

Chapter 3

New Biological Insights into Smoking Cessation

Introduction 125

Cell and Molecular Biology of Nicotine Addiction 125

Literature Review Methods 125

Neurobiology of Nicotine Addiction 125

 Nicotinic Acetylcholine Receptors 126

 Nicotine Reward 128

 Nicotine Withdrawal and Relapse 128

 Animal Models of Nicotine Addiction 129

Molecular Targets of Current Pharmacotherapies 129

Novel Targets for Smoking Cessation 131

 Glutamatergic Signaling 131

 Neuropeptide Systems 132

 The Habenulo-Interpeduncular Pathway 135

Vaccines and Other Immunotherapies as Treatments for Nicotine Addiction 137

Literature Review Methods 137

Design and Mechanism of Action 137

Examining Data from Animals to Confirm Vaccine Activity 138

Clinical Trials of Nicotine Vaccines 138

Next-Generation Vaccines 138

Combining Vaccines with Medications 139

Passive Immunization with Monoclonal Antibodies or Gene Transfer 139

Summary 139

Insights into Smoking Cessation from the Field of Neurobiology 139

Literature Review Methods 140

Methodology of Neuroimaging Studies 141

Differences in Brain Circuitry and Cognitive Constructs in Nicotine Dependence 141

Smoking Cues and Craving Provocation 142

Reward 143

Cognition and Cognitive Control 143

Insights from Neuroimaging for Antismoking Messages 144

Neuronal Circuits and Networks 144

Molecular Imaging 146

Summary 147

Genetic Studies of Smoking Phenotypes 147

Literature Review Methods 148

Candidate Gene Studies 148

Genomewide Association Studies 148

 Examples of Biologically Promising Candidate Genes (*DRD2* and *DAT1*) 148

 Associations Between the *DRD2* and *DAT1* Genes and Smoking Behavior 149

The Moderating Effect of <i>DRD2</i> and <i>DAT1</i> on the Efficacy of Treatment for Smoking Cessation	149
Genetic Risk Scores	149
Examples of Biologically Promising Genes That May Help Optimize Treatment	150
The <i>CHRNA5-A3-B4</i> Gene Cluster	150
Associations with Nicotine Dependence and Smoking Intensity	150
<i>CHRNA5-A3-B4</i> Variants and Smoking Cessation in Absence of Treatment	151
<i>CHRNA5-A3-B4</i> Variants and Smoking Cessation with Pharmacotherapy	151
The <i>CYP2A6</i> Gene and the Nicotine Metabolite Ratio	153
Nicotine Metabolite Ratio and Smoking Behavior	153
Nicotine Metabolite Ratio and Smoking Cessation in Absence of Treatment	154
Smoking Cessation in Treatment Seekers	154
Summary	154
Summary of the Evidence	155
Conclusions	157
References	158

Introduction

The 1988 Surgeon General's report on nicotine addiction was the first in this series to conclude that "[n]icotine is the drug in tobacco that causes addiction" (U.S. Department of Health and Human Services [USDHHS] 1988, p. 9). The biologic mechanisms underlying nicotine addiction continue to be a subject of great research interest, and several promising pharmacotherapeutic targets have emerged. For example, acquisition of basic knowledge about the function of nicotinic acetylcholine receptors (nAChRs) led to the development of targeted smoking cessation medications currently in use, and research would benefit from an additional understanding of molecular mechanisms (USDHHS 2010). The 2010 Surgeon General's report on how tobacco causes disease described the pharmacokinetics of nicotine, the behavioral pharmacology of nicotine addiction, and the known genotypes and receptor subtypes that contribute to nicotine addiction (USDHHS 2010). This chapter focuses on how biology can influence smoking cessation and reviews four areas of intensive research since the publication of the 2010 Surgeon General's report.

1. **Cell and molecular biology of nicotine addiction** focuses on the nAChRs as the primary target of currently available medications and on the following potential targets for medication development: glutamatergic signaling, neuropeptide systems, habenulo-interpeduncular pathway, and noradrenergic system. This section describes the preclinical basis for

understanding nicotine addiction and the ways that this knowledge could be used to enhance smoking cessation.

2. **Vaccines and other immunotherapies as treatments for tobacco addiction** focuses on the conceptual basis of vaccine treatment, vaccine mechanistic design, and vaccine animal studies; progress made and barriers encountered with the early generation vaccines; and approaches to next-generation treatments and passive immunization.
3. **Insights into smoking cessation from the field of neurobiology** describes the brain circuitry involved in nicotine dependence, as understood primarily through advances in brain imaging techniques; the role of stress, craving, and reward; and changes in cognitive control. Findings provide insight into the effects of smoking on the brain and the potential to identify new types of targets for smoking cessation.
4. **Genetic studies of smoking phenotypes** focuses on the further mechanistic understanding gained from the interindividual differences that genetics creates and from some of the methodologic approaches that can be used to examine genetics in humans. Findings provide insight into distinct classes of genes that represent potential targets for novel smoking cessation therapeutics and optimizing choice of treatment.

Cell and Molecular Biology of Nicotine Addiction

Literature Review Methods

For this section of the chapter, PubMed was searched in January 2017 for studies published between 2010 and 2017 that focused on the neurobiologic mechanisms underlying nicotine addiction in model organisms and in human subjects. Such search teams included "nACh" and "nicotinic receptor," and these terms were combined with such terms as "addiction" and "behavior." Studies about nicotinic acetylcholine receptor mechanisms that were cited in these articles were also reviewed to identify primary research articles. These studies and a current search of clinical trials websites were used to identify molecular targets for the development of novel smoking cessation

aids and ongoing clinical trials of relevant therapeutic agents. One reviewer conducted a full review and identified 76 articles for this section. The cited references for preclinical work represent a compilation of the current knowledge base obtained from rodent studies, but the base cannot be considered completely comprehensive because of the large volume of studies in this area.

Neurobiology of Nicotine Addiction

Nicotine, the main addictive constituent of cigarette smoke, binds to nAChRs, a class of ligand-gated ion channels that, following the binding of acetylcholine or

nicotine, open and allow the trafficking of cations (positive ions [e.g., Ca^{++} , Na^+ , K^+]) (USDHHS 2010). nAChRs play an important role in transmitter release, cell excitability, and neuronal integration. Through these processes, nicotine stimulates the release of many different neurotransmitters throughout the brain. In particular, nicotine activates the mesocorticolimbic dopamine system, which can induce both reward or aversion (USDHHS 2010).

The mesocorticolimbic system, which is characterized by the ventral tegmental area (VTA) located in the midbrain, transmits dopamine to two main targets: one cortical, the prefrontal cortex (PFC); and one limbic, the nucleus accumbens (NAc) in the ventral striatum (Figure 3.1). Nicotine increases extracellular dopamine in all of these structures but mainly in the NAc. The reward associated with the release of dopamine is one of the underlying mechanisms of the development of nicotine dependence. In fact, the dopaminergic pathway is targeted by existing pharmacotherapies for smoking cessation. At present, the approved pharmacologic treatments in the United States or Europe are nicotine replacement therapy (NRT), varenicline, and bupropion (U.S. Food and Drug Administration [FDA] 2016). Varenicline (trade names: Chantix, Champix) partially blocks the $\alpha 4\beta 2$ nAChRs, and bupropion (trade names: Wellbutrin, Zyban) is a norepinephrine/dopamine reuptake inhibitor that also can decrease the function of nAChRs by acting as an antagonist of the receptors (Mansvelter et al. 2007). These two medications act indirectly and directly on the dopamine pathway.

Nicotinic Acetylcholine Receptors

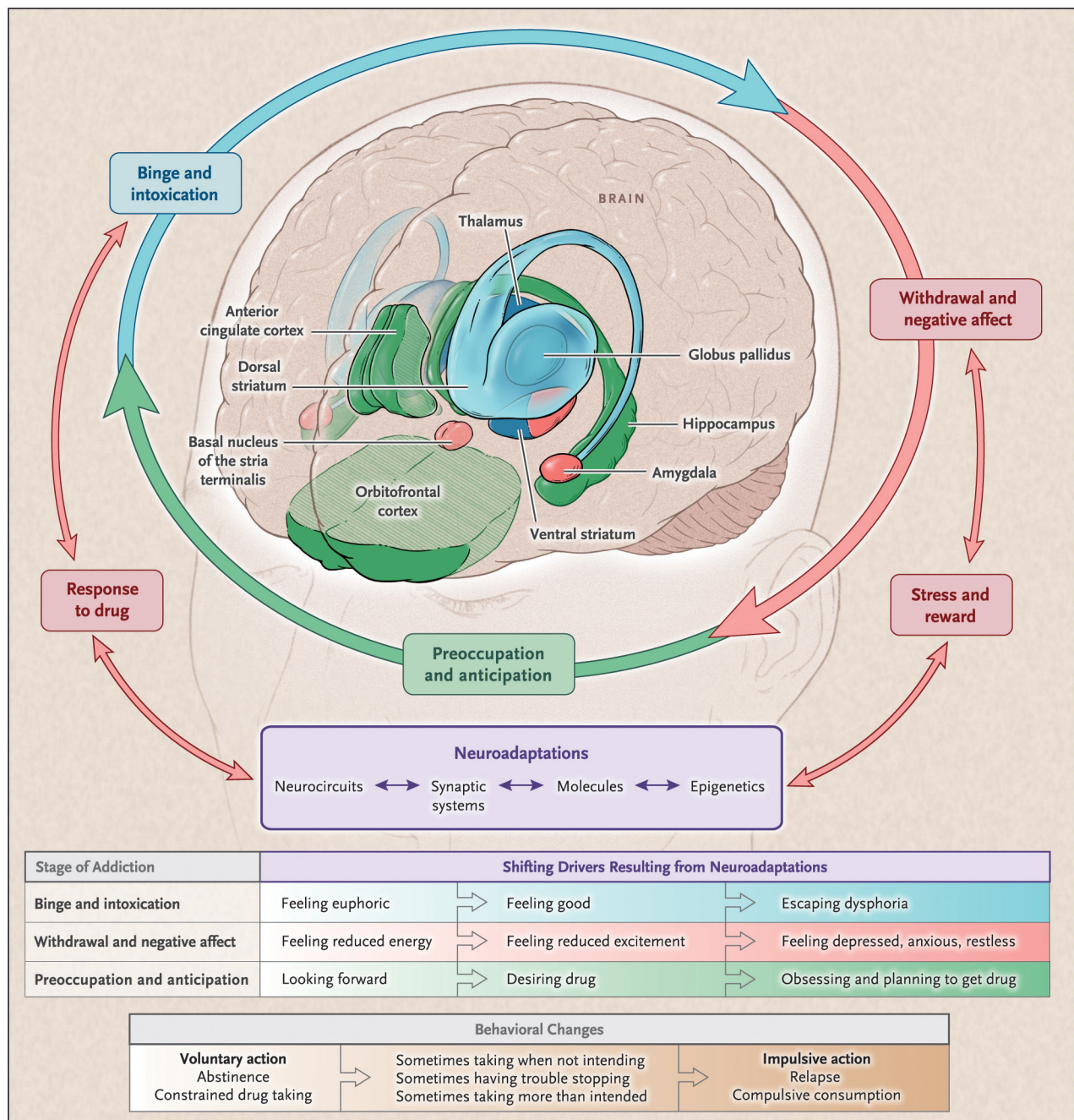
nAChRs are ion channels that normally are activated by the neurotransmitter acetylcholine, but the nicotine in tobacco products “hijacks” nAChRs. In humans, these receptors are assembled from combinations of 17 known subunits, 12 of which are expressed in the brain ($\alpha 2$ – $\alpha 10$ and $\beta 2$ – $\beta 4$) (Picciotto et al. 2008; Picciotto and Kenny 2013). Importantly, co-assembly of specific combinations of subunits results in a set of nAChR subtypes that vary in their properties, location in the brain, and sensitivity to nicotine (Figure 3.2). For example, $\alpha 7$ can form a functional nAChR on its own [$(\alpha 7)^5$], while all other nAChRs contain at least one α subunit and one β subunit [e.g., $(\alpha 4)^2(\beta 2)^3$]. The $\alpha 4$ and $\beta 2$ subunits, which are expressed throughout the brain and body in many types of cells, nearly always assemble together, sometimes with additional subunits, and their interface forms a high-affinity nicotine binding site (Kutlu and Gould 2016). Activation of these $\alpha 4$ - and $\beta 2$ -containing receptors is required for many of the neurobiologic and behavioral effects associated with nicotine reward. The $\alpha 6$ subunit also can associate selectively with

these receptors in dopamine and norepinephrine neurons (Kutlu and Gould 2016).

Nicotine and the endogenous ligand acetylcholine bind to the extracellular interface between two nAChR subunits. Upon binding of either nicotine or acetylcholine, the receptors undergo a structural change that causes the ion channel to open, permitting the influx of cations and membrane depolarization. Cellular responses to nicotine depend on the composition of nAChR subunits and their subcellular localization. For example, activation of nAChRs located on nerve terminals stimulates the release of neurotransmitters, and activation of cell body receptors increases neuronal excitability and can induce action potentials. Nicotine also binds to intracellular receptors in the endoplasmic reticulum and promotes their assembly and trafficking. Long-term exposure to nicotine increases the surface expression of nAChRs, particularly the high-affinity $\alpha 4$ - and $\beta 2$ -containing receptors. Cells in the brains of smokers, therefore, have an increased capacity for nicotine binding, which may result in altered neuronal signaling once nicotine is cleared from the brain and these nAChRs become available for acetylcholine signaling. In fact, heightened expression of nAChRs is observed in the brains of smokers for weeks following cessation; this might contribute to craving and withdrawal symptoms (Cosgrove et al. 2012). Although low levels of nicotine activate nAChRs, leading to nicotine reinforcement, continued exposure to nicotine desensitizes the receptors, which contributes to tolerance. The extent of desensitization varies with the composition of receptors and concentration of nicotine. $\beta 2$ subunit-containing nAChRs, which are required for the rewarding effects of nicotine, desensitize rapidly in response to very low concentrations of nicotine (Picciotto et al. 2008). $\alpha 7$ receptors, however, will continue to respond in the presence of sustained low concentrations of nicotine.

The physiologic consequences of nAChR desensitization are complex and not entirely understood, but chronic exposure to nicotine in the brains of users of tobacco products likely results in phases of activation and desensitization of nAChRs that contribute to nicotine reinforcement and tolerance, respectively. The variability in this balance also may contribute to individual differences in susceptibility to nicotine addiction. In addition, receptors are reactivated once nicotine is removed from the system. Thus, increases in the number of nAChRs and receptor reactivation when nicotine is cleared from the system that last at least 4 weeks after cessation (Cosgrove et al. 2012) result in robust potentiation of nAChR signaling following abstinence, which then contributes to withdrawal symptoms (Millar and Harkness 2008; Picciotto et al. 2008; Changeux 2010).

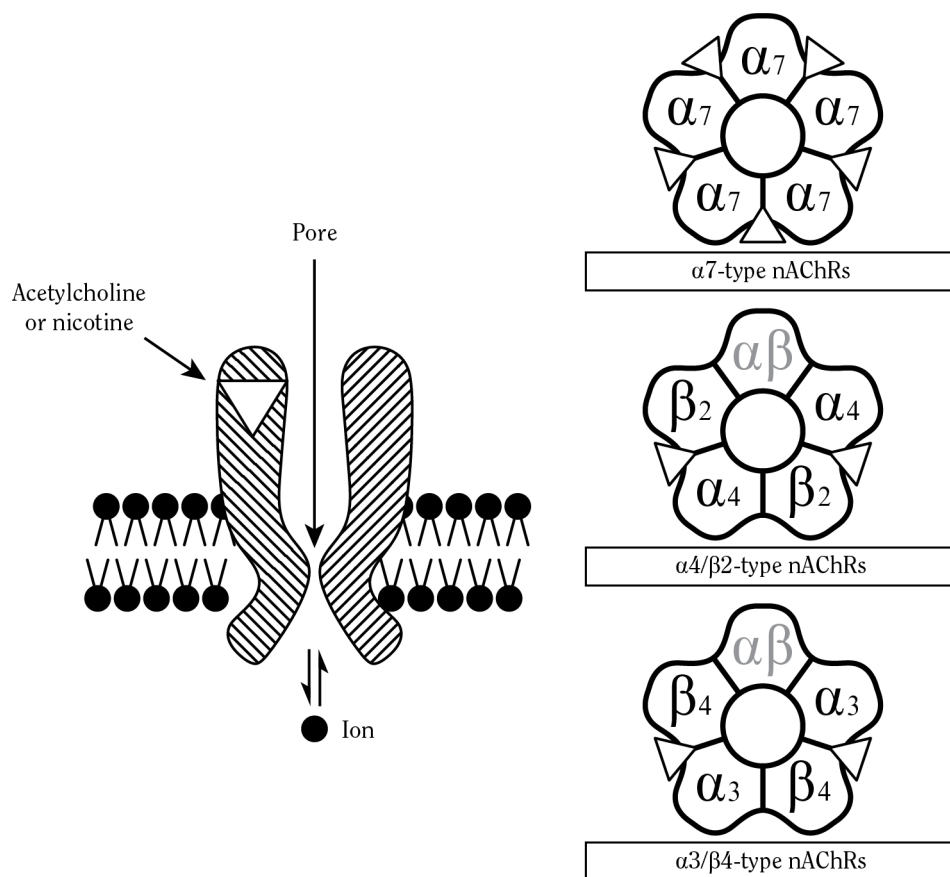
Figure 3.1 Stages of the addiction cycle



Source: From Volkow and colleagues (2016, p. 365). Copyright © 2016 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

Notes: “Binge and intoxication” and “feeling euphoric” are not relevant to nicotine. “During intoxication, drug-induced activation of the brain’s reward regions (in blue) is enhanced by conditioned cues in areas of increased sensitization (in green). During withdrawal, the activation of brain regions involved in emotions (in pink) results in negative mood and enhanced sensitivity to stress. During pre-occupation, the decreased function of the prefrontal cortex leads to an inability to balance the strong desire for the drug with the will to abstain, which triggers relapse and reinitiates the cycle of addiction. The compromised neurocircuitry reflects the disruption of the dopamine and glutamate systems and the stress-control systems of the brain, which are affected by corticotropin-releasing factor and dynorphin. The behaviors during the three stages of addiction change as a person transitions from drug experimentation to addiction as a function of the progressive neuroadaptations that occur in the brain” (Volkow et al. 2016, p. 365).

Figure 3.2 Structure and properties of nAChRs



Source: Created by Marina Picciotto and Megan Miller, Yale University School of Medicine, for this Surgeon General’s report.

Notes: α = alpha; β = beta; nAChR = nicotinic acetylcholine receptor. nAChRs in the brain assemble as pentameric (5-member) structures from 12 subunits: $\alpha 2$ – $\alpha 10$ and $\beta 2$ – $\beta 4$. The most common combinations are formed as homomers (all subunits the same) of the $\alpha 7$ subunit, or heteromers of the $\alpha 4$ and $\beta 2$, or $\alpha 3$ and $\beta 4$ subunits. Many subunit combinations with different properties are possible, with variability particularly at the fifth position in the receptor (indicated in grey as a choice of α or β subunit in this figure). Assembled receptors form a channel through the membrane, with a pore that is closed under resting conditions. The neurotransmitter acetylcholine normally binds to interfaces between subunits in the assembled nAChRs, activating the receptors and allowing ions to flow through the opened pore into cells expressing them. Nicotine binds to the same site in the nAChR as acetylcholine and can open the channel, although with different open times and likelihood of desensitization.

Nicotine Reward

As for all drugs of abuse, the primary reinforcing (i.e., initial rewarding or addictive) effects of nicotine are driven by its activation of the mesolimbic dopamine system, commonly known as the brain’s reward circuit. Nicotine promotes phasic firing of dopamine neurons in the VTA through several nAChR-mediated mechanisms (USDHHS 2010). Activation of $\alpha 4$ - and $\beta 2$ -containing nAChRs on dopamine cell bodies increases their excitability and is required for the reinforcing properties of nicotine. Nicotine also acts through $\alpha 7$ nAChRs located on glutamatergic terminals in the VTA to promote glutamate

release onto dopamine neurons, further enhancing their excitation (USDHHS 2010). Similarly, nicotine stimulation of nAChRs made of the $\alpha 4$, $\beta 2$, and $\alpha 6$ subunits that are found on dopamine terminals promotes the release of dopamine in NAc and other regions (Picciotto and Kenny 2013; Wickham et al. 2013; Picciotto and Mineur 2014).

Nicotine Withdrawal and Relapse

Chronic nicotine use can induce a physical dependence severe enough that cessation induces a series of negative withdrawal symptoms in humans and in laboratory animals (Picciotto et al. 2008; USDHHS 2010). Thus,

in addition to being drawn to the primary reinforcing properties of nicotine, many persons return to smoking to avoid negative effects of abstinence, such as irritability, anxiety, depression, insomnia, and difficulty concentrating. Additionally, environmental cues (sights, sounds, or other sensations) associated with nicotine often elicit drug cravings that can be sufficient to induce relapse to regular smoking after a quit attempt (USDHHS 2010). For example, former smokers who used to have a cigarette with their morning coffee may experience intense nicotine cravings at the smell of coffee, which could trigger relapse to smoking (Bevins and Palmatier 2004). Importantly, drug-paired cues (things in the environment that are associated with nicotine being on board) can become themselves reinforcing after repeated pairings, and this conditioned reinforcement may be at least partially responsible for continuing drug use and relapse. Mechanistically, perseverative drug use and high relapse rates happen because of persistent neurobiologic adaptations (tolerance), particularly within the mesocorticolimbic dopamine system. Thus, although developing therapies aimed at reducing the reinforcing properties of nicotine itself is reasonable, this strategy is unlikely to be completely effective in combating relapse to smoking (USDHHS 2010). For this reason, several research efforts have focused on elucidating the neurobiologic underpinnings of relapse.

Animal Models of Nicotine Addiction

Studies of animal models of disease have contributed to much of our understanding of the neurobiologic basis of nicotine addiction. Although animal models cannot capture the full range of human addiction, mice and rats do develop addiction-like behaviors, and several reliable paradigms have been established to measure specific aspects of the disease in animals. The drugs that animals self-administer correspond well with drugs that have high abuse liability in humans (Carter and Griffiths 2009). As described in detail below, nicotine-dependent animals will work to obtain nicotine and to relieve nicotine withdrawal symptoms (Koob and Simon 2009). Therefore, animal models are useful for measuring the abuse liability of addictive drugs, such as nicotine, and identifying pharmacotherapies that make addictive drugs less reinforcing or that mitigate withdrawal symptoms.

Modeling Nicotine Reward

The conditioned place preference (CPP) and self-administration paradigms are two common models used to evaluate nicotine reinforcement and drug-seeking behavior. CPP is established by repeatedly pairing nicotine administration with exposure to a particular environmental

context. Over time, the animal learns to associate the context with nicotine and develops a preference for that environment over an adjacent, similar environment that is not paired with nicotine. The development of such a preference is considered to be an indication of the rewarding effects of the drug.

In the self-administration model, animals are trained to complete an operant task, such as pressing a lever to receive an infusion of nicotine. Once the task is learned, changes in operant behavior are thought to indicate changes in drug reinforcement or craving. Variations of this task also can be used to measure motivation (i.e., how hard an animal is willing to work for nicotine), extinction, and relapse. Interestingly, self-administration of nicotine is more robust if infusion is paired with a cue versus with the drug alone (Caggiula et al. 2001).

Modeling Nicotine Withdrawal and Relapse

Human smokers often relapse in response to one of three stimuli: exposure to environmental cues associated with nicotine, aversive or stressful life events, or a small amount of the drug (i.e., a “lapse”) (USDHHS 2010). Each of these types of stimuli is also sufficient to induce reinstatement of nicotine-seeking behavior in rodents after forced extinction of the behavior. In the cue-induced reinstatement model, animals are trained to self-administer nicotine that is paired with an innocuous cue, such as a light or a tone. After self-administration of nicotine is acquired, the operant behavior can be extinguished by placing the animals in the same context but in the absence of the drug and the associated cue. Following extinction, animals will resume responding to the cue alone, even in the absence of nicotine. Similar paradigms have been developed to model stress-induced reinstatement and drug-induced reinstatement in animals, all of which may be valid for nicotine relapse in humans (Mantsch et al. 2016). Preclinical studies using these paradigms have been useful in identifying cellular and molecular processes that contribute to drug reinstatement, as discussed in this section.

Molecular Targets of Current Pharmacotherapies

As a consequence of our understanding of the neurobiology of nicotine addiction, several successful pharmacotherapies have been developed to aid in smoking cessation (Table 3.1) (Cochrane Tobacco Addiction Group n.d.), most of which alter nAChR signaling (Cahill et al. 2013, 2016). These include varenicline (a partial agonist of nAChRs) and bupropion (an atypical antidepressant with the ability to block nAChRs). Various forms of NRT—including

Table 3.1 Current pharmacotherapies for smoking cessation

Line	Trade name(s)	Target	Action	FDA approved for smoking cessation: Yes/no	Other information
First-line					
Bupropion	<ul style="list-style-type: none"> Wellbutrin Elontril Zyban 	Catecholamine system/nAChRs (multiple subtypes)	Norepinephrine or dopamine reuptake inhibitor/nAChR antagonist	Yes	Atypical antidepressant; also approved for ADHD and obesity
NRT	<ul style="list-style-type: none"> Nicoderm Commit Nicorette Others 	nAChRs (multiple subtypes)	Agonist	Yes	—
Varenicline	<ul style="list-style-type: none"> Chantix Champix 	nAChRs (multiple subtypes)	Partial agonist	Yes	—
Second-line					
Nortriptyline	<ul style="list-style-type: none"> Sensoval Aventyl Pamelor Norpress Allegron Noritren Nortrilen 	Serotonin and norepinephrine systems	Serotonin or norepinephrine reuptake inhibitor	No	Tricyclic antidepressant
Clonidine	<ul style="list-style-type: none"> Catapres Kapvay Duraclon Nexiclon 	Adrenergic receptors	Agonist	No	Also indicated for high blood pressure, ADHD, anxiety, migraine, withdrawal (opiates, alcohol, and nicotine), and other
Others					
Cytisine	<ul style="list-style-type: none"> Tabex 	nAChR	Partial agonist	No	Popular in Eastern Europe but not available in the United States; relatively inexpensive
Naltrexone	<ul style="list-style-type: none"> Revia Vivitrol 	Opioid receptors (μ , κ)	Antagonist	No	Commonly used to treat alcoholism and opioid dependence

Notes: κ = kappa; μ = mu; **ADHD** = attention-deficit/hyperactivity disorder; **FDA** = U.S. Food and Drug Administration; **nAChR** = nicotinic acetylcholine receptor; **NRT** = nicotine replacement therapy.

the patch, gums, lozenges, and nasal sprays—also act on nAChRs. Varenicline activates nAChRs, although to a lesser extent than nicotine, and blocks the binding of nicotine from tobacco to the nAChR, thereby resulting in reduced withdrawal symptoms and less reward from a lapse to smoking. Although not currently approved for use in the United States, cytisine is another nAChR partial agonist and has been used as an herbal smoking cessation aid for decades in Eastern European countries and Canada (Gómez-Coronado et al. 2018). Repeated efficacy

studies, including a Phase 3 clinical trial in New Zealand, have found cytisine to be effective for smoking cessation at levels similar to varenicline (Etter 2006). Because cytisine is a naturally occurring compound, it is less expensive than currently available cessation aids, making it a potentially promising tool for reducing smoking rates in certain populations, including low-income individuals. With withdrawal-induced negative affect a major problem for smokers trying to quit, antidepressants are often prescribed, and several of these drugs have shown efficacy

in reducing smoking (Hughes et al. 2014). Bupropion can alleviate withdrawal symptoms and reduce the severity of nicotine cravings. Overall, its efficacy for cessation is about double that of placebo (Wu et al. 2006). Notably, bupropion is also an nAChR antagonist that alters nicotine-mediated dopamine responses, which likely contributes to its efficacy in reducing smoking (Mansvelder et al. 2007). Although it has not been approved by FDA for smoking cessation, nortriptyline (trade names: Sensoval, Pamelor, Aventyl, and others), a tricyclic antidepressant and serotonin/norepinephrine reuptake inhibitor, also has shown off-label efficacy in improving rates of smoking cessation (Hughes et al. 2005).

Novel Targets for Smoking Cessation

Glutamatergic Signaling

Although enhanced dopamine signaling is critical for the initial reinforcing properties of nicotine, both the maintenance and reinstatement of nicotine-seeking behavior require long-lasting alterations in the actions of glutamate, the major excitatory neurotransmitter in the brain (Knackstedt and Kalivas 2009; Li et al. 2014; Marchi et al. 2015). Glutamate levels are elevated in both the NAc and the VTA after exposure to nicotine, and glutamate inputs to the VTA mediate the increases in the activity of dopamine neurons in response to nicotine. Repeated exposure to nicotine results in a long-term potentiation (or long-lasting increase in activation) of these synapses, which contributes to elevated excitability of dopamine neurons. Furthermore, sustained low levels of nicotine, as would be observed in the brains of smokers, can desensitize nAChRs located on inhibitory nerve terminals in the VTA. This may reduce the inhibition of dopamine neurons, further shifting the excitatory–inhibitory balance in the VTA. Nicotine dependence also is associated with long-term potentiation of glutamate synapses in the NAc, and disruption of glutamate signaling in this region alters nicotine-mediated physiology and behavior. Thus, chronic use of nicotine causes long-lasting changes to the mesolimbic dopamine system, many of which are driven by alterations in glutamate transmission. Behaviorally, these adaptations sustain drug cravings and contribute to a vulnerability to relapse. Glutamate binds to and activates two types of receptors: ionotropic, which are ion channels that allow current to pass through and activate cell membranes; and metabotropic, which are G-protein-coupled receptors that activate downstream cell signaling cascades. Neuroadaptive mechanisms in the glutamate system, perhaps on both types of glutamate receptors, may be targets for pharmacologic intervention.

Ionotropic Glutamate Receptors

Glutamate signaling through the ionotropic glutamate receptors N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is implicated in the neurobiologic mechanisms of nicotine dependence (Li et al. 2014; D'Souza 2015). Pharmacologic blockade of both NMDA and AMPA receptors in the VTA attenuates nicotine-induced dopamine release in the NAc, and inhibition of NMDA receptors impairs nicotine-seeking behaviors (Kenny et al. 2009; Mao et al. 2011). Conversely, blockade of NMDA receptors in the shell region of the NAc increases the self-administration of nicotine, suggesting that glutamatergic transmission in this region may offset the rewarding effects of nicotine (D'Souza and Markou 2014). The mechanisms underlying this effect are not fully understood, but one hypothesis is that medium spiny neurons in the shell region of the NAc are activated by glutamate, and these medium spiny neurons project to and inhibit dopamine neurons in the VTA (Yang et al. 2018). Regardless, glutamatergic signaling in mesocorticolimbic regions clearly contributes to nicotine reinforcement.

Gipson and colleagues (2013) demonstrated that long-lasting changes in glutamate signaling are central to post-withdrawal reinstatement of nicotine-seeking behavior in rats. Long-term potentiation of glutamatergic synapses in the NAc was apparent after 2 weeks of nicotine withdrawal, with further strengthening observed following cue-induced reinstatement of nicotine seeking. Furthermore, blocking the function of NMDA receptors in the core region of the NAc prevented cue-induced reinstatement of nicotine-seeking behavior. Similar observations have been made with other drugs of abuse, such as cocaine and alcohol. These data suggest that dampening mesolimbic glutamate signaling, potentially by inhibiting the function of NMDA receptors in the core of the NAc, may be a useful strategy for reducing vulnerability to smoking relapse in humans.

Although blockade of ionotropic glutamate receptors is effective in reducing addiction-like behaviors in animal models, systemic use of these drugs in humans is likely not feasible using current pharmacologic agents, given the crucial role of glutamate in the function of the nervous system. Also, because glutamate plays different roles in different regions of the brain, a more targeted, region-specific approach is warranted.

Metabotropic Glutamate Receptors

Metabotropic glutamate receptors (mGluRs) are widely expressed, G-protein-coupled receptors that use second-messenger systems (key distributors of an external signal) to modulate neuronal excitability. Two of these receptors, mGluR5 and mGluR2, have been implicated in

the neurobiology of nicotine addiction. Because pharmacologic manipulation of metabotropic glutamate signaling may have a more subtle effect on the function of the nervous system than do ionotropic drugs, targeting these receptors may be a more feasible clinical approach for smoking cessation (D'Souza 2015; Mihov and Hasler 2016; Acri et al. 2017; Chiamulera et al. 2017). Table 3.2 summarizes novel pharmacologic targets for smoking cessation.

mGluR5. mGluR5 is localized postsynaptically where it signals through the excitatory G-protein $G\alpha_s$, to enhance neuronal excitability. Reducing the function of mGluR5 with MPEP (2-methyl-6-(phenylethynyl pyridine), a negative allosteric modulator (NAM) in the NAC shell, attenuates nicotine self-administration and dopamine release (Paterson et al. 2003; Tronci et al. 2010), suggesting a role for this receptor in the primary reinforcing properties of nicotine. Additionally, both drug- and cue-induced reinstatement of nicotine-seeking behavior are reduced in animals that have been pretreated with mGluR5 antagonists (Bespalov et al. 2005). In humans, studies using selective mGluR5 radiotracers have revealed a significant reduction of binding sites in the brains of persons addicted to nicotine, which is normalized after cessation (Akkus et al. 2013; Hulka et al. 2014). The mechanism for this reduction is not entirely understood, but it may be a compensatory action meant to limit aberrant glutamate signaling in the brains of smokers.

The preclinical efficacy of mGluR5 NAMs in reducing drug-seeking behavior is well documented, but higher doses of the same drugs also have been reported to impair food-seeking behaviors in animals (Mihov and Hasler 2016). Although the curbing of appetite during smoking cessation may seem like an appealing side effect, such overly generalized effects may be dangerous or undesirable. mGluR5 NAMs also have been shown to increase the severity of nicotine withdrawal symptoms (Chiamulera et al. 2017), which could limit their feasibility for clinical use.

mGluR2. In contrast to mGluR5, mGluR2 is expressed on presynaptic glutamate terminals, acting as an autoreceptor that inhibits the release of this neurotransmitter. Therefore, dopamine neurons can be inhibited by the activation of mGluR2 receptors on glutamatergic inputs to the VTA.

Stimulation with the nonselective mGluR2/3 agonist LY379268 can reduce drug- and cue-induced reinstatement of nicotine responding, and these effects can be blocked by an mGluR2 antagonist (Justinova et al. 2016). mGluR2/3 stimulation also can reduce the primary reinforcing properties of nicotine, but these effects are smaller and less consistent than the effects on reinstatement. In nicotine-experienced rats, LY379268 reduced nicotine-induced increases in dopamine levels in the NAC only in the presence of drug-predicting cues (D'Souza et al. 2011),

supporting the hypothesis that mGluR2/3 stimulation is more effective at reducing the conditioned effects of nicotine than its primary reinforcing effects.

Although a selective mGluR2 positive allosteric modulator (PAM) was not shown to improve schizophrenia symptoms in a Phase 2 clinical trial, it has been repurposed as a possible therapy for nicotine addiction because of its good safety profile and preclinical efficacy in reducing nicotine reinstatement (Justinova et al. 2015). A Phase 2 clinical trial of this drug for smoking cessation was completed in January 2017, but results are not yet available. This study enrolled 210 female cigarette smokers and evaluated abstinence from nicotine as a primary endpoint. Although GluR5 and GluR2 have been linked to addiction-like behaviors in animals, Acri and colleagues (2017) argued that mGluR2 may be a more feasible drug target because of its relatively mild side-effect profile compared with mGluR5 antagonists.

Glutamate Transporters

Alterations in the function of glutamate transporters also contribute to nicotine-mediated disruptions in the excitatory–inhibitory balance. Mesocorticolimbic expression of glutamate transporter 1 (GLT-1), the cystine/glutamate exchanger, and excitatory amino acid transporter 3 are all decreased after chronic administration of nicotine in rodents (Knackstedt and Kalivas 2009; Knackstedt et al. 2009; Yoon et al. 2014). In addition, reinstatement of nicotine-seeking behavior is associated with decreased expression of GLT-1 and elevated concentration of extracellular glutamate (Gipson et al. 2013). In mice, upregulation of GLT-1 with ceftriaxone had no effect on CPP acquisition but reduced withdrawal symptoms and significantly attenuated nicotine-primed reinstatement of nicotine CPP (Alajaji et al. 2013). Stimulating cystine/glutamate exchanger activity with N-acetylcysteine also may be effective in reducing nicotine consumption. An open-label pilot study of a combination therapy of varenicline and N-acetylcysteine showed a favorable safety profile. Although the study was not designed to evaluate differences in cessation efficacy, patients receiving both therapies smoked fewer cigarettes than those receiving only varenicline (McClure et al. 2015). In addition, a double-blind, randomized controlled trial (RCT) found that, in combination with group behavioral therapy, N-acetylcysteine was effective in reducing the number of cigarettes smoked and in increasing quit rates versus a placebo control group (Prado et al. 2015).

Neuropeptide Systems

Neuropeptides are a class of short-chain polypeptides that serve as neurotransmitters (Table 3.2). Acting

Table 3.2 Novel pharmacologic targets for smoking cessation

Target	Pharmacology	Expected neurobiologic effect	Expected behavioral outcome	Stage of drug development	Other information
Glutamate system					
mGluR5	NAM	<ul style="list-style-type: none"> Decreased Glu transmission 	<ul style="list-style-type: none"> Decreased nicotine intake Decreased relapse vulnerability 	Preclinical	—
mGluR2	PAM	<ul style="list-style-type: none"> Decreased Glu transmission 	<ul style="list-style-type: none"> Decreased relapse vulnerability Decreased nicotine intake 	Phase 2	—
GLT-1	Agonist	<ul style="list-style-type: none"> Decreased Glu transmission 	<ul style="list-style-type: none"> Decreased relapse vulnerability 	Preclinical	—
xCT	Agonist (N-acetylcysteine)	<ul style="list-style-type: none"> Decreased Glu transmission 	<ul style="list-style-type: none"> Decreased nicotine intake 	Phase 2	—
Neuropeptides					
CRF-1	Antagonist (Paxacerfont)	<ul style="list-style-type: none"> Decreased reactivity to withdrawal Decreased dopamine response 	<ul style="list-style-type: none"> Decreased relapse vulnerability Decreased nicotine intake 	Preclinical	Failed Phase 2 anxiety trial
DOR	Antagonist	<ul style="list-style-type: none"> Decreased dopamine response 	<ul style="list-style-type: none"> Decreased nicotine intake 	Preclinical	Naltrexone is a non-selective opioid receptor antagonist used to treat alcoholism and opioid dependence
KOR	Antagonist	<ul style="list-style-type: none"> Decreased reactivity to withdrawal 	<ul style="list-style-type: none"> Decreased relapse vulnerability 	Preclinical	Naltrexone is a non-selective opioid receptor antagonist used to treat alcoholism and opioid dependence
MHb-IPN pathway					
α 5	PAM	<ul style="list-style-type: none"> Increased nicotine-mediated MHb-IPN activation 	<ul style="list-style-type: none"> Decreased nicotine intake 	Preclinical	—
Noradrenergic system					
α 1	Antagonist	<ul style="list-style-type: none"> Decreased norepinephrine signaling 	<ul style="list-style-type: none"> Decreased nicotine intake Decreased relapse vulnerability 	—	—
α 2	Agonist (clonidine)	<ul style="list-style-type: none"> Decreased norepinephrine signaling 	<ul style="list-style-type: none"> Decreased relapse vulnerability 	Off-label use	Potent side effects include sedation and low blood pressure

Notes: α = alpha; **CRF** = corticotropin-releasing factor; **DOR** = delta (δ) opioid receptor; **GLT** = glutamate transporter; **KOR** = kappa (κ) opioid receptor; **mGluR** = metabotropic glutamate receptor; **MHb-IPN** = medial habenulo-interpeduncular nucleus; **NAM** = negative allosteric modulator; **PAM** = positive allosteric modulator; **xCT** = cystine/glutamate exchanger.

on designated G-protein-coupled receptors, these molecules can modulate neuronal activities. As outlined in the upcoming sections of this chapter, a substantial amount of preclinical evidence suggests that multiple neuropeptide systems can contribute to the development of nicotine dependence. Additionally, because several neuropeptides can modulate mood, manipulating these systems may be an effective strategy for improving success rates for cessation by reducing the severity of negative withdrawal symptoms. Although at least a dozen neuropeptides have been linked to nicotine dependence, this section focuses on two primary promising targets: corticotropin-releasing factor (CRF) and the opioid system.

Corticotropin-Releasing Factor

CRF is a peptide hormone known best for its role in the stress response. Chronic nicotine administration increases CRF levels in the VTA of rats, and genetic knockdown of this peptide attenuates self-administration of nicotine (Grieder et al. 2014). In addition, blockade of the peptide's receptor, CRF1, in rats prevented the normally observed increase in nicotine self-administration following a period of forced abstinence and prevented the aversive effects of withdrawal (Cohen et al. 2015). In an intracranial self-stimulation paradigm, the sensitivity of the brain reward pathway can be assessed by measuring the intensity of a stimulus required to elicit self-stimulation behavior, such that higher stimulation thresholds indicate a less sensitive reward system. Exposure to nicotine (or other drugs of abuse) causes animals to perform for much less intense stimulation (i.e., they have lower thresholds), indicating a drug-induced potentiation of the reward system. Conversely, a period of abstinence from a drug elicits a large increase in the intracranial self-stimulation threshold, indicating reduced excitability of the reward system and signifying a depression-like brain state (reflected in elevations of brain reward thresholds) (Stoker et al. 2012).

In nicotine-dependent animals, withdrawal-induced increases in the intracranial self-stimulation threshold are absent in animals treated systemically with CRF1 receptor antagonists, or only in the central amygdala, a brain region known to regulate mood (Marcinkiewicz et al. 2009; Bruijnzeel et al. 2012). Similarly, withdrawal-induced, anxiety-like behavior is exacerbated by infusion of CRF into the interpeduncular nucleus (IPN), and blockade of CRF1 alleviates this behavior (Zhao-Shea et al. 2015). Thus, CRF signaling, particularly in the amygdala and IPN, contributes to the negative affect associated with nicotine withdrawal. Lastly, inhibition of the CRF1 receptor can block both stress-induced potentiation of nicotine CPP and stress-induced reinstatement of self-administration (Zislin et al. 2007). Together, these studies suggest that CRF

signaling is central to changes in nicotine-seeking behavior in response to stress. Although clinical data regarding the role of CRF in smoking behavior are not available, many studies in animal models of nicotine dependence suggest that CRF antagonists may be useful for reducing smoking in humans (Bruijnzeel 2017).

Notably, several small-molecule CRF ligands can cross the blood–brain barrier. Although most are being used only for preclinical research, several have been evaluated clinically to treat anxiety and depression. In a clinical trial of 260 patients, Paxacerfont (a CRF1 receptor agonist) was no more effective than placebo for treating generalized anxiety disorder (Coric et al. 2010); however, this drug has not been evaluated for smoking cessation.

The Opioid System

Mounting evidence has implicated the endogenous opioid system in both neurobiologic and behavioral responses to nicotine. The opioid system consists of three G-protein-coupled opioid receptors that are activated by endogenous peptide ligands. Delta (δ) opioid receptors (DORs) are activated primarily by enkephalins; kappa (κ) opioid receptors (KORs) are activated by dynorphins; and mu (μ) opioid receptors (MORs) are activated by β -endorphins. Each of these receptor–ligand pairs appears to play a role in nicotine addiction. Nicotine-induced dopamine release is attenuated in mice lacking DORs, and these animals do not acquire a CPP for nicotine (Berrendero et al. 2012). Genetic ablation or pharmacologic blockade of DORs with naltrindole also substantially reduces self-administration of nicotine (Berrendero et al. 2012). Although DORs do not appear to play an important role in the somatic responses to nicotine (Berrendero et al. 2012), animals treated with the KOR antagonist JDTic have diminished physical and affective nicotine withdrawal symptoms (Jackson et al. 2010a).

Interestingly, KOR activity does not appear to be necessary for the initial reinforcing properties of nicotine (Jackson et al. 2010a), but pharmacologic blockade of the receptor reduces the anxiogenic effects of nicotine withdrawal and prevents stress-induced reinstatement of nicotine-seeking behavior (Jackson et al. 2010a; Nygard et al. 2016). In addition, withdrawal-mediated activation of the amygdala was reduced in mice pretreated with the KOR antagonist norbinaltorphimine (Nygard et al. 2016). Together, these data suggest that DORs and KORs play discrete roles in the physiological and behavioral responses to nicotine. Although DOR contributes to dopamine release and nicotine reinforcement, KOR appears to be more involved in the physiologic effects of nicotine withdrawal.

In humans, MORs have been linked to craving and addiction severity among smokers. Compared with

nonsmoking controls, smokers had fewer available MOR-binding sites in the basal ganglia and thalamus, and the number of binding sites in the basal ganglia was negatively associated with baseline craving levels (Nuechterlein et al. 2016). Additionally, the availability of MOR-binding sites in both the basal ganglia and temporal cortex was inversely correlated with the severity of physical dependence on nicotine, as assessed by the Fagerström Test for Nicotine Dependence (FTND) (Kuwabara et al. 2014; Nuechterlein et al. 2016). Interestingly, a MOR gene variant (OPRM1 A118G) was found to be potentially associated with reduced availability of MOR binding (Nuechterlein et al. 2016).

Naltrexone (trade names: Revia, Vivitrol), a nonselective opioid receptor antagonist, is commonly used to treat alcoholism and opioid dependence. A clinical trial of 121 smokers found that combining naltrexone with bupropion was associated with higher rates of abstinence from smoking after 7 weeks of treatment compared with bupropion alone, but these rates did not differ significantly between the bupropion-plus-placebo group and the bupropion-plus-naltrexone group at 6 months (Mooney et al. 2016). Similarly, a Cochrane review of eight trials showed no effect of naltrexone alone or as an adjunct to NRT (David et al. 2013a).

Finally, preclinical studies have implicated orexin/hypocretin peptides, originally thought to be involved mainly in feeding and arousal but now shown to modulate the rewarding effects of nicotine, as potential therapies for smoking cessation (Plaza-Zabala et al. 2010; Hollander et al. 2012). An orexin/hypocretin receptor 2 polymorphism has been associated with nicotine dependence in human smokers (Nishizawa et al. 2015), and in rats the selective receptor 2 antagonist (2-SORA 18) can block both cue-induced reinstatement of nicotine self-administration and motivation to respond to nicotine cues, as determined by a progressive ratio experiment in which animals had to press a lever exponentially more times to receive each successive nicotine-paired cue (Uslaner et al. 2014). Similarly, the orexin/hypocretin receptor 1 antagonist SB-334867 decreased the reward-enhancing effects of nicotine in rats, as well as their cue-induced reinstatement of nicotine-seeking behaviors (Hollander et al. 2008; Plaza-Zabala et al. 2013). Interestingly, stimulation of nAChRs increased the activity of orexin/hypocretin neurons (Zhou et al. 2015), suggesting that stimulation of this system may contribute to the physiologic effects of nicotine.

Summary

Neuropeptide systems play a role in multiple stages of the addiction process. Experiments in animals have shown that CRF and the opioid system, neuropeptide Y,

hypocretin, galanin, ghrelin, and vasopressin and additional peptides not discussed here are associated with nicotine dependence (Bruijnzeel 2017). Thus, modulating the function of neuropeptides may effectively reduce smoking behavior in humans. Even so, the role that neuropeptide systems play in human addiction should be investigated further. Several drugs targeting neuropeptide receptors are already in use for treatment of other disorders, but none are approved by FDA for use in smoking cessation.

The Habenulo-Interpeduncular Pathway

Aversive Effects of Nicotine

As discussed previously, nicotine stimulates dopamine pathways to generate the rewarding effects that contribute to addiction. At the same time, activation of nAChRs in the brain and elsewhere results in highly aversive effects, such as nausea, dizziness, and irregular heartbeat. In fact, most first-time smokers report a largely unpleasant experience with nicotine, and sensitivity to the aversive effects of cigarette smoke is inversely correlated with the likelihood of developing habitual smoking (Sartor et al. 2010).

Animal studies of nicotine withdrawal and aversion have identified a crucial role for the habenulo-interpeduncular pathway in mediating these responses. The medial habenula (MHb) is composed mostly of cholinergic neurons that also express Substance P and co-release glutamate. MHb neurons project to the IPN, and activation of this circuit is required for many of the negative effects associated with exposure to nicotine, including the sedative effects induced by high concentrations of this chemical and negative symptoms of withdrawal. Furthermore, stimulation of the MHb or IPN reduces the reinforcing properties of nicotine, but disrupting neuronal signaling in these connected brain regions has the opposite effect—resulting in increased self-administration of nicotine in rodents (Fowler and Kenny 2014).

Potential Molecular Targets

Nicotinic receptors are highly expressed on MHb and IPN neurons, and these regions have the highest expressions of $\alpha 3$, $\beta 4$, and $\alpha 5$ nAChR subunits in the brain. Several human genomewide association studies (GWAS) have linked variants in the *CHRNA3-CHRNA5-CHRNB4* gene cluster (genes that encode the $\alpha 3$, $\alpha 5$, and $\beta 4$ nAChR subunits, respectively) to susceptibility to tobacco use, and preclinical studies in rodents have revealed an important role for these subunits in moderating nicotine intake. $\alpha 5$ knockout mice lacking the $\alpha 5$ nAChR subunit acquired a CPP for high doses of nicotine, but such doses were aversive to their wild-type littermates (Jackson et al.

2010b). Similarly, $\alpha 5$ knockout animals failed to titrate their responses in a self-administration paradigm when increasing doses of nicotine were offered, and this effect was rescued by expression of $\alpha 5$ in MHb (Fowler et al. 2011). Interestingly, $\alpha 5$ knockout mice were indistinguishable from controls at low doses of nicotine in both CPP and self-administration paradigms (Jackson et al. 2010b; Fowler et al. 2011), indicating that the $\alpha 5$ nAChR subunit is not required for the rewarding properties of nicotine. Furthermore, overexpression of the $\beta 4$ nAChR subunit in MHb resulted in increased aversion to nicotine (Frahm et al. 2011). Mice overexpressing $\beta 4$ nAChRs—with or without a $\beta 4$ mutation, which is associated with decreased risk of smoking in humans—displayed larger nicotine-evoked current amplitudes and enhanced aversive behavior (Slimak et al. 2014). Together, these studies suggest that $\alpha 5$ - and $\beta 4$ -containing nAChRs in the MHb are essential for encoding the aversive properties of nicotine, and they likely serve to limit nicotine intake.

Characterization of the MHb to IPN aversive circuit offers a novel and intriguing approach to addiction pharmacotherapy, in which the goal is to enhance the aversive effects of nicotine rather than to reduce its reinforcing effects. $\alpha 5$ - and $\beta 4$ -containing nAChRs are obvious targets. Unfortunately, continuous stimulation of this aversive pathway likely will warrant the use of full agonists of these receptors and is, therefore, clinically unrealistic, because the $\beta 4$ -containing nAChRs are also highly expressed in the autonomic ganglia and a full agonist would likely be poorly tolerated. Instead, the use of PAMs that would enhance signaling only in the presence of an agonist may be more feasible (Fowler and Kenny 2014). Recent findings show that galantamine, which acts as a positive allosteric modulator of $\alpha 5$ subunit-containing nAChRs at low doses, can reduce nicotine intake in rats and smoking in humans (Ashare et al. 2016), supporting the rationale for developing $\alpha 5$ PAMs as novel smoking cessation agents. Notably, other brain regions and neuronal systems, including the mesocorticolimbic dopamine pathway and autonomic nervous system, also contribute to the aversive effects of nicotine. However, the specific mechanisms by which aversive pathways communicate with reward pathways are uncertain.

The Noradrenergic System

Norepinephrine (also known as noradrenaline) is a monoamine neurotransmitter that signals through $\alpha 1$, $\alpha 2$, and β G-protein-coupled adrenoceptors. Like other neuromodulators, norepinephrine receptors are found throughout the brain, and norepinephrine is well known for its role in arousal and the stress response. The noradrenergic system has also been implicated in neurobiologic

responses to nicotine, contributing to both nicotine reward and reinstatement (Fitzgerald 2013). Nicotine increases activity of adrenergic neurons in the locus coeruleus, resulting in increased levels of norepinephrine in the brain. In animal models of nicotine addiction, blocking the transmission of norepinephrine with prazosin, the $\alpha 1$ receptor antagonist, reduced nicotine-induced dopamine signaling and attenuated nicotine self-administration and reinstatement (Forget et al. 2010). In other studies, reducing the tone of norepinephrine by stimulating $\alpha 2$, an inhibitory autoreceptor, with clonidine or dexmedetomidine diminished stress-induced reinstatement of nicotine-seeking behavior in rats (Zislis et al. 2007; Yamada and Bruijnzeel 2011).

In humans, long-term smoking is associated with reduced expression of $\alpha 2$ - and β -adrenergic receptors, which normalize after a period of abstinence (Klimek et al. 2001). In addition, guanfacine, the $\alpha 2$ agonist, reduced stress-induced nicotine craving and smoking in a study of 33 smokers (McKee et al. 2015). Thus, both clinical and preclinical evidence suggest that nicotine increases noradrenergic activity and that correction of this increase may be an effective strategy for reducing smoking.

Clonidine (trade names: Catapres, Kapvay, Nexiclon), the $\alpha 2a$ receptor agonist, has consistently shown some efficacy in improving cessation rates by alleviating negative withdrawal symptoms (Gourlay et al. 2004), but clonidine is not an FDA-approved cessation aid, and prominent adverse side effects, mainly sedation and low blood pressure, limit its practicality. Notably, bupropion and nortriptyline, the antidepressant smoking cessation aids, are norepinephrine reuptake inhibitors.

Summary

Although current pharmacotherapies are effective in reducing smoking in some persons, many are unable to maintain abstinence. With continued interest in the neurobiologic mechanisms of addiction, preclinical advances have improved considerably our understanding of the pathophysiology of nicotine dependence, withdrawal, and relapse. Correspondingly, dozens of novel targets for pharmacologic intervention have emerged, and further investigation into the role of these targets in human smoking is warranted.

Moving forward, the need to develop individualized, multifaceted approaches to smoking cessation is becoming apparent. For instance, drugs that reduce the initial rewarding properties of nicotine are unlikely to normalize the long-lasting neuroadaptations associated with persistent drug use, which underlie craving, withdrawal, and vulnerability to relapse. Another approach may be combination therapy that targets multiple aspects

of addiction behavior, such as a combination of bupropion with NRT or varenicline, which has been successful in human clinical trials. Current evidence is conclusive that current pharmacotherapies for smoking cessation, including such combination therapies as bupropion with NRT or varenicline, improve quit rates (see Chapter 6) but many persons still relapse to smoking (see Chapter 2).

Finally, the pathophysiology underlying addiction to other drugs of abuse, particularly stimulants like cocaine, is similar to that of nicotine. Thus, research that leads to improved smoking cessation therapies also may benefit the treatment of other addictions. The literature should be mined to identify novel targets for interventions that promote smoking cessation.

Vaccines and Other Immunotherapies as Treatments for Nicotine Addiction

Nicotine vaccines are a new class of medication being developed for smoking cessation; interest in these vaccines stems from their novel mechanism of action. Unlike existing cessation medications that act on neurotransmitter receptors in the brain to reduce the reinforcement or withdrawal associated with the use of tobacco products, vaccines act directly on nicotine, the principal addictive constituent of tobacco (Pentel and LeSage 2014). Vaccines stimulate the immune system to produce antibodies that can bind and retain nicotine in the blood, thereby reducing or slowing its delivery to the brain (LeSage et al. 2006b; Esterlis et al. 2013). Interrupting nicotine delivery to its site of action blocks or reduces its behavioral effects (Jefferson et al. 2004; Goniewicz and Delijewski 2013; Maglione et al. 2014). If it proves feasible for nicotine vaccines to produce very high levels of antibodies in blood, efficacy for this approach to smoking cessation should be possible. Because vaccines act in a different manner than existing medications for smoking cessation, such as varenicline or bupropion, combining a nicotine vaccine with those medications to enhance overall efficacy may be possible. An additional potential benefit of nicotine vaccines is that their effects last for many months (Cornuz et al. 2008; Hatsukami et al. 2011), avoiding the need to take a medication each day or, for some products, even more often (Prochaska and Benowitz 2016).

Literature Review Methods

For this section of the chapter, PubMed was searched in January 2017 for studies published between January 1966 and January 2017 about active or passive immunization against nicotine *in vitro* in animals or humans. The following terms were searched alone or in combination: nicotine, tobacco, smoking, cigarette, vaccine, vaccination, immunogen, immunization, antibody, linker, hapten, conjugate, adjuvant, addiction, dependence, cessation,

and monoclonal. Articles identified in this manner were also reviewed to find additional primary references. One reviewer conducted a full review and identified 35 articles for this section.

Design and Mechanism of Action

The human immune system can recognize foreign (nonhuman) proteins present on infectious agents, such as bacteria or viruses, and can form antibodies to help defend against them. Nicotine is a much smaller molecule than a protein and lacks the structure needed to be recognized as foreign. Even so, nicotine can be chemically linked to a foreign carrier protein to stimulate the production of antibodies against it (Pentel et al. 2000; Isomura et al. 2001; Maurer et al. 2005). This nicotine–protein immunogen is typically administered with an adjuvant, a chemical or mix of chemicals that generally enhances immune responsiveness. Administration of such a vaccine results in the production of antibodies that circulate in the blood and bind nicotine tightly and with high specificity. Because these antibodies do not bind appreciably to anything other than nicotine, they might not disrupt the actions of other drugs or medications, and they might not interfere with normal physiologic functions.

Nicotine vaccines have not shown any serious side effects in animals and humans (Hatsukami et al. 2005; Fahim et al. 2013). Autoimmune reactions from vaccine-generated antibodies have not been observed (Hatsukami et al. 2005). Nicotine-specific antibodies do not bind acetylcholine (the endogenous ligand that nicotine mimics), and nicotine itself is a small molecule that should not be able to cross-link antibodies and form immune complexes (Pentel et al. 2000).

Nicotine-specific antibodies in blood cannot enter the brain because of their large size (Satoskar et al. 2003). In addition, nicotine that binds to an antibody cannot enter the brain to interact with the receptors that mediate its

actions. As a consequence, vaccination can attenuate many of the effects of nicotine, provided a sufficient amount of antibody is present (Lindblom et al. 2002; LeSage et al. 2006a). After vaccination, levels of nicotine-specific antibodies in blood decline slowly, over months, and periodic booster doses of vaccine are needed to maintain high levels of antibody (Cornuz et al. 2008; Hatsukami et al. 2011). Because smoking cessation medications generally are required for only 3–6 months, vaccine efficacy should be obtainable after an initial three or four monthly doses of vaccine to achieve high serum antibody concentrations and perhaps a booster dose 3–6 months after that (Hatsukami et al. 2011).

After vaccination, nicotine in blood exists as an equilibrium between a large amount of nicotine bound to antibody and a much smaller amount that remains unbound. Nicotine that is bound to antibody cannot be metabolized, but the unbound nicotine is metabolized normally. As the concentration of unbound nicotine in blood is reduced by metabolism, bound nicotine dissociates from the antibody to re-establish equilibrium and is, in turn, metabolized. In this manner, nicotine can be eliminated even in the presence of antibody, albeit more slowly than otherwise. For example, in rats, immunization doubled the elimination half-life of nicotine from 1 hour in controls to 2 hours in rats vaccinated against nicotine (Keyler et al. 2005). This process frees the antibody of its bound nicotine so that it is once again available to bind newly delivered nicotine (e.g., from the next cigarette).

Examining Data from Animals to Confirm Vaccine Activity

In rats and mice, nicotine vaccination reduces by up to 80% the delivery of single doses of clinically relevant nicotine (equivalent to one or two cigarettes) to the brain (Cerny et al. 2002; Maurer et al. 2005; Pravetoni et al. 2011). Vaccine efficacy is lower with chronic doses of nicotine that approximate regular smoking, but the entry of nicotine into the brain is still slowed (Hieda et al. 2000). In rats, which are thought to provide the best animal models for smoking behavior in humans, vaccination markedly reduces addiction-relevant behaviors, such as nicotine self-administration (Lindblom et al. 2002; LeSage et al. 2006a). Animal studies consistently show that vaccine efficacy is greatest when the level of nicotine-specific antibodies in the blood is high, maximizing the nicotine-binding capacity provided in relation to the amount of nicotine present (Maurer et al. 2005; Pravetoni et al. 2011). For the same reason, vaccination is more effective in blocking the effects of fewer or lower doses of

nicotine than against regular or higher doses (Keyler et al. 1999). Extrapolating these findings to humans, it appears that nicotine vaccines will be most useful for preventing relapse, which is often triggered by taking just a few puffs or smoking just a few cigarettes, and may be less effective for encouraging smoking cessation among regular smokers who are not motivated to quit.

Clinical Trials of Nicotine Vaccines

Several nicotine vaccines have progressed through Phase 2 or 3 clinical trials (i.e., have been tested for safety, efficacy, and effectiveness relative to other treatments), in combination with standard behavioral counseling (Cornuz et al. 2008; Hatsukami et al. 2011; Fahim et al. 2013; Tonstad et al. 2013). All of these studies provide preliminary evidence of safety, but levels of antibody in the blood have been substantially lower than those achieved in rats or mice. Mean levels of antibody in participants in human studies have reached approximately 40 micrograms per milliliter ($\mu\text{g/mL}$), but levels of 200–500 $\mu\text{g/mL}$ can be produced in mice or rats (Maurer et al. 2005; Keyler et al. 2008). Part of this difference comes from the ability to administer higher doses of immunogens and stronger adjuvants in animals than would be tolerated in humans without producing side effects. Not surprising, therefore, is that the overall efficacy of vaccines for enhancing smoking cessation has not been demonstrated. In several studies, however, participants with the highest levels of serum antibody also had higher rates of smoking cessation compared with those who received a placebo vaccine (Cornuz et al. 2008; Hatsukami et al. 2011). This key observation suggests that the vaccine strategy has merit and has the potential to be effective. At this time, FDA has not approved any nicotine vaccines.

Next-Generation Vaccines

Next-generation vaccines hold promise for producing higher levels of antibody than those studied to date; several approaches are being evaluated:

- Improving the way in which nicotine is attached to its carrier protein to provide tighter binding to the immune cells that initiate antibody production (Moreno et al. 2012);
- Using more immunogenic carrier proteins or designing and synthesizing carrier proteins that are optimized to enhance the interaction of nicotine

with immune cells (McCluskie et al. 2013; Rosenberg et al. 2013; Miller et al. 2014; Jacob et al. 2016);

- Mixing or combining the nicotine–protein immunogen with newer adjuvants (e.g., CpG oligonucleotides, water/lipid emulsions) that enhance the production of antibodies by activating novel molecular pathways, or using combinations of adjuvants that provide additive efficacy (McCluskie et al. 2013; Jacob et al. 2016); and
- Attaching nicotine to synthetic nanoparticle scaffolds that are designed to more precisely control and optimize interactions between nicotine and the immune system (Lockner et al. 2013; Desai and Bergman 2015; Liu et al. 2016).

Combining Vaccines with Medications

Nicotine vaccines can be designed to display different surfaces of the nicotine molecule to the immune system. Because the immune system sees each surface as a distinct stimulus, two or three suitably designed nicotine vaccines can be co-administered to get an additive antibody response (Keyler et al. 2008; de Villiers et al. 2013). Nicotine vaccines also can be combined with small-molecule medications because those drugs act by separate mechanisms. For example, nicotine-specific antibodies can be combined with mecamylamine, a nicotine antagonist that blocks the action of nicotine on its receptors in the brain and has been used experimentally to promote smoking cessation. This combination is more effective in rats than either of these treatments alone for blocking nicotine discrimination, a measure of whether the animal recognizes that it has received nicotine (LeSage et al. 2012). However, a clinical trial of a nicotine vaccine combined with another drug for smoking cessation, varenicline, found no additional effect from vaccination compared with the drug alone (Hoogsteder et al. 2014).

Passive Immunization with Monoclonal Antibodies or Gene Transfer

The amount of antibody produced by vaccination is limited by the capacity of the immune system. Thus, it could be possible to produce nicotine-specific monoclonal antibodies in bacterial cultures or other *in vitro* systems and bypass the need for vaccination by administering the preformed antibodies directly (passive immunization). In animals, this approach mimics vaccination, but greater efficacy is possible because very large doses of antibody can be safely administered (Carrera et al. 2004; Keyler et al. 2005). The main limitations to this approach in humans are its high cost and the likely need to administer the antibodies intravenously (Skolnick 2015). An alternative approach to passive immunization is to administer a harmless virus (not capable of replication) that contains DNA coding for the production of the desired antibody. This virus can take up temporary residence in tissues and produce nicotine-specific antibodies that are independent of the host's immune system. In rodents, extremely high levels of antibody have been achieved using this strategy for periods of up to several months (Hicks et al. 2012). This approach holds promise for human therapies if measures to ensure its safety can be established.

Summary

Animal studies and early clinical trials have provided proof-of-principle that drug-specific antibodies can block the addictive effects of nicotine and serve as an adjunct to smoking cessation. The main benefit of this approach may be preventing relapse. Anticipated progress in vaccine design and enhancement of the immune response should (a) provide substantially more effective vaccines and other approaches to providing nicotine-specific antibodies and (b) create opportunities to better explore their therapeutic potential.

Insights into Smoking Cessation from the Field of Neurobiology

Smokers trying to quit often can maintain abstinence for short periods, ranging from days to weeks. However, quitting smoking usually requires several attempts (USDHHS 2000, 2010; García-Rodríguez et al. 2013). Evidence shows that smokers often require multiple quit attempts (even more than 20, depending on the

metrics used) and many years to obtain long-term (greater than 1 year) smoking abstinence (Chaiton et al. 2016). This clinical observation highlights the often-mistaken assumption made by both practitioners and smokers trying to quit that the absence of the behavior (smoking) reflects the absence of the disease (dependence). Thus, to

enhance treatment outcomes, a better understanding of the neurobiologic basis of the disease is required. Until the development of noninvasive brain imaging (initially positron emission tomography [PET] and more recently and prominently, functional magnetic resonance imaging [fMRI]), such an understanding of affected humans has been difficult to obtain. In contrast, considerable preclinical data (Leslie et al. 2013) have convincingly supported the proposition that chronic self-administration of nicotine—like that of other dependence-producing drugs, including stimulants and opiates—alters specific long-term regional neurobiologic processes that have been hypothesized to explain the high rates of recidivism in persons who are trying to quit smoking (Sutherland and Stein 2018).

During the past two decades, noninvasive brain imaging has repeatedly demonstrated differences in brain structure and function in smokers compared with matched, never-smoking, healthy persons. Thus, it is plausible that such differences might be applied usefully and clinically to develop better behavioral interventions and pharmacologic treatment strategies to improve the current rates of cessation. There are, however, no currently available brain-based neuroimaging biomarkers of treatment outcome, and much of the historic behavioral and personality characterizations that have been shown to differ between smokers and nonsmokers have failed to serve as accurate predictors of treatment success.

Why, after consistent demonstrations of differences in brain and behavior between groups, have these data not been effective in predicting treatment outcomes? One working hypothesis is that the differences are not a result of the addiction process, but rather that they reflect a predispositional trait that preceded drug use and dependence and are more likely to reflect risk factors for addiction than consequences of drug use. If so, it would seem unlikely that differences identified from cross-sectional population studies would or should signal outcome changes in brain circuits.

The alternative hypothesis is that the aforementioned brain differences are indeed caused by chronic drug use and reflect dependence-induced, neuroplastic brain changes. If so, this would suggest that longitudinal, within-participant neuroimaging data collected along the trajectory from the onset of treatment through short- and long-term recovery might serve as a biomarker of current disease severity and, importantly, be predictive of disease remission. Such a biomarker also could determine the possible liability risk for addiction of potential novel pharmacologic agents and help match treatment options with the highest probability of aiding the individual smoker. A review of the neuroimaging literature reveals a miniscule number of studies performed on former smokers

(Neuhaus et al. 2006; Nestor et al. 2011, 2018a,b; Krönke et al. 2015; Zanchi et al. 2015, 2016; Weywadt et al. 2017; Ono et al. 2018), leaving mostly unknown the answer to the question of what a former smoker's brain actually looks like.

Once the data become available in greater numbers, noninvasive brain imaging could:

- Identify differences in brain structure and function between smokers and nonsmokers;
- Follow persons along the course of treatment to identify brain circuits and networks that uniquely change in those whose treatments induce prolonged abstinence versus those who relapse (i.e., whether the above-group differences return to a [presumed] pre-addicted state vs. whether other neurobiological systems strengthen to compensate for the dysregulated brain system and networks);
- Make post hoc predictions of treatment outcomes by using pretreatment data and posttreatment outcomes;
- Develop brain-based biomarkers in clinical trials that predict treatment outcomes;
- Identify intermediate phenotypes of brain circuits and networks that can be used to fractionate the phenotype of the individual smoker to allow for personalized medicine and identify treatments with the highest probability of successful outcomes.

The ultimate goal of this strategy is to develop a system to individualize predictions of health outcomes on the basis of a model developed from group studies (Gabrieli et al. 2015).

Literature Review Methods

For this section of the chapter, PubMed was searched in January 2017 for articles that were published between 2014 and 2017 about studies that focused on the intersection of human neuroimaging and nicotine addiction. The following terms were searched: fMRI, PET, MRI, nicotine, and nicotine addiction. The references cited represent publications in this domain since the 2014 Surgeon General's report. From these articles, some studies conducted between the publication of the 2010 and 2014 Surgeon General's reports were also included. One reviewer conducted a full review and identified 77 articles for this section. Articles were omitted if the studies were

considered to be underpowered or if quality could not be assessed because of incomplete descriptions.

Methodology of Neuroimaging Studies

In contrast to PET technology, which is best suited to identify molecular changes in neurotransmitter systems (for a review, see Lameka et al. 2016), MRI can be used to study brain structure, including gray matter density and cortical thickness, and the microstructure and integrity of white matter tracts (diffusion tensor imaging). MRI also can measure certain biochemical constituents of the brain using magnetic resonance spectroscopy. Finally, fMRI measures changes in brain activity (as inferred from changes in blood flow, blood volume, and oxygenation). The strength of fMRI is that it can measure brain activity while persons perform various cognitive and emotionally laden tasks, linking the behavioral performance of such nicotine addiction-related processes as working memory, attention, cue reactivity, and inhibitory (cognitive) control to the localization and magnitude of brain activity (for a review, see Huettel et al. 2014).

Data from fMRI also can be acquired in the absence of a directed task (i.e., the participant is at rest) (Biswal et al. 1995). Studies using resting-state fMRI have demonstrated that specific brain connections (i.e., circuits and networks) are apparent in the absence of a directed task, with the strength of connections at rest sufficient to predict the strength of subsequent task activation and behavioral performance (Kelly et al. 2008; Baldassarre et al. 2012). Differences in resting-brain circuits may reflect neuropsychiatric disease, including nicotine dependence (Fedota and Stein 2015).

Despite their increasing applicability, neuroimaging studies are inherently correlative. Nevertheless, designs that include a pharmacologic intervention and incorporate a parametric manipulation of the task or drug (dose-response) enable more precise interpretations. Finally, the advent of noninvasive brain stimulation (NIBS) (e.g., transcranial magnetic stimulation and transcranial direct [or alternating] current stimulation) may enable more direct probes of and interventions directed at putative neural circuit plasticity. The rationale for applying NIBS in addiction is that it could enhance circuits related to cognitive control or weaken circuits that are sensitive to provocations from cues. Although these circuits are also targets for many of the behavioral therapies applied in addiction (e.g., cognitive behavioral therapy), brain electrical stimulation has the potential to improve the efficacy of the treatment intervention by directly engaging the

affected circuits. Having achieved some modest success, transcranial magnetic stimulation, an FDA-approved treatment for depression, has been proposed as a treatment for addiction in general (Barr et al. 2008; Gorelick et al. 2014; Dunlop et al. 2017) and for smoking in particular (Fraser and Rosen 2012; Li et al. 2013b; Dinur-Klein et al. 2014; Pripfl et al. 2014). However, the data for NIBS are too preliminary to evaluate its efficacy in smoking cessation.

Differences in Brain Circuitry and Cognitive Constructs in Nicotine Dependence

The neuroimaging studies reviewed in this section have examined the effects of chronic cigarette smoking, acute versus extended abstinence, treatment interventions, and smoking cessation on the major cognitive and affective constructs hypothesized to be involved in nicotine addiction (for a general review of addiction neurobiology, see earlier discussion, Koob and Volkow 2016, and USDHHS 2010). Although different drugs of abuse initially bind to receptors specific to that drug's pharmacology (e.g., opiate receptors [opioids]; psychostimulants [monoamine transporters]; tobacco [various nicotinic receptor subtypes]), the "downstream" neurobiologic circuits and mechanisms generally are believed to share a common substrate across all (or most) addictions. The cyclic nature of addiction and the underlying circuitry and neuroplastic consequences of chronic drug administration provide a theoretical framework to discuss the circuitry of nicotine addiction (Koob and Volkow 2016; Volkow et al. 2016). A better understanding of these neurobiologic mechanisms may yield more effective tools to aid in smoking cessation and also may be achievable using many fewer participants than are necessary in a behavior-only-based clinical trial, because the effect size of a brain response, which is more proximal to the causative mechanism, is significantly greater than the more distal behavioral response (Rasetti and Weinberger 2011). A review of the literature by Menossi and colleagues (2013) summarized the role of neuroimaging in pharmacologic treatment for smoking and nicotine dependence. They identified multiple brain regions—including the anterior and posterior cingulate cortex, orbitofrontal cortex, ventral striatum, amygdala, thalamus, and insula—that are involved in both the maintenance of smoking and processes related to nicotine withdrawal, such that two reasonably efficacious drugs used to treat nicotine dependence, varenicline and bupropion, modulated activity in these areas. In contrast, although NRT improves cognitive symptoms related to withdrawal, it does not generally alter the activity of neural circuits that are associated with nicotine addiction.

Smoking Cues and Craving Provocation

Exposure to cues related to smoking is thought to activate brain circuits related to the salience (i.e., of immediate relevance) of the stimuli and to engage memory, affective, and cognitive processes that promote drug seeking and, in most cases, drug taking. Moreover, smoking cues can directly interfere with the abstinent person's ability to concentrate and to focus attention on performing a task or on a therapeutic intervention that involves behavioral change (Luijten et al. 2011). Accordingly, a better understanding of the brain circuits and neurobiologic mechanisms engaged by cues might lead to novel targets for treatment interventions and potentially the development of a biomarker of outcome efficacy. For example, treatment with bupropion is associated with improved ability to resist cue-induced cravings and a reduction in cue-induced activation of limbic and prefrontal brain regions, including the ventral striatum, medial orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC) (Culbertson et al. 2011). Similarly, responses to varenicline in the medial OFC (as a function of reward) and in the lateral OFC (during reward evaluation) may play a role in a diminished response to smoking cues, which may contribute to the drug's clinical efficacy (Franklin et al. 2011). Consistent with these findings, Hartwell and colleagues (2013) found that successful smoking cessation with varenicline was associated with increased activation, before a quit attempt, in brain areas related to attentiveness and memory while the person resisted the urge to smoke, suggesting the drug may exert its effects by reducing craving and enhancing resistance to urges to smoke during cue-elicited craving.

More mechanistically, activation in the amygdala—a structure long associated with stress processing, reinforcement learning, and risk of relapse—is dampened by both varenicline and nicotine, but a report by Sutherland and colleagues (2013b) found that this was only in a subset of smokers who appeared most susceptible to the negative consequences of nicotine abstinence for behavioral performance (in this case, forced choice reaction time). This finding on individual difference may provide a useful step toward fractionating the smoker phenotype by discrete neurobiologic characteristics, which in turn could lead to differential treatment algorithms. Furthermore, the functional connectivity between the amygdala and insula and, in turn, of the insula to components of the default mode network (DMN) (which is composed of the ventromedial PFC, parahippocampal gyrus, and posterior cingulate cortex [PCC] and is thought to process interoceptive states, ruminations, reflective thoughts, and similar phenomena) is downregulated by both varenicline and nicotine in abstinent (but not sated) smokers, and the circuit

reduction is linked to reduced symptoms of nicotine withdrawal, which may help to promote cessation (Sutherland et al. 2013a).

Consistent with a role for the amygdala and insula in cessation, 3 months of mindfulness treatment was found to reduce both behavioral reactivity and responsivity in both brain regions and to predict successful cessation (Kober et al. 2017). In another study, 2 weeks of meditation training (vs. a relaxation control) resulted in an average 60% reduction in smoking that correlated with increased activity in the ACC and PFC, which are brain areas related to self-control (Tang et al. 2013). Taken together, these studies suggest that reducing DMN-insula-amygdala circuit activity (via pharmacologic or behavioral interventions) may promote abstinence by modulating the interoceptive, negative affective, and ruminatory consequences (i.e., cravings) of cessation and point toward reduced strength of discrete circuit connectivity, contributing in turn to the amelioration of subjective withdrawal symptoms.

Sutherland and colleagues (2012) hypothesized that the balance between various large-scale brain networks modulates both normal and addiction-related behaviors. The three major large-scale brain networks in this model were (1) the DMN; (2) the executive control network, primarily composed of the dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex and thought to be engaged during the cognitive processing of exteroceptive signals; and (3) the salience network, which is anchored by the dorsal anterior cingulate cortex (dACC) and anterior insula and is thought to attribute salience to stimuli and the selection of action during times of conflict. In a test of this hypothesis, Lerman and colleagues (2014) demonstrated that the dynamic interrelationship among these three major large-scale networks is altered during acute 24-hour abstinence (vs. satiety) and predicts the (a) difference in abstinence-induced changes in craving to smoke and (b) reduced cognitive performance and brain activation seen during a working memory task. Independently, a study by Zhang and colleagues (2011) positively correlated cue-elicited activity in the dlPFC with the strength of functional connectivity between the dlPFC and rostral anterior cingulate cortex.

If acute abstinence in fact modulates the circuits and networks described above, intervention strategies aimed at changing their activities might prove efficacious. One such potential cessation treatment uses real-time feedback of the fMRI signal to facilitate volitional control over regions of the brain that regulate craving. In a proof-of-concept study, modulating the strength of functional connectivity between the ACC and medial PFC via feedback was associated with a reduction in craving among heavy smokers (Kim et al. 2015). Furthermore, feedback from the ACC but not the dorsomedial PFC (dmPFC),

which is thought to be more involved in resisting craving, reduced activation to smoking cues, especially in persons with less severe nicotine dependence (Canterberry et al. 2013; Hanlon et al. 2013; Hartwell et al. 2016).

The amount of nicotine presented to the brain via smoking is directly related to the severity of nicotine dependence, which in turn is linked to the severity of cravings during abstinence. Emergent data suggest a genetic link between the rate of nicotine metabolism, success in smoking cessation, pharmacologic efficacy, and brain activity (see “Genetic Studies of Smoking Phenotypes” later in this chapter for a discussion about the influence of nicotine metabolism on dependence). For example, compared with slow metabolizers, persons who are fast nicotine metabolizers demonstrate significantly greater responses to cigarette cues in the amygdala, hippocampus, striatum, insula, and cingulate cortex—supporting the importance of cue-induced craving in relapse and helping to explain why fast metabolizers have lower cessation rates (Tang et al. 2012). In one study, greater activation in the caudate and frontal pole in fast versus normal metabolizers predicted abstinence-induced subjective cravings in response to smoking cues, suggesting that adjunctive behavioral cessation treatment, such as desensitization to repeated exposures to cues, may be useful in faster metabolizing persons (Falcone et al. 2016).

Reward

Like other abused drugs, nicotine, by virtue of its ability to interact with components of the mesocorticolimbic system and to enhance levels of dopamine (Volkow et al. 2015), modulates reward processes in ways that may help perpetuate smoking and limit successful cessation. For example, 24-hour abstinence is associated with increased striatal activation during anticipation of a smoking reward and decreased activation in anticipation of a monetary reward, and greater abstinence-induced decrements in striatal activation during monetary reward are associated with a greater likelihood of relapse (Sweitzer et al. 2016b). Consistently, administration of nicotine during abstinence reduces activity in the ventral striatum when the person is anticipating a win or loss (i.e., reward valence) and increases activity in the dorsal striatum when the person is anticipating the magnitude of a rewarded outcome (Rose et al. 2013; Fedota et al. 2015), suggesting a mechanism influencing the observed continued motivation to smoke and difficulty with cessation when trying to quit. Importantly, chronic dependence on nicotine, but not acute nicotine administration (i.e., NRT), reduced the ventral striatal temporal difference error signal (a learning mechanism construct related to dopamine release) in a

classical conditioning reward paradigm, which is consistent with the inability of NRT to alter reward-related functional properties and perhaps explains its only modest ability to aid in smoking cessation (Rose et al. 2012). In contrast, varenicline blunts the magnitude of mesocorticolimbic dopamine activity when a smoker is processing a reward, likely contributing to the drug’s greater efficacy as pharmacotherapy for smoking cessation (Fedota et al. 2015).

Practically speaking, smokers who show lower prequit brain reactivity to pleasant stimuli than to cigarette-related cues are less likely to be abstinent 6 months after their quit attempt. Therefore, an important factor underlying relapse may be the lack of alternative forms of reinforcement when someone is deprived of nicotine (Versace et al. 2014). Indeed, ambivalence about treatment negatively correlates with cue-related activation in brain areas linked to reward processing, motivation, and attention—including the rostral ACC, medial PFC, and caudate nucleus—thus, supporting the importance of both motivation to quit and expectancy to smoke (Wilson et al. 2012, 2013).

Cognition and Cognitive Control

Cognitive performance and control processes have long been known to regulate so-called top-down control over behaviors, such as the ability to resist the drug-seeking drive following cue presentation, subsequent drug craving, and ultimately drug taking. Such processes may serve as potential markers of sustained abstinence and treatment efficacy. For example, in a study by Krönke and colleagues (2015), former smokers exhibited less Stroop interference, indicating superior cognitive control, compared with current smokers. (Stroop interference is a behavioral task designed to induce a conflict in cognitive processing that leads to a reduction in reaction time to perform the task. One example of this effect requires individuals to identify the color of a word that is incongruent with the word itself [e.g., the word green written in red ink] [Stroop 1935].) Furthermore, when more demanding incongruent trials were contrasted with easier congruent trials in this study, former smokers showed stronger activity in the superior frontal gyrus and ACC than current smokers, suggesting successful smoking cessation may be mediated by enhanced cognitive control (Krönke et al. 2015). Elsewhere, in a study by Froeliger and colleagues (2017), differences in baseline corticothalamic function were predictive of inhibitory control processing and vulnerability to smoking relapse. In another study, greater activation in the inferior frontal gyrus, presupplementary motor area, and basal ganglia during a response inhibition task at pretreatment baseline was associated

with an attenuated association between cravings and subsequent smoking (Berkman et al. 2011).

Externalizing tendencies and/or compromised error processing among subsets of smokers may be relevant factors for the success of smoking cessation. Specifically, higher externalizing tendencies correlated with more performance errors and predicted less recruitment of the insula and dACC following the commission of errors in smokers, and smaller error-related insula activity and less dACC activity correlated with higher craving during abstinence (Carroll et al. 2015). In support of these regional alterations, reduced density of gray matter in the dlPFC of smokers, a structure long implicated in working memory, was associated with cue-elicited activity in the same brain area, suggesting a neurobiologic mechanism for the impaired cognitive control associated with chronic drug use (Zhang et al. 2011). Finally, smoking is associated with a diffuse cortical thinning that accelerates normal age-induced thinning and cognitive decline, which requires approximately 25 years post-cessation for complete cortical recovery (Karama et al. 2015). Although the amount of cortical thinning was related to the amount of nicotine used, as an association, the causation of the thinning is not known. Similarly, Power and colleagues (2015) observed a dose-dependent relationship between smoking and white matter hyperintensities.

Working memory is a sensitive biomarker of nicotine dependence and acute withdrawal (Loughead et al. 2010). Relapse to smoking was highly predictive by decreased dlPFC and increased PCC activation during acute abstinence versus smoking satiety (Loughead et al. 2015). Moreover, acute smoking abstinence was sufficient to reduce dmPFC activity and performance on a working memory task, and because smoking a low-nicotine cigarette did not ameliorate the deficit, NRT may be sufficient to resolve cognitive function during smoking abstinence. In contrast, an attempt to improve withdrawal-induced cognitive deficits by using tolcapone (to inhibit dopamine metabolism) only modestly improved the performance of working memory (Ashare et al. 2013). Similarly, a nicotine vaccine that blocks binding to nicotinic receptors in the brain did not block effectively either cue responsivity or brain activity during a working memory task (Havermans et al. 2014). Thus, like most vaccines, a nicotine vaccine may prove more effective in preventing a disease (i.e., nicotine addiction) because brain circuits that have been modified or dysregulated as a result of nicotine dependence are not likely to return to their pre-addiction state simply by blocking new nicotine from reaching the brain. Indeed, that smoking relapses occur months or even years after smoking cessation suggests that the absence of nicotine alone is insufficient to reverse dependence-induced circuit neuroadaptations.

Insights from Neuroimaging for Antismoking Messages

In addition to providing a salient stimulus to seek out or enhance drug use (Wang et al. 2013), smoking cues could serve, together with appropriate messaging, as a negative reinforcement. For example, analyses using neuroimaging of responses to antismoking ads that were intended to change attitudes toward smoking appeared to predict the severity of subsequent smoking and treatment outcomes (Camenga and Klein 2016). Most persons begin using nicotine and often become nicotine dependent during adolescence (USDHHS 2012; Camenga and Klein 2016). Compared with adult smokers, adolescent smokers exhibited greater craving reduction and greater blunted recruitment of insula and dlPFC in response to package warning labels (Do and Galván 2015). Furthermore, greater dlPFC regulation of limbic regions predicted cigarette craving. These data underscore the prominent role of fronto-insular circuitry in predicting the efficacy of graphic warning labels for reducing craving in adult and adolescent smokers. In adult smokers, activation in the dmPFC in response to persuasive advertisements predicted urine cotinine levels 1 month later (Wang et al. 2013). In smokers trying to quit, the amygdala's response to smoking cessation messages was modulated by genetic variation in the serotonin transporter and was predictive of quitting outcome (Jasinska et al. 2012). Genetic alterations in the dopamine D4 receptor also modulated responsiveness of the amygdala to cues (Xu et al. 2014). A study by Chua and colleagues (2011) supports the hypothesis that tailored health interventions are more effective at eliciting positive behavior change than generic interventions. For example, messages tailored to the individual increased activation of the dmPFC, a region known to be involved in self-related processing, and predicted quitting during a 4-month follow-up. Taken together, these data suggest that fMRI may aid the prerelease evaluation of televised public health ads.

Neuronal Circuits and Networks

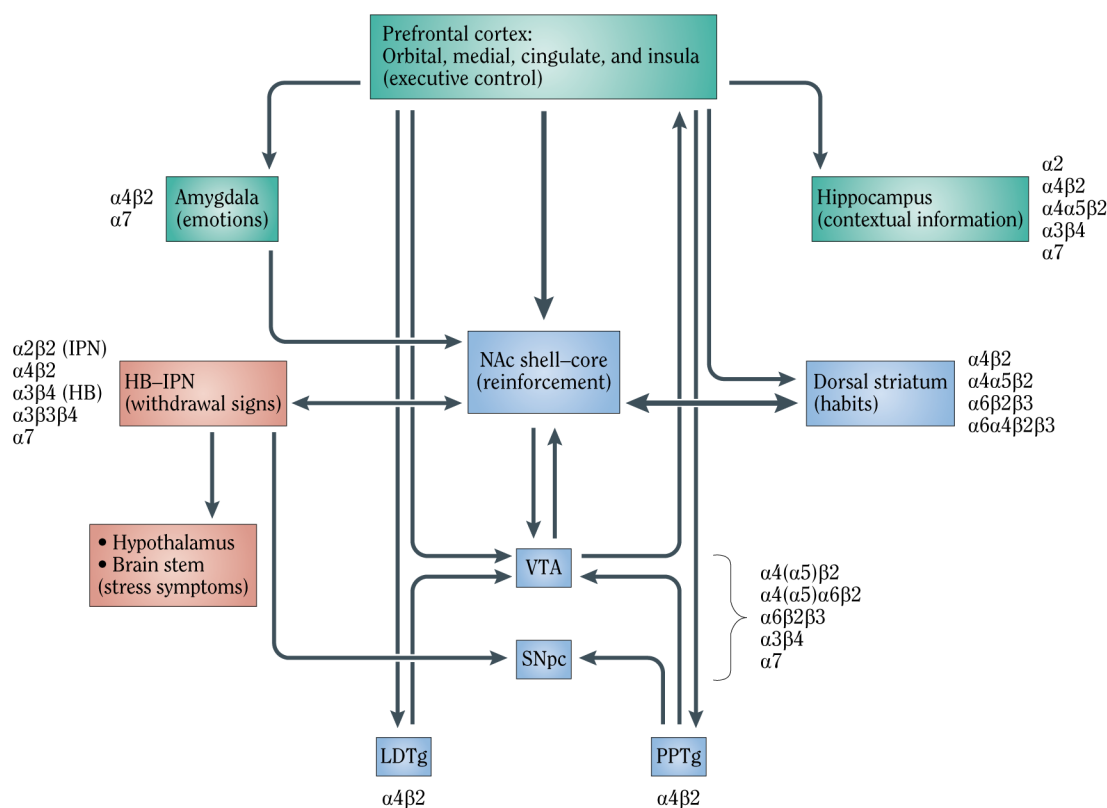
Studies of resting-state functional connectivity have revealed that the ACC, PCC, medial and lateral OFC, ventral striatum, amygdala, thalamus, and insula are all heavily involved in the maintenance of smoking and nicotine withdrawal (Figure 3.3). Varenicline and bupropion modulate activities in these brain areas, providing mechanistic support for their abilities to alleviate withdrawal symptoms and help with smoking cessation. For example, among non-lapsed smokers who were making a 3-week quit attempt,

Sweitzer and colleagues (2016a) observed abstinence-induced increases in connectivity strength between the ventral striatum and a network of regions implicated in addictive disorders, including the insula, superior temporal gyrus, and ACC; the opposite pattern was observed for those who later lapsed. Also in this study, following 24-hour abstinence, decreased connectivity between the dorsal striatum and the medial PFC, PCC, hippocampus, and supplemental motor area was observed across both successful and unsuccessful cessation groups. These findings suggest that modulation of striatal connectivity with

the cingulo-insular network during early withdrawal may be associated with outcomes for smoking cessation.

This potential association is particularly important because a high density of nAChRs has been found in the cingulo-insular network (Picard et al. 2013), and this salience network has been implicated in the switching of cognitive resources during abstinence (vs. satiety) toward more internal bodily processing and nicotine craving (Sutherland et al. 2012; Lerman et al. 2014). Moran-Santa Maria and colleagues (2015) found a psychophysiological interaction between the anterior insula and the

Figure 3.3 Neuronal mechanisms involved in nicotine addiction: A model



Source: Changeux (2010, p. 391), with permission.

Notes: α = alpha; β = beta; **HB-IPN** = habenula-interpeduncular; **LDTg** = laterodorsal tegmental nucleus; **NAc** = nucleus accumbens; **nAChR** = nicotinic acetylcholine receptor; **PPTg** = pedunculopontine tegmental nucleus; **SNpc** = substantia nigra pars compacta; **VTA** = ventral tegmental area. “Many brain areas contain nAChR subunits and are involved in nicotine addiction. First, the somata of the dopaminergic neurons that contribute to nicotine intake and reinforcement are in the VTA of the midbrain: they project to the prefrontal cortex and to limbic areas, in particular the hippocampus and NAc in the striatum [Balfour et al. 2000; Di Chiara 2000; Maskos et al. 2005; Balfour 2009]. These VTA neurons receive cholinergic innervation from the PPTg and the adjacent LDTg [Picciotto and Corrigall 2002; Maskos 2008]. Second, the emergence of a negative emotional state and withdrawal syndrome following smoking cessation—or nicotine deprivation—mobilizes distinct neural circuits that can include the extended amygdala and brain stress systems [Koob 2008], the hypothalamus, hippocampus [Davis and Gould 2009], SNpc, and/or the HB-IPN system [Salas et al. 2009]. Third, the ‘switch’ from voluntary nicotine use to compulsive drug use may represent a global top-down ‘gating’ transition from control by a prefrontal (cortical and insular) global neuronal workspace (BOX 1) to subcortical (striatal) control [Grace 2000; Changeux and Dehaene 2008; Naqvi and Bechara 2009]” (Changeux 2010, p. 391).

precuneus (a part of the DMN)—which are regions known to be involved in self-awareness and interoception, or the sense of internal bodily states—during the presentation of smoking cues. According to Zelle and colleagues (2017), connectivity strength between the anterior insula and dlPFC following provocation from smoking cues predicts the ability to resist smoking after acute abstinence.

Vulnerability to relapse after a quit attempt was associated with weaker connectivity between the posterior insula and primary sensorimotor cortex, suggesting that greater connectivity in this network improves the ability to inhibit a motor response to cigarette cravings when those cravings conflict with the goal to remain abstinent (Addicott et al. 2015). Elsewhere, research has consistently shown that the insula and basal ganglia play a role in addiction to smoking, as revealed by localized stroke lesions in these regions (Naqvi and Bechara 2010; Gaznick et al. 2014), and that local connectivity coherence within the PCC, a key DMN region, can predict the success of cessation (Wang et al. 2017).

In contrast to the insula-based circuits related to the state of nicotine withdrawal and the positive effects of NRT on cognitive processing, NRT does not alter the activity in an ACC-ventral striatal neural circuit that is associated with the severity of trait nicotine addiction (Hong et al. 2009). Further speaking to the role of the ACC and striatum in trait addiction, slow nicotine metabolizers, which presumably have relatively higher nicotine levels in the brain, showed greater functional connectivity in the dACC and ventral striatum, which is negatively associated with the severity of nicotine dependence (Li et al. 2017). Critically, the dACC and ventral striatum are biased by inputs from the insula. Moreover, a similar gene–environment reduction was seen in the dACC and ventral striatum during smoking abstinence when study participants performed a cognitive control response inhibition task and a reward task to probe their function, which were both normalized following NRT. These data suggest that the inherited rate of nicotine metabolism fundamentally changes brain circuits and function, which may, in turn, influence the outcomes of smoking cessation (Li et al. 2017).

The findings that both nicotine trait addiction (long standing) and current state (transient) engage distinct neural mechanisms (dACC and ventral striatum) and circuits (amygdala, insula, and DMN) and that NRT appears to improve cognitive symptoms related to withdrawal but does not alter a measure of disease severity (the FTND), suggest that both nicotinic and non-nicotinic pharmacotherapy may reduce smoking via distinct neural mechanisms of action and thereby endorse the potential value of neuroimaging in the development of new medications and discovery of brain-based biomarkers of early therapeutic response in cigarette smokers (Menossi et al. 2013).

Molecular Imaging

PET imaging has contributed to a better understanding of the biochemical and molecular alterations in nicotine addiction and smoking cessation. Clearly, understanding the mechanisms of action of effective pharmacotherapies for nicotine dependence is critical to the development of better treatments. A PET study using [(11)C]-(+)-PHNO demonstrated that varenicline, the most effective pharmacotherapy currently available, increases levels of striatal dopamine, much as smoking does (Cosgrove et al. 2014), which may contribute to the drug's efficacy (Di Ciano et al. 2016).

An important public health question is whether documented changes in brain structure and function in persons who are dependent on nicotine can be reversed or normalized following extended abstinence. Notably, smoking cessation is accompanied by a decrease in the density of $\alpha 4\beta 2^*$ nAChRs across the brain, suggesting a normalization of the receptors that primarily bind nicotine following intake (Brody et al. 2013). Additionally, smokers with less upregulation of $\alpha 4\beta 2^*$ were found to have a greater probability of quitting smoking than those with greater upregulation, providing a potential biomarker of cessation success (Brody et al. 2014). In a different study (Akkus et al. 2016), compared with recent former smokers, long-term former smokers showed higher mGluR5 binding, most prominently in the frontal cortex and thalamus, suggesting that downregulation of these receptors may be a mechanism underlying nicotine dependence and the high rate of relapse seen in those previously exposed to nicotine. Accordingly, mGluR5 receptor binding may serve as an effective smoking biomarker and a potential target for future medications (Akkus et al. 2016). In contrast, binding at the GABA(A) receptor, a component of the principal brain inhibitory system, does not seem to normalize with sustained abstinence (Stokes et al. 2013).

Sex differences in smoking behavior and brain molecular mechanisms have been reported (Sieminska and Jassem 2014). Consistent with the notions that men smoke cigarettes for their reinforcing properties and women smoke for such reasons as mood regulation and cue reactivity (Perkins et al. 2001; Xu et al. 2008), Cosgrove and colleagues (2014) found, in an analysis of smoking in a PET scanner, that smoking resulted in rapid increases in dopamine in the ventral striatum of men, while dopamine release in women was faster than in men in a subregion of the dorsal putamen. Moreover, smoking-induced alterations in nAChR binding appeared to differ by sex, with receptor upregulation seen in male but not female smokers (vs. nonsmokers, respectively). In contrast, nAChRs are negatively correlated with levels of progesterone, which in turn are positively correlated with symptoms of depression and intensities of cigarette craving and withdrawal (Cosgrove et al. 2012). These data

suggest that female smokers may be best treated by medications that do not interact directly with nicotinic mechanisms. Additionally, a study using fMRI indicated higher reactivity to smoking cues (vs. neutral cues) in males compared with females in specific reward-related regions of the brain (the ventral striatum/ventral pallidum and the ventral medial prefrontal cortex) (Dumais et al. 2017). Brain activation during smoking cues correlated positively with cue-induced subjective craving in males but not in females. These data suggest that, compared with women, men have greater reward-related brain activation to drug cues.

Although these small studies may have been underpowered to definitively distinguish smoking-related, sex-specific differences in the neurochemistry and circuitry in the brain, they add to a growing and important base of literature on sex differences in nicotine addiction. They also underscore the need for more research on sex-specific neurobiology of the etiology and treatment of nicotine dependence.

Summary

The data presented in this section highlight new biologic insights into smoking cessation gained from multiple neuroimaging modalities, including PET and fMRI.

These studies highlight the neurobiologic complexities of nicotine dependence and, in their totality, are sufficient to support the multiple cognitive and affective systems that are dysregulated in persons with this disease, suggesting why persons who are addicted to nicotine are so resistant to treatment even with multiple FDA-approved medications. On a more positive note, these neuroimaging findings have begun to reveal neurobiologic mechanisms and cognitive constructs that may serve as novel targets for future therapeutic developments, including reward processing, cognitive control, and executive functions (such as working memory and inhibitory control processes and affective responses to internal and external cues and stressors). These studies are suggestive of dysregulated brain regions, including various prefrontal and cingulate cortical regions, and their corresponding circuits and interactions with various striatal and insula loci. Almost all studies were cross-sectional—not longitudinal. Therefore, specific causal relationships are difficult to infer in the absence of repeated measurements within subjects. Nevertheless, outcomes for smoking cessation may be improved by using pre- and posttreatment, multimodal neuroimaging measures that are coupled with recent computational advances (e.g., machine learning) to create objective, quantifiable biomarkers that can be used to assess disease severity and treatment efficacy.

Genetic Studies of Smoking Phenotypes

Studies of twins suggest that smoking behaviors are moderately to highly heritable. For example, according to earlier studies, genetic factors explain an estimated 46–84% of the variability in smoking initiation and smoking persistence, up to 75% of the variability in nicotine dependence (Kendler et al. 1999; Vink et al. 2005), and 50–58% of the variability in smoking cessation (Xian et al. 2003; Broms et al. 2006). Two broad approaches to molecular genetics exist: Candidate gene studies identify a specific gene to investigate, on the basis of biologic plausibility, and test the association between the selected genetic variants and the phenotype of interest. In contrast, GWAS are not restricted to individual genes. Instead, they assess the association between hundreds of thousands of variants (and, more recently, several million variants) across the genome with the phenotype of interest.

The 2010 Surgeon General's report summarized studies of candidate genes involved in the dopamine pathway, which at the time was considered a promising target for genetic dissection, with the *DRD2 TaqIA* polymorphism being one focus of interest (USDHHS 2010). Early studies suggested that the A1 allele at this locus was

associated with increased short-term effectiveness of NRT and bupropion. Subsequent studies, however, have not confirmed an association with smoking status (Tobacco and Genetics Consortium 2010) or with response to pharmacotherapy for smoking cessation (Choi and Shin 2015). The 2010 Surgeon General's report also reviewed studies of candidate genes (e.g., *CYP2A6* and *CYP2E1*) involved in nicotine metabolism in relation to smoking phenotypes, but it concluded that findings were not consistent, possibly because of differences in samples across studies (USDHHS 2010). Two later studies used the nicotine metabolite ratio (NMR), which is the ratio of 3'-hydroxycotinine (the product of *CYP2A6* activity) to cotinine, as a phenotypic biomarker for *CYP2A6* activity and concluded that NMR predicts the outcomes of treatment for smoking (Kaufmann et al. 2015; Lerman et al. 2015). This conclusion likely results from better measurement of nicotine metabolism activity gained using a phenotype instead of a genotype, as this gene locus is very complicated and results can be inconsistent because of the different variants being tested. Since the 2010 Surgeon General's report, considerable progress has been made in understanding the genetic basis of smoking phenotypes,

particularly through GWAS. Candidate gene studies and GWAS have identified variants in the *CHRNA5-CHRNA3-CHRNA4* region as promising targets for the study of nicotine dependence and smoking intensity.

Literature Review Methods

For this section of the chapter, MEDLINE was searched for articles that were published between 2000 and 2018 about studies that focused on genetic associations with smoking behavior (including cessation). A combination of controlled vocabulary and keyword terms was used for each of the concepts: smoking cessation, smoking behavior, smoking phenotype, genetics, and precision medicine. Studies were excluded if they did not focus on the underlying biology of smoking behavior and/or smoking cessation. Conclusions were formulated from evidence cited in the 2014 Surgeon General's report on smoking and any newly available evidence. Search results were limited to studies published in English and to original research. Duplicates were deleted, and unique hits were screened. Two independent reviewers conducted a full review and identified 47 articles for this section. From these articles, seven more articles about studies conducted in the 1990s were also included.

Candidate Gene Studies

Candidate gene approaches require some theoretical knowledge of the biologic mechanism underlying the phenotype of interest that points to specific genes. Typically, these approaches focus on genetic variants that result in functional changes (Kwon and Goate 2000). The selected variant is tested for its occurrence in cases and controls (e.g., assigned by smoking status) or for its association with a continuously distributed trait (e.g., nicotine dependence) (Patnala et al. 2013).

Findings from candidate gene studies are difficult to reproduce. This is likely because of the typically small samples used in these studies, the small effect sizes associated with common genetic variants and complex behavioral traits, and the relatively liberal alpha threshold used (Chang et al. 2014). Despite these limitations, candidate gene studies have produced some robust associations, as discussed later in this section.

Genomewide Association Studies

GWAS adopt the same approach as candidate gene studies, but rather than testing the association of one or

a small number of genetic variants with a phenotype of interest, GWAS simultaneously test hundreds of thousands of genetic variants (typically single nucleotide polymorphisms [SNPs]) across the genome. The multiple testing burden implicit in GWAS has led to a consensus that signals have to achieve a very stringent threshold for statistical significance (typically $p < 5 \times 10^{-8}$). This, in turn, requires very large samples or the pooling of data across multiple studies to achieve the necessary sample size to robustly identify the small effects associated with the common genetic variants. Most GWAS also report results from discovery and replication datasets. This combination of large sample sizes, statistical stringency, and replication means that GWAS have been extremely successful in identifying genetic variants associated with a range of complex phenotypes, including variants that would not have been considered previously on the basis of biological function. GWAS have identified novel genetic associations with smoking behaviors, such as *BDNF* for smoking initiation, the *CHRNA5-A3-B4* gene cluster for intensity of smoking, and *DBH* for smoking cessation (Berrettini et al. 2008; Bierut et al. 2008; Thorgeirsson et al. 2008; Tobacco and Genetics Consortium 2010).

As would be expected, one of the limitations of GWAS is their limited ability to detect low-frequency variants. For example, Lindquist and colleagues (2013) estimated the first GWAS to have detected less than 20% of all independent GWAS-detectable SNPs in chronic diseases. More recent GWAS have employed imputation to expand genomic coverage to better capture low-frequency variants. To impute genotypes, data for the microarray are matched to a genome reference panel, which consists of densely sequenced genomic data from multiple persons (e.g., 1,000 Genomes Project Consortium et al. 2010).

Microarrays designed in this manner cover a large portion of all SNPs in the human genome by directly measuring high- and low-frequency variants and by measuring SNPs in linkage disequilibrium (Lindquist et al. 2013). Even so, another limitation of GWAS is that the phenotypes are relatively crude because they are tested in large samples and in the case of smoking behavior, often rely on retrospective self-reports. Carefully defined and well-characterized phenotypes offer greater precision of measurement, increase the genetic signal, and improve the likelihood of replication (Munafò et al. 2012).

Examples of Biologically Promising Candidate Genes (*DRD2* and *DAT1*)

The mesolimbic dopamine system is particularly important in addictive behaviors and is activated by nicotine. As a consequence, genes encoding proteins involved in the neurotransmission of dopamine have been considered plausible candidate genes for nicotine dependence

and smoking cessation, and they have been widely investigated in candidate gene studies. Variations of the dopamine receptor D2 (*DRD2*) and the dopamine transporter *SLC6A3* (also known as *DAT1*) genes have received particular attention (Sullivan and Kendler 1999; Dani 2003; Duan et al. 2003; Li et al. 2003; Dahl et al. 2006; Lerman et al. 2006a; Schnoll et al. 2007).

Associations Between the *DRD2* and *DAT1* Genes and Smoking Behavior

In the *DRD2* gene, rs1800497 (Taq1A) is one polymorphism that is located downstream and in the neighboring ankyrin repeat and kinase domain containing 1 (*ANKKI*) gene (Neville et al. 2004). This polymorphism, which is involved in inhibiting the synthesis and release of dopamine, leads to decreased density of the dopamine receptor (Noble et al. 1991, 1997; Pohjalainen et al. 1998; Jonsson et al. 1999) and, therefore, reduced dopamine binding in the brain (Thompson et al. 1997). Various studies have reported that the A1 allele of the *DRD2* Taq1A polymorphism is associated with being a former or current smoker (Noble et al. 1994; Morton et al. 2006); with age of smoking initiation and duration of abstinence (Comings et al. 1996); and with smoking intensity (Connor et al. 2007). In addition, meta-analyses have reported suggestive evidence of an association of the A1 allele with increased likelihood of smoking persistence (Munafò 2004; Munafò et al. 2004, 2009). Other studies, however, did not yield similar findings (Batra et al. 2000; Bierut et al. 2000).

Other studies have investigated whether *DAT1* variants are associated with smoking behavior. *DAT1* has a polymorphic variable number of tandem repeats sequence that varies from 3 to 11 copies, of which only the 9- and 10-repeat alleles are common (Chen and Reith 2000). *DAT1* plays a key role in regulating the transport of dopamine by regulating its reuptake (Choi and Shin 2015). Timberlake and colleagues (2006) reported that the absence of the 9-repeat allele in *DAT1* (*DAT-9*) was associated with being less likely to be a smoker; other studies have suggested that this association is stronger if the person was also carrying the *DRD2* A2 allele (Lerman et al. 1999), had a later onset of smoking (Lerman et al. 1999; Schmid et al. 2009), had longer quitting attempts (Lerman et al. 1999), or had formally tried smoking cessation (Sabol et al. 1999). However, these associations have not been found in other studies (Bierut et al. 2000; Jorm et al. 2000; Vandenbergh et al. 2002).

Meta-analyses of GWAS conducted by the Tobacco and Genetic Consortium (2010), using data from three GWAS of smoking consortia to evaluate a number of phenotypes, did not find evidence of an association between loci in either *DRD2* or *DAT1* and smoking behavior. Despite these equivocal results, several pharmacogenetic studies

have suggested an association between genes involved in the dopaminergic pathway and response to pharmacotherapy that is aimed at smoking cessation (David and Munafò 2008).

The Moderating Effect of *DRD2* and *DAT1* on the Efficacy of Treatment for Smoking Cessation

Some studies have found that the A1 allele of the *DRD2* gene is associated with better response to NRT (Johnstone et al. 2004; Yudkin et al. 2004; Lerman et al. 2006b), and others have found an association between A2 and better response to bupropion for specific nicotine withdrawal symptoms (David et al. 2003; Swan et al. 2005; David et al. 2007). In contrast, Berlin and colleagues (2005) did not find an association between the *DRD2* genotype and smoking cessation. Additionally, Choi and Shin (2015) did not find an association between *DRD2* polymorphisms and response to therapy for smoking cessation. Finally, in a randomized, placebo-controlled, smoking cessation study of bupropion, Lerman and colleagues (2003) did not find an association between the *DRD2* and *DAT1* genotypes and either the abstinence rate or response to treatment.

These findings suggest that many genes likely play a role in the efficacy of treatment for smoking cessation (David et al. 2013b). Each genetic variant probably explains only a small fraction of the variation in response to medication and success in quitting, and most studies have investigated only a single variant or just a small number of them. A combination of genetic variants in a single genetic risk score may reveal stronger associations with the outcomes of therapies for smoking cessation and support personalized therapy on the basis of a person's score.

Genetic Risk Scores

Additive genetic scores (AGS) are an alternative approach to evaluate the effects of multiple susceptible SNPs for a single phenotype. These scores take into account the collective impact of several variants, on the basis of theoretical knowledge of those included, and provide greater statistical power than single-variant studies (David et al. 2013b). Early approaches developed AGS on the basis of candidate genes of theoretical interest, and recent approaches have generated scores from variants identified via GWAS.

In two randomized clinical trials of bupropion for smoking cessation, David and colleagues (2013b) used an AGS from genes in the dopaminergic system, including *COMT*, *DRD2*, *DRD4*, and *DAT1*. The score was calculated on the basis of the number of alleles considered to promote smoking cessation through bupropion and was estimated

for each participant. The score was not associated with the number of days to first lapse, but evidence from this study indicated that bupropion (vs. placebo) counteracts the propensity to lapse in persons with a higher additive genetic efficacy score.

Uhl and colleagues (2014) studied smokers by using the “v1.0 score,” which is based on 12,058 SNPs (Uhl et al. 2010). Using a randomized controlled clinical trial in which dose of NRT was matched to the smoking intensity of each participant, the study found that the v1.0 score can predict success of quitting.

More recently, Chenoweth and Tyndale (2017) suggested that including environmental effects (e.g., use of estrogen-containing hormonal therapy) into AGS approaches would improve the ability to predict the outcomes of treatment for smoking cessation. At the same time, evaluative tools, such as biomarkers, could lead to tailored or personalized treatment (Bough et al. 2013). Regardless, early approaches to AGS, which used candidate genes, need to be treated with caution in light of the poor reproducibility of many findings for candidate genes.

Examples of Biologically Promising Genes That May Help Optimize Treatment

Both genetic and metabolic biomarkers have the potential to predict outcomes for different treatments for smoking cessation and individual responses to medication. Particularly promising genetic variants include those in the *CHRNA5-A3-B4* gene cluster on chromosome 15 (at 15q25) that encodes 3 (α 3, α 5, β 4) of the 11 (α 2– α 7, α 9, α 10, β 2– β 4) neuronal nAChR subunits (Gold and Lerman 2012). Multiple candidate gene studies and GWAS have verified the small but robust association of this cluster of genes with smoking intensity and nicotine dependence (Saccone et al. 2007; Bierut et al. 2008; Thorgeirsson et al. 2010). Importantly, smoking intensity and nicotine dependence predict the success of cessation (Piper et al. 2006; Transdisciplinary Tobacco Use Research Center on Tobacco and Dependence et al. 2007), and thus the relationship between the *CHRNA5-A3-B4* gene cluster and cessation phenotypes has been investigated (Munafò et al. 2011; Bergen et al. 2013; Tyndale et al. 2015).

NMR is a metabolic predictive biomarker that captures activity of the *CYP2A6* gene. *CYP2A6* plays an important role in nicotine metabolism; up to 80% of nicotine is inactivated to cotinine by the hepatic enzyme cytochrome P450 (CYP) 2A6, with a small contribution (10%) from *CYP2B6*. Most of the cotinine is further metabolized to 3'-hydroxycotinine. NMR is used as a proxy of *CYP2A6* activity and is preferred over assessing the gene itself because *CYP2A6* is characterized by dozens of polymorphisms. A faster NMR reflects higher *CYP2A6* activity and is associated with several smoking phenotypes.

The *CHRNA5-A3-B4* Gene Cluster

Associations with Nicotine Dependence and Smoking Intensity

Saccone and colleagues (2007) authored the first candidate gene study to report an association between the SNP rs16969968 in *CHRNA5* and nicotine dependence. The following year, GWAS conducted separately by Berrettini and colleagues (2008) and Thorgeirsson and colleagues (2008) reported that rs1051730 at the same locus but in *CHRNA3* (and strongly correlated with rs16969968 in samples of European ancestry) was associated with the number of cigarettes smoked per day. *CHRNA5* was not considered a strong candidate gene at the time, given what was then known about the neurobiology of nicotine dependence. Animal experiments had implicated the α 4 and β 2 nicotinic receptor subunits as critical to nicotine's reinforcing effects (Picciotto et al. 1998; Tapper et al. 2004), and α 4 β 2* partial agonists are now known to be one of the most effective treatments available for smoking cessation (Fowler and Kenny 2014). Findings from GWAS have made variants in the *CHRNA5-A3-B4* region promising targets for the study of nicotine dependence and smoking intensity, given their association with response to nicotine and its consequent consumption.

In particular, the rs1051730 SNP in *CHRNA3* is a coding-synonymous variant that does not result in an altered protein, and thus it likely does not have any functional significance. However, the highly correlated variant rs16969968 in *CHRNA5* is functional and presents with a missense mutation that results in an amino acid substitution of aspartate to asparagine in the α 5 subunit protein. Both in vitro and in vivo studies have further characterized the role of the rs16969968 variant. In in vitro studies, α 5 receptor complexes featuring the aspartic acid variant, when exposed to a nicotine agonist, have exhibited a substantially greater maximal response than the α 5 receptor complexes containing the asparagine variant (i.e., the risk variant associated with the number of cigarettes smoked per day and nicotine dependence) (Bierut et al. 2008). A series of animal studies has established the role of the α 5 nAChR subunit by investigating the phenotype via an α 5 knockout mouse model, which is analogous to a reduced α 5 receptor function in humans (i.e., carriers of the rs16969968 risk allele) (Salas et al. 2009; Fowler et al. 2011; Frahm et al. 2011). Salas and colleagues (2009) showed that α 5 knockout mice, when exposed to chronic infusions of nicotine, exhibited withdrawal symptoms comparable to those of saline-infused mice (i.e., a lack of withdrawal symptoms relative to wild-type mice). In the experiment conducted by Fowler and colleagues (2011), both wild-type and mutant mice were trained to press a lever

to obtain nicotine intravenously. All the mice showed the expected inverted U-shaped dose-response curve, with the difference that knockout mice responded more vigorously at high doses. Knockout mice consumed a greater amount of nicotine, and the wild-type mice appeared to titrate the delivery of nicotine to achieve a desired level. Although knockout mice appeared to experience rewarding effects of nicotine similar to those experienced by wild-type mice, the inhibitory effects of the high doses of nicotine on the activity of the rewarding circuitry seemed to be largely altered. The injection of a lentivirus vector into the MHB in $\alpha 5$ knockout mice rescued the expression of $\alpha 5$ subunits in this region and the phenotype.

Similarly, a study by Jackson and colleagues (2010b) showed differential effects of nicotine dose on reward between $\alpha 5$ knockout and wild-type mice using a CPP task. Later, in a study of humans, Jensen and colleagues (2015) found an attenuated aversive response to nicotine administered intravenously in overnight-abstinent smokers who were carriers of the *CHRNA5* rs16969968 risk allele genotype. In summary, high doses of nicotine seem to stimulate the MHB–IPN tract through nAChRs containing $\alpha 5$ subunits and elicit aversion, limiting further intake. This does not happen when the $\alpha 5$ signaling is deficient and, consequently, the negative effects of nicotine are attenuated. Similarly, smokers carrying the rs16969968 risk allele are more likely to smoke more heavily than their counterparts without the risk allele.

Evidence from in vitro and in vivo studies further indicates that the MHB acts as a gatekeeper for nicotine intake. Frahm and colleagues (2011) manipulated the concentration of $\alpha 5$ and $\beta 4$ subunits in vitro, while $\alpha 3$ was kept constant. Nicotine-evoked currents in MHB neurons of wild-type and transgenic Tabac mice (characterized by an overexpression of $\beta 4$) led to a dramatically higher firing rate in the neurons of the Tabac mice. Those mice exhibited a reduced nicotine intake and a strong preference for water rather than low-nicotine-concentration solutions in a two-bottle choice test that compared them with wild-type mice presented with the same volumes of water and the low-nicotine solution. When the expression of the $\alpha 5$ risk variant was elicited by injecting a lentivirus vector into MHB neurons in the Tabac mice, the latter restored their nicotine consumption and their two-bottle choice behavior to a level comparable to that of the wild-type mice. These animal studies show that $\alpha 5$ and $\beta 4$ play an important role and compete in regulating nicotine intake.

In humans, Hong and colleagues (2010) used resting-state functional connectivity to understand the mechanistic link between variation at the *CHRNA5-A3-B4* locus and nicotine addiction. Their study identified a circuit between the dorsal anterior cingulate and the ventral striatum/extended amygdala that distinguished smokers

from nonsmokers and predicted nicotine dependence. Both smokers and nonsmokers with the risk allele had a weaker circuit than those with the more common allele (although the circuit strength was even weaker in smokers), suggesting a trait-like circuit biomarker. A nearly identical circuit was described previously in smokers (Hong et al. 2009) as a function of nicotine dependence. Critically, in that study, circuit strength did not change following NRT, suggesting that it reflected chronic dependence.

***CHRNA5-A3-B4* Variants and Smoking Cessation in Absence of Treatment**

The genetic risk variants associated with nicotine dependence and smoking intensity also were associated with smoking cessation. Interestingly, persons who smoke a greater number of cigarettes per day seem to quit at a later age (Chen et al. 2015). Some studies have shown that *CHRNA5*, in particular the rs16969968 risk variant, has potential clinical significance in predicting delayed smoking cessation. Chen and colleagues (2015) conducted a large meta-analysis to investigate whether rs16969968 plays a role in the age of smoking cessation among smokers without smoking-related disease and patients with lung cancer, chronic obstructive pulmonary disease, or coronary heart disease. Results from 24 datasets in their study showed evidence for an association only for the smokers without a smoking-related disease and the rs16969968 risk allele, with a median delay of 4 years. The heterogeneity of the studies in this meta-analysis shows that a number of factors may moderate genetic risk, such as the presence of disease, use of medication, and environmental risk factors (e.g., having a partner or friend who smokes).

Freathy and colleagues (2009) assessed smoking cessation in a large cohort of women of European ancestry, over the course of their pregnancies. Carriers of the risk variant rs1051730 showed a reduced likelihood of stopping smoking. The effect did not appear to be solely mediated by intensity of smoking, as adjusting the analysis for that variable did not affect the results, although this may have been because the number of cigarettes smoked per day does not fully capture intensity of smoking (e.g., given interindividual differences in depth of smoke inhalation and other measures of smoking topography). Thorgeirsson and Stefansson (2010) replicated this finding in a retrospective study of pregnant women, which found an association between the risk variant rs1051730 and continuing smoking during pregnancy.

***CHRNA5-A3-B4* Variants and Smoking Cessation with Pharmacotherapy**

Several studies have examined whether personalized smoking cessation treatments based on genotype

can improve cessation success. Such treatments require knowledge of whether genetic variants moderate the effects of the available pharmacotherapies for smoking cessation.

Baker and colleagues (2009) studied the effect of haplotypes on the basis of five tagging SNPs (rs680244, rs569207, rs16969968, rs578776, and rs1051730) in the *CHRNA5-CHRNA3-CHRNA4* locus. For participants receiving either bupropion or placebo, the haplotypes were associated with tolerance, craving, and loss of control, but only among persons who had started smoking early in life.

Elsewhere, Munafò and colleagues (2011) found evidence for a weak association between the same locus, looking at the risk variant rs1051730 in *CHRNA3* and at the short-term ability to quit smoking in heavy smokers receiving either the placebo or NRT. Interestingly, the effect size reported in this study was comparable to the effects found in the studies of pregnant women (Freathy et al. 2009; Thorgeirsson and Stefansson 2010) and the study by Baker and colleagues (2009).

Chen and colleagues (2012) conducted a large study to examine genetic associations with age of cessation. *CHRNA5-A3-B4* risk haplotypes (rs16969968 and rs680299, both in *CHRNA5*) were associated with the number of cigarettes smoked per day and a later quitting age; the latter was no longer associated with the haplotypes when the analysis was adjusted for the number of cigarettes smoked per day. This study suggested that intensity of smoking, measured as the number of cigarettes smoked per day, impedes cessation. Furthermore, carriers of the medium- to high-risk haplotypes found abstinence more difficult, but if carriers received pharmacologic treatment (e.g., nicotine patch, nicotine lozenge, bupropion), they showed an increased rate of quitting success.

A meta-analysis by Bergen and colleagues (2013), which included eight RCTs, found that 6 months after a quit attempt, the risk allele rs1051730 was associated with higher rates of abstinence in the NRT group compared with the placebo group. The authors of this study assessed the association of four SNPs with smoking cessation and response to medication at the end of the treatment (8- to 12-weeks post-quit) and after 6 months. The genetic variants were rs1051730, rs578776, and rs588765 in *CHRNA5* and *CHRNA3*, and rs2072661 in *CHRNA2*. *CHRNA2* has been associated with a number of smoking cessation phenotypes, such as abstinence, FTND, and nausea among treatment-seeking smokers randomized to behavioral therapies and prescribed varenicline (Ehringer et al. 2007; Conti et al. 2008; Wessel et al. 2010; Swan et al. 2012). The eight RCTs considered in the meta-analysis employed placebo, NRT, bupropion, varenicline, or a combination of NRT and bupropion (along with a variety of counseling options). Although rs2072661 and rs578776 were not associated with smoking cessation, rs1051730 and, to a

lesser degree, rs588765 were associated with quitting success in persons randomized to NRT and in those who received the placebo. Participants in the placebo conditions were less likely to be abstinent after 6 months, but those who received NRT were more likely to achieve abstinence after that time. Mediation analysis indicated that rs1051730 increased nicotine dependence—a variable that decreases the success of abstinence—and that a further mechanism (speculated to be abstinence-induced impairment in cognitive function) increased abstinence in the NRT group at the 6-month follow-up from the end of drug administration.

Two subsequent studies—a meta-analysis of four studies and a clinical trial—did not confirm these findings. The meta-analysis revealed no evidence at the end of NRT that rs16969968 or rs1051730 were associated with cessation (Leung et al. 2015). The clinical trial, conducted by Tyndale and colleagues (2015), examined the association between *CHRNA5-A3-B4* haplotypes and smoking abstinence, finding no associations between rs16969968, rs578776, and rs588765 and abstinence at 6- or 12-month follow-up in participants who received placebo, NRT, or varenicline.

An important factor in smoking cessation is adherence to treatment. Ware and colleagues (2015), who studied this phenotype in a secondary analysis of data from an RCT of smoking cessation, found an association between rs1051730 and adherence to NRT after 7 days of the quit attempt but not after 28 days. Each copy of the minor allele corresponded to a 2.9% decrease in adherence to the prescribed dose of NRT over 7 days. This association was robust to adjustments made for age, sex, socioeconomic status, trial condition, body mass index at baseline, and daily cigarette consumption at baseline.

Most studies to date have used samples of European ancestry, but a few have examined samples from other populations, including African Americans. For example, in a small deep-sequencing discovery study of African Americans, Hamidovic and colleagues (2011) reported an association between rs12915366 in *CHRNA5* and rs12914385 in *CHRNA3* and smoking persistence. David and colleagues (2012), who performed a genomewide meta-analysis of 13 studies of African Americans, found that rs2036527, which is in linkage disequilibrium with rs1051730, was significantly associated genomewide with the number of cigarettes smoked per day. In another study of African Americans, Zhu and colleagues (2014) failed to find an association between rs16969968 and smoking abstinence in either the placebo or NRT group. In contrast, the minor allele of rs588765 was associated with lower abstinence in the placebo group and greater abstinence in the group receiving NRT during treatment but not after 6 months.

The study by Zhu and colleagues (2014) also reported an association, both during and at the end of treatment, between the risk allele of rs2036527 in *CHRNA5* and lower smoking abstinence in those who received NRT but not in the placebo group. Interestingly, adjusting the analyses for the number of cigarettes smoked per day had a negligible effect. The rs2036527 SNP was in high linkage disequilibrium with rs1051730, and these findings are consistent with the association reported by Munafò and colleagues (2011) for rs1051730 and short-term smoking cessation in their study of a European population. These findings suggest that linkage disequilibrium structures differ between European and African American populations.

Overall, although the association between the *CHRNA5-A3-B4* gene cluster and smoking intensity is robust, its role in smoking cessation needs further investigation, and currently no clear evidence exists that it influences responses to specific pharmacotherapies. Some of the inconsistent results may be due to differences in methods and sampling or to environmental factors that influence each study. AGS could be employed to explore the collective genetic influence of several variants that may exert a role in complex phenotypes, such as smoking behaviors, but more work is required to understand the role of these genes in ethnic groups other than those of European ancestry.

The *CYP2A6* Gene and the Nicotine Metabolite Ratio

Nicotine from cigarette smoke is distributed in the body via the bloodstream (Benowitz et al. 2009). Its elimination half-life is around 2 hours, and up to 90% of nicotine is converted to cotinine, mainly by the metabolic enzyme *CYP2A6*, which, in turn, is solely responsible for the metabolism of cotinine to 3'-hydroxycotinine (Benowitz and Jacob 3rd 1994; Tanner and Tyndale 2017). Nicotine is also metabolized to more minor metabolites by additional enzymes, including *FMO3* and *UGT2B10* (Benowitz et al. 2009). NMR is the ratio of 3'-hydroxycotinine to cotinine; studies of twins have estimated that about 60% of the variation in NMR is due to genetic factors (Swan et al. 2004). Importantly, *CYP2A6* enzyme activity is reflected by NMR (Dempsey et al. 2004; Johnstone et al. 2006; Malaiyandi et al. 2006a). *CYP2A6* is a highly polymorphic gene (with >30 genetic variants), and its numerous variants have an impact on NMR. Grouping variants, however, is possible according to the impact of *CYP2A6* on the rate of NMR (i.e., faster or slower). Importantly, NMR also captures environmental influences (e.g., hormonal therapies and body mass index). Furthermore, NMR values are stable across time and exhibit high test-retest reliability

when measured 2 to 3 weeks apart (Hamilton et al. 2015). Despite no consensus on the cut-off point between faster and slower metabolizers, several studies have used the lowest 25–50% of the NMR distribution to classify slower metabolizers (Lerman et al. 2006b; Ray et al. 2009; Schnoll et al. 2009; Dubroff et al. 2015).

Nicotine Metabolite Ratio and Smoking Behavior

The GWAS by Thorgeirsson and colleagues (2010) found an association between reduced smoking quantity, measured as the number of cigarettes smoked per day, and variants in or near *CYP2A6* that reduce the enzymatic activity of *CYP2A6* (in particular, rs4105144). Later, Loukola and colleagues (2015) conducted a GWAS meta-analysis of current smokers using data from three Finnish cohorts and identified novel genetic variants associated with NMR. Their study detected three strong independent signals in the immediate vicinity of *CYP2A6*: SNPs rs56113850, rs113288603, and rs2663194. Although the functional consequences of the first two SNPs are unknown, the third one is associated with a decreased clearance rate, and the three SNPs captured up to 31% of the total variance in NMR.

NMR has been assessed in several studies to further characterize smoking behavior. In one study, slower metabolizers smoked an average of 6 to 7 fewer cigarettes per day and had an earlier smoking onset by about 1 year (Schoedel et al. 2004). Other studies found slower metabolizers to be less dependent on nicotine, as measured by the FTND (Malaiyandi et al. 2006b; Wassenaar et al. 2011; Sofuoglu et al. 2012), and slower metabolizers took longer to become dependent on nicotine (Audrain-McGovern et al. 2007; Al Koudsi et al. 2010). Fast metabolizers exhibited a higher total cigarette puff volume (Strasser et al. 2011). This finding is consistent with the observation that fast metabolizers require higher levels of nicotine intake than those with a slower nicotine clearance, which is consistent with self-titration by smokers to achieve the desired circulating level of nicotine (Strasser et al. 2007). Adolescents who were slow metabolizers, however, had a higher risk of becoming nicotine dependent compared with fast metabolizers (Chenoweth et al. 2013, 2016). It is not clear if adolescent smokers titrate their level of nicotine intake according to their NMR to maintain desired levels, but Chenoweth and colleagues (2013) found that once adolescents who were slow metabolizers became dependent on nicotine, they smoked fewer cigarettes and were more likely to become adult smokers (Chenoweth et al. 2013). In fact, slow metabolizers who were adults were more likely than fast metabolizers to successfully quit smoking in the absence of pharmacotherapy (Gu et al. 2000; Patterson et al. 2008; Chenoweth et al. 2013).

In a study of mice, Bagdas and colleagues (2014) used an inhibitor of CYP2A5, the mouse ortholog of human CYP2A6, to mimic the slower nicotine metabolism of humans. The effects of this manipulation were illustrated using a CPP task. A low dose of nicotine administered on one side of a box, versus saline administered on the other side, did not induce a CPP in mice in the control group. In contrast, mice treated with the CYP2A5/6 inhibitor before being exposed to nicotine developed a CPP for the nicotine side and showed increased levels of plasma nicotine. Thus, it appears that the treated mice had become more sensitive to the effects of nicotine. Li and colleagues (2013a) reported similar results from a study that measured CPP in *CYP2A4/5* knockout mice that were exposed to nicotine. In addition, Bagdas and colleagues (2014) administered nicotine to naïve mice across 5 days and pretreated half of the mice with the CYP2A5/6 inhibitor; they then tested the somatic signs of withdrawal after nicotine abstinence. The pretreated mice showed a potentiation of the intensity of somatic signs of withdrawal and higher levels of plasma nicotine. In summary, the mice tested in these studies experienced a decrease of nicotine clearance, similar to human slow metabolizers, and a greater exposure to nicotine in these mice enhanced nicotine dependence and affected nicotine withdrawal behaviors.

Nicotine Metabolite Ratio and Smoking Cessation in Absence of Treatment

Gu and colleagues (2000), who compared the likelihood of quitting smoking between slow and fast metabolizers, found that slow metabolizers were almost twice as successful in quitting smoking. Later, in a prospective cohort of adolescents, Chenoweth and colleagues (2016) also assessed the hypothesis that slow metabolizers are more likely to quit smoking than fast metabolizers and found a linear relationship between *CYP2A6* activity and quit rate: slow metabolizers were more than twice as likely as fast metabolizers to quit smoking.

Smoking Cessation in Treatment Seekers

Compared with slow metabolizers, fast metabolizers have a higher NMR and inactivate nicotine quickly. A higher NMR results in lower levels of nicotine in the blood. Lerman and colleagues (2006b) found that a lower NMR was associated with increased odds of abstinence, both at the end of treatment and after 6 months, in persons who received a nicotine patch but not in those who received nicotine in the form of nasal spray, suggesting that, in contrast with transdermal nicotine (for which the dose is fixed), users of nicotine nasal spray may titrate their intake of nicotine. Furthermore, cravings for cigarettes after 1 week of abstinence were more severe in fast

metabolizers who received the transdermal patch. A subsequent study by Lerman and colleagues (2010) found that slow metabolizers benefitted from using the transdermal nicotine patch for an extended period of time (i.e., 6 months vs. the standard 8 weeks).

Some evidence suggests that bupropion enhances the quit success of fast metabolizers and that the nicotine patch enhances the quit success of slow metabolizers. Patterson and colleagues (2008) assessed the baseline NMR in smokers who subsequently participated in a 10-week randomized trial of bupropion versus placebo with counseling support. With placebo, quit rates were lower among fast metabolizers than slow metabolizers, but with bupropion, quit rates were similar between fast and slow metabolizers.

Because slow metabolizers showed no difference in the likelihood of relapse in either the placebo or bupropion conditions, Lerman and colleagues (2015) conducted an NMR-stratified, placebo-controlled, randomized trial of nicotine patch versus varenicline to test whether varenicline had a superior effect compared with placebo. On the basis of evidence for an interaction of NMR by treatment, fast metabolizers receiving varenicline had higher odds of being abstinent. These studies suggest that NMR may be a predictive biomarker that can be used to personalize treatments for smoking cessation.

Summary

This section examined the role in smoking cessation played by candidate genes in the dopamine system (dopamine receptor D2, *DRD2*, and the dopamine transporter, *DAT1*) and variants in the *CHRNA5-A3-B4* gene cluster and the *CYP2A6* gene. Despite early evidence for associations between genetic variation in *DRD2* or *DAT1* and smoking cessation and response to smoking cessation therapy, subsequent studies have failed to replicate these findings. In contrast, the small but robust association between the *CHRNA5-A3-B4* gene cluster and smoking intensity and nicotine dependence has been replicated in several candidate gene studies and GWAS, and smoking intensity and nicotine dependence predict the success of cessation. Whether variants in this gene cluster influence responses to specific pharmacotherapies is still not clear. Investigating polygenic risk scores may better capture the quitting success and variations in responses to medication.

More consistent results have been provided by studies assessing *CYP2A6* or related biomarkers, such as NMR, and smoking cessation (both with and without pharmacologic treatment). A linear relationship exists between *CYP2A6* activity and quit rate: slow nicotine

metabolizers are more likely than fast metabolizers to quit smoking. In addition, studies suggest that bupropion and varenicline enhance the quit success of fast metabolizers, and the nicotine patch enhances the quit success of slow metabolizers.

Schuit and colleagues (2017) published the first Cochrane systematic review and meta-analyses of pharmacogenetic biomarkers for smoking cessation, which included clinical trials with available genetic or NMR data for all approved smoking cessation pharmacotherapies, all genomewide significant SNPs for number of cigarettes smoked per day or smoking cessation, non-SNP polymorphisms with replication, and NMR. Data were available for 18 clinical trials and the following gene variants: nine SNPs (rs1051730 [*CHRNA3*]; rs16969968, rs588765, and rs2036527 [*CHRNA5*]; rs3733829 and rs7937 [in *EGLN2*, near *CYP2A6*]; rs1329650 and rs1028936 [LOC100188947]; and rs215605 [*PDE1C*]), two variable number tandem repeats (*DRD4* and *SLC6A4*), and the NMR biomarker.

The meta-analyses indicated that genotype groups within certain ethnic groups may benefit more from NRT than from placebo (non-Hispanic Black individuals at 6-months with rs16969968 GG genotype, slow metabolizers, non-Hispanic White and non-Hispanic Black individuals at the end of treatment with rs1051730 GA or AA genotype, and rs16969968 GG genotype) and from NRT (non-Hispanic Black individuals with rs2036527 GG genotype), or may benefit less from a combination of bupropion with NRT (non-Hispanic White individuals with rs1329650 TT genotype and non-Hispanic Black individuals with

rs3733829 AG or GG genotype). These results should be interpreted with caution because none of the statistically significant meta-analyses from placebo-controlled trials included more than two trials per genotype comparison, many confidence intervals were wide, and the quality of this evidence was generally moderate. Although evidence existed of superior NRT efficacy for NMR of normal versus slow metabolizers, the authors could not conclude that NRT is more effective in slow metabolizers. Given the number of trials and investigators who did not provide or publish meta-analyzable data, access to additional data is needed, particularly for comparisons of different pharmacotherapies to improve the reliability of meta-analysis and the potential clinical utility of genomic testing to guide treatment choice for smoking cessation.

Benefits may be derived from personalized precision tailoring of interventions based on genetic approaches. The efficacy of treatment could be improved by assigning patients to a specific treatment based on the results of genetic or biomarker testing. However, for a pharmacogenetic approach to be cost-effective, the effect size must be substantially larger in one stratum compared with another stratum. Other considerations, such as the proportion of the population that falls into each stratum, are also relevant. In particular, before pharmacogenetic or biomarker stratification becomes routine in clinical practice, an RCT should be conducted to determine whether this approach improves overall cessation outcomes. Ideally, the RCT would also include a health economic analysis to help determine the cost-effectiveness of this approach.

Summary of the Evidence

Although current pharmacotherapies are effective in increasing quitting, many current smokers want to quit but have been unable to sustain abstinence, so smoking remains one of the leading causes of preventable disease and death globally. Decades of preclinical advances have improved our understanding of the neurobiologic mechanisms underlying nicotine addiction. Although more remains to be understood, this information has identified dozens of novel and promising targets for pharmacologic intervention that remain to be evaluated in humans. Preclinical studies suggest that targeting multiple stages of addiction may be the most effective way to reduce smoking.

Immunotherapies for nicotine dependence offer an alternative therapeutic mechanism, producing antibodies that bind nicotine in blood and reduce nicotine delivery to the brain (see “Vaccines and Other Immunotherapies as Treatments for Tobacco Addiction”). This approach

involves targeting the drug rather than the brain, potentially reducing the side effects of existing medications to treat nicotine dependence and perhaps treating a limited repertoire of smoking behaviors (see “Insights into Smoking Cessation from the Field of Neurobiology”). Immunotherapies are highly effective in animal models for blocking nicotine reinforcement, but they have not yet been effective in Phase 3 clinical trials for smoking cessation, at least in part because of insufficient and variable antibody concentrations in humans.

Multiple cognitive systems (e.g., attention, reward, inhibitory control) and affective processes (negative and positive emotion) are dysregulated in nicotine dependence, which might help to explain poor treatment outcomes. Regions of the brain involved in the maintenance of smoking and nicotine withdrawal include the anterior and posterior cingulate, amygdala, insula, striatum, and orbitofrontal cortex. Large-scale brain networks altered as

a result of nicotine dependence include the default mode, salience, and executive control networks. Circuit and network connections may serve as predictive biomarkers to personalize treatment choices and as predictors of the outcomes of cessation treatment. More longitudinal neuroimaging studies are needed to understand brain alterations as a function of sustained abstinence. Neuroimaging and genetic analyses to fractionate the nicotine addiction phenotype would help to identify novel therapeutic targets. Transcranial magnetic stimulation, an FDA-approved treatment for depression, has been proposed as a treatment for addiction in general, but further evaluation is needed to determine its efficacy for smoking cessation.

Large GWAS are identifying molecular genetic influences on smoking phenotypes. The greater sensitivity of these large studies allows signals to be identified that may inform the search for potential therapeutic targets, but the studies require somewhat blunt phenotypes. The strongest evidence on potential therapeutic targets to date points to variants related to nAChRs (*CHRNA5-A3-B4* gene cluster) and nicotine metabolism (*CYP2A6* gene). Variation in these genes influences intensity of smoking and nicotine dependence, and an increasing amount of evidence suggests that such variation may influence smoking cessation and be useful for personalized optimization of therapeutic choice. Genetic variants associated with smoking behaviors also provide tools that can be used to support stronger causal inference in observational studies—for example, by treating these genetic variants as instrumental variables (a method known as Mendelian randomization, which is predicated on the assumption that because genotype is assigned randomly at meiosis it should not be associated with potential confounders at a population level) (Gage et al. 2016). Emerging evidence suggests that genetic variants may influence responses to smoking cessation treatments, offering the potential for personalized or stratified approaches to treatment. However, this approach requires a randomized clinical trial to determine its efficacy and cost-effectiveness. Future research should focus on assessing smoking cessation outcomes prospectively

(e.g., by routinely collecting genetic data at baseline in RCTs of smoking cessation interventions) and using intermediate phenotypes (e.g., brain circuits that are relevant to nicotine dependence) through modern genetic approaches. Research should also investigate genetic predictors of responses to behavioral and pharmacologic interventions.

From a public health perspective, interventions to achieve smoking cessation must be developed that are more effective than the current options. The development of biologically based biomarkers for diseases involving organ systems has led to the development of successful therapies for a variety of these diseases. However, such biomarker research lags behind in the fields of addiction (in general) and of nicotine dependence (in particular). It will be important to invest in continued efforts to translate findings and observations from animal models of nicotine addiction and apply them to clinical settings to provide novel, mechanistically sound therapies for humans.

Limited ecologic validity and questions about subsequent predictability are limitations to almost all studies summarized in this chapter. Smoking is frequently comorbid with other neuropsychiatric diseases, including schizophrenia, depression, and anxiety disorders. Moreover, persons who abuse nicotine also use other drugs, including alcohol and marijuana. And yet, most research cohorts involving drugs are only on the basis of smoking. Therefore, a better understanding of the connections between nicotine dependence and neuropsychiatric comorbidity dual-drug dependence is warranted. Similarly, responses to smoking pharmacotherapies clearly differ by sex, but to date, little work has focused on these differences, whether in basic neurobiology or in the interactions with pharmacogenetics. For example, some studies suggest that female smokers may be best treated by medications that do not interact directly with nicotinic mechanisms; this should be explored further. Sex differences also should be evaluated further in the pathophysiology of nicotine addiction and be considered when treating patients. A shift toward developing individualized, multifaceted approaches to smoking cessation is critical.

Conclusions

1. The evidence is suggestive but not sufficient to infer that increasing glutamate transport can alleviate nicotine withdrawal symptoms and prevent relapse.
2. The evidence is suggestive but not sufficient to infer that neuropeptide systems play a role in multiple stages of the nicotine addiction process, and that modulating the function of certain neuropeptides can reduce smoking behavior in humans.
3. The evidence is suggestive but not sufficient to infer that targeting the habenulo-interpeduncular pathway with agents that increase the aversive properties of nicotine are a useful therapeutic target for smoking cessation.
4. The evidence is suggestive but not sufficient to infer that vaccines generating adequate levels of nicotine-specific antibodies can block the addictive effects of nicotine and aid smoking cessation.
5. The evidence is suggestive but not sufficient to infer that dysregulated brain circuits, including prefrontal and cingulate cortical regions and their connections with various striatal and insula loci, can serve as novel therapeutic targets for smoking cessation.
6. The evidence is suggestive but not sufficient to infer that the effectiveness of nicotine replacement therapy may vary across specific genotype groups.

References¹

- Acri JB, Cross AJ, Skolnick P. From bench to bedside: mGluR2 positive allosteric modulators as medications to treat substance use disorders. *Psychopharmacology* 2017;234(9–10):1347–55.
- Addicott MA, Sweitzer MM, Froeliger B, Rose JE, McClernon FJ. Increased functional connectivity in an insula-based network is associated with improved smoking cessation outcomes. *Neuropsychopharmacology* 2015;40(11):2648–56.
- Akkus F, Ametamey SM, Treyer V, Burger C, Johayem A, Umbrecht D, Gomez Mancilla B, Sovago J, Buck A, Hasler G. Marked global reduction in mGluR5 receptor binding in smokers and ex-smokers determined by [11C]ABP688 positron emission tomography. *Proceedings of the National Academy of Sciences of the United States of America* 2013;110(2):737–42.
- Akkus F, Treyer V, Johayem A, Ametamey SM, Mancilla BG, Sovago J, Buck A, Hasler G. Association of long-term nicotine abstinence with normal metabotropic glutamate receptor-5 binding. *Biological Psychiatry* 2016;79(6):474–80.
- Al Koudsi N, Hoffmann EB, Assadzadeh A, Tyndale RF. Hepatic CYP2A6 levels and nicotine metabolism: impact of genetic, physiological, environmental, and epigenetic factors. *European Journal of Clinical Pharmacology* 2010;66(3):239–51.
- Alajaji M, Bowers MS, Knackstedt L, Damaj MI. Effects of the beta-lactam antibiotic ceftriaxone on nicotine withdrawal and nicotine-induced reinstatement of preference in mice. *Psychopharmacology* 2013;228(3):419–26.
- 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. *Translational Psychiatry* 2016;6:e713.
- Ashare RL, Wileyto EP, Ruparel K, Goelz PM, Hopson RD, Valdez JN, Gur RC, Loughhead J, Lerman C. Effects of tolcapone on working memory and brain activity in abstinent smokers: a proof-of-concept study. *Drug and Alcohol Dependence* 2013;133(3):852–6.
- Audrain-McGovern J, Al Koudsi N, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. *Pediatrics* 2007;119(1)e264–e274.
- Bagdas D, Muldoon PP, Zhu AZ, Tyndale RF, Damaj MI. Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice. *Neuropharmacology* 2014;85:67–72.
- Baker TB, Weiss RB, Bolt D, von Niederhausen A, Fiore MC, Dunn DM, Piper ME, Matsunami N, Smith SS, Coon H, et al. Human neuronal acetylcholine receptor A5-A3-B4 haplotypes are associated with multiple nicotine dependence phenotypes. *Nicotine and Tobacco Research* 2009;11(7):785–96.
- Baldassarre A, Lewis CM, Committeri G, Snyder AZ, Romani GL, Corbetta M. Individual variability in functional connectivity predicts performance of a perceptual task. *Proceedings of the National Academy of Sciences of the United States of America* 2012;109(9):3516–21.
- Balfour DJ. The neuronal pathways mediating the behavioral and addictive properties of nicotine. *Handbook of Experimental Pharmacology* 2009;(192):209–33.
- Balfour DJ, Wright AE, Benwell ME, Birrell CE. The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. *Behavioural Brain Research* 2000;113(1–2):73–83.
- Barr MS, Fitzgerald PB, Farzan F, George TP, Daskalakis ZJ. Transcranial magnetic stimulation to understand the pathophysiology and treatment of substance use disorders. *Current Drug Abuse Reviews* 2008;1(3):328–39.
- Batra A, Gelfort G, Bartels M, Smolczyk H, Buchkremer G, Riess O, Schols L. The dopamine D2 receptor (DRD2) gene—a genetic risk factor in heavy smoking? *Addiction Biology* 2000;5(4):429–36.
- Benowitz NL, Hukkanen J, Jacob P 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handbook of Experimental Pharmacology* 2009;(192):29–60.
- Benowitz NL, Jacob P 3rd. Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clinical Pharmacology and Therapeutics* 1994;56(5):483–93.
- Bergen AW, Javitz HS, Krasnow R, Nishita D, Michel M, Conti DV, Liu J, Lee W, Edlund CK, Hall S, et al. Nicotinic acetylcholine receptor variation and response to smoking cessation therapies. *Pharmacogenetics and Genomics* 2013;23(2):94–103.
- Berkman ET, Falk EB, Lieberman MD. In the trenches of real-world self-control: neural correlates of breaking the link between craving and smoking. *Psychological Science* 2011;22(4):498–506.
- Berlin I, Covey LS, Jiang H, Hamer D. Lack of effect of D2 dopamine receptor Taq1A polymorphism on smoking cessation. *Nicotine and Tobacco Research* 2005;7(5):725–8.
- Berrendero F, Plaza-Zabala A, Galeote L, Flores A, Bura SA, Kieffer BL, Maldonado R. Influence of delta-opioid receptors in the behavioral effects of nicotine. *Neuropsychopharmacology* 2012;37(10):2332–44.

¹For reference entries that contain URLs, those URLs were active on the access date presented in the respective reference entry.

- Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, Waterworth D, Muglia P, Mooser V. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Molecular Psychiatry* 2008;13(4):368–73.
- Bespalov AY, Dravolina OA, Sukhanov I, Zakharova E, Blokhina E, Zvartau E, Danysz W, van Heeke G, Markou A. 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.
- Bevins RA, Palmatier MI. Extending the role of associative learning processes in nicotine addiction. *Behavioral and Cognitive Neuroscience Reviews* 2004;3(3):143–58.
- Bierut LJ, Rice JP, Edenberg HJ, Goate A, Foroud T, Cloninger CR, Begleiter H, Conneally PM, Crowe RR, Hesselbrock V, et al. Family-based study of the association of the dopamine D2 receptor gene (DRD2) with habitual smoking. *American Journal of Medical Genetics* 2000;90(4):299–302.
- Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Gruzca RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, et al. Variants in nicotinic receptors and risk for nicotine dependence. *American Journal of Psychiatry* 2008;165(9):1163–71.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine* 1995;34(4):537–41.
- Bough KJ, Lerman C, Rose JE, McClernon FJ, Kenny PJ, Tyndale RF, David SP, Stein EA, Uhl GR, Conti DV, et al. Biomarkers for smoking cessation. *Clinical Pharmacology and Therapeutics* 2013;93(6):526–38.
- Brody AL, Mukhin AG, Mamoun MS, Luu T, Neary M, Liang L, Shieh J, Sugar CA, Rose JE, Mandelkern MA. Brain nicotinic acetylcholine receptor availability and response to smoking cessation treatment: a randomized trial. *JAMA Psychiatry* 2014;71(7):797–805.
- Brody AL, Mukhin AG, Stephanie S, Mamoun MS, Kozman M, Phuong J, Neary M, Luu T, Mandelkern MA. Treatment for tobacco dependence: effect on brain nicotinic acetylcholine receptor density. *Neuropsychopharmacology* 2013;38(8):1548–56.
- Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J. Genetic architecture of smoking behavior: a study of Finnish adult twins. *Twin Research and Human Genetics* 2006;9(1):64–72.
- Brujinzeel AW. Neuropeptide systems and new treatments for nicotine addiction. *Psychopharmacology* 2017;234(9–10):1419–37.
- Brujinzeel AW, Ford J, Rogers JA, Scheick S, Ji Y, Bishnoi M, Alexander JC. Blockade of CRF1 receptors in the central nucleus of the amygdala attenuates the dysphoria associated with nicotine withdrawal in rats. *Pharmacology, Biochemistry and Behavior* 2012;101(1):62–8.
- Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA, Sved AF. Cue dependency of nicotine self-administration and smoking. *Pharmacology, Biochemistry and Behavior* 2001;70(4):515–30.
- Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. *Cochrane Database of Systematic Reviews* 2016, Issue 5. Art. No.: CD006103. DOI: 10.1002/14651858.CD006103.pub7.
- Cahill K, Stevens S, Perera R, Lancaster T. Pharmacological interventions for smoking cessation: an overview and network meta-analysis. *Cochrane Database of Systematic Reviews* 2013, Issue 5. Art. No.: CD009329. DOI: 10.1002/14651858.CD009329.pub2.
- Camenga DR, Klein JD. Tobacco use disorders. *Child and Adolescent Psychiatric Clinics of North America* 2016;25(3):445–60.
- Canterberry M, Hanlon CA, Hartwell KJ, Li X, Owens M, LeMatty T, Prisciandaro JJ, Borckardt J, Saladin ME, Brady KT, et al. Sustained reduction of nicotine craving with real-time neurofeedback: exploring the role of severity of dependence. *Nicotine and Tobacco Research* 2013;15(12):2120–4.
- Carrera MR, Ashley JA, Hoffman TZ, Isomura S, Wirsching P, Koob GF, Janda KD. Investigations using immunization to attenuate the psychoactive effects of nicotine. *Bioorganic and Medicinal Chemistry* 2004;12(3):563–70.
- Carroll AJ, Sutherland MT, Salmeron BJ, Ross TJ, Stein EA. Greater externalizing personality traits predict less error-related insula and anterior cingulate cortex activity in acutely abstinent cigarette smokers. *Addiction Biology* 2015;20(2):377–89.
- Carter LP, Griffiths RR. Principles of laboratory assessment of drug abuse liability and implications for clinical development. *Drug and Alcohol Dependence* 2009;105(Suppl 1):S14–S25.
- Cerny EH, Levy R, Mauer J, Mpandi M, Mutter M, Henzlin-Nkubana C, Patiny L, Tuchscherer G, Cerny T. Preclinical development of a vaccine “against smoking”. *Onkologie* 2002;25(5):406–11.
- Chaiton M, Diemert L, Cohen JE, Bondy SJ, Selby P, Philipneri A, Schwartz R. Estimating the number of quit attempts it takes to quit smoking successfully in a longitudinal cohort of smokers. *BMJ Open* 2016;6(6):e011045.
- Chang CQ, Yesupriya A, Rowell JL, Pimentel CB, Clyne M, Gwinn M, Khoury MJ, Wulf A, Schully SD. A systematic review of cancer GWAS and candidate gene meta-analyses reveals limited overlap but similar effect sizes. *European Journal of Human Genetics* 2014;22(3):402–8.

- Changeux J, Dehaene S. The neuronal workspace model: conscious processing and learning. In: Byrne L, editor. *Learning and Memory: A Comprehensive Reference*. Oxford, United Kingdom: Elsevier, 2008:729–58.
- Changeux JP. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nature Reviews: Neuroscience* 2010;11(6):389–401.
- Chen LS, Baker TB, Piper ME, Breslau N, Cannon DS, Doheny KF, Gogarten SM, Johnson EO, Saccone NL, Wang JC, et al. Interplay of genetic risk factors (CHRNA5-CHRNA3-CHRNA4) and cessation treatments in smoking cessation success. *American Journal of Psychiatry* 2012;169(7):735–42.
- Chen LS, Hung RJ, Baker T, Horton A, Culverhouse R, Saccone N, Cheng I, Deng B, Han Y, Hansen HM, et al. CHRNA5 risk variant predicts delayed smoking cessation and earlier lung cancer diagnosis—a meta-analysis. *Journal of the National Cancer Institute* 2015;107(5):dju100.
- Chen N, Reith ME. Structure and function of the dopamine transporter. *European Journal of Pharmacology* 2000;405(1–3):329–39.
- Chenoweth MJ, O’Loughlin J, Sylvestre MP, Tyndale RF. CYP2A6 slow nicotine metabolism is associated with increased quitting by adolescent smokers. *Pharmacogenetics and Genomics* 2013;23(4):232–5.
- Chenoweth MJ, Sylvestre MP, Contreras G, Novalen M, O’Loughlin J, Tyndale RF. Variation in CYP2A6 and tobacco dependence throughout adolescence and in young adult smokers. *Drug and Alcohol Dependence* 2016;158:139–46.
- Chenoweth MJ, Tyndale RF. Pharmacogenetic optimization of smoking cessation treatment. *Trends in Pharmacological Sciences* 2017;38(1):55–66.
- Chiamulera C, Marzo CM, Balfour DJK. Metabotropic glutamate receptor 5 as a potential target for smoking cessation. *Psychopharmacology* 2017;234(9–10):1357–70.
- Choi HD, Shin WG. Lack of association between DRD2 Taq1A gene polymorphism and smoking cessation therapy: a meta-analysis. *International Journal of Clinical Pharmacology and Therapeutics* 2015;53(6):415–21.
- Chua HF, Ho SS, Jasinska AJ, Polk TA, Welsh RC, Liberzon I, Strecher VJ. Self-related neural response to tailored smoking-cessation messages predicts quitting. *Nature Neuroscience* 2011;14(4):426–7.
- Cochrane Tobacco Addiction Group. Our reviews, n.d.; <<http://tobacco.cochrane.org/our-reviews>>; accessed: March 30, 2018.
- Cohen A, Treweek J, Edwards S, Leao RM, Schulteis G, Koob GF, George O. Extended access to nicotine leads to a CRF1 receptor dependent increase in anxiety-like behavior and hyperalgesia in rats. *Addiction Biology* 2015;20(1):56–68.
- Comings DE, Ferry L, Bradshaw-Robinson S, Burchette R, Chiu C, Muhleman D. The dopamine D2 receptor (DRD2) gene: a genetic risk factor in smoking. *Pharmacogenetics* 1996;6(1):73–9.
- Connor JP, Young RM, Lawford BR, Saunders JB, Ritchie TL, Noble EP. Heavy nicotine and alcohol use in alcohol dependence is associated with D2 dopamine receptor (DRD2) polymorphism. *Addictive Behaviors* 2007;32(2):310–9.
- Conti DV, Lee W, Li D, Liu J, Van Den Berg D, Thomas PD, Bergen AW, Swan GE, Tyndale RF, Benowitz NL, et al. Nicotinic acetylcholine receptor beta2 subunit gene implicated in a systems-based candidate gene study of smoking cessation. *Human Molecular Genetics* 2008;17(18):2834–48.
- Coric V, Feldman HH, Oren DA, Shekhar A, Pultz J, Dockens RC, Wu X, Gentile KA, Huang SP, Emison E, et al. Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. *Depression and Anxiety* 2010;27(5):417–25.
- Cornuz J, Zwahlen S, Jungi WF, Osterwalder J, Klingler K, van Melle G, Bangala Y, Guessous I, Muller P, Willers J, et al. A vaccine against nicotine for smoking cessation: a randomized controlled trial. *PLoS One* 2008;3(6):e2547.
- Cosgrove KP, Esterlis I, McKee SA, Bois F, Seibyl JP, Mazure CM, Krishnan-Sarin S, Staley JK, Picciotto MR, O’Malley SS. Sex differences in availability of beta2*-nicotinic acetylcholine receptors in recently abstinent tobacco smokers. *Archives of General Psychiatry* 2012;69(4):418–27.
- Cosgrove KP, Wang S, Kim SJ, McGovern E, Nabulsi N, Gao H, Labaree D, Tagare HD, Sullivan JM, Morris ED. Sex differences in the brain’s dopamine signature of cigarette smoking. *Journal of Neuroscience* 2014;34(50):16851–5.
- Culbertson CS, Bramen J, Cohen MS, London ED, Olmstead RE, Gan JJ, Costello MR, Shulenberg S, Mandelkern MA, Brody AL. Effect of bupropion treatment on brain activation induced by cigarette-related cues in smokers. *Archives of General Psychiatry* 2011;68(5):505–15.
- D’Souza MS. Glutamatergic transmission in drug reward: implications for drug addiction. *Frontiers in Neuroscience* 2015;9:404.
- D’Souza MS, Liechti ME, Ramirez-Nino AM, Kuczenski R, Markou A. The metabotropic glutamate 2/3 receptor agonist LY379268 blocked nicotine-induced increases in nucleus accumbens shell dopamine only in the presence of a nicotine-associated context in rats. *Neuropsychopharmacology* 2011;36(10):2111–24.

- 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. *European Journal of Neuroscience* 2014;39(8):1314–22.
- Dahl JP, Jepsen C, Levenson R, Wileyto EP, Patterson F, Berrettini WH, Lerman C. Interaction between variation in the D2 dopamine receptor (DRD2) and the neuronal calcium sensor-1 (FREQ) genes in predicting response to nicotine replacement therapy for tobacco dependence. *Pharmacogenomics Journal* 2006;6(3):194–9.
- Dani JA. Roles of dopamine signaling in nicotine addiction. *Molecular Psychiatry* 2003;8(3):255–6.
- David SP, Brown RA, Papandonatos GD, Kahler CW, Lloyd-Richardson EE, Munafò MR, Shields PG, Lerman C, Strong D, McCaffery J, et al. Pharmacogenetic clinical trial of sustained-release bupropion for smoking cessation. *Nicotine and Tobacco Research* 2007;9(8):821–33.
- David SP, Hamidovic A, Chen GK, Bergen AW, Wessel J, Kasberger JL, Brown WM, Petruzella S, Thacker EL, Kim Y, et al. Genome-wide meta-analyses of smoking behaviors in African Americans. *Translational Psychiatry* 2012;2:e119.
- David SP, Lancaster T, Stead LF, Evins AE, Prochaska JJ. Opioid antagonists for smoking cessation. *Cochrane Database of Systematic Reviews* 2013a, Issue 6. Art. No.: CD003086. DOI: 10.1002/14651858.CD003086.pub3.
- David SP, Munafò MR. Genetic variation in the dopamine pathway and smoking cessation. *Pharmacogenomics* 2008;9(9):1307–21.
- David SP, Niaura R, Papandonatos GD, Shadel WG, Burkholder GJ, Britt DM, Day A, Stumpff J, Hutchison K, Murphy M, et al. Does the DRD2-Taq1 A polymorphism influence treatment response to bupropion hydrochloride for reduction of the nicotine withdrawal syndrome? *Nicotine and Tobacco Research* 2003;5(6):935–42.
- David SP, Strong DR, Leventhal AM, Lancaster MA, McGeary JE, Munafò MR, Bergen AW, Swan GE, Benowitz NL, Tyndale RF, et al. Influence of a dopamine pathway additive genetic efficacy score on smoking cessation: results from two randomized clinical trials of bupropion. *Addiction* 2013b;108(12):2202–11.
- de Villiers SH, Cornish KE, Troska AJ, Pravetoni M, Pentel PR. Increased efficacy of a trivalent nicotine vaccine compared to a dose-matched monovalent vaccine when formulated with alum. *Vaccine* 2013;31(52):6185–93.
- Dempsey D, Tutka P, Jacob P 3rd, Allen F, Schoedel K, Tyndale RF, Benowitz NL. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clinical Pharmacology and Therapeutics* 2004;76(1):64–72.
- Desai RI, Bergman J. Effects of the nanoparticle-based vaccine, SEL-068, on nicotine discrimination in squirrel monkeys. *Neuropsychopharmacology* 2015;40(9):2207–16.
- Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology* 2000;393(1–3):295–314.
- Di Ciano P, Guranda M, Lagzdins D, Tyndale RF, Gamaledin I, Selby P, Boileau I, Le Foll B. Varenicline induced elevation of dopamine in smokers: a preliminary [(11)C]-(+)-PHNO PET study. *Neuropsychopharmacology* 2016;41(6):1513–20.
- Dinur-Klein L, Dannon P, Hadar A, Rosenberg O, Roth Y, Kotler M, Zangen A. Smoking cessation induced by deep repetitive transcranial magnetic stimulation of the prefrontal and insular cortices: a prospective, randomized controlled trial. *Biological Psychiatry* 2014;76(9):742–9.
- Do KT, Galván A. FDA cigarette warning labels lower craving and elicit fronto-insular activation in adolescent smokers. *Social Cognitive and Affective Neuroscience* 2015;10(11):1484–96.
- Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics* 2003;12(3):205–16.
- Dubroff JG, Doot RK, Falcone M, Schnoll RA, Ray R, Tyndale RF, Brody AL, Hou C, Schmitz A, Lerman C. Decreased nicotinic receptor availability in smokers with slow rates of nicotine metabolism. *Journal of Nuclear Medicine* 2015;56(11):1724–9.
- Dumais KM, Franklin TR, Jagannathan K, Hager N, Gawrysiak M, Betts J, Farmer S, Guthrie E, Pater H, Janes AC, et al. Multi-site exploration of sex differences in brain reactivity to smoking cues: Consensus across sites and methodologies. *Drug and Alcohol Dependence* 2017;178:469–76.
- Dunlop K, Hanlon CA, Downar J. Noninvasive brain stimulation treatments for addiction and major depression. *Annals of the New York Academy of Sciences* 2017;1394(1):31–54.
- Ehringer MA, Clegg HV, Collins AC, Corley RP, Crowley T, Hewitt JK, Hopfer CJ, Krauter K, Lessem J, Rhee SH, et al. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNA2) with subjective responses to alcohol and nicotine. *American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics* 2007;144B(5):596–604.
- Esterlis I, Hannestad JO, Perkins E, Bois F, D'Souza DC, Tyndale RF, Seibyl JP, Hatsukami DM, Cosgrove KP, O'Malley SS. Effect of a nicotine vaccine on nicotine binding to beta2*-nicotinic acetylcholine receptors in

- vivo in human tobacco smokers. *American Journal of Psychiatry* 2013;170(4):399–407.
- Etter JF. Cytisine for smoking cessation: a literature review and a meta-analysis. *Archives of Internal Medicine* 2006;166(15):1553–9.
- Fahim RE, Kessler PD, Kalnik MW. Therapeutic vaccines against tobacco addiction. *Expert Review of Vaccines* 2013;12(3):333–42.
- Falcone M, Cao W, Bernardo L, Tyndale RF, Loughhead J, Lerman C. Brain responses to smoking cues differ based on nicotine metabolism rate. *Biological Psychiatry* 2016;80(3):190–7.
- Fedota JR, Stein EA. Resting-state functional connectivity and nicotine addiction: prospects for biomarker development. *Annals of the New York Academy of Sciences* 2015;1349:64–82.
- Fedota JR, Sutherland MT, Salmeron BJ, Ross TJ, Hong LE, Stein EA. Reward anticipation is differentially modulated by varenicline and nicotine in smokers. *Neuropsychopharmacology* 2015;40(8):2038–46.
- Fitzgerald PJ. Elevated norepinephrine may be a unifying etiological factor in the abuse of a broad range of substances: alcohol, nicotine, marijuana, heroin, cocaine, and caffeine. *Substance Abuse* 2013;7:171–83.
- 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;35(8):1751–60.
- Fowler CD, Kenny PJ. Nicotine aversion: neurobiological mechanisms and relevance to tobacco dependence vulnerability. *Neuropharmacology* 2014;76(Part B):533–44.
- Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. Habenular alpha5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* 2011;471(7340):597–601.
- Frahm S, Slimak MA, Ferrarese L, Santos-Torres J, Antolin-Fontes B, Auer S, Filkin S, Pons S, Fontaine JF, Tsetlin V, et al. Aversion to nicotine is regulated by the balanced activity of beta4 and alpha5 nicotinic receptor subunits in the medial habenula. *Neuron* 2011;70(3):522–35.
- Franklin T, Wang Z, Suh JJ, Hazan R, Cruz J, Li Y, Goldman M, Detre JA, O'Brien CP, Childress AR. Effects of varenicline on smoking cue-triggered neural and craving responses. *Archives of General Psychiatry* 2011;68(5):516–26.
- Fraser PE, Rosen AC. Transcranial direct current stimulation and behavioral models of smoking addiction. *Frontiers in Psychiatry* 2012;3:79.
- Freathy RM, Ring SM, Shields B, Galobardes B, Knight B, Weedon MN, Smith GD, Frayling TM, Hattersley AT. A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNA4) is associated with a reduced ability of women to quit smoking in pregnancy. *Human Molecular Genetics* 2009;18(15):2922–7.
- Froeliger B, McConnell PA, Bell S, Sweitzer M, Kozink RV, Eichberg C, Hallyburton M, Kaiser N, Gray KM, McClernon FJ. Association between baseline corticothalamic-mediated inhibitory control and smoking relapse vulnerability. *JAMA Psychiatry* 2017;74(4):379–86.
- Gabrieli JD, Ghosh SS, Whitfield-Gabrieli S. Prediction as a humanitarian and pragmatic contribution from human cognitive neuroscience. *Neuron* 2015;85(1):11–26.
- Gage SH, Munafo MR, Davey Smith G. Causal inference in developmental origins of health and disease (DOHaD) research. *Annual Review of Psychology* 2016;67:567–85.
- García-Rodríguez O, Secades-Villa R, Flórez-Salamanca L, Okuda M, Liu SM, Blanco C. Probability and predictors of relapse to smoking: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence* 2013;132(3):479–85.
- Gaznick N, Tranel D, McNutt A, Bechara A. Basal ganglia plus insula damage yields stronger disruption of smoking addiction than basal ganglia damage alone. *Nicotine and Tobacco Research* 2014;16(4):445–53.
- Gipson CD, Reissner KJ, Kupchik YM, Smith AC, Stankeviciute N, Hensley-Simon ME, Kalivas PW. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proceedings of the National Academy of Sciences of the United States of America* 2013;110(22):9124–9.
- Gold AB, Lerman C. Pharmacogenetics of smoking cessation: role of nicotine target and metabolism genes. *Human Genetics* 2012.
- Gómez-Coronado N, Walker AJ, Berk M, Dodd S. Current and emerging pharmacotherapies for cessation of tobacco smoking. *Pharmacotherapy* 2018;38(2):235–58.
- Goniewicz ML, Delijewski M. Nicotine vaccines to treat tobacco dependence. *Human Vaccines & Immunotherapeutics* 2013;9(1):13–25.
- Gorelick DA, Zangen A, George MS. Transcranial magnetic stimulation in the treatment of substance addiction. *Annals of the New York Academy of Sciences* 2014;1327:79–93.
- Gourlay SG, Stead LF, Benowitz NL. Clonidine for smoking cessation (2008 review). *Cochrane Database of Systematic Reviews* 2004, Issue 3. Art. No.: CD000058. DOI: 10.1002/14651858.CD000058.pub2.
- Grace AA. Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Research: Brain Research Reviews* 2000;31(2–3):330–41.

- Grieder TE, Herman MA, Contet C, Tan LA, Vargas-Perez H, Cohen A, Chwalek M, Maal-Bared G, Freiling J, Schlosburg JE, et al. VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. *Nature Neuroscience* 2014;17(12):1751–8.
- Gu DF, Hinks LJ, Morton NE, Day IN. The use of long PCR to confirm three common alleles at the CYP2A6 locus and the relationship between genotype and smoking habit. *Annals of Human Genetics* 2000;64(Part 5):383–90.
- Hamidovic A, Kasberger JL, Young TR, Goodloe RJ, Redline S, Buxbaum SG, Benowitz NL, Bergen AW, Butler KR, Franceschini N, et al. Genetic variability of smoking persistence in African Americans. *Cancer Prevention Research* 2011;4(5):729–34.
- Hamilton DA, Mahoney MC, Novalen M, Chenoweth MJ, Heitjan DF, Lerman C, Tyndale RF, Hawk LW Jr. Test-retest reliability and stability of the nicotine metabolite ratio among treatment-seeking smokers. *Nicotine and Tobacco Research* 2015;17(12):1505–9.
- Hanlon CA, Hartwell KJ, Canterbury M, Li X, Owens M, Lematty T, Prisciandaro JJ, Borckardt J, Brady KT, George MS. Reduction of cue-induced craving through realtime neurofeedback in nicotine users: the role of region of interest selection and multiple visits. *Psychiatry Research* 2013;213(1):79–81.
- Hartwell KJ, Hanlon CA, Li X, Borckardt JJ, Canterbury M, Prisciandaro JJ, Moran-Santa Maria MM, LeMatty T, George MS, Brady KT. Individualized real-time fMRI neurofeedback to attenuate craving in nicotine-dependent smokers. *Journal of Psychiatry and Neuroscience* 2016;41(1):48–55.
- Hartwell KJ, Lematty T, McRae-Clark AL, Gray KM, George MS, Brady KT. Resisting the urge to smoke and craving during a smoking quit attempt on varenicline: results from a pilot fMRI study. *American Journal of Drug and Alcohol Abuse* 2013;39(2):92–8.
- Hatsukami DK, Jorenby DE, Gonzales D, Rigotti NA, Glover ED, Oncken CA, Tashkin DP, Reus VI, Akhavan RC, Fahim RE, et al. Immunogenicity and smoking-cessation outcomes for a novel nicotine immunotherapeutic. *Clinical Pharmacology and Therapeutics* 2011;89(3):392–9.
- Hatsukami DK, Rennard S, Jorenby D, Fiore M, Koopmeiners J, de Vos A, Horwith G, Pentel PR. Safety and immunogenicity of a nicotine conjugate vaccine in current smokers. *Clinical Pharmacology and Therapeutics* 2005;78(5):456–67.
- Havermans A, Vuurman EF, van den Hurk J, Hoogsteder P, van Schayck OC. Treatment with a nicotine vaccine does not lead to changes in brain activity during smoking cue exposure or a working memory task. *Addiction* 2014;109(8):1260–7.
- Hicks MJ, Rosenberg JB, De BP, Pagovich OE, Young CN, Qiu JP, Kaminsky SM, Hackett NR, Worgall S, Janda KD, et al. AAV-directed persistent expression of a gene encoding anti-nicotine antibody for smoking cessation. *Science Translational Medicine* 2012;4(140):140ra87.
- Hieda Y, Keyler DE, Ennifar S, Fattom A, Pentel PR. Vaccination against nicotine during continued nicotine administration in rats: immunogenicity of the vaccine and effects on nicotine distribution to brain. *International Journal of Immunopharmacology* 2000;22(10):809–19.
- Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105(49):19480–5.
- Hollander JA, Pham D, Fowler CD, Kenny PJ. Hypocretin-1 receptors regulate the reinforcing and reward-enhancing effects of cocaine: pharmacological and behavioral genetics evidence. *Frontiers in Behavioral Neuroscience* 2012;6:47.
- Hong LE, Gu H, Yang Y, Ross TJ, Salmeron BJ, Buchholz B, Thaker GK, Stein EA. Association of nicotine addiction and nicotine's actions with separate cingulate cortex functional circuits. *Archives of General Psychiatry* 2009;66(4):431–41.
- Hong LE, Hodgkinson CA, Yang Y, Sampath H, Ross TJ, Buchholz B, Salmeron BJ, Srivastava V, Thaker GK, Goldman D, et al. A genetically modulated, intrinsic cingulate circuit supports human nicotine addiction. *Proceedings of the National Academy of Sciences of the United States of America* 2010;107(30):13509–14.
- Hoogsteder PH, Kotz D, van Spiegel PI, Viechtbauer W, van Schayck OC. Efficacy of the nicotine vaccine 3'-AmNic-rEPA (NicVAX) co-administered with varenicline and counselling for smoking cessation: a randomized placebo-controlled trial. *Addiction* 2014;109(8):1252–9.
- Huettel S, Song AW, McCarthy G. *Functional Magnetic Resonance Imaging*. 3rd ed. Cary (NC): Oxford University Press, 2014.
- Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, Lancaster T. Antidepressants for smoking cessation. *Cochrane Database of Systematic Reviews* 2014, Issue 1. Art. No.: CD000031. DOI: 10.1002/14651858.CD000031.pub4.
- Hughes JR, Stead LF, Lancaster T. Nortriptyline for smoking cessation: a review. *Nicotine and Tobacco Research* 2005;7(4):491–9.
- Hulka LM, Treyer V, Scheidegger M, Preller KH, Vonmoos M, Baumgartner MR, Johayem A, Ametamey SM, Buck A, Seifritz E, et al. Smoking but not cocaine use is associated with lower cerebral metabotropic glutamate receptor 5 density in humans. *Molecular Psychiatry* 2014;19(5):625–32.

- Isomura S, Wirsching P, Janda KD. An immunotherapeutic program for the treatment of nicotine addiction: hapten design and synthesis. *Journal of Organic Chemistry* 2001;66(12):4115–21.
- Jackson KJ, Carroll FI, Negus SS, Damaj MI. Effect of the selective kappa-opioid receptor antagonist JDTC on nicotine antinociception, reward, and withdrawal in the mouse. *Psychopharmacology* 2010a;210(2):285–94.
- Jackson KJ, Marks MJ, Vann RE, Chen X, Gamage TF, Warner JA, Damaj MI. Role of alpha5 nicotinic acetylcholine receptors in pharmacological and behavioral effects of nicotine in mice. *Journal of Pharmacology and Experimental Therapeutics* 2010b;334(1):137–46.
- Jacob NT, Lockner JW, Schlosburg JE, Ellis BA, Eubanks LM, Janda KD. Investigations of enantiopure nicotine haptens using an adjuvanting carrier in anti-nicotine vaccine development. *Journal of Medicinal Chemistry* 2016;59(6):2523–9.
- Jasinska AJ, Chua HF, Ho SS, Polk TA, Rozek LS, Strecher VJ. Amygdala response to smoking-cessation messages mediates the effects of serotonin transporter gene variation on quitting. *Neuroimage* 2012;60(1):766–73.
- Jefferson T, Rudin M, Di Pietrantonj C. Adverse events after immunisation with aluminium-containing DTP vaccines: systematic review of the evidence. *Lancet Infectious Diseases* 2004;4(2):84–90.
- Jensen KP, DeVito EE, Herman AI, Valentine GW, Gelernter J, Sofuoglu M. A CHRNA5 smoking risk variant decreases the aversive effects of nicotine in humans. *Neuropsychopharmacology* 2015;40(12):2813–21.
- Johnstone E, Benowitz N, Cargill A, Jacob R, Hinks L, Day I, Murphy M, Walton R. Determinants of the rate of nicotine metabolism and effects on smoking behavior. *Clinical Pharmacology and Therapeutics* 2006;80(4):319–30.
- Johnstone EC, Yudkin P, Griffiths SE, Fuller A, Murphy M, Walton R. The dopamine D2 receptor C32806T polymorphism (DRD2 Taq1A RFLP) exhibits no association with smoking behaviour in a healthy UK population. *Addiction Biology* 2004;9(3-4):221–6.
- Jonsson EG, Nothen MM, Grunhage F, Farde L, Nakashima Y, Propping P, Sedvall GC. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Molecular Psychiatry* 1999;4(3):290–6.
- Jorm AF, Henderson AS, Jacomb PA, Christensen H, Korten AE, Rodgers B, Tan X, Easteal S. Association of smoking and personality with a polymorphism of the dopamine transporter gene: results from a community survey. *American Journal of Medical Genetics* 2000;96(3):331–4.
- Justinova Z, Le Foll B, Redhi GH, Markou A, Goldberg SR. Differential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on nicotine versus cocaine self-administration and relapse in squirrel monkeys. *Psychopharmacology* 2016;233(10):1791–800.
- Justinova Z, Panlilio LV, Secci ME, Redhi GH, Schindler CW, Cross AJ, Mrzljak L, Medd A, Shaham Y, Goldberg SR. The novel metabotropic glutamate receptor 2 positive allosteric modulator, AZD8529, decreases nicotine self-administration and relapse in squirrel monkeys. *Biological Psychiatry* 2015;78(7):452–62.
- Karama S, Ducharme S, Corley J, Chouinard-Decorte F, Starr JM, Wardlaw JM, Bastin ME, Deary IJ. Cigarette smoking and thinning of the brain's cortex. *Molecular Psychiatry* 2015;20(6):778–85.
- Kaufmann A, Hitsman B, Goelz PM, Veluz-Wilkins A, Blazekovic S, Powers L, Leone FT, Gariti P, Tyndale RF, Schnoll RA. Rate of nicotine metabolism and smoking cessation outcomes in a community-based sample of treatment-seeking smokers. *Addictive Behaviors* 2015;51:93–9.
- Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP. Competition between functional brain networks mediates behavioral variability. *Neuroimage* 2008;39(1):527–37.
- Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA. A population-based twin study in women of smoking initiation and nicotine dependence. *Psychological Medicine* 1999;29(2):299–308.
- Kenny PJ, Chartoff E, Roberto M, Carlezon WA Jr, Markou A. NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of the ventral tegmental area and central nucleus of the amygdala. *Neuropsychopharmacology* 2009;34(2):266–81.
- Keyler DE, Hieda Y, St Peter J, Pentel PR. Altered disposition of repeated nicotine doses in rats immunized against nicotine. *Nicotine and Tobacco Research* 1999;1(3):241–9.
- Keyler DE, Roiko SA, Benlhabib E, LeSage MG, St Peter JV, Stewart S, Fuller S, Le CT, Pentel PR. Monoclonal nicotine-specific antibodies reduce nicotine distribution to brain in rats: dose- and affinity-response relationships. *Drug Metabolism and Disposition: The Biological Fate of Chemicals* 2005;33(7):1056–61.
- Keyler DE, Roiko SA, Earley CA, Murtaugh MP, Pentel PR. Enhanced immunogenicity of a bivalent nicotine vaccine. *International Immunopharmacology* 2008;8(11):1589–94.
- Kim DY, Yoo SS, Tegethoff M, Meinlschmidt G, Lee JH. The inclusion of functional connectivity information into fMRI-based neurofeedback improves its efficacy in the reduction of cigarette cravings. *Journal of Cognitive Neuroscience* 2015;27(8):1552–72.
- Klimek V, Zhu MY, Dilley G, Konick L, Overholser JC, Meltzer HY, May WL, Stockmeier CA, Ordway GA. Effects of long-term cigarette smoking on the human

- locus coeruleus. *Archives of General Psychiatry* 2001;58(9):821–7.
- Knackstedt LA, Kalivas PW. Glutamate and reinstatement. *Current Opinion in Pharmacology* 2009;9(1):59–64.
- Knackstedt LA, LaRowe S, Mardikian P, Malcolm R, Upadhyaya H, Hedden S, Markou A, Kalivas PW. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. *Biological Psychiatry* 2009;65(10):841–5.
- Kober H, Brewer JA, Height KL, Sinha R. Neural stress reactivity relates to smoking outcomes and differentiates between mindfulness and cognitive-behavioral treatments. *Neuroimage* 2017;151:4–13.
- Koob GF. A role for brain stress systems in addiction. *Neuron* 2008;59(1):11–34.
- Koob GF, Simon EJ. The neurobiology of addiction: where we have been and where we are going. *Journal of Drug Issues* 2009;39(1):115–32.
- Koob GF, Volkow ND. Neurobiology of addiction: a neuro-circuitry analysis. *Lancet Psychiatry* 2016;3(8):760–73.
- Krönke KM, Wolff M, Benz A, Goschke T. Successful smoking cessation is associated with prefrontal cortical function during a Stroop task: a preliminary study. *Psychiatry Research* 2015;234(1):52–6.
- Kutlu MG, Gould TJ. Nicotinic modulation of hippocampal cell signaling and associated effects on learning and memory. *Physiology and Behavior* 2016;155:162–71.
- Kuwabara H, Heishman SJ, Brasic JR, Contoreggi C, Cascella N, Mackowick KM, Taylor R, Rousset O, Willis W, Huestis MA, et al. Mu opioid receptor binding correlates with nicotine dependence and reward in smokers. *PLoS One* 2014;9(12):e113694.
- Kwon JM, Goate AM. The candidate gene approach. *Alcohol Research and Health* 2000;24(3):164–8.
- Lameka K, Farwell MD, Ichise M. Positron emission tomography. *Handbook of Clinical Neurology* 2016;135:209–27.
- Lerman C, Caporaso NE, Audrain J, Main D, Bowman ED, Lockshin B, Boyd NR, Shields PG. Evidence suggesting the role of specific genetic factors in cigarette smoking. *Health Psychology* 1999;18(1):14–20.
- Lerman C, Gu H, Loughhead J, Ruparel K, Yang Y, Stein EA. Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. *JAMA Psychiatry* 2014;71(5):523–30.
- Lerman C, Jepson C, Wileyto EP, Epstein LH, Rukstalis M, Patterson F, Kaufmann V, Restine S, Hawk L, Niaura R, et al. Role of functional genetic variation in the dopamine D2 receptor (DRD2) in response to bupropion and nicotine replacement therapy for tobacco dependence: results of two randomized clinical trials. *Neuropsychopharmacology* 2006a;31(1):231–42.
- Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, Mroziewicz M, Benowitz N, Tyndale RF. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clinical Pharmacology and Therapeutics* 2010;87(5):553–7.
- Lerman C, Schnoll RA, Hawk LW Jr, Cinciripini P, George TP, Wileyto EP, Swan GE, Benowitz NL, Heitjan DF, Tyndale RF, et al. Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. *Lancet Respiratory Medicine* 2015;3(2):131–8.
- Lerman C, Shields PG, Wileyto EP, Audrain J, Hawk LH Jr, Pinto A, Kucharski S, Krishnan S, Niaura R, Epstein LH. Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. *Health Psychology* 2003;22(5):541–8.
- Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, Benowitz N. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clinical Pharmacology and Therapeutics* 2006b;79(6):600–8.
- LeSage MG, Keyler DE, Hieda Y, Collins G, Burroughs D, Le C, Pentel PR. Effects of a nicotine conjugate vaccine on the acquisition and maintenance of nicotine self-administration in rats. *Psychopharmacology* 2006a;184(3–4):409–16.
- LeSage MG, Keyler DE, Pentel PR. Current status of immunologic approaches to treating tobacco dependence: vaccines and nicotine-specific antibodies. *AAPS Journal* 2006b;8(1):E65–E75.
- LeSage MG, Shelley D, Pravetoni M, Pentel PR. Enhanced attenuation of nicotine discrimination in rats by combining nicotine-specific antibodies with a nicotinic receptor antagonist. *Pharmacology, Biochemistry and Behavior* 2012;102(1):157–62.
- Leslie FM, Mojica CY, Reynaga DD. Nicotinic receptors in addiction pathways. *Molecular Pharmacology* 2013;83(4):753–8.
- Leung T, Bergen A, Munafò MR, De Ruyck K, Selby P, De Luca V. Effect of the rs1051730-rs16969968 variant and smoking cessation treatment: a meta-analysis. *Pharmacogenomics* 2015;16(7):713–20.
- Li L, Jia K, Zhou X, McCallum SE, Hough LB, Ding X. Impact of nicotine metabolism on nicotine's pharmacological effects and behavioral responses: insights from a Cyp2a(4/5)bgs-null mouse. *Journal of Pharmacology and Experimental Therapeutics* 2013a;347(3):746–54.
- Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction* 2003;98(1):23–31.
- Li S, Yang Y, Hoffmann E, Tyndale RF, Stein EA. CYP2A6 genetic variation alters striatal-cingulate circuits,

- network hubs, and executive processing in smokers. *Biological Psychiatry* 2017;81(7):554–63.
- Li X, Hartwell KJ, Owens M, Lematty T, Borckardt JJ, Hanlon CA, Brady KT, George MS. Repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex reduces nicotine cue craving. *Biological Psychiatry* 2013b;73(8):714–20.
- 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;76(Part B):554–65.
- Lindblom N, de Villiers SH, Kalayanov G, Gordon S, Johansson AM, Svensson TH. Active immunization against nicotine prevents reinstatement of nicotine-seeking behavior in rats. *Respiration* 2002;69(3):254–60.
- Lindquist KJ, Jorgenson E, Hoffmann TJ, Witte JS. The impact of improved microarray coverage and larger sample sizes on future genome-wide association studies. *Genetic Epidemiology* 2013;37(4):383–92.
- Liu X, Wang L, Yan H, Chang Y. Assembly and assessment of DNA scaffolded vaccines. *Methods in Molecular Biology* 2016;1404:301–11.
- Lockner JW, Ho SO, McCague KC, Chiang SM, Do TQ, Fujii G, Janda KD. Enhancing nicotine vaccine immunogenicity with liposomes. *Bioorganic and Medicinal Chemistry Letters* 2013;23(4):975–8.
- Loughead J, Ray R, Wileyto EP, Ruparel K, Sanborn P, Siegel S, Gur RC, Lerman C. Effects of the alpha-4beta2 partial agonist varenicline on brain activity and working memory in abstinent smokers. *Biological Psychiatry* 2010;67(8):715–21.
- Loughead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, Lerman C. Working memory-related neural activity predicts future smoking relapse. *Neuropsychopharmacology* 2015;40(6):1311–20.
- Loukola A, Buchwald J, Gupta R, Palviainen T, Hallfors J, Tikkanen E, Korhonen T, Ollikainen M, Sarin AP, Ripatti S, et al. A genome-wide association study of a biomarker of nicotine metabolism. *PLoS Genetics* 2015;11(9):e1005498.
- Luijten M, Veltman DJ, van den Brink W, Hester R, Field M, Smits M, Franken IH. Neurobiological substrate of smoking-related attentional bias. *Neuroimage* 2011;54(3):2374–81.
- Maglione MA, Das L, Raaen L, Smith A, Chari R, Newberry S, Shanman R, Perry T, Goetz MB, Gidengil C. Safety of vaccines used for routine immunization of U.S. children: a systematic review. *Pediatrics* 2014;134(2):325–37.
- Malaiyandi V, Goodz SD, Sellers EM, Tyndale RF. CYP2A6 genotype, phenotype, and the use of nicotine metabolites as biomarkers during ad libitum smoking. *Cancer Epidemiology, Biomarkers and Prevention* 2006a;15(10):1812–9.
- Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F, Tyndale RF. Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. *Molecular Psychiatry* 2006b;11(4):400–9.
- Mansvelder HD, Fagen ZM, Chang B, Mitchum R, McGehee DS. Bupropion inhibits the cellular effects of nicotine in the ventral tegmental area. *Biochemical Pharmacology* 2007;74(8):1283–91.
- Mantsch JR, Baker DA, Funk D, Lê AD, Shaham Y. Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology* 2016;41(1):335–56.
- Mao D, Gallagher K, McGehee DS. Nicotine potentiation of excitatory inputs to ventral tegmental area dopamine neurons. *Journal of Neuroscience* 2011;31(18):6710–20.
- Marchi M, Grilli M, Pittaluga AM. Nicotinic modulation of glutamate receptor function at nerve terminal level: a fine-tuning of synaptic signals. *Frontiers in Pharmacology* 2015;6:89.
- Marcinkiewicz CA, Prado MM, Isaac SK, Marshall A, Rylkova D, Bruijnzeel AW. Corticotropin-releasing factor within the central nucleus of the amygdala and the nucleus accumbens shell mediates the negative affective state of nicotine withdrawal in rats. *Neuropsychopharmacology* 2009;34(7):1743–52.
- Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* 2005;436(7047):103–7.
- Maurer P, Jennings GT, Willers J, Rohner F, Lindman Y, Roubicek K, Renner WA, Muller P, Bachmann MF. A therapeutic vaccine for nicotine dependence: preclinical efficacy, and Phase I safety and immunogenicity. *European Journal of Immunology* 2005;35(7):2031–40.
- McClure EA, Baker NL, Gipson CD, Carpenter MJ, Roper AP, Froeliger BE, Kalivas PW, Gray KM. An open-label pilot trial of N-acetylcysteine and varenicline in adult cigarette smokers. *American Journal of Drug and Alcohol Abuse* 2015;41(1):52–6.
- McCluskie MJ, Pryde DC, Gervais DP, Stead DR, Zhang N, Benoit M, Robertson K, Kim IJ, Tharmanathan T, Merson JR, et al. Enhancing immunogenicity of a 3’aminomethylnicotine-DT-conjugate anti-nicotine vaccine with CpG adjuvant in mice and non-human primates. *International Immunopharmacology* 2013;16(1):50–6.
- McKee SA, Potenza MN, Kober H, Sofuoglu M, Arnsten AF, Picciotto MR, Weinberger AH, Ashare R, Sinha R. A translational investigation targeting stress-reactivity and prefrontal cognitive control with guanfacine for smoking cessation. *Journal of Psychopharmacology* 2015;29(3):300–11.

- Menossi HS, Goudriaan AE, de Azevedo-Marques Perico C, Nicastrì S, de Andrade AG, D'Elia G, Li CS, Castaldelli-Maia JM. Neural bases of pharmacological treatment of nicotine dependence—insights from functional brain imaging: a systematic review. *CNS Drugs* 2013;27(11):921–41.
- Mihov Y, Hasler G. Negative allosteric modulators of metabotropic glutamate receptors subtype 5 in addiction: a therapeutic window. *International Journal of Neuropsychopharmacology* 2016;19(7):pyw002.
- Millar NS, Harkness PC. Assembly and trafficking of nicotinic acetylcholine receptors (review). *Molecular Membrane Biology* 2008;25(4):279–92.
- Miller KD, Roque R, Clegg CH. Novel anti-nicotine vaccine using a trimeric coiled-coil hapten carrier. *PLoS One* 2014;9(12):e114366.
- Mooney ME, Schmitz JM, Allen S, Grabowski J, Pentel P, Oliver A, Hatsukami DK. Bupropion and naltrexone for smoking cessation: a double-blind randomized placebo-controlled clinical trial. *Clinical Pharmacology and Therapeutics* 2016;100(4):344–52.
- Moran-Santa Maria MM, Hartwell KJ, Hanlon CA, Canterberry M, Lematty T, Owens M, Brady KT, George MS. Right anterior insula connectivity is important for cue-induced craving in nicotine-dependent smokers. *Addiction Biology* 2015;20(2):407–14.
- Moreno AY, Azar MR, Koob GF, Janda KD. Probing the protective effects of a conformationally constrained nicotine vaccine. *Vaccine* 2012;30(47):6665–70.
- Morton LM, Wang SS, Bergen AW, Chatterjee N, Kvale P, Welch R, Yeager M, Hayes RB, Chanock SJ, Caporaso NE. DRD2 genetic variation in relation to smoking and obesity in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Pharmacogenetics and Genomics* 2006;16(12):901–10.
- Munafò M. Replication validity of genetic association studies of smoking behavior: what can meta-analytic techniques offer? *Nicotine and Tobacco Research* 2004;6(2):381–2.
- Munafò M, Clark T, Johnstone E, Murphy M, Walton R. The genetic basis for smoking behavior: a systematic review and meta-analysis. *Nicotine and Tobacco Research* 2004;6(4):583–97.
- Munafò MR, Johnstone EC, Walther D, Uhl GR, Murphy MF, Aveyard P. CHRNA3 rs1051730 genotype and short-term smoking cessation. *Nicotine and Tobacco Research* 2011;13(10):982–8.
- Munafò MR, Timofeeva MN, Morris RW, Prieto-Merino D, Sattar N, Brennan P, Johnstone EC, Relton C, Johnson PC, Walther D, et al. Association between genetic variants on chromosome 15q25 locus and objective measures of tobacco exposure. *Journal of the National Cancer Institute* 2012;104(10):740–8.
- Munafò MR, Timpson NJ, David SP, Ebrahim S, Lawlor DA. Association of the DRD2 gene Taq1A polymorphism and smoking behavior: a meta-analysis and new data. *Nicotine and Tobacco Research* 2009;11(1):64–76.
- Naqvi NH, Bechara A. The hidden island of addiction: the insula. *Trends in Neurosciences* 2009;32(1):56–67.
- Naqvi NH, Bechara A. The insula and drug addiction: an interoceptive view of pleasure, urges, and decision-making. *Brain Structure & Function* 2010;214(5–6):435–50.
- Nestor L, McCabe E, Jones J, Clancy L, Garavan H. Differences in “bottom-up” and “top-down” neural activity in current and former cigarette smokers: evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *Neuroimage* 2011;56(4):2258–75.
- Nestor LJ, McCabe E, Jones J, Clancy L, Garavan H. Shared and divergent neural reactivity to non-drug operant response outcomes in current smokers and ex-smokers. *Brain Research* 2018a;1680:54–61.
- Nestor LJ, McCabe E, Jones J, Clancy L, Garavan H. Smokers and ex-smokers have shared differences in the neural substrates for potential monetary gains and losses. *Addiction Biology* 2018b;23(1):369–78.
- Neuhaus A, Bajbouj M, Kienast T, Kalus P, von Haebler D, Winterer G, Gallinat J. Persistent dysfunctional frontal lobe activation in former smokers. *Psychopharmacology* 2006;186(2):191–200.
- Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Human Mutation* 2004;23(6):540–5.
- Nishizawa D, Kasai S, Hasegawa J, Sato N, Yamada H, Tanioka F, Nagashima M, Katoh R, Satoh Y, Tagami M, et al. Associations between the orexin (hypocretin) receptor 2 gene polymorphism Val308Ile and nicotine dependence in genome-wide and subsequent association studies. *Molecular Brain* 2015;8:50.
- Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Archives of General Psychiatry* 1991;48(7):648–54.
- Noble EP, Gottschalk LA, Fallon JH, Ritchie TL, Wu JC. D2 dopamine receptor polymorphism and brain regional glucose metabolism. *American Journal of Medical Genetics* 1997;74(2):162–6.
- Noble EP, St Jeor ST, Ritchie T, Sydulko K, St Jeor SC, Fitch RJ, Brunner RL, Sparkes RS. D2 dopamine receptor gene and cigarette smoking: a reward gene? *Medical Hypotheses* 1994;42(4):257–60.
- Nuechterlein EB, Ni L, Domino EF, Zubieta JK. Nicotine-specific and non-specific effects of cigarette smoking on endogenous opioid mechanisms. *Progress in*

- Neuro-Psychopharmacology and Biological Psychiatry* 2016;69:69–77.
- Nygaard SK, Hourguettes NJ, Sobczak GG, Carlezon WA, Bruchas MR. Stress-induced reinstatement of nicotine preference requires dynorphin/kappa opioid activity in the basolateral amygdala. *Journal of Neuroscience* 2016;36(38):9937–48.
- Ono M, Kochiyama T, Fujino J, Sozu T, Kawada R, Yokoyama N, Sugihara G, Murai T, Takahashi H. Self-efficacy modulates the neural correlates of craving in male smokers and ex-smokers: an fMRI study. *Addiction Biology* 2018;23(5):1179–88.
- Paterson NE, Semenova S, Gasparini F, Markou A. The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology* 2003;167(3):257–64.
- Patnala R, Clements J, Batra J. Candidate gene association studies: a comprehensive guide to useful in silico tools. *BMC Genetics* 2013;14:39.
- Patterson F, Schnoll RA, Wileyto EP, Pinto A, Epstein LH, Shields PG, Hawk LW, Tyndale RF, Benowitz N, Lerman C. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. *Clinical Pharmacology and Therapeutics* 2008;84(3):320–5.
- Pentel PR, LeSage MG. New directions in nicotine vaccine design and use. *Advances in Pharmacology* 2014;69:553–80.
- Pentel PR, Malin DH, Ennifar S, Hieda Y, Keyler DE, Lake JR, Milstein JR, Basham LE, Coy RT, Moon JW, et al. A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats. *Pharmacology, Biochemistry and Behavior* 2000;65(1):191–8.
- Perkins KA, Gerlach D, Vender J, Grobe J, Meeker J, Hutchison S. Sex differences in the subjective and reinforcing effects of visual and olfactory cigarette smoke stimuli. *Nicotine and Tobacco Research* 2001;3(2):141–50.
- Picard F, Sadaghiani S, Leroy C, Courvoisier DS, Maroy R, Bottlaender M. High density of nicotinic receptors in the cingulo-insular network. *Neuroimage* 2013;79:42–51.
- Picciotto MR, Addy NA, Mineur YS, Brunzell DH. It is not “either/or”: activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Progress in Neurobiology* 2008;84(4):329–42.
- Picciotto MR, Kenny PJ. Molecular mechanisms underlying behaviors related to nicotine addiction. *Cold Spring Harbor Perspectives in Medicine* 2013;3(1):a012112.
- Picciotto MR, Mineur YS. Molecules and circuits involved in nicotine addiction: the many faces of smoking. *Neuropharmacology* 2014;76(Pt B):545–53.
- Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* 1998;391(6663):173–7.
- Piper ME, McCarthy DE, Baker TB. Assessing tobacco dependence: a guide to measure evaluation and selection. *Nicotine and Tobacco Research* 2006;8(3):339–51.
- 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;38(9):1724–36.
- 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. *Journal of Neuroscience* 2010;30(6):2300–10.
- Pohjalainen T, Rinne JO, Nagren K, Lehtikoinen P, Anttila K, Syvalahti EK, Hietala J. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Molecular Psychiatry* 1998;3(3):256–60.
- Power MC, Deal JA, Sharrett AR, Jack CR Jr, Knopman D, Mosley TH, Gottesman RF. Smoking and white matter hyperintensity progression: the ARIC-MRI Study. *Neurology* 2015;84(8):841–8.
- Prado E, Maes M, Piccoli LG, Baracat M, Barbosa DS, Franco O, Dodd S, Berk M, Vargas Nunes SO. N-acetylcysteine for therapy-resistant tobacco use disorder: a pilot study. *Redox Report* 2015;20(5):215–22.
- Pravetoni M, Keyler DE, Raleigh MD, Harris AC, Lesage MG, Mattson CK, Pettersson S, Pentel PR. Vaccination against nicotine alters the distribution of nicotine delivered via cigarette smoke inhalation to rats. *Biochemical Pharmacology* 2011;81(9):1164–70.
- Pripfl J, Tomova L, Rieckensky I, Lamm C. Transcranial magnetic stimulation of the left dorsolateral prefrontal cortex decreases cue-induced nicotine craving and EEG delta power. *Brain Stimulation* 2014;7(2):226–33.
- Prochaska JJ, Benowitz NL. The past, present, and future of nicotine addiction therapy. *Annual Review of Medicine* 2016;67:467–86.
- Rasetti R, Weinberger DR. Intermediate phenotypes in psychiatric disorders. *Current Opinion in Genetics and Development* 2011;21(3):340–8.
- Ray R, Tyndale RF, Lerman C. Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. *Journal of Neurogenetics* 2009;23(3):252–61.
- Rose EJ, Ross TJ, Salmeron BJ, Lee M, Shakleya DM, Huestis M, Stein EA. Chronic exposure to nicotine is associated with reduced reward-related activity in the

- striatum but not the midbrain. *Biological Psychiatry* 2012;71(3):206–13.
- Rose EJ, Ross TJ, Salmeron BJ, Lee M, Shakleya DM, Huestis MA, Stein EA. Acute nicotine differentially impacts anticipatory valence- and magnitude-related striatal activity. *Biological Psychiatry* 2013;73(3):280–8.
- Rosenberg JB, De BP, Hicks MJ, Janda KD, Kaminsky SM, Worgall S, Crystal RG. Suppression of nicotine-induced pathophysiology by an adenovirus hexon-based antinicotinic vaccine. *Human Gene Therapy* 2013;24(6):595–603.
- Sabol SZ, Nelson ML, Fisher C, Gunzerath L, Brody CL, Hu S, Sirota LA, Marcus SE, Greenberg BD, Lucas FR 4th, et al. A genetic association for cigarette smoking behavior. *Health Psychology* 1999;18(1):7–13.
- Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Human Molecular Genetics* 2007;16(1):36–49.
- Salas R, Sturm R, Boulter J, De Biasi M. Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *Journal of Neuroscience* 2009;29(10):3014–8.
- Sartor CE, Lessov-Schlaggar CN, Scherrer JF, Bucholz KK, Madden PA, Pergadia ML, Grant JD, Jacob T, Xian H. Initial response to cigarettes predicts rate of progression to regular smoking: findings from an offspring-of-twins design. *Addictive Behaviors* 2010;35(8):771–8.
- Satoskar SD, Keyler DE, LeSage MG, Raphael DE, Ross CA, Pentel PR. Tissue-dependent effects of immunization with a nicotine conjugate vaccine on the distribution of nicotine in rats. *International Immunopharmacology* 2003;3(7):957–70.
- Schmid B, Blomeyer D, Becker K, Treutlein J, Zimmermann US, Buchmann AF, Schmidt MH, Esser G, Banaschewski T, Rietschel M, et al. The interaction between the dopamine transporter gene and age at onset in relation to tobacco and alcohol use among 19-year-olds. *Addiction Biology* 2009;14(4):489–99.
- Schnoll RA, Johnson TA, Lerman C. Genetics and smoking behavior. *Current Psychiatry Reports* 2007;9(5):349–57.
- Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. *Pharmacology, Biochemistry and Behavior* 2009;92(1):6–11.
- Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics* 2004;14(9):615–26.
- Schuit E, Panagiotou OA, Munafo MR, Bennett DA, Bergen AW, David SP. Pharmacotherapy for smoking cessation: effects by subgroup defined by genetically informed biomarkers. *Cochrane Database of Systematic Reviews* 2017, Issue 9. Art. No.: CD011823. DOI: 10.1002/14651858.CD011823.pub2.
- Sieminska A, Jassem E. The many faces of tobacco use among women. *Medical Science Monitor* 2014;20:153–62.
- Skolnick P. Biologic approaches to treat substance-use disorders. *Trends in Pharmacological Sciences* 2015;36(10):628–35.
- Slimak MA, Ables JL, Frahm S, Antolin-Fontes B, Santos-Torres J, Moretti M, Gotti C, Ibanez-Tallon I. Habenular expression of rare missense variants of the beta4 nicotinic receptor subunit alters nicotine consumption. *Frontiers in Human Neuroscience* 2014;8:12.
- Sofuoglu M, Herman AI, Nadim H, Jatlow P. Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology* 2012;37(6):1509–16.
- Stoker AK, Olivier B, Markou A. Involvement of metabotropic glutamate receptor 5 in brain reward deficits associated with cocaine and nicotine withdrawal and somatic signs of nicotine withdrawal. *Psychopharmacology* 2012;221(2):317–27.
- Stokes PR, Benecke A, Myers J, Erritzoe D, Watson BJ, Kalk N, Barros DR, Hammers A, Nutt DJ, Lingford-Hughes AR. History of cigarette smoking is associated with higher limbic GABA_A receptor availability. *NeuroImage* 2013;69:70–7.
- Strasser AA, Benowitz NL, Pinto AG, Tang KZ, Hecht SS, Carmella SG, Tyndale RF, Lerman CE. Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. *Cancer Epidemiology, Biomarkers and Prevention* 2011;20(2):234–8.
- Strasser AA, Lerman C, Sanborn PM, Pickworth WB, Feldman EA. New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure. *Drug and Alcohol Dependence* 2007;86(2–3):294–300.
- Stroop JR. Studies of interference in serial verbal reactions. *Journal of Experimental Psychology* 1935;18(6):643–62.
- Sullivan PF, Kendler KS. The genetic epidemiology of smoking. *Nicotine and Tobacco Research* 1999;1(Suppl 2):S51–S57. discussion S69–S70.
- Sutherland MT, Carroll AJ, Salmeron BJ, Ross TJ, Hong LE, Stein EA. Down-regulation of amygdala and insula functional circuits by varenicline and nicotine in abstinent cigarette smokers. *Biological Psychiatry* 2013a;74(7):538–46.
- Sutherland MT, Carroll AJ, Salmeron BJ, Ross TJ, Hong LE, Stein EA. Individual differences in amygdala reactivity following nicotinic receptor stimulation in abstinent smokers. *Neuroimage* 2013b;66:585–93.

- Sutherland MT, McHugh MJ, Pariyadath V, Stein EA. Resting state functional connectivity in addiction: Lessons learned and a road ahead. *Neuroimage* 2012;62(4):2281–95.
- Sutherland MT, Stein EA. Functional neurocircuits and neuroimaging biomarkers of tobacco use disorder. *Trends in Molecular Medicine* 2018;24(2):129–43.
- Swan GE, Benowitz NL, Jacob P, 3rd, Lessov CN, Tyndale RF, Wilhelmsen K, Krasnow RE, McElroy MR, Moore SE, Wambach M. Pharmacogenetics of nicotine metabolism in twins: methods and procedures. *Twin Research* 2004;7(5):435–48.
- Swan GE, Benowitz NL, Lessov CN, Jacob P, 3rd, Tyndale RF, Wilhelmsen K. Nicotine metabolism: the impact of CYP2A6 on estimates of additive genetic influence. *Pharmacogenetics and Genomics* 2005;15(2):115–25.
- Swan GE, Javitz HS, Jack LM, Wessel J, Michel M, Hinds DA, Stokowski RP, McClure JB, Catz SL, Richards J, et al. Varenicline for smoking cessation: nausea severity and variation in nicotinic receptor genes. *Pharmacogenomics Journal* 2012;12(4):349–58.
- Sweitzer MM, Geier CF, Addicott MA, Denlinger R, Raiff BR, Dallery J, McClernon FJ, Donny EC. Smoking abstinence-induced changes in resting state functional connectivity with ventral striatum predict lapse during a quit attempt. *Neuropsychopharmacology* 2016a;41(10):2521–9.
- Sweitzer MM, Geier CF, Denlinger R, Forbes EE, Raiff BR, Dallery J, McClernon FJ, Donny EC. Blunted striatal response to monetary reward anticipation during smoking abstinence predicts lapse during a contingency-managed quit attempt. *Psychopharmacology* 2016b;233(5):751–60.
- Tang DW, Hello B, Mrozievicz M, Fellows LK, Tyndale RF, Dagher A. Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. *Neuroimage* 2012;60(4):2136–43.
- Tang YY, Tang R, Posner MI. Brief meditation training induces smoking reduction. *Proceedings of the National Academy of Sciences of the United States of America* 2013;110(34):13971–5.
- Tanner JA, Tyndale RF. Variation in CYP2A6 activity and personalized medicine. *Journal of Personalized Medicine* 2017;7(4):pii:E18.
- Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* 2004;306(5698):1029–32.
- Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997;7(6):479–84.
- Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP, Manolescu A, Thorleifsson G, Stefansson H, Ingason A, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* 2008;452(7187):638–42.
- Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, et al. Sequence variants at CHRNB3-CHRNA6 and CYP2A6 affect smoking behavior. *Nature Genetics* 2010;42(5):448–53.
- Thorgeirsson TE, Stefansson K. Commentary: gene-environment interactions and smoking-related cancers. *International Journal of Epidemiology* 2010;39(2):577–9.
- Timberlake DS, Haberstick BC, Lessem JM, Smolen A, Ehringer M, Hewitt JK, Hopfer C. An association between the DAT1 polymorphism and smoking behavior in young adults from the National Longitudinal Study of Adolescent Health. *Health Psychology* 2006;25(2):190–7.
- Tobacco and Genetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nature Genetics* 2010;42(5):441–7.
- Tonstad S, Heggen E, Giljam H, Lagerback PA, Tonnesen P, Wikingson LD, Lindblom N, de Villiers S, Svensson TH, Fagerstrom KO. Nicotine(R), a nicotine vaccine, for relapse prevention: a phase II, randomized, placebo-controlled, multicenter clinical trial. *Nicotine and Tobacco Research* 2013;15(9):1492–501.
- Transdisciplinary Tobacco Use Research Center on Tobacco and Dependence, Baker TB, Piper ME, McCarthy DE, Bolt DM, Smith SS, Kim SY, Colby S, Conti D, Giovino GA, et al. Time to first cigarette in the morning as an index of ability to quit smoking: implications for nicotine dependence. *Nicotine and Tobacco Research* 2007;9(Suppl 4):S555–S570.
- Tronci V, Vronskaya S, Montgomery N, Mura D, Balfour DJ. The effects of the mGluR5 receptor antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) on behavioural responses to nicotine. *Psychopharmacology* 2010;211(1):33–42.
- Tyndale RF, Zhu AZ, George TP, Cinciripini P, Hawk LW Jr, Schnoll RA, Swan GE, Benowitz NL, Heitjan DF, Lerman C, et al. Lack of associations of CHRNA5-A3-B4 genetic variants with smoking cessation treatment outcomes in Caucasian smokers despite associations with baseline smoking. *PLoS One* 2015;10(5):e0128109.
- U.S. Department of Health and Human Services. *The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General*. Atlanta (GA): U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National

- Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 1988. DHHS Publication No. (CDC) 88-8406.
- U.S. Department of Health and Human Services. *Reducing Tobacco Use. A Report of the Surgeon General*. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2000.
- U.S. Department of Health and Human Services. *How Tobacco Smoke Causes Disease—The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010.
- U.S. Department of Health and Human Services. *Preventing Tobacco Use Among Youth and Young Adults: A Report of the Surgeon General*. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2012.
- U.S. Food and Drug Administration. FDA 101: Smoking Cessation Products, 2016; <<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm198176.htm>>; accessed: May 24, 2017.
- Uhl GR, Drgon T, Johnson C, Walther D, David SP, Aveyard P, Murphy M, Johnstone EC, Munafò MR. Genome-wide association for smoking cessation success: participants in the Patch in Practice trial of nicotine replacement. *Pharmacogenomics* 2010;11(3):357–67.
- Uhl GR, Walther D, Musci R, Fisher C, Anthony JC, Storr CL, Behm FM, Eaton WW, Ialongo N, Rose JE. Smoking quit success genotype score predicts quit success and distinct patterns of developmental involvement with common addictive substances. *Molecular Psychiatry* 2014;19(1):50–4.
- Uslaner JM, Winrow CJ, Gotter AL, Roecker AJ, Coleman PJ, Hutson PH, Le AD, Renger JJ. Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement. *Behavioural Brain Research* 2014;269:61–5.
- Vandenbergh DJ, Bennett CJ, Grant MD, Strasser AA, O'Connor R, Stauffer RL, Vogler GP, Kozlowski LT. Smoking status and the human dopamine transporter variable number of tandem repeats (VNTR) polymorphism: failure to replicate and finding that never-smokers may be different. *Nicotine and Tobacco Research* 2002;4(3):333–40.
- Versace F, Engelmann JM, Robinson JD, Jackson EF, Green CE, Lam CY, Minnix JA, Karam-Hage MA, Brown VL, Wetter DW, et al. Prequit fMRI responses to pleasant cues and cigarette-related cues predict smoking cessation outcome. *Nicotine and Tobacco Research* 2014;16(6):697–708.
- Vink JM, Willemsen G, Boomsma DI. Heritability of smoking initiation and nicotine dependence. *Behavior Genetics* 2005;35(4):397–406.
- Volkow ND, Koob G, Baler R. Biomarkers in substance use disorders. *ACS Chemical Neuroscience* 2015;6(4):522–5.
- Volkow ND, Koob GF, McLellan AT. Neurobiologic advances from the brain disease model of addiction. *New England Journal of Medicine* 2016;374(4):363–71.
- Wang AL, Ruparel K, Loughhead JW, Strasser AA, Blady SJ, Lynch KG, Romer D, Cappella JN, Lerman C, Langleben DD. Content matters: neuroimaging investigation of brain and behavioral impact of televised anti-tobacco public service announcements. *Journal of Neuroscience* 2013;33(17):7420–7.
- Wang C, Shen Z, Huang P, Qian W, Yu X, Sun J, Yu H, Yang Y, Zhang M. Altered spontaneous activity of posterior cingulate cortex and superior temporal gyrus are associated with a smoking cessation treatment outcome using varenicline revealed by regional homogeneity. *Brain Imaging and Behavior* 2017;11(3):611–18.
- Ware JJ, Aveyard P, Broderick P, Houlston RS, Eisen T, Munafò MR. The association of rs1051730 genotype on adherence to and consumption of prescribed nicotine replacement therapy dose during a smoking cessation attempt. *Drug and Alcohol Dependence* 2015;151:236–40.
- Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNA4 variation and smoking behaviors and lung cancer risk. *Journal of the National Cancer Institute* 2011;103(17):1342–6.
- Wessel J, McDonald SM, Hinds DA, Stokowski RP, Javitz HS, Kennemer M, Krasnow R, Dirks W, Hardin J, Pitts SJ, et al. Resequencing of nicotinic acetylcholine receptor genes and association of common and rare variants with the Fagerstrom test for nicotine dependence. *Neuropsychopharmacology* 2010;35(12):2392–402.
- Weywadt CR, Kiehl KA, Claus ED. Neural correlates of response inhibition in current and former smokers. *Behavioural Brain Research* 2017;319:207–18.
- Wickham R, Solecki W, Rathbun L, McIntosh JM, Addy NA. Ventral tegmental area alpha6beta2 nicotinic acetylcholine receptors modulate phasic dopamine release in the nucleus accumbens core. *Psychopharmacology* 2013;229(1):73–82.
- Wilson SJ, Creswell KG, Sayette MA, Fiez JA. Ambivalence about smoking and cue-elicited neural activity in quitting-motivated smokers faced with an opportunity to smoke. *Addictive Behaviors* 2013;38(2):1541–9.

- Wilson SJ, Sayette MA, Fiez JA. Quitting-unmotivated and quitting-motivated cigarette smokers exhibit different patterns of cue-elicited brain activation when anticipating an opportunity to smoke. *Journal of Abnormal Psychology* 2012;121(1):198–211.
- Wu P, Wilson K, Dimoulas P, Mills EJ. Effectiveness of smoking cessation therapies: a systematic review and meta-analysis. *BMC Public Health* 2006;6:300.
- Xian H, Scherrer JF, Madden PA, Lyons MJ, Tsuang M, True WR, Eisen SA. The heritability of failed smoking cessation and nicotine withdrawal in twins who smoked and attempted to quit. *Nicotine and Tobacco Research* 2003;5(2):245–54.
- Xu J, Azizian A, Monterosso J, Domier CP, Brody AL, Fong TW, London ED. Gender effects on mood and cigarette craving during early abstinence and resumption of smoking. *Nicotine and Tobacco Research* 2008;10(11):1653–61.
- Xu X, Clark US, David SP, Mulligan RC, Knopik VS, McGeary J, MacKillop J, McCaffery J, Niaura RS, Sweet LH. Effects of nicotine deprivation and replacement on BOLD-fMRI response to smoking cues as a function of DRD4 VNTR genotype. *Nicotine and Tobacco Research* 2014;16(7):939–47.
- Yamada H, Bruijnzeel AW. Stimulation of alpha2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. *Neuropharmacology* 2011;60(2–3):303–11.
- Yang H, de Jong JW, Tak Y, Peck J, Bateup HS, Lammel S. Nucleus accumbens subnuclei regulate motivated behavior via direct inhibition and disinhibition of VTA dopamine subpopulations. *Neuron* 2018;97(2):434–49.e4.
- Yoon HJ, Lim YJ, Zuo Z, Hur W, Do SH. Nicotine decreases the activity of glutamate transporter type 3. *Toxicology Letters* 2014;225(1):147–52.
- Yudkin P, Munafò M, Hey K, Roberts S, Welch S, Johnstone E, Murphy M, Griffiths S, Walton R. Effectiveness of nicotine patches in relation to genotype in women versus men: randomised controlled trial. *BMJ* 2004;328(7446):989–90.
- Zanchi D, Brody A, Borgwardt S, Haller S. Sex effects on smoking cue perception in non-smokers, smokers, and ex-smokers: a pilot study. *Frontiers in Psychiatry* 2016;7:187.
- Zanchi D, Brody AL, Montandon ML, Kopel R, Emmert K, Preti MG, Van De Ville D, Haller S. Cigarette smoking leads to persistent and dose-dependent alterations of brain activity and connectivity in anterior insula and anterior cingulate. *Addiction Biology* 2015;20(6):1033–41.
- Zelle SL, Gates KM, Fiez JA, Sayette MA, Wilson SJ. The first day is always the hardest: functional connectivity during cue exposure and the ability to resist smoking in the initial hours of a quit attempt. *Neuroimage* 2017;151:24–32.
- Zhang X, Salmeron BJ, Ross TJ, Gu H, Geng X, Yang Y, Stein EA. Anatomical differences and network characteristics underlying smoking cue reactivity. *Neuroimage* 2011;54(1):131–41.
- Zhao-Shea R, DeGroot SR, Liu L, Vallaster M, Pang X, Su Q, Gao G, Rando OJ, Martin GE, George O, et al. Increased CRF signalling in a ventral tegmental area-interpeduncular nucleus-medial habenula circuit induces anxiety during nicotine withdrawal. *Nature Communications* 2015;6:6770.
- Zhou WL, Gao XB, Picciotto MR. Acetylcholine acts through nicotinic receptors to enhance the firing rate of a subset of hypocretin neurons in the mouse hypothalamus through distinct presynaptic and postsynaptic mechanisms. *eNeuro* 2015;2(1):e0052.
- Zhu AZ, Zhou Q, Cox LS, David SP, Ahluwalia JS, Benowitz NL, Tyndale RF. Association of CHRNA5-A3-B4 SNP rs2036527 with smoking cessation therapy response in African-American smokers. *Clinical Pharmacology and Therapeutics* 2014;96(2):256–65.
- 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;53(8):958–66.