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A mechanistic hypothesis of the factors that enhance vulnerability to nicotine use in females

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Abstract

Women are particularly more vulnerable to tobacco use than men. This review proposes a unifying hypothesis that females experience greater rewarding effects of nicotine and more intense stress produced by withdrawal than males. We also provide a neural framework whereby estrogen promotes greater rewarding effects of nicotine in females via enhanced dopamine release in the nucleus accumbens (NAcc). During withdrawal, we suggest that corticotropin-releasing factor (CRF) stress systems are sensitized and promote a greater suppression of dopamine release in the NAcc of females versus males. Taken together, females display enhanced nicotine reward via estrogen and amplified effects of withdrawal via stress systems. Although this framework focuses on sex differences in adult rats, it is also applied to adolescent females who display enhanced rewarding effects of nicotine, but reduced effects of withdrawal from this drug. Since females experience strong rewarding effects of nicotine, a clinical implication of our hypothesis is that specific strategies to prevent smoking initiation among females are critical. Also, anxiolytic medications may be more effective in females that experience intense stress during withdrawal. Furthermore, medications that target withdrawal should not be applied in a unilateral manner across age and sex, given that nicotine withdrawal is lower during adolescence. This review highlights key factors that promote nicotine use in females, and future studies on sex-dependent interactions of stress and reward systems are needed to test our mechanistic hypotheses. Future studies in this area will have important translational value toward reducing health disparities produced by nicotine use in females.

Keywords

sex; tobacco; adolescent; withdrawal; dependence; reward

1. Introduction

Tobacco use is the number one cause of preventable deaths in the United States (Center for Disease Control and Prevention, 2011). Epidemiological studies have provided important advances with regard to which populations are especially vulnerable to tobacco use. However, very little is known about the factors that lead to higher rates of tobacco use in these vulnerable populations.

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The overarching goal of our laboratory is to understand the factors that contribute to tobacco use among vulnerable populations. Five years ago, we described a psychobiological hypothesis of adolescent nicotine use for the 35th anniversary of NIDA issue of this journal (O'Dell, 2009). Our hypothesis was that nicotine use in adolescence is driven by strong rewarding effects of nicotine that are inadequately balanced by the negative effects of withdrawal from this drug. This hypothesis was derived largely from rodent studies showing that the rewarding effects of nicotine are higher, whereas the aversive effects of withdrawal are lower in adolescent versus adult rats. Subsequent studies in our laboratory revealed that age differences produced by nicotine withdrawal are mediated via dopaminergic mechanisms in the mesocorticolimbic pathway (Natividad et al., 2010; 2012). This review reflects an extension of our adolescent work to a new hypothesis that describes the interaction of biological systems, including dopamine, that are believed to contribute to nicotine use in females.

Our working hypothesis is that nicotine use in females is driven by stronger positive effects of nicotine and more intense stress produced by withdrawal from this drug as compared to males. This hypothesis was derived from pre-clinical studies showing that the rewarding effects of nicotine are enhanced in female versus male rodents (see Carroll et al., 2009; Perkins et al., 1999; Pogun and Yazarbas, 2009). Also, the short-term aversive effects of nicotine are lower in female versus male rats (Torres et al., 2009). The overall result is that females may seek nicotine because of strong positive effects that are unopposed by short-term aversive effects. We recently observed that nicotine withdrawal produces more intense anxiety-like behavior and stronger biological responses within stress systems in females as compared to males. Thus, females may also seek nicotine because of intense negative affective states that emerge during withdrawal that promote relapse behavior compared to males.

This review also includes a mechanistic hypothesis that describes enhanced nicotine use among females in the context of hormone and stress regulation of mesocorticolimbic dopamine systems. Our hypothesis is that physiological substrates implicated in the rewarding effects of nicotine (dopamine) as well as those that are recruited during withdrawal from this drug (stress systems) promote nicotine use to a greater extent in females than males. Specifically, the enhanced drive to use nicotine in females is mediated, at least in part, by estrogen that facilitates dopamine release in terminal regions of the mesocorticolimbic pathway. During withdrawal, the drive to use nicotine is intensified by the emergence of stress that is stronger in females than males.

Lastly, this review considers the clinical implications of our hypothesis describing nicotine use in females. First, the finding that females experience strong rewarding effects of nicotine suggests that strategies to *prevent* smoking initiation in females are critical. Second, following chronic nicotine use, females display intense stress during nicotine withdrawal that likely promotes relapse behavior during abstinence. Thus, cessation medications that alleviate stress produced by nicotine withdrawal may be more effective in females versus males. Third, recent studies in our laboratory have shown that nicotine withdrawal is lower in adolescent versus adult females. Thus, medications that target withdrawal should not be applied in a unilateral manner across age and sex.

2. Clinical research

It is widely accepted that the motivational properties of tobacco are due, in large part, to the presence of nicotine (see Benowitz, 2010; Picciotto and Kenny, 2013). Nicotine use is motivated by the positive effects of this drug and avoiding negative affective states induced by withdrawal from long-term exposure to nicotine. We hypothesize that in females, strong

positive effects of nicotine fuel this balance to a greater extent than males. In addition, we postulate that females experience more intense negative affective states during nicotine withdrawal than males. Thus, nicotine use in females is motivated by the avoidance of intense withdrawal states that promote relapse behavior during abstinence. Below clinical evidence is provided that supports our hypothesis regarding enhanced vulnerability to nicotine use in females.

2.1. Enhanced rewarding effects of nicotine in females

Clinical studies suggest that women are more sensitive to the rewarding effects of nicotine than men (see Greenfield et al., 2010; Mendelsohn, 2011; Perkins et al., 2009; Pogun and Yazarbas, 2009; Van Voorhees et al., 2012). Women consume more tobacco products and have a harder time quitting smoking than males (Perkins et al., 1999; Zilberman et al., 2003). Moreover, self-reports of positive mood effects following cigarette use are higher in women relative to men smokers (Perkins et al., 2006). In addition, women that use tobacco regularly display higher rates of responding for smoking-related cues than men (Perkins et al., 1999; 2001).

Epidemiological studies have shown that young females are especially vulnerable to nicotine use. Adolescent females are more likely to initiate smoking and are less likely to quit than adolescent males (Anderson and Burns, 2000; James-Walke et al., 2007; Perkins, 2001). External factors also appear to contribute to nicotine use in adolescent females, including appetite suppression and a desire to project a more appealing self-image (Seguire and Chalmers, 2000). The recent rise in smoking initiation among female teenagers is believed to explain the slower decline in smoking prevalence rates in women relative to men, a phenomenon that has been referred to as the *telescoping effect* (Zilberman et al., 2003). In summary, these studies suggest that nicotine is more reinforcing in females relative to males regardless of age.

2.2. Enhanced aversive effects of nicotine withdrawal in females

Clinical studies have shown that women are also more sensitive to the aversive effects of nicotine withdrawal. For example, women exhibit lower rates of quitting and are less likely to benefit from tobacco cessation therapies than men (Cepeda-Benito et al., 2004; Cropsey et al., 2008; Hammond, 2009; Perkins et al., 2001; Perkins and Scott, 2008; Piper et al., 2010; Schnoll et al., 2007). During smoking abstinence, women report greater levels of anxiety, depression, and stress than men (Perkins and Scott, 2008; Schnoll et al., 2007; Xu et al., 2008). Women also report less relief from negative affective states with nicotine replacement therapy than men (Perkins et al., 2001). During abstinence from smoking, women also display higher levels of cortisol (a biological marker of stress in humans) as compared to men (Hogle and Curtin, 2006). Lastly, women report more often than men that the anxiety-reducing effects of cigarettes are the main reason for continued smoking and relapse (Perkins and Scott, 2008; Perkins et al., 2012; 2013; Piper et al., 2010). Adolescent females also report higher levels of stress and depression during nicotine abstinence as compared adolescent males (Colby et al., 2000; Nichter et al., 1997). Taken together, these studies suggest that intense stress experienced during nicotine withdrawal may be a significant factor contributing to relapse behavior in females.

On a related note, much work has illustrated that women use nicotine to cope with anxiety to a larger extent than men (Perkins, 2009; Perkins et al., 2012; Piper et al., 2010). In fact, nicotine has been shown to decrease anxiety elicited by a moderate stressor in women (File et al., 2001). Interestingly, the latter report demonstrated that nicotine increased anxiety following presentation of the moderate stressor in men. There is also evidence that pre-existing stress disorders lead to nicotine use in females. For example, there is a stronger co-

morbid association between anxiety disorders and smoking rates in women than men (Mykletun et al., 2008). In addition, women with a prior history of an anxiety disorder are more likely to develop nicotine dependence later in life than men (Brook et al., 2012). These studies suggest that stress may be a more critical factor contributing to nicotine use and relapse in females as compared to males.

2.3. Other factors that contribute to nicotine use in females

There are a variety of external factors that may also contribute to nicotine use in females. For example, social factors such as having a friend who smokes contributes to smoking initiation in women (Holahan et al., 2012). Hunger suppression and weight control have also been shown to play an important role in smoking initiation among women. In fact, women consider nicotine use as a tool to control appetite and weight gain more than men (French et al., 1995; Meyers et al., 1997; Mendelson et al., 2011; Torchalla et al., 2012). This is consistent with the finding that adolescent females with a stronger desire to be thin are more likely to use nicotine later in life (Austin and Gortmaker, 2001). Lastly, one might argue that nicotine use is higher in females than males given that the marketing of tobacco products targets women. This is based on findings showing that cigarette packages with bright colors are rated as more attractive and associated with positive attributes such as glamour and sophistication among women compared to men (Doxey and Hammond, 2011; White et al., 2012). An online survey also showed that women rate tobacco products with female-oriented packaging as having more appeal, style, and glamour (Hammond et al., 2011). These findings highlight the importance of regulating marketing strategies that target young women, especially given that females initiate smoking to increase sociability, attractiveness and sophistication (Kaufman and Augustson, 2008; Oh et al., 2011).

2.4. Implications for extrapolating from clinical findings

A fundamental assumption in the review is that females are more susceptible to tobacco addiction than males. Our assessment of the literature is based on data collected in the US and other developed nations. It is recognized; however, that epidemiological evidence from around the world suggests that the prevalence of tobacco use is higher in men as compared to women (Ernster et al., 2000; Mackay, 1996; Slama, 2008). This discrepancy is likely related to where the data are collected, as smoking prevalence rates in women are higher in developed versus underdeveloped countries (Mackay, 1994). Nonetheless, regardless of nationality, women find it harder to quit smoking than men (Mackay and Amos, 2003). The smoking prevalence rates in developed versus under-developed countries is likely influenced by cultural norms, socio-economic status, access to tobacco products, and marketing strategies that promote tobacco use in women from developed countries. These factors that influence tobacco use prevalence rates should be carefully considered when extrapolating the patterns of sex differences found in clinical findings to the interpretation of pre-clinical studies using animal models.

3. Pre-clinical rodent models of nicotine reward and aversive effects

Rodent models have been widely used to assess the motivational factors that contribute to nicotine use (Le Foll and Goldberg, 2009; O'Dell and Khroyan, 2009). Intravenous self-administration (IVSA) procedures have been widely used to study the reinforcing effects of nicotine (see Caille et al., 2012; Le Foll and Goldberg, 2009; Levin et al., 2010). The IVSA model is based on reinforcement principles in which the behavioral response is strengthened by presentation of nicotine after the operant response is performed. Place-conditioning procedures have also been used to study the rewarding effects of nicotine (Briellmaier et al., 2008; Calcagnetti and Schechter, 1994; LeFoll and Goldberg, 2005). These procedures involve passive administration of drug on one side of a conditioning apparatus and saline on

an adjacent side. Following repeated drug-environment pairings, animals are allowed free access to both compartments simultaneously in the absence of drug. The nature of the affective properties of the drug is evident on the test day when the environmental cues elicit either conditioned place preference (CPP) or aversion (CPA) to the drug-paired side relative to the neutral side. This is a major advantage of place-conditioning procedures, as the short-term rewarding and aversive effects of nicotine can be assessed in the same study.

Nicotine withdrawal has been widely studied in rats using chronic nicotine administration via subcutaneous osmotic mini-pumps for 5–7 days (Kenny and Markou, 2001; Malin, 2001). Withdrawal from nicotine is produced via removal of the nicotine pump (spontaneous withdrawal) or administration of a nicotinic receptor antagonist (precipitated withdrawal). Using either spontaneous or precipitated methods, nicotine withdrawal produces a behavioral profile comprised of both physical and affective components. The physical signs of nicotine withdrawal in male adult rats include abdominal constrictions, facial fasciculation, writhes, gasps, eye blinks, and ptosis (O'Dell et al., 2004; Shram et al., 2008; Watkins et al., 2000).

The affective properties of nicotine withdrawal have been assessed in procedures that measure anxiety-like behavior (for a review see Bruijnzeel et al., 2012). When rodents experience stress, they spend more time in the enclosed arms of an elevated plus maze or the peripheral areas of an open field as compared to control rats. Previous studies have demonstrated that male adult rats display an increase in time spent in the enclosed arms of the elevated plus maze following precipitated (Tejeda et al., 2012; Wilmouth and Spear, 2006) and spontaneous (Chae et al., 2007; Irvine et al., 2001; Jonkman et al., 2008) withdrawal from nicotine. Male adult rats experiencing nicotine withdrawal also display an increase in the amount of time spent in the peripheral areas of an open field compared to controls (Tzavara et al., 2002). The effects of nicotine withdrawal on anxiety-like behavior in male rodents have also been assessed in the defensive burying paradigm (George et al., 2007), the light/dark exploration test (Jonkman et al., 2005; Stoker et al., 2008) and the startle-response test (Helton et al., 1993).

The negative affective properties of nicotine withdrawal have also been assessed using intracranial self-stimulation procedures (see Malin and Goyarzu, 2009; Paterson and Markou, 2007). Withdrawal from chronic nicotine produces an increase in the brain reward threshold that is thought to reflect a decrease in brain reward function in male adult rats (Bauzo and Bruijnzeel, 2012; Epping-Jordan et al., 1998; Panagis et al., 2000). The need for higher current levels is believed to reflect a decrease in brain reward function during withdrawal. The affective properties of nicotine withdrawal have been assessed using CPA procedures. During conditioning, nicotine-treated animals receive a nicotinic receptor antagonist to precipitate withdrawal in one side of the apparatus. On alternating days they receive saline in the other compartment. Following conditioning, nicotine-dependent male adult rats display a CPA for the compartment where they experienced withdrawal (Ise et al., 2000; O'Dell et al., 2007; Suzuki et al., 1996; Watkins et al., 2000). Collectively, these studies demonstrate that nicotine withdrawal induces negative affective states in rodents.

There are several issues to consider when examining the effects of nicotine in female rodents in the models described above. First, ovarian hormones such as estrogen and progesterone fluctuate across the 4-day estrous cycle in adult female rodents (see McCarthy et al., 2012). The fluctuation of hormones levels may influence the behavioral outcome assessed by these rodent models. To address this issue, some studies test female adult rats during each phase of the estrous cycle. However, this approach quadruples the size of the study. Another approach involves ovariectomy (OVX) procedures and testing the female rats following replacement of key ovarian hormones, such as estrogen and/or progesterone.

Special consideration must be taken when employing OVX procedures in rodent models of motivational behavior, given that OVX procedures alter anxiety-like behavior (Pandaranandaka et al., 2006) and suppress dopamine systems (Bosse and Di Paolo, 1996; Garcia-Munoz et al., 1989; Morissette and Di Paolo, 1993; Russo et al., 2003). Nicotine has also been shown to facilitate learning tasks to a greater extent in females as compared to males (Taylor and Maloney, 2010). A final issue to consider is that female rats have a higher fat/muscle ratio than males, which may influence the absorption, elimination and metabolism of nicotine. These issues should be considered when comparing sex differences in rodent models of nicotine use.

4. Pre-clinical research on the short-term effects of nicotine in females

When examining the abuse liability of nicotine, one must consider that this drug produces initial short-term effects that likely influence whether or not the drug is used in the future. This is particularly important with regards to nicotine, as nicotine produces both positive and negative short-term effects.

4.1. Enhanced rewarding effects of nicotine in females

Similar to the clinical research reviewed above, several lines of pre-clinical evidence suggest that the rewarding effects of nicotine are enhanced in female versus male rodents (see Carroll et al., 2009; Perkins et al., 1999; Pogun and Yararbas, 2009). One of the first major studies in this area demonstrated that female adult rats display faster acquisition rates of nicotine IVSA at lower doses than males (Donny et al., 2000). This study also showed that adult females reach a higher break point for nicotine infusions on a progressive ratio (PR) schedule of reinforcement than males. Subsequent work in the same laboratory demonstrated that female rats display twice as much nicotine intake in the presence of a visual stimulus as compared to males (Chaudhri et al., 2005). In contrast to these reports, another recent study demonstrated that female and male rats display similar levels of nicotine intake, extinction, and stress-induced reinstatement of drug seeking-behavior (Feltenstein et al., 2011). The authors suggest that the lack of sex differences in their study may be related to lower reinforcement requirements as compared to previous reports. Another report showed that nicotine intake is more robust in female rats, as they shown higher and more stable levels of oral nicotine intake than males (Nesil et al., 2011). The latter study also showed that female rats display greater weight suppressant effects of nicotine than males.

Female rodents also display greater rewarding effects of nicotine than males in studies employing place-conditioning procedures. Work in our laboratory demonstrated that female rats display CPP that is larger in magnitude and observed across a wider range of nicotine doses as compared to males (see Figure 1; Torres et al., 2009). The latter study also revealed that OVX females did not display CPP produced by nicotine. These results suggest that ovarian hormones facilitate the rewarding effects of nicotine. Our results are consistent with the finding that nicotine produces CPP in female mice that is larger in magnitude as compared to males (Kota et al., 2008). However, we also acknowledge a report showing that nicotine-induced CPP is larger in male versus female rats (Yararbas et al., 2010). The discrepancy in these reports may be related to differences in the rat strain and/or conditioning parameters. In general, the IVSA and CPP studies provide support for the hypothesis that the rewarding effects of nicotine are enhanced in female versus male rodents.

4.2. Reduced aversive effects of nicotine in females

Emerging lines of evidence suggest that the aversive effects of nicotine are lower in female rodents. Work in our laboratory has shown that high doses of nicotine produce CPA in male rats; however, the magnitude of this effect is lower in females (Figure 1; Torres et al., 2009). Interestingly, the aversive effects of nicotine were similar in male and OVX female rats. This suggests that ovarian hormones protect against the aversive effects of nicotine. Sex differences in the aversive effects of nicotine have also been studied using conditioned taste aversion (CTA) procedures. In contrast to the sex differences observed in CPA studies, a study using CTA procedures demonstrated that female and male rats display a similar aversion to a flavor that was previously paired with a high dose of nicotine (Rinker et al., 2008). More work may be needed to resolve the discrepancy between studies comparing the aversive effects of nicotine with different behavioral procedures in female and male rats.

5. Pre-clinical research on the long-term effects of nicotine in females

Two relevant issues arise when considering the effects of long-term nicotine exposure. First, chronic nicotine exposure produces neuroendocrine effects that alter stress systems. Second, following the removal of chronic nicotine exposure, a withdrawal syndrome emerges that shows clear negative effects, both physical and affective. Both of these long-term effects of nicotine are relevant to our hypothesis regarding greater nicotine use in females.

5.1. Enhanced stress produced by repeated nicotine administration in females

Behavioral studies have demonstrated that females are more sensitive to the effects of nicotine exposure on anxiety-like behavior. For example, female mice display more anxiety-like behavior compared to their male counterparts following chronic oral nicotine intake (Caldarone et al., 2008). Female rats also display a greater increase in nicotine IVSA following a pharmacological stressor as compared to males (Li et al., 2012). The authors of the latter report suggest that females are more sensitive to the effects of stress on nicotine intake as compared to males. Consistent with this, a recent report showed that chronic administration of nicotine in combination with immobilization stress produced a larger decrease in feeding and body weight in females as compared to males (Faraday et al., 2005).

The main neuroendocrine substrate of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis (for a review see Vale et al., 1981). When a stressor is experienced, corticotropin-releasing factor (CRF) is secreted from the hypothalamus and stimulates adrenocorticotropic hormone (ACTH) release from the pituitary gland. ACTH then stimulates the release of corticosterone and other glucocorticoids from the adrenal cortex. Corticosterone serves as a major negative feedback signal that terminates HPA axis activity. Within the hypothalamus, corticosterone binds to nuclear glucocorticoid receptor II subunits causing an inhibition of CRF mRNA synthesis.

Studies comparing biological indices of stress are consistent with behavioral reports. For example, plasma corticosterone levels are increased to a greater extent in female versus male rats following repeated injections (Gentile et al., 2011; Moidel et al., 2006; Rhodes et al., 2001; 2004; Skwara et al., 2012) and continuous delivery (Faraday et al., 2005) of nicotine. In an *in vitro* perfusion system, the presence of nicotine increased CRF and ACTH levels to a greater extent in hypothalamic tissue that was collected from female versus male rats (Mcklveen et al., 2010; Moidel et al., 2006). Overall, these studies suggest that the behavioral and biological effects of nicotine on stress systems are greater in females as compared to males.

5.2. Enhanced effects of nicotine withdrawal in females

Pre-clinical studies have shown that the behavioral effects of nicotine withdrawal are larger in female versus male rats. Although few studies have compared sex differences in the physical signs of withdrawal, one report showed that female adult rats display more physical signs of nicotine withdrawal relative to males (Hamilton et al., 2009). The affective properties of withdrawal also appear to be higher in females. Recent unpublished observations in our laboratory revealed that the ability of nicotine withdrawal to produce CPA is greater in female versus male rats (Figure 2). These studies suggest that the physical and negative affective properties of nicotine withdrawal are greater in female versus male rats.

Recent work in our laboratory has compared sex differences in the behavioral and biological markers of stress during nicotine withdrawal (Figure 3). This work has revealed that females display both an increase in anxiety-like behavior and corticosterone levels that are higher as compared to males. Female rats also displayed an increase in CRF gene expression in the nucleus accumbens (NAcc) as compared to male rats experiencing withdrawal from nicotine. This sex difference in CRF gene expression was not observed in the amygdala or hypothalamus of these rats, suggesting that the NAcc plays a central role in modulating sex differences produced by withdrawal. Our behavioral results are consistent with the finding that female adult mice display more anxiety-like behavior on the elevated plus maze during nicotine withdrawal as compared to males (Kota et al., 2007; 2008). Similarly, Caldarone et al. (2008) showed that female mice display a higher level of anxiety-like behavior following chronic oral nicotine intake as compared to males. Two recent reports also showed that adult female rats display higher plasma corticosterone and ACTH levels during nicotine withdrawal as compared to males (Gentile et al., 2011; Skwara et al., 2012). Taken together, there is strong pre-clinical evidence to suggest that females experience greater stress during nicotine withdrawal as compared to males.

The pre-clinical evidence described above may elucidate some of the factors that contribute to nicotine use in humans. Namely, we hypothesize that the strong rewarding effects of nicotine and robust negative affective states that emerge during nicotine withdrawal both contribute to greater nicotine use in females as compared to males. Our hypothesis relies on strong positive effects of nicotine that promote initial use and activate stress systems during withdrawal to a larger extent in females versus males. Over time, intense stress produced by withdrawal may contribute to a higher incidence of relapse behavior in women.

6. Opponent-process theory as a framework for nicotine use in females

Organisms are equipped with homeostatic mechanisms that regulate internal parameters within upper and lower limits. Opponent processes correct for deviations outside of normal parameters to maintain stability. These adaptive responses oppose the disruption in homeostasis, and are therefore referred to as *opponent processes*. Organisms are also equipped with opponent processes that limit the excessive activation of brain reward systems. This is adaptive to limit an organism from being continuously immersed in activities that activate reward systems, such as eating or sexual behavior.

The opponent-process theory of motivation was originally applied to tobacco use by Solomon and Corbit in (1973). These researchers suggested that tobacco usurps opponent processes that modulate motivated behavior. More recent extensions of this theory postulate that nicotine produces strong motivational effects via activation of brain reward systems that are opposed by stress systems. Following chronic nicotine use, Koob and LeMoal, (2001) suggest that the opponent processes that limit reward function fail to return to a normal homeostatic range. These changes are counteradaptive because they reflect a chronic

deviation from homeostasis in which reward is regulated around a drug-modified set point outside of the normal limits. This alteration is referred to as allostasis, which represents a chronic deviation of the reward set point that causes an enhanced state of vulnerability and a loss of control over drug use. The term *allostatic load* refers to the excessive demand on the organism caused by defending a set point that is outside of a normal range.

The opponent-process theory of addiction postulates that there are two opposing motivational processes (George et al., 2012). The short- and long-term effects of nicotine use are illustrated in Figure 4. The a-process has a quick onset but quick offset, and it decreases with repeated drug exposure. Nicotine produces short-term rewarding effects as well as initial aversive effects. Thus, we propose that the a-process reflects the cumulative mood state that is the net result of the initial positive effects of nicotine minus any aversive effects. Given that nicotine has short-term aversive effects, the intake of this drug is carefully titrated in order to achieve positive mood states. Our interpretation of the a-process may be unique to nicotine, given that other drugs of abuse produce less short-term aversive effects and typically used in a binge-like pattern. Thus, a female that experiences primarily strong rewarding effects of nicotine would display a larger positive hedonic state (larger a-process) as compared to males that experience more initial aversive effects of this drug.

On the other hand, it is postulated that the b-process opposes the a-process, and appears after the a-process has terminated (Koob and Le Moal, 2001). The b-process is sluggish in onset and becomes sensitized with long-term drug exposure. The b-process involves the recruitment of stress systems, which are activated by positive hedonic states produced by nicotine. The recruitment of stress systems is hypothesized to modulate the behavioral and biochemical consequences of withdrawal from chronic nicotine use, and have been referred to as *the dark side of addiction* (Koob, 2010). The magnitude of the positive hedonic state is hypothesized to determine the extent to which the ensuing opponent process is recruited to counter the hedonic state. We propose that females display stronger positive effects of nicotine (a-process) that will recruit stress systems (b-process) to a larger extent than males. Thus, long-term nicotine use produces a larger recruitment of opponent systems in females that results in a greater stress response during nicotine withdrawal as compared to males.

The opponent-process theory of addiction has provided a useful framework for studying the underlying factors that contribute to drug dependence. We postulate that there are important differences in the processes that mediate nicotine use in females as compared to what has been described for nicotine dependence in males. A comprehensive hypothesis describing the rapid downward spiral into drug abuse has been described for females (Becker et al., 2012). Here we extend the opponent-process theory of addiction to include a hypothesis of chronic nicotine use in females of different ages.

6.1. Opponent processes mediating the short-term effects of nicotine in females

It is hypothesized that stronger positive mood states (a-process) contribute to higher nicotine use in females than males (Figure 4). This is based on a large body of evidence showing that the rewarding effects of nicotine are enhanced in females as compared to males, as described previously. The stronger rewarding effects of nicotine may promote nicotine use in females during the initiation, maintenance and relapse phases of nicotine use. It is presently unclear whether the positive rewarding effects of nicotine change with repeated exposure in females. Given that the rewarding effects of drugs of abuse generally decrease over time in adult drug users, we denote the change in the a-process as a gradual decrease over time. However, the possibility exists that the rewarding effects of nicotine increase over time in females, but not males. This is based on the finding that adolescent female rats display an increase in nicotine intake as they enter adulthood, an effect that was not observed in males (Levin et al., 2011).

During short-term use, nicotine can also induce aversive effects. Work in our laboratory demonstrated that adult female rats display less aversive effects of nicotine as compared to males (Figure 1). This finding suggests that females display strong rewarding effects of nicotine that are only slightly offset by aversive effects. Thus, the positive mood state produced by nicotine is larger in magnitude in females as compared to males.

6.2. Opponent processes mediating nicotine withdrawal in females

We suggest that females who experience stronger hedonic mood states following nicotine administration also exhibit a larger recruitment of opponent processes than males. During nicotine withdrawal, we postulate that chronic activation of stress systems produces a greater downward shift in the allostatic set point of females versus males (Figure 4). Thus, the chronic activation of stress systems results in a sensitized endocrine response during nicotine withdrawal, that is larger in females relative to males. This hypothesis is based largely on pre-clinical studies showing that the behavioral and neuroendocrine effects of nicotine withdrawal are greater in females versus males, as described previously. In clinical studies, females also report more than males that the anxiety-reducing effects of nicotine are the main reason for relapse behavior (Perkins and Scott, 2008; Perkins et al., 2009; 2012; Piper et al., 2010). Taken together, these studies support our hypothesis that females are more likely to use nicotine because of the strong rewarding effects of nicotine in combination with an intense desire to avoid stress produced withdrawal from this drug.

Below we provide a biochemical hypothesis regarding the neural substrates that mediate nicotine use in females versus males. Although this framework is derived from rodent models, it is intended to provide a possible explanation for why females are different from males with regard to the role of estrogen and stress systems on dopamine transmission in the mesocorticolimbic system.

7. Hypothesized substrates mediating nicotine use in females

It has been suggested that the allostatic state is fueled by a dysregulation in the brain circuits within the extended amygdala, which includes the NAcc (Koob and Volkow, 2010). Changes within these brain structures are believed to modulate the net hedonic effects of nicotine (a-process), as well as changes in counter-adaptive processes that counteract this response (b-process). Recently, Koob and colleagues postulated that within-system changes in a-processes are modulated by the dopamine system, whereas between-system alterations in b-processes are modulated via CRF stress systems (George et al., 2012). Drugs of abuse are postulated to elicit an opposing neutralizing reaction *within* the same system in which the drug elicits its primary reinforcing actions. Accordingly, nicotine produces an increase in dopamine in the NAcc that is reversed during withdrawal from this drug. Thus, the pattern of changes in dopamine reflects within-system changes. The hedonic states induced by nicotine activate between system changes in CRF systems that sensitize with repeated nicotine exposure. Our hypothesis regarding nicotine use in females is focused on the circuitry within the NAcc that regulates dopamine and CRF release in this region.

7.1. Within-system changes in dopamine

The behavioral effects of nicotine are modulated, in large part, by dopamine transmission in the mesocorticolimbic pathway (Corrigall et al., 1992; Mansvelder and McGehee, 2002). This pathway originates in the ventral tegmental area (VTA) and terminates in the frontal cortex as well as several forebrain structures including the amygdala and NAcc. Behavioral work has also shown that nicotine reward is mediated, in large part, by increasing dopamine levels in the NAcc (Balfour, 2002; Mansvelder et al., 2003). The neurochemical effects of nicotine are reversed during withdrawal from this drug, such that NAcc dopamine levels are

decreased (Carboni et al., 2000; Di Chiara et al., 2000; Hildebrand et al., 1998; Rada et al., 2001). Thus, dopamine in the NAcc is critically important to both nicotine reward and withdrawal from this drug.

With regard to the rewarding effects of nicotine, an estrogen-based mechanism is offered to explain sex differences in the ability of nicotine to increase dopamine levels in the NAcc based on the work described in (Becker, 1999 and 2012). Estrogen receptors are located on the local terminals of medium spiny striatal neurons that have recurrent collaterals on dopamine terminals (Mermelstein et al., 1996). We suggest that estrogen inhibits the activity of the intrinsic medium spiny striatal neurons that modulate dopamine. Medium spiny striatal neurons are primarily of gamma-aminobutyric acid origin (GABA). Thus, it is speculated that the inhibitory effects of estrogen decrease GABA levels via a reduction of calcium currents. This results in a decreased response to GABA at the dopamine terminals that results in increased stimulated dopamine release in the NAcc.

Another mechanism by which estrogen may facilitate dopamine release in the NAcc is that estrogen may act directly on dopamine terminals to down-regulate D2 auto-receptors (Bazzett et al., 1994). In support of our hypothesis, estrogen has been shown to increase nicotine-induced striatal dopamine levels in female, but not male rats (Dluzen and Anderson, 1997). Consistent with this and most relevant to our hypothesis, Pogun (2001) describes data from his laboratory showing that adult female rats display a greater increase in NAcc dopamine following nicotine administration as compared to males. Taken together, these data suggest that nicotine may produce greater increases in NAcc dopamine in the presence of estrogen. Future studies are needed to fully assess the proposed mechanisms.

With regard to within-system changes during withdrawal, dopamine is decreased in the NAcc of adult male rats experiencing nicotine withdrawal (Carboni et al., 2000; Hildebrand et al., 1998; Rada et al., 2001). Recent work in our laboratory has shown that dopamine levels in the NAcc are reduced to a greater extent in adult versus adolescent rats (Natividad et al., 2010). These data suggest that NAcc dopamine modulates age-dependent differences produced by nicotine withdrawal. Here, we extend our hypothesis regarding the difference between adolescents and adults to sex differences, by suggesting that changes in dopamine systems also modulate enhanced nicotine withdrawal in females. Specifically, we suggest that the changes in dopamine transmission produced by withdrawal in females are potentiated by stress systems within the NAcc, as described below.

7.2. Between-system changes in CRF

Pre-clinical studies have shown that CRF systems modulate the motivational effects of stress produced by nicotine withdrawal (for a review see Bruijnzeel, 2012). Work in this area has focused on the effects of nicotine withdrawal in adult male rats. For example, blockade of CRF-1 receptors reduces the escalation of nicotine IVSA in rodents given extended access to this drug (George et al., 2007). Also, nicotine-withdrawal produces a deficit in brain reward function that was prevented by activation of CRF receptors (Bruijnzeel et al., 2007). Activation of CRF receptors also reverses hyperphagia and weight gain produced by withdrawal from chronic nicotine (Kamdi et al., 2009). Blockade of CRF-1 receptors in NAcc reverses the deficits in brain reward function produced by nicotine withdrawal (Marcinkiewicz et al., 2009). This suggests that CRF systems play a role in modulating stress produced by nicotine withdrawal, and that the NAcc is an important neuronal structure implemented in these effects.

The interaction between CRF and dopamine in the NAcc appears to change over time following as a function of chronic stress. For example, initial activation of CRF systems increases dopamine transmission in this region (Chen et al., 2012; Lemos et al., 2012). This

suggests that the relationship between CRF and dopamine in the NAcc is facilitative. However, another report suggests that effects of CRF in the NAcc undergo a dynamic shift following chronic stress. Specifically, intra-NAcc infusions of CRF produce a place preference in naïve rats (Lemos et al., 2012). However, following chronic stress intra-NAcc infusions of CRF produce a place aversion. The later report also showed that the ability of CRF to increase NAcc dopamine is reversed in rats that received chronic stress, an effect that persisted for 90 days after stress exposure. These data suggest that the modulatory role of CRF on dopamine systems within the NAcc is switched such that following chronic stress, CRF undergoes a dynamic change from facilitating hedonic states to inducing negative aversive states.

It is our hypothesis that the shift in the relationship between CRF and dopamine is modulated via GABA systems. Within the NAcc, GABAergic interneurons inhibit dopamine release. We suggest that activation of the CRF –1 receptors results in the increase of GABA levels that inhibit dopamine release in this region. This is based on the finding that activation of CRF –1 receptors increases GABAergic inhibitory post-synaptic potentials in dopamine neurons of the mesocorticolimbic pathway (Beckstead et al., 2009). Thus, chronic exposure to nicotine is expected to enhance the release of CRF. Therefore, we suggest that a greater activation of CRF-1 receptors produces an increase in local GABA levels, which in turn leads to a decrease in dopamine during withdrawal. This hypothesis relies heavily on enhanced GABA levels in the NAcc during withdrawal from nicotine, and future studies are needed to examine the influence of CRF systems on GABA levels in the NAcc during withdrawal. However, there is evidence supporting our hypothesis in cortical terminals of the mesocorticolimbic pathway where GABA levels are increased following chronic CRF administration (Kirby et al., 2008; Sirinathsingji and Heavens, 1989).

With regard to sex differences during nicotine withdrawal, we suggest the ability of CRF to modulate dopamine systems is disproportionately greater in females. We furthermore propose that sex-dependent differences in CRF modulation of dopamine occur within the local circuits of the NAcc. This is based on recent work in our laboratory showing that changes in CRF gene expression were increased in the NAcc of female versus male rats (Figure 3). There are several ways in which females may be more susceptible to stress during withdrawal. Given that females display less negative feedback inhibition of the CRF systems, nicotine withdrawal may produce a stronger and more sustained activation of stress systems as compared to males. This may involve higher numbers of CRF receptors or a higher ratio of CRF-1 receptor to coupling of G-proteins in female versus male rats (Bangasser et al., 2010). Females also display lower levels of beta-arrestin2, an intracellular protein that internalizes CRF-1 receptors (Bangasser et al., 2010). Thus, females may be more responsive to CRF stimulation due to reduced internalization of CRF-1 receptors. By extension, the female CRF system may be more activated by CRF release during nicotine withdrawal as compared to males.

Another possibility for explaining greater female susceptibility to nicotine use is that estrogen may potentiate the effects of stress produced by nicotine withdrawal in females versus males. Viau et al. (2005) showed that CRF mRNA levels are higher in the hypothalamus of female versus male rats. Also, the highest levels of CRF have been observed during the proestrus phase of the estrous cycle, where estrogen levels are highest (Bohler et al., 1990; Nappi et al., 1997). Direct activation of estrogen-beta receptors (ER β) also increases CRF mRNA expression in vitro (Chen et al., 2008; Lalmansingh and Uht, 2008; Zhu and Zhou, 2008). The estrogen gene sequence has also been shown to promote CRF gene transcription (Vamvakopoulos and Chrousos, 1993). These studies suggest that estrogen may also potentiate the female response to stress.

Taken together, we suggest that repeated withdrawal from nicotine produces hyper-activation of CRF stress systems in females. This may occur via a sensitization mechanism or an inability to return to normative stress levels. Given that females display a stronger recruitment of CRF systems during withdrawal, these changes represent an even greater allostatic load for females that display heightened stress during withdrawal as compared to males.

To summarize, we suggest that the acute rewarding effects of nicotine are greater in females via estrogen receptors that reduce GABAergic inhibition of dopamine as well as a down-regulation of D2 auto receptors in the NAcc. However, following chronic activation of stress systems by repeated nicotine withdrawal episodes, CRF systems in the NAcc switch from facilitating dopamine to inhibiting dopamine release in the NAcc. We suggest that this change in CRF modulation on dopamine transmission is the result of increased GABA levels within the NAcc. Furthermore, we hypothesize that this effect is larger in females due to a greater activation of GABA via CRF-1 receptors as compared to males. During withdrawal, one might expect that in females estrogen may serve to produce a larger inhibition of GABA. However, we suggest that the larger effect of CRF in females overrides the inhibitory effects of estrogen on GABA within the NAcc. Our hypothesis with regard to GABA is supported by a recent report suggesting that drug reward and aversive effects are modulated via GABAergic medium spiny neurons that tonically inhibit dopamine release in the NAcc (Carlezon and Thomas, 2009).

8. Hypothesis for nicotine use in adolescent females

An important question for our research program has been whether adolescent females are particularly more vulnerable to nicotine use because they are both adolescent and female. In general, our assessment has been that adolescent females follow an addiction profile that is more closely akin to what has been described for adolescents as opposed to adult females. With regard to the rewarding effects of nicotine, adolescent females display greater rewarding effects of nicotine than adult females (see Figure 7), consistent with other reports in mice (Kota et al., 2008). With regard to nicotine withdrawal, female adults display aversive effects produced by nicotine withdrawal that are absent in adolescent female rats (Figure 2). In addition, female adults also display behavioral and biological indices of stress produced by nicotine withdrawal that are also lower in female adolescents. These data suggest that adolescent females experience stronger rewarding effects of nicotine (denoted as a larger plus sign), but fewer negative effects of withdrawal (denoted with a smaller minus sign) relative to adult females.

	Reward	Withdrawal
Female		
Adults	+	—
Adolescents	+	-

Here, we propose a mechanistic hypothesis describing why adolescent females may experience stronger rewarding effects of nicotine and are impervious to the negative effects of withdrawal as compared to adult females. Our hypothesis is that GABA and glutamate systems in the VTA are organized in a manner that protects adolescents from the decreases in dopamine in NAcc produced by nicotine withdrawal in adult rats (Figure 7). Our work with adult male rats demonstrated that nicotine withdrawal produced a decrease in NAcc dopamine that was the result of an increase in GABA levels and a decrease in glutamate levels in the VTA (Natividad et al., 2010; 2012). However, adolescent rats experienced

lower withdrawal-related deficits in NAcc dopamine than adults. The lack of changes in dopamine levels appears to be related to an absence of changes in GABA and glutamate in the VTA of adolescent rats. To explain the differences between adolescents and adults, we postulated that excitatory regulation of dopamine is enhanced whereas inhibitory regulation of this pathway is reduced. This is based on previous literature showing that adolescence is characterized by heightened excitatory glutamatergic influence in the cortex (de Graaf-Peters and Hadders-Algra, 2006; McDonald and Johnston, 1990; Dunah et al., 1996; Herlenius and Lagercrantz, 2004) and diminished inhibitory GABAergic influences (Cherubini et al., 1991; Paysan et al., 1994; Yu et al., 2006). Thus, it is possible that this age-related imbalance toward excitation serves to buffer against withdrawal-associated decreases in excitatory drive and enhancements in inhibitory signaling.

Our neurochemical hypothesis regarding the special vulnerability of adolescent females to nicotine use also focuses on the contribution of GABA and glutamate systems in the VTA that regulate dopamine release in the NAcc. With regard to nicotine reward, it is suggested that adolescent females display a greater increase in NAcc dopamine as compared to adults following nicotine administration. We hypothesize that this effect is modulated via enhanced excitatory glutamate and reduced inhibitory GABA release in the VTA following nicotine administration. Our hypothesis regarding the role of glutamate systems in age-dependent differences in nicotine use has been described (Placzek et al., 2009). Also, the ability of estrogen to decrease GABA is greater in adolescents because the GABA system at this stage in development is still underdeveloped. Thus, the net result is that nicotine produces a greater increase in NAcc dopamine as compared to adult females.

With regard to nicotine withdrawal, we postulate that adolescent females will display a smaller decrease in NAcc dopamine than adults. We hypothesize that this effect may be modulated via higher glutamate and reduced GABA release in the VTA of adolescent females, as was shown in adolescent males. Moreover, we suggest that in adult females GABA systems play an intermediary role between CRF and decreases in NAcc dopamine produced by withdrawal. However, adolescent female GABA systems are underdeveloped, which means that the ability of CRF to decrease GABA in the NAcc is reduced. In fact, we recently observed that nicotine withdrawal produced an up-regulation of CRF gene expression in the NAcc of female adult, but not female adolescent rats. Given that CRF mRNA in the NAcc is synthesized in GABA interneurons, we suggest that adolescents displayed fewer changes in CRF gene expression due to underdeveloped GABA systems. The GABA interneurons that control CRF production are absent and/or underdeveloped in adolescent female rats. Thus, adolescents may be less sensitive to the decreases in NAcc dopamine due to enhanced excitation of glutamate in the VTA and reduced GABAergic inhibition of dopamine transmission in the VTA and NAcc. This neurochemical profile is consistent with our behavioral finding that adolescent females are less sensitive to stress produced by nicotine withdrawal than adult females.

9. Do the factors that contribute to nicotine use overlap in females of different ages?

A common finding among adolescent rats of both sexes and female adult rats is that they both display *enhanced* rewarding effects of nicotine compared to their respective controls. Adolescent and adult female rats also display reduced short-term aversive effects of nicotine as compared to age-matched control males (Torres et al., 2008; 2009). These findings suggest that populations that are susceptible to nicotine use experience strong rewarding effects of nicotine that promote continued nicotine use. Also, a lack of initial aversive effects of nicotine may facilitate intake of higher amounts of nicotine that would also promote dependence on this drug.

However, a major point of divergence in our work with female adolescent and adult rats has been with regard to nicotine withdrawal. Adolescent females are less sensitive to nicotine withdrawal, whereas adult females display intense stress during withdrawal from this drug. In our neurochemical analyses, different biological substrates have emerged as important for modulating age and sex differences produced by nicotine withdrawal. Our work with adolescent rats has demonstrated that GABA and glutamate systems in the VTA play an important role in modulating age differences to nicotine. In contrast, our work with female adults suggests that stress is a critical factor that modulates sex differences to nicotine. These findings imply that different mechanisms modulate nicotine use in females of different ages. Namely, during adolescence GABA and glutamate systems promote nicotine reward while at the same time they protect from the consequences of withdrawal from this drug. When a female reaches adulthood, the systems that promote nicotine reward are fully developed, such as estrogen facilitation of NAcc dopamine release. Also, adult females outgrow the mechanisms that protected them from withdrawal during adolescence. Importantly, adult females also have developed stress systems that promote nicotine withdrawal, such as CRF systems. Future studies are needed to examine sex differences in the VTA and age differences in the NAcc that may also promote nicotine use in females.

It is difficult to find a single explanation for enhanced vulnerability for nicotine use across age and sex. This is due, in large part, to the paucity of studies that assess the effects of nicotine in female and male rats at different stages of development. However, a study assessing this question revealed that adolescent female rats increase nicotine IVSA as they enter adulthood, whereas males do not display this effect (Levin et al., 2011). Consistent with this, adolescent female rats display a greater increase in nicotine/acetalddehyde IVSA as they enter adulthood as compared to males (Park et al., 2007). These studies suggest that adolescence is a unique period of development characterized by enhanced rewarding effects of nicotine. Adolescent females may also display an increase in nicotine use as they age with fully developed estrogen systems that facilitate dopamine and promote stress responses elicited by nicotine withdrawal.

10. Are the hypothesized mechanisms sufficient to understand sex differences to nicotine?

The proposed framework is intended to provide a testable hypothesis regarding sex differences to the behavioral effects of nicotine. This mechanism involves dopamine transmission in the mesocorticolimbic pathway and its regulation via stress systems in the NAc. With regard to sex differences in adolescent rats, our proposed mechanism involves excitatory and inhibitory mechanism in the cell body region of this pathway. Although this framework focuses on one particular neural system, it is recognized that the proposed mechanisms are not likely sufficient to explain the complex sex and age differences to the behavioral effects of nicotine.

One important factor to consider with regard to the addictive properties of tobacco is the presence of monoamine oxidase (MAO) inhibitors that suppress the breakdown of monoamines, such as dopamine (Fowler et al., 2003). Indeed, MAO inhibitors have been shown to enhance the rewarding effects of nicotine in rodents (Guillem et al., 2006; Kapelewski et al., 2011). With regard to age differences, MAO inhibitors have been demonstrated to promote the anti-depressant effects of nicotine in adolescent rats (Villegier et al., 2010). With regard to sex differences, female rats display greater sensitivity to MAO-B inhibition following L-deprenyl administration (Murphy et al., 1993). Thus, the possibility exists that MAO inhibition may contribute to the anti-depressant effects of tobacco that promote smoking behavior in women.

Another factor to consider with regard to age and sex differences to the behavioral effects of nicotine is the density of nicotine binding sites in the brain. Nicotine binds to a family of cholinergic receptors consisting of pentameric membrane proteins of homomeric or heteromeric complexes of α or β subunits. To date, 12 subunits have been identified ($\alpha 2-10$ and $\beta 2-4$), and the various combinations of these subunits leads to differences in channel activation and desensitization in the presence of nicotine (Leslie et al., 2013). With regard to age differences, it is well established that the emergence of the various nicotine acetylcholine receptors (nAChRs) is developmentally different, and the expression and/or functional properties of these subunits are likely to contribute to behavioral differences to nicotine in adolescent and adults (see Slotkin, 2002). Furthermore, there are several reported long-term effects of adolescent nicotine exposure on cholinergic regulation of transmitter release, neurite outgrowth, cell survival and synaptogenesis (see Dwyer et al., 2008). With regard to sex differences, females display higher nAChR densities in various brain regions; however, chronic exposure to nicotine produces a larger up-regulation of nAChRs in males as compared to female rats (Koylu et al., 1997) and mice (Mochizuki et al, 1998). Accordingly, male smokers display an up-regulation of $\beta 2$ receptors in the striatum during abstinence from chronic tobacco use, an effect that was not observed in females (Cosgrove et al., 2012). However, it is acknowledged that when rats self-administer nicotine, no sex differences in nAChRs were observed (Donny et al., 2000). Future work is needed to systematically examine the contribution of different nAChRs in modulating sex differences produced by nicotine.

11. Concluding statements regarding clinical implications

The hypothesis that vulnerable populations display strong rewarding effects of nicotine implies that prevention strategies are important for discouraging experimentation with tobacco products in young persons. Legislative approaches are also important to limit marketing strategies that promote nicotine use in vulnerable populations, such as tobacco packaging designed to entice women. In fact, a recent report showed that cigarettes in branded packaging were rated as more appealing, better tasting, and more closely associated with style and sophistication in female smokers (White et al., 2012). There have been some limitations on the tobacco industry with regard to marketing to adolescents; however, more legislation is needed to limit marketing strategies that target women.

The hypothesis that nicotine withdrawal is qualitatively different between adolescents and adults implies that medications targeting withdrawal should not be prescribed in a unilateral manner across smokers of different ages. One might also predict that treatments that target withdrawal may be less effective in adolescent versus adult smokers, regardless of sex. Moreover, nicotine replacement therapies in adolescent smokers may increase the probability of nicotine use later in life. Our observation that adult female rats experience intense stress during nicotine withdrawal implies that cessation treatments that are given in combination with anxiolytics medications may be more effective in women versus men smokers. In fact, a recent report showed that sazetidine-A, a selective nicotinic receptor desensitizing agent and partial agonist is less effective at reducing nicotine IVSA in female versus male rats (Johnson et al., 2012). This drug may be less effective in women smokers because it does not target stress systems that are critical in modulating nicotine use in females.

In conclusion, serious consideration should be given to specialized medicines for tobacco cessation in different vulnerable populations. It is important to recognize and respond to differences in the factors that motivate nicotine use in distinct populations. This review posited several empirical questions for future studies. This basic research has translational

value toward developing more effective cessation medications in populations that are especially vulnerable to nicotine use.

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Highlights

- Females display enhanced nicotine reward and stress during withdrawal than males.
- Females display enhanced nicotine reward via estrogen.
- Adolescent females display enhanced nicotine reward and reduced withdrawal.
- Medications targeting withdrawal should not be applied in a unilateral manner.
- Pre-clinical work has translational value for reducing health disparities in females.

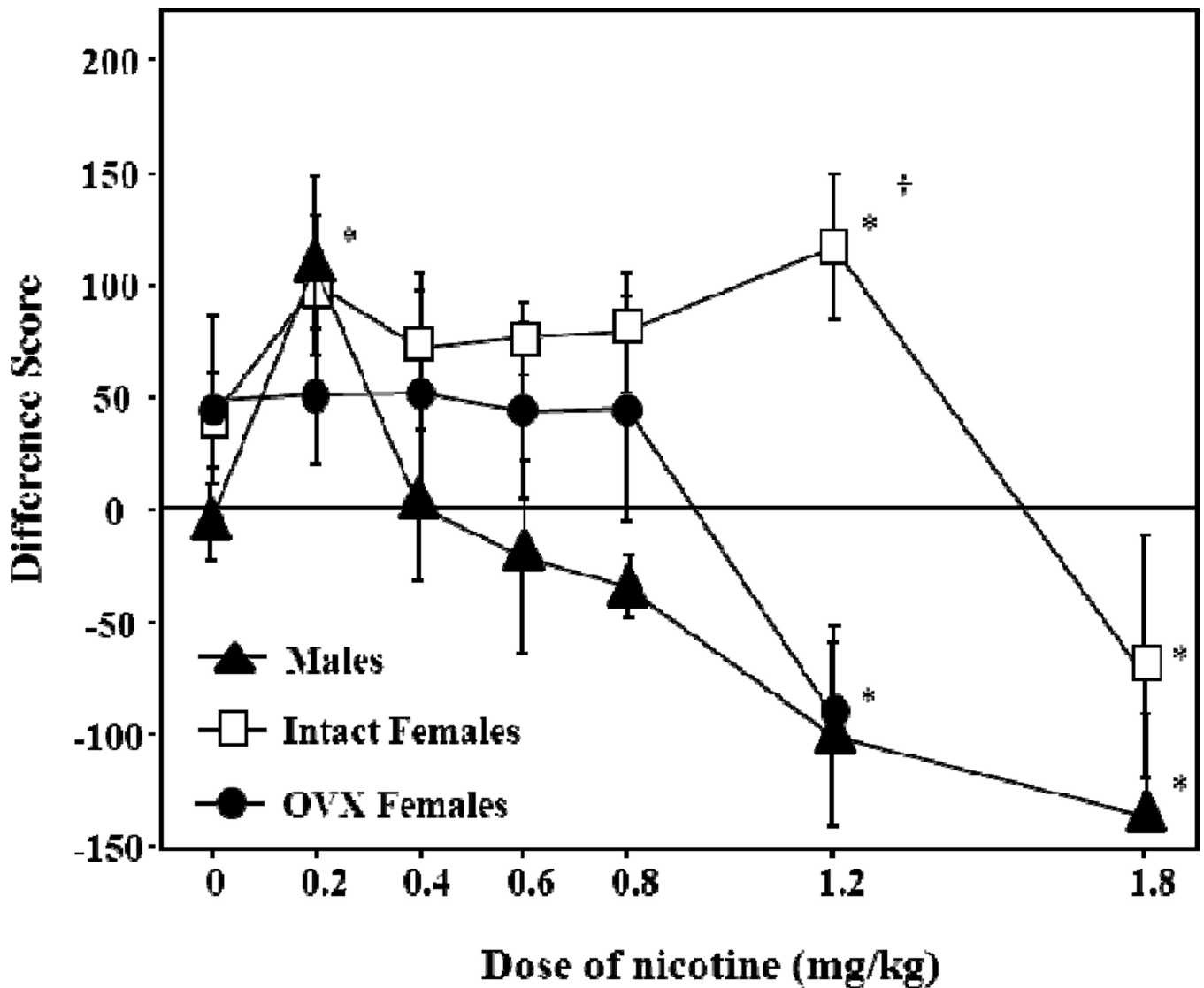


Fig. 1. Enhanced rewarding and reduced aversive effects of nicotine in female versus male rats
 This graph reflects place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, or 1.8 mg/kg, base, sc) in adult male, intact female and OVX female rats. These data are presented as difference scores (\pm SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above “0” reflect a positive shift in preference (i.e., CPP); whereas, values below “0” represent a negative shift in preference (i.e., CPA). The asterisks denote a significant difference from their respective saline controls ($p < 0.05$) and daggers denote a significant difference between treatment groups ($p < 0.05$). These data were previously published in Torres et al. (2008).

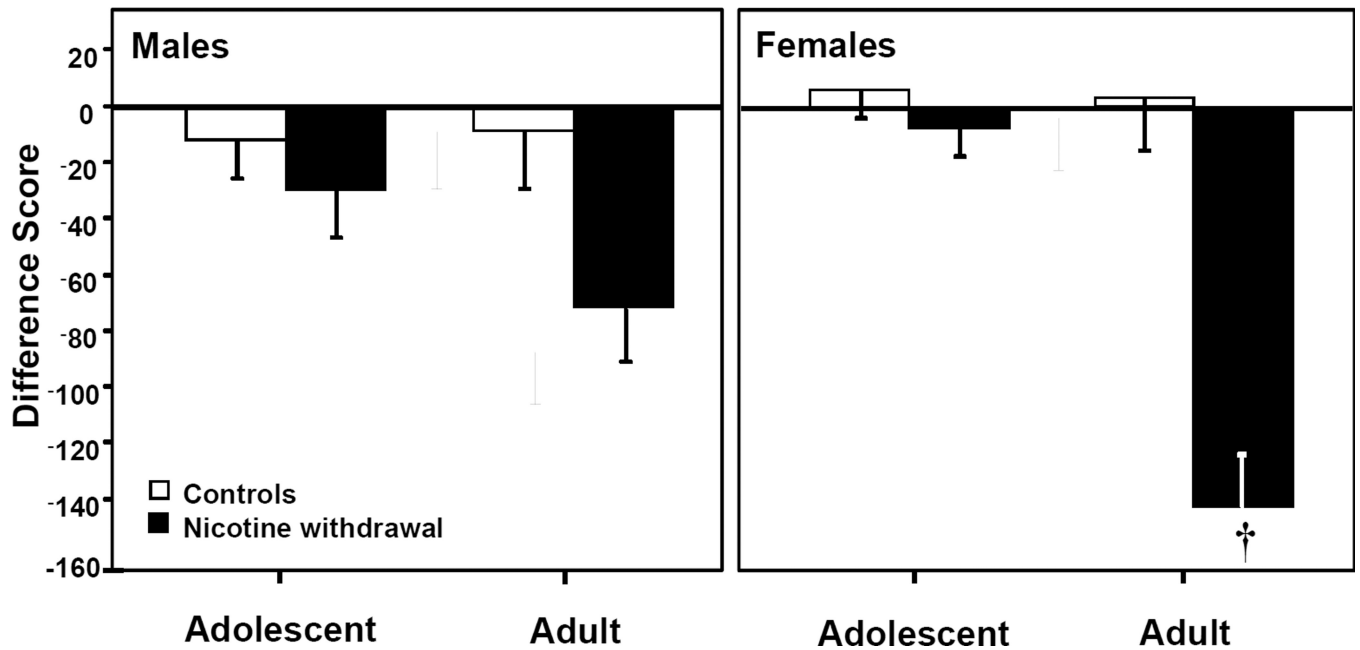


Fig. 2. Enhanced negative affective states produced by nicotine withdrawal in female versus male rats

This graph reflects place aversion produced by nicotine withdrawal in adolescent and adult female and male rats. Rats ($n=7-14$) were tested for initial preference and were then prepared with saline or nicotine pumps. A dose of nicotine was used that produced equivalent plasma cotinine levels (a metabolite of nicotine) across age and sex treatment groups. After 7 days of nicotine exposure, the rats were conditioned with repeated injections of mecamylamine to precipitate withdrawal in their initially preferred side. On alternate days, they received saline in their initially non-preferred side. Following 8 days of conditioning, the rats were re-tested for any shifts in preference behavior. The data reflect difference scores (\pm SEM) that were calculated as time spent in the initially preferred side after minus before conditioning, such that negative values reflect the aversive effects of nicotine withdrawal (i.e., CPA). Female rats displayed a significant decrease in time spent in their withdrawal-paired side that was larger than all other treatment groups ($\dagger p < 0.05$).

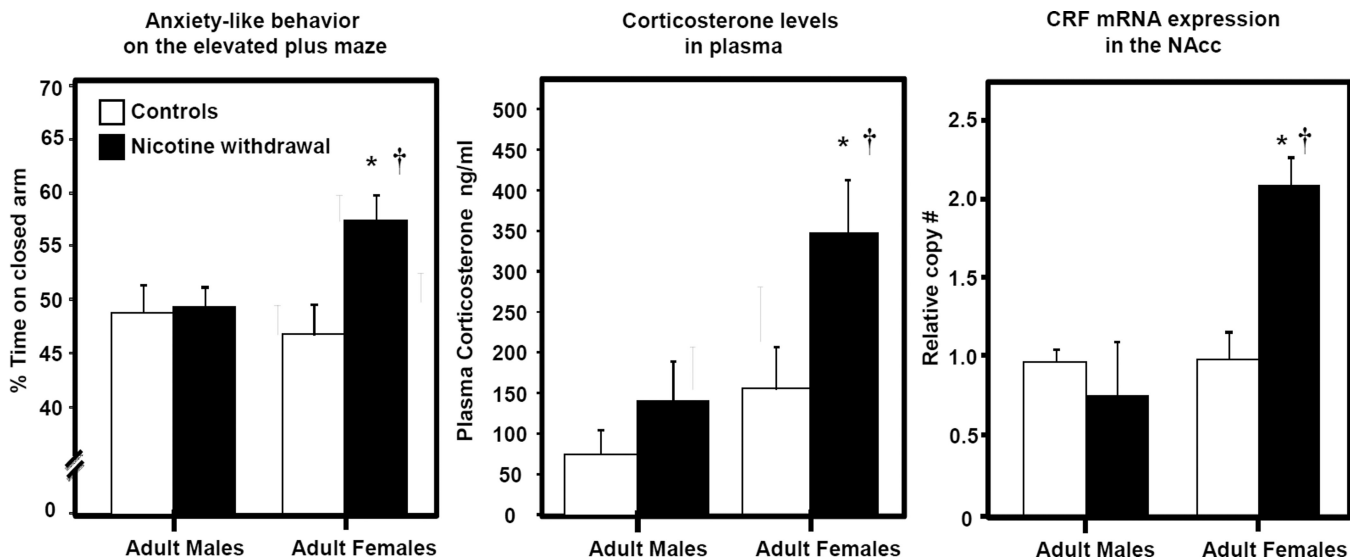
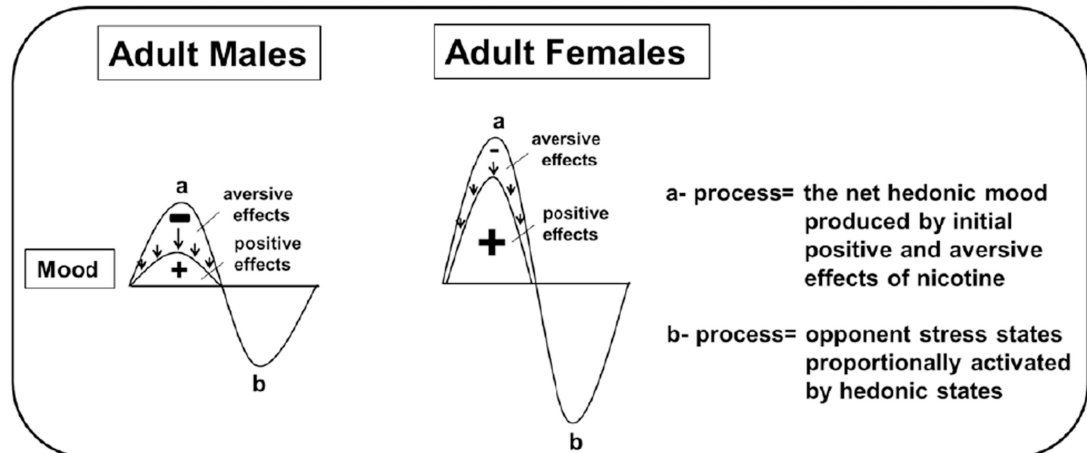


Fig. 3. Enhanced behavioral and biological indices of stress produced by nicotine withdrawal in adult female versus male rats

This graph reflects anxiety-like behavior, plasma corticosterone levels and changes in CRF gene expression in the NAcc during spontaneous withdrawal from nicotine in adult female and male rats ($n=6-8$). Control rats were not exposed to nicotine and received a sham surgery. All nicotine-treated rats displayed similar plasma cotinine levels (a metabolite of nicotine) prior to the removal of nicotine, suggesting that our effects are not confounded by sex differences in nicotine metabolism. The asterisks (*) denote a significant difference from their respective sham controls and the dagger (†) denotes a significant difference between males and females ($p < 0.05$). *These data are under review for publication in another journal.*

Short-term effects of nicotine use



Long-term effects of nicotine use

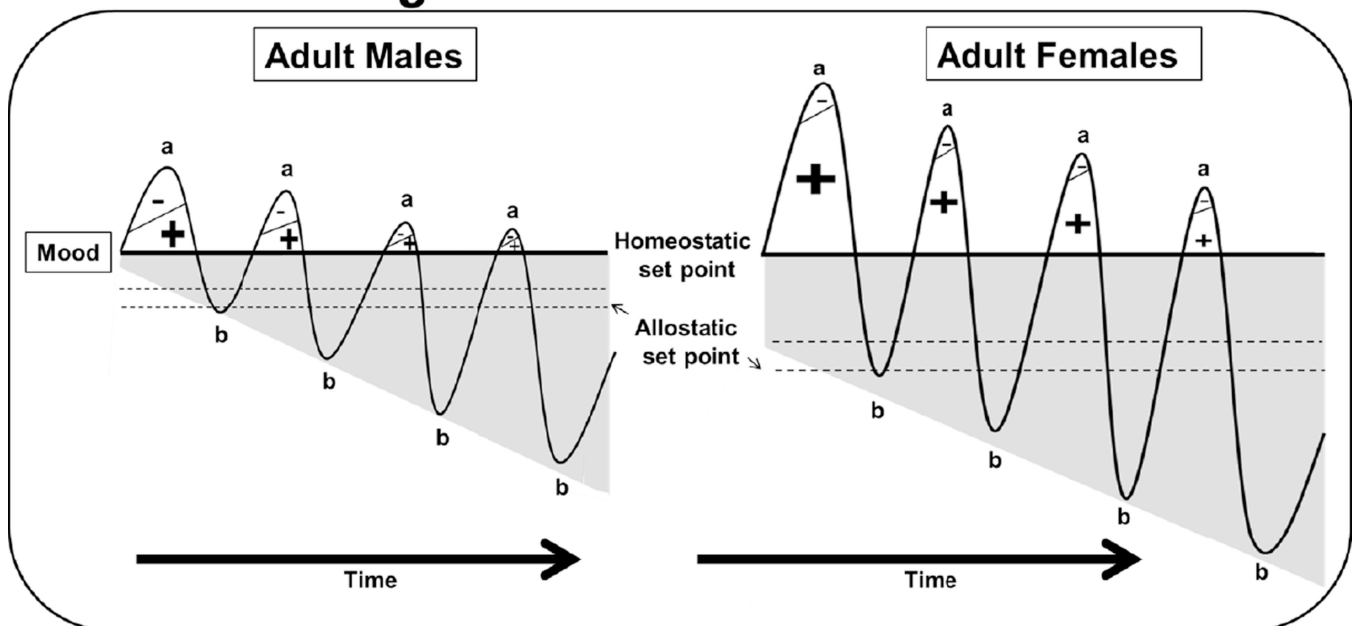


Fig. 4. Opponent-process theory of nicotine use in females

The diagrams denote changes in mood states induced by short-term (top) and long-term (bottom) nicotine use. Our hypothesis regarding enhanced vulnerability to nicotine use in females is derived from the opponent-process theory of addiction that was developed by Koob and Le Moal (2001). These researchers hypothesize that there are two processes that influence the development of drug dependence. These a- and b-processes oppose each other and change over the course of long-term drug use. Following initial nicotine use, the net hedonic state (a-process) induced by nicotine reflects the initial positive effects of nicotine minus any aversive effects. We hypothesize that females experience greater positive mood states (denoted with a larger plus sign) due to stronger rewarding effects of nicotine and

minimal initial aversive effects of this drug as compared to males. The b-process reflects the activation of stress systems that oppose the initial hedonic state elicited by nicotine. Furthermore, the magnitude of the a-process determines the extent to which the opponent b-process is recruited. Thus, we hypothesize that because females experience greater initial hedonic responses to nicotine, they will also experience greater recruitment of opponent stress systems than males. Koob and Le Moal also postulate that following initial drug use, with no prior drug history; ample time is provided for the a- and b-processes to return to a normal homeostatic set point. However, repeated drug use produces a dysregulation in the opponent processes that maintain homeostasis, as reflected by the downward shift in the homeostatic set point to a new allostatic set point. We suggest that chronic nicotine exposure produces a greater sensitization of stress systems in females than males. Thus, during nicotine withdrawal, the cumulative stress effects lower the allostatic set point to a greater extent in females than males. As a result, long-term nicotine use in females is driven to a larger extent by avoidance of intense stress states produced by nicotine withdrawal than males.

Hypothesized substrates mediating sex differences in nicotine use

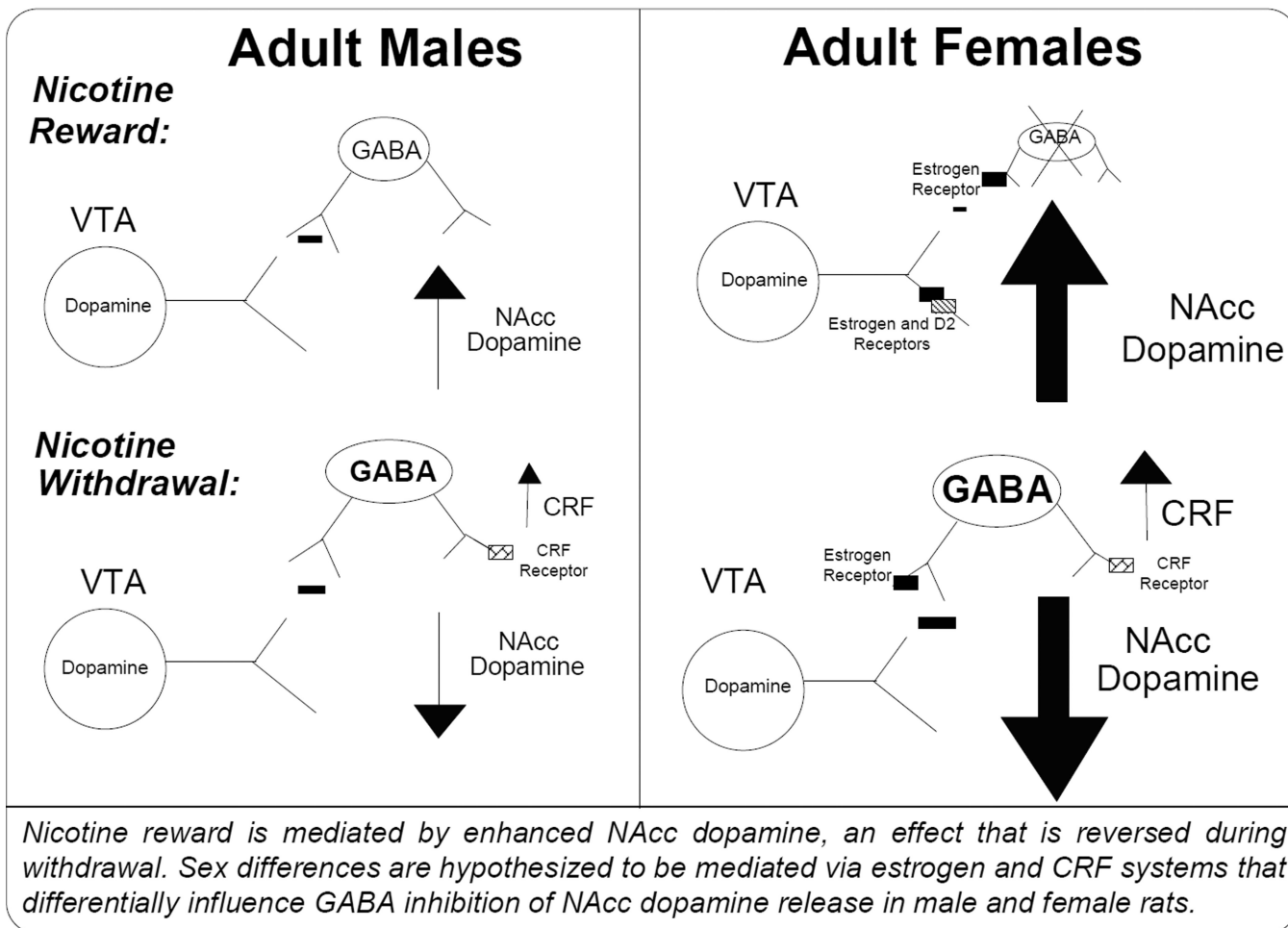


Fig. 5. Hypothesized mechanisms mediating nicotine use in females

The rewarding effects of nicotine are mediated via enhanced dopamine transmission in the NAcc. The dopamine terminals in this region are under inhibitory control via GABA interneurons that have estrogen receptors on them. Estrogen receptor activation is postulated to reduce GABA inhibition of dopamine, producing a greater increase in NAcc dopamine following nicotine administration. During nicotine withdrawal, dopamine levels in the NAcc are decreased, an effect that is hypothesized to be related to enhanced GABA release in this region. We hypothesize that female rats will display a greater suppression of dopamine that is modulated via CRF systems in this region. Thus, sex differences are related to overactive CRF systems that modulate dopamine release in the NAcc.

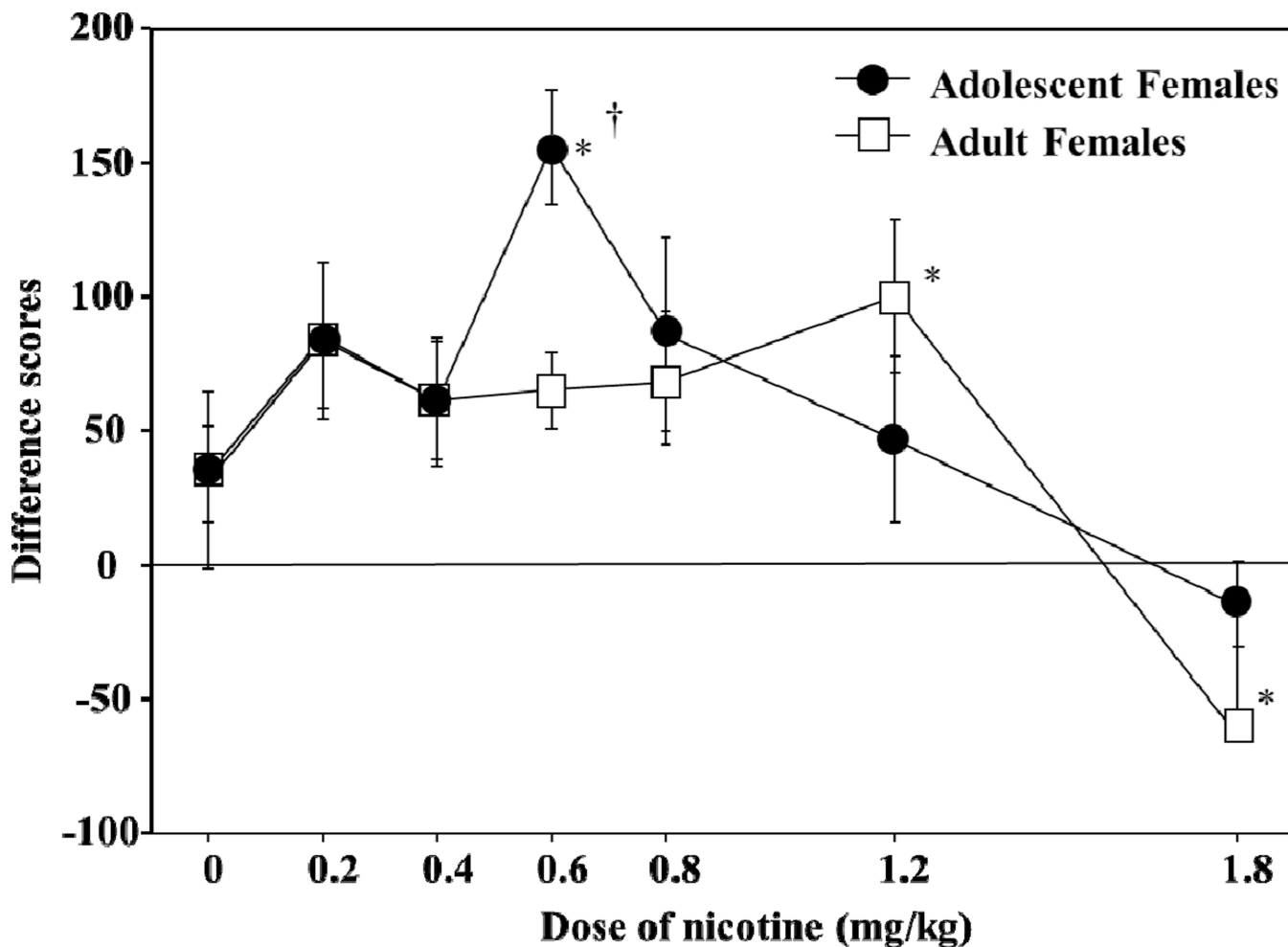


Fig. 6. Enhanced rewarding effects of nicotine in adolescent versus adult female rats

This graph reflects place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, or 1.8 mg/kg, base, sc) in adolescent and adult female rats. These data are presented as difference scores (\pm SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above “0” reflect a positive shift in preference (i.e., conditioned place preference); whereas, values below “0” represent a negative shift in preference (i.e., conditioned place aversion). The asterisks denote a significant difference from their respective saline controls ($p < 0.05$) and the dagger denotes a significant difference between treatment groups ($p < 0.05$). These data were previously published in Torres et al. (2008).

Hypothesized substrates mediating age differences in nicotine use in females

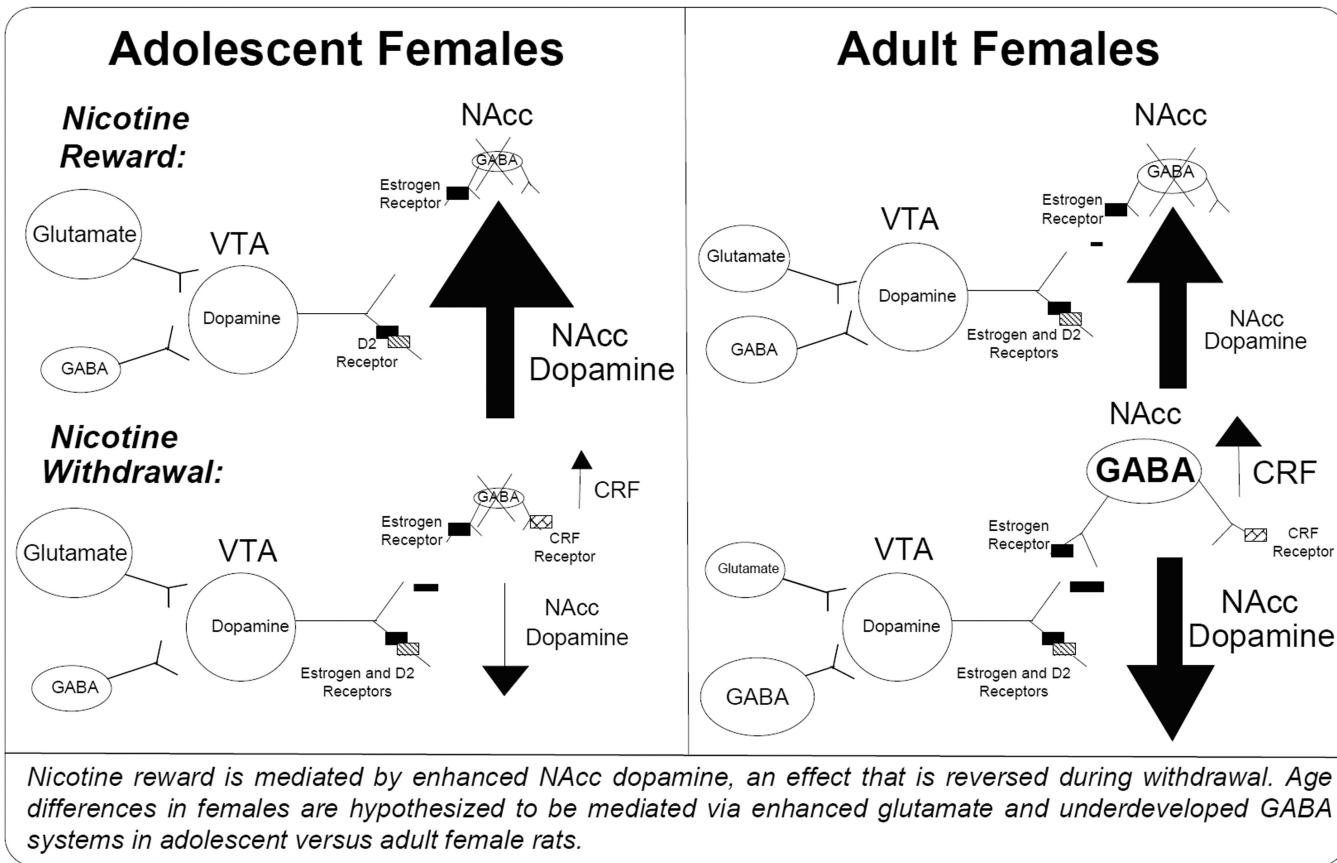


Fig. 7. Hypothesized mechanisms mediating nicotine use in adolescent females

The VTA is under inhibitory control via GABA and excitatory regulation via glutamate release that regulates dopamine transmission in the NAcc. During nicotine withdrawal, dopamine levels in the NAcc are decreased, an effect that is hypothesized to be related to enhanced GABA and reduced glutamate in the VTA. We hypothesize that adolescent rats will display higher increases in NAcc dopamine following nicotine and fewer changes in this measure during withdrawal. We also hypothesize that these age differences are related to overdeveloped glutamate and underdeveloped GABA systems that modulate dopamine cell bodies in the VTA that project to the NAcc.