[81] Schlatter J, Battig K. Differential effects of nicotine and amphetamine on locomotor activity and maze exploration in two rat lines. Psychopharmacology (Berl) 1979;64: 155–61.
[82] Fleming JC, Broadhurst PL. The effects of nicotine on two-way avoidance conditioning in bi-directionally selected strains of rats. Psychopharmacologia 1975;42:147–52.
[83] Schlatter J, Battig K. The adrenergic role in the manifestation of nicotine effects on maze ambulation in Roman High and Roman Low Avoidance rats. Br J Addict 1981;76:199–209.
[84] Dilsaver SC, Peck JA, Overstreet DH. The Flinders Sensitive Line exhibits enhanced thermic responsiveness to nicotine relative to the Sprague–Dawley rat. Pharmacol Biochem Behav 1992;41:23–7.
[85] Tizabi Y, Rezvani AH, Russell LT, Tyler KY, Overstreet DH. Depressive characteristics of FSL rats: involvement of central nicotinic receptors. Pharmacol Biochem Behav 2000;66:73–7.
[86] Auta J, Lecca D, Nelson M, Guidotti A, Overstreet DH, Costa E, et al. Expression and function of striatal nAChRs differ in the flinders sensitive (FSL) and resistant (FRL) rat lines. Neuropharmacology 2000;39:2624–31.
[87] Dobelis P, Marks MJ, Whiteaker P, Balogh SA, Collins AC, Stitzel JA. A polymorphism in the mouse neuronal alpha4 nicotinic receptor subunit results in an alteration in receptor function. Mol Pharmacol 2002;62:334–42.
[88] Picciotto MR, Zoli M, Lena C, Bessis A, Lallemand Y, Le Novere N, et al. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. Nature 1995;374:65–7.
[89] Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, et al. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. J Neurosci 1997;17:9165–71.
[90] King SL, Marks MJ, Grady SR, Caldarone BJ, Koren AO, Mukhin AG, et al. Conditional expression in corticothalamic efferents reveals a developmental role for nicotinic acetylcholine receptors in modulation of passive avoidance behavior. J Neurosci 2003;23:3837–43.
[91] Labarca C, Schwarz J, Deshpande P, Schwarz S, Nowak MW, Fonck C, et al. Point mutant mice with hypersensitive alpha 4 nicotinic receptors show dopaminergic deficits and increased anxiety. Proc Natl Acad Sci USA 2001;98:2786–91.
[92] Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 2004;306:1029–32.
[93] Orr-Urtreger A, Broide RS, Kasten MR, Dang H, Dani JA, Beaudet AL, et al. Mice homozygous for the L250T mutation in the alpha7 nicotinic acetylcholine receptor show increased neuronal apoptosis and die within 1 day of birth. J Neurochem 2000;74:2154–66.
[94] Chen J, Kelz MB, Zeng G, Sakai N, Steffen C, Shockett PE, et al. Transgenic animals with inducible, targeted gene expression in brain. Mol Pharmacol 1998;54: 495–503.
[95] King SL, Caldarone BJ, Picciotto MR. Beta2-subunit-containing nicotinic acetylcholine receptors are critical for dopamine-dependent locomotor activation following repeated nicotine administration. Neuropharmacology 2004;47(Suppl. 1):132–9.
[96] Molles BE, Maskos U, Pons S, Besson M, Guiard P, Guilloux JP, et al. Targeted in vivo expression of nicotinic acetylcholine receptors in mouse brain using lentiviral expression vectors. J Mol Neurosci 2006;30:105–6.
[97] le Novere N, Zoli M, Lena C, Ferrari R, Picciotto MR, Merlo-Pich E, et al. Involvement of alpha6 nicotinic receptor subunit in nicotine-elicited locomotion, demonstrated by in vivo antisense oligonucleotide infusion. Neuroreport 1999;10:2497–501.
[98] Zoli M, Picciotto MR, Ferrari R, Cocchi D, Changeux JP. Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. EMBO J 1999;18:1235–44.
[99] Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, et al. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron 2006;50:911–21.
[100] Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, et al. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 1998;391:173–7.
[101] Grady SR, Meinerz NM, Cao J, Reynolds AM, Picciotto MR, Changeux JP, et al. Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum. J Neurochem 2001;76:258–68.
[102] Villegier AS, Salomon L, Blanc G, Godeheu G, Glowinski J, Tassin JP. Irreversible blockade of monoamine oxidases reveals the critical role of 5-HT transmission in locomotor response induced by nicotine in mice. Eur J Neurosci 2006;24:1359–65.
[103] Shoaib M, Gommans J, Morley A, Stolerman IP, Grailhe R, Changeux JP. The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. Neuropharmacology 2002;42:530–9.

[104] Walters CL, Brown S, Changeux JP, Martin B, Damaj MI. The beta2 but not alpha7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. Psychopharmacology (Berl) 2006;184:339–44.
[105] Brunzell DH, Chang JR, Schneider B, Olausson P, Taylor JR, Picciotto MR. beta2-Subunit-containing nicotinic acetylcholine receptors are involved in nicotine-induced increases in conditioned reinforcement but not progressive ratio responding for food in C57BL/6 mice. Psychopharmacology (Berl) 2006;184:328–38.
[106] Davis JA, Gould TJ. beta2 subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. Psychopharmacology (Berl) 2007;190:343–52.
[107] Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, et al. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. Neuroscience 2004;129:11–24.
[108] Owens JC, Balogh SA, McClure-Begley TD, Butt CM, Labarca C, Lester HA, et al. Alpha 4 beta 2* nicotinic acetylcholine receptors modulate the effects of ethanol and nicotine on the acoustic startle response. Alcohol Clin Exp Res 2003;27:1867–75.
[109] Zachariou V, Caldarone BJ, Weathers-Lowin A, George TP, Elsworth JD, Roth RH, et al. Nicotine receptor inactivation decreases sensitivity to cocaine. Neuropsychopharmacology 2001;24:576–89.
[110] Zanetti L, de Kerchove D'Exaerde A, Zanardi A, Changeux JP, Picciotto MR, Zoli M. Inhibition of both alpha7* and beta2* nicotinic acetylcholine receptors is necessary to prevent development of sensitization to cocaine-elicited increases in extracellular dopamine levels in the ventral striatum. Psychopharmacology (Berl) 2006;187:181–8.
[111] Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the beta4 nicotinic acetylcholine receptor subunit. J Neurosci 2004;24: 10035–9.
[112] Besson M, David V, Suarez S, Cormier A, Cazala P, Changeux JP, et al. Genetic dissociation of two behaviors associated with nicotine addiction: beta-2 containing nicotinic receptors are involved in nicotine reinforcement but not in withdrawal syndrome. Psychopharmacology (Berl) 2006;187:189–99.
[113] Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature 1999;398:805–10.
[114] Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, et al. Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. J Neurosci 2000;20:6431–41.
[115] Butt CM, King NM, Hutton SR, Collins AC, Stitzel JA. Modulation of nicotine but not ethanol preference by the mouse Chrna4 A529T polymorphism. Behav Neurosci 2005;119:26–37.
[116] Kim H, Flanagin BA, Qin C, Macdonald RL, Stitzel JA. The mouse Chrna4 A529T polymorphism alters the ratio of high to low affinity alpha 4 beta 2 nAChRs. Neuropharmacology 2003;45:345–54.
[117] Butt CM, Hutton SR, Stitzel JA, Balogh SA, Owens JC, Collins AC. A polymorphism in the alpha4 nicotinic receptor gene (Chrna4) modulates enhancement of nicotinic receptor function by ethanol. Alcohol Clin Exp Res 2003;27:733–42.
[118] Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM, et al. Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. Eur J Neurosci 2003;17:1329–37.
[119] Parish CL, Nunan J, Finkelstein DI, McNamara FN, Wong JY, Waddington JL, et al. Mice lacking the alpha4 nicotinic receptor subunit fail to modulate dopaminergic neuronal arbors and possess impaired dopamine transporter function. Mol Pharmacol 2005;68:1376–86.
[120] Orb S, Wieacker J, Labarca C, Fonck C, Lester HA, Schwarz J. Knockin mice with Leu9'Ser alpha4-nicotinic receptors: substantia nigra dopaminergic neurons are hypersensitive to agonist and lost postnatally. Physiol Genomics 2004;18:299–307.
[121] Schwarz J, Schwarz SC, Dorigo O, Stutzer A, Wegner F, Labarca C, et al. Enhanced expression of hypersensitive alpha4* nAChR in adult mice increases the loss of midbrain dopaminergic neurons. Feder Am Soc Exp Biol J 2006;20:935–46.
[122] Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. Learn Mem 1998;5:302–16.
[123] Franceschini D, Paylor R, Broide R, Salas R, Bassetto L, Gotti C, et al. Absence of alpha7-containing neuronal nicotinic acetylcholine receptors does not prevent nicotine-induced seizures. Brain Res Mol Brain Res 2002;98:29–40.
[124] Morley BJ, Rodriguez-Sierra JF. A phenotype for the alpha 7 nicotinic acetylcholine receptor null mutant. Brain Res 2004;1023:41–7.
[125] Naylor C, Quarta D, Fernandes C, Stolerman IP. Tolerance to nicotine in mice lacking alpha7 nicotinic receptors. Psychopharmacology (Berl) 2005;180:558–63.
[126] Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron 2000;27:349–57.
[127] Marubio LM, Paylor R. Impaired passive avoidance learning in mice lacking central neuronal nicotinic acetylcholine receptors. Neuroscience 2004;129: 575–82.
[128] Broide RS, Salas R, Ji D, Paylor R, Patrick JW, Dani JA, et al. Increased sensitivity to nicotine-induced seizures in mice expressing the L250T alpha 7 nicotinic acetylcholine receptor mutation. Mol Pharmacol 2002;61:695–705.
[129] Gil Z, Sack RA, Kedmi M, Harmelin A, Orr-Urtreger A. Increased sensitivity to nicotine-induced seizures in mice heterozygous for the L250T mutation in the alpha7 nicotinic acetylcholine receptor. Neuroreport 2002;13: 191–6.
[130] Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou CN, et al. Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 1999;96:5746–51.
[131] Yeh JJ, Yasuda RP, Davila-Garcia MI, Xiao Y, Ebert S, Gupta T, et al. Neuronal nicotinic acetylcholine receptor alpha3 subunit protein in rat brain and sympathetic ganglion measured using a subunit-specific antibody: regional and ontogenic expression. J Neurochem 2001;77:336–46.
[132] Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci 2001;21:1452–63.
[133] Champtiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, et al. Distribution and pharmacology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. J Neurosci 2002;22:1208–17.
[134] Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, et al. The beta3 nicotinic receptor subunit: a component of alpha-conotoxin MII-binding nicotinic

acetylcholine receptors that modulate dopamine release and related behaviors. J Neurosci 2003;23:11045–53.
[135] Richardson CE, Morgan JM, Jasani B, Green JT, Rhodes J, Williams GT, et al. Megacystis-microcolon-intestinal hypoperistalsis syndrome and the absence of the alpha3 nicotinic acetylcholine receptor subunit. Gastroenterology 2001;121:350–7.
[136] Salas R, Cook KD, Bassetto L, De Biasi M. The alpha3 and beta4 nicotinic acetylcholine receptor subunits are necessary for nicotine-induced seizures and hypolocomotion in mice. Neuropharmacology 2004;47:401–7.
[137] Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, et al. Multiorgan autonomic dysfunction in mice lacking the beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptors. J Neurosci 1999;19: 9298–305.
[138] Shiffman S, Ferguson SG, Gwaltney CJ. Immediate hedonic response to smoking lapses: relationship to smoking relapse, and effects of nicotine replacement therapy. Psychopharmacology (Berl) 2006;184:608–18.
[139] Shiffman S, Ferguson SG, Gwaltney CJ, Balabanis MH, Shadel WG. Reduction of abstinence-induced withdrawal and craving using high-dose nicotine replacement therapy. Psychopharmacology (Berl) 2006;184:637–44.
[140] Wang N, Orr-Urtreger A, Chapman J, Rabinowitz R, Nachman R, Korczyn AD. Autonomic function in mice lacking alpha5 neuronal nicotinic acetylcholine receptor subunit. J Physiol 2002;542:347–54.
[141] Salas R, Orr-Urtreger A, Broide RS, Beaudet A, Paylor R, De Biasi M. The nicotinic acetylcholine receptor subunit alpha 5 mediates short-term effects of nicotine in vivo. Mol Pharmacol 2003;63:1059–66.
[142] Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, et al. The beta3 Nicotinic Receptor Subunit: a component of alpha-conotoxin mii-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. J Neurosci 2003;23:11045–53.
[143] Salas R, Pieri F, Fung B, Dani JA, De Biasi M. Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. J Neurosci 2003;23:6255–63.
[144] Caldarone BJ, Harrist A, Cleary MA, Beech RD, King SL, Picciotto MR. High-affinity nicotinic acetylcholine receptors are required for antidepressant effects of amitriptyline on behavior and hippocampal cell proliferation. Biol Psychiatry 2004;56:657–64.
[145] Booker TK, Butt CM, Wehner JM, Heinemann SF, Collins AC. Decreased anxiety-like behavior in beta3 nicotinic receptor subunit knockout mice. Pharmacol Biochem Behav 2007;87:146–57.
[146] Harrist A, Beech RD, King SL, Zanardi A, Cleary MA, Caldarone BJ, et al. Alteration of hippocampal cell proliferation in mice lacking the beta 2 subunit of the neuronal nicotinic acetylcholine receptor. Synapse 2004;54:200–6.
[147] Rabenstein RL, Caldarone BJ, Picciotto MR. The nicotinic antagonist mecamylamine has antidepressant-like effects in wild-type but not beta2- or alpha7-nicotinic acetylcholine receptor subunit knockout mice. Psychopharmacology (Berl) 2006;189:395–401.
[148] Fonck C, Cohen BN, Nashmi R, Whiteaker P, Wagenaar DA, Rodrigues-Pinguet N, et al. Novel seizure phenotype and sleep disruptions in knock-in mice with hypersensitive alpha 4* nicotinic receptors. J Neurosci 2005;25:11396–411.
[149] Fonck C, Nashmi R, Deshpande P, Damaj MI, Marks MJ, Riedel A, et al. Increased sensitivity to agonist-induced seizures, straub tail, and hippocampal theta rhythm in knock-in mice carrying hypersensitive alpha 4 nicotinic receptors. J Neurosci 2003;23:2582–90.
[150] Steinlein OK. Genetic mechanisms that underlie epilepsy. Nat Rev Neurosci 2004;5:400–8.