Review

Targeting the Noradrenergic System for Gender-Sensitive Medication Development for Tobacco Dependence

Terril L. Verplaetse PhD¹, Andrea H. Weinberger PhD², Philip H. Smith PhD¹, Kelly P. Cosgrove PhD^{1,3}, Yann S. Mineur PhD¹, Marina R. Picciotto PhD¹, Carolyn M. Mazure PhD¹, Sherry A. McKee PhD¹

¹Department of Psychiatry and Women's Health Research, Yale University School of Medicine, New Haven, CT;

²Ferkauf Graduate School of Psychology, Yeshiva University, Bronx, NY; ³Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT

Corresponding Author: Sherry A. McKee, PhD, Yale University School of Medicine, 2 Church St South, Suite 109, New Haven, CT 06519, USA. Telephone: 203-737-3529; Fax: 203-737-4243; E-mail: sherry.mckee@yale.edu

Abstract

Introduction: Tobacco use remains the leading cause of morbidity and mortality for both women and men in the United States, and women often experience poorer smoking cessation outcomes than men. Preliminary evidence suggests there are sex differences in medication effectiveness for smoking cessation. However, current medications do not take into account gender-sensitive treatment development and efficacy, underscoring the importance of this underdeveloped area of research.

Methods: We reviewed preclinical and clinical evidence for gender differences in the inability to quit smoking by examining (a) the effect of increased negative affect and stress reactivity on smoking outcomes in women and (b) smoking for nicotine reinforcement in men. We also reviewed the current literature targeting the noradrenergic system as a novel gender-sensitive treatment strategy for tobacco dependence.

Results: We hypothesize that noradrenergic agents that normalize noradrenergic activity may differentially attenuate stress reactivity in women and nicotine-related reinforcement in men, indicating that targeting the noradrenergic system for smoking cessation may be effective for both genders, with benefits operating through sex-specific mechanisms.

Conclusions: Converging lines of preclinical and clinical evidence suggest that gender-sensitive approaches to medication development for smoking cessation are a critical next step for addressing low quit rates and exacerbated health risks among women. Evidence reviewed indicates that smoking activates different brain systems modulated by noradrenergic activity in women versus men, and noradrenergic compounds may preferentially target these gender-sensitive systems.

Introduction

Tobacco use remains the leading cause of morbidity and mortality for both women and men in the United States, with 440,000 deaths per year attributable to smoking-related causes.¹ Compared to male smokers, female smokers appear to be at a greater risk of a number of

health-related consequences including lung cancer,^{2,3} oral cancer,³ and coronary heart disease.⁴ Women also experience sex-specific smoking consequences such as dysmenorrhea, early natural menopause, spontaneous abortion, and preterm delivery.⁵ Thus, women experience significant health-related disparities regarding tobacco use, underscoring the need to develop effective treatments for women smokers.

Smoking cessation can prevent and reduce many of the harmful consequences of smoking, and successful smoking cessation exerts greater cardiovascular⁶ and respiratory⁷ benefits for women compared to men. However, smokers often fail to maintain long-term abstinence. Seventy percent or more of smokers relapse within 1 year even with our most effective treatments.⁸ Despite being more likely to report a quit attempt than men,⁹ women are less successful in quitting smoking^{10,11} (see also Jarvis et al.¹²) and may be more likely to relapse to smoking after a period of abstinence.¹³ Further, some smoking cessation medications have demonstrated differential efficacy across gender. Women may be less responsive to nicotine replacement therapy (NRT) as a cessation aid,^{14,15} and findings for sex differences in bupropion efficacy have been mixed.¹⁶

In light of evidence for sex differences in medication efficacy, sex-sensitive treatments show promise for improving success rates for both women and men. Yet, current treatment guidelines for tobacco dependence¹⁷ provide little guidance for incorporating sex-specific factors into treatment decisions. Supported by the U.S. National Institutes of Health's Office of Research on Women's Health and the Federal Drug Administration's Office on Women's Health, there is a growing awareness, supported by federal regulation, to consider sex and gender in treatment development and efficacy. With regard to smoking cessation, sex-specific studies conducted thus far have focused on behavioral interventions related to weight gain^{18,19} or exercise.¹⁹ Pharmacological development targeted at gender-sensitive aspects of smoking behavior is a critical, yet underdeveloped area of research.

In the following sections, we review the preclinical and clinical evidence for gender differences in the inability to quit smoking examining (a) the effect of increased negative affect and stress reactivity on smoking outcomes in women and (b) smoking for nicotine reinforcement in men. We present preliminary evidence supporting our hypothesis that noradrenergic targets differentially attenuate stress reactivity in women and nicotine-related reinforcement in men, indicating that targeting the noradrenergic system for smoking cessation may be effective for both genders, with benefits operating through sex-specific mechanisms.

Smoking for Negative Affect Regulation

Negative affect and stress are associated with smoking initiation, maintenance, and relapse to cigarette smoking.^{20,21} While stress and negative affect are related to the smoking behavior of women and men, the role of affect is especially critical in women's smoking and the ability of women to maintain abstinence after a quit attempt. In a cohort of ~130,000 smokers, self-reported smoking to alleviate negative affect was a more important dimension of nicotine dependence for women than men, and this sex difference has increased in magnitude over the past 10 years.²²

Women smokers are more likely to experience greater levels of negative affect, including depression,²³ are more likely to expect that smoking will improve their mood,²⁴ and are more likely to report smoking to reduce and manage negative affect compared to men.^{10,25} In human laboratory studies, female smokers smoked more quickly following a negative mood induction compared to males,²⁶ and females with faster accessibility to negative affect reduction beliefs smoked more cigarettes, had longer puff durations, greater puff volume, and shorter inter-puff intervals compared to men²⁷ (see also Fucito and Juliano²⁸).

Our group and others have shown that stress and negative affect (including depression) appear to exert a greater negative impact

on the ability of women to quit smoking compared to men^{29,30} (see also Hitsman et al.³¹). Prior to a quit attempt, women anticipated greater negative affect associated with smoking cessation compared to men, which reduced their odds of successfully quitting by a factor of 4.³² Following a quit attempt, women experience greater increases in negative affect,³³ greater stress-induced distress and cravings to smoke,³⁴ and greater relief of abstinence-induced negative affect by smoking.³⁵ Females, compared to males, also have a higher propensity to relapse following stressful events.²⁹

Gender-sensitive relationships between negative affect and smoking also extend to smokers with depression. In a nationally representative study, both current and lifetime major depressive disorder were associated with a lower likelihood of quitting smoking and a higher probability of relapse.³⁶ As depression is twice as common in women compared to men, these associations had a greater impact on women smokers. In a systematic review of clinical trials examining smoking cessation in depressed smokers, depression had a greater negative impact on treatment outcomes for women than men,³⁷ although it is important to note that only 10% of these studies examined outcomes by gender.

In the preclinical literature, there is a well-established connection between the nicotinic acetylcholine receptor (nAChR) system and depression. Dysregulation of the cholinergic system has been associated with increased negative affect and depression,^{38,39} a key precipitant of smoking in women. Stimulation of the cholinergic system leads to an increase in anxiety-like behavior and depressive symptoms in normal individuals and those diagnosed with mood disorders.⁴⁰ Furthermore, preclinical evidence suggests that rats with increased sensitivity to cholinergic stimulation exhibit an increase in stress reactivity.⁴¹ Similarly, nicotine can produce an anxiogenic-like effect in female versus male mice, whereas females are less sensitive to the psychostimulant properties of nicotine, indicating that there may be sex differences in the activity of the cholinergic system which could contribute to the increased likelihood of smoking relapse in women following abstinence.⁴²

It is important to note that researchers have questioned whether smoking directly relieves stress and negative affect as anticipated by smokers, noting mixed findings in the literature.²⁰ The source of negative affect may be important—although smoking effectively reduces abstinence-induced negative affect,⁴³ its effects on environmentally induced negative affect (e.g., mood-induction procedures in laboratory settings) are less consistent.^{20,43} Regarding sex differences, as previously noted, women experience greater reduction of abstinence-induced negative affect when smoking.³⁵ Sex differences in smoking-related negative affect reduction in response to environmental stressors are not well studied, highlighting an important area for further investigation, as findings may have important clinical implications for smoking cessation.

Regardless of the source, negative affect and stress are clearly related to smoking maintenance and relapse, and these relationships appear stronger for women than for men. Taken together, animal models and human studies point to stress and negative affect as critically important targets for interventions aimed at reducing smoking behavior in women.

Smoking for Nicotine Reinforcement

While negative affect may play a greater role in the smoking behavior of women, nicotine reinforcement may play a more significant role in the smoking behavior of men. It has been suggested that nicotine replacement may not be as effective in women, as women

are less sensitive to the reinforcing effects of nicotine.¹⁵ Laboratory studies have identified that women, compared to men, report greater sensitivity to the negative subjective effects of nicotine (e.g., dizziness,^{44,45} see also Perkins et al.⁴⁶) and less sensitivity to the reinforcing effects of nicotine (e.g., satisfying, “want more”).^{46,47} Perkins and colleagues^{48,49} demonstrated that men are better able than women to discriminate between doses of nicotine, indicating that men are better able to detect the interoceptive cues of nicotine. Further, women, compared to men, report greater craving relief from denicotinized tobacco than men,⁵⁰ experience less of a change in subjective effects across doses of nicotine,⁴⁸ experience less reinforcement from nicotine in the absence of visual and olfactory stimuli,⁵¹ and report greater reward and reinforcement related to verbal information about nicotine content.⁵² Such findings provide supporting evidence for the diminished role that nicotine reinforcement is thought to have in maintaining tobacco use in women and the greater role that nicotine reinforcement is thought to have in men.⁴⁹

Nicotine's reinforcing effects are mediated by the β_2 subunit of the nAChR, leading to the release of dopamine in the ventral striatum and prefrontal cortex (PFC).⁵³ In animal models, deletion of the β_2 subunit-containing nAChRs abolishes reinforcement-related behaviors, including nicotine locomotor sensitization, nicotine self-administration, and conditioned place preference.^{54,55} Preclinical studies also generally support clinical findings showing differential sensitivity in female rodents in response to nicotine suggesting an underlying biological sexual dimorphism. For example, female mice respond more to the locomotor stimulating⁴² and conditioned rewarding properties of nicotine,⁵⁶ whereas male mice respond more to pharmacological properties of nicotine by titrating their consumption to available nicotine dose.⁵⁶ Currently, our group has focused on investigating mechanisms underlying these sex differences focusing on the regulatory effects of nicotine in the brain.⁵⁷⁻⁵⁹

Cosgrove and colleagues⁵⁸ completed a large study imaging availability of the β_2 subunit-containing nAChRs, which have been shown to be higher, or “upregulated” after nicotine administration or tobacco smoking. They found significantly higher numbers of nicotinic receptors in male smokers but not in female smokers when compared to same-sex nonsmokers. These findings provide a neurochemical explanation for why men generally have a preferential therapeutic response to smoking cessation medications which target nicotine receptors. Specifically, NRT may help “wean” their receptors down to nonsmoker levels over time. New technology examining dynamic changes in dopamine release during in vivo smoking has also demonstrated that males activate in response to smoking a cigarette consistently and rapidly in the ventral striatum whereas women respond faster than men in a discrete subregion of the dorsal putamen,⁶⁰ consistent with the established notion that men smoke for the reinforcing properties of nicotine, which are thought to originate in the ventral striatum.

Most medications that are FDA approved for the treatment of tobacco dependence target the nAChR system to some extent, and significant preclinical and clinical data demonstrate that women smokers are less sensitive to the effects of nicotine in tobacco smoke, making the nAChR system a less than optimal therapeutic target for women. In order to treat female smokers effectively, medication development strategies need to target systems other than the nicotinic acetylcholine system and factors that serve to maintain smoking behavior in women, and which underlie poor treatment response. While there are a number of complex factors which facilitate poor treatment response in women, targeting negative affect and stress reactivity is a highly promising avenue for gender-sensitive

medication development. Noradrenergic agents are known to attenuate stress reactivity and nicotine-related reinforcement, and targeting the noradrenergic system for smoking cessation may be effective for both genders, with benefits operating through different gender-sensitive mechanisms.

Targeting the Noradrenergic System to Reduce Stress Reactivity, Attenuate Nicotine Reinforcement, and Improve Smoking Cessation Outcomes

Brief Overview of the Noradrenergic System

The noradrenergic system is widely known to be involved in arousal, anxiety, and, more recently, addiction,⁶¹⁻⁶³ and norepinephrine is extensively distributed throughout the brain. The noradrenergic system is comprised of two main projections: the dorsal noradrenergic bundle (DNB) has cell bodies originating in the dorsal pons (locus coeruleus; LC) and the ventral noradrenergic bundle (VNB) has cell bodies that originate in the brain stem (pons and medulla). Projections from the LC, a nucleus comprised of entirely norepinephrine containing neurons, innervate the cortices and hippocampus and projections from the brain stem innervate the hypothalamus, basal forebrain, and amygdala.⁶³⁻⁶⁵

The noradrenergic receptor system is made up of three main receptor subtypes; α_1 (α_1), α_2 (α_2), and β (β). Alpha-1 adrenoceptors are primarily located postsynaptically⁶¹ in several brain regions, including the LC,⁶⁶ olfactory bulb, cerebral cortex, amygdala, dentate gyrus, and the thalamus,⁶⁷ and can be further subdivided into α_{1a} -, α_{1b} -, and α_{1d} -adrenoceptor subtypes. Alpha-2 adrenoceptors can be subdivided into α_{2a} -, α_{2b} -, and α_{2c} -adrenoceptor subtypes and exist both presynaptically and postsynaptically⁶¹ in the amygdala, LC, and hypothalamus.⁶⁸ Beta-adrenoceptors exist postsynaptically in brain regions previously mentioned,⁶¹ and this class of β -adrenoceptors include β_1 -, β_2 -, and β_3 -adrenoceptor subtypes. Antagonists at α_1 and β receptors reduce central adrenoceptor activity by blocking the binding of norepinephrine to postsynaptic receptors.⁶¹ Similarly, α_2 agonists stimulate inhibitory presynaptic α_2 receptors resulting in a reduction of noradrenergic firing.⁶⁹ The reduction in norepinephrine neurotransmission associated with these pharmacological agents has historically led to their use for the treatment of hypertension, attention-deficit/hyperactivity disorder (ADHD), and more recently, substance abuse.

Nicotine and the Noradrenergic System

Nicotine binds to nAChRs in the brain, and it is known that the cholinergic system interacts with the noradrenergic system. Nicotine administration or smoking can modulate the activity of the noradrenergic system and lead to an increase in peripheral sympathetic activity.^{70,71} In animal models, systemic administration of nicotine increases extracellular norepinephrine levels, and this effect is mediated by the LC, the main source of noradrenergic neurons in the brain.⁷² Nicotine exerts direct effects on the LC, such that nicotine increases the firing rate of LC neurons and stimulates the release of norepinephrine.⁷³ Chronic nicotine exposure in rats can also increase the activity of tyrosine hydroxylase, a precursor for the synthesis of dopamine and norepinephrine, in brain regions innervated by the noradrenergic system but not in dopamine projection areas.⁷⁴ Both acetylcholine and norepinephrine are key neurotransmitters involved in the regulation of the amygdala-PFC pathway, which is critical to stress reactivity and emotional control.⁷⁵

In humans, smoking increases plasma levels and urinary excretion of norepinephrine and epinephrine,^{71,76} and elevated baseline plasma epinephrine and norepinephrine levels seen in smokers decrease during abstinence, suggesting an increased noradrenergic activity as a result of smoking.⁷⁷ Long-term smoking has been associated with decreases in the density of the β -adrenergic receptors in blood cells and α_2 -adrenergic receptor binding in the LC, and this decrease in receptor density normalizes with abstinence to nonsmoker control levels.⁷⁸ In addition, nicotine-induced increases in blood pressure and heart rate^{71,76,79} are attenuated by adrenergic blockade.^{80,81} In a study of nine smokers, labetalol (an α_1 - and β -adrenergic blocker) attenuated nicotine-induced increases in heart rate and enhanced the decrease in symptoms associated with nicotine withdrawal after intravenous nicotine.⁸⁰ Labetalol also increased the subjective feelings of a "head rush" and "strength" of nicotine use in that study. Similarly, carvedilol (a mixed α_1 - and β -adrenergic antagonist) reduced heart rate, systolic and diastolic blood pressure, and subjective ratings of "bad effects" associated with nicotine in male and female smokers but did not affect tobacco withdrawal symptoms.⁸¹ Overall, nicotine induces an increase in noradrenergic activity, and drugs that block or normalize noradrenergic activation could be used as potential pharmacotherapeutic treatments for tobacco dependence.

Noradrenergic Targets Are Effective for Smoking Cessation

Clonidine, which reduces noradrenergic activity by stimulating inhibitory presynaptic α_2 receptors, blunts LC firing in rats undergoing opiate withdrawal and attenuates opiate withdrawal syndrome in opiate dependent human subjects, providing the initial basis for α_2 -adrenergic agonist treatment in drug addiction.⁶⁹ In regular smokers, clonidine has been shown to reduce tobacco craving and other tobacco withdrawal symptoms including anxiety, irritability, restlessness, and tension.⁸² A Cochrane review found that clonidine increased rates of smoking cessation by an odds ratio of 1.63,⁸³ and in studies that stratified by gender, clonidine was more effective in women than men for smoking cessation.⁸⁴ In a meta-analysis ($n = 813$), end-of-treatment quit rates in women were 70% for clonidine versus 18% for placebo. Men did not demonstrate an effect of medication with end of treatment quit rates of 42% for clonidine versus 43% for placebo.⁸⁵ Surprisingly, mechanisms underlying this gender difference in medication response were never pursued. Regarding stress, clonidine has been found to block stress-induced reinstatement of drug-seeking in rodents^{86,87} and reduce subjective measures of stress-induced cocaine craving in nontreatment seeking cocaine users.⁸⁸ While clonidine demonstrates efficacy for smoking cessation and alleviating withdrawal symptoms associated with nicotine dependence, significant adverse events, most notably sedation, have limited its use to a second line medication for smokers who are nonresponsive to other smoking cessation treatments.⁸³

Antidepressants have also shown some efficacy for the treatment of tobacco dependence. While most antidepressants antagonize nicotinic receptors (see Shytle et al.⁸⁹ for a review), it appears that antidepressants with actions on norepinephrine reuptake are particularly effective for smoking cessation. Studies of antidepressant medications for smoking cessation have found that bupropion (risk ratio [RR] = 1.69) and nortriptyline (RR = 2.03) demonstrate efficacy on par with NRT, but that serotonin reuptake inhibitors do not promote smoking cessation⁹⁰ (see also Hitsman et al.⁹¹). Bupropion's mechanism of action on smoking cessation is unclear. There is

evidence that it may increase the ability to quit through blockade of nAChR receptors⁹² but bupropion also modulates dopaminergic and noradrenergic activity. The mechanism of action for nortriptyline on smoking cessation is unknown, but it is interesting to note that nortriptyline reduces noradrenergic activity through antagonism of α_1 receptors. Bupropion has not demonstrated consistent effects across genders⁹⁰ and only two studies with nortriptyline have investigated smoking cessation outcomes by gender with both demonstrating similar efficacy in women and men.^{93,94} Tricyclic antidepressants have been found to block reward threshold elevations and somatic signs associated with nicotine withdrawal and to decrease nicotine self-administration in rodents.⁹⁵

Monoamine oxidase (MAO) inhibitors (MAOIs) have well-characterized antidepressant-like actions and have demonstrated clinical efficacy in treating depression (for a comprehensive review, see Shulman et al.⁹⁶). MAOs are enzymes involved in the regulation and catabolism of monoamine neurotransmitters, such as norepinephrine, dopamine, and serotonin.^{97,98} Two isoenzymes of MAO are well-characterized in mammals: MAO-A and MAO-B.⁹⁷ The MAO-A subtype preferentially metabolizes norepinephrine and serotonin,⁹⁷ and MAO-A methylation has been found to be associated with nicotine dependence in women, but not men.⁹⁹ Indeed, MAOIs have been utilized as a potential pharmacotherapy for the treatment of tobacco dependence, albeit with limited efficacy. Clinical studies have found MAO-A inhibitors to be effective in reducing self-reported abstinence rates, but not abstinence rates verified by plasma cotinine levels or measures of cigarette craving.¹⁰⁰ Likewise, initial studies using MAO-B inhibitors such as selegiline, which produces inhibition of MAO-A at higher doses, exhibited efficacy in reducing self-reported craving for cigarettes¹⁰¹ and achieving biochemically verified abstinence rates,¹⁰² but subsequent studies yielded negative results with regard to the effectiveness of selegiline for tobacco dependence.^{103,104} Further, a selective MAO-B inhibitor was not effective in smoking cessation as measured by end-of-treatment 4-week continuous abstinence rates,¹⁰⁵ suggesting poor efficacy of MAOIs as a treatment option for tobacco dependence.

Norepinephrine reuptake inhibitors reduce abstinence-induced subjective withdrawal symptoms and decrease self-reported smoking urges in nontreatment seeking smokers.¹⁰⁶ This same class of drugs has also been found to decrease the number of cigarettes smoked in nicotine-dependent individuals, although side effects due to treatment resulted in high drop-out rates.¹⁰⁷ High drop-out rates also lead to inconclusive findings of milnacipran, a serotonin-norepinephrine reuptake inhibitor (SNRI), in combination with the nicotine patch on smoking abstinence, but this combination did improve mood and anxiety measures in tobacco-dependent individuals with a history of major depressive disorder.¹⁰⁸ Another SNRI, venlafaxine, is not effective for smoking cessation,⁹⁰ so increasing norepinephrine and serotonin levels in combination may not be an effective strategy.

Medications targeting the noradrenergic system may be useful for the treatment of tobacco dependence. In the following sections, we discuss potential gender-sensitive mechanisms for these effects.

Targeting the Noradrenergic System to Attenuate Stress Reactivity

Stress is defined as a state of threatened homeostasis, which is counteracted by adaptive processes involving physiological and behavioral responses in an attempt to regain homeostasis.¹⁰⁹ This state of threatened homeostasis often refers to hyperarousal and the overactivation of the body's normal processes.¹¹⁰ The array of stress responses is

coordinated by the release of corticotrophin-releasing factor (CRF), and subsequent activation of the hypothalamic–pituitary–adrenal (HPA) axis and the catecholaminergic system (norepinephrine, epinephrine, and dopamine pathways) in response to stress.¹¹¹ In addition, several other neurotransmitter systems such as the serotonin and opioid system, the neuroactive steroids and inhibitory/excitatory amino acid (GABA and glutamate) system, thyroid hormones, and the immune system contribute to the adaptive response to stress (for a comprehensive review, see Tsigos and Chrousos¹¹²). Despite this generalized activation, it is the specific ability of stressors to reliably activate the CRF-HPA axis and catecholamines, including the meso-corticolimbic dopaminergic system, that has been linked to stress-induced increases in self-administration of drugs of abuse.^{110,113} Relevant to this review, in nicotine-exposed animals and humans, acute stress additively increases HPA, catecholamine, and sympathetic responses.^{70,114}

The PFC-amygdala axis is critical for the regulation of emotions and stress reactivity, and noradrenergic projections play a role in the regulation of PFC and amygdala activity. Exposure to even mild uncontrollable stress markedly impairs PFC-dependent cognitive function.¹¹⁵ In response to psychological stress, amygdala projections activate noradrenergic neurons in the LC and the dopaminergic neurons in the ventral tegmental area (VTA) resulting in increased release of norepinephrine and dopamine throughout the forebrain. High levels of norepinephrine and dopamine release in the PFC markedly impair PFC-mediated cognitive functions, while high levels of catecholamine release in subcortical structures such as the amygdala and nucleus accumbens strengthen affective associations and habitual responses,¹¹⁶ such as those underlying substance use.

Preclinical research demonstrates that exposure to acute stress induces relapse to drug-seeking in both nondependent and drug-dependent laboratory animals, particularly in reinstatement paradigms, a phenomenon mediated by brain stress circuits including noradrenergic pathways.^{87,117} Reinstatement paradigms use stress to elicit responding for a drug following extinction and are used to model relapse and craving. Footshock stress has been shown to effectively reinstate nicotine-seeking behaviors in rats.¹¹⁸ Acute challenge with yohimbine, an α_2 -adrenergic antagonist and pharmacological stressor that provokes the norepinephrine system by transiently increasing noradrenergic firing, has demonstrated robust reinstatement of drug-seeking behavior following extinction.¹¹⁹ Conversely, agents that blunt or normalize noradrenergic signaling can block stress-induced reinstatement to drug-seeking. Zislis et al.¹²⁰ demonstrated that clonidine attenuated footshock-induced reinstatement of nicotine-seeking behavior. Consistent with this finding, Yamada and Bruijnzeel¹²¹ demonstrated that stimulation of α_2 -adrenergic receptors with clonidine or dexmedetomidine infused into the central nucleus of the amygdala attenuated stress-induced reinstatement of nicotine seeking. Likewise, α_1 -adrenergic antagonists have been found to block reinstatement of nicotine-seeking by a nicotine prime or a nicotine cue.¹²²

Clinical research also indicates that stress exposure induces craving and relapse to drug taking. Human laboratory studies suggest that stress-induced craving states are associated with increases in cardiovascular activity and dysregulated HPA axis function.^{123,124} In smokers, stress precipitated smoking lapse and increased HPA axis reactivity, and tobacco craving. Moreover, increased cortisol, adrenocorticotrophic hormone (ACTH), and tobacco craving were associated with increased smoking lapse following stress.¹²⁴ In a subsequent study, these stress-related effects on smoking were eliminated in subjects treated with guanfacine, an α_{2a} -adrenergic

agonist.¹²⁵ Specifically, guanfacine attenuated the effect of stress on smoking lapse, ad-lib smoking, tobacco craving, and normalized cortisol responding. Consistent with these findings, noradrenergic targets have shown efficacy in attenuating stress reactivity in other drug addicted populations.

Importantly, α_2 -adrenergic agonists stimulate presynaptic inhibitory autoreceptors and consequently inhibit norepinephrine release, and this action has been found to improve attention, working memory, and inhibitory control in non-human primates.¹²⁶ Specifically, guanfacine reduces excitatory postsynaptic neurotransmission in the medial PFC and bed nucleus of the stria terminalis in rats,¹²⁷ brain areas associated with stress exposure. Provocation of the noradrenergic system elicits a stress response in humans and animals and has been found to increase distractibility and impulsiveness in rodents. Animal studies have also shown α_2 -adrenergic agonists to have anxiolytic-like actions on stress-related behavior associated with mesolimbic prefrontal dopamine turnover,¹²⁸ and improvement of connectivity in the PFC during periods of stress.¹²⁶ Furthermore, several studies have shown that guanfacine is associated with significant improvement in attention, hyperactivity/impulsivity, and frontal executive functioning tasks in children with ADHD and tic disorders^{129,130} as well as in adults with ADHD.¹³¹ Interestingly, our group has recently shown that guanfacine relative to placebo increases prefrontal activation to incongruent stimuli in a Stroop task during functional magnetic resonance imaging sessions in brain areas relevant to attention to inhibitory control.¹²⁵ These data suggest that effects of α_2 -adrenergic agonists on PFC function may underlie decreases in stress-induced relapse to smoking.

Our research group has also found that guanfacine appears to preferentially act through gender-sensitive systems for smoking cessation. We have found that guanfacine significantly and equally reduced the number of cigarettes per day following a quit attempt for both men and women over a 4-week proof-of-concept treatment period. However, when examining mechanisms for these treatment effects, guanfacine decreased smoking lapse, reduced cigarettes smoked, and reduced tobacco craving following stress in women but not men.¹³² Consistent gender findings have been found in cocaine users, where α_2 -adrenergic agonist treatment decreased cocaine and alcohol craving, anxiety, and negative mood following stress imagery in cocaine-dependent women but not men.¹³³

Overall, preclinical and clinical findings indicate that women are generally more likely to smoke cigarettes to alleviate negative affect and reduce stress compared to men. Moreover, women are less likely to succeed in a quit attempt because of perceived stress and anxiety associated with quitting smoking. Medications targeting the noradrenergic system, specifically a reduction in noradrenergic signaling, have been found to reduce stress-induced relapse in animal models and attenuate stress-induced craving in humans. Our preliminary data suggest that these treatment effects appear to be more pronounced in women compared to men, supporting gender-sensitive therapeutic development targeting the relationship between stress and smoking.

Targeting the Noradrenergic System to Attenuate Nicotine Reinforcement

Drugs such as nicotine stimulate dopamine neurons in the mesolimbic pathway, a critical brain pathway underlying the rewarding effects of drugs of abuse. In particular, nicotine stimulates the VTA of the brain which leads to dopamine release in the ventral striatum, also called the nucleus accumbens, and this is critically tied

to the pleasurable effects experienced from the drug. As previously mentioned, the primary reinforcing effects of nicotine are mediated by nAChRs containing the β_2 subunit,⁵³ which are necessary for nicotine to stimulate dopamine release in the ventral striatum and PFC. The mesolimbic dopamine system is modulated by norepinephrine,^{63,65,134} and noradrenergic neurons provide excitatory input to mesolimbic dopamine neurons.⁶³ Burst firing of VTA dopamine neurons is increased by stimulation of the noradrenergic cell bodies in the LC, however, attenuating norepinephrine signaling with noradrenergic agents eliminates and normalizes the firing of VTA neurons.^{135,136}

Consistent with the notion that the noradrenergic system modulates the mesolimbic dopamine system, there is extensive evidence that noradrenergic transmission is involved in reinforcement and the rewarding properties of drugs of abuse.⁶³ The administration of amphetamine has been shown to increase the release and prevent the uptake of catecholamines in adrenergic neurons,¹³⁷ and amphetamine-induced dopamine release in the nucleus accumbens is abolished in α_{1b} -adrenergic receptor knockout mice and in dopamine beta-hydroxylase (DBH) knockout mice lacking norepinephrine.^{138,139} High rates of intracranial self-stimulation (ICSS) are obtained in the medial forebrain bundle (MFB) of the lateral hypothalamus, an area that coincides with major rostral projections of noradrenergic fibers,¹⁴⁰ as well as in the LC and throughout the DNB and VNB pathways,¹⁴¹ and drugs of abuse exert mechanistic actions that summate with rewarding stimulation of the MFB.¹⁴² Conversely, compounds that deplete brain norepinephrine disrupt ICSS.¹⁴³

With regard to the noradrenergic system's involvement in the reinforcing properties of drugs of abuse, depletion of norepinephrine¹⁴⁴ and neurochemical lesions of noradrenergic neurons¹⁴⁵ can suppress voluntary ethanol self-administration in rats. Similarly, DBH knockout mice that cannot synthesize norepinephrine exhibit a reduced preference for ethanol.¹⁴⁶ Noradrenergic agents that block norepinephrine signaling decrease nicotine self-administration and reduce nicotine-evoked dopamine release in the nucleus accumbens in rodents.^{122,147} Similarly, agents that decrease norepinephrine release also reduce conditioned place preference to nicotine¹⁴⁸ and attenuate nicotine withdrawal-induced deficits in brain reward thresholds.¹⁴⁹

Very few studies have examined clinical outcomes for gender-sensitive treatments targeting nicotine reinforcement. Preliminary findings from our group demonstrate that guanfacine reduces smoking-related reinforcement in men but not women in an ad-lib smoking session, and that reductions in smoking-related reinforcement are associated with an increased ability to resist smoking in men, but not women.¹²⁵ These findings are consistent with studies showing that α_2 -adrenergic agonists modulate dopamine neurotransmission in the PFC. For example, guanfacine and clonidine attenuated phencyclidine (PCP)-induced dopamine release in the rat PFC,¹⁵⁰ and clonidine decreased extracellular dopamine in the rabbit caudate nucleus.¹⁵¹

The noradrenergic system plays a key role in mediating drug-motivated behaviors in rodents and in humans, and the reduction of noradrenergic signaling is able to block the reinforcing properties of nicotine, as well as other drugs of abuse. While extensive preclinical literature exists on noradrenergic contributions to nicotine reinforcement, research in clinical populations have been limited. Preliminary data in humans suggests that noradrenergic treatment approaches are able to target gender-sensitive mechanisms underlying tobacco dependence in men smoking for nicotine reinforcement.

Conclusions

Converging lines of preclinical and clinical evidence indicate that gender-sensitive approaches to medication development are needed to address the critical health disparities in cessation rates and exacerbated health risks that female smokers face. Most FDA-approved medications for smoking cessation target the nicotinic/cholinergic system to some extent (nicotine derivatives, nicotinic partial agonists, or compounds with known affinity for nAChRs) and may not directly target factors which maintain smoking behavior in women. The considerable body of data suggesting that women are generally more likely to smoke to regulate negative affect and stress, while men are generally more likely to smoke for the reinforcing properties of nicotine, suggests an important direction in the development of smoking cessation treatments. Substantial preclinical evidence demonstrates that noradrenergic transmission is involved in stress-induced relapse and nicotine-related reinforcement, yet there is a surprising lack of clinical investigations translating these findings to humans. Early work with clonidine demonstrated efficacy for smoking cessation with preferential treatment outcomes for women, which have never been pursued, and recent preliminary evidence suggests that noradrenergic agents, in particular guanfacine, may improve the poorer treatment outcomes seen in women. We hypothesize that smoking activates different brain systems modulated by noradrenergic activity in women (PFC-amygdala axis) and in men (mesolimbic dopamine system), and noradrenergic compounds can preferentially target these gender-sensitive systems. Continued work will determine whether the noradrenergic system holds promise for the identification of new neurobiological targets and the repurposing of existing targets for gender-sensitive therapeutic development.

Funding

This work was funded by National Institutes of Health P50DA033945 (Office of Research on Women's Health [ORWH], National Institute of Drug Abuse [NIDA], FDA Office of Women's Health to SAM); MH077681 (National Institute of Mental Health to MRP and YSM); K02DA031750 (NIDA to KPC); K12DA031050 (ORWH, NIDA, National Institute of Alcohol Abuse and Alcoholism [NIAAA] to CMM and PHS); T32AA015496 (NIAAA to TLV).

Declaration of Interests

None declared.

References

- Centers for Disease Control Prevention. Smoking-attributable mortality, years of potential life lost, and productivity losses--United States, 2000--2004. *Morb Mortal Wkly Rep*. 2008;57:1226.
- Ceribelli A, Pino MS, Cecere FL. Gender differences: implications for clinical trials and practice. *J Thorac Oncol*. 2007;2:S15-S18.
- Kiyohara C, Ohno Y. Sex differences in lung cancer susceptibility: a review. *Gend Med*. 2010;7:381-401.
- Huxley RR, Woodward M. Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. *Lancet*. 2011;378:1297-1305.
- USDHHS. *The Health Consequences of Smoking—50 Years of Progress: 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; 2014. <http://www.legacyforhealth.org/content/download/4428/62627/file/Abrams.SurGenReport.50thAnniv.2.5.14.FIN.pdf>. Accessed November 4, 2014.

6. Mercurio G, Deidda M, Piras A, Dessalvi CC, Maffei S, Rosano GM. Gender determinants of cardiovascular risk factors and diseases. *J Cardiovasc Med*. 2010;11:207–220.
7. Rahmanian SD, Diaz PT, Wewers ME. Tobacco use and cessation among women: research and treatment-related issues. *J Womens Health*. 2011;20:349–357.
8. Jorenby DE, Hays JT, Rigotti NA, et al. Efficacy of varenicline, an alpha-4beta2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA*. 2006;296:56–63.
9. Shiffman S, Brockwell SE, Pillitteri JL, Gitchell JG. Use of smoking-cessation treatments in the United States. *Am J Prev Med*. 2008;34:102–111.
10. Wetter DW, Kenford SL, Smith SS, Fiore MC, Jorenby DE, Baker TB. Gender differences in smoking cessation. *J Consult Clin Psychol*. 1999;67:555.
11. Smith PH, Kasza K, Hyland A, et al. Gender differences in medication use and cigarette smoking cessation: results from the International Tobacco Control Four Country Survey. *Nicotine Tob Res*. In press.
12. Jarvis MJ, Cohen JE, Delnevo CD, Giovino GA. Dispelling myths about gender differences in smoking cessation: population data from the USA, Canada and Britain. *Tob Control*. 2013;22:356–360.
13. Weinberger AH, Pilver CE, Mazure CM, McKee SA. Stability of smoking status in the US population: a longitudinal investigation. *Addiction*. 2014;109:1541–1553.
14. Weinberger AH, Smith PH, Kaufman M, McKee SA. Consideration of sex in clinical trials of transdermal nicotine patch: a systematic review. 2014;22:373–383.
15. Perkins KA, Scott J. Sex differences in long-term smoking cessation rates due to nicotine patch. *Nicotine Tob Res*. 2008;10:1245–1251.
16. Scharf D, Shiffman S. Are there gender differences in smoking cessation, with and without bupropion? Pooled- and meta-analyses of clinical trials of Bupropion SR. *Addiction*. 2004;99:1462–1469.
17. Fiore MC, Jaen CR, Baker TB, et al. A clinical practice guideline for treating tobacco use and dependence: 2008 update. A US Public Health Service report. *Am J Prev Med*. 2008;35:158–176.
18. Copeland AL, Martin PD, Geiselman PJ, Rash CJ, Kendzor DE. Smoking cessation for weight-concerned women: group vs. individually tailored, dietary, and weight-control follow-up sessions. *Addict Behav*. 2006;31:115–127.
19. Spring B, Howe D, Berendsen M, et al. Behavioral intervention to promote smoking cessation and prevent weight gain: a systematic review and meta-analysis. *Addiction*. 2009;104:1472–1486.
20. Kassel JD, Stroud LR, Paronis CA. Smoking, stress, and negative affect: correlation, causation, and context across stages of smoking. *Psychol Bull*. 2003;129:270.
21. Kenford SL, Smith SS, Wetter DW, Jorenby DE, Fiore MC, Baker TB. Predicting relapse back to smoking: contrasting affective and physical models of dependence. *J Consult Clin Psychol*. 2002;70:216–227.
22. Smith PH, Rose JS, Mazure CM, Giovino GA, McKee SA. What is the evidence for hardening in the cigarette smoking population? Trends in nicotine dependence in the US, 2002–2012. *Drug Alcohol Depend*. 2014;142:333–340.
23. Husky MM, Mazure CM, Paliwal P, McKee SA. Gender differences in the comorbidity of smoking behavior and major depression. *Drug Alcohol Depend*. 2008;93:176–179.
24. Brandon TH, Baker TB. The smoking consequences questionnaire: the subjective expected utility of smoking in college students. *Psychol Assessment*. 1991;3:484.
25. Westmaas JL, Langsam K. Unaided smoking cessation and predictors of failure to quit in a community sample: effects of gender. *Addict Behav*. 2005;30:1405–1424.
26. Weinberger AH, McKee SA. Gender differences in smoking following an implicit mood induction. *Nicotine Tob Res*. 2012;14:621–625.
27. Weinberger AH, McKee SA. Mood and smoking behavior: the role of expectancy accessibility and gender. *Addict Behav*. 2012;37:1349–1352.
28. Fucito LM, Juliano LM. Depression moderates smoking behavior in response to a sad mood induction. *Psychol Addict Behav*. 2009;23:546.
29. McKee SA, Maciejewski PK, Falba T, Mazure CM. Sex differences in the effects of stressful life events on changes in smoking status. *Addiction*. 2003;98:847–855.
30. Schnoll RA, Patterson F, Lerman C. Treating tobacco dependence in women. *J Womens Health*. 2007;16:1211–1218.
31. Hitsman B, Borrelli B, McChargue DE, Spring B, Niaura R. History of depression and smoking cessation outcome: a meta-analysis. *J Consult Clin Psychol*. 2003;71:657.
32. McKee SA, O'Malley SS, Salovey P, Krishnan-Sarin S, Mazure CM. Perceived risks and benefits of smoking cessation: gender-specific predictors of motivation and treatment outcome. *Addict Behav*. 2005;30:423–435.
33. Leventhal AM, Waters AJ, Boyd S, Moolchan ET, Lerman C, Pickworth WB. Gender differences in acute tobacco withdrawal: effects on subjective, cognitive, and physiological measures. *Exp Clin Psychopharmacol*. 2007;15:21.
34. al'Absi M. Hypothalamic-pituitary-adrenocortical responses to psychological stress and risk for smoking relapse. *Int J Psychophysiol*. 2006;59:218–227.
35. Xu J, Azizian A, Monterosso J, et al. Gender effects on mood and cigarette craving during early abstinence and resumption of smoking. *Nicotine Tob Res*. 2008;10:1653–1661.
36. Weinberger AH, Pilver CE, Desai RA, Mazure CM, McKee SA. The relationship of major depressive disorder and gender to changes in smoking for current and former smokers: longitudinal evaluation in the US population. *Addiction*. 2012;107:1847–1856.
37. Weinberger AH, Mazure CM, Morlett A, McKee SA. Two decades of smoking cessation treatment research on smokers with depression: 1990–2010. *Nicotine Tob Res*. 2013;15:1014–1031.
38. Mineur YS, Picciotto MR. Nicotine receptors and depression: revisiting and revising the cholinergic hypothesis. *Trends Pharmacol Sci*. 2010;31:580–586.
39. Mineur Y, Obayemi A, Wigstrand MB, et al. Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. *Proc Natl Acad Sci U S A*. 2013;110:3573–3578.
40. Janowsky DS, Overstreet DH. Cholinergic dysfunction in depression. *Pharmacol Toxicol*. 1990;66(suppl 3):100–111.
41. Overstreet DH. The Flinders sensitive line rats: a genetic animal model of depression. *Neurosci Biobehav Rev*. 1993;17:51–68.
42. Caldarone BJ, King SL, Picciotto MR. Sex differences in anxiety-like behavior and locomotor activity following chronic nicotine exposure in mice. *Neurosci Lett*. 2008;439:187–191.
43. Perkins KA, Karelitz JL, Conklin CA, Sayette MA, Giedgowd GE. Acute negative affect relief from smoking depends on the affect situation and measure but not on nicotine. *Biol Psychiatry*. 2010;67:707–714.
44. Evans SE, Blank M, Sams C, Weaver MF, Eissenberg T. Transdermal nicotine-induced tobacco abstinence symptom suppression: nicotine dose and smokers' gender. *Exp Clin Psychopharmacol*. 2006;14:121.
45. Sofuoglu M, Mooney M. Subjective responses to intravenous nicotine: greater sensitivity in women than in men. *Exp Clin Psychopharmacol*. 2009;17:63.
46. Perkins KA, Coddington SB, Karelitz JL, et al. Variability in initial nicotine sensitivity due to sex, history of other drug use, and parental smoking. *Drug Alcohol Depend*. 2009;99:47–57.
47. Perkins KA. Smoking cessation in women. *CNS Drugs*. 2001;15:391–411.
48. Perkins KA, Jacobs L, Sanders M, Caggiula AR. Sex differences in the subjective and reinforcing effects of cigarette nicotine dose. *Psychopharmacology*. 2002;163:194–201.
49. Perkins KA, Donny E, Caggiula AR. Sex differences in nicotine effects and self-administration: review of human and animal evidence. *Nicotine Tob Res*. 1999;1:301–315.
50. Barrett SP. The effects of nicotine, denicotinized tobacco, and nicotine-containing tobacco on cigarette craving, withdrawal, and self-administration in male and female smokers. *Behav Pharmacol*. 2010;21:144–152.
51. 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 Tob Res*. 2001;3:141–150.
52. Perkins KA, Doyle T, Ciccocioppo M, Conklin C, Sayette M, Caggiula A. Sex differences in the influence of nicotine dose instructions on the reinforcing and self-reported rewarding effects of smoking. *Psychopharmacology*. 2006;184:600–607.
53. Picciotto MR, Zoli M, Rimondini R, et al. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998;391:173–177.

54. Besson M, David V, Suarez S, et al. Genetic dissociation of two behaviors associated with nicotine addiction: beta-2 containing nicotinic receptors are involved in nicotine reinforcement but not in withdrawal syndrome. *Psychopharmacology*. 2006;187:189–199.
55. Mineur YS, Brunzell DH, Grady SR, et al. Localized low-level re-expression of high-affinity mesolimbic nicotinic acetylcholine receptors restores nicotine-induced locomotion but not place conditioning. *Genes Brain Behav*. 2009;8:257–266.
56. Isiegas C, Mague SD, Blendy JA. Sex differences in response to nicotine in C57Bl/6: 129SvEv mice. *Nicotine Tob Res*. 2009;11:851–858.
57. Cosgrove KP, Mitsis EM, Bois F, et al. 123I-5-IA-85380 SPECT imaging of nicotinic acetylcholine receptor availability in nonsmokers: effects of sex and menstrual phase. *J Nucl Med*. 2007;48:1633–1640.
58. Cosgrove KP, Esterlis I, McKee SA, et al. Sex differences in availability of $\beta 2^*$ -nicotinic acetylcholine receptors in recently abstinent tobacco smokers. *Arch Gen Psychiatry*. 2012;69:418–427.
59. Esterlis I, McKee SA, Kirk K, et al. Sex-specific differences in GABA(A)-benzodiazepine receptor availability: relationship with sensitivity to pain and tobacco smoking craving. *Addict Biol*. 2013;18:370–378.
60. Cosgrove KP, Wang S, Kim S, et al. Sex differences in the brain's dopamine signature of cigarette smoking. *J Neurosci*. 2014;34:16851–16855.
61. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Rev*. 2003;42:33–84.
62. Levine ES, Joseph Litto W, Jacobs BL. Activity of cat locus coeruleus noradrenergic neurons during the defense reaction. *Brain Res*. 1990;531:189–195.
63. Weinshenker D, Schroeder JP. There and back again: a tale of norepinephrine and drug addiction. *Neuropsychopharmacology*. 2006;32:1433–1451.
64. Koob GF. A role for brain stress systems in addiction. *Neuron*. 2008;59:11–34.
65. Moore R, Bloom F. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu Rev Neurosci*. 1979;2:113–168.
66. Unerstall JR. Localizing the alpha-1 adrenergic receptor in the central nervous system. In: Ruffolo RR, ed. *The Alpha-1 Adrenergic Receptors*. Clifton, NJ: Humana; 1987:71–109.
67. Pieribone VA, Nicholas AP, Dagerlind A, Hökfelt T. Distribution of ~ 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. *J Neurosci*. 1994;14:4252–4252.
68. Tanaka M, Yoshida M, Emoto H, Ishii H. Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. *Eur J Pharmacol*. 2000;405:397–406.
69. Aghajanian G. Central noradrenergic neurons: a locus for the functional interplay between alpha-2 adrenoceptors and opiate receptors. *J Clin Psychiatr*. 1982;43:20–24.
70. Morse DE. Neuroendocrine responses to nicotine and stress: enhancement of peripheral stress responses by the administration of nicotine. *Psychopharmacology*. 1989;98:539–543.
71. Grassi G, Seravalle G, Calhoun DA, et al. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation*. 1994;90:248–253.
72. Mitchell S. Role of the locus coeruleus in the noradrenergic response to a systemic administration of nicotine. *Neuropharmacology*. 1993;32:937–949.
73. Engberg G, Svensson T. Pharmacological analysis of a cholinergic receptor mediated regulation of brain norepinephrine neurons. *J Neural Transm*. 1980;49:137–150.
74. Smith MA, Brady LS, Glowa J, Gold PW, Herkenham M. Effects of stress and adrenalectomy on tyrosine hydroxylase mRNA levels in the locus coeruleus by in situ hybridization. *Brain Res*. 1991;544:26–32.
75. Lin LC, Lewis DA, Sibille E. A human-mouse conserved sex bias in amygdala gene expression related to circadian clock and energy metabolism. *Mol Brain*. 2011;4:1–6.
76. Benowitz NL, Gourlay SG. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. *J Am Coll Cardiol*. 1997;29:1422–1431.
77. West R, Russell M, Jarvis M, Pizzey T, Kadam B. Urinary adrenaline concentrations during 10 days of smoking abstinence. *Psychopharmacology*. 1984;84:141–142.
78. Klimek V, Zhu MY, Dilley G, et al. Effects of long-term cigarette smoking on the human locus coeruleus. *Arch Gen Psychiatry*. 2001;58:821–827.
79. Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N Engl J Med*. 1976;295:573–577.
80. Sofuoglu M, Babb D, Hatsukami DK. Labetalol treatment enhances the attenuation of tobacco withdrawal symptoms by nicotine in abstinent smokers. *Nicotine Tob Res*. 2003;5:947–953.
81. Sofuoglu M, Mouratidis M, Yoo S, Kosten T. Adrenergic blocker carvedilol attenuates the cardiovascular and aversive effects of nicotine in abstinent smokers. *Behav Pharmacol*. 2006;17:731–735.
82. Glassman AH, Jackson WK, Walsh BT, Roose SP, Rosenfeld B. Cigarette craving, smoking withdrawal, and clonidine. *Science*. 1984;226:864–866.
83. Gourlay SG, Stead LF, Benowitz N. Clonidine for smoking cessation. *Cochrane Database Syst Rev*. 2004;3:CD000058.
84. Glassman AH, Stetner F, Walsh BT, et al. Heavy smokers, smoking cessation, and clonidine: results of a double-blind, randomized trial. *JAMA*. 1988;259:2863–2866.
85. Covey LS, Glassman AH. A meta-analysis of double-blind placebo-controlled trials of clonidine for smoking cessation. *Br J Addict*. 1991;86:991–998.
86. Erb S, Hitchcott PK, Rajabi H, Mueller D, Shaham Y, Stewart J. Alpha-2 adrenergic receptor agonists block stress-induced reinstatement of cocaine seeking. *Neuropsychopharmacology*. 2000;23:138–150.
87. Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*. 2003;168:3–20.
88. Jobes ML, Ghitza UE, Epstein DH, Phillips KA, Heishman SJ, Preston KL. Clonidine blocks stress-induced craving in cocaine users. *Psychopharmacology*. 2011;218:83–88.
89. Shytle R, Silver A, Lukas R, Newman M, Sheehan D, Sanberg P. Nicotinic acetylcholine receptors as targets for antidepressants. *Mol Psychiatry*. 2002;7:525–535.
90. Hughes JR, Stead LF, Hartmann-Boyce J, Cahill K, Lancaster T. Antidepressants for smoking cessation. *Cochrane Database Syst Rev*. 2014;1:CD000031.
91. Hitsman B, Pingitore R, Spring B, et al. Antidepressant pharmacotherapy helps some cigarette smokers more than others. *J Consult Clin Psychol*. 1999;67:547–554.
92. Wiley JL, Lavecchia KL, Martin BR, Damaj MI. Nicotine-like discriminative stimulus effects of bupropion in rats. *Exp Clin Psychopharmacol*. 2002;10:129.
93. Hall SM, Reus VI, Muñoz RF, et al. Nortriptyline and cognitive-behavioral therapy in the treatment of cigarette smoking. *Arch Gen Psychiatry*. 1998;55:683–690.
94. Hall SM, Humfleet GL, Reus VI, Muñoz RF, Hartz DT, Maude-Griffin R. Psychological intervention and antidepressant treatment in smoking cessation. *Arch Gen Psychiatry*. 2002;59:930–936.
95. Semenova S, Paterson N, Markou A. Chronic, but not acute, desipramine treatment prevented nicotine withdrawal and decreased nicotine self-administration in rats. *Neuropsychopharmacology*. 2005;30:S214.
96. Shulman KI, Herrmann N, Walker SE. Current place of monoamine oxidase inhibitors in the treatment of depression. *CNS Drugs*. 2013;27:789–797.
97. Lewis A, Miller JH, Lea RA. Monoamine oxidase and tobacco dependence. *Neurotoxicology*. 2007;28:182–195.
98. George TP, Weinberger AH. Monoamine oxidase inhibition for tobacco pharmacotherapy. *Clin Pharmacol Ther*. 2008;83:619–621.
99. Philibert RA, Gunter TD, Beach SR, Brody GH, Madan A. MAOA methylation is associated with nicotine and alcohol dependence in women. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B:565–570.
100. Berlin I, Said S, Spreux-Varoquaux O, et al. A reversible monoamine oxidase A inhibitor (moclobemide) facilitates smoking cessation and abstinence in heavy, dependent smokers. *Clin Pharmacol Ther*. 1995;58:444–452.
101. Houtsmuller EJ, Thornton JA, Stitzer ML. Effects of selegiline (L-deprenyl) during smoking and short-term abstinence. *Psychopharmacology (Berl)*. 2002;163:213–220.

102. George TP, Vessicchio JC, Termine A, Jatlow PI, Kosten TR, O'Malley SS. A preliminary placebo-controlled trial of selegiline hydrochloride for smoking cessation. *Biol Psychiatry*. 2003;53:136–143.
103. Killen JD, Fortmann SP, Murphy GM Jr, et al. Failure to improve cigarette smoking abstinence with transdermal selegiline + cognitive behavior therapy. *Addiction*. 2010;105:1660–1668.
104. Weinberger AH, Reutenauer EL, Jatlow PI, O'Malley SS, Potenza MN, George TP. A double-blind, placebo-controlled, randomized clinical trial of oral selegiline hydrochloride for smoking cessation in nicotine-dependent cigarette smokers. *Drug Alcohol Depend*. 2010;107:188–195.
105. Berlin I, Hunneyball IM, Greiling D, Jones SP, Fuder H, Stahl HD. A selective reversible monoamine oxidase B inhibitor in smoking cessation: effects on its own and in association with transdermal nicotine patch. *Psychopharmacology (Berl)*. 2012;223:89–98.
106. Ray R, Rukstalis M, Jepson C, et al. Effects of atomoxetine on subjective and neurocognitive symptoms of nicotine abstinence. *J Psychopharmacol*. 2009;23:168–176.
107. Silverstone PH, Dadashova R. Atomoxetine treatment for nicotine withdrawal: a pilot double-blind, placebo-controlled, fixed-dose study in adult smokers. *Ann Gen Psychiatry*. 2012;11:6.
108. Berlin I, Aubin H, Mansuy L, Laguerre G. p. 2.c. 002 Efficacy of milnacipran augmentation of nicotine replacement therapy on post-cessation mood and anxiety in smokers with previous history of major depression. *Eur Neuropsychopharm*. 2006;16:S313–S314.
109. Chrousos GP, Gold PW. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *JAMA*. 1992;267:1244–1252.
110. Koob GF. Brain stress systems in the amygdala and addiction. *Brain Res*. 2009;1293:61–75.
111. Glavin GB. Stress and brain noradrenaline: a review. *Neurosci Biobehav Rev*. 1985;9:233–243.
112. Tsigos C, Chrousos GP. The neuroendocrinology of the stress response. In: Hunt WA, Zakhari S, eds. *Stress, Gender, and Alcohol-seeking Behavior*. Diane Publishing Co.; 1995:103–124.
113. Thierry A, Tassin J, Blanc G, Glowinski J. Selective activation of the mesocortical DA system by stress. *Nature*. 1976;263:242–244.
114. Perkins KA, Epstein LH, Jennings JR, Stiller R. The cardiovascular effects of nicotine during stress. *Psychopharmacology*. 1986;90:373–378.
115. Arnsten AF, Goldman-Rakic PS. Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. *Arch Gen Psychiatry*. 1998;55:362–368.
116. Arnsten AF, Lombroso PJ. Genetics of childhood disorders: XVIII. ADHD, Part 2: Norepinephrine has a critical modulatory influence on prefrontal cortical function. *J Am Acad Child Adolesc Psychiatry*. 2000;39:1201–1203.
117. Stewart J. Pathways to relapse: the neurobiology of drug- and stress-induced relapse to drug-taking. *J Psychiatr Neurosci*. 2000;25:125.
118. Buczek Y, Lê A, Wang A, Stewart J, Shaham Y. Stress reinstates nicotine seeking but not sucrose solution seeking in rats. *Psychopharmacology*. 1999;144:183–188.
119. Lee B, Tiefenbacher S, Platt DM, Spearman RD. Pharmacological blockade of α_2 -adrenoceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys. *Neuropsychopharmacology*. 2004;29:686–693.
120. Zislin G, Desai TV, Prado M, Shah HP, Bruijnzeel AW. Effects of the CRF receptor antagonist D-Phe CRF(12–41) and the α_2 -adrenergic receptor agonist clonidine on stress-induced reinstatement of nicotine-seeking behavior in rats. *Neuropharmacology*. 2007;53:958–966.
121. Yamada H, Bruijnzeel AW. Stimulation of α_2 -adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. *Neuropharmacology*. 2011;60:303–311.
122. Forget B, Wertheim C, Mascia P, Pushparaj A, Goldberg SR, Le Foll B. Noradrenergic α_1 receptors as a novel target for the treatment of nicotine addiction. *Neuropsychopharmacology*. 2010;35:1751–1760.
123. Ashare RL, Sinha R, Lampert R, et al. Blunted vagal reactivity predicts stress-precipitated tobacco smoking. *Psychopharmacology*. 2012;220:259–268.
124. McKee SA, Sinha R, Weinberger AH, et al. Stress decreases the ability to resist smoking and potentiates smoking intensity and reward. *J Psychopharmacol*. 2011;25:490–502.
125. McKee SA, Potenza M, Kober H, et al. A translational investigation targeting stress-reactivity and pre-frontal cognitive control with guanfacine for smoking cessation [published online ahead of print December 16, 2014]. *J Psychopharmacol*. doi: 10.1177/0269881114562091
126. Arnsten AF, Jin LE. Guanfacine for the treatment of cognitive disorders: a century of discoveries at Yale. *Yale J Biol Med*. 2012;85:45.
127. Ji XH, Cao XH, Zhang CL, et al. Pre- and postsynaptic beta-adrenergic activation enhances excitatory synaptic transmission in layer V/VI pyramidal neurons of the medial prefrontal cortex of rats. *Cereb Cortex*. 2008;18:1506–1520.
128. Morrow BA, George TP, Roth RH. Noradrenergic α_2 agonists have anxiolytic-like actions on stress-related behavior and mesoprefrontal dopamine biochemistry. *Brain Res*. 2004;1027:173–178.
129. Chappell PB, Riddle MA, Scahill L, et al. Guanfacine treatment of comorbid attention-deficit hyperactivity disorder and Tourette's syndrome: preliminary clinical experience. *J Am Acad Child Adolesc Psychiatry*. 1995;34:1140–1146.
130. Scahill L, Chappell PB, Kim YS, et al. A placebo-controlled study of guanfacine in the treatment of children with tic disorders and attention deficit hyperactivity disorder. *Am J Psychiatry*. 2001;158:1067–1074.
131. Taylor FB, Russo J. Comparing guanfacine and dextroamphetamine for the treatment of adult attention-deficit/hyperactivity disorder. *J Clin Psychopharmacol*. 2001;21:223–228.
132. McKee SA. *Why is it more difficult for women to quit smoking? Translating knowledge into practice*. American Psychological Association Annual Convention; 2013; Honolulu, HI.
133. Fox HC, Morgan PT, Sinha R. Sex differences in guanfacine effects on drug craving and stress arousal in cocaine-dependent individuals. *Neuropsychopharmacology*. 2014;39:1527–1537.
134. Bloom FE. The catecholamine neuron: Historical and future perspectives. *Prog Neurobiol*. 2010;90:75–81.
135. Grenhoff J, Svensson TH. Pharmacology of nicotine. *Br J Addict*. 1989;84:477–492.
136. Grenhoff J, Svensson TH. Prazosin modulates the firing pattern of dopamine neurons in rat ventral tegmental area. *Eur J Pharmacol*. 1993;233:79–84.
137. Carr L, Moore K. Effects of amphetamine on the contents of norepinephrine and its metabolites in the effluent of perfused cerebral ventricles of the cat. *Biochem Pharmacol*. 1970;19:2361–2374.
138. Auclair A, Cotecchia S, Glowinski J, Tassin J-P. D-Amphetamine fails to increase extracellular dopamine levels in mice lacking α_1 -adrenergic receptors: relationship between functional and nonfunctional dopamine release. *J Neurosci*. 2002;22:9150–9154.
139. Schank JR, Ventura R, Puglisi-Allegra S, et al. Dopamine beta-hydroxylase knockout mice have alterations in dopamine signaling and are hypersensitive to cocaine. *Neuropsychopharmacology*. 2005;31:2221–2230.
140. Ungerstedt U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand Suppl*. 1970;367:1–48.
141. Crow TJ, Spear P, Arbuthnott G. Intracranial self-stimulation with electrodes in the region of the locus coeruleus. *Brain Res*. 1972;36:275–287.
142. Wise RA. Addictive drugs and brain stimulation reward. *Annu Rev Neurosci*. 1996;19:319–340.
143. Poschel BP, Ninteman FW. Hypothalamic self-stimulation: its suppression by blockade of norepinephrine biosynthesis and reinstatement by methamphetamine. *Life Sci*. 1966;5:11–16.
144. Davis WM, Smith SG, Werner TE. Noradrenergic role in the self-administration of ethanol. *Pharmacol Biochem Behav*. 1978;9:369–374.
145. Brown Z, Amit Z, Levitan D, Ogren S, Sutherland E. Noradrenergic mediation of the positive reinforcing properties of ethanol: II. Extinction of ethanol-drinking behavior in laboratory rats by inhibition of dopamine-beta-hydroxylase. Implications for treatment procedures in human alcoholics. *Arch Int Pharmacodyn Ther*. 1977;230:76–82.

146. Weinshenker D, Rust NC, Miller NS, Palmiter RD. Ethanol-associated behaviors of mice lacking norepinephrine. *J Neurosci.* 2000;20:3157–3164.
147. Villégier AS, Lotfipour S, Belluzzi JD, Leslie FM. Involvement of alpha1-adrenergic receptors in tranlycypromine enhancement of nicotine self-administration in rat. *Psychopharmacology.* 2007;193:457–465.
148. Forget B, Hamon M, Thiébot MH. Involvement of alpha1-adrenoceptors in conditioned place preference supported by nicotine in rats. *Psychopharmacology.* 2009;205:503–515.
149. Bruijnzeel AW, Bishnoi M, van Tuijl IA, et al. Effects of prazosin, clonidine, and propranolol on the elevations in brain reward thresholds and somatic signs associated with nicotine withdrawal in rats. *Psychopharmacology.* 2010;212:485–499.
150. Jentsch JD, Sanchez D, Elsworth JD, Roth RH. Clonidine and guanfacine attenuate phencyclidine-induced dopamine overflow in rat prefrontal cortex: mediating influence of the alpha-2A adrenoceptor subtype. *Brain Res.* 2008;1246:41–46.
151. Trendelenburg AU, Starke K, Limberger N. Presynaptic alpha 2A-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. *Naunyn Schmiedeberg's Arch Pharmacol.* 1994;350:473–481.