Nicotine Demand Elasticity in Male and Female Rats

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Abstract

According to the Centers for Disease Control, tobacco use is the leading cause of preventable disease and death in the United States. The Family Smoking Prevention and Tobacco Control Act was signed into law in 2009 and authorized the Food and Drug Administration to manage levels of nicotine in tobacco products. To modify cigarette price and/or cigarette nicotine concentration in a way that minimizes addiction acquisition, it is important to understand how price and nicotine concentration affect consumption. This study contributes data to help answer this question.

Rodent intravenous self-administration is one of the most effective models for drug addiction research and allows for analysis of the reinforcing effects of nicotine. A behavioral economics approach can also be used to analyze the reinforcing properties of nicotine; studying relationship between cost and consumption of a product provides insight into the value assigned to the product by the consumer. Elasticity of demand is a measure of the rate of decrease in consumption of a product relative to an increase in price.

We combined these two concepts in this study. Male and female rats self-administered intravenous nicotine solutions of two different concentrations. Demand curves were created. Results showed that demand for the 0.01mg/kg/infusion solution was more elastic in males than females, but demand for the 0.03mg/kg/infusion solution was more elastic in females than males.
Introduction

According to the Centers for Disease Control, tobacco use is the leading cause of preventable disease and death in the United States\(^1\). Cigarette smoking has been linked to numerous diseases, including cancer, respiratory disease, cardiovascular disease, diabetes, and autoimmune disorders. It is estimated that smoking causes a person to lose at least a decade of life, yet smoking cessation by age 40 can reduce this loss of life by 90%\(^2\).

Nicotine is the primary component of cigarettes that contributes to addiction. In 1994, Benowitz and Henningfield proposed that the nicotine threshold of addiction was an intake of approximately 5mg per day\(^3\). Since young people may smoke as many as 30 cigarettes per day, each cigarette should provide no more than 0.17g of nicotine, to minimize addiction. Given that the maximum nicotine bioavailability of a cigarette is approximately 40%, it was concluded that addiction acquisition by new smokers would likely be prevented if the maximum nicotine concentration per cigarette was 0.4 - 0.5 mg\(^3\). Most conventional cigarettes contain 10-15 mg of nicotine per cigarette\(^4\).

The Family Smoking Prevention and Tobacco Control Act was signed into law in the United States in 2009 in an effort to decrease rates of smoking acquisition and promote smoking cessation. It authorized the Food and Drug Administration to regulate the manufacturing, marketing, and distribution of tobacco products, including the ability to manage their levels of nicotine\(^5\). For the FDA to modify cigarette price and/or cigarette nicotine concentration in a way that minimizes addiction acquisition, it is important to understand how price and nicotine concentration affect levels of consumption. This study contributes data to help answer this question.
Several clinical trials have studied smoking behavior. A small trial of smokers expressing interest in smoking cessation showed that participants smoking 0.05 mg nicotine yield cigarettes did not show compensatory smoking behavior and displayed reduced biomarker levels, reduced nicotine dependence, and lower withdrawal scores than participants smoking 0.3 mg nicotine yield cigarettes. After 4 weeks, smoking abstinence rates were higher in those that had smoked the 0.05 mg cigarettes. A large clinical trial showed similar results in smokers who were not interested in smoking cessation; participants assigned to smoke cigarettes with 2.4 mg of nicotine or less per gram of tobacco smoked fewer cigarettes per day than participants assigned to smoke cigarettes with 15.8 mg of nicotine per gram of tobacco after 6 weeks. Participants who smoked cigarettes with 0.4 mg nicotine per gram of tobacco also showed decreased nicotine dependence and were more likely to attempt smoking cessation. Participants given cigarettes with 1.3 or 0.4 mg nicotine per gram of tobacco smoked fewer cigarettes than the 15.8 mg group. These studies all provide support for Benowitz and Henningfield’s idea of a nicotine threshold of addiction.

While clinical trials are certainly vital, several limitations necessitate pre-clinical rodent studies in smoking cessation research. For example, it is difficult to study nicotine addiction acquisition in humans due to ethical concerns. In addition, study participants in clinical research may use non-study tobacco products, even if directed not to do so. In animal studies, restriction to use of study tobacco products is guaranteed, an animal’s history of nicotine exposure is known, and wider nicotine dose range can be administered than in a clinical trial. Rodent intravenous self-administration is one of the most effective models for drug addiction research and allows for analysis of the reinforcing effects of nicotine. Rodent studies corroborate findings of clinical trials that there is a nicotine threshold dose for maintenance of nicotine use, further supporting their validity.
A behavioral economics approach can be used to analyze the reinforcing properties of nicotine, providing important implications for drug policy. Studying the relationship between cost and consumption of a product provides insight into the value assigned to the product by the consumer. Elasticity of demand is a measure of the rate of decrease in consumption of a product relative to an increase in price of that product.

Reinforcers like food are considered inelastic, as there is a minimal decrease in consumption even with a large increase in price. Luxury goods are considered highly elastic, as consumption is dependent on price. Elasticity of a drug can be determined by analyzing a demand curve produced by plotting daily drug consumption as a function of the fixed-ratio (FR) “price” in log-log coordinates.

The present study uses rodent intravenous self-administration of nicotine to measure the demand elasticity of nicotine. Rodents are placed in an operant conditioning chamber and receive a nicotine infusion through a catheter by pressing a lever. The number of presses required for one infusion is the “price” of nicotine. By starting the “price” for an infusion at one lever press (also known as fixed ratio (FR) 1) and gradually increasing it over time, one can see what price initiates a product shift from inelastic to elastic ($P_{max}$).

In drug self-administration studies, Bickel defines unit price as follows:

$$\text{Unit price} = \frac{\text{response requirement}}{\text{reinforcer magnitude}}$$

In this study, response requirement is the number of lever presses required to receive one nicotine infusion. The reinforcer magnitude is the dose of nicotine per infusion. Consumption is
defined as the total amount of drug ingested and depends on unit price\textsuperscript{11}. Increased unit price generally results in decreased consumption\textsuperscript{11}. Since consumption is dependent on unit price as a whole, a proportional change in response requirement (price) or reinforcer magnitude (drug dose) should theoretically produce the same behavior in the consumer\textsuperscript{12}. This has strong implications for drug policy, as theoretically reducing nicotine concentrations of cigarettes or increasing the price of current cigarettes could both result in the same magnitude of decreased cigarette use.

Some studies have shown that consumption of nicotine is consistent at a single unit price, regardless of the dose of nicotine administered\textsuperscript{11,12}. Yet another study shows that increases in nicotine cost and decreases in nicotine dose that result in the same unit price do not produce the same response results\textsuperscript{13}. Due to these inconclusive findings, more research is needed to help reach conclusions about how changes in cigarette price will affect nicotine consumption.

The present study addresses this knowledge gap with rodent intravenous self-administration of two different concentrations of nicotine solution. The goal is to analyze demand elasticity of nicotine and determine how nicotine consumption is affected by both cost and nicotine concentration. The use of both male and female rodents also contributes to the understanding of sex differences in nicotine demand elasticity\textsuperscript{14}. 
Materials and methods

Animals

Male (200-225g) and female (175-200g) Wistar rats (7-8 weeks old) were used for this study. They were housed in a climate-controlled vivarium on reversed 12-hour light-dark cycle. During the study, food intake was slightly restricted to 75-80% of calories consumed during ad libitum feeding.

Experiment

Pre-surgery training

Rats were trained for 10 minutes on the Large Open Field. The following day, they were trained for 5 mins on the Elevated Plus Maze. Then, food training was conducted to teach rats how to press the levers in the operant chambers. Food training was done in the same chambers where drug self-administration was later done, after surgery. Training began on the fixed ratio schedule FR1 TO1-s, then was increased to FR1 TO10-s. This lasted for 9 days. On the 10th day, intravenous catheter implantation surgery was performed.

Surgery

Jugular vein cannulation with vascular access button closure was performed, as described more extensively elsewhere15. The catheter used in this study was made of polyurethane (length 15 cm, inner diameter 0.64 mm, outer diameter 1.0 mm, model 3Fr, Instech Laboratories, Plymouth Meeting, PA). Isoflurane was used to anesthetize the rats. A 2.5 cm catheter was inserted into the jugular vein on the ventral side of the animal. A 2-2.5 cm incision was made on the dorsal side of the animal, between the shoulder blades, where the vascular access button
(VAB) was placed. A trocar was used to thread the catheter from the ventral side of the animal to exit the dorsal incision. The catheter was connected to the VAB, then flushed with heparinized saline and locked with a solution of Heparin/Glycerol.

**Post-surgery intravenous self-administration (IVSA)**

The post-surgery recovery period lasts 5-7 days. Rats received subcutaneous Flunixin injections (2.5mg/kg) twice daily for 48 hours after surgery. Rats received intravenous injections of Gentamycin (2mg/kg) once daily for 7 days after surgery.

Rats began IVSA of nicotine 120-min sessions of FR1 with 10-second time-out, for 10 days. Rats received infusions only when they pressed the right lever. An infusion was accompanied by a 10-second cue light during the time-out period. The left lever was inactive. On the first day, rats were only allowed a maximum of 20 nicotine infusions. After 10 days on FR 1, rats spent 2 days each on 120-minute sessions of FR 2, 3, 6, 9, 15, 30, 60, 120, 240, 480, and 960, with 10 second time outs.

There were two experimental groups. The first group received 0.03 mg/kg/infusion of nicotine, while the second group received 0.01 mg/kg/infusion of nicotine. Each infusion consisted of 0.1 mL of nicotine solution. The experiment was finished when none of the rats received a nicotine infusion.

*Demand curve analysis*

Demand curve analysis was performed using the exponential-demand equation developed by Hursch and Silberberg\textsuperscript{16}. This equation was specifically designed to analyze demand at an individual consumer level, as opposed to market-demand and has been used in other IVSA studies\textsuperscript{17}. It is as follows:
\[ \log Q = \log Q_0 + k(e^{-\alpha Q_0 C} - 1) \]

Where \( Q \) is the quantity consumed, \( Q_0 \) is consumption at zero price, \( k \) is a scaling constant, \( \alpha \) is the rate constant, and \( C \) is the cost per reinforcer (FR value). Nonlinear regression was used to fit the data to the equation. Percentage of variance is indicated by \( R^2 \).

The rate constant \( \alpha \) indicates the elasticity of a commodity by determining the rate of decline in relative consumption (log nicotine consumption) relative to price increase. A higher \( \alpha \) value means greater elasticity and lower essential value of the item. The demand curves were created using a GraphPad Prism template from the Institutes for Behavior Resources in Baltimore, MD, found at http://www.ibrinc.org/index.php?id=181.

\( O_{\text{max}} \) and \( P_{\text{max}} \) for each group were calculated using an Excel template\(^\text{18}\) found at http://hdl.handle.net/1808/14934. \( P_{\text{max}} \) is the price at which responding is at its maximum. When price surpasses \( P_{\text{max}} \), response rate declines. This is the price at which demand for the product shifts from inelastic to elastic. The higher the \( P_{\text{max}} \) value, the longer an item retains its demand, and therefore the lower the elasticity\(^9\). \( O_{\text{max}} \) is the peak response output at a price of \( P_{\text{max}} \)\(^{19}\).

**Statistics**

The first day of nicotine self-administration was limited to 20 infusions, so this data was not included in the analysis. After FR1, rats spent 2 days at each of the subsequent FR schedules. Separate demand analyses were conducted for Day 1 of each FR schedule and Day 2 of each FR schedule. SPSS Statistics 25 and GraphPad Prism 7 for Windows were used to analyze data.

Since sex differences were being studied, the extra sum-of-squares (ESS) F-test and a secondary one-way ANOVA analysis were used to determine if there was a difference in \( \alpha \).
values between male and female rats. Operant chamber lever presses were analyzed with a two-way ANOVA. The within-subject factor was the FR schedule, while the between-subject factor was sex of the rat.
Results

0.03 mg/kg/infusion of nicotine

All rats (14 males, 13 females) received 20 infusions on day 1 of FR-1. On days 2-9 (FR-1 schedule, 2-hour session), there was no effect of sex on nicotine intake (Figure 1A) or lever presses (Figure 1B).

Following FR-1, the rats were kept at each FR schedule for two days. Day 1 and day 2 of each FR schedule are graphed separately. Demand for nicotine at 0.03 mg/kg/infusion was more elastic for females than males on both day 1 and 2, as evidenced by the demand curve and $\alpha$ values. $\alpha$ is a measure of the change in consumption relative to an increase in price, so a higher alpha value indicates greater elasticity. An ESS F-test showed that two curves (day 1 and 2) were better at describing the change in nicotine intake after an increase in price, as opposed to one curve (Day 1, F1,15=48, p<0.0001; Day 2, F1,15 = 101, p<0.0001; Figure 2A and 2B). A secondary ANOVA analysis using values from individual-subject demand curves confirmed that $\alpha$ was higher for females than males on both day 1 (Day 1, Sex: F1,25 = 5.068, p<0.05; Table 1) and day 2 (Day 2, Sex: F1,25 = 4.552, p<0.05; Table 1).

Analysis of day 1 showed that $P_{\text{max}}$ (F1,25=4.421, p<0.05) and $O_{\text{max}}$ (F1,25 = 4.421, p < 0.05) were lower in females than males on both day 1 and day 2 (Table 1). This was also the case for $P_{\text{max}}$ (F1,25 = 5.219, p < 0.05) and $O_{\text{max}}$ (F1,25 = 4.446, p < 0.05) on day 2. There was no difference in $Q_0$ between the sexes on either day (Table 1). The mean $R^2$ value based on group values ranged from 0.96 to 1, demonstrating that the model fitted the data well.
Figure 1: Nicotine self-administration (0.03mg/kg/inf) in male and female rats on FR-1 (days 2-9).

Figure 2: Nicotine self-administration (0.03mg/kg/inf) in male and female rats from FR-2 to FR-960.

<table>
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<tr>
<th>Day</th>
<th>K</th>
<th>α</th>
<th>Q₀</th>
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<td>Females</td>
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<td>0.001118 ± 0.000331*</td>
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<td>0.000046</td>
<td>143 ± 37</td>
<td>42 ± 16*</td>
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<td>852 ± 217</td>
<td>312 ± 123*</td>
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<tr>
<td>Day 2</td>
<td>1.8</td>
<td>0.000332 ± 0.000076</td>
<td>0.001033 ± 0.000332*</td>
<td>24 ± 1</td>
<td>31 ± 4</td>
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<tr>
<td></td>
<td>1.8</td>
<td>0.000041</td>
<td>0.000041</td>
<td>118 ± 30</td>
<td>38 ± 16*</td>
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<td>765 ± 179</td>
<td>301 ± 122*</td>
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Table 1: Behavioral economics of nicotine self-administration in male and female rats with 0.03 mg/kg/infusion. Asterisks indicate significant difference between males and females.
0.01 mg/kg/infusion of nicotine (14 males, 14 females)

All male rats (14) and 12 of 14 female rats received 20 infusions on day 1 of FR-1. On days 2-9 (FR-1 schedule, 2-hour session), there was no effect of sex on nicotine intake (Figure 3C) or right lever (RL) presses (Figure 3D). Female rats made more left lever (LL) presses than male rats (F1,26 = 7.73, p <0.05).

Demand for nicotine at 0.01mg/kg/infusion was more elastic for males than females on both day 1 and 2, as evidenced by the demand curve and \( \alpha \) values. An ESS F-test showed that two curves (day 1 and 2) were better at describing the change in nicotine intake after an increase in price, as opposed to one curve (Day 1, F1,18=23, p<0.0001; Day 2, F1,16 = 10, p<0.0001; Figure 2C and 2D). A secondary ANOVA analysis using values from individual-subject demand curves did not support that \( \alpha \) was higher for males than females.

Analysis showed no difference in \( P_{max} \), \( O_{max} \), or \( Q_0 \) between the sexes on either day (Table 2). The mean \( R^2 \) value based on group values ranged from 0.92 to 1, demonstrating that the model fitted the data well.
Figure 3: Nicotine self-administration (0.01mg/kg/inf) in male and female rats on FR-1 (days 2-9).

Figure 4: Nicotine self-administration (0.01mg/kg/inf) in male and female rats from FR-2 to FR-960.

<table>
<thead>
<tr>
<th>0.01 mg/kg/infusion</th>
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<td>( K )</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Males</td>
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<tr>
<td>Day 1</td>
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<tr>
<td>Day 2</td>
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Table 2: Behavioral economics of nicotine self-administration in male and female rats with 0.01 mg/kg/infusion. Asterisks indicate significant difference between males and females.
Discussion

This study investigated the elasticity demand of two different concentrations of nicotine administered via intravenous self-administration (0.03 mg/kg/infusion and 0.01 mg/kg/infusion). Results of male and female rats were also compared to each other.

The study found that there was no difference in intravenous self-administration between males and females under the FR-1 schedule. A meta-analysis of sex differences in nicotine IVSA suggested that at FR schedules greater than FR-1, females self-administered more nicotine than males\(^\text{20}\). This difference was more apparent in studies that used a light cue when nicotine was self-administered, as was present in our study. Yet another study showed that males and females self-administered similar levels of nicotine, although males acquired self-administration faster\(^\text{14}\). Minimal research on sex differences in initial and long-term self-administration of nicotine necessitates additional rodent studies on this topic\(^\text{14}\).

Our study indicates that females respond more than males to the 0.01 mg/kg/infusion dose, but not the 0.03 mg/kg/infusion dose. This suggests that demand elasticity of nicotine is dose dependent. At 0.03 mg/kg/infusion, the demand for nicotine was more elastic for females than for males. This means that as the price of nicotine increased (FR schedule), the number of lever presses by females, and therefore number of nicotine infusions, declined faster than that of males.

Contrastingly, at 0.01 mg/kg/infusion, the demand for nicotine was more elastic for males than females. This indicates that females have a preference for lower doses of nicotine, while males prefer a standard dose. This has implications for drug policy, as it suggests that changes in nicotine concentration in cigarettes will disproportionally affect the sexes.
Previous studies have also shown conflicting results on the nicotine dose that maintains self-administration in rodents. A study by Corrigall and Coen\textsuperscript{21} showed that there was no significant response difference between 0.01 and 0.03 mg/kg/infusion. A later study showed that while 0.03 and 0.06 mg/kg/infusion maintain responses to IVSA, 0.01 mg/kg/infusion does not\textsuperscript{22}. Our study demonstrates that both 0.01 and 0.03 mg/kg/infusion maintain responses and that response rates depend on the FR schedule and sex of the rat.

**Future research**

Minimal research has been done regarding sex differences in nicotine self-administration in rodents and studies that have been conducted have yielded conflicting results; replications of this study would be useful to help corroborate its findings. The study found that demand for the lower dose of nicotine (0.01 mg/kg/infusion) was less elastic in female rats, implying that a reduction in the nicotine content of cigarettes would likely have greater success in decreasing smoking rates in male humans than females. To confirm this idea, research assessing demand elasticity of low-nicotine concentration cigarettes in male and female humans is warranted.
References


