Review

Preclinical Evidence That Activation of Mesolimbic Alpha 6 Subunit Containing Nicotinic Acetylcholine Receptors Supports Nicotine Addiction Phenotype

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Abstract

Introduction: Nicotine is a major psychoactive ingredient in tobacco yet very few individuals quit smoking with the aid of nicotine replacement therapy. Targeted therapies with more selective action at nicotinic acetylcholine receptors (nAChRs) that contain a $\beta 2$ subunit ($\beta 2^*nAChRs$; *denotes assembly with other subunits) have enjoyed significantly greater success, but exhibit potential for unwanted cardiac, gastrointestinal, and emotive side effects.

Discussion: This literature review focuses on the preclinical evidence that suggests that subclasses of $\beta 2^*nAChRs$ that assemble with the $\alpha 6$ subunit may provide an effective target for tobacco cessation. $\alpha 6\beta 2^*nAChRs$ have a highly selective pattern of neuroanatomical expression in catecholaminergic nuclei including the ventral tegmental area and its projection regions. $\alpha 6\beta 2^*nAChRs$ promote dopamine (DA) neuron activity and DA release in the mesolimbic dopamine system, a brain circuitry that is well-studied for its contributions to addiction behavior. A combination of genetic and pharmacological studies indicates that activation of $\alpha 6\beta 2^*nAChRs$ is necessary and sufficient for nicotine psychostimulant effects and nicotine self-administration. $\alpha 6\beta 2^*nAChRs$ support maintenance of nicotine use, support the conditioned reinforcing effects of drug-associated cues, and regulate nicotine withdrawal.

Conclusions: These data suggest that $\alpha 6\beta 2^*nAChRs$ represent a critical pool of high affinity $\beta 2^*nAChRs$ that regulates nicotine dependence phenotype and suggest that inhibition of these receptors may provide an effective strategy for tobacco cessation therapy.

Introduction

There is a growing need for identification of novel targets for tobacco cessation. Cigarette smoking remains the leading

preventable cause of death in developed countries and continues to be a growing health problem worldwide (World Health Organization, 2008). Nicotine, a major psychoactive ingredient in tobacco, binds to a variety of subclasses of nicotinic receptors in the brain as well as in the periphery. All of these receptors are the molecular target of nicotine replacement therapies (NRT). NRT aids approximately 10% of smokers in quitting smoking. Quit rates are increased approximately threefold with varenicline, a more selective drug that acts as a partial agonist at the high affinity $\beta 2$ subunit containing nicotinic acetylcholine receptors ($\beta 2^*nAChR$; *denotes assembly with other subunits e.g., $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 3$; Coe et al., 2005; Gonzales et al., 2006), suggesting that smoking cessation can be improved using more targeted strategies. This review will summarize a growing literature that suggests that a subtype of $\beta 2^*nAChRs$, which contains the $\alpha 6$ nAChR subunit ($\alpha 6\beta 2^*nAChR$) is a strong candidate for the next wave of tobacco cessation therapeutics. The nicotine addiction field has benefited greatly from the isolation and development of peptides that selectively target nicotinic receptors that contain the $\alpha 6$ subunit (Azam & McIntosh, 2005; Cartier et al., 1996; Dowell et al., 2003; McIntosh et al., 2004), the development of molecular chimeras and concatamers that can test drug efficacy at $\alpha 6\beta 2^*nAChRs$ in vitro (Kuryatov & Lindstrom, 2011; Kuryatov, Olale, Cooper, Choi, & Lindstrom, 2000; for detailed review see Letchworth & Whiteaker, 2011), and from transgenic knockout and knockin technologies and viral-mediated rescue of nAChR subunit genes that enable observation of subunit contributions to neurochemistry and behaviors that are relevant to nicotine and tobacco addiction (Cui et al., 2003; Drenan et al., 2008; Marubio et al., 2003; Maskos et al., 2005; McGranahan, Patzlaff, Grady, Heinemann, & Booker, 2011; Mineur et al., 2009; Picciotto et al., 1998; Pons et al., 2008; Tapper et al., 2004). An accumulation of findings in the preclinical literature show that activation of $\alpha 6\beta 2^*nAChRs$ promotes phenotypic behaviors that support tobacco addiction.

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Nicotine-Stimulated Mesocorticolimbic DA Release

A preponderance of evidence suggests that nicotine addiction behavior is regulated in part by the mesocorticolimbic dopamine (DA) pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), anterior cingulate cortex, hippocampus, and amygdala. Human and animal studies show that nicotine and other drugs of abuse result in elevated DA release in the NAc (Barrett, Boileau, Okker, Pihl, & Dagher, 2004; Benwell & Balfour, 1992; Brody et al., 2004; Di Chiara et al., 2004). Imaging studies in human smokers show that these areas are not only activated by nicotine and tobacco smoke, but are associated with feelings of “high” and “liking” the drug (Barrett et al., 2004; Brody et al., 2004; Stein et al., 1998). It is interesting that cigarette-associated cues also activate mesolimbic projection regions of smokers, demonstrating that cues associated with tobacco ingestion can become conditioned reinforcers that are capable of activating the DA circuitry in the absence of nicotine (Due, Huettel, Hall, & Rubin, 2002; Franklin et al., 2007). Rodent studies show that lesions of DA projections to the NAc attenuate nicotine self-administration (Corrigall, Franklin, Coen, & Clarke, 1992), suggesting that these DA inputs are critical for nicotine reinforcement. Nicotine-associated mesolimbic DA release is regulated in part by nAChRs within the VTA as well as by nicotinic receptors on DA terminals in the dorsal and ventral striatum where $\alpha 6\beta 2^*$ nAChRs are enriched (Champtiaux et al., 2003; Grady et al., 2007; Gotti et al., 2010; Mansvelder, Keath, & McGehee, 2002; Pidoplichko et al., 2004; Salminen et al., 2004, 2005, 2007). Studies have begun to identify mesolimbic $\alpha 6\beta 2^*$ nAChRs for their contributions to nicotine-associated mesolimbic DA release as well as to the behavioral and neurochemical effects of nicotine. This paper will review the evidence which suggests that activation of $\alpha 6\beta 2^*$ nAChRs is critical for nicotine reinforcement and reward, conditioned reinforcement, nicotine withdrawal, and psychoactive effects of nicotine.

How Nicotine Affects DA Release: Building a Nicotinic Receptor Profile

The nAChRs are ion channels made of a combination of five subunits that exist in a variety of functional states including a resting state, an activated state, and a desensitized, unresponsive state (Changeux, Devillers-Thiery, & Chemouilli, 1984; Figure 1). nAChRs reside on neurons that release various types of neurotransmitters including acetylcholine, gamma-aminobutyric acid (GABA), glutamate, serotonin, norepinephrine, and DA. nAChRs reside on the soma and axon terminals of neurons where they promote neurotransmitter release. Binding of nicotine or acetylcholine leads to a conformational change in the nAChR that renders the ion pore permeable to cations that excite the cell (Arias, 2000; Karlin & Akabas, 1995; Leonard, Labarca, Charnet, Davidson, & Lester, 1988). The predominant low affinity nAChRs in brain are homomers made up of five $\alpha 7$ nAChR subunits. The heteromeric nAChR in brain is made of a combination of α and β subunits ($\alpha 3$ – $\alpha 7$; $\beta 2$ – $\beta 4$) but the assembly and neuroanatomical expression of central nervous system nAChRs are tightly regulated.

Figure 1. The putative composition of mesolimbic $\alpha 6\beta 2^*$ nicotinic acetylcholine receptors (nAChRs). nAChRs are pentameric in structure, made up of a combination of five subunits that assemble around an ion pore. The ACh binding site is believed to be at the interface of the $\alpha 6/\alpha 4$ and $\beta 2$ subunits. The accessory subunit, $\beta 3$, is highly expressed in $\alpha 6\beta 2^*$ nAChRs. Agonist binding results in opening of the ion pore and activation of the receptor. Following activation, the bound nAChRs desensitize and become impermeable to cation penetration through the ion pore. The $\alpha 4\alpha 6\beta 2^*$ nAChRs have a higher affinity for agonist binding and are slower to desensitize.

$\alpha 6\beta 2^*$ nAChRs almost exclusively assemble with a $\beta 3$ subunit (Cui et al., 2003; Gotti et al., 2005) and when assembled with $\alpha 4$ have the highest affinity for nicotine and ACh (Salminen et al., 2007). Assembly with $\alpha 4$ also increases the sensitivity of $\alpha 6\beta 2^*$ nAChR to compounds such as varenicline and dihydro-beta-erythroidine (DHBE) which are selective for $\alpha 4\beta 2^*$ nAChRs (Grady et al., 2010; Kuryatov & Lindstrom, 2011). Although the current evidence suggests that two major $\alpha 6\beta 2^*$ nAChRs are expressed in brain ($\alpha 4\alpha 6\beta 2\beta 3$ nAChRs and $\alpha 6\beta 2\beta 3$ nAChRs), we reserve the use of the more conservative nomenclature, $\alpha 6\beta 2^*$ nAChRs, to indicate that other receptor conformations may contribute to the observations presented in this paper.

Pharmacological and genetic studies suggest that nicotine-associated DA release and nicotine reinforcement are regulated by nAChRs that contain the $\beta 2$ subunit (Champtiaux et al., 2003; Corrigall, Coen, & Adamson, 1994; Drenan et al., 2008; Maskos et al., 2005; Picciotto et al., 1998; Pons et al., 2008;

Salminen et al., 2004, 2007; Tapper et al., 2004), making these high affinity receptors an attractive therapeutic target for tobacco cessation (Gonzales et al., 2006; Rollema et al., 2007). The $\beta 2^*$ nAChRs are the primary target of varenicline, the first “receptor-selective” therapeutic for tobacco dependence (Coe et al., 2005; Gonzales et al., 2006) but see (Mihalak, Carroll, & Luetje, 2006). $\beta 2^*$ nAChRs, however, are not only expressed in brain areas that regulate motivation to use cigarettes but also expressed in brain areas that contribute more generally to motivation, mood, and cognition (for review see Brunzell & Picciotto, 2009; Levin, McClernon, & Rezvani, 2006). Varenicline is highly effective in some smokers, but for others may result in unfavorable emotional, cardiovascular, and gastrointestinal side effects (Hays & Ebbert, 2010; Leung, Patafio, & Rosser, 2011; Moore, Furberg, Glenmullen, Maltsberger, & Singh, 2011; Singh, Loke, Spangler, & Furberg, 2011). Fortunately, $\beta 2^*$ nAChRs are also very diverse in their composition so that identification of receptor subunits that assemble with $\beta 2$ but that have a more selective neuroanatomical expression pattern may identify targets for tobacco cessation therapies that have less potential for side effects. One such candidate is the $\alpha 6$ nAChR subunit. Unlike other $\beta 2^*$ nAChRs that do not assemble with $\alpha 6$ (e.g., $\alpha 4\beta 2$ nAChRs and $\alpha 4\alpha 5\beta 2$ nAChRs), the $\alpha 6\beta 2^*$ nAChRs are not expressed in the periphery and show a selective neuroanatomical pattern of expression within catecholaminergic nuclei, retinal ganglion cells, and catecholaminergic and retinal projection regions of the brain (Champtiaux et al., 2002; Cui et al., 2003; Klink, de Kerchove d’Exaerde, Zoli, & Changeux, 2001; Whiteaker, McIntosh, Luo, Collins, & Marks, 2000). Recent in vitro data suggest that $\alpha 6\beta 2^*$ nAChRs, like $\alpha 4\beta 2^*$ nAChRs, are pharmacologically inhibited by the partial agonist properties of varenicline (Grady et al., 2010; Kuryatov, Berrettini, & Lindstrom, 2011). Their enrichment in the mesolimbic dopamine (DA) system, a brain pathway long known to contribute to motivational valence for drug reward (Volkow, Wang, Fowler, & Tomasi, 2011), makes these $\alpha 6\beta 2^*$ nAChRs a promising novel target for tobacco cessation therapies.

Mesolimbic $\alpha 6\beta 2^*$ nAChRs on Soma and Terminals Support Nicotine-Associated DA Release

$\alpha 6$ assembles with $\beta 2$ with a selective neuroanatomical pattern of expression in catecholaminergic nuclei in the brain and on DA neuron axon terminals in catecholaminergic projection areas (Champtiaux et al., 2002; Klink et al., 2001; Le Novere, Zoli, & Changeux, 1996; Marks et al., 2010; Mineur et al., 2009; Whiteaker et al., 2000). Although the focus of this review is on the mesolimbic DA system, $\alpha 6\beta 2^*$ nAChRs are also highly expressed within the nigrostriatal system, the locus coeruleus, and in retinal ganglion cells and the visual system (Champtiaux et al., 2002; Guo, Liu, Sorenson, & Chiappinelli, 2005; Lecchi, McIntosh, Bertrand, Safran, & Bertrand, 2005; Whiteaker et al., 2000). $\alpha 6\beta 2^*$ nAChRs, like $\alpha 3\beta 2^*$ nAChRs are classified according to their sensitivity to the cone snail peptide, α -conotoxin MII (α -CTX MII; Cartier et al., 1996; Champtiaux et al., 2002; Whiteaker et al., 2000). Studies of α -CTX MII binding in $\alpha 6$ and $\alpha 3$ subunit knockout mice reveal that with the exception of the interpeduncular nucleus, the $\alpha 6\beta 2^*$ nAChRs and not $\alpha 3\beta 2^*$ nAChRs predominate in these regions (Champtiaux

et al., 2002; Whiteaker et al., 2002). α -CTX MII binding studies also show that $\alpha 6\beta 2^*$ nAChRs on catecholaminergic terminals are most highly expressed in the dorsal striatum and NAc regions with sparse binding apparent in other DA projection regions including the anterior cingulate cortex, hippocampus, and amygdala (Cartier et al., 1996; Champtiaux et al., 2002; Marks et al., 2010; Mineur et al., 2009; Whiteaker et al., 2000). Nicotinic receptor knockout strategies used in combination with α -CTX MII have enabled researchers to identify that nicotine-stimulated DA release on terminals in the NAc/striatum involves activation of several combinations of $\beta 2^*$ nAChRs including α -CTX MII sensitive ($\alpha 6\beta 2\beta 3$, $\alpha 4\alpha 6\beta 2\beta 3$) and insensitive ($\alpha 4\alpha 5\beta 2$, $\alpha 4\beta 2$) nAChRs (Champtiaux et al., 2003; Salminen et al., 2004, 2007; Figure 2). Cyclic voltammetry studies suggest that $\alpha 6\beta 2^*$ nAChRs support 80% of the nicotine-stimulated DA release in the NAc (Exley, Clements, Hartung, McIntosh, & Cragg, 2008). The $\alpha 6\beta 2^*$ nAChRs are also localized on DA cell bodies in the VTA and activation of these receptors by nicotine is sufficient to stimulate firing of VTA DA neurons (Drenan et al., 2008; Zhao-Shea et al., 2011).

Psychostimulant Effects of Nicotine

The psychostimulant effects of nicotine are thought to be regulated in large part via DA release and an accumulation of evidence suggests that these effects are regulated by $\alpha 6\beta 2^*$ nAChRs (Drenan et al., 2008, 2010). Mice lacking either the $\alpha 6$ or $\beta 2$ subunit fail to show locomotor activating effects of nicotine (King, Caldarone, & Picciotto, 2004; Le Novere et al., 1996; Mineur, Somenzi, & Picciotto, 2007), a phenotype that is rescued by partial reinsertion of $\beta 2$ nAChR subunit messenger RNA (mRNA) into the VTA-nigra region (Mineur et al., 2007). Studies in mice with a single point mutation that renders their $\alpha 6\beta 2^*$ nAChRs hypersensitive to nicotine reveal that subthreshold doses for activation of other nAChRs are sufficient for locomotor activating effects of nicotine in this strain (Drenan et al., 2008, 2010). This phenotype is blocked by systemic DA receptor antagonism and abolished in mice crossed to an $\alpha 4$ knockout background; these studies suggest that $\alpha 4\alpha 6\beta 2^*$ nAChR-stimulated DA release supports the locomotor activating effects of nicotine (Drenan et al., 2008, 2010). These mice also fail to show locomotor suppressant effects of acute nicotine at low doses, unmasking the fact that locomotor suppressant effects of nicotine are regulated by a class of nAChRs that does not contain an $\alpha 6$ subunit. Studies comparing mice with a selective null mutation of the $\alpha 4$ nAChR subunit in DA neurons with global $\alpha 4$ knockouts suggest that locomotor suppressant effects of nicotine appear to be regulated by $\alpha 4$ subunit containing nicotinic receptors that are not expressed on mesolimbic DA neurons (McGranahan et al., 2011). The locomotor stimulant effects of nicotine do not appear to be due to activation of $\alpha 6\beta 2^*$ nAChRs on DA terminals in the NAc shell since local infusion of α -CTX MII in this region does not affect locomotor stimulating effects of nicotine in rats (Brunzell, Boschen, Hendrick, Beardsley, & McIntosh, 2010). This behavior is more likely mediated in the VTA where, unlike the NAc, local administration of nicotine results in hyperactivity (Ferrari, Le Novere, Picciotto, Changeux, & Zoli, 2002). The dorsal striatum receives inputs from the substantia nigra that have $\alpha 6\beta 2^*$ nAChRs on their terminals (Meyer, Yoshikami, & McIntosh, 2008; Perez,

Figure 2. A neuroanatomical summary of $\alpha 6\beta 2^*$ nicotinic acetylcholine receptor (nAChR) contributions to behaviors associated with nicotine and tobacco addiction. A preponderance of the evidence suggests that activation of $\alpha 6\beta 2^*$ nAChRs in the ventral tegmental area (VTA) and on dopamine (DA) terminals in the dorsal and ventral striatum (aka NAc core and shell) supports nicotine-stimulated DA release. Antagonism of $\alpha 6\beta 2^*$ nAChRs in the NAc shell or VTA greatly attenuates nicotine self-administration. $\alpha 6\beta 2^*$ nAChRs in the NAc shell regulate motivation to self-administer nicotine. Mesolimbic rescue of $\alpha 4$, $\alpha 6$, or $\beta 2$ subunits on a null mutant background is sufficient to rescue self-administration phenotype, suggesting that $\alpha 4\alpha 6\beta 2^*$ nAChRs regulate this behavior. IntraVTA infusion of α -conotoxin MII also blocks the rewarding effects of cues paired with a drug reinforcer. Studies in transgenic mice with highly sensitive $\alpha 4$ or $\alpha 6$ subunit containing nAChRs show that nicotine-associated stimulation of VTA DA neurons is at the $\alpha 4\alpha 6\beta 2^*$ nAChRs and the $\alpha 6$ -sensitive mice bred on an $\alpha 4$ knockout background show that $\alpha 4\alpha 6\beta 2^*$ nAChRs regulate the locomotor stimulating effects of nicotine, likely at the level of the VTA and perhaps within the nigrostriatal circuitry that includes projections to the dorsal striatum. Affective but not somatic withdrawal is attenuated by injection of an $\alpha 6\beta 2^*$ nAChR antagonist into the lateral ventricle; the locus of this effect is not clear. Several studies show that antagonism of $\alpha 6\beta 2^*$ nAChRs and $\alpha 4\beta 2^*$ nAChR does not have the same effect as selective antagonism of $\alpha 6\beta 2^*$ nAChRs, perhaps due to expression of $\alpha 4\beta 2^*$ nAChRs on gamma-aminobutyric acid terminals in mesolimbic DA areas. These studies suggest that $\alpha 6\beta 2^*$ nAChRs may be an effective target for tobacco cessation. Increasing evidence suggests that $\alpha 4\alpha 6\beta 2\beta 3$ nAChRs represent a critical pool of high affinity $\beta 2^*$ nAChRs that support nicotine addiction behavior.

Bordia, McIntosh, & Quik, 2010). Lesions of the VTA and substantia nigra greatly attenuate nicotine locomotor activation (Louis & Clarke, 1998). It remains to be determined if $\alpha 6\beta 2^*$ nAChRs on DA terminals in the dorsal striatum, NAc core, or elsewhere contribute to nicotine's psychostimulant effects. It is important to note that although nicotine reinforcement and psychostimulant effects of nicotine both appear to depend on DA release (Boye, Grant, & Clarke, 2001; Cadoni & Di Chiara, 2000; Corrigan & Coen, 1991; Corrigan et al., 1992; Di Chiara et al., 2004; Iyaniwura, Wright, & Balfour, 2001; Kelsey, Gerety, & Guerriero, 2009; Louis & Clarke, 1998), a series of studies have shown a dissociation between the neuroanatomical networks that support nicotine psychostimulant effects versus nicotine reinforcement and reward (e.g., Brunzell et al., 2010; Corrigan et al., 1994).

Nicotine Reinforcement and Reward

Nicotine Reinforcement is measured in rodents using intravenous nicotine self-administration, a model with good face validity and predictive validity for tobacco smoking. Nicotine reward is generally measured using nicotine conditioned place preference (CPP), a Pavlovian paradigm where preference for a nicotine-paired chamber is compared with preference for

a neutral chamber after several exposures to nicotine. Both nicotine self-administration and nicotine CPP are absent in null mutant mice lacking their $\alpha 4$, $\alpha 6$, or $\beta 2$ subunits (Maskos et al., 2005; McGranahan et al., 2011; Picciotto et al., 1998; Pons et al., 2008; Walters, Brown, Changeux, Martin, & Damaj, 2006). Nicotine self-administration is recovered in mice that have a reintroduction of the $\alpha 4$, $\alpha 6$, or $\beta 2$ subunit mRNA in the VTA region (Pons et al., 2008). These 3-day place conditioning studies and acute, single-day tail-vein procedures suggest that $\alpha 4\alpha 6\beta 2^*$ nAChRs may be critical for initiation of tobacco use. Intraventricular injection of a selective $\alpha 6\beta 2^*$ nAChR antagonist also blocks expression of nicotine CPP in adult, wild-type mice that have grown up with their nAChRs intact (Jackson, McIntosh, Brunzell, Sanjakdar, & Damaj, 2009). The possibility that $\alpha 6\beta 2^*$ nAChRs may affect initiation of tobacco use is supported by genetic research in humans that have implicated CHRNA6 and CHRNA3 in sensitivity to initial tobacco exposure (Zeiger et al., 2008; Hoft et al., 2009). Youth with polymorphisms in the genes that encode the $\alpha 6$ or $\beta 3$ nAChR subunits had an elevated risk for tobacco dependence (Hoft et al., 2009) and increased dizziness in response to nicotine (Zeiger et al., 2008). More research is necessary to discover how these genes might impact the development of habitual tobacco use.

It is not clear from genetic rescue and intracerebral ventricular infusion studies in mice if $\alpha 6\beta 2^*$ nAChRs within the VTA or on

terminals in VTA projection regions may regulate self-administration of nicotine or if $\alpha 6\beta 2^*$ nAChRs continue to contribute to nicotine ingestion following chronic exposure. Pharmacological studies which target specific neuroanatomical structures suggest that $\alpha 6\beta 2^*$ nAChRs exert their effects on DA release and self-administration at DA terminals in the NAc shell (Brunzell et al., 2010; Champtiaux et al., 2003; Exley et al., 2008; Kulak, Nguyen, Olivera, & McIntosh, 1997; Salminen et al., 2004, 2007). Studies in naïve rats chronically trained to self-administer intravenous nicotine show that NAc shell infusions of concentrations of α -CTX MII that are capable of blocking nicotine-stimulated DA release (Kulak et al., 1997; Salminen et al., 2004) greatly reduce how hard rats are willing to work for nicotine using a progressive ratio (PR) schedule of reinforcement (Brunzell et al., 2010). The PR schedule requires rats to give an increasing number of responses for a single i.v. infusion of nicotine (Brunzell et al., 2010). Since there are virtually no $\alpha 3\beta 2^*$ nAChRs in the NAc shell, these behavioral data and in vitro studies suggest that activation of $\alpha 6\beta 2^*$ nAChRs on DA terminals in the NAc shell support motivation to self-administer nicotine (Brunzell et al., 2010; Champtiaux et al., 2003; Exley et al., 2008; Kulak et al., 1997; Salminen et al., 2004, 2007). Of import from a therapeutic standpoint, these data further suggest that $\alpha 6\beta 2^*$ nAChRs continue to support self-administration of nicotine following a more chronic dosing paradigm. It is interesting that self-administration of nicotine was not affected following NAc administration of DH β E, a drug that antagonizes both $\alpha 4\beta 2$ nAChRs and $\alpha 4\alpha 6\beta 2^*$ nAChRs (Corrigall et al., 1994; and Brunzell, unpublished findings), raising the possibility that these distinct receptor populations have opposing effects on self-administration behavior in this region, perhaps due to the prevalence of $\alpha 4\beta 2$ nAChRs but not $\alpha 6\beta 2^*$ nAChRs on GABA terminals in the NAc (Drenan et al., 2008; Gotti et al., 2010; Tapper et al., 2004) but see (Yang et al., 2011). This is in contrast to the VTA where infusion of DH β E greatly attenuates nicotine self-administration (Corrigall et al., 1994). Other studies show that local infusion of an $\alpha 6\beta 2^*$ nAChR antagonist, α -conotoxin PIA, (Dowell et al., 2003) into the VTA also attenuates systemic administration of nicotine in rats that were previously trained for food (Gotti et al., 2010). Immunoprecipitation studies suggest that these receptors are $\alpha 4\alpha 6\beta 2\beta 3$ nAChRs (Gotti et al., 2010). Studies that use local self-administration of low-dose nicotine in the VTA of nicotinic subunit knockout mice reveal similar behavioral effects although intraVTA infusion of nicotine is attenuated to a greater extent in $\alpha 4$ subunit knockout mice than in $\alpha 6$ subunit knockouts (Exley et al., 2011), suggesting that within the VTA $\alpha 4\beta 2^*$ nAChRs without an $\alpha 6$ subunit regulate intraVTA administration of nicotine (Exley et al., 2011). These studies also showed that VTA DA neuron activity using this regimen was specifically abolished in $\alpha 4$ KO mice (Exley et al., 2011) and not $\alpha 6$ KO subjects, suggesting that $\alpha 6\beta 2^*$ nAChRs exert their DA-releasing activity (Drenan et al., 2008) elsewhere in the mesolimbic circuitry, namely in the NAc or on terminals in the dorsal striatum (Champtiaux et al., 2003; Drenan et al., 2008; Exley et al., 2008; Gotti et al., 2010; Kulak et al., 1997; Meyer et al., 2008; Salminen et al., 2004, 2007). Evidence suggests that the nM concentrations of nicotine used in the intraVTA study, however, may preferentially desensitize rather than activate nAChRs (Fenster et al., 1999; Grady, Marks, & Collins, 1994; Grady, Wageman, Patzlaff, & Marks, 2012; Lester & Dani, 1995; Lu, Marks, & Collins, 1999; Marks, Grady, Yang, Lippiello, & Collins, 1994; Paradiso & Steinbach, 2003). Recent data sug-

gest that $\alpha 6\beta 2^*$ nAChRs are persistently activated by 300 nM nicotine and in comparison with $\alpha 4\beta 2^*$ nAChRs, $\alpha 6\beta 2^*$ nAChRs are resistant to the desensitizing effects of low-dose nicotine (Grady et al., 2012; Liu, Zhao-Shea, McIntosh, Gardner, & Tapper, 2012). Whereas inhibition of $\alpha 6\beta 2^*$ nAChRs would be expected to reduce VTA DA activity, previous data using slice electrophysiology have shown that desensitization of $\alpha 4\beta 2^*$ nAChRs on GABA terminals in the VTA results in a disinhibition of DA receptors that may increase their sensitivity to further excitatory input (Mansvelder, Mertz, & Role, 2009; Mansvelder et al., 2002; Pidoplichko, DeBiasi, Williams, & Dani, 1997). Studies using systemic administration of independent compounds with antagonist activity at $\alpha 6\beta 2^*$ nAChRs have reported reductions of DA release and nicotine self-administration in rats (Markou & Paterson, 2001; Mogg et al., 2002; Neugebauer, Zhang, Crooks, Dwoskin, & Bardo, 2006; Rahman et al., 2007). Together these findings suggest that ligands that reduce $\alpha 6\beta 2^*$ nAChR-mediated DA release may promote tobacco cessation.

Conditioned Reinforcement

The human and animal literature suggest that tobacco- and nicotine-associated cues play a critical role in tobacco use (Besheer, Palmatier, Metschke, & Bevins, 2004; Caggiula et al., 2002; Donny, Houtsmuller, & Stitzer, 2007). Cigarette cues can elicit craving and precipitate relapse (Carter & Tiffany, 1999). Sensory cues such as taste and the feeling of smoke traveling down one's throat play a large role in how pleasurable an individual reports that it is to smoke a cigarette (Perkins et al., 2001). By virtue of their association with smoking behavior, cigarette-associated cues become capable of activating the same mesolimbic brain structures that are stimulated by nicotine (Due et al., 2002; Schroeder, Binzak, & Kelley, 2001; Stein et al., 1998; Volkow, Fowler, Wang, Swanson, & Telang, 2007). Perhaps, this explains in part why individuals will smoke denicotinized cigarettes, albeit at a reduced rate (Donny et al., 2007). Animal studies support the premise that these cues become conditioned reinforcers capable of regulating behavior on their own, and evidence suggests that conditioned reinforcement is supported by $\alpha 6\beta 2^*$ nAChRs (Brunzell et al., 2006; Olausson, Jentsch, & Taylor, 2004a, 2004b; Lof et al., 2007). Cues greatly increase the level at which animals self-administer nicotine (Caggiula et al., 2002) and nicotine-associated cues can maintain self-administration behavior for weeks after removal of nicotine in rats (Cohen, Perrault, Griebel, & Soubrie, 2005). Human laboratory studies suggest that cue-induced craving increases following protracted abstinence (Bedi et al., 2011), although it is not clear if this translates to nicotine seeking (Perkins, 2011). Other studies show that nicotine also enhances conditioned reinforcement (Brunzell et al., 2006; Olausson et al., 2004a, 2004b) via regulation of the $\beta 2^*$ nAChRs (Brunzell et al., 2006). Recent data using ethanol as a reinforcer suggest that $\alpha 6\beta 2^*$ nAChRs in the VTA may contribute to conditioned reinforcement that supports tobacco addiction. In this study, an ethanol-paired tone but not an unpaired tone, led to elevated DA release in the NAc and supported acquisition of responding reinforced by a previously ethanol-paired cue (Lof et al., 2007). These behavioral effects were blocked by local infusion of α -CTX MII into the VTA, suggesting that acetylcholinergic tone at $\alpha 6\beta 2^*$ nAChRs or perhaps $\alpha 3\beta 2^*$ nAChRs mediates this effect (Lof et al., 2007). Self-administration of nicotine and cues, but

not cues alone, was greatly attenuated by intraaccumbens shell infusion of α -CTX MII (Brunzell et al., 2010); it remains to be determined if this $\alpha 6\beta 2^*$ nAChR-associated behavior is mediated in part by the conditioned reinforcing properties of the cues by virtue of their association with nicotine. In further support of $\alpha 6\beta 2^*$ nAChRs mediating conditioned reinforcement, systemic administration of methylcaconitine, an antagonist of $\alpha 7$ and $\alpha 6\beta 2^*$ nAChRs (Mogg et al., 2002) is also sufficient to block responding for cues associated with sucrose reward (Lof, Olausson, Stomberg, Taylor, & Soderpalm, 2010). Although intraVTA infusion of DH β E blocks nicotine self-administration (Corrigall et al., 1994), this antagonist of $\alpha 6\beta 2^*$ nAChRs and $\alpha 4\beta 2^*$ nAChRs had no effect on responding for a conditioned reinforcer or on cue-associated DA release when infused into the VTA (Lof et al., 2007), suggesting that $\alpha 6\beta 2^*$ nAChRs and not $\alpha 4\beta 2^*$ nAChRs contribute to conditioned reinforcement in this region.

Nicotine Withdrawal

Nicotine withdrawal is characterized by a combination of somatic and affective symptoms that are observable in humans and rodents (Damaj et al., 2004; Donny et al., 2007; Jackson, Martin, Changeux, & Damaj, 2008; Jackson et al., 2009; Kenford et al., 2002; Salas, Pieri, & De Biasi, 2004; Salas, Sturm, Boulter, & De Biasi, 2009; Vann, Balster, & Beardsley, 2006). Intracerebral infusion of a selective $\alpha 6\beta 2^*$ nAChR antagonist (McIntosh et al., 2004) dose dependently blocks conditioned place aversion and withdrawal-precipitated anxiety-like behavior in and elevated plus maze (Jackson et al., 2009) while leaving somatic withdrawal symptoms intact. This is consistent with studies showing that $\beta 2$ KO mice show a selective reduction in affective and not somatic withdrawal (Jackson et al., 2008), however it is likely that other subpopulations of nicotinic receptors regulate withdrawal behavior as well (e.g., $\alpha 4\alpha 5\beta 2$ nAChRs, $\alpha 4\beta 2$ nAChRs, $\alpha 3\beta 4$ nAChRs, and $\alpha 7$ nAChRs; Jackson et al., 2008, 2009; Salas et al., 2004, 2009).

Does Activation or Inhibition of Mesolimbic $\alpha 6\beta 2^*$ nAChRs Support Nicotine Addiction Behavior?

The traditional view is that DA release in the NAc produces rewarding effects of nicotine and to the extent that we can measure DA activity in human subjects this premise has generally held true (Barrett et al., 2004; Brody et al., 2004; Stein et al., 1998). Nicotine-associated DA release is abolished in $\beta 2$ nAChR subunit knockout mice that also fail to self-administer nicotine (Picciotto et al., 1998). Nicotine-mediated elevations in DA release are also inhibited by intraVTA infusion of α -CTX MII (Gotti et al., 2010), suggesting that $\alpha 6\beta 2^*$ nAChRs regulate this behavior. In vitro electrophysiology, synaptosome release assays, and cyclic voltammetry studies in tonic-firing neurons show that nicotine results in elevated DA release that is blocked by antagonism of $\alpha 6\beta 2^*$ nAChRs (Champtiaux et al., 2003; Drenan et al., 2008; Perez, O'Leary, Parameswaran, McIntosh, & Quik, 2009; Perez et al., 2010; Salminen et al., 2004, 2007; Zhang & Sulzer, 2004; Zhao-Shea et al., 2011). In contrast, cyclic voltammetry studies assessing neurons after phasic stimulation show that nicotine, DH β E, or α -CTX MII have similar effects to

increase DA release in neurons that are phasically stimulated (Exley et al., 2008; Rice & Cragg, 2004; Perez et al., 2009, 2010; Zhang & Sulzer, 2004). This preparation using bath application of nicotine and nicotinic antagonists could model the brains of smokers after they have had their first cigarette of the day when most of the receptors would likely be desensitized (Brody et al., 2006; Changeux et al., 1984). These data suggest that under conditions when most of the high affinity $\beta 2^*$ nAChRs are in the desensitized state, behavior that supports phasic activity of DA neurons could be further reinforced by desensitization, rather than excitation, of $\alpha 6\beta 2^*$ nAChRs. It is important to note, however, that following repeated in vivo nicotine exposure, α -CTX MII-sensitive nAChRs in the dorsal striatum no longer have a stimulatory effect on DA release in phasically active DA neurons (Perez, Bordia, McIntosh, Grady, & Quik, 2008). It is unknown how the $\alpha 6\beta 2^*$ nAChRs adapt to chronic nicotine exposure in the NAC.

Relevance to Tobacco Dependence

Regardless of the mechanism, animal models with good predictive validity for tobacco use suggest that antagonism of $\alpha 6\beta 2^*$ nAChRs would be an effective strategy for tobacco cessation. The preponderance of the behavioral data show that nicotine self-administration, nicotine CPP, conditioned reinforcement, and nicotine withdrawal are significantly attenuated by selective antagonism of $\alpha 6\beta 2^*$ nAChRs. From a therapeutic standpoint, it is encouraging that global knockdown or blockade of $\alpha 6\beta 2^*$ nAChRs have thus far had similar effects on these behaviors that are relevant to tobacco addiction. The more selective expression profile of $\alpha 6\beta 2^*$ nAChRs versus other nAChRs makes them an attractive target for tobacco cessation, but consideration should be given to the expression of these receptors in the retinal ganglion cells and the visual system. Development of selective antagonists or partial agonists of $\alpha 6\beta 2^*$ nAChRs that cross the blood brain barrier may lead to effective treatment of tobacco dependence in smokers.

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Declaration of Interests

None declared.

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