INCREASING NICOTINE COST AND DECREASING NICOTINE DOSE ARE NOT EQUIVALENT MANIPULATIONS

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INCREASING NICOTINE COST AND DECREASING NICOTINE DOSE ARE NOT EQUIVALENT MANIPULATIONS

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Introduction: Two factors that have been shown to affect smoking behavior are the cost of cigarettes and the dose of nicotine. Behavioral economics posits that the two factors may be related through the concept of unit price (unit price = cost/reinforcer magnitude). According to this framework, increases in the cost of a reinforcer and decreases in the magnitude of a reinforcer are equivalent manipulations. However, this assumption has not been thoroughly tested. *Method:* Across three studies, a rodent self-administration model was used to assess the relationship between increases in nicotine cost and decreases in nicotine dose. In Aim 1, each rat experienced six unit prices twice: cost was manipulated and dose was held constant across one set of combinations, and cost was held constant and nicotine dose was manipulated across the other set of combinations. In Aim 2, the same procedure was used as in Aim 1 except that very low nicotine doses, hypothesized to below the threshold for maintaining self-administration, were used for the dose manipulation. In Aim 3, the hypothesis that consumption should be the same at a single unit price regardless of the cost/dose combination used to create it was tested. *Results:* Results show that across the range of unit prices that maintain consumption, behavior is more sensitive to nicotine dose than to nicotine cost. However, when above-threshold doses are used, increases in nicotine cost maintain consumption across a smaller range of unit prices than decreases in dose. When very low nicotine doses are used for dose reduction, animals consumed less nicotine than they did for the ratio-escalation manipulation. Finally, nicotine consumption was not equivalent across a variety of unit price combinations forming a single unit price.

Discussion: Results of the present study are the first to show that increases in the cost of nicotine and decreases in the dose of nicotine are not equivalent manipulations, and they raise questions about a fundamental assumption within the behavioral economics framework.

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PREFACE

I would first like to thank my advisor, Dr. Eric Donny, and my co-advisor, Dr. Alan Sved, for their invaluable guidance and support over the last four years. I am incredibly proud of my growth as a scientist since I started at the University of Pittsburgh, and their mentorship has shaped that growth throughout the entire process. I would also like to thank the rest of my dissertation committee, Dr. Saul Shiffman, Dr. Kenneth Perkins, and Dr. Mary Torregrossa, for their feedback on my study design and data analyses. Thank you to my fellow graduate student, Laura Rupprecht, for her insight on my projects and support over the last two years. Finally, thank you to the members of the lab for their extensive help in conducting experimental sessions, including Emily Pitzer, Josh Alberts, Elizabeth Shupe, Samuel Gutherz, Samantha Cwalina, Jessica Pelland, Corina Andriescu, Dora Danko, Hangil Seo, Nicole Silva, Marisa Wallas, Mackenzie Meixner, and Kayla Convry.

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1.0 INTRODUCTION

Urgent action is needed to reduce the prevalence of smoking. While the prevalence of smoking in the United States (US) has declined over the last five decades, 43.8 million people are still smokers (Ingersoll & Cohen, 2005). Cigarette smoking is extremely harmful, causing 443,000 deaths in the United States each year, and accounting for one in every five deaths (USDHHS, 2010), making cigarette smoking the leading cause of preventable death in the US. Reducing the prevalence of smoking is among the most important priorities for public health.

One factor that is known to influence smoking behavior is the cost of cigarettes. Taxation is one of the most historically effective tobacco control interventions. Evidence for the effectiveness of cigarette taxation comes from both studies examining the impact of tobacco taxation across time in the US, and from the variation in the price of cigarettes across different regions (Chaloupka, Yurekli, & Fong, 2012). Increasing the price of cigarettes has been shown to drive down cigarette consumption, with every 10% increase in the cost of cigarettes causing an estimated 4% decline in cigarette consumption (Chaloupka & Warner, 1999; Jha & Chaloupka, 1999). When the price of cigarettes is increased, some of the decrease in cigarette consumption is because the prevalence of smoking decreased (Chaloupka et al., 2012), meaning that a proportion of smokers quit smoking when price is increased. The impact of cigarette taxation has been shown to be increased in low socioeconomic status (SES) groups (Townsend, Roderick, & Cooper, 1994), and in young people . It is hypothesized that cigarette taxation decreases the

proportion of adolescents that transition from experimentation to regular smoking (Chaloupka et al., 2012). Some of the impact of taxation on cigarette consumption is offset by substitution. That is, smokers will switch to another tobacco product if prices are not raised uniformly across products (Laxminarayan & Deolalikar, 2004). However, noncombustible products often carry reduced health risks compared to combustible cigarettes, so the shift to noncombustible products may be desirable in comparison to use of traditional cigarettes.

Another factor that has recently been shown to impact smoking behavior is nicotine content. A reduction in the nicotine content within cigarettes has received attention as a potential strategy for reducing the harm caused by tobacco. In 2009, the FDA was given the authority to regulate cigarette constituents, including nicotine to any non-zero level. Existing nicotine research is promising, with data suggesting there is no increase in toxicant exposure, there is no compensatory smoking at very low nicotine contents past the first few cigarettes, and in some cases there is a decrease in cigarettes per day after a few weeks of use (Benowitz et al., 2012; Benowitz et al., 2007; Donny, Houtsmuller, & Stitzer, 2007; Hatsukami, Kotlyar, et al., 2010; Hatsukami, Perkins, et al., 2010).

One framework, behavioral economics, suggests that the two factors, nicotine cost and nicotine content, may function to change behavior in the same way (Smith, Sved, Hatsukami, & Donny, 2014). Behavioral economics is a subfield of psychology that uses concepts from microeconomics to characterize changes in behavior. According to this framework, changes in the consumption of a reinforcer occur as a function of the unit price of that reinforcer, and unit price combines both the cost and the magnitude (i.e., drug dose) of the reinforcer (unit price = reinforcer cost / reinforcer magnitude). From this perspective, increasing the cost of nicotine and decreases in the magnitude of each nicotine reinforcer should result in an equivalent change in

unit price, and should result in the same change in consumption. For example, if the price per pack of cigarettes were doubled, that increase in unit price should result in the same change in cigarette consumption as if the number of cigarettes in a pack were reduced by half with the cost remaining constant. Thus, if nicotine is the primary reinforcing component of a cigarette, decreasing the content of nicotine may be functionally equivalent to raising the cost of nicotine (or raising the cost of cigarettes) (Smith et al., 2014).

A fundamental concept in the field of behavioral economics is the demand curve. An example is plotted in Figure 1 using data from a single subject in a recently completed study (Smith et al., In Press). A demand curve plots the consumption of a reinforcer as a function of the unit price of that reinforcer (Hursh & Roma, 2013; Hursh & Silberberg, 2008).

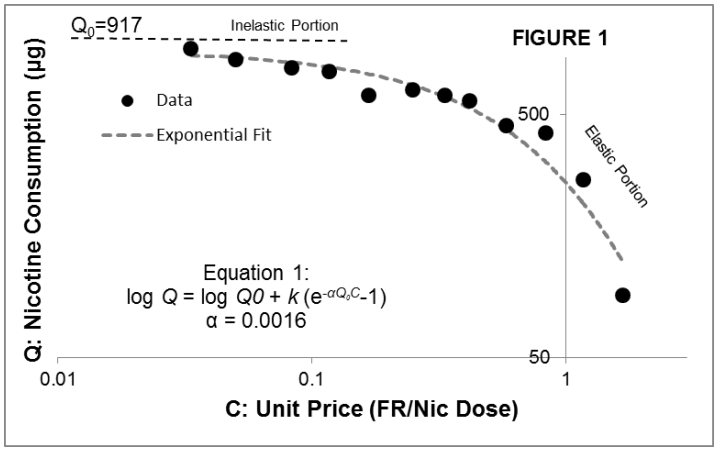

Figure 1. Example demand curve for an individual subject.

From this perspective, the individual cost and reinforcer magnitude components making up a given unit price are irrelevant, with the critical variable being the ratio between the two

components. Demand curves can be used to characterize how behavior changes as a function of unit price. Research shows that demand curves for reinforcers generally follow a uniform pattern (Hursh & Silberberg, 2008): demand curves have a negative slope such that consumption of a reinforcer decreases as unit price of that reinforcer increases. Across a range of low unit prices, demand is inelastic, meaning that the decrease in consumption is proportionally less than the increase in unit price (i.e., the slope of the demand curve is shallower than -1). Over this range of doses, responding for the reinforcer increases with increases in unit price. At some unit price (termed P_{max}), demand becomes elastic, meaning that the decrease in consumption is proportionally more than the increase in unit price (i.e., the slope of the demand curve is steeper than -1). Over this range of prices, responding for the reinforcer decreases with increases in unit price (Hursh & Roma, 2013).

Demand curves have been shown to conform well to an exponential equation (Hursh & Silberberg, 2008) (shown in Figure 1; Equation 1) in which Q is consumption at a given unit price, C, and k is a scaling parameter. Q_0 and α are the two free parameters, with Q_0 being the predicted consumption if the reinforcer were free (graphically the y-intercept), and α is the rate at which consumption changes as a function of unit price (graphically the rate of change in the slope). α has been described as a measure of the essential value of a reinforcer. After the exponential equation has been fit to obtained consumption data, the equation can be used to predict consumption at any unit price.

Behavioral economics has been used for a variety of purposes. One of its primary uses is to characterize changes in self-administration behavior of drugs of abuse (Hursh, 1991). The sensitivity of an organism to changes in unit price might be thought of as a measure of the essential value of the reinforcer, and behavioral economics allows for the estimation of this value

in a single parameter (α) that describes the change in the demand function as unit price is increased (Hursh & Silberberg, 2008). Sensitivity to unit price might be thought of as a measure of drug abuse liability (Hursh, 1991). Demand curves can also be used to characterize changes in consumption of other reinforcers as the unit price of the first reinforcer is manipulated (Hursh & Roma, 2013). For example, behavioral economics could characterize changes in alcohol consumption as the cost of cigarettes is escalated. Researchers have recently suggested that demand curves could be used to describe how agonist and antagonist drug therapies change drug-taking behavior (Hursh & Roma, 2013). Other recent uses for behavioral economics include how changes in income might change cigarette smoking behavior (Koffarnus, Wilson, & Bickel, 2014), predicting compulsive drug-taking behavior in a rodent self-administration model (Bentzley, Jhou, & Aston-Jones, 2014), describing the role of the serotonin transporter gene in alcohol-taking behavior (Lamb & Daws, 2013), and characterizing how subpopulations of individuals differ in drug-taking behavior (i.e., individuals with schizophrenia) (MacKillop & Tidey, 2011).

Behavioral economics has been occasionally used to characterize changes in tobacco or nicotine use. For example, it has been used to characterize changes in cigarette smoking as a function of increases in the cost of cigarettes by manipulating the monetary cost of cigarettes (Acker & Mackillop, 2013; Grace, Kivell, & Laugesen, 2014; MacKillop et al., 2012; MacKillop et al., 2008), and the effort required to obtain cigarette puffs (Shahan, Bickel, Badger, & Giordano, 2001; Shahan, Bickel, Madden, & Badger, 1999). There is one instance in which behavioral economics was used characterize changes in nicotine consumption as a function of changes in nicotine yield. DeGrandpre, Bickel, Hughes, and Higgins (1992) reanalyzed data from 17 studies in which nicotine yield was manipulated either through brand switching (14

studies), shortening cigarettes (two studies) or ventilated filters (one study). Estimated nicotine consumption was plotted as a function of unit price, and in each case appeared to fit an exponential function. This analysis suggests that behavioral economics can be useful for characterizing changes in behavior as a function of changes in nicotine yield. However, in all of the studies reanalyzed by DeGrandpre et al. (1992), manipulations of nicotine yield are likely accompanied by manipulations of other non-nicotine constituents which may influence the value of cigarette reinforcers (Bardo, Green, Crooks, & Dwoskin, 1999; Clemens, Caille, Stinus, & Cador, 2009; Smith et al., In Press). To date, no research has used a behavioral economics analysis to examine changes in nicotine consumption when the nicotine content within cigarettes is directly manipulated.

Behavioral economics has also been mostly neglected as a tool in rodent nicotine self-administration studies, with a couple of recent exceptions. In one paper, a behavioral economics analysis was applied post-hoc to a data set in which the number of responses required to earn a nicotine infusion was increased across sessions (Diergaarde, van Mourik, Pattij, Schoffelmeer, & De Vries, 2012), showing that a measure of impulsivity was related to elasticity of demand for nicotine. Another recently completely study from our lab (Smith et al., In Press) used behavioral economics to characterize how other cigarette constituents might shift sensitivity to cost of nicotine. Only two studies have used behavioral economics to examine changes in rodent self-administration behavior by manipulating the concentration of nicotine (Grebenstein, Burroughs, Roiko, Pentel, & LeSage, 2015; Grebenstein, Burroughs, Zhang, & LeSage, 2013), and nicotine consumption conformed well to Equation 1 (Hursh & Silberberg, 2008), suggesting behavioral economics may be a useful future measure in other nicotine self-administration studies where the concentration of nicotine is manipulated.

An important assumption embedded in the behavioral economics approach is that a reduction in the dose of nicotine is functionally equivalent to an increase in cost. If this assumption holds true, then increases in the price of cigarettes would be functionally equivalent to decreases in the nicotine content in cigarettes, because they are both manipulations of unit price (Smith et al., 2014). In this case, existing literature examining how escalating cost changes cigarette consumption could be leveraged to better understand the likely outcomes of nicotine reduction. For example, changes in cigarette consumption as a function of taxation are inelastic, meaning that the decrease in cigarette consumption is proportionally less than the increase in price (Chaloupka et al., 2012). These data suggest that at the current price of cigarettes, changes in nicotine consumption as a function of small changes in nicotine content are also likely to be inelastic, meaning that decreases in nicotine content will result in increases in smoking behavior. Thus, a large decrease in nicotine content may be required to decrease smoking behavior. We also know that about half of the impact of cigarette price on cigarette consumption is a result of changes in the prevalence of smoking (Chaloupka et al., 2012), which suggests that if nicotine content is decreased a proportion of adults may quit smoking. Finally, if the two manipulations are equivalent, then the impact of a nicotine reduction policy is likely dependent on the cost of cigarettes. Cigarette cost varies widely across the US, and across countries, so it may be appropriate to think of a target unit price for nicotine reduction instead of a target nicotine content (Smith et al., 2014). The price of a pack of Marlboro cigarettes in Norway is $15.11 (USD), but is as low as $0.74 in the Philippines. If cost is an equal determinant of smoking behavior following implementation of a nicotine reduction policy, then the threshold nicotine dose required for maintaining smoking behavior would be approximately 20 times less in the Philippines than in Norway (World Lung Foundation & American Cancer Society, 2012).

If increases in cigarette cost and decreases in nicotine content are equivalent manipulations, we can use research on cigarette taxation to make predictions about individual differences in response to nicotine reduction. For example, cigarette taxation has been shown to be more effective in young people and individuals of low SES (Chaloupka et al., 2012; Townsend et al., 1994), so these individuals may be especially sensitive to decreases in nicotine content. Furthermore, demand parameters derived from hypothetical increases in the cost of cigarettes (i.e., Cigarette Purchase Task) have been shown to be related to clinical outcomes such as nicotine dependence and treatment motivation (Chase, Mackillop, & Hogarth, 2013; MacKillop et al., 2008; MacKillop & Tidey, 2011; Murphy, MacKillop, Tidey, Brazil, & Colby, 2011)). These data suggest that lower dependence and increased motivation to quit smoking may be predictive of better nicotine reduction outcomes. Furthermore, an individual's sensitivity to increases in monetary cost of cigarettes may predict an individual's sensitivity to decreases in nicotine content. Sensitivity to increases in cost may be characterized efficiently using hypothetical tasks like the Cigarette Purchase Task, in which individuals predict how many cigarettes they would smoke if cigarettes were a variety of prices. This task might also be useful for characterizing the sensitivity of vulnerable subpopulations to cost. This information could then be used to predict how those subpopulations would respond to decreases in nicotine dose.

The equivalence of reinforcer cost and reinforcer magnitude relies on the assumption that the critical factor in determining the level of reinforcer consumption is the cost per unit of a reinforcer, but the two manipulations will produce markedly different patterns of reinforcer delivery that may impact the level of reinforcer consumption. When cost is increased, the same bolus of drug is delivered with each reinforcer delivery, but when dose is decreased, smaller infusions of drug are delivered and many reinforcer deliveries are required to earn equivalent

consumption. Thus, even if the same level of reinforcer consumption is obtained following the two manipulations, the reinforcer will be delivered in infrequent boluses for the cost manipulation and in smaller, more frequent boluses for the dose manipulation. Previous research suggests that the duration over which a drug is delivered is a critical variable in determining whether or not that drug is reinforcing, and in determining the level of drug self-administration (Balster & Schuster, 1973; Comer et al., 2009; Panlilio et al., 1998; Schindler, Panlilio, & Thorndike, 2009; Sorge & Clarke, 2009; Wakabayashi, Weiss, Pickup, & Robinson, 2010; Wakasa, Takada, & Yanagita, 1995; Wing & Shoaib, 2013). Thus, increases in cost and decreases in dose may not function to change behavior in the same way because they differentially change the pattern of reinforcer delivery. In addition to the difference in the delivery pattern of the reinforcer, there will be differences in the pattern of cue delivery. Reinforcers are delivered in a complex environment of other stimuli, and some of these stimuli may act as conditioned reinforcers through their pairing with other reinforcers (Pavlov, 1927). When reinforcer cost is increased, the unit price of those conditioned reinforcers is also increased, but when reinforcer magnitude is decreased, the unit price of conditioned reinforcers remains the same because the magnitude of the conditioned reinforcers has not been explicitly manipulated. If the value of a conditioned reinforcer is proportional to the value of the primary reinforcer is has been paired with, over time the value of these conditioned reinforcers should decrease because the magnitude of the primary reinforcer has decreased, but the timeline for this change is unknown.

There is quite a bit of existing research investigating the relationship between cost and reinforcer magnitude using a behavioral economics analysis. A variety of reinforcers have been used to investigate equivalence including food (Collier, Johnson, Hill, & Kaufman, 1986;

DeGrandpre, Bickel, Hughes, Layng, & Badger, 1993; Foster & Hackenberg, 2004; Hursh, Raslear, Shurtleff, Bauman, & Simmons, 1988; Sumpter, Temple, & Foster, 2004), heroin (English, Rowlett, & Woolverton, 1995), morphine (DeGrandpre et al., 1993), dextropropoxyphene (English et al., 1995), codeine (DeGrandpre et al., 1993), and pentazocine (English et al., 1995), pentobarbital (Bickel, DeGrandpre, Higgins, & Hughes, 1990; DeGrandpre et al., 1993), procaine (DeGrandpre et al., 1993), d-Amphetamine (Bickel et al., 1990; DeGrandpre et al., 1993), ethanol (Bickel et al., 1990), phencyclidine (Bickel et al., 1990; Carroll, Carmona, & May, 1991), ketamine (Bickel et al., 1990), methohexital (Bickel et al., 1990; DeGrandpre et al., 1993), and cocaine (Bickel et al., 1990; DeGrandpre et al., 1993; Nader, Hedeker, & Woolverton, 1993; Woolverton & English, 1997). These studies have employed one of two techniques for assessing equivalence: 1) plotting consumption at a single unit price regardless of the cost / reinforcer magnitude combination used to create that unit price and using visual inspection to assess equivalence, or 2) plotting consumption at a variety of unit prices on a single demand curve regardless of the cost / reinforcer magnitude combination used and using visual inspection to confirm that all data points fall on a single demand curve. These techniques are not the most rigorous test of equivalence because they do not directly compare manipulations of cost and dose, and they do not require consumption data to meet any standard of equivalence. The single most thorough investigation thus far reanalyzed data from 10 drug self-administration studies in which at least two FR requirements and two drug doses were tested. Data were re-plotted as drug consumption as a function of unit price, and visual inspection was used to confirm that all combinations fell on a single demand curve (Bickel et al., 1990). The data appeared to roughly fit an exponential function. However, this analysis did not

employ any statistical techniques to assess equivalence, only one subject was used from each previous study, and cost and dose were not directly compared.

One study has evaluated the relationship between cost and dose using cigarettes (Bickel, DeGrandpre, Hughes, & Higgins, 1991). In this study, five participants were given the opportunity to perform an effortful response (plunger pulls) to earn puffs of a cigarette. Across different sessions, the number responses required to earn cigarette puffs (fixed-ratio, FR) and the number of cigarette puffs that could be earned was manipulated. Two FR/puff combinations were created for each of six unit prices. Visual inspection of individual data suggested that consumption was generally equivalent between the two combinations of any unit price. However, there are several limitations associated with this study. Only five participants were tested, visual inspection of consumption data was the primary method of analysis, the manipulation of nicotine yield likely also manipulated non-nicotine cigarette constituents and cue magnitude, and for one of the five participants nicotine consumption was markedly different between the two FR/puff combinations for two of the unit prices.

Thus far, all research investigating the relationship between increasing cost and decreasing drug dose has used drug doses in the range expected to maintain behavior, although researchers have hypothesized that this relationship between drug dose and drug cost may change when doses outside of the range for maintaining behavior are used (DeGrandpre et al., 1993; Hursh & Winger, 1995). If increases in drug cost and decreases in drug dose are not equivalent manipulations when doses in the range that maintain behavior are used, it would be unlikely that they would be equivalent manipulations when very low drug doses are used. However, even if they are equivalent manipulations when drug doses in the range that maintains behavior are used, this relationship may be altered at very low doses because low doses may not

have any reinforcing value above vehicle administration. If very low doses do not have any reinforcing value, consumption may not be maintained even though a unit price approach would predict that it would. However, it is possible that at lost costs, multiple deliveries of a very low drug dose could be earned with very little time in between and equivalent consumption may be maintained.

It is particularly important to extend the equivalence of reinforcer cost and reinforcer magnitude to very low doses for nicotine because a nicotine reduction policy is likely to use very low nicotine contents that are hypothesized to be below the threshold for maintaining smoking behavior (Sofuoglu & Lesage, 2012). Previous nicotine research suggests that nicotine doses at or below 3.75 µg/kg/infusion produce nicotine consumption similar to saline substitution in self-administration paradigms (Smith et al., 2013). However, the determination of this threshold nicotine dose may be dependent on methodological factors. In Smith et al. (2013)'s study, five responses were required to earn each infusion, and a behavioral economics framework would suggest a lower cost, or number of required responses, would lower the threshold dose for maintaining behavior. Additionally, in standard self-administration procedures, each infusion is followed by a time out. These time outs prevent the animal from earning multiple infusions in a short time span, and necessarily lengthen the time over which rats can earn a larger amount of drug. Because the duration of drug delivery has been shown to be a critical variable in determining drug consumption (Wing & Shoaib, 2013), time outs may function to decrease drug consumption. Additionally, when dose is reduced, subjects must increase the number of infusions earned to maintain nicotine consumption. An increase in earned infusions will increase time out presentations, and will reduce time available in a session to earn infusions. Thus, typical

experimental procedures make it difficult to assess whether increases in cost are equivalent to decreases in dose even at very low nicotine doses.

1.1 TRANSLATIONAL ISSUES ASSOCIATED WITH RODENT NICOTINE SELF-ADMINISTRATION

A self-administration paradigm, in which rats receive intravenous infusions of nicotine contingent on their own responding, is ideal for assessing the relationship between increasing the cost of nicotine and decreasing the dose of nicotine because dose and cost can be tightly controlled, and both consumption and responding for nicotine can be precisely measured. In the self-administration paradigm, cost is manipulated using the number of responses that are required to earn an infusion of nicotine (i.e., fixed-ratios or FRs). Self-administration also removes variation in other variables that might contribute to differences in sensitivity to cost for humans (e.g., income, exposure to marketing, social pressure and constraints). However, there are some difficulties associated with translating this research directly to human smokers. First, a behavioral economics approach assumes that each additional response is associated with the same marginal increase in effort and time for the organism. However, it may be that the relationship between response requirement and cost is not linear, and this may vary depending on the typography of the response. For rats in a self-administration paradigm, each additional response may be associated with the same marginal increase in cost. However, for human smokers, each additional cigarette smoked may only be linearly related to cost within a range, and at some threshold level, there may be greater costs associated with additional smoking (i.e., work places offering a set number of smoking breaks, respiratory limitations). The rodent self-

administration model standardizes cost, but results will need to be interpreted with the translational limitations in mind.

Second, in a self-administration paradigm, rats can only change their behavior by altering the number of responses they make or the rate at which they make them. However, human smokers have more flexibility in how their behavior changes. When unit price is increased, smokers may change the number of cigarettes per day that they smoke or they may change the way in which they smoke cigarettes (e.g., changes in puff volume, puff duration, inter-puff interval, breathe hold time). Thus, human smokers have more flexibility in the pattern of nicotine delivery than rats in the self-administration procedure. For example, for rats in the self-administration procedure, an increase in cost likely means that the time between reinforcer deliveries will increase because more time is required to make the increased number of responses. However, for human smokers, the pattern of nicotine delivery does not necessarily change when the price of cigarettes is increased.

Third, human smokers take their nicotine in the context of many other variables including initially neutral cues that are paired with nicotine over time and cigarette constituents that may reinforce behavior on their own or interact with the reinforcing potential of nicotine (Caggiula et al., 2009). The rodent self-administration paradigm models environmental variables using an initially neutral stimulus that is paired with nicotine. However, non-nicotine cigarette constituents may interact with the reinforcing value of nicotine differently in different nicotine dose ranges. For example, constituents that inhibit MAO may selectively increase the value of low nicotine doses. The neutral cue employed in rodent models is unlikely to model this kind of change in value across the nicotine dose-response curve. Because of these differences between human smoking and rodent self-administration procedures, even if the relationship between

increasing cost and decreasing dose is strong in the present experiments, additional barriers may be present when translating this concept to the human smoker.

1.2 THE PRESENT EXPERIMENTS

The present studies aim to evaluate the relationship between increasing the cost of nicotine and decreasing nicotine dose. **Aim 1** evaluated how changes in cost of nicotine and nicotine dose relate to consumption of nicotine using doses within the range expected to maintain self-administration behavior. **Aim 2** evaluated whether the relationship between increasing cost and decreasing dose is maintained at low doses expected to be below the threshold for maintaining behavior. **Aim 3** evaluated whether consumption is the same for a single unit price when combinations are used that employ nicotine doses both above and below the hypothesized threshold for self-administration.

2.0 AIM 1

2.1 PURPOSE

The purpose of Aim 1 was to evaluate whether increases in nicotine cost and decreases in nicotine dose change behavior in the same way when the doses used are above the hypothesized threshold for reinforcing behavior. Previous research from our lab suggests that the threshold for nicotine self-administration is between 7.5 µg/kg/infusion and 3.75 µg/kg/infusion (Smith et al., 2013). Thus, the lowest dose used in the present experiment was 7.5 µg/kg/infusion.

2.2 METHODS

2.2.1 Subjects

Male Sprague-Dawley rats (Harlan-Farms, IN) weighing between 200 and 225 g on the day after arrival were used as subjects (n=44). Data are pooled from two cohorts of rats that completed the experimental procedures at separate times. Rats were housed individually in tub cages on a ventilated rack with an automatic watering system. Temperature in the colony room was kept between 68 and 70 degrees Fahrenheit. Rats were kept on a reverse light-dark 12:12 hour schedule, and testing occurred during the dark phase. Rats received ad libitum chow for the

first seven days while habituating to individual home cages. At least eight days after arrival, rats were implanted with jugular catheters and were changed to a feeding schedule where 20 g/day was delivered after each session. Rats were allowed at least five days of recovery following surgery.

2.2.2 Apparatus

Thirty-eight standard self-administration operant chambers (ENV-008 CT; Med-Associates) were configured as previously described (Smith et al., 2013), and included two nose poke holes below two stimulus lights on one side of the chamber.

2.2.3 Drugs

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) was dissolved in 0.9% saline (doses expressed as free base). All solutions were sterilized by being passed through a 0.22 μm filter. Nicotine was delivered in a volume of 0.1 ml / kg in approximately 1 s.

2.2.4 Procedures

2.2.4.1 Surgery

Procedures for jugular catheterization were as previously described (Smith et al., 2013). For the first five days following surgery, the first cohort of rats had their cannulae flushed once daily with a sterile saline solution containing heparin (3 U), timentin (6.67 mg) and streptokinase (833.3 U) to maintain catheter patency and prevent infection. After this initial post-surgery time

period, the flushing solution contained only the heparin and timentin. The second cohort of rats had the same flushing procedures except that timentin was unavailable after the surgical recovery period, and 10mg cefazolin was substituted. Only data points from rats that passed a patency test consisting of rapid loss of muscle tone to methohexital (5 mg/kg i.v.) are included.

2.2.4.2 General Self-Administration Procedures

Rats were given the opportunity to respond via nosepokes for i.v. infusions. The side of the active nosepoke hole (left vs. right) was counterbalanced across rats. Active pokes resulted in a simultaneous onset of an intravenous infusion, a 3-s cue light presentation and time-out period according to the reinforcement schedule in effect. Active nosepokes during time out and inactive nosepokes were recorded, but had no consequence. Sessions lasted at least two hours and were conducted seven days per week.

Time-out and session length

This set of experiments employed a novel time out procedure. In traditional procedures, the session time is fixed, and each infusion produces a time out from reinforcement (lab standard is 1-min). This creates an inequity between increasing cost and decreasing dose because when unit price is increased, the number of infusions is likely to increase only for the dose reduction, reducing the time available in the session to respond. In the present study, a novel procedure was employed such that the time out period was 3 s, and each time out extended the length of the session by 3 s. This procedure ensures that each rat had two hours of time-in to respond, regardless of the number of infusions earned. A shorter time out than traditionally used was employed to allow rats receiving low nicotine doses at low costs to earn nicotine infusions with a short inter-infusion interval.

2.2.4.3 Acquisition

Rats started self-administration of 60 µg/kg/infusion on an FR2 schedule of self-administration, and reached a training condition of 60 µg/kg/infusion on an FR10 schedule of reinforcement before beginning the unit price manipulation. The two cohorts of rats reached the training condition differently. The first cohort of rats responded for 60 µg/kg/infusion nicotine on an FR2 schedule of reinforcement. After 13 days, self-administration behavior was stable, and the unit price portion of the experiment began. After two unit price combinations, self-administration behavior was very low, likely as a result of large changes in FR requirement, which functioned as extinction when rats never made enough responses to experience the contingency. The "training condition" was then established. Rats were given four sessions on this training procedure before continuing on through the unit price procedure, and experienced a single training condition session in between each unit price combination. At the end of the experiment, the first two unit price combinations were repeated, and only data from those repetitions is included here.

The second cohort of rats experienced an increase in FR across sessions to reach the terminal FR used in the training condition (FR10) before beginning the unit price manipulation. Rats experienced 11 sessions on FR2, 7 sessions on FR5, and 10 sessions on FR10. Rats then began the unit price portion of the experiment described below.

2.2.4.4 Unit Price Procedure

Rats each experienced six unit prices, and each rat experienced each unit price twice, creating 12 total combinations (Table 1). The first six combinations all used the same dose of nicotine, but the number of responses required to earn an infusion (FR, cost) increased across

unit prices. The second six combinations all required the same number of responses to earn an infusion, but the dose of nicotine decreased across unit prices. Nicotine doses in this experiment were chosen to be in a range that is expected to maintain self-administration behavior (Smith et al., 2013). Rats experienced four sessions at each unit price combination, and each rat experienced the combinations in a random order. Rats experienced one session on the training condition (FR10, 60 µg/kg/infusion) in between each unit price combination.

Table 1. FR/Dose combinations used in Aim 1

	UNIT PRICE: FR/NICOTINE DOSE (µG/KG/INFUSION)											
	0.133		0.267		0.4		0.533		0.8		1.33	
	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE
COST ESCALATION	8	60	16	60	24	60	32	60	48	60	80	60
DOSE REDUCTION	10	75	10	37.5	10	25	10	18.75	10	12.5	10	7.5

2.2.4.5 Data Analysis

Analyses focused on comparing: 1) consumption for the two combinations at each unit price, 2) how the two manipulations change consumption across the range of unit prices that maintain consumption, and 3) how the two manipulations change consumption as consumption changes from being maintained to not being maintained. To test for significant differences, paired samples t-tests were used. Because of the theoretical importance of hypothesized equivalence, when no significant differences were found for consumption or for free parameters, two one-sided tests of equivalence were used, with the margin of equivalence (δ) set at 25% of the overall mean. When comparing breakpoints (ordinal data), a Wilcoxon Signed Ranked Test was used. Type 1 error rate (α) was set at 0.05.

For the second objective, evaluating behavior changes across the range of unit prices that maintain consumption, a demand curve analysis was employed. The last two sessions at each unit-price combination were used to calculate consumption. Consumption is the total nicotine

infused (in μg/kg). Two demand curves were created for each subject: one for the dose-reduction and one for the ratio-escalation, and Equation 1 was fit to each demand curve using a GraphPad Prism template provided by the Institute for Behavior Resources at no cost (http://www.ibrinc.org/index.php?id=181). For each rat and each demand curve, data points were excluded if consumption fell below 10% of baseline (average consumption over final two training condition sessions prior to unit price manipulation) or fell to 0.

For the third objective, breakpoint (highest unit price maintaining consumption at or above 10% of baseline) was compared between the two manipulations. Visual inspection of individual demand curves suggested that when breakpoint is reached for ratio-escalation curves, the subsequent change in consumption is often drastic, while the change in consumption for dose-reduction curves is more gradual. However, the change in consumption immediately following breakpoint could not be compared because rats often maintained behavior across the full range of dose-reduction combinations tested. Thus, the maximum instance of elasticity was compared between the two manipulations. For each data point, the proportional change in consumption given the proportional change in unit price was calculated (proportional decrease = 1 – (% decrease in consumption / % increase in unit price), and the lowest value was compared between the two manipulations. Negative values indicate a larger percentage change in consumption than the percentage change in unit price.

2.3 RESULTS

Of the 44 rats that were used in the experiment, 8 failed the first patency test, and no data are included from these rats. Four rats never earned an infusion on the unit price procedure, and

their data are excluded (three in the first cohort were removed from the study after the first four unit prices, the fourth was in the second cohort and completed the study, but data has been excluded). The remaining 32 have usable data for at least some of the unit prices.

2.3.1 Comparing consumption at each unit price

Figures 2, 3, 4 show the average and individual consumption data for Aim 1. Figure 2 shows the average data expressed as a group demand curve (error bars represent standard error).

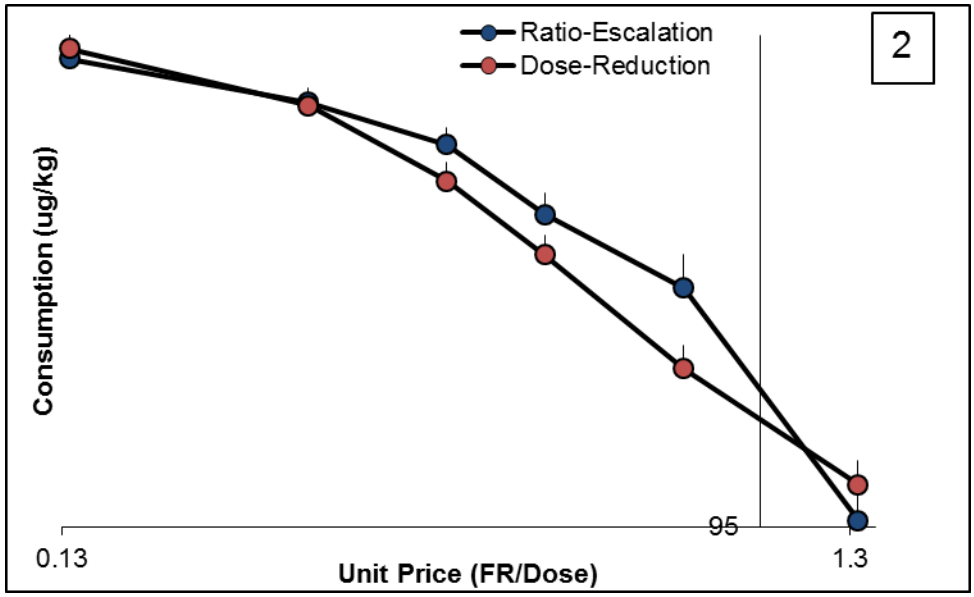

Figure 2. Average consumption for ratio-escalation and dose-reduction unit price combinations.

Figures 3 and 4 show the average and individual data shown for each unit price in a bar graph. In Figure 3 the data are plotted on a common y-axis to emphasize the decrease in consumption across unit prices. In Figure 4, the same data are shown, but each y-axes has been adjusted to reveal any differences in consumption between combinations. Consumption appears qualitatively similar across the lowest and highest unit prices (e.g., 0.133, 0.267, 1.33), but not at intermediate prices. Six paired samples t-tests conducted using consumption at each of the six unit prices were unable to reveal any differences between the two approaches (ps > 0.05). The difference in consumption was significantly less than 25% of the overall mean for the two lowest unit prices: 0.133 and 0.267, but not for any other unit prices (0.133: $\delta = 184.7$, $t\,(26) = 2.208$, $p < 0.05$; 0.267: $\delta = 148.5$, $t\,(25) = 3.345$, $p < 0.05$; 0.4: $\delta = 115.1$; 0.533: δ: 84.5; 0.8: $\delta = 56.4$; 1.33: $\delta = 26.6$). Thus, while a paired-samples t-test was unable to reveal that consumption was different between the two combinations, most also do not meet a reasonable criterion of equivalence.

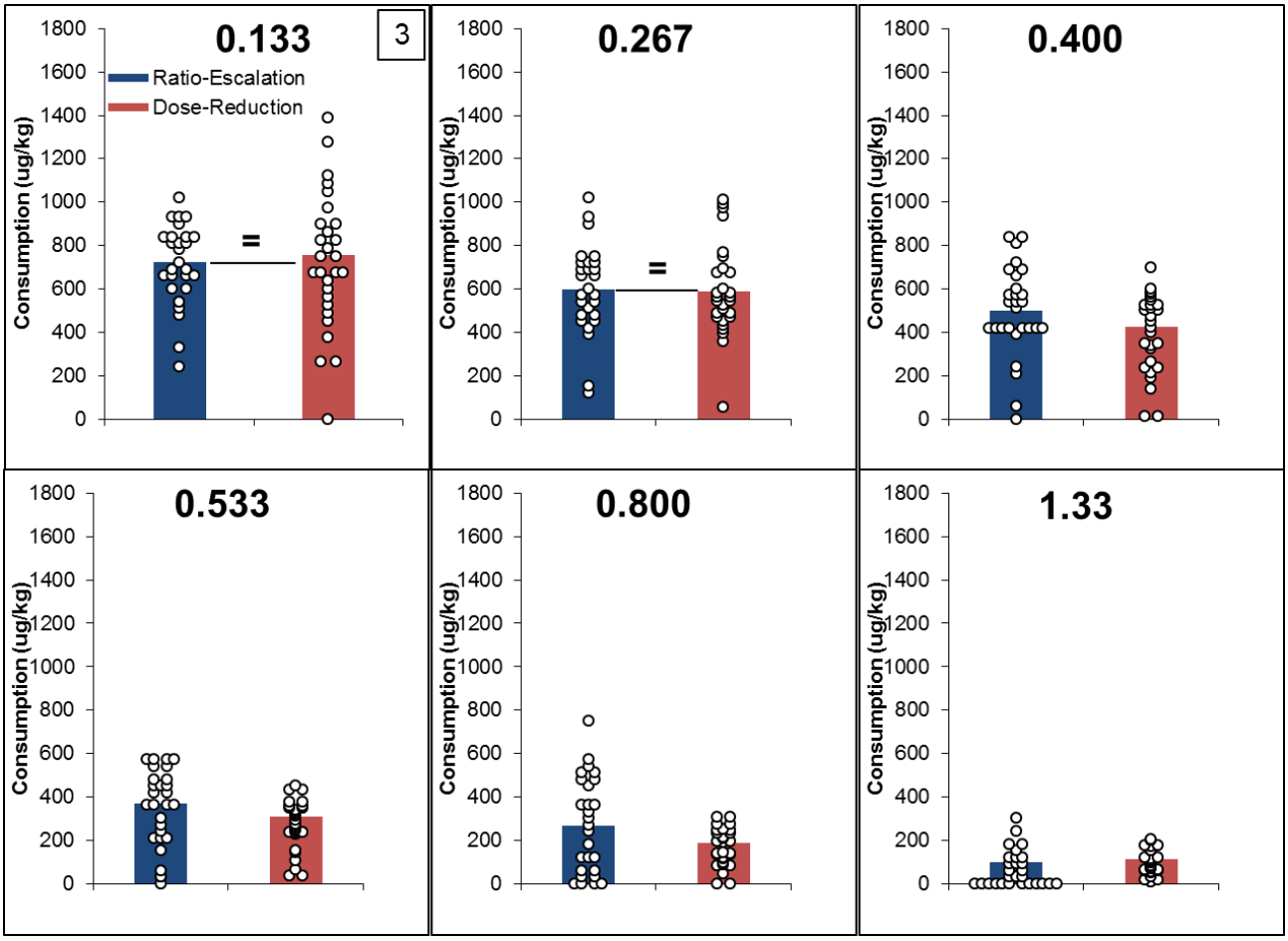

Figure 3. Average and individual consumption data for all 12 unit price combinations with all y-axes on the same scale.

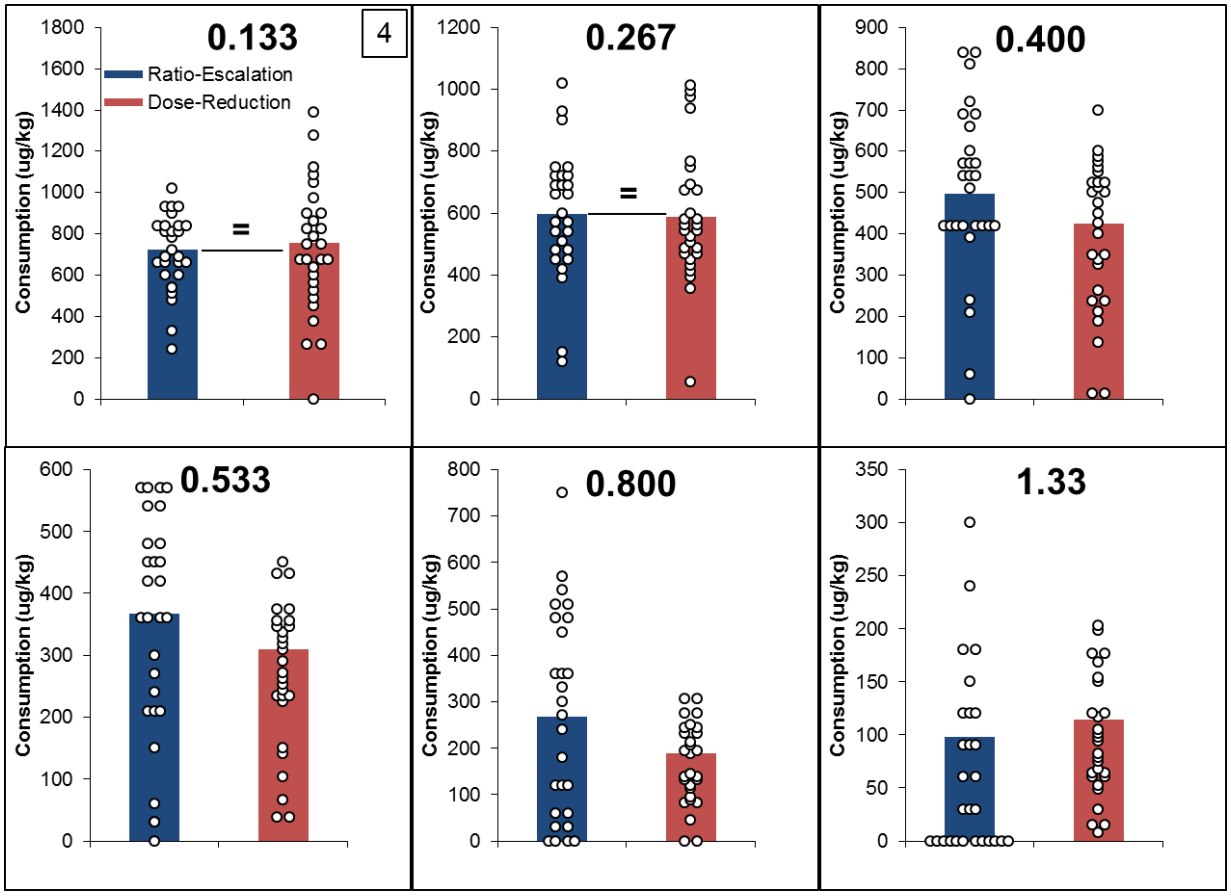

Figure 4. Average and individual consumption data for all 12 unit price combinations with y-axes adjusted for each graph.

2.3.2 Comparing increases in cost and decreases in dose when consumption is maintained

A demand curve analysis was employed to assess whether manipulations of cost and dose change consumption similarly across the range of unit prices that maintain consumption. Only data points where consumption was maintained were included (defined as consumption at or above 10% of baseline) (Figure 5).

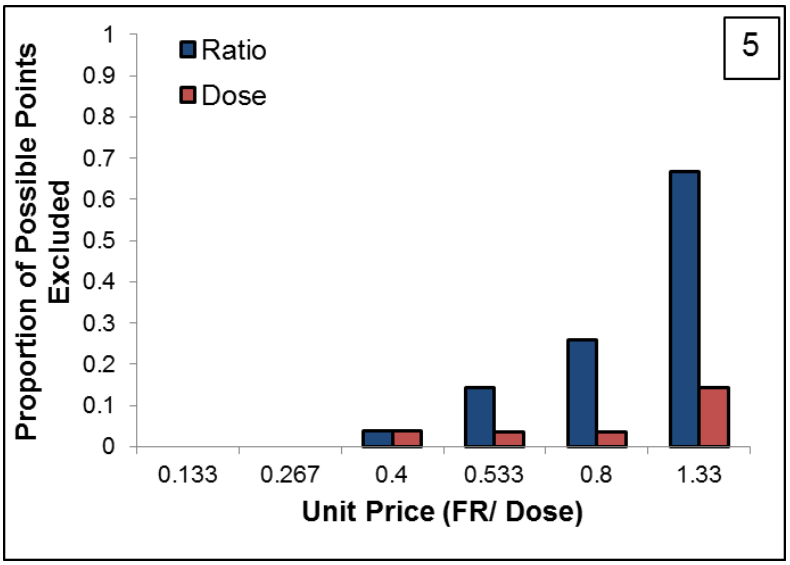

Figure 5. Proportion of data points excluded from demand curve analysis.

Four rats had two or less data points on at least one of the two curves, and were not included in the demand analysis (n=28 for demand analysis). A large proportion of data points were excluded from ratio-escalation curves because consumption was not maintained at high unit prices, suggesting that breakpoints likely differ between the two manipulations. An average demand curve including only data points where consumption was maintained is shown in Figure 6 (error bars represent standard error). While average consumption is qualitatively similarly for the two lowest unit prices tested, consumption appears more sensitive to changes in dose across the remaining four unit prices tested.

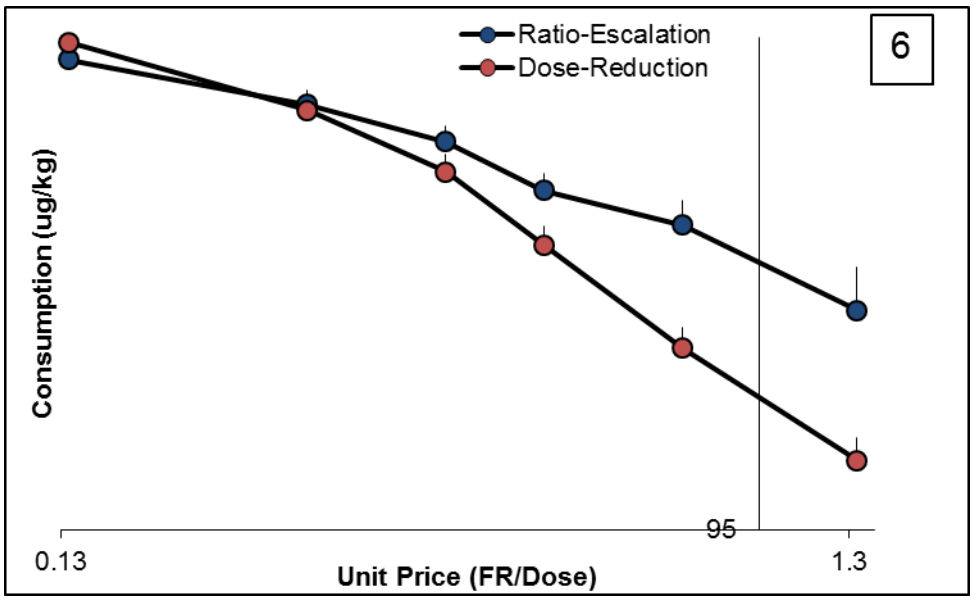

Figure 6. Average consumption for ratio-escalation and dose-reduction unit price combinations including only data points that were included in demand analysis.

Figure 7 shows R^2 values for the demand curve analysis. Fits were good for both ratio and dose fits, but fits were significantly better for dose curves than for ratio curves (t (27) = 2.777, $p < 0.05$).

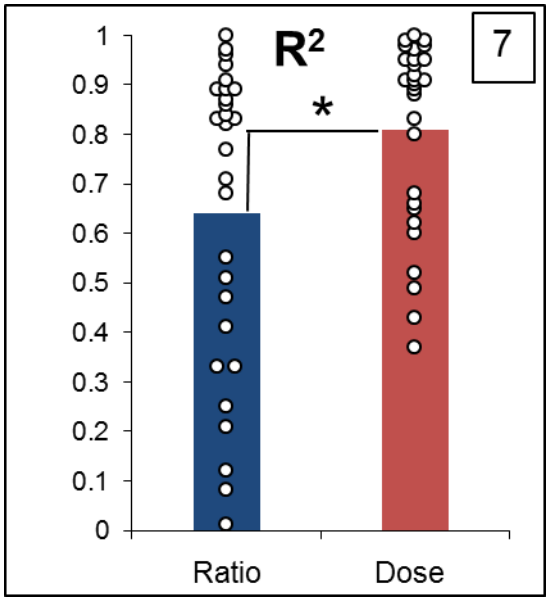

Figure 7. R^2 values for the best fitting functions of Equation 1 for 28 rats included in demand analysis.

Figure 8 shows free parameters from the best fitting functions of Equation 1 for both Q_0 and α. Values more than three standard deviations from the mean were excluded (Q_0: 2 values, α: 1 value). Ratio and dose Q_0 values were not significantly different from each other ($p > 0.05$), and the difference between the two sets of scores was significantly less than 165 µg, approximately 25% of the mean (t (25) = 2.074, $p < 0.05$). This difference is less than three infusions for ratio combinations (180 µg/kg). Dose α scores were significantly greater than ratio α scores (t (26) = 3.411, $p < 0.05$), suggesting that consumption is more sensitive to dose manipulations across the range of unit prices that maintain behavior. Q_0 scores were also highly correlated, but the

correlation of α scores did not meet significance (Figure 9), suggesting that sensitivity to the cost of nicotine is a poor predictor of sensitivity to nicotine dose.

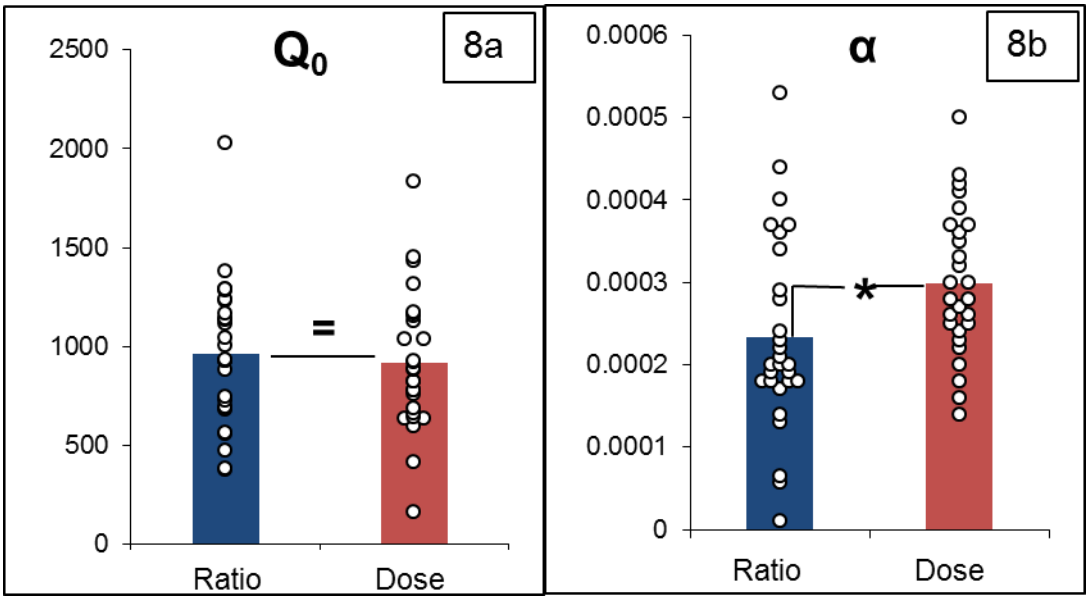

Figure 8. Free parameters Q0 (a) and α (b) from best fitting functions of Equation 1.

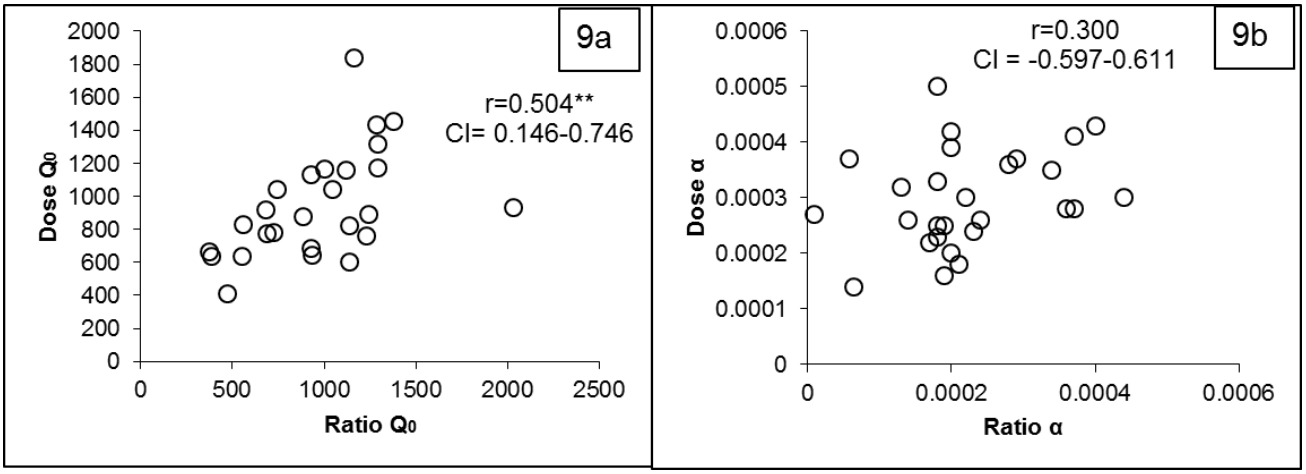

Figure 9. Correlation between free parameters Q0 (a) and α (b).

2.3.3 Comparing increases in cost and decreases in dose when consumption is not maintained

Breakpoint (BP) was defined as the highest unit price at which consumption was maintained at or above 10% of baseline (average of last two training condition sessions prior to start of the unit price manipulation) (Figure 10). This analysis was only conducted for rats that completed the entire procedure (n=26). A Wilcoxon Signed Ranked Test confirmed that BP was significantly higher for the dose procedure than for the ratio procedure ($Z = 3.619$, $p < 0.05$). Sixteen rats had a higher dose BP than a ratio BP, 10 rats had the same BP for both procedures, and 0 rats had a higher ratio BP than a dose BP.

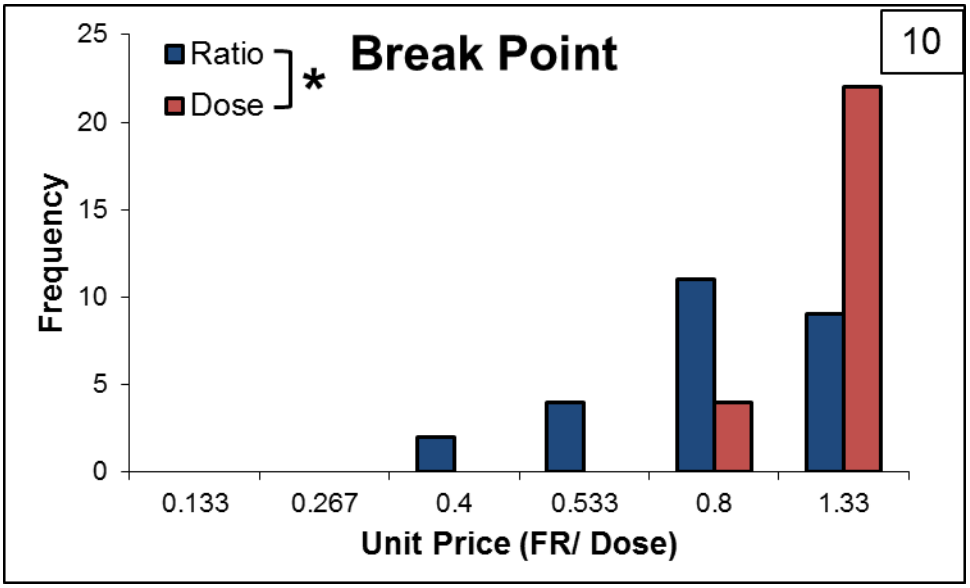

Figure 10. Breakpoint unit prices for each of the 26 rats that completed all 12 unit prices.

Visual inspection of individual demand curves suggested that the ratio escalation curves tended to decrease drastically after reaching a BP, while dose reduction curves showed a more gradual change across unit prices. Three exemplars are shown in Figure 11 (data points falling below graph had a value of 0).

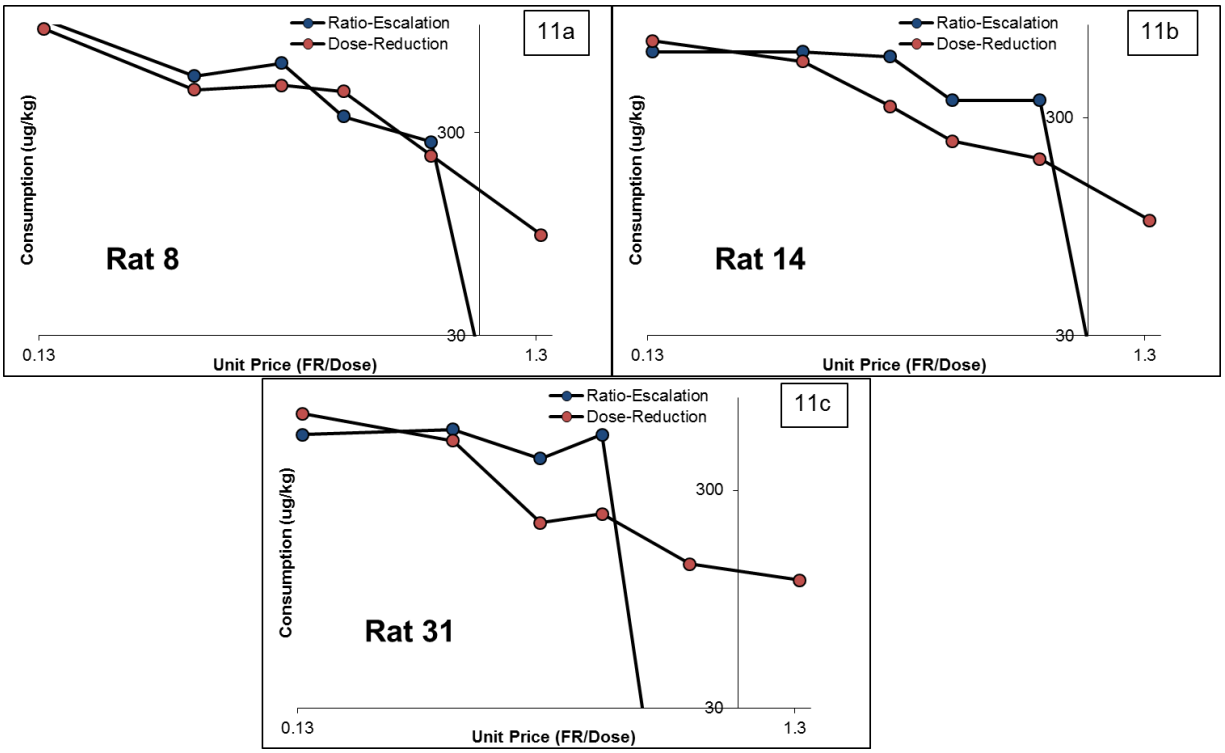

Figure 11. Individual demand curves for rats 8, 14, and 31.

It was not possible to compare the change immediately following breakpoint for all rats, because for most rats, consumption was maintained across all dose-reduction unit price combinations tested. Instead, change in consumption between each unit price was expressed as the proportional decrease in consumption given the proportional change in unit price, and the largest decrease for each rat for each of the two procedures was selected (Figure 12). This analysis might be thought of as quantifying the maximum instance of elasticity for each curve. A paired samples *t*-test confirmed the ratio escalation procedure produced a larger maximum change in consumption given the change in unit price than the dose reduction procedure (t (25) = 4.793, $p < 0.05$).

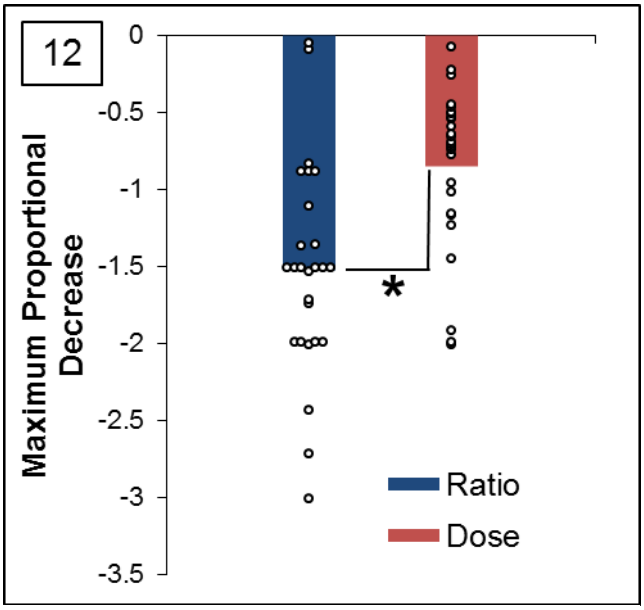

Figure 12. The maximum proportional change in consumption for both the ratio-escalation and dose-reduction demand curves for each rat that completed all 12 unit price combinations (n=26).

2.4 DISCUSSION

The present study is the first to show that decreasing the dose of nicotine and increasing the cost of nicotine do not change behavior in the same way. Across the range of unit prices that maintained consumption, rats were more sensitive to manipulations of nicotine dose than to manipulations of nicotine cost. However, consumption was not maintained across as many unit prices for the ratio escalation manipulation, and across all unit prices the largest instance of elasticity was greater for ratio escalation than dose reduction.

Previous research that suggested manipulations of reinforcer cost and manipulations of reinforcer are equivalent manipulations took different analytical approaches than the one taken here (Bickel et al., 1990; Bickel et al., 1991; Carroll et al., 1991; Collier et al., 1986; DeGrandpre et al., 1993; English et al., 1995; Foster & Hackenberg, 2004; Hursh et al., 1988; Nader et al., 1993; Sumpter et al., 2004; Woolverton & English, 1997). Previous studies most often investigated whether consumption differs at a single unit price when that unit price is created using more than one combination. Absence of a significant difference is taken as confirming equivalence. The present analysis is consistent with those reports in that it also failed to find a significant difference between consumption at two combinations across six unit prices. However, more thorough analyses revealed that behavior is not changed in the same way between the two manipulations. The present study is the first to investigate the equivalence of cost and reinforcer magnitude using nicotine as the reinforcer. It is unclear whether the inequity between nicotine cost and nicotine dose is specific to nicotine, or whether a more thorough analysis of other reinforcers would reveal inequity for other reinforcers as well. The implications of these findings for other drugs of abuse and non-drug reinforcers will be discussed in the General Discussion.

However, these data suggest that a more thorough analysis is warranted for previously tested reinforcers.

The difference in consumption between two different FR/dose combinations was significantly less than a margin of equivalence at the lowest two unit prices tested. It may be that increases in nicotine cost and decreases in nicotine dose change behavior similarly at very low unit prices, but at higher unit prices, the two manipulations have different effects. There is some support for this hypothesis. Bickel et al. (1990) reanalyzed data from 10 different studies using a variety of drugs as reinforcers, and reported that increases in cost and decreases in dose were equivalent manipulations, but acknowledged that equivalence was most clear at low unit prices, and at high unit prices there was some variability in consumption depending on the FR/dose combination used.

Across the range of unit prices that maintain consumption, rats were more sensitive to manipulations of nicotine dose than manipulations of nicotine cost. The inequity between cost and dose over this time period may be the result of differences in the timing of drug delivery. Although the ratio between cost and drug delivery is constant at a given unit price, a given quantity of nicotine is delivered in a larger bolus over the cost combinations, and is delivered over many small boluses for the dose combinations. Many small drug deliveries may have less reinforcing value than one large drug delivery, increasing elasticity for dose manipulations.

Previous nicotine research suggests that the duration over which nicotine is delivered is an important determinant in reinforcer value. In one study, rhesus monkeys responded for nicotine infusions when nicotine was delivered over 6 s or 24 s, but failed to respond if the infusion duration was lengthened to 100 s (Wakasa et al., 1995). Valentine, Hokanson, Matta, and Sharp (1997) also reported that nicotine self-administration was not maintained in rats if the

infusion duration was longer than 2 or 3 seconds. Wing & Shoaib (2013) showed that nicotine self-administration rates dropped sharply when the infusion duration was lengthened from 0.5 s to 5 s or 19.5 s, and that the effect was similar to saline substitution. These studies suggest that longer infusions durations may not hold equivalent reinforcing value as shorter durations, consistent with the hypothesis that rats were more sensitive to decreases in dose because many low-dose infusions do not hold the same value as a similar total nicotine dose delivered in a single infusion.

Rats also reached breakpoint at a lower unit price when ratio was escalated than when dose was reduced, and the maximum instance of elasticity was greater for ratio-escalation than it was for dose-reduction. Combined with the demand analyses, these data indicate that when cost is increased, rats are less sensitive to these changes until a breakpoint is reached, and then consumption is drastically decreased. The inequity between cost and dose at high unit prices may be related to contingencies associated with the cue, which likely functions as a conditioned reinforcer because of its previous pairings with nicotine. When ratio is escalated, the cost associated with cue delivery is also increased. However, when dose is decreased, a cue is delivered along with each smaller drug delivery. The cue has previously been paired with larger nicotine doses and likely maintains reinforcement value across conditions, especially given that rats only experience four sessions at each combination. Frequent cue delivery may maintain self-administration at higher unit prices that would otherwise be the case. Other researchers have shown that cue delivery can maintain nicotine self-administration when behavior would otherwise extinguish (Cohen, Perrault, Griebel, & Soubrie, 2005). Furthermore, it is well established that frequent delivery of a conditioned reinforcer combined with infrequent delivery of a primary reinforcer (i.e., second-order schedule of reinforcement) can maintain behavior in

instances where behavior would not be maintained by the primary reinforcer alone (Kelleher, 1966).

Nicotine has also been shown to increase the value of other reinforcing stimuli, including stimuli that were previously paired with nicotine delivery (Caggiula et al., 2009; Donny et al., 2003; Palmatier et al., 2006), an effect known as enhancement. The threshold nicotine dose for enhancement has not been established, and it may be that in the present study, low nicotine doses increased the value of the cue, which had been previously paired with a higher dose of nicotine, maintaining responding for the cue at higher unit prices than maintain responding in the ratio-escalation combinations where cue delivery is less frequent.

The cue contingencies in the present study are similar to cue contingencies that would be experienced when the price of cigarettes is increased or when nicotine content is decreased. When the price of cigarettes is escalated, the cost of the nicotine-associated cues is escalated also. However, if nicotine content is decreased, the price of nicotine-associated cues remains unchanged. The delivery of these nicotine-associated cues may maintain smoking behavior even at low nicotine contents, even though smoking behavior would not be maintained at equivalent unit prices created with high nicotine contents and high costs. The value of these cues would be expected to decrease with extended experience with low nicotine contents. However, the timeline for this change is unclear (Smith et al., 2014).

3.0 AIM 2

3.1 PURPOSE

The purpose of Aim 2 was to assess whether increases in nicotine cost and decreases in nicotine dose change consumption equivalently when very low nicotine doses, below the hypothesized threshold for maintaining self-administration behavior, are used. The relationship between nicotine dose and nicotine cost may be dependent upon the range of doses tested. Researchers have hypothesized that there may be a threshold nicotine dose below which nicotine does not function as a primary reinforcer (Benowitz & Hennningfield, 1994; Sofuoglu & LeSage, 2012). If nicotine dose is reduced below this threshold, consumption may drop drastically even in instances where a unit price approach predicts that consumption would be maintained. Thus, the relationship between nicotine cost and nicotine dose below threshold cannot be predicted from the relationship between nicotine cost and nicotine dose above threshold. If a nicotine reduction policy is enacted, the reduced nicotine content will be one that is hypothesized to be below the threshold for maintaining smoking behavior (or maintaining nicotine dependence), so the relationship between nicotine cost and very low nicotine doses is particularly important to explore.

3.2 METHOD

3.2.1 Subjects

Male Sprague-Dawley rats (Harlan-Farms, IN) weighing between 200 and 225 g on the day after arrival were used as subjects (n=37). All rats were part of a single cohort of rats. Housing and feeding conditions were the same as in Aim 1.

3.2.2 Apparatus

The same operant chambers were used as in Aim 1.

3.2.3 Drugs

There were no changes from Aim 1, except that after the first unit price manipulation, drug was delivered in a volume of 0.05 ml / kg in approximately 0.5 s to allow rats to take more infusions within a single session.

3.2.4 Procedures

3.2.4.1 Surgery

For the first five days following surgery, the first cohort of rats had their cannulae flushed once daily with a sterile saline solution containing heparin (3 U), timentin (6.67 mg) and streptokinase (833.3 U) to maintain catheter patency and prevent infection. After this initial post-surgery time period, the flushing solution contained only the heparin and timentin. Only data

points from rats that passed a patency test consisting of rapid loss of muscle tone to methohexital (5 mg/kg i.v.) are included.

3.2.4.2 General Self-Administration Procedures

Self-administration procedures, including the time-out and session-length procedures were the same as in Aim 1.

3.2.4.3 Acquisition

Rats experienced an increase in FR across sessions to reach the terminal FR used in the training condition (FR10). Rats experienced 8 sessions on FR2, 5 sessions on FR5, and 7 sessions on FR10. Rats then began the unit price portion of the experiment described below.

3.2.4.4 Unit Price Procedure

The Unit Price procedure was similar to the Unit Price procedure in Aim 1, except that doses 10 times lower than those in Aim 1 were used for the dose-reduction combinations, and rats responded on an FR1 for these combinations. Rats each experienced six unit prices, and each rat experienced each unit price twice, creating 12 total combinations (Table 2). The first six combinations all used the same dose of nicotine, but the number of responses required to earn an infusion (FR, cost) increased across unit prices. The second six combinations all required the same number of responses to earn an infusion, but the dose of nicotine decreased across unit prices. Nicotine doses in this experiment were chosen such that the majority of doses are not expected to maintain self-administration behavior (Smith et al., 2014). Rats experienced four sessions at each unit price combination, and each rat experienced the combinations in a random

order. Rats experienced one session on the training condition (FR10, 60 µg/kg/infusion) in between each unit price combination. During the first unit price manipulations, some rats on the

Table 2. FR/Dose combinations used in Aim 2.

	UNIT PRICE: FR/NICOTINE DOSE (µG/KG/INFUSION)											
	0.133		0.267		0.4		0.533		0.8		1.33	
	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE	FR	DOSE
COST ESCALATION	8	60	16	60	24	60	32	60	48	60	80	60
DOSE REDUCTION	1	7.5	1	3.75	1	2.5	1	1.875	1	1.25	1	0.75

low-dose combinations were coming close to emptying the drug syringe in a single session (approximately 150 infusions). Starting with the training condition between the first and second unit price manipulation, drug solutions were twice as concentrated (nicotine delivered in a volume of 0.05 ml/kg) and delivered over half the duration (~0.5 s). On Day 2 of Unit Price 7, a rat emptied the drug syringe in a single session (257 infusions), and starting with the following session a larger drug syringe was used (10 ml syringe instead of 5 ml), and the infusion speed was adjusted. Following completion of all 12 unit price combinations, rats experienced a 13^{th} condition where they responded on an FR1 for saline along with regular cue conditions (saline + cue condition) immediately followed by two sessions where they responded on an FR1 for saline without the cue (saline + no cue condition).

3.2.4.5 Data Analysis

Analyses were the same as in Aim 1, with one addition. Infusions earned during the saline + cue and saline + no cue conditions were compared to infusions earned in the lowest nicotine dose condition first using paired samples *t*-tests, and then using a two one-sided test of equivalence.

3.3 RESULTS

Although 37 rats were used in the experiment, five rats were excluded from all unit prices because of catheter patency. One rat did not earn any infusions on any of the ratio combinations, and data have been excluded. The remaining 31 rats have data that are included for at least some unit prices.

3.3.1 Comparing consumption at each unit price

Figures 13, 14, and 15 show average and individual consumption across the 12 unit price combinations (average last two sessions at each combination). Figure 13 shows consumption plotted as two demand curves—one for ratio escalation, and one for dose reduction (error bars represent standard error).

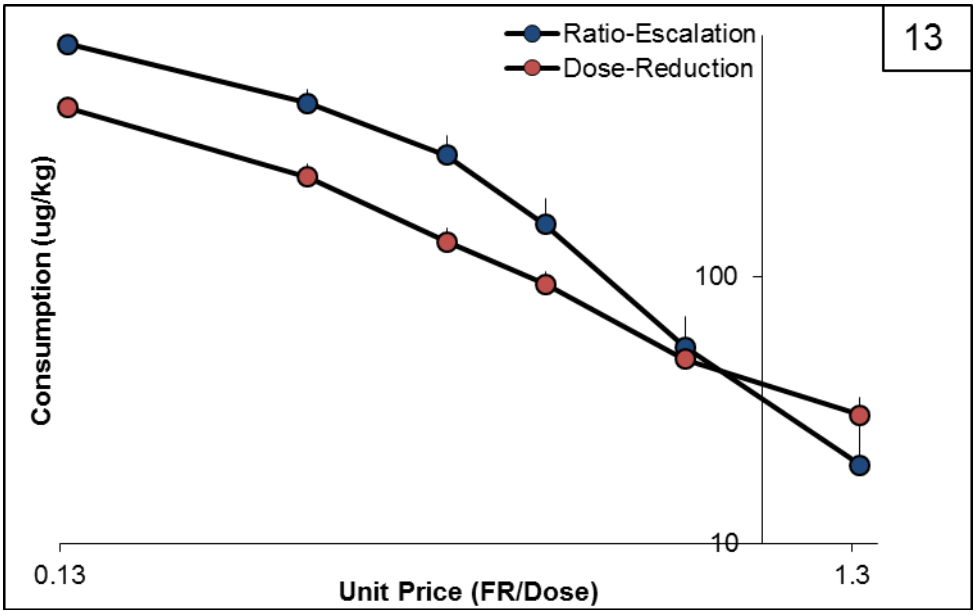

Figure 13. Average consumption for ratio-escalation and dose-reduction unit price combinations.

Consumption only appears similar at the highest unit prices, whereas in Aim 1 consumption was qualitatively similar at high and low unit prices. Paired samples t-tests confirmed that consumption was significantly different between the two manipulations at the three lowest unit prices (0.133: t (28) = 6.909, $p < 0.05$; 0.267: t (30) = 3.863, $p < 0.05$; t (31) = 3.113, $p < 0.05$). At the three higher unit prices, consumption failed to meet criteria for a significant difference (ps > 0.05), but the difference was also not significantly less than 25% of the mean (0.533: $\delta = 30.3$; 0.8: $\delta = 12.5$; 1.33: $\delta = 6.1$).

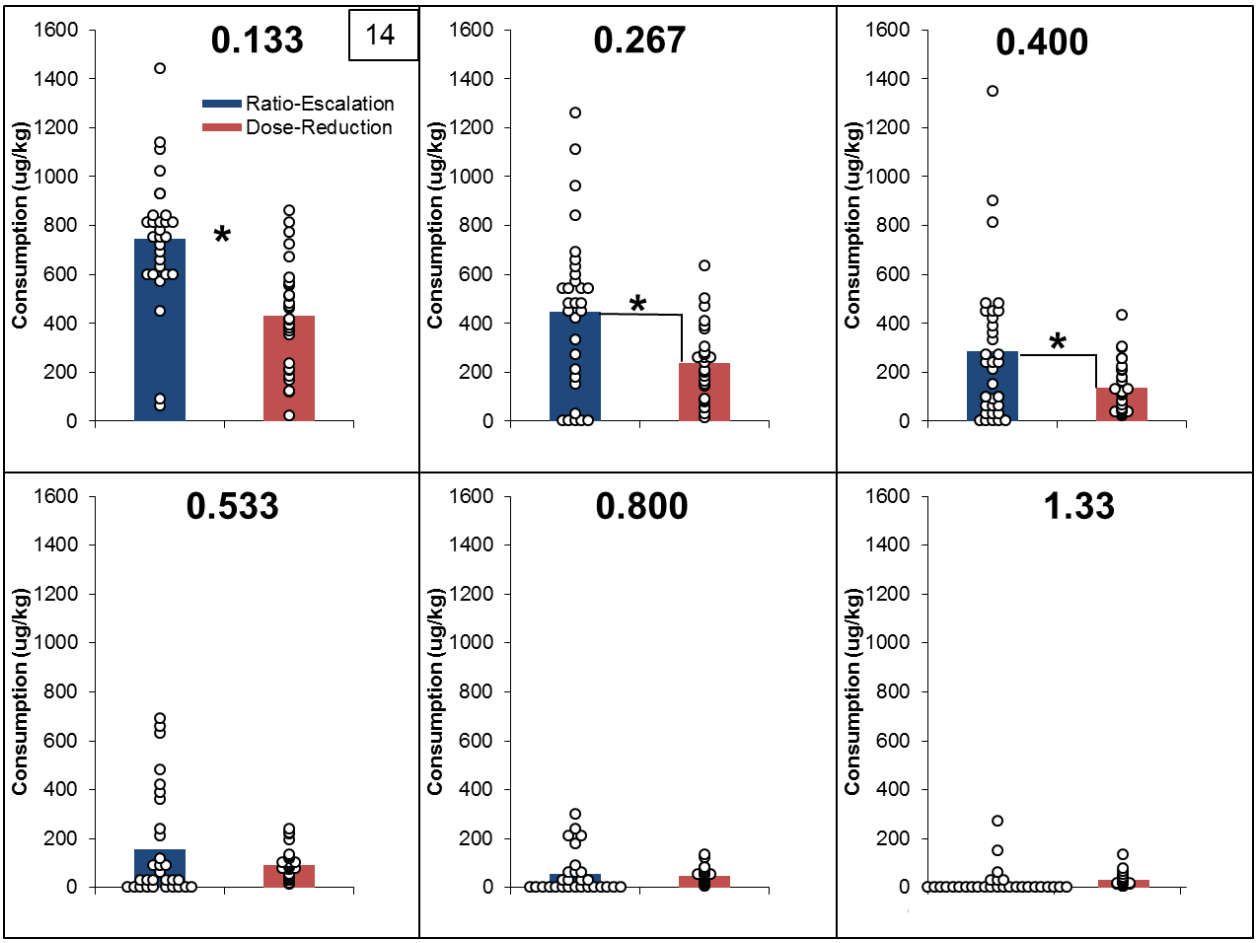

Figure 14. Average and individual consumption data for all 12 unit price combinations with all y-axes on the same scale.

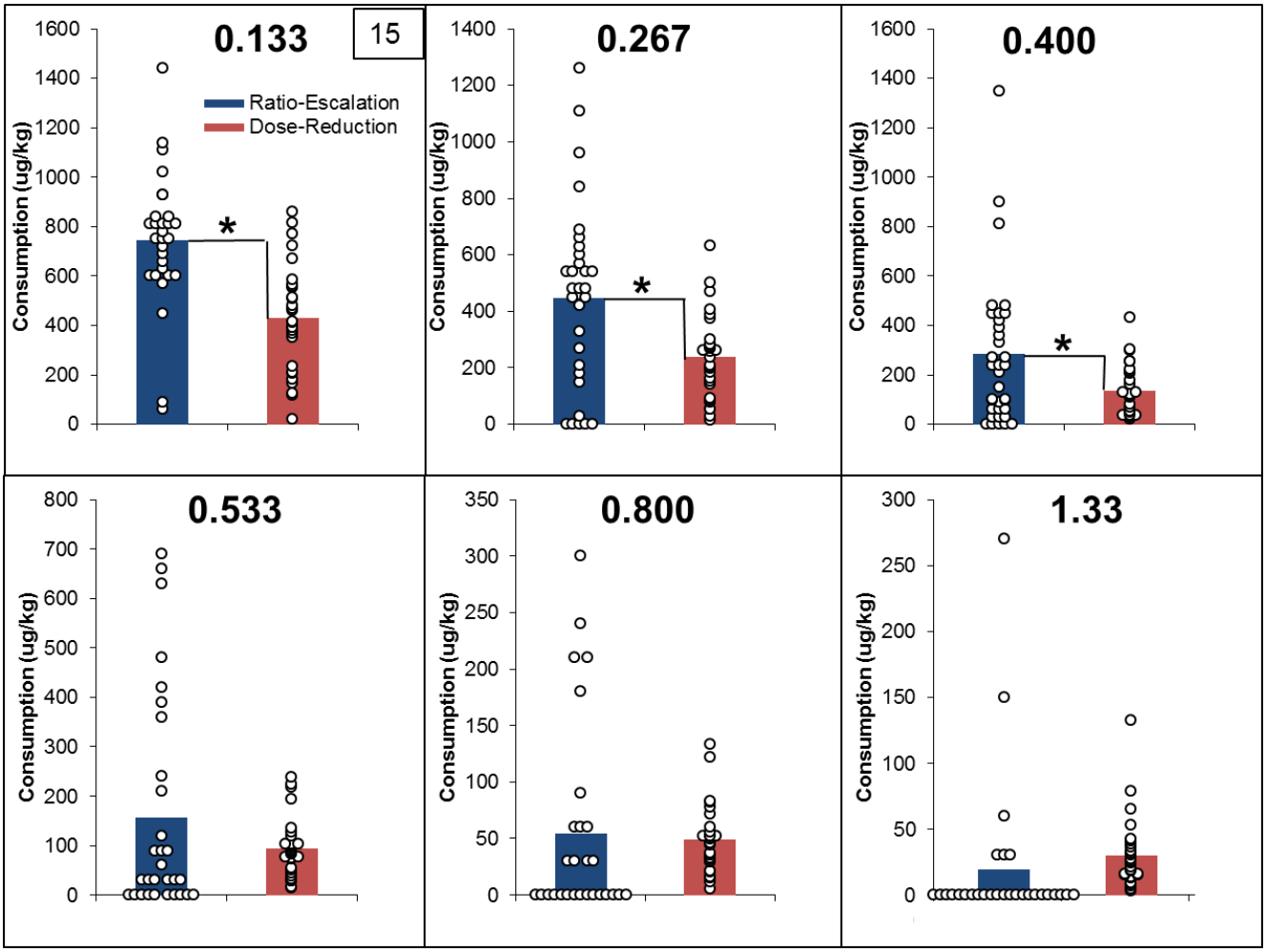

Figure 15. Average and individual consumption data for all 12 unit price combinations with y-axes adjusted for each graph.

3.3.2 Comparing increases in cost and decreases in dose when consumption is maintained

As in Aim 1, a demand analysis was employed to test whether the two manipulations changed behavior differently across the range of unit prices that maintained consumption. Data points were excluded if consumption fell below 10% of baseline (Figure 16).

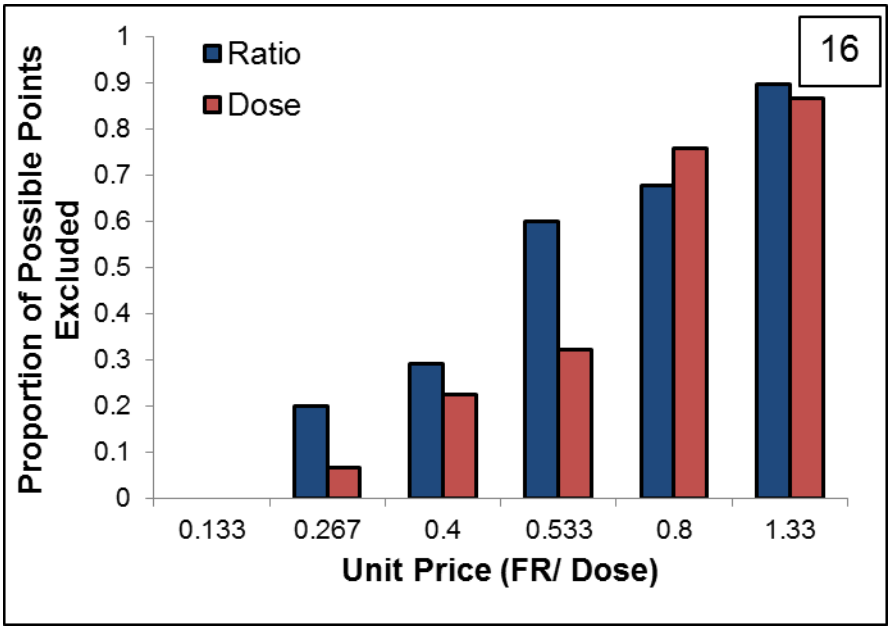

Figure 16. Proportion of data points excluded from demand curve analysis.

After excluding these data points, 13 rats had two or less data points for at least one of the two demand curves, and 18 rats were included in the demand analysis. Figure 17 shows the average demand curves for only those data points that were included in the demand curve analysis (error bars represent standard error). It represents how average consumption changed over the range of unit prices that maintained consumption. The demand curve for ratio-escalation is shifted upwards, suggesting that Q_0 is likely to differ between the two manipulations.

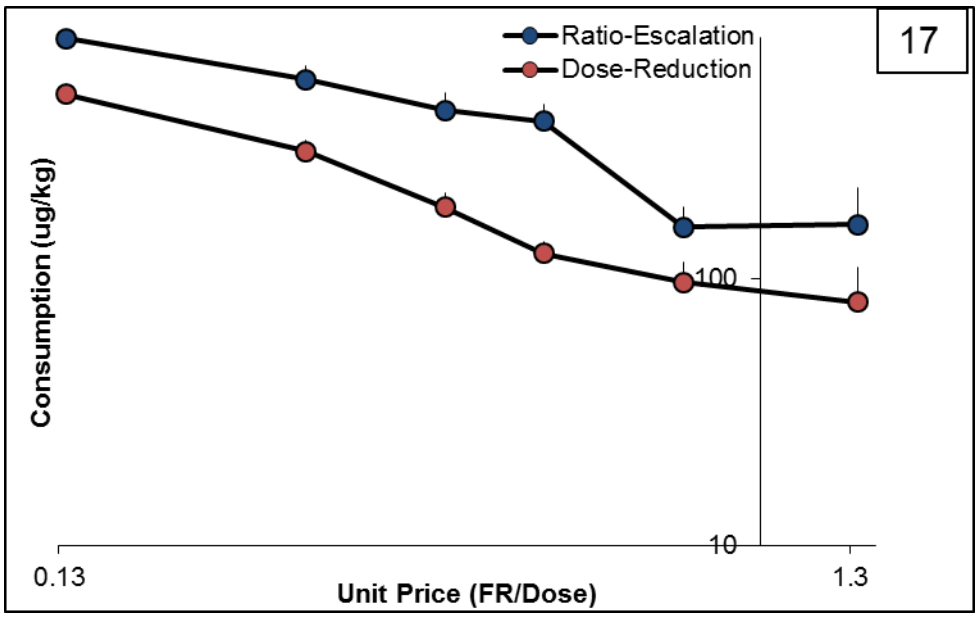

Figure 17. Average consumption for ratio-escalation and dose-reduction unit price combinations including only data points that were included in demand analysis.

Equation 1 normalizes both consumption and price for differences in Q_0, making it possible to test for differences in sensitivity to unit price despite existing differences in Q_0. The difference in Q_0, and the standardization of price in Equation 1 make it difficult to tell from the demand curve shown in Figure 17 whether there is a difference in sensitivity to unit price. Figure 18 is a graphical representation of average demand once consumption and price have been normalized for Q_0. In this figure, it appears that dose is more sensitive than ratio to manipulations of unit price across the majority of unit prices tested.

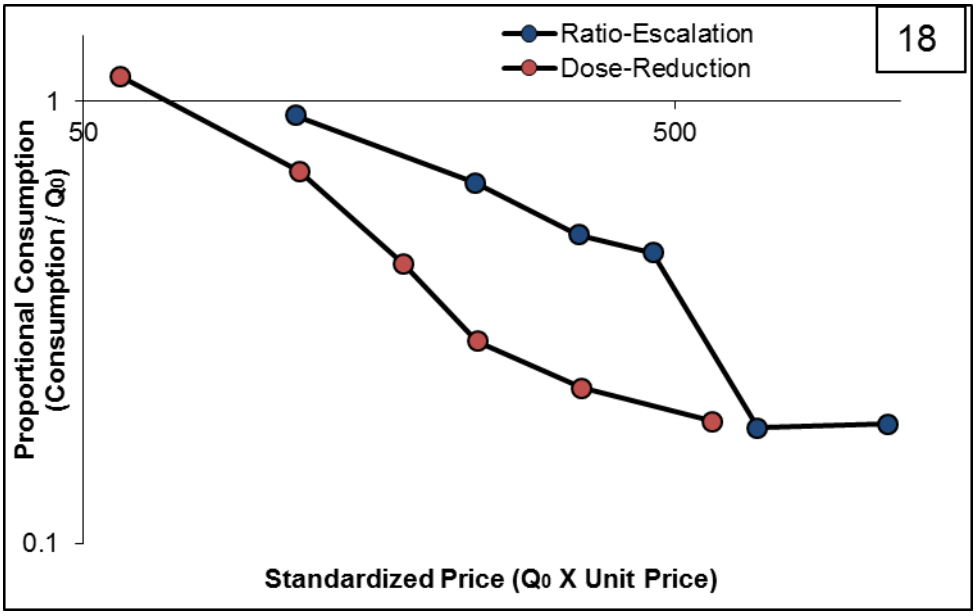

Figure 18. Average demand after normalizing consumption and price for Q_0.

R^2 values for both sets of demand curves are shown in Figure 19. While there were a few low R^2 values, fits were generally good, and there was not a significant difference between ratio-escalation or dose-reduction curves ($p > 0.05$).

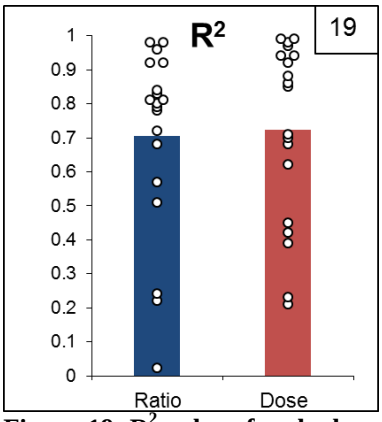

Figure 19. R^2 values for the best fitting functions of Equation 1 for 28 rats included in demand analysis.

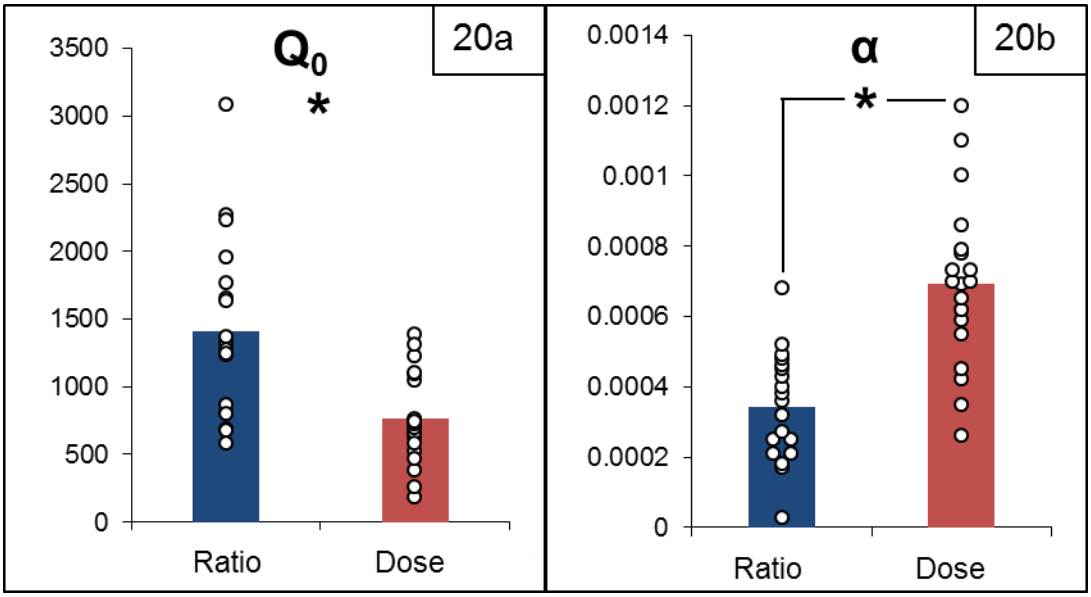

Figure 20. Free parameters Q0 (a) and α (b) from best fitting functions of Equation 1.

Figure 20 shows free parameters from the best fitting functions of Equation 1 for both Q_0 and α. Q_0 was higher for ratio-escalation than dose reduction, and α was lower for ratio-escalation than dose-reduction (Q_0: t (17) = 4.084, $p < 0.05$; α: t (18) = 6.919, $p < 0.05$). Parameters from ratio-escalation and dose-reduction curves were not significantly correlated with each other (Figure 21, ps > 0.05), suggesting that an individual's response to one manipulation is a poor predictor of their response to the other manipulation.

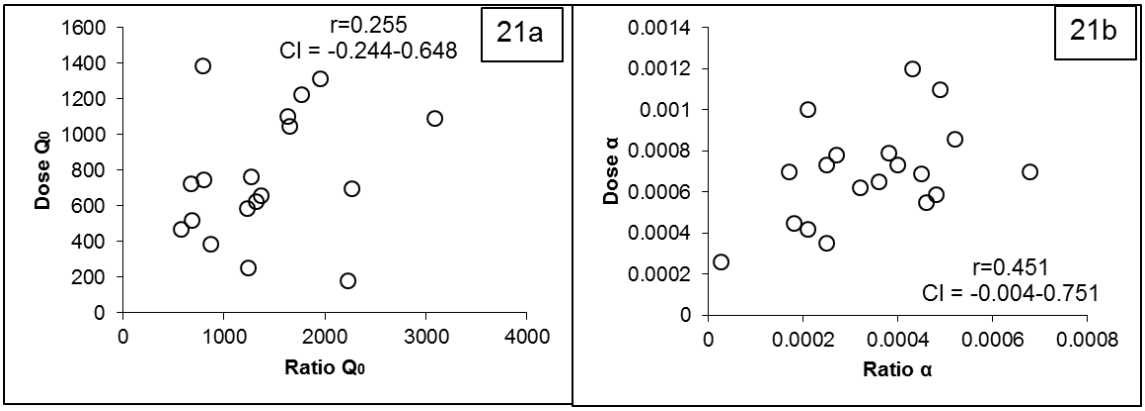

Figure 21. Correlation between free parameters Q_0 (a) and α (b).

3.3.3 Comparing increases in cost and decreases in dose when consumption is not maintained

Breakpoint was calculated for 26 rats that completed all 12 unit price combinations, and had at least one unit price for both curves at or above baseline consumption (Figure 22). A Wilcoxon Signed Ranks test failed to reveal significant differences between the two manipulations ($p > 0.05$). However, the breakpoint for dose was higher than the breakpoint for ratio for 14 rats, the same for both manipulations for eight rats, and higher for ratio than for dose for 4 rats.

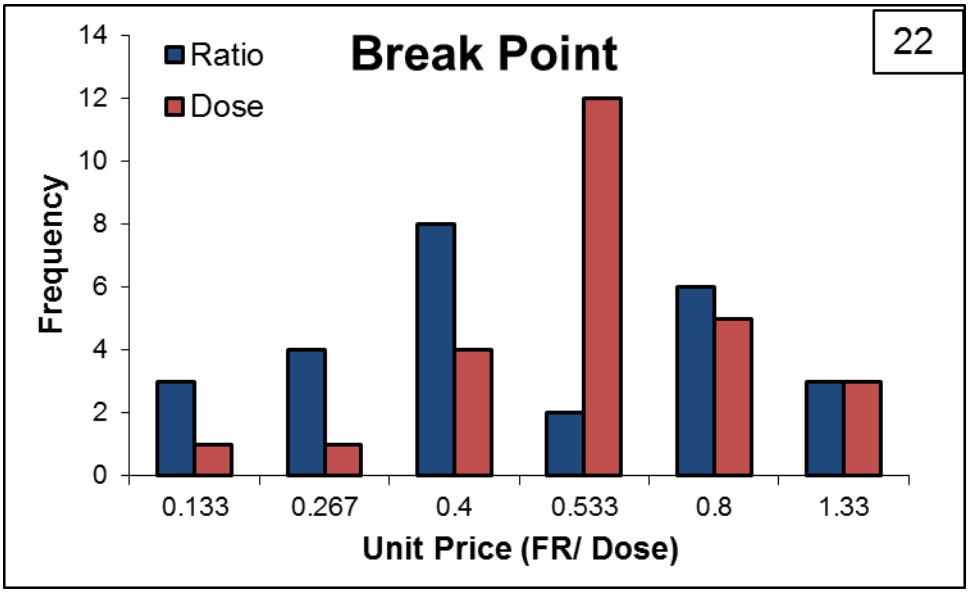

Figure 22. Break point unit prices for each of the 26 rats that completed all 12 unit prices.

As in Aim 1, the maximum instance of elasticity was compared for the ratio-escalation and dose-reduction curves (Figure 23). The maximum decrease was larger for the ratio-escalation manipulation than for the dose-reduction manipulation (t (25) = 4.518, $p < 0.05$).

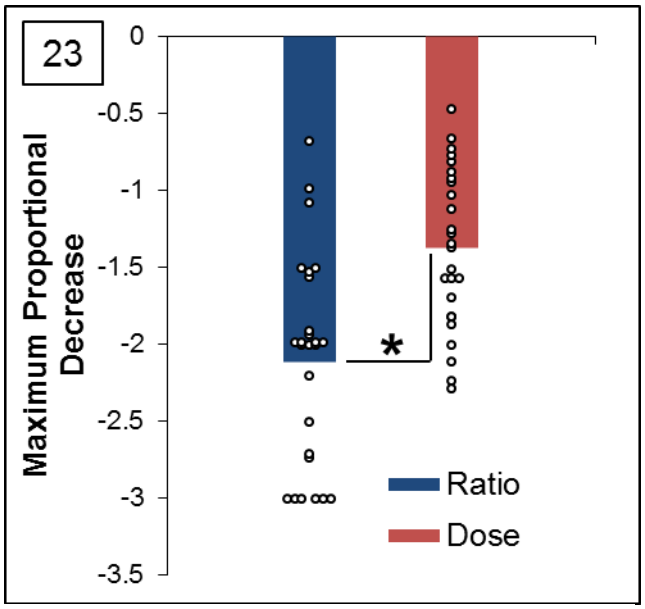

Figure 23. The maximum proportional change in consumption for both the ratio-escalation and dose-reduction demand curves for each rat that completed all 12 unit price combinations (n=26).

3.3.4 Saline conditions

The number of infusions earned in the final two saline conditions (saline + cue, saline + no cue) was compared to the number of infusions earned for the lowest dose condition (Figure 24). Paired samples t-tests failed to reveal a significant difference between the saline + cue condition, but infusions earned in the saline + no cue condition were significantly less than infusions earned in the lowest nicotine dose condition (t (24) = 2.776, $p < 0.05$). The difference in infusions between the lowest nicotine dose condition and the saline + cue condition was not significantly less than 25% of the overall mean ($p > 0.05$, δ=9.59).

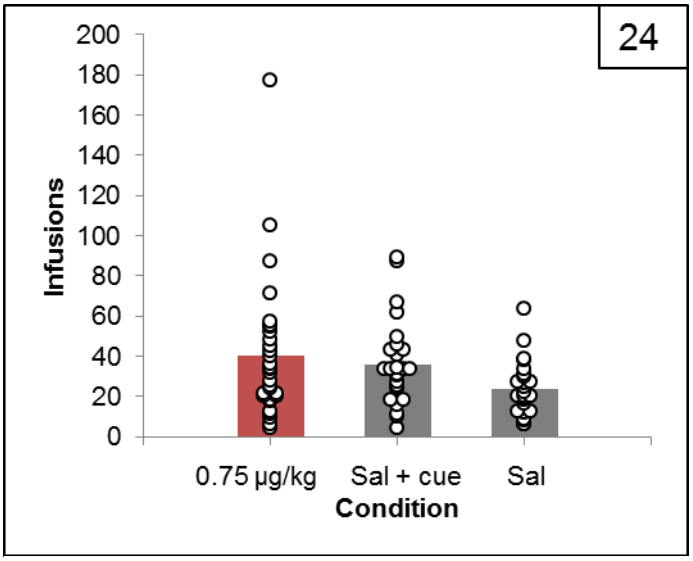

Figure 24. Average infusions earned over the last two sessions of the lowest dose tested in the set of dose-reduction combinations, the saline + cue condition, and the saline + no cue condition.

3.4 DISCUSSION

The present study showed that when very low nicotine doses are used, increases in nicotine cost and decreases in nicotine dose are not equivalent manipulations. Consistent with Aim 1, rats were more sensitive to decreases in dose than they were to increases in cost. Breakpoints were not significantly different, but the maximum decrease in consumption experienced across unit prices was larger for ratio escalation than for dose reduction. Furthermore, ratio-escalation curves had higher Q_0 values than dose-reduction curves.

Q_0 is a free parameter estimating consumption if the reinforcer were free, so higher Q_0 values for ratio-escalation suggest that even if doses in the range used for the dose-reduction curve in the present study were made freely available, rats would take less nicotine than if the dose used in the ratio-escalation curve was made free available.

Breakpoints were not significantly different between ratio-escalation and dose-reduction, but more rats did have higher dose breakpoints than ratio breakpoints (14 vs. 4 rats), and the largest proportional decrease in consumption across unit prices was greater for ratio-escalation than dose-reduction. The high rate of cue delivery for dose-reduction combinations may prevent large changes in consumption when unit price is increased, as discussed in Aim 1.

Evidence that the cue was important in maintaining self-administration behavior at low nicotine doses also comes from the final two saline conditions. Infusions earned in the saline + cue condition did not differ from infusions earned for the lowest dose of nicotine, suggesting that responding at the lowest nicotine dose may be mostly maintained by the cue. Furthermore, the number of infusions earned in the saline + no cue condition was significantly lower than the number of infusions earned in the lowest nicotine dose condition (0.75 μg/kg/infusion + cue). However, several methodological limitations make it difficult to draw conclusions about the role

of the cue from these data. The presentation of the two saline conditions was confounded with the number of sessions since the last nicotine exposure (all rats received the saline + cue condition first with no training condition in between), and rats only experienced two sessions of the saline + no cue condition. Unfortunately, the second session of the saline + no cue condition occurred on December 23rd, 2014, and further experimental testing was not possible. Furthermore, the difference in infusions for the low nicotine dose + cue condition and the saline + cue condition was not significantly less than a reasonable margin of equivalence (25% of overall mean), so it is not possible to conclude that responding at the lowest nicotine dose was entirely cue maintained.

4.0 AIM 3

4.1 PURPOSE

The purpose of Aim 3 was to directly evaluate the hypothesis that consumption at a single unit price should be equivalent regardless of the combination used to create that unit price. The best challenge to this hypothesis is to incorporate some combinations that use very low nicotine doses that may be below the threshold for maintaining self-administration behavior. Using data from Aims 1 and 2, a unit price of 0.533 was chosen. Lower unit prices would not have allowed for doses as low as 1.875 µg/kg/infusion and 3.75 µg/kg/infusion to be included. Higher unit prices may not have maintained self-administration behavior. Because the role of the cue in maintaining self-administration at low nicotine doses is uncertain, one combination was included where rats had the opportunity to respond on an FR1 for saline alone with the cue.

4.2 METHOD

4.2.1 Subjects

Male Sprague-Dawley rats (Harlan-Farms, IN) weighing between 200 and 225 g on the day after arrival were used as subjects (n=42). Data are pooled from two cohorts of rats that

completed the experimental procedures at separate times. Housing conditions were the same as in Aim 1.

4.2.2 Apparatus

The same operant chambers were used as in Aim 1.

4.2.3 Drugs

There were no changes from Aim 1, except prior to the start of the unit price manipulation, the concentration of drug was increased to allow rats to earn more infusions in a single session. Across all unit price manipulations, drug was delivered in a volume of 0.05 ml /kg/infusion delivered in approximately 0.5 s.

4.2.4 Procedures

4.2.4.1 Surgery

The antibiotic used in the flushing solution varied across the two cohorts. The first cohort of rats had their cannulae flushed once daily with a sterile saline solution (0.1 ml) containing heparin (3 U), timentin (6.67 mg) and streptokinase (833.3 U) to maintain catheter patency and prevent infection for the first five days following surgery. After this initial post-surgery time period, the flushing solution contained only the heparin and timentin. Prior to the start of self-administration, timentin became unavailable and 10mg cefazolin was substituted. The second cohort of rats received 10 mg cefazolin as the antibiotic during acquisition, but prior to the start

of the unit price manipulation, the lab switched to gentamicin (1mg). Only data points from rats that passed a patency test consisting of rapid loss of muscle tone to methohexital (5 mg/kg i.v.) are included.

4.2.4.2 General Self-Administration Procedures

Self-administration procedures, including the time-out and session-length procedures were the same as in Aim 1.

4.2.4.3 Acquisition

All rats experienced an increase in FR across sessions to reach the terminal FR used in the training condition (FR10), and drug was delivered in a volume of 0.05 ml/kg/infusion using 10 ml syringes for all unit price manipulations. However, the two cohorts of rats differ in the number of sessions spent at each FR during acquisition, and in how the final drug concentration was reached. The first cohort of rats used 10-ml syringes throughout the experiment. At the start of acquisition, rats responded on an FR2 schedule of reinforcement for eight days using the more concentrated nicotine solution (0.05 ml/kg/infusion), delivered using 10-ml syringes. However, responding and earned infusions were low, and we hypothesized that the highly concentrated solution was interfering with rats learning to respond for nicotine. The solution was diluted to the lab standard concentration (0.1 ml/kg/infusion) for five sessions while rats continued on an FR2, and rates of responding increased. The FR was then increased across sessions (FR5 for six sessions, FR10 for eight sessions). The concentration of the solution was then increased again, and infusions were unchanged, suggesting that the more concentrated solution interferes during initial acquisition only. Rats remained on an FR10 with the more highly concentrated solution for six sessions before beginning the unit price manipulations. The second cohort of rats started

acquisition on an FR2 schedule of reinforcement with drug delivered in the more dilute volume of 0.1 ml/kg/infusion using 5-ml syringes in order not to interfere with learning nicotine administration. The FR was then escalated (FR2 for 18 sessions, FR5 for six sessions, FR10 for five sessions) before the drug syringe was changed to allow rats to earn more infusions within a single session (10 ml syringes for five sessions) and the drug concentration was increased for the same reason (0.05 ml/kg/infusion for 6 sessions). The unit price manipulation then began.

4.2.4.4 Unit Price Procedure

The Unit Price procedure was similar to the Unit Price procedure in Aim 1, except that seven of the eight FR/dose combinations used create equivalent unit prices, allowing for a direct test of the hypothesis that consumption will be equivalent at equivalent unit prices, regardless of the combination used to create that unit price. The eighth combination involved rats responding for saline + the cue, and was added to investigate the role of the cue in maintaining self-administration at the lowest dose combinations used. Combinations are shown in Table 3.

Table 3. FR/Dose combinations used in Aim 3.

UNIT PRICE = 0.533	
FR	**DOSE (µG/KG/INFUSION)**
1	SAL
1	1.875
2	3.75
3	5.625
4	7.5
12	22.5
24	45
32	60

As in the first two experiments, rats experienced four sessions at each combinations, experienced the combinations in a random order, and a single training condition session was

inserted between each combination. Due to an error, rats only experienced three sessions on the seventh unit price combination, and on the third session two of the rats were switched in the operant chambers and experienced the other rat's experimental condition for that session. The data points for those two rats from the seventh combination have been excluded, and for the remaining rats consumption at the second and third combination was averaged for data analysis. Average consumption at the seventh combination appears consistent with remaining data points gathered at other combinations using the third and fourth session.

4.2.4.5 Data Analysis

Data analysis focused on testing whether consumption was equivalent across the seven unit price combinations. Planned comparisons tested whether each combination was different from the highest and lowest dose combination using paired-sample t-tests. In the case of nonsignificant t-tests, two-one sided tests of equivalence tested whether the difference was less than 25% of the mean of those two combinations.

4.3 RESULTS

Of the 42 rats used in Aim 3, 13 rats failed the first patency test conducted, and all data from these rats has been excluded. One rat failed a final patency test, but had passed an earlier one, and data from the last passed patency have been included. Five rats earned less than two infusions at baseline (average of last two training condition sessions) and have been excluded from all analyses.

Figure 25 shows consumption across the seven unit price combinations (saline excluded because consumption cannot be plotted). Qualitatively, there appears to be an inverted-U shape to the graph, such that consumption increases across the first few combinations, is high for the middle combinations, and then is lower at the highest dose combination. Paired samples t-tests using FR1/1.875 µg/kg/infusion as the reference group revealed that consumption was significantly greater at FR2/3.75, FR3/5.625, FR4/7.5, and FR12/22.5 combinations (represented by *, FR2/3.75: $t(23) = 3.92$, $p < 0.05$; FR3/5.625: $t\ (23) = 5.364$, $p < 0.05$; FR4/7.5: $t(22) = 4.765$, $p < 0.05$; FR12/22.5: $t\ (23) = 4.890$, $p < 0.05$). Consumption at the other two combinations did not meet criteria for a significant difference, and the difference was also not significantly less than 25% of the mean (δ) (FR24/45: δ=35.33; FR32/60: δ=30.38). Paired samples t-tests using FR32/60 as the reference group revealed that consumption was significantly greater at FR3/5.625 and FR12/22.5 combinations (represented by **, FR3/5.625: $t(24)=2.215$, $p < 0.05$; FR12/22.5: $t(23) = 2.724$, $p < 0.05$), but did not meet criteria for a significant difference at the other combinations, and the difference was also not significantly less than 25% of the mean (δ) (FR1/1.875: δ = same as above; FR2/3.75: δ=37.14; FR4/7.5: δ=39.58; FR24/45: δ=36.89).

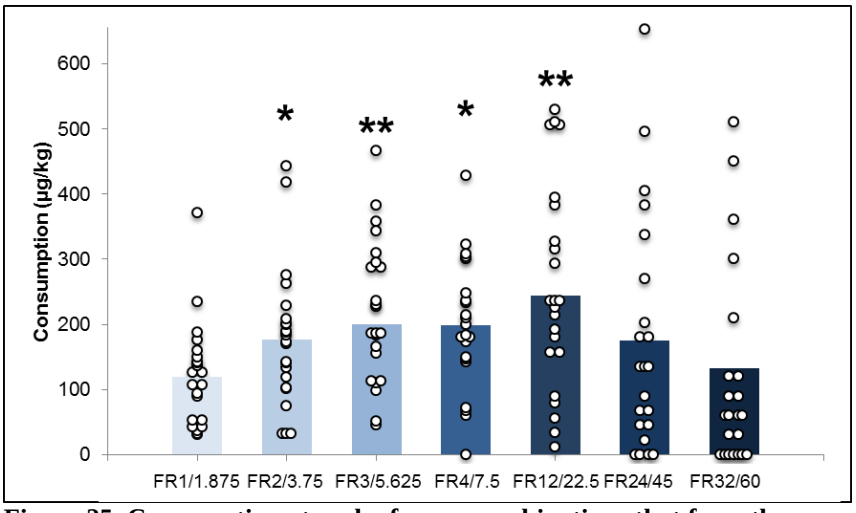

Figure 25. Consumption at each of seven combinations that form the same unit price (0.533).

Figure 26 shows the average number of infusions earned at each of the eight combinations. A paired-samples t-test confirmed that rats earned significantly more infusions at the FR1/1.875 µg/kg/infusion combination than at the FR1/saline combination ($t(21)=3.907$, $p < 0.05$). Differences between other combinations were not tested because the FR varies across combinations, making other differences difficult to interpret.

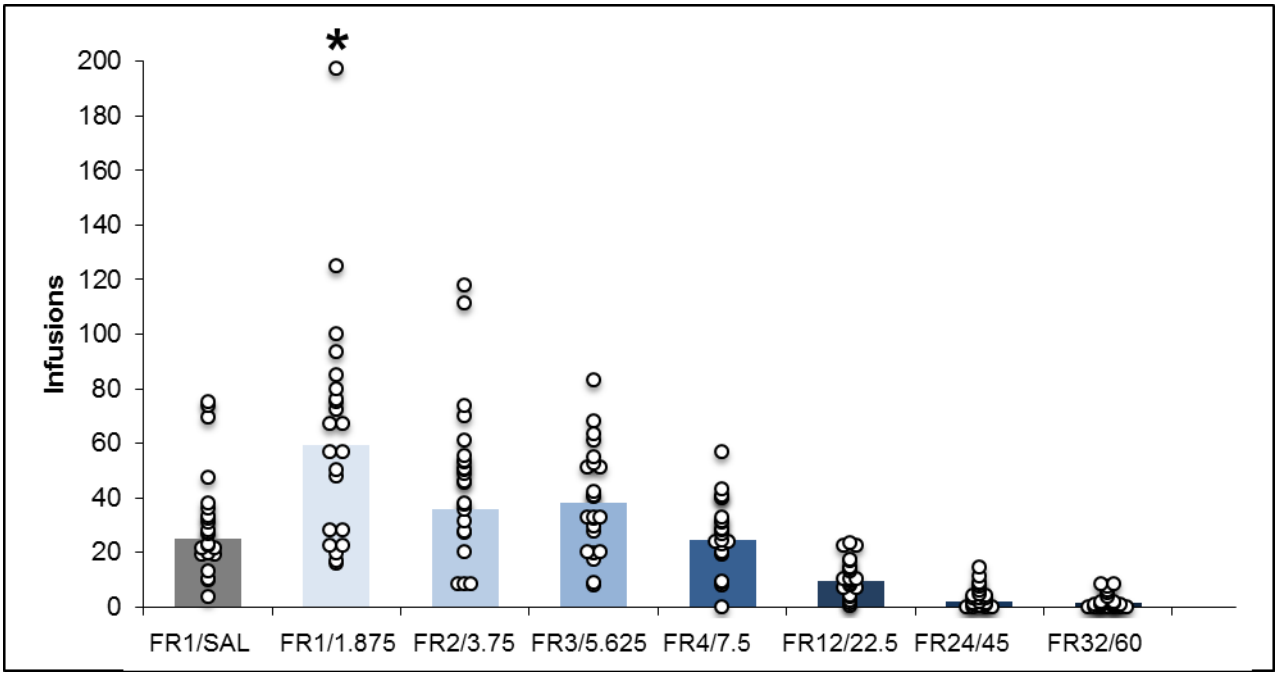

Figure 26. Infusions earned across eight combinations.

4.4 DISCUSSION

The results from Aim 3 show that consumption is not equivalent at a single unit price regardless of the cost/dose combination used to create that unit price. Qualitatively, consumption appeared to be low when low FR/dose and high FR/dose combinations were used, but higher across a middle range of FR/dose combinations. When a low FR and dose was used (FR1/1.875),

consumption was significantly less than four other combinations that employed higher FRs and doses, and consumption at the highest FR and dose combination used (FR32/60) was significantly lower than consumption at two combinations. These data are inconsistent with the behavioral economics hypothesis that consumption should be equivalent at a single unit price regardless of the combination used to create that unit price.

Infusions earned at the FR1/1.875 combination were significantly greater than infusions earned at the FR1/Saline condition, suggesting that the cue is not solely responsible for consumption at the FR1/1.875 combination. It is unclear whether responding at the FR1/1.875 combination is maintained by the primary reinforcing or reinforcement enhancing effects of nicotine. Previous data from our lab (Smith et al., 2013) has shown that the threshold nicotine dose for maintaining self-administration behavior is between 3.75 µg/kg/infusion and 7.5 µg/kg/infusion, but in the present study 1.875 µg/kg infusion maintained self-administration significantly above saline. The procedure used in our previous paper was different in that threshold was evaluated using an FR5 schedule of reinforcement, the time-out following each infusion was 1-min instead of 3-s, and the session length was fixed at one hour. The decreased FR and shortened time out likely contributed to a higher threshold nicotine dose required for maintaining behavior. If the threshold nicotine dose for maintaining behavior is shifted by time-out, that suggests that several small nicotine infusions can be earned in a row, creating a bolus large enough to maintain behavior at a lower dose than would otherwise be possible. However, data from Aims 1 and 2 suggest that while several infusions in a row may create a bolus large enough to maintain behavior, they may be extended over too long a period to be of equal value as that same bolus delivered over a short period. If the threshold nicotine dose for maintaining

behavior is shifted by FR, these data are consistent with the behavioral economics hypothesis that cost is important in determining whether or not behavior will be maintained.

Another possibility is that consumption is increased because a small dose of nicotine enhances the value of the nicotine-associated cue. Previous research has shown that nicotine non-contingently enhances the value of other reinforcers in the environment (Caggiula et al., 2009; Donny et al., 2003; Rupprecht et al., 2015). The cue conditions used in the present study have been paired with nicotine across many sessions. While 1.875 µg/kg/infusion may be below the threshold for primary reinforcement, it may be above threshold for the reinforcement enhancing effects of nicotine. Increased infusions at the FR1/1.875 combination may then reflect an increased value of the cue rather than responding for the nicotine per se. The experimental procedures here make it impossible to dissociate the primary reinforcing and reinforcement enhancing effects of the low nicotine dose.

5.0 GENERAL DISCUSSION

5.1 SUMMARY

The studies reported in this dissertation show that increasing nicotine cost and decreasing nicotine dose are not equivalent manipulations. Behavior was more sensitive to decreases in the dose of nicotine than to increases in the cost of nicotine. The largest decrease in consumption across all unit prices was greater for ratio escalation. When above threshold doses were used, behavior was maintained across a smaller range when cost was increased than when dose was decreased. The results of Aim 3 confirm that consumption is not equivalent at a single unit price regardless of how that unit price is created. Consumption is significantly lower at low FR/dose and high FR/dose combinations than at combinations with moderate FRs and moderate doses. Together, these results suggest that increases in the cost of a reinforcer and decreases in the magnitude of a reinforcer do not change behavior in the same way, and are not manipulations of the same factor, unit price.

5.2 BEHAVIOR IS MORE SENSITIVE TO DECREASES IN DOSE THAN TO INCREASES IN COST

It is unclear why rats are more sensitive to decreases in dose than to increases in cost across the range of prices that maintain behavior. One possibility is that the pharmacological effect of several small doses is not equivalent to one large dose of nicotine, even if they total the same total drug consumption. A series of small drug infusions differ in the overall rate of drug delivery, the volume of vehicle, and the pattern of delivery (continuous versus a series of infusions interspersed with breaks), any of which could result in a different pharmacological effect than a larger continuous bolus.

There is substantial evidence that the rate of drug delivery may be a critical factor in producing any given pharmacological effect, and especially in functioning as a primary reinforcer. The majority of previous research suggests that nicotine is less likely to maintain behavior when delivered over a longer duration. Wing and Shoaib (2013) showed that a dose of nicotine near the peak of the dose-response curve (30 µg/kg/infusion) only maintained behavior when it was delivered over a relatively short duration (0.5 and 1.0 s), and not when that duration was extended (5 s or 19.5 s). Another study showed that rhesus monkeys only self-administered nicotine when the infusion duration was short, but not when it was extended (Wakasa et al., 1995). Furthermore, the highly addictive nature of cigarettes is often attributed to the high rate of drug delivery associated with the route of administration (Benowitz, 1990). In contrast to these studies, one study showed that infusion duration had no effect in mice (Fowler & Kenny, 2011), and another study showed that rats self-administered lower doses of nicotine than previously thought possible when the infusion duration was lengthened (Sorge & Clarke, 2009).

The impact of infusion duration may apply more broadly to all drugs of abuse. Rapid drug delivery has been shown to increase the subjective effects of all drugs of abuse (Abreu, Bigelow, Fleisher, & Walsh, 2001; Marsch et al., 2001; Nelson et al., 2006). Rats have also been shown to escalate cocaine intake when infusions are delivered rapidly, but not when infusions are delivered slowly (Wakabayashi et al., 2010), and faster infusion durations maintain higher response rates in self-administration procedures (Balster & Schuster, 1973; Panlilio et al., 1998). Oxycodone has been shown to support self-administration in humans when it is delivered over a short duration as opposed to a long duration (Comer et al., 2009). Because the duration of drug delivery appears to be an important variable across multiple drugs abuse, the increased sensitivity of behavior to nicotine dose over nicotine cost may apply more broadly to other drugs of abuse.

Faster infusion durations may promote higher rates of drug self-administration through differences in pharmacokinetics or pharmacodynamics, or in differences in learning the contingency. A faster rate of cocaine delivery has been shown to produce a shorter time to peak concentration, but similar peak concentration, and similar concentrations several minutes after injection (Panlilio et al., 1998). These effects were similar when brain concentrations of cocaine were measured. The difference in time to peak drug concentration may affect brain areas that are involved in reward. Researchers have shown that shorter infusion durations increase c-*fos* and *arc* activation in the nucleus accumbens core and shell, and increase the inhibition of re-uptake of dopamine release in the nucleus accumbens core (Samaha, Mallet, Ferguson, Gonon, & Robinson, 2004). Woolverton and Wang (2004) also reported that shorter infusions increased the rate of dopamine transporter binding. A longer duration of reinforcer delivery may also function as a delay between the end of the response requirement and delivery of the reinforcer. Research

has shown that long delays in reinforcer delivery can interfere with learning a contingency, and may decrease responding for the reinforcer (Lattal, 2010).

While the duration of reinforcer delivery has not been investigated with reinforcers that are not drugs of abuse, there is some evidence that the timing of reinforcer delivery is an important variable even when the reinforcer is not a pharmacological drug. It is well established that a reinforcer loses subjective value as the delay to reinforcer delivery is increased, a phenomenon known as delay discounting (Bickel, MacKillop, Madden, Odum, & Yi, 2015; Madden, Begotka, Raiff, & Kastern, 2003). Rats will choose a shorter delay for food reward over a longer delay (Schindler et al., 2009). However, it is unclear whether a large reinforcer delivered after a delay (e.g., after completing a large FR) would have more subjective value than a reinforcer delivered in small increments that extend over a long delay. For example, would people choose to have $80 delivered after 80 days instead of $1/day for 80 days? It seems likely that people would choose to wait and receive the $80. Thus, it is unclear whether the increased sensitivity to dose reduction observed here would extend to non-drug reinforcers.

5.3 BREAKPOINT IS LOWER FOR RATIO ESCALATION THAN DOSE REDUCTION WHEN ABOVE-THRESHOLD NICOTINE DOSES ARE USED

In Aim 1 when above-threshold nicotine doses were used, the unit price that maintained consumption was lower for ratio-escalation than for dose-reduction. While there was not a significant difference between breakpoints in Aim 2, there was a trend such that more rats had lower breakpoints for ratio-escalation than for dose-reduction (14 rats had higher dose breakpoints, only four had higher ratio breakpoints). Furthermore, in both Aims 1 and 2, the

maximum instance of elasticity was larger for ratio-escalation than dose-reduction. Together, these results suggest that when cost is escalated, rats will reach a sudden breakpoint and consumption will drop drastically, whereas when dose is decreased rats reach a breakpoint later.

The ability of lower nicotine doses to maintain consumption at higher breakpoints may be related to differences in cue delivery. In the procedure used here, the cue light was delivered along with each nicotine infusion, so when dose is decreased, rats continue to receive frequent cue delivery. However, when cost is escalated, the cue is delivered less frequently because more responses are required to produce it. The cue likely has value because of its prior pairing with nicotine delivery (Pavlov, 1927). Thus, frequent cue delivery in the dose-reduction combinations may have maintained behavior at unit prices where behavior would not have been maintained by nicotine alone. Previous research has shown that nicotine-associated cues can maintain behavior for at least 55 sessions following substitution of saline for nicotine (Cohen et al., 2005).

Responding for the cue is also likely impacted by reinforcement enhancement. Previous research from our lab has shown that nicotine can noncontingently increase the value of reinforcers (Caggiula et al., 2009; Donny et al., 2003; Rupprecht et al., 2015). The enhancement is synergistic in that responding for nicotine + a cue is usually higher than responding for the cue alone and responding for nicotine alone added together. Thus, as nicotine dose is decreased, rats may continue to respond for a nicotine + cue combination because the low dose of nicotine has increased the value of the cue reward.

A lack of reinforcement enhancement may be the reason that the difference in breakpoints was less drastic in Aim 2. The dose-response relationship for nicotine dose and reinforcement enhancement is untested. Unpublished research from our lab has shown that there is a threshold nicotine dose for reinforcement enhancement, and increasing the nicotine dose

above that threshold does not result in a larger degree of enhancement. However, in this unpublished study nicotine was delivered subcutaneously, so it is not possible to determine which doses used in Aims 1 and 2 are above the threshold for reinforcement enhancement. If low doses of nicotine used in Aim 2 are not above threshold for reinforcement enhancement, then responding at very low nicotine doses may be only maintained by the secondary reinforcing characteristics of the cue along with any primary reinforcing characteristics of the low nicotine dose.

Although the inequity in cue delivery makes it impossible to assess whether breakpoints would have been similar for ratio escalation and dose reduction if the cue were removed, the present procedure in which the cue is delivered along with each infusion more closely models reinforcer delivery in the natural environment. Reinforcers are delivered in the context of other stimuli, which often take on reinforcing value (Conklin & Tiffany, 2002). When the magnitude of each reinforcer delivery is decreased, the presence of other stimuli is unlikely to change, so it is important to assess the equivalence of cost escalation and dose reduction in this context. If the magnitude of the reinforcer is decreased enough, extinction should take place and the cue should cease to have reinforcing value. The four sessions at each combination used in the present studies are likely not enough for extinction, so the studies here most closely resemble a scenario where extinction is not yet complete.

Cue delivery in the present set of experiments also more closely models a scenario of relevance for nicotine reduction. If a nicotine reduction policy is implemented, non-nicotine cues will continue to be delivered along with each smaller delivery of nicotine (Donny et al., 2012). For example, the taste and feel of a cigarette will continue to be paired with each puff of smoke even though the nicotine yield within each puff will be lower. However, when the price of

cigarettes is increased, the nicotine-paired cues are only delivered along with each nicotine delivery after the full cost has been paid (Smith et al., 2014). Thus, the cue conditions arranged in the present experiment are most appropriate for assessing the equivalence between cost escalation and dose reduction.

5.4 CONSUMPTION IS NOT EQUIVALENT ACROSS MULTIPLE COMBINATIONS OF THE SAME UNIT PRICE

Aim 3 showed that consumption is not equivalent at a single unit price regardless of the FR/dose combination used to create that unit price. These results conflict with predictions made by a behavioral economics framework that consumption is a function of unit price, and the FR or dose used for that unit price is not relevant.

Because FR and dose vary together in Aim 3, it is impossible to determine which factor is responsible for decreased consumption at low FR/dose and high FR/dose combinations. However, previous literature can provide some hypotheses. Low consumption at low FR/dose combinations is likely the result of decreased reinforcing value of low nicotine doses. Previous self-administration studies have shown that very low nicotine doses (1.875 and 3.75 µg/kg/infusion) produce lower responding than higher nicotine doses at the same FR (Smith et al., 2013), consistent with the interpretation that in Aim 3 low nicotine doses used in some combinations produced lower consumption. Also consistent with that hypothesis, consumption for a given dose of nicotine is generally higher when low FRs are used (Donny et al., 1998), making it unlikely that the low FRs used in these combinations are responsible for decreased consumption.

Low consumption at high FR/dose combination is likely the result of high FRs used in these combinations. Previous studies have shown that when nicotine dose is increased and FR is held constant, consumption increases (Donny et al., 1999; Donny et al., 2000). Thus, it is unlikely that the high doses contributed to decreased consumption in high FR/dose combination. However, it is well known that high FRs produce lower levels of consumption. Thus, it is likely that low FR/dose combinations produce lower consumption because of the low doses used, and high FR/dose combinations produce lower consumption because of the high FRs used.

5.5 PREVIOUS INVESTIGATIONS OF EQUIVALENCE BETWEEN REINFORCER COST AND REINFORCER MAGNITUDE

The present studies are the first to show that increases in the cost of a reinforcer and decreases in the magnitude of a reinforcer are not equivalent manipulations. There are several differences between this investigation and previous investigations in the analytic strategy that was employed. This investigation used a more rigorous strategy that previous investigations. First, this is the first set of studies to evaluate the two manipulations by fitting demand curves to consumption produced by the two manipulations. The majority of previous investigations have used the strategy undertaken in Aim 3—assessing whether consumption is the same at a given unit price regardless of the cost/dose combination used to assess it. Second, this is the first set of studies to require that consumption meet a margin of equivalence rather than to accept that no significant difference signified equivalence. Third, this is the first set of studies to compare breakpoints or the maximum instance of elasticity between the two manipulations.

The present studies are also the first to investigate this research question by directly manipulating nicotine. The majority of previous investigations have investigated other reinforcers. One other previous report has investigated this research question using human smokers by manipulating the number of cigarette puffs that could be earned (Bickel et al., 1991). However, manipulating the number of cigarettes puffs also manipulates non-nicotine cigarette constituents that may contribute to reinforcing value. Furthermore, manipulating the number of cigarette puffs manipulates delivery of nicotine-paired cues. In the present procedure, the magnitude of the cue was not directly manipulated, which may have contributed to the inequity between cost and dose observed here.

5.6 IMPLICATIONS FOR BEHAVIORAL ECONOMICS

The present results conflict with a fundamental assumption of the behavioral economics approach. Behavioral economics posits that sensitivity to unit price is a measure of "essential value," a construct that is inherent to any given reinforcer and can be used to compare reinforcers (Hursh & Roma, 2013; Hursh & Silberberg, 2008). The present results suggest that sensitivity to cost and sensitivity to dose are not equivalent, and therefore cannot be measures of the same construct, essential value.

The construct of "essential value" as measured by sensitivity to unit price has been posited as a measure drug abuse liability, and as an index if dependence (Bentzley et al., 2014; Hursh & Silberberg, 2008). The majority of previous research validating sensitivity to unit price as a measure of essential value has manipulated cost and held reinforcer magnitude constant (Hursh & Silberberg, 2008). Thus, it seems likely that for any reinforcer, sensitivity to cost may

be useful as an indicator of reinforcer value or even of dependence for drug reinforcers. Manipulations of reinforcer magnitude have not been validated as predicting constructs that might be associated with reinforcer value or drug dependence.

Fits of Equation 1 were good for both manipulations of nicotine cost and nicotine dose, suggesting that consumption is changed exponentially by both increases in nicotine cost and decreases in nicotine dose. These data suggest that behavioral economics may still provide useful tools for assessing how behavior is changed by both manipulations of cost and magnitude. Demand curves may by useful for understanding the relationship between consumption and either cost or reinforcer magnitude. Equation 1 may be useful for quantifying how consumption is changed by each manipulation. However, researchers should be wary of interpreting parameters as measures of any construct, and cannot make inferences about one manipulation based on information about the other manipulation.

5.7 IMPLICATIONS FOR NICOTINE REDUCTION

These data suggest that smoking behavior will not be changed in the same way when nicotine content is reduced as when the price of cigarettes is increased. Furthermore, an individual's sensitivity to changes in the price of cigarettes will be a poor predictor of that individual's sensitivity to decreases in the content of nicotine within a cigarette. However, we may be able to use information about the relationship between increasing cost and decreasing dose to provide us with some information about changes in behavior as a function of nicotine content.

Nicotine consumption is likely to be more sensitive to changes in nicotine content than to changes in the cost of cigarettes, at least over the range of prices that maintain smoking behavior. Prior research on cigarette taxation suggests that for every 10% increase in the price of cigarettes, there is a 4% reduction in cigarette consumption (Chaloupka & Warner, 1999). Thus, the ratio of the change in nicotine consumption to the change in nicotine content is likely to be greater than 0.4 for above-threshold nicotine contents.

The primary objective of nicotine reduction is to reduce the prevalence of smoking—or to reduce nicotine content beyond the unit price that maintains behavior. Results from these studies showed that when very low doses were used, there was a trend towards breakpoints being higher for the dose-reduction manipulation than for the ratio-escalation manipulation. However, this difference was not significant, so breakpoints for dose-reduction may be similar to or even higher than breakpoints for ratio-escalation. One study evaluated breakpoint for the price of cigarettes using a hypothetical purchase task in which participants estimated the number of cigarettes they would smoke if cigarettes were a variety of prices (MacKillop et al., 2012). Participants in these studies reported mean breakpoints of $3.49-$4.88 per cigarette for their usual brand cigarette depending on the cue conditions and the time since they had smoked. Assuming usual brand cigarettes yield approximately 1 mg of nicotine, and an average pack of cigarettes in the US is $6.36 (31.8 cents per cigarette) (World Lung Foundation & American Cancer Society, 2012) a unit price approach would estimate the threshold nicotine yield to be between 0.065 mg and 0.091 mg, depending on deprivation and cue conditions. Based on results presented in this dissertation, we would expect the breakpoint yields to be in that range or even lower. These values are consistent with previous research indicating the threshold nicotine yield may be between 0.05 and 0.1 mg (Donny & Jones, 2009; Hatsukami, Kotlyar, et al., 2010).

Finally, the results from Aim 3 suggest that very low doses and very high costs are likely to suppress consumption more than moderate doses and costs that create the same unit price. Thus, the most effective method for suppressing consumption is likely to combine very low nicotine content with very high costs. Nicotine reduction is likely to be most effective when combined with other tobacco control interventions that increase the cost of obtaining cigarettes.

5.8 FUTURE DIRECTIONS

Future studies should assess the generalizability of these results to other reinforcers. The present study suggests that increasing the cost of nicotine and decreasing the dose of nicotine are not equivalent manipulations. However, it is unclear whether the discrepancy between these results and equality found in previous studies is the result of differences in analytic strategy or differences in the reinforcer investigated. The present studies were the first to conduct a thorough analysis of how consumption is changed as a function of the two manipulations, and the first to directly manipulate nicotine.

It may also be beneficial to assess the role of cue-maintained responding in the relationship between increasing the cost of nicotine and decreasing the dose of nicotine. While the present experiments arranged the cue such that the results would be most relevant to the "natural environment," the underlying assumption of behavioral economics does not include a drug-paired cue that is delivered frequently for one manipulation and infrequently for the other manipulation. Thus, it may be beneficial to understand the role of the cue, if any, in the inequity seen in these experiments.

Future studies may examine whether the relationship between increasing nicotine cost and decreasing dose would be equivalent under other conditions. For example, a 3-s time out was employed in the present studies to prevent rats from taking a dangerous quantity of nicotine. However, the time-out may have played a critical role in the inequity between increasing cost and decreasing dose because rats were unable to earn infusions immediately following one another. Behavioral economics procedures are also typically conducted under extended access conditions, where the session is not limited by time. The present procedure allowed all rats to have two hours within the session to respond. Thus, it may be useful to assess whether increasing reinforcer cost and decreasing reinforcer magnitude are equivalent manipulations under extended-access conditions. However, even when session length is uncapped, time-outs are generally used following each infusion, creating an inequity between cost and dose because rats will have less time in a day to respond when dose is reduced than when cost is increased. The present procedure, in which session time is variable but the total duration of time-in is fixed, may actually allow cost and dose to be more equivalent than traditional procedures. Another potential avenue for future research is whether the two manipulations would have been equivalent, or closer to equivalent, if nicotine was manipulated through changes in the length of earn infusion instead of concentration of the nicotine solution. In this case, duration of nicotine delivery would have differed less between the two manipulations when consumption was equal, although it still would have differed by the length of time required to complete the FRs in the dose-reduction manipulation. However, the procedure used here where concentration of nicotine solution was manipulated is more relevant to nicotine reduction, where the concentration of nicotine within tobacco and within cigarette smoke will be reduced.

5.9 CONCLUSIONS

The present set of studies showed, for the first time, that increases in the cost of nicotine and decreases in the dose of nicotine are not equivalent manipulations. Rats were more sensitive to manipulations of nicotine dose than nicotine cost over the range of unit prices that maintain behavior, but when cost was increased rats reached a breakpoint at a lower unit price than when dose is reduced, and the maximum instance of elasticity was larger was ratio-escalation. Differences in the duration of nicotine delivery may have contributed to increased sensitivity to nicotine dose across a range of unit prices, and differences in cue delivery may have contributed to differences seen in breakpoint and in the maximum instance of elasticity. When very low nicotine doses were used for the dose-reduction manipulation, increases in nicotine cost and decreases in nicotine dose are still not equivalent manipulations, and there were significant differences in consumption at low unit prices and in estimated consumption if the reinforcer were free. Furthermore, at a single unit price, consumption was not equivalent across seven FR/dose combinations, and was significantly less when low nicotine doses or high FRs were used.

These data have important implications of the framework of behavioral economics and a nicotine reduction policy. They call into question a fundamental assumption within behavioral economics. While the results of the present study are specific to nicotine, they may have broader implications for other drugs of abuse or for reinforcers in general. Future researchers should investigate the generalizability of these results. These data also suggest that increases in the cost of cigarettes may not be thought of as functionally equivalent to decreases in nicotine content, and so we cannot use existing information regarding cigarette taxation to make predictions about a nicotine reduction policy.

6.0 BIBLIOGRAPHIC REFERENCES

Abreu, M. E., Bigelow, G. E., Fleisher, L., & Walsh, S. L. (2001). Effect of intravenous injection speed on responses to cocaine and hydromorphone in humans. *Psychopharmacology (Berl), 154*(1), 76-84.

Acker, J., & Mackillop, J. (2013). Behavioral economic analysis of cue-elicited craving for tobacco: a virtual reality study. *Nicotine Tob Res, 15*(8), 1409-1416. doi: 10.1093/ntr/nts341

Balster, R. L., & Schuster, C. R. (1973). Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. *J Exp Anal Behav, 20*(1), 119-129. doi: 10.1901/jeab.1973.20-119

Bardo, M. T., Green, T. A., Crooks, P. A., & Dwoskin, L. P. (1999). Nornicotine is self-administered intravenously by rats. *Psychopharmacology (Berl), 146*(3), 290-296.

Benowitz, N. L. (1990). Pharmacokinetic considerations in understanding nicotine dependence. *Ciba Found Symp, 152*, 186-200; discussion 200-189.

Benowitz, N. L., Dains, K. M., Hall, S. M., Stewart, S., Wilson, M., Dempsey, D., & Jacob, P., 3rd. (2012). Smoking behavior and exposure to tobacco toxicants during 6 months of smoking progressively reduced nicotine content cigarettes. *Cancer Epidemiol Biomarkers Prev, 21*(5), 761-769. doi: 10.1158/1055-9965.EPI-11-0644

Benowitz, N. L., Hall, S. M., Stewart, S., Wilson, M., Dempsey, D., & Jacob, P., 3rd. (2007). Nicotine and carcinogen exposure with smoking of progressively reduced nicotine content cigarette. *Cancer Epidemiol Biomarkers Prev, 16*(11), 2479-2485. doi: 10.1158/1055-9965.EPI-07-0393

Bentzley, B. S., Jhou, T. C., & Aston-Jones, G. (2014). Economic demand predicts addiction-like behavior and therapeutic efficacy of oxytocin in the rat. *Proc Natl Acad Sci U S A, 111*(32), 11822-11827. doi: 10.1073/pnas.1406324111

Bickel, W. K., DeGrandpre, R. J., Higgins, S. T., & Hughes, J. R. (1990). Behavioral economics of drug self-administration. I. Functional equivalence of response requirement and drug dose. *Life Sci, 47*(17), 1501-1510.

Bickel, W. K., DeGrandpre, R. J., Hughes, J. R., & Higgins, S. T. (1991). Behavioral economics of drug self-administration. II. A unit-price analysis of cigarette smoking. *J Exp Anal Behav, 55*(2), 145-154. doi: 10.1901/jeab.1991.55-145

Bickel, W. K., MacKillop, J., Madden, G. J., Odum, A. L., & Yi, R. (2015). Experimental manipulations of delay discounting & related processes: an introduction to the special issue. *J Exp Anal Behav, 103*(1), 1-9. doi: 10.1002/jeab.133

Caggiula, A. R., Donny, E. C., Palmatier, M. I., Liu, X., Chaudhri, N., & Sved, A. F. (2009). The role of nicotine in smoking: A dual-reinforcement model. . In R. A. Bevins & A. R. Caggiula (Eds.), *Nebraska Symposium on Motivation: The Motivational Impact of Nicotine and its Role in Tobacco Use* (Vol. 55, pp. 91-109). New York, NY: Springer Science + Business Media.

Carroll, M. E., Carmona, G. G., & May, S. A. (1991). Modifying drug-reinforced behavior by altering the economic conditions of the drug and a nondrug reinforcer. *J Exp Anal Behav, 56*(2), 361-376. doi: 10.1901/jeab.1991.56-361

Chaloupka, F., & Warner, K. E. (1999). The economics of smoking. *NBER Working Paper No. 7047*.

Chaloupka, F. J., Yurekli, A., & Fong, G. T. (2012). Tobacco taxes as a tobacco control strategy. *Tob Control, 21*(2), 172-180. doi: 10.1136/tobaccocontrol-2011-050417

Chase, H. W., Mackillop, J., & Hogarth, L. (2013). Isolating behavioural economic indices of demand in relation to nicotine dependence. *Psychopharmacology (Berl), 226*(2), 371-380. doi: 10.1007/s00213-012-2911-x

Clemens, K. J., Caille, S., Stinus, L., & Cador, M. (2009). The addition of five minor tobacco alkaloids increases nicotine-induced hyperactivity, sensitization and intravenous self-administration in rats. *Int J Neuropsychopharmacol, 12*(10), 1355-1366. doi: 10.1017/S1461145709000273

Cohen, C., Perrault, G., Griebel, G., & Soubrie, P. (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology, 30*(1), 145-155. doi: 10.1038/sj.npp.1300541

Collier, G. H., Johnson, D. F., Hill, W. L., & Kaufman, L. W. (1986). The economics of the law of effect. *J Exp Anal Behav, 46*(2), 113-136. doi: 10.1901/jeab.1986.46-113

Comer, S. D., Ashworth, J. B., Sullivan, M. A., Vosburg, S. K., Saccone, P. A., & Foltin, R. W. (2009). Relationship between rate of infusion and reinforcing strength of oxycodone in humans. *J Opioid Manag, 5*(4), 203-212.

Conklin, C. A., & Tiffany, S. (2002). Cue-exposure treatment: Time for change. *Addiction, 97*(9), 1219-1221. doi: DOI 10.1046/j.1360-0443.2002.00205.x

DeGrandpre, R. J., Bickel, W. K., Hughes, J. R., & Higgins, S. T. (1992). Behavioral economics of drug self-administration. III. A reanalysis of the nicotine regulation hypothesis. *Psychopharmacology (Berl), 108*(1-2), 1-10.

DeGrandpre, R. J., Bickel, W. K., Hughes, J. R., Layng, M. P., & Badger, G. (1993). Unit price as a useful metric in analyzing effects of reinforcer magnitude. *J Exp Anal Behav, 60*(3), 641-666. doi: 10.1901/jeab.1993.60-641

Diergaarde, L., van Mourik, Y., Pattij, T., Schoffelmeer, A. N., & De Vries, T. J. (2012). Poor impulse control predicts inelastic demand for nicotine but not alcohol in rats. *Addict Biol, 17*(3), 576-587. doi: 10.1111/j.1369-1600.2011.00376.x

Donny, E. C., Caggiula, A. R., Mielke, M. M., Booth, S., Gharib, M. A., Hoffman, A., . . . McCallum, S. E. (1999). Nicotine self-administration in rats on a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl), 147*(2), 135-142.

Donny, E. C., Caggiula, A. R., Mielke, M. M., Jacobs, K. S., Rose, C., & Sved, A. F. (1998). Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. *Psychopharmacology (Berl), 136*(1), 83-90.

Donny, E. C., Caggiula, A. R., Rowell, P. P., Gharib, M. A., Maldovan, V., Booth, S., . . . McCallum, S. (2000). Nicotine self-administration in rats: estrous cycle effects, sex differences and nicotinic receptor binding. *Psychopharmacology (Berl), 151*(4), 392-405.

Donny, E. C., Chaudhri, N., Caggiula, A. R., Evans-Martin, F. F., Booth, S., Gharib, M. A., . . . Sved, A. F. (2003). Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology (Berl), 169*(1), 68-76. doi: 10.1007/s00213-003-1473-3

Donny, E. C., Houtsmuller, E., & Stitzer, M. L. (2007). Smoking in the absence of nicotine: behavioral, subjective and physiological effects over 11 days. *Addiction, 102*(2), 324-334. doi: 10.1111/j.1360-0443.2006.01670.x

Donny, E. C., & Jones, M. (2009). Prolonged exposure to denicotinized cigarettes with or without transdermal nicotine. *Drug Alcohol Depend, 104*(1-2), 23-33. doi: 10.1016/j.drugalcdep.2009.01.021

Donny, E. C., Taylor, T. G., Lesage, M. G., Levin, M., Buffalari, D. M., Joel, D., & Sved, A. F. (2012). Impact of Tobacco Regulation on Animal Research: New Perspectives and Opportunities. *Nicotine Tob Res*. doi: 10.1093/ntr/nts162

English, J. A., Rowlett, J. K., & Woolverton, W. L. (1995). Unit-price analysis of opioid consumption by monkeys responding under a progressive-ratio schedule of drug injection. *J Exp Anal Behav, 64*(3), 361-371. doi: 10.1901/jeab.1995.64-361

Foster, T. A., & Hackenberg, T. D. (2004). Unit price and choice in a token-reinforcement context. *J Exp Anal Behav, 81*(1), 5-25. doi: 10.1901/jeab.2004.81-5

Fowler, C. D., & Kenny, P. J. (2011). Intravenous nicotine self-administration and cue-induced reinstatement in mice: effects of nicotine dose, rate of drug infusion and prior instrumental training. *Neuropharmacology, 61*(4), 687-698. doi: 10.1016/j.neuropharm.2011.05.012

Grace, R. C., Kivell, B. M., & Laugesen, M. (2014). Predicting decreases in smoking with a cigarette purchase task: evidence from an excise tax rise in New Zealand. *Tob Control*. doi: 10.1136/tobaccocontrol-2014-051594

Grebenstein, P. E., Burroughs, D., Roiko, S. A., Pentel, P. R., & LeSage, M. G. (2015). Predictors of the nicotine reinforcement threshold, compensation, and elasticity of demand in a rodent model of nicotine reduction policy. *Drug Alcohol Depend, 151*, 181-193. doi: 10.1016/j.drugalcdep.2015.03.030

Grebenstein, P. E., Burroughs, D., Zhang, Y., & LeSage, M. G. (2013). Sex differences in nicotine self-administration in rats during progressive unit dose reduction: implications for nicotine regulation policy. *Pharmacol Biochem Behav, 114-115*, 70-81. doi: 10.1016/j.pbb.2013.10.020

Hatsukami, D. K., Kotlyar, M., Hertsgaard, L. A., Zhang, Y., Carmella, S. G., Jensen, J. A., . . . Hecht, S. S. (2010). Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. *Addiction, 105*(2), 343-355. doi: 10.1111/j.1360-0443.2009.02780.x

Hatsukami, D. K., Perkins, K. A., Lesage, M. G., Ashley, D. L., Henningfield, J. E., Benowitz, N. L., . . . Zeller, M. (2010). Nicotine reduction revisited: science and future directions. *Tob Control, 19*(5), e1-10. doi: 10.1136/tc.2009.035584

Hursh, S. R. (1991). Behavioral economics of drug self-administration and drug abuse policy. *J Exp Anal Behav, 56*(2), 377-393. doi: 10.1901/jeab.1991.56-377

Hursh, S. R., Raslear, T. G., Shurtleff, D., Bauman, R., & Simmons, L. (1988). A cost-benefit analysis of demand for food. *J Exp Anal Behav, 50*(3), 419-440. doi: 10.1901/jeab.1988.50-419

Hursh, S. R., & Roma, P. G. (2013). Behavioral economics and empirical public policy. *J Exp Anal Behav, 99*(1), 98-124. doi: 10.1002/jeab.7

Hursh, S. R., & Silberberg, A. (2008). Economic demand and essential value. *Psychol Rev, 115*(1), 186-198. doi: 10.1037/0033-295X.115.1.186

Hursh, S. R., & Winger, G. (1995). Normalized demand for drugs and other reinforcers. *J Exp Anal Behav, 64*(3), 373-384. doi: 10.1901/jeab.1995.64-373

Ingersoll, K. S., & Cohen, J. (2005). Combination treatment for nicotine dependence: state of the science. *Subst Use Misuse, 40*(13-14), 1923-1943, 2043-1928. doi: 10.1080/10826080500294817

Jha, P., & Chaloupka, F. J. (1999). *Curbing the Epidemic: Governments and the Economics of Tobacco Control*. Washington, DC, USA: World Bank Publications.

Kelleher, R. T. (1966). Conditioned reinforcement in second-order schedules. *J Exp Anal Behav, 9*(5), 475-485. doi: 10.1901/jeab.1966.9-475

Koffarnus, M. N., Wilson, A. G., & Bickel, W. K. (2014). Effects of Experimental Income on Demand for Potentially Real Cigarettes. *Nicotine Tob Res*. doi: 10.1093/ntr/ntu139

Lamb, R. J., & Daws, L. C. (2013). Ethanol self-administration in serotonin transporter knockout mice: unconstrained demand and elasticity. *Genes Brain Behav, 12*(7), 741-747. doi: 10.1111/gbb.12068

Lattal, K. A. (2010). Delayed reinforcement of operant behavior. *J Exp Anal Behav, 93*(1), 129-139. doi: 10.1901/jeab.2010.93-129

Laxminarayan, R., & Deolalikar, A. (2004). Tobacco initiation, cessation, and change: evidence from Vietnam. *Health Econ, 13*(12), 1191-1201. doi: 10.1002/hec.932

MacKillop, J., Brown, C. L., Stojek, M. K., Murphy, C. M., Sweet, L., & Niaura, R. S. (2012). Behavioral economic analysis of withdrawal- and cue-elicited craving for tobacco: an initial investigation. *Nicotine Tob Res, 14*(12), 1426-1434. doi: 10.1093/ntr/nts006

MacKillop, J., Murphy, J. G., Ray, L. A., Eisenberg, D. T., Lisman, S. A., Lum, J. K., & Wilson, D. S. (2008). Further validation of a cigarette purchase task for assessing the relative reinforcing efficacy of nicotine in college smokers. *Exp Clin Psychopharmacol, 16*(1), 57-65. doi: 10.1037/1064-1297.16.1.57

MacKillop, J., & Tidey, J. W. (2011). Cigarette demand and delayed reward discounting in nicotine-dependent individuals with schizophrenia and controls: an initial study. *Psychopharmacology (Berl), 216*(1), 91-99. doi: 10.1007/s00213-011-2185-8

Madden, G. J., Begotka, A. M., Raiff, B. R., & Kastern, L. L. (2003). Delay discounting of real and hypothetical rewards. *Exp Clin Psychopharmacol, 11*(2), 139-145.

Marsch, L. A., Bickel, W. K., Badger, G. J., Rathmell, J. P., Swedberg, M. D., Jonzon, B., & Norsten-Hoog, C. (2001). Effects of infusion rate of intravenously administered morphine on physiological, psychomotor, and self-reported measures in humans. *J Pharmacol Exp Ther, 299*(3), 1056-1065.

Murphy, J. G., MacKillop, J., Tidey, J. W., Brazil, L. A., & Colby, S. M. (2011). Validity of a demand curve measure of nicotine reinforcement with adolescent smokers. *Drug Alcohol Depend, 113*(2-3), 207-214. doi: 10.1016/j.drugalcdep.2010.08.004

Nader, M. A., Hedeker, D., & Woolverton, W. L. (1993). Behavioral economics and drug choice: effects of unit price on cocaine self-administration by monkeys. *Drug Alcohol Depend, 33*(2), 193-199.

Nelson, R. A., Boyd, S. J., Ziegelstein, R. C., Herning, R., Cadet, J. L., Henningfield, J. E., . . . Gorelick, D. A. (2006). Effect of rate of administration on subjective and physiological effects of intravenous cocaine in humans. *Drug Alcohol Depend, 82*(1), 19-24. doi: 10.1016/j.drugalcdep.2005.08.004

Palmatier, M. I., Evans-Martin, F. F., Hoffman, A., Caggiula, A. R., Chaudhri, N., Donny, E. C., . . . Sved, A. F. (2006). Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. *Psychopharmacology (Berl), 184*(3-4), 391-400. doi: 10.1007/s00213-005-0183-4

Panlilio, L. V., Goldberg, S. R., Gilman, J. P., Jufer, R., Cone, E. J., & Schindler, C. W. (1998). Effects of delivery rate and non-contingent infusion of cocaine on cocaine self-administration in rhesus monkeys. *Psychopharmacology (Berl), 137*(3), 253-258.

Pavlov, I. P. (1927). *Conditioned Reflexes*. New York: Dover Publications.

Rupprecht, L. E., Smith, T. T., Schassburger, R. L., Buffalari, D. M., Sved, A. F., & Donny, E. C. (2015). Behavioral mechanisms underlying nicotine reinforcement. *Curr Top Behav Neurosci, 24*, 19-53. doi: 10.1007/978-3-319-13482-6_2

Samaha, A. N., Mallet, N., Ferguson, S. M., Gonon, F., & Robinson, T. E. (2004). The rate of cocaine administration alters gene regulation and behavioral plasticity: implications for addiction. *J Neurosci, 24*(28), 6362-6370. doi: 10.1523/JNEUROSCI.1205-04.2004

Schindler, C. W., Panlilio, L. V., & Thorndike, E. B. (2009). Effect of rate of delivery of intravenous cocaine on self-administration in rats. *Pharmacol Biochem Behav, 93*(4), 375-381. doi: 10.1016/j.pbb.2009.05.008

Shahan, T. A., Bickel, W. K., Badger, G. J., & Giordano, L. A. (2001). Sensitivity of nicotine-containing and de-nicotinized cigarette consumption to alternative non-drug reinforcement: a behavioral economic analysis. *Behav Pharmacol, 12*(4), 277-284.

Shahan, T. A., Bickel, W. K., Madden, G. J., & Badger, G. J. (1999). Comparing the reinforcing efficacy of nicotine containing and de-nicotinized cigarettes: a behavioral economic analysis. *Psychopharmacology (Berl), 147*(2), 210-216.

Smith, T. T., Levin, M. E., Schassburger, R. L., Buffalari, D. M., Sved, A. F., & Donny, E. C. (2013). Gradual and Immediate Nicotine Reduction Result in Similar Low-Dose Nicotine Self-administration. *Nicotine Tob Res*. doi: 10.1093/ntr/ntt082

Smith, T. T., Schaff, M. B., Rupprecht, L. E., Schassburger, R. L., Buffalari, D. M., Murphy, S. E., . . . Donny, E. C. (In Press). Effects of MAO inhibition and a combination of minor alkaloids, B-carbolines, and acetaldehyde on nicotine self-administration in adult male rats. *Drug and Alcohol Dependence*.

Smith, T. T., Sved, A. F., Hatsukami, D. K., & Donny, E. C. (2014). Nicotine reduction as an increase in the unit price of cigarettes: A behavioral economics approach. *Prev Med*. doi: 10.1016/j.ypmed.2014.07.005

Sofuoglu, M., & Lesage, M. G. (2012). The reinforcement threshold for nicotine as a target for tobacco control. *Drug Alcohol Depend, 125*(1-2), 1-7. doi: 10.1016/j.drugalcdep.2012.04.023

Sorge, R. E., & Clarke, P. B. (2009). Rats self-administer intravenous nicotine delivered in a novel smoking-relevant procedure: effects of dopamine antagonists. *J Pharmacol Exp Ther, 330*(2), 633-640. doi: 10.1124/jpet.109.154641

Sumpter, C. E., Temple, W., & Foster, T. M. (2004). Comparing demand functions when different price manipulations are used: does unit price help? *Learn Behav, 32*(2), 202-212.

Townsend, J., Roderick, P., & Cooper, J. (1994). Cigarette smoking by socioeconomic group, sex, and age: effects of price, income, and health publicity. *BMJ, 309*(6959), 923-927.

USDHHS. (2010). How tobacco smoking causes disease: The biological and behavioral basis for smoking-attributable disease: A report of the surgeon general (Executive Summary).

Valentine, J. D., Hokanson, J. S., Matta, S. G., & Sharp, B. M. (1997). Self-administration in rats allowed unlimited access to nicotine. *Psychopharmacology (Berl), 133*(3), 300-304.

Wakabayashi, K. T., Weiss, M. J., Pickup, K. N., & Robinson, T. E. (2010). Rats markedly escalate their intake and show a persistent susceptibility to reinstatement only when cocaine is injected rapidly. *J Neurosci, 30*(34), 11346-11355. doi: 10.1523/JNEUROSCI.2524-10.2010

Wakasa, Y., Takada, K., & Yanagita, T. (1995). Reinforcing effect as a function of infusion speed in intravenous self-administration of nicotine in rhesus monkeys. *Nihon Shinkei Seishin Yakurigaku Zasshi, 15*(1), 53-59.

Wing, V. C., & Shoaib, M. (2013). Effect of infusion rate on intravenous nicotine self-administration in rats. *Behav Pharmacol, 24*(5-6), 517-522. doi: 10.1097/FBP.0b013e3283644d58

Woolverton, W. L., & English, J. A. (1997). Further analysis of choice between cocaine and food using the unit price model of behavioral economics. *Drug Alcohol Depend, 49*(1), 71-78.

Woolverton, W. L., & Wang, Z. (2004). Relationship between injection duration, transporter occupancy and reinforcing strength of cocaine. *Eur J Pharmacol, 486*(3), 251-257. doi: 10.1016/j.ejphar.2004.01.003

World Lung Foundation & American Cancer Society (2012). *The Tobacco Atlas: Fourth Edition*. Atlanta, GA: Bookhouse Group, Inc.