Molecular Pharmacology Fast Forward. Published on December 17, 2012 as doi: 10.1124/mol.112.083659
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MOL #83659

Title Page

NICOTINIC RECEPTORS IN ADDICTION PATHWAYS

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Molecular Pharmacology Fast Forward. Published on December 17, 2012 as DOI: 10.1124/mol.112.083659
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Running Title Page

NICOTINIC RECEPTORS IN ADDICTION PATHWAYS

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Abbreviations: BLA, basolateral amygdala; hipp, hippocampus; IPN, interpeduncular nucleus; LDTg, lateral dorsal tegmental nucleus; MHb, medial habenula; MS, medial septum; NAc, nucleus accumbens; nAChR, nicotinic acetylcholine receptor; NB, nucleus basalis; PFC, prefrontal cortex; PPTg, pedunculopontine tegmental nucleus; VTA, ventral tegmental area.

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Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that consist of pentameric combinations of α and β subunits. These receptors are widely distributed throughout the brain, and are highly expressed in addiction circuitry. The role of nAChRs in regulating neuronal activity and motivated behavior is complex, and varies both within and across brain regions. The rich diversity of central nAChRs has hampered the characterization of their structure and function using classical pharmacological techniques. However, recent molecular approaches using null mutant mice with specific regional lentiviral re-expression, in combination with neuroanatomical and electrophysiological techniques, have allowed the elucidation of the influence of different nAChR types on neuronal circuit activity and behavior. This review will address the influence of nAChRs on limbic dopamine circuitry and the medial habenula-interpeduncular nucleus complex that are critical mediators of reinforced behavior. Characterization of the mechanisms underlying regulation of addiction pathways by endogenous cholinergic transmission and by nicotine may lead to the identification of new therapeutic targets for treating tobacco dependence and other addictions.

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Introduction

The nicotinic acetylcholine receptor (nAChR) was the first receptor to be extensively studied, following its identification in the early twentieth century as the 'receptive substance' that mediated the actions of synthetic nicotine (Langley, 1905). A series of classic studies then characterized the structure and function of nAChRs at the neuromuscular junction, leading to a detailed understanding of the pharmacology of this ligand-gated ion channel (Dani & Balfour, 2011). Although the behavioral effects of nicotine, the major psychoactive component of tobacco, have long been known, it was not until the early 1980s that the structure and functions of neuronal nAChRs within the brain were addressed. The identification, by Romano & Goldstein (1980), of stereospecific nicotine binding sites within brain homogenates was a landmark finding that served as a gateway to our current extensive knowledge of central nAChRs. Neuronal nAChRs have been shown to be widely distributed throughout the brain (Perry et al., 2002), and have a rich pharmacology resulting from heteropentameric combinations of α2-6 and β2-4 subunits, or homopentameric assemblies of α7-10 (Dani & Bertrand, 2007). Although ligand binding occurs only at α subunits, all subunits contribute to nAChR signaling and can regulate agonist affinity and efficacy, desensitization, channel ion permeability and downstream signaling. In contrast to muscular and ganglionic nAChRs, which mediate fast synaptic transmission, central neuronal nAChRs frequently serve a modulatory role and signal at a distance from the site of transmitter release (Dani & Balfour, 2011). Nonetheless, nAChRs have critical physiological roles in regulating neuronal signaling, particularly within mesolimbic addiction pathways. The complexity of nAChR pharmacology has hampered attempts to date to fully elucidate the functional mechanisms underlying nAChR regulation of addiction circuitry. However, recent technical advances, including in vivo electrophysiological recording, optogenetics and lentiviral re-expression of nAChR subunits in null mutant mice, have facilitated this process, as will be discussed in the current review. By focusing on brain

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regions that have been closely associated with drug-related behaviors, this review will examine the functional properties of nAChRs.

Ventral tegmental area dopamine neurons

The neurocircuitry underlying addiction is broad and complex, and is dependent on both the drug and the stage of the disease process (Koob & Volkow, 2009). However, all drugs of abuse activate mesolimbic dopamine neurons in the ventral tegmental area (VTA), which are a final common pathway for addiction (Figure 1). VTA dopamine neurons express a diverse array of nAChR subunits, including α3-7 and β2-4 (Azam et al., 2002). Whereas most VTA dopamine neurons express nAChRs, the posterior VTA subnuclei which project to the nucleus accumbens (NAc; Ikemoto, 2007) are particularly enriched in α4, α6, and β3 transcripts as compared to the anterior subnuclei (Zhao-Shea et al., 2011). At least two subtypes of α6* nAChRs (where the asterisk denotes the presence of other subunits) have been characterized within posterior VTA dopamine neurons - α6(non-α4)β2* and α6α4β2*. In contrast to α4β2 nAChRs, which desensitize within seconds (Paradiso & Steinbach, 2004), α6α4β2* nAChRs within the VTA remain persistently activated for minutes by nicotine at smoking-relevant concentrations (Liu et al., 2012; Grady et al. 2012). This nAChR type has been shown to be critical for nicotine activation of mesolimbic dopamine neurons (Zhao-Shea et al., 2011).

Dopamine neurons within the VTA receive local inhibitory input from GABA interneurons (Mansvelder et al., 2002). These neurons express fewer types of nAChR subunit transcripts, with the major nAChR population believed to be α4β2, with some α7 (Klink et al., 2001), although α6β2 nAChRs have also recently been described (Yang et al., 2011). Excitatory glutamate inputs from cortex and elsewhere also express α7 nAChRs (Mansvelder et al., 2002; Jones & Wonnacott, 2004). The excitatory input from both lateral dorsal tegmental (LDTg) and pontine pedunculo tegmental (PPTg) nuclei is critical for converting tonic firing of VTA dopamine

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cells to a burst firing pattern (Lodge & Grace, 2006). This switch from tonic to phasic activation is associated with reward-predicting stimuli and results in enhanced dopamine release in terminal regions (Schultz, 2007; Zhang et al., 2009b). Both cholinergic and glutamate projections from the midbrain tegmental nuclei regulate VTA neuronal firing activity. Burst firing of dopamine neurons is eliminated in the β2 subunit knockout mouse, and is restored by viral vector transfection of β2 subunits within the VTA (Mameli-Engvall et al., 2006). VTA expression of α7 subunits is also required for the full firing pattern of dopamine neurons although, in contrast to β2, it is not essential for the fast firing-long bursting mode (Mameli-Engvall et al., 2006).

Nicotine increases the firing rate and burst activity of dopamine neurons, particularly within the posterior VTA (Li et al, 2011; Zhao-Shea et al., 2011). This effect is gradual, reaching a stable plateau within twenty minutes, and is then followed by synchronization of the activity of a subset of dopamine neurons (Li et al, 2011). Synchronous activity may optimize dopamine output and is predicted to be important for reinforcement learning (Joshua et al, 2009). Whereas earlier models had predicted that continued exposure to nicotine, at concentrations seen in smokers' blood, would desensitize the α4β2 nAChRs on GABA interneurons and leave the α7 nAChR-driven glutamate excitatory input to VTA dopamine neurons unopposed, leading to burst firing (Mansvelder et al., 2002), a recent study with cell-specific re-expression of nAChR subunits in knockout animals suggests a more complex model (Tolu et al., 2012). Tolu and colleagues have shown through in vivo recording that nicotine does not immediately desensitize nAChRs on GABA interneurons. Furthermore, restoration of β2* nAChRs in only VTA dopamine cells is not sufficient to restore nicotine-evoked burst firing in β2 knockout mice. Bursting is only restored when β2 subunits are transfected into both VTA dopamine and GABA neurons, indicating that the coordinated action of nAChRs in both cell types is essential for normal dopamine cell function.

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Viral vector re-expression of nAChR subunits in knockout mice has confirmed the importance of VTA nAChRs in mediating the reinforcing effects of nicotine. Intravenous nicotine self-administration is abolished by transgenic elimination of α4, α6 or β2 subunits, and is restored by re-expression of these subunits in the VTA (Pons et al., 2008). In contrast, Exley and colleagues (2011) have shown that the α4 subunit, but not α6, is essential for intracranial self-administration of nicotine into the VTA and for nicotine-induced bursting of VTA dopamine neurons. These discrepancies between findings from studies with differing routes of nicotine administration may reflect the transport of re-expressed VTA α6 subunits to dopamine terminal fields within the nucleus accumbens, where they are essential regulators of nicotine actions (Exley et al., 2011; see below). Cell-specific re-expression has been shown to play a critical role for β2* nAChRs in both VTA dopamine and GABA neurons in inducing not only dopamine neuron burst firing but also sustained intracranial self-administration (Tulo et al., 2012). Selective re-expression of β2* nAChRs within dopamine neurons increases firing rate but not bursting and leads to a transient behavioral reinforcing effect, whereas selective re-expression within GABA cells results in inhibition of dopamine neuron firing and aversion to nicotine intake. The latter finding is consistent with recent evidence that selective activation of VTA GABA neurons drives conditioned place aversion and disrupts rewarded behavior (Tan et al., 2012; van Zessen et al., 2012).

Ventral tegmental area terminal regions

Although nAChRs in the VTA play a major role in regulating dopamine release in limbic brain regions, there are also nAChRs on axonal terminals. These have been shown to have a critical role in controlling local dopamine release (Exley & Cragg, 2008), and exhibit marked differences in subunit composition across brain regions (Livingstone & Wonnacott, 2009). The ventral striatum, or nucleus accumbens, is a major output for reinforced behavior and is the target of VTA mesostriatal dopamine projection neurons. Immunoprecipitation coupled with cell-

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specific lesions has shown that nAChRs on dopamine terminals in the ventral striatum differ from that in the dorsal region (Gotti et al., 2010), even though the subunit expression profile within the cells of origin in the VTA and substantia nigra is largely similar (Azam et al., 2002). Functional studies have also shown critical differences in the probability of dopamine release in the dorsal and ventral striatum (Zhang et al., 2009 a, b) and in the properties of nAChRs that regulate dopamine release in these regions (Exley et al., 2008; Exley et al., 2012).

Throughout the striatum, dopamine terminals are contacted by a rich dendritic arbor from striatal cholinergic interneurons (Zhou et al., 2002). Although cholinergic and dopamine neurons were once thought to have opposing actions, a complex interrelationship has now been demonstrated (Surmeier & Graybiel, 2012). In both dorsal and ventral striatum, presynaptic nAChRs function as frequency-dependent regulators of dopamine release (Exley & Cragg, 2008). Whereas dopamine release probability following a single action potential is quite high, further release by subsequent action potentials in a burst is limited by short-term depression. The role of nAChRs in mediating this 'flattening' of the frequency-response curve has been demonstrated through pharmacological and molecular techniques. When nAChR activity is eliminated, along with short-term depression of dopamine release probability, striatal dopamine release becomes highly sensitive to the activity of the neurons of origin. In this case, nicotine itself acts as an antagonist by desensitizing striatal nAChRs. Using fast-scan cyclic voltammetry to measure action potential-dependent dopamine release from mouse striatal slices, combined with *in vivo* recording of dopamine neuron firing, Zhang et al (2009b) have shown that the probability of basal dopamine release is lower in the NAc shell than the dorsal striatum, and that nicotine enhances the signal-to-noise relationship of dopamine transmission more effectively in the ventral striatum. Exley and colleagues have also demonstrated pharmacological differences in the nAChRs that regulate synaptic dopamine release in dorsal and ventral striatum: nAChRs on dopamine terminals in the nucleus accumbens, but not the caudate putamen, are blocked by

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the α6*-specific antagonist, α-conotoxin-MII (Exley & Cragg, 2008). Studies with subunit-specific knockout mice have since verified this regional difference in nAChR pharmacology (Exley et al., 2012). Whereas α4α6β2β3 nAChRs play a critical role in regulating dopamine release in nucleus accumbens core, α4α5(non-α6)β2 nAChRs predominate in dorsal striatum. Two recent studies, in which cholinergic interneurons were optogenetically driven, have confirmed that nAChRs on dopamine terminals have a key role in mediating the effects of endogenous acetylcholine on synaptic dopamine release (Cachope et al., 2012; Threfell et al., 2012). Whereas frequency dependent modulation by β2* nAChRs was demonstrated in dorsal striatum (Threfell et al., 2012), this was not the case in ventral striatum, although technical issues may have limited the upper frequency range (Cachope et al., 2012). Another difference between the two studies was that glutamate, released either from cholinergic interneurons or by cholinergic stimulation of excitatory inputs, was found to have a role in mediating dopamine release from ventral but not dorsal striatal neurons.

Whereas many midbrain dopamine neurons express α7 nAChRs (Azam et al., 2002), these receptors are not transported to axonal terminals (Livingstone & Wonnacott, 2009; Gotti et al., 2010). However, transmitter release assays using tissue prisms have provided evidence that α7 nAChRs on excitatory inputs regulate dopamine-glutamate cross-talk in both striatum and prefrontal cortex (PFC; Livingstone et al., 2009). In the PFC, which is a critical regulator of executive function and impulse control, complex interactions between glutamate, dopamine, acetylcholine and GABA terminals mediate the output of pyramidal output neurons (Tseng & O'Donnell, 2004). nAChRs on PFC dopamine terminals differ from those found in the nucleus accumbens in that they are β2* nAChRs with no α6 subunit (Cao et al., 2005a; Livingstone et al., 2009). Separate populations of glutamate terminals express α7 and α4β2* nAChRs that regulate cortical release of dopamine and acetylcholine, respectively (Livingstone et al., 2009; Parikh et al., 2008). Finally, both GABA interneurons and pyramidal cells also express nAChRs

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in a layer-specific manner, with differential impact on pyramidal cell activity on superficial versus deep cortical layers (Poorthuis et al., 2012). Thus, nAChRs serve critical and diverse roles in modulating PFC function.

Whereas both hippocampus and basolateral amygdala serve essential roles in associating drug use with context and cues (Koob & Volkow, 2009), there has been little study of nAChR regulation of dopamine release in these regions. One transmitter release study has indicated that hippocampal dopamine release is regulated by α3β4* nAChRs and by another, as yet unidentified, nAChR type (Cao et al., 2005b). Using in vivo recording techniques, Dani and colleagues have also shown that activation of dopamine D1 receptors in the dentate gyrus is essential for the nicotine induction of long-term potentiation within the perforant path (Tang & Dani, 2009). As yet, however, the technical approaches that have yielded such useful information on nAChR regulation of signaling in the VTA and striatum have not yet been applied to the PFC, hippocampus or amygdala, despite the critical function of these brain areas in addiction processes. One reason for this is the limited sensitivity of current methodology to measure low levels of dopamine release in these regions.

Medial habenula – interpeduncular nucleus

Although β2* nAChRs are the most widely distributed throughout the brain, and much research focus has focused on their role in nicotine addiction, α3β4* nAChRs are also increasingly being seen to play an important role. A number of human studies link polymorphisms in the gene cluster encoding α3-α5-β4 nAChR subunits with degree of tobacco dependence and response to cessation therapy (Berrettini et al. 2008; Chen et al 2012). Whereas α3β4* nAChRs are widely expressed in the periphery, they have a more restricted distribution in the brain with highest expression in the medial habenula (MHb), interpeduncular

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nucleus (IPN) and pineal gland (Perry et al., 2002). Recent findings suggest that nAChRs within the MHb-IPN pathway may serve important functional roles in mediating addiction processes.

The habenular complex, at the center of the dorsal diencephalic conduction system, is considered to be an important 'relay station' in the brain (Bianco & Wilson, 2009). The fasiculus retroflexus projection from the MHb to the IPN is a prominent cholinergic pathway that serves as an important link between the limbic forebrain and the midbrain. Among its many targets, the IPN sends afferents to the raphe and ventral tegmental area, thus regulating the activity of serotonergic and dopamine neurons (Klem, 2004; Lecourtier & Kelly, 2007). Recent findings of an optogenetic study indicate that MHb cholinergic neurons express glutamate as a co-transmitter and that the two transmitters are released by different modes of stimulation (Ren et al., 2011). Whereas brief photostimulation produces glutamate-mediated fast excitatory currents in IPN target neurons, tetanic photostimulation generates nAChR-mediated slow inward currents. Similar to midbrain dopamine neurons, MHb and IPN cells express a rich array of nAChR subunits, including α2-α6 and β2-β4 (Grady et al., 2009). Immunoprecipitation studies in wild-type and null mutation animals have shown a diversity of nAChRs within these nuclei, including some novel nAChR subtypes - α2β2*, α4β3β2*, α3β3β4* and α6β3β4*. The α5 subunit is present in a small minority of nAChRs in both MHb and IPN (Grady et al., 2009; Scholze et al., 2012). Of the rich diversity of nAChRs expressed by the MHb, only α3β4* and α3β3β4* stimulate acetylcholine release in the IPN (Grady et al., 2009), whereas α5* nAChRs stimulate glutamate release (Fowler et al., 2011).

An increasing body of work indicates that nAChRs within the MHb-IPN pathway regulate nicotine reinforcement (Figure 2). An α5 nAChR null mutation increases intravenous self-administration of nicotine by decreasing aversion at high dose ranges (Fowler et al., 2011). Increased nicotine consumption in knockout mice was blocked by re-expression of α5 subunits in the MHb (Figure 2A). Microinjection of lenti-α5-shRNA to knock down habenulo-

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interpeduncular α5* nAChRs in rats also increased self-administration of high nicotine doses. Inactivation of the MHb and IPN with lidocaine similarly increased self-administration of high doses of nicotine in mice, leading Fowler and colleagues to conclude that "this circuit acts in a manner opposite to the mesoaccumbens 'positive reward' pathway and instead transmits an inhibitory motivational signal that limits nicotine intake" (Fowler et al., 2011). Recent pharmacological studies have yielded a more complex picture, however. Whereas administration of α3β4* antagonists into the MHb decreases intravenous nicotine self-administration and acute nicotine-induced dopamine release in the nucleus accumbens, injection into the IPN exerts an opposite behavioral action (Glick et al., 2011; McCallum et al., 2012). This finding suggests that α3β4* nAChRs in the MHb may mediate nicotine reinforcement, a conclusion that is supported by recent evidence that self-administration of nicotine is blocked by peripheral administration of AT-1001, an α3β4*-selective nAChR antagonist (Toll et al., 2012).

Recent molecular studies have provided further evidence for a role of α3α5β4* nAChRs in nicotine reinforcement and aversion. In vitro transfection studies have shown that introduction of the α5 subunit reduces the maximal α3β4* nAChR response to agonist activation and shifts the downstream signaling pathways (Tammimäki et al., 2012). Introduction of the D398N α5 subunit variant, that is linked to increased risk for nicotine dependence, further decreases agonist response at the α3β4* nAChR (Frahm et al., 2011; Tammimäki et al., 2012). A transgenic mouse model, Tabac, in which β4 subunit overexpression enhances α3β4* nAChR levels has been shown to increase aversion to nicotine, an effect that is reversed by lentiviral transfection of the D398N α5 subunit into the MHb (Frahm et al., 2011). Thus, contrary to the observation of Fowler et al. (2011), studies with this transgenic mouse model suggest that α5* nAChR subunits in MHb decrease nicotine aversion. This conclusion may be consistent with the findings of a recent study with another transgenic mouse model, in which overexpression of the

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CHRNA5/A3/B4 genomic cluster led to significantly increased β4 * nAChR binding in the MHb and increased acquisition of nicotine self-administration (Gallego et al., 2012). Thus, although the recent literature provides convergent evidence as to a critical role of nAChRs within the MHb-IPN pathway in regulating nicotine intake, much work is left to be done to elucidate the exact mechanisms involved.

Summary

The role of nAChRs in regulating neuronal activity and motivated behavior is complex, and varies both within and across brain regions. Neuronal activity and neurotransmitter release in many brain areas are regulated by endogenous cholinergic activity, and may be influenced differently by exogenous nAChR agonists and antagonists. The rich diversity of nAChR subtypes hampers classical pharmacological analysis of receptor properties. Thus, in combination with neuroanatomical and electrophysiological techniques, molecular pharmacological approaches have allowed the elucidation of the influence of individual receptor subunits on neuronal circuit activity and behavior. Given the role of nAChRs in regulating many converging cellular elements in a single region, future studies with cell-specific subunit deletion or re-expression will be necessary to fully characterize nAChR regulation of addiction pathways. Although rodent nAChRs are not completely homologous to that of humans, the wealth of knowledge provided from such studies has provided a framework that may lead to the identification of new therapeutic targets for treating tobacco dependence and other addictions.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Leslie, Mojica and Reynaga

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Molecular Pharmacology Fast Forward. Published on December 17, 2012 as DOI: 10.1124/mol.112.083659
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Footnotes

This work was supported in part by Tobacco-Related Disease Research Program grant. [#21RT-0136]

Molecular Pharmacology Fast Forward. Published on December 17, 2012 as DOI: 10.1124/mol.112.083659
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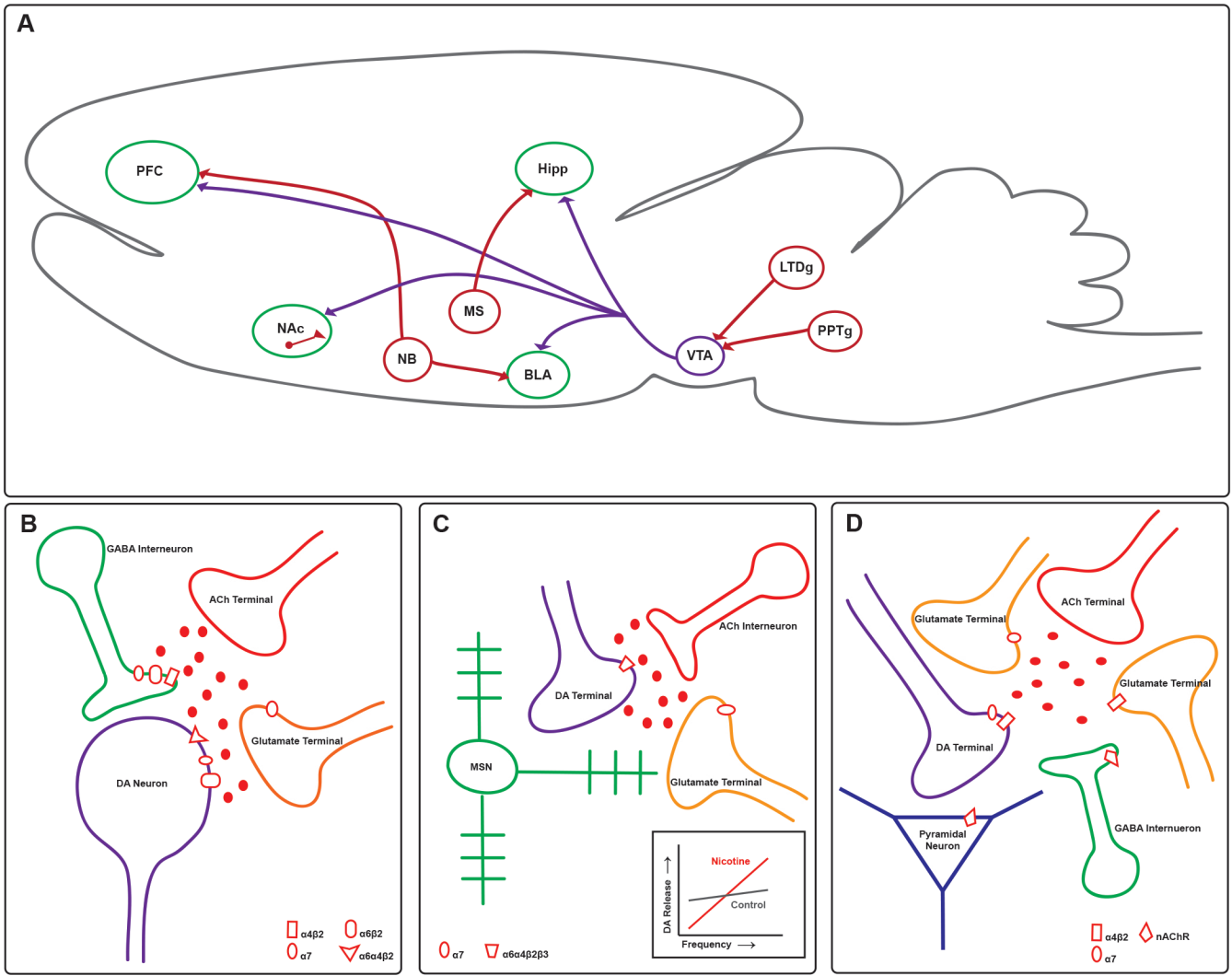
Legends for Figures.

Figure 1. Dopaminergic and cholinergic interactions in addiction circuitry. (A) Interaction of limbic dopamine and cholinergic pathways. BLA, basolateral amygdala; hipp, hippocampus; LTDg, lateral dorsal tegmental nucleus; MS, medial septum; NAc, nucleus accumbens; NB, nucleus basalis; PPTg, pedunculopontine tegmental nucleus; PFC, prefrontal cortex; VTA, ventral tegmental area. (B) Microcircuit of nAChR modulation of dopamine (DA) neuron firing in the VTA via nAChR on DA cell bodies, GABA interneurons and glutamate terminals. (C) Microcircuit of nAChR modulation of DA release in the nucleus accumbens (NAc) via nAChRs on DA and glutamate terminals. Inset illustrates frequency insensitivity of DA release during release that is eliminated by nicotine desensitization of nAChRs (simplified from Exeley et al., 2008). (D) Microcircuit illustration of nAChR modulation DA release and pyramidal neuron activity by nAChRs on pyramidal cell bodies, and on glutamate, GABA and DA terminals.

Figure 2. Modulation of nicotine intake by nAChRs in the medial habenula-interpeduncular nucleus (MHb-IPN) pathway. (A) Fowler and colleagues (2011) showed that mice lacking α5 subunits in the MHb-IPN pathway increase their nicotine intake at high doses, an effect blocked by re-expression of α5 subunits within this pathway. Suggests that α5 subunits regulate inhibitory motivational signal transmitted by this circuit. (B) Glick et al. (2011) and McCallum et al. (2012) showed that α3β4 nAChRs in the MHb-IPN pathway regulate the reinforcing effects of nicotine. Blocking α3β4 nAChRs in MHb in rats decreases self-administration of nicotine and nicotine-induced DA release in the NAc. In contrast, blocking α3β4 nAChRs in the IPN increases nicotine intake. (C) Frahm et al. (2011) showed that transgenic mice with over-expression of α3β4 nAChRs in the MHb-IPN pathway display aversion to nicotine, which is reversed by increasing the expression of α5 subunits in the MHb. Suggests a role of α5 subunits in decreased aversion to nicotine.

Figure 1


A
PFC
Hipp
LTDg
MS
NAc
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NB
VTA
BLA
B
GABA Interneuron
ACh Terminal
Glutamate Terminal
DA Neuron
α4β2
α6β2
α7
α6α4β2
C
ACh Interneuron
DA Terminal
MSN
Glutamate Terminal
Nicotine
Control
DA Release
Frequency
α7
α6α4β2β3
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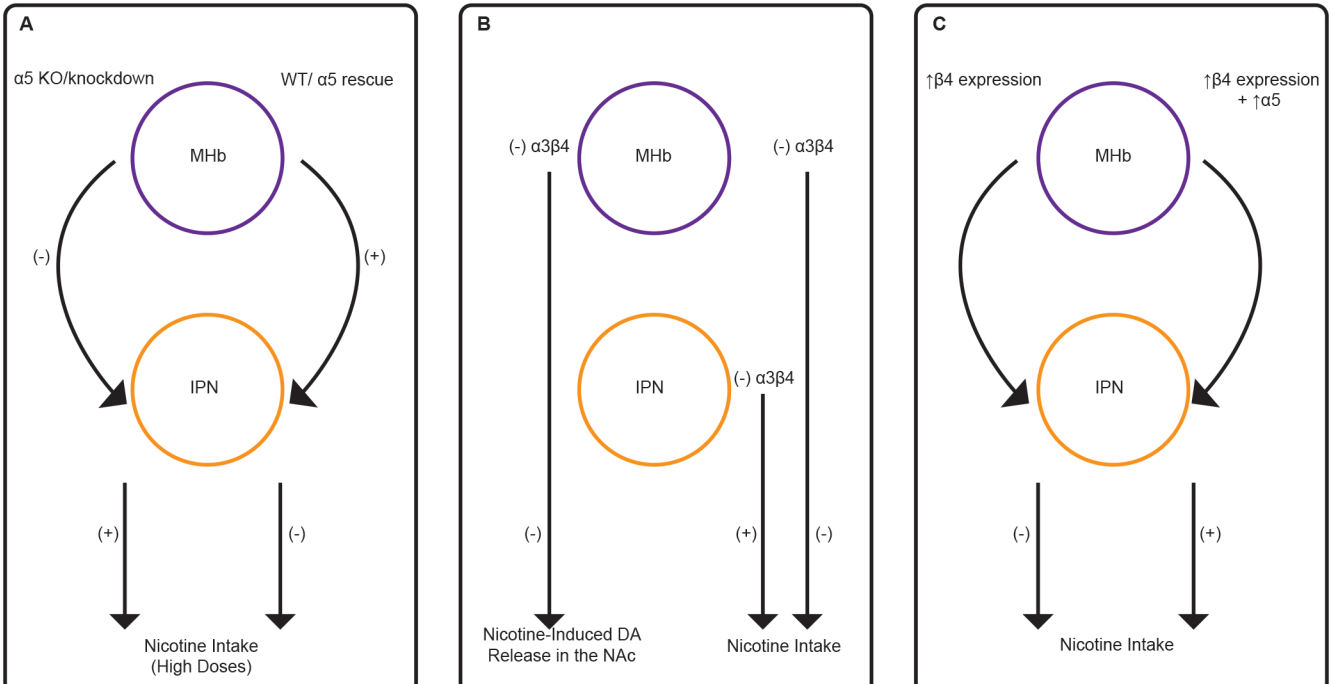
ACh Terminal
Glutamate Terminal
Glutamate Terminal
DA Terminal
Pyramidal Neuron
GABA Internueron
α4β2
nAChR
α7

Figure 2


A
α5 KO/knockdown
WT/ α5 rescue
MHb
(-)
(+)
IPN
(+)
(-)
Nicotine Intake
(High Doses)
B
(-) α3β4
MHb
(-) α3β4
IPN
(-) α3β4
(-)
(+)
(-)
Nicotine-Induced DA
Release in the NAc
Nicotine Intake
C
↑β4 expression
↑β4 expression
+ ↑α5
MHb
IPN
(-)
(+)
Nicotine Intake