Molecular Mechanisms Underlying Behaviors Related to Nicotine Addiction

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Tobacco smoking results in more than 5 million deaths each year and accounts for almost 90% of all deaths from lung cancer. Nicotine, the major reinforcing component of tobacco smoke, acts in the brain through the neuronal nicotinic acetylcholine receptors (nAChRs). The nAChRs are allosterically regulated, ligand-gated ion channels consisting of five membrane-spanning subunits. Twelve mammalian α subunits ($\alpha 2 - \alpha 10$) and β subunits ($\beta 2 - \beta 4$) have been cloned. The predominant nAChR subtypes in mammalian brain are those containing $\alpha 4$ and $\beta 2$ subunits (denoted as $\alpha 4 \beta 2^*$ nAChRs). The $\alpha 4 \beta 2^*$ nAChRs mediate many behaviors related to nicotine addiction and are the primary targets for currently approved smoking cessation agents. Considering the large number of nAChR subunits in the brain, it is likely that nAChRs containing subunits in addition to $\alpha 4$ and $\beta 2$ also play a role in tobacco smoking. Indeed, genetic variation in the *CHRNA5-CHRNA3-CHRNB4* gene cluster, encoding the $\alpha 5$, $\alpha 3$, and $\beta 4$ nAChR subunits, respectively, has been shown to increase vulnerability to tobacco dependence and smoking-associated diseases including lung cancer. Moreover, mice in which expression of $\alpha 5$ or $\beta 4$ subunits has been genetically modified have profoundly altered patterns of nicotine consumption. In addition to the reinforcing properties of nicotine, the effects of nicotine on appetite, attention, and mood are also thought to contribute to establishment and maintenance of the tobacco smoking habit. Here we review recent insights into the behavioral actions of nicotine and the nAChRs subtypes involved, which likely contribute to the development of tobacco dependence in smokers.

NICOTINIC RECEPTOR SUBTYPES INVOLVED IN CONTROL OF THE MESOLIMBIC SYSTEM AND NICOTINE REINFORCEMENT

The mesolimbic dopamine (DA) system is a central mediator of drug reward and reinforcement (Koob 1992). Lesions of the ventral tegmental area (VTA) and its primary projection area, the nucleus accumbens (nAc), greatly attenuate nicotine self-administration and the psychostimulant properties of nicotine (its ability to increase locomotion [Clarke et al. 1988; Corrigall et al. 1992, 1994]). A great deal of progress has been made in identifying the nAChR subtypes expressed in both the dopaminergic and GABAergic neurons of the VTA and on neuronal terminals in the nAc (Klink et al. 2001; Zoli et al. 2002). DA neurons express

heteromeric nAChRs containing the α4, α5, α6, β2, and β3 subunits in various combinations, with the predominant subtypes being α4/β2/α5 and α4/α6/β2/β3. The α6 subunit appears to be selectively expressed in DA neurons (Le Novère et al. 1996; Drenan et al. 2008b), although a recent report has suggested that there may be an effect of α6-containing receptors on GABA transmission in the VTA (Yang et al. 2011). In addition, α7 homomeric nAChRs are expressed in DA neurons (Klink et al. 2001), as well as on neuronal terminals on afferents to the VTA (Mansvelder et al. 2002; Wooltorton et al. 2003).

Electrophysiological studies have shown that nAChRs containing the β2 subunit are essential for the ability of nicotine to depolarize DA cell bodies in the VTA and to increase their firing rate (Picciotto et al. 1998; Zhou et al. 2001). While the predominant inward currents owing to nicotine in these neurons involve β2* nAChRs, nicotine can also modulate the presynaptic input to DA neurons from GABAergic and glutamatergic terminals impinging on them. In a slice preparation, nicotine can potentiate glutamate input to DA neurons through α7 nAChRs, resulting in long-term potentiation of those inputs (Mansvelder and McGehee 2000). In addition, nicotine can desensitize β2* nAChRs on GABAergic inputs to DA neurons, resulting in a shift from mixed excitation and inhibition of DA neurons by nicotine, to a more unmixed stimulation of nAChRs on presynaptic glutamatergic terminals (Mansvelder et al. 2002; Wooltorton et al. 2003).

Evidence from mouse genetic models with knockout or mutations of nAChR subunits suggests that the postsynaptic depolarization of DA neurons is essential for behaviors related to nicotine reward and reinforcement such as nicotine place preference and self-administration. Knockout of the β2 subunit abolishes nicotine-mediated DA release (Picciotto et al. 1998; Grady et al. 2001), nicotine-induced locomotor activation (King et al. 2004), nicotine self-administration (Picciotto et al. 1998; Maskos et al. 2005), and nicotine place preference (Walters et al. 2006; Brunzell et al. 2009; Mineur et al. 2009a). Similarly, knockout of the α4 subunit

abolishes intracerebroventricular (i.c.v.) self-administration of nicotine, consistent with evidence that α4/β2* nAChRs are required for depolarization of DA neurons in the VTA (Exley et al. 2011; McGranahan et al. 2011). Conversely, knockin of a hypersensitive α4 subunit shifts the dose-response curve for nicotine-induced increases in DA neuron firing to the left, and results in nicotine place preference at very low doses of the drug (Tapper et al. 2004). Similarly, expression of a hypersensitive α6 subunit in a bacterial artificial chromosome (BAC) transgenic mouse line potentiates nicotine-induced burst firing in DA neurons, and potentiates nicotine place preference at low doses of nicotine (Drenan et al. 2008a, 2010).

A series of recent studies have provided further support for the involvement of α6* nAChRs in nicotine self-administration. Mice lacking the α6 subunit do not acquire intravenous nicotine self-administration (Pons et al. 2008). Similarly, conotoxins selective for α6/β2* nAChRs disrupt nicotine self-administration in the rat when infused into the VTA (Gotti et al. 2010) and following self-administration training, these conotoxins decrease the motivation to lever press for nicotine on a progressive ratio schedule (Brunzell et al. 2010). In contrast, mice with constitutive knockout of the α6 subunit, intra-VTA self-administration of nicotine is not disrupted, whereas α4* nAChRs are necessary and sufficient for both intra-VTA self-administration, as well as nicotine-induced increases in firing of DA neurons (Exley et al. 2011; McGranahan et al. 2011); however, α4 and α6 subunits are both required for the ability of nicotine to gate DA transmission in the nAc, suggesting that nAChRs in nAc may be more important in motivation to self-administer nicotine (Brunzell et al. 2010; Exley et al. 2011) and that this may affect acquisition of intravenous self-administration behavior (Pons et al. 2008; Gotti et al. 2010), as well as nicotine-dependent locomotor activation (Gotti et al. 2010).

Despite its contribution to nicotine-dependent plasticity in the VTA, knockout of the α7 subunit in mice does not affect nicotine place preference (Walters et al. 2006) or acquisition of nicotine self-administration (Pons et al. 2008);

however, antagonizing α7-type nAChRs in the nAc or anterior cingulate cortex in the rat increases the motivation to self-administer nicotine, whereas infusion of a selective α7 agonist decreases motivation, as measured using a progressive ratio schedule (Brunzell and McIntosh 2012). α7-type nAChRs may modulate, rather than mediate, nicotine reinforcement and therefore the effect of α7 knockout may be more subtle than knockout of β2* nAChRs.

An important role for nAChRs in the VTA in nicotine reinforcement has been shown using both molecular genetic and pharmacological techniques. Selective viral reexpression of β2* (Maskos et al. 2005; Pons et al. 2008) or α4* nAChRs in the VTA is sufficient to support both intra-VTA (Maskos et al. 2005) and systemic nicotine self-administration (Pons et al. 2008), identifying the nAChR subtypes necessary for nicotine reinforcement, as well as demonstrating the importance of nAChRs within the VTA itself for this behavior. This is consistent with previous studies suggesting that nAChRs within the VTA are critical for nicotine reward, because local infusion of a nicotinic agonist into the VTA, but not the nAc, is sufficient for nicotine place preference in the rat (Museo and Wise 1994).

Thus, molecular genetic studies support the idea that α4/α6/β2* nAChRs on DA neurons in the VTA are essential for nicotine reinforcement. These experiments in mice are supported by pharmacological studies in rats, and provide a consistent molecular subtype and neuroanatomical locus for the rewarding and reinforcing effects of nicotine.

NICOTINIC RECEPTORS AND CIRCUITS INVOLVED IN AVERSION AND NICOTINE WITHDRAWAL: FOCUS ON THE HABENULA-INTERPEDUNCULAR PATHWAY

The habenula is a diencephalic structure located on the dorsomedial surface of the caudal thalamus that is segregated into medial (MHb) and lateral (LHb) domains (Lecourtier and Kelly 2007; Hikosaka 2010). The MHb and LHb are anatomically, chemically, and functionally distinct subnuclei, each with different complements of afferent and efferent connections. LHb receives afferent inputs from, and projects extensively to, midbrain and hindbrain sites. In particular, the LHb projects densely to the rostromedial tegmental nucleus (RMTg) (Jhou et al. 2009), and has a well-established inhibitory effect on the firing of midbrain dopamine neurons (Lecourtier and Kelly 2007; Matsumoto and Hikosaka 2009; Hikosaka 2010; Bromberg-Martin and Hikosaka 2011). LHb neurons are excited by omission of anticipated rewards or exposure to aversive stimuli (Lecourtier and Kelly 2007; Matsumoto and Hikosaka 2009; Hikosaka 2010; Bromberg-Martin and Hikosaka 2011). This has prompted considerable interest in the role for LHb neurons in encoding negative motivational states. Unlike the LHb, the MHb projects almost exclusively to the interpeduncular nucleus (IPN) via the fasciculus retroflexus (Fr) (Lecourtier and Kelly 2007; Hikosaka 2010). MHb is comprised of neurons that produce the neurotransmitters acetylcholine or substance P (Cuello et al. 1978; Eckenrode et al. 1987), and a small population that produce the cytokine interleukin-18 (IL-18) (Sugama et al. 2002). However, it is believed that most MHb neurons also produce and corelease glutamate, with this excitatory neurotransmitter considered the major functional transmitter at the MHb-IPN synapse (Mata et al. 1977; Vincent et al. 1980; Girod et al. 2000; Ren et al. 2011). The MHb contains some of the highest densities of nicotine-binding sites in brain (Mugnaini et al. 2002). In particular, the highest density of α5, α3, and β4 nAChR subunit expression in brain is detected in MHb and/or IPN (De Biasi and Salas 2008). Indeed, approximately 90%–100% of MHb neurons express α3, α4, α5, β2, and β4 nAChR subunits (Sheffield et al. 2000), and in mouse brain slices through the MHb, >85% of neurons respond to nicotine with an inward current, and these currents are not altered in mice lacking the β2 nAChR subunit (Picciotto et al. 1995). It is also hypothesized that ∼20% of functional nAChRs in rat MHb neurons that project to IPN contain α5 subunits (Grady et al. 2009).

The fact that the MHb-IPN pathway is enriched in α5, α3, and β4 nAChR subunits is of

particular interest in the context of recent human genetics findings. It has been shown that allelic variation in the $\alpha 5/\alpha 3/\beta 4$ nAChR subunit gene cluster located in chromosome region 15q25 significantly increases the risk of tobacco addiction (Saccone et al. 2007; Berrettini et al. 2008; Lips et al. 2010). For example, a single nucleotide polymorphism (SNP) in *CHRNA5* (rs16969968) that is very common in those of European descent (minor allele frequency = 0.42) increases the risk of tobacco dependence by ~30% in individuals carrying a single copy of the variant, and more than doubles the risk in those carrying two risk alleles (Bierut et al. 2008; Wang et al. 2009); a finding that has been consistently replicated (Berrettini et al. 2008; Bierut et al. 2008; Grucza et al. 2008; Stevens et al. 2008). The rs16969968 risk variant is associated with heavy smoking (Berrettini et al. 2008; Bierut et al. 2008; Grucza et al. 2008; Stevens et al. 2008), early onset of smoking behavior (Weiss et al. 2008), and with "pleasurable buzz" from tobacco (Sherva et al. 2008). In addition, the same genetic variability in *CHRNA5* is also a major risk factor for lung cancer and chronic obstructive pulmonary disease (COPD) in smokers (Amos et al. 2008; Hung et al. 2008; Wang et al. 2010), likely reflecting higher levels of tobacco dependence in individuals carrying risk alleles and consequently greater exposure to carcinogens and toxins contained in tobacco smoke (Le Marchand et al. 2008; Thorgeirsson et al. 2008). In addition to the rs16969968 SNP in *CHRNA5*, there is also increased risk of tobacco dependence in individuals carrying the rs6495308, rs578776, or rs1051730 SNPs in *CHRNA3* (Berrettini et al. 2008; Saccone et al. 2009), and rs1948 in *CHRNB4* (Schlaepfer et al. 2008).

The above findings suggest that nAChRs containing $\alpha 5$, $\alpha 3$, and/or $\beta 4$ nAChR subunits, densely expressed in the MHb-IPN pathway, regulate addiction-related actions of nicotine. Consistent with an important role for $\alpha 5^*$ nAChRs in regulating nicotine intake, it was recently shown that mice with a null mutation for this subunit intravenously self-administered far more nicotine than their wild-type littermates (Fowler et al. 2011). Interestingly,

the knockout mice consumed more nicotine only when higher unit doses of the drug were available (Fowler et al. 2011). By using Fos immunoreactivity as a measure of neuronal activation, it was shown that the MHb-IPN pathway of the knockout mice was far less sensitive to nicotine-induced activation than wild-type mice (Fowler et al. 2011). Moreover, chemical inactivation of the MHb or the IPN using the local anesthetic lidocaine, or disruption of NMDA receptor-mediated glutamatergic transmission in these sites using the competitive antagonist LY2358959, increased nicotine self-administration behavior in rats in a manner similar to what was observed in $\alpha 5$ nAChR subunit knockout mice (Fowler et al. 2011). Virus-mediated reexpression of the $\alpha 5$ nAChR subunit in the MHb-IPN pathway of knockout mice abolished the increased nicotine intake seen at higher doses of nicotine (Fowler et al. 2011). Conversely, RNA interference-mediated knockdown of $\alpha 5$ nAChR subunits in the MHb-IPN pathway in rats resulted in increased nicotine intake at higher unit doses of the drug, very similar to the same behavioral profile detected in the knockout mice (Fowler et al. 2011). Finally, knockdown of $\alpha 5$ nAChR subunits in the MHb-IPN pathway in rats decreased their sensitivity to the reward-inhibiting (i.e., aversive) actions of higher nicotine doses compared with control rats, as measured by nicotine-induced elevations of intracranial self-stimulation (ICSS) reward thresholds (Fowler et al. 2011). Taken together, these findings suggest that nicotine activates the MHb-IPN pathway through stimulatory effects on $\alpha 5^*$ nAChRs. Nicotine-induced activation of the MHb-IPN pathway results in a negative motivational signal that serves to limit further nicotine intake. Hence, disruption of $\alpha 5^*$ nAChR signaling diminishes the stimulatory effects of nicotine on MHb-IPN activity, and thereby permits consumption of greater quantities of nicotine.

In addition to $\alpha 5^*$ nAChRs, evidence suggests that $\beta 4^*$ nAChRs in the MHb-IPN pathway also play an important role in regulating nicotine consumption. Specifically, overexpression of $\beta 4^*$ nAChRs in mice using BAC transgenic technology resulted in greatly diminished

sensitivity to the reinforcing properties of orally consumed nicotine solutions and far less consumption of the drug than wild-type mice (Frahm et al. 2011). This finding suggests that, similar to α5* nAChRs, β4* nAChRs in the MHb-IPN pathway also regulate sensitivity to the aversive effects of nicotine that control the quantities of the drug consumed.

Dependence on tobacco smoking depends not only on the balance between the rewarding and aversive action of nicotine described above, but also on escape from the aversive consequences of nicotine withdrawal (Doherty et al. 1995; Kenny and Markou 2001). Indeed, withdrawal duration and severity predicts relapse in abstinent human smokers (Piasecki et al. 1998, 2000, 2003). The nicotine withdrawal syndrome in abstinent smokers is composed of "physical" or somatic components, and "affective" components. The most common somatic symptoms include bradycardia, gastrointestinal discomfort, and increased appetite. Affective symptoms primarily include depressed mood including anhedonia, dysphoria, anxiety, irritability, difficulty concentrating, and craving (Parrott 1993; Doherty et al. 1995; Kenny and Markou 2001). Similar to α5 subunits, α2 subunits are highly enriched in the IPN (Grady et al. 2009). Recently, it was shown that α5 and α2 subunit knockout mice that were dependent on nicotine (delivered through subcutaneously implanted osmotic minipumps) did not show somatic signs of nicotine withdrawal when withdrawal was precipitated with the nAChR antagonist mecamylamine (Salas et al. 2009). Moreover, direct infusion of mecamylamine into the IPN, but not the VTA, of nicotine-dependent wild-type mice precipitated the expression of somatic withdrawal signs (Salas et al. 2009). This suggests that α5* and α2* nAChRs in the MHb-IPN pathway, and perhaps other nAChR subtypes enriched in this pathway, regulate the expression of somatic signs of nicotine withdrawal. However, little is known concerning the role for nAChRs in the MHb-IPN tract in regulating affective aspects of nicotine withdrawal, and in particular, withdrawal-associated reward deficits that may motivate relapse during periods of abstinence in human smokers.

Taken together, the above findings support a key role for α5 and β4, and perhaps also α2 and α3 nAChRs, which are enriched in the MHb-IPN pathway in regulating nicotine reinforcement and the expression of the nicotine withdrawal syndrome in nicotine-dependent rodents. As such, nAChRs containing these subunits may be important targets for the development of novel therapeutics for smoking cessation.

NICOTINIC INVOLVEMENT IN BEHAVIORS RELATED TO ONGOING SMOKING: EFFECTS OF NICOTINE ON DEPRESSION, APPETITE, AND ATTENTION

Nicotine reinforcement and avoidance of the aversive effects of nicotine withdrawal are clearly fundamental for ongoing smoking, but a number of other factors are also likely to contribute to smoking behavior in humans. nAChRs are expressed throughout the brain on both excitatory and inhibitory neurons, with the ability to increase inhibition of circuits when excitation is high and to increase excitation when circuits are less active (Picciotto 2003). The result of this circuit-level integration is that nicotine can modulate behavioral function bidirectionally, acting as a stimulant and increasing anxiety under some conditions and decreasing activity and anxiety in others (Picciotto 2003).

Some individuals report that they smoke to improve attention (Rusted and Warburton 1992; Warburton et al. 1992), and the ability of smoking to improve attentional function in individuals with schizophrenia (George et al. 2002) is likely to contribute to their extremely high rates of smoking. Similarly, a large proportion of smokers report that they smoke to control symptoms of anxiety and depression (Picciotto et al. 2002), and the rate of smoking in individuals with affective disorders is more than double the rate in the general population (Kalman et al. 2005). The idea that some individuals smoke to self-medicate psychiatric symptoms is thought to underlie the high rate of smoking in individuals with psychiatric illness, and some estimates suggest that ~44% of cigarettes are

sold to individuals with a current psychiatric condition (Lasser et al. 2000).

Effects of nAChRs on Anxiety- and Depression-Like Behaviors

Studies in mouse genetic models have helped identify the nAChR subtypes involved in a number of behavioral effects of nicotine that may affect human smoking. Nicotine is known to have both anxiolytic and anxiogenic effects in rodents (File et al. 2000), and these effects are likely to depend on different nAChR subtypes. For example, chronic administration of nicotine increased anxiety-like behavior in female, but not male, mice (Caldarone et al. 2008), whereas knockout mice lacking the β4 subunit show less anxiety-like behaviors at baseline (Salas et al. 2003) and no difference in anxiety-like behaviors were seen at baseline or following nicotine administration in mice lacking the β2 subunit (Caldarone et al. 2008). Similarly, female, but not male, knockout mice lacking the α5 subunit showed reduced anxiety-like behavior, and this may be related to progesterone effects on α5 subunit expression (Gangitano et al. 2009). These data suggest that stimulation of α5β4* nAChRs is important for the anxiogenic effects of nicotine.

The effects of nicotine on depression-like behavior are also complex. Studies in the Flinders sensitive line of rats have shown that acute nicotine administration is antidepressant-like in the forced swim test and that this effect can be blocked by the nicotinic antagonist mecamylamine, suggesting that activation of nAChRs decreases depression-like behavior in this model (Tizabi et al. 2000). In contrast, the nicotinic antagonist mecamylamine has antidepressant-like effects in mice (Caldarone et al. 2004; Rabenstein et al. 2006; Andreasen et al. 2009), and can be effective as an add-on medication in depressed human subjects who are nonresponsive to an SSRI (George et al. 2008). Similarly, nicotinic partial agonists, that would be expected to decrease activity of acetylcholine at endogenous nAChRs when cholinergic tone is high but increase activity of nAChRs when cholinergic tone is low, are effective in mouse models

of antidepressant efficacy (Mineur et al. 2007, 2009b, 2011b; Rollema et al. 2009; Caldarone et al. 2011) and in human smokers (Philip et al. 2009). These data suggest that inhibition of nAChRs in some neuronal subtypes or brain areas and activation in others may contribute to an antidepressant-like effect of nicotinic drugs, so the cycles of nAChR activation and desensitization experienced by smokers may result in fluctuations in depressive symptoms throughout the day. Both the antagonist mecamylamine (Rabenstein et al. 2006) and the partial agonist sazetidine (Caldarone et al. 2011), as well as the classical antidepressant amitriptyline (Caldarone et al. 2004), are ineffective in mice lacking the β2 subunit, and these knockout mice show decreased depression-like behavior at baseline, suggesting that β2* nAChRs are critical for the antidepressant-like effects of nicotinic drugs; however, mice lacking the α7 subunit are also resistant to the antidepressant-like effects of mecamylamine (Rabenstein et al. 2006), and the effects of the partial agonist sazetidine could be blocked with mecamylamine (Caldarone et al. 2011), suggesting that other nAChR subtypes may also contribute to the antidepressant-like effects of nicotinic drugs, and that activation as well as inhibition of nAChRs can result in antidepressant-like effects.

Effects of nAChRs on Behaviors Related to Attention

In addition to effects on anxiety and depression, nicotine and nicotinic drugs can improve attention in control subjects (Rusted and Warburton 1992) and individuals with schizophrenia (Sacco et al. 2004). Interestingly, after control subjects quit smoking and transition past the acute withdrawal period, their working memory function improves compared with when they were smoking (George et al. 2002). In contrast, individuals with schizophrenia show impaired attentional performance once they quit smoking (George et al. 2002). Genetic and functional studies have implicated α7 nAChRs in prepulse inhibition, a physiological marker associated with schizophrenia (Leonard et al. 2000; Freedman et al. 2003). Mice lacking the α7 subunit

have been shown to have impaired trace eye-blink conditioning (Brown et al. 2010). These data suggest that optimal nAChR stimulation is achieved at baseline in control subjects or wild-type mice with normal α7 nAChR levels, whereas nicotine from tobacco smoke can further improve attention in individuals with schizophrenia.

Studies using knockout mice with lentiviral-mediated reexpression have shown that β2* nAChRs in the prelimbic medial prefrontal cortex (mPFC) are important for normal performance of the five-choice serial reaction time task measuring visual attention. Similarly, rapid acetylcholine transients in the mPFC are correlated with attention to brief cues, and mice lacking the β2, but not the α7, subunit show impaired performance in an attentional task (Parikh et al. 2007, 2008). Studies in rodents have also implicated nAChRs on glutamatergic thalamocortical neurons impinging on layer five pyramidal neurons in the prefrontal cortex as an important site for nAChR control of attention (Lambe et al. 2005; Bailey et al. 2010). Overall, it appears that nAChRs in thalamo-corticothalamic loops are important for regulating glutamate release in this circuit, and for mediating the effects of acetylcholine on attentional function (Heath and Picciotto 2009).

In addition to effects of nicotine on attentional function in adulthood, many studies have shown a role for nAChRs in maturation of circuits important for attention during development (reviewed in Heath and Picciotto 2009). Mice administered nicotine during the adolescent period show deficits in the five-choice serial reaction time task that are associated with decreased expression of mGluR2 receptors, and that are rescued by administration of mGluR2 agonists (Counotte et al. 2011). Similarly, α5/β2* nAChRs on layer six cortical glutamatergic projection neurons to the thalamus are essential in maturation of this circuit and for normal adult performance in passive avoidance, a somatosensory aversive learning task (King et al. 2003; Heath et al. 2010). Electrophysiological studies have shown that currents mediated through α5/β2* nAChRs are maximal in the early postnatal period (Kassam et al. 2008). Nic-

otine administration during this same period alters performance in the passive avoidance task in normal mice as well as in mice with expression of β2* nAChRs exclusively in corticothalamic neurons (Heath et al. 2010), suggesting that disrupting normal acetylcholine signaling through these nAChRs during a critical period has lasting effects on function of the corticothalamic circuit in passive avoidance behavior. Interestingly, modulation of nicotinic function through the lynx1 protein is also important for regulating the critical period for activity-dependent visual system development (Morishita et al. 2010).

Effects of nAChRs on Food Intake

The anorexic effects of smoking have been well-documented in human subjects, and the principal reason cited by female teenagers for why they smoke is weight control (Voorhees et al. 2002). On average, smokers weigh ~5 kg less than nonsmokers and have significantly lower body mass index than nonsmokers (Albanes et al. 1987). Similarly, nicotine decreases feeding in animal models (Grunberg et al. 1987), suggesting that the nicotine in tobacco is important for the effects of smoking on appetite. Whereas β2α4α6* nAChRs are critical for nicotine reward and reinforcement, β4* nAChRs on proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus are necessary for the appetite-suppressing effects of nicotine (Mineur et al. 2011a). There are a number of nAChR subtypes expressed in the hypothalamus (Jo et al. 2002, 2005), and nicotine can stimulate the firing of both POMC neurons, which signal satiety, and neuropeptide Y (NPY) neurons, which stimulate food seeking (Huang et al. 2011). Interestingly, in a slice preparation, the effects of nicotine on firing of POMC neurons persist longer than firing of NPY neurons, showing that at the circuit level, stimulation of nAChRs shifts the balance toward neuronal patterns that signal satiety (Huang et al. 2011).

Although β4* nAChRs on POMC neurons can signal satiety, nAChRs in the mesolimbic dopamine system may be more important for the motivation to work for food. The DA system

is important for the hedonic value of both drugs of abuse, like nicotine, and palatable foods (Kenny 2011). Food or sugar intake can increase acetylcholine release in the VTA (Hajnal et al. 1998; Rada et al. 2000), and withdrawal from binge eating increases acetylcholine release in the nAc (Avena et al. 2008). Interestingly, blocking α7 nicotinic AChRs in the VTA can decrease food seeking (Schilstrom et al. 1998). In contrast, previous nicotine exposure increases the motivation of mice to work for food, and this is attributable to non-β2* nAChRs (Brunzell et al. 2006). Taken together, these data show that, in addition to its effects on satiety mediated through POMC neuron signaling, acetylcholine in the mesolimbic system is also likely to affect motivation to seek palatable foods and to modulate their hedonic value through distinct nAChR subtypes.

CONCLUSIONS

The high-affinity α4β2* nAChRs play a key role in the behavioral actions of nicotine that contribute to the development of tobacco dependence, including its effects on brain circuitries involved in reinforcement, mood, attention, and food consumption. Recent evidence has shed important light on other nAChR subunits that may also be incorporated into the α4β2* nAChRs that regulate these processes. For example, incorporation of α6 and β3 nAChR subunits in α4β2* nAChRs in the mesoaccumbens pathway gives rise to a nAChR subtype (α4α6β2β3*) that appears to play a particularly important role in nicotine reinforcement. In addition, nAChR subtypes containing α5, α3, and/or β4 nAChR subunits have been implicated in regulating the aversive properties of nicotine that control the quantities of the drug consumed and in the development of tobacco dependence. In addition, β4* nAChRs also play an important role in appetite regulation, particularly the inhibitory effects of nicotine on appetite that underlie the anorectic effects of tobacco smoke. A more refined understanding of the precise contribution of discrete nAChR subtypes to these addiction-relevant properties of nicotine may reveal important new targets for the development of novel therapeutics for tobacco dependence. Moreover, such novel therapeutics could also have utility for the treatment of mood and attention disorders and the control of body weight.

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