Gender differences in tobacco smoking dynamics and the neuropharmacological actions of nicotine

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## 1.  ABSTRACT

Previous studies have found gender differences in the dynamics of tobacco smoking and cessation in humans. However the physiological basis for these differences is a subject of much debate.  Animal studies have also revealed some gender-dependant differences in the neuropharmacological actions of acute and chronic nicotine.  The purpose of this article is to review the clinical and basic science studies that have evaluated sex differences in tobacco use/cessation and the animal literature that has provided some clues regarding the possible underlying basis for these differences.  The acute and chronic actions of ovarian steroid hormones on nicotinic receptors could play a direct or indirect role in mediation of gender differences in tobacco use dynamics, however no clear picture has emerged from these studies. The literature supports a general consensus women have a more difficult time with smoking cessation and that perhaps nicotine replacement therapy is less efficacious in female smokers.  This could be due to alterations in nicotine pharmacokinetics mediated by estrogen, ovarian hormones acting as non-competitive nicotinic receptor antagonists, or many additional issues.  Many studies assume that individuals, regardless of gender, have the same motivation for tobacco use and/or cessation.  Individual reasons for smoking may simply override gender differences in the pharmacodynamic/pharmacokinetic actions of nicotine. Many animal studies that have reported gender differences in nicotine sensitivity have not carefully controlled for phase of the estrus cycle, and the results of preclinical studies do not always support conclusions that have been drawn from human studies.  Thus there are still many questions that remain to be answered in this important area of research.

## 2.  INTRODUCTION

The development and maintenance of tobacco addiction is a complex process involving numerous neurobiological, anatomical and psychological determinants.  Smoking cessation and the utility of nicotine replacement therapy (NRT) as a cessation aide also varies substantially between individuals.  Potential gender differences in tobacco addiction and smoking cessation rates have been investigated in animal models as well as human studies, but no clear picture regarding the underpinnings of these differences has emerged.   The purpose of this review is to summarize the current literature in terms of gender differences in cigarette smoking and the psychopharmacological actions of nicotine.  The regulation of tobacco addiction is a multi-factorial process, involving both pharmacological and non-pharmacological factors. For the purpose of this review, it is assumed that nicotine is the major tobacco alkaloid that reinforces the smoking habit.  However it is acknowledged that other tobacco constituents, sensory stimuli and biobehavioral adaptations are likely to also play prominent roles in tobacco addiction.

Neuronal nicotinic receptor (nAChR) subtypes are members of the Cys-loop family of ligand gated ion channels.  Receptors in this superfamily contain 5 homologous protein subunits, each having 4 transmembrane spanning domains.  The M2 transmembrane segments line the pore of the central ion channel, and regulate cation permeability.  In the CNS, two major nAChR subunits termed alpha and beta have been characterized, with 9 different alpha (alpha 2 – alpha 10) and 3 different beta (beta 2 – beta 4) subunits currently identified (1,2).  The alpha 7 - alpha 9 nAChRs are homomeric and have significantly higher calcium

permeability than other nAChR isoforms. Tobacco addiction is likely due to complex interactions between nicotinic alkaloids (and/or metabolites) in tobacco smoke and activation of multiple nAChR subtypes in a variety of different brain regions. While the precise mechanisms of nicotine addiction remain elusive, animal studies have documented the prominent involvement of receptors containing beta 2 subunits. Animals that lack expression of the beta 2 subunit do not readily self administer nicotine, or express nicotine-induced conditioned place preference, an animal model that measures some aspects of nicotine reward (3-6). Several different alpha subunits (most notably alpha 4 and alpha 6) may combine with beta 2 subunits to produce receptors involved with nicotine reward. The primary reinforcing actions of nicotine may be due to presynaptic facilitation of neurotransmitter release in brain reward circuits such as the mesolimbic and mesocortical dopamine projections. In addition to dopamine, nicotine facilitates the presynaptic release of many additional hormones and neurotransmitters that could directly or indirectly participate in brain reinforcement (acetylcholine, norepinephrine, GABA, serotonin, glutamate, etc.; 7,8). Non-pharmacological sensory stimuli may also play an important role in tobacco addiction, but these actions have been difficult to unravel (see below). Tobacco use/nicotine is associated with a wide variety of short-term positive reinforcers (e.g. elevation in mood, decreased anxiety, increased attention, increased analgesia, reduced appetite) and long-term positive reinforcers (e.g. reduced weight gain, improved neuropsychiatric profile). The widespread distribution of nAChR subtypes in the CNS contributes to the complexity of the psychopharmacological actions of tobacco smoking. In addition to nicotine-activated responses in traditional brain reward areas (ventral tegmental area, nucleus acumbens), neurotransmitter release in other brain regions such as the hippocampus, hypothalamus, thalamus, amygdala and spinal cord mediates some of the diverse actions of nicotine on mood and behavior. Since nicotinic mechanisms can affect so many different neurotransmitter systems, it is not surprising that the pattern/outcome of tobacco use and response to smoking cessation might vary substantially from person to person. Individual variability in these factors makes explanations of why men and woman smoke tobacco difficult to generalize.

The literature consistently suggests that the rate of substance abuse and dependence is higher in men, compared to women. In 2004 about 60% of clinically documented cases of substance abuse were in men, 40% in women (9-11). A recent review by Lynch (11) summarizes some reasons that may explain the gender difference in the incidence of substance abuse. Most importantly, sociological influences may make it more "acceptable" for men to be drug abusers, since women are more likely to be the primary childcare provider. However recent evidence suggests that women may be more vulnerable to certain aspects of drug abuse. Following the onset of drug administration, women may progress to drug dependence at a faster rate than men, and also seem more susceptible to relapse following cue-induced drug craving (9-11). In addition, craving and/or relapse due to stress or depression

may be more common in females, and the phase of the menstrual cycle may influence these interactions. Estrogen seems to increase the subjective effects of psychostimulants such as cocaine and amphetamine, but specific effects on sensitivity to nicotine/tobacco smoking have been more difficult to establish (11, 12).

## 3. HUMAN STUDIES ON GENDER AND THE DYNAMICS OF TOBACCO SMOKING

### 3.1. Societal/cultural influences on drug/tobacco use

According to the World Tobacco Atlas, published by the World Health Organization (WHO) in 2002, approximately 35% of males in developed countries, and 50% of males in developing countries use tobacco products; worldwide there are approximately 1 billion male users of tobacco (13). Trends identified in the majority of countries suggest that tobacco-smoking rates in men have peaked, and are slowly decreasing. In comparison, there are on the order of 250 million female smokers worldwide; approximately 22 percent of women in developed countries report smoking tobacco, compared to 9 percent of women in underdeveloped countries. In many developed countries smoking rates are declining in women (Canada, Australia, United Kingdom, United States) however in underdeveloped countries the incidence of smoking in women appears to be on the rise. When comparing gender differences in the prevalence of smoking in specific countries, substantial differences are noted. In many countries, the incidence of smoking in males is over 10 times more common in males, than females (Africa, Middle East, Asia). However in some countries (New Zealand, Norway, Sweden) the rate of tobacco use in females equals that seen in males (13). These findings clearly illustrate that gender differences in tobacco use are not necessarily determined by sex differences in the psychopharmacological properties of nicotine, or other tobacco constituents, and that societal views play an equally important role in determining rates of cigarette smoking, possibly in relation to differences in gender equity across countries (14).

### 3.2. Psychopharmacological reasons for tobacco use in men and women

A key question in this area of research is whether the stimulus properties of nicotine are similar in men and women, and in women whether the subjective effects of nicotine/tobacco smoking may differ over the menstrual cycle. Since a substantial amount of research indicates that men and women use tobacco for different reasons, it is not surprising that gender differences might be present in many facets of tobacco use patterns. Several papers from the Perkins lab, and others, have supported the hypothesis that women are more sensitive to non-nicotine components of tobacco smoking than men, while men smoke primarily for nicotine reinforcement (15-20). Convincing studies have been published that show that women have a more difficult time discriminating the stimulus properties of nicotine. In a study that evaluated nicotine discrimination in men and women using a nicotine nasal spray, males could differentiate varying between low doses of nicotine, but women could not. The lack of ability to discriminate

different doses of nicotine suggests less positive reinforcement obtained from nicotine use, or the use of higher nicotine use to obtain reinforcement. (15). Sensory (non-nicotine based) effects of tobacco smoke may be stronger primary or secondary reinforcers in women compared to men (18-20). Studies that evaluate nicotine discrimination across the menstrual cycle would be interesting and valuable.

Several studies have shown that the reward properties of tobacco smoking/nicotine differ in men and women, and generally women seem to be less sensitive to nicotine reward. The analgesic actions of nicotinic agonists may be one important component of positive reward obtained from tobacco smoking (21). However the results of studies that have evaluated gender differences in tobacco/nicotine-induced analgesia have been inconsistent. Jamner (22) administered nicotine to smoking and non-smoking males and females. Nicotine administration increased pain threshold and pain tolerance in males, but not in females. Nicotine-induced analgesia was similar in smokers and non-smokers, suggesting that tolerance may not readily develop to this effect of tobacco smoking. Girdler (23) found that female smokers showed more analgesia than women non-smokers. Similar results were obtained in male smokers, but the effect depended on the type of pain or stress that was involved. The effects of tobacco smoking on cognition and attention could also vary by gender. One placebo controlled, double blind study, has evaluated the effects of nicotine on attention and sustained focused attention (vigilance) in male and female smokers and non-smokers. Compared to the placebo situation, nicotine delivery increased attentional performance in almost every category evaluated, in all subjects. Differences in attentional enhancement with nicotine treatment were confounded by gender differences in baseline performance (24). Concurrent tobacco use may also influence the stimulus properties of other abused substances. Acheson (25) found that the subjective effects of nicotine alone, and nicotine in combination with alcohol differs between men and women. Nicotine administration was found to increase alcohol consumption in males, but decreased it in females. File *et al.*, (26) used nicotine inhalers to look at changes in mood in male and female tobacco smokers after nicotine (2mg) administration via an inhaler. A moderate stressor increased ratings of anxiety, discontent and aggression in both males and female subjects. However nicotine treatment reduced signs of negative affect in women non-smokers, but enhanced aggression and anxiety in males. These studies illustrate the complexity of gender-dependant changes in neuropsychological function following tobacco administration.

**3.3. Influence of the menstrual cycle on tobacco smoking and withdrawal**

One issue that has undoubtedly contributed to the paucity of information regarding gender differences in tobacco or other substance abuse is that females have often been excluded from clinical psychopharmacological studies due to concerns over pregnancy and possible detrimental effects on fetal development. In addition drug effects may vary significantly with the phase of the menstrual cycle, leading to difficult interpretation of generalized drug response. However the psychological and/or neuroendocrine changes over the cycle that significantly influence drug responsiveness have been difficult to identify. Basal changes in mood/affect across the menstrual cycle could significantly influence drug responsiveness. In addition drug-hormone interactions could potentially cause pharmacokinetic and/or pharmacodynamic changes in drug sensitivity over the menstrual cycle. However these phenomena are difficult to study due to the rapidly changing hormonal environment, individual differences in the timing/magnitude of hormonal changes, in combination with person-to-person variability in baseline drug responsiveness (27). Some studies have evaluated tobacco smoking dynamics across the menstrual cycle, but in general there appear to be no consistent influences on the number of cigarettes smoked or the subjective actions/response to nicotine.

In humans, most studies have evaluated three phases of the menstrual cycle for differences in drug responsiveness. The follicular phase of the cycle (starting on first day of menses to ovulation) is generally associated with low levels of progesterone and rising levels of estrogen and lasts about 2 weeks in humans. The ovulatory phase begins around day 14 of the cycle; ovulation occurs approximately 4 hours after the surge of leutinizing hormone (LH). Estrogen levels are high at this stage of the cycle and progesterone levels are low, but rising. The luteal phase of the cycle typically begins around 4 days after LH surge, and is associated with high levels of progesterone, and declining levels of estrogen (28-29). The potential influences of the rapidly changing hormonal environment in cycling women are vast and diverse. In these situations it is uncertain whether the parent hormone (estrogen, progesterone, LH, follicle stimulating hormone [FSH]) or a hormone metabolite could cause changes in drug responsiveness. On a cellular level, the effects of these hormones could be due to genomic or non-genomic actions of steroid hormone. Genomic effects could increase or decrease nicotinic receptor synthesis and non-genomic actions could alter functional properties of neuronal nAChRs through allosteric interactions (see summary of animal studies below). Finally neuroendocrine changes at different phases of the menstrual cycle could alter the absorption and distribution of metabolism of nicotine, or other tobacco constituents that regulate the subjective effects of tobacco smoking. However the majority of studies suggest that there are minimal differences in the amount of smoking/nicotine consumption or differences in the subjective physiological and psychological actions of tobacco smoking across the various phases of the menstrual cycle (27, 30, 31).

Although there are few well-documented differences in nicotine responsiveness across the menstrual cycle, the literature does consistently indicate that women are less successful in terms of smoking cessation, with or without nicotine replacement therapy (NRT: 32-34). Some studies suggest that women may experience greater

withdrawal symptoms, but not all studies support this hypothesis. A study by Hogle and Curtin (35) compared fear-potentiated startle response in men and women undergoing nicotine withdrawal. Females were slower to show adaptive changes in the startle response and also had higher salivary cortisol following the stress of the fear potentiation paradigm, compared to males. Wetter *et al* (36) reported greater negative affect in women undergoing tobacco withdrawal including more anxiety, depression, irritability and dysphoria, compared to males undergoing tobacco withdrawal. Alleviation of negative affect states may be one factor that leads to more frequent reinstatement of smoking behavior in women (37, 38). Some studies suggest an increase in the rate of tobacco smoking in some women during the premenses period, but not all studies support this conclusion. In addition this type of study is often confounded by other psychological changes in mood and affect that may occur during premenses. Thus increased tobacco/nicotine craving during premensus may be secondary to an increased incidence of negative affect that naturally occurs during this time in some women. Nevertheless it is possible that increased tobacco use during premenses is a strategy that reduces anxiety, depression and other behavioral symptoms that may occur during this time. Sofuoglu (39) administered progesterone to women in the follicular phase of the cycle and assessed smoking behavior. A single dose of progesterone reduced nicotine craving as well as the subjective effects of tobacco smoking in a self-report. There was a trend towards reduced tobacco smoking behavior, but it was not significant.

The vast majority of smoking cessation studies suggest that the process is significantly more difficult for females, as compared to males. Numerous theories have been postulated for this discrepancy including 1) perceived (or real) differences in body weight regulation during smoking cessation, 2) greater negative affect during early smoking cessation 3) gender differences in nicotine pharmacokinetics/pharmacodynamics (15, 27, 29). Smoking may be better at regulating negative affect (depression/anxiety/irritability) than NRT. It is interesting that women use less nicotine, and report less nicotine dependence, however aspects of their withdrawal syndrome may be significantly more deleterious than that seen in men (35). Robinson (40) evaluated the eye-blink response to an acoustic stimulus in men and women undergoing smoking cessation, as a measure of emotional liability. Males and females had a similar response to nicotine nasal spray in this study and little evidence for gender-dependant responses were noted. Due to the inconsistencies that have been reported for gender and smoking cessation, two meta-analyses have been conducted on gender differences in the efficacy of NRT, with opposite results. A meta-analysis performed by Cepeda-Benito (41) concluded that NRT was more effective than placebo for smoking cessation in men at 3, 6 and 12 month outcome measurements. However benefits for women were clear only at the earlier time points (3 and 6 months). Nicotine replacement therapy combined with non-pharmacological approaches (counseling, behavior modification, etc.) seemed much more important in women compared to men. NRT plus low interventional support was effective for men at several

quit intervals, but only for women at the shortest observation points. A meta-analysis of transdermal NRT in men and women performed by Munafo (42) did not support the hypothesis that NRT is not as useful in women. When evaluating data pooled from 11 published studies, a non-significant trend towards more success in men was seen at mid and late evaluation points (6 and 12 months). However NRT appeared to be equally efficacious in men and women, especially at early evaluation periods. Thus it appears that gender alone has a small but potentially significant effect on the efficacy of NRT, but it is clear that women do obtain substantial benefit from NRT. Elucidating the underlying reasons for the apparent gender difference will be difficult unless future clinical trials are designed to specifically address this question. Allen (43) reported no difference in the efficacy of NRT in post-menopausal women who were, or were not, using estrogen replacement therapy. The authors predicted that quit rates would be poorer in women who used estrogen replacement, because of the potential anti-nicotinic actions of estrogen (see below). Because rates of cessation did not differ in the two groups, they speculate that in women using estrogen replacement, the mood enhancing/stabilizing effects of estrogen are beneficial in terms of reducing negative affect produced by smoking cessation. A recent study by Schiffman (44) reports that both the nicotine patch and nicotine lozenges are useful cessation tools in women. In women that have a difficult time quitting using NRT, other pharmacological interventions could be more useful. Gonzales *et al.* (45) reported no differences between males and females in the utility of Buprorion (Zyban®) for smoking cessation. Veranicline (Chantix®), a partial agonist at neuronal nAChRs for smoking cessation also was equally effective for cessation in males and females (46). In addition, the efficacy of Naltrexone (Revia®) for smoking cessation was evaluated by King *et al.* (47), and quit rates were similar between males and females following this intervention.

A comprehensive review article on smoking cessation and the menstrual cycle was recently published by Carpenter *et al.* (29). Several studies that have evaluated possible gender differences in nicotine withdrawal have concluded that males and females do not differ in psychological, physiological or behavioral indices of tobacco withdrawal. However variable study design and other methodological issues seem to contribute significantly to inconsistent conclusions in these studies. Differences in withdrawal assessment confounds some of these studies, and it may also be necessary to pay more careful attention to individual differences in the timing and levels of hormonal changes and the possible influence on tobacco withdrawal. Carpenter *et al.* (24) identified several papers with similar outcome measurements and performed a meta-analysis to reveal any possible differences in smoking cessation across the menstrual cycle. In studies that looked at normal smoking patterns in women across the menstrual cycle there were consistent menstrual-related effects on the severity of tobacco withdrawal and/or a change in nicotine craving over the cycle. The luteal or late luteal phase of the cycle was consistently associated with increased craving and greater withdrawal symptomology

(27, 29, 48). Studies that evaluated smoking abstinence were more variable, but still showed a trend towards increased abstinence symptomology in the luteal phase of the cycle. One major difficulty in these studies is the similarity between pre-menstrual symptoms and signs of nicotine withdrawal, as both are associated with increased anxiety, irritability, depressed affect, and reductions in attention. However these studies do suggest that the luteal phase of the cycle may be associated with more severe premenstrual actions that may make this phase of the cycle very difficult in terms of initiating a smoking cessation program. Women preparing to set a quit date may want to consider these possibilities; however more (and better controlled) studies are needed before specific recommendations could be made with confidence (29).

### 3.4. Effects of ovarian hormones on nicotine pharmacokinetics

In women, nicotine replacement therapy may be influenced by the actions of steroid hormones on nicotine metabolism. Accelerated nicotine metabolism has been reported in pregnant women, possibly because of induction of hepatic cytochrome p450's by progesterone or other steroid hormones/metabolites (49). Nicotine is metabolized by hepatic cytochrome p450's, predominantly by CYP2A6 (49-52). Benowitz *et al.* (50) evaluated nicotine metabolism in men, women taking oral contraceptives, and women not taking estrogen. Women had a significantly higher nicotine clearance rate than males, and women taking oral contraceptives had an even greater rate of nicotine metabolism. Women who used only estrogen as a contraceptive had a higher rate of nicotine metabolism than women taking oral progesterone only, or the combination of estrogen and progesterone. Thus estrogen appears to significantly accelerate nicotine metabolism. However, Hukkanen (51) studied nicotine metabolism in female non-smoking subjects, and concluded that nicotine metabolism (CYP2A6 activity and protein) does not vary across the menstrual cycle. In addition, phase of the menstrual cycle did not influence the hemodynamic response to nicotine, activation of the hypothalamic pituitary adrenal axis or catecholamine secretion response following IV nicotine infusion. Norepinephrine levels were consistently higher in the luteal phase of the cycle, but this did not seem to influence neuroendocrine response to IV nicotine. Thus it appears that physiological levels of ovarian hormones that differ across phases of the cycle are unlikely to explain the observed increase in smoking by women during luteal phase. However higher levels of steroid hormones in pregnant women and in those taking estrogen hormone replacement therapy may have a significant increase in the rate of nicotine metabolism. Mueck and Seeger (53) reported that heavy cigarette smoking can nullify the beneficial actions of oral estrogens on hot flashes, urogenital symptoms and osteoporosis. The main cause for loss of efficacy seems to be elevated hepatic clearance of estrogen. Increasing the dose of oral estrogens in tobacco users does not seem to be a good idea since higher doses of estrogen increase the risk for breast cancer. An *in vitro* study by Gocze (54) studied the effect of nicotine on ovarian hormone metabolism using human granulosa cells obtained from a fertility clinic. Exposure to nicotinic agonists caused a slight but significant, reduction in progesterone synthesis. However estrogen synthesis was not significantly impacted by exposure to nicotinic agonists.

### 4. ANIMAL STUDIES EVALUATING GENDER DIFFERENCES IN NICOTINIC PHARMACOLOGY

Animal studies have identified some significant gender differences in the behavioral, physiological and cellular actions of nicotine. Animal studies have been valuable since they often employ mechanistic approaches to provide information regarding the possible molecular mechanisms that underlie gender differences in response to nicotine. The presynaptic location of nicotinic receptors and the facilitation of neurotransmitter release by nicotinic agonists may underlie a number of potentially significant interactions (7, 8). Previous studies have shown that estrogen increases the rate of cocaine self-administration and that estradiol potentiates dopamine release from the striatum (9-12, 55, 56). Enhancement of dopaminergic activity by estrogen would similarly be expected to affect nicotinic cholinergic mechanisms. The pioneering work of Mody *et al.* (57) demonstrated that female rats express different phenotypes of GABAa receptors at various phases of the estrus cycle, and altered receptor expression leads directly to alterations in neuronal excitability of the hippocampus. During estrus tonic inhibition in the dentate gyrus was reduced by 50% and increases in seizure sensitivity and anxiety were apparent. Although progesterone levels are low during rat estrus, it is not yet known what hormonal changes mediate the effect on GABAa receptor expression. In addition the vast majority of studies with ovarian hormones and central neurotransmitter/receptor expression have evaluated only changes in peripheral hormone levels. It may also be important to look at brain levels of steroid hormones (and/or relevant metabolites) that might be produced at that point in the cycle (58). Changes in reproductive hormones and/or their metabolites might directly or indirectly influence the activity of other neurotransmitter receptors, transporters, enzymes, second messenger signaling pathways, glial cell activity along with a host of other possibilities (57, 58).

### 4.1. Gender differences in acute behavioral sensitivity to nicotine

Although many different types of behavioral studies have evaluated gender differences in sensitivity to nicotine, no clear consistent pattern of results have been found. Consistent with the human literature, some studies have found that female rats are less sensitive to the discriminative stimulus properties of nicotine (59, 60), and that female rodents are generally less sensitive to the behavioral effects of nicotine in locomotor (mice [61, 62]) and cognitive tests (rats [63]). However other studies have concluded that female rats are more sensitive to the analgesic and other behavioral actions of nicotine (64-71). In terms of nicotine-induced analgesia, Elliott *et al.* (72) showed that nicotine caused analgesic actions in males, but not females and Carstens *et al*, (73) reported that males and females had similar nicotine sensitivity in a hot plate test of

analgesia, but males were more sensitive to nicotine in the tail flick test. Some studies have shown gender dependant differences in nicotine-induced neuroendocrine changes (68, 74, 75). Although the available data are limited, the animal literature does not generally support the notion that there are differences in nicotine reinforcement in male and female rats (76). Both sexes acquired the nicotine self-administration task, and response rates across various doses of nicotine were similar except that females tended to take more infusions of nicotine. However in females there were no differences in nicotine self-administration across the various phases of the estrus cycle. Plasma nicotine levels were similar in both genders and the extent of nicotine-induced nAChR up-regulation was also similar in males and females. In support of the Donny *et al.* (76) results, Kuo (77) found no differences in locomotor response to nicotine across the rat estrus cycle.

Since the behavioral effects of nicotine vary with the paradigm employed, it is important to conduct studies where various behavioral responses are evaluated in parallel. Damaj *et al.* (78) performed one of the most exhaustive studies looking at gender differences in nicotine responsiveness using male and female ICR mice. This study evaluated anti-nociceptive testing using both the hot plate, and tail flick tests as well as locomotor, body temperature regulation, incidence of seizures, and tests of anxiety. Females were significantly less sensitive than males in each test of analgesia with nicotine or epibatidine used as agonists. Male mice were also significantly more sensitive to the anxiolytic actions of nicotine in an elevated plus maze paradigm. However no differences were seen in nicotine-induced hypothermia, nicotine induced convulsions or changes in locomotor activity. Pretreatment of the animals with progesterone or estrogen blocked nicotine-induced antinociception. Even at much higher doses testosterone did not affect the response to nicotine, suggesting specificity for ovarian hormones (78).

Nicotine pharmacokinetics was not evaluated in the Damaj study, but it is unlikely that differences in nicotine metabolism underlie the gender differences in sensitivity observed. If males were slower metabolizers of nicotine they might be expected to be more sensitive to nicotine on all of the measures they evaluated. Hatchell and Collins also reported that male and female mice do not differ significantly in nicotine pharmacokinetics (61). Klein (79) reported that female adolescent C57Bl/6J mice consume more oral nicotine than males, when normalized to account for differences in body weight. In spite of the differences in nicotine intake, there were no gender differences in plasma cotinine levels. The authors suggest that female mice may have more rapid elimination of nicotine compared to male mice. In summary while nicotine responsiveness and metabolism may vary somewhat by gender, differences in species/strain tested, dose, route of administration, behavioral task employed and the duration of the exposure are also critical. One consideration in future studies evaluating the acute responsiveness to nicotine in female is to ensure that the phase of the estrus cycle is established and controlled for in all experiments.

## 4.2. Dopamine, ovarian hormones and nicotine sensitization

Gender differences in the long-term adaptive changes to nicotine have also been performed using rat models. Some studies have found that females develop more locomotor sensitization following chronic nicotine intravenous infusions (80-82), however female rats injected subcutaneously with nicotine did not (82). Chronic nicotine infusion for 14 days did not affect the daily pattern or cytology of estrus cycling in female rats (80). The effects of ovarian steroids on dopamine neuron function could contribute to gender differences in nicotine sensitization (9-11, 55). Dluzen (83) showed that estrogen treated females had enhanced release of DA from superfused striatal slices in female animals, but estrogen decreased DA release in males. In a study by Harrod *et al.* (81) increased nicotine locomotor sensitization in females was accompanied by increased dopamine transporter and dopamine D3 receptor expression in the nucleus acumbens. The differences observed in dopamine markers in this study are very intriguing. However no saline controls were included with this study and it is thus not clear whether females have baseline differences in dopamine neurons in the nucleus acumbens or whether a gender-dependant change occurs in females following chronic nicotine infusion. Nevertheless, the results of this study provide a provocative direction to follow in future studies related to gender differences in nicotine sensitivity. In another study utilizing female mice, intraperitoneal injections of nicotine caused locomotor sensitization that was not altered by ovariectomy, estrogen replacement or the phase of the estrus cycle (77). Thus the species used, route/dose of nicotine administration and other interactions may regulate the effects of estrogen on nicotine sensitization.

## 4.3. *In vivo* studies with ovarian hormones and nicotine metabolism

Although most studies have focused on pharmacodynamic differences between males and females, there is some evidence supporting gender differences in nicotine pharmacokinetics from the animal literature. Most studies have found comparable rates of nicotine metabolism in males and females (61). Kyerematen *et al.* (84) measured nicotine metabolism in four different strains of rats, and in each case, nicotine metabolism was faster in males compared to females. Siu *et al.* (85) evaluated mice consuming oral nicotine, and concluded that the rate of nicotine metabolism is highest in mice that consumed the most nicotine. Mice that had the highest amount of nicotine intake had the highest amount of CYP2A6, resulting in the most efficient metabolism of nicotine. However gender differences were not specifically evaluated in this study.

## 4.4. Effects of ovarian steroids of the density/function of neuronal nAChRs

Ovarian steroid hormones have been shown to have acute and chronic regulatory effects on neuronal nAChR density/function in both *in vivo* and *in vitro* paradigms. The first studies reporting this phenomenon were published by Morley (86) and Miller (87-89). Ovariectomy was shown to reduce the density of alpha 7

nAChRs in the hypothalamus, and estrogen replacement increased alpha-bungarotoxin binding to control levels. Similar results have been observed in other brain regions including the amygdala (90) and raphe nucleus (91) and cerebellum (92). The density of non-alpha 7 nAChRs does not appear to be sensitive to regulation by estrogens, or by other hormones. Previous studies have shown that adrenalectomy increases, and corticosterone replacement decreases alpha 7 expression in mouse hippocampus and cerebral cortex (93-95); however gender differences in this regulation have not been evaluated. Svensson (96) reported that chronic estradiol exposure did not affect epibatidine binding to human neuroblastoma cells. However estradiol administration did attenuate nicotine-induced increase in nAChR expression. Some studies have reported that male animals have greater CNS nAChR up-regulation following chronic nicotine treatment (97, 98), however Donny *et al.* (76) found no sex differences in rat brain nAChR density following chronic nicotine self-administration. Nicotinic receptor regulation by ovarian hormones may vary by species, route/duration of nicotine administration, and a variety of other factors. However, in general the number, synaptic location, subtype distribution and up-regulation of nAChRs appear to be fairly consistent in males and females, at least in rodent models that have been utilized to date.

### 4.5. *In vitro* steroid hormone actions on natively expressed receptors

*In vitro* electrophysiological studies evaluating the effects of ovarian steroids on native or heterologously expressed neuronal nAChRs have provided some interesting data that may contribute to gender differences in nicotine psychopharmacology. Glucocorticoids, androgens and estrogen all reduced peak and steady state currents generated by nicotine exposure in a rat superior cervical ganglion preparation (99). Evaluating the effects of pregnenelone sulfate (a neurosteroid found in the CNS) on acetylcholine-induced catecholamine release from cultured adrenal chromaffin cells, Kudo *et al.* (100) concluded that this steroid is non-competitive antagonist of nAChRs expressed on chromaffin cells (most likely nAChRs containing the alpha 3 subunit). Potassium stimulated catecholamine release was not affected by pregnenelone sulfate, indicating a specific interaction with neuronal nAChRs. Similar results were reported by Ke and Lukas (101, 102) who showed that pre-exposure of muscle, ganglionic or neuronal nAChRs to steroids such as progesterone, estradiol, corticosterone, or dexamethasone inhibited rubidium efflux, a functional assay for nAChRs. Bullock *et al.* (103) evaluated the effects of progesterone and its' A-ring reduced metabolites on rubidium efflux from thalamic synaptosomes and DA release from striatal synaptosomes. Progesterone was concluded to be a non-competitive inhibitor of neuronal nAChRs. Results using heterologously expressed receptors support the hypothesis that ovarian steroids are allosteric inhibitors of nAChR function (78, 104). Since progesterone and metabolites can enhance inhibitory transmission via allosteric interactions with GABAa receptors, it is interesting that they seem to attenuate excitatory signaling mediated via nAChrs. However other studies have shown that estrogen potentiates

neurotransmission in human nAChRs expressed in oocytes and HEK cells (105). Estrogen increased the affinity of alpha 4/beta 2 nAChRs for ACh, acting as a positive allosteric modulator. This actions occurred rapidly after E2 exposure, indicating a non-genomic mechanism that was rapid, reversible, and occurred at physiological levels of steroid hormones.

### 4.6. Gender dependant outcomes following prenatal nicotine administration

Prenatal exposure to nicotine (or other drugs) might cause gender-dependant outcomes that influence drug responsiveness in exposed offspring. While there are few human studies that address this possibility, experiments with animals provide some support for this possibility. Male mice (106) and rats (107, 108) exposed to prenatal nicotine are hyperactive as adults compared to females in some studies. Gender dependant outcomes have also been reported in nicotine preference (109) and pre-pulse inhibition (110) following developmental nicotine exposure. Human studies that evaluate interactions between ovarian hormones and nicotine should consider developmental exposure to nicotine as a possible confounding influence in their studies.

### 5. PERSPECTIVE

In spite of a large volume of research published on sex differences in tobacco smoking dynamics and the neuropharmacological actions of nicotine, whether gender per se, plays a prominent role in the regulation of these events is still open to conjecture. Consistent findings in the clinical literature include 1) women have a more time discriminating the nicotine stimulus compared to men 2) women are more sensitive to cue-induced nicotine craving, often possibly leading to recidivism during an attempt to quit 3) NRT may be more efficacious in men and 4) females seem to metabolize nicotine at a higher rate than men. However the role of ovarian hormones in regulation of these differences has not been clearly established. It is possible that estrogen may directly or indirectly accelerate nicotine metabolism, leading to failure of NRT. However it is premature to suggest that women should use higher doses of NRT, due to other safety issues. The animal literature also supports some gender-dependent actions of nicotine, but in many cases conclusions drawn from human studies are not fully supported (e.g. female rats metabolize nicotine more slowly than male rats). Species differences between rats and mice might also contribute to some of the inconsistencies between the human and rodent studies that have been published to date. It is clear that many sex differences in CNS activity and/or adaptations to tobacco smoking are present in males and females. However sex differences are more complicated than simply hormonally modulated responses that differ in men and women, and this concept deserves wider recognition. Sex differences supercede the effects of hormones alone, and extend into other biological, anatomical, social and cultural realms (111). Organizational effects of sex hormones during brain development may sculpt the nervous system into gender specific patterns of neurochemistry and brain circuit activity. Gender differences such as these may affect brain

function through anatomical, cellular and molecular interactions that are just beginning to be understood (112). An interesting recent paper (113) suggests that adolescent female rats may acquire nicotine self-administration more rapidly that adolescent male rats, and additional studies in this area should be conducted, since human studies also suggest that females may progress more rapidly towards tobacco addiction. Individual smokers differ substantially in smoking prevalence, neurobiological affects of smoking, smoking rate, inhalation patterns, transition from nicotine use to dependence and many additional factors. Genetics, race, age, socioeconomic status, cultural influences and psychiatric disposition can all significantly influence patterns of tobacco smoking (114). Thus, gender per se, may only be a superficial reason that leads to individual differences in smoking behaviors in males and females. It is important to not assume that all individuals have the same motivation to use tobacco or other drugs of abuse. As previously discussed, in most developed countries fewer women use tobacco compared to men. In these countries it may be that the ~20% of females that smoke represent the most dependant smokers who are truly using nicotine for purposes of self-medication. It would be very useful to compare the effects of gender on nicotine psychopharmacology and smoking cessation in countries such as Sweden, Norway and New Zealand where smoking rates are similar in men and women.

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