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Sex dependent effects of nicotine on the developing brain

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Abstract

The use of tobacco products represents a major public health concern, especially among women. Epidemiological data have consistently demonstrated that women have less success quitting tobacco use and a higher risk for developing tobacco-related diseases. The deleterious effects of nicotine are not restricted to adulthood, as nicotinic acetylcholine receptors regulate critical aspects of neural development. However, the exact mechanisms underlying the particular sensitivity of women to develop tobacco dependence have not been well elucidated. In this review, we show that gonadal hormone-mediated sexual differentiation of the brain may be an important determinant of sex differences in the effects of nicotine. We highlight direct interactions between sex steroid hormones and ligand-gated ion channels critical for brain maturation, and discuss the extended and profound sexual differentiation of the brain. We emphasize that nicotine exposure during the perinatal and adolescent periods interferes with normal sexual differentiation and can induce long-lasting, sex-dependent alterations in neuronal structure, cognitive and executive function, learning and memory, and reward processing. We stress important age and sex differences in nicotine's effects and emphasize the importance of including these factors in preclinical research that models tobacco dependence.

Graphical Abstract

Sex differences in the effects of nicotine may be due to gonadal hormone-mediated sexual differentiation of the brain during the perinatal and adolescent periods. Exposure to nicotine during these developmental periods can produce long-lasting, sex-dependent changes in neuronal structure and function by inhibiting aromatase or inducing corticosterone release, for example.

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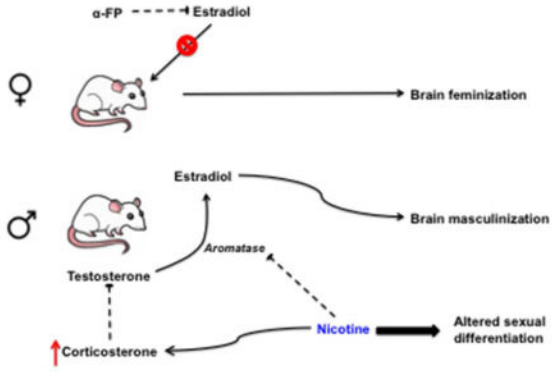
Conflict of Interest Statement

The authors do not have any known or potential conflicts of interest, including any financial, personal, or other relationships within three years of beginning the submitted work, with people or organizations that could inappropriately influence or be perceived to influence their work. No conflicts have been identified.

Role of Authors

SJC, KEL, and FML conceived the work, reviewed and discussed the manuscript, contributed to writing the article, and approved the article in its final form.



Keywords

Nicotinic acetylcholine receptors; hormones; tobacco; prenatal; neonatal; adolescent

Introduction

Nicotine exposure from tobacco products or electronic cigarettes (e-cigarettes) has numerous deleterious effects on overall health. Smoking remains one of the leading causes of preventable death in the United States despite heightened awareness of its dangers (Centers for Disease Control and Prevention 2011), and women seem to be particularly vulnerable. Women have lower success with cessation than men (Perkins 2001; Piper et al. 2010), and also have a higher risk of developing tobacco-related morbidity and mortality (Langhammer et al. 2003; Laviolette et al. 2007; Kiyohara and Ohno 2010; Allen et al. 2014). Preclinical research has also demonstrated that females are more sensitive than males to the rewarding effects of nicotine, as well as more sensitive to the aversive effects of nicotine withdrawal (O'Dell and Torres 2014).

In addition to negatively impacting overall health in adulthood, a large body of literature has indicated that nicotine can have detrimental effects on the brain throughout development, with important sex differences. Nicotine's primary effects are mediated by activation and desensitization of neuronal nicotinic acetylcholine receptors (nAChRs), pentameric ligand-gated ion channels consisting of α2-α10 and β2-β4 subunits (Dwyer et al. 2009). Cholinergic activity at nAChRs is critical for neuronal path-finding, patterning and organization of sensory systems, and regulation of neurochemical systems involved in tobacco addiction (Lipton et al. 1988; Pugh and Berg 1994; Rossi et al. 2001; Feller 2002; Gotti and Clementi 2004; Dwyer et al. 2009). Exposure to nicotine during these critical developmental events can have profound and long-lasting effects. Approximately 10% of women continue to smoke during pregnancy (Tong et al. 2013), despite increased risk for sudden infant death syndrome (SIDS), attentional and cognitive deficits, and drug addiction in offspring (McCartney et al. 1994; Weissman et al. 1999; Buka et al. 2003; Dietz et al. 2010; Goldschmidt et al. 2012). Nicotine's deleterious effects are not limited to the perinatal period, however, but extend to adolescence, a developmental period marked by continued maturation of brain regions critical for reward processing, learning and memory, and

executive function (Yuan et al. 2015). Even brief exposure to nicotine during the adolescent period can have long-lasting effects on neurochemistry and behavior, including decreased cognitive function and enhanced drug reward (Jacobsen et al. 2005; Treur et al. 2015; Yuan et al. 2015).

The exact mechanisms underlying women's enhanced vulnerability to tobacco dependence have not been fully elucidated. However, as gonadal steroids coordinate sexual differentiation of the brain and can interact directly with ligand-gated ion channel receptors, they are likely determinants of sex differences in nicotine reward and reinforcement. The classical organizational-activational hypothesis says that gonadal hormones differentiate the brain into male and female early in development, while activity of estrogens, progesterone, and androgens activate neural circuits involved in reproductive function after puberty (Arnold 2009). However, a large body of literature has emerged in recent years highlighting the important role of gonadal hormones in modulating a variety of behaviors, including learning, memory, and drug reward and reinforcement. Further, in contrast to the short span of sexual differentiation suggested in the original hypothesis, recent data describe continued sexual differentiation throughout puberty and adolescence (Sisk and Zehr 2005; McCarthy and Arnold 2011).

In this review, we discuss critical interactions between gonadal hormones and neurochemical systems mediating nicotine reward and reinforcement. We argue that sexual differentiation, beginning in the perinatal period and persisting into adolescence, is an important determinant of sex differences in brain structure and function that underlie females' unique sensitivity to nicotine.

Sexual differentiation, gonadal steroids, and ligand-gated ion channels

Sex differences in nicotine responses likely first emerge as a result of sexual differentiation of the brain. The earliest stages of sexual differentiation are determined by the SRY gene on the Y chromosome, which initiates testes development during the prenatal period. Absence of the SRY gene leads to the 'default' female phenotype, precluding masculinization and defeminization of the brain. Fetal masculinization then occurs under the control of gonadal hormones, with a surge of androgens that peaks at the end of the embryonic period in males before quickly falling after the first postnatal day (Konkle and McCarthy 2011). Testosterone is converted to estradiol via aromatase, an enzyme that is abundant and active during the perinatal androgen surge (George and Ojeda 1982; Roselli and Resko 1993) and is highly expressed in sexually dimorphic brain regions (Konkle and McCarthy 2011). In contrast, absence of the androgen surge and sequestration of excess estradiol by α-fetoprotein in the female results in early feminization of the brain.

Sex steroid hormones signal predominantly by binding to intracellular receptors, inducing dimerization and translocation to the nucleus where they bind to hormone response elements on DNA to regulate gene transcription (King and Greene 1984; O'Malley and Tsai 1992). However, recent data suggest that estrogens can induce more rapid signaling, likely via membrane-bound ERα receptors (Schultz et al. 2009), and that sex steroid hormones and their metabolites can interact directly with ligand-gated ion channels to influence excitatory

and inhibitory neurotransmission (Hu et al. 2006). Ligand-gated ion channels coordinate multiple aspects of brain maturation and are influenced by gonadal hormones, providing one potential mechanism by which sex affects nicotine responses.

Estrogen and progesterone act as allosteric modulators of GABA, glutamate, and acetylcholine receptors, often with opposing effects. In particular, estrogen is a negative allosteric modulator of $GABA_A$ receptors (Murphy et al. 1998), whereas progesterone and its neuroactive metabolites (i.e., allopregnanalone and pregnanalone) promote inhibitory neurotransmission via enhancement of $GABA_A$ receptor function (Paul and Purdy 1992; Lan and Gee 1994; van Wingen et al. 2008; Gunn et al. 2011; Puia et al. 2012) and inhibition of glutamate-induced excitation and NMDA receptor binding (Smith et al. 1987; Cyr et al. 2000). The specific effects of circulating hormones or their neuroactive metabolites on ligand-gated ion channels may depend on the overall hormone milieu. During puberty, when the hypothalamic pituitary gonadal axis reawakens and cyclical surges in estrogens and androgens begin, progesterone actually acts as a negative allosteric modulator of $GABA_A$ receptors (Smith et al. 2009). This effect of progesterone is only evident during puberty, as pre- and post-pubertal rodents show positive allosteric modulation of $GABA_A$ receptors.

Sex steroids also modulate nicotinic acetylcholine receptor (nAChR) function (Figure 1). Progesterone and its A-ring metabolites dose-dependently inhibit function of α3β4 and α4β2 nAChRs (Valera et al. 1992; Ke and Lukas 1996; Paradiso et al. 2000), although physiological levels of progesterone may not be sufficient to inhibit α4β2 nAChRs (Paradiso et al. 2000). In contrast, progesterone may enhance the function of α5-containing nAChRs by binding to a progesterone response element in the promoter region to increase α5 mRNA levels in cultured cells and in the brains of ovariectomized female rats (Gangitano et al. 2009). 17β-estradiol increases α7 nAChR subunit expression in the dorsal raphe and locus coeruleus of macaques (Centeno et al. 2006) and potentiates human, but not rat, α4β2 nAChRs by direct actions at the C-terminus of the α4 subunit that result in an increase in the probability of open conformation (Figure 2; Paradiso et al. 2000; Paradiso et al. 2001; Curtis et al. 2002; Jin and Steinbach 2015). Both rodent and recombinant human α3β4 nAChRs are noncompetitively inhibited by estrogens, with more profound inhibition occurring after chronic exposure (Ke and Lukas 1996; Nakazawa and Ohno 2001). Alpha 7 nAChRs, as measured by α-bungarotoxin (α-BTX) binding, can be regulated by estradiol, as pre-pubertal gonadectomy in females, but not males, decreases α-BTX binding in the suprachiasmatic nucleus. Binding can be normalized by semi-chronic (3 week) or long-term (5 week) estradiol replacement, while short-term replacement with estradiol has no effect (Miller et al. 1984).

Early sexual differentiation, nAChRs, and perturbation by nicotine

The gestational period is a dynamic and critical developmental window when sexual differentiation is initiated and the nervous system begins to form under the control of the cholinergic system. Even brief exposure to nicotine during this period can produce long-lasting, sex-dependent alterations in neuronal structure and function.

Development of the cholinergic system

Cholinergic activity is observed as early as the gastrulation phase with the detection of choline acetyltransferase (ChAT) and acetylcholinesterase in the fetus (Mansvelder and Role 2006), and is implicated in morphogenic cell movements during early gestation (Lauder and Schambra 1999). Nicotine's actions are mediated primarily by activation and desensitization of nAChRs, which appear during the first trimester in human brain and on the first day the rodent central nervous system forms, gestational day (G) 11. Each nAChR subtype has distinct and evolving patterns of distribution, implicating their differential roles in development and highlighting potential avenues for nicotine-associated perturbation (Dwyer et al. 2008).

nAChR activity is important for a variety of developmental functions and, in many cases, serves a critical ontogenetic role (for review, please see (Dwyer et al. 2009). Although most of the ontogenetic studies of nAChR function do not differentiate between sexes, one early study did note that α-BTX binding to α7 nAChRs in the mouse corticomedial amygdala was higher in males than females at postnatal day (P) 14, a sex difference that was eliminated by neonatal castration of males (Arimatsu 1983). Furthermore, nAChR-mediated excitation of corticothalamic neurons in layer VI of prefrontal cortex is significantly larger in male rats during the first postnatal month when prefrontal circuitry underlying attention is actively maturing (Alves et al. 2010). Sex differences have also been noted in the role of nAChRs in the development of rodent hippocampus (Figure 3). During gestation and the early postnatal period, hippocampal GABA activity evokes an excitatory cell response as a result of a high internal Cl^- concentration produced by predominance of the immature $Na^+/K^+/Cl^-$ cotransporter 1 (NKCC1) over the mature K^+/Cl^- cotransporter 2 (KCC2) (Rivera et al. 1999; Ben-Ari 2002). During the first two postnatal weeks, GABA switches to inhibitory signaling as levels of NKCC1 decline and KCC2 rise, a process that occurs significantly earlier in females than males (Nuñez and McCarthy 2007; Galanopoulou 2008). This switch in cotransporter expression is dependent on calcium influx into the cell, which is controlled by increased depolarization and activation of receptors with high calcium permeability, such as the α7 nAChR, which shows peak expression in hippocampus during this period (Galanopoulou et al. 2003; Galanopoulou 2008). Activation of presynaptic α7 nAChRs during this time also stimulates GABA release, as measured by giant depolarizing potentials (Maggi et al. 2001). Thus, the interaction of nAChR signaling with GABAergic transmission has a sex-dependent impact on early hippocampal development. Similar nAChR regulation of GABA signaling has been observed in other neural regions (Liu et al. 2006), some of which may exhibit sex-dependent transmitter regulation (McCarthy et al. 2002).

Nicotine, sex, and nAChRs

Many studies have examined the long-term consequences of prenatal nicotine exposure (Dwyer et al. 2008), but few have examined potential sex differences in exposure outcomes (Table 1). However, the clinical literature on maternal smoking does suggest that such differences occur (Weissman et al. 1999). Although prenatal nicotine exposure induces complex alterations in brain structure and function, it has been shown to have more significant deleterious effects on cholinergic and serotonergic markers and β2-adrenergic function in males than females (Slotkin et al. 2007). Prenatal nicotine exposure also induces

sex-dependent changes in myelin-associated gene and protein expression (Cao et al. 2013), and in dendritic complexity of the prefrontal cortex and nucleus accumbens (Mychasiuk et al. 2013). Subsequent drug reward is also sex-dependently altered by *in utero* nicotine exposure, with exposed males exhibiting increased preference for oral nicotine but no effect of maternal nicotine in females (Klein et al. 2003). Gestational nicotine may also alter normal sex-dependent nicotine responses by inhibiting aromatase activity (Barbieri et al. 1986). Prenatal nicotine also increases corticosterone levels via direct action on the adrenal gland, which deflates the pivotal testosterone surge during the perinatal period (Figure 4; von Ziegler et al. 1991; Sarasin et al. 2003). This nicotinic alteration of sex hormones levels leads to long-term changes in behavior, such as eliminating sex-specific sucrose preferences (Lichtensteiger and Schlumpf 1985).

Nicotine exposure during the early neonatal period of hippocampal maturation induces increased expression of the KCC2 cotransporter in male pups, a change that may influence the timing of the switch in GABA transmission from excitatory to inhibitory (Figure 3; Damborsky and Winzer-Serhan 2012). The long-term consequence of neonatal nicotine exposure is enhanced excitation in the adult hippocampal CA1, which is greater in males than females (Damborsky et al. 2012). Whereas this group has reported that neonatal nicotine treatment does not affect later cognitive function (Huang et al. 2007), others have noted that perinatal nicotine exposure produces a mild spatial learning deficit in in females (Eppolito and Smith 2006).

Adolescence: a second critical period of brain sexual differentiation and nicotine sensitivity

Continued sexual differentiation of the brain

Adolescence is a developmental period marked by dynamic maturation of limbic regions mediating reward and reinforcement, learning and memory, and executive function (Spear 2000; Yuan et al. 2015). It is also the time of peak onset of tobacco use, with the vast majority of smokers initiating use before the age of eighteen (Substance Abuse and Mental Health Services Administration 2011). The continued organizational effects of gonadal hormones during this period solidify the uniqueness of the male and female brain and are reflected by the emergence of many sex differences in nicotine reward and reinforcement. Although our emphasis in this review is on sex steroid hormones, it is important to note that puberty and adolescence are not synonymous. Puberty lasts approximately 5 years in humans (Sun et al. 2002) and 10–20 days in rodents (Sisk and Zehr 2005; Schneider 2013). Adolescence is a protracted and elaborate developmental period that extends beyond puberty (Spear 2000), when a significant portion of brain maturation occurs independently of pubertal influences (Yuan et al. 2015).

Adolescence is a period of increased responsiveness to both stress- and sex-hormones (Sinclair et al. 2014). Sex hormones continue to have organizational effects, while activational effects also emerge. Multiple brain sexual dimorphisms develop during adolescence, including regions beyond those involved in sexual behavior and reproduction. Both androgens and estrogens modulate white matter volume during adolescence, with

estrogens having an inhibitory influence in both rats (Yates and Juraska 2008) and humans (Herting et al. 2012; Herting et al. 2014), whereas testosterone levels in adolescent boys are positively associated with white matter development (Herting et al. 2012). Both primary visual and medial prefrontal cortices show enhanced cell death in female but not male rodents during adolescence (Nuñez et al. 2001; Willing and Juraska 2015), and this divergence is prevented by pre-pubertal ovariectomy (Nuñez et al. 2001; Nuñez et al. 2002).

Sexual differentiation of major neurotransmitter systems also occurs during this period (Table 2). The noradrenergic locus coeruleus, for example, becomes a female-biased structure during late adolescence, with females exhibiting a more sustained increase in cell number as compared to males (Pinos et al. 2001). This sex difference is eliminated by neonatal androgen treatment of females, but not orchiectomy of males (Guillamón et al. 1988). The cholinergic system becomes sexually diergic almost entirely as a result of pubertal surges in gonadal hormones rather than perinatal hormone effects. Ovariectomy reduces ChAT mRNA and function, as well as choline uptake, in female rats, and this can be reversed by estradiol replacement (Gibbs et al. 1994; Yamamoto et al. 2007). Females also have greater basal acetylcholine release in the medial prefrontal cortex, premotor cortex, and supplementary motor cortex than males, likely due to sex differences in the number of ChAT-positive cells in the nucleus basalis of Meynert (Gibbs 1997; Takase et al. 2007; Takase et al. 2009). Furthermore, endogenous or supplemental estrogen enhances serotonin-mediated increases in acetylcholine release, likely through a 5-$HT1_A$ receptor mechanism (Matsuda et al. 2002).

The dopaminergic system is critically involved in mediating the rewarding properties of drugs of abuse and exhibits prolonged maturation during adolescence (Yuan et al. 2015), as well as a variety of sex differences that are determined at varying times across development. Dopamine neurons in the ventral tegmental area and substantia nigra are more numerous in females than males, a sex difference that is eliminated by gonadectomy of males (Johnson et al. 2010a; Johnson et al. 2010b). Striatal dopamine receptors also exhibit major overproduction followed by pruning in adolescent males, with only a minor increase seen in females. By adulthood, dopamine receptor levels in the dorsal striatum are similar across sexes, whereas males have a higher density of D1 receptors in the nucleus accumbens. Gonadal steroid hormones do not contribute to the pruning of D2 receptors observed during adolescence in males (Andersen et al. 1997; Andersen and Teicher 2000; Andersen et al. 2002; Azam et al. 2007).

While basal levels of dopamine are comparable between males and females, electrical or psychostimulant evoked-dopamine release is greater in the dorsal striatum of females (Walker et al. 2000; Kuhn et al. 2010). Striatal dopamine dynamics are positively modulated by estrogens (Morissette and Di Paolo 1993; Becker 1999; Becker and Hu 2008), whereas androgens have a greater modulatory role over dopamine in the frontal cortex (Adler et al. 1999; Kritzer and Pugach 2001; Kritzer 2003). Interestingly, it seems that genetic sex, rather than gonadal hormones *per se*, determine sex differences in dopaminergic projections to the forebrain (Beyer et al. 1991; Kolbinger et al. 1991). In the prefrontal cortex, glutamatergic inputs have a unique, sex-dependent influence over dopamine function. In males, signaling at AMPA and NMDA receptors stimulate and inhibit, respectively, mesocortical dopamine

release, suggesting that actions at these receptors oppose one another to balance dopamine system regulation. In contrast, both glutamate receptor subtypes appear to drive dopamine release in the female prefrontal cortex (Locklear et al. 2016).

We have shown alterations in the pharmacology of nAChR-mediated striatal dopamine release across the developmental spectrum, from the fetal period on (Azam et al. 2007). In particular, in the transition from adolescence to adulthood, there is a complex pattern of functional maturation of nAChRs in ventral, but not dorsal, striatum. In males, but not females, there are significant changes in both nicotine potency and efficacy during this developmental period. Chronic nicotine treatment during this period has also been shown to produce acute and long-term changes in dopamine systems that are more pronounced in males (Trauth et al. 2001).

The emergence of sex differences in nicotine reward and reinforcement

Continued divergence of the male and female brain during adolescence contributes to the sex differences in nicotine's effects that typically emerge during this period. There is relatively little data directly examining interactions between age and sex on nicotine reward. However, female adolescents have been shown to exhibit greater withdrawal-related craving than males (Dickmann et al. 2009). A slowly growing body of literature in rodents also supports the view that female adolescents have a greater biological vulnerability to nicotine dependence.

Adolescent female rodents have been found to be more likely than males to acquire nicotine self-administration, to acquire the behavior more rapidly, and have higher nicotine intake (Lynch 2009; Li et al. 2014; Sanchez et al. 2014). Furthermore, female rodents display a late-emerging enhancement of motivation, as measured by progressive ratio, compared to males. The enhanced motivation for nicotine is positively correlated with the ratio of estradiol to progesterone and negatively correlated with levels of progesterone alone (Lynch 2009). Other groups reporting increased motivation in females have not found differences in responding across the estrous cycle (Donny et al. 2000; Li et al. 2014). Acetaldehyde, a non-nicotine constituent in tobacco smoke, enhances nicotine self-administration in adolescents of both sexes. However, males display a reduction in responding for the combination of nicotine and acetaldehyde as adolescence proceeds whereas females do not (Belluzzi et al. 2005; Park et al. 2007). A similar age- and sex-dependent interaction has been observed with nicotine alone (Levin et al. 2011).

Whether adolescent females are more sensitive than adult females to nicotine reward is unclear. Torres et al. (2009) did not find a significant age difference in females, whereas adolescent males displayed higher nicotine reward than adult males. A more recent study, using a conditioned place preference (CPP) paradigm, has found adolescent females to be more sensitive to the rewarding properties and less sensitive to the aversive properties of nicotine than adult females (Lenoir et al. 2015). Whereas adolescent females had a higher preference to a mid-range dose (0.4 mg/kg) of nicotine than adolescent males, this sex difference was not apparent in adults. In parallel to the behavioral results, only females displayed an increase in nAChR binding in the nucleus accumbens after 0.4 mg/kg nicotine

and adolescent, but not adult, females had higher nAChR binding in the caudate putamen (Lenoir et al. 2015).

Stress is an important factor in the initiation and maintenance of smoking (Torres and O'Dell 2016), with female smokers showing heightened negative responses to stressful situations (Swan et al. 1993), and an association between smoking and depressive symptoms during adolescence (Jamner et al. 2003). Recent data also demonstrate that female college students are more likely than their male peers to report initiation of tobacco use as a way to alleviate negative mood states (Morrell et al. 2010). Furthermore, nicotine acutely activates the hypothalamic pituitary adrenal (HPA) axis, (al'Absi 2006; Cao et al. 2010), and the anxiolytic effects of acute nicotine are more pronounced in adolescent males than females (Damaj 2001; Cao et al. 2010). The pharmacological stressor, yohimbine, has been shown to stimulate nicotine self-administration and reinforcement efficacy in both male and female adolescent rats (Li et al. 2014). However, adolescent females are more sensitive to this effect, showing significantly increased progressive ratio responding at yohimbine and nicotine infusion doses to which males do not respond.

Anxiety-like and depressive-like behaviors during withdrawal from chronic nicotine are also influenced by age of exposure and sex, although data are equivocal. In an open-field test of anxiety-like behavior, one group reported a late-emerging depression of locomotor activity (P60) in females with nicotine exposure from P30–44, without any change in behavior during drug exposure or in early withdrawal. Exposed males were not different from controls at any time point (Trauth et al. 2000). In contrast, Thanos et al. (2013) observed decreased locomotor activity in females during short-term withdrawal from adolescent nicotine. This effect was normalized after a protracted withdrawal, whereas males displayed enhanced anxiety- and depressive-like behaviors after a 30-day withdrawal. Physical symptoms of nicotine withdrawal are similar in male and female adolescents (Kota et al. 2007; Kota et al. 2008; Torres et al. 2013).

Sustained sex differences in nicotine's effects in adulthood

Sex differences in nicotine responses persist in adulthood. The effects of gonadal hormones on these responses are not always directly assessed, but increasing data emphasize their role. Women have less success with cessation than men (Perkins 2001; Piper et al. 2010), experiencing more intense craving, higher cortisol levels during tobacco abstinence, and higher rates of tobacco withdrawal-associated depression and anxiety (al'Absi 2006; Schnoll et al. 2007; Xu et al. 2008). There is some evidence for interactions between circulating hormones or menstrual cycle phase with smoking behavior and cessation success. In women, estrogen seems to promote smoking behavior while progesterone discourages smoking (Sofuoglu et al. 2009; Lynch and Sofuoglu 2010). As a result, drug reward is greatest during times of high estradiol and low progesterone, such as the follicular phase. Indeed, women report reduced craving and decreased enjoyment or feelings of pleasure from smoking during the luteal phase (Allen et al. 2015; Goletiani et al. 2015). This relationship is mirrored in rodents, where midbrain dopamine neuron firing rate and burst firing is higher during estrous and diestrous compared to proestrous (Zhang et al. 2008), and dopamine release is inhibited by progesterone but promoted by estradiol (Becker and Beer 1986;

Walker et al. 2012). Interestingly, a recent meta-analysis reported greater and more intense withdrawal symptoms during the luteal phase (Weinberger et al. 2015), suggesting that progesterone might contribute to decreased dopamine levels during tobacco withdrawal that are associated with craving.

In rodent models, the influence of sex on nicotine reward and reinforcement in adults is less consistent. Some groups report higher or more rapid acquisition in females (Donny et al. 2000; Lynch 2009), faster acquisition in males (Swalve et al. 2016), or no sex differences (Feltenstein et al. 2012). In the maintenance phase of self-administration, females tend to have higher drug intake than males (Donny et al. 2000; Chaudhri et al. 2005; Rezvani et al. 2008; Grebenstein et al. 2013), although some studies report no effect of sex (Feltenstein et al. 2012; Swalve et al. 2016). Some sex differences are potentially mediated by ovarian hormone-induced changes in nicotine metabolism. Repeated intravenous nicotine produces higher plasma levels of nicotine in females than males, which may contribute to enhanced nicotine reinforcement. This sex difference is eliminated by ovariectomy (Harrod et al. 2007). Non-drug conditioned cues are also more salient to females than males during self-administration, extinction, and potentially withdrawal/craving (Perkins et al. 1999; Chaudhri et al. 2005). In addition, females experience more robust withdrawal from chronic nicotine compared to males (Torres and O'Dell 2016), an effect that seems to be due to ovarian hormones (Torres et al. 2015).

Research examining the rewarding properties of nicotine, frequently measured by CPP, is conflicting. Whereas one group reported that higher doses of nicotine are necessary for the development of CPP in females compared to males (Lenoir et al. 2015), another group reported no CPP in females at any dose tested (Yararbas et al. 2010). These studies are in contrast to Isiegas et al. (2009) who showed that female mice exhibited a greater magnitude of nicotine preference than males, and Torres et al. (2009) who reported that adult females display place preference over a wider range of nicotine doses than males. Interestingly, ovarian hormones seem to be necessary for nicotine reward, as ovariectomy precludes nicotine CPP (Torres et al. 2009). Similarly, ovarian hormones may also protect against the aversive properties of nicotine, as a high dose of nicotine (1.2 mg/kg) induced a significant aversion in intact females, but not ovariectomized females or intact males (Torres et al. 2009).

Nicotine enhances anxiety-like behavior in female rodents, but not males, as measured by elevated plus maze (Elliott et al. 2004; Caldarone et al. 2008) and open-field behavior (Cao et al. 2010). Furthermore, chronic nicotine treatment is anxiogenic in females, but not males (Caldarone et al. 2008). These sex differences may reflect differential sensitivity of the stress system that increases females' vulnerability to tobacco use (Torres and O'Dell 2016). Females have greater expression than males of CRF-1 receptors and hypersecretion of CRF in the locus coeruleus (Curtis et al. 2006; Bangasser et al. 2010; Bangasser et al. 2013), as well as lower levels of β-arrestin2, an intracellular protein that internalizes CRF-1 receptors to inactivate them (Bangasser et al. 2010; Bangasser and Valentino 2012). Furthermore, acute nicotine stimulates HPA axis activity and peripheral corticosterone secretion to a greater extent in adult females than in males (Cao et al. 2010; Gentile et al. 2011).

Although clinical data suggest important interactions between circulating hormones and tobacco smoking, they are correlational. Additionally, the majority of preclinical reports of sex differences in nicotine reward and reinforcement do not see an effect of circulating gonadal hormones (i.e., no variation across the estrous cycle). Instead, pre-pubertal gonadectomy can attenuate or even completely prevent these normal sex differences (Harrod et al. 2007; Torres et al. 2009). Ultimately, this highlights the critical organizational influence of gonadal hormones during perinatal and adolescent development as the primary determinants of sex differences in nicotine's effects.

Conclusion

Tobacco use, and increasingly e-cigarette use, are a major public health concern. Data at epidemiological, clinical, and preclinical levels consistently demonstrate that females are especially sensitive to the aversive effects of nicotine withdrawal. Age interacts strongly with sex in determining responses to nicotine as well, which is evidenced by recent findings that the rate of decline in smoking over the last ten years is slowing in adolescent girls (Allen et al. 2014).

Despite this, preclinical research has predominantly focused on adult males, failing to model a significant portion of the population. As a result, the exact mechanisms underlying females' unique sensitivity to the effects of nicotine are poorly understood. However, sex steroid hormones represent an important potential mediator of nicotine's differential effects in females. Early in development, gonadal hormones begin the extended process of sexual differentiation of the brain, which is predominantly mediated by masculinization and defeminization of the male brain via aromatization of testosterone into estradiol. The process of sexual differentiation is not completed until the end of adolescence, a period also marked by major reorganization of brain regions critical for executive function, reward processing, and motivated behavior. Ultimately, it may be the extended and profound organizational effects of gonadal hormones on neuronal structure, function, and neurochemistry, rather than the acute effects of circulating steroids, that primarily underlie female sensitivity to the effects of nicotine and tobacco.

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Significance Statement

There are substantial sex differences in the effects and outcomes of nicotine exposure, but the exact mechanism underlying these sex differences is unclear. This review highlights the developmental impacts of nicotine on sexual differentiation, and describes how nicotine exposure during the perinatal and adolescent periods can have gender-specific effects on neuronal structure and function. It further emphasizes the importance of including age and sex as factors in preclinical research that models tobacco use.

Hormone	nAChR Subtype	Effect of Hormone	References
Progesterone	α3β4	↓ Dose-dependent inhibition	Ke and Lukas 1996
	α4β2	↓ Dose-dependent inhibition	Paradiso et al. 2000; Valera et al. 1992
	α5	↑ Increased expression	Gangitano et al. 2009
17β-estradiol	α7	↑ Increased expression	Centeno et al. 2006 Miller et al. 1984
	α4β2 (human)	↑ Increased open probability due to direct actions at C-terminus	Paradiso et al. 2001; Curtis et al. 2002; Jin and Steinbach 2015
	α4β2 (rat)	— No effect	Paradiso et al. 2000
	α3β4	↓ Noncompetitive inhibition	Ke and Lukas 1996; Nakazawa and Ohno 2001

Figure 1. Ovarian hormones influence nicotinic acetylcholine receptors

Progesterone and estradiol modulate both expression and function of multiple nAChR subtypes, often with opposing effects. nAChR = nicotinic acetylcholine receptor

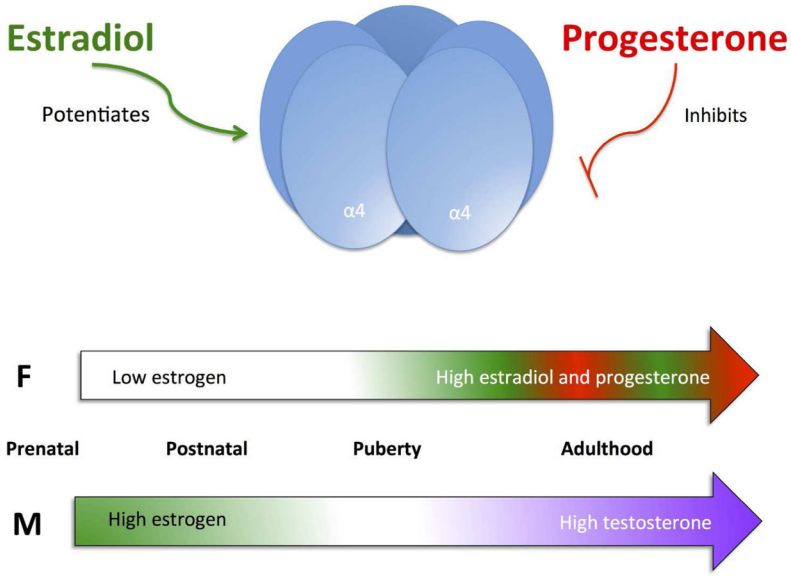

Figure 2. Nicotinic acetylcholine receptor (nAChR) activity is modulated by different sex hormones throughout development

Progesterone allosterically inhibits α4 nAChR subunits via a site outside the membrane pore (Valera et al., 1992), whereas estradiol potentiates the activity of human α4-containing nAChRs by increasing the opening probability (Curtis et al., 2002). The abundance of these steroids changes throughout development and across sexes. How this complex relationship between sex, nAChRs and development affects brain maturation and function is still being elucidated.

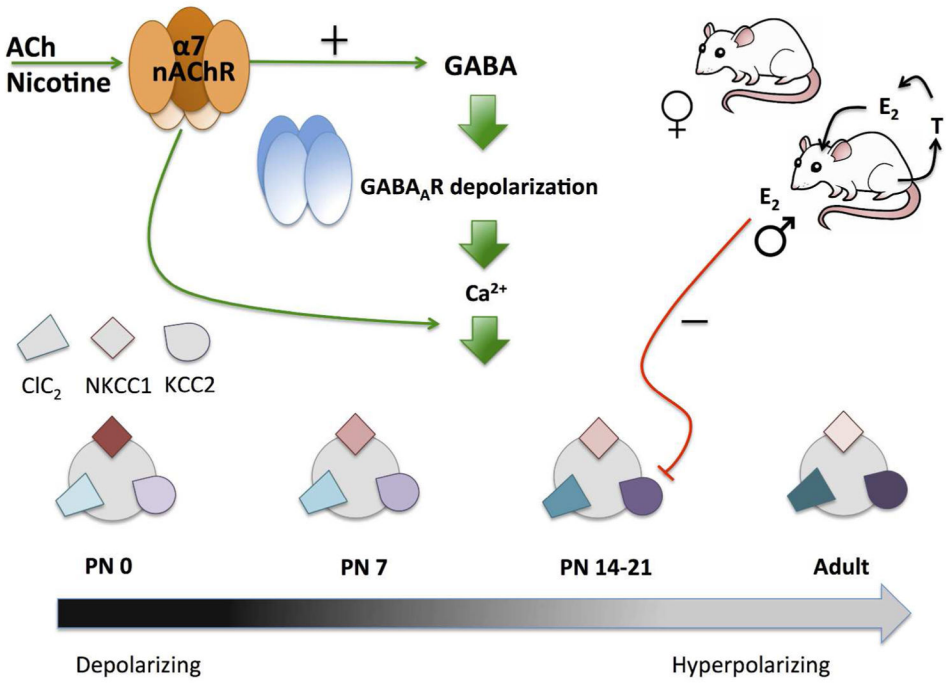

Figure 3. The role of α7 nAChRs in regulating GABA$_A$ receptor signaling in rodent neonatal hippocampus

The GABA$_A$ receptor switches from depolarizing to hyperpolarizing during the early neonatal period as a result of changes in intracellular Cl^-. This change, which is mediated by a shift in the expression of the Cl^- cotransporters from NKCC1 to KCC2, occurs later in males than females as a result of high estradiol in males having an inhibitory effect on KCC2 expression. Levels of the Cl^- channel, ClC_2, and the Cl^- cotransporters NKCC1 and KCC2, are pictured, with darker shades indicating higher expression levels. Activation of α7 nAChRs increases intracellular calcium levels both directly and through increased GABA release and GABA$_A$ receptor-induced depolarization, resulting in higher expression of KCC2. This regulates the switch of GABA$_A$ receptor signaling from depolarization to hyperpolarization. ACh = acetylcholine. T = testosterone. E_2= estradiol.

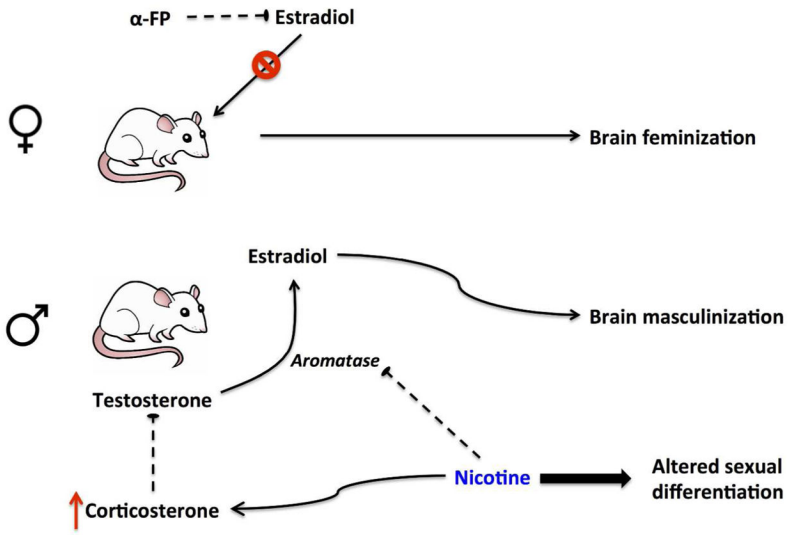

Figure 4. Schematic of fetal brain sexual differentiation and the effects of nicotine

Male sexual differentiation is induced by estradiol, which is produced via aromatization of testosterone. Females are protected from circulating maternal estrogen by α-fetoprotein (α-FP). Nicotine inhibits aromatase activity, leading to a decrease in male brain estradiol. Nicotine also increases corticosterone levels during gestation, which then inhibits testosterone production.

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Table 1

Sex differences in nicotine effects across development in rodents

Developmental period of exposure	Measure	Effect of nicotine	Citations
Gestation	Adrenergic function	Decreased β2-adrenergic-mediated adenylyl cyclase activity in males only	Slotkin et al. 2007
	Cholinergic function	In males, decreased ChAT activity and HC3 binding in the cerebral cortex; increased HC3 binding in the hippocampus; decreased basal adenylyl cyclase signaling. In females, increased HC3 binding in the midbrain; decreased basal adenylyl cyclase signaling (less profound than males)	Slotkin et al. 2007
	Serotonergic function	Reduced 5-HT1A binding and increased 5-HT2 binding in males only	Slotkin et al. 2007
	Myelin-associated gene and protein expression	In males, upregulation of myelin-associated genes (e.g., *Mbp, Plp1, Gjc3, Mobp, Aspa*) in the PFC, CPu, and NAc during adolescence that generally normalized by adulthood. In females, downregulation or normal expression of myelin-associated genes (e.g., *Plp1, Gjc3, Mobp, Mbp, Cnp, Mal, Mog*) in PFC, CPu, and NAc during adolescence. Downregulation in adult CPu of females	Cao et al. 2013
	Dendritic complexity	In males, increased complexity in AID and NAc but decreased complexity in apical field of Cg3; increased spine density everywhere but NAc; increased dendritic length in NAc and basilar Cg3 but decreased length in basilar PAR. In females, increased complexity in NAc but decreased complexity in AID; increased spine density in AID and PAR but decreased in Cg3 and NAc; reduced dendritic length in NAc, Cg3 and PAR	Mychasiuk et al. 2013
	Expression of KCC2 cotransporter	Greater in males	Damborsky and Winzer-Serhan 2012
	Excitation of adult hippocampal CA1	Increased excitation as measured by fEPSP slope at higher voltage inputs in males only	Damborsky et al. 2012
	Sucrose preference	Eliminates normal sex difference (i.e., females have higher preference than males)	Lichtensteiger and Schlumpf 1985
	Nicotine reward	Increased preference for oral nicotine in adolescent males only	Klein et al. 2003
	Cognitive function	Mild spatial deficit in females only	Eppolito and Smith 2006
		No change	Huang et al. 2007
Adolescence	Nicotine self-administration	In females, a greater percentage acquire self-administration, acquisition is more rapid, and intake is higher compared to males	Lynch 2009; Li et al. 2014; Sanchez et al. 2014
		Adolescent rats of both sexes had higher nicotine self-administration than adults but the effect was more pronounced in adolescent males; responding in males decreased with age but did not in females	Levin et al. 2011
		Acetaldehyde enhances nicotine self-administration in both sexes, but only males reduce responding with age	Belluzzi et al. 2005; Park et al. 2007
		Females are more sensitive to yohimbine-induced increases in motivation for nicotine under progressive ratio responding	Li et al. 2014
	Nicotine reward	Adolescent males displayed enhanced nicotine conditioned place preference compared to adults, but adolescent females were more sensitive than adults to low dose nicotine without a difference in magnitude of preference	Torres et al. 2009

Developmental period of exposure	Measure	Effect of nicotine	Citations
		Adolescent males had higher nicotine-induced preference to a mid-range dose of nicotine than adolescent females	Lenoir et al. 2015
	Motivation for nicotine	Females display higher motivation under progressive ratio testing. Greater motivation is positively correlated with the ratio of estradiol:progesterone and negatively correlated with levels of progesterone alone; does not seem to vary across estrous cycle	Lynch 2009; Donny et al. 2000; Li et al. 2014
	Nicotine withdrawal-related craving	Higher in females	Dickmann et al. 2009
	Anxiolytic effects of acute nicotine	More pronounced in males	Damaj et al. 2001; Cao et al. 2010
	Anxiety-like behavior during nicotine withdrawal	Late-emerging (P60) depression of locomotor activity in open-field testing in females with early adolescent nicotine exposure; no difference in males	Trauth et al. 2000
		Decreased locomotor activity during short-term withdrawal from nicotine in females that normalized after 30 days; males had increased anxiety- and depressive-like activity that emerged after 30 days of withdrawal	Thanos et al. 2013
	Physical symptoms of nicotine withdrawal	No sex differences	Kota et al. 2007; Kota et al. 2008; Torres et al. 2013
	Nicotine-induced changes in nAChR binding	Increased nAChR binding in striatum of adolescent females with no difference in adolescent males	Lenoir et al. 2015
Adulthood	Nicotine self-administration	Higher responding and faster acquisition in females	Donny et al. 2000; Lynch 2009
		Faster acquisition in males	Swalve et al. 2016
		No sex differences in acquisition rates	Feltenstein et al. 2012
	Nicotine intake	Higher intake during maintenance phase of self-administration	Donny et al. 2000; Chaudri et al. 2005; Rezvani et al. 2008; Grebenstein et al. 2013
		No sex differences	Feltenstein et al. 2012; Swalve et al. 2016
	Nicotine metabolism	Repeated nicotine produces higher plasma levels in females than males	Harrod et al. 2007
	Salience of non-drug conditioned cues	Higher in females during self-administration, extinction, and possibly withdrawal/craving	Perkins et al. 1999; Chaudri et al. 2005
	Withdrawal	More robust in females than males, likely due to ovarian hormones	Torres et al. 2015; Torres and O'Dell 2016
	Nicotine reward	Female rats are less sensitive than males; higher doses of nicotine are required to produce significant place preference	Lenoir et al. 2015
		Females display place preference over a wider range of nicotine doses than males	Torres et al. 2009
		No significant preference at any tested dose	Yararbas et al. 2010
		Females had greater magnitude of nicotine place preference than males	Igiegas et al. 2009
	Anxiety-like behavior	Greater in females than males in open-field and elevated plus maze	Elliott et al. 2004; Caldarone et al. 2008; Cao et al. 2010

Developmental period of exposure	Measure	Effect of nicotine	Citations
	Corticosterone levels	Nicotine stimulates HPA axis activity and corticosterone release more in females than males	Cao et al. 2010; Gentile et al. 2011

Abbreviations: AID = agranular insular cortex, CPu = caudate putamen, ChAT = choline acetyltransferase, Cg3 = layer III of anterior cingulate cortex, fEPSP = field excitatory postsynaptic potential, HC3 = [^{3}H]hemicholinium, HPA axis = hypothalamic pituitary adrenal axis, KCC2 = K^{+}/Cl^{-} cotransporter 2, nAChR = nicotinic acetylcholine receptor, NAc = nucleus accumbens, PAR = parietal cortex, P = postnatal day, PFC = prefrontal cortex

Table 2

Adolescent sexual differentiation of major neurotransmitter systems

Neurotransmitter system	Sex difference	Measure	Mechanism	Citations
Norepinephrine	**F > M**	Size of locus coeruleus	More sustained increase in cell number. Eliminated by neonatal androgen treatment in females; unaffected by orchiectomy of males	Pinos et al. 2001; Guillamón et al. 1988
Acetylcholine	**F > M**	Basal ACh release in mPFC, premotor, and supplementary motor cortices	Due to sex differences in the number of ChAT-positive cells in the nucleus basalis of Meynert	Gibbs 1997; Takase et al. 2007; Takase et al. 2009
Dopamine	**F > M**	Number of dopamine neurons in the VTA and SN	Androgens suppress midbrain dopamine neuron number; exact mechanism unknown	Johnson et al. 2010a; Johnson et al. 2010b
	M > F	Nucleus accumbens D1 receptor levels	Greater increase in receptor density. Not influenced by gonadal hormones	Andersen et al. 1997; Andersen and Teicher 2000; Andersen et al. 2002; Azam et al. 2007
	F > M	Electrical or psychostimulant-evoked DA release	Faster release than uptake in females that is perturbed by electrical or psychostimulant stimulation	Walker et al. 2000; Kuhn et al. 2010
	F > M	DA dynamics in striatum	Positively modulated by estrogens	Morissette and Di Paolo 1993; Becker 1999; Becker and Hu 2008
	M > F	DA dynamics in mPFC	Positively modulated by androgens	Adler et al. 1999; Kritzer and Pugach 2001; Kritzer 2003
	M − **F +**	NMDA receptor influence on mesocortical DA	Differential NMDAR-mediated activation of PFC pyramidal cells projecting to the VTA, possibly regulated by androgen levels	Locklear et al. 2016
	M > F	Soma size of DA neurons	Determined by genetic sex; not influenced by gonadal hormones	Beyer et al. 1991; Kolbinger et al. 1991
	M > F	nAChR-mediated ventral striatal DA release	Higher maximum nicotine-stimulated [3H]DA release at P30 and higher sensitivity at P40, as compared to adults. No age-related changes in nicotine efficacy or potency in females	Azam et al. 2007

Abbreviations: ACh = acetylcholine, ChAT = choline acetyltransferase, DA = dopamine, F = female, M = male mPFC = medial prefrontal cortex, NMDA/NMDAR = N-methyl-D-aspartate receptor, P = postnatal day, SN = substantia nigra, VTA = ventral tegmental area.