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Examining potential sex differences with treatments to reduce nicotine self-administration in rats

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ABSTRACT

A variety of neural systems are involved in nicotine addiction, presenting the opportunity to develop drugs with different mechanisms of action for reducing nicotine self-administration (SA) and enhancing cessation of tobacco use. Previously, we found that drug treatments affecting several different neurotransmitter systems significantly reduce nicotine SA in female rats. However, potential sex-differences in the effects of these drugs on nicotine SA have not yet been characterized. In this study, we compared the effects of a variety of drugs affecting dopamine, serotonin, histamine, norepinephrine and glutamate systems on nicotine SA in both male and female rats. The drugs tested in male and female Sprague-Dawley rats included lorcaserin, a serotonin $5HT_{2c}$ agonist; SCH-23390, a dopamine D_1 antagonist; pyrilamine, a histamine H_1 antagonist; dextromethorphan, a glutamate N -methyl-D-aspartate receptor (NMDA receptor) antagonist; and amitifadine, a triple dopamine, norepinephrine and serotonin reuptake inhibitor. We directly compared their efficacy in reducing nicotine SA in male and female rats. In addition, we compared the effects of these drugs by sex with regard to food-motivated responding and locomotor activity. We found that all of the above-mentioned drugs except dextromethorphan (5 mg/kg) significantly decreased nicotine SA in both sexes. There were significant sex differences in the timing of expression of drug effects on nicotine SA across the initial and repeated phases of drug treatment. Male rats showed greater effects after repeated treatment and females showed greater effect during initial treatment. These drugs also reduced food self-administration and locomotor activity. However, notably, amitifadine and pyrilamine produced significantly greater effects in reducing nicotine SA than both food self-administration and locomotor activity. Subtle differential effects between male and female rats were seen with drugs acting on monoaminergic transmitter systems with respect to nicotine self-administration.

KEYWORDS: male, female, SCH-23390, lorcaserin, pyrilamine, dextromethorphan, amitifadine.

INTRODUCTION

Tobacco addiction involves a variety of interconnected behavioral processes and neuronal systems. Therefore, treatments to aid cessation of tobacco use can be developed to act through a variety of mechanisms. The most direct actions of cessation aides are through nicotine replacement or inhibition of nicotinic acetylcholine receptors using medications like varenicline. However, these interventions are only effective for a portion of all patients who have the desire to quit smoking, and compounds with other mechanisms of action are needed to address this treatment gap.

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Promisingly, targeting several neurotransmitter systems beyond neuronal nicotinic acetylcholine receptors can significantly reduce nicotine self-administration in rat models. We and others have shown that drugs affecting dopamine, serotonin, histamine, glutamate and norepinephrine systems can each influence nicotine self-administration in rats. This suggests that therapies targeting one of several neuronal systems can be developed to address the needs of different patients.

Dopaminergic systems in particular the dopaminergic innervation of the nucleus accumbens from the ventral tegmental area, are key systems for all drug addictions, including tobacco addiction. In our earlier studies, dopamine D_1 receptors were found to be more relevant drivers of nicotine self-administration than D_2 receptors [1, 2]. D_1 receptors are found not only in the nucleus accumbens but also in the insular cortex and anterior cingulate cortex. The D_1 antagonist SCH-23390, when administered systemically, significantly reduces nicotine self-administration in female rats, as does local infusion of SCH-23390 into the nucleus accumbens, insular cortex and anterior cingulate cortex. In contrast, the D_2 antagonist haloperidol was not found to significantly affect nicotine self-administration when administered either systemically or locally into any of these brain areas [1, 2]. Although SCH-23390 is a proprietary drug and is not in development for clinical applications, its specific modulation of D_1 activity appears to be a promising target for cessation aid development.

Serotoninergic systems are also involved in nicotine self-administration. In particular, serotonin $5HT_{2c}$ receptors appear to play a key role in this phenomenon. For instance, systemic administration of the $5HT_{2c}$ agonist lorcaserin significantly reduces nicotine self-administration in rats [3-5]. Lorcaserin has also been shown to significantly reduce self-administration of other drugs of abuse such as alcohol [6] and cocaine [7]. Recently, lorcaserin has been found to significantly improve tobacco smoking cessation in moderate smokers as well [8]. Lorcaserin is currently in use as a weight loss drug, but appears to have substantial potential for additional uses as a cessation aid for smoking.

In addition to its well-known role in the peripheral nervous system, histamine plays an important role in the brain. We have shown that histamine H_1 receptors are important players in controlling nicotine self-administration. Acute or chronic administration of the H_1 antagonist pyrilamine significantly reduces nicotine self-administration in female rats [9]. Pyrilamine is a well-tolerated antihistamine and is found in several over-the-counter medications, including cold medicines and pain relievers, but may have additional applications in smoking cessation.

Glutamate is the most widespread excitatory neurotransmitter in the brain. Drugs that affect NMDA glutamate receptor activity have been shown by our studies and others to significantly reduce nicotine self-administration. We have shown that D-cycloserine which is a partial agonist of NMDA glutamate receptors, significantly reduces nicotine self-administration after acute or chronic treatment of female rats that have lower than median baseline nicotine self-administration [10]. Dextromethorphan is a complex drug that has actions as an antagonist at NMDA glutamate receptors as well as plays a variety of other roles in the brain, and significantly reduces nicotine self-administration after acute or chronic treatment of female rats [11]. Dextromethorphan is a well-tolerated cough suppressant found in a number of over-the-counter medications, and may have additional applications in smoking cessation.

Although we and others have identified a variety of targets for intervention, more work is needed to understand how sex influences substance abuse and the eventual effectiveness of any proposed treatment. Sex-related differences have been well documented in both humans and laboratory animals in every aspect of drug addiction, including reinforcing effects, craving, relapse and withdrawal [12-16]. Sex differences have been established in the acquisition of intravenous self-administration of addictive drugs such as cocaine, methamphetamine, heroin, and nicotine in rats [17-23]. We have shown further sex differences in nicotine self-administration, with female rats showing a more rapid trailing off of self-administration over the course of the test session [24]. In addition, we found that male rats self-administered more nicotine during adolescence

but that male and female rats self-administered similar amounts of nicotine during adulthood [25]. Others have also shown that adolescent female rats are more sensitive to the acute rewarding effects of nicotine than adolescent male rats [26].

Similar findings on sex differences also have been reported in humans. For example, women who smoke report smoking more cigarettes, a greater withdrawal response, and greater difficulty quitting than their male counterparts [27]. Additionally, women have been reported to exhibit more rapid escalation from drug taking to addiction, a phenomenon also seen in female rats [15, 28]. On the other hand, reports indicate that men may experience more positive effects of a variety of drugs than women [28, 29], and so may be vulnerable to addiction for somewhat different reasons than women. For instance, men have elevated rates of substance abuse relative to women, including smoking. This difference corresponds with a variety of underlying neurobiological and environmental risk and cultural factors as well as sexual dimorphisms [see 28 for review]. These gender-related differences impact virtually every aspect of drug addiction including pharmacokinetics, reinforcing effects, craving, relapse, and withdrawal in both humans and laboratory animals [12-14, 16]. It also has been shown that sex plays a major role in the synthesis, release and uptake of some neurotransmitters such as dopamine [12-15]. For example, *in vivo* microdialysis of castrated male rats and ovariectomized female rats showed that basal level of extracellular dopamine was significantly lower in the nucleus accumbens and dorsal striatum of female rats compared to male rats [29, 30]. Sex differences in D_1 and D_2 dopaminergic receptors in the frontal cortex and dorsal striatum have also been documented [31]. Although environmental factors, mental health problems, availability of drugs, and cultural differences play an important role in substance abuse and addiction in both males and females, it has been shown that sex plays a major role in the neuronal mechanisms mediating drug abuse and addiction as well as the pharmacotherapy of addiction [12, 32].

Given these differences, a strong understanding of how sex differences modulate substance use and treatment will be necessary to give both men and women the best chances for successful medication-assisted smoking cessation. In this respect, one important question that still remains is whether there are sex differences in response to pharmacotherapy for nicotine self-administration. The current study was conducted to determine the effect of a variety of drugs affecting dopamine, serotonin, histamine, norepinephrine and glutamate systems on nicotine self-administration in both male and female rats. To assess the more general behavioral effects of these drug treatments, we also tested the effects of these drugs on food-motivated responding and locomotor activity. Sex differences and similarities in response to nicotine self-administration will provide a more robust understanding of the most effective use of these drugs to facilitate smoking cessation.

MATERIALS AND METHODS

Animals

Young adult male (N = 12) and female (N = 9) Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC, USA) were used. Animals were individually housed in a temperature-controlled vivarium room located adjacent to the nicotine self-administration testing room. Animals were maintained on a 12:12 reversed light-dark cycle (lights off at 7.00 a.m.) so that experimental sessions occurred during the active phase of the rats' diurnal cycle. Animals were given *ad libitum* access to water at all times excluding experimental sessions and were fed daily 20-30 minutes after the completion of their experimental session. Despite being maintained at roughly 85% of their free-feeding weight, the animals progressively and healthily gained weight throughout the study. At the end of each experimental session, catheter patency tests were conducted and the data from animals with obstructed catheters were dropped from statistical analysis. All procedures were approved by the Institutional Animal Care & Use Committee of Duke University.

Drug preparation

Nicotine bitartrate solutions were prepared every 2 weeks in sterilized isotonic saline. The dose of nicotine used for self-administration (0.03 mg/kg/infusion) was calculated as a function of the nicotine-free base molecular weight. The pH of the nicotine solution was adjusted to 7.0 using NaOH and the solution was filtered in a Nalgene

filter (Nalgene Nunc International, Rochester, NY, USA) for sterilization. Between sessions, all nicotine solutions were kept in a dark refrigerator. All other drugs were also prepared in sterilized isotonic saline and kept in a dark refrigerator between experiments.

Drug treatments

Nicotine was available in the self-administration procedure at a dose of 0.03 mg/kg/infusion as described below. The following drugs were administered by subcutaneous (SC) injection 20 min. before the start of the one-hour behavioral test sessions following a repeated measures counterbalanced design: saline vehicle, serotonin $5HT_{2c}$ agonist lorcaserin (0.6 mg/kg), dopamine D_1 antagonist SCH-23390 (0.01 mg/kg), histamine H_1 antagonist pyrilamine (20 mg/kg), NMDA glutamate antagonist dextromethorphan (5 mg/kg) and the triple monoaminergic (dopamine, serotonin and norepinephrine) reuptake inhibitor amitifadine (10 mg/kg). There were two treatment phases, referred to as Phase 1 and Phase 2 henceforth, with each of the drugs and vehicle given in a repeated measures counterbalanced order once, and then the same doses given a second time a week later. These doses were selected based on our previous findings in rats. Drug-free nicotine self-administration sessions without injection were run between each drug test session.

The volume of drug injection (SC) was 1 ml/kg and the time of injection was 20 min. before the start of the test session. To assess the specificity of each drug for nicotine self-administration, following the completion of the drug treatments for nicotine self-administration, the same drugs were tested in the same manner once for their potential effects on food-motivated responding, and then once for their effects on locomotor activity.

Behavioral procedures

Before the start of nicotine self-administration sessions, all animals were trained to lever press in a standard dual-lever experimental chamber (Med Associates, St. Albans, VT, USA) for food reinforcement. Each chamber was equipped with two levers (one active, one inactive), two cue lights located directly above each lever, a house light, and a tone generator. After lever pressing was established, animals experienced three sessions of lever pressing for food under a fixed ratio (FR1) schedule of reinforcement. Following the completion of their final training session with food reinforcement, animals were anesthetized by injection of ketamine (60 mg/kg) and medetomidine hydrochloride (0.15 mg/kg) and a catheter (Strategic Application Inc., Libertyville, IL, USA) was implanted into their jugular vein. The jugular catheter was attached to a harness that could be tethered to the infusion pump during experimental sessions. Animals were given a minimum of 24 hours to recover from the surgery before experiencing nicotine self-administration sessions.

Nicotine self-administration

Following recovery from the surgery, animals experienced 5 experimental sessions where a correct lever press resulted in the delivery of a nicotine infusion (0.03 mg/kg/infusion) on a fixed ratio (FR1) schedule of reinforcement, and the activation of a feedback tone for 0.05 sec. Each infusion was followed by a 20-sec period time-out where the cue lights went off, the house light came on, and correct responses were recorded but not reinforced. Each nicotine self-administration session lasted for 60 min. After establishing a baseline with the initial 5 sessions of nicotine self-administration training, the rats were tested for the effects of different drug treatments on nicotine self-administration.

To keep the catheters patent, they were flushed daily before the experimental sessions with a 100 U/ml heparinized saline solution. After the completion of a test session, any nicotine remaining in the port was removed, and a 0.3-ml sterile lock solution containing 500 U/ml of heparinized saline and 8 mg/ml of gentamicin (American Pharmaceutical Partners, Schaumberg, IL, USA) was infused [33].

Food-motivated responding

To assess the specificity of the drugs, the rats were tested for the effects of the same drug treatments and control vehicle on food-motivated responding. To measure the acute effects of the drugs on food-motivated responding, the rats were administered SC injections of each drug and tested 20 min later in the operant testing apparatus for 45 mg food rewards on an FR-1 schedule for

one hour. The numbers of lever pressing for food was recorded at the end of the session.

Locomotor activity

Testing for locomotor activity was conducted in an enclosed maze apparatus in the shape of a figure-8. The maze consisted of a continuous alley that measured 10 cm x 10 cm, contained within an apparatus measuring 70 cm x 42 cm. Animals were injected SC with each drug or the control vehicle, and 20 minutes later, were allowed to freely explore the entire apparatus. Locomotor activity was assessed by the crossing of eight photobeams located at equal points in the maze alleys. Each locomotor test session lasted 1 h, and photobeam breaks were tallied in 5 min. blocks across the entire session.

Data analysis

The data were assessed for statistical significance by the analysis of variance. Hierarchical analysis of mix variance was performed between subjects and repeated measures. The between-subjects factor was sex, and the within-subjects factor for all behavioral tests was drug treatment. In addition, repetition of drug treatment was designated as a within-subjects factor for nicotine self-administration, and the 5-min block within the one-hour session was a within-subjects factor for locomotor activity testing. In order to equate the baseline for each test, the performance measures in nicotine self-administration and the number of photobeam breaks per 5 min block were converted into a percentage of the control condition means. Interactions with $p < 0.10$ [34] were followed up with tests of the simple main effects of treatment at each level of the interacting factor. Planned comparisons were made between the vehicle control condition and each of the drug treatments. An alpha cutoff of $p < 0.05$ (two-tailed) was used as the threshold for significance.

RESULTS

Nicotine self-administration

The main effect of drug treatment was very significant [$F(5,95) = 22.25$, $p < 0.0005$]. As shown in Figure 1, compared with the control vehicle,

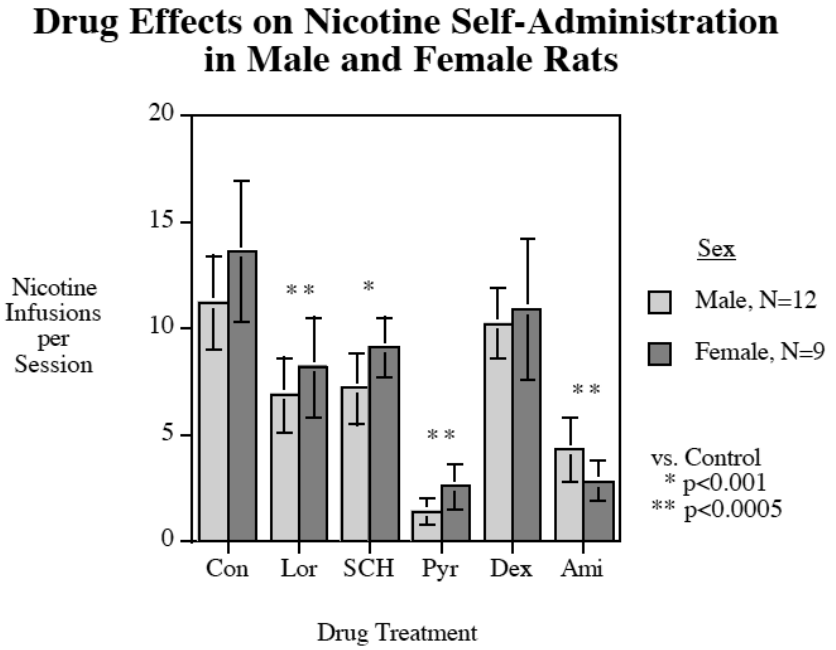

Figure 1. Effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on nicotine self-administration in male and female rats (mean ± sem). Each of the drug treatments except for dextromethorphan produced a significant ($p < 0.05$) decrease in nicotine self-administration relative to the control. No main effect of sex or sex x treatment effects was found. N = 12 males and 9 females.

lorcaserin ($p < 0.0005$), SCH-23390 ($p < 0.005$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$) each significantly decreased nicotine self-administration regardless of sex. Dextromethorphan did not produce a significant effect on nicotine self-administration. Neither the main effect of sex, nor sex x treatment effects was significant. The main effect of the phase of treatment was significant [$F(1,19) = 5.31$, $p < 0.05$] with self-administration of nicotine being higher during the second phase than for the first. There was a significant three-way interaction of drug x sex x phase [$F(5,95) = 2.66$, $p < 0.05$]. Tests of the simple main effects of drug treatment with each sex at each phase (Figure 2) showed that males during Phase 1 showed significant decreases in nicotine self-administration in response to pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.05$), but not the other drugs. The males showed more pervasive effects in Phase 2 with significant reductions in nicotine self-administration with lorcaserin ($p < 0.0005$), SCH-23390 ($p < 0.0005$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$). Females showed a different pattern of effects. In Phase 1, they showed significant declines in nicotine self-administration with lorcaserin ($p < 0.05$), SCH-23390 ($p < 0.001$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$). However, in females, smaller effects were seen with some drugs in Phase 2, with significant declines seen with lorcaserin ($p < 0.05$), pyrilamine ($p < 0.0005$) and amitifadine

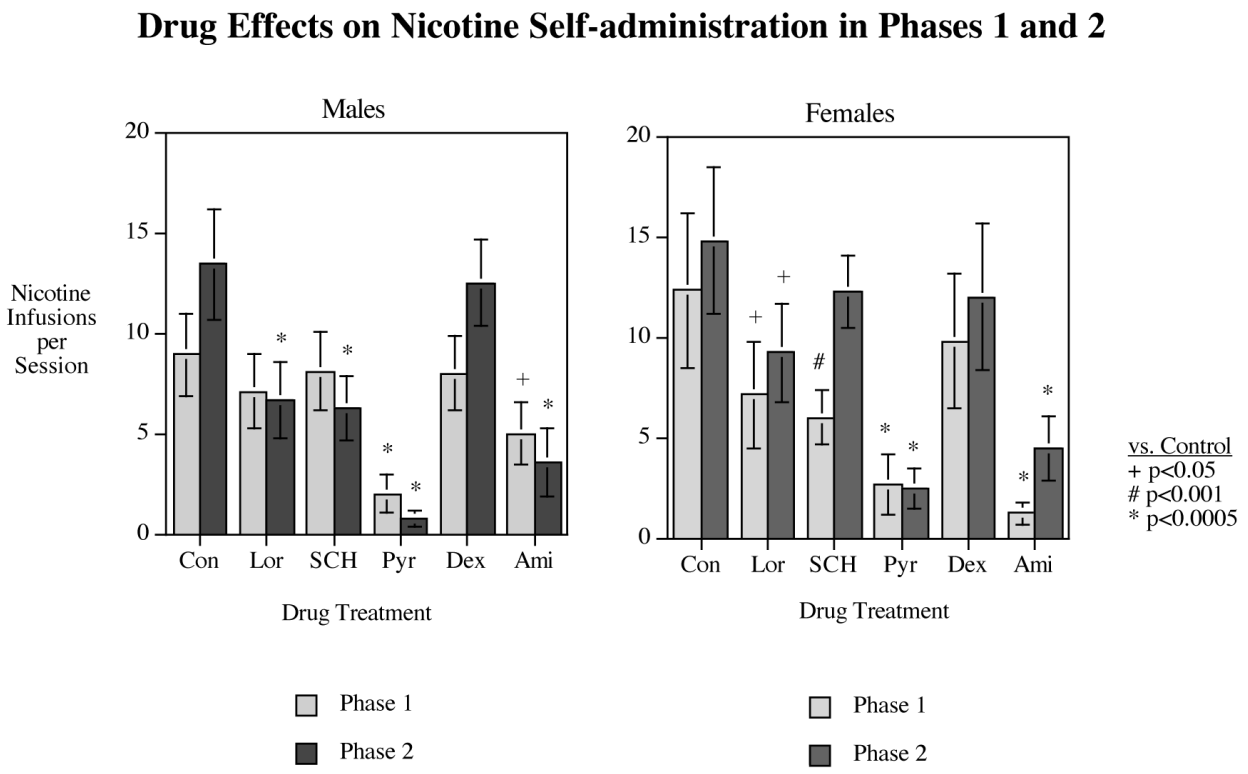

Figure 2. Effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on nicotine self-administration in male and female rats (mean ± sem) during repeated phases of drug administration. There was a significant interaction of drug treatment x sex x phase interaction ($p < 0.05$). Males in Phase 1 showed significant reductions in nicotine self-administration only with pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.05$) while in Phase 2 they showed significant reduction in nicotine self-administration with lorcaserin ($p < 0.0005$), SCH-23390 ($p < 0.0005$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$). In contrast, females in Phase 1 showed significant reductions in nicotine self-administration with lorcaserin ($p < 0.05$), SCH-23390 ($p < 0.001$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$), and in Phase 2 they showed similar effects except that SCH-23390 was no longer active. N = 12 males and 9 females.

($p < 0.0005$). No evidence for carryover of drug effects was seen (Figure 3).

Food-motivated responding

Effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on food-motivated responding in male and female rats were assessed (Figure 4). As hypothesized, a pronounced sex effect was seen [$F(5,95) = 15.33$, $p < 0.001$] with males averaging 241.3 ± 11.7 pellets per session and females averaging 137.1 ± 8.9 pellets per session. There was a significant main effect of drug treatment [$F(5,95) = 15.00$, $p < 0.0005$]. Lorcaserin ($p < 0.05$), SCH-23390 ($p < 0.01$), pyrilamine ($p < 0.0005$) and amitifadine ($p < 0.0005$) all significantly reduced food-motivated responding. No differential sex x treatment effects were seen.

Although some of the drug treatments affected both nicotine self-administration and food-motivated responding, the nicotine self-administration was affected significantly [$F(5,95) = 4.12$, $p < 0.005$] and more pronouncedly. The comparative magnitudes of the effects are shown in Figure 5. To put the effects on an even baseline, scores for all rats in both tests were expressed as a percentage of overall control performance on each test. Thus, control performance averaged 100% for both nicotine self-administration and food-motivated responding. As it is clear from the left panel of Figure 5, there was a greater degree of decrease for nicotine self-administration than for food-motivated responding for several of the drug treatments. The right-hand panel of Figure 5 shows the difference between the effect of each drug with the difference in percent of control performance between nicotine self-administration and food-motivated responding calculated for each rat. For lorcaserin ($p < 0.05$), pyrilamine ($p < 0.005$) and amitifadine ($p < 0.0005$) there were significantly greater reductions of nicotine self-administration than food-motivated responding. Lorcaserin produced a 21.8 ± 11.9% greater decrease in nicotine self-administration than food-motivated responding, pyrilamine produced a 31.7 ± 6.5% greater decrease in nicotine self-administration than food-motivated responding, and amitifadine produced a 40.2 ± 10.1% greater

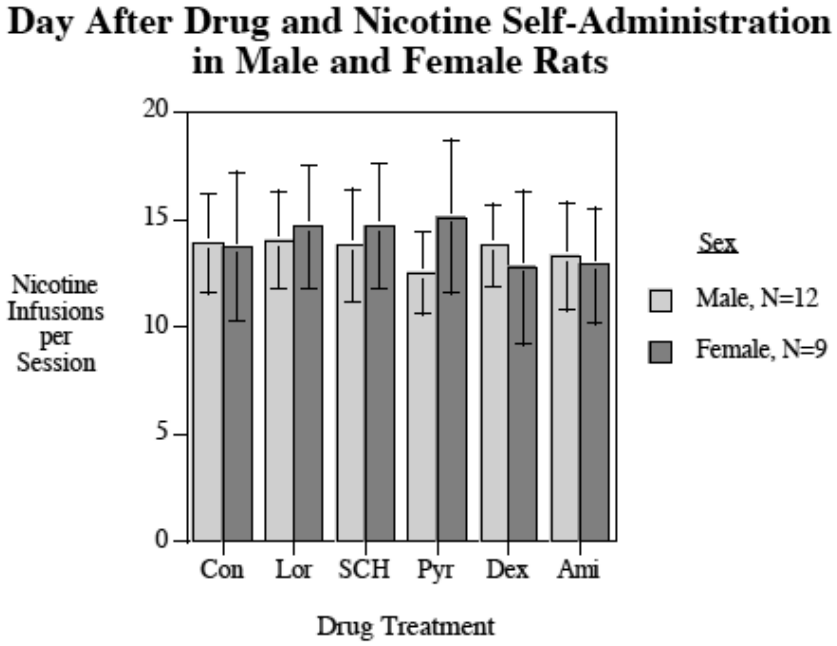

Figure 3. Nicotine self-administration on the day after the treatment with lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine in male and female rats (mean ± sem). In the session following each of the drug treatments, nicotine self-administration had returned to a level not different from the control levels. N = 12 males and 9 females.

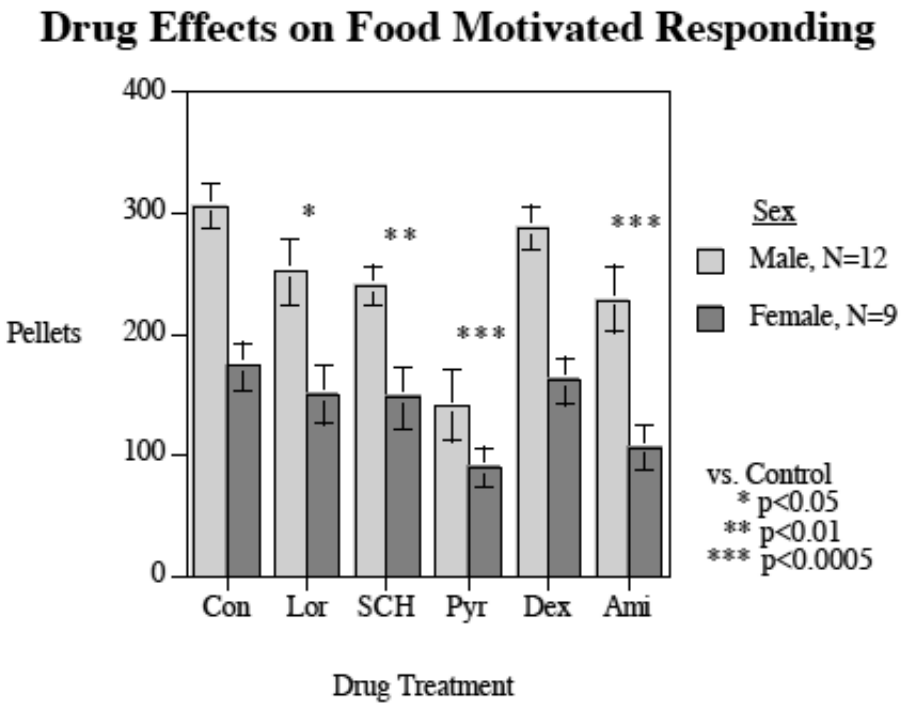

Figure 4. Effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on food-motivated responding in male and female rats (mean ± sem). Each of the drug treatments except for dextromethorphan produced a significant (lorcaserin, $p < 0.05$; SCH-23390, $p < 0.01$; pyrilamine, $p < 0.0005$; amitifadine, $p < 0.0005$) decrease in food self-administration relative to control. There was a significant ($p < 0.001$) main effect of sex, but no differential sex x treatment effects was seen. N = 12 males and 9 females.

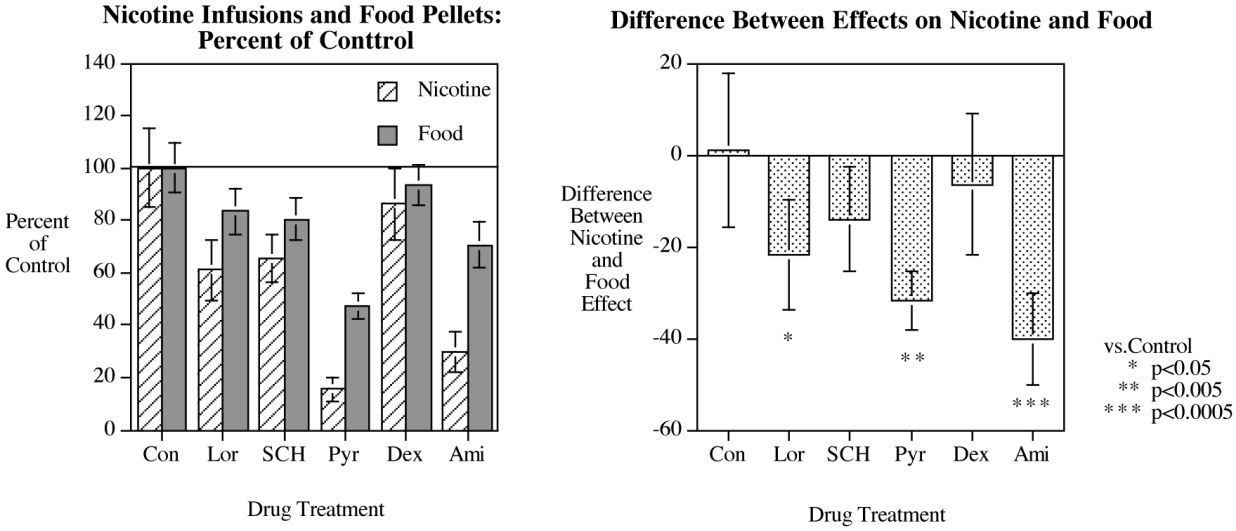

Figure 5. Differential effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on nicotine and food motivated responding (mean ± sem). Lorcaserin ($p < 0.05$), pyrilamine ($p < 0.005$) and amitifadine ($p < 0.0005$) had significantly greater effects on reducing nicotine self-administration than food self-administration. N = 12 males and 9 females.

decrease in nicotine self-administration than food-motivated responding. No carryover effects were seen with either type of testing during the no-treatment sessions that took place after each drug and control session.

Locomotor activity

With locomotor activity, there was a significant [$F(5,95) = 10.55$, $p < 0.0005$] main effect of drug treatment. Each of the drug treatments except for dextromethorphan produced a significant ($p < 0.05$) decrease in locomotor activity relative to the control (Figure 6). There was a significant main effect of sex [$F(5,95) = 10.55$, $p < 0.0005$], but no significant interaction of drug treatment x sex. The main effect of the 5-min block within session was quite significant [$F(11,209) = 68.60$, $p < 0.0005$] with habituation (decreasing activity) over the course of the 12 5-min time blocks over the one hour session. The interaction of time block x drug treatment was significant [$F(55, 1045) = 5.04$, $p < 0.0005$)]. As shown in Figure 7, the habituation of locomotor activity was significantly ($p < 0.005$) decreased by each drug treatment except for dextromethorphan.

To compare the magnitude of effect of the drug treatments on locomotor activity vs. nicotine self-administration, the difference in percent of control response for each measure was calculated in the same way as calculated for the comparison of food-motivated responding and nicotine self-administration. The results of this analysis show that two of the treatments, pyrilamine ($p < 0.05$) and amitifadine ($p < 0.0005$), showed a significantly greater effect of reducing nicotine self-administration than reducing locomotor activity, with pyrilamine producing a 40.5 ± 6.8% greater decrease in nicotine self-administration than locomotor activity and amitifadine producing a 47.6 ± 8.5% greater decrease in nicotine self-administration than locomotor activity (Figure 8).

DISCUSSION

This study tested for sex differences in response to five evaluated drugs that we had previously

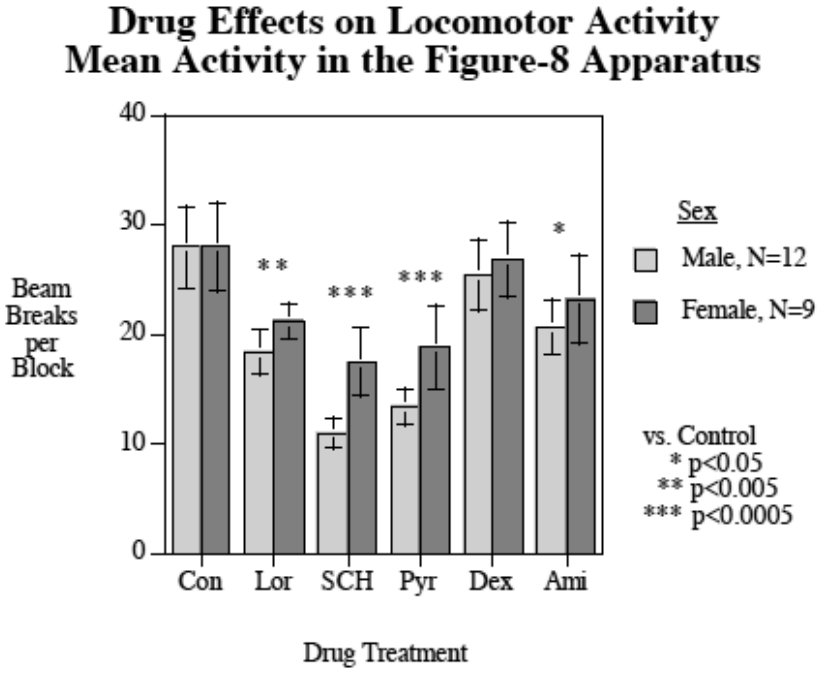

Figure 6. Effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on mean locomotor activity in male and female rats in the Figure-8 apparatus (mean ± sem). Each of the drug treatments except for dextromethorphan produced a significant ($p < 0.05$) decrease in locomotor activity relative to control. No main effect of sex or sex x treatment effects was found. N: 12 males and 9 females.

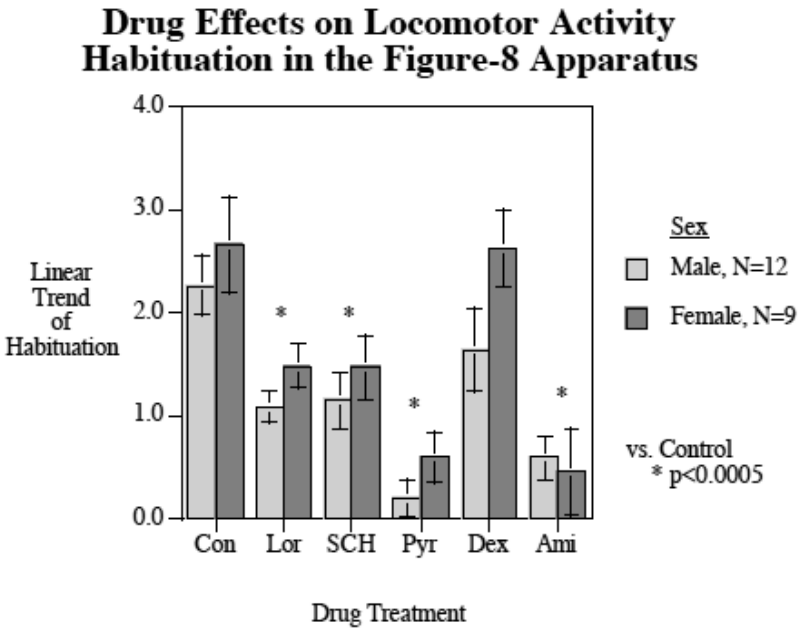

Figure 7. Linear trend of habituation (decreasing activity) over the course of the one-h session (mean ± sem). All of the treatments except for dextromethorphan significantly ($p < 0.0005$) decreased habituation relative to control.

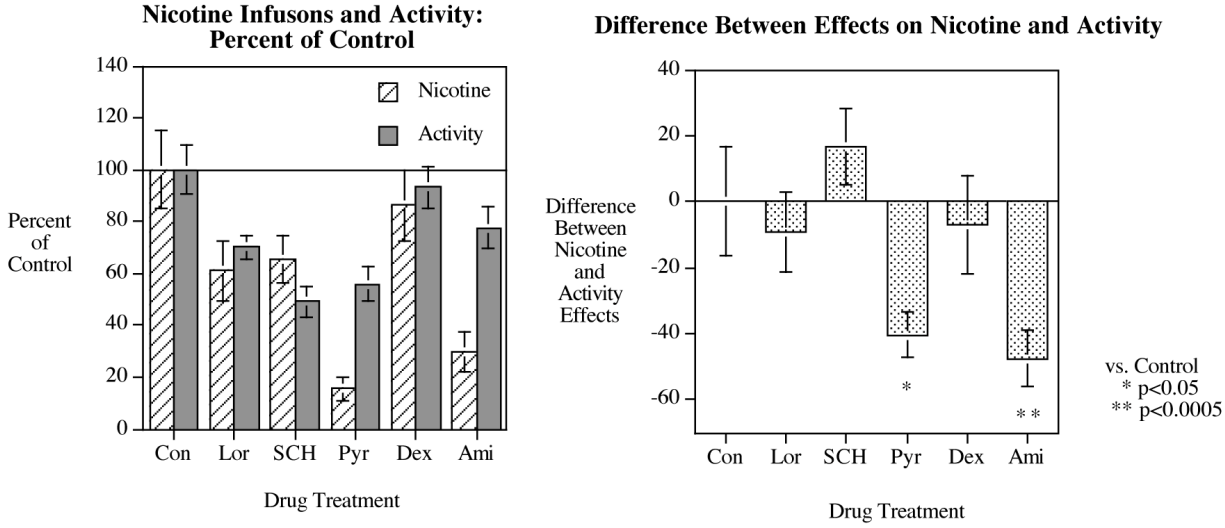

Figure 8. Differential effects of lorcaserin, SCH-23390, pyrilamine, dextromethorphan and amitifadine on nicotine self-administration and locomotor activity (mean ± sem). Pyrilamine ($p < 0.05$) and amitifadine ($p < 0.0005$) had significantly greater effects in reducing nicotine self-administration than locomotor activity. N = 12 males and 9 females.

found to significantly decrease nicotine self-administration in female rats. The dopamine D_1 antagonist SCH-23390, the serotonin $5HT_{2c}$ agonist lorcaserin, the histamine H_1 antagonist pyrilamine and the triple monoaminergic reuptake inhibitor amitifadine were all found to significantly reduce nicotine self-administration regardless of sex over all testing. There were subtle differential

effects of these drugs with respect to their effectiveness across the repeated phases of testing. Dextromethorphan at the 5 mg/kg dose was not found to significantly decrease nicotine self-administration regardless of sex. None of these drugs were found to have persisting effects on nicotine self-administration in the session following each of the drug treatments. The effects of SCH-23390, lorcaserin, pyrilamine and amitifadine, but not dextromethorphan were also seen in food-motivated responding and locomotion, again regardless of sex.

Amitifadine and pyrilamine showed greater effects in reducing nicotine self-administration than the other drugs tested. Furthermore, the analysis of the differential effects of the treatments on nicotine vs. food self-administration showed that lorcaserin, pyrilamine and amitifadine all had significantly greater effects in reducing nicotine self-administration than food self-administration. Among these three compounds, amitifadine had the most robust differential effect.

All four drugs that decreased nicotine self-administration also significantly decreased locomotor activity. However, the analysis of the differential effects of the drug treatments on locomotor activity vs. nicotine self-administration showed that pyrilamine and amitifadine had a significantly greater effect of reducing nicotine self-administration than reducing locomotor activity.

Interestingly, though the overall effectiveness of the drug treatments showed no significant sex differences, there were significantly differential effects of the drug treatments in male and female rats across the successive phases of administration. Males showed more substantial effects during the second round of drug dosing (Phase 2) compared to the initial round (Phase 1). In contrast, females showed the full effects of the drug treatments reducing nicotine self-administration during Phase 1, and some degree of tolerance during Phase 2. This sex difference in the timing of drug therapy may be of importance with regard to chronic drug treatment to aid smoking cessation. Further research is needed to determine the full extent of this effect.

Similar to our previous study [11], the 5 mg/kg dose of dextromethorphan did not have a significant effect on nicotine self-administration, food-motivated responding, or locomotor activity in either male or female rats. However, we had previously found this dose to produce a modest, but significant effect in reducing nicotine self-administration in female rats [11]. The methodological difference between the current study and the earlier one was that in the current study, the rats were treated with other drugs between treatments with dextromethorphan. Thus, it is possible that this inter-concurrent administration of other effective drugs may have attenuated the effects of dextromethorphan in the current study. This interpretation is supported by findings in another earlier study [35] in which 5 mg/kg of dextromethorphan was not found to significantly lower nicotine self-administration when given intercurrently with another effective drug, sazetidine-A. However, notably, this was not the case with all other drugs. Intercurrent administration with the nicotinic channel blocker mecamylamine did not block the effect of dextromethorphan (5 mg/kg) in significantly decreasing nicotine self-administration [36]. Such evidence suggests that there may be complex interactions between dextromethorphan and other drugs, some of which may attenuate its efficacy at this dose. It has been shown that dextromethorphan behaves as a noncompetitive nicotinic receptor antagonist with a preferential activity to $\alpha 3\beta 4^*$ neuronal AChR subtypes [36]. Such evidence suggests that although none of the drugs led to persistent effects on nicotine self-administration, there may still be complex interactions between dextromethorphan and other drugs when administered intercurrently, one or more of which may attenuate its efficacy at the present dose.

Our exploration of sex differences in this study was based on our acknowledgement of the fact that the most prevalent and extensive genetic difference in humans is sex. There are numerous behavioral differences between males and females in both reproductive functions as well as non-reproductive behaviors, such as drug addiction. As such, it is certainly important to determine sex differences where they exist, as well as to determine where they do not. In this study, in which male and female rats went through the same behavioral assessments with the same drug treatments, we only saw subtle sex-based differential effects of these particular drug treatments on

nicotine self-administration, and none on food self-administration and locomotor activity.

Previous studies have established sex differences in response to other drug treatments for reducing nicotine self-administration in rats and smoking in humans. [24, 37]. It is thus important to continue testing for possible sex differences in drug effects and responses because the discovery of differential sex effects would have important implications for the conduct of clinical therapeutic addiction medicine. Sex is the most pervasive polymorphism in humans and many other species. Determining where there are significant sex differences in individuals' responses to potential pharmaceuticals against addiction and where there are not will help guide effective therapeutic treatment for smoking cessation as well as other diseases and disabilities for both sexes.

CONCLUSION

In conclusion, it was found that treatment with lorcaserin, pyrilamine, dextromethorphan, SCH-23390 and amitifadine, significantly decreased nicotine self-administration in both male and female rats. Male rats showed greater effects after repeated treatment and female rats showed greater effect during initial treatment. Although these drugs also reduced food self-administration and locomotor activity, both amitifadine and pyrilamine produced significantly greater effects in reducing nicotine self-administration. Determining sex differences in response to different therapeutic drugs for nicotine addiction as well as other diseases can be useful in finding more effective treatments for each sex.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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