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# Insights into the Neurobiology of the Nicotinic Cholinergic System and Nicotine Addiction from Mice Expressing Nicotinic Receptors Harboring Gain-of-Function Mutations

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***Abstract*——Nicotinic acetylcholine receptors (nAChRs) are ligand-gated, cation-selective ion channels expressed throughout the brain. Although these channels have been investigated for several decades, it is still challenging 1) to identify the important nAChR subunits in cholinergic transmission and nicotine dependence and 2) to develop nAChR subtype-specific ligands. To overcome these challenges, we and others have studied mice expressing mutant, gain-of-function nAChR subunits. In this review, we discuss this research approach and the results it has yielded to date. Gain-of-function mutations, including those in nAChR subunits, provide an approach that is complementary to loss-of-function studies such as gene knockouts; the former allows one to answer questions of sufficiency and the latter addresses questions of necessity. Mutant mice expressing gain-of-function nAChR subunits are commonly produced using traditional gene targeting in embryonic stem cells, but novel approaches such as bacterial artificial chromosome transgenesis have yielded important insights as well. α7 nAChRs were the first nAChRs to be targeted with a gain-of-function mutation, followed by a pair of α4 nAChR gain-of-function mutant mice. These α4 nAChR gain-of-function mice (α4 L9′S mice, followed by α4 L9′A mice) provided an important system to probe α4 nAChR function in vivo, particularly in the dopamine reward system. α6 nAChR gain-of-function mice provided the first robust system allowing specific manipulation of this receptor subtype. Other targeted mutations in various nAChR subunits have also been produced and have yielded important insights into nicotinic cholinergic biology. As nAChR research advances and more details associated with nAChR expression and function emerge, we expect that existing and new mouse lines expressing gain-of-function nAChR subunits will continue to provide new insights.**

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## I. Introduction

Nicotinic acetylcholine receptors (nAChRs[1]) are pentameric ligand-gated ion channels (Itier and Bertrand, 2001). These protein complexes are widely expressed in muscle and neuronal tissues, and their potential utility as drug targets for various therapeutic interventions has been increasingly recognized in recent years (Aubin et al., 2011; Wallace and Porter, 2011; Williams et al., 2011). Heteromeric nAChRs are composed of $\alpha$ and $\beta$ subunits, which assemble around and operate a central, cation-conducting pore domain (Keramidas et al., 2004; Absalom et al., 2009; Zouridakis et al., 2009). Muscle-type nAChRs, including their subunit stoichiometry and role as postsynaptic ACh receptors at the neuromuscular junction (Changeux, 2010a), are well understood. In contrast, neuronal-type nAChRs comprise a larger group of receptor subtypes with a subcellular expression profile and functional role that is not as well understood. Interest in neuronal nAChRs arises from 1) their important natural role in the nicotinic cholinergic system (Maskos, 2010) as well as 2) their activation and/or desensitization by nicotine in cigarette smoke (Picciotto et al., 2008). These two overlapping roles in the brain continue to drive nAChR basic research and drug development efforts involving these receptors.

Research into nAChR expression and function has been hampered by two critical challenges: 1) the diversity of nAChR stoichiometries and expression patterns in the brain, and 2) selectivity, or lack thereof, of specific agonists or antagonists capable of functionally isolating specific nAChR subtypes in vivo. Aside from homomeric-type $\alpha$7 or $\alpha$8 nAChRs, there are three neuronal $\beta$ subunits ($\beta$2-$\beta$4) and at least seven neuronal $\alpha$ subunits ($\alpha$2-$\alpha$6, $\alpha$9, $\alpha$10) that assemble to form heteromeric nAChR pentamers (Keramidas et al., 2004; Dani and Bertrand, 2007; Absalom et al., 2009; Zouridakis et al., 2009). Each of these subunits is expressed in multiple brain areas, often with specific subcellular localization and developmental expression patterns (Marks et al., 1992; Azam et al., 2002). Thus, the "diversity" challenge arises from both 1) the large number of possible subunit combinations and 2) the poorly understood anatomical, subcellular, and developmental expression patterns of each of these subunits. Medium- or high-throughput screening assays in heterologous expression systems are required for discovering subtype-selective therapeutic neuronal nAChR compounds (Lester, 1988), but this problem of diversity has vitiated learning the appropriate subunit combination from the appropriate neuronal type. Even when the relevant subunit combination is known, challenges also arise in functional reconstitution of the particular nAChR subtype in ectopic expression systems (Drenan et al., 2008b).

During the past 10 to 15 years, approaches in mouse genetics have enabled investigators to address and, in some cases, overcome the challenges noted above. Gene-targeting approaches, used mainly to produce nAChR knockout (KO) mice, have provided important new details into nAChR neurobiology (Zhang, 2006; Fowler et al., 2008; Mineur and Picciotto, 2008; Changeux, 2010b). Mice lacking $\alpha$7 nAChR subunits have subtle phenotypes, such as decrements in sustained attention (Young et al., 2004) and impairments in passive avoidance learning (Marubio and Paylor, 2004). Although $\alpha$7 KO mice show alterations in DA neuron firing rates in response to endogenous ACh (Mameli-Engvall et al., 2006), they are largely normal with respect to nicotine and the DA reinforcement pathway (Naylor et al., 2005; Walters et al., 2006).

$\alpha$3 and $\beta$4 nAChR subunits are often coexpressed in the same nAChR pentamer, in both central neurons (Zoli et al., 1999) and peripheral nerves (Yeh et al., 2001). $\alpha$3 KO mice die perinatally, possibly because of autonomic dysfunction (Xu et al., 1999a). $\beta$4 KO mice, however, are grossly normal and may exhibit some compensation by $\beta$2 nAChR subunits (Xu et al., 1999b). These mice have decreased somatic signs of nicotine withdrawal (Salas et al., 2004), which was an important initial observation leading to the hypothesis that $\beta$4 (Frahm et al., 2011) and/or $\alpha$5* (* denotes the possibility that other nAChR subunits may be present in the pentameric receptor) nAChRs (Fowler et al., 2011) in the medial habenula (MHb)/interpeduncular nucleus (IPN) pathway mediate the aversive properties of nicotine. $\alpha$2 and $\alpha$5 KO mice also point to a critical role for these nAChR subunits in nicotine withdrawal (Salas et al., 2009). Fowler et al. (2011) demonstrated that $\alpha$5* nAChRs in MHb and/or IPN are critical regulators of nicotine intake. These results, along with those of Frahm et al. (2011), support the idea that human genetic polymorphisms in the $\alpha$5 nAChR subunit gene (Saccone et al., 2009) may partially underlie susceptibility to nicotine dependence.

$\beta$2 nAChR subunits are essential components of most heteromeric receptors with high affinity for nicotine, and $\beta$2 KO mice exhibit a number of behavioral and physiological phenotypes that reflect this fact. $\beta$2 KO mice are grossly normal (Picciotto et al., 1995), but nicotine-induced firing of DA neurons and nicotine-stimulated DA release is largely abolished (Picciotto et al., 1998; Grady et al., 201). Consequently, $\beta$2 KO mice do not self-administer nicotine (Picciotto et al., 1998), and nicotine is unable to condition a place preference (Walters et al., 2006) or activate locomotion in these mice (King et al., 2004; Villégier et al., 2006). Most $\alpha$4 nAChRs also contain $\beta$2 subunits, and $\alpha$4 KO mice recapitulate many phenotypes of $\beta$2 KO mice. $\alpha$4 KO mice have blunted nicotine-elicited DA release (Marubio et al., 2003), nicotine-stimulated locomotion is altered

[1]Abbreviations: ACh, acetylcholine; ADNFLE, autosomal-dominant nocturnal frontal-lobe epilepsy; BAC, bacterial artificial chromosome; CPP, conditioned place preference; DA, dopamine; ES, embryonic stem; IPN, interpeduncular nucleus; KO, knockout; MHb, medial habenula; nAChR, nicotinic acetylcholine receptor; neo, neomycin; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; WT, wild type.

(Marubio et al., 2003), and nicotine self-administration is abolished in these mice (Pons et al., 2008; Exley et al., 2011).

Interest in α6* and β3* nAChRs has been strong since the demonstration that these subunits exhibit an expression pattern restricted mainly to catecholaminergic and visual system neurons (Deneris et al., 1989; Le Novère et al., 1996; Forsayeth and Kobrin, 1997; Léna et al., 1999; Vailati et al., 2000; Whiteaker et al., 2000; Azam et al., 2002; Champtiaux et al., 2002; Zoli et al., 2002). α6 and β3 KO mice have reduced nicotine-stimulated DA release in striatum (Champtiaux et al., 2002, 2003; Cui et al., 2003). β3 nAChR subunits are important for α6* nAChR biogenesis in the DA system, because α6* nAChR binding and functional expression is dramatically reduced in β3 KO mice (Cui et al., 2003). Acute intravenous nicotine self-administration is eliminated in α6 KO mice and is restored when α6 subunits are selectively re-expressed in the VTA (Pons et al., 2008). In intracranial nicotine self-administration experiments where learning is required, α6 KO mice show a trend (though not a significant difference) toward reduced self-administration compared with control mice (Exley et al., 2011). β3* and/or α6* nAChRs may be involved in anxiety as well, because β3 KO mice exhibit reduced anxiety (Booker et al., 2007).

Heteromeric nAChRs containing α9 and α10 subunits are specialized nAChRs with high $Ca^{2+}$ permeability in auditory hair cells (Elgoyhen and Katz, 2012). α9α10 nAChRs are present at the medial olivocochlear (MOC)-hair cell synapse, and α9α10 nAChR activation by ACh causes $Ca^{2+}$-induced SK $K^+$ channel activation and hyperpolarization of the hair cell membrane (Elgoyhen and Katz, 2012). α9 KO mice have disturbed cochlear responses, and improper synaptic formation in the cochlea (Vetter et al., 1999; Murthy et al., 2009). Work with α10 KO mice demonstrated the essential nature of these subunits to the hair cell nAChR (Vetter et al., 2007).

nAChR KO mouse strains, although an essential research tool for understanding nicotinic cholinergic neurobiology, do not allow investigators to fully address the diversity and selectivity challenges in the nAChR field. In particular, KO animals typically only allow for answering questions of necessity—not sufficiency. We and others have studied several strains of knock-in or transgenic mice that are complementary to nAChR KO animals and to pharmacological studies on native receptors: mice expressing hypersensitive nAChR variants that functionally amplify and isolate the actions of specific nAChR subtypes. In this review, we discuss this approach in detail, including the development of new hypersensitive mouse models, key observations from several of these models, and their unique utility in advancing drug discovery efforts.

## II. Critical Issues in the Production of Useful Nicotinic Acetylcholine Receptor Gain-of-Function Mice

Unlike production of a KO mouse, whereUnlike production of a KO mouse, where removal or inactivation of the gene of interest can be done in many different ways, creation of a mouse with a single point mutation in a nAChR subunit poses two critical challenges: 1) selection of an appropriate mutation and 2) selection of an appropriate genetic targeting strategy. For the former, several amino acid residues in nAChR subunits are known to alter receptor sensitivity when mutated, but the preferred region of the receptor to mutate is the M2 transmembrane domain (Fig. 1) (Lester et al., 2003). The M2 region is an α-helix that contributes to the formation of the ion conducting pore of the pentameric ion channel (Miyazawa et al., 2003). Several highly conserved hydrophobic residues line the channel pore (Fig. 1), and when five contributing subunits are assembled, these hydrophobic residues are thought to create a channel gate that opens upon ligand binding (Miyazawa et al., 2003). Revah et al. (1991) first described mutations in a conserved leucine residue in the chick α7 homomeric receptor that significantly increased receptor sensitivity to agonist. Labarca et al. (1995) later demonstrated a similar effect in the muscle-type nAChR and further demonstrated that the number of nAChR subunits within a pentameric receptor with a sensitizing mutation is roughly proportional to the log of the gain in overall receptor sensitivity. This observation continues to prove useful in interpretation of data derived from nAChR knock-in/transgenic hypersensitive mice. Several studies on M2 domain mutations have since been conducted on many neuronal nAChR subunits, including α3 (Boorman et al., 2000), α4 (Fonck et al., 2005), α5 (Groot-Kormelink et al., 2001; Li et al., 2011), β3 (Boorman et al., 2000), and

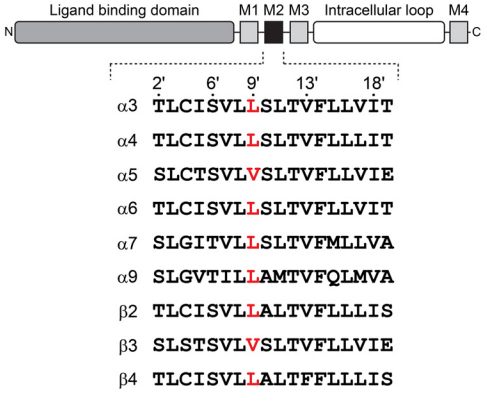


FIG. 1. nAChR M2 domain. nAChRs are composed of an N-terminal ligand binding domain, three adjacent transmembrane segments (M1–M3), a large cytoplasmic intracellular loop, and a fourth transmembrane segment (M4). Mouse M2 transmembrane protein sequences were aligned for the following nAChR subunits between the 2′ and 19′ positions: α3, α4, α5, α6, α7, β2, β3, and β4. The 9′ Leu residue is highly conserved across nAChRs and is often mutated to Ser or Thr when creating nAChR gain-of-function mutant mice.

β4 (Boorman et al., 2000). Although there are conflicting reports on α5 (Groot-Kormelink et al., 2001; Li et al., 2011), results from nearly all these studies are consistent: polar substitutions at the 9′ or 13′ position in the M2 domain (Fig. 1) produce a marked (10- to 100-fold) leftward shift in the concentration-response curve, and thus create a useful feature to better understand these proteins in vitro and in vivo [in the useful "prime" nomenclature, the 1′ position denotes the most N-terminal, cytoplasmic residue in the M2 region (Charnet et al., 1990)]. These mutations also produce biophysical effects that may be unwanted, leading to difficulty in interpretation of experimental results. For example, 9′ serine or threonine mutations may alter receptor desensitization (Revah et al., 1991), create alternative conducting states (Bertrand et al., 1992), abolish or reduce voltage rectification (Revah et al., 1991; Bertrand et al., 1992; Xiu et al., 2009), and increase receptor open time (Labarca et al., 1995). Although it is possible to understand a particular subunit of interest quite well in vitro, it is not often predictable how a specific mutation will perform in vivo.

Once a "hypersensitive" mutation is chosen for incorporation into the genome of a mouse, the second challenge is choosing the appropriate genetic targeting strategy. The knock-in approach uses homologous recombination in embryonic stem (ES) cells to replace one WT allele of the nAChR gene of interest with an allele containing the hypersensitive point mutation (Doyle et al., 2012). This approach has the advantage of being highly precise, only modifying the endogenous nAChR gene and usually leaving behind only one 34-base pair loxP sequence (Doyle et al., 2012). The knock-in approach is more time-consuming, however, usually requiring 1 to 3 years to produce a usable strain. Creation of a transgenic mouse is the alternative approach to a knock-in. In this method, exogenous DNA that directs expression of the hypersensitive nAChR subunit of interest is randomly inserted into the genome (Gong et al., 2003; Doyle et al., 2012). Because the ectopic DNA is capable of incorporating into undesired loci in the genome, possibly disrupting expression of other important genes, several founder strains must be studied at the outset, and misexpression of the nAChR gene of interest in ectopic brain areas must be mitigated. The use of bacterial artificial chromosomes (BACs) containing the nAChR gene of interest along with important regulatory sequences can reduce expression artifacts (Gong et al., 2003). Although the transgenic approach suffers from imprecision, the time to product is much shorter (~3 months), and creation of a targeting vector is simpler compared with the knock-in approach (Gong et al., 2003; Doyle et al., 2012). Regardless of the molecular biological route taken to produce the desired mouse strain, the resulting hypersensitive mutation should produce a substantial leftward shift in the concentration-response curve for the particular receptor of interest (Fig. 2). In the future, newer methods, such as conditional gene knock-in/KO approaches, inducible expression systems (e.g., tetracycline on/off systems), and/or targeted expression of nAChR genes with viral vectors (Molles et al., 2006) could be used to create new mouse models expressing hypersensitive nAChRs.

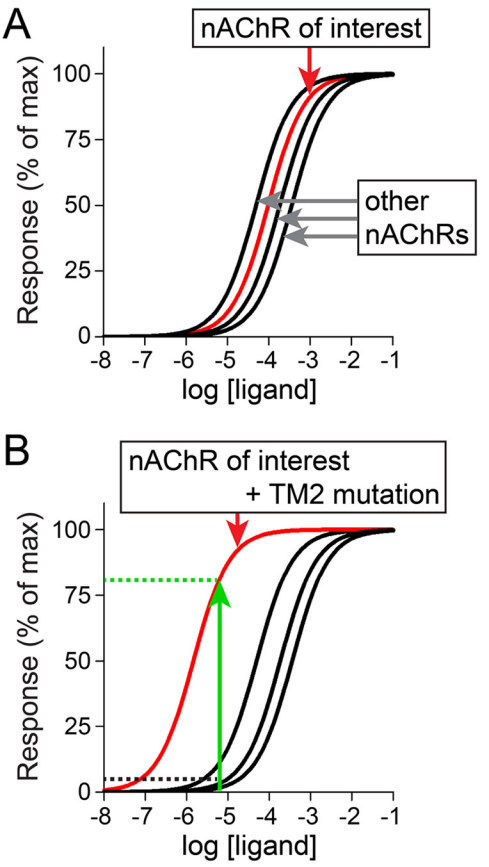


FIG. 2. Effect of a transmembrane (TM) 2 mutation on nAChR sensitivity. A, the particular nAChR subtype of interest will often, broadly speaking, have a sensitivity to ligand similar to that of other nAChR subtypes. In the absence of ligands that specifically activate the nAChR subtype of interest, an alternative approach to isolating the biological action of the receptor is to increase the sensitivity of the receptor by 10- to 100-fold. B, in the presence of a sufficient fraction of nAChR subunits of interest harboring a TM2 sensitizing mutation, receptor sensitivity is dramatically increased and the action of the receptor can be pharmacologically isolated relative to other nAChRs.

## III. Critical Molecular, Neurochemical, and Behavioral Observations from Mice Expressing Hypersensitive Nicotinic Acetylcholine Receptor Subunits

*1. α7 Nicotinic Acetylcholine Receptor Subunits.* To date, mouse α7, α4, α6, and α9 nAChR genes have been targeted using the hypersensitive nAChR approach (Table 1). Orr-Urtreger et al. (2000) first reported the production of a mouse expressing hypersensitive, α7 L250T nAChRs. Mice homozygous for the L250T substitution mutation died perinatally, possibly as a result of excitotoxic cell death resulting from excess $Ca^{2+}$ entry through mutant channels. Because α7-dependent currents in L250T mice were prominent at low concentrations of nicotine, this report suggested that recording

TABLE 1
*Summary of key results obtained from studies on mice with hypersensitive nAChR subunit mutations*

| nAChR Subunit and TM2 Mutation | Key Results | Reference |
|---|---|---|
| α4 | | |
| L9′S | Increased anxiety, poor motor learning, and locomotor hyperactivity in heterozygote mice | Labarca et al., 2001 |
| | Sensitization to the seizure-causing action of nicotine and other nAChR agents | Fonck et al., 2003 |
| | Sensitization to the antinociceptive action of nicotine | Damaj et al., 2007 |
| L9′A | Selective activation of α4* nAChRs is sufficient for nicotine reward, tolerance, and sensitization | Tapper et al., 2004 |
| | Seizure and sleep phenotypes associated with hypersensitive α4 subunits | Fonck et al., 2005 |
| | α4* nAChRs regulate respiratory rhythm | Shao et al., 2008 |
| | Demonstrated strong expression of α4* nAChRs in ventrolateral medial habenula | Fonck et al., 2009 |
| | Selective activation of α4* nAChRs is necessary and sufficient for varenicline's ability to reduce alcohol consumption | Hendrickson et al., 2010 |
| α6 | | |
| L9′S | Selective α6* nAChR activation causes spontaneous and nicotine-elicited locomotor hyperactivity | Drenan et al., 2008 |
| | Altered frequency-dependent DA release in α6 L9′S mice; α4 subunits are required for all significant behavioral effects of the α6 L9′S mutation | Drenan et al., 2010 |
| | Voluntary exercise is inversely correlated with the degree of α6* nAChR hypersensitivity | Cohen et al., 2012 |
| α7 | | |
| L250T | Perinatal death of α7 L250T homozygotes but survival of L250T heterozygotes | Orr-Urtreger et al., 2000 |
| | Hypersensitivity to nicotine-induced seizures | Broide et al., 2002 |
| | Somatodendritic expression of α7 nAChRs on midbrain DA neurons | Wooltorton et al., 2003 |
| α9 | | |
| L9′T | Mutant mice are protected from hearing loss in response to intense noise | Taranda et al., 2009 |

from neurons in brain slices derived from mice with hypersensitive nAChRs is a viable method for assaying functional expression of a channel of interest without the need for complex pharmacological inhibition of other nAChRs. In contrast to homozygous L250T mice, animals heterozygous for the L250T mutation were viable and fertile (Orr-Urtreger et al., 2000). These mice exhibit augmented nicotine-elicited currents in hippocampal neurons, and are hypersensitive to nicotine-induced seizures (Broide et al., 2002). It is noteworthy that Dani and colleagues demonstrated somatodendritic expression of functional α7 nAChRs in VTA and substantia nigra pars compacta (SNc) DA neurons (Wooltorton et al., 2003), implicating native α7 nAChRs in nicotine action at midbrain DA neuron cell bodies (Fig. 3A). In the future, these mice could prove useful in determining the role of heightened α7* nAChR activity in nicotine reward behavior.

*2. α4 Nicotinic Acetylcholine Receptor Subunits.* After the pioneering proof-of-concept studies on α7 L250T mice, knock-in mice expressing α4 L9′S mutant receptors were reported (Labarca et al., 2001). Like homozygous α7 L250T mice, α4 L9′S homozygous mice died perinatally (Labarca et al., 2001), probably from excitotoxic cell death of midbrain DA neurons (Labarca et al., 2001; Orb et al., 2004). α4 L9′S receptor sensitivity in homozygous animals may have been elevated such that normal levels of extracellular choline were able to constitutively activate α4 L9′S receptors (Labarca et al., 2001; Orb et al., 2004), leading to cell death. Heterozygous α4 L9′S mice retaining the neomycin (neo) selection cassette in the α4 gene were viable and fertile, and they also showed deficits in DA neuron survival, specifically in SNc (Labarca et al., 2001; Orb et al., 2004). These mice had reduced expression of the mutant subunit, suggesting that a mixture of α4 L9′S and WT alleles was more compatible with survival (Labarca et al., 2001; Orb et al., 2004). Similar to homozygous versus heterozygous α7 L250T mice (Orr-Urtreger et al., 2000), a mixture of mutant and WT α4 alleles may have produced α4* nAChRs with only modest changes in channel properties (Labarca et al., 2001). This was a key observation leading to the design of BAC transgenic α6 L9′S mice (Drenan et al., 2008a). Heterozygous α4 L9′S mice have a number of behavioral abnormalities, including elevated anxiety, poor motor learning, locomotor hyperactivity, and hypersensitivity to nicotine-induced reduction in ambulation (Labarca et al., 2001). Midbrain DA neurons survive better in α4 L9′S neo-intact heterozygous animals (Labarca et al., 2001), but ~35% of these neurons die, and the remaining neurons exhibit elevated sensitivity to nicotine (Labarca et al., 2001; Orb et al., 2004). In α4 L9′S heterozygote mice injected with a helper-dependent, cre enzyme-producing adenovirus into SNc, α4 L9′S receptor expression was increased because of cre-mediated removal of the neo selection cassette (Schwarz et al., 2006). Increased α4 L9′S receptor expression was associated with 1) increased DA neuron death due to cholinergic excitotoxicity and 2) a reduction in amphetamine-stimulated locomotion (Schwarz et al., 2006). This approach may be a useful way to inducibly destroy midbrain DA neurons similar to the DA neuron loss seen in Parkinson's disease (Schwarz et al., 2006). α4 L9′S mice also highlight the importance of α4* nAChRs in analgesic pathways and mechanisms, because α4 L9′S heterozygotes were 6-fold more sensitive to the antinociceptive effect of nicotine in the hotplate assay (Damaj et al., 2007).

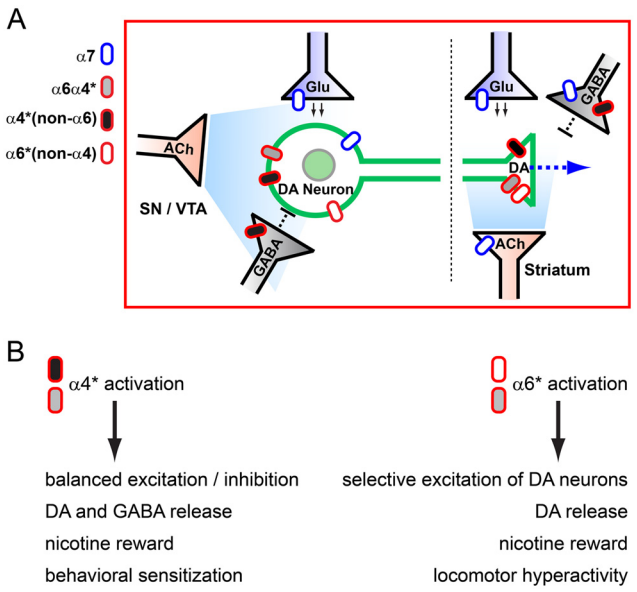


FIG. 3. DA transmission regulated by the nicotinic cholinergic system: insights from hypersensitive nAChR mice. A, DA neurons express both α4*(non-α6), α6*(non-α4), and α4α6* nAChRs on their soma and dendrites, and a fraction of DA neurons also express α7 nAChRs. α7 nAChRs are also known to be expressed on glutamatergic terminals that synapse onto DA neurons. Midbrain GABAergic neurons express α4*(non-α6) nAChRs. Cholinergic input to the ventral midbrain is capable of activating all of these nAChRs. In striatum, DA terminals also express α4*(non-α6), α6*(non-α4), and α4α6* nAChRs, which respond to local ACh to modulate DA release. Striatal α4*(non-α6) nAChRs are known to participate in cholinergic regulation of GABA release. In striatum, α7 nAChRs are expressed in GABAergic and cholinergic cells, but are largely excluded from DA terminals. B, in α4 L9′A mice, selective activation of α4* nAChRs largely recapitulates the action of nicotine, producing both activation of DA neurons via the action of somatodendritic α4* nAChRs and attenuation of DAergic activity through stimulation of inhibitory GABAergic interneurons. Selective α4* activation with nicotine produces nicotine reward as well as behavioral sensitization and tolerance. In α6 L9′S mice, selective activation of α6* nAChRs can specifically activate DA neurons and cause elevated DA release and locomotor hyperactivity.

Because α4 L9′S mice express nAChRs with M2 domain mutations that probably increase α4* nAChR sensitivity in vivo, they are a useful model of human autosomal-dominant nocturnal frontal-lobe epilepsy (ADNFLE) (Scheffer et al., 1994; Scheffer et al., 1995). ADNFLE is a rare disorder caused by M2 domain mutations in either human α4 or β2 nAChR subunits (Sutor and Zolles, 2001). ADNFLE begins in childhood or adolescence and is characterized by seizures occurring during sleep and that probably originate in frontal cortex (Scheffer et al., 1994; Scheffer et al., 1995). Heterozygous α4 L9′S mice exhibit increased sensitivity to nicotine-, epibatidine-, tacrine-, and galanthamine-induced seizures, as well as a pronounced tail dorsiflexion (Straub tail) phenotype at low-to-moderate (0.1–1.0 mg/kg) nicotine doses (Fonck et al., 2003). After these studies, investigators created various knock-in mouse models by expressing the mouse equivalent to human ADNFLE mutations in α4 (Klaassen et al., 2006; Teper et al., 2007) or β2 (Xu et al., 2010) nAChR genes. These models further support the notion that ADNFLE is mediated by mutant nAChRs, possibly as a result of augmented nAChR sensitivity (Rodrigues-Pinguet et al., 2005), changes in α4β2* nAChR stoichiometry (Son et al., 2009), or altered GABAergic network activity (Steinlein, 2010).

To overcome the problems associated with neonatal lethality of α4 L9′S homozygote mice, a new α4 nAChR knock-in strain was created with L9′A mutant subunits (Tapper et al., 2004). The α4 L9′A mutation produces a marked (~50-fold) left-shift in the concentration-response relation, but the mutant nAChRs are not activated by circulating levels of choline as α4 L9′S receptors are. As a result, the α4 L9′A mice do not experience excitotoxic depolarization. The α4 L9′A heterozygous and homozygous mice are viable and fertile, and they have led to several important insights into α4β2* nAChR function. In 2004, a study on α4* nAChRs in nicotine action used the α4 L9′A model to show that α4* nAChRs are sufficient for nicotine reward (Tapper et al., 2004). Selective activation of α4* nAChRs (which may include α4(non-α6)β2 and α4α6β2* nAChRs; Fig. 3A) with low doses of nicotine led to a conditioned place preference (CPP) for the nicotine-paired chamber in CPP assays. Repeated activation of α4* nAChRs in vivo also resulted in behavioral sensitization and/or tolerance to the locomotor-suppressive and hypothermia-producing effects of nicotine in α4 L9′A mice (Tapper et al., 2004, 2007). When combined with parallel experiments on α4 KO mice (Tapper et al., 2007), these studies suggest that α4* nAChRs are necessary and sufficient for critical aspects of nicotine's addictive properties. These results were later corroborated by elegant studies using lentiviral re-expression of α4 subunits in VTA of α4 KO mice (Pons et al., 2008; Exley et al., 2011). Studies in α4 L9′A mice also point to a role for α4* nAChRs in ethanol consumption and to an opportunity to target these receptors in ethanol addiction (Hendrickson et al., 2010, 2011). Parallel experiments in α4 L9′A mice and α4 KO mice show that α4* nAChRs are necessary and sufficient for the ability of varenicline, a α4β2 nAChR partial agonist, to inhibit ethanol consumption (Hendrickson et al., 2010).

Like their α4 L9′S predecessors, α4 L9′A mice are hypersensitive to nicotine-induced seizures (Fonck et al., 2005). α4 L9′A mice also exhibit disturbances in their sleep-wake cycles (Fonck et al., 2005) and respiratory rhythms (Shao et al., 2008). The latter is caused by continuous activation of α4* nAChRs in medullary pre-Bötzinger complex inspiratory neurons by ACh, leading to nicotinic cholinergic modulation of hypoglossal nerve (XIIn) activity (Shao et al., 2008). α4 L9′A mice also demonstrate an imbalance in normal dopamine/acetylcholine cross-talk in the striatum (Zhao-Shea et al., 2010). Challenging α4 L9′A mice with quinpirole, a $D_2$ DA receptor agonist, caused akinesia, rigidity, tremor, and cataplexy (Zhao-Shea et al., 2010). Patch-clamp recordings from striatal cholinergic interneurons in α4 L9′A brain slices suggested that an interaction between $D_2$ DA receptors and α4* nAChRs, which is functionally

augmented in α4 L9′A mice, may lead to this locomotor abnormality (Zhao-Shea et al., 2010). Together, these studies demonstrate the usefulness of α4 hypersensitive mice in understanding the neurobiological and behavioral aspects of nicotine dependence, ethanol consumption, circadian biology, Parkinson's disease, and epilepsy. As approaches such as 1) creation of mice with multiple nAChR gene mutations and 2) ectopic expression of nAChRs in select brain areas continue to mature, we expect α4-hypersensitive mice to continue to be useful for studying complex behaviors.

In addition to their utility in studying complex behaviors, α4 L9′A knock-in mice are also useful in mapping brain circuits that either contain α4* nAChRs, or are activated by α4(+) neurons. Taking advantage of the ability to selectively activate α4* nAChRs, (Fonck et al. (2009)) injected α4 L9′A mice with low doses of nicotine and searched for activated neurons using c-Fos immunohistochemistry. Using this procedure, they discovered a specific group of neurons in the ventrolateral aspect of the MHb that strongly express α4* nAChRs (Fonck et al., 2009). Given the recent appreciation for the MHb and IPN in nicotine consumption (Fowler et al., 2011; Frahm et al., 2011) and cholinergic transmission (Ren et al., 2011), these experiments in α4 L9′A mice suggest a role for α4* nAChRs in regulating neurotransmission in the MHb during nicotine exposure. Zhao-Shea et al. (2011) recently used α4 L9′A mice to map α4* nAChR expression within the VTA, reporting that α4* nAChRs coassemble with α6 subunits preferentially in posterior VTA. As a follow-up, Liu et al. (2012) demonstrated that a population of α4* nAChRs in VTA DA neurons may be resistant to desensitization by nicotine. These nAChRs probably contain both α4 and α6 nAChR subunits (Fig. 3A), and their activation by nicotine may drive VTA DA neuron firing for several minutes during smoking (Liu et al., 2012).

*3. α6 Nicotinic Acetylcholine Receptor Subunits.* Since the discovery that α6 nAChR subunits are selectively expressed in relatively few brain areas (Le Novère et al., 1996; Léna et al., 1999), including midbrain DA neurons (Azam et al., 2002), α6* nAChRs have been the subject of intense basic and translational research. More than a decade after the first comprehensive study (Kuryatov et al., 2000), the ability to reconstitute α6* nAChRs in vitro for biophysical studies or drug screening has been improving (Walsh et al., 2008; Kuryatov and Lindstrom, 2011). Xiao et al. (2011) greatly improved expression of functional α6* nAChRs by 1) coexpressing green fluorescent protein-labeled α6 subunits (to better identify transfected cells) (Drenan et al., 2008b) and 2) substituting mutant β2 subunits with enhanced endoplasmic reticulum export and plasma membrane expression (Srinivasan et al., 2011).

To learn about the role of α6* nAChRs in vivo, and to circumvent the problems associated with α6* in vitro expression, we constructed and studied BAC transgenic mice expressing hypersensitive α6 L9′S subunits. α6 L9′S mice have a number of spontaneous and induced behavioral alterations, including home cage hyperactivity, nicotine-stimulated hyperactivity, and reduced wheel rotations (Drenan et al., 2008a, 2010; Grady et al., 2010; Cohen et al., 2012). Patch-clamp recordings from α6 L9′S midbrain slices containing SNc and VTA demonstrated that SNc DA neurons—but not nearby SNr GABA neurons—can be selectively activated by low doses of nicotine (Drenan et al., 2008a) or ACh (R. Drenan, unpublished observations). Electrochemical recordings of DA release from α6 L9′S striatal slices also demonstrated altered frequency-dependent DA release in these mice, because a 1-pulse stimulus induced less DA release and a four-pulse burst stimulus induced greater release compared with control (Drenan et al., 2010).

α6 L9′S mouse behavioral phenotypes, as well as neurochemical and physiological results, were sensitive to genetic manipulations that affected the sensitivity of the α6* nAChR pool. For example, a line of α6 L9′S mice with fewer copies of the mutant α6 transgene demonstrated normal locomotor activity and agonist-induced DA release from striatal synaptosomes (Cohen et al., 2012). In addition, crossing the α4 KO strain with α6 L9′S mice removed α4 subunits from α6* nAChRs, resulting in 1) a reduced sensitivity of the α6* nAChR pool, 2) normalized frequency-dependent DA release from striatal slices, and 3) attenuation of most of the behavioral alterations seen in α6 L9′S mice (Drenan et al., 2010).

These studies indicate that α6 L9′S mice are or may be very useful in studying behaviors involving DA transmission (drug addiction, Parkinson's disease, schizophrenia, attention-deficit/hyperactivity disorder, etc.), and specifically those DA-dependent behaviors that may be influenced by activation of brain cholinergic systems. Collectively, studies with α6 L9′S mice point to the need to maintain a precise level of endogenous nicotinic cholinergic input to DA neurons, because moderate perturbations in the strength of nAChR activation by ACh can induce substantial alterations in DA-dependent behaviors (Cohen et al., 2012). In addition to endogenous ACh, studies with α6 L9′S mice have begun to show the role of α6* nAChRs in the response to nicotine (Drenan et al., 2008a, 2010; Grady et al., 2010; Cohen et al., 2012). Recent work implicates α6* nAChRs in nicotine reward behavior (Jackson et al., 2009; Brunzell et al., 2010; Gotti et al., 2010; Exley et al., 2011), and α6 L9′S mice will be instrumental in determining whether α6* nAChR activation is sufficient to establish and/or maintain nicotine reward.

*4. α9 and α10 Nicotinic Acetylcholine Receptor Subunits.* As discussed in section I, α9α10 nAChRs are selectively expressed in the cochlea, where they mediate SK2 $K^+$ channel activation and subsequent inhibition of auditory hair cells (Elgoyhen and Katz, 2012). Taranda et al. (2009) produced α9 nAChR subunit L9′T gain-of-

function mice. In hair cells of α9 L9′T mice, ACh-gated nAChR responses are hypersensitive to agonist and desensitize less compared with responses in WT hair cells (Taranda et al., 2009). Synaptic currents are prolonged in hair cells of α9 L9′T mice, and these mice show reduced hearing loss in response to intense noise relative to WT littermates (Taranda et al., 2009). This study, along with parallel studies in α9 KO mice, show that cholinergic synaptic feedback in the cochlea is important in providing protection from acoustic trauma (Taranda et al., 2009). These mice should continue to be useful in understanding the contribution of the auditory system to complex behaviors, including mating and maternal behavior.

## IV. Conceptual Advances in Understanding Dopamine Reinforcement Circuitry

*1. α7* Nicotinic Acetylcholine Receptors.* Mice expressing hypersensitive nAChRs have contributed substantially to our understanding of the DA system. Since the seminal work by Mansvelder and McGehee (2000), α7 nAChRs have been strongly implicated in DA reinforcement. Although their work and that of others (Schilström et al., 1998) have implicated α7 nAChRs primarily on glutamatergic presynaptic terminals that synapse onto DA neurons, other results suggested the involvement of α7 nAChRs directly within DA neurons (Pidoplichko et al., 1997). Work with α7 L250T mice directly demonstrated a role for these receptors in DA neuron somata (Wooltorton et al., 2003) (Fig. 3A). These results were corroborated later by Yang et al. (2009), who used acutely dissociated DA neuron preparations to demonstrate expression of functional, α7 nAChR currents in a substantial fraction of DA neurons. In addition, electrophysiology and pharmacology methods have been used to localize α7 nAChR function to several cell types in striatum (Azam et al., 2003; Xiao et al., 2009) (Fig. 3A). Additional work is required, however, to further define the role of α7* nAChRs in striatum.

*2. α4* versus α6* Nicotinic Acetylcholine Receptors.* The role of β2* nAChRs in DA transmission has also been elucidated in part via mice expressing hypersensitive α4 and α6 nAChR subunits. In particular, α4 L9′A and α6 L9′S mice have revealed subtle but important differences for α4 versus α6 subunits in the DA reward system. α4 L9′A mice lack dramatic spontaneous behavioral phenotypes (Tapper et al., 2004, 2007; Fonck et al., 2005). Most results from α4 L9′A mice injected with α4-specific doses of nicotine (which activate α4(non-α6)β2* and α4α6β2* nAChRs), such as nicotine CPP, behavioral sensitization, locomotor suppression and nicotine-induced seizures, largely recapitulate the effects of nicotine in WT mice (Tapper et al., 2004, 2007). These data suggest that α4* nAChRs function within DA system circuits such that selective activation of these receptors maintains a roughly WT-like balance in excitatory, inhibitory, or modulatory transmission (Fig. 3B). This may be due largely to the expression of α4β2* nAChRs within both DA neurons and the cells that tonically inhibit them, midbrain GABAergic interneurons (Nashmi et al., 2007) (Fig. 3A). Experiments with α4 KO (McClure-Begley et al., 2009) mice also implicate α4* nAChRs in striatal GABA release, which may affect DA transmission via cholinergic signaling (Witten et al., 2010) (Fig. 3A).

In contrast, α6 L9′S mice exhibit various locomotor hyperactivity phenotypes, including spontaneous home cage hyperactivity (Drenan et al., 2008a) (Fig. 3B). In addition, in contrast to results with α4 L9′A mice, repeated daily nicotine administration to α6 L9′S mice does not lead to behavioral tolerance or sensitization (Tapper et al., 2004, 2007; Drenan et al., 2008a) (Fig. 3B). α6* nAChRs are strongly implicated in nicotine reward (Jackson et al., 2009; Brunzell et al., 2010; Gotti et al., 2010; Exley et al., 2011), and experiments in α6 L9′S mice testing the role of these receptors are a major priority. These results, when coupled with the ability of nicotinic agonists to selectively activate α6 L9′S DA neurons without stimulating local, inhibitory GABAergic neurons (Drenan et al., 2008a) (Fig. 3A), suggest differing natural roles for α4 versus α6 subunits in the DA system. α4β2* nAChRs may be general-purpose, high-sensitivity ACh receptors important for modulating circuit activity in response to cholinergic tone. On the basis of their extremely high sensitivity to ACh (Salminen et al., 2007) and peculiar expression pattern in the brain (Le Novère et al., 1996; Léna et al., 1999), α6 nAChR subunits may confer a more specialized property to nAChR pentamers. More experiments are needed to address this hypothesis.

## V. Implications for Development of Therapeutics

nAChR preclinical drug development involves in vitro studies on heterologously expressed ion channels, followed by animal studies to probe both safety and efficacy of the candidate drug. Although the safety of a candidate drug is often reliably tested in animals, efficacy cannot readily be evaluated in the absence of an appropriate assay, where one can be confident that the target receptor is being activated. α4 L9′A and α6 L9′S mice, because of their ability to report selective α4* or α6* nAChR activation, offer a solution to some of the problems associated with in vivo efficacy testing. In particular, assays that use a behavioral or physiological readout that is specific for the nAChR in question provide instant information regarding a drug's bioavailability. Among several behavioral readouts that result from selective α4* nAChR activation in α4 L9′A mice, nicotine-induced hypothermia has proven useful (Tapper et al., 2004). In this assay, mice are implanted with subcutaneous temperature probes that transmit data to a nearby receiver that is connected to a computer (Tapper

et al., 2004, 2007). Mice are administered nicotine or a drug candidate, and body temperature is recorded. Whereas WT mice typically become hypothermic at nicotine doses ranging from 0.5 to 10.0 mg/kg, hypothermia in α4 L9′A mice can be induced selectively through α4* nAChRs at doses of 0.01 to 0.03 mg/kg (Tapper et al., 2004, 2007). We used this assay recently in a secondary screen of a small library of compounds for their ability to activate or block α4* nAChRs in vivo (Grady et al., 2010).

In α6 L9′S mice, α6* nAChRs can be studied in a similar manner using locomotor behavioral assays (Drenan et al., 2008a). In these mice, nicotine selectively activates α6* nAChRs and briefly (5–10 min) induces locomotor activity at doses of 0.02 to 0.15 mg/kg (Drenan et al., 2008a). We found that compounds with α6-selectivity in vitro were able to strongly activate locomotion in vivo (Drenan et al., 2008a). In addition, we used this assay to show that α4-selective drugs can weakly induce locomotor activity in α6 L9′S mice, implicating α4α6* nAChR pentamers in this locomotor response (Drenan et al., 2008a). These results were later corroborated by studies in α6 L9′S mice that were crossed to the α4 KO (Drenan et al., 2010). Such results are useful in providing a detailed description of the important nAChR subtypes giving rise to behavioral effects in these mice.

Studies in α4 and α6 hypersensitive mice, such as those noted above, are generally useful in identifying compounds that activate or antagonize these receptor subtypes and could improve nAChR drug development for several diseases/disorders that implicate nicotinic cholinergic control of dopamine neurotransmission, including nicotine dependence, alcohol dependence, and Parkinson's disease. Varenicline, a cytisine-derived α4β2* and α6β2* nAChR partial agonist (Grady et al., 2010), is used as a smoking cessation aid and has been demonstrated to reduce ethanol consumption in rats (Steensland et al., 2007) and mice (Hendrickson et al., 2010, 2011). Varenicline exerts these effects primarily through VTA neurons and the DA system (Hendrickson et al., 2010, 2011) but has undesirable side effects such as GI disturbances (Hays et al., 2008). These results suggest that future nAChR-directed drug therapies for smoking or alcohol-use cessation could include compounds that more selectively target DA system nAChRs, perhaps by selectively activating α4α6β2* nAChRs that are more selectively expressed in DA neurons (Drenan et al., 2010).

Whereas smoking cessation and reduction of alcohol-use may be effectively treated with nAChR partial agonists, full nAChR agonists may be useful in treating Parkinson's disease. PD involves a reduction in forebrain DA levels, and α6-specific agonists may be very useful in stimulating DA release from residual, surviving DA terminals (Quik and McIntosh, 2006). α6-activating drugs could be combined with levodopa therapy to potentially allow lower doses of levadopa to effectively control PD symptoms. This approach could delay or fully prevent the development of levodopa-induced dyskinesias (Quik et al., 2008).

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**Authorship Contributions**

*Wrote or contributed to the writing of the manuscript:* Drenan and Lester.

**References**

Absalom NL, Schofield PR, and Lewis TM (2009) Pore structure of the Cys-loop ligand-gated ion channels. *Neurochem Res* **34:**1805–1815.
Aubin HJ, Karila L, and Reynaud M (2011) Pharmacotherapy for smoking cessation: present and future. *Curr Pharm Des* **17:**1343–1350.
Azam L, Winzer-Serhan U, and Leslie FM (2003) Co-expression of α7 and β2 nicotinic acetylcholine receptor subunit mRNAs within rat brain cholinergic neurons. *Neuroscience* **119:**965–977.
Azam L, Winzer-Serhan UH, Chen Y, and Leslie FM (2002) Expression of neuronal nicotinic acetylcholine receptor subunit mRNAs within midbrain dopamine neurons. *J Comp Neurol* **444:**260–274.
Bertrand D, Devillers-Thiéry A, Revah F, Galzi JL, Hussy N, Mulle C, Bertrand S, Ballivet M, and Changeux JP (1992) Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc Natl Acad Sci USA* **89:**1261–1265.
Booker TK, Butt CM, Wehner JM, Heinemann SF, and Collins AC (2007) Decreased anxiety-like behavior in β3 nicotinic receptor subunit knockout mice. *Pharmacol Biochem Behav* **87:**146–157.
Boorman JP, Groot-Kormelink PJ, and Sivilotti LG (2000) Stoichiometry of human recombinant neuronal nicotinic receptors containing the β3 subunit expressed in *Xenopus* oocytes. *J Physiol* **529:**565–577.
Broide RS, Salas R, Ji D, Paylor R, Patrick JW, Dani JA, and De Biasi M (2002) Increased sensitivity to nicotine-induced seizures in mice expressing the L250T α7 nicotinic acetylcholine receptor mutation. *Mol Pharmacol* **61:**695–705.
Brunzell DH, Boschen KE, Hendrick ES, Beardsley PM, and McIntosh JM (2010) α-Conotoxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens shell regulate progressive ratio responding maintained by nicotine. *Neuropsychopharmacology* **35:**665–673.
Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Léna C, Clementi F, Moretti M, Rossi FM, Le Novère N, et al. (2003) Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knockout mice. *J Neurosci* **23:**7820–7829.
Champtiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, McIntosh JM, and Changeux JP (2002) Distribution and pharmacology of α6-containing nicotinic acetylcholine receptors analyzed with mutant mice. *J Neurosci* **22:**1208–1217.
Changeux JP (2010a) Allosteric receptors: from electric organ to cognition. *Annu Rev Pharmacol Toxicol* **50:**1–38.
Changeux JP (2010b) Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat Rev Neurosci* **11:**389–401.
Charnet P, Labarca C, Leonard RJ, Vogelaar NJ, Czyzyk L, Gouin A, Davidson N, and Lester HA (1990) An open-channel blocker interacts with adjacent turns of alpha-helices in the nicotinic acetylcholine receptor. *Neuron* **4:**87–95.
Cohen BN, Mackey ED, Grady SR, McKinney S, Patzlaff NE, Wageman CR, McIntosh JM, Marks MJ, Lester HA, and Drenan RM (2012) Nicotinic cholinergic mechanisms causing elevated dopamine release and abnormal locomotor behavior. *Neuroscience* **200:**31–41.
Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, Salminen O, Tritto T, Butt CM, Allen WR, et al. (2003) The β3 nicotinic receptor subunit: a component of α-conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. *J Neurosci* **23:**11045–11053.
Damaj MI, Fonck C, Marks MJ, Deshpande P, Labarca C, Lester HA, Collins AC, and Martin BR (2007) Genetic approaches identify differential roles for α4β2* nicotinic receptors in acute models of antinociception in mice. *J Pharmacol Exp Ther* **321:**1161–1169.
Dani JA and Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* **47:**699–729.
Deneris ES, Boulter J, Swanson LW, Patrick J, and Heinemann S (1989) β3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. *J Biol Chem* **264:**6268–6272.
Doyle A, McGarry MP, Lee NA, and Lee JJ (2012) The construction of transgenic and gene knockout/knockin mouse models of human disease. *Transgenic Res* **21:**327–349.
Drenan RM, Grady SR, Steele AD, McKinney S, Patzlaff NE, McIntosh JM, Marks MJ, Miwa JM, and Lester HA (2010) Cholinergic modulation of locomotion and striatal dopamine release is mediated by α6α4* nicotinic acetylcholine receptors. *J Neurosci* **30:**9877–9889.
Drenan RM, Grady SR, Whiteaker P, McClure-Begley T, McKinney S, Miwa JM, Bupp S, Heintz N, McIntosh JM, Bencherif M, et al. (2008a) In vivo activation of

midbrain dopamine neurons via sensitized, high-affinity α6* nicotinic acetylcholine receptors. *Neuron* **60:**123–136.
Drenan RM, Nashmi R, Imoukhuede P, Just H, McKinney S, and Lester HA (2008b) Subcellular trafficking, pentameric assembly, and subunit stoichiometry of neuronal nicotinic acetylcholine receptors containing fluorescently labeled α6 and β3 subunits. *Mol Pharmacol* **73:**27–41.
Elgoyhen AB and Katz E (2012) The efferent medial olivocochlear-hair cell synapse. *J Physiol Paris* **106:**47–56.
Exley R, Maubourguet N, David V, Eddine R, Evrard A, Pons S, Marti F, Threlfell S, Cazala P, McIntosh JM, et al. (2011) Distinct contributions of nicotinic acetylcholine receptor subunit α4 and subunit α6 to the reinforcing effects of nicotine. *Proc Natl Acad Sci USA* **108:**7577–7582.
Fonck C, Cohen BN, Nashmi R, Whiteaker P, Wagenaar DA, Rodrigues-Pinguet N, Deshpande P, McKinney S, Kwoh S, Munoz J, et al. (2005) Novel seizure phenotype and sleep disruptions in knock-in mice with hypersensitive α4* nicotinic receptors. *J Neurosci* **25:**11396–11411.
Fonck C, Nashmi R, Deshpande P, Damaj MI, Marks MJ, Riedel A, Schwarz J, Collins AC, Labarca C, and Lester HA (2003) Increased sensitivity to agonist-induced seizures, straub tail, and hippocampal theta rhythm in knock-in mice carrying hypersensitive α4 nicotinic receptors. *J Neurosci* **23:**2582–2590.
Fonck C, Nashmi R, Salas R, Zhou C, Huang Q, De Biasi M, Lester RA, and Lester HA (2009) Demonstration of functional α4-containing nicotinic receptors in the medial habenula. *Neuropharmacology* **56:**247–253.
Forsayeth JR and Kobrin E (1997) Formation of oligomers containing the β3 and β4 subunits of the rat nicotinic receptor. *J Neurosci* **17:**1531–1538.
Fowler CD, Arends MA, and Kenny PJ (2008) Subtypes of nicotinic acetylcholine receptors in nicotine reward, dependence, and withdrawal: evidence from genetically modified mice. *Behav Pharmacol* **19:**461–484.
Fowler CD, Lu Q, Johnson PM, Marks MJ, and Kenny PJ (2011) Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* **471:**597–601.
Frahm S, Slimak MA, Ferrarese L, Santos-Torres J, Antolin-Fontes B, Auer S, Filkin S, Pons S, Fontaine JF, Tsetlin V, et al. (2011) Aversion to nicotine is regulated by the balanced activity of β4 and α5 nicotinic receptor subunits in the medial habenula. *Neuron* **70:**522–535.
Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, et al. (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425:**917–925.
Gotti C, Guiducci S, Tedesco V, Corbioli S, Zanetti L, Moretti M, Zanardi A, Rimondini R, Mugnaini M, Clementi F, et al. (2010) Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area α6β2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement. *J Neurosci* **30:**5311–5325.
Grady SR, Drenan RM, Breining SR, Yohannes D, Wageman CR, Fedorov NB, McKinney S, Whiteaker P, Bencherif M, Lester HA, et al. (2010) Structural differences determine the relative selectivity of nicotinic compounds for native α4β2*-, α6β2*-, α3β4*- and α7-nicotine acetylcholine receptors. *Neuropharmacology* **58:**1054–1066.
Grady SR, Meinerz NM, Cao J, Reynolds AM, Picciotto MR, Changeux JP, McIntosh JM, Marks MJ, and Collins AC (2001) Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum. *J Neurochem* **76:**258–268.
Groot-Kormelink PJ, Boorman JP, and Sivilotti LG (2001) Formation of functional α3β4α5 human neuronal nicotinic receptors in *Xenopus* oocytes: a reporter mutation approach. *Br J Pharmacol* **134:**789–796.
Hays JT, Ebbert JO, and Sood A (2008) Efficacy and safety of varenicline for smoking cessation. *Am J Med* **121:**S32–S42.
Hendrickson LM, Gardner P, and Tapper AR (2011) Nicotinic acetylcholine receptors containing the α4 subunit are critical for the nicotine-induced reduction of acute voluntary ethanol consumption. *Channels* **5:**124–127.
Hendrickson LM, Zhao-Shea R, Pang X, Gardner PD, and Tapper AR (2010) Activation of α4* nAChRs is necessary and sufficient for varenicline-induced reduction of alcohol consumption. *J Neurosci* **30:**10169–10176.
Itier V and Bertrand D (2001) Neuronal nicotinic receptors: from protein structure to function. *FEBS Lett* **504:**118–125.
Jackson KJ, McIntosh JM, Brunzell DH, Sanjakdar SS, and Damaj MI (2009) The role of α6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal. *J Pharmacol Exp Ther* **331:**547–554.
Keramidas A, Moorhouse AJ, Schofield PR, and Barry PH (2004) Ligand-gated ion channels: mechanisms underlying ion selectivity. *Prog Biophys Mol Biol* **86:**161–204.
King SL, Caldarone BJ, and Picciotto MR (2004) β2-subunit-containing nicotinic acetylcholine receptors are critical for dopamine-dependent locomotor activation following repeated nicotine administration. *Neuropharmacology* **47:**132–139.
Klaassen A, Glykys J, Maguire J, Labarca C, Mody I, and Boulter J (2006) Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. *Proc Natl Acad Sci USA* **103:**19152–19157.
Kuryatov A and Lindstrom J (2011) Expression of functional human α6β2β3* acetylcholine receptors in *Xenopus laevis* oocytes achieved through subunit chimeras and concatamers. *Mol Pharmacol* **79:**126–140.
Kuryatov A, Olale F, Cooper J, Choi C, and Lindstrom J (2000) Human α6 AChR subtypes: subunit composition, assembly, and pharmacological responses. *Neuropharmacology* **39:**2570–2590.
Labarca C, Nowak MW, Zhang H, Tang L, Deshpande P, and Lester HA (1995) Channel gating governed symmetrically by conserved leucine residues in the M2 domain of nicotinic receptors. *Nature* **376:**514–516.
Labarca C, Schwarz J, Deshpande P, Schwarz S, Nowak MW, Fonck C, Nashmi R, Kofuji P, Dang H, Shi W, et al. (2001) Point mutant mice with hypersensitive α4 nicotinic receptors show dopaminergic deficits and increased anxiety. *Proc Natl Acad Sci USA* **98:**2786–2791.
Le Novère N, Zoli M, and Changeux JP (1996) Neuronal nicotinic receptor α6 subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur J Neurosci* **8:**2428–2439.
Léna C, de Kerchove D'Exaerde A, Cordero-Erausquin M, Le Novère N, del Mar Arroyo-Jimenez M, and Changeux JP (1999) Diversity and distribution of nicotinic acetylcholine receptors in the locus ceruleus neurons. *Proc Natl Acad Sci USA* **96:**12126–12131.
Lester HA (1988) Heterologous expression of excitability proteins: route to more specific drugs? *Science* **241:**1057–1063.
Lester HA, Fonck C, Tapper AR, McKinney S, Damaj MI, Balogh S, Owens J, Wehner JM, Collins AC, and Labarca C (2003) Hypersensitive knockin mouse strains identify receptors and pathways for nicotine action. *Curr Opin Drug Discov Devel* **6:**633–639.
Li P, McCollum M, Bracamontes J, Steinbach JH, and Akk G (2011) Functional characterization of the α5(Asn398) variant associated with risk for nicotine dependence in the α3β4α5 nicotinic receptor. *Mol Pharmacol* **80:**818–827.
Liu L, Zhao-Shea R, McIntosh JM, Gardner P, and Tapper A (2012) Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing α4 and α6 subunits. *Mol Pharmacol* **81:**541–548.
Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, and Faure P (2006) Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* **50:**911–921.
Mansvelder HD and McGehee DS (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* **27:**349–357.
Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, and Collins AC (1992) Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci* **12:**2765–2784.
Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM, McIntosh JM, Rossi F, Champtiaux N, Zoli M, et al. (2003) Effects of nicotine in the dopaminergic system of mice lacking the α4 subunit of neuronal nicotinic acetylcholine receptors. *Eur J Neurosci* **17:**1329–1337.
Marubio LM and Paylor R (2004) Impaired passive avoidance learning in mice lacking central neuronal nicotinic acetylcholine receptors. *Neuroscience* **129:**575–582.
Maskos U (2010) Role of endogenous acetylcholine in the control of the dopaminergic system via nicotinic receptors. *J Neurochem* **114:**641–646.
McClure-Begley TD, King NM, Collins AC, Stitzel JA, Wehner JM, and Butt CM (2009) Acetylcholine-stimulated [$^3$H]GABA release from mouse brain synaptosomes is modulated by α4β2 and α4α5β2 nicotinic receptor subtypes. *Mol Pharmacol* **75:**918–926.
Mineur YS and Picciotto MR (2008) Genetics of nicotinic acetylcholine receptors: relevance to nicotine addiction. *Biochem Pharmacol* **75:**323–333.
Miyazawa A, Fujiyoshi Y, and Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423:**949–955.
Molles BE, Maskos U, Pons S, Besson M, Guiard P, Guilloux JP, Evrard A, Cormier A, Mameli-Engvall M, Cloëz-Tayarani I, et al. (2006) Targeted in vivo expression of nicotinic acetylcholine receptors in mouse brain using lentiviral expression vectors. *J Mol Neurosci* **30:**105–106.
Murthy V, Taranda J, Elgoyhen AB, and Vetter DE (2009) Activity of nAChRs containing α9 subunits modulates synapse stabilization via bidirectional signaling programs. *Dev Neurobiol* **69:**931–949.
Nashmi R, Xiao C, Deshpande P, McKinney S, Grady SR, Whiteaker P, Huang Q, McClure-Begley T, Lindstrom JM, Labarca C, et al. (2007) Chronic nicotine cell specifically upregulates functional α4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. *J Neurosci* **27:**8202–8218.
Naylor C, Quarta D, Fernandes C, and Stolerman IP (2005) Tolerance to nicotine in mice lacking α7 nicotinic receptors. *Psychopharmacology (Berl)* **180:**558–563.
Orb S, Wieacker J, Labarca C, Fonck C, Lester HA, and Schwarz J (2004) Knockin mice with Leu9'Ser α4-nicotinic receptors: substantia nigra dopaminergic neurons are hypersensitive to agonist and lost postnatally. *Physiol Genomics* **18:**299–307.
Orr-Urtreger A, Broide RS, Kasten MR, Dang H, Dani JA, Beaudet AL, and Patrick JW (2000) Mice homozygous for the L250T mutation in the α7 nicotinic acetylcholine receptor show increased neuronal apoptosis and die within 1 day of birth. *J Neurochem* **74:**2154–2166.
Picciotto MR, Addy NA, Mineur YS, and Brunzell DH (2008) It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* **84:**329–342.
Picciotto MR, Zoli M, Léna C, Bessis A, Lallemand Y, Le Novère N, Vincent P, Pich EM, Brûlet P, and Changeux JP (1995) Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature* **374:**65–67.
Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, Fuxe K, and Changeux JP (1998) Acetylcholine receptors containing the β2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391:**173–177.
Pidoplichko VI, DeBiasi M, Williams JT, and Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* **390:**401–404.
Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, Changeux JP, Maskos U, and Fratta W (2008) Crucial role of α4 and α6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* **28:**12318–12327.
Quik M and McIntosh JM (2006) Striatal α6* nicotinic acetylcholine receptors: potential targets for Parkinson's disease therapy. *J Pharmacol Exp Ther* **316:**481–489.
Quik M, O'Leary K, and Tanner CM (2008) Nicotine and Parkinson's disease: implications for therapy. *Mov Disord* **23:**1641–1652.
Ren J, Qin C, Hu F, Tan J, Qiu L, Zhao S, Feng G, and Luo M (2011) Habenula "cholinergic" neurons co-release glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. *Neuron* **69:**445–452.
Revah F, Bertrand D, Galzi JL, Devillers-Thiéry A, Mulle C, Hussy N, Bertrand S,

Ballivet M, and Changeux JP (1991) Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* **353:**846–849.
Rodrigues-Pinguet NO, Pinguet TJ, Figl A, Lester HA, and Cohen BN (2005) Mutations linked to autosomal dominant nocturnal frontal lobe epilepsy affect allosteric $Ca^{2+}$ activation of the $\alpha4\beta2$ nicotinic acetylcholine receptor. *Mol Pharmacol* **68:** 487–501.
Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, et al. (2009) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet* **150B:**453–466.
Salas R, Pieri F, and De Biasi M (2004) Decreased signs of nicotine withdrawal in mice null for the $\beta4$ nicotinic acetylcholine receptor subunit. *J Neurosci* **24:**10035–10039.
Salas R, Sturm R, Boulter J, and De Biasi M (2009) Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *J Neurosci* **29:**3014–3018.
Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, and Grady SR (2007) Pharmacology of $\alpha$-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. *Mol Pharmacol* **71:**1563–1571.
Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann E, Andermann F, Desbiens R, Keene D, and Cendes F (1995) Autosomal dominant nocturnal frontal lobe epilepsy. A distinctive clinical disorder. *Brain* **118:**61–73.
Scheffer IE, Bhatia KP, Lopes-Cendes I, Fish DR, Marsden CD, Andermann F, Andermann E, Desbiens R, Cendes F, and Manson JI (1994) Autosomal dominant frontal epilepsy misdiagnosed as sleep disorder. *Lancet* **343:**515–517.
Schilström B, Svensson HM, Svensson TH, and Nomikos GG (1998) Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of $\alpha7$ nicotinic receptors in the ventral tegmental area. *Neuroscience* **85:**1005–1009.
Schwarz J, Schwarz SC, Dorigo O, Stützer A, Wegner F, Labarca C, Deshpande P, Gil JS, Berk AJ, and Lester HA (2006) Enhanced expression of hypersensitive $\alpha4^*$ nAChR in adult mice increases the loss of midbrain dopaminergic neurons. *FASEB J* **20:**935–946.
Shao XM, Tan W, Xiu J, Puskar N, Fonck C, Lester HA, and Feldman JL (2008) $\alpha4^*$ nicotinic receptors in preBotzinger complex mediate cholinergic/nicotinic modulation of respiratory rhythm. *J Neurosci* **28:**519–528.
Son CD, Moss FJ, Cohen BN, and Lester HA (2009) Nicotine normalizes intracellular subunit stoichiometry of nicotinic receptors carrying mutations linked to autosomal dominant nocturnal frontal lobe epilepsy. *Mol Pharmacol* **75:**1137–1148.
Srinivasan R, Pantoja R, Moss FJ, Mackey ED, Son CD, Miwa J, and Lester HA (2011) Nicotine up-regulates $\alpha4\beta2$ nicotinic receptors and ER exit sites via stoichiometry-dependent chaperoning. *J Gen Physiol* **137:**59–79.
Steensland P, Simms JA, Holgate J, Richards JK, and Bartlett SE (2007) Varenicline, an $\alpha4\beta2$ nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci USA* **104:**12518–12523.
Steinlein OK (2010) Animal models for autosomal dominant frontal lobe epilepsy: on the origin of seizures. *Expert Rev Neurother* **10:**1859–1867.
Sutor B and Zolles G (2001) Neuronal nicotinic acetylcholine receptors and autosomal dominant nocturnal frontal lobe epilepsy: a critical review. *Pflugers Arch* **442:**642–651.
Tapper AR, McKinney SL, Marks MJ, and Lester HA (2007) Nicotine responses in hypersensitive and knockout $\alpha4$ mice account for tolerance to both hypothermia and locomotor suppression in wild-type mice. *Physiol Genomics* **31:**422–428.
Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, and Lester HA (2004) Nicotine activation of $\alpha4^*$ receptors: sufficient for reward, tolerance, and sensitization. *Science* **306:**1029–1032.
Taranda J, Maison SF, Ballestero JA, Katz E, Savino J, Vetter DE, Boulter J, Liberman MC, Fuchs PA, and Elgoyhen AB (2009) A point mutation in the hair cell nicotinic cholinergic receptor prolongs cochlear inhibition and enhances noise protection. *PLoS Biol* **7:**e18.
Teper Y, Whyte D, Cahir E, Lester HA, Grady SR, Marks MJ, Cohen BN, Fonck C, McClure-Begley T, McIntosh JM, et al. (2007) Nicotine-induced dystonic arousal complex in a mouse line harboring a human autosomal-dominant nocturnal frontal lobe epilepsy mutation. *J Neurosci* **27:**10128–10142.
Vailati S, Moretti M, Balestra B, McIntosh M, Clementi F, and Gotti C (2000) $\beta3$ subunit is present in different nicotinic receptor subtypes in chick retina. *Eur J Pharmacol* **393:**23–30.
Vetter DE, Katz E, Maison SF, Taranda J, Turcan S, Ballestero J, Liberman MC, Elgoyhen AB, and Boulter J (2007) The $\alpha10$ nicotinic acetylcholine receptor subunit is required for normal synaptic function and integrity of the olivocochlear system. *Proc Natl Acad Sci USA* **104:**20594–20599.
Vetter DE, Liberman MC, Mann J, Barhanin J, Boulter J, Brown MC, Saffiote-Kolman J, Heinemann SF, and Elgoyhen AB (1999) Role of $\alpha9$ nicotinic ACh receptor subunits in the development and function of cochlear efferent innervation. *Neuron* **23:**93–103.
Villégier AS, Salomon L, Blanc G, Godeheu G, Glowinski J, and Tassin JP (2006) Irreversible blockade of monoamine oxidases reveals the critical role of 5-HT transmission in locomotor response induced by nicotine in mice. *Eur J Neurosci* **24:**1359–1365.
Wallace TL and Porter RH (2011) Targeting the nicotinic $\alpha7$ acetylcholine receptor to enhance cognition in disease. *Biochem Pharmacol* **82:**891–903.
Walsh H, Govind AP, Mastro R, Hoda JC, Bertrand D, Vallejo Y, and Green WN (2008) Up-regulation of nicotinic receptors by nicotine varies with receptor subtype. *J Biol Chem* **283:**6022–6032.
Walters CL, Brown S, Changeux JP, Martin B, and Damaj MI (2006) The $\beta2$ but not $\alpha7$ subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology* (*Berl*) **184:**339–344.
Whiteaker P, McIntosh JM, Luo S, Collins AC, and Marks MJ (2000) [$^{125}$I]-$\alpha$-Conotoxin MII identifies a novel nicotinic acetylcholine receptor population in mouse brain. *Mol Pharmacol* **57:**913–925.
Williams DK, Wang J, and Papke RL (2011) Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. *Biochem Pharmacol* **82:**915–930.
Witten IB, Lin SC, Brodsky M, Prakash R, Diester I, Anikeeva P, Gradinaru V, Ramakrishnan C, and Deisseroth K (2010) Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* **330:**1677–1681.
Wooltorton JR, Pidoplichko VI, Broide RS, and Dani JA (2003) Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci* **23:**3176–3185.
Xiao C, Nashmi R, McKinney S, Cai H, McIntosh JM, and Lester HA (2009) Chronic nicotine selectively enhances $\alpha4\beta2^*$ nicotinic acetylcholine receptors in the nigrostriatal dopamine pathway. *J Neurosci* **29:**12428–12439.
Xiao C, Srinivasan R, Drenan RM, Mackey ED, McIntosh JM, and Lester HA (2011) Characterizing functional $\alpha6\beta2$ nicotinic acetylcholine receptors in vitro: mutant $\beta2$ subunits improve membrane expression, and fluorescent proteins reveal responsive cells. *Biochem Pharmacol* **82:**852–861.
Xiu X, Puskar NL, Shanata JA, Lester HA, and Dougherty DA (2009) Nicotine binding to brain receptors requires a strong cation-pi interaction. *Nature* **458:**534–537.
Xu J, Cohen BN, Zhu Y, Dziewczapolski G, Panda S, Lester HA, Heinemann SF, and Contractor A (2010) Altered activity-rest patterns in mice with a human autosomal-dominant nocturnal frontal lobe epilepsy mutation in the $\beta2$ nicotinic receptor. *Mol Psychiatry* **16:**1048–1061.
Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou CN, Patrick J, Role L, De Biasi M, and Beaudet AL (1999a) Megacystis, mydriasis, and ion channel defect in mice lacking the $\alpha3$ neuronal nicotinic acetylcholine receptor. *Proc Natl Acad Sci USA* **96:**5746–5751.
Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, Patrick JW, Role LW, Beaudet AL, and De Biasi M (1999b) Multiorgan autonomic dysfunction in mice lacking the $\beta2$ and the $\beta4$ subunits of neuronal nicotinic acetylcholine receptors. *J Neurosci* **19:**9298–9305.
Yang K, Hu J, Lucero L, Liu Q, Zheng C, Zhen X, Jin G, Lukas RJ, and Wu J (2009) Distinctive nicotinic acetylcholine receptor functional phenotypes of rat ventral tegmental area dopaminergic neurons. *J Physiol* **587:**345–361.
Yeh JJ, Yasuda RP, Dávila-García MI, Xiao Y, Ebert S, Gupta T, Kellar KJ, and Wolfe BB (2001) 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* **77:**336–346.
Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, and Sharkey J (2004) Nicotine improves sustained attention in mice: evidence for involvement of the $\alpha7$ nicotinic acetylcholine receptor. *Neuropsychopharmacology* **29:**891–900.
Zhang L (2006) Cholinergic receptor knockout mice, in *Animal Models of Cognitive Impairment* (Levin ED, Buccafusco JJ eds) pp 199–222, CRC Press, Boca Raton, FL.
Zhao-Shea R, Cohen BN, Just H, McClure-Begley T, Whiteaker P, Grady SR, Salminen O, Gardner PD, Lester HA, and Tapper AR (2010) Dopamine D2-receptor activation elicits akinesia, rigidity, catalepsy, and tremor in mice expressing hypersensitive $\alpha4$ nicotinic receptors via a cholinergic-dependent mechanism. *FASEB J* **24:**49–57.
Zhao-Shea R, Liu L, Soll LG, Improgo MR, Meyers EE, McIntosh JM, Grady SR, Marks MJ, Gardner PD, and Tapper AR (2011) Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. *Neuropsychopharmacology* **36:**1021–1032.
Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, and Gotti C (2002) Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci* **22:**8785–8789.
Zoli M, Picciotto MR, Ferrari R, Cocchi D, and Changeux JP (1999) Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. *EMBO J* **18:**1235–1244.
Zouridakis M, Zisimopoulou P, Poulas K, and Tzartos SJ (2009) Recent advances in understanding the structure of nicotinic acetylcholine receptors. *IUBMB Life* **61:**407–423.