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Neurobiological Mechanisms of Nicotine Reward and Aversion

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***Abstract*——Neuronal nicotinic acetylcholine receptors (nAChRs) regulate the rewarding actions of nicotine contained in tobacco that establish and maintain the smoking habit. nAChRs also regulate the aversive properties of nicotine, sensitivity to which decreases tobacco use and protects against tobacco use disorder. These opposing behavioral actions of nicotine reflect nAChR expression in brain reward and aversion circuits. nAChRs containing $\alpha4$ and $\beta2$ subunits are responsible for the high-affinity nicotine binding sites in the brain and are densely expressed by reward-relevant neurons, most notably dopaminergic, GABAergic, and glutamatergic neurons in the ventral tegmental area. High-affinity nAChRs can incorporate additional subunits, including $\beta3$, $\alpha6$, or $\alpha5$ subunits, with the resulting nAChR subtypes playing discrete and dissociable roles in the stimulatory actions of nicotine on brain dopamine transmission. nAChRs in brain dopamine circuits also participate in aversive reactions to nicotine and the negative affective state experienced during nicotine withdrawal. nAChRs containing $\alpha3$ and $\beta4$ subunits are responsible for the low-affinity nicotine binding sites in the brain and are enriched in brain sites involved in aversion, including the medial habenula, interpeduncular nucleus, and nucleus of the solitary tract, brain sites in which $\alpha5$ nAChR subunits are also expressed. These aversion-related brain sites regulate nicotine avoidance behaviors, and genetic variation that modifies the function of nAChRs in these sites increases vulnerability to tobacco dependence and smoking-related diseases. Here, we review the molecular, cellular, and circuit-level mechanisms through which nicotine elicits reward and aversion and the adaptations in these processes that drive the development of nicotine dependence.**

***Significance Statement*——Tobacco use disorder in the form of habitual cigarette smoking or regular use of other tobacco-related products is a major cause of death and disease worldwide. This article reviews the actions of nicotine in the brain that contribute to tobacco use disorder.**

I. Introduction

A. Motivational Properties of Nicotine

Tobacco smoking results in more than 5 million deaths each year worldwide (WHO, 2008), and it is predicted that approximately 0.6 billion current smokers will die from smoking-related illnesses (Ezzati and Lopez, 2003; Doll et al., 2004; Coe et al., 2005; Mathers and Loncar, 2006). Even in nonsmokers tobacco can be deadly, with over 880,000 people worldwide estimated to die annually year from diseases related to secondhand smoke exposure (Oberg et al., 2011). According to the Centers for Disease Control and Prevention, an estimated 14% of adults in the United States (~34.1 million people) were current cigarette smokers in 2019 (Statistics, 2018). This level of tobacco use represents an all-time low and the culmination of a trend that has been apparent since the mid-1960s, when approximately 45% of adults in the United States were current smokers. In contrast to this progress in reducing cigarette smoking in adults, use of noncigarette tobacco products, most notably electronic delivery devices, has increased dramatically over the past 10 years, particularly in school-age children. From 2011 to 2019, use of electronic cigarettes (e-cigarettes) among children increased >1500%, with almost 3 million children initiating e-cigarette use (7900 per day) in 2019 alone. Strikingly, ~30% of high school students report use of a tobacco product. These young e-cigarette users show increased propensity to use conventional cigarettes and other combustible tobacco products relative to children who do not use e-cigarettes (Leventhal et al., 2015). As might be expected, habitual tobacco use is associated with diseases of the airways, including lung, larynx, and mouth cancers; chronic obstructive pulmonary disease; and asthma (Collaborators, 2017). Cigarette smoking is responsible for ~90% of all lung cancers in the United States (Mokdad et al., 2004), with more people dying from this smoking-related disease than any other type of cancer. Tobacco smoking and e-cigarette use are also a

ABBREVIATIONS: aVTA, anterior VTA; CB_1, cannabinoid 1; CNS, central nervous system; CPA, conditioned place avoidance; CPP, conditioned place preference; CRF, corticotropin-releasing hormone; DHβE, dihydro-β-erythroidine; Ex-4, exendin-4; GluN1, glutamate ionotropic receptor NMDA subunit 1; GluN3A, glutamate ionotropic receptor NMDA subunit 3A; GLP-1, glucagon-like peptide 1; GPCR, G protein–coupled receptor; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; ICSS, intracranial self-stimulation; IPn, interpeduncular nucleus; LDTg, laterodorsal tegmental nucleus; LHb, lateral habenula; 18-MC, 18-methoxycoronaridine; mHb, medial habenula; MLA, methyllycaconitine; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-D-aspartic acid; nTS, nucleus tractus solitarius/nucleus of the solitary tract; PDE2A, phosphodiesterase 2A; PPTg, pedunculopontine tegmental nucleus; pVTA, posterior VTA; RMTg, rostromedial tegmental nucleus; shRNA, short interfering ribonucleic acid; TCF7L2, transcription factor 7–like 2; VH, ventral hippocampus; VTA, ventral tegmental area; VTA^{MED}, medial VTA; VTA^{LAT}, lateral VTA.

major cause of nonrespiratory system diseases, including type 2 diabetes and cardiovascular disease (Rostron et al., 2014; Collaborators, 2017; Hedman et al., 2018; Xie et al., 2020). Smokers who quit before the onset of tobacco-related illness can largely avoid the increased mortality risk (Doll et al., 1994; Peto et al., 2000). Despite awareness of the dangers of smoking, approximately 80% of current smokers attempting to quit will relapse within the first month of abstinence (Benowitz, 2009). Current smoking cessation medications have limited utility. In smokers attempting to quit, ~23% treated with Chantix (varenicline) and ~16% treated with Zyban (bupropion) remain abstinent after 1 year compared with ~9% of those treated with placebo (Knight et al., 2009). Pharmacotherapy is therefore an effective strategy to aid smoking cessation, but there remains considerable risk of relapse even when using the most effective medications currently available. The development of more efficacious smoking-cessation therapeutics would prevent the premature death of hundreds of thousands of people each year and is perhaps the most cost-saving intervention possible within a modern healthcare system (Knight et al., 2009). Leveraging our growing understanding of the basic neurobiological mechanisms of tobacco use disorder will likely lead to more effective smoking-cessation therapeutics.

Nicotine is the major rewarding component responsible for the reinforcing properties of cigarette smoke, which drive the development of tobacco use disorder (Stolerman and Jarvis, 1995). Nicotine intake produces a subjectively pleasant experience (reward), the obtaining of which increases the likelihood that smoking behavior will occur again (reinforcement) (Fowler and Kenny, 2011). Nicotine has both positive and negative reinforcing properties, meaning that the drug enhances the activity of brain reward circuits (positive reinforcement) while attenuating the activity of brain aversion circuits during withdrawal (negative reinforcement) (Kenny and Markou, 2001). Consistent with a key role in tobacco use disorder, nicotine is volitionally self-administered via intravenous infusions by humans (Harvey et al., 2004), nonhuman primates (Goldberg and Spealman, 1982), dogs (Risner and Goldberg, 1983), rats (Corrigall and Coen, 1989; DeNoble and Mele, 2006), and mice (Fowler and Kenny, 2011). Nicotine self-administration under fixed-ratio schedules of reinforcement produces an inverted U-shaped dose-response curve similar to other reinforcing drugs, such as opioids and cocaine. The shape of the nicotine dose-response curve reflects competing positive and negative effects of nicotine at different doses. Increased responding for nicotine over the ascending portion of the dose-response curve reflects the intensifying rewarding effects of nicotine as the amount of drug per infusion increases. Decreased responding over the descending portion of the curve likely reflects increasing aversive properties of nicotine that motivate avoidance behaviors. Consistent with the notion that nicotine elicits both reinforcing and punishing effects is the observation that nonhuman primates volitionally self-administer the same doses of nicotine that they will work to avoid when they are delivered nonvolitionally (Goldberg and Spealman 1982, 1983; Spealman and Goldberg, 1982). Factors other than aversion also contribute to the descending arm of the dose-response curve seen in animals responding under fixed-ratio schedules. For example, more rapid "satiation" when higher doses are available, which means that lower rates of responding can be sustained yet still achieve the same pharmacological actions as lower drug doses, can contribute to the descending arm of the curve (Fowler and Kenny, 2011). Similarly, disruption of behavioral performance also contributes to decreased responding when higher drug doses are self-administered (Fowler and Kenny, 2011). Obtaining the rewarding effects of nicotine while avoiding its aversive and performance-disrupting effects is thought to play a major role in determining patterns and amounts of tobacco intake in smokers (Fowler and Kenny, 2014). Moreover, nicotine-induced adaptive responses in the brain systems that regulate nicotine reward and aversion likely regulate the establishment and maintenance of the tobacco habit with genetic variation that influences these processes, rendering individuals more or less sensitive to the abuse liability of tobacco products (Jensen et al., 2015). Neuronal nicotinic acetylcholine receptors (nAChRs) are the major substrates in the brain for the rewarding and aversive actions of nicotine. The precise molecular, cellular, and circuit-level mechanisms through which different nAChR subtypes regulate these properties of nicotine are not yet fully characterized, but important new insights into these processes have emerged in recent years. Here, we summarize some of the most recent findings on the mechanisms of nicotine reward and aversion and the role for nAChR subtypes in these processes.

B. Structural Architecture of nAChRs

Neuronal nAChRs are comprised of five distinct membrane-spanning subunits (α and β) that combine to form a functionally mature pentameric receptor complex (Hucho and Changeux, 1973; Deneris et al., 1991; Sargent, 1993; Albuquerque et al., 1995; Lena and Changeux, 1998). The mature receptor pentamer functions as an allosteric complex that can assume inactive, active, or desensitized confirmational states (Changeux and Taly, 2008), with the cognate agonist acetylcholine or other agonists, such as nicotine, stabilizing the active confirmation associated with an open inner transmembrane cationic channel (Fig. 1). Nicotine and other exogenous agonists can also drive nAChRs into a desensitized state (Changeux, 1979; Changeux et al., 1984) with the propensity to enter and exit desensitization related to the subunit composition of the receptor complex and influenced by

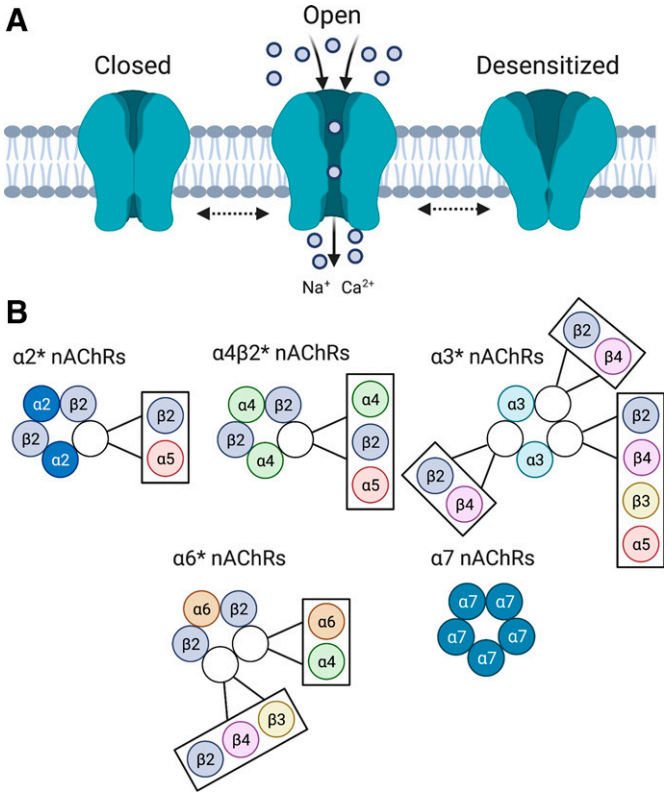

Fig. 1. Structural organization nicotinic acetylcholine receptors. (A) nAChRs are pentameric ligand-gated cationic channels. nAChR agonists, such as acetylcholine and nicotine, stabilize the receptor in the active confirmation associated with an open transmembrane pore permeable to calcium sodium (Na^+), potassium (K^+), and (Ca^{2+}) ions. Prolonged stimulation of the receptor by agonists, such as nicotine, can drive the receptor into an inactive "desensitized" state. (B) Major stoichiometries of homopentameric or heteropentameric nAChRs expressed in the mammalian brain. The open white circles indicate that one of the adjacent subunits contained within the adjacent box are often incorporated into that nAChR subtype.

various post-translational modifications to this complex. The neuronal α subunit exists in nine isoforms (α2–α10), whereas the neuronal β subunit exists in three isoforms (β2–β4) (Elgoyhen et al., 1994, 2001; Le Novere et al., 2002). Assembly of nAChR subunits into a mature receptor is a tightly regulated process that requires appropriate subunit interactions, with only certain subunit stoichiometries able to generate functional nAChRs. The molecular mechanisms that control assembly of nAChR subunits into mature receptor complexes are poorly understood (Gotti et al., 2009), but modern genome-wide screening approaches are beginning to reveal the cellular components and processes involved (Gu et al., 2019). The major stoichiometries of nAChRs found in the mammalian brain are summarized in Fig. 1. Briefly, homomeric nAChRs are comprised of five α7, α8, or α9 subunits, whereas heteromeric nAChRs contain both α and β subunits. Heteromeric nAChRs contain agonist binding sites at the interface between an α and β subunit (Changeux, 1979; Changeux et al., 1984; Gotti et al., 2007). Nicotinic receptors containing α4 and β2 subunits are denoted as $\alpha4\beta2$* nAChRs, with the asterisk signifying that the nAChR contains other unidentified subunits. $\alpha4\beta2$* nAChRs are the most abundant subtype in the mammalian central nervous system (CNS) and account for the high-affinity nicotine binding sites in the brain (Flores et al., 1992). Nicotinic receptors containing α3 and β4 subunits ($\alpha3\beta4$* nAChRs) are abundantly expressed by neurons in the autonomic nervous system and are sometimes referred to as the "ganglionic" nAChRs (Kemp and Morley, 1986). α5 subunits do not contain an agonist binding domain and thus do not form functional homomeric channels or heteromeric channels when coexpressed with β2 or β4 nAChR subunits and instead serve as "accessories" that modify the pharmacology of the receptor complexes into which they incorporate (Ramirez-Latorre et al., 1996; Gotti et al., 2009). β3 also functions as an accessory subunit (Gotti et al., 2009) and plays a particularly important role in facilitating the assembly and function of α6* nAChRs. The role of nAChR subtypes comprised of these different subunits in regulating the behavioral actions of nicotine is reviewed below.

II. Dopamine Mechanisms of Nicotine Reward

A. nAChRs in Brain Dopamine Systems

nAChRs in the CNS are located primarily on presynaptic terminals (Wonnacott, 1997) but can also be found at somatodendritic and postsynaptic locations (Clarke et al. 1986; Sargent, 1993). Atypically, nAChRs are also found on the axons of medial habenula (mHb) neurons that comprise the fasciculus retroflexus tract (Clarke et al., 1986; Mulle et al., 1991; Grady et al., 2009; Passlick et al., 2018). The major function of nAChRs in the CNS is the modulation of neurotransmitter release. Accordingly, nicotine stimulates the release of many transmitters in addiction-relevant regions of the brain and enhances neurotransmission in ventral tegmental area (VTA) and nucleus accumbens (NAc), which have been heavily implicated in nicotine reinforcement processes (Schwartz et al., 1984; Carboni et al., 2000b; Mansvelder and McGehee, 2000; Fu et al., 2003). nAChRs are expressed by midbrain dopamine neurons (Clarke and Pert, 1985), and what it has in common with other major drugs of abuse nicotine is that is enhances dopamine release in the NAc of rodents and human smokers (Imperato et al., 1986; Di Chiara and Imperato, 1988; Marshall et al., 1997; Johnson et al., 2000; Brody et al., 2004, 2006, 2009). This action is thought to reflect the ability of nicotine to stimulate VTA dopamine neurons (Pidoplichko et al., 1997) and increase their burst patterns of firing (Grenhoff et al., 1986; De Biasi and Dani, 2011). The stimulatory actions of nicotine on mesoaccumbens dopamine transmission were thought to exclusively reflect its actions at

nAChRs located in the VTA (Nisell et al., 1994a,b). However, recent findings have highlighted the contributions of nAChRs expressed on the terminals of dopamine neurons in the striatum (Schwartz et al., 1984; Rice and Cragg, 2004; Cachope et al., 2012; Threlfell et al., 2012). As reviewed in detail below, the majority of studies investigating the mechanisms of nicotine reward have focused on the role of mesoaccumbens dopamine neurons in this process. However, it is important to note that nicotine elicits rewarding effects through actions at nAChRs located outside the mesocorticolimbic dopamine system. Indeed, nicotine is self-administered directly into the central linear nucleus and supramammillary nucleus by rats (Ikemoto et al., 2006), yet the mechanisms underlying the reinforcing actions in these sites and the link between these sites and the stimulatory effects of nicotine on VTA dopamine neurons have not been explored. The rewarding effects of nicotine are also blunted in genetically modified mice in which genes encoding μ opioid receptors, opioid peptides, or other related neuropeptide genes have been null mutated, hinting at dopamine-independent mechanisms of nicotine reward (Berrendero et al., 2002, 2005; Walters et al., 2005; Galeote et al., 2009; Sakoori and Murphy, 2009; Trigo et al., 2009; Neugebauer et al., 2011). Nevertheless, remarkably little is known about the role of nAChRs in dopamine-independent mechanisms of nicotine reward.

Nicotine enhances dopamine transmission more robustly in the shell region of the NAc compared with the NAc core region (Nisell et al., 1997; Lecca et al., 2006), with the NAc shell thought to play an important role in nicotine reward (Iyaniwura et al., 2001; Sellings et al., 2008). Dopamine receptor antagonists block the lowering effects of nicotine on intracranial self-stimulation (ICSS) thresholds (Huston-Lyons et al., 1993; Ivanova and Greenshaw, 1997), which reflects the direct stimulatory effects of nicotine on brain reward systems (Wise et al., 1992). Lesioning of midbrain dopamine neurons using the toxin 6-hydroxydopamine abolished the locomotor-stimulating properties of nicotine (Clarke et al., 1988) and reduced intravenous nicotine self-administration in rats (Corrigall et al., 1992; Singer and Wallace, 1984). Blockade of dopamine receptors also reduced nicotine self-administration in rats (Corrigall and Coen, 1991; Corrigall et al., 1992) and attenuated nicotine-induced conditioned place preference (CPP) (Acquas et al., 1989; Spina et al., 2006). These findings suggest that dopamine transmission plays a key role in the motivational properties of nicotine. Notably, however, dopamine receptor and nAChR antagonists increased tobacco consumption in human cigarette smokers (Nemeth-Coslett et al., 1986; Dawe et al., 1995), which is opposite to the effects of such manipulations on nicotine self-administration in rats (Watkins et al., 1999). The reason for this discrepancy is unclear but likely reflects the fact that rodents that self-administer nicotine for a few weeks are less dependent on nicotine than smokers who have been exposed to nicotine for many months or years. Whatever the underlying mechanisms, these findings support an important role for midbrain dopamine neurons, and the nAChRs that regulate mesoaccumbens dopamine transmission, in regulating the motivational properties of nicotine that drive the tobacco habit in human smokers (Corrigall et al., 1992; Fu et al., 2000; Grillner and Svensson, 2000; Tapper et al., 2004; David et al., 2006; Ikemoto et al., 2006).

B. β2 nAChR Subtypes and Nicotine Reward*

$\alpha4\beta2$* nAChRs are the most abundant subtype in the mammalian CNS (Flores et al., 1992). $\alpha4\beta2$ nAChRs occur in two discrete stoichiometries: $(\alpha4\beta2)_2\beta2$ or $(\alpha4\beta2)_2\alpha4$ subtypes (Nelson et al., 2003; Gotti et al., 2009). $(\alpha4\beta2)_2\beta2$ nAChRs are far more sensitive to agonist-induced activation (EC_{50} ~1 mM for acetylcholine) than the $(\alpha4\beta2)_2\alpha4$ subtype (EC_{50} ~100 mM) (Gotti et al., 2009). However, $(\alpha4\beta2)_2\beta2$ nAChRs are also far more sensitive to agonist-induced desensitization than $(\alpha4\beta2)_2\alpha4$ nAChRs (Gotti et al., 2009). In addition to $(\alpha4\beta2)_2(\alpha4)$ and $(\alpha4\beta2)_2(\beta2)$ nAChRs, several other $\beta2$* nAChR stoichiometries exist in the brain and are involved in regulating behavioral responses to nicotine. For example, $\beta2$* nAChRs can incorporate $\alpha2$ or $\alpha6$ subunits in some regions of the brain (Zoli et al., 2002; Salminen et al., 2004; Grady et al., 2009; Gotti et al., 2010). Likewise, $\beta4$* nAChRs can also incorporate $\alpha2$ and $\alpha6$ subunits (Zoli et al., 2002; Salminen et al., 2004; Gotti et al., 2009; Grady et al., 2009; Azam et al., 2010; Dash and Li, 2014). Using polymerase chain reactions (PCRs) to assess nAChR subunit expression in animals after unilateral lesion of VTA dopamine neurons, it was found that mRNA transcripts for $\alpha2$, $\alpha3$, $\alpha5$, $\alpha6$, $\alpha7$ and $\beta4$ subunits were downregulated in the lesioned hemisphere compared with the intact side (Charpantier et al., 1998). By contrast, mRNA for $\alpha4$, $\beta2$ and $\beta3$ subunits was detected after the lesion of dopamine neurons (Charpantier et al., 1998). This suggests that $\alpha2$, $\alpha3$, $\alpha5$, $\alpha6$, $\alpha7$, and $\beta4$ nAChR subunits are expressed by VTA dopamine neurons that project to the NAc, whereas $\alpha4$, $\beta2$, and $\beta3$ subunits are expressed by nondopamine cells in the VTA. Using PCR and single-cell electrophysiological recordings, almost 100% of dopamine and nondopamine neurons in the VTA were shown to express mRNA for the $\alpha4$ subunit (Klink et al., 2001), whereas ~90% of dopamine neurons and 20% of GABA neurons expressed $\beta3$ nAChR subunits (Klink et al., 2001). Further, 70%–75% of VTA dopamine neurons expressed $\alpha5$ and $\alpha6$ subunit mRNAs, but a much lower proportion of GABAergic cells (10%–20%) expressed these subunit transcripts (Klink et al., 2001). A similar proportion of VTA dopamine and GABA neurons (~40%) expressed $\alpha7$ nAChR subunit transcripts (Klink et al., 2001). $\beta4$ mRNA was detected only at low concentrations in VTA

cells. Based on these and related findings, it was proposed that three major subtypes of nAChRs are expressed by VTA dopamine neurons: $(\alpha4\beta2)(\alpha6\beta2)(\alpha5)$, $(\alpha4\beta2)_2(\alpha5)$, and $\alpha7$ (Fig. 2). On VTA GABAergic neurons, it was proposed that two nAChR subtypes predominate: $(\alpha4\beta2)_2(\alpha4)$ and $\alpha7$ nAChRs (Fig. 2). Notably, $\beta3$* nAChRs are not thought to exist in the VTA despite the high concentrations of $\beta3$ mRNA expressed by dopamine neurons (Klink et al., 2001). This is because $\beta3$ subunits are transported to the terminal regions to which VTA neurons project (Forsayeth and Kobrin, 1997), with high $\beta3$ protein concentrations detected in striatum but not VTA (Forsayeth and Kobrin, 1997; Reuben et al., 2000; Salminen et al., 2004). Hence, $\beta3$ nAChR subunits are likely to be incorporated exclusively into the nAChRs on the terminals of VTA neurons in the striatum and elsewhere in the brain, where they regulate the stimulatory effects of nicotine on dopamine transmission (Klink et al., 2001). Immunoprecipitation, ligand-binding, genetic deletion, and targeted lesion studies support the existence of at least four species of nAChRs on the terminals of VTA dopamine neurons in striatum: $(\alpha4\beta2)_2(\alpha4)$, $(\alpha4\beta2)2(\alpha5)$, $(\alpha4\beta2)(\alpha6\beta2)\beta3$, $(\alpha6\beta2)_2\beta2$, and $(\alpha6\beta2)_2\beta3$ (Zoli et al., 2002; Salminen et al., 2004) (Fig. 2). In addition to dopamine neurons, GABA neurons in the VTA also express $\alpha6\beta2$* nAChRs (Yang et al., 2011). There is evidence that $\alpha6$ can occasionally assemble into $\beta4$* nAChRs, with $\alpha6\beta4$* nAChRs involved in regulating norepinephrine release in the hippocampus (Azam et al., 2010) (Fig. 2). However, $\alpha6\beta4$* are thought to be minimally involved in regulating mesoaccumbens dopamine transmission (Azam et al., 2010). It has been speculated that each dopamine neuron in the VTA expresses only one particular nAChR subtype, meaning that these cells can be functionally categorized based on their nAChR expression patterns, with the particular nAChR subtype expressed by each class of dopamine neuron determining their responses to nicotine (Yang et al., 2009).

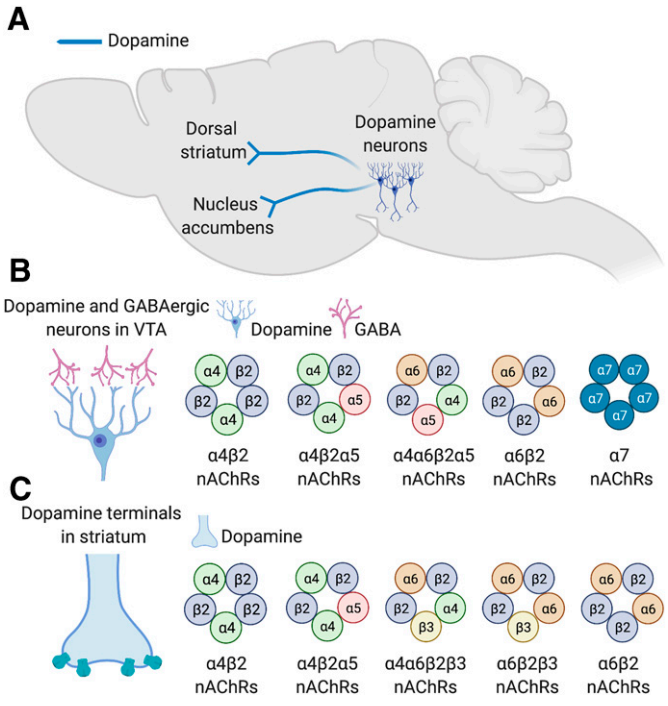

Fig. 2. nAChR subtypes in brain dopamine systems. (A) Ventral midbrain dopaminergic neurons that project to the dorsal striatum or nucleus accumbens are stimulated by nicotine, resulting in increased dopamine transmission in the striatum. (B) Major stoichiometries of nAChRs predicted to be expressed by dopaminergic and GABAergic neurons in the ventral tegmental area. (C) Major stoichiometries of nAChRs predicted to be expressed by on the terminals of dopaminergic in the dorsal striatum and nucleus accumbens.

$\alpha4\beta2$* nAChRs are the major subtype responsible for the stimulatory effects of nicotine on midbrain dopamine neurons (Subramaniyan and Dani, 2015; Thiruchselvam et al., 2017). Nicotine-induced burst firing of midbrain dopamine neurons is abolished in mice lacking the $\beta2$ nAChR subunit (Mameli-Engvall et al., 2006), resulting in attenuated striatal dopamine release in response to nicotine treatment (Picciotto et al., 1998). Accordingly, $\beta2$ subunit knockout mice are almost entirely insensitive to the reinforcing properties of nicotine (Picciotto et al., 1998), with this effect attributed to the diminished stimulatory actions of nicotine on VTA dopamine neurons (Besson et al., 2006; Pons et al., 2008; Orejarena et al., 2012; Grieder et al., 2019; Bagdas et al., 2019). Nicotine stimulates VTA dopamine neurons via $\beta2$* nAChRs located directly on these cells (Durand-de Cuttoli et al., 2018). Nicotine also stimulates populations of $\beta2$* and $\alpha7$ nAChRs on the terminals of glutamatergic inputs to VTA dopamine neurons, resulting in prolonged increases in excitatory transmission in the VTA (Yan et al., 2018; Mansvelder and McGehee, 2000). In parallel, nicotine activates then rapidly desensitizes $\beta2$* nAChRs expressed by VTA GABAergic neurons (Mansvelder et al., 2002; Yang et al., 2011). These findings suggest that the net action of nicotine is an increase in the ratio of excitatory to inhibitory transmission onto VTA dopamine neurons (Durandde Cuttoli et al., 2018), with $\beta2$* nAChRs critical for these actions. Indeed, virus-mediated re-expression of the *Chrnb2* gene, which encodes the $\beta2$ nAChR subunit, in the VTA of $\beta2$ nAChR subunit knockout mice restored the ability of nicotine to evoke burst firing of dopamine neurons in these animals (Maskos et al., 2005; Mameli-Engvall et al., 2006; Naude et al., 2016). *Chrnb2* re-expression in the VTA also restored the reinforcing and locomotor-stimulating properties of nicotine in $\beta2$ knockout mice (Maskos et al., 2005). However, a more recent study showed that virus-mediated *Chrnb2* re-expression in the midbrain dopamine system of $\beta2$ knockout mice did not reinstate their sensitivity to nicotine in a CPP procedure but did reinstate sensitivity to the locomotor-stimulating effects of nicotine (Avale et al., 2008; Mineur et al., 2009). These findings could reflect different components of the midbrain dopamine system being involved in regulating

discrete behavioral responses to nicotine, with these components differentially targeted across studies. Alternatively, it is possible that discrete populations of cells within the VTA regulate behavioral responses to nicotine, with the virus vectors or other factors used across different studies differentially impacting these cell populations. Consistent with both possibilities, nicotine is self-administered only into posterior but not anterior regions of VTA by rats (Ikemoto et al., 2006), and nicotine modulates the balance between excitatory and inhibitory drive onto VTA dopamine neurons in a manner that depends on their precise location within the VTA (Yan et al., 2019). Furthermore, *Chrnb2* re-expression concurrently in both VTA dopamine and GABAergic neurons was required to rescue the sensitivity of β2 subunit knockout mice to the actions of nicotine in CPP and self-administration procedures (Tolu et al., 2013; Grieder et al., 2019). Hence, reward-related behavioral responses to nicotine are likely parsed into discrete anatomic regions of the VTA, and within these regions many different classes of neurons are likely to participate. This concept of spatial and cellular segregation of nicotine reward within the VTA is considered in more detail below.

Systemic or intra-VTA administration of the β2* nAChR antagonist dihydro-β-erythroidine (DHβE) decreased nicotine self-administration in rats (Corrigall and Coen, 1989; Watkins et al., 1999). In addition, DHβE reduced the stimulatory effects of nicotine on brain reward systems, as shown by attenuated nicotine-induced lowering of ICSS thresholds in rats (Ivanova and Greenshaw, 1997; Harrison et al., 2002). As DHβE is relatively selective for β2* nAChRs compared with other classes of nAChRs (Harvey and Luetje, 1996; Harvey et al., 1996), these findings further implicate this nAChR subtype in the motivational properties of nicotine. The novel nAChR compound SSR591813, considered a partial agonist at α4β2* nAChRs, decreased nicotine self-administration in rats (Cohen et al., 2003). Similarly, the novel nAChR compound UCI-30002, a partial agonist at α4β2* nAChRs, also decreased nicotine self-administration in rats (Yoshimura et al., 2007), whereas 5-iodo-A-85380, a putative agonist at β2* nAChRs, was actively self-administered by rats (Liu et al., 2003). Varenicline is a partial agonist at α4β2* nAChRs but a full agonist at α7 nAChRs (Coe et al., 2005; Mihalak et al., 2006; Lerman et al., 2007; Reus et al., 2007; Dwoskin et al., 2009) and can compete with nicotine for binding sites on α4β2* nAChRs in the VTA and elsewhere in the brain. In this manner, varenicline can attenuate the stimulatory action of nicotine on dopamine transmission (Coe et al., 2005; Reperant et al., 2010). Varenicline dose-dependently decreased nicotine self-administration in rats (Rollema et al., 2007; O'Connor et al., 2010), with this action thought to directly reflect the ability of varenicline to attenuate the stimulatory actions of nicotine on VTA dopamine neurons (Harmey et al., 2012). Cytisine and dianicline are both structurally related to varenicline and similarly act as partial agonists at α4β2* nAChRs (Coe et al., 2005). These compounds have shown clinical utility as smoking cessation medications in humans (Barlow and McLeod, 1969; Reavill et al., 1990; Etter, 2006; Rollema et al., 2010). Bupropion has been shown to antagonize α4β2* nAChRs (Alkondon and Albuquerque, 2005), whereas nicotine replacement therapy is thought to facilitate smoking cessation by stimulation α4β2* nAChRs in the midbrain (Harmey et al., 2012). Hence, currently available smoking cessation therapeutics have at least some actions at α4β2* nAChRs. Together, these findings support a major role for α4β2* nAChRs located in midbrain dopamine systems in regulating the reinforcing properties of nicotine.

Nicotine-induced upregulation of nAChR expression has been detected in the brains of rodents and human smokers (Marks et al., 1983; Breese et al., 1997; Staley et al., 2006; Nashmi et al., 2007). nAChR upregulation is thought to modify the subsequent actions of nicotine on brain reward systems and thereby contribute to the development of nicotine dependence (Esterlis et al., 2014, 2016). Indeed, β2* nAChRs expressed by VTA GABAergic neurons are highly sensitive to nicotine-induced upregulation, with this effect correlated with increased sensitivity to the rewarding properties of the drug (Nashmi et al., 2007; Ngolab et al., 2015). Positron emission tomographic imaging of the brains of smokers using a radiotracer to measure densities of α4β2* nAChRs has shown that greater levels of nAChR upregulation are associated with greater difficulty in achieving and maintaining abstinence from tobacco use (Brody et al., 2014). The fact that β2* nAChRs expressed by VTA GABAergic neurons are so sensitive to nicotine-induced upregulation suggests that adaptive responses in VTA GABAergic transmission may play a particularly important role in the development of nicotine dependence. Intriguingly, farnesol, which is often added to nicotine contained in electronic delivery systems to improve its flavor, can increase the activity of VTA dopamine neuron while decreasing the activity of local GABAergic neurons in a manner similar to nicotine (Avelar et al., 2019). Menthol, which is also incorporated into cigarettes to modify their flavor (Ai et al., 2016), also modifies the function of β2* nAChRs in the VTA to enhance the rewarding effects of nicotine (Henderson et al., 2017). This suggests that flavorants added to cigarettes and electronic smoking devices may facilitate nicotine use not only by masking the noxious bitter taste of nicotine but by directly modifying the receptor and cellular substrates in the VTA upon which nicotine acts.

C. α4 nAChR Subtypes and Nicotine Reward*

The most common stoichiometry of β2* heteromeric nAChRs is thought to incorporate two α4 and three β2

subunits, which is denoted as $(\alpha4\beta2)_2(\beta2)$, and contains orthosteric binding sites for acetylcholine, nicotine, and other agonists at the two $\alpha4/\beta2$ interfaces (Zwart and Vijverberg, 1998; Albuquerque et al., 2009). In a less common stoichiometry denoted as $(\alpha4\beta2)_2(\alpha4)$, one of the $\beta2$ subunits is substituted with an $\alpha4$ subunit (Zwart and Vijverberg, 1998; Mazzaferro et al., 2011). This incorporates a third "unorthodox" agonist binding site at the $\alpha4/\alpha4$ interface (Mazzaferro et al., 2011). As noted above, the $(\alpha4\beta2)_2(\alpha4)$ nAChR stoichiometry has lower affinity for nicotine than the $(\alpha4\beta2)_2(\beta2)$ subtype (Moroni et al., 2006; Campling et al., 2013). However, the unorthodox binding site in the $(\alpha4\beta2)_2(\alpha4)$ nAChR stoichiometry results in 3- to 4-fold greater levels of receptor activation by acetylcholine or nicotine when compared with the $(\alpha4\beta2)_2(\beta2)$ stoichiometry (Moroni et al., 2006; Timmermann et al., 2012; Wang et al., 2015; Jain et al., 2016). Hence, the $(\alpha4\beta2)_2(\beta2)$ and $(\alpha4\beta2)_2(\alpha4)$ subtypes can be considered "high-affinity" and "high-efficacy" nAChRs, respectively. Both of these nAChR stoichiometries are thought to be expressed by neurons in the adult mammalian brain (Marks et al., 2007; Gotti et al., 2008), and pharmacological agents have been identified or developed that can discriminate between them (Moroni et al., 2006). The smoking-cessation therapeutics varenicline and cytisine are partial agonists at $(\alpha4\beta2)_2(\alpha4)$ nAChRs but are inactive at $(\alpha4\beta2)_2(\beta2)$ nAChRs (Moroni et al., 2006; Campling et al., 2013). In addition, the $(\alpha4\beta2)_2(\alpha4)$ nAChR positive allosteric modulator NS9283 (Mazzaferro et al., 2019) decreased nicotine self-administration in rats (Maurer et al., 2017). NS9283, cytisine, and varenicline also decreased ethanol intake in rats (Steensland et al., 2007; Bell et al., 2009; Sotomayor-Zarate et al., 2013; Wang et al., 2020). Hence, $(\alpha4\beta2)_2(\alpha4)$ nAChRs likely play an important role in the reinforcing properties of nicotine and other drugs of abuse, and compounds that modulate this nAChR stoichiometry may serve as novel therapeutics for substance use disorders (see Fig. 2).

Similar to $\beta2$ nAChR subunit knockout mice (Walters et al., 2006), deletion of $\alpha4$ nAChR subunits in the ventral midbrain blocked nicotine-induced CPP in mice (Peng et al., 2017). Conditional deletion of $\alpha4$ nAChR subunits from dopamine neurons in mice similarly blocked nicotine-induced CPP (McGranahan et al., 2011). Conversely, mutant mice expressing a hypersensitive $\alpha4$ nAChR subunit are hyper-responsive to nicotine reward, as reflected by the establishment of CPP for very low doses of nicotine that have no detectable effects in wild-type mice (Tapper et al., 2004). The hypersensitive $\alpha4$ mutant mice also showed increased sensitivity to the stimulatory effects of nicotine on midbrain dopamine neurons (Tapper et al., 2004). Using a behavioral procedure in which nicotine is self-administered via the tail vein during a single session, it has been shown that $\beta2$ and $\alpha4$ subunit knockout mice but not $\alpha7$ knockout mice consume markedly less nicotine than their wild-type counterparts (Pons et al., 2008). Lentivirus-mediated re-expression of the $\beta2$ or $\alpha4$ subunit genes in the VTA but not substantia nigra of the respective knockout mice "rescued" their nicotine intake in this acute self-administration procedure (Pons et al., 2008). However, $\alpha4$ nAChR subunit knockout mice did not show any difference in nicotine intake using a more traditional chronic intravenous (jugular catheter) self-administration procedure (Cahir et al., 2011). However, they did show attenuated locomotor suppression in response to injection of a relatively high dose of nicotine (Cahir et al., 2011), a behavioral response thought to reflect the aversive actions of nicotine (Morrison and Stephenson, 1972; Stolerman et al., 1973; Clarke and Kumar, 1983a,b; Hentall and Gollapudi, 1995; Salas et al., 2004a; Frahm et al., 2015; Antolin-Fontes et al., 2020). Hence, it is possible that $\alpha4$* nAChRs play a more prominent role in nicotine aversion than nicotine reward at least under the testing conditions in these experiments (discussed in more detail below) (see Fig. 2).

D. α6 nAChR Subtypes and Nicotine Reward*

Much interest has centered on the potential involvement of $\alpha6$* nAChR subunits in nicotine reward processes. This interest has arisen in large part because of the high concentrations and restricted patterns of expression of mRNA transcripts for $\alpha6$ subunits within the VTA and other catecholaminergic nuclei of the brain (Le Novere et al., 1996; Quik et al., 2000; Azam et al., 2002; Champtiaux et al., 2002; Gotti et al., 2006a). Non-$\alpha4(\alpha6\beta2)_2$* nAChRs are abundantly expressed by mesoaccumbens dopamine neurons, whereas $(\alpha6\beta2)(\alpha4\beta2)$* nAChRs are expressed by nigrostriatal dopamine neurons (Gotti et al., 2010). The stimulatory effects of nicotine on VTA dopamine neurons were attenuated $\alpha6$ nAChR subunit knockout mice (Liu et al., 2012). $\alpha6$ nAChR subunits were also upregulated in rats by chronic intravenous nicotine self-administration (Parker et al., 2004) in both dopaminergic and GABAergic neurons in the VTA (Akers et al., 2020). The magnitude by which $(\alpha6\beta2)(\alpha4\beta2)$* nAChRs were upregulated in the VTA of mice corresponded to their sensitivity to the rewarding effects of nicotine (Akers et al., 2020). However, it was previously reported that $\alpha6$* nAChRs were downregulated by chronic nonvolitional nicotine treatment through a process influenced by the presence or absence of $\beta3$ nAChR subunits (Lai et al., 2005; Mugnaini et al., 2006; Perry et al., 2007; Marks et al., 2014). Hence, the volitional nature of nicotine delivery and their precise subunit composition likely determine whether $\alpha6$* nAChRs are upregulated or downregulated by nicotine. $\alpha6\beta2$* nAChRs are activated by cytisine and varenicline far more efficiently than other $\beta2$* nAChRs (Salminen et al., 2004; Bordia et al., 2012), suggesting that they may contribute to the clinical utility

of these compounds as smoking-cessation therapeutics. Furthermore, allelic variation in the *CHRNB3-CHRNA6* gene cluster on chromosome 8, which encodes the β3 and α6 nAChR subunits, respectively, increases vulnerability to tobacco dependence (Bierut et al., 2007; Thorgeirsson et al., 2010; Wen et al., 2016). Behavioral data have accumulated to support a role for α6* nAChRs in nicotine reinforcement, but their involvement is complex, and their precise contributions remain unclear. α6 nAChR subunit knockout mice did not drink a nicotine-containing solution in a two-bottle choice procedure (Bagdas et al., 2019). Similarly, α6 nAChR subunit knockout mice do not self-administer nicotine using an acute tail-vein procedure during a single session (Pons et al., 2008). Lentivirus-mediated re-expression of the α6 subunit in the VTA of the knockout mice re-established their sensitivity to nicotine reinforcement in this procedure (Pons et al., 2008). Transgenic mice expressing a gain-of-function α6 nAChR mutant subunit showed enhanced sensitivity to the stimulatory effects of nicotine on striatal dopamine transmission and increased locomotor stimulant responses to nicotine (Drenan et al., 2010), with these effects attributed to increased function of (α6β2)(α4β2)* nAChRs (Drenan et al., 2010; Engle et al., 2013). Pharmacological blockade of α6* nAChRs in the VTA or NAc of rodents abolished the stimulatory effects of nicotine on dopamine transmission in the striatum and decreased nicotine self-administration behavior (Brunzell et al., 2010; Gotti et al., 2010; Brunzell, 2012; Sanjakdar et al., 2015), with the α6β2* nAChR subtype hypothesized to play a prominent role in these effects (Whiteaker et al., 2000; Marks et al., 2014). Indeed, the (α4β2)(α6β2)β3 nAChR subtype has the highest sensitivity to nicotine of any native nAChR so far identified (Grady et al., 2007). In addition, the novel nAChR antagonist bPiDDB (*N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide) dose-dependently decreased nicotine self-administration in rats and attenuated the locomotor-stimulating effects of acute and repeated nicotine treatment (Neugebauer et al., 2006). The related α6* antagonist (*N*,*N*-decane-1,10-diyl-*bis*-3-picolinium diiodide) bPiDI decreased intravenous nicotine self-administration in wild-type mice and in mice carrying a mutation in the α4 nAChR subunit that renders it insensitive to mecamylamine and other nAChR antagonists (Madsen et al., 2015). Considering that α4 nAChR subunit knockout mice intravenously self-administer nicotine at similar levels to wild-type mice (Cahir et al., 2011), it was proposed that α6β2* nAChRs are likely to be the critical subtype that regulates the reinforcing properties of nicotine (Madsen et al., 2015); for review, see (Brunzell 2012).

However, recent findings have raised questions about the degree to which α6* nAChRs contribute to the reinforcing properties of nicotine. α4* but not α6* nAChRs regulate nicotine-induced bursting of VTA dopamine neurons (Exley et al., 2011). The novel α6β2* nAChR agonist TC299423 induced only modest rewarding effects in wild-type, which were enhanced in α6 gain-of-function mutant mice (Wall et al., 2017). However, TC299423 had no-effects, no-intravenous-nicotine self-administration in rats (Wall et al., 2017). Using an intra-VTA self-administration procedure, it was shown that α6 nAChR subunit knockout mice will self-administer similar quantities of nicotine in the VTA as wild-type mice, whereas α4 subunit knockout mice self-administer far less nicotine (Exley et al., 2011). This suggests that α6* nAChRs located in the VTA are unlikely to regulate the reinforcing actions of nicotine. One explanation to reconcile these discrepant findings is that α4* nAChRs may dominate the local actions of nicotine in the VTA, whereas α6* nAChRs located on the terminals of dopamine neurons may regulate the local actions of nicotine in the striatum (Exley et al., 2011). Indeed, both α4* and α6* contribute to the stimulatory effects of nicotine on dopamine release in the striatum (Cui et al., 2003; Salminen et al., 2004, 2007; Grady et al., 2007, 2010), with α4β2* and (non-α4)(α6β2)$_2$* nAChRs located on the terminals of dopamine neurons regulating the actions of nicotine in the striatum (Exley et al., 2008, 2011) (Fig. 2). If α6* nAChRs only regulate the actions of nicotine in the striatum and not in the VTA, then nicotine may modulate dopamine release through dissociable actions on dopamine neurons at somatodendritic and terminal brain regions (Reuben et al., 2000). This raises important questions about the function and behavioral significance of dopamine released by nicotine acting at somatodendritic versus terminal locations (discussed in more detail below).

E. β3 nAChR Subtypes and Nicotine Reward*

The β3 nAChR subunit gene is located in the same genomic locus as the α6 subunit gene, and both are thought to be cotranscribed (Moen et al., 2021). Moreover, the β3 subunit is known to facilitate the maturation and expression of α6* nAChRs (Gotti et al., 2006b, 2009; Drenan et al., 2008), and β3 subunit knockout mice demonstrate markedly reduced ($\sim$75% lower) levels of α6* nAChRs in the striatum compared with wild-type mice (Gotti et al., 2005). Hence, the β3 nAChR subunit can be considered an accessory component of the α6* nAChRs that regulate striatal dopamine transmission (Fig. 2). The β3 nAChR subunit has received considerable attention as a possible component of the nAChR subtypes that regulate nicotine reward processes. The nAChR antagonist α-conotoxin MII partially inhibits nicotine-induced dopamine release from striatal synaptosomes (Kulak et al., 1997; Kaiser et al., 1998). α-Conotoxin MII binding in the striatum was shown to depend on the expression of β3* and α6* nAChRs (Champtiaux et al., 2002; Cui et al., 2003). This has led to the proposal that at least two populations of

nAChRs regulate the stimulatory effects of nicotine on dopamine release in the striatum (Kulak et al., 1997) α-conotoxin MII–sensitive and –insensitive components. Subsequent studies suggested that the α-conotoxin MII–sensitive component of nicotine-evoked dopamine release in striatum requires β3* nAChRs that are partially dependent upon α4 subunits (Cui et al., 2003; Salminen et al., 2004), likely representing α6β3β2* and α4α6β3β2* nAChRs. By contrast, the α-conotoxin MII–insensitive component reflects the contributions of α4* nAChRs, likely representing α4β2* and α4β2α5* nAChRs (Salminen et al., 2004). This is consistent with previous work described above, suggesting that mesoaccumbens dopamine neurons express four species of nAChR subtypes on their terminals, two of which contain β3 subunits: α6β3β2* and α4α6β3β2* nAChRs (Zoli et al., 2002).

F. α5 nAChR Subtypes and Nicotine Reward*

Similar to β3, α5 nAChR subunits do not reliably form functional nAChRs containing agonist binding sites when coexpressed with β subunits (Ramirez-Latorre et al., 1996; Gerzanich et al., 1998; Kuryatov et al., 2008; Dash et al., 2012). Instead α5 nAChR subunits are thought to function as accessory subunits in mature nAChR complexes. In the adult mammalian brain, α5 subunits are thought to incorporate most efficiently into α4β2* nAChRs (Perry et al., 2007; Gotti et al., 2007; Kuryatov et al., 2008; Mao et al., 2008). Incorporation of an α5 subunit into α4β2* nAChRs yields the $(\alpha4\beta2)_2\alpha5$ subtype that has the highest known permeability to calcium ions of any nAChR subtype (Ramirez-Latorre et al., 1996; Gotti et al., 2009); for recent review, see (Scholze and Huck, 2020). The presence of an α5 subunit can also alter receptor desensitization and upregulation dynamics in response to agonist exposure. In synaptosome and slice physiology preparations from mouse brain, up to 8-fold higher concentrations of nicotine and other agonists were required to desensitize α4β2* nAChRs that had incorporated an α5 subunit (Grady et al., 2012; Poorthuis et al., 2013; Wageman et al., 2014). Allelic variation in *CHRNA5*, the gene that encodes the α5 nAChR subunit, is heavily associated with vulnerability to nicotine dependence (Berrettini et al., 2008; Bierut et al., 2008; Hung et al., 2008; Thorgeirsson et al., 2008). In particular, the rs16969968 risk variant in *CHRNA5* increases risk of tobacco dependence gives rise to an amino-acid substitution (398D→398N) in the cytoplasmic domain in an amphipathic α helix just preceding the fourth transmembrane domain. This area of the subunit is known to influence channel permeability, particularly to Ca^{2+} ions (Wang et al., 1996, 1998; Gerzanich et al., 1998; Tapia et al., 2007; Kuryatov et al., 2008). This suggests that nAChRs that incorporate the mutant α5 nAChR have reduced function, which is consistent with experimental observations (Bierut et al., 2008). α5 nAChR subunits are expressed by midbrain dopamine neurons (Azam et al., 2002) and can incorporate into α4β2* (but not α6*) nAChRs to form a functional $(\alpha4\beta2)_2\alpha5$ nAChR subtype in these cells. In the VTA, α5 subunits are thought to facilitate the maturation and expression of α4β2 nAChRs and enhance their function (Chatterjee et al., 2013). As might be expected, incorporation of an α5 subunit also increases the resistance of α4β2 nAChRs in the VTA to agonist-induced desensitization (Chatterjee et al., 2013) and renders α4β2α5* nAChRs in the ventral midbrain largely resistant to nicotine-induced upregulation (Mao et al., 2008).

The $(\alpha4\beta2)_2\alpha5$ nAChR subtype is expressed on the terminals of dopamine neurons in the striatum (Salminen et al., 2004; Mao et al., 2008; Grady et al., 2010; Scholze and Huck, 2020) (Fig. 2), where it serves as an important regulator of the stimulatory effects of nicotine on dopamine transmission. $(\alpha4\beta2)_2\alpha5$ nAChRs regulate dopamine release in the striatum in a manner that can be pharmacologically, functionally, and anatomically dissociated from dopamine release regulated by α6β2* and (α6β2)(α4β2)* nAChRs (Salminen et al., 2004; Grady et al., 2010; Exley et al., 2012). For example, $(\alpha4\beta2)_2\alpha5$ nAChRs regulate the stimulatory effects of nicotine on dopamine release largely in dorsal striatum, whereas α6* nAChRs regulate dopamine release in the NAc (Exley et al., 2012). In fact, α5* and α6* nAChRs on dopamine neurons are thought to be nonoverlapping populations that independently regulate striatal dopamine release (Exley et al., 2012). The functional significance of these different mechanisms of dopamine release is not yet clear but likely reflects dissociable dopamine-related behaviors influenced by nicotine. The direct stimulatory effects of nicotine on VTA dopamine neurons are attenuated in α5 subunit knockout mice (Morel et al., 2014; Sciaccaluga et al., 2015) consistent with the attenuated striatal dopamine responses to nicotine in these mice. Virus-mediated re-expression of α5 subunits in VTA dopamine neurons of the α5 subunit knockout mice can "rescue" their responsiveness to nicotine (Morel et al., 2014; Sciaccaluga et al., 2015), further supporting an important role for $(\alpha4\beta2)_2\alpha5$ nAChRs in the dopamine-enhancing actions of nicotine. In contrast to α4β2* nAChRs in the midbrain (Akers et al., 2020), $(\alpha4\beta2)_2\alpha5$ nAChR's system is resistant to nicotine-induced upregulation (Mao et al., 2008), suggesting that adaptive changes in their expression levels do not contribute to the development of nicotine dependence.

G. α7 nAChR Subtypes and Nicotine Reward*

In contrast to the heteromeric nAChRs, behavioral evidence linking α7 homomeric nAChRs to the rewarding and reinforcing actions of nicotine is relatively weak. α7 nAChRs are thought to contribute to the stimulatory effects of nicotine on excitatory glutamatergic inputs to VTA dopamine neurons (Girod et al., 2000;

Mansvelder and McGehee, 2000). Nevertheless, the rewarding effects of nicotine were unaltered in α7 nAChR subunit knockout mice compared with wild-type mice in a CPP procedure (Walters et al., 2006). Similarly, the acquisition of nicotine self-administration was unaltered relative to wild-type mice in an acute tail-vein self-administration procedure (Pons et al., 2008). However, oral nicotine intake gradually extinguished over time in α7 knockout mice relative to wild-type mice using a two-bottle choice procedure (Levin et al., 2009). Female but not male α7 nAChR subunit knockout mice consumed less nicotine that their respective wild-type control groups in a two-bottle choice procedure (Bagdas et al., 2019). Intravenous nicotine self-administration was reduced by the putatively selective α7 nAChR antagonist methyllycaconitine (MLA) (Markou and Paterson, 2001). Complicating this finding is the fact that MLA can antagonize non-α7 nAChR subtypes (Bryant et al., 2002) and retains nicotine-related behavioral effects in α7 subunit knockout mice (Salas et al., 2007). Hence, caution should be exercised when attributing behavioral or physiologic effects of MLA to an action exclusively at α7 nAChRs.

H. Nicotine Modifies Impulse-Dependent and -Independent Accumbal Dopamine Release

The studies exported above often employed in vivo microdialysis or ex vivo brain slice superfusion techniques to investigate the actions of nicotine on mesoaccumbens dopamine transmission (Di Chiara, 2000). Drawbacks of such approaches include the very long sampling times (order of minutes) over large portions of the striatum and often reflect nonsynaptic "spillover" of dopamine that escapes rapid reuptake or breakdown (Parsons and Justice, 1992; Zhou et al., 2001). More complex actions of nicotine on accumbal dopamine transmission have been revealed using more modern approaches that can capture rapid "synaptic" dopamine transmission in the accumbens. Using fast-scan cyclic voltammetry to monitor electrically evoked dopamine release, it was shown that nicotine inhibits action potential-dependent dopamine release in the NAc (Zhou et al., 2001). The nAChR antagonists mecamylamine and DHβE mimicked this action of nicotine (Zhou et al., 2001). These data suggest that nicotine acted by desensitizing β2* nAChRs located on the terminals of dopamine neurons in the accumbens and hint at complex "multimodal" actions of nAChRs on accumbal dopamine transmission rather than the uniform nicotine-induced increases in dopamine release suggested by previous studies. The inhibitory effect of nicotine on dopamine release measured by voltammetry depends on the baseline activity of dopamine neurons. When dopamine release in the accumbens was stimulated using single electrical pulses to recapitulate tonic-like firing patterns (usually 2–5 Hz), nicotine decreased dopamine release in a manner consistent with the desensitization of presynaptically located β2* nAChRs (Rice and Cragg, 2004; Zhang and Sulzer, 2004). However, when dopamine release was stimulated using multiple pulses in a manner that recapitulates burst-like firing patterns (usually 15–100 Hz) thought to occur when rewarding or reward-predictive stimuli are encountered (Schultz, 1986), nicotine instead increased dopamine release (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Based on these findings, it was proposed that nicotine acts as a "high-pass filter" that enhances the contrast between tonic and phasic patterns of dopamine neuron activity, with this action potentially contributing to the reward-enhancing properties of the drug (Rice and Cragg, 2004). Nicotine appeared to act in this manner by blocking short-term inhibitory plasticity in dopamine neurons (Rice and Cragg, 2004), which serves to limit dopamine release during periods of high activity (Cragg, 2003), and instead enhancing short-term calcium-dependent excitatory plasticity (Zhang and Sulzer, 2004).

The population of β2* nAChRs in the accumbens that is desensitized by nicotine to enhance activity-dependent dopamine release was shown to contain α4, α6, and β3 subunits (Exley and Cragg, 2008; Exley et al., 2011, 2012). This likely reflects the high-affinity (α4β2)(α6β2)β3 nAChR subtype. By contrast, α4(non-α6), α6(non-β3), and α6(non-α4) nAChRs play minimal roles in this action of nicotine (Exley et al., 2012). Interestingly, α5 subunit knockout mice did not show a desensitization-like enhancement of activity-dependent dopamine release in the accumbens, suggesting that they are not those nAChRs desensitized by nicotine to promote impulse-dependent dopamine release (Exley et al., 2012). However, the knockout mice showed greater sensitivity to the enhancing effects of the α3/α6* nAChR antagonist α-conotoxin MII on activity-dependent dopamine release in the dorsal striatum (Exley et al., 2012), suggesting that α5* nAChR deficiency resulted in upregulation in the expression and function of (α4β2)(α6β2)β3 nAChRs in dorsal striatum, likely to compensate for deficits in the function of $(\alpha4\beta2)_2\alpha5$* nAChRs thought to specialize in regulating dopamine transmission in dorsal striatum (Champtiaux et al., 2002, 2003; Marubio et al., 2003; Salminen et al., 2004; Grady et al., 2010; Exley et al., 2011, 2012) (Fig. 2). The mechanisms by which desensitization of (α4β2)(α6β2)β3 nAChRs can alleviate short-term inhibitory and promote short-term excitatory plasticity in dopamine neurons to facilitate activity-dependent dopamine release are unclear but appear to involve dopamine D1 receptor signaling (Goutier et al., 2016). It is important to note that squirrel monkeys treated chronically with nicotine (3 weeks of drinking a nicotine-containing solution) showed attenuated accumbal dopamine release in response to low (tonic) or high (phasic) frequency

electrical stimulation and had abolished sensitivity the effects of α-conotoxin MII on dopamine release (Perez et al., 2012). This raises questions about the nature of nicotine-induced adaptive responses in α6β2* nAChR function in the accumbens of human smokers and whether phasic dopamine release is increased or decreased by chronic nicotine exposure in smokers.

The fact that nAChRs located on dopamine terminals exert such a robust inhibitory influence on activity-dependent dopamine release suggests that endogenous acetylcholine derived from striatal cholinergic neurons controls the degree to which dopamine transmission is increased in the striatum when these cells fire in reward-relevant burst patterns of activity (Zhou et al., 2001; Brimblecombe et al., 2018). Indeed, optical stimulation of cholinergic interneurons in the striatum was shown to increase dopamine release in an impulse-independent manner through a direct action of acetylcholine at β2* nAChRs located on the terminals of dopamine neurons (Cachope et al., 2012; Threlfell et al., 2012). Hence, nAChR-mediated cholinergic transmission in the striatum is likely to facilitate dopamine release when midbrain dopamine neurons engage in tonic firing patterns but inhibit dopamine release when these cells engage in burst firing patterns (Cachope et al., 2012; Threlfell et al., 2012). By desensitizing nAChRs in the striatum, nicotine may facilitate reward-related burst firing in the accumbens to enhance brain reward function. Conversely, stimulation of nAChRs in accumbens may enhance dopamine release even when dopamine neurons are tonically firing, which may also contribute to reward-related behaviors (Cover et al., 2019). Precisely how these different sources of nAChR-related dopamine release interact and their relevance to discrete behavioral responses to nicotine are currently unclear.

III. Dopamine Mechanisms of Nicotine Aversion

In addition to its rewarding effects, nicotine also has aversive effects that motivate avoidance behaviors in humans, nonhuman primates, rats, and mice (Shoaib et al., 1997; Spealman, 1983; Sartor et al., 2010; Fowler et al., 2011). The same doses of nicotine that laboratory animals will work to obtain in self-administration experiments also have punishing properties that animals will work to avoid (Spealman, 1983). Whether nicotine is rewarding or aversive depends on whether the drug is consumed volitionally or delivered nonvolitionally, respectively (Spealman and Goldberg, 1978; Goldberg et al., 1981; Goldberg and Spealman, 1982; Spealman, 1983). Individuals who are less sensitive to the aversive effects of nicotine are more likely to be heavy smokers (Jensen et al., 2015). Similarly, those who suffer from psychiatric disorders associated with heavy smoking are often less sensitive to nicotine aversion (Williams et al., 2013), which likely contributes to their high levels of tobacco smoking. Sensiti-vity to nicotine aversion is thought to influence the likelihood of transitioning from occasional to regular tobacco use (Sartor et al., 2010; Fowler and Kenny, 2014). Interestingly, menthol and other additives contained in cigarettes and non-nicotine components of tobacco smoke can attenuate the aversive effects of nicotine (D'Silva et al., 2018; Harris et al., 2019). Hence, a better understanding of the neurobiological mechanisms of nicotine aversion may reveal new insights into genetic and environmental factors that influence vulnerability to tobacco dependence and the brain systems that undergo nicotine-induced adaptions to establish and maintain the tobacco smoking habit. As described above, the stimulatory effects of nicotine on midbrain dopamine neurons contribute to the rewarding properties of the drug that motivate tobacco smoking. However, dopamine transmission also regulates avoidance behaviors (Acquas et al., 1989; Shippenberg et al., 1993; Bromberg-Martin et al., 2010). This raises the possibility that midbrain dopamine neurons contribute to the aversive actions of nicotine. Consistent with this possibility, allelic variation in the genes that encode the dopamine D4 and D2 receptors has been shown to influence aversion-related responses to nicotine delivered by nasal spray in humans (Perkins et al., 2008). Lesioning the pedunculopontine tegmental nucleus (PPTg), which provides a major cholinergic projection to the VTA (Good and Lupica, 2009), blocked the rewarding effects of nicotine injected directly into the VTA and enhanced its aversive effects (Laviolette et al., 2002) (see Fig. 2). Infusion of a β2* nAChR antagonist or a mixed α7/α6* nAChR antagonist into the VTA blocked the aversive effects of nicotine in a place conditioning procedure (Laviolette and van der Kooy, 2003b). These findings suggest that endogenous cholinergic transmission in the VTA derived from PPTg inputs and acting through locally expressed β2* nAChRs regulates the rewarding and aversive properties of nicotine. Infusion of an NMDA glutamate receptor antagonist into the VTA similarly blocked the aversive effects of nicotine in a place conditioning procedure (Laviolette and van der Kooy, 2003b). Antagonism of NMDA receptors also rendered previously rewarding doses of nicotine aversive in rats, as measured using an ICSS procedure (Kenny et al., 2009). Nicotine-enhanced NMDA receptor-mediated glutamatergic transmission in the VTA contributes to the stimulatory effects of nicotine on accumbal dopamine release, with the role for NMDA receptor-mediated transmission in nicotine-enhanced dopamine release particularly important at higher doses of nicotine known to have aversive behavioral effects (Schilstrom

et al., 1998; Fu et al., 2000). Hence, glutamatergic transmission in the VTA regulates components of dopamine transmission involved in both the rewarding and aversive effects of nicotine.

Focal lesion of dopamine inputs to the NAc shell accomplished by local infusion of the toxin 6-hydroxydopamine decreased the CPP response to intravenous nicotine injections in rats (Sellings et al., 2008). Conversely, lesion of dopamine inputs to the NAc core enhanced nicotine-induced CPP in rats (Sellings et al., 2008). NAc core dopamine lesions also attenuated conditioned taste avoidance triggered by intravenous nicotine infusions (Sellings et al., 2008). Systemic or intra-NAc injections of the mixed dopamine D1 and D2 receptor antagonist α-flupenthixol blocked only the aversive effects of nicotine in a place conditioning procedure (Laviolette and van der Kooy, 2003a). Similarly, blockade of D2 dopamine receptors in the shell region of the NAc or D1 dopamine receptors in the NAc core abolished the aversive properties of nicotine injected directly into the VTA (Laviolette et al., 2008; Grieder et al., 2012). These findings provide compelling evidence that the rewarding and aversive effects of nicotine are encoded by VTA-derived dopamine transmission in the accumbens, with the NAc core playing a prominent role in nicotine aversion. Notably, the aversive effects of nicotine were abolished in α5 nAChR subunit knockout mice in a manner that phenocopies the effects of dopamine receptor antagonists (Grieder et al., 2017). This suggests that the component of nicotine-enhanced dopamine transmission in the striatum mediated by $(\alpha4\beta2)_2\alpha5$ nAChRs may signal the aversive but not the rewarding effects of nicotine.

IV. Cellular Mechanisms in VTA of Nicotine Reward and Aversion

A. Balance between nAChR Signaling in VTA Dopaminergic and GABAergic Neurons

Recent findings have shed important light on the cellular mechanisms in the VTA that regulate nicotine aversion. Using mice with floxed alleles of the *Chrna4* gene, it was shown that Cre recombinase-mediated conditional deletion of α4 nAChR subunits in the ventral midbrain increased nicotine self-administration (oral intake) only when high-concentration nicotine solutions were available (Peng et al., 2017). This pattern of nicotine self-administration behavior is thought to occur when the punishing properties of higher nicotine doses are attenuated, which disinhibits self-administration of aversive doses that would otherwise suppress intake (Fowler et al., 2011). α4 nAChR subunits were knocked down in both dopamine and GABAergic neurons in the VTA of mice in this study (Peng et al., 2017), raising the possibility that at least some fraction of those VTA dopamine neurons that express α4* nAChR regulate the aversive reactions to nicotine. As noted above, virus-mediated re-expression of the *Chrnb2* gene in VTA GABAergic neurons rescued nicotine-induced CPP in β2 subunit knockout mice (Grieder et al., 2019). This study also reported that virus-mediated re-expression of *Chrnb2* only in VTA dopamine neurons failed to rescue nicotine reward in β2 subunit knockout mice but instead rendered the knockout mice sensitive to nicotine aversion (Grieder et al., 2019). Virus-mediated re-expression of *Chrnb2* simultaneously in VTA dopamine and GABA neurons but not in either cell type alone was necessary to "rescue" nicotine-induced burst firing of VTA dopamine neurons and reconstitute sensitivity to the reinforcing properties of nicotine in β2 subunit knockout mice, as measured using an intra-VTA self-administration procedure (Tolu et al., 2013). However, when *Chrnb2* was re-expressed only in VTA GABA neurons in β2 subunit knockout mice, these cells were persistently activated by nicotine, and only aversive behavioral responses to nicotine were detected (Tolu et al., 2013). This is consistent with the observation that VTA GABA neurons are more robustly activated by aversive than rewarding doses of nicotine (Dehkordi et al., 2018). These findings support a complex regulatory mechanism whereby concerted actions by nicotine on β2* nAChRs expressed by both VTA dopamine and GABA neurons are required to trigger reward-relevant burst firing of dopamine neurons, with this action necessary to experience the reinforcing properties of nicotine that support self-administration behavior. By contrast, nicotine acting on VTA dopamine or GABA neurons alone can promote aversion (Tolu et al., 2013; Grieder et al., 2019), likely by blocking the ability of dopamine neurons to engage in burst firing.

B. Anterior-Posterior Domains of the VTA

The fact that VTA dopamine neurons regulate both the rewarding and aversive properties of nicotine reflects the remarkable functional heterogeneity of these cells, with discrete populations likely specializing in positive or negative reinforcement processes (Lammel et al., 2011, 2012, 2014). Dopamine neurons located in the posterior VTA (pVTA) but not the anterior VTA (aVTA) regulate the rewarding properties of nicotine that support self-administration behavior (Ikemoto et al., 2006) (Fig. 3). Similarly, ethanol, cocaine, opioids, and cholinergic agonists are all volitionally self-administered into the pVTA but are not reliably self-administered into the aVTA (Rodd-Henricks et al., 2000; Ikemoto and Wise, 2002; Zangen et al., 2002; David et al., 2004; Rodd et al., 2005). The pVTA but not aVTA also regulates the locomotor-stimulating properties of these drugs (Ikemoto et al., 2003; Sanchez-Catalan et al., 2009). aVTA and pVTA dopamine neurons are distinguished by distinct morphologic features (Zhao-Shea et al., 2011) and project

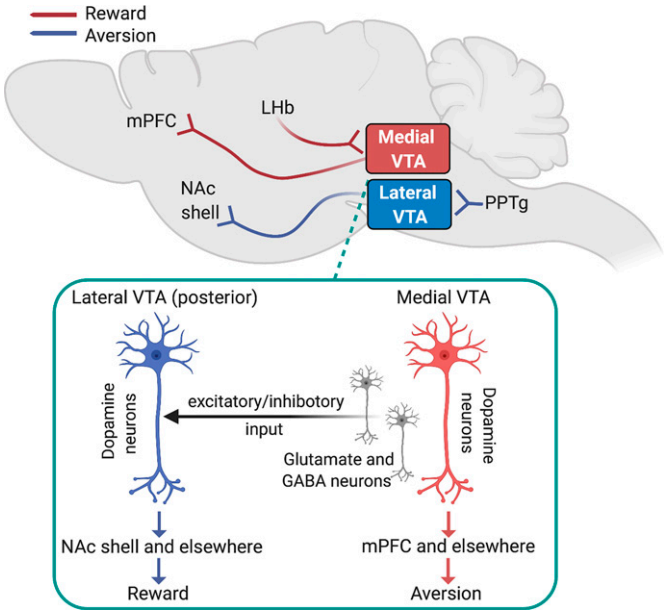

Fig. 3. Dopamine mechanisms of nicotine reward and aversion. Dopamine neurons located in the medial VTA that receive input from the LHb and project to the mPFC regulate aversion-related behaviors. Dopamine neurons located in the lateral VTA that receive input from the PPTg and project to the medial portion of the NAc shell regulate reward-related behaviors. Shown in the insert is the putative nAChR-regulated excitatory and inhibitory input from the medial VTA to the lateral VTA, particularly in posterior (caudal) VTA, that may regulate reward-related responses to nicotine.

to different regions of the striatum (Ikemoto et al., 2006). Specifically, pVTA dopamine neurons project predominantly to medial NAc shell and medial olfactory tubercle, whereas aVTA neurons instead project to the accumbens core, lateral tubercle, and dorsal striatum (Ikemoto et al., 2006). Dopamine neurons in the pVTA show greater responsiveness to nicotine than those in the aVTA, as measured by nicotine-induced cFos immunoreactivity (Zhao-Shea et al., 2011), consistent with the fact that pVTA contains denser concentrations of nAChRs than aVTA (Zhao-Shea et al., 2011). Moreover, pVTA dopamine neurons are activated by rewarding doses of nicotine, whereas aVTA dopamine neurons are activated only by high concentrations of nicotine that have aversive properties (Fonck et al., 2005; ZhaoShea et al., 2011). Within the pVTA, α4* and α6* nAChR subtypes are thought to regulate the rewarding and reinforcing properties of nicotine (ZhaoShea et al., 2011; Exley et al., 2012; Liu et al., 2012; Engle et al., 2013).

C. Medial-Lateral Domains of the VTA

In addition to antero-posterior heterogeneity, VTA neurons also demonstrate a medial-lateral functional gradient (Lammel et al., 2011, 2012, 2014) (Fig. 3). Indeed, neurons in the medial VTA (VTA^{MED}) are more likely to be GABAergic or glutamatergic than neurons in the lateral VTA (VTA^{LAT}), where the majority of the dopamine neurons are concentrated (Lammel et al., 2008; Hnasko et al., 2012; Root et al., 2014a,b; Ntamati and Luscher, 2016; Yan et al., 2018, 2019). Projections from the PPTg to the VTA are known to regulate the rewarding properties of nicotine (Corrigall et al., 2002; Alderson et al., 2006, 2008; Maskos, 2008). These PPTg inputs synapse preferentially onto neurons in lateral domains of the VTA (PPTg-VTA^{LAT} neurons), which in turn project to the NAc medial shell (Lammel et al., 2012). Optical stimulation of PPTg-VTA^{LAT} neurons can elicit reward-related behaviors that are blocked by dopamine receptor antagonists infused into the NAc shell (Lammel et al., 2012). Rewarding doses of nicotine are known to preferentially increase dopamine transmission in the NAc shell (Nisell et al., 1997; Lecca et al., 2006), which may reflect the recruitment of this PPTg-VTA-NAc shell reward circuit (Fig. 3). In addition to reward-relevant VTA^{LAT} dopamine neurons, cholinergic inputs from PPTg and the adjacent laterodorsal tegmental nucleus (LDTg) also project to VTA^{MED} glutamatergic neurons that have been heavily implicated in aversion-related behavioral states (Root et al., 2014a; Lammel et al., 2015; Qi et al., 2016; McGovern et al., 2020) and are known to express functional α4* and α6* nAChRs (Yan et al., 2018, 2019). Recent data have revealed that PPTg- and LDTg-derived cholinergic transmission in the VTA bidirectionally modulates reward and aversion behaviors (Dautan et al., 2016). This suggests that the rewarding and aversive effects of nicotine may reflect the nonphysiologic recruitment of these processes, which are normally controlled by endogenous cholinergic transmission acting at local nAChRs in the VTA (Fig. 3).

In contrast to PPTg inputs to the VTA, which predominately but nonexclusively target VTA^{LAT} neurons, inputs from the lateral habenula synapse preferentially onto neurons in the VTA^{MED} [lateral habenula (LHb)-VTA^{MED} neurons] (Fig. 3). VTA^{MED} neurons targeted by the LHb in turn project to the medial prefrontal cortex (mPFC) (Lammel et al., 2012) (Fig. 3). Optical stimulation of LHb-VTA^{MED} neurons elicits aversion-related behaviors that are blocked by dopamine receptor antagonists infused in the mPFC (Lammel et al., 2012). Nicotine is known to increase dopamine release in the mPFC (Rossi et al., 2005), which could reflect the engagement of this LHb-VTA-mPFC aversion circuit. VTA^{MED} dopamine neurons send a reciprocal projection back to the LHb, with these VTA^{MED} dopamine neurons known to corelease both glutamate and GABA (Root et al., 2014b) (Fig. 3). VTA-derived glutamate transmission in the LHb regulates aversion-related behaviors (Root et al., 2014a), whereas VTA-derived GABA transmission in the LHb regulates reward-related behaviors (Stamatakis et al., 2013). Notably, nicotine increases both glutamatergic and GABAergic transmission in the LHb, likely by stimulating $\alpha4\beta2$*

and α6* nAChRs expressed by VTA dopamine inputs to the habenula (Zuo et al., 2016). This suggests that nicotine bidirectionally modulates the activity of aversion-relevant neurons in the LHb by altering the balance of VTA-derived glutamatergic and GABAergic transmission. In addition to their projections to aversion-related neurons in VTA^{MED}, LHb neurons also send a prominent projection to GABA neurons in the rostromedial tegmental nucleus (RMTg) (Jhou et al., 2009b), also known as the "tail" of the VTA. In turn, RMTg GABA neurons project to the VTA where they can inhibit dopamine neurons to elicit aversion-related behaviors (Jhou et al., 2009a,b; Hong et al., 2011; Barrot et al., 2012). Nicotine markedly enhances the activity of RMTg neurons (Lecca et al., 2011), providing another mechanism by which nicotine can modulate the activity of reward-related dopamine neurons in the VTA. Hence, nAChR-induced modulation of synaptic inputs to VTA^{LAT} and VTA^{MED} neurons and modulation of the projections from VTA^{LAT} and VTA^{MED} neurons to downstream brain sites likely contribute to the reinforcing actions of nicotine (Fig. 3).

Recent studies have begun to reveal the complex interplay between dopamine neurons located in medial and lateral domains of the VTA and how nicotine modifies these interactions (Fig. 3). Specifically, VTA^{MED} neurons were shown to provide both excitatory and inhibitory input to VTA^{LAT} dopamine neurons (Yan et al., 2019). Interestingly, a discrete subpopulation of the VTA^{MED} neurons expresses β2* nAChRs and coreleases both glutamate and GABA onto VTA^{LAT} dopamine neurons (Yan et al., 2019). Nicotine had bidirectional effects on VTA^{MED}-derived excitatory glutamatergic transmission in VTA^{LAT} dopamine neurons, increasing excitatory input to approximately half of the recorded neurons in VTA^{LAT} and decreasing excitatory input to the other half (Yan et al., 2019). By contrast, nicotine uniformly decreased VTA^{MED}-derived inhibitory transmission in VTA^{LAT} dopamine neurons (Yan et al., 2019). These findings suggest that nicotine-induced inhibition of VTA^{MED}-derived GABAergic input and simultaneous stimulation of VTA^{MED}-derived glutamatergic input to VTA^{LAT} "reward" dopamine neurons contribute to the rewarding properties of the drug, whereas nicotine-induced inhibition of VTA^{MED}-derived glutamatergic input to VTA^{LAT} reward neurons contributes to the aversive properties of the drug. More broadly, these findings suggest that complex modulation of excitatory and inhibitory transmission onto VTA^{MED} and VTA^{LAT} dopamine neurons by nicotine acting through local neurons within the VTA and on long-range inputs to the VTA determines whether nicotine has rewarding or aversive effects. Further investigation will be required to better define the complex effects of nicotine on local and long-range synaptic inputs to VTA^{MED} and VTA^{LAT} dopamine neurons and the consequences of these actions on downstream brain sites.

The findings described above suggest that VTA-derived dopamine, glutamate, and GABA transmission in the accumbens, LHb, mPFC, and elsewhere in the brain likely contribute to aversion-related responses to nicotine. However, dopamine receptor antagonists block nicotine aversion only in animals with a limited history of nicotine exposure (Tan et al., 2009). By contrast, the same dopamine manipulations block the rewarding effects of nicotine in animals that have previously been exposed to the drug (Tan et al., 2009). This apparent switch in the role for dopamine transmission that occurs with repeated exposure to nicotine from initially regulating nicotine aversion to instead regulating nicotine reward coincides with an increase in the incidence of nicotine-induced burst firing of VTA dopamine neurons and a decrease in the baseline activity of VTA GABAergic neurons (Tan et al., 2009). Hence, VTA dopamine neurons may participate in aversive reactions to nicotine that influence the likelihood of transitioning from initial tobacco use to regular intake (de Wit & Phillips 2012). Furthermore, adaptive responses in dopamine-mediated aversion systems driven in part by alterations in the function of VTA GABA neurons may contribute to this transition process. However, these findings also suggest that dopamine transmission is unlikely to participate in aversive responses to nicotine once regular nicotine use has been established and that other aversion-related brain systems influence patterns and amounts of tobacco smoking in smokers (Fowler and Kenny, 2014). This raises the important issue of the identity of dopamine-independent brain systems that regulate nicotine aversion in tobacco smokers and nicotine-experienced laboratory animals, which is considered in more detail below.

V. Nondopamine Mechanisms of Nicotine Aversion

A. Human Genetics Reveal nAChR Subtypes that Regulate Nicotine Intake

As noted above, nicotine has rewarding properties that motivate intake and aversive properties that motivate avoidance. These competing positive and negative effects likely explain the inverted U-shape of the dose-response curve for self-administered nicotine seen in humans, nonhuman primates, and laboratory rodents responding under fixed-ratio schedules. Lower doses elicit primarily rewarding effects that motivate self-administration, whereas higher doses elicit mixed rewarding/aversive effects that necessitate careful titration of intake (Goldberg and Spealman, 1982; Risner and Goldberg, 1983; Corrigall and Coen, 1989; Harvey et al., 2004; DeNoble and Mele, 2006; Fowler and Kenny, 2011). One potential explanation for these opposing motivational properties of nicotine and the shape of the dose-response curve is that the same population of nAChRs regulates both the

rewarding and aversive effects of nicotine, with lower doses activating these nAChRs and higher doses desensitizing and thereby inactivating these nAChRs (Pidoplichko et al., 1997). An alternative explanation is that lower unit doses of nicotine engage high-affinity nAChRs located in brain reward circuits to motivate nicotine self-administration, whereas higher nicotine doses engage low-affinity nAChRs in brain aversion circuits that motivate nicotine avoidance. According to this framework, nicotine intake is titrated to maximize the activation of reward-related nAChRs while minimizing the activation of aversion-related nAChRs. The mesoaccumbens dopamine system is enriched in $(\alpha4\beta2)(\alpha6\beta2)\beta3$ nAChRs (Grady et al., 2007), which have the highest sensitivity to nicotine of any native nAChR so far identified (Grady et al., 2007) and are thought to contribute to nicotine reward. Until recently, little was known about the identity of the putative "low-affinity" nAChRs activated by higher nicotine doses or the contributions of the brain regions in which they are located to the control of nicotine intake.

The *CHRNA5-CHRNA3-CHRNB4* gene cluster located in chromosome region 15q25 encodes the $\alpha5$, $\alpha3$, and $\beta4$ nAChR subunits, respectively. nAChRs that incorporate subunits encoded by this gene cluster are known as "low-affinity" nAChRs because they bind nicotine far less efficiently than the so-called high affinity $\beta2$* nAChRs (Zoli et al., 1998). They are also known as the ganglionic nAChRs because of their dense expression in neurons of the autonomic nervous system (Kemp and Morley, 1986). Large-scale human genetics studies have shown that allelic variation across the entire *CHRNA5-CHRNA3-CHRNB4* gene cluster is associated with increased vulnerability to tobacco dependence and higher numbers of cigarettes smoked per day (Amos et al., 2008; Ware et al., 2011; Marques-Vidal et al., 2011; Gallego et al., 2013; Liu et al., 2018; Perez-Morales et al., 2018). In particular, individuals who carry risk alleles in *CHRNA5* are less sensitive to the aversive effects of nicotine (Jensen et al., 2015) and more likely to be heavy smokers (Saccone et al., 2007; Berrettini et al., 2008; Lips et al., 2009; Ohi et al., 2019). A particular variant in C*HRNA5* (rs16969968) that gives rise to an amino-acid substitution ($\alpha5$-398D$\rightarrow\alpha5$-398N) thought to decrease the function of $\alpha5$* nAChRs that incorporate the mutant subunit increases tobacco dependence risk by ~30% in individuals who carry a single copy of the gene variant and doubles the risk in individuals who carry two copies of the variant (Bierut et al., 2008; Wang et al., 2009). Alleles in *CHRNA5* are also 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). Hence, low-affinity $\alpha3\beta4$* nAChRs and $\alpha5$* nAChR subtypes are of considerable interest in the context of nicotine aversion.

B. α5, α3*, and β4* nAChRs Regulate Nicotine Avoidance*

As noted above, the so-called "ganglionic" $\alpha3\beta4$* nAChRs are expressed in the CNS, where they account for low-affinity nicotine binding sites (Flores et al., 1992). They are far less abundant than $\alpha4\beta2$* nAChRs and are concentrated in just a few brain regions, most notably the medial habenula and interpeduncular nucleus (Clarke et al., 1985; Cimino et al., 1992). In heterologous expression systems, $\alpha5$ subunits can incorporate into the $\alpha3\beta4$* nAChRs, which modifies their sensitivity to agonist-induced desensitization (Wang et al., 1996; Mao et al., 2008; Gotti et al., 2007; Perry et al., 2007; Kuryatov et al., 2008). However, $\alpha5$ subunits incorporate more efficiently into $\alpha4\beta2$ nAChRs (Gotti et al., 2007; Perry et al., 2007; Kuryatov et al., 2008; Mao et al., 2008). Similar to $\alpha5$, $\beta3$ can also functions as an accessory subunit in $\alpha3\beta4$* and $\alpha3\beta2$* nAChRs (Gotti et al., 2009). $\beta3$ has a dominant-negative function when incorporated into $\alpha3\beta4$* nAChRs, rendering the resulting receptor complex nonfunctional (Palma et al., 1999; Broadbent et al., 2006). Less frequently, $\alpha3$ nAChRs can coassemble with $\beta2$ subunits to form a functional $\alpha3\beta2$* nAChR subtype.

$\alpha5$ nAChR subunit knockout mice and their wild-type littermates intravenously self-administer nicotine according to an inverted U-shaped dose-response curve (Fowler et al., 2011), as expected based on previous findings in humans and laboratory animals (Goldberg et al., 1981; Henningfield and Goldberg, 1983; Corrigall and Coen, 1989; Le Foll et al., 2007). Levels of nicotine self-administration were similar in $\alpha5$ subunit knockout and wild-type mice on the "ascending" portion of the dose-response curve when lower unit doses of nicotine were available (Fowler et al., 2011). By contrast, responding was much higher in the knockout mice than their wild-type littermates when higher unit doses of nicotine were on the "descending" portion of the dose-response curve (Fowler et al., 2011). Transgenic rats that express a mutant form of the $\alpha5$ nAChR subunit gene modified to contain the same amino-acid substitution caused by the rs16969968 risk allele also self-administered greater quantities of nicotine than wild-type rats but only when higher unit doses of nicotine on the descending portion of the dose-response curve were available (Forget et al., 2018). Enhanced responding for nicotine as the unit dose increases is thought to reflect an intensification of the reinforcing properties of the drug, thereby motivating higher levels of intake (Lynch and Carroll, 2001). Diminished responding for nicotine as the dose increases reflects greater restraint over intake to avoid the increasingly aversive effects of higher drug doses (Henningfield and Goldberg, 1983; Lynch and Carroll, 2001), more rapid

development of drug satiation (Lynch and Carroll, 1999, 2001), or the manifestation of performance-disrupting actions of the drug (Lynch and Carroll, 1999, 2001). Hence, deletion of α5 nAChR subunits has dissociable effects on the motivational processes that control nicotine intake. The stimulatory effects of nicotine on brain reward systems that motivate nicotine use were unaltered by α5 subunit knockout, whereas the aversive effects of nicotine that limit its self-administration were attenuated by α5* nAChR deficiency. Consistent with this interpretation, α5 nAChR subunit knockout mice had similar sensitivity to the rewarding effects of nicotine as wild-type mice but markedly reduced sensitivity to the aversive effects of nicotine, as measured using CPP and ICSS procedures (Jackson et al., 2010; Fowler et al., 2011, 2013; Grieder et al., 2017). More recently, *Chrna3*$^{tm1.1Hwrt}$ hypomorphic mice, which express much lower levels of α3 nAChR subunits than wild-type mice (Caffery et al., 2009), were shown to self-administer greater numbers of intravenous nicotine infusions than their wild-type littermates, with this effect most apparent when nicotine doses on the descending portion of the dose-response curve were available (Elayouby et al., 2021). Similarly, β4 nAChR subunit knockout mice also self-administered greater numbers of nicotine infusions directly into the VTA when higher nicotine doses were available (Husson et al., 2020), although these animals self-administered less nicotine across all doses tested when the drug was delivered by intravenous infusion (Harrington et al., 2016). Transgenic mice that overexpressed β4 nAChR subunits in only those neurons that constitutively express β4 subunits showed enhanced nicotine aversion and decreased oral nicotine intake (Frahm et al., 2011). Similarly, lentivirus-mediated expression in the brains of mice of mutant gain-of-function β4 nAChR subunits, which incorporated *CHRNB4* variants associated with reduced risk of tobacco dependence, increased aversion to nicotine (Slimak et al., 2014). Together, these findings suggest that α5*, α3*, and β4* nAChRs regulate nicotine aversion.

C. Low-Affinity nAChRs Are Enriched in Medial Habenula and Interpeduncular Nucleus

α3 and β4 nAChR subunit transcripts are expressed at low levels in the midbrain dopamine system (Klink et al., 2001; Azam et al., 2002), and functional α3* and β4* nAChRs have not been reliably detected in the VTA. α5 subunit transcripts are expressed at modest levels in the VTA, although functional $(\alpha4\beta2)_2\alpha5$ nAChRs are thought to be expressed by dopamine and nondopamine neurons in the VTA (Fig. 2). By contrast, high densities of α5, α3 and α4 nAChR subunit transcripts are detected in the mHb, interpeduncular nucleus (IPn), and nucleus of the solitary tract (nTS) (Marks et al., 1992; Winzer-Serhan and Leslie, 1997; Sheffield et al., 2000; De Biasi and Salas, 2008; O'Leary et al., 2008; Grady et al., 2009; Frahm et al., 2011; Tuesta et al., 2011, 2017; Hsu et al., 2013; Antolin-Fontes et al., 2015; Morton et al., 2018; Husson et al., 2020). The mHb is located immediately adjacent to the LHb in the epithalamus, projects almost exclusively to the IPn, and is subdivided into four functional domains: superior, inferior, central, and lateral nuclei (Aizawa et al., 2012). Neurons in the inferior, central, and lateral domains produce acetylcholine, whereas those in the superior portion produce substance P (neurokinin 1) or the cytokine interleukin-18 (Contestabile et al., 1987; Andres et al., 1999; Aizawa et al., 2012; Kobayashi et al., 2013; Quina et al., 2017). All mHb neurons are thought to produce and corelease glutamate, which is considered the major functional transmitter at the habenula-IPn synapse (Ren et al., 2011; Aizawa et al., 2012; Aizawa and Zhu, 2019). The cholinergic neurons in ventral portions of the mHb project to central, rostral, and intermediate domains of the IPn (Kimura et al., 1981; Houser et al., 1983; Qin and Luo, 2009) where they assume highly unusual "serpentine" patterns of innervation (Ramón y Cajal, 1953; Lenn, 1976; Herkenham and Nauta, 1979; Lenn et al., 1983), whereas substance P–positive neurons in dorsal MHb neurons project to the lateral domains of the IPn (Qin and Luo, 2009). nAChRs located on the presynaptic terminals of mHb inputs to the IPn regulate neurotransmitter release into the IPn, and the mHb-IPn synapse has long served as a model system for nAChR signaling in the brain (Sastry, 1978; Brown et al., 1983; McGehee et al., 1995). In addition, postsynaptic nAChRs located on IPn neurons can generate retrograde signaling molecules, including endocannabinoids and nitric oxide, that provide a source of feedback inhibition onto mHb inputs (Rodrigo et al., 1994; Ables et al., 2017; Quina et al., 2017; Melani et al., 2019). Little is currently known about the function of the mHb-IPn circuit, although available data suggest that mHb-derived cholinergic, glutamatergic, and substance P transmission in the IPn modulated by $GABA_B$, cannabinoid 1 (CB1), or glycine receptors regulates the encoding of memories related to aversive stimuli or events (Thompson, 1960; Donovick et al., 1970; Agetsuma et al., 2010; Yamaguchi et al., 2013; Soria-Gomez et al., 2015; Zhang et al., 2016; Koppensteiner et al., 2017; Geng et al., 2019; Melani et al., 2019). The mHb-IPn circuit may also coordinate adaptive physiologic and behavioral responses to stressful or anxiety-provoking stimuli (Lisoprawski et al., 1981; Murray et al., 1994; Shumake et al., 2003; Hsu et al., 2012, 2016; Xu et al., 2018; Duncan et al., 2019; Sherafat et al., 2020).

D. nAChR Signaling in the mHb-IPn Circuit Regulates Nicotine Avoidance

Considering the high densities of α5, α3 and α4 nAChR subunits in the habenula-IPn circuit (Marks et al., 1992; De Biasi and Salas, 2008) and the role for this circuit in regulating responses to aversive

stimuli, it is perhaps not surprising that evidence has accumulated over the past decade implicating the mHb-IPn circuit in coordinating aversive behavioral responses to nicotine. Infusion of the local anesthetic lidocaine into the mHb or IPn of rats increased their nicotine intake via intravenous infusions (Fowler et al., 2011), suggesting that the mHb-IPn circuit exerts an inhibitory influence over nicotine-taking behavior. IPn neurons that receive excitatory inputs from the mHb are perhaps the most nicotine-responsive cells in the brain, as measured by nicotine-induced cFos immunoreactivity (Ren and Sagar, 1992; Pang et al., 1993; Salminen et al., 2000). Nicotine-induced cFos expression in IPn was greatly reduced in α5 subunit knockout mice compared with wild-type mice (Fowler et al., 2011). nAChR function was also decreased in the mHb and IPn of α5 subunit knockout mice, as measured by the rubidium efflux assay (Fowler et al., 2011). By contrast, nicotine-induced activation of the VTA was similar in wild-type and α5 knockout mice (Fowler et al., 2011). This suggests that α5* nAChRs are an important functional subtype that regulates the stimulatory effects of nicotine on the mHb-IPn circuit. The elevated level of nicotine self-administration in α5 subunit knockout mice was normalized by virus-mediated re-expression of α5 subunits specifically in the mHb-IPN circuit (Fowler et al., 2011). Conversely, lentivirus-mediated expression of short interfering RNAs (shRNAs) to knock down α5 or α3 nAChR subunits in the mHb or IPn increased nicotine self-administration in rats (Fowler et al., 2011; Elayouby et al., 2021), with these effects most apparent when higher unit doses were available. Conversely, lentivirus-mediated expression of mutant gain-of-function β4 nAChR subunits in the mHb of mice increased nicotine aversion and decreased their nicotine intake (Slimak et al., 2014). Infusion of the α3β4* nAChR antagonists 18-methoxycoronaridine (18-MC) or α-conotoxin AuIB into the mHb or IPn but not the α3β2* nAChR antagonist α-conotoxin MII increased nicotine intake in rats (Fowler et al., 2011; Elayouby et al., 2021). By contrast, infusion of 18-MC into the VTA had no effect on nicotine self-administration in rats (Glick et al., 2011). These findings suggest that stimulatory effects of nicotine on the mHb-IPn circuit mediated by α5* and α3β4* nAChRs decrease the motivation to consume nicotine.

E. nAChR Signaling in mHb-IPn Circuit Regulates the Reward-Inhibiting Effects of Nicotine

Using an ICSS procedure, it was shown that shRNA-mediated knockdown of α5 nAChR transcripts in the mHb-IPn circuit of rats had no effects on the threshold-lowering effects of lower nicotine doses but attenuated the threshold-elevating effects of higher nicotine doses in rats (Fowler et al., 2011). These findings in rats recapitulate the same pattern of effects described above in α5 nAChR subunit knockout mice (Fowler et al., 2013). The ICSS threshold-lowering doses of nicotine are thought to reflect nicotine-induced enhancement of brain reward activity (Kenny and Markou, 2006). Conversely, the ICSS threshold-elevating effects of nicotine are through to reflect the reward-inhibiting effects of the drug that contribute to nicotine aversion and motivate nicotine avoidance (Schaefer and Michael, 1986; Fowler et al., 2011). Rats regulate their intravenous nicotine self-administration to achieve maximal lowering of ICSS thresholds (Kenny and Markou, 2006), suggesting that nicotine intake is titrated to obtain this action of nicotine while avoiding the threshold-elevating effects of the drug. Hence, disruption of α5* nAChR-mediated signaling in the mHb-IPn circuit likely increases nicotine intake by attenuating the reward-inhibiting effects of nicotine and extending the range of nicotine doses that are reward-enhancing (Fowler et al., 2011). This is consistent with the observation that aversive responses to nicotine are attenuated in individuals who carry the rs16969968 polymorphism in C*HRNA5* (Jensen et al., 2015). In contrast to dopamine-mediated mechanisms of nicotine aversion, which were detected only when animals had limited previous exposure to nicotine (Tan et al., 2009) (see above), the aversion mediated by the mHb-IPn circuit was observed even in animals that had experienced extended periods of daily access to high unit doses of nicotine (Fowler et al., 2011; Elayouby et al., 2021). This suggests that nAChR transmission in the mHb-IPn circuit is likely to control nicotine intake in human smokers even after the habit has been established. Indeed, functional brain imaging studies have shown that nicotine modulates habenular activity in both nonsmokers and habitual smokers (Flannery et al., 2019; Jennings et al., 2020). Notably, mHb neurons receive excitatory and inhibitory synaptic input almost exclusively from the posterior septum via the stria medullaris (Sperlagh et al., 1998; Qin and Luo, 2009; Yamaguchi et al., 2013; Otsu et al., 2018), with ATP thought to function as a neurotransmitter at the septo-mHb synapse (Edwards et al., 1992; Sperlagh et al., 1995). Nevertheless, little is known about function of these septal inputs to the mHb, and their potential involvement in behavioral responses to nicotine has not been explored.

F. Stoichiometries of nAChRs Expressed in the mHb-IPn Circuit

The mHb contains some of the highest concentrations of nAChRs in the brain, particularly those that account for the "low-affinity" nicotine binding sites (i.e., do not contain β2 subunits) (Clarke et al., 1985; Wada et al., 1989; Marks et al., 1992; Zoli et al., 1998). As noted above, the highest concentrations of α5, α3, and α4 nAChR subunits in the brain are

detected in the mHb and IPn. nAChRs expressed by mHb neurons are highly calcium-permeable (Mulle et al., 1992; Guo and Lester, 2007) and are expressed along the entire length of mHb neurons, including their soma, axons, and terminals in the IPn (Mulle et al., 1991; Lena et al., 1993; Passlick et al., 2018). Electrophysiological recordings of mHb neurons revealed two discrete nAChR-mediated currents, suggesting that at least two populations of nAChRs are expressed in the mHb (Mulle et al., 1991; Connolly et al., 1995). Both nAChR populations are desensitized by nicotine (Lester and Dani, 1995; Hicks et al., 2000; Duncan et al., 2019). Neurons in the ventrolateral portion of the mHb are most responsive to nicotine (Fonck et al., 2009; Shih et al., 2014), with $\alpha 3$ and $\beta 4$ nAChR subunits expressed by most neurons in this region (Shih et al., 2014). Nicotine-responsive neurons in ventrolateral mHb provide synaptic inputs to rostral and dorsomedial domains of the IPn (Mulle et al., 1991; Shih et al., 2014). Blockade of $\alpha 3\beta 4$* nAChRs has been shown to greatly reduce but not eliminate the stimulatory effects of nicotine on mHb neurons (Quick et al., 1999; Elayouby et al., 2021). Immunoprecipitation studies support these findings and suggest that relatively uncommon nAChR subtypes are expressed by mHb neurons (Fig. 4). Specifically, mHb neurons express high concentrations of $\alpha 3\beta 4$* nAChRs but also $\alpha 3\beta 2$*, $\alpha 3\alpha 4\beta 4$*, and $\alpha 4\beta 2$*; nAChRs (Grady et al., 2009); and much smaller populations of $\alpha 2\beta 2$* and $\alpha 6\beta 2$* nAChRs (Fig. 4). A large proportion of these nAChRs contained $\alpha 5$ accessory subunits, which associate primarily with $\beta 2$* nAChR stoichiometries (Grady et al., 2009). A large proportion of the nAChRs in the mHb also contained $\beta 3$ accessory subunits, which were most often found in combination with $\beta 4$* nAChRs (Grady et al., 2009). This contrasts with ventral midbrain dopamine neurons, in which $\beta 3$ subunits are exclusively almost expressed within $\alpha 6$* nAChRs. In the IPn, $\alpha 2\beta 2$*, $\alpha 3\beta 2$*, $\alpha 4\beta 2$*, $\alpha 3\beta 4$*, $\alpha 4\beta 4$*, and $\alpha 6\beta 4$* nAChRs have been detected (Grady et al., 2009). Again, a large proportion of these contained $\alpha 5$ or $\beta 3$ accessory subunits, with $\alpha 5$ subunits associating primarily with $\beta 2$* nAChRs and $\beta 3$ with $\beta 4$* nAChRs (Grady et al., 2009) (Fig. 4).

As noted above, a large proportion of nAChRs in the mHb contain $\alpha 5$ accessory subunits (Grady et al., 2009) and nAChR function in the mHb was markedly reduced in $\alpha 5$ subunit knockout mice when measured using rubidium efflux from habenular synaptosomes (Fowler et al., 2011). Habenular synaptosomes generated from $\beta 2$ but not $\beta 4$ knockout mice also showed striking deficits in nAChR function (Grady et al., 2009). These findings suggest that $\alpha 5$* nAChRs play an important role in the mHb (Fig. 4). However, $\alpha 5$ subunit mRNA transcripts are expressed at very low levels by mHb neurons,

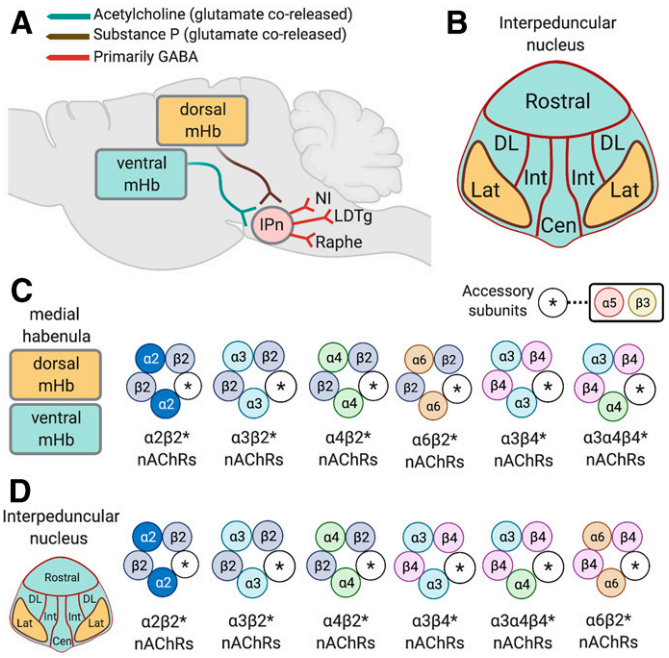

Fig. 4. Organization and nAChR subtypes of the habenula-interpeduncular nucleus circuit. (A) Substance P–expressing neurons in in the dorsal region of the mHb and cholinergic neurons in the ventral mHb corelease glutamate and project to the IPn. The IPn send predominately GABAergic projections to the raphe nuclei, LDTg, and the nucleus incertus (NI). (B) Graphical representation of subregions of the interpeduncular nucleus, including the rostral, dorsolateral (DL), intermediate (I), and central (Cen) nuclei that receive input from cholinergic neurons in the ventral mHb. (C) Major stoichiometries of nAChRs predicted to be expressed presynaptically and postsynaptically in the mHb. (D) Major stoichiometries of nAChRs predicted to be expressed presynaptically and postsynaptically in the mHb in the IPn.

with far higher concentrations found in the IPn (Sheffield et al., 2000; Hsu et al., 2013; Morton et al., 2018), although $\alpha 5$ nAChR subunit protein is detected in the mHb (Grady et al., 2009). As nAChR signaling in synaptosomes primarily reflects presynaptic nAChRs, these data suggest that $\alpha 4\beta 2\alpha 5$* nAChRs are the major functional nAChR subtype expressed on the terminals of synaptic inputs to mHb neurons. Consistent with this notion, nicotine increases the intrinsic excitability of mHb neurons through an action that depends on $\alpha 5$* nAChRs contained on presynaptic terminals in the mHb (Dao et al., 2014), with these presynaptic $\alpha 5$* nAChRs regulating the release of neurokinins into the mHb, which act at postsynaptic neurokinin receptors located on mHb neurons (Dao et al., 2014).

This raises the important question of which nAChR subtypes into which $\alpha 5$ subunits assemble in the mHb-IPn circuit to regulate behavioral responses to nicotine. In heterologous expression systems, $\alpha 5$ nAChR subunits can coassemble into $\alpha 4\beta 2$, $\alpha 3\beta 2$, and $\alpha 3\beta 4$ nAChR subtypes (Fucile et al., 1997; Gerzanich et al., 1998; Tapia et al., 2007). However, in the brain $\alpha 5$ subunits preferentially assemble into $\alpha 4\beta 2$* nAChR subtypes and are rarely detected in $\alpha 3$* nAChRs (Gotti et al., 2007; Perry et al., 2007; Kuryatov et al., 2008;

Mao et al., 2008). Moreover, α5 subunits were shown to incorporate far more readily into β2* nAChRs than β4* nAChR in the mHb-IPn circuit of rats and mice (Grady et al., 2009). In *Xenopus* oocytes, only the function of α4β2α5* nAChRs but not α3β4α5* or α3β2α5* nAChRs was affected by incorporation of mutant α5 subunits modified to include the major smoking-related *CHRNA5* risk allele (D398N), as measured by agonist-evoked calcium influx (Kuryatov et al., 2011). This suggests that α4β2α5* nAChRs likely play a major role in regulating the stimulatory actions of nicotine on the mHb-IPn circuit. However, as noted above, pharmacological blockade of α3β4* nAChRs attenuated the stimulatory effects of nicotine on the mHb-IPn circuit and increased nicotine intake in rats (Glick et al., 2011; Elayouby et al., 2021), whereas overexpression of these receptors in the mHb-IPn circuit increased nicotine aversion and decreased nicotine intake in mice (Frahm et al., 2011; Slimak et al., 2014). Hence, both α4β2α5* and α3β4* nAChRs in the mHb-IPn circuit appear to regulate nicotine aversion. Nicotine enhances both glutamate and acetylcholine release from habenular terminals in the IPn (Ren et al., 2011), with both neuro-transmitters coreleased from the same mHb cholinergic neurons (Ren et al., 2011). Acetylcholine also facilitates the packaging of glutamate into synaptic vesicles in mHb cholinergic neurons, thereby indirectly facilitating glutamatergic transmission in the IPn (Frahm et al., 2015). Discrete firing patterns of habenular cholinergic neurons are required to stimulate glutamate versus acetylcholine release (Ren et al., 2011), with brief optogenetic stimulation sufficient to elicit glutamate release but more persistent (tetanic) optogenetic stimulation required to trigger acetylcholine release (Ren et al., 2011). Intriguingly, α3β4* nAChRs are thought to exclusively regulate acetylcholine release in the mHb-IPn circuit (Grady et al., 2001; Hussain et al., 2008; Grady et al., 2009), whereas α4β2α5* nAChRs regulate glutamate transmission (Girod et al., 2000). These findings suggest that multiple parallel yet pharmacologically distinct streams of information originate from habenular inputs to the IPn. The first is a glutamate-regulated signal modulated by α4β2α5* nAChRs, and the second is an acetylcholine-regulated signal modulated by α3β4* nAChRs. In addition, a third stream of information is likely to originate from substance P–expressing neurons in dorsal portions of the mHb that project to the lateral IPn (see below), but remarkably little is known about the function of these substance P cells. How these putatively dissociable signals function together and independently to regulate the motivational properties of nicotine is unknown.

G. nAChRs in IPn Regulate Nicotine Reward and Aversion

The mHb projects almost exclusively to the IPn via the fasciculus retroflexus (Qin and Luo, 2009), where mHb neurons corelease glutamate and acetylcholine (Ren et al., 2011). Nevertheless, relatively little is known about how IPn neurons regulate behavioral responses to nicotine. Aversive doses of nicotine decrease then increase locomotor activity of rats in a time-dependent manner (Morrison and Stephenson, 1972; Stolerman et al., 1973; Clarke and Kumar 1983a,b). Locomotor depression occurs during the first 15–20 minutes after administration of higher nicotine doses followed by locomotor stimulation that can persist for >60 minutes. The locomotor-depressing effects of nicotine are thought to reflect nicotine-induced malaise and are mediated at least partly by mHb cholinergic neurons (Salas et al., 2004a; Frahm et al., 2015; Antolin-Fontes et al., 2020). Excitotoxic lesion of the IPn attenuated the initial locomotor-depressing but enhanced the later locomotor-stimulating effects of nicotine (Hentall and Gollapudi, 1995). This suggests that stimulation of excitatory inputs from the mHb to the IPn and direct actions on IPn neurons (Mulle et al., 1991; Lena et al., 1993) contributes to aversive behavioral responses to nicotine. Immunoprecipitation techniques have established that significant populations of β2* nAChRs that combine with α3 or α4 subunits and sparse populations of β2* nAChRs that combine with α6 subunits are contained within the IPn (Grady et al., 2009). High concentrations of β4* nAChRs are also detected in the IPn, with the majority containing α3 subunits and smaller populations containing α4 or α6 subunits (Grady et al., 2009). As noted above, knockdown of α3 nAChR subunits or pharmacological blockade of α3β4* nAChRs in the IPn increased nicotine self-administration in rats (Glick et al., 2011; Elayouby et al., 2021). However, blockade of α3β4* nAChRs in the VTA located bilaterally to the IPn had no effects on nicotine self-administration in rats (Glick et al., 2011). This suggests that α3β4* nAChRs expressed by IPn neurons regulate the motivational properties of nicotine. High concentrations of α5* nAChRs are detected in the IPn (Morton et al., 2018), particularly in rostral, central, and the intermediate regions that receive input from ventral portions of the mHb (Fonck et al., 2009; Shih et al., 2014; Quina et al., 2017). Optogenetic stimulation of α5 nAChR-expressing neurons in the IPn elicits aversion-related behaviors in mice, with these behaviors facilitated by pretreatment with nicotine (Morton et al., 2018). Translating ribosomal affinity profiling combined with RNA sequencing has been used to transcriptionally profile α5* nAChR-expressing ($\alpha5^{+}$) neurons in the IPn (Ables et al., 2017). This analysis identified at least two transcriptionally dissociable and spatially segregated populations of $\alpha5^{+}$ neurons (Ables et al., 2017). $\alpha5^{+}$ neurons that express *Amigo1* ($\alpha5^{Amigo1}$ cells) also coexpress high concentrations of somatostatin and nitric oxide synthase 1, are concentrated rostral nucleus of the IPn, and

send efferent input to the median raphe and LDTg (Ables et al., 2017). By contrast, $\alpha5^{+}$ neurons that express *Epyc* ($\alpha5^{Epyc}$ cells) are concentrated in the intermediate nucleus but also present in the rostral nuclei of the IPn and do not send long-range projections but instead are scattered locally within the IPn and to a lesser extent in the raphe nuclei (Ables et al., 2017). *Amigo1* and *Epyc* encode cell adhesion proteins, the function is poorly understood, but their expression is useful for distinguishing between these subpopulations of $\alpha5^{+}$ neurons in IPn. As might be expected, $\alpha5^{Amigo1}$ cells concentrated in the rostral IPn, which receives dense input from the ventrolateral mHb, showed robust increases in activity in response to nicotine treatment (Ables et al., 2017). $\alpha5^{Epyc}$ cells located in the rostral IPn also showed nicotine-induced increases in activity, whereas those located in the intermediate nucleus were relatively insensitive to nicotine (Ables et al., 2017). This suggests that $\alpha5^{Epyc}$ cells are a collection of at least two further subpopulations of $\alpha5^{+}$ neurons. Excitatory inputs to the IPn from mHb cholinergic were inhibited by nitric oxide–mediated retrograde signaling presumably derived from the $\alpha5^{Amigo1}$ cells (Ables et al., 2017). Furthermore, expression of a genetically encoded toxin to inhibit neurotransmitter release and thereby silence $\alpha5^{Amigo1}$ cells attenuated nicotine-induced CPP and decreased oral intake of the drug, whereas silencing of $\alpha5^{Epyc}$ cells had no effects of nicotine reward (Ables et al., 2017). shRNA-mediated knockdown of nitric oxide synthase 1 in the IPn also attenuated nicotine reward (Ables et al., 2017). These findings suggest that $\alpha5^{Amigo1}$ cells are activated by nicotine and, presumably, by acetylcholine and glutamate derived from mHb inputs from cholinergic neurons in ventral mHb, which results in the generation of nitric oxide–mediated inhibitory feedback inhibition onto mHb inputs to these cells. This feedback inhibition of mHb inputs by $\alpha5^{Amigo1}$ cells contributes to the rewarding properties of nicotine. It is notable that the rewarding and not the aversive properties of nicotine were attenuated by silencing of $\alpha5^{Amigo1}$ cells (Ables et al., 2017), a finding similar to the attenuated nicotine reward seen in mice after genetic deletion of choline acetyltransferase in mHb cholinergic neurons (Frahm et al., 2015). This suggests that the mHb-IPn circuit not only regulates nicotine aversion but also nicotine reward. The fact that only the aversive properties of nicotine were in impacted in $\alpha5$ nAChR subunit knockout mice suggests that $\alpha5$* nAChR-independent signaling mechanisms in the mHb-IPn circuit regulate nicotine reward (Jackson et al., 2010; Fowler et al., 2011, 2013; Grieder et al., 2017). In addition to $\alpha3$* and $\alpha5$* nAChRs, the IPn also contains the highest concentrations of $\alpha2$ nAChR subunit transcripts in the rodent brain (Wada et al., 1988). Allelic variation in *CHRNA2*, the gene that encodes the $\alpha2$ nAChR subunit, is associated with increased vulnerability to physical dependence on nicotine (Wang et al., 2014) and cannabis (Demontis et al., 2019). $\alpha2$ nAChR subunit knockout mice show higher levels of intravenous nicotine self-administration during the acquisition phase when nicotine-taking behavior is being established but not after stable intake has been established (Lotfipour et al., 2013). In the IPn, $\alpha2$ nAChRs incorporate into in $\beta2$* nAChRs primarily (Grady et al., 2009). Hence, $\alpha2\beta2$* nAChRs in the IPn are likely to regulate the acquisition of nicotine-taking behaviors.

H. Efferent Projections from IPn Regulate Nicotine Reward and Aversion

Intra-mHb infusion of the $\alpha3\beta4$* nAChR antagonists 18-MC or α-conotoxin AuIB blocked the stimulatory effects of nicotine but not morphine or *d*-amphetamine on dopamine release in the NAc shell (McCallum et al., 2012). Further, dopamine levels were elevated in the accumbens, and VTA dopamine cells appeared more sensitive to the stimulatory effects of nicotine in $\beta4$ nAChR subunit knockout mice compared with wild-type mice (Harrington et al., 2016). These dopamine-related effects were reversed by virus-mediated re-expression of $\beta4$ subunits in the IPn of the knockout mice (Harrington et al., 2016). This regulatory action of the mHb-IPn circuit over nicotine-induced increases in accumbal dopamine transmission may explain why manipulations of neurons in the mHb-IPn circuit can modify both the rewarding and aversive properties of nicotine (Jackson et al., 2010; Fowler et al., 2011, 2013; Frahm et al., 2015; Ables et al., 2017; Grieder et al., 2017). Unlike the LHb, the mHb does not provide any direct projections to midbrain dopamine neurons, nor does it project to the accumbens (Aizawa et al., 2012; Viswanath et al., 2014). Similarly, IPn neurons do not send projections to the VTA (Lima et al., 2017; Quina et al., 2017; Metzger et al., 2019). How then does the mHb-IPn circuit regulate dopamine transmission and the reward effects of nicotine? Recent findings have begun to shed some light on this issue. The IPn sends inhibitory and excitatory inputs from anatomically segregated subnuclei to the LDTg (Lima et al., 2017; Quina et al., 2017), a component of which arises from the $\alpha5^{Amigo1}$ GABAergic neurons in rostral IPn (Ables et al., 2017) (Fig. 5). IPn inputs to LDTg are predominately GABAergic, as expected considering the majority of IPn neurons synthesize GABA. However, a small component of IPn input to the LDTg is glutamatergic, likely reflecting efferent projections from the sparse populations of neurons in IPn that synthesize glutamate (Quina et al., 2017). The LDTg sends reciprocal GABAergic and cholinergic projections to the IPn (Bueno et al., 2019). Nicotine acts at presynaptic terminals to enhance GABAergic and glutamatergic transmission in the LDTg (Ishibashi et al., 2009), with at least a portion of these responses

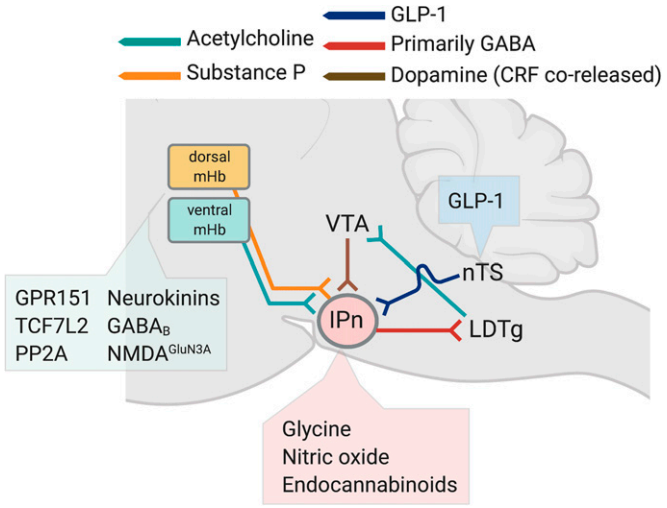

Fig. 5. Habenula-interpeduncular mechanisms of nicotine aversion. Neurons in dorsal and ventral mHb express genes implicated in nicotine aversion and other aversion-related behavioral states. These genes include the orphan G-protein coupled receptor (GPCR) GPR151, the transcription factor TCF7L2, the phosphatase PP2A, neurokinins and their receptors, $GABA_B$ receptors, and NMDA receptors that contain NR3A subunits. The IPn receives inputs from the nTS that release the neuropeptide GLP-1, which facilitates excitatory transmission in the IPn and thereby enhances nicotine aversion. The IPn also receives inputs from VTA neurons that release dopamine and CRF, both of which increase IPn neural activity. Excitatory transmission in the IPn derived from excitatory inputs is regulated by locally released glycine, nitric oxide, and endocannabinoids. The IPn sends an inhibitory projection to the LDTg that inhibits cholinergic projections from the LDTg to the VTA, which results in decreased activity of VTA dopamine neurons. See main text for further details.

presumably reflecting the actions of nicotine on IPn terminals in the LDTg. The LDTg also sends cholinergic projections to the VTA that regulate the tonic and phasic activity of local dopamine neurons through LDTg-derived cholinergic transmission acting act β2* nAChRs (Lodge and Grace, 2006; Mameli-Engvall et al., 2006; Chen and Lodge, 2013; Faget et al., 2016) (Fig. 5). Hence, LDTg cholinergic projections to the VTA control VTA-derived dopamine release in the accumbens (Blaha et al., 1996; Forster and Blaha, 2000). LDTg cholinergic, GABAergic, and glutamatergic afferents can also bypass the VTA to directly innervate the accumbens (Dautan et al., 2014; Coimbra et al., 2019). These LDTg inputs to the VTA and accumbens play major roles in controlling reward- and aversion-related behaviors (Dautan et al., 2016; Xiao et al., 2016; Steidl et al., 2017b; Coimbra et al., 2019) in a manner that depends on accumbal dopamine transmission (Steidl and Veverka, 2015; Steidl et al., 2017a). Moreover, the LDTg-VTA circuit is known to regulate behavioral responses to nicotine (Alderson et al., 2005; Maskos, 2008; Ishibashi et al., 2009). Hence, the MHb-IPn circuit may influence mesoaccumbens dopamine transmission through an indirect mechanism that involves IPn projections to the LDTg. Consistent with this possibility, nicotine stimulates β2* nAChRs on IPn neurons to increase inhibitory GABAergic transmission in LDTg neurons (Wolfman et al., 2018). Moreover, optical stimulation of IPn terminals in the LDTg elicited avoidance behaviors, whereas optical inhibition of the IPn-LDTg circuit attenuated nicotine aversion (Wolfman et al., 2018). These findings suggest that an mHb-IPn-LDTg-VTA circuit regulates nicotine reward and aversion, in part by modulating mesoaccumbens dopamine transmission.

In addition to the LDTg and raphe nuclei, the IPN also projects to other hindbrain sites, including the dorsolateral tegmental nucleus and nucleus incertus (Smaha and Kaelber, 1973; Shibata and Suzuki, 1984; Goto et al., 2001; Olucha-Bordonau et al., 2003). The IPn also provides ascending projections to septal and hippocampal sites, although whether IPn neurons are the source of these ascending projections has been questioned (Quina et al., 2017). There is a small population of serotonergic (5-hydroxytryptophan) neurons in apical regions of the IPn (IPn^{5HT} cells) known to provide ascending input to the hippocampus and septum (Groenewegen and Steinbusch, 1984; Sherafat et al., 2020), which are activated by aversive stimuli (Chen et al., 2003). Chemogenetic inhibition of the IPn^{5HT} cells that project to ventral hippocampus (IPn^{5HT}-VH cells) enhanced escape behaviors and increased sucrose intake in mice (Sherafat et al., 2020). However, chemogenetic inhibition of IPn^{5HT}-VH cells did not alter responding for intravenous nicotine infusions in mice even when a higher unit dose of nicotine on the descending portion of the dose-response curve was available (Sherafat et al., 2020). This suggests that IPn^{5HT}-VH cells are not involved in regulating nicotine intake, although it is unclear whether projections from IPn^{5HT} cells to other addiction-relevant brain sites may be involved. It is important to note that the apical portion of the IPn, which contains serotonergic and other ascending projections to limbic brain regions is functionally and anatomically distinct from the IPn proper (Quina et al., 2017). It has been argued that this apical region may in fact represent an extended portion of the median raphe nucleus (Quina et al., 2017). If so, the serotonergic neurons in apical IPn regions may not be participate in behaviors directly regulated by the mHb-IPn circuit. As noted above, the IPn sends dense projections to the nucleus incertus in the hindbrain. Indeed, it was shown that $\alpha5^+$ IPn neurons send GABAergic projections to the nucleus incertus (Morton et al., 2018), and the sparse population of IPn glutamatergic neurons also project to this site (Quina et al., 2017). Nucleus incertus neurons in turn project to the hippocampus and elsewhere in the brain (Goto et al., 2001; Szőnyi et al., 2019). Optogenetic stimulation of nucleus incertus neurons that project to hippocampus blocked the encoding of fear memories in mice, whereas their

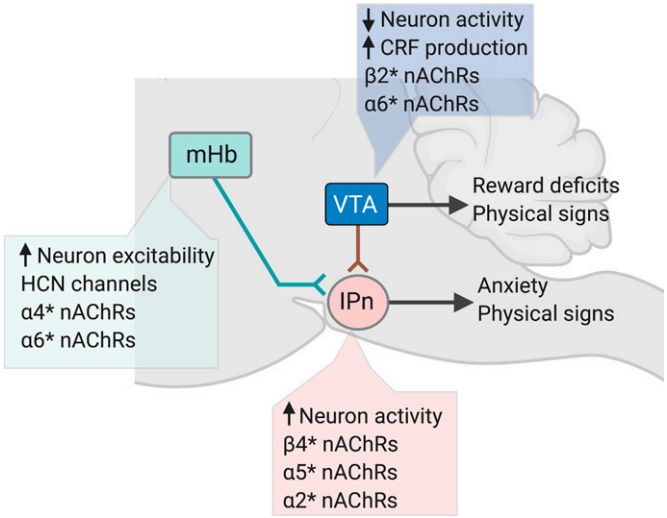

Fig. 6. VTA, habenula, and interpeduncular nucleus contributions to nicotine withdrawal. Neurons in ventral mHb show increased excitability after chronic nicotine treatment, and inhibitors of HCN pacemaker channels can precipitate withdrawal in nicotine-dependent animals. In addition, α4* and α6* nAChR function in the mHb is upregulated in nicotinedependent animals. In the IPn, nicotine withdrawal is associated with increased activity of local GABAergic neurons that express somatostatin, and upregulated expression/function of β4*, α5*, and α2* nAChRs is thought to contribute to the expression of nicotine withdrawal. In the VTA, nicotine withdrawal is associated with decreased activity of dopamine neurons that project to the nucleus accumbens, increased production and release of the stress hormone CRF, and upregulated expression/function of β2*, α6* nAChRs.

inhibition markedly enhanced the encoding of fear memories (Szőnyi et al., 2019). This suggests that bidirectional modulation of nucleus incertus activity by inhibitory and excitatory inputs from the IPn potentially contributes to the rewarding and aversive actions of nicotine, respectively, a possibility that has not yet been explored.

I. Afferent Projections to IPn Regulate Nicotine Reward and Aversion

The IPn receives inputs from brain sites other than the mHb known to encode reward and aversion states and likely to influence behavioral responses to nicotine. Recent evidence suggests that dopamine neurons in the VTA project to the IPn (VTA-IPn neurons) (Molas et al., 2017; DeGroot et al., 2020) (Fig. 5). Optogenetic stimulation of VTA-IPn neurons decreased anxiety-related behaviors in mice (DeGroot et al., 2020). Similarly, infusion of a D1 dopamine receptor agonist into the IPn decreased anxiety-related behaviors whereas a D1 receptor antagonist had the opposite effect (DeGroot et al., 2020). Hence, nicotine acting on VTA dopamine neurons that project to the IPn, potentially through α5* nAChRs expressed by these cells, may contribute to its well known anxiety-promoting actions and other aversive responses that promote nicotine avoidance (File et al., 1998, 2000; Ouagazzal et al., 1999a,b; Kenny et al., 2000). In addition to the mHb-IPn circuit, α5, α3, and α4 nAChR subunit transcripts are also densely expressed by neurons in the nTS. Nicotine dose-dependently activates nTS neurons, as reflected by increases in cFos immunoreactivity, with this effect most apparent in nTS neurons that express the neuropeptide glucagon-like peptide-1 (GLP-1) (Tuesta et al., 2017). Using a line of *Chrna5*-tdTomato reporter mice, GLP-1 neurons were shown to express α5 nAChR subunits (Tuesta et al., 2017). Systemic administration of the GLP-1 receptor agonist exendin-4 or the dipeptidyl peptidase 4 inhibitor sitagliptin, which inhibits GLP-1 breakdown, decreased nicotine self-administration in mice (Tuesta et al., 2017). Using transgenic mice that express Cre recombinase in GLP-1 neurons in nTS, it was shown that chemogenetic stimulation of these cells reduced nicotine self-administration (Tuesta et al., 2017). Conversely, GLP-1 receptor knockout mice self-administered greater quantities of nicotine than their wild-type littermates (Tuesta et al., 2017). Some of the highest densities of GLP-1 receptor binding sites in the brain are detected in the IPn (Goke et al., 1995), suggesting that GLP-1–expressing nTS neurons project to the IPn and thereby regulate nicotine intake. Indeed, GLP-1–immunoreactive fibers were detected in the IPn of mice. Furthermore, optical stimulation of the terminals of GLP-1 neurons in the IPn enhanced local excitatory transmission through a mechanism involving increased glutamate release from mHb inputs (Tuesta et al., 2017) (Fig. 5). Consistent with this mechanism of action, infusion of exendin-4 into the IPn or habenula decreased nicotine intake, whereas IPn infusion of the GLP-1 receptor antagonist exendin-(9-39)-amide or shRNA-mediated knockdown of GLP-1 receptor mRNA in the mHb-IPn circuit increased nicotine self-administration in rats (Tuesta et al., 2017). GLP-1 neurons are known to be activated by gastric expansion to induce feelings of satiety and trigger cessation of food consumption (van Bloemendaal et al., 2014). GLP-1 neurons also regulate feelings of malaise and nausea when food is consumed past satiety (Chang and Scott, 1984; Hayama et al., 1985; Scott et al., 1986; Garcia-Diaz et al., 1988; Monnikes et al., 1997; Willing and Berthoud, 1997; Rinaman et al., 1998; Gutzwiller et al., 1999; Schwartz, 2000; Qin et al., 2005; Appleyard et al., 2007; Bello and Moran, 2008). Hence, it was proposed that activation of GLP-1 neurons by nicotine engages IPn circuits to elicit satiety-like responses to the drug that promote nicotine avoidance behaviors and thereby terminate nicotine self-administration (Tuesta et al., 2017).

J. Other Reward and Aversion Signaling Mechanisms in the mHb-IPn Circuit

In addition to nAChRs, other receptors and signaling mechanisms in the mHb-IPn circuit have been shown to regulate behavioral responses to nicotine. As described above, GLP-1 facilitates excitatory transmission in the mHb-PN circuit to promote nicotine avoidance behaviors (Tuesta et al., 2017). GLP-1 receptors

are G-protein–coupled receptors (GPCRs) that, when activated, enhance the production of cAMP. In pancreatic β cells, GLP-1–enhanced cAMP signaling results in the phosphorylation and nuclear translocation of β-catenin, which dimerizes with the transcription factor TCF7L2, and TCF7L2 is considered a core component the GLP-1 signaling cascade (Yi et al., 2005; Liu and Habener, 2008; Vazquez-Roque et al., 2011; Chiang et al., 2012; Shao et al., 2013). TCF7L2 is densely expressed in mHb cholinergic neurons (Duncan et al., 2019). Using a line of genetically modified rats that express a loss-of-function TCF7L2 variant, it was shown that TCF7L2 deficiency markedly reduced aversive behavioral responses to nicotine and increased nicotine self-administration behavior (Duncan et al., 2019). Similarly, CRISPR/CRISPR-associated protein 9–mediated genomic cleavage of the *Tcf7l2* gene in the mHb of mice or shRNA-mediated knockdown of TCF7L2 mRNA transcripts in the mHb of rats increased nicotine self-administration behavior (Duncan et al., 2019). The stimulatory effects of nicotine on mHb inputs to the IPn were diminished in the TCF7L2-deficient rats, as reflected by reductions in mHb-derived excitatory transmission in IPn slices evoked by nicotine (Duncan et al., 2019). The diminished sensitivity of the mHb-IPn circuit to nicotine was explained by a deficit in the function but not the expression of nAChRs in mHb neurons (Duncan et al., 2019). Deficient TCF7L2-mediated transcriptional activity in the mHb of the TCF7L2 mutant rats resulted in striking deficits in the production of cAMP in mHb neurons, which reduced activity of the cAMP-regulated protein kinases that positively regulate nAChR function in mHb neurons (Huganir et al., 1986; Paradiso and Brehm, 1998; Giniatullin et al., 2005). This abnormality in cAMP signaling explained the deficits in habenular nAChR function and the elevated nicotine intake seen in TCF7L2-deficient animals (Duncan et al., 2019). These findings highlight the importance of cAMP and likely other intracellular signaling processes that regulate nAChR function in the mHb-IPn pathway in the reinforcing actions to nicotine.

GPR151 is orphan GPCR that is highly enriched in habenular neurons in rodents and humans, particularly mHb neurons that coexpress α3* nAChRs (Quina et al., 2009; Kobayashi et al., 2013; Broms et al., 2015; Wagner et al., 2016; Broms et al., 2017; Antolin-Fontes et al., 2020). The stimulatory effects of nicotine on mHb neurons are attenuated in GPR151 knockout mice (Antolin-Fontes et al., 2020). The knockout mice also had attenuated aversive behavioral responses to nicotine and self-administered greater numbers of intravenous nicotine infusions than wild-type mice, particularly when a higher unit dose on the descending portion of the dose-response curve was available (Antolin-Fontes et al., 2020). GPR151 appears to be negatively coupled to adenylyl cyclase activity, as levels of cAMP were elevated in mHb tissue collected from GPR151 knockout mice compared with wild-type mice (Antolin-Fontes et al., 2020). Hence, GPR151 may modulate behavioral responses to nicotine in a manner similar to TCF7L2 by regulating the cAMP-dependent kinases that control nAChR function in the mHb-IPn circuit. GPR151 is thought to be expressed almost exclusively on the terminals of mHb neurons in the IPn (Antolin-Fontes et al., 2020). Hence, GPR151 may influence nicotine intake by regulating the function of presynaptically located nAChRs on mHb terminals and the neurotransmitter release machineries in mHb terminals known to be controlled by cAMP-dependent signaling processes (Azhderian et al., 1994; Chen and Regehr, 1997; Duncan et al., 2019).

GPR139 is another orphan GPCR that is densely expressed in the mHb and is also found in the LHb, septum, and striatum (Liu et al., 2015; Wagner et al., 2016). Allelic variation in the *GPR139* gene influences body mass index and blood pressure in a manner that depends on smoking status (current smoker, ex-smoker, or nonsmoker) (Justice et al., 2017; Sung et al., 2018). GPR139 modulates μ opioid receptor function in the mHb to regulate the rewarding actions of opioids (Wang et al., 2019) through a mechanism that likely involves regulation of cAMP signaling dynamics in mHb neurons (Stoveken et al., 2020). The role for GPR139 in regulating the motivational properties of nicotine has not yet been explored. However, the novel GPR139 agonist TAK-041 blocks the stimulatory effects of nicotine on accumbal dopamine release (Schiffer et al., 2020), suggesting that GPR139 located in the mHb-IPn circuit and striatum may modulate the rewarding and aversive actions of nicotine, a possibility that awaits further investigation.

Phosphodiesterase 2A (PDE2A) metabolizes cAMP and is robustly expressed by neurons in the mHb and IPn as well as the hippocampus, striatum, globus pallidus, and substantia nigra (Stephenson et al., 2009, 2012). Atrial natriuretic peptide activates PDE2A in mHb neurons, thereby inhibiting the release of glutamate into the IPn (Hu et al., 2012). This inhibitory action of atrial natriuretic peptide on mHb-derived glutamatergic transmission in the IPn was prevented by PDE2A inhibition or by a chemical activator of cAMP-regulated protein kinase A (Hu et al., 2012). These findings further highlight the importance of cAMP signaling in regulating mHb-IPn circuit function and suggest that PDE2A modulators that influence mHb-IPn circuit responses to nicotine may represent a novel class smoking-cessation therapeutics.

As described above, $\alpha5\beta2$* nAChRs enhance neurokinin signaling in the mHb, which increases the intrinsic excitability of mHb neurons (Dao et al., 2014). The source of neurokinins in this action is unclear but may

derive from noncholinergic neurons in dorsal mHb that synthesize substance P (neurokinin A) and project to lateral IPn (Contestabile et al., 1987; Qin and Luo, 2009; Aizawa et al., 2012; Melani et al., 2019). Substance P–expressing mHb neurons in dorsal mHb that project to lateral IPn also express CB_1, $GABA_B$, and glycine receptors, all of which regulate the encoding or extinction of fear memories (Soria-Gomez et al., 2015; Zhang et al., 2016; Melani et al., 2019) (Fig. 5). Stimulation of neurons in dorsal mHb neurons increased excitatory glutamatergic transmission in the lateral IPn, which in turn stimulated local inhibitory glycine-containing neurons in the IPn (Melani et al., 2019) (Fig. 5). Glycine released in this manner is thought to serve a negative feedback role by counterbalancing the local actions of mHb-derived glutamate and thereby opposing glutamate-driven long-term synaptic potentiation in the IPn (Melani et al., 2019). Strychnine-mediated inhibition of glycinergic transmission in lateral IPn disrupted this counter-regulatory response and enhanced the release of glutamate from mHb terminals, which in turn facilitated the induction of long-term synaptic potentiation at the dorsal mHb-lateral IPn synapse (Melani et al., 2019). This synaptic potentiation was blocked by neurokinin 1 receptor antagonism (Melani et al., 2019), suggesting that glycine released from lateral IPn neurons acts by inhibiting substance P and glutamate corelease from mHb terminals in the IPn (Melani et al., 2019). Injection of a neurokinin 1 receptor antagonist into lateral IPn, which blocked the actions of substance P released by dorsal mHb inputs, impaired the ability of mice to extinguish fear memories (Melani et al., 2019), suggesting that dorsal mHb–lateral IPn synapse controls the processing and gradual extinction of behavioral responses to aversive stimuli. Notably, CB_1 receptor antagonism also blocked activity-dependent synaptic potentiation at the dorsal mHb-lateral IPn synapse, an effect mimicked by a $GABA_B$ receptor antagonist (Melani et al., 2019) (Fig. 5). Conditional deletion of CB_1 receptors from mHb neurons decreased fear-conditioned freezing behavior and abolished conditioned odor aversion in mice but did not alter behavioral responses to neutral or appetitive stimuli Soria-Gomez et al. (2015). These findings suggest that glycine, endocannabinoid, and neurokinin transmission in the IPn regulate the acquisition, expression, and extinction of behavioral responses to aversive stimuli (Fig. 5). Interestingly, mHb neurons express an uncommon NMDA receptor subtype that contains GluN1/GluN3A subunits, which are activated by glycine (Otsu et al., 2019). Activation of these GluN1/GluN3A NMDA receptors by glycine increases the activity of mHb neurons, which triggers avoidance-related behaviors (Otsu et al., 2019) (Fig. 5). It is likely that glycine, endocannabinoid, and neurokinin transmission in lateral IPn regulate aversive responses to nicotine in a manner that involves GluN1/GluN3A NMDA receptors on the terminals of mHb neurons. This possibility has not yet been explored.

K. Nicotine Actions in the Periphery Impact Reward and Aversion Behaviors

The nTS is the major site to which the vagus nerve (cranial nerve X) delivers sensory information to the brain from peripheral organs involved in processing the interoceptive actions of nicotine contained in cigarette smoke (Ogawa et al., 1984; Hines et al., 1994). This raises the possibility that nicotine acts not only at nAChRs located directly in the nTS but also at nAChRs located in peripheral organs, with nicotine-related sensory information contributing to nTS activation and nicotine avoidance behaviors. Indeed, nAChRs are expressed in tissues that come into direct contact with nicotine in tobacco smoke, including nAChRs in the mouth and lungs. Sensory information related to the actions of nicotine in the oral cavity, airways, and elsewhere in the body (Cain, 1980; Ahijevych et al., 2015) activate nTS neurons through vagal inputs (Lemon and Smith, 2005; Simons et al., 2006). Nicotine-related sensory information is then relayed from the nTS to higher-order midbrain and corticothalamic circuits involved in reward and avoidance behaviors. This nicotine-related sensory information contributes to the interoceptive properties of tobacco smoke that smokers use to titrate their intake to avoid its aversive properties (Iwasaki and Sato, 1981; Feyerabend et al., 1982; Rose et al., 1993; Pritchard et al., 1996; Dahl et al., 1997; Ming et al., 1998; Fowler and Kenny, 2014). Nicotine acting peripherally can also precipitate craving in smokers during periods of abstinence (Naqvi et al., 2007; Gray and Critchley, 2007; Naqvi and Bechara, 2010; Fowler and Kenny, 2014). Moreover, blocking the sensory properties of nicotine in cigarette smokers attenuates the rewarding properties of cigarettes and decreases smoking behavior (Rose et al., 1985, 1993, 2016; McClernon et al., 2016). In rodents, actions of nicotine outside the brain contribute to many of the behavioral and physiologic responses to the drug (Kiyatkin, 2014). Despite the considerable evidence linking peripheral actions of nicotine to tobacco use disorder, remarkably little is known about the underlying mechanisms of such brain-body interactions. However, a number of groups have used the modified nicotine analog nicotine pyrrolidine methiodide (nicotine-PM), which is a full nAChR agonist that does not cross the blood-brain barrier (Lenoir and Kiyatkin, 2011; Kiyatkin, 2014) to map brain circuits and cells activated by nicotine acting at peripheral organs (Dehkordi et al., 2015; Rose et al., 2016). It was found that nicotine and nicotine-PM increased cFos immunoreactivity in overlapping brain regions, including the IPn and other sites involved in the reward and aversion, such as the LDTg, PPTg, LHb, accumbens, and VTA (Dehkordi et al., 2015; Rose et al., 2016). Within the VTA, GABAergic but not dopaminergic

cells were almost exclusively activated by a higher aversive but not by a lower rewarding dose of nicotine (Dehkordi et al., 2018), suggesting that these cells may transduce aversive sensory properties of peripherally acting nicotine. Further studies will be required to explore this possibility and to determine whether nTS inputs to VTA IPn, LDTg, PPTg, LHb, or accumbens explain their sensitivity to the peripheral actions of nicotine.

VI. Mechanisms of Nicotine Withdrawal

A. Nicotine Withdrawal Syndrome in Humans and Rodents

Tobacco use disorder depends not only on the rewarding properties of nicotine that underlie its positive reinforcing properties but also on escape from the aversive consequences of nicotine withdrawal that motivate tobacco smoking according to negative reinforcement processes (Doherty et al., 1995; Kenny and Markou, 2001). Chronic nicotine exposure results in the development of a dependent state in smokers such that cessation of intake elicits an aversive nicotine withdrawal syndrome (Shiffman and Jarvik, 1976; Hughes et al., 1991). This withdrawal syndrome is attenuated by nicotine replacement therapy (Schneider and Jarvik, 1984; Fagerstrom et al., 1993; Molander et al., 2000), confirming that nicotine plays a major role in its induction and expression. Conversely, smoking cigarettes with reduced nicotine content can elicit a withdrawal syndrome in smokers that is accompanied by a reduction in plasma nicotine levels (West et al., 1984). The duration and severity of the nicotine withdrawal syndrome in abstinent smokers accurately predict their likelihood of relapse to tobacco use (Piasecki et al., 1998, 2000, 2003). Conversely, the efficacy of nicotine replacement therapy in certain individuals is related to its ability to prevent the onset and reduce the duration of nicotine withdrawal (Fagerstrom, 1988; Sachs and Leischow, 1991). Hence, it is important to understand the brain mechanisms that regulate nicotine withdrawal–associated aversive states.

Nicotine withdrawal in abstinent smokers is comprised of physical and affective components. The most common physical symptoms include bradycardia and gastrointestinal discomfort, suggesting that autonomic nervous system function is disrupted in smokers during withdrawal (Niedermaier et al., 1993). Affective symptoms primarily include irritability, depressed mood, anxiety, difficulty concentrating, and craving (Parrott, 1993; Doherty et al., 1995; Kenny and Markou, 2001). Escape from affective components of withdrawal is thought to play a particularly important role in the maintenance nicotine dependence (Koob and Le Moal, 1997; Markou et al., 1998; Kenny and Markou, 2001). Nicotine withdrawal is associated with physical and affective abnormalities in laboratory rodents. Withdrawal from nicotine and other drugs of abuse is thought to reflect compensatory adaptations in the same neurobiological substrates that regulate acute responses to the drug (Koob and Bloom, 1988; Koob and Le Moal, 1997). Thus, it is likely that the same nAChRs that regulate the rewarding and aversive effects of acutely administered nicotine undergo adaptations upon prolonged exposure to nicotine, which contributes to the development of nicotine dependence and the expression of withdrawal-associated physical and affective components of nicotine withdrawal (Kenny and Markou, 2001).

B. β2 nAChRs Regulate Affective but Not Physical Components of Nicotine Withdrawal*

Spontaneous withdrawal from nicotine, accomplished by the surgical removal of subcutaneously implanted osmotic minipumps that delivered nicotine continuously for >7 days, was associated with the expression of a physical withdrawal syndrome in rats (Malin et al., 1992; Epping-Jordan et al., 1998). Systemic administration of mecamylamine precipitated physical withdrawal signs in nicotine-dependent rats (Watkins et al., 2000). Systemic or intracerebroventricular administration of the nAChR antagonist chlorisondamine, which does not readily cross the blood-brain barrier, also precipitated physical withdrawal signs in nicotine-dependent rats (Watkins et al., 2000). These findings suggest that nAChRs in the brain regulate physical withdrawal from nicotine. They also suggest that peripheral ganglionic nAChRs located outside the brain contribute to the physical withdrawal syndrome. Indeed, expression of physical withdrawal signs in rats undergoing spontaneous nicotine withdrawal were attenuated by the peripherally acting nAChR agonist tetramethylammonium (Hildebrand et al., 1997). By contrast, β2* nAChR-preferring antagonist DHβE did not precipitate physical withdrawal signs in nicotine-dependent rats (Epping-Jordan et al., 1998). This suggests that β2* nAChRs in the brain are unlikely to contribute to physical dependence on nicotine (see Fig. 6).

In addition to a physical syndrome, spontaneous and antagonist-precipitated nicotine withdrawal is also associated with an affective withdrawal syndrome in rats and mice, which can be measured by elevations of ICSS reward thresholds, the induction of a conditioned place avoidance (CPA), decrements in sucrose consumption, increases in anxiety-related behaviors, and other behavioral abnormalities consistent with the manifestation of a negative affective state (see Kenny and Markou, 2001; Fowler et al., 2008). In nicotine-dependent rats, spontaneous withdrawal or withdrawal precipitated by mecamylamine or DHβE, elevated ICSS thresholds (Ivanova and Greenshaw, 1997; Epping-Jordan et al., 1998; Watkins et al., 2000; Harrison et al.,

2002). Similarly, systemic administration of chlorisondamine at doses unlikely to cross the blood-brain barrier or its direct intracerebroventricular administration also elevated ICSS thresholds in nicotine-dependent rats (Epping-Jordan et al., 1998; Watkins et al., 2000). This suggests that nAChRs located in brain reward circuits, including β2* nAChRs, are contribute to the development of "affective dependence" on nicotine. Surprisingly, ganglionic nAChRs located outside the brain may also contribute to this process, although very little is known about the contribution of peripherally located nAChRs to affective components of withdrawal.

β2* nAChR subunit knockout mice, which are insensitive to the rewarding properties of nicotine (see above), demonstrated a robust physical nicotine withdrawal syndrome (Besson et al., 2006), but withdrawal-induced elevations in ICSS thresholds were absent in these animals (Stoker et al., 2015). Withdrawal-induced increases in anxiety-related behaviors and the establishment of a CPA for an environment paired with antagonist-precipitated nicotine withdrawal were also absent in β2 knockout mice but readily apparent in wild-type mice (Jackson et al., 2008). Deficits in fear conditioning typically observed in wild-type mice experiencing nicotine withdrawal were also attenuated in β2 knockout mice (Portugal et al., 2008; Raybuck and Gould, 2009). Most recently, it was shown that β2 subunit knockout mice were resistant to nicotine withdrawal-induced deficits in sucrose consumption and increases in anxiety-related behaviors (Alkhlaif et al., 2017). Hence, β2* nAChRs regulate affective but not physical signs of nicotine withdrawal (see Fig. 6).

C. Other nAChR Subtypes Contribute to Affective Components of Nicotine Withdrawal

Similar to β2 nAChR subunit knockout mice, β3 and α6 nAChR subunit knockout mice showed attenuated sucrose drinking and anxiety-related behavioral abnormalities during nicotine withdrawal, whereas physical withdrawal signs were unaltered in these animals (Alkhlaif et al., 2017; Jackson et al., 2019). Furthermore, an α6* nAChR-selective antagonist blocked the expression of a nicotine withdrawal-induced CPA and withdrawal-associated increases in anxiety-related behavior but did not modify the expression of physical withdrawal signs in mice (Jackson et al., 2009). These findings suggest that the same $\alpha6\beta2\beta3$* nAChR subtype known to regulate the stimulatory effects of nicotine on striatal dopamine release (see above) is likely to undergo nicotine-induced adaptations in function and expression during chronic nicotine exposure, which contributes to the development of affective but not physical dependence on nicotine. The role for α7 nAChRs in affective components of nicotine withdrawal is less clear. Deficits in context-related encoding of fear memories typically seen in mice experiencing nicotine withdrawal were unaltered in α7 knockout mice, whereas similar withdrawal-related cognitive deficits were absent in β2 subunit knockout mice (Portugal et al., 2008). The α7 nAChR antagonist MLA did not precipitate a CPA or other affective signs of withdrawal in nicotine-dependent mice (Markou and Paterson, 2001; Jackson et al., 2008). Nor did MLA precipitate withdrawal-related cognitive deficits in nicotine-dependent rats (Shoaib and Bizarro, 2005). The novel α7 nAChR partial agonist encenicline did not attenuate nicotine withdrawal-associated cognitive deficits in abstinent human smokers, as reflected by similar deficits in treated and untreated abstinent smokers when tested using the Conners Continuous Performance Task to assess attention and response inhibition and using the N-Back task to measure working memory (Schuster et al., 2018). Also, the α7 nAChR agonist ABT-107 did not attenuate cognitive deficits (encoding of fear memories) detected in mice undergoing nicotine withdrawal (Yildirim et al., 2015). However, the α7 nAChR agonist PNU282987 attenuated nicotine withdrawal–induced increases in anxiety-related behavior in mice (Jackson et al., 2018). Furthermore, the deficits in attention typically seen in wild-type mice undergoing nicotine withdrawal were absent in α7 knockout mice (Higa et al., 2017). Infusion of MLA into the VTA of nicotine-dependent rats decreased accumbal dopamine release in a manner similar to mecamylamine infusion (Nomikos et al., 1999), suggesting that α7 nAChRs may contribute to deficits in dopamine transmission during nicotine withdrawal (Nomikos et al., 2000). Together, these findings suggest that α7 nAChRs may play a role in affective components of nicotine withdrawal, but its precise contributions remain unclear (see Fig. 6).

D. Dopamine Transmission Contributes to Affective Components of Nicotine Withdrawal

Consistent with a role for mesoaccumbens dopamine transmission in nicotine withdrawal, extracellular dopamine levels were markedly reduced in rats undergoing nicotine withdrawal compared with nicotine-naıve animals (Hildebrand et al., 1998), opposite to the stimulatory effects of acutely administered nicotine on accumbal dopamine release (see above). Nicotine withdrawal–related deficits in accumbens dopamine release are less severe in adolescent than adult rats (Natividad et al., 2010), which may explain the attenuated withdrawal-related behaviors seen in adolescent versus adult rats (O'Dell et al., 2004, 2007). Mecamylamine delivered into the VTA decreased extracellular dopamine levels in the accumbens and precipitated withdrawal-like physical signs in nicotine-dependent but not nicotine-naıve control rats (Hildebrand et al., 1999). Similarly, infusion of DHβE into the VTA (Bruijnzeel and Markou, 2004) but not the central nucleus of the amygdala or bed nucleus of the stria terminalis (Jonkman and Markou, 2006) precipitated withdrawal-associated elevations of ICSS thresholds in nicotine-dependent but not control rats. Hence, the VTA serves as a key

neuroanatomical substrate in the regulation of affective components of nicotine withdrawal.

In contrast to the decreased dopamine release detected in the NAc during nicotine withdrawal, dopamine levels were dramatically increased in the mPFC during withdrawal (Carboni et al., 2000a). This effect is similar to the increased cortical dopamine transmission seen in rodents undergoing withdrawal from opioids (Bozarth, 1994; Bassareo et al., 1995; Espejo et al., 2001). Hence, it is unlikely that the reward deficits and other affective components of withdrawal from nicotine and other major drugs of abuse reflect a generalized state of depressed activity of midbrain dopamine neurons. Instead, the activity of some populations of dopamine neurons are decreased, whereas the activity of others is increased. This likely reflects the same type of partitioning of withdrawal-relevant dopamine neurons in the VTA into functional domains as described above for the reward-related actions of nicotine. Hence, it is an interesting possibility that withdrawal from nicotine and other drugs of abuse is associated with decreased activity of VTA^{LAT} dopamine neurons that project to the NAc shell and increased activity of VTA^{MED} dopamine neurons that project to the mPFC.

A population of dopamine neurons that express corticotropin-releasing factor (CRF) has been identified in the posterior VTA (Grieder et al., 2014). Chronic nicotine treatment upregulated CRF expression in these VTA dopamine neurons, which blocked the stimulatory effects nicotine on local GABAergic transmission in the VTA (Grieder et al., 2014). Blockade of CRF signaling in the VTA during nicotine withdrawal restored the stimulatory actions of nicotine on VTA GABA transmission and attenuated affective components of nicotine withdrawal in rats (Grieder et al., 2014). Notably, the majority of these CRF-positive neurons were located in posterior regions of the VTA immediately adjacent to the IPn (Grieder et al., 2014). Moreover, the increased CRF mRNA levels detected in the VTA of nicotine-dependent rats coincided with decreased CRF immunoreactivity in the IPn of the same animals (Grieder et al., 2014). This suggests that posterior VTA neurons that synthesize CRF may project to the IPn, a circuit known to regulate anxiety-related behaviors in rodents and release CRF into the IPn to drive affective components of nicotine withdrawal. Consistent with this possibility, it was shown that increased anxiety-related behaviors in mice during nicotine withdrawal were associated with increased activity of neurons located in the intermediate nucleus of the IPn, in which $\alpha5^{Epyc}$ neurons are known to be located (see above) (Zhao-Shea et al., 2015). This effect was regulated by CRF derived from VTA inputs (Zhao-Shea et al., 2015). Furthermore, blockade of CRF transmission in or optogenetic inhibition of mHb inputs to the intermediate nucleus of the IPn attenuated nicotine withdrawal–induced increases in anxiety-related behavior (Zhao-Shea et al., 2015). Further supporting an important role for the IPn in nicotine withdrawal-related increases in anxiety-related behavior, it was shown that chronic nicotine treatment increased the sensitivity of nAChRs in the mHb to nicotine (Pang et al., 2016; Arvin et al., 2019), with habenular $\alpha4$* and $\alpha6$* nAChRs located in the ventroinferior portion of the mHb being particularly sensitive to this action (Shih et al., 2015; Pang et al., 2016). Moreover, pharmacological blockade of $\alpha4\beta2$* or $\alpha6\beta2$* nAChRs but not $\alpha3\beta4$* nAChRs in the mHb alleviated nicotine withdrawal-related increased in anxiety-related behaviors in mice (Pang et al., 2016). Most recently, it was shown that a history of intravenous nicotine self-administration similarly upregulated nAChR function in the mHb of rats (Jin et al., 2020). Finally, it was shown that a metabotropic glutamate 2/3 receptor antagonist infused systemically or directly into the VTA attenuated the elevations of ICSS thresholds in rats undergoing spontaneous nicotine withdrawal (Kenny et al., 2003). These findings suggest that adaptive responses in $\beta2$*, $\beta3$*, and $\alpha6$* nAChRs in the VTA, leading to alterations in dopamine, CRF, GABA, and glutamate signaling in the accumbens, IPn, and other reward and aversion relevant brain sites, drives the expression of affective but not physical components of nicotine withdrawal.

E. β4 nAChRs Regulate Physical but Not Affective Components of Nicotine Withdrawal*

$\beta4$* nAChRs appear to regulate physical not affective signs of nicotine withdrawal, opposite to the contributions of $\beta2$* nAChRs described above. Indeed, $\beta4$ nAChR subunit knockout mice had decreased physical signs in response to spontaneous or mecamylamine-precipitated nicotine withdrawal (Salas et al., 2004b; Stoker et al., 2012). However, mecamylamine-precipitated nicotine withdrawal elevated ICSS thresholds by a similar magnitude in wild-type and $\beta4$ subunit knockout mice (Stoker et al., 2012), although the onset of threshold elevations during spontaneous nicotine withdrawal was delayed in $\beta4$ knockout mice relative to wild-type mice (Stoker et al., 2012). Similar to $\beta4$ knockout mice, $\alpha5$ subunit knockout mice also demonstrated a loss of nicotine withdrawal–induced physical withdrawal signs compared with wild-type mice (Jackson et al., 2008; Salas et al., 2009). By contrast, withdrawal-induced increases in anxiety-related behaviors were unaltered in $\alpha5$ knockout mice (Jackson et al., 2008). The role for $\alpha2$ nAChRs in nicotine withdrawal is complex, however, as one study reported that physical withdrawal signs were attenuated in $\alpha2$ nAChR subunit knockout mice (Salas et al., 2009), and another reported that physical withdrawal was exacerbated in these animals (Lotfipour et al., 2013). The role for $\alpha7$ nAChRs in physical aspects of nicotine withdrawal is also unclear.

Some studies have shown that physical withdrawal signs were attenuated in α7 subunit knockout mice (Salas et al., 2007), whereas others have shown no difference between knockout and wild-type mice (Stoker et al., 2012). The α7 nAChR antagonist MLA did not precipitate physical signs of withdrawal in nicotine dependent mice (Markou and Paterson, 2001; Jackson et al., 2008). However, the α7 nAChR agonist PNU282987 attenuated nicotine withdrawal–induced increases in anxiety-related behaviors and physical withdrawal signs in mice (Jackson et al., 2018). Overall, these findings suggest that β4* and α5* nAChRs and perhaps α2* and α7 nAChRs regulate physical dependence on nicotine.

F. nAChR Signaling in mHb-IPn Circuit Regulates Nicotine Withdrawal

Considering the high concentrations of β4, α5, and α2 nAChR subunits in the mHb-IPn circuit, it is perhaps not surprising that this circuit has been implicated in physical components of the nicotine-withdrawal syndrome. Direct infusion of mecamylamine into the mHb or IPn but not the cortex, VTA, or hippocampus precipitated physical withdrawal signs and increased anxiety-related behaviors in nicotine-dependent mice (Salas et al., 2009; Zhao-Shea et al., 2013). Mecamylamine infused into the IPn also increased physical signs of withdrawal and anxiety-related behaviors in nicotine-dependent male and female rats (Correa et al., 2019). The onset of nicotine withdrawal was associated with increased activity of IPn GABAergic neurons, as reflected by increased cFos expression and spontaneous excitatory currents in IPn neurons (Zhao-Shea et al., 2013). Pharmacological blockade of mHb-derived glutamatergic transmission in the IPn attenuated, whereas the optical stimulation of IPn GABAergic neurons precipitated, withdrawal-related physical signs in mice (Zhao-Shea et al., 2013). Chronic nicotine treatment upregulated the expression of β4 nAChR subunits in somatostatin-expressing IPn neurons in dorsal portions of the IPn (Zhao-Shea et al., 2013), which may reflect the $\alpha5^{Amigo}$ cells that regulate nitric oxide–mediated retrograde inhibition of mHb inputs to the IPn (see above) (Ables et al., 2017). This suggests that adaptions in β4* nAChR signaling in the IPn contribute to the development of nicotine dependence. Consistent with this interpretation, infusion of the α3β4* nAChR antagonist SR 16584 into the IPn of nicotine-dependent mice precipitated physical signs of nicotine withdrawal (Zhao-Shea et al., 2013). Together, these findings suggest that β4* nAChRs and perhaps α5* and α2* nAChRs located in the mHb-IPn circuit regulate physical signs of nicotine withdrawal. However, the mHb-IPn circuit also regulates the increases in anxiety-related behaviors that occur during nicotine withdrawal, although β4*, α5*, and α2* nAChRs do not appear to be involved. This may instead reflect the contribution of β2* nAChR-expressing dopamine neurons in the VTA that express CRF and project to the IPn to modulate anxiety-related states (Grieder et al., 2014; Zhao-Shea et al., 2015) (see above).

In addition to IPn neurons, mHb neurons also show adaptive alterations in their activity in response to chronic nicotine exposure, which contribute to the development of nicotine dependence and expression of the aversive nicotine withdrawal syndrome (Gorlich et al., 2013). In slice recordings, mHb neurons show tonic trains of action potentials (Kim and Chang, 2005; Gorlich et al., 2013), which likely reflects "pacemaker" activity driven by the high concentrations of HCN4 channels expressed by these cells (Monteggia et al., 2000; Santoro et al., 2000; Gorlich et al., 2013; Oyrer et al., 2019). This pattern of tonic cellular activity contrasts with LHb neurons, which show much lower levels of baseline activity but a greater propensity to engage in burst firing (Kim and Chang, 2005). Infusion of an HCN antagonist into the mHb of nicotine-dependent mice precipitated physical signs of withdrawal and increased anxiety-related behavior (Gorlich et al., 2013). No change in the rate of tonic firing of mHb neurons was detected in mice after chronic nicotine treatment (Gorlich et al., 2013). However, the ability of nicotine to stimulate mHb neurons was markedly enhanced during nicotine withdrawal in a manner that depended on α3β4* nAChRs (Gorlich et al., 2013). Hence, modulatory actions of nicotine on mHb neurons could alleviate signs of withdrawal regulated by the mHb-IPn circuit and thereby serve as a source of motivation to seek and consume the drug during periods of abstinence, which contributes to relapse vulnerability. Overall, these findings support a major role for the mHb-IPn circuit in regulating behavioral abnormalities associated with nicotine withdrawal.

VII. Summary

The diversity of nAChR subtypes and their dense expression in brain systems involved in reward, aversion, mood regulation, and cognition explain the complex actions of nicotine contained in tobacco products on these behavioral processes. By carefully titrating their nicotine intake to dynamically control the activity of nAChRs in these brain systems, tobacco users can powerfully modulate the motivational, mood, and cognitive processes that drive the initiation, establishment, and maintenance of regular tobacco use. Better understanding of the nAChR subtypes involved in the actions of nicotine in the brain and periphery and identification of the precise circuits, cells, and molecules through which nAChRs transduce responses to nicotine will reveal fundamental new insights into the mechanisms of tobacco dependence. Moreover, because nAChRs have also been implicated in dependence on other classes of abused drugs, including alcohol, opioids, and cocaine, and tobacco smoking is

highly comorbid with other neuropsychiatric disorders, such as schizophrenia, improved understanding of the mechanisms of tobacco use disorder may provide key insights into other substance use and neuropsychiatric disorders. Ultimately, it is hoped that such information will catalyze the development of "next-generation" therapeutics to combat these disorders.

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Author Contributions

Wrote or contributed to the writing of the manuscript: Wills, Ables, Braunscheidel, Caligiuri, Elayouby, Fillinger, Ishikawa, Moen, Kenny.

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