Neurobiological and Neurophysiological Mechanisms Underlying Nicotine Seeking and Smoking Relapse

Heath D. Schmidt, Laura E. Rupprecht, Nii A. Addy

Department of Biobehavioral Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, PA, USA; Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA; Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA; Interdepartmental Neuroscience Program, Yale Graduate School of Arts and Sciences, New Haven, CT, USA

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.

Keywords
Electrophysiology · Synaptic plasticity · Tobacco · Relapse · Reinstatement · Self-administration · Neuroadaptations

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...
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 self-administration is used primarily in laboratory animals and humans as 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 EMQMC 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
Mechanisms of Nicotine Seeking

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 GA-BAergic regulation of reward-related activity [60, 118, 119]. With respect to nicotine-seeking behavior, systemic administration of the GABA<sub>B</sub> receptor agonist CGP44532 has been shown to attenuate cue-induced reinstatement of nicotine-seeking behavior [120]. Consistent with these effects, the GABA<sub>B</sub> 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<sub>B</sub> receptors have fewer adverse effects than full agonists. PAMs of GABA<sub>B</sub> 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<sub>B</sub> receptors, attenuates cue-induced reinstatement of drug seeking in rats [125]. Collectively, these findings indicate an important role for GABA<sub>B</sub> receptors in nicotine seeking and suggest that the efficacy of PAMs of GABA<sub>B</sub> 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<sub>A</sub> receptor subunits α1 and γ2 in synaptic membrane fractions from the medial prefrontal cortex (mPFC) [126]. Furthermore, inhibiting surface expression of GABA<sub>A</sub> receptors in the dorsal, but not ventral, mPFC increases nicotine seeking, suggesting a region-specific role of mPFC GABA<sub>A</sub> receptors in relapse [126]. Consistent with these findings, infusions of muscimol, a GABA<sub>A</sub> receptor antagonist, into the mPFC attenuates cue-induced reinstatement of nicotine seeking [126]. Thus, it has been proposed that increased GABA<sub>A</sub> receptor signaling in the mPFC may represent a compensatory response to reduce nicotine seeking [126].

Endocannabinoids

Given the critical involvement of increased mesolim-bic dopamine signaling in nicotine reinforcement and behavior, preclinical studies have begun to study endocannabinoi'd 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-arachidonoylglyc-erol. 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 oleylethanolamide (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
Mechanisms of Nicotine Seeking

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 [166] 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 mesocumbens 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 Segmental 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 to 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-
Mediated 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]. 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 afferents 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 has been shown to reduce daily ciga-

DOI: 10.1159/000494799

178 Mol Neuropsychiatry 2018;4:169–189

Schmidt/Rupprecht/Addy
Mechanisms of Nicotine Seeking

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 exposure. 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<sub>B</sub> 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 neuuropeptide 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.

Acknowledgements

L.E.R. and N.A.A. were supported by the Yale Tobacco Center of Regulatory Science (TCORS) grant P50 DA036151 from the National Institute on Drug Abuse (NIDA) and the Federal Drug Administration (FDA) Center for Tobacco Products (CTP). H.D.S. was supported by grants DA037897 and DA039393 from the NIDA. The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIH or the FDA.

Disclosure Statement

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

References

1 Arrazola RA, Singh T, Corey CG, Husten CG, Nefli, 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, Nefli IJ, Whitmill J, Babb SD, Graffunder CM. Current Cigarette Smoking Among Adults - United States, 2005-2014. MMWR Morb Mortal Wkly Rep. 2016 Nov 11;65(4):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, Ramasundarathigiti 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 to treat smoking-related morbidity and mortality. 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):869–97.
10 Cahill K, Stevens S, Lancaster T. Pharmacological treatments for smoking cessation. JAMA. 2014 Jan;311(2):193–4.

11 Lerman C, Le Sage MG, Perkins KA, O’Malley SS, Siegel J, 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 Ettet JF, Eisenberg T. Dependence levels in users of electronic cigarettes, nicotine gum and tobacco cigarettes. Drug Alcohol Depend. 2015 Feb;147:68–75.
16 Boyle RG, Jensen J, Hatsuakumi DK, Severson IH. Measuring dependence in smokeless tobacco users. Addict Behav. 1995 Jul-Aug;20(4):443–50.
17 Fagerström K, Eisenberg 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 acetalddehyde on nicotine self-administration in adult male rats. Drug Alcohol Depend. 2015 Oct;155:243–52.

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. Neuropsychopharmacology. 2014 Oct;49:293–304.
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. 2016 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, Khoryan 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, Corrigan WA. Nicotine self-administration in animals and humans: similarities and differences. Psychopharmacology (Berl). 1997 Mar;130(1):28–40.
Mechanisms of Nicotine Seeking

39 Epstein DH, Preston KL, Stewart J, Shaham Y.
35 de Wit H, Stewart J. Reinstatement of co-
29 Gourlay SG, Benowitz NL. Arteriovenous dif-
31 Coen KM, Adamson KL, Corrigall WA. Med-
37 Hopkins TJ, Rupprecht LE, Hayes MR,
28 Rupprecht LE, Smith TT, Schassburger RL,
33 George O, Lloyd A, Carroll FI, Damaj MI,
40 O'Connor EC, Parker D, Rollema H, Mead

53 Mansvelder HD, McGehee DS. Long-term
potentiation of excitatory inputs to brain re-
ard areas by nicotine. Neuron. 2000 Aug;
27(2): 349–57.
44 Liu L, Zhao-Shao R, McIntosh JM, Gardner
PD, Tapper AR. Nicotine persistently acti-
votes ventral tegmental area dopaminergic
neurons via nicotinic acetylcholine receptors
containing α4 and α6 subunits. Mol Pharma-
col. 2012 Apr;81(4):541–8.
51 Benwell ME, Balfour DJ, Lucchi HM. Influ-
ence to function. Physiol Rev. 1998 Jan;
78(1): 189–225.
183
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, Justinoza 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 JH, 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. Phenotypic characterization of neurons expressing the dopamine D3 receptor in the rat brain. *Neuroscience.* 1995 Apr;65(3):731–45.

77 Bouthenet ML, Soulé 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, 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.
Mechanisms of Nicotine Seeking

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.

106 Anderson DJ, Malysz J, Grønlien JH, El Kouhen R, Häkerud M, Wetterstrand C, et al. Stimulation of dopamine release by nicotinic acetylcholine receptor ligands in rat brain areas and smokes 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):1306–16.

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 galanin and its analogues. Pharmacol Ther. 1995;68(1):113–28.

110 Kosass T, Kuruya Y, Matsuki T, 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(1-2):7–13.

111 Kimmey BA, Rupprecht LE, Hayes MR, Schmidt HD. Donepezil, an acetylcholine receptor modulator, 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 self-administration and reinstatement of nicotine seeking in rats. Addict Biol. 2014 Jul;19(4):539–51.

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;222(3–4):413–20.

115 Lougheed J, Willeyto EP, Puparel K, Falcone M, Hopson R, Gur R, et al. Working memory-related neural activity predicts future smoking relapse. Neuropsychopharmacol. 2015 May;40(6):1311–20.

116 Patterson F, Jepson C, Lougheed 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 gamma-aminobutyric acid A 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.

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. Repeat administration of the GABAB receptor agonist CGP44532 decreased nicotine self-administration, and acute administration decreased cue-induced reinstatement of nicotine-seeking in rats. Neuropsychopharmacol. 2005 Jan;30(1):119–28.

121 Fattore L, Spano MS, Cossu G, Schermia 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, Kiliaan AM, Coope J, 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 DJ. Clinical potential of GABAB receptor modulators. CNS Drug Rev. 2005;11(3):317–34.

124 Urvylevy S, Pozza MF, Lingenhoechl K, Mosbach J, Lampert C, Froestl W, et al. N,N'-Dicyclopropyl-2-methylsulfanyl-5-nitro-pyridine-4,6-diamine (GS59783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acid B receptor function. J Pharmacol Exp Ther. 2003 Oct;307(1):322–30.

125 Vlachou S, Guery S, Froestl W, Banerjee S, Dunois D, Ferré Y, et al. Suppression of conditioned nicotine and sucrose seeking by the cannabimimetic CB2 receptor antagonist AM4116A. Behav Brain Res. 2005 Jun;161(1):164–8.

126 Lubbers BR, van Mourik Y, Schetters D, Smit AB, De Vries TJ, Spijker S. Preclinical 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, Schoffeleer 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, Schoffeleer AN. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. Behav Brain Res. 2009 Jan;199(1):58–63.

130 Schindler CW, Redhi GH, Vemuri K, Makriyannis A, Le Foll B, Bergman J, et al. Blockade of Nicotine and Cannabinoid Re-inforcement and Relapse by a Cannabinoid CB1-Receptor Neutral Antagonist AM4113 and Inverse Agonist Rimonabant in Squirrel Monkeys. Neuropsychopharmacology. 2016 Aug;41(9):2283–93.

131 Shoab M. The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats. Neuropharmacology. 2008 Feb;54(2):438–44.

132 Vleugels A, de Vries W, Raase H, Schoffeleer AN, De Vries TJ. Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor antagonist Rimonabant (SR141716A). Neuropharmacology. 2010 Oct;58(5):712–6.

133 Gamaleldin 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 Vlachou S, Vanillo V, 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. Neuropsychopharmacol. 2015 Aug;40(9):2185–97.

135 Justinova Z, Mangieri RA, Bortolato M, De Serrano M, Charif SI, Mukhin AG, Clapper JR, et al. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3-carboxamidobutyric acid β-phenyl-3-yl ester (URB597): effects on anandamide and oleyloylethanolamide deactivation. J Pharmacol Exp Ther. 2005 Apr;313(1):352–8.
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.

186 Mol Neuropsychiatry 2018;4:169–189
DOI: 10.1159/000494799

Schmidt/Rupprecht/Addy
Mechanisms of Nicotine Seeking

172 Egecioglu E, Engel JA, Jerhag E. The glau- cow-like peptide 1 analogue Exendin-4 at- tentuates 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 ha- bunular avoidance circuits to control nicot- nine intake. *Nat Neurosci.* 2017 May;20(5): 708–16.

174 Corrigall WA, Coen KM, Adamson KL. Self-administered nicotine activates the me- solimbic dopamine system through the ven- tral 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 sys- tema nigrostriatal self-administration. *Neurosci.* 2008 Nov;28(47):12318–27.

176 Mansvelder HD, Mertz M, Role LW. Nico- tense. *Annu Rev Neurosci.* 1999 Oct; 846(1):137–43.

177 Gould TJ, Leach PT. Cellular, molecular, and genetic substrates underlying the im- pact of nicotine on learning. *Neurobiol Learn Mem.* 2014 Jan;107:108–32.

178 Mansvelder HD, Mertz M, Role LW. Nico- tonic coupling. *Neuroscience.* 1983 Oct; 10(2):333–48.

179 Grace AA, Bunney BS. Intracellular and ex- tracellular electrophysiology of nigral dopa- minergic neurons—3. Evidence for electro- tonic coupling. *J Neurosci.* 1983 Oct; 10(2):333–48.

180 Radcliffe KA, Dani JA. Nicotinic stimula- tion enhanced by low concentrations of nico- tine. *Nature.* 1996 Oct; 383(6602):713–16.

181 Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicoti- nic activation of alpha4 receptors: sufficient for reward, tolerance, and sensitization. *Science.* 2004 Nov;306(5698):1029–32.

182 Mansvelder HD, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-in- duced excitability of brain reward areas. *Neuron.* 2002 Mar;33(6):905–19.

183 Exley R, Cragg SJ. Presynaptic nicotinic re- ceptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmis- sion. *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 re- ceptor subtypes in midbrain dopamine ar- eas. *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 es- culation. *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 ef- fects 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. Nicoti- nic activation of alpha4 receptors: sufficient for reward, tolerance, and sensiti- zation. *Science.* 2004 Nov;306(5698):1029–32.

189 Grace AA, Bunney BS. Intracellular and ex- tracellular electrophysiology of nigral dopa- minergic neurons—3. Evidence for electro- tonic coupling. *J Neurosci.* 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 dopa- mine cells in the freely moving rat. *Neuro- science.* 2002;114(2):475–92.

192 Phillips PE, Stuber GD, Heien ML, Wight- man RM, Carelll RM. Subsecond dopamine release promotes cocaine seeking. *Nature.* 2003 Apr;422(6932):614–8.

193 Stuber GD, Wightman RM, Carelli RM. Ex- tinction of cocaine self-administration re- veals functionally and temporally distinct dopaminergic signals in the nucleus accu- bens. *Neuron.* 2005 May;46(4):661–9.

194 Solecki W, Wickham RJ, Behrens S, Wang J, Grenhoff J, Aston-Jones G, Svensson TH. Facilitative effects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berl).* 1998 Mar;136(2):183–90.

195 Schwartz RD, Kellar KJ. Nicotinic cholin- ergic receptor binding sites in the brain: regu- lation in vivo. *Science.* 1983 Apr;220(4593):214–6.

196 Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholin- ergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regu- lated by chronic nicotine treatment. *Mol Pharmacol.* 1992 Jan;41(1):31–7.

197 Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzel E, Frohlich E, Perry E, et al. Hu- man tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetyl- choline receptors than nonsmokers. *J Neu- rosci.* 2006 Aug;26(34):8707–14.

198 Nashmi R, Dickinson ME, McKinney S, Jar- eb M, Labarca C, Fraser SE, et al. Assembly of alpha4beta2 nicotinic acetylcholine re- ceptors assessed with functional fluores- cence-labeled subunits: effects of localization, trafficking, and nicotine-induced up- regulation in cloned mammalian cells and in cultured midbrain neurons. *J Neurosci.* 2003 Dec;23(37):11554–67.

199 Mignaini M, Tessari M, Tarter G, Merlo Pich E, Chiamulera C, Bunnemann B. Up- regulation of [3H]methyllycaconitine bind- ing 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.

200 Ngolab J, Liu L, Zhao-Shea R, Gao G, Gard- ner PD, Tapper AR. Functional Upregula- tion of alpha4* Nicotinic Acetylcholine Recep- tors in VTA GABAergic Neurons Increases Sensitivity to Nicotine Reward. *J Neurosci.* 2015 Jun;35(22):8570–8.

201 Zhou FM, Liang Y, Dani JA. Endogenous nicotinic cholinergic activity regulates do- pamine release in the striatum. *Nat Neu- rosci.* 2001 Dec;4(12):1224–9.

202 Tolu S, Eddine R, Marti F, David V, Graupner M, Pons S, et al. Co-activation of VTA DA and GABA neurons mediates nic- toline reinforcement. *Mol Psychiatry.* 2013 Mar;18(3):382–93.

203 Hukkanen J, Jacob P 3rd, Benowitz NL. Met- abolism and disposition kinetics of nicotine. *Pharmacol Rev.* 2005 May;57(1):79–115.

204 Rose JS, Dierker LC, Donny E. Nicotine de- pendence symptoms among recent onset adolescent smokers. *Drug Alcohol Depend.* 2010 Jan;106(2-3):S126–32.

205 Nashmi R, Caggiula AR, Mielke MM, Ja- cobs KS, Rose C, Sved AF. Acquisition of nicotine self-administration in rats: the ef- fects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berl).* 1998 Mar;136(2):183–90.

206 Donny EC, Caggiula AR, Rowell PP, Gharib MA, Maldivan V, Booth S, et al. Nicotine self-administration in rats: estrous cycle ef- fects, sex differences and nicotinic receptor binding. *Psychopharmacology (Berl).* 2000 Sep;151(4):392–405.

207 Schwartz RD, Kellar KJ. Nicotinic cholin- ergic receptor binding sites in the brain: regu- lation in vivo. *Science.* 1983 Apr;220(4593):214–6.

208 Nashmi R, Dickinson ME, McKinney S, Jar- eb M, Labarca C, Fraser SE, et al. Assembly of alpha4beta2 nicotinic acetylcholine re- ceptors assessed with functional fluores- cence-labeled subunits: effects of localization, trafficking, and nicotine-induced up- regulation in cloned mammalian cells and in cultured midbrain neurons. *J Neurosci.* 2003 Dec;23(37):11554–67.
219 Salminen O, Drapeau JA, McIntosh JM, Exley R, Maubourguet N, David V, Eddine
188
212 Zhang T, Zhang L, Liang Y, Siapas AG, Zhou FM, Dani JA. Dopamine signaling dif-
214ferences in the nucleus accumbens and dorsal striatum exploited by nicotine. J Neuro-
213sci. 2009 Apr;29(13):4035–46.
215
214 Exley R, Clements MA, Hartung H, McIntosh JM, Cragg SJ. Alpha6-containing nico-
tinic acetylcholine receptors dominate the nicotine control of dopamine neurotrans-
munication in nucleus accumbens. Neuropsychopharmacology. 2008 Aug;33(9):2158–
66.
216 Exley R, Maubourguet N, David V, Eddine R, Evrard A, Pons S, et al. Distinct contribu-
tions of nicotinic acetylcholine receptor subunit alpha4 and subunit alpha6 to the re-
inferring effects of nicotine. Proc Natl Acad Sci USA. 2011 May;108(18):7577–82.
217 Perez XA, McIntosh JM, Quik M. Long-term nicotine treatment down-regulates a6β2* nico-
tinic receptor expression and function in nucleus accumbens. J Neuro-
213chem. 2013 Dec;127(6):762–71.
218 Exley R, McIntosh JM, Marks MJ, Maskos U, Cragg SJ. Striatal a5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. J Neurosci. 2012 Feb;32(7):
219 2352–6.
220 McCallum SE, Parameswaran N, Bordia T, Fan H, McIntosh JM, Quik M. Differential regula-
tion of mesolimbic alpha 3/alpha 6 beta 2 and alpha 4 beta 2 nicotinic acetyl-
choline receptor sites and function after long-term oral nicotine to monkeys. J Phar-
221 macol Exp Ther. 2006 Jul;318(1):381–8.
222 Schultz W. Responses of midbrain dopa-
mine neurons to behavioral trigger stimuli in the monkey. J Neurophysiol. 1986 Nov;
223 56(5):1439–61.
224 Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, Grady SR. Pharma-
cology of alpha-conotoxin MII-sensitive subtypes of nicotinic acetylcholine recep-
tors isolated by breeding of null mutant mice. Mol Pharmacol. 2007 Jun;71(6):
225 1563–71.
226 Al-Awqatiq X, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, et al. Distribution and pharma-
cology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. J Neurosci. 2002 Feb;22(4):
227 1208–17.
228 Wickham R, Solecki W, Rathbun L, McInt-
229 osh JM, Addy NA. Ventral tegmental area a6β2 nicotinic acetylcholine receptors modu-
late phasic dopamine release in the nucleus accumbens core. Psychopharmacology (Berl). 2013 Sep;229(1):73–82.
230 Brunzell DH, Boschen KE, Hendrick ES, Beardsley PM, McIntosh JM. Alpha-cono-
toxin MII-sensitive nicotinic acetylcholine receptors in the nucleus accumbens reg-
Mechanisms of Nicotine Seeking

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, Spikker 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. Postsynaptic 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. Neuropsychopharmacology. 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. Nicot 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.