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Neurobiological and Neurophysiological Mechanisms Underlying Nicotine Seeking and Smoking Relapse

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Abstract
Tobacco-related morbidity and mortality continue to be a significant public health concern. Unfortunately, current FDA-approved smoking cessation pharmacotherapies have limited efficacy and are associated with high rates of relapse. Therefore, a better understanding of the neurobiological and neurophysiological mechanisms that promote smoking relapse is needed to develop novel smoking cessation medications. Here, we review preclinical studies focused on identifying the neurotransmitter and neuromodulator systems that mediate nicotine relapse, often modeled in laboratory animals using the reinstatement paradigm, as well as the plasticity-dependent neurophysiological mechanisms that facilitate nicotine reinstatement. Particular emphasis is placed on how these neuroadaptations relate to smoking relapse in humans. We also highlight a number of important gaps in our understanding of the neural mechanisms underlying nicotine reinstatement and critical future directions, which may lead toward the development of novel, target pharmacotherapies for smoking cessation.

Introduction

Approximately 16% of American adults smoke traditional cigarettes, and the prevalence of noncombustible tobacco use is rising dramatically. The use of electronic cigarettes (e-cigarettes) has tripled in middle- and high-school students in the United States in the last 4 years [1, 2]. Tobacco smoking remains the leading cause of preventable death in the United States, accounting for approximately 1 out of every 5 deaths annually [3]. On average, smokers die 13.5 years earlier than nonsmokers [4] and cigarette smoking-related illnesses cost more than USD 300 billion annually in the United States (i.e., USD 156 billion in lost productivity and USD 170 billion in health care expenditures) [5]. While smoking cessation at any age significantly increases life expectancy [6], only 3% of smokers are able to quit successfully on their own [7]. The majority of smokers who relapse do so during the first week of a quit attempt [8, 9]. Unfortunately, current FDA-approved medications for smoking cessation have modest efficacy and are associated with high rates of relapse [10]. Thus, there is a critical need to develop novel and more effective smoking cessation pharmacotherapies. Successful development of novel smoking cessation medications requires translational studies that advance and accelerate

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preclinical findings into pilot clinical trials [11, 12]. Preclinical studies utilizing rodent models of addiction-like behaviors are critical towards identifying molecular substrates that could serve as targets for new smoking cessation pharmacotherapies. Indeed, this drug discovery process can be guided by an improved understanding of the neurobiology that mediates smoking relapse.

Nicotine is the principal psychoactive chemical in tobacco that mediates the reinforcing effects of cigarette smoking and other forms of tobacco [13–17]. Therefore, the vast majority of biomedical research investigating the neurobiological and neurophysiological bases of smoking cessation focuses primarily on nicotine – although more recent studies have begun to investigate the role of other tobacco constituents and byproducts [18–23]. Intravenous drug self-administration in laboratory animals and humans is a behavioral paradigm used commonly to measure the reinforcing efficacy of nicotine [24, 25]. Nicotine self-administration has the highest degree of face validity of all animal models of nicotine addiction, primarily because it mimics voluntary tobacco consumption in humans [25]. Therefore, the drug self-administration model is a critical component of key studies examining the neural mechanisms underlying voluntary nicotine taking and seeking in rodents [24, 26, for review see, 27, 28]. While nicotine self-administration studies in human subjects typically involve cigarette-smoking behavior, the majority of nicotine self-administration studies in rodents entail intravenous administration immediately following an operant response (i.e., lever press or nose poke). Intravenous self-administration is used primarily because this route simulates the rapid rise in arterial nicotine and rapid distribution of nicotine into the brain that occurs via the typical pulmonary route of exposure in humans [29]. Plasma nicotine levels are similar in animals self-administering nicotine and human tobacco smokers, further validating this animal model of nicotine addiction [30]. In addition, the predictive validity associated with altered nicotine self-administration and the effectiveness of smoking cessation medications appears to be relatively high [11]. For example, all three FDA-approved treatments for smoking cessation (i.e., varenicline, nicotine replacement therapies, and bupropion) decrease nicotine self-administration in rodents [31–33]. Finally, it is thought that nicotine self-administration has good construct validity as an emerging literature indicates that similar changes in dopaminergic, cholinergic, GABAergic, glutamatergic, serotonergic, and cannabinoid systems mediate the reinforcing effects of nicotine in both humans and laboratory animals [25].

Smoking relapse is typically modeled in animals using the nicotine self-administration/extinction/reinstatement paradigm. Briefly, nicotine seeking can be reinstated after a period of nicotine self-administration and the subsequent extinction of the drug-reinforced behavior. The same stimuli that precipitate smoking relapse (i.e., stress, exposure to drug-associated stimuli and re-exposure to the drug itself) during periods of abstinence in humans can be used to reinstate nicotine-seeking behavior in rodents [34–36]. For example, following extinction of nicotine self-administration, systemic injections of relatively low doses of nicotine and/or cues previously paired with nicotine taking reinstate operant responding in the absence of nicotine reinforcement in rodents [37, 38]. The validity of the nicotine reinstatement model as an in vivo medication screen appears promising for smoking relapse [39]. Indeed, varenicline, an FDA-approved smoking cessation pharmacotherapy, attenuates the reinstatement of nicotine seeking in rats [40, 41] as well as smoking relapse in humans [42]. However, despite robust effects on nicotine reinstatement in rats, the efficacy of smoking cessation pharmacotherapies (i.e., varenicline and bupropion) in humans remains modest. This is likely due to the complexity of smoking behavior and relapse in humans as well as an incomplete understanding of the neurobiological mechanisms underlying relapse. It is important to note that the reinstatement paradigm, which is used to model relapse in human smokers, does not require nicotine dependence, as measured by somatic signs of physical withdrawal. Therefore, it is possible that some of the neuroadaptations that underlie smoking relapse are distinct from the mechanisms mediating physical withdrawal from nicotine. This presents a distinct advantage of preclinical research aimed at identifying specific neuroadaptations that promote or facilitate nicotine-seeking behavior and smoking relapse. However, the lack of physical dependence also highlights a potential limitation, as nicotine seeking in humans is likely due to both avoidance of withdrawal syndrome and relapse mechanisms. Thus, this is a consideration of preclinical studies of nicotine seeking that requires additional attention.

To better understand the mechanisms mediating nicotine addiction-like behaviors, including relapse, several researchers have investigated nicotine-mediated changes in neurophysiology and plasticity using in vitro biochemical, electrochemical, and electrophysiological preparations [43–47]. The goal of this article is to review an emerging literature examining the neurotransmitter systems and the plasticity-dependent neurobiological and

neurophysiological mechanisms underlying nicotine-seeking behavior in rodents, particularly as it relates to relapse in human smokers. We will also highlight important areas of consideration for future investigations. A more complete understanding of how nicotine-induced neuroadaptations alter neural circuits to produce nicotine-seeking behavior could lead to the development of novel, targeted pharmacotherapies for smoking cessation.

Neurotransmitter Mechanisms Regulating Nicotine Seeking

Nicotine reinforcement is mediated through different neurotransmitter systems, and many investigations of nicotine-seeking behavior (in animal models) and relapse behavior (in human smokers) have focused on neurotransmitter mechanisms of drug-taking and drug-seeking behavior. The reinforcing effects of nicotine are mediated, in large part, by activation of neuronal nicotinic acetylcholine receptors (nAChRs) in the mesoaccumbens dopamine pathway [26, 48, 49]. Indeed, systemic administration of nicotine increases extracellular dopamine levels in the nucleus accumbens (NAc) [50–52], specifically in the shell subregion [53]. Moreover, direct infusions of nicotine into the ventral tegmental area (VTA) [52, 54] and NAc [54, 55] also increase dopamine release in the accumbens. Furthermore, acute and chronic nicotine exposures produce dynamic changes in dopamine levels in the NAc that are associated with nicotine reinforcement [53, 56]. The ability of nicotine to activate mesoaccumbens dopamine projections relies on β2-containing nAChRs expressed on VTA dopamine neurons [57] and α7* nAChRs (* indicates the possible presence of additional, unidentified subunit[s]) expressed on glutamatergic afferents in the VTA [43]. There is clear evidence that neuronal α4β2* nAChRs (* indicates the possible presence of additional subunits) play a critical role in nicotine reinforcement [26, 58, 59], which also supports the utility of the ongoing development of novel smoking cessation pharmacotherapies targeting this receptor population. It is also clear that glutamate transmission in the brain plays a critical role in nicotine addiction [60–62]. Specifically, nicotine exposure increases extracellular glutamate levels in the VTA [63] and promotes excitatory tone in the VTA, both of which can enhance dopamine cell firing to facilitate nicotine reinforcement. Given this literature, many studies of nicotine seeking and smoking relapse have focused on dopaminergic, cholinergic, and glutamatergic mechanisms [36, 60]. However, more recent studies reveal novel roles for other neurotransmitter and neuropeptide mechanisms. In this section, we will review how these systems contribute to nicotine seeking in rats, and possibly to relapse behavior in humans.

Dopamine

As noted above, nicotine effects on the mesolimbic reward system, and on dopamine transmission in particular, are known to mediate nicotine reinforcement. There are five dopamine receptor subtypes classified as D1-like (D1 and D5) or D2-like (D2, D3, and D4), based on sequence homology and pharmacology [64–66]. Systemic administration of either a D1-like or D2-like dopamine receptor antagonist is sufficient to reduce cue-induced reinstatement of nicotine seeking [67]. While these findings indicate that dopamine receptors play an important role in nicotine seeking, they do not identify roles for individual dopamine receptor subtypes in the reinstatement of nicotine seeking. Importantly, however, human genetics studies have identified single nucleotide polymorphisms in the dopamine D4 receptor gene that correlate with cue reactivity in human smokers [68–70]. The systemic administration of a dopamine D4 receptor antagonist attenuates both cue- and nicotine-induced reinstatement of drug seeking [71]. These findings suggest that dopamine D4 receptors could serve as potential targets for novel smoking cessation pharmacotherapies. In addition to these dopamine D4 receptor-mediated effects, systemic administration of selective dopamine D3 receptor antagonists reduces drug-seeking behavior elicited by either an acute priming injection of nicotine [72, 73] or re-exposure to conditioned stimuli that were paired with nicotine taking prior to withdrawal [74]. Repeated systemic nicotine administration produces locomotor sensitization, which is associated with increased expression of dopamine D3, but not D1 and D2, receptor subtypes in the NAc shell [75]. Dopamine D3 receptors are expressed at high levels in the NAc shell, specifically in medium spiny GABAergic projection neurons [76–78]. However, it is not clear if similar D3 mechanisms in the accumbens mediate nicotine seeking during abstinence. Recent evidence indicates that pharmacological blockade of dopamine D3 receptors in the basolateral amygdala (BLA) and lateral habenula, but not the NAc, regulate the reinstatement of nicotine-seeking behavior [74]. Thus, future studies are required to identify the precise role of dopamine receptor subtypes in neurocircuits known to mediate nicotine seeking.

Endogenous fatty acid amides function as ligands of alpha-type peroxisome proliferator-activated receptors (PPAR-α) and are metabolized by fatty acid amide hydrolase (FAAH). Inhibition of FAAH prevents nicotine-induced elevations in extracellular dopamine levels in the NAc shell and reduces nicotine-induced excitation of VTA dopamine neurons [79, 80]. Moreover, pharmacological inhibition of FAAH attenuates nicotine seeking, and these effects are due, in part, to activation of PPAR-α [80, 81]. Consistent with the effects of FAAH inhibitors, PPAR-α agonists decrease nicotine-induced excitation of VTA dopamine neurons, thereby blocking nicotine-induced dopamine release in the NAc shell, and attenuate nicotine- and cue-induced reinstatement of nicotine-seeking behavior [82, 83]. These data indicate that activating PPAR-α is sufficient to prevent nicotine seeking by decreasing dopaminergic tone.

Glutamate

Several pieces of evidence point to a strong role for glutamatergic mechanisms underlying nicotine-seeking behavior. For example, extracellular glutamate levels in the NAc increase following cue-induced nicotine reinstatement [84]. Additionally, nicotine-seeking behavior is associated with changes in the cystine-glutamate exchanger, a heterodimeric protein complex that regulates extrasynaptic glutamate levels [85]. Specifically, withdrawal following voluntary nicotine taking reduces expression of the catalytic subunit (xCT) of the cystine-glutamate exchanger in the VTA and NAc [86]. Together, these results suggest that reversing or restoring nicotine-induced decreases in extracellular glutamate levels in the brain may prevent the reinstatement of nicotine-seeking behavior. However, strategies aimed at increasing extracellular glutamate levels in the brain during nicotine withdrawal have produced mixed effects on nicotine-seeking behavior. For example, systemic administration of N-acetylcysteine, a cystine pro-drug that increases the activity of the xCT resulting in increased extracellular glutamate levels, has been shown to attenuate [87] or have no effect [88] on the reinstatement of nicotine seeking. With regard to the clinical literature, N-acetylcysteine does not produce a therapeutically robust response in human smokers. For example, N-acetylcysteine decreases self-reported number of cigarettes smoked per day but does not produce concurrent reductions in breath carbon monoxide levels and fails to prevent relapse in human smokers [86, 89]. Thus, the efficacy of N-acetylcysteine in reducing nicotine seeking in both rodents and humans appears weak at best.

At the receptor level, withdrawal following repeated nicotine taking is associated with increased glutamate receptor expression. Specifically, nicotine withdrawal increased protein expression of AMPA receptor GluA1 subunits and NMDA receptor GluN2A and GluN2B subunits, as well as reduced protein expression of the glutamate transporter-1 in the NAc core [84, 86]. Pharmacological inhibition of GluN2A- and GluN2B-containing NMDA receptors in the NAc core decreases cue-induced reinstatement of nicotine seeking [84]. Interestingly, a nonselective NMDA receptor antagonist increases cue-induced nicotine seeking when infused directly into the NAc core [90]. It is hypothesized that this increase in drug seeking when NMDA receptors are inhibited is due, in part, to activation of non-NMDA glutamate receptors (e.g., mGlu5 receptors) following cue-induced increases in extracellular glutamate levels in the NAc core [90]. Consistent with this hypothesis, administration of the mGlu5 receptor antagonist MPEP attenuates cue-induced nicotine-seeking behavior [91]. Interestingly, systemic administration of the mGlu1 receptor antagonist EMQMCM also attenuates cue- and nicotine-induced reinstatement of nicotine seeking [92]. Due to adverse effects of AMPA and NMDA receptor antagonists in humans, attention has focused on developing type I mGlu (mGlu1) receptor antagonists for smoking cessation [36]. In addition to these mGlu1 effects, repeated nicotine exposure and subsequent withdrawal reduce mGlu2/3 receptor function in the VTA and NAc [93]. Furthermore, systemic administration of the mGlu2/3 receptor agonist LY379268 attenuates cue-induced reinstatement of nicotine seeking [93]. However, mGlu2/3 receptor agonists are nonselective and tolerance develops with repeated administration [93]. For these reasons, focus has shifted to selective positive allosteric modulators (PAMs) of mGlu2 receptors. Recent studies show that systemic administration of a PAM of mGlu2 receptors reduces nicotine- and cue-induced reinstatement of nicotine-seeking behavior, and that these effects are associated with reduced dopamine release in the NAc [94, 95]. Based on these studies, a phase II clinical trial examining the efficacy of the mGlu2 receptor PAM AZD8529 in human smokers is currently underway [96], with preliminary results forthcoming.

Acetylcholine and Nicotinic Acetylcholine Receptors

With respect to nAChR subtypes, a growing literature continues to delineate a role for α4β2* and α7* nAChRs in nicotine-seeking behavior and suggests that these two subpopulations of nAChRs may play differential roles in

drug- versus cue-primed reinstatement of nicotine seeking. Indeed, varenicline, currently the best-in-class treatment for smoking cessation, was developed based on its action as a partial agonist at α4β2* nAChRs [97]. Interestingly, varenicline appears to have differential effects on the reinstatement of nicotine seeking depending upon the stimulus used to elicit drug seeking. For example, varenicline attenuates nicotine seeking following an acute priming injection of nicotine [40]. However, varenicline has no effect on cue-induced reinstatement of nicotine seeking [40] and even enhances cue-induced nicotine seeking at high doses [98]. These results suggest that α4β2* nAChRs mediate nicotine primed, but not cue-induced, reinstatement of drug seeking. This is further supported by a study showing that cue-induced reinstatement of nicotine seeking is attenuated following pretreatment with an α7*, but not α4β2*, nAChR antagonist [99]. Interestingly, chronic nicotine exposure promotes formation of α7* nAChR-NMDA receptor heterodimeric protein complexes in the hippocampus, and blocking this interaction attenuates cue-induced nicotine seeking [100]. Taken together, these findings indicate that nicotine priming-induced reinstatement of drug seeking is mediated by α4β2* nAChRs and that cue-induced reinstatement of nicotine seeking is mediated by α7* nAChRs.

The role of other nAChR subtypes in nicotine seeking is less clear. In light of varenicline's modest efficacy in promoting long-term abstinence [11], more recent studies have focused on newer subtype-specific nAChR agonists. Systemic administration of the α7* nAChR agonist ABT-107, and the α4β2*/α6β2* agonist ABT-089 attenuates the reinstatement of nicotine seeking indicating an important role for α7* nAChRs and α4-, α6-, and β2-containing nAChRs, respectively, in this behavioral response [101]. A more recent study found that systemic administration of the α3β4* nAChR ligand AT-1001 decreases nicotine priming-induced reinstatement of drug seeking [102]. This emerging literature expands our understanding of the role of nAChR subtypes in nicotine seeking and identifies potential new targets for novel smoking cessation pharmacotherapies.

In addition to agonists of α4β2* nAChRs, PAMs of α4β2* nAChRs may also represent potential smoking cessation medications. PAMs bind nAChRs at allosteric sites that are distinct from the orthosteric binding site for nicotine. By enhancing receptor activity, as well as the probability of nicotine-induced channel opening, PAMs can substantially increase and prolong α4β2* nAChR responses to nicotine from tobacco smoke [103]. Based on this unique mechanism, PAMs of α4β2* nAChRs may reduce the amount of nicotine consumed in daily smokers and reduce the likelihood that a smoking lapse may lead to relapse. α4β2* nAChRs can assemble into two stoichiometrically and functionally different protein complexes characterized by the α4:β2 subunit ratio. The 2(α4)3(β2) and 3(α4)2(β2) subtypes represent channels with high or low sensitivity, respectively, to acetylcholine [104, 105]. Nicotine and varenicline bind to and activate both high- and low-sensitivity populations of α4β2 nAChRs [106]. While the relevance of these findings to the therapeutic effects of nicotine and varenicline is unknown, it raises the intriguing possibility that compounds targeting high- or low-sensitivity α4β2 nAChRs may be more efficacious anti-smoking medications [107]. Recently, NS9283, a PAM that binds selectively to low-sensitivity 3(α4)2(β2) nAChRs, was shown to attenuate nicotine seeking when administered systemically to rats [108]. These findings indicate that stoichiometry-selective PAMs of nAChRs, specifically 3(α4)2(β2) nAChR subtypes, may prevent smoking relapse.

Given nicotine's demonstrated actions at endogenous nicotine acetylcholine receptors (nAChRs), activation of nAChRs has appropriately been a large focus of nicotine-mediated behavioral investigations. However, it is also apparent that altering acetylcholine levels is sufficient to alter nicotine-mediated behaviors – particularly nicotine-seeking behavior. Several studies have been performed with acetylcholinesterase inhibitors (AChEIs), which increase extracellular levels of acetylcholine in the brain and augment cholinergic transmission through inhibition of acetylcholinesterase, a catabolic enzyme responsible for metabolizing acetylcholine in the synapse [109, 110]. Systemic administration of the AChEIs galantamine [37] and donepezil [111] attenuates nicotine seeking in rats, suggesting that enhanced cholinergic tone is sufficient to reduce nicotine consumption and smoking relapse. The translational implications of these findings are supported by a small clinical trial showing a significant reduction in total daily cigarettes smoked and the subjective measures of smoking in cigarette smokers treated with galantamine [112]. Moreover, systemic galantamine administration improves cognitive performance in rodents and humans during nicotine withdrawal [113, 114]. Given that cognitive deficits during nicotine withdrawal predict smoking relapse [115, 116], these results suggest that AChEIs may improve cognitive performance in abstinent smokers and prevent smoking relapse. Taken together, these studies suggest that AChEIs could be re-purposed as pharmacotherapies for smoking cessation [117].

GABA

GABA is the canonical inhibitory neurotransmitter and an increasing number of studies are delineating GABAergic regulation of reward-related activity [60, 118, 119]. With respect to nicotine-seeking behavior, systemic administration of the $GABA_B$ receptor agonist CGP44532 has been shown to attenuate cue-induced reinstatement of nicotine-seeking behavior [120]. Consistent with these effects, the $GABA_B$ receptor agonist baclofen decreases nicotine priming-induced reinstatement of drug seeking [121]. While baclofen also reduces total cigarettes smoked per day and tobacco craving in human smokers [122], compliance is limited by adverse effects including cognitive impairments, sedation, muscle relaxation and tolerance [123]. In contrast, PAMs of $GABA_B$ receptors have fewer adverse effects than full agonists. PAMs of $GABA_B$ receptors potentiate GABA signaling only in brain regions and at times when endogenous GABA is released [124]. Systemic administration of BH177, a PAM of $GABA_B$ receptors, attenuates cue-induced reinstatement of drug seeking in rats [125]. Collectively, these findings indicate an important role for $GABA_B$ receptors in nicotine seeking and suggest that the efficacy of PAMs of $GABA_B$ receptors in smoking cessation should be explored further.

In addition to the effects of pharmacological agents of GABA receptors on nicotine seeking, recent studies have begun to examine the effects of chronic nicotine exposure and subsequent withdrawal on the endogenous GABA system. Cue-induced reinstatement of nicotine seeking is associated with increased expression of the $GABA_A$ receptor subunits α1 and γ2 in synaptic membrane fractions from the medial prefrontal cortex (mPFC) [126]. Furthermore, inhibiting surface expression of $GABA_A$ receptors in the dorsal, but not ventral, mPFC increases nicotine seeking, suggesting a region-specific role of mPFC $GABA_A$ receptors in relapse [126]. Consistent with these findings, infusions of muscimol, a $GABA_A$ receptor agonist, into the mPFC attenuates cue-induced reinstatement of nicotine seeking [126]. Thus, it has been proposed that increased $GABA_A$ receptor signaling in the mPFC may represent a compensatory response to reduce nicotine seeking [126].

Endocannabinoids

Given the critical involvement of increased mesolimbic dopamine signaling in nicotine reinforcement and behavior, preclinical studies have begun to study endocannabinoid regulation of both the dopamine system and nicotine-seeking behavior. Indeed, studies have focused on cannabinoid-1 (CB1) receptors due to their ability to regulate VTA dopamine cell firing and dopamine release in the NAc [127, 128]. In addition, systemic administration of CB1 receptor antagonists and inverse agonists attenuates the reinstatement of nicotine-seeking behavior [81, 129–132]. Consistent with these effects, activation of CB1 receptors promotes nicotine seeking during withdrawal [133]. In contrast to CB1 antagonist effects, CB2 receptor antagonists do not affect reinstatement of nicotine seeking [133]. Overall, these findings highlight a role for the endocannabinoid system, specifically CB1 receptors, in the reinstatement of nicotine seeking.

There are two endogenous ligands for the cannabinoid receptors: anandamide (AEA) and 2-arachidonoylglycerol. Pharmacological inhibition of FAAH, the primary catabolic enzyme that breaks down AEA, attenuates the reinstatement of nicotine seeking, reduces nicotine-induced excitation of VTA dopamine neurons and decreases nicotine-induced increases in extracellular dopamine levels in the NAc shell of rats [79–81]. Consistent with these effects, FAAH inhibitors also decrease nicotine reinstatement in non-human primates [134]. Inhibition of FAAH increases levels of the endocannabinoid AEA as well as the noncannabinoid fatty acid ethanolamides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (see the section Dopamine above for a discussion of OEA and PEA in nicotine seeking) [135, 136]. The effects of FAAH inhibitors on nicotine seeking are mediated, in part, by activation of CB1 receptors [134, 137]. Consistent with these effects, systemic administration of AM404, an inhibitor of the AEA transporter, reduces nicotine-induced elevations in extracellular dopamine levels in the NAc shell and attenuates nicotine-seeking behavior, effects likely due to increased levels of AEA in the brain [138–140]. Thus, novel pharmacotherapies targeting FAAH/CB1 receptor signaling may represent promising smoking cessation medications.

Norepinephrine

In addition to nicotine and nicotine-associated cues, stress exposure can also elicit nicotine seeking during withdrawal. The effects of stress on drug seeking are mediated by numerous neurotransmitter and neuromodulator systems including norepinephrine transmission [141]. Systemic administration of the α2 adrenergic receptor agonist clonidine attenuates stress-induced reinstatement of nicotine seeking [142]. These effects are due, in part, to activation of α2 adrenergic receptors in the central nucleus of the amygdala (CeA) [143]. Systemic administration of the α1 adrenergic receptor antagonist

prazosin attenuates nicotine- and cue-induced reinstatement of nicotine seeking as well as nicotine-induced release of dopamine in the NAc shell [144]. These studies suggest that α1 and α2 adrenergic receptor agonists may reduce smoking relapse in human smokers. While no studies have examined the efficacy of α1 adrenergic receptor agonists in human smokers, α2 adrenergic receptor agonists have been shown to reduce stress- and cue-induced nicotine craving in human smokers [145–147]. β-Adrenergic receptors have also been shown to play a role in nicotine seeking. For example, administration of the nonselective β-adrenergic receptor antagonist propranolol transiently reduces nicotine reinstatement [148]. Consistent with these effects, propranolol decreases nicotine craving in human smokers [149]. These exciting translational studies suggest that α2 adrenergic receptor agonists and β-adrenergic receptor antagonists could be efficacious smoking cessation medications.

Serotonin

In addition to the norepinephrine system, central serotonin signaling has also been examined in relapse models. Systemic administration of the 5-HT2A receptor antagonist M100907 attenuates nicotine seeking and these effects may be due to reduced NAc dopamine release following a priming injection of nicotine and/or re-exposure to conditioned stimuli [150]. Consistent with these effects, activation of 5-HT2A receptors is associated with increased release of dopamine in the NAc and prefrontal cortex [151–153]. While 5-HT2A receptor antagonists on their own do not affect dopamine levels, these compounds attenuate psychostimulant-induced increases in extracellular dopamine levels in the brain [154, 155]. Recent studies have also examined the efficacy of serotonin (5-HT) 2C receptor agonists in the nicotine reinstatement model. Similar to 5-HT2A receptor antagonists, 5-HT2C receptor agonists attenuate nicotine seeking in rats [150]. Thus, selective ligands of 5-HT receptor subtypes have been proposed as novel smoking cessation medications based on their ability to regulate dopamine transmission in the brain [156].

Neuropeptides

Nicotine interacts with many neuropeptide systems to promote reinforcement and reinstatement (for review see [157]). For example, dysregulation of dynorphin/kappa opioid receptor signaling in the brain may contribute to stress-induced reinstatement of drug seeking [158, 159]. A recent study showed that systemic administration of the selective kappa opioid receptor antagonist nor-binaltorphimine attenuates stress-, but not cue-, induced reinstatement of nicotine seeking [160]. Interestingly, systemic administration of the mixed mu/kappa opioid receptor antagonist naltrexone reduces cue-induced reinstatement of nicotine seeking [161]. Taken together, these results suggest that opioid receptor subtypes may play differential roles in nicotine seeking with mu opioid receptors involved in cue-induced nicotine seeking and kappa opioid receptors involved in stress-induced nicotine seeking.

Stress-induced reinstatement of nicotine seeking is also mediated by corticotropin-releasing factor 1 (CRF1) receptors. For example, intracerebroventricular infusions of the nonselective CRF1/CRF2 receptor antagonist D-Phe $CRF_{(12–41)}$ attenuates stress-induced reinstatement of nicotine seeking [142]. These effects appear to be due to pharmacological inhibition of CRF1 receptors as selective antagonists of CRF1, but not CRF2, receptors decrease stress-induced reinstatement of nicotine seeking [162–164].

Increased hypocretin transmission mediates the reinforcing effects of nicotine [165] and may produce a state of hyperarousal that promotes drug seeking [166]. In agreement, an infusion of hypocretin directly into the ventricles reinstates nicotine seeking [163]. Moreover, systemic administration of the hypocretin-1 receptor antagonist SB334867 reduces cue-induced nicotine seeking and these effects are due, in part, to increased protein kinase C signaling in the NAc [167]. In contrast, the role of hypocretin-2 receptors in nicotine seeking is not clear. One study found no effect of the hypocretin-2 receptor antagonist TCSOX229 on cue-induced nicotine seeking [167]. However, another study showed that systemic administration of the hypocretin-2 receptor antagonist 2-SORA attenuates cue-, but not nicotine-, induced reinstatement of drug seeking [168]. Thus, these preliminary studies suggest that hypocretin transmission may play an important role in cue-induced nicotine seeking. In addition to kappa opioid, CRF and hypocretin systems, there is emerging evidence that other neuropeptide systems may play an important role in nicotine seeking [157]. For example, systemic administration of the melanocortin 4 receptor antagonists HS014 and HS024 attenuates stress-induced reinstatement of nicotine seeking [169]. Taken together, these findings clearly identify a role for neuropeptide systems in nicotine reinstatement and suggest the neuropeptide-based therapies may prevent stress-induced nicotine seeking.

Recent studies have begun to examine the role of metabolic factors in regulating nicotine-mediated behaviors

[170]. For example, glucagon-like peptide-1 (GLP-1), an incretin hormone, has been shown to regulate both food and drug reinforcement [171]. With regard to nicotine addiction, systemic administration of a GLP-1 receptor agonist attenuates nicotine-induced increases in extracellular dopamine levels in the NAc and reduces the rewarding properties of nicotine [172]. GLP-1 receptor agonists also reduce nicotine self-administration indicating that activation of GLP-1 receptors is sufficient to reduce the reinforcing efficacy of nicotine [173]. The role of GLP-1 signaling in nicotine seeking, however, is unclear. From a translational perspective, it will be important to determine the efficacy of GLP-1 receptor agonists in reducing nicotine withdrawal-mediated behaviors including the reinstatement of nicotine seeking to support clinical trials of GLP-1 receptor agonists in human smokers.

Neurophysiological Mechanisms Underlying Nicotine-Seeking Behavior

As noted in the section Neurotransmitter Mechanisms Regulating Nicotine Seeking above, nAChRs in the mesoaccumbens dopamine system are known to play a critical role in nicotine-taking and -seeking behaviors [57, 58, 174, 175]. Activation of nAChRs in other brain regions such as the amygdala, hippocampus, and PFC are also known to impact nicotine taking, particularly in the context of reward learning and cognition [176, 177, as reviewed in, 178]. Over the last 20 years, seminal studies from different laboratories have revealed important insights into the nAChR mechanisms underlying nicotine-induced plasticity. Early electrophysiology studies focused on nicotine-mediated synaptic plasticity in the hippocampus and in the VTA [43, 45, 179–182]. In subsequent years, electrochemical investigations also revealed plasticity-related mechanisms through which nicotine enhances phasic dopamine signaling within the NAc [46, 47, 183]. In many of these investigations, the authors often suggested that nicotine-induced plasticity in specific brain regions could mediate nicotine reinforcement, nicotine-mediated reward learning, and nicotine-seeking behavior. Many of the electrophysiological and electrochemical investigations have been performed using acute, single nicotine exposure to provide key insights into the mechanisms underlying these phenomena. Of particular note, the use of in vitro slice application of nicotine doses that mimic nicotine exposure levels in plasma of human smokers has facilitated significant advancements in our understanding of nAChR plasticity [184]. Increasingly, these in vitro investigations have begun to use repeated nicotine exposure, a model that more closely mirrors repeated exposure in human smokers, especially with respect to the mechanisms underlying nicotine withdrawal-induced behavioral deficits [185, 186]. Indeed, the mechanisms underlying nicotine-mediated plasticity may differ from those mediating acute nicotine effects. In this section, we will review key findings on nicotine-mediated plasticity and discuss the implications of such findings on our understanding of nicotine-seeking behavior and smoking relapse. We will also highlight areas where additional study can provide greater understanding of these neurobiological mechanisms and can potentially provide new insights for novel therapeutic interventions.

Ventral Tegmental Area

As noted above, VTA nAChRs are necessary and sufficient for nicotine reinforcement, as measured by nicotine self-administration in rodents [58, 175, 187, 188]. Thus, several slice electrophysiological studies have examined the mechanisms through which VTA nAChRs mediate neuronal activity and signaling in the mesolimbic dopamine system. Dopamine neurons in the VTA exhibit two types of firing patterns distinguished as tonic (3–7 Hz) or burst (15–20 Hz) firing [189–191]. Burst firing of VTA dopamine neurons in particular, is known to play a critical role in drug-taking and drug-seeking behaviors [192–194]. Previous work revealed that even in the absence of nicotine, β2* nAChRs are required for dopamine burst firing, whereas spontaneous burst firing is still observed in mutant mice lacking α7 nAChRs [57]. The presence of nicotine has been shown to enhance burst firing of VTA dopamine neurons [57, 195, 196], an effect that is absent in mutant mice that do not express the β2 nAChR subunit [57]. The role of α7 nAChRs in nicotine-induced burst firing, however, appears to be more complex. While there are no overall differences in nicotine-induced burst firing of VTA dopamine neurons between α7 nAChR knockout mice and wild-type controls, the emergence of both inhibitory (in ½ of tested neurons) and enhanced (in ½ of tested neurons) dopamine responses to nicotine are seen in α7 nAChR knockout mice [57]. Therefore, it has been proposed that β2* nAChRs play a role in regulating dopamine neuronal excitation, while α7* nAChRs play a role in fine-tuning dopamine neuronal responses [57]. However, important questions regarding the role of nAChR subtype-specific regulation of VTA dopamine cell firing in nicotine-seeking behavior remains to be tested.

In addition to activation of nAChRs, nicotine exposure causes desensitization of nAChRs, which may in part explain acute tolerance to nicotine [197, 198]. Notably, α7* and β2* nAChRs desensitize at different rates [184]. Specifically, acute nicotine exposure in slice, at concentrations thought to persist in the brain hours after cigarette smoking (20–80 nM) enhances VTA dopamine burst firing by preferentially desensitizing β2* nAChRs on GABAergic interneurons, and not affecting (or limited desensitization of) presynaptic α7* nAChRs on glutamatergic afferents [43, 182, 184, 199]. This differential desensitization of nAChRs results in decreased GABAergic inhibition of dopamine neurons and increased glutamatergic drive onto dopamine neurons, resulting in increased VTA dopamine neuron firing. A relatively recent study demonstrated that β2* nAChRs on GABAergic interneurons are required for intracranial nicotine self-administration [200]. The role of desensitization of these specific receptors in nicotine seeking remains to be tested. A more recent body of work has demonstrated that α6* nAChRs on VTA neurons are more resistant to desensitization, allowing for prolonged dopamine cell depolarization by nicotine [44]. However, the role of α6* nAChRs is complicated by evidence that this receptor population is also expressed on GABAergic afferents in apposition to VTA dopamine neurons [199]. Further understanding of the precise role of nAChR subtypes and the desensitization kinetics of these receptor subpopulations in response to acute and chronic nicotine exposure in nicotine-seeking behavior is still needed.

It is important to note that the concentrations of nicotine used in many of the slice electrophysiology experiments described above were selected because they fall within the range typically found in the plasma of human smokers (50–500 nM) [184, 201]. We also note that the concentrations of plasma nicotine in a human smoker are likely much higher than nicotine concentrations in the brain [22, 201–203]. For example, the concentration of nicotine in the rodent brain is approximately 0.3% of the plasma nicotine levels measured in rats following nicotine self-administration [22]. Thus, it is possible that the nicotine concentrations used in some slice experiments are higher than would be identified in brain tissue of human smokers and of rodents self-administering nicotine. Due the potential nicotine dose-related issues indicated above, an ongoing consideration for the field is to identify mechanistic insights from nicotine electrophysiological studies in VTA slices and translate them to nicotine-related behavior. Electrophysiological investigations in rodents that have undergone abstinence after nicotine self-administration, and re-exposure to nicotine and nicotine-related cues, are needed to understand the role of region-specific nAChR activation and desensitization in nicotine seeking.

Electrophysiological experiments in which an animal is first exposed to acute or repeated nicotine can offer unique insights into the consequences of such exposure on both synaptic plasticity and nicotine-related behavior. Both acute and chronic nicotine administration can alter nAChR expression profiles in the VTA. However, the chronicity and route of administration, as well as dose, likely have differential impacts on neuroadaptations, as these factors drastically impact nicotine-seeking behaviors. Broadly, nAChRs are upregulated in the VTA following chronic and self-administered nicotine in rats [204–206]. The nAChR upregulation observed in human smokers is long lasting, even during smoking abstinence [207] and is thought to be a compensatory change in response to the nAChR desensitization described above. Incubation of nicotine in cultured VTA cells results in the upregulation of α4β2* nAChRs [208]. Likewise, chronic subcutaneous nicotine exposure in rats increases α4β2* and α7* nAChR binding sites in the VTA [209]. Nicotine-mediated upregulation of nAChRs is likely to influence behavior, as functional upregulation of α4* nAChRs in VTA GABAergic neurons has been shown to increase sensitivity to nicotine reward, measured by conditioned place preference [210]. While upregulation of nAChRs impacts reward-related behaviors, the role of nAChR upregulation on nicotine seeking is not well studied. It has been proposed that persistent upregulation of nAChRs during nicotine abstinence may promote craving, resulting in relapse [205]. However, additional work is needed to more fully test this hypothesis.

In addition to altering nAChR expression, nicotine can also alter glutamate receptor profiles in the VTA, thereby impacting synaptic strength. For instance, an acute nicotine injection followed by a period of abstinence increases the AMPA/NMDA ratio in VTA dopamine neurons [45]. More specifically, nicotine application in brain slices increases AMPA/NMDA ratio, which occurs via α7* but not α4β2* nAChR mechanisms [199]. Extensions of this published work evaluating AMPA/NMDA ratio in VTA slice from rats following self-administration of nicotine, and during abstinence following self-administration, will provide further understanding of the impact of glutamatergic synaptic plasticity on nicotine-seeking behaviors. Indeed, increased synaptic strength, via altered nAChR and glutamate receptor-me-

diated plasticity, may contribute to both the maintenance of nicotine-taking behavior and to nicotine-seeking behavior.

Nucleus Accumbens

The NAc receives direct dopaminergic projections from the VTA. Many of the VTA nAChR mechanisms noted in the previous section also regulate NAc dopamine signaling, which is known to play a critical role in reward-related behavior. However, the role of NAc nAChRs in mediating nicotine reinforcement was initially unclear, given that blockade of NAc nAChRs failed to produce an attenuation of nicotine self-administration [183]. However, more recent work has revealed complex effects of nicotine and NAc nAChRs on dopamine signaling, which provides some clarity for the previously observed lack of NAc nAChR effects on nicotine self-administration. The current evidence suggests that nicotine modulates dopamine release in the NAc, and in the dorsal striatum, in a frequency-dependent manner via accumbal nAChR desensitization [46, 47]. Specifically, nicotine decreases dopamine release evoked by tonic-like firing activity, but maintains, and in some instances increases, dopamine release during phasic-like frequency stimulation – thereby acting as a frequency filter for dopamine release [46, 47, 211, 212]. This frequency gating is dependent on presynaptic β2* [47], α6* [213–215], α4* [214], and β3* nAChRs [216, 217]. Mechanistically, it has been proposed that this frequency filtering of tonic and phasic mediated dopamine release could underlie the encoding of salient, nicotine-associated stimuli [46, 47]. This has important behavioral implications, given the impact of both nicotine-paired cues and the encoding of such cues in guiding reward- or drug-seeking behavior [47, 218]. However, whether this nicotine-mediated frequency filtering directly contributes to cue-induced reinstatement remains untested.

Approximately 50% of nAChRs expressed on dopamine terminals in the striatum are of the α6α4β3β2* subtype [219]. Evidence suggests that the α6* nAChR is important for striatal dopamine release and nicotine-related behaviors [220, 221]. Indeed, intra-NAc shell infusion of α-conotoxin-MII, an α6* nAChR antagonist, reduced motivation to self-administer intravenous nicotine infusions in combination with associated cues [222]. To our knowledge, further parsing of whether α6* nAChRs mediate nicotine seeking has not been conducted, and this presents an interesting opportunity for future work. In addition to the effects of direct nicotine exposure, withdrawal from nicotine delivered via drinking water has been shown to increase basal dopamine concentrations in the NAc shell and to decrease phasic and tonic dopamine release, but with increased contrast between tonic and phasic release [223]. This change was accompanied by decreased control of β2* nAChRs over dopamine release in the NAc, as inhibition of β2* nAChRs was ineffective in attenuating phasic dopamine release in mice withdrawn from nicotine exposure [223]. Extending these studies to investigations of accumbal dopamine release during nicotine abstinence or withdrawal and subsequent cued reinstatement would provide further understanding of the role of nAChR subtypes in the mechanisms by which conditioned cues facilitate relapse.

In addition to the aforementioned effects of nicotine on NAc dopamine levels, nicotine also increases extracellular glutamate levels in the NAc [224, 225]. Nicotine-induced glutamate release can occur independently of dopamine-related mechanisms, as dopamine receptor antagonism in the NAc and denervation of dopamine efferents to the NAc had no effect on glutamate release stimulated by nicotine [225]. More recent work has shown that glutamate release in the NAc sensitizes over repeated nicotine exposures when delivered intravenously [226]. Additionally, chronic nicotine injections, at doses that result in locomotor sensitization, have been shown to increase dendritic length and spine density in the NAc shell [227], which could contribute to changes in glutamate release. These changes in dendrites and spines may underlie long-lasting behavioral sensitization to chronic nicotine exposure and could be important for nicotine-seeking behaviors. Nicotine has also been shown to regulate glutamate receptor expression. Nicotine self-administration may result in increased NR2B NMDA subunit expression in NAc shell, although these changes did not reach significance [228]. In contrast, nicotine exposure has been shown to downregulate NR2B-containing NMDA subunits on nAChR-expressing dopaminergic terminals in NAc synaptosomes [229]. Moreover, nicotine self-administration is associated with decreased mGlu2/3 receptor expression in the NAc shell [93]. Additional changes in the cysteine/glutamate exchange and glial glutamate exchange systems, important regulators of glutamate release, have also been observed after nicotine exposure [84, 86, 88]. For instance, acute activation of the cystine/glutamate exchange (xCT) system with N-acetylcysteine attenuates cue-induced nicotine reinstatement [87], effects that are mediated by mGluR 2/3 [88], although results describing the effective dose of N-acetylcysteine to suppress reinstatement are mixed. Importantly, N-acetylcysteine has been shown to reduce daily ciga-

rettes smoked in smokers [86], especially if combined with varenicline [89]. Work in human smokers testing the efficacy of N-acetylcysteine on relapse and smoking cue-related tasks is warranted. Withdrawal from nicotine self-administration also reduces expression of xCT and glutamate transporter-1 in the NAc [86]. Interestingly, reductions of xCT are not observed during withdrawal if chronic nicotine is delivered via minipump. This could indicate that nicotine-induced reduction in xCT requires phasic daily nicotine exposure, as opposed to constant nicotine exposure [86]. However, it is also possible that the xCT reduction may act as an interoceptive cue predicting the availability of nicotine. This remains to be tested.

The above nicotine-induced changes in glutamate receptor expression are consistent with the glutamate hypothesis of addiction, that drug-induced alterations in glutamate homeostasis to the NAc are critical in controlling drug-seeking behavior [62]. One body of work in particular has demonstrated that reinstatement of nicotine seeking is mediated by glutamatergic plasticity in the NAc core [84]. This work specifically demonstrated that a cue-induced reinstatement test increased extracellular glutamate, spine head diameter, and AMPA/NMDA ratio in the NAc. Withdrawal from nicotine self-administration resulted in increased expression of NR2A and NR2B subunit protein expression in the NAc core, while pharmacological inhibition of these subunits attenuated cue-induced reinstatement. These results suggest that rapid glutamatergic plasticity in the accumbens contributes to nicotine-seeking behavior elicited by re-exposure to conditioned cues previously paired with nicotine taking.

Hippocampus

One facilitator of relapse is that a drug-paired context or cue serves as a memory for the drug itself and promotes drug-seeking behavior. Therefore, nicotine-induced plasticity in regions that regulate memory consolidation and learning may also mediate nicotine-seeking behavior. Nicotine has been shown to both improve working memory and to enhance hippocampal-dependent learning [230]. In contrast, chronic nicotine administration, when delivered at high doses, enhances hippocampal neurogenesis, the growth of new neurons, specifically in the dentate gyrus region of the hippocampus [231–233]. Hippocampal neurogenesis has been observed after daily 21-h long-access sessions, but not daily 1-h short-access sessions, of nicotine self-administration [234]. In contrast, short access to nicotine self-administration has been reported to increase cell death and decrease neurogenesis in the dentate gyrus [235]. However, the relationship between changes in neurogenesis and nicotine-seeking behavior and cognition are not known. As hippocampal neurogenesis is suggested to play a role in learning and memory [236], one hypothesis is that nicotine-induced increases in neurogenesis may facilitate learned associations between nicotine and paired environmental cues, and thus facilitate cue-induced nicotine seeking during abstinence.

Nicotine induces hippocampal long-term potentiation (LTP) in vitro and in vivo, which is thought to be a neurophysiological correlate of learning and memory [237]. Specifically, acute and chronic nicotine exposure produces LTP in the CA1 [238] and dentate gyrus [239] regions of the hippocampus. In addition, in vitro activation of nAChRs, using local acetylcholine application, potentiates excitatory activity via presynaptic and postsynaptic nAChR mechanisms in hippocampal slices [240]. Systemic experimenter-administered nicotine is also sufficient to increase synaptic LTP in the dentate gyrus [241]. This systemic nicotine-induced LTP can be blocked by dopamine D1-receptor blockade, indicating that nicotine-induced hippocampal plasticity requires dopaminergic signaling [241]. This mechanism may be important for nicotine seeking, as dopamine D1 receptor blockade also blocks nicotine-conditioned place preference, which is dependent on nicotine-paired context and/or cues [241]. In the hippocampus, nAChRs are densely expressed on inhibitory interneurons [242], and nicotine-induced hippocampal LTP is at least in part dependent on local disinhibition of granule cells [243]. Nicotine-mediated hippocampal plasticity and its role in learning and plasticity have been discussed extensively [230, 244, 245].

Amygdala

The amygdala is associated with drug taking, drug reward, and relapse. While not as extensively studied as the hippocampus, the amygdala also plays an important role in the neurocircuitry mediating nicotine-taking and -seeking behaviors [246]. Like other brain regions discussed, nicotine induces synaptic plasticity in the amygdala. Chronic nicotine administered via drinking water in mice facilitates LTP in the amygdala, a process that depends on activation of α7* and β2* nAChRs [247]. Further, nicotine-induced LTP in the amygdala is long-lasting, up to 72 h following nicotine cessation. Given that these changes remain following cessation of nicotine exposure, it is possible that they are important in mediating nicotine-seeking behavior during abstinence, although

this has not been tested. Recent work has also established the basolateral amygdala (BLA) and central amygdala (CeA) as important subregions mediating nicotine seeking and craving. Stress-induced reinstatement of nicotine-seeking behavior is attenuated by activation of α2 adrenergic receptors in the CeA [143]. Similarly, blockade of dopamine D3 receptors in the BLA attenuates cue-induced reinstatement of nicotine seeking [248]. These findings suggest that CeA α2 adrenergic receptors and BLA dopamine D3 receptors differentially mediate reinstatement of nicotine seeking elicited by stress and cues. Further, inhibition of nicotine-associated neuronal ensembles, using Daun02 in the BLA [249] and CeA [250], reduced drug-seeking behavior, indicating the importance of these regions in nicotine seeking, and perhaps in nicotine craving. Future research on these neuronal circuits will provide more insights into the role of the amygdala in nicotine-seeking behaviors.

Prefrontal Cortex

The prefrontal cortex (PFC) is known to mediate drug reinforcement, extinction learning, and drug-seeking behavior. In the PFC, plasticity depends on correlative activity at pre- and postsynaptic sites, referred to as spike-timing-dependent plasticity [251, 252]. Nicotine increases the threshold for this spike-timing-dependent plasticity in medial PFC [253], while also increasing glutamatergic drive to these same regions via activation of presynaptic nAChRs [254, 255]. Both β2* and α7* nAChRs appear to be important in modulating glutamate release, as measured using PFC synaptosomes, though via different mechanisms [256]. PFC β2* nAChRs regulate glutamate release through voltage-gated calcium channels, whereas α7* nAChRs regulate glutamate release through intracellular calcium stores [256]. Chronic noncontingent nicotine administration results in the upregulation of β2* and downregulation of GluA1 and GluA2/3 in the PFC [257]. Likewise, chronic nicotine enhances NMDA currents in PFC [258]. Investigating the impact of self-administered nicotine, and its abstinence, on glutamate and nicotinic receptor expression in the PFC is an important next step to these experiments, as important differences have been noted between contingent (i.e., self-administration) and noncontingent procedures [28, 259]. Little attention has focused on the effects of withdrawal and abstinence from nicotine on plasticity or receptor function in the PFC and this should be a focus of future research questions to better understand the mechanisms underlying nicotine seeking.

Concluding Remarks and Future Considerations

An ongoing challenge for the nicotine and tobacco fields is the need to identify and develop novel, efficacious smoking cessation pharmacotherapies, especially given the modest efficacy of current tobacco cessation methods and the high rates of smoking relapse. As we have emphasized throughout this review, the identification of novel therapeutic targets can be strongly informed and guided by preclinical investigations in rodent models of nicotine addiction, including the reinstatement model of smoking relapse. Indeed, much of our understanding of the dopaminergic, glutamatergic, and cholinergic mechanisms underlying nicotine seeking and relapse behavior has been delineated from research in rodent models [67, 71, 87, 91, 92, 102]. Mechanistically, different neurotransmitter systems also interact to influence nicotine-seeking behavior and smoking relapse. As we have described, previous studies have already examined cholinergic and endocannabinoid influences on the dopamine system and how such processes mediate relapse behavior [46, 47, 128, 129, 132]. In many of the preclinical studies to date, investigators have demonstrated the efficacy of regulating extracellular neurotransmitter levels or manipulating specific neurotransmitter receptors with pharmacological antagonists or agonists (both full and partial agonists) to alter cue and/ or nicotine-primed reinstatement of drug seeking in rodents. More recently, preclinical studies have begun to screen the efficacy of PAMs of nAChRs in attenuating nicotine seeking [108]. By enhancing endogenous tone of neurotransmitter levels in the brain, PAMs may have greater efficacy than full and partial agonists in reducing nicotine seeking and produce less adverse effects. PAMs of α4β2* nAChRs and $GABA_B$ receptors have recently been shown to attenuate nicotine reinstatement, results that support pharmacological approaches towards increasing endogenous acetylcholine and GABA tone, respectively, at these receptor populations during smoking cessation [108, 125]. Moreover, as the role of specific nAChR subtypes in nicotine seeking becomes clearer, stoichiometry-selective compounds targeting specific populations of nAChRs may represent efficacious smoking cessation therapies. Consistent with this strategy, a recent study showed that a PAM of nAChRs containing three α4 subunits and two β2 subunits reduces nicotine seeking in rats [108]. From a translational perspective, these preclinical studies identify novel pharmacological approaches that attenuate nicotine-seeking behavior in rodent models, and may also be used to decrease relapse behavior in human smokers.

In addition to cue- and nicotine-induced reinstatement studies, and their therapeutic implications for smoking relapse, the field will greatly benefit from additional investigations of neurotransmitter mechanisms underlying stress-mediated relapse behavior. Preclinical studies thus far revealed important roles for the norepinephrine, endocannabinoid, and neuropeptide systems (including opiate receptor and CRF mechanisms) in stress-primed reinstatement [142, 150, 160, 260]. Another critical consideration for ongoing research will be a closer examination of gender differences and their influence on stress-mediated relapse. Indeed, emerging data from multiple studies continue to provide evidence for gender-specific differences in successful quit attempts in smokers [19] as well as sex-specific effects regarding the role of stress in nicotine use and relapse in preclinical models and in humans [261–263]. Evidence from smokers suggests that men are more sensitive to the pharmacological effects of nicotine, where women are more sensitive to smoking-related cues [264, 265], and these gender differences have important implications for smoking relapse and cessation strategies [266]. It is intriguing to think that sex-specific mechanisms underlying stress-mediated relapse and cue reactivity may be differentially targeted by novel smoking cessation pharmacotherapies to reduce relapse in male and female smokers.

In addition to these behavioral studies, significant mechanistic insight has been gained through in vitro investigations of the neuroadaptations associated with nicotine exposure. These observed neuroadaptations include nicotine-mediated upregulation of subtype-specific nAChRs [204–206], nicotine-induced synaptic plasticity [43, 181, 239, 247], nicotine-mediated frequency filtering of dopamine signaling [46, 47], nicotine-induced alterations in NAc dendritic spine length and density [227], and nicotine-mediated hippocampal neurogenesis [231–233]. The behavioral implications of these findings are often interpreted based on the brain regions in which they are identified. Specifically, nicotine-mediated neuroadaptations in specific loci are thought to play a role in nicotine reinforcement (VTA and NAc), nicotine-related learning (PFC and hippocampus), nicotine craving (VTA, NAc, amygdala, and PFC), and cue-mediated nicotine-taking and nicotine-seeking behaviors (NAc, hippocampus, and amygdala). A critical factor in these mechanistic studies is a consideration of whether the in vitro experiments were performed using behaviorally relevant nicotine doses. Admittedly, it is challenging to translate relevant nicotine doses from a behavioral in vivo experiment to physiologically relevant nicotine doses in an in vitro slice experiment. In particular, we note that nicotine concentrations used in some in vitro slice experiments may be higher than would be observed in the brain of a rodent self-administering nicotine. In some previous in vitro slices studies, lower nicotine concentrations (20–80 nM) have also been used as an estimate of nicotine concentrations that may exist in the brains of human smokers hours after cigarette smoking [184]. We note that the challenges associated with in vitro nicotine dose will continue to be an important consideration for future studies. As in vitro studies continue to be performed, we also argue that an effective approach for gaining more direct mechanistic insight into nicotine seeking will be the examination of nicotine-induced neuroadaptations in rodents, or in rodent brain slices, after nicotine self-administration and subsequent abstinence. Similarly, important insights can be gained from performing in vitro slice studies in tissue following stress-, drug-, and cue-primed reinstatement of nicotine seeking. Unfortunately, there is a paucity of literature investigating synaptic plasticity that occurs during the reinstatement of nicotine seeking. Clearly, these experiments are not trivial. Thus, such approaches will benefit from, and likely require, collaborations between in vivo- and in vitro-focused nicotine researchers. This presents an exciting opportunity for the nicotine and tobacco field that has the potential to greatly enhance our understanding of nicotine seeking and craving-induced relapse behavior.

Here, we have presented both in vivo and in vitro preclinical investigations and their importance for guiding and informing clinical research aimed at providing improved pharmacotherapies to prevent nicotine relapse. However, one challenge in translating preclinical findings to clinical investigations is the possibility that individual differences in subpopulations of tobacco users might lead to differential neuroadaptations, and therefore differential efficacy of smoking cessation pharmacotherapies [266, 267]. Therefore, these are aspects that must also be considered in the experimental design of preclinical investigations, to better inform clinical investigation (e.g., how might age or sex moderate reinstatement due to stress, drug, or environmental cues).

Another highly relevant consideration is the changing landscape of tobacco use. While rates of smoking traditional cigarettes continue to decline, use of noncombustible products (electronic cigarettes; snus) is increasing [268]. Thus, one remaining question is whether subpopulations of tobacco product users have different neurophysiological mechanisms driving nicotine seeking. Thus, researchers should keep the tobacco product popu-

lation, modeled in their studies, in mind. This could provide important information for cessation treatment across populations of tobacco users.

Developing the next generation of smoking cessation medications depends on advancing our understanding of the neurobiological and neurophysiological mechanisms underlying nicotine seeking and smoking relapse. Here, we have highlighted important strategies that could be used to develop novel smoking cessation medications. Identifying more efficacious pharmacotherapies to treat smoking relapse are needed to further decrease tobacco-related morbidity and mortality.

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Disclosure Statement

The authors do not have any conflicts of interest to declare.

References

1 Arrazola RA, Singh T, Corey CG, Husten CG, Neff LJ, Apelberg BJ, et al.; Centers for Disease Control and Prevention (CDC). Tobacco use among middle and high school students - United States, 2011-2014. MMWR Morb Mortal Wkly Rep. 2015 Apr;64(14):381–5.

2 Jamal A, King BA, Neff LJ, Whitmill J, Babb SD, Graffunder CM. Current Cigarette Smoking Among Adults - United States, 2005-2014. MMWR Morb Mortal Wkly Rep. 2016 Nov 11;65(44):1205-1211.

3 National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. Preventing Tobacco Use Among Youth and Young Adults: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US); 2012.

4 Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN, et al. 21st-century hazards of smoking and benefits of cessation in the United States. N Engl J Med. 2013 Jan;368(4):341–50.

5 Xu X, Bishop EE, Kennedy SM, Simpson SA, Pechacek TF. Annual healthcare spending attributable to cigarette smoking: an update. Am J Prev Med. 2015 Mar;48(3):326–33.

6 Taylor DH Jr, Hasselblad V, Henley SJ, Thun MJ, Sloan FA. Benefits of smoking cessation for longevity. Am J Public Health. 2002 Jun; 92(6):990–6.

7 Benowitz NL. Nicotine addiction. N Engl J Med. 2010 Jun;362(24):2295–303.

8 Garvey AJ, Bliss RE, Hitchcock JL, Heinold JW, Rosner B. Predictors of smoking relapse among self-quitters: a report from the Normative Aging Study. Addict Behav. 1992; 17(4):367–77.

9 Hughes JR. Tobacco withdrawal in self-quitters. J Consult Clin Psychol. 1992 Oct;60(5): 689–97.

10 Cahill K, Stevens S, Lancaster T. Pharmacological treatments for smoking cessation. JAMA. 2014 Jan;311(2):193–4.

11 Lerman C, LeSage MG, Perkins KA, O'Malley SS, Siegel SJ, Benowitz NL, et al. Translational research in medication development for nicotine dependence. Nat Rev Drug Discov. 2007 Sep;6(9):746–62.

12 Le Foll B, Pushparaj A, Pryslawsky Y, Forget B, Vemuri K, Makriyannis A, et al. Translational strategies for therapeutic development in nicotine addiction: rethinking the conventional bench to bedside approach. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Jul; 52:86–93.

13 Henningfield JE, Heishman SJ. The addictive role of nicotine in tobacco use. Psychopharmacology (Berl). 1995 Jan;117(1):11–3.

14 Stolerman IP, Jarvis MJ. The scientific case that nicotine is addictive. Psychopharmacology (Berl). 1995 Jan;117(1):2–10; discussion 14-20.

15 Etter JF, Eissenberg T. Dependence levels in users of electronic cigarettes, nicotine gums and tobacco cigarettes. Drug Alcohol Depend. 2015 Feb;147:68–75.

16 Boyle RG, Jensen J, Hatsukami DK, Severson HH. Measuring dependence in smokeless tobacco users. Addict Behav. 1995 Jul-Aug; 20(4):443–50.

17 Fagerström K, Eissenberg T. Dependence on tobacco and nicotine products: a case for product-specific assessment. Nicotine Tob Res. 2012 Nov;14(11):1382–90.

18 Hall BJ, Wells C, Allenby C, Lin MY, Hao I, Marshall L, et al. Differential effects of non-nicotine tobacco constituent compounds on nicotine self-administration in rats. Pharmacol Biochem Behav. 2014 May;120:103–8.

19 Smith TT, Schaff MB, Rupprecht LE, Schassburger RL, Buffalari DM, Murphy SE, et al. Effects of MAO inhibition and a combination of minor alkaloids, β-carbolines, and acetaldehyde on nicotine self-administration in adult male rats. Drug Alcohol Depend. 2015 Oct;155:243–52.

20 Caine SB, Collins GT, Thomsen M, Wright C, Lanier RK, Mello NK. Nicotine-like behavioral effects of the minor tobacco alkaloids nornicotine, anabasine, and anatabine in male rodents. Exp Clin Psychopharmacol. 2014 Feb;22(1):9–22.

21 Arnold MM, Loughlin SE, Belluzzi JD, Leslie FM. Reinforcing and neural activating effects of norharmane, a non-nicotine tobacco constituent, alone and in combination with nicotine. Neuropharmacology. 2014 Oct;85:293–304.

22 Smith TT, Rupprecht LE, Cwalina SN, Onimus MJ, Murphy SE, Donny EC, et al. Effects of Monoamine Oxidase Inhibition on the Reinforcing Properties of Low-Dose Nicotine. Neuropsychopharmacology. 2016 Aug;41(9): 2335–43.

23 Harris AC, Mattson C, Lesage MG, Keyler DE, Pentel PR. Comparison of the behavioral effects of cigarette smoke and pure nicotine in rats. Pharmacol Biochem Behav. 2010 Aug; 96(2):217–27.

24 Goodwin AK, Hiranita T, Paule MG. The Reinforcing Effects of Nicotine in Humans and Nonhuman Primates: A Review of Intravenous Self-Administration Evidence and Future Directions for Research. Nicotine Tob Res. 2015 Nov;17(11):1297–310.

25 O'Dell LE, Khroyan TV. Rodent models of nicotine reward: what do they tell us about tobacco abuse in humans? Pharmacol Biochem Behav. 2009 Feb;91(4):481–8.

26 Tuesta LM, Fowler CD, Kenny PJ. Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behavior. Biochem Pharmacol. 2011 Oct;82(8):984–95.

27 Rose JE, Corrigall WA. Nicotine self-administration in animals and humans: similarities and differences. Psychopharmacology (Berl). 1997 Mar;130(1):28–40.

28 Rupprecht LE, Smith TT, Schassburger RL, Buffalari DM, Sved AF, Donny EC. Behavioral mechanisms underlying nicotine reinforcement. Curr Top Behav Neurosci. 2015;24:19–53.
29 Gourlay SG, Benowitz NL. Arteriovenous differences in plasma concentration of nicotine and catecholamines and related cardiovascular effects after smoking, nicotine nasal spray, and intravenous nicotine. Clin Pharmacol Ther. 1997 Oct;62(4):453–63.
30 Shoaib M, Stolerman IP. Plasma nicotine and cotinine levels following intravenous nicotine self-administration in rats. Psychopharmacology (Berl). 1999 Apr;143(3):318–21.
31 Coen KM, Adamson KL, Corrigall WA. Medication-related pharmacological manipulations of nicotine self-administration in the rat maintained on fixed- and progressive-ratio schedules of reinforcement. Psychopharmacology (Berl). 2009 Jan;201(4):557–68.
32 Rauhut AS, Neugebauer N, Dwoskin LP, Bardo MT. Effect of bupropion on nicotine self-administration in rats. Psychopharmacology (Berl). 2003 Aug;169(1):1–9.
33 George O, Lloyd A, Carroll FI, Damaj MI, Koob GF. Varenicline blocks nicotine intake in rats with extended access to nicotine self-administration. Psychopharmacology (Berl). 2011 Feb;213(4):715–22.
34 Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl). 2003 Jul;168(1-2):3–20.
35 de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl). 1981;75(2):134–43.
36 Stoker AK, Markou A. Neurobiological Bases of Cue- and Nicotine-induced Reinstatement of Nicotine Seeking: Implications for the Development of Smoking Cessation Medications. Curr Top Behav Neurosci. 2015;24:125–54.
37 Hopkins TJ, Rupprecht LE, Hayes MR, Blendy JA, Schmidt HD. Galantamine, an acetylcholinesterase inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors, attenuates nicotine taking and seeking in rats. Neuropsychopharmacology. 2012 Sep;37(10):2310–21.
38 Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, et al. Cue dependency of nicotine self-administration and smoking. Pharmacol Biochem Behav. 2001 Dec;70(4):515–30.
39 Epstein DH, Preston KL, Stewart J, Shaham Y. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology (Berl). 2006 Nov;189(1):1–16.
40 O'Connor EC, Parker D, Rollema H, Mead AN. The alpha4beta2 nicotinic acetylcholine-receptor partial agonist varenicline inhibits both nicotine self-administration following repeated dosing and reinstatement of nicotine seeking in rats. Psychopharmacology (Berl). 2010 Feb;208(3):365–76.
41 Le Foll B, Chakraborty-Chatterjee M, Lev-Ran S, Barnes C, Pushparaj A, Gamaleddin I, et al. Varenicline decreases nicotine self-administration and cue-induced reinstatement of nicotine-seeking behaviour in rats when a long pretreatment time is used. Int J Neuropsychopharmacol. 2012 Oct;15(9):1265–74.
42 Hays JT, Ebbert JO, Sood A. Efficacy and safety of varenicline for smoking cessation. Am J Med. 2008 Apr;121(4 Suppl 1):S32–42.
43 Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron. 2000 Aug;27(2):349–57.
44 Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR. Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing α4 and α6 subunits. Mol Pharmacol. 2012 Apr;81(4):541–8.
45 Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron. 2003 Feb;37(4):577–82.
46 Zhang H, Sulzer D. Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci. 2004 Jun;7(6):581–2.
47 Rice ME, Cragg SJ. Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci. 2004 Jun;7(6):583–4.
48 Pierce RC, Kumaresan V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev. 2006;30(2):215–38.
49 Laviolette SR, van der Kooy D. The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. Nat Rev Neurosci. 2004 Jan;5(1):55–65.
50 Damsma G, Day J, Fibiger HC. Lack of tolerance to nicotine-induced dopamine release in the nucleus accumbens. Eur J Pharmacol. 1989 Sep;168(3):363–8.
51 Benwell ME, Balfour DJ, Lucchi HM. Influence of tetrodotoxin and calcium on changes in extracellular dopamine levels evoked by systemic nicotine. Psychopharmacology (Berl). 1993;112(4):467–74.
52 Nisell M, Nomikos GG, Svensson TH. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse. 1994 Jan;16(1):36–44.
53 Pontieri FE, Tanda G, Orzi F, Di Chiara G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature. 1996 Jul;382(6588):255–7.
54 Ferrari R, Le Novère N, Picciotto MR, Changeux JP, Zoli M. Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. Eur J Neurosci. 2002 Jun;15(11):1810–8.
55 Mifsud JC, Hernandez L, Hoebel BG. Nicotine infused into the nucleus accumbens increases synaptic dopamine as measured by in vivo microdialysis. Brain Res. 1989 Jan;478(2):365–7.
56 Rahman S, Zhang J, Engleman EA, Corrigall WA. Neuroadaptive changes in the mesoaccumbens dopamine system after chronic nicotine self-administration: a microdialysis study. Neuroscience. 2004;129(2):415–24.
57 Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, et al. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron. 2006 Jun;50(6):911–21.
58 Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, et al. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature. 1998 Jan;391(6663):173–7.
59 Changeux JP. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. Nat Rev Neurosci. 2010 Jun;11(6):389–401.
60 Li X, Semenova S, D'Souza MS, Stoker AK, Markou A. Involvement of glutamatergic and GABAergic systems in nicotine dependence: Implications for novel pharmacotherapies for smoking cessation. Neuropharmacology. 2014 Jan;76 Pt B:554-65.
61 Liechti ME, Markou A. Role of the glutamatergic system in nicotine dependence : implications for the discovery and development of new pharmacological smoking cessation therapies. CNS Drugs. 2008;22(9):705–24.
62 Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 2009 Aug;10(8):561–72.
63 Fu Y, Matta SG, Gao W, Brower VG, Sharp BM. Systemic nicotine stimulates dopamine release in nucleus accumbens: re-evaluation of the role of N-methyl-D-aspartate receptors in the ventral tegmental area. J Pharmacol Exp Ther. 2000 Aug;294(2):458–65.
64 Civelli O, Bunzow JR, Grandy DK. Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol. 1993;33(1):281–307.
65 Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev. 1998 Jan;78(1):189–225.
66 Seeman P, Van Tol HH. Dopamine receptor pharmacology. Curr Opin Neurol Neurosurg. 1993 Aug;6(4):602–8.
67 Liu X, Jernigen C, Gharib M, Booth S, Caggiula AR, Sved AF. Effects of dopamine antagonists on drug cue-induced reinstatement of nicotine-seeking behavior in rats. Behav Pharmacol. 2010 Mar;21(2):153–60.
68 Hutchison KE, LaChance H, Niaura R, Bryan A, Smolen A. The DRD4 VNTR polymorphism influences reactivity to smoking cues. J Abnorm Psychol. 2002 Feb;111(1):134–43.
69 McClernon FJ, Hutchison KE, Rose JE, Kozink RV. DRD4 VNTR polymorphism is associated with transient fMRI-BOLD responses to smoking cues. Psychopharmacology (Berl). 2007 Nov;194(4):433–41.

70 Munafò MR, Johnstone EC. Smoking status moderates the association of the dopamine D4 receptor (DRD4) gene VNTR polymorphism with selective processing of smoking-related cues. Addict Biol. 2008 Sep;13(3-4):435–9.
71 Yan Y, Pushparaj A, Le Strat Y, Gamaleddin I, Barnes C, Justinova Z, et al. Blockade of dopamine d4 receptors attenuates reinstatement of extinguished nicotine-seeking behavior in rats. Neuropsychopharmacology. 2012 Feb;37(3):685–96.
72 Andreoli M, Tessari M, Pilla M, Valerio E, Hagan JJ, Heidbreder CA. Selective antagonism at dopamine D3 receptors prevents nicotine-triggered relapse to nicotine-seeking behavior. Neuropsychopharmacology. 2003 Jul;28(7):1272-80.
73 Micheli F, Bonanomi G, Blaney FE, Braggio S, Capelli AM, Checchia A, et al. 1,2,4-triazol-3-yl-thiopropyl-tetrahydrobenzazepines: a series of potent and selective dopamine D(3) receptor antagonists. J Med Chem. 2007 Oct;50(21):5076–89.
74 Khaled MA, Farid Araki K, Li B, Coen KM, Marinelli PW, Varga J, et al. The selective dopamine D3 receptor antagonist SB 277011-A, but not the partial agonist BP 897, blocks cue-induced reinstatement of nicotine-seeking. Int J Neuropsychopharmacol. 2010 Mar;13(2):181–90.
75 Le Foll B, Diaz J, Sokoloff P. Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. Synapse. 2003 Mar;47(3):176–83.
76 Diaz J, Lévesque D, Lammers CH, Griffon N, Martres MP, Schwartz JC, et al. Phenotypical characterization of neurons expressing the dopamine D3 receptor in the rat brain. Neuroscience. 1995 Apr;65(3):731–45.
77 Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res. 1991 Nov;564(2):203–19.
78 Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature. 1990 Sep;347(6289):146–51.
79 Scherma M, Panlilio LV, Fadda P, Fattore L, Gamaleddin I, Le Foll B, et al. Inhibition of anandamide hydrolysis by cyclohexyl carbamic acid 3′-carbamoyl-3-yl ester (URB597) reverses abuse-related behavioral and neurochemical effects of nicotine in rats. J Pharmacol Exp Ther. 2008 Nov;327(2):482–90.
80 Melis M, Pillolla G, Luchicchi A, Muntoni AL, Yasar S, Goldberg SR, et al. Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. J Neurosci. 2008 Dec;28(51):13985–94.
81 Forget B, Coen KM, Le Foll B. Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration—comparison with CB(1) receptor blockade. Psychopharmacology (Berl). 2009 Sep;205(4):613–24.
82 Mascia P, Pistis M, Justinova Z, Panlilio LV, Luchicchi A, Lecca S, et al. Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. Biol Psychiatry. 2011 Apr;69(7):633–41.
83 Panlilio LV, Justinova Z, Mascia P, Pistis M, Luchicchi A, Lecca S, et al. Novel use of a lipid-lowering fibrate medication to prevent nicotine reward and relapse: preclinical findings. Neuropsychopharmacology. 2012 Jul;37(8):1838–47.
84 Gipson CD, Reissner KJ, Kupchik YM, Smith AC, Stankeviciute N, Hensley-Simon ME, et al. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. Proc Natl Acad Sci USA. 2013 May;110(22):9124–9.
85 Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, et al. The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities. Antioxid Redox Signal. 2013 Feb;18(5):522–55.
86 Knackstedt LA, LaRowe S, Mardikian P, Malcolm R, Upadhyaya H, Hedden S, et al. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. Biol Psychiatry. 2009 May;65(10):841–5.
87 Ramirez-Niño AM, D'Souza MS, Markou A. N-acetylcysteine decreased nicotine self-administration and cue-induced reinstatement of nicotine seeking in rats: comparison with the effects of N-acetylcysteine on food responding and food seeking. Psychopharmacology (Berl). 2013 Jan;225(2):473–82.
88 Moro F, Orrù A, Marzo CM, Di Clemente A, Cervo L. mGluR2/3 mediates short-term control of nicotine-seeking by acute systemic N-acetylcysteine. Addict Biol. 2018 Jan;23(1):28-40.
89 McClure EA, Baker NL, Gipson CD, Carpenter MJ, Roper AP, Froeliger BE, et al. An open-label pilot trial of N-acetylcysteine and varenicline in adult cigarette smokers. Am J Drug Alcohol Abuse. 2015 Jan;41(1):52–6.
90 D'Souza MS, Markou A. Differential role of N-methyl-D-aspartate receptor-mediated glutamate transmission in the nucleus accumbens shell and core in nicotine seeking in rats. Eur J Neurosci. 2014 Apr;39(8):1314–22.
91 Bespalov AY, Dravolina OA, Sukhanov I, Zakharova E, Blokhina E, Zvartau E, et al. Metabotropic glutamate receptor (mGluR5) antagonist MPEP attenuated cue- and schedule-induced reinstatement of nicotine self-administration behavior in rats. Neuropharmacology. 2005;49 Suppl 1:167–78.
92 Dravolina OA, Zakharova ES, Shekunova EV, Zvartau EE, Danysz W, Bespalov AY. mGlu1 receptor blockade attenuates cue- and nicotine-induced reinstatement of extinguished nicotine self-administration behavior in rats. Neuropharmacology. 2007 Feb;52(2):263–9.
93 Liechti ME, Lhuillier L, Kaupmann K, Markou A. Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. J Neurosci. 2007 Aug;27(34):9077–85.
94 Justinova Z, Panlilio LV, Secci ME, Redhi GH, Schindler CW, Cross AJ, et al. The Novel Metabotropic Glutamate Receptor 2 Positive Allosteric Modulator, AZD8529, Decreases Nicotine Self-Administration and Relapse in Squirrel Monkeys. Biol Psychiatry. 2015 Oct;78(7):452–62.
95 Li X, D'Souza MS, Niño AM, Doherty J, Cross A, Markou A. Attenuation of nicotine-taking and nicotine-seeking behavior by the mGlu2 receptor positive allosteric modulators AZD8418 and AZD8529 in rats. Psychopharmacology (Berl). 2016 May;233(10):1801–14.
96 Acri JB, Cross AJ, Skolnick P. From bench to bedside: mGluR2 positive allosteric modulators as medications to treat substance use disorders. Psychopharmacology (Berl). 2017 May;234(9-10):1347-1355.
97 Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. J Med Chem. 2005 May;48(10):3474–7.
98 Wouda JA, Riga D, De Vries W, Stegeman M, van Mourik Y, Schetters D, et al. Varenicline attenuates cue-induced relapse to alcohol, but not nicotine seeking, while reducing inhibitory response control. Psychopharmacology (Berl). 2011 Jul;216(2):267–77.
99 Liu X. Effects of blockade of α4β2 and α7 nicotinic acetylcholine receptors on cue-induced reinstatement of nicotine-seeking behaviour in rats. Int J Neuropsychopharmacol. 2014 Jan;17(1):105–16.
100 Li S, Li Z, Pei L, Le AD, Liu F. The α7nACh-NMDA receptor complex is involved in cue-induced reinstatement of nicotine seeking. J Exp Med. 2012 Nov;209(12):2141–7.
101 Lee AM, Arreola AC, Kimmey BA, Schmidt HD. Administration of the nicotinic acetylcholine receptor agonists ABT-089 and ABT-107 attenuates the reinstatement of nicotine-seeking behavior in rats. Behav Brain Res. 2014 Nov;274:168–75.
102 Cippitelli A, Wu J, Gaiolini KA, Mercatelli D, Schoch J, Gorman M, et al. AT-1001: a high-affinity α3β4 nAChR ligand with novel nicotine-suppressive pharmacology. Br J Pharmacol. 2015 Apr;172(7):1834–45.

103 Williams DK, Wang J, Papke RL. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. Biochem Pharmacol. 2011 Oct;82(8):915–30.
104 Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. Mol Pharmacol. 2003 Feb;63(2):332–41.
105 Moroni M, Zwart R, Sher E, Cassels BK, Bermudez I. alpha4beta2 nicotinic receptors with high and low acetylcholine sensitivity: pharmacology, stoichiometry, and sensitivity to long-term exposure to nicotine. Mol Pharmacol. 2006 Aug;70(2):755–68.
106 Anderson DJ, Malysz J, Grønlien JH, El Kouhen R, Håkerud M, Wetterstrand C, et al. Stimulation of dopamine release by nicotinic acetylcholine receptor ligands in rat brain slices correlates with the profile of high, but not low, sensitivity alpha4beta2 subunit combination. Biochem Pharmacol. 2009 Oct;78(7):844–51.
107 Harmey D, Griffin PR, Kenny PJ. Development of novel pharmacotherapeutics for tobacco dependence: progress and future directions. Nicotine Tob Res. 2012 Nov; 14(11):1300–18.
108 Maurer JJ, Sandager-Nielsen K, Schmidt HD. Attenuation of nicotine taking and seeking in rats by the stoichiometry-selective alpha4beta2 nicotinic acetylcholine receptor positive allosteric modulator NS9283. Psychopharmacology (Berl). 2017 Feb;234(3):475–84.
109 Harvey AL. The pharmacology of galanthamine and its analogues. Pharmacol Ther. 1995;68(1):113–28.
110 Kosasa T, Kuriya Y, Matsui K, Yamanishi Y. Inhibitory effects of donepezil hydrochloride (E2020) on cholinesterase activity in brain and peripheral tissues of young and aged rats. Eur J Pharmacol. 1999 Dec; 386(1):7–13.
111 Kimmey BA, Rupprecht LE, Hayes MR, Schmidt HD. Donepezil, an acetylcholinesterase inhibitor, attenuates nicotine self-administration and reinstatement of nicotine seeking in rats. Addict Biol. 2014 Jul;19(4): 539–51.
112 Ashare RL, Kimmey BA, Rupprecht LE, Bowers ME, Hayes MR, Schmidt HD. Repeated administration of an acetylcholinesterase inhibitor attenuates nicotine taking in rats and smoking behavior in human smokers. Transl Psychiatry. 2016 Jan; 6(1):e713.
113 Wilkinson DS, Gould TJ. The effects of galantamine on nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6 mice. Behav Brain Res. 2011 Sep;223(1):53–7.
114 Sofuoglu M, Herman AI, Li Y, Waters AJ. Galantamine attenuates some of the subjective effects of intravenous nicotine and improves performance on a Go No-Go task in abstinent cigarette smokers: a preliminary report. Psychopharmacology (Berl). 2012 Dec;224(3):413–20.
115 Loughead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, et al. Working memory-related neural activity predicts future smoking relapse. Neuropsychopharmacology. 2015 May;40(6):1311–20.
116 Patterson F, Jepson C, Loughead J, Perkins K, Strasser AA, Siegel S, et al. Working memory deficits predict short-term smoking resumption following brief abstinence. Drug Alcohol Depend. 2010 Jan;106(1):61–4.
117 Ashare RL, Schmidt HD. Optimizing treatments for nicotine dependence by increasing cognitive performance during withdrawal. Expert Opin Drug Discov. 2014 Jun; 9(6):579–94.
118 van Zessen R, Phillips JL, Budygin EA, Stuber GD. Activation of VTA GABA neurons disrupts reward consumption. Neuron. 2012 Mar;73(6):1184–94.
119 Morales M, Margolis EB. Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev Neurosci. 2017 Feb; 18(2):73–85.
120 Paterson NE, Froestl W, Markou A. Repeated administration of the GABAB receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. Neuropsychopharmacology. 2005 Jan;30(1):119–28.
121 Fattore L, Spano MS, Cossu G, Scherma M, Fratta W, Fadda P. Baclofen prevents drug-induced reinstatement of extinguished nicotine-seeking behaviour and nicotine place preference in rodents. Eur Neuropsychopharmacol. 2009 Jul;19(7):487–98.
122 Franklin TR, Harper D, Kampman K, Kildea-McCrea S, Jens W, Lynch KG, et al. The GABA B agonist baclofen reduces cigarette consumption in a preliminary double-blind placebo-controlled smoking reduction study. Drug Alcohol Depend. 2009 Jul; 103(1-2):30–6.
123 Ong J, Kerr DI. Clinical potential of GABAB receptor modulators. CNS Drug Rev. 2005; 11(3):317–34.
124 Urwyler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, et al. N,N′-Dicyclopentyl-2-methylsulfanyl-5-nitropyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor function. J Pharmacol Exp Ther. 2003 Oct;307(1):322–30.
125 Vlachou S, Guery S, Froestl W, Banerjee D, Benedict J, Finn MG, et al. Repeated administration of the GABAB receptor positive modulator BHF177 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine seeking in rats. Psychopharmacology (Berl). 2011 May; 215(1):117–28.
126 Lubbers BR, van Mourik Y, Schetters D, Smit AB, De Vries TJ, Spijker S. Prefrontal gamma-aminobutyric acid type A receptor insertion controls cue-induced relapse to nicotine seeking. Biol Psychiatry. 2014 Nov; 76(9):750–8.
127 Wiskerke J, Pattij T, Schoffelmeer AN, De Vries TJ. The role of CB1 receptors in psychostimulant addiction. Addict Biol. 2008 Jun;13(2):225–38.
128 Panagis G, Mackey B, Vlachou S. Cannabinoid Regulation of Brain Reward Processing with an Emphasis on the Role of CB1 Receptors: A Step Back into the Future. Front Psychiatry. 2014 Jul;5:92.
129 De Vries TJ, de Vries W, Janssen MC, Schoffelmeer AN. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. Behav Brain Res. 2005 Jun;161(1):164–8.
130 Diergaarde L, de Vries W, Raasø H, Schoffelmeer AN, De Vries TJ. Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor antagonist Rimonabant (SR141716A). Neuropharmacology. 2008 Oct;55(5):712–6.
131 Schindler CW, Redhi GH, Vemuri K, Makriyannis A, Le Foll B, Bergman J, et al. Blockade of Nicotine and Cannabinoid Reinforcement and Relapse by a Cannabinoid CB1-Receptor Neutral Antagonist AM4113 and Inverse Agonist Rimonabant in Squirrel Monkeys. Neuropsychopharmacology. 2016 Aug;41(9):2283–93.
132 Shoaib M. The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats. Neuropharmacology. 2008 Feb;54(2):438–44.
133 Gamaleddin I, Zvonok A, Makriyannis A, Goldberg SR, Le Foll B. Effects of a selective cannabinoid CB2 agonist and antagonist on intravenous nicotine self administration and reinstatement of nicotine seeking. PLoS One. 2012;7(1):e29900.
134 Justinova Z, Panlilio LV, Moreno-Sanz G, Redhi GH, Auber A, Secci ME, et al. Effects of Fatty Acid Amide Hydrolase (FAAH) Inhibitors in Non-Human Primate Models of Nicotine Reward and Relapse. Neuropsychopharmacology. 2015 Aug;40(9):2185–97.
135 Justinova Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, Clapper JR, et al. Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. Biol Psychiatry. 2008 Dec;64(11):930–7.
136 Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G, et al. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. J Pharmacol Exp Ther. 2005 Apr; 313(1):352–8.

137 Forget B, Guranda M, Gamaleddin I, Goldberg SR, Le Foll B. Attenuation of cue-induced reinstatement of nicotine seeking by URB597 through cannabinoid CB1 receptor in rats. Psychopharmacology (Berl). 2016 May;233(10):1823–8.
138 Gamaleddin I, Guranda M, Goldberg SR, Le Foll B. The selective anandamide transport inhibitor VDM11 attenuates reinstatement of nicotine seeking behaviour, but does not affect nicotine intake. Br J Pharmacol. 2011 Nov;164(6):1652–60.
139 Gamaleddin I, Guranda M, Scherma M, Fratta W, Makriyannis A, Vadivel SK, et al. AM404 attenuates reinstatement of nicotine seeking induced by nicotine-associated cues and nicotine priming but does not affect nicotine- and food-taking. J Psychopharmacol. 2013 Jun;27(6):564–71.
140 Scherma M, Justinová Z, Zanettini C, Panlilio LV, Mascia P, Fadda P, et al. The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. Br J Pharmacol. 2012 Apr;165(8):2539–48.
141 Mantsch JR, Baker DA, Funk D, Lê AD, Shaham Y. Stress-Induced Reinstatement of Drug Seeking: 20 Years of Progress. Neuropsychopharmacology. 2016 Jan;41(1):335–56.
142 Zislis G, Desai TV, Prado M, Shah HP, Bruijnzeel AW. Effects of the CRF receptor antagonist D-Phe CRF(12-41) and the alpha2-adrenergic receptor agonist clonidine on stress-induced reinstatement of nicotine-seeking behavior in rats. Neuropharmacology. 2007 Dec;53(8):958–66.
143 Yamada H, Bruijnzeel AW. Stimulation of α2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. Neuropharmacology. 2011 Feb-Mar;60(2-3):303–11.
144 Forget B, Wertheim C, Mascia P, Pushparaj A, Goldberg SR, Le Foll B. Noradrenergic alpha1 receptors as a novel target for the treatment of nicotine addiction. Neuropsychopharmacology. 2010 Jul;35(8):1751–60.
145 McKee SA, Potenza MN, Kober H, Sofuoglu M, Arnsten AF, Picciotto MR, et al. A translational investigation targeting stress-reactivity and prefrontal cognitive control with guanfacine for smoking cessation. J Psychopharmacol. 2015 Mar;29(3):300–11.
146 Fox HC, Seo D, Tuit K, Hansen J, Kimmerling A, Morgan PT, et al. Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. J Psychopharmacol. 2012 Jul;26(7):958–72.
147 Fox HC, Morgan PT, Sinha R. Sex differences in guanfacine effects on drug craving and stress arousal in cocaine-dependent individuals. Neuropsychopharmacology. 2014 May;39(6):1527–37.
148 Chiamulera C, Tedesco V, Zangrandi L, Giuliano C, Fumagalli G. Propranolol transiently inhibits reinstatement of nicotine-seeking behaviour in rats. J Psychopharmacol. 2010 Mar;24(3):389–95.
149 Xue YX, Deng JH, Chen YY, Zhang LB, Wu P, Huang GD, et al. Effect of Selective Inhibition of Reactivated Nicotine-Associated Memories With Propranolol on Nicotine Craving. JAMA Psychiatry. 2017 Mar;74(3):224–32.
150 Fletcher PJ, Rizos Z, Noble K, Soko AD, Silenieks LB, Lê AD, et al. Effects of the 5-HT2C receptor agonist Ro60-0175 and the 5-HT2A receptor antagonist M100907 on nicotine self-administration and reinstatement. Neuropharmacology. 2012 Jun;62(7):2288–98.
151 Ichikawa J, Kuroki T, Kitchen MT, Meltzer HY. R(+)-8-OH-DPAT, a 5-HT1A receptor agonist, inhibits amphetamine-induced dopamine release in rat striatum and nucleus accumbens. Eur J Pharmacol. 1995 Dec;287(2):179–84.
152 Pehek EA, McFarlane HG, Maguschak K, Price B, Pluto CP. M100,907, a selective 5-HT(2A) antagonist, attenuates dopamine release in the rat medial prefrontal cortex. Brain Res. 2001 Jan;888(1):51–9.
153 Yan Q, Reith ME, Yan S. Enhanced accumbal dopamine release following 5-HT(2A) receptor stimulation in rats pretreated with intermittent cocaine. Brain Res. 2000 Apr;863(1-2):254–8.
154 Auclair A, Blanc G, Glowinski J, Tassin JP. Role of serotonin 2A receptors in the D-amphetamine-induced release of dopamine: comparison with previous data on alpha1b-adrenergic receptors. J Neurochem. 2004 Oct;91(2):318–26.
155 Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdère P, Caccia S, et al. 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. Neuropsychopharmacology. 2002 Mar;26(3):311–24.
156 De Deurwaerdere P, Di Giovanni G. Serotonergic modulation of the activity of mesencephalic dopaminergic systems: therapeutic implications. Prog Neurobiol. 2017 Apr;151:175-236.
157 Bruijnzeel AW. Neuropeptide systems and new treatments for nicotine addiction. Psychopharmacology (Berl). 2017 May;234(9-10):1419-1437.
158 Shippenberg TS, Zapata A, Chefer VI. Dynorphin and the pathophysiology of drug addiction. Pharmacol Ther. 2007 Nov;116(2):306–21.
159 Van't Veer A, Carlezon WA Jr. Role of kappa-opioid receptors in stress and anxiety-related behavior. Psychopharmacology (Berl). 2013 Oct;229(3):435–52.
160 Grella SL, Funk D, Coen K, Li Z, Lê AD. Role of the kappa-opioid receptor system in stress-induced reinstatement of nicotine seeking in rats. Behav Brain Res. 2014 May;265:188–97.
161 Liu X, Palmatier MI, Caggiula AR, Sved AF, Donny EC, Gharib M, et al. Naltrexone attenuation of conditioned but not primary reinforcement of nicotine in rats. Psychopharmacology (Berl). 2009 Mar;202(4):589–98.
162 Bruijnzeel AW, Prado M, Isaac S. Corticotropin-releasing factor-1 receptor activation mediates nicotine withdrawal-induced deficit in brain reward function and stress-induced relapse. Biol Psychiatry. 2009 Jul;66(2):110–7.
163 Plaza-Zabala A, Martín-García E, de Lecea L, Maldonado R, Berrendero F. Hypocretins regulate the anxiogenic-like effects of nicotine and induce reinstatement of nicotine-seeking behavior. J Neurosci. 2010 Feb;30(6):2300–10.
164 George O, Ghozland S, Azar MR, Cottone P, Zorrilla EP, Parsons LH, et al. CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. Proc Natl Acad Sci USA. 2007 Oct;104(43):17198–203.
165 Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. Proc Natl Acad Sci USA. 2008 Dec;105(49):19480–5.
166 de Lecea L, Jones BE, Boutrel B, Borgland SL, Nishino S, Bubser M, et al. Addiction and arousal: alternative roles of hypothalamic peptides. J Neurosci. 2006 Oct;26(41):10372–5.
167 Plaza-Zabala A, Flores Á, Martín-García E, Saravia R, Maldonado R, Berrendero F. A role for hypocretin/orexin receptor-1 in cue-induced reinstatement of nicotine-seeking behavior. Neuropsychopharmacology. 2013 Aug;38(9):1724–36.
168 Uslaner JM, Winrow CJ, Gotter AL, Roecker AJ, Coleman PJ, Hutson PH, et al. Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement. Behav Brain Res. 2014 Aug;269:61–5.
169 Qi X, Yamada H, Corrie LW, Ji Y, Bauzo RM, Alexander JC, et al. A critical role for the melanocortin 4 receptor in stress-induced relapse to nicotine seeking in rats. Addict Biol. 2015 Mar;20(2):324–35.
170 Engel JA, Jerlhag E. Role of appetite-regulating peptides in the pathophysiology of addiction: implications for pharmacotherapy. CNS Drugs. 2014 Oct;28(10):875–86.
171 Hayes MR, Schmidt HD. GLP-1 influences food and drug reward. Curr Opin Behav Sci. 2016 Jun;9:66–70.

172 Egecioglu E, Engel JA, Jerlhag E. The glucagon-like peptide 1 analogue Exendin-4 attenuates the nicotine-induced locomotor stimulation, accumbal dopamine release, conditioned place preference as well as the expression of locomotor sensitization in mice. PLoS One. 2013 Oct;8(10):e77284.
173 Tuesta LM, Chen Z, Duncan A, Fowler CD, Ishikawa M, Lee BR, et al. GLP-1 acts on habenular avoidance circuits to control nicotine intake. Nat Neurosci. 2017 May;20(5):708–16.
174 Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res. 1994 Aug;653(1-2):278–84.
175 Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, et al. Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. J Neurosci. 2008 Nov;28(47):12318–27.
176 Subramaniyan M, Dani JA. Dopaminergic and cholinergic learning mechanisms in nicotine addiction. Ann N Y Acad Sci. 2015 Sep;1349(1):46–63.
177 Gould TJ, Leach PT. Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. Neurobiol Learn Mem. 2014 Jan;107:108–32.
178 Mansvelder HD, Mertz M, Role LW. Nicotinic modulation of synaptic transmission and plasticity in cortico-limbic circuits. Semin Cell Dev Biol. 2009 Jun;20(4):432–40.
179 Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA. Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature. 1996 Oct;383(6602):713–6.
180 Radcliffe KA, Dani JA. Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission. J Neurosci. 1998 Sep;18(18):7075–83.
181 Fujii S, Ji Z, Morita N, Sumikawa K. Acute and chronic nicotine exposure differentially facilitate the induction of LTP. Brain Res. 1999 Oct;846(1):137–43.
182 Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. Neuron. 2002 Mar;33(6):905–19.
183 Exley R, Cragg SJ. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. Br J Pharmacol. 2008 Mar;153(S1 Suppl 1):S283–97.
184 Wooltorton JR, Pidoplichko VI, Broide RS, Dani JA. Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. J Neurosci. 2003 Apr;23(8):3176–85.
185 De Biasi M, Dani JA. Reward, addiction, withdrawal to nicotine. Annu Rev Neurosci. 2011;34(1):105–30.
186 Grieder TE, Herman MA, Contet C, Tan LA, Vargas-Perez H, Cohen A, et al. VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. Nat Neurosci. 2014 Dec;17(12):1751–8.
187 Corrigall WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology (Berl). 1992;107(2-3):285–9.
188 Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science. 2004 Nov;306(5698):1029–32.
189 Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons—3. Evidence for electrotonic coupling. Neuroscience. 1983 Oct;10(2):333–48.
190 Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci. 1984 Nov;4(11):2877–90.
191 Hyland BI, Reynolds JN, Hay J, Perk CG, Miller R. Firing modes of midbrain dopamine cells in the freely moving rat. Neuroscience. 2002;114(2):475–92.
192 Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM. Subsecond dopamine release promotes cocaine seeking. Nature. 2003 Apr;422(6932):614–8.
193 Stuber GD, Wightman RM, Carelli RM. Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. Neuron. 2005 May;46(4):661–9.
194 Solecki W, Wickham RJ, Behrens S, Wang J, Zwerling B, Mason GF, et al. Differential role of ventral tegmental area acetylcholine and N-methyl-D-aspartate receptors in cocaine-seeking. Neuropharmacology. 2013 Dec;75:9–18.
195 Grenhoff J, Aston-Jones G, Svensson TH. Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol Scand. 1986 Nov;128(3):351–8.
196 Schilström B, Rawal N, Mameli-Engvall M, Nomikos GG, Svensson TH. Dual effects of nicotine on dopamine neurons mediated by different nicotinic receptor subtypes. Int J Neuropsychopharmacol. 2003 Mar;6(1):1–11.
197 Pidoplichko VI, DeBiasi M, Williams JT, Dani JA. Nicotine activates and desensitizes midbrain dopamine neurons. Nature. 1997 Nov;390(6658):401–4.
198 Lester RA, Dani JA. Time-dependent changes in central nicotinic acetylcholine channel kinetics in excised patches. Neuropharmacology. 1994 Jan;33(1):27–34.
199 Yang K, Buhlman L, Khan GM, Nichols RA, Jin G, McIntosh JM, et al. Functional nicotinic acetylcholine receptors containing α6 subunits are on GABAergic neuronal boutons adherent to ventral tegmental area dopamine neurons. J Neurosci. 2011 Feb;31(7):2537–48.
200 Tolu S, Eddine R, Marti F, David V, Graupner M, Pons S, et al. Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. Mol Psychiatry. 2013 Mar;18(3):382–93.
201 Hukkanen J, Jacob P 3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev. 2005 Mar;57(1):79–115.
202 Rose JS, Dierker LC, Donny E. Nicotine dependence symptoms among recent onset adolescent smokers. Drug Alcohol Depend. 2010 Jan;106(2-3):126–32.
203 Donny EC, Caggiula AR, Mielke MM, Jacobs KS, Rose C, Sved AF. Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. Psychopharmacology (Berl). 1998 Mar;136(1):83–90.
204 Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldovan V, Booth S, et al. Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. Psychopharmacology (Berl). 2000 Sep;151(4):392–405.
205 Schwartz RD, Kellar KJ. Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. Science. 1983 Apr;220(4593):214–6.
206 Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. Mol Pharmacol. 1992 Jan;41(1):31–7.
207 Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzler E, Frohlich E, Perry E, et al. Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. J Neurosci. 2006 Aug;26(34):8707–14.
208 Nashmi R, Dickinson ME, McKinney S, Jareb M, Labarca C, Fraser SE, et al. Assembly of alpha4beta2 nicotinic acetylcholine receptors assessed with functional fluorescently labeled subunits: effects of localization, trafficking, and nicotine-induced upregulation in clonal mammalian cells and in cultured midbrain neurons. J Neurosci. 2003 Dec;23(37):11554–67.
209 Mugnaini M, Tessari M, Tarter G, Merlo Pich E, Chiamulera C, Bunnemann B. Upregulation of [3H]methyllycaconitine binding sites following continuous infusion of nicotine, without changes of alpha7 or alpha6 subunit mRNA: an autoradiography and in situ hybridization study in rat brain. Eur J Neurosci. 2002 Nov;16(9):1633–46.
210 Ngolab J, Liu L, Zhao-Shea R, Gao G, Gardner PD, Tapper AR. Functional Upregulation of α4* Nicotinic Acetylcholine Receptors in VTA GABAergic Neurons Increases Sensitivity to Nicotine Reward. J Neurosci. 2015 Jun;35(22):8570–8.
211 Zhou FM, Liang Y, Dani JA. Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci. 2001 Dec;4(12):1224–9.

212 Zhang T, Zhang L, Liang Y, Siapas AG, Zhou FM, Dani JA. Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. J Neurosci. 2009 Apr;29(13):4035–43.
213 Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ. Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. Neuropsychopharmacology. 2008 Aug;33(9):2158–66.
214 Exley R, Maubourguet N, David V, Eddine R, Evrard A, Pons S, et al. Distinct contributions of nicotinic acetylcholine receptor subunit alpha4 and subunit alpha6 to the reinforcing effects of nicotine. Proc Natl Acad Sci USA. 2011 May;108(18):7577–82.
215 Perez XA, McIntosh JM, Quik M. Long-term nicotine treatment down-regulates α6β2* nicotinic receptor expression and function in nucleus accumbens. J Neurochem. 2013 Dec;127(6):762–71.
216 Exley R, McIntosh JM, Marks MJ, Maskos U, Cragg SJ. Striatal α5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. J Neurosci. 2012 Feb;32(7):2352–6.
217 McCallum SE, Parameswaran N, Bordia T, Fan H, McIntosh JM, Quik M. Differential regulation of mesolimbic alpha 3/alpha 6 beta 2 and alpha 4 beta 2 nicotinic acetylcholine receptor sites and function after long-term oral nicotine to monkeys. J Pharmacol Exp Ther. 2006 Jul;318(1):381–8.
218 Schultz W. Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. J Neurophysiol. 1986 Nov;56(5):1439–61.
219 Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, Grady SR. Pharmacology of alpha-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. Mol Pharmacol. 2007 Jun;71(6):1563–71.
220 Champtiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, et al. Distribution and pharmacology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. J Neurosci. 2002 Feb;22(4):1208–17.
221 Wickham R, Solecki W, Rathbun L, McIntosh JM, Addy NA. Ventral tegmental area α6β2 nicotinic acetylcholine receptors modulate phasic dopamine release in the nucleus accumbens core. Psychopharmacology (Berl). 2013 Sep;229(1):73–82.
222 Brunzell DH, Boschen KE, Hendrick ES, Beardsley PM, McIntosh JM. Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens shell regulate progressive ratio responding maintained by nicotine. Neuropsychopharmacology. 2010 Feb;35(3):665–73.
223 Zhang L, Dong Y, Doyon WM, Dani JA. Withdrawal from chronic nicotine exposure alters dopamine signaling dynamics in the nucleus accumbens. Biol Psychiatry. 2012 Feb;71(3):184–91.
224 Toth E, Sershen H, Hashim A, Vizi ES, Lajtha A. Effect of nicotine on extracellular levels of neurotransmitters assessed by microdialysis in various brain regions: role of glutamic acid. Neurochem Res. 1992 Mar;17(3):265–71.
225 Reid MS, Fox L, Ho LB, Berger SP. Nicotine stimulation of extracellular glutamate levels in the nucleus accumbens: neuropharmacological characterization. Synapse. 2000 Feb;35(2):129–36.
226 Lenoir M, Kiyatkin EA. Intravenous nicotine injection induces rapid, experience-dependent sensitization of glutamate release in the ventral tegmental area and nucleus accumbens. J Neurochem. 2013 Nov;127(4):541–51.
227 Brown RW, Kolb B. Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. Brain Res. 2001 Apr;899(1-2):94–100.
228 Lim WK, Wang K, Lefebvre C, Califano A. Comparative analysis of microarray normalization procedures: effects on reverse engineering gene networks. Bioinformatics. 2007 Jul;23(13):i282–8.
229 Salamone A, Zappettini S, Grilli M, Olivero G, Agostinho P, Tomé AR, et al. Prolonged nicotine exposure down-regulates presynaptic NMDA receptors in dopaminergic terminals of the rat nucleus accumbens. Neuropharmacology. 2014 Apr;79:488–97.
230 Kutlu MG, Gould TJ. Nicotinic modulation of hippocampal cell signaling and associated effects on learning and memory. Physiol Behav. 2016 Mar;155:162–71.
231 Shingo AS, Kito S. Effects of nicotine on neurogenesis and plasticity of hippocampal neurons. J Neural Transm (Vienna). 2005 Nov;112(11):1475–8.
232 Scerri C, Stewart CA, Breen KC, Balfour DJ. The effects of chronic nicotine on spatial learning and bromodeoxyuridine incorporation into the dentate gyrus of the rat. Psychopharmacology (Berl). 2006 Mar;184(3-4):540–6.
233 Van Kampen JM, Eckman CB. Agonist-induced restoration of hippocampal neurogenesis and cognitive improvement in a model of cholinergic denervation. Neuropharmacology. 2010 May;58(6):921–9.
234 Cohen A, Soleiman MT, Talia R, Koob GF, George O, Mandyam CD. Extended access nicotine self-administration with periodic deprivation increases immature neurons in the hippocampus. Psychopharmacology (Berl). 2015 Jan;232(2):453–63.
235 Abrous DN, Adriani W, Montaron MF, Aurousseau C, Rougon G, Le Moal M, et al. Nicotine self-administration impairs hippocampal plasticity. J Neurosci. 2002 May;22(9):3656–62.
236 Deng W, Aimone JB, Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci. 2010 May;11(5):339–50.
237 Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993 Jan;361(6407):31–9.
238 Fujii S, Ji Z, Sumikawa K. Inactivation of alpha7 ACh receptors and activation of non-alpha7 ACh receptors both contribute to long term potentiation induction in the hippocampal CA1 region. Neurosci Lett. 2000 Jun;286(2):134–8.
239 Hamid S, Dawe GS, Gray JA, Stephenson JD. Nicotine induces long-lasting potentiation in the dentate gyrus of nicotine-primed rats. Neurosci Res. 1997 Sep;29(1):81–5.
240 Ji D, Lape R, Dani JA. Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. Neuron. 2001 Jul;31(1):131–41.
241 Tang J, Dani JA. Dopamine enables in vivo synaptic plasticity associated with the addictive drug nicotine. Neuron. 2009 Sep;63(5):673–82.
242 Yakel JL, Shao Z. Functional and molecular characterization of neuronal nicotinic ACh receptors in rat hippocampal interneurons. Prog Brain Res. 2004;145:95–107.
243 Zhang TA, Tang J, Pidoplichko VI, Dani JA. Addictive nicotine alters local circuit inhibition during the induction of in vivo hippocampal synaptic potentiation. J Neurosci. 2010 May;30(18):6443–53.
244 Kenney JW, Gould TJ. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. Mol Neurobiol. 2008 Aug;38(1):101–21.
245 Yakel JL. Nicotinic ACh receptors in the hippocampus: role in excitability and plasticity. Nicotine Tob Res. 2012 Nov;14(11):1249–57.
246 Koob GF. The role of the striatopallidal and extended amygdala systems in drug addiction. Ann N Y Acad Sci. 1999 Jun;877:445–60.
247 Huang YY, Kandel ER, Levine A. Chronic nicotine exposure induces a long-lasting and pathway-specific facilitation of LTP in the amygdala. Learn Mem. 2008 Aug;15(8):603–10.
248 Khaled MA, Pushparaj A, Di Ciano P, Diaz J, Le Foll B. Dopamine D3 receptors in the basolateral amygdala and the lateral habenula modulate cue-induced reinstatement of nicotine seeking. Neuropsychopharmacology. 2014 Dec;39(13):3049–58.
249 Xue YX, Chen YY, Zhang LB, Zhang LQ, Huang GD, Sun SC, et al. Selective Inhibition of Amygdala Neuronal Ensembles Encoding Nicotine-Associated Memories Inhibits Nicotine Preference and Relapse. Biol Psychiatry. 2017 Dec;82(11):781–93.

250 Funk D, Coen K, Tamadon S, Hope BT, Shaham Y, Lê AD. Role of Central Amygdala Neuronal Ensembles in Incubation of Nicotine Craving. J Neurosci. 2016 Aug; 36(33):8612–23.
251 Bi GQ, Poo MM. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J Neurosci. 1998 Dec;18(24):10464–72.
252 Markram H, Lübke J, Frotscher M, Sakmann B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science. 1997 Jan;275(5297):213–5.
253 Couey JJ, Meredith RM, Spijker S, Poorthuis RB, Smit AB, Brussaard AB, et al. Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. Neuron. 2007 Apr;54(1):73–87.
254 Gioanni Y, Rougeot C, Clarke PB, Lepousé C, Thierry AM, Vidal C. Nicotinic receptors in the rat prefrontal cortex: increase in glutamate release and facilitation of mediodorsal thalamo-cortical transmission. Eur J Neurosci. 1999 Jan;11(1):18–30.
255 Vidal C, Changeux JP. Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. Neuroscience. 1993 Sep;56(1):23–32.
256 Dickinson JA, Kew JN, Wonnacott S. Presynaptic alpha 7- and beta 2-containing nicotinic acetylcholine receptors modulate excitatory amino acid release from rat prefrontal cortex nerve terminals via distinct cellular mechanisms. Mol Pharmacol. 2008 Aug;74(2):348–59.
257 Pistillo F, Fasoli F, Moretti M, McClure-Begley T, Zoli M, Marks MJ, et al. Chronic nicotine and withdrawal affect glutamatergic but not nicotinic receptor expression in the mesocorticolimbic pathway in a region-specific manner. Pharmacol Res. 2016 Jan; 103:167–76.
258 Ávila-Ruiz T, Carranza V, Gustavo LL, Limón DI, Martínez I, Flores G, et al. Chronic administration of nicotine enhances NMDA-activated currents in the prefrontal cortex and core part of the nucleus accumbens of rats. Synapse. 2014 Jun;68(6): 248–56.
259 You ZB, Wang B, Zitzman D, Wise RA. Acetylcholine release in the mesocorticolimbic dopamine system during cocaine seeking: conditioned and unconditioned contributions to reward and motivation. J Neurosci. 2008 Sep;28(36):9021–9.
260 Higgins GA, Silenieks LB, Rossmann A, Rizos Z, Noble K, Soko AD, et al. The 5-HT2C receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. Neuropsychopharmacology. 2012 Apr;37(5): 1177–91.
261 O'Dell LE, Torres OV. A mechanistic hypothesis of the factors that enhance vulnerability to nicotine use in females. Neuropharmacology. 2014 Jan;76 Pt B:566-80.
262 Torres OV, O'Dell LE. Stress is a principal factor that promotes tobacco use in females. Prog Neuropsychopharmacol Biol Psychiatry. 2016 Feb;65:260–8.
263 Verplaetse TL, Weinberger AH, Smith PH, Cosgrove KP, Mineur YS, Picciotto MR, et al. Targeting the noradrenergic system for gender-sensitive medication development for tobacco dependence. Nicotine Tob Res. 2015 Apr;17(4):486–95.
264 Perkins KA. Smoking cessation in women. Special considerations. CNS Drugs. 2001; 15(5):391–411.
265 Perkins KA. Nicotine discrimination in men and women. Pharmacol Biochem Behav. 1999 Oct;64(2):295–9.
266 Lerman C, Kaufmann V, Rukstalis M, Patterson F, Perkins K, Audrain-McGovern J, et al. Individualizing nicotine replacement therapy for the treatment of tobacco dependence: a randomized trial. Ann Intern Med. 2004 Mar;140(6):426–33.
267 Perkins KA. Acute responses to nicotine and smoking: implications for prevention and treatment of smoking in lower SES women. Drug Alcohol Depend. 2009 Oct; 104 Suppl 1:S79–86.
268 Warren GW, Alberg AJ, Kraft AS, Cummings KM. The 2014 Surgeon General's report: "The health consequences of smoking—50 years of progress": a paradigm shift in cancer care. Cancer. 2014 Jul;120(13): 1914–6.